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Nitrogen fixation in the East China Sea and southern Yellow Sea during summer 2006

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ABSTRACT: Nitrogen fixation in the subtropical East China Sea (ECS) and the southern Yellow Sea (YS) were measured using $^{15}N_2$ tracer assay during June and July 2006. Depth-integrated nitrogen fixation (2 to 221 µmol N m^{-2} d^{-1}) was highest in the oceanic main path of the Kuroshio Current in the northeastern ECS, and in the mesohaline (surface salinity 30 to 34) stations. Very little N_2 fixation was encountered in the low-salinity (surface salinity <30) area, and we suggest that N_2 fixation was hindered by the nutrient conditions (lack of 'excess' phosphate relative to nitrate, $xsPO_4 = [PO_4] - [NO_3]/16$) near the Changjiang (Yangtze River) mouth. In the mesohaline waters, N_2 fixation was positively correlated with the vertical density (σ_t) gradient in the upper water column (30 m), indicating that N_2 fixation can also be controlled by physical regime, and enhanced water column stratification may promote N_2 fixation during summer. N_2 fixation met 0.01 to 4.6% of nitrogen demand by primary production, suggesting that N_2 fixation was not a major contributor to primary production in the study area. Estimated new N flux by N_2 fixation to the ECS continental shelf (13 Gg N) during summer is at the same order of magnitude as atmospheric deposition, but much lower than either the Kuroshio subsurface water upwelling or the Changjiang riverine input.

KEY WORDS: Nitrogen fixation · East China Sea · Southern Yellow Sea · $^{15}N_2$ assay

INTRODUCTION

Marine pelagic N_2 fixation is of great importance because it not only provides significant levels of combined nitrogen to the environment, but also regulates the sequestration of atmospheric CO_2 to a large extent (Karl et al. 2002). Recent studies suggest that N_2 fixation may be much more widespread in marine environments than previously thought, thus its importance in nitrogen biogeochemical cycles may have been underestimated, especially in the tropical and subtropical marginal sea areas (Voss et al. 2006, Subramaniam et al. 2008, Riemann et al. 2010).

The subtropical East China Sea (ECS) is the largest marginal sea in the western Pacific with a broad continental shelf. The ECS and the adjacent Yellow Sea (YS) are bounded by the Chinese mainland to the west, the Kuroshio on the slope side, and the Korean Peninsula to the northeast (see Fig. 1). The Kuroshio, the Changjiang (Yangtze River) runoff, and the East Asia monsoons are the dominant factors affecting the circulation in the ECS (Liu et al. 2003). The Kuroshio, a strong western boundary current of the Pacific Ocean (the northeasterly flowing continuation of the Pacific North Equatorial Current) with a width of about 100 km, flows northeastward approximately along the 200 m isobath into the ECS and plays a key role in the current system and nutrient budget for the ECS continental shelf (Chen et al. 1995, Chen et al. 1999, Lee & Matsuno 2007). To the west, the Changjiang, the third largest river in terms of freshwater discharge in the world, empties large nutrient loads into the shelf waters in wet seasons (Tian et al. 1993, Zhang 1996, Ichikawa & Beardsley 2002). In summer,

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the southwest monsoon may extend the plume area affected by the Changjiang Diluted Water (CDW).

N_2 fixation in the ECS and the YS, 2 marginal seas bordering the western Pacific, has rarely been studied, especially for directly measured rates. Previous studies have shown that *Trichodesmium* spp. is relatively abundant in the Kuroshio water and the adjacent ECS continental shelf water (Nagasawa & Marumo 1967, Marumo & Asaoka 1974, Marumo & Nagasawa 1976, Saino 1977, Minagawa & Wada 1986, Chang et al. 2000, Suzuki et al. 2007, Shiozaki et al. 2010). On the ECS shelf and the bordering YS, *Trichodesmium* has been found almost all year round and occasionally forms blooms (Zhou 1962, Chen 1982, Yang 1998, Chang et al. 2000, Wang 2002, Yan et al. 2002, Bai et al. 2007, Ding 2009). The diatom-symbiotic cyanobacteria *Richelia intracellularis* are also present in the Kuroshio-influenced ECS (Ding 2009, Shiozaki et al. 2010). Stable nitrogen isotope (^{15}N) evidence from either the blooming organisms (Minagawa & Wada 1986) or nitrate (Liu et al. 1996) indicate N_2 fixation in the ECS.

Unfortunately, directly measured rates of N_2 fixation in the ECS are sparse and confined to part of the Kuroshio water in the vicinity of the Japanese islands (Saino 1977, Shiozaki et al. 2010). To our knowledge, no N_2 fixation rates have been reported for the YS. To improve basic understanding of the biogeochemical cycle in the marginal seas, it is important to measure N_2 fixation in the ECS and the YS. In this study, we measure N_2 fixation and consider some possible physico-chemical controlling factors in the ECS and the YS during summer. In addition, the importance of new nitrogen provided by N_2 fixation in supporting local primary production and the nitrogen budgets on the continental shelf during summer will be evaluated.

