

Joint effect of
freshwater plume and
coastal upwelling

Y.-F. Tseng et al.

Joint effect of freshwater plume and coastal upwelling on phytoplankton growth off the Changjiang River

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Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

Changjiang River discharges vast amount of unbalanced nutrients (dissolved inorganic nitrogen (N) and phosphorus (P) with $N/P > 80$ in general) into the East China Sea during summertime. To explore nutrient dynamics and P stress potential for phytoplankton, a cruise was conducted in the Changjiang plume during summer 2011. With 3-D observations of nutrients, chlorophyll *a* (Chl *a*), and bulk alkaline phosphatase activity (APA), we concluded that the Changjiang Diluted Water (CDW) and coastal upwelling significantly influenced the horizontal and vertical heterogeneities of phytoplankton P-deficiency in the plume. Allochthonous APA was detected at nutrient-enriched freshwater end. Excessive N (~ 10 to $112 \mu\text{M}$) was observed throughout the entire plume surface. In the plume fringe where featured by stratification and excess N, diapycnal phosphate supply was blocked to stimulate APA for phytoplankton growth. We observed upwelling outcrops just attaching the turbidity front at seaward side, where Chl *a* peaked yet much less APA was detected. An external phosphate supply from subsurface, which stimulated phytoplankton growth but inhibited APA, was suggested and the supply was likely sourced from the Nearshore Kuroshio Branch Current. In such hydrographically complicated Changjiang plume, phosphate supply instead of its concentration was more important determining the expression of APA. Meanwhile, allochthonous APA may also alter the usefulness of APA as a P-stress indicator.

1 Introduction

Rapid urbanization in past decades along coastline has introduced massive loadings of nutrients and organics to rivers causing dramatic changes of nutrient status in adjacent estuarine and coastal ecosystems (Jickells, 1998; Galloway et al., 2004). These rivers are well known to carry nutritional water with high ratios of dissolved inorganic nitrogen (DIN): dissolved inorganic phosphorus (DIP), or N/P ratios. Such massive yet disproportionate riverine nutrient input has caused an obvious “excess N” (the concentration

BGD

10, 10363–10397, 2013

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Joint effect of
freshwater plume and
coastal upwelling**Y.-F. Tseng et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

of DIN in excess of DIP) phenomenon in nearby estuarine ecosystems (Galloway et al., 2004). Ultimately, the continuing increases in N-loading are potentially going to transfer marine ecosystems from N-limitation to P-limitation. In fact, seasonal P-limitation is becoming evident in some estuarine and coastal ecosystems (Howarth and Marino, 2006). The altered nutrient dynamics would subsequently change the ecosystem structures and functions and threaten the safety of aquatic environments. Evidences also showed that unbalanced nutrient composition may stimulate the growth of harmful algae (Varkitzi et al., 2010) and modulate the amount of carbon sequestration due to the adjustment of cellular C/P ratios of plankton living in varying N/P conditions (Geider and Roche, 2002). The ambient nutrient concentrations that determine the exchange rate between the cell and its medium may vary along the mixing gradient from river to sea as processes such as uptake, transformation, remineralization, and diapycnal supply proceeded. The ecosystem responses to nutrient loading had been demonstrated; however, the information about the dynamic nutrient pattern of excess N versus the influence of nutrient stoichiometry on phytoplankton growth around riverine plume and adjacent coastal ecosystems require more explorations.

Under N excess condition, P generally limits phytoplankton growth in plume and coastal areas where light is sufficient. P-limitation in ecosystems can be determined by surrounding nutrient concentrations coupled with the N/P ratios in dissolved inorganic form (Krom et al., 1991; Dortch and Whitedge, 1992). However, most phytoplankton species have very high affinity with N and P, thus, the ambient N or P concentration may be below the analytical detection while growth is limited. Recently, a physiological indicator of phytoplankton P-deficiency – alkaline phosphatase (AP) activity (APA) has been widely used (Mather et al., 2008; Duhamel et al., 2010). AP is an inducible extracellular enzyme generally existed in freshwater and marine plankton that can cleave phosphate from organic P molecules when surrounding or intercellular phosphate concentrations are low (Perry, 1972). Growing evidence has reconfirmed that under P-depletion, phytoplankton could utilize DOP via generating AP (Hoppe, 2003). Such

characteristic makes APA as a good indicator of P-stress or P-deficiency of phytoplankton in aquatic ecosystems (Dyhrman and Ruttenberg, 2006; Lomas et al., 2010).

The East China Sea (ECS) is one of the largest marginal seas in the north-western Pacific Ocean. According to history records ECS was considered as a N-limited ecosystem as other marine ecosystems in the world (Chen et al., 2001). The Changjiang (CJ) River holds large water discharge (annual mean $\sim 924 \text{ km}^3 \text{ yr}^{-1}$; Beardsley et al., 1985; Tian et al., 1992) and high nutrient concentrations serving as the major nutrient sources to ECS (Zhang et al., 2007). Since 1950s, rapid growing anthropogenic activities such as fertilization application has caused $\sim 8\times$ increase in N concentrations and fluxes in CJ River (Wang, 2006; Li et al., 2007). In addition, CJ River is recorded to be a high N/P ratio system (Harrison et al., 1990; Liu et al., 2009) and “excess N” was found in surface water covering more than one-third of ECS in summer (Wong et al., 1998). Obviously, these increased riverine nutrients have induced major changes in nutrient stoichiometry in coastal zones (Chai et al., 2006) and seems that the increasing trend and the expansion of influenced areas are non-stopping. As a consequence, vast areas around CJ River mouth and ECS shelf are suggested to be P-limitation for phytoplankton growth (Harrison et al., 1990; Wang et al., 2003). Likely, such P-limitation will be more serious in plume regions during summer period when river discharge and productivity are both promoted.

In this study, the Changjiang plume (CJ plume) was selected to explore the consistency among chemical and physiological indicators such as phosphate concentration, N/P ratio, and APA in representing a P-limitation environment. To our knowledge, none study was conducted for the distributions of APA in this region, particularly, during flood period. Moreover, in summer time the Nearshore Kuroshio Branch Current (NKBC) brings nutrient-enriched subsurface water with low N/P ratio up onto ECS shelf even approaching CJ estuary (Chen et al., 2003; Yang et al., 2012, 2013). NKBC intrusion may compensate the excess N in plume region and further complicate the unbalanced nutrient status in CJ plume system. In this study, we presented the 3-D structures of hydrographic parameters, nutrients, Chl *a*, and applied APA assays to probe whether

BGD

10, 10363–10397, 2013

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



phytoplankton physiologically endure P-stress in such N-replete and hydrographically-complicated CJ plume region. A conceptual model was constructed and the properness of usage of various P-limitation indicators was discussed from different perspectives.

2 Materials and methods

2.1 Study area and sampling

Our cruise was conducted during 14–24 August 2011, just about one week after the invasion of super typhoon Muifa (27 July–9 August; highest wind speed $\sim 175 \text{ km h}^{-1}$; see more details in Hsiao et al., 2013). Total 28 sampling stations were set in the field of $30\text{--}32^\circ \text{ N}$ and $121\text{--}124^\circ \text{ E}$ (Fig. 1) where CJ plume significantly influenced. Five stations were deployed in the Qiantang (QT) estuary also. Vertical profiles of temperature, salinity, and fluorescence were recorded with a CTD profiler (SBE-9/11 plus, SeaBird) and packaged sensors at sampling interval of 3 Hz. Water samples were collected starting from $\sim 3 \text{ m}$ deep to the near bottom layer using a rosette sampler assembly with 10 L Go-Flo bottles for chemical analyses.

