PROCEDURES FOR PARTICULATE CARBON, NITROGEN, PHOSPHORUS AND TOTAL MASS ANALYSES USED IN THE US-JGOFS HAWAII OCEAN TIME-SERIES PROGRAM

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1. Summary

A deep-water hydrostation has been established near Oahu, Hawaii as one component of the U.S. Joint Global Ocean Flux Study (U.S.-JGOFS) research program. Since October 1988, oceanographic cruises have been conducted for the purpose of studying the time-varying rates of several biogeochemical processes, including primary production of organic matter and particulate matter export from the euphotic zone. This contribution describes briefly our sampling and analysis protocols with an emphasis on the measurements of the carbon (PC), nitrogen (PN) and phosphorus (PP) concentrations of particulate matter suspended in the water column and the downward vertical flux of PC, PN, PP and total mass (TM) as measured using free-drifting sediment traps.

2. Introduction

In June 1988, a team of scientists at the University of Hawaii received funding from the NSF Global Geosciences Program to establish and maintain a deep-water hydrographic station at a site near the island of Oahu, Hawaii (22° 45'N, 158° W). The Hawaii Ocean Time-Series (HOT) program was funded initially for a five-year period but is expected to continue for at least 10-15 years, and possibly longer. This hydrostation will serve as the open ocean benchmark for combined JGOFS- and WOCE-related research activities, and will comprise the single most extensive investigation of its kind in the Pacific Ocean. The HOT site has been named Station ALOHA (A Long-term Oligotrophic Habitat Assessment) to reflect our primary focus on low-nutrient environments.

Data collected during the first 12 cruises of HOT, from October 1988 to November 1989 have been published [Chiswell et al., 1990] along with a detailed field and laboratory protocols manual which outlines, in addition to the analytical methods employed, the time-series sampling strategy and the HOT program quality control/quality assurance programs [Karl et al., 1990].

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This contribution discusses our sampling procedures for suspended and rapidly sinking particles and outlines the post-collection treatments and analytical detection systems used for the measurements of particulate carbon (PC), nitrogen (PN), phosphorus (PP) and total mass (TM) in the HOT program. Blanks become very important when conducting low-level measurements of PC, PN and PP.

3. Definitions and Caveats

We define "particles," operationally for all but total mass analysis, as those materials which are retained by a Whatman micro-fine glass fiber filter, catalog #GF/F 0.7 μm nominal retention [Sheldon, 1972], acknowledging the fact that some particulate material is known to pass through this filter. The particle retention characteristics are also known to vary with filter loading and, hence, may not be constant even during the filtration of a single water sample. We also know that certain dissolved compounds are likely to adsorb to the glass fiber filter matrix, or bind to or react with the collected particulate matter, thereby contributing to the total "particle" concentration. While sample blank treatments can be designed to evaluate the approximate magnitude of these effects, no approach is universally acceptable, or accepted.

In presenting our methods, we have focused on total elemental masses and mass fluxes without consideration as to the chemical form of the given element. Consequently, we derive estimates of PC, PN and PP which are not taken to be equivalent to the organically-bound fraction. Methods currently in use to distinguish inorganic from organic C, N and P cannot rigorously distinguish these important particle classes. Additional research will be required in order to resolve these outstanding issues.

In recent years, an important scientific controversy regarding the accuracy of dissolved organic carbon determinations has emerged [Williams and Druffel, 1988]. Historical measurements of C [Sugimura and Suzuki, 1988] and N [Suzuki et al., 1985] dissolved in seawater may be in error due to the presence of as yet unidentified compounds that are not fully oxidized except in the presence of a platinum catalyst. By analogy to

these recalcitrant dissolved materials, it is conceivable that similar compounds are present in the particulate or colloidal fractions of seawater. If so, it may be necessary to re-evaluate the "standard methods" of catalysis which have been used historically for the measurements of PC, PN and PP. However, until this present controversy is resolved, or at least until the higher DOC and DON estimates are confirmed by independent laboratories, it may be inappropriate to call into question, a priori, the entire historical literature.

4. General Precautions

Because suspended particle concentrations and particle fluxes in oligotrophic habitats are expected to be low, special attention must be paid to the preparation and processing of water samples and sediment trap collections in order to reduce potentially contaminating particles. For example, sediment traps should be capped immediately after filling and immediately after retrieval. Particular attention should be paid to airborne and/or shipboard particulate contamination sources. In addition, the time interval between trap retrieval and subsample filtration should be minimized in order to limit the inclusion of extraneous abiotic particles and the post-collection solubilization of particles. For water sample analysis, it is important to process large volumes of water in order to increase the signal-to-noise ratio and precision estimates of the determinations.

