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An Electron Microscopic Study of Picoplanktonic Organisms from a Small Lake

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Abstract. Picoplankton, both prokaryotic and eukaryotic, are distinguished from other aquatic organisms by their small size (0.1–2.0 μm). Such organisms were recovered from waters of a small oligotrophic lake using screens, filters, and high-speed centrifugation. The majority of the picoplankton were unable to form visible colonies on common media. Cells examined in thin sections by electron microscopy showed that 60–75% of the cells had an average diameter after dehydration of 0.48–0.51 μm. The maximum dimensions of the rest of the cells ranged from 0.56–1.81 μm. Using details of ultrastructure, cells were classified as prokaryotic or eukaryotic. Phototrophs present included two cyanobacterial morphotypes (5–6%) and two eukaryotic algae (less than 1%). The arrays of intracytoplasmic membranes in 18–20% of the cells were suggestive of methanotrophic rods and chemoautotrophs. Relatively few prosthecate bacteria were observed in the water column samples. The smallest cells (1–2%) contained magnetosomes, the presence of which were confirmed by x-ray spectroscopy. Iron was also detected in the envelopes of some rod shaped cells by the same technique. The study of in situ picoplankton populations using TEM coupled with other techniques may provide better understanding of picoplankton biomass.

Introduction

The recognition of bacterial-sized plankton as an important part of the biomass in oceanic waters was made by Johnson and Sieburth [24] and Waterbury et al. [51]. Picoplankton have been recognized for making a significant contribution to primary productivity [48], as a principle food source for a wide variety of protozooplankton [39] and as part of the food web in the oceans. Picoplankton have also been recognized in large lakes [9, 17, 43, 46, 47, 50].

Johnson and Sieburth [25] explored oceanic picoplankton using electron microscopic methods to examine cells recovered from large volumes of seawater by centrifugation after screening and filtration to remove cells larger than 2 μm. This approach allows one to compare relative abundance of morphological and ultrastructural types of picoplankton [44] collected at different depths of various bodies

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of water and has been used to examine both seawater [26] and brackish waters [2]. Larsson et al. [28] used the TEM to study bacteria concentrated by centrifugation from a small eutrophic lake. The value of the approach is to circumvent cultural problems in a study of indigenous organisms in natural environments [4].

Our recent studies have focused on the microbiological quality of waters from a group of small lakes situated in the Black Rock Forest near Cornwall, NY. All but one of the seven small lakes serve a reservoir system supplying freshwater to several towns in the region. Because pollution is minimal and the nutrient level of the waters is quite low [1], we visualized the opportunity to examine the microflora of small oligotrophic lakes [38] using the in situ procedure proposed by Johnson and Sieburth [25].

Methods

Separation of picoplankton from other aquatic organisms was carried out with screens, filters, and high speed centrifugation, following the procedure developed by Johnson and Sieburth [25]. Three-liter water samples were collected from Arthur's Lake once a month from May through November, 1990. Single surface collections were made at Sutherland Lake and Alec Meadow Lake in July, 1990, for comparative purposes. Surface samples were taken by bucket. A second sample was taken with a Niskin bottle at a depth of 13 feet, one foot above the sediment surface to provide a deep sample. The temperature and pH value for each sample were recorded, as were viable counts for heterotrophs and cyanobacteria. The latter were estimated by membrane filtration using 0.2-μm Nuclepore filters (47 mm diam.) in triplicate. Appropriate dilutions were filtered and filters placed onto the surfaces of suitable media. mHPC agar [3] was used for heterotrophic counts after incubation for 48 hours and 1 week at 35°C. For oxygenic phototrophs, filtered dilutions were mounted onto modified Fitzgeralds mineral salts agar [5]. Incubation in the light (60 $u^E/m^2/sec$) was at 25°C for at least two weeks before final colony counts were made. The heterotrophic count provided a simple estimation of water quality, reflecting the relative amounts of organic nutrients present.

Total counts of picoplankton were carried out using glutaraldehyde-fixed samples filtered onto small (25 mm) Nucleopore filters (0.2 μm pore size pretreated with Irgalan Black). The filters were stained with acridine orange and counted by epifluorescent microscopy [21].

The glutaraldehyde (3%)-fixed concentrate was stained with O_sO_4 [36] and then embedded in EPON [29]. Thin sections were cut with a diamond knife, post-stained with uranyl acetate [45] and examined in the TEM (Hitachi-H7000) at 75 kV. Cell size was determined by measuring cells sectioned medially. We recognize that dehydrated cells measure less than living cells. Measuring cells at the light microscope level is difficult because of the extremely small dimensions of cells.

Suspected magnetite particles that appeared in some cells were analyzed using the electron microscope operating in the STEM mode at 75 kV in conjunction with a PGT System 4 energy dispersive x-ray spectrometer (EDAX). The beam in the spot mode was placed on the item of interest to generate the x-ray spectrum. The system was also used to confirm polyphosphate bodies [5] and to examine composition of other structural parts, pertinent to the present study [23]. Polybetahydroxy-

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Table 1. Comparison of viable and total counts of picoplankton from Arthur's Lake

	Samples Collected			
Viable Counts	Surface (CFU/ml)		Deep (CFU/ml)	
Heterotroph[a]	333	8.0%[b]	455	8.89%[b]
Cyanobacteria[a]	850	20.42%[b]	717	14.01%[b]
	Cell Count/ml		Cell Count/ml	
Total Count[c]	4,162	100%	5,118	100%

[a]Average of five monthly determinations
[b]Percentage of corresponding total count
[c]Average of three monthly determinations

alkanoate (PHA) inclusions in thin sectioned cells were identified solely on a cytological basis, which we have found to be fairly reliable [12].

Results

Determination of Viable and Total Cell Numbers

The viable counts of heterotrophic and cyanobacteria were estimated and compared with the total, epifluorescent, microscopic counts of water samples collected at surface and deep locations in the water column (Table 1). pH values at every sampling period were pH 6.8–7.0. We account for only about 25% of the total count as colony-forming (CFU) heterotrophs and blue greens. Full-strength and 1/4-strength nutrient agar and incubation of heterotrophic plates at 20, 25, and 35°C gave similar quantitative heterotrophic counts.

Over a nine-month period, the heterotrophic count ranged from 330–450 CFU/ml. The isolates were favored by aerobic conditions, and no more than five colony types were observed. All of the heterotrophic isolates grew on glucose as a sole carbon and energy source as well as on nutrient agar. All were Gram-negative rods. Two of the isolates produced large yellow and whitish slimy colonies, whereas the other colony types were flat, bright yellow, orange and pink-pigmented. The same colony types were observed at each plating, although in relatively different numbers. Exopolymer-producing cells may be more abundant than is apparent in the plated picoplankton samples. Certainly many exopolymer-producing organisms might be lost from view if one restricts observation of water samples to those passed through 5–10-μm screens and 2-μm filters. Some samples of biomass were recovered for sectioning after filtration through the 20 μm Nitex screen. The omission of the 2 μm filtration had no apparent effect on the viable or microscopic counts of picoplankton.

