Title: Consortial brown tide - picocyanobacteria blooms in Guantánamo Bay, Cuba

Nathan S. Hall[1], R. Wayne Litaker[2], W. Judson Kenworthy[3], Mark W. Vandersea[2], William G. Sunda[4], James P. Reid[5], Daniel H. Slone[5], Susan Butler[5]

[1] University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell St. Morehead City, NC 28557 USA. Email: nshall@email.unc.edu. Tel: +1 252-726-6841 ext. 228

[2] National Oceanic and Atmospheric Administration, National Ocean Service, Center for Coastal Fisheries and Habitat Research, 101 Pivers Island Rd. Beaufort, NC 28516 USA

[3] 109 Holly Lane, Beaufort, NC 28516

[4] University of North Carolina, Department of Marine Sciences, 292 Old Piedmont Circle Chapel Hill, NC 27516

[5]U.S. Geological Survey, Wetland and Aquatic Research Center, Gainesville, Florida, 32653 USA

Graphical Abstract:

Caption: True color satellite image of brown tide bloom in Guantánamo Bay, Cuba on 21 January 2013 showing high bloom biomass in the northern regions of the bay contrasted strongly against the clear Caribbean waters to the south (DigitalGlobe 2013).

Highlights:

- Consortial blooms of the pelagophyte *Aureoumbra lagunensis* and the picocyanobacterium *Synechococcus* documented in Guantánamo Bay, Cuba
- The *A. lagunensis* bloom was short-lived (<10 months), unlike a similar bloom of this species in Laguna Madre, Texas, which lasted 8 years
- Strong hypersalinity is not required for *A. lagunensis* brown tide formation
- *A. lagunensis* dominance does not necessarily lead to low biomass of microzooplankton grazers
- *A. lagunensis* and picocyanobacteria co-occur during brown tide events, with dominance often shifting between the two groups, indicating brown tides may be considered as complex pelagophyte/cyanobacterial blooms rather than just brown tide blooms.

Abstract:

A brown tide bloom of *Aureoumbra lagunensis* developed in Guantánamo Bay, Cuba during a period of drought in 2013 that followed heavy winds and rainfall from Hurricane Sandy in late October 2012. Based on satellite images and water turbidity measurements, the bloom appeared to initiate in January 2013. The causative species (*A. lagunensis*) was confirmed by microscopic observation, and pigment and genetic analyses of bloom samples collected on May 28 of that year. During that time, *A. lagunensis* reached concentrations of 900,000 cells ml^{-1} (28 ppm by biovolume) in the middle portion of the Bay. Samples could not be collected from the northern (Cuban) half of the Bay because of political considerations. Subsequent sampling of the southern half of the Bay in November 2013, April 2014, and October 2014 showed persistent lower concentrations of *A. lagunensis*, with dominance shifting to the cyanobacterium *Synechococcus* (up to 33 ppm in April), an algal group that comprised a minor bloom component

on May 28. Thus, unlike the brown tide bloom in Laguna Madre, which lasted 8 years, the bloom in Guantánamo Bay was short-lived, much like recent blooms in the Indian River, Florida. Although hypersaline conditions have been linked to brown tide development in the lagoons of Texas and Florida, observed euhaline conditions in Guantánamo Bay (salinity 35-36) indicate that strong hypersalinity is not a requirement for *A. lagunensis* bloom formation. Microzooplankton biomass dominated by ciliates was high during the observed peak of the brown tide, and ciliate abundance was high compared to other systems not impacted by brown tide. Preferential grazing by zooplankton on non-brown tide species, as shown in *A. lagunensis* blooms in Texas and Florida, may have been a factor in the development of the Cuban brown tide bloom. However, subsequent selection of microzooplankton capable of utilizing *A. lagunensis* as a primary food source may have contributed to the short-lived duration of the brown tide bloom in Guantánamo Bay.

Keywords:

Aureoumbra lagunensis, *Synechococcus*, nutrients, residence time, microzooplankton, Guantánamo Bay

1. INTRODUCTION

Aureoumbra lagunensis and *Aureococcus anophagefferens* (Pelagophyceae) are nanoplanktonic (2- 5 μm diameter) pelagophytes that can form dense, nearly monospecific "brown tide" blooms in coastal lagoons and estuaries (Gobler and Sunda, 2012). Although brown tides pose no known risk to human health, they can have severe negative impacts on ecosystem

functions leading to their classification as ecosystem disruptive algal bloom (EDAB) species (Sunda et al., 2006). Frequently, growth and survival of a wide range of benthic and planktonic grazers is drastically reduced during brown tide blooms (Jakobsen et al., 2001; Buskey and Hyatt, 1995; Gobler and Sunda, 2012; Gobler et al., 2013) with resultant declines in biomass and diversity of the grazer communities and reduced carbon flow to higher trophic levels (Buskey and Stockwell, 1993). The severe light attenuation associated with these blooms can also cause mortality of seagrass beds that provide important ecological functions such as sediment stabilization, food sources for grazer communities, and nursery and foraging habitat for a host of invertebrate and vertebrate taxa (Dennison et al., 1989; Onuf, 1996; Phlips et al., 2015). Due either to the toxicity or poor nutritional value of brown tide cells, commercially important shellfish stocks such as clams and scallops have been decimated by brown tide blooms (Tracey, 1988; Gobler and Sunda, 2012; Gobler et al., 2013).

Compared to larger microplankton competitors, nanoplankton and picoplankton generally have faster growth rates (Banse, 1976), and their greater surface area to biomass makes them superior competitors under conditions of low light and nutrient availability (Gobler and Sunda, 2012; Sunda and Hardison, 2010). Generally, the growth advantages of small size are offset by increased susceptibility to microzooplankton grazing (Kiørboe, 1993; Thingstad et al., 2005; Sunda and Hardison, 2010) which stabilizes populations of very small phytoplankton at low biomass levels (Cushing, 1989; Barber and Hiscock, 2006). Contrary to the general trend toward higher maximum growth rates with decreasing cell size, the maximal growth rates of brown tide species are relatively low compared to microplankton competitors (e.g. diatoms) (Muhlstein and Villareal, 2007; Sunda and Hardison, 2007, 2010). The current conceptual model of EDAB development including brown tides postulates that lower growth rate is attributed to the cells

diverting a greater part of their fixed carbon into compounds that deter grazing (Ward et al., 2000; Buskey et al., 2001; Sunda and Hardison, 2010; Kang et al., 2015), thereby providing EDAB species a competitive advantage that compensates for their slow rates of intrinsic growth (Gobler and Sunda, 2012, Sunda and Shertzer, 2012).

Another aspect of the EDAB model concerns the rate at which nutrients become available prior to and during a bloom. Though a large nutrient supply is required to build high biomass blooms of any species, high supply rates of labile nutrients favor competitors of EDABs with higher maximum rates of nutrient uptake and growth (e.g. diatoms) (Keller and Rice, 1989; LaRoche et al., 1997; Gobler et al., 2002). EDABs are instead favored when the ratio of nutrient supply to uptake demand keeps ambient nutrient concentration sufficiently low that the EDAB species have a competitive advantage when combined with inherently low grazing rates (Sunda et al., 2006). In addition, growth of the EDAB population is favored when nutrients are largely in organic forms, which are readily utilized by EDAB species and less accessible to competing phytoplankton (Sunda et al., 2006; Gobler et al., 2004a; Gobler et al., 2011). Once EDABs are established, reduced grazing losses, coupled with the ability to compete well with other phytoplankton at low nutrient and light levels as the bloom intensifies, allow EDAB species to sustain exceptionally high biomass (~10^9 cells L^{-1}) for months to years in systems where dilution losses do not exceed their relatively low growth rates (Cosper et al., 1987; Buskey et al., 2001; Sunda et al., 2006).

Consistent with this theoretical framework, blooms of *Aureococcus anophagefferens* have been documented in poorly flushed, temperate bays and lagoons along the U.S. east coast from Rhode Island to Virginia (Dennison et al., 1989); Saldanha Bay, South Africa (Probyn et al., 2010); and the Bohai Sea, China (Zhang et al., 2012). Until recently, blooms of *A. lagunensis*

had only been reported from Texas lagoons (Gobler et al., 2013; DeYoe et al., 1997), but low cell concentrations were detected in lagoonal estuaries along the coast of the Gulf of Mexico from south Texas to the western coast of Florida (Villareal et al., 2004). During the summer of 2012, a bloom of *A. lagunensis* occurred within the Indian River Lagoon, Florida (Gobler et al., 2013, Phlips et al., 2015), and expanded the known bloom range of this species to the U.S. east coast. During late 2012 to early 2013, a brown tide bloom developed in Guantánamo Bay, Cuba (Koch et al., 2014) (Fig. 1). Because brown tides appear to be occurring with greater frequency, it is important from a management standpoint to understand how and why these blooms develop, how they are sustained, and what might be done to mitigate their adverse effects.

Samples documenting the most recent bloom in Guantánamo Bay were collected in conjunction with an ongoing project designed to map and monitor seagrasses, track manatee movements, and investigate habitat use by manatees in Guantánamo Bay (MIPR number N60514-12-MP-001GO, Slone et al., 2015). Water samples and hydrographic data were obtained throughout the southern, U.S.-controlled region of the bay four times between May 2013 and October 2014. The goals of this study were to: 1) identify and quantify the distribution of the causative organism of the Guantánamo Bay bloom, 2) document the physical, chemical, and biological conditions associated with the bloom, and 3) use these findings to evaluate current ideas regarding brown tide development and persistence as described above.

2. METHODS AND MATERIALS

2.1 Study area. Guantánamo Bay is located near the southeastern tip of Cuba (~ 20˚N, 75˚W) (Fig. 1A). The region has a tropical climate with average winter and summer temperatures of ~20 and 30 ˚C, respectively. The bay's watershed is largely a dry savannah in the rain shadow of the Sierra Maestra mountain range. Freshwater inputs to the bay are minimal (Chiappone et al.,

2001), but thunderstorms occur episodically from May through October and the occasional passage of tropical cyclones can deliver significant freshwater inputs to the bay (D.O.N., 2006). Geographically and politically, the bay is divided by a narrow strait into a northern section controlled by Cuba and a southern section controlled by the U.S. Naval Station Guantánamo Bay (NSGB). Río Guantánamo (Guantánamo River) is the only major river discharging directly into the southern portion of Guantánamo Bay, entering at the southwestern corner close to the bay mouth (Fig. 1A). The southern portion of the bay has an average depth of 6.5 m (Table 1) with the central portion having a depth >10 m and littoral areas, which include numerous sub-embayments, generally less than 5 m deep. The northern (Cuban controlled) portion of the bay is similar in surface area to the southern portion and receives freshwater inputs from three rivers, the Río Guaso, Río Hondo, and Río Salado (Fig. 1A; Risk et al., 2014). Desalinization of bay water provides the primary water supply for NSGB. The bay also provides recreational resources for the NSGB staff, and contains important benthic marine habitats, such as seagrasses, macroalgae and corals. These are critical for fish and wildlife, including several endangered marine species, including sea turtles and Antillean manatees (*Trichechus manatus manatus*) (D.O.N., 2006).

