

# Inorganic and Organic Nitrogen Use by Phytoplankton Along Chesapeake Bay, Measured Using a Flow Cytometric Sorting Approach

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**Abstract** Two different approaches to measuring phytoplankton nitrogen (N) use were compared in late summer 2004 along the main axis of Chesapeake Bay. Uptake of  $^{15}\text{N}$ -labeled ammonium and nitrate and dual-labeled ( $^{15}\text{N}$  and  $^{13}\text{C}$ ) urea and dissolved free amino acids (DFAA) were measured in surface water samples from upper, mid, and lower bay stations. Two distinct methods were used to assess the relative uptake of N substrates by phytoplankton and correct for bacterial artifacts: (1) traditional filtration using Whatman glass fiber (GF/F) filters and (2) flow cytometric (FCM) sorting of chlorophyll-containing cells. The concentration of dissolved inorganic N (DIN) decreased with distance south along the bay, whereas dissolved organic N (DON) concentrations were relatively constant. Absolute N uptake rates measured using the traditional approach exceeded those of FCM-sorted phytoplankton, thereby suggesting the possibility of bacterial “contamination.” Ammonium was the dominant N form used throughout the transect, although FCM-sorted phytoplankton relied more on urea and DFAA as the ratio of DON/DIN increased toward the bay mouth. Overall, ammonium comprised  $74\pm 17\%$ , urea

$10\pm 9\%$ , DFAA  $9\pm 7\%$ , and nitrate  $7\pm 12\%$  of total measured N uptake by phytoplankton. Results suggest that bacteria relied primarily on DFAA and ammonium for N nutrition but also used N from urea at a rate similar to that of phytoplankton, whereas bacterial nitrate uptake was insignificant. On average, phytoplankton uptake of ammonium, urea, and DFAA was overestimated by 61%, 53%, and 135%, respectively, as a result of bacterial retention on GF/F filters.

**Keywords** Nitrogen uptake · Flow cytometry · Phytoplankton · Bacteria · Dissolved organic nitrogen · Dissolved inorganic nitrogen

## Introduction

Despite extensive research on the roles of phytoplankton and heterotrophic bacteria in nitrogen (N) cycling, relatively little is known about how these two groups compete when forced to share limited N resources. Traditionally, phytoplankton were believed to use primarily dissolved inorganic N (DIN), such as ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ), to meet their N demand, followed by release of dissolved organic N (DON), which fueled bacterial production and remineralization (e.g., Pomeroy 1974). However, DON substrates such as urea, dissolved free amino acids (DFAA), dissolved combined amino acids, and humics can contribute substantially to phytoplankton N nutrition (Bronk et al. 2007; Glibert et al. 1991; See et al. 2006). Furthermore, heterotrophic bacteria supplement their DON consumption with uptake of  $\text{NH}_4^+$  (Kirchman 2000; Wheeler and Kirchman 1986) or  $\text{NO}_3^-$  (Allen et al. 2002; Kirchman 2000; Kirchman and Wheeler 1998).

The factors controlling DON versus DIN consumption by heterotrophic bacteria and its effect on phytoplankton

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dynamics are important but unresolved pieces of the marine N cycle. Bacterial DIN use, for example, may be relatively high in estuarine waters (e.g., Middelburg and Nieuwenhuize 2000), depending on the supply of carbon (C)-rich, terrestrially derived organic matter (Gardner et al. 1996; Goldman and Dennett 1991). Others have suggested that bacterial  $\text{NH}_4^+$  use increases from estuarine to coastal waters as amino acid availability decreases (Hoch and Kirchman 1995; Kirchman 2000). Regardless, competition between phytoplankton and bacteria for DIN in Chesapeake Bay likely peaks in late summer when warmer water temperatures enhance metabolic activity, surface water DIN concentrations are minimal, and phytoplankton productivity and biomass are N-limited (Fisher et al. 1999; Kemp et al. 2005). As such, increased bacterial DIN use may exacerbate phytoplankton N limitation, diminish primary productivity or biomass accumulation (e.g., Joint et al. 2002), or exert a selective pressure favoring phytoplankton taxa that can either compete effectively with bacteria for DIN or use available DON (Kirchman 2000).

Phytoplankton and bacteria play distinct, yet equally significant, roles in N cycling and energy transfer; however, the methodological difficulty in separating these two groups continues to limit our understanding of their N nutrition. The preferred technique to date has been filtration, targeting the size difference between phytoplankton and bacteria (e.g., Allen et al. 2002; Kirchman and Wheeler 1998; Wheeler and Kirchman 1986), often using glass fiber filters (e.g., Whatman GF/F) to retain phytoplankton. However, GF/F filters, which have a nominal pore size of 0.7  $\mu\text{m}$ , also typically retain over 50% of the bacterial community in coastal and estuarine waters (Gasol and Morán 1999; Lee and Fuhrman 1987). An alternative approach is to distinguish between prokaryotic and eukaryotic N assimilation using chemicals that selectively inhibit protein synthesis (Middelburg and Nieuwenhuize 2000; Wheeler and Kirchman 1986). The value of this method is weakened, however, by inadequate effectiveness and specificity of the inhibitors (Oremland and Capone 1988), which can lead to inconsistent results (Veuger et al. 2004). Molecular techniques that identify the presence and expression of N assimilation genes in various microbial groups are promising (reviewed in Zehr and Ward 2002) but provide qualitative rather than quantitative estimates of N uptake. None of these approaches are ideal for accurately quantifying phytoplankton-specific or bacteria-specific N use in marine ecosystems.

A newer approach to physically separate phytoplankton and bacteria is flow cytometric (FCM) sorting of pigmented (i.e., autotrophic) cells from concentrated field samples. Flow cytometry was first applied to marine planktology over two decades ago to rapidly and precisely estimate picophytoplankton and nanophytoplankton abundance (Yentsch et al. 1983). Although FCM has been used extensively to describe microbial community structure by enumeration of phyto-

plankton (e.g., Campbell et al. 1994), heterotrophic bacteria (e.g., Monger and Landry 1993), and marine viruses (Marie et al. 1999), it has also been used to analyze marine particulate organic matter (Minor et al. 1998) and estimate grazing rates by zooplankton and bivalves (reviewed in Olson et al. 1991). Using FCM sorting, one can isolate microorganisms of interest based on specific cellular properties, such as size or pigment autofluorescence. Paaou et al. (1979) were the first to separate algal cells from bacteria using this approach, and others have similarly quantified primary production (Li 1994), bacterial activity (Servais et al. 1999), phytoplankton growth rates (Casey et al. 2007; Pel et al. 2004), and N assimilation (Lipschultz 1995) on a per-cell basis.

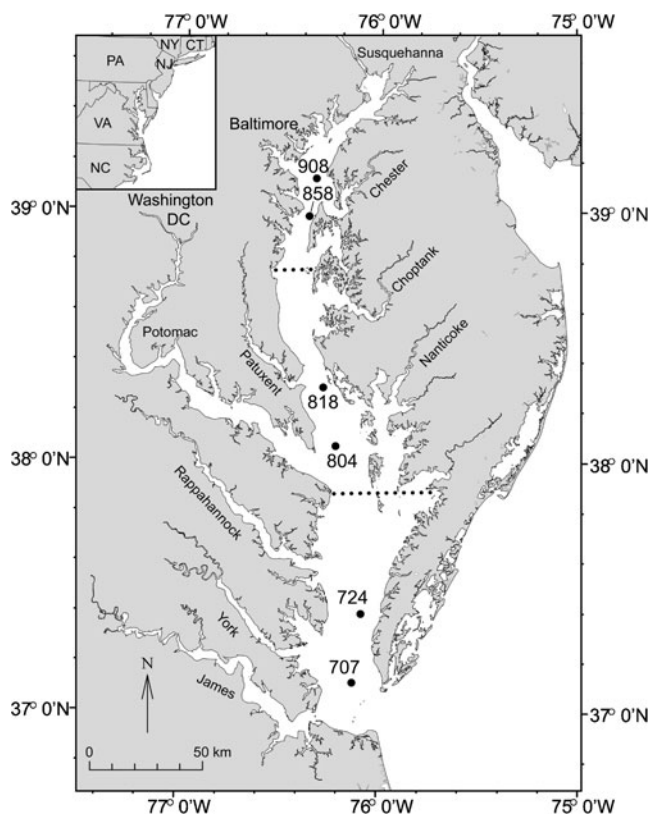
In the present study, we used  $^{15}\text{N}$  tracer techniques with both traditional filtration and FCM sorting of phytoplankton cells to measure uptake of different DIN and DON substrates in Chesapeake Bay. The goals were to: (1) examine the use of DIN and DON by phytoplankton in Chesapeake Bay surface waters during the N-limited late summer and (2) use FCM sorting to evaluate the effect of bacterial retention on uptake rates measured using the traditional approach. We hypothesized that FCM sorting more accurately measures true phytoplankton uptake, whereas traditional filtration overestimates N uptake by phytoplankton due to inclusion of bacteria. We also hypothesized that phytoplankton N use shifts with availability, from DIN-based in the upper bay to more DON-based in the lower bay.