MATERIALS AND METHODS

Sampling

A total of 18 stations were occupied along 5 transects in the ECS and the southern YS (25 to 35° N) from 28 June to 15 July (southwest monsoon season) 2006 on board RV 'Dongfanghong 2'. The southernmost station S1010 (1769 m) is located in the Kuroshio main path, northeast of Taiwan, while the northernmost (35° N) transect S03 is located in the southern YS, and the remaining stations are located on the shallow shelf with a depth less than 100 m in the ECS (Fig. 1). Seawater samples were generally collected from standard depths (mainly from 1, 10, 30, 50, and 75 m) at 14 stations, using Go-Flo bottles mounted on a CTD rosette. At Stn S1010, seawater was only sampled for the upper 50 m due to the sampling constraints, which may lead to an underestimated depth-integrated rate. At Stns S0402, S0404, FJ07, and S0504, only surface water (about 1 m) was sampled. Note that at Stn S1006, primary production was sampled only at the surface. Nutrient concentrations were not measured at Stn FJ07.

Incubation experiments

N_2 fixation was measured using the $^{15}N_2$ tracer assay (Montoya et al. 1996). In brief, duplicate sam-

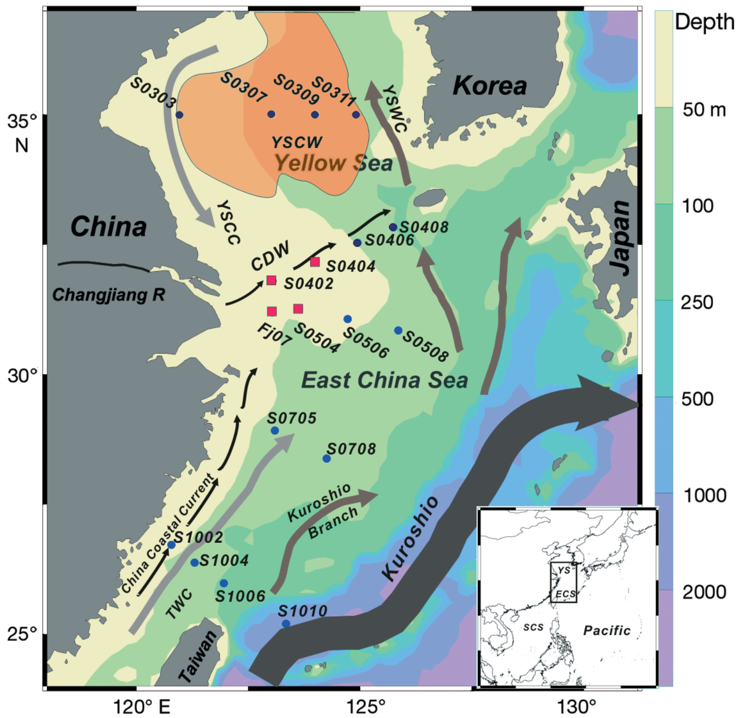


Fig. 1. Sampling locations (n = 18) in the East China Sea (ECS) and the southern Yellow Sea (YS) during summer 2006. The symbols indicate the stations that were either (●) vertically sampled or (■) only surface-sampled. The arrows represent the schematic major currents in summer: Kuroshio, the Kuroshio branch, the Taiwan Warm Current (TWC), the China Coastal Current, the Changjiang Diluted Water (CDW), the Yellow Sea Coastal Current (YSCC) and the Yellow Sea Warm Current (YSWC). The locations of the currents are taken from Guan (1994). The orange shaded area in the Yellow Sea represents the approximate maximum extent of the Yellow Sea Cold Water (YSCW) during summer (Weng et al. 1989)

ples were filled bubble-free into 600 ml transparent glass bottles and sealed, then spiked with 1.0 ml $^{15}N_2$ (99 atom% ^{15}N) via a septum using a gastight syringe (VICI, 1 ml), with the pressure across the septum balanced by another syringe. The bottles were gently shaken for several minutes before incubation. Primary production was measured using the ^{14}C method (Wolfe & Schelske 1967). Water samples were immediately transferred into three 125 ml glass bottles, two assigned as light bottles and one as dark, and 0.8 μCi $NaH^{14}CO_3$ was added to each bottle. All of the bottles for N_2 fixation and primary production measurements were fitted with appropriate screens to simulate the light densities (Table 1) and incubated on deck in flowing surface water. After 24 h incubation, N_2 fixation and primary production samples were gently (<100 mm Hg) filtered onto precombusted (450°C, 4 h) Whatman GF/F filters or mixed cellulose ester membranes (0.2 μm), respectively, before being stored frozen. Natural ^{15}N abundance in suspended particulate organic matter was measured for calculating the ^{15}N enrichment during incubation.

Analyses

On return to the land laboratory, filters for particulate organic nitrogen (PON) and ^{15}N measurements were dried at 60°C and pelletized in tin capsules. PON and its nitrogen isotopic abundance were measured using an elemental analyzer (Carlo Erba NC 2500) coupled to a Finnigan MAT Delta^{plus} XP isotope ratio mass spectrometer. Reproducibility of nitrogen isotope analysis (in the convention of δ notation) was within 0.2‰. The volumetric N_2 fixation rate (*NF*, nmol N m^{-3} h^{-1}) was calculated following Montoya et al. (1996):

$$NF\ (\text{nmol N m}^{-3}\ \text{h}^{-1}) = \frac{1}{\Delta t}\left(\frac{A_{PN_f} - A_{PN_0}}{A_{N_2} - A_{PN_0}}\right)\frac{PON_0 + PON_f}{2} \quad (1)$$

where Δt is the incubation time, PON is the concentration of particulate organic nitrogen, and A_{PN_f}, A_{PN_0} and A_{N_2} are absolute abundance ratios of final and natural ($t = 0$) particulate organic nitrogen and the N_2 substrate in the incubation bottle (originally dissolved plus manually injected), respectively.

