

A shipboard natural community continuous culture system for ecologically relevant low-level nutrient enrichment experiments

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Abstract

Inputs of low concentrations of new and regenerated forms of nitrogen ($\leq 1 \mu\text{M}$) have large impacts on phytoplankton community structure and ocean biogeochemistry in the oligotrophic central gyres. However, current manipulative experimental methods cannot effectively simulate low-level, continuous supplies of nitrate, such as those from upwelling or eddy events, or steady-state inputs of regenerated ammonium and dissolved organic nitrogen from zooplankton grazing. Using a new shipboard continuous culture system based on laboratory chemostat methodology, we compared the effects of a continuous supply of $1 \mu\text{M}$ nitrate, ammonium, and urea-N at a dilution rate of 0.5 d^{-1} on algal community composition in the North Atlantic. In the Gulf Stream, continuous inputs of $1 \mu\text{M}$ nitrate dramatically changed phytoplankton community structure from dominance by cyanobacteria to dominance by diatoms. Equimolar continuous supplies of ammonium resulted in much smaller increases in total phytoplankton biomass, and favored a community co-dominated by diatoms and cyanobacteria, and promoted the growth of pelagophytes. In the Sargasso Sea, continuous $1 \mu\text{M}$ urea-N inputs greatly increased the biomass and dominance of *Synechococcus* relative to the initial community and compared with control and $1 \mu\text{M}$ nitrate additions. The shipboard natural community continuous culture system is uniquely suited for realistically simulating inputs of low levels of limiting nutrients, allowing new types of prognostic enrichment experiments that give novel insights into the processes that control phytoplankton community structure in the ocean.

Phytoplankton productivity and community structure are regulated to a large extent by the availability of essential nutrients required for growth. Nitrogen is usually considered to be the limiting nutrient in the oligotrophic central gyres (Thomas 1970a, 1970b; Eppley et al. 1973), whereas iron is limiting in high-nutrient, low chlorophyll (HNLC) areas (Martin et al. 1991; Geider and LaRoche 1994; Wells et al. 1995). Recent evidence, however, suggests that climate change may have driven oligotrophic gyres into phosphorus limitation (Karl 2000).

Oligotrophic systems are dominated by small phytoplankton species that grow rapidly, and that rely on remineralization and recycling of these limiting nutrients to sustain growth. When nutrient limitation is relaxed through new inputs, there is often a shift in community structure toward

larger, heavier cells (Letelier et al. 2000; Vaillancourt et al. 2003). Shifts toward larger species increase the potential for vertical export and sequestration of carbon in the ocean (Boyd and Newton 1999; DiTullio et al. 2000), thus impacting global carbon cycling (Falkowski et al. 1998).

Both shipboard and in situ addition methods have been used to experimentally examine the effects of nutrient limitation on natural communities. Bottle experiments or growouts (Martin and Gordon 1988; Hutchins and Bruland 1998; Caron et al. 2000; Kudela and Dugdale 2000) and open-ocean fertilization experiments (Martin et al. 1994; Coale et al. 1996; Boyd et al. 2000) effectively demonstrate that the addition of limiting nutrients increases the biomass and growth rates of phytoplankton communities. Unfortunately, the technology of nutrient-addition bioassay experiments remains relatively primitive.

Bottle and open-ocean fertilization experiments are good diagnostic indicators of nutrient limitation, but they may not be realistic models of real world nutrient inputs. High, single additions of nutrients to experimental bottle incubations usually result in massive blooms that are not typically seen in the natural environment. Nutrient supply rates and phytoplankton physiology, growth rates, biomass, and community

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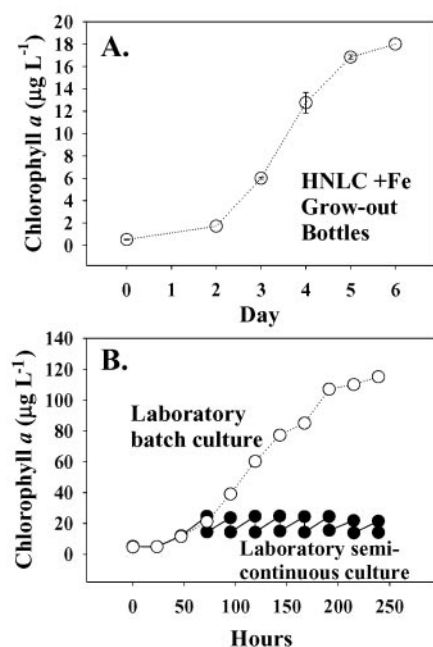


Fig. 1. Typical growth curve of a shipboard “growout” experiment (A) compared with laboratory batch and semi-continuous cultures (B). (Hutchins unpubl. data unref.).

composition are not in steady state, and thus growout experiments closely resemble a laboratory batch culture (Fig. 1A).

Mesoscale open-ocean fertilization experiments usually require repeated replenishment of the fertilized waters with relatively large pulses of iron to prevent iron from quickly becoming limiting again (Coale et al. 1996; Boyd et al. 2000). Again, these multiple discrete pulses of iron are usually at higher concentrations than those that are introduced by natural supply processes. In this respect, open-ocean addition experiments can be compared with laboratory semi-continuous cultures, in which phytoplankton are maintained in exponential growth phase by repeated dilutions with fresh culture medium (Fig. 1B). Although more realistic than batch cultures, biomass and limiting nutrient supply rates and concentrations in semi-continuous cultures are still in a non-steady-state condition, and total phytoplankton biomass is typically maintained at artificially high levels.

