Reprinted from *Estuarine and Coastal Marine Science* (1979) **8**, 279–293

The Seeding of two Red Tide Blooms by the Germination of Benthic *Gonyaulax tamarensis* Hypnocysts[a]

D. M. Anderson[b] and F. M. M. Morel

Department of Civil Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, U.S.A.

Received 23 December 1977 and in revised form 17 April 1978

Keywords: cysts; red tide; resting spores; trace elements; toxicity; shellfish fisheries; Cape Cod

Complementary laboratory and field data are presented that demonstrate the seeding of a spring and a fall bloom of the toxic dinoflagellate *Gonyaulax tamarensis* by the temperature-induced germination of benthic hypnocysts. Nutrient, salinity, temperature and rainfall data collected before, during, and after a spring bloom in a Cape Cod salt pond indicate that germination of the overwintering hypnocysts was initiated by a temperature increase. Laboratory experiments with hypnocysts exposed to a gradual temperature increase after storage at 5 °C for four months verified this result. The fall bloom was apparently seeded by hypnocyst germination as well, but with excystment initiated by a temperature decrease from the summer level of 20–22 °C. This result was also confirmed in the laboratory using hypnocysts stored at 22 °C and subjected to a gradual decrease in temperature. The reasons for the eventual decline of both blooms are not known, but it is proposed that the motile populations were transformed into new hypnocysts that settled to the sediments. Immediately after both bloom peaks, there was a sharp decrease in the percentage of hypnocysts that germinated during laboratory incubation after isolation from fresh, unstored sediment samples. The duration of these periods of limited excystment after the declines suggests that cold storage (or overwintering) is not a required conditioning mechanism prior to germination but that a prolonged period of conditioning at higher temperatures will suffice. Finally, data are presented that suggest that the extreme localization of the blooms within the salt pond was due to limited tidal advection of the motile population and the favourable trace metal chemistry of the estuarine environment compared to the adjacent coastal waters.

Introduction

Despite extensive research into the various chemical, physical, and biological factors that affect the growth of the toxic dinoflagellates *Gonyaulax tamarensis* and *G. excavata* (e.g., Prakash, 1967; Yentsch *et al.*, 1975; Mulligan, 1975; Hartwell, 1975), the basic mechanisms underlying the erratic outbreaks of paralytic shellfish poisoning along major portions of

[a]Research supported in part by the Office of Sea Grant in the National Oceanic and Atmospheric Administration, United States Department of Commerce, through grant number 04-7-158-44079, in part by National Science Foundation grant number 77-09000, and in part by International Copper Research Association Project Number 252.

[b]Present address: Woods Hole Oceanographic Inst., Woods Hole, Mass. 02543, U.S.A.

0302–3524/79/030279+05 $02.00/0

varied throughout the study, averaging 23 and 26·5‰, respectively [Figure 2(c)]. Rainfall was heaviest in March when a total of 11·4 cm fell in a two week period [Figure 2(d)]. Nitrate concentrations at the surface of Perch Pond were very high in March (56 μM), gradually decreasing to 5 μM in May [Figure 2(e)]. The same gradual decrease was observed in the bottom waters, although the initial and final concentrations were 10·7 and 0·6 μM, respectively. Phosphate followed no apparent trend over the 8 week study period [Figure 2(f)]; concentrations were low, ranging between 0·5 and 0·04 μM at the surface, and between 0·21 and 0·055 μM at the bottom. Surface and bottom concentrations of silicate were very similar in Perch Pond, with both profiles peaking near 30 μM on 22 April and decreasing thereafter to a final value of approximately 2·5 μM [Figure 2(g)]. The total iron concentration was generally constant near 1 μM throughout the study except for one peak on 22 April when the bottom water contained 2·2 μM [Figure 2(c)].

