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Diversity and distribution of tintinnid ciliates along salinity gradient in the Pearl River Estuary in southern China

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ABSTRACT

Tintinnid community structure in the Pearl River Estuary were investigated 6 times (10, 29 October 2014; 11, 30 June 2015 and 15 March, 1 April 2017) from upstream freshwaters to polyhaline waters. A total of 43 tintinnid species in 15 genera were identified. Freshwater, brackish and marine species occurred in sequence along salinity gradient. *Tintinnopsis mayeri*, *T. tubulosa* and *Tintinnidium fluviatile* were freshwater species. Fourteen and 15 tintinnid species were considered as brackish and marine species, respectively. The preferred salinity of freshwater species was < 3, and the abundance of freshwater species decreased along salinity gradient. Preferred salinity of brackish species ranged in 3–23. High abundance of brackish species occurred at mesohaline regions and decreased towards both oligohaline and polyhaline regions. Marine species abundance dropped sharply with the decrease of salinity, and seldom appeared when salinity was < 15. Tintinnid species richness was low at low salinity areas. With the increase of salinity, tintinnid species richness showed different variation patterns in different seasons. Tintinnid abundance showed bimodal-peak along salinity gradient. The first abundance peak was mainly contributed by brackish species and occurred where salinity was < 15. While the second abundance peak appeared where salinity was around 25 or > 30, which was mainly contributed by marine species. Low salinity, brackish and coastal tintinnid communities were divided based on the abundance percentage variations of different tintinnid types. Complexities of those communities increased along salinity gradient.

1. Introduction

Estuaries are important to humans and marine lives. Estuarine environment is characterized by strong interaction of freshwater outflow and seawater intrusion. The mixture of seawater and freshwater present challenges to the physiology of the organisms. Therefore, estuaries support unique communities of organisms that live at the margin of the sea (Elliott and McLusky, 2002; McLusky and Elliott, 2004). The variability of environmental features, especially salinity, is the main factor controlling the plankton distribution in an estuary (Laprise and Dodson, 1994). Estuarine zooplankton tend to occupy relatively well-defined salinity ranges in relation to their own osmoregulatory abilities

(Modéran et al., 2010). Species composition of both phytoplankton and zooplankton varied along salinity gradient in estuarine areas (Marshall and Alden, 1990, 1993; Sautour and Castel, 1995; Tackx et al., 2004; Calliari et al., 2005; Islam et al., 2005; Muylaert et al., 2009). Accordingly, different zooplankton and phytoplankton communities were divided along salinity gradient in the estuarine waters (Marshall and Alden, 1990, 1993; Huang et al., 2004; Tan et al., 2004; Muylaert et al., 2009; Modéran et al., 2010).

Tintinnids (Ciliophora: Spirotrichea) are planktonic ciliates with durable lorica (Lynn, 2008), that could be found in marine habitats all over the world. According to tintinnid genera occurrence in the global ocean, tintinnids were divided into five biogeographical patterns

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(cosmopolitan, neritic, warm-water, boreal, and austral) in genera level (Pierce and Turner, 1993; Dolan and Pierce, 2013). Tintinnids mainly occur in marine environments, but also in freshwaters (Kofoid and Campbell, 1929; Nie, 1933; Chiang, 1956; Petz and Foissner, 1993) and brackish waters (Sniezek et al., 1991; Snyder and Brownlee, 1991; Smith et al., 2018). Most studies of tintinnids in brackish waters mainly reported the occurrence salinity in limited sites (Balkis, 2004; Urrutxurtu, 2004). There was no report on those tintinnids distribution variations along salinity gradient around the world. In this case, we are not sure whether those species reported in brackish waters were brackish species or just expatriated from their indigenous areas.

As the largest estuary in southern China, the Pearl River Estuary is a dynamic complex estuary with 8 river inlets on its western boundary and has lots of freshwater input channels from the Pearl River and its branches (Wong et al., 2004). The mean annual discharge of the Pearl River is about $10,000 \text{ m}^3 \text{ s}^{-1}$ and the discharge is about four times greater in the rainy season (April to September) than in the dry season (October to March) (Dong et al., 2004). The mean discharges of the Pearl River Estuary are about 8000, 19,000 and $6000 \text{ m}^3 \text{ s}^{-1}$ in March, June and October, respectively (Zheng et al., 2012). In the Pearl River Estuary, phytoplankton was divided into estuarine, mixed water and coastal communities (Huang et al., 2004), the zooplankton was divided into estuarine, tropical neritic, warm-temperate nearshore and pelagic communities (Tan et al., 2004). In this study, we investigated tintinnid community variation along transects where salinity varied from 0.1 to

34.1. Our aims were to 1) find out whether there are freshwater or brackish tintinnid species, 2) determine the trends of tintinnid species richness and abundance from the freshwater to seawater, 3) understanding the mixing of the different tintinnid communities.

2. Materials and methods

Surface waters were collected onboard R/V “Nanfeng” in transects approximately along the axis of Pearl River Estuary in dry season (Transect A on 10 October 2014; Transect B on 29 October 2014), rainy season (Transect C on 11 June 2015; Transect D on 30 June 2015) and transitional season (Transect E on 15 March 2017; Transect F on 1 April 2017). All transects had the same innermost station (Stns. A1, B1, C1, D1, E1 and F1) in Xinzhou Wharf. However, they had different lengths and different number of stations: Transects A, B, C, D, E and F had 19, 41, 36, 26, 29 and 30 stations, respectively (Fig. 1). Surfer (Version 12, Golden Software Inc., USA) was used to present the sampling stations.

At each station, 80 L of surface water were taken with a bucket while the ship was moving, and gently filtered through a $10 \mu\text{m}$ mesh-size net. The samples (ca. 150 cm^3) in the cod end of the net were transferred into bottles and immediately fixed with Lugol's solution (1% final concentration). Surface water temperature ($^{\circ}\text{C}$) and salinity (measured using the Practical Salinity Scale) were determined using a portable water quality analyzer YSI EC300A (Professional Plus, USA).

In the laboratory, two subsamples (each 25 cm^3) from each original

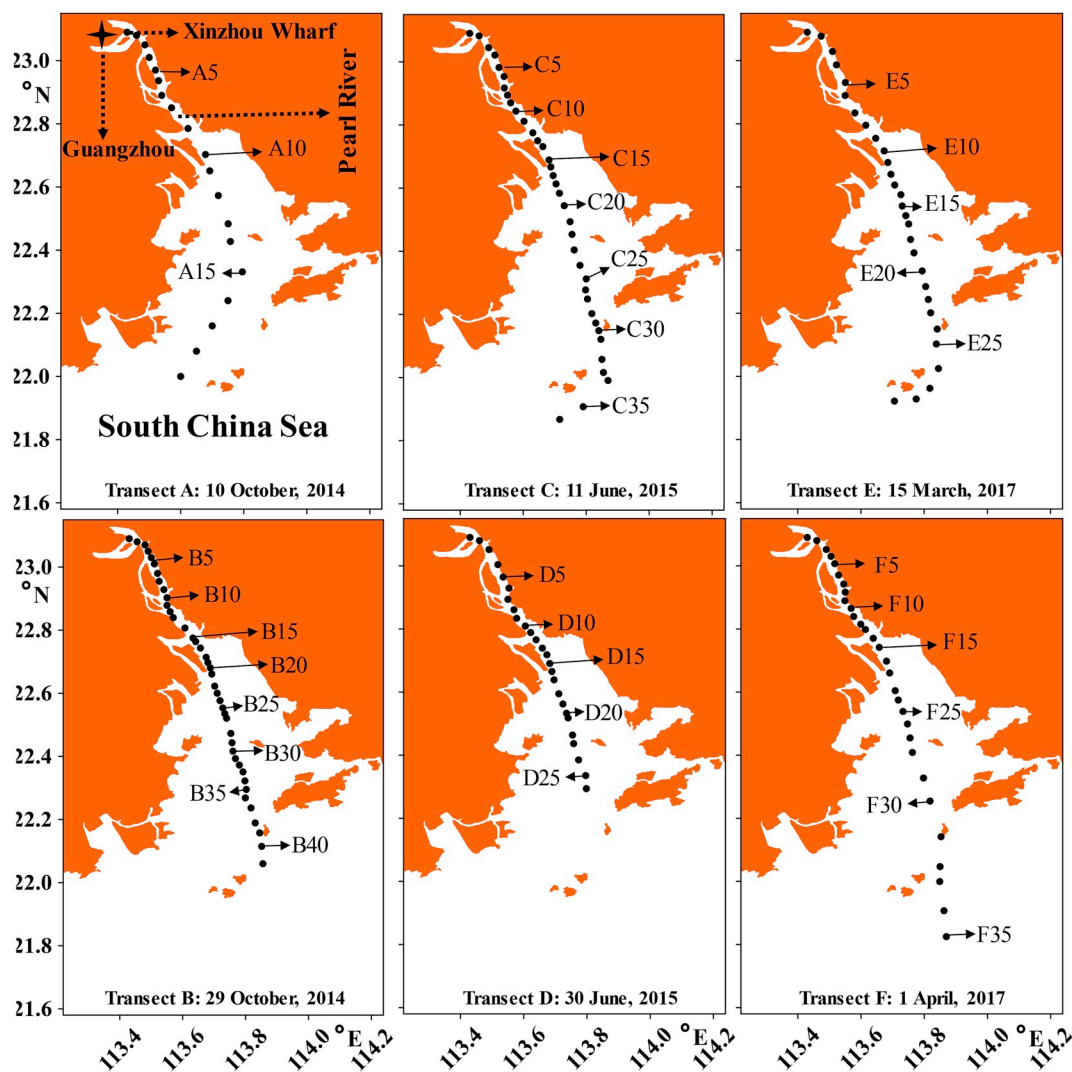


Fig. 1. Stations and sampling days of each transect in the Pearl River Estuary.

