

## Spatial and seasonal variations in the planktonic ciliate community and its relationship with environmental factors in Daya Bay, the South China Sea

by

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### Abstract

Planktonic ciliate composition, abundance and its response to environmental change were investigated during four seasons (winter of 2013, spring, summer and autumn of 2014) in Daya Bay, the South China Sea. A total of 41 species belonging to eight orders were identified, 14 of which were dominant. Planktonic ciliate communities showed a distinct seasonal pattern in ciliate abundance and a clear seasonal shift in the taxonomic composition. The largest number of ciliate species occurred in summer, whereas the highest abundance peaked in spring, mainly due to oligotrichids. In terms of spatial distribution, ciliate species were abundant in the area of artificial reefs, and ciliate abundance was higher in the Dapeng Cove aquaculture area and lower at the Daya Bay Nuclear Power Station. Clustering analysis demonstrated that the seasonal variations of the ciliate community structure were more obvious than spatial variations. Multivariate and univariate analyses illustrated that ciliate abundance was significantly correlated with the water nutrient level and chlorophyll-*a* concentration, temperature, salinity and dissolved oxygen. Moreover, the dominant abiotic environmental factors affecting the spatial pattern of ciliate communities varied between seasons.

**Key words:** ciliate community, environmental factors, relationship, Daya Bay

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## Introduction

Planktonic ciliates are a significant component of microplankton communities in the ocean and they play a crucial role in microbial food webs (Azam et al. 1983). They have long been considered mediators of production and energy transfer from primary producers (e.g. pico- and nano-phytoplankton) to higher trophic levels (Ellumi et al. 2006; Xu et al. 2008). Moreover, ciliates respond more quickly to environmental factors compared to other organisms, because of their sensitivity, higher reproduction rates, and delicate cell membranes (Madoni et al. 2005; Kchaou et al. 2009). Therefore, changes in ciliate communities are integral to aquatic habitats, especially to chemical and physical conditions in aquatic ecosystems (Xu et al. 2009; Jiang et al. 2011a; Kim et al. 2012).

Daya Bay is a shallow semi-enclosed bay with an area of approximately 600 km<sup>2</sup>, located in the northwestern part of the South China Sea. Daya Bay and its surrounding area have been listed as an important economic development district and mariculture areas in Guangdong Province, China. Mariculture has developed rapidly since the 1990s and is responsible for the increasing nutrient loadings (Wang et al. 2008). Petrochemical, plastic, printing and other industries, as well as harbors are present in the province (Song et al. 2004). Additionally, the Daya Bay Nuclear Power Station (DNPS) is located in the bay, which has operated since 1994 and discharges heated water at the rate of  $2.9 \times 10^7 \text{ m}^3 \text{ yr}^{-1}$  (Liu et al. 2006). Another nuclear

power station, the Lingao Nuclear Power Station, has operated since 2002. All these activities generate an increasing anthropogenic impact on the ecological environment of Daya Bay, and environmental changes (e.g. nutrients, water temperature, salinity and pH) cause significant changes in the abundance, biomass, diversity and the community structure of microplanktonic organisms in Daya Bay (Wang et al. 2009; Ma et al. 2014).

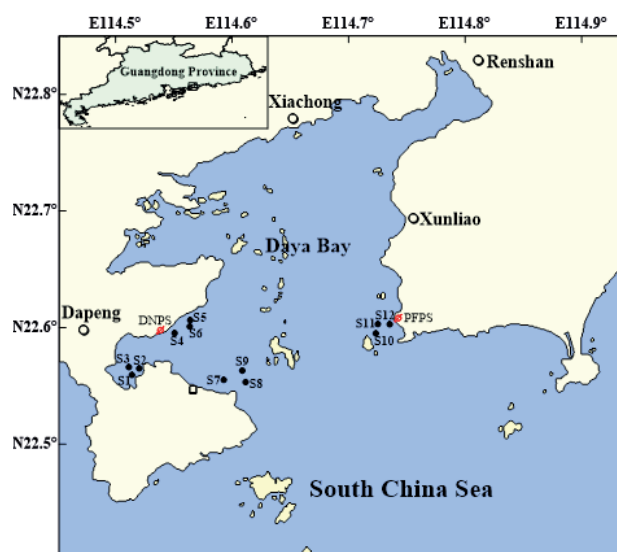
There have been a number of studies dealing with plankton community dynamics in Daya Bay (Song et al. 2004; Sun et al. 2011, Wang et al. 2014a; Li et al. 2014). However, there are no data available on planktonic ciliate community structures. In this study, we investigated the spatial and seasonal variation in the species composition and abundance. The main objectives were to clarify the spatial and seasonal pattern of planktonic ciliate communities on a local scale and to identify the most important environmental factors affecting the spatial and seasonal pattern.

## Materials and methods

### Study sites and sampling

Twelve sampling sites were located in four areas of Daya Bay where different types of human activities occur (Fig. 1). Sampling sites 1-3 were located in the Dapeng Cove aquaculture area (DC), sites 4-6 near the Daya Bay Nuclear Power Station (DNPS), sites 7-9 at artificial reefs (ARs), and sites 10-12 near the water outlets of the Pinghai fossil fuel-fired Power Station (PFPS).

