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Urea uptake and urease activity in the Chesapeake Bay

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ABSTRACT

The importance of urea in supplying the nitrogen (N) required by planktonic communities has long been recognized, notably by James J. McCarthy in studies as early as the 1970s. Utilization of urea involves a two-step enzymatic process in phytoplankton, with urea first entering the cell via transport (i.e., urea uptake), followed by the conversion of urea into ammonium by the enzyme urease. This article describes a series of field observations and experiments conducted in the Chesapeake Bay, USA, from 2001 through 2018, aimed at understanding the relationship between urea uptake and urease activity and the role of environmental factors on that relationship. Principal component analysis revealed a few patterns. Urea uptake, for example, was consistently positively related to combined variables that included urea concentrations. Similarly, urease activity was consistently positively related to combined variables that included temperature. Contrary to findings in culture studies, however, relationships with environmental factors within different phytoplankton taxa in the field were not clear. This suggests that factors other than those examined may be involved in the regulation of urea uptake and urease activity. New insights into the role of the urea cycle in phytoplankton nitrogen dynamics suggest that the regulation of urease may not be directly impacted by environmental factors, but indirectly regulated by different metabolic pathways responding to nutrient availability, light, and temperature conditions.

Keywords: Urea, urea uptake, urease, environmental factors

1. Introduction

James J. McCarthy was a pioneer in recognizing the importance of urea utilization in meeting the nitrogen (N) demand of phytoplankton and bacteria (McCarthy 1972a, 1972b; McCarthy, Taylor, and Taft 1977). The work of many in more recent times (Glibert et al. 2006; Solomon, Alexander, and Glibert 2007; Solomon and Glibert 2008; Solomon et al. 2010; Belisle et al. 2016; Morando and Capone 2018) owes a debt to McCarthy's early insights in the contribution of urea to the total nitrogen (N) pool and how N-uptake rates reflect the relative availability of each N form (NO_3^-, NH_4^+, and urea).

Traditionally, rates of urea uptake have served as a means to measure urea utilization rates. The urea utilization pathway (sensu acquisition; Berges and Mulholland 2008) in microbes

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involves first the transport of urea into the cell via various urea transporters, such as *URT* and *DUR3* (i.e., urea uptake), and then the accumulation of urea in the cell before being converted to NH_4^+ and CO_2, most commonly by the enzyme urease (reviewed in Solomon et al. 2010). The NH_4^+ produced is then assimilated via the glutamine synthetase/glutamate (GS/GOGAT) pathway (Capone 2000; Mulholland and Lomas 2008). Rates of urea uptake are often reported to comprise close to or more than 50% of total N uptake in coastal regions around the world (reviewed in Kudela and Cochlan 2000; Mulholland and Lomas 2008), suggesting the importance of urea in N dynamics in phytoplankton.

McCarthy was among the first to observe that urea uptake may be regulated by the presence of NO_3^- or NH_4^+ (Horrigan and McCarthy 1982). Urea uptake rates of phytoplankton grown in culture under N-starved and/or urea-replete conditions decrease after the addition of NH_4^+ and/or NO_3^- to cultures (Rees and Syrett 1979; Lund 1987; Lomas 2004; Jauzein et al. 2008a, 2008b; Lee et al. 2018) or are lower when grown on NH_4^+ or NO_3 as the sole N source (Lomas 2004). Inhibition of urea uptake by NH_4^+ can occur within hours and can be up to 84% in diatoms such as *Phaeodactylum tricornutum* and *Skeletonema costatum* (Lund 1987; Molloy and Syrett 1988). In natural phytoplankton communities, urea uptake rates can be inversely related to ambient NH_4^+ concentrations (Tamminen and Irmisch 1996).

Urea uptake rates also vary among different phytoplankton taxa. McCarthy examined 35 species of phytoplankton for their capacity to utilize urea as part of his dissertation work (McCarthy 1971). He later measured urea uptake in seven clones of neritic diatoms that had different abilities to utilize urea (McCarthy 1972a). Although diatoms can utilize urea, higher urea uptake rates have since been observed during blooms of both dinoflagellates and cyanobacteria than of other phytoplankton taxa (Kudela and Cochlan 2000; Collos et al. 2004; Glibert et al. 2004, 2006; Belisle et al. 2016).

The ability to examine relationships between urea utilization and environmental factors within different phytoplankton taxa has been fostered by continual improvements in various methods. McCarthy (1970) was an early leader who developed the protocol to measure urea concentrations that involved using urease. This method was complicated by temperature sensitivity of the enzyme. A more direct determination of urea in field samples, including saltwater samples, was first tested by Price and Harrison (1987) and further developed by Revilla, Alexander, and Glibert (2005) resulting in removal of the temperature effects. This protocol was later optimized by Chen et al. (2015) to measure trace levels of urea in the ocean.

Although McCarthy (1970) used urease to measure urea concentrations, he did not measure urease activity in vivo. Peers, Milligan, and Harrison (2000) developed a protocol to measure urease activity that worked well in laboratory cultures at constant temperatures but that was not able to detect the smaller changes in NH_4^+ that typify field samples. Solomon, Alexander, and Glibert (2007) modified the method to remove high background levels of NH_4^+ and recommended filtering a minimum amount of chlorophyll *a* (Chl *a*) on GF/F filters to reduce inhibitors of urease that may be present in cells. There is now promise in the DNA stable isotope probing method that can trace the utilization of urea into microbial cells

(Wawrik, Callaghan, and Bronk 2009; Connelly et al. 2014; Morando and Capone 2018). At present this method does not capture all taxonomic groups such as dinoflagellates, which may be major consumers of urea, because of the complexity of their genomes (Solomon et al. 2010; Jing et al. 2017; Morando and Capone 2018).

A few recent studies have used measurements of urease activity as a proxy for urea utilization rates (Peers, Milligan, and Harrison 2000; Fan et al. 2003; Lomas 2004) following earlier work by others (Leftley and Syrett 1973; Bekheet and Syrett 1977). Urease activity rates have been measured in a few phytoplankton species (Oliveria and Anita 1986; Collier, Brahamsha, and Palenik 1999; Dyhrman and Anderson 2003; Fan et al. 2003; Lomas 2004; Solomon and Glibert 2008) and in few natural communities (Dyhrman and Anderson 2003; Glibert et al. 2004; Heil et al. 2007; Belisle et al. 2016; Table 1). Little is understood about how rates of urease activity and urea utilization compare and which rate is a more accurate reflection of urea use by phytoplankton and bacteria.

There appear to be only two field studies that have simultaneously measured both urea uptake and urease activity (Glibert et al. 2004; Heil et al. 2007). Often urea uptake rates are measured in conjunction with NO_3^- and NH_4^+ uptake rates but are not connected with enzymatic activity (McCarthy 1972a, 1972b; McCarthy, Taylor, and Taft 1977; Kristiansen 1983; Kokkinakis and Wheeler 1987; Twomey, Piehler, and Pearl 2005). Rates of urease activity are most frequently reported as specific activity (fmol-N cell^{-1} h^{-1}, μmol-N Chl a^{-1} h^{-1}), not as bulk measurement units (μmol-N L^{-1} h^{-1}) in which urea uptake rates are often reported (Table 1). Unless Chl *a* concentrations or cell numbers are available, the differing units adds complexity to the comparison of the two rates.

Before more was understood about the role of the urea cycle (e.g., Allen et al. 2011; Bender, Parker, and Armbrust 2012), it was suggested that there might be some similarities in factors that regulate urea uptake and urease activity. Like urea uptake, urease activity can be inversely correlated with inorganic N and positively correlated with organic N (Solomon 2006; Glibert et al. 2004; Heil et al. 2007). Similarly, urease activity rates were also shown to be higher when NO_3^- and NH_4^+ concentrations were low during a bloom of the dinoflagellate *Alexandrium* sp., compared with conditions prior to the bloom in the western Gulf of Maine (Dyhrman and Anderson 2003). Higher rates of urease activity were also observed in a bloom of the cyanobacterium *Synechococcus elongatus* in Barnes Key in Florida Bay compared with nearby areas that had higher Dissolved Inorganic Nitrogen (DIN) concentrations (Glibert et al. 2004). On the West Florida Shelf, Heil et al. (2007) found the highest urease activity at the mouth of the Peace and Shark Rivers where urea and Dissolved Organic Nitrogen (DON) levels were higher than offshore sites (Table 1).

Urease activity also appears to be related to the taxonomic composition of the phytoplankton community. For example, higher urease activity rates have been observed during blooms of both dinoflagellates and cyanobacteria compared with other phytoplankton (Glibert et al. 2004, 2006; Belisle et al. 2016). Urease activity rates in a dinoflagellate culture (*Prorocentrum minimum*) were observed to be higher on a per cell basis than in a diatom and a pelagophyte culture (Fan et al. 2003). In a bloom of *P. minimum* in the Corsica River,

Table 1. Comparison of urease activity rates in cultures and field studies. Urea uptake rates are reported if available. ATP, adenosine triphosphate.

