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RECENT DEVELOPMENTS IN PARALYTIC SHELLFISH POISONING RESEARCH¹

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ABSTRACT

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The finding that *Gonyaulax catenella*, the organism that produces much of the paralytic shellfish poison (PSP) in the Pacific, undergoes diel vertical migration offers greater insight into the distribution of both the toxic organism and toxicity in shellfish. This insight points to useful avenues of research to develop a predictive model for PSP and suggests modifications of shellfish culturing practices which may improve monitoring for PSP or reduce losses due to PSP.

The occurrence of a heavy infestation of *G. catenella* by a dinoflagellate parasite during the decline of a dense bloom of *G. catenella* suggests the parasite may play an important role in controlling *G. catenella* populations and might be useful as a biological control agent when cultured and introduced into certain confined bays.

INTRODUCTION

One of the factors to be considered in the development and management of bivalve aquaculture projects is a negative one, namely, the probability of periodic restrictions on harvesting of the cultured shellfish because of their accumulation of unacceptable levels of paralytic shellfish poisons (PSP). The accumulation of PSP in bivalves is a natural phenomenon resulting from their consumption of large numbers of dinoflagellates which produce highly potent neurotoxins. Poisonings of humans who have eaten toxic shellfish have been reported from various parts of the world for several centuries. Approximately one-sixth of the cases have ended in death (Dale and Yentsch, 1978).

Protection of the public against the health hazards of toxic shellfish has been accomplished by governmental imposition of various types of closures of sports and commercial harvesting. For example, along the coastline of the northeastern Pacific Ocean, harvesting of shellfish in some regions is subject to blanket closures of wide areas for a few months or year-round, even though toxic shellfish may occur in only parts of the closed areas. Such blanket closures can result in underutilization of stocks. This has been particularly true

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in Alaska where vast natural shellfish resources remain unexploited because of PSP. In other areas monitoring programs permit the restriction of harvesting, closures to those areas and times when shellfish are known to have unacceptable levels of toxicity. In British Columbia testing for PSP is done after harvesting and before selling the shellfish.

During the last 15 years, in many parts of the world, occurrences of PSP in shellfish have spread to geographical areas previously unaffected. Furthermore, in many areas with a prior history of PSP, recent outbreaks have been of longer duration and, in some cases, have involved much higher toxin levels than previously experienced. This trend has had considerable effect on the aquaculture industries in the Pacific Basin. The culture of scallops in Japan has suffered a severe setback because high levels of PSP have prevented harvesting for as much as 10 months each year (S. Itoh, personal communication, 1982). In Washington the area affected by PSP closures has more than tripled in the last decade, with losses felt by mussel, oyster, and clam growers. In California several cases of paralytic shellfish poisoning in 1980 resulted from consumption of commercial oysters, and two deaths were caused by rock scallops and mussels harvested for personal use. The subsequent public fear of shellfish resulted in losses to commercial growers in California, Oregon, and Washington in excess of \$600,000 (F. Conte, personal communication, 1982). In the last 10 years PSP outbreaks have occurred in new areas in Chile, Mexico, and New Guinea.

The adverse effects of PSP on the aquaculture industry can be ameliorated to some extent through the efforts of governmental monitoring agencies, research institutions, and the industry itself. These efforts should include rigorous monitoring programs in all commercial growing areas to prevent any sales of PSP-contaminated shellfish, and thus to avert adverse publicity and public fear of shellfish products. The development of a faster, more sensitive method for assaying PSP in shellfish than the mouse bioassay currently used will be highly beneficial to these programs. Research on the behavior, growth, and ecology of the causative organism will lead to increased understanding of the factors related to the onset, intensity, and small-scale distribution of PSP in shellfish. Such information will be valuable both in developing more rigorous monitoring programs and in learning how to extend periods of harvesting safe shellfish in areas with recurring PSP problems.

In all PSP outbreaks in the coastal areas of the Pacific Ocean, with the exception of those in Mexico and New Guinea, the toxin-producing organism belongs in the genus *Gonyaulax*. The taxonomy of the several poisonous species of this genus has not been satisfactorily resolved (F.J.R. Taylor, personal communication, 1982), and uncertainty exists about the specific name of the causative organism in certain Pacific areas. However, *G. catenella* is the species considered responsible for at least some of the PSP outbreaks in Chile, Japan, and Alaska, and is the only known cause of PSP in shellfish in Washington and California.

