



Urea Inputs Drive Picoplankton Blooms in Sarasota Bay, Florida, U.S.A.

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Abstract: Recent increases in global urea usage, including its incorporation in slow-release fertilizers commonly used in lawn care in Florida, have the potential to alter the form and amount of nitrogen inputs to coastal waters. This shift may, in turn, impact phytoplankton community diversity and nutrient cycling processes. An autonomous water quality monitoring and sampling platform containing meteorological and water quality instrumentation, including urea and phycocyanin sensors, was deployed between June and November of 2009 in Sarasota Bay, Florida. This shallow, lagoonal bay is characterized by extensive and growing urban and suburban development and limited tidal exchange and freshwater inputs. During the monitoring period, three high-biomass (up to $40 \ \mu g$ chlorophyll-*a*·L⁻¹) phytoplankton blooms dominated by picocyanobacteria or picoeukaryotes were observed. Each bloom was preceded by elevated (up to 20 μ M) urea concentrations. The geolocation of these three parameters suggests that "finger canals" lining the shore of Sarasota Bay were the source of urea pulses and there is a direct link between localized urea inputs and downstream picoplankton blooms. Furthermore, high frequency sampling is required to detect the response of plankton communities to pulsed events.

Keywords: autonomous water quality monitoring platform; eutrophication; "finger canals"; nutrient pulses; picocyanobacteria; urea

1. Introduction

Eutrophication of estuarine and coastal waters is a significant global problem [1–5]. Elevated nutrient loading, typically in the form of dissolved nitrate and phosphate from agricultural sources [6,7], results in increases in estuarine and coastal phytoplankton biomass and productivity [8,9], which can lead to degraded and esthetically unappealing water quality. Other detrimental impacts include the development of harmful algal blooms (HABs), loss of seagrasses and bottom habitat, development of hypoxic zones, decreases in economically important aquatic species, changes to the local habitat biodiversity, and widespread impacts to regional tourism economies [1–3,10–16].

Globally, urea has increasingly replaced nitrate as the agricultural nitrogen (N) source in fertilizer over the past 30 years [17]. It is often used in animal feeds and manufacturing processes, to the point that it now accounts for more than 50% of global nitrogen fertilizer use [13,17,18]. How much of this

urea is volatilized at the application site and how much is transported to the coastal ocean is unknown. Recent studies on urea in coastal waters suggest that allochthonous urea inputs can be significant, particularly in areas adjacent to agriculture systems during rain-driven runoff events (summarized in Glibert et al. [17] and Switzer [18]) or urban wastewater [19]. Generally present in concentrations <1 μ M N in coastal systems [20–23], urea concentrations can be locally elevated and sufficient enough to represent a large portion of the dissolved organic nitrogen (DON) pool. Within tributaries of the Chesapeake Bay, urea concentrations have been reported as high as 25 to 50 μ M N [13,24] and elevated concentrations have also been reported in nearshore waters adjacent to the heavily fertilized Yaqui Valley, Mexico [17,25].

Within Florida, urea is used extensively as a fertilizer for animal feeds, citrus crops, and lawns [26]. It has been shown to readily leach from both soluble and slow-release forms of fertilizer [27]. In southwest Florida, some of this urea is transferred to coastal waters. Glibert et al. [17] documented elevated urea concentrations (up to 4 μ M) at the mouths of the Caloosahatchee and Shark rivers after Hurricane Charley in 2004. During low runoff conditions these rivers typically have urea concentrations <1 μ M [22]. Urea concentrations of up to 1.4 μ M have been noted in Florida Bay during managed flow processes out of the Everglades [28], and between 2.2 and 6.4 μ M in Florida Bay's adjacent canals and reef track [29].

Urea has long been known to be a significant N source for phytoplankton [15,30–32] and these allochthonous urea inputs may contribute significant N to coastal phytoplankton communities [33–35]. Urea is readily available for uptake by marine bacteria and phytoplankton that possess the enzyme urease (see Solomon et al. [34] for review). Its influence on the promotion of coastal HAB species has been suggested by Glibert et al. [17] based on global maps of HAB distributions and urea inputs, although Gowen et al. [10] and Davidson et al. [36] argue that there are currently insufficient data to evaluate this relationship. Urea has been shown to select for non-heterocytous cyanobacteria and chlorophytes in freshwater phytoplankton populations [37] and dinoflagellates in estuarine aquaculture ponds [38] and lagoonal waters [33]. Blooms of cyanobacteria and dinoflagellates often follow large inputs of urea to coastal waters [28,39–41]. Additionally, Kudela et al. [42] have demonstrated that many of the HAB species found on California's coast will preferentially use urea as a N source, even when urea concentrations are at or below 24 μ g N L⁻¹.

In southwest Florida shelf waters, urea has been shown to be a preferred N source for dinoflagellates and cyanobacteria [22]. Much of the coastal areas of southwest Florida are bordered by extensive resort-like development, which includes residential housing, lawns or parks, and golf courses [43,44]. This has raised public concern as to the fate of fertilizers, especially urea-based lawn fertilizers, in coastal waters and their role in supporting toxic Karenia brevis blooms, which have been shown to preferentially take up urea in southwest Florida coastal waters [23,45]. Here we report on the deployment of an autonomous water quality monitoring and sampling platform, which included urea and phycocyanin (a pigment commonly used as a proxy for detecting cyanobacteria) sensors, and the relationship between in situ urea concentrations, rainfall, wind events, and picoplankton blooms in Sarasota Bay. Sarasota Bay, located in southwest Florida, is a coastal lagoonal system bordered by extensive suburban development, including man-made "finger canals", a land development feature utilized since the 1960s to make more waterfront properties [44], but which often results in degraded water quality, particularly in summer months [46]. Man-made waterways such as these are known to act as natural incubators for numerous HAB species and have been a "hot spot" for HABs along the US east coast [47–50], including cyanobacteria HABs (cyanoHABs). Managing cyanoHABs for human and environmental safety has become a critical focus of scientists and policymakers alike over the past decade. As summarized in Bullerjahn et al. [51], agricultural nutrient loading is a major driver of cyanoHABs, and may be tightly coupled with predicted climate changes (increases in global temperature and major storm events [52]). There are few studies on the harmful properties of picocyanobacteria; however, Sliwińska-Wilczewska et al. [53,54] have shown that picocyanobacteria populations thrive under the conditions predicted to occur with global climate change and produce both allelopathic compounds

that alter the phytoplankton community structure and secondary metabolites that harm aquatic flora and fauna.

