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# Final technical report for NSF grant #OCE 75-20241, entitled Identification and role of the ultraplankton of the lower Chesapeake Bay region

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# Identification and Role of the Ultraplankton of the Lower Chesapeake Bay Region

by

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## INTRODUCTION

Attempts were made to determine the numbers and identities of eucaryotic ultraplankters (cells which pass through a 15μm mesh screen) in the lower Chesapeake Bay. Their measured abundances and diversities were then related to salinity, temperature, chlorophyll *a*, phaeophytin, time of day, time of year, dissolved oxygen, and $^{14}C$-fixation rates. By analysis of those data, comparison with $^{14}C$-fixation by plankters in unfiltered sea water, and observations of pure cultures, attempts are being made to determine the relative roles of the autotrophic ultraplankters in the Chesapeake Bay.

## MATERIALS AND METHODS

Eight cruises were conducted in the study area during the months of February, May, August, and October of 1976 and 1977. During each cruise at the mouth of the York River, samples of estuarine water were obtained at surface (1 meter), mid-water, and near bottom every 4 hours for 24 hours. At 3 other stations (Johns Hopkins stations 707Ø, 701A, and 707V) only 1 sampling was conducted at the 3 depths during each cruise.

Temperature, salinity, oxygen, and fluorometric profiles were made of the water column at each sampling period, then water samples were collected in Niskin sterile bag samplers at 1 meter, mid-water, and near bottom depths. The "mid-water" depth was selected to correspond to the strongest discontinuity in fluorometer or temperature values along the water column transect. Bottom samples were taken about 1 meter from the bottom. Light transmission was measured with a Li-Cor instrument

with a 400-700nm quantum sensor suspended at various levels in the water column.

Inorganic nutrients (ammonia, nitrate, nitrite, and phosphate) were analyzed by standard manual methods, chlorophyll a and phaeopigments by standard fluorometric methods, and productivity at various light levels using the $^{14}CO_2$ method and a multi-intensity artificial light incubator.

For cell counts and cell identifications water samples were passed through a 15µm Nitex mesh and fixed in Lugol's solution ($I_2$, 5g; KI, 10g; glacial acetic acid, 10ml; $H_2O$, 100ml) where 99ml of filtered sea water was added to 1ml of the fixative. A high diversity of other fixatives, including gluteraldehyde, formaldehyde, acrolein, $O_sO_4$, HgCl, and $KMnO_4$, were tried, but Lugol's classic fixative was found to yield samples with the largest number of autotrophs. Heterotrophs were fixed best in glutaraldehyde buffered with sodium cacodylate; however, since autotrophs were of primary interest in this study, Lugol's solution was used. The superiority of Lugol's solution as a fixative was not discovered until after cruise 3. Since cruises 1-3 were conducted using 3% gluteraldehyde and since lower cell counts were obtained, it was necessary to determine and use correction factors for autotrophic flagellates, diatoms, autotrophic nonflagellates, dinoflagellates, heterotrophic flagellates, and non-flagellated heterotrophs.

For cell identification only water samples were also fixed, after concentration by centrifugation (800xg, 5 min.), in $O_sO_4$ fumes and 3%

glutaraldehyde at pH 7.2 in 0.1M sodium cacodylate. Whole mounts were prepared for transmission (TEM) and scanning electron microscopy with the $O_sO_4$ fume-fixed cells. Sections were prepared for TEM and whole wet mounts for light microscopy from material fixed in glutaraldehyde.

As a means of becoming familiar with the species involved, interference, phase, and bright field microscope observations were made of fresh samples during the cruise. In attempts to culture the cells, water samples, concentrated by centrifugation, were inoculated into a high diversity of culture media which included Erd-Schreiber's medium (Throndsen, 1969. Nor. J. Bot. {16:164}), Guilliard's $F_2$ medium, and Provasoli's ES media.

After each cruise cells were counted in a Petroff-Hauser counting chamber at 400X magnification using phase contrast optics. Three replicate cell counts were made for each water sample. The Petroff-Hauser counting chamber was used rather than the more popular settling chamber (Utermöhl) method, because 1) comparison of the two methods yielded no significant differences in all cell categories counted and 2) better phase optics were obtained with the Petroff-Hauser counting chamber using the microscopes available in our laboratories.

Unialgal cultures were established from cells isolated by means of hand-held capillary pipettes. Attempts at establishing species identities were made using observations of 1) fixed, whole cells by light and transmission electron microscopy, 2) living cells from pure

and mixed cultures by light microscopy, and 3) thin-sectioned cells from cultures and uncultured sea water by TEM. Cell volumes were estimated using measurements obtained from light microscope observations at 1000X magnification. Dimensions were applied to the appropriate formulae for various solid geometric figures (i.e. solid cone, prolate spheroid, oblate spheroid, etc.).

