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Differences in planktonic ciliate spatial distribution in spring and autumn in the southern Yellow Sea

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Abstract

Seasonal variation of marine plankton spatial distribution is important in understanding the biological processes in the ocean. In this study, we studied spatial distribution of planktonic ciliate abundance and biomass in the central deep area (station depth greater than 60 m) and the coastal shallow area (station depth less than 60 m) of the southern Yellow Sea (32°-36.5°N, 121°-125°E) in spring (April) and autumn (October-November) of 2006. Our results showed that both ciliate abundance and biomass in the surface waters were higher in spring ((1 490±2 336) ind./L; $(4.11\pm7.81) \mu g/L$) than in autumn ((972±823) ind./L; $(1.11\pm1.18) \mu g/L$, calculated by carbon). Ciliate abundance and biomass in the surface waters of the coastal shallow area were similar in spring and autumn. However, in the central deep area, those values were much higher in spring ((1 878±2 893) ind./L; (5.99±10.10) μg/L) than in autumn ((738±373) ind./L; (0.74±0.76) μg/L). High values of ciliate abundance and biomass occurred in the central deep area in spring and in the coastal shallow area in autumn. Mixotrophic ciliate Laboea strobila was abundant in the central deep area in spring, when a phytoplankton bloom occurred. However, in autumn, L. strobila was abundant in the coastal shallow area. Boreal tintinnid Ptychocyli obtusa was found in spring. Both L. strobila and P. obtusa were concentrated in the surface waters when their abundance was more than 1 000 ind./L. Peaks of these species were in the subsurface waters when their abundance was less than 400 ind./L. This study showed that both high abundance and biomass of ciliates occurred in different areas in southern Yellow Sea seasonally.

Key words: ciliates, abundance, biomass, southern Yellow Sea

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1 Instruction

Marine planktonic ciliates are a group of single-celled protozoan in marine ecosystem. They are divided into loricate (tintinnids) and aloricate (naked) forms. With length from 5 μ m to 200 μ m, they are important members of microzooplankton. As key components of the marine microbial food web, they play an important role in plankton ecosystem and act as the primary consumers of the pico/nano-producers, major food sources of mesozooplanktons and larval fish (Pierce, 1992; Montagnes et al., 2010). Therefore, they are linkages between microbial food web and classical food chain (Edwards and Burkill, 1995). They are also important nutrient regenerators (Laval-Peuto et al., 1986; Pierce and Turner, 1994) in the marine ecosystems. There are seasonal changes of abundance in currents and stratifications in the coastal seas. However, little information was reported about the spatial distribution in different seasons, particularly in coastal seas.

The Yellow Sea (average station depth of 44 m) is a coastal sea located on the continental shelf of the western Pacific Ocean.

Here a significant seasonal phenomenon is the formation and dissipation of the Yellow Sea Cold Bottom Water (YSCBW), a basin-scale water mass with low temperature under thermocline in the warm period of a year (Ho et al., 1959; Yu et al., 2006). Another character is the spring phytoplankton bloom (Chl a concentration greater than 4 µg/L) from April to May in the central Yellow Sea (Tang et al., 2013). Spatial distribution of planktonic ciliates has been studied in different parts and seasons. Horizontal distribution of planktonic ciliates in the southern Yellow Sea was limited in April, August (Ding and Xu, 2012) and June (Zhang et al., 2002). High abundance area of planktonic ciliates and anchovy larvae was overlapped in June (Zhang et al., 2002). Jellyfish grazing on ciliates was ascribed as possible reason for low ciliate abundance in some area of the Yellow Sea (Ding and Xu, 2012). The spatial distributions of large tintinnid were well correlated with hydrology (Zhang et al., 2008, 2009). The monthly variation of ciliate abundance in two transects in YSCBW showed that there was low ciliate abundance in the YSCBW while high

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ciliate abundance occurred in the tidal front at the edge of YSCBW (Yu et al., 2014). The response of ciliates to phytoplankton bloom was not studied before in the Yellow Sea. rDNA sequence diversity has been studied to demonstrate ciliate distribution pattern in the neritic Bohai Sea and the Yellow Sea in June and November, 2011 (Dong et al., 2014). In this paper, we studies the spatial distribution of planktonic ciliates in the southern Yellow Sea in spring (with phytoplankton bloom and without YSCBW) and autumn (with YSCBW). We also highlighted the distribution of the mixotrophic ciliate *Laboea strobila* and the biogeographically boreal tintinnid species *Ptychocylis obtusa* in the southern Yellow Sea.

