Journal of Plankton Research Vol.22 no.7 pp.1311–1327, 2000

Factors regulating germination of resting cysts of the spring bloom dinoflagellate *Scrippsiella hangoei* from the northern Baltic Sea

Anke Kremp and Donald M.Anderson[1]

Department of Ecology and Systematics, Division of Hydrobiology, P.O. Box 17, 00014 University of Helsinki, Finland and [1]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

Abstract. The role of cyst germination as a factor in bloom initiation was investigated for the dinoflagellate *Scrippsiella hangoei* (Schiller) Larsen from the northern Baltic Sea. This species blooms in very cold, often ice-covered waters, and is responsible for a significant fraction of the production in that region. Dormancy, temperature, oxygen and light were studied as factors potentially controlling the germination of *S.hangoei* resting cysts. Laboratory-stored and field-collected cysts began to germinate in December following a mandatory dormancy period lasting 6 months. Germination after this maturation interval was maximal when temperatures were within a 0–9°C 'window'. Mandatory dormancy is therefore the primary factor regulating the timing of germination in this species, as temperatures in the natural environment normally fall within this range at the time when *S.hangoei* cysts deposited the preceding year have matured. Non-optimal temperatures, darkness and low oxygen conditions all maintain a state of quiescence in the cysts. Cysts could germinate in darkness, but the rate of excystment was significantly higher in the light. Likewise, excystment was completely inhibited in anoxic conditions and was reduced under severe hypoxia, with normal germination under moderate hypoxic concentrations. Temporary exposure to high sulfide concentrations permanently reduced germination potential, indicating that *S.hangoei* cysts have low resistance to oxygen deficiency. Prolonged periods of anoxia at the sediment surface, as frequently occurs in the study area, might reduce the size of the viable cyst pool and thus, alter the magnitude of the inoculum for *S.hangoei* bloom initiation. Together, these internal and external regulatory factors play important roles in the bloom dynamics of this important dinoflagellate.

Introduction

Many dinoflagellate species produce resting cysts as a part of their sexual life cycle (Dale, 1983). There are many benefits from this strategy, as outlined by Wall (Wall, 1975), including the evolutionary or adaptive value of genetic recombination, but survival through adverse conditions and the subsequent re-population of the water column in highly seasonal environments are often viewed as the most important and immediate benefits. Although the 'seed bank' hypothesis (Wall, 1975; Steidinger, 1975) has been confirmed as a general strategy for bloom initiation, several studies have revealed that germination physiology and behavior differ greatly among species [e.g. (Anderson *et al.*, 1983; Pfiester and Anderson, 1987; Ishikawa and Taniguchi, 1996; Anderson, 1998; Rengefors, 1998)]. It is therefore necessary to document the species-specific endogenous and exogenous requirements for germination if we are to understand the bloom dynamics of a particular dinoflagellate species in a specific habitat. Here, we report such information for *Scrippsiella hangoei.*

One of the factors that must be characterized is the duration of the mandatory dormancy period through which newly formed cysts must proceed before they are physiologically capable of germination. The duration of this 'maturation' process is highly variable among species, ranging from 12 h to 12 months [e.g.

(Pfiester, 1977; Pfiester and Anderson, 1987; Perez *et al.*, 1998)], and it can differ within a single species when storage conditions change (Anderson, 1980; Montresor and Marino, 1996). Once maturation is completed, cysts remain in a resting state, termed 'quiescence', until external factors are favorable for germination. Temperature is considered to be a primary regulatory factor that can prolong quiescence when it is non-optimal, or break it when it is 'permissive' for excystment [e.g. (Huber and Nipkow, 1923; Anderson and Morel, 1979; Bravo and Anderson, 1994)]. However, external and internal constraints can reduce, and even prevent germination when the ambient temperature is within the permissive range. Light (or specifically the lack of light) is an important factor in this regard. Though total inhibition of the germination process in the absence of light is reported for only few species (Binder and Anderson, 1986; Anderson *et al.*, 1987; Rengefors and Anderson, 1998), darkness clearly reduces the germination rate in many others (Anderson *et al.*, 1987; Cannon, 1993; Rengefors and Anderson, 1998). Oxygen has been specified as an absolute requirement for germination in nearly all species so far examined (Anderson *et al.*, 1987; Rengefors and Anderson, 1998), but temporary exposure to anoxic conditions does not seem to affect ultimate germination success as cysts stored for months, and even years, in anoxic sediments can be successfully germinated after re-exposure to oxygenated water (Keafer *et al.*, 1992). An endogenous, annual clock has been documented as an additional regulatory mechanism that will take cysts out of quiescence and back into dormancy, such that germination will not occur even though the cysts are mature and the external environment is favorable (Anderson and Keafer, 1987; Perez *et al.*, 1998; Rengefors and Anderson, 1998).

Scrippsiella hangoei (Schiller) Larsen is one of the most important species of the phytoplankton community in the coastal areas of the northern Baltic Sea during winter and spring (Larsen *et al.*, 1995). The ongoing eutrophication in the Baltic Sea provides favorable growth conditions for the vernal phytoplankton, but this production leads to a decreasing oxygen supply in both deep and shallow areas and thus, represents a serious threat to the benthic biota (Cederwall and Elmgren, 1990; Bonsdorff, 1992). The environmental, physiological and behavioral factors that regulate the *S.hangoei* life cycle processes, which lead to an alternation between the pelagic and the benthic environment, are particularly important to understand as much of the production and fate of the biomass during spring is governed by this single species (Lignell *et al.*, 1993; Heiskanen and Kononen, 1994).

Cyst formation of *S.hangoei* and its effect on bloom termination has been described by Heiskanen (Heiskanen, 1993) and Kremp and Heiskanen (Kremp and Heiskanen, 1999). The present study examined the factors which could potentially control cyst germination and, in turn, affect bloom initiation of this species. Dormancy requirements, as well as the effects of temperature and light, were investigated. Furthermore, a laboratory study was conducted to determine whether oxygen deficiency in surface sediments could affect the germinability of *S.hangoei* cysts. The seasonal germination patterns of cysts collected from a well oxygenated and a temporarily anoxic sampling site were investigated to complement the laboratory results.