sample were settled in two Utermöhl counting chambers (25 cm³) for at least 24 h. Tintinnid were identified and counted using an inverted microscope (Olympus IX 71) at magnification of 100 × or 400 ×, photo of each tintinnid species was taken by a software (Olympus cellSens Standard 1.17) connecting the microscope and computer. Tintinnid species were determined according to lorica morphology and size based on literature (Kofoid and Campbell, 1929, 1939; Hada, 1937, 1938; Bakker and Phaff, 1976; Yoo et al., 1988; Yoo and Kim, 1990; Zhang et al., 2012, 2014). Distributional data were presented as scatter diagrams and bar charts by Grapher (Version 12, Golden Software Inc., USA).

3. Results

3.1. Hydrography

Surface salinity changed greatly along the Pearl River Estuary in each season. In dry season, surface salinity fell in the range of 0.2–32.6 and 0.3–34.1 at Transects A and B, respectively. In rainy season, it fell in the range of 0.1–24.4 and 0.1–12.7 at Transects C and D, respectively. In transitional season, surface salinity ranged in 0.1–32.5 and 0.1–34.3 at Transects E and F, respectively (Fig. 2).

Salinity variation trends along each transect had obvious differences. At Transect A, salinity stayed at 0.2 at the innermost 5 stations and then increased slowly with small fluctuations. At Transect B, salinity increased continuously from upstream to downstream with large fluctuation between Stns. B19 and B28. The salinity fluctuations at Transects A and B were caused by freshwater discharge from branches of the Pearl River. Because of the largest freshwater discharge in rainy season, salinity kept its minimum value (0.1) at upstream stations at both Transects C and D. Salinity increased slower at Transects C and D than the other 4 transects. At Transect E, salinity increased continuously before reaching a plateau at the last three stations. At Transect F, salinity stayed at 0.1 or 0.2 before Stn. F10, then increased rapidly (Fig. 2).

Temperature fluctuations were small (< 3 °C) along each transect. Temperatures were the highest in rainy season, ranged in 28.2–30.8 °C

and 29.0–31.8 °C at Transects C and D, respectively. In dry season, temperatures fell in the range of 27.5–28.8 °C and 26.0–28.2 °C at Transects A and B, respectively. In transitional season, temperatures were the lowest, fell in ranges of 18.2–19.7 °C and 20–22.3 °C at Transects E and F, respectively (Fig. 2).

3.2. Salinity preference of tintinnid species

Totally 43 tintinnid species belonging to 15 genera were identified at 6 sampling transects. *Tintinnopsis* (23 species) was the most diverse genus followed by *Codonellopsis* (3 species), *Tintinnidium* (2 species), *Leprotintinnus* (2 species), *Amphorides* (2 species), and *Eutintinnus* (2 species). Other genera each contained one species (Table 1).

In dry season, 32 species belonging to 12 genera occurred (30 species in 11 genera and 26 species in 8 genera at Transects A and B, respectively). In rainy season, 23 species belonging to 8 genera occurred (16 species in 6 genera and 16 species in 4 genera at Transects C and D, respectively). In transitional season, 26 species belonging to 8 genera occurred (19 species in 6 genera and 22 species in 7 genera at Transects C and D, respectively). For individual species, 11 species occurred in all 3 seasons. While 16 species occurred in only 1 season (Table 1).

Salinity ranges were determined for 32 species occurred in ≥ 5 stations at all transects. These species were divided into freshwater species, brackish species and marine species according to their preferred salinity (salinity with the highest abundance occurred) and abundance variations along salinity gradient (Figs. 3–5 and S1–S3, Table 1 and S1). Eleven species occurred in < 5 stations were occasional species (Fig. S4). We could not assign salinity preference because of their low abundance and occurrence frequencies (Table 1 and S1).

3.2.1. Freshwater species

Three species (*Tintinnopsis mayeri*, *T. urnula* and *Tintinnidium fluviatile*) with the highest abundance in oligohaline (salinity < 3) zone were freshwater species. *T. mayeri* was the only freshwater species that appeared at all 6 transects in 3 seasons. *T. urnula* occurred at 5 transects in 3 seasons and *T. fluviatile* was identified at 3 transects in dry and

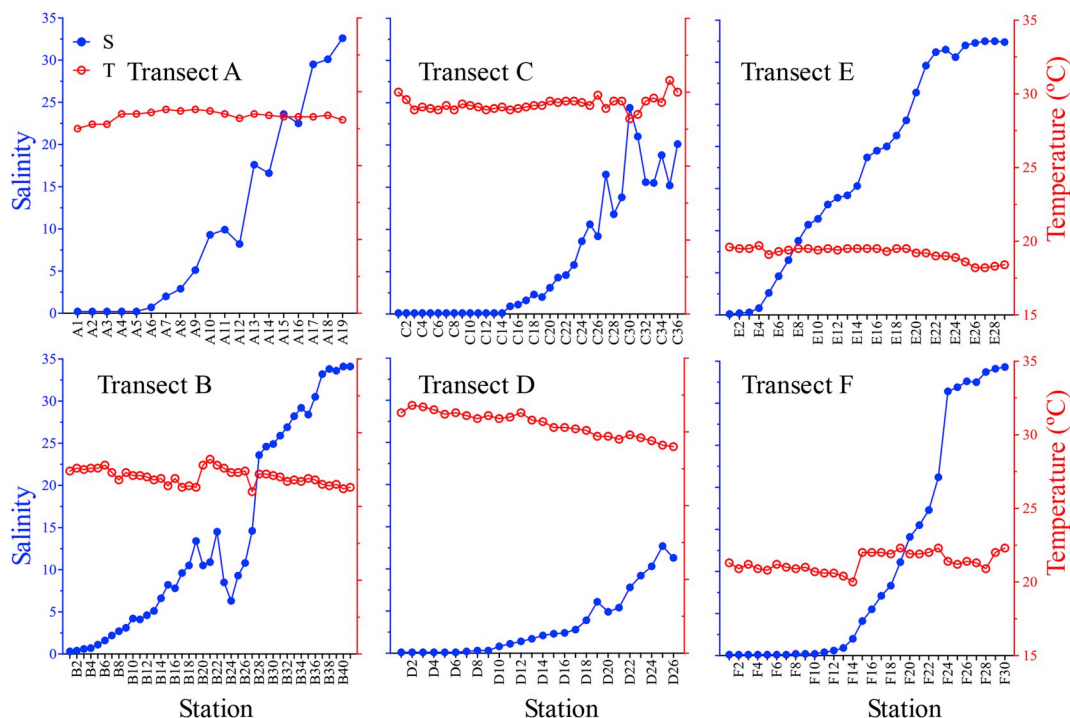


Fig. 2. Variation of salinity and temperature (°C) at each transect in the Pearl River Estuary.

Table 1

Species list and their occurrence frequencies (times) at Transects A, B, C, D, E and F. Nineteen, 41, 36, 26, 29 and 30 stations were conducted at Transects A, B, C, D, E and F, respectively.