This article presents the results of studies of the behavior and estuarine ecology of *G. catenella* and their implications for aquaculture.

METHODS

Studies to determine the environmental factors associated with changes in density of *G. catenella* and PSP in shellfish were carried out in Puget Sound, a fjord in Washington State. The study area is a portion of the main basin of Puget Sound having both a deep channel area which is well mixed during much of the year and a shallower bay, Quartermaster Harbor, which becomes thermally stratified more frequently (Fig. 1).

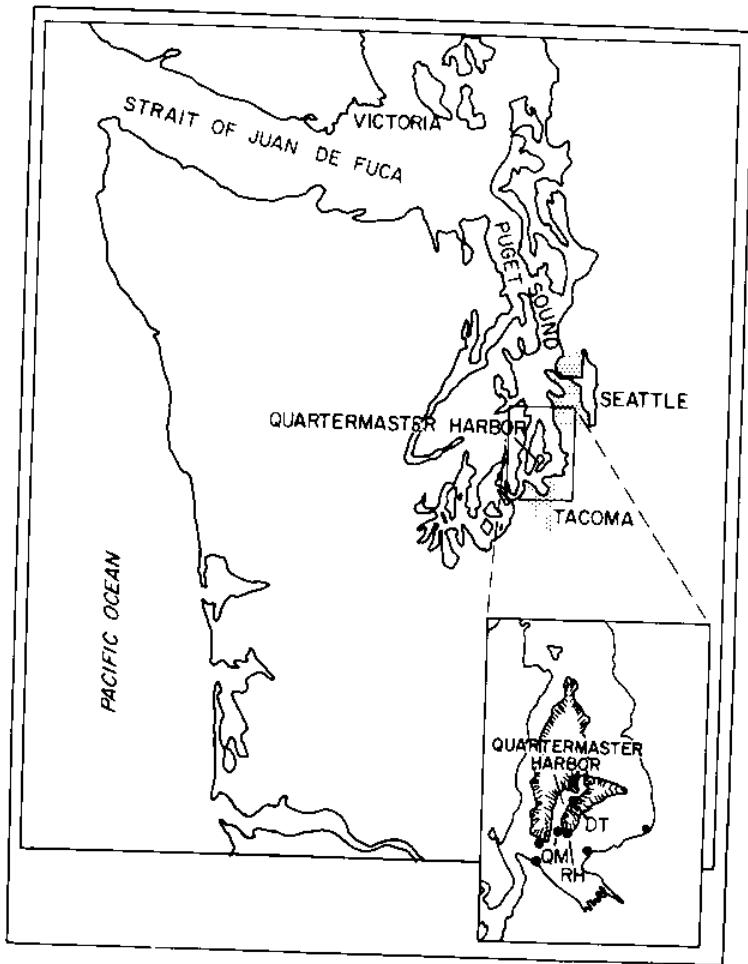


Fig. 1. Map of western Washington. Insert shows sampling sites in the channel and Quartermaster Harbor.

Weekly sampling was conducted, using standard techniques to determine physical and nutrient conditions and density of *G. catenella*. Samples of bay mussels (*Mytilus edulis*) were analyzed for toxin content by the State Public Health Laboratory, using the mouse bioassay.

During a bloom of *G. catenella* in Quartermaster Harbor, a study was conducted to determine whether this species undergoes diel vertical migration. Both drogue and transect studies were conducted in an area near the head of the bay. Temperature and salinity were measured and Van Dorn water samples for cell counts were taken at 1-m depth intervals in time series through two days and three nights during the decline of the bloom.

Also during a bloom, the rate of infestation of *G. catenella* by the endoparasitic dinoflagellate *Amoebophyra ceratii* was studied using acetocarmine staining.

RESULTS

Seasonal and short-term changes in the depth of the thermally stratified layer (here defined as lowest depth at which $\Delta t \geq 1^\circ\text{C}/\text{m}$), temperature at 0.5 m depth, and the PSP levels of bay mussels at two stations in April-July 1981 are shown in Fig. 2. Data for station DT are similar to those for the other station near the head of the bay. Data for temperature and depth of thermal stratification for station QM are representative of other channel stations; however, PSP levels for the several channel stations show inconsistencies.