Planktonic ciliate samples were collected with a Niskin water sampler at a depth of 0.5 m from Daya Bay in January, April, August and November 2014, representing the temporal ciliate community in winter, spring, summer and autumn, respectively. Seawater samples of 2500 ml (fixed with 1% formaldehyde in plastic bottles) were used for quantitative analysis and identification of ciliates. A total of 48 ciliate samples were fixed and analyzed. Environmental factors, such as pH, salinity (Sal.), and temperature (Temp. °C) were measured in situ at sampling sites, using a multi-parameter sensor (YSI Professional Plus, USA). Dissolved oxygen concentration (DO, mg l<sup>-1</sup>), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>, μmol l<sup>-1</sup>), nitrite nitrogen (NO<sub>2</sub><sup>-</sup>, μmol l<sup>-1</sup>), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>, μmol l<sup>-1</sup>) and soluble reactive phosphate (PO<sub>4</sub><sup>3-</sup>, μmol l<sup>-1</sup>) were determined using a UV-visible spectrophotometer (UV2450, Shimadzu) according to marine monitoring specifications (GB 17378.4-2007, 2007). DIN is the sum of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup>. Chlorophyll-*a* (Chl<sub>*a*</sub>, μg l<sup>-1</sup>)



**Figure 1**

Location of the study area and the sampling sites of planktonic ciliates in Daya Bay

concentrations were measured using a Fluor-meter (Turner-10-AU).

### Identification and counting

Formaldehyde fixed samples were settled for at least 48 h and then concentrated to 50 ml (Utermöhl 1958). Each time, 0.1 ml of a well-mixed concentrated sample was placed on a microscope slide and ciliates were counted under a light microscope at 200× or 400×. Ten slide replicates were examined for each concentrated sample. Tintinnids were identified on the basis of lorica morphology and species description provided by Kofoid and Campbell (1929, 1939) and Lynn (2008). Other ciliates were identified following Song et al. (2008). The taxonomic scheme mainly refers to Lynn (2008). All ciliates were finally identified to the lowest possible taxa.

### Data analysis

The number of species and ciliate abundance were calculated for each sample. The dominance ( $Y$ ) in samples was calculated according to the following formulae:

$$Y = \left( \frac{n_i}{N} \right) \times f_i$$

where  $n_i$  is the abundance of the  $i^{\text{th}}$  species,  $N$  is the total number of individuals,  $f_i$  is the occurrence frequency of the  $i^{\text{th}}$  species. The criterion for dominant species was  $Y \geq 0.02$ .

Cluster analysis from the Primer v5.0 software package was used to investigate the ciliate community structure (Clarke and Gorley 2001). Bray-Curtis similarity matrices were computed on the fourth-root transformed ciliate abundance data and the Euclidean distance matrix was computed on  $\log(x+1)$ -transformed environmental data (including Chl  $a$ ). The group-average clustering was used to divide ciliate communities into several assemblages. Differences between groups of samples were tested by the routine ANOSIM (global  $R$ -values range from 0 to 1, and a higher global  $R$  value demonstrates more significant variance). Sample relationships based on a comparison of similarity matrices were displayed using multi-dimensional scaling (MDS). BIOENV (biota and/or environment matching, using Spearman rank correlations) was used to explore the potential relationships between environmental parameters and biotic data.

Detailed relationships between ciliate communities and environmental factors were analyzed by CANOCO 4.5 (ter Braak and Smilauer 2002). To prevent

any disproportionate influence of rare species in the subsequent analysis, only species with incidence >10% in all samples (or total sites) and abundance >1.0% of the total ciliate abundance were considered. First, detrended correspondence analysis (DCA) was employed on the ciliate abundance data to decide whether linear or unimodal ordination methods should be applied. If the length of the gradient was greater than 3, Canonical Correspondence Analysis (CCA) was used, otherwise Redundancy Analysis (RDA) was applied. CCA or RDA analysis was conducted based on  $\log(x+1)$ -transformed species abundance and environmental data (except for pH). In addition, Spearman correlation analysis between ciliate communities and environmental factors was computed by the SPSS v20.0 statistical program.

## Results

### Environmental parameters

Mean values of ten environmental factors were summarized in Table 1. Water temperature followed a clear seasonal pattern, ranging from 16.93°C to 27.75°C (mean 23.58°C), and the maximum was recorded in DNPS areas in four seasons. Salinity peaked in summer (33.94 PSU). Values of pH ranged from 8.07 to 8.27, on average 8.21. Values of DO varied inversely with temperature, and the maximum was recorded in winter. Concentration of  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and DIN peaked in summer, and was relatively low in winter.

**Table 1**

Environmental variables for Daya Bay water samplings in winter 2013, spring, summer and autumn 2014

Parameters	Unit	Winter	Spring	Summer	Autumn
Temp.	°C	16.93	24.73	27.75	24.89
Sal.	PSU	32.83	31.86	33.94	33.80
pH		8.27	8.31	8.07	8.20
DO	mg l <sup>-1</sup>	7.21	5.98	5.45	5.46
$\text{NO}_2^-$	μmol l <sup>-1</sup>	0.39	0.27	0.63	0.20
$\text{NO}_3^-$		1.57	1.73	7.05	2.09
$\text{NH}_4^+$		1.53	1.74	3.12	1.78
DIN		3.49	3.74	10.8	4.08
$\text{PO}_4^{3-}$		0.29	0.20	0.41	0.22
Chl $a$	μg l <sup>-1</sup>	1.78	2.07	3.92	2.48

Soluble reactive phosphate ( $\text{PO}_4^{3-}$ ) ranged from 0.20  $\mu\text{mol l}^{-1}$  to 0.41  $\mu\text{mol l}^{-1}$ , which was relatively high in summer. Chl *a* concentration peaked in summer (4.42  $\mu\text{g l}^{-1}$ ), but decreased in winter (1.78  $\mu\text{g l}^{-1}$ ).