## Study Location

During a cruise aboard the R/V *Cape Henlopen*, a total of six stations were sampled along the main axis of Chesapeake Bay from 29 August to 02 September 2004 (Fig. 1). At each station, water was collected near the surface (2 m) using a Niskin rosette, and depth profiles of salinity, temperature, oxygen, and fluorescence were characterized with a Sea-Bird Electronics 911 Plus CTD. Samples were taken in the morning at each station, starting with the two upper bay stations, 858 and 908, on 30 August, followed by the lower bay stations, 707 and 724, on 31 August, and the mid bay stations, 818 and 804, on 01 September.

## Methods

**Dissolved and Particulate N and C Concentrations** Samples for nutrient analyses were filtered using combusted (450°C for 2 h) Whatman GF/F filters and kept frozen at  $-20^\circ\text{C}$ . Ammonium concentrations were measured manually with the phenol-hypochlorite method (Koroleff 1983), and a Lachat



**Fig. 1** Stations sampled during a north–south transect of Chesapeake Bay. Dotted lines delineate the upper, mid, and lower bay regions

QuikChem 8500 autoanalyzer was used with the Parsons et al. (1984) colorimetric technique to measure  $\text{NO}_3^-$  and nitrite ( $\text{NO}_2^-$ ) concentrations. Urea was determined using the manual monoxime method (Price and Harrison 1987), whereas DFAA concentrations were measured as total DFAA using the fluorometric *o*-phthalaldehyde method (Parsons et al. 1984). Total dissolved N was measured using the persulfate oxidation technique (Bronk et al. 2000). Concentrations of DON were determined as the difference between total dissolved N (TDN) and DIN; the errors from the TDN,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NO}_2^-$  measurements were propagated to provide a standard error for DON estimates. A Shimadzu TOC-V was used with high-temperature combustion to measure dissolved organic C (DOC) concentrations (Hansell et al. 1997). Particulate N (PN) and organic C (POC) concentrations were determined from filters used to terminate isotopic tracer experiments on a Europa Geo 20/20 isotope ratio mass spectrometer equipped with an Automated Nitrogen and Carbon Analyzer for Solids and Liquids sample processing unit.

**Uptake Experiments** Stable isotope tracer techniques were used to quantify uptake rates of inorganic and organic N by distinct components of the microbial community. To this end, the following four substrates were added separately to

duplicate water samples:  $^{15}\text{N}$ -labeled  $\text{NH}_4^+$  and  $\text{NO}_3^-$  and dual-labeled ( $^{15}\text{N}$ ,  $^{13}\text{C}$ ) urea and DFAA (an algal extract containing 16 amino acids; Cambridge Isotope Laboratories, Andover, MA, USA). Tracer-level additions (<10% of ambient concentrations) of  $^{15}\text{NH}_4^+$  and  $^{15}\text{NO}_3^-$  were estimated from historic data provided by the Chesapeake Bay Program ([www.chesapeakebay.net](http://www.chesapeakebay.net)). Additions of labeled urea and DFAA were based on published data for Chesapeake Bay and adjacent coastal waters. To correct  $\text{NH}_4^+$  uptake rates for isotope dilution,  $\text{NH}_4^+$  was isolated from the post-incubation filtrate using solid-phase extraction (Brzezinski 1987; Selmer and Sörensson 1986), the product was analyzed using mass spectrometry, and the isotopic enrichment of the substrate pool was calculated according to Glibert et al. (1982). Isotope dilution of the  $\text{NO}_3^-$ , urea, and DFAA pools was not measured. Although uptake of these substrates may therefore be underestimated, the comparison between the two different methodological approaches, which is the primary focus of this paper, is unaffected by isotope dilution. Furthermore, given the low  $^{15}\text{N}$  enrichment values for cells in the  $\text{NO}_3^-$ , urea, and DFAA incubations, even extensive dilution of these substrates would not change the primary conclusions presented here.

Immediately after sampling and CTD retrieval, eight 500-mL polyethylene (PETG) bottles (four substrates, in duplicate) were filled with surface water and spiked with  $^{15}\text{N}$  tracer. The PETG bottles were then incubated on deck for 1–3 h in flow-through incubators under simulated in situ light and temperature conditions. Incubations were terminated using varying filtration approaches to examine different components of the microbial community. A portion (150 mL) of each bottle was filtered through a combusted 25 mm GF/F filter to obtain uptake rates for what has traditionally been referred to as phytoplankton, although as noted above, this fraction also contains some percentage of the bacterial community. Another 150 mL of sample was first passed through 35- $\mu\text{m}$  mesh to remove larger plankton that could clog the flow cytometer, and these cells were then washed onto a GF/F filter in order to measure their biomass and isotopic enrichment. All GF/F filters were immediately frozen and stored at  $-20^\circ\text{C}$  until isotopic analysis on the mass spectrometer. The <35- $\mu\text{m}$  filtrate was concentrated over a 47-mm, 0.2- $\mu\text{m}$  Supor filter to a final volume of 5–12 mL, which was then transferred to a centrifuge tube. The Supor filter was occasionally rinsed during concentration using a 10-mL pipette and also by placing the rolled filter into the centrifuge tube with the concentrated sample and inverting gently several times. The concentrated sample was preserved with paraformaldehyde at a final concentration of 0.2% (Campbell 2001) and then frozen in liquid N.

An analysis of this concentration technique was conducted in the York River, a tributary of Chesapeake Bay, and showed that little phytoplankton material, if any, is lost

to the Supor filter. The amount of chlorophyll *a* (Chl *a*) in samples concentrated from 100–200 to 10 mL averaged  $95\pm 3\%$  of whole-water (unconcentrated) Chl *a*, whereas the Supor filter retained  $3\pm 1\%$  of Chl *a*. Increasing the concentration factor using an initial volume of 300 mL (twice that of this study) resulted in a greater loss of Chl *a* to the Supor filter, with  $89\pm 3\%$  in the concentrated sample and  $12\pm 3\%$  of the Chl *a* remaining on the Supor filter (Bradley, unpublished data).

**FCM Sorting of Phytoplankton** Duplicate samples for FCM sorting were kept at  $-80^{\circ}\text{C}$  until analysis, whereupon they were thawed at room temperature. Phytoplankton cells were identified and sorted based on their chlorophyll autofluorescence using an Influx V-GS flow cytometer (Becton Dickinson) located at the Bermuda Institute of Ocean Sciences. The Influx V-GS is designed for stable, high-speed sorting, which enabled high phytoplankton yield at sort rates of 2,000 to 10,000 cells per second. Phytoplankton cells were sorted into polypropylene tubes and filtered onto GF/F filters, which were stored at  $-20^{\circ}\text{C}$  until isotopic analysis on the Europa mass spectrometer described above. To obtain N masses sufficiently above the Europa's detection limit for reliable  $^{15}\text{N}$  atom percent enrichment values,  $1\text{--}2\ \mu\text{g}$  N of carrier  $[(\text{NH}_4)_2\text{SO}_4]$  was added to the filters prior to analysis. The original sample's N mass, which ranged from 0.3 to  $3.3\ \mu\text{g}$  N, was then determined using a standard carrier correction by deducting the carrier amount from the total measured N mass. Isotopic enrichment of the original sample was calculated using the proportion of total N mass as sample versus carrier, as well as the respective isotopic enrichments of these two components.