Species or location	Conditions	Urea uptake or transport rates if reported	Urease activity	Source
Cyclotella cryptica (diatom)	In presence of different additions (e.g., ATP, Ni, hydroxyurea)		0.03–0.33 μM urea hydrolyzed min^{-1} mg $protein^{-1}$	Oliveira and Anita (1986)
Synechococcus sp. WH7805 (cyanobacteria)			16.5 units (mg total $protein^{-1}$)	Collier, Brahamsha, and Palenik (1999)
Aureococcus anophagefferens (pelaophyte), *Prorocentrum minimum* (dinoflagellate), *Thalassiosira weissflogii* (diatom)	Semibatch *f*/2 cultures on different N sources (NO_3^-, NH_4^+, urea)		*A. anophagefferens*: 6.03–6.54 fg at N $cell^{-1}$ h^{-1} *P. minimum:* 48.5–65.0 fg at N $cell^{-1}$ h^{-1} *T. weissflogii*: 19.8–45 fg at N $cell^{-1}$ h^{-1}	Fan et al. (2003)
Thalassiosira weissflogii (diatom)	Semibatch *f*/2 cultures on different N sources (NO_3^-, NH_4^+, urea) then "challenged" with a different N substrate	75–275 fg at N $cell^{-1}$ h^{-1}	0–90 fg at N $cell^{-1}$ h^{-1}	Lomas (2004)
Prorocentrum minimum, *Karlodinium veneficum, Hetrocapsa triquetra* (dinoflagellates), *Storeatula major* (cryptophyte), *Isochrysis* sp. (haptophyte)	Semibatch *f*/20 cultures on different N sources (NO_3^-, NH_4^+, urea); V_{max} rates reported		*P. minimum*: 23.7–43.4 fmol $cell^{-1}$ h^{-1} *K. veneficum*:15.1–36.7 fmol $cell^{-1}$ h^{-1} *H. triquetra:* 4.71–12.1 fmol $cell^{-1}$ h^{-1} *S. major* : 8.46–9.23 fmol $cell^{-1}$ h^{-1} *Isochrysis* sp.: 1.15–2.24 fmol $cell^{-1}$ h^{-1}	Solomon and Glibert (2008)
Alexandrium sp. (dinoflagellate), Gulf of Maine			Culture: 0–100 fmol $cell^{-1}$ h^{-1} Field, May: 8.5 ± 0.5 fmol $cell^{-1}$ h^{-1} Field, June: 92.5 ± 63.9 fmol $cell^{-1}$ h^{-1}	Dhyrman and Anderson (2003)
Florida Bay	*Synechococcus elongatus* (cyanobacterial) bloom; urea concentrations increased from west to east transect	No reported urea uptake, but urea and amino acid uptake was substantial during the bloom	0–0.4 μg at N L^{-1} h^{-1} (0–0.55 μg at N chl a^{-1} h^{-1})	Glibert et al. (2004)
Peace, Caloosahatchee, Shark Rivers, Western Florida Bay	Most of urease activity associated with 0.6–3 μm biomass	0.01–0.26 μmol L^{-1} h^{-1} 0.005–0.45 h^{-1}	0.06–0.15 μmol L^{-1} h^{-1}	Heil et al. (2007)
Lake Erie	Only urea concentrations reported		ND–1.963 μmol L^{-1} h^{-1}	Belisle et al. (2016)

Maryland, urease activity rates were fourfold higher than outside the bloom (Salerno 2005). On the West Florida Shelf, regions with high urease activity were dominated by dinoflagellates, including *Karenia brevis*, and cyanobacteria as opposed to southern regions on the shelf with lower urease activity and a higher percentage of diatoms in the phytoplankton community (Heil et al. 2007). Because of similar behaviors between urea uptake and urease activity, it would be expected that both are regulated by the same environmental factors in the same phytoplankton species.

If urea uptake and urease activity are tightly coupled, rates would be expected to follow gradients of NO_3^- and NH_4^+ that exist in coastal ecosystems (Antia, Harrison, and Oliveria 1991; Kirchman 2000). Gradients in Chesapeake Bay and its tributaries provide an ideal location to test this relationship. If particular phytoplankton species are major users of urea, urea uptake and urease activity would be expected to also vary with phytoplankton community composition. To test these hypotheses, temperature, N availability (NO_3, NH_4^+, and urea concentrations), phytoplankton composition, urea uptake, and urease activity in these coastal ecosystems were measured and compared.

2. Materials and methods

a. Sites

Freshwater flow into the Chesapeake Bay establishes a gradient in N availability (Fisher et al. 1992; Glibert et al. 1995; Bronk et al. 1998) and plankton community structure (Adolf et al. 2006). The upper reaches of the estuary are dominated by oxidized forms of N (NO_3^- and NO_2^-; Fisher et al. 1992; Kemp et al. 2005), and diatoms are abundant throughout the year (Adolf et al. 2006). Reduced forms such as NH_4^+, urea, and DON become progressively more important from the upper to the lower parts of the estuary (Glibert et al. 1995; Bronk et al. 1998) where summer dinoflagellate and cyanobacterial blooms often occur (Adolf et al. 2006).

Samples were collected from three stations along the main stem of the Chesapeake Bay in the spring, summer, and fall from 2001 to 2004, with the exception of one station (Mid Bay), which was only sampled in summer of 2003 (Fig. 1a). The first station was in the upper reaches of the bay (Upper Bay; 39.34° N, 76.18° W), the second site was in the middle of the bay (Mid Bay; 38.56° N, 76.44° W), and the third site was near the mouth of the bay (Lower Bay; 37.26° N, 76.15° W).

Samples were also collected from two tributaries of Chesapeake Bay, the Choptank and Anacostia Rivers. The Choptank River has been impacted by anthropogenic activity, especially by N inputs from agriculture (Fig. 1c; Fisher et al. 1988, 2006; Staver et al. 1996). The predominately agricultural region of the Choptank River watershed produces corn, soybeans, wheat, fruit, and vegetables (Goel, McConnell, and Torrents 2005; Fisher et al. 2006). Total N concentrations in the river are strongly correlated with freshwater discharge through groundwater (Staver et al. 1996) and peak twice a year in late fall or winter and late spring (Fisher et al. 1988; Whitall et al. 2010). Groundwater supplies mostly NO_3^-,

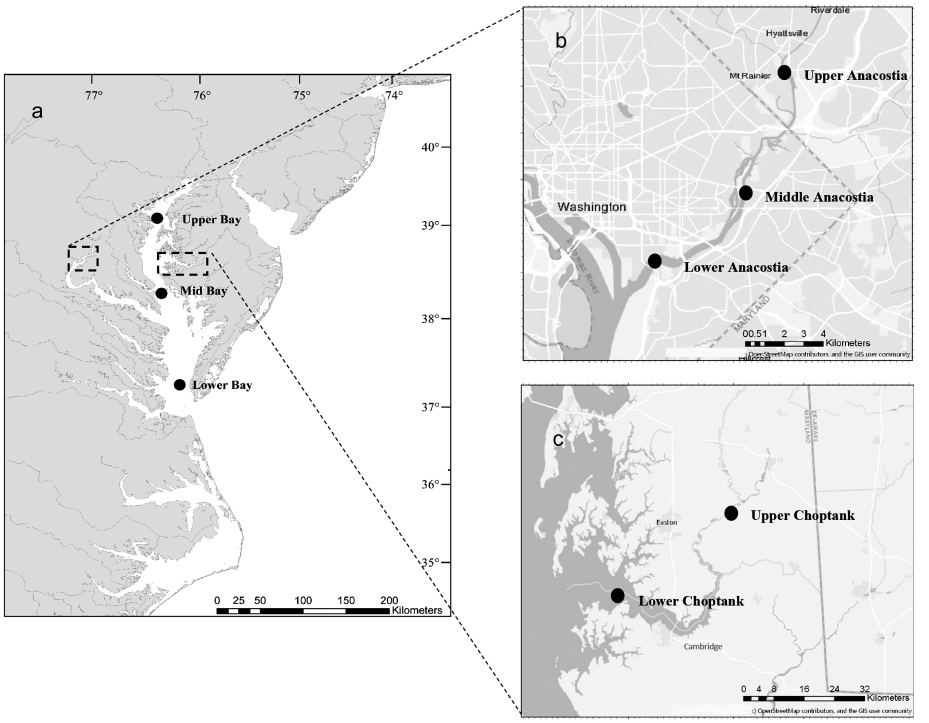

Figure 1. Sampling sites throughout the Chesapeake Bay. (a) Locations of sampling sites in the Chesapeake Bay: Upper Bay, Mid Bay, and Lower Bay and the general locations of the Anacostia and Choptank Rivers. (b) Three sampling sites on the Anacostia River: Upper Anacostia, Middle Anacostia, and Lower Anacostia. (c) Two sampling sites on the Choptank River: Upper Choptank and Lower Choptank.

which has been increasing annually since 1980, possibly because of lags in leaching from agricultural lands enriched with N-based fertilizers (Fisher et al. 1988; Hively et al. 2011). On average, NH_4^+ represents a much smaller portion of the total N pool than NO_3^- (4%; Fisher et al., 1988, 2006). Samples were collected from two sites in the Choptank River in April, July, or August and October from 2001 to 2004. Additional sampling was done during February (2002 and 2003), June (2002–2004), September (2002), and December (2001 and 2003). The first site was located downstream of the confluence of the Tuckahoe and Choptank Rivers (Upper Choptank), and the second site was located near the mouth of the river where it enters the Chesapeake Bay (Lower Choptank). Water was collected from the near surface and the near bottom using a diaphragm pump to minimize damage to plankton. Water was stored in acid-washed (with 10% HCl and repeated deionized water washes) Nalgene carboys for transport to the lab ($<$1 h) for processing.