2 Materials and methods

Two cruises were conducted in spring (8-29 April 2006) and autumn (from 16 October to 3 November 2006) in the southern Yellow Sea (32°-36.5°N, 121°-125°E) (Fig. 1) on board R/V *Beidou*. Study area covered the deep central trough (maximum station depth of 89 m in Sta. 13) and the coastal shallow Subei Bank (minimum station depth of 17 m in Sta. 23). Sampling stations were not completely the same. There were totally 38 stations in spring cruise and 34 stations in autumn cruise, respectively. These stations formed six transects (Transects 1 to 6).

Vertical profiles of water temperature and salinity were measured with Seabird 911 CTD system. Water samples were collected by rosette water sampler at several depths of each station. To determine chlorophyll *a* (Chl *a*) concentration, 1 L of sea water was filtered through GF/F glass fiber filters, which was then extracted with 90% acetone at -20°C in darkness for 24 h. Chlorophyll *a* concentrations were determined using a Turner Designs fluorometer (Strickland and Parsons, 1972). One-liter water for ciliate sample was fixed with Lugol's solution to a final concentration of 1%. A total of 181 ciliate samples were collected. Samples were kept in cool dark place before analysis.

Ciliate samples were processed according to Utermöhl



Fig. 1. Station and Transect map in the southern Yellow Sea. T1 to T6 represent Transect 1 to Transect 6. The black dot represents the sampling station both in spring and autumn and the red triangle the sampling stations in spring only. The contours are isobaths (m).

(1958). Supernatant (about 900 mL) were siphoned out after 48 h sedimentation. The rest 100 mL were well mixed and 20 mL subsample were transferred into settlement chamber. Ciliates in the chamber were examined and counted under Olympus CKX41 inverted microscope with a magnification of \times 100 to estimate ciliate abundance. All analysis was finished within two months. Taxa of tintinnid were determined to species according to Zhang et al. (2012, 2015).

For aloricate ciliates, their body length and width were measured as the similar geometrical shape (e.g., cylinder, sphere and pyramids) to obtain the volume. For tintinnids, body volume was equal to 1/3 of loricate volume. Ciliate carbon biomass was calculated based on cell volume with a conversion factor of 0.19 $pg/\mu m^3$ (Putt and Stoecker, 1989). Correlation analysis was performed by software SPSS (Version 16).

3 Results

3.1 Hydrographic conditions, Chl a and ciliates in spring

3.1.1 Horizontal distribution

Chlorophyll *a* concentration of the surface waters ranged from 0.89 μ g/L (Sta. 2) to 15.38 μ g/L (Sta. 11). High value was in the central deep area (station depth greater than 60 m) where a phytoplankton bloom occurred. The other area where high value appeared was Sta. 34, which was near the Changjiang (Yangtze River) Estuary (Fig. 2a).

Surface temperature (6.7–14.4°C) decreased gradually from south to north, with peak value in Sta. 34 near the Changjiang Estuary (Fig. 2b). A warm water tongue intruded along the 70 m isobath. Surface salinity (25.91–34.18) decreased from the center to coastal stations with peak value in Sta. 18 (Fig. 2c).

Ciliate abundance and biomass in the surface waters were (1 490±2 336) (0–10 267) ind./L and (4.11±7.81) (0–32.29) µg/L, respectively. There were two high value areas (Figs 2d, g). One was in the central deep area, where peak abundance (7 080-10 267 ind./L) and biomass (16.03–32.29 µg/L) were in Sta.10 and Sta. 8, respectively. Another one was in Sta. 34 (abundance 6 450 ind./L, biomass 9.68 µg/L). Values of both abundance and biomass were higher in the north than south, and their distribution pattern coincided with that of Chl *a*.

3.1.2 Vertical distribution

Waters were vertically well mixed without stratification in the six transects (Figs 3 and 4). High Chl *a* occurred in the subsurface waters of Transects 1 and 4 and the surface waters of other transects. Ciliates abundance peaks were in the 20 m waters of Transects 4 and 5 and the surface waters of Transects 1–3 and 6.

3.2 Hydrographic conditions, Chl a and ciliates in autumn

3.2.1 Horizontal distribution

High values of Chl *a* in the surface waters were outside YSCBW and peaked in Sta. 23 ($3.03 \mu g/L$) and Sta. 32 ($2.84 \mu g/L$) (Fig. 5a). Surface temperature ranged from 18.1°C (Sta. 3) to 24.7°C (Sta. 38) and decreased gradually from south to north (Fig. 5b). Surface salinity was in a range of 30.86 (Sta. 23) to 33.87 (Sta. 36) (Fig. 5c). Bottom temperature and salinity showed that the range of YSCBW (bottom temperature less than 12°C) was approximately limited in waters with station depth greater than 60 m (Figs 5e, f).