Inorganic carbon on the primary production sample filters was removed by acid fuming. The assimilated ^{14}C radioactivity was measured on a liquid scintillation counter (Perkin-Elmer TriCarb 2900TR). Primary production (*PP*) was calculated as:

$$PP\ (\text{mmol C m}^{-3}\ \text{h}^{-1}) = \frac{(R_S - R_B) \cdot TCO_2}{R \cdot \Delta t} \quad (2)$$

where R_S and R_B are the radioactivities of ^{14}C (μCi) in light and dark bottles after correction for quenching, respectively, R is the added radioactivity of $NaH^{14}CO_3$ (0.8 μCi), and TCO_2 is the total carbon dioxide (mmol C m^{-3}) in seawater.

Table 1. Sampling date, longitude, latitude, bottom depth, and light intensity setting

Station	Date (mo/d/yr)	Longitude (°E)	Latitude (°N)	Bottom (m)	Sampling layer (irradiance level setting, %)
S0303	7/15/2006	121.00	35.00	37	1 m (100), 10 m (50), 33 m (0.1)
S0307	7/14/2006	123.03	35.00	78	1 m (100), 10 m (50), 30 m (10), 50 m (0.1)
S0309	7/14/2006	124.01	35.01	82	1 m (100), 10 m (50), 30 m (10), 60 m (0.1)
S0311	7/14/2006	124.91	35.01	88	1 m (100), 10 m (50), 30 m (10), 75 m (0.1)
S0402	7/10/2006	123.04	31.80	43	1 m (100)
S0404	7/11/2006	123.99	32.16	44	1 m (100)
S0406	7/11/2006	124.94	32.53	59	1 m (100), 10 m (50), 20 m (10), 30 m (0.1)
S0408	7/11/2006	125.74	32.83	94	1 m (100), 10 m (50), 30 m (10), 75 m (0.1)
FJ07	7/7/2006	123.04	31.20	66	1 m (100)
S0504	7/7/2006	123.63	31.26	108	1 m (100)
S0506	7/7/2006	124.72	31.06	51	1 m (100), 10 m (50), 30 m (10), 48 m (0.1)
S0508	7/12/2006	125.85	30.85	75	1 m (100), 10 m (50), 30 m (10), 50 m (1), 65 m (0.1)
S0705	7/4/2006	123.09	28.92	70	1 m (100), 10 m (50), 30 m (10), 50 m (0.1)
S0708	7/4/2006	124.26	28.38	96	1 m (100), 10 m (50), 30 m (30), 50 m (10), 75 m (0.1)
S1002	6/28/2006	120.79	26.72	50	1 m (100), 5 m (75), 10 m (50), 20 m (30), 30 m (10)
S1004	6/28/2006	121.30	26.38	79	1 m (100), 10 m (40), 20 m (30), 30 m (10), 50 m (1), 78 m (0.1)
S1006[a]	6/30/2006	121.95	25.98	104	1 m (100), 10 m (50), 30 m (30), 50 m (10), 75 m (0.1)
S1010	7/1/2006	123.35	25.20	1769	1 m (100), 10 m (50), 30 m (30), 50 m (10)

[a]Vertically sampled for N_2 fixation only

The depth-integrated N_2 fixation rate (µmol N m^{-2} d^{-1}) and primary production (mmol C m^{-2} d^{-1}) were estimated by trapezoidal integration over the sampling depths. Temperature, salinity and density were recorded by Sea Bird 911 Plus CTD sensors during the cast. Nitrate was determined by the standard pink azo dye method, while phosphate and silicate were determined by the standard molybdenum blue method onboard, immediately after sampling. All the methods and procedures were in accordance with those recommended by Hansen & Koroleff (1999). The precisions for determining nitrate, phosphate and silicate were ±0.1, ±0.02 and ±0.2 µM, respectively.

RESULTS

Hydrographic conditions

The surface distributions of salinity (SSS, 27.4 to 34.3), temperature (SST, 21.4 to 29.6°C), nitrate (0 to 14.9 µM), phosphate (0 to 0.13 µM) and silicate (0 to 18.6 µM for SiO_3^{2-}) in the study area are shown in Fig. 2. The intrusion of the Kuroshio water onto the ECS continental shelf was evidenced by the SSS tongues in the southeastern part of the study area, because high salinity surface waters in the ECS originate mainly from the Kuroshio (Chen et al. 1995), while the less saline northward flow of Taiwan Warm Current water and the large freshwater discharge from the Changjiang result in low salinities in coastal areas (Beardsley et al. 1985). We thus classified our sampling stations into 3 categories according to their SSS: the low-salinity group with SSS <30 (n = 6), which is found approximately along the boundary of the Changjiang river plume (Gong et al. 1996), the mesohaline group with SSS between 30 and 34 (n = 11) and the oceanic Kuroshio mainstream in the northeastern ECS (Stn S1010).