Species	Occurrence frequency					
	A	B	C	D	E	F
Freshwater species						
<i>Tintinnidium fluviatile</i>	2	7	0	3	0	0
<i>Tintinnopsis mayeri</i>	8	11	29	21	24	9
<i>Tintinnopsis urnula</i>	1	0	6	14	10	15
Brackish species						
<i>Codonellopsis lusitanica</i>	0	0	0	0	6	4
<i>Leptotintinnus nordqvisti</i>	4	1	8	3	0	0
<i>Leptotintinnus simplex</i>	0	2	7	0	3	0
<i>Nolaculus bicornis</i>	0	0	0	5	0	0
<i>Tintinnidium sp.</i>	3	2	0	2	0	0
<i>Tintinnopsis amoyensis</i>	6	15	15	13	13	11
<i>Tintinnopsis chinglanensis</i>	0	0	0	0	6	7
<i>Tintinnopsis fimbriata</i>	5	16	11	11	5	3
<i>Tintinnopsis nana</i>	2	13	2	3	9	8
<i>Tintinnopsis nucula</i>	5	7	0	0	0	1
<i>Tintinnopsis parva</i>	4	14	8	5	11	15
<i>Tintinnopsis tentaculata</i>	7	21	13	12	0	1
<i>Tintinnopsis tubulosa</i>	12	21	10	12	16	15
<i>Tintinnopsis sp.1</i>	0	0	0	5	0	0
Marine species						
<i>Amphorellopsis acuta</i>	5	4	6	0	8	7
<i>Amphorides amphora</i>	0	0	0	0	1	8
<i>Codonellopsis morchella</i>	0	1	1	0	0	4
<i>Codonellopsis ostenfeldi</i>	0	0	1	0	0	4
<i>Dadayella garymedes</i>	3	0	0	0	0	3
<i>Tintinnopsis beroidea</i>	1	1	0	0	3	3
<i>Tintinnopsis butschlii</i>	3	1	0	0	1	0
<i>Tintinnopsis estuariensis</i>	1	4	0	0	0	0
<i>Tintinnopsis glans</i>	5	6	0	0	3	3
<i>Tintinnopsis plagiostoma</i>	4	16	0	0	2	0
<i>Tintinnopsis radix</i>	6	5	1	0	0	0
<i>Tintinnopsis schotti</i>	4	13	0	0	1	0
<i>Tintinnopsis tocantinensis</i>	7	11	0	0	0	0
<i>Tintinnopsis tubulosoides</i>	6	9	0	0	16	8
<i>Wangiella dicollaria</i>	5	11	0	0	10	3
Occasional species						
<i>Amphorides minor</i>	0	0	0	0	0	3
<i>Epiplocytilis sp.</i>	0	0	0	0	0	2
<i>Eutintinnus lusus-undae</i>	2	1	1	0	0	0
<i>Eutintinnus stramentus</i>	1	0	0	0	0	0
<i>Favella sp.</i>	1	2	0	0	0	0
<i>Helicostomella longa</i>	1	0	1	0	0	0
<i>Metacyclis jorgensenii</i>	1	0	0	0	0	0
<i>Protorhabdonella curta</i>	3	0	0	0	0	0
<i>Tintinnopsis cylindrica</i>	0	0	0	4	0	0
<i>Tintinnopsis sp.2</i>	0	0	0	2	0	0
<i>Tintinnopsis sp.3</i>	0	0	0	4	0	0

rainy seasons (Fig. 3, Table 1). The highest abundance of *T. mayeri* (230 ind. L⁻¹) was higher than *T. urnula* (20 ind. L⁻¹) and *T. fluviatile* (9 ind. L⁻¹) (Table S1). The highest abundances of *T. mayeri*, *T. urnula* and *T. fluviatile* appeared at salinity of 2.8, 0.9 and 1.6, respectively (Fig. 3).

Salinity tolerances of the 3 freshwater species had slight difference. *T. fluviatile* only appeared in salinity ≤12.7, while both the salinity range of *T. mayeri* and *T. urnula* was 0.1–32.5 (Table S1). *T. mayeri* merely appeared in salinity ≤6.1 at Transects A, B, D and F. At Transects C and E, *T. mayeri* was identified at high salinity regions and even to 32.5 at Transect E. Maximum abundance of *T. mayeri* was highest in rainy season, which were 150 and 230 ind. L⁻¹ at Transects C and D, respectively. In dry and transitional seasons, maximum abundance were extremely lower than in rainy season, which were 37, 15, 12 and 5 ind. L⁻¹ at Transects A, B, E and F, respectively (Fig. 3).

Tintinnopsis urnula did not appear at Transect B. It only occurred in salinity ≤4.1 at Transects A, C, D and F. At Transect E, it appeared in salinity ranged in 6.5–32.5. Maximum abundance of *T. urnula* was the highest (20 ind. L⁻¹) at Transect F and the lowest (7 ind. L⁻¹) at Transect E (Fig. 3). *T. fluviatile* did not occur at transitional season. In dry season, it only appeared in salinity ≤2.9 and 5.1 at Transects A and B, respectively. At Transect D, it occurred in salinity 10.3–11.3. Maximum abundance of *T. fluviatile* was the highest (7 ind. L⁻¹) at Transect B and the lowest (3 ind. L⁻¹) at Transect D. Maximum abundance of *T. fluviatile* was 5 ind. L⁻¹ at Transect A (Fig. 3).

3.2.2. Brackish species

Codonellopsis lusitanica, *Leptotintinnus nordqvisti*, *L. simplex*, *Nolaculus bicornis*, *Tintinnidium sp.*, *Tintinnopsis amoyensis*, *T. chinglanensis*, *T. fimbriata*, *T. nana*, *T. nucula*, *T. parva*, *T. tentaculata*, *T. tubulosa*, and *Tintinnopsis sp.1* were defined as brackish species. Salinity ranges of these species were different, but the preferred salinity ranged in 3–23. With the increase of salinity, abundances of most brackish species increased at first, then decreased (Fig. 4).

Tintinnopsis amoyensis and *T. fimbriata* were the most abundant species, whose highest abundance were 2202 and 1657 ind. L⁻¹, respectively. Both of them occurred at all transects in 3 seasons. Swarm (abundance > 1000 ind. L⁻¹) of *T. amoyensis* and *T. fimbriata* occurred at Transect D when salinity ranged in 5.4–9.2 and 3.9–6.1, respectively. At other 5 transects, maximum abundance of *T. amoyensis* and *T. fimbriata* were ≤200 ind. L⁻¹. Salinity range of *T. amoyensis* and *T. fimbriata* were 0.2–26.4 and 0.2–24.4, and the preferred salinity of *T. amoyensis* and *T. fimbriata* were 5.4 and 3.9, respectively (Fig. 4).

Maximum abundance of *N. bicornis*, *T. nana*, *T. parva*, *T. tentaculata*, *T. tubulosa*, and *Tintinnopsis sp.1* were in the range of 50–1000 ind. L⁻¹, which occurred when salinity were 10.3, 6.6, 7.1, 11.3, 5.4 and 3.9, respectively. *T. nana*, *T. parva* and *T. tubulosa* occurred at all transects in 3 seasons, with salinity ranged in 0.1–19.5, 0.1–30.6 and 0.1–32.6, respectively. *T. tentaculata* appeared at 5 transects in 3 seasons, with salinity ranged in 1.4–33.8. *N. bicornis* and *Tintinnopsis sp.1* only occurred at Transect D in rainy season, with salinity ranged in 7.8–12.7 and 2.1–12.7, respectively (Fig. 4).

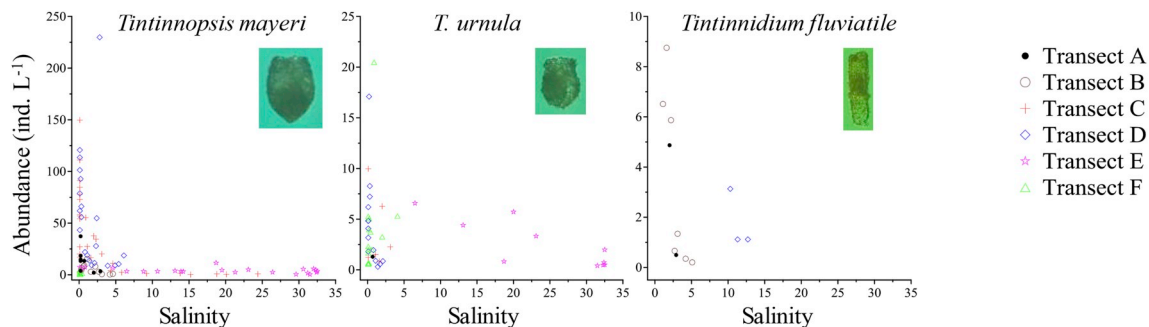


Fig. 3. Abundance (ind. L⁻¹) variation of freshwater tintinnids along salinity gradient at each transect.