Areas where new nutrients are delivered primarily from below, like the equatorial Pacific HNLC area, have been suggested to resemble neither a batch culture nor a semi-continuous system, but rather a continuous culture or chemostat (Frost and Franzen 1992; Dugdale and Wilkerson 1998). An argument can be made that increased nutrient concentrations from vertical displacement of the nutricline during eddy events in oligotrophic systems represent a similar steady-state, low-level enhancement of N and P availability. In these ecosystems, cell growth rates are determined by vertical supply rates (in upwelling systems) or increased concentrations (during eddy events) of limiting nutrients and are often in approx-

imate balance with cell-removal processes such as grazing, sinking, and advection. Chemostats have the potential to mimic these natural processes by providing limiting nutrients continuously at low concentrations, allowing growth rates to reach equilibrium with loss rates to dilution. Chemostats can consequently achieve a steady-state condition for nutrients, growth rates, and biomass (Novick and Szilard 1950).

Adaptation of a continuous culture system for shipboard use to introduce limiting nutrients to natural plankton communities offers the possibility of effectively simulating natural changes in nutrient supplies under controlled experimental conditions. Rather than simply testing the effects of the presence or lack of an essential nutrient, a shipboard chemostat can also examine how nutrient input rates at realistic concentrations affect algal growth and productivity. Unlike laboratory unialgal chemostats, continuous culture experiments with mixed natural communities examine the effects of nutrient inputs on species dominance and succession. Natural loss processes can be simulated by cells washing out through the chemostat outflows; under any particular set of experimental conditions, those species with the fastest net growth rates will come to dominate the community as slower-growing species are washed out. This method has been used with natural communities in shore-based continuous culture systems (Harrison and Davis 1979; Turpin and Harrison 1979), but to our knowledge has not been previously applied in a shipboard setting.

Here, we present a new shipboard chemostat method and use it to examine changes in phytoplankton community structure at realistic degrees of nitrogen limitation alleviation, providing new insights into how nitrogen-driven changes in algal species composition may affect marine biogeochemical cycles. Our intention was not to carry out diagnostic experiments to demonstrate nitrogen limitation; it has been well established that nitrogen is often limiting in oligotrophic areas. Instead, we used our new shipboard continuous culture method to carry out prognostic experiments to simulate the effects of natural variations in nitrogen supplies on the taxonomic composition of phytoplankton communities.

Materials and procedures

Shipboard continuous culture system—The mechanics of the shipboard continuous culture system are similar to those of laboratory chemostats. However, the shipboard chemostat uses a natural plankton community instead of monospecific laboratory cultures. A schematic of the shipboard chemostat system is shown in Fig. 2. Near-surface water (10 to 15 m) was collected using a trace metal clean pump system (Hutchins et al. 1998). An acid-washed, 0.2- μm in-line cartridge filter was used to fill one 50-L seawater medium reservoir for each experimental treatment; the 2.7-L polycarbonate chemostat bottles were filled at the same time with unfiltered seawater containing the intact plankton community. The medium reservoirs were secured in the ship’s laboratory under a laminar flow hood in a class-100 clean area (<100 particles per cubic meter).

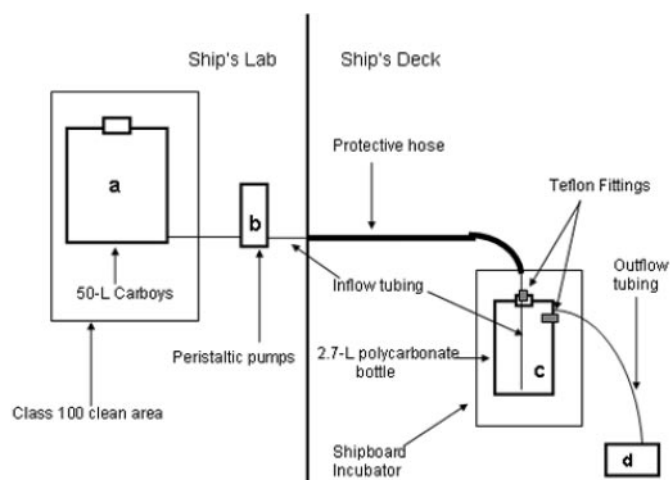


Fig. 2. Schematic diagram of the shipboard natural community continuous culture system. (a) Inside a class-100 clean area, three 50-L carboys contained amended or unamended cleanly collected 0.2- μm filtered seawater. Each carboy served as the medium source for replicate sample bottles for that treatment. (b) Adjustable peristaltic pumps were used to pump medium from the carboys through Teflon tubing outside the ship's laboratory across the deck (inside a protective hose) to the shipboard incubator. (c) The shipboard incubator was adapted with a Plexiglas holder that held nine 2.7-L polycarbonate bottles. Each bottle had Teflon fittings at the top for the inflow and at the neck for the outflow. Each sample bottle held approximately 2.5 L of seawater containing the natural community. The treated media was delivered to the bottom of the bottle and periodically shaken to keep cells evenly suspended. (d) The outflow was collected in sampling and waste collection bottles outside the shipboard incubator.

