Marine Biology 76, 179–189 (1983)

Printed in Germany

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Importance of life cycle events in the population dynamics of *Gonyaulax tamarensis* *

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Abstract

Life cycle changes that allow populations of the toxic dinoflagellate *Gonyaulax tamarensis* Lebour to inhabit the benthos and the plankton alternately are important factors regulating the initiation and decline of blooms in restricted embayments. When the dynamics of these estuarine populations were monitored during "bloom" and "non bloom" years, it was shown that: (1) each year, germination of benthic cysts inoculated the overlying waters during the vernal warming period, but a large residual population remained in the sediments throughout the blooms; (2) the resulting planktonic population began growth under suboptimal temperature conditions; (3) the populations developed from this inoculum through asexual reproduction until sexuality (and cyst formation) were induced; (4) encystment was not linked to any obvious environmental cue and occurred under apparently optimal conditions; and (5) an increase in the number of non-mitotic swimming cells (planozygotes, the precursors to dormant cysts) accompanied the rapid decline of the planktonic population. Thus encystment, in combination with hypothesized losses due to advection and grazing, contributed substantially to the decline of the vegetative cell population. We conclude that the encystment/excystment cycle temporally restricts the occurrence of the vegetative population and may not be optimized for rapid or sustained vegetative growth and bloom formation in shallow embayments. The factors that distinguish "bloom" from "non-bloom" years thus appear to be operating on the growth of the planktonic population.

Introduction

The toxic dinoflagellate *Gonyaulax tamarensis* Lebour commonly occurs in estuarine and coastal waters along the northeast coast of North America, resulting in widespread, recurrent outbreaks of paralytic shellfish poisoning (PSP). The life cycle of this motile organism is extremely important in the bloom dynamics. Normal population growth occurs through asexual division, but a transition to sexual reproduction can occur whereby gametes are formed that fuse to produce a motile, zygotic cell (planozygote). This large, distinctive cell does not divide but continues swimming for a few days before becoming a cyst (hypnozygote) (Anderson, 1980). Following a mandatory dormant period, the cyst remains in a quiescent state during the winter before germinating (excysting) to liberate a large swimming cell (planomeiocyte), which subsequently divides to produce the asexual vegetative cells that are the inoculum for the overlying waters (Steidinger, 1975; Dale, 1977; Anderson and Wall, 1978; Anderson, 1980). This alternation of generations allows populations to inhabit both the benthos and the plankton and may prove to be one of the dominant factors regulating the initiation and decline of blooms of this species.

Unfortunately, little is known of the mechanisms that control the timing of encystment and excystment in dinoflagellates (reviewed in Pfiester and Anderson, in press). In laboratory cultures, excystment often follows a maturation period of several months, sometimes occurring spontaneously if normal culture conditions are maintained (von Stosch, 1973; Pfiester, 1975, 1976, 1977). For nearly all species examined however, cold storage suppresses germination until temperatures rise, at which time excystment is rapid and well-synchronized (Huber and Nipkow, 1922; 1923; von Stosch, 1973; Pfiester, 1977). This temperature mechanism was proposed to explain the appearance of motile *Gonyaulax tamarensis* cells during the vernal warming of coastal embayments (Anderson and Wall, 1978; Anderson and Morel, 1979).

Encystment has been induced in laboratory cultures through nutrient depletion, and more specifically, nitrogen starvation (von Stosch, 1973; Pfiester, 1975, 1976, 1977; Turpin *et al.*, 1978; Walker and Steidinger, 1980). Phosphorus limitation (Pfiester, 1976; Watanabe *et al.*, 1982; Anderson, unpublished data) and simultaneous variation

* Contribution No. 5201 from the Woods Hole Oceanographic Institution

of temperature, photoperiod, and light intensity have also been successfully employed (von Stosch, 1964). In most studies to date, the objective has been to describe the sexual phase of the life cycle, not to determine the factors that induce it.

Field studies of cyst formation are equally inconclusive regarding the mechanism of induction. Wall and Dale (1968) monitored cyst abundance during the late stages of blooms of three dinoflagellates, but gave no background data to explain the life cycle changes. Anderson and Morel (1979) demonstrated the formation of new cysts during a bloom of *Gonyaulax tamarensis,* but could not suggest an explanation for the timing of encystment since no nutritional deficiency or environmental cue was apparent. Despite these observations and those on several species that seem to require no external stimulus prior to sexuality and encystment (Wall *et al.,* 1970; Zingmark, 1970; Beam and Himes, 1974, 1977; Morey-Gaines and Ruse, 1980), a common assumption has been that dinoflagellates form resting cysts in response to adverse conditions, most notably nutrient limitation.

Given this confusion and the potential importance of life cycle phenomena, a principle objective of this study was to examine the factors controlling encystment and excystment in natural *Gonyaulax tamarensis* populations. We chose to focus on shallow Cape Cod salt ponds (Anderson and Wall, 1978; Anderson and Morel, 1979) because the unique hydrographic characteristics of these basins in conjunction with the patchy cyst distribution in the region (Anderson *et al.,* 1982) result in isolated populations, which can be studied throughout the stages of bloom initiation, development, and decline. It is possible therefore to examine the typical phytoplankton population losses and gains due to growth, advection and grazing, while also examining the causes and timing of encystment and excystment and the relative impact of these processes on the bloom sequence. Although the environmental conditions within salt ponds differ from those in nearshore waters subject to coastal blooms, certain of the possible controlling factors are common to both environments (e.g. temperature and light variations, mixing events, nutrient availability). In effect, the salt ponds can serve as microcosms for the more widespread, coastal blooms of this species.

The overall objective of this study is to describe the population dynamics of *Gonyaulax tamarensis* in three of these embayments over two years – 1980 when toxic blooms occurred, and 1981 when *G. tamarensis* was present, but below quarantine concentrations. It was hoped that a comparative study of the various patterns of bloom development would clarify the common mechanisms underlying life cycle changes, while also permitting an assessment of the magnitude of these changes relative to growth, grazing, and advection of the population.

