Extracellular amino acid oxidation by microplankton: a cross-ecosystem comparison

Margaret R. Mulholland^{1,*}, Patricia M. Glibert², Gry Miné Berg²,
Laurie Van Heukelem², Silvio Pantoja^{3,**}, Cindy Lee³

¹University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, PO Box 38, Solomons, Maryland 20688, USA

²University of Maryland Center for Environmental Science, Horn Point Laboratory, PO Box 775, Cambridge, Maryland 21613, USA

³Marine Sciences Research Center, SUNY, Stony Brook, New York 11794-5000, USA

ABSTRACT: Rates of extracellular amino acid oxidase activity in natural phytoplankton, cyanobacterial, and bacterial assemblages were measured using a fluorescent analog of the amino acid lysine. Activity was measured in a variety of ecosystems with different levels of nutrient enrichment and diverse community composition. Sites included a station in Shinnecock Bay, Long Island Sound, New York (USA); the Chesapeake Bay, Maryland (USA); the NW Atlantic Ocean near the Bahamas and the Caribbean Sea; Brazilian coastal waters; and 2 estuarine mesocosms. Highest rates of amino acid oxidase activity (25 to 30 nM h^{-1}) were found in the summer mesocosm experiments when NH_4^+ concentrations were near the limit of detection, and biomass levels were indicative of an algal bloom. Lower rates of amino acid oxidase activity were found during a bloom of *Aureococcus anophagefferens* and in oligotrophic oceanic waters. High rates of amino acid oxidase activity (up to 20 nM h^{-1}) were also found in oceanic samples enriched with colonies of the diazotrophic cyanobacteria *Trichodesmium*. No activity was observed in samples from oligotrophic environments that were pre-fractionated through $1.0 \text{ }\mu\text{m}$ filters; however, when amended with glucose or an amino acid mixture, oxidation rates of up to 8 nM h^{-1} were observed. No activity was found during a diatom-dominated, autumnal bloom in Chesapeake Bay. Overall, amino acid oxidation represented a higher percentage of NH_4^+ uptake in the oligotrophic waters (up to 10%) than in the coastal waters studied. In oligotrophic waters, where ambient inorganic nitrogen concentrations are low and consequently uptake rates are low, this pathway appears to represent a potentially important source of nitrogen for phytoplankton and the diazotrophic cyanobacteria *Trichodesmium*.

KEY WORDS: Amino acid oxidase activity · Nitrogen uptake · Marine phytoplankton · Marine cyanobacteria · Oxidative deamination

INTRODUCTION

Regenerated production (sensu Dugdale & Goering 1967) is commonly estimated as NH_4^+ uptake in most marine and estuarine systems. Recently, dissolved organic nitrogen (DON), including amino acids, has been identified as another important source of regenerated nitrogen in some marine systems (Wheeler & Kirchman 1986, Bronk & Glibert 1993, Bronk et al.

1994); however, its uptake has been measured far less routinely in assessments of nitrogen based production. A variety of marine and estuarine phytoplankton and bacteria can use DON as a source of nitrogen (Antia et al. 1991, Keil & Kirchman 1991, Glibert 1993). Uptake of dissolved free amino acids (DFAA) and other specific organic nitrogen compounds (e.g. primary amines, and nucleotides) has been demonstrated using isotopically labeled substrates in a variety of aquatic ecosystems and in cultures of microorganisms (Wheeler et al. 1974, Suttle et al. 1991, Jørgensen et al. 1993). Reported rates are typically very low relative to rates of inorganic nitrogen uptake, although urea

*E-mail: margie@cbl.umces.edu

**Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

uptake can be a major contributor to phytoplankton nitrogen nutrition seasonally in coastal and estuarine regions (Glibert et al. 1991).

Isotopic methods are available for estimating cross-membrane transport and incorporation of labeled compounds into particulate organic material or biomass. Due to the experimental and analytical ease of measuring incorporation of radioactive label into particulate material, ^{14}C and ^3H labeled organic substrates have been commonly used to assess uptake rates of organic compounds by bacterial and algal cells. These measurements have been considered as direct measures of nitrogen uptake based on the assumption that the entire compound is taken up by cells. Less commonly, ^{15}N and ^{13}N labeled substrates have been used to measure uptake or metabolism of organic substrates (Schell 1974, Wheeler & Kirchman 1986); however, due to methodological and analytical complexities, these studies are more limited.

Recently an extracellular mechanism for scavenging nitrogen from organic substrates has been observed in a wide variety of taxonomically diverse cultured algal cells and in size-fractionated natural microbial populations (Palenik et al. 1989, Palenik & Morel 1990a, b, Pantoja & Lee 1994). Cell surface enzymes such as alkaline phosphatases, responsible for the regeneration of organic phosphorus, have been observed in a variety of algal and bacterial taxa (Hoppe 1983, Ammerman 1991, Chróst 1991). Extracellular deamination of amino acids is also associated with cell surface amino acid oxidases rather than with enzymes liberated into the environment or with other inorganic deaminating processes (Palenik et al. 1989, Pantoja & Lee 1994). Amino acid oxidation results in the liberation of equimolar concentrations of NH_4^+ , hydrogen peroxide (H_2O_2) and a deaminated organic acid. The NH_4^+ released is then available for uptake by microorganisms.

The observation that H_2O_2 was produced by phytoplankton and natural assemblages in the Sargasso Sea (Palenik et al. 1987, Palenik & Morel 1988) led to the investigation of the occurrence of amino acid oxidases in marine phytoplankton (Palenik & Morel 1990a, b). Earlier observations of H_2O_2 production by cyanobacteria grown in culture did not suggest the process responsible for this phenomenon (Stevens et al. 1973). Methodological constraints limit the widespread assessment of amino acid oxidase activity in natural populations by measuring product formation (e.g. H_2O_2 , NH_4^+ , or keto and hydroxy acids). In natural aquatic systems H_2O_2 and other products of amino acid oxidation are produced and consumed in a variety of organic and inorganic reactions, and this rapid cycling confounds estimates of any particular pathway. To overcome these problems, Pantoja et al. (1993) synthesized

a more generally applicable fluorescent amino acid analog, Lucifer Yellow anhydride-labeled lysine (LYA-lysine), amenable to measuring amino acid oxidase activity in natural waters. These investigators verified that the compound was not taken up by cells, that products could be separated using high performance liquid chromatography (HPLC) at concentrations relevant to aquatic ecosystems, and that the specific compound was generally applicable for measuring enzyme activity. Pantoja & Lee (1994) went on to show that amino acid oxidase activity can be an important pathway of nitrogen acquisition in natural populations of phytoplankton and bacteria-size organisms in Long Island Sound, New York (USA). By comparing rates of lysine oxidation with uptake rates of ^{14}C -labeled amino acids, they determined that oxidation rates could be as much as 40% of the total removal rate of amino acids.