Method

Study area

The field work was conducted in the vicinity of the Tvärminne Zoological Station, southeast of the Hanko peninsula near the entrance of the Gulf of Finland, Baltic Sea (Figure 1). The study area is strongly influenced by the motions of different water masses, due to changing meteorological conditions. Salinities are typically 5–6 psu in surface waters and 6–8 psu in bottom waters (Niemi, 1975). During winter, the area is usually covered by ice for 2 months on average. The sampling station, Storgadden (S; 59°47′N, 23°19′E), is situated in the open sea zone in a relatively large basin with an average depth of 50 m (Heiskanen and Leppänen, 1995). It is characterized by the periodical occurrence of low oxygen concentrations in bottom waters, resulting in oxygen depleted surface sediments. A second sampling site, representing well mixed and oxygenated sediments (Viitasalo and Katajisto, 1994), Tvärminne Storfjärden (SF; depth 34 m), is located in the archipelago zone at the northern end of a furrow stretching from the open Gulf of Finland towards the archipelago.

Planktonic population

Phytoplankton samples were taken monthly from the water column at the Storgadden station from May 1995 to June 1996 using a Limnos water sampler.

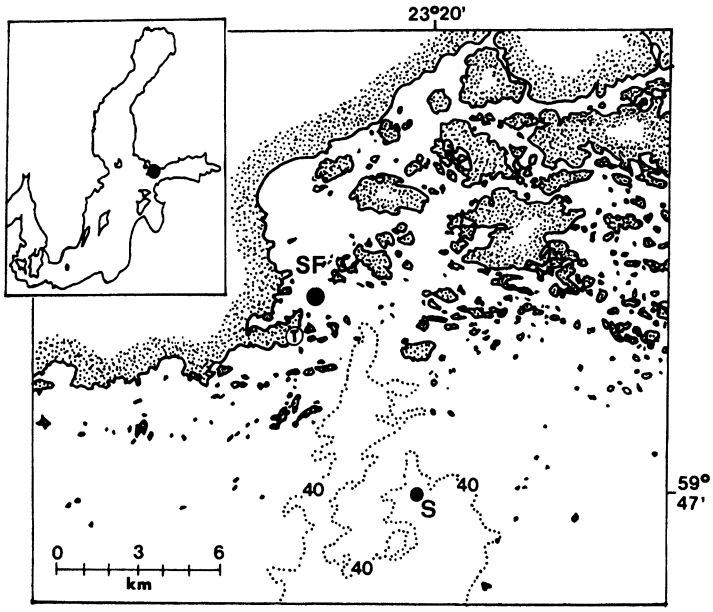

Fig. 1. Location of the study area on the SW coast of Finland, Baltic Sea (small map). The sampling stations Storgadden (S) and Storfjärden (SF) are marked. The depth contour of 40 m is depicted by a dotted line.

Integrated samples (0–20 m) were preserved with acid Lugol's solution and counted under an inverted microscope (Leitz Labovert) after 24 h of sedimentation in 50 ml settling chambers. Water temperatures were obtained by CTD.

Dormancy and seasonal germination pattern

For dormancy experiments, newly formed cysts were collected during the termination of the vernal *S.hangoei* bloom in May and June, 1995 and 1996, using a cylindrical sediment trap moored at 20 m depth at Storgadden. After 3 weeks of trap deployment, the settled material was collected, sieved through a 120 μm and a 50 μm net to remove larger zooplankton and phytoplankton, and then through a 20 μm net. Collected material was then suspended in sea water and transferred to a 1 l plastic container in 1995. This nearly pure suspension of *S.hangoei* cysts was stored in the laboratory at 3°C in total darkness. No precautions were taken to prevent cyst germination by anoxic conditions in the storage container. In 1996, the cyst suspension was divided into 10 ml portions and stored in cryovials under anoxic conditions, according to the method of Rengefors and Anderson (Rengefors and Anderson, 1998). Sub-samples for monthly germination experiments were cleaned by sonication (Branson 2200 bath, 5 min) and sieved on a nylon net, keeping the 20–50 μm fraction in Whatman GF/F filtered sea water.

Sub-samples of the cyst suspension were placed into five replicate wells of a 24-well tissue culture plate and incubated at 3°C and 100 $\mu E\ m^{-2}\ s^{-1}$ under a 12:12 L:D (light:dark) cycle for 1 and 2 weeks in 1995/96, and 3 and 5 weeks in 1996/97. Chlorophyll fluorescence in the cysts (Anderson and Keafer, 1985) was examined in 1995/96 in a separate portion of the prepared cyst suspension, prior to incubation, using an epifluorescence microscope (Leitz Fluovert) with blue light excitation. At least five replicate sub-samples of the cyst preparation were fixed with Lugol's solution, immediately after processing, for microscopic determination of the initial percentage of empty cysts. Empty and full cysts were counted in the culture wells immediately after termination of the experiment. The percentage of germination was determined from the increase in the fraction of empty cysts.

Sediment samples were taken monthly using a Limnos gravity corer at the Storgadden and Storfjärden sampling stations, between January and December 1998, to characterize the seasonal germination pattern of field-collected cysts. The flocculent surface layer of the sediment core was vacuumed into a sampling container and suspended in sea water. A sub-sample was sonicated and sieved, and prepared culture plates were placed at 3°C (12:12 L:D). Cyst germination was quantified after 4 weeks of incubation, as described above.

Temperature

A temperature gradient bar (Watras *et al.*, 1982) was used to study the effect of temperature on germination, following the methods of Bravo and Anderson (Bravo and Anderson, 1994). This device consisted of an aluminium bar with insertion holes for test tubes. The bar was heated at one end and cooled at the

other, providing a gradient of temperatures. Replicate sets of test tubes (four replicates for every temperature), each containing a 25 ml suspension of mature cysts (collected by sediment traps in spring 1996 and matured under cold and dark conditions) in filtered sea water, were incubated for 7 weeks at 15 temperatures ranging from 0 to 18°C. Temperatures in the test tubes were measured at the beginning and at the end of the experiment. Germination was calculated from the difference between the initial (10 replicate test tubes processed and counted at the beginning of the experiment) and final number of empty cysts.

