Physiology and Bloom Dynamics of Toxic *Alexandrium* Species, with Emphasis on Life Cycle Transitions

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1. Introduction

Of the many genera of phytoplankton associated with harmful algal blooms (HABs), the dinoflagellate genus *Alexandrium* includes the largest number of toxic species. At least 8 species in this genus (*A. acatenella*, *A. catenella*, *A. cohorticula*, *A. fundyense*, *A. ostenfeldii*, *A. minutum*, *A. tamarense* (= *A. excavatum*) and *A. tamiyavanichi)* produce saxitoxin - the suite of compounds associated with paralytic shellfish poisoning (PSP) in humans (Cembella, this volume). An eighth species, *A. monilatum*, produces a poorly characterized toxin capable of killing fish but which appears to be unrelated to the PSP toxins (Aldrich *et al.* 1967).

This diversity of toxic species is matched by a diversity among strains of those species with respect to temperature requirements, toxicity, bioluminescence, and genetics. Some, such as *Alexandrium tamarense*, can be found in sub-Arctic and temperate zones (e.g., Taylor 1984; Cembella *et al.* 1988) as well as in tropical regions (Reyes-Vasquez *et al.* 1995). Although some of these globally distributed strains possess the same external morphology, they are genetically different. A comprehensive overview of the biogeography and population biology of the *Alexandrium* genus is provided elsewhere (Scholin (this volume), Scholin *et al.*, (1995), and Gallagher (this volume)). Here the focus will be on the autecological features that underlie many *Alexandrium* blooms, based predominantly on the small number of species that have been well-studied in the laboratory and the field. This effort will necessarily emphasize life cycle transformations and their quantitative effect on bloom dynamics, for it is in this specific area that *Alexandrium* blooms have been especially well-characterized and where differences from other HAB species become apparent. A concept that will recur throughout this discussion is that *Alexandrium* blooms have a "life-span" - a relatively short period of time in which these species are in the water column as motile vegetative cells. *Alexandrium* cells do not persist throughout the year, as do those of species such as *Gymnodinium breve* (Steidinger *et al.* this volume). Most *Alexandrium* species can be considered "background" bloom species, in that they are often outnumbered by co-occurring phytoplankton. High-biomass, monospecific *Alexandrium* blooms that discolor the water do occur, such as those of *A. minutum* in south Australia (Hallegraeff *et al.* 1988), but these are the exceptions rather than the rule.

2. The *Alexandrium* life cycle

The life histories of most *Alexandrium* species that have been studied involve an

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alternation between asexual and sexual reproduction (Fig. 1). Repeated divisions (binary fission) lead to the proliferation of motile, vegetative cells as blooms develop. This is an asexual process that terminates when sexuality is induced. Sexuality begins with the formation of gametes which fuse to form swimming zygotes (planozygotes) which in turn become dormant, resting cysts (hypnozygotes). A useful compilation of the morphology of known *Alexandrium* cysts can be found in Bolch *et al.* (1991). Hereafter, the term "cyst" will refer to hypnozygotes formed through sexuality. Most species also produce another resting stage called a "temporary cyst" when motile, vegetative cells are exposed to unfavorable conditions such as mechanical shock or a sudden change of temperature or salinity. When conditions become favorable again, temporary cysts quickly re-establish a vegetative, motile existence. The temporary resting state thus allows the cells to withstand short-term environmental fluctuations.

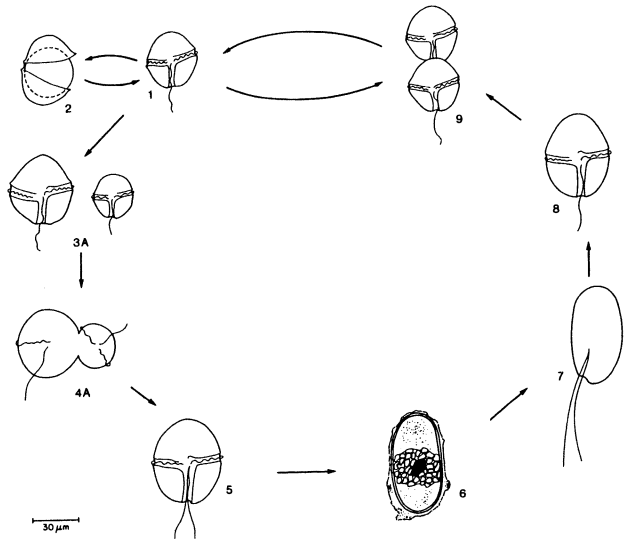

Fig. 1. Life cycle diagram of *Alexandrium tamarense*. Stages are identified as follows: (1) vegetative, motile cell; (2)temporary or pellicle cyst; (3) anisogamous "female" and "male" gametes; (4) fusing gametes; (5) swimming zygote or planozygote; (6) resting cyst or hypnozygote; (7&8) motile, germinated cell or planomeiocyte; and (9) pair of vegetative cells following division. Adapted from Anderson *et al.* 1996.

2.1 Mating mechanisms

In *A. tamarense*, both unequal-sized and equal-sized gametes have been reported (anisogamy and isogamy; Turpin *et al.* 1978; Anderson 1980), and generally, two different mating types are needed for a successful mating. As demonstrated by Destombe and Cembella (1990), however, self-recognition and fusion of gametes of *A. tamarense* clones does occur, although the zygote produced is not viable. These authors also demonstrated that there were not just two mating types in an assemblage of *A. tamarense* clones (i.e. that mating types appear not to be strictly bi-polar but rather are part of a continuum of mating affinity). Fusion between different "species" within *Alexandrium* is also possible, depending of course on one's definition of a species. Anderson *et al.* (1994) showed that *A. fundyense* and *A. tamarense* could

produce viable cysts, but they also argue that these two species should be considered variants of a single species. Attempts to mate *A. tamarense* with *A. catenella* have not been successful to date (Y. Ishida, pers. comm.).

