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Community assembly and seasonal succession of marine dinoflagellates in a temperate estuary: The importance of life cycle events

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Abstract

Dinoflagellate successional strategies and community structure were investigated in Perch Pond, a temperate estuary on the North American east coast by field surveys as well as laboratory investigations on growth rates, cyst maturation period, and cyst germination temperature thresholds. The dominant species were those predicted by the Smayda and Reynolds Rules of Assembly life form model. Three successional strategies were characterized: (1) holoplanktonic, (2) meroplanktonic (i.e., germinated from cysts), and (3) introduced by advection. The seasonal succession of the meroplanktonic dinoflagellates that were studied reflects the differential lengths of their mandatory dormancy periods as well as differences in their temperature thresholds or ''windows'' for germination. The holoplanktonic species present at low densities year-round in Perch Pond had a wide temperature tolerance for growth and thus did not need a cyst stage to survive seasonal extremes. Another non–cyst-forming species relied solely on advection to inoculate the salt pond; thus, blooms in successive years would be expected to be more stochastic in nature than for the other two strategies. The timing of cyst formation and population decline for meroplanktonic species corresponded on several occasions to an increase in grazers, suggesting that grazing might have contributed to bloom decline from cyst formation. This timing also suggests the possibility of encystment as a predator avoidance strategy. We suggest that seasonal succession of cyst-forming dinoflagellates is not stochastic. Instead, the appearance of these species in the plankton is predictable on the basis of measurable physiological responses to both endogenous and exogenous factors that they experience during dormancy and quiescence.

The factors regulating seasonal succession and bloom events of marine dinoflagellates, especially harmful algal bloom species, are of great interest both from an ecological perspective and for bloom management. Despite years of monitoring and experimental studies, successional patterns and bloom events are largely unpredictable. In contrast, marine diatom blooms have recurrent and expected patterns (Smayda 1980). Likewise, seasonal succession of phytoplankton in eutrophic deep lakes are largely predictable and can be explained by a combination of abiotic factors and grazing effects, as proposed in the Phytoplankton Ecology Group model (Sommer et al. 1986). For dinoflagellates, on the other hand, it is argued that the unpredictability of blooms is due to stochastic selection within a species pool (Huisman and Weissing 2001; Smayda and Reynolds 2001, 2003). Huisman and Weissing (2001) used modeling studies with hypothetical species to argue that even with full knowledge of species traits, it would be impossible to forecast the outcome of competition.

The key to understanding, if not entirely predicting seasonal succession of marine dinoflagellates is to examine the structure of the species pool. In a recent paper, Smayda and Reynolds (2001) showed that the Reynolds Intaglio Model, aimed at explaining freshwater phytoplankton assemblages, is also applicable to dinoflagellate bloom species. This concept states that phytoplankton form associations on the basis of adaptive strategies evolved to survive suboptimal conditions. The adaptive strategies include being either good competitors (C), stress-tolerant (S), or disturbance tolerant (R; the C-S-R concept; Reynolds 1988). Smayda and Reynolds (2001) showed that marine dinoflagellate species can be divided into these different strategies and further split into nine life form types, each having both a distinctive morphotype (size and shape) and habitat preference. Subsequently, Smayda and Reynolds (2003) introduced five Rules of Assembly for marine dinoflagellate communities. The Assembly Rules state that there are specific habitat conditions for specific life form types and that these are mainly a result of abiotic factor (turbulence and nutrient availability) selection. In other words, the selection of life forms (i.e., the potential groups that can bloom) is not stochastic and determines which species are present in the species pool.

The composition of a species pool in a specific habitat can be determined by three main sources or successional strategies: (1) tolerant species present at low numbers in the water column year-round, (2) introduction via advection, and

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(3) inoculation through germination of resting cysts. The third source of seeding a species pool is especially likely to be important in dinoflagellate succession. Many dinoflagellate species, including harmful algal bloom species, have a life cycle of an alternating planktonic vegetative state and a benthic resting cyst (Wall 1971; Anderson and Wall 1978; Dale 1983). One consequence is that the planktonic presence of these species is discontinuous (i.e., they can be present in high numbers for a short period and later disappear completely from the water column; Boero 1996). Consequently, life cycle transitions can have a major effect on succession patterns (Hansson 1995; McQuoid and Hobson 1995; Rengefors and Anderson 1998).

Life cycle transitions (i.e., encystment and excystment) thus determine when a cyst-forming dinoflagellate is present in the water column. Encystment determines the end of planktonic growth, typically induced through nutrient depletion in the laboratory (von Stosch 1973; Pfiester 1975; Anderson et al. 1985). Nevertheless, cyst formation in the field cannot generally be explained by nutrient depletion (e.g., Anderson et al. 1983; Kremp and Heiskanen 1999). Cyst germination, on the other hand, has been shown to be regulated by both endogenous and exogenous factors. Dinoflagellate cysts undergo a mandatory dormancy period (maturation) that lasts from days to months depending on species as well as storage temperature (Anderson 1980; Binder and Anderson 1987; Kremp and Anderson 2000). After dormancy, cysts enter a period of quiescence, during which excystment can occur given a specific favorable environment (Pfiester and Anderson 1987). Otherwise, the resting state continues. One of the main factors regulating germination for a variety of freshwater and marine dinoflagellates appears to be temperature (Huber and Nipkow 1922; Binder and Anderson 1987; Bravo and Anderson 1994). Rengefors and Anderson (1998) showed that the length of the cyst dormancy period and the temperature ''window'' conducive for germination together could explain the appearance in plankton of two freshwater dinoflagellates. This window defines the range over which germination occurs, with inhibition occurring at both low and high temperatures.

Here, we describe an effort to identify the successional strategies of nine dinoflagellate species occurring in an estuarine habitat subject to recurring dinoflagellate blooms. For the cyst formers, an additional aim was to determine whether the length of cyst dormancy (maturation) and the temperature window for germination could together explain the observed seasonal succession patterns. In addition, we applied Smayda and Reynold's life form types and Assembly Rules to our data, to assess whether these concepts could be used to explain the observed dinoflagellate community composition.

Materials and methods

Study site—Perch Pond is a shallow coastal embayment ~75,000 m^2 with a mean depth of 1.5 m at low tide and an average salinity of 25. It is located in Falmouth, Massachusetts, and connects to the ocean and nearby Vineyard Sound via Great Pond, a distance of ~2 km. Freshwater discharge at the northern end of Perch Pond significantly reduces the salinity in this small section compared with the majority of the embayment. At its southern point, Perch Pond is connected to Great Pond via a narrow, shoaled inlet, which restricts the exchange of water between the two ponds. Nonetheless, 40% of the salt pond's volume can be replaced by tidal mixing over a period of 24 h (Garcon et al. 1986). Perch Pond has relatively high nutrient levels (>12 μmol L^{-1} nitrate and 0.15 μmol L^{-1} phosphate in the period March–June; Anderson et al. 1983). Furthermore, the pond is subjected to recurrent blooms of the toxic dinoflagellate *Alexandrium tamarense* (Anderson et al. 1983) and other dinoflagellate species (Jacobson 1987).

Phytoplankton sampling—On about a weekly basis, from January through December 1983, water samples were collected at five equally spaced locations along a north–south transect through the central portion of the pond. Phytoplankton samples were also taken ~3 km away in Vineyard Sound for a separate study. For the Perch Pond samples, a polyvinyl chloride tube (4 cm internal diameter, 2.2 m length), whose end could be closed with a stopper was used to capture an integrated sample through the water column extending from the surface to ~20 cm above the bottom. The five integrated samples were pooled together in a carboy. On return to the laboratory, the carboy was gently mixed, and aliquots were removed and preserved for species identification and enumeration. At one station, near the middle of the pond, temperature and salinity measurements were taken with a YSI model 33 S-C-T meter (Yellow Springs Instrument).

A 50-ml subsample preserved in 2% (v/v) glutaraldehyde was settled overnight in a Utermöhls counting chamber and then viewed at ×250 magnification on a Zeiss inverted microscope. A sufficient volume of sample was examined so that all of the dominant microplanktonic organisms, with emphasis on dinoflagellate species, were properly identified and enumerated.

