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Autotrophic Picoplankton: Their Presence and Significance In Marine and Freshwater Ecosystems[1]

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During the first half of the 20th century, scientists collecting plankton specimens would use nets having different sized apertures to selectively obtain organisms within various plankton categories. As these net apertures were reduced in size, it was realized that there were numerous microscopic cells capable of passing through the smallest openings of these nets (Lohmann, 1911). The presence of these very small cells was later reported at numerous freshwater sites (Rodhe, 1955, Bailey-Watts et al., 1968; Pennak, 1968; Votintsev et al., 1972; Pearl, 1977) and marine locations (Van Baalin, 1962; Saijo, 1964; Saijo and Takesue, 1965; Reynolds, 1973; Banse, 1974; Berman, 1975; etc.). In this early literature, various terms were used to describe these cells (e.g. ultraplankton, olive green cells, μ-algae, nanoplankton, etc.), but it wasn't until Sieburth et al. (1978) established a plankton reference classification system based on size, that the term picoplankton began to be used collectively for these microscopic cells. The standard definition of picoplankton refers to cells within the size range of 0.2 to 2.0 microns. This term has since been generally accepted as the category to assign plankton cells that occur singly or within colonies that are within this size range. However, one of the initial concerns in algal studies was the inability to distinguish many of the bacteria, cyanobacteria, and eukaryotes in this category with similar characteristics, and to specifically identify the heterotrophs from autotrophs when limited to standard light microscopy protocols.

A major contribution regarding the identification of picoplankton components came from Johnson and Sieburth (1979) and Waterbury et al. (1979). Using epifluorescence microscopy, they identified abundant and widely distributed phycoerythrin containing cyanobacteria as the common component of the picoplankton community in the world oceans. These were chroococcalean taxa which were identified as *Synechococcus* and occurring at concentrations up to 10^4 cells/mL . They recognized the high abundance and broad distribution of these cells in marine waters and suggested their likely importance as a food source for micozooplankton. This significance was also indicated by productivity studies in the tropical north Atlantic equatorial current by Gieskes et al. (1979). They reported 20-30% of the productivity and 43-53% of the chlorophyll *a* measured was passing through a 1.0μ filter, which indicated a major portion of the total productivity from this region was derived from cells <1.0μ in size. Other investigations followed that supported the wide spread presence of picoplankton in freshwater lakes (Chang, 1980; Cronberg and Weibull, 1981; Craig, 1984). Similar picoplankton studies using epifluorescence procedures with fluorochrome dyes were able to further distinguish the heterotrophic bacteria from the prokaryotes and eu-

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karyotes within water samples (Porter and Feig, 1980; Davis and Sieburth, 1982; Caron, 1983), with the term autotrophic picoplankton, or picophytoplankton commonly used for the autotrophic cells (Fogg, 1986). Review articles on marine and freshwater picoplankton include those by Fogg (1986), Joint (1986), Stockner and Anita (1986), Stockner (1988; 1991), and Stockner et al. (2000).

Although the term picoplankton is applied to cells 0.2 to 2.0 μ in size, there is the general acceptance by investigators to include cells having a larger size range, but still capable of passing through a 2.0 μ pore filter. A broader size range of cells, for instance <3μ in size, was generally considered a more natural upper size limit to characterize these cells, many of which were referred to as unidentified cells (e.g. cells <3μ) when using standard Utermöhl protocols (Saijo, 1964; Reynolds, 1973, Munawar and Fahnenstiel,1982; Marshall, 1982; 1983). The majority of these cells are considered somewhat pliable, and may be ellipsoid, spherical, or rod shaped, with cell diameters <2μ , but with their length often longer, and yet still be capable of passing through a 2.0μ filter. In their measurements of 14 chroococcales picoplankton isolates, Smarda and Smajs (1999) noted variability in their dimensions and recorded the average cell length was from 0.8 to 2.9 , but having widths between 0.6 and 2.0 μ. However, as more taxa within this category were discovered, it became evident that there were large concentrations of cells at the lower ranges of this size category. Joint (1986) would favor the picoplankton definition to be expanded to identify more specifically cell groups within this size range. For instance to recognize those cells that can pass through a 1.0 μ pore size filter from larger cells, so the significance of these much smaller cells can be established. A further breakdown of this size fraction would provide more specific information regarding their contribution to productivity and prey relationships. Viewpoints of other investigators consider in addition to size, that there are other criteria that may be applied to taxa described as picoplankton. For instance, there are numerous and common freshwater colonial cyanobacteria with cells <2.0μ in size (e.g. *Aphanothece, Aphanocapsa, Merismopedia*, etc.). Komarek (1996) also indicates the term should be limited to those unicellular taxa that appear singly in the water column, rather than as colonial clusters. This is in consideration of the different ecological relationships and descriptive taxonomy that may be associated with these different colonial species. In addition, the gelatin matrix within which these cells are found may interfere with their passage through the smaller apertures of the filters used, producing an incomplete representation of this category. Komarek (1996) recommends species identification should go beyond morphology and genetic makeup, and include specific ecophysiological relationships unique to the taxon. Many investigators will combine standard Utermöhl procedures in determining the composition and abundance of the colonial picoplankkton, and the epifluorescence protocols for recording the single cell taxa among the picoplankton (Marshall and Nesius,1993; 1996; Marshall and Burchardt, 1998; among others).

