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CONTROL OF GERMINATION OF *ALEXANDRIUM TAMARENSE* (DINOPHYCEAE) CYSTS FROM THE LOWER ST. LAWRENCE ESTUARY (CANADA)[1]

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ABSTRACT

Cysts of the toxic dinoflagellate Alexandrium tamarense *(Lebour) Balech 1992 from the lower St. Lawrence estuary were used in a test of the following hypotheses: (1) cyst germination is triggered by a change in temperature, and (2) germination rate varies throughout the year and is controlled by a circannual internal biological clock. Results show that cyst germination was not affected significantly by temperature of incubation over the range 1°–16° C, and light showed no significant stimulation of germination. This is supported by the lack of effect of cyst incubation conditions during evaluation of the seasonal changes in germination rate (two temperatures: 4° and 15° C, and two light conditions: darkness and 150 μmol photons·m^{-2}·s^{-1}). Thus, direct environmental control through short-term increases in temperature and exposure to light has no effect on the germination of the cysts tested. The rate of germination, observed monthly over a 16-month period, showed low germination (<20%) over most of the period tested, except for a maximum reaching more than 50% germination in August to October of the second year of the experiment. This pattern was observed for cysts both from monthly field collections and from laboratory-stored cysts kept under constant environmental conditions (4° C, in the dark). The peak in germination observed under constant environmental conditions (in the laboratory), the almost coincidental increase in cyst germination observed for the field-collected cysts, and the absence of effects of temperature and light during incubation could be explained either by a temperature-controlled cyst maturation period (the time-temperature hypothesis of Huber and Nipkow 1923) or by the presence of an internal biological clock. However, the large decline in the rate of germination 2 months after the maximum provides strong support for the biological clock hypothesis. The* ca. *12-month maturation (dormancy) period observed for the laboratory-stored cysts is the longest reported for this species to our knowledge; this might be related to the low storage temperature (4° C), which is close to bottom temperatures generally encountered in this environment (0° to 6° C). Similar field and laboratory storage temperatures could explain the coincidental increase in germination rate in the fall of the second year if cyst maturation is controlled by temperature. A fraction of the laboratory-stored cysts did not follow a rhythmic pattern: A rather constant germination rate of about 20% was observed throughout the year. This continuous germination of likely mature cysts may supplement the local blooms of this toxic dinoflagellate, as these often occur earlier than peak germination observed in late summer. It seems that two cyst germination strategies are present in the St. Lawrence: continuous germination after cyst maturation, with temperature controlling the length of the maturation period, and germination controlled by a circannual internal rhythm.*

Key index words: Alexandrium tamarense*; circannual rhythm; cyst germination; cyst maturation; light; temperature; toxic dinoflagellate*

Many dinoflagellates produce resting cysts as part of their sexual life cycle (von Stosch 1973, Pfiester 1975, Dale 1977, Anderson and Wall 1978, Walker and Steidinger 1979). After a dormancy period, these cysts germinate into vegetative cells that can act as potential seed populations for initiating red tide events (Prakash 1967, Steidinger 1975, Anderson and Wall 1978). The length of the dormancy period ranges from 12 h to several weeks or months (Wall and Dale 1968, von Stosch 1973, Pfiester 1975, Anderson 1980, Pfiester and Anderson 1987) and seems to be under physiological control. Huber and Nipkow (1923) and Dale (1983) suggested that there is a basic time-temperature relationship that governs the duration of cyst dormancy (i.e. lower temperature = longer maturation time), whereas Steidinger and Haddad (1981) speculated that internal storage product levels could determine the timing of excystment. Once cysts are mature, their

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germination may be triggered by various environmental factors. von Stosch (1973) and Pfiester (1975) proposed that a cold treatment of several weeks (followed by return to light and higher temperature) enhanced and synchronized germination. Anderson and Morel (1979) and Anderson (1980) showed that a change in temperature caused excystment in *Alexandrium tamarense* once dormancy was over. Past studies on the effect of light on cyst germination have lead to equivocal conclusions. In most studies (and species), germination did not differ significantly between light and dark treatments (Huber and Nipkow 1923, Anderson and Wall 1978). However, light clearly increased both the rate of germination and total number of cells excysted in five dinoflagellates tested by Anderson et al. (1987). Light stimulation of germination is especially striking in *Scrippsiella trochoidea* (Binder and Anderson 1986). Beside temperature and light, the effects of other environmental factors on dinoflagellate cyst germination are poorly understood (Pfiester and Anderson 1987). No clear effects of the chemical composition of the growth media were found in the study of Huber and Nipkow (1923) on freshwater *Ceratium hirundinella,* nor in that of Anderson and Wall (1978) on marine *A. tamarense.* However, An et al. (1992) suggested that decreasing salinity and increasing nutrient concentrations were associated with increased germination rate of *Scrippsiella* and *Gonyaulax* species. External concentration of nutrients may also affect the duration of the cyst maturation period if it influences the levels of internal cyst storage products (Steidinger and Haddad 1981). However, it is clear that anoxia prevents germination in most cyst species (Anderson et al. 1987, Ishikawa and Taniguchi 1994).

