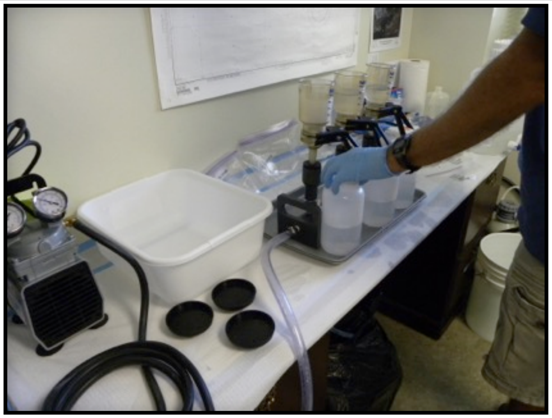

DETERMINING SOURCES AND HISTORY OF EUTROPHICATION ON NEARSHORE REEFS AT NAVAL STATION GUANTANAMO BAY, CUBA

PHASE II FINAL REPORT

Prepared for the U.S. Navy at Guantanamo Bay Naval Station, Cuba for fulfillment of Cooperative Agreement #W9126G-14-2-0028

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Executive Summary

Nutrient pollution from land-based sources is a primary threat to marine environments and impedes the sustainable use of its coastal resources. Of these, wastewater is a major and increasing source of nitrogen (N) and phosphorus (P) pollution. A common symptom of wastewater-driven eutrophication in tropical and subtropical coastal waters is increased concentrations of phytoplankton and the development of macroalgal blooms in coastal areas, including on coral reefs. In 2013, the first documented occurrence of the brown tide, caused by the pelagophyte *Aureoumbra lagunensis*, occurred in Guantanamo Bay, Cuba (*cf* 1). Because the coral reefs in and adjacent to Guantanamo Bay flourish in oligotrophic waters, they are susceptible to low-level increases in nutrient concentrations.

Previous monitoring of coral reefs at U.S. Naval Station Guantanamo Bay (or the Base) raised concerns that increasing macroalgal abundance could be linked to escalating land-based nutrient inputs. This monitoring included 2011 studies by Lapointe and Risk that demonstrated that nutrient inputs from the Base and upstream Cuban waters (Guantanamo River and Upper Guantanamo Bay) were impacting local reefs. Recognizing that it was important to establish temporal and spatial boundaries for the influence of the Base and river discharges, macroalgae and gorgonians were analyzed from a variety of locations within the Bay and along the coastal reefs. The results showed a strong correlation between reef health and proximity to the Guantanamo River plume, the upper portion of the Bay, and Base discharges. Because of the level of nutrient pollution identified in 2011, more extensive water quality monitoring was recommended to better understand the ongoing eutrophication at the Base (Lapointe and Herren, 2013). While the value of long-term records of nutrient entry were established in gorgonians that live in the relatively clean waters of the reefs, records in more ubiquitous species, such as bivalve shellfish, were also recommended to trace and document potential sources of the relatively recent stressors to the reefs in the nearshore waters of the Base.

In response to these recommendations, the U.S. Navy supported a two-part study aimed at: 1) capturing a more detailed assessment of the status of land-based nutrient inputs through a 12-station water quality monitoring network for chlorophyll *a*, dissolved nutrients (N and P), and stable isotopic analysis of carbon (C) and N sources (δ^{13}C, δ^{15}N) to phytoplankton 2) understanding historical changes in pollution sources through isotopic and trace element records encoded in the shells of long-lived clams. Ancient clams from remnant Pleistocene (~126,000 ± 5,000 y BP) reefs served as the baseline for pre-urbanized conditions because shell deposition occurred prior to human occupation, while modern shells from seven sites, coincidental with water quality monitoring stations, were used to typify the range of modern conditions.

Results suggest that high N inputs are conveyed to Guantanamo Bay through both Cuban activities (via the Guantanamo River and upper Bay) and U.S. activities along the Windward side

of the Base. This was especially true near Corinaso and Caravella Points, which are both adjacent to known wastewater outfalls (*cf* 2). High P inputs are also conveyed to Guantanamo Bay from Cuba via the Guantanamo River. The highest chlorophyll *a* concentrations were documented in Guantanamo Bay near the Cuba-USA boundary (Watergate and Port Palma) and, to a lesser extent, the upper water quality sampling station in the Guantanamo River. Nitrogen stable isotope analysis indicates wastewater contamination entering the Guantanamo Bay from upstream of the Base via the Guantanamo River and Upper Guantanamo Bay as well as from the developed Windward shoreline of the Base, adjacent to known wastewater outfalls and septic systems (*cf* 2). Trace element analyses additionally suggest there are multiple sources of contamination to the Guantanamo Bay system, only some of which are related to nutrient inputs, likely including industrial and human wastewater and aquaculture or agriculture activities.

Because the general condition of coastal reefs at the Base is still very good, continued monitoring of land-based nutrient and contaminant inputs in combination with plans for wastewater/stormwater management will help sustain these important resources. To begin to take steps needed to guide future management actions, however, it is important to conduct studies targeted to address the remaining fundamental unanswered question: what is the relative importance of inputs from the Base, as opposed to those from mainland Cuba? The results of this study provide essential data to inform these future studies.

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1.0 Introduction

Nutrient pollution from land-based sources is a primary threat to marine environments of the Caribbean region and impedes the sustainable use of its coastal resources (Windom, 1992; UNEP, 1994; Siung-Chang, 1997; MEA, 2005; UNEP, 2006). Sources of nutrient pollution include human wastewater, stormwater runoff, fertilizers, aquaculture activities, deforestation, soil loss, and atmospheric deposition (Lapointe *et al.*, 1990; NRC, 2000; Likens, 2001; Barile and Lapointe, 2005; Lapointe *et al.*, 2005; Lapointe and Bedford, 2011). Of these, wastewater is a major and increasing source of nitrogen (N) and phosphorus (P) pollution and contributes to eutrophication and public health threats along many tropical and subtropical coastlines (Caperon *et al.*, 1971; Windom, 1992; Siung-Chang, 1997, Wear and Vega Thurber, 2015). Wear and Vega Thurber (2015) show that wastewater pollution, commonly thought of as a single stressor, is in actuality a stressor with multiple agents including pathogens, endocrine disrupters, and heavy metals in addition to inorganic nutrients. A common symptom of wastewater-driven eutrophication in tropical and subtropical coastal waters is increased concentrations of phytoplankton (Caperon *et al.*, 1971; Laws and Redalje, 1979) and the development of macroalgal blooms in coral reef communities (Pastorok and Bilyard, 1985). Waters upstream and downstream of the Cuba-USA border in Guantanamo Bay, experienced the first documented prolonged brown tide, caused by the pelagophyte *Aureoumbra lagunensis* (Koch *et al.*, 2014). This algal bloom was similar to those documented in Laguna Madre along the Gulf coast of Texas and, more recently, in Florida's Indian River Lagoon (Lapointe *et al.*, 2015). Because the coral reefs along the Cuban coastline flourish in oligotrophic tropical and subtropical waters, they are susceptible to low-level increases in nutrient concentrations (Johannes, 1975; Bell, 1992; Dubinsky and Stambler, 1996; NRC, 2000).

Previous monitoring of coral reefs at U.S. Naval Station Guantanamo Bay (or Base) raised concerns that increasing macroalgal abundance could be linked to escalating land-based nutrient inputs. This monitoring included Phase I of this study, conducted in 2011, when macroalgal tissue samples from a network of stations were analyzed for C:N:P content (Lapointe and Herren, 2013), and macroalgae and skeletons of gorgonians were analyzed for carbon (C) and N stable isotope ratios (Lapointe and Herren, 2013; Risk *et al.*, 2014). Although previous studies did not address sources or historical patterns of nutrient inputs, the 2011 results from Phase I demonstrated that land-based nutrient inputs from the Base and Cuban waters upstream of the Base (Upper Guantanamo River and Upper Guantanamo Bay) contributed to the eutrophication on adjacent coastal reefs. The documented "bottom-up" effects of increasing macroalgae due to nutrient enrichment within Guantanamo Bay explains the appearance of brown tides within the Base, which cannot otherwise be explained by "top-down" changes in grazing by populations of herbivorous fishes or invertebrates (DoN, 2005).

The ecological effects of land-based nutrient enrichment documented during Phase I was based on multiple lines of evidence. In particular, spatial patterns and absolute values of stable N and C isotope ratios in macroalgae and gorgonians indicated wastewater influence even on coastal reefs that were previously considered "unaltered". The relatively high tissue C:N:P ratios for the coastal reef macroalgae demonstrated that these populations were nutrient-limited for both N and P, so that any increase in availability of these nutrients (especially P), such as from river discharges or wastewater inputs, would enhance macroalgal growth and biomass. The 2011 reef site most vulnerable to direct nutrient input (End-of-Runway) was directly impacted by the plume of the Guantanamo River and had little living coral. The stable N isotope values in macroalgae at adjacent reef sites (Phillips Shallow and Chapman) also confirmed enrichment from land-based sources; most likely discharges from the Guantanamo River at Phillips Shallow and wastewater at Chapman Beach. Based on the presence of discolored (elevated CDOM, chlorophyll *a*) coastal water at all reef sites relative to oligotrophic offshore Caribbean "blue water" during the sampling (Corredor *et al.*, 1984; Rajendran *et al.*, 1991), it was apparent that all reef sites were impacted to some extent by land-based runoff during the wet season sampling.

Urbanization and other land-use changes in the Guantanamo Bay watershed increase nutrients to the Bay as well as convey other contaminants such as trace metals (e.g., from wastewater or industrial discharge, agriculture, shrimp and fish ponds, mining activities). Like nutrients, some trace elements are naturally occurring in the marine environment and are essential to basic biological and physiological processes. For example, Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), and other trace elements are necessary for the growth of algae and are correlated with algal blooms (Fitzwater *et al.*, 1982; Sunda, 2006). Because all N transformations require trace metals (metalloenzymes), metal contamination may exacerbate eutrophication (Morrel and Price, 2003). In excess, these elements also can have other negative effects on habitat quality and physiology of organisms in receiving waters (Gupta and Gupta, 1998; Islam *et al.*, 2003; Yilmaz, 2006).

Trace element profiles are capable of revealing a remarkably detailed signature of contamination entering an area because each type of industry produces characteristic chemical fingerprints. Elements such as Cadmium (Cd), Cu, and Zn are broadly associated with urban industrial and human wastewater, along with Mercury (Hg), Mn, Nickel (Ni), and Pb (Helz *et al.*, 1975; Kawasaki *et al.*, 1998; Kassir *et al.*, 2012), while oil-derived pollution may be characterized by higher concentrations of Pb and Vanadium (V) (Al-Arfaj and Alam, 1993; Chiffoleau *et al.*, 2003; Carmichael *et al.*, 2012; Li *et al.*, 2013). Agricultural activities also have distinct signatures due to applications of pesticides and fungicides that contain Arsenic (As), Chromium (Cr), Cu, Pb, Hg and Zn (Navratil and Minarik, 2005; Risk *et al.*, 2010). Similarly, aquaculture feeds and husbandry, including Caribbean shrimp farms, are associated with higher concentrations of Cd, Cobalt (Co), Cr, Cu, Fe, and Zn (Prapaiwong and Boyd, 2014; Tang, 2015). Hence, quantifying the combinations of trace elements in a system can provide valuable information to define sources of contamination that mediate ecosystem health. "Fingerprinting" different combinations or ratios of trace elements has high potential for differentiating Base inputs from up-river (Cuban) inputs. The first peer-reviewed publication from this work (Risk *et al.*, 2014) emphasized the role of the Guantanamo River as a source of pollution, and suggested that this has increased through time.

Knowledge of the unique combinations of trace elements can be combined with organic stable isotope ratios to better identify and trace pollution sources from land to the Guantanamo Bay system. Previous work with gorgonians on the reefs near the Base demonstrated the value of using organic stable isotope signatures as records of contamination and to distinguish material carried in by rivers (Risk *et al.*, 2014). While the spatial distribution of gorgonians is limited, more ubiquitous (and often better preserved) bivalve shellfish, such as clams, provide records of water quality spanning decades, centuries (Risk *et al.*, 2009), and even millennia (Darrow, 2015; Oczkowski *et al.,* in press). Bivalve shell, unlike soft tissues, is not actively remobilized (Putten *et al.*, 2000). Hence, bivalves record longer-term environmental conditions in the organic and inorganic matter deposited in shell during growth, making them excellent sentinels of nutrient and other types of anthropogenic pollution. Stable isotope ratios in bivalve shell reliably trace land-derived C and anthropogenic N such as from human wastewater into coastal embayments (Carmichael *et al.*, 2008; Kovacs *et al.*, 2010; Darrow, 2015). A similar approach has been used to trace human influence and watershed changes through time using trace element signatures (Carroll *et al.*, 2009; Risk *et al.*, 2010). A major advantage of using bivalves is that stratigraphic control is typically superb, with growth increments corresponding to years, months, and even days. With modern laser-ablation methods, most of the elements in the periodic table can be determined with a precision of a few ppm or better. Because bivalves assimilate environmental elements largely from their food (typically phytoplankton and other suspended particulate organic matter), the combination of these elemental analyses can help decipher the sources of contamination to a system and determine if some elements may be working with nutrients to fuel phytoplankton growth and subsequent eutrophication.

Considering the level of land-based nutrient pollution identified during Phase I, Lapointe and Risk suggested more extensive monitoring of the ongoing eutrophication problem at the Base. Because of the overriding importance of nutrient inputs from the Guantanamo River and the recent brown tide (Fig. 1) in Upper and Lower Guantanamo Bay, it was suggested that the Navy consider a two-part project for Phase II. Part 1 included monthly monitoring for chlorophyll *a* (Chl *a*), dissolved nutrients (N and P), and C and N sources through stable isotope analysis (δ^{13}C, δ^{15}N) of phytoplankton to quantify spatial and temporal patterns of nutrients within the Base (including areas near the Cuba-USA border and nearby coastal reefs). Part 2 focused on stable isotope ratios and trace metals in clams to better understand the spatial and temporal patterns of nutrient inputs to the Bay and begin to characterize sources of pollution. It was also suggested that the water quality parameters (nutrients, isotopes, trace metals) identified for Parts 1 and 2 be quantified at known nutrient sources, including wastewater treatment plants, septic tank-to-discharge systems, and stormwater outfalls to allow characterization of major storm events (e.g., hurricanes, worst case scenario) and nutrient sources. The goal of Phase II was to provide data useful to estimate and distinguish the effects of nutrient loads from the Guantanamo River and Upper Guantanamo Bay relative to nutrient inputs from the Base.

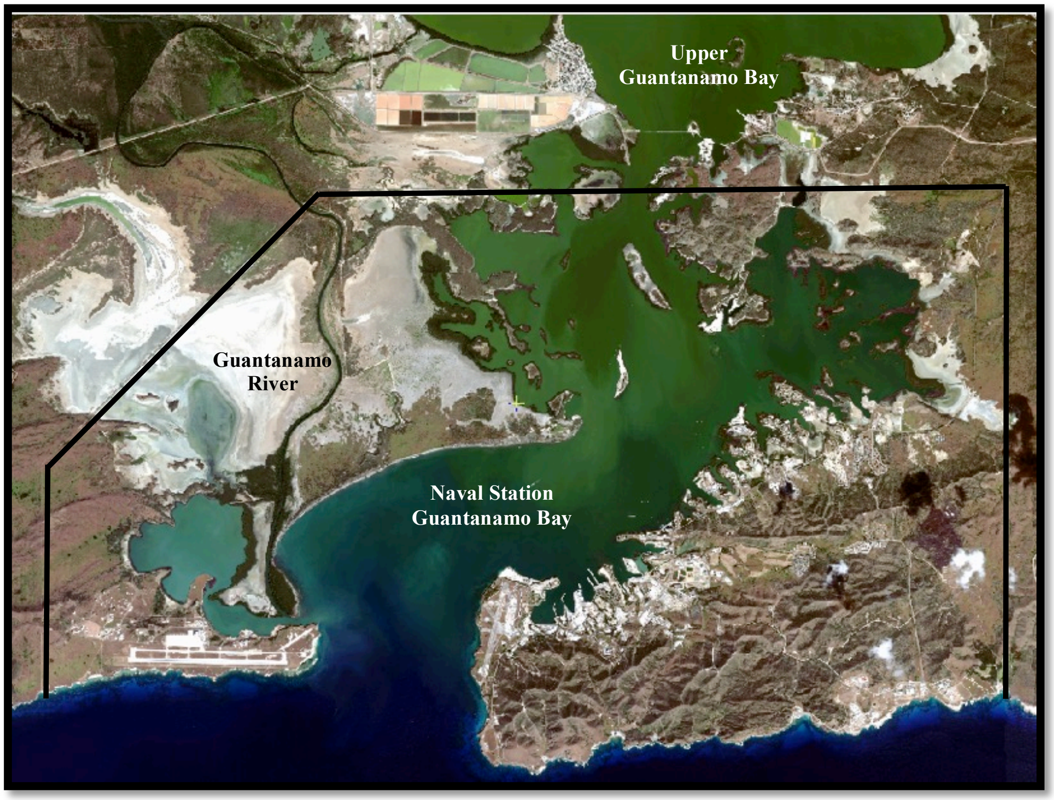


Fig. 1. Harmful algal bloom (brown tide) in Upper and NS Guantanamo Bay. This image was taken in January 2013. Note the green coloration of the algae bloom.

2.0 Materials and Method

2.1 Study sites and sampling rationale – Twelve water quality monitoring stations (Fig. 2, Table 1) and eight bivalve collection sites (Fig. 3, Table 2) were established in NS Guantanamo Bay and along the adjacent Caribbean Sea coastline in 2014. These sampling stations were positioned to capture potential nutrient and contaminant inputs from hypothesized sources, including Mahomilla Bay, Guantanamo River, NS Guantanamo Bay, urbanized areas on the Base, and the Caribbean Sea. Initial water sampling and all bivalve collection took place 24-26 September 2014. With the exception of December 2014 and January 2015, Navy personnel collected monthly water samples at the 12 fixed stations between September 2014 and August 2015. As part of an ongoing precipitation monitoring program, daily rainfall data for the Base were also provided by the U.S. Navy to help delineate between the Wet and Dry seasons during the study. Water quality and phytoplankton stable isotope data are presented by: 1) rain period including Wet (September-November 2014 and February 2015) and the Dry (March – August 2015), 2) season including Fall (September – November 2014), Winter (February-March 2015), Spring (April-May 2015), and Summer (June-August 2015), and 3) individual station.

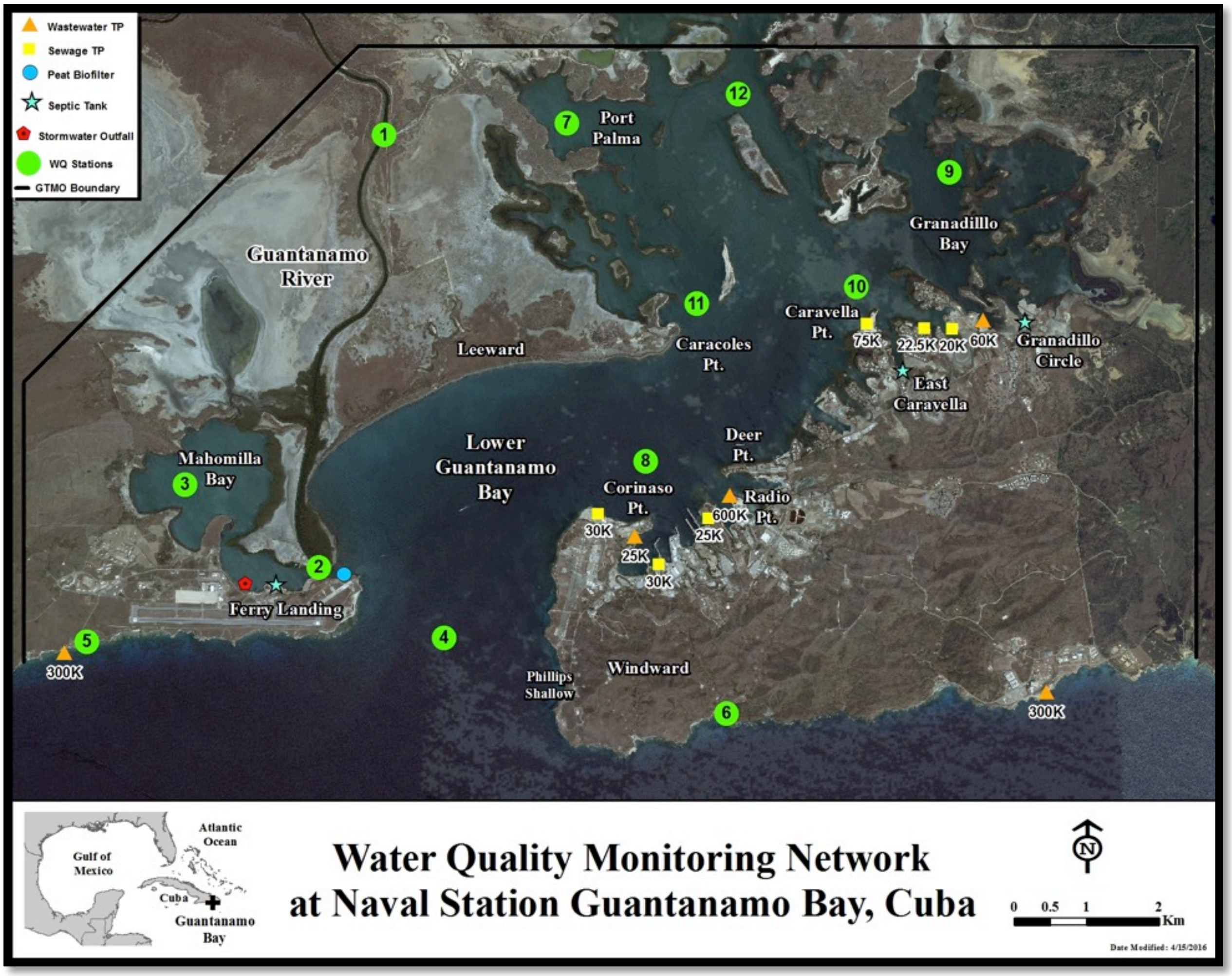


Fig. 2. Dissolved nutrient monitoring network established at U.S. Naval Station Guantanamo Bay, Cuba in September 2014. Outfalls are broken down by category (TP= Treatment Plant) and include the mean gallons per day of discharge into the system.

Table 1. Location of dissolved nutrient monitoring stations established at U.S. Naval Station Guantanamo Bay, Cuba in September 2014. Phase II water quality stations that best align with Phase I macroalgae collection sites are embolden.

Region	Station	Geographic Description	Latitude	Longitude
Guantanamo River	1	Upstream River	19.96367	-75.18738
	2	**River Mouth**	19.91296	-75.19553
Mahomilla Bay	**3**	**Mahomilla Bay**	19.92266	-75.21216
Bay Mouth	4	Bay Mouth	19.90469	-75.17997
Nearshore Reefs	**5**	**Chapman Beach**	19.90422	-75.22434
	6	**Cuzco Beach**	19.89581	-75.14486
Guantanamo Bay	7	Port Palma	19.96508	-75.16466
	8	Corinaso Point	19.92540	-75.15490
	9	Granadillo Bay	19.95934	-75.11716
	10	Caravella Point	19.94587	-75.12877
	11	Caracoles Point	19.94387	-75.14856
	12	Watergate	19.96851	-75.14345

For bivalve collection, the widest possible variety of clam species of a range of sizes was collected at each of the eight sites shown in Table 2 (Fig. 3), including ancient shell from two remnant coral reef outcroppings on the Base and live animals and whole shell remains from waters at the other sites. A total of two species (n = 2 to 11, depending on species) were collected from the two ancient reefs (Fig. 3, OR). Of these, the most abundant (hard clam, *Periglypta listeria*; Portell *et al.* 2008) was selected for analysis to represent pre-historical and pre-urbanization time periods (Fig. 4, left). A total of eight species (n = 3 to 26 individuals per site, depending on availability) were collected among the other sites to represent the range of modern conditions. From these species, the Caribbean thick lucine (*Lucina pectinata* aka *Phacoides pectinatus*) was chosen for analysis because of its relative similarity to the ancient hard clam and because this species was the only bivalve found at all of the sites to allow uniform comparison of shell composition among sites (Fig. 4, right). At the Ferry Landing site (Table 2) the cockle *Anadara notabilis* was also analyzed due to low sample number and isotopic similarity to *L. pectinata*.

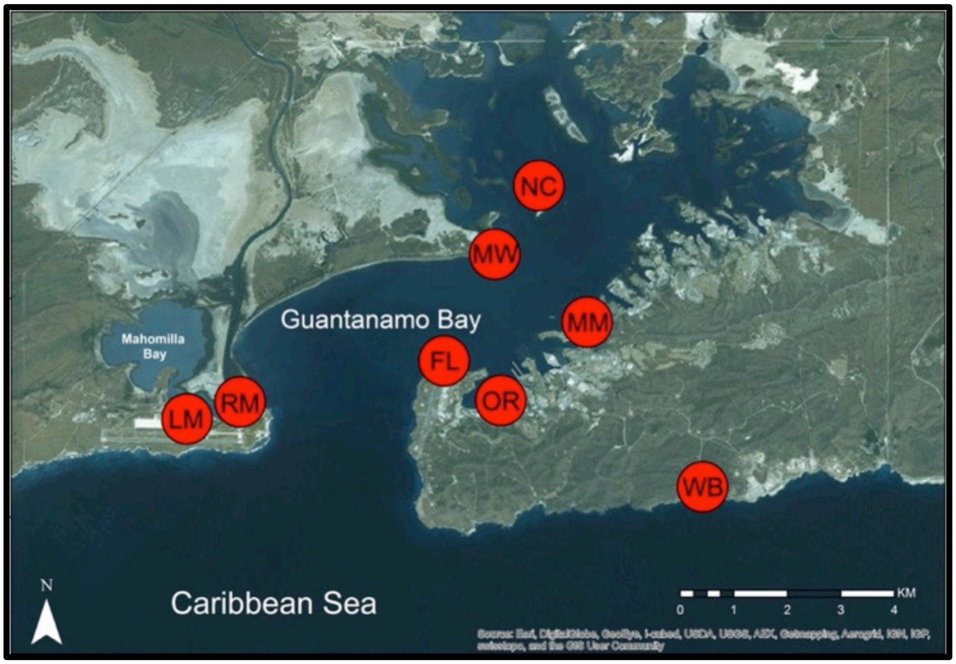


Fig. 3. Location of bivalve collection sites at NS Guantanamo Bay, Cuba, corresponding to Table 2. (Google Earth sources; ESRI, DeLorme, NAVTEQ, TomTom, Intermap, increment P Corp, GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, Kadaster NL, Ordnance Survey, Esri Japan, METI, and the GIS User Community).

