NBP-90-31

Characterization of "Brown-Tides" & Other Bloom Forming

Picoalgae, & their Interactive Effects on Phytoplankton 42 pp

Hargraves (URI)

Narragansett Bay Estuary Program

Characterization of "Brown Tides" and Other Bloom-Forming Picoalgae, and Their Interactive Effects on Phytoplankton

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FOREWORD

The United States Congress created the National Estuary Program in 1984, citing its concern for the "health and ecological integrity" of the nation's estuaries and estuarine resources. Narragansett Bay was selected for inclusion in the National Estuary Program in 1984 and designated an "estuary of national significance" in 1988. The Narragansett Bay Project (NBP) was established in 1985. Under the joint sponsorship of the U.S. Environmental Protection Agency and the Rhode Island Department of Environmental Management, the NBP's mandate is to direct a five-year program of research and planning focussed on managing Narragansett Bay and its resources for future generations. The NBP will develop a comprehensive management plan by December, 1990, which will recommend actions to improve and protect the Bay and its natural resources.

The NBP has established the following seven priority issues for Narragansett Bay:

* management of fisheries
* nutrients and potential for eutrophication
* impacts of toxic contaminants
* health and abundance of living resources
* health risk to consumers of contaminated seafood
* land-based impacts on water quality
* recreational uses

The NBP is taking an ecosystem approach to address these problems and has funded research that will help to improve our understanding of various aspects of these priority problems. The Project is also working to expand and coordinate existing programs among state agencies, governmental institutions, and academic researchers in order to apply research findings to the practical needs of managing the Bay and improving the environmental quality of its watershed.

This report represents the technical results of an investigation performed for the Narragansett Bay Project. The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement #CX812680 to the Rhode Island Department of Environmental Management. It has been subject to the Agency's and the Narragansett Bay Project's peer and administrative review and has been accepted for publication by the Management Committee of the Narragansett Bay Project. The results and conclusions contained herein are those of the author(s), and do not necessarily represent the views or recommendations of the NBP. Final recommendations for management actions will be based upon the results of this and other investigations.

EXECUTIVE SUMMARY

Characterization of "Brown Tides" and Other Bloom-Forming Picoalgae,and Their Interactive Effects on Phytoplankton

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This research had three main objectives: first, the isolation and cultivation of dominant picoplankton-sized algae from Narragansett Bay to determine their diel division patterns and seasonal life cycle phenomena, including resting cell formation and other life history stages; and relative abundance of different species in natural water samples; second, ultrastructural determination of species identities and morphology of picoplankton-sized algae in Narragansett Bay and other U.S. east coast locations; third, examination of possible biological competitive interactive effects in vitro (promotive or inimical) among these algae, with emphasis on taxa related to Chlorella, Minutocellus, and Synechococcus.

Morphology and species determination were accomplished primarily by standard techniques of transmission electron microscopy with supplemental observations by high-resolution phase contrast, interference contrast, and epifluorescence light microscopy. Samples and cultures from other investigators were also obtained. Clonal isolation and cultivation were done using permutations of basal seawater medium, nutrient combinations, and culture vessel substance and shape. Using media preconditioned by growth of typical and bloom species, re-enriched in various ways, the interactive effects among bloom species were evaluated by comparative growth rate measurements.

Several hundreds of picoalgae isolates were made, as well as periodic samplings of local embayments. The causative organism of the 1985 bloom, Aureococcus anophagefferens was not detected although it was present in adjacent waters. Some picoalgae were shown to influence the growth of their cohorts in vitro.

The results of this research have suggested further lines of research which are being pursued in an exploratory way without formal funding to assess their suitability for future funding requests: 1) despite several decades of research the flora of Narragansett Bay is incompletely known, yet such knowledge is necessary for an adequate monitoring program, and to determine future environmental modifications; 2) the ability of picoalgae to modify the growth of

their cohorts suggests that the extent and nature of their bioactive compounds should be examined for potential biomedical application (i.e. bactericidal/fungicidal properties); 3) the apparent global increase in noxious and toxic blooms, including those caused by picoalgae, theoretically may have some relationship to global increases in UV light and/or global warming trends, and the role of increased temperature and UV in stimulating/inhibiting bloom algae needs investigation.

In recent years, picoplankton-sized species have shown their capability of disrupting "normal" ecosystem events by blooming in estuaries and coastal embayments of the U.S. east coast. Considerable economic dislocation has resulted. This work characterized the several specis responsible in Narragansett Bay; further, defined some of the interactions which may influence their abundance and impact. These tasks have been defined as necessary goals by a consortium of scientists and managers, and can be viewed as a major part of multi-institutional research on nuisance blooms.

TITLE: CHARACTERIZATION OF 'BROWN TIDES' AND OTHER BLOOM-FORMING PICOALGAE, AND THEIR INTERACTIVE EFFECTS ON PHYTOPLANKTON

PRINCIPAL INVESTIGATOR: Paul E. Hargraves

START DATE: 1 July 1988 END DATE: 30 JUNE 1989

BACKGROUND

The importance of nanoplankton (2.0-20.0μm) and picoplankton (0.2-2.0) sized primary producers in marine waters has been of considerable interest in the last decade. In cell numbers, carbon biomass, and nutrient dynamics these normally overlooked organisms frequently dominate estuarine, coastal, and oceanic waters. When environmental conditions are suitable, they can overwhelmingly dominate normal populations of primary producers. Such blooms are well known from northeastern U.S. coastal areas (e.g. Ryther, 1954; Ryther et al., 1958) and differ from blooms of many larger phytoplankton in a variety of ways, among which are their higher concentration, apparently faster growth rate, lack of toxicity to humans, presumed unsuitability as food to marine herbivores, and lack of knowledge of their biology. The lack of even the most basic information is a serious problem: new species are frequently found, even in areas rich in a tradition of phytoplankton research (Hargraves & Guillard, 1974; Johnson & Sieburth, 1982; Sieburth, Johnson & Hargraves, 1988). Moreover, the apparent increases in "red tides" and links to a "greenhouse effect" have sparked worldwide interest in these phenomena.

In 1985, massive summer blooms of 2μm-sized organisms provoked considerable interest because of extensive water discoloration ['brown tides'], mass mortality of filter-feeding shell fish, and dislocation of "normal" plankton communities, particularly on Long Island, NY and Narragansett Bay, RI. The organisms responsible included:

Aureococcus anophagefferens Hargraves & Sieburth
Minutocellus polymorphus (Hargraves & Guillard) Hasle
Synechococcus spp.
Nannochloris / Chlorella spp.