All available physical and biological measurements for each sample, including cell count, cell volume, salinity, temperature, dissolved oxygen, chlorophyll a, and phaeophytin, etc., have been incorporated into a multivariate data set accessible interactively by APL functions and stored on disc at The College of William and Mary. An interactive data management system has been developed using APL as the basis. The data is stored in variable length records to eliminate empty missing value space and is in the form of APL matrices. This makes possible use of the data by APL functions without conversion. Functions for converting the data to EIBCDIC-FORTRAN format have been generated to enable use of other available programs for analysis. This system is in the process of being published for William and Mary system users.

RESULTS

Within the Bay autotrophic ultraplankters were most numerous in the late spring to late summer time period, varying from means of 3,800 cells/ml to 35,800 cells/ml during the 8 cruises at the York River mouth and 1,800 to 23,300 cells/ml in the southern part of the Chesapeake Bay. The shift in maximum cell numbers from August in 1976 to May-August in 1977 may be correlated with the warm winter of 1975-76 and the very cold winter of 1976-77, but with the available data such a hypothesis cannot be tested. Minimum numbers of cells were found in May, 1976 and February, 1977 corresponding to the shift to an earlier maximum in 1977.

Off the Bay mouth autotrophic ultraplankters are less numerous than in the Bay ranging from 294 to 9,700 cells/ml with maximum numbers appearing at station 701A (directly off the Bay mouth) in August, 1976 and May, 1977 as in the Bay whereas at station 707V the first maximum shifted from August to October, 1976 and the second peak remained the same (Fig. 1).