5. Collection and Processing of Suspended Particles

5.1. Collection

Suspended particulate matter is collected from discrete water depths using a SeaBird CTD-rosette system with external temperature, conductivity, dissolved oxygen and fluorescence sensors and an internal pressure sensor. A General Oceanics 24-place pylon and an aluminum rosette containing twenty four 12 L sample bottles are used to obtain water from predetermined depths. The CTD and rosette are deployed on a 3-conductor cable allowing for the real-time display of data and enabling the collection of water samples from areas of interest relative to hydrographic and biochemical features. The CTD system takes 24 samples per second and the raw data are stored both in the computer and, for redundancy, on VHS-format video tapes. CTD data manipulation and sensor calibration are presented elsewhere [Chiswell et al., 1990; Karl et al., 1990].

Generally, 10-12 consecutive casts to 1000 m are made over a 36 hr period, at approximately 3 hr intervals, to span the local inertial period (~ 31 hr) and three semi-diurnal tidal cycles. This sampling is performed so that tidal or near-inertial variability can be estimated and removed, thereby minimizing aliasing by these components. During this 36 hr intensive sampling period, most of the water samples for the GOFS program objectives are collected. To the extent possible, depth profiles of specific chemical constituents are collected from a single or from contiguous casts. This is done in order to produce profiles which minimize the effects of time-dependent density structure variation within the water column.

The HOT Program 24-place aluminum rosette was manufactured by Scripps Institution of Oceanography's Oceanographic Data Facility (ODF). The 12 L polyvinylchloride (PVC) sample bottles, also manufactured by SIO-ODF, are equipped with Buna-N rubber O-rings, Teflon-coated steel springs and standard General Oceanics sampling valves.

5.2. Processing

Seawater is collected in 12 L sample bottles and transferred directly to acid-cleaned filtration bottles. The samples are transferred via Tygon tubing which incorporates an in-line 202 μm Nitex screen prefilter to remove zooplankton or other rare particles which might otherwise affect the precision of the estimate. The filtration bottles are 4 and 12 L polyethylene aspirator bottles fitted with a valve assembly and tubing connection.

Once the rosette sampler is on deck, the vent valve of each sample bottle is opened and one end of the drawing tube is attached to the outflow spigot of the sampling bottle and the other end to the tubing connector on the cap of the filtration bottle. The filtration bottle valve is opened and 100-200 mL is run through the transfer tube and valve assembly to rinse the filtration bottle. The bottle and cap are rinsed three times. During the rinsing and filling operation, the filtration bottle cap valve is used to control and direct the sample flow. After rinsing, the cap is placed over the bottle mouth (without tightening), the valve opened and the polyethylene bottle filled to the calibration mark.

After filling, the filtration bottles are inverted and placed into the filtration rack. The contents are then pressure filtered (4-7 psi nitrogen gas) through combusted in-line 25 mm GF/F filters. Filters used for the determination of PP are combusted and acid-washed. Following filtration, clean forceps are used to transfer each filter to a clean plastic petri dish containing a ~ 6 cm^2 piece of combusted foil or, in the case of PP,

into combusted 12 x 75 mm glass test tubes covered with foil. The sample is labeled and stored frozen, at -20° C, in preparation for the final measurement of PC, PN and PP. Combusted filters and, in the case of PP, combusted/acid-washed filters, are used as procedural blanks.

6. Collection and Processing of Sinking Particles

6.1. Collection

Sinking particles are collected using a free-drifting "Knauer-design" sediment trap array [Knauer et al., 1979; Martin et al., 1987]. Twelve individual 0.0039 m^2 mouth opening polycarbonate particle interceptor traps (PITs) with honeycomb baffles are attached to polyvinylchloride (PVC) cross members and are positioned on the 1/2" polypropylene line at depths of 150, 300 and 500 m. A spar buoy is equipped with a VHF radio transmitter, an Argos satellite transmitter and two xenon strobe lights. Generally, deployments are made for a 72 hr period on each monthly cruise.