In Narragansett Bay, Aureococcus was the dominant organism (in excess of 10^9/L), with Minutocellus (a diatom) becoming abundant as Aureococcus declined. There is strong inferential evidence that the Long Island bloom was similar. A complex of Synechococcus/Nannochloris/Chorella and several other 2μm phytoplankters were also abundant, but less so. During 1986 a similar bloom occurred on Long Island, and Barnegat Bay but in Narragansett Bay the bloom did not approach the abundance or distribution of the previous year. In 1987, these picoalgae did not bloom in Narragansett Bay, although massive

outbreaks continued during the summer in Long Island. The organisms responsible were probably the same, although differentiation among the species is difficult without superior optical equipment and technique. Some of the bloom organisms (Minutocellus, Synechococcus, Nannochloris/Chlorella) are easily amenable to lab cultivation (pers.obs.) while Aureococcus is not. Only one clone of the latter has been isolated (E. Carpenter & E. Cosper, pers. com.); and this clone appears identical to the Aureococcus from Narragansett Bay.

RESEARCH FORMAT

1. Biological Characterization of Bloom Species.

The primary goal in characterization is to determine the identity of the organism; secondarily to define to some extent its biological characteristics and interactions with other organisms which may have an impact on its fate in the environment.

Identification of phytoplankton species may be viewed as a four-tiered system (Taylor, 1980): consultations of taxonomic keys by comparatively unskilled investigators; morphological analyses of non-living samples by experienced microscopists; study of the variability in living material (natural and cultured) using light and/or electron microscopy; and determination of the molecular and genetic bases for species recognition. The first tier is unsuitable for any purpose, and the last has great application for phylogenetic and sensu stricto taxonomic studies, but was outside the scope of this project. The third level, still more advanced than many published studies, is both desireable and attainable. It is mandatory to know if we are dealing with the same (or a number of) species in the blooms which have been occurring in Narragansett Bay or elsewhere, and how proportions of similar-sized organisms change over time. Since they are picoplankton (about 2μm in size), distinctions are not readily made without resort to high resolution light microscopy (including phase contrast, Nomarski interference contrast, and epifluorescence) and transmission electron microscopy (TEM). We used all these techniques to characterize bloom organisms, and to determine in a semi-quantitative way (by low magnification, high resolution TEM thin-sectioning of carefully concentrated samples) the relative proportions of different picoplankton species as blooms wax and wane. This technique has already proved useful (Sieburth, Johnson & Hargraves, 1988) in tracing the decline of the new anorexia-inducing bloom organism Aureococcus in Narragansett Bay, and suggested an unsuspected possible contributory cause for its decline (i.e. virus infestations). An improved method for quantitative epifluorescent light microscopy (Geider, 1987) shows considerable promise in its application to this research as well. Because similar problems occur in contiguous waters, we also examined similarly prepared bloom samples from other adjacent bloom areas, using TEM, to identify bloom organisms.

2. Isolation/Cultivation of Bloom Organisms.

The optimum in vitro growth conditions for each phytoplankton species are different. Many bloom-forming species, paradoxically, are fastidious under cultivation, and require a specific combination of light, temperature, macronutrients, micronutrients, and habitat conditions for successful maintenance. Moreover, physiological infraspecific "races" may appear at different times of the year. The subdominants of the Narragansett Bay bloom picoplankton are easily cultivatable. The dominant, Aureococcus, is not, although attempts to isolate and grow it from in tributaries of Narragansett Bay were unsuccessful, since Aureococcus was absent or in such low abundance as to be undetectable by any of our techniques. Special emphasis was placed on basal medium quality (filter-sterilized vs. autoclaved water vs. bloom water; artificial seawater), low to high surface: volume ratio), and chemical microenrichment (organic vs. inorganic N and P sources). There is some evidence that the Stony Brook isolate Aureococcus is enhanced by addition of organic phosphate to growth media. New refinements in cultivation techniques (e.g. Keller & Guillard, 1985) have pointed out potential approaches in cultivating fastidious species.

Emphasis was on isolates from Narragansett Bay and its associated embayments, primarily Pt. Judith Pond, Wickford Harbor, and Greenwich Bay. Collection trips to Woods Hole and eastern Long Island were made to isolate picoplankton bloom-forming organisms from these contiguous areas. Long Island has experienced perhaps more severe picoplankton blooms in 1985 through 1988 than Narragansett Bay, while the Woods Hole area has had none. We hoped to determine this via isolations and observations by TEM of natural picoplankton populations.

It is likely that Aureococcus, and certainly its consort picoalgae, are present in extremely low numbers throughout much of the year, either planktonic or epibenthic. Since 1988 proved to be a year in which no bloom developed, as a contingency we artificially enriched outdoor aquarium tanks (300 liter), containing 10 µm filtered Bay water, with organic and inorganic N and P sources. The purpose was be to encourage the growth of picoalgae, in the absence of natural blooms, to provide enough material for diel division pattern studies, presence of resting stages, and TEM of natural populations.

3. Interactive effects.

Another type of characterization is interactive. Chemical and biological competition among phytoplankton has been long known (Pratt, 1966; Sieburth, 1962). The bloom in Narragansett Bay during 1985 caused some dislocation in the "normal" pattern of summer species. Was this due to competition (for nutrients or light) or a more active inhibition? The dominant bloom organism, Aureococcus, produces an exocellular organic material, presumably polysaccharide, which we believe has a role in the anorexia-inducing effect on Mytilus [Tracey et al., 1988]. This material may also affect its phytosynthetic

competitors. We examined growth rates of typical summer phytoplankton (e.g. Heterosigma akashiwo (= Olisthodiscus luteus), Skeletonema costatum, Asterionella glacialis) against bloom picoplankton species (Minutocellus, Chlorella, Synechococcus) in the following way. In a suitable growth medium, the picoplankton selected are grown to senescence, and the medium filtered gently to remove all cells. The medium is re-enriched and inoculated with a typical summer species. Controls are medium enriched in the same way, in which no picoplankton has been grown. Cell division rates over time gave an indication of whether picoplankton cell exudates have a stimulatory or inhibitory effect on growth of other species. Although physiological/ecological data exists for Minutocellus and Synechococcus (Glover, 1986; Hargraves & Guillard, 1974), there are no data available on any potential inhibitory effects. Apart from the potential effects on plankton dynamics, these picoplankton (and larger species) also have the potential to serve as sources of biomedically active antibiotic agents, a research area poorly investigated (e.g. Kellam & Walker, 1989).

The problems of ecological significance of these picoplankton blooms is a regional one; other interactive studies, presumably currently and to be supported by Sea Grant, are also ongoing. Since information on one aspect of these blooms may impact research directions of another aspect, it is logical to share research results prior to their formal presentation, and this sharing of information among other Sea Grant investigators will be implemented, as well as collaboration with EPA-ERL on effects of picoplankters on larval and adult invertebrates of commercial importance.

