Cycling of suspended particulate phosphorus in the redoxcline of the Cariaco Basin

Erin McParland^{a,b}, Claudia R. Benitez-Nelson^{b*}, Gordon T. Taylor^c, Robert Thunell^b, Anna Rollings^b, Laura Lorenzoni^d

a. Marine and Environmental Biology Program, 3616 Trousdale Parkway, University of Southern California, Los Angeles, California, 90089, USA

b. Marine Science Program & Department of Earth and Ocean Sciences, University of South Carolina, Columbia, SC, 29208, USA

c. School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY, 11794, USA

d. College of Marine Science, University of South Florida, St. Petersburg, Florida, USA

*Corresponding author: Marine Science Program & Department of Earth and Ocean Sciences, University of South Carolina, Columbia, SC, 29208, USA

Email address: cbnelson@geol.sc.edu

Abstract: The Cariaco Basin, located off the coast of Venezuela, is anoxic below ~250 m and has a productive redoxcline where several chemical gradients support multiple biogeochemical reactions. Thus, the Cariaco Basin is an ideal study site for investigating how redox chemistry influences phosphorus (P) biogeochemistry across oxic/anoxic boundaries. While sinking particles in the Cariaco Basin have been investigated previously for P composition and flux, this is the first study to describe P within suspended particulate particles, a key phase connecting particulate and dissolved P pools. Analysis of samples collected between April 2008 and April

2012, indicates that secondary peaks in total suspended particulate P (TSPP) concentrations (88 ± 60 nM) were present in the redoxcline and were similar in magnitude to those measured in the upper 100 m (84 ± 77 nM TSPP). However, the composition of TSPP was significantly different between the surface and the redoxcline. Suspended soluble reactive phosphorus (SSRP) was on average 3 ± 2 times greater in the redoxcline than observed in surface waters. Furthermore, composition of the TSPP pool significantly changed from less biologically available P forms (~45% organic P) above and below the redoxcline to more biologically available P forms (~80% loosely/oxide-bound P) within the redoxcline. We argue that the abrupt increases and transformations in the TSPP pool within the redoxcline result from both abiotic reactions associated with a manganese and iron redox shuttle and biotic reactions associated with a large and diverse chemoautotrophic prokaryotic community. These results suggest that the biogeochemical cycling of P may be altered within oxygen depleted water columns in unexpected ways.

Keywords: Suspended phosphorus, oxygen-depleted water columns, Cariaco Basin, euxinic basin, polyphosphate

Introduction

Large-scale dissolved oxygen depletions have profound influences on marine biogeochemistry, ranging from changes in food web structure to nutrient availability and the recycling of organic matter at depth (Levin 2003, Lam and Kuypers, 2011). Marine environments contain several major systems where extreme changes in oxygen with depth significantly impact biogeochemical cycling of major and minor elements: oxygen minimum zones, euxinic basins and seas, eutrophic estuaries and fjords (Spencer and Brewer 1971, Emerson et al. 1983, Stramma et al., 2010, Lam and Kuypers 2011, Kalvelage et al., 2013).

Oxygen minimum zones (OMZs) occur along major ocean basin boundaries, typically at water depths that range from 100 – 1000 m, and emanate from regions characterized by highly productive surface waters (Wyrski 1962). Limited mixing, coupled with oxygen consumption during remineralization of sinking organic matter, reduces oxygen concentrations far below equilibrated surface water concentrations (Codispoti et al., 2005, Karstensen et al., 2008, Paulmier & Ruiz-Pino 2009). Over the past several decades, open ocean systems have experienced a significant decline in dissolved oxygen concentrations, resulting in the expansion of OMZs worldwide (Stramma et al., 2008; Deutsch et al., 2011, 2014). Such expansions are expected to continue in response to climate induced changes in ocean temperature and circulation, which result in decreased oxygen solubility, a decline in vertical mixing, and reduced subduction and advection of deep waters (Matear and Hirst 2003, Stramma et al., 2008). In coastal marine systems, increased fertilizer runoff from rivers and atmospheric nitrogen deposition from fossil fuel burning have also contributed to the global expansion of OMZs via intensified eutrophication, which results in large-scale increases in organic matter export that significantly stimulates microbial respiration in the subsurface (Jahnke 1992, Paerl 1997, Correll

1998, Bennett et al., 2001, Doney 2010). Indeed, coastal OMZs have spread exponentially since the 1960's (Diaz and Rosenberg 2008).

Euxinic basins, while less prevalent than OMZs, provide invaluable test beds for examination of redox chemistry and include three major sites: the Cariaco Basin, the Black Sea and the Baltic Deep. Euxinic basins are characterized by minimal mixing with the upper ocean and shallow sills that restrict vertical circulation below the surface waters, creating anoxic, sulfidic bottom waters (Diaz et al. 2012). Transition zones between oxic and anoxic waters are usually characterized by large hydrographic and chemical gradients due to large scale changes in density structure, intense elemental cycling, and elevated microbial activity (e.g., Taylor et al., 2001; Murray and Yakushev 2006; Wakeham et al. 2012, Li et al. 2012). Stratified waters create multiple layers of biological activity fueled by a variety of redox reactions that rely on specific elements, such as sulfur, nitrogen, iron and manganese (Karl 1978, Madrid et al. 2001, Taylor et al. 2006). These redox reactions are similar to those found in marine sediments, except that they tend to occur over scales of tens of meters, rather than centimeters, enabling redox processes to be studied in more detail. Finally, these basins are relatively small, and their enclosed nature is such that climate forcing tends to be more evident in basin-wide physical, chemical and biological processes (Murray et al. 2006).

The majority of research in these oxygen-depleted environments has understandably focused on the biogeochemical cycling of elements with clear redox chemistry, such as nitrogen, carbon, sulfur and trace elements (Spencer and Brewer 1971, Emerson et al. 1983, Stramma et al., 2010, Lam and Kuypers 2011, Kalvelage et al., 2013). Less attention has been given to the macronutrient phosphorus (P). P is a vital nutrient utilized by all marine organisms in essential biomolecules, including nucleic acids, phospholipids, ATP and other energy-conserving

compounds (Benitez-Nelson et al., 2007, Diaz et al., 2012). While P is often thought to limit global primary production over geologic timescales, recent evidence suggests that P availability may also influence phytoplankton cellular composition, community structure, growth and production in the modern ocean (Benitez-Nelson 2000, Paytan and McLaughlin 2007, Karl 2014, Silkin et al., 2014). This has led to more focused investigations regarding P speciation and cycling over short time scales (e.g., Diaz et al., 2012, Martin et al., 2014). As natural and anthropogenically-induced climatic changes occur, biological production has been hypothesized to become increasingly P limited (Fanning 1989, Wu et al., 2000, Karl et al., 2001, Benitez-Nelson et al., 2004, Karl 2014).

Until recently, P was not considered to have extensive reduced forms in natural environments, with the exception of phosphine gas (PH_3) potentially produced via microbial activity under strongly reducing conditions (Pasek et al., 2013). Newer studies, however, have now shown that P exists in a variety of reduced P phases (phosphonates, phosphite, and hypophosphite) that are cycled rapidly in both marine and freshwater regimes (Pasek et al., 2014, Van Mooy et al., 2015). Thus P biogeochemistry may be significantly influenced by redox chemistry in unanticipated ways.

Many studies focusing on marine sediments and sinking particles argue for enhanced regeneration of dissolved P forms from particulate matter under anoxic conditions, mainly as a result of inorganic P release via metal oxide reduction (Dellwig et al. 2010); although the magnitude of P regeneration, particularly in comparison to carbon, continues to be debated (e.g., Anderson et al., 2001). Other studies suggest that under reducing conditions, microbial activity is instrumental in controlling P biogeochemistry by either preferentially releasing P storage compounds (e.g., polyphosphate), or by converting soluble P into insoluble apatite forms

(Sannigrahi and Ingall 2005, Goldhammer et al., 2010). Again, the magnitude of this release may vary given the rapid transformation of polyphosphate into apatite, e.g., sink switching (Diaz et al., 2008; Goldhammer et al., 2010). Observations of enhanced P remobilization across oxic/anoxic boundaries in both lacustrine and marine environments imply that over geologic timescales, P remobilization has contributed to redox stabilization of the atmosphere and oceans (Van Cappellen and Ingall 1994). On shorter timescales, enhanced P regeneration in oxygen deficient zones perturbs ecosystems via increasing eutrophication, or by changing relative nutrient availability (Diaz and Rosenberg 2008). Despite increasing evidence that P biogeochemistry is significantly influenced by changing oxygen conditions, mechanisms of these processes and their participants remain poorly understood (Diaz et al., 2008).

Continental shelves may only occupy a small area of the oceans, but they are responsible for 15 - 30% of global marine production and more than 40% of seabed carbon sequestration; hence, climate-induced changes in these marginal seas are likely to be more readily apparent than in their more open ocean counterparts (Yool and Fashman 2001, Muller-Karger et al., 2005, Taylor et al., 2012). The Cariaco Basin, located on the northern continental margin of Venezuela, is a euxinic basin having remained anoxic below 250 - 350 m over the past millennia (Black et al., 2004; Muller-Karger et al., 2010; Scranton et al., 2014). Cariaco Basin surface waters also experience wind-induced seasonal upwelling, a characteristic similar to that of many OMZs (Helly and Levin 2004, Karstensen et al., 2008). Within the Cariaco Basin, water density is relatively homogenous below 200 m and thus has little influence on structuring the geochemical and biological properties or vertical particle transport through the redoxcline and below (Taylor et al., 2001). Rather, distributions of elemental species and transitions between particulate and dissolved phases within the redoxcline result from an array of reactions catalyzed by a diverse

community of heterotrophic and chemoautotrophic microorganisms (Taylor et al., 2001; 2006, Edgcomb et al., 2011). Previous research has shown that a chemoautotrophic community residing within the Cariaco redoxcline assimilates dissolved inorganic carbon on the same order of magnitude as surface photoautotrophic production (Taylor et al., 2001, Wakeham et al., 2012).

