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Vertical distribution of planktonic ciliates in the oceanic and slope areas of the western Pacific Ocean

Chaofeng Wang[a,b,c,d], Haibo Li[a,b,d], Li Zhao[a,b,d], Yuan Zhao[a,b,d], Yi Dong[a,b,d], Wuchang Zhang[a,b,d,*], Tian Xiao[a,b,d]

[a] CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China
[b] Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, PR China
[c] University of Chinese Academy of Sciences, Beijing 100049, PR China
[d] Center for Ocean Mega-Science, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, PR China

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ABSTRACT

Ciliates are important grazers in the planktonic food web, and spatial distribution information is the key to understanding their function. However, our understanding of their vertical distribution in the euphotic zone of oceanic waters is limited. In this study, we investigated the vertical distribution of ciliates in the western Pacific Ocean and the northern South China Sea. The ciliates showed a bimodal distribution, with abundance peaks in surface waters and the deep chlorophyll maximum (DCM) in the western Pacific, but a single surface peak in the northern South China Sea slope. At stations influenced by shelf water, the surface abundance was much greater than in slope waters. Within the ciliates, the vertical distribution of tintinnid species groups I and V had higher abundances overall and showed surface and DCM peaks, respectively. We speculate that aloricate ciliates might also have surface peak and DCM peak groups. The overall vertical distribution patterns showed that the planktonic food web may *function* differently within the surface waters and the DCM.

1. Introduction

Planktonic ciliates are mainly composed of the Oligotrichia and Choreotrichia (phylum Ciliophora, class Spirotrichea; Lynn, 2008). They consist of tintinnids with lorica and aloricate ciliates. They are primary consumers of pico- (0.2–2 μm) and nano- (2–20 μm) sized producers, as well as important food sources of metazoans and fish larvae (Stoecker et al., 1987; Dolan et al., 1999; Gomez, 2007). Therefore, they play an important role in material circulation and energy flow from the microbial food web into the traditional food chain (Azam et al., 1983; Pierce and Turner, 1992; Calbet and Saiz, 2005).

Spatial distribution information is a key to understanding the functioning of ciliates in planktonic ecosystems. The full vertical depth distribution of ciliates has been studied in oceanic waters of the Pacific Ocean (Leakey et al., 1996; Yang et al., 2004; Gomez, 2007; Sohrin et al., 2010); however, no data are available regarding tintinnid vertical distribution patterns. Although recent molecular tools have been used to assess ciliate distribution (Bachy et al., 2013; Santoferrara et al., 2014, 2016; Grattepanche et al., 2016), these molecular methods have not been applied in studies on vertical distribution. The vertical distribution of tintinnids in the oceanic waters of the southern Adriatic Sea (to 1200 m) showed layering of tintinnids through the water column (Kršinić, 1982, 1998), but these studies did not examine their distribution in the euphotic zone in detail.

In tropical and subtropical oceans, the euphotic zone is usually well stratified. There is a deep chlorophyll maximum (DCM), which is deepest in the tropics and becomes shallower towards the subtropics (McGowan, 1967; Wolf and Woods, 1988; Sohrin et al., 2010). In continental slope areas, surface tongues of low salinity coastal waters shoal the DCM (Estrada et al., 1993). Members of the planktonic food web (e.g. ciliates, radiolaria, flagellates, bacteria, *Synechococcus*, *Prochlorococcus* and picoeukaryotes) show a characteristic vertical distribution in the euphotic zone (Venrick, 1988; West and Scanlan, 1999; Ishitani et al., 2014; Zhao et al., 2017). Maximum abundances of *Prochlorococcus* and picoeukaryotes occur in the DCM, whereas maximal *Synechococcus* and bacteria occur above the DCM (Zhao et al., 2017). Therefore, we hypothesized that ciliates should have maximum abundance peaks similar to other components of the planktonic food web (i.e. maximum abundance in the DCM). Ciliate abundance showed a maximum in the DCM, as well as a positive correlation with Chlorophyll

* Corresponding author at: CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China.
E-mail address: wuchangzhang@qdio.ac.cn (W. Zhang).

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a (Chl *a*) concentration in the Catalan Sea (western Mediterranean) (Dolan and Marrasé, 1995).

The South China Sea (SCS) is the largest semi-enclosed basin in the western Pacific Ocean (Su, 2004), and hosts various physico-chemical environments and diverse phytoplankton (Li et al., 2012). Many investigations have been conducted on ciliate abundance and species diversity in the SCS (Liu et al., 2010, 2016; Zhang et al., 2010; Zheng et al., 2012; Feng et al., 2013; Yu et al., 2014). Ciliates were also studied in the western Pacific Ocean (Taniguchi, 1984; Yang et al., 2004; Gomez, 2007; Sohrin et al., 2010; Kim et al., 2012). However, most of the studies did not provide details on ciliate vertical distribution in the euphotic zone. Thus, the aims of this study are to (1) analyze the vertical distribution of tintinnids, (2) investigate ciliate vertical distribution in the euphotic zone of oceanic and slope waters in the northern SCS (nSCS), and (3) determine the influence of shelf water intrusions (He et al., 2016) on the vertical distribution of ciliates in the nSCS slope waters.

2. Materials and methods

Sampling was conducted along an oceanic transect (DY) in the western Pacific from 28 November to 31 December 2015 aboard R/V *Kexue* (Fig. 1). Water depths at all stations were deeper than 3000 m. Another cruise was conducted in the slope area of the nSCS in early summer (10 June to 1 July 2015) aboard R/V *Nanfeng*. The sampling of ciliates was conducted at 16 stations along four transects with water depths ranging from 110 m (St. L01) to > 3000 m (St. L04).

