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ORIGINAL RESEARCH ARTICLE

# Summer distribution patterns of *Trichodesmium* spp. in the Changjiang (Yangtze River) Estuary and adjacent East China Sea shelf

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Kuroshio

**Summary** To examine the distribution of *Trichodesmium* relative to physicochemical factors during summer in the Changjiang (Yangtze River) Estuary and adjacent East China Sea shelf, three cruises were conducted separately in June 2009, July 2011, and August 2009. *Trichodesmium* species found were *T. thiebautii*, *T. erythraeum*, and *T. hildebrandtii*. The population was dominated by *T. thiebautii*, which accounted for >85% of the samples found. Most of them were free trichomes. Colonial forms were rarely observed (approximately 10% of our samples), occurring only in offshore waters. The depth integrated abundances of *Trichodesmium* were  $308 \times 10^3$ ,  $1709 \times 10^3$ , and  $3448 \times 10^3$  trichomes  $m^{-2}$  in June, July, and August, respectively. *Trichodesmium* was distributed abundantly in the southern or southeastern part of our study area,

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where nutrients were low and light penetration, temperature, and salinity were high, which were influenced by the Taiwan Warm Current (TWC) and Kuroshio. *Trichodesmium* was found in low abundance in inshore, eutrophic, low-salinity waters, which were mainly controlled by the Changjiang Diluted Water (CDW) and coastal current. These results suggest that spatiotemporal changes in the summer *Trichodesmium* distribution correlate highly with the variations in physicochemical properties that are primarily controlled by the TWC, Kuroshio, and CDW. The summer  $N_2$  fixation rate of *Trichodesmium* was estimated at  $12.3 \mu\text{mol N m}^{-2} \text{d}^{-1}$  in our study area, contributing >50% of biological  $N_2$  fixation.

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## 1. Introduction

*Trichodesmium* spp. (hereafter, *Trichodesmium*) are diazotrophic filamentous nonheterocystous cyanobacteria that live as colony-forming or individual filaments. *Trichodesmium* is broadly distributed in the photic zones of tropical and subtropical seas with low nutrients, clear waters, and deep light penetration (Capone et al., 1997). *Trichodesmium* is the main primary producer and likely the major contributor of  $N_2$  fixation in these oligotrophic water regiments (typically in temperatures  $>20^\circ\text{C}$ ). This  $N_2$  fixation relieves the restraining of the nitrogen limitation to the marine primary production and produces more biological carbon, which enhances the efficiency of the biological pump in oligotrophic oceans (Falkowski, 1997; Hutchins et al., 2015). *Trichodesmium* plays an essential role in the carbon and nitrogen biogeochemical cycles and is therefore of great interest regarding global warming and increased  $p\text{CO}_2$  (Capone et al., 1997; Das and Mangwani, 2015; Hutchins et al., 2015; Jiang et al., 2015b; Karl et al., 1997).

During the summer, the East China Sea (ECS), which is the largest marginal sea in the western North Pacific Ocean, undergoes significant changes in circulation and water mass (Fig. 1; Chen, 2009; Jiang et al., 2015a; Su and Yuan, 2005; Zhou et al., 2015). Many studies have found that *Trichodesmium* is relatively abundant and occasionally blooms in warm months in the ECS and Kuroshio areas (Chang et al., 2000; Chen et al., 2014; Ding, 2009; Marumo and Asaoka, 1974; Shiozaki et al., 2010, 2015; Yang, 1998; Zhang et al., 2014). *Trichodesmium* populations and especially blooms may contribute considerably to the local carbon and nitrogen budget of the ECS during summer stratification with water temperatures above  $20^\circ\text{C}$ , particularly in the offshore waters controlled by the Taiwan Warm Current (TWC) and Kuroshio (Saino, 1977; Shiozaki et al., 2010, 2015; Zhang et al., 2012). Although abundant *Trichodesmium* in the Changjiang Estuary (CE) and adjacent ECS is conveyed by the TWC and Kuroshio, their distribution is limited by low-salinity and -temperature water masses, including the Changjiang Diluted Water (CDW), coastal current, and Yellow Sea Cold Water Mass (YSCWM). Additionally, the eutrophic, low-salinity CDW and coastal current promote diatom and dinoflagellate blooms (Jiang et al., 2015a; Zhu et al., 2009), which are not conducive to *Trichodesmium* growth or  $N_2$  fixation because of the contemporary principles of phytoplankton physiological and ecological trade-offs (Carpenter, 1983; Fu and Bell, 2003). However, previous studies have indicated

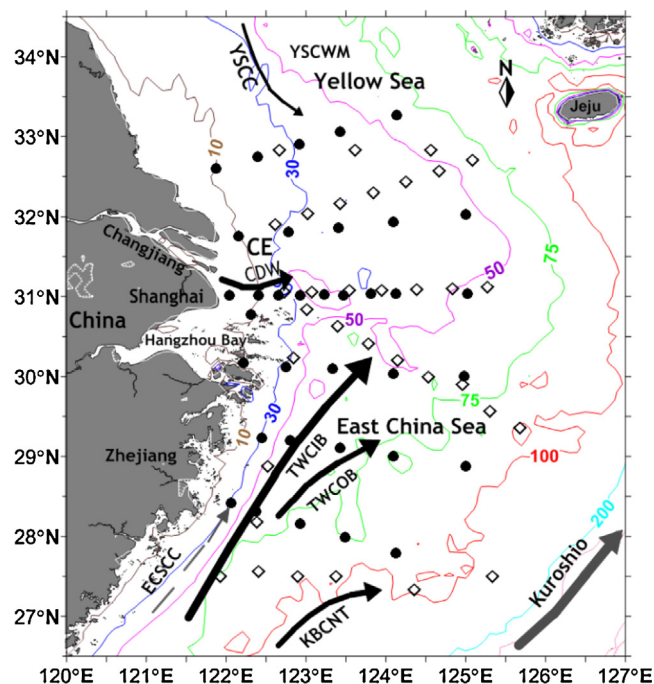
that large rivers, such as the Amazon (Olson et al., 2015; Subramaniam et al., 2008) and Mekong (Grosse et al., 2010; Voss et al., 2006), enhance the biomass and  $N_2$  fixation of diazotrophic cyanobacteria (e.g., *Richelia* and *Trichodesmium*) in the shelves influenced by the riverine plume. These reports have revealed that the water column stability of and micronutrients and trace metals (e.g., Fe, Mo, and Ni) in the river plumes may enhance cyanobacterial growth. Therefore, investigating the distribution patterns and  $N_2$  fixation of *Trichodesmium* in the CE and adjacent ECS (which is directly subjected to a considerable amount of riverine freshwater and terrestrial materials) is necessary.

In summer, spatiotemporal changes are apparent in the physicochemical properties of the CE and adjacent ECS, including variations in river discharge associated with abundant nutrients, the turning and extension of the CDW, seawater temperature elevation, and an increase in the incursion of the TWC because of the prevailing southwestern monsoon (Jiang et al., 2015a; Su and Yuan, 2005; Zhou et al., 2015). In this context, we hypothesized that summer *Trichodesmium* populations may exhibit significant spatiotemporal changes in the CE and adjacent ECS. However, the effects of environmental factors on *Trichodesmium* in summer remain poorly documented (Yang, 1998). Regional accelerated warming (Jiang et al., 2014; Tang et al., 2009), the increasing transport of the Kuroshio and TWC (Tang et al., 2009), and enhanced stratification (which prevents the supplementation of upwelling bottom nitrogen) may affect the distribution boundary, growth, and  $N_2$  fixation of *Trichodesmium*. However, the effects of these factors remain largely undetermined, and their contributions to phytoplankton biomass and  $N_2$  fixation are also unclear. We observed how the changing physicochemical properties in the CE and adjacent ECS shelf during summer affected the *Trichodesmium* population. Our objectives were to (1) examine the *Trichodesmium* species composition and population structure, (2) explore its distribution patterns in relation to environmental factors, and (3) estimate its contributions to phytoplankton biomass and biological  $N_2$  fixation.