Similar to the observed expansion of OMZs in marine systems associated with rising global temperatures (Oschlies et al., 2008, Stramma et al., 2010), a number of changes have been observed in the Cariaco Basin over the past two decades. Intrusions of dense, deep water from the Caribbean Sea have declined, causing a decrease in density and a reduction in the width of the suboxic zone (Scranton et al., 2014). At the same time, as the density in the Cariaco Basin is strongly controlled by temperature, continued increases in surface temperature (Astor et al. 2013) have the potential to enhance stratification and decrease exchange between surface and deep waters. This is further exacerbated by declining surface wind strength caused by climatic shifts in the Intertropical Convergence Zone (ITCZ), which has increased water stratification over time (Taylor et al., 2012). As a result, a large scale shift in the biological community structure from larger to smaller taxa, including nitrogen fixing organisms that have moved deeper into the water column, has also occurred in the basin (Taylor et al., 2012, Montes et al., 2013, Pinckney et al., 2015).

Investigations of P geochemistry within sinking particles in the Cariaco Basin suggest that preferential remineralization of particulate P to the dissolved pool alternates between inorganic and organic P phases, depending on oxygen availability (Benitez-Nelson et al., 2007). Furthermore, a large fraction of the sinking particulate P pool appears to be comprised of phosphonates that are also preferentially remineralized with increasing depth in the water column (Benitez-Nelson et al., 2004). The source of the phosphonates remains ambiguous, but may be

due to the presence of cyanobacteria in Cariaco Basin surface waters, which have recently become more abundant (Dyhrman et al., 2009, Montes et al., 2013, Pinckney et al., 2015).

Suspended particulate P may serve as an important link between sinking particulate P and dissolved P phases, especially across the redoxcline where an active microbial community has been consistently observed. This study is the first to investigate the composition and transformation of suspended particulate P across the Cariaco Basin redoxcline. The results suggest that suspended particulate P composition is also significantly influenced by oxygen availability and is likely controlled by both abiotic and biotic processes.

Methodology

Sample Site and Collection

The Cariaco Basin is the largest, truly marine, semi-permanent anoxic basin in the world (Scranton et al., 2001). Located off the north coast of Venezuela, it is surrounded by a relatively undeveloped coastline that includes several major rivers: the Unari, Tuy, and Neveri. Water exchange with the Caribbean Sea is restricted to the upper 90-140 m by a sill and is focused through the Tortuga and Centinela channels (Percy et al., 2008, Lorenzoni et al., 2009). The eastern Cariaco Basin is the site of the joint Venezuela - United States CARIACO Ocean Time Series Program, which has collected monthly data on hydrography, primary production, nutrient and carbonate chemistry, sinking flux of particulate matter and many other biogeochemical variables since November 1995 (Thunell et al., 2007, Muller-Karger et al., 2010, Taylor et al., 2012). Enhanced seasonal primary production over the Cariaco Basin is driven by wind-induced coastal upwelling. From December to May, the annual southern migration of the ITCZ causes Trade Winds in the southeastern Caribbean to intensify (Benitez-Nelson et al., 2007, Muller-

Karger et al., 2010, Taylor et al., 2012). During this period of coastal upwelling, surface productivity averages $\sim 1.7 \text{ g C m}^{-2} \text{ d}^{-1}$ in contrast to less than $\sim 0.99 \text{ g C m}^{-2} \text{ d}^{-1}$ during non-upwelling months (Muller-Karger et al., 2001). High surface productivity combined with restricted water exchange results in anoxic waters below $\sim 250 \text{ m}$. The oxic/anoxic interface fluctuates between 250 and 320 m of depth due to both lateral intrusions of oxygenated waters from the Caribbean Sea and variations in upwelling intensity (Astor et al., 2003, Li et al., 2012, Astor et al., 2013, Scranton et al., 2014). Recent data suggest that the depth of the oxic/anoxic interface has shoaled over the 19 years of the CARIACO Ocean Time Series (Scranton et al., 2014). In this study we define the redoxcline to be the depth range starting from where oxygen begins to exponentially decline to where oxygen concentrations fall below detection.

As part of the CARIACO Ocean Time Series Program, microbial abundance and suspended particulate matter samples were collected monthly from April 2008 to April 2012 at 19 different depths from 1 - 1310 m (Table 1). Heterotrophic bacterial net production and dark dissolved inorganic carbon (DIC) assimilation rates were evaluated semi-annually during the same time period and over the same depth range.

Microbial abundance, bacterial net production, dark dissolved inorganic carbon assimilation

For microbial abundance, whole-water samples (200 mL) were preserved with 2% (final concentration) borate-buffered formaldehyde and stored at 5°C. Bacteria were enumerated with epifluorescence microscopy using standard acridine orange-stained slides prepared on dark 0.2 Nuclepore polycarbonate membranes (Taylor et al., 2006).

Heterotrophic bacterial net production (BNP) was estimated by ^3H -leucine incorporation into protein following the methods of Kirchman (1993) and Taylor et al. (2001). Using a gas-

209 tight syringe, triplicate samples in 40 mL Pierce septa vials with no headspace were immediately
210 spiked with 50 μ l of N₂-purged ³H-leucine [20 nM final concentration; L-(4,5-³H[N])-leu; 52 Ci
211 mmol⁻¹]. Samples were incubated in on-deck water baths for 6-8 hours at ambient temperature in
212 darkness. Following incubation, samples were fixed with cold trichloroacetic acid (TCA; 5%
213 final concentration) and refrigerated until processing immediately after the cruise. Filter blanks
214 from samples fixed with 5% TCA immediately after ³H-leucine addition were used to correct for
215 ³H-leucine incorporation into protein over time. In the lab, samples were processed according to
216 Kirchman (1993) and ³H-protein precipitates were captured on 0.22 μ m cellulosic filters
217 (Osmonics). Filters were then dissolved in 0.5 ml ethyl acetate, suspended in Hionic-Fluor
218 scintillation cocktail and radioassayed. BNP was calculated using a conversion factor of 3.1 kg C
219 mole⁻¹ of leucine incorporation (Kirchman 1993).

220 Chemoautotrophic assimilation of dark DIC was measured by ¹⁴C-bicarbonate
221 incorporation into particles. Triplicate samples were dispensed into 40 mL ground glass
222 stoppered bottles and injected with 200 μ l of chilled N₂-purged ¹⁴C-bicarbonate in an alkaline
223 brine (pH 9.5; S=60 on practical salinity scale) at the bottom before sealing without a headspace
224 (Tuttle and Jannasch 1973). ¹⁴C-bicarbonate samples were incubated in parallel with BNP
225 samples for 14-20 hours under conditions minimizing potential oxygen contamination and light
226 exposure. After incubation, particles were collected on 0.22 μ m cellulosic filters (Osmonics) and
227 then rinsed twice with 5 mL of filtered seawater adjusted to pH 3.5. Unassimilated ¹⁴C was
228 further purged from filters in a saturated HCl atmosphere for >1 hour. Filters were then dried and
229 suspended in Hionic-Fluor scintillation cocktail and radioassayed. Non-biological sorption was
230 corrected for by samples processed immediately after introduction of the radiotracer. Rates of

dark dissolved inorganic carbon (DIC) assimilation were normalized to $\mu\text{M C d}^{-1}$ with values of total DIC derived from pH, temperature and alkalinity (Taylor et al., 2001).

Suspended particulate matter

Water samples for suspended particulate matter analysis were collected in 2 L opaque polyethylene bottles, immediately filtered through acid-cleaned and pre-combusted (450°C at 5 hours) Whatman glass fiber filters ($0.7\ \mu\text{m}$ nominal pore size), and frozen in the dark until analysis. Total suspended particulate P (TSPP) and suspended particulate inorganic P were determined on all samples using a modification of the Aspila phosphomolybdate method (Aspila et al., 1976, Benitez-Nelson et al., 2007). Briefly, samples for suspended particulate inorganic P were extracted using weak hydrochloric acid, while samples for TSPP were combusted at 550°C to convert any organic P to inorganic P prior to extraction (Aspila et al., 1976). Suspended particulate organic P was determined as the difference between TSPP and suspended particulate inorganic P. To monitor run to run variability and validate analytical accuracy, standard reference materials of tomato leaves (NIST# 1573a) and estuarine sediment (NIST# 1646a) were analyzed with each run (Benitez-Nelson et al., 2007). During analysis of suspended particulate inorganic P, it is possible for some organic P compounds, e.g., simple sugars, to be hydrolyzed during analysis, thus inflating suspended particulate inorganic P concentration estimates. We have therefore more appropriately termed this pool suspended soluble reactive P (SSRP). It is also possible that suspended particulate organic P concentrations may contain non-reactive inorganic compounds, such as inorganic polyphosphates, and we have therefore termed this pool as suspended soluble non-reactive P (SSNP) (Benitez-Nelson 2000). Rather than deciphering the difference between inorganic and organic P, these methods separate P into pools of different

chemical reactivity. The more reactive pool, SSRP, is presumably more widely bioavailable than SSNP (Benitez-Nelson 2000). SSRP and TSPP were measured with 3% precision and SSNP, with 5% precision.