At every station, vertical profiles of temperature, salinity and chlorophyll *a* in-vivo fluorescence were obtained from the surface to 2000 m (or 10 m above the bottom at stations with depths < 2000 m) using a conductivity-temperature-pressure sensor (Sea-Bird Electronics, Bellevue, WA, USA). Water samples were collected at six to 13 depths using 12 L Niskin bottles attached to a rosette. The depths sampled were surface, 15, 30, 50, 75, 100, 150, 200, 300, 500, 1000, 1500 (only in the nSCS) and 2000 m. Around the DCM, the sampling depths were changed to sample the DCM if it was within 10 m of any sampling depth. Water samples (1 L) from each depth were fixed with 1% acid Lugol's iodine and stored at < 4 °C in the dark.

In the laboratory each water sample was concentrated to ~ 100 mL by gently siphoning out the supernatant after settling for at least 48 h. The settling and siphoning processes were repeated to concentrate each sample to a final volume of 50 mL (concentrated sample). The concentrated sample was settled in Utermöhl (1958) counting chambers (25 mL each) for at least 24 h and examined using an Olympus IX 71 inverted microscope (100 × or 400 ×).

We counted the entire volume (two counting chambers) of each concentrated sample. Because mechanical and chemical disturbance associated with our collection and fixation procedures could provoke detachment of the protoplasma from a loricae (Paranjape and Gold, 1982; Alder, 1999), empty tintinnid lorica were counted as living cells in our study. Since some lorica might have been empty when they were sampled (Kato and Taniguchi, 1993; Dolan and Yang, 2017), our tintinnid abundance results may have been overestimated.

For each species, size (e.g. length and width, oral diameter of tintinnids) of the cells (aloricate ciliate) or lorica (tintinnid) were measured from at least 20 individuals. According to loricae morphology and size, tintinnids were identified to species level (Kofoid and Campbell, 1929, 1939; Lynn, 2008; Zhang et al., 2012). Ciliate volumes were estimated using appropriate geometric shapes (cone, ball and cylinder). Tintinnid carbon biomass was estimated using the equation: C = lorica volume (μm^3) × 0.053 + 444.5 (Verity and Langdon, 1984). The conversion factor of carbon biomass for aloricate ciliates used was 0.19 pg C μm^{-3}, as defined by Putt and Stoecker (1989).

The biogeography of tintinnid genera (e.g. neritic, cosmopolitan and warm water types) were derived according to Dolan et al. (2013). The autotrophic *Mesodinium rubrum* (Yih et al., 2004) and large mixotrophic ciliates (*Laboea strobila*, *Tontonia appendiculariformis* and *T. gracilima*) were counted according to their distinctive morphologies as in Dolan and Marrasé (1995) and Zhang et al. (2015).

The dominance index (*Y*) of tintinnid species in one assemblage was calculated using the following formula (Xu and Chen, 1989): $Y = n_i / N \times f_i$, where n_i is the number of individuals of species *i* in all samples, f_i is the occurrence frequency of species *i* in all samples and *N* is the total number of all species. Species with $Y > 0.02$ represented the dominant species in an assemblage. The vertical distribution group of individual tintinnid species was defined according to Kršinić (1982).

3. Results

3.1. Intrusion of shelf water and shoaling of the DCM in nSCS slope water

The water column was well stratified at all stations (Fig. 2). Surface temperatures in the western Pacific (25.8–28.9 °C) were lower than in the nSCS (29.4–31.2 °C). In the western Pacific, the surface mixed layer was 50 m, with temperatures above 26 °C. The thermocline was deeper than 100 m. There was no well-defined surface mixed layer in the nSCS slope water. Temperatures decreased from > 30 °C in surface waters to 14 °C at 200 m.

Salinity showed significant stratification with depth in both the western Pacific and the nSCS slope water. The surface salinity in the western Pacific (33.8–34.8) was higher than the nSCS slope water (31.4–34.0). In the western Pacific, the halocline was deeper than 50 m and salinities > 35 were recorded from 70 m to 130 m. In transects A and B on nSCS slope, salinity increased with depth to 150 m, then decreased to 200 m. There was a low salinity band (surface salinity < 33 in St. L09, L10, L11, L12, L13 and L16) in transects C and D, which indicated that shelf waters had advected into the slope area (Fig. 2). These stations were regarded as influenced by nSCS shelf water and the other stations were regarded as dominated by nSCS oceanic water (Fig. 2). Therefore, we characterized three study areas: western Pacific,

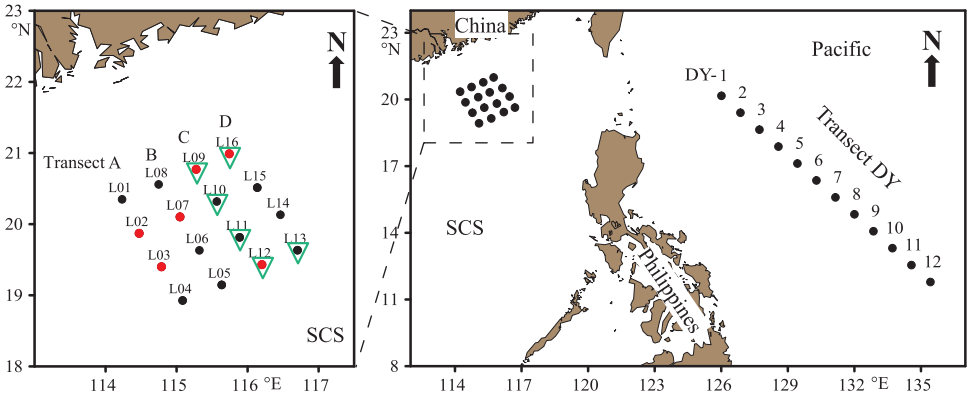

Fig. 1. Map of stations in the western Pacific and the northern South China Sea (SCS). Red dots: stations where neritic tintinnid occurred. ▽: shelf water stations with surface salinity lower than 33.

