Deep-Sea Research I 56 (2009) 1266–1283

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

# Hydrolytic ectoenzyme activity associated with suspended and sinking organic particles within the anoxic Cariaco Basin

Gordon T. Taylor [a,*], Robert Thunell [b], Ramon Varela [c], Claudia Benitez-Nelson [b], Mary I. Scranton [a]

[a] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000, USA
[b] Department of Geological Sciences, University of South Carolina, Columbia, SC 29208, USA
[c] Estación de Investigaciónes Marinas de Margarita, Fundación la Salle de Ciencias Naturales, Apartado 144, Punta de Piedras, Edo. Nueva Esparta, Venezuela

ARTICLE INFO

*Article history:*
Received 9 January 2008
Received in revised form
11 February 2009
Accepted 25 February 2009
Available online 9 March 2009

*Keywords:*
Biogenic debris
Decomposition
Ectohydrolase
Anoxic basin
Bacteria
Particle flux
Seston

ABSTRACT

Ectohydrolase activities of suspended microbiota were compared to those associated with sinking particles (sed-POM) retrieved from sediment traps deployed in the permanently anoxic Cariaco Basin. In shore-based assays, activities of aminopeptidase, β-glucosidase, chitinase and alkaline phosphatase were measured in samples obtained from oxic and anoxic depths using MUF- and MCA-labeled fluorogenic substrate analogs. Hydrolysis potentials for these enzymes in the seston varied widely over the nine cruises sampled (8 Nov 1996–3 May 2000) and among depths (15–1265 m); from $<10$ to over 1600 nM $d^{-1}$ hydrolysate released, generally co-varying with one another and with suspended particulate organic carbon (POC) and particulate nitrogen (PN). Hydrolytic potentials, prokaryotic abundances and POC/PN concentrations in sinking debris were $400–1.3\times10^7$ times higher than in comparable volumes of seawater. However when normalized to PN, hydrolytic potentials in sediment trap samples were not demonstrably higher than in Niskin bottle samples. We estimate that PN pools in sediment trap samples were turned over 2–1400 times (medians = 7–26*x*) slower by hydrolysis than were suspended PN pools. Median prokaryotic growth rates (divisions $d^{-1}$) in sinking debris were also ~150 times slower than for bacterioplankton. Hydrolytic potentials in surface oxic waters were generally faster than in underlying anoxic waters on a volumetric basis (nM hydrolysate $d^{-1}$), but were not significantly ($p>0.05$) different when normalized to PN or prokaryote abundances. Alkaline phosphatase was consistently the most active ectohydrolase in both sample types, suggesting that Cariaco Basin assemblages were adapted to decomposing phosphate esters in organic polymers. However, phosphorus limitation was not evident from nutrient inventories in the water column. Results support the hypothesis that efficiencies of polymer hydrolysis in anoxic waters are not inherently lower than in oxic waters.

© 2009 Elsevier Ltd. All rights reserved.

## 1. Introduction

Sinking particulate organic matter (POM) is believed to be the primary mechanism by which fixed carbon is transported from the epipelagic to the mesopelagic zone and below (Alldredge and Silver, 1988; Simon et al., 2002). The efficiency with which POM is delivered to mesopelagic depths determines how effectively the 'Biological Pump' sequesters atmospheric $CO_2$ in the ocean's interior (Longhurst, 1991; Sarmiento et al., 2004). Sinking POM, primarily macroaggregates and fecal pellets, is rare relative to suspended POM, yet global fluxes of this pool

* Corresponding author. Tel.: +1 631 632 8688; fax: +1 631 632 8820.
*E-mail address:* gordon.taylor@stonybrook.edu (G.T. Taylor).

0967-0637/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dsr.2009.02.006

may be on the order of ~10–16 GT C annually and comparable to anthropogenic $CO_2$ emissions (Falkowski et al., 2000; Boyd and Trull, 2007). Consequently, understanding the dynamics of sinking POM is pivotal to oceanic carbon flux budgets.

Total POM pools (suspended+sinking) are much smaller than those of dissolved organic matter (DOM). For example, the long-term mean POM concentration in the upper 100 m at station ALOHA in the N. Pacific Central Gyre is equivalent to about 2% of the local DOM pool (Karl, 1999; Hebel and Karl, 2001). However at this same location, net annual production of POM and DOM are nearly equal, 0.8–1.3 and 0.5–1.3 mol C $m^{-2}$ $yr^{-1}$, respectively (Emerson et al., 1997). Similar POM and DOM production rates and the relatively small POM inventories imply that these biogenic particles are far more dynamic and mobile than DOM in the water column. While POM distributions are affected by gravitational settling, >90% of the POM produced in offshore euphotic zones is decomposed in the upper 500 m of the water column, the rate of which depends on physical regime, productivity and plankton community structure (Martin et al., 1987; Michaels and Silver, 1988; Taylor and Karl, 1991; Armstrong et al., 2002).

POM decomposition by disaggregation, dissolution and remineralization is controlled by a variety of physical, chemical and biological processes. However, microbial decomposition and animal consumption are commonly believed to dominate POM loss processes (Simon et al., 2002). Paradoxical depth-dependent trends observed in microbial distributions and growth suggest that sinking particles themselves are not particularly productive microhabitats, failing to directly support burgeoning microbial assemblages as they age (Taylor et al., 1986; Karl et al., 1988). Rather this biogenic debris seems to support modest-sized assemblages of copiotrophic prokaryotes that digest away their habitat using ectohydrolases and thereby support a resident microbial loop, complete with protozoa and viruses (Taylor et al., 1986, 2003a; Taylor, 1989; Simon et al., 2002). Inefficient capture of low molecular weight hydrolysates by resident microbial assemblages permits diffusion of polymers, oligomers and monomers away from particle surfaces, creating an organic-rich "detritosphere" around suspended particles or organic-rich plumes trailing behind sinking particles (Azam and Smith, 1991; Kiørboe and Jackson, 2001; Simon et al., 2002). Colloidal and dissolved organic matter escaping into the detritosphere can be further hydrolyzed by free prokaryotes, allowing transmembrane uptake of the released oligomers and monomers. Smith et al. (1992) estimated that about 97% of the hydrolysates produced diffuse away from aggregates leaving only 3% behind for the aggregate residents to utilize. These released materials apparently provide sufficient energy and nutrients to support reasonable growth rates of microheterotrophic assemblages throughout the mesopelagic zone (Cho and Azam, 1988; Azam and Long, 2001; Kiørboe and Jackson, 2001).

SCUBA-collected macroaggregates and solid-water interfaces are known to possess intensified levels of hydrolysis for at least seven classes of polymers (Smith et al., 1992; Karner and Herndl, 1992; Taylor and Gulnick, 1996). Presumably large individually collected particles represent a portion of the sinking flux. To the best of our knowledge, however, hydrolytic activity associated with the entire sinking POM flux has only been directly compared with contemporaneous planktonic activity from shallow waters in an Arctic polynya (Vetter and Deming, 1994; Huston and Deming, 2002). In the present study, a unique opportunity was provided by the CARIACO Time Series Program to repeatedly assay freshly collected sediment trap material from four depths (225–1205 m) contemporaneously with Niskin bottle samples for leucine aminopeptidase (LAPase), chitinase (CHIase), β-glucosidase (β-GLUase) and alkaline phosphatase (APase) activities.

The CARIACO Time Series provides an excellent natural laboratory for studying fluxes and transformations of biogenic particles in neritic waters. Sustained upwelling along the northern coast of Venezuela in January–May produces an annual cycle oscillating between high and low productivity with the plankton dominated by large and small phytoplankters, respectively (Muller-Karger et al., 2001; Goñi et al., 2003). Furthermore, potential trap biases imposed by hydrodynamic shear across trap openings and invasion by "swimmers" are reduced in this relatively quiescent, anoxic environment (Baker et al., 1988; Gust et al., 1994; Steinberg et al., 1998). Nekton and macrozooplankton are known to vertically migrate deep into the Cariaco Basin's sulfidic waters on a diel basis, but their role in particle translation between layers and in influencing trap collections are unknown (Baird et al., 1973; Love et al., 2004).

While surface productivity over the Cariaco Basin is similar to that of many coastal seas, the deeper waters are permanently anoxic from ⩾260 m to the seabed (⩽1400 m), which may affect POM delivery to the seabed. Whether POM decomposition is significantly altered by this >1 km sulfidic layer is an open question. Hydrolysis rates of polymers, and by extension particle disaggregation, can be accelerated by catalysis, extreme pH and elevated temperatures, but the process does not require dissolved $O_2$. Therefore, lower hydrolysis rates are not necessarily predicted for anoxic systems. On the other hand, anaerobes are known to have a more limited repertoire of useable substrates compared to aerobes and they possess thermodynamically imposed lower growth yields, so decomposition may be limited by downstream processes. In this study, we compare for the first time hydrolytic rates associated with suspended and sinking POM in oxic and anoxic waters during upwelling and relaxation periods.

## 2. Methods

### 2.1. Site description and sampling

The CARIACO time series station is located near the center of the Cariaco Basin's eastern sub-basin in almost 1400 m of water (10.50°N, 64.66°W). All results presented are from this single site sampled between 8 Nov 96 and 3

May 00 (Table 1). Water column sampling and sediment trap servicing were conducted on separate cruise legs, aboard the 23-m long R/V *Hermano Gines*, operated by Estación de Investigaciónes Marinas (EDIMAR), Fundación la Salle de Ciencias Naturales, Margarita Island, Venezuela. Water samples were collected with a SeaBird rosette accommodating 12 TFE-lined, 8-l Niskin bottles. For hydrographic profiling, the rosette was equipped with a SeaBird CTD, YSI oxygen probe, Chelsea Instruments fluorometer for chlorophyll *a* estimates and Sea Tec c-beam transmissometer (660 nm). All samples were withdrawn from Niskin bottles under a $N_2$ atmosphere to prevent oxygenation [see Taylor et al. (2001) for sampling details]. Samples for microbial abundances were collected directly in 250-ml polyethylene bottles containing filtered, borate-buffered formaldehyde (2% final conc.), and refrigerated. All samples used for biological rate measurements were transferred from Niskin bottles to HCl-washed 1-l TFE-stoppered glass bottles and sealed without headspace after overflowing ~1–2 volumes. Samples for $^3$H-leucine uptake were dispensed from these 1-l TFE-stoppered glass bottles under $N_2$ pressure and assayed immediately onboard (described below). Because space and time constraints precluded shipboard measurements of ectohydrolase activity, separate 1-L samples from selected depths were refrigerated (2–4 °C) in sealed TFE-stoppered glass bottles, returned to the EDIMAR shore laboratory and assayed within 24 h of collection. While untested, we assume that chilling samples slowed metabolism of mesophilic communities, preserved substrates and nutrients, and that effects were fully reversible upon returning samples to environmental temperatures. Depths were selected to represent oxic, transitional (redoxcline) and sulfidic waters as well as to coincide with deployment depths of sediment traps (Table 1).

### 2.2. Dissolved $O_2$ and $H_2S$

Continuous dissolved $O_2$ concentration profiles were obtained from the rosette's YSI electrode and validated with discrete samples using standard Winkler titrations after fixation of samples in the field (Aminot, 1983). Samples for $H_2S$ were collected by syringe, avoiding atmospheric contact, and immediately transferred to vials containing zinc chloride to form ZnS precipitates. ZnS was derivatized and measured colorimetrically according to Cline (1969).

### 2.3. Vertical flux of biogenic debris

The sediment trap mooring was located in the deepest portion (~1400 m) of the eastern basin (10°30′N, 64°40′W) and consisted of four automated traps positioned at depths of approximately 225, 405, 840 and 1205 m (Thunell et al., 2007). Traps have a 0.5 $m^2$ opening at the top and 13 collection cups at the bottom, each programmed to sequentially collect samples over 2 week intervals. Prior to their 6-month deployments, collection cups were filled with buffered formaldehyde (2% final conc.) in filtered seawater to preserve accumulating organic matter.

In four deployments, preservative was intentionally omitted from the last cup in the series (cup 13) at each depth (Table 1). These cups contained freshly filtered ($<$0.22 μm) seawater only. Cups collecting living material over the 9–12 d immediately preceding trap retrieval were subsampled after gentle mixing and placed in 44-ml septa vials without headspace and refrigerated (2–4 °C). As with water column samples, trap materials were assayed for ectohydrolytic activity at the EDIMAR laboratory within 24 h of retrieval. Material remaining in the collection cups was preserved with buffered formaldehyde (2% final conc.), sealed, and refrigerated for subsequent elemental analysis. Shortly after trap retrieval, water column samples were obtained at the four trap depths using the same collection and storage methods described above and served as diluent for the live trap samples (see below).

