Cyst formation: an important mechanism for the termination of *Scrippsiella trochoidea* (Dinophyceae) bloom

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Received June 10, 2006; accepted in principle October 18, 2006; accepted for publication December 7, 2006; published online January 19, 2007

Communicating editor: K.J. Flynn

A sediment trap study was conducted at Daya Bay, South China Sea, to investigate the relationships between encystment and population dynamics of *Scrippsiella trochoidea* from December 1999 to January 2001. A dense bloom of *S. trochoidea* occurred during the study period from August to September 2000, with the maximum cell number of $3.18 \times 10^4$ cells mL$^{-1}$. Two morphotypes of cysts, one with a thick calcareous wall (calcified cyst) and another without the obvious calcareous cover (non-calcified cyst), were observed during this investigation. The morphological and excystment characteristics of these two cyst types were studied as well. Mass encystments of *S. trochoidea*, with the maximum of $3.05 \times 10^5$ cysts m$^{-2} \text{d}^{-1}$ for calcified cyst, and $1.54 \times 10^7$ cysts m$^{-2} \text{d}^{-1}$ for non-calcified cyst, coincided with the maximum abundance of the vegetative cells. Encystment caused the transfer of a total of $2.24–4.49 \times 10^8$ cells m$^{-2}$ vegetative cells from the water column to the sea bottom during the bloom and resulted in a considerable loss of the bloom population. High assemblages of cysts of *S. trochoidea* were detected in the surface sediments as well. This rich ‘seed bed’ in the surface sediments caused by the high efficiency of encystment after blooms acting as a benthic reservoir for future vegetative population, together with the short dormant period (15–26 days) and high germination rate (50–90%), may explain the repeated occurrence of *S. trochoidea* blooms in Daya Bay.

**INTRODUCTION**

Approximately 13–16% living dinoflagellates have been documented to have the ability of forming cysts in their life cycles (Head, 1996). Formation of resting cysts has various ecological functions such as genetic recombination, dispersal, seedling or regulation of seasonal succession of dinoflagellates (Dale, 1983). Cyst formation allows them alternatively to inhabit either the water column as motile cells or the benthos as cysts and is usually explained as an adaptation to adverse environmental conditions (Dale, 1983; Fryxell, 1983; Piester and Anderson, 1987).

The importance of resting stage of dinoflagellates in bloom dynamics has been demonstrated by many species. Cyst germination provides the inoculum for a new planktonic population (Anderson, 1998; Rengefors, 1998; Kremp and Anderson, 2000). Massive encystments decrease the population density of vegetative cells in the water column, thus contributing to the collapse of the blooms (Matsuoka and Takeuchi, 1995; Kremp and Heiskanen, 1999).

Encystment has been well studied using cultured dinoflagellate populations (von Stosch, 1973; Binder and Anderson, 1987; Piester and Anderson, 1987; Brackburn *et al.*, 1989; Park and Hayashi, 1993; Blanco,
These studies showed that culture media depleted of nutrients induce sexuality and cyst formation; endogenous processes are also involved in encystment, with temperature and light irradiance influencing the rate of encystment to some extent.

_Scrippsiella trochoidea_ is a cosmopolitan dinoflagellate species in coastal waters and widely distributes in China (Wang et al., 2004a). Blooms of this species were reported in Japan, Korea and China (Adachi, 1972; Park et al., 1989; Ishikawa and Taniguchi, 1996; Qi et al., 2004). _Scrippsiella trochoidea_ is reported from a wide variety of environmental conditions and is able to proliferate in a broad range of temperature (5–30°C) and salinity (5–55) (Kim and Han, 2000). This species displays a high efficiency for producing cysts, which helps to explain its success in the neritic environment, where cyst accumulation in the sediments acts as a benthic reservoir of the vegetative population (Sgrosso et al., 2001; Wang et al., 2004b).

Daya Bay is a shallow embayment, located in the northeast part of the South China Sea. It is an important aquacultural area. Nutrient enrichment has been greatly accelerated by human activities since 1990s, and harmful algal blooms occurred frequently in this area (Wang et al., 2006). From the first occurrence of _S. trochoidea_ bloom in September 1998, blooms of this species have occurred almost every year in the bay (SOA, 1998–2004; Qi et al., 2004).