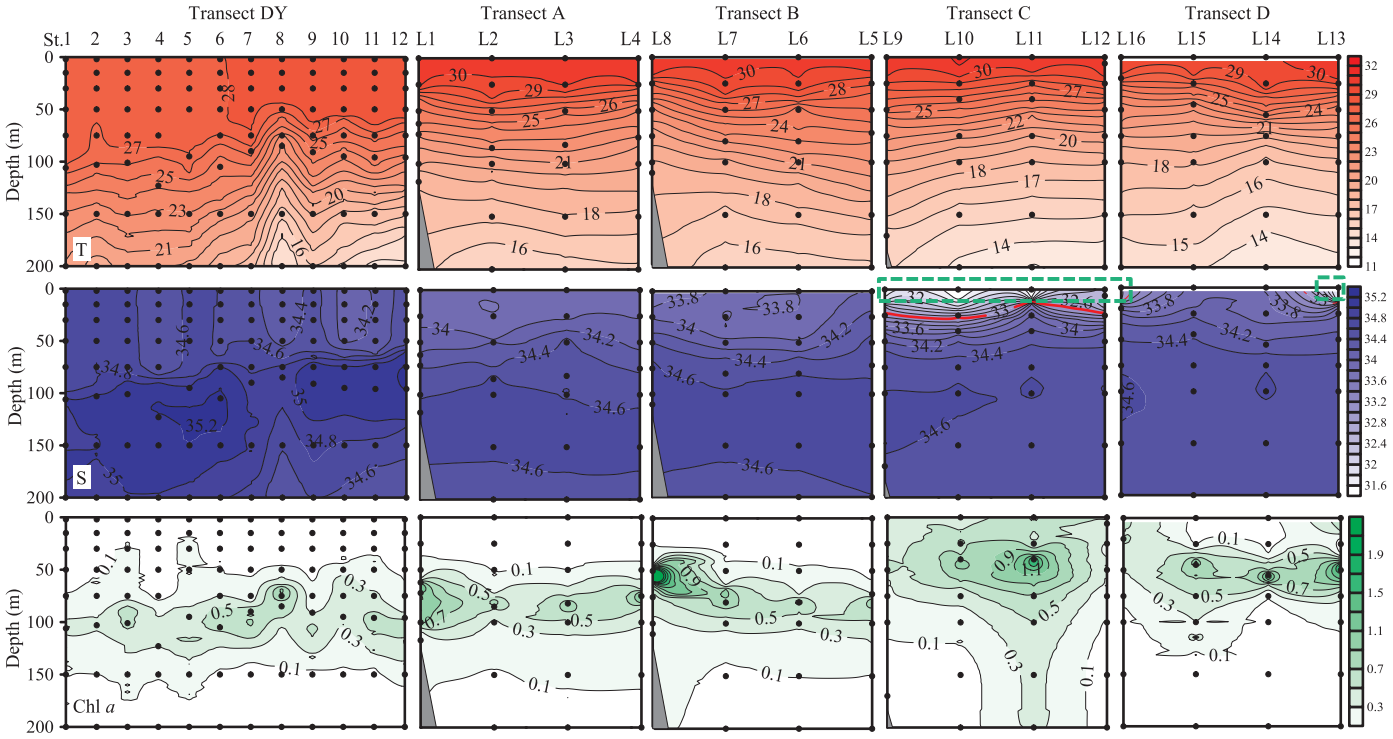

Fig. 2. Vertical distribution of temperature (T) (°C), salinity (S) and Chlorophyll *a* in vivo fluorescence (Chl *a* in arbitrary units) in 0–200 m along transects in the western Pacific and the nSCS. Dots are depths sampled.: nSCS shelf water stations with surface salinity lower than 33.

nSCS oceanic water and nSCS shelf water.

The nSCS shelf water had higher surface Chl *a* concentrations (0.52 ± 0.06, n = 6) than the nSCS (0.05 ± 0.02) and western Pacific (0.05 ± 0.02) oceanic water (Fig. 2). A DCM was found in both the western Pacific and nSCS oceanic water. In the western Pacific DCM, Chl *a* concentrations (0.66 ± 0.18) were about half those of nSCS oceanic waters (1.35 ± 1.15). The DCM depth in the western Pacific (101 ± 17 m) was deeper than the nSCS oceanic water (68 ± 13 m). The maximum Chl *a* depth in nSCS shelf water (38 ± 9 m) were shallower than those in the western Pacific and nSCS oceanic water (Fig. 2).

3.2. Ciliate abundance and biomass

Ciliate abundance ranged from 0 to 443 ind L^{-1} in the western Pacific and 2–809 ind L^{-1} in the nSCS (Fig. 3). Ciliate biomass ranged from 0 to 0.68 μg C L^{-1} in the western Pacific and 0–1.38 μg C L^{-1} in the nSCS (Fig. 3). The highest ciliate abundance and biomass were observed in the upper 200 m and then decreased with depth. *Laboea strobila* and *Mesodinium* spp. were not found and *Tontonia* spp. occurred in nine samples in the upper 75 m with maximum abundances of 6 ind L^{-1} (representing less than 3% of the total abundance).

3.3. Tintinnid species composition

Tintinnid abundance ranged from 0 to 104 ind L^{-1} and decreased to below detection limits below 500 m. In total, 70 tintinnid species from 28 genera were recorded in the western Pacific and the nSCS (Table S1). Fifty-six tintinnid species from 28 genera were identified in the western Pacific. The four dominant tintinnid species were *Salpingella faurei* (dominance index $Y = 0.21$, maximum abundance $A_{max} = 8$ ind L^{-1}), *Steenstrupiella gracilis* ($Y = 0.05$, $A_{max} = 11$ ind L^{-1}), *S. steenstrupii* ($Y = 0.04$, $A_{max} = 5$ ind L^{-1}) and *Protorhabdonella simplex* ($Y = 0.04$, $A_{max} = 4$ ind L^{-1}). Fifty-three tintinnid species in 27 genera occurred in the nSCS slope water with four dominant tintinnid species: *S. faurei* ($Y = 0.09$, $A_{max} = 20$ ind L^{-1}), *S. acuminata* ($Y = 0.08$, $A_{max} = 13$ ind L^{-1}), *Dadayiella ganymedes* ($Y = 0.05$, $A_{max} = 23$ ind L^{-1}) and *P. parva* ($Y = 0.05$, $A_{max} = 10$ ind L^{-1}).