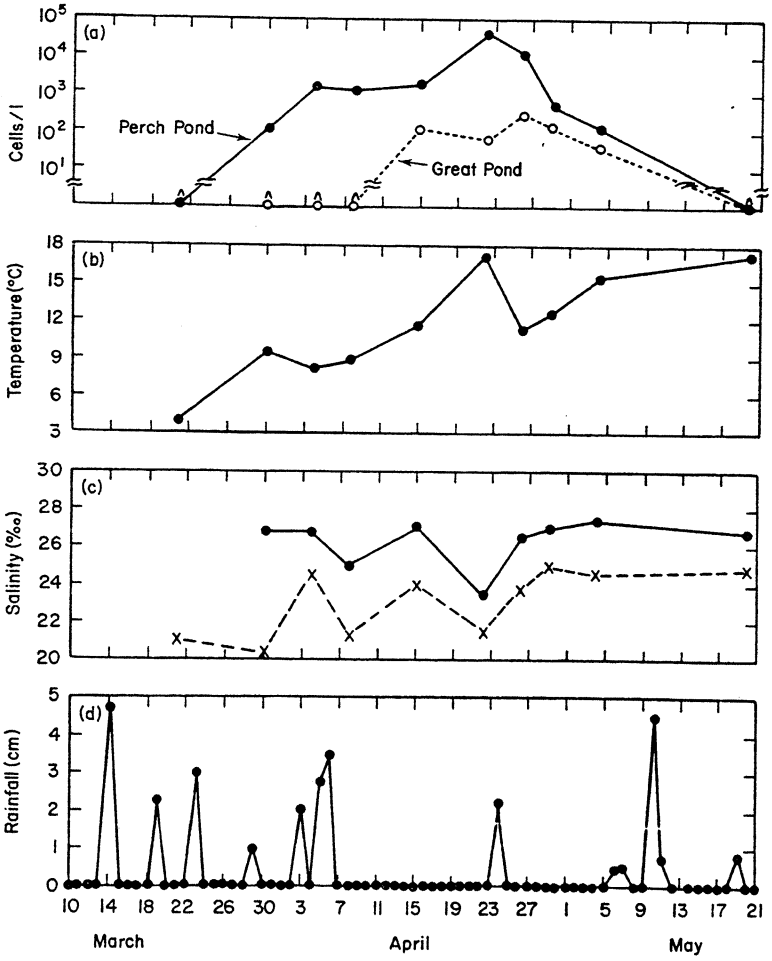

Figure 2. Spring bloom time series. (a) Cell densities (at 2·5 m) in Perch Pond and Great Pond. Data points with 'hats' are those below counting detection limits (5–10 cells l^{-1}). (b) Bottom water temperature (2·5 m); (c) Salinity of surface (X – – – – X) and bottom waters (●——●); (d) Rainfall in West Falmouth; (e–h) Surface (X – – – – X) and bottom water (●——●) concentrations of nitrate (e), phosphate (f), silicate (g) and total iron (h), (next page).

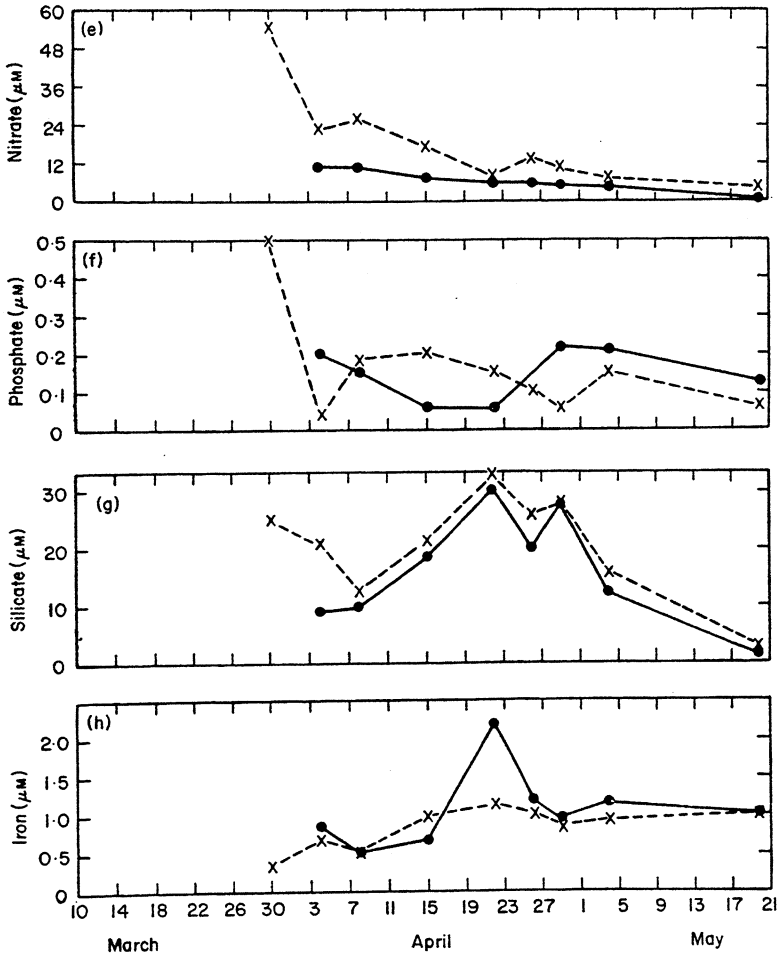

Fig. 2. (e - h)

Average surface solar radiation for the months of February, March, April and May were 210, 302, 443, and 508 ly day^{-1} respectively, corresponding to mean values of 99, 147, 206, and 228 ly day^{-1} over the 2·5 m water column in Perch Pond (estimated using extinction coefficients from subsurface measurements on the ten sampling days). Based on Riley's (1967) estimate of 40 ly day^{-1} as the critical light threshold needed to support a spring bloom in a temperate zone estuary, it would appear that the *G. tamarensis* bloom in late March, early April could have occurred over a month earlier if light was the critical limiting parameter.

On each sampling date, the *G. tamarensis* cell densities were significantly higher in the Perch Pond bottom waters than at the surface. This observation was confirmed by more complete counts taken on three different occasions (Figure 3). In each case there was a bottom maximum in cell density and salinity, although the largest salinity differential was only 3·5‰ from top to bottom. No systematic variation of temperature with depth was apparent. Note that samples were not taken continuously throughout one complete day and that Perch Pond is quite shallow, so these results may not be indicative of general vertical migration patterns of *G. tamarensis*.