Two cyanobacteria and at least one eukaryotic alga were subcultured from single colony isolates. All of these were contaminated with heterotrophic bacteria. The young blue-green cultures appeared to be free of contaminants when examined in the electron microscope, but older ones were clearly associated with heterotrophs. The eukaryotic algae were persistently associated with eubacteria.

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Transmission Electron Microscopy of Picoplankton

Low magnification electron micrographs of picoplankton concentrates are shown in Fig. 1. The range of morphotypes and sizes of organisms are characteristic of so called deep-water samples collected near the water–sediment interface of Lake Arthur. Measurements of cells on such electron micrographs showed that 76.9% of the cells were less than 0.5 μm in diameter. In contrast, the largest cells had an average size range of only 1.3–1.8 μm and represented about 14% of the total (Table 2).

Almost all cells had a typical Gram-negative cell wall structure, but because of other ultrastructural features could be arranged into several subcategories. Examination of cells in section showed numerous bacteria with internal membranes. The arrangements observed are shown in Figs. 2 and 3.

The cyanobacteria were distinguished not only by internal membranes, but also by the presence of polyhedral bodies (Figs. 4A and 5A). Two morphologically distinctive species are shown in Figs. 4A and 5A. Other bacterial morphotypes are shown in Fig. 5, both rod and coccoid forms, each with distinctive or characteristic features: multilayered envelope (Fig. 5B), intracellular inclusions (Fig. 5E), and a square-ended cell with subpolar inclusions perhaps of a lipid nature (Fig. 5C). Cells containing poly-B-hydroxyalkanoate (Fig. 5D) were relatively numerous.

Cell envelopes, including multilayered walls of the various Gram-negative cells contrast with the thick walls of the eukaryotic picoplankton (Fig. 6C and D), are distinctive and, together with cytoplasmic features, make them recognizable as groups. The most abundant of the smallest picoplankton cells have a multilayered envelope, the outer component of which is a much-looped, delicate barrier; and a polyphosphate body that occupies a large part of the cell protoplast. These are displayed prominently in Fig. 1.

Electron-dense, complex surface components (Fig. 7A) observed in a few cells were found by EDAX analysis to be rich in iron (Fig. 7B). Encapsulated cells were observed (Fig. 7C) in relatively small numbers, but many of these probably escape detection, having been removed by sieving and filtration. Cells not bound to the voluminous polymer might pass into the picoplankton fraction, and colonies of exopolymer-producing cells were routinely recognized as a part of the heterotrophic plate count of both filtered and raw water samples.

Iron-accumulating properties of cell surfaces seemed relatively rare, but 1–2% of the picoplankton were found to have magnetite crystals. These small coccoid rods measured 0.38–0.62 μm (Fig. 6A) and characteristically contained 5–6 magnetite granules/cell that were aligned across the cell diameter. The individual crystals measured 80–90 nm. EDAX analysis (Fig. 6B) of these bodies showed their iron content. Control spectra of envelopes and cytoplasm of such cells were negative for iron. The cytoplasm contained no polyphosphate bodies.

A dominant feature was the presence of polyphosphate bodies in slightly less than half of the cells in thin section regardless of morphotype or size. The polyphosphate bodies are particularly numerous in the cyanobacteria shown in Fig. 2 and Fig. 4. Four to six bodies were commonly present (Fig. 5A), in contrast to other cells that usually showed 1–2 bodies per cell, as in those with arrays of intracellular membranes (Figs. 1 and 2) and in the eukaryotic algae (Fig. 6C and D). The group of very small bacterial cells, part of the size group that is most numerous (Fig. 1A

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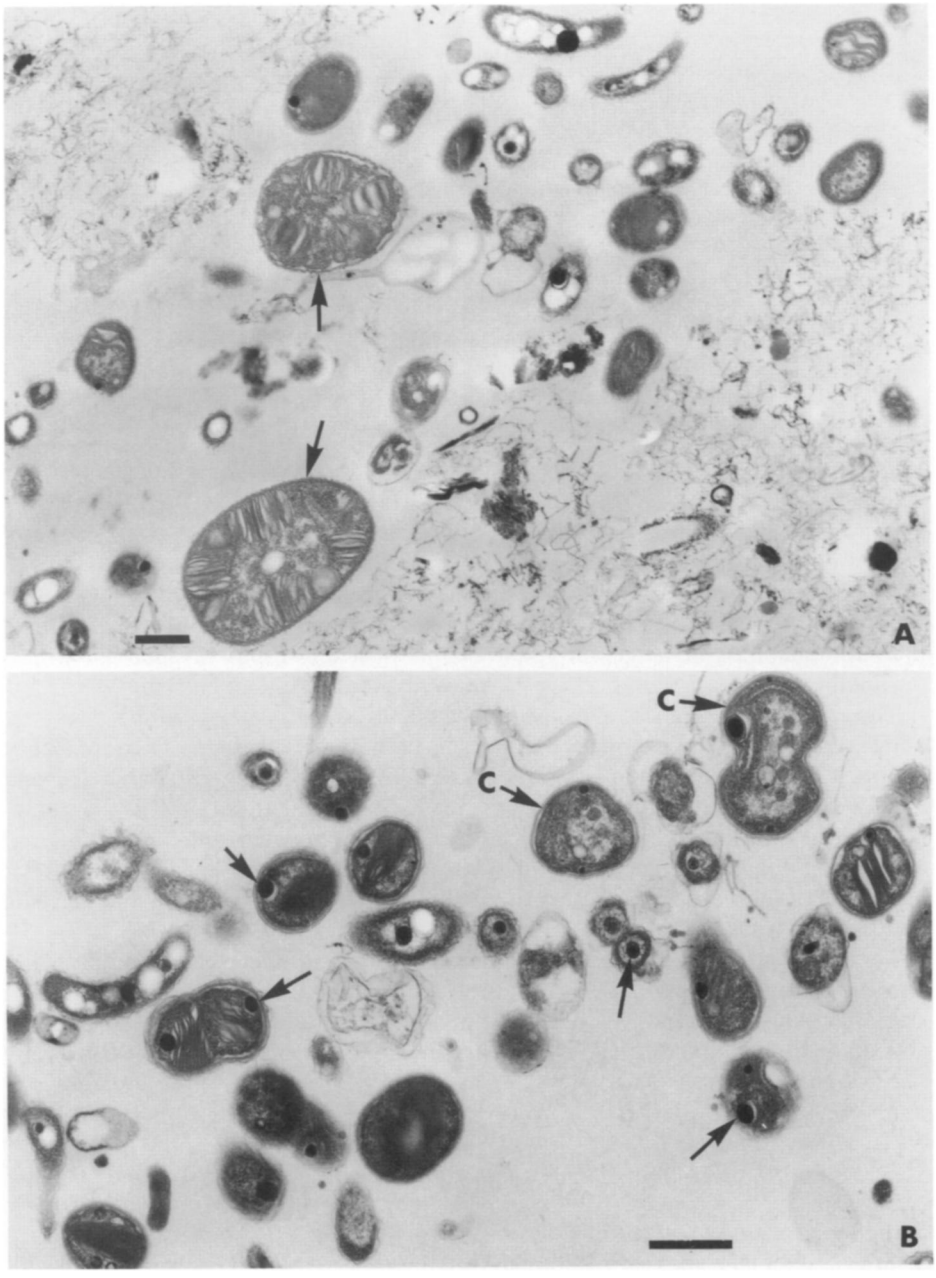

Fig. 1. Low-power electron micrographs of thin-sectioned picoplankton biomass (deep sample) showing the diversity of organisms of numerical importance. **A**: Two prominent intracytoplasmic membrane-bearing cells (arrows) are shown, as well as various types of other bacteria. The bar represents 0.5 μm. **B**: Variety of bacterial types are visible, including cyano-bacteria (**C**). The cells possess numerous polyphosphate bodies (arrows). The bar represents 0.5 μm.