### 2.4. Particulate C, N, and P analyses

Samples for suspended particulate organic carbon (POC) and particulate nitrogen (PN) from Niskin bottles were filtered through precombusted GF/F filters shipboard. For preserved sediment trap samples, most of the supernatant from each cup was discarded, along with all obvious swimming organisms not considered part of the passive particle flux. Samples were then split into quarters

**Table 1**
Summary of dates and depths sampled for ectohydrolase activity associated with suspended (Niskin bottles) and sinking assemblages (sediment trap) at the CARIACO time series station.

| Cruise no. | Niskin bottle Date | Collections Depths sampled (m) | Sediment trap Dates | Collections Depths sampled (m) |
|---|---|---|---|---|
| CAR-13 | 8 Nov 1996 | 15, 100, 220, 260, 320, 500 | n.c.[a] | |
| CAR-19 | 8 May 1997 | 15, 100, 220, 260, 320, 500 | n.c. | |
| CAR-25 | 14 Nov 1997 | 15, 100, 310, 350 | 23 Oct–3 Nov 1997 | 225, 405, 840, 1205 |
| CAR-29 | 10 Mar 1998 | 15, 100, 255, 330, 1200 | n.c. | |
| CAR-32 | 7 Jul 1998 | 15, 320, 450, 900, 1265 | n.c. | |
| CAR-36 | 7 Nov 1998 | 15, 315, 341, 360, 451, 946 | n.c. | |
| CAR-42 | 8 May 1999 | 35, 250, 305, 435, 900, 1225 | 24 Apr–3 May 1999 | 225, 405, 840, 1205 |
| CAR-48 | 7 Nov 1999 | 235, 275, 455, 930, 1225 | 21 Oct–2 Nov 1999 | 225, 405, 840, 1205 |
| CAR-54 | 3 May 2000 | 35, 260, 455, 930, 1225 | 20 Apr–2 May 2000 | 225, 405, 840, 1205 |

Sediment trap dates correspond to time interval beginning when unpoisoned collection cups opened and ending with trap retrieval.
[a] n.c., unpoisoned samples not collected during this period.

using a precision rotary splitter. The quarter sample used for analysis was rinsed with deionized water a total of three times, frozen, dried, and ground (Thunell et al., 2000; Goñi et al., 2003). POC and PN analyses were conducted according to the methods described in Thunell et al. (2000).

Total particulate (TPP) and particulate inorganic (PIP) phosphorus were measured using an adaptation of the Aspila method (Aspila et al., 1976) as described in Benitez-Nelson et al. (2007). Particulate organic P (POP) is estimated by difference (TPP–PIP). As such, each fraction is operationally defined and the PIP fraction may contain some acid-labile organic P-containing molecules, such as simple sugars, whereas the POP may contain inorganic compounds, such as pyrophosphates (Benitez-Nelson, 2000).

### 2.5. Preparation of model substrates

Fluorogenic substrate analogs were selected to assay activities of ectohydrolases acting on five major classes of macromolecules present in natural waters; leucine-methylcoumarinylamide for proteins, methylumbelliferyl β-D-glucoside for cellulose, MUF-N-acetyl-β-D-glucosaminide for chitin, MUF-palmitate for lipids, and MUF-phosphate for phosphate-bearing organics. Results from MUF-palmitate assays were unreliable for reasons discussed in Taylor et al. (2003b) and are therefore not presented here. Solutions (500 μM) of substrate analogs were prepared in 10:1 sterile water:methylcellosolve solutions to promote complete dissolution (Hoppe, 1993). Methylcellosolve was omitted from MUF-phosphate, which readily dissolved in water alone. Effects of methylcellosolve on enzymatic activities and plankton growth have been tested previously and appear to be negligible (Hoppe, 1983; Taylor, unpubl. data). Working solutions were stored frozen, thawed just prior to use and purged with $N_2$ to remove dissolved $O_2$.

### 2.6. Ectohydrolase activity assays

In order to maintain ambient redox conditions in sediment trap samples, unpoisoned 44-ml concentrates from each collection cup were diluted into unfiltered seawater retrieved from trap depths by transferring sample to 1-l transfer bottles using a 60 $cm^3$ syringe and Tygon tubing. Subsequently, both unaltered seawater samples and diluted sediment trap samples were dispensed under $N_2$ pressure from stoppered transfer bottles into acid-washed, 32-ml septa vials and sealed with laminated TFE-butyl rubber septa (Pierce Inc.) without headspace after overflowing. Care was taken to agitate transfer bottles during dispensing to minimize variability among subsamples. For all samples, 0.2 ml aliquots of 2–5 individual fluorogenic analogs were syringe-injected into separate septa vials. Samples were incubated in darkened water baths and maintained at in situ temperatures; 17–18 °C for deep samples and 24–27 °C for shallow samples (<250 m).

To test linearity of hydrolysis reactions, time courses were initially conducted using water column samples from 15 m (typically most active depth), and rates were found to be constant for at least 15 h (not presented). Subsequent assays were end point experiments lasting from 6 to 14 h, consisting of $T_0$, $T_x$ and poisoned (0.5 ml sat'd. $HgCl_2$ solution) samples. To avoid artifacts caused by varying redox conditions, vials were not subsampled through time. In all experiments, each point represents either a single sacrificed 32-ml sample or mean of duplicates. Variations between duplicate vials were low, always yielding ranges $\leqslant$5% of the mean and analytical variation among multiple subsamples was <1%.

We were unable to conduct kinetics experiments with a range of analog concentrations as suggested by Hoppe (1993) because of the number and volume of samples required to maintain in situ redox conditions. The final 3.1 μM analog concentration was chosen based on the need to inject small volumes of the analog working stock into samples. This concentration approximates that employed by Karner and Herndl (1992) to derive hydrolytic rates of LAPase and α- and β-GLUase in concentrated marine snow samples. In the present study, substrate depletion was never apparent in either seawater or diluted sediment trap samples, because median turnover rates for each tracer varied between 0.43 and 2.2% $d^{-1}$ based on fluorescent hydrolysate production and the maximum never exceeded 69% $d^{-1}$ in the 323 samples analyzed.

Fluorescence was measured on a Shimadzu RF-551 Fluorescence HPLC monitor. To calibrate fluorescence response and compensate for varying background fluorescence among samples, 20 μl internal standards of 5 μM MUF or MCA (free fluorochrome) were repetitively added to appropriate samples during each run of measurements. Standard addition calibration curves were generated for both fluorochromes (MUF and MCA) by regressing fluorochrome concentrations added against fluorescent yield and the slope was used to compute concentration from fluorescence. Ectoenzyme activities are expressed as hydrolysate production rates (% hydrolysate $d^{-1}$) and calculated by dividing fluorochrome production between $T_x$ and $T_0$ by incubation time ($x$) and analog concentration (3.1 μM). Values were corrected for abiotic hydrolysis by subtracting fluorochrome production in Hg-killed controls for each analog. Rates represent potential, rather than in situ rates, because ambient polymeric substrate and total hydrolysate concentrations required for such calculations were not measured. In sediment trap assays, rates were corrected for the seawater diluent contributions on a case by case basis; median activity in diluents was only ~3% of total measured hydrolytic activity (range = 0–85%; $n = 32$).

### 2.7. Microbial abundances and activity

For Niskin bottle samples, standard DAPI-stained slides were prepared by vortex-mixing preserved whole water and capturing cells on dark 0.2 μm Poretics™ polycarbonate membranes for enumeration of prokaryotes by epifluorescence microscopy (Porter and Feig, 1980). Preserved sediment trap subsamples were processed as described in Taylor et al. (2003a). Briefly, aliquots from

vigorously stirred samples were diluted 100-fold with 2% buffered formaldehyde in seawater. Diluted samples were sonicated for 20 min in a Bransonic Model 5200 ultrasonic bath to dislodge cells from particles. Methods optimization experiments demonstrated that this treatment did not detectably reduce recovery of fluorescent bacterioplankton in seawater samples. Sonicated suspensions were further diluted approximately 10-fold in particle-free seawater or 1 mM saline tetrasodium pyrophosphate buffer (35 g $L^{-1}$ NaCl) within the 25 mm diameter filtration funnels prior to staining. Previous experience demonstrated that dislodging and dispersing cells from particles did not vary between seawater or pyrophosphate buffers, ranging in strength from 0.5 to 9 mM. From this point, protocols for enumeration of prokaryotes were the same as described for Niskin bottle samples. For all samples, a minimum of 300 individuals and 10 random fields were enumerated. Prokaryotic C biomass was determined microscopically as previously described (Taylor et al., 2006). Detection efficiency of particle-associated prokaryotes by our methods is certainly less than 100% and probably varied among samples. Available data and methods are inadequate to calculate effective recoveries.

In the CARIACO Program, we routinely estimate bacterial net production (BNP) from incorporation of $^3$H-leucine into protein (Kirchman, 1993). Briefly, triplicate samples from each depth were dispensed into 44-ml septa vials, immediately spiked with 50 μl of $N_2$-purged $^3$H-leucine (10 nM final conc.; L-(4,5-$^3$H(N))-leu; 52 Ci $mmol^{-1}$), and incubated shipboard at ambient temperature in darkness in water baths for 8–12 h. Following incubation, samples were fixed and processed as described in Taylor et al. (2001). The live dilute sediment trap samples were subjected to this same assay in parallel with ectohydrolase assays at the EDIMAR lab. BNP was estimated using a conversion factor of 3.1 kg C $mol^{-1}$ of leucine incorporated (Kirchman, 1993). Specific growth rates (divisions $d^{-1}$) were estimated by dividing BNP by prokaryotic C biomass.

### 2.8. Primary production

Chlorophyll *a* (Chl *a*) concentrations were measured in methanol-extracted samples collected from eight depths in the upper 100 m. Net primary productivity (NPP) was measured at the same depths using the $^{14}$C-bicarbonate method and in situ incubations. Details of both methods appear in Muller-Karger et al. (2001). Values integrated over the upper 100 m are used for statistical comparisons.

### 2.9. Major nutrients analyses

Nutrient samples (ammonium, nitrite, nitrate, and phosphate) are routinely collected as part of the CARIACO time series, methods of which are described in detail in Scranton et al. (2006). Briefly, samples were filtered through a 0.8 μm glass fiber filter into clear 60 ml polycarbonate bottles and frozen within minutes of collection. After transport back to the laboratory, samples were analyzed using an ALPKEM RFA II nutrient autoanalyzer following the recommendations of Gordon et al. (1993) for nitrate, nitrite, phosphate, and silicate analysis and standard techniques described by Strickland and Parsons (1972). Detection limits were: 0.07 μM for ammonium, 0.02 μM for nitrite, 0.06 μM for nitrate, and 0.03 μM for phosphate. Precision of triplicate measurements were about ±5% for concentrations greater than 1 μM.

### 2.10. Statistics

Least squares regressions, Mann–Whitney rank sum test, Kruskal–Wallis one-way ANOVA, Dunn's multiple pair-wise comparisons and Pearson product–moment correlations were performed with SigmaPlot 10.0 and SigmaStat 3.5 (SPSS). For correlation tables, the group-wide type-I error rate (the probability that significant correlations arose by chance alone) was evaluated using the sequential Bonferroni test and a table-wide significance level, $\alpha$, of 0.05 to yield conservative estimates of significance (Rice, 1989).

## 3. Results

### 3.1. Water column conditions

Seston samples were collected every 3–6 months between November 1996 and May 2000 to profile ectohydrolase activities during upwelling and relaxation periods. Our sampling program included primary productivities varying from 38 to 276 mmol C $m^{-2}$ $d^{-1}$ (0.5–3.3 g C $m^{-2}$ $d^{-1}$) and phytoplankton standing stocks of 19–126 mg Chl *a* $m^{-2}$, but only captured one major upwelling event in March 1998 (Fig. 1). Levels of NPP and Chl *a* measured throughout the photic zone (eight depths) were not statistically different among the nine sampling dates ($p > 0.8$ and $p > 0.3$, respectively; Kruskal–Wallis one-way ANOVA). Among the four sediment trap deployments sampled, POC and PN fluxes to the 225 m traps varied between upwelling and non-upwelling seasonal from 1.8 to 5.5 mmol C $m^{-2}$ $d^{-1}$ (20–660 mg C $m^{-2}$ $d^{-1}$) and 0.2–0.7 mmol N $m^{-2}$ $d^{-1}$ (2.4–8.4 mg N $m^{-2}$ $d^{-1}$), respectively (Fig. 1).

During all cruises, Chl *a* approached detection limits below 80 m and subsurface maxima were frequently observed between 25 and 55 m (Fig. 2A). For clarity's sake, only data from cruises for which sediment trap samples were available are presented in Fig. 2 and are representative of all nine cruises. Prokaryotic biomass tended to have bimodal distributions with maxima in the photic zone and within the redoxcline on most dates (Fig. 2B). Seston, which also includes microbiota and is measured as POC and PN, was usually most abundant in the upper 80 m, but secondary midwater enrichments were also evident at or near the $O_2/H_2S$ interface (Figs. 2B, C). Among all seston samples, POC and PN concentrations varied from 1.3 to 59 μM C, and 0.01 to 4.0 μM N, and were highly correlated with one another ($r = 0.87$, $p < 0.0001$) (Table 2). Because PN pools generally appear to be more labile and are remineralized faster than POC pools,

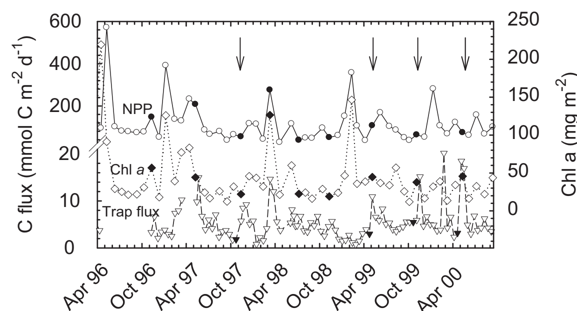


**Fig. 1.** Monthly rates of net primary production (NPP) and POC flux to the 225 m sediment traps (trap flux) and phytoplankton standing stock as integrated chlorophyll *a* (Chl *a*) inventories at the CARIACO time series station. Photic zone integrations are from 0 to 100 m. Solid symbols and arrows denote sampling dates of water column and sediment traps, respectively.