Cyst collection—Near-surface plankton from Perch Pond was collected in late summer by pumping seawater through an 80-μm Nitex mesh to remove large predators while concentrating the phytoplankton on a 20-μm mesh. In the lab, the 80–20-μm fraction was diluted with two parts filtered (0.45 μm) Perch Pond water, placed in 2-liter Erlenmeyer flasks, and enriched with nutrients (f/30 final concentration) and soil extract (5 ml L^{-1}). After 3 weeks of incubation under a 14 : 10 light : dark (LD) cycle at 15°C, flasks were harvested by scraping and resuspending the material off the bottom and concentrating it by sieving through a 10-μm mesh. The cyst material was split into two aliquots and stored at 2°C and 15°C in the dark until experiments were performed. Microscopic examination of that material revealed the production of three dominant cyst species: *Scrippsiella* species 1 (see following), *Gonyaulax rugosum* Wailes, and *Gyrodinium uncatenum* Hulburt (pending transfer or renaming).

Although vegetative cells of *Lingulodinium polyedrum* were present in the original plankton sample, cysts of that species were not produced in sufficient quantity for experimentation. As a result, *L. polyedrum* cysts were produced in culture by incubating vegetative cells at 26°C for 1 month

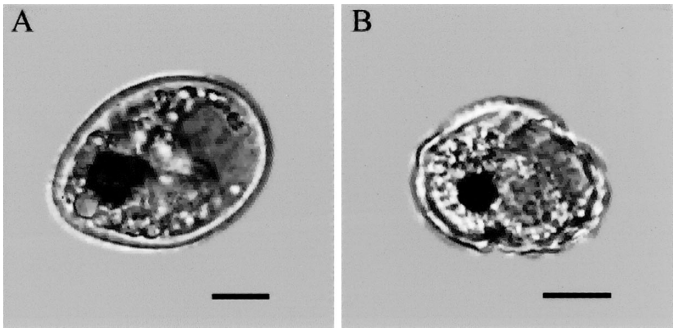

Fig. 1. Light micrographs of cysts of (A) *Scrippsiella* sp. 1 and (B) *G. rugosum.* Scale bar is 10 μm in both cases.

in f/2 growth medium with reduced (f/30) levels of nitrate and phosphate. Newly formed cysts were harvested from the bottom of a culture tube, split into two aliquots, and stored at 2°C and 15°C as above.

Cyst-forming species—Several of the cysts investigated here are well described in the literature. These include *A. tamarense,* described from Perch Pond by Anderson and Wall (1978); *Gonyaulax verior* (Matsuoka et al. 1988); *Gyrodinium uncatenatum* (Coats et al. 1984); *L. polyedrum* (originally described as *Lingulodinium machaerophorum* by Deflandre and Cookson (1955), with other descriptions by Wall (1967) and Kokinos and Anderson (1995)); and *Scrippsiella trochoidea* (Watanabe et al. 1982). The cyst of *Heterocapsa triquetra* has been reported by Braarud and Pappas (1951) and Yamochi and Joh (1986). This designation is problematic, however, because cysts have only been observed in culture. Some (K. Matsuoka pers. comm.), including ourselves, question whether this species produces true resting cysts, and we suspect that the reports by Braarud and Pappas (1951) and Yamochi and Joh (1986) are of temporary cysts.

Cyst descriptions and motile stage affinities for several other cyst types are not yet published, so the following details are provided.

Scrippsiella sp. 1 is an unornamented, elongate cyst ~20 × 15 μm in size, tapering to a slightly narrow, rounded point at one apex. Contents include a red accumulation body, starch grains, and small particles in Brownian motion (Fig. 1A). Two cell walls are clearly visible. This cyst is not any of those described by Lewis (1991) in her review of several cyst types in *Scrippsiella.* Nevertheless, the motile stage that emerges from this cyst is of typical *S. trochoidea* morphology with its prominent apical horn. It cannot be distinguished from other *S. trochoidea* morphotypes without detailed examination. Until further description of this species is available, it will be referred to simply as *Scrippsiella* sp. 1. *Scrippsiella* sp. 2 is similar to sp. 1, but produces an unornamented cyst that is round in shape. It also produces a typical *S. trochoidea* cell on germination.

G. rugosum produces an unornamented cyst with an irregular outer wall (Fig. 1B). Cyst size is ~15 × 10 μm. A red- or brown-pigmented accumulation body is visible inside the cyst, as are numerous small starch grains and small particles in Brownian motion. The motile cell that emerges is *G. rugosum* Wailes.

Cyst maturation—The dormancy or maturation periods of cysts of *Scrippsiella* sp. 1, *G. rugosum* Wailes, *G. uncatenum* Hulburt, and *L. polyedrum* Stein (Dodge 1989) were determined. Cysts were produced from either field samples or cultures as described above. Approximately every 3 weeks over a period of 216 d, 24 cysts of each species were isolated into 96-well tissue culture plates containing modified K media (10^{-3} μmol L^{-1} Tris in diluted Sargasso seawater base). Examination of the plate under an inverted microscope at ×100 confirmed the placement of one cyst in each well. To determine germination potential, plates were incubated at 22°C under cool white fluorescent light (200 μmol quanta m^{-2} s^{-1}; 14 : 10 LD). After ~2 weeks of incubation, each individual well was re-examined. Germination was determined to be positive if an empty cyst wall was present in the well or if swimming cells originating from the isolated cysts were observed. Germination was recorded as negative if the cyst remained intact and did not germinate after the incubation period. Germination was determined as the percentage of positive wells relative to the number of total wells containing isolated cysts. The maturation period was defined as the time needed for 50% of the cysts to germinate.

Germination—Temperature window experiment: The effect of temperature on germination was tested on mature cysts of *A. tamarense, G. verior, G. rugosum, G. uncatenum, L. polyedrum,* and *Scrippsiella* sp. 1. Cysts were incubated for 3 months at 20 different temperatures ranging between 0 and 30°C with the use of a temperature gradient bar (TGB) described by Watras et al. (1982). The device consists of an aluminum bar with a heat source connected at one end, and a cooling device at the other, thereby producing a temperature gradient. Holes in the bar allow for the insertion of four test tubes (diameter = 25 mm) for each of the 20 different temperatures along the gradient. Temperature measurements along the TGB were made in simulated sample tubes containing equivalent volumes of seawater and mud as the treatment tubes. A light source with cool-white fluorescent lamps was positioned underneath the test tubes and regulated to a 14 : 10 LD cycle. In this experiment, both light and dark treatments were performed along the gradient. The data presented here are only for dark conditions, however, because light is so rapidly attenuated in the sediments that most cysts in bottom sediments are essentially germinating in the dark, even if located in shallow waters (Anderson et al. 2005). To prevent light penetration in the dark treatments, the tubes and caps were wrapped in two layers of aluminum foil.

Cyst material for the experiments was collected from the surface sediments of Perch Pond and temporarily stored at 2°C in the dark. At the start of the experiment, the sediment was diluted 10 times with cold, filtered (0.45 μm) Perch Pond water. The sediment slurry was portioned into temperature-equilibrated tubes in the TGB that contained 15 ml of f/2 medium (Guillard and Ryther 1962) with reduced nutrients (3 μmol L^{-1} nitrate and 0.3 μmol L^{-1} phosphate). Frequent mixing and rapid removal of 2.5 ml of the slurry ensured that homogeneous slurry was dispensed into each tube.

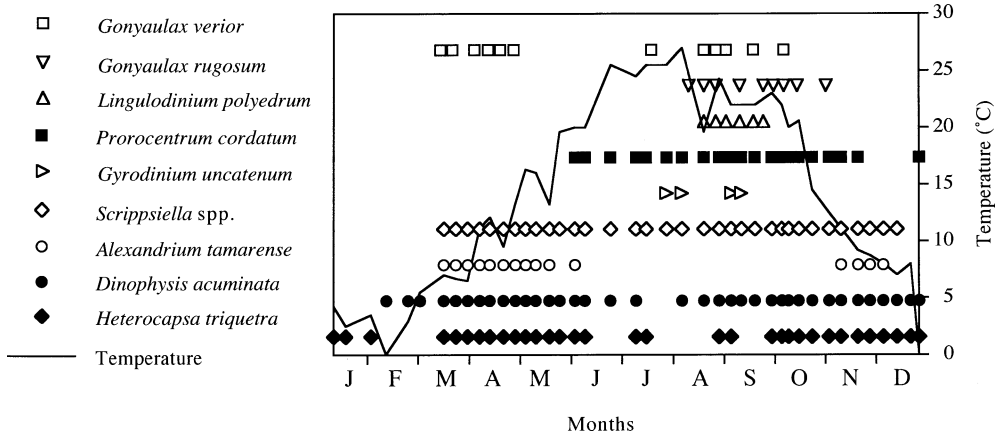

Fig. 2. The figure shows presence versus absence of dinoflagellate motile cells in water column samples from Perch Pond during 1983. Cyst formers are indicated by open symbols, whereas non–cyst formers are shown as closed symbols. Symbols indicate the presence of cells only, with no reference to concentrations.

