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An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*

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Blooms of the toxic dinoflagellate *Gonyaulax tamarensis* (synonyms *Protogonyaulax tamarensis*[1] and *Alexandrium tamarense*[2]) cause outbreaks of paralytic shellfish poisoning (PSP) in coastal waters throughout the world. In the Gulf of Maine, episodes occur between April and November, a seasonality due in part to life-cycle alternations between motile, vegetative cells and resting cysts which overwinter in bottom sediments[3,4]. Newly formed cysts have a mandatory 2–6 month dormancy period during which germination is not possible[5], but once mature, the resting state will continue if temperatures are unfavourable[5] or oxygen is unavailable[6]. We now report another factor controlling germination of cysts of *G. tamarensis* from deep coastal waters—an endogenous annual clock that can override an otherwise favourable environment for germination. Similar annual variability in germination has not been observed for cysts of this species from shallow estuaries. These results represent the first conclusive demonstration of an endogenous circannual rhythm in a marine plant. They are evolutionarily and ecologically significant because an endogenous annual clock can lead to the release of motile cells into deep and relatively invariant bottom waters at those times when temperature and light at the surface are suitable for growth. In shallow waters where seasonal variability is large and extends to bottom sediments, a strategy similar to that of the seeds of terrestrial plants would be more appropriate, namely a direct coupling between germination and the external environment.

The annual rhythm was first observed in cysts from freshly collected sediment cores. Regardless of depth of burial within the sediment or overlying water depth, germination frequency of cysts from the Gulf of Maine oscillated systematically during the two-year study (Fig. 1*b*). Cysts of other species from the Gulf of Maine and of the same species from a shallow Cape Cod estuary had high germination frequencies in the same tissue culture plates and medium.

The germination rhythm was also evident in monthly subsamples from an August core stored in the dark at 2 °C (Fig. 1*c*). Thus the reduced germination success from August to December is not due to the primary dormancy of immature cysts; maturation would be evidenced by an increasing trend in germination frequency through time, remaining constant and high thereafter. Persistence of the germination rhythm for two years under constant storage conditions indicates endogenous control.

Annual cycles have long been recognized in plants and animals[7], but many of the rhythms occur in response to external cues and disappear with storage in constant conditions. True endogenous control of annual rhythms is most easily demonstrated in animals[8] and is difficult to document in plants[9]. The germination of some plant seeds can vary over an annual cycle, in that seeds can be alternately responsive and unresponsive to the same germination conditions[10,11], but this rhythm typically disappears with storage in constant conditions. The phenomenon is thus termed secondary dormancy—the environmentally mediated re-induction of a state of deep dormancy. The germination rhythm in *G. tamarensis* cysts isolated from monthly core samples (Fig. 1*b*) could be attributed to secondary dormancy as the 'storage' environment could not be controlled. However, persistence of the rhythm for two years under constant conditions in the laboratory (Fig. 1*c*) is consistent with endogenous regulation.

Suggestions of annual rhythms are found in three studies of *G. tamarensis*. Yentsch and Mague[12] report evidence suggestive