Though _in situ_ cyst formation, germination and population dynamics of _S. trochoidea_ have been observed in Japan and Korea, most of these studies focused on the influence of encystment on bloom initiation (Ishikawa and Taniguchi, 1994; 1996; Kim and Han, 2000). Kremp and Heiskanen (Kremp and Heiskanen, 1999) observed encystment of _S. hangoei_ in coastal northern Baltic Sea and suggested that encystment was an important factor in bloom termination. In order to investigate the relationships between cyst formation and population dynamics of _S. trochoidea_, an annual survey was undertaken by sediment trap in Daya Bay from December 1999 to January 2001. During the period of survey, a dense bloom of _S. trochoidea_ covering about 30 km² occurred from August to September 2000. In the course of the investigation, an interesting phenomenon of the disappearance of the calcareous outer layer of _S. trochoidea_ cyst was found, which has hitherto only been observed in laboratory studies on other _Scrippsiella_ species (Olli and Anderson, 2002). The present study discusses the results of seasonal changes in population dynamics and cyst formation of _S. trochoidea_ in order to elucidate the role of encystment in bloom

**METHOD**

**Site description**

Daya Bay, situated at 114°29′–114°49′ E, 22°30′–22°52′ N, is a semi-enclosed shallow embayment with an area of ca. 650 km² and maximum water depth of about 20 m and is separated into several small sub-basins.

The climate of Daya Bay is mild, with wet subtropical to tropical weather. The study site is located in the Aotou area, in the north-west part of the bay, which is the most extensive aquacultural site. Nutrient concentrations are much higher in the bay than that in other regions, and the maximum dissolved inorganic nitrogen, dissolved inorganic phosphorus and dissolved silicate concentrations in 1998 were 23.2, 2.4 and 126.2 μM, respectively (Wang et al., 2006). The map of Daya Bay with the location of the sediment trap is illustrated in Fig. 1.

![Fig. 1. Location of sediment trap array station in Daya Bay.](https://academic.oup.com/plankt/article-abstract/29/2/209/1556707)
Sediment trap and surface sampling
The sediment trap was set to collect settling materials from December 1999 to January 2001. The trap consisted of a cylindrical PVC collector (height 35 cm, diameter 7 cm, height/diameter ratio 5), a flexible tube holder, a subsurface buoy, a surface buoy marker and an anchor. The trap was moored at 1.0 m from the sea bottom (the depth at the deployment location was about 6 m). Settled materials were collected monthly. An additional trap was moored in 1–28 September 2000 during the bloom of *S. trochoidea*, and the contents of the trap were emptied once a week. The settled material was suspended in 2 L seawater, and two 500 mL subsamples of this suspension were taken out for counting and for germination, respectively. Material for counting was fixed with 4% buffered formalin, and material for germination experiments was stored in the dark at 4°C. The fixed subsample was concentrated to 50 mL by sedimentation.

Surface sediment samples were taken monthly with a TFO (Tokyo University Fisheries Oceanography Lab.) gravity corer at the same site and the same time as trap samples. The top 2 cm of each sediment core was cut and transferred to a beaker with filtered seawater, then fixed in buffered formalin.

The samples (both trap samples and surface sediment samples) were gently sonicated (50 Hz) for 30 s. The sonicated material was thereafter sieved through 125 and 20 μm metallic screens to remove larger particles and detritus, and the slurry remaining on the 20 μm screen was washed into a beaker and collected into a plastic tube to a final sample volume of 10 mL with filtered seawater. In samples containing a lot of heavy sand particles in the sieved slurry, the materials from the 20 μm screen were then transferred to a watch glass. By panning on the watch glass, cysts and light particles were separated from heavier grains and the suspension was collected into a plastic tube. This process was repeated for three to four times until all residues except sand particles were collected. All suspensions were pooled together and concentrated to 10 mL by sedimentation.

For observation, 0.1–0.5 mL aliquot of sample was placed on a 1 mL counting chamber and diluted with appropriate distilled water. Observation was under an inverted light microscope (Olympus IX70) at magnifications of ×100–400. Only living cysts, which have not germinated and contain a lot of cellular inclusions, were counted. At least triple observations were conducted for each sample until a minimum of 200 dinoflagellate cysts were observed. Mean daily cyst fluxes (cysts m⁻² day⁻¹) and cysts per cubic cm (cysts cm⁻³) were calculated for trap samples and surface sediments, respectively.