RESULTS OF THE RESEARCH

At present the picoplankton flora of Narragansett Bay consists of 15 taxa of which eight are diatoms (Table 1). This is certainly a conservative estimate, because of the problems in species designations in Chlorella and Synechococcus (see Appendix B) and because almost certainly some species are present in such low numbers as to escape detection (as was most probably the case for Aureococcus in 1998-1989; our sample size on a given date represents approximately 10^{-12}% of the Bay, and one would expect that some species are overlooked). Of nearly 800 primary isolations of picoplankton algae from 1987-1989, about 95% were of the Chlorella and Synechococcus groups, the remainder being small diatoms, and no Aureococcus. Three 300 liter tanks of 10 µm-filtered water separately enriched with organic phosphate, nitrate, and inorganic phosphate salts and seeded with 50 liters of East Greenwich Cove water (the presumed initiatory locus of the 1985 "brown tide" which recurred to a minor extent in 1986) yielded blooms of Chlorella, Synechococcus, Euglena proxima, and the coccolithophorid Cricosphaera roscoffensis, but no Aureococcus. Additionally, no water blooms attributable to Aureococcus were observed throughout the period. It therefore seems likely that Aureococcus had little impact on the plankton of Narragansett Bay in 1987, 1988, and (to date) 1989.

Table 1. Known autotrophic picoplankton from Narragansett Bay.

Bacillariophyceae
- *Chaetoceros tenuissimus* Meunier
- *Extubocellus spinifer* (Hargraves et Guillard) Hasle, v. Stosch et Syvertsen
- *Fragilaria rotundissima* Hargraves et Guillard
- *Leptocylindrus minimus* Gran
- *Minutocellus polymorphus* (Hargraves et Guillard) Hasle, v. Stosch et Syvertsen
- *Minidiscus trioculatus* (Taylor) Hasle
- *Thalassiosira mala* Takano
- *Thalassiosira pseudonana* Hasle et Heimdal

Chrysophyceae
- *Aureococcus anophagefferens* Hargraves et Sieburth

Prymnesiophyceae
- *Chrysochromulina* sp.

Prasinophycee
- *Micromonas pusilla* (Butcher) Manton et Parke
- "scaled prasinophyte"

Chlorophyceae
- *Chlorella* spp.
- *Nannochloris* spp.

Cyanobacteria
- *Synechococcus* spp.

Picoplankton abundance varies considerably throughout the year, but in general (based on relative abundances in TEM thin sections and cell counts of antifluorescent cells) Chlorella and Synechococcus maintain minimum populations of 10^4-10^5 throughout the year, with variable maxima (Table 2 and Table 3). Further discussion of these results is presented in Appendix 2.

Field samples from Pt. Judith Pond, East Greenwich Cove, and Wickford harbor were taken up to May 1989 for TEM sectioning and fluorescence counting. Additional samples were obtained from eastern Long Island (to September 1988) and embayments in the Woods Hole region (Eel Pond, Green Pond) to April 1989. The usual group of picoplankton, dominated by Chlorella and Synechococcus were present, with Aureococcus seen only in the Long Island samples. A cooperative effort with M. Levandowsky to compare remotely sensed images with simultaneous field samples was begun, and will continue in 1989 with supplemental funding apart from NBP and Sea Grant.

Effects of picoplankton exudates on larger, common microplankters are shown in Table 4. The cyanobacteria Synechococcus (clone M-11) exerted no significant negative influence on any of the three microplankters. On the contrary, growth of Skeletonema was significantly enhanced when grown in both logarithmic phase and senescent phase exudates of Synechococcus. Exudates from Chlorella (clone PJ8C) had no effect on growth while in log phase, and none on Heterosigma in exudates from senescent cultures. Results for exudates from senescent phase were not obtainable for Skeletonema. Exudates from Minutocellus (clone NML-100) negatively affected all three microplankters. Senescent exudates inhibited growth in the other two diatoms; log phase exudates inhibited one diatom (Asterionella) and the flagellate Heterosigma. Moreover, only in Minutocellus was there a difference in growth between log phase and senescent exudates: both Skeletonema and Heterosigma had lower growth rates in senescent exudates relative to log phase.

The microplankton chosen to test the effect of picoplankton exudates represent differing niches in Narragansett Bay. Skeletonema is the dominant diatom at most times of the year, while Asterionella usually peaks in winter or late summer (Karentz and Smayda, 1984; Hargraves, 1988). Heterosigma akashiwo (frequently in litt. as Olisthodiscus luteus) is a frequent summer bloom-forming flagellate, whose pattern of reciprocal dominance with Skeletonema may be due to either presence of inhibitory substances or patterns of grazing (Tomas, 1980). That picoplankton exudates affect these three test organisms in vitro is not surprising, given the wide variety of photosynthetic end products produced and exuded (Fabregas et al., 1987; Fogg, 1983; Hino, 1988; Murakami et al., 1988; Shelef and Soeder, 1980). Allelopathy among marine microalgae has received some attention (e.g. Chan et al., 1980; Goldman et al., 1981; Maestrini and Bonin, 1981) but the specific compounds active in promotion or inhibition are still mostly unknown. There is evidence that Aureococcus has no effect on, or enhances the growth of its microplankton cohorts (Cosper et al., 1988), but its exudates are autoinhibitory

Table 2. Abundant autotrophic picoplankton taxa from Narragansett Bay: their analogues and approximate maximum abundance, 1985-1989.

1. *Aureococcus anophagefferens* Hargraves et Sieburth
   maximum concentration: > 10^9/L
   similar taxa: *Pelagococcus subviridis* Norris (in Lewin et al., 1977)

2. *Minutocellus polymorphus* (Hargraves et Guillard) Hasle, v. Stosch et Syvertsen
   maximum concentration: ~10^8/L
   similar taxa: several species in the genera *Arcocellus*, *Leyanella*, *Papiliocellus* and *Minutocellus* can resemble this species (see Hasle et al., 1983)

3. *Synechococcus* spp.
   maximum concentration: 10^8/L
   similar taxa: *Synechococcus* cannot be differentiated into species using morphological criteria alone; biochemical criteria have not been adequately developed (see Waterbury et al., 1986)

4. *Chlorella* spp.
   maximum concentration: ~10^7/L
   similar taxa: morphological criteria are generally inadequate for generic and specific determination; closely related (possibly synonymous) taxa include *Mychonastes ruminatus* (Simpson & Van Valkenburg, 1978); *Nanochlorum eucaryotum* (Wilhelm et al., 1982) and *Nannochloris* (Brown & Elfman, 1983; Sarokin & Carpenter, 1982). Biochemical criteria for marine taxa have not been adequately developed (e.g., Kessler, 1982, 1984)

5. *Chaetoceros tenuissimus* Meunier
   maximum concentration: 10^6/L
   similar taxa: the systematics of < 10µm *Chaetoceros* species is chaotic; similar forms have, almost randomly, been called *C. calcitrans*, *ceratosporus*, *galvestonensis*, *gracilis*, *minutissimus*, *pumilus*, *salsugineum*, *simplex*, and *socialis* (see Rines & Hargraves, 1988)

Table 3. Picoplankton detected in Narragansett Bay and adjacent waters.