COMPOSITION

Following the publications of Johnson and Sieburth (1979) and Waterbury et al. (1979), it was common practice in freshwater and marine studies to identify the picocyanobacteria as *Synechoccocus*. However, many of these cells exhibited different morphological features and were collected from extremely diverse habitats. Questions regarding the possible presence of other taxa within this assemblage were

aided during the past two decades by the application of autofluorescence, electron microscopy, and genetic analysis. These approaches have been essential in the identification of various strains and species within the *Synechococcus* complex, in addition to other picoplankton species (Rippka and Cohen-Bazere, 1983; Waterbury and Rippka, 1989; Leppard et al., 1989; Corpe and Jensen, 1992; Hall, 1991; Olson et al., 1993; Komarek, 1994, 1996, 1999; Komarek and Cepak,. 1998; etc.). In general, the most common and abundant taxa within the autotrophic picoplankton category are cyanobacteria (e.g. in the genera *Synechococcus, Cyanobium, Cyanothece*). If the colonial cyanobacteria are included in the category, species from the genera *Aphanocapsa, Aphanothece, Chroococcus, Merismopedia*, etc., would be added. In addition, among these taxa are differences in the type and amount of pigments they contain. For instance, phycoerythrin-rich prokaryotes are in high concentrations within oceanic regions (Mousseau et al. (1996), whereas, phycocyanin-rich taxa are more common in estuaries (Ray et al., 1989; Lacouture et al., 1990), and when passing through salt water-freshwater transition zones (Bertrand and Vincent, 1994). In these areas, the ratio between phycoerythrin and phycocyanin rapidly decreases.

Another major phylogenetic category is represented by the eukaryotes. These include the chlorophytes (e.g. *Chlorella* spp., *Micromonas pusilla*) and other major phytogenetic groups (e.g. chrysophytes, haptophytes, prasinohytes, etc.) that collectively contribute to the picoplankton biomass and productivity (Thomsen, 1986; Stockner, 1988; Hargraves et al., 1989). Also reported from the world oceans are the prochlorophytes (Chisholm et al., 1988), with *Prochlorococcus marinus* a common species (Chisholm et al., 1992). These are among the smallest photosynthetic organisms known. This is a unique prokaryotic group with species that contain divinyl derivatives of chlorophylls *a* and *b*, *a*-carotene and zeaxanthin, with a thylakoid feature of having them closely arranged in the cell (similar to the original Type II cell described by Johnson and Sieburth, 1979, Alberte et al., 1984; and others). These cells are 0.6-0.8 X 1.2-1.6 μ in size and have been recorded in coastal and oceanic waters (Chisholm et al., 1988, 1992; Shimada et al., 1995), and appear to be ubiquitous within the photic zone of tropical and sub-tropical oceans. Jochem (1995) also reports prochlorophytes as common in the lower extent of the euphotic zone and as dominant flora below the chlorophyll maximum regions, with *Synechococcus* abundant from sub-surface waters to the bottom of the euphotic zone.

Since the picoplankton cells lack many of the morphological features necessary for distinguishing between species, a more exact genetically-based approach is to use polymerase chain reactions (PCR) (Krienitz et al., 1999; among others). This method provides for the amplification and identification of specific gene sequences that are used to identify these species and to resolve questions regarding whether taxa with similar phenotypic or morphological characteristics represent one or more species, or strains of a species. Through this type of analysis additional strains of common prokaryote species, plus an array of new eukaryote species, including haptophytes, chlorophytes, prasinophytes, and others have been identified (Moon-van der Staay et al., 2000, 2001; Lopez-Garcia et al., 2001). Examples from fresh water habitats include: *Nannochloropsis limnetica* (eustigmatophyte) and *Pseudodictyosphaerium jurisii* (chlorophyte) by Krienitz et al. (1999, 2000); and from marine waters *Chrysoichromulina fragaria* (prymnesiophyte) by Eikrem and Edvardsen (1999), plus a new class and species designation of *Bolidomonas* spp. (Polidophyceae) by Guillou

et al. (1999), and *Pelagomonas calceolata* (Pelagophyceae) by Anderson et al. (1993). A listing of many common picocyanobacteria is given by Stockner et al. (2000).