## 2. Material and methods

### 2.1. Study area and sample collection

During summer, the Kuroshio mainstream flows northeastward along the ECS shelf break (200-m isobath) and the Kuroshio branch northeast of Taiwan flows northeastward



**Figure 1** Sampling stations in the Changjiang Estuary (CE) and adjacent East China Sea (ECS) shelf in summer (after [Chen, 2009](#); [Su and Yuan, 2005](#)). Circle: sampling stations in June 2009 and August 2009; Diamond: sampling stations in July 2011. CDW: Changjiang Diluted Water; YSCC: Yellow Sea Coastal Current; ECSCC: ECS Coastal Current; YSCWM: Yellow Sea Cold Water Mass; TWC: Taiwan Warm Current; TWCIB: TWC inshore branch; TWCIB: TWC offshore branch; KBCNT: Kuroshio Branch Current northeast of Taiwan.

along the 100-m isobaths ([Fig. 1](#)). The inshore and offshore branches of the TWC flow northeast during the southwest monsoon season. Concurrently, the ECS receives substantial freshwater input from the Changjiang, which forms the strong CDW. Thus, the CE and adjacent ECS shelf are mainly controlled by eutrophic, low-salinity, inshore and oligotrophic, high-salinity, offshore current systems.

Three cruises were conducted in the CE and adjacent ECS shelf during early (June 1–11, 2009), middle (July 7–17, 2011), and late (August 14–24, 2009) summer ([Fig. 1](#)). We established 35 stations in June and August and 34 stations in July. At each station, temperature, salinity, depth, and density ( $\sigma_t$ ) were measured in situ with a CTD recorder (SBE 917 Plus, Seabird Co.). Surface (2- or 3-m depth) water samples for measuring nutrient concentrations, suspended particulate matter (SPM), and chlorophyll *a* (Chl *a*) were collected in 12-L NOEX bottles attached to a CTD rosette.

## 2.2. Biogeochemical analysis

Water samples for dissolved inorganic nitrogen (DIN) and phosphorus (DIP) were filtered through a 0.45- $\mu\text{m}$  cellulose acetate filter. Nutrient concentrations were determined using a continuous flow analyzer (Skalar San<sup>+</sup>, Netherlands) with colorimetric methods described by [Grasshoff et al. \(1999\)](#). For the SPM analysis, the filters were dried at 105°C to a constant mass and weighed after the samples (1–5 L seawater) were filtered through preweighed filters (Whatman GF/C), as described in [Jiang et al. \(2015a\)](#). To measure the Chl *a* content, 100–250-mL seawater samples were filtered through a GF/F filter and stored at –20°C until

analysis. Chl *a* samples were extracted with 10 mL of 90% acetone at –20°C and measured using a Turner Designs 10-AU fluorometer (Sunnyvale, CA, USA).

## 2.3. *Trichodesmium* collection and analysis

*Trichodesmium* samples were collected vertically from the bottom to the surface by using a 76- $\mu\text{m}$  mesh net (0.1 m<sup>2</sup> in acreage of the mouth) at a tow speed of 0.5 m s<sup>–1</sup> at each station, although the collection lost some individual free trichomes ([Chang, 2000](#); [Jiang et al., 2015b](#); [Taboada et al., 2010](#)). Our data exhibited no significant difference between the depth integrated abundances (DIAs, with average of  $1709 \times 10^3$  and  $1755 \times 10^3$  trichomes m<sup>–2</sup>, respectively) of *Trichodesmium* collected by two methods (76- $\mu\text{m}$  mesh net and NOEX bottles mounted on a CTD rosette sampler) in the ECS shelf during July 2011 (Figs. S1 and S2 in Supplementary material). The nets were fitted with a digital flow meter (model 438115, Hydro-Bios, Germany) to estimate the total volume of water passing through the net. All collected samples were preserved with 4% formalin. To count the trichomes and colonies, we added 1 mL of acetic acid to collapse the gas vesicles in the *Trichodesmium* cells before the sedimentation process. After at least 48 h of sedimentation in a laboratory, preserved samples were concentrated to 100–1000 mL by slowly siphoning off the supernatant. To prevent the loss of floating *Trichodesmium* samples, the supernatant was filtered through a 10- $\mu\text{m}$  nylon membrane (Millipore). *Trichodesmium* species were identified and counted on a 1-mL scaled slide by using a Leica DM3000B microscope, according to the trichome morphological characteristics ([Guo, 2004](#); [Janson et al., 1995](#)).

## 2.4. Data analysis

The strength of the vertical stratification (water column stratification index,  $\Delta\sigma_t$ ) was estimated by applying the difference in  $\sigma_t$  between the bottom and surface layers. We assumed that the environmental conditions observed in July 2011 approximated those observed in July 2009 and thereby we compared the monthly changes in the *Trichodesmium* in relation to the physicochemical observations in June and August 2009 as well as July 2011. We used SPSS 20.0 for the data analysis. Because the data did not satisfy the assumptions of normality and homogeneity, a Kruskal–Wallis test was performed for significant differences in *Trichodesmium* population and environmental variables in June, July, and August. Spearman's rank correlation was used to determine the relationship between the *Trichodesmium* abundance and environmental variables in the surface layer. Figures depicting the distribution of environmental variables, Chl *a*, *Trichodesmium* abundance, and abundance versus temperature and salinity were constructed using ODV 4.5. Some of the data sets on physicochemical parameters used in the present analysis have been published previously, including the temperature, salinity, and nutrient data recorded in June and August 2009 (Jiang et al., 2015a).

## 3. Results

### 3.1. Environmental factors

Because of the increase in solar irradiation and southern warm-water intrusion, the sea surface temperature increased significantly ( $P < 0.001$ ) in summer, with averages of  $21.4 \pm 1.8^\circ\text{C}$  (June),  $25.9 \pm 2.7^\circ\text{C}$  (July), and  $27.9 \pm 1.9^\circ\text{C}$  (August; Table 1). However, the surface and bottom average salinities did not differ significantly. The  $\Delta\sigma_t$  (surface density–bottom density) in July ( $4.95 \text{ kg m}^{-3}$ ) and August ( $4.29 \text{ kg m}^{-3}$ ) were markedly higher than that in June ( $3.03 \text{ kg m}^{-3}$ ), indicating increased water column stratification. The SPM in June ( $22.4 \text{ mg L}^{-1}$ ) was significantly ( $P < 0.01$ ) higher than that in August ( $12.6 \text{ mg L}^{-1}$ ). The DIN and DIP distributions were similar in the low-salinity

CDW. The nutrient concentrations did not differ significantly among the different months (Table 1).

The surface cold tongues in summer moved southeastward in the northern part of the CE, indicating an intrusion of the Yellow Sea Coastal Current (YSCC) (Fig. 2A–C). The north-eastern part of the study area was influenced by the YSCWM, which represented a cold tongue with a bottom temperature approximating  $10^\circ\text{C}$  (data not shown). The surface warm tongues with salinities above 34 moved northward along the Zhejiang coast (Fig. 2), indicating an intrusion of the TWC and Kuroshio branch. The bottom salinity isohaline of 34.5 moved northward, suggesting an increased TWC and Kuroshio from June to August. The CDW (inner part of the isohaline of 31) dominated the upper water column in our study area, especially in the CE and adjacent northern ECS shelf (Fig. 2D–F).

### 3.2. Species composition and trichome form of *Trichodesmium*

Our samples contained the following species: *T. thiebautii*, *T. erythraeum*, and *T. hildebrandtii*. Most of the population (>85%) consisted of *T. thiebautii* (Table 2). Free trichomes accounted for most of the *Trichodesmium* population were found; their dominances were 90.2%, 92.0%, and 88.6% in June, July, and August, respectively (Table 3). The colonial forms were rarely observed. Those that we found often were composed of few trichomes, generally fewer than 20 trichomes per colony.