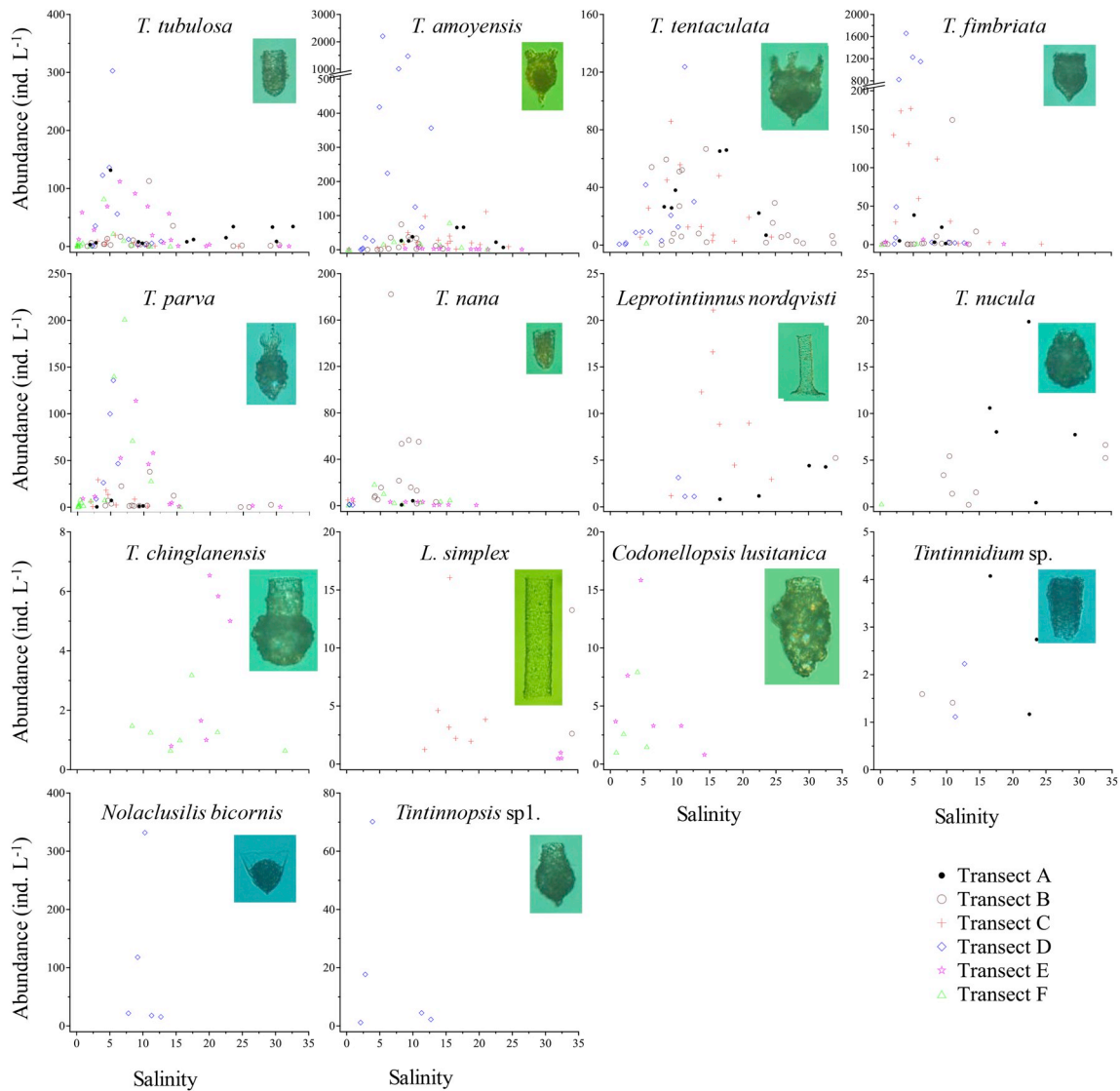


Fig. 4. Abundance (ind. L⁻¹) variation of brackish tintinnids along salinity gradient at each transect.

Maximum abundance of *C. lusitanica*, *L. nordqvisti*, *L. simplex*, *Tintinnidium* sp., *T. chinglanensis* and *T. nucula* were < 25 ind. L⁻¹. The preferred salinity of the 6 species ranged in 4.6–22.5. Salinity ranges of *C. lusitanica*, *L. nordqvisti*, *L. simplex*, *Tintinnidium* sp., *T. chinglanensis* and *T. nucula* were 0.8–14.2, 9.2–34.1, 11.8–34.1, 6.3–23.6, 8.3–31.4 and 0.2–34.1, respectively. Among them, *L. nordqvisti* occurred at 4 transects in dry and rainy seasons, *Tintinnidium* sp. occurred at Transects A, B and D in dry and rainy seasons, *L. simplex* occurred at Transects B, C and E in 3 seasons, *T. nucula* occurred at Transects A, B and F in dry and transitional seasons, while *C. lusitanica* and *T. chinglanensis* occurred at Transects E and F in transitional season (Fig. 4).

3.2.3. Marine species

Fifteen species with the highest abundance occurred at polyhaline (salinity > 23) regions were considered as marine species: *Amphorellopsis acuta*, *Amphorides amphora*, *C. morchella*, *C. ostenfeldi*, *Dadayiella ganymedes*, *T. beroidea*, *T. butschlii*, *T. estuariensis*, *T. glans*, *T. plagiostoma*, *T. radix*, *T. schotti*, *T. tubulosoides*, *T. tocantinensis* and *Wangiella dicollaria*. Their abundance decreased sharply with salinity decrease from downstream to upstream. They rarely appeared in low

salinity (< 15) waters. No marine species was identified at Transect D (Fig. 5, Table S1).

Maximum abundance of *W. dicollaria* (305 ind. L⁻¹) and *T. tocantinensis* (107 ind. L⁻¹) were > 100 ind. L⁻¹. Salinity ranges of *W. dicollaria* and *T. tocantinensis* were 15.3–34.1 and 16.6–34.1, respectively. *A. acuta*, *C. morchella*, *C. ostenfeldi*, *D. ganymedes*, *T. plagiostoma*, *T. radix* and *T. tubulosoides* had maximum abundance ranged in 20–100 ind. L⁻¹, with salinity tolerance ranges in 15.2–34.1, 17.3–34.3, 21.0–34.3, 29.5–34.3, 6.3–34.1, 16.6–34.1 and 11.1–34.1, respectively. Maximum abundances of *A. amphora*, *T. beroidea*, *T. butschlii*, *T. estuariensis*, *T. glans* and *T. schotti* were < 20 ind. L⁻¹. They occurred in salinity ranges of 21.2–34.3, 17.3–34.1, 29.5–34.1, 16.6–34.1, 14.6–34.3 and 22.5–34.3, respectively (Fig. 5, Table S1).

Amphorellopsis acuta and *C. morchella* occurred in 3 seasons. But *A. acuta* appeared at Transects A, B, C, E and F, while *C. morchella* appeared at Transects B, C and F. *T. beroidea*, *T. glans*, *T. tubulosoides* and *W. dicollaria* occurred at 4 transects in dry and transitional seasons. *A. amphora* only appeared at 2 transects in transitional season, while *T. estuariensis* and *T. tocantinensis* only appeared at 2 transects in rainy season. *T. butschlii*, *T. plagiostoma*, *T. radix* and *T. schotti* occurred at 3

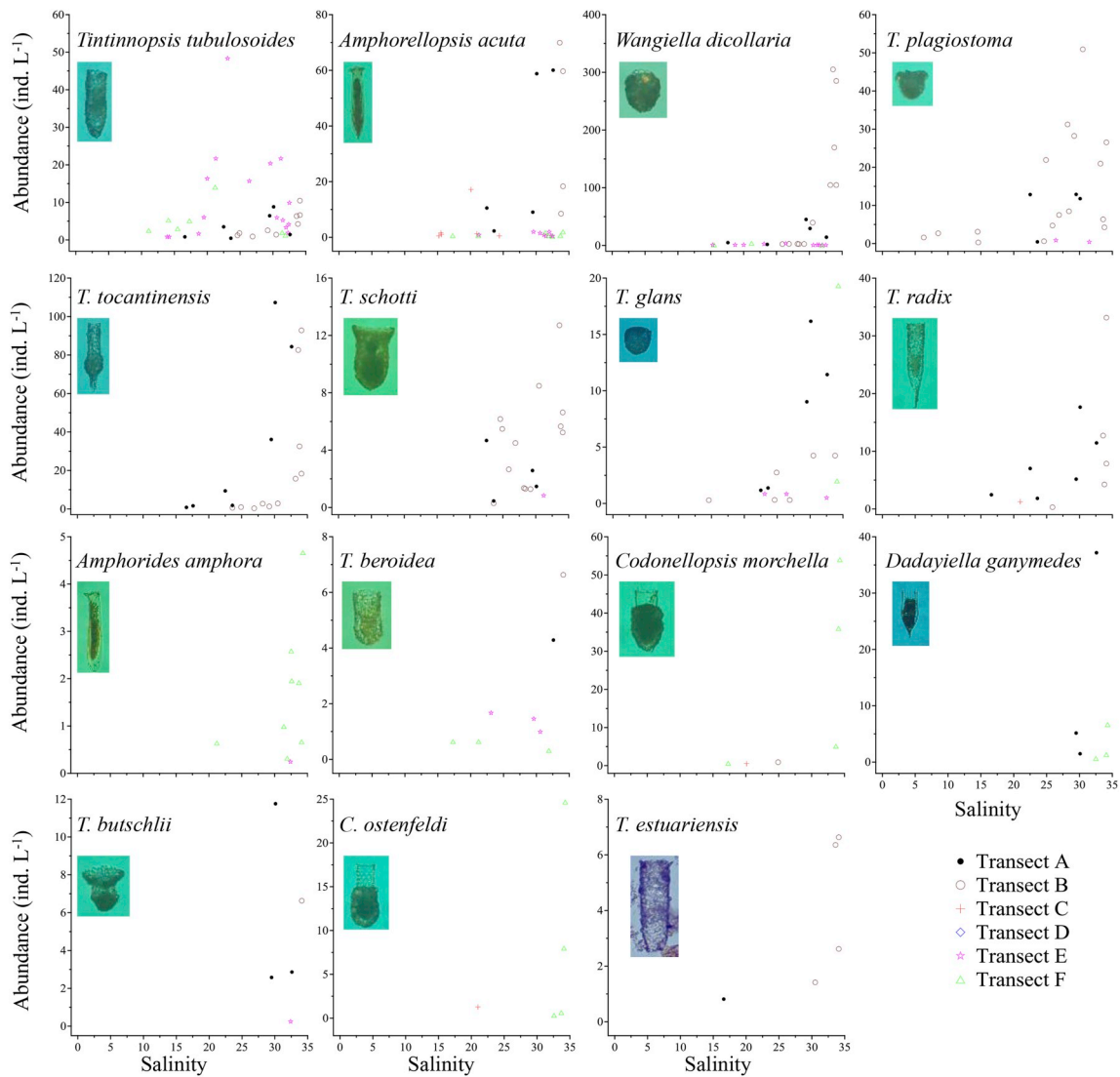


Fig. 5. Abundance (ind. L⁻¹) variation of marine tintinnids along salinity gradient at each transect.

transects, while *C. ostenfeldi* and *D. ganymedes* occurred at 2 transect in different seasons (Fig. 5, Table 1).