Table 2. Bivalve collection sites at the U.S. Naval Station Guantanamo Bay, Cuba, including coastal waters of the Caribbean Sea, NS Guantanamo Bay, and remnant ancient coral reefs. Urbanized sites in NS Guantanamo Bay include locations adjacent to human activity associated with the Base (sites FL, MM, LM; *cf* Fig. 2) and receiving putative inputs from mainland Cuba via the Guantanamo River (RM, NC). Reference sites include ocean waters (WB) and an ancient reef site (OR) that represents 2 separate reef outcrops at this general location. Phase II bivalve collection sites that best align with Phase I macroalgae collection sites are shown in bold.

Region	Site	Site Name	Latitude	Longitude
Coastal (Caribbean Sea)	**WB**	**Windmill Beach**	19.89883	-75.11790
Guantanamo Bay				
Open water	NC	North Medio Cay	19.94946	-75.14542
	MW	**Main Bay West (Caracoles Pt.)**[1]	19.93789	-75.15285
Urbanized shoreline	**MM**	**MWR Marina**[2]	19.92649	-75.13729
	RM	**River Mouth**	19.91306	-75.19567
	FL	**Windward Ferry Landing**	19.91119	-75.20560
Tidal lake	**LM**	**Mahomilla Bay (Leeward FL)**[3]	19.91022	-75.20459
Ancient reef	OR	Naval base reefs	19.91333	-75.15181

[1]Main Bay West corresponds to Caracoles Point, [2]MWR Marina is located between Corinaso and Caravella Points, and [3]Mahomilla Bay corresponds to the Leeward Ferry Landing Phase I and Phase II water quality stations.

Fig. 4. Ancient *Periglypta listeri* (left) and modern *Phacoides* sp. (right) bivalves sampled at the U.S. Naval Station Guantanamo Bay, Cuba

2.2 Dissolved nutrients and chlorophyll a analysis – MONTHLY water samples were collected in triplicate (n=3) at the 12 fixed monitoring stations into clean 250 mL HDPE bottles and placed on ice until return to the wet laboratory (established September 2014 in the Public Works Building). Field measurements of salinity, conductivity, water temperature, and dissolved oxygen were collected *in situ* with an YSI85 field meter and water transparency was measured using a Secchi disk (Yentsch *et al*., 2002). Water transparency was not measured at Stations 5 and 6 (nearshore reefs) because of the shallowness of these sites. These sites were within close proximity to the beach and the Secchi disk was visible while resting on the bottom and therefore not a good indication of transparency. In the Base laboratory, the water samples were filtered through 0.7 µm GF/F filters into clean 125 mL HDPE bottles and frozen until analysis at the University of Georgia's Center for Applied Isotope Studies (UGA-CAIS). The associated 25 mm GF/F filters were also frozen for chlorophyll *a* analysis. At UGA-CAIS, samples were thawed, homogenized, and subsampled for either persulfate digestion (TDN/TDP) or direct analyses (NOx, NH4, and SRP). For TDN/TDP persulfate digestion, a 5 mL subsample was digested with 1 mL persulfate reagent and autoclaved until all N was oxidized to nitrate and all P was oxidized to orthophosphate. Once digested, all nutrient forms (TDN, TDP, NOx, NH4, and SRP) in the samples were analyzed on an Alpkem 300 series nutrient autoanalyzer using EPA standard methods (4500-NH_3 G, 4500-NO_3^- F, and 4500-P F). For chlorophyll *a* analysis, GF/F filters were frozen until extracted. To extract, the filters were placed in a 15 mL centrifuge tube with 10 mL of 96% ethanol, and allowed to extract for 24 h in the dark under refrigeration. After extraction, the samples were removed from refrigeration, warmed to room temperature and particulates settled by gravity. The samples were measured fluorometrically for active chlorophyll a with a Turner Designs TD700 fluorometer equipped with a blue lamp, 436 nm excitation filter and 680 nm emission filter. After project completion, rainfall data recorded on the Base were analyzed to accurate divide the results into Wet and Dry seasons.

2.3 Stable isotopes and C:N ratios in phytoplankton – One liter bottles of surface water were collected MONTHLY in triplicate (n=3) along with the dissolved nutrient and chlorophyll *a* samples at the 12 fixed water quality monitoring stations, placed on ice, and returned to the Base laboratory. In the wet laboratory, the samples were filtered through 47 mm GF/F filters to capture the phytoplankton and sent to the UGA-CAIS for stable C ($\delta^{13}C$) and N ($\delta^{15}N$) isotopes and C:N analysis. At UGA-SIEL, the "phytoplankton filters" were frozen and freeze-dried. The filter was then analyzed for stable C and N isotopes and total µg C and N on a Thermo Delta V Isotope Ratio Mass Spectrometer (IRMS) coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo Conflo III Interface. While the resulting data are referred to as "phytoplankton," the values also reflect contributions from other forms of suspended particulate matter in the water column. Regardless, the resulting stable isotope and C:N data are used to identify nutrient sources and characterize temporal and spatial variation in phytoplankton nutrient status, which allow for inferences regarding nutrient availability in relation to various natural and anthropogenic nutrient sources and climate-related phenomena.

2.4 Bivalve sectioning and aging - Each shell was thoroughly cleaned to remove dirt, and in the case of live animals, soft tissues were removed and subsequently archived. Shells were then allowed to air dry. Each shell was sectioned, photographed, and macroscopic growth lines were used to determine age. Internal macroscopic growth lines were revealed by radially sectioning each valve using a Buehler ISOMET low-speed saw with a diamond wafer blade. The shells were cut straddling the umbo to create a 4 mm wide strip (thick section) of shell (Fig. 5; Carmichael *et al.,* 2004). Sections were polished as needed with a Beuhler Beta Grinder Polisher 600 grit silicon carbide and cleaned again after polishing. Growth layers were counted and measured blind to collection site by at least two independent researchers. A Zeiss Stereodiscovery V12 stereomicroscope, linked to a 1.4 megapixel Axiocam with Axiovision software and a Z-stack module, was used to produce high-resolution images for analysis.

Fig. 5. Ancient (left) and modern (right) bivalves sectioned for aging and elemental analyses.

Growth rates were determined by age-at-length relationships for modern clams and by age-at-height (or thickness) for ancient clams. Growth rates were defined as the slope of the best-fit line to these relationships. Regression analyses were performed in StatPlus:Mac LE v.5

2.5 C and N stable isotope ratios in bivalve shell –The thick section of one valve was ground into a powder using a Dremel rotary grinding tool equipped with a diamond sanding drum. Work was performed under a Misonix workstation, and the powder was collected into an acid washed glass dish. To remove carbonate that interferes with organic stable isotope determination, shells were decalcified by incubation in dilute acid using methods established by Carmichael (Carmichael *et al.,* 2008; Kovacs *et al.,* 2010; Darrow, 2015).

For modern bivalves, 750 mg of homogenized shell powder was treated with 1mL of a 0.5% $PtCl_2$ solution in a 1N HCl and vortexed to mix. This treatment was repeated 4 times to a total volume of 4 mL, with the solution allowed to react with the sample (indicated by bubbling) between additions. For Midden shells, 2 g of shell powder was used, and the acid was added in increments of 3 mL for a total volume of 9 mL, with gentle vortexing between acid additions. Samples were centrifuged at 5000 rpm for 5 minutes, and the supernatant was discarded without disturbing the pellet. The process was repeated and after the second centrifugation, the resulting organic pellet was gently rinsed with ultrapure water, lightly vortexed, and centrifuged to remove acid residue. Water was decanted, and the remaining pellet was dried at 40°C to a constant weight. To determine whether acidification affected stable isotope ratios and test whether organic content of *Phacoides* sp. may be sufficient to avoid acidification, a subset of shells from each site was analyzed as raw (unacidified) powder. Values were compared among samples for quality control. Samples were analyzed at the University of California at Davis Stable Isotope Facility by continuous flow isotope ratio mass spectrometry (IRMS; 20–20 mass spectrometer, PDZ Europa) after sample combustion to CO_2 and N_2 in an online elemental analyzer (PDZ Europa). Gases were separated on a Carbosieve G column (Supelco) before introduction to the IRMS. Analytical precision was 0.2 ‰ for N.

2.6 Trace elements in bivalve shell – The thick section of one valve was used for trace element analysis by laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS; ESI NWR213 coupled to an Agilent 7700). To determine elemental concentrations in shell, two horizontal transects 1-2 mm apart were sampled in each section, with the external surface facing up and running perpendicular to the lines of growth to capture elemental variation throughout life (this approach will enable future analyses to assess ontogenetic or temporally explicit environmental change and interannual contaminant variation without the need for resamping). A total of 18 trace elements (As, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Sn, Sr, Ti, V, Zn) were targeted to identify potential contaminant signatures associated with the different hypothesized sources and environmental variation (*cf* Table 7 on page 42).

Helium flow rate of 1 L min^{-1} carried ablated material to the ICP, followed by 0.56 L min^{-1} of Ar. A 40 μm laser beam of 5 mm depth was used at a scan speed of 10 μm s^{-1}. Laser energy was 45% (~3 J cm^{-2}), with a laser repetition rate of 10 Hz. Each sample was pre-ablated with an 80 mm beam at 30 µm s^{-1} to clean the sample surface. NIST SRM612 glass and MACS-3, which has matrix similarity to bivalve shell, were used as standards. The MACS-3 standard was referenced before first and after last sample ablation and approximately every hour during sample collection, which corresponded to following each ancient shell ablated or every two modern shells ablated.

Data were exported as counts per unit time for sample and reference materials. Background (10-20 seconds of gas blank) data, collected before ablation of standards or samples, were averaged and subtracted from mean counts for each element in each sample. After background subtraction, negative values (very close to zero) were equated to a value of zero. Calcium (Ca^{44}) was used as an internal reference. Counts were then converted to concentrations using a one point calibration to the mean of all transects (variation ≤ 2%) of MACS-3 reference material. One-way analyses of variance (ANOVA) followed by a Tukey's post-hoc test was used (PRISM 6) to compare the mean (± S.E.) concentration of each element from each shell transect among sampling sites.

To begin to identify sources of nutrient and metal pollution to the Guantanamo Bay system, we compared concentrations of source-specific combinations of trace elements among sites and identified elements that were present in the Guantanamo Bay system in excess of naturally occurring sources. To do this, we divided the concentration of each element at the test sites by the concentration of the same elements in the estimated background samples (in this case the mean concentration in shells from the ancient reefs and Windmill Beach, which represent pre-urbanization and modern open ocean water signatures that should not be significantly affected by human activities). Assuming these samples represent background or natural signatures, then any values above 1 should reflect trace element concentrations above background likely due to human activities in the water or on the adjacent watershed. One-way analyses of variance (ANOVA) followed by a Tukey's post-hoc test was used (PRISM 6) to compare the mean (± S.E.) concentrations above background for each element among sampling sites.

3.0 Results

3.1 Rainfall – The rainfall data recorded at U.S. Naval Station Guantanamo Bay, Cuba in 2014 and 2015 support the notion that 2015 was an exceptionally dry El Niño year (Fig. 6). The Wet season included the first four sampling events (September - November 2014 and February 2015) and the Dry season included the last six consecutive sampling events (March – August 2015; Fig. 6). No major storms impacted the Base during the study. However, increased rainfall associated with Hurricane Cristobal was recorded weeks prior to the first sampling event (August 2014) and Tropical Storm Erika hit immediately following the last sampling event in August 2015. Hurricane Joaquin impacted this area two months (October 2015) following the last collection.

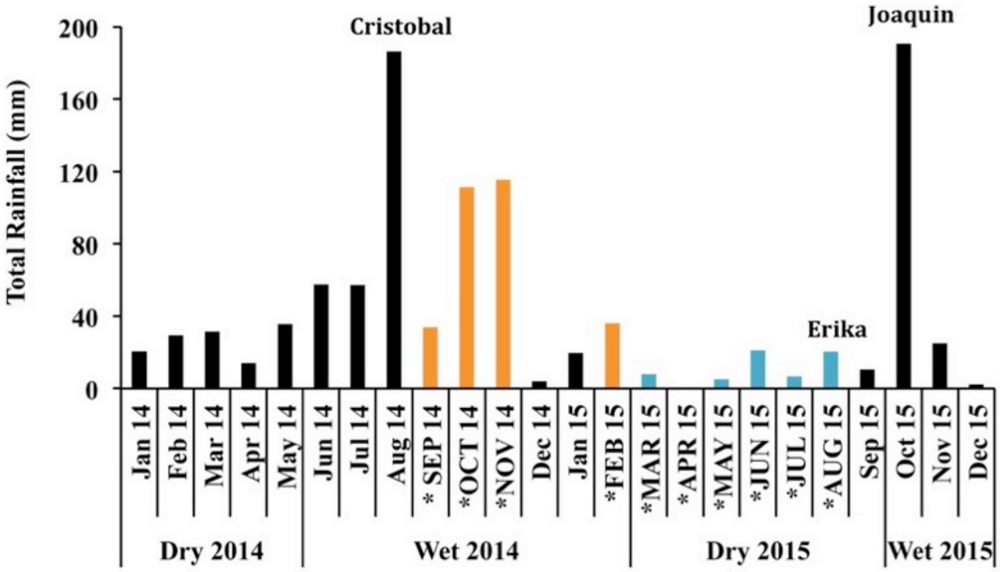


Fig. 6. Monthly total rainfall (mm) throughout 2014 and 2015. Ten sampling events were conducted during months denoted by an asterisk. Hurricane Cristobal, Tropical Storm Erika, and Hurricane Joaquin were the largest storms to pass near Cuba these two years. Bars are colored orange (Wet season) and blue (Dry season) to denote the different rain period sampling events. No samples were collected in December 2014 or January 2015.

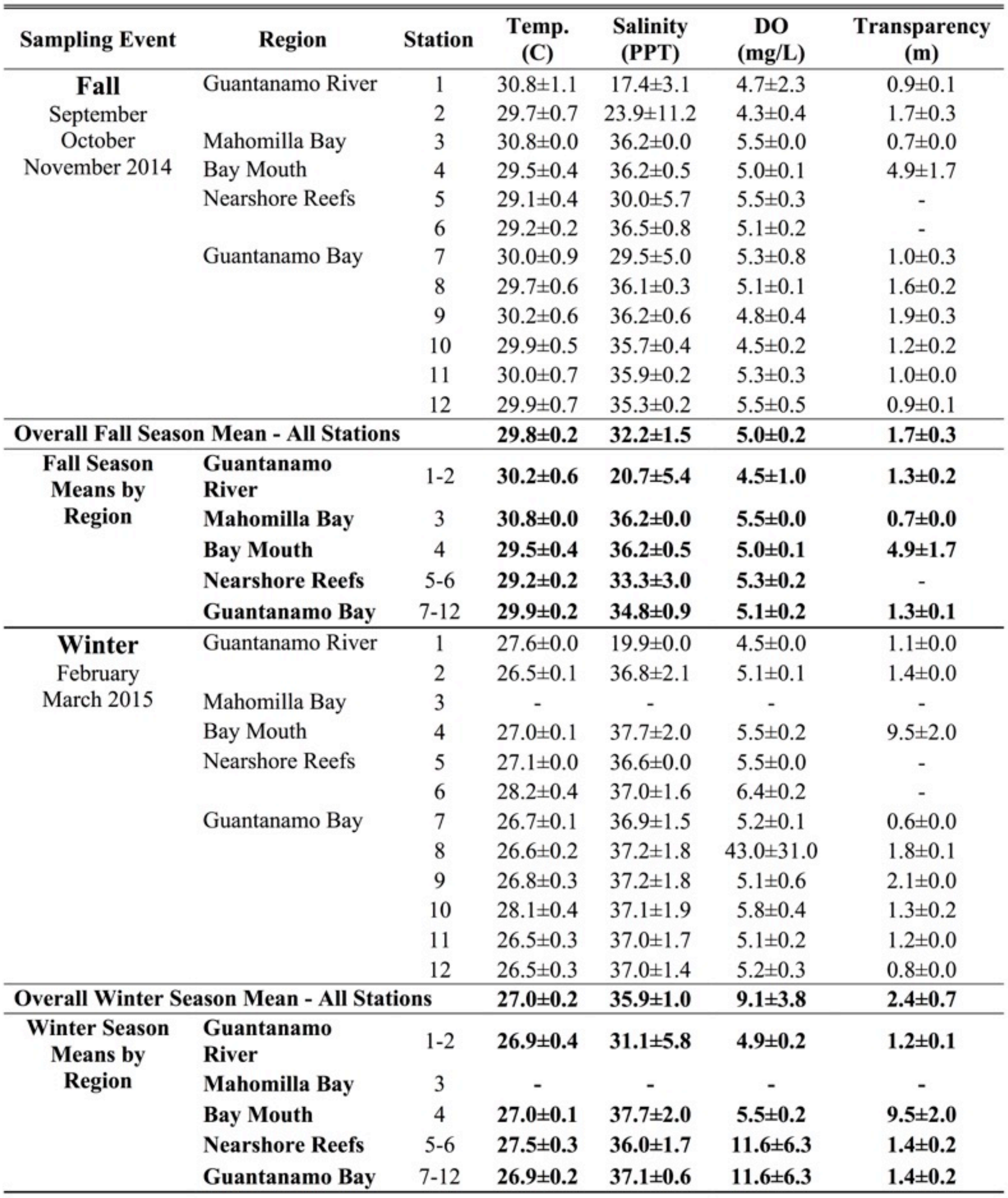
3.2 Environmental data – Environmental measurements for temperature, salinity, dissolved oxygen (DO), and transparency varied by location and sampling event (Table 3A-C). The project-wide mean (± S.E.) temperature was 29.4 ± 0.1°C with the highest overall mean temperatures recorded in Mahomilla Bay (30.5 ± 0.7°C) and the upstream station in the Guantanamo River (Station 1; 30.5 ± 0.6°C; Fig. 7a, Table 3C). The overall mean temperatures were highest during the Summer (30.5 ± 0.1°C) and lowest during the Winter (27.0 ± 0.2°C). Overall, the temperature varied little between the Wet (29.2 ± 0.2°C) and the Dry (29.5 ± 0.2°C) seasons.

Project-wide, the mean (± S.E.) salinity was 34.3± 0.6 ppt with the highest overall mean salinities recorded in Mahomilla and Guantanamo Bays (both ~36.5 ppt) followed by the Bay Mouth (~36 ppt), the nearshore reefs (~34.5 ppt), and the Guantanamo River (~26 ppt; Table 3C). Overall, the salinity was significantly higher during the Dry season (35.2 ± 0.6 ppt) than the Wet season (32.8 ± 1.2 ppt); further confirming an accurate Wet and Dry season delineation (Fig. 7b). Seasonally, the salinity was highest during the Winter (35.9 ± 1.0 ppt) when salinities ≥ 37 ppt were widespread and lowest during the Fall (32.2 ± 1.5 ppt) when salinities were generally ≤ 36 ppt (Table 3A,B). Caracoles Point (Station 11) had the highest mean salinity (37.3 ± 0.6 ppt) over the course of the study followed by Ganadillo Bay (Station 9; 36.9 ± 0.6 ppt), Watergate (Station 12; 36.7 ± 0.6 ppt), and Mahomilla Bay (Station 3; 36.5 ± 1.3 ppt; Table 3C).

The overall mean (± S.E.) dissolved oxygen (DO) concentration was 4.7 ± 0.1 mg/L, with the highest concentrations along the nearshore reefs (Stations 5-6), Bay Mouth (Station 4), and Guantanamo Bay (Stations 7-12; all ~5 mg/L), followed by the Guantanamo River (Stations 1-2; ~4 mg/L) and Mahomilla Bay (Station 3; ~3 mg/L; Table 3C). Overall, DO concentrations were higher during the Wet (5.1 ± 0.2 mg/L) than the Dry (4.5 ± 0.1 mg/L) season (Fig. 7c). Seasonally, the mean DO concentrations were highest in Winter (5.3 ± 0.1 mg/L) when concentrations ≥ 5 mg/L were documented project-wide and lowest in Summer (4.1 ± 0.2 mg/L) when concentrations ranged between 2 and 5 mg/L (Table 3A,B). When comparing individual sites, the two nearshore reef sites and Bay Mouth had the highest overall DO concentrations and Mahomilla Bay had the lowest (Table 3C).

Water transparency varied by region (Fig. 7d). The overall project mean (± S.E.) was 1.8 ± 0.2 m with the highest transparency in the Bay Mouth (~6.5 m), followed by Guantanamo Bay and the Guantanamo River (both ~1.5 m), and Mahomilla Bay (~0.6 m; Table 3C). With the exceptions of the higher transparency documented at Stations 7 (Port Palma) and 8 (Corinaso Point) during the Dry season, there was little difference in the transparency between the Wet (1.9 ± 0.4 m) and Dry (2.0 ± 0.3 m) seasons (Fig. 7d). The highest transparency was seen in Winter (2.4 ± 0.7 m) and the lowest in Spring (1.6 ± 0.4 m; Tables 3A,B).

Table 3A. A comparison of mean (± S.E.) sea surface temperature, salinity, dissolved oxygen (DO), and transparency throughout NS Guantanamo Bay and along nearshore reefs recorded during three Fall and two Winter season sampling events. Data are summarized by fixed station and by geographic region. No transparency data were collected at Stations 5 and 6.



Sampling Event	Region	Station	Temp. (C)	Salinity (PPT)	DO (mg/L)	Transparency (m)
Fall September October November 2014	Guantanamo River	1	30.8±1.1	17.4±3.1	4.7±2.3	0.9±0.1
		2	29.7±0.7	23.9±11.2	4.3±0.4	1.7±0.3
	Mahomilla Bay	3	30.8±0.0	36.2±0.0	5.5±0.0	0.7±0.0
	Bay Mouth	4	29.5±0.4	36.2±0.5	5.0±0.1	4.9±1.7
	Nearshore Reefs	5	29.1±0.4	30.0±5.7	5.5±0.3	-
		6	29.2±0.2	36.5±0.8	5.1±0.2	-
	Guantanamo Bay	7	30.0±0.9	29.5±5.0	5.3±0.8	1.0±0.3
		8	29.7±0.6	36.1±0.3	5.1±0.1	1.6±0.2
		9	30.2±0.6	36.2±0.6	4.8±0.4	1.9±0.3
		10	29.9±0.5	35.7±0.4	4.5±0.2	1.2±0.2
		11	30.0±0.7	35.9±0.2	5.3±0.3	1.0±0.0
		12	29.9±0.7	35.3±0.2	5.5±0.5	0.9±0.1
Overall Fall Season Mean - All Stations			**29.8±0.2**	**32.2±1.5**	**5.0±0.2**	**1.7±0.3**
Fall Season Means by Region	**Guantanamo River**	1-2	**30.2±0.6**	**20.7±5.4**	**4.5±1.0**	**1.3±0.2**
	Mahomilla Bay	3	**30.8±0.0**	**36.2±0.0**	**5.5±0.0**	**0.7±0.0**
	Bay Mouth	4	**29.5±0.4**	**36.2±0.5**	**5.0±0.1**	**4.9±1.7**
	Nearshore Reefs	5-6	**29.2±0.2**	**33.3±3.0**	**5.3±0.2**	-
	Guantanamo Bay	7-12	**29.9±0.2**	**34.8±0.9**	**5.1±0.2**	**1.3±0.1**
Winter February March 2015	Guantanamo River	1	27.6±0.0	19.9±0.0	4.5±0.0	1.1±0.0
		2	26.5±0.1	36.8±2.1	5.1±0.1	1.4±0.0
	Mahomilla Bay	3	-	-	-	-
	Bay Mouth	4	27.0±0.1	37.7±2.0	5.5±0.2	9.5±2.0
	Nearshore Reefs	5	27.1±0.0	36.6±0.0	5.5±0.0	-
		6	28.2±0.4	37.0±1.6	6.4±0.2	-
	Guantanamo Bay	7	26.7±0.1	36.9±1.5	5.2±0.1	0.6±0.0
		8	26.6±0.2	37.2±1.8	43.0±31.0	1.8±0.1
		9	26.8±0.3	37.2±1.8	5.1±0.6	2.1±0.0
		10	28.1±0.4	37.1±1.9	5.8±0.4	1.3±0.2
		11	26.5±0.3	37.0±1.7	5.1±0.2	1.2±0.0
		12	26.5±0.3	37.0±1.4	5.2±0.3	0.8±0.0
Overall Winter Season Mean - All Stations			**27.0±0.2**	**35.9±1.0**	**9.1±3.8**	**2.4±0.7**
Winter Season Means by Region	**Guantanamo River**	1-2	**26.9±0.4**	**31.1±5.8**	**4.9±0.2**	**1.2±0.1**
	Mahomilla Bay	3	**-**	**-**	**-**	**-**
	Bay Mouth	4	**27.0±0.1**	**37.7±2.0**	**5.5±0.2**	**9.5±2.0**
	Nearshore Reefs	5-6	**27.5±0.3**	**36.0±1.7**	**11.6±6.3**	**1.4±0.2**
	Guantanamo Bay	7-12	**26.9±0.2**	**37.1±0.6**	**11.6±6.3**	**1.4±0.2**

Table 3B. A comparison of mean (± S.E.) sea surface temperature, salinity, dissolved oxygen (DO), and transparency throughout NS Guantanamo Bay and along nearshore reefs recorded during two Spring and three Summer season sampling events. Data are summarized by fixed station and by geographic region. No transparency data were collected at Stations 5 and 6.