### Species composition and seasonal succession of dominant species

The taxonomic composition of ciliate communities observed during the study period is summarized in Table 2. A total of 41 planktonic ciliate species representing 22 genera and eight orders (Tintinnida, Oligotrichida, Euplotida, Cyclotrichida, Haptorida, Pleurostomatida, Prostomatida, Pleuronematida) were recorded during the one-year survey. Tintinnida and Oligotrichida were represented by the highest number of species, accounting for 70% and 15% of the total number of species, respectively (Fig. 2a). However, Oligotrichida was the most abundant order and accounted for up to 54.35% of the total ciliate abundance, followed by Tintinnida and Cyclotrichida (Fig. 2b).

The dominant species (dominance  $Y \geq 0.02$ ) in each season are listed in Table 3. Ciliate communities were dominated by *Tintinnopsis minuta*, *Strombidium globosaneum*, *Tintinnopsis beroidea* and *Strombidium conicum* in winter. *S. conicum* and *S. globosaneum* were still dominant in spring, accompanied by two new dominant species – *Spirotontonia turbinata* and *Laboea strobila*. In summer, five new dominant species, *Amphorellopsis acuta*, *Eutintinnus stramentus*, *Tintinnopsis tocaninencis*, *Tintinnopsis corniger*, *Helicostomella longa*, occurred along with *S. conicum*, *S. globosaneum* and *S. turbinata*. Additionally, *H. longa* was the most dominant species in summer. In autumn, the dominant species from summer were partially replaced by new species, i.e. *T. minuta*, *Tintinnopsis nucula*, *Wangiella dicollaria* and *Mesodinium rubrum*. *S. conicum* and *S. globosaneum* were still the most dominant species in autumn.

### Seasonal and spatial variation in the number and abundance of ciliate species

The total number of ciliate species ranged from 6 to 32 (Fig. 3a). The smallest number of species was observed in spring, and the highest one in summer. In terms of spatial distribution, the maximum number of species was recorded at ARs and the minimum at DC (Fig. 3a).

The abundance of ciliates showed a marked seasonal change, with the highest values observed in spring and the lowest in summer (Fig. 3b). In spring, their abundance ranged from 350 to 3120 ind.  $\text{l}^{-1}$ ,

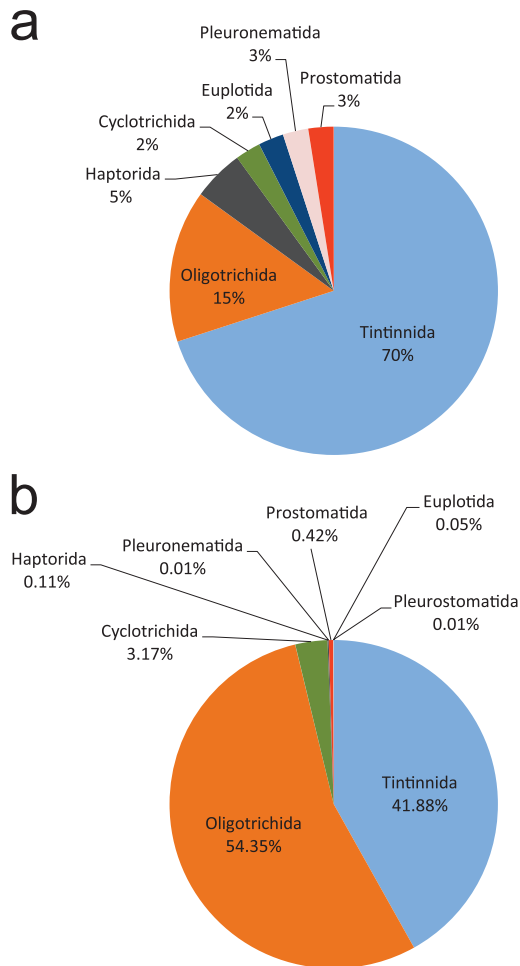
**Table 2**  
List of ciliate species encountered in Daya Bay

