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Annual variation of *in situ* sedimentation of planktonic Ciliophora cysts in a temperate bay

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Marine planktonic Ciliophora serve as a key component of the plankton food web. The formation of cysts is of common occurrence among planktonic Ciliophora, and encystment plays an important role in the persistence and diffusion of population. However, studies on the seasonal pattern of encystment of planktonic Ciliophora in natural environments were limited. Here, we investigated the sedimentation of Ciliophora cysts, and explored the seasonal differences of encystment between aloricate Oligotrichea and Tintinnina in Bohai Bay. Ciliophora cysts were collected monthly with a sediment trap from July 2019 to June 2020 at a fixed station, and identified according to the morphological characteristics of cysts by fluorescence microscopy. Ten types of aloricate Oligotrichea cysts were identified and only three species of Tintinnina cysts were recognized, namely, *Favella* sp., *Helicostomella longa* and *Tintinnopsis* sp. There were obvious seasonal differences of encystment between aloricate Oligotrichea and Tintinnina. Encystment of Tintinnina mainly occurred in summer, while encystment of aloricate Oligotrichea was found at all seasons and the seasonal patterns varied among species. The production rate of several types of cysts showed significant positive correlations with water temperature and Chlorophyll a concentration, and a significant negative correlation with salinity. Our study exhibited that the seasonal pattern of encystment of Ciliophora varied greatly from species to species, and assessing seasonal patterns of encystment will aid our ability to understand the mechanism of vegetative population dynamics.

KEYWORDS

planktonic Ciliophora, cyst, sedimentation, seasonal variation, Bohai Bay

1 Introduction

Planktonic Ciliophora, mainly including aloricate Oligotrichea and Tintinnina, comprise a major part of microzooplankton in various marine environments (Yang et al., 2008; Montagnes et al., 2010; Romano et al., 2021) and they play an important role in material recycling and energy flow through pelagic marine ecosystems (Pierce and Turner, 1992; Calbet, 2008). Similar to Bacillariophyceae, Dinophyceae and other microorganisms, the formation of cysts is of common occurrence among planktonic Ciliophora. Generally, encystment is considered as a protection strategy against ‘unfavorable environmental conditions’, and therefore playing an important role in the persistence and diffusion of population (Lennon and Jones, 2011; Verni and Rosati, 2011; Li et al., 2022).

Knowledge on cysts of planktonic Ciliophora in marine environments was limited, although Ciliophora serve as a key component of the plankton food web (Calbet, 2008). To the best of our knowledge, about 17 species of aloricate Oligotrichea and 61 species of Tintinnina (Belmonte and Rubino, 2019; Ganser et al., 2022; Yu et al., 2022) were recorded to produce cysts in marine environments. Assessing seasonal patterns of encystment will aid our ability to understand the mechanism of vegetative population dynamics. At present, few studies focused on the seasonal pattern of encystment in natural waters. Mass encystment of *Strombidium crassulum* (Reid, 1987) and *Cyrtostrombidium boreale* (Kim et al., 2002) were both found in spring. Mass encystment of *Strombidium capitatum* occurred in spring in Onagawa Bay and in autumn in Masan Bay (Kim et al., 2008). Encystment of Tintinnina was primarily a late summer/autumn event (Paranjape, 1980; Reid and John, 1981; Davis, 1985). These previous studies showed that the seasonal pattern might vary from species to species, and from region to region. More investigations are required to further study the seasonal patterns of encystment of planktonic Ciliophora.

Bohai Bay is a typical temperate semi-closed bay (mean depth of 12 m) on the continental shelf of the western Pacific Ocean.

Seasonal variation of cyst bank of planktonic Ciliophora was investigated in surface sediments of Bohai Bay (Yu et al., 2022). However, the seasonal patterns of the formation of cysts in the water column are not clear. Here, we reported annual variation of encystment through continuous recording the production rate of cysts in the waters, and explored the seasonal differences of encystment between aloricate Oligotrichea and Tintinnina in Bohai Bay. Consequently, assessing seasonal patterns of encystment will aid our ability to understand the mechanism of vegetative population dynamics.

2 Materials and methods

Ciliophora and the sedimentation of their cysts were investigated monthly from July 2019 to June 2020 at a fixed station (118°02' E, 39°02' N, with less human disturbance due to far from marine ranching and seaway) in Bohai Bay (Figure 1). Water depth was 8 to 9 m according to tide. Surface water temperature and salinity were measured by YSI Professional Plus instrument. For total Chlorophyll *a* (Chl *a*) determination, 250 ml of surface water was filtered (GF/F) and filters were frozen at -20°C. Chl *a* was extracted with 90% acetone at -20°C in the dark for 24 h. Chl *a* concentration was determined using a spectrophotometer.

For sampling of Ciliophora vegetative cells, 1 L of surface water was fixed (1% acid Lugol's iodine, Harris et al., 2000) and stored in dark until analysis. Samples were concentrated and examined following Utermöhl method (Utermöhl, 1958) under inverted microscope (Olympus IX71). Due to the lack of infraciliature, aloricate Oligotrichea were only identified to the genus level according to the morphological characteristics and the common species recorded in Bohai Bay. For Tintinnina, species were identified based on lorica morphology (Hada, 1937; Yin, 1952; Zhang et al., 2012).

Sinking Ciliophora cysts were collected with a cylindrical sediment trap of 56 cm in height and 14 cm in inner diameter

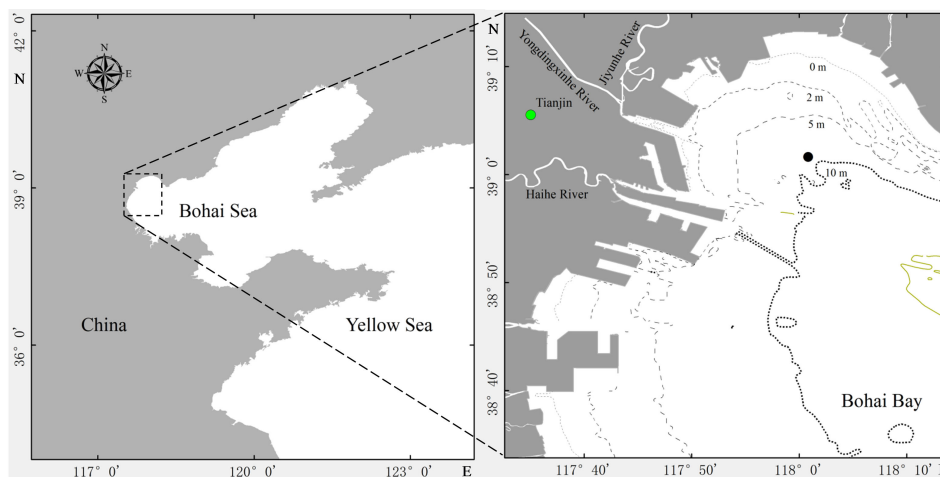


FIGURE 1
Position of the sampling station (●) in Bohai Bay with isobaths in meters.