Toxin in mussels at DT remained at low or undetectable levels until mid-June when it increased rapidly to a peak value of 1916 $\mu\text{g}/100$ g meat. The increase in density of *G. catenella* in daytime samples taken at 0.5 m depth at DT was parallel to the rise in toxicity in mussels, the density increasing nearly ninefold in 3 days (Fig. 3). The rapid increase in both the density of *G. catenella* and PSP in mussels at DT followed a 5-week period in which temperatures at 0.5 m depth exceeded 14°C and the thermally stratified layer was 4 m in depth on sampling days.

In contrast, temperatures of the channel waters (Fig. 2, station QM) were approximately $1.5\text{-}3^\circ\text{C}$ lower than those at DT, and no thermal stratification occurred during the period prior to the development of the bloom at DT. PSP levels at station RH, the channel shore station near QM, remained low during the development of the bloom at DT. During the decline of the bloom, the PSP levels at RH and at the station on the other side of the mouth of Quartermaster Harbor rose significantly.

Two other periods of high density of *G. catenella* and high PSP in mussels, which occurred at the head of the bay in September of both 1980 and 1981, were associated with conditions similar to those of the June 1981 bloom, that is, temperatures of at least 14°C and marked thermal stratification. Following the bloom at DT in 1980, the PSP level at RH increased as it did in July 1981. (The station on the other side was not sampled at that time, nor was either of those stations sampled following the September 1981 bloom).

Although there were frequent marked inconsistencies in levels of PSP among the five channel stations, a seasonal temperature-related pattern of PSP was observed. With one exception, PSP levels higher than 80 $\mu\text{g}/100$ g mussel meat occurred only when the water temperature exceeded 13°C , or during the seasonal cooling in October. (In the United States, shellfish may not be harvested if toxin levels exceed 80 $\mu\text{g}/100$ g meat.)

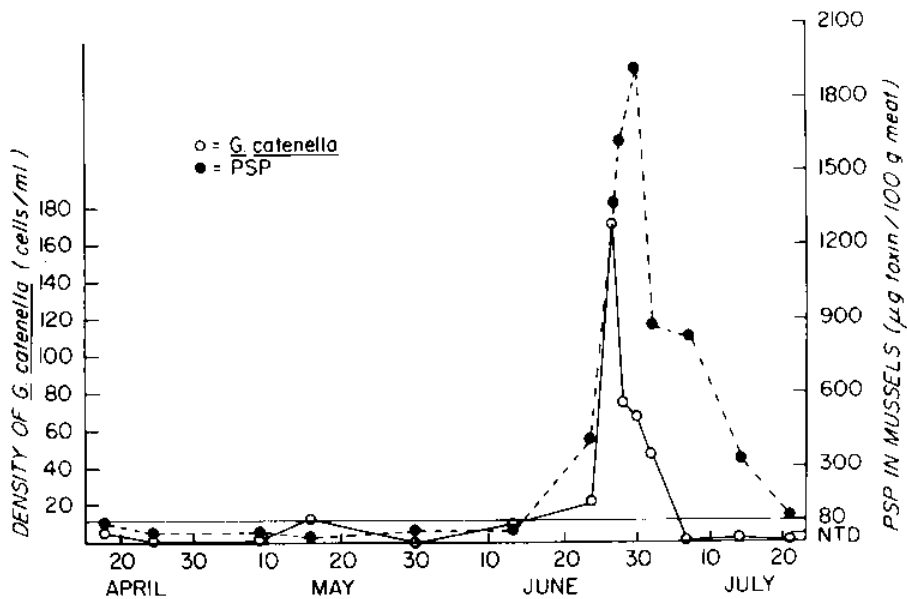


Fig. 3. Changes in PSP in mussels and density of *G. catenella* in afternoon samples at 0.5 m depth, Station DT, April-July 1981.

When the water was thermally stratified (Fig. 4, except 1225 h), the vertical spread was 2-3 m and the nighttime depth was 4-7 m. When the water was mixed by wind-driven turbulence (Fig. 4, 1225 h) or by strong tidal currents (Fig. 5), the vertical spread and depth of migration were more variable. From these data, we estimate the downward migration started at ~1800 h and ended at ~0100 h, with an average rate, in vertically stable water conditions, of approximately 0.5 m/h, and the upward migration started at ~0200 h and ended at ~1300 h, with a rate of ~0.3 m/h.