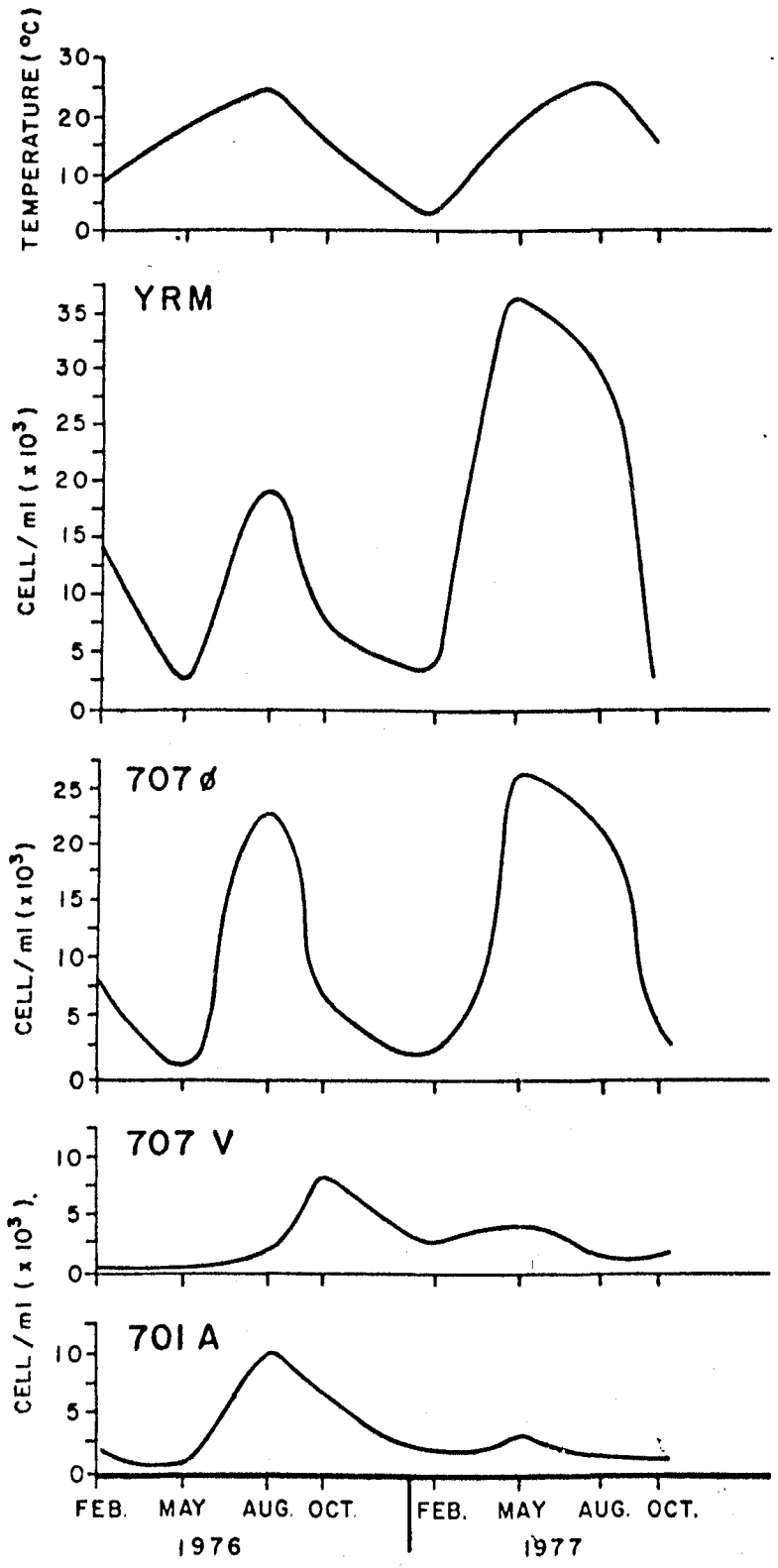


Fig. 1-Number of autotrophic, ultraplankton cells found in the study area as related to temperature and time of year. YRM=York River mouth; 707Ø=lower Chesapeake Bay; 707V=mouth of Chesapeake Bay, north station; and 701A=mouth of Bay, south station. Numbered stations are standard stations of Johns Hopkins University.

When the major categories of autotrophs (other flagellated autotrophs, cryptomonads, dinoflagellates, diatoms, and non-flagellated autotrophs) are examined, it can be seen that the "other" flagellated autotrophs (non-dinoflagellate, non-cryptomonad flagellates) are the primary cell types which caused the August, 1976 and May, 1977 maxima. Cryptomonads become more dominant after the maxima. Diatoms were unpredictable becoming the largest part of the population in mid-winter of both years as well as late summer in 1976. They ranged widely from 5-51% of the population whereas "other" flagellated autotrophs were more stable ranging from 32-74% of the population. With the exception of February and May, 1976, where there were large numbers of Prorocentrum minimum, dinoflagellates comprised only 2-6% of the cells.

The major species and cell types which comprise 1% or more of the total cells counted during the study are listed in Table I. Together the cells comprise 62% of the total population. Cell groupings I and II consisted of a mixture of non-flagellated heterotrophs which had no distinguishing features other than size. Both were spheroidal or pyriform with group I falling in the range of 2-3μm in longest axes and group II, less than 2μm. Blue-green algae as well as small flagellates which had lost flagella undoubtedly were included in the general categories along with Chlorella spp. The latter were known to be present on the basis of pure cultures established from the cruise water samples.

There were 448 autotroph cell types and species and 65 heterotrophs based on light microscope observations. The list is suspected to contain

Table I.-Species which comprised 1% or more of total cell population during study period

| Species or Cell Type | % of Total Numbers | # of Sample Occurrences | Individual Cell Volume ($\mu^3$) | Volume Rank |
|---|---|---|---|---|
| *Isochrysis galbana* | 10.5 | 58 | 20 | 21 |
| non-flagellate group I | 9.6 | 170 | 14 | 30 |
| *Chroomonas lateralis* | 4.5 | 157 | 29 | 33 |
| *Cyclotella caspia* and *C. atomus* | 4.4 | 121 | 72 | 16 |
| non-flagellate group II | 4.2 | 131 | 4 | 102 |
| *Skeletonema costatum* | 4.0 | 168 | 136 | 11 |
| *Cryptomonas acuta* | 3.4 | 130 | 167 | 10 |
| *Prorocentrum minimum* | 3.1 | 102 | 1989 | 1 |
| non-flagellate III | 2.7 | 105 | 91 | 19 |
| uniflagellate I | | 154 | 17 | 58 |
| *Thalassiosira bioculata* | 2.2 | 91 | 161 | 15 |
| uniflagellate II | 1.9 | 71 | 14 | 74 |
| biflagellate I | 1.4 | 113 | 102 | 29 |
| *Katodinium rotundatum* | 1.2 | 156 | 134 | 25 |
| biflagellate II | 1.1 | 122 | 18 | 93 |
| biflagellate III | 1.0 | 93 | 15 | 103 |

many more designations than there are species, because of naturally occurring polymorphism and cell damage from handling. Through use of transmission and scanning electron microscopy and light microscopy of preserved or dried samples collected during the cruises and fresh samples obtained from the York River, cell identifications are being obtained. It will not be possible to identify all of the cell types recorded; however, identities of a significant portion (>50%) of the 100 most common species is expected to be obtained. New species and new range records are emerging as well as identifications of previously established species.

Characterization of species in unialgal cultures is proving to be the most significant technique for establishing which species are found in the study area. Table II lists the species established in unialgal culture where identity to genus or genus and species is known.