(HYDRO-BIOS, Saarso). The trap was deployed at 7 m depth and retrieved after 70 h, except February 2020 when the trap was deployed for only 50 h because of a strong wind. After retrieval of the trap, supernatant water was gently siphoned after settling at 4°C for 12 h, and the trapped particles were frozen at -20°C until analysis (Figure 2).

Cyst samples were treated and analyzed in accordance with Ichinomiya et al. (2004). An aliquot (1 g wet weight) of the sample was suspended in ca. 20 ml of filtered seawater and sonicated for 30 s. The fraction between 20 to 125 µm was retained on a sieve, and diluted with 10 ml of filtered seawater. The sieved fraction was treated with glutaraldehyde (2.5% final concentration) and stored in a refrigerator for 24h. For the enumeration of cysts, 1 ml aliquot of the suspension (concentrated to 5 ml) was placed on a counting slide and the whole slide was examined under blue-light excitation (450 to 480 nm) using a fluorescence microscope (Olympus BX53). Cysts of aloricate Oligotrichea were classified according to the shape, size and the characteristics of cyst wall (Kim and Taniguchi, 1995; Kim et al., 2002; Ichinomiya et al., 2004; Agatha et al., 2005; Kim et al.,

2008). Tintinnina cysts were identified by the characteristics of lorica, since the cyst was inside the lorica. All the examinations were replicated 3 times.

Annual data were presented as line and bar charts by Grapher (Version 18, Golden Software Inc., USA). Mantel test was performed between cyst compositional and environmental data given geographic distance (9,999 permutations) using the vegan R software package.

3 Results

3.1 Annual cycle of water temperature, salinity and Chl *a* concentration

Water temperature was lowest in January (2.35°C) and peaked in July (29.60°C). In contrast, salinity was lower in the summer-autumn and highest in December (32.11). There were two peaks in Chl *a* concentration: one in March (5.53 µg/L), and another in July (15.29 µg/L, Figure 3).



FIGURE 2
Photographs of sediment trap after retrieval. (A): supernatant water was released through the faucet; (B): collecting bottle; (C): trapped particles.

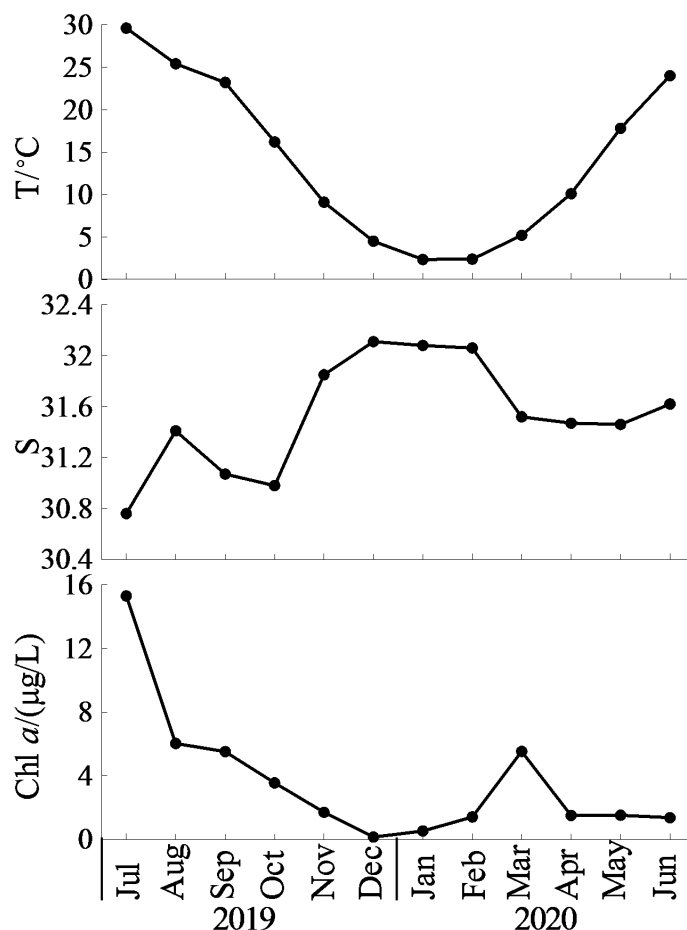


FIGURE 3

Monthly variation of surface water temperature (T), salinity (S), Chlorophyll a (Chl a) concentration.

3.2 Annual cycle of vegetative population of Ciliophora

The Ciliophora community was dominated by aloricate Oligotrichea, accounting for an average $71.4 \pm 32.1\%$ of the total abundance of Ciliophora. A total of eight taxa of aloricate Oligotrichea were identified, among which *Strombidium* spp. were dominant with an average abundance ratio of $90.3 \pm 10.0\%$ (Figure 4). Twenty-one Tintinnina species were recognized, and the species composition of Tintinnina exhibited obvious seasonal variation (Figure 5). The dominant Tintinnina were *Leprotintinnus simplex*, *Leprotintinnus nordqvisti* and *Tintinnidium primitivum*.

There were two peaks of aloricate Oligotrichea abundance: one in April (3266 ind/L), and another in August (6748 ind/L). One peak of Tintinnina abundance occurred in July (8440 ind/L). There were two peaks of total Ciliophora abundance: one in April (3266 ind/L), and another in July (8577 ind/L). The average contribution of Tintinnina to the total Ciliophora through the whole year was $28.6 \pm 32.6\%$, and they dominated over aloricate Oligotrichea only in May (72.8%), June (53.5%) and July (98.4%, Figure 6).