	1	2	3	4	5	6
N. Bay 3/88-6/88	-	+	+	-	-	-
N. Bay 7/88-5/89	-	+	+	+	+	+
Wood Hole region 4/88-4/89	-	+	+	+	-	+
Long Island embayments 4/88-9/88	+	+	+	+	-	+
N. Bay 7/88-9/88 (enrichments)	-	+	+	+	+	+

1 = *A. anophagefferens*
2 = *Synechococcus* spp.
3 = *Chlorella* spp.
4 = *Minutocellus polymorphus*
5 = *Chaetoceros tenuissimus*
6 = *Micromonas pusilla*
7 = "scaled prasinophyte" (Johnson & Sieburth, 1982)

Table 4. Effect of picoplankton exudates from logarithmic growth and senescent cultures on growth rates of autotrophic microplankton from Narragansett Bay, expressed as doublings/day (std. dev. in parentheses). Growth rates significantly different from controls ($P \leq 0.05$; ANOVA) are underlined. Significant differences between log/senescent phases are overlined.

	Control	M11 (log)	M11 (sen)	NML 100(log)	NML 100(sen)	PJ8C(log)	PJ8C(sen)
Skeletonema costatum	0.76 (±0.08)	1.02 (±0.14)	0.90 (±0.02)	0.90 (±0.11)	0.55 (±0.07)	0.86 (±0.04)	D.I.
Asterionella glacialis	0.96 (±0.05)	0.88 (±0.12)	0.87 (±0.03)	0.64 (±0.07)	0.53 (±0.03)	0.91 (±0.12)	0.74 (±0.09)*
Heterosigma akashiwo	0.66 (±0.08)	0.49 (±0.11)	0.59 (±0.02)	0.85 (±0.06)	0.64 (±0.03)	0.59 (±0.10)	0.77 (±0.13)

D.I. = data incomplete; * = based on control growth of 0.82 (±0.05)

M11 = *Synechococcus*

NML 100 = *Minutocellus polymorphus*

PJ8C = *Chlorella*

in vitro. Somewhat surprising was the inhibition of all tested microplankters by exudates of the diatom Minutocellus. Allelopathic exudates by diatoms are uncommon; Skeletonema (Chan et al., 1980) and several pennate diatoms (Maestrini and Bonin, 1981; but see Goldman et al., 1981) have been implicated, but most studies (including this one) suffer from using cell concentrations of the interacting species which may be environmentally unrealistic (~10^6/ml), and from the undefined nature of the exudate responsible. Recently, domoic acid exudates from natural populations of the pennate diatom Nitzschia pungens have been implicated in plankton and fish kills in Atlantic Canada (Subba Rao et al., 1988); and mortality in salmon aquaculture facilities due to Chaetoceros (species uncertain) have been reported from Puget Sound, Washington (Horner, 1988).

Almost certainly the exudate results in these experiments cannot be generally applicable, and must be viewed as preliminary. The nature and concentration of exudates changes with the physiological condition of the bloom organism. Likewise, other phytoplankters differ in their responses interspecifically and according to physiological state. We designed our experiments to maximize the likelihood of detecting a response. Moreover we maintain that, given the morphological and physiological evidence of intergeneric and interspecific variability in the Chlorella and Synechococcus complexes (and to a lesser extent, Minutocellus; Hargraves and Guillard, 1974), considerably expanded permutations will be necessary to assess the role of allelopathy in picoplankton bloom effects. It would also be useful to evaluate the possibility that these exudates may also be bactericidal or bacteristatic. Recent research (Kellam & Walker, 1989) has shown that many common phytoplankton exhibit antibacterial activity.

SUMMARY OF ACCOMPLISHMENTS

1. The causative organism of the 1985 bay bloom was formally described and named as: Aureococcus anophagefferens Hargraves & Sieburth (published as Sieburth, Johnson & Hargraves, 1988);

2. The multiyear "brown tide" organism plaguing Long Island waters continually since 1985 was shown to be conspecific with the Narragansett Bay "brown tide";

3. Aureococcus was undetectable in Narragansett Bay waters in 1987-1989 by a variety of techniques;

4. Viruses and microplankton grazers were suggested as major constraints on picoplankton algae in Narragansett Bay (in Sieburth, Johnson & Hargraves, 1988);

5. At least 15 algae are members of the picoplankton community of Narragansett Bay, of which 8 are diatoms (in Hargraves, Vaillancourt & Jolly, in press);

6. The total known phytoplankton flora of Narragansett Bay was expanded from 138 to well over 200 species, with numerous others as yet unidentified (published in Hargraves, 1988);

7. Preliminary experiments indicated that exudates from picoplankton algae can have significant effects on the growth of their larger phytoplankton cohorts, adding another dimension to the plankton dynamics of local waters (in Hargraves, Vaillancourt & Jolly, in press).

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Appendix A.

Table 15.1 from Hargraves 1988, "Phytoplankton in Narragansett Bay"

TABLE 15.1
Checklist of Phytoplankton from Narragansett Bay. Includes confirmed species, regardless of prior published lists. Excludes most tychopelagic species unless their presence in the plankton is fairly constant. Includes autotrophic and heterotrophic species.