3.3. Number of tintinnid species variations along salinity gradient

Number of freshwater tintinnid species was ≤ 2 at each station. Although the number of freshwater tintinnid species was low, the variation trends were quite clear except for Transect E (and partly Transect C) (Fig. 6). Freshwater species disappeared when salinity > 5 at Transects A, B and F. They occurred discontinuously when salinity > 5 at Transects C and D. However, they occurred at high salinity regions at Transect E (Fig. 6).

Number of brackish tintinnid species increased at first, then decreased along salinity gradient at Transects A, B, and F. It was difficult to determine the variation trends at Transects C and D because of the narrower salinity range (Fig. 6). The maximum numbers fell in the range of 5–8 at each transect, occurred when salinity ranged in 5–25 (Fig. 6).

At Transects A and B, number of marine tintinnid species decreased from polyhaline to mesohaline (salinity ranged in 3–23) stations, the maximum number (10 in both Transects A and B) occurred at stations with salinity > 30 (Fig. 6). At Transects E and F, number of marine tintinnid species was ≤ 6 , higher numbers occurred when salinity > 20

and decreased sharply when salinity < 20 (Fig. 6). Number of marine tintinnid species was extremely low at Transect C where the highest salinity was 24.4. No marine species occurred at Transect D (Fig. 6).

Tintinnid species richness ranged in 1–15, 1–13, 1–7, 2–11, 1–7 and 2–8 at Transects A, B, C, D, E and F, respectively. It increased along salinity gradient when salinity < 5 at each transect. Different variation trends of tintinnid species richness were observed when salinity > 5 in different seasons. Tintinnid species richness was high when salinity > 30 at Transects A and B. It stayed stable at mesohaline regions at Transects C, E and F (Fig. 6).

3.4. Tintinnid abundance variations along salinity gradient

Abundances of freshwater species were high when salinity was extremely low and decreased sharply with the increase of salinity at each transect except Transect E (Fig. 7). Maximum abundances ranged in 12–230 ind. L⁻¹ at each transect. Maximum abundance was much higher in rainy season (160 and 230 ind. L⁻¹ at Transects C and D, respectively) than in dry season (38 and 22 ind. L⁻¹ at Transects A and B, respectively) and transitional season (12 and 21 ind. L⁻¹ at Transects E and F, respectively) (Fig. 7).

Brackish species abundances increased at first and then decreased along salinity gradient at each transect, high abundance occurred at

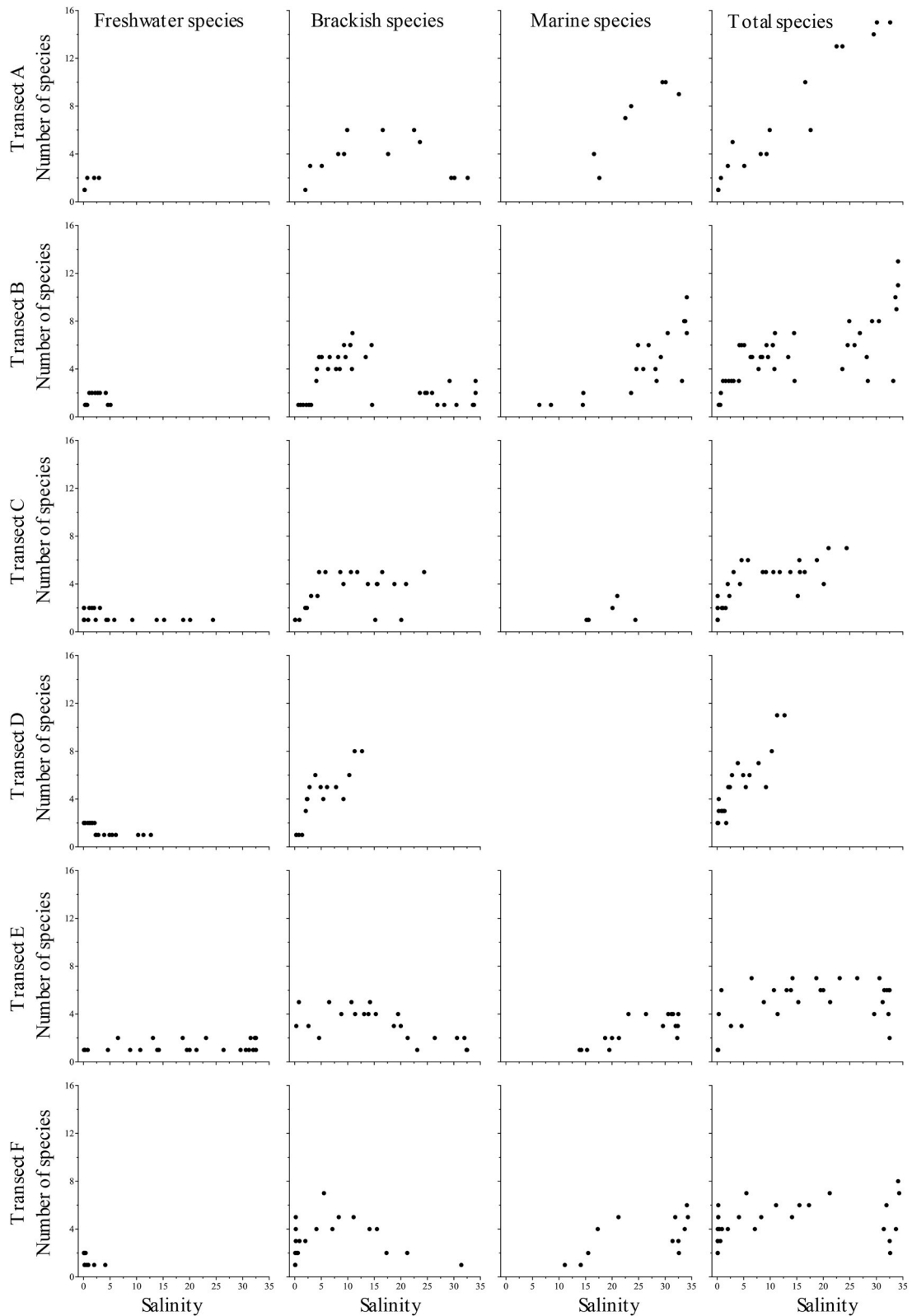


Fig. 6. Number of species variation of different type tintinnids along salinity gradient at each transect. Note that total species including freshwater species, brackish species, marine species and occasional species.

regions with salinity ranged in 5–20. Maximum abundance was extremely higher at Transect D (2683 ind. L^{-1}) than at the other 5 transects (177–376 ind. L^{-1}) (Fig. 7).

At polyhaline regions, marine species abundance increased rapidly with the increase of salinity. Maximum abundances were higher in dry

season (247–531 ind. L^{-1} at Transects A and B, respectively) than in transitional season (53–109 ind. L^{-1} at Transects E and F, respectively) and rainy season (< 20 ind. L^{-1} at Transect C) (Fig. 7).

Total tintinnid abundances were in the ranges of 4–285, 2–557, 2–235, 10–2693, 1–215 and 1–239 ind. L^{-1} at Transects A, B, C, D, E