Material and methods

Study areas. Three salt ponds were monitored in 1980 (Perch Pond, Salt Pond, and Mill Pond) and two (Perch, Salt) in 1981. All three are on Cape Cod and are a maximum of 70 km apart. These embayments are connected to the ocean by up to 8 km of intervening estuary, but each has a narrow, shallow inlet channel with wide sandy ebb and flood tidal deltas, which restrict flushing. The important features of these salt ponds are listed in Table 1. The reduced salinities are largely a result of groundwater flow, although Perch Pond has a small stream inflow as well.

Table 1. Average characteristics of the study areas during spring

	Perch Pond	Salt Pond	Mill Pond
Location	Falmouth, MA	Eastham, MA	Orleans, MA
Area (m^2)	66 400	82 200	160 000
Volume (m^3)*	98 500	278 000	–
Average depth* (m)	1.48	3.4	–
Exchange ratio**	0.25	0.29	–
Salinity (‰)	26	31	31

* Low tide
** Ketchum (1951), measured by Anderson *et al.* (in preparation)

Sampling. Water samples were collected from all the ponds on the same day on approximately a weekly basis from March through June in 1980 and 1981. Most methods did not change between years, but two exceptions are significant. Throughout 1980, integrated samples were taken at a single station using a hand pump and a hose lowered at a constant rate through the water column. In 1981, six stations were sampled in each pond using a rigid PVC sampling tube, which was lowered to the bottom and sealed, thus capturing an integrated parcel of water. The six water samples were pooled and subsampled for analysis. Thus, the data for 1980 are integrated over depth only, while 1981 data are integrated over depth and area as well.

In 1980, duplicate zooplankton samples were collected with a 73-μm mesh net (30 cm diameter) equipped with a flow meter. The net was towed at the surface behind a skiff rowed for 60–75 s, resulting in filtration volumes of 0.6 to 2.2 m^3. In 1981, whole water samples were taken with a Kemmerer-style PVC sampling bottle from the surface, mid-depth and bottom at a single station. These were pooled and subsampled before counting.

During both years, 200-ml subsamples for phytoplankton cell counts were fixed in the field. Those needed for *Gonyaulax tamarensis* measurements were placed unpreserved in the dark in a cooler during transit to the laboratory. Nutrient samples were filtered in the field through acid-washed glass fiber filters, poured into 100-ml acid-washed LPE bottles and kept on ice until they could be frozen. 1980 samples for ammonia analysis from Salt and Mill Ponds were spiked with reagents in the field in an effort to minimize contamination, but blanks were highly variable and the data of little use. Ammonia values for the pond closest to the laboratory in 1980 (Perch), and

for Perch and Salt Ponds in 1981 were filtered in the field and processed entirely in the laboratory. Reactive silicate, phosphate and nitrate concentrations were determined using the methods of Strickland and Parsons (1972), and ammonia as described by Solorzano (1969).

Salinity was measured with a salinometer. Weather data was collected and/or compiled by R. E. Payne at the Woods Hole Oceanographic Institution.

Phytoplankton samples were preserved in Utermohls solution and counted with an inverted microscope. Phytoplankton carbon (μg C l^{-1}) was calculated for each species as the product of cell plasma volume, cell concentration, and the empirically determined conversion factor, F, given by Edler (1979; F=0.13 for armored dinoflagellates; =0.11 for diatoms and naked dinoflagellates).

Gonyaulax tamarensis cells were counted and measured using the following procedure: 500 ml of unpreserved sample were gravity filtered through a 10-μm Nitex filter, which was immediately washed with 5 ml of filtered seawater from a squeeze bottle. Checks with laboratory cultures indicate that this is a reliable means to concentrate *G. tamarensis* quantifiably while at the same time producing non-motile cells with intact thecae and no size distortion from fixatives. The concentrated sample was promptly examined in a Palmer-Maloney slide, thus permitting examination and manual manipulation of the cells for positive identification and measurement under 250× magnification. Approximately 50 cells were measured each time the natural cell concentration was sufficiently high.

The size measurements were used to distinguish two different life cycle stages from the normal vegetative cells. Based on previous observations, all cells with a length greater than or equal to 43 μm were operationally defined to be either newly-germinated cells (planomeiocytes) or swimming zygotes before cyst formation (planozygotes) (Anderson and Wall, 1978; Anderson, 1980). These two stages could then be distinguished from each other, since the planozygotes are pigmented a dark brown color, while the planomeiocytes contain a dark red accumulation body. Although this feature is retained by one daughter cell after a planomeiocyte divides, this cell is reduced in size and thus can be distinguished from the parent cell. A large cell with an accumulation body is thus considered to be a planomeiocyte prior to division. Gametes were not monitored because they could not be distinguished from vegetative cells on the basis of size.

It should be noted that the species *Gonyaulax tamarensis* is the subject of considerable taxonomic controversy at this time. Although the valid synonyms *Protogonyaulax tamarensis* (Taylor, 1979) and *Gessnerium tamarensis* (Loeblich and Loeblich, 1979) have been proposed, we have chosen to use the more widely recognized name *G. tamarensis* until such time as one designation is generally accepted.

Sediment cores were collected from each pond using a hand-coring device. These cores were then agitated slightly and water plus the surface flocculent layer poured off for processing, using the methods of Wall and Dale (1968). This is a qualitative technique, but it allows a determination of the percentage of newly-formed *Gonyaulax tamarensis* cysts relative to the total for that species in the surface sediments. The self-consistency of the data leads us to believe that these data are not biased by resuspension and burial. New cysts were easily distinguishable by their characteristic contents [filled completely with lipid or starch reserves, with little or no visible microgranular cytoplasm in motion at each pole as described by Anderson (1980)].