Here we report on the results of studies of amino acid oxidase activity, measured using LYA-lysine, from a variety of ecosystems, ranging from eutrophic to oligotrophic and dominated by a range of phytoplankton populations, including diatoms, chrysophytes and cyanobacteria. In some of these studies, we compared the rate of LYA-lysine oxidation with the rate of NH_4^+ uptake, as measured using stable isotope ($^{15}\text{NH}_4^+$) techniques, to estimate the relative contribution of amino-acid-oxidase-derived NH_4^+ to total NH_4^+ uptake. Size-fractionated incubations were conducted at some of the study sites to determine the distribution of amino acid oxidase activity between the bacterial size-fraction and the size-fraction dominated by phytoplankton-sized organisms.

METHODS

Overview of study sites and experimental design.

We measured rates of amino acid oxidase activity in 4 natural systems (Fig. 1), including 2 coastal Atlantic estuaries, coastal waters off Brazil, and oligotrophic waters of the Caribbean Sea, as well as in estuarine mesocosms. The estuarine sites included Shinnecock Bay, one of the bar-built estuaries along the south shore of Long Island, New York, USA, and the Chesapeake Bay, Maryland, USA, at a mid-Bay station. Water depths at these sites were about 6 and 21 m, respectively. Samples from Shinnecock Bay were collected during a 'brown tide' event, caused by the chrysophyte *Aureococcus anophagefferens* (Cosper et al. 1987, Dzurica et al. 1989). Further description of Shinnecock Bay and the brown tide can be found in Lomas et al. (1996) and Berg et al. (1997). The Chesapeake Bay study was conducted during an autumnal diatom bloom. The coastal site off Brazil was located in an island channel near Ubatuba, south of Rio de

Fig. 1. Map of study sites with expanded views of Shinnecock Bay, Long Island Sound, NY, USA, the Chesapeake Bay, USA, the western N. Atlantic and eastern Caribbean Sea, and the Brazilian coastal region

Janeiro in about 25 m of water. Further description of the site and the phytoplankton and water column dynamics can be found in Metzler et al. (1997). Oligotrophic sites were sampled during a cruise to the west-

ern North Atlantic (Bahamas) and eastern Caribbean Sea (henceforth, for brevity, we refer to all sites from this cruise as E. Caribbean). Cyanobacteria, *Trichodesmium* spp., were found in 3 study sites, Brazilian coastal waters, the Bahamian waters of the Atlantic Ocean and the Caribbean Sea near St. Martin. A summary of dates of sample collection are given in Table 1. Details of the protocols at each site are given below.

Abiotic control incubations were done in parallel with experiments in the Caribbean Sea and separately in Sargasso seawater incubated at the Chesapeake Biological Laboratory to verify that there was no abiotic oxidation of the substrate. The amount of the LYA-lysine added to incubations was initially 30 nM in the Chesapeake Bay experiments. This addition was selected based on previous experience. However, since we saw no oxidation there, higher amounts were used for subsequent experiments. These later experiments were all carried out before the samples were analyzed, and before it was determined that less substrate could be used. Thus, all substrates were added at saturating rather than tracer levels. Even though any concentration of LYA-lysine could be considered saturating, since this compound is not found in nature, many amino acids compete

with it for enzyme active sites (see Pantoja et al. 1993, Pantoja & Lee 1994). Incubations that included *Trichodesmium* spp., or that were from environments where *Trichodesmium* spp. were abundant, were inoc-

Table 1. Summary of sampling dates and some environmental parameters. LYA-lysine: Lucifer Yellow anhydride-labeled lysine

Study area	Sampling dates	Vessel used	Mean water temp. (°C)	General comments
Chesapeake Bay	21–22 Oct 1994	RV 'Cape Henlopen'	17.5	Sampled just after destratification event
Shinnecock Bay	24–26 Jul 1995	Small boat	18–18.5	Sampled during 'brown tide' event
East Caribbean	3–25 Jan 1995	RV 'Seward Johnson'	26.5	Oligotrophic waters with accumulations of <i>Trichodesmium</i> spp.
Brazilian coastal waters	1–6 Dec 1995	RB 'Albacore'	25–26	Intrusion of nutrient-rich cooler water on 5 Dec, surface slicks of <i>Trichodesmium</i> spp.
Mesocosm tanks	20–31 Oct 1994		22–23	Inorganic N depletion prior to LYA-lysine sampling
Mesocosm tanks	1–10 Sep 1995		22–23	Inorganic N depletion; high algal biomass

ulated with larger additions based on higher estimated cell biomass per incubation and estimates of amino acid and DON cycling in *Trichodesmium*-dominated communities (Capone et al. 1994, Glibert & Bronk 1994).

All size-fractionations were performed using gentle filtration (<125 mm Hg) through Nucleopore filters. The LYA-lysine used in all of the studies reported here was synthesized as reported by Pantoja et al. (1993). All subsamples withdrawn for measurements of LYA-lysine oxidation were extracted using clean syringes and then filtered through 0.2 μ m Acrodisc filters. The filtrates were frozen at -20°C until analysis. Uptake rates of nitrogen were estimated using highly enriched ^{15}N substrates ($\sim 99\%$) (Glibert & Capone 1993). Samples for ^{15}N isotopic analysis and particulate nitrogen (PN) analysis were collected by gentle filtration onto precombusted (450°C for 2 h) GF/F filters and frozen until analysis. Filtrates were collected and frozen (-20°C) for nutrient analyses.

All sample incubations (LYA-lysine and ^{15}N) were done in acid-cleaned polycarbonate bottles. Neutral density screening was used to simulate the light level at collection depths during field incubations. Deck incubators with flow-through seawater were used to maintain temperature.

