Zooplankton Elemental Composition in the Amazon River Plume Region

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Abstract

The Amazon River generates an extensive surface plume, the Amazon River Plume (ARP), that reaches hundreds of kilometers into the Western Tropical North Atlantic. Within the ARP, distinct phytoplankton habitats are generated by the interplay of various physical and chemical factors. The impact of these factors on higher trophic levels has not been studied. We measured the elemental composition of five size fractions of zooplankton, a critical link between phytoplankton and upper trophic levels in the ocean. Specifically, we measured the nitrogen, carbon and phosphorus content, in addition to the isotopic composition ($\square^{15}N$ and $\square^{13}C$) of zooplankton collected during cruise EN614 in spring 2018. We found statistically significant differences in zooplankton phosphorus content, C:P and N:P ratios between planktonic habitats and day vs. night samples. Further, we will integrate these results with stable isotope data to assess the degree and nature of nutrients limiting secondary production among each habitat.

Introduction

The Amazon River contributes nearly one fifth of all riverine freshwater to the Western Tropical North Atlantic (Vorosmarty et al. 2000). The complex physical and chemical interactions in the Amazon River Plume (ARP) generates distinct phytoplankton habitats defined by biogeochemical factors, including surface salinity, temperature and nutrient distributions (Weber et al. 2019, Fig. 1). The resulting distinct diazotroph communities among habitats can affect the amount of biologically available nitrogen, contributing to varying elemental ratios (Weber et al. 2017).

Marine plankton typically produce biomass with an elemental composition following the Redfield ratio of C:N:P 106:16:1 (Redfield 1958). Plankton require carbon (C), nitrogen (N) and phosphorus (P) to successfully synthesize organic matter, nucleic acid, lipids and proteins. If one of these elements is in short supply relative to the others, it is known as the 'limiting nutrient' and can constrain phytoplankton biomass production. Departures from Redfield stoichiometry thus provide insight into the nature and degree of nutrient limitation of planktonic growth.

In the ocean, nitrogen is present in multiple forms and oxidation states, interconnected by multiple pathways, most of which are biologically mediated (Montoya 2008). One method of tracking the movement of nitrogen through these pathways is by measuring the isotopic composition of nitrogen pools in comparison to known fractionation (Sigman and Casciotti 2001). Specifically, the natural abundance of ^{15}N ($\delta^{15}N$) within an animal's tissue reflects both the sources of N supporting biological production and the animal's trophic position. Variations of ^{15}N in the marine web were first recorded by Hoering (1955) and Parwel et al. (1957), followed by targeted studies on nitrogen isotopic abundances soon thereafter (Miyake and Wada 1967; Wada et al. 1975; Wada and Hattori 1976; Minagawa and Wada 1984). Similarly, the movement of C through the ocean's biogeochemical cycles is reflected in the natural abundance of ^{13}C ($\delta^{13}C$).

The two stable isotopes together can provide critical insight into the movement of N and C in the mesozooplankton community, a critical link between phytoplankton and higher trophic levels in the marine webs (Montoya 2002; Landrum et al. 2011). In this study, we quantified the phosphorus content of zooplankton collected from distinct phytoplankton habitats in the ARP. We combined these novel P measurements with mass spectrometric assessments of zooplankton