2.2 Meteorological data. Hourly temperature and daily total precipitation data at Leeward Point Airfield, NSGB meteorological station (site MUGM) were collected by the United States National Weather Service and downloaded from MesoWest (http://mesowest.utah.edu, accessed: 14 September 2015) for the period 16 February 2008 through 1 November 2014.

2.3 Tidal amplitude, bathymetry and calculation of residence time. Tidal amplitude estimates for the southern portion of the bay were produced by the U.S. National Oceanic and Atmospheric Administration (NOAA) (http://tidesandcurrents.noaa.gov/noaatidepredictions/NOAATidesFacade.jsp?Stationid=TEC4667, accessed 13 November 2013). Raster bathymetry data from NOAA were spatially integrated to determine bay volume and surface area at mean water level. Tidal amplitude was estimated from the average predicted semidiurnal tidal amplitudes over the period 1 January 2013 through 1 November 2013. Tidal amplitude was multiplied by surface area of the bay to estimate the tidal prism (P) (Monsen et al., 2002). Average residence time (τ) for the bay was then calculated by the tidal prism method as:

Equation 1: $$\tau = \mathrm{T}\ \frac{\mathrm{V}+0.5\mathrm{P}}{\mathrm{P}}$$

where V is the mean bay volume, P is the tidal prism, and T is the half day tidal period (Dyer, 1997).

2.4 Sampling and* in situ *measurements. Seven main transect stations along the north to south axis of the southern portion of the bay were sampled on four occasions: 1) 28 May 2013, 2) 18 November 2013, 3) 15 April 2014, and 4) 14 October 2014 (Fig. 1B; latitude and longitude provided in Supplemental Information Table SI-1). The transect began at the southern boundary (SB) where the bay opens to the Caribbean Sea, ran northwest to the mouth of the Guantánamo River (GR), extended northward through the middle of the bay to stations at Buoy 5 (B5), Mid Bay (MB), Granadillo Bay (GB), and Port Palma (PP), and ended at a point near the northern extent of the southern section of the bay referred to as the northern boundary (NB). No samples were collected in the upper bay within Cuban territory.

At each station, water transparency was measured as Secchi disk depth, and depth profiles of temperature, salinity, and dissolved oxygen were obtained down to ~ 4 m using a YSI model 30 in tandem with a YSI-55 (Yellow Springs Inc., Yellow Springs, Ohio). Measurements of Secchi disk depth were also made at a number of locations throughout the bay from 18-23 October 2012 prior to the brown tide bloom. Locations of the October 2012 Secchi depth measurements did not exactly match any of the main transect stations but the closest measurement was chosen to represent each main transect station. Actual locations are shown in Table SI-1. Secchi disk measurements were also made on 7 May 2013 at all main transect stations except the Mid Bay station. Turbidity was measured approximately daily at the intake of the desalinization plant using an Aurora nephelometer (Ecotech Environmental Monitoring Solutions, Knoxfield, Australia) and provided key information on bloom chronology. During late summer 2013, dissolved oxygen was measured at several stations throughout the bay by NSGB staff using a YSI Pro2030 multi-parameter sonde (Yellow Springs Inc., Yellow Springs, Ohio).

Samples for plankton identification and enumeration, HPLC photopigments, and dissolved nutrient concentrations were collected from the surface into sample-rinsed 2 L polyethylene bottles. Sample processing was completed shipboard, immediately upon collection. Aliquots of 60 mL were preserved with 1% Lugol's solution for microscopic plankton identification and DNA analysis. For HPLC pigments, duplicate aliquots of 200 mL were filtered through 47 mm Whatman ^{TM} GF/F filters (nominal pore size 0.7 μm; GE Healthcare Bio-Sciences, Pittsburgh, Pennsylvania) under subdued light conditions using a manual vacuum pump. Duplicate filters were sealed in 2 mL o-ringed, screw-cap microcentrifuge tubes that were then sealed in plastic bags. Subsamples of filtrate were retained in 60 mL conical centrifuge vials

for nutrient analyses. Both filters and nutrient samples were kept on ice prior to freezing at -20 °C once on shore.

2.5 Nutrient analysis. Total dissolved nitrogen (TDN), nitrate ($NO_3^- + NO_2^-$, reported as NO_3^-), ammonium (NH_4^+), and soluble reactive phosphorus (SRP) were measured at all stations during the study. On 28 May 2013, when maximum brown tide biomass was observed, total dissolved phosphorous and dissolved silicate (DSi) were also measured. Nutrient analytes were determined colorimetrically on a Lachat QuikChem 8000 (Loveland, CO) flow injected autoanalyzer using methods described in Wetz et al., (2011). Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were determined as TDN minus dissolved inorganic nitrogen (DIN = sum of nitrate and ammonium) and TDP minus SRP. Detection limits were: TDN = 0.75; NO_3^- = 0.05; NH_4^+ = 0.24; TDP = 0.80; SRP = 0.02; DSi = 0.09 μmol L^{-1}.

2.6 Phytoplankton and microzooplankton abundance and community composition. For all four sampling dates, cell abundances of the brown tide picoeukaryote and of the picocyanobacterium, *Synechococcus* sp., were determined microscopically with a 0.1 mL hemocytometer on a Nikon Eclipse E800 microscope (Tokyo, Japan) at 400 times magnification. Depending on which quantity was reached first, either 400 cells of the dominant picophytoplankter or 100 fields were counted per sample. Abundances of larger phytoplankton and microzooplankton were determined using 5-15 mL settling chambers and inverted microscopy on a Leica DMIRB inverted microscope (Wetzlar, Germany). For each sample, 100 fields were counted at 400 times magnification for the < 20 μm fraction (2.1 % of total sample counted), and again at 200 times magnification for the > 20 μm fraction (8.4% of total sample counted). The median (range) of

cell numbers counted for ciliates, dinoflagellates, and diatoms were respectively 11 (0-43), 84 (17-229), and 28 (3-407). Dimensions of microplankton were measured using the divisions of an ocular Whipple grid. Biovolumes of simply shaped phytoplankton and microzooplankton were calculated using appropriate geometric formulae (e.g. sphere, ellipsoid, cylinder, parallelepiped, cone) while biovolumes of more complicated shaped cells (e.g. *Ceratium sp*.) were approximated based on cell size category according to values provided in Olenina et al., (2006). Biovolumes of picoplanktonic brown tide and *Synechococcus* sp. cells, and a small filamentous cyanobacterium were determined using diameters measured on expanded versions of calibrated digital photomicrographs of thirty cells each. Biovolumes are expressed as ppm; 1 ppm = 10^6 μm^3 mL^{-1}.

Frozen filters for chlorophyll *a* and accessory pigments were shipped on ice in insulated packaging from Guantánamo Bay to the University of North Carolina at Chapel Hill, Institute of Marine Sciences in Morehead City, NC. Samples arrived cold as evidenced by presence of ice in the packaging and were immediately refrozen at –20 ˚C. Samples were extracted in 100% acetone, sonicated, and stored at -20ºC for approximately 24 h. Following procedures described by Pinckney et al. (1998), acetone extracts (200 µl) were then analyzed via high performance liquid chromatography (HPLC) on a Shimadzu LC-20AB HPLC coupled to an in-line photodiode array spectrophotometer (Shimadzu-Benelux, Antwerp, Belgium). Pigment identification and quantification were based on elution time and absorbance spectra comparisons against commercially obtained pigment standards (DHI, Denmark). For all samples measured by HPLC, Chemtax matrix factorization (Mackey et al., 1996) was used to determine the proportion of total chlorophyll *a* contained within each of the phytoplankton taxonomic classes that were microscopically observed. Methods and results for Chemtax analyses are presented in the Supplemental Information (Fig. SI-1).

2.7 Species-specific PCR assay design. Molecular methods were used to identify single cell isolates established from brown tide samples. Briefly, the numerically dominant cells obtained from Guantánamo Bay exhibited a morphology consistent with the pelagophyte *Aureoumbra lagunensis*. Single cells exhibiting this morphology were picked using a drawn Pasteur pipette and placed in culture medium consisting of filtered Gulf Stream seawater, salinity 36, 12 mmol NH_4Cl, 2 mmol Na_2HPO_4, 40 mmol Na_2SiO_3, 10 nmol Na_2SeO_3, vitamins (0.074 nmol vitamin B12, 0.4 nmol biotin, and 60 nmol thiamin), and an ethylenediaminetetraacetic acid (EDTA)–trace metal buffer (100 mmol EDTA, 1 mmol Fe, 50 nmol Mn, 100 nmol Zn, 40 nmol Cu, and 40 nmol Co). Light was provided at an intensity of 100 μmol quanta m^2 s^{-1} from fluorescent bulbs (Vita-Lite) on a 14:10 light: dark cycle. Approximately 5 ml aliquots from two cultures were concentrated by filtration onto a 47 mm, 0.6 μm pore size Nuclepore™ polycarbonate filter (Whatman, Clifton, New Jersey). Genomic DNA was extracted from the filters using the Mo Bio Laboratories PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, California) following the manufacturer's protocol except that 350 μL of cell lysate rather than the prescribed 450 μL was processed. The DNA extracts were eluted from the mini columns using 50 μL of elution buffer and stored at 4°C.

The SSU rDNA gene region from the extracted DNA samples was PCR amplified using *Aureoumbra lagunensis* specific forward (AlagF1-3'GTATAAATGACTTTGTACAGTGAAA5') and reverse primers (AlagR1-3'GCGTGCTAAAGTCCGAGGATT5'). The PCR amplification reaction mixtures contained 20 mM Tris-HCl, pH 8.4, 3 mM $MgCl_2$, 50 mM KCl, 25 pmoles of each primer, 2.5 mM of each deoxynucleoside triphoshate, 0.2 units Platinum® *Taq* DNA polymerase (Invitrogen™ Life

Technologies, Rockville, Maryland), in a total reaction volume of 50 μL. An MJ Research MiniCycler® (MJ Research, Waltham, Massachusetts) was used to conduct the PCR with the following cycling conditions: 2 min. at 95 ºC, 35X (30 s denaturation at 95 ºC, 40 s annealing temperature at 64 ºC and an extension of 1.5 min at 72 ºC), and a final 5 min extension at 72 ºC. The resulting amplicons were analyzed using a 1% TAE agarose gel to confirm their size (~2000 base pairs), and were then cloned and sequenced as described in Vandersea et al., (2012). BLAST searches using the National Center for Biotechnology Information tool confirmed the sequence identities.