2.2 Nutrients, total suspended matter, and Chl *a* analyses

Water samples for nutrients analyses were filtered through Whatman GF/F filters and analysed immediately on board for DIN ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) and phosphate following the standard colorimetric protocols (Parsons et al., 1984). The analyses were conducted with a continuous flow injection autoanalyzer (Technicon AA3-HR; SEAL Analytical). The detection limits for DIN and phosphate with 1-cm Quartz tubes were $0.1 \mu\text{M}$ and $0.05 \mu\text{M}$, respectively. Chl *a* samples were filtered onto Whatman GF/F filters under pressure $< 100 \text{ mmHg}$ and then kept in liquid N_2 before analysis. In the laboratory, Chl *a* samples were extracted in dark at 4°C with 100% acetone for 12–16 h and then measured with a fluorometer (10-AU, Turner designs) using a non-acid method (Welschmeyer, 1994). Total suspended matter (TSM) samples were filtered

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



onto Whatman GF/F filters, folded and warped by pre-combusted aluminium foils after salt washing, and then freeze-dried for 24 h for weighing in laboratory.

2.3 Bulk APA

Samples for bulk APA assays were filtered through a 100 μm mesh to remove large zooplankton and then collected with 2 L acid-cleaned polycarbonate bottles. The zooplankton free samples were smoothly pre-concentrated onto 0.2 μm polycarbonate filters and then resuspended in 50 mL filtered seawater ($< 0.2 \mu\text{m}$) from the same sampling depth. APA was measured using a sensitive fluorometric protocol similar to Perry (1972) with 3-*o*-methylfluorescein phosphate substrate (3-*o*-MFP; Sigma). For each sample, 6 mL triplicate sample waters and 0.75 mL 3-*o*-MFP substrate were mixed in a 10 mL glass tube to yield a final substrate concentration of $\sim 250 \text{ pmol mL}^{-1}$. Then samples were incubated in dark at 25 $^{\circ}\text{C}$ for 4 h. We use a fluorometer (TD-700; Turner Designs) equipped with a set of filters (excitation wavelength = 475 nm; emission wavelength = 515 nm) to determine 3-*o*-MF concentrations initially and after 4 h incubation.

3 Results

3.1 Spatial distribution patterns of measured parameters in the surface CJ plume

Surface temperature ranged from 23.9 $^{\circ}\text{C}$ to 29.5 $^{\circ}\text{C}$ decreased eastward basically with the highest value in the river mouth (Fig. 2a). Two cold center were observed at around the stations Y3 and Y12, respectively. Surface salinity (0.3–30.5) showed an eastward increasing pattern with the lowest value in CJ River mouth (Fig. 2b). The distributions of DIN and phosphate were quite similar (Fig. 2c, d). For DIN, the concentrations decreased from $> 120 \mu\text{M}$ near the river mouth (Fig. 2c) to $< 10 \mu\text{M}$ in the plume fringe with salinity of ~ 30 (Fig. 2b and c). For phosphate, $> 2 \mu\text{M}$ can be seen near the river mouth

BGD

10, 10363–10397, 2013

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



and then decreased significantly towards the plume fringe to $< 0.1 \mu\text{M}$ (Fig. 2d). Surface TSM ranged from as high as $170\text{--}592 \text{ mg L}^{-1}$ near CJ River mouth then decreased rapidly then jumped again at salinity ~ 17 and then decreased offshore (Fig. 2e). Surface excess N (excess N = $[\text{DIN}] - 16 \times [\text{Phosphate}]$) existed throughout the whole study area with > 100 values in CJ estuary and < 10 values near the plume fringe (Fig. 2f). For biological parameters Chl *a* and APA, we observed distinctive patchy distributions (Fig. 2g, h). For Chl *a* ($2.20\text{--}24.97 \text{ mg Chl m}^{-3}$; Fig. 2g), we saw the first bloom patch appeared at the river mouth (station Y0b) and the second bloom appeared in the center (stations Y9 and Y18). The landward boundary of Chl *a* patch was found to parallel with the turbidity front (red dashed curve in Fig. 2g). The surface distribution of APA (Fig. 2h) differs from those of nutrient and Chl *a*. Higher APA values were found in the river mouth (9.7 nM h^{-1} at station Y0) as well as the plume fringe (11.5 nM h^{-1} at station Y7). Note that around the outer plume the isopleth of 2 nM h^{-1} of APA was closely related to the isolines of $0.1 \mu\text{M}$ phosphate and $10 \mu\text{M}$ excess N. Note that a clear front for TSM was observed in shallow water at around $5\text{--}10 \text{ m}$ (see bathymetry in Fig. 1b), and then two outcrops of low temperature happened with N–S direction along the 20 and 30 m isobaths with concentrated Chl *a* and low APA. Such distribution pattern was likely driven by hydrodynamics due to rugged topographic changes underneath.

From salinity, nutrient, and TSM distribution patterns we can see QT River mouth is atypical, with high nutrients accompanying with high salinity. The extremely high TSM ($1812\text{--}2990 \text{ mg L}^{-1}$) appeared in the shallow river mouth of QT River indicating a strong resuspension had occurred, while both Chl *a* and APA remained low. In Sect. 3.3, we will have more discussion for parameters for QT River versus the entire water body. Below, we illustrated the distributions along the offshore plume transect.

3.2 Offshore transect along CJ plume

During summer, the Changjiang Diluted Water (CDW) flows predominantly eastward (Lie et al., 2003). Therefore, CJ transect (see Fig. 1b, stations Y0–Y6) was chosen to illustrate the influence of CDW plume on the distributions of environmental parameters.

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



High temperature and low salinity water was restricted in the river mouth (Fig. 3a, b). From salinity pattern, we saw clearly wedge-like plume dispersing outward. The stratification was created by the density difference when the bottom depth deeper than 20 m. A feature of upwelling intrusion, as mentioned earlier caused by NKBC, can be judged by the bulged thermocline around stations Y9 and Y9a where topographic change is significant (Fig. 3a). DIN showed a vertically well-mixed pattern in nearshore shallow water, yet, a significant vertical difference with lower DIN in surface and higher DIN in deep can be observed at plume fringe. Phosphate concentrations were observed $> 0.2 \mu\text{M}$ throughout the entire section (Fig. 3d) except the surface of outer plume ($< 0.2 \mu\text{M}$). Interestingly, phosphate pattern resembled that of temperature showing an intrusion-like pattern but outcropping between the stations Y2 and Y9. TSM in freshwater end was $170\text{--}261 \text{ mgL}^{-1}$, it increased dramatically to $> 2000 \text{ mgL}^{-1}$ within 45 km. This turbidity maximum occurred at salinity around 15–20 near stations Y0d and Y0e (Fig. 3e). Note that TSM dropped to $< 10 \text{ mgL}^{-1}$ between stations Y2 and Y9 where low temperature water came up from the subsurface. Excess N existed throughout the entire transect except the bottom of outer plume (stations Y6 and Y7; Fig. 3f). Zero excess N indicated an external supply of phosphate from the bottom to compensate the imbalanced nutrient ratios of this plume system. Two clear Chl *a* blooms developed, one at the river mouth stations (Y0a and Y0b) and the other near the surface of upwelling outcrops (Y2 through Y9a). The latter one held concentrations more than 16 mgChl m^{-3} in the patch centers (Fig. 3g). APA also showed patchy distribution; however, locations of APA patches were inconsistent with those of Chl *a* (Fig. 3g, h). High APA values emerged in the river mouth (station Y0) and the surface of plume fringe (stations Y6 and Y7). Note, high surface APA in the plume fringe coexisted with low phosphate concentrations while excess N still high (Fig. 3d, f, h).