The technology and platforms that we use to detect, track, and predict these blooms, especially cyanoHABs, are currently limited in spatial and temporal resolution. Data from the water quality monitoring platform described here demonstrate how autonomous high-frequency temporal sampling can detect and monitor picoplankton blooms that are not routinely captured through traditional monitoring programs. This effort also provides further evidence for resource managers of this sub-tropical lagoonal system that nutrient reductions, particularly reductions in urea-based fertilizers, must be achieved in order to reduce the threat from cyanoHABs within Sarasota Bay.

2. Methods

2.1. Sampling Stations

An autonomous water quality monitoring and sampling platform was deployed from 16 June 2009 to 10 November 2009 in 3 m of water in an area adjacent to Long Boat Key, FL in Sarasota Bay, on the mid-central Gulf coast of Florida, U.S.A. (Station 3, Figure 1). The deployment site is a shoal region with minimal boat traffic and situated immediately offshore of a large golf course. The platform was serviced biweekly, coincident with in situ sampling at the platform site and six additional stations in Sarasota Bay (Figure 1) to provide validation of the autonomous platform data and a "snapshot" of biweekly conditions within the Bay. A YSI 6600 Sonde (YSI, Inc., Yellow Springs, OH, USA) was used to profile over the depth of each station for temperature, salinity, pH, dissolved oxygen, turbidity, phycocyanin fluorescence, and chlorophyll-*a* (Chl-*a*) fluorescence. At Station 3, additional samples collected biweekly at approximately 10 cm below the surface using a modified Nisken bottle included inorganic nutrients (NO₃⁻, NH₄⁺, PO₄⁻, and SiO₄), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), particulate carbon (C), nitrogen (N) and phosphorus (P), and whole water urease activity. Whole water samples were collected for plankton analyses. All water samples were stored at ambient water temperature in a cooler and returned within two hours to the lab for processing.



Figure 1. Map of Sarasota Bay showing the autonomous platform (MARVIN) station mooring site (Station 3) and six additional stations monitored throughout the study.

2.2. Autonomous Water Quality Platform

Water measurements at Station 3 were conducted using an autonomous water quality platform called the MERHAB Autonomous Research Vessel In-situ (MARVIN) [55,56]. The MARVIN platform consisted of a 14' by 8' aluminum pontoon boat with a mast and two 6'chests bolted on the deck that housed instruments (Figure 2). Water quality parameters measured by the instruments are listed in Table 1. A peristaltic pump was used to collect a surface water sample at the top of the hour and a bottom sample at the half hour using Tygon[®] (US Plastic Corporation, Lima, OH, USA) tubing fitted with 280-µm mesh screen on the intake. The near-surface tubing was lowered to approximately 0.5 m below the platform while the near-bottom tubing was kept approximately 0.5 m above the bottom. The pumped water samples were passed through a YSI 6600 sonde (YSI, Inc.) to measure salinity, temperature, Chl-a fluorescence, phycocyanin fluorescence, pH, dissolved oxygen (DO), and turbidity. Following the methods of Rahmatullah and Boyd [57] and Glibert et al. [58], a Microlab sensor (EnviroTech, Chesapeake, VA, USA) measured urea concentrations of the sampled water every three hours. Urea sampling interval was limited by the 164 min sample processing time. A Vaisala meteorological sensor (Vaisala, Woburn, MA, USA) mounted on the mast measured wind speed, wind direction, air temperature, barometric pressure, relative humidity, and precipitation. A Sontek XR ADP (Xylem, San Diego, CA, USA) mounted on the platform hull in a downward direction measured three-dimensional current speed and direction every hour and Li-Cor LI-190 and LI-192 meters (Li-Cor Biosciences, Lincoln, NE, USA) were used to measure irradiance at the surface and 1 m below the surface. The entire monitoring platform system and its component instrumentation was integrated and controlled using a Campbell CR1000 datalogger (Campbell Scientific, Logan, UT, USA). Data were downloaded every hour using a cellular modem. Power was provided by two 80 W solar panels with four 80 deep-cycle marine batteries for night and low light periods. Date and time data were recorded in GMT.



Figure 2. The MERHAB Autonomous Research Vessel In-situ (MARVIN) deployed in Sarasota Bay.

| Instrument | Measurement | Final Output | Sample Location | | | |
|----------------------|------------------------------|---|--------------------|--|--|--|
| | Chlorophyll-a | $\mu g L^{-1}$ | Surface, Bottom | | | |
| | Salinity | PSU | Surface, Bottom | | | |
| | Temperature | °C | Surface, Bottom | | | |
| VSI 6600 Data Sanda | pH | pН | Surface, Bottom | | | |
| 131 0000 Data 30110e | Phycocyanin | cells L^{-1} | Surface, Bottom | | | |
| | Dissolved Oxygen | $mg L^{-1}$ | Surface, Bottom | | | |
| | Dissolved Oxygen | Percent saturation | Surface, Bottom | | | |
| | Turbidity | Nephloid Turbidity Units | Surface, Bottom | | | |
| YSI 9600 | Nitrate | $\mu g L^{-1}$ | Surface, Bottom | | | |
| Envirotech | Urea | $\mu g L^{-1}$ | Bottom | | | |
| | Wind Speed | ${ m m~s^{-1}}$ | 4 m above surface | | | |
| | Temperature | °C | 4 m above surface | | | |
| Vaisala WXT-520 | Precipitation | inches h^{-1} | 4 m above surface | | | |
| | Barometric Pressure | inches hg | 4 m above surface | | | |
| | Relative Humidity | percent | 4 m above surface | | | |
| Licor LI-190 | Licor LI-190 Downwelling PAR | | 4 m above surface | | | |
| Licor LI-192 | Downwelling PAR | μ einsteins m ⁻² s ⁻¹ | Surface, 1 m | | | |
| | Current Flows | m s ⁻¹ | horizontal X and Y | | | |
| Sontek ADCP | Current Flows | ${ m m~s^{-1}}$ | vertical | | | |
| | Water depth | m | Water column | | | |

Table 1. Instruments, with associated parameters measured, final output, and sampling depth, installed on the MARVIN platform during the 2009 Sarasota Bay deployment.