The St. Lawrence gulf and estuary are characterized by seasonal blooms of the PSP-producing dinoflagellate *A. tamarense* leading to annual closure of shellfish harvesting (Prakash et al. 1971, Therriault et al. 1985, Cembella et al. 1988, Cembella and Therriault 1989) as well as intoxication of fish larvae (Gosselin et al. 1989, Robineau et al. 1993). In an attempt to improve our understanding of the life cycle of this species, we examine the factors that could potentially govern cyst germination in this region, where high concentrations of cysts have been reported (Turgeon et al. 1990). Two major hypotheses have been proposed for this species concerning excystment: (1) A change in temperature that can be stimulated by light (Anderson et al. 1987) triggers cyst germination once the mandatory dormancy period is over (Anderson and Morel 1979, Anderson 1980). (2) An internal biological clock induces cyst germination at specific times of the year, regardless of local environmental conditions (Anderson and Keafer 1987). The latter was demonstrated for *A. tamarense* cysts from the Gulf of Maine, which germinate only during specific months of the year—a circannual rhythm (Anderson and Keafer 1987). In the present study, we examine these two hypotheses for *A. tamarense* cysts from the lower St. Lawrence estuary. The first hypothesis was tested by submitting local cysts to a range of temperatures under conditions of light and darkness; the second was examined by comparing the rate of germination of cysts collected monthly in the field with that of cysts kept under constant conditions over a period of 16 months.

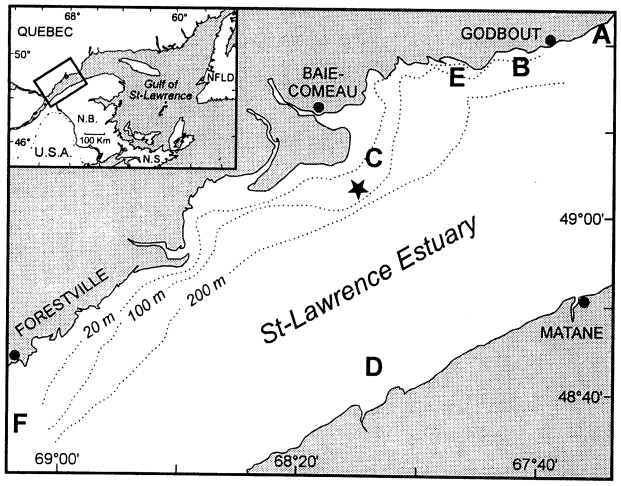

Fig. 1. Location of the study area on the north shore of the St. Lawrence estuary, Québec, Canada. The main sampling station is illustrated with a star. Letters refer to other stations surveyed at the beginning of the study.

MATERIALS AND METHODS

The sampling station was located near Baie-Comeau on the north shore of the lower St. Lawrence estuary (Fig. 1). This region is considered a seedbed for local blooms of *A. tamarense* because of its high cyst concentration (Cembella et al. 1988). Sampling took place on 8 April 1991 for the temperature test described below and on a monthly basis from 10 May 1992 to 25 October 1993 (with the exception of the winter months, when ship work was not possible) for examination of the annual variations of cyst germination rates. As cyst concentration was very low in spring 1992, other stations were surveyed on 30 May 1992 to determine whether this was a general feature of the region at this time (Fig. 1).

Surface sediments were collected using two methods: (1) Van Veen grab for the quantitative determination of cyst concentration in the sediment and (2) dragging a plankton net across the sediment surface (Wall and Dale 1968), which maximizes cyst concentration. This last method was used for cyst collection for all germination experiments described below.