### 3.3. Distribution of *Trichodesmium* abundance

*Trichodesmium* abundance varied significantly ( $P < 0.001$ ) by month, with averages of  $4.08 \times 10^3$ ,  $20.70 \times 10^3$ , and  $53.31 \times 10^3$  trichomes  $\text{m}^{-3}$  in June, July, and August, respectively. Their occurrence frequency increased from 71.4% in June to 94.3% in August (Table 2). The DIA of *Trichodesmium* also varied significantly ( $P < 0.001$ ) by month (Table 3).

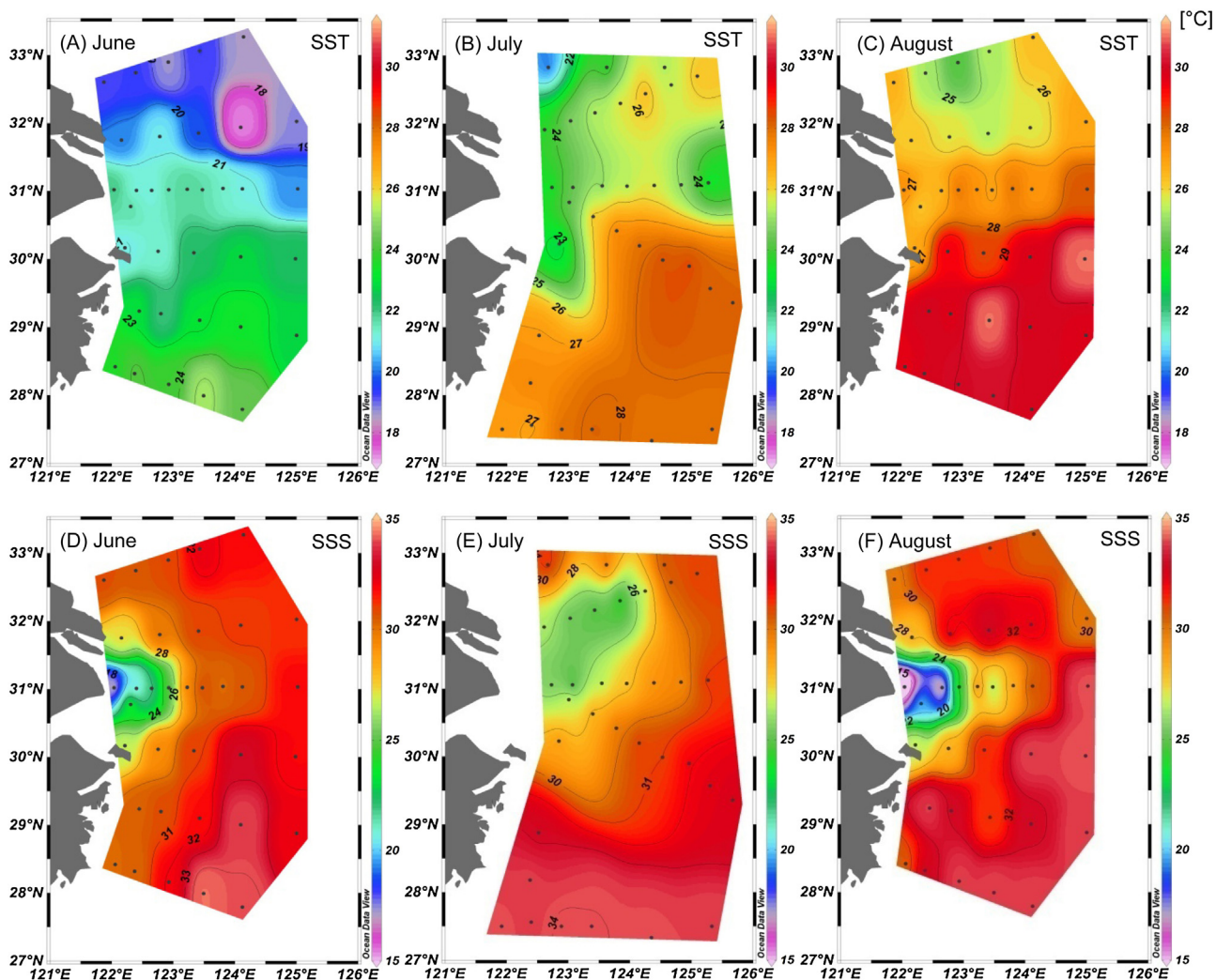
In summer, the spatial variability in *Trichodesmium* abundance was generally southern > northern, offshore > inshore (Fig. 3). In June, *Trichodesmium* was concentrated in the

**Table 1** Environmental parameters in the CE and adjacent ECS shelf in summer.

| Environmental parameters                             | June 2009           |            | July 2011          |            | August 2009          |             |
|--|---------------------|------------|--------------------|------------|----------------------|-------------|
|  | Mean $\pm$ SD       | Range      | Mean $\pm$ SD      | Range      | Mean $\pm$ SD        | Range       |
| Depth [m]  | $47.1 \pm 23.7^a$   | 10.0–98.0  | $60.9 \pm 22.7^b$  | 28.1–110.0 | $47.1 \pm 23.5^a$    | 11.0–99.0   |
| Surface temperature [ $^\circ\text{C}$ ]             | $21.4 \pm 1.8^a$    | 17.3–25.1  | $25.9 \pm 2.7^b$   | 19.7–28.6  | $27.9 \pm 1.9^c$     | 24.1–31.0   |
| bottom temperature [ $^\circ\text{C}$ ]              | $18.2 \pm 2.2^a$    | 11.2–21.8  | $17.6 \pm 4.1^a$   | 10.4–20.7  | $21.8 \pm 3.4^b$     | 12.0–26.5   |
| Surface salinity                                     | $29.6 \pm 3.9^a$    | 16.4–34.3  | $29.9 \pm 0.4^a$   | 22.6–34.1  | $29.9 \pm 5.0^a$     | 13.2–34.0   |
| Bottom salinity                                      | $32.3 \pm 3.6^a$    | 16.8–34.8  | $33.5 \pm 0.5^a$   | 30.8–34.6  | $32.5 \pm 3.7^a$     | 16.6–34.8   |
| $\Delta\sigma_t$ [ $\text{kg m}^{-3}$ ]              | $3.13 \pm 2.41^a$   | 0.01–9.00  | $4.95 \pm 2.15^b$  | 0.22–10.37 | $4.29 \pm 3.11^{ab}$ | 0.08–14.10  |
| Surface SPM [ $\text{mg L}^{-1}$ ]                   | $22.4 \pm 50.7^a$   | 1.6–243.6  | –                  | –          | $12.6 \pm 27.1^b$    | 1.1–128.3   |
| Surface DIN [ $\mu\text{mol L}^{-1}$ ]               | $17.13 \pm 20.52^a$ | 1.85–78.31 | $11.67 \pm 2.84^a$ | 0.71–53.75 | $22.86 \pm 34.72^a$  | 0.63–139.66 |
| Surface DIP [ $\mu\text{mol L}^{-1}$ ]               | $0.32 \pm 0.34^a$   | 0.08–1.30  | $0.41 \pm 0.29^a$  | BDL–3.64   | $0.33 \pm 0.42^a$    | BDL–1.62    |
| Depth integrated Chl <i>a</i> [ $\text{mg m}^{-2}$ ] | $44.4 \pm 26.4^a$   | 6.0–126.1  | $71.1 \pm 75.9^a$  | 27.5–440.8 | $68.3 \pm 45.5^a$    | 5.6–187.7   |

SPM: suspended particulate matter; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus;  $\Delta\sigma_t$  = bottom density–surface density; Chl *a*: chlorophyll *a*; BDL: below the detection limit. Different superscripted letters indicate significant differences at  $P < 0.05$  among different months.