N and C elemental and isotopic composition to explore nutrient limitation of zooplankton communities in this region.
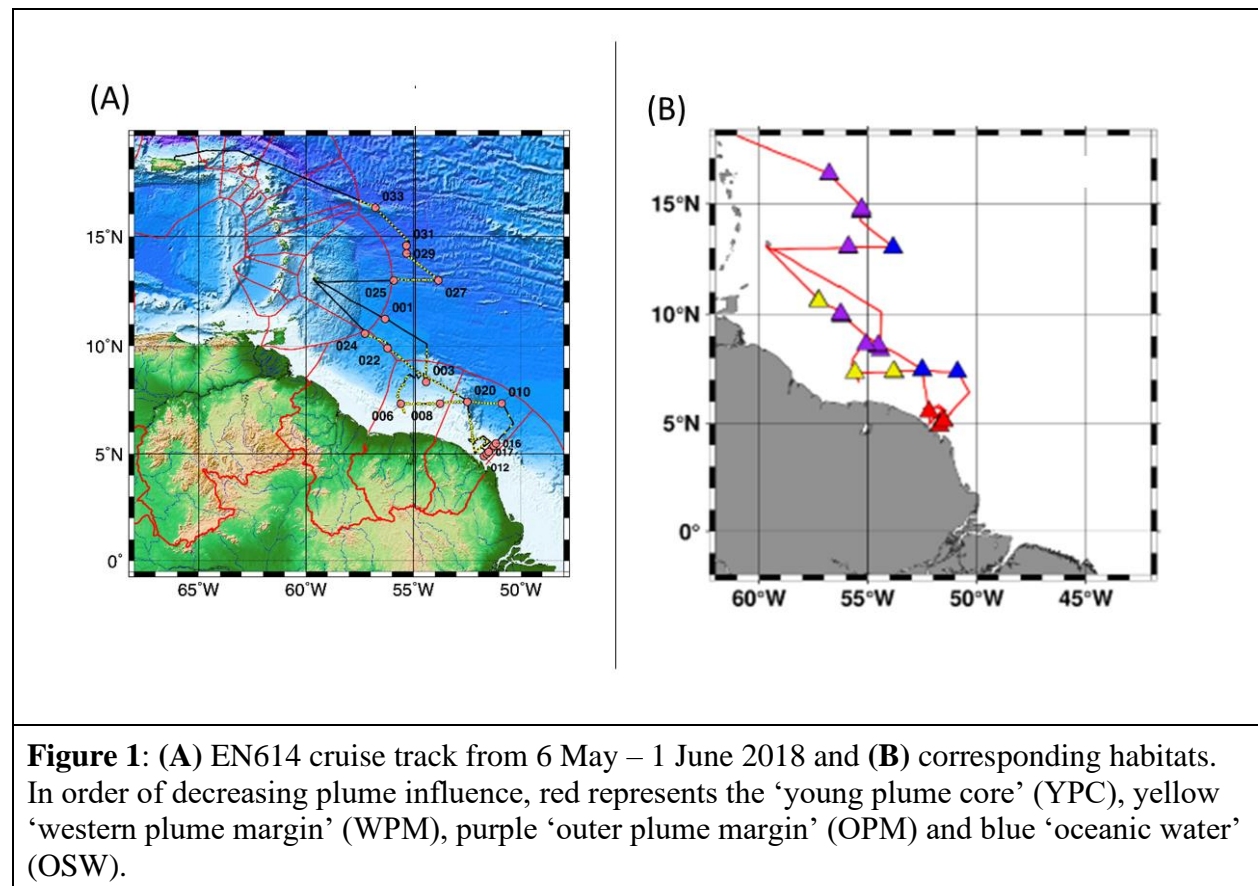
Methods

We sampled four phytoplankton habitats within the ARP on research cruise EN614 in spring 2018 (Fig. 1). We collected zooplankton with either a 1 m diameter ring net or a 1 m^2 MOCNESS (multiple opening and closing net and environmental sampling system). Zooplankton samples were separated into five size fractions by sequential passage through a graded series of Nitex sieve: 200-500 µm, 500-1000 µm, 1000-2000 µm, 2000-4000 µm, and > 4000 µm.

For this study, we focused on nine tows from six stations that encompassed four phytoplankton habitats in the ARP. Three of the six stations contained paired day and night tows to capture diel zooplankton migration.