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Table 2. Picoplankton in Arthur's Lake

Size category	Size range[a] (μm)	Average dimensions (μm)	Relative abundance (%)
Large	1.28–2.24 × 0.96–1.92	1.81 × 1.31	13.85
Intermediate	0.96–1.15 × 0.45–0.64	1.11 × 0.56	9.23
Small	0.44–0.77 × 0.32–0.64	0.57 × 0.48	76.92

[a]Cells sectioned medially and observed in micrographs were counted and measured.

and B), had only one polyphosphate body per cell, but the latter occupied a major part of the cell's internal volume.

Discussion

Oligotrophic, Lake Arthur

Preliminary study of the microbiology of Lake Arthur showed that the CFU/ml of heterotrophs and photoautotrophs yielded a small number when compared with the total epifluorescent count. Surface samples collected from Sutherland and Alec Meadowlakes were virtually identical. There were no blooms of algae and no dramatic qualitative changes in viable counts of heterotrophs or photoautotrophs during the whole sampling period. We may have missed the microbial bloom.

The fact that the total count exceeded the viable count by a large number was not altogether unexpected, as many bacteria in natural environments fail to produce visible colonies on common laboratory media. Witzel [52] suggested that as many as 99% of bacteria in natural habitats may not grow on agar plates. The phenomenon caused Johnson and Sieburth [25] to question the nature of such organisms and to develop the system of study that we have used in the present work. The phenomenon observed in soil also motivated Bae et al. [4] to use in situ electron microscopy to study soil microflora.

The lakes in the Black Rock Forest are environmentally protected and probably poor in organic nutrients. The lakes are looked upon as oligotrophic, following criteria outlined by Poindexter [38]. The organisms contained in the waters presumably have high affinity uptake systems enabling them to be maintained in a low nutrient environment.

Study of the Picoplankton Biomass with the Electron Microscope

Sampling and study of picoplankton biomass using the TEM generally confirmed that the assemblage showed no dramatic changes over a period of 9 months. About 75% of the picoplankton did not form colonies on common media (Table 1). The vast majority of the cells visualized in thin sections (Fig. 1) were intact, healthy-appearing cells; clearly not dead. Most importantly, one is able to recognize distinctive features in some that are reminiscent of organisms that have been seen in the literature and described ultrastructurally, and even physiologically and biochemically [14].

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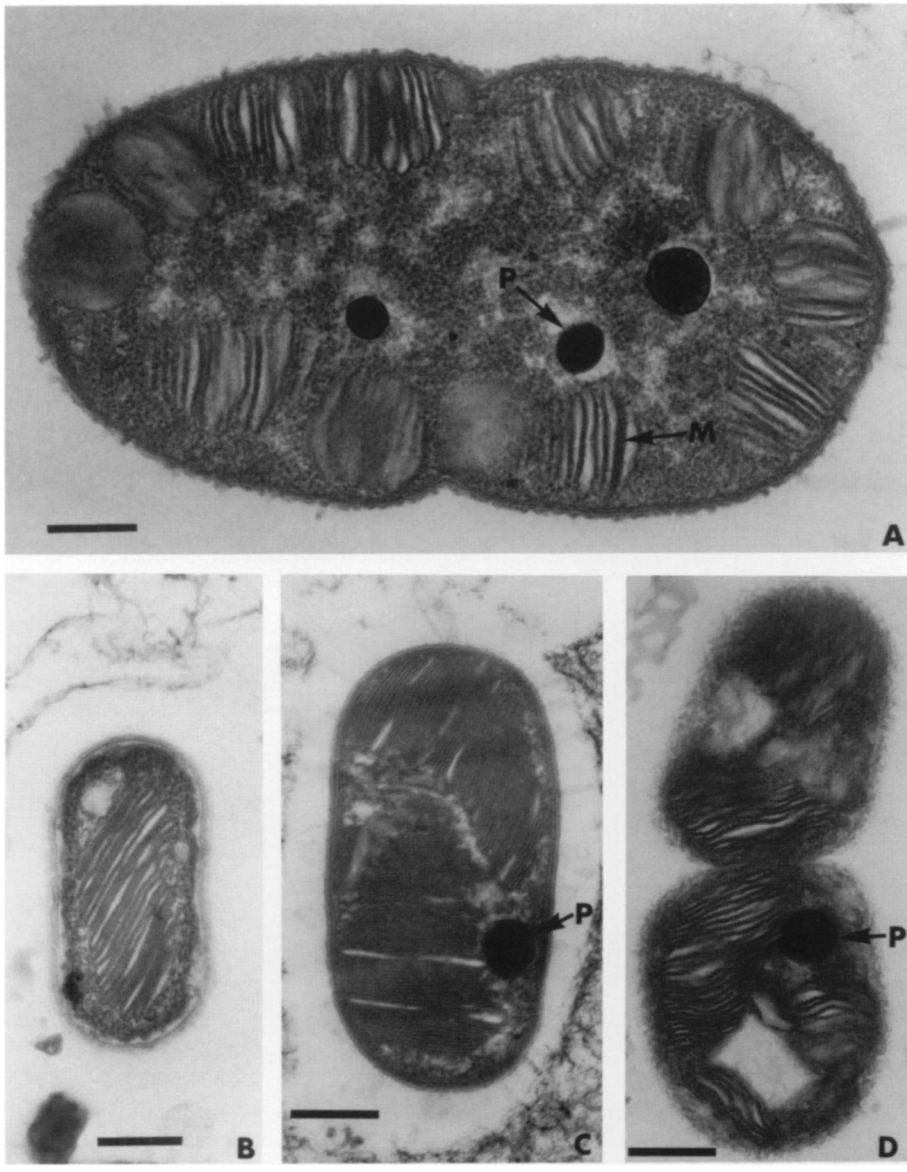

Fig. 2. Thin-sectioned picoplankton cells of a deep sample biomass displaying various intracytoplasmic membrane arrangements. **A**: Probable methanotroph. In addition to the prominent membrane system (M), several polyphosphate bodies can be seen (P). Bar represents 0.2 μm. **B**: Small bacterium with numerous membranes. Bar represents 0.2 μm. **C**: Bacterium with numerous membranes and a polyphosphate body (P). Bar represents 0.2 μm. **D**: Dividing bacterium with numerous membranes and a polyphosphate body (P). Bar represents 0.2 μm.