Little is known of the manner in which gametes locate each other and fuse. Sawayama *et al.* (1993) showed that concanavalin A (a lectin) and tunamycin (an inhibitor of glycoprotein synthesis) prevented sexual attachment in *A. catenella.* A cell-to-cell recognition system involving agglutinins similar to those found in *Chlamydomonas* is thus a possibility for *Alexandrium.* The location of these recognition sites on the cell surface and the details of the biochemical interaction between receptor and ligand remain unknown.

2.2 Induction of sexuality

In *Alexandrium* cultures, sexuality has been induced by the imposition of nutrient limitation - typically a decrease in nitrogen or phosphorus (Turpin *et al.* 1978; Anderson *et al.* 1984; Anderson and Lindquist 1985). Non-optimal temperature or light, and cessation of growth in nutrient-replete batch cultures due to over-crowding or carbon limitation do not result in cyst formation to any significant extent, yet there are occasional reports of spontaneous cyst formation in high nutrient cultures of other dinoflagellates (Morey-Gaines and Ruse 1980; S. Blackburn, pers. comm.). Yoshimatsu (1981) performed crossing experiments with *A. catenella* in nutrient-replete medium containing 1,400 μM nitrate and 11 μM phosphate, and observed zygote formation. The latter value is well above levels where Anderson *et al.* (1984) found virtually no cyst formation in *A. tamarense* cultures.

Numerous issues concerning the induction of sexuality remain to be resolved. The nutrient levels at which sexuality is induced are not known, nor is it known whether ambient concentrations or the size of internal nutrient pools triggers the transition. Regulatory mechanisms also remain a mystery. The pathways involved are especially perplexing since nitrogen-, phosphorus-, and iron-limitation can all induce sexuality (Anderson *et al.* 1984; Doucette *et al.* 1989). Finally, if gametes are formed when nutrients are depleted, how then does the zygote obtain sufficient nutrients to support prolonged dormancy, quiescence, germination and growth? One possibility is that gametes form before internal pools are completely exhausted. Another is that nutrient depletion is not required, but that sexuality is instead controlled by an endogenous clock similar to that shown to regulate excystment in *A. fundyense* (Anderson and Keafer 1987). Alternatively, perhaps nutrient uptake occurs during the planozygote stage. The latter suggestion derives from nutrient-limited laboratory batch cultures in which nutrients are exhausted very quickly. A large proportion of the cells fuse and become planozygotes but are arrested at that stage and never become cysts (Anderson *et al.* 1985; Anderson and Lindquist 1985). It is not known why this occurs, but one possibility is that only the first planozygotes to form are able to complete the transition to cysts, perhaps because they are able to take up nutrients before concentrations become too low in the batch cultures to permit significant uptake.

2.3. Dormancy and quiescence

The planozygotes that develop after the fusion of gametes swim for up to a week before falling to the sediment as resting cysts to begin dormancy. "Dormancy" is defined as the suspension of growth by active endogenous inhibition, and "quiescence" as the suspension of growth by unfavorable environmental (i.e. exogenous)

conditions. Thus dormant cysts cannot germinate, even under optimal environmental conditions, whereas quiescent cysts are competent to germinate, but are inhibited from doing so by some environmental factor. *Alexandrium* cysts typically proceed through a mandatory dormancy period before they are capable of germination. The duration of this interval, which is generally considered a time for physiological "maturation", varies considerably among dinoflagellate species (12 hrs to 6 months; Anderson *et al.* 1996). For a single species, this dormancy can vary with storage temperature (Anderson 1980). Despite its critical importance to the ecology of all cyst-forming *Alexandrium* species, the duration of this interval is known only for *A. tamarense*. Cysts of *A. tamarense* stored at 4 °C mature in 4-6 months, whereas storage at warmer temperatures shortens the mandatory interval to 1-3 months (Anderson 1980). The duration of this process can have a significant effect on the timing of recurrent blooms, as cysts with a long maturation requirement may only seed one or two blooms per year, whereas those that can germinate in less time may cycle repeatedly between the plankton and the benthos and contribute to multiple blooms in a single season. Hallegraeff (this volume) argues that the short maturation time of *Gymnodinium catenatum* reduces the effect of cysts on motile cell dynamics, as the cysts germinate gradually throughout the year rather than as a single, synchronized pulse.

Once a cyst is mature and the dormancy interval is complete, the resting state will continue if external conditions are unfavorable for growth. Thus a quiescent cyst cannot germinate until an applied external constraint (such as cold temperature) is removed. The factors that break the quiescence of mature cysts are not known for most *Alexandrium* species. A primary stimulus for excystment of *A. tamarense* from temperate waters (Anderson and Morel 1979) and *A. minutum* from Australia (Bolch *et al.* 1991) is a shift in temperature to favorable levels, as occurs in seasonal warming or cooling. Cysts stored at cold temperatures remain quiescent until the temperature is increased. Likewise, cysts held at high temperatures maintain quiescence and germinate only when temperatures decrease to a favorable level (Anderson and Morel 1979; Anderson 1980). The absence of germination at these cold and warm extremes defines a permissive temperature "window" within which quiescent cysts will germinate, but outside of which they will continue their resting state. For *A. tamarense* from Cape Cod, the window ranges from 5 to 21 °C (Fig. 2). This does much to explain the occurrence of two discrete *A. tamarense* blooms each year in shallow embayments on Cape Cod, one in the spring and one in the fall (Anderson and Morel 1979). Mature, over-wintering cysts germinate when temperatures warm in the spring and a bloom results, depositing new cysts in the sediments. Those cysts need a month or more to mature, at which time the salt pond water is above 21 °C and thus is too warm to permit germination. As water temperatures decrease in the fall, germination is again possible, leading to another bloom. Temperature thus can maintain quiescence for extended periods, determine the duration of dormancy after cyst formation, synchronize or entrain cyst populations for more uniform germination, and initiate the excystment process. It is thus a major factor in the dynamics of dormancy, quiescence and germination of *Alexandrium* species, or at least for temperate species which have been the only ones studied in this regard. Species or strains from tropical waters where temperature fluctuations are less dramatic might not be as reliant on temperature cues, but this speculation awaits further research. In support of this concept, *A. catenella* strains from subtropical Sydney Harbor (winter temperature >15 °C) have a dormancy period of only 1-2 weeks (G. Hallegraeff, pers. comm.).