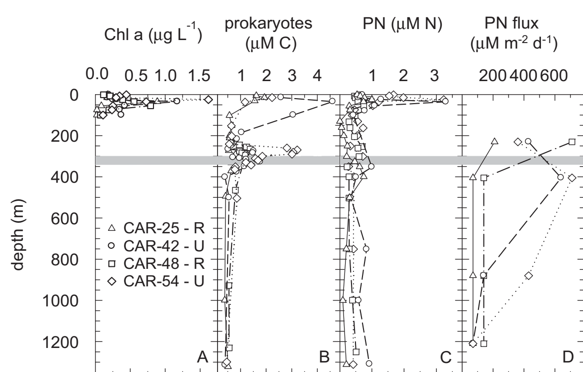


**Fig. 2.** Vertical profiles of Chl *a* (A), prokaryotic biomass (B), and particulate nitrogen, PN (C) concentrations as well as PN fluxes to sediment traps (D) obtained from cruises when both suspended and sinking particles were assayed for ectohydrolytic activity. Shaded horizontal band represents depth range of $O_2$ disappearance during these cruises to station CARIACO. "U" and "R" after cruise numbers signify upwelling and relaxation periods, respectively.

subsequent discussion will focus on PN pools. Sestonic PN concentrations were significantly correlated ($p \ll 0.001$) with Chl *a* concentrations and prokaryotic biomass in the upper 100 m; $r = 0.76$ for both. One-way ANOVAs suggest that vertical distributions of prokaryotes and PN varied significantly among the cruises; $p<0.05$ and $p<0.005$, respectively. This outcome is largely influenced by CAR-19 and CAR-25 supporting significantly lower inventories than the other seven cruises.

Two patterns of vertical PN flux were observed; the typical power function decay evident during CAR-25 and CAR-48 and a unimodal pattern evident during CAR-42 and CAR-54 (Fig. 2D). While PN (and POC) fluxes to all four depths were not statistically different among the four deployments ($p>0.1$), the power of this statistic is quite low due to small sample size.

### 3.2. Vertical distributions of ectohydrolase activity

During eight of the nine sampling periods, a distinct interface was evident where concentrations of $O_2$ and $H_2S$ both fell below detection limits at the same depth (e.g., Fig. 3A), the depth of which varied between 260 and 350 m. A suboxic zone was evident during CAR-48 (7 Nov 99), where $O_2$ was detectable above 255 m and $H_2S$ appeared below 295 m (Fig. 3B). Potential ectohydrolytic rates (% hydrolysate $d^{-1}$) in Niskin bottle samples tended to be highest in surface waters and lower in deeper waters, with profiles commonly resembling those observed for the 6 May 99 (CAR-42) cruise (Fig. 3A). Activities of selected ectohydrolases either varied little with depth or exhibited maxima in sulfidic waters, such as observed for APase during CAR-48 (Fig. 3B). However, these variations in activity do not appear to be explained by seasonality. Although POC concentrations and C/N ratios were slightly higher ($p<0.001$; ANOVA) during upwelling collections, ectohydrolase activities, PN concentrations and all other biotic variables were not statistically higher than in non-upwelling collections ($p>0.05$; ANOVA).

Hydrolysis rates associated with trap collections are not easily compared to rates measured on suspended material collected by Niskin bottles. Rates obtained from trap materials were corrected for activity in the seawater used to dilute them, then multiplied by a dilution factor ($22.7 = 1000/44$) to yield "equivalent" volumetric rates (% hydrolysate $d^{-1}$) for the material in the collection cups 1 d after retrieval. This approach is internally consistent among all trap samples. However, it does not account for the fact that the traps collect particles settling through a cross-sectional collection area ($0.5\,m^2$), the fact that particles are diluted by sterile seawater in the collection cups nor the fact that collection takes place over a 9–12 d period. This estimation method also disregards the fact that materials that entered the traps at the start of the collection period are probably less labile at the time of the assays than those that entered just prior to trap retrieval. These constraints are addressed in more detail below.

Like PN flux (Fig. 2D), two ectohydrolytic activity patterns were exhibited in our sediment trap collections. During CAR-42 and CAR-54, both upwelling periods, samples from the 405 m trap exhibited higher activities for all four ectohydrolases than samples retrieved from the 225 m trap or the deeper traps (e.g., Fig. 4A). In contrast, during CAR-25 and CAR-48, both during relaxation periods, samples from the shallowest trap exhibited the highest activity and ectohydrolase activities were generally diminished at depth (e.g., Fig. 4B).

### 3.3. Ectohydrolase activities and environmental variables

Potential rates of LAPase, CHIase, and APase were strongly correlated with POC and PN concentrations in Niskin bottle samples, while those of β-GLUase were not (Table 2). C/N atomic ratios in seston varied from 3.8 to 16.4, averaged 8.9, and did not vary predictably with depth. LAPase activity was the only variable correlating with the sestonic C/N ratio, although weakly ($r = -0.31$, $p<0.05$) (not presented). We note that particulate phosphorus was not measured in Niskin bottle samples. LAPase and CHIase activities covaried with concentrations of Chl *a*

**Table 2**
Pearson product–moment correlation matrix comparing potential ectohydrolase activities (% hydrolysate– $d^{-1}$) with relevant bulk chemical and microbiological variables in seston samples throughout the water column.

| | PN (μM N) | NPP ($gCm^{-2} d^{-1}$) | Chl *a* ($mg m^{-2}$) | [prokary] (# $L^{-1}$) | BNP (μM C $d^{-1}$) | LAPase (% $d^{-1}$) | CHIase (% $d^{-1}$) | β-GLUase (% $d^{-1}$) | APase (% $d^{-1}$) | $PO_4^{3-}$ (μM) | DIN (μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| POC | **0.87**$^{***}$ (48) | 0.82$^{*}$ (9) | 0.85$^{**}$ (9) | **0.67**$^{***}$ (48) | **0.87**$^{***}$ (32) | **0.53**$^{***}$ (48) | **0.54**$^{***}$ (42) | Ns (48) | 0.48$^{**}$ (31) | −**0.56**$^{***}$ (32) | ns (40) |
| PN | | 0.77$^{*}$ (9) | 0.74$^{*}$ (9) | **0.76**$^{***}$ (48) | **0.95**$^{***}$ (32) | **0.66**$^{***}$ (48) | **0.61**$^{***}$ (42) | ns (48) | 0.47$^{**}$ (31) | −**0.61**$^{***}$ (32) | ns (40) |
| NPP | | | 0.87$^{**}$ (9) | ns (9) | 0.94$^{**}$ (9) | 0.75$^{*}$ (9) | 0.84$^{*}$ (8) | ns (9) | ns (6) | ns (8) | ns (8) |
| Chl *a* | | | | ns (9) | 0.94$^{**}$ (9) | 0.67$^{*}$ (9) | **0.96**$^{**}$ (8) | ns (9) | ns (6) | ns (8) | ns (8) |
| [prokary] | | | | | **0.73**$^{***}$ (32) | **0.53**$^{***}$ (48) | 0.39$^{*}$ (42) | ns (48) | 0.38$^{*}$ (31) | −**0.73**$^{***}$ (32) | −**0.54**$^{**}$ (40) |
| BNP | | | | | | **0.72**$^{***}$ (32) | **0.74**$^{***}$ (26) | ns (32) | ns (15) | −0.60$^{*}$ (16) | ns (24) |
| LAPase | | | | | | | **0.89**$^{***}$ (42) | **0.45**$^{**}$ (48) | 0.51$^{**}$ (31) | −**0.68**$^{***}$ (32) | −0.32$^{*}$ (40) |
| CHIase | | | | | | | | ns (42) | ns (31) | −0.52$^{**}$ (32) | ns (40) |
| β-GLUase | | | | | | | | | **0.61**$^{***}$ (31) | −0.47$^{*}$ (32) | ns (40) |
| APase | | | | | | | | | | −**0.58**$^{**}$ (32) | ns (31) |
| $PO_4^{3-}$ | | | | | | | | | | | **0.59**$^{***}$ (32) |

Statistical significance presented as follows: $^{***}p$-values $<0.0001$; $^{**}p<0.005$; $^{*}p<0.05$; ns $= p>0.05$ (not significant). Boldface correlations are those passing sequential Bonferroni test, indicating that correlations are highly significant and unlikely to have arisen by chance alone (group-wide type-I error). Numbers of samples compared are presented in parentheses. Photic zone assumed to be well-mixed, thus integrated NPP and Chl *a* are compared to $PO_4^{3-}$ and DIN concentrations at base (100 m) and with shallow ectohydrolase activities at discrete depths.

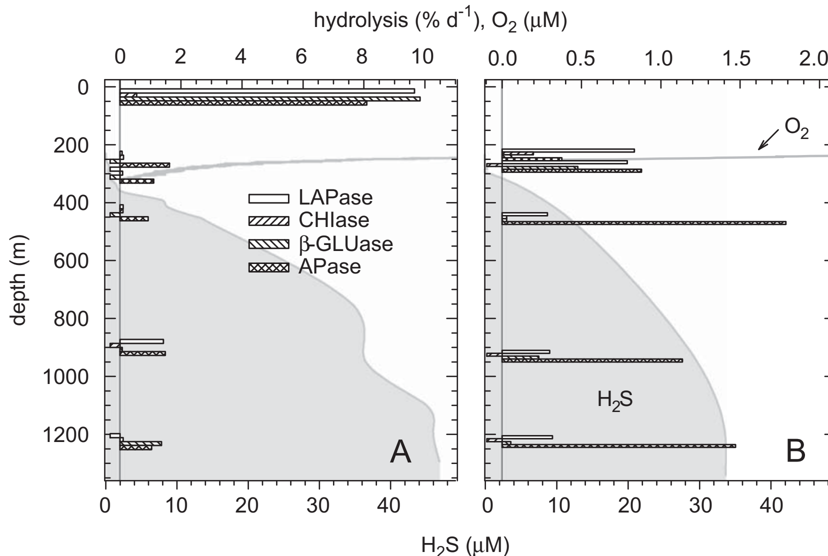


**Fig. 3.** Vertical profiles of potential ectohydrolytic activities (bars) in seston and concentrations of dissolved $O_2$ and hydrogen sulfide ($H_2S$ = shaded area). Activities expressed as first-order rate constant, i.e., hydrolysate production rate divided by total analog added (3.1 μM). Samples in panels A and B were collected during the 8 May '99 (CAR-42) and 7 Nov '99 (CAR-48) cruises, which represent upwelling and relaxation periods, respectively. All samples were assayed at the shore-based laboratory one day after retrieval. Negative hydrolysis rates denote samples below detection limits.

and prokaryotes and with rates of net primary production and BNP. While β-GLUase and APase were not consistently correlated with these variables, they did covary with one another. Correlations between dissolved organic carbon (DOC) concentrations and ectohydrolase activities were not found, but this may be because the quality and availability of DOC data during these cruises were inadequate for robust statistical comparisons (not presented).

In contrast to seston samples, activity potentials of all four ectohydrolases appeared to strongly covary with POC

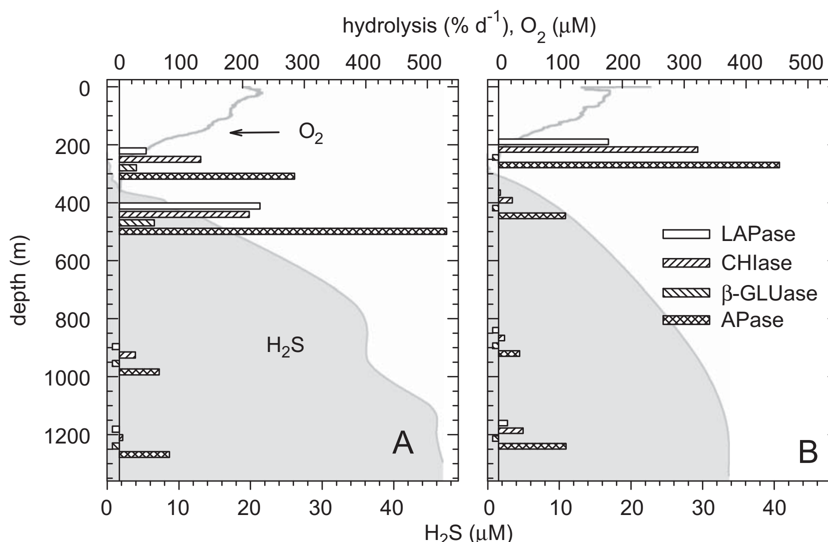


**Fig. 4.** Potential ectohydrolytic activities (bars) associated with sinking particles. Activities expressed as first-order rate constants corrected for sample dilution during incubations, i.e., observed % hydrolysate d$^{-1}$ × 22.7. Samples in panels A and B were collected over intervals of 24 Apr–3 May '99 and 21 Oct–2 Nov '99, immediately preceding CAR-42 and CAR-48 cruises, respectively (Fig. 3). All samples were assayed at the shore-based laboratory one day after retrieval. Negative hydrolysis rates denote samples below detection limits.