Ciliate species			
1	<i>Amphorellopsis acuta</i>	22	<i>Tintinnopsis schotti</i>
2	<i>Codonellopsis morchella</i>	23	<i>Tintinnopsis tentaculata</i>
3	<i>Codonellopsis ostenfeldi</i>	24	<i>Tintinnopsis tubulosa</i>
4	<i>Dadayiella ganymedes</i>	25	<i>Tintinnopsis turgida</i>
5	<i>Eutintinnus fraknoii</i>	26	<i>Tintinnopsis vosmaeri</i>
6	<i>Eutintinnus stramentus</i>	27	<i>Wangiella dicollaria</i>
7	<i>Favella companula</i>	28	<i>Helicostomella longa</i>
8	<i>Leptotintinnus nordqvisti</i>	29	<i>Strombidium conicum</i>
9	<i>Leptotintinnus simplex</i>	30	<i>Strombidium globosaneum</i>
10	<i>Salpingacantha minutissima</i>	31	<i>Strombidium major</i>
11	<i>Metacylis tropica</i>	32	<i>Strombidium sp.</i>
12	<i>Metacylis oviformis</i>	33	<i>Spirotontonia turbinata</i>
13	<i>Stenosemella pacifica</i>	34	<i>Laboea strobila</i>
14	<i>Tintinnopsis minuta</i>	35	<i>Euplotes euryostomus</i>
15	<i>Tintinnopsis radix</i>	36	<i>Mesodinium rubrum</i>
16	<i>Tintinnopsis tocaninencis</i>	37	<i>Didinium nasutum</i>
17	<i>Tintinnopsis beroidea</i>	38	<i>Lacrymaria sp.</i>
18	<i>Tintinnopsis butschlii</i>	39	<i>Amphileptus sp.</i>
19	<i>Tintinnopsis corniger</i>	40	<i>Cyclidium sp.</i>
20	<i>Tintinnopsis digita</i>	41	<i>Prorodon sp.</i>
21	<i>Tintinnopsis nucula</i>		

with an average of  $1409.1 \pm 92.6$  ind.  $\text{l}^{-1}$ . Interestingly, *S. conicum* and *S. turbinata* were primarily responsible for the peak, reaching the abundance of 7750 ind.  $\text{l}^{-1}$  and 6220 ind.  $\text{l}^{-1}$ , respectively. In terms of spatial distribution, higher abundance of ciliates was recorded in the DC area, whereas lower values were determined in the DNPS area.

### Ciliate community structure

Cluster analysis divided the ciliate communities into four groups at a similarity level of 40% in terms of their temporal distribution in Daya Bay during the study period (Fig. 4). Group 1 comprised some of the samples collected in autumn. Group 2 included all winter samples, while Group 3 consisted of all spring samples except for the sample collected from site 3 in autumn. Group 4 contained all the remaining autumn



**Figure 2**  
Proportions of the number of species (a) and abundance (b) of planktonic ciliates in Daya Bay

**Table 3**

Dominant ciliate species in each season in Daya Bay (species dominance,  $Y \geq 0.02$ )

Dominant species	Y			
	Winter	Spring	Summer	Autumn
<i>Tintinnopsis minuta</i>	0.5716	-	-	0.0215
<i>Tintinnopsis beroidea</i>	0.0863	-	-	-
<i>Strombidium conicum</i>	0.0387	0.4583	0.0336	0.1792
<i>Strombidium globosaneum</i>	0.1165	0.0538	0.0413	0.1481
<i>Spirotontonia turbinata</i>	-	0.3678	0.0656	0.0443
<i>Laboea strobila</i>	-	0.0687	-	-
<i>Amphorellopsis acuta</i>	-	-	0.1321	-
<i>Eutintinnus stramentus</i>	-	-	0.0407	-
<i>Tintinnopsis tocaninensis</i>	-	-	0.0200	0.0447
<i>Tintinnopsis corniger</i>	-	-	0.0235	-
<i>Helicostomella longa</i>	-	-	0.3221	-
<i>Tintinnopsis nucula</i>	-	-	-	0.0937
<i>Wangiella dicollaria</i>	-	-	-	0.0507
<i>Mesodinium rubrum</i>	-	-	-	0.0948

and all summer samples. The multidimensional scaling test (MDS) produced a similar result as mentioned above (Fig. 5). ANOSIM demonstrated that there were significant differences between each group (global  $R = 0.836$ ,  $p < 0.001$ ), implying an obvious seasonal pattern of ciliate communities. Although the interrelationship within each group differed, the difference between groups were more obvious than within groups, thus seasonal variation in ciliate communities was more significant than the spatial variation.

### Correlation between ciliate communities and environmental factors

BIOENV analysis revealed that ciliate communities were significantly correlated with abiotic environmental factors, i.e. temperature, salinity,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  (Table 4). Spearman correlation analysis demonstrated that ciliate abundance was significantly positively correlated with pH and DO, and negatively correlated with water salinity (Table 5). Similarly, there was a significant negative correlation between ciliate abundance and salinity in spring. In summer, temperature, DO,  $\text{NO}_2^-$  and  $\text{PO}_4^{3-}$  were the main factors affecting the ciliate abundance, whereas  $\text{NH}_4^+$  was significantly positively correlated with ciliate abundance in autumn. As can be observed in the CCA plot of four seasons (Fig 6a), samples collected in winter were generally gathered on positive Axis 2, indicating higher optima for DO,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-$  and DIN, and lower values for temperature and pH. Samples collected in summer were entirely on positive Axis 1, indicating higher optima for temperature and  $\text{NH}_4^+$ , whereas lower optima for pH,  $\text{NO}_3^-$ , DIN and DO. Spring and autumn samples were located on negative Axis 2, indicating higher optimum values for temperature and pH, but lower values for  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , DO and DIN. Results of RDA implied that environmental variables significantly affecting ciliate communities during four seasons were different. Environmental variables that significantly affected the spatial pattern of ciliate communities in winter were DO, Chl $a$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and salinity (Fig. 6b). The highest DO concentration was determined at site Wi1 where the ciliate abundance peaked. Similarly, the highest Chl $a$  concentration was determined at site Wi10 where ciliate abundance was relatively high. In spring, the significant variables were temperature,  $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-$ , salinity and pH. High temperature,  $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-$ , and low salinity, pH were determined at site Sp1 and Sp3 (DC area) where ciliate abundance peaked (Fig. 6c). In contrast, the highest values of pH and salinity were determined at site Sp12 (PFPS area), where the ciliate abundance and species number were the lowest.