**Figure 2** Sea surface temperature (SST) [ $^{\circ}\text{C}$ ] and salinity (SSS) in the CE and adjacent ECS shelf in summer.

**Table 2** Abundance (mean  $\pm$  SD) [ $\times 10^3$  trichomes  $\text{m}^{-3}$ ] and occurrence frequency (OF) of *Trichodesmium* species in the CE and adjacent ECS shelf in summer.

| Species                   | June 2009         |       | July 2011           |       | August 2009         |       |
|---------------------------|-------------------|-------|---------------------|-------|---------------------|-------|
|                           | Abundance         | OF    | Abundance           | OF    | Abundance           | OF    |
| <i>T. thiebautii</i>      | $3.86 \pm 6.32^a$ | 71.4% | $20.00 \pm 28.68^b$ | 91.2% | $45.76 \pm 58.38^c$ | 94.3% |
| <i>T. erythraeum</i>      | $0.17 \pm 0.42^a$ | 14.3% | $0.62 \pm 1.44^b$   | 50.0% | $5.22 \pm 11.87^b$  | 31.4% |
| <i>T. hildebrandtii</i>   | $0.06 \pm 0.16^a$ | 17.1% | $0.07 \pm 0.18^a$   | 23.5% | $2.40 \pm 4.10^a$   | 34.3% |
| <i>Trichodesmium</i> spp. | $4.08 \pm 6.74^a$ | 71.4% | $20.70 \pm 29.50^b$ | 94.1% | $53.31 \pm 69.97^c$ | 94.3% |

Different superscripted letters indicate significant differences at  $P < 0.05$  among different months.

southwestern part of our study area with the highest density of  $2508 \times 10^3$  trichomes  $\text{m}^{-2}$ , and the northern part of the CE had no trichome (Fig. 3A and D). In July, *Trichodesmium* presented a high abundance (with the highest DIA of  $10,167 \times 10^3$  trichomes  $\text{m}^{-2}$ ) in the southern part of our study area and low abundance in the northeastern part, despite the patchiness of distribution (Fig. 3B and E). In August, *Trichodesmium* was abundantly distributed in our investigation area (with the highest DIA of

$17,031 \times 10^3$  trichomes  $\text{m}^{-2}$ ) except in the CE. The low abundance area was consistent with the low-salinity tongue of the CDW (Fig. 3C and F).

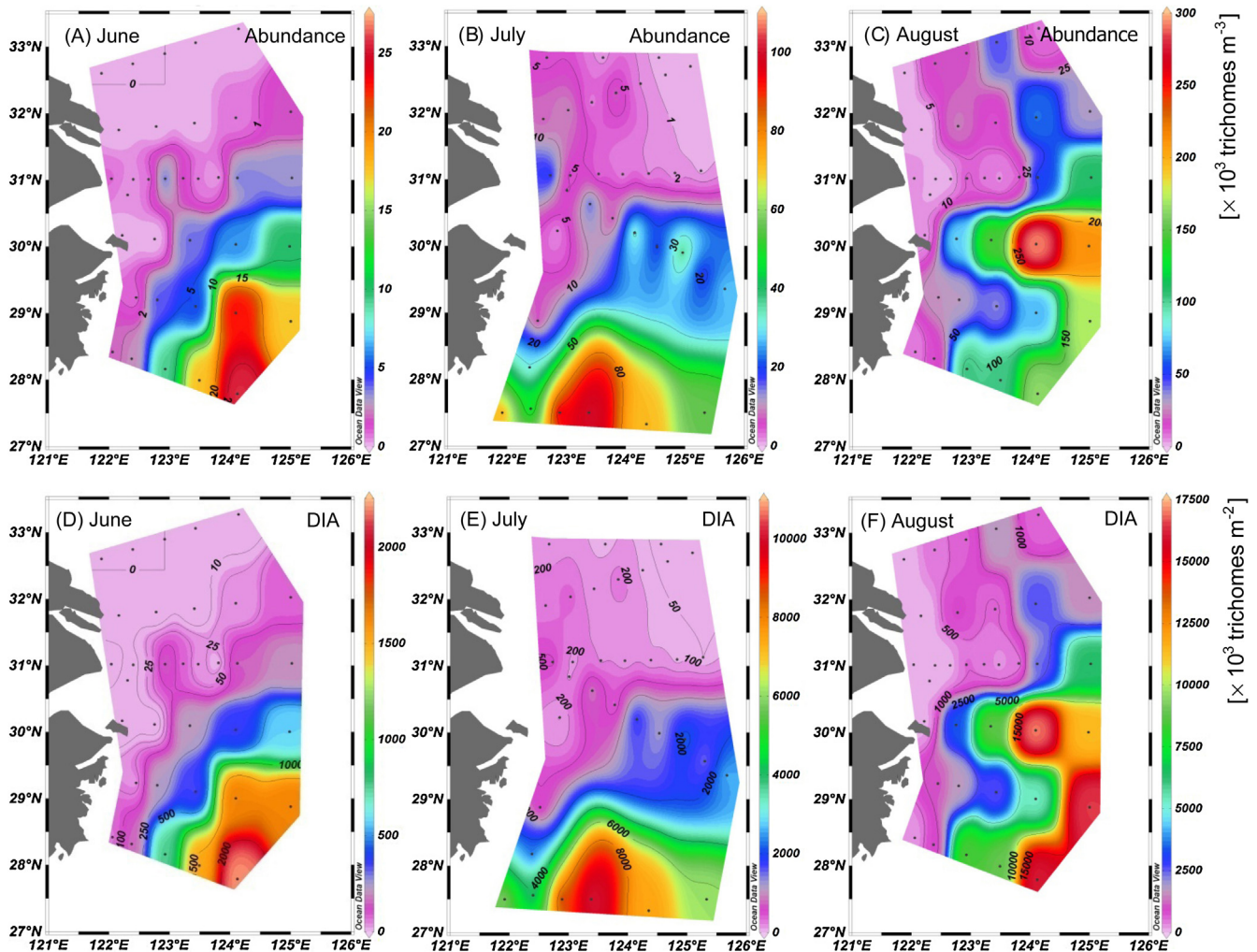
### 3.4. Relationship between *Trichodesmium* and environmental factors

Overall, *Trichodesmium* abundance correlated significantly ( $P < 0.01$ ) and positively with the surface temperature,

**Table 3** Depth integrated abundance (DIA) [ $\times 10^3$  trichomes  $m^{-2}$ ] of *Trichodesmium* in the CE and adjacent ECS in summer.

| Parameters                           | June                       |         | July                         |         | August                       |         |
|--------------------------------------|----------------------------|---------|------------------------------|---------|------------------------------|---------|
|                                      | Mean $\pm$ SD              | Maximum | Mean $\pm$ SD                | Maximum | Mean $\pm$ SD                | Maximum |
| DIA of free <i>Trichodesmium</i>     | 278 $\pm$ 526 <sup>a</sup> | 2321    | 1572 $\pm$ 2631 <sup>b</sup> | 9270    | 3054 $\pm$ 4223 <sup>c</sup> | 14,330  |
| DIA of colonial <i>Trichodesmium</i> | 30 $\pm$ 107 <sup>a</sup>  | 497     | 137 $\pm$ 261 <sup>a</sup>   | 975     | 394 $\pm$ 950 <sup>a</sup>   | 3630    |
| Total DIA                            | 308 $\pm$ 595 <sup>a</sup> | 2508    | 1709 $\pm$ 2839 <sup>b</sup> | 10,167  | 3448 $\pm$ 5055 <sup>c</sup> | 17,031  |

Different superscripted letters indicate significant differences at  $P < 0.05$  among different months.