Site-specific experimental techniques. Chesapeake Bay: Water was collected at several times of day from the surface (2 m) and near the bottom (20 m) in Niskin bottles during CTD casts. All water was shaded from direct sunlight during transfers and manipulations. Aliquots of the sampled water were placed directly into incubation bottles while a portion of the sample was filtered (1 μ m) and measured into incubation bottles. Bottles were then inoculated with 30 nM LYA-lysine. Samples were withdrawn from the incubation bottles using a clean syringe over a 2 to 16 h time-course. Uptake rates of $^{15}\text{NH}_4^+$, $^{15}\text{NO}_2^-$, $^{15}\text{NO}_3^-$, and ^{15}N -urea on samples collected from the same station and depth several days before the LYA-lysine experiments were conducted using trace additions (10 to 20 atom % enrichment) of the ^{15}N substrates. Incubations were under the same conditions as described above. The duration of the incubations for these ^{15}N uptake measurements was less than 1 h.

Shinnecock Bay: Samples were collected from just below the surface using a hand-held water bottle, and returned to the dock in a darkened carboy. Whole water was prescreened through a 10 μ m Nitex screen, dispensed into 50 ml incubation bottles and inoculated with 60 nM LYA-lysine. A time zero sample was extracted immediately and subsamples were withdrawn over a 4 h time course.

Samples for ^{15}N uptake were also prescreened and dispensed into 50 ml bottles. They were inoculated

with 10 μM concentrations (saturating) of $^{15}\text{NH}_4^+$, $^{15}\text{NO}_3^-$, ^{15}N -urea or ^{15}N -lysine. Subsamples were withdrawn at time intervals between 0 and 60 min. Samples for PN and dissolved nutrients were also collected.

Eastern Caribbean: A first set of incubation studies was conducted on size-fractionated samples. Water was collected in Niskin bottles during CTD casts at 3 depths, twice during the daylight and twice at night. Depths were selected based on the location of the fluorescence maximum as determined from a CTD cast: above the fluorescence maximum (25 to 35 m), near the top of the fluorescence maximum (65 to 75 m), and near the bottom of the fluorescence maximum (90 to 95 m). Whole, <1.0, <0.7, or <0.2 μ m water (50 ml) was prepared (see above), and incubation bottles were inoculated with 150 nM LYA-lysine. Subsamples from each incubation were collected along a 2 to 16 h time-course. For the E. Caribbean, no parallel ^{15}N measurements were made.

A second set of incubations was conducted to determine the effect of the cyanobacteria *Trichodesmium* spp. on LYA-lysine oxidation. *Trichodesmium* spp. were concentrated in a 202 μ m mesh plankton net that was towed at 15 to 20 m depth for up to 10 min at speeds of <1 knot. Water for the incubations was collected in Niskin bottles from the same depth and was size-fractionated (<1.0, <0.7 or <0.2 μ m) and measured into incubation bottles. Twenty *Trichodesmium* spp. (*T. erythraeum* and *T. thiebautii*) colonies (estimated to be 10 μg -atom PN) were added to half of the bottles for each size fraction using an inoculating loop. Incubations were inoculated with 150 nM LYA-lysine to initiate experiments and a time zero sample withdrawn immediately. Samples were placed in deck incubators (see above) and aliquots were extracted over a 16 h time course.

Brazilian coastal waters: Three types of experiments were performed. First, on 3 occasions, samples were collected from near surface (4 m) and near bottom (30 m) using Nansen bottles. Samples (unfractionated) were transferred to incubation bottles and 150 nM LYA-lysine added. Samples were incubated in bags made from neutral density screening, simulating the original light level, that were hung over the side of the vessel. Subsamples were removed over a 4 to 8 h period.

In a second experiment, water collected near the surface (4 m) on 3 occasions was size-fractionated and the <1.0 μ m water transferred into 3 incubation bottles. One bottle received a 1.0 μM glucose addition; another received a 1.0 μM amino acid addition (commercial amino acid mixture, Pierce 20089); and the third bottle received no addition (control). These bottles were incubated in neutral density screen bags placed in the surface water. After 5 h, an aliquot from each treat-

ment bottle was dispensed into an incubation bottle, inoculated with 150 nM LYA-lysine, and a time zero sample was withdrawn. Bottles were replaced in the screen bags and samples were withdrawn over a 4 to 8 h period.

The third experiment was to determine the effect of *Trichodesmium* spp. on rates of LYA-lysine oxidation. Dense slicks of *Trichodesmium* spp. (primarily *T. erythraeum* and *T. thiebautii*) were observed at the water surface during the study period. Cells were collected using clean buckets, and a slurry of cells was added to incubation bottles filled with preparations of <1.0 µm surface water and inoculated with 150 nM LYA-lysine. Oxidation rates were determined as above over a 4 h period. Chlorophyll was measured to determine the total biomass added to the incubation bottles since the aggregations were too dense to count individual colonies.

Uptake of $^{15}\text{NH}_4^+$ was measured in parallel by inoculating additional incubation bottles with tracer concentrations (i.e. 10% of the ambient NH_4^+ concentration, or 0.03 µM if ambient concentrations of NH_4^+ were at the limit of detection) of $^{15}\text{NH}_4^+$ in parallel incubations. Incubations were approximately 1 h in duration.

Estuarine mesocosms: Indoor mesocosms of differing size and shape were sampled during October 1994 and September 1995. The mesocosms are part of the Multiscale Experimental Ecosystem Research Center (MEERC), located at the Horn Point Environmental Laboratory on the eastern shore of Chesapeake Bay. The dimensions and environmental conditions of the mesocosms are given in Table 2.

At the beginning of each experiment, the mesocosms were filled with unfiltered water from the Choptank River, a tributary of the Chesapeake Bay. Subsequently, water in the mesocosms was exchanged at a dilution rate of 10% of mesocosm volume per day, using filtered (<2.0 µm) Choptank River water. The experiment was run under a 12 h light:12 h dark cycle, with light provided by a combination of cool-white fluorescent bulbs and incandescent lights. Mesocosms were well mixed with paddle stirrers during the experiment. The mixing time (t_{mix}) was adjusted to achieve a vertically homogenous distribution of dissolved properties (Table 2).

During the October experiment, samples were removed to measure rates of LYA-lysine oxidation on Days 1, 3, 6, and 10. During the following September, samples were removed for LYA-lysine oxidation experiments on Days 5, 12, 14, and 16. The environmental conditions, light, temperature, and inorganic nitrogen, were similar in both years. In both studies, water was siphoned from the tanks into incubation bottles. A 60 nM LYA-lysine addition was made to each bottle, and subsamples were extracted along a 4 h time course to determine rates of LYA-lysine oxidation. Rates of NH_4^+ uptake were measured in parallel experiments using whole water collected at the same time. Tracer additions of $^{15}\text{NH}_4^+$ were equivalent to 10% of the ambient concentrations in the tanks. Incubations were 1 h in duration.