The accuracy of autotrophic sorting was periodically verified by collecting and reanalyzing the sorted and waste streams, and the presence of bacteria in sorted samples was quantified via epifluorescence microscopy using acridine orange direct counts (Sherr et al. 2001). To evaluate the extent of any negative effects of the FCM sorting method on cellular integrity or retention of  $^{15}\text{N}$  label, a simple experiment was conducted at three stations using boiling distilled water to deliberately rupture phytoplankton cells during filtration for both GF/F and FCM-sorted fractions. Before the GF/F or Supor filters went dry, 25 mL of boiling distilled water was added, and the filtration or FCM concentration procedures were completed. These samples were later processed identically to those described above. To assess whether exposure to boiling water caused additional loss of internal N from preserved and sorted cells, absolute N uptake rates were calculated for GF/F and FCM samples as described below, but with the PN measured from filters analyzed on the mass spectrometer. If preservation and FCM sorting caused cells to rupture, the uptake rates for the control and boiled treatments would be

statistically equal in the FCM-sorted samples and significantly different in the GF/F fraction.

**Calculation of N Uptake Rates** Specific ( $V$ ,  $\text{h}^{-1}$ ) and absolute ( $\rho$ ,  $\mu\text{mol N L}^{-1} \text{h}^{-1}$ ) N uptake rates were calculated using the equations of Dugdale and Goering (1967). Specific rates are useful when comparing N uptake patterns and preferences among phytoplankton and bacteria for various substrates, whereas absolute rates illustrate how including bacterial biomass on GF/F filters can skew measurements of phytoplankton N uptake.

To examine the accuracy of GF/F-based measurements of autotrophic N use, total phytoplankton-only (Phyto) uptake rates were calculated by combining absolute uptake rates in the FCM and  $>35\text{-}\mu\text{m}$  fractions. Concentrations of PN were measured directly in the  $>35\text{-}\mu\text{m}$  and GF/F fractions. However, because of the uncertainty in volume of original FCM sample sorted, it was not possible to obtain direct, accurate measurements of autotrophic PN from the mass spectrometer. Therefore, Phyto PN was estimated by correcting GF/F PN for bacterial biomass retained on these filters. Bacterial abundance was measured in surface samples from each station using epifluorescence microscopy (K. Wang, unpublished data) and was converted to total bacterial biomass using a N content of 12 fg N per cell (Vrede et al. 2002). Assuming that 50% of bacteria were retained on GF/F filters, Phyto PN was estimated by subtracting 50% of total bacterial biomass from PN measured for the GF/F fraction.

The assumption that GF/F filters retained 50% of the bacterial biomass is supported in the literature for various ecosystems (e.g., Gasol and Morán 1999; Lee et al. 1995). In addition, Glibert et al. (1995) estimated that 50% of bacteria are retained by GF/F filters in Chesapeake Bay, and recent measurements in the York River, one of its tributaries, ranged from 53% to 71% (Bradley, unpublished data). Furthermore, 50% is a conservative estimate because bacterial biomass in Chesapeake Bay tends to peak in late summer, and the above studies examined retention of bacteria by abundance rather than biomass. Presumably, larger bacteria are captured by the GF/F matrix and have proportionally more biomass than smaller cells that pass through the filter.

## Results

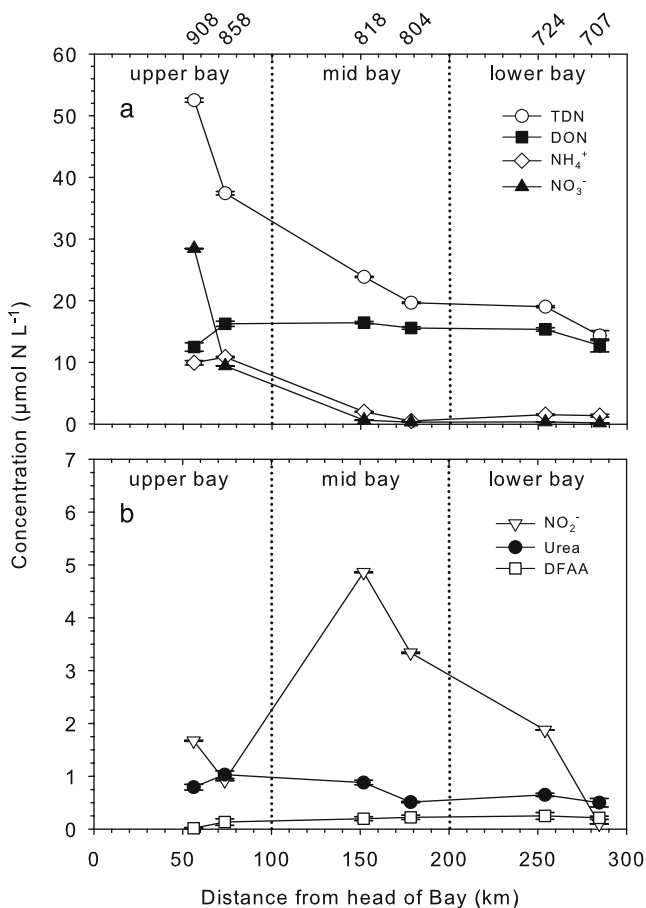
### Environmental Conditions

Depth profiles captured by CTD varied considerably along Chesapeake Bay, with surface salinity increasing from 4 at stations 908 to 24 at the Bay mouth (station 707). Surface temperatures varied relatively little throughout the Bay, from  $25.7^{\circ}\text{C}$  to  $27.6^{\circ}\text{C}$ , with the coldest values measured at

the mouth. Tropical Storm *Gaston*, which passed through the Bay on the night of 30 August, was a likely cause of stratification differences between the three bay regions. For example, station 858, which was sampled the morning of 30 August, had a strong pycnocline at 9- to 12-m depth. In contrast, the lower bay stations, which were sampled the following morning, had virtually no change in salinity, temperature, or oxygen with depth. The mid bay stations, 818 and 804, were moderately stratified at depths of 15–20 m, and it seems likely that the storm had weakened and deepened these pycnoclines. The effects of this disruption on N transformations and bioavailability are discussed in further detail below.

### Concentrations of Dissolved Nutrients and Biomass

Total dissolved N concentrations decreased by 73% from the northernmost station (908) to the mouth (station 707), concomitant with steady declines in ambient  $\text{NH}_4^+$  and



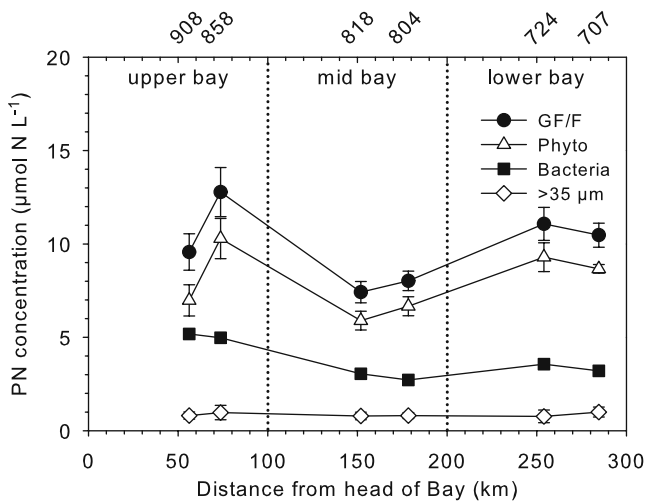
**Fig. 2** Concentrations ( $\mu\text{mol N L}^{-1}$ ) of **a** total dissolved nitrogen (TDN), dissolved organic N (DON), ammonium ( $\text{NH}_4^+$ ), and nitrate ( $\text{NO}_3^-$ ) and **b** nitrite ( $\text{NO}_2^-$ ), urea, and dissolved free amino acids (DFAA) along the main axis of Chesapeake Bay. Station numbers are shown at the top of the graph, and vertical dotted lines delineate the upper, mid, and lower bay regions. Error bars represent  $\pm 1$  SD of the mean

$\text{NO}_3^-$  along this transect (Fig. 2a). Accordingly, DIN comprised 76% and 11% of TDN at these two endpoints, respectively. Nitrate alone comprised 54% of TDN at station 908 but decreased southward to a minimum of  $0.2 \mu\text{mol N L}^{-1}$  at station 707. Surface water  $\text{NO}_2^-$  concentrations were relatively high throughout the bay and even dominated the mid bay DIN pool (Fig. 2b). Mid bay bottom waters are often hypoxic or anoxic during summer, creating low redox conditions in the sediment and water column that enhance  $\text{NH}_4^+$  flux from the former and inhibit nitrification in the latter (Kemp et al. 2005). The mixing, and subsequent nitrification, of  $\text{NH}_4^+$ -rich bottom water with oxygenated surface water during *Gaston* likely accounts for the high  $\text{NO}_2^-$  concentrations observed (Bronk et al. 1998; McCarthy et al. 1984).