The tidal freshwater Anacostia River flows through Washington, D.C., to the Potomac River, one of the major tributaries of the Chesapeake Bay (Fig. 1b). The urban Anacostia

River is a sewage-influenced ecosystem with varying anthropogenic land uses (Solomon, Jackson, and Glibert 2019). With a relatively small watershed (456 km^2), the width of the Anacostia River varies from 60 to 500 m, with a minimum depth of 1.2 m near Bladensburg Water Park to a maximum depth of 5.6 m near the confluence of the Potomac River (Behm, Buckley, and Schultz 2003). The volume of the Anacostia River at mean tide is approximately 1×10^{10} L (Behm, Buckley, and Schultz 2003). The Anacostia River is slow moving, with an average water residence time of 23–28 days, but increases to 2–3 months during extreme low-flow conditions (Metropolitan Washington Council of Governments 2009). Samples were collected from the surface via a bucket at low tide at three sites along the Anacostia River, starting at Bladensburg Water Park (Bladensburg, MD) and ending near where the Anacostia meets the Potomac River biweekly from March to November during 2013–2018 in coordination with the Anacostia Riverkeeper.

b. Environmental factors

Salinity, dissolved oxygen, and temperature data were collected by a YSI Pro 2030 probe in the Anacostia River, a YSI 85 probe for the Choptank River, and by a Seabird 911 CTD (conductivity-temperature-depth) on a General Oceanic 1015 rosette aboard the R/V *Cape Henlopen* in the Chesapeake Bay. Yearly stream flow data into the Anacostia, Choptank, and Chesapeake Bay were obtained from the U.S. Geological Survey (USGS; https://waterdata.usgs.gov) and were converted from $ft.^3\ s^{-1}$ to $m^3\ s^{-1}$. Data for the Anacostia River were obtained from USGS 01650500 near Colesville, MD; for Choptank River, from USGS 01491000 at Greensboro, MD; and for Chesapeake Bay, from computations of stream flow measurements from the Susquehanna, Potomac, and James Rivers (https://www.usgs.gov/centers/cba).

For nutrient analyses, sample water from each site was filtered through precombusted GF/F filters (450°C for 1 h) into acid-washed bottles and then frozen for later determination of nutrients in the laboratory. For samples from the Choptank River and Chesapeake Bay, concentrations of NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-} (DIP, Dissolved Inorganic Phosphate) were determined with a Technicon Autoanalyzer II (Lane et al. 2000). For samples from the Anacostia, concentrations of NO_3^- were analyzed according to the vanadium (II) reduction method (Miranda, Espey, and Wink 2001; Doane and Horwáth 2003), and NH_4^+ by the method of Parsons, Maita, and Lalli (1984). Urea concentrations were measured by the urease method described by Parsons, Maita, and Lalli (1984) until April 2004. Samples taken afterward (the rest of 2004 for Choptank and Chesapeake Bay samples in addition to the Anacostia samples) were measured by the diacetylmonoxime method, which had a smaller salt interference than the urease method (Mulvenna and Savidge 1992; Revilla, Alexander, and Glibert 2005).

Phytoplankton composition and biomass were determined via Chl *a* and pigment analyses. Sample water was filtered through precombusted GF/F filters (450°C for 1 h), and the filters were immediately frozen (−20°C). Once back in the laboratory, samples

were immediately transferred to an −80°C freezer until analysis. Chl *a* samples were analyzed according to Parsons, Maita, and Lalli (1984), using a 10-AU Turner Designs flourometer. Pigment analyses was conducted according to van Heukelem et al. (1994) and van Heukelem and Thomas (2001) using a Hewlett Packard high-performance liquid chromatograph (Model 110) system. Phytoplankton community composition was characterized by using pigment ratios; the ratio of fucoxanthin:Chl *a* was used as an indicator of diatoms, and zeaxanthin:Chl *a*, Chl *b*:Chl *a*, and alloxanthin:Chl *a* were used as measures of cyanobacteria, chlorophytes, and cryptophytes, respectively (Jeffrey and Wright 1994; Jeffrey and Vesk 1997; Glibert et al. 2004). For the Chesapeake Bay stations, phytoplankton community composition analysis was supplemented by microscopy of water samples preserved in Lugol's solution, 4% glutaraldehyde, or 2% formalin.

c. Biochemical processes

Samples for whole water measurement of both urea uptake and urease activity samples were collected from all sites. The station water was filtered through precombusted GF/F filters (450°C for 1 h). Urea uptake rates were determined using ^{15}N tracer techniques (Glibert and Capone 1993). For the Anacostia samples, incubations were conducted in 100 mL acid-washed polycarbonate bottles with 0.5 μM ^{15}N-urea (resulting in an atom % enrichment of 6% to 100%) and incubated on a windowsill for 30 min. For the Choptank and Chesapeake Bay samples, incubations were conducted in 1 L acid-washed polycarbonate bottles with 0.5 μM ^{15}N-urea (resulting in atom % enrichment of 13.1% to 100%) under 60% natural irradiance using neutral-density screening for 30 min. After the incubations, samples were filtered onto precombusted GF/F filters and immediately frozen. Samples were dried at 50°C, packed into tin boats, and analyzed on either a SerCon mass spectrometer or a Carlo Erba 1110 Elemental Analyzer coupled to a Thermo Delta V IRMS (isotope ratio mass spectrometry).

For urease activity, particulate matter was collected immediately onto filters in a similar fashion as for the urea uptake samples, and the filters were frozen in liquid N_2 until analysis. Samples from the Chesapeake Bay and the Choptank River were analyzed for urease activity within 1 week of sampling using the method of Peers, Milligan, and Harrison (2000) as modified by Fan et al. (2003). Beginning in August 2003, urease activity samples were analyzed using an optimized assay for field samples (Solomon, Alexander, and Glibert 2007) that involved grinding the filter samples in buffers to extract urease. Samples were then exposed to saturating levels of urea (e.g., V_{max} assays) at in situ temperatures. Rates of urease activity were calculated from beginning and final concentrations of NH_4^+. Conversions of data prior to August 2003 were made using an equation developed from samples collected from a range of sites and seasons analyzed by both methods (Solomon, Alexander, and Glibert 2007). Samples from the Anacostia River were analyzed within a year using the method of Solomon, Alexander, and Glibert (2007).

d. Statistical analysis

Both environmental (temperature and nutrient concentrations) and phytoplankton taxa data were first tested for normality using histograms in R. If the data were skewed, then the data were transformed before performing principal component analysis (PCA) in R (Clarke et al. 2014). Multivariable community measures were created via PCA that included temperature and nutrient data (or phytoplankton taxa) and then were analyzed for statistical correlations with urea uptake and/or urease activity. Multivariable community measures were created separately for temperature and nutrient data from phytoplankton taxa because there were more environmental data available.

3. Results

a. Environmental patterns

Across all sites and times samples, a large gradient in stream flow, temperatures, and salinities was encountered (Table 2). The highest stream flow for the Anacostia River was observed in 2014 (1.04 ± 0.86 m^3 s^{-1}), whereas other years remained on average between 0.44 and 0.67 m^3 s^{-1}. Stream flow in the Choptank River and Chesapeake Bay was below average during 2001 and 2002, considered dry years (<5 m^3 s^{-1} and $<1{,}700$ m^3 s^{-1}, respectively), below the range of normal stream flow (4 m^3 s^{-1}, the long-term average from 1949 to 2018 for the Choptank, USGS 2019; 1,800–2,500 m^3 s^{-1}, the long-term average for the Chesapeake Bay from 1935 to 2018; https://www.usgs.gov/centers/cba). Average stream flow into Chesapeake Bay in 2003 and 2004, considered wet years, exceeded 3,000 m^3 s^{-1} and reduced salinity levels by approximately 5 throughout Chesapeake Bay. The timing of the spring freshet differed each year, which influenced nutrient concentrations during sampling in April. In 2001, 2003, and 2004, the freshet occurred prior to or during the spring sampling period. However, in 2002, the spring freshet occurred after the spring sampling.