2.8 DNA analysis of field samples. The *A. lagunensis* species-specific PCR primers were then used to screen eight Lugol's preserved field samples from Guantánamo Bay (Fig. 1B; Table SI-1). One hundred ml of each field sample was filtered and extracted as described above, then screened using the species-specific primer set. One μl of each extract was then PCR amplified as described above. The amplicons were analyzed on a 1% TAE agarose gel and the size and intensity of the resulting band used to determine the relative concentrations of *A. lagunensis* present. Actual densities were determined using light microscopy as described above.

3. RESULTS

3.1 Environmental Setting

3.1.1 Local meteorology. From 22-24 October 2012, Hurricane Sandy impacted the Guantánamo Bay basin with wind speeds reaching 120 km h^{-1}, strong wave energy (Cooke and Marx, 2015), and rainfall totals exceeding 18 cm over the three day period (Fig. 2A). Following this storm, the area experienced an unusually prolonged period of low precipitation (Fig. 2A).

Cumulative rainfall during the ten-month period from November 2012 to August 2013 was ~9 cm, less than 10% of the normal average. The *Aureoumbra lagunensis* bloom was believed to have initiated during the early part of this dry period (from November 2012 to January 2013) (see graphical abstract), and lasted until early fall 2013 (see below) when normal rainfall patterns resumed in late September 2013. From spring through summer 2013, air temperatures were below normal by about 1 ˚C (Fig. 2B). Air temperature and rainfall from fall 2013 through fall 2014 were near long-term mean values with low winter and higher summer precipitation (Fig. 2A, B).

3.1.2 Salinity, temperature, and dissolved oxygen. With the exception of brackish conditions in the Guantánamo River plume (GR), salinity in the bay increased from ~35 at northern stations to ~36 at the bay mouth (Table 2). Significant salinity based stratification was only measured at the mouth of the Guantánamo River (Table SI-2) with typical seawater salinities underlying a brackish surface layer (salinity 15-30 salinity). Temperature ranged between 28.5 and 30.5˚C in surface waters of the bay during all sampling events (Table 2). When measured, surface water dissolved oxygen was near saturation (+/- 10%) except at station GR where under saturated conditions were observed (Table 2).

3.1.3 Residence time of Guantánamo Bay. The U.S. controlled southern portion of the bay was estimated to have a volume of 2.15×10^8 m^3, a surface area of 3.33×10^7 m^2, an average depth of 6.5 m, and a mean tidal amplitude of 0.26 m. Based on a tidal prism of 8.66×10^6 m^3, the average water residence time for the southern portion of the bay is estimated to be 13 days (Table 1). Average discharge of the major freshwater input to the bay, the Guantánamo River, is 2.1 m^3 s^{-1} (Borrero, 2006). Assuming that the combined discharge of the other three small rivers

is similar, flushing due to freshwater inputs alone would result in a residence time of ~600 d. Therefore, tidal flushing largely determines the water residence time of the bay.

3.2 Bloom Conditions

***3.2.1 Identification of the bloom organism as Aureoumbra lagunensis*.** Microscopic examination of the "brown tide" water samples collected in May 2013 revealed high densities of a small spherical, eukaryotic alga with mean diameter of 3.9 μm ±0.6 (±sd, N = 30). The cells contained single, cup shaped, peripheral chloroplasts that are characteristic of *A. lagunensis* (DeYoe et al., 1997). Water samples from five bay sampling sites (Port Palma, Mid Bay, Lizard Island, and Fuel Dock Cove) collected from 24-28 May and from the Northern Boundary on 18 November 2013 amplified strongly using the *A. lagunensis* species-specific primers, consistent with *A. lagunensis* dominating the bloom at that time. Independent confirmation of the bloom organism was provided by a species-specific immunoassay and sequencing of 18S rRNA genes from cell isolates collected on 20 May 2013 (Koch et al., 2014). Pigment data corroborated the PCR results, indicating that *A. lagunensis* was the dominant bloom organism. Strong correlations existed between cell counts of the dominant phytoplankter and particulate concentrations of chlorophyll *a* and the pelagophyte pigment 19'butanoyloxyfucoxanthin (19'but) (Fig. 3A-B). Additionally, the ratio of the regression slopes of 19'but and chlorophyll *a* on cell abundance shown in Fig. 3 (1.52×10^{-9}: 4.33×10^{-8}) provides a 19'but: chlorophyll *a* mass ratio of 0.035 which is consistent with the range of pigment ratios (0.01-0.04) measured in natural populations and cultures of *A. lagunensis* (DeYoe et al., 1997).

3.2.2 Bloom chronology and spatial distribution. Beginning in January 2013, personnel at NSGB began to observe signs of an unusual phytoplankton bloom including abnormally-

brown water color, decreased water transparency, and unusually high turbidity values at the intake of the desalinization plant (Koch et al., 2014; Fig. 2C). With the exception of two peaks in turbidity, one following Tropical Storm Isaac (26 August 2012), and a second following Hurricane Sandy (25 October 2012), nephalometric turbidity units (NTU) at the intake of the desalinization plant generally ranged from ~1.5 to 3.5 throughout 2012. In mid-January 2013, turbidity began increasing steadily and reached a mid-April peak of ~ 8 NTU, more than twice the ambient values observed in 2012. A satellite photo on 21 January 2013 confirmed the presence of a well-developed algal bloom in the northern and mid portion of bay (Graphical abstract). Based on these observations, the bloom likely initiated during the period between Hurricane Sandy and January 2013, and likely peaked prior to the 28 May 2013 sampling. This chronology of bloom initiation is corroborated by comparatively deep Secchi disc depths in Oct. 2012 before Hurricane Sandy (median = 4.0 m), minimum Secchi depths on 7 May 2013 (median = 0.8 m) (Fig. 4) and slightly deeper Secchi depths on 28 May 2013 (median = 1.2 m) when high levels of *A. lagunensis* were actually measured (Fig. 5A).

Increased water clarity (Fig. 4), decreased turbidity at the desalinization plant (Fig. 2C) and lower phytoplankton biomass throughout the bay in November 2013 (Fig. 5B; Supplemental Information [SI] Fig. SI-1) indicate that the brown tide abated sometime prior to fall 2013. A decline in turbidity at the desalinization plant (Fig. 2C) and a marked decline of dissolved oxygen levels at several stations within the bay in early September 2013 may have coincided with the eventual collapse of the brown tide bloom (Fig. 6). The low oxygen conditions were coincident with a fish kill that affected a wide variety of fish species (Fig. SI-3). NSGB staff were not aware of any previous fish kills in the bay from 2007 to 2014 (Montalvo, J. Natural Resources Program Manager, pers. comm.).

In May 2013, biomass of *A. lagunensis* showed a strong decreasing gradient from the northern reaches of the U.S. portion of the bay (28 ppm biovolume, ~900,000 cells mL^{-1}) to the bay mouth (3 ppm biovolume, ~100,000 cells mL^{-1}) (Fig. 5A). Samples collected in the Granadillo Bay (GB) sub-embayment had lower cell densities than sites within the main axis of the bay. The lowest *A. lagunensis* concentrations were observed in the low salinity waters at the mouth of the Guantánamo River where other taxa (*Synechococcus* sp., diatoms, dinoflagellates, and a chloromonad) collectively dominated the phytoplankton biomass. With the exception of the Guantánamo River station, *A. lagunensis* accounted for 50-95% of phytoplankton biomass with the remainder split nearly evenly between *Synechococcus* sp. and other phytoplankton taxa (Fig. 5A). Biomass of *Synechococcus* sp. and other phytoplankton showed the same general north to south gradient as *A. lagunensis*.

By November 2013, the *A. lagunensis* bloom had diminished and the phytoplankton community was dominated by the cyanobacterium *Synechococcus* sp. with biomass values up to ~10 ppm biovolume or ~450,000 cells mL^{-1} (Fig. 5B). Similar to the *Aureombra* distribution in May 2013, *Synechococcus* sp. abundance exhibited a clear decreasing gradient from north to south in the bay (Fig. 5A,B). The distribution and quantitative dominance of *Synechococcus* sp. in November 2013 was corroborated by pigment analyses (SI, Fig. SI-1) that showed dominance by cyanobacteria with a decreasing north to south gradient. PCR analyses of a sample from station NB collected on 18 November 2013 confirmed that *A. lagunensis* was still present but at much lower concentrations (1,700-44,000 cells mL^{-1} as determined by microscopy). Pigment analyses corroborated the minimal contribution of *A. lagunensis* to total phytoplankton biomass (Fig. SI-1).

By April 2014, *Synechoccocus* cell densities had increased at all stations. *Synechococcus* biovolumes at stations PP (33 ppm or 1,200,000 cells mL^{-1}), NB (20 ppm or 740,000 cells mL^{-1}), and GB (18 ppm or 680,000 cells mL^{-1}) were similar to biovolume values observed for *A. lagunensis* the preceding May. Elevated turbidity with turbidity spikes > 5 NTU was observed at the intake of the Guantanamo Bay desalinization plant suggesting that the *Synechoccocus* bloom developed in late March and declined in late May 2014. There was an unambiguous north to south decreasing cell concentration gradient in the bay (Fig. 5C). Pigment patterns confirmed dominance by cyanobacteria and the north to south latitudinal gradient in cyanobacterial biomass (SI, Fig. SI-1). *A. lagunensis* was still present throughout the bay, but at low densities (4,000 - 83,000 cells mL^{-1}), and constituted less than ten percent of total phytoplankton biovolume (Fig. 5C).

By October 2014, the cyanobacterial bloom had diminished, but moderate concentrations of *Synechococcus* sp. (150,000 cells mL^{-1}) occurred at the two most northern stations (NB, PP) (Fig. 5D). *A. lagunensis* was detected microscopically at five of the seven stations (all but MB and SB), but at low concentrations (800 – 14,000 cells mL^{-1}) which comprised a negligible fraction of total phytoplankton biomass.

3.2.3 Water clarity. Prior to the bloom and Hurricane Sandy in October 2012, the bay water was more transparent with Secchi depths of 2 to 6 m in most areas (Fig. 4, Fig. SI-2). With the exception of the bay mouth station (SB), during the *A. lagunensis* bloom, Secchi depths in the bay were less than 1 m on 7 May and ranged from < 1 m at northern stations (NB, PP) to ~ 2 m at southern stations on 28 May 2013. Secchi depths generally followed an inverse pattern with bloom density indicating that bloom biomass was the dominant light attenuating factor (Figs. 4 & 5; Table 2). In November 2013 when the *Aureoumbra* bloom had receded and was replaced by

a lower biomass bloom of *Synechococcus*, Secchi depths increased at all stations, and ranged from 1.5 to 3 m within the bay and 11.2 m at the bay mouth (SB) (Fig. 4; Table 2). With high biomass of *Synechococcus* sp. at the northern stations in April 2014 (Fig. 5C), Secchi disc values decreased to values (~0.5 m) similar to those during the brown tide bloom (Table 2). With lower *Synechococcus* cell densities in Oct 2014, Secchi disc depths increased again to a median value of 2 m. Over the study period, water clarity did not return to levels observed in October 2012 prior to Hurricane Sandy and the initiation of the brown tide bloom (Fig. 4).