**Table 3**
Pearson product–moment correlation matrix comparing potential ectohydrolase activities (% hydrolysate d$^{-1}$) with relevant bulk chemical and microbiological variables in sediment trap samples.

| | PN (μM N) | PIP (μM P) | POP (μM P) | [prokary] (# l$^{-1}$) | BNP (μM C d$^{-1}$) | LAPase (% d$^{-1}$) | CHIase (% d$^{-1}$) | β-GLUase (% d$^{-1}$) | APase (% d$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| POC | **0.98**$^{***}$<br>(16) | −0.58$^{*}$<br>(14) | −0.62$^{*}$<br>(14) | 0.56$^{*}$<br>(16) | ns<br>(8) | **0.92**$^{***}$<br>(16) | 0.64$^{*}$<br>(16) | **0.76**$^{**}$<br>(16) | **0.84**$^{**}$<br>(12) |
| PN | | −0.59$^{*}$<br>(14) | −0.62$^{*}$<br>(14) | 0.56$^{*}$<br>(16) | ns<br>(8) | **0.89**$^{***}$<br>(16) | 0.66$^{*}$<br>(16) | 0.72$^{**}$<br>(16) | 0.76$^{**}$<br>(12) |
| PIP | | | ns<br>(14) | ns<br>(14) | ns<br>(14) | −0.54$^{*}$<br>(14) | ns<br>(14) | −0.54$^{*}$<br>(14) | ns<br>(14) |
| POP | | | | ns<br>(14) | 0.88$^{*}$<br>(7) | −0.53$^{*}$<br>(14) | ns<br>(14) | ns<br>(14) | ns<br>(14) |
| [prokary] | | | | | ns<br>(8) | ns<br>(16) | 0.67$^{**}$<br>(16) | ns<br>(16) | ns<br>(12) |
| BNP | | | | | | ns<br>(16) | 0.74$^{*}$<br>(8) | ns<br>(16) | ns<br>(12) |
| LAPase | | | | | | | 0.57$^{*}$<br>(16) | **0.85**$^{***}$<br>(16) | **0.91**$^{***}$<br>(12) |
| CHIase | | | | | | | | ns<br>(16) | 0.64$^{*}$<br>(12) |
| β-GLUase | | | | | | | | | **0.90**$^{***}$<br>(12) |

Statistical significances presented as in Table 2. Numbers of samples compared are presented in parentheses.

and PN concentrations in trap collection cups (Table 3). C/N atomic ratios in sed-POM were generally lower than for seston, varying unpredictably with depth from 3.6 to 8.8, and averaging 6.9. LAPase and APase potentials were the only biotic variables that correlated with C/N ratios in sed-POM, $r = 0.51$ and 0.69 ($p < 0.05$), respectively (not presented). Unlike seston samples, only CHIase was positively correlated with prokaryotic abundances and BNP. The lower number of trap samples undermines confidence in some of these correlations, as suggested by the Bonferroni test, but the trends are clear. Patterns of ectohydrolytic activity and C/N ratios suggest that polymer composition differed between suspended and sinking pools. Furthermore, the comparatively weak relationships between enzyme activity and prokaryotic abundance in trap material indicate that cell-specific ectohydrolase activities within microbial assemblages varied sharply among samples.

### 3.4. Normalized ectohydrolase activities

On the presumption that compounds contributing to particulate nitrogen pools are more biologically labile

than the total particulate organic carbon pool, we normalized potential hydrolysate production rates (nM hydrolysate $d^{-1}$) by PN, which permits direct comparisons of hydrolytic activity between seston and sinking debris. As an example, LAPase activity calculated in this manner yields potential release rates of free leucine per unit of particulate nitrogen (Fig. 5). Because prokaryotic biomass covaried with PN (Tables 2 and 3), normalizing activities by prokaryotic biomass or cell number did not significantly alter observed patterns (not presented). However, we prefer to normalize by PN, because proportions of cells expressing a specific ectohydrolase and relative activities among competent cells are likely to vary widely among samples and the calculation implies uniformity. In order to compare ectohydrolase activities within similar biogeochemical zones among cruises, sample depths in Figs. 5–8 are expressed relative to disappearance of $O_2$, because position of the $O_2/H_2S$ interface and redoxcline shifted temporally.

In five of the nine cruises where LAPase activity was measured in seston, PN-specific hydrolysis potentials were as high as 50–300 (mmol leu) $(\text{mol PN})^{-1}\,d^{-1}$ in surface waters and were usually lower at depth, approaching detection limits (Fig. 5A). CAR-29 and CAR-36 were exceptions, where potential rates across the interface appeared to be higher than at depths above or below. A different pattern was observed during CAR-13 and CAR-19, where PN-specific potentials in samples from 150 m above the interface were higher than in shallower or deeper samples (560–670 compared to 40–300 (mmol leu)

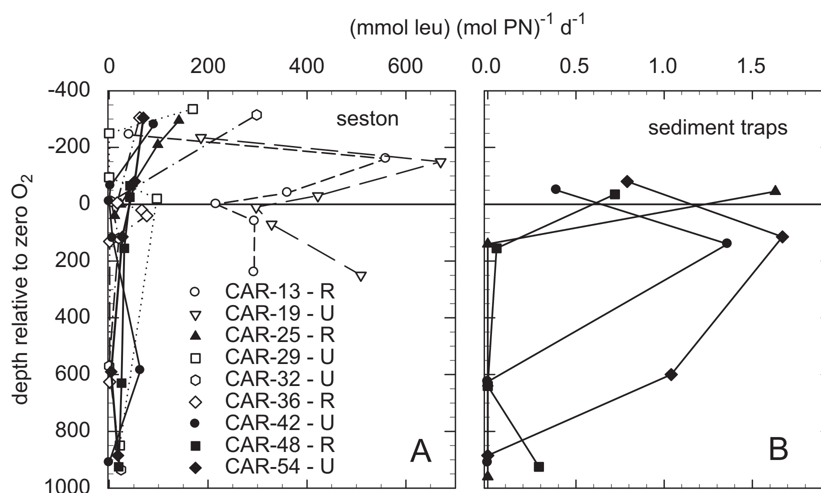


**Fig. 5.** Depth profiles of PN-normalized leucine production resulting from leu-aminopeptidase (LAPase) activity in Niskin bottle (A) and sediment trap (B) samples relative to the disappearance depth of $O_2$ (horizontal line) for all collections. "U" and "R" after cruise numbers signify upwelling and relaxation periods, respectively.

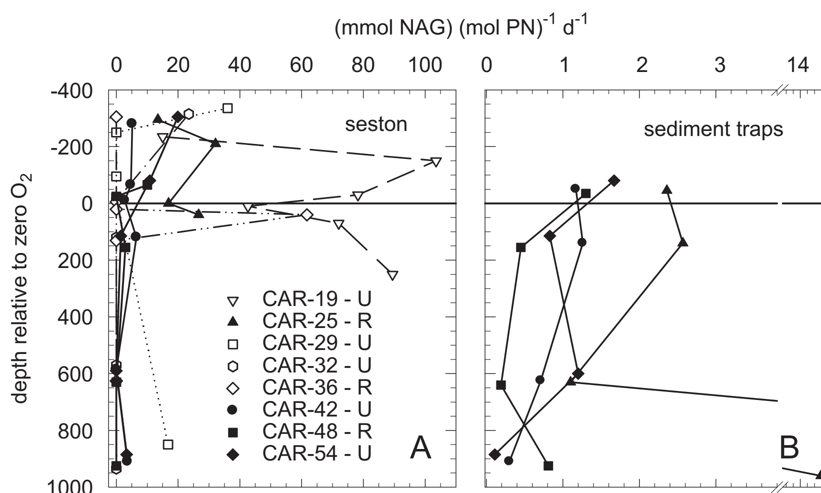


**Fig. 6.** Depth profiles of PN-normalized n-acetylglucosamine (NAG) production resulting from chitinase (CHIase) activity in Niskin bottle (A) and sediment trap (B) samples relative to the disappearance depth of $O_2$ (horizontal line) from same samples presented in Fig. 5.

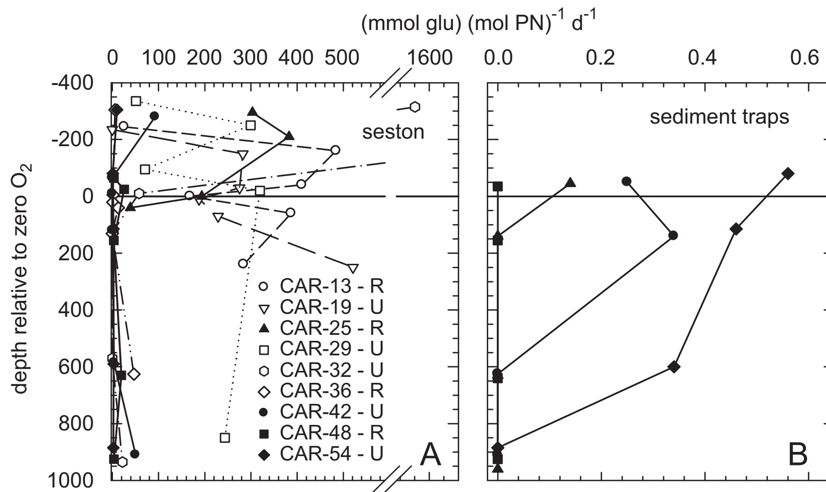


**Fig. 7.** Depth profiles of PN-normalized glucose production resulting from β-glucosidase (β-GLUase) activities in Niskin bottle (A) and sediment trap (B) samples relative to the disappearance depth of $O_2$ (horizontal line) from same samples presented in Fig. 5.

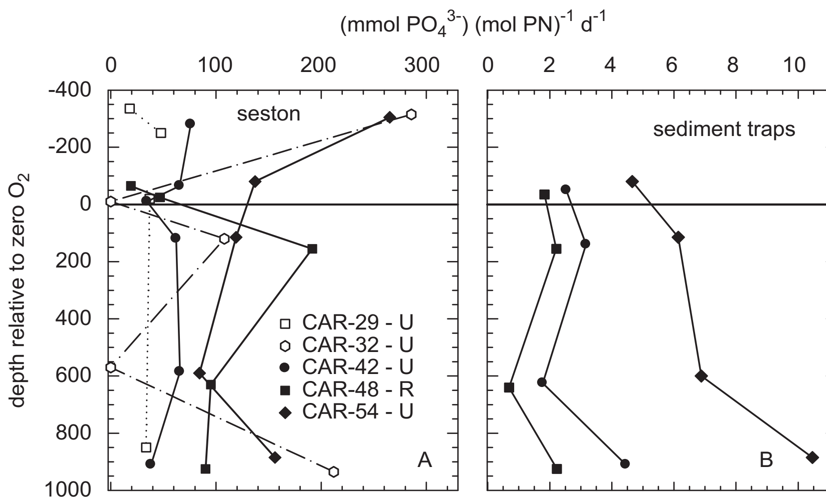


**Fig. 8.** Depth profiles of PN-normalized phosphate production resulting from alkaline phosphatase (APase) activities in Niskin bottle (A) and sediment trap (B) samples relative to the disappearance depth of $O_2$ (horizontal line) from same samples presented in Fig. 5.

(mol PN)$^{-1}$ d$^{-1}$) and specific rates increased again below the interface. These alternative LAPase activity patterns bear no obvious relationship to upwelling or productivity. For example, even though primary productivities during CAR-13 and CAR-19 were relatively high, 150 and 208 mmol C m$^{-2}$ d$^{-1}$, they were not the highest in our data set. Furthermore, upwelling and relaxation periods could exhibit the same pattern, e.g., CAR-19 vs CAR-13 or CAR-29 vs CAR-36 (Fig. 5A).

In the four available sediment trap data sets, the PN-specific potentials (Fig. 5B) exhibited the same two depth-related patterns observed for bulk rates (Fig. 4). During relaxation periods (CAR-25 and 48), specific rates were modest in shallow, oxic collections (225 m), varying from 0.7 to 1.6 (mmol leu) (mol PN)$^{-1}$ d$^{-1}$, and were near detection limits in deeper, anoxic traps (Fig. 5B). During upwelling periods (CAR-42 and 54), specific rates in trap material collected below the interface (405 m) were more than double those observed in 225 m traps and lower rates were observed in deeper traps (Fig. 5B). We interpret the first pattern, which resembles the dominant pattern in the seston, as resulting from decomposition of euphotic zone-derived proteins, while the second pattern suggests a community response to inputs of fresh protein below the 225 m trap.

PN-specific CHIase potentials, i.e., *n*-acetyl-glucosamine (NAG) production rates, were below detection limits in >40% of our seston assays (Fig. 6A). In six of the eight cruises assayed, PN-specific CHIase potentials in the water column decreased by 2–30-fold from surface waters to depth. During CAR-19 and CAR-25, however, CHIase activities exhibited subsurface maxima, decreasing toward the interface, then increasing below it. Unlike CAR-25, CHIase and LAPase activity profiles for CAR-19 were

very similar (Figs. 5A and 6A). In sinking debris, specific CHIase rates varied unpredictably with depth, sometimes exhibiting relatively high values at depth (Fig. 6B). Variations in PN-specific CHIase activity patterns for seston and sinking particles do not correspond to productivity or upwelling status.