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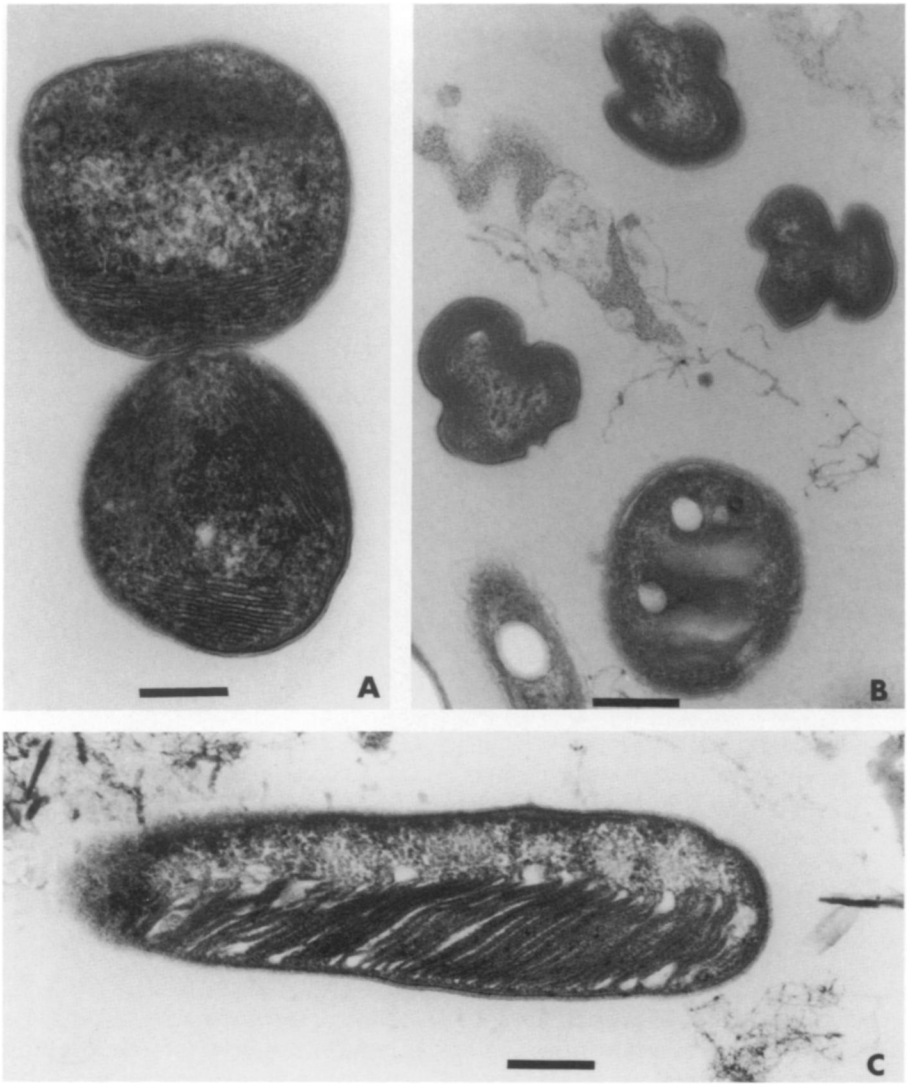

Fig. 3. Thin-sectioned picoplankton cells that display a range of morphotypes and intracytoplasmic membrane arrangements. **A**: Two bacterial cells showing membranes running parallel to the plasma membrane. The bar marker represents 0.2 μm. **B**: Three small bacterial cells with membranes running parallel to the plasma membrane. The bar marker is 0.2 μm. **C**: Bacterial cell showing membranes running at an angle to plasma membrane. The bar marker represents 0.2 μm.

Picoplankton with Intracytoplasmic Membranes

Bacteria-sized eukaryotic algae (Fig. 7D) are similar to those described by Johnson and Sieburth [25]. They may be members of the genus *Chlorella* and are apparently ubiquitous, having been observed in brackish water environments [2] and by Fahnenstiel et al. [17] in waters of Lake Superior.

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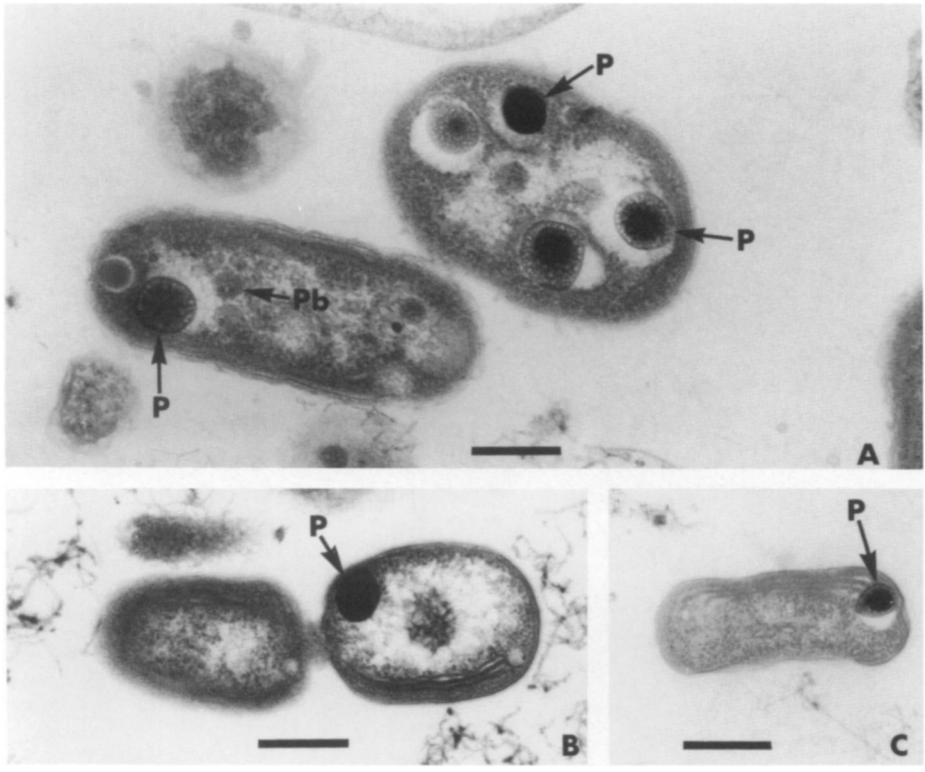

Fig. 4. Thin-sectioned picoplankton cells. **A**: Two cyanobacteria morphotypes, rod and coccoid rod each with multiple polyphosphate bodies (P) and polyhedral bodies (Pb). The bar marker represents 0.2 μm. **B**: Bacterial cells with intracytoplasmic membranes that are parallel to the cell envelope on one side of the cell. A polyphosphate body is also present in one of the cells (P). The bar marker represents 0.2 μm. **C**: Small bacterial cell with membranes running parallel to the plasma membrane. A polyphosphate body (P) is also present. The bar marker represents 0.2 μm.