Only two species (Table S1) of neritic tintinnids were found in six samples at six stations (Fig. 1) in the nSCS with a maximum abundance (5 ind L^{-1}) at 50 m of St. L02 (Table S1). In all other samples, the neritic species abundance was 1 ind L^{-1} (proportion < 1%).

3.4. Tintinnid vertical distribution groups

Based on the vertical distribution of tintinnid species abundance, tintinnids were divided into four groups (Table S1, Fig. 4): Group I only occurred from 0 to 100 m, Group II occurred from 50 to 200 m, Group III from > 100 m, and Group V was found throughout the water column. Because we did not find tintinnids below 500 m, there was no Group IV, which has been defined as occurring in waters below 600 m (Kršinić, 1982, 1998). The most abundant six species comprised more than 50% of the total group abundance at each depth interval.

Group I included 20 and 16 species in the western Pacific and the nSCS, respectively (Table S1). Among them, nine species (*Ascampbelliella armilla*, *Epiplocyloides reticulata*, *E. apertus*, *E. lusus-undae*, *E. stramentus*, *P. simplex*, *R. sanyahensis*, *S. gracilis* and *S. steenstrupii*) were found during all cruises. *S. gracilis* was classified as Group I, although it also occurred in one sample deeper than 100 m (Fig. 5). *S. gracilis* was the most abundant species in the western Pacific and *E. stramentus* was the most abundant species in the nSCS. *P. simplex* and *E. apertus* were among the most abundant six species and occurred in all cruises.

Group II included eight and seven species in the western Pacific and the nSCS, respectively, but no species from this group were found during all cruises. *P. aculeata* was the most abundant species in the nSCS, and *S. curta* was the most abundant species in the western Pacific (Table S1). Group III included eight and five species in the western Pacific and the nSCS, respectively. Among these, three species (*Ormosella apsteini*, *O. bresslaui* and *Dictyocysta spinosa*) were found in all cruises. *Amphorellopsis acantharus* and *O. bresslaui* were the most abundant species in the western Pacific and nSCS, respectively. *O. bresslaui* and *O. apsteini* were among the most abundant six species and

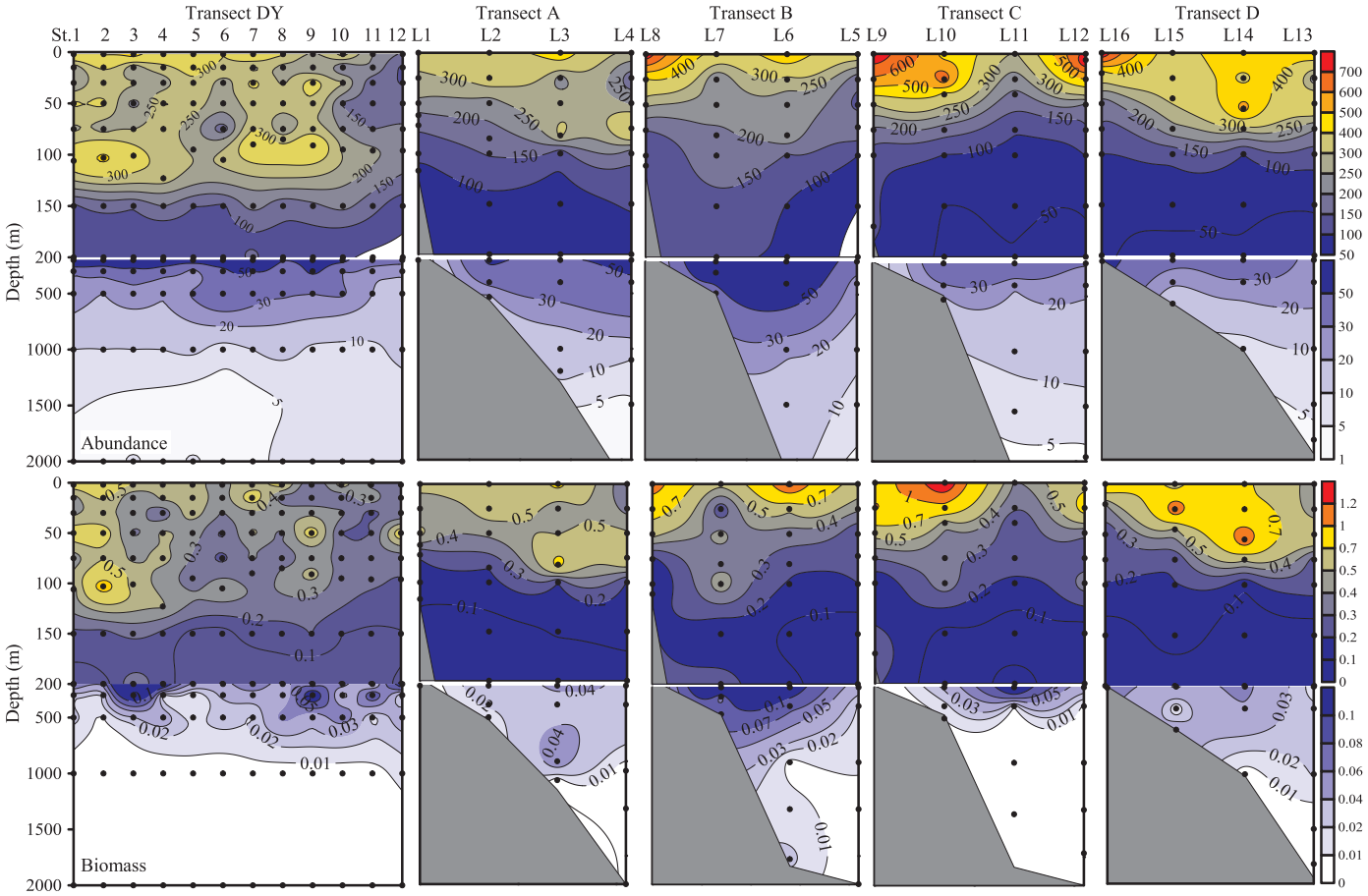

Fig. 3. Vertical distribution of the ciliate abundance (ind L^{-1}) and biomass (μg C L^{-1}) from the surface to bottom (or 2000 m with depth > 2000 m) along transects for the two cruises. Dots are depths sampled.

occurred in all cruises.