Analytical techniques. Nutrient and biomass measurements: Samples were collected from all sites to characterize ambient inorganic and organic nitrogen concentrations, and biomass. Inorganic nitrogen concentrations were determined on samples filtered through GF/F filters, either by Technicon Auto-Analyzer (mesocosm and Caribbean Sea), or manual analyses (Shinnecock Bay, Chesapeake Bay and Brazil coastal waters: Parsons et al. 1984). Concentrations of DON were determined using persulfate oxidation (Valderrama 1981). Chlorophyll concentrations were determined by fluorometry (Parsons et al. 1984), and PN concentrations were determined using an elemental analyzer.

^{15}N analysis: Filters were prepared for mass spectrometry following a protocol similar to that described by Fiedler & Proksch (1975). The mass spectrometer used was a Nuclide 3 inch 60 sector analyzer with dual mass collection, as described in Glibert et al. (1991). Calculations of uptake rates were according to Glibert & Capone (1993).

LYA-lysine analysis: LYA-lysine and its oxidation products were separated and quantified using HPLC as described by Pantoja et al. (1993). Solvent A was 0.05 M KH_2PO_4 , pH 4.5; solvent B was either 50:50 solvent A:acetonitrile or 50:50 deionized water:acetonitrile. The solvent gradient and flow rate varied depending on the HPLC system and analytical column used. The following configurations were used for

Table 2. Estuarine mesocosm dimensions. Experiments conducted during October 1994 used Tanks D and E, whereas experiments conducted in September 1995 used Tank C. t_{mix} : mixing time; I_0 : light intensity

Tank	Volume (m ³)	Depth (m)	Radius (m)	Surface area:volume	t_{mix} (min)	Temperature (°C)	I_0 (µE m ⁻² s ⁻¹)
C	1	1.0	0.57	1.00	20	23 ± 0.4	150
D	10	2.15	1.22	2.15	68	23 ± 0.1	150
E	10	1.0	1.78	1.00	26	22 ± 0.3	150

analyses: either an Alltech Adsorbosphere 250 × 4.6 mm, 5 µm, C-18 column or Waters Novapac 150 × 3.9 mm, 4 µm, C-18 column was used with a Waters HPLC system at the Chesapeake Biological Laboratory, and a Waters µBondapak 250 × 4.6 mm, 10 µm, C-18 column was used with either a Waters or a Hewlett Packard HPLC system at Horn Point Laboratory. The detection limit for these analytical conditions was less than 1.0 pmol at a signal:noise ratio of 2.

Oxidation of LYA-lysine was verified using commercially available amino acid oxidase (AAO) purchased from Sigma (#A9253). Distilled water and filtered seawater were inoculated with LYA-lysine and 2 units of AAO, and the disappearance of LYA-lysine and appearance of LYA-labeled products were monitored over time. Reproducibility and sample stability were established by repeat injections, and quality control standards were run to determine changes in response and retention time over time. Amino acid oxidase activity was determined by measuring the decrease in LYA-lysine over time. While oxidation products were not quantified, increases in peak height and area were noted in conjunction with the decrease in the LYA-lysine parent compound.

RESULTS

Abiotic controls

No LYA-lysine oxidation was found in abiotic controls conducted on 2 separate occasions. Previous investigation by Pantoja et al. (1993) also showed no abiotic oxidation.

Chesapeake Bay

Our study in Chesapeake Bay was conducted following the fall diatom bloom. Total dissolved nitrogen was 20 to 30 µM during the study period. Phytoplankton depended primarily on NH_4^+ for their nitrogen nutrition (data not included), which is typical for this time of year in the Chesapeake Bay (Bronk et al. 1998, in this issue). No oxidation of LYA-lysine was found in any sample collected during this period from mid Chesapeake Bay.

Shinnecock Bay

Shinnecock Bay was experiencing a 'brown tide' event when these samples were collected (Lomas et al. 1996). Concentrations of P_N averaged 20 µM. Combined inorganic nitrogen concentrations were <1 µM;

NH_4^+ constituted >80% of the inorganic nitrogen pool, with concentrations of 0.45 to 0.57 µM. Concentrations of DON were about 3.5 µM. Chlorophyll levels averaged 39 µg l⁻¹ during this time.

Using experimentally determined kinetic coefficients (V_{max} and K_s ; Lomas et al. 1996), ambient rates of nitrogen uptake were determined to be 2.9 µM h⁻¹ for urea, 1.5 µM h⁻¹ for NH_4^+ , 0.03 µM h⁻¹ for NO_3^- , and 0.1 µM h⁻¹ for lysine. The mean oxidation rate of LYA-lysine was 13.2 nM h⁻¹, while the maximum rate was 42 nM h⁻¹. These results agree well with those of Pantoja & Lee (1994) from Long Island Sound. The oxidation of LYA-lysine represented ~1% of the $^{15}\text{NH}_4^+$ uptake rate and ~14% of the ^{15}N -lysine uptake.

Eastern Caribbean

The E. Caribbean during January 1995 had water quality characteristics indicative of oligotrophic conditions: ambient inorganic concentrations were below the limits of detection for standard wet chemistry, and chlorophyll concentrations were below 0.1 µg l⁻¹. Under these conditions phytoplankton are generally thought to depend primarily on regenerated nitrogen for growth.

While results of LYA-lysine oxidation experiments were quite varied, several overall patterns emerged. Rates of LYA-lysine oxidation measured in whole water samples showed little change with depth during the day, but at night, values in the upper water column were much lower (Fig. 2). No measurable activity was detected in samples from <1.0 µm size fractions (Table 3). Oxidation rates in unfractionated samples ranged from 0 to 13.5 nM h⁻¹ (mean 2.7 nM h⁻¹). Highest rates overall were observed for samples to which 20 colonies of *Trichodesmium* spp. were added (Table 3). Using values for NH_4^+ previously determined for the E. Caribbean for the same time of year (P. Gilbert unpubl. data), the mean oxidation of LYA-lysine determined here would represent 5 to 20% of the NH_4^+ uptake rate.