Surface nitrate and silicate distributions generally mirrored the distribution of SSS. Mean values of surface nitrate (6.4 µM, 0.6 to 14.9 µM, n = 5) and silicate (12.5 µM, 4.6 to 18.6 µM, n = 5) in the low-salinity waters are the highest among the sub-regions, while surface nitrate (0.3 µM, 0 to 1.4 µM, n = 11) decreased below the detection limit at 7 of 11 stations in the mesohaline waters or the Kuroshio. Surface silicate (3.1 µM, 0 to 13.5 µM, n = 11) also decreased dramatically in the mesohaline waters. In contrast, the differences in surface phosphate concentrations among the 3 sub-regions were smaller (Table 2).

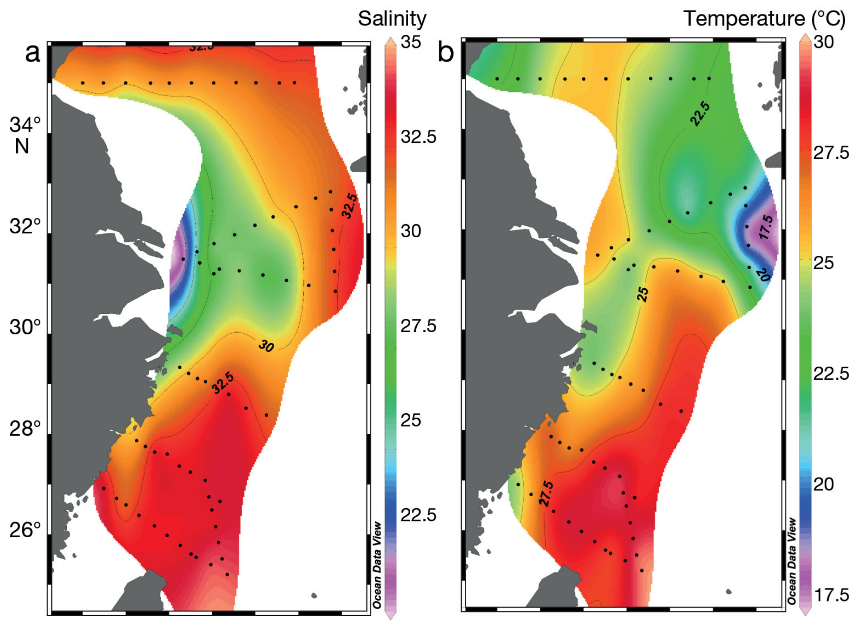
N_2 fixation

The surface N_2 fixation rate (nmol N m^{-3} h^{-1}) was very low for the low-salinity stations (4.2 ± 5.6 nmol N m^{-3} h^{-1}), and became much more active at the mesohaline stations (38.3 ± 48.3 nmol N m^{-3} h–1) (Fig. 3). The Kuroshio station had the highest surface N_2 fixation rate (193 nmol N m^{-3} h^{-1}) among all stations. It is interesting to see that the southern YS was characterized by a higher surface N_2 fixation rate compared to the ECS continental shelf (72 versus 12 nmol N m^{-3} h^{-1}). Depth profiles showed that N_2 fixation rates appeared higher at the surface or subsurface (10 to 20 m), and showed less strong variation than those for primary production (Fig. 4). It is interesting to note that even higher N_2 fixation rates occurred at the near-bottom layers of Stns S0303 and S0307. Although the exact cause for such an 'anomaly' is still unknown, there are 2 possible reasons. First, there are unicellular diazotrophs that actively fix N_2 at relatively low light levels (Montoya et al. 2004, Church et al. 2009). Second, there may be benthic N_2 fixers present. This has been shown for the Sanya Bay of the northern South China Sea, where benthic cyanobacteria can fix N_2 at a high rate during summer (Dong et al. 2008). However, this will not be discussed further because we do not have direct data to test these possibilities.

Depth-integrated N_2 fixation rates ranged between 2 and 221 µmol N m^{-2} d^{-1} in the whole area (Fig. 5A). The mean depth-integrated N_2 fixation rate of low-salinity stations (7 ± 6 µmol N m^{-2} d^{-1}, n = 2) was much lower than that of the mesohaline stations (53 ± 47 µmol N m^{-2} d^{-1}, n = 11) or the Kuroshio station (221 µmol N m^{-2} d^{-1}). We also found that depth-integrated N_2 fixation rate along 35° N was much higher than that of the ECS continental shelf.