Oxygen conditions and light

The effects of anoxia and hypoxia on *S.hangoei* cyst germination were tested by incubating mature cysts (obtained from sediment trap material from May 1996) in filtered sea water which was made anoxic or hypoxic. Oxygen levels used at the six different treatments (five replicates) were: normoxic (11–13 mg O_2 l^{-1}), hypoxic (2, 1.5 and 0.5 mg O_2 l^{-1}), anoxic (0.001 mg O_2 l^{-1}) and anoxic with 30 mmol l^{-1} NaS (0.01 mg O_2 l^{-1}). The preparation of different oxygen concentrations in the filtered sea water (buffered with Na_2CO_3 and set to pH 7.8) followed the methods of Lutz *et al.* (Lutz *et al.*, 1992) as modified by Rengefors and Anderson (Rengefors and Anderson, 1998). To one set of anoxic vials, 1 ml of 1 mol l^{-1} NaS was added. For initial cyst abundance, 15 of the prepared vials were fixed with Lugol's. The remaining vials were incubated in the dark for 21 days at 3°C. One additional series of the normoxic and anoxic treatments was exposed to light for the incubation period. After the incubation, all vials were measured for oxygen content using a gas chromatograph, and the initial and final oxygen concentrations in the vials were calculated (Lutz *et al.*, 1992; Rengefors and Anderson 1998).

One vial of each treatment was used to test the germination success of the surviving cysts. Replicate 2 ml sub-samples were transferred into wells of a tissue culture plate and incubated at 3°C (12:12 L:D cycle). Full and empty cysts were recorded every third day for 40 days. The contents of the other vials were preserved with Lugol's for cyst counts. Germination was determined from the increase in empty cysts. Results were analyzed using the Student's *t*-test.

Results

Planktonic population

The phytoplankton survey 1995/1996 showed that vegetative cells of *S.hangoei* remained at undetectable levels during summer and autumn when water temperatures were in excess of 6°C. The first vegetative cells were observed in January 1996 (Figure 2), but cell concentrations remained low (10^3–10^4 cells l^{-1}) until March when a dense bloom formed under the ice and the abundance increased to the maximum of 2.3×10^5 cells l^{-1}. Thereafter, cell numbers decreased and remained low during April until after the break-up of the ice cover in early May, when a new bloom was established (1.3×10^5 cells l^{-1}) which then declined in the warming water at the beginning of June.

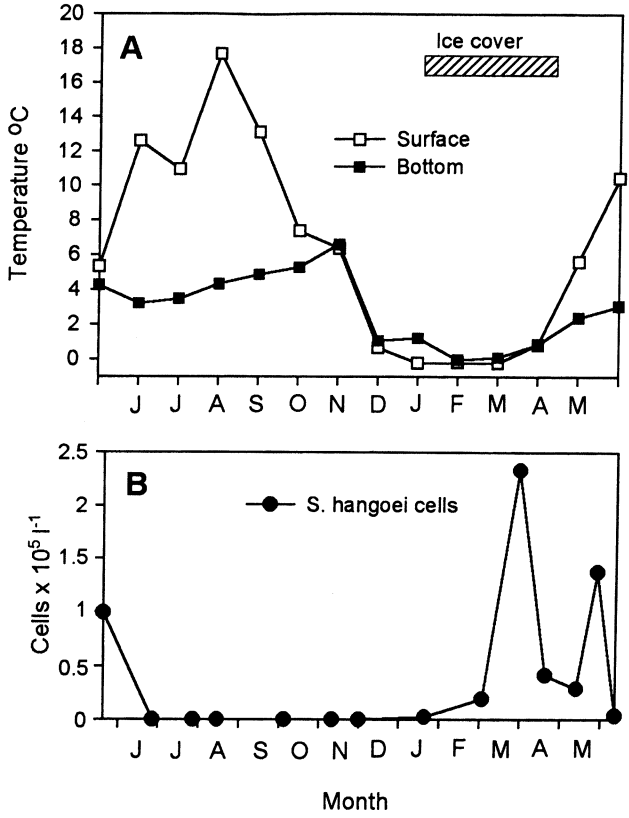

Fig. 2. (**A**) Temperatures in surface and bottom waters and (**B**) the vegetative population (cells l^{-1}) of *Scrippsiella hangoei* at Storgadden sampling station from May 1995 to June 1996.

Dormancy and seasonal germination pattern

Resting cysts of *S.hangoei* that were formed in late May or early June 1995 and stored in darkness at 3°C started to germinate in December. After 7 days of incubation, 58% of the cysts had germinated (Figure 3). The loss of Chl *a* autofluorescence in these cysts soon after the transfer to the storage container (Figure 3) indicated that the cysts were newly formed and that chlorophyll was degrading as they entered dormancy. The reappearance of fluorescence in a major fraction of the cysts in December (70%) was evidence of the synthesis of photosynthetic pigment and thus, the physiological readiness of the cysts for germination (Anderson and Keafer, 1985).

The numbers of empty cysts compared with initial levels increased significantly after 7 days of incubation during experiments conducted from December to March ($P < 0.05$, Student's *t*-test). Longer incubation times (14 days) resulted in significantly higher final numbers of empty cysts compared with the 7-day incubation interval ($P < 0.05$). Between February and May (Figure 3), however,

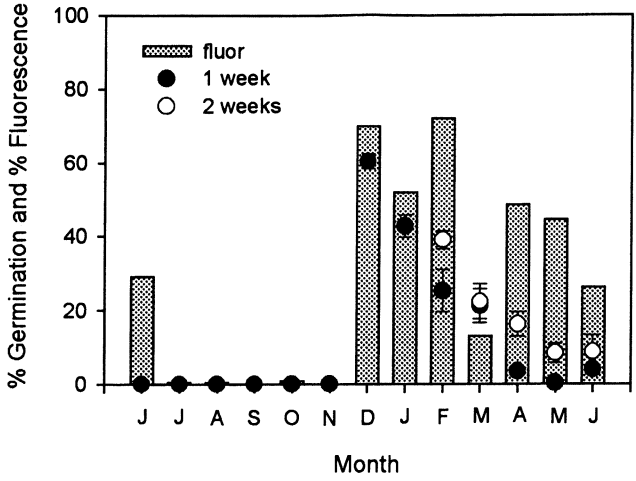

Fig. 3. Germination pattern in *Scrippsiella hangoei* cysts. Germination percentage (after 1 and 2 weeks of incubation in 3°C, 100 μE m^{-2} s^{-1} and 12:12 L/D, circles) and Chl *a* fluorescence (% of fluorescent cysts, columns) collected by sediment traps during May and June 1995 and stored under constant laboratory conditions (3°C and darkness). Error bars indicate the SD.

germination decreased steadily, irrespective of the incubation time, until the end of spring.