In all water column profiles, results from the Aspila TSPP measurements show a large peak in suspended particulate P within the redoxcline, usually located directly below the depth where oxygen began to decrease exponentially. These peaks equaled or surpassed the suspended P concentrations within the upper 100 m. A subset of these samples was selected from within, above and below the redoxcline TSPP peak for additional sequential extraction analyses. “Within the redoxcline” refers to samples collected between the depths of 230 and 300 m, depending on the depth profile of oxygen concentrations measured for a given month. These samples contained the highest TSPP concentrations. “Above redoxcline” and “Below redoxcline” samples ranged between depths of 160 – 230 m and 260 – 350 m, depending on the given month’s redoxcline depth range. TSPP concentrations within these zones dropped by > 30% from the within redoxcline concentrations. Due to limited sample material availability, filters were combined across monthly cruises to ensure adequate material for analysis. “Within redoxcline” samples contained at most two samples from different cruises, whereas “Above redoxcline” and “Below redoxcline” samples could contain up to five samples from different cruises. Samples were combined across similar depths, years and hydrographic conditions (e.g., upwelling versus non-upwelling periods as defined by Astor et al., 2003). Table S1 catalogues the individual filters combined and used for the analyses described below.

A sequential extraction method, SEDEX, was performed on these selected sub-samples from April 2009 to June 2011 to quantify five different inorganic forms of P: 1) loosely-bound P, 2) oxide-bound P, 3) authigenic P, 4) detrital P, and 5) organic P (Ruttenberg 1992). Due to

small sample sizes and high run to run variability, we report the first two steps as a combined fraction, loosely/oxide-bound P. SEDEX TSPP refers to the sum of both inorganic and organic P fractions (Steps 1 – 5), SEDEX SSRP refers to the sum of inorganic P fractions (Steps 1 – 4; loosely/oxide-bound, authigenic and detrital P) and SEDEX SSNP refers to only the organic P fraction (Step 5; organic P). A standard reference material of tomato leaves (NIST# 1646a) and estuarine sediment (NIST# 1573a) were analyzed with each run (Benitez-Nelson et al., 2007). The extractions were analyzed for P concentrations using a modification of the ash hydrolysis technique as described earlier (Aspila et al., 1976, Benitez-Nelson et al., 2007).

Aspila TSPP and SEDEX TSPP concentrations agreed within $\pm 10\%$ and there was no significant difference between Aspila and SEDEX measured TSPP concentrations ($p > 0.3$). Furthermore, a least squares linear regression between the two different analyses had a slope of 1.02 indicating a $\sim 1:1$ relationship. Composition of the P pools, however, differed between the extraction methods. SEDEX SSRP was consistently 10% lower than Aspila SSRP, suggesting either incomplete digestion of the inorganic P by SEDEX or overestimation of SSRP by Aspila due to oxidation of the organic P fraction. SEDEX SSNP and Aspila SSNP were poorly correlated due to relatively low concentrations and likely loss of SSNP components to other SEDEX fractions. On average, run to run variability in SEDEX was less than 10% based on repeat measurements of NIST standards. Variability between replicate SEDEX P measurements within a single run averaged less than 5%.

Suspended particulate organic carbon (SPOC) and suspended particulate nitrogen (SPN) concentrations were determined on the same filters as the P analyses following the procedures outlined in Froelich (1980). Briefly, approximately $\frac{1}{4}$ to $\frac{1}{2}$ of the filter was acidified (1 M

H₃PO₄) to remove the particulate inorganic carbon (i.e., carbonates) and then combusted at 1000°C in a Perkin Elmer 2400 elemental analyzer (Thunell et al., 2007).

Results

Bacteria

From April 2008 to April 2012, average prokaryoplankton concentrations tended to follow a bimodal pattern with depth across all seasons (described previously by Taylor et al., 2006), but were highly variable (Figure 1A, 1B and 1C). The median surface prokaryote peak from 0 – 100 m was 1 µM C (25th percentile = 0.7 µM C and 75th percentile = 2 µM C) and the median in redoxcline depths (230 – 290 m) was 0.7 µM C (25th percentile = 0.6 µM C and 75th percentile = 0.9 µM C) (Figure 1A). Bacterial net production in the surface peaked at 35 ± 38 m and dark DIC assimilation rates peaked within the redoxcline at 268 ± 26 m. The median dark DIC assimilation rate was 0.2 µM C·day⁻¹ (25th percentile = 0.02 µM C·day⁻¹ and 75th percentile = 0.5 µM C·day⁻¹) within the redoxcline (Figure 1).

For the most part, prokaryoplankton depth profiles complement dissolved oxygen and TSPP profiles, similar to those depicted in the example provided from December 2010: as oxygen becomes depleted, TSPP concentrations increase to form a secondary peak, prokaryotic biomass forms one or more slightly shallower peaks (Figure 2A), while peaks in dark DIC assimilation and BNP are nearly coincident with that of TSPP (Figure 2B). Incongruities between peak positions for prokaryoplankton biomass and chemoautotrophic and heterotrophic production (Figures 2A and 2B) are interpreted as evidence for higher mortality rates within the most productive layer. Enhancements in dark DIC assimilation in the upper ~100 m likely are caused by anaplerotic reactions occurring in the tricarboxylic acid cycle, a pathway present in all

non-fermenting organisms (Taylor et al., 2001). As the euphotic zone contains the greatest biomass, contributions of anaplerotic reactions are expected to dominate dark carbon fixation in this layer. The majority of TSPP peaks (93%, n=44) occurred between 240 and 270 m, as did 70% of the peaks in prokaryotic biomass (n=43) (Figure 2A inset) and 75% of the peaks in dark DIC assimilation rates (n=8) (Figure 2B inset).

Suspended Particulate P

Suspended P concentrations in the upper 100 m (n = 44) were highest at 25 ± 17 m (average ± 1 standard deviation) and averaged 84 ± 77 nM TSPP, 17 ± 23 nM SSRP and 68 ± 57 nM SSNP (Figures 3A and 3B). Below the upper 100 m, concentrations steadily decreased until reaching the redoxcline. Secondary peaks were always observed between 230 and 290 m (mean depth = 256 ± 13 m) and TSPP, SSRP, and SSNP averaged 88 ± 60 , 54 ± 53 and 34 ± 23 nM, respectively. Suspended P declined throughout the deeper anoxic waters (> 300 m).

The magnitude of the redoxcline P peaks was not significantly different between upwelling and non-upwelling seasons (Student t-test, $p > 0.05$). However, the composition of the TSPP maximum in the upper 100 m and the redoxcline was significantly different (Figure 3A and 3B). Average SSRP peak concentrations in the redoxcline (54 ± 53 nM) were as much as 5 (3 ± 2) times greater than those associated with the peak in surface waters (17 ± 23 nM) (Student t-test, $p < 0.00006$). In contrast, upper 100 m SSNP peak concentrations (68 ± 57 nM) were as much as 4.5 (2 ± 2.5) times greater than in the redoxcline peaks (34 ± 23 nM) (Student t-test, $p < 0.0007$). Thus, SSNP dominated in the surface, while SSRP dominated in the redoxcline.

SEDEX Speciation

From April 2008 to June 2011, TSPP concentrations were measured in a subset of samples from above, within and below the redoxcline using SEDEX (n = 24). As SEDEX samples were analyzed for a shorter time period, average P concentrations differ from those averaged over the entire dataset. SEDEX TSPP concentrations were significantly higher within the redoxcline (143 ± 26 nM) than those above and below the redoxcline (81 ± 22 and 71 ± 57 nM, respectively) (Student t-test, $p < 0.002$). Loosely/oxide-bound P dominated SEDEX TSPP pools within the redoxcline, averaging 111 ± 24 nM ($79 \pm 12\%$ of SEDEX TSPP) for all observations, and were significantly different from those above and below the redoxcline (Student t-test, $p < 0.002$; Figure 4A and 4B). In contrast, SEDEX TSPP pools above and below the redoxcline were almost equally comprised of loosely/oxide-bound P, 39 ± 7 nM ($49 \pm 6\%$ of SEDEX TSPP) above and 43 ± 39 nM ($52 \pm 23\%$ of SEDEX TSPP) below, and organic bound P, 35 ± 7 nM ($44 \pm 8\%$ of SEDEX TSPP) above and 19 ± 8 nM ($34 \pm 18\%$ of SEDEX TSPP) below (Figure 4A and 4B). Authigenic P concentrations did not vary significantly with depth, contributing only 13 ± 14 nM ($9 \pm 10\%$ of SEDEX TSPP) within the redoxcline, 5 ± 6 nM ($6 \pm 7\%$ of SEDEX TSPP) above, and 9 ± 19 nM ($13 \pm 8\%$ of SEDEX TSPP) below the redoxcline. Detrital P was almost undetectable, accounting for 0.5 ± 1.5 nM ($0.3 \pm 1\%$ of SEDEX TSPP) within the redoxcline, 1 ± 2 nM ($1.5 \pm 2\%$ of SEDEX TSPP) above, and 0.2 ± 0.4 nM ($0.3 \pm 0.6\%$ of SEDEX TSPP) below the redoxcline. Although SEDEX data is limited, depth-dependent trends in P composition were similar during upwelling (December to April) and non-upwelling seasons, with no significant difference in P speciation.