Temperature, salinity, and nutrient concentrations (nitrate, nitrite, phosphate, and silicate) were sampled with 5-L Niskin bottles, at 1 m below the surface and 1 m above bottom on every cruise between May 1992 and October 1993. Temperature was measured with a thermometer in water from the Niskin bottle immediately on collection. Salinity was determined with an Autosal® instrument. Nutrient concentrations were analyzed with a Technicon Auto-Analyzer, according to the methods described in Parsons et al. (1984). Both the salinity and the nutrient determinations were done at the Maurice-Lamontagne Institute of the Department of Fisheries and Oceans Canada (DFO). Light penetration was estimated using a Secchi disk. The concentration of vegetative cells of *A. tamarense* in the water column was determined from vertical plankton hauls using a 0.5-m-diameter net with a mesh size of 30 μm. Lugol-fixed samples were counted under an inverted microscope after 24 h sedimentation in a 25-

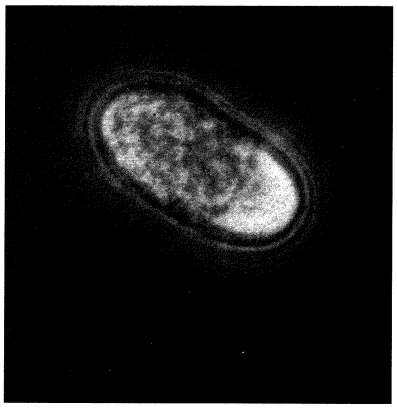

Fig. 2. Photograph of a cyst from *A. tamarense* obtained from bottom sediments at the sampling station. Scale bar = 30 μm.

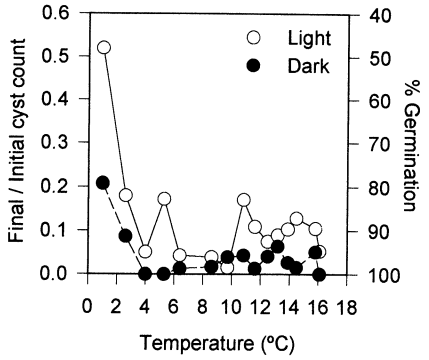

Fig. 3. Effect of temperature on the germination rate of sedimentary cysts of *A. tamarense* collected in April 1991. Open circles = samples incubated in the light. Filled circles = samples incubated in darkness (wrapped in aluminum foil).

mL settling chamber (tubular plankton chamber, Hydro-Bios, Kiel, Germany).

Cyst concentration in the top 1 cm^3 of sediment (from the Van Veen grab sample) and in the other sediment samples was determined from triplicate 1-cm^3 samples suspended in 150 mL of filtered seawater (collected at the sampling station in May 1992, double-filtered on Whatman GFF filters, and stored at 4° C in the dark; all further manipulations used this water). This cyst suspension was sonified (Branson S-75 microprobe, 45 s, for April 1991, and Branson Swest B-12 bath, 4 min, for 1992–93) to separate cysts from sand and sieved on Nitex, keeping the 15–70-μm fraction and transferring it into filtered seawater. Microscopic cyst identification and counts were done on this fraction using a Palmer-Maloney counting chamber.

Germination rates were determined from the decrease in number of living cysts after an incubation period of about 1 month. Only cysts with full cytoplasm were counted (Fig. 2). Cyst suspensions, prepared as described above, were poured into glass culture tubes. The volume was completed to 13 mL with filtered seawater, to which was added f/2 concentrations of vitamins and metals but only 3 μM of nitrate and 0.3 μM of phosphate, according to Anderson et al. (1987). Cyst concentration was adjusted to a minimum value of 100 cysts per tube. All cyst handling was done in a 4° C cold room under minimal light (<0.3 μmol photons·m^{-2}·s^{-1}, from a flashlight).

For the temperature test, performed 2 weeks after sediment collection in April 1991, 30 glass tubes (150 × 23 mm) were incubated for 38 days in a temperature-gradient bar (Watras et al. 1982) cooled at one end and heated at the other (ranging from 1° to 16° C). Half the total number of tubes were put in darkness by wrapping the tubes completely with aluminum foil, and the other half were exposed to 170 μmol photons·m^{-2}·s^{-1}. Because sonication is sometimes questioned as a potential artefact affecting germination (Dale 1983), a test was done using 2 cm^3 of sediment put directly into four tubes with 15 mL of filtered seawater from the sampling station and without presieving or sonication. These tubes were incubated alongside the others, two at 15.5° C and two at 3° C.