ABUNDANCE WITHIN COASTAL AND DEEP OCEAN REGIONS

Numerous marine studies have indicated a positive correlation of picoplankton abundance to temperature. These include those by Murphy and Haugen (1985), El Hag and Fogg (1986), Waterbury et al. (1986), Jochem (1988), Iriarte and Purdie (1994), etc., plus in estuary studies by Malone et al. (1991), Marshall and Nesius (1993, 1996), Marshall (1995), and Davis et al. (1997). In California coastal areas, Krempin and Sullivan (1981) found the picoplankton abundance lowest in winter, then gradually rising through spring to peak in fall (10^7 cells/mL). Davis et al. (1985) found similar findings in Narragansett Bay with highest concentrations from spring through fall. In the north Atlantic, picoplankton concentrations decrease moving from the coastal waters to less nutrient and more stable regions seaward at the surface and within the euphotic zone (Platt et al., 1983; Murphy and Haugen, 1985; Glover et al., 1985b). In a transect between the Gulf of Maine and the Sargasso Sea, Glover et al. (1985a) found cyanobacteria the most abundant picoplankter in the eutrophic region over the continental shelf (88-98%). Concentrations of the (phycoerythrin-rich) cells ranged from 1.3×10^3 to 1.5×10^5 cell/mL, with the eukaryotes ranging from 0.3×10^3 to 4.4×10^4 cell/mL. The cyanobacteria abundance in comparison to other categories within the picoplankton, was greatest in the least productive regions (representing 91% of the autotrophic picoplankton), and lowest (65.7%) in the well mixed and more productive sites where they co-existed with other abundant taxa (eukaryotes). However, seaward into the open ocean the eukaryotes became more dominant in the lower regions of the photic zone. At one station, with light transmission levels of 4%, 1%, and 0.5%, the eukaryotes represented 73%, 78%, and 70% of the picoplankton respectively. The autotrophic picoplankton represented 70-97% of the phytoplankton chlorophyll and 73-78% of the autotrophic picoplankton at the deep chlorophyll maximum, showing an increased ratio between eukaryotes and picocyanobacteria with depth.

A similar pattern was reported by Murphy and Haugen (1985) with the picoplankton more abundant at coastal sites in the North Atlantic (10^7 to 10^8 cells/mL), with decreasing abundance seaward (10^6 to 10^7 cells/mL). In the surface waters, the cyanobacteria were more abundant than the eukaryotes, but the eukaryotes were often in greater concentrations with increased depth, being dominant at the lower ranges of the photic zone (150-200m). These eukaryotes and the prokaryotes were in high concentrations at and below the compensation depth. Often high concentrations of eukaryotes (up to 50% of the picoplankton population) have been reported within the oceans (e.g. Hall and Vincent, 1990). Also, Zubkov et al. (2000) recorded *Prochlorococcus* at concentrations of 10^6 cells/mL dominating the other two categories in the oligotrophic gyre regions of the Atlantic Ocean, but within the more productive waters of the equatorial region, both the eukaryotes and pico-cyanobacteria were more abundant. Summer prokaryote concentrations in the Baltic were reported by Sondergaard et al. (1991) to be at 10^6 to 10^8 cell/mL. Within a polynesian atoll, Charpy and Blanchot (1998) recorded the picoplankton showed a diurnal variation in their size, being smaller before sunrise, and becoming larger by the afternoon hours. Within these waters the average size for the pico-eukaryotes, *Prochlorococcus*, and *Synechococcus*

were 3.11, 0.89, and 0.62 μ respectively. Dimensions in the literature vary for these categories. However, common ranges reported for these groups have included up to 3.26 μ for the pico-eukaryotes, 0.55 to 1.0 μ for *Prochlorococcus,* and 0.6 to 2.2 μ for *Synechococcus* spp.

Although picoplankton cell abundance typically decreases with depth at near shore and ocean sites, a sub-surface zone of increased abundance is commonly found. Krempin and Sullivan (1981) recorded this at 30 m off the California coast, and high cell concentrations were also associated with the chlorophyll maximum layer in the north Pacific at 60 m depth (Takahashi and Hori, 1984). At this depth, more than 70% of the chlorophyll was represented by autotrophic picoplankton (<3 μ), with the two dominant taxa being a *Chlorella*-like eukaryote species (1.2 - 1.5μ) and a prokaryote. (0.5 - 2.0 μ). Other picoplankters included prasinophyte and haptophyte taxa, and non-thecate dinoflagellates. Within the Kiel Bight region, Jochem (1988) reported peak cyanobacteria picoplankton abundance in summer and increasing more in the eutrophic areas (10^8 cells/mL), than in less eutrophic regions. During summer months, 8-52% of the total phytoplankton carbon, and up to 97% of the autotrophic picoplankton carbon came from the picocyanobacteria. High cell concentrations occurred during the late summer or early fall (e.g. eukaryotes abundance at 10^6 cells/mL, Hargraves et al., 1989). Deeper presence of picoplankton was reported by Li and Wood (1988) in the central north Atlantic to depths of 220 m, in addition to high concentrations (>90%) of eukaryotes. They also report a a sub-surface maximum at 70 m, then a decrease in cell abundance with increased depth. The contribution of the autotrophic picoplankton to total carbon production in the oligotrophic and less disturbed regions of the ocean is estimated as 50-90 % , in contrast to the meso-eutrophic coastal regions where it much lower, 2-25% (Stockner and Antia, 1986). Due to the lesser abundance of the larger phytoplankters in these open oceanic areas of low nutrient input, and where there occurs periods of extended stratifications and reduced nutrient entry, the percent contribution of picoplankton as a primary producer becomes greater.