Results

Overview

During 1980, all three study areas developed blooms of *Gonyaulax tamarensis* sufficiently dense to cause shellfish toxicity and a ban on harvesting, while during 1981, toxicity was undetectable and cell counts in the two ponds that were monitored showed that *Gonyaulax tamarensis* was present, but in low concentrations (Figs. 1–3).

Throughout the 1980 bloom development period, nutrient concentrations remained relatively high, with nitrate always above 12, 0.8 and 0.6 μM, and phosphate above 0.15, 1.3, and 0.7 μM for Perch, Salt and Mills Ponds respectively. This favorable nutritional environment is further evidenced by the relatively high levels of dinoflagellate and diatom cell carbon throughout this period (Figs. 1–3 G). In 1981, nutrient levels were also high throughout the very slow *Gonyaulax tamarensis* population increase.

Throughout each sampling season, the macrozooplankton community in all three ponds was dominated by calanoid copepods (predominantly *Acartia hudsonica*) and the larvae of polychaete annelids (tentatively *Polydora* sp.) (Figs. 1 F–3 F). Although sampling methodologies differed betweeen 1980 and 1981, zooplankton abundance in 1981 in both Perch and Salt Ponds during the bloom development period appeared to be several times higher than in 1980 (Figs. 1 F, 2 F). The phytoplankton community was more complex, although the dominant organisms in all three ponds were generally the dinoflagellate *Heterocapsa triquetra* and the diatoms *Thalassiosira nordenskioldii* and *Skeletonema costatum.* When all species are tabulated using carbon per cell estimates (Figs. 1–3 G), we see a phytoplankton standing crop dominated throughout the spring of both years by dinoflagellates in Perch Pond. Salt and Mill Ponds had occasional dominance by both algal classes.

In general, there is no obvious explanation that might account for the large disparity in the population size of *Gonyaulax tamarensis* between the two years. Meteorologically, notable differences between 1980 and 1981 can be seen in Fig. 4 A. Although cumulative precipitation (Fig. 4 B) was similar for both years, the timing of the spring rains was quite different with the heaviest rain during March in 1980 and during February in 1981.

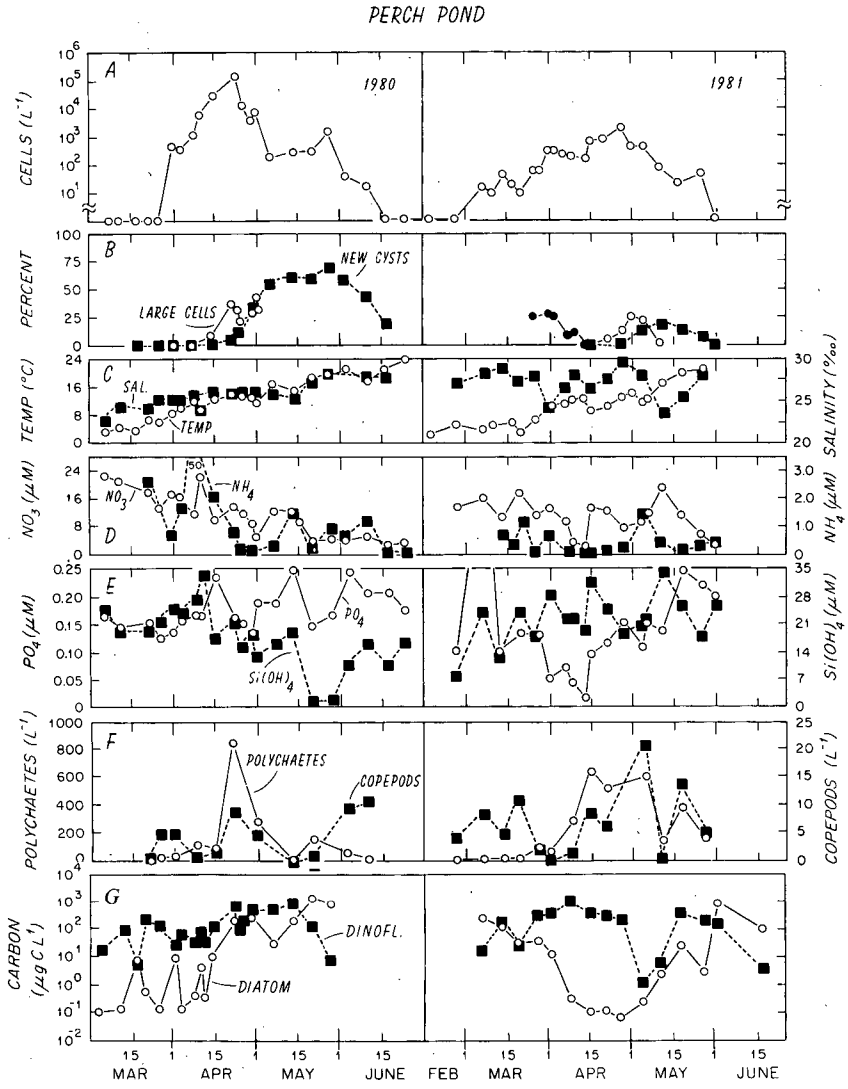

Fig. 1. *Gonyaulax tamarensis.* Spring bloom time series for Perch Pond in 1980 and 1981. (A) *G. tamarensis* cell densities; (B) Planomeiocytes (●—●) and planozygotes (○—○) as a percent of the total cell concentration; new cysts (■ ---■) as a percent of total cysts in surface sediments; (C) Temperature (○—○) and salinity (■ ---■); (D) Nitrate (○—○) and ammonium (■ ---■); (E) Phosphate (○—○) and reactive silicate (■ ---■); (F) Copepods (copepodites plus adults) (■ ---■) and polychaete larvae (○—○); (G) phytoplankton carbon as dinoflagellates (■ ---■) and diatoms (○—○)