3.2.4 Nutrients. During the observed brown tide maximum on 28 May 2013, nitrate concentrations were at or below the detection limit (≤ 0.05 L^{-1}) at the four northern bay stations (NB, PP, MB, and GB) where *A. lagunensis* abundance was highest. Measureable but low nitrate (~0.2-1 μmol L^{-1}) was observed at stations closer to the mouth of the bay (Table 2). Ammonium was lowest at the bay mouth but otherwise showed no clear spatial patterns. Dissolved organic nitrogen (DON) and soluble reactive phosphorus (SRP) both displayed a similar decreasing north to south gradient with two-fold higher values (16 vs 8 μmol L^{-1} for DON and 0.28 vs 0.14 for SRP) at stations NB and PP than at the bay mouth. Likewise, DSi decreased from 48 to 15 μmol L^{-1} along a similar north-south gradient (Table SI-2). DIN to SRP molar ratios ranged from 3.5-10.4 with an average of ~7. Total dissolved phosphorus concentrations were mostly below detection levels (0.8 μmol L^{-1}), but a few measurements at stations in the mid to upper bay allowed calculation DOP levels of ~0.7 μmol L^{-1}.

During the other three sampling dates, nitrate was similar to that during the brown tide with most samples being below the detection level (<0.05 μmol L^{-1}) except within the Guantánamo River plume (Table 2). During fall 2013, ammonium, DON, and SRP were much higher (50-400%) than during the brown tide bloom. Ammonium was still high (mean 6.7 μmol

L^{-1}) during the *Synechococcus* bloom in spring 2014 but SRP was consistently low, (~ 0.2 μmol L^{-1}) and DIN to SRP ratios were greater than 20. By fall 2014, DIN ranged from 1-4 μmol L^{-1} and SRP was less than 0.2 μmol L^{-1} with the exception of elevated DIN and SRP at the river mouth station (GR).

3.2.5 Microzooplankton grazers. In May 2013, at the peak of the *A. lagunensis* bloom, the abundance and biomass of ciliates and dinoflagellates was generally highest at northern stations where the bloom was densest (Fig. 7A). Dinoflagellates were numerically dominant but larger ciliates dominated biomass (Fig. 7B). In November 2013, ciliate abundance was approximately an order of magnitude lower than in May (Fig. 7C) leading to biomass dominance by dinoflagellates (Fig. 7D). During the *Synechococcus* sp. bloom in April 2014, dinoflagellate biomass was similar to that during the brown tide, but ciliate abundance and biomass were more than an order of magnitude lower than during the brown tide bloom (Fig. 7A, B, E, F). In October 2014, ciliate biomass was negligible (Fig. 7G, H). On average, the abundance and biomass of dinoflagellates in October was similar to that during April 2014 but with a much less pronounced decreasing north to south gradient (Fig. 7G, H). When viewed collectively across sampling dates and stations, both dinoflagellate (Fig. 8A) and ciliate abundance (Fig. 8B) exhibited significant positive correlations with the abundance of *A. lagunensis*. The abundance of the obligate heterotrophic dinoflagellate, *Oxyrrhis marina*, showed a strong correlation with that of *A. lagunensis*. (R = 0.75; Fig. 8C).

4. DISCUSSION

This study documented the environmental and biological factors associated with a bloom of the nanoplanktonic brown tide species *Aureoumbra lagunensis* followed by a bloom of the

picoplanktonic cyanobacteria, *Synechococcus* sp. in Guantánamo Bay, Cuba. Comparing the environmental conditions associated with these "small form" blooms in Guantánamo Bay to conditions during previously documented blooms in other systems provides insight into the characteristics that make coastal systems susceptible to bloom formation and persistence.

Compared to the eight year-long brown tide bloom in Laguna Madre, the brown tides in Guantánamo Bay and Indian River Lagoon were comparatively short-lived, lasting only several months (Buskey et al., 2001; Phlips et al., 2015). As occurred during brown tides in the Laguna Madre and Indian River Lagoon, the Guantánamo Bay brown tide was associated with a significant decrease in water transparency (Stockwell et al., 1993; Gobler et al., 2013; Phlips et al., 2015). However, unlike these other systems (Onuf, 1996; Phlips et al., 2015), severe negative impacts on seagrass were not observed in Guantánamo Bay (Slone et al., 2015). Resiliency of seagrasses in Guantánamo Bay may have been due to dominance by *Thalassia testudinum* (Slone et al., 2015). Compared to the dominant seagrasses of the Indian River Lagoon and Laguna Madre (*Halodule wrightii* or *Syringodinium filiforme* {Virnstein and Carbonara, 1985; Onuf, 1996}), *Thalassia* allocates a larger portion of its resources to below ground energy reserves, which makes it more tolerant of short periods of light deprivation (Unsworth et al., 2015). The Guantánamo Bay brown tide was associated with a hypoxic episode and a fish kill as was also observed in the Indian River Lagoon, but not in the Laguna Madre (Gobler et al., 2013). Ecosystem impacts from *A. lagunensis* blooms can therefore vary by system and may largely depend on bloom intensity and longevity, and ecosystem structure (i.e. dominant sea grass species) prior to the bloom (Slone et al., 2015). A commonality among all these *A. lagunensis* blooms, and the *Aureococcus anophagefferens* brown tide blooms of the mid-Atlantic U.S. coast, is the observed co-dominance or alternating dominance with picocyanobacteria (*Synechococcus*

sp.) (Buskey et al., 2001; Gobler et al., 2004a; Phlips et al., 2015; Kang et al., 2015). Hence, it is more appropriate to consider brown tide blooms as a consortium of pelagophyte and cyanobacterial species.

4.1 Aureoumbra lagunensis bloom conditions. Understanding the sources of nutrients that fueled the brown tide is critical for mitigating future blooms in the bay. Since data on nutrient loading was unavailable, we can only speculate on the potential sources of nutrients. The ideal scenario for brown tide development is a source of bioavailable nitrogen and phosphorus (e.g. ammonium, orthophosphate, or biologically available organic N and P compounds) supplied at rates that can support the net growth of the emerging brown tide bloom (Gobler and Sunda, 2012). In October 2012, rain from Hurricane Sandy led to high freshwater discharge (Slone et al., 2015) and likely resulted in a significant external input of inorganic and organic nutrients from inflowing rivers and the surrounding watershed (Paerl et al., 2014). Additionally, strong wave action (Cooke and Marx, 2015) likely mobilized additional nutrients and organic matter by scouring and resuspending bay sediments and benthic flora/ fauna (Slone et al., 2015; Crosswell et al., 2014). As discussed below, the pulsed nutrient load would likely not have favored *A. lagunensis* directly but may have favored blooms of other high-nutrient adapted species (e.g. diatoms), as often occur in blooms of the brown tide species *Auroecoccus anofhagefferens* (Gobler and Sunda, 2012). Subsequent grazer-mediated recycling of nutrients from the initial bloom(s) of other species and remineralization of bloom organic matter and organic matter mobilized by the storm could have provided a slow-release supply of nutrients for subsequent brown tide development (Sunda et al., 2006). Such a scenario is consistent with the observed 2-3

month time lag between Hurricane Sandy and detection of the brown tide by satellite imagery and elevated turbidity at the desalinization plant.

A second candidate for a slow-release source of available nutrients are the small rivers that drain into the bay. The bay's arid watershed and euhaline salinities within the bay suggest that freshwater inputs are minimal. However, river nutrient loads could still be significant if river nutrient concentrations were high enough. The largest freshwater rivers, the Río Guantánamo and Río Guaso, both drain highly developed industrial and residential areas surrounding Guantánamo City (population 250,000) (O.N.E 2006). The prevalence of water-borne diarrheal diseases and hepatitis A infections (Borrero, 2006) along these rivers indicates contamination by raw or poorly treated sewage that is generally associated with high concentrations of nutrients and organic matter (Corcoran et al., 2010). Significant sewage contamination is also consistent with the observed low dissolved oxygen concentrations measured at the Guantánamo River mouth (Table 2), and is corroborated by strong stable N isotope signatures of sewage waste in macroalgae (Lapointe and Herren, 2013) and corals (Risk et al., 2014) within the bay. Thus, the rivers also could have provided a steady supply of nutrients to fuel growth of *A. lagunensis* and other low-nutrient adapted phytoplankton such as *Synechococcus* sp. that achieved dominance following the brown tide bloom.

During the brown tide bloom in May 2013, low inorganic nutrient concentrations throughout the bay (~2 μmol L^{-1} ammonium and ~0.2 μmol L^{-1} SRP), dominance of the dissolved N and P pools by organic forms, and extremely low nitrate levels (Table 2) suggest that remineralization or grazer mediated cycling was the major nutrient source sustaining the bloom. Ratios of DIN: SRP less than the Redfield ratio (16), particularly at station NB (DIN: SRP < 4) where brown tide biomass was highest, suggest potential N-limited conditions (Table

2). However, sample contamination from a variety of sources, cell lysis during filtration, and unaccounted sample blanks can lead to substantial overestimation of ammonium concentrations in low ammonium samples the using the traditional colorometric methods employed here (Aminot et al., 1997; Sunda and Hardison, 2007). Consequently, ammonium concentrations and N:P ratios may have been much lower than measured values in the northern Bay stations during the bloom. Indeed nitrate+nitrite, which unlike ammonium is not prone to contamination and blank problems, had measured concentrations in the northern Bay stations during the brown tide bloom of <0.05 μmol L^{-1}, much lower than those for ammonium, even though nitrate is not known to be utilized by *A. lagunensis* (DeYoe and Suttle, 1994; Kang et al., 2015). Similar undetectable nitrate concentrations were observed during the 2012 *A. lagunensis* bloom in the Indian River Lagoon, FL (Gobler et al., 2013), but ammonium levels were not reported. Efficient nutrient uptake at low concentrations and the capacity to utilize a wide range of organic N and P forms (Muhlstein and Villareal, 2007; Gobler and Sunda, 2012) provides brown tide species a competitive advantage when grazer mediated recycling and remineralization are the primary nutrient sources. Conversely, when pulsed external loads are significant, nutrient levels are generally high and nitrate is often the dominant form of bioavailable nitrogen (Turner et al., 2003; Paerl and Piehler, 2008). Lacking nitrate reductase (DeYoe and Suttle, 1994), nitrate pulses should be unavailable to *A. lagunensis*, but favor competing phytoplankton that often have higher maximum growth rates than *A. lagunensis* (Sunda and Hardison, 2007, 2010). Similar low levels of inorganic nutrients were observed during the *A. lagunensis* brown tide of the Laguna Madre (Stockwell et al., 1993), *Aureococcus* brown tides of the northeastern U.S (Gobler and Sunda 2012; Mulholland et al., 2009; Glibert et al., 2007), and *Synechococcus* blooms in Florida Bay (Glibert et al., 2004) and are all consistent with competition for recycled nutrients playing a

major role in the competitive dominance of potential EDAB species. It seems no coincidence that brown tide blooms in the Indian River Lagoon, Laguna Madre, and now Guantánamo Bay have all occurred during drought conditions (Phlips et al., 2015; Buskey et al., 1998) when external nutrient inputs are minimal and the relative proportion of internally supplied reduced forms of nitrogen (ammonium and DON) increases (LaRoche et al., 1997).

