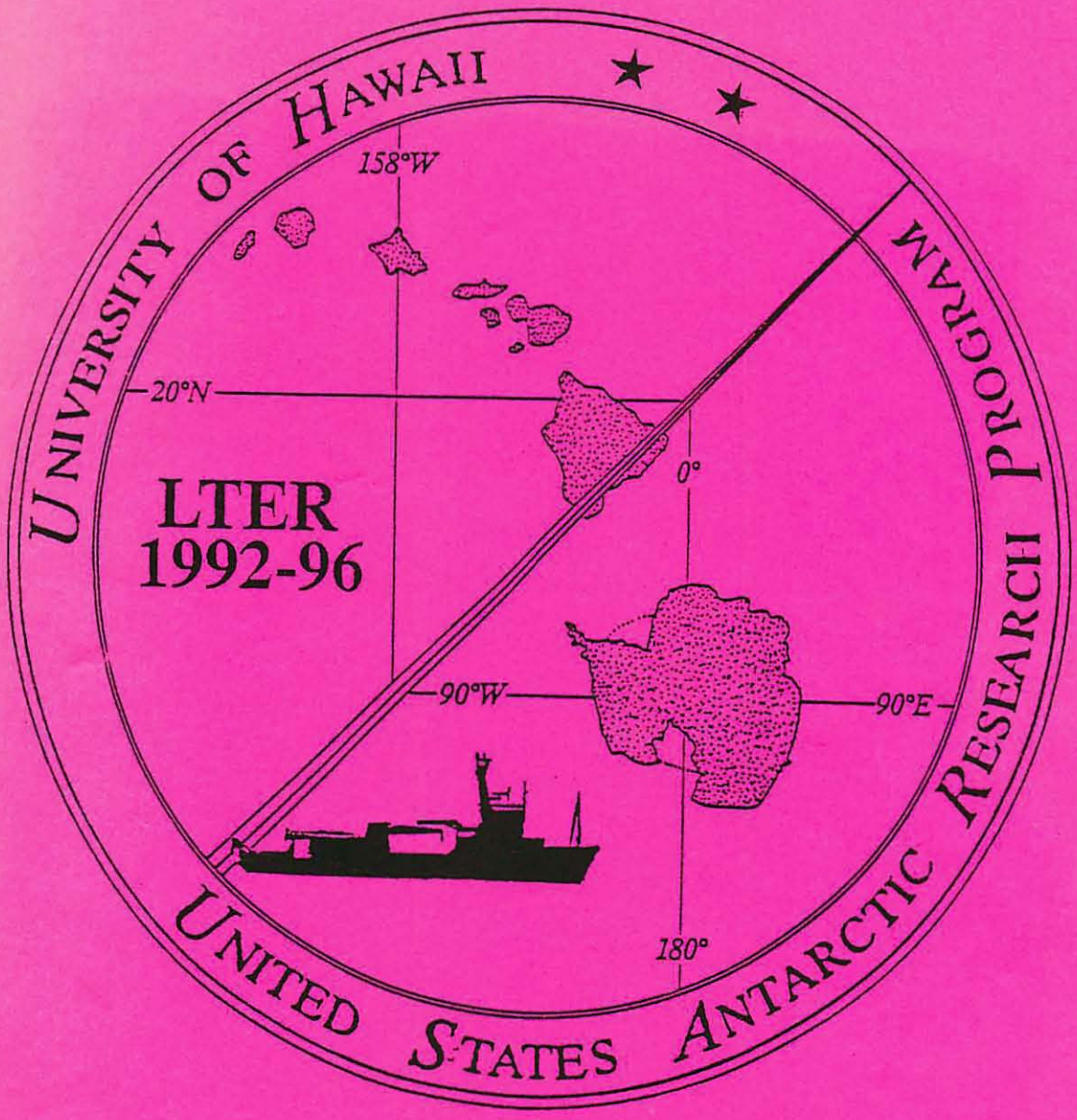
PALMER PENINSULA LONG-TERM ECOLOGICAL RESEARCH (LTER) PROGRAM



PROJECT S-046 UNIVERSITY OF HAWAII FIELD AND LABORATORY PROTOCOLS MANUAL

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Chapter 1
PREFACE

This document was prepared as a field guide to the measurements and experiments conducted by project S-046 participants. Although the specific sampling strategies and schedules vary from cruise to cruise depending upon the number of personnel, this protocol manual presents the rationale and step-by-step procedures for our core parameters.

Chapter 2
PALMER LTER PROGRAM OVERVIEW
(the following text is excerpted from a paper by R. Ross and L. Quetin)

1. Long-term Ecological Research: LTER

LTER recognizes that some ecological phenomena occur on time scales of decades or centuries, and that investigations on these time scales are not routinely supported by funding agencies. Without an understanding of interannual variability over the long term, interpretation of ecological experiments and distinguishing long-term trends from cyclic changes in natural ecosystems is difficult. The LTER Network, sponsored by the National Science Foundation, has grown during the last decade to a total of eighteen sites in ecosystems ranging from tall grass prairies to tundra. To facilitate comparison and the ability to construct ecological generalities, all sites are required to set up research efforts in five core areas:

- pattern and control of primary production
- spatial and temporal distribution of populations representing trophic structures
- pattern and control of organic matter accumulation
- pattern of inorganic inputs and movements of nutrients
- pattern and frequency of disturbance to the research site

The Palmer LTER, established in the fall of 1990, focuses on the pelagic marine ecosystem in Antarctica, and the ecological processes which link the extent of annual pack ice to the biological dynamics of different trophic levels. Pack ice may be a major physical factor affecting the structure and function of polar biota. Interannual cycles and/or trends in the annual extent of pack ice are hypothesized to impact all levels of the food web, from total annual primary production to breeding success in seabirds. In the region around Palmer Station (64°40'S, 64°W) west of the Antarctic Peninsula (see Figure 1), the maximum extent of pack ice varies from near zero to halfway across Drake Passage and appears to vary on a six- to eight-year cycle. Satellite data on the maximum extent of pack ice in the Weddell Sea sector shows cold winters with heavy pack ice in 1973, and 1980 and 1981, and personal observations confirm that winters of 1980 and 1981, and 1986 and 1987 had heavy ice cover in the region around Palmer Station. The overall objectives of the Palmer LTER are:

- to document interannual variability in the development and extent of the annual pack ice and in life-history parameters of primary producers and populations of "key" species from different trophic levels
- to quantify the processes that underlie natural variation in these representative populations
- to construct models that link ecosystem processes to physical environmental variables and that simulate the spatial/temporal relationships between representative populations
- to employ such models to predict and validate the impacts of altered periodicities in the annual extent of pack ice on ecosystem dynamics

To achieve these program objectives, data will be obtained on a variety of spatial and temporal scales including, but not limited to, continuous remote sensing of a variety of environmental parameters at representative locations within the general study area, annual cruises at approximately the same time each year (~10 days in duration) to ascertain the interannual variabiltity and spatial gradients of key oceanographic and biological parameters and at least two process-oriented cruises (~6 weeks in duration). The following section describes, in more detail, the S-046 (D. Karl, P.I.) subcomponent of the Palmer LTER.

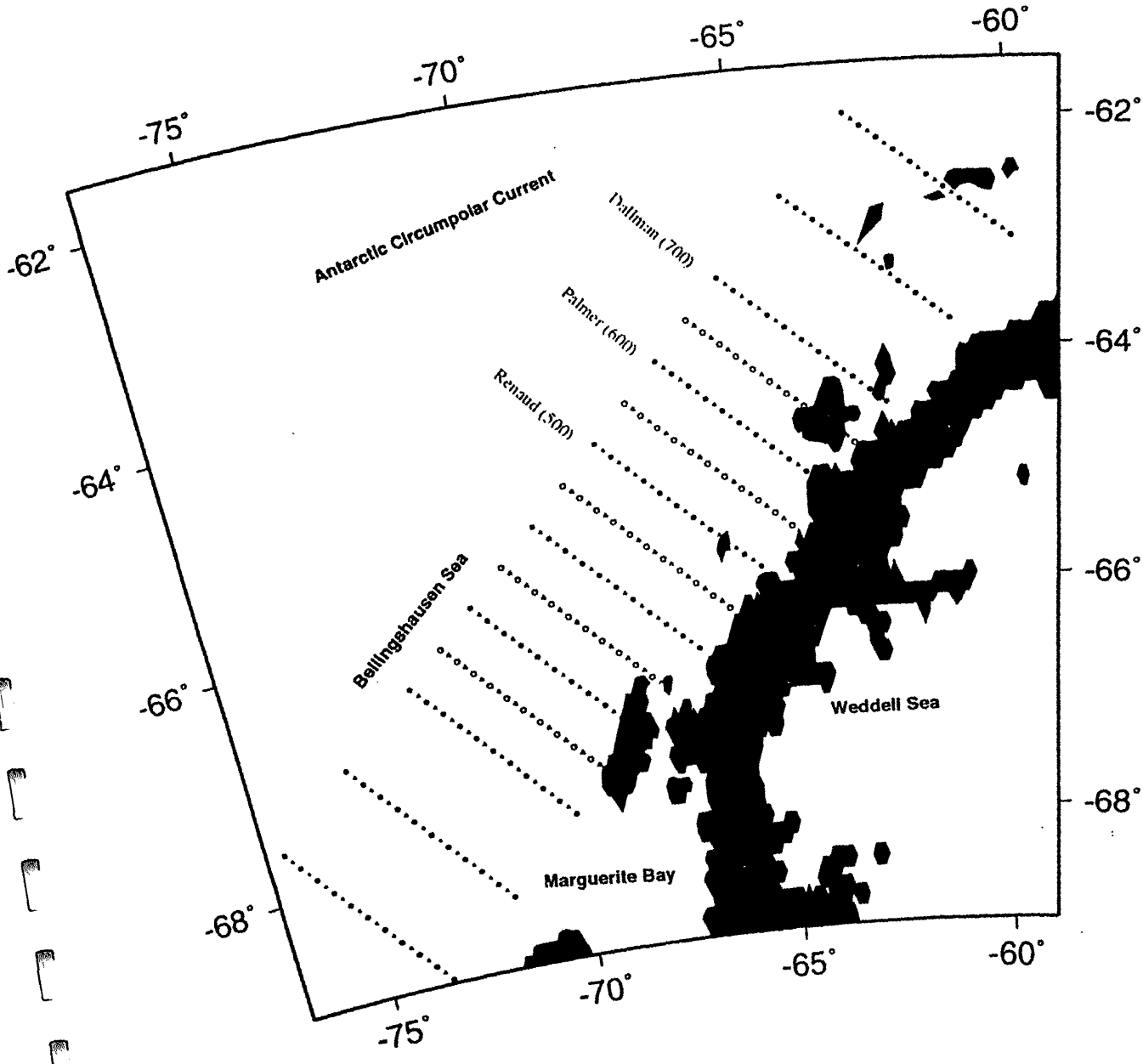

Figure 1: Map of the Antarctic Palmer LTER region.

Chapter 3
LTER: MICROBIOLOGY AND CARBON FLUX
(PROJECT S-046)

Within the general, but integrating hypothesis of the Palmer Station LTER program (stated here as a null hypothesis):

> H_o: interannual variation in the extent of pack ice does not affect the vitality of ice-edge phytoplankton, bacterioplankton and associated krill and seabird populations,

We propose to focus specifically on microheterotrophic populations (especially the heterotrophic bacterioplankton) and on the quantification of several important carbon cycling rate processes. The approach will be two-pronged, including:

- the measurement of time (days to years) and space (1-100 km) variability in a suite of core carbon pools and particle fluxes, and
- experimental verification of carbon flux pathways and rates.

We hypothesize that during the seasonal spring bloom in the ice-edge region of the LTER study area (typically, Nov-Dec), microbial loop processes consume only a negligible amount of organic matter. However, during post-bloom conditions (typically, Jan-Feb), microbial loop processes dominate the euphotic zone carbon cycle. This temporal uncoupling of phytoplankton and bacterial processes has numerous potential implications to the carbon cycle of antarctic coastal ecosystems (as discussed below), yet the reason(s) for this uncoupling are not well understood.

We will evaluate several ecological predictions of this seasonal uncoupling hypothesis in an attempt to determine the cause(s) and to predict further some of the consequences of these microbial processes. Among the potential causes of this uncoupling are:

- DOC-Bacterial Interactions
- Temperature Effects
- Direct Competition and Chemical Antagonism

DOC-Bacterial Interactions: Prior to and during the intensive spring bloom of phytoplankton in the LTER region, bacterial populations are low and relatively inactive because of organic carbon substrate limitation, low temperature, or both. The net heterotrophic overwintering period has all but exhausted utilizable DOC. The initial, ungrazed autotrophic component of the spring bloom is very efficient in converting dissolved inorganic carbon to phytoplankton carbon with minimal losses as low molecular weight DOC. This further restricts bacterial biomass accumulation and also suppresses protozoan populations. The net effect is an accumulation of phytoplankton biomass. No DOM time-series or molecular weight distributions have been reported for the antarctic coastal habitat. However, based upon dissolved TCO_2 and O_2 gas stoichiometries (Karl et al., 1991b, Karl and Hebel 1990) it can be deduced that there is little heterotrophic consumption of O_2 during the bloom. As the bloom matures, DOM is produced by a combination of excretion, leakage, cell lysis and grazing processes. The accumulation of DOM stimulates microheterotrophic processes and, eventually, the entire microbial loop. An alternative to the uncoupled autotrophic production-microheterotrophic utilization hypothesis is the possibility that high molecular weight (HMW) DOM produced during the initiation phases of the spring bloom is subsequently hydrolyzed by bacterial exoenzymes, thereby converting this "unavailable" DOM pool into one which is readily assimilated by microheterotrophs. In the opinions of Lancelot et al. (1989) and Billen and Becquevort

(1991), this "molecular uncoupling" is the fundamental cause of the temporal uncoupling between the phytoplankton and bacterial populations during bloom conditions.

Temperature Effects: The active microbial components of most antarctic marine environments are, by definition, either psychrophilic or psychrotrophic (*sensu* Morita 1975). Despite the apparent ubiquity of psychrophilic bacteria in the Southern Ocean, it is difficult to ascertain whether the various heterotrophic bacterial assemblages are dominated by psychrophiles or psychrotrophs; the difference being whether the organisms are uniquely adapted for optimal growth at low temperatures (i.e., psychrophiles) or are simply cold-tolerant organisms which require warmer temperatures (15-20°C) for optimal growth (i.e., psychrotrophs). Several independent antarctic field studies have evaluated the heterotrophic response of natural microbial assemblages to variations in incubation temperature. Hodson et al. (1981) reported that bacterial assemblages collected from McMurdo Sound have temperature optima for the assimilation of a variety of organic substrates at or near the ambient seawater temperatures (0-2°C); i.e., a psychrophilic response. In contrast to these results, Morita et al. (1977) and Hanson and Pope (1981) concluded that increased incubation temperature over the range 0-15°C, resulted in an increased metabolism and growth of microbial assemblages collected from the near-surface waters of the Southern Ocean; i.e., a psychrotrophic response. These differences may reflect a regional variability in the temperature response of indigenous microheterotrophic assemblages. In any case, the relative importance of psychrophiles vs. psychrotrophs in the Southern Ocean remains an open question.

In an attempt to reconcile the high rates of metazoan secondary productivity which are often observed during the spring bloom period in cold-water habitats, Pomeroy and Deibel (1986) suggested that environmental temperature might play a fundamental role in food web function and ecosystem efficiency. Although their studies were directed towards obtaining a general understanding of productivity in high northern latitudes, they may also have direct application to the microbial assemblages of the Southern Ocean. In their ecosystem model, Pomeroy and Deibel (1986) hypothesized that low temperature suppression of bacterial activity, relative to photosynthesis, might be an important factor in restricting energy flow through the microbial loop and, therefore, allowing a greater amount of carbon and energy to be transferred to higher (metazoan) trophic levels. Implicit in this model is the fact that bacteria functioning within the microbial loop comprise a "sink" rather than a trophic "link" for carbon and energy (Pomeroy 1974; Pomeroy and Wiebe 1988). Because of the greater seasonal variation in seawater temperature, the evolutionary selection pressures for either Newfoundland coastal waters or Bedford Basin might not be the same as those expected to occur in Southern Ocean habitats. Nevertheless, the Pomeroy-Deibel hypothesis does provide a framework for the design and conduct of relevant field experiments on the effects of temperature on autotrophic and microheterotrophic processes.