In contrast to DIN, DON concentrations varied relatively little (Fig. 2a). Urea concentrations were significantly higher in the upper half of the Bay (stations 908, 858, and 818;  $p < 0.001$ ; Fig. 2b), whereas DFAA concentrations were significantly higher in the lower half (stations 804, 724, and 707;  $p < 0.01$ ; Fig. 2b). The contribution of DON to TDN increased southward, from 24% at station 908 to 89% at station 707, as available  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were biologically removed in the upper and mid bay. The DIN/DON ratio thus decreased from 3.2 at station 908 to 0.1 at station 707. The profile of DOC concentrations was similar to that of DON (data not shown), in that concentrations were highest in the mid bay ( $282.4 \pm 5.5 \mu\text{mol L}^{-1}$ ), followed by the upper bay ( $251.9 \pm 4.7 \mu\text{mol L}^{-1}$ ) and lower bay regions ( $232.9 \pm 7.0 \mu\text{mol L}^{-1}$ ). Ratios of DOC to DON were roughly similar along the bay, with values of  $17.9 \pm 3.6$ ,  $17.7 \pm 0.7$ , and  $16.7 \pm 1.7$  for the upper, mid, and lower bay, respectively.

Chlorophyll *a* values decreased from a high of  $38.1 \mu\text{g L}^{-1}$  at station 908 to  $5.9$ – $8.0 \mu\text{g L}^{-1}$  at the lower four stations (W. Coats, unpublished data; not shown), but PN values did not exhibit any distinct trends along Chesapeake Bay (Fig. 3). Mean PN in the GF/F fraction was  $9.9 \pm 2.0 \mu\text{mol N L}^{-1}$ . Phytoplankton (Phyto: FCM +  $>35 \mu\text{m}$  cells) PN was 73% to 84% of GF/F PN and averaged  $8.0 \pm 1.7 \mu\text{mol N L}^{-1}$ . Bacterial abundance averaged  $5.9 \pm 0.2 \times 10^6$ ,  $3.4 \pm 0.3 \times 10^6$ , and  $3.9 \pm 0.3 \times 10^6$  cells per milliliter in the upper, mid, and lower bay, respectively (K. Wang, unpublished data; not shown). Bacterial PN, as estimated from bacterial abundance, averaged  $3.8 \pm 1.0 \mu\text{mol N L}^{-1}$  across all six stations. Particulate N concentrations in the  $>35$ - $\mu\text{m}$  fraction were considerably lower, with an overall mean of  $0.9 \pm 0.3 \mu\text{mol N L}^{-1}$ .

Concentrations of POC were measured on GF/F filters from urea and DFAA uptake samples in order to calculate POC/PN ratios. As for PN, Phyto POC concentrations were derived from GF/F filters by correcting for the assumed 50% of bacterial biomass these filters retain (see “Methods”) using bacterial abundance and a cellular C content of 65 fg C



**Fig. 3** Particulate N (PN) concentrations ( $\mu\text{mol N L}^{-1}$ ) measured along the main axis of Chesapeake Bay, with station numbers shown at the top of the graph. Phytoplankton (Phyto) PN was derived by correcting GF/F PN for retention of bacterial biomass, and bacterial PN values were estimated from bacterial abundance. Error bars represent  $\pm 1$  SD of the mean

per cell (Fukuda et al. 1998 and references therein; Vrede et al. 2002). The mean ratio of POC/PN in phytoplankton (Phyto) was  $8.7 \pm 1.1$ . The corresponding ratio from GF/F filters was  $7.3 \pm 0.8$ , which indicates that other constituents retained by these filters, such as bacteria or heterotrophic protists, were relatively N rich. The contribution of bacteria to particulate C/N ratios from GF/F filters was assessed using Phyto and bacterial PN, as proportions of total PN, as well as the above-mentioned C/N ratios for the Phyto and GF/F fractions. Overall, bacterial C/N averaged  $4.5 \pm 0.8$  across all six stations, which agrees well with values reported in the literature (Kirchman 2000).

#### FCM Methodological Considerations

In order to obtain sufficient N biomass for analysis on a mass spectrometer, a large number of phytoplankton cells (roughly  $5 \times 10^6$ ) must be sorted. As such, FCM sorting requires a compromise between purity and yield in the sorted sample. In this study, we favored a higher yield of sorted phytoplankton, resulting in the inclusion of a small percentage ( $7 \pm 3\%$  based on cell counts) of the bacterial community. Using a conversion factor of 12 fg N per cell (Vrede et al. 2002), this represents a bacterial biomass of  $0.28 \mu\text{mol N L}^{-1}$ , or 4% of phytoplankton PN, which was then diminished (by  $\sim 50\%$ ) with filtration of sorted samples onto GF/F filters. Therefore, bacterial influence on uptake rates calculated for FCM-sorted phytoplankton was minimal and likely  $< 2\%$  by mass.

An additional concern with the FCM method is that preserving, freezing, and sorting may damage cell mem-

branes, thus decreasing apparent uptake rates due to loss of N that was taken up during the incubation but not yet utilized. If this were the case, FCM-sorted rates would be a function of assimilation (i.e., N incorporated into biomass) rather than N uptake and would not differ between treatments, based upon the reasonable assumption that the tracer additions do not lead to short-term changes in growth rate. However, samples exposed to boiling distilled water had significantly lower ( $p < 0.05$ ) estimated absolute uptake rates than the control treatments for both the GF/F and FCM-sorted fractions (data not shown). This suggests that cellular integrity of FCM-sorted cells was similar to that of cells on GF/F filters and was only minimally compromised, if at all, by the method. Similarly, in methodological trials conducted using surface water collected from the York River, there was no significant difference ( $p = 0.147$ ) in N uptake rates measured from FCM samples sorted fresh versus after preservation, freezing, and thawing (data not shown). These results agree with the conclusions of Rivkin et al. (1986) that the cellular integrity of phytoplankton remains intact (i.e., no radioisotope is lost) during FCM sorting. It is unclear, however, to what extent this resilience varies between phytoplankton taxa and how it may fluctuate with changes in phytoplankton community structure over an annual cycle.

#### Specific and Absolute Uptake Rates

Efforts to keep  $^{15}\text{N}$  tracer additions under 10% of ambient concentrations (Dugdale and Goering 1967) had varying results. Addition of  $^{15}\text{NO}_3^-$  ranged from 2% to 34% (mean of 17%), added urea label was 10–20%, and DFAA additions exceeded 40% of ambient concentrations at all stations. Although some of these enrichments could raise concerns about artificial stimulation of uptake rates due to increased availability, the amount of tracer added ( $0.05$ – $0.10 \mu\text{mol N L}^{-1}$ ) and the low uptake rates for these three substrates relative to those for  $\text{NH}_4^+$  suggest that this would not affect our conclusions. For example,  $^{15}\text{NO}_3^-$  tracer additions were highest relative to ambient concentrations in the mid and lower bay. Though this addition may have resulted in enhanced uptake,  $\text{NO}_3^-$  contributed  $< 2\%$  of total N uptake by the GF/F fraction. Similarly,  $^{15}\text{N}$  enrichment from DFAA tracer was highest in the upper bay, where DFAA uptake rates were lowest. Labeled  $\text{NH}_4^+$  additions were 10% or less of ambient concentrations for all stations except 804 (43%). However, due to relatively high  $\text{NH}_4^+$  regeneration rates at station 804 ( $3.2 \pm 0.1 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ ; data not shown), isotopic enrichment of the  $\text{NH}_4^+$  substrate pool at the end of the incubation did not exceed 8%. Regeneration rates were similarly high at station 858 ( $3.1 \pm 0.1 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ ), moderate at station 908 ( $1.0 \pm 0.4 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ ), and low ( $< 0.4 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ ) at the remaining three stations (data not shown).