Table 2. Environmental characteristics when life cycle transitions occurred. Values in parenthesis are for the preceding week; NM = not measured; undet = undetectable

	Motile cells					Planozygotes				
	Perch Pond		Salt Pond		Mill Pond	Perch Pond		Salt Pond		Mill Pond
	1980	1981	1980	1981	1980	1980	1981	1980	1981	1980
First appearance	1 Apr	6 Mar	1 Apr	1 Apr	8 Apr	15 Apr	21 Apr	13 May	4 May	13 May
Temperature (°C)	8.5 (6.5)	4.0 (6.0)	5.0 (5.5)	10.0 (5.7)	9.0 (6.7)	12.3 (11.7)	11.0 (9.5)	12.7 (12.0)	9.0 (10.5)	11.4 (12.5)
Salinity (‰)	25.3 (25.5)	22.3 (22.5)	31.9 (30.1)	33.5 (31.4)	32.3 (29.6)	26.9 (26.0)	25.0 (24.1)	31.3 (31.5)	31.5 (31.8)	31.4 (30.0)
NO_3^- (μM)	17.3 (13.1)	15.9 (13.3)	0.3 (1.3)	0.6 (2.4)	3.0 (1.1)	9.6 (11.7)	12.3 (13.4)	1.1 (0.7)	0.7 (0.4)	0.50 (1.71)
NH_4^+ (μM)	0.72 (undet)	NM (NM)	NM (NM)	NM (2.93)	NM (NM)	2.1 (5.0)	0.09 undet	NM (NM)	1.8 (2.5)	NM (NM)
PO_4^{3-} (μM)	0.137 (0.13)	0.46 (0.11)	1.92 (0.56)	1.07 (0.65)	0.66 (0.34)	0.24 (0.19)	0.12 (0.09)	1.35 (1.41)	0.9 (1.0)	0.72 (0.65)

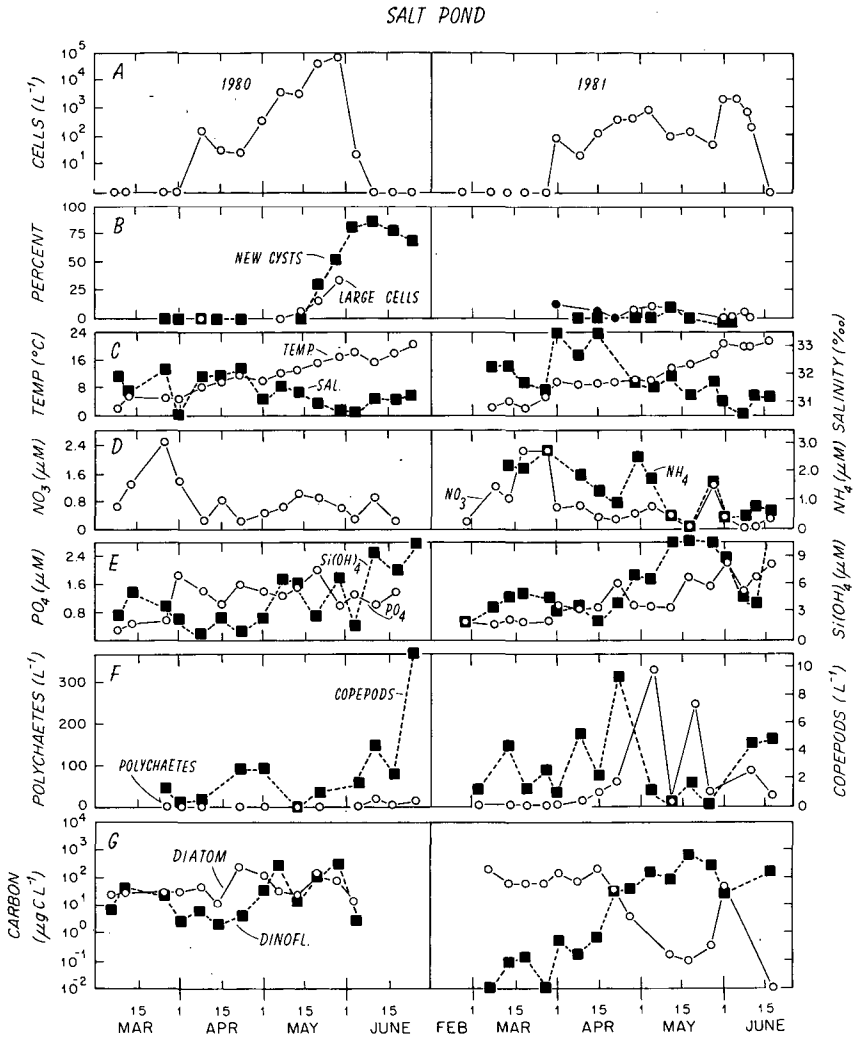

Fig. 2. *Gonyaulax tamarensis.* Spring bloom time series for Salt Pond in 1980 and 1981. Symbols as in Fig. 1

March, 1981 had only 1.7 cm of precipitation, the lowest monthly total in 15 yr of records. Cumulative solar radiation was the same between years (Fig. 4 D).

If we examine characteristics and events within the ponds themselves at this general level, Salt and Mill Ponds are seen to be comparable to each other but significantly different from Perch Pond. All three ponds warmed at the same rate ($0.17\,°C \pm 0.02\,C°\ d^{-1}$), but Perch was roughly 3 C° warmer on any given day than the other two (Watras *et al.*, 1982). As shown in Table 1 and Figs. 1–3, Perch Pond is also consistently less saline, with more nitrate and less phosphate than the other two ponds. The other noteworthy difference is that *Gonyaulax tamarensis* bloom development was delayed in Salt and Mill Ponds in 1980, peaking approximately 35 d after the maximum in Perch Pond, even though cells first appeared in the water at approximately the same time (Table 2).