Primary production

The primary production rate decreased with depth at all stations, and much higher rates were observed at Stns S0508 and S0705 (Fig. 4). The depth-integrated primary production (mmol C m^{-2} d^{-1}) was 489 at Stn S0508 and 534 at Stn S0705 (Fig. 5B). Such values are much higher than those of either the remaining stations in this study, or the published mean rates of around 100 mmol C m^{-2} d^{-1} on the ECS continental shelf during summer (Hama et al. 1997, Gong et al. 2003, Chen et al. 2004), indicating that these 2 stations were likely under phytoplankton bloom conditions. We suggest that the blooms at Stn S0508 may



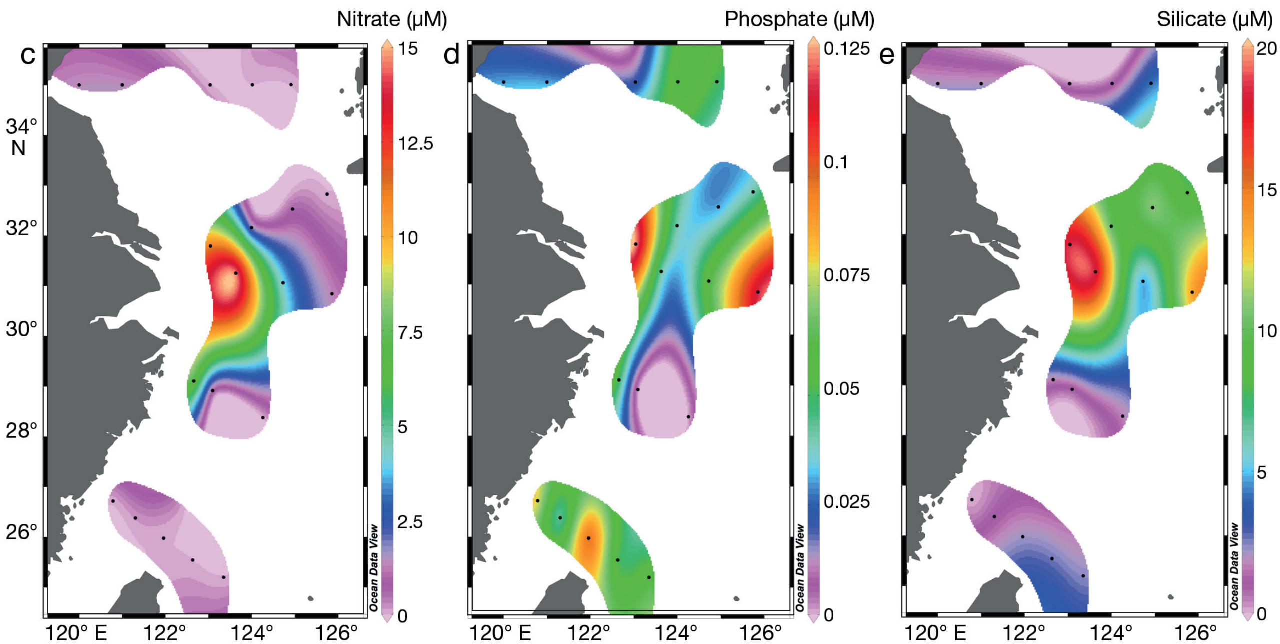


Fig. 2. Distributions of sea surface (A) salinity, (B) temperature, (C) nitrate, (D) phosphate, and (E) silicate in µM in the East China Sea and the southern Yellow Sea. Note that there are more hydrographic sampling stations (n = 64 for salinity and temperature, n = 21 for nutrients) than in the incubation experiment. The additional hydrographic data were provided by the Program 'Marine Physical Variations in Eastern Marginal Seas of China and their Environmental Impacts' (www3.ouc.edu.cn/973/)

have been triggered by the intensified cold eddy (30 to 32° N, 124 to 127° E) in summer (Chen et al. 1994, Yuan & Guan 2007). Similarly, Stn S0705 is located in the well-known Min-Zhe coastal upwelling region (caused by the southwest monsoon that prevails in summer acting together with the northward Taiwan Warm Current and China Coastal Current; Chen et al. 2004), which creates the most important fishing ground (Zhoushan fishing ground) in China. The Kuroshio was the least productive among all the stations, with a depth-integrated primary production of 32 mmol C m^{-2} d^{-1}. In addition, the mean depth-integrated primary production for the low-salinity stations (121 ± 16 mmol C m^{-2} d^{-1}, n = 2) was close to that of the non-blooming mesohaline stations (136 ± 28 mmol C m^{-2} d^{-1}, n = 8).

DISCUSSION

Variations in N_2 fixation

Our results show that the low-salinity waters are a suitable environment for N_2 fixation, and this is con-

Table 2. Summary of means and standard deviations of variables in the 3 regions. $xsPO_4$: excess PO_4; ND: not detected

Region	No. of stations	Surface: Salinity	Temperature (°C)	NO_3^- (µM)	SiO_3^{2-} (µM)	PO_4^{3-} (µM)	$xsPO_4$ (µM)	N_2 fixation rate (nmol N m^{-3} h^{-1})	Depth-integrated N_2 fixation rate (µmol N m^{-2} d^{-1})
Low salinity	6	28.67 ±0.99	24.07 ±1.44	6.4 ±6.2[a]	12.5 ±6.0[a]	0.06 ±0.04[a]	−0.34 ±0.37[a]	4.2 ±5.6	7.8 ±7.7[b]
Mesohaline	11	31.97 ±0.89	25.02 ±2.50	0.3 ±0.5	3.1 ±4.1	0.05 ±0.04	0.03 ±0.05	38.3 ±43.3	53.4 ±43.0
Oceanic	1	34.29	29.58	ND	2.3	0.06	0.06	193.1	220.8