Direct Competition and Chemical Antagonism: In our previous studies of organic substrate uptake by microorganisms in waters of the Antarctic Peninsula, we were careful not to attribute all glutamate and thymidine uptake exclusively to the heterotrophic bacteria, but rather to "microheterotrophs", a term which also includes algae and microzooplankton (Karl et al. 1991a, Bird and Karl 1991). This precaution was necessary following the demonstration of uptake and incorporation of organic substrates by antarctic microalgae (Rivkin and Putt 1987). These authors showed that diatoms can assimilate dissolved amino acids and glucose at ambient concentrations in both light and dark incubations. Therefore, it is possible that some unknown portion of the observed incorporation might have been the result of mixotrophic growth of algae.

Studies from December 1986 to March 1987 showed that waters of Gerlache Strait and the southwestern Bransfield Strait support a rich seasonal bloom of microorganisms, consisting primarily of phytoplankton. Bacterial-C concentrations never exceeded 20 μg C l^{-1} and were relatively invariant throughout the study area despite strong onshore-to-offshore gradients in chl *a*, total ATP and in the uptake of labeled organic substrates (Karl et al. 1991a, Bird and Karl 1991). Possible causes for the uncoupling of phytoplankton and bacterioplankton population dynamics, at high phytoplankton

concentrations ($\geq$2.5 *ug* chl *a* l^{-1}) might include: (1) bacteriostatic effects due to the production of extracellular products such as acrylic acid (Sieburth 1960), (2) disproportionately higher bacterial loss rates (e.g. grazing) compared to algal populations or (3) direct competition between algae and bacteria for growth limiting substrates. Because all of our stations with low-to-moderate chl *a* concentrations (i.e. <2.5 *ug* chl *a* l^{-1}) were within the 95% confidence limits of the previously published empirical models, we have no reason to suspect altogether different coupling mechanisms between phytoplankton and bacterioplankton in south polar regions. This makes the observations of apparent uncoupling at high chl *a* concentrations that much more intriguing.

1. Experimental Design and Procedures

The goals of the microbiology and carbon flux program are two-fold: (1) to conduct systematic, core measurements of relevant biogeochemical properties of the Antarctic marine habitat and (2) to test explicit hypotheses regarding the role of microheterotrophs and the cycling of major bioelements.

Our primary objectives are to determine the mechanisms and rates of coupling between photoautotrophic and microheterotrophic processes and to measure mass fluxes among the various dissolved inorganic, dissolved organic and particulate organic carbon pools. To accomplish this task and to test various hypotheses regarding controls on bacterial biomass and metabolic activity, we will obtain quantitative data on the following pools and fluxes: (1) dissolved inorganic carbon (ΣCO_2) and pCO_2 (from alkalinity and pH), (2) dissolved organic carbon (DOC), (3) microbial biomass (ATP and DNA), (4) bacterial biomass (lipopolysaccharide, epifluorescence microscopy and flow cytometry), (5) total microbial productivity (^{3}H-adenine incorporation into DNA), (6) bacterial production (^{3}H-leucine incorporation into protein and ^{3}H-thymidine incorporation into DNA), (7) dissolved oxygen and (8) dissolved inorganic and organic nutrients. Sediment traps will also be deployed to measure the downward flux of biogenic matter and for microscopic identification of the collected particulate materials. Samples will be made available to LTER program colleagues for a variety of complementary experiments. Finally, a variety of specialized experiments will be performed including, but not limited to: (1) algal cell sinking rates, (2) production of DOC, (3) exoenzymatic activity, (4) bacterial nitrification and (5) bacterial grazing rate studies.

2. Data Management and Modeling

All of the core data derived from cruises in the Palmer LTER study area will be available for general distribution to the core LTER P.I.s and will also be published as part of the annual LTER Data Report. Ideally, the sampling program for the microbiology and carbon flux program will be coordinated with the other water column components of the LTER program (especially the phytoplankton biomass and primary productivity) so that the two resultant data records will be entirely synoptic.

An important component of the Palmer LTER program will be the development of more accurate coupled physical-biological models for ecological processes at the antarctic ice-edge. The data obtained in the microbiology and carbon flux component will be used to model interactions between dissolved inorganic and organic carbon cycles and to provide a more comprehensive model of the role of the microbial loop in carbon cycle dynamics. Eventually, a simulation model similar to that developed by Fasham et al. (1990) for the seasonal cycle of plankton nutrients and dissolved organic matter in the North Atlantic Ocean will be developed for microbial loop in the Palmer LTER study area.

3. Significance of Proposed Research

Sea-ice dominates polar marine ecosystems and, in Antarctica, the annual acretion and ablation of sea-ice impacts approximately 50% of the Southern Ocean habitat. This natural, annual cycle affects the structure of the marine food web and the metabolic activities of nearly all species of marine organisms, from bacteria to top-level predators. The recent establishment of a Long-Term Ecological Research (LTER)

site near Palmer Station provides a unique opportunity to conduct a systematic and comprehensive investigation of ecosystem dynamics in an area that is representative of the sea-ice impacted marine environment. The development of ecosystem models will help focus the research efforts on important, testable hypotheses that are probably beyond the scope of individual P.I.-directed research efforts. In this way, the LTER program provides an invaluable opportunity for research on the Antarctic coastal ecosystem (see *Antarctic Journal of the United States*, Dec 1990, vol. XXV, pp. 12-15 for more details on the LTER program).

The research proposed in this LTER "satellite" project will complement the LTER "core" efforts already in progress in several ways. First, the establishment of two time-series sediment trap stations in the Palmer LTER study area and the routine measurements of oceanic carbon pools (total CO_2, pCO_2 and DOC) will provide a strong connection to the Joint Global Ocean Flux Study (JGOFS) program which has as its primary goal (Scientific Committee on Ocean Research [SCOR] report of 1987 Paris meeting):

> "To determine and understand on a global scale the processes controlling the time-varying fluxes of carbon and associated biogenic elements in the ocean, and to evaluate the related exchanges with the atmosphere, sea floor and continental boundaries."

Time-series observations at a representative site, extending over several years remains as the only strategy whereby long-term cycles and trends in the ocean can be observed and, eventually, understood. Furthermore, Antarctica figures prominently in global models of atmospheric-ocean CO_2 and productivity. Increased involvement and proactive participation in both LTER and JGOFS programs were recently cited as two key initiatives of the Polar Biology and Medicine Program of the Division of Polar Programs (in A Long Range Science Plan for the Division of Polar Programs of the National Science Foundation, April 1990). Of the core measurements being proposed, the dissolved inorganic carbon system parameters (TCO_2 and pCO_2) are particularly rare from antarctic environments; measurements of DOC by high temperature combustion methods are non-existent. These measurements will comprise an invaluable data base not only in support of other core LTER projects but for the developing models of the role of antarctic ecosystems in global carbon flux.

Finally, heterotrophic microorganisms are now recognized as having a major role in ocean productivity and nutrient regeneration and, therefore, in the oceanic carbon cycle. The establishment of a microbiology component to the LTER program will provide an opportunity to establish the role of the "microbial loop" in Southern Ocean habitats and to quantify carbon fluxes through this poorly characterized component of the food web.

4. Relationship to the Ongoing LTER Program

The University of California at Santa Barbara is the lead institution for the Palmer LTER. Drs. R. Ross and L. Quetin will be the project coordinators and will be largely responsible for integrating the various research components. An Executive Committee will decide upon program directions and changes therein. An annual meeting of P.I.s will be held at UC Santa Barbara. A centralized multi-disciplinary data base will be established and distributed to program participants through an INTERNET or BITNET computer network.

The microbiology and carbon flux subcomponent of LTER is coordinated with the existing LTER program, but would not be part of the "core" project. The P.I., however, is responsible for meeting all of the LTER requirements on pre-cruise planning and post-cruise data reporting, and is expected to contribute to the annual reports of the LTER program. In return, the LTER data base will be made available to provide complementary information to that obtained in this proposed "satellite" project. The goals and objectives of this current proposal have been explicitly discussed among the members of the Palmer LTER Executive Committee. Drs. Ross and Quetin have subsequently encouraged the P.I. to pursue his research foci as

an extension of the present LTER program. It was generally agreed that a satellite component on microbiology and carbon flux would be a welcomed addition to this interdisciplinary research program.

5. Note on Safety

Please, above all else, exercise extreme caution when working aboard the *Polar Duke*. The weather is extreme and the heavy clothing results in loss of dexterity and coordination on the deck. Do not step into the bight of a line; Do not use the hero platform without a floatcoat; Do not spit in the wind; etc.... All joking aside, I want you all to enjoy a very safe trip, so please be careful at all times.

Second only to precautions regarding personal injury, is the precaution that should be taken to avoid loss of the equipment that we have on board. Please handle it carefully and be sure that all items are secure at all times.

Another important consideration is the fact that you are collectively representing the U.S. Antarctic Research Program, NSF and Project S-046... in that order. You are expected to maintain a high level of professional behavior in your day-to-day interactions with the master and crew of the *Polar Duke*, with the Palmer crew and, perhaps most importantly, with the other LTER colleagues and NSF and ASA personnel. Remember, our cruise is an interdisciplinary program and the success of the field experiment requires interdisciplinary and interinstitutional cooperation. Please lend a hand to members of the other programs whenever possible.

6. Note on Use of Radioisotopes

6.1. *General Comments*: *N.B. PALMER* and *POLAR DUKE* isotope activities are to be restricted to the HELO DECK LAB. Samples may be stored (out of necessity) in the main deck walk-in freezer but only after proper packing (be very careful here).

6.2. *HELO Deck Lab Operations*: We must package all wastes, both liquid and solid. For solid wastes (pipette tips, LSC vials-empty, kimwipes, etc.), accumulate in lab in small plastic bag. When you have a bag-full of stuff, place it into the waste drum on HELO deck marked "SOLID WASTE"; be sure to enter these wastes in the disposal record. The liquid waste that we generate is one of two types: (1) primary waste -- the effluent or filtrate from our incubation experiments and (2) secondary waste -- from bottle rinsing. Be sure to keep all of our waste (tritium) separate from that generated by other investigators (carbon-14), unless instructed by the MPC to combine them.

All of our liquid primary waste will first appear in our 20 l glass carboy. When this is approximately 50-75% full (please do not run the risk of overflowing this carboy or else we are in trouble -- or should I say I am in trouble with ASA and NSF and you are in trouble with me), transfer the waste to one of the two plastic carboys (tritium) in the lab. This transfer should be done by pressurizing the bottle with the pump. The bottle can, and should, remain stationary for this procedure. Two people are required for this operation. Simply disconnect the tube which leads from the pump to the manifold (use the quick disconnect which is in-line) and place the end leading from the waste bottle into the plastic carboy. Next, remove the tubing from the vacuum outlet on the pump and attach to the pressure outlet. Be sure that the pressure is not on full blast -- turn the pump on. The glass bottle should pressurize and push the waste into the plastic container. Do not walk away during this 5 min operation but rather be awake, aware and careful to keep all tubing where it (they) belong. We do not want a nuclear blowout!!!

6.3. *Bottle Rinsing Procedure and Secondary/Tertiary Waste*: During the cruise, you will be required to re-use the 60 ml polyethylene bottles (LEU) that have previously contained radioisotopes. Each bottle is marked with a permanent label. Please keep these labels on so that we re-use the bottles for the

same isotopes. Additional, temporary labels can, and should, be placed on the bottles for each individual experiment. It is important that the bottles are not cross-contaminated because we cannot conduct a thorough rinsing between each use (*i.e.* no sink!!!).

To wash the bottles, fill with approximately 10-15 ml of tap water (this must be transported to the HELO DECK LAB from the HOLD LAB), shake, decant liquid into the tritium carboy. Repeat once with distilled water. Replace cap. Bottle is now ready for use. This procedure generates additional waste but is, under the present circumstances, an acceptable compromise situation. Between cruises, give the bottles an extra distilled water rinse and store open/upsidedown (be sure to put paper towels underneath). Be sure to segregate the tops to prevent cross-contamination.

Be extremely careful about spills... avoid these at all costs. If a spill does occur, take the appropriate corrective measures to contain and clean. Be especially alert in heavy seas.

7. Note on Labeling Samples and Entering Data in Notebook

It is important that all samples are labeled carefully, logically and consistently. Please follow a logical, complete labeling procedure. This will eliminate confusion back at the lab when the samples are analyzed. The stations should be labeled consecutively in the order worked... NOT BY STATION NUMBER DESIGNATION. Of course, the station number should also be entered in the notebook along with the date and time of arrival on that station. The latter is important because we can (if confusion arises) compare our station number to the master log of the ship. Both GMT and local times should be entered as GMT____, L____.

8. Note on Use of This Manual

This S-046 "training" manual is intended to familiarize the cruise participants with our field and subsequent laboratory operations. Much of the information on detailed protocols, etc. is not of immediate interest to those of you who will primarily be assisting in sample colection and field processing. The information is included here primarily for the use of those individuals who will complete the processing once the samples are returned to Hawaii.

The most important thing is to be aware of what is expected of you in the field. Please read this manual (at least chapters 1-3 and Appendix I) carefully before you arrive at the dock in Punta Arenas! I will run through the procedures on the *Polar Duke* prior to sailing on 01 Jan 1994. At that time, and after the initial set-up of the various shipboard laboratories, we will provide additional more detailed and much more practical listings of exactly what to do for each type of sample analysis.

9. References

Billen, G. and S. Becquevort. 1991. Phytoplankton-bacteria relationship in the Antarctic marine ecosystem. Pro Mare Symposium on Marine Arctic Ecology, Trondheim, Norway.

Bird, D. F. and D. M. Karl. 1991. Spatial patterns of assimilation of exogenous glutamate and thymidine in the western Bransfield Strait, Antarctica, during and following the austral spring bloom. *Deep-Sea Research*, in press.

Fasham, M. J. R., H. W. Ducklow and S. M. McKelvie. 1990. A nitrogen-based model of plankton dynamics in the oceanic mixed layer. *Journal of Marine Research*, **48**, 1-49.

Hanson, R. B. and D. H. Pope. 1981. Bacterioplankton adaptation and growth in different zones of the southern ocean. *Antarctic Journal of the United States*, **16**, 130-132.

Hodson, R. E., F. Azam, A. F. Carlucci, J. A. Fuhrman, D. M. Karl and O. Holm-Hansen. 1981. Microbial uptake of dissolved organic matter in McMurdo Sound, Antarctica. *Marine Biology*, **61**, 89-94.

Karl, D. M. and D. V. Hebel. 1990. RACER: Dissolved oxygen and nitrate dynamics during the 1989 austral spring bloom. *Antarctic Journal of the United States*, **25**, 149-151.

Karl, D. M., O. Holm-Hansen, G. T. Taylor, G. Tien and D. F. Bird. 1991a. Microbial biomass and productivity in the western Bransfield Strait, Antarctica during the 1986-87 austral summer. *Deep-Sea Research*, **38**, 1029-1055.

Karl, D. M., B. D. Tilbrook and G. Tien. 1991b. Seasonal coupling of organic matter production and particle flux in the Bransfield Strait, Antarctica. *Deep-Sea Research*, **38**, 1097-1126.

Lancelot, C., G. Billen and S. Mathot. 1989. Ecophysiology of phyto- and bacterioplankton growth in the Southern Ocean. Belgian Scientific Research Programme on Antarctica Scientific Results of Phase One (Oct 85 - Jan 89), Volume 1: Plankton Ecology, 97 pp.

Morita, R. Y. 1975. Psychrophilic bacteria. *Bacteriological Reviews*, **39**, 144-167.

Morita, R. Y., R. P. Griffiths and S. S. Hayasaka. 1977. Heterotrophic activity of microorganisms in Antarctic waters. In: *Adaptations within Antarctic Ecosystems: Proceedings of the Third SCAR Symposium on Antarctic Biology*, G. A. Llano, editor, Smithsonian Institution, pp. 99-113.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. *BioScience*, **24**, 499-504.

Pomeroy, L. R. and D. Deibel. 1986. Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters. *Science*, **233**, 359-361.

Pomeroy, L. R. and W. J. Wiebe. 1988. Energetics of microbial food webs. *Hydrobiologia*, **159**, 7-18.

Rivkin, R. B. and M. Putt. 1987. Heterotrophy and photoheterotrophy by Antarctic microalgae: light-dependent incorporation of amino acids and glucose. *Journal of Phycology*, **23**, 442-452.

Sieburth, J. M. 1960. Acrylic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters. *Science*, **132**, 676-677.