Sampling Event	Region	Station	Temp. (C)	Salinity (PPT)	DO (mg/L)	Transparency (m)
Spring April May 2015	Guantanamo River	1	29.8±0.1	19.3±6.2	4.7±0.0	1.2±0.1
		2	29.2±0.5	31.5±2.8	3.7±0.3	1.5±0.3
	Mahomilla Bay	3	28.1±0.0	39.0±0.0	4.2±0.0	0.6±0.0
	Bay Mouth	4	28.5±0.1	34.8±0.0	5.6±0.4	7.0±0.0
	Nearshore Reefs	5	27.5±0.4	37.6±0.5	5.2±0.2	-
		6	29.1±1.8	35.2±0.4	5.7±0.6	-
	Guantanamo Bay	7	30.3±0.3	37.2±0.9	5.2±0.8	0.5±0.0
		8	29.0±0.1	35.0±0.1	4.8±0.2	2.5±0.0
		9	30.1±0.3	37.3±1.1	4.6±0.4	1.7±0.1
		10	8.7±0.9	36.6±0.2	4.0±0.2	1.4±0.3
		11	29.4±0.1	36.8±1.2	4.6±1.6	0.9±0.1
		12	29.4±0.3	36.7±0.8	5.0±0.6	0.7±0.2
Overall Spring Season Mean - All Stations			**29.1±0.2**	**34.5±1.2**	**4.8±0.2**	**1.6±0.4**
Spring Season Means by Region	**Guantanamo River**	1-2	**29.5±0.3**	**25.4±4.5**	**4.2±0.3**	**1.3±0.2**
	Mahomilla Bay	3	**28.1±0.0**	**39.0±0.0**	**4.2±0.0**	**0.6±0.0**
	Bay Mouth	4	**28.5±0.1**	**34.8±0.0**	**5.6±0.4**	**7.0±0.0**
	Nearshore Reefs	5-6	**28.3±1.0**	**36.4±0.8**	**5.4±0.4**	**-**
	Guantanamo Bay	7-12	**29.5±0.2**	**36.6±0.4**	**4.7±0.2**	**1.3±0.2**
Summer June July August 2015	Guantanamo River	1	31.6±0.9	22.1±2.2	3.5±0.6	1.0±0.1
		2	30.3±0.3	34.9±1.6	4.0±0.2	2.1±0.3
	Mahomilla Bay	3	31.1±0.6	35.7±2.0	2.0±0.2	0.6±0.2
	Bay Mouth	4	30.0±0.3	35.8±2.0	4.6±0.1	4.0±0.0
	Nearshore Reefs	5	30.0±0.4	34.3±1.9	5.1±0.3	-
		6	29.7±0.3	35.0±2.2	4.6±0.4	-
	Guantanamo Bay	7	30.8±0.5	38.2±1.0	3.8±0.4	0.6±0.1
		8	30.2±0.3	35.8±2.0	4.9±0.0	5.1±0.7
		9	30.7±0.4	37.1±1.4	4.7±0.2	2.4±0.4
		10	30.7±0.4	36.3±1.3	3.8±0.6	2.1±0.4
		11	30.2±0.3	39.3±0.3	4.4±0.4	1.5±0.3
		12	30.4±0.4	37.8±1.3	3.5±0.6	0.8±0.2
Overall Summer Season Mean - All Stations			**30.5±0.1**	**35.2±0.8**	**4.1±0.2**	**2.2±0.4**
Summer Season Means by Region	**Guantanamo River**	1-2	**30.9±0.5**	**28.5±3.1**	**3.7±0.3**	**1.6±0.3**
	Mahomilla Bay	3	**31.1±0.6**	**35.7±2.0**	**2.0±0.2**	**0.6±0.2**
	Bay Mouth	4	**30.0±0.3**	**35.8±2.0**	**4.6±0.1**	**4.0±0.0**
	Nearshore Reefs	5-6	**29.9±0.2**	**34.7±1.3**	**4.8±0.3**	**-**
	Guantanamo Bay	7-12	**30.5±0.2**	**37.4±0.5**	**4.2±0.2**	**2.1±0.4**

Table 3C. A comparison of comprehensive, project-wide means (± S.E.) for sea surface temperature, salinity, dissolved oxygen (DO), and transparency throughout NS Guantanamo Bay and along nearshore reefs broken down by station and by geographic region. No transparency data were collected at Stations 5 and 6.

Sampling Event	Region	Station	Temp. (C)	Salinity (PPT)	DO (mg/L)	Transparency (m)
Comprehensive Project Means by Station	Guantanamo River	1	30.5±0.6	19.7±1.7	4.3±0.7	1.0±0.0
		2	29.1±0.5	31.3±3.4	4.2±0.2	1.7±0.2
	Mahomilla Bay	3	30.5±0.7	36.5±1.3	3.1±0.7	0.6±0.1
	Bay Mouth	4	28.9±0.4	36.1±0.7	5.1±0.2	6.4±1.2
	Nearshore Reefs	5	28.8±0.4	33.2±2.0	5.3±0.2	-
		6	29.1±0.4	35.9±0.7	5.3±0.3	-
	Guantanamo Bay	7	29.6±0.6	35.1±1.8	4.8±0.4	0.7±0.1
		8	29.1±0.5	36.0±0.7	12.6±7.6	2.9±0.5
		9	29.6±0.5	36.9±0.6	4.8±0.2	2.0±0.2
		10	29.5±0.4	36.3±0.5	4.5±0.3	1.5±0.5
		11	29.2±0.5	37.3±0.6	4.8±0.2	1.2±0.1
		12	29.3±0.5	36.7±0.6	4.7±0.4	0.8±0.1
Comprehensive Project Means by Region	**Guantanamo River**	1-2	**29.8±0.4**	**25.8±2.4**	**4.3±0.3**	**1.4±0.1**
	Mahomilla Bay	3	**30.5±0.7**	**36.5±1.3**	**3.1±0.7**	**0.6±0.1**
	Bay Mouth	4	**28.9±0.4**	**36.1±0.7**	**5.1±0.2**	**6.4±1.2**
	Nearshore Reefs	5-6	**29.0±0.3**	**34.6±1.1**	**5.3±0.2**	**-**
	Guantanamo Bay	7-12	**29.4±0.2**	**36.4±0.4**	**6.0±1.3**	**1.5±0.1**
Overall Project-Wide Means			**29.4±0.1**	**34.3±0.6**	**5.4±0.7**	**1.8±0.2**

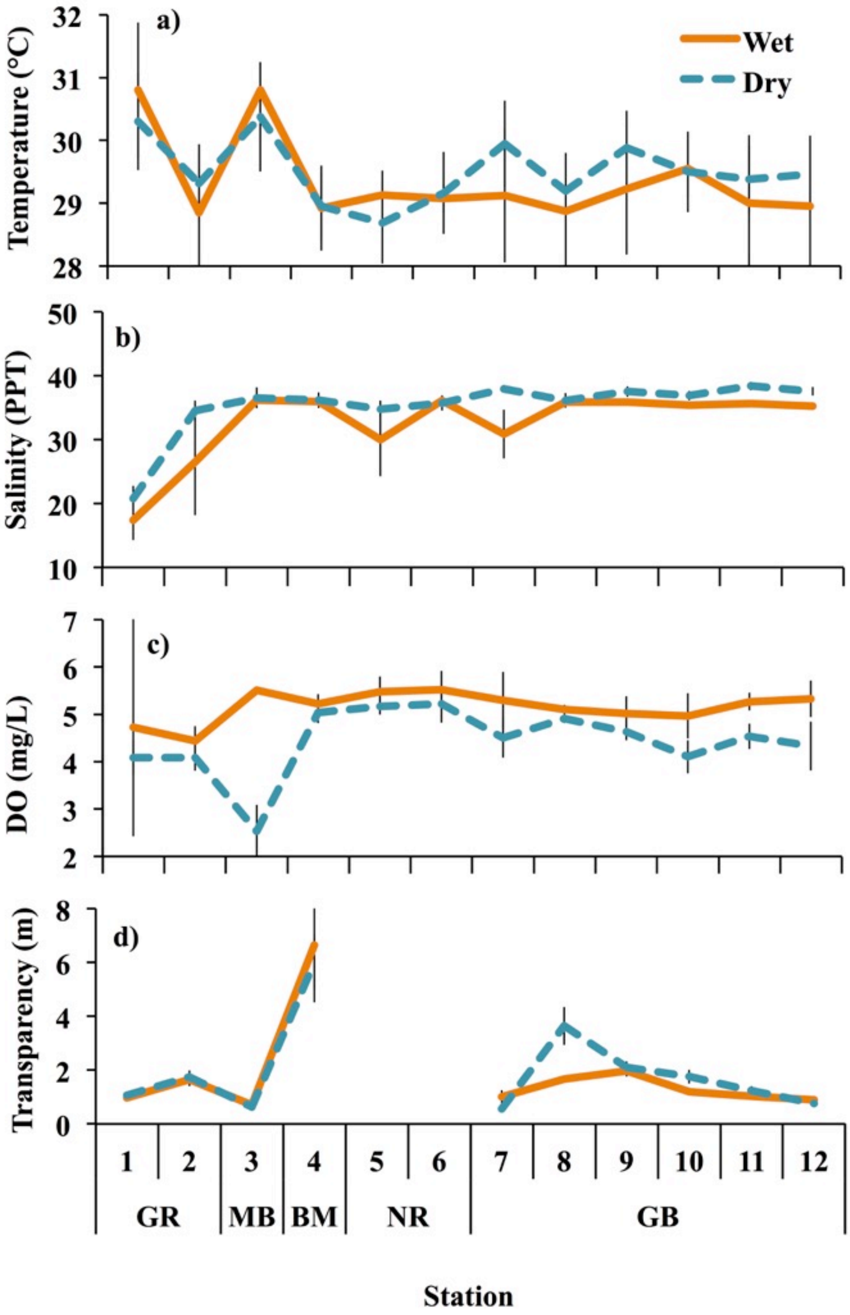


Fig. 7. Mean (± S.E.) a) temperature, b) salinity, c) dissolved oxygen (DO), and d) transparency at the 12 fixed water quality monitoring stations on U.S. Naval Station Guantanamo Bay during the Wet and Dry seasons. No transparency data were collected at Stations 5 and 6. Stations are divided by the Guantanamo River (GR), Mahomilla Bay (MB), the Bay mouth (BM), nearshore reefs (NR), and lower Guantanamo Bay (GB).

3.3 Dissolved nutrients and chlorophyll a *concentrations* – Macroalgae and phytoplankton primarily assimilate the dissolved inorganic forms of N and P from the water column so high concentrations of these forms can help fuel and sustain harmful algal blooms. Inorganic N comes in the form of ammonium, nitrate, and to a lesser extent, nitrite. Dissolved inorganic nitrogen (DIN) is the cumulative concentration of all three forms of N available to potential bloom formers. Unlike N, there is only one inorganic form of phosphorus, soluble reactive phosphorus (SRP). Some macroalgae and phytoplankton have enzymes that allow them to utilize organic forms of P when SRP is not readily available. Ratios of DIN:SRP and TDN:TDP serve as a proxy for nutrient limitation, where ratios greater than 16 are indicative of P-limitation and those less than 16 of N-limitation. If a system is P-limited, then small increases in P concentration have the ability to prompt phase shifts in macroalgae and/or phytoplankton abundance. As in this study, chlorophyll *a* is usually measured along with dissolved nutrients as an indicator of phytoplankton biomass and eutrophication.

Dissolved nutrient and chlorophyll *a* concentrations varied by station, season, and rain period. The most reduced form of N is ammonium (NH_4) as it is typically converted through the nitrification process to nitrate (NO_3) during the secondary sewage treatment process if adequate aeration is provided. Thus, high concentrations of ammonium near sewage outfalls can indicate partially-treated sewage discharge to receiving waters. The overall project mean (± S.E.) for ammonium (NH_4) at all 12 stations was 4.0 ± 0.6 µM with the highest mean concentrations in the Fall (8.2 ± 1.7 µM) and lowest in the Spring (1.7 ± 0.2 µM; Tables 4A-E). Overall, mean NH_4 concentrations were higher during the Wet (6.6 ±1.3 µM) than the Dry (2.3 ± 0.3 µM) season. This was especially true at the two Guantanamo River stations and three stations in Guantanamo Bay; Port Palma (Stations 7), Corinaso Point (Station 8), and Caravella Point (Station 10; Fig. 8a; Table 4E). When comparing project-wide regional means, the Guantanamo River had the highest mean NH_4 concentration (5.3 ± 1.1 µM) where seasonal means were especially high during the Fall (9.7 ± 2.0 µM) and Summer (5.0 ± 2.9 µM; Table 4A-E). The individual stations with the overall highest NH_4 concentrations (> 7 µM) were located near Corinaso Point (Station 8) and Caravella Point (Station 10; Table 4E). These two areas (located adjacent to wastewater outfalls) had exceptionally high NH_4 concentrations (> 20 µM) in the Fall; 2x the next highest concentration of 10 µM documented in the upper Guantanamo River (Station 1; Table 4A). Project-wide, approximately 40% and 35% of the DIN documented in the Guantanamo River and Guantanamo Bay, respectively, was NH_4.

Nitrate (NO_3), which can also be enriched in secondarily treated wastewater effluent, had an overall project mean (± S.E.) of 8.1 ± 0.9 µM with the highest means in the Summer (17.2 ± 2.1 µM) and Fall (7.7 ± 1.6 µM; Tables 4A-E). Project-wide, the collective Guantanamo Bay stations had the highest mean NO_3 concentration (9.1 ± 1.5 µM) where seasonal means were especially high during the Summer (18.3 ± 3.3 µM) and Fall (10.3 ± 3.0 µM; Table 4A-E). Although not as high as the Guantanamo Bay stations, project-wide, the Guantanamo River also had high NO_3 concentrations (7.7 ± 1.4 µM). The overall mean (± S.E.) NO_3 concentration was lower during the Wet (6.5 ±1.2 µM) than the Dry (9.1 ± 1.2 µM) season. With the exception of a few stations during the Wet season, NO_3 concentrations were exceptionally high (> 5 µM) throughout the study; especially near Port Palma (Station 7), Corinaso Point (Station 8), and Caravella Point (Station 10) in Guantanamo Bay (Fig. 8b). The areas with the overall highest NO_3 concentrations (> 11 µM) were again Corinaso Point (Station 8) and Caravella Point (Station 10) adjacent to wastewater outfalls, with the highest concentrations (15-27 µM) documented in the Fall and Summer (Table 4A,D,E). Approximately 65% of the DIN documented in Guantanamo Bay and 60% in the Guantanamo River was comprised of NO_3.

The overall project mean (± S.E.) for dissolved inorganic nitrogen (DIN), the cumulative sum of N able to be assimilated by phytoplankton and macroalgae, at all 12 stations was 12.1 ± 1.3 µM with slightly higher concentrations during the Wet (13.1 ± 2.5 µM) than the Dry (11.5 ± 1.4 µM) season. Overall seasonal means (± S.E.) were significantly higher in the Summer (20.1 ± 2.4 µM) and Fall (15.9 ± 3.3 µM) than in the Winter (3.8 ± 0.3 µM) and Spring (2.6 ± 0.3 µM; Tables 4A-E). Project-wide, the stations in Guantanamo Bay had the highest mean DIN concentration (13.9 ± 2.3) followed by the Guantanamo River (12.9 ± 2.3; Table 4E). Within Guantanamo Bay, the highest DIN concentrations (> 30 µM) were documented near Port Palma (Station 7), Corinaso Point (Station 8), and Caravella Point (Station 10) in the Fall or Wet season (Fig. 9a; Table 4A). In the Guantanamo River, the DIN concentrations were highest in the Fall and Summer (both averaging ~18 µM; Tables 4A,D).

Soluble reactive phosphorus (SRP) concentrations were elevated, with a project-wide mean (± S.E.) of 1.0 ± 0.2 µM for all 12 stations, indicating sufficient amounts to support macroalgae and phytoplankton growth. Lower SRP concentrations were consistently recorded in the Wet (0.4 ± 0.1 µM) rather than in the Dry (1.4 ± 0.3 µM) rain period (Fig. 9b). Project-wide, mean concentrations were higher during the Summer (2.3 ± 0.6 µM) than the other three seasons (all < 0.5 µM; Tables 4A-E). Overall, the Guantanamo River had the highest mean SRP concentration (2.7 ± 1.0); nearly 3x higher than the next highest inputs of ~1.1 µM in Granadillo Bay (Station 9) and near Caracoles Point (Station 11; Table 4E). In the Guantanamo River, the upstream (Station 1) concentration was an average of 5x higher than that documented downstream (Station 2) over the course of the study indicating an upstream to downstream dilution effect (Table 4E). Because of an unusual spike to 35 µM at Station 1 in July 2015, the Guantanamo River had an exceptionally high seasonal mean concentration (12.5 ± 5.7 µM) during the Summer (Table 4D). In addition to the high SRP concentrations in the upper Guantanamo River, Corinaso Point (Station 8), Granadillo Bay (Station 9), and Caracoles Point (Station 11) in Guantanamo Bay also had exceptionally high inputs (> 7 µM) of SRP in July 2015 (Fig. 9b).

Overall, the mean DIN:SRP ratio (± S.E.), an indicator of relative N vs. P limitation, at the 12 stations was 49.7 ± 11.7 with the lowest mean DIN:SRP ratios (most P or least P-limited) in the

Guantanamo River and adjacent Mahomilla Bay (~24 in each; Table 4E) and the highest ratios (least P or most P-limited) in the Bay Mouth and nearshore reefs (~143 and ~55, respectively). Project-wide, DIN:SRP ratios ranged from 5.1 ± 1.0 in the Guantanamo River to 142.9 ± 107.1 at the Bay Mouth (Table 4E). Seasonally, the overall mean DIN:SRP ratios (± S.E.) were higher (more P-limited) in the Fall (~95) and Winter (~72) than they were Spring (~12) and Summer (~18; Tables 4A-D). When looking at the rain period, the mean (± S.E.) DIN:SRP ratios were significantly higher indicating more P-limitation during the Wet (109.2 ± 30.4) than the Dry (14.2 ± 1.7) season. This was especially true near Corinaso Point (Station 8) in Guantanamo Bay and at the Bay Mouth (Station 4; Fig. 9c). The range of DIN:SRP ratios was significantly wider during the Wet (9 to 368) than the Dry (3 to 32) season. The low ends of the ranges (9 in the Wet season, 3 in the Dry season) were both documented in the upper Guantanamo River (Station 1).

Total dissolved nitrogen (TDN) concentrations are comprised of both the inorganic forms of DIN mentioned above as well as pooled concentrations of organic N produced during nutrient cycling of the living flora and fauna in the system. Project-wide, the stations in the Guantanamo River (113.9 ± 24.8 µM) and Mahomilla Bay (111.1 ± 45.8 µM) had the highest mean TDN concentrations (± S.E.; Table 4E). Like DIN, mean TDN concentrations (± S.E.) were significantly higher during the Summer (211.5 ± 24.5 µM) and Fall (47.5 ± 3.8 µM; Tables 4A-D). When looking at the rain period, all stations had significantly higher mean TDN concentrations during the Dry season (part of Winter, Spring and Summer; 122.1 ± 13.8 µM) than the Wet season (Fall and part of Winter; 41.9 ± 3.0 µM; Fig. 10a). Among the six Guantanamo Bay stations, the highest TDN concentrations (>200 µM) were documented in Port Palma (Stations 7) and at Caravella Point (Station 10) in the Summer or Dry season (Table 4D); far exceeding Florida's numeric nutrient criteria of 17.86 µM for similar patchy coral reef habitat in the Back Bay region of Florida Bay (see 62-302.532 F.A.C.). Similarly, the TDN concentrations in the Guantanamo River were highest (both stations >220 µM) in the Summer or Dry season (Table 4D).

Similar trends seen for SRP were documented for total dissolved phosphorus (TDP). Like TDN, TDP is the sum of both dissolved inorganic SRP and the organic pools of P produced through nutrient cycling. As with SRP, the highest project-wide TDP means (± S.E.) were documented during the Summer (9.5 ± 1.3 µM; Tables 4D,E). Project-wide, the Guantanamo River and adjacent Mahomilla Bay had the highest mean TDP concentration (6.5 ± 1.7 µM and 5.1± 1.8 µM, respectively; Table 4E). Also like SRP, the TDN concentration in the upper Guantanamo River (Station 1) was an average of 2x higher than that documented downstream (Station 2) over the course of the study indicating an upstream to downstream dilution effect. Because of an unusual spike to 57 µM at Station 1 in July 2015, the Guantanamo River had an exceptionally high seasonal mean concentration (20.3 ± 9.5) during the Summer (Table 4D). In addition to the high TDP concentrations in the upper Guantanamo River, Mahomilla Bay (Station 3), the Bay Mouth (Station 4), and Watergate (Station 12) also had relatively high concentrations (> 3.7 µM) of TDP in July 2015. Overall project means for stations in Guantanamo Bay, the Bay Mouth, and Nearshore Reefs ranged from 4-5 µM (Table 4E); again far exceeding Florida's numeric nutrient criteria of 0.29 µM for similar patchy coral reef habitat in the Back Bay region of Florida Bay (see 62-302.532 F.A.C.). When comparing rain periods, the highest TDP concentrations were consistently recorded during the Dry season regardless of the station (Fig. 10b). The mean (± S.E.) TDP concentrations during the Dry and Wet seasons were 5.8 ± 0.7 µM and 2.7 ± 0.4 µM, respectively.

Like DIN:SRP, the lowest mean project-wide TDN:TDP ratio (most N-limited), which is also used as a proxy for relative N vs. P limitation, was documented in the Guantanamo River (~23) and the highest ratios (most P-limited) was in the Bay Mouth (~53; Table 4E). Unlike DIN:SRP ratios, seasonal overall mean TDN:TDP ratios (± S.E.) were lower (more N-limited) in the Winter (~14) and Spring (~20) than they were during the Fall (~42) and Summer (~36; Tables 4A-D). When looking at the rain period, the mean (± S.E.) TDN:TDP ratios were significantly higher (more P-limited) during the Wet season (35.0 ± 7.6 µM) than the Dry season ratios (26.7 ± 1.2 µM; Fig. 10c). The Wet season ratios ranged from 18 in the Guantanamo River to 103 at the Bay Mouth and the Dry season ratios from 20 in the Guantanamo River and Bay Mouth to 33 at Mahomilla Bay and Caravella Point (Station 10).

Chlorophyll *a*, an indicator of phytoplankton biomass and eutrophication, had an overall project mean of 8.6 ± 0.6 µg/L. This value is considerably higher than the "trigger" values for impaired coral reefs (< 0.5 µg/L) documented by Bell (1992) and Florida's numeric nutrient criteria of 0.3 µg/L established for similar coral reef habitat in the Back Bay region of Florida Bay (see 62-302.532 F.A.C.). Project-wide, the highest mean concentrations were seen in Guantanamo Bay followed by Mahomilla Bay, the Guantanamo River, Bay Mouth, an the nearshore reefs, respectively (Table 4E). Overall, the highest concentrations (>10 µg/L) were seen in the upper reaches of the Base at Watergate (Station 12), Port Palma (Station 7), and Caracoles Point (Station 11) and, to a lesser extent, in the upper Guantanamo River (Station 1), respectively (Table 4E). Seasonally, the chlorophyll *a* concentrations were highest in the Fall (~12 µg/L) and gradually decreased through the Winter (~8 µg/L), Spring (~8 µg/L), and Summer (~6 µg/L) sampling events (Tables 4A-D). Regardless of the station, the chlorophyll *a* concentrations were consistently higher during the Wet than during the Dry season, where the mean concentrations (± S.E.) were 11.3 ± 1.0 µg/L and 6.8 ± 0.6 µg/L, respectively (Fig. 11).