As mentioned previously, there is a difference in the ratio of phycocyanin and phycoerythrin pigments in picocyanobacteria cells found in the estuaries and pelagic regions. Phycoerythrin-enriched cells are more dominant in the oceanic and coastal regions (Waterbury et al., 1979; Johnson and Sieburth, 1979; Takahashi and Hori, 1984; Murphy and Haugen, 1985), with the phycocyanin-enriched cells more dominant in the less saline regions of tidal rivers and bays (Ray et al., 1989; Lacouture et al., 1990; Bertrand and Vincent, 1994). In the York River estuary (Virginia), Ray et al. (1989) determined the autotrophic picoplankton (0.2-3.0μ) represented 7% of the phytoplankton biomass and 9% of the primary production. The picoplankton was dominated by phycocyanin-enriched cyanobacteria, which were 8 times greater in abundance than the phycoerythrin-enriched cyanobacteria cells. The combined concentrations of these cells were 10^5 cells/mL and they represented 51% of the picoplankton biomass. The remaining biomass was by flagellates, diatoms, etc. This study also recognized increased abundance of the picoplankton coincided with the spring neap tide, a period where the water column is more stable and stratified. In the Patuxent River, a Chesapeake Bay tributary, the cyanobacteria were the major component of the autotrophic picoplankton, having a seasonal abundance maximum after the spring diatom bloom (Lacouture et al., 1990). They accounted for up to 50% of the summer

phytoplankton productivity during this period. Affronti and Marshall (1993) conducted diel studies during August and January at the Chesapeake Bay entrance. The phycocyanin enriched *Synechococcus* was the dominant prokaryote in August and the highest concentrations occurred during ebb tide. The average August pico-abundance was 8.84 X 10^5 cells/mL and 1.43 X 10^5 cells/mL, above and below the pycnocline respectively. There were greater concentrations of the phycocyanin enriched cyanobacteria above the pycnocline, in contrast to higher concentrations of the phycoerythrin enriched cells below the pycnocline in the deeper more saline waters entering the Bay. In January, the mean picoplankton abundance was 3.65 X 10^4 cells/mL and 4.66 10^4 cells/mL, above and below the pycnocline respectively. At this time the phycoerythrin enriched *Synechococcus* cells were dominant throughout the water column. In a 41 month study of picoplankton concentrations at 7 stations in Chesapeake Bay, Marshall (1995) reported their cell abundance was closely associated to temperature, forming a single annual maximum that peaked in July or August. The monthly means ranged from a 9.6 X 10^3 (February) to 907.0 X 10^3 (August) cell/mL. The sub-pycnocline concentrations were less than those above the pycnocline between May and November, but were slightly higher from December through May. In several tidal tributaries to the Chesapeake Bay, the autotrophic picoplankton produced typical summer maxima and winter population lows where their monthly concentrations ranged from 3-5 X 10^3 to 10^5 cells/mL (Lacouture et al., 1990; Davis et al., 1997; Marshall and Burchardt, 1998). Campbell et al. (1983) in comparisons between *Synechococcus* and *Synechocystis* presence, found phycocyanin rich *Synechococcus* cells a minor component in Great Bay, N.Y. during spring and summer, being dominated by phycoerythrin rich *Synechococcus* cells from summer through late autumn.

Thus, picoplankton composition decreases in abundance, moving from off shore and continental shelf regions seaward (Murphy and Haugen, 1985; Stockner and Anita, 1986). Although they are in lesser abundance in these open oceanic regions in comparison to their concentrations in coastal waters, they contribute a greater percentage of the total algal productivity in comparison to those algal cells larger than 2-3 μ, and represent a significant contributor to the total productivity in these less nutrient enriched regions (Stockner and Anita, 1986). Due to their ability to better utilize low intensities of light and existing nutrients in comparison to larger size phytoplankters, they are abundant throughout the photic zone, with regions of high concentrations at the surface, and in a sub-surface maximum, plus being abundant at the lower range of the euphotic zone. The picocyanobacteria generally are found in greater concentrations than the eukaryote species, especially in oligotrophic regions. Seasonal periods associated with peak abundance of picoplankton in temperate waters occur during summer and/or early fall, whereas, in tropical waters the concentrations are more constant. The typical concentrations in oceanic waters are approximately 10^3, 10^4, and 10^5 cells/mL for eukaryotes, cyanobacteria, and prochlorophytes respectively (Fogg, 1986; Stockner, 1988; Caron et al., 1985), and represent a common range of 2-25% and 50-80% of the total oceanic primary production in eutrophic coastal waters and oligotrophic regions respectively.