Three 1/8-inch Teflon lines leading out of each reservoir supplied triplicate chemostat bottles for each treatment. Medium was pumped through these supply lines using adjustable peristaltic pumps, which were located nearby on the lab bench where flow rates could be easily monitored and calibrated. The supply lines then traveled through the wall of the laboratory and across the deck of the ship (enclosed in a heavy-duty protective hose) to a standard flow-through deckboard incubator. Incubator light levels were adjusted using spectrally correct blue plastic at 40% of incident intensity (Hutchins et al. 1998).

The chemostat bottles were secured in a Plexiglas rack inside the incubator (Fig. 3). Supply lines entered each chemostat bottle through the top, and incoming medium flowed down a Teflon tube to be released near the bottom of the bottle. Outflow from the bottles was through gravity-fed 1/2-inch tubing in the shoulders of the bottles; tubes exited the incubator through a port in the side, and overflow was collected outside in receiving bottles. All components of the system were made of polycarbonate or Teflon and were rigorously cleaned before the experiments by pumping 10% HCl through the entire system, followed by clean-filtered seawater rinses.

Quantitative, continuous removal of biomass through the outflow is needed to operate a continuous culture system, so it is essential that the cells remain suspended in the chemostat bottles during the experiments. In the experiments presented



Fig. 3. Photograph of the chemostat bottles in the holder rack inside the incubator, showing inflow tubing entering through the top of the bottles and outflows at the shoulders of the bottles.

here, we accomplished this by frequent (hourly) manual agitation of the bottles around the clock. A newer version of the shipboard chemostat keeps the cells suspended using a compressed air-driven system that rotates the entire Plexiglas rack holding the chemostat bottles inside the incubator on a timed cycle, usually once every 5 to 15 min (Hare and Hutchins unpubl. data unref.). Details of the new compressed air-driven cell suspension system are available from the corresponding author on request.

Daily sampling from the chemostat bottles was limited to ~10% to 15% of bottle volume to avoid significant perturbations to the steady state. To obtain samples, the bottles were thoroughly shaken, and the peristaltic pumps were turned on full until the desired volume had been collected. Pumps were then turned off for an appropriate short interval (2 to 2.5 h) to compensate for the rapid flow rate during collection, before being reset at the experimental flow rates. The sampling protocol thus represents a minor but unavoidable perturbation of the continuous culture system. Sampling perturbations of the equilibrium system are a consideration in all chemostat experiments, making it imperative to choose sampling volumes that are as low as possible for the required analyses.

Analytical methods—Size-fractionated chlorophyll *a* (Chl *a*; 0.2 and 3.0 μm) was measured daily in both experiments. Samples were filtered onto 47-mm polycarbonate filters, extracted in 90% acetone overnight in the freezer, and Chl *a* was estimated on a Turner 10 AU fluorometer using the nonacidified method of Welschmeyer (1994). Nutrient samples (NO_3^- , PO_4^{3-} , and SiOH_4) from each daily sampling and from initial and final samples from the reservoir carboys were frozen at sea and analyzed on shore using standard autoanalyzer methods. Limits of detection for these nutrients using our autoanalyzer system were 0.15 μM (NO_3^-), 0.02 μM (PO_4^{3-}), and 0.03 μM (SiOH_4).

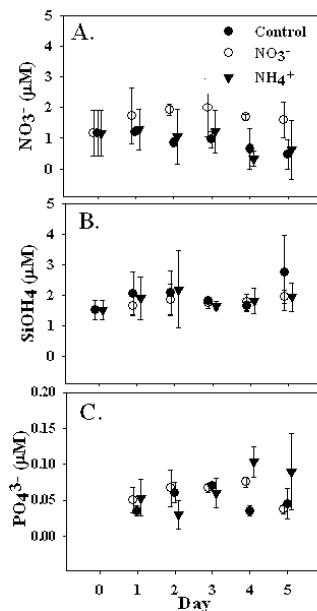


Fig. 4. Major inorganic nutrient concentrations in the Gulf Stream shipboard chemostat experiment in controls receiving continuous inputs of unamended filtered seawater, or seawater amended with 1 μM nitrate or ammonium. Error bars represent the standard deviations of the triplicate or replicate bottles. Initial values from the collected seawater from day 1 on the measurements are from the chemostat bottles. (A) Nitrate; (B) Silicic acid; (C) Phosphate.

Analyses of concentrations of our “regenerated” nitrogen additions of ammonium and urea were not available, but typical ambient levels of these in this region are at or below detectable levels (i.e., $<0.1 \mu\text{M}$; Price and Harrison 1988).