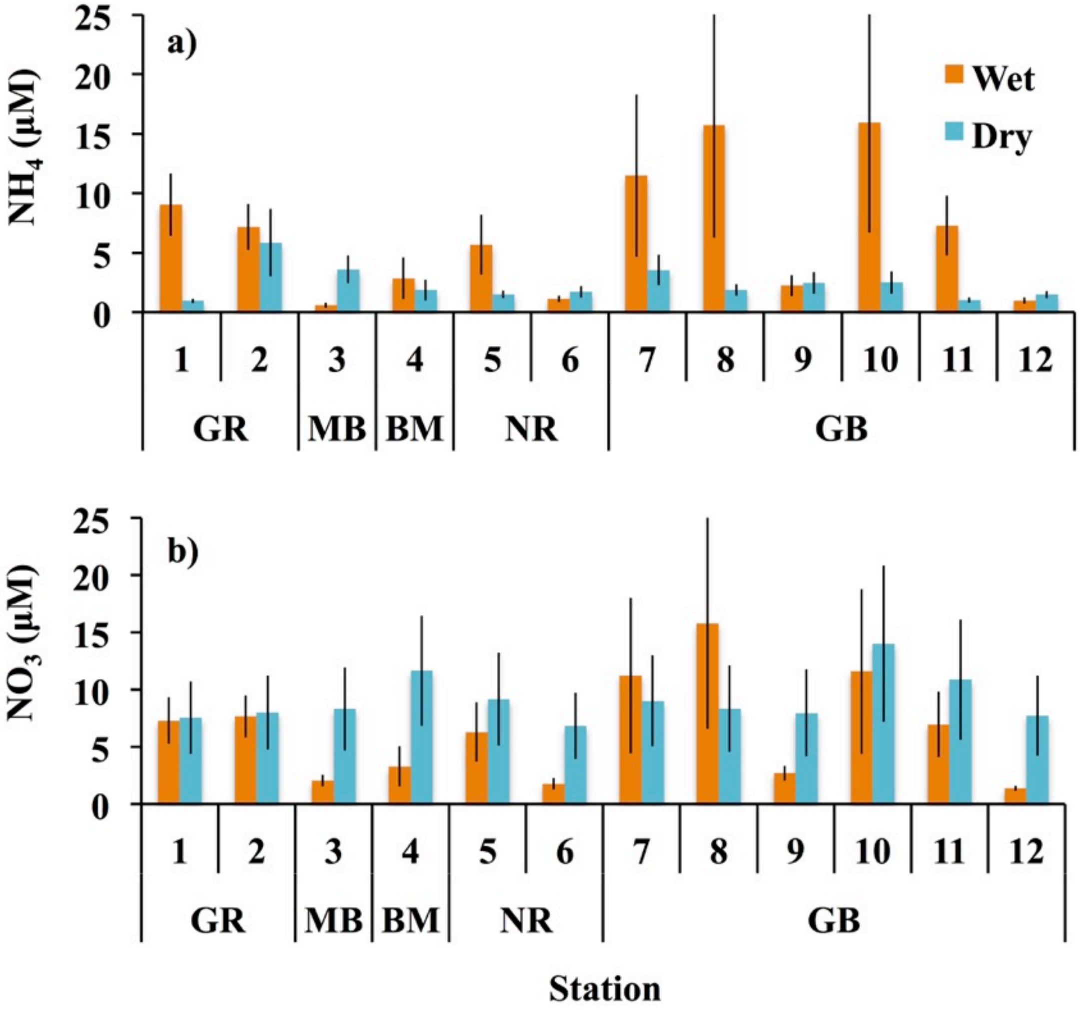


Fig. 8. Mean concentrations (± S.E.) of a) ammonium (NH_4) and b) nitrate (NO_3) at the 12 fixed water quality monitoring stations on U.S. Naval Station Guantanamo Bay during the Wet and Dry seasons. Stations are divided by the Guantanamo River (GR), Mahomilla Bay (MB), the Bay mouth (BM), nearshore reefs (NR), and lower Guantanamo Bay (GB).

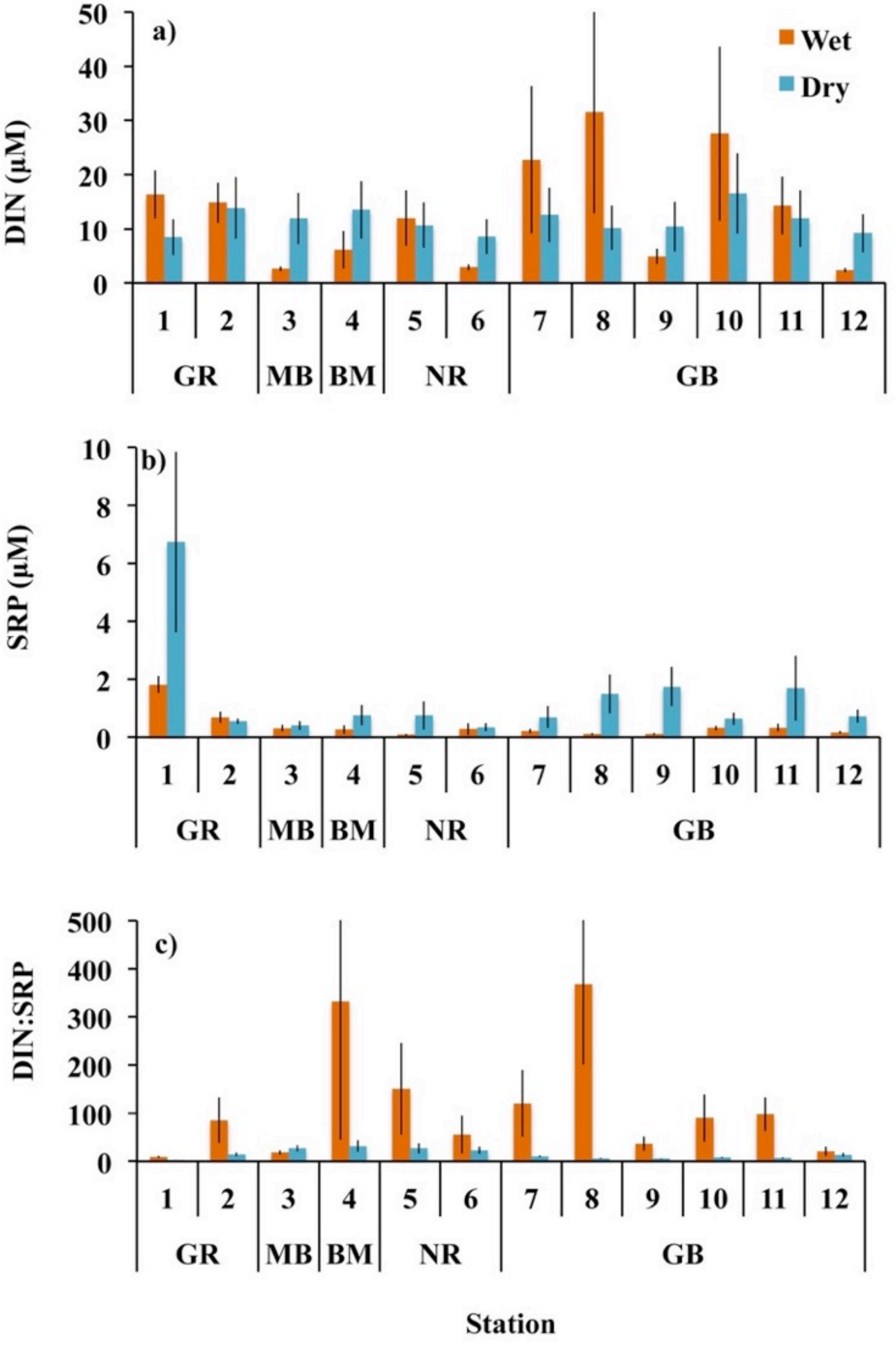


Fig. 9. Mean concentrations (± S.E.) of a) dissolved inorganic nitrogen (DIN), b) soluble reactive phosphorus (SRP), and c) the DIN:SRP ratio at the 12 fixed water quality monitoring stations on U.S. Naval Station Guantanamo Bay. Stations are divided by the Guantanamo River (GR), Mahomilla Bay (MB), the Bay mouth (BM), nearshore reefs (NR), and lower Guantanamo Bay (GB).

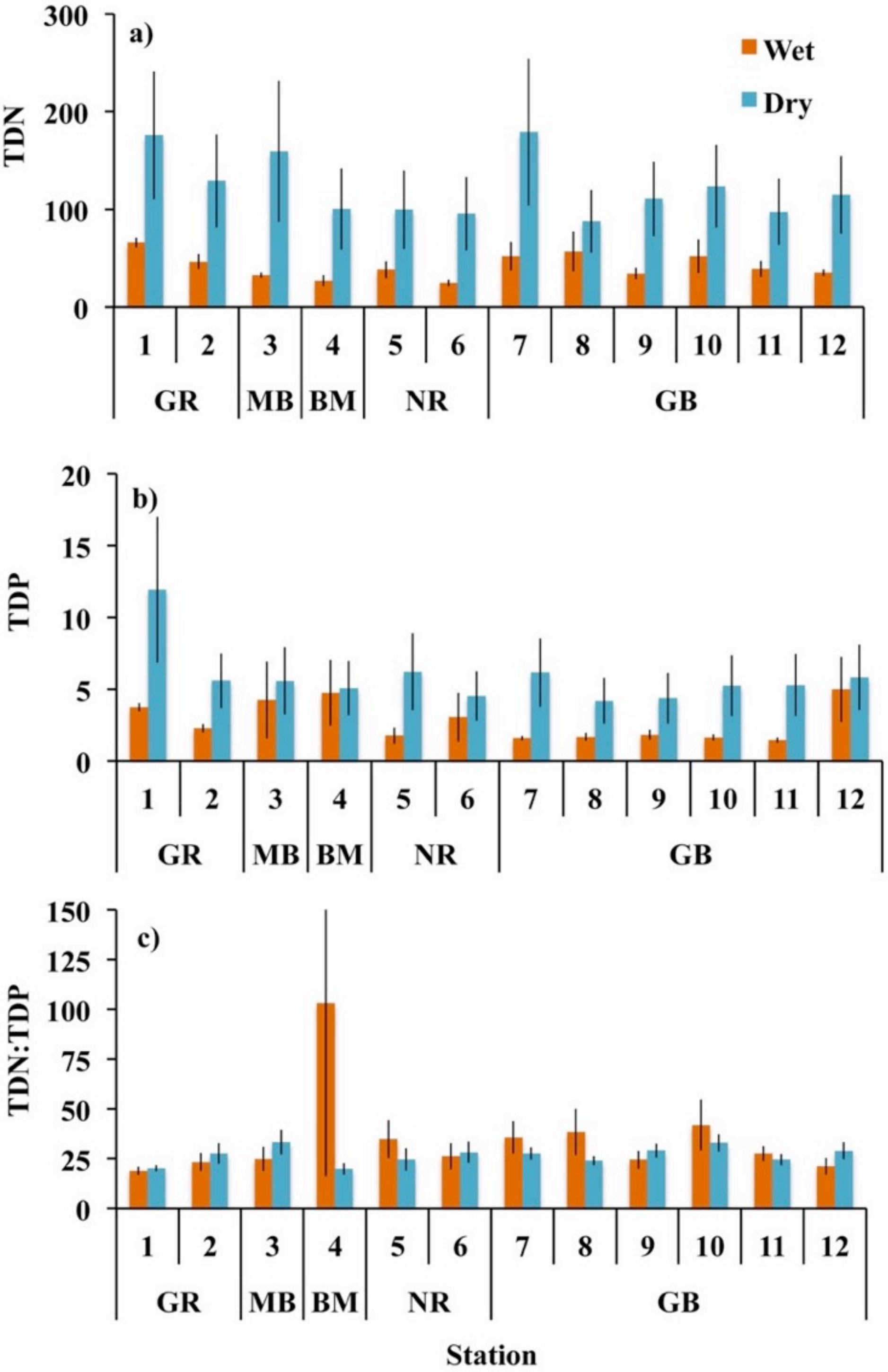


Fig. 10 Mean concentrations (± S.E.) of a) total dissolved nitrogen (TDN), b) total dissolved phosphorus (TDP), and c) the TDN:TDP ratio at the 12 fixed water quality monitoring stations within U.S. Naval Station Guantanamo Bay. Stations are divided by the Guantanamo River (GR), Mahomilla Bay (MB), the Bay mouth (BM), nearshore reefs (NR), and lower Guantanamo Bay (GB).

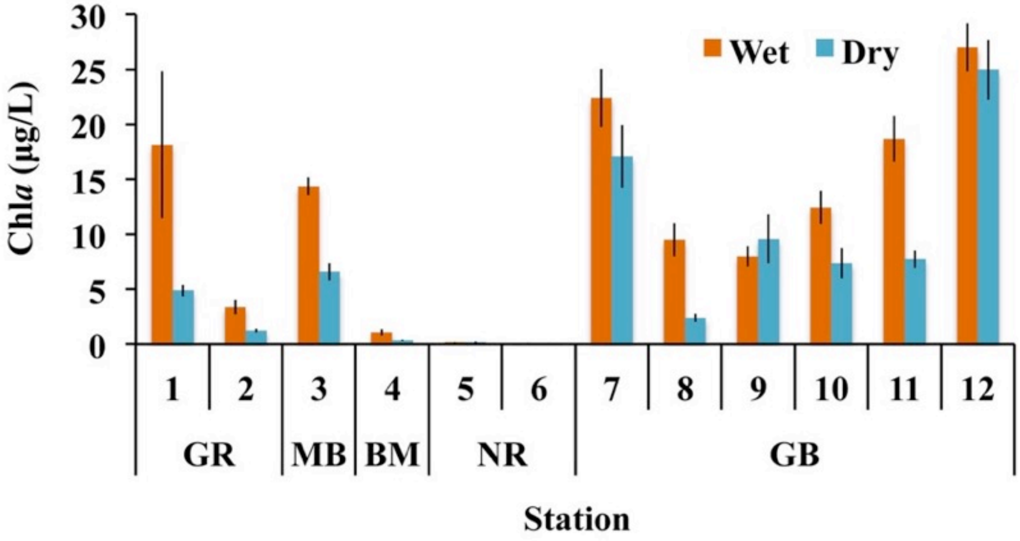


Fig. 11. Mean concentrations (± S.E.) of chlorophyll *a* at the 12 fixed water quality monitoring stations within U.S. Naval Station Guantanamo Bay. Stations are divided by the Guantanamo River (GR), Mahomilla Bay (MB), the Bay mouth (BM), nearshore reefs (NR), and lower Guantanamo Bay (GB).

Table 4A. A comparison of mean (± S.E.) surface water dissolved nutrient and chlorophyll *a* concentrations throughout NS Guantanamo Bay and along nearshore reefs recorded during the three Fall season sampling events at each fixed station and by geographic regions.

Sampling Event	Region	Station	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)	Chla (µg/L)
Fall September October November 2014	Guantanamo River	1	10.5±0.3.4	7.9±2.7	18.4±5.8	2.0±0.4	9.4±2.4	70.0±6.1	4.0±0.3	18.7±2.6	51.6±4.6	2.1±0.5	23.2±8.3
		2	8.9±2.2	9.0±2.3	17.9±4.5	0.8±0.2	108.1±61.6	54.8±8.2	2.5±0.4	26.5±5.6	36.8±4.4	1.7±0.4	3.8±0.9
	Mahomilla Bay	3	0.8±0.3	1.2±0.3	1.9±0.2	0.4±0.2	17.5±6.2	34.9±3.3	5.3±3.7	26.8±8.4	33.0±3.1	5.0±3.6	15.3±0.9
	Bay Mouth	4	3.3±2.3	3.3±2.4	6.6±4.6	0.3±0.2	45.4±15.7	31.0±6.9	5.9±3.0	133.5±115.8	24.5±3.2	5.6±2.9	1.4±0.3
	Nearshore Reefs	5	6.8±3.3	7.9±3.3	14.7±6.6	0.1±0.0	165.9±108.3	45.4±10.2	2.0±0.7	41.8±11.8	30.7±4.7	1.9±0.7	0.2±0.0
		6	1.1±0.3	1.1±0.2	2.2±0.2	0.4±0.3	14.3±4.0	25.1±4.5	3.6±2.3	29.2±8.6	22.9±4.5	3.2±2.0	0.1±0.0
	Guantanamo Bay	7	15.3±9.2	15.0±9.1	30.3±18.3	0.3±0.1	120.2±69.3	60.5±19.6	1.6±0.2	41.7±10.4	30.2±2.1	1.3±0.2	21.6±3.5
		8	20.4±12.4	19.8±12.2	40.2±24.6	0.1±0.0	368.3±167.7	68.2±26.4	1.8±0.4	45.9±14.7	27.9±3.3	1.6±0.4	10.4±1.9
		9	3.0±1.1	2.4±0.8	5.3±1.8	0.2±0.0	36.3±14.5	37.3±7.3	1.8±0.5	27.9±5.6	31.9±6.0	1.7±0.4	8.3±1.2
		10	21.0±12.5	15.4±9.7	36.4±21.6	0.3±0.0	100.5±54.1	61.8±23.2	1.6±0.3	51.3±16.7	25.4±4.7	1.3±0.3	14.2±1.4
		11	8.2±3.3	8.8±3.7	16.9±7.0	0.2±0.0	123.9±44.0	44.6±10.7	1.4±0.2	31.7±4.0	27.7±4.2	1.2±0.2	22.1±1.3
		12	1.0±0.3	1.5±0.3	2.4±0.5	0.2±0.1	24.5±14.5	38.5±4.1	6.1±3.0	22.9±5.5	36.1±3.8	6.0±2.9	25.7±2.9
Overall Fall Season Mean - All Stations			**8.2±1.7**	**7.7±1.6**	**15.9±3.3**	**0.4±0.1**	**95.4±20.4**	**47.5±3.8**	**3.1±0.5**	**41.5±10.1**	**31.6±1.4**	**2.7±0.5**	**12.3±1.2**
Fall Season Means by Region	**Guantanamo River**	1-2	**9.7±2.0**	**8.4±1.7**	**18.1±3.6**	**1.4±0.3**	**58.8±32.2**	**62.4±5.3**	**3.3±0.3**	**22.6±3.1**	**44.2±3.6**	**1.9±0.3**	**13.5±4.7**
	Mahomilla Bay	3	**0.8±0.3**	**1.2±0.3**	**1.9±0.2**	**0.4±0.2**	**17.5±6.2**	**34.9±3.3**	**5.3±3.7**	**26.8±8.4**	**33.0±3.1**	**5.0±3.6**	**15.3±0.9**
	Bay Mouth	4	**3.3±2.3**	**3.3±2.4**	**6.6±4.6**	**0.3±0.2**	**45.4±15.7**	**31.0±6.9**	**5.9±3.0**	**133.5±115.8**	**24.5±3.2**	**5.6±2.9**	**1.4±0.3**
	Nearshore Reefs	5-6	**3.9±1.8**	**4.5±1.8**	**8.5±3.6**	**0.2±0.1**	**96.0±60.3**	**35.2±5.9**	**2.8±1.2**	**35.5±7.3**	**26.8±3.3**	**2.5±1.1**	**0.2±0.0**
	Guantanamo Bay	7-12	**11.2±3.3**	**10.3±3.0**	**21.5±6.2**	**0.2±0.0**	**132.3±34.8**	**51.5±6.8**	**2.4±0.5**	**36.5±4.3**	**30.0±1.7**	**2.2±0.5**	**17.1±1.2**

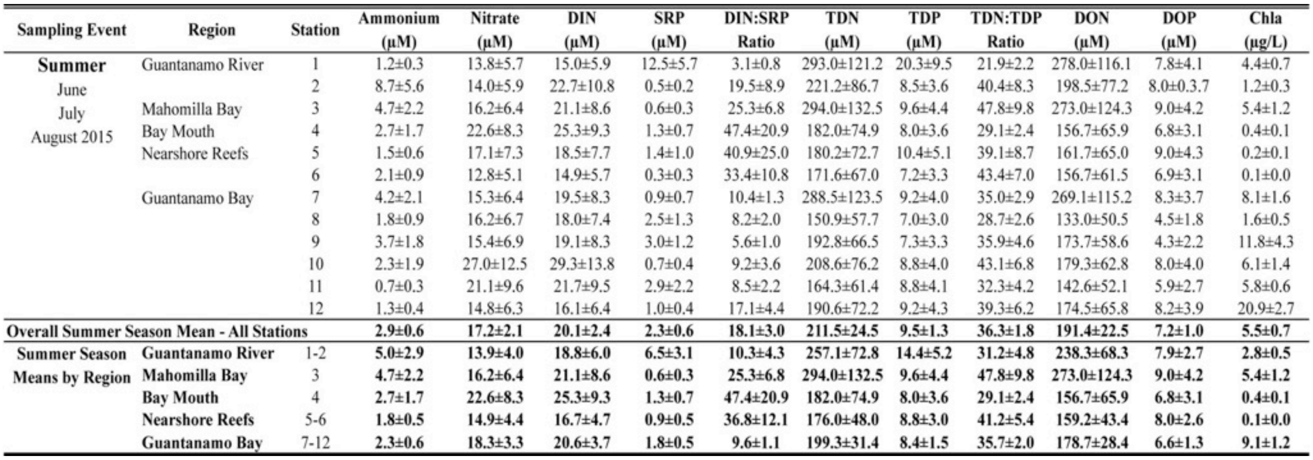
Table 4B. A comparison of mean (± S.E.) surface water dissolved nutrient and chlorophyll *a* concentrations throughout NS Guantanamo Bay and along nearshore reefs recorded during the two Winter season sampling events at each fixed station and by geographic regions.

Sampling Event	Region	Station	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)	Chla (µg/L)
Winter	Guantanamo River	1	2.5±1.0	3.9±1.0	6.4±1.9	1.1±0.2	5.5±1.3	52.0±4.7	2.8±0.2	19.0±2.3	45.6±5.2	1.1±0.2	3.2±0.8
February		2	3.5±1.1	2.9±0.4	6.4±1.0	0.5±0.1	14.5±4.0	22.4±0.8	2.4±0.4	10.5±1.5	16.0±0.5	1.0±0.1	1.7±0.3
March 2015	Mahomilla Bay	3	0.6±0.2	2.4±0.9	3.0±0.7	0.3±0.0	12.4±4.0	23.0±2.3	1.9±0.3	14.3±2.9	20.0±2.0	0.8±0.2	10.4±1.3
	Bay Mouth	4	1.6±0.7	2.1±0.7	3.6±0.6	0.3±0.1	503.1±483.2	13.6±0.9	1.4±0.2	10.2±1.2	10.0±1.2	0.8±0.1	0.5±0.1
	Nearshore Reefs	5	2.0±0.4	0.9±0.2	2.9±0.5	0.0±0.0	27.8±13.8	13.3±1.7	1.5±0.2	9.7±1.9	10.4±1.6	0.6±0.3	0.2±0.1
		6	1.4±0.4	2.3±1.0	3.7±0.9	0.1±0.0	113.8±73.3	19.2±2.5	1.8±0.2	12.1±2.8	15.5±2.3	0.8±0.4	0.1±0.0
	Guantanamo Bay	7	2.0±0.5	0.8±0.2	2.8±0.3	0.1±0.1	15.4±3.2	25.9±2.0	1.8±0.2	15.3±2.4	23.1±2.2	0.9±0.3	26.6±0.9
		8	1.7±0.7	1.9±1.2	3.7±0.8	0.3±0.1	3.6±1.1	20.9±2.0	1.4±0.1	14.6±1.0	17.2±1.8	1.0±0.2	5.6±0.5
		9	1.4±0.6	2.2±0.9	3.5±0.5	0.3±0.1	7.4±2.8	21.8±1.8	1.7±0.1	13.3±1.0	18.3±1.7	1.2±0.3	5.2±0.9
		10	1.9±0.3	1.1±0.2	3.0±0.5	0.3±0.1	6.1±0.8	24.1±1.7	1.4±0.1	17.2±1.0	21.1±1.4	0.8±0.2	9.7±1.7
		11	3.3±0.8	0.9±0.3	4.2±1.0	0.6±0.2	13.1±4.7	21.6±1.3	1.6±0.2	14.2±1.4	17.4±1.3	0.6±0.1	7.6±0.8
		12	1.2±0.4	0.9±0.1	2.2±0.4	0.2±0.0	11.2±2.6	24.1±1.0	1.8±0.1	13.9±1.1	21.9±1.3	0.8±0.3	28.5±1.3
Overall Winter Season Mean - All Stations			**1.9±0.2**	**1.9±0.2**	**3.8±0.3**	**0.3±0.0**	**72.2±53.1**	**23.5±1.3**	**1.8±0.1**	**13.7±0.6**	**19.7±1.2**	**0.9±0.1**	**8.3±1.1**
Winter Season	**Guantanamo River**	1-2	**3.0±0.7**	**3.4±0.6**	**6.4±1.0**	**0.8±0.1**	**10.0±2.5**	**37.2±5.0**	**2.6±0.2**	**14.7±1.8**	**30.8±5.1**	**1.0±0.1**	**2.4±0.5**
Means by Region	**Mahomilla Bay**	3	**0.6±0.2**	**2.4±0.9**	**3.0±0.7**	**0.3±0.0**	**12.4±4.0**	**23.0±2.3**	**1.9±0.3**	**14.3±2.9**	**20.0±2.0**	**0.8±0.2**	**10.4±1.3**
	Bay Mouth	4	**1.6±0.7**	**2.1±0.7**	**3.6±0.6**	**0.3±0.1**	**503.1±483.2**	**13.6±0.9**	**1.4±0.2**	**10.2±1.2**	**10.0±1.2**	**0.8±0.1**	**0.5±0.1**
	Nearshore Reefs	5-6	**1.7±0.3**	**1.6±0.5**	**3.3±0.5**	**0.0±0.0**	**85.1±49.9**	**16.3±1.7**	**1.7±0.1**	**10.9±1.7**	**12.9±1.5**	**0.7±0.2**	**0.2±0.0**
	Guantanamo Bay	7-12	**1.9±0.2**	**1.3±0.3**	**3.2±0.3**	**0.3±0.1**	**10.0±1.5**	**23.1±0.7**	**1.6±0.1**	**14.8±0.6**	**19.8±0.7**	**0.9±0.1**	**13.9±0.7**

Table 4C. A comparison of mean (± S.E.) surface water dissolved nutrient and chlorophyll *a* concentrations throughout NS Guantanamo Bay and along nearshore reefs recorded during the two Spring season sampling events at each fixed station and by geographic regions.