Two chroococcoid cyanobacteria morphotypes were observed in water from Lake Arthur, isolated in axenic cultures and identified as *Synechococcus* species of two different morphotypes. The cultures, however, were not free of heterotrophic-bacteria. This organism has also been seen in other natural waters [24, 47, 51]. The prokaryotic and eukaryotic phototrophs may be one of the main sources of carbon that sustains what appears to be a balanced microbial consortium. They are certainly intimately associated with heterotrophs and may supply not only their cell bodies, but possibly soluble, reduced carbon compounds during active growth. Booth et al. [8] demonstrated by electron microscopy the direct association of bacteria with blue-green and diatom surfaces in a eutrophic freshwater lake. Paerl [35] also reported this phenomenon in several freshwater lakes.

About 16% of the picoplankton that have arrays of intracytoplasmic membranes are neither of the blue-green type nor do they contain vesicular, mesosome inclusions. Several of them (Figs. 2B, C, D, 3A, B, C, and 4B, C) are similar in some

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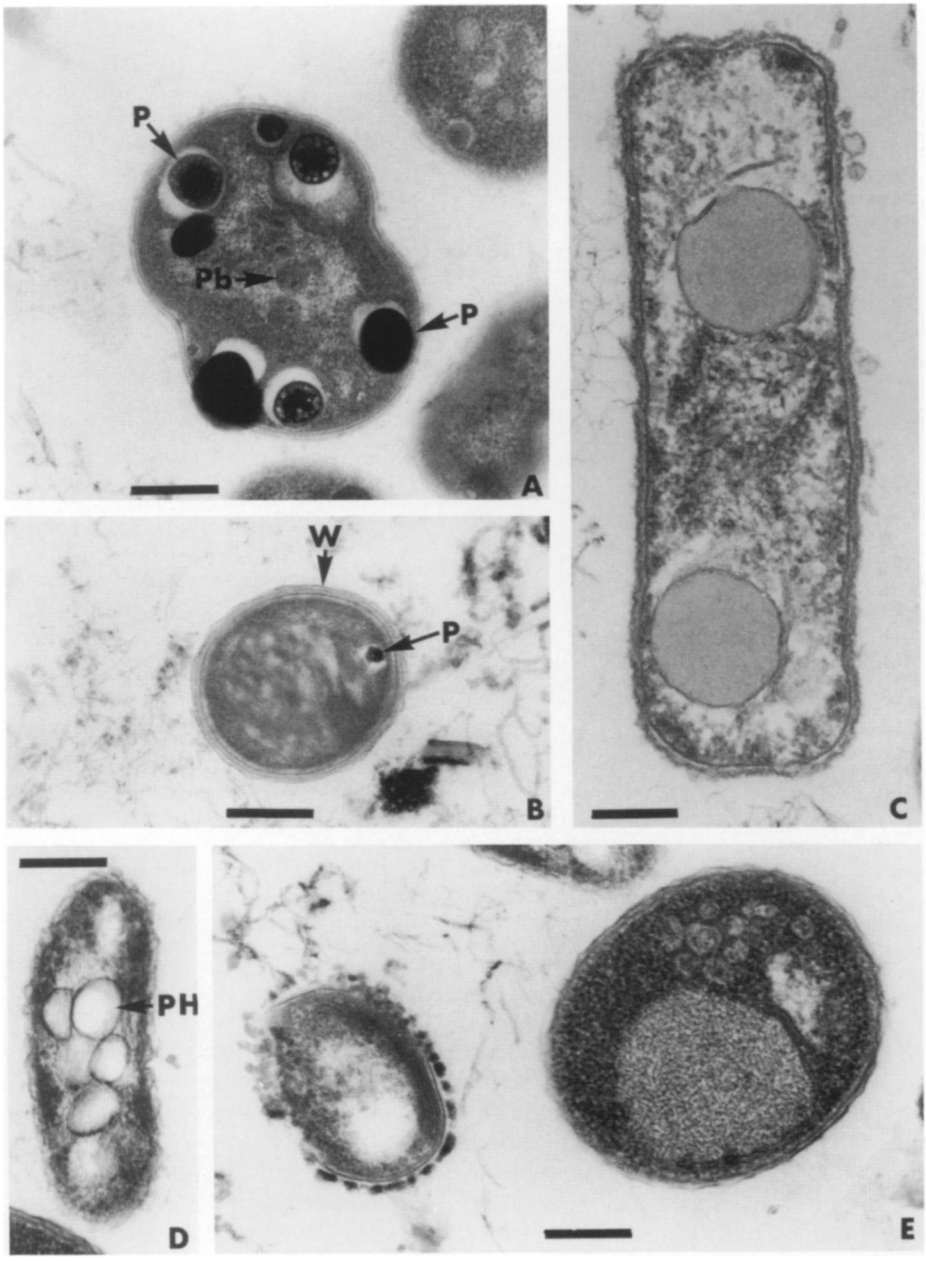

Fig. 5. Various picoplankton morphotypes. **A**: Blue green bacterium with many polyphosphate bodies (P) and polyhedral bodies (PH). The bar marker represents 0.2 μm. **B**: A coccus ovoid cell with a multilayered wall (W) and single polyphosphate body (PP). The bar marker represents 0.2 μm. **C**: A long rod-shaped bacterium with square ends and cytoplasmic granular storage bodies possibly lipid in character. The bar marker represents 0.2 μm. **D**: Rod shaped bacterial cell containing many granules of poly-B-hydroxyalkanoate (PH). The bar marker represents 0.2 μm. **E**: Two bacterial cells. The larger contains a granular area. The smaller cell has capsular material that resembles that of *Methylosinus trichosporium*. The bar marker represents 0.2 μm.