Group V included 20 and 23 species in the western Pacific and the nSCS, respectively, and nine of these species (*D. ganymedes*, *D. reticulate*, *Epiplocylis constricta*, *E. fraknoii*, *Proplectella claparedei*, *R. parvula*, *S. acuminata*, *S. decurtata* and *S. faurei*) occurred in all cruises. *S. faurei* was the most abundant species in the western Pacific and nSCS. *S. faurei*, *S. acuminata* and *D. ganymedes* were among the most abundant species and occurred in all cruises (Table S1).

Overall, the groups had different vertical abundance profiles. The Group I total abundance was highest in the surface waters of the western Pacific and nSCS oceanic water, whereas Group II had the highest abundance in the DCM. Group III had its highest abundance at 100 m and decreased with depth, while the highest abundance of Group V was in the DCM (Fig. 4). Groups I and V were the dominant groups, accounting for 50–100% in each depth interval (Fig. 4). From the surface to 100 m, the percentage of Group I decreased while Group V increased. Every abundant species (defined as abundance ≥ 4 ind L^{-1} in at least one depth sampled) in groups I and V had maximum abundance layers in both the western Pacific and nSCS (Fig. 5).

3.5. Differences in ciliate vertical distribution in the western Pacific and nSCS oceanic water

The vertical distribution profiles of average ciliate abundance and biomass was determined by the average DCM depth in each studied region. For example, in the western Pacific, when the 100 m layer at a station was near (within < 10 m) the DCM, the 100 m layer was incorporated into the DCM layer. The vertical profiles of ciliate abundance and biomass showed a bimodal distribution in the western Pacific (Fig. 6) with peaks in the surface waters and DCM layers. The average ciliate abundance and biomass in the DCM were 295 ± 74 ind L^{-1} and 0.4 ± 0.2 μg C L^{-1}, respectively. The abundance in the DCM was 1.3 times higher than in the adjacent 75 m layers (218 ± 59 ind L^{-1}). The average biomass in the DCM layers was 1.2 times higher than at 75 m (0.3 ± 0.1 μg C L^{-1}). In the nSCS, the ciliate average abundance and biomass showed surface peak pattern: abundance and biomass in waters above the DCM were higher than in DCM itself, with the maximum abundance in the surface waters (Fig. 6). The maximum depth of Group V and total tintinnids was deeper in the western Pacific than nSCS slope water (Fig. 4). The species abundances also showed the same pattern (Fig. 5).

3.6. Influence of shelf water intrusion on ciliate vertical distribution in nSCS slope water

In the nSCS shelf water, groups I and V and total tintinnids had lower abundances at the surface than in the subsurface (Fig. 4). Group I abundance in surface waters was less than Group V's, with the percentage of Group I lower than in the nSCS oceanic water (Fig. 4). While ciliate average abundance and biomass showed surface peak pattern in both nSCS oceanic water and shelf water, shelf water had higher surface abundance than oceanic water. The vertical distribution of average ciliate abundance below 75 m was similar in oceanic and shelf waters. However, the average abundance in the upper 50 m in shelf water was higher than in oceanic water (Fig. 6). In the upper 50 m, the average abundance in shelf water (313 ± 128 ind L^{-1}) was 1.3 times higher than in oceanic water (234 ± 52 ind L^{-1}). In the upper 25 m, the average abundance in shelf water was 1.8 times higher than in oceanic

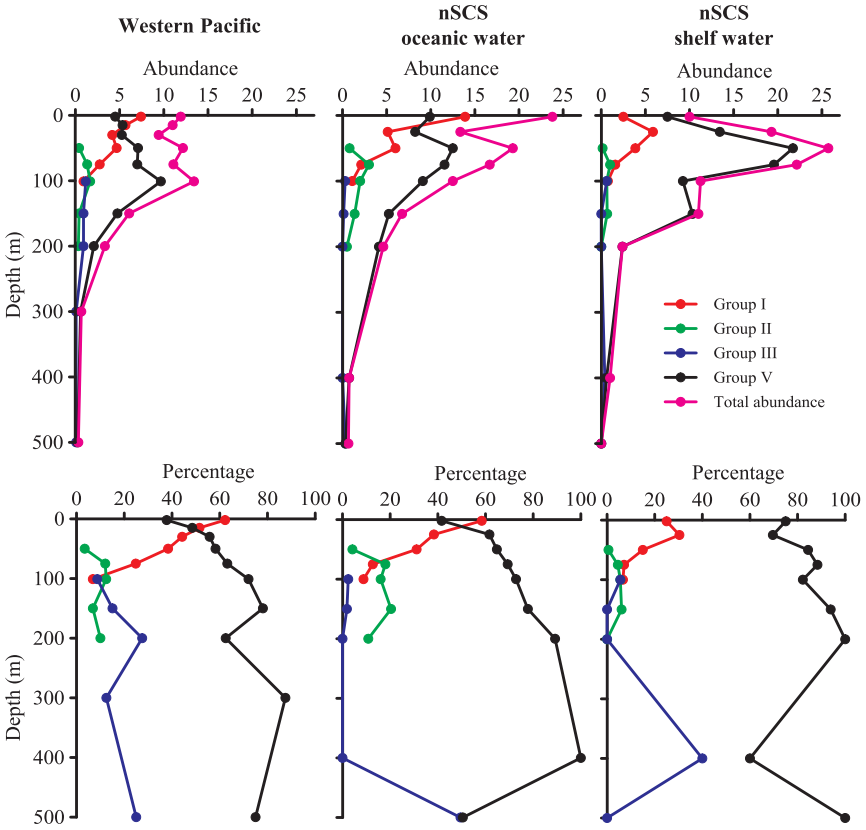

Fig. 4. Vertical distribution of different groups of tintinnid abundance (ind L^{-1}) and percentage (%) in 0–500 m in all stations. Group I: tintinnids that only appeared in 0–100 m, Group II: tintinnids that only appeared in 50–200 m, Group III: tintinnids that only appeared in at depths > 100 m, Group V: tintinnids appeared throughout the water column.

water. Some oceanic stations (e.g. St. L03, L04 and L05) showed a bi-modal distribution pattern similar to the western Pacific.