From each collection event, we measured out three ground zooplankton samples per size fraction range, equaling a total of 135 samples. We measured the total dissolved phosphorus content (by mass fraction) of each sample by the persulfate oxidation method to transform organic phosphorus into inorganic phosphorus (Menzel & Corwin 1965). We then analyzed samples on

Figure 1: **(A)** EN614 cruise track from 6 May – 1 June 2018 and **(B)** corresponding habitats. In order of decreasing plume influence, red represents the 'young plume core' (YPC), yellow 'western plume margin' (WPM), purple 'outer plume margin' (OPM) and blue 'oceanic water' (OSW).

an automated Lachat Quikchem 8500 flow injection analysis system. Additionally, dried zooplankton samples were pelletized into tin capsules and analyzed on a continuous-flow isotope ratio mass spectrometer (CF-IRMS) to measured C and N content and δ^{15}N and δ^{13}C values measured relative to atmospheric N_2 and VPDB, respectively (Equations 1 and 2).

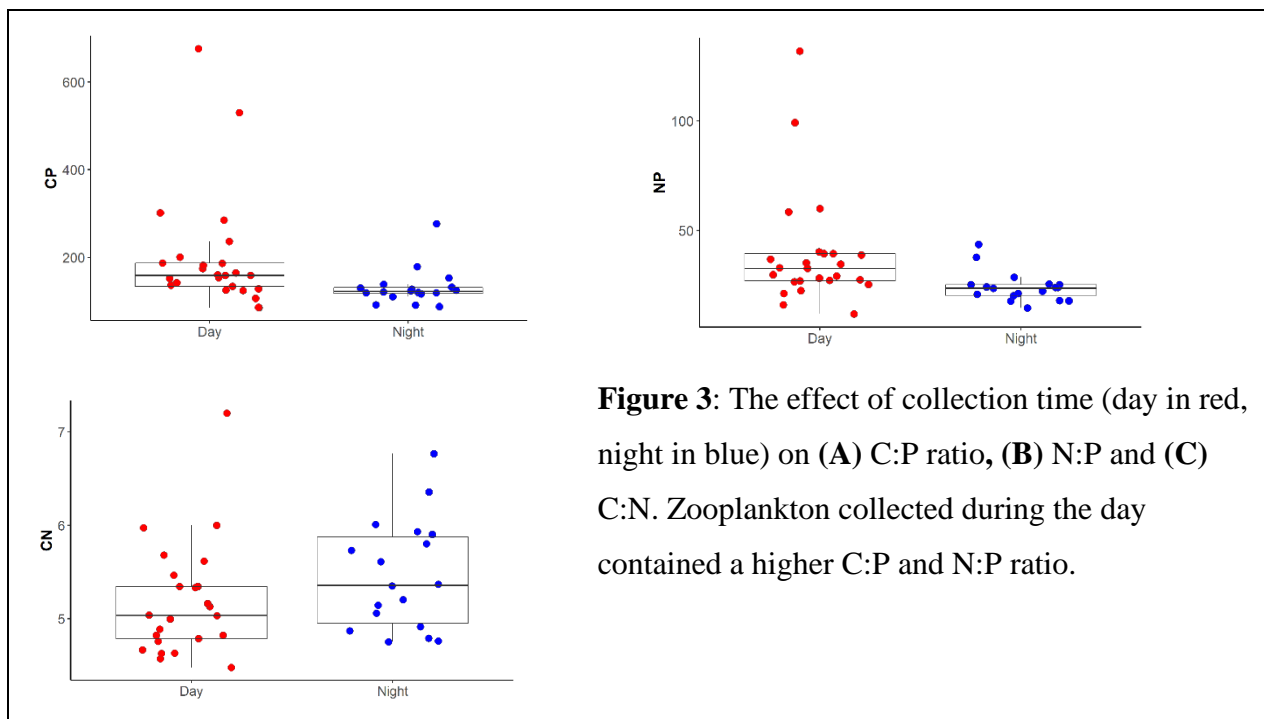$$\delta^{15}N(\text{\textperthousand}) = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \tag{1}$$

