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# Ecological thresholds of phytoplankton community across environmental gradients in the harmful algal blooms-frequently-occurring, subtropical coastal waters, East China Sea

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**Abstract**

Phytoplankton communities can response immediately and directly to environmental changes, and thus have been applied as reliable biotic indicators in aquatic systems. This study provided insights into the relationships concerning ecological thresholds of phytoplankton communities and individual taxon in response to environmental changes in coastal waters of northern Zhejiang Province, East China Sea. Results demonstrated that there existed seasonal variations of phytoplankton community ecological thresholds of which spring being higher than those in summer. As for individual species, *Prorocentrum donghaiense* and *Noctiluca scintillans* were identified as the most tolerant and sensitive indicator species in spring and summer, respectively. They exhibited strong indications in response to environmental changes. These findings highlighted that phytoplankton community structure in this region was stable when environmental gradients were below the thresholds of sensitive species, whereas potential harmful algal blooms may occur when environmental gradients exceeded the thresholds of tolerant species.

**Key words:** ecological thresholds, phytoplankton community, environmental gradients, indicator species, coastal waters of northern Zhejiang Province

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## 1 Introduction

Ecological thresholds can be defined as a point or zone of relatively abrupt change point occurs between alternate ecological conditions in relation to one or more ecological factors (Zhao et al., 2007; Baker and King, 2010). Identification of thresholds for ecological communities is of crucial importance since such change points pose practical applications upon eco-disaster pre-warning, natural resources conservation, and ecosystem balance. Many organisms, such as phytoplankton (Kovalenko et al., 2017; Taylor et al., 2018), zooplankton (Yang et al., 2018), fish (Milardi et al., 2018), macroinvertebrates (Sultana et al., 2019), and bacteria (Simonin et al., 2019) have been applied to research stressor-response patterns across environmental gradients, resulting in different ecological thresholds. However, selecting which ecological community to better indicate environmental changes in a certain ecoregion still remains controversy.

Algae, particularly phytoplankton, are the key primary producers in most aquatic ecosystems, supplying the essential energy for senior food chains. Phytoplankton is ubiquitously distributed in transitional, estuarine, and coastal environments and acting as carriers for nutrients fixation and transition in terms of biogeochemical cycling in these areas. As widely acknowledged, estuarine and coastal waters is susceptible to multiple environmental stresses (e.g., eutrophication, harmful algal blooms (HABs), and hypoxia) that mainly caused by human activities (Wang, 2006; Ye et al., 2017). Given the short generation times, marine phytoplankton species responses immediately and sensitively to environmental variations, especially to nutrient inputs derived from riverine discharge. This makes it suitable as an indicator to nutrient over-enrichment and being an early-warning signal of environmental changes in response to external pressures (McCormick and Cairns, 1994; Zhou et al., 2008; Jiang et al., 2014). Moreover, as a routine item of ecological monitoring, field observations and laboratory identifications of phytoplankton are cost-effective and easy (McCormick and Cairns, 1994). All these evidence that phytoplankton possesses excellent attributes for the indications of environmental changes and ecological successions.

The coastal waters of northern Zhejiang Province, a subtropical region that has experienced multiple stresses from anthropogenic perturbations for decades (Wang, 2006; Zhou et al., 2008; Jiang et al., 2014; Ye et al., 2017), is ceaselessly suffering a great deal of ecological problems. Large amounts of nutrient and organic matter loadings are transported into marine environments via terrestrial runoff, among which the Changjiang Diluted Water (CDW) has been recognized as the most critical source (Gao et al., 2012). Excessive nutrient inputs inevitably lead to severe eutrophication and subsequently facilitate frequent occurrences of HABs under certain circumstances, making coastal waters of

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northern Zhejiang Province as one of the most notable regions of HABs outbreaks in China (Wang and Wu, 2009; Liu et al., 2013; Zeng et al., 2019; Wang et al., 2019). In addition, the complex hydrological and hydrodynamic conditions, especially in wet seasons (Zhou et al., 2017a), also enable this region to be substantially affected by the complicated environmental changes. Hence, the coastal waters of northern Zhejiang Province seems to become an ideal region to study ecological thresholds of phytoplankton community in response to environmental changes.

Recent publications have documented various ecological thresholds of phytoplankton communities along environmental gradients. Most of them focused on freshwater ecosystems, such as stream (Smucker et al., 2013; Taylor et al., 2018), river (Porter-Goff et al., 2013), reservoir (Tang et al., 2016), wetland (Mazzei and Gaiser, 2018), and lake (Cao et al., 2016; Kovalenko et al., 2017), little knowledge is known in coastal marine environment. Therefore, this study aims to figure out: (1) the thresholds of phytoplankton community responses to environmental gradients; (2) the thresholds of individual species responses to environmental gradients; and (3) seasonal community responses of ecological thresholds between spring and summer.

## 2 Materials and methods

### 2.1 *Sampling strategy and sites*

Field investigations were conducted twice a year (in May and August) from 2015 to 2018, including 19 sites in 2015, 20 sites in 2016, 22 sites in 2017, and 18 sites in 2018, respectively. Due to the adjusted sampling strategy, monitoring networks differed annually despite seasonal sampling sites remained the same within each year in terms of numbers and geographic positions (Fig. 1).

### 2.2 *Sample collections and analyses*

Samples for physicochemical parameters were collected by a CTD system (Seabird 19 Plus, USA) through water collectors fixed on it at different layers based on water depth of each site (AQSIQ and Standardization Administration of China, 2008a). Water temperature (WT), water clarity (secchi depth, SD), salinity (Sal), pH, nitrate ($NO_3^-$), nitrite ($NO_2^-$), ammonium ($NH_4^+$), phosphate ($PO_4^{3-}$), silicate ($SiO_3^{2-}$), total phosphorus (TP), and total nitrogen (TN) concentrations were measured following the standard methods (AQSIQ and Standardization Administration of China, 2008b). All the parameters displayed in this work were the average values of all the sampling layers in each site.

Phytoplankton samples were collected from bottom to surface using plankton net with a 77 μm mesh size and preserved with 5% formalin. After at least 24 h of sedimentation, samples were concentrated to 100–150 mL and then, 0.5 mL sample was uniformly siphoned onto the counting plate for counting and identification through light microscopy (Olympus bx41, Japan). Samples were identified into species level at which an accurate identification could be confirmed. All the analyses were carried out according to the standard methods (AQSIQ and Standardization Administration of China, 2008c).