Class Bacillariophyceae

Actinoptychus senarius Ehr.
Astrionella bleakleyi W. Sm.
A. glacialis Castr.
A. notata Grun.
Attheya decora West
Bacteriastrum delicatulum Cl.
B. hyalinum Laud.
Cerataulina pelagica (Cl.) Hend.
Chaetoceros affinis Laud.
C. amanita Cl.-Eul.
C. atlanticus Cl.
C. borealis Bail.
C. brevis Sch.
C. ceratosporus Ost.
C. compressus Laud.
C. constrictus Gran
C. convolutus Castr.
C. coronatus Gran
C. costatus Pav.
C. crinitus Sch.
C. curvisetus Cl.
C. danicus Cl.
C. debilis Cl.
C. decipiens Cl.
C. densus Cl.
C. diadema (Ehr.) Gran.
C. didymus Ehr.
C. eibenii (Gran) Meun.
C. fallax Proschk.-Lavr.
C. holsaticus Sch.
C. ingolfianus Ost.
C. laciniosus Sch.
C. lauderi Ralfs
C. lorenzianus Grun.
C. pelagicus Cl.
C. perpusillus Cl.
C. peruvianus Bright.
C. pseudocrinitus Ost.
C. pseudocurvisetus Man.
C. radicans Sch.
C. rostratus Laud.
C. seiracanthus Gran
C. septentrionalis Oestr.
C. similis Cl.
C. simplex Ost.
C. socialis Laud.
C. subtilis Cl.
C. teres Cl.
C. tenuissimus Meunier
C. spp.
Corethron criophilum Castr.
Coscinodiscus asteromphalus Ehr.
C. centralis Ehr.
C. concinnus W. Sm.
C. granii Gough
C. oculus-iridis Ehr.
C. wailesii Gran
Cyclotella caspia Grun.
C. meneghiniana Kütz.
C. striata (Kütz.) Grun.
C. closterium (Ehr.) Reim. & Lew.
Detonula confervacea (Cl.) Gran
D. pumila (Castr.) Sch.
Ditylum brightwellii (West) Grun.
Eucampia zoodiacus Ehr.
Guinardia flaccida (Castr.) Per.
Hemiaulus sinensis Grev.
Lauderia annulata Cl.
Leptocylindrus danicus Cl.
L. mediterraneus (Per.) Hasle
L. minimus Gran
Lithodesmium undulatum Ehr.
Minidiscus trioculatus (Tayl.) Hasle
Minutocellus polymnorphus (Harg. & Guill.) Hasle, Stosch & Syv.
Nitzschia pseudodelicatissima (Hasle) Hasle
N. pungens Grun.
N. seriata Cl.
Odontella sinensis (Grev.) Grun.
Paralia sulcata (Ehr.) Cl.
Porosira glacialis (Grun.) Jorg.
Rhizosolenia alata Brightw.
R. calcar-avis M. Sch.
R. delicatula Cl.
R. fragilissima Berg.
R. imbricata Brightw.
R. pungens Cl.-Eul.
R. setigera Brightw.
R. stolterfothii Per.
R. styliformis Brightw.
Roperia tesselata Grun.
Skeletonema costatum (Grev.) Cl.
Stephanopyxis palmeriana (Grev.) Grun.
S. turris (Grev. & Arn.) Ralfs.
Thalassionema nitzschioides Grun.
Thalassiosira anguste-lineata (Schm.) Fryx. & Hasle
T. binata Fryx.
T. bioculata (Grun.) Ost.
T. constricta Gaard.
T. decipiens (Grun.) Jorg.
T. delicatula Ost.
T. eccentrica (Ehr.) Cl.
T. gravida Cl.
T. mala Tak.
T. nordenskioldii Cl.
T. oestrupii (Ost.) Hasle em. Fryx.
T. profunda (Hend.) Hasle
T. pseudonana Hasle & Heim.
T. rotula Meun.
T. solitaria Gay.
T. weissflogii (Grun.) Fryx. & Hasle.
T. spp.
Thalassiothrix frauenfeldii Grun.

Class Dinophyceae

Amphidinium carteri Hulb.
A. sphenoides Wisl.

TABLE 15.1 *Continued*
Checklist of Phytoplankton from Narragansett Bay.

A. sp.
Cochlodinium spp.
Ceratium furca (Ehr.) Clap. & Lachm.
C. fusus (Ehr.) Duj.
C. lineatum (Eyhr.) Cl.
C. longipes (Bail.) Gran
C. minutum Jorg.
C. tripos (Müll.) Nitz.
Dinophysis acuminata Clap. & Lachm.
D. caudata Sav.-Kent
D. norvegica Clap. & Lachm.
D. rotundata Clap. & Lachm.
Dissodinium pseudolunula Swift
Gonyaulax digitale (Pouch.) Kof.
G. polyedra Stein
G. sp.
Gymnodinium abbreviatum Kof. & Swezy
G. splendens Leb.
G. spp.
Gyrodinium aureolum Hulb.
G. spirale (Berg.) Kof. & Swezy
G. uncatenum Hulb.
G. spp.
Helgolandinium subglobosum Stosch
Heterocapsa triquetra (Ehr.) Stein
Katodinium rotundatum (Lohm.) Fott
Oxyrrhis marina Duj.
Paulsenella chaetoceratis (Paul.) Chatt.
Polykrikos schwarzii Butsch.
Prorocentrum balticum (Lohm.) Loeb.
P. gracile Schutt
P. micans Ehr.
P. minimum (Pav.) Schill.
P. scutellum Schr.
P. triestinum Schill.
Protogonyaulax tamarensis (Leb.) Tayl.
Protoperidinium bipes (Paul.) Bal.
P. conicum (Gran) Bal.
P. depressum (Bail.) Bal.
P. excentricum (Paul) Bal.
P. granii (Ost.) Bal.
P. leonis (Pav.) Bal.
P. minutum (Kof.) Loeb.
P. steinii (Jorg.) Bal.
P. spp.
Scrippsiella trochoidea (Stein) Loeb.

Class Ebriophyceae
Ebria tripartita (Schu.) Lemm.
Hermesinum adriaticum Zach.

Class Chrysophyceae
Aureococcus anophagefferens Harg. & Sieb.
Apedinella spinifera (Throud.) Throud.
Dictyocha fibula Ehr.
Dinobryon balticum (Schutt) Lemmn.
Distephanus speculum (Ehr.) Haeck.
Mesocena polymorpha Lemmn
Ochromonas sp.
Paraphysomonas sp.
Pseudopedinella pyriformis Cart.

Class Prymnesiophyceae
Chrysochromulina ericina Parke & Mant.
C. parkae Green & Leadb.
C. spp.
Coccolithus pelagicus (Wall.) Schill.
Cricosphaera roscoffensis (Dan.) Gay. & Fres.
Isochrysis sp.
Pavlova gryrans Butch.
P. sp.
Phaeocystis pouchetii (Har.) Lag.

Class Cryptophyceae
Chroomonas salina (Wisl.) Butch.
C. spp.
Cryptomonas spp.
Hemiselmis sp.

Class Prasinophyceae
Micromonas pusilla (Butch.) Mant. & Parke
Nephroselmis rotunda (Cart.) Fott
N. sp.
Pedinomonas minor Korsch.
Pterosperma sp.
Pyramimonas amylifera Conr.
P. torta Conr. & Kuff.
P. sp.
Tetraselmis spp.