Additional subsamples ($n = 5$) were immediately counted to provide an estimate of the initial cyst concentration for each species. At the end of the incubation period, all incubated sediment-containing tubes were harvested and counted within 1 week. The unpreserved samples were sonicated with a Branson Sonifier 250 at a constant 40-W output for 1 min, sieved to yield a clean 20–80-μm size fraction, and resuspended into 5 ml of filtered seawater (Anderson et al. 2003). A 1-ml subsample was loaded into a Sedgewick–Rafter counting slide, and each species was enumerated at $\times 100$ total magnification with a Zeiss light microscope. Germination was defined to have occurred when the final cyst number was less than the initial mean minus one standard deviation.

Threshold germination experiment: In a second set of germination experiments, the effect of a slowly increasing temperature on excystment was tested on mature cysts collected from Perch Pond. This experiment attempted to mimic the temperature rise in the spring in Perch Pond. Five replicate sediment cores (2–3 m water depth) were collected in December when the ambient bottom temperature was 7.5°C. A sediment slurry was made from the combined top 4 cm of those cores (347 ml) by diluting the sediment with cold, filtered Perch Pond seawater (total volume = 1,200 ml) and stored at 2°C in the dark. The slurry was portioned into 50 replicate 125-ml Erlenmeyer flasks by adding 5 ml of the slurry to 45 ml of cold f/2 medium with reduced nutrients (3 μmol L^{-1} nitrate and 0.3 μmol L^{-1} phosphate). As above, five replicates were counted to determine initial cyst abundance. The flasks were incubated at 200 μmol quanta m^{-2} s^{-1} with cool white fluorescent light initially starting at 2°C. Subsequently, the temperature was increased at the rate of 1.5°C per week up to 18.5°C (12 weeks total). Three flasks were harvested as replicates each week for microscopic counting and processed as above. Cyst germination threshold was assessed by analyzing cyst number decline compared with initial values. The threshold temperature was determined statistically with a *t*-test by SPSS 10 statistical software for Macintosh.

Temperature growth optimum—The temperature growth optima of *H. triquetra* (clone Ht984), *A. tamarense* (clone GtPP-A), and *G. uncatenum* (clone Gyro-3) were determined with the TGB setup described above. Cultures were grown in triplicate tubes in f/2 medium as prepared in Anderson et al. (1984). They were equilibrated for at least 10–20 generations at 18 of 20 temperatures available in the TGB before growth rate determination. Growth was monitored daily by measurement of in vivo fluorescence with a Turner Designs, model 10 fluorometer (Brand et al. 1981). Growth rates at each temperature were determined by the change in fluorescence from the exponential portion of the growth curve according to the equations of Guillard (1973).

Zooplankton sampling—Zooplankton were collected on a weekly basis during the same time period as the phytoplankton (*see* Jacobson [1987] for details).

Results

Seasonal succession—The autotrophic dinoflagellate community in Perch Pond was dominated by ten species (Fig. 2): *A. tamarense* (Lebour) Balech, *Dinophysis acuminata* Claparède and Lachman, *G. rugosum* Wailes, *G. verior* Sournia, *H. triquetra* (Ehrenberg) Stein, *G. uncatenum* Hulburt, *L. polyedrum* (Stein) Dodge, *Prorocentrum cordatum* (Ostenfeld) Dodge (previously *Prorocentrum minimum* (Pavillard) Schiller), and *Scrippsiella* spp. (at least two species, 1 and 2). Of these, some were present in the water all year round, whereas others appeared only periodically (Fig. 2). The Vineyard Sound phytoplankton community was dominated by diatoms and cryptophytes, with very few dinoflagellates (data not shown).

The early winter community (January–February) with water temperatures <5°C, was characterized by a declining bloom of *H. triquetra* (Fig. 3A). The only other species within our group of study organisms that was detected in the water at the time was *D. acuminata*. By March, *A. tamarense* and *G. verior* started increasing in the water (Fig. 4), where-

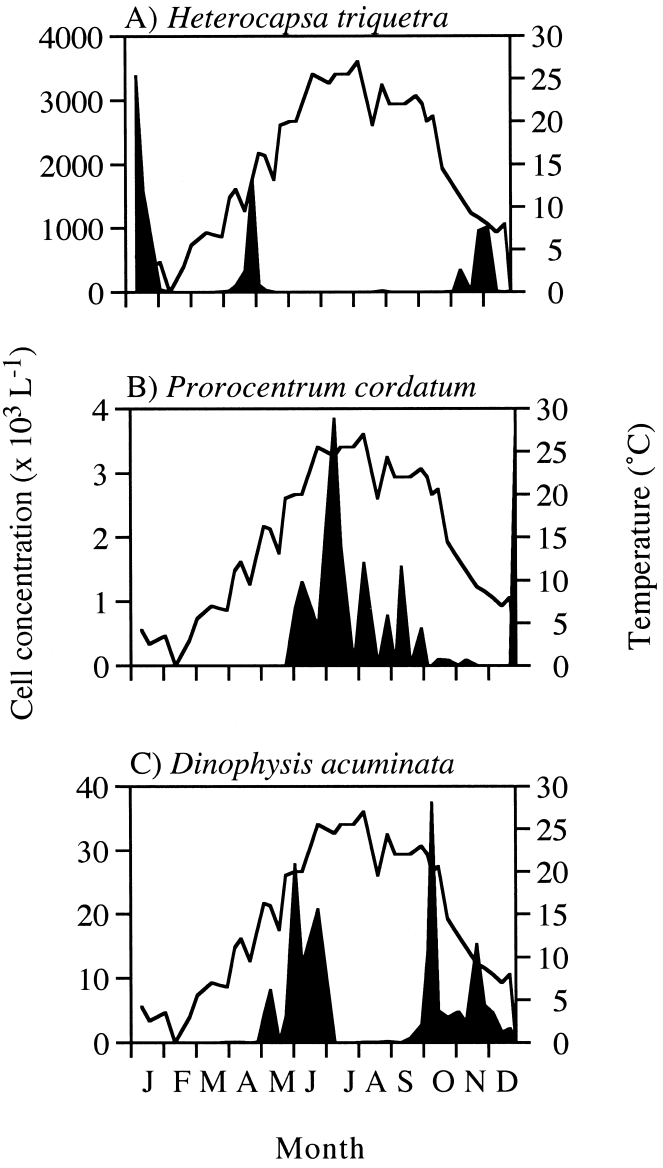

Fig. 3. Dynamics of non–cyst-forming dinoflagellate motile cells in the water column (black shaded area) coupled with temperature (solid line) development in Perch Pond during 1983.

as all other targeted dinoflagellates were either below detection or present in low numbers. During this period, the water temperature increased rapidly from 5°C to 15°C. The peak of both *A. tamarense* and *G. verior* abundances occurred in early May, reaching almost 16,000 and 15,000 cells L^{-1}, respectively (Fig. 4A,F). Simultaneously, *H. triquetra* had a second bloom, peaking at 1.75×10^6 cells L^{-1}, and *Scrippsiella* spp. reached a cell maximum of 15,000 cells L^{-1}. The *A. tamarense* and *G. verior* peaks declined rapidly, and by early June, cells of these species were not detected in the water column.