**Figure 3** Distribution of *Trichodesmium* abundance [ $\times 10^3$  trichomes  $m^{-3}$ ] and DIA [ $\times 10^3$  trichomes  $m^{-2}$ ] in the CE and adjacent ECS shelf in summer.

salinity, and depth, whereas correlations with DIN, DIP, SPM, and Chl *a* were negative (Table 4), suggesting that *Trichodesmium* preferred the offshore oligotrophic, clear, warm, high-salinity environments (which are controlled by the TWC and Kuroshio). The temperature was the most crucial factor in the correlation coefficients (except for in August, when it was the salinity). Fig. 4 demonstrates that *Trichodesmium*

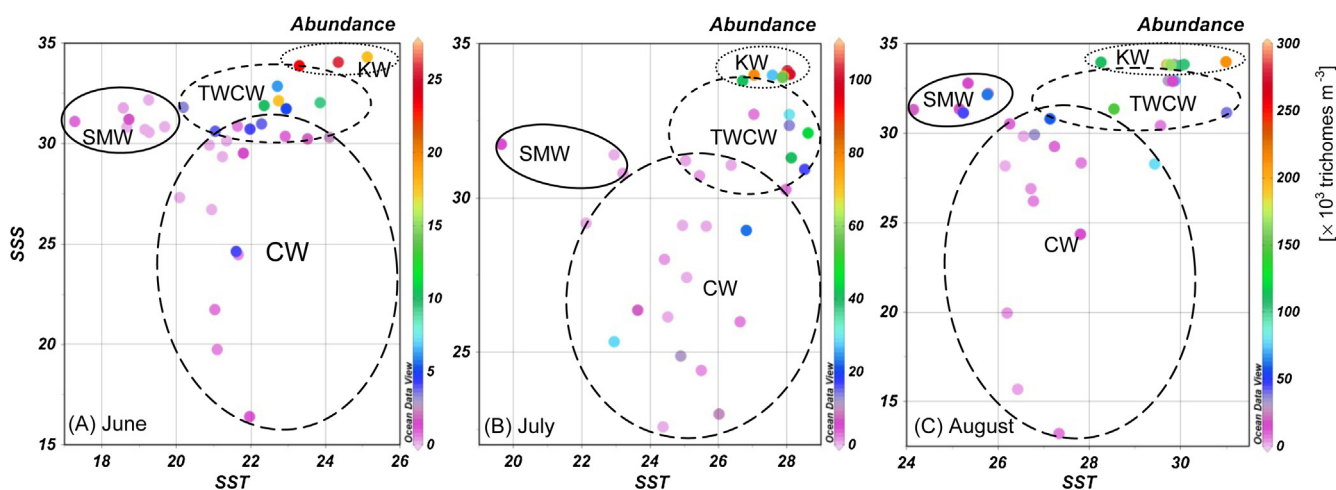
thrived in the offshore high-temperature and -salinity waters beyond the eutrophic, low-salinity CDW front (isohaline 31).

#### 4. Discussion

Generally, the CE and adjacent ECS shelf in summer are characterized by eutrophic, low-salinity, inshore (mainly

**Table 4** Correlation coefficients (Spearman's  $r$ ) between *Trichodesmium* abundance and surface environmental factors in the CE and adjacent ECS shelf in summer.

| Month  | Temperature | Salinity | SPM     | Depth    | DIN       | DIP      | Chl $a$   |
|--------|-------------|----------|---------|----------|-----------|----------|-----------|
| June   | 0.741***    | 0.545*** | −0.255  | 0.750*** | −0.642*** | −0.460** | −0.489**  |
| July   | 0.700***    | 0.628*** | −       | 0.526*** | −0.568*** | −0.154   | −0.517*** |
| August | 0.523**     | 0.781*** | −0.432* | 0.717*** | −0.625*** | −0.119   | −0.619*** |

\*  $P < 0.05$ .\*\*  $P < 0.01$ .\*\*\*  $P < 0.001$ .**Figure 4** *Trichodesmium* abundance [ $\times 10^3$  trichomes  $m^{-3}$ ] with the SST [ $^{\circ}C$ ] and SSS in the CE and adjacent ECS shelf in summer. Each dot indicated a measurement point of abundance. Different water masses in the CE and adjacent ECS were determined by the temperature and salinity properties (Chen, 2009; Su and Yuan, 2005). CW: coastal water dominated by CDW; TWCW: TWC Water; KW: Kuroshio Water; SMW: Shelf Mixed Water.

the CDW and coastal current) and oligotrophic, high-salinity, offshore (mainly the TWC) current systems (Fig. 1; Chen, 2009; Su and Yuan, 2005; Zhou et al., 2015). The CDW is the most significant hydrographic property in summer in our study area, because of its large-area plume and substantial runoff associated with particulate matter and macro- and micro-nutrients (Bai et al., 2014; Chen, 2009; Su and Yuan, 2005). In our study, the CDW in July 2011 extended offshore toward the northeast (Fig. 2E), which is consistent with previous observations in our study area (Su and Yuan, 2005). Similarly, the CDW in July 2009 transported north-eastward (Bai et al., 2014), which is the same as July 2011. Because the monthly average runoff ( $35,977 \text{ m}^3 \text{ s}^{-1}$ ) from the Changjiang in June 2009 approximated to the fresh-water flux ( $33,253 \text{ m}^3 \text{ s}^{-1}$ ) in June 2011, as recorded at Datong station, the extension pathway and strength of the CDW were similar in the July of both 2009 and 2011. Therefore, the environmental conditions observed in July 2011 approximated those observed in July 2009. In this context, we compared the monthly changes in the *Trichodesmium* in relation to the physicochemical observations.

#### 4.1. Effects of environmental factors on *Trichodesmium* abundance

Our study found that *Trichodesmium* generally occurred in areas with a surface temperature of  $>20^{\circ}C$ . The highest

abundance was at the temperature range of  $22\text{--}25^{\circ}C$  in June,  $27\text{--}28.5^{\circ}C$  in July, and  $29.5\text{--}31^{\circ}C$  in August (Fig. 4). This result supports the hypothesis that seawater temperature sets a physiological constraint to the geographic distribution of *Trichodesmium*, and  $20^{\circ}C$  is the general lowest temperature for their normal growth (Capone et al., 1997). Laboratory experimental results also demonstrated that IMS-101, a strain of *Trichodesmium*, has adapted to optimal growth at temperatures ranging from  $24^{\circ}C$  to  $30^{\circ}C$ , can tolerate temperatures from  $20^{\circ}C$  to  $34^{\circ}C$ , and even survive at  $17^{\circ}C$  for several weeks (Breitbarth et al., 2007). Fu et al. (2014) demonstrated that their thermal limits range from  $18^{\circ}C$  to  $32^{\circ}C$ , with optimal growth temperatures at approximately  $26^{\circ}C$ . The temperature for present abundant *Trichodesmium* in the ECS was within the optimal growth temperature range.

The occurrence frequency and abundance of *Trichodesmium* increased significantly ( $P < 0.001$ ; Tables 2–4) with the temperature (Table 1), and the abundance in the southern or southeastern part of our study area (relatively high temperatures) was also substantially higher than that in the northern part (relatively low temperatures) (Figs. 2 and 3). Other field investigations have reported similar relationships (Chen et al., 2003; Fernández et al., 2010; Zhang et al., 2014).

Our study shows that abundant *Trichodesmium* filaments in summer generally occur at surface salinities above 31 (Fig. 4). We observed a highly significant ( $P < 0.001$ ) positive



correlation between *Trichodesmium* abundance and salinity (Table 4). Figs. 2 and 3 illustrate that high densities of *Trichodesmium* were observed in offshore areas with high salinity. Zhang et al. (2014) also determined a highly significant ( $P < 0.01$ ) positive correlation between *Trichodesmium* abundance and salinity in summer in the northern ECS. These findings for the ECS agree with those of reports on other shelf seas influenced by river plumes (Olson et al., 2015; Subramaniam et al., 2008; Voss et al., 2006). Navarro et al. (2000) reported that *Trichodesmium* in the eastern Caribbean Sea was suppressed by Orinoco River discharge, although the abundance was not significantly correlated with salinity. The physiological effects of salinity cause these distribution patterns; Fu and Bell (2003) found that the maximal growth of *Trichodesmium* occurred with salinities in the range 33–37. Salinity seems to play a crucial role in the *Trichodesmium* distribution in the large estuaries and adjacent shelves.