Brazilian coastal waters

In the Brazilian coastal region, ambient concentrations of inorganic nitrogen were higher than those found in the oligotrophic E. Caribbean. Concentrations of NO_3^- ranged from 0.08 to 0.26 µM. Concentrations of NH_4^+ ranged from 0.46 to 1.05 µM in the near-surface waters, with slightly higher concentrations near the bottom (30 m). During the sampling period, an intrusion of cooler, nutrient-rich South Atlantic water was observed (Metzler et al. 1997). The <1 µm size fraction

Fig. 2. Oxidation rates of LYA-lysine (Lucifer Yellow anhydride-labelled lysine; nM h^{-1}) as a function of depth for samples collected from the Eastern Caribbean Sea (A) during the day and (B) during the night

contained <20% of the total chlorophyll before the intrusion, but this fraction increased to about 35% after the intrusion and subsequent stratification of the water column. This was due to an increase in <1 μm phytoplankton, most likely *Synechococcus* sp. (P. M. Metzler, P. M. Glibert, S. A. Gaasta & J. M. Ludlam unpubl.). Total chlorophyll concentrations ranged from 0.21 to 0.32 $\mu\text{g l}^{-1}$ in surface waters and from 1.36 to 1.44 $\mu\text{g l}^{-1}$ at 30 m. Large but localized aggregations of *Trichodesmium* spp. were observed during the study. The highest rates of oxidation of LYA-lysine were found to occur on the 2 sampling days prior to the nutrient intrusion (Fig. 3). Rates of LYA-lysine oxidation were consistently higher at 30 m than near the surface and ranged from 1 to 6% of the $^{15}\text{NH}_4^+$ uptake rate in water collected from 30 m depth. After the intrusion and subsequent stratification, rates were undetectable in surface waters.

Higher rates of LYA-lysine oxidation were found in samples containing *Trichodesmium* spp. than whole water samples without *Trichodesmium* spp. (Table 3). No concurrent measurements were made of NH_4^+ uptake on the samples containing *Trichodesmium* spp., although it has previously been shown that *Trichodesmium* spp. have very low rates of NH_4^+ uptake, and depend primarily on nitrogen fixation to obtain their required nitrogen (Carpenter & McCarthy 1975, Glibert & Banahan 1988).

Rates of LYA-lysine oxidation were also measured in 1 μm -filtered Brazilian surface water amended with glucose and an amino acid mixture (Fig. 4). Rates of LYA-lysine oxidation in the 1 μm -filtered control samples were undetectable. However, rates increased substantially after the addition of each substrate (Fig. 4). Uptake

Fig. 3. Oxidation rates of LYA-lysine (nM h^{-1}) for samples collected in Brazilian coastal waters. Light bars represent samples collected near surface (4 m) and dark bars represent samples collected at 30 m. Nutrient enrichment of the water column and stratification occurred between December 2 and 6. Circles super-imposed on the bar graph are the percent contribution of LYA-lysine oxidation relative to $^{15}\text{NH}_4^+$ uptake

of $^{15}\text{NH}_4^+$ did not vary much between treatments and the controls. Rates ranged from 0.38 to 0.80 $\mu\text{M h}^{-1}$ in the controls, 0.10 to 0.79 $\mu\text{M h}^{-1}$ in glucose-amended samples, and 0.15 to 0.55 $\mu\text{M h}^{-1}$ in amino acid-amended samples. Rates of NH_4^+ uptake were lowest in the experiments conducted after the onset of stratification. Rates of LYA-lysine oxidation were <1% of the measured NH_4^+ uptake in all experiments before stratification, but increased to 2–6% of NH_4^+ uptake in the glucose- and amino acid-amended samples after the nutrient intrusion and stratification.

Estuarine mesocosms

During the October 1994 mesocosm study, which was conducted concurrently with the October Chesapeake Bay sampling, nutrient and biomass concentra-

Table 3. Oxidation rates of LYA-lysine for whole water or size-fractionated samples and samples to which colonies of *Trichodesmium* spp. were added. In the E. Caribbean, 20 colonies of *Trichodesmium* spp. were added per sample; in coastal Brazil, a slurry from a near-surface slick of *Trichodesmium* spp. was added (see 'Methods'). Number in parentheses is the number of measurements made per experimental treatment (not replicates). ND: LYA-lysine oxidation was not detected

Location	Size-fraction	LYA-lysine oxidation rate (nM h^{-1})			
		without <i>Trichodesmium</i> spp.		with <i>Trichodesmium</i> spp.	
E. Caribbean	<0.2 μm	ND	(4)	ND–2.7	(4)
E. Caribbean	<0.7 μm	ND	(1)	10.0–20.0	(2)
E. Caribbean	<1.0 μm	ND	(4)	ND–14.6	(6)
E. Caribbean	Whole water	ND–13.5	(5)	ND–19.4	(6)
Coastal Brazil	Whole water	ND–7.8	(12)	2.8–12.7	(4)

Fig. 4. Oxidation rates of LYA-lysine (nM h^{-1}) for samples collected from Brazilian coastal waters, filtered through $1.0 \mu\text{m}$ filters, and amended as indicated. Samples were collected (A) before water column stratification and (B) after water column stratification. Circles super-imposed on the bar graph are the percent contribution of LYA-lysine oxidation relative to $^{15}\text{NH}_4^+$ uptake

tions within the tanks were quite different from those in the Bay, probably because the indoor facility was maintained at a temperature that was considerably warmer than the natural environment (Table 2). In both mesocosms sampled, there was a decrease in total ambient inorganic nitrogen concentrations, from $0.05 \mu\text{M}$ (Tank D) and $0.43 \mu\text{M}$ (Tank E) to below the analytical limit of detection over the days that we sampled. During this same period, there was also a marked decrease in the rate of NH_4^+ uptake (data not shown). Concurrently, the rate of LYA-lysine oxidation in the tanks increased to a level that was 7 (Tank D) and 4 (Tank E) times higher than the initial values (Fig. 5A, B). The contribution of extracellular oxidation to total NH_4^+ utilization increased over time to a high of 5% in both tanks by the time the experiment was terminated (Fig. 5A, B).

In the September 1995 mesocosm study, ambient NH_4^+ concentrations were consistently near the analytical limit of detection for wet chemistry, while concentrations of DON were $\sim 10 \mu\text{M}$. Rates of LYA-oxidation and NH_4^+ uptake averaged ~ 5 -fold higher than those measured in the October study (Fig. 5C). Consistent with that study, rates of LYA-oxidation increased over the course of the experiment, and represented from <1 to 3.5% of the rate of NH_4^+ uptake (Fig. 5C).