DISCUSSION

Environmental factors and PSP levels

If the patterns found during this 1-year study are typical annual patterns for Puget Sound, they indicate that gradual changes in PSP levels in shellfish are associated with seasonal changes in the water temperature and that sudden increases in toxicity are associated with microscale changes in environmental conditions. To date, there is no evidence of subsurface accumulation and transport of *G. catenella*, such as that reported by Yentsch and Incze (1982) for *G. tamarensis* in the Gulf of Maine. The strong vertical mixing in the main basin of Puget Sound (Barnes and Ebbesmeyer, 1978) precludes, for much of the year, the development of a sharp thermocline with which such subsurface phytoplankton maxima are associated (Pingree *et al.*, 1975; Holligan, 1979).

The finding of a seasonal temperature pattern with PSP levels in excess of 80 µg/100 g meat occurring only after the summer warming of the top mixed

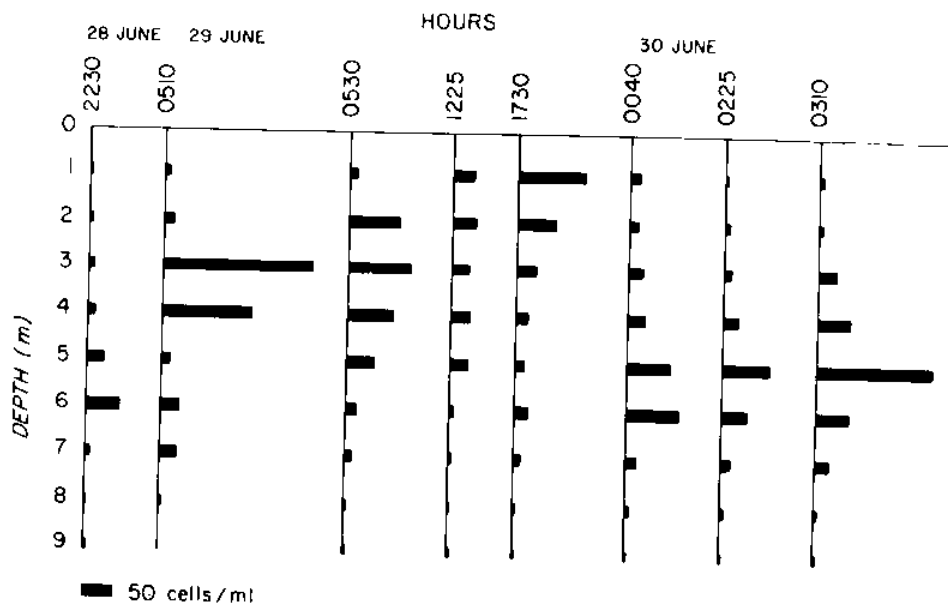


Fig. 4. Changes in vertical distribution of *G. catenella* over time, during thermally stratified water conditions, Quatermaster Harbor, June 28-30, 1981. Dawn at 0511 h.