Another factor essential in maintaining brown tide blooms is a low rate of dilution. The estimated water residence time in the southern portion of Guantánamo Bay (~13 d) would lead to a net population loss due to dilution of ~0.07 d^{-1}. This level of dilution loss is an order of magnitude higher than that for Laguna Madre and five times greater than in the Indian River Lagoon, but is comparable to dilution losses in Chincoteague Bay where *Aureococcus anophagefferens* forms recurrent summertime blooms (Table 1). Though higher than other systems that support brown tides, a dilution loss of ~0.07 d^{-1} is still an order of magnitude lower than the maximum growth rate of *A. lagunensis* (0.87 d^{-1} at 30^{o}C; Gobler and Sunda, 2012). Thus, based on hydrodynamics alone, Guantánamo Bay can support an autochthonous *A. lagunensis* bloom, but blooms are less likely to persist than those observed in the lagoons of Florida and Texas.

This residence time estimate is only for the southern portion of Guantánamo Bay, and does not account for incomplete mixing of bay and ocean water over a tidal cycle (Dyer, 1997). Therefore, even within the southern portion of the bay, the residence time farther from the bay mouth would be longer than 13 d and that closer to the mouth would be significantly less. Unfortunately, bathymetry and tide data are not available for the northern, Cuban-controlled section of the bay. However, the northern portion of Guantánamo Bay has approximately the same area and likely a similar volume. Increased distance from the bay mouth and flow

restriction by the narrow strait that separates the northern and southern bay suggests that residence time in the northern portion of the bay is likely significantly longer than the southern bay studied here (Dyer, 1997). With a longer residence time and nutrient inputs from three small rivers, the northern bay may be an incubator for blooms. Phytoplankton biomass maxima at the northern most stations during all four transects and the January 2013 satellite image of the brown tide bloom (Graphical abstract) are also consistent with blooms originating in the northern region and then spreading to the southern bay with increasing dilution of bay water with low biomass Caribbean water.

In this study, salinities were 34.8-36.4 (excluding the river mouth station, GR) during all four sampling transects even after significant rainfall events. Euhaline salinities and bay temperatures of ~30 °C are optimal for *A. lagunensis* growth (Buskey et al., 1998) but unlike many other phytoplankton and microzooplankton grazers, *A. lagunensis* can grow near its maximum rate at salinities near 70 (Buskey et al., 1998). Acting alone or in concert, a tolerance of high salinity and reduced grazing pressure on *A. lagunensis* are two factors hypothesized to promote *A. lagunensis* bloom formation (Buskey et al., 1998; Gobler et al., 2013; Kang et al., 2015). It is possible that hypersaline conditions existed between our sampling events, in restricted subembayments, or in the northern portion of the bay. Koch et al., (2014) reported weak hypersalinity (salinity 38) for water samples collected near the desalinization plant on 1 May 2013. The occurrence of strong hypersalinity (> 60) during the brown tide's development seems very unlikely because the euhaline measurements made in May should have represented maximum salinities during the bloom's development due to the previous period of intense drought. Additionally, it seems highly unlikely that strongly hypersaline conditions occurred in the northern, Cuban side of the bay because in the southern, U.S. side, salinities were lowest near

the strait (station NB) that connects the two sides. The relatively short residence time of Guantánamo Bay imparted by tidal flushing could account for why strong hypersaline conditions did not develop within the bay. These observations suggest that strong hypersalinity may promote *A. lagunensis* bloom formation, as is believed to have occurred in the Laguna Madre (Buskey et al., 1998), but it is likely not a prerequisite.

4.2 Microzooplankton grazers. The observed poor growth and survival of many microzooplankters grown on *A. lagunensis* prey (Buskey and Hyatt, 1995; Jakobsen et al., 2001) and the observed lower grazing rates on this species than on competing phytoplankton during the Florida Indian River bloom (Kang et al., 2015) are believed to promote brown tide blooms by minimizing grazing losses (Buskey and Stockwell, 1993; Sunda et al., 2006; Sunda and Schertzer, 2012). In Guantánamo Bay, densities of the dominant microzooplankton groups, ciliates and dinoflagellates, were higher during the May 2013 brown tide sampling event compared to either non-bloom autumn sampling events or during the *Synechococcus* sp. bloom of April 2014. Ciliates dominated microzooplankton biomass in May 2013 and the average ciliate abundance (54 cells mL^{-1}) was higher than the ciliate abundance of all eleven study sites reported in Buskey's (1993) intersystem comparison of ciliate abundance within estuaries, bays, and coastal waters of the Western Atlantic coast, including the Gulf of Mexico and Caribbean Sea. Similarly, dinoflagellate and ciliate micrograzers also increased during peak *A. lagunensis* abundance in the Indian River Lagoon (Phlips et al., 2015), and there the brown tide bloom was at least partly related to lower specific grazing rates on *A. lagunensis* than on competing species of phytoplankton (Kang et al., 2015). During the spring of 1990 in Laguna Madre, Texas, microzooplankton abundance declined as *A. lagunensis* formed its initial bloom, but strong

hypersalinity (salinity >60) also developed during the same period and was thought to be largely responsible for the decline in microzooplankton populations (Buskey and Stockwell, 1993; Buskey et al., 1997). In the absence of strong hypersalinity, microzooplankton abundance increased as the brown tide recovered following a brief period of decline caused by high freshwater inputs (Buskey et al., 2001). Collectively, evidence from Guantánamo Bay and the lagoons of Texas and Florida indicate that during the initiation of a bloom, microzooplankton likely favor other phytoplankton species, giving *A. lagunensis* a competitive advantage, but that over time a large microzooplankton biomass can develop.

Several microzooplankton species are known to graze and grow well on a diet dominated by *A. lagunensis* (Jakobsen et al., 2001; Buskey et al., 1997; Buskey and Hyatt, 1995). The heterotrophic dinoflagellate, *Oxyrrhis marina*, is one such organism (Buskey et al., 1998) and the positive correlation between its abundance and that of *A. lagunensis* in Guantánamo Bay (Fig. 8C) may reflect bottom-up control of this grazer population by *A. lagunensis* availability. The net biomass response of the micrograzer community is likely determined by the relative proportions of grazers that respond positively (i.e., graze and grow well) or negatively (graze and/or grow poorly) to increasing *A. lagunensis* abundance. Such selection within the micrograzer population has been hypothesized for brown tide blooms of *Aureococcus anophagefferens* (Deonarine et al., 2006). Thus, although initial preferential grazing on other phytoplankton species may favor brown tide bloom formation, intensifying grazing pressure may have played a role in the short-lived (<10 month) nature of the Guantánamo Bay brown tide bloom. Further investigations into the role of grazers in shaping brown tide blooms are warranted, and particular emphasis should be placed on 1) determining whether grazer communities shift toward species more capable of

utilizing brown tide species as a food source, and 2) determining the role that hypersalinity may play in influencing shifts in microzooplankton grazer communities.

4.3 Synechococcus bloom. The universal co-occurrence, and often alternating co-dominance, between *A. lagunensis* and *Synechococcus* suggest they share comparable habitat requirements and fill similar ecological niches. Similar to *A. lagunensis*, the *Synechococcus* strains that form high biomass coastal blooms exhibit relatively slow growth rates, and reduced grazing pressure compared to faster growing *Synechococcus* strains (Goleski et al., 2010; Kang et al., 2015). Small cell size makes *Synechoccocus* a good competitor under nutrient and light limited conditions, and like *A. lagunensis*, *Synechococcus* has the ability to use a wide range of organic N and P substrates when inorganic forms are depleted (Glibert et al., 2004; Fu et al., 2006). Thus, *Synechoccocus* also is favored in poorly flushed systems where recycling provides the major nutrient input, and phytoplankton uptake rates can maintain low nutrient concentrations. Based solely on its small cell size, *Synechococcus* should have a competitive advantage under conditions of low, growth limiting nutrient concentrations (Sunda and Hardison, 2010), and its capacity for direct utilization of nitrate (which *A. lagunensis* appears to lack; DeYoe and Suttle, 1994) provides an advantage to *Synechococcus* when external N inputs occur (Kang et al., 2015). However, strong allelopathic growth inhibition of *Synechococcus* by bloom densities of *A. lagunensis* (Kang and Gobler, *in press*) could explain the low biomass fraction comprised by *Synechoccocus* (<10 %) during the Guantanamo Bay brown tide (Figure 5), and suggests that termination of brown tides may be a prerequisite for subsequent blooms of *Synechococcus*.

Based on the available data, other factors that prompted the switch from *A. lagunensis* to *Synechococcus* in Guantánamo Bay can only be speculated. One possibility for the switch may

have been related to bottom-up controls mediated by elevated terrestrial inputs of nutrients (including nitrate) upon a return to normal rainfall conditions. In the Indian River Lagoon, experimental nitrate additions occasionally enhanced the growth of *Synechococcus* but never stimulated *A. lagunensis* growth (Kang et al., 2015). High DIN levels (Table 2) during the fall of 2013 (median ~11 μmol L^{-1}) and during the observed peak *Synechococcus* biomass in April 2014 (median = 8.9 μmol L^{-1}) are consistent with elevated nitrogen inputs but the ammonium dominated DIN pool should also have been available to *A. lagunensis*. Although high DIN: SRP ratios indicative of P-limitation were observed when *Synechococcus* was dominant, P limitation was not likely the cause of the observed shift to *Synechococcus*. *Synechococcus* has a higher P requirement than *A. lagunensis* and consequently should not have a competitive advantage when the overall assemblage is P-limited (DeYoe et al., 2006).