Prior to deployment, each trap is rinsed thoroughly with tap water then with deionized distilled water (DDW) before filling with a high density solution to prevent advective-diffusive loss of extractants and preservatives during the deployment period and to eliminate flushing of the traps during recovery [Knauer et al., 1979]. The trap solution is prepared by dissolving 50 g of NaCl in each liter of surface seawater. This brine solution is pressure filtered through a 0.5 μm filter cartridge prior to the addition of 10 mL of 100% formalin per liter of solution. The individual traps are filled and a portion of the trap solution is saved for analysis of the solution blanks.

6.2. Processing: PC, PN and PP

Upon recovery, individual traps are capped and transported to the shipboard portable laboratory for analysis. Care is taken not to mix the higher density trap solutions with the overlying seawater. Trap samples are processed from deep to shallow in order to minimize potential cross contamination. The depth of the interface between the high density solution and the overlying seawater is marked on each trap. The overlying seawater is then aspirated with a plastic tube attached to a vacuum system in order to avoid disturbing the high density solution. Because some sinking particulate material collects near the interface between the high density solution and the overlying seawater, the seawater is removed only to a depth that is 5 cm above the previously identified interface. After the overlying seawater has been removed from all the traps at a given depth, the contents of each trap is passed through a 335 μm Nitex screen to remove the so-called "swimmers" (i.e., contaminating zooplankton and micronekton which entered the traps in a living state and are not truly part of the passive flux). Immediately before this sieving process, the contents of each trap are mixed to disrupt large amorphous particles. The traps are rinsed with a portion of the less than 335 μm sample in order to recover all particulate matter, and the 335 μm Nitex screen is examined to determine whether residual material, in addition to the "swimmers", is present. If so, the screens are rinsed again with a portion of the 335 μm filtrate. After all traps from a given depth have been processed, the 335 μm screen is removed and placed into a vial containing 20 mL of formalin-seawater solution, and stored at 4° C for subsequent microscopic examination and organism identification and enumeration.

6.3. Processing: Total Mass

Three of the 12 traps at each water depth are used for the determination of mass flux. At our shore-based laboratory, triplicate 250 mL subsamples of the time-zero high density trap solution (procedural blank) and equivalent volumes from individual traps (starting with the deepest depth and working up), are vacuum filtered through tared 25 mm 0.2 μm Nuclepore membrane filters prepared as follows: (1) rinse filters three times with distilled water and place rinsed filter on a ~6 cm^2 foil square (to reduce static electricity) in a plastic 47 mm petri dish, (2) fold the foil in half over the filter and place the petri dish in a drying oven with the lid ajar for 2 hours at 55° C, remove and cool in desiccator for 30 minutes, (3) weigh filter to constant weight (i.e., repeat oven drying, cooling and weighing until a relative standard deviation of less than 0.01% is achieved), on a microbalance capable of 0.1 μg resolution, record weights (to the nearest 0.1 μg) on label tape placed on top of the petri dish.

After the last of the sample has passed through the tared filter, the walls of the filter funnel are washed with three consecutive 5 mL rinses of an approximately isotonic (1 M) ammonium formate solution to remove seawater salts (note: during each rinse, allow the ammonium formate solution to completely cover the filter). Return the processed filter to its petri dish, record sample number (on the dish and data sheet), and place in a drying oven at 55° C for 8 hours (or alternatively, store in a desiccator, if an oven is not

Table 3-1.
Equipment, Supplies and Reagents for Particulate C and N

Water sampling bottles and optional rosette/ CTD system	Low pressure filtration apparatus (4-7 psi nitrogen gas), or equivalent
Perkin-Elmer Model #2400 (or equivalent)	Carbon/Nitrogen analyzer with integrator
Cahn (or equivalent) electronic balance	O_2 and He gases
Muffle furnace (for 450° C)	Drying oven
Combusted 2.5 cm GF/F filters (450° C, 4 hours)	Ethanol-cleaned forceps for handling filters
Combusted foil (450° C, 4 hours)	Aluminum foil for covering work area
Vacuum filtration system	Clean petri dishes
Acetanilide standard	Formalin (100%)
NaCl	

immediately available), and dry to constant weight (as above).

7. Particulate Carbon (PC) and Nitrogen (PN)

7.1. Principle

Both PC and PN can be measured using commercially-available instruments which detect the gaseous products of high-temperature combustion. The Perkin-Elmer model 2400 CHN analyzer used in the GOFS project, combines the classical Pregal and Dumas methods for the determination of carbon and nitrogen, respectively. The samples are combusted in a pure oxygen environment, the gases mixed and separated by frontal chromatography. The separated gases are determined stepwise by a thermal conductivity detector.