Ciliate abundance and biomass in the surface waters were (972 ± 823) (35–3 885) ind./L and (1.11 ± 1.18) (0.14–5.330) µg/L,



Fig. 2. Horizontal distribution of surface Chl *a* (μ g/L) (a), temperature (°C) (b), salinity (c), ciliate abundance (ind./L) (d), bottom temperature (°C) (e), salinity (f), and biomass (μ g/L) (g) in spring.

respectively. Abundance peaked in Sta. 30 and biomass peaked in Stas 30, 35 and 37 (4.08–5.33 μ g/L). They were all distributed outside YSCBW region (Figs 5d, g).

3.2.2 Vertical distribution of ciliates

The YSCBW spanned from Transects 1 to 4 (34° - 36.5° N) where station depth was greater than 60 m with apparent stratification. Unlike the other four transects, waters in Transect 5 and Transect 6 were vertically well mixed (Figs 6 and 7). Chlorophyll *a* concentration was high in the surface waters in Transects 2 and 6 and the subsurface waters in other transects. Ciliate abundance peaked in the surface waters of Transects 2, 5 and 6 and the subsurface layers of the rest transects. There were some common features on the distribution of ciliates in Transects 1 to 4: peak values of ciliate abundance were in the coastal stations, where Chl *a* and temperature was relatively high and salinity was low.

3.3 Correlation analysis

Correlation analysis was conducted between biological and environmental data in spring and autumn, separately (Table 1). In spring, significant negative correlations were identified between ciliate abundance/biomass and surface/bottom temperature (P<0.01). There is a significant positive correlation between autumn ciliate biomass and Chl *a* concentration in surface (P<0.01). Surface ciliate biomass and temperature difference between surface and bottom waters (ΔT) were negatively related in spring and autumn (P<0.05). In autumn, ciliate biomass was high where bottom temperature was low (P<0.05).

3.4 Laboea strobila

In spring, *L. strobila* abundance was (952 ± 2573) ind./L and was mainly distributed in northern Transects 1–4. Higher values (1 125–10 000 ind./L) were in the surface waters of Stas 8, 9 and



Fig. 3. Vertical distribution of temperature (°C), salinity, Chl *a* (μ g/L), ciliate abundance (ind./L) and biomass (μ g/L) of Transects 1, 2 and 3 in spring of 2006. a1. Temperature, b1. salinity, c1. Chl *a*, d1. ciliate abundance and e1. biomass of Transect 1; a2. temperature, b2. salinity, c2. Chl *a*, d2. ciliate abundance and e2. biomass of Transect 2; a3. temperature, b3. salinity, c3. Chl *a*, d3. ciliate abundance and e3. biomass of Transect 3. Dots mean the sampling positions.

10, where average station depth was 74 m (Fig. 8a). In autumn, its abundance dropped dramatically to (228 ± 121) ind./L and was mainly concentrated outside of YSCBW (Fig. 8b). High abundance (350 ind./L) occurred in Stas 7 and 32, where average station depth was only 53.5 m. Abundance of *L. strobila* accounted for 1%–97% of total ciliate abundance in the water columns.

When abundance of *L. strobila* (Fig. 9a) was greater than 1 000 ind./L in water column, its peak abundance occurred in the surface waters as in Stas 8, 9 and 10 (Figs 9b1-b3). When its abundance was less than 400 ind./L, peak values appeared in the subsurface waters (Figs 9c1-c6).

3.5 Ptychocyli obtusa

With higher abundances (1 437-2 895 ind./L) in the surface

waters of Stas 8, 13 and 9, *P. Obtusa* (Fig. 10a) was only detected in spring with abundance of (503±874) ind./L. Its distribution was limited in deep stations with station depth of ~76.3 m (Fig. 8c) and surface temperature less than 10°C. The contribution of *P. obtusa* to total ciliate abundance ranged from 1% to 64% that peaked at Sta. 12. Peak of *P. obtusa* abundance was in the surface waters when its abundance was greater than 1 000 ind./L (Figs 10b1-b3). Abundance peaks were beneath 20 m when its abundance was less than 400 ind./L (Figs 10c1-c4).

