Cooccurrence of Elevated Urea Levels and Dinoflagellate Blooms in Temperate Estuarine Aquaculture Ponds†

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In hybrid striped bass aquaculture ponds, dinoflagellate blooms were found on 10 of 14 occasions to co-occur with concentrations of urea in excess of 1.5 μM nitrogen. When urea levels were <1.5 μM nitrogen, on seven occasions, no evidence of dinoflagellate blooms was observed in these ponds.

Phytoplankton ecologists have long grappled with the fundamental question of what factors determine the differential growth of species in phytoplankton communities which may result in the dominance, or bloom, of one particular species at a particular time. The availability of different forms of nitrogen and their relative rates of utilization are important factors contributing to the relative success and productivity of different phytoplankton (2, 12, 18). Typically, fast-growing diatoms have been found to be highly correlated with large and/or frequent additions of NO_3^- (16, 20, 33). By contrast, microflagellates (including dinoflagellates) have been correlated with low nitrate concentrations and high rates of NH_4^+ or dissolved organic nitrogen (DON) supply (2, 7, 11, 28). Recent studies of enriched coastal areas also suggest that while phytoplankton production may increase quantitatively with overall nitrogen availability, the DON component may contribute disproportionately to the alteration of phytoplankton succession and the triggering of harmful algal blooms (2, 7, 28). In the past few years, notable advances have been made in the study of the DON pool. The fluxes and composition of this large and complex pool are now characterized to a much better extent than just a few years ago (4, 5, 24, 25).

The factors contributing to the differential growth of phytoplankton species are important issues for aquaculturists as well. Aquaculturists aim to have stable phytoplankton blooms, and unfavorable blooms may cause off flavors or may threaten the survival and marketability of the cultured species (32). In recent years, harmful algal blooms, in particular those of the icthyotoxic dinoflagellates *Gyrodinium galatheanum* and *Pfiesteria piscicida*, have caused massive mortality of fish in natural riverine systems along the east coast of the United States and in cultured fish ponds (6). Aquaculture ponds are typically eutrophic or hypereutrophic, as fertilizer and feed additions are made routinely (10). Indeed, it has been suggested that aquaculture systems, due to their enriched conditions, may actually promote the growth of harmful algal species not previously detected in the source water body (15).

In early 1997 we began an investigation of the nitrogen dynamics and phytoplankton succession in a commercial hybrid striped bass aquaculture facility to determine the role that nitrogen, and in particular DON, plays in development of dinoflagellate blooms in these ponds. Our hope was to identify either specific components of the nitrogen pool or other characteristics of the nitrogen supply that were related to the dinoflagellate blooms and thus could serve as predictors of such harmful blooms.

Samples were collected from commercial ponds located at HyRock Farms on the Manokin River, a tributary of Chesapeake Bay. At the time of sampling, the ponds contained commercial densities of 1-year-old hybrid striped bass (striped bass, *Morone saxitilis*, and white bass, *Morone chrysops*). Sampling was conducted biweekly from 6 June to 15 August 1997, and in addition, one sample was collected in December 1997. In total, three ponds were sampled on seven occasions each, six times on a biweekly basis during the summer and on one occasion in early winter. Samples were collected with acid-cleaned buckets and immediately returned to the laboratory on ice, where they were filtered through precombusted (550°C for 2 h) Whatman GF/F filters, and the filtrates were frozen for later determination of nutrient content (within 2 weeks). Inorganic-nutrient concentrations were determined by standard autoanalyzer methods (29), while concentrations of urea were determined in triplicate by the urease method (23). The filters were retained for analysis of pigment and particulate composition (29). Samples for phytoplankton identification and enumeration were preserved in Lugol's solution and enumerated with a hemacytometer under phase-contrast microscopy (14).

Water temperatures ranged from 19 to 33°C during the midsummer growing season and from 3 to 7°C in early winter. Salinity ranged from 6 to 11.5 PSU. Of the 21 sampling periods, 9 in summer and 1 in fall had dinoflagellate blooms sufficiently developed to require water quality treatment by the grower. Dinoflagellate blooms were classified as a minimum of 5×10^3 cells ml^{-1}. The summer dinoflagellate blooms were largely composed of *G. galatheanum*, *Gymnodinium nelsonii*, and *Prorocentrum minimum*, and the winter bloom was composed of *Katodinium* sp. When dinoflagellates were not abundant, the dominant phytoplankton group was diatoms.

The range of water quality factors observed over the growout cycle of 1997 (Table 1) reflect the "boom-bust" cycles of hypereutrophic systems, in which phytoplankton blooms develop and crash over time scales of several days. Concentrations of NH_4^+, while typically being <2 μM, did exceed 70 μM on two occasions, and NO_3^- also became elevated above 50 μM on several occasions. Concentrations of chlorophyll *a* typically exceeded 100 μg liter^{-1}, reflecting the high phytoplankton density typical of these systems.