ABUNDANCE WITHIN FRESHWATER LAKES

The autotrophic picoplankton have long been recognized as an abundant and ubiquitous component of freshwater lakes (Hawley and Witton, 1991; among others). Stockner (1991) indicates that concentrations of autotrophic picoplankton in several oligotrophic lakes in Canada becomes greater as the lake pH, conductivity, and productivity increases. However, as these lakes become eutrophic, there is also the accompanying increase in the biomass and productivity of the larger (>3μ) phytoplankton populations, so the percent contribution of the picoplankton to the total phytoplankton biomass and total photosynthesis decreases (Vörös et al., 1991; Burns and Stockner, 1991; Petersen, 1991). These larger phytoplankton taxa will proportionally represent the greater biomass and more primary producers than the picoplankton as the lake's eutrophic state increases. In contrast, the autotrophic picoplankton populations in oligotrophic lakes would be less abundant than in the eutrophic waters, but they would contribute a greater proportion to the lake's productivity and phytoplankton biomass than the less abundant and larger algal taxa. Picoplankton species within each of these lake types will typically be represented by both prokaryote and eukaryote taxa, but not the prochlorophytes. Coccoid cyanoprokaryote are typically ubiquitous, dominant in abundance, and may be represented by one or several taxa, or strains of a species (e.g. the *Synechococcus* complex). Depending on the investigators definition of picoplankton, colonial forms may also be included if the cells fall within the accepted size range. Peak development of these colonial taxa would be during the summer/early fall period, being more characteristic of eutrophic waters. Eukaryotes, are also ubiquitous, favoring cooler waters, and are more common in eutrophic and dystrophic lakes, with increased nutrients, and the pH <6.2 (Stockner, 1991).

Weisse (1988), in a vertical assay of picoplankton in Lake Constance (a meso-eutrophic lake), reported concentrations up to 10^5 cells/mL, with the highest concentrations at 12-16 m depth, and then decreased with increased depth to 10^4 cells/mL at 140m.. Within this lake the cells were grazed actively by ciliates and heterotrophic nanoflagellates. Picoplankton was also reported in Lake Baikal by Boraas et al. (1991). Highest concentrations were at 5-10 m and decreased to 250m, with their abundance 9.8×10^4 and 4.2×10^3 cell/mL respectively, with mean water column values at 2.7×10^4 cells/mL. The abundance of the prokaryotes in lakes has been correlated directly to temperature, with peaks occurring during summer and early fall (Caron et al., 1985). Many of the eukaryotes, and some of the prokaryotes, will also have an earlier bloom during mid- to late spring, followed by another bloom in late summer after stratification (Szelag-Wasilewska, 1997, 1999). These eukaryotes are typically 1.2-2.0μ in size, and usually larger than most of the prokaryotes. Pick and Agbeti (1991) reported autotrophic cyanoprokaryote abundance peaks in oligo-mesotropohic lakes in late summer at 10^5 cell/mL, and representing 1-9% of the phytoplankton biomass. The eukaryotes peak concentrations were less abundant (10^3 cell/mL). The eukaryotes accounted for about half of the phytosynthetic picoplankton biomass in the "colored" lakes, and less than 20% in the clear water lakes. The major eukaryotes were *Chlorella* and *Nannochloris* spp. Exceptions to single summer periods for the cyanoprokaryote blooms have also been reported by Weisse and Kenter (1991) in Lake Constance. Over four consecutive years they noted spring and late summer blooms that were dominated by cyanobacteria. The range of abundance was 10^2 to 10^6 cells/mL, with the horizontal

differences across the lake as high as a factor of 3 in abundance and biomass during summer, and with more seasonal changes occurring in the upper eight meters of depth. They found differences in cell size seasonally, being larger in summer and fall, and with increasing depth (also Caron et al., 1985). Although the initiation and duration of these seasonal bloom periods may vary year to year among the various lakes, a typical pattern of abundance peaks during spring and late summer/early fall is common, as described by Szelag-Wasielewska (1998, 1999) in several Polish lakes. Decreasing abundance typically follows from late fall into winter and early spring.

Fahnenstiel and Carrick (1992) reported in Lakes Huron and Michigan that the autotrophic picoplankton was composed of 59% cyanobacteria and 21% eukaryotes with surface concentrations at 10^3 cells/mL. This represented 10% of the autotrophic plankton biomass with 17% of the primary production coming from the <1.0μ fraction, and 40% from the <3μ fraction. Picoplankton concentrations in Lake Ontario ranged from 10^3-10^5 cells/mL (Pick and Caron, 1987). In a small shallow and oligo-mesotrophic lake in Poland, Szelag-Wasielewska (1999) reported the autotrophic picoplankton was dominated by cyanobacteria which had concentrations of 10^5-10^6 cells/mL (these counts included colonial cyanobacteria). The dominant eukaryotes were species of *Chlorella* and *Choricystis*. Grazers included mixotrophic flagellates and ciliates. Wehr (1990) noted the development of pico- and nanoplankton were favored during summer periods of phosphorus limitation in a small eutrophic lake. Wehr (1991)also reports that most autotrophic picoplankton had greater biomass and abundance in phosphorus limiting systems, but were more influenced by nitrogen limitation, and seldom with phosphorus. However, Stockner and Shortreed (1989) noted in fertilized treated lakes, that the picoplankton abundance increased with added phosphorus. In Lake Tahoe, considered to be a phosphorus limited oligotrophic lake, the picoplankton represented 34-72% of the total productivity (Chang and Petersen, 1995). In another approach, evaluating the percent abundance of picoplankton to total algal biomass in 12 lakes of different trophic status, Szelag-Wasielewska (1997) found a negative relationship, with the range of abundance in these lakes from 3.2×10^3 to 1.16×10^6 cells/mL. In reference to what may happen to many of the picoplankton cells that settle out of the water column, Eguchi et al. (1996) found viable phototrophic picoplankton cells in the surface sediment of Lake Biwa (Japan). They indicated these may represent a seed population source that when re-suspended would produce further development of this population in the water column.

PRODUCTIVITY

A major significance of the oceanic picoplankton community is their contribution to primary production (Gieskes et al., 1979). In studies within temperate coastal waters, this percentage has been commonly 20-30% (Larsson and Hagström, 1982; Joint et al., 1986), and in other estuaries up to 10% of the total production (Jochem, 1988; Ray et al., 1989). Iriarte and Purdie (1994), in waters of Southampton estuary reported autotrophic picoplankton abundance at 10^4 cells/mL, and that the <3μ fraction was responsible for 17-20% of the total production, with the <1μ fraction producing 6% of the total production. However, the highest values have been reported in the oligotrophic regions of oceans (e.g. up to 80% by Li et al., 1983; 77-82% by Takahashi and Bienfang, 1983)).

Teixeira and Gaeta (1991) estimated the autotrophic picoplankton (cells 0.45-1.0μ) productivity in the equatorial waters of Brazil in estuaries 3.0-28.5%, coastal regions 18.5-40.4 %, and oceanic 6.7-100%, of the total phytoplankton production. In the Southern ocean this percentage for the <1μ fraction ranges seasonally 0-32% of the primary production (Weber and El Sayed, 1987). In an extensive review of primary production and abundance of autotrophic picoplankton (<3μ) in the Mediterranean Sea, Magazzu and Decembrini (1995) reported the abundance of the picocyanobacteria and the eukaryotes ranged from 10^2-10^5 cells/mL, and the prochlorophytes at 10^4 cells/mL. The picoplankton productivity contribution was 44% and 71% for neritic and pelagic waters respectively. Bienfang et al. (1984) found in the tropical Pacific that the chlorophyll maximum area consisted mainly (60-80%) of cells < 3μ , and that they represented 71% of the total production in the photic zone, and 77% of the chlorophyll. In sub-Antarctic waters, Vanucci and Mangoni (1999) reported the picoplankton was dominated by chroococcoid cyanobacteria (*Synechococcus* spp.), with the eukaryotes about one order of magnitude less abundant. Overall, in comparison to the total phytoplankton present, the picoplankton represented 46% of the chlorophyll and 53% of the primary productivity. In general, the autotrophic picoplankton represents up to 80% of the total primary production in marine waters.

In three tidal tributaries to the Chesapeake Bay, Marshall and Nesius (1993) reported the major phytoplankton peaks in productivity were enhanced by the autotrophic picoplankton when their peaks coincided with the summer productivity maximum. A similar pattern of a summer/fall enhancement of productivity by the increased picoplankton abundance was repeated over a four year period in the Chesapeake Bay (Marshall and Nesius,1996). During the seasonal maxima the pico-plankton concentrations in late summer and early fall were 10^5 and 10^6 cells/mL respectively. Within the southern Chesapeake Bay, the percent contribution of the picoplankton productivity to the total production during the spring/summer months of 2001 ranged from 6.4% (June) to 57% (July) (K. Nesius personal communication). The productivity rates for this period ranged from a river entrance site of 1.31- 28.46 μgC/L/hr to 1.55 - 36.97 μgC/L/hr at the Bay entrance. In their August diel study at the entrance to Chesapeake Bay, Affronti and Marshall (1993) reported the average picoproductivity rate above the pycnocline was 6.27μgC/L/hr, with lower productivity occurring in the morning, and below the pycnocline (0.77 μg C/L/hr). In January, these productivity rates above and below the pycnocline were 0.134 and 0.153 μgC/L/hr respectively. Using frequency of dividing cells to estimate growth rate and productivity at the Chesapeake Bay entrance, the picoplankton growth rates varied from 0.23/day to 1.10/day, with highest rates occurring in summer (Affronti and Marshall, 1994). These results indicate the picoplankton contribution to the total Bay productivity ranged from winter values of 2.2-2.3% to 53.4-55.6% in summer (July). Ray et al. (1989) determined a summer picoplankton production mean rate within a tidal river to be 2.5 μgC/gChla/hr, and responsible for 9% total primary production, with a mean abundance of 2.75 X 10^5 cells/mL. In the northern, less saline, and more nutrient rich section of Chesapeake Bay, Malone et al. (1991) reported winter/spring productivity lows and summer highs, with picoplankton productivity rates over a two year period in August at 50-70 μgC/L/hr, and a high the third year at 120 μgC/L/hr. During the summer the picoplankton contributed 20% of the total production in this