In the nSCS shelf water, the average tintinnid abundance percentage in the surface layer (1.5%) was lower than in the western Pacific (6%) and the nSCS oceanic water (5.9%). In the 25 m layer and deeper, the tintinnid abundance percentages in shelf water were similar to the western Pacific and nSCS oceanic water (Fig. 6).

4. Discussion

4.1. Vertical distribution of different tintinnid species

The majority of studies on tintinnid vertical distributions has been limited to the upper 100 m (Boltovskoy et al., 1991; Paranjape, 1987; Thompson and Alder, 2005; Thompson et al., 1999, 2001). Tintinnid vertical distribution data from deeper water are scarce, but it has been analyzed through 1200 m in the Adriatic Sea (Kršinić, 1982, 1998) and to 800 m in the Otranto Strait (Kršinić and Grbec, 2002). However, our

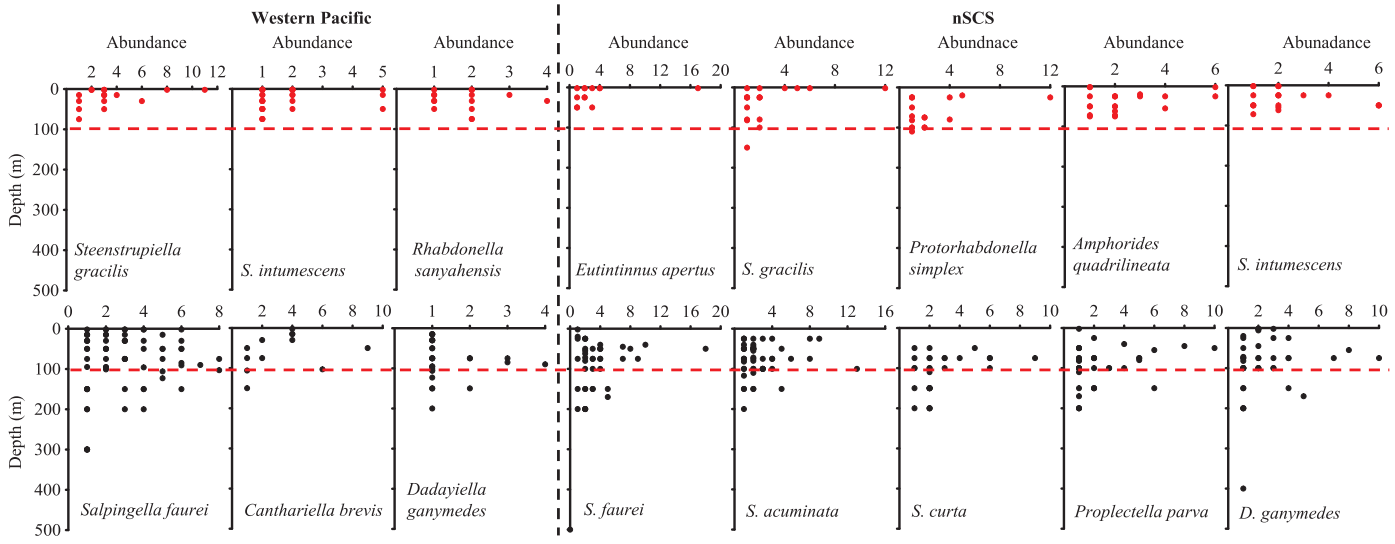

Fig. 5. Two groups of abundant tintinnids (with abundance ≥ 4 ind L^{-1} in at least one depth sampled) vertical distribution patterns in 0–500 m in all stations. red dots: tintinnids belonging to Group I; black dots: tintinnids belonging to Group V. vertical black dashed line: boundary between the western Pacific and the nSCS. horizontal red dashed line: 100 m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

