

Project Title: Nutrient concentration and isotopic analysis of samples associated with discharge from Piney Point

DEP P.O. No: BA1F45

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Final Report and Deliverables: 11/1/2021 – 6/30/2022

Program Goals and Objectives: Samples were collected from the Tampa Bay region on four sampling dates: 21 April 2021, 29 April 2021, 27 May 2021, and 22 June 2021.

Goal 1: NH₄⁺ concentration

From the samples collected 157 NH₄⁺ concentration measurements were made (1 sample had inadequate sample volume to complete analysis). The concentration of NH₄⁺ in the samples ranged from 0.03 μM to 3.65 μM (Fig. 1C, 1H, 1M, and 1R), consistent with the range expected for the Tampa Bay region, and much lower than the concentration of NH₄⁺ observed immediately after the discharge event (i.e., often >20 μM up to 20 mM). Over the course of the four sampling events, the average (± 1 S.D.) NH₄⁺ concentration decreased, from 0.7 ± 0.3 μM on 21 April, to 0.5 ± 0.8 μM on 29 April, to 0.3 ± 0.4 μM on 27 May, and finally to 0.2 ± 0.4 μM on 22 June 2021. No significant difference in NH₄⁺ concentration within the sampling region were observed (Fig. 1C, 1H, 1M, and 1R). When samples were collected at two depths in the water column, the concentration of NH₄⁺ was not consistently or significantly higher in samples collected at the bottom of the water column compared to surface water samples. The method used to measure the NH₄⁺ concentration of samples was the fluorescence/OPA method (Holmes et al., 1999). Samples were calibrated by a dilution series made daily from a 20 mM NH₄⁺ stock solution that ranged from 0 to 5 μM. The coefficient of variation (C.V.) was 21% which reflects the relatively low concentration of NH₄⁺ in these samples as well as the ease with which NH₄⁺ concentration samples can be contaminated. We note that the C.V. decreased over subsequent sample sets to 5-7% for the May and June samples, potentially a result of lower environmental concentrations that supported the ability to not cross-contaminate samples in the field and/or improved sample handling in the lab during analysis.

Goal 2: TDN concentration:

A total of 158 Total Dissolved Nitrogen (TDN) concentration measurements were made. The concentration of TDN in samples ranged from 7 to 30 μM, again, broadly similar to "what may be expected in the Tampa Bay region", and were considerably lower than immediately after the discharge event, when TDN concentrations exceeded 100 μM. Inorganic forms of nitrogen, including NO₃⁻, NO₂⁻, and NH₄⁺, were low in these samples, indicating that dissolved organic nitrogen was the dominant form of TDN. Over the course of the four sampling events the average (± 1 S.D.) TDN concentration was highest on the first sampling date, 18.7 ± 3.6 μM on 21 April, and then decreased to similar mean concentrations for the remaining three sampling dates, 15.3 ± 3.4 μM on 29 April, 15.9 ± 3.6 μM on 27 May, and 16.2 ± 3.5 μM on 22 June 2021. The distribution of TDN concentration did not show a consistent spatial pattern, with concentrations typically near 15 ± 5 μM within the sampling region (Fig. 1D, 1I, 1N, and 1S), and were similar at both surface and deep depths when multiple depths were collected at the same location. TDN concentrations were measured using the persulfate oxidation method which oxidizes NH₄⁺, NO₂⁻, and DON in a sample to NO₃⁻ (Knapp et al., 2005). Consequently, TDN concentration samples were analyzed as and calibrated with NO₃⁻ standards that bracketed the

concentration range, from 0 to 30 μM . Additionally, as an internal check on the completeness of the persulfate oxidation method, DON standards were included as “unknowns” in each set of persulfate oxidation, in this case USGS 40 (L-glutamic acid) and IAEA 600 (caffeine). The C.V. for TDN concentration analysis was 7%, typical for this analysis.

Goal 3: TDN $\delta^{15}\text{N}$:

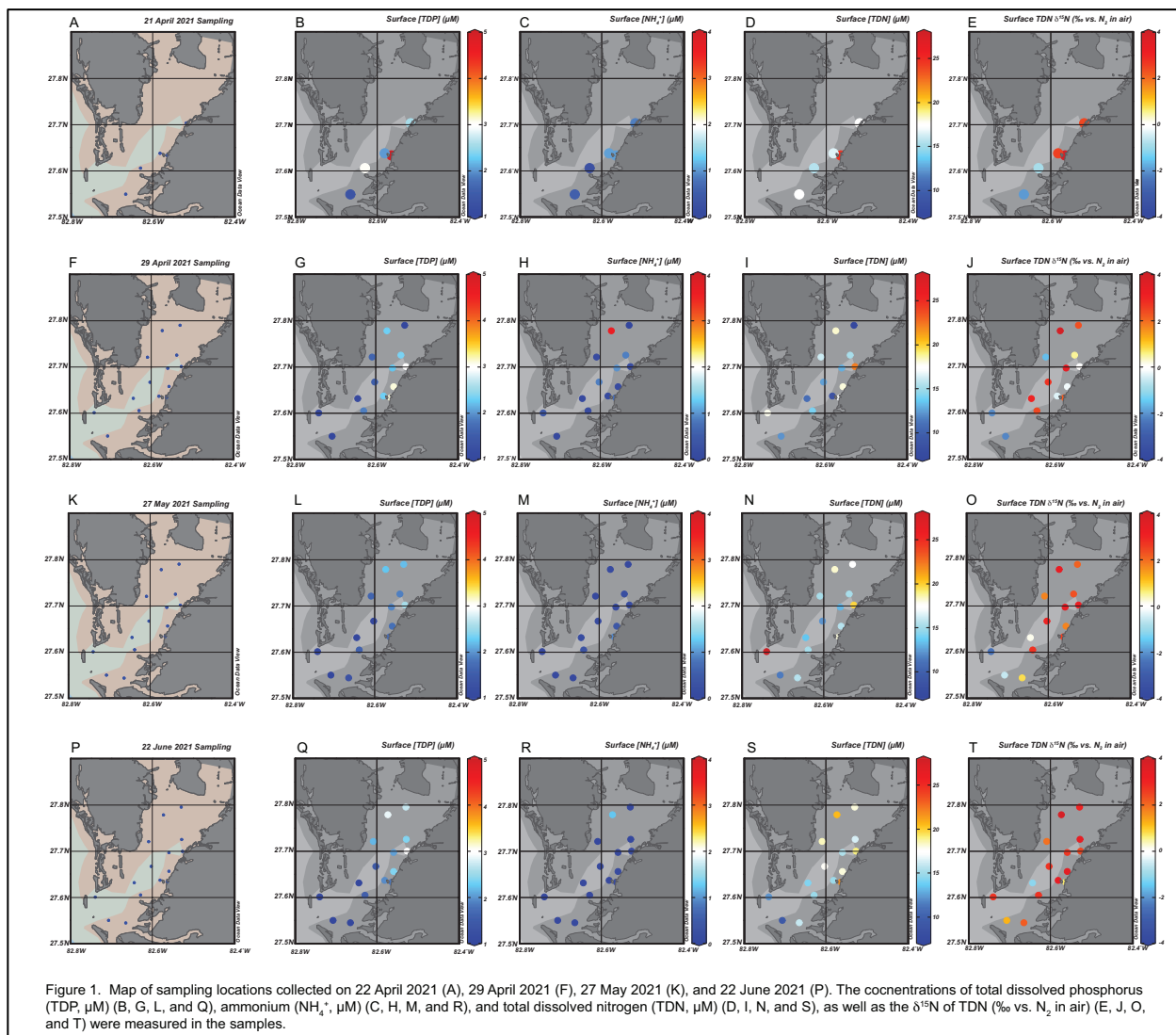
The $\delta^{15}\text{N}$ of TDN was measured in 158 samples and ranged from -14‰ to 4.0‰ (Fig. 1E, 1J, 1O, and 1T). The mean (± 1 S.D.) TDN $\delta^{15}\text{N}$ was similar over the four sampling dates, 1.1 \pm 3.0‰ on 21 April, 0.8 \pm 3.2‰ on 29 April, 2.1 \pm 1.4‰ on 27 May, and 2.4 \pm 1.9‰ on 22 June 2021. Similar to TDN concentration, the TDN $\delta^{15}\text{N}$ did not show variability in its spatial distribution, but was relatively consistent within the sampling region. Additionally, no significant differences were observed between surface and deep samples collected at the same site, consistent with a well-mixed, shallow water column. No correlation between the $\delta^{15}\text{N}$ and concentration of TDN in samples was observed. The method used to measure the $\delta^{15}\text{N}$ of TDN was the persulfate oxidation of the TDN to NO_3^- , with the resulting NO_3^- analyzed by the denitrifier method (Knapp et al., 2005). Consequently, the TDN $\delta^{15}\text{N}$ analyses were calibrated with NO_3^- $\delta^{15}\text{N}$ reference materials, IAEA N3 ($\delta^{15}\text{N} = 4.7\text{‰}$) and USGS 34 ($\delta^{15}\text{N} = -1.8\text{‰}$), as described in McIlvin and Casciotti (2011). However, an internationally calibrated DON $\delta^{15}\text{N}$ reference material, here USGS 40 ($\delta^{15}\text{N} = -4.52\text{‰}$) and IAEA 600 ($\delta^{15}\text{N} = 1.0\text{‰}$), was analyzed with the samples as an internal “check” to ensure completeness of the persulfate oxidation method. The typical standard deviation of replicate TDN $\delta^{15}\text{N}$ samples was $<0.8\text{‰}$.

