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Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration

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Abstract

Tangential-flow ultrafiltration was used to isolate particulate and high-molecular-weight dissolved material from seawater collected at various depths and geographic regions of the Pacific and Atlantic Oceans. Ultrafiltration proved to be a relatively fast and efficient method for the isolation of hundreds of milligrams of material. Optical and electron microscopy of the isolated materials revealed that relatively fragile materials were recovered intact. Depth-weighted results of the size distribution of organic matter in seawater indicated that ~75% of marine organic carbon was low-molecular-weight (LMW) dissolved organic carbon (< 1 nm), ~24% was high-molecular-weight (HMW) dissolved organic carbon (1–100 nm), and ~1% was particulate organic carbon (> 100 nm). The distribution of carbon in surface water was shifted to greater relative abundances of larger size fractions, suggesting a diagenetic sequence from macromolecular material to small refractory molecules. The average C:N ratios of particulate organic matter (POM) and HMW dissolved organic matter (DOM) were 7.7 and 16.7, respectively. Differences in C:N ratios between POM and HMW DOM were large and invariant with depth and geographic region, indicating that the aggregation of HMW DOM to form POM must be of minor significance to overall carbon dynamics. The stable carbon isotope composition ($\delta^{13}C$) of POM averaged −22.7‰ in surface water and −25.2‰ in subsurface water. Several possible explanations for the observed isotopic shift with depth were explored, but we were unable to discern the cause. The $\delta^{13}C$ of HMW DOM samples was relatively constant and averaged −21.7‰, indicating a predominantly marine origin for this material. The $\delta^{15}N$ values of POM were highly variable (5.8–15.4‰), and the availability of nitrate in surface waters appeared to be the major factor influencing $\delta^{15}N$ values in the equatorial Pacific. In the upwelling region nitrate concentrations were relatively high and $\delta^{15}N$ values of POM were low, whereas to the north and south of the upwelling nitrate concentrations were low and $\delta^{15}N$ values were high. The $\delta^{15}N$ values of HMW DOM reflected the same trends observed in the POM fraction and provided the first such evidence for biological cycling of dissolved organic nitrogen (DON). Using the observed $\delta^{15}N$ values and an estimate of meridional advection velocity, we estimated a turnover time of 0.3 to 0.5% day^{-1} for HMW DON. These results suggest a major role for DON in the upper ocean nitrogen cycle.

Keywords: ultrafiltration; dissolved and particulate organic matter; stable carbon and nitrogen isotopes

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1. Introduction

The world ocean is a major global reservoir of organic carbon. Only a small fraction of the organic carbon in the ocean is associated with living organisms; most marine organic carbon exists as relatively small molecules in fairly uniform distribution and very dilute concentration (Carlson et al., 1985; Benner et al., 1992; Ogawa and Ogura, 1992). While there has been considerable progress in understanding the cycling of organic matter in the ocean (e.g. Druffel et al., 1992), many approaches to studying the origin, composition, and reactivity of marine organic matter have been hampered by the lack of methods for the concentration and isolation of suitable quantities of material for detailed characterization.

Tangential-flow or cross-flow ultrafiltration has recently emerged as a relatively fast and efficient method for concentrating and isolating materials from seawater for organic and inorganic analyses (Moran and Moore, 1989; Whitehouse et al., 1990; Benner et al., 1992; Guo et al., 1995). Initial studies employing tangential-flow ultrafiltration for the isolation of organic carbon indicate that 20–40% of the organic carbon in seawater can be isolated using filters with a pore-size of ~ 1 nm (Benner et al., 1992; Guo et al., 1995). Ultrafiltration membranes with pore sizes ≥ 1 nm (1,000 Dalton nominal molecular weight cutoff) do not retain sea salts, so it is possible to recover materials that are relatively rich in organic carbon and thereby amenable to chemical and isotopic characterization.

Polysaccharides are the major biochemical component of dissolved organic matter (DOM) isolated from seawater by tangential-flow ultrafiltration (Benner et al., 1992). Polysaccharides comprise a much greater fraction of the ultrafiltered DOM from surface waters than deep waters, indicating that they are largely produced and consumed in the upper ocean. The aldose composition of seawater polysaccharides is distinct from that observed in particulate materials and is relatively rich in galactose and deoxy sugars (McCarthy et al., 1996). Studies of the bioavailability of ultrafiltered DOM indicate that it is an important carbon and energy source for bacteria and a major reactive component of the ocean carbon cycle (Amon and Benner, 1994, 1996).

In the present study tangential-flow ultrafiltration was used to isolate two size fractions of organic matter from large volumes (200–1150 L) of seawater collected at various depths and geographic regions of the Pacific and Atlantic Oceans. Suspended particulate material (< 60 μm > 0.1 μm) was isolated from 16 samples and the high-molecular-weight fraction of dissolved material (1–100 nm) was isolated from 24 samples, representing the largest collection of oceanic samples undertaken to date. Isolated materials were examined by various types of microscopy to provide visual characterization, and the organic carbon and nitrogen concentrations and stable isotopic compositions were measured to provide additional information about the origin and reactivity of marine organic matter. These represent the first measurements of the stable nitrogen isotopic composition of marine dissolved organic matter. Additional characterizations of all or a subset of these samples have either been presented (Benner et al., 1992; McCarthy et al., 1993; McCarthy et al., 1996) or will be presented in the near future (Opsahl and Benner, 1997; Skoog and Benner, 1997).

2. Materials and methods

2.1. Sample collection

Water samples were collected from the Pacific and Atlantic Oceans and the Gulf of Mexico during four cruises on the RV *Alpha Helix* (April, 1991), the RV *John Vickers* (February–March 1992), the RV *Weatherbird* (May 1992), and the RV *Longhorn* (August 1991). A general description of the sampling locations is given in Table 1. Samples 17, 18, and 19 were collected from the Hawaii time-series station (ALOHA), and samples 20, 21, and 22 were collected from the Bermuda time-series station (BATS: see Table 1). During the RV *Alpha Helix* and RV *Longhorn* cruises all water samples were collected using Niskin bottles equipped with Teflon- or epoxy-coated closure springs. During the RV *John Vickers* and RV *Weatherbird* cruises surface water samples were collected with high-volume pumping systems and all other samples were collected using Niskin bottles equipped with Teflon- or epoxy-coated closure springs. Analysis of DOC concentrations (see

below) in water samples collected from the surface water pumping systems on both ships and samples collected from surface water using Niskin bottles were similar (±5%), indicating that the pumping systems were non-contaminating for bulk DOC. Immediately following collection, water samples were passed through a Nitex sieve (60 μm pore size) to remove large organisms and stored in 50 L or 200 L barrels for ultrafiltration. The high-density polypropylene storage barrels (Nalgene), silicone tubing (Masterflex), and pumps (peristaltic and diaphragm) were thoroughly rinsed with dilute HCl and found to be non-contaminating for bulk DOC, although trace levels of specific contaminants, such as phthalates, were detected by gas chromatography and mass spectrometry (van Heemst et al., 1993).

2.2. Tangential-flow ultrafiltration

Ultrafiltration of samples began aboard ship immediately following collection except for the three samples (#20–22) collected at the Bermuda time-series station (BATS). The BATS samples were transported to the Bermuda Biological Station where they were ultrafiltered within 48 h of collection. Two size fractions were isolated by ultrafiltration during the RV *John Vickers* cruise. The 0.1–60 μm size fraction was isolated using an Amicon DC10L ultrafiltration system with a polysulfone hollow fiber filter (H5MP01). This size fraction is referred to as ultrafiltered particulate organic matter (UPOM). The filtrate from the DC10L ultrafiltration system was fed directly into an Amicon DC30 ultrafiltration

Table 1
Sample descriptions, dissolved organic carbon (DOC) concentrations, and mass-balance results for tangential-flow ultrafiltration experiments. CF (concentration factor) = ratio of the initial sample volume to the volume of retentate at the end of filtration; % Initial DOC = 100 $(DOC_{retentate} + DOC_{permeate})(DOC_{initial\ water})^{-1}$; * indicates depth of the oxygen minimum layer

Sample #	Depth (m)	Collection date	Latitude	Longitude	DOC (μM)	Volume (L)	CF	% DOC retained	% Initial DOC
Pacific Ocean									
1	2	02/21/92	33°17′N	119°01′W	74	1000	1095	25	95
2	2	02/24/92	22°47′N	130°05′W	87	1000	1018	22	103
3	2	02/25/92	18°47′N	133°50′W	78	1000	1028	30	–
4	2	02/27/92	10°14′N	140°00′W	70	1000	979	30	107
5	2	02/28/92	5°35′N	140°00′W	76	1000	1018	26	–
6	2	03/17/92	5°16′N	140°00′W	71	400	475	34	96
7	2	02/29/92	1°30′S	140°00′W	72	600	562	35	87
8	100	03/14/92	2°00′S	140°00′W	68	980	1029	26	111
9	400 *	03/16/92	2°00′S	140°00′W	56	975	983	24	81
10	400 *	03/01/92	2°00′S	140°02′W	51	800	861	26	81
11	4000	03/15/92	1°57′S	140°03′W	44	985	1003	20	99
12	2	03/06/92	12°12′S	134°40′W	82	1010	1049	38	103
13	100	03/04/92	11°58′S	135°07′W	85	995	855	27	89
14	200	03/05/92	12°06′S	134°55′W	57	1025	1043	23	103
15	375 *	03/07/92	12°19′S	134°26′W	53	1020	1018	19	92
16	4000	03/03/92	12°00′S	135°00′W	45	1020	1018	22	85
17	10	04/05/91	22°45′N	158°00′W	82	200	222	33	110
18	765 *	04/05/91	22°45′N	158°00′W	38	200	211	25	118
19	4000	04/05/91	22°45′N	158°00′W	41	200	203	22	106
Atlantic Ocean									
20	1	05/24/92	31°50′N	64°10′W	72	600	602	23	97
21	900 *	05/20/92	31°50′N	64°10′W	47	600	729	18	105
22	2400	05/25/92	31°50′N	64°10′W	46	380	390	23	126
Gulf of Mexico									
23	10	08/17/91	27°07′N	95°30′W	95	1050	1059	30	101
24	750 *	08/18/91	27°07′N	95°30′W	48	1150	966	24	128