3.3 Cyst types

A total of 10 types of aloricate Oligotrichea cysts were identified based on their morphological characteristics under regular light and epifluorescent light.

Type I: similar to *Cyrtostrombidium boreale*, same as Type I in earlier study of Bohai Bay (Yu et al., 2022).

Type II: similar to *Strombidinopsis* sp., same as Type II in earlier study of Bohai Bay (Yu et al., 2022).

Type III: similar to *Strombidium biarmatum*, same as Type III in earlier study of Bohai Bay (Yu et al., 2022).

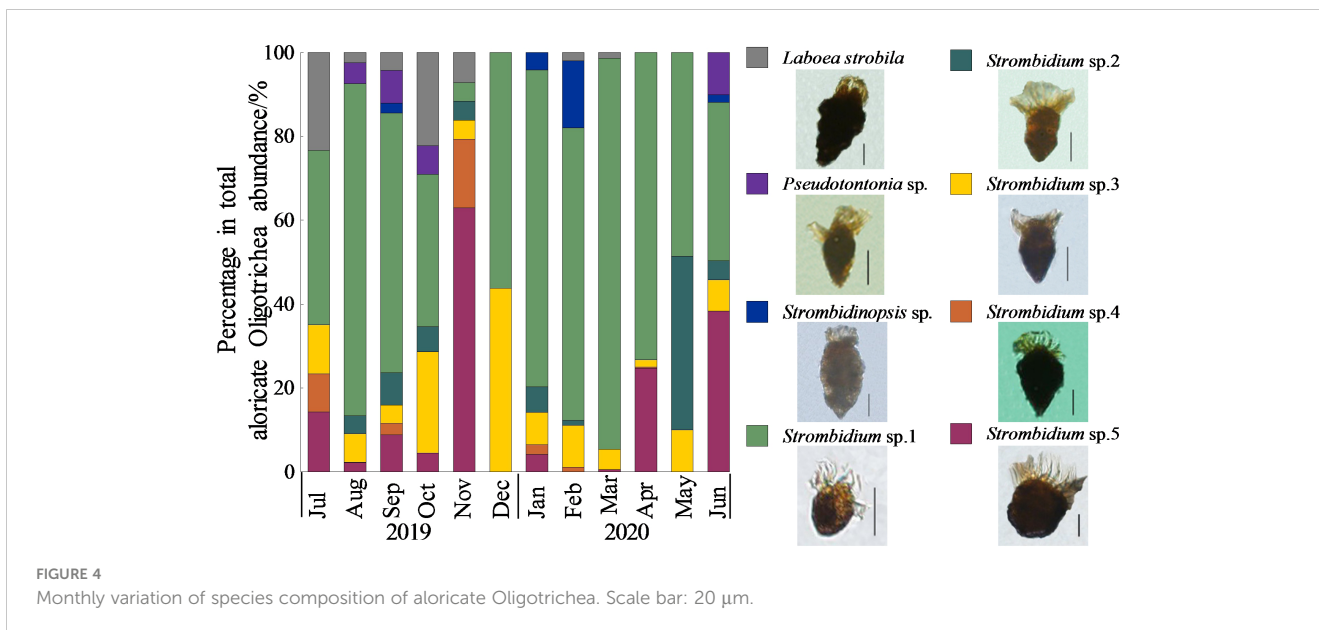
Type IV: similar to *Strombidium capitatum*, same as Type IV in earlier study of Bohai Bay (Yu et al., 2022).

Type V: similar to *Strombidium conicum*, same as Type V in earlier study of Bohai Bay (Yu et al., 2022).

Type VI: same as Type IX in earlier study of Bohai Bay (Yu et al., 2022).

Type VII: same as Type X in earlier study of Bohai Bay (Yu et al., 2022).

Type VIII: an elliptical shape with a short collar at one end, closed by a hyaline, elliptical papula (Figure 7A, a). Size varies



from 27–42 μ m in total length and 20–32 μ m in width. The papula was 7–8 μ m in height and 5–6 μ m in width. The cyst wall enclosing the cytoplasm of dense granular structure is single and hyaline.

Type IX: a nearly spherical body with a short collar at one end, closed by a hyaline papula (Figure 7B, b). Size varies from 45–54 μ m in total length and 41–49 μ m in width. The papula was 2–3 μ m in height and 10–11 μ m in width. The cyst wall is composed of two layers, and the inner cyst wall encloses the cytoplasm of dense granular structure.

Type X: a nearly spherical body with a short collar at one end, closed by a hyaline papula (Figure 7C, c). Size varies from 33–45 μ m in total length and 30–40 μ m in width. The papula was 2 μ m in height and 9–10 μ m in width. The cyst wall enclosing the cytoplasm of dense granular structure is single and hyaline.