### 2.3 *Data analysis*

This study used Kruskal-Wallis tests to examine seasonal differences for environmental parameters. Species data were lg ($x$+1) transformed prior to community data analysis. To visualize seasonal dissimilarity of phytoplankton community composition, Principal Coordinate Analysis (PCoA) was employed using Bray-Curtis dissimilarity matrices. Analysis of Similarity (ANOSIM) was thereafter applied to evaluate the significant differences between the two seasons. Relationship between phytoplankton community and environmental viarbles was conducted via Redundancy Analysis (RDA).

Once the most significant environmental factors were selected, Threshold Indicator Taxa Analysis (TITAN) (Baker and King, 2010) was then performed to identifiy ecological thresholds at the commnity and individual level along enviromnemtal gradients, respectively. This approach integrates indicator species analysis (Dufrêne and Legendre, 1997) with nonparametric change-point analysis (King and Richardson, 2003; Qian et al., 2003) to determine indicator species scores across binary partitions of samples for detecting congruence in taxon-specific changes of abundance and occurrence frequency across environmental gradients as evidences of ecological thresholds (Baker and King, 2010, 2013; King and Baker, 2010). After positive taxa (z+) and negative taxa (z–) are identified, bootstapping is used to assess the purity and reliability of each species. Two key parameters are employed to estimate whether an indicator species is statistically reliable. Purity is the mean proportion correct taxa direction (z– or z+) assignment, reliability is the mean proportion of $P<0.05$. Only species that characterized with purity⩾0.95 and reliability⩾0.95 will be defined as reliable indicator species. Species data used in TITAN were lg (x+1) transformed and only species with⩾3 occurrences were included (Baker and King, 2010).

All the analyses were performed in R 3.3.1 (R Development Core Team, 2016), with package“vegan” (Oksanen et al., 2010) for PCoA, ANOSIM, and RDA and package “TITAN2” (Baker and King, 2010) for TITAN.

## 3 Results

### 3.1 *Dynamics of environmental conditions*

As predicted by seasonal patterns, WT ranged from 16.94°C to 22.40°C and 22.40°C to 30.70°C in spring and summer, respectively; Sal varied from 23.25 to 32.58 and 21.48 to 33.99 in spring and summer, respectively; pH ranged from 7.96 to 8.42 and 7.84 to 8.38 in the two seasons, separately. For nutrients, concentrations of TP were detected from 0.028 5 mg/L to 0.281 3 mg/L (mean=0.083 0 mg/L) and 0.026 4 mg/L to 0.259 5 mg/L (mean=0.102 4 mg/L) during spring and summer periods, respectively, while TN were measured from 0.469 mg/L to 1.943 mg/L (mean=1.103 mg/L) and 0.423 mg/L to 2.100 mg/L (mean=0.883 mg/L) in spring and summer, respectively (Table 1). Characteristics of other parameters were also displayed in Table 1.

Overall, WT (Fig. A1a), pH (Fig. A1d) and nutrients (Figs A1e–f, h–i), except for $PO_4^{3-}$, exhibited significant seasonal differences. On the contrary, Sal and SD did not show any remarkable fluctuations within the two seasons (Figs A1b–c).

### 3.2 *Phytoplankton community composition and α-diversity*

In general, a total of 130 species belonging to 5 phyla (Bacillariophyta, Pyrrophyta, Chlorophyta, Chrysophyta and Cyanophyta) were recorded in the two seasons; and 77 species of 36 genera and 101 species of 72 genera were detected in spring and summer, respectively. *Noctiluca scintillans* (41.5%) was identified as the most abundant species for the former and *Pseudo-nitzschia pungens* (41.2%) for the latter.

Regarding the α-diversity, Shannon-Weiner diversity index ($H'$), Pielou evenness index ($J$) and Margalef richness index ($d$) varied from 0 to 2.58, 0 to 0.93 and 0 to 2.01, respectively in spring. Meanwhile, they maintained round at 1.51, 0.54 and 1.15 during summertime (Table 1). Marked seasonal dissimilarities of

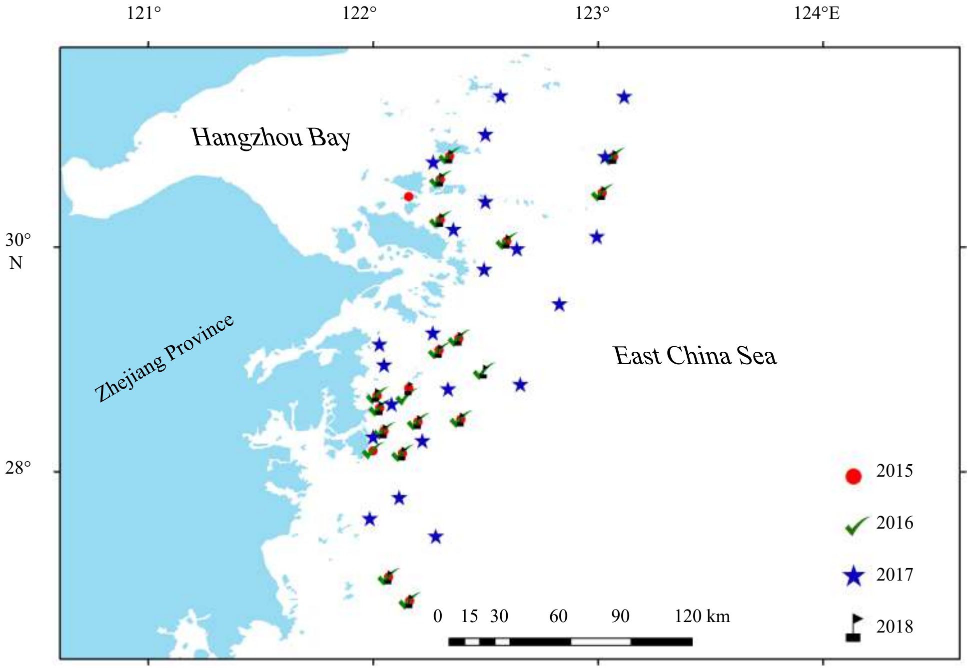

**Fig. 1.** Geographic positions of sampling sites from 2015 to 2018.

*H*' and *d* were observed (Figs A1j, l), whereas *J* demonstrated little temporal variations (Fig. A1k).

### 3.3 *Patterns of seasonal differences of phytoplankton community and its relationship with environmental variables*

A relative strong seasonal dissimilarity of phytoplankton community was discovered via PCoA plot (Fig. 2, ANOSIM test, $R$=0.300, $p$<0.001), indicating a season-to-season separation was acceptable across the study area.