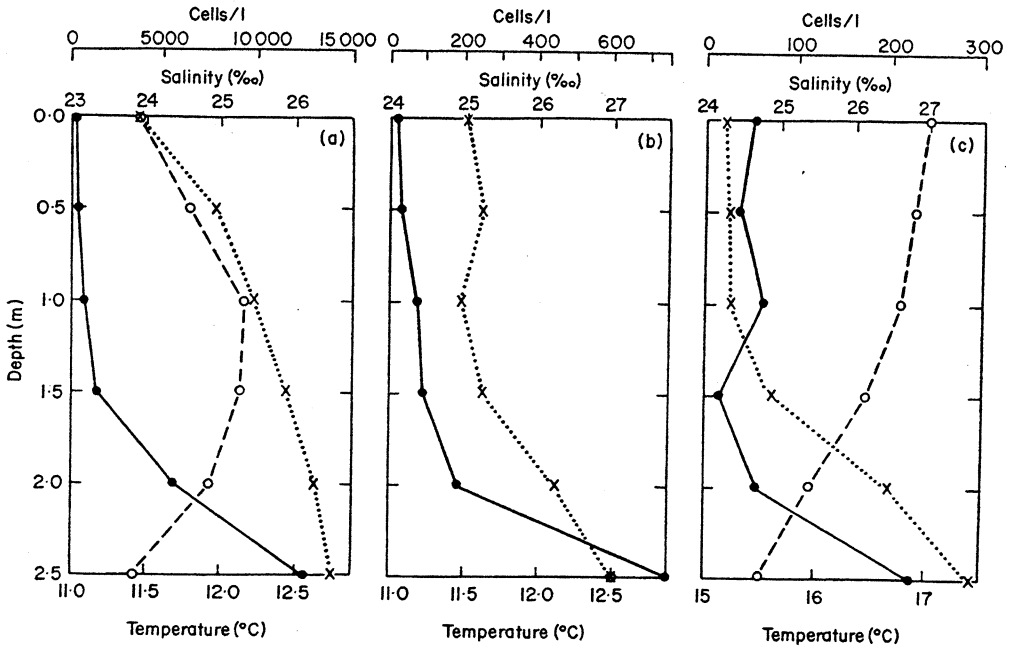

Figure 3. Vertical profiles of *G. tamarensis* cell density (●——●), temperature (○ – – – – ○) and salinity (X · · · · · X) during the spring bloom in Perch Pond. (a) 26 April, 9.00 am, measured incident radiation 750 and 65 μeinsteins $m^{-2} s^{-1}$ at surface and bottom; (b) 29 April, 12.00 noon, surface 1300, bottom 210 μeinsteins $m^{-2} s^{-1}$, temperature data at one depth only; (c) 4 May, 3.30 pm, surface 270, bottom 32 μeinsteins $m^{-2} s^{-1}$.

Fall bloom

A small bloom of *G. tamarensis* also occurred in Perch Pond in the fall [Figure 4(a)]. Although sampling was limited, cell densities as high as 1050 cells l^{-1} were observed. Nutrient data were not collected but measured bottom water temperatures reveal a sharp decrease (6 °C) from the summer levels during the onset of the bloom [Figure 4(b)].

Excystment of hypnocysts from unstored sediment samples

One series of hypnocyst germination experiments was conducted at 16 °C using sediment samples taken directly from Perch Pond without storage [Figure 4(c)]. In late March and early April, over 90% of these unstored hypnocysts germinated successfully, while after 15 April, there was a steady decrease to a low of 27%. Thereafter the trend reversed, increasing to 89% excystment in mid-September, followed once again by a decrease through October and an increase back to 90% level in November. Figure 4 shows the temperature of the bottom water (and therefore that of the sediment sample) immediately prior to cleaning, isolation and incubation. *G. tamarensis* cell densities in Perch Pond during this March to November period are also shown in Figure 4.

Excystment of hypnocysts after controlled-temperature conditioning

Figure 4(c) represents a series of excystment results from a sediment sample collected on 3 June and stored in the dark at 16 °C in an attempt to elucidate the effect of temperature