The cysts originating from the subsequent spring bloom in 1996 and stored in anoxic conditions started to germinate after 6 months (Table I). However, these cysts needed a considerably longer incubation time, and reached much lower ultimate germination frequencies (a maximum of 26% of the cysts germinated after 5 weeks of incubation in December), than the cysts from the previous year which had been supplied with oxygen (when sampled) during storage (Figure 3).

The germination frequency of cysts collected from the constantly well oxygenated sediment surface at the Storfjärden sampling station during 1998

Table I. Germination of *Scrippsiella hangoei* resting cysts collected after the spring bloom 1996 (SD in parentheses). Cysts were stored under anoxic conditions in the laboratory at 3°C in the dark and incubated at 3°C, 100 μE m^{-2} s^{-1}, 12:12 L/D under normal oxygen conditions for 3 and 5 weeks, respectively

Date	% Germination	
	3 weeks	5 weeks
September	0.4 (± 0.2)	0.2 (± 0.1)
October	0.3 (± 0.2)	0.7 (± 0.3)
November	<0.1 (± 0.03)	0.3 (± 0.2)
December	2.5 (± 0.3)	26.4 (± 1.7)
January	0.4 (± 0.2)	2.0 (± 0.5)
February	0.8 (± 0.2)	9.1 (± 0.5)
March	0.7 (± 0.2)	0.7 (± 0.3)

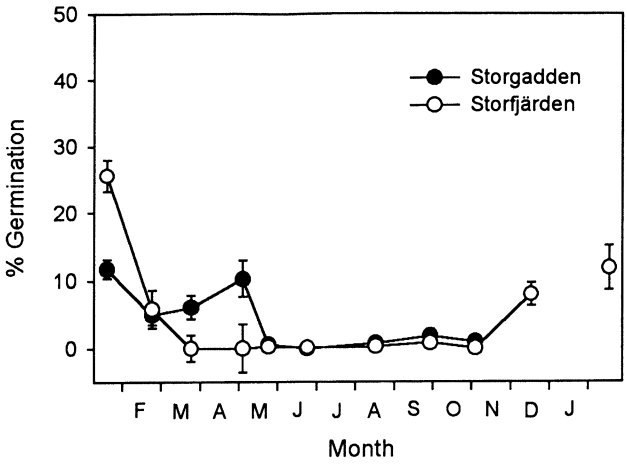

Fig. 4. Germination success (mean % ± SD) of cysts collected from the sediment surface and incubated at 3°C in the light (12:12 L/D) for 4 weeks in 1998. Filled circles = cysts from Storgadden sampling station (with periodic anoxia in bottom waters); open circles = cysts from Storfjärden (well oxygenated sediments).

decreased from 25% in January to 6% in February. No significant germination ($P > 0.05$) was detected from February until late autumn (Figure 4). The seasonal germination pattern differed for cysts collected from the periodically anoxic Storgadden sediments, where germination occurred over a period of 4 months. However, the maximum germination frequency (around 10%) was lower than in the cysts from Storfjärden (Figure 4).

Temperature

The temperature gradient bar experiment showed that *S.hangoei* cysts require low temperatures for germination (Figure 5); 29% of the cysts germinated at –0.7°C (in frozen samples), and temperatures between 0.5 and 4°C were optimal for excystment, resulting in a germination success >90% after 7 weeks of incubation. Germination success decreased between 4 and 8°C and was suppressed completely above this threshold.

Oxygen conditions and light

Cysts of *S.hangoei* did not germinate under anoxic conditions, nor did they germinate in the vials to which sulfides were added (Figure 6), although the initial oxygen concentration in the latter (0.01 mg O_2 l^{-1}) slightly exceeded the level of oxygen in the anoxic sea water (0.001–0.002 mg O_2 l^{-1}). After 3 weeks of dark incubation, the fraction of empty cysts had increased significantly ($P < 0.05$) in all hypoxic vials. However, the germination frequency differed considerably among the three hypoxic concentrations (Figure 6). The increase in germination from

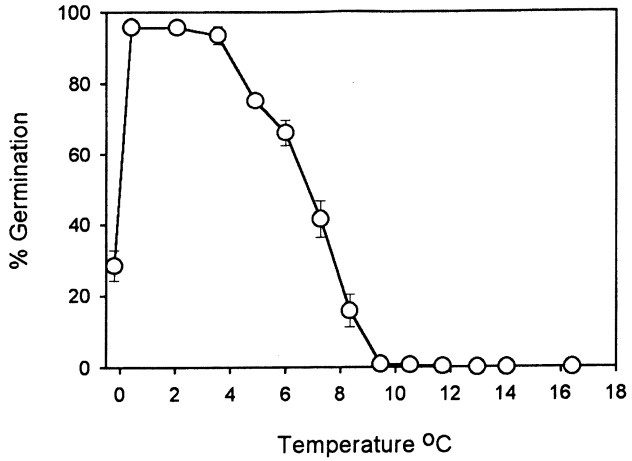

Fig. 5. Effect of temperature on the germination of *Scrippsiella hangoei* cysts collected after the 1996 spring bloom and stored at 3°C in the dark until December 1996 (error bars indicate SD).

2% at 0.5 mg O_2 l^{-1} (initial minus final concentration) to 16% at 1.5 mg O_2 l^{-1} and 25% at 2 mg O_2 l^{-1} (which was the same percentage as at normoxic concentrations of 11–13 mg O_2 l^{-1}), indicated that a 15 to 20% oxygen saturation is sufficient for *S.hangoei* cysts to germinate.