Fig. 5. Oxidation rates of LYA-lysine (nM h^{-1}) for samples collected during the mesocosm experiments conducted (A, B) in October 1994, and (C) in September 1995. (A) Results from Tank D; (B) results from Tank E. Circles super-imposed on the bar graph are the percent contribution of LYA-lysine oxidation relative to $^{15}\text{NH}_4^+$ uptake

DISCUSSION

Although amino acid oxidases have been identified in a variety of taxa, investigations examining the importance of this group of enzymes in the acquisition of growth-limiting nitrogen in natural aquatic systems are limited. Consequently, it is not known how widespread the occurrence of extracellular oxidation of amino acids is in marine or freshwater ecosystems, what the environmental conditions are that affect the synthesis and activity of extracellular amino acid oxidases, and what the capacity of cells *in situ* is for using this pathway to acquire cell nitrogen. Our results are discussed below relative to environmental parameters, community microbial composition and utilization of nitrogen from various sources.

Relationships of oxidation to environmental parameters

Pantoja & Lee (1994) found that amino acid oxidation rates occurred only above 20°C . Our findings in

Chesapeake Bay are not inconsistent with this observation; no activity was found in the Chesapeake Bay waters where temperatures were 17 to 18°C. Measurable rates of amino acid oxidation were found in the E. Caribbean Sea and Brazilian coastal waters, as well as our mesocosm studies, consistent with this pattern; temperatures for all of these studies were in excess of 22°C. Amino acid oxidase activity occurred during a brown tide in Shinnecock Bay when water temperatures were 18 to 18.5°C. Furthermore, Palenik & Morel (1990a, b) found activity in cultures maintained at about 20°C. More data are clearly needed from a variety of assemblages growing at lower temperatures before the effect of temperature can be clearly elucidated for a broad spectrum of phytoplankton communities.

We measured LYA-lysine oxidation at a variety of depths within the euphotic zone at several sites to determine whether activity might be affected by light or position in the water column. Muñoz-Blanco et al. (1990) previously observed that amino acid oxidase activity in *Chlamydomonas reinhardtii* decreased at low light levels. Our results from the E. Caribbean and coastal Brazil revealed no consistent pattern with depth in the water column. However, activity in the surface waters in the E. Caribbean went down at night suggesting a possible link to photosynthesis. The distribution with depth may also reflect changes in the community composition with depth, changes in the distribution of cells over a diel cycle, or other interactive factors not yet fully understood.

Amino acid oxidase activity appeared to be related to ambient inorganic nitrogen concentrations. Ambient inorganic nitrogen levels were low in the oligotrophic ocean sites and in Brazilian coastal waters prior to stratification when amino acid oxidase activity was observed. Consistently, in the mesocosm tanks, amino acid oxidase activity increased as the inorganic nutrient supply was exhausted. Palenik & Morel (1990a) also found an increase in phytoplankton amino acid oxidase activity in cultures depleted in NH_4^+ . Summarizing data from all the sites studied here, it appears that the lowest range in LYA-lysine oxidation rates were from more oligotrophic sites while higher rates were associated with estuarine and coastal sites (Fig. 6). At low NH_4^+ concentrations the relative importance of labile DON as a nitrogen source may be greater. Thus, in low NH_4^+ areas, higher observed LYA-lysine oxidation rates may occur. In mesocosm tanks, a direct relationship between decreasing dissolved inorganic nitrogen (DIN), increasing DON and increasing LYA-lysine oxidation was demonstrated. In coastal Brazil before and after the cold-water intrusion this effect was also observable. However, for oligotrophic areas, low levels of

Fig. 6. LYA-lysine oxidation rates expressed in nM h^{-1} by study site. Bars represent the entire range of values reported for each site. Long Island Sound data include values reported by Pantoja & Lee (1994)

nutrients and rapid recycling of labile components precludes the accumulation of nutrients, and this may mask correlations between nutrient concentrations and amino acid oxidase activity. Measurements made over a range of DIN and DON concentrations are necessary to determine whether a correlation can be made between DON and NH_4^+ concentrations and amino acid oxidase activity.

High rates of amino acid release and high concentrations of amino acids in the microenvironment created by *Trichodesmium* spp. colonies have been reported (Capone et al. 1994, Glibert & Bronk 1994). In our experiments, higher rates of LYA-lysine oxidation were associated with samples containing *Trichodesmium* spp., consistent with higher substrate levels. In addition, higher substrate concentrations may induce amino acid oxidase synthesis or activity, while high concentrations of NH_4^+ may inhibit activity. This sort of environmental regulatory mechanism has been implicated for the synthesis and activity of other ectoenzymes (Chróst 1991).

Experiments conducted using $<1 \mu\text{m}$ filtered Brazilian surface waters showed that glucose and amino acid additions stimulated amino acid oxidase activities relative to controls. No biomass measurements were made to determine whether this effect was due to stimulation of bacterial growth in amended samples. However, incubations were fairly short. Regulation of amino acid oxidase activity has been examined in *Neurospora crassa* (Sikora & Marzluf 1982, Prade & Terenzi 1985). These authors found nitrogen derepression and induction by amino acids to be necessary for enzyme activity. These authors also noted a requirement for organic carbon in the media. In our nutrient amendment experiments, both induction of amino acid oxidase by added amino acids and carbon limitation of microbial growth were possible.

Relationship of oxidation to microbial composition

Palenik & Morel (1990a, b) found no amino acid oxidase activity associated with the diatom *Thalassiosira weissflogii*. Consistently, in the Chesapeake Bay during the autumnal diatom bloom, we found little or no activity. Since water temperatures were also low, we were unable to determine whether the absence of activity was a temperature effect or whether the community lacked the capacity for amino acid oxidation. Diatom communities characteristically thrive in environments when water temperatures are low and NO_3^- concentrations are high and, therefore, may not gain any competitive advantage from synthesizing and using this pathway for nitrogen acquisition.

We detected amino acid oxidase activity during a bloom of *Aureococcus anophagefferens*. This organism grows well in organic-rich media (Dzurica et al. 1989), and short-term uptake measurements with 6 nitrogen substrates (NH_4^+ , NO_3^- , urea, lysine, glutamic acid, and algal extract) have shown that reduced nitrogen represents up to 95% of overall nitrogen uptake, with 70% of this due to organic nitrogen (Berg et al. 1997). A high capacity for utilization of organic nitrogen may allow this organism to outcompete other species, leading to complete dominance in the assemblage. Our data suggest that rates of direct uptake of amino acids are greater than rates of LYA-lysine oxidation. However, further studies are necessary to determine whether our rates reflect the full range of the physiological capability of *A. anophagefferens* for cell surface amino acid oxidation.