In nearly half the seston profiles, PN-normalized β-glucosidase potentials exhibited subsurface maxima in the oxic layer, decreased toward the interface, then increased below it (Fig. 7A). In the remainder of profiles, β-GLUase activities were highest in the photic zone, attaining potentials as high as 1560 (mmol glu) (mol PN)$^{-1}$ d$^{-1}$, and declined at depth. β-GLUase activities in the shallowest traps varied from undetectable to 0.14 (mmol glu) (mol PN)$^{-1}$ d$^{-1}$ during relaxation periods (CAR-25 and 48) and between 0.25 and 0.55 (mmol glu) (mol PN)$^{-1}$ d$^{-1}$ during upwelling (CAR-42 and 54) (Fig. 7B). However, these activities did not directly correlate with primary production measured during the same period. β-GLUase activity was undetectable in all CAR-48 trap samples. During CAR-42, the 405 m trap material produced the highest β-GLUase activities. In general, specific β-GLUase rates in sinking debris decreased with depth, approaching detection limits anywhere from 150 to 900 m below the interface, depending on activities apparent in shallowest traps.

Generally, PN-specific APase potentials in seston samples varied less with depth than any of the other three ectohydrolases (Fig. 8A). APase potentials in these samples ranged from undetectable to 285 (mmol $PO_4^{3-}$) (mol PN)$^{-1}$ d$^{-1}$ with no consistent pattern relating to depth or plankton productivity. In contrast, a consistent pattern was evident in sediment trap samples (Fig. 8B), with APase activity generally increasing with depth in sinking debris relative to PN pools. Samples from 840 m traps repeatedly had lower than expected rates based on apparent trend lines. Consistent with the other three ectohydrolases, PN-normalized APase potentials in all trap material were significantly lower than contemporaneously collected seston samples ($p<0.001$; ANOVA).

## 4. Discussion

### *4.1. Oxic versus anoxic decomposition*

Whether water column anoxia in the present or geologic past promotes higher preservation efficiencies for sinking POM remains controversial (see Deuser, 1975; Demaison and Moore, 1980; Calvert et al., 1991; Lee, 1992; Thunell et al., 2000). As in open waters, factors controlling decomposition of particles suspended or falling through anoxic waters are critical in determining carbon sequestration and preservation efficiencies at the seafloor. Our experiments allowed direct comparison of ectohydrolytic activities among aerobic and truly anaerobic planktonic assemblages fueled by materials of the same provenance. In the closest example of which we are aware, hydrolysis rates of LAPase, β-GLUase and APase were not detectably altered within the oxygen minimum zone ($\leqslant$0.1 mg $O_2$ l$^{-1}$) of the Arabian Sea relative to waters above and below (Hoppe and Ullrich, 1999). Hoppe et al. (1990) demonstrated that subjecting aerobic assemblages to sulfidic conditions can depress apparent ectohydrolytic activity. However, this effect was probably indicative of downstream metabolic responses of aerobes in the original sample subjected to reducing conditions, rather than indicative of intrinsic differences in ectohydrolase functionality in oxic and euxinic systems. In the present study, pair-wise comparisons (ANOVA) of oxic and anoxic samples failed to demonstrate statistical differences attributable to presence or absence of dissolved oxygen for any of the ectohydrolases (Fig. 9). Relationships between oxic and anoxic hydrolytic rates were also very similar when normalized to prokaryotic abundance or biomass (not presented). Our sediment trap sample size is insufficient to statistically test for activity differences between sed-POM in oxic (225 m only) and anoxic traps ($\geqslant$405 m).

The absence of demonstrable quantitative differences between specific hydrolytic potentials in oxic and anoxic data sets is interesting for two reasons. First, heterotrophs living below Cariaco Basin's $O_2/H_2S$ interface have nontrivial energetic constraints: labile substrates and oxidants appear to be in short supply. In the absence of $O_2$, $NO_3^-$, and $NO_2^-$, organisms can ferment susceptible substrates, respire sulfate or oxides of Mn and Fe. Competitive inhibition experiments have suggested that sulfate reduction rates are quite slow in the Cariaco Basin's water column (Ho et al., 2002) and little is known about the importance alternate respiratory pathways or fermentation in this system. The second consideration is that aerobic assemblages are living in water that is generally ~9 °C warmer than the anaerobes that inhabit the constant 17.5 °C anoxic environment. Bacterioplankton metabolism is believed to possess $Q_{10}$ responses lying between 2 and 3 (Shiah et al., 2000). For Arctic bacterioplankton, $Q_{10}$ responses of several ectohydrolases were observed to vary between 1 and 8 (Vetter and

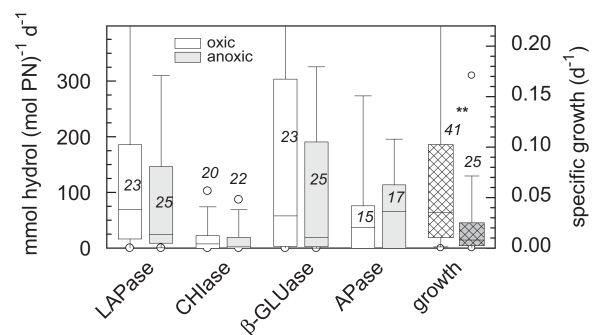


**Fig. 9.** Activity potentials of LAPase, CHIase, β-GLUase, and APase and growth rates of heterotrophic prokaryotes in Niskin bottle collections from oxic and anoxic waters during nine cruises. Ectohydrolase rates are normalized by PN concentrations. Specific growth rate = prokaryotic carbon production ($^3$H-leucine technique) divided by prokaryotic carbon biomass to yield mean biomass turnover. Boxes enclose the 25th–75th percentiles of all observations, lines within boxes represent medians, error bars enclose 10th–90th percentiles and circles represent the 5th–95th percentiles. Sample sizes (*n*) are associated with each bar. Significant differences determined by ANOVA and Dunn's multiple pairwise comparisons are denoted as * ($p<0.05$) and ** ($p<0.001$).

Deming, 1994). We have observed that $Q_{10}$ ratios for LAPase activity in temperate bacterioplankton assemblages lie between 2 and 5 (unpubl. data). Therefore, the slightly lower activities observed for most ectohydrolases in deep anaerobic assemblages may reflect a temperature response more than anoxia effects. If held at the same temperature as surface aerobic assemblages (25–27 °C), activities of deep anaerobic assemblages could be nearly 2–3-fold higher than observed, thereby exceeding those measured for aerobes. We conclude that anoxia does not directly hamper polymer hydrolysis by resident microbial assemblages. Therefore, impediments to organic matter remineralization imposed by anoxia on prokaryotes must act at the membrane or intracellular levels, meaning expression of appropriate transporters and catabolic pathways, or availability of oxidants.

In contrast to hydrolytic activity, prokaryotic-specific growth rates were significantly lower ($p<0.001$) in anoxic than oxic waters (Fig. 9). The apparent disconnect between ectohydrolytic activities and depressed prokaryotic growth suggests that some classes of released monomers and oligomers (substrates) may accumulate in anoxic waters faster than they can be metabolized. Alternatively, oxic–anoxic differences in growth may be a methodological artifact of BNP measurements. If fewer anaerobes are capable of $^3$H-leucine uptake than aerobes, then growth rate estimates will be biased towards aerobes. At single oxic and anoxic depths in the Cariaco Basin, Lee (1992) observed that several radiolabeled substrates, including an amino acid mix, were assimilated at similar rates, but turned over more slowly in anoxic waters. At a single depth in the Cariaco Basin's redoxcline (270 m), Lin et al. (2007) observed that only 30% of the prokaryotic assemblage incorporated $^3$H-leucine with entire proteobacterial classes exhibiting no $^3$H-leucine uptake. However, we are unaware of any studies specifically demonstrating systematic differences in $^3$H-leucine uptake between aerobes and anaerobes. The relatively low growth rates estimated for both aerobic and anaerobic assemblages in this study suggest that large fractions of both planktonic assemblages are either not growing or are incapable of $^3$H-leucine uptake.

### *4.2. Seston versus sed-POM—quantitative differences*

Several previous studies of hand-collected macroaggregates compared ectohydrolytic potentials and prokaryotic growth in a cm$^3$ of particles with a cm$^3$ of bulk seawater, yielding volume concentration factors (VCF). Reported VCF for as many as seven different ectohydrolases varied from 0 to $\infty$, but were typically between about 10 and 50,000 and indicate the degree to which activities were intensified within macroaggregates (e.g., Karner and Herndl, 1992; Smith et al., 1992). Our VCF estimates for ectohydrolase activity associated with sed-POM are significantly higher, varying from 400 to $1.5\times10^6$ and medians between 66,000 and 365,000 (Fig. 10). These high values are a likely consequence of PN, POC and prokaryotic biomass being on the order of $2.3\times10^6$, $1.8\times10^6$, and $0.4\times10^6$ times more concentrated in sed-POM than in surrounding waters, respectively. For hand-collected macroaggregates, DOC, carbohydrates, amino acids, $NH_4^+$, $PO_4^{3-}$, and prokaryotes have all been reported to be tens to thousands of times more concentrated than in surrounding waters in a number of studies (reviewed in Simon et al., 2002). The higher VCFs reported for our rapidly sinking particulate pools may simply be a consequence of their higher compaction and lower porosity compared to macroaggregates, and does not necessarily translate to qualitative differences in the organic matter.

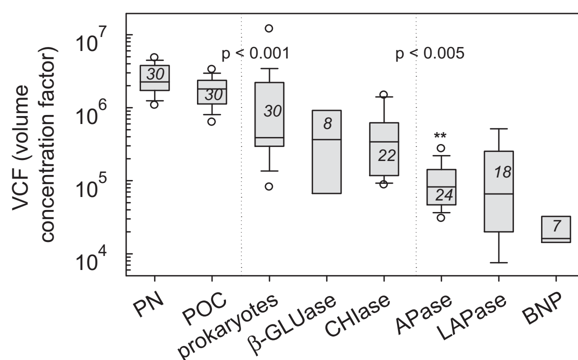


**Fig. 10.** Box plot of volume concentration factors (VCFs) of key inventory and activity measurements measured in 1 cm$^3$ of sinking particles compared to those measured in 1 cm$^3$ of seawater including seston. Box plot presentation and statistics same as in Fig. 9. Vertical dotted lines separate significantly different groups of variables (ANOVA and Dunn's multiple pair-wise comparisons) at levels denoted. ** indicates that APase activity is significantly ($p<0.005$) more concentrated than BNP.

While VCFs of many measured variables were high in trap material, Dunn's multiple pair-wise comparisons indicate that prokaryotic activities in sed-POM were significantly less enhanced ($p<0.001$) than their potential substrates, measured as PN or POC (Fig. 10). These disparities in enrichment factors suggest that smaller proportions of sinking PN and POC were labile compared to equal volumes of seawater containing dissolved, colloidal and suspended particulate organic matter. Among the activity measurements, VCFs of β-GLUase and CHIase significantly exceeded those of APase, LAPase and BNP. The divergence of VCFs among ectohydrolytic potentials suggest that sed-POM is enriched in polysaccharides and chitin relative to seston.

When normalized to available resources (PN), microbial activities in sed-POM actually appeared to be significantly lower than in seston samples (Fig. 11). For example, median LAPase, CHIase, β-GLUase and APase activities in trap samples were approximately 150, 4, 430, and 28-fold lower per unit PN than in seston samples, respectively (Fig. 11). These findings appear to contradict earlier findings for hand-collected macroaggregates (e.g., Smith et al., 1992). Our findings for trap material, however, are not completely unprecedented. In shallow sediment traps deployed in an Arctic polynya, ectohydrolase activities normalized to sinking POM dry weight were not significantly different from those associated with seston (Vetter and Deming, 1994). Thus, it appears that

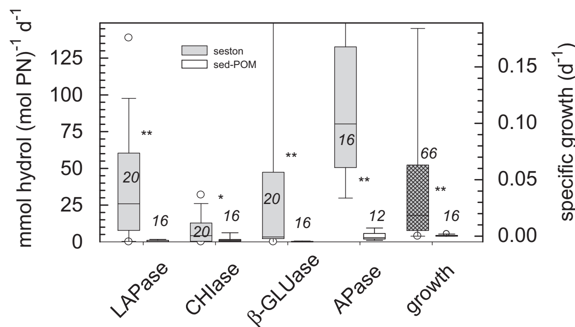


**Fig. 11.** Comparison of potential activities of LAPase, CHIase, β-GLUase, APase, and heterotrophic prokaryotic growth rate in Niskin bottle and sediment trap samples. Ectohydrolase activities are normalized by PN concentrations. Box plot presentation and statistics are same as in Fig. 9.

polymers in sed-POM are not necessarily turned over more readily by hydrolysis than those in the seston.