Chapter 4
SAMPLING SCHEDULE AND STRATEGY

SUMMARY: *During LTER, data will be collected by project S-046 participants using CTD-rosette-Niskin bottle sampling. Two modes of station occupation (transect and time-series) will define our sampling strategy.*

This cruise, designated PD 94-01, will combine the research efforts of several independent but complementary projects. The primary objectives of the UH (S-046) effort are: (1) to obtain data on nutrient and biomass distributions in the LTER study region, (2) conduct physiological/ecological studies of microbial growth and grazing and (3) conduct studies of organic matter turnover including photolytic degradation. The S-046 field party will include 6 scientists.

You should all have received a copy of the "Personnel Manual." Please read it carefully. You will be briefed about shipboard etiquette and safety shortly after we depart from Punta Arenas on 01 Jan 1994. Detailed assignments of wire time, distribution of collected water samples and other details of the field work will be decided once we arrive in Antarctica. What follows is a broad-brush cruise plan highlighting only the major events and ship movements. Last minute changes, by definition, occur at the last minute. A fully revised plan will be posted aboard the ship before we sail from Punta Arenas.

1. Tentative Cruise Plans

These will be distributed and discussed at the dock in Punta Arenas in late Dec.

Chapter 5
DISSOLVED OXYGEN

SUMMARY: *Seawater is collected from known depths using CTD-rosette sampling protocols. Subsamples are drawn into precalibrated iodine flasks and dissolved oxygen is chemically bound by the formation of a manganous hydroxide floc. The floc is subsequently dissolved under acidic conditions which stochiometrically converts the original dissolved oxygen (DO) to triiodide. The latter is quantitatively titrated with sodium thiosulfate to either a visual starch or potentiometric end-point.*

1. Principle

The oxygen content of seawater is a fundamental measurement in oceanography providing information which can elucidate water mass movements, net primary productivity, atmosphere-ocean interactions and carbon remineralization processes. The oxygen content of a seawater sample is largely determined by a balance between: (a) the exchange of atmospheric oxygen with the upper mixed layer, (b) net increases due to photosynthetic processes and (c) net decreases due to respiratory demands and heterotrophic processes. From an oceanographic perspective, the measurement of dissolved oxygen is a parameter of fundamental importance.

Even with recent advances in analytical chemistry, the classical Winkler titration procedure for the measurement of dissolved oxygen (DO) remains the most accurate and reliable method for oceanographic applications. In this procedure, a divalent manganese solution and a strong alkali are added to the water sample which results in the formation of a floc. When the sample is subsequently acidified, iodine, equivalent to the original oxygen concentration, is liberated. The iodine is titrated with a standardized thiosulfate solution and the oxygen concentration calculated from the quantity of thiosulfate consumed.

2. Precautions

Careful subsampling is especially important if accurate and precise results are to be obtained. Care must be taken to ensure that the subsample is drawn as soon as possible after sample collection, and that bubbles are excluded from the sample flask. Also, sample storage can be a problem. If the water samples cool significantly or if the seal dries during storage, air can infiltrate the flask contaminating the sample. Therefore, after resuspending the floc, the rim of the flask is filled with seawater and the flask rim covered with a plastic cap (if available). Samples are stored near room temperature in a location where fluctuations are minimized.

If the water sample temperature at the time of collection differs from the *in situ* potential temperature, then it is necessary to use the temperature at the time of collection to convert DO concentrations to per unit mass (*i.e.* μmol kg^{-1}). Therefore, both the *in situ* water and the sub-sampling temperatures must be known.

3. Field Sampling

3.1. Sample collection

3.1.1. Subsamples are drawn as soon as the rosette arrives on deck. DO samples are the first

samples drawn from the water sampling bottle.

3.1.2. The drawing tube is flushed with sample and any bubbles are displaced. The tube is then inserted (with water continuously flowing) almost to the bottom of the sample flask. The sample stream should not generate too much turbulence in the flask in order to prevent the intrusion of atmospheric oxygen.

3.1.3. The flask is overflowed with approximately three volumes of sample, being careful to prevent bubbles from adhering to the walls of the sample flask. The fluid flow rate is timed to estimate required flushing time.

3.1.4. The tube is slowly withdrawn from the flask while the sample is flowing into the bottle so that the bottle remains brimful when the tube is completely withdrawn.

3.2. Sample fixation

3.2.1. Before fixing the sample, the iodine flask is carefully examined for any bubbles that may have adhered to the walls. If any bubbles are present, the sample is discarded and drawn again.

3.2.2. Immediately after confirmation of adequate sampling, 1 ml each of the two fixing reagents ($MnSO_4 \cdot H_2O$ and alkaline iodide) are added simultaneously using two 5 ml autopipettes mounted side-by-side. The pipette tips are positioned at least 3 cm below the surface of the sample. Care is taken so that bubbles are not added in this process.

3.2.3. The glass stopper is carefully inserted so that no bubbles are trapped in the flask, and so that the water in the neck, which might be contaminated with atmospheric oxygen, is displaced.

3.2.4. The flask is shaken vigorously for at least 20 seconds with a rapid wrist action (it is extremely important that the floc be dispersed throughout the flask).

3.3. Storage

3.3.1. Approximately 20 minutes after the samples are collected, the sample bottles are shaken again to resuspend the floc. The flared mouth of the flask is then filled with seawater. The box of bottles are stored in a cool location where temperature fluctuations are minimized.

4. Analysis

4.1. KIO_3 standards and thiosulphate concentrations determination

An iodine flask is filled to below the neck with dH_2O. 1 ml of 10 N H_2SO_4 is added and mixed thoroughly. 1 ml of NaOH/KI is added and mixed, then 1 ml of $MnSO_4 \cdot H_2O$. Precipitating agents are added in reverse order to sample fixing. 5 ml of 0.0100 N KIO_3 standard is added with a calibrated volumetric pipet. Any solution on the flask neck is rinsed down with a squirt bottle and the flask contents are titrated using Letelier's potentiometric endpoint titration program. Repeat 3-5 times. Solve for unknown using:

$$V_1 * N_1 = V_2 * N_2$$

where: V_1 = blank corrected volume of thiosulfate to reach end-point
N_1 = thiosulfate normality
V_2 = volume of potassium iodate added

N_2 = potassium iodate normality

4.2. Blank determination

As in 4.1, except to titrated flask contents add 5 additional ml of KIO_3 standard. Repeat titration.

$$\text{Blank} = V_1 - V_2 \text{ (can be + or -)}$$

where: V_1 = initial titrant volume
V_2 = second titrant volume

4.3. Titrate samples

Bring fixed DO samples to ambient temperature. Rinse electrode with dH_2O. Wick drops from electrode with Kimwipe. Aspirate water from rim of iodine flask. Be very consistent in these manipulations to achieve greatest precision. Add 1 ml of the sulfuric acid reagent (10 N) to each iodine flask. Drop a clean teflon-coated magnetic stir bar into the flask and place on magnetic stirrer. Dissolve floc and titrate to pale straw color. Complete titration with Letelier's potentiometric endpoint titration program.

5. Calculations

Calculate the DO concentration using the following formula:

$$\mu\text{mol } O_2\, l^{-1} = \frac{(V_t - V_b) * N_t * E}{V_f} - R_{DO}$$

where: V_t = volume of titrant (μl)
V_b = volume of blank (μl)
N_t = normality of titrant (μeq μl^{-1})
E = 0.2500 (μmol O_2 μeq^{-1})
V_f = volume of flask (l)
R_{DO} = dissolved oxygen content of the reagents, 0.804 (0.14/V_f) (μmol O_2 l^{-1})

From: Carpenter 1965

0.018 ml O_2 l^{-1} = DO content of fixing reagents when 1 ml of each reagent is used in 140 ml of sample

6. Precision and Accuracy

The method as outlined above is capable of a precision of 0.1% or less (as defined by the coefficient of variation for triplicate samples), when conducted by an experienced analyst. The accuracy of the Winkler titration procedure, when the Carpenter modifications are employed, has been determined to be 0.1% (Carpenter, 1965).

7. Equipment

5 ml Dosimat titrator (Brinkmann)
calibrated iodine titration flasks
teflon-coated magnetic stir bars and retriever

squirt bottle with deionized distilled water (DDW)
reagent dispensers
magnetic stir plate
calibrated volumetric pipettes and flasks

8. Reagents

Manganese sulfate reagent (2.2 M)

Dissolve 365 g of manganese sulfate ($MnSO_4 \cdot H_2O$) in 800 ml distilled water and make up to 1 liter in a volumetric flask.

Alkaline iodide reagent

Dissolve 500 g sodium hydroxide (NaOH) in 500 ml distilled water and, separately, dissolve 300 g potassium iodide in 450 ml distilled water. Mix the two solutions 1:1, by volume.

Sulfuric acid reagent (10 N)

Mix 280 ml of concentrated sulfuric acid into distilled water using a 1 liter volumetric flask packed in ice as reaction is exothermic

Sodium thiosulfate reagent

Use commercial solution (Fisher Scientific Co.) and check normality, as described.

Potassium iodate reagent (0.1000 N)

Dissolve 0.3567 g of dry (100°C, 2 hours) KIO_3 into 800 ml distilled water and bring up to 1 liter in a volumetric flask. Calibrate against Wako KIO_3 standard solution.

9. References

American Public Health Association. 1981. *Standard Methods for the Examination of Water and Wastewater*, 15th edition.

Carpenter, J. H. 1965. The accuracy of the Winkler method for dissolved oxygen analysis. *Limnology and Oceanography*, **10**, 135-141.

Carpenter, J. H. 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. *Limnology and Oceanography*, **10**, 141-143.

Strickland, J. D. H. and T. R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, Ottawa, 167.

Chapter 6
DISSOLVED INORGANIC CARBON AND ALKALINITY

SUMMARY: *Seawater samples are collected at discrete depths using CTD-rosette sampling protocols. Subsamples for dissolved inorganic carbon are collected, immediately preserved with $HgCl_2$ and stored for subsequent analysis in the laboratory using a commercial CO_2 coulometer.*

1. Principle

The accurate and precise determination of dissolved inorganic carbon (DIC) concentrations over annual and interannual time scales is central to the achievement of LTER objectives. In the central ocean basins, DIC concentration in surface seawater is believed to be controlled by air-sea exchange reactions. However, physical processes and biological activity also influence the concentration of DIC in surface waters. Beneath the mixed layer the concentration of DIC increases as a result of the decomposition of organic material.

DIC concentrations are determined using a commercial CO_2 coulometer. The coulometric determination of carbon dioxide has the unique distinction of performing with a high degree of both precision and accuracy while maintaining relatively high sample throughput. The coulometric determination of carbon dioxide involves the stripping of an acidified seawater sample with a carbon dioxide-free air stream and subsequent absorption of the carbon dioxide by a solution of ethanolamine. The weak acid generated by carbon dioxide absorbed in ethanolamine is titrated by a strong base produced electrolytically. The equivalence point is detected photometrically with thymolphthalein as the indicator. The number of coulombs required to reach the end-point is proportional to the quantity of carbon dioxide evolved from the sample.

2. Precautions

DIC samples should be the first samples taken from the water bottle unless dissolved oxygen (DO) is also sampled from the same hydrocast, in which case DIC samples are collected immediately after the DO samples.

Careful subsampling is especially important for all dissolved gases. Samples should be taken as soon as possible and in the same manner as DO samples (*i.e.* no bubbles, low turbulence with adequate flushing).

3. Water Sampling

DIC samples are currently collected in the same manner as prescribed by Keeling-Guenther (SIO) for their total carbon sampling regime.

3.1. Drawing the sample

3.1.1. Samples are drawn into clean 300 ml glass reagent bottles (combusted, not acid washed) as soon as the rosette arrives on deck.

3.1.2. The drawing tube is completely filled with sample by raising the end of the drawing tube.

Bubbles are simultaneously dislodged by tapping and manipulation of the tube. The drawing tube is flushed and inserted to the bottom of the sample bottle.

3.1.3. The sample bottle is overflowed with at least two volumes of sample.

3.1.4. The tube is slowly withdrawn from the bottle while the sample is flowing into the bottle so that the bottle remains brimful when the tube is completely withdrawn.

4. Preserving the Sample

4.1. Some of the sample is removed from the reagent bottle using a plastic pipette equipped with a rubber bulb. Enough water is removed so that approximately 1 ml of air is contained in the bottle when the glass stopper is inserted.

4.2. 100 μl to 1 ml (depending on current biological activity in seawater) of saturated $HgCl_2$ is added to each sample. The tapered ground glass bottle neck is dried with a Kimwipe wrapped on an applicator stick. The bottle is sealed with a ground glass stopper coated with a light covering of Apiezon grease. The stopper is pressed firmly into the bottle to make a good seal. The stopper is secured with polyethylene tape or a large rubber band.

4.3. The samples are stored in a cool location, in the dark.

5. Coulometric Determination of DIC

5.1. Maintenance of extraction and analysis system

5.1.1. The glassware used in the extraction system is combusted (450°C, 3 hours) on a regular basis in order to prevent the buildup of organic films within the extraction system.

5.1.2. The titration cell and rubber stopper are dried overnight at 55°C before use.

5.1.3. Silica gel drying tubes are renewed frequently to prevent water vapor from passing from the extractor to the titration cell.

5.2. Analysis

5.2.1. Sample bottles to be analyzed are brought to 25°C by placing them in a temperature-controlled water bath.

5.2.2. A glass pipette of predetermined volume is carefully filled with sample at 25°C. The pipette is flushed with at least 1.5 volumes of sample and inspected to be certain that no air bubbles are lodged in the pipette.

5.2.3. Five ml of 6% phosphoric acid is added to the extractor and the acid is purged for 2-5 minutes with carbon dioxide-free carrier gas.

5.2.4. The coulometer is reset and the pipette is emptied into the extraction vessel containing 5 ml of carbon dioxide-free phosphoric acid. The bulb is allowed to drain for 1 minute before the stopcocks are closed.

5.2.5. After an additional 2 minutes, the lower portion of the bulb and delivery tube are backwashed with the acidified sample solution and the bulb allowed to drain.

5.2.6. The acidified sample is purged with carrier gas. Successive coulometer readings are integrated at 1 minute intervals until they differ by less than 0.05%.

6. Determination of the Coulometer Blank

The coulometer blank is determined at the beginning of each day by allowing the coulometer to titrate a CO_2-free air stream. The blank is taken as the μg C per minute value detected by the coulometer when a steady-state reading is achieved.

7. Coulometer Calibration

Although the digital coulometer output is fairly accurate, the coulometer response per unit carbon may vary with time. In order to achieve maximum accuracy, it is necessary to calibrate the coulometer with samples containing known quantities of inorganic carbon. We are presently using both wet and dry carbon standards for calibration. Wet standards are comprised of high purity anhydrous sodium carbonate dissolved in distilled deionized water. These standards are prepared in stoppered volumetric flasks and run immediately in order to avoid errors due to the absorption of atmospheric CO_2. Anhydrous sodium carbonate is also used as a standard. The dried (270°C for 3 hours) reagent is carefully weighed on a microbalance to the nearest 0.1 μg and introduced into a degassed acidified sample solution in a combusted aluminum boat through a port in the side of the extractor. Recoveries for both wet and dry standards are generally slightly less than 100%. We are currently preparing to employ ultrahigh purity, calibrated gas standards, as well, for our routine calibrations.

8. Coulometer Data Reduction and Calculations

In order to compute the absolute concentration of DIC in a water sample, the integrated reading given at the titration endpoint must be corrected for both the coulometer blank and the recovery of wet and dry standards. These corrections are made by multiplying the blank μg C min^{-1} by the time taken to reach the endpoint and subtracting this value from the integrated reading. This value is then corrected for the recovery of standards by dividing by the average percentage recovery of known standards run on the day of analysis.

9. Coulometer Precision and Accuracy

The precision of our DIC analyses has steadily improved during the first year of field work. As of the beginning of year 2, three replicate samples from a single Niskin bottle generally yield a coefficient of variation of less than 0.1%. With the dry standard procedure now being used, our accuracy is approximately 0.3 to 0.4%. The additional error in our estimates of accuracy are presumably due to variability introduced by drying, weighing and handling the dry sodium carbonate prior to introduction to the coulometer extractor. We anticipate that the accuracy of the analysis will improve significantly with the addition of a gas sample loop for the introduction of known quantities of high purity carbon dioxide.

10. Titration Alkalinity

Total alkalinity will be measured using subsamples of the water collected for total DIC (300 ml samples) or from replicate samples (60 ml). Standard Gran titration procedures will be employed using a computer-assisted system.