Class Chlorophyceae
Carteria sp.
Chlamydomonas sp.
Chlorella sp.
Dunaliella sp.
Nannochloris sp.
Oltmannsiella virida Harg. & Steele [1]

Class Euglenophyceae
Euglena proxima Dang.
E. spp.
Eutreptia scotica Butch.
Eutreptiella hirudoidea Butch.
E. sp.
Heteronema acus (Ehr.) Stein
Urceolus sp.

Class Raphidophyceae
Fibrocapsa japonica Tor. & Tak.
Olisthodiscus luteus Cart. [2]

Class Cyanophyceae
Spirulina subsalsa Oerst.
Synechococcus sp.

[1] *Oltmannsiella virida* has been renamed by Chihara (1987; Arch. Protistenk. 132: 313–324) as *Oltmannsiellopsis viridis*.

[2] The organism from Narragansett Bay known as *Olisthodiscus luteus* may be a species of *Heterosigma* Hada. (see Throndsen J., 1983, Working Party on Taxonomy in the Akashiwo Mondai Kenkyukai, Tokyo, pp. 1–62).

Appendix B.

Text of Hargraves et al., 1989 (in press) "Autotrophic Picoplankton in Narragansett Bay and their Interaction with Microplankton"

AUTOTROPHIC PICOPLANKTON IN NARRAGANSETT BAY AND THEIR INTERACTION WITH MICROPLANKTON

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ABSTRACT

The picoplankton flora of Narragansett Bay consists of at least 15 species of which eight are diatoms. Maximum abundances of these taxa range from 10^6 to 10^9 per liter. The current state of taxonomic knowledge is insufficient to characterize many non-diatoms to species based on morphological criteria alone. Initial examination of the effect of exudates from three representative local picoplankters on three typical larger autotrophic phytoplankters suggests that some inhibition of in vitro growth rates, particularly by the diatom Minutocellus polymorphus, can occur.

INTRODUCTION

There is abundant evidence that picoplankton (approximately 0.2-2.0 μm; Sieburth et al., 1978) [or ultraplankton, approximately 0.5-8.0 μm; Murphy and Haugen, 1985] are present in northeastern U.S. coastal waters continually at 10^6-10^9/L depending on season, with cyanobacteria (primarily Synechococcus) and eucaryotic algae at typical concentrations of 10^4-10^6 and 10^3-10^5/L respectively (Carpenter and Campbell, 1988; Glover et al., 1985; Johnson and Sieburth, 1979, 1982; Murphy and Haugen, 1985; Waterbury et al., 1986). Moreover, these tiny autotrophs have caused extensive blooms in estuaries and coastal waters sporadically for many years, particularly under conditions of eutrophication (Malone, 1977; Ryther, 1954; and

pers. obs.). Until about ten years ago the occurrence of these organisms in Narragansett Bay was based primarily on anecdotal observations. In 1979 and 1982 Johnson and Sieburth presented evidence that extensive populations of cyanobacteria and eucaryotic cells were continually present, and in 1985 a major "brown tide" of Aureococcus anophagefferens Hargraves and Sieburth (in Sieburth et al., 1988) significantly altered the normal summer phytoplankton pattern and caused extensive mortality in some filter-feeding invertebrates (Durbin and Durbin, this volume; Smayda, this volume; Tracey et al., 1988)

In contrast to the situation on Long Island, the recurrence of Aureococcus was diminutive in 1986 and subsequently. Since Aureococcus did not bloom prior to 1985, and is unlikely to have appeared de novo, an investigation was undertaken to examine local waters for Aureococcus during non-bloom periods, to characterize other picoplankton autotrophs with the latent ability to form blooms, and to determine whether potential biological interaction of picoplankton autotrophs (i.e., allelopathic growth inhibition through production of exudates) with representative and typical phytoplankton from Narragansett Bay could play a part in altering phytoplankton communities.

MATERIALS AND METHODS

Clonal cultures were isolated and maintained in nutrient dilutions of "f" medium (Guillard and Ryther, 1962) with nutrients and seawater autoclaved separately in Teflon containers. The clones were isolated from local or contiguous waters and included: Synechococcus sp. (clone M11); Chlorella sp. (clone PJ8C); Minutocellus polymorphus (clone NML-100); Asterionella glacialis Castr. (clone E45); Skeletonema costatum (Grev.) Cl. (clone E47) and Heterosigma akashiwo (Hada) Hada (clone Olisth; from Long Island Sound, via P-GCCMP).

For electron microscopy, field samples (1-2 L) and clones were fixed in 2% glutaraldehyde, post-fixed in osmium, dehydrated through an alcohol series to propylene oxide, embedded in Spurr's epoxy resin, sectioned, post-strained with uranyl acetate or lead citrate, and examained in either a JEOL-STEM or Zeiss EM-9S/2 electron microscope. Field samples were

collected from embayments tributary to Narragansett Bay where the 1985 Aureococcus bloom reached in excess of 10^9 cells/L; East Greenwich Cove, Wickford Harbor, and Point Judith Pond. Samples from the Woods Hole region came from Eel pond, Great Pond and Green Pond in April and June. Samples from Long Island embayments were collected and fixed by Dr. J. J. Lee or EPA-ERL personnel and processed in our lab.

In the interaction experiments, selected picoplankton (clones M-11, PJ8C and NML-100; Synechococcus, Chlorella, and M. polymorphus respectively, see Figure 1) were grown in one-liter flasks, harvested by gentle filtration and centrifugation in both logarithmic and senescent phases, and the supernatants (with dissolved exudates) enriched to f/2 nutrients and inoculated with logarithmic growth phase cultures of E-45, E-47 and Olisth respectively, in triplicate 125 ml flasks. Growth rates of Asterionella, Skeletonema and Heterosigma were calculated as suggested by Guillard (1973) based on multiple Sedgewick-Rafter counts on days 0, 4 and 10. The experimental matrix yielded 189 counted samples.

Large volume enrichment cultures from Narragansett Bay in July 1988 were established by adding 50 liters of 10μm-filtered seawater from East Greenwich Bay (the presumed initial locus of the 1985 brown tide bloom) to each of three 250 liters of 1 μm filtered Narragansett Bay water enriched with inorganic phosphate, organic phosphate or nitrate at 25 μM concentration, with samples withdrawn for electron microscopy at approximately two week intervals until September, with one re-enrichment in August.

RESULTS

Based on whole mount and thin-section electron microscopy, supplemented by light microscopy, a minimum of 15 autotrophic taxa fall into the picoplankton size range in Narragansett Bay, although most also may attain sizes larger than 2 μm. Of these, the majority (Table 1) are diatoms, distributed through seven genera. Those that may be chain-forming (E. spinifer, E. rotundissima, L. minimus, M. polymorphus) are sometimes retained in net tows,

since chain lengths may exceed net mesh size. The remaining organisms are distributed through five classes and are primarily unicells.