Light had a positive effect on germination. Significantly more empty cysts

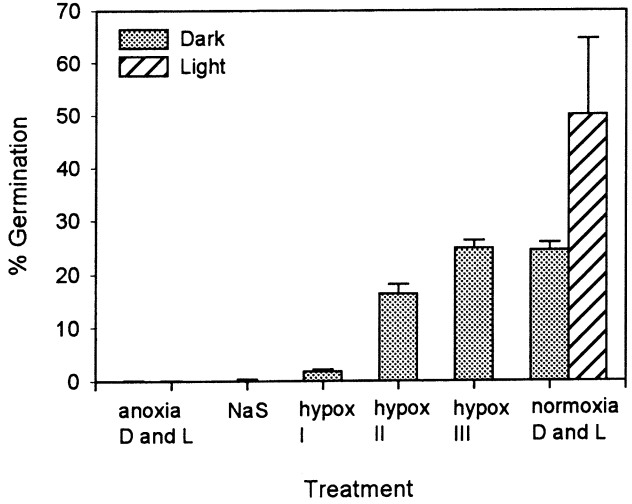

Fig. 6. Effect of oxygen concentration on *Scrippsiella hangoei* cyst germination. Germination success (%) after 3 weeks of incubation at six different O_2 concentrations: anoxic (0.001 mg O_2 l^{-1}), anoxic + NaS (0.01 mg O_2 l^{-1}), hypoxic I (0.5 mg O_2 l^{-1}), hypoxic II (1.5 mg O_2 l^{-1}), hypoxic III (2 mg O_2 l^{-1}) and normoxic (11–13 mg O_2 l^{-1}). All treatments were incubated in total darkness; one anoxic and one normoxic incubation series were placed in the light.

($P < 0.05$) were counted after 3 weeks in the vials incubated in light (resulting in a germination success of 50%) than in vials incubated in darkness (Figure 6).

The effect of periodic exposure to anoxic conditions on the germinability of *S.hangoei* cysts is shown in Figure 7. Normoxic dark incubated cysts attained 65% germination 7 days after re-exposure to normal conditions (light and normal oxygen concentrations), subsequently increasing to an ultimate germination frequency of 82%. Germination of the cysts from the anoxic vial was delayed, but the percentage of excystment (72%) after 34 days of normal incubation did not differ significantly from the normoxic controls. An even stronger delay in germination was observed with the anoxic treatment with sulfide addition. Significant germination occurred only after 20 days of re-exposure to normal conditions; after 34 days, 29% of the cysts had germinated and the ultimate germination success did not exceed 50%.

Discussion

This study demonstrates that the seasonal appearance of *S.hangoei* in the coastal northern Baltic is largely regulated by physiological and environmental constraints on the germination of its cysts. A long mandatory dormancy period (endogenous control) of approximately 6 months appears to be the primary factor determining the timing of germination in this species. Temperature can potentially control excystment once this maturation process is complete and the cysts enter the stage known as 'quiescence' (exogenous control). However, under the environmental conditions in the study area, temperatures are in the optimal or permissive 'window' for germination at the time the dormancy process is completed during mid-winter. For most other dinoflagellate species studied thus far, the mandatory dormancy interval is shorter and thus, quiescence is often

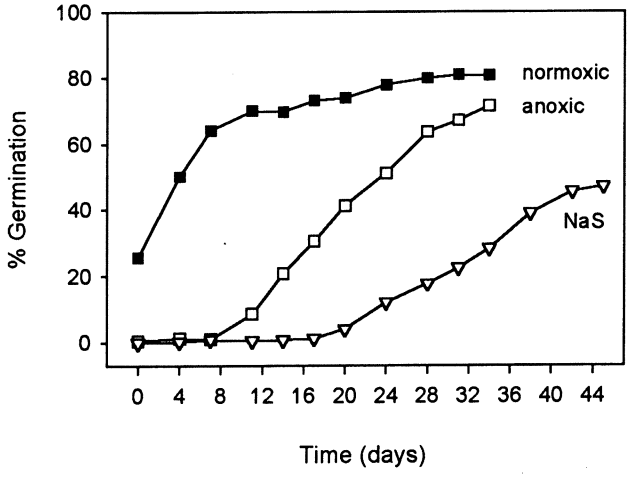

Fig. 7. Survival of *Scrippsiella hangoei* cysts after anoxic and normoxic treatment. Post-treatment incubation was carried out at 3°C, 12:12 h L/D cycle with ample oxygen.

maintained due to unfavorable ambient temperatures [reviewed in (Pfiester and Anderson, 1987)]. The experiments presented here revealed that oxygen and light conditions can also modulate the germination process by retarding it, decreasing the germination success or totally suppressing excystment. These factors are therefore of significance to our understanding of the germination patterns observed in nature.

Dormancy, quiescence and the seasonal germination pattern

Scrippsiella hangoei cysts require a relatively long maturation period of 6 months when stored at low temperatures. The length of this mandatory dormancy interval remained stable in cysts from subsequent blooms stored under laboratory conditions (3°C), and was consistent with results for cysts collected from the field where temperatures at the sediment surface ranged from 0 to 6°C throughout the year. It is not clear which mechanisms regulate the duration of mandatory dormancy in *S.hangoei.*

Long dormancy intervals seem to be typical in marine dinoflagellates from high latitude waters (Anderson, 1998; Perez *et al.*, 1998) and may be explained by low metabolic activity at low temperatures resulting in a prolonged maturation process. However, the effects of temperature on dormancy are more complex than the hypothesis that lower temperatures result in longer dormancy periods (Dale, 1983) suggests. For example, the cold stenothermic freshwater dinoflagellate, *Peridinium aciculiferum*, has a short mandatory dormancy interval and remains quiescent for several months (Rengefors and Anderson, 1998), although the climatic conditions in the Scandinavian lake where *P.aciculiferum* was studied do not differ much from the conditions in the northern Baltic where we studied *S.hangoei.* Both species are adapted to life in cold water (Larsen *et al.*, 1995; Rengefors, 1998), so their metabolism is expected to work equally well at low temperatures. In contrast, the Mediterranian *Scrippsiella* species, *S.rotunda*, needs 17–25 weeks for maturation (Nuzzo and Montresor, 1999), which is an unexpectedly long period given the high temperatures (14–20°C) prevailing in the sediments of the Gulf of Naples. These species-specific differences in dormancy characteristics are presumably controlled genetically (Perez *et al.*, 1998) as a manifestation of different life cycle or survival strategies.

The completion of the maturation period in December was marked by a sudden increase of Chl *a* fluorescence just prior to germination of the *S.hangoei* cysts. This suggests that the cysts had entered a new physiological state with higher metabolic activity (Yentsch *et al.*, 1980; Binder and Anderson, 1990). This internally-stimulated physiological activation might explain the rapid germination observed once cysts were exposed to light, despite the very low incubation temperature (3°C). In December, approximately 60% of the cysts had germinated after an incubation interval of only 7 days.