$$\delta^{13}C(\text{\textperthousand}) = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \tag{2}$$
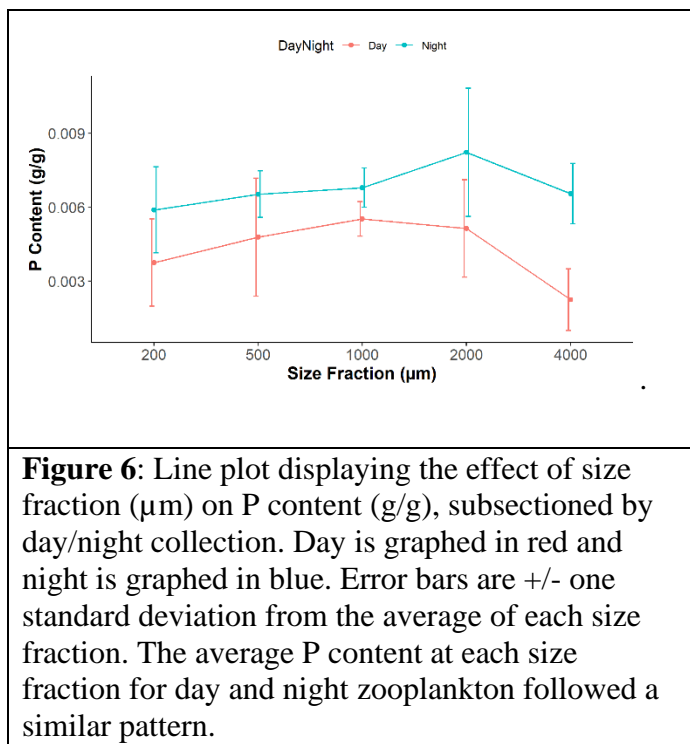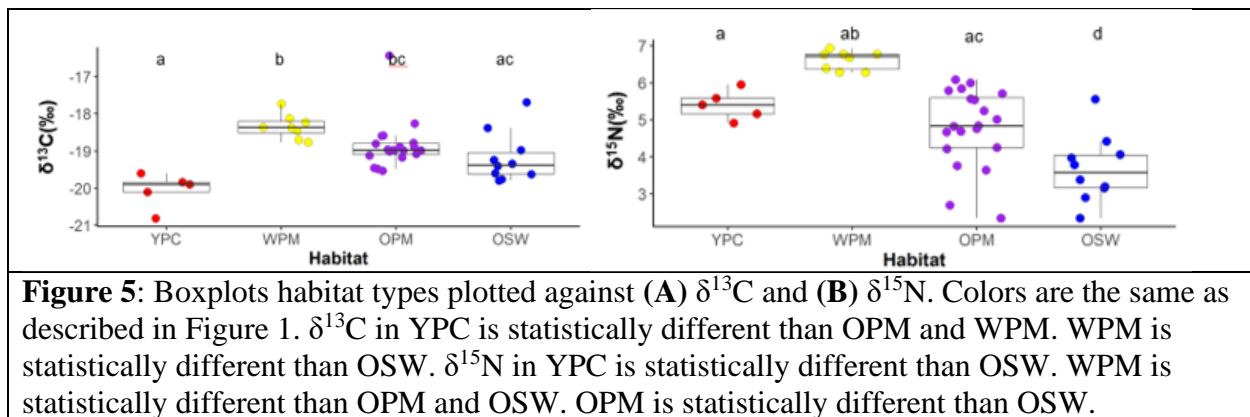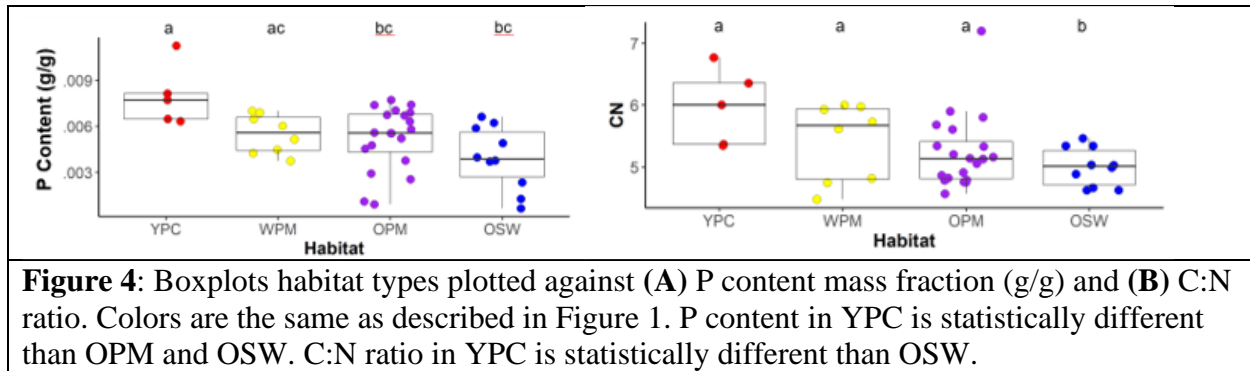
We conducted statistical analyses in R-Studio v4.13 to assess elemental and isotopic variations within and among stations. For each dependent variable measured, we first ran a Shapiro–Wilk test for normality. Depending on the results, we ran either a Kruskal-Wallis or ANOVA analysis to test for significant differences in elemental ratios, P content and δ^{13}C and δ^{15}N values among habitat types and between day/night tows. We then ran a Tukey post-hoc test on data with statistically significant results. Finally, we conducted a paired t-test between day/night samples, pairing by size fraction.