All sites exhibited an annual pattern in both temperature and salinity (Table 2). The coldest temperatures observed were recorded in spring (8.45°C to 15.0°C), and the warmest temperatures in summer (24.9°C to 27.3°C). Salinity was not measured in the Anacostia as this river consists of mostly fresh water (on average 0.26; Anacostia Watershed Toxics Alliance and Anacostia Watershed Restoration Commission 2015), whereas there was a difference of 8–11 in salinity between the two Choptank River stations. Salinity increased from <9 at the mostly upper station to >16.7 in the lower station of the Chesapeake Bay. Salinity was lower in 2003 and 2004 in the Chesapeake Bay than in 2001 and 2002 (Table 2).

Concentrations of N forms followed a gradient from the upper to lower portions at all sites (Choptank River and Chesapeake Bay: Table 3; Anacostia River: Solomon, Jackson, and Glibert 2019). Of the three N substrates, NO_3^- concentrations were the highest during the spring, and often increased again during the fall months. NO_3^- concentrations were, on average, >30 μM in the Anacostia River but were often higher during the spring as opposed to summer and declined at downstream stations under both low and high flow conditions

Table 2. Average stream flow, temperature, and salinity at Anacostia,[a] Choptank, and Chesapeake Bay stations. Temperature values are the season average ± standard deviation.

Average annual stream flow ($m^3\ s^{-1}$)	2001	2002	2003	2004	2013	2014	2015	2016	2017	2018
Choptank	4.33	2.89	9.11	5.27						
Chesapeake Bay	1,416	1,700	3,964	3,115						
Anacostia					0.64 ± 0.42	1.04 ± 0.86	0.64 ± 0.50	0.62 ± 0.50	0.44 ± 0.35	0.67 ± 0.28[c]
Temperature (°C)										
Choptank										
Spring	15.3 ± 0.91	12.7 ± 0.70	11.6 ± 0.62	9.96 ± 0.49						
Summer	27.0 ± 0.45	26.7 ± 0.29	26.3 ± 0.97	26.3 ± 0.35						
Fall	18.1 ± 0.21	23.7 ± 0.32	20.8 ± 0.07	23.0 ± 0.08						
Chesapeake Bay										
Spring	8.45 ± 1.08	10.6 ± 0.82	8.90 ± 0.39	8.98 ± 0.35						
Summer	27.3 ± 0.33	27.0 ± 0.53	26.9	26.4 ± 1.10						
Fall	18.8 ± 1.46	23.5 ± 1.29	19.7 ± 1.51	21.3 ± 1.67						
Anacostia										
Spring					12.0 ± 4.16	14.49 ± 6.62	15.1 ± 5.51	13.6 ± 2.41	14.9 ± 2.66	11.1 ± 5.77
Summer					26.0 ± 3.65	25.71 ± 1.58	26.4 ± 2.06	27.3 ± 2.35	26.1 ± 3.06	24.9 ± 2.10
Fall					19.8 ± 5.19	18.24 ± 5.12	17.7 ± 4.02	20.5 ± 4.93	21.4 ± 3.14	19.9 ± 7.6
Salinity										
Upper Choptank	0.58 ± 0.20	3.68 ± 0.27	0.29 ± 0.12	0.98 ± 0.19						
Lower Choptank	12.0 ± 0.78	15.1 ± 0.60	9.43 ± 0.70	10.33 ± 0.19						
Upper Bay	2.76 ± 1.98	4.53 ± 4.64	0.13 ± 0.00[b]	0.26 ± 0.19						
Middle Bay	13.2 ± 2.80	15.2 ± 3.37	9.27 ± 2.75	7.89 ± 2.00						
Lower Bay	22.1 ± 0.58	22.6 ± 2.51	17.2 ± 0.30[b]	16.9 ± 0.26						

Notes: [a] Data were downloaded from U.S. Geological Survey and converted from $ft.^3\ s^{-1}$ to $m^3\ s^{-1}$. [b] Samples were not collected in summer 2003 in Upper Bay and Lower Bay, so the range reflects spring and fall values. [c] The 2018 value for the Anacostia River is from January to June.

Table 3. Distribution of nitrogen (μg at N L^{-1}) in Chesapeake Bay during three seasons from 2001 to 2004. Values represent the mean of 2 years ± standard deviation. Distribution of nitrogen for the Anacostia River can be found in Solomon, Jackson, and Glibert (2019).

		2001–2002 (Dry years)			2003–2004 (Wet years)		
Station	Season	NO_3^-	NH_4^+	Urea	NO_3^-	NH_4^+	Urea
Upper Choptank	Spring	54.9 ± 19.8	15.3 ± 0.28	1.19 ± 1.11	147.2 ± 99.5	5.67 ± 0.85	1.22 ± 0.15
	Summer	39.4 ± 31.0	1.56 ± 1.15	0.58 ± 0.44	43.9 ± 36.5	2.94 ± 4.15	0.76 ± 0.73
	Fall	24.83 ± 3.39	1.82 ± 1.14	1.46 ± 2.53	45.3 ± 58.7	3.00 ± 2.54	0.39 ± 0.26
Lower Choptank	Spring	3.76 ± 5.25	2.51 ± 3.56	0.41 ± 0.01	45.0 ± 12.5	7.68 ± 2.12	0.32 ± 0.12
	Summer	0.06 ± 0.07	0.62 ± 0.50	0.41 ± 0.27	6.10 ± 6.36	1.14 ± 0.82	0.52 ± 0.21
	Fall	0.17 ± 0.28	1.16 ± 0.82	0.24 ± 0.27	3.00 ± 4.12	2.90 ± 3.00	0.42 ± 0.18
Upper Bay	Spring	73.9 ± 20.9	8.24 ± 0.22	1.06 ± 1.10	85.7 ± 12.6	4.83 ± 2.04	0.92 ± 0.60
	Summer	15.5 ± 6.29	5.22 ± 1.42	0.11 ± 1.20	25.5	9.7	0.89
	Fall	21.7 ± 3.49	5.74 ± 4.98	0.61 ± 0.32	82.8 ± 27.0	3.8 ± 2.81	0.66 ± 0.32
Mid Bay	Spring	23.8 ± 27.2	1.87 ± 0.82	0.40 ± 0.00	47.7 ± 16.3	4.61 ± 2.22	0.36 ± 0.07
	Summer	0.40 ± 0.04	0.77 ± 0.33	0.31 ± 0.14	3.45 ± 4.38	2.96 ± 0.29	1.27 ± 0.43
	Fall	1.64 ± 1.00	0.75 ± 0.66	0.26 ± 0.05	21.2 ± 18.6	4.8 ± 5.64	0.55 ± 0.41
Lower Bay	Spring	0.84 ± 1.00	0.39 ± 0.07	0.40 ± 0.01	13.2 ± 0.83	0.43 ± 0.49	0.32 ± 0.45
	Summer	0.71 ± 0.69	1.67 ± 0.76	0.31 ± 0.28	0.34	0.64	0.87
	Fall	0.39 ± 0.47	1.04 ± 1.47	0.47 ± 0.40	0.52 ± 0.54	1.29 ± 0.26	0.79 ± 0.59

(Solomon, Jackson, and Glibert 2019). Concentrations of NO_3^- were higher during the wet years (2003–2004) than the dry years (2001–2001) in both the Choptank River and the Chesapeake Bay. The Upper Choptank had higher NO_3^- concentrations than the Lower Choptank River, as much as 30-fold greater during the wet years. The same was true for the Chesapeake Bay, with Upper Bay concentrations 4-fold higher than the Lower Bay during the dry years as opposed to 15-fold higher during the wet years. Concentrations of NH_4^+ and urea also had seasonal trends with higher concentrations during the spring, declining in summer, and then increasing again in fall at all stations. The percentage contribution of NH_4^+ to total available N increased downstream (Table 3). In the Anacostia, NH_4^+:NO_3^- ratios increased from upstream to downstream, were highest during the summer months, and were significantly greater during high than low flow conditions (Solomon, Jackson, and Glibert 2019), while in the Choptank River the contribution of NH_4^+ and urea to the total N pool was often >80% during the summer and fall, except in 2003 (Solomon 2006). A gradient in contribution of the sum of NH_4^+ and urea relative to the total inorganic N pool in the Chesapeake Bay ranged from <15% in the Upper Bay, 39% in the Mid Bay, and 62% in the Lower Bay (data not shown).

Phytoplankton biomass (Chl *a*) followed different trends at various sites (Table 4). Concentrations of Chl *a* in the Anacostia River were highly variable both with year and station, averaging 8.73–31.5 $\mu g\ L^{-1}$ on an annual basis. Higher Chl *a* values, >75 $\mu g\ L^{-1}$, were observed in the summer to early fall and under conditions of low flow as compared with high flow (Solomon, Jackson, and Glibert 2019). Concentrations of Chl *a* in the Choptank River increased from spring to summer and then decreased again in the fall.