RDA showed that significant factors explained 9.3% of the total variation and TN, pH and TP were the driving factors controlling phytoplankton community in spring. There was 15.6% of the total variation which was explained by six remarkable parameters, namely WT, TN, TP, Sal, $SiO_3^{2-}$ concentration and pH in summer (Table 2). Accordingly, this study suggested that TN, TP and pH were the leading factors structuring phytoplankton community in both spring and summer.

### 3.4 *Community-level responses to environmental gradients*

TITAN revealed that in spring, pH fluctuated drastically between 8.00 and 8.28 (Fig. 3a) resulting in the negative response threshold of 8.03 and positive response threshold of 8.25, respectively (Table 3). TN declined between 0.9 mg/L and 1.3 mg/L and increased intensively from 0.7 mg/L to 1.4 mg/L (Fig. 3b), leading to 0.966 mg/L and 1.553 mg/L as thresholds of negative and positive taxa, respectively (Table 3). Meanwhile, the thresholds of TP were 0.043 3 mg/L and 0.098 7 mg/L for negative and positive responders, separately (Table 3 and Fig. 3c).

During summertime, pH decreased sharply between 8.0 and 8.2 and increased drastically from 7.9 to 8.05 (Fig. 3d), which provided the thresholds of 7.98 and 8.12 for negative taxa and positive taxa, respectively (Table 3). For nutrients, the thresholds of TN were 0.706 mg/L and 1.008 mg/L for negative taxa and positive taxa, respectively (Table 3 and Fig. 3e) while thresholds of TP occurred at 0.035 5 mg/L and 0.146 4 mg/L (Table 3) for negative taxa and positive taxa, separately (Fig. 3f).

### 3.5 *Taxon-level responses to environmental gradients*

In spring, this study identified 3, 5 and 4 reliable taxa as tolerant indicator taxa along pH, TN and TP gradient. *Prorocentrum donghaiense* was repeatedly observed and intensively responding to the increasing environmental parameters (Table A1, Figs 4a and b). Meanwhile, only one reliable species was detected as sensitive indicator species in response to both TN and TP, respectively (Table A1, Figs 4a and b), whereas no sensitive responder was found in relation to pH.

In summer, more indicator taxa were detected in response to environmental gradients, of which 11, 12 and 3 reliable taxa were recorded as tolerant indicator taxa that increased with pH value and TN, TP concentrations. In contrast, only 3, 7 and 3 reliable taxa were recorded as sensitive indicator taxa that declined with increasing pH value and TN, TP concentrations (Table A1, Figs 4c–e). *Noctiluca scintillans* occurred frequently as negative responder across pH and TN gradients (Figs 4c and d). Moreover, *Ceratium* spp., *Chaetoceros* spp. corresponded positively to pH; *Skeletonema costatum*, *Coscinodiscus* spp. and *Ceratium* spp. responded positively to TN and TP, respectively (Figs 4d and e).

## 4 Discussion

### 4.1 *Ecological thresholds for phytoplankton community*

Identifying thresholds for ecological communities are of great importance for ecological application and management (Townsend et al., 2008; Martin and Kirkman, 2009). In this study, TITAN revealed that phytoplankton community in the coastal waters of northern Zhejiang Province, East China Sea substantially responded to TN, TP and pH gradients in a nonlinear way (Fig. 3), suggesting the existence of complex stressor-response patterns. Both negative (z–) and positive (z+) thresholds were identified in the two seasons (Table 3). Previous studies have also recorded the thresholds of phytoplankton community across multiple environmental gradients (Smucker et al., 2013; Cao et al., 2016; Taylor et al., 2018). Tang et al. (2016) suggested that

**Table 1.** Statistical summaries for environmental variables and α-diversity indices of phytoplankton community

| | Spring | | | | Summer | | | |
|---|---|---|---|---|---|---|---|---|
| | Minimum | Median | Mean | Maximum | Minimum | Median | Mean | Maximum |
| WT/°C | 16.94 | 18.29 | 18.71 | 22.40 | 22.40 | 25.35 | 25.90 | 30.70 |
| Sal | 23.25 | 29.27 | 29.31 | 32.58 | 21.48 | 29.91 | 29.68 | 33.99 |
| SD/m | 0.1 | 1.75 | 3.21 | 14.5 | 0.1 | 2.0 | 2.9 | 13.5 |
| pH | 7.96 | 8.10 | 8.13 | 8.42 | 7.84 | 8.09 | 8.07 | 8.38 |
| TP/(mg·L$^{-1}$) | 0.028 5 | 0.074 8 | 0.083 0 | 0.281 3 | 0.026 4 | 0.097 6 | 0.102 4 | 0.259 5 |
| TN/(mg·L$^{-1}$) | 0.469 | 0.999 | 1.103 | 1.943 | 0.423 | 0.742 | 0.883 | 2.100 |
| $PO_4^{3-}$/(mg·L$^{-1}$) | 0.000 9 | 0.025 8 | 0.025 6 | 0.055 4 | 0.005 9 | 0.022 0 | 0.024 7 | 0.074 0 |
| DIN/(mg·L$^{-1}$) | 0.172 | 0.538 | 0.555 | 1.520 | 0.183 | 0.447 | 0.465 | 1.327 |
| $SiO_3^{2-}$/(mg·L$^{-1}$) | 0.358 | 0.976 | 1.000 | 1.970 | 0.239 | 0.887 | 0.898 | 1.918 |
| *H′* | 0 | 1.50 | 1.28 | 2.58 | 0.32 | 1.59 | 1.51 | 2.77 |
| *J* | 0 | 0.60 | 0.54 | 0.93 | 0.11 | 0.59 | 0.54 | 0.92 |
| *d* | 0 | 0.76 | 0.82 | 2.01 | 0.40 | 1.04 | 1.15 | 2.51 |

Note: WT, water temperature; Sal, salinity; SD, secchi depth; TP, total phosphorus; TN, total nitrogen; $PO_4^{3-}$, phosphate concentration; DIN, dissolved inorganic nitrogen concentration; $SiO_3^{2-}$, silicate concentration; *H′*, Shannon-Weiner diversity index; *J*, Pielou evenness index; *d*, Margalef richness index.