Bloom initiation

The first motile *Gonyaulax tamarensis* cells were generally observed in the first week of April when temperatures had risen from winter levels (2°–4 °C in February) to 8 °C (Table 2). Cells were first seen in Perch Pond in early March in 1981 when the water was 4 °C, but measurements the preceding week indicated that temperatures of 5.5 °C or higher had already occurred. Weekly examination of surface sediments in each pond revealed large numbers of viable cysts, none of which were new. Nutrient concentrations were generally high in each pond as excystment occurred. Nitrate was approximately 16, 1.5, and 2 μM, and phosphate 0.15, 1.0 and 0.5 μM in Perch, Salt and Mill Ponds respectively (Table 2).

The impact of germinating cysts as an inoculum is indicated by the numbers of planomeiocytes during the early stages of the blooms. Although they were seen in 1980, they were most noticeable in 1981 when these distinctive cells constituted nearly 30 percent ($90\,l^{-1}$) of the Perch Pond population (Fig. 1). Since a fixed number of cells were examined and measured from each sample, the very small number of planomeiocytes (less than 2 percent of counted cells) in 1980 does not negate their importance as an inoculum, but indicates instead that they were rapidly outnumbered by dividing vegetative cells.

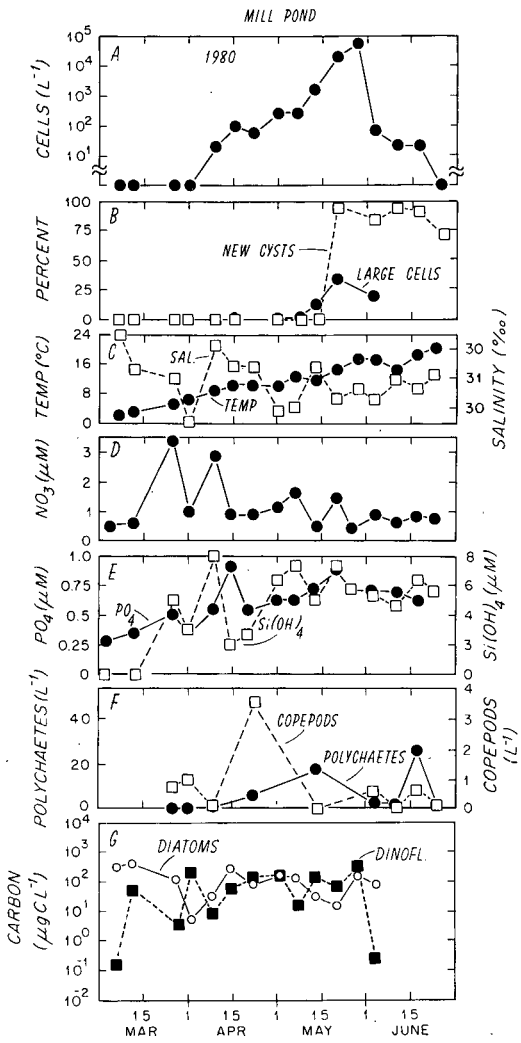

Fig. 3. *Gonyaulax tamarensis.* Spring bloom time series for Mill Pond in 1980. Symbols as in Fig. 1

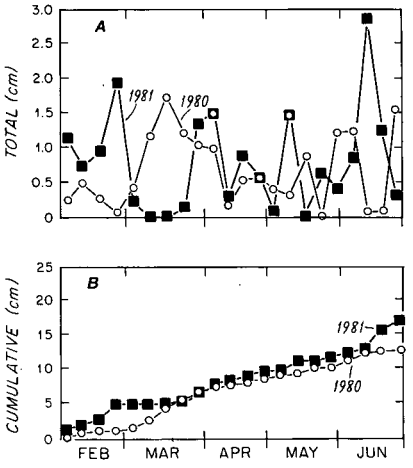

Fig. 4. Precipitation in West Falmouth in 1980 and 1981. (A) Weekly total; (B) Weekly cumulative

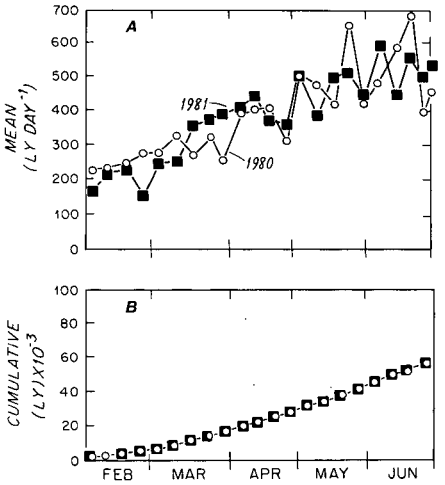

Fig. 5. Solar radiation in West Falmouth in 1980 and 1981. (A) Weekly mean; (B) Weekly cumulative

Bloom development

As mentioned above, in 1980, *Gonyaulax tamarensis* first appeared in the three ponds at approximately the same time, but population development was much slower in Salt and Mill Ponds than in Perch Pond. Specific growth rates calculated from ln-linear regression of the weekly cell counts (thus including losses due to grazing and advection) are 0.30, 0.16, and 0.16 d^{-1} for Perch, Salt and Mill Ponds respectively. In 1981, the equivalent values are 0.09 and 0.07 d^{-1} for Perch and Salt Ponds.

In 1980, the first large cells seen in any numbers were the planozygotes, and these appeared in all three ponds while the *Gonyaulax tamarensis* population was still numerically increasing (Figs. 1–3 B). These distinctive cells eventually comprised 35–40 percent of the total population. The timing of planozygote appearance was approximately one month later in Mill and Salt Ponds than in Perch Pond, consistent with the 35-d lag in bloom development.