area. In the Mediterranean, Magazzu and Decembrini (1995) had mean productivity rates within the coastal regions of 1.19 µgC/L/hr, representing 31% of the total production, compared to the open water regions of 1.73 µgC/L/hr, with a 92% contribution to total productivity. Picoplankton growth rates associated with the Great Lakes were 0.8-1.5/day, whereas in Pacific regions these varied from 0.97 to 3.62/day (Bienfang et al., 1984; Fahnenstiel et al., 1986; Bienfang and Takahashi, 1983; Iturriaga and Mitchell, 1986). Growth rates for *Prochlorococcus marinus* were reported by Moore et al. (1995) were from 0.53 to 0.63/day.

Numerous studies have also identified autotrophic picoplankton as major contributors to algal productivity in freshwater habitats (Rodhe et al., 1958; Holmes and Anderson, 1963; Kalff, 1972; Shiomoto et al., 1997; Steitz and Velinirov, 1999; Han and Furuya, 2000; among others). In the North American Great Lakes, Fahnenstiel et al. (1986) attributed 50% of the phytoplankton productivity in Lake Superior to cells <3µ size, and were composed of 20% chroococcoid cyanobacteria at concentrations of 10^3 cells/mL. Of note, was that cells filtered through a <1.0µ filter represented 20% of the primary productivity. In other studies, productivity rates within a variety of lakes of different eutrophic status were rather similar. These include the oligotrophic Great Central Lake (0.10-0.73 $mgCm^3h^1$), Lake Superior (0.58 $mgCm^3h^1$ and 0.31$mgCm^3h^1$), and in eutrophic Lake Kinneret (0.01-1.5 $mgCm^3h^1$) (Costella et al., 1979; Munawar and Fahnenstiel, 1982; Fahnenstiel et al., 1986; Malinsky-Rushansky et al., 1997).

TOXIC AND HARMFUL AUTOTROPHIC PICOPLANKTON

Although due to its colonial nature and community interactions, Komarek (1996) would exclude the *Microcystis* complex from the picoplankton category, strains within this group are known to be toxin producers (Hughes et al., 1958). They produce metabolites described as microcystins which are toxic to fish (Zimba et al., 2001; and others). Skulberg et al. (1993) and Codd (1995) makes reference to a variety of cyanobacteria as toxin producers that includes picoplankton strains within *Synechococcus* and *Synechocystis*. Toxicity associated with various strains of these genera are noted by Lincoln and Carmichael (1981) and Mitsui et al. (1989). These same two genera form a symbiotic association with several marine heterotrophic dinoflagellates (Gordon et al., 1994) . The cells were found attached to the outer surface of these dinoflagellates in tropical and sub-tropical waters during periods of nitrogen limitation. Imai and Nishitani (2000) noted a similar relationship where unidentified picoplankton cells (1-2µ) were on the surface of the toxic marine dinoflagellates *Dinophysis acuminata* and *D. fortii*. These cells are a suggested food source for these heterotrophs and may be a source of toxicity in the *Dinophysis* spp. Glasgow and Burkholder (1998) found amoeboid and zooplankton stages of the toxic dinoflagellate *Pfiesteria piscicida* readily consumed *Synechococcus* cells. Similar uptake has been studied for these and other dinoflagellates by Marshall (unpublished), and others.

Since the mid-1980s, the chrysophyte *Aureococcus anophagefferens*, a spherical, unicellular organism (2-3µ), has produced annual blooms (called brown tides) in bays and inlets along the U.S. northeastern coast (Bricelj and Lonsdale, 1997). The blooms are associated with rising temperatures of spring (> 20°C), salinities >28 ‰, and reduced flushing rates within these inlets associated with reduced spring rains. Bloom concentrations are in excess of 1.0 X 10^6 cells/mL. The blooms coincide with the

growth season of *Zostera marina* producing extended periods of light attenuation to impact its development, and is associated with mass mortality among suspension feeding bivalves (mussels, bay scallops) by inhibition of their feeding. To date, no toxin has been linked to this species.