Because of phytoplankton physiological and ecological trade-offs (Carpenter, 1983; Fu and Bell, 2003), low abundance of *Trichodesmium* has been observed in coastal eutrophic waters where diatoms and dinoflagellates absolutely dominated in phytoplankton community (Jiang et al., 2015a; Zhu et al., 2009). The high abundance of *Trichodesmium* occurs in offshore oligotrophic and low-Chl *a* waters. Table 4 indicates that *Trichodesmium* abundance is negatively correlated with DIN, DIP, Chl *a*, and depth. In June and August, *Trichodesmium* abundance was negatively related to SPM, implying that *Trichodesmium* growth was significantly affected by light penetration. Their photosynthetic apparatus is adapted for a high-light regime, which needs a high transparent environment to grow and propagate (Capone et al., 1997). Therefore, *Trichodesmium* is generally encountered in high abundance in waters characterized by low nutrient concentrations, clear waters, and deep light penetration (Capone et al., 1997).

#### 4.2. Effects of water masses on *Trichodesmium* distribution

During summer, the TWC and Kuroshio flowed into the ECS, resulting in abundant *Trichodesmium*. However, their growth was suppressed by the eutrophic and low-salinity coastal waters (especially the CDW in the upper water column) or low-temperature YSCWM (Figs. 2 and 3), despite the obvious monthly changes in the *Trichodesmium* distribution. In June and July, the bottom isohaline of 33 moved northward near the CE (31°N; data not shown), indicating TWC incursion. The CDW (inner part of the isohaline of 31) in the upper water column moved northeastward and joined with the south-eastward YSCC, indicating a low surface salinity and temperature (Fig. 2) with high DIN and DIP. Concurrently, the low-temperature YSCWM (bottom temperature  $< 12^{\circ}\text{C}$ ) beyond the coastal waters inhibited the *Trichodesmium* distribution. The varying properties of these water masses resulted in a high density of *Trichodesmium* in the southern part of our study area and an extremely low density in the northern part, especially in the waters near the CE (Fig. 3). In August, the enhanced TWC dominated in the lower water column, and the CDW dominated in the upper water column, resulting in a high concentration of *Trichodesmium* in the southeastern

part and a low concentration in the CDW plume. The summer distribution patterns of *Trichodesmium* agree with those of previous studies on the ECS shelf (Ding, 2009; Yang, 1998). Our findings indicate that variations in water masses (especially the CDW, TWC, and Kuroshio) control the spatiotemporal changes in the *Trichodesmium* distribution. Similarly, Navarro et al. (2000) noted *Trichodesmium* to be absent at the Caribbean Sea Time Series station, where waters are strongly influenced by the Orinoco River in summer.

In summer, the abundance of *Trichodesmium* increased significantly ( $P < 0.001$ ) from  $4.08 \times 10^3$  trichomes  $\text{m}^{-3}$  in June to  $53.31 \times 10^3$  trichomes  $\text{m}^{-3}$  in August (Table 2). We assume that the enhanced TWC and increased water column stratification ( $\Delta\sigma_t$ ) contributed to the abundance increase, in addition to the increased temperature discussed in Section 4.1. Under the prevailing southwestern monsoon, the TWC was enhanced obviously from June to August and moved northward (Jiang et al., 2015a; Zhou et al., 2015), resulting in the transport of more *Trichodesmium* filaments and a suitable environment (relatively low-nutrient, high-temperature, and high-salinity water regiments) for their growth. The increased stratification (Table 1) in the CE and adjacent ECS shelf (which is caused by the upper buoyant CDW floating on the lower, stronger TWC) prevents the supplementation of upwelling bottom nutrients for upper phytoplankton growth (Jiang et al., 2015a) and is therefore also favorable for the  $\text{N}_2$ -fixing *Trichodesmium*. Because of the presence of gas vesicles, *Trichodesmium* can migrate vertically for nutrient acquisition (particularly phosphorus) by regulating its buoyancy in a stratified water column (Capone et al., 1997; Olson et al., 2015; Villareal and Carpenter, 2003).

The present maximum density of *Trichodesmium* in August occurred at the station adjacent to the river plume mixing with the offshore TWC with relatively low DIN ( $< 2 \mu\text{mol L}^{-1}$  on the surface layer) and high DIP (approximately  $0.5 \mu\text{mol L}^{-1}$  at a 30 m-depth). This is consistent with previous studies in the CE and adjacent shelf (Ding, 2009; Yang, 1998; Zhang et al., 2014) and in the Mekong River Estuary (Grosse et al., 2010). We assume that dissolved trace metal elements (e.g., Fe, Ni, and Mo) in the river plume might stimulate the growth of *Trichodesmium*, as indicated by previous reports on the plumes of the Amazon (Olson et al., 2015; Tovar-Sanchez and Sañudo-Wilhelmy, 2011) and Mekong Rivers (Voss et al., 2006). The CDW deeply influenced the *Trichodesmium* distribution in our study area.

#### 4.3. Comparison of *Trichodesmium* species and abundance in other seas

*Trichodesmium* species in the CE and adjacent ECS shelf consisted of *T. thiebautii*, *T. erythraeum*, and *T. hildebrandtii*. *T. thiebautii* accounted for  $> 85\%$  of *Trichodesmium* abundance (Table 2). This finding is consistent with previous observations in the ECS shelf (Ding, 2009; Yang, 1998) and is similar to reports in other China marginal seas (Marumo and Asaoka, 1974; Wang et al., 2012; Yang, 1998), Indo-Pacific area north of Australia, western Gulf of Mexico (Villareal and Carpenter, 2003), Caribbean and Sargasso Seas (Carpenter and Price, 1977), and tropical North Atlantic Ocean (Carpenter et al., 2004).



**Table 5** Abundance [ $\times 10^3$  trichomes  $m^{-3}$ ] and DIA [ $\times 10^3$  trichomes  $m^{-2}$ ] of *Trichodesmium* in China's marginal seas and adjacent areas in summer.

| Study areas   | Time                          | Sample methods         | Abundance/DIA | References             |
|---|-------------------------------|------------------------|---------------|------------------------|
| Southern Yellow Sea (32°–34°N, 120.5°–124.5°E)      | Jun–Aug 1978                  | Net (64 $\mu$ m)       | 1.13          | Yang (1998)            |
| ECS (28°–32°N, 122°–127°E)                          |                               |                        | 17.93         |                        |
| Northern ECS (~30°–32.5°N, 122°–127°E)              | Jul–Aug 2006                  | Net (76 $\mu$ m)       | 35.70         | Zhang et al. (2014)    |
| Southern ECS  | Jul 1995                      | Net (20 $\mu$ m)       | 8.8           | Chang (2000)           |
|   | Aug 1994                      |                        | 19.7          |                        |
| ECS and adjacent Kuroshio near Japan                | Summer 2006–2010              | Bottle (surface water) | 32            | Shiozaki et al. (2015) |
| ECS (~27.5°–33.3°N, 122.0°–125.5°E)                 | Jun and Aug 2009 and Jul 2011 | Net (76 $\mu$ m)       | 26.03         | This study             |
| Northern SCS (18°–22.5°N, 111.5°–123°E)             | Summer 2000–2002              | Bottle (1 L)           | 87.78         | Chen et al. (2003)     |
| South East Asia Time Series Station, Northern SCS   | Summer 2004–2006              | Bottle (1.2 or 2.4 L)  | 15.5          | Chen et al. (2011)     |
| Mesoscale cyclonic eddy, SCS                        | Aug–Sep 2007                  | Bottle (10 L)          | 330–850       | Zhang et al. (2011)    |
| Kuroshio off Taiwan (18.2°–25.8°N, 120.8°–129.2°E)  | Jul 1997                      | Net (76 $\mu$ m)       | 15.27         | Wang et al. (2012)     |
| Sanya Bay, SCS (18°11′–18°18′N, 109°20′–109°30′E)   | Aug 2004                      | Net (20 $\mu$ m)       | <b>2755.6</b> | Dong et al. (2008)     |
| Northern SCS (18°–22.5°N, 111.5°–123°E)             | Jul 2000–2002                 | Bottle (1 L)           | <b>5267</b>   | Chen et al. (2003)     |
| SCS (10°–17°N, 108°–115°E)                          | Aug 2007                      | Bottle (3 L)           | <b>8383.2</b> | Ding (2009)            |
| Kuroshio in southern ECS                            | Sep 1995                      | Bottle (1 L)           | <b>610</b>    | Chang et al. (2000)    |
| Upstream Kuroshio (19°–24°N, 121°–124°E)            | Summer 2002–2006              | Bottle (1.2 or 2.4 L)  | <b>34,610</b> | Chen et al. (2008)     |
| Northern SCS basin (18°–22°N)                       | Summer 2004–2007              |                        | <b>4870</b>   |                        |
| CE and adjacent ECS (~27.5°–33.3°N, 122.0°–125.0°E) | Aug 2006                      | Bottle (3 L)           | <b>765.0</b>  | Ding (2009)            |
| CE and adjacent ECS (~27.5°–33.3°N, 122.0°–125.5°E) | Jun and Aug 2009 and Jul 2011 | Net (76 $\mu$ m)       | <b>1821.9</b> | This study             |