During the dry years (2001–2002) at the Upper Bay station, Chl *a* biomass peaked in the summer. In contrast, during 2003 and 2004, the highest Chl *a* biomass was found in spring. Concentrations of Chl *a* at the Mid Bay station were highest in spring during the dry years, but in summer in the wet years. Chl *a* biomass did not exceed 6.98 μg Chl *a* L^{-1} during 2001–2002 at the Lower Bay station but was generally greater (between 5.00 and 18.1 μg Chl *a* L^{-1}) during 2003–2004 (data not shown).

The composition of the phytoplankton community (based on pigment:Chl *a* ratios) exhibited some consistent trends among the Anacostia and Choptank Rivers and the Chesapeake Bay (Table 4). The spring assemblages had proportionately more diatoms (based on fucoxanthin:Chl *a* ratios), but overall Chl *a* levels were lowest at this time. The relative contribution of diatoms in the Anacostia River and some locations in Chesapeake Bay declined through the summer and increased in late summer or fall (Solomon, Jackson, and Glibert 2019), whereas in the Choptank River the relative contributions of diatoms continued to decline or remained constant throughout the year (Table 4). The relative contribution of cyanobacteria (based on zeaxanthin:Chl *a* ratios) in the Anacostia River increased both with station downstream and with season, and it was substantially higher in late summer and early fall in the middle of the river relative to the upper and lower sites (Solomon, Jackson, and Glibert 2019). The contribution of cyanobacteria in the Choptank River was almost nonexistent, with zeaxanthin:Chl *a* ratios <0.07, whereas in the Chesapeake Bay it increased from the

Table 4. Average abundance of Chl *a* (μg chl *a* L^{-1}) $\pm$ standard deviation and average pigment ratios (pigment:Chl *a*) $\pm$ SD. The various pigments include fucoxanthin (representative of diatoms), zeaxanthin (representative of cyanobacteria), chlorophyll *b* (representative of chlorophytes), and alloxanthin (representative of cryptophytes). Distribution of pigments for the Anacostia River can be found in Solomon, Jackson, and Glibert (2019).

		2001–2002 (Dry years)					2003–2004 (Wet years)				
Station	Season	Chl *a*	Fuco:Chl *a*	Zea:Chl *a*	Chl *b*:Chl *a*	Allo:Chl *a*	Chl *a*	Fuco:Chl *a*	Zea:Chl *a*	Chl *b*:Chl *a*	Allo:Chl *a*
Upper Choptank	Spring	7.33 ± 2.60	0.25 ± 0.09	0.01 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	13.6 ± 3.13	0.27 ± 0.01	0.01 ± 0.00	0.03 ± 0.04	0.02 ± 0.00
	Summer	20.3 ± 7.59	0.20 ± 0.11	0.05 ± 0.04	0.06 ± 0.02	0.03 ± 0.02	25.1 ± 18.0	0.21 ± 0.07	0.02 ± 0.01	0.05 ± 0.01	0.06 ± 0.04
	Fall	16.6 ± 9.78	0.20 ± 0.12	0.03 ± 0.02	0.06 ± 0.02	0.03 ± 0.02	26.8 ± 31.0	0.10 ± 0.15	0.01 ± 0.01	0.05 ± 0.04	0.06 ± 0.03
Lower Choptank	Spring	11.3 ± 9.68	0.29 ± 0.07	0.00 ± 0.00	0.06 ± 0.06	0.01 ± 0.00	4.01 ± 0.63	0.15 ± 0.07	0.02 ± 0.00	0.13 ± 0.13	0.03 ± 0.00
	Summer	11.8 ± 2.20	0.19 ± 0.17	0.07 ± 0.07	0.04 ± 0.02	0.01 ± 0.00	25.2 ± 12.9	0.22 ± 0.13	0.05 ± 0.04	0.03 ± 0.01	0.04 ± 0.02
	Fall	11.0 ± 4.00	0.19 ± 0.05	0.03 ± 0.01	0.06 ± 0.03	0.03 ± 0.01	12.2 ± 7.44	0.13 ± 0.18	0.020.02	0.04 ± 0.02	0.08 ± 0.01
Upper Bay	Spring	1.39 ± 0.90	0.22 ± 0.03	0.03 ± 0.01	0 ± 0.00	0.02 ± 0.00	3.81 ± 2.14	0.24 ± 0.01	0.01 ± 0.01	0.06 ± 0.02	0.020.00
	Summer	10.8 ± 0.20	0.07 ± 0.03	0.03 ± 0.00	0.09 ± 0.03	0.05 ± 0.01	3.36	0	0	0.03	0.02
	Fall	5.32 ± 0.00	0.07 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	3.05 ± 3.20	0.12 ± 0.13	0.05 ± 0.04	0.09 ± 0.05	0.04 ± 0.02
Mid Bay	Spring	6.32 ± 0.76	0.28 ± 0.02	0.002 ± 0.001	0.03 ± 0.03	0.02 ± 0.02	9.97 ± 5.51	0.19 ± 0.02	0.00 ± 0.00	0.02 ± 0.02	0.03 ± 0.01
	Summer	5.58 ± 2.53	0.16 ± 0.04	0.15 ± 0.03	0.05 ± 0.01	0.03 ± 0.00	12.16 ± 3.44	0.17 ± 0.22	0.01 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
	Fall	3.90 ± 0.00	0.24 ± 0.00	0.12 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	6.22 ± 2.41	0.16 ± 0.23	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.01
Lower Bay	Spring	2.85 ± 1.31	0.23 ± 0.05	0.01 ± 0.00	0.06 ± 0.01	0.02 ± 0.00	12.8 ± 6.09	0.38 ± 0.01	0.00 ± 0.00	0.03 ± 0.04	0.00 ± 0.00
	Summer	3.85 ± 1.56	0.10 ± 0.04	0.33 ± 0.12	0.07 ± 0.04	0.03 ± 0.00	6.06	0.00	0.00	0.05	0.02
	Fall	2.73 ± 0.00	0.17 ± 0.00	0.24 ± 0.00	0.09 ± 0.00	0.01 ± 0.00	11.2 ± 1.50	0.20 ± 0.28	0.02 ± 0.03	0.03 ± 0.02	0.03 ± 0.00

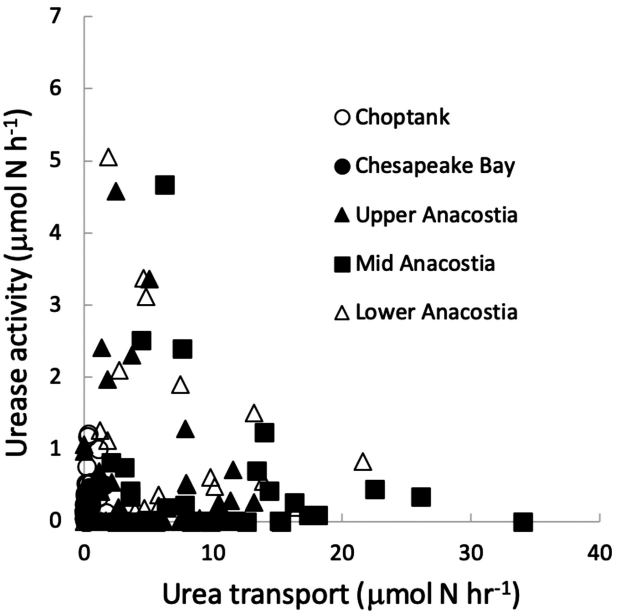

Figure 2. The relationship between urea uptake and urease activity rates in the Choptank River (two sites), Chesapeake Bay (three sites), and the Anacostia River (three sites).

Upper Bay to Lower Bay and peaked during the summer only during the dry years. The relative contribution of chlorophytes (based on Chl *b*:Chl *a* ratios) in the Anacostia River was higher in the summer and early fall relative to spring (Solomon, Jackson, and Glibert 2019) but remained mostly <0.06 in the Choptank River and the Chesapeake Bay, with increased contributions in the Lower Choptank in the spring during wet years and Upper Bay in summer during the dry years. Cryptophyte abundance (based on alloxanthin:Chl *a* ratios) in the Anacostia River increased steadily with season upriver, and the increases late in the year were most pronounced in midriver with variable abundances downstream (Solomon, Jackson, and Glibert 2019). These trends are in contrast with the Choptank River and Chesapeake Bay with small relative contributions of cryptophytes at all sites through seasons and years.

b. Seasonal and spatial patterns in and between urea uptake and urease activity rates

Urea uptake rates were not significantly correlated with urease activity rates at any of the sites studied when all data were considered ($r^2 = -0.11$, n = 226; Anacostia River: $r^2 = -0.05$, n = 161; Choptank River and Chesapeake Bay: n = 65, $r^2 = -0.28$; Fig. 2). Only in the Chesapeake Bay were urea uptake and urease activity positively related bay-wide (n = 31, $r^2 = 0.47$, $P < 0.05$). The relationship was strongest in Upper Bay (n = 11, $r^2 = 0.68$, $P < 0.05$) and Lower Bay (n = 11, $r^2 = 0.59$, $P < 0.05$).