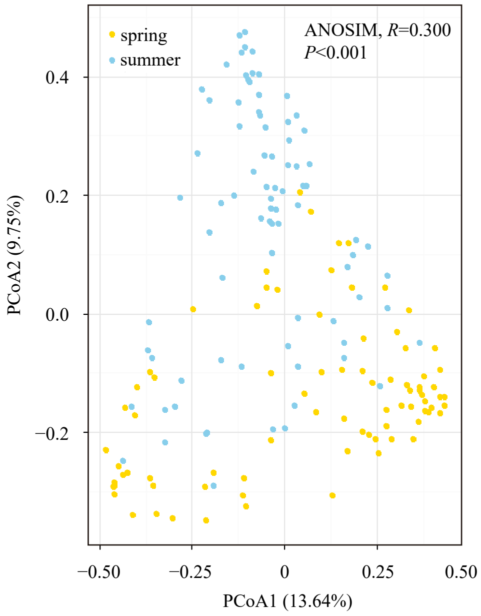

**Fig. 2.** Principal coordinate analysis (PCoA) plots of phytoplankton community with One-way analysis of similarity (ANOSIM, 9999 permutations) visualize seasonal dissimilarity.

thresholds of epilithic diatom assemblages in responses to TN and TP in the Three Gorges Reservoir were 0.382 mg/L (z–), 1.298 mg/L (z+) and 0.016 0 mg/L (z–), 0.065 0 mg/L (z+) in spring, respectively. Results of Mazzei and Gaiser (2018) documented thresholds of diatom assemblages in response to TP gradient were 0.048 7 mg/L (z–) and 0.265 0 mg/L (z+), 0.045 3 mg/L (z–) and 0.364 8 mg/L (z+) in spring and summer, respectively. In the Laurentian Great Lakes, USA, phytoplankton community change-points along $NO_3^-$ and TP gradients reached 0.285 mg/L (z–), 0.460 mg/L (z+) and 0.001 7 mg/L (z–), 0.005 3 mg/L (z+) in spring, respectively while they approximately turned to be twice lower than that in summer (Kovalenko et al., 2017). These thresholds derived from freshwater environments were lower compared with the findings of this study, probably ascribed to the different aquatic environments. In freshwater ecosystems, phytoplankton community generally received nutrients from univariate sources (e.g., agricultural wastes and domestic sewage) within a single watershed despite characterizing with high-level concentrations. On the contrary, in marine systems, especially the coastal waters of northern Zhejiang Province, one of the most severely eutrophic and environmentally heterogeneous areas (Ye et al., 2017), it may be quite different. Given the complex hydrodynamic conditions, mixed riverine inputs (e.g., Changjiang River, Qiantang River, Cao'e River and Yongjiang River) posed considerable effects upon biological communities. Phytoplankton community in this area was significantly influenced by CDW and Taiwan Warm Current (TWC) in wet seasons (Jiang et al., 2015; Zhou et al., 2017a). Moreover, global climate changes continually played an unnegligible role in structuring marine phytoplankton community composition (Harding et al.,

**Table 2.** Results of RDA

| Season | Significant variables | $R^2$ | Cumulated $R^2$ | Adjusted cumulated $R^2$ | *F*-test | *P* |
|---|---|---|---|---|---|---|
| Spring | TN | 0.057 | 0.057 | 0.045 | 4.824 | 0.002 |
| | pH | 0.039 | 0.096 | 0.073 | 3.434 | 0.012 |
| | TP | 0.030 | 0.127 | 0.093 | 2.718 | 0.032 |
| Summer | WT | 0.050 | 0.050 | 0.038 | 4.171 | <0.001 |
| | TN | 0.048 | 0.097 | 0.074 | 4.182 | <0.001 |
| | TP | 0.047 | 0.144 | 0.111 | 4.260 | <0.001 |
| | Sal | 0.027 | 0.172 | 0.128 | 2.551 | 0.015 |
| | $SiO_3^{2-}$ | 0.026 | 0.198 | 0.145 | 2.468 | 0.015 |
| | pH | 0.021 | 0.219 | 0.156 | 2.029 | 0.037 |

Note: WT, water temperature; Sal, salinity; TP, total phosphorus; TN, total nitrogen; $SiO_3^{2-}$, silicate concentration.