Water samples
One thousand millilitres of water samples for phytoplankton observation were collected in surface waters (0.5 m) monthly from December 1999 to June 2000, and every other 3 days from 27 June 2000 to 31 January 2001 and fixed immediately with neutralized formalin to a final concentration of 4%. The fixed water samples were finally concentrated to 20 mL by sedimentation. Phytoplankton species were counted from aliquots of 0.2–1.0 mL subsample using a microscope (Olympus IB41) at magnifications of ×100–400. The phytoplankton abundance was presented as cell numbers per millilitre (cells mL⁻¹).

Hydrographic parameters
Hydrographic properties including water temperature, salinity and dissolved oxygen (DO) were measured monthly using a DO and conductivity meter (YSI 85, YSI Incorporated, USA). Transparency was measured with a Secchi disk at the same time.

Cyst germination experiments
About 0.2–0.5 mL of non-fixed trap sample collected during the bloom was placed in a Sedwick–Rafter chamber, and living intact cysts were isolated by micropipetting under an inverted microscope (Olympus IX70). The isolating and culturing of cysts were completed within 2 days after sampling. Cysts were inoculated into the individual wells of 96-well tissue culture plates filled with f/10 medium (Guillard and Ryther, 1962) without silica, then cultured at 100 μmol photons m⁻² s⁻¹ cool-white illumination under 12:12 h light : dark (L:D) cycle in 20°C. Plates were inspected for cyst germination every day in the first 20 days and once a week until the end of experiments (75 days). The germination ratio was calculated as a ratio of cumulative excystment to the number of inoculated cysts. Fifty calcified cysts and 50 non-calcified cysts were isolated for germination experiment, respectively.

To obtain cultured *S. trochoidea*, calcified cysts from trap sample collected on 7 September 2000 were isolated and incubated in 96-well-culture plate. Upon germination, a single motile cell was isolated with a micropipette and a clonal culture was established. Culture stocks were maintained in glass test tubes filled with 30 mL f/2 medium minus silica, and incubated under standard conditions.

Newly formed cysts were harvested from the culture and incubated in three temperatures (15°C, 20°C and 25°C). For each batch of germination experiments,
24 cysts were isolated. The experiment was run for 150 days; germination of the cysts was checked daily at the beginning of 25 days and every 4–7 days at the rest of experiment.

RESULTS

Hydrographic parameters

Figure 2 illustrated the seasonal changes in water temperature, salinity, DO and transparency. Water temperature went through an annual cycle characterized by a minimum of 14.3°C (28 January 2001) and a maximum of 31°C (31 July 2000). Temperatures were usually over 20°C, started to decrease in November, dropped to less than 20°C in December and then maintained between 15°C and 20°C until the end of March. Salinities remained almost stable from 26.5 to 32.1. DO was from 4.2 mg L⁻¹ (31 August 2000) to 7.9 mg L⁻¹ (27 February 2000), with a mean value of 6.3 mg L⁻¹. High DO values were recorded in cold winter months, whereas low values in hot summer months.

Cyst characterization

Two cyst morphotypes of *S. trochoidea* were observed in this study, one with the thick calcareous wall and another without obvious calcareous wall (Fig. 3). These two cyst morphotypes were identified as cysts of *S. trochoidea* by germination experiments.

The calcified cysts (25.5–42.5 mm long excl. spines, 23.5–34.6 mm wide excl. spines, 20 specimens) were dark greyish in colour and covered by numerous calcareous spines, with one or two bright red accumulation bodies (Fig. 3a). Germinations of the calcified cysts occurred successively after 2–4 weeks of dormant period in laboratory conditions. The archeopyle was a circular split at one end of the cyst forming a cap-shaped operculum which remains attached to the main body of the cyst (Fig. 3b). After germination, the accumulation red body was still inside the empty cyst (Fig. 3b and d). The thick calcareous cover together with the calcareous spines dissolved (Fig. 3c and d) after processing by a palynological procedure using hydrochloric acid and hydrofluoric acid (Cho and Matsuoka, 2001).

The non-calcified cysts were of the same shape and size (23.5–37.9 μm long, 20.4–32.6 μm wide, 20 specimens) as the calcified ones, although they lacked an evident calcareous thick wall and spines. One or two bright red accumulation bodies and a lot of fat and starch granules were presented in the cyst body (Fig. 3e and f). After germination, the cysts produced *S. trochoidea* vegetative cells (Fig. 3 g) when incubated in f/10 medium. The shapes of non-calcified cysts were unchanged after palynological procedure.