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TABLE 1. Phytoplankton biomass (as chlorophyll *a*) and nutrient ranges in striped bass aquaculture ponds during the 1997 grow-out period in midsummer and early winter

Analyte	Midsummer range	Early-winter range
Chlorophyll *a* (μg liter^{-1})	10–338	20–83
Urea (μM N)	0.4–9.9	0.4–3.6
Total dissolved nitrogen (μM N)	33–252	31–59
DON (μM N)	24–180	29–45
NH_4^+ (μM N)	0.6–71	1.6–9.4
$NO_3^- + NO_2^-$ (μM N)	<0.03–70	0.3–4.5
PO_4^{3-} (μM N)	0.04–0.72	0.11–0.45

All dinoflagellate blooms were observed to co-occur with elevated levels of urea (>1.5 μM nitrogen). In total, we found 14 instances of elevated urea levels, of which 10 co-occurred with dinoflagellate blooms. In all cases where urea was <1.5 μM nitrogen (seven occasions), no dinoflagellate blooms were found (Fig. 1). The occurrence and/or abundance of dinoflagellate blooms was not found to be correlated significantly with any other nutrient parameter.

Urea constitutes only a small percentage of total DON in eutrophic and oligotrophic waters; in estuarine fish ponds it is typically <1% of the DON pool. However, urea has been shown to contribute from 60 to 80% of the nitrogen utilized much of the year in the plume of the Chesapeake Bay and up to 50% of the nitrogen utilized in many other coastal regions (13, 17, 19).

The correlation between urea availability and the development of dinoflagellate blooms has implications for phytoplankton ecology in general as well as for the management of aquaculture fish ponds. First, these results are consistent with observations suggesting that some flagellates may have a preference for the uptake of urea over NO_3^- (21, 28). In addition, the strong correlation between dinoflagellate blooms and elevated levels of urea may also suggest that release of urea may be stimulated directly, or indirectly, by the presence of dinoflagellates themselves. Dinoflagellates are known to irritate the gills of finfish, which may lead to the loss of nitrogenous waste products other than ammonia. In teleost fish exposed to other stressors, blood urea nitrogen levels have been found to increase substantially (27). Urea regeneration also occurs from zooplankton as well as bacteria, and rates are dependent on both DON concentration and bacterial activity and/or growth rate (3, 8, 9, 26, 30). The combination of high capacity for urea uptake by dinoflagellates and the potential for enhanced release of urea by the fish exposed to high concentrations of dinoflagellates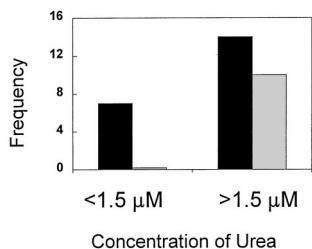, or to enhanced bacterial regeneration, could lead to conditions favorable for sustaining a dinoflagellate bloom if other environmental conditions remain favorable.

There is some evidence that urea may directly stimulate toxin production in dinoflagellates. *Gymnodinium breve*, for example, has been shown to increase its production of brevetoxin to up to six times that of the control when exposed to urea levels of 0.5 to 1.0 mM nitrogen in culture (31).

Finally, aquaculturists have an important interest in maintaining high water quality and minimizing the development of harmful algal blooms. After-the-fact treatment of dinoflagellate blooms is very costly, in terms of loss of marketable product and also in the cost of chemical treatment, often permanganate. These results suggest that an early warning signal for aquaculturists, and perhaps coastal managers as well, may be available by monitoring urea concentrations. By monitoring urea levels and aggressively treating blooms when they appeared during the 1998 growth season at the study site, dinoflagellate blooms similar to those observed in 1997 were avoided and levels of urea were maintained at <1.5 μM nitrogen. A chemical test to detect elevated levels of urea would be more feasible to implement in the field than microscopic analyses of phytoplankton composition, particularly when dealing with the small, poorly known dinoflagellates that are of increasing concern in aquaculture operations.

These results further underscore the need to incorporate urea, and likely other organic nitrogen compounds, into our models of nitrogen flow and our estimates of nitrogen incorporation by primary producers (1). This is especially true for coastal and estuarine ecosystems, which receive nutrient inputs from river flow, sewage, and runoff, including aquaculture pond effluent. The appearance of harmful dinoflagellates, especially *P. piscicida*, in the Pocomoke River, a tributary of the Chesapeake Bay, during the summer of 1997 was correlated with increased organic loading (22). Nutrient reduction efforts in coastal waters will need to take these forms of nitrogen, among other factors, into consideration if a reduction in the occurrence of harmful algal blooms is to be achieved.

FIG. 1. Frequency diagram of number of sampling periods in which urea concentrations were <1.5 or >1.5 μM N (solid bars) and number of sampling periods in which dinoflagellate blooms developed (shaded bars) in commercial hybrid striped bass (*M. saxitilis* and *M. chrysops*) aquaculture ponds in Maryland in 1997.

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