In 1981 , the planomeiocytes seen in the early stages of the bloom decreased in number relative to the normal vegetative cells, to be followed several weeks later by the equally large but morphologically distinct planozygotes, which are a product of sexual reproduction. This double-peaked succession of the larger life cycle stages was seen in both Perch and Salt Ponds in 1981 (Figs. 1 B, 2 B). In all of the ponds, the first planozygotes were generally followed one week later by the appearance of new cysts in the surface sediments.

Bloom peak and decline

During the 1980 bloom year, the *Gonyaulax tamarensis* population in Perch Pond reached peak densities 35 d before the maxima occurred in Salt and Mill Ponds. In all three ponds, the decline was very rapid despite favorable nutrient, temperature and salinity regimes. Temperatures were 14.5°, 16.5° and 17 °C for Perch, Salt and Mill Ponds

as the populations peaked, well within the broad optimum established for our Mill Pond clone of this species (Watras *et al.*, 1982).

During this interval, planozygotes remained a significant fraction of the population until the counted population was too small to detect them. Their fate is seen in the rapid increase of new cysts in surface sediments. By 3 June, new cysts constituted between 65 and 95 percent of the total cysts in sediment samples from the three ponds in 1980. [The eventual decline of these percentages is due to the maturation process whereby new cysts become indistinguishable from mature cysts (Anderson, 1980)]. In contrast, during 1981 new cysts were less than 20 percent of the encysted population in surface sediments, presumably due to the lower recruitment from a reduced planktonic population.

Even in 1980 when new cysts were a large percentage of the total in surface sediments, our weekly observations and other quantitative cyst counts (Anderson, unpublished data) indicated that many mature cysts remained quiescent in the benthos. This included a large number of those in both the surface sediments sampled for this study and in subsurface accumulations several cm deep where the cyst concentrations were the highest (Anderson *et al.*, 1982). Clearly there is a large residual cyst population carried over from year to year without germination, even during bloom years.

Discussion

This study demonstrates the importance of life cycle changes in *Gonyaulax tamarensis* bloom dynamics. Excystment initiated the population development, although many cysts did not germinate and those that did encountered a temperature environment that was suboptimal for rapid vegetative growth. The induction of sexuality leading to new cyst formation was not clearly linked to a deterioration of growth conditions, but instead occurred early in the bloom when nutritional and environmental conditions were apparently favorable. These observations suggest that the excystment/encystment cycle is not optimized for rapid or sustained vegetative growth and bloom formation in the salt pond environment.

Our data also suggest that the switch from asexual to sexual reproduction was a major factor in the decline of the blooms due to the formation of a large number of nondividing gametes and planozygotes, which eventually fell to the sediments as cysts. Coupled with losses from grazing and advection, the bloom populations rapidly declined.

Bloom initiation

As with nearly every dinoflagellate species tested (Pfiester and Anderson, in press), quiescence in *Gonyaulax tamarensis* is maintained by cold temperatures but can be broken by a temperature increase (Anderson and Wall, 1978; Anderson, 1980). In our study, the first motile cells appeared after winter temperatures had risen to 5.5 °C or higher (Table 2). We do not have accurate estimates of the number of cysts that germinated each year, but the quantities of mature cysts observed in sediment samples every week and the low concentrations of planomeiocytes in the water column both suggest that the percentage was small. We conclude that the numerical abundance of cysts has little bearing on the magnitude of subsequent blooms.

Since the mature cysts could be germinated in the laboratory with 80 to 100 percent success (D. M. Anderson, unpublished data), it is also evident that either: (a) sonication and other standard processing and incubation techniques in the laboratory (Wall and Dale, 1968) introduce artifacts that obscure the actual control of excystment; and/or (b) there are factors that can override the temperature stimulus and suppress germination at favorable temperatures. In any case, significant numbers of viable cysts did not germinate under conditions thought to be favorable for excystment. Given the durability and longevity of dinoflagellate cysts (Huber and Nipkow, 1922, 1923; Dale, 1979), this carryover population is available to seed future blooms and thus helps the species to survive through "non-bloom" years. Perhaps there is an optimal set of conditions that can stimulate maximal germination, leading to the occasional widespread "red tides" caused by this species.

Given that dinoflagellate cysts are the result of sexual fusion and genetic recombination and that many cysts do not germinate each year, we would expect a heterogeneous population in the sediments. If this variability were reflected in the factors needed to induce germination and in the growth response of the motile cells, sexuality in *Gonyaulax tamarensis* would insure that: (a) some cells would be likely to excyst and survive after excystment each year; and (b) that a quiescent population would remain in the sediments to inoculate blooms in subsequent years. It follows that there should be less genetic variability in the motile, bloom population than in the cyst population in the underlying sediments. This hypothesis could be tested using the methods of Brand *et al.* (1981) to compare cultures established from isolates of motile cells during a bloom with others established from cysts germinated in the laboratory.

Bloom development and decline

In an attempt to understand the population dynamics of *Gonyaulax tamarensis* once vegetative cells were present in the water, we have focussed on four key regulatory processes of concern: vegetative growth; advection; mortality (due to grazing) and encystment. The first three are the subjects of other papers and further study and thus are only briefly discussed here as a reference for our assessment of the impact of encystment on bloom decline.

Vegetative growth. Data from the early stages of the *Gonyaulax tamarensis* blooms in 1980 suggest that cyst germination inoculated the salt ponds with a population of

planomeiocytes, which was quickly transformed into and outnumbered by vegetative cells produced through asexual reproduction. The first measurements in 1980 were predominantly of vegetative cells and included very few of the distinctive planomeiocytes (Fig. 1–3 B). Likewise, our sampling schedule gave no indication that a burst of germination occurred that might have provided a large, one-time input early in the bloom. In contrast, during the non-bloom year of 1981, planomeiocytes were a significant fraction of the population (approximately 30 percent of the total over a two week interval in Perch Pond), presumably because of reduced vegetative growth.