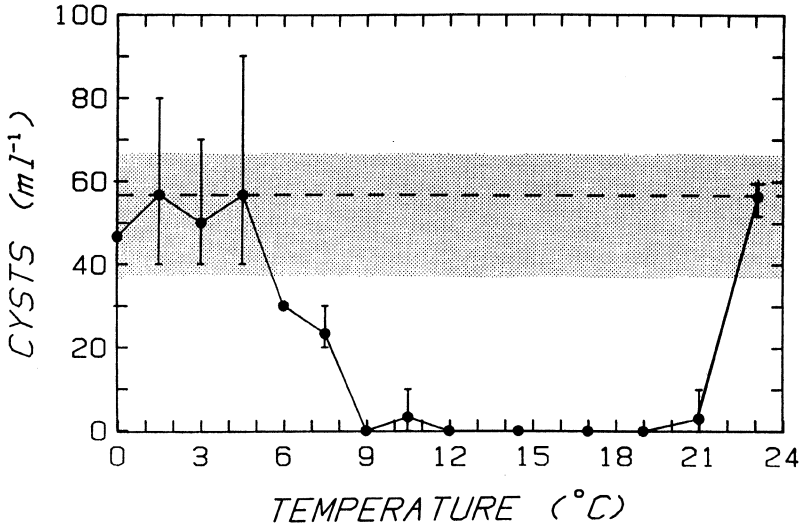

Fig. 2. Temperature "window" for *A. tamarense* cyst germination. Sediment from Perch Pond, (Cape Cod, MA USA) was incubated for three weeks at the indicated temperatures and germination determined by counting the remaining intact cysts. The means of three replicate counts of ungerminated cysts are plotted, and error bars indicate the range of those replicates. Dashed line and shaded area represent mean of initial counts ± SE. Differences between the initial mean and the final counts are assumed to represent germinated cysts.

The effects of other environmental factors on dormancy and excystment are less-studied, not just for *Alexandrium*, but for all dinoflagellate species. Nutrient concentrations do not seem to affect the success or rate of germination (Cannon 1993), but light, salinity, and oxygen are important to varying degrees. *Alexandrium tamarense* cysts did not germinate after 7 weeks of incubation in total darkness, although the presence of chlorophyll fluorescence in those cysts suggests that germination would eventually have occurred (Anderson *et al.* 1987). Light is thus not required for germination, but does accelerate the process. Likewise, very few Australian *A. minutum* cysts germinated in complete darkness, but high rates were observed at light levels as low as 20 $\mu E\ m^{-2} sec^{-1}$ (Cannon 1993) Germination of *A. minutum* occurred between 14-26 PSU, the salinity range of the waters in which the organism occurs in south Australia.

Oxygen (or the lack thereof) can have a dramatic effect on cyst germination. Among *Alexandrium* species, only *A. tamarense* has been tested thus far (Anderson *et al.* 1987), but it and most other dinoflagellate species examined have an absolute requirement for oxygen during germination. Cysts that are buried deep in the sediment can thus remain quiescent for years, their fate being either eventual death if anoxia persists, or germination should they be transported to the sediment surface or overlying water. The longevity of buried cysts is difficult to determine, but quantitative cyst profiles, radioisotope measurements, and a simple model suggest that the half-life of *A. tamarense* in anoxic sediments is approximately 5 years (Keafer *et al.* 1992).

2.3.1 Endogenous clock regulation

The dormancy and germination story can be more complicated than indicated above. Some dinoflagellate species can alternate between dormancy and quiescence through time, with the interval when germination is possible being determined by an endogenous, annual clock. Mature, quiescent cysts of *A. tamarense* from sediments in the Gulf of Maine did not germinate to any significant extent during fall and winter months, but did excyst in high numbers during the spring and summer (Anderson and Keafer 1987). An endogenous clock of this type would allow cysts to germinate even when deposited in deep waters where seasonal environmental cues such as temperature or daylength are small or nonexistent. In contrast, germination of *A. tamarense* cysts from shallow Cape Cod salt ponds showed no sign of clock control, suggesting that strains inhabiting those waters were regulated by the external environment (Anderson and Keafer 1987). This strategy makes sense in hindsight, given the variability in shallow, estuarine waters where a long winter or early spring could be detrimental to an organism restricted by an internal clock to a fixed time for germination.

The existence of endogenous annual clocks has not yet been confirmed in other strains or species of *Alexandrium*, but several studies hint that such control might exist. Kim (1994) suggest that endogenous clock-controlled germination can explain the variability in germination success seen in cysts from Jinhae Bay, Korea. In the St. Lawrence estuary in Canada, a study of cysts isolated from a sediment sample kept in the laboratory under constant conditions again suggests that there is an endogenous germination rhythm in those cysts, that the period is about one year, and that the germination "window" lasts about two months (Perez *et al.* in press).

It is noteworthy that in the Perez *et al.* study, as well as in those of Kim (1994) and Anderson and Keafer (1987), a small percentage (10 - 20%) of the cysts germinated during the interval when the clock control was inhibiting germination of the bulk of the cyst population. Two possibilities for this duality in response are suggested. First, the cyst population in the stored sediment samples may not represent a single genotype, but instead may include at least two strains with different germination physiology. Alternatively, clock control may not be operative in all cells within a population, perhaps as an adaptive strategy to ensure that germination is variable within that population. Work is clearly needed to better characterize the nature of endogenous control of *Alexandrium* germination, given the importance of excysted cells to overall bloom dynamics.

3. Bloom dynamics

Studies of the bloom dynamics of *Alexandrium* species are remarkably few, despite the importance of toxic species within this genus. The "tamarense" group of *Alexandrium* has been best-studied in this regard, but even there, most studies are descriptive and lack autecological detail. The following discussion will therefore focus on two hydrographic systems which are commonly associated with *Alexandrium* blooms and which demonstrate the extent to which life cycle transformations influence bloom dynamics in different habitats. The two regimes to be addressed are shallow, restricted embayments with localized blooms, and open coastal waters with widespread blooms.