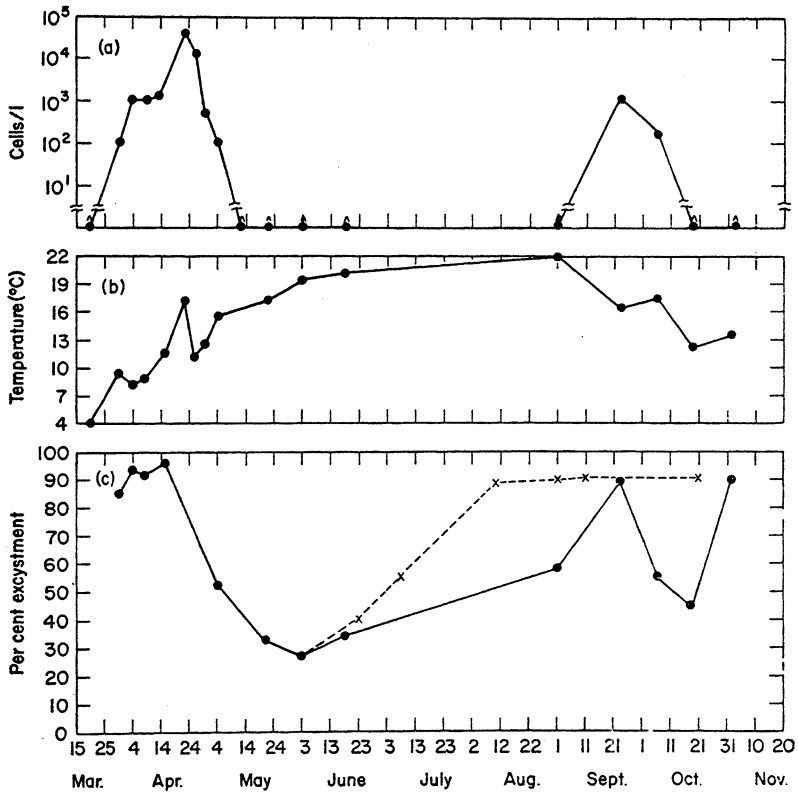

Figure 4. Time series, March through November in Perch Pond: (a) *Gonyaulax tamarensis* cell density (2·5 m), with data points below counting detection limits (5–10 cells l^{-1}) indicated with 'hats'; (b) Bottom water temperature (2·5 m); (c) Excystment success of hypnocysts from fresh sediment samples (●——●) and from one sample, collected 3 June and stored in the dark at 16 °C (X – – – – X). Cleaning and isolation were at room temperature, incubation at 16 °C for 5 days.

on cyst conditioning. Hypnocysts conditioned by this constant temperature became more viable with time, reaching 86% excystment success in early August, in contrast to the more gradual increase in viability of those exposed to the warmer natural temperature [Figure 4(c)]. This conclusion hinges on one data point (Sept. 1) however.

When hypnocysts stored at 5 °C in the dark for four months (after collection on 3 June, water temperature 19·4 °C) were carefully cleaned and isolated at 5 °C and then subjected to a gradual temperature increase (*ca.* 0·4 °C day^{-1}), the first motile cells were seen when the temperature was 7–8 °C [Figure 5(a)]. Excystment of the population was gradual, taking place over a 6-day period, despite the fact that all cysts were exposed to the same conditions and that the temperature was increasing. No excystment was observed in a control culture maintained in the light at 5 °C for 15 days.

A similar experiment (two months dark storage, then cleaning and isolation, all at 22 °C, using samples collected from 22 °C water on 1 September) demonstrated that excystment could also be initiated by a gradual *decrease* in temperature (*ca.* 0·4 °C day^{-1}) from the pre-conditioned level [Figure 5(b)]. In this case, motile cells were first seen when the temperature reached 18–19 °C with excystment occurring over an 11 day period. No germination was observed in the control culture maintained at 22 °C. Further, when the

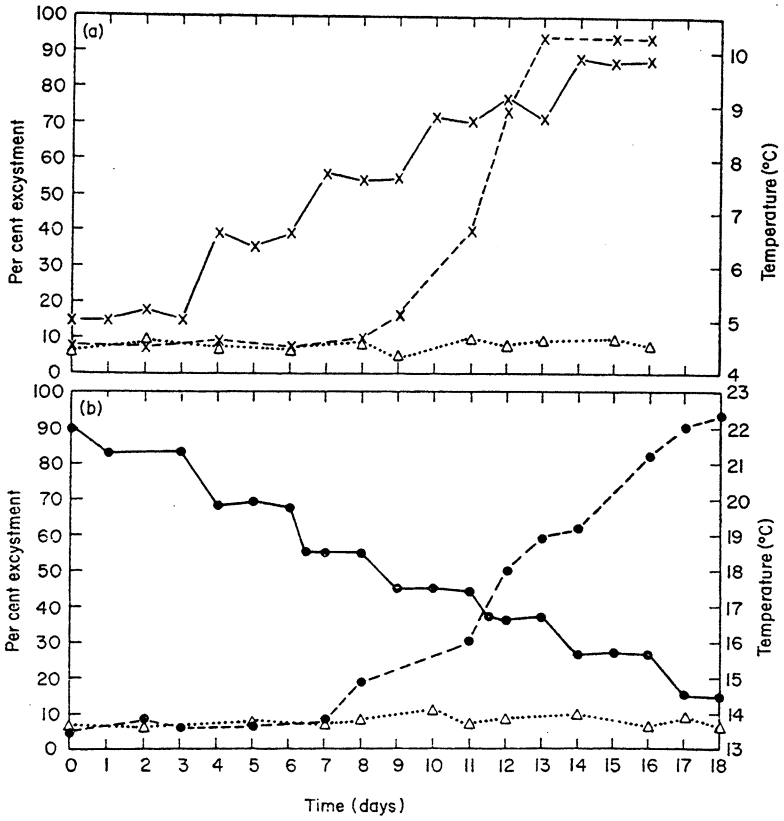

Figure 5. Excystment response to gradual temperature change (*ca.* 0·4 °C day^{-1}). (a) Gradual temperature increase. Hypnocysts from 3 June sediment sample, stored at 5 °C for 4 months, then cleaned and isolated at 5 °C. Per cent excystment (X – – – – X) with increasing temperature (X —— X); Per cent excystment (Δ · · · · · Δ) at constant 5 °C; (b) Gradual temperature decrease. Hypnocysts from 1 September sediment sample, stored at 22 °C for two months, then cleaned and isolated at 22 °C. Per cent excystment (●– – – –●) with decreasing temperature (●——●); Per cent excystment (Δ – – – – Δ) at constant 22 °C.