As the spring blooms declined, *D. acuminata* increased and reached maximum densities at >25,000 cells L^{-1} in June as the water temperature approached 20°C (Fig. 3C). *P. cordatum* was first observed in the salt pond at that time and reached up to 3,800 cells L^{-1} by the beginning of July when a maximum temperature of 25°C was recorded (Fig. 3B). Cell numbers of the other species remained low or undetectable in July, except for a short bloom of *Scrippsiella* spp., reaching 38,000 cells L^{-1} in the first week of August (Fig. 4B). In July and August *G. uncatenum* was also detected, reaching a maximum of almost 17,000 cells L^{-1} in early August (Fig. 4C). The end of August was characterized by a new peak of *Scrippsiella* spp. (91,000 cells L^{-1}), when water temperature was still high (20–25°C).

At the end of August, *L. polyedrum* and *G. rugosum* were first detected in the water. *L. polyedrum* peaked at 16,000 cells L^{-1} at the beginning of September (Fig. 4D,E). By the end of September a new bloom of *Scrippsiella* spp. was detected, this time reaching up to 325,000 cells L^{-1}. Simultaneously, *G. rugosum* reached a maximum of 970,000 cells L^{-1}. A week later, *D. acuminata* had a second bloom that peaked at 37,000 cells L^{-1} (Fig. 3C).

In November, several fall peaks occurred, starting with *Scrippsiella* spp. the first week (20,000 cells L^{-1}), followed by *D. acuminata* (15,000 cells L^{-1}) together with a second appearance of *A. tamarense* (400 cells L^{-1}) in mid-November. By the end of November, a third major peak of *H. triquetra* reached 1×10^6 cells L^{-1}. During November, the temperature declined from 12°C to 8°C. In December, the water temperature had dropped to zero, and only cells of *H. triquetra* and *P. cordatum* were detected in the water (Fig. 2).

Dormancy period—The dormancy or maturation periods were determined for *G. rugosum, G. uncatenum, L. polyedrum,* and *Scrippsiella* sp. 1. *G. rugosum* germinated equally well at both storage temperatures (2°C and 15°C), in contrast to the other species. The maturation period for this species was relatively short, being only 20 and 15 d following storage at 2°C and 15°C, respectively (Fig. 5A). *G. uncatenum,* on the other hand, had a maturation period close to 60 d in duration at 15°C storage, with the first germination observed on day 22 (Fig. 5B). The germination success of cysts stored at 2°C never exceeded 30%. *L. polyedrum* showed yet another pattern. In this species, no germination was observed for cysts stored at 2°C. With 15°C storage, however, the maturation period was determined to be 55 d (Fig. 5C). At 2°C storage, the germination of cysts of *Scrippsiella* sp. 1 was also very low, reaching no more than 30% after 141 d of incubation at favorable growth temperature (Fig. 5D). At 15°C storage, cysts started germinating after only 22 d (15%), and by 119 d, all cysts had germinated. The maturation period was determined to be 55 d.

Germination—The temperature window for germination was determined for *A. tamarense, G. rugosum, G. verior, G. uncatenum, L. polyedrum,* and *Scrippsiella* sp. 1. In *A. tamarense,* germination occurred between 6°C and 21°C, with no germination above or below this temperature range during the allotted incubation time (Fig. 6A). *G. rugosum* had a window for germination between 8°C and 25°C. No germination occurred below or above (26–29°C) this interval (Fig. 6B). *G. verior* had a temperature window similar to *G. rugosum,* 6°C to 25°C (Fig. 6C). Above 25°C, little or no germination had occurred. The pattern for *G. uncatenum*

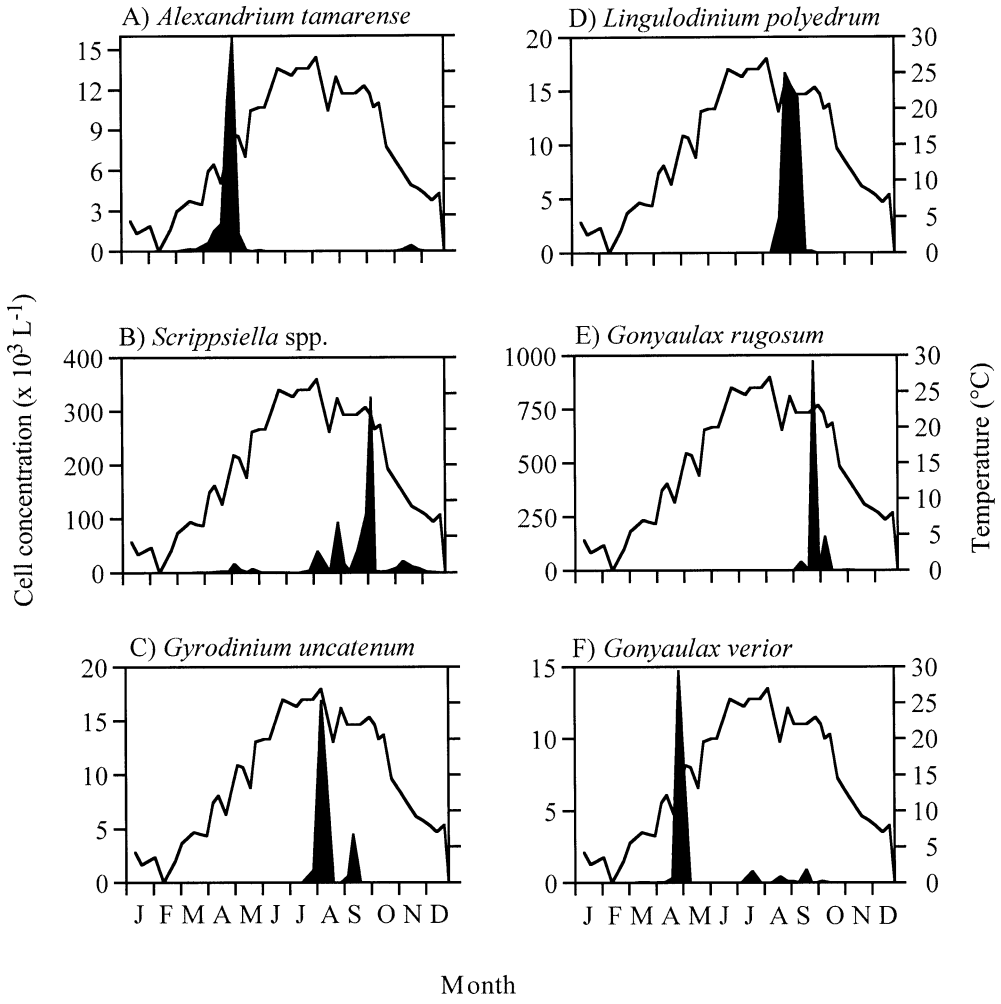

Fig. 4. Dynamics of cyst-forming dinoflagellate motile cells in the water column (black shaded area) coupled with temperature (solid line) development in Perch Pond during 1983.

suggests that cysts germinated both above 13°C and below 6°C (Fig. 6D) because the observed cyst abundances after incubation at these temperatures were less than the initial, preincubation values. However, at temperatures of ≤8°C, numerous dead cysts were found in the samples, suggesting that many cysts did not survive the low temperatures. Thus, we suggest a temperature window of ≥13°C (at least to 29°C) for *G. uncatenum.* A similar pattern was observed for *L. polyedrum,* in that many dead and empty cysts were found at low temperatures, again suggesting that the low cyst abundance observed below 18°C was because of death rather than germination. At ≥19°C, germination must have occurred (Fig. 6E). At low temperatures, dead cysts of *Scrippsiella* sp. 1 were also encountered. In this species, we defined the germination window to be ≥13°C (Fig. 6F).