Unlike Pantoja & Lee (1994) for Long Island Sound, we found no measurable activity in the bacterial size-fraction in natural samples in the E. Caribbean or in Brazilian waters not amended with glucose or the amino acid mixture. We did find activity in $<1.0 \mu\text{m}$ samples when incubated with amino acids or glucose for a 5 h period. As mentioned above, induction by amino acids may be necessary for activity in some species.

We found high rates of amino acid oxidase activity associated with the cyanobacteria *Trichodesmium* spp. *Trichodesmium* colonies provide microhabitats that may host a variety of microorganisms including other cyanobacterial genera, such as *Synechococcus*, as well as bacteria, phytoplankton and zooplankton (Paerl et al. 1989, Sellner 1992). *Synechococcus* has intracellular amino acid oxidases (Meyer & Pistorius 1989, Engels et al. 1992); indirect evidence for extracellular activity in *Synechococcus* and other cyanobacteria is that they produce H_2O_2 in culture (Stevens et al. 1973). We were unable to determine from our investigations whether *Trichodesmium* itself or some other organism living in close association with colonies was responsible for the measured rates of amino acid oxidase activity. How-

ever, it is likely that in these organic-rich microhabitats, a number of consortial members might be adapted to scavenge organic nitrogen by cell surface oxidation. *Trichodesmium* has been shown to release up to 50% of recently fixed N_2 as organic nitrogen, primarily as glutamate and glutamine (Capone et al. 1994, Glibert & Bronk 1994). In *Trichodesmium*-dominated communities, DON may be a primary source of nitrogen available both in the microenvironment provided by colonies themselves and for the ecosystem in general.

At the Brazilian site, where high activity was observed on a number of days in samples amended with amino acids and glucose, a significant fraction of the chlorophyll was found in the $<1 \mu\text{m}$ size fraction. *Synechococcus* or some other cyanobacteria or prochlorophytes were likely the dominant organisms in this size fraction. Palenik & Morel (1990a, b) did not examine cyanobacteria for the presence of amino acid oxidases in their culture studies; however, based on our findings and the results of Engels et al. (1992) and Stevens et al. (1973), cyanobacteria may have the capacity for extracellular amino acid oxidase activity.

Contribution of amino acid oxidation to NH_4^+ regeneration and uptake

Our studies suggest that extracellular amino acid oxidation may be a small but important pathway of NH_4^+ regeneration and uptake in oligotrophic and low-nutrient systems enriched in labile DON. Bronk et al. (1994) reported that DON release from phytoplankton averaged between 25 and 41% of DIN uptake (NH_4^+ and NO_3^-). These authors estimated NH_4^+ uptake rates for the Caribbean Sea at 20 to 30 nM h^{-1} and DON release rates of 7.8 to 13 nM h^{-1} . In this oligotrophic site, amino acid oxidation rates as measured by LYA-lysine oxidation ranged from 0 to 15 nM h^{-1} . Thus, cell surface oxidation of amino acids was occurring at rates similar to the previously reported rates of DON release and at rates 5 to 20% of NH_4^+ uptake rates. Rates of LYA-lysine oxidation measured in the oligotrophic E. Caribbean Sea were low relative to rates observed in more productive coastal waters. However, because total DIN concentrations and uptake rates are also lower in the Caribbean, the relative contribution of this nitrogen acquisition pathway may be higher than for more eutrophic areas. LYA-lysine oxidation contributed 0 to 6% of the NH_4^+ uptake at other sites studied.

Summary

We have shown that amino acid oxidase activity is widespread across a variety of coastal and oceanic

ecosystems. Under low inorganic nutrient conditions amino acid oxidation appears to represent a potentially significant, and usually ignored, source of nitrogen for phytoplankton and cyanobacteria. This adds further support to the growing recognition that labile DON is cycled rapidly (Antia et al. 1991, Bronk et al. 1994). Failure to consider this mechanism of DON utilization can result in significant (up to 20%) underestimates of DON utilization and nutrient recycling in general. Future studies are needed to address the specific nature of this group of enzymes in order to generate an understanding of how specific these enzymes are for particular organic compounds, how they are regulated, and how they function relative to the heterogeneous DON pools characteristic of natural systems.

Acknowledgements. We thank J. Zehr and D. G. Capone for ship time aboard the RV 'Seward Johnson' in the Caribbean Sea and Atlantic Ocean and SA 'Gaeta' for ship time in the coastal Brazil region. Ship time in the Chesapeake Bay was funded through the NSF Land Margin Ecosystem Research program (BSR 88-14272). We also thank M. Lomas, J. Ludlam and P. Metzler for help in collecting and analyzing samples and D. Kennedy for graphics. This work was supported by NSF grants to P.M.G. (INT-9314610 and OCE-9521254) and D. G. Capone (OCE-9314252; DEB-9633510), and by the EPA Multiscale Ecosystem Experimental Research Center Project (R819640). LC Resources, Inc. provided the DryLab software for the HPLC system. This is contribution no. 3069 from the University of Maryland Center for Environmental Science and 1086 from the Marine Sciences Research Center, SUNY Stony Brook.