Although motile cells appeared in the three ponds at approximately the same time in 1980, the population increased much faster in Perch Pond and eventually peaked 35 d earlier than in Salt and Mill Ponds. One explanation for this differential can be found in differences in the temperature and salinity regimes in the ponds (Figs. 1–3 C). In experiments with laboratory cultures, (Watras *et al.*, 1982), we have found that the combination of higher salinities and lower temperatures (as in Salt and Mill Ponds) results in significantly lower *Gonyaulax tamarensis* growth rates than those for lower salinity, warmer waters (i.e. Perch Pond). Using our initial 1980 cell counts, the empirically determined relationships between growth rates, temperature and salinity, and the measured temperature and salinity, we were able to predict population development in the ponds up to the point of peak abundance – including the 35-d time differential (Watras *et al.*, 1982). One surprising result is the excellent fit of this simple model without consideration of losses due to grazing and advection.

The laboratory studies on temperature regulation of *Gonyaulax tamarensis* growth emphasize an important concept concerning the timing of cyst germination. The first cells were seen when water temperatures rose to approximately 6 °C, a temperature where growth rates are less than 30 percent of the maximum which occurs between 12° and 20 °C (Watras *et al.*, 1982). In fact, the entire interval of population increase occurred at suboptimal temperatures (Figs. 1–3 A, B). It thus appears that excystment is not a strategy that places a large number of cells in an optimum environment for rapid vegetative growth in the shallow salt ponds. It may play a different role, however, in deeper coastal waters where there is a larger temperature variation over the water column.

Advection and grazing. The advection of *Gonyaulax tamarensis* cells from these restricted salt ponds is a function of tidal transport and, we think, vertical migration behavior. This is the subject of a separate study (Anderson *et al.*, in preparation), but it should be noted that the loss of *G. tamarensis* on outgoing tides is considerably lower than would be predicted based solely on seawater exchange (Ketchum, 1951). For example, during two 24-h studies, more than 50 percent of the high tide volume moved through the inlet channel each day, while the net *G. tamarensis* loss was approximately 7 percent.

Grazing impacts are particularly difficult to estimate because of the spatial and temporal variability in the densities of both the zooplankton and the *Gonyaulax tamarensis* populations in the ponds and diel variability in grazing activity. The limited data we have collected thus far has been for non-bloom years, but we have established that both the dominant macrozooplankters in the ponds, *Acartia hudsonica* and larvae of the polychaete *Polydora* sp. do consume *G. tamarensis* in the presence of other phytoplankton species (Turner and Anderson, submitted). An abundant microzooplankter, the tintinnid *Favella ehrenbergii*, also feeds on *G. tamarensis* within rather narrow size limits (Stoecker *et al.*, 1981). Our estimates of short-term, whole community grazing rates on *G. tamarensis* in the ponds have shown a strong diel component with rates as high as 10 percent per hour (at certain times of the day).

Although limited, these data indicate that advection and, particularly, grazing could inflict significant losses on the vegetative populations. This is, however, at odds with the good agreement we found between observed and predicted population development ignoring these loss terms (Watras *et al.*, 1982). We can only suggest that either (i) field populations of *Gonyaulax tamarensis* may actually grow faster than those in laboratory cultures, (ii) the vertical migration behavior of this species is very effective in minimizing losses due to advection and grazing, or (iii) the high grazing rates were actually responsible for the fact that *G. tamarensis* did not reach high concentrations in 1981 when these rates were determined, and that grazing pressure was much lower in 1980.

Encystment. The environmental triggers for the encystment process remain elusive, but this study has at least eliminated some possible mechanisms and revealed the potential importance of others. The first planozygotes appeared when the *Gonyaulax tamarensis* population as a whole was increasing rapidly (Figs. 1–3), suggesting that differentiation and fusion of gametes occurred in the relatively early stages of bloom development. This is further evidenced by the consistent appearance of new cysts in surface sediments before the actual peak in *G. tamarensis* cell numbers. The conditions at the time of the first planozygote appearance as well as those one week earlier when gamete formation is likely to have begun are listed in Table 2. These data do not reveal a common mechanism for the induction of sexuality in *G. tamarensis* in the three ponds. Although nutrient limitation is generally implicated as the encystment stimulus in laboratory studies (e.g. von Stosch, 1973; Pfiester, 1975; Walker and Steidinger, 1980; Pfiester and Anderson, in press), this is unlikely in the salt ponds, considering the relatively high nutrient concentrations measured week after week (Figs. 1–3). Likewise, a temperature stress is unlikely since planozygotes appeared in all three ponds when temperatures had risen to approximately 12 °C, which is the lower limit of the optimal temperature range for vegetative growth (Watras *et al.*, 1982).

Many reproductive rhythms in higher plants and algae are regulated by photoperiod, but we can eliminate this as a single factor in *Gonyaulax tamarensis* sexuality since the timing of planozygote formation differed substantially between ponds, all of which experience the same photoperiod. It is of interest, however, that in both 1980 and 1981, the first planozygotes in the ponds accompanied the especially high tides of the spring series in both April and May. This evidence is circumstantial at best, but we cannot rule out the possibility that sexuality was affected by a lunar cycle. Lunar periodicities have been observed in the release of gametes and spores by macroalgae (Tahara, 1909; Hoyt, 1927; Hollenberg, 1936), which have been shown to release gametes only during the lowest spring tides (Smith, 1947). The synchronous release of many gametes into relatively shallow water theoretically increases the probability of fertilization.