11. Equipment/Supplies

Model 5011 Coulometer (UIC Inc.) and modified glassware

300 ml ground glass stoppered reagent bottles
kimwipes and applicator sticks
large plastic pipette
Apiezon grease
Cahn microbalance
volumetric flasks
analytical balance / pan balance
muffle furnace
carbon dioxide-free carrier gas
data acquisition system
water bath

12. Reagents

distilled deionized water
high purity sodium carbonate
potassium iodide
coulometer cathode solution (UIC Inc.)
coulometer anode solution (UIC Inc.)
orthophosphoric acid

13. References

Huffman, E. W. D., Jr. 1977. Performance of a new automatic carbon dioxide coulometer. *Microchemical Journal*, **22**, 567-573.

Johnson, K. M., A. E. King and J. McN. Sieburth. 1985. Coulometric TCO_2 analysis for marine studies; An introduction. *Marine Chemistry*, **16**, 61-82.

Lindberg, A. O. 1978. Automatic coulometric titration with photometric end-point detection. Part II. Coulometric determination of nanomole amounts of carbon dioxide by non-aqueous titration. *Analytica Chimica Acta*, **96**, 327-333.

Chapter 7
DISSOLVED ORGANIC CARBON

SUMMARY: *Surface and water column samples are collected directly into sterile polypropylene (PP) tubes from Niskin bottles. Samples are stored at -20°C. Before analysis, dissolved inorganic carbon (DIC) is removed by acidification and purging. DOC is measured by high temperature catalytic oxidation (HTCO) using the Ionics 555 TC/TOC analyzer system.*

1. Principle

DOC in the ocean constitutes the largest reduced carbon pool on earth and accounts for about half the mass of the ocean's dissolved organic matter. Thus, knowing the magnitude of this pool and its dynamics is important not only to traditional biological oceanography, but also to the earth's carbon budget and the effects of global environmental change.

The recently developed HTCO method yields DOC concentrations that are 2x or greater than the historical wet chemical or UV oxidation procedures. The HTCO reaction mechanism is not fully understood, but is suspected to be more effective in breaking carbon bonds in complex refractory molecules than previously employed techniques.

Seawater samples are first acidified to convert DIC to CO_2. The sample is purged of CO_2 by bubbling with high purity O_2, effectively removing all DIC. The sample is injected onto a Pt catalyst at ~800°C with O_2 as the carrier gas. DOC is converted to CO_2 and passes through a gas-liquid separator, a Sn trap (to remove HCl generated during combustion), a condensation trap, a dessicating Mg $(ClO_4)_2$ trap and a Balston particle filter. The CO_2 then enters a non-dispersive infrared (IR) detector. The integrated signal is recorded.

2. Precautions

Due to the high sensitivity of the HTCO method and the exceedingly low concentrations of organic matter in seawater, contamination is of the greatest concern. All equipment and reagents should be handled using "sterile" technique (*i.e.* such that no surface that contacts sample, such as cap lining or threads, is exposed to a non-sample substance). Gloves should be worn. For this reason, Tygon tubing and Nitex screens are not used for sample collection from the Niskin bottle as prior handling will have fingerprint contamination.

3. Sampling, Standards, Spikes, Blanks and Replicates

3.1. Water column samples are collected directly from Niskin bottle spout, *i.e.* without tubing or screen. Gloves should be worn. Allow sw to flow briefly to rinse spout. Fill a labelled, 15 ml, sterile PP tube with >10 ml of sw without rinsing. Cap and store at -20°C, upright.

3.2. For an occasional surface sample or depth profile, a set of internal standards is made using the sterile sucrosesolution provided. For fast grid or depth profile, spike triplicate subsamples. For each fast grid or depth profile, take triplicate samples from the same Niskin, or different Niskins from same depth.

3.3. This assay has no field nor analytical blank.

4. Analysis

The Ionics 555 is stabilized at ~800°C for ~48 hrs. O_2 gas flow is set to 125 cc/min. The thawed sample is agitated vigorously and 10 ml transferred using a combusted glass pipet to a 20 ml combusted glass scintillation vial. 50 μl of 50% H_3PO_4 is added. The vial is covered with combusted foil and purged >10 min with research grade O_2. A 100 μL volume is injected in triplicate and peak areas recorded.

A standard curve between 0-200 μM added sucrose is run with unknowns and standards and spikes prepared in the field.

5. Calculations

Slope is determined from the regression of peak area on sucrose concentration. Unknown concentration is equal to the peak area divided by the slope. The linear regression constant (intercept) is not used in the calculation. Carbon is reported in molar units and calculations are conveniently done by spreadsheet.

6. Equipment

Field:

15 ml sterile PP tubes and racks
gloves
adjustable pipet
sterile, acid washed pipet tips
12 mM carbon standard, ultrapure sucrose

Lab:

Ionics 555
HP 3396A integrator
O_2, research grade (<1 ppm CO_2/CO)
20 ml glass scintillation vials, combusted
adjustable pipet
sterile, acid washed pipet tips
H_3PO_4, 50%
combusted glass 10 ml pipet

7. References

Peltzer, E. P., P. G. Brewer, Y. Suzuki and Y. Sugimura. 1991. Measurement of dissolved organic carbon in seawater by high temperature catalytic oxidation. Submitted to *Journal of Marine Research*.

Sugimura, Y. and Y. Suzuki. 1988. A high temperature catalytic oxidation method of non-volatile dissolved organic carbon in seawater by direct injection of liquid samples. *Marine Chemistry*, **24**, 105-131.

Suzuki, Y., Y. Sugimura and T. Itoh. 1985. A catalytic oxidation method for the determination of total nitrogen dissolved in seawater. *Marine Chemistry*, **16**, 83-97.

Chapter 8
NITRATE, NITRITE and DISSOLVED ORGANIC NITROGEN

SUMMARY: *Seawater is collected from known depths using CTD-rosette sampling protocols. Subsamples are drawn, filtered and stored frozen in acid-washed polyethylene bottles. Nitrate/nitrite is measured with an azo dye either before (nitrite) or after (nitrite plus nitrate) subsamples are passed through a cadmium reduction column. Dissolved organic nitrogen is determined after quantitative conversion to inorganic N by exposure to UV radiation.*

1. Principle

In seawater the forms of dissolved nitrogen of greatest interest are, in order of decreasing oxidation state: nitrate, nitrite, ammonium and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N_2), are biochemically interconvertible and are components of the biological nitrogen cycle.

In this method nitrate is quantitatively reduced to nitrite in a copperized cadmium reduction column. The nitrite thus produced, along with any nitrite present in the original sample, is coupled with an aromatic amine, which in turn is reacted with a second aromatic amine to produce an azo dye. The extinction due to the dye is then read spectrophotometrically. A second subsample is analyzed without prior reduction in order to determine the nitrite level. Nitrate is calculated by difference between the [nitrate+nitrite] and nitrite concentrations, using standard solutions. For surface water samples (<100 m) where the [nitrate+nitrite] concentration is generally $\leq 0.05\ \mu$M, we have employed a low-level assay procedure which is based on the production and detection of nitrous oxides.

Total dissolved nitrogen (TDN) is determined by UV oxidation of the sample and subsequent analysis for dissolved inorganic nitrogen (DIN = nitrite + nitrate + ammonia). Dissolved organic nitrogen (DON) is computed from the relationship DON = TDN - DIN, where TDN is total dissolved nitrogen after UV oxidation and DIN is the sum of the dissolved inorganic nitrogen species before UV oxidation. As an alternative to the UV oxidation method, Walsh (1989) has described a high-temperature (1100°C) combustion method which has been applied to open ocean samples collected in the North Pacific Ocean. No significant differences were observed between these two procedures (Walsh, 1989).

2. Precautions

Contamination is the primary concern with these samples. This is particularly true with samples collected from the euphotic zone, where inorganic nutrient concentrations are extremely low ($\leq 0.2\ \mu$M). In order to avoid contamination, sample bottles must be meticulously cleaned with dilute HCl and rinsed with deionized distilled water (DDW) before use. The filtration step is the most likely point of contamination. Samples are stored frozen until analysis, generally within1-2 weeks of sample collection.

3. Sampling Collection and Storage

NOTE: The currently held "dogma" in the oceanographic literature is that seawater samples must be processed fresh and on board ship for high-precision, low-level inorganic nutrient analyses (Morse *et al.*, 1982; Venrick and Hayward, 1985). However, extensive results from automated analyses of nutrients in tropical seawaters (Ryle *et al.*, 1981) and the VERTEX program (D. Karl and S. Moore, unpubl. results) which included direct comparisons of [NO_3+NO_2], PO_4 and SiO_2 determinations in fresh vs. frozen

samples would suggest otherwise. Provided that caution is taken to collect and store the samples in an environment free of potential contamination, we found no significant treatment effect. A similar conclusion was presented by Walsh *et al.* (manuscript) following the analysis of a wide range of seawater samples that were either analyzed fresh or frozen and stored for varying periods of time. They conclude that, "Despite published and voiced opinions to the contrary, there appears to be no adequate basis either from the literature or from our experiments for across-the-board dismissal of high-precision nutrient analyses undertaken on properly stored seawater samples." As we are not able to take an autoanalyzer to sea during RACER, we have focused our attention on maintaining a contamination-free environment during collection and storage of nutrient samples.

3.1. Sample collection

3.1.1. Rinse the nutrient sample bottle (acid-washed, 125 ml polyethylene bottle) 3 times before filling. Fill to approximately 2/3 full, tighten cap and freeze.

3.1.2. Record cruise, cast and Niskin bottle number on the bottle and data sheet.

4. Sample Analysis

4.1. Nitrate (NO_3^-) plus Nitrite (NO_2^-)

$[NO_3^- + NO_2^-]$ analyses are performed on a four-channel Technicon Autoanalyzer II^R continuous flow system. The automated wet chemistries generally follow the standard methods of seawater analysis as given by Technicon (1977), which involve: (1) reduction of nitrate to nitrite using a copperized cadmium reduction column, (2) reaction of nitrite with sulfanilamide for diazotization and (3) coupling with N-1-napthylethylenediamine dihydrochloride (NED) forming a purple azo dye (Armstrong *et al.*, 1967). The dye absorbance is read through a 15 mm pathlength flowcell at 550 nm. The reduction column is looped in line using a Hamilton 4-way valve and can be by-passed for nitrite analysis only. Both nitrate and nitrite standards are run to check column efficiency. If speciation is desired, nitrite is determined separately by omitting the reduction step. Nitrate is calculated by difference.

4.2. Dissolved Organic Nitrogen (DON)

The method for DON involves initial UV digestion of a seawater sample followed by autoanalysis of the digestion products for [nitrate+nitrite], as above, and ammonium using the Berthelot (indophenol) method. The modified photooxidation technique (Armstrong *et al.*, 1966) utilizes a 24 hour irradiation. Details are given in Walsh (1989). Periodic calibration checks of the UV lamp efficiency are made using a dissolved organic nitrogen (2,2-bypyridyl) standard. As a general rule, the UV lamp is replaced after approximately 700-800 hr of use. DON is calculated by difference between the sum of [nitrate+nitrite+ammonium] before and after UV treatment.

5. Calibration, Data Reduction and Calculations

5.1. Calibration stocks and regression standards

The calibration of dissolved inorganic nutrients in the autoanalysis of seawater samples is performed using standard solutions containing N, P and Si. A nutrient stock solution is prepared by dissolving dried (65°C, 72 hours) analytical grade reagent chemicals with distilled-deionized water in 1 liter glass volumetric flasks containing 1 ml of chloroform. Once dissolved, this stock solution is immediately transferred into 1 liter amber polypropylene bottles and stored at 4°C. The reagent chemicals and concentrations are: phosphate (KH_2PO_4, 1 mM), nitrate (KNO_3, 5 mM) and silica (Na_2SiF_6, 4 mM).

Working standards are prepared daily by volumetric dilutions of the stock using glass pipettes and a plastic (polymethylpentene; PMP) volumetric flask. All pipettes and PMP flasks are acid-washed (1 M HCl) and gravimetrically calibrated prior to use. The daily regression standards are prepared by diluting the working standard with low nutrient natural seawater diluent (SWDIL). The SWDIL is filtered open ocean surface seawater that is stored in a carboy at room temperature. By using this technique all standards are matrix-matched with the seawater samples and any cross-nutrient interference effect should be accounted for.

Cross-nutrient interference and reagent contamination was evaluated by preparing separate solutions, as above, but with one of the three standards omitted. Only phosphate showed a slightly measurable increase (+0.014 μM) in the presence of 40 μM-NO_3 and 160 μM-Si. The linear regressions of the standards were applied to all seawater sample peaks for calculating each batch of cruise samples. Typical correlations produced r^2 values that were between 0.9999 and 0.99999.

5.2. Blank corrections

All seawater standard absorbance peaks were corrected for the absorbance of the seawater diluent (SWDIL). All seawater sample peaks were corrected for the refractive index absorbance for each unique nutrient detection system. The refractive index corrections (in apparent μM units) ranged from approximately 0.13 (for P), 0.23 (for N) to 2.41 (for Si), and represent the increase in absorbance that is due strictly to the presence of dissolved salts in seawater when compared to the distilled-deionized water baseline. These corrections are made running seawater (35 o/oo salinity) through the autoanalyzer with DDW only in reagent lines. The Levor surfactant used routinely in the phosphate channel was omitted from the DDW lines during the refractive index measurement because Levor reacts erratically with seawater in the absence of the acidic color reagent.

6. Accuracy and Precision

The detection limit for nitrate plus nitrite is approximately 0.03 μM with a coefficient of variation for field-collected replicates of 0.3%. The detection limit for DON is 0.05 with a coefficient of variation of 4%.

7. Equipment/Supplies

Niskin bottles and rosette/CTD unit
acid-washed, 125 ml polyethylene bottles
Autoanalyzer (Technicon Corp.) and accessories
UV oxidizer unit
nitrogen oxide analyzer (Antek model #720, operated in vacuum mode)
reaction tube, cold finger and drying tube glassware array

8. Reagents

glass distilled deionized water (DDW)
1 M HCl for cleaning
concentrated H_2SO_4 (36 N)
Ammonium Chloride Reagent: Dissolve 10 g of ammonium chloride in DDW, adjust pH to 8.5 with concentrated ammonium hydroxide and dilute to 1 liter. Add 0.5 ml Brij-35 (Technicon No. T21-0110).
Color Reagent: To approximately 1500 ml of distilled water, add 200 ml of concentrated phosphoric acid and 20 g of sulfanilamide. Dissolve completely (heat if necessary). Add 1 g of N-1-naphthylethylenediamine dihydrochloride and dissolve. Dilute to 2 liters. Add 1.0 ml of Brij-35.

Store in a cold, dark place. Stability: one month.

Cadmium Powder (Technicon No. T11-5063): Use coarse cadmium powder (99% pure). Rinse the powder once or twice with a small quantity of clean diethyl ether and 1 M HCl to remove grease and dirt. Follow with a DDW rinse. Allow the metal to air-dry and store in a well-stoppered bottle.

Ferrous Ammonium Sulfate (4% w/v): Dissolve 4 g of reagent grade ferrous ammonium sulfate in 100 ml DDW. Prepare fresh daily.

Ammonium molybdate (2% w/v): Dissolve 2 g reagent grade ammonium molybdate in 100 ml DDW. Prepare fresh daily.

Sulfanilamide (1% in 10% HCl). Dissolve 1 g reagent grade sulfanilamide in 100 ml of 10% HCl.

Sodium hydroxide (6 M): Dissolve 240 g of reagent grade sodium hydroxide and make up to 1 liter with DDW.

Preparation of Reduction Column: See Technicon Industrial System, 1977.

— Stock Standard (1000 μM): Dissolve 0.101 g of potassium nitrate in DDW and dilute to 1 liter. Store in a dark bottle. (Add 1 ml of chloroform as a preservative.)

— Working Standard (50 uM): Dilute 5 ml of stock standard in a volumetric flask with DDW or seawater diluent.

9. References

Armstrong, F. A. J., C. R. Sterns and J. D. H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. *Deep-Sea Research*, **14**, 381-389.

Armstrong, F. A. J., P. M. Williams and J. D. H. Strickland. 1966. Photo-oxidation of organic matter in seawater by ultraviolet radiation, analytical and other applications. *Nature*, **211** 481-483.

Cox, R. D. 1980. Determination of nitrate at the parts per billion level by chemiluminescence. *Analytical Chemistry*, **52**, 332-335.

Garside, C. 1982. A chemiluminescent technique for the determination of nanomolar concentrations of nitrate and nitrite in seawater. *Marine Chemistry*, **11**, 159-167.