[a]n = 5; [b]n = 2

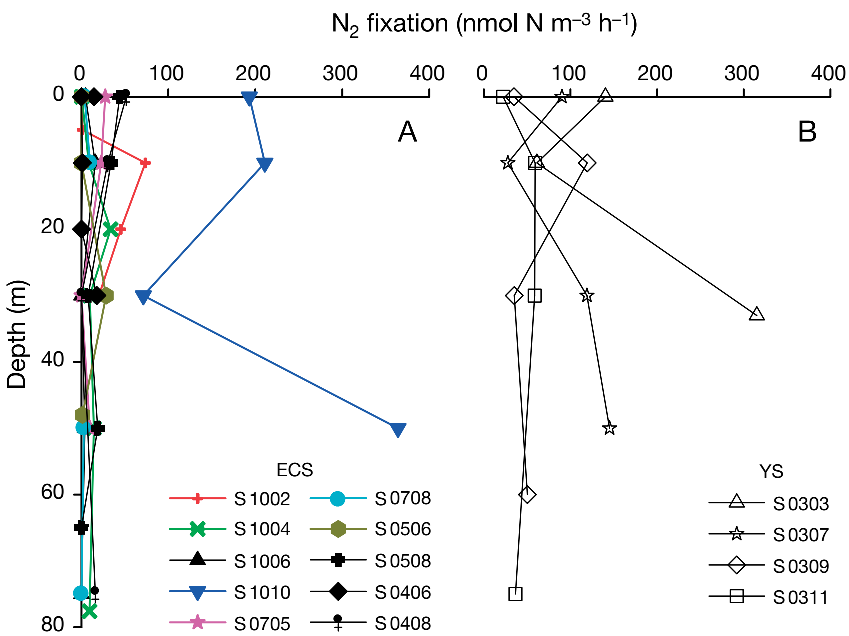


Fig. 3. Depth profiles of N_2 fixation rates in (A) the East China Sea (ECS) and (B) the southern Yellow Sea (YS). Note that only 2 profiles at Stns S0406 and S0506 fall within the low-salinity category

sistent with the reported *Trichodesmium* distribution in the Changjiang river plume in summer (Yang 1998), or in the tropical low-salinity waters near the Amazon River mouth (Subramaniam et al. 2008). Depth-integrated N_2 fixation rates observed at the mesohaline stations generally fell in line with the published rates for subtropical sea areas, such as the Vietnamese upwelling region in the southern South China Sea (Voss et al. 2006, Grosse et al. 2010) and the North Pacific subtropical gyre (Karl et al. 1997, Church et al. 2009). A high N_2 fixation rate at Stn S1010 was also consistent with the few available published results for the Kuroshio and adjacent waters. Shiozaki et al. (2010) reported an average $^{15}N_2$ assay-based N_2 fixation rate of 232 µmol N m^{-2} d^{-1} for the northeastern ECS Kuroshio mainstream during the summers of 2006 and 2007, which is very close to our result. Both results are higher than the N_2 fixation rates previously measured via acetylene reduction assay of *Trichodesmium* (141 µmol N m^{-2} d^{-1}, Saino & Hattori 1980), or solely estimated from its abundance (59 µmol N m^{-2} d^{-1}, Chang et al. 2000).

For the first time, our direct results show that the southern YS, where the Yellow Sea Cold Water resides (and becomes most prominent in summer), is also a favorable environment for N_2 fixation. This is partly consistent with published results showing that *Trichodesmium* can be observed all year round, and sometimes displays high abundance, dominating the phytoplankton species in the southern YS (Yang 1998, Bai et al. 2007).

Impact of nutrient conditions and water stratification on N_2 fixation

We suggest that the absence of N_2 fixation in the low-salinity waters may largely arise from the nutrient conditions (Table 2). The Changjiang runoff contains very high concentrations of nitrate (33 µM) and silicate (100 µM) but relatively low phosphate (0.59 µM, Zhang 1996); however, non-N_2-fixing phytoplankton in the ECS consume N and P with a Redfield ratio of 16, in common with other areas in the world oceans (Chen et al. 1996, Wang et al. 2003), thus non-N_2-fixing phytoplankton should have more than enough nitrate to fully consume all available phosphate (P-limiting) in the ECS low-salinity waters (Wong et al. 1998, Wang et al. 2003). It is unlikely that N_2 fixers will have an advantage over the fast-growing non-N_2-fixing phytoplankton