Observations from 1985 to 1988 show that five of these picoplankters (Table 2) can reach high population levels in the Bay, either annually (Synechococcus and Minutocellus) or in unpredictable major bloom proportions (Aureococcus). Because of the many problems associated with species limits in Chlorella and Synechococcus it is infeasible to state how many species actually are present, either in Narragansett Bay or contiguous waters, but their continuing abundance has been documented (Sieburth et al., 1988). Aureococcus, after a major bloom (> 10^9/L) in 1985, was present at about two orders of magnitude less in 1986, and TEM thin sections from various places in the Bay in summers of 1987 and 1988 failed to demonstrate its presence. Minutocellus, Chlorella and Synechococcus are persistent features of summer picoplankton, although apparently not responsible for water discoloration. The occurrence of C. tenuissimus is more problematic. Based on light microscopy of field populations, it appears sporadically in the summer, sometimes in excess of 10^6/L, but is more difficult to identify in TEM if thin sections do not include portions of the setae, which are themselves variable in their development.

Although nonquantitative, TEM thin sections are useful for determining presence or absence of picoplankton autotrophs, particularly where routine light microscopy is marginally adequate for critical determination except under ideal conditions. Information for Narragansett Bay and adjacent regions is presented in Table 3. The species clusters of the cyanobacterium genus Synechococcus and the chlorophyte genus Chlorella appear widespread and ubiquitous. The diatom M. polymorphus was present throughout the spring and summer in the Long Island and Woods Hole regions, but was undetected in spring samples in Narragansett Bay. Micromonas pusilla followed a similar pattern of occurrence. Supporting the observations of other investigators, Aureococcus was undetectable in Narragansett Bay and in the Woods Hole region in spring, but was commonly seen in their sections from Long Island through spring and early summer. Somewhat in contrast to Johnson and Sieburth (1982), the "scaled prasinophyte" was only seen once, in a late spring sample from Narragansett Bay. Chaetoceros tenuissimus confirmed earlier observations of confinement to warmer conditions. The enrichment tanks were set up to determine whether routine sections from field material were overlooking the presence of

Aureococcus. However, enrichment with inorganic phosphate, organic phosphate and nitrate in separate tanks failed to reveal the presence of Aureococcus. Moreover, the pattern of occurrence of other autotrophic picoplankters was the same in enrichment tanks as in field samples from the three Narragansett Bay locations.

Effects of picoplankton exudates on larger, common microplankters are shown in Table 4. The cyanobacteria Synechococcus (clone M-11) exerted no significant negative influence on any of the three microplankters. On the contrary, growth of Skeletonema was significantly enhanced when grown in both logarithmic phase and senescent phase exudates of Synechococcus. Exudates from Chlorella (clone PJ8C) had no effect on growth while in log phase, and none on Heterosigma in exudates from senescent cultures. Results for exudates from senescent phase were not obtainable for Skeletonema. Exudates from Minutocellus (clone NML-100) negatively affected all three microplankters. Senescent exudates inhibited growth in the other two diatoms; log phase exudates inhibited one diatom (Asterionella) and the flagellate Heterosigma. Moreover, only in Minutocellus was there a difference in growth between log phase and senescent exudates: both Skeletonema and Heterosigma had lower growth rates in senescent exudates relative to log phase.

DISCUSSION

The picoplankton causing blooms in Narragansett Bay remain incompletely described for three reasons; the bases for their systematics are insufficiently developed; some blooms are ephemeral and highly localized in small embayments within Narragansett Bay; and their inception and demise is unpredictable.

The problem in identifying and assigning a name to picoplankton organisms can be viewed as a two-tiered one: first, the problem of determining how a species is to be circumscribed; second, integrating the available and new information to make a rational decision as to what to call it. The need for specific identification is most acute in the Chlorella complex and the Synechococcus complex. Traditionally, Chlorella has been separated from Nannochloris

on the basis of the mode of cell division (into four or two cells respectively) and the presence of a parent wall during division in Chlorella (autospore formation). But Sarokin and Carpenter (1982) reported strains of "Nannochloris" with parent cell walls, and multiple autospore formation. Other criteria such as size and pyrenoid formation are inter- and intra-specifically variable and are of limited utility (Andreoli et al., 1978; Dodge, 1973; Sarokin and Carpenter, 1982). Brown and Elfman (1983) report N. atomus as having a pyrenoid; some Chlorella species lack one. Moreover, the original description of Nannochloris by Naumann (1921) is vague enough to include many species now included in Chlorella. The related taxon, Nanochlorum eucaryotum (Wilhelm et al., 1982) appears not to be sufficiently distinct from Chlorella to merit a new genus, and was invalidly published (no type designation). Mychonastes ruminatus (Simpson and Van Valkenburg, 1978) differs from Chlorella only in the rugosity of the cell wall, which varies during the life cycle. At least one species of Nannochloris, N. oculata (Droop) Hibberd, is apparently a member of a different class, the Eustigmatophyceae (Antia et al., 1975).

Multiple clones of Chlorella isolated from Narragansett Bay and seen in thin sections from adjacent regions vary in presence or absence of pyrenoids, and in ultrastructural details of the chloroplast and cell wall, suggesting at least a multiplicity of species. In this report they have all been referred to Chlorella, although some will almost certainly be eventually referrable to Nannochloris. As has been frequently pointed out, the delimitations of species and separation of genera is unlikely to be accomplished solely on morphological bases, but rather on a combination of ultrastructure with life cycle observations (e.g., Brown and Elfman, 1983; Sarokin and Carpenter, 1982) and chemotaxonomic/molecular analysis approaches (e.g. Kessler, 1982, 1984). The bloom proportions reached by these algae in the New Jersey (Olsen, 1980), New York (Hulburt, 1963; Malone, 1977) and upper Narragansett Bay (pers. obs.) suggests that it would be useful to know what they are.

A similar situation obtains with Synechococcus. In ultrastructure, morphological variation is on a generally continuous gradient among multiple clones, and distinct "breaks" in morphology make delimitation of species (and genera) presently infeasible. Considerable physiological/biochemical variation among clones is well documented, however (e.g. Glöver,

1985; Waterbury et al., 1986) and eventually will provide a firmer basis for taxonomic groupings.