Results

We found significant differences in C:P and N:P ratios between bulk zooplankton collected in the day versus night (x^2=9.0206, p=0.00267; x^2=10.401, p=0.00126, Figure 3). However, C:N did not produce a statistically significant difference (x^2=3.1418, p=0.07631, Figure 3).

Figure 3: The effect of collection time (day in red, night in blue) on **(A)** C:P ratio**, (B)** N:P and **(C)** C:N. Zooplankton collected during the day contained a higher C:P and N:P ratio.

An ANOVA test revealed significant differences in P content among habitat types (F=4.994, p=0.005). The highest P content was found in the YPC habitat and the lowest in the OSW habitat (Figure 4a). A Kruskal-Wallis test showed significant differences in the C:N ratio among habitat types, with C:N decreasing as plume influence decreased (x^2=9.2642, p=0.02598, Figure 4b). Additionally, an ANOVA revealed significant differences in δ^{13}C and δ^{15}N among habitat types (F=9.295, p<0.005; F=17.69, p<0.005, Figure 5). δ^{13}C and δ^{15}N were both highest in the WP habitat.

Figure 4: Boxplots habitat types plotted against **(A)** P content mass fraction (g/g) and **(B)** C:N ratio. Colors are the same as described in Figure 1. P content in YPC is statistically different than OPM and OSW. C:N ratio in YPC is statistically different than OSW.

Figure 5: Boxplots habitat types plotted against **(A)** δ^{13}C and **(B)** δ^{15}N. Colors are the same as described in Figure 1. δ^{13}C in YPC is statistically different than OPM and WPM. WPM is statistically different than OSW. δ^{15}N in YPC is statistically different than OSW. WPM is statistically different than OPM and OSW. OPM is statistically different than OSW.

A paired t-test showed a significant difference in P content between zooplankton collected during the day and night, when paired by size fraction (t=4.95, p<0.005). Zooplankton of all size fractions collected at night contained more P per unit mass than zooplankton collected during the day (Figure 6).

Figure 6: Line plot displaying the effect of size fraction (µm) on P content (g/g), subsectioned by day/night collection. Day is graphed in red and night is graphed in blue. Error bars are +/- one standard deviation from the average of each size fraction. The average P content at each size fraction for day and night zooplankton followed a similar pattern.