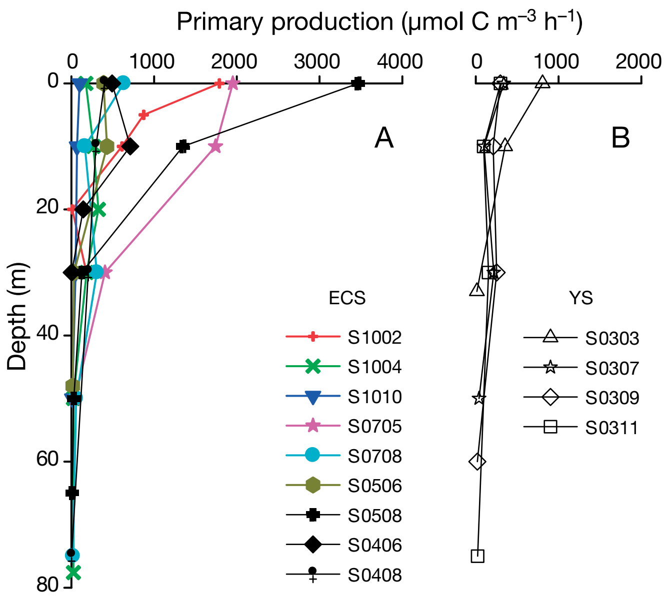


Fig. 4. Depth profiles of primary production in (A) the East China Sea (ECS) and (B) the southern Yellow Sea (YS)

(dominated by a high abundance of the diatoms *Chaetoceros* and *Rhizosolenia* during summer, Luo et al. 2007) under such P-deficient nutrient conditions (Sañudo-Wilhelmy et al. 2001, Mills et al. 2004). With more mixing of coastal water with the oceanic water, the relative deficiency of phosphate was alleviated in the surface water of the mesohaline stations, as indicated by the 'excess' phosphate (expressed as $xsPO_4 = ([PO_4^{3-}] - [NO_3^-]/16)$ under Redfield assumptions, Deutsch et al. 2007), and nutrient conditions turn more favorable for potential N_2 fixation (Table 2).

Analysis of the physical regime suggests that N_2 fixation in the mesohaline waters may have also been influenced by the degree of stratification in the upper water column, and N_2 fixation can be promoted by enhanced stratification. This idea is supported by a positive correlation between N_2 fixation and the vertical density (σ_t) gradients in the upper 30 m of the mesohaline waters ($y = -12.3 + 590x$, $r^2 = 0.54$, $p = 0.01$; Fig. 6). It is consistent with the previous finding that *Trichodesmium* blooms on the ECS continental shelf and the YS mostly occur in summer, when the water column is highly stratified by riverine freshwater input and solar heating on the ECS continental shelf (Zhou 1962, Marumo &

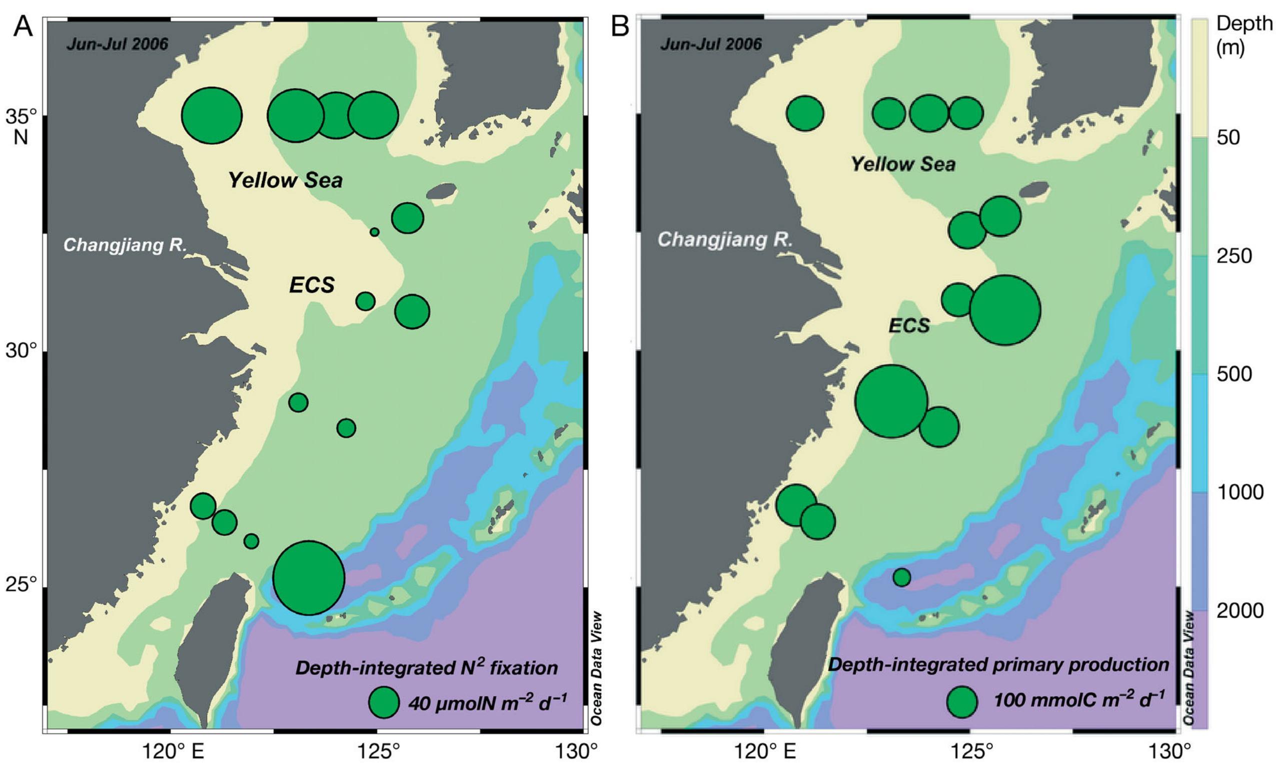


Fig. 5. Distributions of depth-integrated (A) N_2 fixation and (B) primary production rates in the East China Sea (ECS) and the southern Yellow Sea. Circle area is proportional to rates as shown in the figure legend