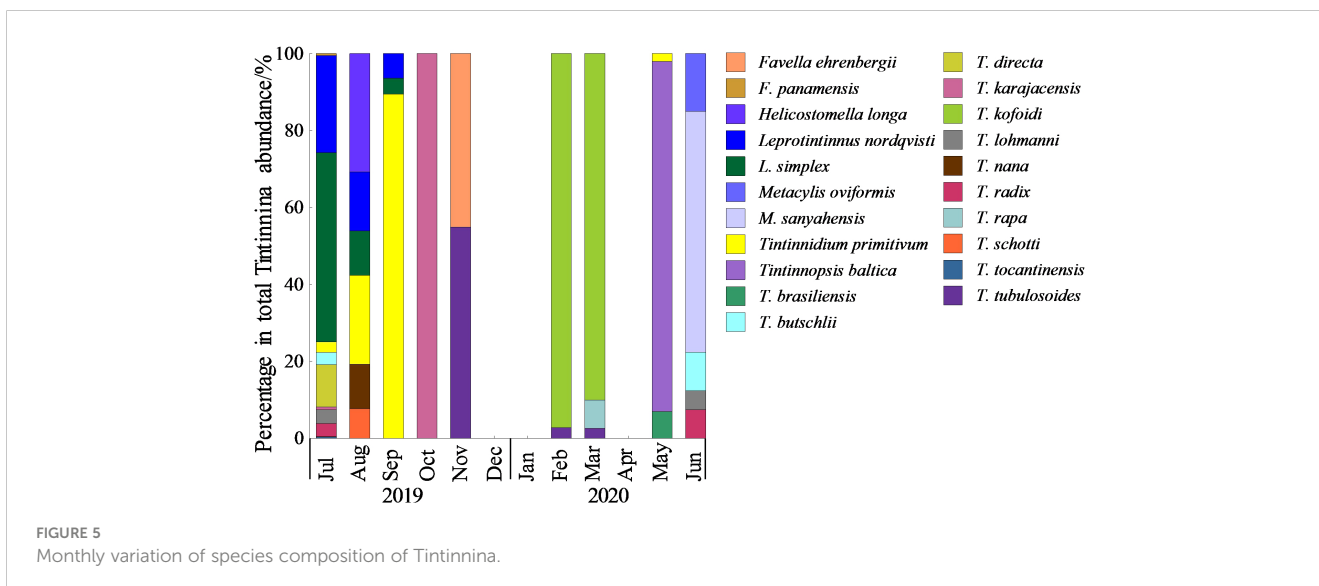
Only three types of Tintinnina cysts were recognized, namely, *Favella* sp., *Helicostomella longa* and *Tintinnopsis* sp.

Cyst of *Favella* sp.: same as Type VI in earlier study of Bohai Bay (Yu et al., 2022).

Cyst of *Helicostomella longa*: same as Type VII in earlier study of Bohai Bay (Yu et al., 2022).

Cyst of *Tintinnopsis* sp.: a spherical cyst, surrounded by a thin layer, within a lorica (Figure 7D, d). Size varies from 16–18 μ m in diameter. The cyst wall enclosing the cytoplasm of dense granular structure is hyaline.

The community of Ciliophora cysts was dominated by aloricate Oligotrichea cysts, with an average contribution of $96.2 \pm 6.0\%$ through the whole year. The dominant types were Type VIII, Type III, Type IX and Type X, and they accounted for $33.3 \pm 17.2\%$, $15.9 \pm 13.4\%$, $12.7 \pm 7.3\%$ and $11.5 \pm 10.4\%$ of the total cyst production through the whole year, respectively (Figure 8).



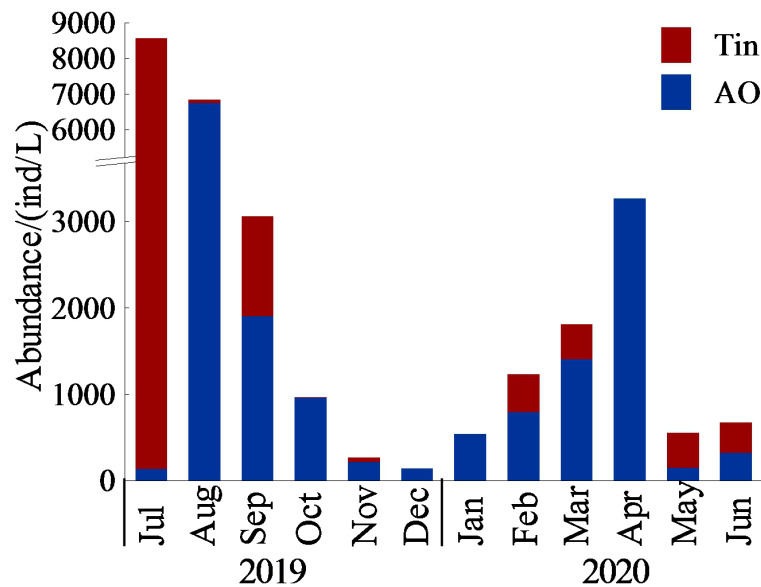


FIGURE 6 Monthly variation of aloricate Oligotrichea (AO) and Tintinnina (Tin) abundance.

3.4 Annual cycle of sedimentation of Ciliophora cysts

The production rate of aloricate Oligotrichea cysts ranged from 23.2×10^3 – 263.9×10^3 cysts $m^{-2} d^{-1}$ (on average $92.9 \times 10^3 \pm 72.5 \times 10^3$ cysts $m^{-2} d^{-1}$). The production rate was lowest in April and higher in January, July and October. The production rate of Tintinnina cysts ranged from 0 – 20.0×10^3 cysts $m^{-2} d^{-1}$ (on average $4.2 \times 10^3 \pm 6.5 \times 10^3$ cysts $m^{-2} d^{-1}$). No production of Tintinnina cysts was detected from January to June, and the production peaked in July and August. In the case of total Ciliophora cysts, the production rate ranged from 23.2×10^3 – 269.8×10^3 cysts $m^{-2} d^{-1}$ (on average

$97.1 \times 10^3 \pm 74.4 \times 10^3$ cysts $m^{-2} d^{-1}$), and the production exhibited the same annual trend with aloricate Oligotrichea cysts (Figure 9).

The production of different types of cysts exhibited different seasonal patterns, and could be divided into three classes. In Class I, the production peaked in one single season. For instance, the production of three types of Tintinnina cysts all peaked in summer (Figures 10A–C); the production of Type III and VII both peaked in autumn (Figures 10D, E); the production of Type VIII peaked in winter (Figure 10F). In Class II, the production peaked in two seasons. For example, the production of Type IV, V and X peaked in both summer and autumn (Figures 10G–I); the production of Type II and IX peaked in both autumn and winter