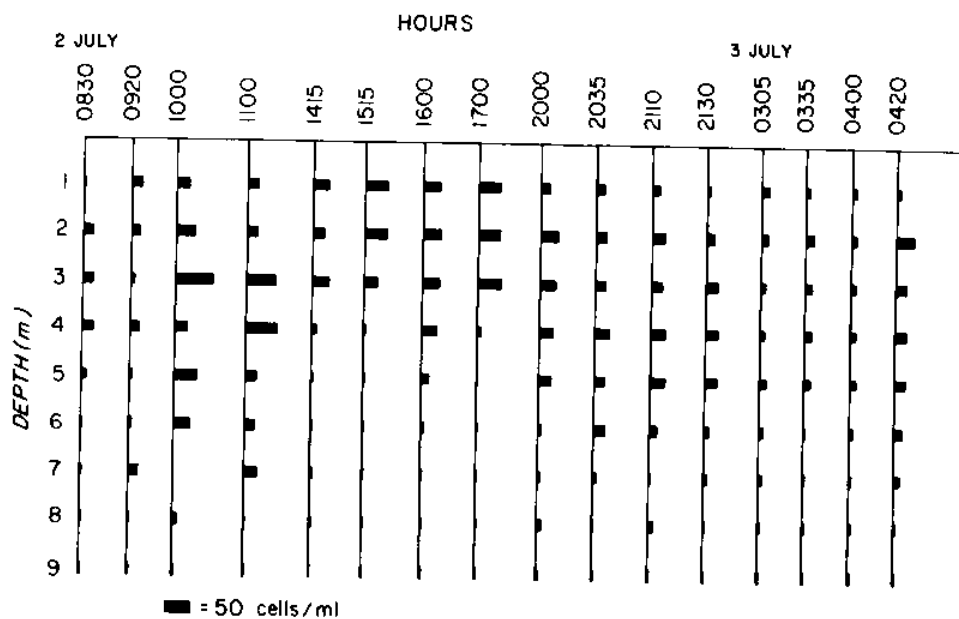


Fig. 5. Changes in vertical distribution of *G. catenella* over time, during spring-tide conditions, Quatermaster Harbor, July 2-3, 1981. Dawn at 0514 h.

layer to 13°C is consistent with results of laboratory studies in which optimal temperatures for growth of this species were found to be 13-17°C (Norris and Chew, 1975). The microscale changes in environmental conditions with which growth and accumulation of *G. catenella* and increases in PSP appear to be closely related are a reduction of turbulence and an increase in water temperature. The reduction of turbulence has a twofold effect on density of *G. catenella* and PSP levels in shellfish. It reduces the depth of the layer through which both the solar heat absorbed by the surface water and the *G. catenella* cells are physically dispersed. As the turbulence abates, greater accumulation of *G. catenella* cells in the upper water column can occur by means of upward migration, and the increased temperature in the upper layer enhances the growth rate of those cells. Thus, the numbers of *G. catenella* in the upper water layer may rapidly increase, causing a rapid rise in PSP in shellfish in the intertidal and shallow nearshore areas, in which most bivalve aquaculture is conducted.

C.M. Yentsch (personal communication, 1982) found that, in the estuaries of Maine also, increases in PSP in shellfish are associated with microscale meteorological events. In contrast to the estuarine situation, increases in PSP on the open coasts and offshore islands of Maine are associated with macroscale events and their effects on the subsurface band of maximum density *G. tamarensis*: the seasonal warming of surface water offshore in the Gulf of Maine in spring and cooling in fall, and the resultant development and erosion of the pycnocline with which the subsurface maximum is associated; the disruption of the pycnocline by a major storm at sea; and the onshore movement of the subsurface populations as a result of an extended period of offshore winds. Dinoflagellate ecologists believe that similar physical phenomena and resultant PSP problems can be expected to occur in certain open coastal areas of the Pacific Ocean also (C.M. Yentsch and P. Holligan, personal communications, 1981).

Breeding bays and implications for aquaculture

The importance of reduced turbulence to the development of dense populations of *G. catenella* points to the potential importance of sheltered bays where wind-driven turbulence is least likely to occur. Margalef *et al.* (1979) referred to certain shallow, protected bays as "breeding grounds" for dense populations of dinoflagellates. The results of the studies reported here suggest that this term could be appropriately used to describe the head end of Quartermaster Harbor, which supported three blooms in 13 months. The contrast between that area and the channel stations in terms of timing and levels of toxin in shellfish suggests that a "breeding bay" concept may be useful to aquaculturists in selection of growing areas. A potential new growing site should be evaluated to determine the degree of probability that it will function as a breeding bay and, thus, be subject to frequent closures. Shellfish farmers with established growing areas only in bays that have a tendency to act as breeding bays may find that diversification of growing areas to include a channel site would be advantageous at times. The channel growing area could permit continued production with fewer harvesting closures and could provide

an area of less dense *G. catenella* in which to purge toxin from shellfish grown in the bay.

The breeding-bay concept could also be useful in designing a monitoring program. It suggests that such a bay may be a good primary sampling station to provide early warning of PSP outbreaks. Increases in toxicity would be expected when thermal stratification of the water occurs, surface temperatures are in excess of 13°C, and tidal magnitude is low to moderate. The exportation of *G. catenella* from breeding bays, suggested in this study by rises in PSP at channel stations at the mouth of Quartermaster Harbor following blooms within the bay, also has implications for a monitoring program. The amount of exportation from a given bay can be expected to be dependent on many factors, including the length and bathymetry of the bay, tidal action, and wind direction and velocity. D. Anderson (personal communication, 1982) has found that, of the *G. tamarensis* cells produced in a Massachusetts salt pond, the percentage exported varies widely with weather and tidal conditions. To the extent that exportation from a particular breeding bay is significant, a monitoring site in the channel at the mouth of that bay may be useful as a secondary sampling site, with increases in toxicity to be expected following increases within the bay and subsequent tidal flushing or following a seasonal increase in the temperature of the surface layer of the channel to ~13°C.