22 °C pre-conditioned cysts were incubated at 16 °C directly (a 6° decrease), excystment was rapid (75% in 5 days), yet none germinated when incubation was at 28 °C (a 6° increase).

Trace metal toxicity

On 15 April, hypnocysts from a fresh sediment sample were isolated into filtered seawater from each of the three sampling sites. Table 1 shows the excystment and motility results for the raw seawater samples and for the samples plus EDTA. In the raw samples, excystment success was approximately the same in each case, while the motility of the germling cells varied considerably between the estuarine sites (Perch Pond, Great Pond) and the coastal site (Vinyard Sound). Addition of EDTA did not affect excystment, but did increase the percentage of cells that reached motility in all three cases. The largest change occurred in Vinyard Sound sample where the addition of the chelator increased motility from 6 to 70%.

TABLE 1. Cyst revival in natural water samples[a]

Seawater sample	Excystment	Motility
Perch Pond, raw	94%	69%
Perch Pond+EDTA[b]	96%	83%
Great Pond, raw	86%	72%
Great Pond+EDTA[b]	91%	81%
Vinyard Sound, raw	91%	6%
Vinyard Sound+EDTA[b]	94%	70%

[a]Sediment and water samples were collected 15 April 1977. Motile cells were counted immediately before the first cell divisions. A minimum of 50 hypnocysts were incubated in each water sample.
[b]5×10^{-5}M EDTA.

Discussion

The complementary field and laboratory data presented here support the contention that a spring and a fall bloom of the toxic dinoflagellate *G. tamarensis* were initiated by the temperature-induced germination of dormant hypnocysts as sediments warmed in the spring and cooled in the fall.

Bloom initiation

Looking only at the field data for the spring bloom in Perch Pond, it is evident that there is insufficient information to conclude that the initiation of *G. tamarensis* growth was triggered by favourable interactions between one particular parameter or set of parameters and either motile cells or hypnocysts. The data give no indication that nutrients, salinity, light, photoperiod (Yentsch *et al.*, 1975), or other factors were important other than in providing a suitable environment into which the benthic cysts revived as a seed population.

The first cell counts from Perch Pond in March did not include *G. tamarensis*. Since the counting procedure would not detect densities lower than 5–10 cells l^{-1}, the possibility cannot be ruled out that a small motile population was present to seed the bloom (either an indigenous motile population or an external population carried to the pond by tidal currents). After the *G. tamarensis* cells were first found in Perch Pond, however, it was three weeks before detectable concentrations were seen in Great Pond (less than 200 m away), and none were ever counted in the Vinyard Sound samples. In light of these data, it is noteworthy that the hypnocyst density in Perch Pond sediments was extremely high in the fall 1976 and the spring 1977, whereas the density in Great Pond was so low as to be nearly undetectable (Anderson & Wall, 1978; Wall, personal communication). Further, as shown in Figure 4, 90% of the hypnocysts isolated from Perch Pond in late March excysted when incubated in the laboratory. The benthic population, though dormant, was ready for exystment given the proper stimulus. Figure 5(a) shows that cysts 'conditioned' to 5 °C in the laboratory for 4 months began their revival after a warming of 2–3 degrees and that the temperature threshold for germination within the population was relatively large (i.e., excystment occurred over a 6-day period even as the temperature was increased further). It is thus not surprising to find that motile *G. tamarensis* cells were first seen in Perch Pond on 30 March when the water temperature was 9·6 °C, an increase from 4 °C just 9 days earlier. This was also the first day that some hypnocysts in the sediment samples had the

cell contents and pigmentation typical of cysts immediately prior to excystment (Anderson & Wall, 1978).

The contention that increasing temperature was the critical parameter in the initiation of the spring excystment process supports the findings of Huber & Nipkow (1923) who studied the cysts of the freshwater dinoflagellate *Ceratium hirundinella*, and Anderson & Wall (1978), who worked with *G. tamarensis* and *G. excavata*. In each case, excystment was found to be relatively independent of medium composition and light regime, but clearly linked to a temperature increase. Indirect evidence in support of the temperature trigger can also be seen in the shellfish toxicity records for Cape Cod (Anderson & Wall, 1978). The first toxicity records occurred in the fall of 1972 during a massive coastal bloom, yet from 1974 through 1977, toxicity peaked in the late spring—the time of vernal warming of the waters and sediments—with no toxicity in the fall anywhere on Cape Cod. (Hence, the decision not to take extensive fall data.) The very low cell density actually observed during the fall bloom (1050 cells l^{-1}) may explain this lack of historical toxicity.

The striking feature of this small bloom is that it was apparently seeded by the revival of hypnocysts whose germination was triggered by cooling. To the authors' knowledge, this mechanism has not been reported previously. Figure 5(b) provides laboratory confirmation of this phenomenon, as hypnocysts germinated when incubated at gradually decreasing temperatures after two months of 22 °C storage. No excystment was observed in the control maintained at 22 °C. It is again not surprising (in retrospect of course) that motile *G. tamarensis* cells were detected 22 September when the water temperature was 16·2 °C, a decrease of 6 degrees from the 22 °C temperature on 1 September. It is also noteworthy that 90% of the hypnocysts collected on 22 September excysted on laboratory incubation.