Discussion

Day/Night Contrasts

Every night, a significant portion of the oceanic zooplankton community migrates to the upper water column, then returns to deeper water upon sunrise. This diel vertical migration (DVM) is the largest synchronized movement of animal biomass in the world (Hays 2003). Previous DVM research has focused on the ecophysiological consequences on these migratory zooplankton, but few studies have tracked the movement of nutrients in the water column resulting from DVM. A previous study found that freshwater *Daphnia* transported phosphorus to the upper water column via their excretions (Haupt et al. 2010). In concurrence, our study found migratory zooplankton had a higher P content (lower N:P and C:P ratios) than non-migratory zooplankton. When migratory zooplankton travel to the upper water column at night, they excrete more P per unit mass than P stressed zooplankton, leading to a net upward flux of P, against the biological pump.

Phytoplankton could benefit from migratory zooplankton transporting P into the upper water column. According to previous studies, zooplankton play a role in regulating phytoplankton populations through macro and micronutrient (nitrogen, phosphorus and iron) recycling (Sterner 1986; Vanni 2002; Tovar-Sánchez et al. 2007). For example, a 2017 study found that zooplankton metabolites increased phytoplankton net growth and ammonia removal rates in Southern Ocean phytoplankton communities (Coello-Camba et al. 2017). Our data offers a new mechanism of phosphorus recycling by migratory zooplankton, possibly increasing primary production at the surface. *add estimate of total N and P in zoops at surface (day vs night).

Habitat Comparisons

As the influence of the Amazon River Plume decreased in our study, C:N ratio and P content both decreased, possibly reflecting a general diminution of phosphorus entering the ocean from the Amazon River. A variety of field studies have found that the Amazon River stimulates primary production both directly via nutrient input (e.g., Muller-Karger et al. 1995), and indirectly by promoting diazotrophy (e.g., Subramaniam et al. 2008). This influence can extend hundreds of km into the open ocean. As plume influence decreases, phytoplankton are likely more nutrient limited, as evidenced by our study's findings that zooplankton contained the highest C:N ratio and P content in the YPC region.

A previous study found that the WPM region was dominated by a variety of diazotrophic phytoplankton (N_2-fixers). These organisms typically produce fixed nitrogen with low $\delta^{15}N$ values, a pattern evident in the surface $\delta^{15}N$ values of Weber et al. (2016). Interestingly, we observed high $\delta^{15}N$ values in zooplankton collected in the WPM habitat, despite the importance of diazotrophs in that habitat. A variety of biogeochemical factors and processes could generate this mismatch between the isotopic composition of suspended particles and zooplankton. The simplest explanation is that animal tissues integrate diet over a period that reflects the rate of production of new biomass, so changes in the $\delta^{15}N$ of the available food will propagate into the animals more slowly than the initial perturbation at the base of the food web (Montoya 2008). Animals act as a low pass filter for isotopic perturbations, and our zooplankton data suggest that the large blooms of diatom-diazotroph associations (DDAs) and *Trichodesmium* documented in the WPM habitat are episodic and/or patchy enough to not dominate the integrated diet of zooplankton collected there.

The highest zooplankton $\delta^{13}C$ values we measured were also in animals collected in the WPM habitat, possibly reflecting the important contribution of large phytoplankton in this habitat. In large cells (e.g., DDAs) and colonies (e.g., *Trichodesmium*), CO_2 fixation becomes diffusion limited, resulting in high $\delta^{13}C$ values in the biomass formed (Goericke et al. 1994; Laws et al. 1995; Rau et al. 1996). Weber et al. (2016) previously observed a strong correlation between blooms of *H. hauckii*, a large and fast-growing diatom, and high $\delta^{13}C$ values in the WPM.

Conclusions

Nutrients limiting zooplankton growth in the ARP play an important role in the marine food web, yet much is still unknown about phosphorus cycling in relation to the biological pump. Our data suggests a potential mechanism for transporting phosphorus into surface waters, potentially increasing the action of the biological pump and carbon sequestration by phytoplankton. To further investigate this, more zooplankton samples from additional cruises need to be analyzed.

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