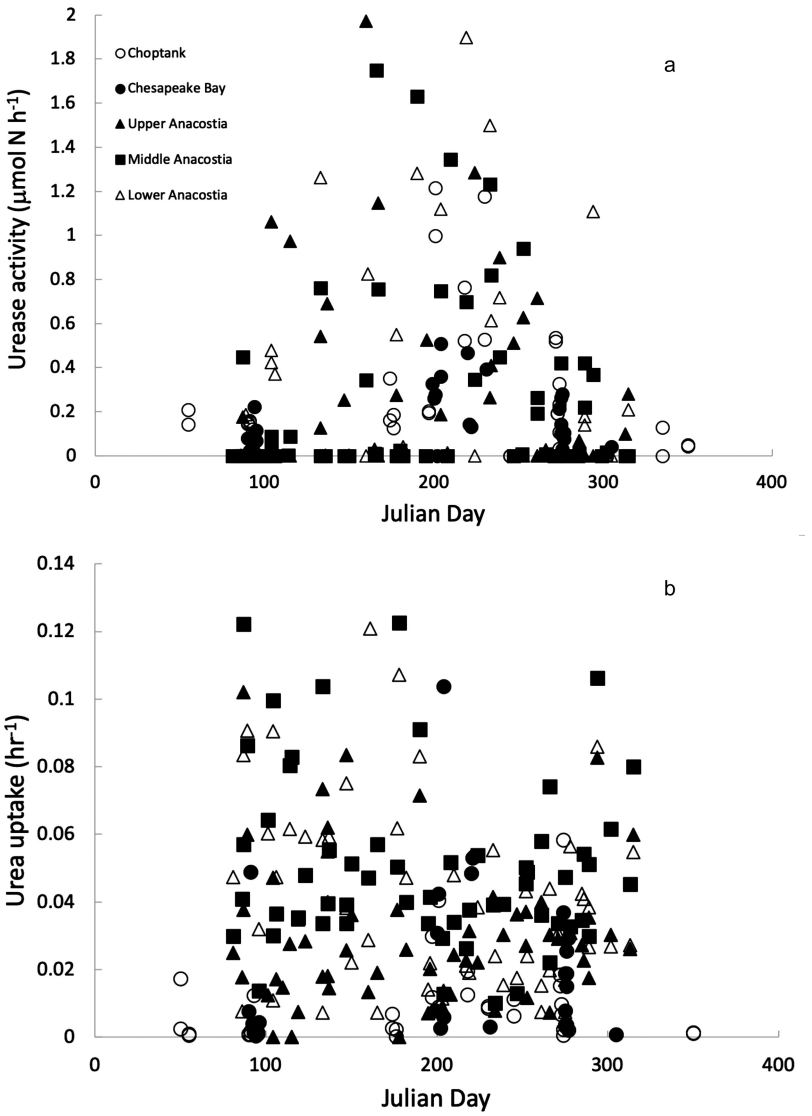

Figure 3. Seasonal and spatial patterns in urea uptake (a) and urease activity (b) at all sites in Chesapeake Bay during all years of the study: Choptank River and Chesapeake Bay (2001–2004) and Anacostia River (2013–2018).

Urea uptake and urease activity in surface waters exhibited seasonal differences in all systems. Urease activity had more of a peak in the summer months than urea uptake at all sites (Fig. 3a and b). Both urea uptake and urease activity rates in the Middle and Lower Anacostia River were generally higher than the other sites, despite the large variability in rates.

c. Relationships between temperature, nitrogen availability, phytoplankton community composition, and urea uptake and urease activity

The PCA analysis created several combined principal components based on temperature and nutrient availability. When data from the Anacostia and Choptank Rivers and the Chesapeake Bay were analyzed together, PC1 was defined by a negative relationship with temperature, PC2 by a positive relationship with NH_4^+, PC3 by a negative relationship with NO_3^-, and lastly PC4 with a positive relationship with urea (Fig. 4a; Table 5). Urea uptake had a significant negative relationship with PC2 ($P < 0.001$) and a positive relationship with PC4 ($P < 0.001$), suggesting that urea uptake decreased with increasing NO_3^- concentrations and increased with increasing urea concentrations (Table 6). Urease activity had a negative relationship with PC1 ($P < 0.01$) and a positive relationship with PC2 ($P < 0.05$), suggesting that urease activity increased with temperature and NO_3^- concentrations (Table 6).

When examining these relationships on a local level, the combined variables for the Anacostia River changed slightly, with Anacostia River PC2 (APC2) being defined mostly by a positive relationship with NH_4^+ and Anacostia River PC3 (APC3) being defined mostly by a negative relationship with NO_3^- (Fig. 4b, Table 5). Urea uptake had a significant relationship with all combined variables ($P < 0.001$), whereas urease activity only had a significant relationship with Anacostia River PC1 (APC1; Fig. 4b, Table 6). This suggests that urea uptake in the Anacostia River was negatively related to temperature and positively related to NO_3^-, NH_4^+ , and urea concentrations, whereas urease activity was positively related to temperature. In the Choptank River and the Chesapeake Bay, the combined variables were defined by the same factors except with some inverse relationships in Chesapeake PC2 (CPC2; negative relationship with NO_3^-) and Chesapeake PC4 (CPC4; negative relationship with urea; Fig. 4c). Both urea uptake and urease activity were negatively correlated with Chesapeake PC1 (CPC1; $P < 0.01$) suggesting that those rates were positively related to temperature (Table 5).

The PCA analysis also created several combined PCAs based on phytoplankton community composition using pigment ratios (Fig. 5, Table 7). When data from the Anacostia and Choptank Rivers and the Chesapeake Bay were analyzed together, pigment PC1 (PPC1) was defined by a negative relationship with diatoms and positive relationship with chlorophytes; pigment PC2 (PPC2), by a negative relationship with chlorophytes; pigment PC3 (PPC3), by a positive relationship with cryptophytes; and pigment PC4 (PPC4), by a positive relationship with cyanobacteria (Fig. 5a, Table 7). Urea uptake had a significant positive relationship with PPC1 ($P < 0.05$) and PPC4 ($P < 0.01$), suggesting that urea uptake decreased with more diatoms present and increased with more chlorophytes and cyanobacteria present (Table 8). However, urease activity did not demonstrate any significant relationship with any of the pigment combined variables (Table 8).

As for temperature and nutrient availability on the local level, the combined variables for phytoplankton community in the Anacostia River differed by Anacostia pigment PC2

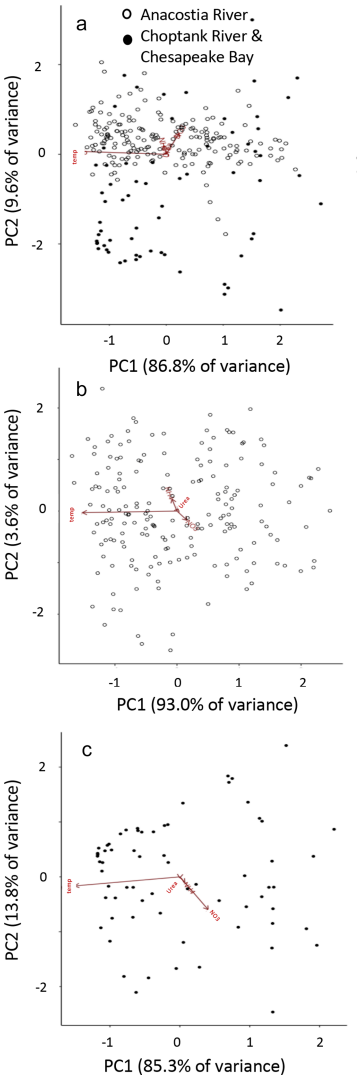

Figure 4. Principal component analysis showing the first two principal components for nutrient and environmental factors for all sites (a), Anacostia River (b), and Choptank River and Chesapeake Bay (c). The third and fourth principal components are not shown because of low proportion of variance.

(APPC2) being defined mostly by a negative relationship with cryptophytes and Anacostia pigment PC3 (APPC3) being defined mostly by negative relationships with diatoms and cryptophytes and a positive one with cyanobacteria (Fig. 5b, Table 7). Urea uptake and urease activity had no significant relationships with all the pigment combined variables (Table 8).

Table 5. Proportion of variance and loadings for multivariable (principal component analysis) analysis for temperature and N concentrations. PC indicates combined variables when analyzing data from all locations, APC indicates combined variables from Anacostia River, and CPC indicates combined variables from Choptank River and Chesapeake Bay.