Shifts in the dominant small form phytoplankton species could also be controlled by top-down changes in mortality rates due to grazing or viral infection (Sieracki et al., 2004; Gobler et al., 2004b). Interestingly, ciliates dominated microzooplankton biomass during the *A. lagunensis* bloom (Fig. 7), but when *Synechococcus* was dominant, ciliate abundance was minimal, and dinoflagellates were the dominant microzooplankton. A decline in ciliate abundance was also observed in the Laguna Madre during a similar alternation between *A. lagunensis* and *Synechococcus sp.* dominance (Buskey et al., 2001). Finer temporal sampling resolution might help determine whether these shifts in dominance among microzooplankton guilds is a driver or response to shifts in the dominant group of phytoplankton. Nevertheless, these shifts in dominant predators and prey are consistent with a tight linkage between the populations of very small phytoplankton and microzooplankton grazers (Cushing, 1989; Barber and Hiscock, 2006).

4.4 Management implications. Combining field observations from Guantánamo Bay, Laguna Madre, and the Indian River Lagoon with results from laboratory and modeling studies provides information useful for managing brown tide bloom formation. Even though small brown tide organisms are well adapted to low nutrient concentrations, reducing nutrient loads, particularly loads of reduced and/or organic forms may reduce the severity of *A. lagunensis* blooms. Enhancing exchange of bay waters with the ocean would likely also increase flushing rates and may moderate the accumulation of biomass and the reduced forms of nitrogen (including organic nitrogen) that favor brown tide dominance. Increasing flushing rates should also lessen the frequency and severity of hypersalinity that favors *A. lagunensis* over other phytoplankton, and can reduce top down control by microzooplankton grazers. Any use of freshwater flow management to enhance flushing and prevent hypersalinity has the potential for worsening the brown tide blooms due to associated increases in nutrient loading (Sunda and Shertzer, 2012).

In Guantánamo Bay, low concentrations of *A. lagunensis* cells were observed more than a year after the bloom collapse. This small population could serve as an inoculum for the recurrence of brown tide blooms, as has occurred after the initial brown tide of 2012 in the Indian River Lagoon (Koch et al., 2014), and following disruptions of the brown tides in the Laguna Madre (Buskey et al., 2001). Enhancing the flushing rate is likely not a viable option for bloom management in Guantánamo Bay due to the rugged topography of its shores and limited supply of freshwater (Chiappone et al., 2001). Consequently, reducing nutrient loads to Guantánamo Bay will likely be the most effective means of minimizing recurrence of such blooms. Most of the nutrient sources to Guantánamo Bay likely occur outside the borders of NSGB because the population of NSGB is only about one percent (D.O.N., 2011) of the half million Cuban residents within the bay's watershed (Borrero, 2006). Additionally, wastewater

from NSGB is treated, while that from the Cuban controlled watershed is generally not treated before being discharge into waterways (Cueto and De Leon, 2010).

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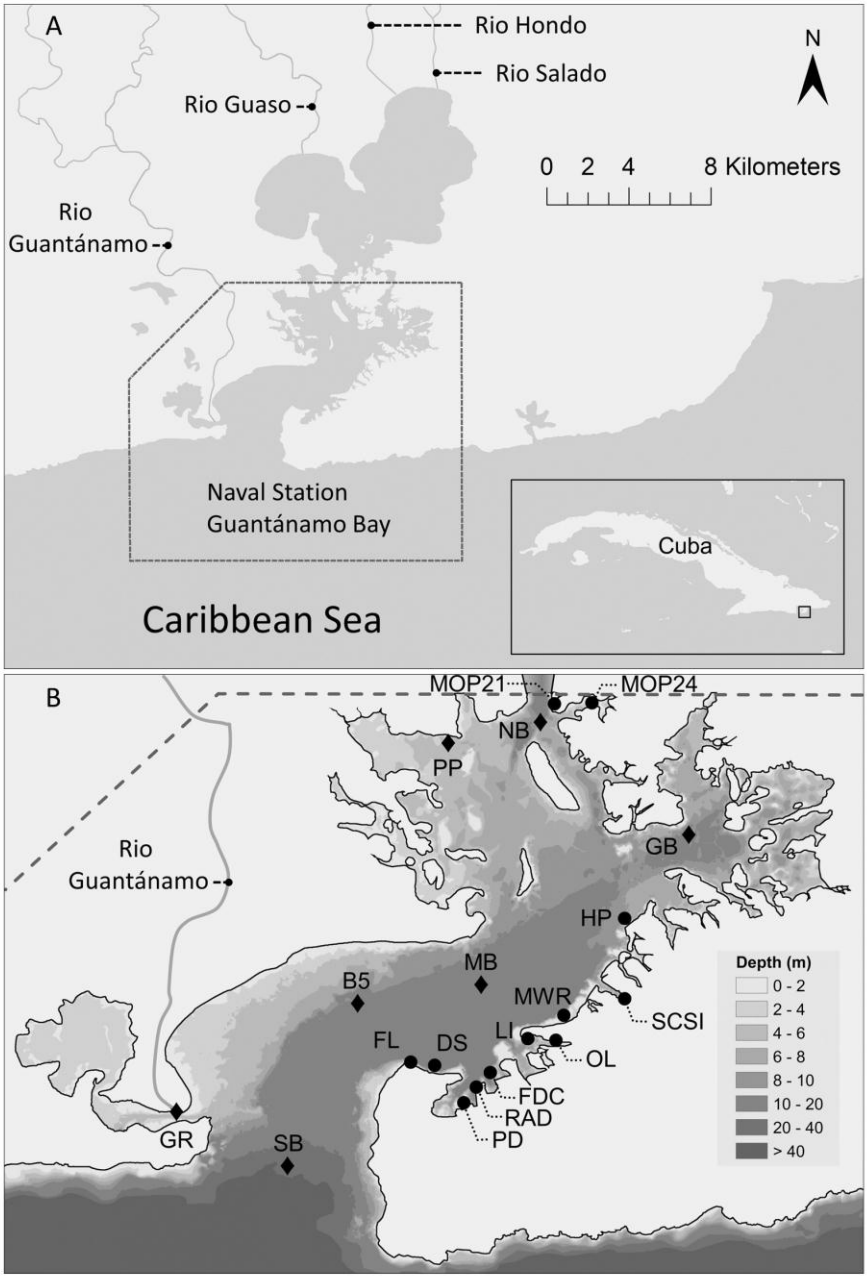

Figure 1. Map showing the location of Guantánamo Bay, Cuba and its major tributaries (A) and the bathymetry and sampling stations in the southern, U.S. controlled region of Guantánamo Bay, Cuba (B). The seven main transect stations are indicated by black square symbols. Other stations are shown as black circles. SB = Southern Boundary; GR = Guantánamo River; B5 = Buoy 5; MB = mid Bay; GB = Granadillo Bay; PP = Port Palma; NB = Northern Boundary; FDC = Fuel Dock Cove; LI = Lizard Island; DS = desalinization plant; FL= Ferry Landing; RAD= Radio NSGB ramp; OL=Officer's Landing; MWR=MWR Marina; PD=Pier D; HP=Hospital Pier; SCSI = pier at Satellite Communications Systems Inc.; MOP21= Marine Observation Post 21; MOP24= Marine Observation Post 24.

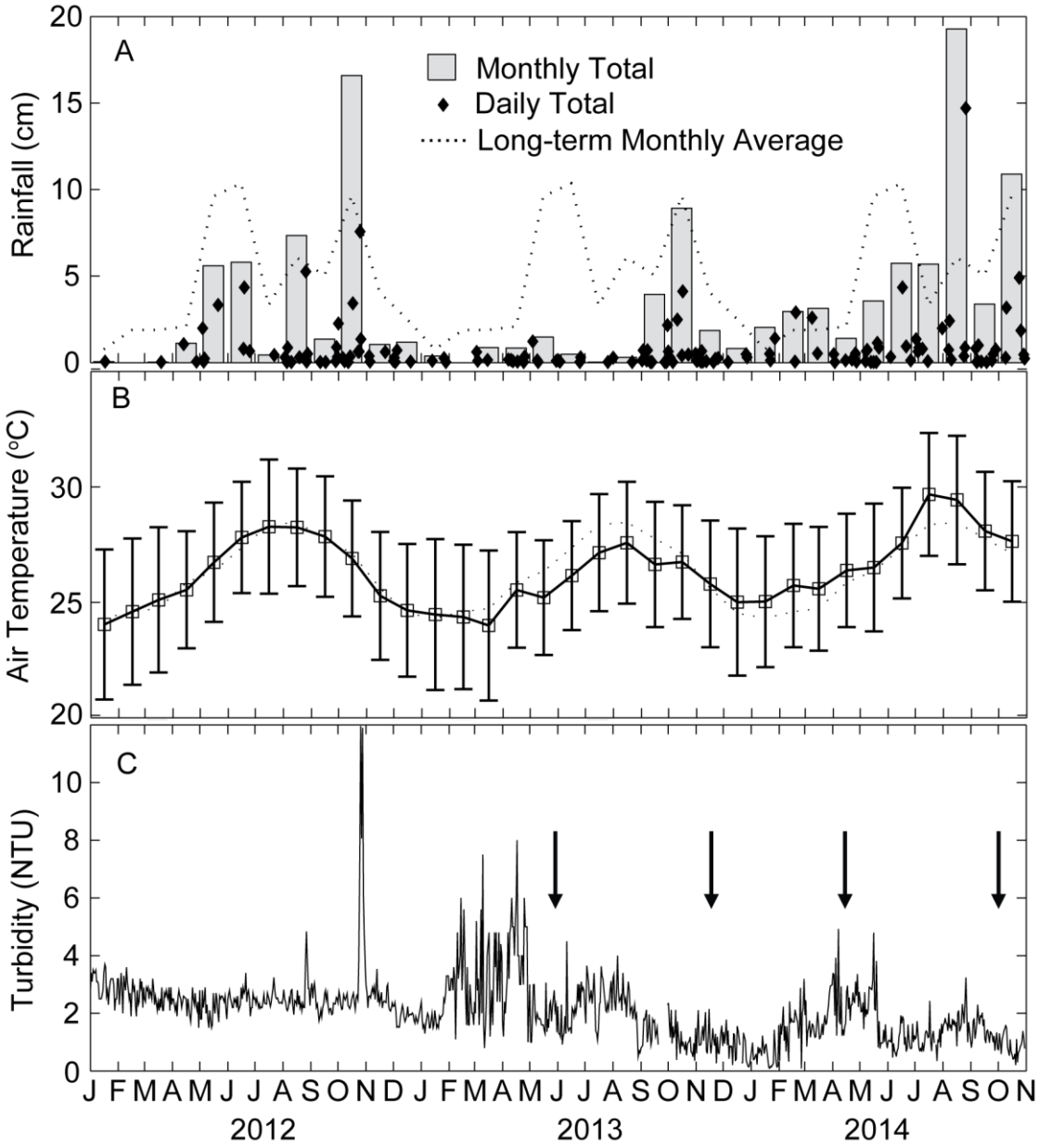

Figure 2. Comparison of air temperature and rainfall during the study period with the long term averages and daily turbidity values at the desalinization plant. A) Monthly total, daily total, and long-term average monthly total rainfall. B) Long-term average (Feb. 2008- Oct 2014) monthly temperature (dashed line) and average monthly temperature during the study period (squares). Error bars are standard deviation and include diel variation in temperature. C) Daily turbidity measurements at the desalinization plant. X-axis tick marks are on the first day of each month. Arrows indicate sampling dates when transects of Guantánamo Bay were conducted.