PREDATION

There exist different opinions as to the relationships between the components of the microbial (food) loop and the predators in the metazoan food chain. Fogg (1995) considers the microbial loop more of a self-contained system that basically perpetuates itself by minimizing losses outside of the loop. Predation loss to metazoans is not extreme. Hagström et al. (1988) considers that only 6% of its biomass passes on to the higher tropic levels. Any losses outside the loop can be recaptured through nutrient enhancement from waste or decomposition products in the water column. Within this microbial loop, the microzooplankton predators would include protozoa, and a variety of mixotrophic and heterotrophic nanoflagellates (Caron et al., 1991; Kuuppo-Leinikki et al., 1994; Hadas et al., 1999; Sanders et al., 2000). The changing role of the autotrophic picoplankton is also directly related to trophic status. Weisse (1991) discusses the implications resulting from a shift from the smaller picoplankters to the larger eukaryotes and how this transition will be more beneficial to the metazoa. Safi and Hall (1999) used fluorescently labeled bacteria and microspheres as picophytoplankton sized particles to evaluate grazing by mixotrophic and heterotrophic nanoflagellates, and others. They found that the mixotrophic and heterotrophic nanoflagellates had a preference for the picoplankton sized particles over the bacteria when grazing on these artificial prey. The common nanoflagellates predators to picoplankton in oceans would include representative taxa within the pyrmnesiophytes, choanoflagellates, raphidophytes, dinoflagellates, chrysophytes, and euglenoids. Of note is that Cynar et al. (1985) report predaceous nanoplankton were present in both 0.4 and 0.6μ water filtrates. In a study of herbivory in Newfoundland coastal waters, Putland (2000) found the microzooplankton consumed 25-30% of the *Synechococcus* standing crop daily. In the oligotrophic north Pacific, the coccoid cyanobacteria were the most abundant autotrophic picoplankton (64%) (Iturriaga and Mitchell, 1986). They estimated 30-40% of this cyanobacteria standing crop were consumed daily. The micro-grazers were represented by a diverse assemblage of protozoa, copepod larvae, and chaetognaths. The growth rates of these picoplankton cells was at 1.6/day.

Simek et al. (1995) studied protozoan grazing during summer within a eutrophic reservoir in Bohemia. The grazing rates were 560 picoplankton cells/hr for *Vorticella aquadulcis*. However, the dominant predators were oligotrichs, with an ingesting rate of 76-210 cells/hr. Other grazers of autotrophic picoplankton, and bacteria, included chrysomonads, choanoflagellates, ciliates, and bodonids. (Simek et al., 1997). At an oligo-mesotrophic lake, Pernthaler et al. (1996) identified the importance of heterotrophic nanoflagellates as grazers of picocyanobacteria and bacteria. They were responsible for ~90% of the grazing with the remaining 10% attributed to ciliates

The status of the picoplankton dominance within lakes will influence carbon utilization moving through the upper trophic levels. For instance, Stockner and Shortreed (1989) describe two contrasting oligotrophic lakes in British Columbia, which have different primary producers and predatory relationships that resulted in

trophic biomass differences. The picoplankton based food web results in a longer series of trophic steps to reach the higher trophic levels (e.g. fish). In contrast, the other lake has nanoplankton and microplankton phytoplankton as the primary producers, and is preyed in turn by larger zooplankton and reaches the fish consumption level in fewer steps, being more productive than the other lake. Considering the abundant and ubiquitous presence of picoplankton cells in these waters, Stockner (1988) considers a "top down" control of their abundance is very likely. This predation pressure appears to be caused mainly by various phytoflagellates and ciliates.

ANALYSIS OF AUTOTROPHIC PICOPLANKTON

There are significant limitations in light microscopy usage in the identification and enumeration of the various taxa within the picoplankton category. In addition to scanning electron microscopy and PCR analysis, two common approaches used today involve epifluorescence microscopy and flow cytometer analysis. References for these methods include: Davis and Sieburth (1982), Caron (1983), Wood et al. (1985), Pick and Caron (1987), Booth (1987), Weisse (1988), MacIsaac and Stockner (1993), Chisholm et al. (1988), Li and Wood (1988), Chisholm et al. (1992), Fahnenstiel et al. (1991), Olson et al. (1993), among others.

SUMMARY

Since the original identification of *Synechococcus* in the world oceans (Johnson and Sieburth, 1979; Waterbury et al.,1979), the composition of these ubiquitous picoplankton assemblages in both freshwater and marine locations has expanded. This has been accomplished through the utilization of more sophisticated instrumentation, along with the added scientific interest directed to this community over the past two decades. Although cyanobacteria are the most common and typically the more abundant representative within the picoplankton, there are a variety of eukaryote phylogenetic groups represented in both oceanic and freshwater habitats, with the prochlorophytes broadly distributed in the oceans. However, there remains questions as to what size groups should be included, and whether to exclude those colonial picoplankton from this category. These issues become important when attempting to compare data when investigators use different size categories to define their picoplankton , attribute productivity rates to this group, or if both prokaryotes and eukaryotes are included in studies.

In both freshwater and marine habitats the autotrophic picoplankton's percent contribution to the total primary production decreases moving from the less nutrient rich oligotrophic waters (e.g. off shore waters, open ocean) to the more eutrophic coastal regions (Craig, 1984; Fahnenstiel et al., 1986; Stockner, 1988; Stockner and Anita, 1986; Vörös et al. 1991; and Bell and Kalff, 2001). This occurs even as the picoplankton abundance increases into the more eutrophic waters. The major influence to this pattern is the greater concentrations of the larger eukaryotes ($> 3\mu$) present in these more nutrient enriched waters in comparison to the picoplankton, in contrast to a reverse relationship at the oligotrophic sites. Their production is essential to the microzooplankton within the microbial loop in lakes and oceans, but linkages to predators that would bring this productivity to the higher trophic levels are limited and need further study and clarification.

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