Grasshoff, K., M. Ehrhardt, and K. Kremling. 1983. Methods of Seawater Analysis. Verlag Chemie.

Morse, J. W., M. Hunt, J. Zullig, A. Mucci, and T. Mendez. 1982. A comparison of techniques for preserving dissolved nutrients in open ocean seawater samples. *Ocean Science and Engineering*, **7**, 75-106.

Ryle, V. D., H. R. Mueller, and P. Gentien. 1981. Automated analysis of nutrients in tropical sea waters. *Australian Institute of Marine Science Technical Bulletin, Oceanography Series No. 3.*

Standard Methods for the Examination of Water and Wastewater, 15th Edition.

Strickland, J. D. H. and T. R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, 167.

Technicon Industrial Systems. 1977. Nitrate & Nitrite in Water and Seawater. Autoanalyzer II ^{R} Industrial Method No. 155-71W. W. Tarrytown, New York 10591.

Venrick, E. L. and T. L. Hayward. 1985. Evaluation of some techniques for preserving nutrients in stored seawater samples. *CalCOFI Report*, **26**, 160-168.

Walsh, T. W. 1989. Total dissolved nitrogen in seawater: A new high temperature combustion method and a comparison to photo-oxidation. *Marine Chemistry*, **26**, 295-311.

Chapter 9
ORTHOPHOSPHATE AND DISSOLVED ORGANIC PHOSPHORUS

SUMMARY: *Seawater is collected from known depths using CTD-rosette sampling protocols. Subsamples are drawn, filtered and stored frozen in acid-washed polyethylene bottles. Soluble reactive phosphorus (SRP) is measured spectrophotometrically following the formation of phosphomolybdic acid. Total dissolved phosphorus (TDP) is measured in a separate sample after exposure to ultraviolet (UV) light. Dissolved organic phosphorus (DOP) is estimated by difference.*

1. Principle

Phosphorus (P) is one of several macronutrients required for the growth of marine organisms. In open ocean marine ecosystems, P is often present in low and, perhaps, limiting concentrations for microalgal and bacterial populations. Therefore, P is a central element in oceanic biogeochemical cycles.

In seawater, inorganic phosphorus (also referred to as orthophosphate or soluble reactive phosphorus, SRP) occurs chiefly as ions of HPO_4^{2-}, with a small percentage present as PO_4^{3-}. Dissolved organic phosphorus (DOP) exists in a variety of forms (primarily P-esters) which result from excretion, decomposition, death and autolysis.

In this analytical procedure, we make direct measurements of SRP and total dissolved phosphorus (TDP). The latter includes all organic and inorganic phosphorus compounds. Bound phophorus is released from organic matter by ultraviolet light (UV) oxidation and the liberated orthophosphate is reacted with an acidified molybdate reagent and potassium antimonyl tartrate. The resulting compound, a heteropoly acid (phosphomolybdic acid), is reduced to the intensely colored molybdenum blue by ascorbic acid and measured spectrophotometrically. SRP samples are prepared in the same manner as TDP samples, but without the prior oxidation step. DOP is calculated by difference (*i.e.* TDP-SRP).

2. Precautions

Contamination is the primary concern with P determinations. This is particularly true with samples collected from the euphotic zone, where SRP concentrations are extremely low ($\leq 0.2\,\mu$M). In order to avoid contamination, sample bottles must be meticulously cleaned with dilute HCl and rinsed with deionized distilled water (DDW) before use.

3. Sample Collection and Storage

3.1. Sample collection (also see "NOTE" in previous chapter on dissolved nitrogen)

3.1.1. Rinse the nutrient sample bottle (acid-washed, 125 ml polyethylene bottle) 3 times before filling. Fill to approximately 2/3 full, tighten cap and freeze.

3.1.2. Record cruise, cast and Niskin bottle number on the bottle and data sheet.

4. Sample Analysis

Phosphorus analyses are performed on a four-channel Technicon Autoanalyzer II continuous flow system. The automated wet chemistries generally follow the standard methods of seawater analysis as given by Technicon (1973). Slight modifications have been incorporated to achieve the optimum range and sensitivity for each nutrient at concentration levels specific for the Station ALOHA seawaters.

4.1. Soluble Reactive Phosphorus (SRP)

This method employs a single color reagent consisting of an acidified solution of ammonium molybdate, ascorbic acid and antimony-tartrate. The blue phosphomolybdenum complex is read colorimetrically through a 50 mm pathlength flowcell at 880 nm (Murphy and Riley, 1962). The specific automated method used is described in Technicon (1973) with the following modifications: sample pump tube size is increased to allow for a flow rate of 0.8 ml min^{-1}, and the color reagent solution concentration is diluted by a factor of 2.

4.2. Total Dissolved Phosphorus (TDP)

The method for TDP involves initial UV digestion of a seawater sample followed by autoanalysis of the digestion product (SRP; see Chapter 8, section 4.1). The modified photoxidation technique (Armstrong *et al.*, 1966) utilizes a 2 hour UV irradiation period. Exact details of the photoxidation unit are described in Walsh (1989). Periodic calibration checks of the UV lamp efficiency are made using dissolved organic phosphorus (beta-glycerolphosphate) standards. As a general rule, the UV lamp is replaced after approximately 700-800 hr of use.

5. Calibration, Data Reduction and Calculations

5.1. Calibration stocks and regression standards

The calibration of dissolved inorganic nutrients in the autoanalysis of seawater samples is performed using standard solutions containing N, P and Si. A nutrient stock solution is prepared by dissolving dried (65°C, 72 hours) analytical grade reagent chemicals with distilled-deionized water in 1 liter glass volumetric flasks containing 1 ml of chloroform. Once dissolved, this stock solution is immediately transferred into 1 liter amber polypropylene bottles and stored at 4°C. The reagent chemicals and concentrations are: phosphate (KH_2PO_4, 1 mM), nitrate (KNO_3, 5 mM) and silica (Na_2SiF_6, 4 mM).

Working standards are prepared daily by volumetric dilutions of the stock using glass pipettes and a plastic (polymethylpentene; PMP) volumetric flask. All pipettes and PMP flasks are acid-washed (1M HCl) and gravimetrically calibrated prior to use. The daily regression standards are prepared by diluting the working standard with low nutrient natural seawater diluent (SWDIL). The SWDIL is filtered open ocean surface seawater that is stored in a carboy at room temperature. By using this technique all standards are matrix-matched with the seawater samples and any cross-nutrient interference effect should be accounted for.

Cross-nutrient interference and reagent contamination were evaluated by preparing separate solutions, as above, but with one of the three standards omitted. Only phosphate showed a slightly measurable increase (+0.014 μM) in the presence of 40 μM-NO_3 and 160 μM-Si. The linear regressions of the standards were applied to all seawater sample peaks for calculating each batch of cruise samples. Typical correlations produced r^2 values that were between 0.9999 and 0.99999.

5.2. Blank corrections

All seawater standard absorbance peaks were corrected for the absorbance of the seawater diluent (SWDIL). All seawater sample peaks were corrected for the refractive index absorbance for each unique nutrient detection system. The refractive index corrections (in apparent μM units) ranged from approximtely 0.13 (for P), 0.23 (for N) to 2.41 (for Si), and represent the increase in absorbance that is due strictly to the presence of dissolved salts in seawater when compared to the distilled-deionized water baseline. These corrections are made by running sampling seawater (35 o/oo salinity) through the autoanalyzer with DDW only in reagent lines and also with all reagents except the color producing reagent. The Levor surfactant used routinely in the phosphate channel was omitted from the DDW lines during the refractive index measurement.

6. Accuracy and Precision

The detection limit for phosphorus is approximately 0.02 μM with a coefficient of variation for field-collected replicates of 0.3% For DOP the detection limit is 0.02 μM with a coefficient of variation of 1%.

7. Equipment/Supplies

Niskin bottles and rosette/CTD unit
acid-washed, 125 ml polyethylene sample bottles
Autoanalyzer (Technicon Corp.) and accessories

8. Reagents

glass distilled deionized water (DDW)
HCl (1M) for cleaning
concentrated H_2SO_4
ultrapure NaOH and HCl for low-level P determinations
Ammonium molybdate solution: Dissolve 40 g of ACS grade ammonium paramolybdate [$(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$] into 800 ml DDW and dilute to 1 liter in a volumetric flask. Store in plastic bottle in the dark. Solution is stable indefinitely.
Ascorbic acid solution (Prepare fresh): Dissolve 1.8 g of ACS ascorbic acid into 100 ml DDW (1.8% wt/vol). NO!
Antimony potassium tartrate solution: Dissolve 3.0 g of ACS antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$) into 800 ml DDW and dilute to 1 liter in a volumetric flask. Solution is stable for several months.
Mixed reagent (Prepare fresh): Mix together in the following order 15 ml ammonium molybdate, 50 ml 5 N sulfuric acid, 30 ml 1.8% ascorbic acid and 5 ml potassium antimony tartrate.
Stock phosphate standard solution (1 mM): Dissolve 0.1361 g of dry (65°C for 72 hours) potassium phosphate monobasic (KH_2PO_4) into 800 ml of DDW and dilute to 1 liter in a volumetric flask. Store in a dark bottle with 1 ml of chloroform.
Working phosphate standard (40 μM): Dilute 4.0 ml of the stock standard to 100 ml (use volumetric flask). Then dilute the working standard to prepare a series of standards to cover a range of P concentrations.

9. References

Armstrong, F. A. J., P. M. Williams and J. D. H. Strickland. 1966. Photo-oxidation of organic matter in seawater by ultraviolet radiation, analytical and other applications. *Nature*, **211**, 481-483.

Grasshoff, K., M. Ehrhardt and K. Kremling. 1983. Methods of Seawater Analysis. Verlag Chemie.

Johnson, D. L. 1971. Simultaneous determination of arsenate and phosphate in natural waters. *Environmental Science and Technology*, **5**, 411-414.

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytical Chimica Acta*, **27**, 30.

Strickland, J. D. H. and T. R. Parsons. 1972. A Practical Handbook of Seawater Analysis. *Fisheries Research Board of Canada*, 167.

Technicon Industrial Systems. 1973. Orthophosphate in water and seawater. Autoanalyzer II^{R} Industrial Method No. 155-71W. W. Tarrytown, New York 10591.

Walsh, T. W. 1989. Total dissolved nitrogen in seawater: A new high temperature combustion method and a comparison to photo-oxidation. *Marine Chemistry*, **26**, 295-311.

Walsh, T. W., R. E. Young and S. V. Smith. Seawater nutrient storage and analysis. Manuscript.

Chapter 10
DISSOLVED SILICIC ACID

SUMMARY: *Seawater is collected from known depths using CTD-rosette sampling protocols. Subsamples are drawn, filtered and stored frozen in acid-washed polyethylene bottles. Soluble reactive silica is measured spectrophotometrically following the formation of silico- molybdic acid from the reaction of ammonium molybdate and silica at acidic pH.*

1. Principle

Silicon is the second most abundant element in the Earth's crust but is rarely present as elemental Si in nature. The most abundant forms include dissolved silicic acid $Si(OH)_4$, biogenic particulate Si (opal) and a variety of silicate minerals (quartz, feldspars, etc.). Subaerial weathering processes produce orthosilicic acid $Si(OH)_4$ which eventually is deposited in the oceans. In seawater, various groupsof organisms (diatoms, radiolarians, silicoflagellates, sponges and some fungi) utilize dissolved silica as a structural component. However, all living organisms require trace amounts of Si which is a co-factor for DNA polymerase.

The analysis of soluble reactive silica (by analogy to soluble reactive phosphorus) is based upon the formation of yellow silicomolybdic acid from the reaction of ammonium molybdate and silica at low pH. Phosphate also reacts to produce a positive interference due to the formation of molybdophosphoric acid. The addition of oxalic acid eliminates the phosphate interference. The sensitivity of the analysis is increased by a further reduction of the yellow silicomolybdic acid using ascorbic acid, in order to produce "molybdenum blue."

2. Precautions

Contamination is the primary concern with these samples. This is particularly true with samples collected from the euphotic zone, where inorganic nutrient concentrations are extremely low. In order to avoid contamination, all sample bottles must be meticulously cleaned with dilute HCl and rinsed with deionized distilled water (DDW) before use. It is important to realize that silica is leached from glass at seawater pH. Therefore, plastic should be used for sample handling and storage. Finally, special care must be taken when performing dissolved Si analyses on frozen seawater samples (Macdonald *et al.*, 1986).

3. Sample Collection and Storage (also see "NOTE" in section on dissolved nitrogen)

3.1. Rinse the nutrient sample bottle (acid-washed, 125 ml polyethylene bottle) 3 times before filling. Fill to approximately 2/3 full, tighten cap and freeze.

3.2. Record cruise, cast and Niskin bottle number on the bottle and data sheet.

4. Sample Analysis

Si analyses are performed on a four-channel Technicon Autoanalyzer II continuous flow system. The automated wet chemistries generally follow the standard methods of seawater analysisas given by Technicon (1977). This method involves a reaction of the sample with oxalic acid, molybdate, and

ascorbic acid. The absorbance is read at 660 nm using a 15 mm pathlength flowcell.

5. Calibration, Data Reduction and Calculations

5.1. Calibration stocks and regression standards

The calibration of dissolved inorganic nutrients in the autoanalysis of seawater samples is performed using standard solutions containing N, P and Si. Nutrient stock solution "A" is prepared by dissolving dried (65°C, 72 hours) analytical grade reagent chemicals with distilled-deionized water in 1 liter glass volumetric flasks containing 1 ml of chloroform. Once dissolved, this stock solution is immediately transferred into 1 liter amber polypropylene bottles and stored at 4°C. The reagent chemicals and concentrations are: phosphate (KH_2PO_4, 1 mM), nitrate (KNO_3, 5 mM) and silica (Na_2SiF_6, 4 mM).

Working standards are prepared daily by volumetric dilutions of the stock using glass pipettes and a plastic (polymethylpentene; PMP) volumetric flask. All pipettes and PMP flasks are acid-washed (1 M HCl) and gravimetrically calibrated prior to use. The daily regression standards are prepared by diluting the working standard with low nutrient natural seawater diluent (SWDIL). The SWDIL is filtered open ocean surface seawater that is stored in a carboy at room temperature. By using this technique all standards are matrix-matched with the seawater samples and any cross-nutrient interference effect should be accounted for.

Cross-nutrient interference and reagent contamination was evaluated by preparing separate solutions, as above, but with one of the three standards omitted. Only phosphate showed a slightly measurable increase (+0.014 μM) in the presence of 40 μM-NO_3 and 160 μM-Si. The linear regressions of the standards were applied to all seawater sample peaks for calculating each batch of cruise samples. Typical correlations produced r^2 values that were between 0.9999 and 0.99999.

5.2. Blank corrections

All seawater standard absorbance peaks were corrected for the absorbance of the seawater diluent (SWDIL). All seawater sample peaks were corrected for the refractive index absorbance for each unique nutrient detection system. The refractive index corrections (in apparent μM units) ranged from approximately 0.13 (for P), 0.23 (for N) to 2.41 (for Si), and represent the increase in absorbance that is due strictly to the presence of dissolved salts in seawater when compared to the distilled-deionized water baseline. These corrections were measured by sampling seawater (35 o/oo salinity) with DDW only in reagent lines and also with all reagents except the color producing reagent. The Levor surfactant used routinely in the phosphate channel was omitted from the DDW lines during the refractive index measurement.

6. Accuracy and Precision

The detection limit for dissolved Si is approximately 0.3 μM. The coefficient of variation of field-collected replicates is 6%.

7. Equipment/Supplies

Niskin bottles and rosette/CTD unit
acid-washed, 125 ml polyethylene bottles
Autoanalyzer (Technicon Corp.) and accessories

8. Reagents

Because Si is the principle component of glass, all solutions should be made up and contained in plastic. Glass distilled deionized water will have minimal silica leaching due to the low pH of distilled water.

Glass distilled deionized water (DDW)

Ammonium molybdate solution: Dissolve 10 g of ammonium molybdate into 1 liter of sulfuric acid (0.1 N). Filter and store in an amber plastic container.

Oxalic acid (0.56 M): Dissolve 50 g of oxalic acid into 900 ml of DDW and dilute to 1 liter.

Ascorbic acid solution (1.76% wt/vol): Dissolve 17.6 g of ACS quality ascorbic acid in 500 ml of DDW containing 50 ml of acetone. Mix and dilute to 1 liter with DDW. Add 0.5 ml of Levor V per liter of reagent.