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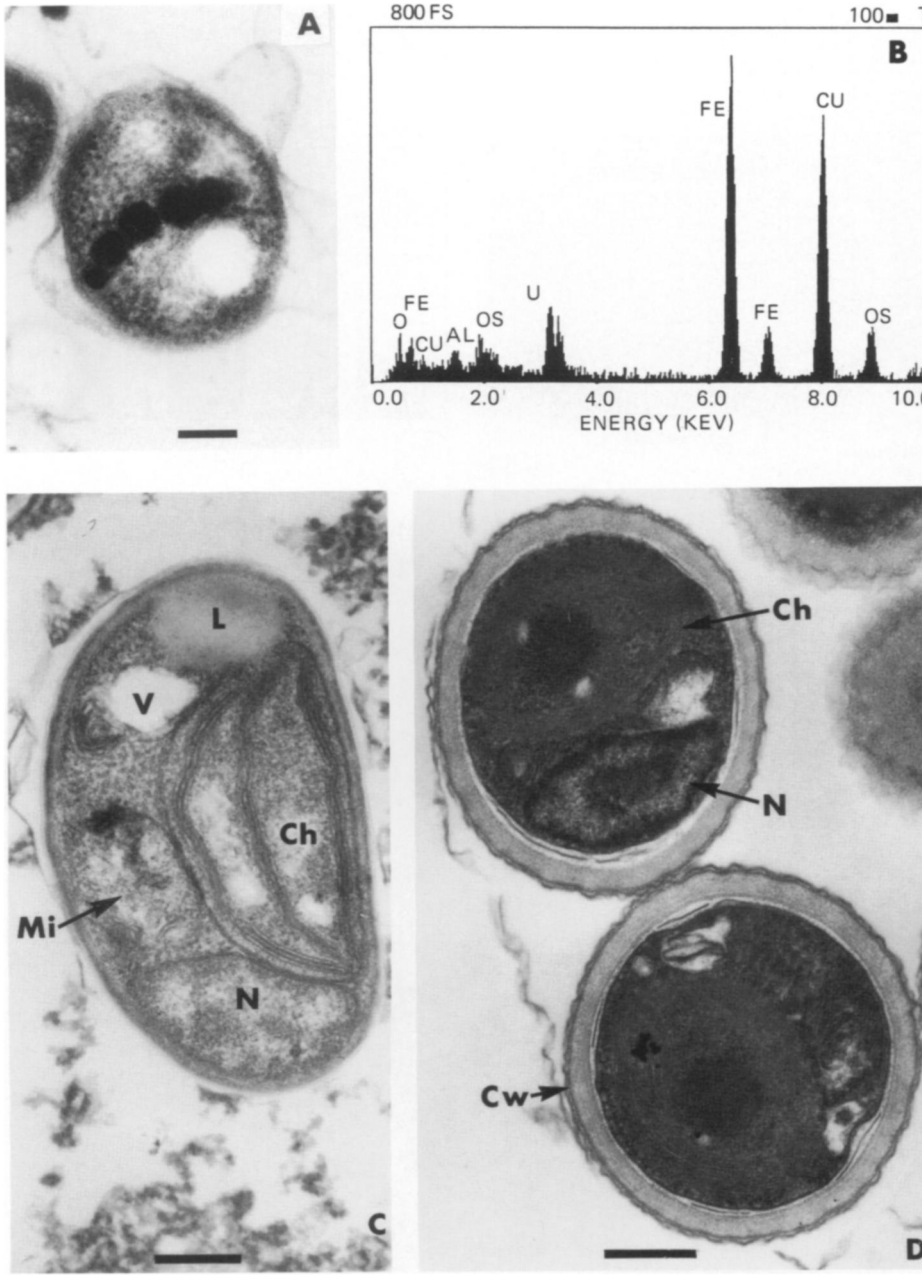

Fig. 6. Picoplankton morphotypes in thin section. **A**: Coccoid bacterium with chain of 5 dense granules across the cell diameter. The bar marker represents 0.1 μm. **B**: EDAX spectrum of dense granules depicted in (A). Prominent iron (Fe) peaks were detected. **C**: Eukaryotic phototrophic picoplankton cell. Cell has a nucleus (N), chloroplast (Ch), mitochondrion (Mi), vacuole (V) and a lipid deposit (L). The bar marker represents 0.2 μm. **D**: Eukaryotic phototrophic organism. The prominent cell wall (Cw) indicates a *Chlorella*-like organism. A nucleus (N) and a chloroplast (Ch) are visible in the cells. The bar marker represents 0.2 μm.

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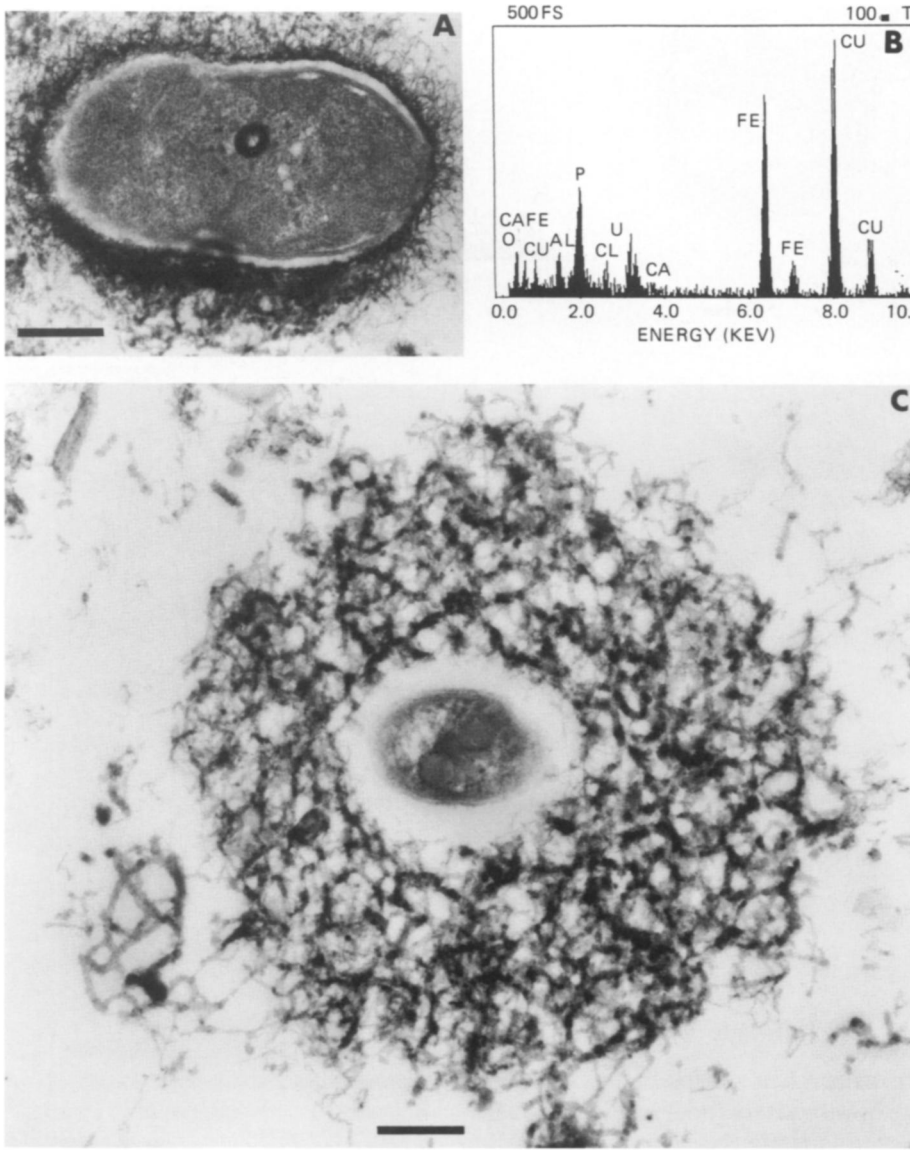

Fig. 7. Picoplanktonic bacterial cell with extracellular polymer deposits. **A**: Fibrous exopolymer-bearing rod-shaped bacterium. The bar marker represents 0.2 μm. **B**: EDAX spectrum of envelope showing substantial amounts of iron (Fe). Section was mounted on a copper grid and stained with uranyl acetate. **C**: A small coccoid shaped bacterium surrounded by a matrix of exopolymer. The bar marker represents 0.2 μm.

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respects to various methanotrophs and chemoautotrophs that have been described in the literature [6, 23].

