

RESEARCH NOTE

Comparison of zooxanthellae densities from upside-down jellyfish, *Cassiopea xamachana*, across coastal habitats of The Bahamas

Densidad de zooxantelas presentes en la medusa invertida, *Cassiopea xamachana*, en los hábitats costeros de Las Bahamas

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Abstract.- Anthropogenic disturbances may drive jellyfish blooms, and previous studies have suggested this is the case for upside-down jellyfish (*Cassiopea xamachana*). *Cassiopea* were found to have higher mean zooxanthellae densities in human-impacted areas on Abaco Island, The Bahamas, suggesting that nutrient loading in impacted sites may be one factor driving zooxanthellate jellyfish blooms. Gut contents from *Cassiopea* medusae were positively correlated to zooxanthellae densities, indicating that heterotrophically-derived nutrition may be an important factor in facilitating increased zooxanthellae population densities. Understanding the mechanisms driving jellyfish blooms is crucial for developing effective management strategies in impacted coastal ecosystems.

Key words: Benthic, facilitation, human impacts, heterotrophy, mutualism

INTRODUCTION

Jellyfish blooms may be increasing in both magnitude and frequency in certain parts of the world since the 1970s, likely due to various anthropogenic disturbances (Condon *et al.* 2013). Some of the main drivers for jellyfish blooms include nutrient loading, overfishing, global climate change, development of artificial marine structures, introduction of nonindigenous jellyfish species, and sedimentation (Graham *et al.* 2001, Brodeur *et al.* 2008, Hoover & Purcell 2009, Condon *et al.* 2012, Purcell 2012, Riisgard *et al.* 2012, Stoner *et al.* 2014). Recently, Stoner *et al.* (2011) suggested that populations of the benthic, zooxanthellate upside-down jellyfish, *Cassiopea* spp. Péron & Lesueur, 1810, are larger and more abundant in human-impacted coastal systems in The Bahamas. This pattern suggests that human activity may be initiating or facilitating blooms of this relatively little-studied epibenthic jellyfish.

One mechanism suggested to contribute to *Cassiopea* spp. blooms in anthropogenically-disturbed habitats may be increased nutrient availability, driving higher zooxanthellae densities in jellyfish tissues (Stoner *et al.* 2011). Because zooxanthellae are nutrient limited (by both nitrogen and phosphorus), increased nutrient supply may result in increased primary production, thereby providing higher levels of photosynthate (*i.e.*, autotrophic nutrition) to their hosts

(Muscatine *et al.* 1989, Falkowski *et al.* 1994, Koop *et al.* 2001). Higher zooxanthellae densities may enhance host growth and fitness, as has been well-documented in coral reefs (Muscatine & Porter 1977, Berkelmans & van Oppen 2006, Yellowlees *et al.* 2008).

In this study, we explored whether there was a difference in zooxanthellae densities from *Cassiopea xamachana* Bigelow, 1892 (hereafter *Cassiopea*) between anthropogenically-disturbed and relatively-pristine coastal sites on Abaco Island, The Bahamas. It was hypothesized that zooxanthellae densities in the tissue of *Cassiopea* would be higher in sites adjacent to human population centers, likely as a function of elevated nutrient concentrations.

MATERIALS AND METHODS

This study was conducted in nearshore habitats on Abaco Island, The Bahamas, from June to July 2011. Study sites were located within mangrove wetlands, embayments, or low energy coastlines < 2 m in depth at low tide. All sites were characterized by silty-sandy sediment (~0.05 mm particle size, as classified by the USDA soil classification triangle; Schoeneberger *et al.* 2002), which suggests low localized water velocities. Following Stoner (2011), sites were categorized *a priori* as being adjacent

to high or low human population densities. The number of buildings (the proxy for human impact) within a 3 km radius from the mid-point of each site was estimated using Google Earth v. 5.1 (Google Inc. 2010). High human population density sites (hereafter referred to as HP sites) had a mean number of 563 residential buildings (range of 84–1712 buildings; Little Harbour, Marsh Harbour, Treasure Cay, Cherokee and Little Abaco South), while low human population density sites (hereafter referred to as LP sites) had a mean number of 4 residential buildings present (range of 0–10 buildings; Snake Cay, North Bight of Old Robinson, Twisted Bridge, and Little Abaco North) (Stoner *et al.* 2011).