LITERATURE CITED

- Ammerman JW (1991) Role of ecto-phosphohydrolases in phosphorus regeneration in estuarine and coastal ecosystems. In: Chróst R (ed) *Microbial enzymes in aquatic environments*. Springer, New York, p 165–186
- Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology, and ecology. *Phycologia* 30:1–89
- Berg GM, Glibert PM, Lomas MW, Burford M (1997) Organic nitrogen uptake and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar Biol* 129:377–387
- Bronk DA, Glibert PM (1993) Application of a ^{15}N tracer method to the study of dissolved organic nitrogen uptake during spring and summer in Chesapeake Bay. *Mar Biol* 115:501–508
- Bronk DA, Glibert PM, Malone TC, Banahan S, Sahlsten E (1998) Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. *Aquat Microb Ecol* 15: 177–189
- Bronk DA, Glibert PM, Ward BB (1994) Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science* 265:1843–1846
- Capone DG, Ferrier MD, Carpenter EJ (1994) Amino acid cycling in colonies of the planktonic marine cyanobacterium *Trichodesmium thiebautii*. *Appl Environ Microbiol* 60:3989–3995
- Carpenter EJ, McCarthy JJ (1975) Nitrogen fixation and uptake of combined nitrogenous nutrients by *Oscillatoria (Trichodesmium) thiebautii* in the western Sargasso Sea. *Limnol Oceanogr* 20:389–401
- Chróst RJ (1991) Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Chróst R (ed) *Microbial enzymes in aquatic environments*. Springer, New York, p 29–59
- Cosper EM, Dennison WC, Carpenter EJ, Bricelj VM, Mitchell JG, Kuenstner SH (1987) Recurrent and persistent brown tide blooms perturb coastal marine ecosystem. *Estuaries* 10:284–290
- Dugdale RC, Goering JJ (1967) Uptake of new and regenerated forms of nitrogen in primary production. *Limnol Oceanogr* 12:196–206
- Dzurica S, Lee C, Cosper EM, Carpenter EJ (1989) Role of environmental variables, specifically organic compounds and macronutrients, in the growth of the chrysophyte *Aureococcus anophagefferens*. In: Cosper EM, Bricelj VM, Carpenter EJ (eds) *Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms*. Springer-Verlag, New York, p 229–252
- Engels DH, Engels A, Pistorius EK (1992) Isolation and partial characterization of an L-amino acid oxidase and of photosystem II complexes from the cyanobacterium *Synechococcus* PCC7942. *Z Naturf* 47c:859–866
- Fiedler R, Proksch G (1975) The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: a review. *Analytica Chim Acta* 78:1–62
- Glibert PM (1993) The interdependence of uptake and release of NH_4^+ and organic nitrogen. *Mar Microb Food Webs* 7:53–67
- Glibert PM, Banahan S (1988) Uptake of combined nitrogen sources by *Trichodesmium* and pelagic microplankton in the Caribbean Sea: comparative uptake capacity and nutritional status. *EOS* 69: 1089
- Glibert PM, Bronk DA (1994) Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium* spp. *Appl Environ Microbiol* 60:3996–4000
- Glibert PM, Capone DG (1993) Mineralization and assimilation in aquatic sediment and wetland systems. In: Knowles R, Blackburn TH (eds) *Nitrogen isotope techniques in plant, soil, and aquatic systems*. Academic Press, New York, p 243–272
- Glibert PM, Garside C, Fuhrman JA, Roman MR (1991) Time-dependent coupling of inorganic and organic nitrogen uptake and regeneration in the plume of the Chesapeake Bay estuary and its regulation by large heterotrophs. *Limnol Oceanogr* 36:895–909
- Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar Ecol Prog Ser* 11: 299–308
- Jørgensen NOG, Kroer N, Coffin RB, Yang XH, Lee C (1993) Dissolved free amino acids, combined amino acids and DNA as sources of carbon and nitrogen to marine bacteria. *Mar Ecol Prog Ser* 98:135–148
- Keil RG, Kirchman DL (1991) Contribution of dissolved free amino acids and ammonium to the nitrogen requirements of heterotrophic bacterioplankton. *Mar Ecol Prog Ser* 73: 1–10
- Lomas MW, Glibert PM, Berg GM, Burford M (1996) Characterization of nitrogen uptake by natural populations of *Aureococcus anophagefferens* (Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. *J Phycol* 32:907–916
- Metzler PM, Glibert PM, Gaeta SA, Ludlam JM (1997) New

- and regenerated production in the South Atlantic off Brazil. *Deep Sea Res* 42:363–384
- Meyer R, Pistorius EK (1989) Partial characterization of an arginine-metabolizing enzyme from the thermophilic cyanobacterium *Synechococcus* sp. *Biochim Biophys Acta* 975:80–87
- Muñoz-Blanco J, Hidalgo-Martinez J, Cardenas J (1990) Extracellular deamination of L-amino acids by *Chlamydomonas reinhardtii* cells. *Planta* 182:194–198
- Paerl HW, Bebout BM, Prufert LE (1989) Bacterial associations with marine *Oscillatoria* sp. (*Trichodesmium* sp.) populations: ecophysiological implications. *J Phycol* 25: 773–784
- Palenik B, Morel FMM (1990a) Comparison of cell-surface L-amino acid oxidases from several marine phytoplankton. *Mar Ecol Prog Ser* 59:195–201
- Palenik B, Morel FMM (1990b) Amino acid utilization by marine phytoplankton: a novel mechanism. *Limnol Oceanogr* 35:260–269
- Palenik BP, Kieber DJ, Morel FMM (1989) Dissolved organic nitrogen use by phytoplankton: the role of cell-surface enzymes. *Biol Oceanogr* 6:347–354
- Palenik BP, Morel FMM (1988) Dark production of H₂O₂ in the Sargasso Sea. *Limnol Oceanogr* 33:1606–1611
- Palenik BP, Zafiriou OC, Morel FMM (1987) Hydrogen peroxide production by a marine phytoplankter. *Limnol Oceanogr* 32:1365–1369
- Pantoja S, Lee C (1994) Cell-surface oxidation of amino acids in seawater. *Limnol Oceanogr* 39:1718–1726
- Pantoja S, Lee C, Marecek JF, Palenik BP (1993) Synthesis and use of fluorescent molecular probes for measuring cell-surface enzymatic oxidation of amino acids and amines in seawater. *Analyt Biochem* 211:210–218
- Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford
- Prade RA, Terenzi HF (1985) Control of L-amino acid oxidase in *Neurospora crassa* by different regulatory circuits. *Arch Microbiol* 143:37–41
- Schell DM (1974) Uptake and regeneration of free amino acids in marine waters of Southeast Alaska. *Limnol Oceanogr* 19:260–270
- Sellner K (1992) Trophodynamics of marine cyanobacteria blooms. In: Carpenter EJ, Capone DG, Rueter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other diazotrophs. Kluwer Academic Publishers, Dordrecht, p 75–94
- Sikora L, Marzluf GA (1982) Regulation of L-amino acid oxidase and of D-amino acid oxidase in *Neurospora crassa*. *Mol Gen Genet* 186:33–39
- Stevens SE Jr, Patterson COP, Myers J (1973) The production of hydrogen peroxide by blue-green algae: a survey. *J Phycol* 9:427–430
- Suttle CA, Chan AM, Fuhrman JA (1991) Dissolved free amino acids in the Sargasso Sea: uptake and respiration rates, turnover times, and concentrations. *Mar Ecol Prog Ser* 70:189–199
- Valderrama JC (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar Chem* 10:109–122
- Wheeler PA, Kirchman DL (1986) Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol Oceanogr* 31:998–1009
- Wheeler PA, North BB, Stephens GC (1974) Amino acid uptake by marine phytoplankters. *Limnol Oceanogr* 19: 249–259

Editorial responsibility: Farooq Azam,
La Jolla, California, USA

Submitted: April 15, 1997; Accepted: February 2, 1998
Proofs received from author(s): June 8, 1998