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Mass sedimentation of picoplankton embedded in organic aggregates

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Abstract

During a survey cruise crossing the Subtropical Front over the Chatham Rise east of New Zealand, we made the first observation in High Nutrient Low Chlorophyll waters of massive sedimentation of picoplankton embedded in large (>0.5 mm diam.) organic aggregates (OAs) sensu Riley (1963). We estimate 9.3 mg C m^{-2} yr^{-1} of prokaryotic picoplankton biomass alone may be transported below the euphotic zone via this mechanism. Using confocal microscopy, we made direct observations of picoplankton within undisrupted individual OAs, collected in sediment traps fitted with acrylimide gels, which largely conserved particle structure. Prokaryotic picoplankton autofluorescence was well-preserved and concentrations were extremely high within large rapidly sedimenting aggregates, ranging from 1.06×10^8 ml^{-1} in the 120 m sediment traps in subantarctic waters to 7.5×10^6 ml^{-1} at 550 m in subtropical waters, yielding Enrichment Factors of 10^3-10^5 relative to picoplankton concentrations in the water column. Aggregate picoplankton concentrations showed a well-constrained exponential decline with depth, which we speculate may represent an estimate of protozoan grazing rate within the aggregates. Picoplankton were found within heterotrophic flagellates, within copepod fecal pellets, and within organic matrices, all of which were incorporated in OAs. The key event of picoplankton incorporation into initial particles occurs in the upper water column, very likely through grazing with low assimilation efficiency. Once OAs are formed, their changing porosity and reduction in picoplankton cell numbers via heterotrophy is likely to be a key factor mediating picoplankton carbon fluxes in moderately productive ecosystems, and in determining the overall particle structure of sedimenting OAs.

Recent studies on sedimentary fluxes in the southwest Pacific near the Subtropical Front indicate that the region may be a moderate biologically-mediated sink for atmospheric CO_2 (Murphy et al. 1991; Nodder 1997). The physical oceanography of the region is highly complex (Heath 1985) and the ecosystem function diverse and complex (Bradford-Grieve et al. 1999). The present study outlines data on the sedimentation of picoplankton and microplankton embedded in sinking organic aggregates (OAs). Prokaryotic picoplankton are ubiquitous in the open oceans of the world (Joint 1986) and feature prominently in the phytoplankton of the front (Bradford-Grieve et al. 1997). However, because of their small size (<2 μm, Stockner and Antia 1986) picoplankton are not thought to sediment individually from surface waters in large numbers (Pedros-Alio et al. 1989). Significant vertical transport of organisms like the prokaryotic picoplankter *Synecchococcus* sp. can only occur when they become associated with larger particles (Fowler and Knauer 1986; Silver et al. 1986), generally via grazing (Madin 1982; Urban et al. 1993). Prokaryotic picoplankton have even been used to trace the fate of salp fecal pellets through benthic foodwebs because of their high resilience and the persistence of their distinctive phycoerythrin fluorescence (Pfannkuche and Lochte 1993).

In such situations picoplankton sedimentation via incorporation into large organic particles, such as fecal pellets, can contribute significantly to inputs of particulate organic carbon (POC) to the deep sea (1.17 mg C m^{-2} yr^{-1} to >3000 m; Turley and Mackie 1994; 1995, see also Wiebe et al. 1979; Iseki 1981). These organisms also contribute a distinctive biochemical signature to deep waters including a high protein quota (Glover et al. 1986), specific trace organic compounds (Wiebe et al. 1979), fatty acids and lipids (Matsueda et al. 1986) and a unique genetic signature (Turley and Mackie 1995).

Techniques used to study the formation, structure and composition of organic aggregates must balance the need to sample the delicate particles containing picoplankton without disruption, while estimating flux quantitatively, over rea-

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sonable vertical scales. Sampling particles with submersible cameras, though quantitative, cannot fully resolve structures within individual aggregates. Sediment traps collect sinking particles in a semi-quantitative manner (Knauer et al. 1979; Ducklow et al. 1982; US GOFS Report 10, 1989; Martin et al. 1993), but yield no individual particle resolution since fluxes are consolidated into a slurry in the trap (Silver et al. 1986). Upper ocean tracers such as ^{234}Th are fully quantitative, but yield no particle-specific data since measurements are integrated over the entire mixed layer (Buesseler et al. 1995). SCUBA collection has been very effective in sampling aggregates while maintaining particle structure (Trent et al. 1978), and especially when paired with confocal microscopy (Holloway and Cowen 1997), yields excellent high resolution images of particle substructure. However, this sampling is of necessity limited to larger particles and limited to depths accessible by diving.

Here we present data on aggregate sedimentation utilizing sediment trap gels (Lundsgaard 1995) and confocal microscopy which yields semi-quantitative (i.e., quantification dependent on the semi-quantitative nature of the sediment trap technique, see Buesseler et al. 1994), high-resolution particle measurements over a depth of 550 m. Using this method we can resolve individual aggregates up to 2 mm in diam., and image the spatial distribution of individual phytoplankton, including picoplankton and microplankton, within these aggregates.

We present measurements of picoplankton fluxes to floating sediment traps suspended at water depths of 120, 300, and 550 m, in two different water masses on either side of the Subtropical Front east of New Zealand. We make direct observations of the picoplankton clusters within individual organic aggregates which sank into the sediment traps, and estimate the picoplankton concentrations within the large (0.5–2 mm) size fraction. We discuss mechanisms by which picoplankton numbers might have become so high in the organic particles collected, relative to picoplankton concentrations in the water column. We also consider more briefly the role of microplankton in the same particles.

Materials and Methods

Study area and cruise plan—The Subtropical Front is a zone of high oceanic productivity occurring throughout the Southern Ocean where cool, low salinity Subantarctic High Nutrient Low Chlorophyll (HNLC) waters are mixed with warmer, high-salinity subtropical waters (Heath 1985). East of New Zealand, the front coincides with Chatham Rise, a narrow sea-floor feature that rises to within 400 m of the sea surface and extends 1500 km east from the NZ coast into the Southwest Pacific Ocean (Fig. 1).