For the internal clock hypothesis, a total of 25 cyst-containing tubes (same model as above) were prepared monthly from both the field-collected and the laboratory-stored sediments. Five of those were counted immediately (initial cyst counts), and the 20 tubes remaining were randomly split into four groups, incubated for 30 days under the following four conditions: (1) light and 15° C, (2) light and 4° C, (3) darkness and 15° C, and (4) darkness and 4° C. Light intensity (cool-white fluorescent tubes) was 150 μmol photons·m^{-2}·s^{-1}, with a 14:10 h light:dark (L:D) photoperiod. All cyst incubations (field collected and laboratory stored) were done alongside in the same cold rooms (15° and 4° C). The laboratory-stored sediment came from the sample collected on 31 July 1992, which contained 105 cysts·cm^{-3} (grab sample). On retrieval from the water, this sediment was mixed manually and poured into glass Mason jars, filled to the top. These were individually covered with aluminum foil, put into plastic coolers, and stored in a 4° C cold room. One jar was removed each month for germination experiments. Counting errors were generally <15% CV (coefficient of variation) for both initial and final cyst counts. The composite variable Final/initial counts was used to present the data graphically. Means and standard errors for this variable were calculated according to Commissariat à l'Energie Atomique (1978).

RESULTS

The temperature-gradient bar experiment shows that, for temperatures above 2° C, germination rates varied between 82% and 99% in light and between 91% and 99% in darkness (Fig. 3). Temperature did not affect germination in the range 2°–16° C. A decrease in germination rate was observed only at 1° C, mostly in the light. Light did not show a stimulatory effect on germination. In fact, rates were significantly higher in darkness (Student's *t*-test, $P < 0.001$). Results from the temperature test in April 1991 show that germination can take place with little if any temperature increase and can in fact occur at the low temperatures observed in bottom sediments of this region (see below). Presieving and sonication showed no significant effect: Cysts present in the sediment that was not sonicated germinated, respectively, at rates of 94% and 89.5% at 15.5° C and 94.8% and 100% at 3° C.

At the beginning of the field sampling period in spring 1992, cyst concentrations in surface sediment were low (Fig. 4A). This situation was not the result of a patchy distribution as low concentrations were found at six other stations surveyed in May 1992 (Table 1; Fig. 1). Furthermore, whenever cysts were abundant, their surface sediment concentration varied little in the vicinity of the main sampling station: eight stations each located 1 km away from the main sampling station gave a CV of <6% on two occasions (1 and 27 October 1992). Sampling errors (CV) at each station (triplicate samples of the same sediment) were <10%. Increasing cyst concentrations were observed in late June and July 1992, following a bloom of *A. tamarense* in late June 1992 (Fig. 4B).

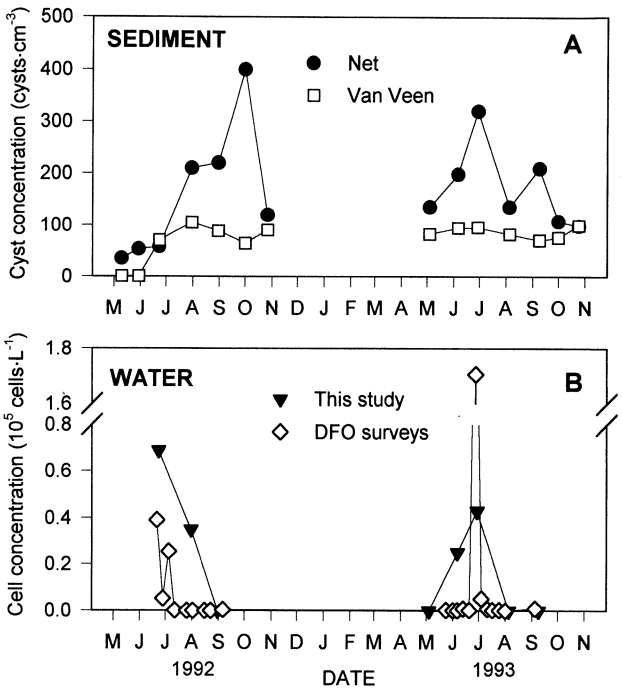

Fig. 4. Concentration of cysts in (A) surface sediments (filled symbols = collected using a plankton net dragged at the surface of sediment; open symbols = collected using a Van Veen grab) and (B) vegetative cells in the water column (filled symbols = results from this study; open symbols = surface samples from a nearby station surveyed in the monitoring program of harmful algae, Maurice Lamontagne Institute, DFO, Canada). Months, on the abscissa, are indicated by their first letter.

Thus, the sediment sample set aside for laboratory storage (31 July 1992) likely contained cysts that originated mostly from the algal bloom near the end of June 1992.

The Van Veen grab samples generally gave lower cyst concentrations than the net method, probably because of a loss of the watery sludge at the very surface of the sediment on retrieval from the water. The net method is less appropriate for quantitative estimates, but it is better at collecting the surface layer. Sediment cyst concentrations from both sampling methods generally increased during or after blooms of *A. tamarense* in the water column (Fig. 4) in both years. No decrease in cyst concentration was observed prior to *A. tamarense* blooms.