Scrippsiella hangoei cysts germinated over a relatively long period of 5 months, from December to April. In February and April, longer incubation times (14 days) resulted in significantly higher germination frequencies, presumably due to the effect of cyst age on germination (Binder and Anderson, 1987). Similarly,

Rengefors and Anderson (Rengefors and Anderson, 1998) found that incubation time increased with cyst age in the freshwater dinoflagellates *Ceratium hirundinella* and *Peridinium aciculiferum*. However, irrespective of the incubation time, the germination frequency decreased steadily towards the end of spring. There are two possible explanations for this trend. First, the germination potential of the cysts might have decreased with time, meaning that fewer viable cysts were available for germination every subsequent month. Due to continuous germination in the storage container, the initial number of empty cysts increased with progressing time. The remaining cysts might therefore have been of lower quality, requiring longer incubation times for germination as a consequence of ageing, so that excystment could not be detected within the given incubation intervals (7 and 14 days). The decrease in the percentage of fluorescing cysts from these samples, coinciding with the fluorescence pattern observed in a natural cyst population (A.Kremp, unpublished data), supports the idea of a reduced potential for germination towards the end of the annual cycle. The other explanation for the observed decreases in germination and fluorescence could be the existence of an endogenous rhythm, as has been described for *Alexandrium tamarense* (Anderson and Keafer, 1987). However, the data presented here do not yet provide conclusive evidence for an annual, endogenous clock in *S.hangoei* cysts.

Temperature

For most dinoflagellate species, temperature has been found to be the most important external factor controlling the germination of quiescent cysts (Pfiester and Anderson, 1987). The results of the temperature gradient bar experiment show that *S.hangoei* cyst germination is inhibited above 9°C. High germination frequencies occurred at the lower end of the temperature gradient. Given the long incubation time of 50 days, it is not clear to what extent the observed responses conceal differences in the rates of germination, as low germination rates at non-optimal temperatures can result in high ultimate germination percentages after long incubation periods (Binder and Anderson, 1987). However, taking into account that 60% of the cysts germinated within 7 days in the incubation experiment at 3°C (Figure 3), it is likely that germination proceeds rapidly at low temperatures.

A considerable fraction of cysts germinated in frozen samples, indicating a high tolerance to extremely low temperatures. The ability to germinate at temperatures below 0°C has been described only for polar dinoflagellates living in the brine channels of sea ice (Stoecker *et al.*, 1998).

The temperature 'window' for germination of *S.hangoei* cysts is unique with respect to the environment in which these cysts occur. The temperatures at which germination was optimal (0–6°C) are present at the sediment surface throughout the year at the study site. Therefore, we conclude that excystment occurs spontaneously as a natural consequence of the completion of mandatory dormancy, without any need for an external stimulus or trigger. This is also demonstrated by the excystment of laboratory-stored cysts exposed to the same temperature (3°C) during incubation as during storage. This clearly differs from

many other dinoflagellate species, where the end of the maturation period usually precedes favorable temperatures and thus, a seasonal shift into the temperature 'window' acts as the trigger for excystment (Huber and Nipkow, 1923; Anderson and Morel, 1979; Anderson, 1980; Bravo and Anderson, 1994; Rengefors and Anderson, 1998). The cysts of *Alexandrium tamarense* from a cold water environment showed a similar combination of long maturation time and a wide temperature window (Perez *et al.*, 1998). These observations support the hypothesis that in species from high latitudes, internal factors such as maturation and/or an endogenous clock play a more important role in controlling germination than external cues (Anderson, 1998).

Oxygen conditions and light

Germination of *S.hangoei* cysts was completely inhibited under anoxic conditions. The requirement for oxygen seems to be obligatory for dinoflagellate excystment, as all species studied so far under truly anoxic conditions responded by total suppression of excystment to the lack of oxygen (Anderson *et al.*, 1987; Rengefors and Anderson, 1998). However, some species can germinate successfully under very low oxygen concentrations. For example, Rengefors and Anderson (Rengefors and Anderson, 1998) found that the germination success of *Ceratium hirundinella* cysts incubated under severe hypoxia (5% oxygen-saturated medium) did not differ significantly from normoxic controls. In contrast, the germination of *S.hangoei* cysts was very low (2%) after incubation in 0.5 mg O_2 l^{-1} (being equivalent to 5% saturation). Normal germination, present in the third hypoxic treatment, occurred only with moderate hypoxia (>20% oxygen saturation). At 17% oxygen saturation, germination was still significantly reduced ($P < 0.05$). Thus, *S.hangoei* cysts appear to be less tolerant to oxygen deficiency than other dinoflagellate species (Anderson *et al.*, 1987).

For several dinoflagellate species, it has been shown that cysts can be successfully germinated after long periods of burial in anoxic sediments, typically associated with high sulfide concentrations (Huber and Nipkow, 1923; Dale, 1983; Keafer *et al.*, 1992). However, in *S.hangoei*, short-term exposure to high sulfide concentrations strongly retarded germination and decreased germination potential. Although the applied sulfide concentration of 30 mmol l^{-1} was extremely high compared with concentrations in anoxic sediments (Fenchel, 1969), the response of the cysts might help to explain the somewhat unexpected reduced and delayed germination of cysts stored in anoxic and sulfide-rich sediments (Table I). It is possible that the differences in the germination pattern of cysts from the two different sampling sites (i.e. retarded and more gradual germination in the cysts from periodically anoxic sediments at Storgadden compared with cysts from continuously oxygenated Storfjärden) are caused by the effects of oxygen deficiency. The low resistance of *S.hangoei* cysts to anoxia might be due to the thin cyst wall (Larsen *et al.*, 1995). A possible correlation between a reduced viability and a thin cyst wall was discussed by Wall and Dale (Wall and Dale, 1969).

Light significantly increased germination frequency in *S.hangoei* cysts, but

incubation in darkness did not prevent germination. This result is consistent with our observation of spontaneous excystment of laboratory-stored cysts kept in darkness. We cannot rule out, however, that a short exposure to low light occurred during handling and that this stimulated excystment, as described by Binder and Anderson (Binder and Anderson, 1986). Total darkness could not be maintained during all steps of the cyst processing and sampling. However, the increase in the fraction of empty cysts during winter and spring in sediment samples collected from 30 and 50 m depth, where light is totally absent, suggests that germination does occur in natural sediments without any light stimulus.