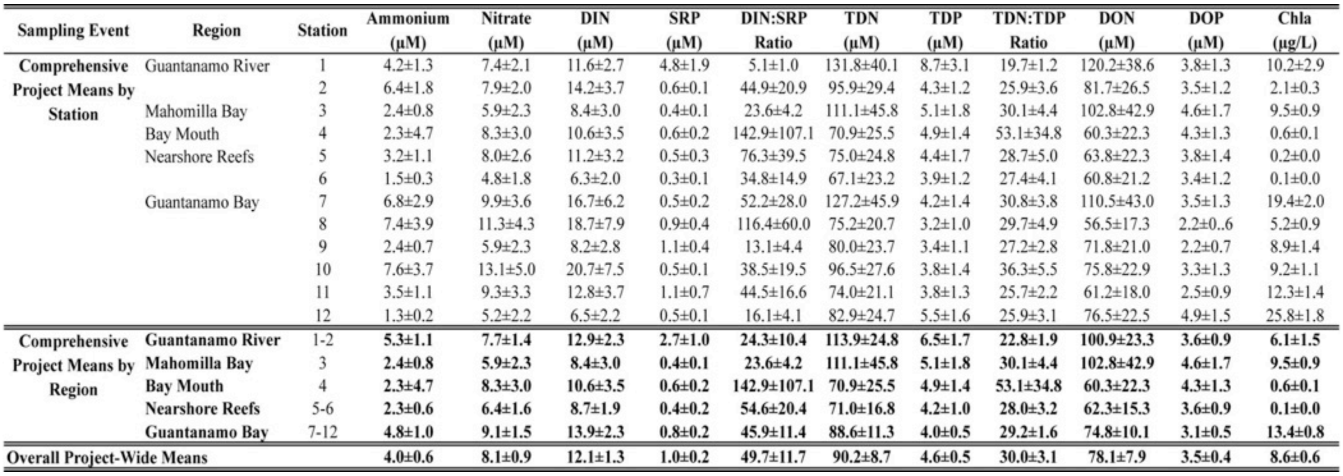
Sampling Event	Region	Station	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)	Chla (µg/L)
Spring	Guantanamo River	1	0.9±0.2	0.8±0.5	1.7±0.6	1.1±0.2	1.5±0.4	62.6±11.7	3.9±1.3	18.4±2.6	60.9±11.1	2.8±1.1	6.3±0.8
April		2	2.0±0.5	1.9±0.3	3.9±0.7	0.6±0.1	10.0±4.5	43.3±5.8	2.4±0.1	18.4±2.6	39.4±6.5	1.8±0.1	1.2±0.2
May 2015	Mahomilla Bay	3	3.1±0.7	0.4±0.1	3.5±0.6	0.2±0.1	40.9±13.1	26.6±4.5	1.1±0.2	23.7±2.3	23.1±5.0	1.0±0.2	7.2±0.8
	Bay Mouth	4	0.8±0.3	0.5±0.3	1.3±0.5	0.2±0.0	23.8±18.3	21.0±2.7	2.4±0.5	11.5±3.4	19.7±2.8	2.2±0.5	0.1±0.0
	Nearshore Reefs	5	1.5±0.4	1.7±0.8	3.1±0.9	0.2±0.0	17.5±4.5	23.±2.8	2.2±0.4	12.2±2.3	19.8±2.6	2.0±0.4	0.1±0.0
		6	1.2±0.5	0.8±0.7	2.0±1.1	0.5±0.0	3.9±2.2	21.0±4.0	1.7±0.3	16.0±5.2	19.1±3.2	1.2±0.3	0.1±0.0
	Guantanamo Bay	7	2.8±2.1	1.3±0.9	4.1±3.0	0.6±0.2	6.4±3.0	23.0±11.2	2.3±0.7	23.2±4.4	45.4±8.4	1.7±0.6	28.8±2.4
		8	2.0±0.9	0.6±0.1	2.7±0.9	0.5±0.1	5.0±0.6	21.1±2.8	1.3±0.2	22.0±4.0	23.9±2.1	0.8±0.2	2.5±0.1
		9	0.5±0.3	0.4±0.1	1.0±0.4	0.4±0.1	3.8±1.7	49.5±4.0	1.3±0.1	26.9±5.6	32.1±3.8	0.9±0.2	9.4±0.5
		10	3.4±0.6	1.2±0.2	4.5±0.6	0.7±0.1	6.9±0.9	26.6±4.3	2.0±0.3	25.2±3.2	42.3±4.1	1.3±0.3	6.7±3.5
		11	1.2±0.4	0.8±0.1	2.0±0.4	0.5±0.1	4.8±1.4	33.1±3.2	1.9±0.1	18.0±1.0	33.1±3.5	1.4±0.1	11.7±1.0
		12	1.7±0.7	0.6±0.3	2.3±0.7	0.5±0.1	11.5±8.0	46.9±7.0	2.6±0.6	22.2±5.4	44.5±7.7	2.2±0.7	30.5±6.6
Overall Spring Season Mean - All Stations			**1.7±0.2**	**0.9±0.1**	**2.6±0.3**	**0.5±0.0**	**11.5±2.4**	**35.9±2.2**	**2.1±0.2**	**19.7±1.1**	**33.3±2.1**	**1.6±0.2**	**8.1±1.3**
Spring Season	**Guantanamo River**	1-2	**1.4±0.3**	**1.3±0.3**	**2.8±0.6**	**0.8±0.1**	**5.8±2.5**	**53.0±6.8**	**3.1±0.6**	**18.4±1.8**	**50.2±6.9**	**2.3±0.6**	**3.7±0.9**
Means by Region	**Mahomilla Bay**	3	**3.1±0.7**	**0.4±0.1**	**3.5±0.6**	**0.2±0.1**	**40.9±13.1**	**26.6±4.5**	**1.1±0.2**	**23.7±2.3**	**23.1±5.0**	**1.0±0.2**	**7.2±0.8**
	Bay Mouth	4	**0.8±0.3**	**0.5±0.3**	**1.3±0.5**	**0.2±0.0**	**23.8±18.3**	**21.0±2.7**	**2.4±0.5**	**11.5±3.4**	**19.7±2.8**	**2.2±0.5**	**0.1±0.0**
	Nearshore Reefs	5-6	**1.3±0.3**	**1.3±0.5**	**2.6±0.7**	**0.3±0.1**	**10.7±3.1**	**22.0±2.4**	**1.9±0.3**	**14.1±2.8**	**19.5±2.0**	**1.6±0.3**	**0.1±0.0**
	Guantanamo Bay	7-12	**1.9±0.4**	**0.8±0.1**	**2.7±0.4**	**0.5±0.0**	**6.4±1.5**	**39.1±2.5**	**1.9±0.2**	**22.9±1.7**	**36.4±2.3**	**1.4±0.2**	**14.2±2.3**

Table 4D. A comparison of mean (± S.E.) surface water dissolved nutrient and chlorophyll *a* concentrations throughout NS Guantanamo Bay and along nearshore reefs recorded during the three Summer season sampling events at each fixed station and by geographic regions.



Sampling Event	Region	Station	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)	Chla (µg/L)
Summer	Guantanamo River	1	1.2±0.3	13.8±5.7	15.0±5.9	12.5±5.7	3.1±0.8	293.0±121.2	20.3±9.5	21.9±2.2	278.0±116.1	7.8±4.1	4.4±0.7
June		2	8.7±5.6	14.0±5.9	22.7±10.8	0.5±0.2	19.5±8.9	221.2±86.7	8.5±3.6	40.4±8.3	198.5±77.2	8.0±0.3.7	1.2±0.3
July	Mahomilla Bay	3	4.7±2.2	16.2±6.4	21.1±8.6	0.6±0.3	25.3±6.8	294.0±132.5	9.6±4.4	47.8±9.8	273.0±124.3	9.0±4.2	5.4±1.2
August 2015	Bay Mouth	4	2.7±1.7	22.6±8.3	25.3±9.3	1.3±0.7	47.4±20.9	182.0±74.9	8.0±3.6	29.1±2.4	156.7±65.9	6.8±3.1	0.4±0.1
	Nearshore Reefs	5	1.5±0.6	17.1±7.3	18.5±7.7	1.4±1.0	40.9±25.0	180.2±72.7	10.4±5.1	39.1±8.7	161.7±65.0	9.0±4.3	0.2±0.1
		6	2.1±0.9	12.8±5.1	14.9±5.7	0.3±0.3	33.4±10.8	171.6±67.0	7.2±3.3	43.4±7.0	156.7±61.5	6.9±3.1	0.1±0.0
	Guantanamo Bay	7	4.2±2.1	15.3±6.4	19.5±8.3	0.9±0.7	10.4±1.3	288.5±123.5	9.2±4.0	35.0±2.9	269.1±115.2	8.3±3.7	8.1±1.6
		8	1.8±0.9	16.2±6.7	18.0±7.4	2.5±1.3	8.2±2.0	150.9±57.7	7.0±3.0	28.7±2.6	133.0±50.5	4.5±1.8	1.6±0.5
		9	3.7±1.8	15.4±6.9	19.1±8.3	3.0±1.2	5.6±1.0	192.8±66.5	7.3±3.3	35.9±4.6	173.7±58.6	4.3±2.2	11.8±4.3
		10	2.3±1.9	27.0±12.5	29.3±13.8	0.7±0.4	9.2±3.6	208.6±76.2	8.8±4.0	43.1±6.8	179.3±62.8	8.0±4.0	6.1±1.4
		11	0.7±0.3	21.1±9.6	21.7±9.5	2.9±2.2	8.5±2.2	164.3±61.4	8.8±4.1	32.3±4.2	142.6±52.1	5.9±2.7	5.8±0.6
		12	1.3±0.4	14.8±6.3	16.1±6.4	1.0±0.4	17.1±4.4	190.6±72.2	9.2±4.3	39.3±6.2	174.5±65.8	8.2±3.9	20.9±2.7
Overall Summer Season Mean - All Stations			**2.9±0.6**	**17.2±2.1**	**20.1±2.4**	**2.3±0.6**	**18.1±3.0**	**211.5±24.5**	**9.5±1.3**	**36.3±1.8**	**191.4±22.5**	**7.2±1.0**	**5.5±0.7**
Summer Season Means by Region	**Guantanamo River**	1-2	**5.0±2.9**	**13.9±4.0**	**18.8±6.0**	**6.5±3.1**	**10.3±4.3**	**257.1±72.8**	**14.4±5.2**	**31.2±4.8**	**238.3±68.3**	**7.9±2.7**	**2.8±0.5**
	Mahomilla Bay	3	**4.7±2.2**	**16.2±6.4**	**21.1±8.6**	**0.6±0.3**	**25.3±6.8**	**294.0±132.5**	**9.6±4.4**	**47.8±9.8**	**273.0±124.3**	**9.0±4.2**	**5.4±1.2**
	Bay Mouth	4	**2.7±1.7**	**22.6±8.3**	**25.3±9.3**	**1.3±0.7**	**47.4±20.9**	**182.0±74.9**	**8.0±3.6**	**29.1±2.4**	**156.7±65.9**	**6.8±3.1**	**0.4±0.1**
	Nearshore Reefs	5-6	**1.8±0.5**	**14.9±4.4**	**16.7±4.7**	**0.9±0.5**	**36.8±12.1**	**176.0±48.0**	**8.8±3.0**	**41.2±5.4**	**159.2±43.4**	**8.0±2.6**	**0.1±0.0**
	Guantanamo Bay	7-12	**2.3±0.6**	**18.3±3.3**	**20.6±3.7**	**1.8±0.5**	**9.6±1.1**	**199.3±31.4**	**8.4±1.5**	**35.7±2.0**	**178.7±28.4**	**6.6±1.3**	**9.1±1.2**

Table 4E. A comparison of comprehensive, project-wide means (± S.E.) for surface water dissolved nutrient and chlorophyll *a* concentrations throughout NS Guantanamo Bay and along nearshore reefs broken down by station and by geographic region.



Sampling Event	Region	Station	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)	Chla (µg/L)
Comprehensive Project Means by Station	Guantanamo River	1	4.2±1.3	7.4±2.1	11.6±2.7	4.8±1.9	5.1±1.0	131.8±40.1	8.7±3.1	19.7±1.2	120.2±38.6	3.8±1.3	10.2±2.9
		2	6.4±1.8	7.9±2.0	14.2±3.7	0.6±0.1	44.9±20.9	95.9±29.4	4.3±1.2	25.9±3.6	81.7±26.5	3.5±1.2	2.1±0.3
	Mahomilla Bay	3	2.4±0.8	5.9±2.3	8.4±3.0	0.4±0.1	23.6±4.2	111.1±45.8	5.1±1.8	30.1±4.4	102.8±42.9	4.6±1.7	9.5±0.9
	Bay Mouth	4	2.3±4.7	8.3±3.0	10.6±3.5	0.6±0.2	142.9±107.1	70.9±25.5	4.9±1.4	53.1±34.8	60.3±22.3	4.3±1.3	0.6±0.1
	Nearshore Reefs	5	3.2±1.1	8.0±2.6	11.2±3.2	0.5±0.3	76.3±39.5	75.0±24.8	4.4±1.7	28.7±5.0	63.8±22.3	3.8±1.4	0.2±0.0
		6	1.5±0.3	4.8±1.8	6.3±2.0	0.3±0.1	34.8±14.9	67.1±23.2	3.9±1.2	27.4±4.1	60.8±21.2	3.4±1.2	0.1±0.0
	Guantanamo Bay	7	6.8±2.9	9.9±3.6	16.7±6.2	0.5±0.2	52.2±28.0	127.2±45.9	4.2±1.4	30.8±3.8	110.5±43.0	3.5±1.3	19.4±2.0
		8	7.4±3.9	11.3±4.3	18.7±7.9	0.9±0.4	116.4±60.0	75.2±20.7	3.2±1.0	29.7±4.9	56.5±17.3	2.2±0..6	5.2±0.9
		9	2.4±0.7	5.9±2.3	8.2±2.8	1.1±0.4	13.1±4.4	80.0±23.7	3.4±1.1	27.2±2.8	71.8±21.0	2.2±0.7	8.9±1.4
		10	7.6±3.7	13.1±5.0	20.7±7.5	0.5±0.1	38.5±19.5	96.5±27.6	3.8±1.4	36.3±5.5	75.8±22.9	3.3±1.3	9.2±1.1
		11	3.5±1.1	9.3±3.3	12.8±3.7	1.1±0.7	44.5±16.6	74.0±21.1	3.8±1.3	25.7±2.2	61.2±18.0	2.5±0.9	12.3±1.4
		12	1.3±0.2	5.2±2.2	6.5±2.2	0.5±0.1	16.1±4.1	82.9±24.7	5.5±1.6	25.9±3.1	76.5±22.5	4.9±1.5	25.8±1.8
Comprehensive Project Means by Region	**Guantanamo River**	1-2	**5.3±1.1**	**7.7±1.4**	**12.9±2.3**	**2.7±1.0**	**24.3±10.4**	**113.9±24.8**	**6.5±1.7**	**22.8±1.9**	**100.9±23.3**	**3.6±0.9**	**6.1±1.5**
	Mahomilla Bay	3	**2.4±0.8**	**5.9±2.3**	**8.4±3.0**	**0.4±0.1**	**23.6±4.2**	**111.1±45.8**	**5.1±1.8**	**30.1±4.4**	**102.8±42.9**	**4.6±1.7**	**9.5±0.9**
	Bay Mouth	4	**2.3±4.7**	**8.3±3.0**	**10.6±3.5**	**0.6±0.2**	**142.9±107.1**	**70.9±25.5**	**4.9±1.4**	**53.1±34.8**	**60.3±22.3**	**4.3±1.3**	**0.6±0.1**
	Nearshore Reefs	5-6	**2.3±0.6**	**6.4±1.6**	**8.7±1.9**	**0.4±0.2**	**54.6±20.4**	**71.0±16.8**	**4.2±1.0**	**28.0±3.2**	**62.3±15.3**	**3.6±0.9**	**0.1±0.0**
	Guantanamo Bay	7-12	**4.8±1.0**	**9.1±1.5**	**13.9±2.3**	**0.8±0.2**	**45.9±11.4**	**88.6±11.3**	**4.0±0.5**	**29.2±1.6**	**74.8±10.1**	**3.1±0.5**	**13.4±0.8**
Overall Project-Wide Means			**4.0±0.6**	**8.1±0.9**	**12.1±1.3**	**1.0±0.2**	**49.7±11.7**	**90.2±8.7**	**4.6±0.5**	**30.0±3.1**	**78.1±7.9**	**3.5±0.4**	**8.6±0.6**

3.4 Stable isotopes and C:N in phytoplankton – Stable isotopes of δ^{13}C and δ^{15}N are routinely used to track sources of C and N, respectively. The lighter (more negative) the C signal, the more indicative that the C originated from a land-based source in the watershed whereas the heavier signals (less negative) suggest a marine-based source of C. Documentation of stable N isotope (δ^{15}N) ratios in macroalgae and phytoplankton allow one to discriminate among natural (upwelling, N-fixation) and anthropogenic (wastewater, fertilizer) nutrient sources (Risk *et al.*, 2008). Because natural N-fixation source values are close to 0 $^{o}/_{oo}$ (Heaton, 1986; France *et al.*, 1998), offshore upwelled nitrate is ~ 2.0 $^{o}/_{oo}$ (Knapp *et al.*, 2008), atmospheric N typically ranges from -3 $^{o}/_{oo}$ to +1 $^{o}/_{oo}$ (Paerl and Fogel, 1994) and synthetic fertilizer N ranges from -2 $^{o}/_{oo}$ to +2 $^{o}/_{oo}$ (Bateman and Kelly, 2007), all these N sources are depleted relative to enriched values of +3 $^{o}/_{oo}$ to +19 $^{o}/_{oo}$ for human wastewater (Heaton, 1986; Costanzo *et al.*, 2001; Table 1).

Stable isotopes ($\delta^{13}C$, $\delta^{15}N$) varied by station, season, and rain period. The overall mean (± S.E.) isotopic signature for $\delta^{13}C$ at all 12 stations was -21.5 ± 0.2 $^{o}/_{oo}$ with the lightest values indicating a more terrestrial-based C source in the Fall (-22.3 ± 0.5 $^{o}/_{oo}$) and only slightly heavier values in the Winter, Spring, and Summer (all ~21 $^{o}/_{oo}$; Tables 5A-C). Overall, mean $\delta^{13}C$ values did not change between the Wet (-21.8 ± 0.4 $^{o}/_{oo}$) and Dry (-21.2 ± 0.3 $^{o}/_{oo}$) seasons, but most individual stations, especially in Guantanamo Bay, were slightly heavier during the Dry season (Fig. 12a). When comparing project-wide regional means, the Guantanamo River had the lightest isotopic signature (~ -27 $^{o}/_{oo}$) followed by Mahomilla Bay (~ -25 $^{o}/_{oo}$), the Bay Mouth (~ -23 $^{o}/_{oo}$), Guantanamo Bay (~ - 21 $^{o}/_{oo}$), and the heaviest signature was documented on the nearshore reefs (~ -14 $^{o}/_{oo}$); a trend consistently seen each of the four seasons data were collected (Tables 5A-C, Fig. 12a). The individual stations with the overall lightest $\delta^{13}C$ values (< -22 $^{o}/_{oo}$) were in the Guantanamo River (Stations 1 and 2), Mahomilla Bay (Station 3), and the Bay Mouth (Station 4; Table 5C). This was especially true for the upper Guantanamo River (Station 1), which had significantly lighter isotopic ratios than all other stations during each season except Watergate (Station 12) during the Spring (Tables 5A,B). The heaviest ratios were consistently seen at Chapman Beach (Station 5; Tables 5A,B).

The overall mean (± S.E.) isotopic signature for phytoplankton $\delta^{15}N$ at all 12 stations was 2.8 ± 0.2 $^{o}/_{oo}$ with depleted values in the Fall (2.2 ± 0.3 $^{o}/_{oo}$) and Spring (1.9 ± 0.5 $^{o}/_{oo}$) suggesting a mixture of atmospheric and wastewater N sources compared to the more enriched values documented in the Winter (3.3 ± 0.3 $^{o}/_{oo}$) and Summer (3.8 ± 0.4 $^{o}/_{oo}$) when both means exceeded the lower threshold of +3 $^{o}/_{oo}$ for wastewater N (Tables 5A-C). Overall, the mean $\delta^{15}N$ isotopic signature was more depleted during the Wet (2.0 ± 0.2 $^{o}/_{oo}$) than the Dry (3.4 ± 0.3 $^{o}/_{oo}$) season. This was especially true for Mahomilla Bay (Station 3), the nearshore reefs (Stations 5-6), and all stations in Guantanamo Bay (Stations 7-12; Fig. 12b). The most consistency between rain periods was observed in the Guantanamo River, especially the upstream station (Station 1) where the overall mean isotopic ratios were nearly 3x the overall project mean; Fig.12b). When comparing project-wide regional means, the Guantanamo River (~ +5 $^{o}/_{oo}$) and Guantanamo Bay (~ +3 $^{o}/_{oo}$) had the heaviest isotopic signature suggesting impacts from wastewater N in both regions (Table 5C). Mahomilla Bay, the Bay Mouth, and the nearshore reefs all had $\delta^{15}N$ signatures suggesting predominantly an atmospheric N source (0 to +2 $^{o}/_{oo}$; Table 5C). Seasonal regional means show the strongest wastewater N signal in the Guantanamo River in the Fall and Summer (both > +6 $^{o}/_{oo}$; Tables 5A,B). Other regions with a seasonal wastewater signal include Guantanamo Bay in Winter and Summer (both ~ +4 $^{o}/_{oo}$), Mahomilla Bay during the Summer (~

+5 $^{o}/_{oo}$), and the Bay Mouth (~ +5 $^{o}/_{oo}$) in the Winter (Tables 5A,B). Overall, the individual stations with the most enriched δ^{15}N values (> +3 $^{o}/_{oo}$) suggesting phytoplankton uptake of wastewater N were in the upper Guantanamo River (Station 1) and in Guantanamo Bay's Corinaso Point (Station 8), Granadillo Bay (Station 9), Caracoles Point (Station 11), and Watergate (Station 12; Table 5C). This was especially true for the upper Guantanamo River (Station 1), which consistently had the most enriched isotopic ratios (> +7 $^{o}/_{oo}$) during each of the four seasons except Winter (Tables 5A,B). The most depleted ratios were primarily documented along the nearshore reef (Stations 5-6; Tables 5A,B; Fig. 12b).

The C:N ratios documented in phytoplankton tissue during this study showed weak N limitation. Based on Redfield's (1953) widely accepted atomic C:N:P ratios of 106:16:1 for plankton in seawater, C:N ratio > 6.63 indicate more N-limiting conditions whereas ratios < 6.63 indicate generally high concentrations of bio-available N in the system to support primary producers like phytoplankton and macroalgae. The mean (± S.E.) C:N ratio at all 12 stations was 7.3 ± 0.2 with the lowest mean ratios (most tissue N) in the Spring and Summer (6.3 during each season; Table 5A,B). Overall, the mean C:N ratio was lower (higher tissue N) during the Dry (6.3 ± 0.2) than the Wet (8.7 ± 0.3) season, reflecting more concentrated N in the phytoplankton and particulate matter during this dry period (Fig. 13). This was especially true for Chapman Beach (Station 5) during the Wet season (Fig. 13). The overall regional mean (± S.E.) C:N ratios were lowest (most tissue N) in the Guantanamo River, Guantanamo Bay, and the Bay Mouth and highest (least tissue N) in Mahomilla Bay and along the nearshore reefs (Table 5C). Seasonal regional means show the lowest C:N ratio (most tissue N) in phytoplankton collected at the Bay Mouth in the Spring and Summer (both ~4.5; Tables 5A,B). The individual stations with the lowest overall C:N ratio, or highest tissue N, were Cuzco Beach (Station 6) followed by the stations in Guantanamo Bay and the Bay Mouth (6 to 7; Table 5C), which are influenced by the plume of the Guantanamo River or wastewater outfalls. Thus, even though the nearshore reefs had high C:N ratios (low tissue N) as a collective region, the two individual reef stations greatly differed by having the two project extremes; the project's highest C:N ratio at Chapman Beach (Station 5) in the Wet season and the project's lowest C:N ratio documented at Cuzco Beach (Station 6) in the Dry season (Fig. 13). Except for the Spring 2015, the phytoplankton collected at Chapman Beach (Station 5) consistently had the highest C:N ratio (lowest tissue N) each season whereas Cuzco Beach (Station 6) consistently had the lowest C:N ratio (highest tissue N); Tables 5A,B; Fig. 13).

Table 5A. A comparison of mean (± S.E.) particulate organic matter (phytoplankton) throughout NS Guantanamo Bay and along nearshore reefs recorded during the three Fall and two Winter season sampling events. Data are presented by station, geographic region, and overall seasonal means.