We assessed phytoplankton community structure in our natural community chemostat experiments using pigment high-performance liquid chromatography (HPLC). To measure the concentration of algal taxon-specific pigments, three replicate samples were filtered onto GF/F filters from the initial water (5 to 6 L) and from each of the replicate chemostat bottles at the end of the incubations (1.2 to 2.2 L). The filters were immediately frozen in liquid nitrogen and shipped back to the lab for acetone extraction and analysis by HPLC. Pigments were separated on an automated Hewlett Packard 1050 HPLC system using a reversed-phase Waters Symmetry C-8 column and a solvent gradient containing methanol, aqueous pyridine, acetone, and acetonitrile (Zapata et al. 2000). A diode array detector recorded pigment spectra every 5 s over the wavelengths 350 to 600 nm and was set to record continuous chromatograms at 410, 440, and 465 nm. An HP 1046A fluorescence detector with excitation of 421 nm and emission at 666 nm (optimized for Chl *a*) was also employed to identify and quantify Chl *a* and *c*, as well as Chl degradation products, such as chlorophyllide, phaeophorbides, and phaeophytin (DiTullio and Geesey 2002). The system was calibrated by repeated injections of pigment standards isolated from a variety of unialgal

cultures maintained in the laboratory. The limit of detection was approximately 1 to 5 ng per injection, depending on the pigment.

Assessment

To assess the method, two shipboard continuous culture experiments comparing the effects of different nitrogen sources on algal community composition were carried out in the oligotrophic subtropical Atlantic. These experiments compared nitrate with ammonium (in the Gulf Stream) and nitrate with urea additions (in the Sargasso Sea), using low-level (1 μM total N) continuous enrichments. Both experiments included controls supplied with the same filtered surface seawater at the same dilution rate, but without nitrogen additions. Because our intention was to examine just the effects of nitrogen speciation on phytoplankton community structure, all treatments (including the controls without added N) were supplemented with silicic acid (1 μM) and phosphate (0.0625 μM , in Redfield proportion to the added N). Both experiments were run for 5 d and used the same experimental dilution rate of 0.5 d^{-1} .

Gulf Stream experiment—This experiment compared the effects of continuous inputs of 1 μM nitrate, 1 μM ammonium, or ambient nutrients (unenriched controls). Water was collected from the Gulf Stream at $\sim 15\text{-m}$ depth on 27 May 2000 at $37^{\circ}19.6'\text{N}$, $71^{\circ}57.5'\text{W}$. Sea surface temperature at this station was 26.3°C and salinity was 36.25. Controls and NO_3^- treatments were run with duplicate chemostat bottles, and NH_4^+ treatments were run in triplicate. Triplicate samples were taken for all analyses of the initial water and community.

Concentrations of inorganic nutrients measured in the Gulf Stream experiment are shown in Fig. 4. Initial (day 0) concentrations are those of the collected seawater before nutrient amendments (day 0 phosphate samples were lost and are not presented), whereas the subsequent time points show nitrate, silicic acid, and phosphate concentrations in the chemostat bottles. Initial nitrate concentrations were $\sim 1.2 \mu\text{M}$, and remained fairly steady in the control and NH_4^+ addition bottles, with a slight decline to $\sim 0.6 \mu\text{M}$ during the last 2 d of the experiment. Higher steady-state nitrate concentrations in the $+\text{NO}_3^-$ bottles throughout the experiment (1.6 to 2.0 μM) reflected the continual supply of this nutrient from the chemostat inflows (Fig. 4A). Silicic acid (1.5 to 2.5 μM , Fig. 4B) and phosphate (0.04 to 0.1 μM , Fig. 4C) concentrations did not vary systematically between treatments, but also were maintained at relatively constant concentrations in the bottles throughout the experiment. The chemostat system successfully replicated natural low-level, continuous nutrient inputs, without the large fluctuations in nutrients levels typically observed in batch growout incubations. The steady availability of 1 to 2 μM nitrate to the phytoplankton community is very similar to levels measured during natural mesoscale eddy events in oligotrophic waters (Vaillancourt et al. 2003).

Initial fluorometrically determined total Chl *a* ($>0.2 \mu\text{M}$) in the collected water was $\sim 0.1 \mu\text{g L}^{-1}$ and remained close to this

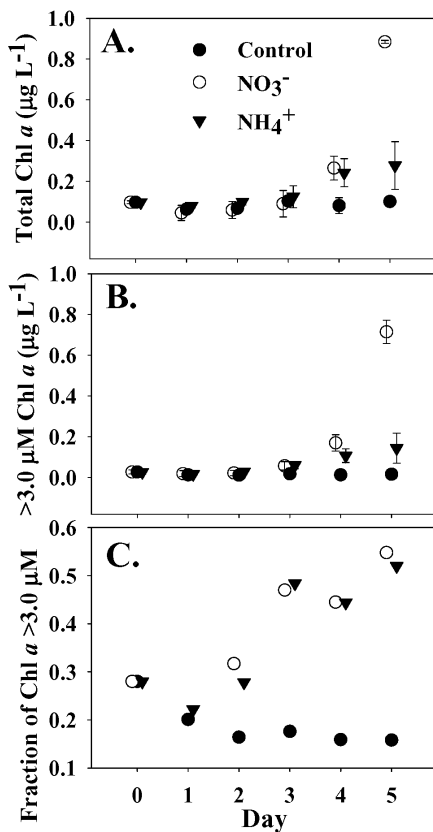


Fig. 5. Fluorometrically determined Chl *a* from the Gulf Stream shipboard chemostat experiment. Error bars as in Fig. 4. (A) Total (>0.2 µM) Chl *a*. (B) Large size class (>3.0 µm) Chl *a*. (C) Fraction of total Chl *a* in the large size class.

value throughout the experiment in the control treatment (Fig. 5A). Increased total Chl *a*, however, was seen in the N-amended bottles by the fourth and fifth days of the experiment, with concentrations approaching 0.3 µg L⁻¹ (+NH₄⁺) and 0.9 µg L⁻¹ (+NO₃⁻). The same pattern was evident with large-size class Chl *a* (>3.0 µm), which changed very little from the initial value of 0.03 µg L⁻¹ in the controls, but increased in the +NH₄⁺ and +NO₃⁻ treatments by the last 2 d, particularly in the latter (Fig. 5B). Although equimolar amounts of nitrogen (1 µM) were supplied at identical rates in both +N treatments, final increases in both total and >3.0 µm Chl *a* were much larger (~3×) when NO₃⁻ was used as the N source.