Site	Variables	PC1	PC2	PC3	PC4
All (PC)	Proportion of variance	0.868	0.095	0.031	0.005
	Temperature	−0.989	0.124	0.080	0.014
	NO_3^-	0.145	0.912	0.383	−0.007
	NH_4^+	−0.028	0.377	−0.890	−0.255
	Urea	0.008	0.104	−0.233	0.967
Anacostia (APC)	Proportion of variance	0.930	0.036	0.027	0.007
	Temperature	−0.993	−0.102	−0.059	0.025
	NO_3^-	0.106	−0.599	−0.791	−0.069
	NH_4^+	−0.053	0.775	−0.570	−0.269
	Urea	0.018	0.177	−0.216	0.960
Choptank & Chesapeake Bay (CPC)	Proportion of variance	0.853	0.138	0.007	0.001
	Temperature	−0.965	−0.263	0.001	0.010
	NO_3^-	0.260	−0.950	0.174	0.017
	NH_4^+	0.045	−0.166	−0.982	0.079
	Urea	−0.001	−0.032	−0.075	−0.997

Table 6. Correlations between combined variables (temperature and N concentrations) and urea uptake and urease activity. PC indicates combined variables when analyzing data from all locations, APC indicates combined variables from Anacostia River, and CPC indicates combined variables from Choptank River and Chesapeake Bay. Asterisk (*) denotes significance at $P < 0.05$.

Site	Variables	PC1	PC2	PC3	PC4
All (PC)	Urea uptake	−0.118	−0.238	−0.020	0.294
	P value	0.06	<0.001*	0.91	<0.001*
	Urease activity	−0.220	0.134	0.069	0.074
	P value	<0.001*	0.03*	0.61	0.34
Anacostia (APC)	Urea uptake	0.188	0.282	−0.341	0.691
	P value	<0.001*	<0.001*	<0.001*	<0.001*
	Urease activity	−0.240	−0.188	−0.002	0.075
	P value	<0.01*	0.06	0.85	0.41
Choptank & Chesapeake Bay (CPC)	Urea uptake	−0.330	0.075	−0.143	−0.208
	P value	<0.01*	0.59	0.35	0.08
	Urease activity	−0.340	−0.007	0.185	−0.075
	P value	<0.01*	0.90	0.14	0.42

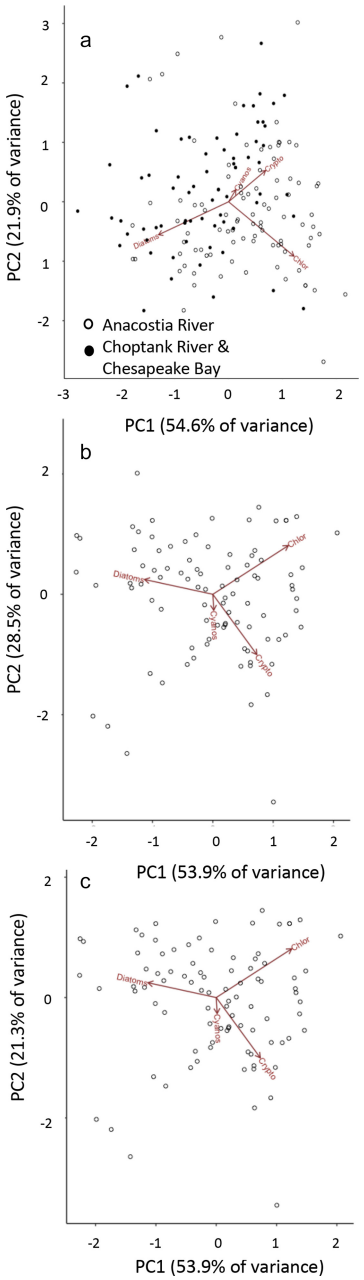

Figure 5. Principal component analysis showing the first two principal components for pigment:Chl *a* for all sites (a), Anacostia River (b), and Choptank River and Chesapeake Bay (c). The third and fourth principal components are not shown because of low proportion of variance.

Table 7. Proportion of variance and loadings for multivariable (principal component analysis) analysis for pigment:Chl *a* ratios. PPC indicates pigment combined variables when analyzing data from all locations, APPC indicates pigment combined variables from Anacostia River, and CPPC indicates pigment combined variables from Choptank River and Chesapeake Bay.

Site	Variables	PC1	PC2	PC3	PC4
All (PPC)	Proportion of variance	0.546	0.219	0.150	0.086
	Diatoms	−0.680	−0.459	0.493	0.290
	Cyanobacteria	0.071	0.169	−0.296	0.937
	Chlorophytes	0.635	−0.756	0.101	0.120
	Cryptophytes	0.360	0.435	0.812	0.151
Anacostia (APPC)	Proportion of variance	0.539	0.285	0.142	0.033
	Diatoms	−0.616	0.184	−0.638	0.424
	Cyanobacteria	0.008	−0.199	0.496	0.845
	Chlorophytes	0.681	0.605	−0.282	0.301
	Cryptophytes	0.397	−0.748	−0.517	0.123
Choptank & Chesapeake Bay (CPPC)	Proportion of variance	0.559	0.213	0.137	0.091
	Diatoms	−0.896	0.338	−0.085	0.275
	Cyanobacteria	0.130	0.358	0.888	0.257
	Chlorophytes	0.365	0.839	−0.402	0.033
	Cryptophytes	0.217	−0.230	−0.207	0.926

Table 8. Correlations between pigment combined variables (pigment:Chl *a*) and urea uptake and urease activity. PPC indicates pigment combined variables when analyzing data from all locations, APPC indicates pigment combined variables from Anacostia River, and CPPC indicates pigment combined variables from Choptank River and Chesapeake Bay. Asterisk (*) denotes significance at $P < 0.05$.

Site	Variables	PC1	PC2	PC3	PC4
All (PPC)	Urea uptake	0.217	0.045	0.093	0.229
	P value	0.05*	0.54	0.36	<0.001*
	Urease activity	0.120	−0.516	−0.052	−0.022
	P value	0.25	0.81	0.71	0.82
Anacostia (APPC)	Urea uptake	0.039	−0.184	−0.041	0.044
	P value	0.64	0.15	0.60	0.68
	Urease activity	0.059	0.092	0.124	−0.129
	P value	0.48	0.54	0.31	0.36
Choptank & Chesapeake Bay (CPPC)	Urea uptake	0.175	0.069	0.391	−0.132
	P value	0.13	0.78	<0.01*	0.23
	Urease activity	0.086	−0.089	0.030	0.028
	P value	0.82	0.26	0.88	0.68

In the Choptank and the Chesapeake Bay, the pigment combined variables were defined differently in that chlorophytes contributed positively to Chesapeake pigment PC1 (CPPC1), chlorophytes contributed positively to Chesapeake pigment PC2 (CPPC2), cyanobacteria contributed positively to Chesapeake pigment PC3 (CPPC3), and cryptophytes contributed positively to Chesapeake pigment PC4 (CPPC4; Fig. 5c). Urea uptake was significantly positively related to CPPC3, whereas there were no significant relationships of urease activity with any of the pigment combined variables (Table 8). This suggests that urea uptake was positively related to the presence of cyanobacteria.

4. Discussion

a. Seasonal changes in rates of urea uptake and urease activity

McCarthy was the first to measure urea concentrations and urea uptake rates in the Chesapeake Bay (McCarthy, Taylor, and Taft 1977). Lomas et al. (2002) built on this foundation using data collected between 1972 and 1998. Their reported urea uptake rates were on average higher during the summer months in surface samples, which is consistent with observations reported here from 2001 to 2004 in the Chesapeake Bay. Both urea uptake and urease activity rates showed seasonal patterns with rates generally higher during the summer at all sites, including the Chesapeake Bay (Fig. 3). Urea uptake and urease activity were often positively correlated to combined variables that included warmer temperatures. The results held whether analyzed overall or within the different locales.

Higher rates of urea uptake in the warmer months have previously been measured in temperate estuaries (Kristiansen 1983; Glibert et al. 1991; Bronk et al. 1998; Lomas et al. 2002; Moschonas et al. 2017). The importance of temperature in urea metabolism is suggested by positive relationships between urea uptake and temperature observed in diatom-dominated assemblages. This contrasts to negative relationships between NO_3^- uptake and temperature for similar diatom-dominated assemblages (Lomas and Glibert 1999). Where diatoms dominate, high urea uptake rates during spring blooms have also been measured in colder regions (Sanderson et al. 2008; Simpson et al. 2013; Moschonas et al. 2017). Although the Anacostia River does not experience spring blooms like other temperate tidal rivers (Solomon, Jackson, and Glibert 2019), urea uptake rates were often higher during earlier parts of the year in the downstream reaches of the river and were not positively related to temperature (APC1) and weakly correlated with diatom abundance (APPC3).

Urea uptake may be regulated by temperature within different phytoplankton groups within phytoplankton communities, especially cyanobacteria that thrive during the warmer months (Paerl and Huisman 2008). Regulation of cyanobacterial urea transporters, such as AmtR and NtcA, by temperature and urea availability may differ from eukaryotic phytoplankton transporters (Solomon et al. 2010). Cyanobacteria were consistently related to urea uptake rates when analyzed over all locations in the Chesapeake Bay, the Lower Anacostia, and Lower Bay (Tables 3, 7, and 8). Similar field studies have found that cyanobacteria are either associated with higher urea uptake and urease activity rates or have been identified

as consumers of urea (Florida Bay and Western Florida Shelf: Glibert et al. 2004; Heil et al. 2007; Wawrik, Callaghan, and Bronk 2009; Lake Erie: Belisle et al. 2016). Utilization of urea is regulated differently in cyanobacteria than other eukaryotic phytoplankton because some species do not contain the genes coding for urease and a complete urea cycle (Collier, Brahamsha, and Palenik 1999; Solomon et al. 2010).