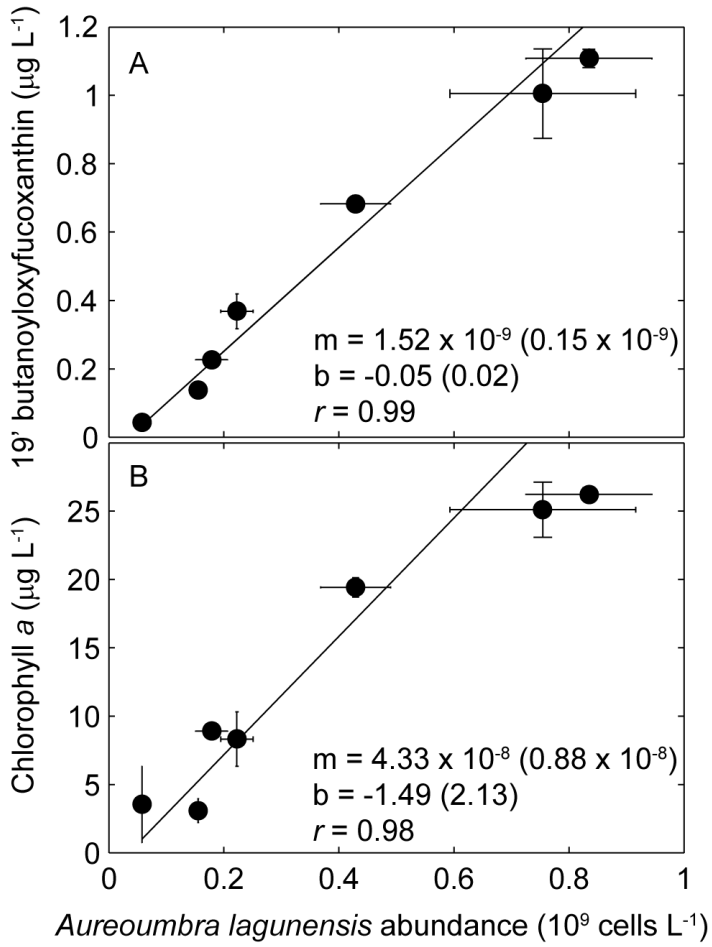

Figure 3. Relationship between 19'butanyoloxyfucoxanthin (but) and chlorophyll *a* (chl *a*) concentration with cell density of *Aureoumbra lagunensis* on 28 May 2013. Data points are labeled by station abbreviation. Station abbreviations are provided in Figure 1. Error bars represent the standard deviation of duplicate pigment measurements and hemocytometer cell counts. m, b, and r are the slope, intercept, and correlation coefficient for a model II regression of cell abundance and pigment concentration. Values in parentheses are the standard deviations of the regression coefficients.

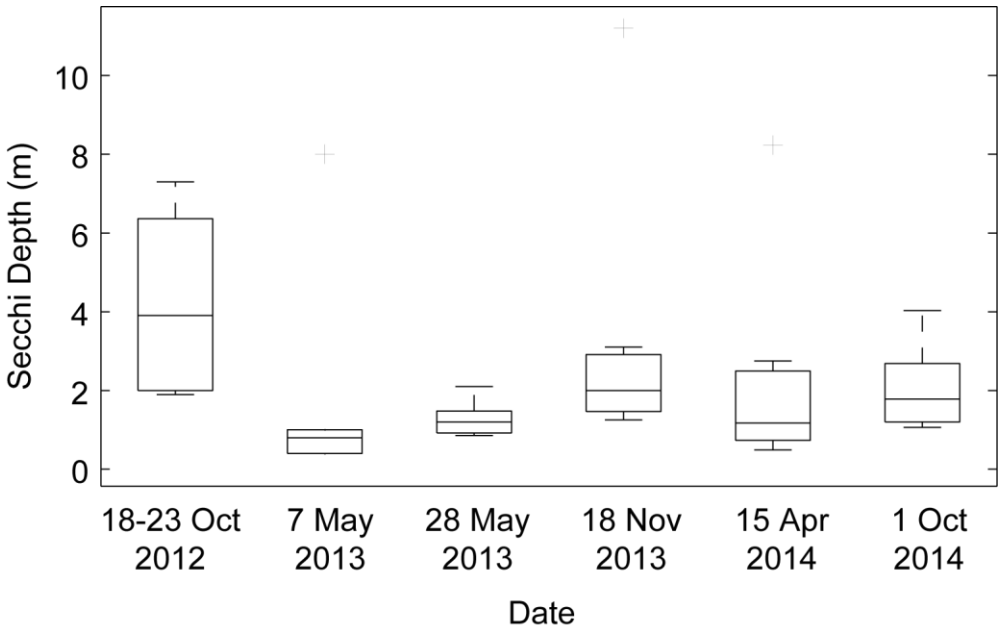

Figure 4. Box plots of water clarity measured by Secchi disk depth at or near the seven main transect stations prior to and during the *Aureoumbra lagunensis* and *Synechococcus sp.* blooms. Boxes represent the interquartile range with the median indicated by the solid line. Whiskers indicate the most extreme measurement within 1.5 times the interquartile range from the median, and "+" symbols indicate outliers that occurred at the Southern Boundary.

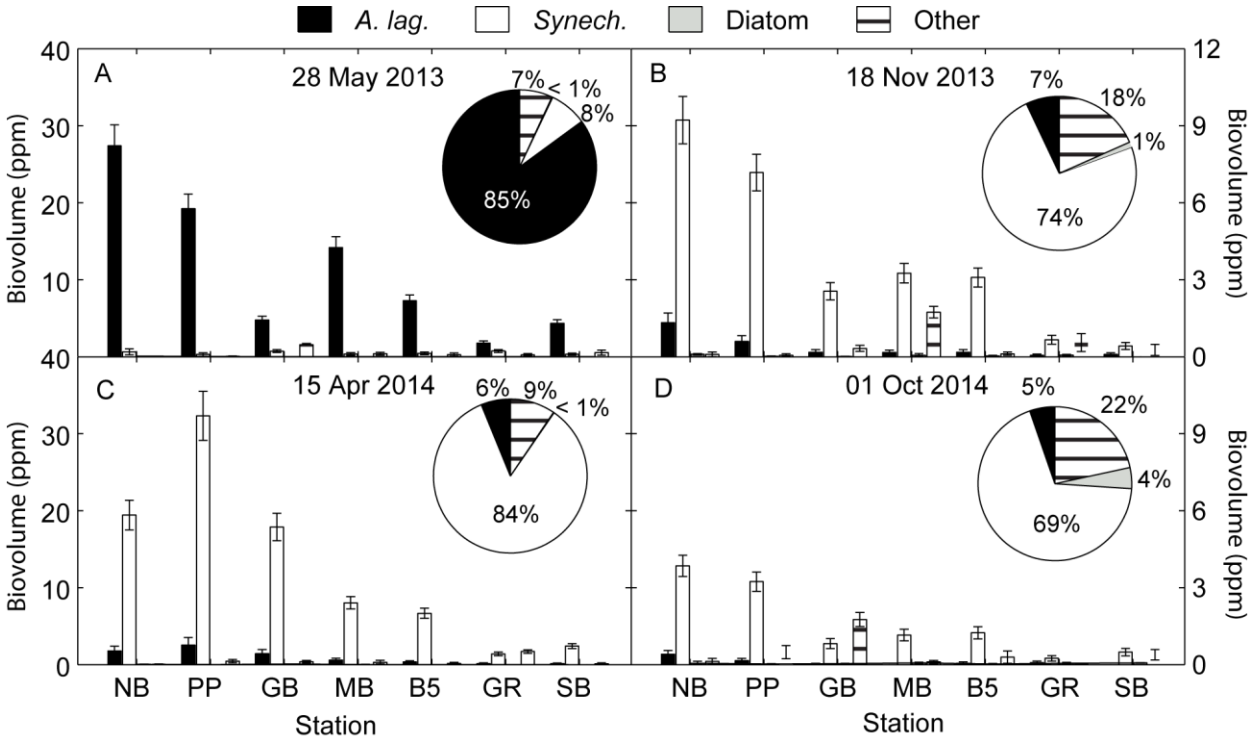

Figure 5. Biovolumes of the dominant phytoplankton on four sampling dates along a north to south transect in Guantánamo Bay. Error bars represent the standard deviations derived from duplicate microscopic counts. Note change in scale for samples collected 18 Nov 2013 and 01 Oct 2014. Inset pie charts depict the average percent biomass at the seven stations. Station acronyms are described in Figure 1.

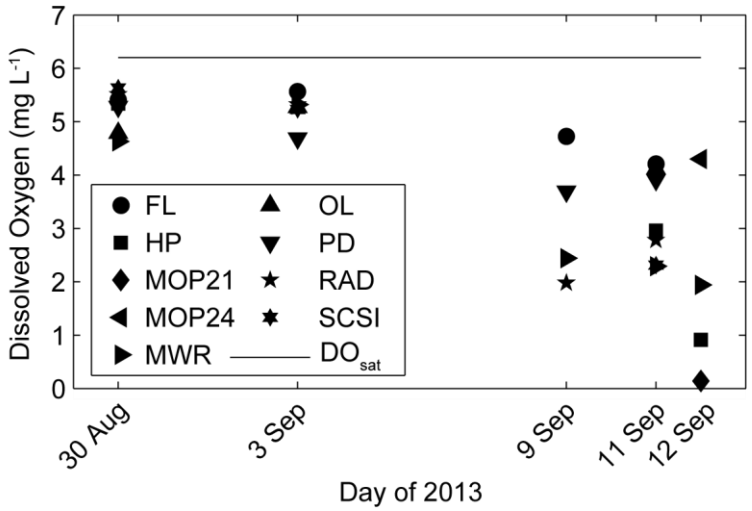

Figure 6. Time series of dissolved oxygen from 30 August to 12 September, 2013 at several stations in Guantánamo Bay. Solid line represents the average dissolved oxygen saturation value (DO_{sat}) at observed temperatures and a salinity of 36. Station locations are shown in Figure 1.