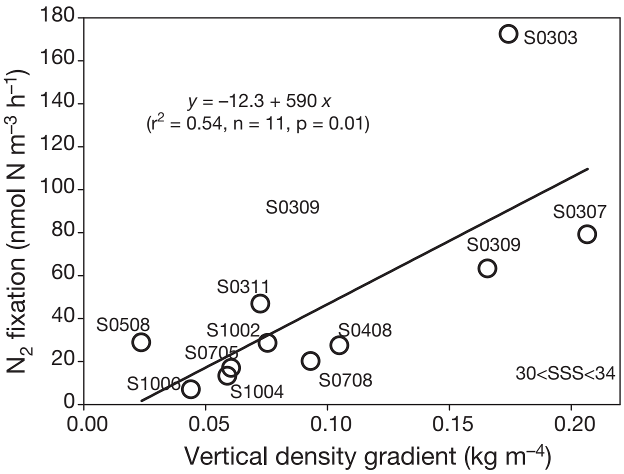


Fig. 6. Relationship between N_2 fixation and vertical density (σ_t, kg m^{-3}) gradient in the upper water column (<30 m) of the mesohaline waters. The density gradient is the averaged density difference between the surface and 30 m. SSS: sea surface salinity

Asaoka 1974, Chen 1982, Minagawa & Wada 1986, Yang 1998, Chang et al. 2000, Bai et al. 2007). In the southern YS, water column stratification can be enhanced by the existence of the Yellow Sea Cold Water, a water mass below seasonal thermocline regarded as a remnant of winter cooling and mixing (Hu & Wang 2004). Enhanced upper water column stratification may have weakened vertical nitrate injection and promoted N_2 fixation, as observed in the North Pacific subtropical gyre and the central Atlantic (Karl et al. 1995, Sañudo-Wilhelmy et al. 2001). Meanwhile, the intrusion of the Kuroshio branch (Yellow Sea Warm Current, Mask et al. 1998) probably also aided N_2 fixation, possibly by delivering oceanic diazotrophs.

Biogeochemical importance of N_2 fixation as a new N source to the ECS continental shelf in summer

The contribution of N_2 fixation to the N demand of primary production on the ECS continental shelf (0.01 to 0.16%, n = 8) and the southern YS (0.42 to 0.78%, n = 4) are much lower than the Kuroshio (4.6%), suggesting that N_2 fixation plays a minor role in supporting phytoplankton N demand on the ECS continental shelf and the YS. Such values fell in the range of previous estimates (0.2 to 6%) for the Kuroshio-influenced water in the ECS (Marumo & Asaoka 1974, Saino & Hattori 1980, Chang et al. 2000), or the upstream Kuroshio (20° N) and the adjacent South China Sea basin (Chen et al. 2008).

The major fixed N supplies to the ECS continental shelf include biological N_2 fixation, Changjiang riverine input, upwelling of Kuroshio subsurface water, and atmospheric (dry and wet) deposition (Zhang 1996, Chen & Wang 1999, Nakamura et al. 2005, Uno et al. 2007). However, no study has ever compared the relative importance of these sources, and such comparison will undoubtedly improve our basic understanding of the biogeochemical cycle in the ECS. Note that the Kuroshio area and the southern YS were not included in the evaluation here due to insufficiency of data.

If we adopt a daily N_2 fixation rate of 21 µmol N m^{-2} d^{-1} (n = 9) measured on the ECS continental shelf and extrapolate to the whole ECS (area = 7.5 × 10^5 km^2) continental shelf (<200 m, comprising 70% the whole ECS area), then N_2 fixation may add 13 Gg new N to the ECS continental shelf during summer. The Changjiang freshwater discharge in flood seasons (June to August) contributes to about 64% of the annual value of 925 km^3 (Bureau of Hydrology, Changjiang Water Resources Commission; www.cjh.com.cn/). We adopt a riverine input of inorganic nitrogen (nitrate + ammonium) of 275 Gg N in summer in combination with the concentration values in the freshwater discharge ($[NO_3^-]$ = 32.9 µM and $[NH_4^+]$ = 14.6 µM; Zhang 1996). Ammonium and nitrate are the most dominant species in the atmospheric deposition with an estimated total (nitrate + ammonium) flux of 430 Gg N in the whole ECS (Nakamura et al. 2005). As model results show negligible seasonal variations for atmospheric inorganic nitrogen deposition (Uno et al. 2007), we adopt a summertime atmospheric deposition total N flux of 75 Gg to the ECS continental shelf. Chen & Wang (1999) estimated that an annual flux of 2072 Gg N nutrients is transported to the ECS as the Kuroshio subsurface waters upwell onto the ECS continental shelf, and a conservative value of 518 Gg N was adopted in our comparison. It is clear that N_2 fixation does not contribute significantly to the sequestration of atmospheric CO_2 on the ECS continental shelf (Tsunogai et al. 1999, Chen et al. 2004).

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