3.1. Localized blooms in salt ponds, bays, and lagoons

Alexandrium blooms frequently occur in shallow salt ponds and coastal bays (e.g., Anderson *et al.* 1983; Su *et al.* 1993; Ho, and Hodgkiss 1993; Takeuchi *et al.* 1995;

Giacobbe *et al.* 1996). In some instances, blooms in these waters are simply a nearshore manifestation of large-scale coastal blooms, but in many cases, such as those discussed below, the blooms are localized, "point-source" events, where the coupling between cysts in the sediments and blooms in overlying waters is direct.

3.1.2. Seedbeds and excystment dynamics

A common assumption is that cyst "seedbeds" provide the inoculum for many *Alexandrium* blooms. The concept of a discrete seedbed is not appropriate in many locations, however, due to widespread distribution of cysts and the likelihood that germination will occur over a large area. The strongest evidence for localized cyst accumulations being linked to subsequent blooms comes from salt ponds on Cape Cod, MA., U.S.A. (Anderson *et al.* 1983), where blooms and PSP are confined to the immediate vicinity of the embayments where cyst are abundant (Anderson *et al.* 1982). Cysts are also important in larger bays, but the linkage is more difficult to quantify.

Encystment and excystment dynamics are determined by the interplay between physiological processes (such as maturation) and environmental controls on dormancy and quiescence. In the temperate Cape Cod salt ponds, *A. tamarense* cysts remain quiescent during the winter months since bottom temperatures are often near 0 °C and are thus below the lower limit of the permissive temperature "window" for germination (Fig. 2). Germination is possible once waters warm to 5-6 °C (Fig. 3), but the only cysts which excyst are those at the sediment surface where oxygen is available (Anderson *et al.* 1987). This temperature threshold is first marked by the appearance of red fluorescence due to chlorophyll that is synthesized in germinating cysts (Anderson and Keafer 1985), and subsequently by the appearance of distinctive germling cells (planomeiocytes) in the water column (Anderson *et al.* 1983). No fluorescence is observed in cysts from anoxic sediments (Fig. 3).

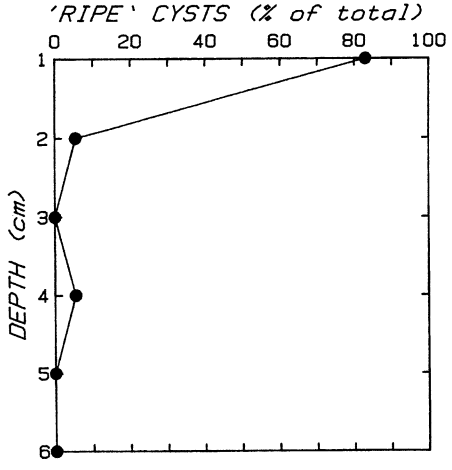

Fig. 3. Vertical profile of *Alexandrium tamarense* cyst fluorescence in Perch Pond. Cysts showing visible red chlorophyll fluoresence are plotted as a percentage of the total *A. tamarense* cysts present at each depth in the core.

The number of cysts that contribute to the bloom initiation process is small relative to the total number in the sediments. One reason is shown in Fig. 4 which depicts a set of vertical profiles of *A. tamarense* cysts from different locations in Perch Pond, a Cape Cod salt pond. Although the vertical distributions differ throughout the

embayment, one pattern is clear - on average, only 20% of the cysts are in the top cm where oxygen is available. As seen in Fig. 3 from the same embayment, "ripe" or fluorescing cysts were only observed in the surface layer. These values, in conjunction with areal estimates of *A. tamarense* cyst abundance in Perch Pond, allow a calculation of the input or inoculum from cyst germination. Given an average density of 4.5 x 10^7 cysts m^{-2} (in the top 6 cm of sediment; Anderson *et al.* 1982) over an area of approximately 30,000 m^2, there would be 1.35 x 10^{12} total cysts in the sediments. Only 20% are in the oxygenated surface layer and only 80% of those will germinate based on their fluorescence (Fig. 3). The total input of planomeiocytes from germination would thus 2 x 10^{11} cells. If all cysts germinated simultaneously, this would yield a concentration of planomeiocytes in the water column of 3,500 cells l^{-1}, which would be a significant inoculum indeed. Germination is not simultaneous, however, but occurs over about a one-month interval in Perch Pond (Fig. 5). Thus the input of planomeiocytes would be about 100 cells l^{-1} per day, on average.

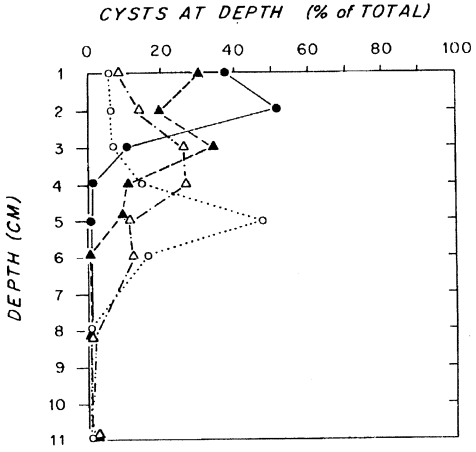

Fig 4. Vertical distribution of *Alexandrium tamarense* cysts from four different locations in Perch Pond.

Direct counts of the number of planomeiocytes inoculated into the water column at the onset of blooms are difficult to obtain since planomeiocytes divide to produce vegetative cells and thus the sustained inoculum of new cells from the sediment is not be easy to detect after a few generations. Anderson *et al.* (1983) observed up to 90 planomeiocytes l^{-1} (30% of the motile cell population) early in a Perch Pond bloom of *A. tamarense*. Takeuchi et al (1995) suggest that *A. catenella* cysts in Tanabe Bay (Japan) sediments germinate to yield an inoculum of 10-100 planomeiocytes l^{-1}. The consistency between these initial planomeiocyte concentrations in Tanabe Bay and the observed and calculated concentrations for Perch Pond is noteworthy, but extrapolation to other systems should be approached with caution.

Anderson *et al.* (1983) concluded that factors which lead to "bloom" versus "non-bloom" years within Cape Cod salt ponds depend more on the growth of the planktonic population, than on the size of the cyst inoculum. This may be true for that study, but the conclusion probably does not apply to all situations. A low inoculum of 10 cells l^{-1} from excystment would require three extra divisions and several more weeks of growth to achieve a bloom with biomass equivalent to one

started from 100 cells l^{-1} at the onset of a growth season. The quantitative dynamics of cyst populations clearly requires further study.