3.3 Parameters along the salinity gradient

In this section, we illustrated TSM, DIN, phosphate, N/P ratio, Chl *a*, and APA distributions along the salinity gradient over the entire water body we monitored. In Fig. 4a, we

can see high TSM, so called turbidity maximum, at moderate salinity. The high TSM zone for QT estuary distributed in a wider salinity range (5–21) comparing with that in CJ estuary (17–21). Nevertheless, a sharp TSM front appeared at salinity of ~ 21 .

In Fig. 4b, c, DIN and phosphate basically displayed linear decreasing trends when salinity ranged from 0 to 20, indicating that conservative mixing predominated the nutrient status at low salinity side. However, the trend in QT estuary was above that of CJ estuary at the given salinity. When the salinity is greater than 21, scattered data points with significant drawdown of DIN and phosphate can be seen. The phosphate uptake was apparent, yet, high phosphate values can be seen at salinity of 34.5 occupied by P-replete bottom water.

In Fig. 4d, we can see N/P ratios decreased continuously as salinity increased. Theoretically, if the plume mixed with nutrient-free water during plume dispersion outward, the ratios would not be salinity dependent. However, the N/P ratios descended from 70 at freshwater end to ~ 16 at salinity of ~ 34.5 . At salinity ~ 22 –32, N/P ratios surged even up to ~ 200 . In Fig. 4e, we saw QT estuary samples hold significantly lower Chl *a* comparing with that of CJ estuary at given salinity probably due to light inhibition. While for CJ estuary samples, a sharp increase can be observed in Chl *a* from salinity of 0 to 5, where TSM remained at low concentrations. Chl *a* decreased afterward toward higher salinity of 20–22, meanwhile, TSM reached its maximum. Note that Chl *a* peaked again at salinity of ~ 22 to 32 and gradually decreased toward high salinity end of 34.5.

In Fig. 4f, we can see high APA values at both low and high salinity ends. Similar to Chl *a*, QT estuary presented lower APA when compared with CJ estuary. At the freshwater end of CJ estuary, interestingly, high APA accompanies with low Chl *a*; this high APA was apparently allochthonous from CJ upstream. From salinity of 0 through 5, APA decreased as the increasing of both Chl *a* and salinity. When salinity greater than 22, APA occupied a wide range from 0 to 12 nMh^{-1} . Note that APA values surged at salinity of 22–32, where higher N/P ratios and Chl *a* had occurred; at the high salinity side the low values of N/P ratio, Chl *a*, and APA were from deeper water samples.

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

4 Discussion

4.1 P-limitation potential from system aspect

The regions we focused were mainly influenced by CDW (CDW was defined as salinity < 3–32; Gong et al., 1996; Fig. 2b) with high nitrate concentrations and N/P ratios (Lie et al., 2003; Shen and Liu, 2009). Excess N was found throughout the entire study water column except the bottom of peripheral plume (Figs. 2f and 3f), implying that P will be used up earlier than N when Redfield uptake (Redfield, 1958) is applied as phytoplankton growth.

In terms of system perspective, the entire CDW-influenced shelf areas will endure P-stress ultimately if no external P supply was provided during dispersion. However, the upwelling phenomenon caused by topography uplifting near stations Y9 and Y9a (Fig. 3a) and a zero excess N in the bottom of plume fringe (Fig. 3f) indicated some extra P supply to this system. According to temperature (< 21 °C) and salinity (> 34) characteristics of the bottom water at plume fringe (Fig. 3a, b), we suspected this external P source is from the Kuroshio Intermediate Water, which contains high nutrient but low N/P ratio (~ 14), may fuel the primary production of CJ plume during summer (Zhang et al., 2007). In fact, the intrusion of the Kuroshio Intermediate Water near CJ plume was first reported by Kondo (1985). Chen (1996) further addressed the significance of this external P source for ECS productivity. By using the Regional Ocean Modeling System (ROMS) and observational data, Yang et al. (2012) well proved bottom-hugging NKBC would transport phosphate-rich water during summer from the southern tip of ECS toward north over the shelf to our study area around 30.5° N (Fig. 1a). According to the discussions above, such external long-range P supply from subsurface will make up the P shortage of plume systems. Besides CJ plume, in northeastern Monterey Bay of the coastal California, Mackey et al. (2012) also reported that coastal upwelling transported additional phosphate to the surface water and caused 10-fold increase of phosphate concentration to draw down the N/P ratio to ~ 5.

**Joint effect of
freshwater plume and
coastal upwelling**

Y.-F. Tseng et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[⏪](#)[⏩](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

From Fig. 5a, we plot DIN against phosphate for all data points. Two significant linear relations with regression coefficient $R^2 > 0.85$ were obtained. The slopes were as high as 69 and 125, respectively, for CJ and QT, and the regression lines have intercepts at phosphate axis. Meanwhile, we added a line of N/P of 16 for reference and found many datapoints in high salinity region (salinity of 22–34.5) fell above the reference line following roughly the N/P slope of 16. The two samples collected at the boundaries of CJ and QT turbidity fronts were taken as the beginning source (see Fig. 5a), then, we assumed CDW front waters started to mix with oligotrophic surface water (zero nutrient) as the plume expansion. Consequently, two conservative mixing lines can be obtained (solid lines in Fig. 5a). If any biological Redfieldian uptake had happened in the water parcel on the mixing lines (16 : 1 reference arrow and star symbol in Fig. 5a), the distribution of observed datapoints should fall into the left zone of the mixing lines, where N availability (N excess) was higher. Yet, only limited datapoints fell into the left of the mixing lines implying biological uptake indeed happened. These datapoints surely will create very high N/P ratios. Note that most datapoints fell into the right zone indicating an extra contribution from water source with lower N/P ratios.

We blowed up the distribution field of low concentration samples (blue triangles in Fig. 5b) for discussion. If we mix the two frontal waters (CJ front, QT front) with a hypothetical source, i.e., NKBC (Fig. 5b), we may obtain the gray zone to represent the full field of mixing products from the three endmembers. The hypothetical source was supported by Yang et al. (2012), who found that NKBC was primarily sourced from the 120–250 m of the Kuroshio Intermediate Water, of which nitrate and phosphate contents were reported to be 11–17 μM and 0.7–1.1 μM , respectively (Chen et al., 1995). Based on this mixed water mass, most of the datapoints can be explained when Redfieldian uptake was followed (star in Fig. 5b). This estimate reconfirmed the importance of P supply from the subsurface in CJ plume region.