Sampling Event	Region	Station	δ^{13}C (‰)	δ^{15}N (‰)	C:N Ratio
Fall September October November 2014	Guantanamo River	1	-31.9±0.5	8.5±0.6	10.0±0.9
		2	-24.5±0.3	3.8±0.6	7.6±0.2
	Mahomilla Bay	3	-25.0±0.1	1.3±0.1	8.9±0.1
	Bay Mouth	4	-23.2±0.1	1.7±0.3	9.4±0.6
	Nearshore Reefs	5	-11.0±0.5	-2.7±0.8	14.6±2.5
		6	-18.2±1.3	-1.6±0.9	4.7±0.5
	Guantanamo Bay	7	-22.3±0.1	2.2±0.4	9.2±0.2
		8	-22.5±0.2	2.2±0.3	8.6±0.2
		9	-22.4±0.2	2.2±0.3	7.0±1.4
		10	-22.1±0.0	3.0±0.4	5.7±0.8
		11	-22.5±0.1	2.7±0.2	7.3±1.2
		12	-21.9±0.0	2.3±0.3	7.5±1.1
Fall Season Means by Region	**Guantanamo River**	1-2	**-28.2±0.9**	**6.2±0.7**	**8.8±0.5**
	Mahomilla Bay	3	**-25.0±0.2**	**1.3±0.1**	**8.9±0.1**
	Bay Mouth	4	**-23.2±0.1**	**1.7±0.3**	**9.4±0.6**
	Nearshore Reefs	5-6	**-14.6±1.1**	**-2.1±0.6**	**9.9±1.8**
	Guantanamo Bay	7-12	**-22.3±0.1**	**2.4±0.1**	**7.5±0.4**
Overall Fall Season Mean - All Stations			**-22.3±0.5**	**2.2±0.3**	**8.4±0.4**
Winter February March 2015	Guantanamo River	1	-29.3±0.2	4.5±0.5	6.6±0.1
		2	-21.5±0.3	1.1±0.6	7.8±0.4
	Mahomilla Bay	3	-24.9±0.2	1.1±0.7	9.5±0.2
	Bay Mouth	4	-21.2±1.2	5.1±2.3	6.6±1.5
	Nearshore Reefs	5	-12.0±1.4	2.0±1.6	11.9±5.0
		6	-13.0±1.3	3.0±0.9	5.7±2.6
	Guantanamo Bay	7	-22.0±0.1	3.4±0.8	7.9±0.4
		8	-20.9±0.2	4.7±0.9	7.8±0.7
		9	-19.9±0.1	3.7±0.6	7.3±0.4
		10	-20.7±0.2	3.8±0.9	8.8±0.5
		11	-20.4±0.1	3.5±0.9	7.4±0.1
		12	-21.5±0.1	4.0±0.5	6.5±0.3
Winter Season Means by Region	**Guantanamo River**	1-2	**-25.4±1.2**	**2.8±0.6**	**7.2±0.3**
	Mahomilla Bay	3	**-24.9±0.2**	**1.1±0.7**	**9.5±0.2**
	Bay Mouth	4	**-21.2±1.2**	**5.1±2.3**	**6.6±1.5**
	Nearshore Reefs	5-6	**-12.5±0.9**	**2.5±0.9**	**8.8±2.9**
	Guantanamo Bay	7-12	**-20.9±0.1**	**3.9±0.3**	**7.6±0.2**
Overall Winter Season Mean - All Stations			**-21.0±0.5**	**3.3±0.3**	**7.8±0.5**

Table 5B. A comparison of mean (± S.E.) particulate organic matter (phytoplankton) throughout NS Guantanamo Bay and along nearshore reefs recorded during the two Spring and three Summer season sampling events. Data are presented by station, geographic region, and overall seasonal means.

Sampling Event	Region	Station	$\delta^{13}C$ ($^{o}/_{oo}$)	$\delta^{15}N$ ($^{o}/_{oo}$)	C:N Ratio
Spring	Guantanamo River	1	-27.7±0.7	7.1±0.7	6.9±0.2
April		2	-23.8±2.0	-3.9±3.0	5.0±1.2
May 2015	Mahomilla Bay	3	-24.5±0.5	0.8±0.8	6.6±0.9
	Bay Mouth	4	-20.0±0.2	2.1±0.6	4.8±2.3
	Nearshore Reefs	5	-14.7±0.7	2.1±0.7	5.0±3.9
		6	-21.0±1.3	-1.0±1.8	5.2±1.1
	Guantanamo Bay	7	-20.4±0.2	1.0±1.6	8.4±0.1
		8	-21.9±0.2	2.5±0.4	5.3±0.5
		9	-20.0±0.3	2.8±0.2	6.7±0.1
		10	-19.9±1.0	2.9±1.0	6.7±0.3
		11	-20.9±0.3	2.6±0.5	6.3±0.2
		12	-28.2±0.1	3.9±0.2	6.9±0.3
Spring Season	**Guantanamo River**	1-2	**-25.7±1.2**	**1.6±2.2**	**6.0±0.6**
Means by Region	**Mahomilla Bay**	3	**-24.5±0.5**	**0.8±0.8**	**6.6±0.9**
	Bay Mouth	4	**-20.1±0.2**	**2.1±0.6**	**4.8±2.3**
	Nearshore Reefs	5-6	**-14.2±0.9**	**0.1±1.3**	**5.2±1.4**
	Guantanamo Bay	7-12	**-20.7±0.2**	**2.6±0.4**	**6.7±0.2**
Overall Spring Season Mean - All Stations			**-21.1±0.5**	**1.9±0.5**	**6.3±0.3**
Summer	Guantanamo River	1	-30.4±0.5	7.9±0.4	6.6±0.3
June		2	-24.8±0.4	7.3±3.3	5.7±0.5
July	Mahomilla Bay	3	-24.7±0.3	4.8±0.5	8.7±0.5
August 2015	Bay Mouth	4	-23.3±1.2	0.1±1.9	4.3±0.6
	Nearshore Reefs	5	-15.3±1.4	0.2±0.4	8.8±0.8
		6	-15.9±1.9	1.1±0.5	4.5±0.8
	Guantanamo Bay	7	-20.8±0.1	3.8±0.6	6.9±0.4
		8	-19.1±0.1	2.9±1.0	5.7±0.7
		9	-18.3±0.9	4.9±0.2	6.2±0.2
		10	-18.8±0.4	1.7±1.9	5.1±0.5
		11	-19.6±0.8	5.5±0.4	6.4±0.2
		12	-21.3±0.0	4.6±0.3	6.5±0.1
Summer Season	**Guantanamo River**	1-2	**-27.6±0.8**	**7.6±1.6**	**6.2±0.3**
Means by Region	**Mahomilla Bay**	3	**-24.7±0.3**	**4.8±0.5**	**8.7±0.5**
	Bay Mouth	4	**-23.3±1.2**	**0.1±1.9**	**4.4±0.6**
	Nearshore Reefs	5-6	**-15.5±1.1**	**0.6±0.3**	**7.1±0.8**
	Guantanamo Bay	7-12	**-19.6±0.3**	**3.9±0.4**	**6.1±0.2**
Overall Summer Season Mean - All Stations			**-21.2±0.5**	**3.8±0.4**	**6.3±0.2**

Table 5C. A comparison of comprehensive, project-wide means (± S.E.) for particulate organic matter (phytoplankton) throughout NS Guantanamo Bay and along nearshore reefs broken down by station, geographic region, and overall project mean.

Sampling Event	Region	Station	$\delta^{13}C$ ($^{0}/_{00}$)	$\delta^{15}N$ ($^{0}/_{00}$)	C:N Ratio
Comprehensive Project Means by Station	Guantanamo River	1	-30.1±0.4	7.2±0.4	7.7±0.4
		2	-23.8±0.5	2.8±1.4	6.6±0.4
	Mahomilla Bay	3	-24.8±0.1	2.2±0.4	8.5±0.3
	Bay Mouth	4	-22.5±0.5	1.7±0.8	6.6±0.6
	Nearshore Reefs	5	-12.9±0.7	-0.2±2.6	11.0±1.5
		6	-16.0±0.8	0.2±0.6	5.0±0.7
	Guantanamo Bay	7	-21.5±0.1	2.7±0.4	8.1±0.2
		8	-20.8±0.3	3.0±0.4	6.9±0.4
		9	-20.5±0.4	3.5±0.3	6.7±0.4
		10	-20.4±0.3	2.8±0.6	6.3±0.4
		11	-20.8±0.4	3.7±0.3	6.9±0.4
		12	-21.4±0.1	3.7±0.2	6.9±0.4
Comprehensive Project Means by Region	**Guantanamo River**	1-2	**-27.0±0.5**	**5.0±0.8**	**7.1±0.3**
	Mahomilla Bay	3	**-24.8±0.1**	**2.2±0.4**	**8.5±0.3**
	Bay Mouth	4	**-22.5±0.5**	**1.7±0.8**	**6.6±0.6**
	Nearshore Reefs	5-6	**-14.4±0.6**	**0.0±0.4**	**8.1±1.0**
	Guantanamo Bay	7-12	**-20.9±0.1**	**3.2±0.2**	**7.0±0.2**
Overall Project-Wide Means			**-21.5±0.2**	**2.8±0.2**	**7.3±0.2**

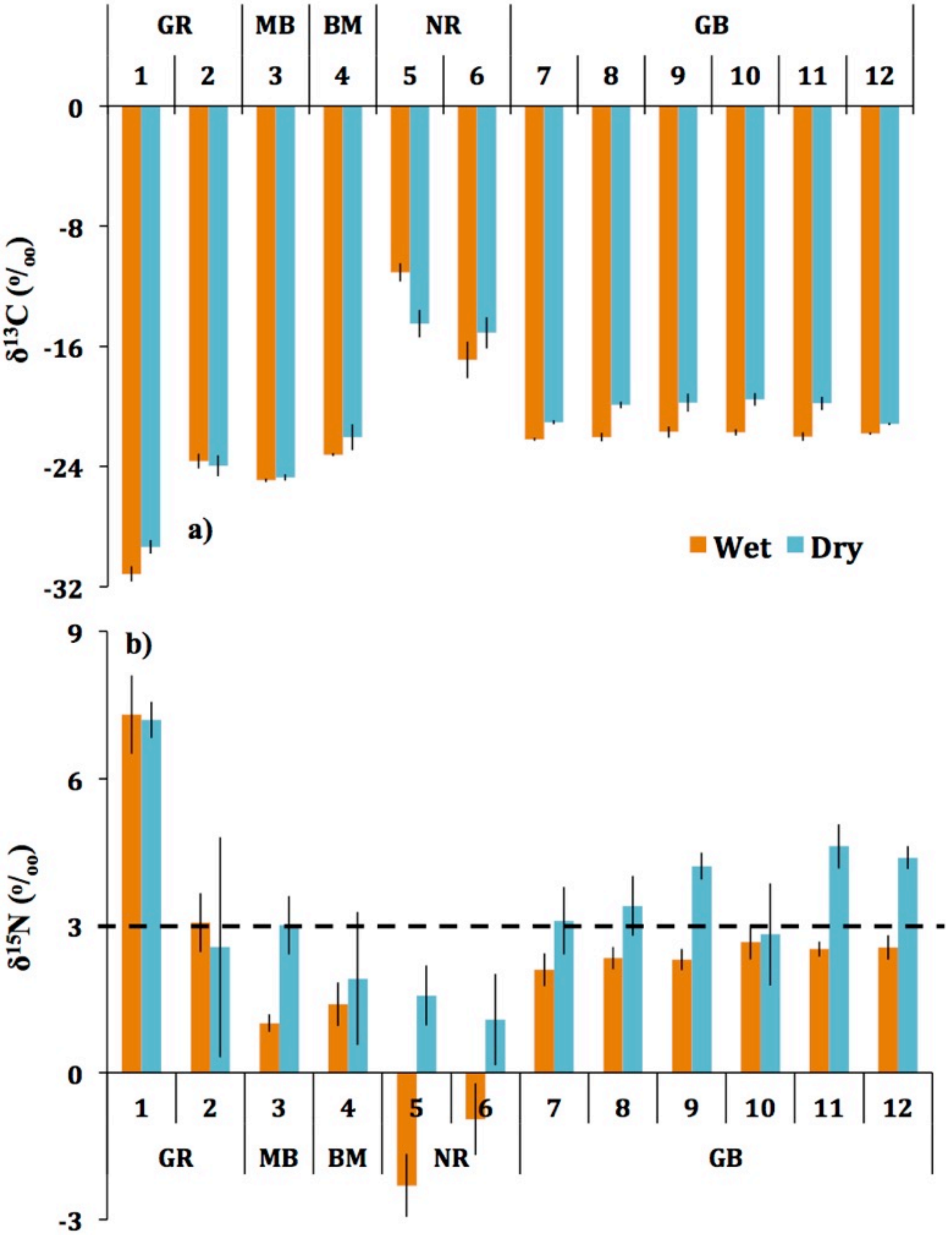


Fig. 12. Mean (± S.E.) values of phytoplankton a) $\delta^{13}C$ and b) $\delta^{15}N$ at the 12 fixed water quality monitoring stations within U.S. Naval Station Guantanamo Bay. Note: The lighter the $\delta^{13}C$ isotopic signature, the more land-based the C source and the more enriched $\delta^{15}N$ values around +3 $^{o}/_{oo}$ are in the lower range reported for wastewater N. Stations are divided by the Guantanamo River (GR), Mahomilla Bay (MB), the Bay mouth (BM), nearshore reefs (NR), and lower Guantanamo Bay (GB).

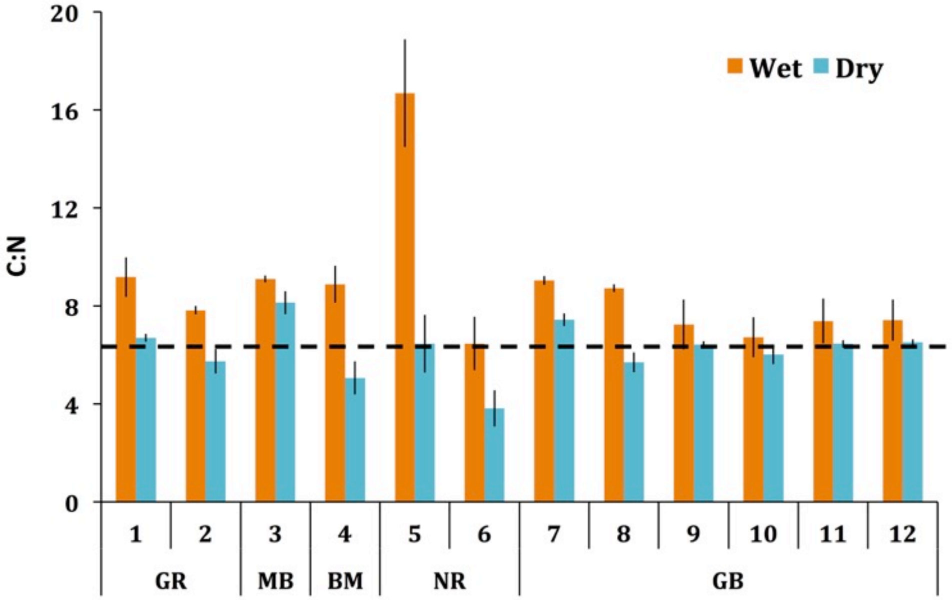


Fig. 13. Mean (± S.E.) C:N ratios in phytoplankton collected at the 12 fixed water quality monitoring stations within U.S. Naval Station Guantanamo Bay. C:N ratios > 6.63 suggest N-limiting conditions. Stations are divided by the Guantanamo River (GR), Mahomilla Bay (MB), the Bay mouth (BM), nearshore reefs (NR), and lower Guantanamo Bay (GB).

3.5 Bivalve age—Estimated ages of sampled bivalves ranged from 0.5 – 27 years and showed different growth patterns between species (Fig. 14). Modern *Phacoides pectinatus* had a smaller maximum size (58 mm) compared to ancient *Periglypta listeri* (88 mm). These findings are consistent with known growth patterns and morphology in these and similar species (Bieler *et al.*, 2004; Carmichael *et al.*, 2004). A sufficient range of age and size classes of *Phacoides pectinatus* were available among sites to allow fitting a logarithmic growth model to the combination of age-at-length data from all sites that reflected a generalized local growth rate. All *Periglypta listeri* were similar length, regardless of age, and length data could not be used to define growth rate for the population. For these ancient clams, we found that shell height and thickness (across the neck of the shell, just below the umbo) provided better metrics of growth-at-age, showing logarithmic and linear relationships respectively (Fig. 15). Future work should include analyses of distances among individual internal growth lines to further define site-specific growth rates, analyses of free amino acid concentrations to determine time since death and align interannual growth rates to specific years (Powell *et al.* 1998), or additional sampling within reef outcrops to find and analyze animals of other size classes to spatially and temporally relate changes in growth to pollution inputs on a finer temporal scale.

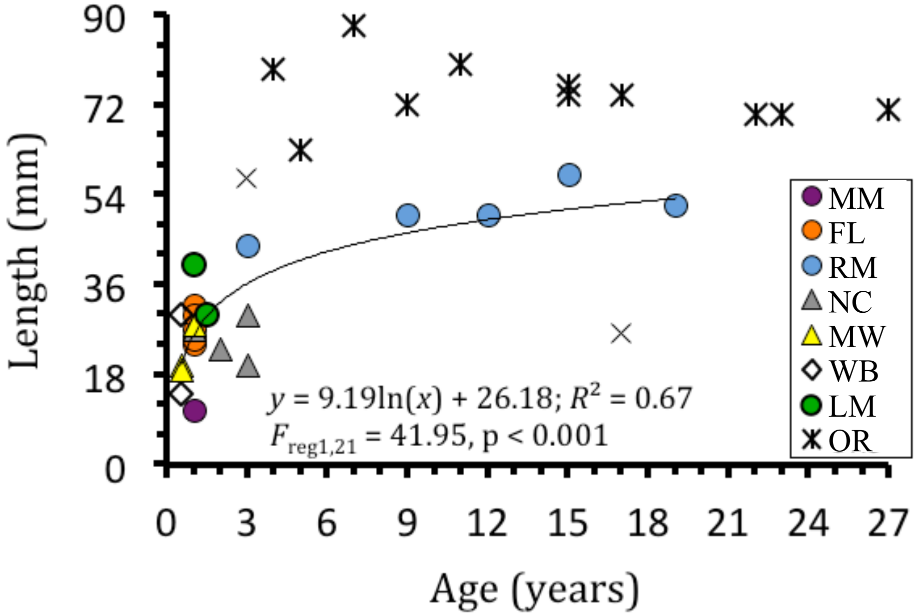


Fig. 14. Length at age relationships for modern (*Phacoides pectinatus*) and ancient (*Periglypta listeri*; OR) bivalves sampled at U.S. Naval Station Guantanamo Bay, Cuba. × = outliers to the regression. Sites correspond to locations in Fig. 3, Table 2.

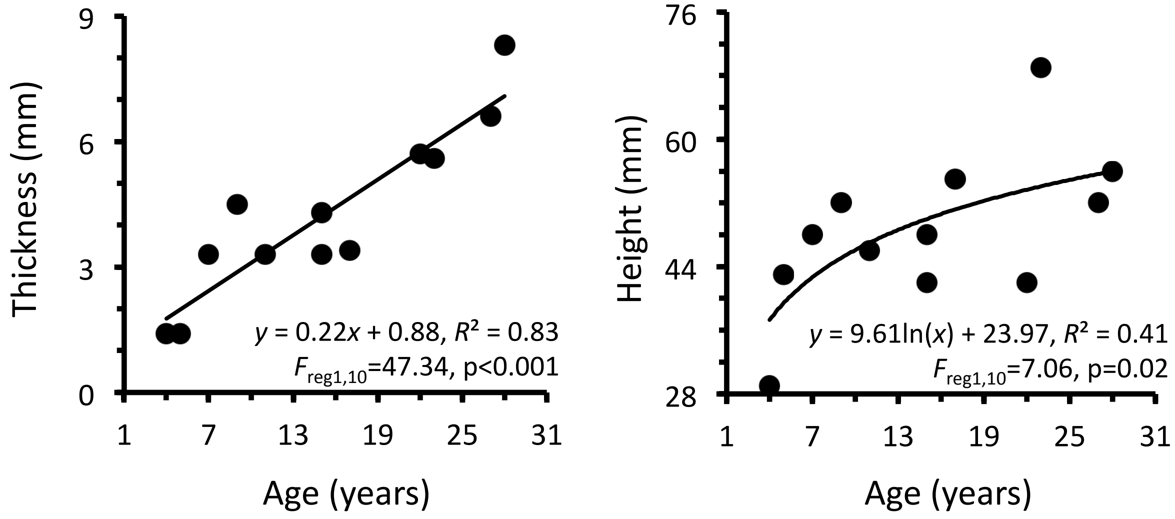


Fig. 15. Thickness (left) and height (right) at-age relationships for ancient (*Periglypta listeri*) bivalves sampled at U.S. Naval Station Guantanamo Bay, Cuba (site OR; Fig. 3, Table 2).

3.6 C and N stable isotope ratios in bivalve shell—Organic C and N stable isotope ratios in bivalve shell varied within and among sites (Fig. 16). Values ranged from 0.4 ‰ to 9‰ for δ^{15}N and -23‰ to -17‰ for δ^{13}C, reflecting a range of N and C sources to the area. In general, bivalves sampled from sites near urbanized areas (MWR Marina (MM), Windward Ferry Landing (FL), Guantanamo River mouth (RM)) had heavier δ^{15}N values than sites in the main part of Guantanamo Bay (North Medio Cay (NC), Main Bay West at Caracoles Point (MW)). These areas, in turn, had heavier values than bivalves from the tidal lake (Mahomilla Bay (LM)) and coastal Caribbean Sea (Windmill Beach (WB)). Among the sites potentially affected by urbanization, bivalve shell from MWR Marina (MM), located between Corinaso and Caravella Points, showed the greatest evidence of human influence by processed wastewater (typically associated with groundwater inputs from septic systems, treated wastewater, or a combination of these sources, which tend to have heavier N stable isotope ratios). On average, δ^{15}N values in ancient bivalves (from site OR) resembled modern bivalves in the main part of NS Guantanamo Bay. Values in ancient shell overlapped with ratios in shells from all other sites except MWR Marina (MM). Overall, the δ^{13}C values in sampled bivalves were similar to values in suspended particles available as food in each area (Caribbean Sea vs. Guantanamo Bay) and sampled as part of the water quality portion of this study (Fig. 16). δ^{13}C values were heavier in bivalve shell at marine influenced sites (Windmill Beach (WB), Mahomilla Bay (LM)) and lightest in shell from the northernmost part of the bay (North Medio Cay (NC)). These findings are consistent with likely greater freshwater inputs and associated terrestrial influence upstream.

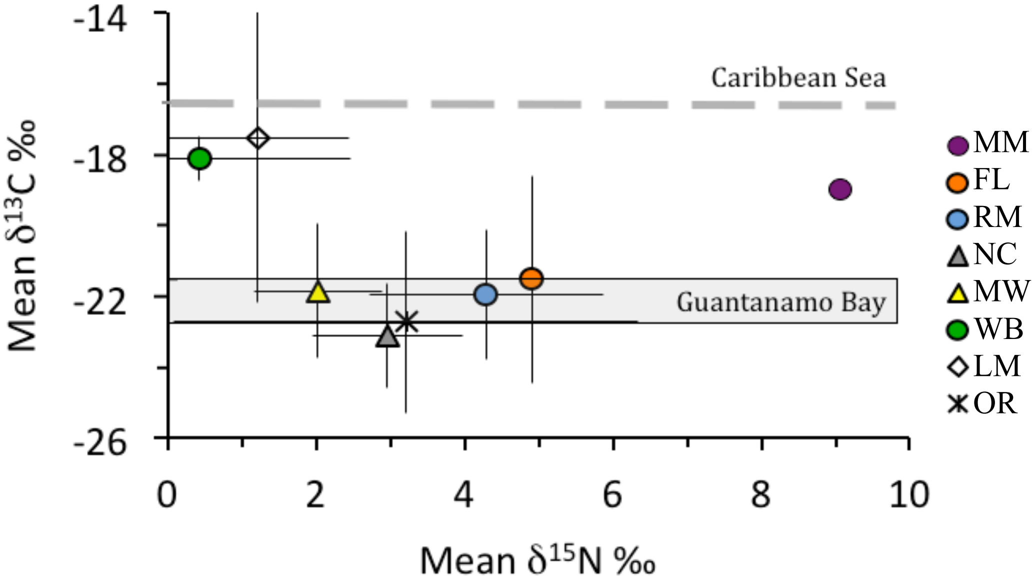


Fig. 16. C and N stable isotope ratios in shell of ancient (OR) and modern bivalves collected at U.S. Naval Station Guantanamo Bay, Cuba. Grey dashed line and shaded box show mean and range of values measured in suspended particles among sites in the Caribbean Sea and NS Guantanamo Bay, respectively. Sites correspond to locations in Fig. 3, Table 2.

3.7 Trace elements in bivalve shell – Concentrations of the 18 trace elements varied among sites and between modern and ancient bivalves (Table 6). Elemental ratios are displayed as concentrations of key individual elements (Fig. 17), combinations of elements that are characteristic to 'elemental signatures' in wastewater or fertilizer discharges and industrial discharge (Fig. 18), and concentrations above estimated naturally occurring background signatures (Fig. 19). Most elements in ancient shells from the Naval base reef site (OR3) were found at comparable or lower concentrations than in modern shells, except for Barium that was higher in ancient shells than in modern shells from other sites (Fig. 17). This finding supports the use of elemental signatures in ancient shells as a pre-urbanization baseline.

Individual elements that may be considered toxic or associated with industrial or wastewater contamination were detectable in shell at all sites (Fig. 17; Tables 6 & 7). MWR Marina (MM), Mahomilla Bay (LM), and Windmill Beach (WB) sites had the greatest frequency of individual elements at high concentrations among sites (Table 6), with Arsenic (As), Cobalt (Co), Chromium (Cr), Mercury (Hg), and Zinc (Zn) found at high concentrations in shell from at least two of these sites. In addition, Copper (Cu), Tin (Sn), Titanium (Ti), and Vanadium (V) concentrations were significantly higher in shells from Mahomilla Bay (LM) than all other sites (Fig. 17). In contrast, shells from Ferry Landing (FL), the Guantanamo Bay River mouth (RM), and ancient shell from remnant reefs (OR) typically had lower concentrations of most elements compared to other sites (Table 6), with the exception of Magnesium (Mg) at Ferry Landing, Lead (Pb) and Zn at the River mouth and Barium (Ba) in ancient shell (Fig. 17). Nickel (Ni) and Cadmium (Cd) did not differ among sites or between ancient and modern shells.

In this study, concentrations of elements specific to wastewater and industrial discharge were elevated at Mahomilla Bay (LM) and North Medio Cay (NC), driven by elevated Cu and Manganese (Mn) or Hg concentrations (Fig. 18). The Guantanamo River (RM) and MWR Marina (MM) sites, which are potential locations of relatively direct discharge from mainland Cuba and the Base, respectively, had generally lower concentrations of trace elements characteristic to raw and industrial wastewater signatures previously reported in the literature (Fig.18). It is noteworthy, however, that the MWR Marina (MM) site had relatively high concentrations of minor elements such as As, Cadmium (Cd), Co and Cr, which are more common to agriculture and urban runoff, compared to most other sites (Fig. 18, *cf* Fig. 19).