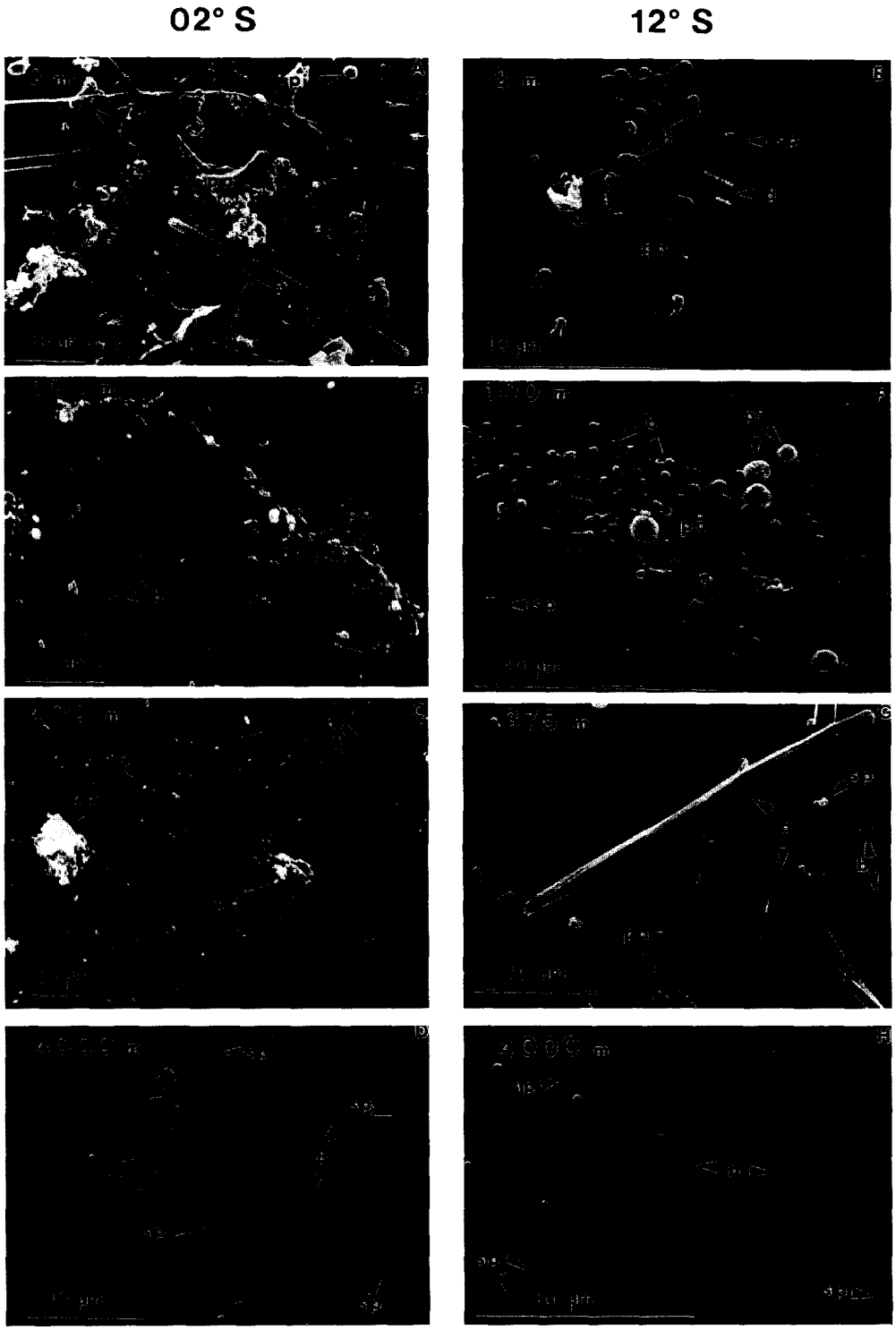
system with nine spiral-wound polysulfone filters (S10N1). The S10N1 filters have a pore size of ~ 1 nm with a molecular weight cutoff of 1,000 Daltons. After concentration to a volume of ~ 10 L the sample was transferred to the DC 10L system with two S10N1 filters and concentrated to a final volume of ~ 1 L. This size fraction (1–100 nm) is referred to as ultrafiltered dissolved organic matter (UDOM) or high-molecular-weight (HMW) DOM. Only one size fraction of organic matter was isolated during the other cruises. Water samples were pumped (peristaltic pump) through polycarbonate cartridge filters (0.2 μm pore size; Nuclepore) directly into the DC30 or DC10L ultrafiltration systems. The 1–200 nm size fraction is also referred to as UDOM or HMW DOM because there was no discernable difference in the concentration of DOC in seawater passed through the 0.2 μm or 0.1 μm filters.

The procedures and operating conditions for the isolation of different size classes of organic matter were similar to those described previously (Benner, 1991; Benner et al., 1992). Operating pressures for the DC10L system with the H5MP01 filter were 20–25 psi at the inlet and 10–15 psi at the outlet. Operating pressures for the DC10L and DC30 systems with S10N1 filters were 50–55 psi at the inlet and 42–48 psi at the outlet. Filtration rates were 60–90 L h^{-1} with the H5MP01 filter, 13–18 L h^{-1} with two S10N1 filters on the DC10L system, and 60–70 L h^{-1} with nine S10N1 filters on the DC30 system. Seawater temperatures ranged from 25–35°C during ultrafiltration in the DC30 system. During concentration of the final 10 L of sample in the DC10L system seawater temperatures ranged from 40–50°C. The total filtration time was less than 20 h for all samples. The filters and filtration system were washed and rinsed after every use. The H5MP01 filter was washed with 0.1 N NaOH, and the S10N1 filters were washed with 60 mM Na_3PO_4. After washing, the filters and filtration system were rinsed 3 times with Milli-Q water. The final rinse water had a neutral pH, indicating that all of the alkaline wash solution was removed.

After concentration all samples were diafiltered with 9–18 L of Milli-Q water to remove sea salts. The extent of carbon loss during diafiltration is dependent upon the concentration factor (see Eq. (1) below), with greater carbon losses occurring at lower concentration factors. Typically < 5% of the carbon retained in seawater during ultrafiltration was lost during diafiltration at the high concentration factors used in the present study. Diafiltered concentrates (~ 1 L) were placed in polycarbonate bottles and stored frozen. The frozen concentrates were brought to the laboratory in Port Aransas where they were thawed and reduced in volume to ~ 100 ml by rotary evaporation at 45–48°C. The samples were then dried under vacuum in a Savant SVC200 SpeedVac concentrator. The dried material was scraped from the flask, weighed, and stored in a freezer for chemical and isotopic analyses.

A mass balance of organic carbon was measured for every ultrafiltered sample to determine whether carbon was lost or gained during filtration. In ultrafiltration, the solution passing through the membrane is referred to as the permeate, and the solution containing the species retained by the membrane is referred to as the retentate. During the collection of particulate materials, the concentration of particulate organic carbon (POC) and bacterial abundance (see below) were measured in the initial water (< 60 μm sieve) and in the retentate from the H5MP01 filter. During the collection of dissolved materials, the concentration of DOC was measured in the initial water (< 0.2 or 0.1 μm pore size), the retentate from the S10N1 filters, and the permeate. The entire

Fig. 1. Scanning electron micrographs of particulate matter collected by tangential-flow ultrafiltration from several depths at 2°S, 140°W (micrographs A–D) and 12°S, 135°W (micrographs E–H). (A) Sample from 2 m with a large particle aggregate (pa), an intact pennate diatom and some diatom frustules (d). Picophytoplankton (pi), possibly a cyanobacterium. (B) Sample from 100 m with an intact comet-shaped aggregate, numerous bacteria (b), and submicron particles (sp). (C) Sample from 400 m with particle aggregates, bacteria, and submicron particles. (D) Sample from 4000 m with bacteria and submicron particles. (E) Sample from 2 m with an aggregate with some associated doughnut-shaped bacteria. (F) Sample from 100 m containing an aggregate with numerous attached bacteria and some picophytoplankton, possibly prochlorophytes. (G) Sample from 375 m contains an intact pennate diatom and the frustule of another diatom as well as bacteria and submicron particles. (H) Sample from 4000 m with bacteria and submicron particles.


02° S
12° S

volume of permeate was saved and subsampled for measurement of DOC because the concentration of DOC in the permeate increases during filtration as the concentration of DOC in the retentate increases.

2.3. Microscopy

Unfiltered seawater samples (5 ml) for the enumeration of bacteria were preserved with 2% (final concentration) of 0.2 μm-filtered formaldehyde and stored at 4°C. Samples were analyzed within a month of collection. Bacterial abundances were determined by epifluorescence microscopy at 1260 × magnification of DAPI-stained samples collected on 0.2 μm black Nuclepore filters, and 25–50 fields of view were examined on each filter (Porter and Feig, 1980). Conversion factors of 20×10^{-15} g C cell^{-1} and 5×10^{-15} g N cell^{-1} were used to estimate bacterial C and N in water samples (Lee and Fuhrman, 1987).

Samples of the UPOM concentrate were preserved with 4% glutaraldehyde in 1 M cacodylate buffer for examination by epifluorescence and electron microscopy. Epifluorescence microscopy (1260 ×) was performed with 0.1 ml samples by the same method as described above for the enumeration of bacteria in seawater samples. A Zeiss Universal microscope equipped with UV, blue and green light excitation filters was utilized. Samples (0.1–0.3 ml) for scanning electron microscopy (SEM; 5000–20,000 ×) were gently filtered onto 0.1 μm Nuclepore filters, dehydrated in an ethanol series, critical point dried, mounted on aluminum stubs with silver conducting paint and coated with gold/palladium. Blank filters were prepared using the same procedure. SEM was performed using a Phillips 515 microscope. Samples (20–30 μl) for transmission electron microscopy (TEM; 40,000–110,000 ×) were directly pipetted onto formvar coated TEM copper grids, desalted by addition of 15 μl of filtered distilled water, stained with 3 μl of filtered aqueous solution of 0.1% uranyl acetate, and dehydrated by successive additions of 50% ethanol and 100% ethanol. Between each of the preceding stages of sample preparation, the samples were allowed to settle on the grid and air dry for 30 minutes at room temperature. Blank grids were prepared using the same procedures. TEM was performed on a Phillips 300 microscope.

2.4. Measurements

Seawater samples (2–5 liters) were vacuum filtered through organic-free (450°C) Whatman GF/F filters for measurement of particulate organic carbon (POC). The carbon contents of filters and the carbon and nitrogen contents of dried samples of ultrafiltered materials were measured after vapor phase acidification using a Carlo Erba 1108 CHN analyzer (Hedges and Stern, 1984). Concentrations of dissolved organic carbon (DOC) were determined using a high temperature combustion method with a Shimadzu TOC 5000 analyzer (Benner and Strom, 1993). Stable C and N isotope compositions of dried and homogenized ultrafiltered materials were measured at Coastal Science Laboratories, a commercial laboratory in Austin, TX. Samples were combusted in evacuated quartz tubes at 850°C. Isotopic compositions are reported as

$$\delta^{N}E = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right) - 1\right] \times 1{,}000,$$

where ^{N}E is the heavy isotope of an element (^{13}C or ^{15}N) and R is the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N in the sample and the standard (PDB carbonate and atmospheric nitrogen). Typical reproducibility of analyses was ±0.1‰ for δ^{13}C and ±0.2‰ for δ^{15}N.

3. Results

3.1. Microscopic examination of ultrafiltered material

Samples of UPOM collected during the equatorial Pacific cruise were subjected to various types of microscopy to provide visual characterization of particles and colloids. Epifluorescence microscopy revealed that surface waters at 2°S had a greater abundance of larger phytoplankton (both centric and pennate diatoms) than surface waters at 12°S. Surface waters at both stations, however, had large numbers of picophytoplankton that were composed of cyanobacteria (confirmed by characteristic reddish–orange fluorescence under green light excitation) and prochlorophytes (not confirmed but see Chavez et al., 1990). The abundance of organisms and particles was greatly reduced in samples from the deep sea (4000 m).

Scanning electron microscopy (SEM) revealed further details of the components of UPOM. These results should be viewed as qualitative, because fields of view were patchy in terms of the distribution of organisms as well as particles. Nevertheless, they are of considerable value for making comparisons between the composition of UPOM from different water columns and depths. Similar to the observations made using epifluorescence microscopy, SEM showed that the surface waters were characterized by a relatively high diversity of both organisms and particles.

At 2°S, surface water UPOM consisted of larger phytoplankton (diatoms), picophytoplankton, bacteria and large aggregates (Fig. 1). Just below the euphotic zone comet-shaped aggregates could be observed. Such aggregates are characteristic of waters underlying productive euphotic zones (Alldredge and Silver, 1988). Samples from the oxygen minimum layer consisted of many small aggregates of amorphous particles and bacteria. In the deep sea there were fewer particles as well as bacteria. Particles in the deep sea were typically small (< 1 μm), and such particles could be found throughout the water column. We refer to these as submicron particles (see Koike et al., 1990).

At 12°S, the surface UPOM was characterized by picophytoplankton, bacteria and aggregates (Fig. 1).

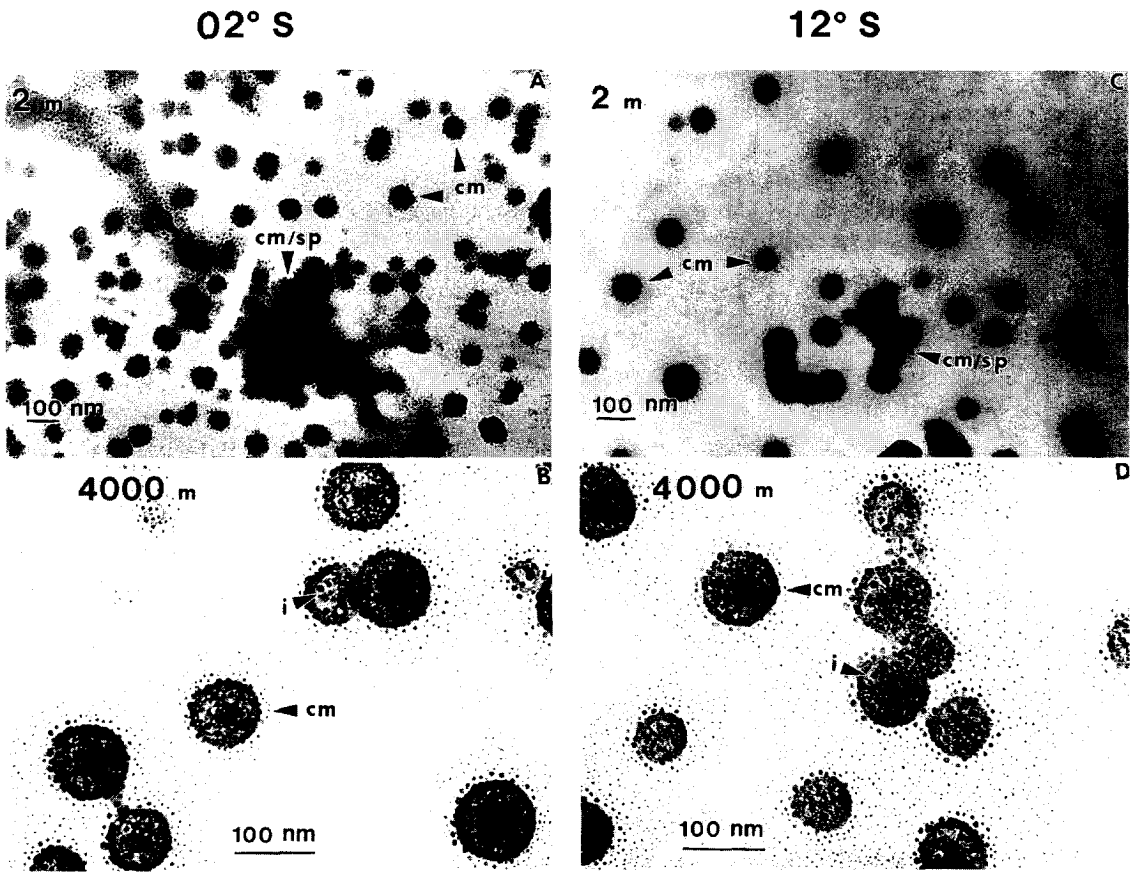


Fig. 2. Transmission electron micrographs of particulate material collected by tangential-flow ultrafiltration from surface and deep water at 2°S, 140°W (micrographs A–B) and 12°S, 135°W (micrographs C–D). (A) Sample from 2 m showing colloidal matter (cm) or submicron particles (sp). (B) Sample from 4000 m showing the composition of the ~ 100 nm sized colloidal matter. Note that the colloidal matter appears to contain smaller 5–15 nm inclusions (i). (C) Sample from 2 m showing colloidal matter or submicron particles. (D) Sample from 4000 m showing colloidal matter that contains smaller colloidal inclusions.

There were few large phytoplankton (> 10 μm), such as diatoms. Particle aggregates with associated doughnut-shaped bacteria were also observed. Below the euphotic zone aggregates were chiefly composed of detrital material with numerous attached bacteria and picophytoplankton. Some phytoplankton, apparently still viable as inferred from their chlorophyll autofluorescence under epifluorescence microscopy, were seen in association with aggregates in samples from the oxygen minimum layer. It appears they were transported below the mixed layer along with the sinking aggregates. In the deep sea there were generally fewer bacteria and submicron particles were relatively abundant.

Transmission electron microscopy (TEM) revealed even greater details of the physical composition of UPOM (Fig. 2). Again these observations are qualitative because the distribution of particles was highly patchy. At these high magnifications few bacteria were encountered and the "microscape" appeared to be exclusively composed of ~ 100 nm sized colloidal matter (see Wells and Goldberg, 1991). The larger-sized colloidal matter was in the same size range as the submicron particles described by Koike et al. (1990) and those visualized by us as submicron particles under the SEM (Fig. 1). Thus, the observed differences between submicron particles and colloidal matter may simply result from different techniques of visualization.

Samples from both stations revealed similar and uniform compositions. Colloidal matter was observed to be composed of even smaller 5–15 nm sized colloidal inclusions (Fig. 2b, d). These micrographs provide independent confirmation of the TEM observations of colloids in seawater that were made by Wells and Goldberg (1991). Whereas Wells and Goldberg (1991) centrifuged the colloids from seawater onto TEM grids, we have directly deposited material that was concentrated by ultrafiltration onto TEM grids. That these two different approaches of gathering material from seawater yielded similar results could indicate that colloids exist in seawater in the form and abundances visualized under the TEM and that ultrafiltration retains a fairly representative fraction of the colloidal matter present in seawater.

3.2. Abundance and distribution of UPOM

Recovery efficiencies of POC (0.1–60 μm) by tangential-flow ultrafiltration with a hollow-fiber filter (H5MP01) were less than 100% in surface waters when compared to the retention of POC on a Whatman GF/F filter (0.7 μm nominal pore-size; Table 2). The average (±SD) percentage of surface water

Table 2
Concentrations of POC, UPOC, the percentage of POC recovered by ultrafiltration, bacterial abundances, the percentage of bacteria recovered by ultrafiltration, and the percentages of UPOC and UPON in bacterial biomass. See Table 1 for sample descriptions; nd = not determined

Sample #	Depth (m)	POC (μM)	UPOC (μM)	%POC recovered	Bacteria (10^8 cells liter^{-1})	Bacteria (% recovered)	Bacterial C (% UPOC)	Bacterial N (% UPON)
1	2	nd	2.69	nd	1.63	21.7	2.2	3.5
2	2	1.35	0.67	50	0.85	58.4	12.4	22.8
3	2	1.95	0.66	34	1.51	55.2	21.0	36.4
4	2	1.18	0.63	54	1.01	52.5	14.0	24.3
5	2	1.56	0.83	53	1.06	41.7	8.9	15.0
7	2	1.82	1.02	56	1.13	55.5	10.2	16.9
8	100	0.95	0.46	49	2.46	8.7	7.7	11.6
9	400	0.32	0.51	159	0.85	12.5	3.5	5.5
10	400	0.53	0.71	134	0.71	62.1	10.3	17.9
11	4000	0.32	0.35	109	0.12	36.0	2.1	3.3
12	2	1.88	0.83	44	1.13	8.1	1.8	3.0
13	100	1.30	0.82	63	1.28	33.4	8.7	13.9
14	200	0.42	0.32	77	2.43	3.2	4.0	6.6
15	375	0.47	0.43	91	1.17	4.6	2.1	3.9
16	4000	0.28	0.32	115	0.14	39.4	2.7	4.1

POC recovered by ultrafiltration was 48.5 ± 8.2%. However, the relative recovery of POC by ultrafiltration increased to values exceeding 100% of the POC retained on a glass fiber filter in deeper waters (Table 2). This difference in the recovery efficiencies of UPOC from surface and deep waters does not appear to be due to enhanced recovery of POC in deep water, because the recovery efficiencies of bacterial cells were similar in surface (42%) and deep (36%) waters (Table 2). It appears that the enhanced recoveries of POC in deep water by ultrafiltration relative to the retention of POC on a glass fiber filter resulted from a shift in the size spectrum of POC from surface to deep waters. The ultrafilter retains particles ≥ 0.1 μm in size whereas the glass fiber filter retains particles ≥ 0.7 μm. Deep water appears to have a greater fraction of POC as submicron or colloidal particles in the 0.1 to 0.7 μm size range than do surface waters. The average (±SD) concentrations of UPOC and UPON in surface waters was 1.05 ± 0.74 μM C and 0.13 ± 0.10 μM N (Table 3). Deep water concentrations were considerably lower and averaged 0.34 μM C and 0.05 μM N (Table 3).

The incomplete recovery of bacteria and POC by ultrafiltration is caused by adsorption of materials to the membrane. Permeates were examined for bacterial abundance by epifluorescence microscopy and were found to be essentially free of cells, indicating that cell losses were due to adsorption. Another indication that materials were adsorbing to the filter was the observed reduction of filtration rates as concentration factors increased. The recovery of adsorbed materials can be enhanced at the end of filtration by increasing the pressure differential between the filter inlet and outlet (fast forward flush) or by reversing the flow through the membrane (back flush; Amicon). We routinely used the fast forward flush method to enhance recoveries, but the effectiveness of this method was highly variable as indicated by the wide range of recoveries (3–62%) of bacterial cells (Table 2). The contributions of bacterial carbon and nitrogen to UPOC and UPON averaged (±SD) 7.4 ± 5.6% and 12.6 ± 9.9%, respectively (Table 2).

3.3. Concentration and isolation of DOM by ultrafiltration

Tangential-flow ultrafiltration was used to isolate the high-molecular-weight components of DOM to investigate their chemical and isotopic composition. The retention of solutes during ultrafiltration is primarily dependent upon their size and shape, but a variety of other factors including the type of membrane and the operating conditions affect retention

Table 3
Concentrations and stable isotope compositions of carbon and nitrogen in ultrafiltered particulate organic matter (UPOM) from the Pacific Ocean. See Table 1 for sample descriptions; nd = not determined

Sample #	Depth (m)	Dry wt. (mg)	Wt. %C	Wt. %N	UPOC (μM)	UPON (μM)	C/N (atom)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
1	2	250	12.87	2.01	2.69	0.36	7.5	−22.2	10.6
2	2	134	5.97	0.81	0.67	0.08	8.7	−23.5	5.8
3	2	194	4.09	0.59	0.66	0.08	8.0	−22.9	7.8
4	2	218	3.47	0.50	0.63	0.08	8.1	−22.6	nd
5	2	267	3.73	0.55	0.83	0.10	7.9	−23.0	10.4
7	2	157	4.70	0.71	1.02	0.13	7.7	−22.1	7.2
8	100	179	3.06	0.51	0.46	0.07	7.0	−25.4	7.9
9	400	170	3.51	0.56	0.51	0.07	7.4	−24.9	nd
10	400	112	6.10	0.88	0.71	0.09	8.0	−24.4	8.6
11	4000	175	2.36	0.38	0.35	0.05	7.2	−26.0	9.7
12	2	215	5.16	0.78	0.83	0.11	7.7	−22.8	14.6
13	100	148	6.58	1.03	0.82	0.11	7.4	−24.2	13.6
14	200	131	3.02	0.46	0.32	0.04	7.6	−25.9	15.4
15	375	115	4.56	0.62	0.43	0.05	8.6	−26.2	10.7
16	4000	145	2.72	0.46	0.32	0.05	7.0	−24.8	8.5

(Cheryan, 1986; Buesseler et al., 1996). The ultrafiltration membranes and operating conditions used in this study were closely monitored to ensure that comparable results were obtained. Flow rates, inlet and outlet pressures, and water temperatures were recorded during each isolation, and molecular-weight standards were analyzed periodically to determine that the retention characteristics of the membranes remained constant.

The percentage of DOM isolated by ultrafiltration and the size spectrum of retained solutes is influenced by the ratio of the initial sample volume to the final retentate volume. This ratio is referred to as the concentration factor (CF) and is defined as:

$$\text{Concentration Factor} = \frac{\text{Initial sample volume}}{\text{Retentate volume}}. \quad (1)$$

The retention of solutes during ultrafiltration can be modeled if the probability that a solute will pass through a membrane is known (Cheryan, 1986). The probability a solute will pass through a membrane is dependent on the extent of rejection (σ), which is defined as:

$$\text{Rejection}(\sigma) = 1 - (C_p/C_r), \quad (2)$$

where C_p is the concentration of solute in the permeate and C_r is the concentration of solute in the retentate. Natural waters likely contain a large mixture of components with varying rejection coefficients ranging from near 0 for low-molecular-weight (LMW) components to near 1.0 for high-molecular-weight (HMW) components. We modeled the behaviour of DOM containing three fractions with varying rejection coefficients to demonstrate how the size spectrum of DOM in the retentate changes during ultrafiltration. We assumed that components of DOM with MWs greater than 5,000 Daltons were completely rejected ($\sigma = 1$) by the 1,000 molecular-weight cutoff (MWCO) membrane, components with MWs ranging from 500 to 5,000 Daltons had a rejection coefficient of 0.5, and components less than 500 Daltons freely passed the membrane ($\sigma = 0$). The concentrations of solutes during ultrafiltration were modeled using the following equation:

$$C = C_o(\text{CF})^{\sigma} \quad (3)$$

where C is the concentration of solute at any time during the filtration, C_o is the initial concentration of the solute, CF is the concentration factor (Eq. (1)), and σ is the rejection coefficient (Eq. (2)).

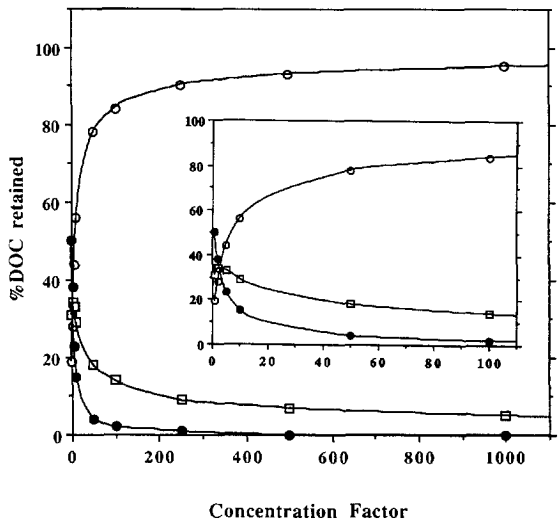


Fig. 3. Modeled retention at varying concentration factors of a mixture of three components of DOC with different membrane rejection coefficients during tangential-flow ultrafiltration. The high-molecular-weight component (open circles) with a rejection coefficient of 1.0 accounted for 20% of the initial DOC. The intermediate-molecular-weight component (open squares) with a rejection coefficient of 0.5 accounted for 30% of the initial DOC, and the low-molecular-weight component (solid circles) with a rejection coefficient of 0.0 accounted for 50% of the initial DOC.

The concentration of low-molecular-weight (LMW) components that freely pass the membrane remains constant in the retentate during ultrafiltration, but the percentage of carbon in the retentate as LMW components rapidly decreases as the concentration of retained components and the CF increases (Fig. 3). We assumed that LMW components comprised 50% of the initial DOC. At a CF of 100, the LMW components accounted for only 2% of the retained carbon. Given that a CF of 200 or greater was used for all DOM isolations from natural waters (Table 1), we assumed that LMW components contributed minimally to the isolated DOM. Intermediate MW components with 50% rejection initially accounted for 30% of the DOC, but by a concentration factor of 100 they accounted for only 14% of the DOC. The HMW components with 100% rejection accounted for 95% of the retained DOC at a CF of 1,000. These results indicated that the predominant components in UDOM collected at high CFs are

HMW materials that are completely rejected by the 1,000 MWCO membrane.

The CFs used in this study are much higher than those commonly used to estimate the molecular weight distribution of DOM (e.g., Carlson et al., 1985). It is obvious from the above example, however, that at CFs less than 10 the retentate contained a significant fraction (> 15%) of LMW DOM that could freely pass the membrane. Therefore, when estimating the molecular weight distribution of DOM by ultrafiltration a correction is normally made to account for the components in the retentate that could pass through the membrane (e.g., Carlson et al., 1985). The effects of CF on the retention of DOC for the above 3-component example are shown in Fig. 4. The percentage of DOC retained decreases rapidly as the CF increases from 2 to 10. At CFs greater than 100, however, the percentage of DOC retained remains fairly constant at around 20%. Even though the modeled results represent an oversimplification of the size spectrum of naturally occurring DOM, they are similar to those we observed during the ultrafiltration of samples discussed herein. Retention of DOC became independent of CF at the high CFs used in the present study, indicating that the components in the retentate consisted predominantly of HMW DOM (i.e., > 1,000 Dalton).

3.4. Abundance and distribution of UDOM

Water samples were collected from a wide range of oceanic environments ranging from highly productive surface waters at the equatorial upwelling to oligotrophic waters in the Pacific and Atlantic gyres and from the euphotic zone to the deep ocean. Surface water DOC concentrations ranged from 70 to 95 μM C with an average value of 78 μM C (Table 1). Deep water (> 500 m) DOC concentrations ranged from 38 to 48 μM C (Table 1). The average deep water DOC concentration was 44 μM C. These DOC concentrations are typical for oceanic waters and similar to values recently measured in the same oceanic regions (Sharp et al., 1993; Sharp et al., 1995).

The percentage of surface water DOC retained by ultrafiltration using the 1,000 Dalton MWCO filter ranged from 22 to 38% with an average ($\pm$SD) value of 30 ± 5.2% (Table 1). Consistently lower percentages of DOC, ranging from 18 to 25%, were isolated from deep waters (Table 1). An average ($\pm$SD) of 22 ± 2.4% of deep water DOC was isolated. Mass balance calculations indicated that an average ($\pm$SD) of 101 ± 12.9% of the DOC in the initial water samples was accounted for in the retentate and permeate fractions (Table 1). These results indicate that carbon contamination and adsorption during sample handling and processing were minimal. The processing of large volume samples produced substantial quantities (0.5–1.0 g) of dried material with an organic carbon content of about 20 weight % (Table 4). The first samples we collected, samples 17–19, 23 and 24, were diafiltered with 9 to 12 liters of Milli-Q water and had considerably lower weight percentages of carbon due to the presence of sea salts (Table 4). All other samples were diafiltered with 18 liters of Milli-Q water and had higher weight percentages of organic carbon (Table 4).

The isolation of seawater DOM as a dry powder with a substantially reduced salt content makes the analysis of carbon and nitrogen content in an elemental (CHN) analyzer fairly routine. The concentrations of UDOC and UDON recovered from surface waters were similar in all environments and averaged

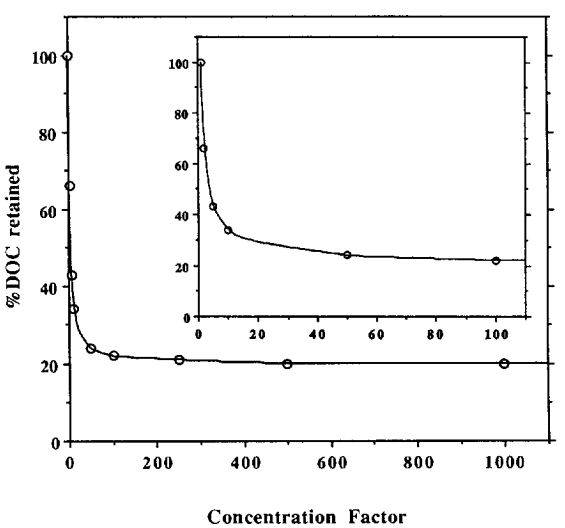


Fig. 4. Modeled retention of total dissolved organic carbon (DOC) at varying concentration factors during tangential-flow ultrafiltration of a mixture of three components with different membrane rejection coefficients (as in Fig. 3).

Table 4
Concentrations and stable isotope compositions of carbon and nitrogen in ultrafiltered dissolved organic matter (UDOM). See Table 1 for sample descriptions. nd = not determined

Sample #	Depth (m)	Dry wt. (mg)	Wt. %C	Wt. %N	UDOC (μM)	UDON (μM)	C/N (atom)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Pacific Ocean									
1	2	922	22.6	1.67	17.3	1.10	15.8	−21.9	7.4
2	2	1853	12.1	0.85	18.7	1.12	16.6	−21.9	7.4
3	2	874	25.4	1.80	18.5	1.12	16.4	−21.7	7.8
4	2	1006	22.3	1.48	18.7	1.06	17.5	−21.7	7.9
5	2	930	24.3	1.79	18.8	1.19	15.9	−21.4	8.1
6	2	490	19.0	1.35	19.4	1.18	16.4	−21.7	7.7
7	2	791	19.7	1.43	21.7	1.35	16.1	−21.6	7.1
8	100	716	23.6	1.66	14.4	0.87	16.6	−21.7	6.7
9	400	612	21.1	1.45	9.14	0.54	17.0	−21.7	nd
10	400	745	15.1	1.05	11.7	0.70	16.7	−21.6	7.5
11	4000	504	18.8	1.19	8.02	0.44	18.4	−21.8	7.3
12	2	928	29.0	2.02	22.2	1.33	16.8	−21.4	8.3
13	100	989	26.8	2.00	22.2	1.42	15.6	−21.8	9.7
14	200	774	20.1	1.45	12.6	0.78	16.2	−21.5	8.5
15	375	705	18.4	1.27	10.6	0.63	16.9	−21.7	7.9
16	4000	678	14.6	0.95	8.08	0.45	18.4	−21.6	7.8
17	10	842	5.95	0.46	20.9	1.38	15.3	−21.6	8.7
18	765	1091	1.70	0.09	7.73	0.35	22.5	−21.3	8.6
19	4000	1097	1.77	0.11	8.09	0.43	19.6	−21.4	8.3
Atlantic Ocean									
20	1	615	19.2	1.31	16.4	0.96	17.1	−22.2	6.6
21	900	319	18.6	1.39	8.25	0.53	15.6	−21.7	8.5
22	2400	399	10.5	0.68	9.20	0.51	18.1	−22.1	8.9
Gulf of Mexico									
23	10	1483	17.9	1.18	21.1	1.19	17.8	nd	9.5
24	750	1867	6.98	0.48	9.45	0.56	16.9	−21.3	10.2

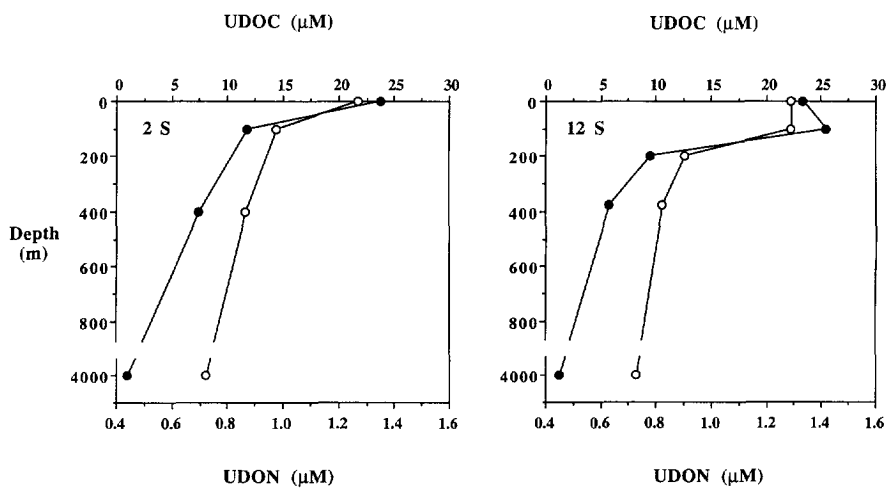


Fig. 5. Concentrations of ultrafiltered dissolved organic carbon (UDOC; open circles) and nitrogen (UDON; closed circles) in the water columns at 2°S 140°W and 12°S 135°W. UDOC and UDON are also referred to as high-molecular-weight (HMW) DOC and DON.

(±SD) 19.4 ± 1.84 μM C and 1.10 ± 0.05 μM N (Table 4). Concentrations of UDOC and UDON declined rapidly with depth in the upper 200 m of the water column (Fig. 5a, b). The three deep water (4000 m) samples from the Pacific Ocean had very consistent UDOC (8.06 ± 0.04 μM C) and UDON (0.44 ± 0.01 μM N) concentrations. Based on our limited data, deep water (> 500 m) UDOC and UDON concentrations in the Atlantic (8.73 μM C and 0.52 μM N) appear to be higher than those in the Pacific (Table 4), but additional sampling is needed to confirm this trend. Previous studies have noted higher concentrations of DOC in deep waters of the Sargasso Sea relative to those in the North Central Pacific (Druffel et al., 1992). The average (±SD) deep water UDOC and UDON concentrations in all environments were 8.52 ± 0.64 μM C and 0.49 ± 0.05 μM N (Table 4).

3.5. C and N concentrations and isotopic compositions of UPOM and UDOM

The C:N ratios of UPOM were similar in surface and deep waters; values ranged from 7.0 to 8.7 and averaged (±SD) 7.7 ± 0.5 (Table 3). The C:N ratios of UDOM were more variable and ranged from 15.3–22.5 with an average (±SD) value of 17.1 ± 1.6 (Table 4). The C:N values of UPOM and UDOM from two depth profiles in the Pacific Ocean are presented in Fig. 6. The C:N values of UDOM were considerably higher than values for UPOM, indicating a major difference in the bulk chemical composition of the two size fractions of organic matter. The C:N values of UDOM were slightly higher (18.4) in deep waters than in surface waters (16.5) indicating a selective removal of nitrogen.

The stable carbon isotope compositions ($\delta^{13}C$) of UPOC were considerably heavier in surface waters than in subsurface waters. The average (±SD) $\delta^{13}C$ values of UPOC in surface and subsurface waters were −22.7 ± 0.5‰ and −25.2 ± 0.8‰, respectively (Table 3). The $\delta^{13}C$ values of UPOC are all depleted in ^{13}C relative to values for UDOC. The average (±SD) $\delta^{13}C$ value of UDOC was −21.7 ± 0.2‰ (Table 4). Two depth profiles of the $\delta^{13}C$ values of UPOC and UDOC are presented in Fig. 7. The $\delta^{13}C$ values of UDOC remained fairly constant with depth, whereas values for UPOC became lighter in subsurface waters. The average $\delta^{13}C$ value of UPOC from subsurface waters was 3.6‰ lighter than the average value for UDOC.

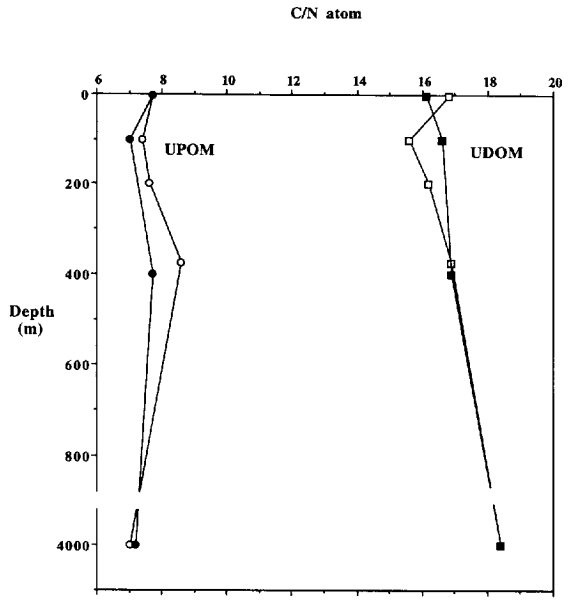


Fig. 6. Atom ratios of C:N in ultrafiltered particulate and dissolved organic matter (UPOM and UDOM) collected from 2°S 140°W (solid circles) and 12°S 135°W (open circles).

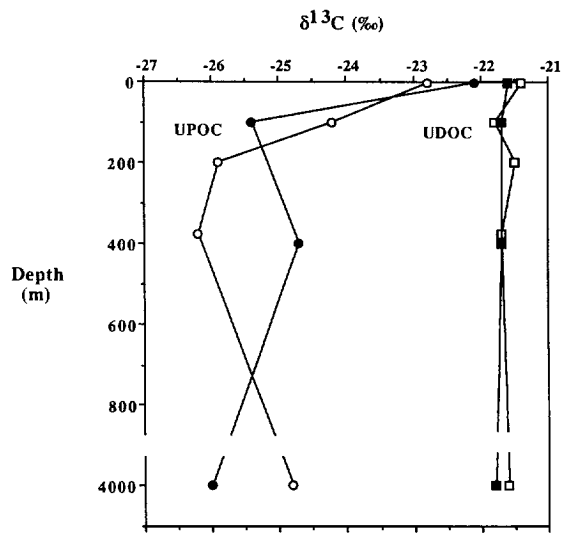


Fig. 7. Stable carbon isotopic compositions of ultrafiltered particulate and dissolved organic matter (UPOM and UDOM) collected from 2°S 140°W (solid circles) and 12°S 135°W (open circles).

Stable nitrogen isotope compositions ($\delta^{15}N$) of UPON were variable in surface waters, with values ranging from 5.8 to 15.4‰ (Table 3). The UPON from the region of the equatorial upwelling generally had the lightest $\delta^{15}N$ values (7–9‰), whereas UPON from the oligotrophic gyre south of the upwelling region had the heaviest $\delta^{15}N$ values (11–15‰). These values are somewhat higher than those reported by Altabet and Francois (1994), and the difference could reflect seasonal variability or variability among different size classes of suspended POM.

The $\delta^{15}N$ values of UDON were also spatially variable. The lightest $\delta^{15}N$ values (6.7 and 7.1‰) for UDON samples from the Pacific Ocean were found in samples from the surface and the chlorophyll maximum (100 m) in the region of the equatorial upwelling (Table 4). Surface and deep water $\delta^{15}N$ values for UDON from areas outside of the equatorial upwelling in the Pacific Ocean averaged ($\pm$SD) 7.9 ± 0.4‰ and 8.0 ± 0.6‰, respectively. Surface water UDON from the BATS station in the Sargasso Sea had a considerably lighter $\delta^{15}N$ value (6.6‰) than deep water UDON (8.5 and 8.9‰), suggesting a possible contribution from recently fixed nitrogen or recycled nitrogen in surface waters (Altabet, 1988). The two UDON samples from the Gulf of Mexico had heavier $\delta^{15}N$ values of 9.5 and 10.2‰.

The $\delta^{15}N$ values of particulate samples were consistently heavier than the $\delta^{15}N$ values of dissolved samples collected at the same station, with the exception of sample # 2 (Tables 3 and 4). A comparison of the $\delta^{15}N$ values of UPON and UDON for two depth profiles is shown in Fig. 8. The $\delta^{15}N$ values of UPON and UDON were similar in the upper water column at the 2°S station, whereas the $\delta^{15}N$ values of UPON in the upper water column at the 12°S station were 2.8 to 6.9‰ heavier than values for UDON samples. The $\delta^{15}N$ values of UPON and UDON at the 12°S station were heavier than the respective values for the 2°S station, with the exception of the $\delta^{15}N$ of UPON samples from 4000 m.

4. Discussion

4.1. Size continuum of organic matter in the ocean

The present study demonstrated the practicality of tangential-flow ultrafiltration for the concentration and isolation of different size classes of material for microscopic, chemical, and isotopic characterization. The ultrafiltration methods used in the present study were relatively fast, clean, and efficient, and they resulted in the isolation of hundreds of milligrams of

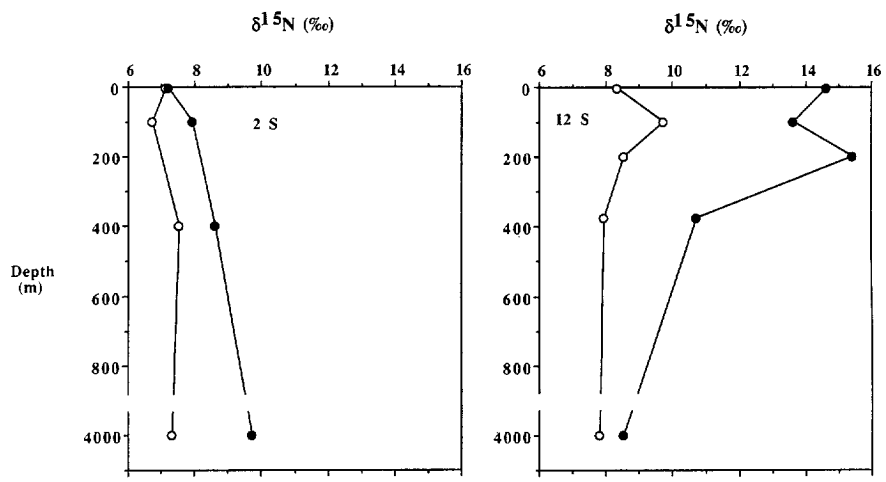


Fig. 8. Stable nitrogen isotopic compositions of ultrafiltered particulate organic matter (UPOM; solid circles) and ultrafiltered dissolved organic matter (UDOM; open circles) collected from 2°S 140°W and 12°S 135°W.

material for further characterization. Ultrafiltration was effective in the isolation of the major types of marine particles, including phytoplankton and other microorganisms, amorphous aggregates and colloidal matter, as well as components within the operationally-defined dissolved pool. Microscopic examination of ultrafiltered materials indicated that relatively fragile components were recovered intact without any apparent alteration.

Microscopy also revealed that marine particles exist in a size continuum from recognizable cellular material to submicron particles and colloids. Comparison of the present study to previous studies indicates that nearly all the particle types described by earlier workers have been visualized here. Particle aggregates of the type described by Gordon (1970), Wiebe and Pomeroy (1972) and Alldredge and Silver (1988) were recorded to the depths of the oxygen minimum layer. Micron and submicron particles of the type described by Koike et al. (1990) and Wells and Goldberg (1992) were visualized by SEM at all depths in the water column. Colloidal matter as described by Wells and Goldberg (1991) was also visualized by TEM throughout the water column. Submicron particles appeared as colloidal matter under TEM, suggesting a common source for these materials.

The observed size distribution of organic carbon in the ocean is skewed towards greater abundances in the smaller size classes. Results from the present study of the abundance and size distribution of particulate and dissolved organic matter indicate that ~ 75% of marine organic carbon resides in low-molecular-weight DOM (< 1 nm), ~ 24% resides in high-molecular-weight DOM (1–100 nm), and ~ 1% resides in suspended particles (> 100 nm). The 1–100 nm size class (i.e. HMW DOM) likely includes colloidal as well as dissolved organic matter, but the quantitative contribution of these physically distinct materials was not determined. The distribution of carbon in the surface ocean was shifted to greater relative abundances of larger size fractions. In the surface ocean ~ 69% of organic carbon resides in low-molecular-weight DOM (< 1 nm), ~ 29% resides in high-molecular-weight DOM (1–100 nm), and ~ 2% resides in suspended particles (> 100 nm). Similar size distributions of organic carbon have been observed in the coastal ocean (Carlson et al., 1985; Ogawa and Ogura, 1992; Guo et al., 1995).

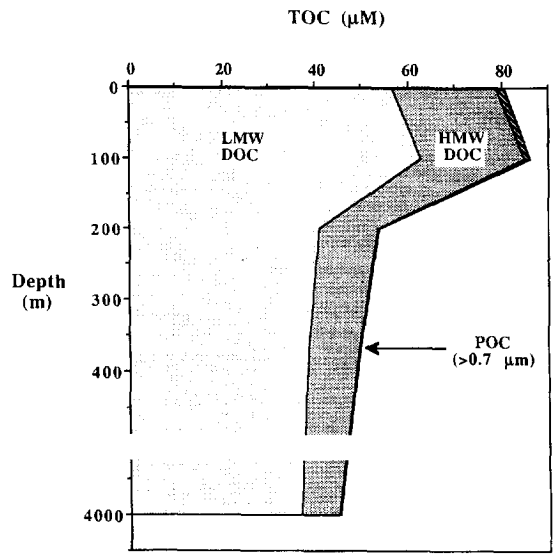


Fig. 9. Depth distribution of organic carbon in three size classes of organic matter at 12°S 135°W. Low-molecular-weight dissolved organic carbon (LMW DOC; < 1 nm), high-molecular-weight dissolved organic carbon (HMW DOC; > 1 nm < 700 nm), particulate organic carbon (POC; > 700 nm).

The depth distribution of organic carbon in three size classes of organic matter in the water column of the open ocean is presented in Fig. 9. Concentrations of low-molecular-weight DOC range from 60 μM in surface water to 37 μM in deep water. Concentrations of HMW DOC range from 22 μM in surface water to 8 μM in deep water. Particulate organic carbon (POC) concentrations range from 1.9 μM C in surface water to 0.3 μM C in deep water. Assuming that deep water concentrations of organic carbon are representative of refractory carbon throughout the water column, the "excess" carbon found in surface water is representative of reactive carbon. By this calculation, a total of 39 μ M C is reactive. Of the reactive carbon, 23 μM (59%) is LMW DOC, 14 μM (37%) is HMW DOC, and 1.6 μM (4%) is POC. It is interesting to note that this calculation indicates that small molecules comprise the largest fraction of reactive carbon even though this size class is the least reactive overall. Only 38% of the LMW DOC in surface waters is reactive, whereas 64% of HMW DOC and 85% of POC are reactive.

These observations are suggestive of a general diagenetic progression from larger particles to smaller molecules during organic matter decomposition in the ocean.

4.2. C:N ratios and potential interactions between POM and DOM

The concentration of nitrogen in the POM samples collected on glass fiber filters were too low to provide reliable data, but there was sufficient particulate material in the ultrafiltered samples (UPOM) for C and N analysis. The average C:N value of 7.7 for UPOM is similar to values measured previously for particulate matter in the ocean (e.g., Bishop et al., 1977) and to expected values based on Redfield stoichiometry. There are relatively few measurements of the C:N composition of DOM, and the reported values are variable with most falling between 10 and 20 (Jackson and Williams, 1985; Hansell et al., 1993; Hansell and Waterhouse, 1997; Williams, 1995). The C:N values measured for HMW DOM (15–22) fall within the range of values reported for marine DOM, indicating that ultrafiltration recovers a similar fraction of total DOC and DON.

Phytoplankton can produce carbon-rich DOM (Williams, 1995; Biddanda and Benner, 1997) and heterotrophic processes can preferentially remineralize nitrogen-rich components of DOM. The relative significance of these processes in determining the observed C:N ratios in DOM is presently unknown. If phytoplankton are directly responsible for the high C:N values in DOM, estimates of new production and carbon export from the upper ocean could be significantly underestimated. If heterotrophic processes are responsible for the high C:N values in DOM, the microbial loop may play a greater role regulating the composition and concentration of organic matter and nutrients in the ocean than previously recognized. The constancy of C:N values in HMW DOM from different oceanographic regions suggests common origins and diagenetic processes for this material.

Differences in C:N and $\delta^{13}C$ values between UPOM and UDOM were relatively large and geographically invariant. If all UPOM formed UDOM, the average C:N value of UDOM would change from 16.7 to 15.8, which is within the range of measured values for UDOM. Likewise, changes in the $\delta^{13}C$ of UDOM ($-21.7‰$ to $-21.8‰$) would be minimal if UPOM were solubilized. Therefore, these data put no constraints on the dissolution of UPOM and the formation of UDOM. However, these data do provide constraints on the possible aggregation of UDOM to form UPOM. If only 5% of the UDOM aggregated to form UPOM, the average C:N value of UPOM would change from 7.7 to 10.6, a value considerably higher than those measured for UPOM. The average $\delta^{13}C$ of UPOM would change from $-24.1‰$ to $-22.9‰$, a value that is enriched in ^{13}C relative to all other subsurface UPOM samples. These data do not exclude the potential aggregation of UDOM to form UPOM, but they indicate that this process is of minor significance to overall carbon dynamics assuming the process is not chemically selective.

4.3. C and N isotopes and the origin and reactivity of POM

A significant decrease in the $\delta^{13}C$ values of UPOC below the mixed layer was observed at both stations in the equatorial Pacific. Previous studies attributed similar changes in the isotopic composition of POC to the selective decomposition of isotopically heavy components, such as carbohydrates and amino acids, and the relative enrichment of the remaining material in isotopically light lipids (Eadie and Jeffrey, 1973; Eadie et al., 1978; Jeffrey et al., 1983). Other studies have not observed such clearly defined depth-dependent isotopic shifts for marine POC (Williams and Gordon, 1970; Bishop et al., 1977; Druffel et al., 1997). Differences among $\delta^{13}C$ values of POC in these studies could also result from spatial and temporal variations in planktonic communities.

A few studies have observed significant variability in $\delta^{13}C$ values among different size classes of POC. Smaller size classes of POC are generally depleted in ^{13}C by several permil relative to larger size classes (Bishop et al., 1977; Altabet, 1990; Rau et al., 1990). Rau et al. (1990) suggested that such variability reflects the nature of carbon and nitrogen cycling and subsequent isotopic fractionation within microbial food webs. This suggestion is consistent with the observation of systematic increases in $\delta^{13}C$ and in $\delta^{15}N$ as a function of trophic level (Rau et al., 1983; Minagawa and Wada, 1984). The size

spectrum of particles isolated in the present study clearly includes smaller particles than those isolated previously. The concentrations of POC isolated from Pacific deep water in the present study were about 50% higher than those recovered on quartz fiber filters (0.8 μm pore size; Druffel et al., 1997). Likewise, Altabet (1990) found that about 40% of the POC in Sargasso Sea water passed a glass fiber filter (GF/F; 0.7 μm pore size) and was retained on a 0.2 μm pore-size filter. Particle retention is dependent upon sample loading when using glass and quartz fiber filters, so studies using the same type of filter can recover different sizes of particles. Differences in $\delta^{13}C$ values of POC among studies could simply reflect differences in particle sizes sampled, with the lighter isotopic values representing smaller particles.

It is also possible that light $\delta^{13}C$ values for POC are indicative of a terrestrial origin for the smaller particles existing in the open ocean. Chesselet et al. (1981) found that atmospheric particles over the north Pacific and Atlantic Oceans had uniform $\delta^{13}C$ values in the range of -26.5 to -26.7‰ indicating a terrestrial origin. Most ($>80\%$) of the carbon in atmospheric particles is associated with very small (<0.5 μm) particles (Hoffman and Duce, 1977; Chesselet et al., 1981). Such small particles would largely be missed by filtration through glass and quartz fiber filters and thereby included in the "dissolved" fraction of marine organic matter. Small particles within the operationally defined DOC pool account for less than 1% of the carbon, so they would not significantly influence the bulk isotopic composition of DOC.

The nitrogen isotopic composition of UPOM showed no consistent relationship with the carbon isotopic composition as might be expected if this material was primarily terrestrially-derived. Instead, the nitrogen isotope compositions of UPOM appeared to reflect differences in the availability of nitrate supporting phytoplankton production. The equatorial region was experiencing El Niño conditions at the time we collected samples in February–March 1992, but nitrate concentrations were ~ 2.5 μM in surface waters indicating that shallow upwelling was occurring (Murray et al., 1994). In contrast, nitrate concentrations in surface waters at the 12°S station were ~ 0.3 μM. A relatively low $\delta^{15}N$ value (7.2‰) was measured for UPOM from surface water in the upwelling region, whereas a high $\delta^{15}N$ value (14.6‰) was measured for UPOM from 12°S.

Such an inverse relationship between nitrate concentrations and $\delta^{15}N$ values of surface particulate matter has been observed previously (Wada and Hattori, 1976), including in the same region of the equatorial Pacific (Altabet and Francois, 1994). Phytoplankton discriminate against $^{15}NO_3$ relative to $^{14}NO_3$, so phytoplankton are depleted in ^{15}N relative to nitrate, and the $\delta^{15}N$ of nitrate increases as the concentration of nitrate decreases (Wada and Hattori, 1978). The $\delta^{15}N$ of nitrate in upwelled waters should be 6–7‰, the isotopic composition of subsurface nitrate in the region (Liu and Kaplan, 1989). Altabet and Francois (1994) observed high nitrate concentrations (~ 6 μM) in equatorial surface waters and low $\delta^{15}N$ values for suspended particulate matter (~ 2‰) during August–September of 1992 when El Niño conditions subsided. At the same time they observed low nitrate concentrations (~ 0.4 μM) and a high $\delta^{15}N$ value (~ 14‰) for particulate matter at 12°S. Our results and those of Altabet and Francois (1994) are consistent with each other, and they demonstrate that large isotopic shifts in particulate nitrogen can occur over relatively short spatial and temporal scales. These large and rapid shifts in $\delta^{15}N$ reflect the dynamic nature of POM in the surface ocean.

The N isotopic signature of PON in surface water is evident in suspended PON collected directly below the euphotic zone (see Fig. 8). Likewise, Saino and Hattori (1987) demonstrated that the $\delta^{15}N$ of PON from below the euphotic zone was correlated with its surface water source in a series of 13 profiles collected from various regions of the Pacific Ocean. These observations suggest that suspended PON in the upper ocean is largely derived from surface water production. However, relatively large shifts in the $\delta^{15}N$ of PON with depth were also observed by Saino and Hattori (1980, 1987) and are evident in the profiles in the present study (Fig. 8). Saino and Hattori (1980, 1987) typically observed increases in the $\delta^{15}N$ of PON with depth, and they attributed the enrichment of ^{15}N in deep water PON to isotopic fractionation during decomposition. We observed a shift to lighter isotopic values with depth at the 12°S station (Fig. 8), and isotopic fractionation

during decomposition cannot explain this depth-related trend. It appears that some of the suspended PON in deeper waters is produced in situ or is transported along isopycnals from other geographic regions as suggested previously (Altabet et al., 1991).

4.4. C and N isotopes and the origin and reactivity of DOM

The stable carbon isotopic composition of DOC is relatively invariant with depth and geographic region in the ocean. The vast majority of measurements of the $\delta^{13}C$ of marine DOC fall in the narrow range of -20 to $-22‰$ (Williams, 1968; Williams and Gordon, 1970; Eadie et al., 1978; Williams and Druffel, 1987; Bauer et al., 1992; Druffel et al., 1992). The $\delta^{13}C$ values of all UDOM samples isolated in the present study are similar and fall in a narrow range of -21 to $-22‰$. These $\delta^{13}C$ values indicate a predominantly marine origin for HMW DOC in the open ocean because they are similar to values for marine plankton and POM (Fry and Sherr, 1984), and they are enriched in ^{13}C relative to $\delta^{13}C$ values (-27 to $-29‰$) of riverine POC and DOC (Hedges et al., 1994). The similarity between $\delta^{13}C$ values of HMW DOC and the total DOC pool indicates that LMW DOC must also have a similar carbon isotopic composition and origin. Radiocarbon measurements of DOC in the deep ocean ($>$ 1,000 m) yield apparent ages of 4,000–6,000 years indicating it is relatively old and resistant to oxidation (Williams and Druffel, 1987; Bauer et al., 1992). Given the observed carbon isotopic compositions for DOC in the deep ocean, it appears that most refractory DOC is of marine origin.

The $\delta^{15}N$ values for HMW DON presented in this study are the first such values reported for any fraction of DON in the ocean. They are particularly exciting because they reveal much more about the reactivity of DOM than can be discerned from stable carbon isotopic compositions. The observed range of $\delta^{15}N$ values (6.6–10.2‰) for UDON appears principally to reflect variability in the isotopic composition of the sources of DON rather than isotopic fractionation during decomposition, because there was no significant correlation between the $\delta^{15}N$ of UDON and UDON concentrations. The $\delta^{15}N$ values of UDON were less variable than those for UPON, suggesting that the smaller PON reservoir is more dynamic than the larger DON reservoir. Atmospheric deposition of DON to the surface ocean can be a significant source of new nitrogen, but this material is mostly of low molecular weight and highly variable isotopic composition (Cornell et al., 1995) and does not appear to be an important component of the HMW DON measured in the present study.

The lowest $\delta^{15}N$ value (6.6‰) was observed in HMW DOM collected in low-nutrient surface waters at the Bermuda time-series station. One possible source for the ^{15}N-depleted DON in this sample is nitrogen fixing cyanobacteria, such as *Trichodesmium* sp., which are important components of the phytoplankton in this area (Carpenter and Price, 1977). There is little isotopic fractionation during nitrogen fixation (Hoering and Ford, 1960; Minagawa and Wada, 1986), so recently fixed nitrogen should have a $\delta^{15}N$ near 0‰. *Trichodesmium* sp. has been shown to release recently fixed nitrogen as DON (Glibert and Bronk, 1994). Nitrate, which has a $\delta^{15}N$ of 3.5‰ in these waters, is thought to be a major source of new nitrogen at the Bermuda time series station (Altabet, 1988), so the low $\delta^{15}N$ in HMW DON could also reflect inputs from new production based on nitrate.

The most dynamic feature revealed by the $\delta^{15}N$ data was in the equatorial Pacific where the upwelling of nitrate-rich waters resulted in $\delta^{15}N$ values for UPON and UDON that were depleted in ^{15}N relative to values for organic matter to the north and south of the upwelling. Variations in the $\delta^{15}N$ of UPON were reflected in the $\delta^{15}N$ of UDON across the region of equatorial upwelling (Fig. 10). The lowest $\delta^{15}N$ values occurred in the region of the equatorial upwelling where surface water nitrate concentrations were highest and isotopic fractionation by phytoplankton is expected to be greatest. The $\delta^{15}N$ values increased to the north and south of the equatorial upwelling where nitrate concentrations in surface waters were much lower.

Water upwelled in the equatorial region (1°N–1°S) of the eastern Pacific Ocean is derived from relatively shallow depths ($\leq$ 100 m) of the eastward flowing Equatorial Undercurrent (Murray et al., 1994). Upwelled waters flow to the west and spread meridionally to the north and south producing a region of relatively high productivity driven by upwelled nutrients. Meridional transport is most evi-

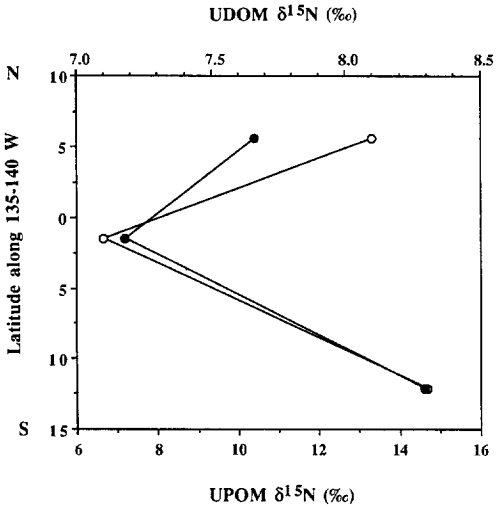


Fig. 10. Meridional variations in stable nitrogen isotopic compositions of UPOM (solid circles) and UDOM (open circles) from equatorial surface waters.

dent within the region from 5°N to 5°S. Concentrations of DOC and DON are lowest in the equatorial upwelling and increase to the north and south indicating that DOM is biologically produced during meridional transport (Peltzer and Hayward, 1996; Libby and Wheeler, 1997). The observed latitudinal increases in $\delta^{15}N$ of UDON support the concept of active biological production of DOM during meridional transport.

A rough estimate of the turnover time of HMW DON in equatorial waters can be calculated using the observed $\delta^{15}N$ values of UDON and an estimate of the meridional advection velocity. For this calculation we assume the following: the $\delta^{15}N$ of UDON collected from 1°30′S (7.1‰) is representative of HMW DON being exported from the region of upwelling, the $\delta^{15}N$ of UPON collected from 5°35′N (10.4‰) is representative of HMW DON produced outside the region of upwelling, a meridional advection velocity of 10 cm sec^{-1} (Toggweiler and Carson, 1995). Using $\delta^{15}N$ values of 7.7 and 8.1‰ for UDON collected at 5°16′N and 5°35′N, we estimate a turnover time of 0.3 to 0.5% day^{-1} for HMW DON. These turnover times for HMW DON indicate nitrogen remineralization rates of 4–6 nM day^{-1}, or enough nitrogen to support 5–8% of daily phytoplankton demand (Murray et al., 1994). If we assume the turnover times of HMW DON are representative for the total DON reservoir (7–8 μM; Libby and Wheeler, 1997), then the remineralization of DON could support 30–50% of daily phytoplankton nitrogen demand.

Relatively large changes in the stable nitrogen isotopic composition of HMW DON within the same geographic region clearly indicate a rapidly cycling component of DON. Recent ^{15}N-tracer addition experiments have demonstrated that a substantial fraction of the inorganic nitrogen taken up by phytoplankton is rapidly released as DON (Bronk et al., 1994). Our results using the natural abundance of nitrogen isotopes as a tracer of DON dynamics in the surface ocean support the concept of a rapidly cycling DON component and are suggestive of a major role for DON in the upper ocean nitrogen cycle. It is unlikely that heterotrophic utilization of DON would specifically remove nitrogen atoms, so these results are also indicative of a rapidly cycling component of DOC in the upper ocean. Most bulk characteristics of DOM reveal little about its reactivity, but this first look at the stable nitrogen isotopic composition of DOM indicates that isotopic analyses of DON have the potential to greatly expand knowledge of organic matter cycling in the ocean.

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