7.2. Analysis

Standard procedures for instrument warm-up are followed. Primary PC/PN standards are prepared using acetanilide (C_8H_9NO; mol. wt. = 135.16). Standards are made to encompass the range ~ 30-600 μg per sample. Instrument conditions include: combustion temperature, 925° C; reduction temperature, 640° C; O_2 gas, 15 psig; He gas, 20 psig. The O_2 used is ultrapure grade (99.995 mole % minimum). The equipment, supplies and reagents are listed in Table 3-1.

8. Particulate Phosphorus (PP)

8.1. Principle

The procedure presented here is a modification of one used by the Hawaii Institute of Marine Biology Analytical Services laboratory at the University of Hawaii. The method relies on the release of organically-bound phosphorus compounds as orthophosphate, by high temperature combustion at 450-500° C. The orthophosphate is then extracted with 0.5 N HCl at 90° C and neutralized with 6 M sodium hydroxide. The liberated orthophosphate is reacted with a mixed reagent of molybdic acid, ascorbic acid and trivalent antimony to form phosphomolybdic acid. This heteropoly acid is then reduced to the colored molybdenum blue complex by ascorbic acid and the solution is measured spectrophotometrically. This procedure measures all forms of reactive phosphorus which can be released by combustion and acid hydrolysis.

8.2. Analysis

Samples are combusted in precombusted, acid-rinsed 12 x 75 mm test tubes at 475-500° C for 3 hours in a muffle furnace. The samples are then allowed to cool and are immersed in 3 mL of 0.5 M HCl. The test tube is then heated for 90 minutes at 90° C in a water bath.

The samples are allowed to cool, vortexed for 20 seconds and vacuum filtered through a combusted acid-washed GF/F filter attached to an acid cleaned syringe. Vortexing and filtration is repeated with three additional 3 mL aliquots of 0.5 M HCl and washes are combined in special acid washed test tubes marked volumetrically at the 20 mL level. After processing all samples, 1 drop of phenolphthalein solution and approximately 1 mL of 6 M NaOH is added to each test tube. While mixing, 0.5 M NaOH is added dropwise to produce a light pink color. The tubes are then filled to the 20 mL mark with DDW.

Two mL of mixed reagent are added to samples and standards. These are mixed thoroughly. Color is developed for 10 minutes and absorbance is read at 860 nm against a DDW reference. Standards are corrected for absorbance of reagent blanks, and samples are corrected for absorbance of filter or procedural blanks. Equipment, supplies and reagents for particulate P are listed in Table 3-2.

Table 3-2.
Equipment, Supplies and Reagents for Particulate Phosphorus

Water sampling bottles and optional rosette /CTD system	Varian DMS-100S spectrophotometer and 1-cm cuvette, or equivalent
Low pressure filtration apparatus (4-7 psi nitrogen gas), or equivalent	Acid-washed 12 x 75 mm glass tubes, combusted (450° C, 3 hr)
Water bath (for 90° C)	Combusted, acid-rinsed GF/F filters
Acid-rinsed glassware	Acid-rinsed vacuum filtering assembly
Glass distilled deionized water (DDW)	Muffle furnace (for 450° C)

Reagents

HCl (0.5 M and 1 M)	
NaOH (0.5 M and 6 M)	
H_2SO_4 (2.5 M)	
ammonium molybdate solution:	Dissolve 15 g of ACS grade ammonium paramolybdate [$(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$], in 500 mL DDW. Store in plastic bottle in the dark.
ascorbic acid solution:	Dissolve 0.5 μg of ACS ascorbic acid in 10 mL DDW (5.4% wt/vol). Prepare fresh.
potassium antimonyl-tartrate solution:	Dissolve 0.34 g of ACS potassium antimonyl-tartrate (tartaremetic), in 250 mL DDW. Solution is stable for many months.
mixed reagent:	Mix together 10 mL ammonium molybdate, 25 mL 2.5 M H_2SO_4, 10 mL 5.4% ascorbic acid and 5 mL potassium antimony tartrate. Prepare fresh.
phosphate standard solution (1000 μM):	Dissolve 0.1361 g of dry (110° C for 2 hrs) KH_2PO_4, in 1000 mL of DDW. NOTE: Store in a dark bottle with 1 mL of chloroform.
working phosphate standard (100 μM):	Dilute 10 mL of the stock standard to 100 mL, using a volumetric flask. Dilute the working standard to prepare a series of standards to cover the range from 0.05-10 μM.
phenolphthalein indicator	