2.3. Laboratory Analyses

Whole water samples used for phytoplankton identification and enumeration (125 mL) were preserved with 5% unacidified Lugol's iodine solution for micro- and nano-plankton and with 25% glutaraldehyde solution for picoplankton. Picoplankton and phytoplankton species, including ~70 harmful species commonly monitored by the State of Florida (see Heil and Steidinger [59]), were identified and enumerated in each preserved sample using either an Olympus IX 71 (Olympus America, Center Valley, PA, USA) or Zeiss Axiovert 25 (Carl Zeiss, Thornwood, NY, USA) inverted microscope following the modified Utermöhl technique detailed in Corcoran et al. [60] in which a minimum of 300 cells were identified and enumerated in at least 20 optical fields at a 400× magnification. Uncommon species within the sample were enumerated when examining the entire settling chamber at a $100 \times$ magnification. Phytoplankton were identified to the lowest taxonomic group possible using Wehr and Sheath [61], Tomas [62], and Steidinger et al. [63]. For data analyses, taxa were binned into the following phytoplankton functional groups: Bacillariophytes, Chlorophytes, Cryptophytes, and Dinophytes. Picoplankton samples were processed according to the method of Marshall [64] by gravity filtering 1–2 mL of glutaraldehyde preserved whole water samples onto 0.45 µm black cellulose Nuclepore filter membranes (Sigma-Aldrich, St. Louis, MO, USA) and examining the filters using the epifluorescent module on the Olympus IX 71 microscope at 1000× magnification. A minimum of 300 autofluorescent cells $< 2 \mu m$ were enumerated in a minimum of 20 optical fields and categorically binned into cyanobacteria and eukaryotic components as described in Affronti and Marshall [65].

Whole water samples for size-fractionated Chl-*a* were filtered through Whatman GF/F (Sigma-Aldrich and 3.0 μ m Nuclepore filters (Sigma-Aldrich), immediately frozen, and analyzed within two weeks of collection according to Holm-Hansen [66]. Additional water was filtered through a 3.0 μ m Nuclepore filter and immediately frozen for subsequent analysis of inorganic nutrients (NO₃⁻, NH₄⁺, PO₄⁻, and SiO₄) and for determination of urea, particulate phosphorus (P), biogenic silica, total dissolved phosphorus (TDP), and total dissolved nitrogen (TDN) using an Alpkem RFA II segmented-flow nutrient analyzer (Xylem). The filter was retained and frozen for determination

of biogenic silica. Samples for particulate P were filtered through precombusted (2 h, 450 °C) Whatman GF/F filters, rinsed twice with 2 mL of 0.17 Na₂SO₄ and frozen with 2 mL of 0.017 MgSO₄ in precombusted (2 h, 450 °C) scintillation vials until analyzed. Samples for particulate N and C analyses were filtered through a pre-combusted (2 h, 450 °C) Whatman GF/F filter, washed with a 10% HCl solution made with Whatman GF/F filtered seawater, rinsed thoroughly with Whatman GF/F filtered seawater, and frozen until analysis using a Carlo-Erba Elemental Analyzer NA 1500 Series 2 instrument (CE Elantech, Inc., Lakewood, NJ, USA). Particulate P and TDP were analyzed using the methods of Solarzano and Sharp [67], TDN according to Bronk et al. [68], and biogenic silica according to Paasche [69]. Urea was analyzed following the methods of Koroleff [70], while urease activity was measured following the method of Solomon et al. [71]. Dissolved organic phosphorus (DOP) was defined as TDP less PO₄⁻. Dissolved inorganic nitrogen (DIN) was defined as NO₃⁻ plus NH₄⁺ and dissolved inorganic phosphorus (DIP) is represented by PO₄⁻.

2.4. Urea Enrichment Experiments

Between 2 October and 22 October, additional water was collected from Station 3, returned to the laboratory within two hours, and immediately used to conduct bioassays examining the response of ambient picoplankton communities to urea additions. Water samples were first screened to remove larger grazers using a <150 μ m mesh, then size-fractionated by gravity filtration through 1.0 and 12.0 μ m Nuclepore (Sigma-Aldrich) filters. Duplicate 250 mL samples from each size-fraction and the original whole water sample were amended with urea additions (final concentration of 1 μ M urea) while duplicate unamended samples of each fraction served as controls. All treatments were incubated at 25 °C in a Revco plant growth chamber (ThermoFisher Scientific, Waltham, MA, USA) with cool white fluorescent lights (Ecolux F40C50, Philips F40T12) set to a 12:12 L:D cycle at a saturation irradiance of 80 mmol photons m² s⁻¹. Picoplankton concentrations and categorization were determined as described above in each bioassay sample before and after a three-day incubation period.

2.5. Historical Data

Additional historical water quality data (Chl-*a*, NO₃⁻, NH₄⁺, PO₄⁻, SiO₄) collected at 40 randomly selected stations within Sarasota Bay monthly from 1998 through 2009 were provided by Sarasota County (https://sarasota.wateratlas.usf.edu/). The stations, while not coinciding with the current study's stations due to our targeted selection of stations to monitor locations with potential runoff sources did provide a broad assessment of biogeochemical properties in Sarasota Bay over a coincident time period. Southwest Florida Water Management District rainfall data (https://www.swfwmd.state.fl.us/data), which covered a larger geographical area than the region in which the MARVIN platform collected data, were also used to provide a more comprehensive dataset for analyses.

2.6. Statistical Analysis of Wind Influence

Statistical analyses were performed on the time series data collected by the MARVIN platform using SigmaPlot (Systat Software, Inc., San Jose, CA, USA). A second order locally estimated scatterplot smoothing regression was used to minimize noise and display long term trends in the data [72]. A subset of the data was used to test whether wind events could pull water out of the sheltered "finger canals". To create this subset, wind, current, and coincident urea data were filtered to remove urea values that might be due to runoff by selecting periods that had no rainfall within 72 h prior to sampling. Additionally, urea values were filtered to remove any values below the median. The vector directions for these higher values were plotted on a compass diagram to estimate abiotic influences on urea concentrations.

3. Results

3.1. Time Series Trends

Water quality during the 2009 MARVIN platform deployment was characterized by a decrease in salinity from 38.3 to 36.2 that coincided with increased rainfall in the region (Figure 3). As the salinity declined, both urea and Chl-*a* values increased; Chl-*a* reached two maximums of 23 μ g L⁻¹ and 31 μ g L⁻¹ which were preceded by short-term (2–3 day) spikes in urea concentration, in one case this spike exceeded 12.5 μ M.



Figure 3. MARVIN deployment data from 29 July to 9 November 2009. Phytoplankton blooms are represented by chlorophyll-*a* values. Associated precipitation, urea and salinity data recorded by MARVIN sensors are also plotted. The time series data collected at Station 3, where the MARVIN platform was deployed in Sarasota Bay, indicate that several blooms occurred following rain events which altered ambient urea and salinity concentrations.

During the periods of highest observed Chl-*a* values, the plankton community was dominated by chlorophytes (<10 μ m) or dinophytes (>10 μ m) and picoplankton (<2 μ m) (Figure 4). While dinoflagellates and chlorophytes were present throughout the sampling period, their concentrations never exceeded 1.9 × 10⁴ and 5.0 × 10⁴ cells L⁻¹, respectively, and no cryptophytes were noted. A prolonged precipitation event, resulting in 74.1 mm of rain, was recorded by the MARVIN platform from 20 August to 29 August. During this period, salinity decreased by 0.2 PSU and a pulse of urea was detected which reached a maximum of 6.2 μ M N on 23 August. After this urea pulse the Chl-*a* concentration increased from the minimum of 2.2 μ g L⁻¹ on 18 August, to a high of 24.5 μ g L⁻¹ on 4 September. Salinity decreased again between 9 September and 17 September by 0.17 PSU due to rainfall of 59 mm during this period. At the same time, urea concentration peaked at 12.6 μ M N and then declined. Following this second decrease in salinity and increase in urea, Chl-*a* began to increase on 20 September and reached a maximum value of 32.0 μ g L⁻¹ on 12 October. Coincident with this second pulse of urea the Chl-*a* concentration reached 23 μ g L⁻¹, salinity was reduced by 0.5 PSU, and urea concentrations reached a maximum of 5.5 μ M N.



Figure 4. Phytoplankton functional groups identified and enumerated using a modified Utermöhl microscopy technique. Picoplankton concentrations are based on enumeration of cells in optical fields using epifluorescent microscopy.

The annual rainfall in Sarasota Bay, calculated for the seven years proceeding this study, was 1080.5 mm yr⁻¹, while the mean of the last 96 years was 1324.2 mm yr⁻¹. An autonomous water quality buoy offshore of Sarasota Bay (http://comps.marine.usf.edu:81/) recorded average salinity values of 35.5 PSU during the deployment period. MARVIN sensors measured salinity values of 38.2 PSU during the first week of the deployment (17–24 June) and then declining values thereafter to a low of 34.7 PSU on 22 October. After 22 October salinity values rose to 36.1 PSU.

3.2. Phytoplankton Data

In the early part of the study only the settling chamber technique [60] was employed to examine the plankton community. Unfortunately, this methodology was not adequate for evaluating picoplankton populations. The subsequent use of epifluorescent microscopy [64] allowed for a more accurate assessment of the picoplankton community. Abundant picoplankton populations were detected when the water temperature was above 30 °C and declined when the water temperature dropped below 20 °C (Figure 4). Epifluorescent microscopy of picoplankton samples indicated that the Sarasota Bay picoplankton community was comprised of both prokaryotic and eukaryotic taxa. In September, when water temperatures exceeded 30 °C, the picoplankton community was dominated by picocyanobacteria (maximum concentration of 4.0×10^7 cells L⁻¹). On 2 October, total picoplankton concentrations of 2.40×10^9 cells L⁻¹. On 22 October, during the declining phase of the bloom (as indicated by Chl-*a* concentration), the total picoplankton concentration was 1.13×10^9 cells L⁻¹ and the eukaryotic picoplankton concentration was 7.94×10^8 cells L⁻¹. During the initial phase of the picoplankton bloom, when picocyanobacteria dominated, diatom concentration was lower and did not increase until the picoplankton community shifted to mix of both eukaryotic and prokaryotic taxa.

3.3. Urea Enrichment Experiments

Results of the urea bioassays conducted with replicates of size-fractionated water samples (150 μ m screened, <12 μ m, <1 μ m; n = 6) from the 2 October bloom are presented in Figure 5. These samples were used to determine the effect of a 1 μ M urea addition during a three-day incubation period. All three size-fractionated water samples exhibited increases in the cell concentrations of both picocyanobacteria and picoeukaryotes in the urea enriched samples compared to the control. Increases were greater in the smaller size-fractionated water samples and the picoeukaryotes exhibited greater increases in cell concentrations within these smaller fractions, approximately double those of the picocyanobacteria.



Figure 5. Growth of picocyanobacteria and picoeukaryote algae in different size-fractionated water samples during a three-day bioassay. Station 3 water samples, collected on 2 October 2009 during a picoplankton bloom, were amended with 1 μ M urea additions.

3.4. Discrete Sample Data

A comparison of biweekly size-fractionated Chl-*a* data and the MARVIN Chl-*a* fluorescence sensor data indicated that only one Chl-*a* maximum was captured by the discrete biweekly in situ sampling which began on 16 June (Figure 6). Beginning on 27 July the <3 μ m Chl-*a* size-fraction was greater than the >3 μ m fraction. This coincided with a decline in salinity and increase in urea concentrations immediately prior to the first observed Chl-*a* peak. This change in Chl-*a* fraction dominance also followed a large peak in phycocyanin fluorescence (an indicator of cyanobacteria) on 17 July. The maximum difference between the two fractions was 1.16 μ g L⁻¹ for the >3 μ m fraction and 10.47 μ g L⁻¹ for the <3 μ m size-fraction on 2 October, which occurred during the bloom period. Phycocyanin fluorescence also indicated a peak that mirrored the shape of the Chl-*a* fluorescence peak during the October 2009 bloom. The phycocyanin sensor was not available between 10 August and 17 September but exhibited a peak of 3300 relative cells L⁻¹ on 17 July when Chl-*a* fluorescence was low and when the <3 μ m Chl-*a* concentration first increased above the >3 μ m fraction in value.



Figure 6. The time series of chlorophyll concentrations and phycocyanin fluorescence from the MARVIN platform compared to values determined in discrete size-fractionated samples.

Water samples exhibited high urease activity in the summer coincident with higher water temperatures and low urease activity in the fall after the water temperature declined (Figure 7). Urease activity had a value of 259.01 ng N μ g Chl- a^{-1} h⁻¹ on 13 July, coincident with a phycocyanin fluorescence value of 2200 relative cells L⁻¹. Urease activity was also elevated during the next three biweekly sampling periods, but due to the phycocyanin sensor failure there are no comparative data available. The urease activity on 10 August and the subsequent two biweekly sampling events coincided with increased urea concentrations, peaking at 241 ng N μ g Chl- a^{-1} h⁻¹ on 8 September. Urease activity declined in October after a 3 °C drop in water temperature, despite elevated urea concentrations. Although water temperature rapidly increased to just above 30 °C and phycocyanin fluorescence values were coincidentally high in early October, the urease activity was low. A second fall urea peak was detected on 20 October. During this time urease activity and Chl-a fluorescence values were low while phycocyanin fluorescence was high.





Figure 7. MARVIN sensor data showing the relationship of temperature, chlorophyll *a* and urea concentrations. Urease measurements were made on discrete water samples collected biweekly.

Dissolved inorganic nitrogen concentrations were generally low throughout the MARVIN deployment; NO₃⁻ concentrations were all within one standard deviation of $0.63 \pm 0.06 \mu$ M (Table 2). The NH₄⁺ concentrations averaged $0.39 \pm 0.34 \mu$ M, with a maximum of 1.42μ M NH₄⁺ following the first phycocyanin fluorescence peak on 27 July and a minimum of 0.07μ M during the October bloom period. Concentrations of PO₄⁻ were generally low, < $0.05 \pm 0.03 \mu$ M, with a maximum of 0.13μ M on 22 October during the bloom. PO₄⁻ concentrations were 0.01μ M or below the detection limit preceding the start of the wet season in June. The concentration of SiO₄ was $14.82 \pm 10.63 \mu$ M with a peak of 29.79 μ M on 8 September and a minimum of 2.29 μ M on 22 October.

| Table 2 | Analysis of nutrients | s from discrete wate | er samples collected fr | om the MARVIN | site (Station 3) | during the 2009 | deployment in S | arasota Bay. 1 | Missing d | lata for 30 |
|---------|---|----------------------|-------------------------|---------------|------------------|-----------------|-----------------|----------------|-----------|-------------|
| Septem | ber were due to equip | pment failure. | | | | | | | | |

| Date Sampled | ΤΝ μΜ | TC µM | TP µM | Urea µM N/L | NO3 μM | NO2 μM | Si µM | NH4 μM | PO4- μM | TDN uM | DON µM | TDP µM | DOP µM | Biogenic Si μM | Urease N µg ⁻¹ Chl-a ⁻¹ h ⁻¹ |
|-----------------------|-------|--------|-------|----------------|-----------|-----------|-------|-----------|------------|-----------|-----------|-----------|-----------|-------------------|--|
| 6/16/2009 | 15.01 | 121.58 | 0.94 | 1.19 | 0.44 | 0.02 | 6.30 | 0.29 | 0.05 | 24.81 | 24.06 | 0.54 | 0.50 | 17.80 | |
| 6/23/2009 | 12.54 | 99.23 | 1.00 | 0.44 | 0.64 | 0.01 | 4.24 | 0.48 | 0.04 | 23.51 | 22.37 | 0.41 | 0.37 | 22.51 | 58.71 |
| 7/8/2009 | 10.85 | 78.27 | 0.78 | 1.82 | 0.64 | 0.01 | 7.28 | 0.27 | 0.01 | 22.53 | 21.61 | 0.45 | 0.44 | 12.29 | 54.99 |
| 7/13/2009 | 10.06 | 69.33 | 0.72 | 0.81 | 0.65 | 0.01 | 10.34 | 0.29 | 0.01 | 22.03 | 21.07 | 1.06 | 1.06 | 13.04 | 259.01 |
| 7/27/2009 | 11.63 | 79.84 | 0.73 | 0.21 | 0.66 | 0.14 | 15.92 | 1.42 | 0.03 | 23.51 | 21.30 | 0.42 | 0.39 | 6.33 | 82.18 |
| 8/10/2009 | 11.74 | 86.59 | 0.63 | 0.33 | 0.65 | 0.02 | 20.91 | 0.34 | 0.06 | 23.03 | 22.02 | 0.45 | 0.39 | 3.88 | 295.08 |
| 8/25/2009 | 11.57 | 113.19 | 0.73 | 0.51 | 0.66 | 0.02 | 20.91 | 0.21 | 0.04 | 23.48 | 22.59 | 0.41 | 0.36 | 7.86 | 464.09 |
| 9/8/2009 | 11.95 | 93.78 | 0.77 | 1.11 | 0.64 | 0.03 | 29.79 | 0.47 | 0.05 | 26.97 | 25.83 | 0.65 | 0.60 | 3.33 | 241.23 |
| 9/22/2009 | 13.03 | 115.55 | 0.85 | 0.21 | 0.63 | 0.02 | 23.66 | 0.07 | 0.03 | 24.80 | 24.07 | 0.61 | 0.58 | 4.57 | 0.80 |
| 9/30/2009 | 18.45 | 149.75 | 0.32 | 1.15 | | | | | | 30.70 | | 0.09 | | | |
| 10/6/2009 | 18.64 | 171.31 | 0.19 | 0.52 | 0.64 | 0.02 | 36.07 | 0.07 | 0.04 | 24.74 | 24.02 | 0.09 | 0.05 | 2.09 | 0.00 |
| 10/22/2009 | 9.44 | 74.22 | 0.63 | 0.23 | 0.66 | 0.02 | 2.29 | 0.29 | 0.13 | 21.32 | 20.35 | 0.73 | 0.60 | 12.97 | 18.06 |
| 11/3/2009 | 11.51 | 99.70 | 0.71 | 0.92 | 0.66 | 0.14 | 6.66 | 0.45 | 0.05 | 30.74 | 29.49 | 0.63 | 0.58 | 13.16 | 24.17 |
| 11/17/2009 | 9.34 | 67.68 | 0.61 | 0.64 | 0.69 | 0.03 | 8.40 | 0.39 | 0.08 | | | 0.39 | 0.31 | 7.63 | 0.23 |
| Average | 12.55 | 101.43 | 0.69 | 0.72 | 0.63 | 0.04 | 14.83 | 0.39 | 0.05 | 24.78 | 23.23 | 0.50 | 0.48 | 9.80 | 124.88 |
| Standard Deviation | 2.92 | 30.59 | 0.22 | 0.47 | 0.06 | 0.05 | 10.63 | 0.34 | 0.03 | 3.00 | 2.52 | 0.25 | 0.23 | 6.11 | 152.17 |

Organic and particulate nutrient concentrations exhibited high variability at the MARVIN site (Table 2). TDN values averaged 24.78 \pm 3.00 μ M with maximum values of 30.70 μ M on 30 September and 30.74 μ M on 3 November. DOP values averaged 0.48 \pm 0.23 μ M with maximum of 1.06 μ M on 13 July and a minimum of 0.05 μ M on 6 October, corresponding to the picoplankton bloom. This low DOP concentration resulted in a DON:DOP ratio of 462.0 during the October bloom period. The average values of particulate C, N, and P were 101.43 \pm 30.59 μ M, 12.55 \pm 2.92 μ M, and 0.69 \pm 0.22 μ M, respectively. Excluding the period during the late October picoplankton bloom, the particulate N:P ratio was 15.35 \pm 1.49 (Figure 8). During the late September to early October bloom the ratio was 57.64, with a maximum value of 98.11 on 6 October.



Figure 8. Chlorophyll-a data from the MARVIN deployment with associated particulate N:P ratio. The red line indicates the Redfield Ratio value of 16.

3.5. Abiotic Data Relationships to Urea Concentrations

The physical data at the MARVIN site covaried with urea concentrations outside of the salinity values. The data were filtered to remove values above 20 μ M N urea concentrations since these exceeded the manufacturer stated concentration range of the instrument. The median values of the resulting urea data were 0.83 μ M N. A preliminary nonlinear regression analysis indicated there was a three-day lag between peak urea concentration values and decreases in salinity, so a filter was created to include only sample data collected when there was no rain in the previous 72 h and urea values above 0.83 μ M N. Within this reduced data set, 78.2% of the values occurred when the wind direction was in a quadrant from 90° to 180° (Figure 9). A line heading at 133° was found to be directly parallel to the shore off Long Boat Key near the MARVIN deployment site (Station 3). The current direction when the urea concentration was above the median value was ebbing in a direction >333° or less than 133° for 79% of the urea values above the median level (Figure 10).



Figure 9. Compass diagram showing the urea concentration (green dots) and associated prevailing wind direction, in relation to the MARVIN platform and Sarasota Bay bathymetry during the summer and fall 2009 deployment. Quadrant of prevailing wind direction is indicated in red. Black arrow indicates a line heading of 133°. Background image, Google Earth 2009.



Figure 10. Compass diagram showing the urea concentration (green dots) and associated water current direction, in relation to the MARVIN platform, measured during the 2009 deployment. The red hemisphere represents all the current directions away from the shore. Background image, Google Earth 2009.

4. Discussion

4.1. Species Composition Shifts

Three picoplankton blooms were recorded in Sarasota Bay during the 2009 MARVIN deployment. The first bloom occurred following a period of high rainfall in August. This was followed by a second bloom after a period of rainfall (September) and a third bloom at the end of the wet season period (October) (see Figures 2 and 3). The first two blooms appeared to be fueled by organic N inputs, including high urea concentrations; however, the final and largest bloom occurred during a period of both increased organic nutrient inputs and phosphate limitation (as indicated by elevated DON:DOP and DIN:DIP ratios). Phycocyanin fluorescence data indicate that the first two blooms were dominated by picocyanobacteria taxa with high urease activity, while the last was co-dominated by picoeukaryote and picocyanobacteria taxa with low urease activity and high particulate N:P and DON:DOP ratios. Even within major picoplankton groups, different genera react differently to N- and P-limitation [73] and some picoplankton can alter their physiology/genotypic expression to align with ambient nutrient conditions [74,75]. The dominant dissolved N form may also play a role in selecting for certain plankton groups, with urea selecting for cyanobacteria, picoeukaryotes and dinoflagellates [28,76,77]. In southwest Florida shelf waters, Heil et al. [22] have shown that urea favors cyanobacteria and dinoflagellates (as indicated by pigments) while nitrate favors diatom dominance. In Florida Bay, urea uptake is positively correlated with the percentage of the phytoplankton community composed of the picocyanobacterium Synechococcus sp. [28]. The MARVIN data suggest that this relationship is happening on much shorter time scales then previously demonstrated, with phytoplankton community shifts occurring in response to pulsed urea inputs on a scale of days to weeks. This time interval may be outside the resolution of most traditional, discrete water quality sampling programs. Indeed, only one of the events captured by the MARVIN platform was evident in the coincident biweekly in situ sampling. Excluding times of K. brevis blooms, historically the phytoplankton community in this region has been dominated by small diatom species, such as *Skeletonema costatum*, *Chaetoceros* spp., and naviculoid pennates, and peridinin-containing dinoflagellates [78,79]. Future efforts to monitor the temporal trends in picoplankton and nutrient concentrations should aim to collect more discrete taxonomic data so more accurate community responses to changing environmental conditions can be assessed. In some cases, a combination of several techniques, such as the modified Utermöhl and epifluorescence microscopy techniques used in the current study, coupled with fluorescence measurements, may be needed to better document phytoplankton community composition.

4.2. Nutrient Fluxes

The period of high salinity recorded by the MARVIN platform coincided with an unusual period of drought for the region. Typically, South Florida has a seasonal pattern of less rainfall during the winter months followed by greater rainfall during the summer. However, rainfall preceding the deployment period was significantly lower than is typical for these months. The resulting lack of flushing and runoff may have caused an accumulation of organic matter on land or within canals, as was demonstrated by Hicks et al. [80] and Zieliński et al. [81]. When the more typical "rainy season" commenced in the summer, the first significant precipitation event increased flushing and resulted in increased urea concentrations in adjacent waters, which in turn increased in cyanobacteria concentrations (as indicated with phycocyanin fluorescence measurements). Intermittent pulses of high rainfall followed this initial event. The shifts in nutrient and salinity concentrations in the region of the MARVIN platform during these low rainfall events were likely exacerbated by the increasing urbanization of the Sarasota Bay watershed and the lagoonal nature of Sarasota Bay, which is shallow (average depth of 1.9 m, https://sarasotabay.org/sarasota-bay/), and lacks a major freshwater tributary. The atypical drought that occurred prior to the MARVIN deployment may have potentially magnified the effects of these episodic pulses of N inputs.

Orthophosphate and DOP are generally elevated in Southwest Florida coastal environments due to local P deposits within the karst bedrock layer and associated P mining activities in central Florida which can transport P to the coast via the regional rivers [82]. The particulate N:P ratio of 98 measured at the MARVIN site on October 6, however, suggests that P may be potentially limiting phytoplankton growth in this system during certain periods. Sarasota Bay has limited exchange with the Gulf of Mexico through three passes, one at the narrow northern end of the Bay and two at the southern end. Freshwater inputs into Sarasota Bay are limited since much of the freshwater available through creek tributaries is diverted for agriculture in rural regions of the watershed and landscaping within urban environments [83]. Using the Sarasota Bay Conditions Report [84] total N and P concentrations measured during the 2009 MARVIN deployment were compared to the historical annual means of Sarasota Bay waters. In 2009, TP concentrations were below the annual mean while TN concentrations were above the annual mean. Taken in combination, these data suggest that regions of low circulation within Sarasota Bay may be periodically P limited and respond to pulses of N with increases in growth of phytoplankton species such as picocyanobacteria that are physiologically adapted to take up urea under low P concentrations, as has been demonstrated in the marine environment by Smith [85], Moore et al. [74], and Lin et al. [75].

4.3. Response to Urea Pulses

Each time there was an observed maximum in urea concentration as the result of rain-driven runoff events, Chl-*a* concentrations increased in response within a week. While the limited urea enrichment experiment conducted with ambient phytoplankton communities from the MARVIN site confirmed that urea inputs can result in both picocyanobacteria and picoeukaryote growth over a three-day period, the long-term impacts of urea additions were not evaluated with laboratory studies. The lag period between the urea maximum and the observed Chl-*a* peak in field measurements may indicate that urea is not always the preferred N source for these populations, or a shift in the dominant picoplankton species occurred that were not adequately identified during the course of this study with the binning method utilized.

Abiotic factors such as wind also influence the urea cycle in Sarasota Bay by driving the movement of water in and out of the smaller "finger canals" that line much of the shoreline. "Finger canals", like those in the area where the MARVIN platform was moored, have high residence times [46,86] and thus may serve as DON reservoirs [80]. In Sarasota Bay, when wind direction is perpendicular to the mouth of these canals, nutrient-rich water from the canal is flushed into the Bay. The accumulation of DON within these canals on Long Boat Key, adjacent to the MARVIN site, may be related to the extensive use of fertilizers in the surrounding residential areas and golf courses to maintain landscaping, as urbanization has been demonstrated to cause of nutrification in coastal waters throughout the U.S. [3–5,12].

Whether the observed episodic picoplankton blooms are a normal occurrence in Sarasota Bay or whether they are enhanced by the urea inputs from the "finger canals" is not clear. In 2013, Cannizzaro et al. [87] documented a shift in the plankton community from dinoflagellates to picoplankton following autumnal rain events in Old Tampa Bay, a similar heavily urbanized, shallow waterway immediately north of Sarasota Bay. The Sarasota Bay data suggest that changes in the timing of the wet/dry season can affect the magnitude, duration, and potential speciation of local algal bloom events. Scenarios that alter regional weather and rain patterns are likely to occur in the near future [52] and several other authors (e.g., O'Neil et al. [88], Paerl et al. [5,89], Gobler et al. [90], Trainer et al. [91]) have suggested that this will intensify HABs in both concentration, duration, and toxicity, necessitating more robust HAB monitoring systems. It may be that lower precipitation during the winter months, followed by higher precipitation in the spring/summer/fall season make high concentration picoplankton blooms a more common occurrence. A shift from a base autotrophic community of diatoms to picoplankton blooms has already occurred, with deleterious impacts on seagrasses and sponges in Florida Bay [92–95]. Śliwińska-Wilczewska et al. [53] have shown that picocyanobacteria, which at times made up a majority of the phytoplankton community in Sarasota Bay, can produce allelopathic compounds that alter phytoplankton community structure. Picocyanobacteria can also produce secondary metabolites that negatively impact other algae and the production of these metabolites is influenced by nutrient availability [54]. We noted that during blooms dominated by picocyanobacteria diatom concentrations were lower; however, the presence of allelopathic compounds was not measured during this study. The ability of many picoplankton populations to thrive in elevated water temperatures [54,74] presents an additional challenge to resource managers trying to improve or maintain water quality under increased coastal eutrophication and global climate change pressures.

4.4. Possible Urea Sources

While P inputs are likely to continue due to changes in hydrology and transport from the P-rich central Florida region, the increase in urea concentrations within Sarasota Bay may arise from the use of timed-release fertilizers that utilize urea as a N source. Given the shallow depth of and high DON concentrations present in Sarasota Bay (e.g., maximum of 29.49 μ M during this study), it is possible that DON may be susceptible to photodegradation. Urea is a labile component of the DON pool [96] with a turnover time of days [97]. Because of local government "rainy season" bans on fertilizer use, many private citizens and lawn care companies have shifted to the application of urea-based timed-release fertilizers. Thus, the fertilizer ban may have actually increased the observed total N flux into Sarasota Bay during these rainy periods [98,99]. Urea breaks down over time into NO₃⁻ that is readily taken up by most phytoplankton. However, certain cyanobacteria and picoeukaryotes are able to take up and assimilate urea directly via the enzyme urease [34,35]. This competitive advantage may also result in population shifts from diatoms to picoplankton in local systems subject to transient urea inputs.

4.5. Mitigation

Using nitrogen stable isotopes, Dillon and Chanton [100,101] traced most N inputs into Sarasota Bay to stormwater runoff. Sarasota County has proactively tried to mitigate runoff into the Bay through wastewater system upgrades and the construction of swales and retention ponds; however, this may not be sufficient to completely prevent runoff [100,102]. Swales are grass lined shallow ditches that act as particle traps, while retention ponds allow particulates to settle out and nutrients to be taken up by local vegetation. If N and P compounds are being flushed at high rates and in high volumes into Sarasota Bay during major storm events, which have increased in south Florida by 33% over the past decade [103], then swales may not have time to sufficiently absorb these nutrients. While the retention ponds are designed to act as particle traps, they are also known reservoirs of HAB species and can act as conduits for HAB populations to move into larger bodies of water through tidal action and flooding [48,104]. In an effort to lessen the impact of failing septic systems on the Bay, Sarasota County initiated a septic tank replacement program in 2001. At the time of this study most of these septic tanks had been replaced [105] and a subsequent study by Lapointe et al. [50] demonstrated that this program reduced N pollution in Sarasota Bay by 64%. The location of most of the remaining septic tanks in the county are along Phillippi Creek, which outflows into Roberts Bay and does not supply freshwater inflows to Sarasota Bay. Despite these activities, the mitigation methods employed by Sarasota County may not impact the "finger canals" of Sarasota Bay's shoreline, which are characterized by low flushing rates and long retention times that allow N constituents to accumulate during dry and low flow periods.

4.6. Potential Trophic Cascades

A shift to picoplankton dominance within Sarasota Bay could potentially impact the entire food web. Picocyanobacteria can produce compounds that are deleterious to resident aquatic flora and fauna and alter the existing phytoplankton community by impacting the photosystems of diatoms and chlorophytes [53,54,106]. Additionally, because of their small size, picocyanobacteria are considered less desirable food items than diatoms and chlorophytes and have been shown to have negative impacts on the nutrition, reproduction, and behavior of organisms at higher trophic levels [107,108].

Disruptions in the food web, which can start with alterations in picoplankton-microzooplankton grazing activities [109], can affect the quantity and distribution of higher trophic animals, such as popular estuarine sport fish like speckled trout or red fish, a reduction of which would have a negative economic impact on the region's tourism industry [110,111]. The quality of important agricultural species, such as oysters and clams, of which Florida ranks as the 5th most productive region in the United States' USD 1.5 billion aquaculture industry [112], would also likely suffer.

4.7. Autonomous Sampling

Like other recent studies, summarized in Stauffer et al. [113], the MARVIN platform deployment in Sarasota Bay demonstrates the importance of appropriate sampling intervals when studying the influence of episodic events on water quality. In the current study, discrete sampling at two-week intervals missed several episodic nutrient pulses and ephemeral picoplankton bloom events that were captured by the MARVIN instrumentation. Discrete station sampling was limited by a two-week sampling schedule and weather events, which included periods of high rainfall when sampling was not possible due to safety concerns (i.e., frequent high winds and lightning storms). Of equal concern is the possibility that one of these episodic events may have been captured by discrete sampling and subsequently considered representative of normal conditions. This has significant implications for the management of water quality within an estuary since either a significant event may be overlooked or an episodic event considered normal. High temporal resolution data from this autonomous platform, combined with a routine discrete sampling program that incorporated a large battery of measurements, provided for a more complete, temporally-relevant representation of episodic events, specifically the influence of pulsed urea inputs, their links to picocyanobacteria blooms, and the significance of these blooms to ecosystem health.

4.8. Conclusions

We observed through autonomous instrumentation that anthropogenic nutrient inputs influenced phytoplankton community biodiversity in Sarasota Bay, altering the dominant plankton group. The urea data collected during this study suggests that the seasonal fertilizer application ban in Sarasota County may have potentially increased the flux of urea into Sarasota Bay. These pulses of N, in the form of urea, combined with changes in geomorphology and nutrient inputs caused by the construction of extensive "finger canal" networks, may have resulted in increased nutrient storage within Sarasota Bay. Pulsed nutrient inputs into the Bay were observed during the study following anomalies in the region's normal wet-dry seasons. With the predicted climate changes to Florida's typical wet-dry seasons in the coming decades, phytoplankton community compositional shifts, such as we observed in Sarasota Bay, may become more frequent and lead to environmental conditions in which more toxic and longer lasting HABs occur. Regional resource managers should consider the role that alterations in N- versus urea-based fertilizers have played and will play in Sarasota Bay as the region undergoes further climatic anomalies that create conditions in which ecosystem-disruptive phytoplankton species can thrive.

As with any scientific endeavor, this study raised as many questions as it answered regarding phytoplankton dynamics in Sarasota Bay. Through this temporally limited study, physical drivers such as wind, temperature, and rainfall had a large influence on the phytoplankton community. A better understanding is needed of the localized effects of these drivers over an extended time period. Conducting research, such as that described here, over the course of several seasons within Sarasota Bay and other impaired tropical, urban lagoonal waters (e.g., Tampa Bay and Florida Bay) would provide the opportunity to examine how seasonal and climatic changes are influencing plankton community dynamics, especially since it has been noted by other researchers [33,114,115] that seasonal availability of N constituents can be a driver of population changes. A more detailed understanding of the role of both organic and inorganic N within shallow, subtropical bays is needed for management purposes. In Sarasota Bay, is urea a primary driver of the observed species composition shift, or just an indicator of

higher DON concentrations? What species are involved in this shift in phytoplankton diversity? Is the observed shift becoming more common? All of these questions have implications for coastal water quality as well as ecosystem management, but they cannot be answered with the one season of data explored here. This study supports the need for high temporal resolution autonomous water quality data over a long monitoring period. Start up and maintenance costs are higher with autonomous water quality used by water quality monitoring programs. However, high resolution temporal sampling provides data on the influence of ephemeral events on the physical, chemical, and biological aspects of water quality that are crucial when making current and future resource management decisions, yielding a high return on investment.

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