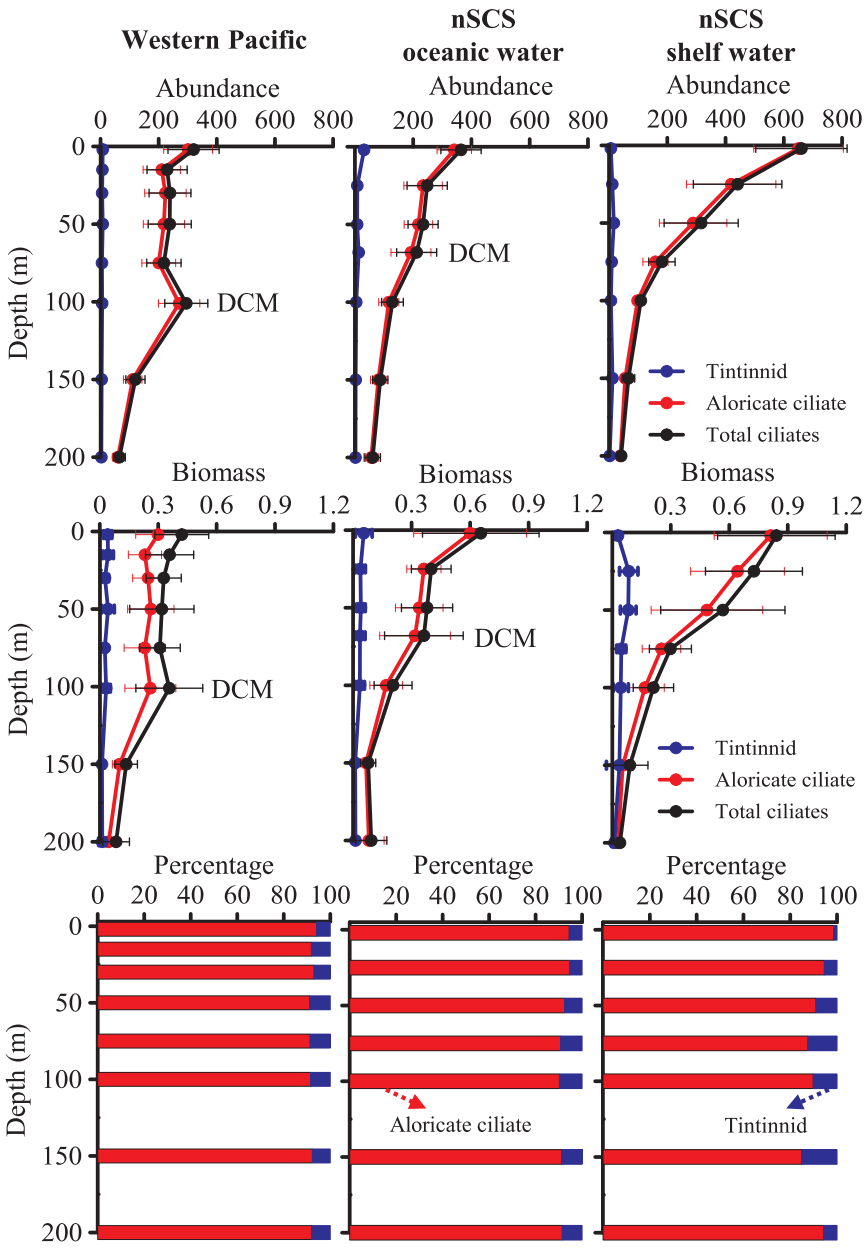

Fig. 6. Vertical distribution of planktonic ciliate (aloricate ciliate and tintinnids) abundance (ind L^{-1}) and biomass (μg C L^{-1}) at all depths (< 200 m) and average abundance percentage among all stations. DCM: deep chlorophyll maximum layer.

study is the first to present deep water data outside of the Mediterranean.

Our sampling method (whole water samples) was different from that of Krsinic and coauthors (53 μm pore size nets towed at different depths). According to the presence or absence of one tintinnid species, tintinnids were divided into different vertical distribution groups. Kršinić (1982) divided tintinnids in the Adriatic Sea into five groups: Group I (0–100 m), II (50–200 m), III (> 100 m), IV (> 600 m) and V (all depths). Kršinić (1998) divided tintinnids into four groups: Group I (0–50 m), II (0–300 m), III (> ; 100 m) and IV (> 600 m). Our data are most similar with that of Kršinić (1982).

The differences in sampling methods could influence the results. Net towing could collect some large but rare species, which were missed by our water sampling. In contrast, our sampling could collect some small < 53 μm) species which are missed by the net tows. The difference in sampling methods is likely one of the causes in the species list differences between our work and Kršinić's (1982). However, 24 tintinnid species from 13 genera were found in both the Adriatic Sea and our study area (Table S2), and of these, eight species (*Amphorides amphora, Codonella amphorella, Epiplocylis undella*, *E. apertus*, *E. lusus-undae*, *E. stramentus*, *S. steenstrupii* and *Xystonellopsis brandti*) had the same vertical distribution pattern. The remaining 16 common species were classified into different groups. For example, *D. ganymedes* and *E. fraknoii* were placed in Group I in the Adriatic Sea, but Group V in this study. We cannot explain this difference, but more study is needed to determine if shifts in vertical distribution of the grouped species are due to differences in location or time.

Our sampling resulted in vertical abundance profiles similar to coastal areas (Boltovskoy et al., 1991; Paranjape, 1987). However, these comparisons do not work well when tintinnid abundance is low. For example, the total tintinnid abundance was below 1 ind L^{-1} in waters deeper than 500 m. Low abundance is often accompanied by low appearance frequency (Dolan et al., 2009). Therefore, vertical distribution patterns are only reliable when abundance is high, which is the main reason we did not have Group IV (> 600 m). For a particular species, we only determined a vertical profile when it had abundances

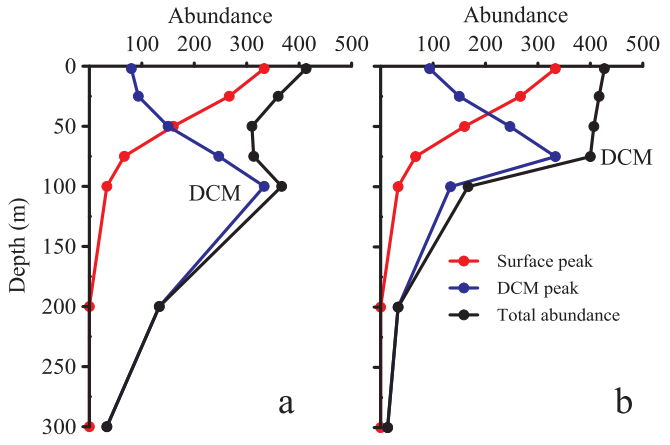

Fig. 7. Schematic for ciliate abundance (ind L^{-1}) vertical distribution patterns with a: a deep DCM layer and b: a DCM layer that is shallow in the nearshore area. We hypothesize that there exists surface peak and DCM peak groups with stable abundance. When the DCM layer is deep (as in the western Pacific; model a), total ciliate abundance shows both surface and DCM maxima (bimodal distribution). When the DCM layer is shallow (in the slope; model b), maximum ciliate abundance is in the surface.

≥ 4 ind L^{-1} (Fig. 6). Our results showed that groups I and V had higher abundance than other groups, which differs from the Mediterranean Sea where Group I comprised more than 90% of the population (Kršinić, 1982).