In the germination threshold experiment, the lowest temperature suitable for germination during a simulated seasonal warming was defined as the temperature at which cyst number was significantly (95% level) lower than the initial cyst counts. Cysts from *A. tamarense, G. rugosum, G. verior, G. uncatenum, L. polyedrum,* and *Scrippsiella* spp. 1 and 2 were analyzed. For *A. tamarense* and *G. verior,* the threshold for germination occurred at 6.5°C (*t*-test, $p = 0.03$ and 0.015, respectively; Fig. 7A,C), and cyst number continued to decline up to 18.5°C (maximum temperature). In *G. rugosum,* the temperature threshold for germination also occurred at 6.5°C ($p = 0.015$). However, between 14°C and 17°C, the cyst number did not differ significantly from the initial counts, whereas it did again at 18°C ($p < 0.001$; Fig. 7B). *G. uncatenum* had a temperature threshold for germination at 9.5°C ($p = 0.007$; Fig. 7D). At two temperatures (14°C and 15.5°C), the cyst number was on the borderline for significance at the 5% level ($p = 0.053$). In contrast to the other species, no threshold temperature could be determined for *L. polyedrum* because cyst numbers never differed significantly from initial cyst counts (Fig. 4E). This reflected the large variance in both the initial cyst counts and the abundances after incubation. *Scrippsiella* sp. 1 had a temperature threshold of 9.5°C ($p = 0.046$) and continued to decline with time and increased temperature. At 14°C and 15.5°C, cell numbers did not differ significantly from initial counts at the 5% level but did at the 10% level (Fig. 7F). *Scrippsiella* sp. 2 had a temperature threshold as low as 5°C ($p = 0.004$; Fig. 7G).

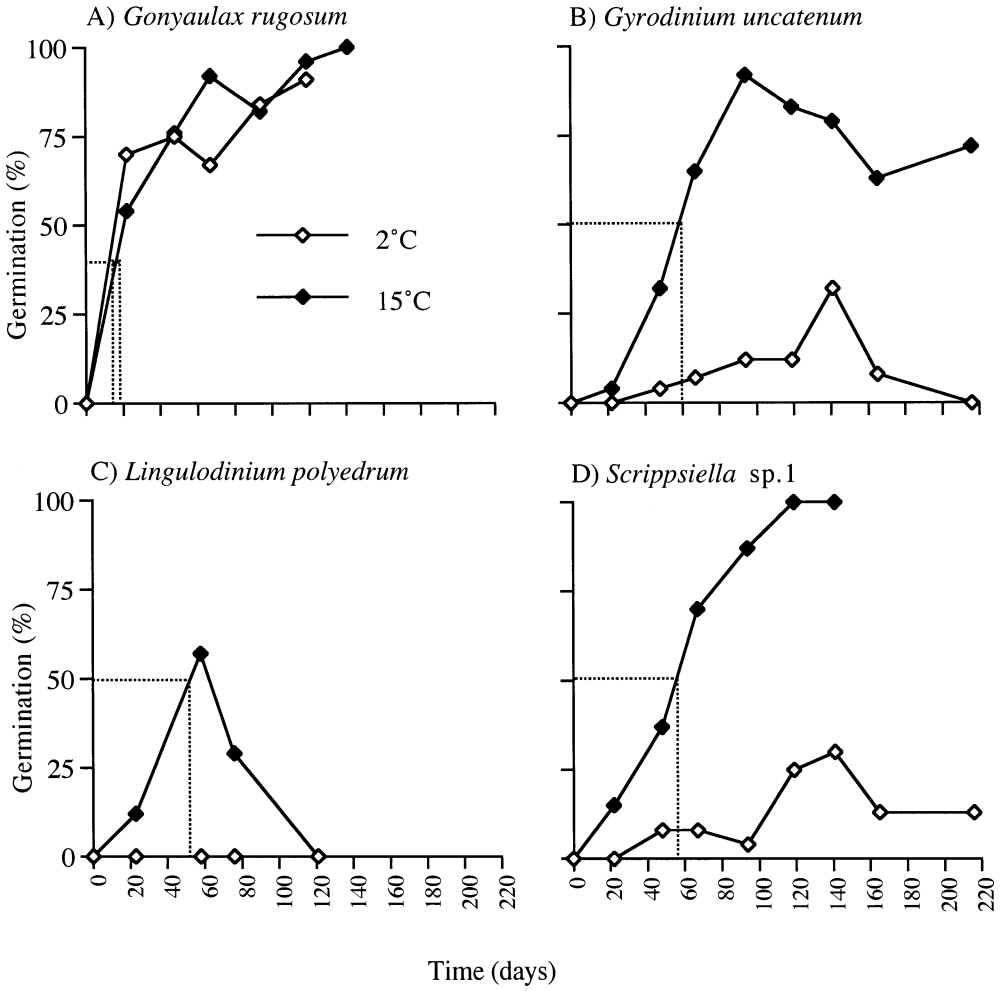

Fig. 5. Germination of cysts stored at 2°C and 15°C over time (200 d). Maturation period was defined at 50% germination and is indicated with a dotted line.

Temperature growth optimum—The temperature growth optimum experiment with *A. tamarense* showed that this species could grow between 2.5°C and 22.5°C (Fig. 8). However, the growth rate was slow at low temperatures and was optimal between 18°C and 22°C, when it reached 0.9 divisions d^{-1}. *G. uncatenum*, in contrast, started growing only above 12°C and had its optimum between 22°C and 28°C, with 0.5 divisions d^{-1} (Fig. 8). *H. triquetra* had an even wider temperature tolerance than *A. tamarense*, and grew between 2°C and 26°C. At 27.5°C, growth ceased altogether (Fig. 8). The temperature optimum for growth was between 16°C and 22°C, at which the growth rate was slightly in excess of 1 division d^{-1}.

Zooplankton—The potential grazers on dinoflagellates in Perch Pond were copepods, annelid larvae, and the ciliate *Favella.* Zooplankton abundance was very low during the winter months (December–February; Fig. 9). Annelid larvae formed the first peak in the spring (April), reaching >200 individuals L^{-1}. The abundance of both copepods and annelid larvae was highest in June when both groups contributed, with >300 individuals L^{-1}. Zooplankton abundance declined throughout the summer, but in October–November, *Favella* formed another peak of high biomass, which reached >700 individuals L^{-1}.

Discussion

In this study, the seasonal succession of several cyst-forming dinoflagellate species is shown to reflect the differential lengths of their mandatory dormancy periods as well as differences in the temperature thresholds and ''windows'' for germination. In other words, the germination, and thereby initiation of blooms of the cyst-forming species we studied, do not appear stochastic. Instead, the appearance of these species in the plankton is predictable on the basis of measurable physiological responses to both endogenous and exogenous factors that they experience during dormancy and quiescence. The temporal patterns of species abundance also highlights alternative successional strategies for non–cyst formers, including a reliance on advection from neighboring waters or a broad thermal tolerance that allows species to populate the pond throughout the year without reliance on a cyst stage. Furthermore, our data revealed that the dominant

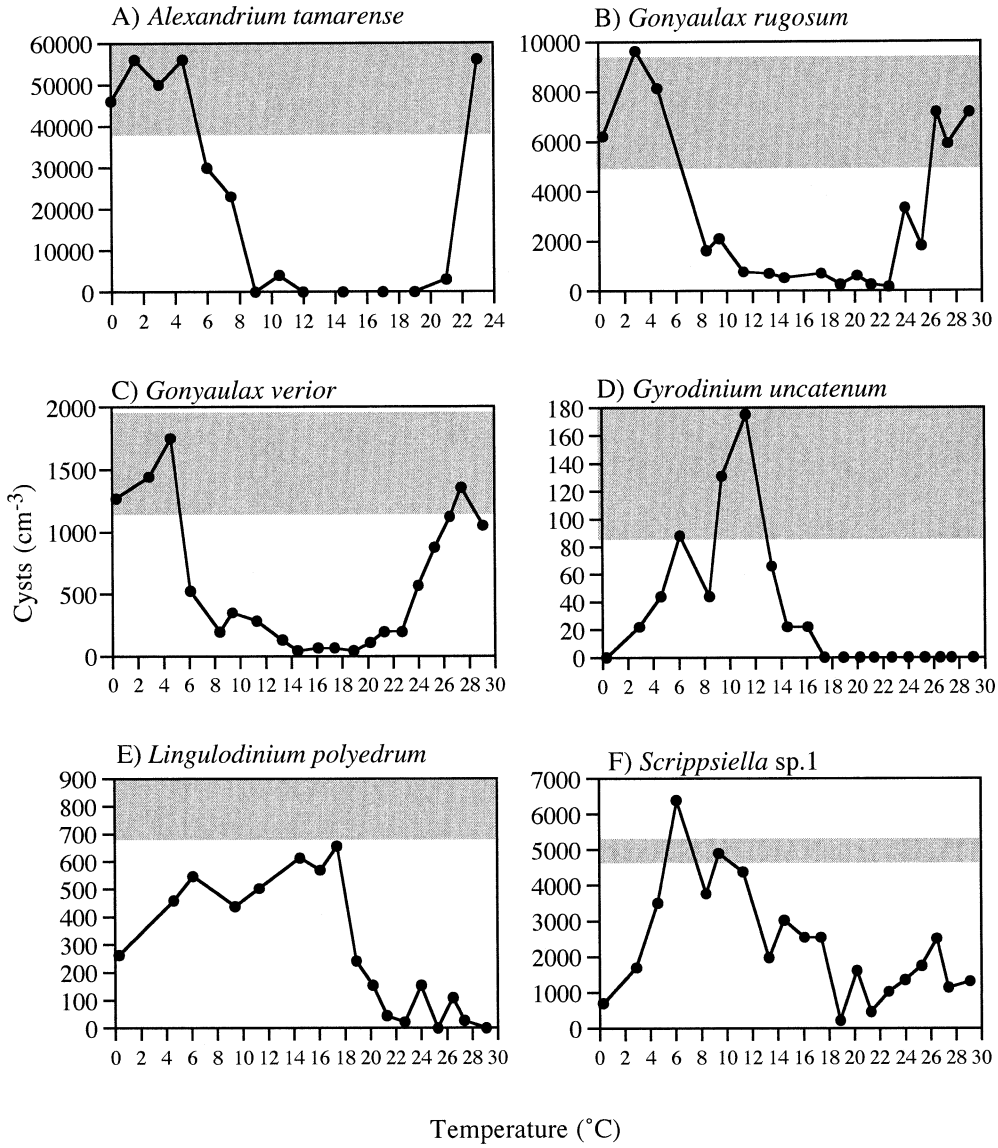

Fig. 6. Temperature window for germination showing germination as a function of temperature at which cysts were incubated for 3 months. The shaded area shows the mean initial cyst number ± one standard deviation. Germination was defined to occur when cyst number was below the shaded area.

species were those predicted by the life form model of Smayda and Reynolds (2001).

Seasonal succession—In Perch Pond, we found that the presence of many of the dominant dinoflagellates could be explained by three successional strategies: (1) tolerant, holoplanktonic species (non–cyst formers) present in low numbers in the water column year-round; (2) species introduced from adjacent waters via advection; and (3) meroplanktonic species inoculated through germination of resting cysts.

The majority of the dominant dinoflagellates found in Perch Pond are cyst producers (*A. tamarense, G. uncatenum, G. rugosum, G. verior, L. polyedrum, Scrippsiella* spp.). *H. triquetra* is reported to produce a cyst (Braarud and Pappas 1951), though we were never able to observe the resting cells of this species during this study despite careful examination of many different sediment samples. The presence and temporal sequence of the blooms of the cyst-forming species in Perch Pond can largely be explained through the timing of excystment for each species, regulated by the length of its mandatory dormancy period and the temperature range over which its cysts could germinate. The timing of the termination of blooms is more complex and was not directly addressed in these studies. These excystment results complement those found for the freshwater dinoflagellates *Peridinium aciculiferum* and *Ceratium hirundinella* (Rengefors and Anderson 1998) and the brackish water species *Scrippsiella hangoei* (Kremp and Anderson 2000) and represent the first comprehensive examination of cyst-forming dinoflagellate emergence and succession in marine waters.

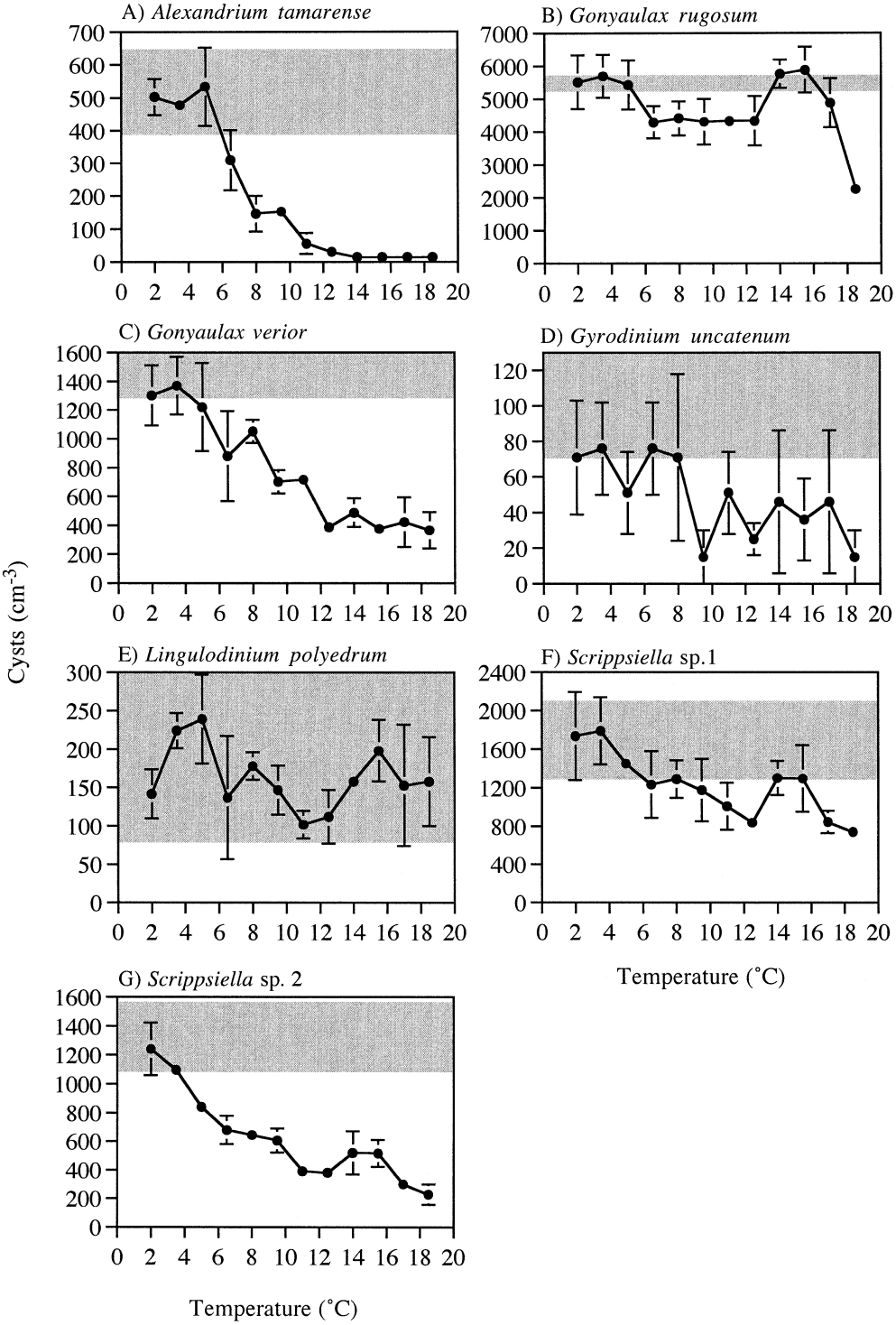

Fig. 7. Threshold temperature for cyst germination in an experiment mimicking the water temperature increase in Perch Pond during the spring. The shaded area shows the mean initial cyst number ± one standard deviation. Germination was defined to occur when cyst number was below the shaded area.

Laboratory germination studies reveal the mechanisms underlying the recurrent and bimodal pattern of *A. tamarense* blooms in Perch Pond. In both this and in former studies (Anderson and Morel 1979; Anderson et al. 1983), *A. tamarense* appeared as vegetative cells in March when water temperature was 6°C, consistent with the observed threshold for germination of *A. tamarense* cysts at 6–6.5°C (Table 1). The spring bloom declined quickly in mid-May in all of

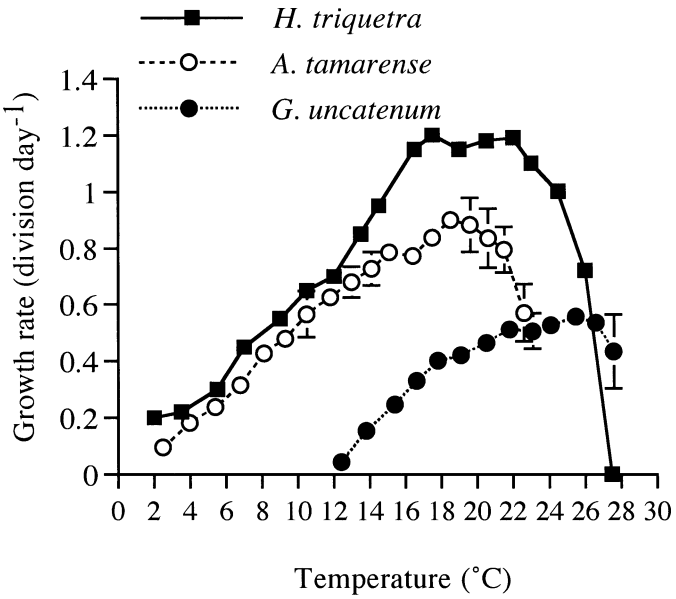

Fig. 8. Growth rate (divisions day^{-1}) of *A. tamarense, G. uncatenum,* and *H. triquetra* as a function of temperature. Error bars in *A. tamarense* and *G. uncatenum* signify standard deviation. Standard deviation values are not available for *H. triquetra.*

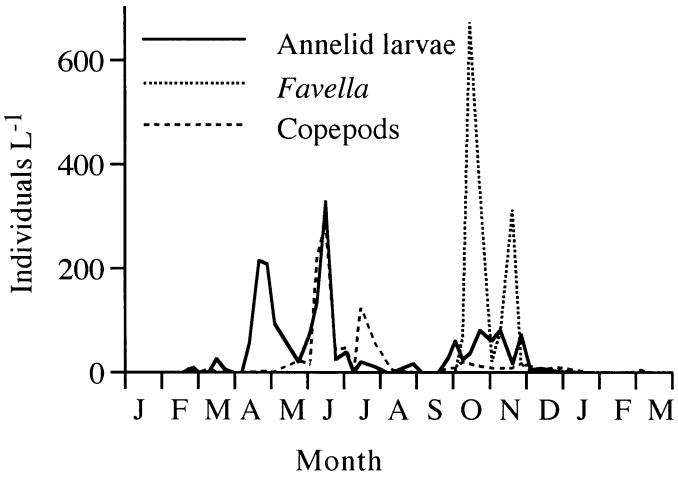

Fig. 9. Abundance of copepods (*Acartia*), ciliates (*Favella*), and annelid larvae, which are potential predators of dinoflagellates, in Perch Pond during spring 1983.

these studies, coinciding with cyst formation (Anderson et al. 1983). The long dormancy period of the cysts of this species (103 d after storage at 15°C; Table 1) explains why *A. tamarense* did not reappear in the water during the summer. These newly formed cysts should, however, have been mature and ready to germinate by the end of summer, but *A. tamarense* cells were not observed until much later (early November). The temperature window for *A. tamarense* excystment accounts for this delay because the period between mid-August and early November had water temperatures well above 20°C—a temperature shown to inhibit germination of mature *A. tamarense* cysts (Fig. 6A). By November, water temperatures had dropped to 12°C, well within the permissive windows for germination and for growth; thus, cells appeared in the water column. The decline of the fall bloom by December is most likely due to low temperature and light levels. *A. tamarense* can grow at 2°C (Fig. 8), but its growth rate at that temperature is very low (<0.1 divisions d^{-1}). Grazing, and of course cyst formation, were also probable factors in bloom decline.

The pattern of *Scrippsiella* spp. is more difficult to explain because this species complex is composed of several species that are indistinguishable in their vegetative phase by the

Table 1. Summary of the maturation period, as well as the temperature thresholds and window of germination for cyst-forming dinoflagellates. The temperature of maximum growth for several of these species is also included, as determined from the literature.

Species	Dormancy period (50% germination) (d) at 15°C	Threshold for germination (°C)	Germination window (°C)	Temp. for maximum growth rate (°C)
Cyst formers				
Alexandrium tamarense	103*	6.5	6–21	20†
Gonyaulax rugosum	15	6.5	8–25	
G. verior	n.a.	6.5	6–24	
Gyrodinium uncaternum	60	9.5	≥13	
Lingulodinium polyedrum	55	No germination	≥19	
Scrippsiella sp. 1 (clear)	55	9.5	≥13	
Scrippsiella sp. 2 (round)	n.a.	5	n.a.	
S. trochoidea	25‡	n.a.	≥4‡	10–18‡
Non–cyst formers				
Dinophysis acuminata				
Heterocapsa triquetra				17–23
Prorocentrum cordatum				

* Extrapolated from Anderson (1980).
† From Anderson et al. (1984).
‡ From Binder and Anderson (1987).

cell-counting methods employed here (Lewis 1991). The cell abundance data in Fig. 2 is thus a composite of several *Scrippsiella* species, two of which form cysts that were studied here and that were shown to have somewhat different characteristics. On the basis of the information available for *Scrippsiella* sp. 2, we know that cysts germinate at ≥4°C, explaining the early appearance of this species in March when water temperature was ~7°C. The remaining pattern, however, can only be unraveled once methods to accurately identify the different vegetative stages are available.

G. uncatenum had two peaks in abundance, in the beginning of August and September, and was not present in Perch Pond during the rest of the year. This species had a relatively high germination threshold (9.5°C) and temperature window (≥13°C), consistent with its absence from the water column in the spring. Water temperature was 22–25°C when it was first observed. A dormancy period of 60 d explains why a second bloom could not occur in the fall because by then, the temperature had dropped below 13°C. Furthermore, our growth rate data show that this species does not grow below 12°C and has a temperature optimum for growth above 22°C, consistent with its occurrence in the late summer.

An even more extreme case of a temperature-sensitive species is *L. polyedrum.* This species also occurred during a very limited period (September) when the water temperature was between 20°C and 25°C. The temperature gradient experiment (TGB) showed that *L. polyedrum* cysts germinated only above 19°C, which coincides with its late summer bloom in Perch Pond. Furthermore, the dormancy period of the cysts was 55 d, thus preventing germination of newly formed cysts in the fall and winter. We note that *L. polyedrum* occurs in coastal waters throughout the world, in many cases in waters colder than those in which it bloomed in Perch Pond. It would appear that the local populations of *L. polyedrum* are adapted to life in a shallow, warm environment.

It is also of note that the cysts of both *G. uncatenum* and *L. polyedrum* appeared to die at storage temperatures below 8°C or 10°C (Fig. 6D,E). This raises an interesting issue—namely that Perch Pond is not an ideal habitat for these two dinoflagellates because bottom temperatures drop to 4°C and lower during winter. Even though some cysts would survive, the mortality of many newly deposited cysts would deplete the inoculum for subsequent blooms. It could be that the local strains of these species are at the northern edge of their biogeographic distributions and are encountering temperature extremes that limit further expansion.

The plankton dynamics of *G. verior* also showed a pattern that could be predicted from our germination data. This species had a broad temperature window for germination (6–24°C) and, as expected, appeared in the water in early March at low temperatures and formed both a spring bloom and several minor late summer and fall blooms. It is likely that *G. verior* has a long dormancy period because it was absent from the water between May and the end of July.

In contrast to the consistencies noted above between laboratory and field data, germination results for *G. rugosum* could not explain its dynamics in Perch Pond. The temperature gradient bar experiments showed that this species germinates between 8°C and 25°C. It also had a short maturation time (15 d). Nevertheless, it was absent from the water except from mid-August until early November. A spring peak of this species would be expected, but this was not observed for two consecutive spring periods, 1983 (Fig. 4E) and 1984 (data not shown). One possible explanation lies with the temperature threshold data (Fig. 7B), which shows only limited germination below 18°C. These data are consistent with the observed bloom in late summer but are inconsistent with the broader germination window suggested by the temperature gradient bar results. Clearly, experiments on the cysts of this species need to be repeated. On the basis of the plankton data, we would predict that this species will prove to be one that requires warm temperatures for germination and thus will bloom in the summer or early fall.