C:N:P Ratios

From April 2008 to April 2012, average concentrations of suspended particulate organic carbon (SPOC) and suspended particulate nitrogen (SPN) in the upper 100 m ($n = 44$) were highest at 25 ± 17 m and averaged $10 \pm 9 \mu\text{M}$ and $1.0 \pm 0.9 \mu\text{M}$, respectively (Table 1). Average concentrations of SPOC and SPN within the redoxcline maximum (256 ± 12 m) were $5 \pm 2 \mu\text{M}$ and $0.7 \pm 0.3 \mu\text{M}$, respectively. Both SPOC and SPN were significantly lower within the redoxcline compared to the upper 100 m (Student t-test, $p < 0.002$ and $p < 0.0005$, respectively).

With the exception of the upper 100 m and redoxcline, SPOC:TSPP and SPN:TSPP ratios were much higher than Redfield (106 C: 16 N: 1P) and increased slowly with depth to ~ 400 m, consistent with preferential remineralization of P (Figure 5). Within the redoxcline, however, suspended SPOC:TSPP and SPN:TSPP ratios declined sharply, with significantly lower SPOC:TSPP and SPOC:SSNP ratios averaging 52 ± 50 and 208 ± 221 , respectively, before increasing back to near surface values (Student t-test, $P < 0.005$). SPOC:SPN ratios were not significantly different within the redoxcline (7.5 ± 4), as compared to above (8.3 ± 4) and below (6.3 ± 4). The dramatic depth-dependent decreases in SPOC:TSPP and SPN:TSPP ratios are mainly due to the abrupt increase in suspended P across the redox boundary.

Discussion

Large scale variability in dissolved and particulate P concentrations has been observed in other redoxclines similar to that of the Cariaco Basin (e.g., Black Sea and Baltic Sea) and attributed to: 1) microbial activity, which converts dissolved into particulate P during biological growth and vice versa during decomposition (Taylor et al., 2001; 2009) and 2) delivery of iron and manganese, whose colloidal and particulate oxides adsorb and co-precipitate P or remobilize P into the dissolved phase upon oxide reduction (Dellwig et al. 2010, Diaz et al. 2012). Our

results suggest that suspended P biogeochemistry is significantly influenced by both of these biotic and abiotic processes within the redoxcline of the Cariaco Basin.

Biotic transformations of suspended P

TSPP concentrations within the Cariaco Basin redoxcline on average were similar or slightly greater than those measured in the upper 100 m (Figure 3A and 3B). For one cruise (December 2008), TSPP was observed to be almost five times higher in the redoxcline than in the upper 100 m. These large scale enrichments in the redoxcline are similar to patterns observed in other systems, e.g., Effingham Inlet (Diaz et al., 2012). Lorenzoni et al. (2013) showed that while DOC concentrations remain relatively constant across the Cariaco Basin redoxcline, dissolved organic P (DOP) concentrations are elevated, exceeding waters immediately above and below the redoxcline by $\sim 20 - 40$ nM. In contrast, dissolved inorganic P concentrations, or phosphate, steadily increase below the mixed layer and across the redox transition, with no obvious peak in phosphate concentrations observed at the redoxcline (Scranton et al., 2014).

Microbial biosynthesis is likely a major factor in DOP, SSRP, and SSNP enrichment within the redoxcline. Dark DIC assimilation rates within the redoxcline are often of the same order of magnitude as primary production in the euphotic zone (Taylor et al., 2001), suggesting that microbial production in this layer plays a critical role in P dynamics. While a significant factor of two enrichment is observed in the redoxcline's SSNP pool relative to that measured immediately above and below, the same is not observed for SPOC or SPN, which results in a sharp decline in suspended SPOC:TSPP ratios within the redoxcline (Figure 5A, 5B and 5C). The lack of an observable redoxcline SPOC or SPN peak is at odds with the clear 1-2 μM increase in SPOC concentrations observed within the Cariaco Basin redoxcline in November

2007 (Wakeham et al., 2012) and other studies reporting enriched SPOC and SPN concentrations across redox boundaries in other euxinic basins and OMZs (e.g., Wishner et al., 1995, Çoban-Yıldız et al., 2006; Pitcher et al., 2011, Diaz et al., 2012). Closer examination of individual profiles for SPOC and SPN suggest that redoxcline peaks in SPOC and SPN concentrations do exist in the Cariaco Basin, but are obscured by high cruise to cruise variability in peak magnitude and depth relative to suspended SPOC and SPN concentrations measured immediately above and below. Regardless, the measured increase in SPOC and SPN periodically observed within the redoxcline is much lower than the increase expected using measured SSNP concentrations and calculating SPOC and SPN using the 106C:16N:1P ratio from Redfield et al. (1963). This argues for preferential accumulation of SSNP in the redoxcline (Table 1 and Figure 5).

The decoupling of SSNP from SPOC and SPN may be due to the presence of inorganic polyphosphate. Inorganic polyphosphate is formed during microbial growth within both P-rich and poor environments primarily as intracellular P storage, but it also has been shown to be involved in gene regulation and can serve as ballast material in *Trichodesmium* (Romans et al., 1994, Brown et al., 2004, Orchard et al., 2010). Only recently has polyphosphate been emphasized as a critical player in the marine P cycle (Diaz et al., 2008, Bjorkman 2014, Martin et al., 2014). Genes encoding for enzymes critical for polyphosphate metabolism (ppk1, ppk2, and ppkx) commonly occur in the Global Ocean Sampling (GOS) metagenomic database, suggesting broad geographic distribution of polyphosphate utilization (Temperton et al., 2011). Furthermore, prevalence of ppk1 and ppkx genes in the GOS database appear to be inversely proportional to phosphate (P_i) concentrations in surrounding waters, suggesting that microbial communities subjected to low or intermittent P_i supply are more reliant on polyphosphate sequestration strategies than communities in P_i -replete environments (Temperton et al., 2011).

Compared to the oxygenated surface ocean, very little is known about redox-sensitive remobilization of inorganic polyphosphate. In anoxic sediments, however, intracellular polyphosphate has been shown to be hydrolyzed and released back into the dissolved phase (Gächter and Meyer 1993, Sannigrahi and Ingall 2005, Schulz and Schulz 2005, Hupfer et al., 2007), although some sulfide-oxidizing bacteria have been shown to convert this released phosphate into apatite (Goldhammer et al., 2010). In Effingham Inlet, concentrations of total particulate phosphorus per bacterial cell increased up to five fold in the redoxcline and polyphosphate was shown to compose a substantial amount of this pool despite high phosphate concentrations (Diaz et al., 2012).

We do not have direct evidence of polyphosphate in our samples. Polyphosphate is normally described as a part of the operationally defined organic P fraction in Aspila SSNP and in the SEDEX analysis (Diaz et al., 2012). However, based on the chemical structure and reactivity of polyphosphate, we argue that some of the shorter chain polyphosphate compounds are likely to be observed in the loosely/oxide-bound P fractions as well, given their reaction with citrate bicarbonate solution. Thus, the observed increase in the loosely/oxide-bound P and within the SSNP pool in the redoxcline is consistent with the presence of a polyphosphate pool (Figures 3 and 4).

Furthermore, if we assume an average cellular C:P atomic ratio of 41 ± 16 for prokaryotes not producing polyphosphate (Vrede et al., 2002), then prokaryotic P-biomass (nucleic acids, phospholipids, etc.) accounts for $77\% \pm 44$, $136\% \pm 135$, $54\% \pm 42$, and $102\% \pm 107$ of TSPP in the euphotic, disphotic, redoxcline and euxinic layers, respectively. Prokaryotic P-biomass accounts for a significantly smaller proportion of the TSPP inventory ($p < 0.001$, ANOVA, $n = 47$) in the redoxcline than in other layers. In the disphotic and euxinic layers,

prokaryotic P-biomass essentially accounts for all TSPP, while phytoplankton in the euphotic layer easily account for the residual 23% of the TSPP inventory not attributable to prokaryotes. Thiotrophic chemoautotrophic ϵ -proteobacteria dominate in the Cariaco Basin redoxcline and are known from the GOS database to carry polyphosphate metabolizing genes (Lin et al., 2006, 2008, Temperton et al., 2011). These results are supported further by a recent study of intact polar lipids (IPLs) within the Cariaco Basin, which found that phospholipids, although less than 1 nM of the TSPP pool, accounted for up to 90% of IPLs measured within the redoxcline, and are typically associated with a suite of organisms capable of polyphosphate synthesis and remineralization (Wakeham et al., 2012). These facts, coupled with the 46% shortfall between P-biomass and TSPP inventories in the Cariaco Basin redoxcline, are suggestive of a chemoautotrophic community residing in this layer that likely relies, at least in part, on polyphosphate. It is unclear why else polyphosphate would reside in the redoxcline despite high concentrations of phosphate and DOP (Lorenzoni et al., 2013), but it may be associated with “luxury” uptake and storage (Diaz et al., 2008, Crocetti et al., 2000, Orchard et al., 2010, Goldhammer et al., 2010, Diaz et al., 2012).