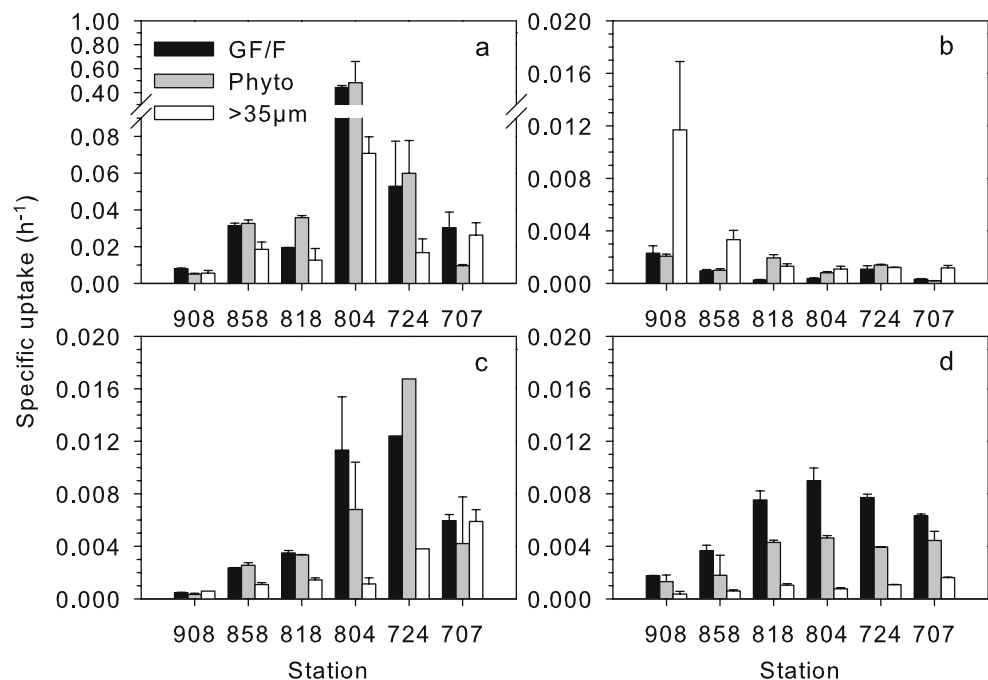
Specific uptake rates in the GF/F and FCM fractions were not significantly different across all stations for  $\text{NH}_4^+$  ( $p=0.969$ ) or urea ( $p=0.915$ ), and although  $\text{NH}_4^+$  and urea uptake rates by the  $>35\text{-}\mu\text{m}$  fraction were consistently lower than GF/F rates, the differences were not significant overall ( $\text{NH}_4^+$ :  $p=0.299$ , urea:  $p=0.111$ ; Fig. 4a, c). For  $\text{NO}_3^-$ , specific uptake by FCM-sorted phytoplankton always equaled or exceeded that of the GF/F fraction, yet  $\text{NO}_3^-$  uptake rates were highest in the  $>35\text{-}\mu\text{m}$  fraction at stations 908, 858, 804, and 707 (Fig. 4b). The opposite trend held true for DFAA; uptake rates in the GF/F fraction were significantly higher than both FCM uptake ( $p<0.05$ ) and  $>35\text{-}\mu\text{m}$  uptake ( $p<0.001$ ; Fig. 4d). With respect to spatial trends, specific uptake of  $\text{NO}_3^-$  by the  $>35\text{-}\mu\text{m}$  fraction generally decreased toward the bay mouth, whereas urea uptake by the FCM and  $>35\text{-}\mu\text{m}$  fractions generally increased southward, and  $\text{NH}_4^+$  uptake rates exhibited a mid-bay peak.

In order to compare GF/F rates with those of phytoplankton only, absolute uptake rates were calculated for the Phyto fraction from the sum of FCM and  $>35\text{-}\mu\text{m}$  rates (Fig. 5). Except for  $\text{NO}_3^-$ , absolute uptake rates were relatively minor in the  $>35\text{-}\mu\text{m}$  fraction due to low total biomass of these larger cells. Although absolute uptake rates for all substrates were consistently higher in the GF/F versus Phyto fraction, the differences across all stations were only significant for DFAA ( $p<0.01$ ), which had rates in the GF/F fraction that were two to three times those of Phyto. On average, absolute  $\text{NH}_4^+$  uptake rates were more than ten times higher than those of the other three substrates

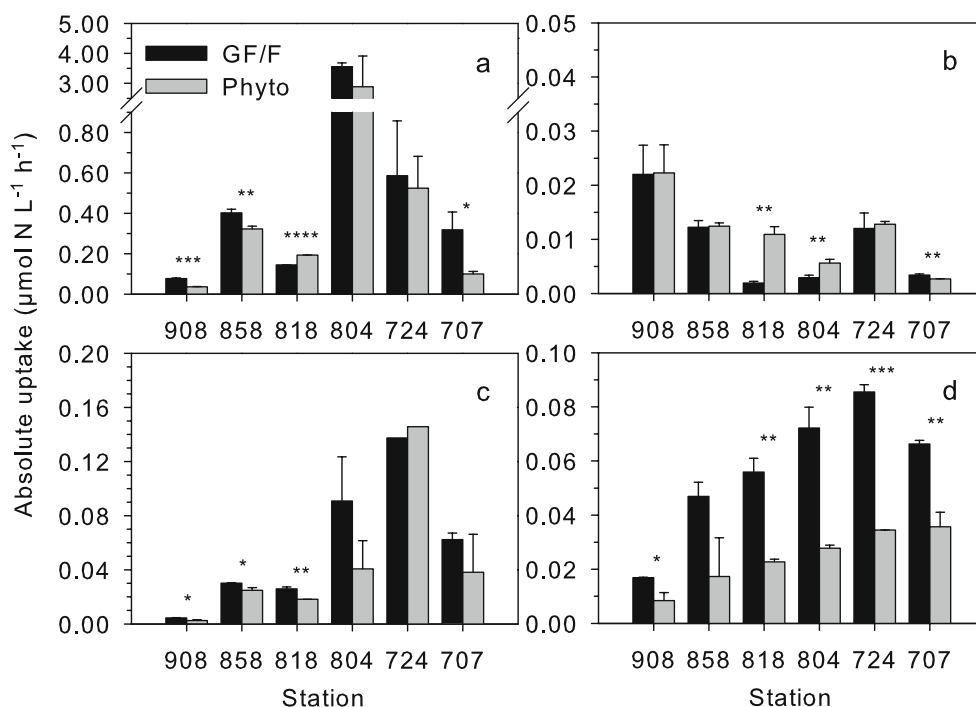
and comprised 74% of total measured N uptake in both the GF/F and Phyto fractions. With the exception of the northernmost station, 908, absolute  $\text{NO}_3^-$  uptake rates were the lowest of the four substrates used and generally decreased toward the bay mouth (Fig. 5b). Absolute uptake rates for urea and DFAA, on the other hand, were highest in the lower half of Chesapeake Bay (Fig. 5c, d). Overall, phytoplankton in the FCM and  $>35\text{-}\mu\text{m}$  fractions relied slightly more on  $\text{NO}_3^-$  and urea and slightly less on DFAA than did the mixed assemblage retained by GF/F filters (Fig. 6). Furthermore, the importance of  $\text{NO}_3^-$  to these two fractions tended to decrease southward; in contrast, urea and DFAA together comprised 9–17% of total Phyto uptake in the upper bay and 22–47% in the lower bay.