In April–May 1997, we conducted a cruise as part of New Zealand's National Institute of Water and Atmospheric Research (NIWA) Ocean Fronts Program. The cruise track of the R.V. *Tangaroa* comprised several N–S transects across the Chatham Rise at roughly 178°30′E longitude (Fig. 1). As part of the survey of processes affecting benthic–pelagic coupling, NIWA scientists estimated phytoplankton biomass, primary production, and sedimentary carbon fluxes to 120, 300 and 550 m using floating sediment trap arrays, and measured benthic respiration and organism abundance. The sediment traps were deployed as free-floating arrays similar in design to the MULTI-trap/PIT deployments off Hawaii (Karl et al. 1996) and Bermuda (Lohrenz et al. 1992).

Before the cruise we prepared the acrylimide-based gel according to the method described in Lundsgaard (1995), and filled forty 50 mm petri dishes half-full with the gel. On board, these petri dishes were affixed inside the removable base of the sediment traps using Velcro® dots. The surface layer of the polymer dissolves partially in seawater, forming a density gradient at the bottom of the trap. This allows the aggregates to slow gently as they sink, finally becoming suspended in dense polymer without losing their structure. Small volumes of the polymer containing individual aggregates can then be gently removed from the trap for microscopic enumeration.

All sediment traps were filled with 0.5 μm filtered surface sea water immediately before deployment. We simultaneously deployed duplicate arrays of free-floating sediment traps in each water mass (subtropical and subantarctic) for 48 h. The arrays were deployed approximately 5 nautical miles apart to minimize the effect of physical discontinuities, since Chiswell (1994) had observed no coherence between currents measured via moorings deployed 7 nautical miles apart on the Chatham Rise. Each array consisted of three cross-frames, with the frames attached at 120 m, 300 m, and 550 m from the surface. Each frame contained 12 baffled sediment traps (7 cm diam., 58 cm height, aspect ratio 8.3), 3 of which had gels affixed in the bottom as described above. The 9 non-gel traps on each frame were used to measure the overall fluxes of total mass as carbon, chlorophyll and bacteria (results to be presented elsewhere). No poisons or preservatives were used for these short-term deployments (e.g., Gardner 1977; US GOFS Report 10, 1989).

Upon recovery, sea water inside the gel traps was aspirated down to 20 cm above the bottom, and left overnight to settle. On the following day, we carefully removed residual water from the gel traps by inserting the aspirator nozzle between the petri-dish and the sediment trap wall and siphoning all water very gently from the trap. The sediment trap bottoms were then removed and the gels were covered and left to dry for 12 h on a laboratory bench.

We examined each gel on board through a Nikon Stereoscopic SMZ-U dissecting microscope. Gels were more or less uniformly covered with organic aggregates (*sensu* Riley 1963). We used an attached CCD camera and SONY Videocam to scan the gel at three different magnifications (field of view = 14 × 10 mm; 4 × 2 mm, and 1.2 × 1 mm). Once each gel was scanned, we removed 5–10 of the largest visible aggregates (0.5–2 mm diam.) from the gel for quantitative picoplankton analysis. We used a micropipette with a wide pipette tip and very gentle suction, so that each aggregate rolled gently up into the pipette tip while still embedded in the gel. These large aggregates were mounted in 6-well slides for future analyses, sealed with nail polish, and stored in the dark at 4°C.

Onshore, the gel videos were analysed using the Optimas image analysis program, to yield the cross-sectional area of all the particles embedded in the gel. We examined the large

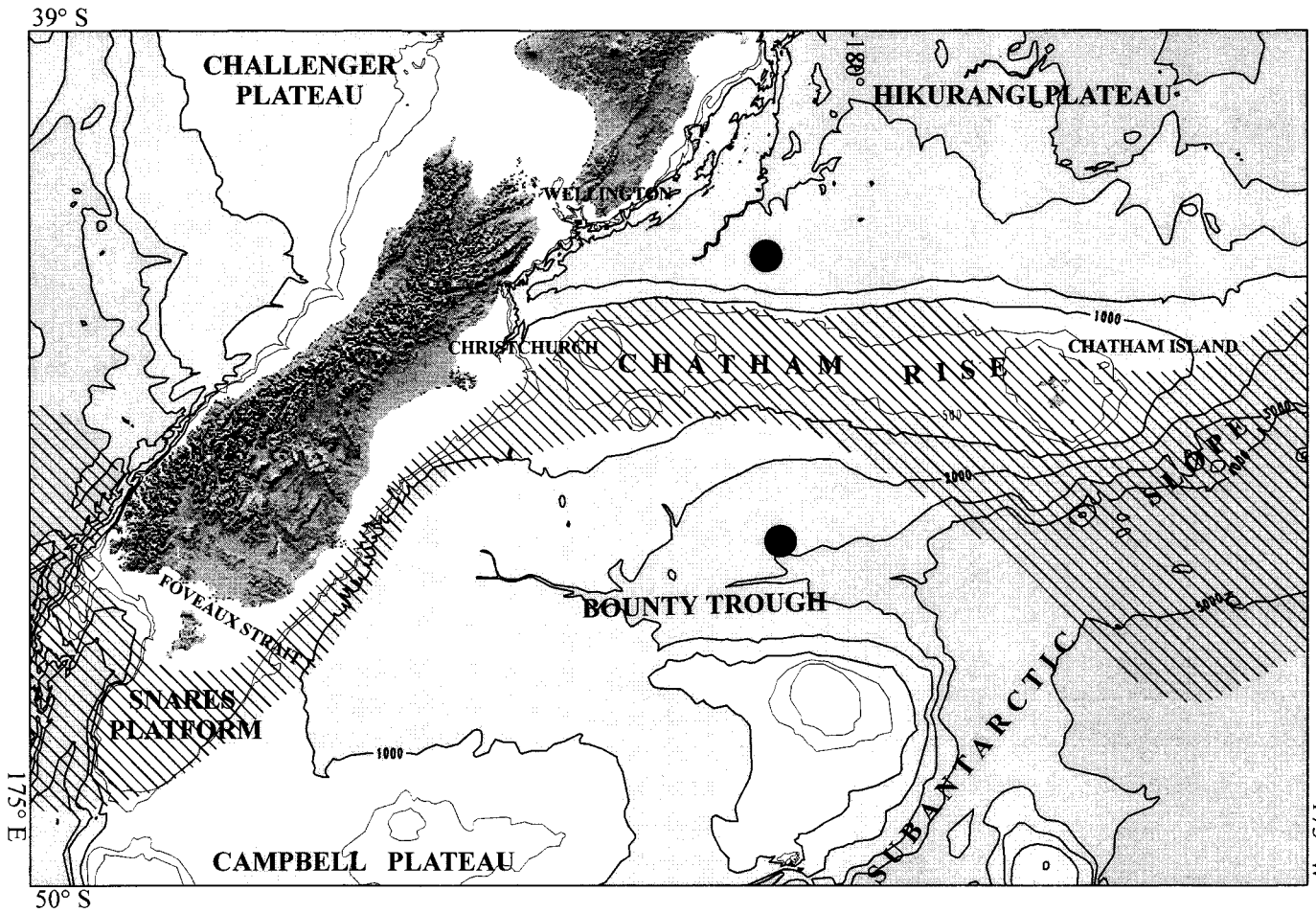

Fig. 1. Map of coastal waters E of New Zealand. Filled circles indicate sediment trap deployments N (in Subtropical waters) and S (in Subantarctic waters) of the Subtropical Front, which is marked as a hatched area.

aggregate slides using a Leica confocal microscope, under both visible light and epifluorescence. Prokaryotic picoplankton (*Synecchococcus* sp.) were visible as bright orange phycoerythrin-containing particles under green light. Eukaryotic picoplankton were identified by red fluorescence from chlorophyll *a* under blue excitation (Hall 1991). Microplankton were recorded as individual species counts to the genus or species level depending on the specimen quality. We enumerated picoplankton per ml of aggregate volume, and made confocal images of the organisms within the aggregates under transmitted light, and then under green and blue excitation to observe chlorophyll and phycoerythrin fluorescence.

Results

Dissecting microscope overviews of intact individual gels showed gels evenly covered with particles of a variety of sizes and shapes (Figs. 2A–2E). Many particles resembled the large round, fluffy organic aggregates (OAs) of fecal origin described as flocs by Pomeroy and Diebel (1980), and the fecal products of pelagic pteropods or salps (Bruland and Silver 1981). The coiled form of many organic aggregates (e.g., 2A) is suggestive of, but not identical to, Bruland and Silver's (1981) image of *Corolla spectabilis* pellets. We direct the reader to our website: *http://www.cwr.uwa.edu.au/cwr/overview/biolpart/images/jpgindex.html* where these images and others are presented in colour.

Our images reveal that the core material of these submillimeter organic particles can be either dense and nonporous (Fig. 2A) or more diffuse (Figs. 2B–2C). Particles larger than this seem to be composed of several individual 0.2–0.5 mm subunits in clusters (Fig. 2C) with channels between the subunits (Fig. 2D). Also present in our gel samples in smaller numbers were individual large euphausiid fecal pellets, smaller copepod fecal pellets, and individually visible protozoa such as foraminifers and radiolaria (Fig. 2B) which we often observed both embedded within and associated marginally with the OAs in the gels. Large particles (>0.5 mm diam.) represented ~20% of the total biovolume in the gels as estimated via image analysis.

High concentrations of picoplankton were contained in all organic aggregates (Figs. 3A, 3C, 3E–3G). The concentrations of prokaryotic picoplankton as quantified in the large OAs only (ca. 0.5–2 mm in diam.) ranged from 1.06×10^8 ml^{-1} of aggregate volume in the 120 m traps in subantarctic

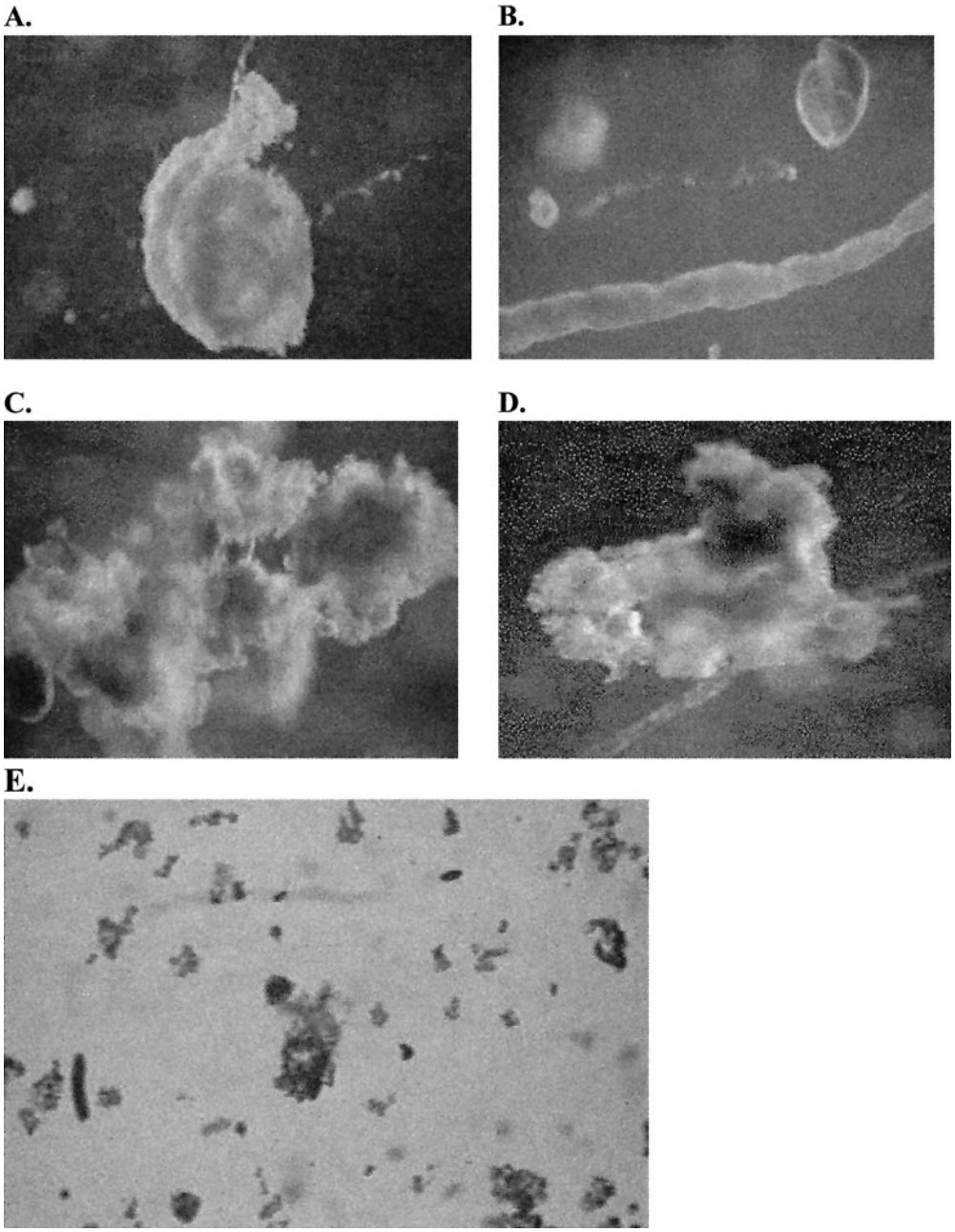

Fig. 2. (A) Large (0.5 mm diam.) organic aggregate (OA). (B) Euphausiid fecal pellet and foraminifer (Field of View (FOV): 1.5 mm × 1 mm). (C),(D) Large organic aggregates (FOV: 1.5 mm × 1 mm). Plane view of gel under dissecting microscope, low resolution (FOV: 7 mm × 3 mm). All particles have been videotaped embedded in gels recovered from a 550 m sediment trap, Subantarctic waters. Individual video frames were then acquired using a frame grabber and the Optimas image analysis package.

waters, to 7.53×10^6 ml^{-1} in aggregates found at 550 m in subtropical waters (Table 1, Fig. 4). We also observed large numbers of picoplankton in OAs embedded within crustacean fecal pellets and within heterotrophic flagellates (Figs. 3B–3C and 3D–3E). In subantarctic waters, prokaryotic picoplankton were incorporated into OAs at about 10 times higher concentrations than in subtropical waters, and replication was excellent between duplicate arrays deployed in the same water mass (Fig. 4). Water column prokaryotic picoplankton concentrations during our study were approximately 10^3 cells ml^{-1} in subantarctic waters and 10^4 cells ml^{-1} in subtropical waters (Fig. 5).

Concentrations of eukaryotic picoplankton and microplankton in the same large (0.5 mm to 2 mm) OAs were an order of magnitude lower and overall more variable than prokaryotic picoplankton. There were up to 25 times higher

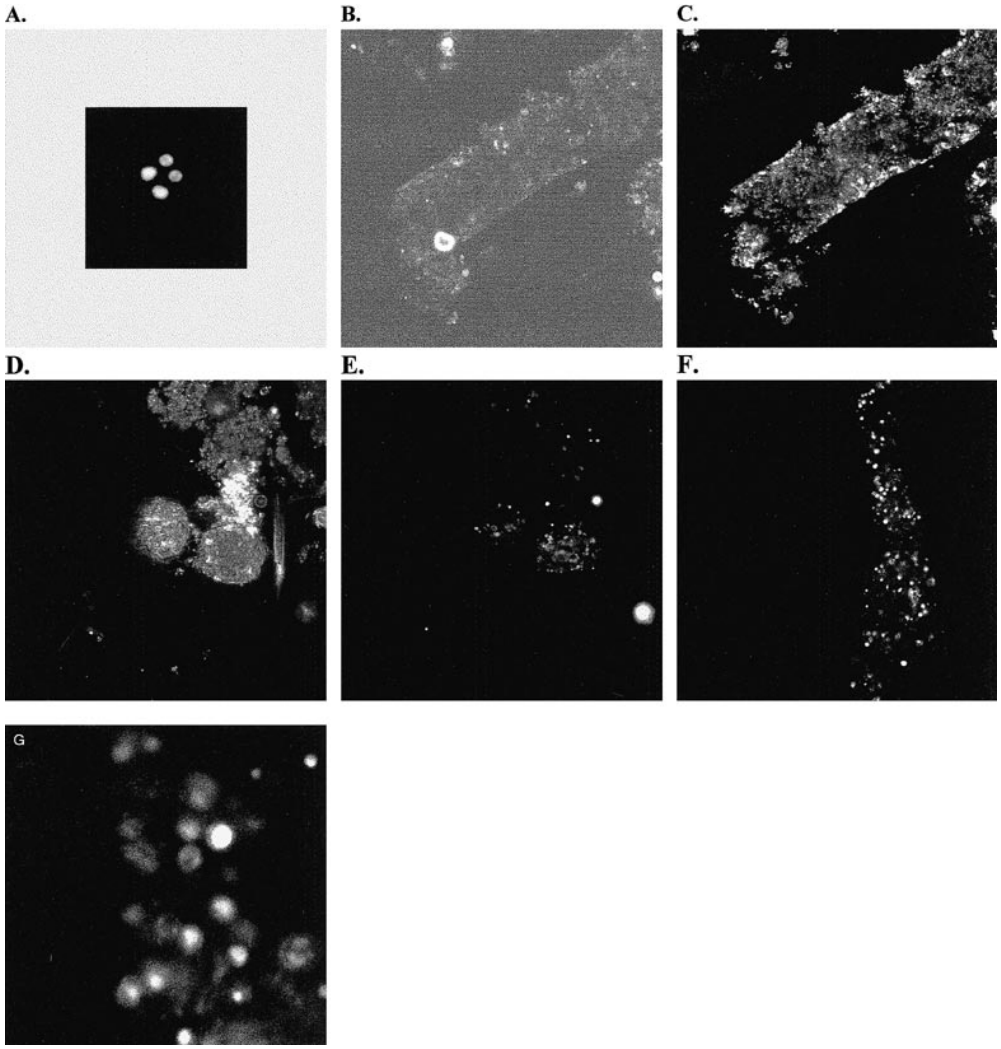

Fig. 3. Confocal microscopy of picoplankton embedded in organic aggregates (OAs) and fecal pellets. (A) Four picoplankton (each cell 2 μm diam.) embedded within an organic aggregate. Image shows phycoerethrin fluorescence only (orange fluorescence). (B) Visible light image of showing outline of a section of a copepod fecal pellet, FOV: ca. 2 mm × 2 mm. (C) Red fluorescent image of B., showing picoplankton embeded throughout the pellet. (D) Light image of segment of an OA containing two dinoflagellates and a diatom frustule (probably *Rhizosolenia* sp.), FOV: ca. 250 μm × 250 μm. (E) Red fluorescent image of D., showing picoplankton embedded within the dinoflagellates which are in turn incorporated within an OA. (F) Fluorescent image of OA (FOV 200 μm × 200 μm) entirely filled with embedded picoplankton. (G) Close up of F, FOV: 60 μm × 60 μm All images were taken on a Leica Confocal Microscope.

aggregate concentrations of eukaryotic picoplankton in subantarctic waters than in subtropical waters (Fig. 6). There was also a greater variety of microplankton species within the aggregates in subantarctic waters, including several species of diatoms not found in OAs from subtropical waters (Table 1). Subtropical aggregates were dominated by dinoflagellates and contained almost no diatoms, while subantarctic aggregates contained an equal mixture of diatoms and dinoflagellates. The most common diatom species were pennate Naviculoids, while the most common dinoflagellates in both water masses were *Gymnodinium* and *Gyrodinium* sp. (Table 1). Comparisons of pico- and micro-phytoplankton concentrations between large (0.5–2 mm) OAs and smaller (<0.5 mm) OAs indicated anecdotally both groups had similar concentrations, but no data set was generated from the smaller group.

Eukaryotic picoplankton were approximately 2×10^3 cells ml^{-1} in both subantarctic and subtropical water masses (Fig. 5). Microplankton concentrations were approximately 10 cells ml^{-1} in subtropical waters and 2 cells ml^{-1} in subantarctic waters. Dinoflagellates, especially *Gymnodinium* sp. and *Gyrodinium* sp. dominated microplankton numbers in subtropical waters (6 : 1; dinoflagellates : diatoms, in total numbers) while the two groups were roughly identical in concentration in subantarctic waters.

There was an exponential decrease in aggregate prokary-

Table 1. Concentrations of picoplankton and microplankton embedded in organic aggregates caught in simultaneously deployed duplicate sediment trap arrays at three depths in two water masses. All numbers are in cells per ml of aggregate volume. Standard deviations represent deviations between mean aggregate cell concentrations at the same depth in the two arrays ($n = 2$), with the exception of microplankton in subtropical waters.

	120-m Mean	STD	300-m Mean	STD	500-m Mean	STD
Subtropical						
Prokaryotic picoplankton	2.53E + 07	1.76E + 06	1.51E + 07	5.38E + 06	7.53E + 06	2.92E + 06
Eukaryotic picoplankton	5.18E + 05	5.51E + 05	2.45E + 06	1.67E + 06	1.06E + 06	1.47E + 06
Microplankton	1.84E + 06	1.322E + 06	7.04E + 05	2.261E + 05	1.00E + 06	N/A
Abundant species	*Dinoflagellates:* *Gymnodinium sp.* *Dictyocha sp.* *Tintinnids*		*Dinoflagellates:* *Chrysochomulina* *Gymnodinium sp.* *Tintinnids*		*Dinoflagellates:* *Gymnodinium sp.*	
Subantarctic						
Prokaryotic picoplankton	1.06E + 08	6.87E + 06	6.24E + 07	3.56E + 07	4.05E + 07	7.67E + 06
Eukaryotic picoplankton	1.40E + 07	1.78E + 05	6.11E + 06	2.89E + 06	1.45E + 07	1.78E + 07
Microplankton	1.02E + 07	1.926E + 06	N/A		N/A	N/A
Abundant species	*Dinoflagellates:* *Gymnodinium sp.* *Gyrodinium sp.* various cysts *Coccolithophores* *Diatoms:* *Nitzchia sp.* *Navicula spp.* *Tintinnids*		*Dinoflagellates:* *Gymnodinium sp.* *Gyrodinium sp.* *Dictyocha sp.* various cysts *Coccolithophores* *Diatoms:* *Nitzchia sp.* *Navicula spp.* *Rhizosolenia setigera* *Chaetoceros sp.* *Tintinnids*		*Dinoflagellates:* *Gymnodinium sp.* *Dictyocha sp.* *Diatoms:* *Nitzchia sp.* *Navicula spp.* *Leptocylindrus sp.* *Tintinnids*	

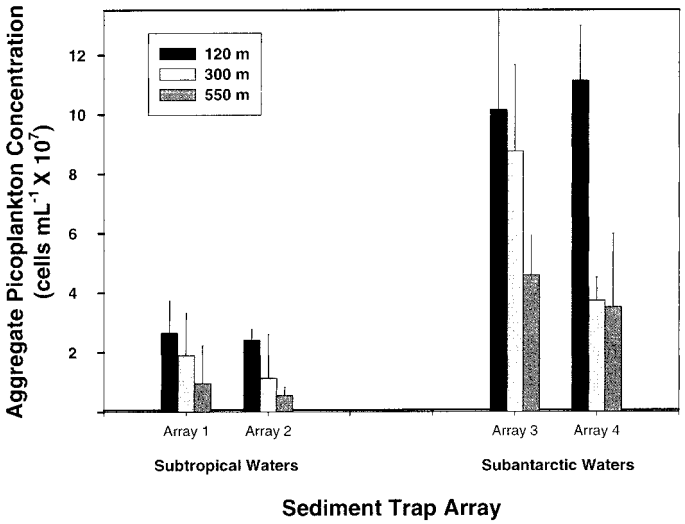

Fig. 4. Prokaryotic picoplankton concentrations within large (0.5–2 mm diam.) heterogeneous aggregates collected in gels, in floating sediment traps deployed to the north of the subtropical front east of NZ (Subtropical Waters) or to the south of the front (Subantarctic Waters). Replicate sediment trap arrays were deployed simultaneously, within 5 nautical miles of each other in each water mass. Error bars represent within-array standard deviation, i.e., SD of picoplankton concentrations in up to 20 aggregates from 3 gels at each depth from each array.

otic picoplankton concentrations in the large OAs with depth (Fig. 4). We fit an exponential decay curve to these data of the form $C(z) = \alpha e^{-\beta z}$ where C is the concentration of prokaryotic picoplankton at a given sediment trap depth and z is depth. We used the independent data points from the two arrays to yield a regression analysis with $n = 6$ for each water mass using a linear transformation $\ln(C) = -\beta z + \ln(\alpha)$. This yielded the following results for subtropical waters:

$$\ln(C) = -0.290(z) + 17.1$$
$$(\text{i.e., } \ln(\alpha) = 17.1)$$

This regression is highly significant at $p < 0.01$ and describes 84% of the variance of the concentrations of picoplankton with depth.

In subantarctic waters the regression yielded:

$$\ln(C) = -0.223(z) + 18.67$$
$$(\text{i.e., } \ln(\alpha) = 18.67)$$

This regression is also significant ($p < 0.05$), but describes only 66% of the variance in picoplankton concentration with depth. Note the similarity of the exponential decay coefficient β between both water masses. Patterns for eukaryotic picoplankton and microplankton were not as clear.

Based on this large size category alone we estimate 2.83×10^8 picoplankton m^2 yr^{-1} sank below the euphotic zone in subantarctic waters (to the 120 m trap), approximately 9.3 mg C m^{-2} yr^{-1} (Table 2). In subtropical waters prokaryotic picoplankton contributed approximately 1 mg m^{-2} yr^{-1} (Ta-

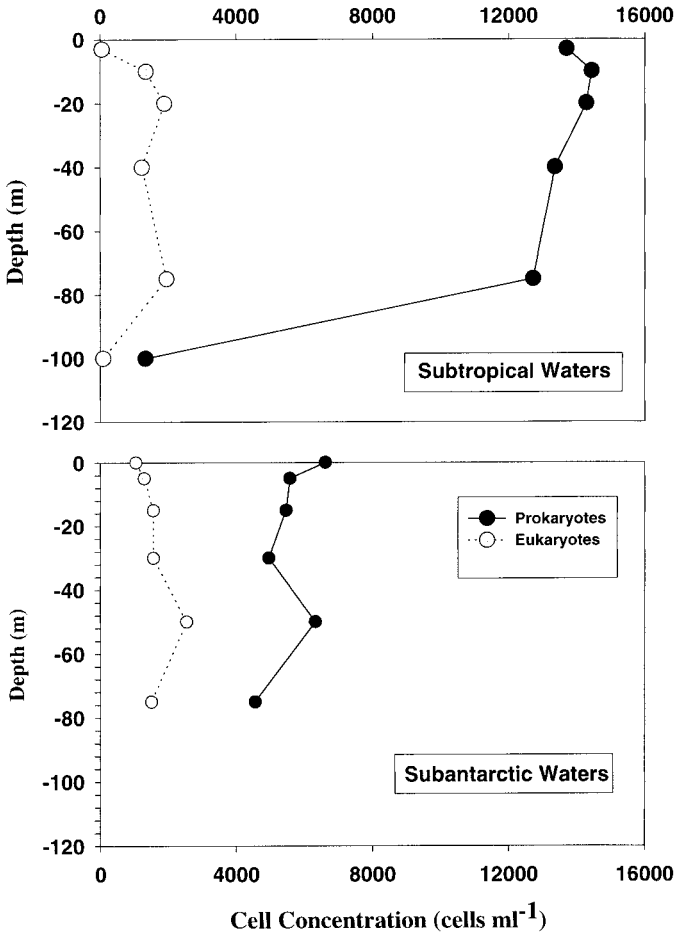

Fig. 5. Water column picoplankton concentrations. Single counts at each depth are given for prokaryotic and eukaryotic picoplankton. Note that prokaryotes are 5 times more concentrated in Subtropical waters while eukaryote concentrations are identical in both water masses.

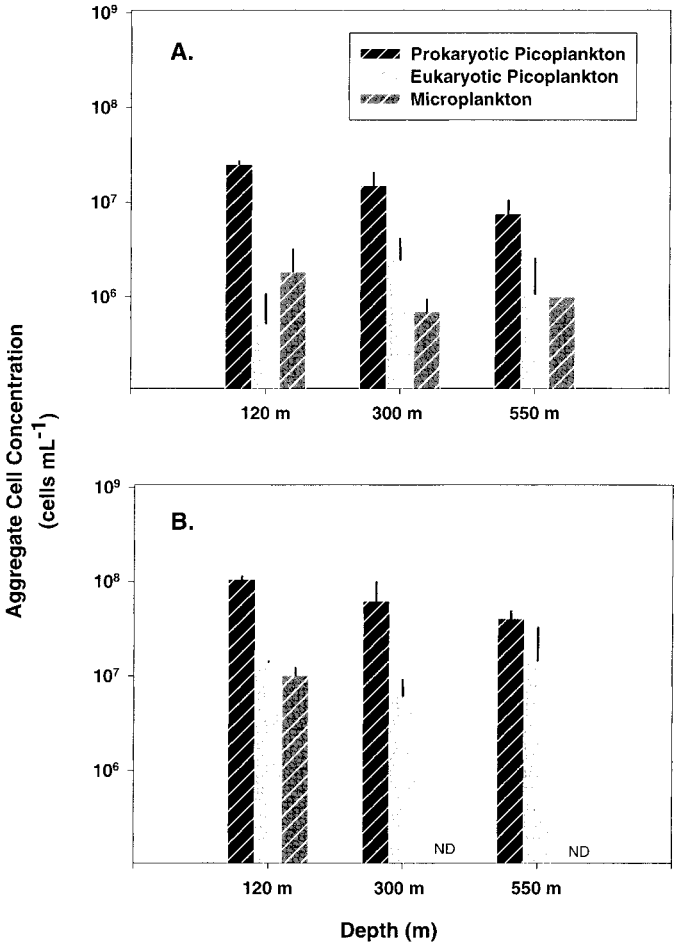

Fig. 6. Mean prokaryotic picoplankton, eukaryotic picoplankton, and microplankton concentrations within large (0.5–2 mm) organic aggregates collected at three depths in both Subtropical and Subantarctic waters. Error bars represent standard deviation of two simultaneously deployed arrays. A. Subtropical waters. B. Subantarctic waters.

Table 2. Flux estimations to 120 m sediment traps for prokaryotic picoplankton embedded in large aggregates only (0.5–2 mm diameter). We estimate the average biovolume of large aggregates in the gel and calculate carbon flux using a value of 8.10×10^{-8} μg C per picoplankter (Turley and Mackie, 1995). Standing stock is estimated by integrating numbers in Figure 5 over depth. We assume a picoplankton growth rate of 0.3 d^{-1} in surface waters. Total C flux per year are from unpublished sediment trap data from S. Nodder.

Variable	Subantarctic waters	Subtropical waters
Average number of large OAs/gel	14.6	6.2
Biovolume in large aggregates (ml per gel):	1.46E−02	6.17E−03
Picoplankton/OA biovolume, 120 m (cells ml^{-1})	1.06E+08	2.53E+07
Picoplankton flux to 120 m per 48 h	1.55E+06	1.56E+05
Total picoplankton flux (cells m^{-2} yr^{-1})	1.15E+11	1.16E+10
Total picoplankton C flux (mg C m^{-2} yr^{-1})	9.29	0.94
Total C flux (mg C m^{-2} yr^{-1})	6,200.0	11,100.0
% Total C flux as prokaryotic picoplankton	0.150	0.008
Standing stock (cells m^{-2})	1.03E+12	3.98E+11
Picoplankton flux as % Standing Stock d^{-1}	0.031	0.008
Integrated production (cells d^{-1}), growth = 0.3 d^{-1}	3.1E+11	1.2E+11
Picoplankton flux as % New Cells d^{-1}	0.10	0.03

ble 2). Eukaryotic picoplankton would contribute an order of magnitude less biomass. We observed microplankton embedded in the OAs at similar concentrations to those of the eukaryotic picoplankton, but because of their much larger size, microplankton would have represented 10^4 to 10^5 times the carbon of either other group.

Discussion

A. Gel videography and particle origin—Over the last two decades, studies have indicated that two key mechanisms govern picoplankton incorporation into organic aggregates, (1) the incorporation of picoplankton into phytodetritus and marine snow via particle aggregation (Silver and Alldredge 1981), and (2) the incomplete digestion and incorporation into fecal pellets of picoplankton grazed by herbivores including salps whose fecal pellets contain large numbers of picoplankton and sink rapidly to the sea floor (Caron et al. 1989; Pfannkuche and Lochte 1993; Noji et al. 1997).

We observed that both the mechanisms 1 and 2 may have played an important role in the formation of the organic aggregates (OAs). However, based on extensive examination we favour the explanation that a large fraction of the particles are fecal in origin. When particles have a low porosity and flow-through of water during sedimentation, small particles tend only to contact other small particles (Hunt 1982), and so it would be difficult for the assimilation of picoplankton into the large organic aggregates to occur via settling (Stolzenbach and Elimelech 1994; Jackson and Lochmann 1993; Jackson 1989). Li and Logan (1997) also argue that collection efficiencies of small particles by large sinking aggregates are $<0.2\%$. The more closely-packed structure of some of the particles (e.g., Figs. 2C,D) suggests that any aggregation of such fecal pellets into larger organic aggregates might have been a reaction-limited, not diffusion-limited process (Logan and Wilkinson 1990).

Given the size and morphology of the most common particles, many of the OAs are likely to have been produced by small salps or other tunicates, though the exact grazer(s) responsible for their production remains unclear. One candidate is the salp *Thalia democratica* and/or appendicularia which were observed in contemporaneous zooplankton samples in subtropical and subantarctic waters (Bradford-Grieve, pers. comm.). The large volumes of water filtered by salps, and their often inefficient digestion (Pomeroy et al. 1984; Wangersky 1984; Gonzalez and Biddanda 1990), result in a concentration of ambient microflora into copious fecal pellets similar to many of those we observed (Matsueda et al. 1986). The low assimilation rate of feeding salps (0.8%, Madin 1974) and the large size range over which they feed using a non-specific feeding net (<1 μm to >1 mm), would explain the wide range of particles we found embedded in the OAs, including individual clusters of prokaryotic and eukaryotic picoplankton, copepod fecal pellets, and microplankton including diatoms and dinoflagellates (Table 1).

It is highly likely that some of the organisms associated with the OAs represent the protozoan population colonizing OAs to feed on incorporated bacteria and picoplankton (e.g., Fenchel 1977; Gonzalez and Biddanda 1990; Shanks and Edmondson 1990; Lampitt et al. 1993*a*,*b*), rather than being incorporated via grazing.

The picoplankton concentrations we observed in OAs are 3–4 orders of magnitude higher than those found by investigators examining aggregated marine snow particles (Silver and Alldredge 1981). In fact the very highest concentrations we saw in our samples may have occurred via a two-step process involving initial grazing by copepods followed by incorporation of copepod fecal pellets into larger OAs. Unlike salps, copepods have very selective and species-specific feeding behaviour when consuming prokaryotic phytoplankton (Burns and Xu 1990). Even though picoplankton can be ingested in large numbers by copepods, they are poorly assimilated as a food source, leading to a high fraction of intact cells surviving in fecal pellet. The magnitude of this fraction is highly dependent on the nutritional value, age, and taste of particular prey organisms, as well as the size and the particular species of copepod grazing the prey (Burns and Xu 1990). Our observations suggested anecdotally that the highest concentrations of picoplankton occurred within regions of OAs resembling whole or partial copepod fecal pellets embedded within the OA matrix. The crustacean fecal pellets may thus have represented the source of highest variability in picoplankton concentrations within the OAs, but our data are currently not conclusive.

Enrichment factors (EFs) can be used to express the ratio of organisms in particles to free-living organisms, giving a useful estimate of the relative density of organisms in aggregate microhabitats (Silver et al. 1986; Biddanda and Gonzalez 1990). The prokaryotic picoplankton numbers in aggregates give an EF of 10^3 to 10^5, up to 3 orders of magnitude greater than those estimated by other investigators (Trent et al. 1978; Alldredge and Cox 1982). Eukaryotes in aggregates had an EF of 10^2 and microplankton EF of 10^4 to 10^5. This indicates that the OAs are a site for the intensified biological processes and enhanced sedimentation for all the phytoplankton within them. Being particularly enriched in prokaryotic picoplankton, OAs are especially important sites for the acceleration of ecologically important processes such as grazing and sinking. Their rapid sedimentation via OAs would be especially significant since there is no other mechanism by which prokaryotic picoplankton can make significant contributions to vertical flux.

B. Grazing and Sedimentation of OAs—Because of the high resilience of prokaryotic picoplankton fluorescence in cool dark conditions (several weeks; Pfannkuche and Lochte 1993), we suspect that intra-aggregate grazing by attached protozoa may be the primary cause of the decline of picoplankton numbers with depth. Fecal pellets of most organisms are colonized rapidly by a succession of bacteria and protozoa (Iseki 1981; Pomeroy and Diebel 1980). Experimental work indicates an exponential loss of carbon and chlorophyll over time in newly produced particles (up to 75% of chlorophyll lost in 48 h; Gonzalez and Biddanda 1990). If we use depth in our study as a proxy for time, this trend is shown clearly in the exponential decline of picoplankton with depth in all arrays across both water masses, with an exponential decay coefficient of 0.223 m^{-1} (subantarctic waters) and 0.290 m^{-1} (subtropical waters).

In our study, probable grazers would include the radiolaria, foraminifers, tintinnids and heterotrophic flagellates we observed, which were directly associated with the OAs under confocal microscopy. These numbers represent direct in situ estimates of picoplankton loss rates in OAs. The strong similarity of the decay rates between the water masses also indicates that whatever the process governing this decline, it was occurring similarly in both subtropical and subantarctic waters.

Ship-board grazing experiments showed that microzooplankton grazing rates were comparable to or greater than growth rates (Safi and Hall, unpubl. data), suggesting that microzooplankton grazing can be intense enough to rapidly process picoplankton at a rate comparable to or greater than their growth rate. This would be consistent with the inference that rapid grazing on the picoplankton within the OAs was the primary cause of their decline with depth. Other studies using bead ingestion experiments have also suggested that there may be vertical gradients in protozoan grazing pressure (McKenzie et al. 1995), and ship-board dilution experiments in the NW Indian Ocean indicated grazing rates similar to those suggested by our study if the loss rates do represent estimates of protozoan grazing rates (Burkill et al. 1993).

OAs the size of those we observed (0.5–2 mm) are in the range of the smallest observed salp fecal pellets (Madin 1982). Their maximum sinking rates are thus likely to be close to the slowest measured by Madin (1982), who indicated that pellets formed by *Pegea socia* and small individuals of *Salpa maxima* of equivalent size to those observed in our study, could sink between 300 and 600 m d^{-1}. Organic particles such as pelagic tunicate fecal pellets have been shown to have a highly variable density, from slow-sinking diffuse particles (Pomeroy and Diebel 1980) to extremely dense, rapidly sinking particles (Wiebe et al. 1979; Matsueda et al. 1986). Experimental work indicates tunicate pellets are initially much more dense than those produced by crustacean grazers (Madin 1982) but that once produced, their density is rapidly reduced via consumption by microbial and protozoan populations colonizing the pellets (Yoon et al. 1996) while their size remains roughly constant (loss of substance, not bulk, Gonzalez and Biddanda 1990). The residence time of the OAs we observed might therefore have ranged from a single day to several weeks, depending on their initial density and sinking rate, and the relative speed with which the protozoan grazers processed the material within the OA.

If OAs sink more rapidly than they are grazed, we should see no significant decline in picoplankton concentrations with depth. If grazing rates are high in comparison to sinking rates, and grazing is the primary cause of picoplankton decline, picoplankton concentrations will decline with depth. The decay rate we observed in the OAs therefore indicates the relative importance of loss terms such as grazing within them, in comparison to their sinking time scale. If we assume that OAs of 0.2 to 1 mm reaching the 550 m sediment trap intact have escaped large metazoan grazers, and that the grazing rates of protozoans on picoplankton are thus roughly equivalent to those we measured in bottle experiments, we can calculate that the 30% reduction in picoplankton concentration per 100 m we documented in our traps should occur in about 15 h. This yields a sinking rate of 300 m d^{-1}, which is a reasonable estimate based on previous work (above).

Sinking aggregates contain enough picoplankton (and microplankton) for them to represent a significant flux of material to the deeper traps. Our estimates (0.94–9.29 mg C m^{-2} yr^{-1}; Table 2) are in the same range as the prokaryotic picoplankton fluxes estimated by Turley and Mackie (1995) in the deep NE Atlantic (1.17 mg C m^{-2} yr^{-1}), based on correlations between pulses of carbon and bacterial and picoplankton numbers arriving in sediment traps.

Despite the small size of picoplankton and despite heavy grazing on them in OAs, a measurable fraction of prokaryotic picoplankton carbon exits the euphotic zone intact (0.1% of estimated daily prokaryotic picoplankton production). Overall, this represents 0.15% of the total estimated annual C flux to 120 m, similar to the fraction estimated by Turley and Mackie (1994). We deduce that prokaryotic picoplankton form part of a complex food web in which they are passed between organisms in the water column, are packaged in fecal pellets, and become highly concentrated in aggregates of multiple origin where they enter an in situ food web. Such mediation of the export process could be significant in High Nutrient Low Chlorophyll (HNLC) regions of the ocean where picoplankton dominate the phytoplankton. Our measurement of a flux of ca. 1.15×10^{11} prokaryotic picoplankton cells m^{-2} yr^{-1} in OAs in HNLC waters further highlights the importance of such fluxes. Such inferences of picoplankton export have also been made from the presence of labile chlorophylls and carotenoids in deep trap or pump samples (Turley and Carstens 1991; Llewellyn and Mantoura 1996; Nodder and Gall 1998).

These data represent the first measurements of picoplankton fluxes in organic aggregates in HNLC environments, since most other field measurements have been made in the NE Atlantic (Turley and Mackie 1994) or in coastal waters (Alldredge and Cox 1982).

Conclusions

In this paper we have used a new combination of techniques including sediment trap gels, videography, and confocal microscopy to investigate the composition and sedimentation of organic aggregates (OAs). Based on their structure, OAs are likely to be salp or other tunicate fecal material. Data from the Subtropical Front indicate annual picoplankton fluxes via OA sedimentation are at least 9.29 mg C m^{-2} yr^{-1} to 120 m, and that a significant fraction picoplankton are incorporated into the OAs via inefficient grazing. Well-defined decay rates in picoplankton concentrations in the OAs with depth of collection may represent estimates of protozoan grazing within OAs.

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