Abiotic transformation of P

Trace metal redox reactions are also likely to play an important role in the enrichment of TSPP through the redoxcline of the Cariaco Basin, particularly with regards to the SSRP pool (Dellwig et al., 2010, Li et al., 2012). The “redox shuttle” refers to the formation of P-associated colloids and particulates when reduced metals, particularly manganese and iron, diffuse upward through the redoxcline and rapidly oxidize and scavenge both phosphate and organic P compounds (Dellwig et al., 2010, Ruttenger and Sulak 2011). As these oxidized particles sink

back through the redoxcline, they are reduced abiotically thereby releasing the associated P (Dellwig et al., 2010). Recent evidence suggests that microbial activity may also enhance these oxidation/reduction reactions (Cosmidis et al., 2014). Closer examination of the TSPP pool reveals that a switch occurs from SSNP dominated waters (81%) at the surface to SSRP dominated waters (61%) within the redoxcline (Figure 3A). The majority of the SSRP enrichment is attributable to loosely/oxide-bound P, which comprises ~ 80% of the SSRP pool in the redoxcline compared to $\leq 50\%$ above and below (Figure 4B). Comparing average concentrations of loosely/oxide-bound P above and below the redoxcline (39 ± 7 nM and 43 ± 39 nM, respectively) with the much higher average concentrations of loosely/oxide-bound P within the redoxcline of 111 ± 24 nM, indicates potential *in situ* production and subsequent loss of ~70 nM of this highly labile redoxcline P pool (Figure 4A). Such contrasts in P speciation and large enrichments in loosely/oxide-bound P, suggest that redox reactions play a significant role in inorganic P accumulation in the redoxcline.

It is possible that some of the measured increase in redoxcline TSPP pools is due to remineralization of sinking particles and lateral transport from the nearshore rather than due to *in situ* formation. However, we argue that this is unlikely. For example, Lorenzoni et al. (2009) measured the composition of several intermediate and bottom nepheloid layers derived from the near shore across the continental shelf of the Cariaco Basin. They found a significant enrichment in SSRP with SSNP concentrations similar to that measured in the overlying water column. At the same time, however, SPOC concentrations were also higher, resulting in elevated SPOC/SSNP ratios within the nepheloid plumes. This is the opposite of that observed within the redoxcline in our study (Figure 5).

Disaggregation and dissolution of large sinking particles from the euphotic zone could also play a role in the observed increase in TSPP concentrations within the redoxcline. Indeed, attenuated particulate P fluxes within the Cariaco Basin below the redoxcline have been described previously for sinking particles (Benitez-Nelson et al., 2004; 2007). However, disaggregation processes would also significantly increase redoxcline SPOC and SPN pools as well, and this is not observed in our dataset. Furthermore, surface suspended P concentrations in the Cariaco Basin have been shown to vary considerably through time, with higher surface water concentrations occurring during upwelling versus non-upwelling periods coincident with higher rates of primary production (Benitez-Nelson et al., 2015). Within the redoxcline, however, suspended P did not significantly vary with time, confirming a decoupling of the surface and redoxcline particles.

The fate of suspended P

While much of the TSPP formed within the redoxcline is likely recycled there, it is quite possible that at least a fraction of the redoxcline TSPP escapes to deeper depths. Taylor et al. (2001) found that the flux of sinking particulate organic carbon captured in sediment traps below the redoxcline frequently exceeded that measured in waters from above and argued that mid-water chemoautotrophic production was an important secondary source of sinking organic carbon in the water column. Other research using a variety of biomarkers suggests that sinking particles and sediments commonly contain organic matter derived from mid-water chemoautotrophic processes in OMZs and euxinic basins, such as the Arabian and Black Seas and the Peru upwelling zone (Wakeham et al., 2007, Saenz et al., 2011).

While absolute fluxes of sinking inorganic and organic P decline with increasing depth in the water column in the Cariaco Basin (Benitez-Nelson et al., 2007), there are a number of instances when sediment trap P fluxes below the redoxcline (~ 400 m) also exceed those measured directly above (~ 225 m) during our study period (~ 40% of samples, data not shown). While these differences in sediment trap fluxes could be due to hydrodynamic issues associated with sediment trap collections (e.g., Buesseler et al., 2007), the enclosed nature of the Cariaco Basin produces a relatively quiescent setting that minimizes those effects. Therefore, it is quite possible that biologically mediated coagulation of suspended particles within the redoxcline enhances the observed “excess” sinking particulate P fluxes measured at depth. Diverse protist communities, now recognized to play major roles in particle coagulation (Sherr and Sherr 1987, Richardson and Jackson 2007), are known to exist within the redoxcline of the Cariaco Basin (Edgcomb et al., 2011) and zooplankton migration and repackaging of material has been observed within the redoxcline of other OMZs (Wishner et al., 2008).

Further evidence for an additional redoxcline source of sinking particulate P at depth is derived from comparison of nutrient ratios. Scranton et al. (2014) noted a dichotomy between the dissolved inorganic N:P ratios measured in Cariaco Basin deep waters (approximately 16:1) relative to the rate of nutrient accumulation (11:1) and the overall ratio of total N:P in the sinking particulate flux (approximately 5:1 to 12.5:1). These authors hypothesized that the ratio difference was due to changes in the relative proportions of sinking particulate source material enriched in P relative to C and N and argued that both terrestrial and redoxcline sources were the likely culprits. Our relatively low C/P ratios within the redoxcline suspended pool support the potential for a redoxcline P source to depth.

Conclusions

While there are a number of studies that have focused on the role of anoxia on the sinking particulate and sedimentary P pools, few have focused on P biogeochemistry within water column redoxclines (Ingall et al., 1993, Benitez-Nelson et al., 2007, Jilbert et al., 2013). In this study, suspended particulate P and its composition within the redoxcline of the Cariaco Basin was examined for the first time. A lack of seasonal variation in redoxcline TSPP dynamics indicates a decoupling from surface processes and *in situ* production. The significant switch from biologically less available (SSNP) to more labile P forms (SSRP) within the redoxcline suggests suspended P cycling in the Cariaco Basin is redox sensitive and is cycled through a combination of both a trace metal redox shuttle and polyphosphate accumulation/remobilization. Furthermore, combined with sediment trap data above and below the redoxcline, our results indicate a mid-water redoxcline source of particulate P (Scranton et al., 2014).

The Cariaco Basin has undergone a number of physical and biogeochemical changes over the past two decades that have been assessed through the Cariaco Time-Series (Scranton et al., 2014, Astor et al., 2013, Taylor et al., 2012). It is unclear whether the changes in surface processes (e.g., plankton community structure) influence suspended P cycling within the redoxcline (Scranton et al., 2014). However, the processes of suspended P transformation presented here suggest independent *in situ* production of suspended P and contribution by coagulation of suspended P to sinking P particles. The reduction in oxygen intrusions and size of the redox zone within the Cariaco Basin, however, may influence the magnitude of suspended P cycling within the redoxcline by changing the bacterial community structure and lowering the concentrations of trace elements available for redox scavenging. As such, mid-water cycling of suspended P and contributions to the sinking particle phase may be reduced, removing an

important sink of P within the system. This would create a positive feedback to eutrophication via the diffusion of P enriched waters back to the surface and contribute to the expansion of OMZs in the future.

Acknowledgements: We thank Eric Tappa for all C and N analyses and Wendy Plessinger for assistance in all P analyses. We also thank two anonymous reviewers for their thorough reviews that greatly improved the resulting publication. We are indebted to the personnel of the Fundación La Salle de Ciencias Naturales, Estación de Investigaciones Marinas, Isla Margarita (FLASA/EDIMAR), who were responsible for the collection of samples and analyses of some of the data presented here. In particular, we'd like to thank Yrene Astor, Jesus Narvaez, Glenda Arias, Aitzol Arrellano, Javier Campano, Jesus Escalona, Gustavo Fuentes, Laurencia Guzman, Luis Medina, and Jaimie Rojas, who provided invaluable field and laboratory support. We also thank the crew of the R/V *Hermano Ginés* for their able support at sea. This work was supported by the National Science Foundation, Ocean Sciences Division, Chemical Oceanography Program (NSF, Grants OCE-0752139, OCE-0752014, OCE-1259110, OCE-9216626, OCE-9729284, OCE-9401537, OCE-9729697, OCE-9415790, OCE-9711318, OCE-0963028 and OCE- 0326268), the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT, Venezuela, Grant 96280221) and the Fondo Nacional de Ciencia y Tecnología (FONACIT, Venezuela, Grant 2000001702 and 2011000353).