Bloom development and decline

In general, the data in Figure 2 indicate that conditions were favourable for the support of a bloom in late March. Nutrients were sufficiently high, salinities within the optimum range for *G. tamarensis* (Prakash, 1967), and the temperature increasing. Rainfall was heavy in the 2 weeks prior to the bloom. This is noteworthy because the resulting terrestrial runoff has often been implicated in dinoflagellate blooms. It can result in a physical transport process (Hartwell, 1975), or be a source of nutrients, iron and other organically bound trace metals (e.g., Prakash & Rashid, 1968; Doig & Martin, 1974; Kim & Martin, 1974; Prakash, 1975) or of organic material capable of chelating and thus detoxifying metal ions (Martin & Martin, 1973; Anderson & Morel, 1978).

It is not clear why the spring *G. tamarensis* population eventually declined after its gradual growth. Phosphate concentrations were as low as 0·05 μM in the bottom water at the peak of the bloom, while sharp decreases in salinity, temperature, and iron were observed as the bloom began its decline. One possible explanation for both spring and fall declines would be that the motile populations were eventually transformed into new hypnocysts that settled to the sediments. Initially it was thought that as a bloom developed and the temperature trend continued, there would be a time when essentially no viable cysts would be left in the sediments. This was not the case as sediment samples continually contained numerous hypnocysts throughout the study, perhaps because of delayed germination due to anoxic conditions, temperature gradients or other unknown inhibitory mechanisms possibly present in the benthos.

Little is actually known of the mechanisms involved in the formation of marine dinoflagellate cysts. Turpin *et al.* (1978) report that aclonal *G. tamarensis* cells undergo sexual fusion and form zygotic cysts when placed in nitrogen-free medium. Wall & Dale (1968) state that encystment in marine populations occurs after the exponential growth phase. Virtually all other research into cyst formation has involved freshwater dinoflagellates where cysts have been induced by the use of nitrogen-free medium (e.g., Pfiester, 1975, 1976; von Stosch, 1973) and by temperature and photoperiod changes (von Stosch, 1973). Nitrate concentrations in Perch Pond were well above micro-molar during the spring bloom, so if new hypnocysts were formed, some other mechanism than nitrogen starvation was probably involved.

A change was observed in the viability of the natural cyst populations whereby the excystment success (normally 90% in March and early April), dropped sharply near the peak of the spring bloom and again after the fall bloom (Figure 4). One reason for these declines might be that the temperature differential between experimental incubation (and isolation) and the natural sediment temperature was insufficient to stimulate the entire population to excystment. However, this reasoning cannot fully explain the different excystment percentages obtained at specific temperatures (e.g., 94, 32, and 56% excystment of cysts isolated from 17 °C sediments). It is more probable that the decline in viability following each bloom peak is a reflection of the dormancy characteristics of newly-formed cysts. Studies of freshwater dinoflagellates by von Stosch (1965, 1967, 1973) and Huber & Nipkow (1922, 1923) suggest that a critical factor in the excystment process is the age of the cyst, whereby excystment is inhibited for several weeks after formation but becomes progressively more rapid after a resting period. Although some newly formed cysts of *Woloszynskia apiculata* and *Gymnodinium pseudoplaustre* were able to germinate after several weeks at 15 °C, von Stosch (1973) emphasized the need for four weeks of 'cold conditioning' (3–6 °C) for more complete and better synchronized germination. The gradual increase in *Gonyaulax tamarensis* cyst viability through the summer implies that if in fact new hypnocysts were formed in the spring, an extended resting period at warm temperatures provides sufficient conditioning for complete germination and that cold temperatures are not necessary. It would then appear that cold conditioning shortens the requisite dormancy period since cyst viability increased faster in November than during the summer [Figure 4(c)]. This may also be inferred from the difference between the time sequence of excystment results for the same initial cyst population after 16 °C conditioning and natural summer conditioning (20–22 °C) in Figure 4(c).

One difficulty in the interpretation of the data in Figure 4 arises from the possible superposition of a dormant population having a range of excystment thresholds and a motile population that encysts in an unknown manner. With this type of overlap, some sediment samples collected during this study could have contained both hypnocysts from a previous bloom and new hypnocysts from the ongoing bloom. Existing techniques for hypnocyst collection and isolation are not sufficiently quantitative to reveal numerical changes in the benthic population with time, nor was there ever a visible physical difference between the cysts in the successive samples that could be distinguished from normal variations within a population (Anderson & Wall, 1978). Apparently many cysts remained dormant throughout both blooms.

Bloom localization

It would appear that the extreme localization of the *G. tamarensis* blooms within Perch Pond was due to the combined effects of physical, biological, and chemical factors.