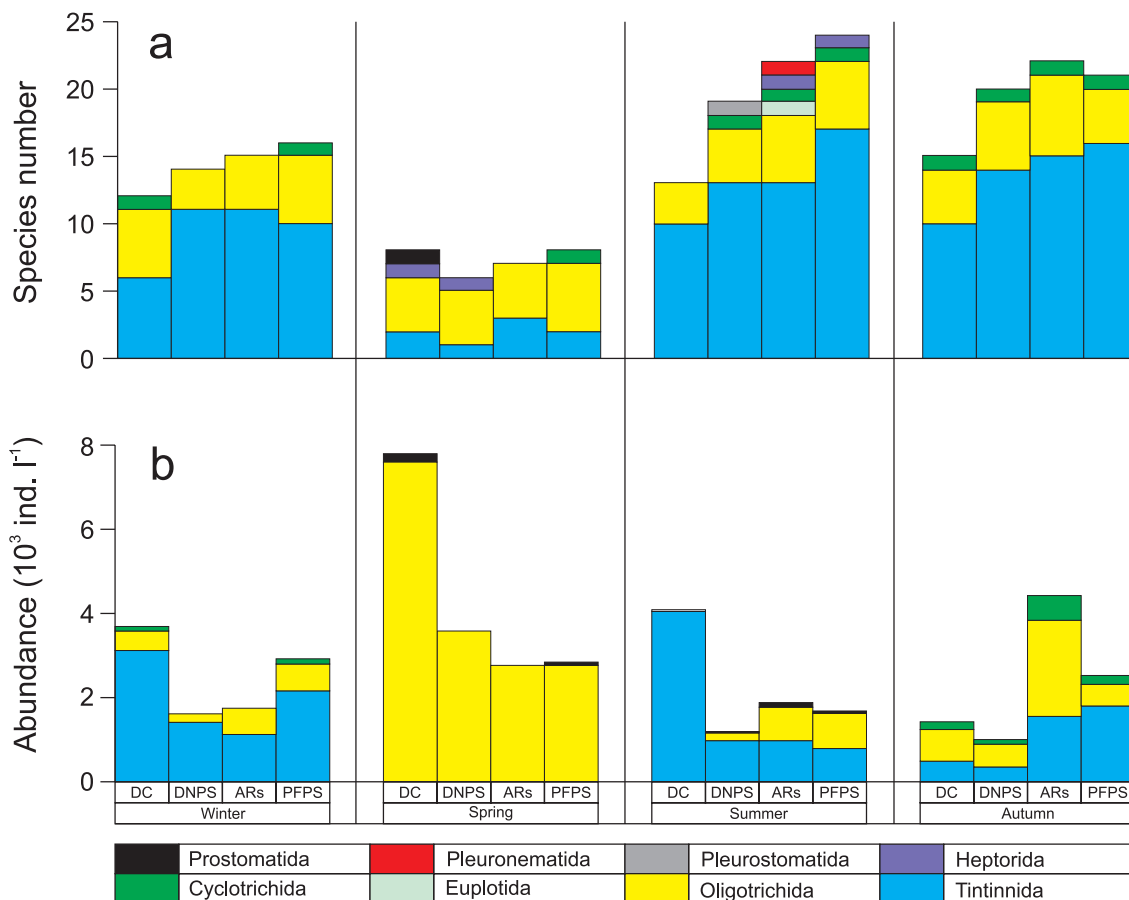


Figure 3

Variation in the number of species (a) and abundance (b) of planktonic ciliates in Daya Bay