As we mentioned in the Introduction, the N versus P fluxes had increased 8 times. Here we attempt to give an estimate for limiting nutrient on system scale by considering the nutrient inputs from NKBC and CJ plume. According to Yang et al. (2012, 2013),

**Joint effect of
freshwater plume and
coastal upwelling**Y.-F. Tseng et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[⏪](#)[⏩](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

the modeled and observational W–E cross section near CJ plume revealed a 100 km wide 20 m thick bottom hugging current with northward velocity of 0.5 m s^{-1} . This gave a flow of 1 Sv (Q_{NKBC}), which is slightly higher than that (0.4 Sv) given for intrusion flow of the Kuroshio Intermediate Water by Chen et al. (1995). In contrast, the peak CJ water discharge (Q_{CJ}) was reported to be $50\,000 \text{ m}^3 \text{ s}^{-1}$ (0.05 Sv), which equaled to that applied in ECS box model by Chen (1996). By utilizing the mean nutrient reported in 1960s and 2008 and assuming NKBC persisted at the same magnitude over the past, we can estimate the fractional contributions of N and P from CJ River as well as the N/P ratios in 1960s and 2008 (Table 1). The estimated fractional contribution of N from CJ River increased from 8–19 to 36–58 % in past 4 decades. Similarly, P contribution from CJ River increased from 1–4 to 11–24 %. The importance of nutrient supply from CJ River had increased significantly, however, NKBC is still the most important P supplier to this area. This estimate is rough, however, telling us that CJ plume system has a tendency toward P-limitation and it seems that we have passed the threshold of 16 (N/P = 19–25 in present day, Table 1).

Standing on this point, we recognized that understandings about water exchange and input–output budget of nutrients in the entire summer plume system are crucial since the supply and relative contribution of N and P from CJ River and NKBC are changing seasonally and interannually, thus, the nutrient status and stoichiometry. Therefore, we can hardly say CJ plume system was P-limited even N-excess phenomenon was significant during our summer cruise. On the other hand, evidences of high APA in the surface near the plume fringe may only tell us that P-limitation had occurred at that specific site where P consumption rate is greater than P supply rate at that specific time period (see Fig. 8, conceptual model). Nevertheless, Chen et al. (2001) found that N instead of P in summer time limited phytoplankton growth over entire ECS where surface salinity > 32 . Their nutrient addition bioassay experiments further supported the importance of NKBC in P supply in term of system perspective.

4.2 Joint effect of CDW and coastal upwelling on phytoplankton P-stress in CJ plume

P-limitation of phytoplankton has been observed in other over-exploited coastal ecosystems. For instance, Sylvan et al. (2006) founded that excess N loading to the Mississippi River watershed has periodically enhanced the P-limitation in the downstream Louisiana Shelf. Fisher et al. (1992) also reported that during the period of high runoff in spring and autumn, the Chesapeake Bay was limited by phosphate with the observation of high N/P ratios, high APA, and short P turnover time. Phytoplankton growth in the surface water of the Mediterranean Sea during the stratified summer season was also limited by P-availability (Vaulot et al., 1996). However, aforementioned studies applied bioassays and APA rather than solely depended on N/P ratios or absolute concentration. In fact, previous studies had indicated that nutrient standing stock should not be taken as a reliable indicator of nutrient limitation/availability (Dodds, 2006; Turner and Rabalais, 2013).

In this study, significant excess N, detectable P, and APA were observed throughout the water column. Their spatial patterns and distributions against salinity gradient were either coupled or decoupled with Chl *a*. The complicated distributions were attributable to the hydrodynamically associated processes, such as freshwater plume with allochthonous input, TSM resuspension, and coastal upwelling. Below we try to entangle the complex correlations among parameters associated with phytoplankton growth.

Low Chl *a* was observed in QT estuary with extremely high TSM (Fig. 2e, g) implying phytoplankton growth was completely inhibited in QT estuary. Thus CJ transect was used for discussion. For the inner estuary of CJ transect, we sampled one single depth from Y0 through Y1 due to shallow water depth, thus, not allowing us to discuss depth profiles. Beside turbid estuary, vertical profiles for stations Y2 through Y6 with salinity > 22 were shown in Fig. 6.

For the inner estuary, APA decreased as the increasing of salinity (Fig. 4f) indicating that dilution of allochthonous APA had occurred; meanwhile, increase in Chl *a* con-

BGD

10, 10363–10397, 2013

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



centrations had happened at the same time (Fig. 4e). Under such condition of high nutrient availability, the inverse relation between APA and Chl *a* indicated phytoplankton growth did not suffer from P-limitation. As the plume kept dispersing seaward, Chl *a* was dropped down gradually as TSM jumped up (Fig. 4a), underlining the importance of light availability to phytoplankton growth. In highly anthropogenic influenced coastal ecosystem, turbidity is one of the major factors restricting phytoplankton growth by reducing light transmission in water column (Chen et al., 2004; Pei, et al., 2009). Therefore, phytoplankton do not bloom in turbid coast where excess nutrients are supplied. Our observation showed that the Chl *a* patch attached to the turbidity front (red dashed curve; Fig. 2g) providing strong support that light could limit the bloom development near CJ coastal stations. The result is consistent with the previous study of light limitation in turbid estuaries (Cloern, 1999).

Contrary to Chl *a*, APA also increased seaward and remained fairly high when compared with those samples in outer plume (Fig. 4f). Such high APA in turbidity maximum zone was likely sourced from heterotrophic bacteria instead of phytoplankton for digesting dissolved organic matters (DOM) in such nutrient-enriched but light-limited environments. Many studies have confirmed that bacteria would excrete AP to utilize the carbon from DOM in order to satisfy their C-demand instead of P-demand (Kirchman et al., 2000; Wambeke et al., 2002). The non-phytoplankton induced APA subsequently caused high Chl *a* normalized APA (APA_{Chl}) in CJ estuary under high phosphate status.

As mentioned earlier, when salinity > 21 the water contained less TSM (Fig. 4a), then Chl *a* and APA started to build up (Fig. 4e, f). Below, we discuss the vertical distribution of phosphate and biological parameters. Meanwhile, we used APA_{Chl} for representing the P-stress on cell physiological level. From the vertical structures in Fig. 6, we saw low Chl *a* but high phosphate for station Y2 throughout the water column. Yet, APA was above 2 nMh^{-1} . This APA we believed was partly from bacteria and partly from allochthonous APA since this station just located at the TSM front (Fig. 2e). Nevertheless, phytoplankton might have some contribution in surface samples according to

BGD

10, 10363–10397, 2013

**Joint effect of
freshwater plume and
coastal upwelling**

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



higher Chl *a* was observed, however, it is difficult for us now to separate the contribution from phytoplankton.

As for stations Y9 and Y9a, water column APA decreased seaward (Fig. 6). Likely, the effect of allochthonous APA still remained at station Y9. Amazingly, APA was almost non-detectable at station Y9a throughout the water column. The blank APA zone can be seen in Fig. 3h also. In contrast to APA, Chl *a* was very high in the surface and it decreased rapidly as water depth increased, reasonably been controlled by light availability. Moreover, phytoplankton activity was going deeper at station Y9a (judging by Chl *a* concentrations), being farther away from the turbidity front. Interestingly, in the surface water at these two stations with very high phytoplankton activity, phosphate concentrations were both around $\sim 0.2 \mu\text{M}$, which already reached the P-limitation criterion of $0.2 \mu\text{M}$ (Dortch and Whitledge, 1992); however, the bloom was unlikely P-limited according to low APA. Such high Chl *a* co-existed with low phosphate concentrations (also seen in Fig. 3d, g), indicating that phytoplankton would rapidly consume phosphate during the formation of algal blooms. This fast nutrient consumption would also cause high N/P ratios in the surface (70–125; Fig. 4d).