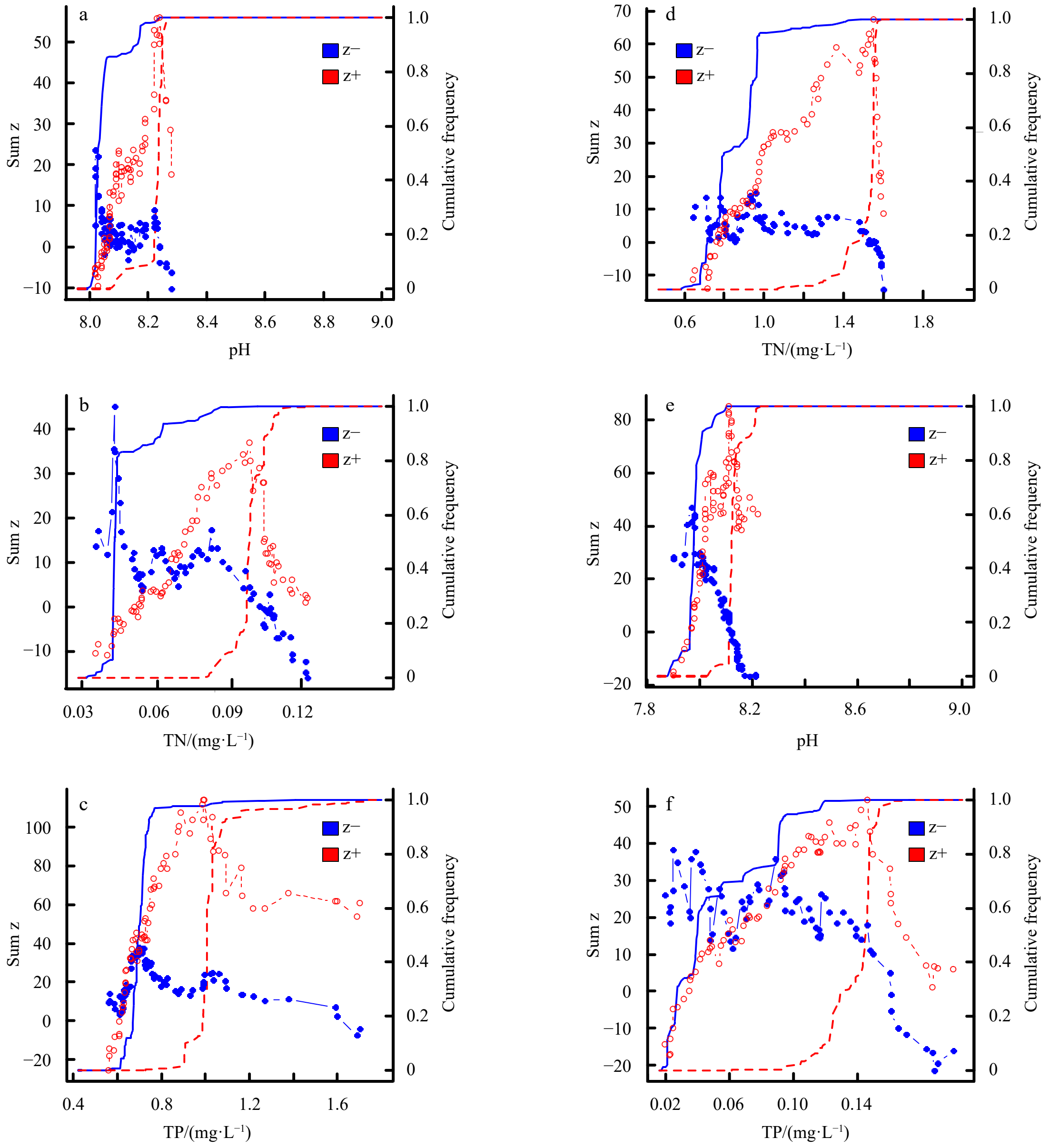

**Fig. 3.** Threshold indicator taxa analysis of phytoplankton community in response to pH, TN and TP (*n*=82 in both spring and summer). Figures referred to the thresholds for the negative response (z–, blue) and positive response (z+, red) of phytoplankton community in spring (a–c) and summer (d–f), respectively. The dashed blue and red lines showed the cumulative frequency distribution of thresholds among 500 bootstrap replicates for sum (z–) and sum (z+), respectively.

**Table 3.** Community-level thresholds in response to environmental gradients

| Season | Environmental gradient | Responder | Threshold |
|---|---|---|---|
| Spring | pH | z– | 8.03 |
| | | z+ | 8.25 |
| | TN (mg·L$^{-1}$) | z– | 0.966 |
| | | z+ | 1.553 |
| | TP/(mg·L$^{-1}$) | z– | 0.043 3 |
| | | z+ | 0.098 7 |
| Summer | pH | z– | 7.98 |
| | | z+ | 8.12 |
| | TN/(mg·L$^{-1}$) | z– | 0.706 |
| | | z+ | 1.008 |
| | TP/(mg·L$^{-1}$) | z– | 0.035 5 |
| | | z+ | 0.146 4 |

Note: Responder meant taxa that either negatively (z–) or positively (z+) responded to environmental gradients.

2016; Conde et al., 2018). These findings implied that phytoplankton community in coastal waters experienced multi-cumulative stresses, which consequently responded more complicated and unpredictable to environmental variability than that in freshwater systems. However, the results of this study did not coincide with a recent study which showed that the thresholds proposed for TN and TP in the region outside Changjiang River Estuary and coastal Zhoushan waters approached to 0.27–0.29 mg/L and 0.023–0.028 mg/L, respectively (Yang et al., 2019). Differences between the two studies may be attributable to the analytical methods used. In the present study, this study concluded the ecological thresholds by taking into account the biological re-