The size distribution of fluorometrically determined Chl *a* in our bottles changed over the course of the experiment (Fig. 5C). Initially, large cells (>3.0 µm) contributed about 28% of the total Chl *a*. This ratio declined slightly over the first 2 d in the controls, then held steady at about 16%. In contrast, both treatments with added N underwent a large shift in the size distribution of community Chl *a*, with the final >3.0 µm contribution reaching 52% and 55% of the total in the +NH₄⁺ and +NO₃⁻ bottles, respectively. Continuous enrichment of Gulf Stream water with realistic levels of two different forms

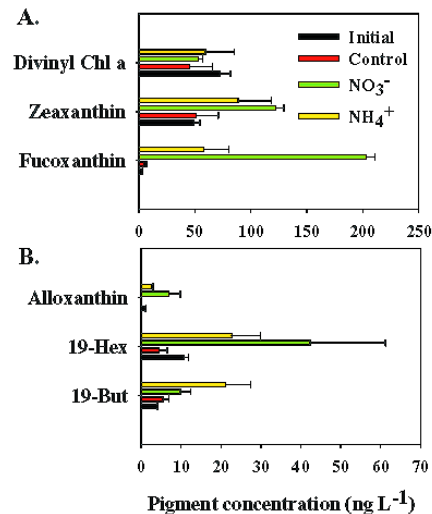


Fig. 6. Phytoplankton pigments in the Gulf Stream shipboard chemostat experiment, including (A) divinyl Chl *a* (prochlorophytes), zeaxanthin (all cyanobacteria), and fucoxanthin (primarily diatoms), and (B) alloxanthin (cryptophytes), 19-hexanoyloxyfucoxanthin (19-hex, prymnesiophytes), and 19-butanoyloxyfucoxanthin (19-but, pelagophytes). Shown are values for the initial water and for the final controls and final nitrate and urea additions. Error bars are the standard deviations of triplicate or replicate samples.

of nitrogen thus resulted in a major shift in the community toward larger phytoplankton species.

Changes in taxon-specific pigment concentrations for major phytoplankton groups are presented in Fig. 6. The initial samples were dominated by the cyanobacterial pigments zeaxanthin (found in both *Synechococcus* and *Prochlorococcus*) and divinyl Chl *a* (*Prochlorococcus* only, Fig. 6A). The initial ratio of divinyl Chl *a* to total Chl *a* (g:g) was nearly 2:1, suggesting dominance of the community by *Prochlorococcus*. Minor contributors to the initial pigment pool were the eukaryotic pigments 19-hexanoyloxyfucoxanthin (19-hex, prymnesiophytes), 19-butanoyloxyfucoxanthin (19-but, pelagophytes), and alloxanthin (cryptophytes) (Fig. 6B). The diatom pigment fucoxanthin was also initially present, but in very low concentrations (Fig. 6A).

At the final time point (day 5), pigment concentrations in the control bottles were either unchanged or only slightly different than levels in the initial water, suggesting very little change in abundance of all of the phytoplankton groups in the chemostat bottles supplied with unamended seawater (Fig. 6). The chemostat system successfully maintained the pre-existing community structure in the controls, suggesting that bottle artifacts were not large.

In contrast, the nitrogen additions resulted in large shifts in the taxonomic composition of the community. Cyanobacteria did benefit from the N additions, because levels of zeaxanthin increased 1.8× (+NH₄⁺) to 2.5× (+NO₃⁻) relative to the control. However, divinyl Chl *a* did not change substantially in either of the N treatments, suggesting that most of the increase in

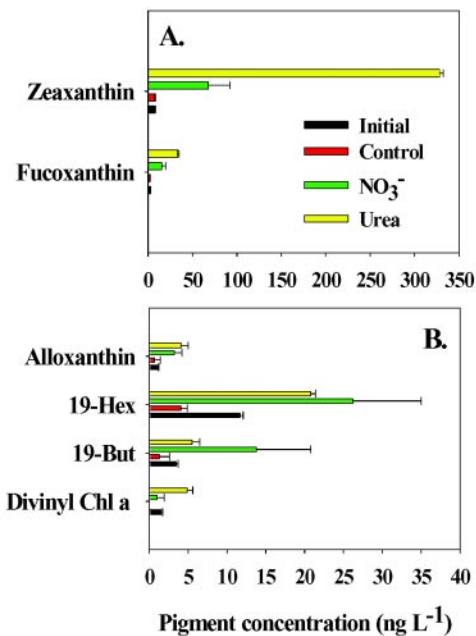


Fig. 7. Phytoplankton pigments in the Sargasso Sea shipboard chemostat experiment, including (A) zeaxanthin (all cyanobacteria), and fucoxanthin (primarily diatoms), and (B) alloxanthin (cryptophytes), 19-hexanoyloxyfucoxanthin (19-hex, prymnesiophytes), 19-butanoyloxyfucoxanthin (19-but, pelagophytes), and divinyl Chl *a* (prochlorophytes). Shown are values for the initial water and for the final controls and final nitrate and urea additions. Error bars as in Fig. 6.