Ecological implications

The vegetative population of *S.hangoei* is present in the water column from January to May, often forming two separate peaks during this bloom period (Larsen *et al.*, 1995). The length of the mandatory dormancy period determines the timing of *S.hangoei* cyst germination and the appearance of vegetative cells, in that the release from this internal constraint to germination coincides with the presence of favorable growth conditions, particularly temperature. This is important as most cysts germinate immediately after maturation is completed, and the resulting inoculum population must therefore survive in the water column until optimal growth conditions, provided by stratification, are present (e.g. under the ice during winter and later in spring). As meteorological conditions are highly unpredictable during winter and spring in the northern Baltic, it would seem to be advantageous for *S.hangoei* to be present in the water column at any time when ice formation and stratification are possible after December, despite the poor light conditions at this time of the year (~100 $\mu E\ m^{-2}\ s^{-1}$ at 1m depth, and ~20 $\mu E\ m^{-2}\ s^{-1}$ under snow-covered ice). The species has been observed to grow successfully in microcosms at light intensities as low as 5–10 $\mu E\ m^{-2}\ s^{-1}$ and we assume that it has evolved mechanisms of low light adaptation, similar to the winter dinoflagellate *Peridinium aciculiferum* (Rengefors and Anderson, 1998). However, it could be possible that the germination of cysts later in February/March, though at lower rates, is substantially more effective for establishing a blooming population because better light conditions favour vegetative growth of the germinated cells.

Furthermore, the ability of *S.hangoei* cysts to germinate in sea ice might be advantageous in severe winters when resuspended cysts are incorporated into the newly formed ice. There, they might germinate in the brine channels and release vegetative cells into the water layer underneath. Favorable light conditions in the ice and direct release into the water column, where growth takes place, could cause a more synchronized and rapid germination, greatly aiding bloom development compared with the more extended recruitment from the sediments.

Eutrophication of the northern Baltic Sea has led to a general decrease of oxygen supply in both deep and shallow areas (Cederwall and Elmgren, 1990). When bottom waters are periodically oxygen deficient and anoxia (in combination with high levels of H_2S) occurs at the sediment surface, we would expect a reduction of germination potential and consequently, less successful initiation

of a new vegetative *S.hangoei* population. If the inoculum of vegetative cells decreases in size, bloom development will be retarded, leading to smaller blooms, as discussed by Nehring (Nehring, 1996) for dinoflagellates from the North Sea and the western Baltic. Consequently, the entire vernal pelagic community in the coastal northern Baltic would be affected, as *S.hangoei* is one of the main primary producers during spring.

The germination of *S.hangoei* cysts provides an inoculum of vegetative cells at the beginning of the growth period that is sufficient to initiate a bloom when growth conditions in the water column become optimal. Excessive vegetative growth and subsequent mass encystment (Heiskanen, 1993; Kremp and Heiskanen, 1999) compensate for the loss of viable cells in sediments due to factors such as sulfide toxicity and rapidly decreasing germination potential towards the end of the annual cycle.

Acknowledgements

We thank T.Sjölund for assistance in the field and Drs A.-S.Heiskanen and H.Kuosa for valuable discussion and comments on the manuscript. Thanks are due to the staff of the Tvärminne Zoological Station/University of Helsinki and the Institute of Limnology at Uppsala University. This study was financed in part by the Finnish Culture Fund, the Walter and Andreé de Nottbeck Foundation and the Nordic Council of Ministers. Support was also provided (to D.M.A.) from the National Science Foundation through grant OCE-9415536. Contribution No. 10051 from the Woods Hole Oceanographic Institution.

References

Anderson,D.M. (1980) Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (Dinophyceae) hypnozygotes. *J. Phycol.*, **16**, 166–172.

Anderson,D.M. (1998) Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In Anderson,D.M., Cembella,A.D. and Hallegraeff,G.M. (eds), *Physiological Ecology of Harmful Algal Blooms*. NATO ASI series, Springer-Verlag, Berlin, Heidelberg, Vol. G 41, pp. 29–48.

Anderson,D.M. and Keafer,B.A. (1985) Dinoflagellate cyst dynamics in coastal and estuarine waters. In Anderson,D.M., White,A.W. and Baden,D.G. (eds), *Toxic Dinoflagellates. Proceedings of the Third International Conference.* Elsevier, New York, pp. 219–224.

Anderson,D.M. and Keafer,B.A. (1987) An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis. Nature*, **325**, 616–617.

Anderson,D.M. and Morell,F.M.M. (1979) The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. *Estuarine Coastal Mar. Sci.*, **8**, 279–293.

Anderson,D.M., Chrisholm,S.W. and Watras,C.J. (1983) Importance of life cycle events in the population dynamics of *Gonyaulax tamarensis. Mar. Biol.*, **76**, 179–189.

Anderson,D.M., Taylor,C.D. and Armbrust,E.V. (1987) The effects of darkness and anaerobiosis on dinoflagellate cyst germination. *Limnol. Oceanogr.*, **32**, 340–351.

Binder,B.J. and Anderson,D.M. (1986) Green light mediated photomorphogenesis in a dinoflagellate resting cyst. *Nature*, **322**, 659–661.

Binder,B.J. and Anderson,D.M. (1987) Physiological and environmental control of germination in *Scrippsiella trochoidea* (Dinophyceae) resting cysts. *J. Phycol.*, **23**, 99–107.

Binder,B.J. and Anderson,D.H. (1990) Biochemical composition and metabolic activity of *Scrippsiella trochoidea* (Dinophyceae) resting cysts. *J. Phycol.*, **26**, 289–298.

Bonsdorff,E. (1992) Drifting algae and zoobenthos. *Neth. J. Sea Res.*, **30**, 57–62.