Dual-labeled ( $^{15}\text{N}$  and  $^{13}\text{C}$ ) urea and DFAA tracers provided a means of calculating C uptake for these two organic substrates. Although phytoplankton and bacteria used the N from urea, there was no  $^{13}\text{C}$  enrichment in any of the samples, which suggests that the C was immediately respired (as  $\text{CO}_2$ ) following cleavage of N groups by urease. In contrast,  $^{13}\text{C}$ -DFAA uptake rates in the FCM fraction ranged from zero in the upper bay to  $0.028\text{ }\mu\text{mol C L}^{-1}\text{ h}^{-1}$  in the lower bay, with a mean of  $0.012\pm 0.012\text{ }\mu\text{mol C L}^{-1}\text{ h}^{-1}$  (data not shown). Rates from GF/F filters were  $0.062\text{--}0.270\text{ }\mu\text{mol C L}^{-1}\text{ h}^{-1}$ , with an overall mean of  $0.195\pm 0.068\text{ }\mu\text{mol C L}^{-1}\text{ h}^{-1}$ . The order of magnitude difference in mean  $^{13}\text{C}$ -DFAA uptake rate between fractions reflects the contribution of heterotrophic bacteria to GF/F-based rates and, relative to N uptake rates, more clearly defines the trophic roles of phytoplankton and bacteria.

**Fig. 4** Specific uptake rates ( $\text{h}^{-1}$ ) of **a**  $\text{NH}_4^+$ , **b**  $\text{NO}_3^-$ , **c** urea, and **d** DFAA for the GF/F, FCM, and  $>35\text{-}\mu\text{m}$  fractions. Specific uptake rates for the FCM fraction were calculated from FCM-sorted phytoplankton. Note the axis break and much higher scale for  $\text{NH}_4^+$ . Error bars represent  $\pm 1$  SD of the mean



**Fig. 5** Absolute uptake rates ( $\mu\text{mol N L}^{-1} \text{h}^{-1}$ ) of **a**  $\text{NH}_4^+$ , **b**  $\text{NO}_3^-$ , **c** urea, and **d** DFAA for the GF/F and phytoplankton-only (Phyto) fractions. Absolute uptake rates for the latter represent the combined uptake of FCM-sorted and  $>35\text{-}\mu\text{m}$  phytoplankton and were calculated using the estimated Phyto particulate N concentrations shown in Fig. 3. Asterisks denote significant differences between fractions, as determined using Student's *t* tests (\* $p < 0.1$ , \*\* $p < 0.05$ , \*\*\* $p < 0.01$ , \*\*\*\* $p < 0.001$ ). Note the difference in y-axis scales. Error bars represent  $\pm 1$  SD of the mean



## Discussion

Flow cytometric sorting is a powerful tool for distinguishing between phytoplankton and bacterial cellular activity. Nitrogen uptake rates measured using the traditional GF/F method, versus FCM sorting, are discussed below both in the context of how the former often overestimates phytoplankton N use and also with respect to N cycling by phytoplankton and heterotrophic bacteria in Chesapeake Bay.

### Interpretation of FCM and GF/F Uptake Rates

The use of GF/F filters to separate phytoplankton from the microbial community can produce ambiguous results when determining autotrophic N uptake and assimilation because they capture a mixed assemblage of phytoplankton and heterotrophic bacteria. However, uptake rates measured using GF/F filters have traditionally been attributed to phytoplankton despite the fact that a substantial fraction of the bacterial community is also retained. The inclusion of bacterial biomass increases PN and, by extension, absolute uptake rates when measured using GF/F filters, thereby potentially overestimating phytoplankton N uptake.

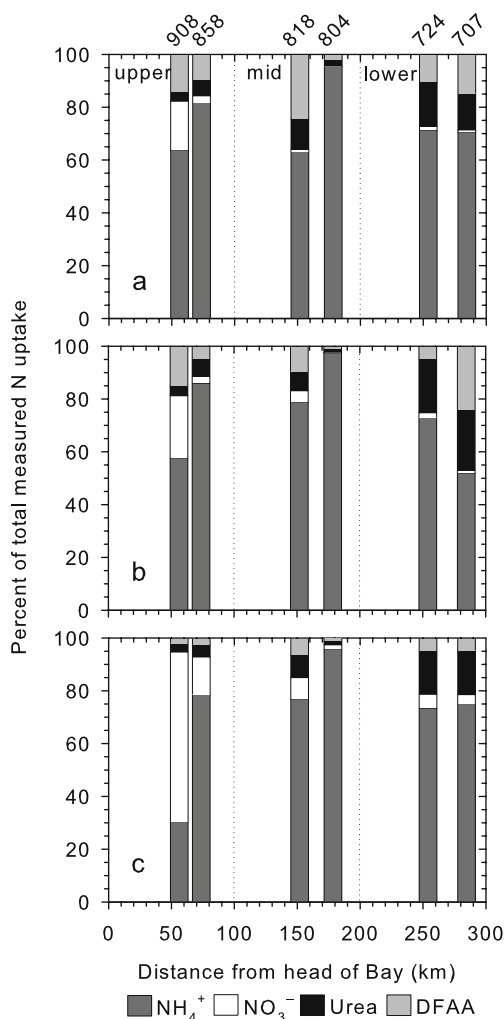
Specific N uptake rates provide a means of comparing the physiological ability of different size fractions or different types of cells to use  $^{15}\text{N}$  tracers and are not affected by varying biomass, as are absolute uptake rates. Therefore, a unique perspective of phytoplankton and bacterial N use can be obtained by comparing specific uptake rates from the fractions studied here. If bacteria retained on GF/F filters

have a strong preference for a given N substrate compared to that of phytoplankton, specific N uptake rates calculated from these filters will be higher than those of phytoplankton only (e.g., FCM fraction). Conversely, the inclusion of relatively  $^{15}\text{N}$ -deficient bacteria (due to low uptake of a substrate) on GF/F filters will dilute the isotopic signal in the PN pool, resulting in lower specific uptake rates and the underestimation of the actual phytoplankton N uptake rate for that substrate.

Application of this analysis to the data presented here for Chesapeake Bay provides insight that varies by substrate. The highest specific  $\text{NO}_3^-$  uptake rates were measured in the FCM-sorted phytoplankton and  $>35\text{-}\mu\text{m}$  fractions (Fig. 4). Relatively low specific uptake rates in the GF/F fraction were due to a lack of bacterial  $\text{NO}_3^-$  use and isotopic dilution of the PN pool by unlabeled ( $^{14}\text{N}$ ) bacterial biomass on these filters. In contrast to  $\text{NO}_3^-$ , specific DFAA uptake rates were highest in the GF/F fraction as a result of strong bacterial preference for this organic substrate relative to that of phytoplankton. The trends for  $\text{NH}_4^+$  and urea, however, were not as consistent across all samples. Overall, specific uptake rates of these two reduced N substrates were roughly equal between the GF/F and FCM fractions, suggesting that bacteria and phytoplankton utilized each substrate similarly at most stations.

In contrast to specific uptake rates, two separate factors can affect measurements of absolute N uptake by phytoplankton using GF/F filters: (1) the enrichment (or dilution) of  $^{15}\text{N}$  in the PN pool due to bacterial uptake (or lack





**Fig. 6** Percent contribution of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , urea, and DFAA to total measured N uptake by the **a** GF/F, **b** FCM-sorted phytoplankton, and **c** >35- $\mu\text{m}$  fractions in Chesapeake Bay surface waters. Vertical dotted lines delineate the upper, mid, and lower bay regions

thereof), as previously discussed, and (2) the overestimation of phytoplankton PN as a result of bacterial retention on GF/F filters. The former can bias uptake rates in either direction, but the latter is unidirectional; in other words, retention of bacteria on a GF/F will always produce erroneously higher values of phytoplankton PN and therefore increase the reported absolute uptake rate. Furthermore, this overestimation of phytoplankton PN as a result of bacterial retention on GF/F filters offsets, to some extent, underestimations of phytoplankton uptake due to low bacterial N use. For example, specific uptake rates indicate that bacteria in Chesapeake Bay were not using  $^{15}\text{NO}_3^-$  as much as phytoplankton were, yet absolute uptake rates were roughly equal between the GF/F and Phyto fractions at most stations (Fig. 5b) as a result of the compensatory effect of bacterial biomass on PN values from GF/F filters. Theoretically, however, absolute uptake rates by the Phyto fraction cannot

exceed those of the GF/F fraction as it did at stations 818 and 804; this may have been due to analytical error associated with isotopic measurements just above detection limits. Nonetheless, using specific uptake rates, one can examine the effect of both bacterial N use and bacterial biomass on absolute uptake rates determined from GF/F filters.