When site-specific elemental concentrations were normalized to mean background concentrations based on trace element signatures in shell from ancient reefs (pre-historical/ pre-urbanization) and the Caribbean Sea (estimated open water sources), more targeted evidence of human influence was detectable (Fig. 19). Most notably, the higher concentrations of some key elements (i.e.; Cu, Hg) were still readily apparent at the Mahomilla Bay (LM) and North Medio Cay (NC) sites. For example, Cu at Mahomilla Bay is estimated at levels ~20x background and Hg is 1.4x background. Higher ratios of Tin (Sn) and Vanadium (V) also emerged in shells from these sites (~13x and 9x background, respectively) as well as V at the River mouth (RM) and Ferry Landing (FL) sites. Despite lower ratios that reflected the overall lower concentration of elements at the River mouth site (RM), the combination of elements above background generally followed patterns found in Mahomilla Bay (LM), except for Pb and Zn (which were 1-2x background at RM but not LM). Small but elevated concentrations of As, Cd, Co, and Cr persisted in shells from the MWR Marina (MM) site, ranging from 1-4x background (Fig. 19).

Table 6. Mean ± S.E. (range) and statistical outputs comparing concentrations (ppm) of 18 trace elements among 8 sites in NS Guantanamo Bay. Site names correspond to locations in Fig. 3, Table 2. Bold values in orange indicate concentrations of individual elements that are significantly higher than at other sites except values shown in yellow, which are statistically similar to orange, but not necessarily different from other sites. N = number of individual elements at each site, found at the highest concentrations.

	Site								ANOVA statistics	
	MM	FL	RM	NC	MW	LM	WB	OR	$F_{7,2126}$	P
As	**0.17±0.55 (0-5.1)**	0.04±0.07 (0-0.3)	0.05±0.12 (0-1.7)	0.05±0.08 (0-0.58)	**0.15±0.59 (0-4.6)**	0.09±0.22 (0-1.6)	0.04±0.07 (0-0.32)	0.04±0.09 (0-1.2)	8.32	<0.001
Ba	1.2±2.3 (0-23)	9.1±14.0 (0.24-65)	5.4±3.4 (0-19)	0.9±0.8 (0-3.9)	1.7±1.3 (0-6.9)	2.0±1.1 (0-5.1)	2.1±1.3 (0-9.5)	**58.0±74.0 (0-514)**	81.98	<0.001
Cd	0.11±0.32 (0-2.5)	0.07±0.13 (0-0.61)	0.05±0.12 (0-1.0)	0.08±0.01 (0-0.50)	0.09±0.25 (0-1.9)	0.07±0.14 (0-0.91)	0.06±0.12 (0-0.79)	0.06±0.16 (0-2.4)	1.88	0.07
Co	0.44± 0.36 (0.03-2.7)	0.26±0.18 (0.04-1.7)	0.24±0.55 (0-7.2)	**0.53±0.27 (0.02-1.3)**	0.45±0.28 (0-1.6)	0.21±0.18 (0.04-1.6)	**0.58±0.43 (0-1.9)**	0.17±0.13 (0-298)	54.71	<.001
Cr	**1.7±1.8 (0-7.3)**	0.8±1.4 (0.05-14)	0.7±0.4 (0-3.2)	0.7±0.7 (0.08-6.7)	0.7±0.8 (0-7.4)	0.7±0.8 (0-4.8)	**1.4±1.2 (0-5.3)**	0.8±1.2 (0-33)	17.71	<.001
Cu	0.7±1.3 (0-12)	1.0±1.0 (0-4.7)	3.5±7.6 (0-91)	8.8±17.0 (0-101)	3.8±4.9 (0-29)	**24.0±89.0 (0-663)**	1.6±2.2 (0-13)	0.8±7.5 (0-238)	18.65	<0.001
Fe	1162±313 (477-1824)	857±94 (636-1080)	632±102 (26-1134)	**1486±572 (721-2823)**	1356±892 (519-9484)	644±175 (420-2076)	**1572±952 (35-3224)**	605±134 (223-1840)	248.60	<0.001
Hg	5.2±6.4 (0-26)	3.7±3.3 (0-14)	0.8±1.7 (0-13)	3.8±8.4 (0-73)	2.0±3.8 (0-23)	**8.2±25.0 (0-189)**	**9.0±10 (0-39)**	2.9±7.7 (0-205)	19.77	<0.001
Mg	115±37 (58-208)	**300±105 (46-582)**	211±94 (1-700)	203 ±67 (72-458)	259±145 (44-914)	220±86 (53-754)	229±89 (110-442)	218±81 (71-617)	62.03	<0.001
Mn	4.1±2.2 (0.93-16)	3.5±1.8 (0.92-9.9)	3.1±3.4 (0-21)	**10.0±7.4 (0.85-29)**	5.2±8.0 (0-51)	5.5±9.9 (0.77-81)	3.1±3.8 (0.03-34)	0.6±0.7 (0-11.06)	116.20	<.001
Ni	0.56±0.53 (0-3.4)	0.30 ±0.28 (0-1.29)	0.80 ±4.4 (0-77)	0.52±0.39 (0.03-1.8)	0.60 ±0.87 (0-8.5)	0.41±0.56 (0-3.7)	0.65±0.45 (0-3.7)	0.48±1.8 (0-41)	1.00	0.43
Pb	0.2±0.8 (0-6.9)	0.2±0.2 (0-0.85)	**0.4±0.9 (0-11)**	0.06±0.08 (0-0.35)	**0.4±0.8 (0-8.4)**	0.06±0.13 (0-0.86)	0.1±0.2 (0-0.94)	0.3±1.0 (0-17)	5.51	<0.001
Sn	0.5±0.9 (0-6.8)	0.3±0.6 (0-496)	0.3±2.6 (0-54)	2.3±6.1 (0-40)	0.7± 1.1 (0-7.7)	**4.7±13.0 (0-89)**	0.4±0.6 (0-3.3)	0.3±3.6 (0-115)	17.71	<0.001
Sr	1782±780 (105-6063)	1414±342 (356-2628)	2162±604 (0-4756)	1608±533 (869-4756)	2269±878 (356-4347)	2074±565 (741-4711)	1606±509 (141-4047)	2078±922 (754-11609)	22.88	<0.001
Ti	0.9 ± 2.7 (0-16)	0.1± 0.3 (0-1.92)	0.9±5.8 (0-70)	0.5±1.9 (0-15)	0.3± 0.7 (0-4.6)	1.7± 8.6 (0-73)	0.9±4.4 (0-40)	0.4±2.4 (0-44)	2.59	<0.01
V	0.11±0.24 (0-1.4)	0.01± 0.02 (0-0.07)	0.15±0.33 (0-2.3)	0.04±0.05 (0-0.3)	0.02± 0.07 (0-0.49)	**0.28 ±1.60 (0-13)**	0.05±0.21 (0-1.6)	0.02±0.77 (0-33)	10.41	<.001
Zn	1.3± 2.5 (0-14)	0.4± 0.7 (0-4.0)	**1.6± 6.4 (0-107)**	0.3± 0.7 (0-4.6)	0.5±0.9 (0-4.7)	0.8±1.5 (0-8.7)	1.5±1.8 (0-8.5)	0.7±1.7 (0-17)	4.98	<0.001
N	**5**	**1**	**2**	**3**	**4**	**7**	**5**	**3**		

Table 7. Potential sources of elements (symbol) commonly associated with anthropogenic pollutants (data from Helz *et al.*, 1975; GESAMP 1985; Windom *et al.,* 1989; DelValls *et al.*, 1998; Kawasaki *et al.*, 1998; Al-Arfaj and Alam, 1993; Neff 2002, Chiffoleau *et al.*, 2003; Navratil and Minarik, 2005; Garelick *et al.*, 2009; Purves 2012, Kassir *et al.*, 2012; Li *et al.*, 2013; Prapaiwong and Boyd, 2014; Tang, 2015).

Element	Anthropogenic sources to the environment
Arsenic (As)	Glass ceramics, textiles, paints, vessel traffic, and pesticides. Some semiconductors and microchips. Mobilized during mining activities. Potentially toxic.
Barium (Ba)	Fabric dye, paper manufacturing, and rubber. Fluorescent lamps, paint, bricks, tiles, and glass. Oil and gas industries (drilling fluid, wastewater). Abundant and readily dispersed.
Cadmium (Cd)	Industrial (smelting), oil, and sewage discharges, insecticides, fungicides, aquaculture, and urban runoff. Potentially toxic.
Chromium (Cr)	Metal and leather industries. Urban wastewater, oil products, pesticides, aquaculture. Some forms toxic.
Cobalt (Co)	By-product of Ni and Cu (among other) mining activities; Cuba is a major Ni producer. Oil refining. Potentially carcinogen.
Copper (Cu)	Wastewater, precipitation from fossil fuel combustion. Mining wastes, phosphate fertilizer production, fungicides, coal ash effluent, anti-fouling paints, aquaculture, industrial discharges and urban runoff. Linked to algal production.
Iron (Fe)	Industrial and urban wastes, aquaculture. Linked to algal production.
Manganese (Mn)	Iron and steel production, industrial wastewater. Linked to algal production.
Mercury (Hg)	Fertilizers and other agricultural activities, industrial wastewater disposal, paints and medical waste. Waste incineration and fossil fuel combustion. Mining activities. May biomagnify.
Molybdenum (Mo)	Ceramic glaze and lubricants. Petroleum refining. Nuclear energy production, missile and aircraft parts, metals processing. Potentially toxic.
Nickel (Ni)	Sewage, oil refining. Mined extensively in Cuba. Linked to brown tides.
Tin (Sn)	Vessel traffic, mining, pesticides, antifouling paints, synthetic rubber, and waste disposal. Trace amounts in canning, dyeprinting, and laundry waste.
Lead (Pb)	Batteries, paints, pesticides, sewage, stormwater runoff, and gasoline. Mining by-products and oil products. Computer screens, sheeting cables, lead crystal glassware, pipes, and ammunitions.
Vanadium (V)	Oil products, burning fossil fuels.
Zinc (Zn)	Industrial wastewater and industrial-based fertilizer, pesticides, mining, coal and waste combustion, aquaculture, and steel processing. Linked to algal production.

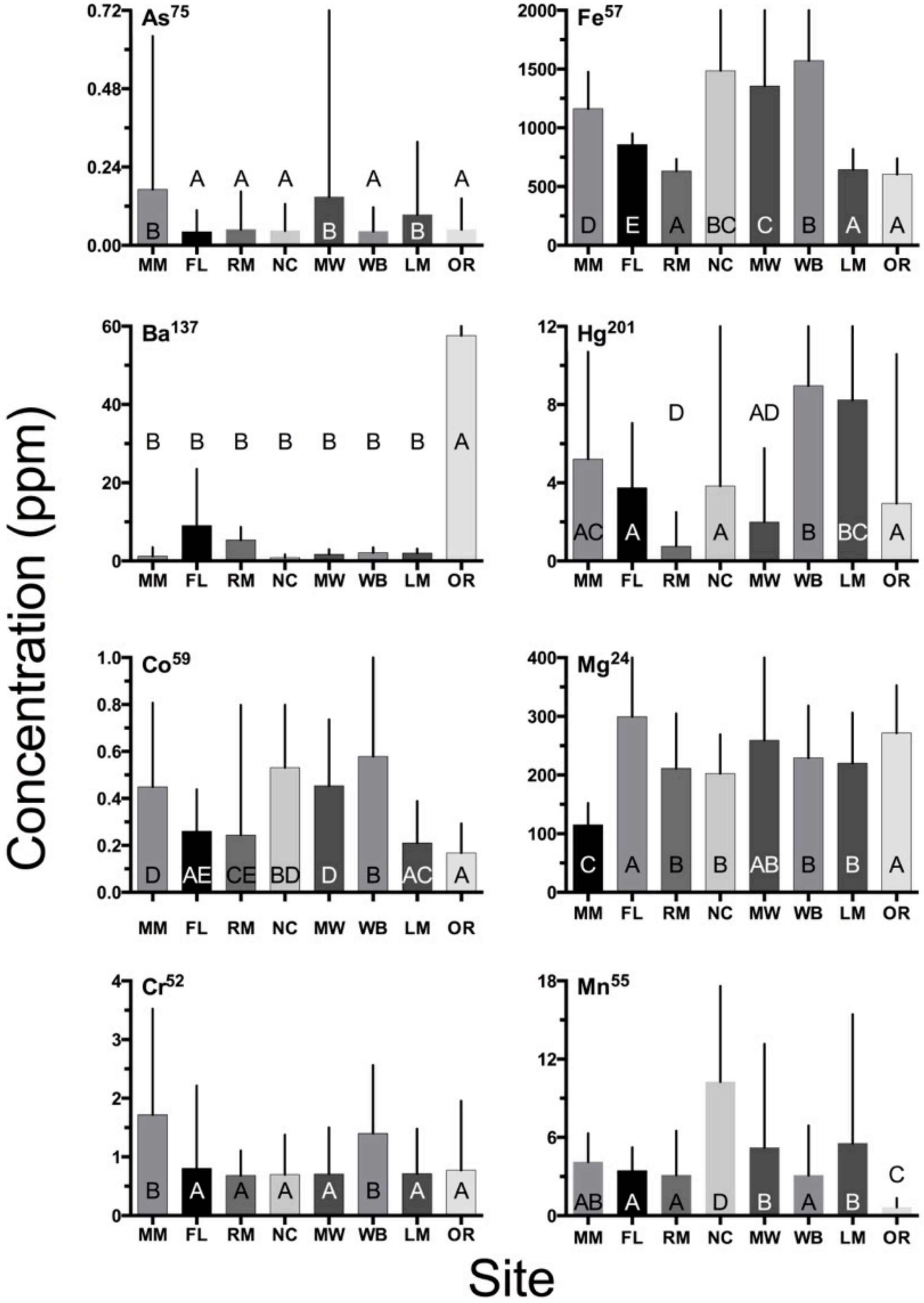
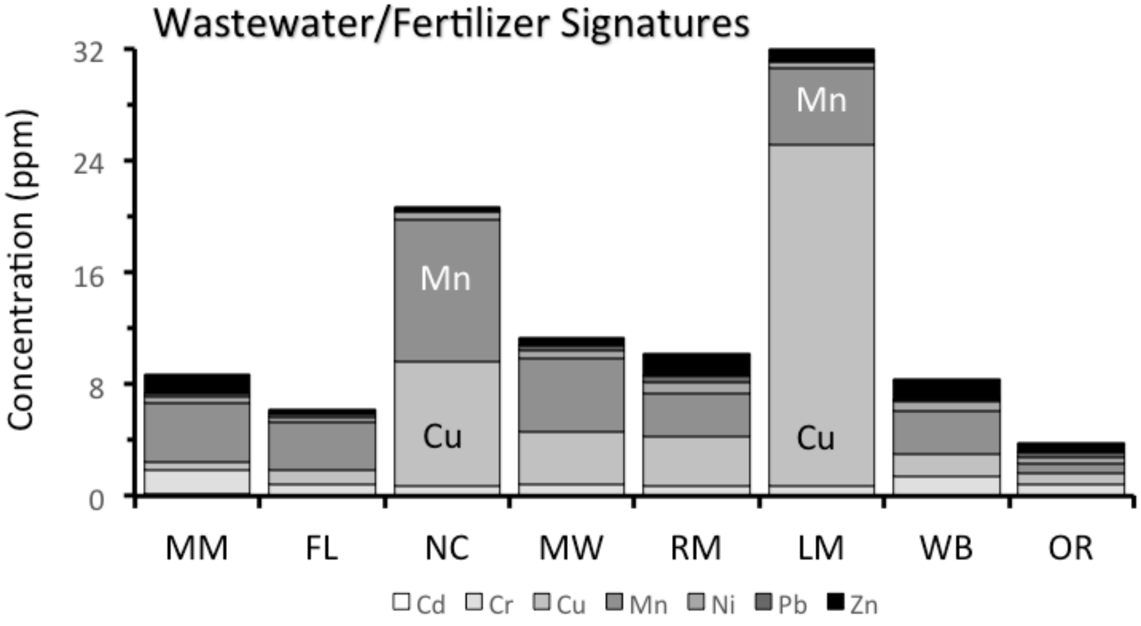


Fig. 17. Concentrations in parts per million (ppm) of selected trace elements in modern and ancient (OR) bivalve shells from sites in NS Guantanamo Bay, Cuba. Letters indicate statistical similarity among sites for a given element (Tuckey's post-hoc results; $p < 0.05$ for all comparisons). Site names correspond to locations in Fig. 3, Table 2.



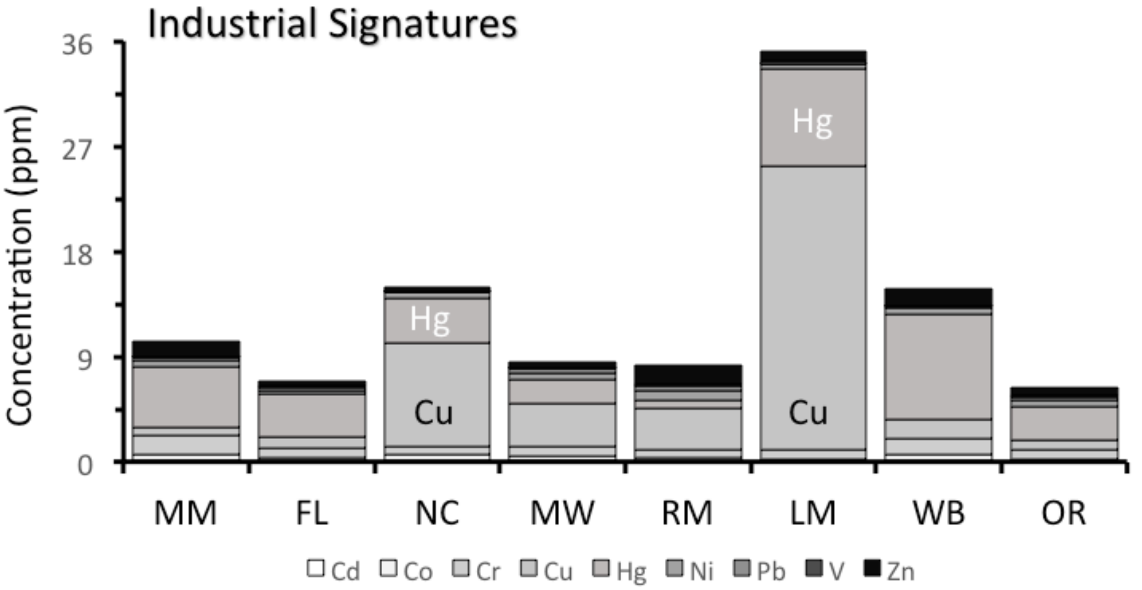


Fig. 18. Concentrations in parts per million (ppm) of trace elements typical to wastewater and fertilizer sources (top) and industrial discharge (bottom) measured in modern and ancient (OR) bivalves from sites in NS Guantanamo Bay, Cuba. Site names correspond to locations in Fig. 3, Table 2. Values for individual elements are shown in Table 6 and Fig. 17.

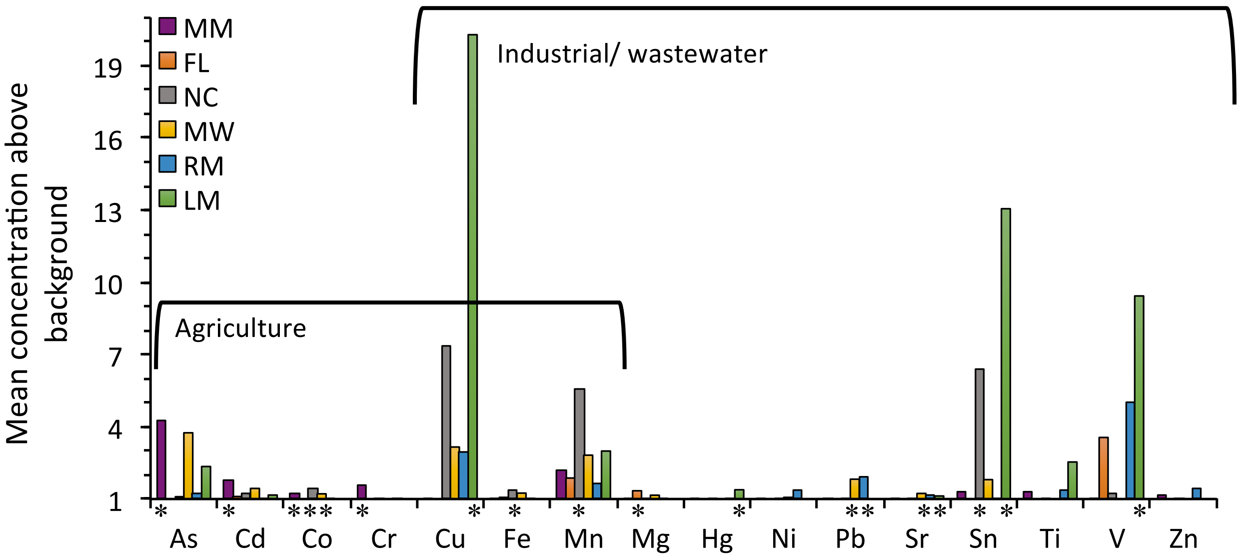


Fig. 19. Mean concentrations of trace elements in bivalve shell at 6 sites in NS Guantanamo Bay Cuba, normalized for estimated background (naturally occurring) elemental signatures. Site names correspond to locations in Fig. 3, Table 2. Background was defined by the mean concentration of each element in shells from ancient reefs (OR) and open ocean waters (WB). * indicates statistically significant highest mean values for each element; more than one asterisk indicates highest values were shared by multiple sites. Brackets indicate combinations of elements that are generally characteristic of agriculture activities (e.g., pesticides) or industrial and urban wastewater.

4.0 Discussion

4.1 Current Water Quality – Coral reefs flourish in oligotrophic tropical and subtropical waters and are susceptible to eutrophication as a result of low-level increases in nutrient concentrations (Johannes, 1975; Bell, 1992; Dubinsky and Stambler, 1996; NRC, 2000). At U.S. Naval Station Guantanamo Bay, nutrients (N and P) primarily enter into the coastal waters from a variety of sources, including: 1) sources upstream of the U.S. jurisdictional boundary (Upper Guantanamo Bay and upper Guantanamo River), 2) wastewater and sewage treatment plants and outfalls on the Base (*cf* 2), 3) groundwater from the Base, 4) stormwater runoff from the Base, and 5) atmospheric deposition. While some marine autotrophs with the enzyme alkaline phosphatase are capable of recycling organic P (Sun *et al.*, 2012), dissolved inorganic N (DIN; ammonium + nitrate) and P (soluble reactive phosphorus or SRP) are the forms of nutrients most readily assimilated by bloom-forming species of macroalgae and phytoplankton and therefore have the ability to promote phase shifts to macroalgae and phytoplankton blooms. When nutrients are abundant, excessive biomass of macroalgae and phytoplankton are referred to as harmful algal blooms (HABs), which can impair the health of coastal ecosystems (ECOHAB, 1995). Based on nutrient-gradient case studies conducted by Lapointe (1997), the tipping point for such phase shifts to macroalgae on Caribbean coral reefs can occur at very low concentrations of ~ 1 µM DIN and 0.1 µM SRP.

During this Phase II (2014-2015) study, the average DIN concentration throughout the sampling network was ~12 µM. While this is 12x higher than the established 1.0 µM threshold for promotion of phase shifts to macroalgal blooms and eutrophication on coral reefs, these high concentrations largely reflect nutrient enrichment of NS Guantanamo Bay proper from the surrounding watersheds and hence the relevance of these values to reef health may be debated. During the most extreme conditions in Fall 2015, mean DIN concentrations increased to > 30 µM (or 30x this threshold) in Port Palma (Station 7) and along Corinaso and Caravella Points (Stations 8 and 10, respectively; Table 4A). As demonstrated in Fall 2015, the highest overall ammonium and nitrate (and therefore DIN) concentrations were also documented at these three stations. Port Palma is a partially enclosed embayment with less circulation and Stations 8 and 10 are closest to the wastewater outfalls along the Windward side of the Base (Fig. 1). In addition to the high concentrations at these stations, DIN (especially ammonium) was, to a lesser extent, also entering into the system from the Guantanamo River (Fig. 7). When comparing nearshore reef stations, the ammonium and nitrate (and therefore DIN) concentrations were still above the 1.0 µM threshold, and consistently 2x higher at Chapman Beach (Station 5) than at Cuzco Beach (Station 6; Table 4E). Although wastewater treatment plants discharge an estimated 300 gallons per day on both sides of the Bay Mouth, Chapman Beach is within closer proximity to an outfall than Cuzco Beach and this is likely driving the nutrient levels up in this area (Fig. 2).

Unlike DIN, which appears to originate primarily from the wastewater outfalls on the Base and the Guantanamo River, the highest inputs of soluble reactive phosphorus are primarily being transported down the Guantanamo River and, to a lesser extent, is accumulating in Granadillo Bay (Station 9) and the waters around Caracoles Point (Station 11; Table 4E, Fig. 2). The project-wide mean SRP concentration (± S.E.) was 1 µM. Similar to DIN, this level is 10x that of the established threshold of 0.1 µM for community phase shifts in such tropical areas (Lapointe, 1997). Also unlike DIN, SRP concentrations were consistently lower during the Wet (0.4 ± 0.1 µM) than the Dry (1.4 ± 0.3 µM) season (Figs. 8, 9b). This is likely explained by the elevated NH_4 concentrations and TDN:TDP ratios (36-41) during the Wet season that foster strong P-limitation. The increased stormwater and groundwater inputs during the rainy season are characterized by higher N:P ratios, which causes macroalgae to become saturated with N, limited by P, and therefore scavenge the available P from the system. This results in lower concentrations of SRP (the primary form of P used by macroalgae and phytoplankton) during the Wet season as seen during this study.