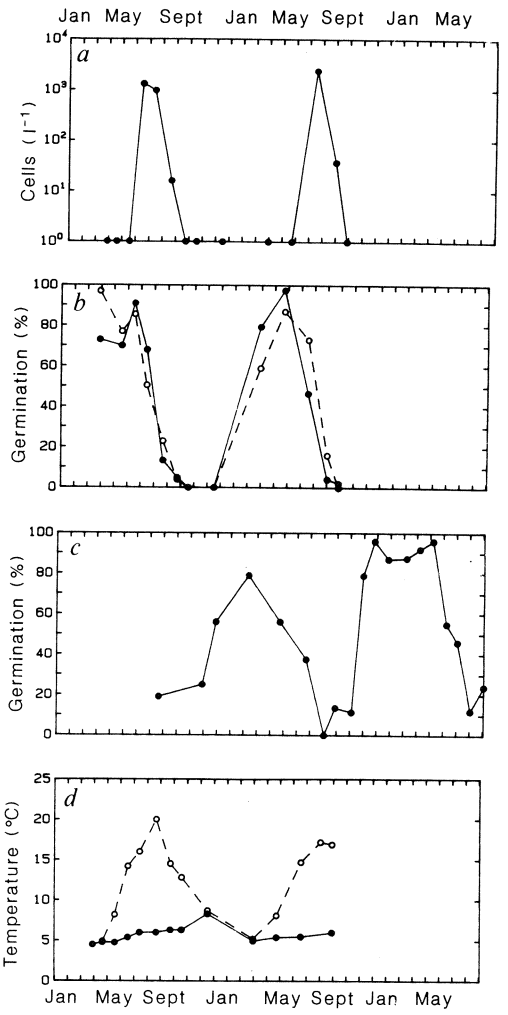

Fig. 1 Population dynamics of cysts and motile cells of *Gonyaulax tamarensis*. *a*, Average motile cell concentration in the top 30 m of the water column from continuously pumped samples at Station 29 (160 m deep, 43° N 70°19′ W) and Station 30 (60 m deep, 43°01′ N, 70°33′ W) in the Gulf of Maine. *b*, Average germination frequency of cysts isolated from (●) top 2 cm and (○) 5- and 6-cm depths in freshly collected cores from Station 29 and Station 30. *c*, Germination frequency of cysts isolated from sediment stored in the laboratory under constant conditions. Sediment from the 3–6-cm depth interval of cores from Station 29 on 17 August 1984. *d*, Bottom (●) and surface (○) water temperature at Station 29. **Methods.** Cores were extruded on board ship, the outer circumference discarded, and the central portion of each depth interval stored in the dark at the temperature of the bottom water during transit to the laboratory. Within 48 h, the samples were sonicated, sieved[17], and the 20–80-μm fraction resuspended in germination medium. To maintain constant germination conditions, all cyst suspensions and germination medium used sea water from the same two polyethylene carboys of Sargasso Sea water, filtered through a 0.2-μm filter and diluted 10% with distilled, deionized water. This was considered more consistent in composition and more conducive to germination than artificial sea water. Germination medium was 'f/2' of Guillard and Ryther[18]. Each data point in *b* and *c* represents the germination success of at least 48 cysts that were isolated and placed individually in separate wells of a 96-well tissue culture plate in 130 μl of medium. Plates were taped to minimize evaporation and incubated on a 14:10 h light to dark photoperiod at 15 °C and 150 μE m^{-2} s^{-1} (cool-white fluorescent light) for one month. Excystment was successful if the germling cell was able to emerge completely from the cyst wall. The germination data in *c* were obtained using small aliquots removed from the central portion of a large sediment sample collected in August 1984 from Station 29. Subsamples were processed and cysts isolated and incubated as described above. The bulk mud sample was stored in the dark at 2 °C.

of an annual cycle in maximum growth rate and cell yield. The data are not conclusive, however, as they were compiled from the control cultures of a series of experiments conducted in different growth chambers by several investigators. Dale *et al.*[13] and Fukuyo *et al.*[14] could not germinate *G. tamarensis* cysts from Gulf of Maine and Ofunato Bay sediments during the fall months. Both studies attributed the lack of germination to immature cysts, but it is also possible that both used cysts in the unresponsive phase of their annual cycle. Interestingly, germination of the cysts of a closely related strain (or species[1,2]) of *Gonyaulax* did not follow a rhythmic pattern during the Ofunato Bay study[14]. Similarly, during this study and others[15], we observed high germination frequencies for cysts of *G. tamarensis* from a shallow estuary throughout the year. This implies that the annual rhythm is maintained only in certain populations or strains of this organism.

Control of cyst germination by an endogenous clock would be most useful in deep coastal waters where bottom temperatures are relatively invariant and photoperiodic cues non-existent. Without endogenous regulation, cyst germination would occur throughout the year, because bottom temperatures in the Gulf of Maine remain within a permissive 4–6 °C range (Fig. 1*d*). Instead, we observe a cyst germination rhythm with the same seaonality as the motile cell blooms (Fig. 1*a*). If further studies verify that there is no similar timing mechanism in shallow water strains of *G. tamarensis*, an environmental separation of genotypes is implied. This would not be surprising as a genetic basis for circadian rhythms has been reported in the fruit fly *Drosophila*[16]. Shallow water sediments experience considerable environmental variability from year to year, so inflexible endogenous control of germination might be a disadvantage compared to a strategy whereby germination is directly controlled by the environment. Shallow water populations of *G. tamarensis* may thus be comparable to terrestrial plants whose seeds are responsive only to the external environment for the breaking of dormancy.

More work is needed to explain the observed annual rhythm and to understand differences between strains or populations. If endogenous annual rhythms are found in the germination patterns of other cyst-forming dinoflagellates or in the growth or metabolism of phytoplankton in general, such information would have profound implications with respect to bloom dynamics and the temporal succession of algal species.

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