Physically, the shallow inlet leading to Great Pond apparently acted as an effective barrier to the tidal advection of the motile cells during the spring bloom. Vertical profiles of cell densities taken on three separate dates during the bloom indicate that very few cells were sufficiently close to the surface to be flushed through the inlet by tidal currents. This observation, though based on limited data, is consistent with the absence of *G. tamarensis* from early cell counts in Great Pond, but not with the observations of Eppley *et al.* (1968) and Seliger *et al.* (1970, 1975) who described vertical migration patterns for the dinoflagellates, *G. polyhedra* and *Pyrodinium bahamense*, *Gymnodinium nelsoni* and *Ceratium furca*, respectively.

Another localization factor could be that the trace metal chemistry of the estuarine environment favors the viability of the germling cells whereas a more coastal location (as exemplified by Vinyard Sound) does not (Table 1). Excystment did not vary in the three different water samples (in agreement with data from Anderson & Wall, 1978) but only in the estuarine waters of Perch Pond and Great Pond were most excysted cells capable of motility. Addition of a chelator improved the suitability of the Vinyard Sound water significantly—presumably through the chelation of toxic metal ions (Sunda & Guillard, 1976) or the solubilization of metals as nutrients (e.g., iron). *G. tamarensis* has been shown to be extremely sensitive to copper ions, with loss of mortality given as one indicator of toxicity (Anderson & Morel, 1978). It is thus conceivable that the higher organic content of estuarine waters provides the natural chelators needed for detoxification of metal ions, and that the observed localization of *G. tamarensis* (hypnocysts and motile populations) reflects this trace metal influence.

Overview

The encystment and excystment processes, trace metal sensitivity, and physical concentration mechanisms described are all consistent with the observed pattern of toxic outbreaks in the Cape Cod region. Certain results are germane to such red tide phenomena throughout the world, however. For example, two different temperature 'triggers' for excystment have been described, each determined by the previous temperature conditioning of the cyst. Thus the seeding of blooms by cyst-forming dinoflagellates in tropical regions may involve germination initiated by slight cooling processes, whereas cysts in much colder areas may only revive in response to temperature increases. Water temperatures and toxicity records in eastern Canada fit well into the latter category, as shellfish toxicity (caused by *G. tamarensis*) typically occurs throughout summer (peak surface temperatures 11·8 °C), beginning in June when the water averages 7·1 °C, up from 4·9 °C in May (Prakash, 1967). Although both warming and cooling excystment 'triggers' could be operating in such an environment, their separate influences cannot be distinguished as they can be in a more temperate climate where widely separated spring and fall blooms can occur.

The experiments and observations reported here are only a first step towards the confirmation of the hypothesized role of benthic cysts in the initiation and spreading of toxic dinoflagellate blooms. Clearly, there is a need for further research into the various chemical and biological processes affecting the encystment/excystment cycle in natural waters throughout the world.

Acknowledgements

We thank D. Wall, J. J. McCarthy, and S. W. Chisholm for helpful discussions, R. E. Payne for daily light measurements and C. Russell for boat and dock facilities.

References

Anderson, D. M. & Wall, D. 1978 The potential importance of benthic cysts of *Gonyaulax tamarensis* and *Gonyaulax excavata* in initiating toxic dinoflagellate blooms. *Journal of Phycology* **14,** 224–234.

Anderson, D. M. & Morel, F. M. M. 1978 Copper sensitivity of *Gonyaulax tamarensis*. *Limnology and Oceanography* **23,** 283–295.

Braarud, T. 1945 Morphological observations on marine dinoflagellate cultures (*Porella perforata, Goniaulax tamarensis, Protoceratium reticulatum*). *Avhandlinger ugitt av det Norske Videnskaps-akademi i Oslo, Mat. Naturv.Kl.* 1944, **11,** 1–18.

Dale, B. 1977 Cysts of the toxic and red-tide dinoflagellate *Gonyaulax excavata* (Braarud) Balech from Oslofjorden, Norway. *Sarsia* **63,** 29–34.

Doig, M. T. & Martin, D. F. 1974 The effect of naturally occurring organic substances on the growth of a red tide organism. *Water Research* **8,** 601–606.

Eppley, R. W., Holm-Hansen, O. & Strickland, J. D. H. 1968 Some observations on the vertical migrations of dinoflagellates. *Journal of Phycology* **4,** 333–340.

Hartwell, A. D. 1975 Hydrographic factors affecting the distribution and movement of toxic dinoflagellates in the western Gulf of Maine. In *Toxic Dinoflagellate Blooms* (LoCicero, V. R., ed.). *Proceedings of the International Conference (1st) Massachusetts Science and Technology Foundation,* pp. 47–68.

Huber, G. & Nipkow, F. 1922 Experimentelle untersuchungen über die entwicklung von *Ceratium hirundinella* O.F.M., *Zeitschrift für Botanik* **14,** 337–371.

Huber, G. & Nipkow, F. 1923 Experimentelle untersuchungen über die entwicklung und formbildung von *Ceratium hirundinella* O.F.M. *Flora, Jena* **116,** 114–215.

Kim, Y. S. & Martin, D. F. 1974 Interrelationship of Peach River parameters as a basis of the iron index: a predictive guide to the Florida red tide. *Water Research* **8,** 607–616.

Martin, D. F. & Martin, B. B. 1973 Implication of metal-organic interactions in red tide outbreaks. In *Trace Metals and Metal–organic Interactions in Natural Waters* (Singer, P. C., ed.). Ann Arbor Science, pp. 339–361.