Figure 2A shows a bacterium with intracellular membrane arrangements similar to the obligate methanotroph *Methylomonas* sp. described by DeBoer and Hazeau [16]. These organisms were most numerous in water samples taken near the water sediment interface. Presumably, methane generated in the sediments becomes available in the water column in large enough amounts to sustain a small population of methanotrophic bacteria [18]. Davies and Whittenbury [15] have found that ammonia- and methane-oxidizing bacteria have extensive intracytoplasmic membrane arrays and indicate that both types oxidize a broad spectrum of compounds, including ammonia and methane.

The arrangement of internal membranes of methane oxidizers can vary greatly depending on growth conditions [10, 22, 41, 42, cf. 23]. For these reasons, and perhaps others, none of the cells observed in the present work can be specifically identified by electron microscopic images alone.

Patt et al. [37] isolated a pink-pigmented facultative methylotroph (PPFM) that oxidized methane from Lake Mendota; the loss of a plasmid resulted in loss of the ability to use methane but not methanol or other carbon sources. One of the five colony types of heterotrophs isolated from Lake Arthur and Sutherland Lake was a PPFM and in isolation contained membranous inclusions identified with that group [12].

Magnetite Storage

The storage of iron as magnetite inclusions was observed only in a group of very small (0.38 μm × 0.62 μm) coccoid rods with a loose undulated outer membrane (Fig. 6A). No magnetotactic bacteria [7] of this size have been described, as far as we can determine. Our cells have not been observed in the living state, so we have no information concerning their motility or magnetotactic capacity. A magnetotactic coccoid organism isolated by Moench and Konetzka [32] measured 1.6 μm, and has been named *B. magnetotacticus* [33]. An important feature of the species was the accumulation of sulfur in a cytoplasmic inclusion, which was not observed in the Lake Arthur cells.

Iron was also detected in the surface of a rod-shaped bacterium (Figs. 7A and B). Whether this indicates that soluble iron is concentrated by interaction with a specific cell surface component or that the cell surface has aggregated minute, iron-bearing filamentous particles concentrated with the picoplankton biomass from lake water (Jensen TE, Corpe WA, 1991, Abstr Soc Ind Microbiol p 96) has not yet been determined. A study of exopolymer-bearing cells in thin sections (Fig. 5B) showed no evidence of iron concentration, so the cell represented in Fig. 7A is probably a unique type. Figure 5E shows a cell with a capsule not unlike that produced by vegetative cells of methane-oxidizing *Methylosinus trichosporium* [49].

Polyphosphate bodies in Picoplankton

The presence of polyphosphate bodies (PPB) was recognized in about half of the thin sections of picoplankton from Lake Arthur. The abundance of PPB reflects an

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ability of a large proportion of the cells to store Pi. Accumulations are reported to be correlated with exogenous nutrient limitations coupled with low endogenous rates of metabolism [27]. Nitrogen may be in short supply in Lake Arthur [1], resulting in unbalanced growth and phosphorous storage as PPBs. The dependence of the heterotrophic population on dissolved organic matter generated by the photoautotrophs and chemoautotrophs would probably still be inadequate for more than a modest development of that consortium.

The functions of polymetaphosphate are not clear, although the subject has been reviewed on numerous occasions [19, 27, 40]. All reviewers seem to place strong emphasis on PPB being an intracellular reserve, although most are quick to point out that evidence is developed with study of only a few species. Recent work in our laboratories [5; Jensen TE, Corpe WA, 1991, Abstr ASM Annu Meeting) has shown that the polyphosphate sequesters a variety of cations.

The Problem of Unusually Small Cells

The identity of very small cells that occur in the Lake Arthur picoplankton and their environmental importance has not been determined; but because they represent a large part of the total, their isolation and characterization should be anticipated at some stage. The observation of small (0.3 μm or less) bacteria in natural populations has been reported for oceanic waters [11, 25, 31]. The origin of the cells may be in part due to miniaturization of normal cells produced as a result of starvation [30, 34]. Studies of miniaturized cells were not accompanied by display of their ultrastructural features, with the exception of those reported by Johnson and Sieburth [25]. Results of their study of oceanic biomass were very similar to our biomass from lake water (Table 2, Fig. 1). Resuscitation of ultramicrobacterial cells of marine origin [30] as heterotrophs was accomplished using dilute nutrient broth and agar. We will attempt controlled experiments of the type described by MacDonnell and Hood [30].

The Value of the in situ TEM Study

In the present work, we have emphasized the value of TEM microscopy in resolving details of intracellular structure of cells in thin sections permitting presumptive recognition of some cell types (methanotrophs) and determination of their relative abundance. The scanning of sections and then use of the spot mode of the EDAX has confirmed the presence of iron in some cell types and established the presence of polyphosphate bodies in a large proportion of the cells.

This kind of information, when used with now commonly employed biochemical methods, will provide direction in resolution of the specific microorganisms concerned with primary productivity and heterotrophic activity. Other specific techniques may be used in conjunction with the TEM [13, 20] that may make the microscopic approach even more useful.

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References

1. Adirondack Lake Survey Reports (1987) Adirondack Lake Survey Corporation, Ray Brook, NY, 12977
2. Andreoli CN, Rascio F, Dalla Vecchia, Talarico L (1989) An ultrastructural research on natural populations of picoplankton from two brackish water environments in Italy. J Plankton Res 11:1067–1074
3. American Public Health Association (1989) Standard methods for the examination of water and waste water. 17th Ed., American Public Health Association, Washington, DC
4. Bae HC, Cota-Robles SH, Casida LE (1972) Microflora of soil as viewed by transmission electron microscopy. Appl Microbiol 23:637–648
5. Baxter M, Jensen TE (1980) Uptake of magnesium, strontium, barium and manganese by *Plectonema boryanum* (Cyanophyceae) with special reference to polyphosphate bodies. Protoplasma 104:81–89
6. Beveridge TJ (1989) The structure of bacteria. In: Poindexter JS, Leadbetter ER (eds) Bacteria in nature, vol. 3: Structure, physiology and genetic adaptability. Plenum Press, New York, pp 1–15
7. Blakemore RP, Blakemore NA, Bazylinski DA, Moench TT (1989) Magnetotactic bacteria In: Staley JT, Bryant MA, Pfennig W, Holt JG (eds) Bergey's manual of systematic bacteriology, Vol. 3, Section 20E. Williams and Wilkins, London, pp 1882–1889
8. Booth KN, Sigee DC, Belliger E (1987) Studies on the occurrence and elemental composition of bacteria in freshwater plankton. Scanning Microscopy 1:2033–2042
9. Caldwell DE (1977) The planktonic microflora of lakes. CRC Critical Reviews in Microbiology, pp. 305–370
10. Chetina EV, Suzina NW, Fikhte BA, Trotsenko YA (1981) Influence of culture conditions on organization of the membrane apparatus in *Methylomonas methanica*. Microbiology 51:257–254
11. Colwell RR, Brayton PR, Grimes DJ, Roszal DB, Huq SA, Palmer LM (1985) Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: Implications for the release of genetically engineered microorganisms. Biotechnology 3:817–820
12. Corpe WA, Jensen TE (1986) Fine structure of cytoplasmic inclusions of some methylotrophic bacteria from plant surfaces. Arch Microbiol 145:107–112
13. Corpe WA, Jensen TE (1991) Major antigens in *Methylobacterium* species and their location in cells using immunoelectron microscopic methods. Cytobios 67:117–126
14. Costerton JW (1979) The role of electron microscopy in the elucidation of bacterial structure and function. Ann Rev Microbiol 33:459–479
15. Davies SL, Whittenbury R (1970) Fine structure of methane and other hydrocarbon utilizing bacteria. J Gen Microbiol 61:227–232
16. DeBoer WE, Hazeau W (1972) Observations on the fine structure of a methane oxidizing bacterium. Ant Van Leeuwenhoek J Microbiol Serol 38:33–47
17. Fahnenstiel GL, Sicko-Goad L, Scavia D, Stoermer E (1986) Importance of picoplankton in Lake Superior. Can J Fish Aquat Sci 43:235–240
18. Hanson RS, Wattenberg EV (1991) Ecology of methylotrophic bacteria. In: Goldberg I, Rokem JS (eds) Biology of methyotrophs. Butterworth-Heineman Publishing, Boston, pp 325–348
19. Harold FM (1966) Inorganic polyphosphates in biology: Structure, metabolism and function. Bacteriol Rev 30:772–794
20. Herman EM (1988) Immunocytochemical localization of macromolecules with the electron microscope. Ann Rev Plant Physiol Plant Mol Biol 39:139–155
21. Hobbie JE, Daley RJ, Jaspar S (1977) Use of nuclepore filters for counting bacteria by epifluorescence microscopy. Appl Environ Microbiol 33:1225–1228
22. Hyder SL, Meyers A, Cayer ML (1979) Membrane modulation in a methylotrophic bacterium *Methylococcus capsulatus* (Texas) as a function of growth substrate. Tissue and Cell 11:597–610
23. Jensen TE, Corpe WA (1991a) Ultrastructure of methylotrophic microorganisms. In: Goldberg I, Rokem JS (eds) Biology of methylotrophs. Butterworth-Heinemann Publishing, Boston