9. Data Reduction and Calculations

9.1. Suspended Particles: PC and PN

Calculate μg per sample of PC or PN from standard curve using:

$$\mu g = (x-b)/m$$

where:

x = (sample counts - blank counts)
b = y intercept of regression line
m = slope of regression line

Calculate μg C L^{-1} or μg N L^{-1} using:

$\mu g\ L^{-1}$ = (μg sample^{-1}) / (volume filtered in liters)

9.2. Suspended Particles: PP

Calculate $\mu M\ L^{-1}$ of PP from standard curve using:

$$\mu M\ L^{-1} = (x - b)/m$$

where:

x = corrected absorbance of sample
b = y intercept of regression line
m = slope of regression line

Calculate μg P-$P0_4\ L^{-1}$ using:

$\mu g\ P\ L^{-1}$ in sample extract =
$\mu M\ L^{-1}$ sample x 30.97376 $\mu g\ \mu M^{-1}$
$\mu g\ P\ mL^{-1}$ = ($\mu g\ P\ L^{-1}$ in extract) /(1000 mL L^{-1})
μg P-$PO_4\ L^{-1}$ = ($\mu g\ P\ mL^{-1}$ x 20.0 mL of extract) / (vol in L filtered)

9.3. Sinking Particles: PC, PN and PP

The quantities of particulate C, N and P in the screened trap solutions are determined using methods described above. Six replicate traps are used for C/N

determinations and three additional traps for P. Typically, 1.5-2 L are used for a single C/N or P measurement. Equivalent volumes of the time-zero sediment trap solution, filtered through the appropriate filters are used as C, N or P blanks.

C, N and P flux is calculated as follows:

$$\text{mg C (or N, P) m}^{-2}\,\text{d}^{-1} = \frac{[(C_s - C_b)] * V_t}{V_f * A_t * t}$$

where:

C_s = carbon (mg) in sample
C_b = carbon (mg) in blank
V_t = volume of trap (L)
V_f = volume filtered (L)
A_t = cross-sectional area of trap (m^2)
t = deployment period (d)

9.4. Sinking Particles: Mass

Mass flux is calculated as follows:

$$\text{mg (dry wt.) m}^{-2}\,\text{d}^{-1} = \frac{[(W_a - W_b) - W_{bl}] * V_t}{V_f * A_t * 1000 * t}$$

where:

W_a = filter weight after filtration (μg)
W_b = filter weight before filtration (μg)
W_{bl} = net weight of blank solution (μg)
V_t = volume of trap (L)
V_f = volume filtered (L)
A_t = cross-sectional area of trap (m^2)
1000 = conversion factor ($\mu g\ mg^{-1}$)
t = deployment period (d)

10. Precision and Accuracy

Estimates of the precision of our suspended PC, PN and PP measurements are based on a number of replicate samples taken during the first year of the HOT program. The standard deviation of replicate determinations (or the difference if n=2) is divided by the mean to yield a coefficient of variation (cv; expressed as percent); the mean cv for the determinations of PC, PN and PP are 12%, 10% and 18%, respectively. In addition, the accuracy obtained with the PE-2400 CHN analyzer has been estimated from determinations of the C and N contents of reference standards analyzed along with samples during each analytical run. This accuracy, expressed as the mean difference between the analytical determination and the known C and N contents of the standards, is found to be 6% for PC and 13% for PN. Note that standards containing less than 10 μg N and/or less than 70 μg C are not included in this latter calculation; the performance of the PE-2400 is suspect at such low levels, thus sample volumes and trap deployment times must be kept large enough to avoid entering this lower range.

The precision of our PC, PN, PP and TM flux determinations is estimated from replicate traps deployed at each given depth during year one of the HOT program. Again, an overall mean cv is calculated for each analysis. The resulting precisions are 23%, 28%, 32% and 18% for fluxes of PC, PN, PP and TM, respectively. Note that at the lowest fluxes, the analytical blank produces a sizeable fraction of the total signal; similarly, the blank can be large in the analyses of suspended particles from deep in the water column where concentrations are very low. Tabulated below are the typical ranges in analytical blanks encountered during year one, expressed as percentages of the total signal produced by a sample in the course of a given analysis:

Suspended PC:	5-17%
Suspended PN:	5-17%
Suspended PP:	6-29%
PC Flux:	17-54%
PN Flux:	13-55%
PP Flux:	12-43%
TM Flux:	18-41%

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