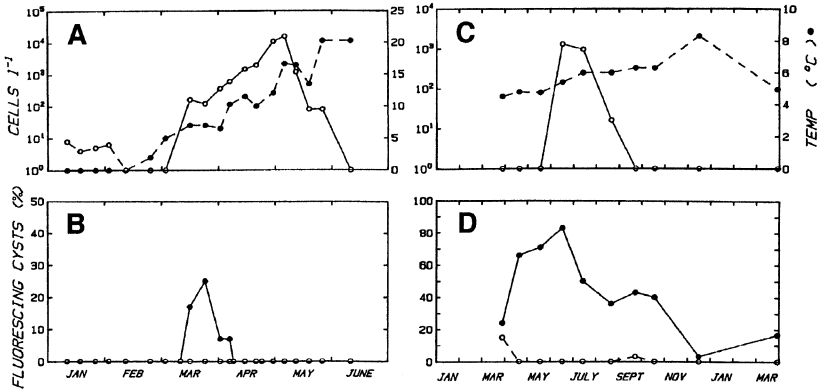

Fig 5 Motile cell and cyst dynamics two different hydrographic and environmental regimes - Perch Pond, a shallow Cape Cod embayment (A,B), and Sta. 29 near Cape Ann, a 160 m deep station in the Gulf of Maine (C,D). (A) Perch Pond motile cells (o) and temperature (●); (B) Perch Pond cyst fluorescence in the top cm of sediment (●) and at 5-6 cm depths in anoxic sediment (o); (C) Gulf of Maine motile cells (o) and temperature (●); and (D) Gulf of Maine cyst fluorescence in the top cm of sediment (●) and at 5-6 cm depths in anoxic sediment (o). From Anderson and Keafer (1985).

3.1.3. Bloom development and encystment

No effort will be made to generalize on the bloom dynamics of motile *Alexandrium* populations in shallow embayments other than to emphasize that such blooms are heavily dependent upon local hydrography and the manner in which it interacts with cell behavior, especially vertical migration. Shimada *et al.* (1996) demonstrated how *A. tamarense* blooms in Funka Bay, Japan are initiated from *in situ* cyst populations, with the distribution and abundance of the motile cells being markedly affected by the timing and strength of the Oyashio current as it flows into the bay. Studies of *A. minutum* in a Mediterranean lagoon by Giacobbe *et al.* (1996) demonstrated that the spring appearance of the species coincided with enhanced rainfall and freshwater runoff, and with stabilization of the water column. Watras *et al.* 1982 conducted laboratory growth studies and used the results to paramaterize a simple model which indicated that for the blooms in Cape Cod salt ponds, the development of *Alexandrium* populations depends solely on salinity-dependent temperature regulation of cell division rates. The same model produced a poor prediction of Bay of Fundy *Alexandrium* bloom dynamics, presumably because physical forcings are more influential in population accumulation in those open, tidally stirred waters.

Another example of physical/biological coupling and the importance of stratification in embayments was seen in Perch Pond, a salt pond which has a shallow entrance sill that restrict outflowing water to the low density surface layer (Anderson and Stolzenbach 1985). The diel vertical migration pattern of *A. tamarense* kept cells below that depth during the night, and even when the cells migrated close to the surface during the day, they remained deep enough to avoid transport out of the embayment with the outflowing surface layer (optimal light levels of 100-150 μE m^{-2}

sec^{-1} occur at 1.5 m or deeper). A density-driven exchange mechanism rapidly flushes water from these salt ponds, but the residence time of the *Alexandrium* cells is much longer due to the limited vertical extent of the migration. This coupling between organism behavior and the hydrography of the system restricts the extent to which cells and cysts can colonize adjacent waters and allows *Alexandrium* populations to accumulate to levels where toxicity becomes dangerous.

The duration of the blooms that have been followed in bays and salt ponds is generally two to three months or less (Anderson *et al.* 1983; Han *et al.* 1992; Shimada *et al.* 1996; Takeuchi *et al.* 1995). Giacobbe *et al.* (1996) describe an *A. minutum* bloom in a Mediterranean lagoon over a six month period, but the concentrations were at bloom levels for only two months - April and May. In Cape Cod, most of the bloom development occurs at water temperatures which are non-optimal for rapid growth of vegetative cells. Perch Pond isolates of *Alexandrium* grow fastest at 15-20 °C in the laboratory, but once the water reaches those temperatures in the field, blooms are typically on the decline and new cysts are already falling to the sediments (Anderson *et al.* 1983). Similarly, Han *et al.* (1992) found that *A. tamarense* disappears from the water column of Chinhae Bay, Korea at temperatures well below those that support optimal growth in the laboratory. The implication is that the induction of sexuality precludes the long-term persistence of vegetative *Alexandrium* cells in the plankton. This is in contrast to the long duration of *Gymnodinium breve* and *G. nagasakiense* populations (Steidinger *et al.* this volume; Gentien, this volume), for example, which remain in the water column for extended periods. The encystment/excystment cycle thus restricts the longevity of the *Alexandrium* vegetative cell population and appears not to be optimized for rapid or sustained vegetative growth. Whether this generalization from Cape Cod salt ponds applies to other areas of the world remains to be seen, but if so, it highlights an important aspect of *Alexandrium* autecology.

Laboratory studies suggest that the induction of sexuality in *Alexandrium* occurs as a result of nutrient limitation, yet this is not well-supported by field measurements. One problem in this regard is that gametes are not easily distinguished from vegetative cells in natural populations, and fusing gametes, though distinctive, are rarely observed. Gametes have thus never been enumerated in field studies. However, it is possible to recognize large, darkly-pigmented planozygotes (Anderson 1980) and to tabulate their abundance through time. Salt ponds are once again ideal for this type of time-series measurement. Studies in three Cape Cod salt ponds over two bloom seasons demonstrated that planozygote formation did not coincide with an obvious decrease in ambient nutrients (Anderson *et al.* 1983). In fact, planozygotes in the plankton and new cysts at the sediment surface were first observed when external nutrients were at or above concentrations equivalent to those measured during the earlier stages of bloom development when vegetative growth was rapid. It may be that as the ambient temperature increased during the blooms, the rates of uptake and metabolism of nutrients increased as well. Thus nutrient concentrations that were sufficient for balanced (but slow) growth at colder, early-bloom temperatures may not have been sufficient to maintain balanced growth when waters warmed and the *A. tamarense* growth rate increased. A gradual decrease in internal nutrient pools would thus occur, leading to nutrient limitation. Another possibility is that micronutrient limitation occurred, but was not measured (e.g. iron stress; Doucette *et al.* 1989). Alternatively, given the discovery of endogenous control of cyst germination for *A.*

tamarense (Anderson and Keafer 1987), endogenous or "clock"-regulated sexuality must also be considered. A final explanation is suggested by the remarkable consistency in the number of net cell divisions that occurred between the time the first *A. tamarense* cells were observed and the time that planozygotes appeared in the salt pond studies of Anderson *et al.* (1983). Despite a one-month differential in the onset of the blooms in the three salt ponds, zygotes appeared approximately six divisions after the first vegetative cells were seen (neglecting advection and grazing losses). This regularity is consistent with the gradual depletion of a stored product that eventually triggers sexuality, with replenishment of that stored reserve during the non-dividing planozygote stage. If verified, this again points to a temporal limit to *Alexandrium* blooms, perhaps under endogenous control.

Only two studies have attempted to enumerate *Alexandrium* planozygotes during blooms in order to quantify the importance of encystment in bloom decline (Anderson *et al.* 1983; Takeuchi *et al.* 1995). Both show that sexuality is induced well before the size of the bloom population peaks, and that during this late stage of bloom development, planozygotes can comprise 20-40% of the motile population. This number underestimates the total percentage of cells that become cysts, however, since it cannot account for the dynamic nature of the zygote sub-population. Each day, some planozygotes fall to the sediments as cysts, but new planozygotes appear following gamete fusion. The estimates do suggest that a large fraction of the bloom population encysts, and thus that bloom decline may be linked more to life cycle transitions than to grazing or other loss factors.

3.2 Coastal blooms

Another prominent habitat for *Alexandrium* blooms is in open coastal waters or large estuaries. The two regions that have been best studied in this regard are the St. Lawrence estuary in Canada and the Gulf of Maine in the U.S.

3.2.1. Seedbeds and excystment dynamics

Discrete seedbeds are difficult to identify in open coastal waters, since mapping surveys typically document widely distributed cyst populations. Quantitative cyst maps are available for *A. tamarense* (e.g., White and Lewis 1982; Anderson and Keafer 1985; Cembella *et al.* 1988), *A. catenella* (e.g., Yamaguchi *et al.* 1995), *A. minutum* (Erard *et al.* 1993) and *A. ostenfeldii* (Mackenzie *et al.* 1996), but few investigators have been able to obtain the data needed to demonstrate that a single location provides the bulk of the motile cell inoculum for a regional bloom. In fact, cyst distribution and abundance did not correlate with shellfish toxicity patterns along the coast of Maine (Thayer *et al.* 1983). Examples of discrete cyst seedbeds that lead to large-scale blooms do exist, however. For example, Cembella *et al.* (1988b) argue that *A. tamarense* cysts along the northern shore of the St. Lawrence estuary near the Manicouagan and Aux-Outardes rivers are responsible for toxic blooms which cause PSP on the south shore and further downstream in the estuary. On the northeast coast of Britain, *A. tamarense* cyst accumulations in the Firth of Forth have been linked to toxic blooms in the adjacent coastal waters to the north (Lewis *et al.* 1995). Evidence for the existence of a regional seedbed is also found in studies in the southwestern Gulf of Maine where *A. tamarense* blooms are confined to a buoyant coastal current formed from the outflows of two rivers in southern Maine (see below). Cyst surveys document a widespread distribution both nearshore and offshore (e.g., Lewis *et al.*

1979; Thayer *et al.* 1983; Anderson and Keafer 1985), but a shallow water source region or "initiation zone" has been identified in the eastern Casco Bay region just "downstream" from the mouths of the rivers that produce the coastal current waters. In two successive years (1993 and 1994), *A. tamarense* cells were generally absent in early spring at all stations in a large study area except those near the Casco Bay region (D. Anderson, unpub. data). Initial concentrations were low (approximately 50-100 cells l^{-1}), but quickly increased within the low salinity coastal current.

The size of the inoculum from cysts in coastal or estuarine systems is not known, as the large scales of the blooms have prevented detailed study, and planomeiocytes are rarely seen. Given the widespread cyst distribution typical of coastal areas, two scenarios are suggested with respect to bloom initiation. One involves the synchronized germination of cysts throughout the region, with only those cells which emerge into favorable growth conditions being responsible for blooms. Alternatively, rapid and synchronized germination in localized areas (e.g., shallow bays) might seed the blooms, leaving cysts that germinate more gradually in other areas with little quantitative impact. One indication of the low magnitude and protracted nature of the inoculum from deep water cyst germination is seen in the chlorophyll fluorescence of cysts in sediments 160 m deep in the Gulf of Maine (Anderson and Keafer 1985). As in the shallow salt ponds and embayments, the majority of the cysts were buried below the sediment surface, and thus were prevented from germinating by anoxia (Keafer *et al.* 1992). Germination of these deep water cysts appears to be controlled by an endogenous annual clock (Anderson and Keafer 1987). The percentage of fluorescing cysts in surface sediments increases sharply in the spring and then decreases during the summer and fall, remaining positive and significant for nearly 8 months (Fig. 3). Deep water cysts thus germinate over a much longer interval than the duration of the coastal bloom in those waters (~ two months), and much longer than the germination interval in the shallow salt ponds described above. Clearly, the cold temperatures and darkness of deep waters extend the excystment process, with only a fraction of the viable cysts in surface sediments actually participating in the bloom. For coastal blooms in the Gulf of Maine, we now suspect that cysts in shallow coastal waters or bays provide a larger and more synchronized inoculum than do the offshore cyst deposits, which may only be sinks where most cysts accumulate and eventually die, with little effect on overall bloom dynamics.

3.2.2. Bloom development and encystment

The complexities of *Alexandrium* blooms in dynamic coastal or estuarine systems are far from understood. One common characteristic of such phenomena is that physical forcings play a significant role in both bloom dynamics and the patterns of toxicity. The coupling between physics and biological "behavior" such as swimming, vertical migration, or physiological adaptation holds the key for understanding these phenomena, yet this is perhaps where our knowledge of this genus is weakest.

Once vegetative cells enter the water following cyst germination, their net growth and transport are heavily affected by circulation, nutrients, stratification, and other chemical or physical factors. Although many of these interactions remain uncharacterized, blooms of several *Alexandrium* species have been linked to particular water masses. In the St. Lawrence estuary, for example, patterns of PSP toxicity and *A. tamarense* cell distributions have been linked to the plume produced by the Manicouagan and Aux-Outardes rivers (Therriault *et al.* 1985). This flow generates a

frontal zone extending into the estuary which is associated with high phytoplankton production, particularly of dinoflagellates. Examples of the importance of fronts in HAB bloom dynamics are many (e.g., Pingree *et al.* 1975; Simpson *et al.* 1979). The key issue here is that the freshwater plume generates a highly stratified water column which favors proliferation and retention of vertically migrating phytoplankton such as *Alexandrium.* A fraction of the vegetative cells retained in that zone are subject to transport across to the south shore of the estuary, where they are entrained into the Gaspé current which carries them towards the Gulf of St.. Lawrence. The frontal system at the Manicouagan and Aux-Outardes plume thus serves as an initiation zone and the Gaspé current as a transport pathway. Similar initiation zones and coastal current transport have also been indentified for *Alexandrium* blooms in the western Gulf of Maine (Franks and Anderson 1992a; see below).

The physical system is not the entire story, however. Although the Manicouagan and Aux-Outardes plume is essential for *A. tamarense*, this species is most abundant during mid- to late-summer, even though the characteristics of the plume and the front are well-established for a much longer interval. Clearly, other factors are regulating *A. tamarense* dynamics. Therriault *et al.* (1985) suggest that *A. tamarense* blooms in the St. Lawrence develop only when the proper combination of meteorological and hydrodynamic factors coincide to produce high temperatures, maximum water column stability, low nutrients, and low winds.

The frontal zone of the Manicouagan and Aux-Outardes rivers is also a site of enhanced cyst deposition (Cembella *et al.* 1988b). Unfortunately, no information is available on the timing or magnitude of cyst formation or the mechanisms underlying sexual induction in these waters.

Another example of the importance of freshwater in *Alexandrium* bloom dynamics is found in the southwestern Gulf of Maine, where the temporal and spatial pattern of persistent PSP outbreaks have been linked to a buoyant plume or coastal current originating in several rivers that empty into the Gulf near Casco Bay (Franks and Anderson 1992a; Anderson in press). Concentrations of the toxic dinoflagellate *Alexandrium tamarense* are much higher within the lower salinity waters of the plume than without, and toxicity in coastal shellfish rises and falls with the movement of the plume. This is in turn driven by the local wind stress, by rainfall and snow melt patterns, and by the general circulation of the Gulf. Downwelling conditions are conducive to toxicity development, since such conditions trap the plume and its associated cells tightly against the coast and accelerate them to the south. In contrast, upwelling favorable winds push the plume offshore, spreading it out laterally and dramatically decreasing the concentration of *A. tamarense* in nearshore waters due to the upwelling of deeper, saltier waters that contain no toxic cells.

The early season bloom dynamics of that area are not the only issue, however. Given a persistent southward flow of the coastal current, the red tide problem "downstream" should gradually diminish year after year as the sediments near the origin of the coastal current are depleted of cysts. In other words, once the *A. tamarense* cells leave the area and travel south in the coastal current, there is no hydrographic pathway that will bring them (or their cysts) back to the bloom initiation zone. Since the toxic blooms have been an annually recurrent event for over 25 years (Franks and Anderson 1992b), there must be a mechanism by which the cyst

seedbeds are replenished. One possible explanation is suggested by the observation of late-season populations of *Alexandrium* in Casco Bay in 1993 and 1994, long after the initial pulses of cells had initiated a bloom in the early spring (unpub. data). With much-reduced rainfall and no snowmelt to drive the coastal current at that time, transport out of the region was limited and localized blooms (and presumably cyst deposition) occurred. Much remains to be clarified concerning the existence and dynamics of this putative initiation zone or seedbed.

As in other open coastal systems, no estimates are available on the extent to which encystment contributes to the decline of the blooms in the coastal current, nor is it known how sexuality is induced. Nutrient measurements during blooms are few, and planozygotes and newly formed cysts are rarely observed. As in other stratified systems, the high nutrient concentrations below the pycnocline (e.g. Franks and Anderson 1992a) would be accessible to vertically migrating *A. tamarense* cells, so it is not clear whether nutrient limitation actually occurs while the cells are associated with the buoyant coastal current.

Another important unknown in the coastal blooms concerns the possible stimulation of *A. tamarense* growth by the unique chemistry of the freshwater plumes. More cells are typically found within the low salinity plumes (e.g., Therriault *et al.* 1985; Franks and Anderson 1992a), but this could simply be a result of small-scale physics interacting with the cells migration behavior, or it could be a reflection of higher growth rates within the plume. Freshwater runoff from the heavily forested watershed contains significant levels of dissolved and particulate organic matter as well as metals and other micronutrients. It appears likely that some component of this mixture could be critical to the rapid growth of *A. tamarense* cells. Iron is a likely candidate for a stimulatory micronutrient, as Wells *et al.* (1991) showed that bioavailable iron was elevated in nearshore waters characteristic of the coastal current, and depleted offshore in the Gulf of Maine. The measured iron levels were within the range of those that stimulated or limited *A. tamarense* growth in laboratory cultures.

3.2.3. General environmental forcings

The large number of *Alexandrium* species involved in harmful events throughout the world makes it difficult to generalize about general environmental controls of bloom dynamics. The nutrition of these organisms is not unusual, although mixotrophy has been reported for some *Alexandrium* species (Jacobson and Anderson 1996) and more are probably capable of this strategy. Like most phytoplankton species, *Alexandrium* will respond to anthropogenic nutrient inputs, but there is no evidence that these species are preferentially stimulated compared to other phytoplankters, nor is there compelling evidence of any increase in *Alexandrium* bloom magnitude or frequency as a direct result of pollution. Indeed, *Alexandrium* blooms, including many toxic ones, occur in remote and relatively pristine waters, such as those in Alaska (Hall 1982) or southern Argentina (Benavides *et al.* 1995). A strong association with freshwater inputs is often seen (e.g. Franks and Anderson 1992a; Therriault et al. 1985), but this presumably reflects the importance of stratification and the supply of natural humic substances, trace elements, and other materials that might serve as growth stimulants.

On a larger scale, some workers have attempted to discern the influence of mesoscale weather patterns and lunar forcings on *Alexandrium* bloom timing. Balch (1981) observed a synchrony between seasonal *A. tamarense* blooms off the coast of Maine

and maximum tidal ranges of major spring tides. Spring tides increase the height of the bottom mixed layer, erode the seasonal thermocline, and inject nutrients into surface waters, each of which could influence cyst germination and motile cell growth and accumulation. White (1987) examined patterns of PSP toxicity caused by *A. fundyense* for over 40 years and looked for correlations with environmental factors during pre-bloom months and summer toxicity episodes. A correlation with an 18.6 year cycle of lunar tidal modulation was observed, as was a relationship with salinity, windspeed and tidal energy dissipation. A possible relationship between El Niño/Southern Oscillation (ENSO) events and *Alexandrium* blooms on the west coast of the U.S. was suggested by Erickson and Nishitani (1985). These events affect sea surface temperature, winds, and sunlight over a large scale, and hence might be expected to influence the vertical stability of the water column, and thus dinoflagellate blooms. Clearly, local and regional *Alexandrium* blooms can be influenced by mesoscale circulation patterns, and thus basin-scale phenomena such as ENSO events can be important in the patterns of toxicity. A careful evaluation of these factors in the context of PSP dynamics would likely be a fruitful exercise in both Pacific and Atlantic waters.

3.2.4 Population biology

Much of the foregoing discussion treats large-scale, regional populations of *Alexandrium* as if they were composed of individual strains or genotypes, yet this is probably not the case. *Alexandrium* cells in the western Gulf of Maine coastal current or the plume of the Manicouagan and Aux Outardes rivers would appear to represent discrete, genetically uniform populations, yet biochemical and genetic studies are now revealing that considerable heterogeneity exists. (Details are provided elsewhere in this volume by Scholin and Gallagher and by Anderson *et al.* 1994; Cembella and Destombe 1996). It is now clear, for example, that two and perhaps three genetically distinct strains of *A. tamarense* occur within the Gulf of Maine and areas to the south. If these strains have different growth characteristics and physiology, fine-scale autecological understanding of the details of bloom dynamics will not be possible until the different genotypes within a region are characterized. For now, we must recognize that considerable genetic variability can exist within regional populations of *Alexandrium*, and that this variability is not easily detected in routine monitoring surveys. A major priority for future research should be to characterize the nature of the genetic variability within *Alexandrium* cells in a given region, and to determine under what conditions the different genotypes become dominant.

4.0 Summary

The ability of *Alexandrium* species to colonize multiple habitats and to persist over large regions through time is testimony to the adaptability and resilience of this important organism *Alexandrium* species are not known for rapid or "explosive" growth rates. Maximal rates in laboratory cultures are typically 0.5 to 0.7 divisions day^{-1} (e.g., Anderson *et al.* 1984; Su et al. 1993; Yamamoto and Tarutani 1996; Chang and McClean 1997), although rates near one division day^{-1} are reported for Australian *A. minutum* (G. Hallegraeff, pers. comm.). Population growth is typically not reflected in monospecific blooms but rather in moderate biomass levels and co-occurrence with other species. Blooms are not particularly long-lasting, and seem restricted in time by life cycle transitions. The cyst stage is clearly important in *Alexandrium* population dynamics, both with respect to bloom initiation and termination, but the nature of this linkage varies among habitats. In shallow

embayments, cysts and motile cell blooms are tightly coupled, whereas in large temperate estuaries and open coastal waters, the linkage is more difficult to define and quantify. In both of these habitats, most of the cysts in the sediments do not germinate due to bioturbation, burial, and inhibition of germination by anoxia. Even when only the cysts in surface sediments are considered, the bulk of the widely distributed cysts in deeper waters may germinate too slowly or too far from suitable growth conditions to be a factor in coastal blooms. In one sense, *Alexandrium* species appear to use a type of r-selection strategy, producing many "offspring" in the form of cysts, only a few of which ever germinate to inoculate blooms. On the other hand, a complex life history and a low growth rate are often considered K-strategies. This group of dinoflagellates does not easily fit into such fixed categories.

Estimates of the inoculum size from excystment are small - on the order of tens to hundreds of cells l^{-1}, suggesting that major blooms require multiple, sustained vegetative divisions that in turn depend greatly on environmental conditions affecting motile cells. Nevertheless, the size of an excystment inoculum can have a bearing on the magnitude of a bloom, especially if that bloom is limited temporally due to seasonal temperatures or to some form of endogenous regulation of excystment and encystment. In small-scale blooms in embayments and in widespread coastal blooms, physical/biological coupling is a critical feature of population accumulation, growth, and dispersal. Behavioral adaptations such as vertical migration are important features in this regard. Bloom termination is clearly linked to life cycle transitions, although the relative importance of encystment relative to grazing or other loss factors has not been explicitly investigated. Overall, the *Alexandrium* species that have been studied in detail have proven to be remarkably resilient and capable of colonizing a spectrum of habitats and hydrographic regimes. It is thus of no surprise that the biogeographic range of these species has expanded considerably in recent times (Anderson *et al.* 1994; Scholin, this volume) and that PSP outbreaks remain a significant global problem.

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