The potential importance of exportation of *G. catenella* from breeding bays to the PSP levels in shellfish in channel waters may easily be underestimated because the dilution factor would appear to be too great in deep channels which are vertically mixed for much of the year. However, in periods of reduced turbulence, vertical dilution of cells decreases and accumulation in the upper waters of the channels can occur. Under those conditions the cells exported from one breeding bay could, if spread horizontally, cause closure levels of PSP in shellfish in a channel area several times greater than the area of the breeding bay. Our data indicate that a density of *G. catenella* as low as 10 cells per milliliter is sufficient to permit accumulations of 80 µg toxin/100 g mussel meat.

Potential implications of vertical migration for aquaculture

The pattern of migration of *G. catenella* determined in this study, namely, starting downward in the evening before dark and swimming upward to the top few meters by early afternoon, is similar to that found for *G. polyedra* by Eppley *et al.* (1968). The nighttime depth and the rate of migration estimated for *G. catenella* (7-8 m and 0.5 m/h) are somewhat less than those estimated for *G. polyedra* (10 m and 1 m/h).

A preliminary pattern of the vertical distribution of *G. catenella* over 24 h was developed in order to consider the implications of diel vertical migration in terms of both the exposure of *G. catenella* to different environmental conditions (temperature, salinity, and nutrients) and the exposure of shellfish to *G. catenella*. The pattern (Fig. 6A), which is based on data shown in Figs. 3 and 4, represents the changes in depth of a band containing 80% of the *G. catenella* in the water column during a midsummer period of thermal stratification and neap tides.

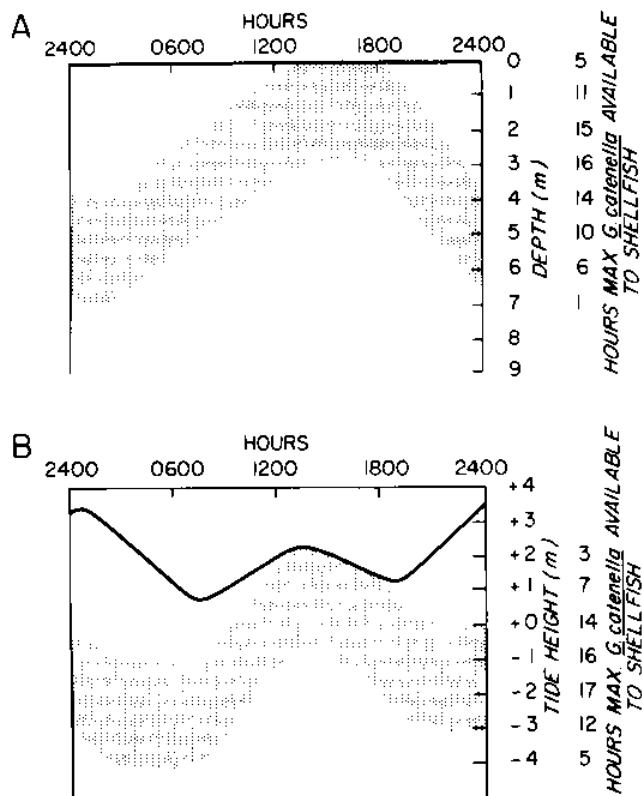


Fig. 6. Projected pattern of changes, over 24 h, in depth of the band containing 80% of the *G. catenella* in the water column, and estimated number of hours per day shellfish in two types of culture would be exposed to that band. Based on migration pattern of *G. catenella* in Quartermaster Harbor, June-July 1981. (A) Shellfish hanging from raft. (B) Shellfish at fixed tide heights on shore bed.

According to this pattern the length of exposure to *G. catenella* of shellfish in a raft culture would vary with their position on the strings below the raft, as indicated in Fig. 6A. Those immediately under the raft would be exposed for 5 h, those at 2-4 m for three times as long, and those at 7 m for only 1 h. The toxicity can be expected to vary with the length of exposure to *G. catenella*.