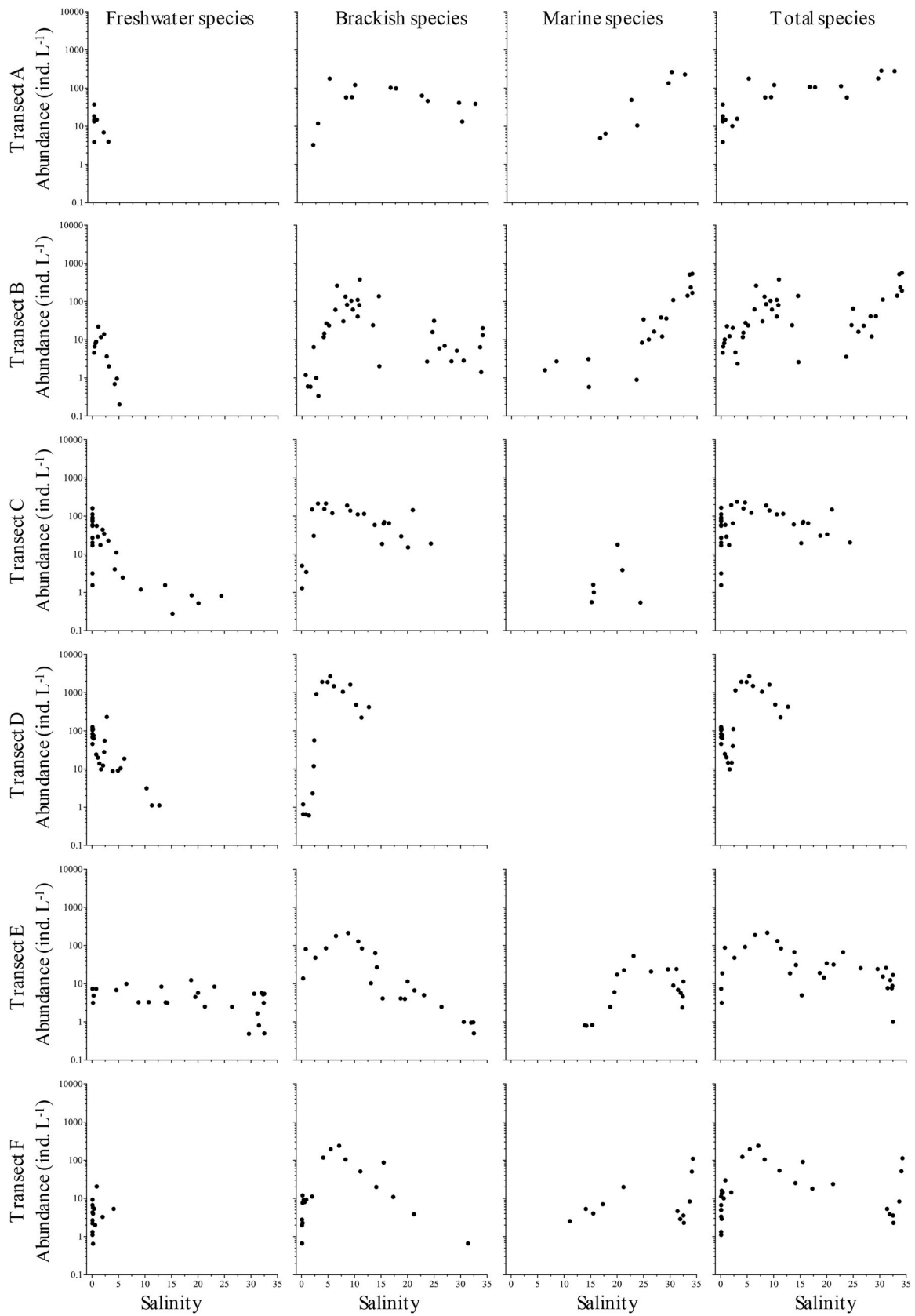


Fig. 7. Abundance (ind. L⁻¹) variation of different type tintinnids along salinity gradient at each transect. Note that total species including freshwater species, brackish species, marine species and occasional species.

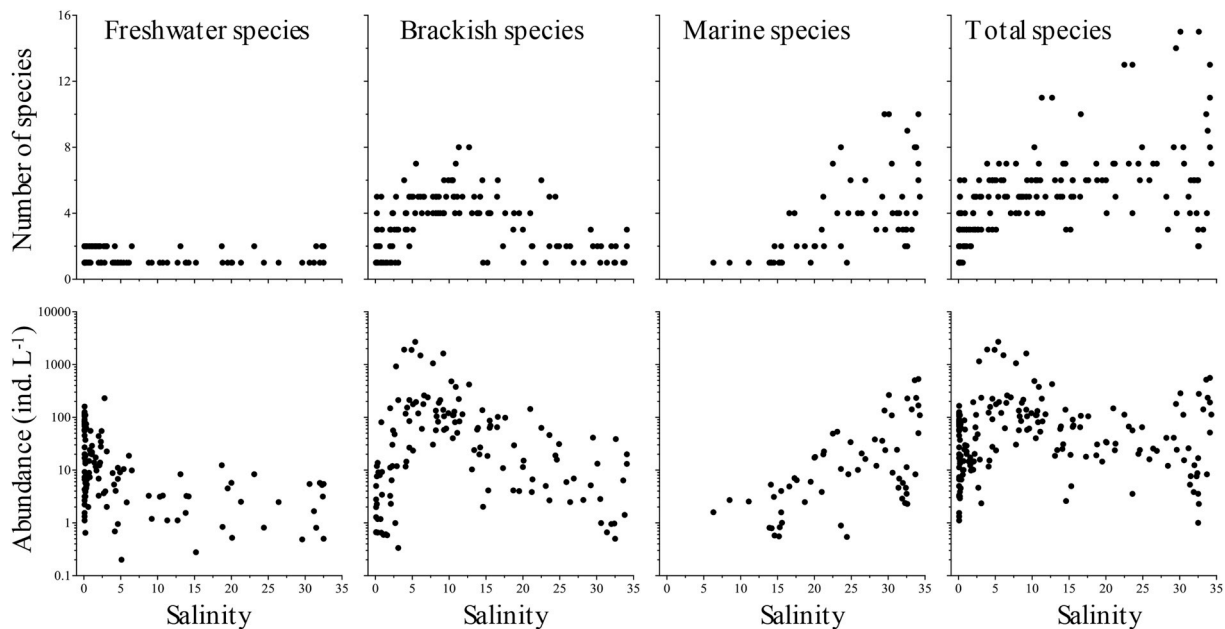


Fig. 8. Cumulative number of species and abundance (ind. L⁻¹) variation of different type tintinnids along salinity gradient. Note that total species including freshwater species, brackish species, marine species and occasional species.

and F, respectively (Fig. 7). Variation tendency of total tintinnid abundance were similar when salinity was < 15 except at Transect A: along with the increasing salinity, it increased at first before reaching a peak, then decreased to low values (Fig. 7).

Total tintinnid abundances showed bimodal-peak along salinity gradient at 4 transects (Transects A, B, E and F). First abundance peaks occurred when salinity was in the range of 4–11 for all transects, which were contributed by brackish species. The second abundance peaks occurred when salinity > 30 at transects A, B and F, and with salinity about 25 at Transect E. Those peaks were contributed by marine species (Fig. 7).

3.5. Tintinnid community variations along salinity gradient

Cumulative data of the 6 transects showed that number of species and abundance of brackish tintinnids had peaks in mesohaline region. Number of species and abundance of marine tintinnids increased with salinity. There were only 3 freshwater species which was too low to show richness trend (Fig. 8). Freshwater species abundance decreased from oligohaline to mesohaline regions. Total species richness was low at oligohaline regions and high at polyhaline regions. Total tintinnid abundance showed peaks at mesohaline and polyhaline regions along salinity gradient (Fig. 8).

Three tintinnid communities (low salinity, brackish and coastal communities) were divided based on the dominance of freshwater, brackish and marine tintinnids (Fig. 9). Low salinity community, dominated by the freshwater species, mainly appeared when salinity was < 2.5 (Fig. 9). At Transects A, B, C and D, the division between low salinity and brackish communities were at the salinity of 2.9, 4.2, 2.0 and 2.4, respectively. The lowest salinity when coastal community first occurred was 29.5, 24.9, 20.1, 19.5 and 21.2 at Transects A, B, C, E and F, respectively (Fig. 9). Therefore, the three communities were generally divided at salinity ranges of 2.0–4.2 and 19.5–29.5, respectively.

The community variations in different season were different: The boundary between different communities were clear cut at Transects A, B, C and D. However, at Transects E and F, low salinity community did not have a clear cut separation with brackish community: freshwater tintinnids bounced back to dominance for several times (Fig. 9).

4. Discussion

4.1. Salinity preference of tintinnid species

Both freshwater and marine organisms could be found at estuaries (McLusky and Elliott, 2004). There were different opinions on whether true brackish species existed at estuaries. Some reports insisted that brackish species were really marine or freshwater species that can adapt brackish conditions (Cognetti and Maltagliati, 2000; Atrill and Rundle, 2002). Most researches agreed that there were true brackish species in mesohaline regions of estuaries (McLusky and Elliott, 2004; Elliott and Quintino, 2007; Whitfield et al., 2012). “(True) brackish species” (Barnes, 1989; McLusky and Elliott, 2004) and “(true) estuarine species” (Carter and Dadswell, 1983; Elliott and Quintino, 2007) were commonly used in estuarine studies with the same meaning (Whitfield et al., 2012). We chose to use “brackish species” in our study.

To our knowledge, brackish tintinnid species was first proposed by Bakker and Phaff (1976). There were lots of tintinnid taxonomic references about freshwater (Kofoid and Campbell, 1929; Nie, 1933; Chiang, 1956; Petz and Foissner, 1993), brackish (Sniezek et al., 1991; Snyder and Brownlee, 1991; Smith et al., 2018) and marine (Kofoid and Campbell, 1939; Zhang et al., 2012) species. However, variation of community composition by those tintinnids along salinity gradient in a specific estuary has not been studied before. Based on the intrusion ability from marine to freshwaters in the Lake Shinji-Ohashi River-Lake Nakaumi brackish-water system, tintinnids were divided into high salinity and wide salinity species, while no freshwater or brackish species was divided (Godhantaraman and Uye, 2003).