The germination rate of cysts collected monthly in the field shows values decreasing from ca. 25% to close to zero in 1992 (Fig. 5). In 1993, germination rates remained low (<25%) from April to August, increased to 30%–50% in September and October, and decreased again to near 0% in November. The same temporal pattern was observed under the four experimental conditions tested. Significant germination (i.e. significant differences between final and initial cyst counts, determined from *t*-tests and shown as filled circles in Fig. 5) was observed only in September and October 1993 under all conditions tested. These various incubation conditions

TABLE 1. *Cyst concentration (cysts·cm⁻³) from surface sediment sampled on 30 May 1992 at various stations along the north shore of the St. Lawrence (station locations indicated in Fig. 1). Cysts were collected either with a plankton net, dragged at the sediment surface (NET), or with a Van Veen grab (VAN VEEN). nd = no data.*

Station identification	Station depth (m)	Net	Van Veen
Main	45	53	0
A	74	0	0
B	140	37	nd
C	70	0	nd
D	51	0	nd
E	61	0	nd
F	79	0	0

(Fig. 5A–D) had no significant effects on germination (Friedman analysis of variance on ranks, $P > 0.05$). Lack of effects of temperature and light observed here over several months confirms the observations made in the temperature-gradient bar experiment, with cysts collected in April 1991.

For cysts stored under constant conditions in the laboratory, germination rates were around 20% between August 1992 and July 1993, increased to 60%–

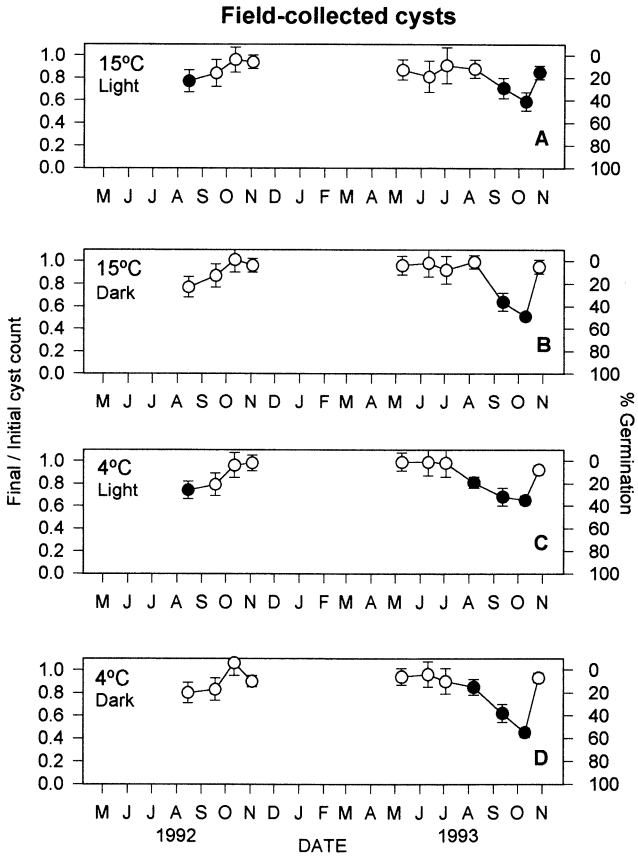

Fig. 5. Germination rates for monthly field-collected cysts (mean ± SE). Filled circles = significant difference between final and initial cyst counts. Open circles = nonsignificant germination. (A) Incubated at 15° C in the light, (B) incubated at 15° C in the dark, (C) incubated at 4° C in the light, (D) incubated at 4° C in the dark.