9. References

Grasshoff, K., M. Ehrhardt, and K. Kremling. 1983. Methods of Seawater Analysis. Verlag Chemie.

Macdonald, R. W., F. A. McLaughlin and C. S. Wong. 1986. The storage of reactive silicate samples by freezing. *Limnology and Oceanography*, **31**, 1139-1142.

Standard Methods for the Examination of Water and Wastewater, 15th Edition.

Strickland, J. D. H. and T. R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, 167 p.

Technicon Industrial Systems. 1977. Silicates in Water and Seawater. Autoanalyzer II ^{R} Industrial Method No. 155-71W. W. Tarrytown, New York 10591.

Chapter 11
MICROBIAL ATP

SUMMARY: *ATP, an obligate constituent of all living organisms, is extracted from viable microorganisms in boiling phosphate buffer following sample concentration by vacuum filtration. The extracted ATP is analyzed in a photometer by the firefly bioluminescence reaction, and the ATP content is related to total living (biomass) microbial carbon by the application of a laboratory-derived extrapolation factor.*

1. Principle

In field studies it is often desirable to determine the total amount of living cellular material (biomass). Conventional methods (*i.e.* fresh or dry weight determinations, rate of increase of cell numbers, etc.) usually cannot be used owing to (a) lack of sensitivity in the analytical procedures, (b) the presence of a heterogeneous assemblage of organisms, (c) the presence of dead cells and (d) the presence of detrital (non-living) organic material which is not associated with the living cells. Estimation of cellular biomass by measurement of adenosine triphosphate (ATP) is not limited by any of these considerations.

The rationale for using ATP to estimate biomass is the ubiquitous distribution of ATP in all living cells, the rapid loss of ATP from dead cells and the fairly uniform concentration of ATP in the protoplasm of all microbial cells. Data on ATP concentrations can thus be extrapolated to biomass parameters, such as cellular organic carbon or dry or fresh weight (Holm-Hansen, 1973). ATP is extracted from cells using boiling phosphate buffer and is stored frozen (-20°C) prior to analysis by firefly bioluminescence.

2. Precautions

ATP samples must be processed as rapidly as possible, because the ATP content of microorganisms can change rapidly when cells are stressed. Furthermore, a phenomenon referred to as the "filtration effect" causes a loss of ATP when cells are exposed to dessicating conditions immediately after the water is drawn through the filter pad (Karl and Holm-Hansen, 1978). For this reason it is very important that the samples are filtered immediately upon sampling and extracted immediately upon filtration; any delay will cause a decline in ATP content. Because the firefly bioluminescence assay is inhibited by metals, it is also important to use clean stainless steel forceps when handling the filters. It is also essential that the extraction buffer is boiling (100°C), as inefficient extraction results at temperatures ($\leq$95°C). Buffer boiling must be confirmed before starting the filtration process.

3. Sampling, Filtration, Extraction and Storage

3.1. Samples for ATP determinations are collected in clean Niskin bottles attached to the rosette/CTD unit.

3.2. Samples are drawn into 4-liter polyethylene bottles that are rinsed 3 times with approximately 100-200 ml seawater from the appropriate depth through Tygon w/o Nitex filter. Filtration is begun immediately (be sure heating block is on and has achieved a temperature $\geq$110°C; time required ~1 hour).

3.3. Filter triplicate samples at each depth through 25 mm GF/F filters. Total volume required per sample will depend on depth.

3.4. As soon as the last few drops of water have passed through the filter, remove the filter tower from the base, fold the filter in half then in half again and plunge the folded filter into 5 ml of boiling phosphate buffer (pH 7.4; 60 mM) which is kept partially covered to eliminate evaporative volume loss. Try to avoid "bumping" of the buffer (caused by superheating of the buffer).

3.5. After a 5 minute extraction period, remove the tubes from the heating block, allow to cool to approximately room temperature, secure the rubber stoppers and freeze (-20°C) in upright position.

3.6. In order to minimize sample cross-contamination it is best to start with deepest sample, which, in most cases, contains the lowest concentration of ATP.

4. Analysis

4.1. Prepare enough firefly lantern extract (Sigma Chemical Co., FLE-50) to process all samples and at least ten external ATP reference standards. Lyophilized FLE-50 should be reconstituted in 5 ml distilled water and allowed to "age" at room temperature for at least 6 hours (but no longer than 24 hours) in order to reduce the background luminescence. Approximately 1 hour before starting the assay, dilute each 50 mg vial of reconstituted FLE-50 with 15 ml of sodium arsenate buffer (0.1 M, pH 7.4) and 15 ml of $MgSO_4$ (0.04 M). Immediately before use, filter the FLE-50 mixture through a GF/F filter.

4.2. Turn on ATP photometer at least 30 minutes before use.

4.3. Prepare set of ATP reference standards ranging from 0.1-100 ng ATP ml^{-1}.

4.4. Using the automatic injector and computer-assisted photometer, analyze the peak height of light emission (0-15 seconds) for each sample and standard.

5. Preparation of ATP Standards

A primary (reference) ATP standard is prepared by dissolving exactly 10 mg of high purity (99.9%) ATP (sodium salt) into exactly 10 ml of sterile distilled water. Be sure to weigh out an amount equivalent to 10 mg of free acid form of ATP (not 10 mg of hydrated Na-ATP) taking into account the cation contribution and average hydration state of the molecule. This latter information is supplied by the manufacturer. Immediately prepare 1/10 and 1/100 dilutions of the primary stock, place a portion of each solution into a 1 cm quartz spectrophotometercuvette and measure the absorbance at 259 nm. Calculate the exact concentration as follows:

$$A = E/c$$

where: A = absorption at 259 nm
E = ATP molar extinction coefficient (15.4×10^3)
l = path length (cm)
c = concentration of ATP in moles per liter

After calculating the precise concentration of ATP in the primary standard, dilute the stock (gravimetrically) into sterile-filtered (0.2 μm) 60 mM phosphate buffer pH 7.4 to yield an ATP standard solution of exactly 1 μg ml^{-1}. Compare with "old" standard and if different by > |1%|, repeat the dilution step. Store frozen in 1 ml aliquots.

Working standards, ranging from 0.1-100 ng ATP ml^{-1}, are prepared by diluting a vial of the primary

standard with freshly-prepared phosphate buffer just prior to use. These working standards are discarded at the end of the day.

6. Data Reduction and Calculations

6.1. Plot ATP standard data and calculate linear regression statistics for the standard curve. Use LOTUS spreadsheet to calculate ATP concentration (ng l^{-1}) from data on peak height (relative to standards), volume of extract and volume of sample filtered.

6.2. ATP concentrations can be related to total biomass by applying the extrapolation factor; 250 x ATP = C (Karl, 1980). This relationship is based upon direct laboratory and field analyses performed over the past two decades. While there is a range in the C/ATP ratio for microorganisms grown under a variety of environmental conditions, the relationship of C/ATP = 250 appears to be reasonable for samples collected from the oligotrophic North Pacific Ocean (Laws *et al.*, 1987).

7. Equipment/Supplies

Niskin bottles and rosette
sample bottles and tygon tubing
heating block and test tubes
ATP photometer (Biospherical Instrument Co.)
stainless forceps
glass fiber filters (Whatman; 47 mm GF/F)

8. Reagents

phosphate buffer (60 mM, pH 7.4)
magnesium sulfate (0.04 M)
sodium arsenate buffer (0.1 M; pH 7.4)
firefly lantern extract (FLE-50; Sigma Chemical Co.)
reagent ATP (Sigma Chemical Co.)

9. References

Holm-Hansen, O. 1973. Determination of total microbial biomass by measurement of adenosine triphosphate. In: *Estuarine Microbial Ecology*, L. H. Stevenson and R. R. Colwell, editors, University of South Carolina Press, Columbia, pp. 73-89.

Karl, D. M. 1980. Cellular nucleotide measurements and applications in microbial ecology. *Microbiological Reviews*, **44**, 739-796.

Karl, D. M. and O. Holm-Hansen. 1978. Methodology and measurement of adenylate energy charge ratios in environmental samples. *Marine Biology*, **48**, 185-197.

Laws, E. A., D. G. Redalje, L. W. Haas, P. K. Bienfang, R. W. Eppley, W. G. Harrison, D. M. Karl and J. Marra. 1984. High phytoplankton growth and production rates in oligotrophic Hawaiian coastal waters. *Limnology and Oceanography*, **29**, 1161-1169.

Chapter 12
PARTICULATE CARBON AND NITROGEN

SUMMARY: *Seawater samples are collected at discrete depths in 12-liter Niskin bottles or from sediment trap collections. The water samples are prefiltered (202 μm) and vacuum filtered onto combusted GF/F filters and stored frozen for subsequent analysis. In the laboratory, the filters are dried and analyzed for C and N using a Perkin-Elmer model 2400 analyzer.*

1. Principle

Particulate carbon (PC) and particulate nitrogen (PN), including both inorganic and organic forms, are present in seawater primarily as by-products of biological activity. Living organisms also contribute a variable amount (< 1% in deep waters to ≥ 50% in the euphotic zone) to the total particulate carbon and nitrogen content of seawater.

Both PC and PN can be measured using commercially-available instruments which detect the gaseous by-products of high-temperature combustion. The Perkin-Elmer model 2400 CHN analyzer used in the LTER 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.

2. Precautions

Care must be taken to ensure that contamination with PC and PN is avoided. Samples should, therefore, be kept away from paper, wood, food or other carbon-containing compounds. The filtration system that is set up to collect these samples is constructed of plastic. Plastic contains carbon and, therefore, is a potential source of contamination.

3. Sampling, Filtration and Storage

3.1. Seawater samples are collected in 12-liter Niskin bottles or from sediment trap collections. Niskin bottle samples are drawn through tubing with 202 μm Nitex screen into polyethylene deck collection bottles.

3.2. A known volume is then vacuum filtered onto combusted 25 mm GF/F filters.

3.3. Following filtration, clean forceps are used to transfer each filter to a clean plastic petri dish containing a 1" square piece of combusted foil. The sample is labeled and stored frozen (-20°C).

4. Analysis

Follow 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 20-500 μg/sample. Blanks are prepared by analyzing combusted GF/F "field filters" and the mean value subtracted from the sample value.

5. Data Reduction and Calculations

External standard data are used to prepare a standard curve of C (or N) versus corrected signal counts, and linear regression statistics are calculated. A LOTUS/EXCEL spreadsheet is used to calculate PC and PN (μ g l^{-1}) for each sample based on standard curve, corrected signal counts and volume of seawater filtered.

6. Equipment/Supplies

PE-2400 (or equivalent) Carbon/Nitrogen analyzer with integrator
Cahn (or equivalent) electronic balance
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
clean petri dishes

7. Reagents

acetanilide standard

8. References

Sharp, J. H. 1974. Improved analysis for particulate organic carbon and nitrogen from seawater. *Limnology and Oceanography*, **19**, 984-989.

Chapter 13
PARTICULATE PHOSPHORUS

SUMMARY: *Seawater samples are collected at discrete depths in 12-liter Niskin bottles or sediment trap collections. The water samples are prefiltered (202 μm) and vacuum filtered through combusted acid-rinsed GF/F filters and stored frozen for subsequent analysis. In the laboratory, the filters are combusted at 450-500°C and the concentration of the resulting inorganic phosphorus is determined by colorimetric analysis.*

1. Principle

This 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 sodium hydroxide (6 M). 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 phosphorus which can be released by combustion and acid hydrolysis.

2. Precautions

Contamination is the primary problem to be avoided with these samples, therefore, combusted acid rinsed filters are used. All sampling bottles, forceps, tubing and filtration bottles are also acid rinsed.

3. Sampling, Filtration and Storage

3.1. Seawater samples from Niskin bottles are collected in polyethylene bottles through tubing with 202 μm Nitex screen.

3.2. A known volume is then vacuum filtered onto combusted acid-rinsed 25 mm GF/F filters.

3.3. Following filtration, clean forceps are used to transfer each filter to a 10 ml Nalgene film bag and heat sealed. Each sample is labeled and stored frozen (-20°C).

4. Blank Determination

Standards are corrected for reagent blanks while samples are corrected for field filter blanks.

4.1. *Standards*: At least 2 reagent blanks are prepared and individual standard absorbances are corrected by the mean blank value.

4.2. *Samples*: The mean absorbance from three field filter blanks, stored and processed in the same manner as samples, are used to correct individual sample absorbances for filter, reagent and systematic procedural contamination.

5. Analysis

5.1. Samples are combusted in 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.

5.2. The samples are allowed to cool, vortexed for 20 seconds and vacuum filtered through a combusted acid- washed GF/F filter attached to acid cleaned syringe. Vortexing and filtration is repeated with 3 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 20 ml mark with DDW.

5.3. 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.

6. Data Reduction and Calculations

6.1. Calculations

6.1.1. Calculate μ mol l^{-1} of phosphate from standard curve using:

$$\mu \text{mol } l^{-1} = (x - b)/m$$

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

6.1.2. Calculate μg P-PO_4 l^{-1} using:

$$\mu \text{mol } l^{-1} \text{ smpl} \times 30.97376 \, \mu \text{g umol}^{-1} = \mu \text{g P } l^{-1} \text{ in sample extract}$$

$$(\mu \text{g P } l^{-1} \text{ in extract}) / (1000 \text{ ml } l^{-1}) = \mu \text{g P ml}^{-1}$$

$$(\mu \text{g P ml}^{-1} \times 20.0 \text{ ml of extract}) / \text{vol in liters filtered} = \mu \text{g P-PO}_4 \, l^{-1}$$

7. Equipment/Supplies

Niskin bottles and rosette/CTD unit
low pressure filtration apparatus (4-7 psi)
muffle furnace
water bath
combusted acid rinsed GF/F filters
acid-rinsed glassware
acid-rinsed vacuum filtering assembly
spectrophotometer (Varian DMS-100S) and 1-cm cuvette
combusted (450°C, 3 hours), acid-washed 12 x 75 mm glass tubes

8. Reagents

Glass distilled deionized water (DDW)
0.5 M HCl
Phenophthalein indicator
HCl for cleaning (1 M)
NaOH (0.5 M and 6 M)
Ammonium molybdate solution: Dissolve 15 g of ACS grade ammonium paramolybdate [$(NH4)_6 Mo_7O_{24} \cdot 4H_2O$], in 500 ml DDW. Store in plastic bottle in the dark. Solution is stable indefinitely.
Sulfuric acid solution (5 N)
Ascorbic acid solution: Dissolve 0.54 g 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 5 N sulfuric acid, 10 ml 5.4% ascorbic acid and 5 ml potassium antimony tartrate. Prepare fresh.
Stock phosphate standard solution (1000 μM): Dissolve 0.1361 g of dry KH_2PO_4, in 1000 ml of DDW. 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.

9. References

Strickland, J. D. H. & T. R. Parsons. 1972. *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, 167 p.

Chapter 14
PARTICULATE Si

SUMMARY: *Depth profile samples are collected from Niskin bottles through tubing and 202 μm Nitex screen. Sediment trap samples are decanted into PC bottles. A known volume is concentrated onto a 0.8 μm Nuclepore filter, dried and stored at -20°C. In the laboratory, particulate Si is dissolved in a basic solution, with heat, over time. Time course subsamples are measured colorimetrically.*

1. Principle

Silicon (Si), present in seawater primarily as $Si(OH)_4$, is a macronutrient required by certain algae (diatoms) and protozoans (radiolarians) and is a required trace nutrient for all organisms. The uptake of $Si(OH)_4$ from seawater during the growth of diatoms results in the formation of biogenic particulate Si (opal).

Silicious particles dissolve in basic solutions, with heat, over time. Biogenic Si dissolves more readily than Si compounds of mineral origin and can be separated by time course reactions. Aliquots are reacted with an ammonium molybdate solution, chemically reduced, incubated and absorbance read at 810 nm.

2. Precautions

Glass, a Si product, is the most likely contamination source. Therefore, samples are collected in plastic bottles, filtered in plastic units onto PC filters and stored in PP tubes.

3. Sampling, Standards, Spikes, Blanks and Replicates

3.1. Water column samples are collected from Niskin bottles through tubing and 202 μm Nitex screen into a PE bottle. Sediment trap contents are decanted into a PE bottle.

3.2. At the time of sediment trap deployment, triplicate 100 ml portions of T_0 sediment trap solution are filtered as described below. For water column samples, triplicate filter blanks are prepared.