Boldfaced numbers indicate the DIA. SCS: South China Sea.

We collected historical data on *Trichodesmium* abundance in summer in China's marginal seas by using plankton net and bottle collection methods (Table 5). Based on historical records, the summer average abundance of *Trichodesmium* in the ECS was markedly higher than that in the southern Yellow Sea, but lower than those in the South China Sea (SCS) and Kuroshio areas. Chang (2000) found that the two methods generated similar distribution patterns for *Trichodesmium* along a transect in the southern ECS despite the estimation bias of *Trichodesmium* abundance. Therefore, *Trichodesmium* may have latitudinal distribution patterns, indicating that the abundance increases from high to low latitudes in China's marginal seas.

In the present study, the summer average abundance of *Trichodesmium* ( $26.03 \times 10^3$  trichomes  $m^{-3}$ ) in the ECS was higher than that in a previous investigation ( $17.93 \times 10^3$  trichomes  $m^{-3}$ ) in the summer of 1978. This change can be attributed to the regional increased seawater temperature, enhanced transports of the Kuroshio and TWC (Jiang et al., 2014; Tang et al., 2009), and northern distribution boundary shifting of *Trichodesmium* resulting from accelerated global warming. Several studies have found that the summer dominances of high-temperature and -salinity phytoplankton species (e.g., *Trichodesmium*) have increased in the CE (Jiang et al., 2014) and adjacent Hangzhou Bay (Zhang et al., 2015) during the past decades. Similarly, one offshore tropical-subtropical copepod species, *Temora turbinata*, which first dominated in the CE in summer 2004, indicating TWC incursion and regional warming (Zhang et al., 2010). Spatharis et al. (2012) first observed a *T. erythraeum* bloom in the Mediterranean Sea, which was attributed to climate change. However, other factors, such as enhanced acidification, increased stratification, and decreased mixed layer depth, may also provide *Trichodesmium* with a competitive advantage over other planktonic populations (Levitan et al., 2007; Spungin et al., 2014).

#### 4.4. Contributions of *Trichodesmium* to Chl *a* and nitrogen fixation

The average contribution of *Trichodesmium* to Chl *a* in the CE and adjacent ECS shelf in June, July, and August was 0.3%, 1.1%, and 2.3% with maximums of 2.7%, 7.0%, and 13.7%, respectively, based on the Chl *a* content of free and colonial *Trichodesmium* measured by Letelier and Karl (1996). These contributions are substantially lower than those reported for the tropical North Atlantic Ocean (11–62%; Carpenter et al., 2004), Caribbean Sea (61%; Carpenter and Price, 1977), Sargasso Sea (5%; Carpenter and Price, 1977), and Station ALOHA (18%; Letelier and Karl, 1996). However, the highest contribution of 13.7% in August occurred in offshore waters, and this contribution is close to that in other seas.

Most of the *Trichodesmium* that we found presented as free trichomes, not colonial forms (which accounted for approximately 10%; Table 3). This finding is consistent with previous observations in the SCS (Wu et al., 2003), ECS and Kuroshio areas (Chang et al., 2000; Saino and Hattori, 1980), and Station ALOHA (Letelier and Karl, 1996). However, in the North Pacific subtropical gyre (Sohm et al., 2011) and tropical North Atlantic Ocean (Carpenter et al., 2004), >70% and ≥89% of the total trichomes were colonies, respectively. The

present per colony numbers (generally <10) of *Trichodesmium* in the ECS matched the values in the SCS (Wu et al., 2003), but were considerably lower than those reported for the Red Sea (Post et al., 2002), north coast of Australia (Sohm et al., 2008), subtropical eastern North Pacific Ocean (Letelier and Karl, 1996), and tropical (Carpenter et al., 2004) and subtropical North Atlantic Ocean (Taboada et al., 2010).

To estimate the summer input of nitrogen from *Trichodesmium*  $N_2$  fixation in the CE and adjacent ECS shelf, the  $N_2$  fixation rate of free trichomes was assumed to be one-third of that of colonial trichomes. This estimation is based on reported averages of 37% (Saino and Hattori, 1982) and 28% (Letelier and Karl, 1998) of trichome to colony fixation rate comparisons. If we accept the assumption of Chang et al. (2000) that the  $N_2$  fixation rate of free *Trichodesmium* in the ECS is  $5.6 \text{ pmol N trichome}^{-1} \text{ d}^{-1}$ , the average  $N_2$  fixation rate of colonial *Trichodesmium* is  $16.8 \text{ pmol N trichome}^{-1} \text{ d}^{-1}$ . Besides, we assume that their  $N_2$  fixation rate would be stable in daylight in our study area, despite the influence by changeable environmental variables (e.g., nutrients, temperature, salinity, and irradiance; Berman-Frank et al., 2001; Breitbarth et al., 2007; Capone et al., 2005; Fu and Bell, 2003). Therefore, based on the present free and colonial *Trichodesmium* DIA, the estimated  $N_2$  fixation rate of *Trichodesmium* in our study area was 2.1, 11.1, and  $23.7 \text{ } \mu\text{mol N m}^{-2} \text{ d}^{-1}$  in June, July, and August, respectively. The summer average  $N_2$  fixation rate of *Trichodesmium* was  $12.3 \text{ } \mu\text{mol N m}^{-2} \text{ d}^{-1}$  in the CE and adjacent ECS, which is close to the values measured in the Sanya Bay and SCS basin but lower than those in the upwelling area in the southern ECS and other areas in the North Pacific and North Atlantic Oceans (Table 7). Zhang et al. (2012) reported that the summer  $N_2$  fixation rate was approximately  $21 \text{ } \mu\text{mol N m}^{-2} \text{ d}^{-1}$  in the ECS shelf as determined using  $^{15}N_2$  tracer method. According to this rate, *Trichodesmium* was estimated to contribute approximately 58.6% of the biological  $N_2$  fixation.