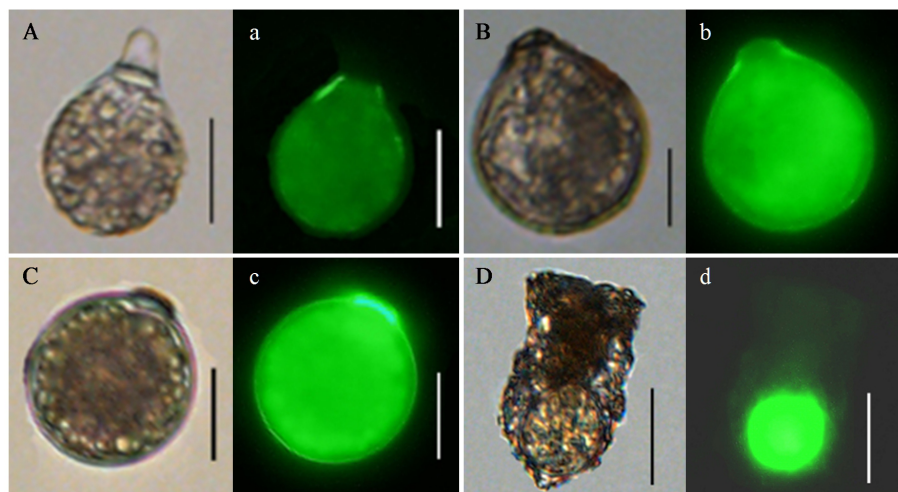


FIGURE 7 Microphotographs of Ciliophora cysts under regular light (A–D) and epifluorescent light (a–d). (A, a): Type VIII; (B, b): Type IX; (C, c): Type X; (D, d): cyst of *Tintinnopsis* sp. Scale bar: 20 μ m.

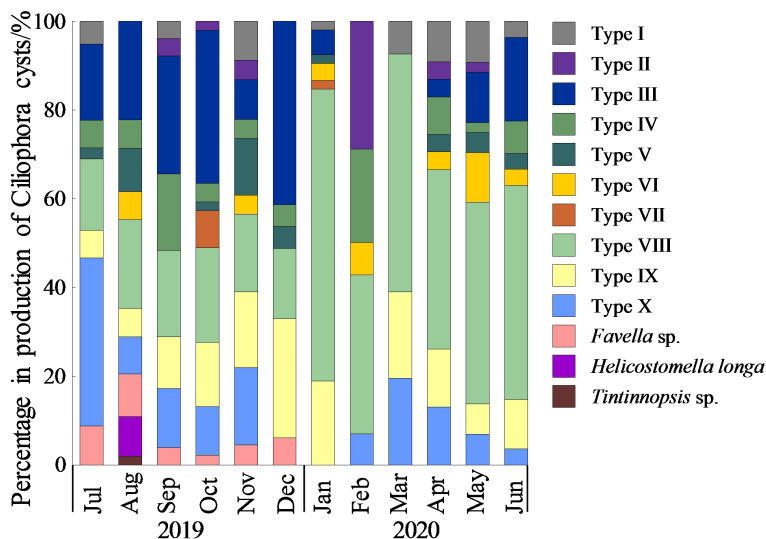


FIGURE 8 Monthly variation of species composition of Ciliophora cysts.

(Figures 10J, K). In Class III, the production occurred throughout the year without obvious seasonal pattern, such as Type I and VI (Figures 10L, M).

3.5 Relationships between production rate of Ciliophora cysts and environmental factors

The production rate of two types of aloricate Oligotrichea cysts, including Type IV and Type X, showed significant positive

correlations with water temperature and Chl *a* concentration. The production rate of Type III, Type IV and Type X showed a significant negative correlation with salinity. The production rate of Type II, tentatively identified as the cyst of *Strombidinopsis* sp., showed a significant positive correlation with the abundance of *Strombidinopsis* sp. (Table 1).

In Tintinnina cysts, the production rate of cyst of *Favella* sp. showed significant positive correlations with water temperature and Chl *a* concentration, and a significant negative correlation with salinity. The production rate of cyst of *Favella* sp. showed a significant positive correlation with the abundance of *Favella*

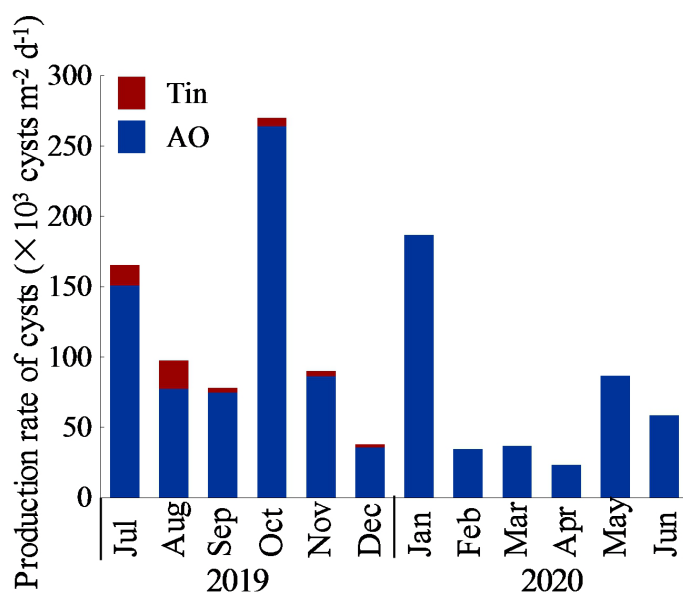


FIGURE 9 Monthly variation of production rate of aloricate Oligotrichea (AO) and Tintinnina (Tin) cysts.