3.3. After agitating the bottle, triplicate subsamples (200 ml for water column samples, 100 ml for sediment trap samples) are filtered onto 0.8 μm pore size 47 mm diameter Nuclepore filters in a 250 ml Nalgene plastic filter unit. Sides of filter chimney are rinsed to recover all particles.

3.4. When filtration is complete, the filter is placed in a labelled 50 ml sterile PP centrifuge tube. The cap is loosely tightened and the filter is dried ~12 hrs at 60°C in a drying oven. The cap is then firmly tightened and the tube is stored at -20°C.

4. Analysis

4.1. 25 ml of 1% Na_2CO_3 is added to each tube. T_0 solution blank and filter blank, a + control (diatom culture), a - control (quartz sand) and a standard curve ranging from 0-250 μM of Na_2SiF_6 (also in 1% Na_2CO_3) are included in each assay.

4.2. The tube is placed in 85°C water bath.

4.3. At T = 1,2,3,5 and 24 hrs, the tube is vortexed and 500 μl removed to a PS tube and covered with parafilm.

4.4. Analysis is by method of Koroleff. The addition of the ammonium molybdate reaction mix, oxalic acid and ascorbic acid result in the formation of a blue silicomolybdate compound. After an incubation period of 60 min the absorbance is read at 810 nm in 1 cm cells.

5. Calculations

5.1. Slope is determined from the regression of peak area on Na_2SiF_6 concentration. Unknowns are corrected for dilution if necessary. T_0 solution and filter blanks are subtracted from appropriate samples. Unknown absorbances are divided by slope.

5.2. Biogenic Si dissolves in 2-3 hrs, as shown by the diatom culture + control. T = 2 or 3 hr time points of unknowns and standards to calculate biogenic Si molarity. Other more refractory Si-minerals begin to dissolve at ~T = 5 hrs and continue to dissolve beyond T = 24 hrs, as shown by the sand-control.

6. Equipment, Supplies

Field:

Nalgene 250 ml filter unit
0.8 μm 47 mm Nuclepore filters
50 ml sterile PP centrifuge tubes

Lab:

PS tubes
Na_2SiF_6
sand
diatom culture
Na_2CO_3
oxalic acid dihydrate
ascorbic acid

7. References

Koroleff, F. 1983. Determination of silicon. *In*: Grasshof, Erhardt and Kremling, eds. Methods of Seawater Analysis. Verlag Chemie, 419 pp.

DeMaster, D. J. 1981. The supply and accumulation of silica in the marine environment. *Geochimica Cosmochimica Acta* 45: 1715-1732.

Chapter 15
DISSOLVED AND PARTICULATE DNA and RNA

SUMMARY: *Water column samples are filtered through a 0.22 μm Millipore filter to remove particulate matter. The filter is stored frozen for the analysis of particulate nucleic acids and the filtrate is retained for the analyses of dissolved nucleic acids. Under appropriate reaction conditions, dissolved DNA (D-DNA) and dissolved RNA (D-RNA) are efficiently removed from solution with the addition of cetyltrimethylammonium bromide (CTAB) and subsequent formation of insoluble CTA-nucleic acid salts. The insoluble salts are collected, by filtration, onto glass-fiber filters and analyzed for DNA and RNA with fluorometric and colorimetric procedures, respectively.*

1. Principle

Nucleic acids (DNA and RNA) are ubiquitous components of the dissolved and particulate organic matter pools of all oceanic environments analyzed to date. Particulate DNA (P-DNA) and RNA (P-RNA) have been shown to correlate well with particulate carbon and have been used as a measure of microbial biomass (Holm-Hansen et al. 1968). Dissolved nucleic acid pools have only recently been reported from the marine environment (DeFlaun et al. 1986; Karl and Bailiff 1989).

The occurrence of dissolved nucleic acids (D-DNA and D-RNA) and their reported rapid rates of uptake suggests a significant flux of C, N and P through these pools. Detrital nucleic acids are believed to exist in dynamic equilibrium between dissolved and particulate forms. Consequently, measurements of particulate DNA and RNA provide complementary information to the dissolved pools described here. Principal pathways for D-DNA and D-RNA production include excretion and exudation, losses due to inefficient feeding or digestion, cell death and autolysis. The removal of D-DNA and D-RNA from oceanic waters is most likely mediated by viable cells or cell-associated nuclease activity.

2. Sampling and Filtration

2.1. Water samples are collected using the CTD-rosette system. Depth profiles at Station A are used for P/D-DNA and P/D-RNA measurements.

2.2. Samples are filtered through 0.22 μm GS Millipore filters and the filtrate is collected into a clean, glass side-armed vacuum flask. The filters are retained for the measurement of P-DNA and P-RNA and stored frozen (-20°C) prior to analysis (see section 5, below).

2.3. Mix filtrate with clean glass-distilled water in a ratio of 1:1; use between 100-1000 ml of each solution. Add CTAB (cetyltrimethylammonium bromide) in proportion to the total volume selected (use 2 ml of "stock" CTAB [0.5 g of CTAB dissolved in 100 ml of 0.5 M NaCl] for every 100 ml of sample). Place sample into freezer and freeze solid.

2.4. Prepare DNA and RNA internal standards, along with samples to estimate the efficiency of recovery.

2.5. Thaw frozen samples to room temperature and filter through combusted 25 mm Whatman GF/F filter. When filtration is complete, the filter is placed in a labelled scintillation vial, and stored at -20°C.

3. D-DNA Determination

3.1. DNA concentrations are measured with the 3,5-diaminobenzoic acid (DABA) fluorometric reaction. The filters are dried at 60°C for 1 h. To the center of each dried filter is added 100 μl of a purified DABA solution prepared by dissolving reagent grade DABA (Sigma Chemical Co.; No. D-1891) in distilled water at a concentration of 400 mg ml^{-1}. For low level ($\leq 0.5\ \mu$g of DNA per sample) determinations, it is necessary to purify the DABA reagent by activated charcoal treatment (Karl and Bailiff 1989). Subsequent to adding DABA, the samples are covered tightly and again placed at 60°C for 1 h followed by addition of 3 ml of HCl (1 M). The samples are vortexed for 1 min, quantitatively transferred to 12- x 75-mm disposable glass test tubes, and the fluorescence determined (excitation, 405 nm; emission, 520 nm).

3.2. An external standard curve is prepared with commercially available DNA (Eastman Kodak Co.; No. 11453). RNA does not react with DABA, so no correction is necessary. After the DABA-DNA complex fluorescence is measured, the samples are quantitatively transferred to clean 12 x 75-mm glass culture tubes, centrifuged, and exactly 1.5 ml of each sample is transferred to a 16- x 150-mm test tube for subsequent determination of RNA.

4. D-RNA Determination

4.1. RNA concentrations are measured with the cupric ion-catalyzed orcinol colorimetric reaction. Each sample (1.5 ml) is diluted with an equal volume of orcinol reagent, which is prepared by dissolving 1 g of reagent grade orcinol (Sigma Chemical Co.; No. O-1875) and 0.15 g $CuCl_2 \cdot 2H_2O$ into 100 ml of concentrated HCl (11.6 M). The samples are tightly sealed and incubated at 100°C for 30 min and then allowed to cool for about 30 min before the absorbance at 666 nm is recorded.

4.2. An external standard curve is prepared with commercially available yeast RNA (Sigma Chemical Co.; No. R-7125). The reaction of DNA with the orcinol reagent is <10% that of an equivalent amount of RNA and is considered to be negligible in the present study. As both DNA and RNA are measured by this technique, however, it is possible to determine the precise contribution of DNA for each sample and to make the appropriate correction. In certain natural environments or laboratory samples where the concentration of DNA greatly exceeds that of RNA this correction may be necessary.

5. P-DNA and P-RNA Determinations

5.1. The Millipore filters are first dissolved in 5 ml acetone (4°C, 100%) by vigorous agitation followed by centrifugation. A small amount (25 mg) of diatomaceous earth (Celite) is added to the tube immediately prior to centrifugation to visualize the pellet. At this point, the samples are treated as in sections 3 and 4 (above) for the quantitative ve determinations of DNA and RNA.

6. Equipment and Supplies

Field:

filters (47 mm diameter, 0.22 μm Millipore and 25 mm diameter Whatman GF/F)
vacuum system and side-armed receiving flasks
freezer storage containers (8 l jugs)
CTAB (0.5 g per 100 ml of 0.5 M NaCl)
standard RNA/DNA solutions

Lab:

fluorometer
spectrophotometer
heating block
orcinol reagent

7. References

Holm-Hansen, O., W. H. Sutcliffe, Jr. and J. Sharp. 1968. Measurement of deoxyribonucleic acid in the ocean and its ecological significance. *Limnology and Oceanography* 13: 507-514.

Karl, D. M. and M. D. Bailiff. 1989. The measurement and distribution of dissolved nucleic acids in aquatic environments. *Limnology and Oceanography* 34: 543-558.

Chapter 16
LIPOPOLYSACCHARIDE

SUMMARY: *LPS, an obligate constituent of the cell walls of bacteria and cyanobacteria, varies in proportion to total bacterial biomass. Particulate (bound) and dissolved LPS samples are collected from standard hydrocasts and are measured using the Limulus amoebocyte lysate (LAL) reaction. Bound-LPS can be extrapolated to bacterial biomass using laboratory-derived LPS/C relationships.*

1. Principle

The outer membrane of all gram-negative bacteria, including photosynthetic prokaryotes (*e.g.* sulfur and nonsulfur purple bacteria, green sulfur bacteria, cyanobacteria) contains lipopolysaccharide (LPS). Total LPS (T-LPS) exists in seawater in both a particulate or bound form (P-LPS) and a dissolved form (D-LPS). The measurement of P-LPS has been suggested as an indicator of bacterial biomass in the marine environment (Watson *et al.*, 1977; Watson and Hobbie, 1979). Watson has adapted the *Limulus* amoebocyte lysate (LAL) test for the *in vitro* detection of bacterial LPS as endotoxin (or pyrogen) and a commercially-available automatic system now exists for the quantitative determination of LPS. During the assay, LPS reacts with LAL which causes an increase in the optical density of the seawater sample. The rate of optical density increase is proportional to the pyrogen concentration. In this analytical procedure, the rate of optical density increase is converted to LPS concentration using lipopolysaccharide standards made up in pyrogen-free seawater. Further extrapolation of LPS to total prokaryote biomass relies upon empirically-determined LPS-to-carbon relationships.

2. Precautions

Contamination is the primary concern with the LPS assay, and great care should be used to ensure that the samples are not contaminated. All glass and plastic sampling gear must be pyrogen free. Niskin bottles should be acid-washed before use. The primary source of contamination is from fingertips, therefore plastic gloves should be worn when collecting samples at sea and when analyzing the samples in the laboratory.

3. Sampling and Storage

3.1. Total LPS (T-LPS)

3.1.1. A 10-12 ml sample is collected directly from each Niskin bottle into a new, 15 ml plastic centrifuge tube. Do not use a drawing tube as this too is a potential site for LPS contamination. It is important that only pyrogen-free, disposable tubes are used. It is not necessary to rinse the centrifuge tubes.

3.1.2. As soon as the samples have been collected, pipette a 1 ml aliquot from each 15 ml centrifuge tube into a 1.5 ml microcentrifuge tube. Use a new, clean pipette tip for each sample and wear gloves during this operation. After the 1 ml subsample has been removed, tighten the cap securely on the 15 ml sample and store at -20°C for subsequent laboratory analysis. The 1 ml subsample contained in the centrifuge tube is then immediately processed for D-LPS.

3.2. Dissolved LPS (D-LPS)

3.2.1. The 1.0 ml subsample is immediately centrifuged for 10 minutes in a Beckman microfuge. The supernatant is then decanted into a second pyrogen-free microcentrifuge tube and stored frozen (-20°C) until assayed.

4. Lab Procedures

4.1. Turn on computer-assisted LAL-5000 and follow menu to set up the instrument. The LAL-5000 must reach the appropriate operating temperature before the assay can begin, this can take up to 2 hours. The instrument should, therefore, be turned on and set up to accept samples before samples are prepared for analysis.

4.2. Dilute the commercially-available pyrotell-GT reagent with 5 ml of pyrogen-free seawater, as described on the label.

4.3. Set up an endotoxin standard curve in combusted 10x75 mm glass culture tubes. The standard curve should contain two pyrogen-free blanks and one tube each of the following concentrations: 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1 ng endotoxin ml^{-1}. Each tube should contain a total of 0.4 ml of the appropriate solution.

4.4. Dilute three replicate aliquots of each sample into a combusted 10x75 mm glass culture tube. Dilute each sample 1:20 with pyrogen-free seawater to make a total of 0.4 ml of diluted sample per tube (*i.e.* 380 μl of pyrogen free seawater and 20 μl of sample). Extreme caution must be taken to avoid contamination. Use new pipette tips for each sample and wear gloves throughout the sample preparation procedure.

4.5. Immediately before positioning the samples and standards in the LAL-5000 for analysis, add 100 μl of diluted pyrotell-GT to each tube and vortex for approximately 10 seconds. Process each tube sequentially and immediately place the tube in the instrument after vortexing. Try to do this part of the assay as reproducibly as possible. Variability between samples in this procedure will directly contribute to variability in measured LPS concentrations between replicates.

5. Data Reduction and Calculations

The quantity of LPS in each sample is determined by the computer software program. LPS concentrations are corrected for dilution in these calculations. P-LPS is calculated by difference between "total" and "dissolved" determinations.

6. Equipment/Supplies

new, pyrogen-free 15 ml and 1.5 ml centrifuge tubes
LAL-5000 (Associates of Cape Cod) and MS-DOS computer
automatic pipettes and tips
combusted 10x75 mm glass culture tubes and appropriate rack
parafilm

7. Reagents

pyrogen-free seawater (prepared by UV irradiation of surface seawater)
pyrotell-GT (Associates of Cape Cod)
endotoxin standard (Associates of Cape Cod)

8. References

Watson, S. W. and J. E. Hobbie. 1979. Measurement of bacterial biomass as lipopolysaccharide. In: *Native Aquatic Bacteria: Enumeration, Activity and Ecology*, J. W. Costerton and R. R. Colwell, editors, American Society for Testing and Materials, Baltimore, pp. 82-88.

Watson, S. W., T. J. Novitsky, H. L. Quinby and F. W. Valois. 1977. Determination of bacterial number and biomass in the marine environment. *Applied and Environmental Microbiology*, **33**, 940-946.

Chapter 17
BACTERIA

SUMMARY: *Bacterial populations are subsampled from seawater collected in Niskin bottles. The cells are fixed in the field by the addition of glutaraldehyde and, in the laboratory, stained with Hoechst 33342 (a DNA specific dye) and enumerated by dual-beam flow cytometry.*

1. Principle

Flow cytometric enumeration is based on the method of Monger and Landry (1993). This method increases substantially the precision of bacterial counts, relative to epifluorescence microscopy. Hoechst 33342 is used because binding to DNA substantially alters its fluorescence spectrum, which facilitates separation of cell fluorescence signals from the background fluorescence of the unbound dye. A 225 mW UV laser is aligned colinearly with a 1 W visible (488 nm) laser to permit enumeration of both heterotrophic and autotrophic picoplankton.

2. Precautions

Because this is a procedure for enumerating preserved cells, sterilization is not a guarantee against contamination. However new, sterile plastic containers tend to be among the cleanest containers available. All reagents (preservative and stain) must be prefiltered through 0.2 *u*m filters and the sample should not come in contact with fingertips or other potentially contaminating surfaces. No drawing tubes are used.

Because of the small sample volume (1 ml), frozen samples thaw quickly. Therefore they should be kept in liquid nitrogen during transport from the ship to the shore-based laboratory.

3. Sampling

3.1. A small volume of sample (10-12 ml) is drawn from the Niskin bottle into a sterile 15 ml plastic centrifuge tube. Drawing tubes are not used.

3.2. 0.9 ml subsamples are drawn from the sample and placed in 2 ml cryovials containing 0.1 ml of 10% glutaraldehyde (final concentration 0.2%).

3.3. Cryovials are let to sit for 10 minutes and then quick frozen in liquid nitrogen. They are then stored frozen at -20° C or colder until analyzed.

4. Analysis

4.1. Samples are stained with freshly prepared, prefiltered Hoechst 33342 (Molecular Probes Inc.) to a final concentration of 0.5 *u*g/ml. Samples stain for 2 h at room temperature in the dark.

215 *u*L sample
25 *u*L Hoechst 33342 (5 *u*g/ml)
10 *u*L fluorescent beads (internal standard)

4.2. Samples are analyzed by colinear dual-beam flow-cytometry using a Coulter EPICS flow instrument (Monger and Landry, 1993). Prior to analyzing samples, fluorescent beads are run to check the volume calibration and the alignment of the instrument.