Bloom development and cyst deposition

Peak abundance of *S. trochoidea* was observed from August to September 2000 when a dense bloom of this species occurred (Fig. 4a). The bloom started at the beginning of August reached the maximum at the end of August and then declined in the middle of September (Fig. 4b). The maximum abundance of 31 760 cells mL⁻¹ occurred on August 31, and this high abundance lasted about 10 days. During the peak stage of the bloom, the proportions of *S. trochoidea* in the phytoplankton community reached almost 100%.

Fig. 2. Seasonal changes in hydrographic parameters. (a) Water temperature and salinity. (b) DO and transparency.
Fig. 3. Light micrographs of cysts and vegetative cell of *S. trochoidea*. (a) Living calcified cyst, with dark grey calcareous outer layer and numerous spines; (b) empty calcified cyst; (c) living calcified cyst treated by palynological technique, showing a obvious red accumulation body; (d) empty calcified cyst treated by palynological technique, with the red body still inside the cyst; (e) living non-calcified cyst, with similar size and shape with calcified cyst; (f) empty non-calcified cyst; (g) vegetative cell. Arrow indicates the archeopyle in empty cyst.

Fig. 4. Numbers of vegetative cells of *S. trochoidea*. (a) Seasonal changes from December 1999 to January 2001. (b) Cell numbers during the bloom from 1 August to 30 September.

Fig. 5. Cyst fluxes of *S. trochoidea*. (a) Seasonal changes from December 1999 to January 2001 and (b) during the bloom from 1 August to 30 September.
was high only during the bloom and shortly after the bloom, with little if any development in other months (Fig. 5a).

Figure 5b illustrated fluxes of calcified cyst and non-calcified cyst during the bloom. Cyst flux in August was relatively low. As the development of the bloom, massive cyst formations occurred, and the maximum fluxes ($3.05 \times 10^5$ cysts m$^{-2}$ day$^{-1}$ for calcified cyst, $1.54 \times 10^7$ cysts m$^{-2}$ day$^{-1}$ for non-calcified cyst) coincided the decline stage of the bloom between September 7 and 14. High cyst fluxes were maintained until the end of September. Cysts produced during the bloom were largely non-calcified ones, whose concentration was about 20–50 times higher than that of calcified cysts.

**Cysts in surface sediments**

The composition of the cyst assemblages showed few differences between the surface sediments and sediment traps. Twenty-four and 25 morphotypes of cysts were observed in samples from the sediment trap and surface sediments, respectively. Cysts of *S. trochoidea* were the most predominant species, and its percentages were over 50% in all samples, with cysts of other species, such as *Gonyaulax spinifera*, *Lingulodinium polyedrum*, *Pyroplaxus steinii*, *Phaeopolykrikos hartmannii*, *Polykrikos schwarzii*, *Protoperidinium leonis* and *P. subinerme*, also observed frequently in this study.

Cyst abundances in surface sediments ranged from 195 cysts cm$^{-3}$ in June 2000 to 7000 cysts cm$^{-3}$ in September 2000 for calcified cysts and from 145 cysts cm$^{-3}$ in January 2000 to 78 900 cysts cm$^{-3}$ in September 2000 for non-calcified cysts (Fig. 6). Abundance showed similar seasonal changes to cyst flux in sediment trap. Cysts occurred sporadically before the bloom except for a low peak of calcified cyst in March 2000. However, peak abundances of both calcified and non-calcified cysts occurred in September coincided with the bloom, but decreased rapidly in October.

**Cyst dormancy and germination**

The germination of calcified and non-calcified cysts isolated from trap samples are shown in Fig. 7a. Non-calcified cysts started to germinate at day 6 after incubation, whereas calcified cysts on day 15. Considering the period of trap setting (7 days during the bloom), the mandatory dormancy periods of non-calcified and calcified cysts were 6–13 and 15–22 days, respectively. The final germination ratio of non-calcified cysts was 40% after 27 days of incubation, and a bit higher in calcified cysts, 50% within 38 days of incubation.

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**Fig. 6.** Cyst numbers of *S. trochoidea* in surface sediments.