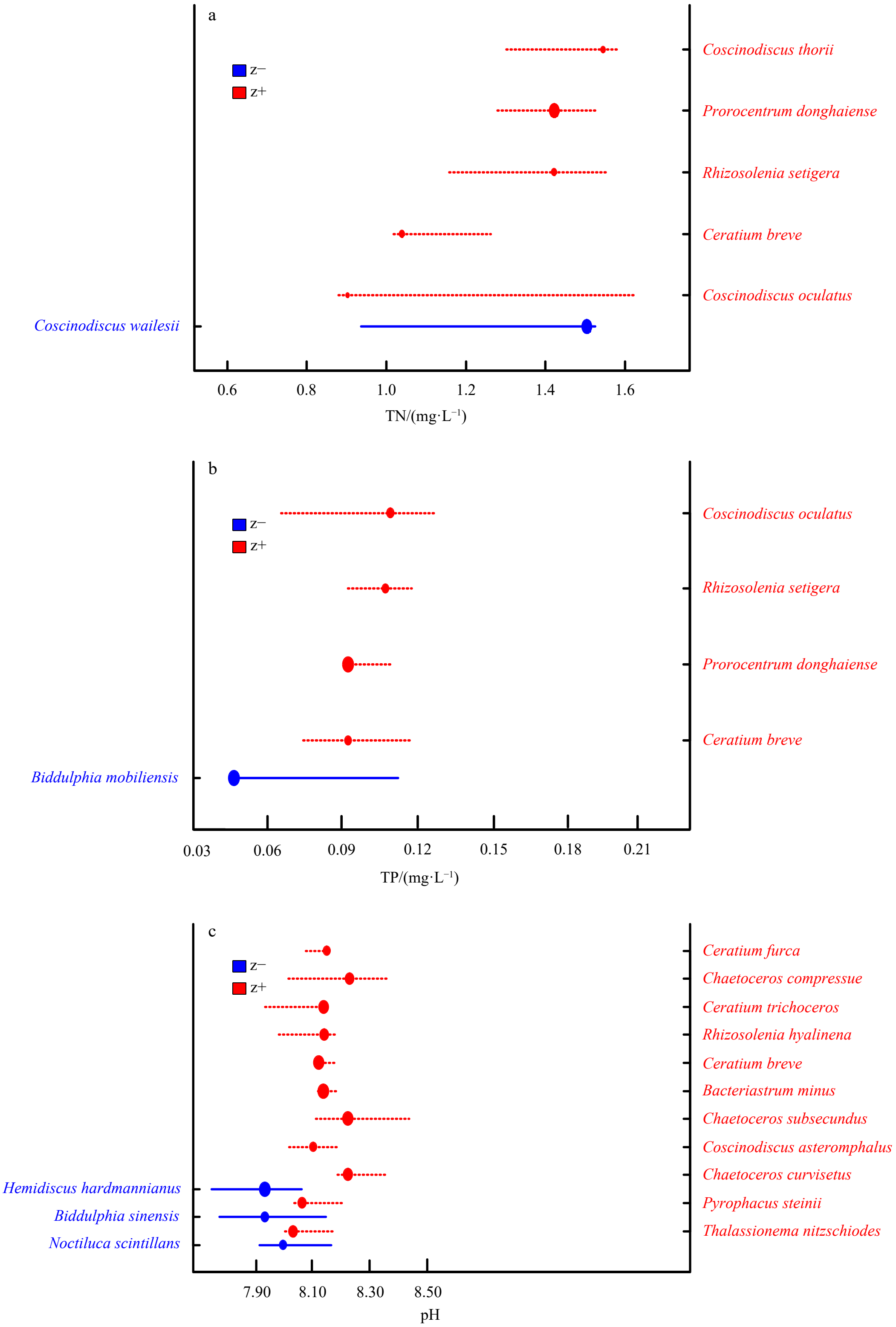

**Fig. 4.**

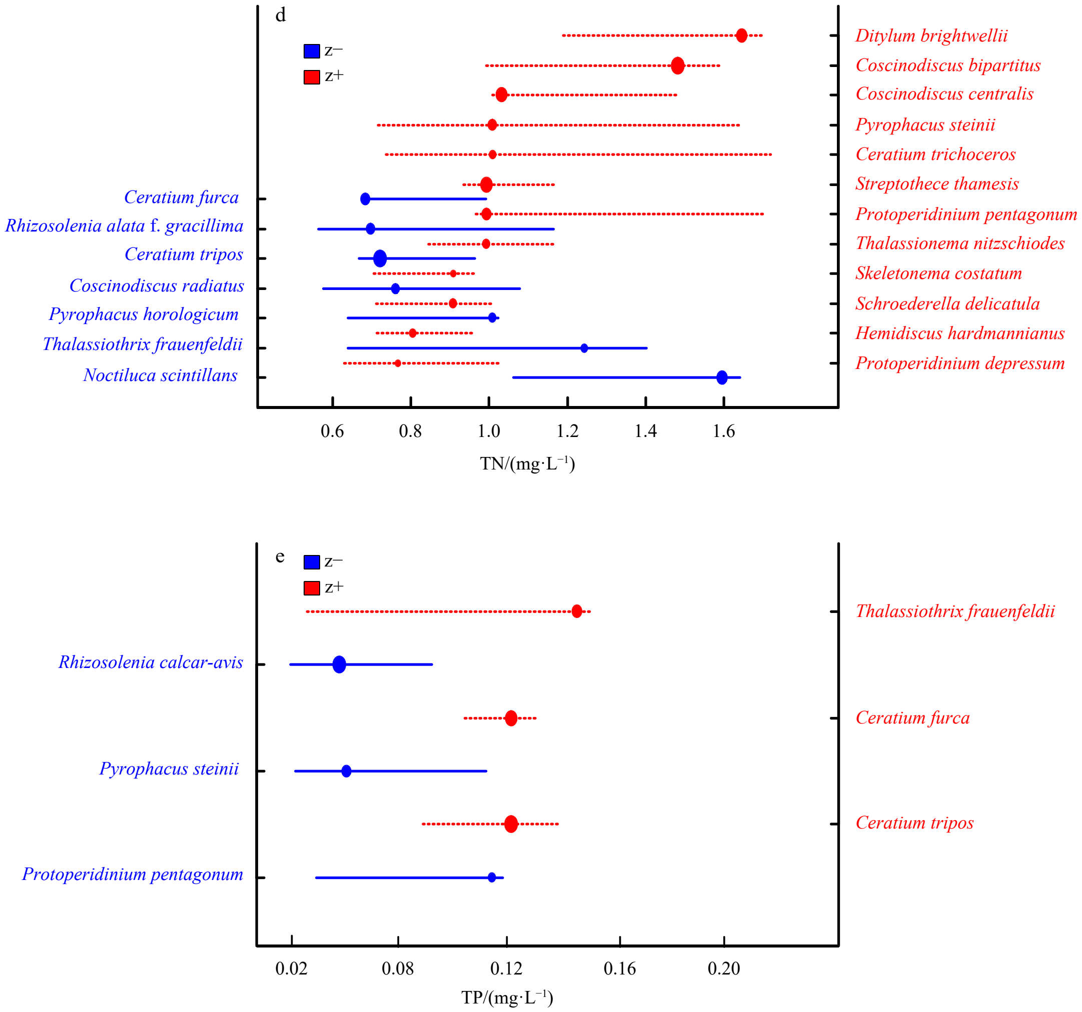

**Fig. 4.** Threshold indicator taxa analysis of individual taxon in response to pH, TN and TP ($n$=82 in both spring and summer). Only statistically significant indicator taxa were plotted (a and b in spring; c, d and e in summer). Blue symbols represented the negative taxa (z−) while red symbols represented the positive taxa (z+). Symbols corresponded to the thresholds across environmental gradients of each taxon and were sized according to the magnitude of the response (z-score). Horizontal lines referred to the 5th and 95th quantiles among 500 bootstrap replicates. TP, total phosphorus; TN, total nitrogen.

sponses to environmental stresses; while Yang et al. (2019) applied frequency distribution approach (US EPA, 2001) to establish nutrient criteria. Despite they yielded numeric nutrient thresholds by collecting long-term dataset in such a complex region in terms of environmental heterogeneity, they merely emphasized on the stressors (concentrations of nutrients) and neglected the effects on responders (biological communities).

### 4.2 *Individual taxa succession along environmental gradients*

Indicator taxa can be utilized to monitor and predict environmental variations in coastal waters that sharply altered by anthropogenic activities. The thresholds of them are thus used to quantify potential ecological risks as one of early-warning signals (McCormick and Cairns, 1994). The results of this study showed that *P. donghaiense* responded positively and substantially to all the three significant environmental parameters, which meant that it was the most crucial indicator species in spring. Earlier research recorded that *P. donghaiense* blooms had been frequently observed in the coastal waters of Zhejiang Province recently in late spring (Xu et al., 2010; Li et al., 2011). This study found the tolerant thresholds of both community level and *P. donghaiense* in response to pH approached to 8.25 and 8.22 (Table 1), respectively. This was almost in accordance with He (2010) which suggested the pH value for the pre-forming stage of HABs in coastal water of Xiamen, southern East China Sea, should be 8.26. Rather, being capable of storing nutrients higher than diatoms, dinoflagellates, especially for *Prorocentrum* spp. (Lv and Li, 2006), need more nutrients (mainly nitrogen and phosphorus) for their growth. The thresholds of *P. donghaiense* in response to TN (1.519 mg/L) and TP (0.097 5 mg/L) were higher than the mean values of them (Table 1). This indicated that great attention should be paid on the potential outbreaks of *P. donghaiense*-caused blooms in the early-warning stage despite nutrient conditions were relatively not enough to trigger bloom-forming. As long as the nutrient concentrations exceeded the thresholds, various tolerant taxa would replace the sensitive taxa and thus dominant the community, leading to the potential risk of forming HABs.