100 *u*L subsamples are analyzed for forward angle light scatter (FALS), right angle light scatter (RALS), blue (DNA) fluorescence (BF) and red (chlorophyll) fluorescence (RF). The scatter parameters are a function of cell size and are used to separate bacteria from beads and other blue fluorescence signals. Marine bacteria form a distinct cluster on a scatter plot of BF vs. RALS (Monger and Landry, 1993). BF and RF signals are used to distinguish heterotrophic (unpigmented) from autotrophic (chlorophyll-containing) cells.

A known concentration of beads (0.98 *u*m, Polysciences) are used as an external standard for volume calibration. Beads (0.46 *u*m, Polysciences) are also added to each sample as an internal standard for per cell blue fluorescence. A record of cruise-to-cruise variation in fluorescence per cell relative to beads is maintained.

4.3. Data are stored in list (ASCII) files. Statistical analysis is carried out on an 80486 microcomputer using the CYTOPC program developed by Dr. Daniel Vaulot (Station Biologique, Roscoff-sur-mer, France). This is not presently available commercially; see Vaulot (1989) for a description of the program. Further calculations (correction for dilution by preservative and stain) are done on a Microsoft Excel spreadsheet.

5. Reagents

Hoechst 33342 (5 *u*g/ml; prepared fresh)
glutaraldehyde (10%)

6. Equipment and Supplies

15 ml sterile polypropylene centrifuge tubes
2 ml sterile cryovials
sterile plastic syringes and Acrodisc filters
1 ml autopipet and tips
liquid nitrogen and container
Coulter EPICS flow cytometer with UV and visible lasers
IBM-compatible 80486 microcomputer and software
0.5 *u*m fluorescent beads

7. References

Monger, B. C. and M. R. Landry. 1993. Flow cytometric analysis of marine bacteria with Hoechst 33342. *Applied and Environmental Microbiology*, **59**, 905-911.

Vaulot, D. 1989. CYTOPC: Processing software for flow cytometric data. *Signal and Noise*, **2**, 8.

Chapter 18
HETEROTROPHIC PRODUCTION

SUMMARY: *The productivity of micro- and picoheterotrophic organisms is estimated by incorporation of the radiolabeled precursor ^{3}H-leucine into cellular protein, respectively. Water samples are collected and incubated either in situ or on board ship under "simulated" (e.g. temperature and light) conditions. Following incubation, the samples are filtered and stored frozen for laboratory-based isolation, purification and liquid scintillation counting of specific macromolecular fractions. Bacterial production is extrapolated from empirical relationships between bacterial biomass accumulation and isotope incorporation using manipulated (size fractionated or diluted) ecosystems.*

1. Principle

Heterotrophic microorganisms comprise a substantial fraction of total biomass carbon in oligotrophic habitats. In recent years, their collective role in ecosystem processes has become well-recognized through a careful and quantitative elaboration of the "microbial loop" (Azam et al. 1983). In this new food web paradigm, small ($\leq 1\ \mu$m) heterotrophic organisms utilize the carbon and energy contained within the large ambient pool of dissolved organic matter. During growth and cell division of these secondary producers, new particulate matter is formed and is made available to protozoan and micrometazoan predators. This newly recognized pathway for carbon and energy flow in marine ecosystems is believed to be responsible for channeling at least 25% and perhaps in excess of 60% of net primary production in most habitats. The accuracy of these estimates, however, is largely dependent upon the reliability of the methods used to estimate heterotrophic production. In theory, these methods are predominantly thought to measure the production of heterotrophic bacteria, but this remains an untested assumption in most field studies.

2. Cleaning and Preparation

The measurement of open ocean heterotrophic production is as susceptible to trace element inhibition as is the measurement of primary production. Consequently "trace metal-free" precautions must be employed throughout this procedure.

3. Isotope Stock

3.1. The preparation of the isotope stock is performed wearing polyethylene gloves both to prevent contamination and to protect the scientist from radiation exposure.

3.2. Stock isotope:
L-leucine, ^{3}H-labeled in either the [3,4], [4,5], [3,4,5] or [2,3,4,5] positions (specific activity $\geq$ 70 Ci mmol^{-1}, DuPont/New England Nuclear Corp. NET-460, or equivalent)

3.3. The working isotope solution is prepared from stocks in a 25 ml acid-washed teflon bottle using filter-sterilized double-distilled water. Working solutions should be approximately 100-250 μCi ml^{-1}.

4. Subsampling, Isotope Addition and Incubation

4.1. 25 ml subsamples collected from the depth profiles are placed into the appropriate 60 ml polyethylene bottles, labeled and color coded.

4.2. The radioisotopic tracers are added (for leucine: final concentration of 10-15 nM; for thymidine: final concentration of 5-10 nM) and mixed thoroughly. As soon as possible the samples are returned to the on deck incubator. Be sure to keep the samples cold at all times. Note T_0 time on incubation log.

4.3. The samples are incubated for 6 hr in the dark at *in situ* temperature.

4.4. Independent experiments will be done to evaluate the effects of time, light and isotope concentration on radioisotope incorporation.

5. Filtration and Processing

5.1. After incubation, the entire sample is filtered onto a 25 mm Whatman GF/F filter. The filters are placed into 3 ml film bags, sealed and stored at -20°C. Note time when sample is filtered (T_{end}).

5.2. Prior to analysis, the filters with collected particulate matter are extracted for at least 4 hr in cold (4°C) TCA (5%, wt/vol), and the ^{3}H-RNA, ^{3}H-DNA and ^{3}H-protein are isolated by differential acid-base hydrolysis.

6. References

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil and F. Thingstad. 1983. The ecological role of water column microbes in the sea. *Marine Ecology Progress Series*, **10**, 257-263.

Brittain, A. M. and D. M. Karl. 1990. Catabolism of tritiated thymidine by aquatic microbial communities and incorporation of tritium into RNA and protein. *Applied and Environmental Microbiology*, **56**, 1245-1254.

Chin-Leo, G. and D. L. Kirchman. 1988. Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. *Applied and Environmental Microbiology*, **54**, 1934-1939.

Fuhrman, J. and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. *Applied and Environmental Microbiology*, **39**, 1085-1095.

Karl, D. M. 1982. Selected nucleic acid precursors in studies of aquatic microbial ecology. *Applied and Environmental Microbiology*, **44**, 891-902.

Moriarty, D. J. W. and P. C. Pollard. 1981. DNA synthesis as a measure of bacterial productivity in seagrass sediments. *Marine Ecology Progress Series*, **5**, 151-156.

Chapter 19
DRIFTING SEDIMENT TRAP PROTOCOLS

SUMMARY: *Passively sinking particulate matter is collected using a free-floating sediment array and, after prescreening (335 um) to remove zooplankton and micronekton carcasses, the sample materials are analyzed for C, N, P and mass flux (mg m^{-2} d^{-1}).*

1. Principle

Although most of the particulate matter both on the seafloor and in suspension in seawater is very fine, recent evidence suggests that most of the benthic material arrives via relatively rare, rapidly sinking large particles (McCave, 1975). Therefore, in order to describe adequately the ambient particle field and to understand the rates and mechanisms of biogeochemical cycling in the marine environment, it is imperative to employ sampling methods that enable the investigator to distinguish between the suspended and sinking pools of particulate matter. This universal requirement for a careful and comprehensive analysis of sedimenting particles has resulted in the development, evaluation and calibration of a variety of *in situ* particle collectors or sediment traps. The results, after nearly a decade of intensive field experiments, have contributed significantly to our general understanding of: (1) the relationship between the rate of primary production and downward flux of particulate organic matter, (2)mesopelagic zone oxygen consumption and nutrient regeneration, (3) biological control of the removal of abiogenic particles from the surface ocean and (4) seasonal and interannual variations in particle flux to the deep-sea. Future sediment trap studies will, most likely, continue to provide novel and useful data on the rates and mechanisms of important biogeochemical processes.

During LTER, we plan to deploy a free-drifting sediment trap array with 12 individual collectors positioned at 5-7 discrete depths. The deployment period is 24 hrs. The passively sinking particles are subsequently analyzed for a variety of chemical properties, including: total mass, C, N and P.

2. Precautions

Because particle fluxes in antarctic habitats can often be low, special attention must be paid to the preparation of individual sediment trap collector tubes so that they are clean and free of dust and other potentially contaminating particles. Traps should be capped immediately after filling and immediately after retrieval. Pay particular attention 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.

3. Field Operations

3.1. Hardware

Our free-floating sediment trap array is patterned after the MULTITRAP system pioneered by Knauer *et al.* (1979) and used extensively in the decade-long VERTEX program. Twelve individual sediment trap collectors (0.0039 m^2) are typically deployed at 5-7 depths. The traps are affixed to a PVC cross attached to 1/2" polypropylene line. The traps are tracked using VHF radio and Argos satellite transmitters, strobelight and radar reflector.

3.2. Trap solutions

Three trap solutions will be prepared: LIVE (no preservative); FORM (formalin); and ATP (acid solution for in situ extraction) depending upon the experiment to be conducted. The LIVE solution is sequentially filtered through 1 μm and 0.5 μm cartridges. Each precleaned trap is filled 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). A portion of the trap solution is saved for analysis of solution blanks. Traps are rinsed with ship's fresh H_2O between deployments.

3.3. Post-recovery processing

3.3.1. Upon recovery, baffles are removed and 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 contamination.

3.3.2. The depth of the interface between the high density solution 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 overlying seawater is removed only to a depth that is 5 cm above the previously identified interface.

3.3.3. 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^R screen to remove 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 <335 μm sample in order to recover all particulate matter, and the 335 μm Nitex^R screen is examined to determine whether residual material, in addition to the so-called "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.

4. Determination of Mass Flux

4.1. Trap solution from 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 (blank) and equivalent volumes individual traps (start with the deepest depth and work up), are vacuum filtered through tared 25 mm 0.2 μm Nuclepore membrane filters (see sections 4.1.4 to 4.1.3). The tared filters are prepared as follows:

4.1.1. Rinse filters three times with distilled water. Place rinsed filter on a 2.5 cm^2 foil square (to reduce static electricity) in a plastic 47 mm petri dish.

4.1.2. 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 dessicator for 30 minutes.

4.1.3. Weigh filter to constant weight (*i.e.* repeat oven drying, cooling and weighing until a relative standard deviation of <0.005% 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.

4.2. After the last of the sample has passed through the filter, the walls of the filter funnel are washed with three consecutive 5 ml rinses of an isotonic (1 M) ammonium formate solution to remove seawater salts. During each rinse, allow the ammonium formate solution to completely cover the filter.

4.3. 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. Alternately, store in a dessicator, if an oven is not immediately available. Dry to constant weight (see section 4.1.3).

4.4. Mass flux is calculated as follows:

$$\text{mg (dry wt.) } m^{-2}\, d^{-1} = \frac{[(W_a - W_b) - W_{bl}] * V_t}{V_f * 0.0039 * 1000 * t}$$

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)
0.0039 = cross-sectional area of trap (m^2)
1000 = conversion factor (μg mg^{-1})
t = deployment period (d)

5. Determination of C, N and P Flux

5.1. The quantities of particulate C, N and P in the prescreened trap solutions are determined using methods described in previous chapters of this report. Six replicate traps are used for C/N determinations and three additional traps for P. Typically, 1.5-2 liters are used for a single C/N or P measurement. An equivalent volume of the time-zero sediment trap solution, filtered through the appropriate filters is used as a C, N or P blank.

5.2. C, N and P flux is calculated as follows:

$$\text{mg C (or N, P) } m^{-2}\, d^{-1} = \frac{[(C_s - C_b)] * V_t}{V_f * 0.0039 * t}$$

where: C_s = carbon (mg) in sample
C_b = carbon (mg) in blank
V_t = volume of trap (liters)
V_f = volume filtered (liters)
0.0039 = cross-sectional area of trap (m^2)
t = deployment period (d)

6. Equipment/Supplies/Reagents

Nuclepore 25 mm 0.2 μm membrane filters
petri dishes and pre-cut foil squares
vacuum filtration apparatus and glassware

Cahn electronic microbalance or equivalent
graduate cylinders
sediment trap array (spar buoy, radiotransmitter, strobe light, floats, trap supports, collector tubes)
forceps
335 μm Nitex^{R} screen
ammonium formate solution (1 M)

7. References

Knauer, G. A., J. H. Martin and K. Bruland. 1979. Fluxes of particulate carbon, nitrogen and phosphorus in the upper water column of the Northeast Pacific Ocean. *Deep-Sea Research*, **26**, 97-108.

McCave, I. N. 1975. Vertical flux of particles in the ocean. *Deep-Sea Research*, **22**, 491-502.

Chapter 20
BOTTOM-MOORED SEDIMENT TRAPS

SUMMARY: *In order to measure the export of C and associated bioelements from the euphotic zone, and to determine both the seasonal and interannual variability in particle flux, time-series sediment traps will be deployed at various locations within the PALMER LTER study area. In Nov 1992, triplicate moorings were deployed in Palmer Basin in approximately 300-350 m of water. The traps will be recovered (hopeully) in Mar 1993 and redeployed for an additional year.*

The sediment trap moorings will employ MK7-21 time-series sediment traps (Honjo and Doherty 1987) which enable the collection of 21 discrete samples during the deployment period. Based upon the results of previous sediment trap deployments in the Southern Ocean (Wefer 1989, Honjo 1990, Karl and Asper 1990, Karl et al. 1991), we expect the particle flux signal to be highly variable in time with a majority of the annual export coinciding with the spring-summer phytoplankton bloom. Consequently, we will program our sediment traps so as to increase the temporal resolution of the particle flux signal during this period. The mooring hardware (anchor, anti-chafing chain, wire rope, glass floats with protective polycarbonate exteriors, acoustical release, spar buoy with radio and strobelight) will all be similar to that described by Honjo and Spencer (1991).

Prior to deployment, the collection bottles will be filled with a hypersaline solution (50 g NaCl per liter of filtered seawater) containing 2% formalin. Due to the relatively long deployment periods and potential for large mass fluxes, we are concerned about the effectiveness of this preservation procedure. Therefore, upon recovery, a small volume subsample of each collection will be incubated with a standard mixture of ^{3}H-labeled amino acids (Azam and Holm-Hansen 1973), with and without the addition of formalin, in order to confirm the effectiveness of the *in situ* preservation procedures.

Following recovery, the samples will be examined under a dissecting scope for the presence of large macrozooplankton (i.e., swimmers; Lee et al. 1988) before processing for total mass, particulate carbon (PC), nitrogen (PN), phosphorus (PP), silica (PSi) [biogenic opal and total-Si] and total dissolved nitrogen (TDN), phosphorus (TDP) and silica (TDSi) according to standard procedures that are currently in use in my laboratory. It is essential to measure both solute and solid phases (US-GOFS Planning Report #10) if one desires accurate estimates of total flux.

References

Azam, F. and O. Holm-Hansen. 1973. Use of tritiated substrates in the study of heterotrophy in seawater. *Marine Biology*, **23**, 191-196.

Honjo, S. 1990. Particle fluxes and modern sedimentation in the Polar Oceans. In: *Polar Oceanography. Part B: Chemistry, Biology and Geology*, W. O. Smith, Jr., editor, Academic Press, Inc., San Diego, CA, pp. 687-739.

Honjo, S. and K. W. Doherty. 1987. Large aperture time-series sediment traps: Design objectives, construction, and application. *Deep-Sea Research*, **35**, 133-149.

Honjo, S., and D. W. Spencer. 1991. Sediment trap intercomparison experiment in the Panama Basin, 1979. *Deep-Sea Research*, in press.

Karl, D. M. and V. L. Asper. 1990. RACER: Particle flux measurements during the 1989-90 austral summer. *Antarctic Journal of the United States*, in press.

Karl, D. M., B. D. Tilbrook and G. Tien. 1991. Seasonal coupling of organic matter production and particle flux in the Bransfield Strait, Antarctica. *Deep-Sea Research*, in press.

Lee, C., S. G. Wakeham and J. I. Hedges. 1988. The measurement of oceanic particle flux -- Are "swimmers" a problem? *Oceanography Magazine*, 1, 34-36.

Wefer, G. 1989. Particle flux in the ocean: Effects of episodic production. In: Productivity of the Ocean: Present and Past, W. H. Berger, V. S. Smetacek and G. Wefer, editors, John Wiley & Sons Ltd., 139-153.