Goal 4: TDP concentration:

The concentration of total dissolved phosphorus (TDP) was measured in 158 samples, and ranged from below detection to 5.2 μM (Fig. 1B, 1G, 1L, AND 1Q), much lower than in samples collected immediately after the discharge event, when TDP concentration was >87 μM . While the TDP concentrations of several samples were higher than typically observed in seawater (i.e., >4 μM), most concentrations were typical for these near-shore samples. The mean TDP concentration (± 1 S.D.) for the 21 April sampling date was 2.8 \pm 1.4 μM and decreased to similar values for the three subsequent sampling dates, 1.8 \pm 0.9, 1.5 \pm 0.6, and 1.7 \pm 0.9 μM for 29 April, 27 May, and 22 June, respectively (Fig. 1), with the majority of TDP present as PO_4^{3-} (PO_4^{3-} concentration data are from Dr. Buck’s lab at USF). The concentration of TDP in shallower and deeper samples collected at the same site were similar, consistent with observations for TDN and NH_4^+ concentration and TDN $\delta^{15}\text{N}$, as may be expected for a shallow, well-mixed water column. TDP concentrations were measured using the ash-hydrolysis method, with the oxidation of TDP to PO_4^{3-} (Monaghan and Ruttenberg, 1999). Consequently, samples were calibrated by a PO_4^{3-} dilution series made daily from a 5 mM stock solution that ranged from 0 to 5 μM . Additionally, three “internal checks” were run in each set of TDP analysis, including adenosine triphosphate, a polyphosphate compound, glyphosate, a phosphonate compound, and a 0.2 μm filtered oligotrophic surface water from the Gulf of Mexico to check the consistency of the TDP concentration analysis over time. The C.V. of analyses from replicate sample bottles was 5%.

Goal 5: NH_4^+ $\delta^{15}\text{N}$:

Given the low concentration of NH_4^+ in the samples (< 2 μM , see Goal 1 above), there was insufficient sample volume to measure NH_4^+ $\delta^{15}\text{N}$ in these samples.



Summary

Compared with samples collected from 7-12 April 2021, immediately following the Piney Point discharge event, the concentrations of NH_4^+ , TDN, and TDP in these samples was significantly lower in the water column of the Tampa Bay sampling region. The mean concentrations of NH_4^+ , TDN, and TDP were higher for the 21 April sampling event than the three subsequent sampling events, where the mean concentrations of each analyte were similar. Additionally, the $\delta^{15}\text{N}$ of TDN was similar across all four sampling events, indicating that the DON was broadly similar in these samples. These results indicate that the fate of NH_4^+ , DON, PO_4^{3-} , and DOP discharged into Tampa Bay during the Piney Point discharge event was not to accumulate in the dissolved phase in the Tampa Bay water column. Instead of accumulating and persisting in the Tampa Bay water column, potential fates of these inorganic and organic nutrients include: 1) consumption by phytoplankton and export to the sediments; and/or, 2) advection in the dissolved and/or particulate (i.e., phytoplankton biomass) phase out of Tampa Bay. Data from the 7-12 April sampling events suggests that significant fractions of the NH_4^+ , DON, PO_4^{3-} were consumed by autotrophs, although that inference will best be tested by comparing with abundances of

phytoplankton at the same sampling locations. Additionally, both the 7-12 April 2021 sampling data and the geochemical data presented here together with concurrent physical oceanographic/circulation measurements as well as benthic/sedimentary geochemical data can help evaluate the fate of the nutrients discharged to the Tampa Bay region. However, there is no evidence for nitrification of the NH_4^+ and/or DON to NO_3^- that persisted in the water column. It is possible that some of the NH_4^+ and/or DON was nitrified to NO_3^- but was consumed too quickly to be detected during the 21 April, 29 April, 27 May, and 22 Jun 2021 sampling events.

References

- Casciotti, K. L., Sigman, D. M., Hastings, M. G., Bohlke, J. K. and Hilkert, A. (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Anal. Chem.*, **74**, 4905-4912.
- Holmes, R.M., Aminot, A., Kerouel, R., Hooker, B.A., and Peterson, B.J. (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.*, **56**, 1801-1808.
- Knapp, A. N., Sigman, D. M. and Lipschultz, F. (2005) N isotopic composition of dissolved organic nitrogen and nitrate at the Bermuda Atlantic time-series study site. *Global Biogeochem. Cycles*, **19**, GB1018.
- McIlvin, M. R. and Casciotti, K. L. (2011) Technical Updates to the Bacterial Method for Nitrate Isotopic Analyses. *Anal. Chem.*, **83**, 1850-1856.
- Monaghan, E. J., & Ruttenberg, K. C. (1999). Dissolved organic phosphorus in the coastal ocean: Reassessment of available methods and seasonal phosphorus profiles from the Eel River Shelf. *Limnol. Oceanogr.*, *44*(7), 1702–1714.
<https://doi.org/10.4319/lo.1999.44.7.1702>
- Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M. and Bohlke, J. K. (2001) A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.*, **73**, 4145-4153.