One goal for this research was to assess the extent to which GF/F filters overestimate autotrophic uptake of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , urea, and DFAA. Percent overestimation was calculated by subtracting absolute Phyto uptake rates from absolute GF/F rates, then dividing by absolute Phyto uptake rates and expressing the quotient as a percentage. On average, use of GF/F filters overestimated phytoplankton uptake of  $\text{NH}_4^+$ , urea, and DFAA by 61%, 53%, and 135%, respectively (Table 1). As discussed above, Phyto absolute uptake rates should not exceed those of GF/F filters (exceptions shown as negative values in Table 1). Although this was the case for  $\text{NO}_3^-$  at every station except 707, Phyto absolute rates were only significantly higher than GF/F rates at stations 804 and 818 ( $p < 0.05$ ), perhaps as a result of very low measured uptake rates. Removal of these two stations results in an overestimation of phytoplankton  $\text{NO}_3^-$  uptake of  $5 \pm 15\%$  by GF/F filters.

#### Phytoplankton and Bacterial N Use in Chesapeake Bay

Several conclusions can be drawn from the results of this study with respect to traditional views of phytoplankton and bacterial N preferences. Concentrations of DIN tend to vary considerably between bay segments and across seasons, but our range of 1.6–40.0  $\mu\text{mol N L}^{-1}$  for DIN agrees well with historical measurements for summer (e.g., Harding 1994). The TDN pool was dominated by  $\text{NO}_3^-$  at the northernmost station and by DON in the mid and lower bay, yet  $\text{NH}_4^+$  contributed most to total N uptake along the entire transect. Indeed, Chesapeake Bay surface waters are typically dominated by heterotrophic processes during summer, with regeneration of reduced N forms exceeding autotrophic uptake (Bronk et al. 1998; Smith and Kemp 1995). Ammonium uptake was particularly high at station 804 ( $3.6 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ ) but was balanced by a regeneration rate of  $3.2 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ . This uptake rate is higher than previous measurements in Chesapeake Bay for August (e.g., Bronk et al. 1998), but not as high as some rates measured elsewhere (Twomey et al. 2005). The nearly exclusive use of  $\text{NH}_4^+$  at station 804 was also measured in samples taken from a dense algal bloom located just north of station 818. Ammonium uptake rates measured using GF/F filters on samples from this algal bloom were nearly double those of station 804 ( $6.7 \pm 0.3 \mu\text{mol N L}^{-1} \text{ h}^{-1}$ ) and comprised 94% of total measured N uptake. These results, which are not presented in further detail because samples

**Table 1** Percent overestimation of absolute ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), urea, and dissolved free amino acid (DFAA) uptake rates by phytoplankton using GF/F filters

Bay segment	Station	$\text{NH}_4^+$	$\text{NO}_3^-$	Urea	DFAA
Upper	908	114	-1	73	100
	858	25	-2	21	171
Mid	818	-25	-82	42	146
	804	23	-48	123	160
Lower	724	12	-6	-6	148
	707	219	27	63	86
	Mean $\pm$ 1 SD	61 $\pm$ 90%	-19 $\pm$ 39%	53 $\pm$ 45%	135 $\pm$ 34%

Data were calculated by subtracting the Phyto (FCM + >35  $\mu\text{m}$ ) absolute uptake rate from that of the GF/F fraction and expressing this difference as a percentage of the Phyto absolute uptake rate. Positive values represent overestimations of phytoplankton N uptake by GF/F filters

for FCM sorting were not taken, suggest that the N dynamics at station 804 may be symptomatic of a similar bloom. Flagellated algal blooms, especially those of the dinoflagellate *Prorocentrum minimum*, are common in Chesapeake Bay and its tributaries during summer (Glibert et al. 2001). The mean  $\text{NH}_4^+$  uptake rate from stations other than 804 was 0.3  $\mu\text{mol N L}^{-1} \text{h}^{-1}$ , which is similar to rates from other studies for late summer (Bronk et al. 1998; Glibert et al. 1991).

Phytoplankton are believed to use  $\text{NH}_4^+$  preferentially over  $\text{NO}_3^-$  when both are available, primarily due to the energetic cost of  $\text{NO}_3^-$  assimilation. Furthermore,  $\text{NH}_4^+$  can inhibit the uptake and assimilation of  $\text{NO}_3^-$ , particularly at  $\text{NH}_4^+$  concentrations exceeding 1  $\mu\text{mol N L}^{-1}$  (Cochlan and Bronk 2003; Dortch 1990; Lomas and Glibert 1999). With the exception of the >35- $\mu\text{m}$  fraction at station 908,  $\text{NO}_3^-$  uptake was always lower than that of  $\text{NH}_4^+$ , even in the upper bay where  $\text{NO}_3^-$  concentrations were highest. This may suggest that  $\text{NH}_4^+$  was inhibiting  $\text{NO}_3^-$  uptake in Chesapeake Bay surface waters during this study; however,  $\text{NO}_3^-$  uptake was more closely related to  $\text{NO}_3^-$  availability than that of  $\text{NH}_4^+$ . Specific  $\text{NO}_3^-$  uptake rates in the >35- $\mu\text{m}$  fraction were strongly correlated with ambient  $\text{NO}_3^-$  concentrations along the bay (Pearson's correlation,  $r^2=0.99$ ,  $p<0.0001$ ). This relationship was also significant for the GF/F fraction ( $r^2=0.85$ ,  $p<0.01$ ), but not for FCM-sorted phytoplankton ( $r^2=0.29$ ,  $p=0.268$ ). In contrast, the relationship between specific  $\text{NO}_3^-$  uptake and ambient  $\text{NH}_4^+$  concentrations was not significant for any fraction. These results suggest that although  $\text{NH}_4^+$  may have inhibited  $\text{NO}_3^-$  uptake, it is also possible that only large phytoplankton (>35  $\mu\text{m}$  and GF/F filters, which include the >35- $\mu\text{m}$  fraction) had a strong preference for  $\text{NO}_3^-$ , whereas smaller phytoplankton (i.e., FCM-sorted cells) preferred  $\text{NH}_4^+$  (e.g., Lipschultz 1995).

Concentrations of DON varied little along the transect relative to DIN, and our mean value of 14.8  $\mu\text{mol N L}^{-1}$  was somewhat low compared to values in excess of

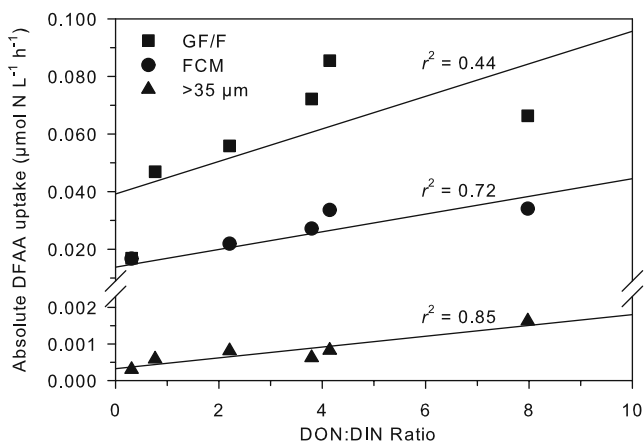
40  $\mu\text{mol N L}^{-1}$  measured by Bronk et al. (1998) but was more consistent with those of other studies (Bronk 2002; McCarthy et al. 1977). Although a large fraction of the DON pool is likely unavailable for phytoplankton use (Bronk et al. 2007), uptake of urea, for example, has been shown to support phytoplankton N nutrition in Chesapeake Bay and its plume, especially under DIN-limited conditions (Glibert et al. 1991; McCarthy et al. 1977). Urea concentrations presented here were 0.50–1.03  $\mu\text{mol N L}^{-1}$ , and most values have historically fallen within this range for Chesapeake Bay (Lomas et al. 2002). Although urea availability generally decreased toward the bay mouth, the contribution of this organic substrate to total measured N uptake by the Phyto fraction increased from 4% at station 908 to 22% at station 707. Furthermore, there was a significant positive correlation between specific urea uptake by the >35- $\mu\text{m}$  fraction and the ratio of DON/DIN ( $r^2=0.83$ ,  $p<0.05$ ). Together with previously discussed results, this suggests that urea and perhaps other organic sources (see below) replace  $\text{NO}_3^-$  in the N nutrition of large phytoplankton as DON becomes relatively more abundant.