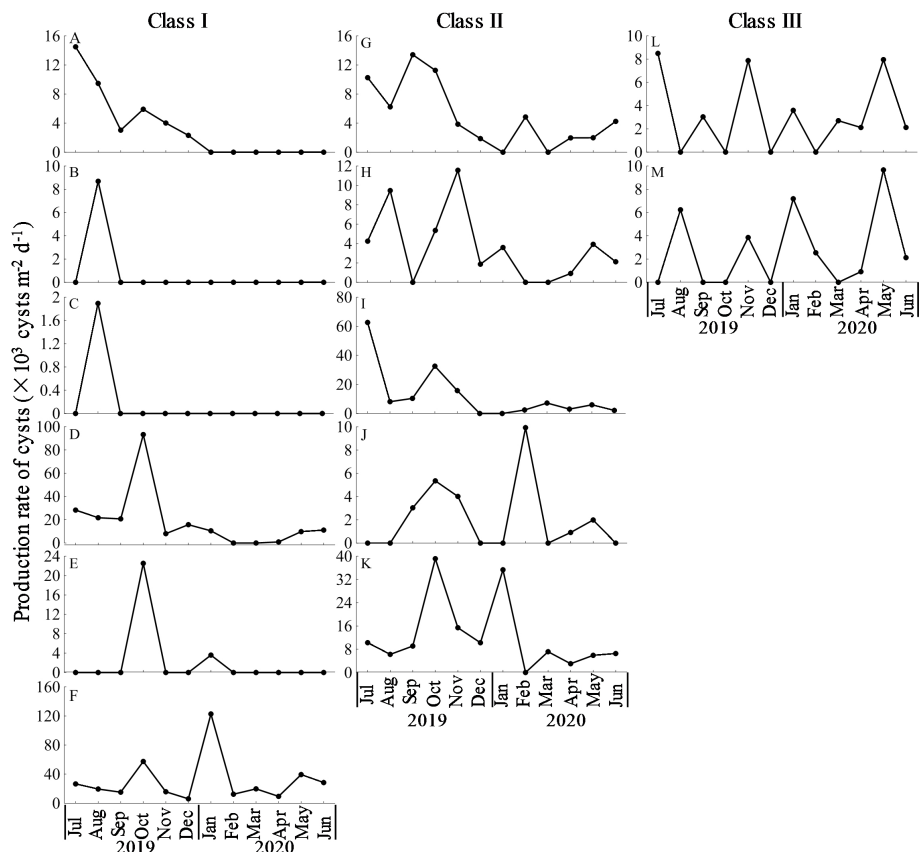


FIGURE 10
 Monthly variation of production rate of Ciliophora cysts. (A): cyst of *Favella* sp.; (B): cyst of *Helicostomella longa*; (C): cyst of *Tintinnopsis* sp.; (D): Type III; (E): Type VII; (F): Type VIII; (G): Type IV; (H): Type V; (I): Type X; (J): Type II; (K): Type IX; (L): Type I; (M): Type VI.

TABLE 1 Correlation between the production rate of aloricate Oligotrichea cysts and water temperature, salinity, Chlorophyll a (Chl a) concentration, and abundance of vegetative stages of aloricate Oligotrichea.

| Production rate of cysts ($\times 10^3$ cysts $m^{-2} d^{-1}$) | T (°C) | S | Chl a ($\mu g/L$) | Abundance of vegetative stages of aloricate Oligotrichea (ind/L) | | | | | | | | Total |
|--|---------------|-----------------|---------------------|--|--------|----------------|--------|--------|--------|--------|--------|--------|
| | | | | AO1 | AO2 | AO3 | AO4 | AO5 | AO6 | AO7 | AO8 | |
| Type I | 0.169 | -0.158 | 0.247 | -0.212 | -0.205 | -0.169 | -0.219 | -0.153 | -0.276 | 0.060 | -0.101 | -0.247 |
| Type II | -0.229 | 0.099 | -0.210 | 0.084 | -0.082 | 0.591** | -0.174 | -0.095 | -0.067 | 0.185 | -0.034 | -0.150 |
| Type III | 0.269 | -0.456* | 0.142 | 0.525** | 0.213 | -0.188 | 0.028 | 0.180 | 0.154 | -0.047 | -0.145 | 0.046 |
| Type IV | 0.495* | -0.559** | 0.419* | 0.479* | 0.347 | 0.091 | 0.065 | 0.299 | 0.099 | 0.298 | -0.090 | 0.102 |
| Type V | 0.139 | -0.022 | 0.027 | 0.209 | 0.107 | -0.221 | -0.046 | 0.223 | 0.322 | 0.066 | 0.029 | 0.004 |
| Type VI | -0.021 | 0.212 | -0.210 | -0.166 | 0.134 | 0.034 | 0.150 | 0.220 | 0.004 | 0.004 | -0.072 | 0.130 |
| Type VII | 0.005 | -0.319 | -0.042 | 0.732** | 0.026 | -0.126 | -0.135 | 0.017 | 0.359 | -0.214 | -0.105 | -0.084 |
| Type VIII | -0.173 | 0.125 | -0.129 | 0.149 | -0.126 | -0.068 | -0.206 | -0.056 | 0.051 | -0.058 | -0.232 | -0.211 |
| Type IX | -0.130 | -0.055 | -0.074 | 0.356 | -0.035 | -0.198 | -0.195 | -0.032 | 0.111 | 0.105 | -0.183 | -0.179 |
| Type X | 0.407* | -0.550** | 0.640** | 0.293 | -0.031 | -0.169 | -0.117 | -0.063 | 0.059 | 0.124 | -0.131 | -0.110 |
| Total | 0.182 | -0.374 | 0.211 | 0.529** | 0.040 | -0.198 | -0.179 | 0.063 | 0.167 | 0.036 | -0.264 | -0.159 |

*Significant difference at the 0.05 level; **Highly significant difference at the 0.01 level. T, Water temperature; S, salinity; Chl a, Chl a concentration. AO1, *Laboea strobila*; AO2, *Pseudotontonia* sp.; AO3, *Strombidinopsis* sp.; AO4, *Strombidium* sp.1; AO5, *Strombidium* sp.2; AO6, *Strombidium* sp.3; AO7, *Strombidium* sp.4; AO8, *Strombidium* sp.5. Bold font: correlation is significant.

TABLE 2 Correlation between the production rate of Tintinnina cysts and water temperature, salinity, Chlorophyll *a* (Chl *a*) concentration, and abundance of vegetative stages of Tintinnina.