Bravo,I. and Anderson,D.M. (1994) The effects of temperature, growth medium and darkness on

excystment and growth of the toxic dinoflagellate *Gymnodinium catenatum* from northwest Spain. *J. Plankton Res.*, **16**, 513–525.
Cannon,J. (1993) Germination of the toxic dinoflagellate, *Alexandrium minutum* from sediments of the Port River, South Australia. In Smayda,T. and Shimizu,Y. (eds), *Toxic Phytoplankton Blooms in the Sea.* Elsevier, pp. 103–112.
Cederwall,H. and Elmgren,R. (1990) Biological effects of eutrophication in the Baltic Sea, particularly the coastal zone. *Ambio*, **19**, 109–112.
Dale,B. (1983) Dinoflagellate resting cysts: 'benthic plankton'. In Fryxell,G.A. (ed.), *Survival Strategies of the Algae.* Cambridge University Press, Cambridge, pp. 69–136.
Fenchel,T. (1969) The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem. *Ophelia*, **6**, 1–82.
Heiskanen,A.-S. (1993) Mass encystment and sinking of dinoflagellates during a spring bloom. *Mar. Biol.*, **116**, 161–167.
Heiskanen,A.-S. and Kononen,K. (1994) Sedimentation of the vernal and late summer phytoplankton communities in the coastal Baltic Sea. *Arch. Hydrobiol.*, **131**, 175–198.
Heiskanen,A.-S. and Lepparen,J.-M. (1995) Estimation of export in the coastal Baltic Sea: effect of resuspension and microbial decomposition of sedimentation measurements. *Hydrobiologia*, **316**, 211–224.
Huber,G. and Nipkow,F. (1923) Experimentelle Untersuchungen über Entwicklung und Formbildung von *Ceratium hirundinella* O.F. Müller. *Flora*, **116**, 114–215.
Ishikawa,A. and Taniguchi,A. (1996) Contribution of benthic cysts to the population dynamics of *Scrippsiella* spp. (Dinophyceae) in Onagawa Bay, northeast Japan. *Mar. Ecol. Prog. Ser.*, **140**, 169–178.
Keafer,B.A., Buesseler,K.O. and Anderson,D.M. (1992) Burial of living dinoflagellate cysts in estuarine and nearshore sediments. *Mar. Micropaleont.*, **20**, 147–146.
Kremp,A. and Heiskanen,A.-S. (1999) Sexuality and cyst formation of the spring-bloom dinoflagellate *Scrippsiella hangoei* in the coastal northern Baltic Sea. *Mar. Biol.*, **134**, 771–777.
Larsen,J., Kuosa,H., Ikävalko,J., Kivi,K. and Hällfors,S. (1995) A redescription of *Scrippsiella hangoei* (Schiller) comb. nov.—a 'red tide' forming dinoflagellate from the northern Baltic. *Phycologia*, **34**, 135–144.
Lignell,R., Heiskanen,A.-S., Kuosa,H., Gundersen,K., Kuuppo-Leinikki,P., Pajuniemi,R. and Uitto,A. (1993) Fate of a phytoplankton spring bloom: sedimentation and carbon flow in the planktonic food web in the northern Baltic. *Mar. Ecol. Prog. Ser.*, **94**, 239–252.
Lutz,R.V., Marcus,N.H. and Chanton,J.P. (1992) Effects of low oxygen concentrations on the hatching and viability of eggs of marine calanoid copepods. *Mar. Biol.*, **114**, 241–247.
Montresor,M.and Marino,D. (1996) Modulating effect of cold-dark storage on excystment in *Alexandrium pseudogonyaulax* (Dinophyceae). *Mar. Biol.*, **127**, 55–60.
Nehring,S. (1996) Recruitment of planktonic dinoflagellates: importance of benthic resting stages and resuspension events. *Int. Revue Ges. Hydrobiol.*, **70**, 527–46.
Niemi,Å. (1975) Ecology of phytoplankton in the Tvärminne area, SW coast of Finland. II. Primary production and environmental conditions in the archipelago and the sea zone. *Acta Bot. Fennica*, **105**, 1–73.
Nuzzo,L. and Montresor,M. (1999) Different excystment patterns in two calcareous cyst-producing species of the dinoflagellate genus *Scrippsiella*, *J. Plankton Res.*, **21**, 2009–2018.
Perez,C.C., Roy,S., Levasseur,M. and Anderson,D.M. (1998) Control of germination of *Alexandrium tamarense* (Dinophyceae) cysts from the lower St Lawrence Estuary (Canada). *J. Phycol.*, **34**, 242–249.
Pfiester,L.A. (1977) Sexual reproduction of *Peridinium gatuense* (Dinophyceae). *J. Phycol.*, **13**, 92–95.
Pfiester,L.A. and Anderson,D.M. (1987) Dinoflagellate reproduction. In Taylor,F.J.R. (ed.), *The Biology of Dinoflagellates.* Blackwell Scientific Publications, Oxford, pp. 611–648.
Rengefors,K. (1998) Seasonal succession of dinoflagellates coupled to the benthic cyst dynamics in Lake Erken. *Arch. Hydrobiol. Spec. Issues, Adv. Limnol.*, **51**, 123–141.
Rengefors,K. and Anderson,D.M. (1998) Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates. *J. Phycol.*, **34**, 568–577.
Steidinger,K.A. (1975) Basic factors influencing red tides. In LoCicero,V.R. (ed.), *Toxic Dinoflagellate Blooms.* Proceedings of the First International Conference on Toxic Dinoflagellate Blooms, Massachusetts Sci. Tech. Found, Wakefield, Mass. pp. 153–162.
Stoecker,D.K., Gustafson,D.E., Black,M.D.and Baier,C.T. (1998) Population dynamics of microalgae in the upper land-fast sea ice at a snow free location. *J. Phycol.*, **34**, 60–69.
Viitasalo,M. and Katajisto,T. (1994) Mesozooplankton resting eggs in the Baltic Sea: identification and vertical distribution in laminated and mixed sediments. *Mar. Biol.*, **120**, 455–465.

Wall,D. (1975) Taxonomy of cysts of red tide dinoflagellates. In LoCicero,V.R. (ed.), *Toxic Dinoflagellate Blooms.* Proceedings of the First International Conference on Toxic Dinoflagellate Blooms, Massachusetts Sci. Tech. Found, Wakefield, Mass. pp. 249–255.

Wall,D. and Dale,B. (1969) The 'Hystrichosphaerid' resting spore of *Pyrodinium bahamense*, Plate, 1906. *J. Phycol.*, **5**, 140–149.

Watras,C.J., Chisholm,S.W. and Anderson,D.M. (1982) Regulation of growth in an estuarine clone of *Gonyaulax tamarensis* Lebour: salinity-dependent temperature responses. *J. Exp. Mar. Biol. Ecol.*, **62**, 25–37.

Yentsch,C.M., Lewis,C.M. and Yentsch,C.S. (1980) Biological resting in the dinoflagellate *Gonyaulax excavata. Bioscience*, **30**, 251–254.

Received on September 9, 1999; accepted on January 19, 2000