**Fig. 7.** Germination ratios of calcified and non-calcified cysts of *S. trochoidea*. (a) Germination of cysts isolated from the trap samples incubating in 20°C. (b) Germination of cysts obtained from the batch culture incubating under three incubation temperatures.
Only calcified cysts were observed in the culture of *S. trochoidea*. The dormancy period varied from 15 to 26 days and was extended at lower temperature (Fig. 7b). The final germination ratios (80–90%) were evidently higher compared with cysts collected from field samples and showed no significant differences between the three incubation temperatures. Germination proceeded quickly at 20°C and 25°C and was completed within 60 days. However, at 15°C, after the first excystment at day 26, no other cysts germinated until day 75 (Fig. 7b).

### DISCUSSION

#### Cyst sedimentation and the bloom

Cyst formation of *S. trochoidea* occurred throughout the year in this study, although it was apparent that the major production of cysts occurred during and following the peak bloom period. Mass cyst production also followed the peak number of vegetative cell in the same strain of *S. trochoidea* grown in nutrient-replete cultures in laboratory studies (Cao et al., 2006). Thus, the same phenomenon was observed in both batch cultures and natural sea waters (Heaney et al., 1983; Heiskanen, 1993; Monstresor et al., 1998; Kim and Han, 2000; Olli and Anderson, 2002; Joyce and Pitcher, 2004). Olli and Anderson (Olli and Anderson, 2002) discussed this issue when they observed high encystment frequency (almost 100%) of *S. lachrymosa* shortly following the peak in cell numbers under high-nutrient batch cultures growing in full f/2 medium and suggested that the onset of encystment was related to the proportion of vegetative cell yield to the total amount of nutrient, and once this critical nutrient concentration was reached, encystment occurred. Though nutrients are usually sufficient in Daya Bay, nutrient limitation occurred during the bloom and peak abundance of phytoplankton (Wang et al., 2006). The massive encystment during the bloom in this study might be caused by nutrient limitation per cell as suggested by Olli and Anderson (Olli and Anderson, 2002). Further laboratory study showed that moderate nitrogen limitation promoted cyst formation of *S. trochoidea* (Cao et al., 2006).

Cyst formation apparently played an important role in the termination of *S. trochoidea* blooms. Encystments transformed a large part of the vegetative population into non-dividing cysts, comprising calcified and non-calcified cysts. Encystment caused a maximum loss of $4.49 \times 10^8$ cells m$^{-2}$ vegetative cells from the water column during the bloom (Table I). Recent study by Kremp and Parrow (Kremp and Parrow, 2006) showed that the asexual resting stage commonly occurred in *S. hangoei* and suggested that the asexual resting stage might be a more common feature of dinoflagellate life cycles than previously thought. We do not know if the cysts observed in this study were sexual or asexual products. There were still $\sim 2.24 \times 10^8$ cells m$^{-2}$ vegetative cells settling down the sea bottom through encystment during the bloom even if all cysts were haploid. The importance of encystment in bloom termination was also reported for a *S. hangoei* bloom in the coastal northern Baltic Sea; encystment caused 40% loss of the entire vegetative population in that case (Kremp and Heiskanen, 1999).

High fluxes of calcified cysts were observed in both winters (December 1999 and January 2001), which indicated that the high winter cyst yield might be a more regular annual circle other than the result of resuspension. However, there were few vegetative cells recorded in surface water in winter. Because phytoplankton data were only recorded per litre of surface water, the results may not give a full representation of the appearance of certain species in the water column. In contrast, cysts in sediment trap are those formed by motile cells in the entire upper water column within the sampling interval. Thus, the cyst study could be considered as giving a more integrated record of the phytoplanktonic population. Moreover, the winter temperature in Daya Bay is over 15°C, still suitable for the growth of *S. trochoidea* (Deng et al., 2004; Xu et al., 2004). High winter cyst production revealed that vegetative cells of *S. trochoidea* were present in the water column, though they were not detected by surface phytoplankton analyses. The high winter cyst production was supposed to be caused by decreasing daylength coupled with still-warm temperature conditions, as suggested by Sgrosso et al. (Sgrosso et al., 2001) after studying the impact of these variables on the formation of calcareous cysts. The late summer–autumn peaks of calcareous cyst