In our study, the estimated contribution of *Trichodesmium* to biological  $N_2$  fixation in the CE and adjacent ECS is consistent with the observations (contribution >50%) in the Kuroshio and ECS near Japan (Saino, 1977; Shiozaki et al., 2010) but considerably higher than those in the northern SCS and upstream Kuroshio (Chen et al., 2014), western equatorial Pacific Ocean (Bonnet et al., 2009), western and central oligotrophic North Pacific Ocean (Shiozaki et al., 2010), and North Pacific Subtropical Gyre west of Hawaii (Sohm et al., 2011). Our unpublished data in the same area in August 2014 also show that fractions >10  $\mu\text{m}$  (*Trichodesmium* and diatom endosymbiont *Richelia intracellularis*) contributed >50% to  $N_2$  fixation of the water column, as determined using the  $^{15}N_2$  tracer method. We deemed that the contribution of *Trichodesmium* to biological  $N_2$  fixation was near 50%, because the *R. intracellularis* was scarcely found in the CE and adjacent ECS (Ding, 2009). Bonnet et al. (2009) found that fractions >10  $\mu\text{m}$  (mainly *Trichodesmium*) contributed the major  $N_2$  fixation at the stations close to Papua New Guinea, whereas fractions <10  $\mu\text{m}$  (unicellular cyanobacterial diazotrophs) contributed markedly more to total  $N_2$  fixation off this island than fractions >10  $\mu\text{m}$  did. Similar results in the Pacific Ocean and marginal seas have been reported by Shiozaki et al. (2010, 2014) and Sohmi et al. (2011). Shiozaki et al. (2015) observed that *Trichodesmium* bloomed near the

**Table 6** Estimated N<sub>2</sub> fixation rate (NFR) [ $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ] of free and colonial *Trichodesmium* in the CE and adjacent ECS in summer.

| NFR                           | June 2009     |      | July 2011       |      | August 2009     |       |
|-------------------------------|---------------|------|-----------------|------|-----------------|-------|
|                               | Mean $\pm$ SD | Max. | Mean $\pm$ SD   | Max. | Mean $\pm$ SD   | Max.  |
| Free <i>Trichodesmium</i>     | 1.6 $\pm$ 2.9 | 13.0 | 8.8 $\pm$ 14.7  | 51.9 | 17.1 $\pm$ 23.7 | 80.3  |
| Colonial <i>Trichodesmium</i> | 0.5 $\pm$ 1.8 | 8.4  | 2.3 $\pm$ 4.4   | 16.4 | 6.6 $\pm$ 16.0  | 61.0  |
| Total                         | 2.1 $\pm$ 4.3 | 16.1 | 11.1 $\pm$ 18.4 | 67.0 | 23.7 $\pm$ 38.2 | 135.5 |

**Table 7** Summer NFR [ $\mu\text{mol N m}^{-2} \text{d}^{-1}$ ] of *Trichodesmium* in China marginal seas and other global seas/oceans determined by different methods.

| Study areas  | Time                          | Method                        | NFR   | Reference            |
|--|-------------------------------|-------------------------------|-------|----------------------|
| CE and adjacent ECS ( $\sim 27.5^\circ\text{--}33.3^\circ\text{N}$ , $122.0^\circ\text{--}125.5^\circ\text{E}$ ) | Jun and Aug 2009 and Jul 2011 | CV                            | 12.3  | This study           |
| Upwelling in southern ECS ( $25.5^\circ\text{--}26^\circ\text{N}$ )  | Jul 1994                      | CV                            | 33.6  | Chang et al. (2000)  |
| SE ECS ( $10^\circ\text{--}25^\circ\text{N}$ )   | Summer 1977                   | C <sub>2</sub> H <sub>2</sub> | 126   | Saino (1977)         |
| Sanya Bay, SCS ( $18.2^\circ\text{--}18.3^\circ\text{N}$ , $109.3^\circ\text{--}109.5^\circ\text{E}$ )           | Aug 2004                      | C <sub>2</sub> H <sub>2</sub> | 10    | Dong et al. (2008)   |
| Northern SCS basin ( $18^\circ\text{--}22^\circ\text{N}$ )   | Summer 2004 and 2006          | <sup>15</sup> N <sub>2</sub>  | 12.6  | Chen et al. (2008)   |
| Kuroshio ( $19^\circ\text{--}24^\circ\text{N}$ , $121^\circ\text{--}124^\circ\text{E}$ )                         | Summer 2002                   | <sup>15</sup> N <sub>2</sub>  | 168.1 |                      |
| Subtropical gyre, N. Pacific ( $18^\circ\text{--}28^\circ\text{N}$ , $170^\circ\text{E--}154^\circ\text{W}$ )    | Jul–Aug 2003                  | C <sub>2</sub> H <sub>2</sub> | 55    | Sohm et al. (2011)   |
| Station BATS ( $31^\circ 50'\text{N}$ , $64^\circ 10'\text{W}$ ),  | Summer 1995–1996              | <sup>15</sup> N <sub>2</sub>  | 153.8 | Orcutt et al. (2001) |
| Tropical North Atlantic ( $\sim 14^\circ\text{--}25^\circ\text{N}$ , $\sim 50^\circ\text{--}75^\circ\text{W}$ )  | May–Jun 1994                  | C <sub>2</sub> H <sub>2</sub> | 898   | Capone et al. (2005) |
| Tropical North Atlantic ( $\sim 3^\circ\text{--}14^\circ\text{N}$ , $\sim 42^\circ\text{--}58^\circ\text{W}$ )   | Jun–Aug 2011                  | C <sub>2</sub> H <sub>2</sub> | 59    |                      |
| Northwestern Gulf of Mexico  | Jul 2000                      | <sup>15</sup> N <sub>2</sub>  | 84.5  | Holl et al. (2007)   |

CV, calculated value according to C<sub>2</sub>H<sub>2</sub> measurement conducted in Kuroshio near southern Japan.

Miyako Islands, and the abundance and N<sub>2</sub> fixation in the Kuroshio and ECS (near islands) were considerably higher than those in the Philippine Sea (far from islands). Apparently, *Trichodesmium* generally dominate in N<sub>2</sub> fixation in the waters near continents and islands. Shiozaki et al. (2010, 2014, 2015) have concluded that *Trichodesmium* abundance and N<sub>2</sub> fixation in the Pacific Ocean tends to be high in areas close to continents and isolated islands but low in the areas remote from landmasses, and this might be related to high terrestrial input (e.g., iron and phosphorus).

Our study demonstrates that free-living trichomes contributed >70% of *Trichodesmium* N<sub>2</sub> fixation throughout the summer (Table 6). This finding indicates that the abundant individual filaments were a vital source of the areal N<sub>2</sub> fixation in the ECS, although they did so with a lower per-cell rate than when present in the colony morphology (Letelier and Karl, 1998; Saino and Hattori, 1982). Similarly, previous works conducted at Station ALOHA (Letelier and Karl, 1996, 1998) and BATS (Orcutt et al., 2001) have reported that free trichomes represent a crucial segment of their populations, contributing up to 75% of *Trichodesmium*-instigated N<sub>2</sub> fixation.

## 5. Conclusions

This study revealed the significant spatiotemporal changes of *Trichodesmium* in relation to physicochemical factors and estimated their N<sub>2</sub> fixation rate in the CE and adjacent ECS shelf during summer. *Trichodesmium* population was dominated by *T. thiebautii* and their spatiotemporal distribution

was primarily controlled by the changes of water masses, particularly the TWC and CDW. Their abundance, occurrence frequency, and contribution to phytoplankton biomass increased significantly with increased temperature, stratification, and the TWC. In addition to the abundance shown in historical data, *Trichodesmium* abundance in summer has increased substantially under regional warming and hydrographic alteration. *Trichodesmium*, particularly the free living trichomes, were clearly the dominant diazotrophs in the CE and adjacent ECS during summer, potentially contributing at least half of areal biological N<sub>2</sub> fixation. However, to accurately calculate the N<sub>2</sub> fixation of *Trichodesmium* and elucidated their biogeochemical significance, direct measurements (<sup>15</sup>N<sub>2</sub> tracer technique) must be performed during field investigations in the CE and ECS shelf.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.oceano.2017.02.001](https://doi.org/10.1016/j.oceano.2017.02.001).

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