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24. Johnson PW, Sieburth JMcN (1979) Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse photo biomass. Limnol Oceanogr 24:928–935
25. Johnson PW, Sieburth JMcN (1982) In situ morphology and occurrence of eukaryotic phototrophs of bacterial size in the picoplankton of estuarine and oceanic waters. J Phycol 18:318–327
26. Joint IR, Pipel RK (1984) An electron microscope study of a natural population of picoplankton from the Celtic Sea. Mar Ecol Prog Ser 20:1123–1118
27. Kulaev IS, Vagabod VM (1983) Polyphosphate metabolism in microorganisms. Adv Microbiol Physiol 24:83–171
28. Larsson KC, Weibull C, Gronberg G (1978) Comparison of light and electron microscopic determinations of the number of bacteria and algae in lake water. Appl Environ Microbiol 35:397–404
29. Luft JH (1961) Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol 9:409–414
30. MacDonnell M, Hood MA (1982) Isolation and characterization of ultramicrobacteria from a gulf coast estuary. Appl Environ Microbiol 43:566–571
31. Morita RY (1982) Starvation-survival of heterotrophs in the marine environment. Adv Microbiol Ecol 6:171–197
32. Moench TT, Konetzka WA (1978) A novel method for isolation and study of a magnetotactic bacterium. Arch Microbiol 119:203–212
33. Moench TT (1988) *Bilophococcus magnetotacticus*, new genus, a motile magnetic coccus. Antonie van Leeuwenhoek 54:483–496
34. Novitsky JA, Morita RY (1976) Morphological characterization of small cells resulting from nutrient starvation of a psychrophilic marine vibrio. Appl Environ Microbiol 32:617–622
35. Paerl HW (1980) Attachment of microorganisms to living and detrital surfaces in fresh water systems. In: Bitton C, Marshall KC (eds) Adsorption of microorganisms to surfaces. Wiley and Sons, New York, pp 375–402
36. Pankratz HS, Bowen CC (1963) Cytology of blue-green algae. I. The cells of *Symploca muscorum*. Am J Bot 50:387–389
37. Patt TE, Cole GC, Bland J, Hanson RS (1974) Isolation and characterization of bacteria that grow on methane and organic compounds as sole sources of carbon and energy. J Bacteriol 120:955–964
38. Poindexter JS (1981) Oligotrophy, fast and famine existence. Adv Microbiol Ecology 5:63–69
39. Pomeroy LR (1984) Significance of microorganisms in carbon and energy flow in marine systems. In: Klug MJ, Reddy CA (eds) Current perspectives in microbiol ecology. Amer Soc Microbiol, Washington DC, pp 405–411
40. Preiss J (1989) Chemistry and metabolism of intracellular reserves. In: Poindexter JS, Leadbetter ER (eds), Bacteria in nature, vol. 3: Structure, physiology and genetic adaptability. Plenum Press, New York, pp 189–258
41. Proctor HM, Norris JR, Ribbons DW (1969) Fine structure of methane utilizing bacteria. J Appl Bacteriol 32:118–121
42. Scott D, Brannan J, Higgins IJ (1981) The effect of growth conditions on intracytoplasmic membranes and methane monooxygenase activities in *Methylosinus trichosporium* OB3b. J Gen Microbiol 125:63–72
43. Sicko-Goad L, Stoermer EF (1984) The need for uniform terminology concerning phytoplankton cell size fractions and examples of picoplankton from the Laurentian Great Lakes. J Great Lakes Res 10:90–93
44. Sieburth JMcN, Johnson PW (1989) Picoplankton ultrastructure: A decade of preparation for the brown tide alga, *Aureococcus anophageferrens*. In: Cosper EM, Bricelj VM, Carpenter EJ (eds) Novel phytoplankton blooms: Causes and impacts of recurrent brown tides and other unusual blooms. Coastal and Estuarine Studies, Springer-Verlag, pp 1–21
45. Stempak JB, Ward RT (1964) An improved staining method for electron microscopy. J Cell Biol 22:697–701
46. Stockner JG (1988) Phototrophic picoplankton: An overview from marine and freshwater systems. Limnol and Oceanogr 33:765–775
47. Stockner JG, Antia NG (1986) Algal picoplankton from marine and freshwater ecosystems: A multidisciplinary perspective. Can J Fish Aquat Sci 43:2472–2503

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48. Takahashi M, Kikuchi K, Hara Y (1985) Importance of picocyanobacteria biomass (unicellular, blue green algae) in the phytoplankton population of the coastal waters off Japan. Mar Biol 89:63–69
49. Titus JA, Reed WM, Pfister RM, Dugan PR (1982) Exospore formation in *Methylosinus trichosporium*. J Bacteriol 149:354–360
50. Vatintsev KK, Meshceryakova AI, Popvskaya GI (1972) The importance of ultrananno planktonic algae in the primary production of Lake Baikal in the summer. Gidrobiol Zh 8:21–27
51. Waterbury JB, Watson SW, Guillard RRL, Brand LW (1979) Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature 277:293–294
52. Witzel KP (1990) Approaches to bacterial population dynamics. In: Overbeck J, Chrost RJ (eds) Aquatic microbial ecology, biochemical and molecular approaches. Springer Verlag, New York, pp 96–128

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