This study found that urease activity was highest during the summer months in the Anacostia and Choptank Rivers and the Chesapeake Bay, and this was supported by significant relationships with combined variables associated with warmer temperatures when all data were examined together and on a local level. Urease activity was found to be higher when growth temperatures were similar to temperatures observed during the summer months (20°C–30°C) for three phytoplankton species, *Aureococcus anophagefferens, Thalassiosira weissflogii*, and *Prorocentrum minimum* (Fan et al. 2003). In field studies, Siuda and Chróst (2006) found a positive relationship between temperature and urease in lakes in the Mazurian Lake District in Poland, but in Lake Erie, there was no significant increase in urease activity with temperature despite one with ambient urea concentrations (Belisle et al. 2016).

b. Regulation of urea uptake and urease activity by NO_3^- and NH_4^+ availability

The results from the Anacostia and Choptank Rivers and the Chesapeake Bay support a growing body of evidence that urea uptake is suppressed or inhibited by NH_4^+. NH_4^+ exhibited significant negative correlations with combined variables when examining data from all sites. Urea uptake rates of phytoplankton grown under N-replete conditions have been shown to decrease after the addition of NH_4^+ and/or NO_3^- in culture (McCarthy 1972a; Horrigan and McCarthy 1981, 1982; Lund 1987; Lomas 2004) or in field incubations (Tamminen and Irmisch 1996). Urea uptake rates are often low in the field when NH_4^+ concentrations are higher, further supporting this relationship. For example, in a study in the Neuse River estuary, North Carolina, NH_4^+ concentrations exceeding 40 μM-N were associated with low urea uptake rates in the upper portion of the estuary (Twomey, Piehler, and Pearl 2005), and Kristiansen (1983) found that urea uptake was inhibited by NH_4^+ concentrations higher than 1–2 μM-N in Oslofjord, Norway.

In contrast to urea uptake, the information available about regulation of urease activity by NO_3^-, NH_4^+, or urea suggests a more complex relationship. Urease activity in this study did not have any significant relationships with combined variables consisting of different N forms with the exception of NO_3^-. Diatoms grown on NO_3^- in culture have lower urease activity than when grown on NH_4^+ or urea (Peers, Milligan, and Harrison 2000; Lomas 2004). Diatoms, mostly large centric species, have been found to be the main utilizers of urea within the mixed layer of the Southern California Blight when NO_3^- and NH_4^+ concentrations were <0.25 μM (Morando and Capone 2018). Likewise, as shown herein, rates of urease activity were generally lower during spring when NO_3^- concentrations were high and diatoms dominated the phytoplankton community than during the summer. Urease activity

rates are consistently the highest during the summer months, despite difficulties in identifying the main consumers of urea among different phytoplankton communities (Solomon 2006; Belisle et al. 2016). Cyanobacteria, which dominated the summer community during the dry years in the Chesapeake Bay and downstream in the Anacostia River, have been shown to have lower urease activity when grown on NH_4^+ than NO_3^- or urea in culture (Collier, Brahamsha, and Palenik 1999; Fan et al. 2003; Solomon and Glibert 2008). In the West Florida Shelf, a coastal marine system characterized by lower inorganic N (NO_3^- and NH_4^+) concentrations than organic urea and dissolved primary amines, *Synechococcus*, which is also prevalent in the Anacostia, has been shown to use urea (Glibert et al. 2004; Wawrik, Callaghan, and Bronk 2009). Diatoms and cryptophytes, which were prevalent during the summer months in the wet years in both Upper Bay and Mid Bay and the Upper Anacostia River during the spring months, do not have lower urease activity when grown on NH_4^+ in culture (Peers, Milligan, and Harrison 2000; Lomas 2004). Thus, the higher summer NH_4^+ concentrations during the wet years in Chesapeake Bay or years with high flow in the Anacostia River may not repress urease activity in diatoms and cryptophytes.

The understanding of regulation of urease activity that is emerging from culture studies has not yet translated into a better understanding of field populations as it is often impossible to differentiate which phytoplankton group is responsible for higher urease activity rates (Solomon 2006; Belisle et al. 2016). Temperature, more than any other environmental factor or phytoplankton taxa, is correlated with urease activity in the Anacostia and Choptank Rivers and Chesapeake Bay. Urease activity may be regulated more by other factors such as nickel availability or light; however, these were not addressed in this analysis. Urease contains a nickel metallocenter (Oliveira and Anita 1986; Mobley and Hausinger 1989; Mobley, Island, and Hausinger 1995), and diatoms have been shown to have higher growth and urease activity when grown on urea and Ni^+ in culture (Rees and Bekheet 1982; Price and Morel 1991; Egleston and Morel 2008).

Urease activity is thought to be conservative and is often expressed on a basal level (Solomon et al. 2010; Allen et al. 2011). Intracellular urea is produced consistently as a metabolite via the ornithine-urea cycle under exponential growth in diatoms (Allen et al. 2011). These two factors may complicate any response to different environmental conditions. With the discovery of a complete urea cycle in diatoms (Armbrust et al. 2004; Bowler et al. 2008), new questions have emerged regarding the role of urea, and subsequently urease, in phytoplankton cells. McCarthy (1972a) did note that some diatoms that did not grow on urea exhibited some short-term uptake of urea and suggested that utilization processes for urea in these diatoms was lacking. We now understand that it is not missing, but that urease, considered part of the urea utilization pathway, may be regulated more by light than external N sources (Bender, Parker, and Armbrust 2012). In addition to being transported into the cell via various cell transporters, urea is being generated as a metabolic by-product of the ornithine-urea cycle that is closely coupled with the TCA (tricarboxylic acid) cycle (carbon fixation) and GS/GOGAT pathway (N assimilation; Allen et al. 2011; Bender, Parker, and Armbrust 2012), potentially explaining the regulation of urease by light conditions. This

may be true for other phytoplankton that have been found to also have a complete urea cycle (Solomon et al. 2010). Further studies on the regulation of urease need to take into consideration the influence of the various metabolic pathways in the phytoplankton cell rather than just exogenous N sources alone.

c. Urease activity as a proxy for urea utilization

Urea uptake and urease activity rates followed different patterns over similar seasonal and nutrient gradients suggesting that the urea transporters (e.g., *URT, DUR3*, and *SLC14A*) and urease (e.g., *ureABC*; Baker, Gobler, and Collier 2009; Solomon et al. 2010) are regulated by different environmental and cellular factors. This observation is supported by the lack of a statistically significant relationship between urea uptake and urease activity rates (Fig. 2). Urea uptake rates seem to be more closely related to the availability and contribution of ambient urea to the total N pool, whereas urease activity may be more sensitive to internal pools of urea generated by the ornithine-urea cycle, nickel availability, and light. Although urease has one gene with several accessory genes, there are at least three known urea transporters that are present and regulated differently in various phytoplankton species (Solomon et al. 2010) that contribute to a phytoplankton community.

5. Conclusion

McCarthy began the examination of the role of urea in the metabolic demand of phytoplankton in Chesapeake Bay (McCarthy 1972b) and La Jolla Bay (McCarthy and Kamykowski 1972) as a natural laboratory. McCarthy, Taylor, and Taft (1977) measured urea concentrations and urea uptake in the Chesapeake Bay and found that often phytoplankton preferred to use NH_4^+ and urea over NO_3^- and that uptake rates were consistently related to N availability. Since McCarthy's original studies, methods for measuring urea concentrations and urease activity in the field have been refined. Because of these improvements, there is now a better understanding of how a range of phytoplankton and bacteria can use urea to meet their N metabolic demand, in both N-limiting and nonlimiting waters (Anita, Harrison, and Oliveria 1991; Kirchman 2000; Collos and Harrison 2014; Glibert et al. 2016). The ability of those organisms to utilize urea depends on the regulation of urea uptake and urease activity in natural waters. Despite the complexity of covarying environmental factors in the field, urea uptake rates are consistently related to ambient urea concentrations, temperature, and specific phytoplankton taxa relationships, as they were in previous culture studies. Recent discoveries related to the presence of the urea cycle in diatoms and other eukaryotic phytoplankton suggest that urease is regulated differently from urea uptake because of close coupling with carbamoyl phosphate synthease, the urea cycle, the TCA cycle, and the GS/GOGAT pathway (Allen et al. 2011, Bender, Parker, and Armbrust 2012). In light of those findings, examining the regulation of urease is just beginning, as is our understanding of the role of urea in bacteria and phytoplankton N metabolism.

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