We note that our activity estimates for sed-POM are likely to be conservative because our assays were performed on material remaining in traps after 9–12 d collections. From PN flux data, we can estimate a plausible range of material loss occurring during trap deployment. Excluding depth intervals where PN fluxes increased and using first-order decay kinetics, PN loss ($\delta F_{PN}/\delta z$), or vertical decay, varied between 0.000 and 0.005 m$^{-1}$. Assuming that mean particle sinking velocities, $\nu$, lie between 25 and 250 m d$^{-1}$ through the water column (Berelson, 2002), $\delta F_{PN}/\delta z$ estimates yield median decay rates of 0.01–0.14 d$^{-1}$ ($\nu \cdot \delta F_{PN}/\delta z$) for slowly and rapidly sinking particles, respectively. This exercise suggests that on average 1–14% of material collected by our unpoisoned traps was decomposed by the time of retrieval. Thus, our hydrolysis rates reflect activities associated with materials varying in age and stages of decomposition, the lability of which can depend upon arrival time. Then again, the same argument can be made for seston captured in Niskin bottles because residence times and labilities of suspended particles are unknown and likely to vary widely also. Correcting rates for decomposition is not feasible with available information because ectohydrolase activities are not likely to scale predictably with particle mass or age in such complex materials that are delivered to depth at varying rates. Our assays may not have captured hydrolysis of the most labile POM fractions. However, PN-specific rates reveal that on balance polymeric pools in traps samples are turned over at significantly lower rates than POM suspended in the Cariaco Basin.

In the present study, prokaryotic production (μM C d$^{-1}$) was intensified the least of all variables measured, attaining a median VCF of only ~16,000 (Fig. 10). Cell-specific growth rates (d$^{-1}$) tended to be almost 150 times slower in sed-POM samples than in seston (Fig. 11). Even in hand-collected macroaggregates and fresh diatom debris, prokaryotes' specific growth rates can be lower than those of free-living prokaryotes (Alldredge et al., 1986; Simon et al., 1990; Unanue et al., 1998). Measured growth rates were low in both seston and sed-POM, signifying that large portions of these prokaryotic assemblages were either inactive or did not assimilate extracellular $^{3}$H-leucine. The disparity between VCFs for abundance and production ($p<0.001$; ANOVA) suggests that particle-associated prokaryotes, in particular, were less active than bacterioplankton on a per cell basis or that measured BNP rates were artificially low. As suggested by Ploug and Grossart (1999), diffusive transport into particles' interiors can limit microbial access to radiotracers in such incubations and lead to underestimates. While we cannot eliminate this possibility, mixing of these diluted incubations and the 8–12-h incubations should have been sufficient for tracer equilibration. It is also unlikely that rates were significantly biased because of dilution of the 10 nM $^{3}$H-leucine tracer by ambient dissolved leucine because concentrations varied from 0 to 6 nM and 0.1 to 1.4 nM in sed-POM and seston incubations, respectively (Taylor, unpubl. data). Clearly this approach has its limitations, but low prokaryotic growth rates in sed-POM have been measured previously by independent in situ techniques and thus do not appear to be uncommon (Taylor et al., 1986; Karl et al., 1988).

Consistent differences in the relative importance of specific ectohydrolases and their depth profiles (Figs. 3–8) suggest that microbial assemblages associated with seston and sed-POM samples are adapted to hydrolyzing different pools of polymers. Individual ectohydrolase activities varied significantly within seston and sed-POM samples as well as between them, when normalized to PN (Fig. 11). Rank order of median activities in sinking particles was APase > CHIase > LAPase > β-GLUase. In contrast, activities in seston samples ranked APase > LAPase > β-GLUase > CHIase (Fig. 11). Activity rankings in seston are based on combined oxic and anoxic observations from nine cruises, and yielded medians of 66 (range = 0–268), 42 (0–670), 32 (0–1561), and 3.5 (0–104) (mmol hydrolysate) (mol PN)$^{-1}$ d$^{-1}$ for APase, LAPase, β-GLUase, and CHIase, respectively. These rankings remain intact whether normalization is by PN or prokaryotic cell abundances. Our rank orderings of ectohydrolase activity are similar to those of other marine studies (e.g., Smith et al., 1992; Martinez et al., 1996).

### 4.3. Methodological considerations

A limitation of most ectoenzyme assays is that ambient concentrations of target polymers are necessary to calculate actual hydrolysis rates, but are difficult to measure and consequently seldom known. Lacking detailed information on macromolecular composition, investigators have resorted to incubating samples with saturating concentrations of fluorogenic analog to estimate maximum potential rates as a reasonable compromise (e.g., Chróst and Velimirov, 1991). To evaluate whether our rates represent maximum potential rates, we examined LAPase activity with respect to protein pools. Particulate and dissolved combined amino acids (PCAA and DCAA) concentrations are unavailable for Cariaco Basin Niskin bottle samples. However, combined these fractions contributed 31% and 18% to the total POC mass flux into the 225 and 405 m sediment traps

recovered immediately before the CAR-54 (6 May 2000) cruise. PCAA and DCAA contributed 33–41 and 7–11 μM total amino acids as peptides to our experimental dilutions and 2.3–3.2 and 0.5–0.7 μM specifically as leucine residues, respectively (data courtesy of L. Abramson and C. Lee). Thus, in samples representing the highest PN mass flux and protein concentrations of the entire study, our analog addition (3.1 μM) was essentially equivalent to total ambient pools of the residue hydrolyzed by LAPase. Therefore, actual rates of LAPase hydrolysis in our most protein-rich samples could potentially be a factor of 2 higher than the reported rates for shallow trap samples. However, in other trap and all seston samples possessing lower protein contents, we expect that leu-MCA was added at saturating concentrations.

In addition to relative tracer and substrate concentrations, community kinetic parameters also influence apparent activities. For example, Azúa et al. (2003) noted that half-saturation constants, $K_m$, in particle-associated assemblages were higher than those associated with seawater alone for all ectohydrolases tested. These results imply that at low substrate concentrations ectohydrolases in free-living assemblages were more responsive than those associated with aggregates. In the literature, fluorogenic analogs have been added at a single level or as a range of concentrations, varying from $<0.5$ to 200 μM (e.g., Hoppe, 1993; Sala et al., 2001). Sebastián and Niell (2004) demonstrated that MUF-pho concentrations $\leqslant 5$ μM yielded saturating hydrolysis rates, $V_{max}$ (nM d$^{-1}$), for subtropical plankton assemblages in the eastern N. Atlantic, and higher analog additions actually depressed apparent APase rates. While kinetics in the present study are unknown, our analog additions approximate those yielding $V_{max}$ for APase in Sebastián and Niell's (2004) study, this being our most active ectohydrolase. Therefore, we hypothesize that the same analog concentrations added to estimate rates of less active ectohydrolases should also approximate their $V_{max}$. Clearly, all rates must be viewed as potential rather than actual (Chróst and Velimirov, 1991).

### 4.4. *LAPase activity in perspective*

Current theory holds that uptake of organic substrates and subsequent microbial production in aquatic systems are generally limited by rates of particle and polymer hydrolysis because concentrations and residence times of most labile monomers and oligomers are generally too low to support observed production (Fuhrman and Ferguson, 1986; Hoppe et al., 1988, 1993; Azam and Smith, 1991). Furthermore, relative activities of specific ectohydrolases have been used to infer which classes of polymers are important to bacterioplankton nutrition in a variety of environments (Christian and Karl, 1995; Koike and Nagata, 1997; Sala et al., 2001). Community level polymer hydrolysis has been related to decomposition, nutrient and energy acquisition, and secondary production in terms of an optimal resource allocation model (Sinsabaugh et al., 1997). The foundation of this model is that osmotrophic assemblages will optimize their energy expenditures by expressing high levels of particular ectohydrolases only if polymeric substrates are abundant and if the monomeric hydrolysates required for growth are scarce.

LAPase activity in the current study appears to be a prime example of optimal resource allocation. Bulk analyses suggest that freshly collected marine plankton is about 50–60% particulate combined amino acids (PCAA) or protein, 20–25% carbohydrates and 20–30% lipids by weight (Hedges et al., 2001). Similarly, PCAA accounts for 40–50% of sed-POM mass in the upper 3500 m of the equatorial Pacific (Hedges et al., 2001). In contrast, the proteinaceous fraction in seston from the equatorial Pacific declines steeply with depth to $<20\%$ by weight in the upper 1000 m (Sheridan et al., 2002). Thus, protein appears to be the most abundant identifiable class of macromolecules in source material, and becomes more depleted in seston than in sed-POM at depth in marine systems.

From the consumer's perspective, proteins and peptides are important, estimated to support between 10% and 45% of C demand and ~40% to $>100\%$ of N demand for bacterioplankton in surface waters (Kroer et al., 1994). Not unexpectedly, strong covariances between LAPase activity, leucine uptake and dissolved free amino acids have been observed widely in aquatic environments (Hoppe et al., 1988, 1998; Chróst et al., 1989; Müller-Niklas et al., 1994; Taylor et al., 2003b). Among all Cariaco Basin samples, LAPase activity correlated with PN concentrations more robustly than any other ectohydrolase, suggesting a microbial community response to protein availability in both seston and sed-POM (Tables 2 and 3). Consistent with microbial utilization of protein hydrolysates, leucine uptake (BNP) was also strongly correlated with PN concentrations and LAPase potentials among seston samples (Table 2).

Aminopeptidases are typically among the most active of ectohydrolases assayed in aquatic systems (Karner and Herndl, 1992; Smith et al., 1992; Sinsabaugh et al., 1997). They are believed to be important for carbon, nitrogen and energy acquisition and appear to be widely expressed among marine prokaryotes (Chróst, 1991; Kroer et al., 1994; Christian and Karl, 1995; Martinez et al., 1996). Depth trends for LAPase activity in both sed-POM and seston samples (Fig. 5) were consistent with general trends for PCAA observed in other ocean regimes (e.g., Hedges et al., 2001; Sheridan et al., 2002; Gupta and Kawahata, 2003). This consistency and strong correlations with PN (Tables 2 and 3) suggest prokaryotic expression of LAPase activity was responsive to PCAA availability. However, LAPase's subordinate ranking in sed-POM samples suggests the possibility that other proteolytic enzymes also participate in protein decomposition in sinking POM and escaped detection in our assays.

### 4.5. *Chitinase activity in sinking and suspended POM*

The enhanced expression of CHIase activity associated with sed-POM relative to seston in our samples may be

driven by the varying composition of polymeric pools. Exuviae and peritrophic membrane-encased fecal pellets from planktonic crustacea are common in sedimenting materials and are expected to be the primary sources of chitin in this ecosystem (Small et al., 1987; Sasaki et al., 1988; Taylor, 1989). Perhaps behavioral patterns of macrozooplankton, such as vertical migration, explain variations in CHIase activity distributions. Other sources of chitin to this system may be cell walls of chlorophytes and extracellular material produced by some diatoms and prymnesiophytes (Cottrell et al., 1999). Therefore, this POM pool may preferentially select for growth of microbial populations expressing CHIase or may induce CHIase up-regulation. Consistent with the prediction of Hoppe et al. (1998), CHIase activity in the seston was highly correlated with phytoplankton abundance and production (Table 2). We interpret this as a microheterotrophic response to enhanced availability of chitinous substrates, produced either directly by phytoplankton or by crustacean herbivores. The n-acetylglucosamine released from chitin hydrolysis may be utilized as N, C and energy resources or directly for synthesis of new prokaryotic peptidoglycan cell wall.

### 4.6. *β-Glucosidase activity in sinking and suspended POM*

Activity of β-GLUase, a low-specificity enzyme that hydrolyzes many forms of polysaccharides produced by microalgae and vascular plants (Chróst, 1991) and releases energy-bearing sugars, was nearly always detectable in our samples. In all but three seston sample sets, activity of this ectohydrolase declined markedly with depth (Fig. 7). Previously, Koike and Nagata (1997) observed that α- and β-GLUase activities in central Pacific deep waters ($>1000$ m) were usually $<1\%$ of those observed in surface layers. These trends suggest that either labile polysaccharides are rapidly depleted with depth or that other sources of energy and carbon are more accessible to microbial assemblages at depth. In general, carbonaceous compounds are remineralized more slowly than nitrogenous compounds as indicated by POC/PN ratios increasing from 5.6 in fresh plankton tows to as high as 16.4 in Cariaco Basin seston and averaging 8.9 (Benitez-Nelson et al., 2004, 2007; this study). Furthermore, β-GLUase potentials have been observed to be 10–1000-fold lower than those of LAPase here and elsewhere, implying either that polysaccharides breakdown is significantly slower than protein or that polysaccharides are less abundant (Smith et al., 1992; Huston and Deming, 2002; this study).