<table>
<thead>
<tr>
<th>Time interval</th>
<th>$\Delta t$</th>
<th>$C_{cal}$</th>
<th>$C_{non-cal}$</th>
<th>$C_{cumul}$</th>
<th>$N_{cumul}$</th>
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<tr>
<td>September 1–7</td>
<td>7</td>
<td>17</td>
<td>528</td>
<td>3815</td>
<td>3815–7630</td>
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<tr>
<td>September 7–14</td>
<td>7</td>
<td>31</td>
<td>1536</td>
<td>10969</td>
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<td>September 14–21</td>
<td>7</td>
<td>197</td>
<td>895</td>
<td>7644</td>
<td>7644–15288</td>
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<tr>
<td>September 1–21</td>
<td>21</td>
<td>62</td>
<td>986</td>
<td>22428</td>
<td>22428–44856</td>
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$I_t$, time interval (days); $C_{cal}$, $C_{non-cal}$, flux of calcified cysts and no-calcified cysts ($\times 10^8$ cysts m$^{-2}$ day$^{-1}$); $C_{cumul}$, cumulative cyst flux ($\times 10^8$ cysts m$^{-2}$) during the time period; $N_{cumul}$, cumulative number of vegetative cells ($\times 10^8$ cells m$^{-2}$) moved by cyst formation from the water column during the time period, based on the assumption that all cysts were by either sexual or asexual reproduction.
production recorded in the Gulf of Naples (Monstresor et al., 1998), and early autumn peaks of *S. trochoidea* encystment in Onagawa Bay in Japan (Ishikawa and Taniguchi, 1994, 1996) also showed the linkage between decreasing daylength coupled with still-warm temperature on the encystment process.

Several methodological aspects deserve further consideration. (i) Time interval to empty the trap. Trap samples were usually collected and fixed once a month. Grazers most probably entered the trap and ingested cysts; the cyst flux might thus have been underestimated. However, most data used in this study for analyses and discussion were obtained during the bloom, when materials were sampled and fixed weekly. (ii) Resuspension of cysts from the sediments. Resuspension is definitely a factor that may influence measurement of the cyst flux. However, its effects in the present study are not likely significant since the trap was set in an enclosed bay. Furthermore, wind speeds remained lower during the bloom. (iii) Time discrepancy between planozygote and resting cyst. The planozygotes need 3 days to 1 week to lose motility and become cysts, subsequently sinking out of the surface layer (Heiskanen, 1993). Given the resultant temporal discrepancy, the most realistic estimate of the total cyst number is obtained by the cumulative cyst production (Kremp and Heiskanen, 1999). For this study, the cumulative cyst production was calculated by summing up the cyst fluxes during the bloom.

**Cysts in surface sediments and bloom re-occurrence**

As planozygotes and resting cysts may be transported with currents, cysts seldom accumulate in the site where they formed. Cysts have similar hydrodynamic properties to fine silt particles, and higher cyst abundance occurs in sediments with higher clay contents (Dale, 1983). The sediments in Daya Bay are mainly composed of grey–black silt rich in organic matters, which are appropriate for cyst deposition. As a result, high numbers of *S. trochoidea* cysts are recorded at the surface of the sediment after the bloom.

Cysts in sediments could provide a rich ‘seed bed’ for initiation of new blooms (Pati *et al*., 1999; Joyce *et al*., 2005). Although Daya Bay is a shallow inshore area, light cannot penetrate to the bottom because of the low transparency (less than 5% surface light attained 3.5 m). Therefore, cysts in sediments cannot germinate before they are resuspended to the upper water column. Owing to the typhoon season between August and September in the northern South China Sea, storms cause strong turbulent mixing in the surface sediments and resuspend cysts. Thus, cysts have the ability to germinate and to be recruited successfully to the vegetative population after undergoing their short dormant period. The newly germinated cells propagate quickly under the conditions with rich nutrients and favourite temperature in Daya Bay. Meanwhile, the monsoon and storms in this season are mostly southwestward, which facilitates the accumulation of vegetative cells from southwest offshore areas into northeast inshore areas, and result in the occurrence of discolouration blooms in inshore inner bay. A rich ‘seed bed’ in the surface sediments caused by the high efficiency of encystment after blooms, resuspension of cysts and immediate excystment, together with other characteristic of *S. trochoidea*, such as the ability to tolerate a wide variety of environmental conditions (Kim and Han, 2000), likely all contribute to the repeated occurrence of blooms of this species in Daya Bay.