## DISCUSSION

In attempts to determine the numbers, identities, and roles of autotrophic ultraplankters in the lower Chesapeake Bay progress has been made. Obviously, any effort to enumerate ultraplankters in natural waters is a difficult problem due to their small size, lack of distinguishing characteristics in the light microscope, and ease with which they lyse. It should be possible to count cells in the transmission electron microscope (TEM) so that relative numbers of cells can be obtained; however, our attempts yielded poor correlation between results from the light and TEM. The limiting factor appears to lie in the need to rinse

Table II.-Species established in unialgal culture

Bacillariophyceae

*Ankistrodesmus falcatus*

*Biddulphia granulata*

*Chaetocerus septentrionalis*

*Cyclotella atomus*

*C. caspia*

*Nitzschia acicularis*

*N. communis*

*Skeletonema costatum*

*Synedra fragelloides*

*Thalassiosira bioculata*

Chlorophyceae

*Chlamydomonas* sp.

Chrysophyceae

*Chrysococcus* sp.

Cryptophyceae

*Cryptomonas* spp. I, II, III, IV

*C. pseudobaltica*

*Hemiselmis* sp.

Table II. (continued)

Dinophyceae

*Katodinium rotundatum*

*Prorocentrum minimum*

Euglenophyceae

*Euglena* sp.

Haptophyceae

*Hymenomonas carterae*

*Pavlova* sp.

*P. gyrans*

Prasinophyceae

*Pseudoscourfieldia* sp.

*Pyramimonas* sp. I

*Pyramimonas* sp. II

*P. amylifera*

*P. virginica*

cells before drying on TEM grids. Such rinsing causes loss of certain cell types. When our scanning electron microscope is installed the possibility of using it to obtain estimates of absolute numbers of cells present in natural waters will be investigated. By eliminating the problems caused by grid bars obscuring views of the cells, absolute rather than relative numbers can be obtained, but the problem associated with loss of cells during rinsing will still need to be resolved.

Since the distinguishing features of many ultraplankters are based on fine structure, it will be necessary to incorporate electron microscopy into attempts at enumeration of ultraplankters to species. Otherwise, workers using a light microscope will have to accept assessments in which only some of the organisms are identified and others are grouped into major categories (i.e. autotrophic flagellates, diatoms, etc.). Complete characterizations of species in the study area would permit future workers to count cells in the light microscope with a much higher level of certainty than is now possible when striving for species identity. Despite the high resolution attainable, electron microscopy introduces the problems associated with small sample sizes and excessive specimen handling; therefore, it probably cannot be used alone.

We shall continue in our efforts to describe at both the light and electron microscope levels the structure of ultraplankters and to identify or describe the species found. An atlas will result which will permit future workers to maximize their efforts at counting cells. Even if the TEM and SEM do not prove feasible to use as counting tools, an atlas

incorporating fine structure will be useful, because once the fine structure is known a competent microscopist can more readily identify ultraplankters seen in the light microscope since poorly resolved structures then assume meaning not otherwise apparent.

A wealth of data is now available as a result of this study on ultraplankton populations in the lower Chesapeake Bay. Data digestion now in progress relating cell numbers, volumes, and volume-to-surface area ratios to physico-chemical parameters is expected to yield useful insights into population dynamics. Pure culture studies in progress on the physiology of dominant ultraplankters is expected to yield further information on the roles of those species. Such studies will continue with financial support from the Commonwealth of Virginia and other federal agencies. Later a proposal for further support from the National Science Foundation will be submitted.

PUBLICATIONS

No publications from this study have been completed thus far; however, several manuscripts will be completed and submitted for publication in the coming year. General titles will be the following:

1. Fixation and counting methodology for enumeration of ultraplankton in natural waters.
2. Fine structure and taxonomy of Cryptophyceae from Chesapeake Bay. I. Cryptomonas acuta.

3. Fine structure and taxonomy of Cryptophyceae from Chesapeake Bay. II. Taxonomic significance of cell surface patterns induced by trichocysts.

4. Fine structure and taxonomy of Prasinophyceae from Chesapeake Bay.

SCIENTIFIC COLLABORATORS

One Master's degree candidate, Steve Hastings, was supported by the grant. The title of his thesis will be "Aspects of diel variation of $^{14}CO_2$ uptake in Chlorella sp." Three other graduate students, Barry Kilch, Don Hayward, and Alyce Thomson, received partial support from the grant. Graduate students Mark Kowalski, William Rizzo, Larry Pastor, and Ed Matheson, in addition to the above-mentioned students, participated in the cruises and thereby received ship-board and research experience. Colleagues Drs. Richard Wetzel and Larry Haas of VIMS participated in the cruises and research, working on related projects.

PERMANENT EQUIPMENT PURCHASED

1. Polaron Model E3000 Critical Point Drying Apparatus (Serial #525.3.77) $1150

2. Ladd Tilting Variable Speed Rotary Coater for vacuum evaporation $ 471

3. Manostat Cassette Pump Model 72-510-000 $ 256