4 Discussion

4.1 Ciliate abundance and biomass

The values of ciliate abundance and biomass in our study



Fig. 4. Vertical distribution of temperature (°C), salinity, Chl *a* (μ g/L), ciliate abundance (ind./L) and biomass (μ g/L) of Transects 4, 5 and 6 in spring of 2006. a1. Temperature, b1. salinity, c1. Chl *a*, d1. ciliate abundance and e1. biomass of Transect 4; a2. temperature, b2. salinity, c2. Chl *a*, d2. ciliate abundance and e2. biomass of Transect 5; a3. temperature, b3. salinity, c3. Chl *a*, d3. ciliate abundance and e3. biomass of Transect 6. Dots mean the sampling positions.

were similar with previous reports. In Ding and Xu (2012), ciliate abundance and biomass in the Yellow Sea were (2 034 \pm 2 026) (0-7 440) ind./L and (8.04 \pm 11.01) (0-42.21) µg/L, respectively, in April. In June, total ciliate abundance was between 40 and 3 420 ind./L and biomass in surface waters was in a range of 0.15-6.76 µg/L (Zhang et al., 2002). Yu et al. (2014) reported annual ranges of ciliate abundance and biomass of 0-3 989 ind./L and 0-6.77 µg/L, respectively, in two transects.

4.2 Ciliates in central deep area and coastal shallow area

The April cruise was carried out during the spring phytoplankton bloom (Chl *a* concentration greater than 4 μ g/L) period, which started from April to May each year (Tang et al., 2013). The maximum Chl *a* concentration (16.19 μ g/L) was near but lower than the recorded 30–35 μ g/L (Tang et al., 2013).

In spring, high surface Chl *a* concentration (>3 μ g/L) area was approximately limited in the stations where station depth was greater than 60 m. In autumn, YSCBW, where bottom water temperature was less than 12°C (Yu et al., 2014), was also limited in the same area. Therefore, we considered this area as the central deep area while that with station depth less than 60 m as the coastal shallow area.

In our study, horizontal distributions of ciliate showed different patterns in spring and autumn. Ciliate peaks in surface waters occurred in the central deep area in spring, while they appeared in the coastal shallow area in autumn.

Chlorophyll *a*, ciliate abundance and biomass in the central deep area of spring was much higher than that of autumn. On the



Fig. 5. Horizontal distribution of surface Chl a (µg/L) (a), temperature (°C) (b), salinity (c), ciliate abundance (ind./L) (d), bottom temperature (°C) (e), salinity (f), and biomass (µg C/L) (g) in autumn.

contrary, they were almost equal in autumn. In spring, peak values of Chl *a*, ciliate abundance and biomass were higher in the central deep area than those in coastal waters, while they were higher in coastal waters in autumn.

Ding and Xu (2012) found that ciliate abundance in Transect 3 (35°N) and Transect 4 (34°N) was higher in April than that in August, while it was opposite in Transect 5 (33°N). It described the same pattern as ours that in spring peak value of ciliates was in north part of southern Yellow Sea, while in autumn it peaks in south. In our study, spring ciliate abundance in the surface of Transects 3 (35°N), 4 (34°N) and 5 (33°N) was (750±954) ind./L, (656±526) ind./L and (160±130) ind./L, respectively. While in autumn, they were (1 020±550) ind./L, (665±177) ind./L and (1 372±1 441) ind./L. Ding and Xu (2012) attributed the sharp decrease of ciliate abundance in Transect 3 (35°N) and Transect 4 (34°N) from April to August to the predation pressure from the

increasing number of large jellyfish. In October, there was very little number of large jellyfish (Sun et al., 2015). Therefore, the reason for the phenomenon was not due to large jellyfish in our study.

The difference in the positions of high ciliate abundance and biomass in different seasons might result from the taxonomic difference. We cannot identify aloricate ciliates to species by light microscopy and Lugol's-fixed samples. The taxonomic information could be obtained from molecular method. For example, Dong et al. (2014) found that the terminal-restriction fragment (T-RF) richness of ciliates was generally higher in the southern Yellow Sea than that in the Bohai Sea.

4.3 Laboea strobila

The mixotrophic ciliate *L. strobila* was both found in spring and autumn. This species is wide spread in temperate coastal wa-



Fig. 6. Vertical distribution of temperature (°C), salinity, Chl *a* (μ g/L), ciliate abundance (ind./L) and biomass (μ g/L) of Transects 1, 2 and 3 in autumn of 2006. a1. Temperature, b1. salinity, c1. Chl *a*, d1. ciliate abundance and e1. biomass of Transect 1; a2. temperature, b2. salinity, c2. Chl *a*, d2. ciliate abundance and e2. biomass of Transect 2; a3. temperature, b3. salinity, c3. Chl *a*, d3. ciliate abundance and e3. biomass of Transect 3. Dots mean the sampling positions.

ters. Maximum abundances of 1 700, 135, and 1 800 ind./L were found in the Tyrrhennian Sea (Modigh, 2001), Catalan Sea (Dolan and Maarassé, 1995) and Chesapeake Bay (Dolan, 1991), respectively. The first record in the Yellow Sea was in June 2000 with a maximum abundance of 640 ind./L in the subsurface waters (Zhang et al., 2002). To our knowledge, its peak abundance (10 000 ind./L) in this study was the highest recorded abundance.

Vertical distribution of this species in our studies coincided with previous studies (Dale and Dahl, 1987; Stoecker et al., 1989), which demonstrated that *L. stobila* was concentrated in the waters where depth greater than 10 m in spring and could only be detected in the waters where depth greater than 30 m in autumn. The reason could be that *L. strobila* is a mixotrophic ciliate. It can implement photosynthesis for survival by feeding phytoplankton and gobbling up their pigments (Stoecker et al., 1988). Therefore it is crucial to live in the surface water to capture sunlight.

The average Chl *a* concentration per cell of this species was 82 pg (Putt, 1990). Accordingly, the highest concentration of Chl *a* in *L. strobila* was 0.820 µg/L (surface water of Sta. 10) and 0.017 µg/L (subsurface water of Sta. 24) in spring and autumn, respectively. The ratio of Chl *a* contributed by *L. strobila* to total Chl *a* ranged from 0% to 7.11% and from 0% to 4.95% in spring and in autumn, respectively. These values are similar to 2%–3% in temperate coastal Long Island Sound, USA (McManus and Fuhrman, 1986).

4.4 Ptychocyli obtusa

The occurrence of P. obtusa in the southern Yellow Sea is ex-



Fig. 7. Vertical distribution of temperature (°C), salinity, Chl a (µg/L), ciliate abundance (ind./L) and biomass (µg/L) of Transects 4, 5 and 6 in autumn of 2006. a1. Temperature, b1. salinity, c1. Chl a, d1. ciliate abundance and e1. biomass of Transect 4; a2. temperature, b2. salinity, c2. Chl a, d2. ciliate abundance and e2. biomass of Transect 5; a3. temperature, b3. salinity, c3. Chl a, d3. ciliate abundance and e3. biomass of Transect 6. Dots mean the sampling positions.

Table 1. Spearman's rank correlation coefficient between ciliate variables (abundance and biomass) and environmer	tal variables
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Season	Ciliate variables	T-Sur	T-Bot	riangle T	S-Sur	S-Bot	Chl a-Sur
Spring	abundance	-0.541**	-0.512**	-0.283	0.047	0.098	0.337
	biomass	-0.548**	-0.544**	-0.373*	-0.154	-0.135	-0.261
Autumn	abundance	-0.100	-0.046	-0.076	-0.139	-0.135	0.345
	biomass	0.333	-0.455*	-0.407*	0.321	-0.048	0.496**

Note: Bot represents bottom, Sur surface, *T* temperature, *S* salinity, Chl *a* chlorophyll *a* concentration, and $\triangle T$ temperature difference between surface and bottom waters. * Correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed).

traordinary. This species was not found previously in the southern Yellow Sea. The size of this species is 100-125 (average 105) μ m in length and 60-67 (average 65) μ m in width, which could not be caught in the plankton net (mesh pore size 76 μ m) used in

the studies of Zhang et al. (2008, 2009). *Ptychocyli* is biogeographically a boreal genus which mainly inhabited in areas with low temperature, such as Arctic Ocean (Gold and Morales, 1975). It was transported from boreal waters southward by the Oyashio



Fig. 8. Horizontal distribution of maximum abundance in water column of *Laboea strobila* in spring (a), *L. strobila* in autumn (b) and *Ptychocyli obtusa* in spring (c). The unit of legends is ind./L.



Fig. 9. Vertical distribution of *Laboea strobila* in spring and autumn. a. Photo of *L. strobila*; b1-b3. vertical distribution of *L. strobila* in spring and c1-c6. in autumn in different stations.



Fig. 10. Vertical distribution of *Ptychocyli obtusa* in spring. a. Photo of *P. obtusa*; b1-c4. vertical distribution of *P. obtusa* in spring in different stations.

Current (Kato and Taniguchi, 1993; Gómez, 2007). In our study, this species was found in waters with temperature less than 10°C. Because the warm current Kuroshio flows northward to the Tsushima Strait and Pacific Ocean west of Japan, it seemed that Oyashio had no chance to intrude into the southern Yellow Sea. Indeed, no boreal genera were found in the year-round monitoring of tintinnid assemblage in coastal waters of the Tsushima Strait for several years (Kim et al., 2012). Therefore, we postulated that *P. obtusa* might be an indigenous species in the southern Yellow Sea.

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