The projection of the effect of diel vertical migration on PSP levels in shellfish at fixed tide heights, as on pilings or a beach, requires consideration of the changing tidal height of the water surface and the position of the migrating band beneath the surface. The tidal conditions and the projected vertical distribution of 80% of the *G. catenella* in the water column during a neap tide period is represented in Fig. 6B. This indicates that, in this particular tidal situation, shellfish at the +1-m tide height might be expected to have twice as much exposure, and presumably toxin, as those at +2 m, and those at 0 to -2 m twice as much as those at +1 m. In some tidal conditions the exposure to *G. catenella* of shellfish at +1- to +2-m tide height would be expected to be

less than that of shellfish at the top of strings under a raft.

The projected differences in toxicity with tide height, or depth under a raft, suggest that PSP monitoring samples should be taken at the tide height, or depth, of maximum toxicity. They also suggest that both diversification of culture methods to include both raft and shore cultures and diversification of species grown to include one which thrives at higher tide heights may be advantageous in some areas.

Because different growing sites vary widely in wind patterns, bathymetry, and tidal currents, and hence, in degree of turbulence, the extent to which these projected differences in toxicity will actually occur may also be expected to vary considerably, and can best be tested on a site-by-site basis.

Potential role of parasites in controlling G. catenella and PSP problems

The relative importance of the infestation by *Amoebophyra ceratii*, the advective washout during the spring-tide series, and the greatly reduced concentrations of nutrients as causal factors in the decline of the June-July 1981 bloom cannot be determined from the data available. However, intuitively, there seems to be a high probability that the parasite, which consumes the nucleus of its host cell and which infested 47% of the *G. catenella* cells, had a major impact not only on the rate of decline of the bloom but on the ability of the remaining population to rebuild to a significant density when major advective removal diminished and nutrient concentrations increased. Further work is needed to determine the importance of the parasite in controlling natural populations of *G. catenella* and, thereby, PSP levels, and the potential value of the rate of infestation as one factor in a model for predicting timing and intensity of PSP outbreaks.

Taylor (1968) suggested using *A. ceratii* as a biological control agent to reduce PSP problems, following his observations of this parasite in *G. catenella* in three plankton samples collected from one bay over a 3-month period. The documentation of the increase in rate of infestation throughout the development and decline of the June-July 1981 bloom underscores the desirability of investigating the potential value and the acceptability of using *A. ceratii* for this purpose. This species has several attributes that would be desirable in a biological control agent: It has a very high reproductive rate, with several hundred infesting dinospores being produced in each host cell (Cachon, 1964), it appears to have a relatively high selectivity for *G. catenella* (Taylor, 1968), and it destroys the nuclei of *G. catenella* cells adjacent in the chain to the cell initially infested (Wakeman and Nishitani, 1982).

Biological control would be logistically impossible in large bodies of water but may be feasible in small confined bays. To the extent that such bays serve as breeding bays and export cells to channel waters, the control of *G. catenella* by massive introduction of the parasite into the small bays could contribute to the reduction of PSP problems in the channels as well.

In a fjord system long-term benefits could also potentially accrue from any reduction in *G. catenella* buildup. In Puget Sound, for example, part of the phytoplankton approaching the exit from the fjord is refluxed back into the fjord basin (Barnes and Ebbesmeyer, 1978). A potential result of this could be

a long-term continued maintenance of a comparatively high population of *G. catenella* within Puget Sound. Thus, reduction of numbers of *G. catenella* in bays permitting greatest reproduction could have an influence contrary to that of the physical system.

CONCLUSIONS

The findings of this 1-year study in Puget Sound indicate that temperature and vertical stability of the water column are major factors related to changes in PSP levels in shellfish and can, therefore, be useful in predicting PSP outbreaks in Puget Sound, and presumably, in other estuaries with similar characteristics. Protected bays in which strong thermal stratification occurs relatively frequently may at times serve as "breeding bays" for *G. catenella*. The pattern of vertical migration of *G. catenella* indicates that shellfish at middle depths under rafts and at intermediate tide height on shore may be expected to have greater toxin levels than those either above or below.

Both the breeding-bay concept and the projected differences in toxicity with depth or tide height because of vertical migration imply that certain changes in aquaculture practices and monitoring programs could potentially enable the aquaculturist in an area of recurring PSP problems to extend the periods of harvesting safe shellfish and to reduce his losses due to closures.

An earlier suggestion that *A. ceratii* might be useful as a biological control agent of *G. catenella* has been strengthened by the strong association observed between the rapid rise in the rate of infestation of *G. catenella* by this parasite and the decline of the bloom, and by the breeding-bay concept.

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