### 4.7. *Phosphorus dynamics*

In Cariaco Basin seston samples, variations in total dissolved inorganic nitrogen concentrations (DIN = $NH_4^+$+ $NO_3^-$+$NO_2^-$) did not correlate with other biotic variables, except weakly with prokaryotic abundances. Consequently, DIN explained almost no variation in planktonic inventories or activities. In stark contrast, variations in dissolved $PO_4^{3-}$ concentrations were inversely correlated with all measured rates and inventories, except for integrated NPP and Chl *a* and probably because of the small sample size (Table 2). Findings were not significantly altered by comparing these variables at discrete depths (not presented). We interpret these trends as suggestive of dissolved phosphorus depletions in samples possessing high microbial biomass and production and that these depletions induce up-regulation of APase. APase hydrolyzes phosphate esters at alkaline pH (7.2–9.8; Kuenzler and Perras, 1965), releasing phosphate for subsequent assimilation from sugar phosphates and other phosphomonoesters (Dyhrman, 2005). Inverse correlations between APase activity and dissolved $PO_4^{3-}$ concentrations have been observed widely in aquatic environments, suggesting that planktonic expression of this ectohydrolase is a response to phosphorus "stress" or P-limitation (Siuda and Chróst, 1987, Ammerman, 1991; Cotner et al., 1997; Sala et al., 2001). For example, Chróst and Rai (1993) observed high levels of APase in nutrient-impoverished mesocosms, while β-GLUase became more important in systems replete with inorganic nutrients. In other words, when inorganic nutrient limitation is relaxed, prokaryotes are capable of expressing higher capacities for carbon and energy-acquiring ectohydrolases, such as β-GLUase.

It is becoming increasingly evident that P-limitation is intermittently operative over vast regions of the world's ocean (Karl, 2002). Therefore, APase and other P-regenerating ectohydrolases (e.g., 5′-nucleotidase) appear to be important for P retention in the epipelagic and are intimately linked to the cycling of P, N, and C. In oceanic systems, APase activities sometimes exceed those of other ectohydrolases and are the least diminished with depth (Smith et al., 1992; Koike and Nagata, 1997). In fact, APase activities in the Arabian Sea were reported to increase with depth, suggesting that this enzyme is important in degrading POP below the photic zone (Hoppe and Ullrich, 1999). Unlike the other three ectohydrolases examined in the Cariaco Basin, PN-normalized APase tended to increase with depth in sed-POM during all three collections and consistently maintained relatively high, though variable, potentials throughout the water column (Fig. 8). This pattern indicates that inorganic P is regenerated faster than N from sed-POM and at varying rates compared to N in seston, suggesting that the Cariaco Basin may experience P-stress.

Benitez-Nelson et al. (2004) previously reported that Cariaco Basin sed-POM was severely depleted in organic phosphorus relative to the Redfield ratio; PN/POP averaging 37. Based on a systematic analysis of 9 years of biweekly sediment trap data and dissolved nutrient inventories from the CARIACO time series station, Benitez-Nelson et al. (2007) reported that phosphorus is regenerated from sed-POM faster than C and N at all times and is fastest during the upwelling season. These authors observed that PIP within sinking material was preferentially lost with depth below 225 m, i.e., total N/P ratios increased systematically below the $O_2/H_2S$ interface. In trap samples analyzed in the current study, PIP and POP were negatively correlated with POC and PN, illustrating that particulate C/N/P ratios were relatively high during periods of high particle production (Table 3). Despite the

suggestion of P-limitation inferred from elemental ratios and high APase potentials in sed-POM, APase activities did not correlate with PIP or POP inventories within sediment traps (Table 3).

Paradoxically, the argument that relatively high APase expression throughout the Cariaco Basin is indicative of phosphorus limitation is not supported by dissolved nutrient stoichiometry in the water column. At the time series station, the euphotic zone varies from 30 to 100 m and typically exhibits depleted inventories of all dissolved inorganic nutrients, and $PO_4^{3-}$ concentrations have always been $\leqslant 1.0\,\mu M$ over the last decade (http://www.imars.usf.edu/CAR/). However, dissolved N/P ratios ($NO_3^-$+$NO_2^-$+$NH_4^+$)/$PO_4^{3-}$ in the upper 30 m have been below the Redfield ratio (16) in 88% of all monthly observations ($n = 382$) and averaged 7.4, suggesting plankton communities are most often N-limited. In the redoxcline (~220–350 m), the second most productive layer in this water column, $PO_4^{3-}$ concentrations have always been between 1 and 3 $\mu M$ over the last decade. In this layer, dissolved N/P ratios were below 3.0 in >94% of all observations ($n = 395$) and averaged 1.4, again implying N-limitation for those microbial assemblages. Below 350 m, dissolved $PO_4^{3-}$ concentrations increased monotonically toward the seafloor to ~4.0 $\mu M$ and N/P ratios averaged 4.2 ($n = 534$). N/P ratios recomputed to include dissolved organic N and P are slightly elevated in the upper 100 m, but are not significantly altered at depth. However, applicability of total dissolved N/P ratios is uncertain, because bioavailability of DON and DOP pools is unknown. Cellular N/P ratios in bacterioplankton appear to be ~16 in P-limited cultures, but are lower under C and N-limited and nutrient-replete conditions, varying between 6.4 and 9.2 (Vrede et al., 2002). Bacterioplankton cell N/P ratios are therefore higher than dissolved ratios generally observed below the euphotic zone and actually suggest that organisms inhabiting most of the water column are more likely to be N-limited than P-limited. Nonetheless, organisms in this system, whether planktonic or associated with sed-POM, appear adapted to hydrolyzing phosphomonoesters, effectively regenerating inorganic $PO_4^{3-}$ throughout the water column, whether it is oxic or anoxic.

## 5. Conclusions

Microbial productivity and ectohydrolytic activity associated with hand-collected macroaggregates have been explored in the literature. However, the present study is one of the few to examine these processes in materials directly captured during their downward transport. As in macroaggregates, ectohydrolase activities and prokaryotic production in sinking biogenic debris were spatially intensified over comparable volumes of seawater ($400–1.5 \times 10^6$-fold). However, our results suggest that when normalized to inventories of PN or prokaryotes, hydrolytic potentials associated with sed-POM are not demonstrably higher than sestonic activities. Our findings also suggest that suspended POM inventories are turned over by hydrolysis more rapidly than sed-POM.

Of the ectohydrolases assayed, alkaline phosphatase generally remained most active at all depths in both seston and sed-POM samples. This observation coupled with previous reports and inverse correlations between phosphorus inventories and microbial abundance, productivity and activity measurements, suggest that microbial assemblages commonly regenerate dissolved inorganic phosphate in the water column. Elemental ratios of sed-POM suggest that associated microorganisms are P-limited, which may explain APase potentials increasing with depth in sediment trap samples. In the seston, however, dissolved nutrient inventories acquired over the last decade and published N/P cell quotients argue against the prevalence of P-limitation for suspended microorganisms in the Basin. We hypothesize that high APase activities observed in the seston may be more reflective of microbial acquisition of carbon and energy from labile phosphate esters rather than demonstrating phosphorus acquisition.

As expected, activity potentials of all ectohydrolases were highest in samples from warm shallow waters, where labile polymers abound in relatively young particles. However, normalizing activity potentials by PN or prokaryotic inventories reduced differences between depths. While bacterial growth rates in oxic seston samples were significantly faster than in anoxic samples, no statistical differences between oxic and anoxic samples could be demonstrated for any ectohydrolase. Furthermore, factoring in the 8–9 °C temperature difference between oxic and anoxic suggests that ectohydrolytic potentials of distinct aerobic and anaerobic microbial assemblages are not fundamentally different within the Cariaco Basin's water column.

## Acknowledgments

We thank the captain and crew of the R/V *Hermano Gines* and the staff of the Fundacion La Salle de Ciencias Naturales for their assistance during our field work in Venezuela. Our gratitude goes to F. Muller-Karger for his tireless efforts to make Project CARIACO a success. We are particularly grateful to Y. Astor for logistical support and provision of hydrographic and dissolved oxygen data. We are also indebted to M. Iabichella-Armas, J. Nichols, J. Gulnick, and A. Modanesi for technical support in the field and laboratory. Analyses of PCAA and DCAA from sediment trap samples were graciously provided by L. Abramson and C. Lee (Stony Brook University). This research was supported by grants from US NSF (OCE9711318, OCE9730278, and OCE 03-26175; M.I.S. and G.T.T. and OCE9729284, OCE9729697, OCE0326313; R.C.T.) and from Venezuela FONACIT (nos. 96280221 and 2000001702; R.V.). The authors are indebted to the three anonymous reviewers whose incisive comments helped immensely in improving this communication. The corresponding author also acknowledges the Institut de Ciéncies del Mar, Barcelona, and Spanish Ministry of Education and Science for their generous sabbatical support during preparation of this paper. School of Marine and Atmospheric Sciences contribution #1381.

## References


Alldredge, A.L., Silver, M.W., 1988. Characteristics, dynamics and significance of marine snow. Progress in Oceanography 20, 41–82.

Alldredge, A.L., Cole, J.J., Caron, D.A., 1986. Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters. Limnology and Oceanography 31, 68–78.

Ammerman, J.W., 1991. Role of ecto-phosphohydrolases in phosphorus regeneration in estuarine and coastal ecosystems. In: Chróst, R.J. (Ed.), Microbial Enzymes in Aquatic Environments. Springer, Berlin, pp. 25–59.

Aminot, A., 1983. Dosage de l'oxygene dissous. In: Aminot, A., Chaussepied, M. (Eds.), Manuel des Analyses Chimiques en Milieu Marin. Centre National pour L'Exploitation des Oceans (CNEXO), France, pp. 75–92.

Aspila, K.I., Agemian, H., Chau, A.S., 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst 101, 187–197.

Armstrong, R.A., Lee, C., Hedges, J.I., Honjo, S., Wakeham, S.G., 2002. A new, mechanistic model for organic carbon fluxes in the ocean: a quantitative role for the association of POC with ballast minerals. Deep-Sea Research II 49, 219–236.

Azam, F., Smith, D.C., 1991. Bacterial influence on the variability in the ocean's biogeochemical state: a mechanistic view. In: Demers, S. (Ed.), Particle Analysis in Oceanography. Springer, New York, pp. 213–236.

Azam, F., Long, R.A., 2001. Sea snow microcosms. Nature 414, 495–498.

Azúa, I., Unanue, M., Ayo, B., Artolozaga, I., Arrieta, J.M., Iriberri, J., 2003. Influence of organic matter quality in the cleavage of polymers by marine bacterial communities. Journal of Plankton Research 25, 1451–1460.

Baird, R.C., Wilson, D.F., Milliken, D.M., 1973. Observations on *Bregmaceros nectabanus* Whitley in the anoxic, sulfurous water of the Cariaco Trench. Deep-Sea Research 20, 503–504.

Baker, E.T., Milburn, H.B., Tennant, D.A., 1988. Field assessment of sediment trap efficiency under varying flow conditions. Journal of Marine Research 46, 573–592.

Benitez-Nelson, C.R., 2000. The biogeochemical cycling of phosphorus in marine systems. Earth-Science Reviews 51 (1–4), 109–135.

Benitez-Nelson, C.R., O'Neill, L., Kolowith, L.C., Pellechia, P.J., Thunell, R.C., 2004. Phosphonates and particulate organic phosphorus cycling in an anoxic marine basin. Limnology and Oceanography 49, 1593–1604.

Benitez-Nelson, C.R., O'Neill Madden, L.P., Styles, Thunell, R.C., Astor, Y., 2007. Inorganic and organic sinking particulate phosphorus fluxes across the oxic/anoxic water column of Cariaco Basin, Venezuela. Marine Chemistry 105, 90–100.

Berelson, W.M., 2002. Particle settling rates increase with depth in the ocean. Deep-Sea Research II 49, 237–251.

Boyd, P.W., Trull, T.W., 2007. Understanding the export of biogenic particles in oceanic waters: is there a consensus? Progress in Oceanography 72, 276–312.

Calvert, S., Karlin, R., Toolin, L., Donahue, D., Southon, J., Vogel, J., 1991. Low organic carbon accumulation rates in Black Sea sediments. Nature 350, 692–695.

Cho, B.C., Azam, A., 1988. Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature 332, 441–443.

Christian, J.R., Karl, D.M., 1995. Bacterial ectoenzymes in marine waters: activity ratios and temperature responses in 3 oceanographic provinces. Limnology and Oceanography 40, 1042–1049.

Chróst, R.J., 1991. Microbial ectoenzymes in aquatic environments. In: Chróst, R.J. (Ed.), Microbial Enzymes in Aquatic Environments. Springer, New York, pp. 29–54.

Chróst, R.J., Rai, H., 1993. Ectoenzyme activity and bacterial secondary production in nutrient-impoverished and nutrient-enriched freshwater mesocosms. Microbial Ecology 25, 131–150.

Chróst, R.J., Velimirov, B., 1991. Measurement of enzyme kinetics in water samples: effect of freezing and soluble stabilizer. Marine Ecology Progress Series 70, 93–100.

Chróst, R.J., Munster, U., Rai, H., Albrecht, D., Witzel, P., Overbeck, J., 1989. Photosynthetic production and exoenzymatic degradation of organic matter in the euphotic zone of a eutrophic lake. Journal of Plankton Research 11, 223–242.

Cline, J.D., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnology and Oceanography 14, 454–458.

Cotner, J.B., Ammerman, J.W., Peele, E.R., Bentzen, E., 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. Aquatic Microbial Ecology 13, 141–149.

Cottrell, M.T., Moore, J.A., Kirchman, D.L., 1999. Chitinases from uncultured marine microorganisms. Applied and Environmental Microbiology 65, 2553–2557.