| Production rate of cysts ($\times 10^3$ cysts $m^{-2} d^{-1}$) | T (°C) | S | Chl <i>a</i> ($\mu g/L$) | Abundance of vegetative stages of Tintinnina (ind/L) | | | | | | | | | | | | | | | | | |
|--|---------|---------|----------------------------|--|---------|---------|--------|--------|---------|---------|---------|---------|--------|---------|---------|---------|--------|---------|---------|--------|---------|
| | | | | Tin1 | Tin2 | Tin3 | Tin4 | Tin5 | Tin6 | Tin7 | Tin8 | Tin9 | Tin10 | Tin11 | Tin12 | Tin13 | Tin14 | Tin15 | Tin16 | Total | |
| <i>Favella</i> sp. | 0.505* | -0.495* | 0.652** | 0.172 | 0.541** | 0.509* | -0.169 | -0.118 | 0.567** | 0.567** | 0.600** | 0.538** | -0.248 | 0.590** | 0.567** | 0.584** | -0.159 | 0.119 | 0.600** | -0.214 | 0.585** |
| <i>Helicostomella longa</i> | 0.250 | -0.060 | 0.143 | -0.043 | -0.050 | 0.272 | -0.062 | -0.043 | -0.065 | -0.062 | -0.062 | -0.049 | -0.091 | -0.066 | -0.043 | -0.068 | -0.058 | 0.747** | -0.061 | -0.078 | -0.079 |
| <i>Tintinnopsis</i> sp. | 0.250 | -0.060 | 0.099 | -0.043 | -0.050 | 0.950** | -0.062 | -0.043 | -0.065 | -0.062 | -0.062 | -0.049 | -0.091 | -0.066 | 1.000** | -0.068 | -0.058 | -0.063 | -0.061 | -0.078 | -0.073 |
| Total | 0.586** | -0.458* | 0.639** | 0.119 | 0.430* | 0.679** | -0.183 | -0.128 | 0.442* | 0.472* | 0.472* | 0.427* | -0.268 | 0.461* | 0.572** | 0.455* | -0.172 | 0.478* | 0.473* | -0.231 | 0.450* |

*Significant difference at the 0.05 level; **Highly significant difference at the 0.01 level. T, Water temperature; S, salinity; Chl *a*, Chl *a* concentration. Tin1, *Favella ehrenbergii*; Tin2, *F. panamensis*; Tin3, *Helicostomella longa*; Tin4, *Tintinnopsis baltica*; Tin5, *T. brasiliensis*; Tin6, *T. butschlii*; Tin7, *T. directa*; Tin8, *T. karajacensis*; Tin9, *T. kofoidi*; Tin10, *T. lohmanni*; Tin11, *T. nana*; Tin12, *T. radix*; Tin13, *T. rapae*; Tin14, *T. schottii*; Tin15, *T. tocanthensis*; Tin16, *T. tubulosoides*. Bold font: correlation is significant.

panamensis. The production rate of cyst of *Tintinnopsis* sp. showed a significant positive correlation with the abundance of *Tintinnopsis nana* (Table 2).

Mantel test showed that there was no significant correlation between aloricate Oligotrichea cyst community and environmental factors. In the case of Tintinnina cysts, Mantel test showed that water temperature ($R=0.184$, $P=0.024$) and Chl *a* concentration ($R=0.331$, $P=0.025$) were the main environmental factor driving the annual change of Tintinnina cyst community (Table 3).

4 Discussion

In our study, a total of 13 types of Ciliophora cysts were identified by morphological characteristics using fluorescence microscopy. This method may lead to inaccuracy in species identification, which has been mentioned in previous papers (Yu et al., 2022). It would be necessary to apply molecular biology techniques, such as DNA metabarcoding, to ecological studies of Ciliophora cysts in the future.

Water depth was shallower than 10 m and only surface water was sampled in our study. No significant difference was observed in water temperature and salinity between surface and bottom layers in coastal waters of Bohai Bay (Shen, 1999; Song, 2009), and thus water temperature and salinity in surface water could be used as a reference for bottom layer in our study.

4.1 Production rate of Ciliophora cysts

To the best of our knowledge, investigations on the seasonal pattern of encystment of Ciliophora in natural waters were limited, except for few studies in Bedford Basin (Paranjape, 1980), the North Atlantic and North Sea (Reid and John, 1981), Conception Bay and Placentia Bay (Davis, 1985), the western English Channel (Reid, 1987), Onagawa Bay (Kim et al., 2002), Masan Bay (Kim et al., 2008) and north coast of Taiwan island (Chao et al., 2013). In early study, Paranjape (1980) collected water samples directly to observe tintinnid cysts. Tintinnid cysts were found from plankton samples by CPR (Continuous Plankton Recorder, Reid and John, 1981) or closing net hauled vertically (Davis, 1985). Later, sediment traps were applied to collect sinking cysts in the water, which are the most convenient tool to obtain qualitative information, such as production rate of cysts and timing of encystment (Kim et al., 2008).

In Bohai Bay, the production rate of Ciliophora cysts varied greatly from species to species, and the maximum value ranged from 1.9×10^3 cysts $m^{-2} d^{-1}$ (cysts of *Tintinnopsis* sp.) to 1.2×10^5 cysts $m^{-2} d^{-1}$ (Type VIII). Our data is similar to that in previous studies (Reid, 1987; Kim et al., 2002; Kim et al., 2008). The maximum production rate of cysts of *Strombidium crassulum* was almost 3.5×10^4 cysts $m^{-2} d^{-1}$ in the western English Channel (Reid, 1987). Peak production rate of cysts of *Cyrtostrombidium boreale* was 5.7×10^3 cysts $m^{-2} d^{-1}$ in Onagawa Bay (Kim et al., 2002). The production of cysts of *Strombidium capitatum* reached a maximum of 1.4×10^5 cysts $m^{-2} d^{-1}$ in Onagawa Bay, and about

TABLE 3 Mantel test between Ciliophora cyst community (measured as Bray–Curtis dissimilarity) and environmental factors.

| Environmental factor | Aloricate Oligotrichea cysts | | Tintinnina cysts | |
|----------------------|------------------------------|----------|------------------|--------------|
| | <i>P</i> | <i>R</i> | <i>P</i> | <i>R</i> |
| T (°C) | 0.255 | 0.038 | 0.024 | 0.184 |
| S | 0.068 | 0.118 | 0.086 | 0.139 |
| Chl <i>a</i> (µg/L) | 0.946 | -0.150 | 0.025 | 0.331 |

T, Water temperature; S, salinity; Chl *a*, Chl *a* concentration. Bold font: correlation is significant at the 0.05 level.

9.0×10^3 cysts $m^{-2} d^{-1}$ in Masan Bay (Kim et al., 2008). The maximum production of two dominant cysts was 1.8×10^4 and 2.4×10^4 cysts $m^{-2} d^{-1}$, respectively in north coast of Taiwan island (Chao et al., 2013).