Another inference based on the timing of sexuality in the three ponds deserves comment. If we generate a log-linear regression of the net *Gonyaulax tamarensis* population increase in each pond in 1980 and calculate the number of divisions required to transform the initial cell counts into the densities present when planozygotes appeared, we find values of 5.8, 5.6, and 6.3. Thus, despite the one month differential in population development between ponds, zygotes appeared approximately six divisions after the first vegetative cells were seen (neglecting advection and grazing). Here we must consider the possibility that the gradual depletion of a stored product following cyst germination somehow initiates sexuality and that the replenishment of this stored reserve occurs during the non-dividing planozygote stage.

All of our observations on the timing of encystment are consistent with the hypothesis of Wall *et al.* (1970), who suggested that encystment is a naturally occurring stage in the dinoflagellate life history that is not a reaction to adverse conditions but rather is favored by optimal conditions for vegetative growth in natural waters. In our study, not only were nutrient levels relatively high, but temperatures had just risen to the optimum levels for vegetative growth (Watras *et al.*, 1982) when the first zygotes appeared. Although most laboratory studies have used nutrient-depleted culture medium for the study of dinoflagellate sexuality, the concept discussed above is supported by a few reports of spontaneous sexuality and encystment in "optimum condition" cultures (Zingmark, 1970; Beam and Himes, 1974; Morey-Gaines and Ruse, 1980).

The numerical impact of the transition from asexual fission to sexual fusion is largely due to the presence of non-dividing gametes and planozygotes and their loss to the sediment as cysts. Gamete formation has not been described for *Gonyaulax tamarensis,* but the results of von Stosch (1973) and Walker and Steidinger (1980) suggest one or two divisions of each vegetative cell after sexual induction, leaving the liberated gametes to fuse or die. In addition to their loss as potential dividing cells, gametes are associated with an additional loss term since two fuse to form each planozygote.

If, as might be expected, all vegetative cells do not produce gametes at the same time, the population following sexual induction would consist of both dividing vegetative cells and non-dividing gametes and planozygotes. *In-situ* 1980 growth rates estimated for *Gonyaulax tamarensis* using the mitotic index approach gave a value of 0.14 d^{-1} six days prior to the Perch Pond population maximum and 0.09 d^{-1} six days after (Rubin, 1981). Both rates are lower than the net population increase of 0.3 d^{-1} estimated earlier in the bloom from weekly cell counts, and both were calculated on the non-planozygote fraction of the *G. tamarensis* population. Given the apparently favorable growth conditions, the implication is that the population included a significant number of non-dividing gametes, which we could not distinguish from vegetative cells.

Over one-third of the *Gonyaulax tamarensis* cells in each pond were planozygotes in 1980 as the bloom maxima approached. The duration of this life cycle stage is not known for *G. tamarensis,* but Figs. 1–3 B suggest that one week is a reasonable estimate based on the lag between the first planozygotes and the first cysts in the sediments. We can thus estimate a loss rate to the sediment of 15–20 percent d^{-1} within the planozygotes fraction of the population, or approximately 6 percent d^{-1} of the total.

The overall effect of sexual induction was to reduce the net growth rate of the entire *Gonyaulax tamarensis* population by introducing these additional "loss" terms. It is intriguing to us that encystment may have prevented the population from reaching true "red tide" proportions through continued asexual reproduction under seemingly favorable conditions. Coupled with excystment when waters were too cold for rapid division, it seems that the *G. tamarensis* excystment/encystment strategy is not keyed to rapid, sustained growth and bloom formation in our study area.

Absence of blooms in 1981. While all three ponds had relatively high *Gonyaulax tamarensis* concentrations and were closed for shellfish harvesting in 1980, in 1981 the cell concentrations were one to two orders of magnitude lower although the general pattern of population dynamics was similar. An excystment inoculum was provided, but the populations increased very slowly (less than 0.1 d^{-1} in both Perch and Salt Ponds) despite favorable nutrient concentrations, salinities, temperatures, and normal sunlight. Other phytoplankton were present, but did not differ in magnitude (as cell number or carbon) from 1980. The low *G. tamarensis* concentrations were not limited to our study areas. In 1981 there was only one brief PSP closure on Cape Cod, compared to 8 in 1980. It is thus reasonable to expect that any explanation for the "non-blooms" in 1981 would be tied to parameters that are not site-specific.

One noteworthy difference between years was the lack of rainfall in March, 1981 compared to the large amount in 1980 (Fig. 4 A). Here again we find an example of a

dinoflagellate bloom linked circumstantially to rainfall and terrestrial runoff (e.g. Prakash, 1975). The causative mechanisms remain obscure, however, and in this case we would need to identify a factor that inhibits *Gonyaulax tamarensis* growth while allowing other species to bloom. Trace metal inhibition and differential sensitivity between phytoplankton species (Anderson and Morel, 1978) are consistent with this observation and with the difference in rainfall and terrestrial runoff, but this is only one conjecture and other constraints are likely.

In summary, our comparative study of three separate embayments emphasizes the importance of life cycle changes in the bloom dynamics of cyst-forming dinoflagellates. In both bloom and non-bloom years, excystment and encystment established the temporal limits of the population. Factors controlling these processes remain obscure, however, as do those that determine the magnitude of the motile population. Clearly our understanding of the development and succession of blooms of many dinoflagellates will require more emphasis on life cycle stages and systematic investigation into the subtle factors that regulate their occurrence in natural waters.

Acknowledgements. Research supported in part by the Office of Sea Grant in the National Oceanic and Atmospheric Administration through grants NA80AA-D-00077 (R/B-41) to the Woods Hole Oceanographic Institution, and NA79AA-D-00101 (R/E-1) to the Massachusetts Institute of Technology Sea Grant College Program, and by the International Copper Research Association Project no. 307. We thank D. Kulis, J. Lively, C. Rubin, J. Turner, E. M. Hulbert and E. Santelmann for their assistance.

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Date of final manuscript acceptance: March 17, 1983.
Communicated by R. O. Fournier, Halifax