zeaxanthin was due to increases in *Synechococcus* biomass rather than *Prochlorococcus* (Fig. 6A). The final ratios of divinyl Chl *a* to total Chl *a* in the nitrogen-supplemented bottles were ~1:3 (+NH₄⁺) and >1:10 (+NO₃⁻), demonstrating a large community shift away from the initial *Prochlorococcus*-dominated community. By far, the greatest response to the nitrogen inputs was by the diatom pigment fucoxanthin, which increased 20× when ammonium was added and 70× in the nitrate additions (Fig. 6A).

Of the other initially minor eukaryotic pigments, 19-hex and alloxanthin showed the same pattern as fucoxanthin, with increased concentrations in the final ammonium treatments compared with the initial values, but much larger increases in the nitrate additions. Only 19-but increased more in the +NH₄⁺ final samples (5.8×) than in the final +NO₃⁻ samples (2.7×), suggesting that pelagophytes may have responded preferentially to ammonium-N (Fig. 6B). In general, the Gulf Stream pigment data suggested that nitrate favored increased biomass of all groups (except possibly pelagophytes) more than ammonium, and continuous nitrate additions, in particular, resulted in a dramatic shift in community dominance from cyanobacteria to diatoms.

Sargasso Sea experiment—Water for this shipboard chemostat experiment comparing the effects of nitrate and urea additions was collected on 2 June 2000 at 38°28.7'N, 65°41.9'W;

ambient sea surface temperature was 24.8°C and salinity was 36.85. Treatments were 1 μM nitrate, 0.5 μM urea (e.g., a 1-μM addition of urea-N), and an unamended control. Controls and urea treatments were duplicated, and the nitrate treatment and initial samples were triplicated.

Inorganic nutrients and total fluorometrically determined Chl *a* in the Sargasso Sea chemostat experiment showed similar trends to those observed in the Gulf Stream experiment (data not shown). Inorganic nutrients remained relatively steady throughout the 5 d of the experiment, whereas total Chl *a* increased in both the +NO₃⁻ and +urea treatments but not in the control. Dominance by the large-size class was less pronounced, however, in the final +NO₃⁻ samples in this experiment. No switch toward larger cells was observed in the controls or +urea treatments.

The HPLC pigment data suggested that the highest levels of initial pigments were from cyanobacteria (Fig. 7A) and prymnesiophytes, with smaller contributions by the other eukaryotic groups (Fig. 7B). Fucoxanthin (Fig. 7A) was at very low concentrations in the initial water; similarly low levels of divinyl Chl *a* (Fig. 7B) suggested that most of the cyanobacteria present were *Synechococcus* rather than *Prochlorococcus*. The initial ratio of divinyl Chl *a* to total Chl *a* was only 0.05, supporting this idea. Concentrations of 19-hex, 19-but, and divinyl Chl *a* declined somewhat in the final control samples, whereas all of the other pigments in the controls were relatively unchanged after 5 d. As in the Gulf Stream experiment, changes in community structure in the Sargasso chemostat controls were minor, suggesting that continuous supply of unamended surface water to the bottles closely reproduced the dominant in situ community.

As in the first experiment, continuous supply of 1 μM nitrogen as either nitrate or urea resulted in growth of most of the members of the Sargasso Sea community. Prymnesiophytes (19-hex) and pelagophytes (19-but) produced higher final pigment levels from nitrate additions than from urea, but there was no difference in the two nitrogen sources for cryptophytes (alloxanthin). The prochlorophyte pigment divinyl Chl *a* did not respond to the nitrate enrichment, but increased slightly after urea additions; nevertheless, prochlorophytes were never more than a minor component of the community (Fig. 7B). The final ratio of divinyl Chl *a* to total Chl *a* in the nitrogen-amended bottles was very low (0.008 to 0.01).

In this experiment, the dramatic shift from cyanobacteria to diatoms seen in the Gulf Stream chemostat experiment did not occur. In fact, pigment data suggested that the final community was heavily dominated by cyanobacteria (presumably *Synechococcus*, because very little divinyl Chl *a* was present). However, equimolar inputs of nitrate-N and urea-N did not produce equal amounts of cyanobacterial pigments, with the final level of zeaxanthin being 2.5× initial values in the +nitrate treatment but 42× initial values in the final +urea bottles (Fig. 7A). Although diatoms were only a minor component of the final community, fucoxanthin also responded

preferentially to urea (12× initial pigment values) compared with nitrate (5.6× increase).

These demonstration experiments showed that the shipboard chemostat system can be used to evaluate the effects of very low, continuous inputs of nitrogen that cannot be examined using traditional batch culture methods. The lack of major changes in community structure in the controls supplied with unamended surface seawater strongly supports the validity of the dramatic effects on algal floristics seen in bottles supplemented with nitrate, ammonium, or urea. Although these results must be considered preliminary, they demonstrate that natural community continuous culture systems are a useful new tool to answer questions about the taxon-specific effects of nitrogen sources that cannot be realistically addressed using batch shipboard culture methods.