Use of TEM thin sections in picoplankton bloom studies is helpful in examining the types of organisms present, and can be used to determine relative abundancese (Johnson and Sieburth, 1982). It is not a quantitative technique and has the drawback of long lead times for sample processing and analysis. A variety of light microscope techniques (Thomsen, 1986) including epifluorescence (e.g. Davis et al., 1985) are more suitable for quantification, but each of these also has disadvantages. Except under the best conditions of optical resolution it is difficult to progress beyond the categories of "cyanobacteria", "chlorophyte" or diatom. One might argue that "Synechococcus" and "Chlorella" from thin sections do not represent a substantial advance beyond the more general terms, but here the difficulty lies more with the adequacy of knowledge for circumscription of species, and not the ability to separate morphological characters. A partial solution may be immunofluorescence relatedness (Campbell et al., this volume; Anderson et al., this volume); however, this technique currently is more applicable to analysis of relatedness of interclonal identities, rather than the more general problems of classification and nomenclature.

The consequences of inadequate criteria for species delimitation are not trivial. Biochemical and physiological inter- and intraspecific variation in Chlorella and Synechococcus complexes have implications in food web dynamics and many questions based on taxonomic variation may be posed in terms of suitability as food for grazers, comparative ability to bloom as opportunists, and relative toxicity and allelopathy, among many others.

Among the bloom-forming picoplankton there is considerable interregional and annual variation in abundance in our coastal waters. Johnson and Sieburth's (1982) "scaled prasinophyte" was only seen in spring samples from Narragansett Bay although it has a much wider distribution (Silver et al., 1986), and Chaetoceros tenuissimus only in summer samples. Aureococcus was only seen in Long Island samples. Chlorella and Synechococcus vary seasonally (Johnson and Sieburth, 1982; Waterbury et al., 1986) but cause winter discolorations infrequently in Narragansett Bay, mostly in the upper Bay and small embayments. Apart from chemical and physical constraints on natural populations, the effects of grazing and pathogens

probably also have significant impact. Many protists feed on picoplankton (Caron, this symposium; Johnson et al., 1988; Verity and Villareal, 1986; Waterbury et al., 1986) with some specificity; and the extant unpurified clone of Aureococcus contains a bicoecid grazer (pers. obs.). Viruses are well known pathogens of cyanobacteria (Padan and Shilo, 1973) and eucaryotic picoplankton (Preisig and Hibberd, 1984; Reisser et al., 1986; Sieburth et al., 1988): their interrelationships are a fruitful ground for investigation.

The microplankton chosen to test the effect of picoplankton exudates represent differing niches in Narragansett Bay. Skeletonema is the dominant diatom at most times of the year, while Asterionella usually peaks in winter or late summer (Karentz and Smayda, 1984; Hargraves, 1988). Heterosigma akashiwo (frequently in litt. as Olisthodiscus luteus) is a frequent summer bloom-forming flagellate, whose pattern of reciprocal dominance with Skeletonema may be due to either presence of inhibitory substances or patterns of grazing (Tomas, 1980). That picoplankton exudates affect these three test organisms in vitro is not surprising, given the wide variety of photosynthetic end products produced and exuded (Fabregas et al., 1987; Fogg, 1983; Hino, 1988; Murakami et al., 1988; Shelef and Soeder, 1980). Allelopathy among marine microalgae has received some attention (e.g. Chan et al., 1980; Goldman et al., 1981; Maestrini and Bonin, 1981) but the specific compounds active in promotion or inhibition are still mostly unknown. There is evidence that Aureococcus has no effect on, or enhances the growth of its microplankton cohorts (Cosper et al., 1988, and this volume) but its exudates are autoinhibitory in vitro. Somewhat surprising was the inhibition of all tested microplankters by exudates of the diatom Minutocellus. Allelopathic exudates by diatoms are uncommon; Skeletonema (Chan et al., 1980) and several pennate diatoms (Maestrini and Bonin, 1981; but see Goldman et al., 1981) have been implicated, but most studies (including this one) suffer from using cell concentrations of the interacting species which may be environmentally unrealistic, and from the undefined nature of the exudate responsible. Recently, domoic acid exudates from natural populations of the pennate diatom Nitzschia pungens have been implicated in plankton and fish kills in Atlantic Canada (Subba Rao et al., 1988); and mortality in salmon aquaculture facilities due to Chaetoceros (species uncertain) have been reported from Puget Sound, Washington (Horner, 1988).

Almost certainly the exudate results in these experiments cannot be generally applicable, and must be viewed as preliminary. The nature and concentration of exudates changes with the physiological condition of the bloom organism. Likewise, other phytoplankters differ in their responses interspecifically and according to physiological state. We designed our experiments to maximize the likelihood of detecting a response. Moreover we maintain that, given the morphological and physiological evidence of intergeneric and interspecific variability in the Chlorella and Synechococcus complexes (and to a lesser extent, Minutocellus; Hargraves and Guillard, 1974), considerably expanded permutations will be necessary to assess the role of allelopathy in picoplankton bloom effects.

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Appendix C.

RESEARCH PUBLICATIONS AND PRESENTATIONS

Hargraves, P.E. Phytoplankton in Narragansett Bay. IN: Freshwater and Marine Plants of Rhode Island. R. Sheath & M. Harlin, eds. pp. 136-142, 1988.

Sieburth, J. Mcn., P.W. Johnson & P.E. Hargraves. Ultrastructure and ecology of *Aureococcus anophagefferens gen. et sp. nov.* (Chrysophyceae); the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, summer, 1985. J. Phycol. 24:416-425, 1988.

Johnson, P.W., J.McN. Sieburth & P.E. Hargraves.. Ultrastructure and ecology of Calycomonas ovalis Wulff 1919 (Chrysophyceae) and its redescription as a testate rhizopod Paulinella ovalis n. comb. (Filosea: Euglyphina). J. Protozool. 35:618-626, 1988.

Hargraves, P.E., R.D. Vaillancourt and G.A. Jolly. Autotrophic picoplankton in Narragansett Bay and their interaction with microplankton. In press, E. Cosper & E. Carpenter, eds. *Novel Phytoplankton Blooms.* Lect. Notes Coast. Est. Stud. --:------. Springer Verlag, Heidelberg, 1989.

PRESENTATIONS SUPPORTED BY THIS PROJECT

Hargraves, Vaillancourt & Jolly. Autotrophic picoplankton in Narragansett Bay and their interaction with microplankton. Symposium on Novel Phytoplankton Blooms, Stony Brook, NY, October 1988.

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PUBLICATIONS INDIRECTLY RESULTING FROM SUPPORT FROM THIS PROJECT

Tracey, G.A., P.W. Johnson, R.W. Steele, P.E. Hargraves & J.McN. Sieburth. A shift in photosynthetic picoplankton composition and its effect on bivalve mollusc nutrition: the 1985 "brown tide" in Narragansett Bay, Rhode Island. J. Shellfish Res. 7:671-675, 1988.

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