Demaison, G., Moore, G., 1980. Anoxic environments and oil source bed genesis. American Association of Petroleum Geologists Bulletin 64, 1179–1190.

Deuser, W., 1975. Reducing environments. In: Riley, J.P., Skirrow, G. (Eds.), Chemical Oceanography. Academic Press, New York, pp. 1–37.

Dyhrman, S., 2005. Ectoenzymes in *Prorocentrum minimum*. Harmful Algae 4, 619–627.

Emerson, S., Quay, P., Karl, D., Winn, C., Tupas, L., Landry, M., 1997. Experimental determination of the organic carbon flux from open-ocean surface waters. Nature 389, 951–954.

Falkowski, P., and many others, 2000. The global carbon cycle: a test of our knowledge of Earth as a system. Science 290, 291–296.

Fuhrman, J.A., Ferguson, R.L., 1986. Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. Marine Ecology Progress Series 33, 237–242.

Goñi, M.A., Aceves, H.L., Thunell, R.C., Tappa, E., Black, D., Astor, Y., Varela, R., Muller-Karger, F., 2003. Biogenic fluxes in the Cariaco Basin: a combined study of sinking particulates and underlying sediments. Deep-Sea Research I 50, 781–807.

Gordon, L.I., Jennings Jr., J.C., Ross, A.A., Krest, J.M., 1993. A suggested protocol for continuous flow automated analysis of seawater nutrients. In: WOCE Operation Manual. WHP Office Report 90-1. WOCE Report 77, No. 68/91, pp. 1–52.

Gupta, L.P., Kawahata, H., 2003. Biogeochemical processes and labile composition of settling particulate organic matter in the south-west Pacific Ocean. Marine and Freshwater Research 54, 259–270.

Gust, G., Michaels, A.F., Johnson, R., Deuser, W.G., Bowles, W., 1994. Mooring line motions and sediment trap hydromechanics: in situ intercomparison of three common deployment designs. Deep-Sea Research I 41, 831–857.

Hebel, D.V., Karl, D.M., 2001. Seasonal, interannual and decadal variations in particulate matter concentrations and composition in the subtropical North Pacific Ocean. Deep-Sea Research II 48, 1669–1695.

Hedges, J.I., Baldock, J.A., Gélinas, Y., Lee, C., Peterson, M., Wakeham, S.G., 2001. Evidence for non-selective preservation of organic matter in sinking marine particles. Nature 409, 801–804.

Ho, T.-Y., Scranton, M.I., Taylor, G.T., Thunell, R.C., Varela, R., Muller-Karger, F., 2002. Acetate cycling in the water column of the Cariaco Basin: seasonal and vertical variability and implication for carbon cycling. Limnology and Oceanography 47, 1119–1128.

Hoppe, H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Marine Ecology Progress Series 11, 299–308.

Hoppe, H.G., 1993. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. In: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp. 423–430.

Hoppe, H.G., Ullrich, S., 1999. Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. Aquatic Microbial Ecology 19, 139–148.

Hoppe, H.G., Kim, S.J., Gocke, K., 1988. Microbial decomposition in aquatic environments: combined process of extracellular enzyme activity and substrate uptake. Applied and Environmental Microbiology 54, 784–790.

Hoppe, H.G., Gocke, K., Kuparinen, J., 1990. Effect of H2S on heterotrophic substrate uptake, extracellular enzyme activity and growth of brackish water bacteria. Marine Ecology Progress Series 64, 157–167.

Hoppe, H.G., Ducklow, H., Karrasch, B., 1993. Evidence for dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. Marine Ecology Progress Series 93, 277–283.

Hoppe, H.G., Geisenhagen, H.C., Gocke, K., 1998. Changing patterns of bacterial substrate decomposition in a eutrophication gradient. Aquatic Microbial Ecology 15, 1–13.

Huston, A.L., Deming, J.W., 2002. Relationships between microbial extracellular enzymatic activity and suspended and sinking particulate organic matter: seasonal transformations in the North Water. Deep-Sea Research II 49, 5211–5225.

Karl, D.M., 1999. A sea of change: biogeochemical variability in the North Pacific subtropical gyre. Ecosystems 2, 181–214.

Karl, D.M., 2002. Nutrient dynamics in the deep blue sea. Trends in Microbiology 10, 410–418.

Karl, D.M., Knauer, G.A., Martin, J.H., 1988. Downward flux of particulate organic matter in the ocean: a particle decomposition paradox. Nature 332, 438–441.

Karner, M., Herndl, G., 1992. Extracellular enzymatic activity and secondary production in free-living and marine-snow-associated bacteria. Marine Biology 113, 341–347.

Kiørboe, T., Jackson, G.A., 2001. Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. Limnology and Oceanography 46, 1309–1318.

Kirchman, D.L., 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publ., Boca Raton, FL, pp. 509–512.

Koike, I., Nagata, T., 1997. High potential activity of extracellular alkaline phosphatase in deep waters of the central Pacific. Deep-Sea Research II 44, 2283–2294.

Kroer, N., Jørgensen, N.O.G., Coffin, R.B., 1994. Utilization of dissolved nitrogen by heterotrophic bacterioplankton: a comparison of three ecosystems. Applied and Environmental Microbiology 60, 4116–4123.

Kuenzler, E.J., Perras, J.P., 1965. Phosphatases of marine algae. Biological Bulletin 128, 271–282.

Lee, C., 1992. Controls on organic carbon preservation: the use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. Geochemica et Cosmochimica Acta 56, 3323–3335.

Lin, X., Scranton, M.I., Varela, R., Chistoserdov, A.Y., Taylor, G.T., 2007. Compositional responses of bacterial communities to redox gradients and grazing in the anoxic Cariaco Basin. Aquatic Microbial Ecology 47, 57–72.

Longhurst, A.R., 1991. Role of marine biosphere in the global carbon cycle. Limnology and Oceanography 36, 1507–1526.

Love, R.H., Fisher, R.A., Wilson, M.A., Nero, R.W., 2004. Unusual swimbladder behavior of fish in the Cariaco Trench. Deep-Sea Research I 51, 1–16.

Martin, J.H., Knauer, G.A., Karl, D.M., Broenkow, W.W., 1987. VERTEX: carbon cycling in the northeast Pacific. Deep-Sea Research 34, 267–285.

Martinez, J., Smith, D.C., Steward, G.F., Azam, F., 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquatic Microbial Ecology 10, 223–230.

Michaels, A.F., Silver, M.W., 1988. Primary production, sinking fluxes and the microbial food web. Deep-Sea Research II 35, 473–490.

Muller-Karger, F., Varela, R., Thunell, R., Scranton, M., Bohrer, R., Taylor, G., Capelo, J., Astor, Y., Tappa, E., Ho, T.-Y., Walsh, J.J., 2001. Annual cycle of primary production in the Cariaco Basin: response to upwelling and implications for vertical export. Journal of Geophysical Research 106, 4527–4542.

Müller-Niklas, G., Schuster, S., Kaltenböck, E., Herndl, G.J., 1994. Organic content and bacterial metabolism in amorphous aggregates of the northern Adriatic Sea. Limnology and Oceanography 39, 58–68.

Ploug, H., Grossart, H.-P., 1999. Bacterial production and respiration in suspended aggregates—a matter of the incubation method. Aquatic Microbial Ecology 20, 21–29.

Porter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting microflora. Limnology and Oceanography 25, 943–948.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Sala, M.M., Karner, M., Marrasé, C., 2001. Measurement of ectoenzyme activities as an indication of inorganic nutrient imbalances in microbial communities. Aquatic Microbial Ecology 23, 301–311.

Sarmiento, J.L., Gruber, N., Brzezinski, M.A., Dunne, J.P., 2004. High-latitude controls of thermocline nutrients and low latitude biological productivity. Nature 427, 56–60.

Sasaki, H., Hatori, H., Nishizawa, S., 1988. Downward flux of particulate organic matter and vertical distribution of calanoid copepods in the Oyashio Water in summer. Deep-Sea Research 35, 505–515.

Scranton, M.I., Taylor, G.T., Astor, Y., Muller-Karger, F., 2006. Temporal variability in the nutrient chemistry of the Cariaco Basin. In: Neretin, L.N. (Ed.), Past and Present Water Column Anoxia, NATO Science Series. Springer, Netherlands, pp. 139–160.

Sebastián, M., Niell, F.X., 2004. Alkaline phosphatase activity in marine oligotrophic environments: implications of single-substrate addition assays for potential activity estimations. Marine Ecology Progress Series 277, 285–290.

Sheridan, C.C., Lee, C., Wakeham, S.G., Bishop, J.K.B., 2002. Suspended particle organic composition and cycling in surface and midwaters of the equatorial Pacific Ocean. Deep-Sea Research I 49, 1983–2008.

Shiah, F.-K., Gong, G.-C., Chen, T.-Y., Chen, C.-C., 2000. Temperature dependence of bacterial specific growth rates on the continental shelf of the East China Sea and its potential application in estimating bacterial production. Aquatic Microbial Ecology 22, 155–162.

Simon, M., Grossart, H.-P., Schweitzer, B., Ploug, H., 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquatic Microbial Ecology 28, 175–211.

Simon, M., Alldredge, A.L., Azam, F., 1990. Bacterial carbon dynamics on marine snow. Marine Ecology Progress Series 65, 205–211.

Sinsabaugh, R.L., Findlay, S., Franchini, P., Fischer, D., 1997. Enzymatic analysis of riverine bacterioplankton production. Limnology and Oceanography 42, 29–38.

Siuda, W., Chróst, R.J., 1987. The relationship between alkaline-phosphatase (APA) activity and phosphate availability for phytoplankton and bacteria in eutrophic lakes. Acta Microbiologie Polonica 36, 247–257.

Small, L.F., Knauer, G.A., Tuel, M.D., 1987. The role of sinking fecal pellets in stratified euphotic zones. Deep-Sea Research 34, 1705–1712.

Smith, D., Simon, M., Alldredge, A.L., Azam, F., 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid dissolution. Nature 359, 139–142.

Steinberg, D.K., Pilskaln, C.H., Silver, M.W., 1998. Contribution of zooplankton associated with detritus to sediment trap 'swimmer' carbon in Monterey Bay, California, USA. Marine Ecology Progress Series 164, 157–166.

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

Taylor, G.T., 1989. Variability in the vertical flux of microorganisms and biogenic material in the epipelagic zone of a North Pacific central gyre station. Deep-Sea Research 36, 1287–1308.

Taylor, G.T., Gulnick, J.D., 1996. Enhancement of marine bacterial growth by mineral surfaces. Canadian Journal of Microbiology 42, 911–918.

Taylor, G.T., Karl, D.M., 1991. Biotic and biogenic fluxes in the eastern North Pacific implications for productivity and biogeochemical cycling. Global Biogeochemical Cycles 5, 289–303.

Taylor, G.T., Karl, D.M., Pace, M.L., 1986. Impact of bacteria and zooflagellates on the composition of sinking particles: an in situ experiment. Marine Ecology Progress Series 29, 141–155.

Taylor, G.T., Scranton, M.I., Iabichella, M., Ho, T.-Y., Thunell, R.C., Muller-Karger, F., Varela, R., 2001. Chemoautotrophy in the redox transition zone of the Cariaco Basin: a significant midwater source of organic carbon production. Limnology and Oceanography 46, 148–163.

Taylor, G.T., Hein, C., Iabichella, M., 2003a. Temporal variations in viral distributions in the anoxic Cariaco Basin. Aquatic Microbial Ecology 30, 103–116.

Taylor, G.T., Way, J., Yu, Y., Scranton, M.I., 2003b. Patterns of hydrolytic ectoenzyme activity among bacterioplankton communities in the lower Hudson River and Western Long Island Sound estuaries. Marine Ecology Progress Series 263, 1–15.

Taylor, G.T., Iabichella-Armas, M., Varela, R., Muller-Karger, F., Lin, X., Scranton, M.I., 2006. Microbial ecology of the Cariaco Basin's oxic/anoxic interface: the US–Venezuelan CARIACO times series program. In: Neretin, L.N. (Ed.), Past and Present Water Column Anoxia, NATO Science Series. Springer, Netherlands, pp. 473–499.

Thunell, R., Varela, R., Llano, M., Collister, J., Muller-Karger, F., Bohrer, R., 2000. Organic carbon fluxes and regeneration rates in an anoxic water column: sediment trap results from the Cariaco Basin. Limnology and Oceanography 45, 300–308.

Thunell, R., Benitez-Nelson, C., Varela, R., Astor, Y., Müller-Karger, F., 2007. Particulate organic carbon fluxes along upwelling-dominated continental margins: rates and mechanisms. Global Biogeochemical Cycles 21, GB1022.

Unanue, M., Azúa, I., Arrieta, J.M., Labirua-Iturburu, A., Egea, L., Iriberri, J., 1998. Bacterial colonization and ectoenzymatic activity in phytoplankton-derived model particles: cleavage of peptides and uptake of amino acids. Microbial Ecology 35, 136–146.

Vetter, Y.A., Deming, J.W., 1994. Extracellular enzyme activity in the Arctic Northeast Water polynya. Marine Ecology Progress Series 114, 23–34.

Vrede, K., Heldal, M., Norland, S., Bratbak, G., 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. Applied and Environmental Microbiology 68, 2965–2971.