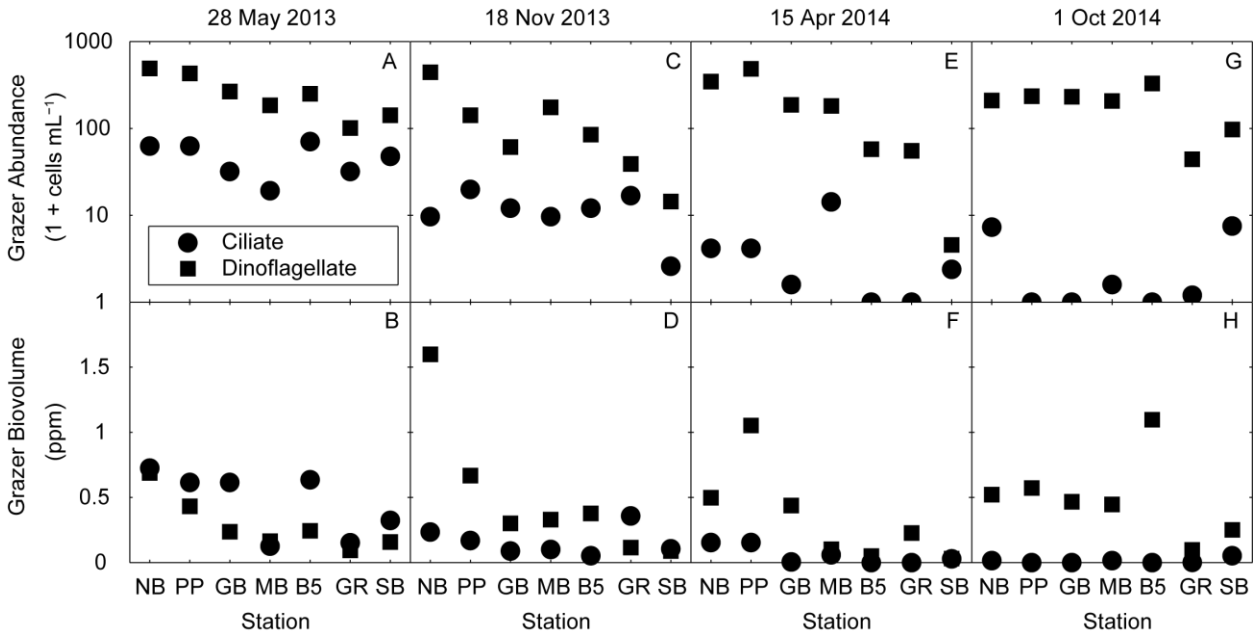

Figure 7. Microzooplankton grazer abundance and biomass. Spatial distribution of ciliate and dinoflagellate grazer abundance on 28 May and 18 November 2013, 15 April and 1 Oct 2014 (A, C, E, G). Distribution of ciliate and dinoflagellate grazer biomass as biovolume (B, D, F, H). Stations are ordered north to south and abbreviations are given in Figure 1.

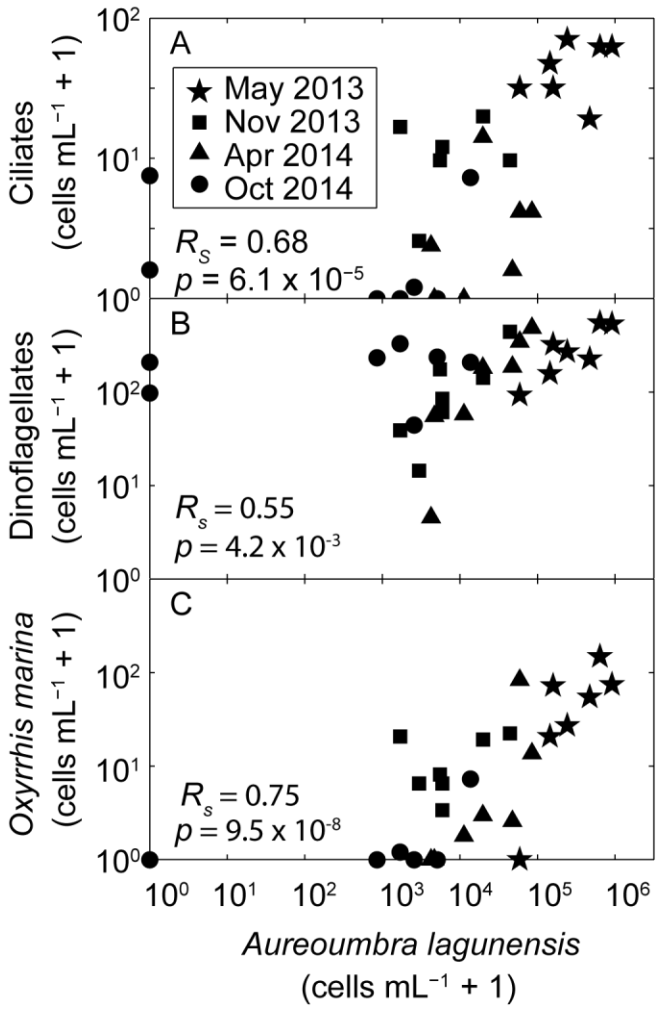

Figure 8. Relationship between microzooplankton and *Aureoumbra lagunensis* abundance during four surveys of seven main transect stations in Guantánamo Bay. R_s and p are the Spearman's rank correlation coefficients and p-values for a test of no rank correlation.

Table 1. Estimated average water residence times (τ) and average depths (Z) for Guantánamo Bay, Cuba and six other estuaries which have experienced brown tide blooms caused by *Aureoumbra lagunensis* or *Aureococcus anophagefferens*.

Estuary	**Species**	**τ (d)**	**Z (m)**
Guantánamo Bay, Cuba	*A. lagunensis*	13[*]	6.5[*]
Laguna Madre, TX	*A. lagunensis*	300[e]	1[e]
Indian River Lagoon, FL	*A. lagunensis*	75[f]	2[f]
Great South Bay, NY	*A. anophagefferens*	100[a]	1.5[b]
Peconic Bay, NY	*A. anophagefferens*	60[c]	5[c]
Chincoteague Bay, VA/MD	*A. anophagefferens*	13[d]	1[d]

[*]This study, southern seaward half of the bay only; [a]Kinney and Valiela 2011; [b]Nixon et al., 1994; [c]Hardy 1976; [d]Boynton et al., 1996; [e]Buskey et al., 2001; [f]Phlips et al., 2014

Table 2. Physical and chemical conditions in surface waters along the main transect in Guantánamo Bay. T = temperature (°C); S = salinity; SD = Secchi depth (m); DO = surface dissolved oxygen (% of saturation) Nutrient (μmol L^{-1}) acronyms are provided in the methods section. For 28 May 2013 samples, values are means of duplicate measurements with standard deviations in parentheses. For other sampling dates, duplicate measurements were not conducted.

Date	Site	T	S	SD	DO	NO_3^-	NH_4^+	DON	PO_4^{3-}	DIN: SRP	DOP
5/28/13	NB	29.2	35.5	0.9	NA	0.06[a]	0.91 (0.53)	16.9 (2.9)	0.27 (0.02)	3.6	BDL
5/28/13	PP	29.4	35.5	0.9	NA	BDL[b]	1.89 (1.84)	16.2 (2.0)	0.28 (0.06)	6.8	0.76[a]
5/28/13	GB	29.5	36.4	1.4	NA	BDL	1.87 (1.39)	14.6 (1.1)	0.18 (0.07)	10.4	0.72 [a]
5/28/13	MB	29.1	36	1.2	NA	BDL	1.81 (1.23)	10.9 (3.0)	0.18 (0.05)	10.1	0.70 [a]
5/28/13	B5	28.9	36.1	1.5	NA	0.24 (0.14)	1.15 (0.76)	10.0 (2.2)	0.16 (0.04)	8.7	BDL
5/28/13	GR	29.6	15.6	1.1	NA	1.05 (0.20)	2.00 (0.51)	14.8 (1.7)	0.51 (0.03)	6.0	BDL
5/28/13	SB	28.6	36.1	2.1	NA	0.30 (0.12)	0.72 (0.24)	8.0 (0.8)	0.14 (0.00)	7.3	BDL
11/18/13	NB	28.9	34.8	1.3	100	BDL	3.61	8.53	0.18	20.1	NA
11/18/13	PP	29.2	35.4	1.3	109	BDL	10.6	26.9	0.58	18.3	NA
11/18/13	GB	28.9	36	2.4	96	BDL	5.15	16.9	0.26	19.8	NA
11/18/13	MB	29.1	35.5	1.9	99	0.99	18.6	46.9	0.70	28.0	NA
11/18/13	B5	29.1	35.7	2.0	99	BDL	8.29	35.1	0.46	18.0	NA
11/18/13	GR	29	29.1	3.1	64	3.56	8.64	23.9	0.78	15.6	NA
11/18/13	SB	29.1	36.2	11.2	100	BDL	15.6	27.5	0.60	26.0	NA
4/15/14	NB	29.6	35.8	0.5	93	BDL	4.86	12.6	0.19	25.6	NA
4/15/14	PP	29.6	36.1	0.6	110	BDL	8.93	13.2	0.19	47.0	NA
4/15/14	GB	30.3	36.1	2.8	99	BDL	3.24	7.94	0.16	20.3	NA
4/15/14	MB	29.5	36.4	1.1	108	BDL	10.4	11.5	0.42	24.8	NA
4/15/14	B5	29.3	36.4	1.2	NA	BDL	10.1	12.6	0.40	25.3	NA
4/15/14	GR	NA	NA	1.7	NA	0.81	8.07	9.55	0.41	21.7	NA
4/15/14	SB	NA	NA	8.2	NA	BDL	1.52	1.49	0.17	8.9	NA
10/1/14	NB	30.2	35.2	1.2	103	1.18	1.96	6.22	0.17	18.5	NA
10/1/14	PP	30.3	35.2	1.1	107	BDL	2.64	6.24	0.16	16.5	NA
10/1/14	GB	30.3	36.1	2.8	107	BDL	1.54	7.91	0.20	7.7	NA
10/1/14	MB	30.1	35.7	1.8	102	1.50	2.89	8.04	0.19	23.1	NA
10/1/14	B5	30	35.8	2.5	92	BDL	1.95	12.5	0.17	11.5	NA
10/1/14	GR	29.7	19	1.2	41	2.01	5.73	17.5	0.80	9.7	NA
10/1/14	SB	30	35.7	4.0	92	BDL	0.81	4.45	0.18	4.5	NA

For each date, stations ordered from north to south. [a]Value represents a single measurement because one of the two duplicate measures was below the detection limit of 0.80 μmol L^{-1}. [b]BDL signifies below detection limit. NA indicates "not analyzed".