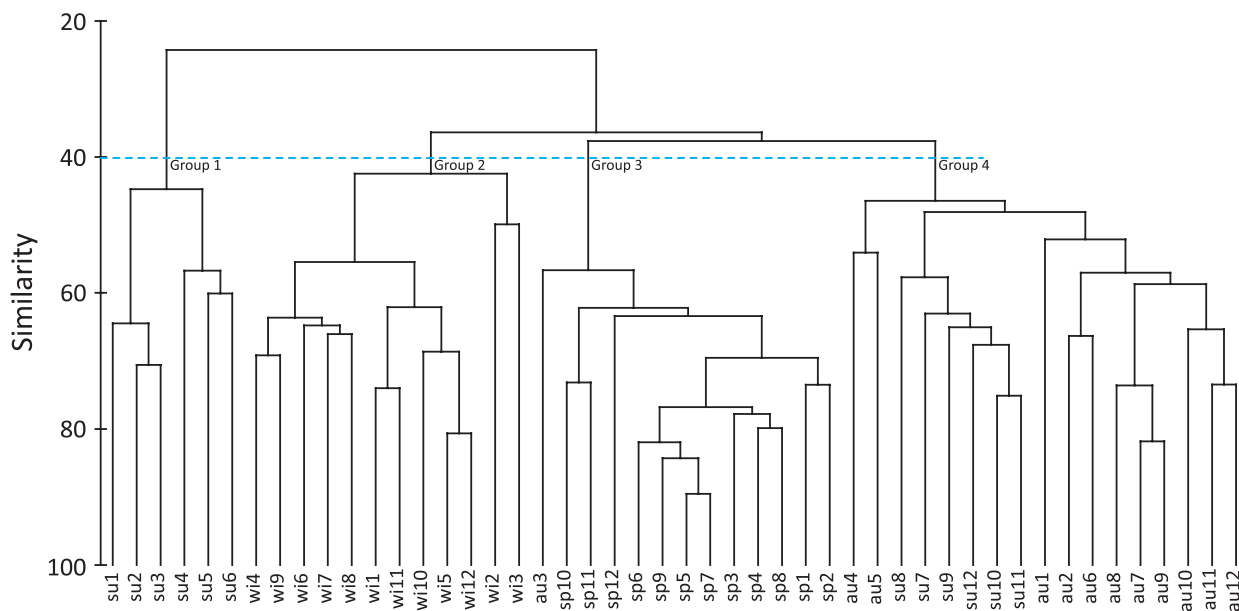


Figure 4

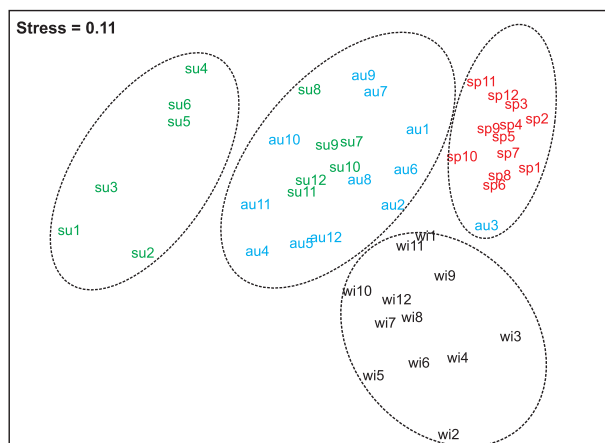
Cluster analysis of ciliate communities on the Bray-Curtis similarity matrix from the fourth-root transformed biotic data of 48 surface samples from Daya Bay. Wi: winter; Sp: spring; Su: summer; Au: autumn

**Table 4**

Summary of results from biota-environment (BIOENV) analysis, with the top ten correlations corresponding to different variables (R is Spearman correlation coefficient)

Rank	R	Environmental variables
1	0.391	Sal., pH
2	0.388	Sal., NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup> , PO <sub>4</sub> <sup>3-</sup>
3	0.387	DO, NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup> , PO <sub>4</sub> <sup>3-</sup>
4	0.386	Temp., Sal., pH
5	0.386	Temp., NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup> , PO <sub>4</sub> <sup>3-</sup>
6	0.386	Sal., DO, NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup>
7	0.384	pH, NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , NH <sub>4</sub> <sup>+</sup> , PO <sub>4</sub> <sup>3-</sup>
8	0.384	Sal., pH, NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup>
9	0.383	Temp., Sal., NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup>
10	0.383	pH, DO, NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup>

The most significant environmental variables in summer were NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NH<sub>4</sub><sup>+</sup>, salinity and DO (Fig. 6d). The levels of NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and NO<sub>2</sub><sup>-</sup> were higher at sites Su1, Su2 and Su3, where ciliate abundance was relatively high. Environmental factors significantly affecting the spatial pattern of ciliate communities in autumn were Chl*a*, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup> (Fig. 6e). High levels of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and DIN were determined at sites Au7, Au8 and Au9, where ciliate abundance and species number were relatively high. However, lower values of ciliate abundance were recorded at sites Au4, Au5 and Au6, where Chl*a* concentration had the lowest value and temperature – the highest value.

**Figure 5**

Multidimensional scaling (MDS) analysis of ciliate communities on the Bray-Curtis similarity matrix from the fourth-root transformed biotic data of 48 surface samples in Daya Bay. Wi: winter; Sp: spring; Su: summer; Au: autumn