The top 2 cm sediments correspond to material sediments over a 1-year interval, considering the silt accumulation rate of 0.94–1.42 cm year$^{-1}$ recorded in Daya Bay (Huang *et al*., 1999). However, there was a rapid decline in cyst numbers just 2 months after the bloom. Two factors might account for this drop: (i) Disturbance of surface sediments. The study area is in a shallow embayment where disturbance occurs frequently by aquaculture and other human activities. Furthermore, the activity of benthic organisms might cause the mixture of upper sediments. (ii) Grazing by benthic animals. Benthic grazers such as shellfishes and invertebrates may ingest cysts, especially the non-calcified cysts.

**Cyst characterization and dormancy**

Two morphotypes of cysts of *S. trochoidea*, calcified and non-calcified cysts, were observed in our study. Olli and Anderson (Olli and Anderson, 2002) observed two types of cysts (they referred to thick- and thin-wall cysts, respectively) produced in batch cultures of *S. cf. lachrymosa* as well. The thin wall cysts were smaller in size and were produced in the later stages when the growth medium was depleted of nutrients. They suggested that the thin-wall cyst should be the true hypnozygote due to the presence of a large red accumulation body and long mandatory dormancy period. The non-calcified cyst in our study has morphological characteristics similar to the thin-wall cyst in their study, i.e. the overall shape, the size and the presence of accumulation body, and exhibits a dormancy period of 6–13 days. The non-calcified cysts were hypothesized by us to be true resting cysts, based on the fact that they had a dormancy period, compared with the temporary cysts which lack a true dormancy and function as short-term
survival stages in response to sudden adverse conditions. However, Kremp and Parrow (Kremp and Parrow, 2006) recently investigated both sexual and asexual resting cysts of *S. hangoei* with identical morphology by comparative measurement of the DNA contents of resting cysts and vegetative cells and found that up to 95% of resting cysts were haploid reproduction. It is hence hard to say whether cysts (both calcified and non-calcified cysts) observed in the present study were in sexual or asexual stage without additional detailed examination. Meanwhile, the non-calcified cysts are never observed in cultures in laboratory conditions, and their formation and characteristics are still unknown. Further investigations are needed to fully understand the encystment conditions and physiological characteristics of non-calcified cyst.

Many dinoflagellate cysts have an endogenously controlled mandatory period lasting from a few hours (Pfiester, 1977) to ca. 1 year (Perez et al., 1998). The dormancy period for *S. trochoidea* calcified cysts in this study ranged from 15 to 26 days, a period comparable with that found by Binder and Anderson (Binder and Anderson, 1987) but shorter than the dormancy period (about 60 days) of the strain of *S. trochoidea* isolated from the Korean southeastern coast (Kim and Han, 2000). Our results indicated that the dormancy period was affected by the incubation temperatures, being extended at 15°C. In previous study, Binder and Anderson (Binder and Anderson, 1987) indicated that the mandatory dormancy period of *S. trochoidea* was not affected by temperature. In contrast, Anderson (Anderson, 1980) found that the length of the dormant period of cysts of *Alexandrium tamarense* (formerly *Gonyaulax tamarensis*) was not affected by the incubation temperatures, being extended at 15°C. In previous study, Binder and Anderson (Binder and Anderson, 1987) indicated that the mandatory dormancy period of *S. trochoidea* was not affected by temperature. In contrast, Anderson (Anderson, 1980) found that the length of the dormant period of cysts of *Alexandrium tamarense* (formerly *Gonyaulax tamarensis*) was influenced by storage temperature. In that study, rapid germination was observed at 22°C but dormancy was longer at 5°C.

In our study, germination occurred rapidly in high temperatures (20°C and 25°C), whereas germination ratios showed a lag period at 15°C. It has been reported that germination of mature cysts, once transferred to favourable conditions, occurred synchronously within a few days (Binder and Anderson, 1990; Kim and Han, 2000; Kremp, 2001; Olli and Anderson, 2002). The longer lag period at 15°C in this study (about 50 days) might be explained by: (i) low incubation temperature was not opportune for germination of *S. trochoidea* cyst isolated form the subtropic South China Sea, which resulted in asynchronous occurrence of excystments, and (ii) the dormancy period might be not longer than 26 days, which was the dormancy length of the first germinated time. As *S. trochoidea* cysts commonly appeared in cultures even during the exponential growth phase (Cao et al., 2006), the cysts used in our experiments could have been produced over a relatively wider time lag and the first germinated cyst was possibly one of the first to be produced.

**ACKNOWLEDGMENTS**

This research project was supported by National Natural Science Foundation of China: Nos U0633006 and 40473046.

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