References

- Anderson, L., Delaney, M. 2001. Carbon to phosphorus ratios in sediments: Implications for nutrient cycling. *Global Biogeochemical Cycles*. 15: 65-79.
- Aspila, K., Agemian, H., Chau, A. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *The Analyst*. 101: 187-197.
- Astor, Y., Muller-Karger, F., Scranton, M. 2003. Seasonal and interannual variation in the hydrography of the Cariaco Basin, Venezuela: implications for basin ventilation. *Continental Shelf Research*. 23: 125-144.
- Astor, Y., Lorenzoni, L., Thunell, R., Varela, R., Muller-Karger, F., Troccoli, L., Taylor, G., Scranton, M., Tappa, E., Rueda, D. 2013. Interannual variability in sea surface temperature and fCO₂ changes in the Cariaco Basin. *Deep Sea Research Part II*: 93: 33-43.
- Astor, Y., Lorenzoni, L., Scranton, M. Eds. 2014. Handbook of methods for the analysis of oceanographic parameters at the CARIACO Time-series station. Eds. Cuadernos FLASA, Serie Ciencia y Tecnología N° 12. Fundación La Salle de Ciencias Naturales, Caracas, Venezuela.
- Black, D., Thunnell, R., Kaplan, A., Peterson, L., Tappa, E. 2004. A 2000-year record of Caribbean and tropical North Atlantic hydrographic variability. *Paleoceanography*. 19: 1-11.
- Benitez-Nelson, C. 2000. The biogeochemical cycling of phosphorus in marine systems. *Earth Science Reviews*. 51: 109-135.
- Benitez-Nelson, C.R., O'Neill Madden, L., Kolowitz, L., Pellechia, P., Thunnell, R. 2004. Phosphonates and particulate organic phosphorus cycling in an anoxic marine basin. *Limnology and Oceanography*. 49: 1593-1604.
- Benitez-Nelson, C., O'Neill Madden, L., Styles, R., Thunnell, R., Astor, Y. 2007. Inorganic and organic sinking particulate phosphorus fluxes across the oxic/anoxic water column of Cariaco Basin, Venezuela. *Marine Chemistry* 105:90-100.
- Benitez-Nelson, C.R., Thunell, R., Pinckney, J., Lorenzoni, L., Montes, E., Muller-Karger, F., Scranton, M., Taylor, G., Varela, R., Astor, Y. 2015. Elemental Composition (C, N and P) of Sinking and Suspended Particulate Matter in the Cariaco Basin, Venezuela. February 2015 ASLO Winter Meeting Granada, Spain
- Bennett, E., Carpenter, S., Caraco, N. 2001. Human impact on erodible phosphorus and eutrophication: A global perspective. *BioScience*. 51: 227-234.
- Bjorkman, K. 2014. Polyphosphate goes from pedestrian to prominent in the marine P-cycle. *Proceedings of the National Academy of Sciences*. 111: 7890-7891.
- Brown M., Kornberg A. 2004. Inorganic polyphosphate in the origin and survival of species. *Proceedings of the National Academy of Sciences*. 101: 16085–16087.
- Buesseler, K., Antia, A., Chen, M., Fowler, S., Gardner, W., Gustafsson, Ö., Harada, K., Michaels, A., Rutgers v. d. Loeff, M., Sarin, M., Steinberg, D., Trull, T. 2007. An assessment of the use of sediment traps for estimating upper ocean particle fluxes. *Journal of Marine Research*. 65: 345-416.
- Çoban-Yıldız, Y., Altabet, M., Yilmaz, A., Tuğrul, S. 2006. Carbon and nitrogen isotopic ratios of suspended particulate organic matter (SPOM) in the Black Sea water column. *Deep Sea Research Part II*. 53: 1875-1892.
- Codispoti, L., Flagge, C., Kelly, V., Swift, J. 2005. Hydrographic conditions during the 2002

- SBI process experiments. *Deep Sea Research II*. 52: 3199-3126.
- Correll, D. 1998. The role of phosphorus in the eutrophication of receiving waters: a review. *Journal of environmental quality*. 27: 261-266.
- Cosmidis, J., Benzerara, K., Morin, G., Busigny, V., Lebeau, O., Jézéquel, D., Noëla, V., Dublet, G., Othmane, G. 2014. Biomineralization of iron-phosphates in the water column of Lake Pavin (Massif Central, France). *Geochimica et Cosmochimica Acta*. 126: 78-96.
- Crocetti, G., Hugenholtz, P., Bond, A., Schuler, J., Keller, D., Jenkins, Blackall, L. 2000. Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantification. *Applied Environmental Microbiology*. 66: 1175–1182
- Dellwig, O., Leipe, T., März, C., Glockzin, M., Pollehne, F., Schnetger, B., Yakushev, E., Böttcher, M., Brumsack, H. 2010. A new particulate Mn-Fe-P shuttle at the redoxcline of anoxic basins. *Geochimica et Cosmochimica Acta*. 74: 7100-7115.
- Deutsch, C., Brix, H., Ito, T., Frenzel, H., Thompson, L. 2011. Climate forced variability of ocean hypoxia. *Science*. 333: 336-339.
- Deutsch, C., Berelson, W., Thunell, R., Weber, T., Tems, C., McManus, J., Crusius, J., Ito, T., Baumgartner, T., Ferreira, V., Mey, J., van Geen, A. 2014. Centennial changes in North Pacific anoxia linked to tropical trade winds. *Science*. 345: 665-668.
- Diaz, R., Rosenberg, R. 2008. Spreading dead zones and consequences for marine ecosystems. *Science*. 321: 926-929.
- Diaz, J., Ingall, E., Benitez-Nelson, C., Paterson, D., de Jonge, M., McNulty, I., Brandes, J. 2008. Marine polyphosphate: A key player in geologic phosphorus sequestration. *Science*. 320: 652-655.
- Diaz, J., Ingall, E., Snow, S., Benitez-Nelson, C., Taillefert, M., Brandes, J. 2012. Potential role of inorganic polyphosphate in the cycling of phosphorus within the hypoxic water column of Effingham Inlet, British Columbia. *Global Biogeochemical Cycles*. 26: 1-13.
- Dyhrman, S., Benitez-Nelson, C., Orchard, E., Haley, S., Pellechia, P. 2009. A microbial source of phosphonates in oligotrophic marine systems. *Nature Geoscience*. 2: 696-699.
- Doney, S. 2010. The growing human footprint on coastal and open ocean biogeochemistry. *Science*. 328: 1512-1516.
- Edgcomb, V., Orsi, W., Taylor G., Vdacny, P., Taylor, C., Suarez, P., Epstein, S. 2011. Accessing marine protists from the anoxic Cariaco Basin. *ISME J*. 5: 1237-1241.
- Emerson, S., Jacobs, L., Tebo, T. 1983. The behavior of trace metals in marine anoxic waters: Solubilities at the oxygen-hydrogen sulfide interface. *Trace Metals in Sea Water*. 9: 579-608.
- Fanning, K. 1989. Influence of atmospheric pollution on nutrient limitation in the ocean. *Nature* 339: 460-463.
- Froelich, P. 1980. Analysis of organic carbon in marine sediments. *Limnology and Oceanography*. 25: 564-572.
- Gächter, R., Meyer, J. 1993. The role of microorganisms in mobilization and fixation of phosphorus in sediments. *Hydrobiologia*. 253: 103-121.
- Goldhammer, T., Brüchert, V., Ferdelman, T., Zabel, M. 2010. Microbial sequestration of phosphorus in anoxic upwelling sediments. *Nature Geoscience*. 3: 557-561.
- Helly, J., Levin, L., 2004. Global distribution of naturally occurring marine hypoxia on continental margins. *Deep-Sea Research I* 51, 1159–1168.
- Hupfer, M., Gloess, S., Grossar, H. 2007. Polyphosphate-accumulating microorganisms in

- aquatic sediments. *Aquatic microbial ecology: international journal*. 47: 299-311.
- Ingall, E., Bustin, R., Van Cappellen, P. 1993. Influence of water column anoxia on the burial and preservation of carbon and phosphorus in marine shales. *Geochimica et Cosmochimica Acta*. 57: 303-316.
- Jahnke, R. 1992. The phosphorus cycle. In: Butcher, S., Charlson, R., Orians, G., Wolfe, G., (Eds). *Global Biogeochemical Cycles*. New York:Academic Press
- Jilbert, T., Slomp, C. 2013. Iron and manganese shuttles control the formation of authigenic phosphorus minerals in the euxinic basins of the Baltic Sea. *Geochemica et Cosmochimica Acta*. 107: 155-169.
- Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Arteaga, L., Loscher, C., Oschlies, A., Paulmier, A., Stramma, L., Kuypers, M. 2013. Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone. *Nature Geoscience*. 6: 228-234.
- Karl D. 1978. Distributions, abundance, and metabolic states of microorganisms in the water column and sediments of the Black Sea. *Limnology and Oceanography*. 23:936-49.
- Karl, D., Dore, J., Fujieki, L., Hebel, D., Houlihan, T., Letelier, R., Tupas, L. 2001. Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. *Deep Sea Research Part II*. 48: 1529-1566.
- Karl, D. 2014. Microbially mediated transformations of phosphorus in the sea: New views of an old cycle. *Annual Review of Marine Science*. 6: 279-337.
- Karstensen, J. Stramma, L., Visbeck, M. 2008. Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans. *Progress in Oceanography*. 77: 331-350.
- Kirchman, D. 1993. Leucine incorporation as a measure of biomass reduction by heterotrophic bacteria, p. 509-512. In P.F. Kemp, B.F. Sherr, E. B. Sherr, and J.J. Cole [eds.], *Handbook of methods in aquatic microbial ecology*. Lewis.
- Lam, P., Kuypers, M. 2011. Microbial nitrogen cycling processes in oxygen minimum zones. *Annual Reviews of Marine Science*. 3: 317-345.
- Levin, L. 2003. Oxygen minimum zone benthos: Adaptation and community response to hypoxia. *Oceanography and Marine Biology: An Annual Review*. 41: 1-45.
- Li, X., Cutter, G., Thunell, R., Tappa, E., Gilhooly, W., Lyons, T., Astor, Y., Scranton, M. 2011. Particulate sulfur species in the water column of the Cariaco Basin. *Geochimica et Cosmochimica Acta*. 75: 148-163.
- Li, X., Taylor, G., Astor, Y., Varela, R., Scranton, M. 2012. The conundrum between chemoautotrophic production and reductant and oxidant supply: A case study from the Cariaco Basin. *Deep Sea Research I*. 61: 1-10.
- Lin, X., Wakeham, S., Putnam, I., Astor, Y., Scranton, M., Taylor, G. 2006. Vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and Black Sea compared using fluorescent in situ hybridization (FISH). *Applied Environmental Microbiology*. 72: 2679-2690.
- Lin, X., Scranton, M., Chistoserdov, A., Varela, R., Taylor, G. 2008. Spatiotemporal dynamics of bacterial populations in the anoxic Cariaco Basin. *Limnology and Oceanography*. 53: 37-51.
- Lorenzoni, L., Thunell, R., Benitez-Nelson, C., Hollander, D., Martinez, N., Tappa, E., Varela, R., Asor, Y., Muller-Karger, F. 2009. The importance of subsurface nepheloid layes in transport and delivery of sediments to the eastern Cariaco Basin, Venezuela. *Deep Sea Research I*. 56: 2249-2262.
- Lorenzoni, L., Taylor, G., Benitez-Nelson, C., Hansell, D., Montes, E., Masserini, R.,