## Discussion

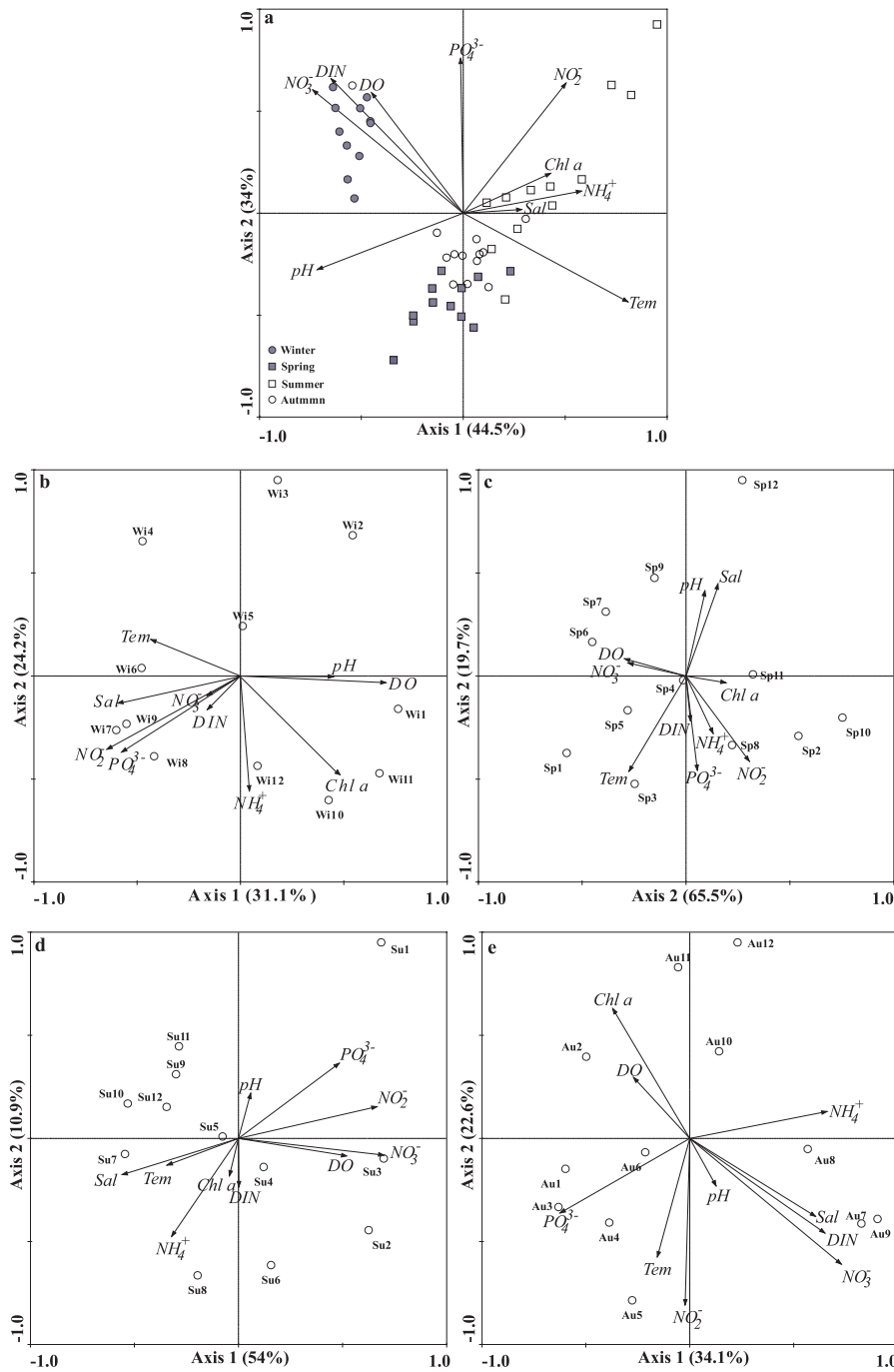
### Seasonal and spatial variation of ciliates

Our study revealed that planktonic ciliate communities exhibited an obvious seasonal and spatial variation in terms of species composition and abundance in Daya Bay. The number of ciliate species was high in summer and autumn, while low in winter and spring. In contrast, the ciliate abundance in spring was significantly higher compared to other three seasons. Similar results were obtained in other marine

**Table 5**

Correlation between environmental variables and ciliate abundance (*N*), the number of species (*S*) in four seasons in Daya Bay

parameters	Winter		Spring		Summer		Autumn	
	<i>N</i>	<i>S</i>	<i>N</i>	<i>S</i>	<i>N</i>	<i>S</i>	<i>N</i>	<i>S</i>
Temp.	-0.448	0.088	0.374	-0.181	0.282	-0.751**	-0.470	-0.083
Sal.	-0.606*	0.441	-0.840**	-0.103	0.881**	-0.720**	0.231	0.431
pH	0.748**	-0.537	-0.448	-0.061	0.342	-0.241	0.309	-0.169
DO	0.672*	-0.352	-0.379	-0.330	0.757**	-0.560	-0.214	-0.064
NO <sub>2</sub> <sup>-</sup>	-0.469	0.454	0.331	-0.088	0.772**	-0.549	0.499	0.395
NO <sub>3</sub> <sup>-</sup>	-0.489	0.185	0.341	-0.02	-0.346	0.049	-0.161	-0.511
NH <sub>4</sub> <sup>+</sup>	-0.113	0.543	0.434	-0.105	-0.088	0.225	0.590*	0.688*
DIN	-0.484	0.247	0.468	-0.091	0.179	-0.155	0.474	0.338
PO <sub>4</sub> <sup>3-</sup>	-0.329	0.693*	0.547	-0.165	0.677**	-0.278	-0.423	-0.794**
Chl <i>a</i>	0.395	0.179	0.244	0.432	0.026	0.047	-0.065	-0.298



**Figure 6**

Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA) ordination plots show the relationship between ciliate communities and environmental factors during four seasons (a), in winter (b), in spring (c), in summer (d) and in autumn (e). The length of vectors indicates the marginal effects of environmental variables they represent. The sample points can be projected perpendicularly onto the line overlaying the arrow of a given environmental variable. The sample points are arranged according to the predicted increase in values of a given environmental variable and the predicted increase occurs in the direction indicated by the arrow. (a) expresses mainly seasonal variation in ciliate communities in relation to environmental factors. (b)-(e) express spatial variation in ciliate communities in each season in relation to environmental factors. Wi: winter; Sp: spring; Su: summer; Au: autumn



systems, such as the Bay of Biscay (Urrutxurtu et al. 2003) and the Bay of Baisha (our unpublished data), where one peak of ciliate abundance was observed in spring. However, in the Helgoland Roads (the North Sea), Yang et al. (2015) reported two peaks of ciliate abundance in spring and summer, respectively. Yu et al. (2013) reported two peaks of ciliates in spring and autumn on Zhangzi Island (the northern Yellow Sea). Jiang et al. (2011b) found that the abundance was high in summer and winter, while low in spring and autumn in Jiaozhou Bay. These differences in temporal variation patterns of ciliate communities may result from different locations of the study sites, different sampling seasons or different study methods applied. For example, samples in our study were collected from the surface water (at a depth of 0.5 m), while the water depth of ciliate samples in another study (Yu et al. 2013) was between 32 m and 58 m. In addition, environmental factors in marine regions were different, for example Jiang et al. (2011b) reported three peaks in *Chla* concentrations: in late August, in mid-January and mid-March. On the other hand, there was one peak in *Chla* concentration in our study – in summer.