4.2 Seasonal differences of encystment between aloricate Oligotrichea and Tintinnina

The seasonality of encystment could be speculated by observing sinking cysts in the water periodically and continuously. Most previous studies focused on the seasonal pattern of encystment of single species (Paranjape, 1980; Davis, 1985; Reid, 1987; Kim et al., 2002; Kim et al., 2008). Reid and John (1981) recorded seasonal occurrence of several types of Tintinnina cysts. Chao et al. (2013) concerned about the seasonal dynamics of the entire Ciliophora cyst community (including 5 types of aloricate Oligotrichea cysts and one type of Tintinnina cysts). In this study, we carried out annual change study in production of 10 types of aloricate Oligotrichea cysts and 3 types of Tintinnina cysts, and compared seasonal differences of encystment between aloricate Oligotrichea and Tintinnina in Bohai Bay.

In Bohai Bay, the encystment of *Favella* sp. mainly occurred in July–August, with minor occurrence from September to December. The sedimentation of cysts of *Helicostomella longa* and *Tintinnopsis* sp. were only found in August. Our data is consistent with previous studies that encystment of Tintinnina was primarily a late summer/autumn event (Paranjape, 1980; Reid and John, 1981; Davis, 1985). The abundance of cysts of *Helicostomella subulata* was high in September, October and November in Bedford Basin (Paranjape, 1980). Tintinnid cysts were most abundant in the late summer/autumn in the North Atlantic and North Sea (Reid and John, 1981). Cysts of *Acanthostomella norvegica* were mainly found in October and November in Placentia Bay (Davis, 1985). Cysts of these species, which may function as “overwinter stages”, could help Tintinnina to survive in “unfavorable” environmental conditions of winter (Paranjape, 1980; Reid, 1987). The encystment in late summer/autumn, which could provide a means of hibernating through the winter, may have important implications for population persistence (Lennon and Jones, 2011).

In terms of aloricate Oligotrichea, encystment was found at all seasons and the seasonal patterns varied among species in Bohai Bay. Most species could encyst in late summer/autumn, which is

similar to *Strombidium capitatum* whose encystment peaked in autumn in Masan Bay (Kim et al., 2008). These cysts formed in late summer/autumn, similar to Tintinnina cysts, could help ciliates survive “unfavorable” conditions in winter (Müller and Wünsch, 1999). In addition, there were three types of aloricate Oligotrichea (Type II, Type VIII and Type IX) with mass encystment of cysts in January/February. The mass encystment in winter, to our knowledge, has not been recorded in previous investigations. Further studies should be carried out to figure out the role of the winter cysts in population persistence of Ciliophora.

Also, the production of two types of aloricate Oligotrichea (Type I and Type VI) occurred throughout the year without obvious seasonal pattern in our study. Cyst formation of these ciliates maybe part of the regular life cycle and does not require a specific environmental stimulus (Verni and Rosati, 2011). Combination of field investigations and laboratory culture experiments would be needed to confirm this assumption.

4.3 Encystment of Ciliophora and environmental factors

Ciliophora cysts are probably produced in response to environmental stresses, like food shortage, water temperature and salinity, tidal rhythm, etc. However, factors acting in natural environments are still largely unknown (Verni and Rosati, 2011; Kamiyama, 2013). In this study, we found production rate of several types of cysts (Type IV, Type X and *Favella* sp.) exhibited a significant positive correlation with water temperature, and a significant negative correlation with salinity. This suggested that the variation of temperature and salinity might play important roles in cyst production of Ciliophora, which was consistent with previous studies. The encystment of *Cyrtostrombidium boreale* in early spring may be triggered by a rapid increase of temperature in Onagawa Bay (Kim et al., 2002). High temperature could impede cyst production of *Pelagostrombidium fallax* during the summer in freshwater Lake Constance (Müller and Wünsch, 1999). Chao et al. (2013) suggested that variations of salinity might excite Ciliophora to enhance encystment in a coastal ecosystem of subtropical western Pacific. All of these speculations were based on data observed in natural environments, and have not been tested experimentally. Culture experiments in laboratory are needed for a better understanding of the relationships between temperature and salinity and encystment of Ciliophora.

In our study, most species exhibited no significant correlation between cyst production and the presence of vegetative stages, which was consistent with the study in the Mar Piccolo of Taranto (Rubino and Belmonte, 2021). On the one hand, this absence of correspondence was due to identification difficulties of cysts (which cysts belong to which species). A combination of microscopic morphology observation and single-cell sequencing would assist in linking the identity of cysts to vegetative stages (Rubino and Belmonte, 2021). Another reason for this lack of connection was that vegetative stages were active in the past and temporarily absent at the time of cyst study (Rubino and Belmonte, 2021). This time-lag between vegetative stages and cysts was found in both laboratory culture (Müller, 1996) and field investigation (Müller and Wünsch, 1999; Kim et al., 2002; Kim et al., 2008). Cyst abundance of the freshwater ciliate *Pelagostrombidium fallax* began to increase after the vegetative cells reached a high abundance value in laboratory culture (Müller, 1996). Highest production of *Limnostrombidium viride* cysts (Müller and Wünsch, 1999), *Cyrtostrombidium boreale* cysts (Kim et al., 2002) and *Strombidium capitatum* cysts (Kim et al., 2008) were all recorded shortly after maximum development of the motile population in field investigation. A combination of laboratory culture experiment and field investigation is needed to find out the correspondence between vegetative stages and cysts, and explore the mechanism of vegetative population proliferation on Ciliophora encystment.

5 Conclusions

For now, studies on encystment of planktonic Ciliophora in natural environments were limited. Our study indicates that the production of Ciliophora cysts exhibited obvious seasonal variation, and there were obvious seasonal differences of encystment between aloricate Oligotrichea and Tintinnina in Bohai Bay. The variation of temperature and salinity might play important roles in encystment of various types of Ciliophora. Culture experiments in lab would be required for a better understanding of the role of environmental factors on Ciliophora encystment.

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Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

YY: ciliate taxonomy and counting, data analysis, writing original draft. WZ: conceptualization, ciliate taxonomy, writing original draft. ZL: field sampling, figure drawing, writing original draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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