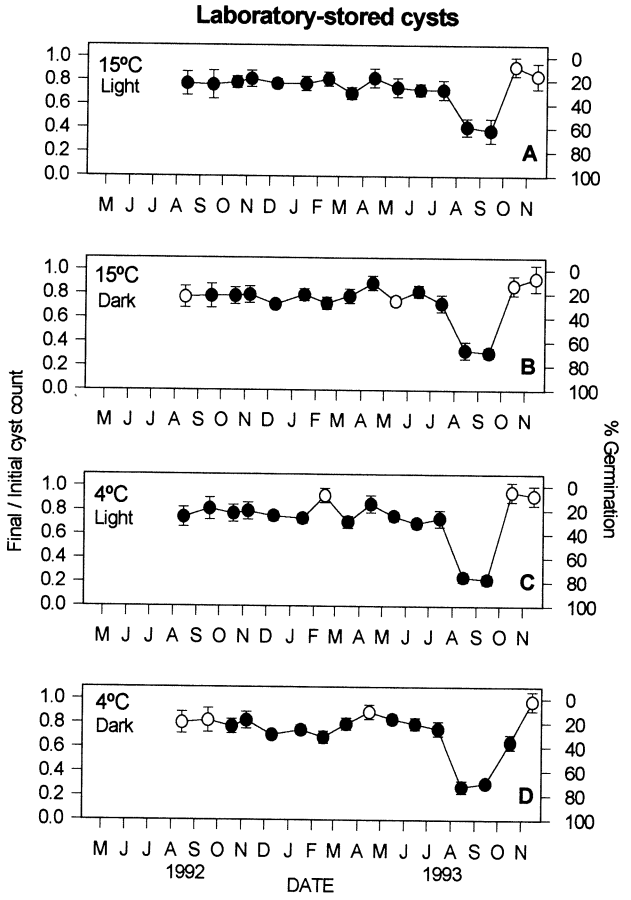

Fig. 6. Germination rates for laboratory-stored cysts (mean ± SE). Filled circles = significant difference between final and initial cyst counts. Open circles = nonsignificant germination. (A) Incubated at 15° C in the light, (B) incubated at 15° C in the dark, (C) incubated at 4° C in the light, (D) incubated at 4° C in the dark.

78% in August and September 1993, and decreased generally below 20% in October and November 1993 (Fig. 6). All months showed a significant germination rate except August 1992 (Dark, both temperatures), September 1992 (Dark, 4° C), February 1993 (Light, 4° C), April 1993 (Dark, 4° C), May 1993 (Dark, 15° C), and the last two months (only Dark, 4° C, showed significant germination). Again, no significant effects of the incubation conditions (Fig. 6A–D) were detected (Friedman analysis of variance on ranks, $P > 0.05$).

The physicochemical characteristics of bottom waters from the main sampling station varied relatively little: Temperature ranged from −0.5° to 4° C in 1992 and from 0° to 6° C in 1993, whereas salinity varied from 29.8 to 32.3 in 1992 and from 27.1 to 32.7 in 1993 (Fig. 7). Phosphate and silicate concentrations in bottom waters ranged from 0.5 to 1.2 μM and from 5.0 to 13.3 μM for 1992 and 1993, respectively (Table 2). Both were never depleted. Nitrate concentrations reached undetectable values on only one occasion, in October 1992, although

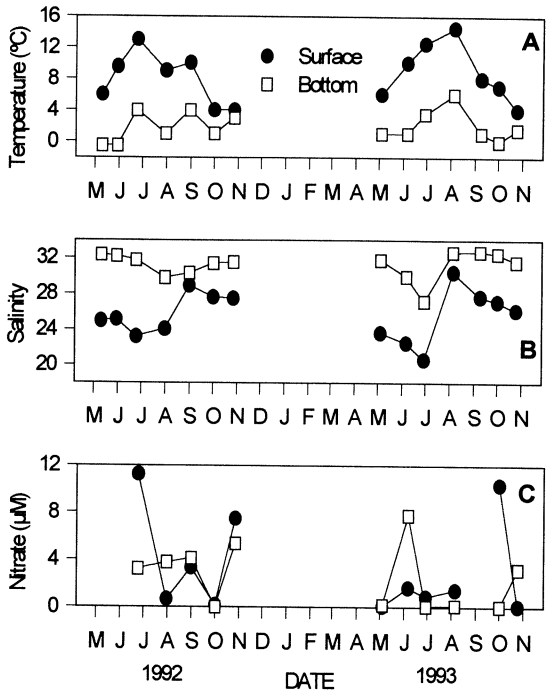

Fig. 7. Environmental conditions during the sampling period: (A) temperature, (B) salinity, (C) nitrate concentration. Filled symbols = surface samples. Open symbols = bottom samples.

values less than 0.2 μM were observed on four occasions in 1993 (Fig. 7C). In general, surface water conditions followed similar trends as bottom waters; these are shown in Figure 7 and Table 2.