During summertime, reliable indicator species shifted from *P. donghaiense* to *N. scintillans* with the latter declining with increasing pH value and TN. *Noctiluca scintillans* is able to form tremendous blooms in subtropical nearshore waters in summer and can cause significant ecological disaster during bloom forming and after-bloom stage (Huang and Qi, 1997; Harrison et al., 2011). However, *N. scintillans* was detected as sensitive species in the present study. This indicated that the structure of *N. scintillans* population was ecologically stable and healthy when pH value and TN concentration were below 7.99 mg/L and 1.595 mg/L, respectively. It has been reported that *N. scintillans* blooms associated more with complicated and unpredictable climate changes than with deterministic mechanisms (Huang and Qi, 1997; Miyaguchi et al., 2006; Harrison et al., 2011). Moreover, other studies have also confirmed that *N. scintillans* was voracious phagotrophs and could feed on a wide range of food proxies, such as bacteria (Kirchner et al., 1996), diatoms (Tsai et al., 2018), and zooplankton (Quevedo et al., 1999). Unfortunately, these climatic factors and biological grazing factors were not taken into consideration and thereby no thresholds of them were identified, which need further study.

There were still numerous species that identified as “non-responders” along environmental gradients. This could probably ascribe to the rigorous statistical procedures that identified as reliable indicator species in TITAN. For instance, *N. scintillans* and *P. pungens* were not detected as reliable indicator species in spring and summer, respectively though they were measured as the most abundant species in each season. They may exist at the minority of sites with high abundance but not be observed in the majority of sites. Consequently, these species could not be robustly identified as indicators despite the fact that they related positively or negatively to environmental gradients (Dufrêne and Legendre, 1997; Simonin et al., 2019).

### 4.3 *Seasonal differences of ecological thresholds*

This study found the thresholds of phytoplankton community in response to environmental gradients differed seasonally. Generally, thresholds for the community-level in spring were much higher than that in summer (Table 3, except for the tolerant thresholds in response to TP). pH value and TN concentrations were significantly higher in spring compared with that in summer (Figs A1d, f) whereas TP in spring markedly outnumbered that in summer (Fig. A1e). Moreover, PCoA also showed significant seasonal differences of phytoplankton community composition (Fig. 2). These results illustrated that given the remark differences between the seasons in terms of community composition and environmental variation, it was not surprising that phytoplankton community thresholds across the environmental gradients changed temporally (Kovalenko et al., 2017). Furthermore, different species will respond differently to environmental dynamics given the fact that the succession of dominant (indicator) species shifted seasonally in coastal waters of northern Zhejiang Province (Wang and Wu, 2009; Zhou et al., 2017b; Zeng et al., 2019).

## 5 Conclusions

This study was the frontal work that focused on the ecological thresholds of marine phytoplankton community in response to environmental gradients in the coastal waters of northern Zhejiang, East China Sea. The ecological thresholds of community-level along environmental gradients differed seasonally with that in spring being higher compared with summer. As the most tolerant and sensitive indicator species identified in spring and summer, *P. donghaiense* and *N. scintillans*, respectively, responded intensively along environmental gradients and thus possessed significant indications for environmental variations. The thresholds of both community-level and taxon-level are useful for coastal HABs early-warning monitoring and management, as well as establishing phytoplankton-based nutrient criteria.

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## Appendix:

**Table A1.** Detail results of TITAN for significant taxa (purity⩾0.95, reliability⩾0.95)

| Season | Taxon | Environmental gradient | Threshold | Direction | Purity | Reliability |
|---|---|---|---|---|---|---|
| Spring | *Prorocentrum donghaiense* | pH | 8.22 | z+ | 1.000 | 0.996 |
| | *Rhizosolenia setigera* | | 8.28 | z+ | 0.988 | 0.976 |
| | *Ceratium fusus* | | 8.22 | z+ | 1.000 | 1.000 |
| | *Prorocentrum donghaiense* | TN/(mg·L$^{-1}$) | 1.519 | z+ | 1.000 | 1.000 |
| | *Rhizosolenia setigera* | | 1.423 | z+ | 0.994 | 0.982 |
| | *Ceratium breve* | | 1.040 | z+ | 0.998 | 0.994 |
| | *Coscinodiscus oculatus* | | 1.602 | z+ | 1.000 | 0.988 |
| | *Coscinodiscus thorii* | | 1.545 | z+ | 0.980 | 0.984 |
| | *Coscinodiscus wailesii* | | 1.504 | z– | 0.982 | 0.978 |
| | *Prorocentrum donghaiense* | TP/(mg·L$^{-1}$) | 0.097 5 | z+ | 1.000 | 1.000 |
| | *Rhizosolenia setigera* | | 0.100 8 | z+ | 0.976 | 0.956 |
| | *Ceratium breve* | | 0.098 7 | z+ | 0.996 | 0.994 |
| | *Biddulphia mobiliensis* | | 0.043 3 | z– | 1.000 | 0.972 |
| | *Coscinodiscus oculatus* | | 0.105 6 | z+ | 1.000 | 0.976 |
| Summer | *Noctiluca scintillans* | pH | 7.99 | z– | 0.994 | 0.998 |
| | *Hemidiscus hardmannianus* | | 7.90 | z– | 1.000 | 0.986 |
| | *Biddulphia sinensis* | | 7.90 | z– | 0.990 | 0.986 |
| | *Chaetoceros curvisetus* | | 8.22 | z+ | 0.994 | 0.994 |
| | *Thalassionema nitzschiodes* | | 8.04 | z+ | 1.000 | 0.998 |
| | *Ceratium furca* | | 8.15 | z+ | 0.978 | 1.000 |
| | *Ceratium breve* | | 8.12 | z+ | 0.986 | 1.000 |
| | *Ceratium trichoceros* | | 8.14 | z+ | 1.000 | 1.000 |
| | *Coscinodiscus asteromphalus* | | 8.11 | z+ | 0.994 | 0.992 |
| | *Chaetoceros subsecundus* | | 8.22 | z+ | 1.000 | 0.998 |
| | *Chaetoceros compressue* | | 8.22 | z+ | 1.000 | 0.986 |
| | *Rhizosolenia hyalinena* | | 8.14 | z+ | 1.000 | 0.960 |
| | *Bacteriastrum minus* | | 8.14 | z+ | 0.996 | 0.988 |
| | *Pyrophacus steinii* | | 8.08 | z+ | 1.000 | 0.988 |
| | *Noctiluca scintillans* | TN/(mg·L$^{-1}$) | 1.595 | z– | 0.988 | 0.992 |
| | *Thalassiothrix frauenfeldii* | | 1.243 | z– | 0.968 | 0.960 |
| | *Ceratium furca* | | 0.684 | z– | 0.988 | 0.982 |
| | *Rhizosolenia alata* f. *gracillima* | | 0.564 | z– | 0.998 | 0.970 |
| | *Ceratium tripos* | | 0.634 | z– | 1.000 | 1.000 |
| | *Coscinodiscus radiatus* | | 0.586 | z– | 0.998 | 0.986 |
| | *Pyrophacus horologicum* | | 0.993 | z– | 0.990 | 0.990 |
| | *Schroederella delicatula* | | 0.909 | z+ | 1.000 | 0.998 |
| | *Skeletonema costatum* | | 0.909 | z+ | 0.976 | 0.964 |
| | *Hemidiscus hardmannianus* | | 0.816 | z+ | 1.000 | 1.000 |
| | *Thalassionema nitzschiodes* | | 1.075 | z+ | 0.994 | 0.996 |
| | *Protoperidinium depressum* | | 0.767 | z+ | 0.982 | 0.996 |
| | *Ditylum brightwellii* | | 1.645 | z+ | 0.988 | 0.992 |
| | *Ceratium trichoceros* | | 1.720 | z+ | 0.958 | 0.984 |
| | *Coscinodiscus bipartitus* | | 1.090 | z+ | 1.000 | 1.000 |
| | *Coscinodiscus centralis* | | 1.164 | z+ | 1.000 | 0.992 |
| | *Protoperidinium pentagonum* | | 1.483 | z+ | 1.000 | 0.994 |
| | *Pyrophacus steinii* | | 1.720 | z+ | 1.000 | 1.000 |
| | *Streptotheca thamesis* | | 1.720 | z+ | 1.000 | 1.000 |
| | *Rhizosolenia calcar-avis* | TP/(mg·L$^{-1}$) | 0.033 4 | z– | 1.000 | 1.000 |
| | *Protoperidinium pentagonum* | | 0.112 9 | z– | 0.998 | 0.964 |
| | *Pyrophacus steinii* | | 0.035 5 | z– | 1.000 | 0.976 |
| | *Thalassiothrix frauenfeldii* | | 0.146 4 | z+ | 0.994 | 1.000 |
| | *Ceratium furca* | | 0.126 0 | z+ | 0.998 | 1.000 |
| | *Ceratium tripos* | | 0.126 0 | z+ | 0.998 | 1.000 |