H. triquetra was present in water samples from Perch Pond throughout the year (Fig. 2). We have not observed cysts of this species, either directly in the sediment or following sediment incubations, but older studies by Braarud and Pappas, (1951) and Yamochi and Joh (1986) report a cyst stage. The latter authors observed vegetative cells emerging from sediment samples and assumed that a resistant resting cyst stage was present, but it is possible that temporary cysts or protoplasts were present instead. The lack of any literature report of a true *H. triquetra* resting cyst calls into question whether such a life history stage exists for this species, and others agree that the cysts reported in the literature might be temporary cysts (K. Matsuoka pers. comm.). For the purposes of this study, we must nevertheless qualify our conclusions with the possibility that this species did produce cysts in Perch Pond and that the observed bloom dynamics reflect the characteristics of those resting stages. We note, however, that *H. triquetra* has such a wide temperature range for growth that refugee cells can exist at all times in the water, even under the ice during the winter, ready to seed a bloom when a niche opens. This species grows maximally between 17°C and 23°C (Table 1) but also grows at temperatures of ≤2°C (Fig. 8). A cyst stage is therefore not needed for overwintering or oversummering but would be of value in terms of genetic recombination and adaptation. The decline in the *H. triquetra* blooms in Perch Pond might be from grazing by the heterotrophic dinoflagellate *Oblea rotunda* (Jacobson 1987). Jacobson (1987) observed that a bloom of *O. rotunda* followed a massive bloom of *H. triquetra* in both 1983 and 1984. Moreover, Jacobson (1987) also found that *O. rotunda* feeds on *H. triquetra* in culture.

D. acuminata was also present in Perch Pond samples throughout most of the year, forming two to three peaks at widely different temperatures. *D. acuminata* does not grow in culture, so laboratory-derived temperature tolerance limits are not available (Maestrini 1998). This species clearly has a very broad temperature tolerance, however, because it was observed throughout the year in Perch Pond, at both winter and summer temperature extremes (Fig. 2). *D. acuminata* does not form a resting cyst (Matsouka and Fukuyo 2003), so inoculation from the sediments is not a possibility. An inoculation to Perch Pond through advection can also be ruled out because this species was not seen in samples from nearby Vineyard Sound, as was also the case for *H. trique-*

tra. These two species appear to use a survival strategy that relies on broad temperature tolerance.

D. acuminata formed a major bloom in mid-May, whereas other spring-blooming species declined sharply at that time. An explanation for this successional pattern might be grazing pressure. Simultaneous with the decline of *A. tamarense, Scrippsiella,* and *G. verior,* copepods and polychaetes reached maximal abundance (Jacobson 1987; Fig. 9). Thus, a new niche opened up, with low dinoflagellate cell concentration (= reduced competition) and high predation pressure. One species that responded was *D. acuminata,* which is known to be essentially ungrazed by the copepod *A. hudsonica* and the polychaete *Polydora* sp. in Perch Pond (Turner and Anderson 1983).

Cysts have not been described for *P. cordatum* (Matsuoka and Fukuyo 2003), and no potential cysts were found in Perch Pond sediment. Because this species was not observed in the water in winter and spring, we consider it to have been introduced by advection. Indeed, small numbers of *P. cordatum* cells were observed in the nearby Vineyard Sound samples. Although it cannot be excluded that populations of the cyst-forming species highlighted here were also advected into the pond, the presence of viable cysts of these species in Perch Pond sediments suggests that germination provided the inocula for the salt pond populations. Furthermore, none of these species were observed in the Vineyard Sound samples.

A question that still remains unanswered and was not addressed experimentally in this study is why cyst formation and a sharp population decline occur in *A. tamarense, Scrippsiella* spp., and *G. verior* in mid-May. Anderson et al. (1983) found that cyst formation occurred in *A. tamarense* in late spring in several Cape Cod salt ponds despite apparently favorable nutrient, temperature, and salinity regimes in both 1980 and 1981. However, in this study, the increase of grazers corresponded to the timing of cyst formation and population decline of the species mentioned (Fig. 9). An alternative, and for marine dinoflagellates, new hypothesis is that predation pressure induces cyst formation, as has been suggested for freshwater dinoflagellates (Rengefors et al. 1998). This is clearly speculative but needs to be explored as a possible contributing factor in the dynamics of marine cyst-forming dinoflagellates.

Assessment of the community composition in Perch Pond in relation to Smayda and Reynolds' Rules of Assembly—Smayda and Reynolds (2003) stated that within a certain habitat, the conditions select for specific life forms (Assembly Rule I), and that the life forms are selected primarily on abiotic factors (turbulence, irradiance, nutrients). Consequently, the dinoflagellate composition in Perch Pond should be composed of life forms associated with this habitat. Perch Pond, is a nearshore, shallow habitat with relatively high nutrient levels (Anderson et al. 1983) but is well-mixed because of efficient tidal flushing (Garcon et al. 1986). According to Smayda and Reynolds (2001), this nearshore habitat should be dominated by species characterized as invasive, small, competitive, and fast growing (types I and II; Smayda and Reynolds 2001). *H. triquetra, S. trochoidea,* and *P. cordatum* all represent type II life forms. Both *H. triquetra* and *S. trochoidea* were abundant in Perch Pond, and *P. cordatum* also formed blooms. Thus, the presence of these species agreed well with the Assembly Rules. Likewise, offshore species typically found in tropical oceans characterized by low nutrient availability did not occur in Perch Pond.

Several species not typical of nearshore habitats, such as *A. tamarense,* were also abundant in Perch Pond. How are these findings consistent with the Assembly Rules? *A. tamarense* is considered by Smayda and Reynolds (2001) to be a type IV (= Frontal Zone Taxa) species, a species that is tolerant to shear/stress (S-strategist) and adapted to acquire sufficient light in turbulent conditions. *A. tamarense* blooms in open coastal areas and frontal zones (e.g., Townsend et al. 2001), and it flourishes in enclosed, nutrient-enriched habitats, such as Perch Pond (e.g., Anderson et al. 1983). In addition, in Perch Pond, the blooms of *A. tamarense* have been observed at the frontal convergence formed near the inlet of the flood tide (Garcon et al. 1986), which is consistent with its tolerance to turbulence. Nevertheless, we favor the view that the strains of *A. tamarense* that inhabit the Cape Cod salt ponds are genetically different from those that occur in open coastal waters and that were used by Smayda and Reynolds (2001) to categorize this species. This contention is supported by regional analyses of toxin composition among strains (Anderson et al. 1994) and of the presence or absence of an endogenous annual clock to control cyst germination (Anderson and Keafer 1987). Our results suggest that the Perch Pond strain of this species relies more on cysts for reinoculation of the water column than on tolerance to turbulence; thus, it is found in shallow habitats where a meroplanktonic existence is favored.

We have documented three inoculation strategies that can lead to dinoflagellate blooms in the Perch Pond system. The first strategy is based on extreme tolerance to temperatures (the hidden flora concept), such that the holoplanktonic species (e.g., *D. acuminata* and possibly *H. triquetra*) can maintain a presence in the water column at all times and respond to favorable growth conditions as they occur. The second strategy is more complex and involves life history transformations through the formation and germination of benthic resting cysts. The succession of these meroplanktonic dinoflagellate species (e.g., *A. tamarense, G. verior, G. rugosum, L. polyedrum, Scrippsiella* spp., and *G. uncatenum*) is predictable on the basis of measurable thresholds and physiological responses during dormancy and quiescence. The final inoculation strategy is via simple advection of cells from adjacent waters (e.g., *P. cordatum*). This introduces cells that do not require cysts or extreme environmental tolerances for survival and is clearly a stochastic process that would lead to highly variable bloom timing in successive years.

The dinoflagellate dynamics observed in Perch Pond suggest that Smayda and Reynolds' Rules of Assembly are consistent with much of the observed community composition. However, we question the assignment of the Perch Pond strain of *A. tamarense* as a type IV species and do not agree that seasonal succession is completely stochastic within a given species pool. Instead, our data indicate that several factors that are endogenous (the duration of cyst maturation)

and exogenous (temperature thresholds and windows for germination) govern the presence and succession of cyst-forming dinoflagellate species. Within these cyst formers, the apparent random succession of species is seen to be understandable and predictable.

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