Amino acids are generally not considered to be important to autotrophic N nutrition, despite the fact that phytoplankton can actively transport DFAA into the cell (Antia et al. 1991; Bronk 2002). There are other amino acid uptake mechanisms in addition to direct uptake, such as amino acid oxidation and peptide hydrolysis by means of proteolytic enzymes, and these pathways can play important roles in phytoplankton N nutrition (Mulholland et al. 2003; Palenik and Morel 1990). Specific DFAA uptake rates presented here for the FCM and >35- $\mu\text{m}$  fraction were not insignificant and actually exceeded those of  $\text{NO}_3^-$  in the FCM fraction at all but one station (908). Clearly, phytoplankton were using DFAA, albeit to a lesser extent than bacteria were, as indicated by comparison with the GF/F rates. Cells in the >35- $\mu\text{m}$  fraction were enriched with  $^{13}\text{C}$ -DFAA at only one station (707), and  $^{13}\text{C}$ -DFAA uptake by the FCM fraction was relatively low compared to  $^{15}\text{N}$ -DFAA uptake by FCM

phytoplankton and  $^{13}\text{C}$ -DFAA uptake by the GF/F fraction. In other words, phytoplankton were using the N, but not always the C, from the individual amino acids. This is likely a result of amino acid oxidation, whereby  $\text{NH}_4^+$  is enzymatically cleaved from DFAA molecules and thus made available for cellular assimilation (Palenik and Morel 1990).

Uptake of amino acid N by phytoplankton increased with distance south along the bay and contributed most to total N uptake at the mouth. Furthermore, there was a strong correlation between the ratio of DON/DIN and absolute DFAA uptake by FCM-sorted phytoplankton ( $r^2=0.72$ ,  $p<0.05$ ) and the  $>35\text{-}\mu\text{m}$  fraction ( $r^2=0.85$ ,  $p<0.01$ ), but this relationship was not significant for the GF/F fraction ( $r^2=0.44$ ,  $p=0.150$ ; Fig. 7). This pattern suggests that a physiological control may exist whereby phytoplankton DON use is triggered by the relative abundance of DON and DIN, a hypothesis supported by studies from various marine ecosystems, for both amino acids (Middelburg and Nieuwenhuize 2000; Mulholland et al. 1998) and urea (Glibert et al. 1991; Mulholland et al. 2002, Bradley et al. 2010). Although it is possible that DFAA uptake by the  $>35\text{-}\mu\text{m}$  fraction was due to the activity of particle-attached bacteria rather than large phytoplankton, this is unlikely for several reasons. First, the rinsing procedures and GF/F filters used when collecting the  $>35\text{-}\mu\text{m}$  fraction may have removed the majority of any attached bacteria. Also, if bacteria were contributing significantly toward DFAA uptake by the  $>35\text{-}\mu\text{m}$  fraction, there would have been measurable  $^{13}\text{C}$  enrichment, as in the GF/F fraction. Finally, large phytoplankton are known to use amino acids in Chesapeake Bay (Mulholland et al. 2003; Stoecker and Gustafson 2003).

Bacterial N use was not directly measured in this study using FCM sorting because of the lengthy processing required to obtain sufficient biomass for isotopic analysis,



**Fig. 7** Correlation between the ratio of DON to DIN and absolute DFAA uptake rates ( $\mu\text{mol N L}^{-1} \text{h}^{-1}$ ) in the GF/F, FCM-sorted phytoplankton, and  $>35\text{-}\mu\text{m}$  fractions in mainstem Chesapeake Bay surface waters

as well as the difficulty in isolating bacteria from detritus and other background particles. However, general conclusions can be drawn by comparing specific and absolute uptake rates between fractions. Bacteria are believed to use amino acids and  $\text{NH}_4^+$  preferentially over other N forms, such as  $\text{NO}_3^-$ , urea, and dissolved DNA (Kirchman 2000), and this was likely the case in the present study as well. On average, specific DFAA uptake by the GF/F fraction was roughly twice that of phytoplankton in the FCM and  $>35\text{-}\mu\text{m}$  fractions. Also, the percent contribution of DFAA to total absolute uptake increased from  $9\pm 7\%$  for Phyto to  $13\pm 7\%$  in the GF/F fraction as a result of bacterial use. Specific uptake rates for  $\text{NH}_4^+$  were generally equal between the GF/F and FCM fractions throughout Chesapeake Bay, which indicates that bacterial uptake was sufficiently high to maintain rates in the GF/F fraction equal to those of phytoplankton only. Assuming that specific  $\text{NH}_4^+$  uptake rates by phytoplankton and bacteria were similar, then the contribution of bacteria to total  $\text{NH}_4^+$  uptake averaged  $32\pm 6\%$ . Furthermore, at station 908,  $\text{NH}_4^+$  comprised 52% of Phyto N uptake but 64% of uptake by cells in the GF/F fraction, and similar results were found at station 707. This indicates that in some cases bacteria outcompeted phytoplankton for available  $\text{NH}_4^+$ . Given that DOC/DON was relatively high (15.5–20.5), as was the ratio of POC/PN in the mixed GF/F assemblage ( $7.3\pm 0.8$ ), bacteria likely used  $\text{NH}_4^+$  to complement the respiration of relatively C-rich organic matter in Chesapeake Bay.

Therefore,  $\text{NH}_4^+$  and DFAA were more important to bacteria than urea, whereas  $\text{NO}_3^-$  uptake was insignificant. Contrary to traditional belief, urea can contribute significantly to bacterial N demand in marine ecosystems (Jørgensen 2006; Sanderson et al. 2008; Bradley, unpublished data). Although it could not be quantified here, urea uptake by bacteria was significant relative to phytoplankton and the other substrates studied. Bacterial  $\text{NO}_3^-$  uptake, on the other hand, was minimal but is known to contribute substantially to  $\text{NO}_3^-$  uptake in other marine ecosystems (Allen et al. 2002; Kirchman 2000; Kirchman and Wheeler 1998).

## Conclusions

Chesapeake Bay is a highly dynamic system with biogeochemical and ecological characteristics that vary over time and space (e.g., Kemp et al. 2005). This study sought not only to examine phytoplankton N use along mainstem Chesapeake Bay during late summer but also to compare true (FCM-sorted) phytoplankton N uptake rates with traditional GF/F-based measurements. Dissolved inorganic N comprised most of the TDN pool in the upper bay but decreased rapidly toward the mouth due to biotic uptake. Ammonium was the dominant form of N used by phyto-

plankton and bacteria throughout the bay. Uptake of  $\text{NO}_3^-$ , on the other hand, was highest in the upper bay but relatively low overall. The uptake of urea and DFAA by phytoplankton increased as DON became relatively more abundant toward the bay mouth, and significant correlation between the ratio of DON/DIN and DFAA uptake suggests that this relative availability may trigger the use of DON by phytoplankton. Results suggest that phytoplankton and bacteria expressed similar preference for  $\text{NH}_4^+$  and urea, whereas bacterial DFAA use was higher and  $\text{NO}_3^-$  use generally lower than that of phytoplankton.

Using FCM sorting, it was determined that GF/F filters overestimated phytoplankton uptake of  $\text{NH}_4^+$ , urea, and DFAA by 61%, 53%, and 135%, respectively, as a result of bacterial retention. While the differences for many pairwise comparisons are marginally significant ( $p \leq 0.1$ ) due to high variability, the trend is clearly for overestimation of phytoplankton uptake. Future application of this FCM technique will allow for a more in-depth analysis of the ecological interactions between phytoplankton and bacteria with respect to N utilization under conditions of limited DIN availability.

Attributing N uptake by a mixed phytoplankton-bacterial assemblage on GF/F filters to phytoplankton alone can skew our understanding of coastal and oceanic ecosystems. For example, natural resource managers seeking to reduce anthropogenic nutrient loading to coastal waters require accurate modeling of nutrient budgets, including the effects of different N forms on plankton communities. In both coastal and oceanic systems, the efficiency with which energy is transferred to higher trophic levels depends partly on phytoplankton and bacterial dynamics and the N sources fueling their production. Furthermore, the  $f$ -ratio and vertical export of particulate matter in the ocean may be underestimated as a result of bacterial contributions to GF/F-measured uptake of reduced N or potentially overestimated under conditions of increased bacterial  $\text{NO}_3^-$  use.

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