Higher abundance of ciliates occurred in the DC area. Intensive aquaculture activities led to elevated nutrient concentrations (Wang et al. 2009) and phytoplankton biomass (*Chla*) stays high in the DC area (Song et al. 2004; Li et al. 2014). These primary producers are grazed by higher groups in the trophic chain (e.g. ciliates, zooplankton and shellfish) (Azam et al. 1983; Pitta et al. 2009; Silva et al. 2012). Thus, phytoplankton abundance was positively correlated with ciliate abundance (Wang et al. 2014b). In contrast to DC area, there was a lower ciliate abundance in the DNPS area, which may be directly or indirectly affected by a thermal discharge from DNPS. Thermal effluents can reduce the phytoplankton biomass, abundance and chlorophyll-*a* (Chuang et al. 2009; Li et al. 2014), which lead to a lack of food resources for ciliate and indirectly affect ciliate abundance. Additionally, thermal effluents can also directly lead to reduced survival of the entrained zooplankton (Hoffmeyer et al. 2005; Kartasheva et al. 2008). Thus, the thermal discharge of DNPS also directly caused a decline in ciliate abundance.

### Relationship between ciliate communities and environmental factors

Multivariate correlation analysis demonstrated that seasonal and spatial variation in planktonic ciliate communities were significantly related to environmental variations, especially water temperature, pH,

dissolved oxygen, nutrients (e.g.  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ ) and *Chla*. These findings are consistent with previous reports for planktonic ciliate in Jianzhou Bay (Jiang et al. 2011b), Beibu Gulf (Wang et al. 2013; Wang et al. 2014c), and Shenhu Bay (Wang et al. 2014b). Furthermore, we found that the dominant abiotic factors affecting the spatial pattern of ciliate communities varied between seasons. For instance, ciliate abundance was significantly and positively correlated with *Chla* in winter. It is a well-known fact that water temperature was one of the most important environmental variables affecting the growth and reproduction of aquatic organisms (Langford 1990). Therefore, the phytoplankton growth and abundance were lower during winter. As most ciliates feed on phytoplankton, the phytoplankton abundance (chlorophyll-*a* concentration) in winter was one of the important factors influencing the community and abundance of ciliates. Consistent with our results, the previous studies indicated that there was a significant and positive correlation between the abundance of ciliates and the abundance of phytoplankton (Wang et al. 2014b; Yang et al. 2015). Therefore, these results suggested that food availability is an important factor influencing the seasonal variation in the ciliate community.

In addition, the BIOENV analyses, spearman correlation analysis and CCA have suggested that ciliate communities in our study were significantly correlated with nutrient levels (e.g.  $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ). Consistent with our results, previous studies indicated that the total abundance of ciliates was significantly affected by the level of phosphorus and nitrogen (Wang et al. 2013, 2014c). Simultaneously, Jiang et al. (2011b) suggested that the ciliate community variation in Jiaozhou Bay was correlated with  $\text{NO}_3^-$  and soluble reactive phosphates. Therefore, these findings demonstrated that the nutrient level is also an important factor influencing the seasonal and spatial variations in ciliate abundance. Moreover, other factors such as salinity, DO and water temperature also played an important role in determining both the spatial and seasonal pattern of ciliate communities in Daya Bay.

Furthermore, it is worth noting that the nutrient level and *Chla* concentration was relatively low in spring. However, the ciliate abundance was the highest in four seasons during the study. This may be related to the ciliate community, especially the ciliate species composition structure. In spring, Oligotrichida was the most abundant order in terms of both the number of species and their abundance. Generally, tintinnids were heterotrophic, whereas oligotrichs were autotrophic or heterotrophic (Tan et al. 2010). Heterotrophs feed on phytoplankton and bacteria, thus low nutrient

levels in spring affect the growth of phytoplankton, and then the total abundance of ciliates was indirectly affected (Sanders 1995). By contrast, oligotrich ciliates can absorb nutrients directly rather than rely only on phytoplankton and although Chl<sub>a</sub> concentration was low in spring, the ciliate abundance was still high. Interestingly, we also found that the genus *Strombidium*, especially *Strombidium conicum* and *Spirotontonia turbinata*, predominated in spring, thus the two ciliate species should be taken into account for future ecological studies.

## Conclusions

In conclusion, the ciliate communities show a significant seasonal and spatial variation in species composition and abundance in surface waters of Daya Bay. In addition, environmental factors, such as the nutrient level, Chl<sub>a</sub> concentration, water temperature, salinity and DO played a crucial role in controlling the ciliate communities in Daya Bay, and the dominant abiotic environmental factors (especially the nutrient level and Chl<sub>a</sub> concentration) affecting the spatial pattern of ciliate communities varied between seasons.

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