DISCUSSION

Environmental Control of Cyst Germination

Temperature conditions tested during this study were selected to verify whether this variable had an effect on cyst germination and whether this temperature effect varied throughout the year, considering the possible presence of various subpopulations of *A. tamarense* (Anderson et al. 1994). The 4° and 15° C temperatures chosen are close to the local maxi-

TABLE 2. *Nutrient concentration in surface and bottom waters collected at the main station on the same dates as the cyst samplings. PO_4^{-3} = phosphate concentration (μM), SI = silicate concentration (μM), S = surface, B = bottom, nd = no data.*

Date	PO_4^{-3}-S	PO_4^{-3}-B	SI-S	SI-B
24 Jun 92	0.9	0.8	21.6	10.0
31 Jul 92	0.5	0.5	10.1	5.5
30 Aug 92	0.4	0.6	12.6	5.0
1 Oct 92	0.6	1.0	9.0	8.2
27 Oct 92	0.7	0.7	11.4	8.2
4 May 93	0.0	0.5	23.7	6.1
6 Jun 93	0.9	1.2	8.2	13.3
29 Jun 93	0.7	0.7	14.5	10.5
5 Aug 93	0.2	1.0	8.4	5.9
9 Sep 93	nd	nd	nd	nd
1 Oct 93	1.0	0.6	17.2	10.2
25 Oct 93	0.3	0.5	8.0	5.4

mum temperatures observed near the sediment surface and water surface, respectively (Silverberg and Sundby 1990; Fig. 7). Incubation at 4° C can represent an increase in temperature of up to 4°–5° C for these field cysts (depending on the time of the year), a fluctuation considered sufficient to trigger germination in environmentally controlled cysts (Anderson 1980). Alternatively, incubations at 15° C can represent the conditons encountered in summer by cysts transported into the upper water column during storm events.

Our results show that incubation temperature had no significant effect on cyst germination for both field-collected and laboratory-stored cysts at any time of the year. Increases in temperature did not initiate excystment: When excystment did occur, it took place under all test conditions. Thus, the hypothesis that a change in temperature triggers germination does not hold for these St. Lawrence cysts. This hypothesis has been criticized by Dale (1983), who believes that excystment simply occurs when a permissive temperature window is reached, once maturation is over, without the need for a particular stimulus. Figure 3 shows that such a temperature window could be wide for our local cysts: Germination took place at all temperatures tested, from 1° to 16° C. Theoretically, this would allow germination in all seasons, except perhaps for part of winter and early spring, when temperatures can drop below 0° C (cf. 10 May 1992, soon after ice breakup, in Fig. 7). Indeed, results in Figure 3 suggest a gradual decrease of the germination rate at temperatures below 2° C.

Light did not stimulate germination of *A. tamarense* cysts in this study. Light intensity being very low at 50 m ($<10^{-4}$ of the surface values) on all sampling dates, it is perhaps not surprising that this factor should be of little importance in the local regulation of cyst germination. These results are at odds with those of Anderson et al. (1987) showing a clear light stimulation of both the rate of germination and the total number of cells excysted in five dinoflagellates, including *A. tamarense.* However, it is interesting to note that in that study signs of excystment were visible by the end of the 7-week study, suggesting that darkness did not totally suppress germination.

Although our protocol was not specifically designed to test their influence, changes in *in situ* salinity and nutrient concentrations were examined for possible relationships with the germination of field cysts. In 1993, bottom salinity showed a large increase between July and August, ca. 1 month before cyst germination began. But germination took place at about the same time in the stored cysts, which were not exposed to these environmental changes. Thus, we do not believe that salinity had a significant effect on germination in our experiment. There is also no apparent relationship between nitrogen and phosphate supply or depletion and cyst formation and germination in our study. Surface nitrate and phosphate concentrations were relatively high in July 1992, at the time when most of the cysts were formed. When germination took place in late summer 1993, bottom phosphate levels were still relatively high, but nitrates had reached low levels 1 month before. Again, we do not believe that this had any special significance with respect to cyst germination because excystment occurred for the stored cysts as well, and these were not exposed to the changes in nutrient concentrations observed in the field.

The Time-Temperature Hypothesis

Although an increase in the incubation temperature does not seem to affect germination, temperature could still control the timing of excystment by determining the duration of the cyst maturation period (Huber and Nipkow 1923, Anderson 1980, Dale 1983). A survey of the literature shows that the length of the cyst maturation period for *A. tamarense* does increase when the temperature decreases: 1 month at 22° C (Anderson 1980), 2 months at 17° C (Turpin et al. 1978), and 4 months at 5° C (Anderson 1980). Fukuyo et al. (1982) report a 6-month dormancy in Japan, but without corresponding temperature data. Results from the stored cysts experiments (Fig. 6), which we consider freshly formed as very few cysts were present in surface sediments for 2 months before, suggest a maturation time of ca. 1 year at 4° C—to our knowledge the longest period reported for this species. If the maturation time of cysts is indeed temperature dependent, the maturation period may be best expressed by integrating the time spent by cysts at various temperatures. Values (in degree-time units) should then be similar for a particular species if this relationship is linear. For *A. tamarense,* these values vary: 20°-month (excystment after 4 months at 5° C), 22°-month (1 month at 22° C; both from Anderson 1980), and 34°-month (2 months at 17° C; Turpin et al. 1978). Our data show nearly 12 months maturation for cysts stored at 4° C (=48°-month) and 13–14 months for the field cysts (integrated taking into account the various bottom temperatures measured monthly = 33°-month). The 20°–48°-month range seems higher than one would expect for a single species. We think that this wide range is indicative of a latitudinal trend, the highest values being observed for the northernmost region (St. Lawrence estuary). Whether this apparent latitudinal trend results solely from the nonlinearity of the time-temperature relationship (Fig. 8) or is genetically controlled remains undetermined.