Discussion

This study adapted a laboratory chemostat system to a shipboard design, allowing us to examine how low concentrations of limiting nutrients entering a water parcel can affect community structure. In the subtropical Atlantic experiments presented here, we were able to use this new incubation system to introduce nitrate by a slow, continuous process that realistically simulated many natural input processes for new nitrogen. The regenerated nitrogen sources ammonium and urea were also introduced in a similar manner, simulating continuous inputs from grazing processes. In contrast, most previous “batch” manipulative experiments have necessarily added concentrations of limiting nutrients far higher than those that are typically introduced naturally by these processes, because realistic levels of nutrients added in single pulses are almost immediately depleted in batch incubation bottles.

Our shipboard chemostat system experiments presented here were short (5 d each, due to ship-time limitations), and thus did not realize the full potential of the method. Longer incubations can allow both biological and biogeochemical parameters to reach a steady-state condition, such as in laboratory chemostat systems. At steady-state, inputs by the chemostat pumps and net growth rates of the plankton are balanced by losses through the outflows, and biomass and nutrients both reach a stable level. Although nutrients were at apparent equilibrium during these 5-d experiments, Chl *a* biomass had obviously not equilibrated with losses and was still increasing rapidly in the +N bottles.

We have since performed other similar shipboard chemostat experiments in which we were able to attain and maintain steady-state biomass, community structure, and nutrient biogeochemistry for periods of up to 3 weeks (Hare and Hutchins unpubl. data unref.). In fact, once steady-state is achieved, shipboard chemostat experiments can theoretically be extended virtually indefinitely, and the length of a particular experiment is apparently limited only by the eventual growth of fouling organisms on the walls of the bottles. Although we did not observe surface growth in the bottles or tubing during

these short experiments, in very long experiments (>3 weeks) the establishment of fouling communities can result in misleading results, because these organisms are not removed by dilution and thus constitute a “batch” culture growing within the chemostat system. Keeping the medium reservoirs chilled also may help to reduce the growth of bacterial or other fouling organisms during the experiment.

A chemostat can thus closely reproduce the low, relatively constant biomass often observed in HNLC regions and oligotrophic areas, where algal growth rates are often in approximate balance with loss rates to grazing, sinking, and advection. The shipboard continuous culture system allows interspecific competition and microzooplankton grazing to determine the composition of the final community. Cells are “sorted” by net growth rate, by selecting for species that grow fastest under a particular nutrient input regime, whereas slower growing species are removed by dilution. Despite the short duration of the nitrogen addition experiments presented here, they can provide important and unique information about the initial responses of phytoplankton community structure to small, continuous increases in the availability of specific nitrogen sources.

In the strictest terms, the experiments presented here are probably not true chemostat experiments. In a true chemostat, the limiting nutrient is undetectable in the medium, which was not true, at least for the nitrate addition experiments presented here (Fig. 4A). A more accurate term for this particular experiment would be an “auxostat,” because biomass accumulation may have been controlled by the pumping rate rather than the limiting nutrient inflow rate. Laboratory chemostats also attempt to maintain cultures under invariant environmental conditions of continuous light, temperature, etc. Another possible designation for our experiment would be a “cyclostat,” because light varied under a natural light/dark regime in the deckboard incubators. For the sake of simplicity, however, we will continue to refer to the system as a shipboard chemostat or continuous culture system.

It is important to recognize that as in all manipulative experiments (including even *in situ* experiments; Boyd et al. 2002), shipboard chemostat incubations are subject to potential experimental artifacts. Therefore, results must be interpreted cautiously. All enclosed shipboard experiments are subject to some degree to “bottle effects,” such as a decrease in turbulence, removal from vertical movements, different light environments, and potential stimulatory or inhibitory effects from the walls of the bottles (Geider and La Roche 1994). Despite this, previous comparisons of enclosed shipboard growout and *in situ* Fe enrichments have produced remarkably similar outcomes (Coale et al. 1996; Boyd et al. 2000). The lack of significant changes in community composition in our chemostat controls also suggests that bottle artifacts do not seriously compromise interpretation of results.

In the shipboard chemostat, continual dilution removal of biomass ensures that those species with the fastest net growth rates in each treatment will eventually come to dominate the

community. In nature however, algal abundance is a function of both absolute growth rates and of loss rates (primarily from grazing). Exclusion of grazers and the loss term they represent has been a major criticism of previous enclosed incubation experiments (Banse 1991).

Most incubation experiments such as growouts do not include a loss term at all; in this respect, our chemostat represents a marked improvement over earlier methods. It is important to recognize, however, that the chemostat imposes a single dilution loss rate on the entire community. Larger cells (e.g., diatoms) may be favored because they are subject only to dilution losses, as their grazers are not well represented in the bottles. Smaller species (e.g., picoplankton) may however be subject to both dilution losses and simultaneous grazing by micrograzers. Relatively high phaeopigment concentrations (not shown) suggest that micrograzers were active in our chemostat bottles. Because grazing rates and abundance of mesozooplankton are typically very low in the oligotrophic Atlantic, however, we suggest that differential grazing was probably not a significant artifact in the experiments presented here. Nevertheless, relief of grazing pressure on large cells should be considered in all shipboard incubations, especially in regimes where larger metazoan zooplankton exert a strong influence on phytoplankton community biomass and structure.