- Fanning, K., Varela, R., Astor, Y., Guzman, L., Muller-Karger, F. 2013. Spatial and seasonal variability of dissolved organic matter in the Cariaco Basin. *Journal of Geophysical Research: Biogeosciences*. 118: 951-962.
- Madrid V., Taylor G., Scranton M., Chistoserdov A. 2001. Phylogenetic diversity of Bacterial and Archaeal communities in the anoxic zone of the Cariaco Basin. *Applied Environmental Microbiology*. 67:1663-74.
- Martin, P., Dyhrman, S., Lomas, M., Poulton, N., Van Mooy, B. 2014. Accumulation and enhanced cycling of polyphosphate by Sargasso Sea plankton in response to low phosphorus. *PNAS*. 111: 8089-8094.
- Matear, R., Hirst, A. 2003. Long term changes in dissolved oxygen concentrations in the ocean caused by protracted global warming. *Global Biogeochemical Cycles*. 17: 1125.
- Montes, E., Thunell, R., Muller-Karger, F., Lorenzoni, L., Tappa, E., Troccoli, L., Astor, Y., Varela, R. 2013. Sources of $\delta^{15}\text{N}$ variability in sinking particulate nitrogen in the Cariaco Basin, Venezuela. *Deep Sea Research Part II*. 93: 96-107.
- Muller-Karger, F., Varela, R., Thunell, R., Scranton, M., Bohrer, R., Taylor, G., Capelo, J., Astor, Y., Tappa, E., Ho, T.Y., Iabichella, M., Walsh, J., Diaz, J. 2000. Sediment record linked to surface processes in the Cariaco Basin. *Eos, Transactions, American Geophysical Union*. 81(45): 529,534-535.
- Muller-Karger F., Varela, R., Thunell, R., Scranton, M., Bohrer, R., Taylor, G., Capelo, J., Astor, Y., Tappa, E., Ho, T., Walsh, J. 2001. Annual cycle of primary production in the Cariaco Basin: Response to upwelling and implications for vertical export. *Journal of Geophysical Research*. 106: 4527-4542.
- Muller-Karger F., Varela, R., Thunell, R., Luerssen, R., Hu, C., Walsh, J. 2005. The importance of continental margins in the global carbon cycle. *Geophysical Research Letters*. 32: 1-4.
- Muller-Karger, F., Varela, E., Thunell, R., Scranton, M., Taylor, G., Astor, Y., Benitez-Nelson, C., Lorenzoni, L., Tappa, E., Goñi, M., Rueda, D., Hu, C. 2010. The CARIACO Oceanographic Time Series. In: Carbon and Nutrient Fluxes in Continental Margins: A Global Synthesis. JGOFS Continental Margins Task Team (CMTT) Editors: K.-K. Liu, L. Atkinson, R. Quinones, L. Talaue-McManus. Springer-Verlag New York. Pp 454-463.
- Murray, J., Yakushev, E. 2006. The suboxic transition zone in the Black Sea. In: Neretin, L.N. (Ed.) Past and Present Water Column Anoxia, NATO Sci Ser., Springer, Netherlands, p.105-138.
- Orchard, E., Benitez-Nelson, C., Pellechia, P., Lomas, M., Dyhrman, S. 2010. Polyphosphate in Trichodesmium from the low phosphorus Sargasso Sea. *Limnology and Oceanography*. 55: 2161-2169.
- Oschlies, A., Schultz, K., Riebesell, U., Schmittner, A. 2008. Simulated 21st century's increase in oceanic suboxia in CO₂-enhanced biotic carbon export. *Global Biogeochemical Cycles*. 22: 1-10.
- Paerl, H. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnology and Oceanography*. 42: 1154-1165.
- Paulmier, A., Ruiz-Pino, D. 2009. Oxygen minimum zones in the modern ocean. *Progress in Oceanography*. 80: 113-128.
- Pasek, M.A., Harnmeijer, J., Buick, R., Maheen, G., Atlas, Z. 2013. Evidence for reactive

- reduced phosphorus species in the early Archean ocean. *Proceedings of the National Academy of Science USA*. 110: 10089-10094.
- Pasek, M., Sampson, J., Atlas, Z. 2014. Redox chemistry in the phosphorus biogeochemical cycle. *Proceedings of the National Academy of Sciences*. 111: 15468-15473.
- Paytan, A., McLaughlin, K. 2007. The oceanic phosphorus cycle. *Chemical Reviews*. 107: 563-576.
- Percy, D., Li, X., Taylor, G., Astor, Y., Scranton, M. 2008. Controls on iron, manganese and intermediate oxidation state sulfur compounds in the Cariaco Basin. *Marine Chemistry*. 111: 47-62.
- Pinckney, J., Benitez-Nelson, C., Thunell, R., Muller-Karger, F., Lorenzoni, L., Troccoli, L., Varela, R. 2015. Phytoplankton community structure and depth distribution changes in the Cariaco Basin between 1996 and 2010. *Deep Sea Research I*. 101: 27-37.
- Pitcher, A., Villanueva, L., Hopmans, E., Schouten, S., Reichart, G., Damste, J. 2011. Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone. *The ISME Journal*. 5: 1896-1904.
- Redfield, A. 1958. The biological control of chemical factors in the environment. *American Scientist*. 46: 205-222.
- Redfield, A., Ketchum, B., Richards, F. 1963. The influence of organisms on the composition of sea-water. In *The Sea*, vol. 2, edited by M.N. Hill, p 26-77, Interscience, New York.
- Richardson, T., Jackson, G. 2007. Small phytoplankton and carbon export from the surface ocean. *Science*. 315: 838-840.
- Romans, K., Carpenter, E., Bergman, B. 2004. Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*: Ultrastructure and storage of carbohydrate, polyphosphate and nitrogen. *Journal of Phycology*. 30: 935-942.
- Ruttenberg, K. 1992. Development of a sequential extraction method for different forms of phosphorus in marine sediments. *Limnology and Oceanography*. 37: 1460-1482.
- Ruttenberg, K., Sulak, D. 2011. Sorption and desorption of dissolved organic phosphorus onto iron (oxyhydro)oxides in seawater. *Geochimica et Cosmochimica Acta*. 75: 4095-4112.
- Saenz, J., Summons, R., Eglinton, T., Wakeham, S., 2011. Distribution of bacteriohopanepolyols in marine anoxic environments: new constraints on the provenance of hopanoids in the marine geologic record. *Organic Geochemistry*. 42: 1322-1351.
- Sannigrahi, P., Ingall, E. 2005. Polyphosphates as a source of enhance P fluxes in marine sediments overlain by anoxic waters: Evidence from ³¹P NMR. *Geochemical Transactions*. 6: 52-59.
- Scranton, M., Astor, Y., Bohrer, R., Ho, T., Muller-Karger, F. 2001. Controls on temporal variability of the geochemistry of the deep Cariaco Basin. *Deep Sea Research I*. 48: 1605-1625.
- Scranton, M., Taylor, G., Thunell, R., Benitez-Nelson, C., Muller-Karger, F., Fanning, K., Lorenzoni, L., Montes, E., Varela, R., Astor, Y. 2014. Interannual and decadal variability in the nutrient geochemistry of the Cariaco Basin. *Oceanography*. 27: 148-159.
- Schulz, H., Schulz, H. 2005. Large sulfur bacteria and the formation of phosphorite. *Science*. 307: 416-418.
- Sherr, E., Sherr, B. 1987. High rates of consumption of bacteria by pelagic ciliates. *Nature*. 325: 710-711.

- Silkin, V., Pautova, L., Pakhomova, S., Lifanchuk, A., Yakushev, E., Chasovnikov, V. 2014. Environmental control on phytoplankton community structure in the NE Black Sea. *Journal of Experimental Marine Biology and Ecology*. 461:267-274.
- Spencer, D., Brewer, P. 1971. Vertical advection diffusion and redox potentials as controls on the distribution of manganese and other trace metals dissolved in waters of the Black Sea. *Journal of Geophysical Research*. 76: 5877-5892.
- Stramma, L., Johnson, G., Sprintall, J., Mohrholz, V. 2008. Expanding oxygen minimum zones in the Tropical Oceans. *Science*. 320: 655.
- Stramma, L., Schmidtko, S., Levin, L., Johnson, G. 2010. Ocean oxygen minima expansions and their biological impacts. *Deep Sea Research Part I: Oceanographic Research Papers*. 57: 587-595.
- Taylor, G., Iabichella, M., Ho, T., Scranton, M., Thunell, R., Muller-Karger, F., Varela, R. 2001. Chemoautotrophy in the redox transition zone of the Cariaco Basin: A significant midwater source of organic carbon production. *Limnology and Oceanography*. 46: 148-163.
- Taylor, G., Iabichella-Armas, M., Varela, R., Muller-Karger, F., Lin, X., Scranton, M. 2006. Microbial ecology of the Cariaco Basin's redoxcline: The U.S.-Venezuela CARIACO Time Series Program. *In* Microbial Ecology of the oxic/anoxic interface.
- Taylor, G., Thunell, R., Varela, R., Benitez-Nelson, C., Scranton, M. 2009. Hydrolytic ectoenzyme activity associated with suspended and sinking organic particles within the anoxic Cariaco Basin. *Deep Sea Research I*. 56: 1266-1283.
- Taylor, G., Muller-Karger, F., Thunell, R., Scranton, M., Asor, Y., Varela, R., Ghinaglia, L., Lorenzoni, L., Fanning, K., Hameed, S., Doherty, O. 2012. Ecosystem responses in the southern Caribbean Sea to global climate change. *PNAS*. 109: 19315-19320.
- Temperton, B., Gilbert, J. ., Quinn, J., McGrath, J. 2011. Novel analysis of oceanic surface water metagenomes suggests importance of polyphosphate metabolism in oligotrophic environments. *PLoS ONE* 6(1): e16499. doi:10.1371/journal.pone.0016499
- Thunell, R., Benitez-Nelson, C., Varela, R., Astor, Y., Müller-Karger, F. 2007. Particulate organic carbon fluxes along upwelling-dominated continental margins: Rates and mechanisms. *Global Biogeochemical Cycles*. 21: GB1022, doi:10.1029/2006GB002793.
- Tuttle, J., Jannasch, H. 1973. Sulfide and thiosulfate-oxidizing bacteria in anoxic marine basins. *Marine Biology*. 20: 64-70.
- Van Cappellen, P., Ingall, E. 1994. Benthic phosphorus regeneration, net primary production, and oceanic anoxia: A model of the coupled marine biogeochemical cycles of carbon and phosphorus. *Paleoceanography*. 9: 677-692.
- Van Mooy, B.A.S., Krupke, A., Dyhrman, S.T., Fredricks, H.F., Frischkorn, K.R., Ossolinski, J.E., Repeta, D.J., Rouco, M., Seewald, J.D., Sylva, S.P. 2015. Major role of planktonic phosphate reduction in the marine phosphorus redox cycle. *Science*, 348: 783-785.
- Vrede, K., Heldal, M., Norland, S., Bratbak, G. 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. *Applied Environmental Microbiology*. 68: 2965-2971.
- Wakeham, S., Amann, R., Freeman, K., Hopmans, E., Jørgensen, B., Putnam, I., Schouten, S., Sinninghe Damste', J., Talbot, H., Woebken, D. 2007. Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study. *Organic Geochemistry*. 38: 2070-2097.
- Wakeham, S., Turich, C., Schubotz, F., Podlaska, A., Li, X., Varela, R., Astor, Y., Saenz, J.,

- 865 Rush, D., Sinninghe Damste, J., Summons, R., Scranton, M., Taylor, G. Hinrichs, K.
866 2012. Biomarkers, chemistry and microbiology show chemoautotrophy in a multilayer
867 chemocline in the Cariaco Basin. *Deep Sea Research I*. 63: 133-156.
- 868 Wishner, K., Ashjian, C., Gelfman, C., Gowing, M., Kann, L., Levin, L., Mullineaux, L.,
869 Saltzman, J. 1995. Pelagic and benthic ecology of the lower interface of the Eastern
870 Tropical Pacific oxygen minimum zone. *Deep Sea Research Part I*. 42: 93-116.
- 871 Wishner, K., Gelfman, C., Gowing, M., Outram, D., Rapien, M., Williams, R., 2008. Vertical
872 zonation and distributions of calanoid copepods through the lower oxycline of the
873 Arabian Sea oxygen minimum zone. *Progress in Oceanography*. 78: 163–191.
- 874 Wright, Kaye. 2013. Proceedings of the Microbial Ecology and Biogeochemistry of Oxygen
875 Deficient Marine Waters Meeting.
- 876 Wyrski, K. 1962. The oxygen minima in relation to ocean circulation. *Deep Sea*
877 *Research*. 9: 11-23.
- 878 Wu, J. E, Sunda, W., Boyle, E.A., Karl, D. 2000. Phosphate depletion in the western
879 North Atlantic Ocean. *Science*. 289: 759-762.
- 880 Yool A, Fashman, M. 2001. An examination of the ‘continental shelf pump’ in an open ocean
881 general circulation model. *Global Biogeochemical Cycles*. 15:831–844.

Table and Figure Captions

Table 1: Average concentrations at each sampling depth of TSPP (nM), SSRP (nM), SSNP (nM), SPOC (μM) and SPN (μM) for all months between April 2008 to April 2012. Redoxcline depth range (230 – 290 m) is highlighted in grey. Sample size for each depth is presented as n.

Figure 1: Box and whisker plot of A) prokaryoplankton, B) bacterial net production, and C) dark DIC assimilation rate weighted means binned from 1-100, 101-229, 230-290 and > 300 m. Weighted means were computed from the layer's integral divided by the layer's depth interval (n represents the number of weighted means represented in each box). Boxes, internal lines, error bars and circles represent the 25th to 75th (= interquartile range), medians, 10th to 90th, and 5th to 95th percentiles of all observations, respectively.

Figure 2: Prokaryote distributions and production with depth relative to P and DO. A) Example profile from December 2010 showing prokaryoplankton concentration ($\mu\text{M C}$), TSPP (nM) and DO (μM), Inset: Frequency of TSPP maximum peaks (black bars) and prokaryoplankton maximum peaks (grey bars) at each depth within the redoxcline from April 2008 to April 2012, B) Example profile from December 2010 showing heterotrophic bacterial net production, dark DIC assimilation rate, TSPP (nM) and DO (μM). Inset: Frequency of TSPP maximum peaks (black bars) and dark DIC assimilation rate maximum peaks (grey bars) at each depth within the redoxcline from April 2008 to April 2012.

Figure 3: Suspended particulate P concentrations with depth. A) Depth profile of SSRP (nM). Grey dots represent individual samples measured for SSRP, while black solid line represents

average SSRP. Dashed line represents average TSPP to illustrate contribution of SSRP to TSPP
B) Depth profile of SSNP (nM). Grey dots represent individual samples measured for SSNP,
while black solid line represents average SSNP. Dashed line represents TSPP to illustrate
contribution of SSNP to TSPP.

Figure 4: Phosphorus composition across the redoxcline. A) Average concentrations of loosely/
oxide-bound P, authigenic P, detrital P and organic P (nM) within the redoxcline (light grey
bars), above the redoxcline (black bars) and below the redoxcline (dark grey bars). Error bars
represent the standard deviation, B) Percent average concentrations of loosely/oxide-bound P,
authigenic P, detrital P and organic P (nM) within the redoxcline (light grey bars), above the
redoxcline (black bars) and below the redoxcline (dark grey bars). Error bars represent the
standard deviation.

Figure 5: Elemental ratios of suspended particulate matter with depth. A) SPOC:TSPP (black
squares) and SPOC:SSNP (white squares), B) SPN:TSPP (black squares) and SPN:SSNP (white
squares), and C) SPOC:SPN (black squares). The redoxcline is denoted by the area between the
dashed lines. Standard deviations of each ratio (not shown) are on average $\pm 100\%$, reflecting
the high variability of the system.

Figure 1

Figure 2A

Figure 2Ainsert

Figure 2B

Bacterial Net Production and Dark DIC Assimilation December 2010

FIG2Binsert.tiff

Frequency of TSP and Dark DIC Assimilation Peaks within the Redoxcline

FIG3AB.tiff

Absolute TSPP Concentrations from SEDEX

Relative TSPP Concentrations from SEDEX

Table 1.pdf

n	Depth (m)	TSPP (nM)	SD	SSRP (nM)	SD	SSNP (nM)	SD	SPOC (μM)	SD	SPN (μM)	SD
29	7	60.5	74.3	13.7	26.8	46.8	49.3	8.3	8.1	1.0	0.8
33	25	70.2	72.2	20.0	30.5	50.2	44.7	8.7	6.0	1.2	0.9
34	35	45.2	22.6	11.5	14.5	33.8	18.3	6.5	3.4	0.9	0.5
33	55	32.7	18.6	7.0	6.3	25.7	16.1	6.0	1.9	0.9	0.3
30	75	26.9	18.8	7.5	8.8	19.0	13.2	4.7	1.6	0.7	0.3
37	100	18.5	9.4	5.0	4.8	13.6	7.5	4.9	1.5	0.7	0.3
29	130	14.8	7.1	4.8	5.1	9.5	5.1	4.1	1.6	0.6	0.3
28	160	14.5	9.4	4.3	4.6	9.3	7.6	4.0	1.8	0.6	0.3
37	200	18.0	15.5	5.0	4.7	11.9	14.5	4.8	2.0	0.6	0.3
3	230	48.9	31.6	24.1	23.4	24.8	9.3	5.2	1.7	0.7	0.3
36	250	67.5	49.8	38.2	46.2	29.4	22.2	5.5	1.4	0.7	0.2
7	260	67.9	91.2	41.1	76.7	26.8	15.2	5.9	1.2	0.9	0.2
14	270	53.7	30.5	26.1	23.7	27.6	18.2	5.3	1.3	0.8	0.3
39	300	21.7	11.3	6.1	6.7	15.3	8.2	8.1	8.8	1.1	1.3
35	350	17.3	7.4	4.7	5.9	11.8	7.2	6.3	3.7	0.8	0.4
38	400	14.9	6.5	4.7	5.5	9.1	5.8	8.2	7.8	1.1	1.2
36	500	18.1	28.9	2.8	3.7	14.3	27.6	7.3	6.3	1.1	1.0
34	750	13.2	9.4	3.8	5.5	9.0	6.2	5.5	2.6	0.8	0.4
34	1310	14.0	7.6	3.7	6.3	10.0	4.3	4.9	2.3	0.7	0.3