Freshwater species and marine species may be expatriated to mesohaline areas due to freshwater discharge and oceanic water intrusion, respectively, into estuaries. Those freshwater and marine species might be found in an expatriate state in mesohaline waters (Angel, 1993). Brackish species usually have wide salinity tolerance (Whitfield et al., 2012). Thus, it was inaccurate to determine salinity preference of tintinnid species according to sporadic report of their occurrence. Therefore, it was more suitable to determine whether a tintinnid species is a freshwater, brackish or marine species according to the preferred salinity and abundance variation along salinity gradient.

According to the division standard above, we divided the 32 tintinnid species into freshwater (3 species), brackish (14 species) and

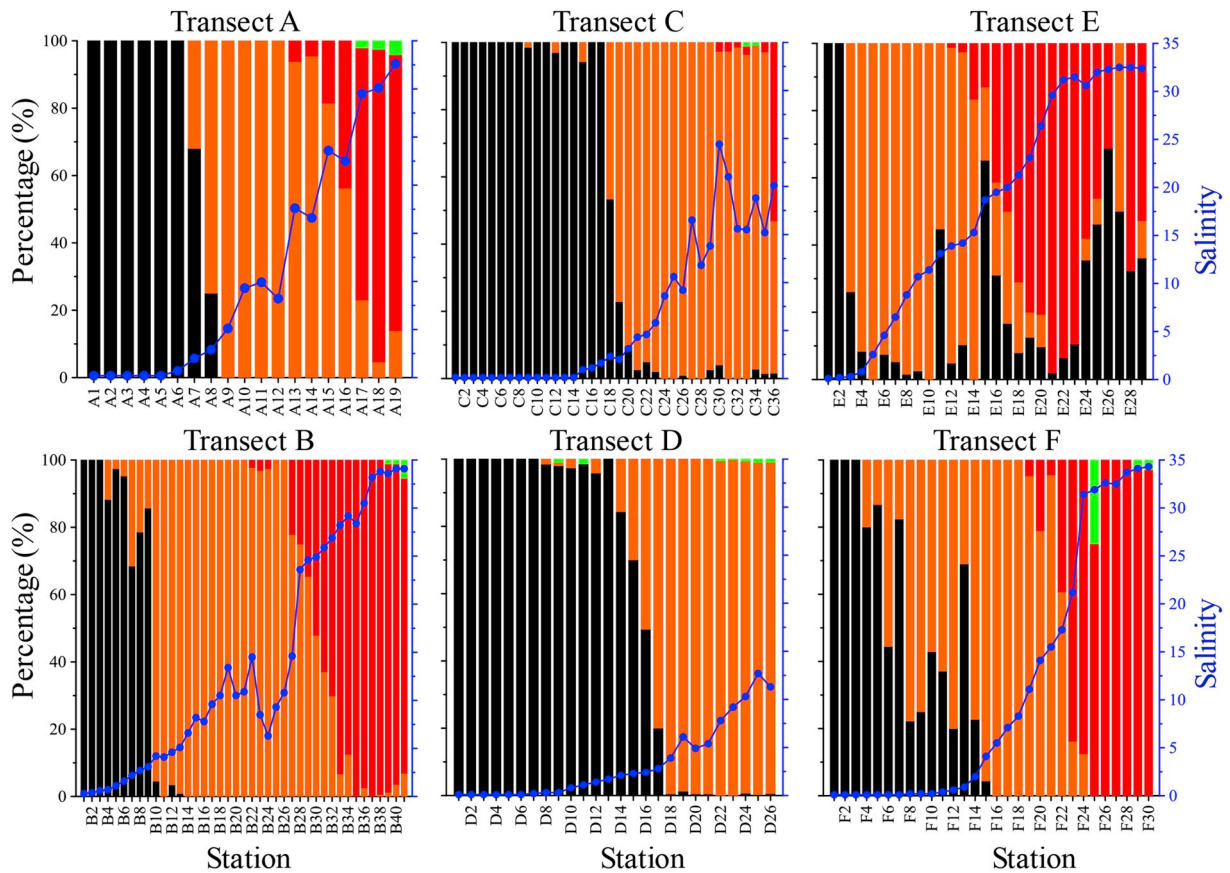


Fig. 9. Abundance percentage (%) of different type tintinnids at each station. ● Salinity, ■ freshwater species, ■ brackish species, ■ marine species, ■ occasional species.

marine (15 species) species (Figs. 3–5). This was consistent with salinity adaptability of phytoplankton in the Pearl River Estuary that freshwater, half-fresh-water and oceanic phytoplankton species were identified (Huang et al., 2004).

4.1.1. Freshwater species

Thirty freshwater tintinnid species have been identified before, and most freshwater species belonging to genera *Tintinnopsis* (20 species) and *Tintinnidium* (6 species) (Zhang et al., 2012). In our study, *Tintinnidium fluviatile*, *Tintinnopsis mayeri* and *T. urnula* were identified as freshwater species with preferred salinity values < 3 (Fig. 3). *T. fluviatile* was considered as a freshwater species in previous studies (Chiang, 1956; Zhang et al., 2012). *T. mayeri* and *T. urnula* were not classified as freshwater species before.

Tintinnidium fluviatile occurred in freshwater lakes in Jiangsu Province, China (Chiang, 1956). It also occurred in the Dock of Woods Hole (Gold and Morales, 1976) and the Río de la Plata estuary (Gómez et al., 2009). *T. mayeri* was found in coastal sites of Jiaozhou Bay without any information of salinity (Yin, 1952). However, a 10-year survey in the central Jiaozhou Bay (salinity > 24.6) did not find this species (Feng et al., 2018). In the present study, although *T. mayeri* appeared when salinity was > 30 in the Pearl River Estuary, the stations *T. mayeri* appeared were not far from the coast. *T. mayeri* may discharged to higher salinity areas by river runoff or the branches of the river in expatriate state and die out soon. However, the 10-year survey in Jiaozhou Bay were conducted in the central area of Jiaozhou Bay far from the coast, *T. mayeri* might not reach those areas even in expatriate state. Our result that *T. mayeri* was a freshwater species may contribute to explain its presence in Yin (1952) and absence in Feng et al. (2018). *T. urnula* mainly occurred at low salinity regions and disappeared at the

outermost sites in the Hooghly (Ganges) River Estuary (Rakshit et al., 2016a).

In the present study, we confirmed that freshwater tintinnids can sometimes be brought to high salinity (32.5) regions. However, we did not understand why freshwater tintinnids were expatriated to high salinity in transitional season rather than in rainy season with largest freshwater discharge.

4.1.2. Brackish species

Four brackish species were reported in previous studies: *Dartintinnus alderae* (Smith et al., 2018), *Nolaculisilis bicornis* (Snyder and Brownlee, 1991), *N. hudsonicus* (Sniezek et al., 1991) and *T. fimbriata* (Bakker and Phaff, 1976). In our study, 14 brackish species were identified (Fig. 4). Among them, *N. bicornis* and *T. fimbriata* were reported in previous studies. *N. bicornis* was first identified in oligohaline and mesohaline regions with salinity ranged in 0.7–16.4 in the Chesapeake Bay (Snyder and Brownlee, 1991). *T. fimbriata* mainly appeared at mesohaline region in Netherlands coastal waters (Bakker and Phaff, 1976) and the Chesterfield Inlet (Rogers et al., 1982). The abundance of *T. fimbriata* reached 1.08×10^6 ind. L^{-1} at salinity of about 15 and caused a yellowish colouration in the Kiel Bight, Baltic Sea, in March 1999 (Agatha, 2008). In this study, swarm of *T. fimbriata* appeared at salinity ranged in 3.9–6.1 and declined both towards the polyhaline and oligohaline waters (Fig. 4).

Other species were not defined as brackish species, although there were reports of some species occurring in brackish waters. *T. amoyensis* is a common and sometime dominant species at estuarine regions. In the Weihe River mouth of Laizhou Bay, *T. amoyensis* was the most dominant species with maximum abundance of 6700 ind. L^{-1} (Zhang and Wang, 2000). *T. amoyensis* (synonym of *T. uruguayensis*) was also a

major tintinnid species at estuarine environment in southwest coast of India (Sivasankar et al., 2018). In this study, swarm of *T. amoyensis* occurred at salinity 5.4–9.2 (Fig. 4). In our study, swarm of *T. amoyensis* and *T. fimbriata* occurred at Transect D caused the extreme high abundance of brackish species at transect D (Figs. 4 and 7).

Tintinnopsis tubulosa was considered as a wide salinity species in the Hooghly (Ganges) River Estuary with the highest salinity of 26 (Rakshit et al., 2016a). *Leptotintinnus nordqvisti* was considered as a high salinity species in a brackish-water system in Japan (Godhantaraman and Uye, 2003). *T. tentaculata*, *T. parva*, and *T. nana* were mostly found at regions with salinity range of 3.2–14.4 in the Hooghly (Ganges) River Estuary (Rakshit et al., 2016a). *T. nucula*, *T. chinglanensis*, *L. simplex*, and *Codonellopsis lusitanica* were sporadically reported at coastal or estuarine environments (Zhang and Wang, 2000; Li et al., 2016; Rakshit et al., 2016b) without detailed salinity range.