In fact, phytoplankton bloom contributed by harmful algae has been frequently recorded near stations Y9 and Y9a since 2000 (Shen et al., 2011; Liu et al., 2013). The enhanced nutrient concentrations caused by increasing anthropogenic activities had stimulated red tide outbreaks around the plume fronts (Zhao et al., 2004; Chai et al., 2006; Tang et al., 2006; Wang, 2006; Zhou et al., 2008) where both light and nutrient are sufficient for algae growth. At stations Y9 and Y9a, TSM concentrations decreased dramatically (Fig. 3e) while nutrient concentrations still remained (Fig. 3c, d) forming an ideal condition for phytoplankton growth. Additionally, the hydrodynamic convergence around the plume front may facilitate algae aggregation (Tang et al., 2006; Zhou, 2010).

For stations Y7 and Y6, Chl *a* values were high in the surfaces but not as high as in stations Y9 and Y9a surfaces, and phytoplankton activities were going more deeper comparing with station Y9a according to Chl *a* concentrations (Fig. 6). On the contrary,

BGD

10, 10363–10397, 2013

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

APA values in the surfaces at stations Y7 and Y6 were much higher than that at stations Y9 and Y9a. For stations Y7 and Y6 surfaces, phosphate concentrations were lower than $0.1 \mu\text{M}$. Meanwhile, clear downward decreasing patterns in APA can be seen. Phosphate profiles mirror imaged with that of APA also illustrated the importance of phosphate supply from subsurface. Comparing with station Y9a, APA of deeper water samples was higher at these two plume fringe stations. In fact, we observed subsurface nitrite and ammonium maximums at around 20 m deep (Hsiao et al., 2013) implying more intensified biological activities had occurred in subsurface layer. Therefore, we hypothesized that APA in deepwater samples was induced by bacteria relied on sinking particles from the surface.

Based on above discussions we concluded that in coastal zone APA_{Chl} can be altered by allochthonous APA, while in upwelling zone P supply might compromise P consumption to maintain the bloom even though the standing stock of phosphate stayed low. In plume fringe, APA was enhanced by limited phosphate supply due to stratification. Such deficient concentrations of phosphate induced phytoplankton producing AP to obtain additional P from DOP for sustaining their growth. Therefore, excess N coupled with low phosphate concentrations, high N/P ratios, and high APA_{Chl} provided here make a strong case for P-stress in CJ plume fringe.

Around CJ plume, *Prorocentrum donghaiense*, *Skeletonema costatum*, *Prorocentrum dantatum*, and *Noctiluca scientillan* are observed as the major red-tide species (Liu et al., 2013). Among these species, *P. donghaiense* holding high affinity with DOP can adapt well to P-limited conditions (Ou et al., 2008). Huang et al. (2005) had confirmed that *P. donghaiense* can utilize DOP by producing AP to sustain their growth. Accordingly, *P. donghaiense* was predominant in offshore P-limited stations (Liu et al., 2013). In our study, we observed high APA in phosphate-depleted surface water at plume fringe (Fig. 2d, h), although we do not have DOP measurements, previous study indicated that DOP concentrations was sufficient at the plume fringe (0.15 to $0.25 \mu\text{M}$; Hung et al., 2003).

4.3 Applicability of P-deficient indicators in CJ plume

Phosphate concentration, N/P ratio, and APA_{Chl} are broadly used for describing the P-deficiency of phytoplankton (Krom, 1991; Dyhrman et al., 2006, Mather et al., 2008; Duhamel et al., 2010). The applicability of these three parameters as P-deficient indicators was discussed by using Fig. 7, in which only surface data were applied. Also, we separated all surface samples into three categories, turbidity-influenced, upwelling-influenced, and plume fringe. Theoretically, APA is produced while phosphate concentration is depleted (Hoppe, 2003). Therefore, an inverse expression of APA and phosphate concentration is expected; in our case, apparently, CJ River discharged vast amount of phosphate and allochthonous APA to alter this correlation (open circles in Fig. 7a). On the other hand, upwelled phosphate though rapidly consumed may inhibit APA formation even in the condition of phosphate $< 0.2 \mu\text{M}$, the criterion suggested by Dortch and Whitedge (1992).

Hu et al. (1989) specified that plankton growth is P-limited when surrounding N/P ratios are higher than 30 (reference vertical line in Fig. 7b) based on in situ enrichment experiment. If the ratio is applicable, we should observe a positive correlation between APA and N/P ratio. However, APA results seemed to refute the appropriateness of N/P ratio as P-limitation indicator since low APA values corresponding to high N/P ratios (20–120) caused by upwelling (Fig. 7b). Even a pseudo positive relation was seen for turbidity-influenced samples, we clearly know this relation was caused by allochthonous APA input. In the past, most of researchers used N/P ratio to circumscribe the chemically-defined space for P-limitation of phytoplankton in CJ estuary and ECS. Therefore, it should be careful when only use N/P ratio for identifying nutrient status in CJ estuary.

On the other hand, an inverse expression of APA_{Chl} and phosphate concentrations is expected (Perry, 1972; Dyhrman and Ruttenberg, 2006). In our study, such negative correlation was basically observed for turbidity-influenced samples (Fig. 7c). APA_{Chl} and phosphate concentrations presented out of synchronization in the upwelling region

BGD

10, 10363–10397, 2013

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



since the supply and biological consumption of phosphate are equally fast. Therefore, even APA_{Chl} would not be a reliable indicator for tracing P-stress of phytoplankton in dynamic water. Similarly, no correlation can be found between APA_{Chl} and N/P ratio (Fig. 7d). To now, all chemical and physiological indicators were evident to be improper in such system affected jointly by freshwater plume and upwelling. Raymont (1980) proposed that the presence of remaining nitrate together with undetectable phosphate in surface water within euphotic zone could be used as a major line of evidence for P-limitation in marine waters. In this study, only two among the three stations in the plume fringe offered credible evidence for P-limitation. Moreover, this evidence happened only on the surface.

4.4 Conceptual model for CJ plume

According to the interpretation of field data, we proposed a conceptual scheme of phytoplankton growth dynamics under the joint effect of CDW and coastal upwelling over CJ plume (Fig. 8). In the river mouth end surface turbidity is not high, thus surface phytoplankton growth is substantially independent of light confinement. In this area some phosphate is even released into the water from sediment resuspension. APA in this section is mainly transported by the river-side source. The APA may be mainly produced by bacteria and independent of phosphate regulation. When the river water disperses seaward the plume encounters energy from ocean, TSM reaches the maximum. In such turbid area light is the priority limiting factor controlling phytoplankton growth. The slightly increasing APA is likely released from the suspended particles. At the junction front of CDW and seawater, sudden drop in topography allows the reduction in turbidity, meanwhile, the upwelling outcrops. In this transition zone CDW and upwelled NKBC co-dominate the nutrient status that phytoplankton in the upwelling zone is frequently immersed in additional phosphate supply from subsurface for easing the P-deficiency and sustaining the bloom. Simultaneously strong biological activities accelerate the utilization of phosphate and promote the N/P ratios up to extremely high values. In the plume fringe, strong stratification inhibits the upward transportation

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



of nutrient. Meanwhile, biological activities continuously consume the available phosphate leading to serious P-limitation. In such P-deficient area, phytoplankton will turn to utilize ambient DOP by increasing APA to overcome the P-deficiency. In addition, dead phytoplankton are decomposed via zooplankton grazing and bacterial remineralization, producing abundant nitrite and ammonia accumulated in the subsurface (Hsiao et al., 2013).