One of the differences between the results from the field-collected and the laboratory-stored cysts is a more or less continual but low rate of excystment of the stored cysts between August 1992 and July 1993, just before the strong increase in germination rate observed in late summer. This is difficult to ex-

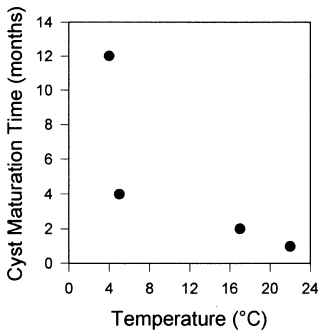

Fig. 8. Influence of temperature on the duration of cyst maturation (in months) for *A. tamarense*. Data are from the literature and this study (see text).

plain, as both types of cysts were treated similarly during the experiments. The rate of germination (ca. 20%) is close to the fraction of cysts that were present before the July 1992 increase in bottom sediments (from 58 to 210 cysts·cm^{-3}). This suggests that the fraction of cysts formed prior to July 1992 were mature and could germinate when removed from anoxic sediment storage conditions. As it was always the same lot of sediment that was repetitively sampled in the laboratory, similar rates of germination indicate the same proportion of mature cysts ready to excyst when put in oxic conditions. In the field, sediment reworking and inputs of new cysts probably diluted these mature cysts such that no significant germination was observed generally in 1992 and 1993 prior to the main germination event in late summer 1993. Unfortunately, microscopic observations were not detailed enough to note structural differences between these types of cysts. If this interpretation is valid, it suggests that once mature cysts are exposed to appropriate (e.g. oxic) conditions, they can germinate during all months of the year. This would lend support to the time-temperature hypothesis rather than to a biological clock controlling the occurrence of germination only at specific months of the year. This could also explain why the bloom of vegetative cells in early July 1993 was 1–2 months earlier than peak rates of cyst germination and the uncoupling between high germination rates in April 1991 (Fig. 3) and the absence of blooms observed for the northern part of the St. Lawrence Estuary in 1991 (M. Levasseur, DFO surveys data). If this is the case, then maximum cyst germination will have little control over the timing of blooms in the water column. Continual germination once cysts are mature would be a more important mechanism to provide seed population for the increase of vegetative cells of *A. tamarense* in the St. Lawrence, as has been reported for *Scrippsiella* spp. in Japan (Ishikawa and Taniguchi 1994).

Annual Variations of Cysts Germination Rates

An important aspect of our results that the time-temperature hypothesis fails to explain is the limited 2-month period of maximum germination observed both in the field and in the laboratory. Once maturation is reached, excystment seems to proceed for only about 60 days in our experiment (field and stored cysts). After this 2-month period, germination rates decrease abruptly. This is difficult to explain using the time-temperature hypothesis because cysts, once mature, should be able to survive and germinate after long periods in anoxic sediments (Dale 1983) and because the "mature" cysts in storage germinated over nearly 12 months. Thus, this limited time span for maximum cyst germination rate suggests that annual variations in germination rates also exist. Although these results advocate the existence of some sort of internal biological clock, a real test of this hypothesis would necessitate longer time series, showing reproducible germination at specific times of year over several years (cf. Anderson and Keafer 1987). Other results, such as increased germination under constant laboratory conditions and similarity in timing of excystment between field and stored cysts, could be explained as well by the time-temperature model as by the internal clock hypothesis.

In a cold environment such as the St. Lawrence estuary and gulf, compliance with the time-temperature model would probably result in a climate control of cyst germination for *A. tamarense* that would exert its action in at least two ways: (1) determining the duration of the maturation period (e.g. colder years, longer maturation) and (2) determining the timing of resuspension events (storms) that remove cysts from their anoxic environment. Endogenous control of excystment could also have a number of advantages, namely, to ensure the potential development of the population through cyst germination regardless of climatic conditions. Whether these two strategies apply here, as suggested by the results of this study, will need to be verified.

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