There is a great deal of potential flexibility in the types of experiments that can be carried out with the shipboard continuous culture system. In the demonstration experiments presented here, we chose to focus on the effects of nitrogen speciation on community structure by examining different N sources added at identical rates and concentrations. Obviously, similar experiments can be carried out using other limiting nutrients, such as various chemical forms of Fe (with a trace metal clean version of the system; Hare and Hutchins unpubl. data unref.) or P supplied to phytoplankton, or even using different dissolved organic carbon (DOC) sources to examine effects on bacterioplankton community structure.

There are however many other possible ways to use the system. We have used our shipboard chemostat system to examine inputs of the same nutrient at the same dilution rate but at different concentrations (simulating different source waters), and identical nutrient concentrations supplied at different dilution rates (simulating differences in upwelling or input rates). This method is the only one presently available to examine the effects of nutrient speciation, concentration, or supply rate on changes in long-term, steady-state phytoplankton community structure. Such a steady-state system can be used to approximate a late successional phytoplankton community growing in a stratified water column, such as the North Atlantic Central Gyre.

The system can however also be used to simulate nonequilibrium phytoplankton communities such as those encountered during spring blooms, or following stochastic events such as deep storm mixing that inject nutrient pulses into surface waters (DiTullio and Laws 1991). To simulate this non-steady-state con-

dition, the shipboard chemostat can be used in a "pulsed" mode, where fresh medium is rapidly pumped into the bottles for a short period, followed by a period of "batch" growth without further dilutions. In this type of experiment, the shipboard culture system is employed in a manner analogous to a semi-continuous culture method.

There is evidence that the species composition effects of continuous nutrient supplies differ from those observed when supplies are added intermittently. In shore-based natural community continuous culture system experiments in Saanich Inlet, British Columbia, Turpin and Harrison (1979) demonstrated that homogenous (continuous) inputs of ammonia favored dominance by the diatom genus *Chaetoceros*, whereas patchy (daily) inputs favored the genus *Skeletonema*. Intermediate supply modes resulted in mixed communities of both genera. Chemostat systems are ideally suited for experiments comparing differing nutrient input modes, and for determining how changes in nutrient regimes structure phytoplankton assemblages.

The experiments presented here were intended as preliminary demonstrations of the capabilities of the method, rather than definitive investigations of nitrogen source influence on community structure. Nevertheless, our early results with the system raise some intriguing questions. It is notable that nitrogen speciation had a great effect on community development, despite the fact that added molar nitrogen concentrations and supply rates were the same in each treatment. Although equimolar additions of both nitrate and ammonium promoted a switch to larger cells in the Gulf Stream experiment, the absolute biomass of Chl *a* and most photosynthetic pigments produced were much higher with nitrate-N inputs. The presumed higher grazing rate by microzooplankton on *Synechococcus* compared with mesozooplankton grazing rates on diatoms in our bottles may partially explain the increase in fucoxanthin concentrations in the nitrate treatment. The fact that we did not observe a similar result in the $+NH_4^+$ treatment, however, suggests that diatom growth can be preferentially stimulated with nitrate. These results deserve further investigation.

Our Gulf Stream results strongly suggested that diatoms benefit most from nitrate additions. While the concentration of cyanobacterial pigments increased slightly over time, concentrations of diatoms pigments increased 60-fold. On the other hand, *Synechococcus* was strongly favored over eukaryotes in the Sargasso Sea experiment by urea inputs. These results suggest that the idea that diatoms carry out new production, whereas picoplankton are responsible for most regenerated production (Dugdale and Wilkerson 1998), may have a physiological basis. *Prochlorococcus* was able to increase its relative contribution to community biomass in our Sargasso Sea experiment when nitrogen was supplied as urea, but not when it was added as nitrate. These results are consistent with laboratory studies showing cultured prochlorophytes cannot use nitrate (Rippka et al. 2000), and in fact lack nitrate

reductase (Moore et al. 2002). Although lab experiments have shown that both *Synechococcus* and *Prochlorococcus* spp. can use urea (Moore et al. 2002), our Sargasso Sea experiment revealed that urea addition preferentially stimulated *Synechococcus* growth (Fig. 6).

Alleviation of phytoplankton nutrient limitation by processes such as upwelling, cold core eddies, and periodic mixing events has important consequences for phytoplankton productivity and community structure. In turn, algal community composition largely controls marine food web structure and the flux of carbon out of the euphotic zone. However, current manipulative incubation methods can only crudely approximate natural nutrient supply processes, and the extrapolation of their results to predictive ocean models is therefore uncertain. Future studies need to take a more sophisticated and subtle approach by examining changes in community structure using prognostic experiments closely based on actual supply modes, rather than with diagnostic experiments using unrealistically high inputs of limiting nutrients. The shipboard natural community continuous culture system offers oceanographers a versatile new tool to carry out such prognostic experiments, and to thus further our understanding of the effects of nutrient limitation on ocean biology and biogeochemistry.

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