Harrison et al. (2008) has proposed a conceptual diagram of phytoplankton growth in the Pearl River estuary. He pointed out that in a large number of phytoplankton growth period, the running out of phosphate and the remaining nitrogen potential may enhance P-limitation. Physiologically, phytoplankton are not P-deficient during blooming period since APA_{Chl} is low. The P-stress occurs mainly during late stage of blooming. However, in our case, we found that algal bloom appeared in contact off the turbidity front and the lasting of algae blooms mainly due to the rich phosphate supply from the upwelling induced by the NKBC. In CJ plume case we suggest that the scale of Chl *a* bloom and phytoplankton P-deficiency were closely associated with the upwelling intensity, which might be controlled by the size of CDW (Chen, 2000).

Besides nutrient-replete plume, in southern ECS, oligotrophic surface water, Liu et al. (2010) explored the P-stress of microphytoplankton in the transition boundary between upwelling and the Kuroshio Current by using APA and the maximum quantum efficiency of photosynthesis (F_v/F_m) assays. Similarly, low APA was observed in the surface of transition boundary; however lateral mixing rather than diapycnal mixing served as P-supply. Although their study area located in the southern ECS more than 500 km from CJ plume, both studies agree well that physical process plays a major role in APA expression. Note that, the values of APA_{Chl} in our study are much lower than those reported in the southern ECS due to methodology. In our study, we analyzed the bulk APA by concentrating the water sample directly; while their analysis in ECS was majorly for large phytoplankton collected by phytoplankton net. More studies are required to discern the major contributors to APA and Chl *a* to explore phytoplankton driven P dynamics.

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

5 Conclusion

Combining 3-D structures of nutrient concentrations and ratios, Chl *a*, and APA parameters, our results specified that phytoplankton growth in CJ plume with excessive N was basically controlled by light availability and P supply, while the coastal upwelling from NKBC is an important phosphate source. A conceptual scheme of Chl *a*, APA, and nutrient dynamics under the joint effect of CDW and coastal upwelling over CJ plume was constructed. Allochthonous APA and upwelled P strongly influenced the applicability of APA and APA_{Chl} as physiological P-stress indicators. Neither phosphate concentration nor N/P ratio was proper as a chemically-defined P-stress indicator also. Only limited surface area in the plume fringe suffered from P-limitation with excess N, where diapycnal P supply was prohibited and phytoplankton growth was low. The question “P-limitation” turns to be a system-dependent question. Nevertheless, more studies, such as the qualitative cell-specific assay, enzyme labelled fluorescence (ELF) method, were encouraged to examine the real contributors responsible for APA. We also suggested that the scale of Chl *a* bloom and phytoplankton P-deficiency in CJ plume were closely associated with the upwelling strength of NKBC, which might be regulated by the size of CDW. Based on our estimation and reasonable assumption, CJ plume is likely passing over the threshold of P-limitation in term of system perspective. If anthropogenic nutrient inputs continually increase, CJ plume and potentially ECS shelf will be opposite to other common oceans becoming P-limitation in the future.

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BGD

10, 10363–10397, 2013

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



References

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Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

- Mather, R., Reynolds, S., Wolff, G., Williams, R. G., Torres-Valdes, S., Woodward, E. M. S., Landolfi, A., Pan, X., Sanders, R. W., and Achterberg, E.: Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres, *Nat. Geosci.*, 1, 439–443, 2008.
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Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Varkitzi, I., Pagou, K., Graneli, E., Hatzianestis, I., Pyrgaki, C., Pavlidou, A., Montesanto, B., and Economou-Amilli, A.: Unbalanced N:P ratio and nutrient stress controlling growth and toxin production of the harmful dinoflagellate *Prorocentrum lima* (Ehrenberg) Dodge, Harmful Algae, 9, 304–311, 2010.
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5

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Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

Table 1. Estimations of the fractional contributions of N and P from the Changjiang River in 1960s and 2008.

Year	Q_{CJ}	Q_{NKBC}	N_{CJ}	N_{NKBC}	P_{CJ}	P_{NKBC}	N/P	CJ contribution	
	(Sv)		(μM)					% N	% P
1960s	0.05	0.4	25	13.5	0.3	1	16	19	4
1960s	0.05	1.0	25	13.5	0.3	1	15	8	1
2008	0.05	0.4	150	13.5	2.5	1	25	58	24
2008	0.05	1.0	150	13.5	2.5	1	19	36	11

Q_{CJ} : runoff of Changjiang (CJ); Q_{NKBC} : transport of the Nearshore Kuroshio Branch Current (NKBC); N_{CJ} : DIN for CJ; N_{NKBC} : DIN for NKBC; P_{CJ} : phosphate for CJ; P_{NKBC} : phosphate for NKBC.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)

[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)


Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

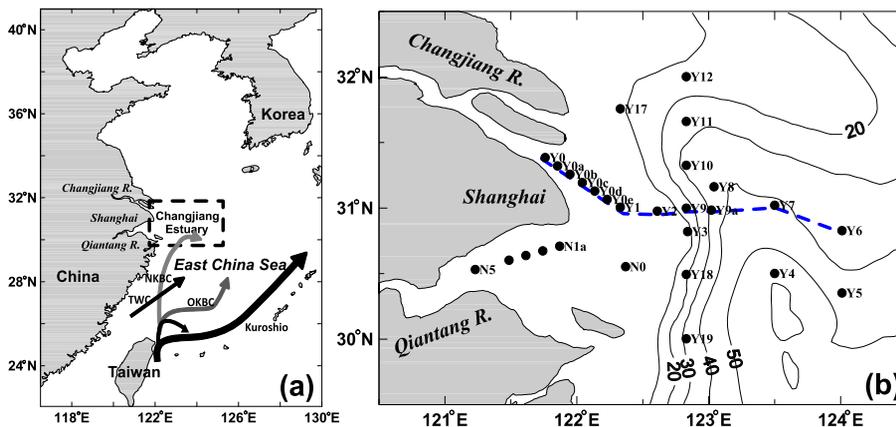


Fig. 1. Location maps of the study site of the Changjiang Estuary and the East China Sea **(a)** and sampling stations with bathymetry (m, solid curves) off the Changjiang River mouth **(b)**. CJ transect (dashed line) comprises estuary stations from Y0 through Y11, then Y2, Y9, Y9a, Y7, and Y6.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

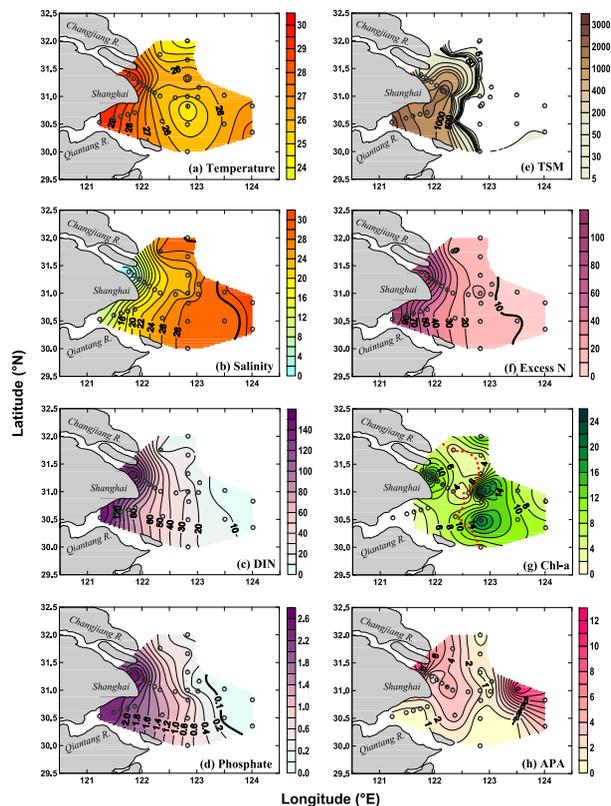


Fig. 2. Spatial distributions of parameters in surface water (3 m) in the Changjiang plume. **(a)** Temperature ($^{\circ}\text{C}$), **(b)** salinity, **(c)** DIN (μM), **(d)** phosphate (μM), **(e)** TSM (mgL^{-1}), **(f)** excess N, **(g)** Chl a (mgChl m^{-3}), and **(h)** APA (nMh^{-1}). The red dashed curve stands for TSM isoline of 50 mgL^{-1} .

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

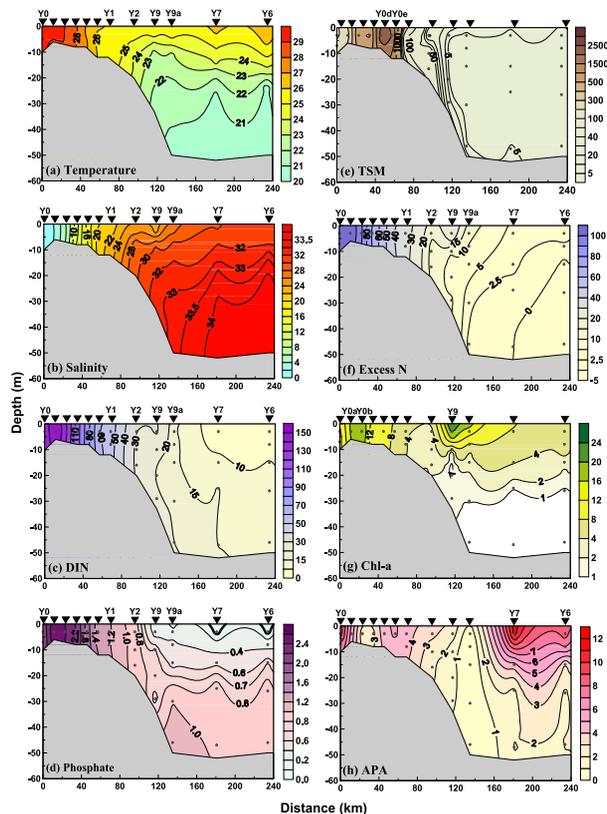


Fig. 3. Vertical distributions of parameters along CJ transect. **(a)** Water temperature ($^{\circ}\text{C}$), **(b)** salinity, **(c)** DIN (μM), **(d)** phosphate (μM), **(e)** TSM (mgL^{-1}), **(f)** excess N (μM), **(g)** Chl *a* (mgChlm^{-3}), and **(h)** APA (nMh^{-1}). The dots denote the discrete sampling over various depths. The inverted triangles denote the stations.

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

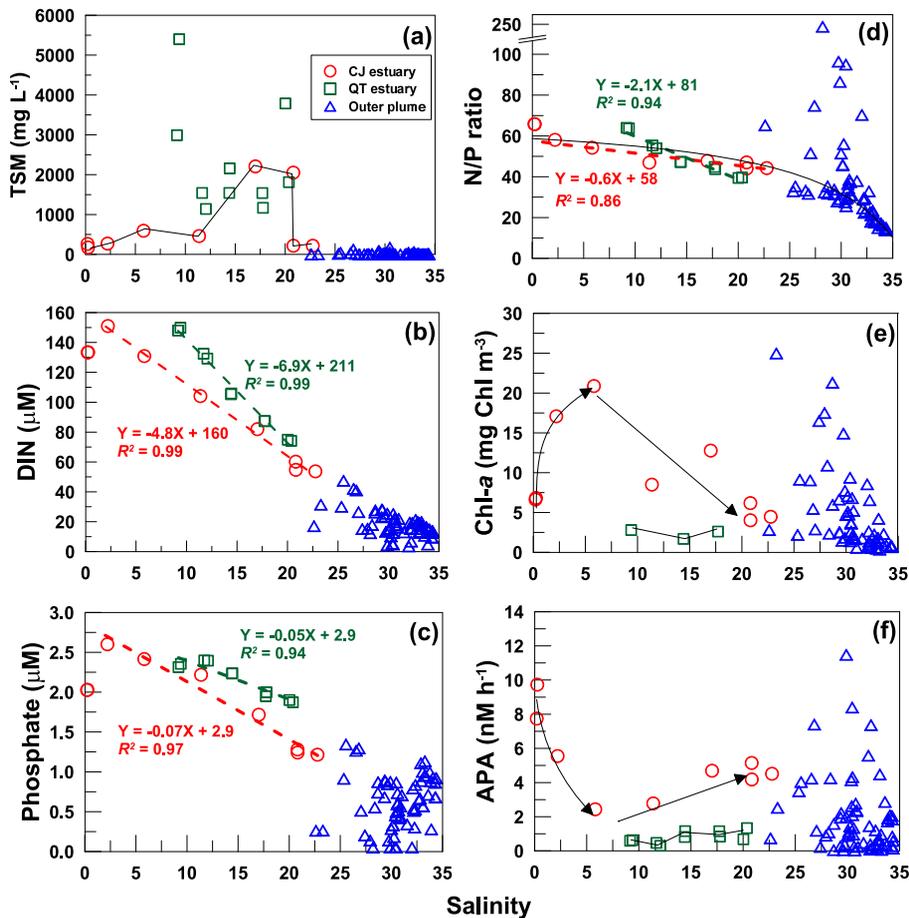


Fig. 4. The variations of TSM (a), DIN (b), phosphate (c), N/P ratio (d), Chl-a (e), and APA (f) in whole water column along estuarine salinity gradient over CJ plume.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

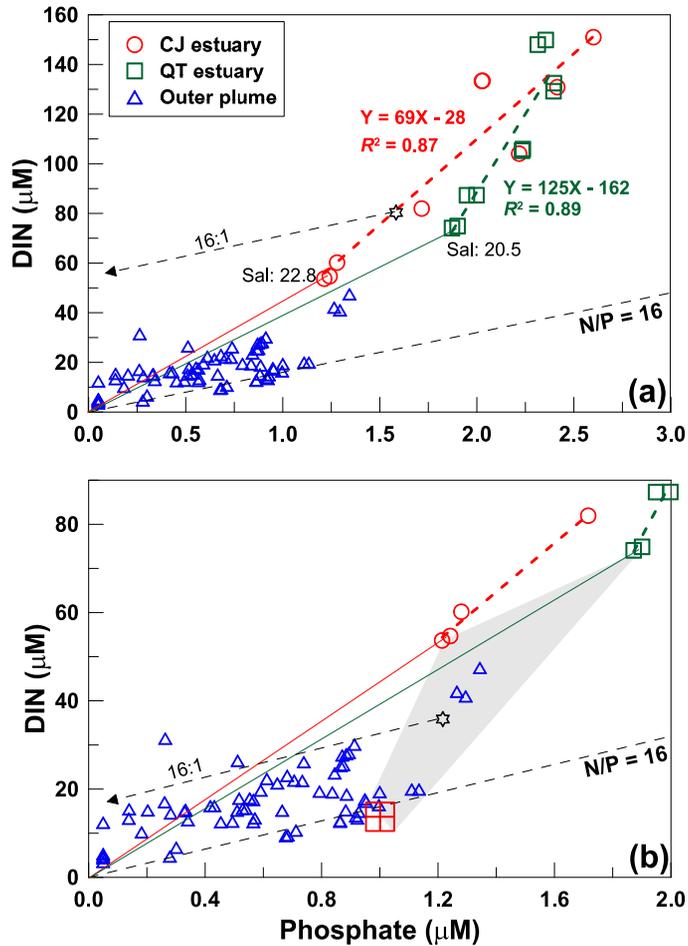


Fig. 5. Correlations between DIN and phosphate in whole water column **(a)** and low concentration samples **(b)** of CJ plume.

Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

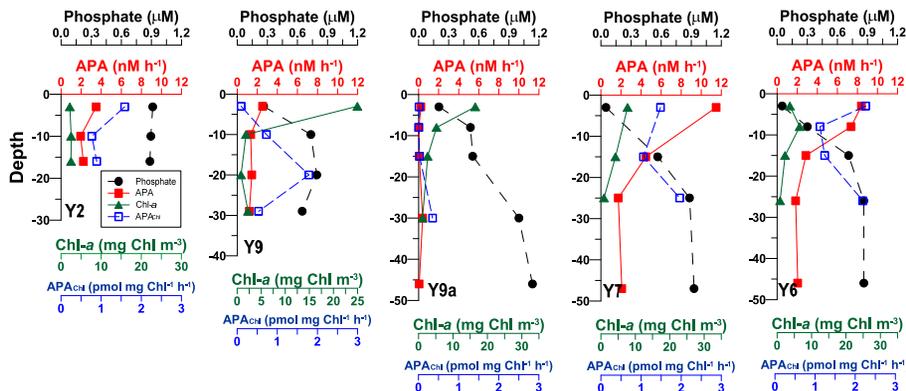


Fig. 6. Vertical profiles of phosphate, APA, Chl *a*, and APA_{Chl} for stations along CJ transect off the turbidity front.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Joint effect of freshwater plume and coastal upwelling

Y.-F. Tseng et al.

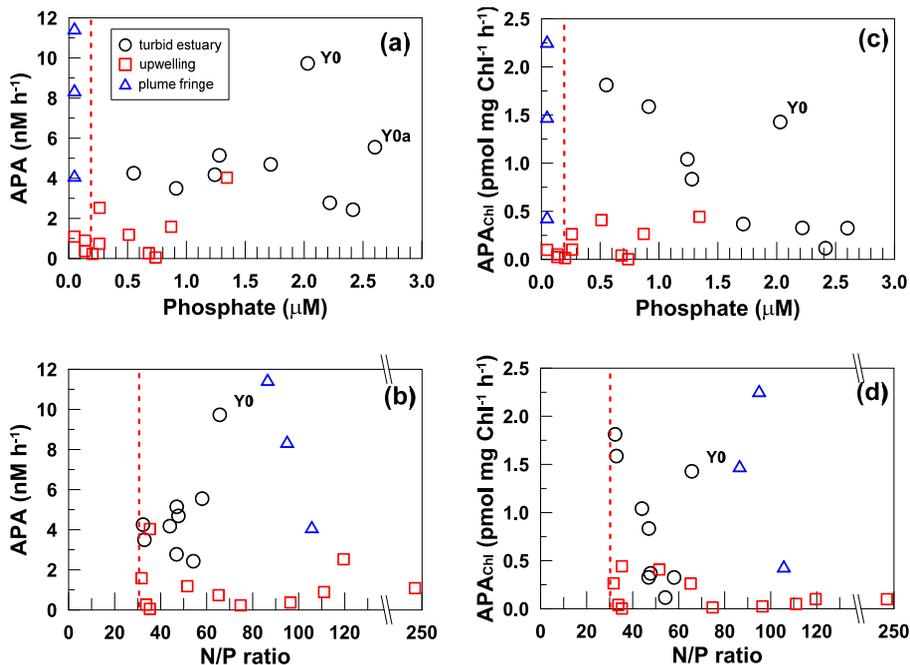


Fig. 7. Scatter plots for APA against phosphate **(a)** and N/P ratio **(b)** and Chl *a* normalized APA (APA_{Chl}) against phosphate **(c)** and N/P ratio **(d)** for surface samples except Qiantang estuary. The stations influenced by turbidity were represented by open circles. Red squares and blue triangles represent upwelling-influenced and plume fringe stations, respectively.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

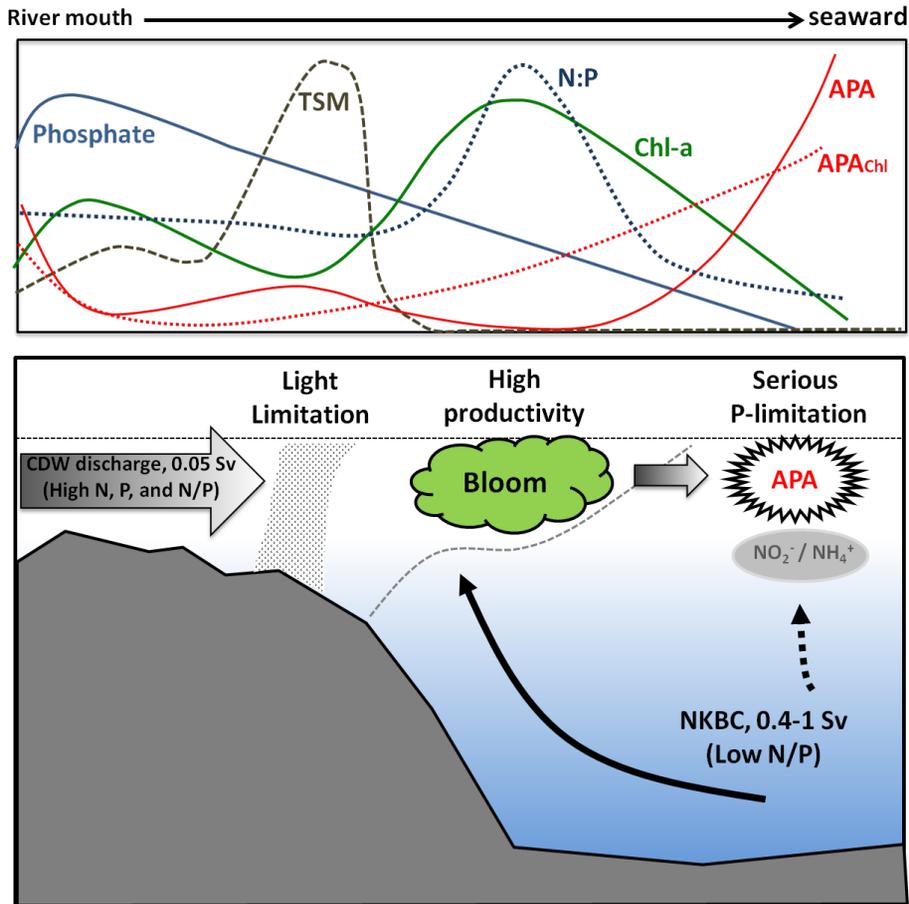


Fig. 8. Conceptual diagram for the interaction between the Changjiang Diluted Water (CDW) and coastal upwelling NKBC on algal bloom and P-limitation of the Changjiang plume. The offshore variations of associated parameters are shown on the top.