Note: Direction meant taxa that either negatively (z–) or positively (z+) responded to environmental gradients. Purity was the mean proportion correct taxa direction (z– or z+) assignment, reliability was the mean proportion of $P<0.05$. TP, total phosphorus; TN, total nitrogen.

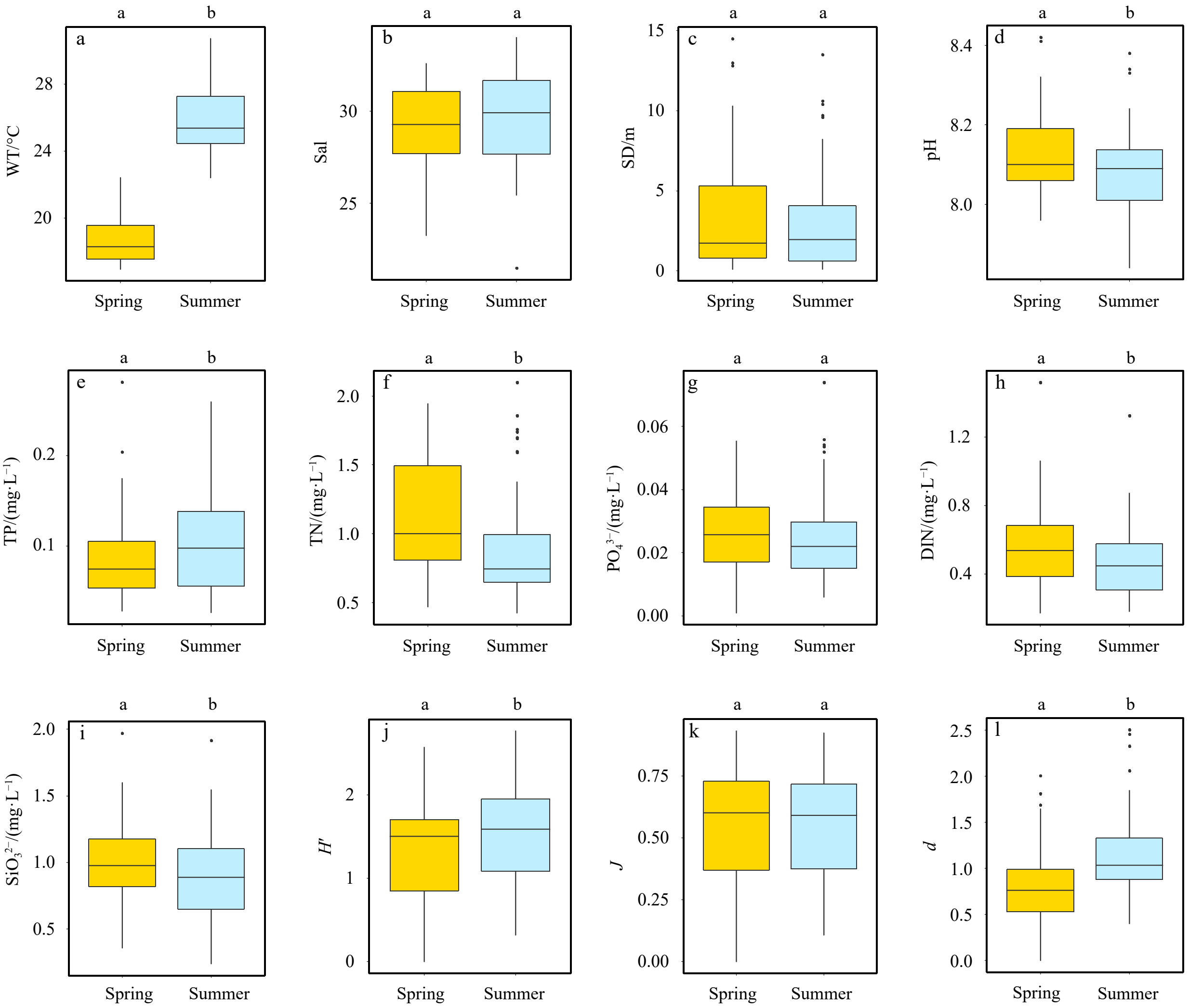

**Fig. A1.** Boxplots of environmental variables and $\alpha$-diversity indices of phytoplankton community in spring and summer. Seasons with the same letter on the top are not significantly different from each other while two seasons without a letter in common are significantly different (Kruskal-Wallis test, $P < 0.05$). WT, water temperature; Sal, salinity; SD, secchi depth; TP, total phosphorus; TN, total nitrogen; $\mathrm{PO}_4^{3-}$, phosphate concentration; DIN, dissolved inorganic nitrogen concentration; $\mathrm{SiO}_3^{2-}$, silicate concentration; $H'$, Shannon-Weiner diversity index; $J$, Pielou evenness index; $d$, Margalef richness index.