4.2. Bimodal distribution in tropical oceanic waters

In oceanic water we found that total ciliates and aloricate ciliates had peak abundances in the surface and DCM, or a bimodal distribution. This pattern is different from the single peak in the DCM found in the Catalan Sea (Dolan and Marrasé, 1995). This phenomenon has also appeared in some stations in the western Pacific (Yang et al., 2004), equatorial central Pacific (Sohrin et al., 2010) and equatorial central Indian Ocean (Sorokin et al., 1985), but these studies largely ignored this because their average abundances did not show this pattern. Some of our offshore stations of the SCS transects showed a bimodal distribution as well.

The relationship between ciliate abundance and Chl *a* concentrations has been studied (Tsuda et al., 1989; Dolan and Marrasé, 1995; Suzuki and Taniguchi, 1998; Gomez, 2007; Santoferrara et al., 2016), but most of these studies did not find a correlation with Chl *a* in the Pacific (Tsuda et al., 1989; Suzuki and Taniguchi, 1998; Gomez, 2007). However, ciliate abundance showed significant correlations with Chl *a* concentration in the Mediterranean (Dolan and Marrasé, 1995) and off the Rhode Island coast (Santoferrara et al., 2016). Our study showed that ciliate abundance in oceanic water did not have positive relationship with Chl *a*. Therefore, Chl *a* was not an important factor for ciliate abundance there.

Since tintinnids were grouped according to their vertical distribution, we hypothesized that there might also be group divisions based on the vertical distribution of aloricate ciliates. Some species are surface dwellers with high abundances in surface waters and some species are distributed throughout the water column, with high abundances in the DCM. If these two groups of species had high enough abundances, the combined total abundance will show a bimodal distribution with peaks in both surface waters and the DCM.

The surface dwellers could be mixotrophic. There are more than 10 mixotrophic ciliate species (Laval-Peuto and Rassoulzadegan, 1988; Stoecker et al., 1988; Pitta and Giannakourou, 2000; McManus et al., 2004; Wang et al., 2016), but most mixotrophic ciliates could not be identified in the Lugol's fixed samples, except for *Mesodinium rubrum* and large *Laboea strobila* and *Tontonia* spp. that have distinctive morphologies (Dolan and Marrasé, 1995). However, mixotrophic ciliate abundances are rarely studied. We found very few *Tontonia* spp. in the upper 75 m. Yang et al. (2004) also did not find any mixotrophic species in the northeastern equatorial Pacific. Dolan and Marrasé (1995) found higher abundances of these species in the subsurface water of the Mediterranean Sea. A maximum of 10% of the total ciliates in the equatorial Pacific Ocean were mixotrophic (Stoecker et al., 1996) and did not show a distinctive vertical distribution pattern.

The phenomenon that different groups had maximum abundances at different depths is not limited to ciliates. Other groups in the planktonic food web also exhibit vertical distribution patterns in euphotic waters. In the western Pacific *Prochlorococcus* and picoeukaryotes have maximum abundances in the DCM while *Synechococcus* and bacterial abundance maxima occur above the DCM (Zhao et al., 2017). *Prochlorococcus* has high light and low light ecotypes that inhabit different depths (Moore et al., 1998). These results suggest that the planktonic food web in the two layers might differ in structure and function.

4.3. Differences in ciliate vertical distribution in the western Pacific and nSCS oceanic water

In nSCS oceanic water a few stations showed a bimodal ciliate distribution pattern. However, the average abundance of total ciliates indicated a surface peak. There are two hypotheses to explain this surface peak pattern in nSCS slope water. One hypothesis is the influence of surface current. Surface waters in the shelf area had higher ciliate abundances than in the oceanic area. In the nSCS slope, the surface current flows offshore (Chen et al., 2016):, i.e. from the shelf to the slope. Thus, surface ciliate abundance in the nSCS slope had higher abundance than in the deeper waters.

Another explanation could be the shoaling of the DCM in the slope area. We assumed that the ciliates had two main groups as in the case of tintinnids, one with a surface peak and another one with a DCM peak (Fig. 7). When the DCM was deep (e.g. 100 m), total abundance of the two groups showed a bimodal distribution (Fig. 7a). However, when the DCM shoaled to 75 m (Fig. 7b), the maximum abundance of the DCM group also shoaled. Total abundance will show a surface peak pattern. The DCM layer becomes shallower from the equatorial Pacific to subarctic waters and nearshore areas (McGowan, 1967; Wolf and Woods, 1988; Sohrin et al., 2010). Therefore, the bimodal distribution pattern may gradually disappear from tropical to subtropical areas and from offshore to nearshore areas.

4.4. Differences in ciliate vertical distribution in the nSCS shelf and oceanic waters

Our results showed that areas influenced by shelf water caused significant decreases in tintinnid abundances but increases in aloricate and total ciliate abundances in surface waters. These results are consistent with Yu et al. (2016). In East China Sea shelf water, stations influenced by freshwater from the Yangtze River had high aloricate ciliate and low tintinnid abundances (Yu et al., 2016), but this pattern has not been reported from other areas.

In our study, neritic tintinnids comprised a very small proportion of tintinnids overall (Table S1). This result is also consistent with findings from the East China Sea where few neritic species were found in waters deeper than 80 m (Yu et al., 2016). Due to this low abundance, neritic tintinnids were not indicator species of shelf waters. Neritic tintinnids have less of a correlation with shelf water and may be regarded as residue of previous mixing of shelf and oceanic waters.

Ciliates are important food items of mesozooplankton. High surface ciliate abundance means more food for mesozooplankton and therefore, for fishes. The intrusion of shelf water might be one of the mechanisms for the slope area to support abundant mesopelagic fish. Moreover, the surface peak of ciliates in the slope area might induce stronger vertical

migration of mesopelagic fish than the bimodal distribution pattern.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr2.2018.08.002.

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