Depending on the station, this system has an exceptionally wide range of N:P ratios. The reduced SRP concentrations that resulted from Wet season scavenging ultimately promoted high DIN:SRP ratios. This is most obvious in areas with a combination of high N inputs from stormwater, treatment plant outfalls, and septic tanks (i.e., Stations 2, 5, 8 10) and low SRP concentrations. Conversely, lower ratios were seen in the upper Guantanamo River where there was both relatively high N concentrations and consistent P loading with no macroalgae (only phytoplankton) to assimilate the SRP.

Unlike the inorganic forms of N and P, TDN and TDP concentrations were consistently higher during the Dry than the Wet season (Fig. 10a,b). The total form is comprised of both inorganic (NH_4, NO_3, and SRP) and organic forms of N and P and is strongly affected by biological nutrient cycling. For example, macroalgae readily assimilate inorganic N and P forms, but can also excrete organic forms. Accordingly, macroalgae grow and assimilate inorganic nutrients into biomass during Wet season, reducing the amount of available DIN and SRP in the water column. In the Dry season, macroalgae typically experience nutrient-limited growth (reduced inorganic nutrient loads to fuel growth) so the macroalgae excrete more dissolved organic N and P, ultimately increasing those TDN and TDP concentrations in the water column. The highest TDN concentrations were generally documented in relatively sheltered areas, such as the upper Guantanamo River, Mahomilla Bay, and Port Palma that are potentially allowing dissolved organic nitrogen (DON) to pool over time. This phenomenon has also been documented along the east coast of Florida in the poorly flushed northern Indian River Lagoon (Lapointe *et al.*, 2015). More consistently than TDN, TDP is elevated throughout the study area and again suggests potential pooling of nutrients during the Dry season. These higher TDN and TDP concentrations generally result in lower TDN:TDP ratios as seen in this study (Fig. 10c).

During Phase I (initiated in 2011), macroalgae were used as “bio-observatories” to help identify patterns of nutrient limitation within NS Guantanamo Bay and the nearshore reefs through C:N, C:P, and N:P ratios (Lapointe and Herren, 2013). Macroalgae C:N ratios were > 12, suggesting N-limitation along the nearshore reefs and at all stations within NS Guantanamo Bay except the River Mouth (Station 2 in Phase II). These C:N ratios in benthic macroalgae were higher than those documented in phytoplankton during Phase II where, conversely, phytoplankton C:N ratios were all close to the Redfield ratio of 6.63, which is considered by some to be N replete, thus suggesting weak N-limitation (Fig. 13). One consistency between the two types of algae was that the C:N ratios were low for both macroalgae (9.8) and phytoplankton (6.6) at the River Mouth, suggesting persistent N inputs to the system from the Guantanamo River. This conclusion was supported by the dissolved nutrient data associated with Phase II discussed above. The highest DIN concentrations were documented in the Guantanamo River and Port Palma (Station 7), Corinaso Point (Station 8) and Caravella Point (Station 10); all of which also had low phytoplankton C:N ratios. Unlike this correlation between high DIN and lower C:N ratios for the Guantanamo River and Guantanamo Bay, the opposite was seen for the nearshore reefs. During Phase II, DIN concentrations were consistently 2x higher at Chapman Beach (Station 5) than Cuzco Beach (Station 6), yet the C:N ratios in phytoplankton were consistently the highest at Chapman Beach (11), suggesting low N inputs, and lowest at Cuzco Beach (5), suggesting high N inputs, whereas the C:N in macroalgae at these two stations were both ~19 (Lapointe and Herren, 2013).

In addition to nutrient limitation, macroalgae (Phase I) and phytoplankton (Phase II) were also used to identify the sources of N fueling harmful algal blooms within NS Guantanamo Bay and the nearshore reefs through $\delta^{15}N$ stable isotope analysis. Of most interest from a management standpoint is the identification of human-impacted areas such as those influenced by wastewater (> +3 $^{o}/_{oo}$) or fertilizers (-2 to +2; values that overlap with atmospheric sources). In Phase I, the macroalgae collected in all areas of Guantanamo Bay, Mahomilla Bay, and the River Mouth had an isotopic signature > +3 $^{o}/_{oo}$, suggesting wastewater influence in each of these regions (Lapointe and Herren, 2013). The signatures were especially enriched in macroalgae collected at the Leeward Ferry Landing (+11 $^{o}/_{oo}$) and MWR Marina, Caracoles Point, and Radio Point (all > +7 $^{o}/_{oo}$); all areas located near wastewater outfalls (Fig. 2). During Phase II, the project-wide mean $\delta^{15}N$ values in phytoplankton were most enriched in the Guantanamo River (~ +5 $^{o}/_{oo}$) and Guantanamo Bay (~ +3 $^{o}/_{oo}$) suggesting impacts from wastewater N in both regions, especially during the Summer, Fall, and Winter seasons (Table 5A-C). These mean values are at the lower range reported for wastewater-derived N and suggest minimal processing. The generally lower $\delta^{15}N$ values in phytoplankton (Phase II) compared to macroalgae (Phase I) could reflect generally greater amounts of detrital material and/or sediments in the phytoplankton samples, which would tend to favor more depleted values. In addition, phytoplankton undergo isotopic fractionation during the uptake of ammonium and nitrate, especially during bloom formation in nutrient enriched areas like NS Guantanamo Bay, that would also tend to produce more depleted values (Pennock *et al.*, 1996).

Additional water and phytoplankton samples were collected at apparent bloom sites in June (near MWR Marina) and July (near Pier 33) to the north and south of Deer Point (Fig. 2), respectively, and a third site just offshore of the hospital on Caravella Point in August 2015. The $\delta^{15}N$ signal in the phytoplankton collected at these sites were exceptionally enriched, +9.4 $^{o}/_{oo}$ at MWR Marina, +8.5 $^{o}/_{oo}$ at Pier 33, and +5.9 $^{o}/_{oo}$ near the hospital, implying assimilation of processed N from the adjacent wastewater outfall on Caravella Point and the East Caravella septic tanks (Fig. 2). Lapointe and Herren (2013) also reported enriched $\delta^{15}N$ values (> +7 $^{o}/_{oo}$) for macroalgae at MWR Marina (near Deer Point) and Radio Point during Phase I. Along with Caracoles Point and the Leeward Ferry Landing, the macroalgae collected at these two sites were the most enriched during the Phase I study. Accordingly, the enriched signature seen in the phytoplankton in Phase II is consistent with Phase I results for this area in NS Guantanamo Bay. The enriched phytoplankton $\delta^{15}N$ values were further supported by the elevated ammonium concentrations in the additional water samples collected near the MWR Marina, Radio Point, and the hospital compared to other water samples collected at the twelve fixed stations in the monitoring network during the same sampling period. The mean NH_4 concentrations (± S.E.) were 1.4 ± 0.8 µM at

MWR Marina in June, 14.4 ± 2.3 μM at Pier 33 in July, and 3.4 ± 0.8 μM near the hospital in August.

Wastewater is a major and increasing source of N and P pollution and contributes to eutrophication and public health threats along many tropical and subtropical coastlines (Caperon *et al.*, 1971; Windom, 1992; Siung-Chang, 1997; Wear and Vega Thurber, 2015). Wastewater generated at U.S. Naval Station Guantanamo Bay is currently managed through eleven outfalls; five associated with wastewater treatment plants and six with sewage treatment plants, as well as three septic systems and one peat boiler (Fig. 2). Of the five wastewater treatment plants, two discharge a total of 600,000 gallons per day to the nearshore reefs and three discharge 685,000 gallons per day directly into Windward side of NS Guantanamo Bay between the Windward Ferry Landing and Granadillo Bay (~ 600 gallons per day through the outfall on Radio Point alone; Fig. 2). In addition to the wastewater treatment plants, six sewage treatment plants release ~ 200,000 gallons per day to the Windward side of NS Guantanamo Bay (Fig. 2). These secondary treatment activated sludge and trickling filter processes incorporate a separation phase to remove settleable solids and a biological process to remove dissolved and suspended organic compounds such as sugars, fats, detergents, human and food waste. In accordance with the Overseas Environmental Baseline Guidance Document for NS Guantanamo Bay, these processing plants are not required to, nor do they, incorporate disinfection, chlorination, or fecal coliform monitoring. The three septic tanks are located at Granadillo Circle, East Caravella, and the Leeward Ferry Landing and the peat boiler is located at the east end of the Leeward Ferry Landing (Fig. 2). There is also one stormwater outfall, which likely does not contribute to the wastewater loading, located by the Leeward Ferry Landing. Considering the enriched stable N isotope data presented above for both Phase I macroalgae and Phase II phytoplankton compared to other areas on the Base, the outfalls between the Windward Ferry Landing near Corinaso Point and Radio Point, as well as the outfalls and the septic tanks between Caravella Point and Granadillo Circle, are impacting the water quality on the Base (Fig. 2). This is especially apparent near Radio Point where the majority (88%) of the wastewater is being discharged into NS Guantanamo Bay. The presence of Clionid sponges, biological indicators of sewage pollution, inhabiting this area provided addition evidence of wastewater impacts (Ward-Paige *et al.*, 2005; Risk *et al.*, 2014).

Another common symptom of wastewater-driven eutrophication in tropical and subtropical coastal waters is the development of macroalgal blooms in coral reef communities (Pastorok and Bilyard, 1985; Lapointe and Herren, 2013) and increased concentrations of phytoplankton (Caperon *et al.*, 1971; Laws and Redalje, 1979). Early studies in Kaneohe Bay, Hawaii, documented how reef corals were overgrown by the green "bubble alga" *Dictyosphaeria cavernosa* as a result of small increases in N and P concentrations from wastewater pollution (Banner, 1974; Smith *et al.*, 1981). Lapointe and Herren (2013) documented elevated δ^{15}N values in the range of wastewater N in macroalgae collected in NS Guantanamo Bay (+6.1 $^{o}/_{oo}$)

and the nearshore reefs (average +3 $^{o}/_{oo}$). The mean δ^{15}N value in NS Guantanamo Bay was similar to the δ^{15}N value documented in the wastewater-impacted Indian River Lagoon (+6.3 $^{o}/_{oo}$) along the east coast of Florida (Lapointe *et al.* 2013). In the Phase I macroalgae study, it was hypothesized that the while N was likely entering into the system through storm and wastewater inputs, the primary land-based N source to NS Guantanamo Bay and adjacent Caribbean coastline was identified as the Guantanamo River. A more in depth look at water chemistry in the 2014-2015 Phase II study suggests that while both N and, especially, P enter the system from the Guantanamo River, significant contributions are also coming from the Windward side of the Base. Port Palma (Station 7, a poorly circulated area) and the stations adjacent to a high density of wastewater outflows and septic tanks at Corinaso (Station 8) and Caravella (Station 10) have exceptionally high N concentrations likely associated with the nearby outfalls and groundwater inputs.

Despite the nutrient hotspots in the Guantanamo River, Port Palma, and the areas near the outfalls and septic tanks along the Windward side of the Base, chlorophyll *a* concentrations, an indicator of HAB biomass, were highest along the northern boundary. Throughout Phase II, chlorophyll *a* concentrations were consistently > 20 µg/L at Port Palma (Station 7) and Watergate (Station 12), the northernmost stations in Guantanamo Bay. This suggests that the algal blooms are originating in the upper Bay north of the boundary, migrating downstream during the ebbing tide, then potentially being sustained by nutrients inputs from the Base (Fig. 1). An algal bloom was also seen in the upper station in the Guantanamo River in the Fall, where mean concentrations reached 18 µg/L during the Wet season (Fig. 11). Based on the lack of development in this area, it is likely that the nutrients fueling the bloom (high P concentrations) in the Guantanamo River were also originating upstream of the U.S. boundary.

The first bloom of the pelagophyte *Aureoumbra lagunensis* D.A. Stockwell, DeYoe, Hargraves and P.W. Johnson to be recorded in NS Guantanamo Bay occurred in 2013 (Fig. 1; Koch *et al.*, 2014); one year after the first recorded bloom of this species along the east coast of Florida (Gobler *et al.*, 2013). According to Gobler and Sunda (2012), coastal waters most susceptible to brown tides are relatively warm, shallow, and hypersaline, with little tidal exchange (high residence times). In addition to these factors, Lapointe *et al.* (2015) documented high ammonium and TDN enrichment (61–82 µM) during the Florida bloom with correspondingly high TDN:TDP ratios (49:1 to 71:1). Regardless of the station, the mean TDN concentrations in and adjacent to NS Guantanamo Bay were consistently > 100 µM, but because of relatively high P concentrations (> 5 µM) throughout the system, N:P ratios were < 50:1. Although high N:P ratios generally indicate P-limitation, some phytoplankton have high growth rates at high N:P ratios (> 70) while experiencing strong P-limitation (Terry *et al.*, 1985; Sun *et al.* 2012). For example, *A. lagunensis*, which formed persistent and damaging blooms in Laguna Madre, TX, is capable of growing under a wide range of N:P ratios (Liu *et al.*, 2001) and actually formed dense blooms when the water column N:P ratio increased to high levels (140; Rhudy *et al.*, 1999). This is partially because of the species ability to use an enzyme, alkaline phosphatase, to cleave P from organic forms when inorganic P is limited (Muhlstein and Villareal, 2007; Sun *et al.,* 2012). Similarly, the cyanobacterium *Synechococcus* spp., which formed severe and damaging blooms in Florida Bay between 1991 and 1996 following increased freshwater flows and N-loading from Everglades runoff (Brand, 2002; Lapointe and Barile, 2004), can outcompete other phytoplankton species under high N:P ratios and P-limited conditions (Lavrentyev *et al.*, 1998; Richardson, 2009).

4.2 Historical changes in water quality and pollution sources –The combination of stable isotope ratios and trace metal concentrations in bivalve shells were useful to identify historical changes in pollution sources to the Guantanamo Bay system. Using $\delta^{15}N$ ratios in shells from ancient reefs as a baseline for nutrient sources, our data demonstrated that modern bivalves at the MWR Marina (MM) site likely incorporated N into their shells more so from processed human wastewater (septic and treated wastewater sources) than at any other site. This finding is consistent with the high nutrient concentrations, enriched $\delta^{15}N$ ratios in suspended particulate matter, and known land use in the area between Corinaso and Caravella Points (Fig. 2), including the 600 gallon per day discharge at Radio Point, which is immediately adjacent to the MWR Marine (MM) sampling station. Similarity between $\delta^{15}N$ values in ancient clams and those at other sites could indicate that nutrients are conveyed from natural sources (less human influence) to these areas or that the sites are influenced by raw (unprocessed) wastewater or a combination of modern sources that isotopically resemble ancient N sources to the area. The high nutrient concentrations and $\delta^{15}N$ values in suspended particles documented during some water quality sampling periods in the Guantanamo River were not clearly reflected in shells of modern clams sampled at the River mouth (RM site). This lack of correspondence could be due to variation in C and N stable isotope ratios between water quality and bivalve sampling stations, driven by dilution or mixing of N sources down stream toward the River mouth.

Trace element ratios in ancient clams provided an additional measure of anthropogenic influence to the Guantanamo Bay system. Overall, average concentrations of most elements (Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr, V, Zn) in bivalve shell were within the range found in sediments, biota, and some water samples previously measured in the Caribbean (Fernandez *et al.* 2007), which tend to be higher than measured elsewhere (e.g.; Risk *et al.* 2010, Kuman 2010, El-Sorgy 2012, Li & Xie 2016). Cu and Hg were found at most sites at concentrations higher than previously reported in shell or coral skeletons in other studies (Risk *et al.* 2010, Kuman 2010, El-Sorgy 2012, Lamborg & Carmichael unpublished data), except in known polluted areas (Trombini *et al.* 2003). The work of Bourgoin (1990) and Risk established that a typical ratio of shell values to tissue values was 1:10 (i.e., shells may record 1/10 of the tissue levels), meaning that live clams at NS Guantanamo Bay could have an order of magnitude higher concentration of Hg than measured in shell. Measurement of metal concentrations in soft tissues of clams or other animals at NS Guantanamo Bay was not part of the scope of this study, however, and requires additional research to confirm this relationship. For this reason Cu and Hg, which are known to be highly toxic, in particular, warrant further study in this system. Some elements were on average lower than previously measured in Caribbean sediments and biota (As, Cd, Co, Mg), but were comparable to values measured elsewhere (Risk *et al.* 2010, Kuman 2010, El-Sorgy 2012). Human presence in and adjacent to receiving waters, including industrial, urban, agriculture and recreational activities has been implicated as contributing to the consistently high concentrations of trace elements measured at sites throughout the wider Caribbean region and has prompted a call for enhanced bio-toxicological sampling to inform environmental management in the area (Fernandez *et al.* 2007).

The low trace element concentrations and the low variance in concentrations among groups of elements known to be associated with specific sources of pollution (i.e., human or industrial wastewater, agriculture) in ancient shells compared to modern shells, corroborated ancient shells as a baseline for conditions of little or no human influence. Unlike other elements that more directly indicate pollution sources, variation in barium values, which were relatively elevated in ancient shells, could be due to physiological differences between species, differences in water column concentrations of Ba through time or differences in diet (available or selected) among ancient and modern species (Barat *et al.* 2009). In particular, high barium concentrations in bivalve shell have been associated with a higher diatom diet in some species (Thébault *et al.* 2009). While high Barium concentrations in fossil shell have also been attributed to weathering and diagenesis (Pilkey and Goodell 1964), values comparable to those in this study were reported in modern marine hard clams (*Mercenaria mercenaria*) and surf clams (*Spisula solidissima*) (Stecher *et al.* 1996), suggesting that although higher than in modern shells we measured, these values are not unusual. Elevated Ba values are also known to reflect terrestrial input from soil erosion (McCulloch et al. 2003), which could be recorded in shell due to historical changes in sea level. Trace element signatures in shells from Windmill Beach (WB) were assumed to represent background signatures of trace elements from open waters of the Caribbean Sea that mix with waters of NS Guantanamo Bay. Concentrations of some elements characteristic to anthropogenic sources (Co, Cr, Fe, Hg) were on average highest at Windmill Beach, however, suggesting that either open waters are a potentially significant source of these elements to Guantanamo Bay or that an undefined anthropogenic source may influence this nearshore sampling location. As a result, estimates of elemental contamination above background reported in Fig. 19 are likely conservative due to the use of trace element values in shell from Windmill Beach in combination with values in ancient shell to represent background. Given the potential for high background elemental concentrations in this region (Fernandez *et al.* 2007), however, this approach provided the best opportunity to unambiguously identify trace elements that are present in the NS Guantanamo Bay at relatively elevated concentrations and most likely from land-derived, anthropogenic sources. Accordingly, when elemental concentrations in modern clams at the remaining sites were normalized to estimated naturally occurring background, in every case, we found some elements at concentrations above background, with distinct spatial distributions relative to industrial and urban wastewater compared to agriculture and runoff sources.

In all, the site-specific distributions of combined $\delta^{15}N$ and trace element data produced evidence of at least five different potential sources of pollution to the Guantanamo Bay system, including: 1) industrial and urban wastewater (Mahomilla Bay, North Medio Cay, Guantanamo River mouth), 2) processed wastewater (MWR Marina, River mouth, Ferry Landing), 3-5) pesticides, urban runoff (e.g., stormwater), and vessel traffic (MWR Marina, western NS Guantanamo Bay).

The spatial distribution of elemental signatures indicates that pollution likely originates from a combination of Base and mainland Cuba. Elevated Cu and Mn or Hg in shells of bivalves from Mahomilla Bay and North Medio Cay, in particular, are characteristic of discharges of industrial and urban wastewater (Helz *et al.*, 1975; Kawasaki *et al.*, 1998; Kassir *et al.*, 2012) that receives lower level or no treatment and/or leachate from antifouling paints historically used on Navy ships (Kaznoff and Rudroff, 2000). The proximity of North Medio Cay to the northern boundary of NS Guantanamo Bay, however, makes it likely that elemental values at this site reflect waste from mainland Cuba that is diluted by open water from the Caribbean Sea as it moves further south in NS Guantanamo Bay. High concentrations of elements in Mahomilla Bay could result from retention of pollutants (discharged from upstream in the Guantanamo River or directly dumped to the site; there is an adjacent stormwater outfall) that have been retained in the relatively enclosed embayment, if the site is not well flushed. Presence of Fe, Sn, Sr, and V also suggest that industrial or oil-derived contaminants (Helz *et al.*, 1975; Al-Arfaj and Alam, 1993; Kawasaki *et al.*, 1998; Carmichael *et al.*, 2012) may be delivered to Mahomilla Bay. Presence of a similar suite of elements but at lower concentrations (except Pb and V) in shells from the River mouth suggest this site is also somewhat influenced by industrial and urban wastewater, potentially from the same origin as Mahomilla Bay, but that the river either a) has not been a significant source of trace metal pollution to the Guantanamo Bay system or b) is flushed past the mouth sufficiently to avoid accumulation of higher concentrations of elements, at least in the past 3-19 years represented by the bivalves we collected. In contrast, the elements derived from processed wastewater (such as delivered by septic systems and some wastewater treatment facilities), vessel traffic, and urban runoff at the MWR Marina most likely originate from the Base. MWR Marina is located in an area of high boat activity, central to the major wastewater outfalls on the Base, including the single treatment facility with the largest daily outflow. Pesticides or other chemicals may enter this site from the adjacent watershed through surface runoff from adjacent impervious surfaces associated with the Marina and nearby urbanization. Additional study of hydrodynamic patterns in Guantanamo Bay system and determination of stable isotope and elemental signatures in effluents from known sources would help decipher the origin and movement of these contaminants in the system.

Regardless of origin, the patterns in quantity, type and spatial distribution of pollution to the Guantanamo Bay system were traceable in the food web. Modern bivalves were larger and represented a broader range of age classes, including older clams at the Guantanamo River mouth site, where nutrient and chlorophyll *a* concentrations currently are high. Because phytoplankton is the major food source for bivalve shellfish, which are suspension feeders, this finding indicates that nutrient inputs to this location not only fueled primary production, but also contributed to secondary production (increased local shellfish growth) at least at the level of this primary consumer. Clams from the MWR Marina, where chlorophyll *a* concentrations were modest, were among the smallest at age. This finding suggests that food resources may be limiting for clams in this area despite high wastewater-derived nutrient inputs. Concentrations of the trace elements known to enhance phytoplankton growth were also low at the MWR Marina (with the exception of Fe), but several potentially toxic elements typically associated with pesticides and agriculture activities were significantly above background (As, Cd, Co, Cr) and may act to increase physiological stress and limit bivalve growth and survival at the site (Luoma and Phillips, 1988). The net effect of combined nutrient and elemental pollution on phytoplankton and primary consumers such as bivalves is not well studied and results are conflicting, but previous study indicates there is potential for both complementary (enhancement of production) and limiting (increased physiological stress and reduced production) effects (Breitburg *et al*., 1999).

5.0 Summary

In summary, the results of the water quality and bivalve monitoring effort in NS Guantanamo Bay and on the adjacent nearshore reefs suggest high N and, especially P, inputs to the system from three main regions: 1) upstream of the Base through the Guantanamo River, 2) Upper Guantanamo Bay upstream of Watergate, and 3) local wastewater outfalls and septic tanks on the Base. The high N concentrations documented near the outfalls explain the enriched $\delta^{15}N$ signature (> +3 $^{o}/_{oo}$) recorded in macroalgae (Phase I) and phytoplankton (Phase II) along the developed portion of the windward shoreline. In addition to the wastewater N signature around the outfalls, enriched $\delta^{15}N$ signatures were documented in phytoplankton collected from the Upper Guantanamo River, indicating relatively constant wastewater contamination from upstream of the U.S. border. The highest chlorophyll *a* concentrations were documented at the north boundary and the associated phytoplankton were likely entering onto the Base from upstream of the U.S. border in both Guantanamo River and Guantanamo Bay. Once such blooms move downstream onto the Base, they are likely maintained by nutrient inputs from the local outfalls.

We found significant site-specific patterns in pollution sources, including increases in the concentration and relative distribution of elements, entering the Guantanamo Bay system in recent years. Modern sources of pollution that were identified included **industrial and urban**

wastewater, **processed wastewater, pesticides, urban runoff, and vessel traffic**, including leachate from anti-fouling paints. Areas of high nutrient input did not necessarily coincide with areas of high metal contamination. In particular, the combination of moderate $\delta^{15}N$ values and lower trace element concentrations in clam shells at the Guantanamo River mouth suggest that the Guantanamo River may convey anthropogenic nutrients, but may be less of a conduit for other pollutants than initially suspected. In contrast, areas near Mahomilla Bay (a semi-enclosed embayment) and North Medio Cay (in the northern part of the Bay) showed evidence of high historical and current industrial and unprocessed urban wastewater from unknown sources possibly including mainland Cuba. These sites also had significantly higher Cu concentrations than any other locations, suggesting possible additional inputs associated with antifouling paint. The MWR Marina and adjacent waters appeared to receive nutrients and trace elements associated with land use at the Base, particularly processed human wastewater and small quantities of some potentially toxic elements from agriculture and runoff.

Trace metals have potential to influence the magnitude of algal blooms as well as impart physiological stress on local organisms. Hence, the net effect of interactions between nutrients and trace metals has significant potential to affect recovery of a system from eutrophication if only one type of pollution is managed (Breitburg *et al*., 1999). Potential for ecosystem level interactions between nutrients and trace metals were detectable in the Guantanamo Bay system in the form of variation in bivalve growth-at-age relative to the combination of nutrient, trace metal and chlorophyll *a* concentrations. Additional study of stable isotope ratios and elemental signatures in effluents from known sources along with study of the hydrodynamics of the Guantanamo Bay system would improve understanding of the origin and movement of these contaminants in the system.

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