In taxonomical studies, brackish tintinnid species were less frequently targeted in brackish waters, where in turn the most recently discovered species and genera, such as *D. alderae* (Smith et al., 2018), *N. hudsonicus* (Sniezek et al., 1991) and *N. bicornis* (Snyder and Brownlee, 1991), have been found. We find that preferred salinity of brackish species were in the range of 3–23, which could be helpful for taxonomist to find more brackish tintinnid species.

4.1.3. Marine species

Most marine tintinnids in our study were common species in coastal and estuarine waters around the world (Godhantaraman and Uye, 2003; Urrutxurtu, 2004; Li et al., 2016; Feng et al., 2018; Sivasankar et al., 2018). Their abundances plummeted sharply with the decrease of salinity in the Pearl River Estuary (Fig. 5).

There were some reports about decreasing abundances of some species from coast to inner part of estuaries. For example, *Wangiella dicollaria* was restricted at regions with salinity > 24 in the Hooghly (Ganges) River Estuary (Rakshit et al., 2016a). *T. berodea* only appeared when salinity was > 18 in the Nervion River estuary, its high abundance occurred when salinity was > 25 (Urrutxurtu, 2004). *T. radix* appeared when salinity ranged in 19.7–20 in the Sea of Marmara (Balkis, 2004) and 29.17–32.89 in Southern Yellow Sea (Zhang et al., 2008). It was the dominant species at all stations in Shenhu Bay of China with salinity ranged in 31–33.6 (Wang et al., 2014). *T. estuariensis* was a species first recorded in the Changjiang River Estuary with salinity ranged in 3.9–29.8 (Zhang et al., 2014).

4.2. Tintinnid composition variation along salinity gradient

In the present study, different tintinnid species occupied different salinity ranges with drastic abundance variations (Figs. 3–5). Variations of tintinnid composition along salinity gradient in the present study were consistent with previous studies of phytoplankton and zooplankton. Estuarine plankton community structure was strongly affected by salinity. Species composition and abundance of both phytoplankton and zooplankton varied along salinity gradient (Marshall and Alden, 1990, 1993; Sautour and Castel, 1995; Tackx et al., 2004; Calliari et al., 2005; Islam et al., 2005; Muylaert et al., 2009). Correspondingly, communities of zooplankton and phytoplankton varied along salinity in estuaries (Marshall and Alden, 1990, 1993; Huang et al., 2004; Tan et al., 2004; Muylaert et al., 2009; Modéran et al., 2010).

4.2.1. Variation of tintinnid species richness along salinity gradient

In estuary regions, number of freshwater and marine macrozoobenthos were higher than brackish species, freshwater and marine macrozoobenthos decreased in diversity with a progression from both upstream freshwater and downstream marine ends, caused the lowest species richness when salinity ranged in 5–8 (Remane, 1934; McLusky and Elliott, 2004; Whitfield et al., 2012). The salinity range of 5–8 was considered as critical salinity zone or horohalimum (Khebovich and

Abramova, 2000). Planktonic protists showed an inverse trend along salinity gradient compared with macrozoobenthos, while the highest species richness occurred in the horohalimum in the Baltic Sea estuaries (Telesh et al., 2011a,b; 2013).

In the present study, 15 marine, 14 brackish and 3 freshwater tintinnid species were identified (Table 1). It was understandable because only 30 freshwater tintinnids while about 900 marine tintinnid species have been reported till now (Zhang et al., 2012). As a result, species richness in mesohaline waters was higher than in oligohaline waters.

Tintinnid species richness were higher in mesohaline than in oligohaline regions in all transects in our study, while the values in polyhaline waters were higher than in mesohaline waters in some transects and lower in other transects (Fig. 6). This was not so consistent with that of planktonic protists in the Baltic Sea estuaries, where the highest species richness occurred in horohalimum (Telesh et al., 2011a,b; 2013). In the Chesapeake Bay with salinity ranged in 9–27, higher numbers of tintinnid species were found in the intermediate stations (salinity ranged in 14–17), low values were found at oligohaline and polyhaline stations (Dolan and Gallegos, 2001). This tendency was similar with that of Transects E and F in our study.

4.2.2. Variation of tintinnid abundance along salinity gradient

In our study, two tintinnid abundance peaks appeared at Transects A, B E and F, only one abundance peak appeared at Transects C and D (Fig. 7). This might be because salinity ranges of Transects C and D were the narrowest, if sampled at a larger salinity range, tintinnid abundance may showed bimodal-peak, too.

In the present study, peaks of tintinnid abundance occurred at mesohaline and polyhaline regions along salinity gradient. This abundance distribution pattern was inconsistent with that in the Bay of Biscay, where tintinnids showed a seaward distribution and were restricted to regions with salinity > 25 (Urrutxurtu et al., 2003). Bimodal-peak of tintinnid abundance along salinity gradient made it difficult to find the correlation between tintinnid abundance and salinity in our study (Fig. 8), which was consistent with that no significant correlation between tintinnid abundance and salinity (ranged in 11.3–33.2) was found in Hiroshima Bay (Kamiyama and Tsujino, 1996).

4.2.3. Tintinnid community variations along salinity gradient

According to the abundance percentage variations of freshwater, brackish and marine zooplankton species along salinity gradient, a distinct brackish zooplankton community could be divided between freshwater and marine zooplankton communities (Cognetti and Maltagliati, 2000). In our study, low salinity, brackish and coastal tintinnid communities were divided using the same method as Cognetti and Maltagliati (2000) (Fig. 9).

Tintinnid community division in this study was similar to previous studies of phytoplankton (Huang et al., 2004) and zooplankton (Tan et al., 2004) in the Pearl River Estuary. Indeed, estuarine, mixed water and coastal phytoplankton communities (Huang et al., 2004), whereas estuarine, tropical neritic, warm-temperate nearshore and pelagic zooplankton communities (Tan et al., 2004) were divided in the Pearl River Estuary. The similar variation tendencies of zooplankton communities were also observed in the St. Lawrence Estuary (Laprise and Dodson, 1994; Winkler et al., 2003) and upper Ariake Bay of Japan (Islam et al., 2006). Those results indicated that it was a common phenomenon that plankton varied from low salinity to brackish to coastal communities along salinity gradient in estuarine regions.

In the present study, low salinity tintinnid community was absolutely dominant by 3 freshwater species. Brackish tintinnid community was shaped by several abundant brackish species such as *T. tubulosa*, *T. amoyensis*, *T. tentaculata*, *T. fimbriata*, *T. parva*, and *T. nana*. While coastal tintinnid community was much more complex, it was composed by more species with low abundance (Figs. 3–5). This distribution pattern was consistent with that of zooplankton in the Gironde estuary, where the upstream zone was exclusively dominated by one copepod,

the median zone was co-dominated by 2 copepods, while the downstream zone presented the highest diversity (David et al., 2016). The similar pattern was also observed in the Chikugo Estuary (Islam et al., 2005) and the Sendai Bay (Kazama and Urabe, 2016). The similar variation patterns of tintinnid and plankton communities along salinity gradient demonstrated that with the increase of salinity, structure of plankton communities become more complex.

4.3. Seasonal variations of tintinnid composition

The waxing and waning of different species within the system was the main reason causing the seasonal changes in tintinnid species compositions (Dolan and Pierce, 2013). The present study showed that 11 in 43 species occurred in 3 seasons, while 16 species only appeared in 1 season. This was consistent with previous studies that most tintinnid species have obvious seasonal occurrence (Modigh and Castaldo, 2002; Dolan and Pierce, 2013; Feng et al., 2018). A 4-year study sampled weekly in the Gulf of Naples indicated that none of the 10 most common species occurred even half the time and most occurred less than a third of the time (Modigh and Castaldo, 2002). A 10-year study sampled monthly at 4 stations in the Jiaozhou Bay showed that 10 species had a year-round distribution, 2 species occurred in 11 months while the other 14 species were found in less than 7 months (Feng et al., 2018).

As plenty of studies showed very distinct seasonal changes in the occurrence of individual tintinnid species, we sampled at 3 seasons (dry, rainy and transitional) in order to collect more tintinnid species in the Pearl River Estuary. The purpose of this study was to show tintinnid community variation along salinity gradient. Therefore we did not discuss too much on seasonal variation of tintinnid community in the Pearl River Estuary.

5. Conclusions

In this study, diversity and distribution variations of tintinnid along salinity gradient from 0.1 to 34.1 in the Pearl River Estuary were investigated. We found that freshwater, brackish and marine tintinnid species appeared successively along salinity gradient. Preference salinity of freshwater, brackish and marine tintinnid species ranged in < 3, 3–23 and > 23, respectively. The alternation of freshwater, brackish and marine tintinnid species caused the variation of tintinnid species richness and abundance. Tintinnid species richness was low at oligohaline regions in 3 seasons. Variation tendency of tintinnid species richness along salinity gradient were different in different seasons. Two abundance peaks appeared at mesohaline and polyhaline regions. According to the dominance of freshwater, brackish and marine tintinnid species, low salinity, brackish and coastal communities were divided in the Pearl River Estuary. Future research of tintinnid community variation along large salinity gradient in other estuaries is needed in order to elucidate whether there is different characteristics in other estuaries.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2019.106268>.

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