Morel, F. M. M., Westall, J. C., Rueter, J. G. & Chaplick, J. P. 1975 Description of the algal growth media 'Aquil' and 'Fraquil'. *Technical Note* no. 16, Massachusetts Institute of Technology, Department Civil Engineering, Ralph M. Parsons Laboratory of Water Resources and Hydrodynamics, 33 pp.

Mulligan, H. F. 1975 Oceanographic factors associated with New England red-tide blooms. In *Toxic Dinoflagellate Blooms* (LoCicero, V. R., ed.). *Proceedings of the International Conference (1st) Massachusetts Science and Technology Foundation,* pp. 23–40.

Pfiester, L. A. 1975 Sexual reproduction of *Peridinium cinctum* f. *ovoplanum* (Dinophyceae). *Journal of Phycology* **11,** 259–265.

Pfiester, L. A. 1976 Sexual reproduction of *Peridinium willei* (Dinophyceae). *Journal of Phycology* **13,** 92–95.

Prakash, A. 1967 Growth and toxicity of a marine dinoflagellate *Gonyaulax tamarensis*. *Journal of the Fisheries Research Board of Canada* **24,** 1589–1606.

Prakash, A. 1975 Land drainage as a factor in 'red tide' development. *Environmental Letters* **9,** 121–128.

Prakash, A. & Rashid, M. A. 1968 Influence of humic substances on the growth of marine phytoplankton: dinoflagellates. *Limnology and Oceanography* **13,** 598–606.

Riley, G. A. 1967 The plankton of estuaries. In *Estuaries* (Lauff, G. H., ed.). Am. Assoc. Adv. Sci. Publication no. 83, pp. 316–326.

Segar, D. A. & Cantillo, A. Y. 1975 Direct determination of trace metals in seawater by flameless atomic absorption spectrophotometry. In *Analytical Methods in Oceanography* (Gibb, T. R. P. Jr., ed.). Advances in Chemistry Series 147. American Chemical Society, pp. 56–81.

Seliger, H. H., Carpenter, J. H., Loftus, M. & McElroy, W. D. 1970 Mechanisms for the accumulation of high concentrations of dinoflagellates in a bioluminescent bay. *Limnology and Oceanography* **15,** 234–245.

Seliger, H. H., Loftus, M. E. & Subba Rao, D. V. 1975 Dinoflagellate accumulations in Chesapeake Bay. In *Toxic Dinoflagellate Blooms* (LoCicero, V. R., ed.). *Proceedings of the International Conference (1st) Massachusetts Science and Technology Foundation.*

Sousa E. S., Estela de. 1962 Some observations on marine dinoflagellate cultures. III. *Goniaulax spinifera* (Clap. and Lach.) Dies., *Goniaulax tamarensis* Leb. and *Peridinium trochoideum* (Stein) Lemm. *Notase Estudos do Instituto de Biologia Maritima* **26,** 1–24.

Steidinger, K. A. 1975 Basic factors influencing red tides. In *Toxic Dinoflagellate Blooms* (LoCicero, V. R., ed.). *Proceedings of the International Conference (1st) Massachusetts Science and Technology Foundation,* pp. 153–162.

Strickland, J. D. H. & Parsons, T. R. 1972 A practical handbook of seawater analysis, 2nd ed. *Bulletin of the Fisheries Research Board of Canada* no. 167.

Sunda, W. G. & Guillard, R. R. L. 1976 Relationship between cupric ion activity and the toxicity of copper to phytoplankton. *Journal of Marine Research* **34,** 511–520

Turpin, D. H., Dobell, P. E. R. & Taylor, F. J. R. 1978 Sexuality and cyst formation in Pacific strains of the toxic dinoflagellate *Gonyaulax tamarensis*. *Journal of Phycology* **14**, 235–238.

von Stosch, H. A. 1965 Sexualität bei *Ceratium cornutum* (Dinophyta). *Naturwissenschaffen* **52**, 112–113.

von Stosch, H. A. 1967 Dinophyta. In (Ettl, H., Muller, D. G., Newmann, K., von Stosch, H. A. & Weber, W., eds), Vegetative fortpflanzung, parthenogense und apogamie bei algen. Handb. Pfl. Physiol. **18**, 626–636.

von Stosch, H. A. 1973 Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. *British Phycological Journal* **8**, 105–145.

Wall, D. 1975 Taxonomy and cysts of red tide dinoflagellates. In *Toxic Dinoflagellate Blooms* (LoCicero, V. R., ed.). *Proceedings of the International Conference (1st) Massachusetts Science and Technology Foundation*, pp. 249–256.

Wall, D. & Dale, B. 1968 Modern dinoflagellate cysts and evolution of the Peridiniales. *Micropaleontology* **14**, 265–304.

Yentsch, C. M., Cole, E. J. & Salvaggio, M. G. 1975 Some of the growth characteristics of *Gonyaulax tamarensis* isolated from the Gulf of Maine. In *Toxic Dinoflagellate Blooms* (LoCicero, V. R., ed.). *Proceedings of the International Conference (1st) Massachusetts Science and Technology Foundation*, pp. 163–180.

Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset