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Shellfish Toxicity and Dormant Cysts in Toxic Dinoflagellate Blooms

DONALD M. ANDERSON

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Several of the dinoflagellates responsible for toxic blooms include a dormant cyst stage in their life cycles. These resistant cells have a variety of potential functions in the overall ecology of the toxic dinoflagellates. As Wall (1) discussed, cysts can theoretically act as "seed" populations to initiate blooms, as a survival mechanism through environmental extremes, as agents for species dispersal, and as means for genetic recombination through sexuality. Two additional functions can be added to this list: cysts can be direct sources of toxicity, and their formation can be a major factor in bloom termination. The purpose of this paper is to examine our present knowledge of the cysts of the toxic Gonyaulax species to see whether these hypothetical considerations are valid representations of the actual roles cysts play in toxic dinoflagellate blooms and shellfish toxicity.

Nearly 100 years ago, dinoflagellate resting cysts were first observed in plankton samples, but nearly 50 years passed before their affinity to motile dinoflagellates was demonstrated (2,3). Early researchers investigating paralytic shellfish poisoning episodes (PSP) speculated that a benthic cyst population germinating at specific times could explain the seasonal nature of toxic dinoflagellate blooms (4-7), but it was only in the last few years that true resting cysts were finally identified and described for toxic dinoflagellates (8-10).

It is now generally accepted (10, 30, 39, 40) that the toxic Gonyaulax species reproduce asexually through vegetative division (Figure 1), but that this process can switch to sexual reproduction through the formation of gametes which fuse together (Figure 2) to yield a large, swimming zygote (planozygote; Figure 3). This presumably diploid cell swims for up to a week before it is transformed into the thick-walled resting cyst or hypnozygote (Figure 4). Upon germination, the cyst releases one cell (Figure 6) which

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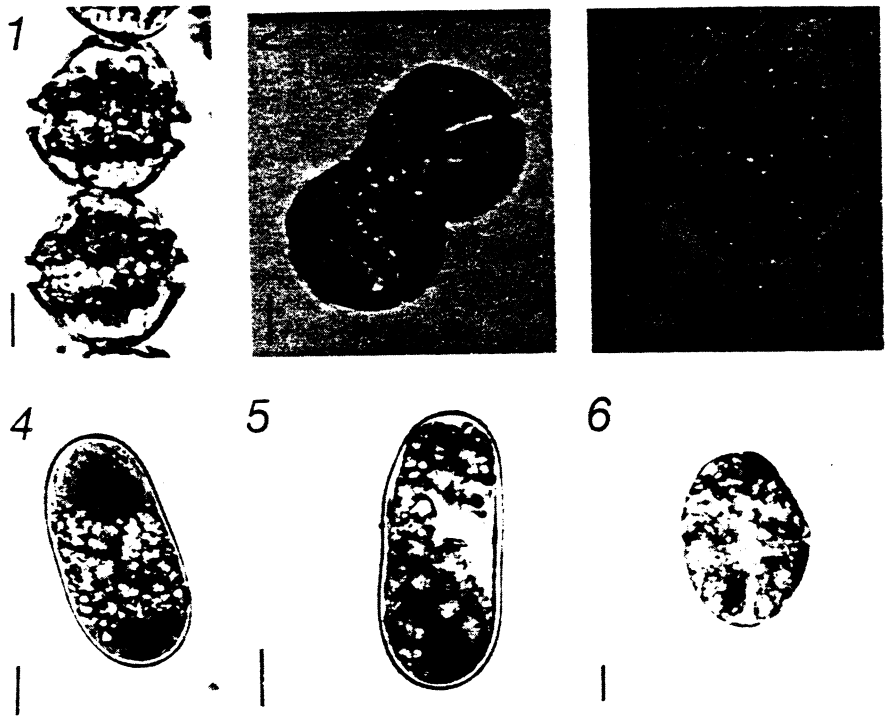

Figures 1-6. Life cycle stages of Gonyaulax tamarensis. Error bars are 10 µm in all cases. Figure 1. Recently divided vegetative cell showing parallel girdle or singulum orientation; Figure 2. Fusing gametes with obliquely-oriented singula; Figure 3. Large, deeply-pigmented planozygote formed from fused gametes; Figure 4. Fully mature resting cyst with starch and lipid accumulations in a central band and pigmented cytoplasm at each pole; Figure 5. Intact, but dead, cyst isolated from fecal pellet of the mussel Mytilus edulis; Figure 6. Recently-germinated cell (planomeiocyte) with faintly-visible pigmented accumulation body lower-left.

divides to yield daughter cells that are haploid and capable of mitotic, asexual division once again.

The discovery of a cyst stage for the toxic Gonyaulax species provided a new set of explanations for various aspects of the toxic bloom phenomenon, all based on the unique characteristics of the resistant, dormant cyst. In an early review by Wall (1), one of the pioneers of studies on this topic, the ecological roles of cysts were presented, but these discussions were largely speculative since so little was known about living cysts at the time. Wall argued that cysts could function: a) as "seed" populations to inoculate overlying waters and initiate blooms; b) as a survival mechanism to permit the species to withstand environmental extremes; c) as agents for population dispersal into new regions; and d) as means for genetic recombination through sexual reproduction. Recent research suggests that cysts are important in two additional ways: e) they represent direct sources of toxicity through their ingestion by shellfish; and f) they can be a major factor in the decline of bloom populations in much the same way that germination is important in bloom initiation.

In the years since these largely hypothetical roles were first proposed, considerable effort has gone into studies of cysts of toxic dinoflagellates. It is the objective of this paper to discuss the importance of cysts in shellfish toxicity episodes with respect to our present state of knowledge. It soon becomes clear from this type of exercise that despite significant progress in certain areas, many of the hypothetical links between cysts and shellfish toxicity remain probable but unverified.

This discussion will focus on two dinoflagellates responsible for PSP (Gonyaulax tamarensis and G. catenella (= Protogonyaulax tamaranesis or excavata (11). The other toxic dinoflagellates known to form cysts (12) are Gonyaulax monilata, Pyrodinium bahamense, and Gonyaulax polyedra (a species that may in fact not be toxic). Studies on the cysts of these latter species are descriptive, however, with no field or laboratory experiments to provide perspective on their ecological importance. Another important toxic species, Ptychodicus brevis (= Gymnodinium breve) has been reported to undergo sexual reproduction, but no resting cyst has yet been observed either in cultures or sediments (13).

Cysts As "Seed" Populations

It seems obvious that cysts would be important in inoculating overlying waters with motile cells through germination. It is, however, essentially impossible to prove that blooms actually originate from cysts since the advection of even a single cell from adjacent waters can theoretically start a bloom. Much of the evidence linking cysts to bloom initiation is thus suggestive but not conclusive, with the most direct evidence coming from shallow estuarine areas where sediments are more easily sampled.

Distributional studies of cysts in sediments provide one indication of inoculum potential. For example, qualitative sediment surveys (i.e., presence vs. absence of cysts) demonstrated a close correlation between sites subject to shellfish toxicity and the presence of G. tamarensis cysts on Cape Cod (10, 14). This asso-

ciation was strengthened by subsequent quantitative cyst surveys documenting large cyst accumulations in sediments of certain key embayments with concentrations falling rapidly to undetectable levels in adjacent areas (15). These distributions suggest "point source" origins for G. tamarensis blooms and supports the contention that cysts provide the initial bloom inoculum. However, the data provide no direct evidence and are only applicable to shallow estuaries at the southern limit of the G. tamarensis geographic distribution in New England, since it is in this region that the blooms and the cyst distributions are patchy and localized. Recent surveys in Maine where cysts are more widespread have not shown a good correlation between cyst distributions and the patterns of PSP outbreaks (16).

Perhaps the most compelling evidence that cysts do function in bloom initiation is based on the observation that newly-germinated G. tamarensis cells are morphologically distinct (Figure 6) from actively-dividing vegetative cells (Anderson and Wall, 1978). In a recent field study, these "planomeiocytes" (17) were observed during the early stages of G. tamarensis blooms, in one instance comprising more than 30% of the initial cell counts (18). This represents the first conclusive evidence that early bloom populations can include recently-germinated cells, but it is also important to recognize that the inocula were relatively small (a few hundred cells L^{-1}). In these instances then, germination of cysts supplied an inoculum, but the magnitude of this input was small relative to the subsequent rapid proliferation of asexually dividing cells.

The link between cysts and bloom initiation becomes more tenuous in regions where the cysts are widespread in estuarine and nearshore sediments (16, 19, 20). For example, given the need for 5-8°C temperatures for germination (18, 21), the fate of cysts deposited in 100 m or deeper coastal water remains an open question since bottom temperatures can be relatively invariant near 4°c (22). Cyst germination from the deep water "seed bed" may thus require resuspension of sediments and advective transport to warmer depths. A further complication is that many cysts are buried by benthic animal activity. Since large members of viable cysts can be found several cm below the sediment surface in deeper coastal waters (Figure 7B), germination (if it occurs) would only follow warming of bottom sediments above 5° C or vertical transport of the cysts to the sediment surface. Unfortunately, the biological and physical processes at the sediment/water interface have not yet been studied in the context dinoflagellate population dynamics.

It is important to recognize that the foregoing discussion is not simply an intellectual exercise attempting to demonstrate the seemingly obvious connection between cysts and bloom initiation. We must recognize that the "easy" answers provided by this dormant life cycle stage do not apply to all situations. In colder temperate waters, it is a reasonable assumption that cyst germination probably introduces the first toxic Gonyaulax cells into the water in some locations, but the subsequent transport of these cells over long distances must be considered a viable "seeding" alternative to the direct input of new cells from underlying sediments.

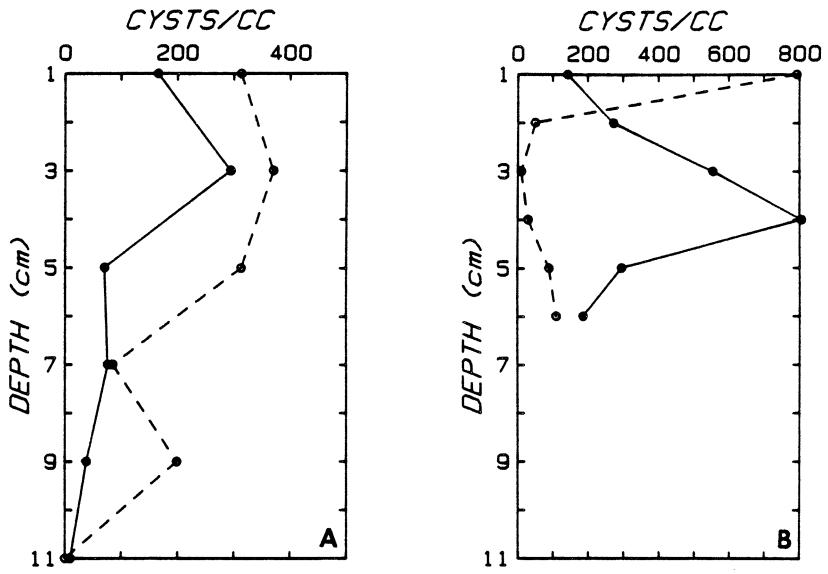

Figure 7. Vertical distribution of Gonyaulax tamarensis cysts in estuarine and nearshore sediments. Each point represents a 1-cm interval above specified depth. A. Two stations in Perch Pond, a shallow Cape Cod salt pond. Note the large number of cysts buried 6-11 cm deep. B. Two coastal stations near Cape Ann, Mass., both approximately 150 m deep. One has a distinct surface maximum in cyst abundance while the other has a peak at 4 cm.

Cysts as Survival Mechanisms

It is well documented that cysts (in general) can be highly resistant to environmental extremes (1, 12, 23). In the context of this discussion, however, the relevant extremes are those to which motile cells would have been exposed had they not encysted. In the dynamic coastal environment, this would include changes in such factors as temperature, salinity, nutrients, and light.

It is important to know the tolerances of motile cells to these parameters in order to evaluate whether encystment truly is a survival mechanism. Unfortunately, the relevant information is incomplete or available only from casual observations. Temperature, for example, is certainly a critical factor for G. tamarensis and G. catenella given their presence in temperate, waters, yet we do not know how many vegetative cells would survive winter temperatures if encystment had not occured. Most studies have examined temperature effects on growth rate, and these generally indicate that division does not occur below 5°C (5, 24-26). We do know, however, that some motile cells persist at temperatures as low as 0-2°C in laboratory cultures (26, 27), but the duration of this survival has not been determined. Winter temperatures in coastal waters can be highly variable, but they often drop to this same 0-2°C range (5, 28). Summer survival would not be a problem in most areas since G. tamarensis growth has been reported at temperatures as high as 24°C (25, 26), a temperature above the maximum for most coastal waters. There is thus the potential for small, motile cell populations to persist in certain temperature regions throughout the year.

Those field studies that include water samples collected during winter months all show undetectable G. tamarensis concentrations (5, 18, 21). Given the relatively small volumes of water typically collected and counted, this does not preclude the presence of a few cells (the "hidden flora"), but it does indicate that motile populations are extremely small at best. Furthermore, since the growth rate of G. tamarensis is essentially zero at very low temperatures, the appearance of even a few hundred cells L^{-1} in early spring when waters are still very cold suggests that it is excystment and not division of surviving motile cells that initiates the bloom development.

The obvious problem with this discussion is that it is circular - that encystment does occur and thus significantly decreases the size of motile cell populations. Low or non-existent wintertime concentrations of G. tamarensis may thus reflect either a low probability of survival or the removal of many motile cells by cyst formation and deposition.

The fate of the toxic Gonyaulax species in low-nutrient environments is also poorly understood. On the one hand, G. tamarensis cells can persist in older cultures for months without dividing, presumably under impoverished nutrient conditions. On the other hand, encystment has been observed in natural waters at relatively high nutrient concentrations (18). We thus have no firm foundation on which to base the conclusion that toxic Gonyaulax species encyst to survive the temperature or nutrient variations of temperate coastal waters. Clearly there are many phytoplankton

species that bloom year after year without dormancy - that rely instead on broad environmental tolerances or advection from more suitable waters.

One line of evidence that supports the view that encystment is not primarilly a strategy to survive through short-term stress is that sexuality is not induced in laboratory cultures by a variety of adverse conditions but instead seems to occur under the relatively specific conditions of nutrient limitation (29, 30). For example, decreasing either temperature or light intensity alone has not successfully induced sexuality in G. tamarensis cultures (26). In fact, if temperature is dropped from optimal levels to that where growth rate begins to decrease (12°C), cyst formation is actually inhibited under low nutrient conditions that typically induce sexuality.

The only stress that has consistently yielded cysts in G. tamarensis cultures to date is nutrient limitation (26). In this context, it is important to recognize that encystment is not a rapid process. The formation and fusion of gametes and the development of a swimming zygote requires up to a week for many dinoflagellates, including G. tamarensis (18, 29, 30). It appears that sexual induction is not a response to absolute nutrient starvation but instead occurs in anticipation of impending limitation, perhaps cued by the decreasing size of internal nutrient pools.

The general picture that emerges is that of encystment as a response to a set of conditions that do not represent an immediate threat to survival. Motile cells could persist well beyond the time when cysts form, with a good possibility for additional blooms as conditions improve. There is no doubt that an indirect result of encystment is that the species survives in relatively large numbers through a variety of environmental stresses that may severely deplete a motile cell population. Those hostile conditions may, however, occur long after encystment has been completed. The value of encystment as a survival strategy thus rests on the numbers of motile cells that would survive each year if encystment did not occur, relative to the number of cysts that successfully germinate. Unfortunately, the latter number may be relatively small and the former is unknown.

Here again we find ourselves tempted to accept the 'easy answer' that encystment is a necessary mechanism developed by a species to ensure survival through environmental extremes. If it is true that the species would have been capable of survival in many areas without resorting to dormancy, the ecological justification for encystment may then lie in more subtle factors, possibly those associated with genetic recombination during sexuality.

Cysts as Dispersal Agents

There are a variety of ways that toxic Gonyaulax cells could be introduced to areas with no previous history of PSP, and most of these involve cysts. The most common claim is that transport of an established motile population by tidal and large-scale circulation patterns permits a species to deposit cysts in new areas as "seeds" for future blooms (1, 9, 10, 31). Within this framework, the cyst is most important in those areas where advection of

existing blooms is not a common occurrence. Thus massive PSP outbreaks are often credited with the dispersal of toxic Gonyaulax to new regions.

The most striking example is perhaps the 1972 New England red tide which caused extensive shellfish harvesting closures in Maine, New Hampshire and Massachusetts, the latter two states having no previous history of shellfish toxicity (32). In the more than 10 years since that event, Massachusetts has had PSP-related closures every year (14).

The absence of PSP events in the past does not imply the absence of toxic Gonyaulax cells however, since other factors may be involved including the lack of conditions suitable for dense population growth, low toxicity strains of the causative organism (33), or a shortage of harvestable shellfish resources (14). Nevertheless, there is little doubt that the 1972 bloom introduced G. tamarensis cells into some areas that were previously unaffected in southern New England. The recurrent nature of the toxic episodes and the dangerously high PSP levels measured in popular clamming areas since then both suggest a significant change. The importance of cysts is this spreading event is seen in the subsequent PSP patterns for the southern portion of this region where outbreaks are highly localized and patchy within estuaries, with no offshore populations of G. tamarensis as a source of advected cells (21).

A similar scenario holds for the Seattle area of Washington state. Historically, G. catenella has been a hazard along the open coast of Washington, British Columbia and Alaska, but not within Puget Sound until 1975. A major bloom in 1978 apparently introduced the problem to the southern part of Puget Sound, where it has since persisted year after year (34). One common attribute of both the Seattle and New England PSP problems is that recurrent blooms in previously unaffected areas began in the years immediately following major, catastrophic blooms. There is little doubt then that the persistence of the problem is linked to the deposition of cysts from advected population during the initial bloom events.

The resistant nature of dormant cysts makes them likely candidates for dispersal via dredging operations, shellfish transplants, or boat ballast as well. Each of these is theoretically possible, but there is no direct evidence that demonstrates conclusively that such mechanisms have operated in the past.

Dredging is perhaps the most likely cause for concern, especially in regions where the toxic Gonyaulax cysts are not widespread. In southern New England, for example, the very characteristics of certain estuaries that result in accumulation of cysts (shallow, narrow inlets, reduced tidal flushing, high productivity) are those that create the need for dredging. The advection of sediment resuspended during dredging is a definite concern, as is the distant disposal of larger volumes of sediment at dredge spoil dumping sites. Both of these mechanisms could transport cysts to new environments, but they also serve to provide suitable germination conditions. Evidence is now accumulating that newly-deposited dinoflagellate cysts are buried into the sediments by benthic animal activity (Figure 7), often resulting in significant subsurface maxima (15, 20). Many of these sediments are

anoxic just below the surface, and recent experiments indicate that G. tamarensis germination is severely inhibited in the absence of oxygen (although the cysts do remain viable; 35). A dredging operation could thus introduce many cysts to oxygenated surface waters, leading to subsequent blooms in nearby areas. The only data suggesting that this may have happened in the past is associated with the 1972 New England red tide, which began shortly after a major dredging operation in the Merrimac River (36).

The transport of cysts via shellfish transplants or relays is even more difficult to evaluate. Not only is it possible that the sediment on the shells of seed shellfish contains cysts, but ingested cysts may even survive ingestion and germinate following defecation. Many cysts fed to soft-shell clams and mussels are viable following isolation from fecal pellets (35), but experiments have yet to be performed that mimic the conditions associated with prolonged residence in the intestines of shellfish during inter or intra-state transport.

Only one piece of evidence suggests that shellfish seeding may have introduced PSP to an area, and that was in Perch Pond, Falmouth MA which developed PSP in 1976, one year after the salt pond was seeded with quahogs of unknown origin (10). It is also of interest that the Perch Pond strain of G. tamarensis is morphologically distinct from others on Cape Cod (variety tamarensis versus excavata; (10, 37), and thus it is unlikely that advective transport of motile populations introduced the species to that estuary.

In general, it may never be possible to prove that species dispersal is facilitated by dredging, shellfish transplants,or boat traffic. Here again we are faced with mechanisms that are theoretically possible but that may be of minor practical concern relative to the introduction of cysts to new areas through advective transport of established blooms. There is little doubt that this latter mechanism has been, and will continue to be, of major importance to the geographic distribution of the toxic Gonyaulax species.

Cysts and Genetic Recombination

Many studies in recent years have demonstrated a link between sexuality and cyst formation in dinoflagellates (38). It is now evident that both G. tamarensis and G. catenella form cysts following the sexual fusion of gametes (31, 39, 40), but only the latter species has been proven to be heterothallic thus far. The ecological advantage of this process stems from genetic recombination during fusion of self-sterile gametes that should, in theory at least, create heterogeneous cyst and motile cell populations.

It is, of course, difficult to demonstrate that such variability exists and to demonstrate the resulting ecological advantages. Genetic variability has been documented among diatoms and dinoflagellates (41, 42), but no definitive studies have been completed on the toxic Gonyaulax species. The G. tamarensis and G. catenella species assemblages are quite diverse, however, and this provides indirect evidence of recombination. For example, Schmidt and Loeblich (37) found nearly every possible combination

of bioluminescence, toxicity, and possession of a ventral pore among nine G. tamarensis isolates, all having identical thecal tabulation. Other workers have demonstrated different toxin content on a per cell basis (33, 43) and different toxin composition between strains of toxic Gonyaulax species (44, 45).

What these differences mean in terms of overall species success is not known, but they imply that there may be other genetic differences that are more difficult to identify that could affect the growth characteristics and environmental tolerances in these species. If this variability were reflected, for example, in the factors affecting germination or motile cell growth rate, we would expect that not all cysts would germinate at the same time under the same conditions or that some motile cells would survive better than others (18). In this way, cyst-forming dinoflagellates might maintain a viable, quiescent seed population in the sediments year after year while optimizing the growth and proliferation of motile cells as well.

These considerations are clearly speculative, but they are suggestive of the tremendous benefits (albeit gradual) that might accrue to sexually-reproducing dinoflagellates. It may be that the ultimate goal of encystment is simply to insure that nuclear fusion occurs. Other benefits of encystment (survival, dispersal, etc.) could be indirect and somewhat coincidental.

Cysts as Direct Sources of Toxicity

Toxicity in deep water scallops throughout the year has been recognized for many years (44, 46, 47). More importantly, the levels of toxicity in the scallop digestive gland were shown to increase dramatically (sometimes by a factor of 2 or 4) during winter months when G. tamarensis motile cell populations were low or undetectable. Bourne (4) postulated that cysts were the toxin source, an opinion also favored by Jamieson and Chandler (47) in a more recent study. It has since been confirmed that G. tamarensis cysts are indeed toxic, and this has led to a proliferation of explanations for toxicity episodes based on cyst ingestion.

The original report of toxicity in cysts claimed that they could be an order of magnitude more toxic than motile cells (48). This contention has since been both confirmed (49) and refuted (20) by studies which compared the toxicities of cysts and motile cells of this species. It is, however, the magnitude and not the existence of toxin in cysts that is in question.

Despite this progress, the evidence linking cysts to shellfish toxicity remains circumstantial and care should be exercised before attributing toxin increases to this mechanism. The major problem is that it has yet to be demonstrated that shellfish can remove toxin from cysts. The feeding studies mentioned earlier (which do not yet include scallops; 35) indicate that many viable G. tamarensis cysts can be isolated from the fecal pellets of mussels and soft-shelled clams fed cyst suspensions. There is certainly some cyst mortality as well (Figure 5), but whether this is also associated with toxin retention by the shellfish has yet to be demonstrated. It is reasonable to expect that the assimilation of toxin from cysts will not be a highly efficient process.

Data are now available that permit a preliminary assessment of the cyst concentrations available for ingestion in deeper waters. White and Lewis (20) mapped the abundance of G. tamarensis cysts in Bay of Fundy sediments in the same regions where toxic scallops have been studied (4, 47). Cyst densities as high as 8000 cc^{-1} were found in the top two cm of sediment, with abundance falling to less than 100 cc^{-1} in rocky, gravelly areas. This latter environment is, however, the one favored by scallops, (47), presumably because the high currents in these areas enhance filter feeding.

If we conservatively assume toxicities of approximately 100 m.u. $cyst^{-1}$ (20) and complete assimilation of toxin (with no depuration), it would require consumption of as many as 100 million cysts to achieve the toxin levels recorded in deep water scallops. In areas of highest cyst abundance, this is equivalent to one scallop ingesting all cysts in the top cm of material over one square meter of sediment. If the lower cyst concentrations reported for the rocky or gravelly bottom are used, the removal must cover an area 100 times larger. (Scallop densities in these regions average 2-3 animals m^{-2}; (50). Arguments by Jamieson and Chandler (47) that areas with high bottom currents would provide a steady supply of cysts advected from high deposition sites nearby may be justified, but this explanation requires that a substantial portion of the cyst population remains resuspended in bottom waters throughout the year. Unfortunately, studies of the dynamics of cyst populations have not reached the stage where this possibility can be evaluated.

Another problem with wintertime toxicity data is that toxin levels are reported per 100 gm of tissue. Studies of the deep sea scallop Placopecten magellanicus indicate that the size of the digestive gland can vary through the year (51). Thus a constant amount of toxin in a gland would look variable when normalized to 100 gm of tissue, with the highest relative toxicity during winter months when the tissues are the smallest. It would appear, however, that this error is small (perhaps 20-30%) relative to the 2 to 4-fold toxin increases typically reported between seasons in scallops (4, 47).

The purpose of the foregoing discussions is to argue for caution in assigning toxic events to cyst ingestion. Once again, this may be an easy explanation that precludes investigations of other mechanisms. It seems highly probable that cysts do contribute to wintertime or deep water shellfish toxicity at some level, but there are other factors that might contribute, including slow toxin depuration or delivery of toxin via fecal pellets, temporary cyst stages, or deep mixing of motile populations.

Cysts as a Factor in Bloom Decline

The prevailing view of the dynamics of many phytoplankton blooms is that they often terminate due to nutrient exhaustion, increased grazing pressure, and/or physical dispersal. Evidence is now accumulating that G. tamarensis blooms have ended when grazing and advection were low and nutrients were above detection limits (18, 21). Instead of persisting without division as nutrients disappear

and grazers multiply, asexual reproduction was replaced by sexuality and a substantial portion of the motile population became non-dividing gametes and planozygotes (18). In much the same way that cyst germination may initiate bloom development in many areas, sexuality and cyst formation appeared to dictate the dynamics of bloom decline.

As discussed previously, it is not known how long some portion of the motile populations could remain in the water column if encystment did not occur, but it is evident that it would be longer than is the case with sexuality. The important unknowns in evaluating this process are the magnitude and duration of encystment once initiated. Although up to 35% of all motile cells in the late stages of some G. tamarensis blooms have been planozygotes (the swimmming precursors to cysts), the status of the remainder of the population was not known (18). Other data indicate that those cells that do not encyst can remain in the water for weeks or months after the major surge of encystment (35). This process is undoubtedly mediated by the nutritional environment of the population, with persistance of some fraction of the bloom facilitated by fluctuations in the supply of nutrients.

Nevertheless, the process of encystment dictates when a substantial fraction of a Gonyaulax population leaves the water column, regardless of the ability of motile cells to persist for extended periods. The encystment/excystment cycle can thus define the temporal limits of some blooms. More work is needed to evaluate the importance of this process relative to bloom termination from factors such as grazing or advection that decrease the numbers of motile cells.

Overview

The preceding discussion was intended as an objective evaluation of what has been clearly established (as opposed to what we would like to believe) concerning the relationship between cysts and shellfish toxicity. Clearly a great deal of work is needed if we are to verify the circumstantial evidence that supports most of the attributes ascribed to cysts. The most difficult task is to determine why encystment actually occurs (which is a different question than what initiates it). Certain of the roles of cysts (e.g. survival, seeding) may simply be indirect benefits of a more fundamental drive - that designed to ensure the adaptive benefits accompanying sexuality and genetic recombination for example. Resting cysts are undoubtedly important in many aspects of the PSP phenomenon, but we must not overlook more subtle or complex biological and physical explanations for our observations.

Acknowledgments

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