For each site, 15 jellyfish between 8 to 10 cm in bell diameter were collected. *Cassiopea* of this size were comparable to the range of bell diameters of *Cassiopea* examined for zooxanthellae densities in previous studies (Table 1). All jellyfish had 8 oral arms with no tears or deformities on the bell, as any injury to the animal may affect zooxanthellae population densities or the ability of the animal to acquire prey. To enumerate zooxanthellae densities, we first selected the ‘outpocketings’ or secondary and tertiary mouths from the primary oral arms following protocol outlined by Estes *et al.* (2003). We chose oral arms as they have been found to have the highest zooxanthellae densities in *C. xamachana* (Estes *et al.* 2003). Oral arms were then homogenized using a mortar and pestle. We extracted 10 µl of jellyfish homogenate, which we added to 1 ml of seawater and mixed for 45 s using a vortex. Zooxanthellae densities in each sample were determined from 3 aliquots (10 µl per sample), which were viewed using a Neubauer hemocytometer under a plain light microscope at 400x. Zooxanthellae density data were averaged across replicates per specimen for each site. Zooxanthellae density across LP and HP sites were analyzed using a nested univariate analysis of variance (ANOVA) with site nested within disturbance regime (*i.e.*, HP and LP sites) (IBM SPSS v. 20.0).

Nutrient concentrations (NH₄⁺, total phosphorus, and %P) were evaluated to elucidate whether elevated nutrients may be a potential driver in changes to zooxanthellae populations from *Cassiopea* in human-impacted sites. To analyze ammonium (NH₄⁺) concentrations, water samples were collected and filtered with 0.45 µm nylon membrane filters. Unfiltered water samples were also collected for total phosphorus (TP) analysis in acid rinsed HDPE bottles, placed on the dark on ice, and stored in the freezer until analysis. In addition, %P was also analyzed from seagrass tissue, as nutrients from seagrass may reflect nutrient availability in a system over a longer time period (*i.e.*, weeks), whereas ambient water nutrient concentrations represent a ‘snapshot’ of nutrient concentrations in a given system (Allgeier *et al.* 2010, Stoner *et al.* 2011). To analyze

blade tissue %P, *Thalassia testudinum* K.D. Koenig, 1805 (turtle grass) leaves were harvested, then scraped to remove epiphytes and dried at 80°C for 48–72 h. Dried seagrass was ground into a fine powder, and both water (TP) and seagrass (%P) samples were analyzed using dry oxidation acid hydrolysis extraction in addition to the use of a colorimetric analysis (Fourqurean *et al.* 1992). Ammonium samples were processed using the Indophenol blue method in addition to the use of the colorimetric analysis.

Gut content weight (*i.e.*, the dry weight of food in the gut) was also examined, as we wanted to explore the potential relationship between zooxanthellae densities and the amount of food that individual *Cassiopea* had consumed. It has been suggested that heterotrophically-derived nutrition may affect zooxanthellae densities and cell division as a result of increased energy translocated to the symbionts (McAuley & Cook 1994, Szmant-Froelich & Pilson 1984). In other words, nutrients acquired heterotrophically may be utilized by the zooxanthellae, potentially stimulating zooxanthellae productivity. Gut contents were removed from the stomach of each jellyfish by carefully removing the bell from the oral arms and extracting any food items present. Gut contents were then analyzed to obtain wet and dry weight for each specimen. Gut content dry weight across LP and HP sites was analyzed using a nested ANOVA (IBM SPSS v. 20.0). Pearson correlations (bivariate) were then run to evaluate the relationship between mean zooxanthellae densities, mean gut dry weight (g d⁻¹) and nutrient concentrations across sampling locations (IBM SPSS v. 20.0).

RESULTS AND DISCUSSION

Mean zooxanthellae densities from both site types (HP and LP) were found to be somewhat lower than previously reported for *Cassiopea* (Table 1). We found a significant difference between zooxanthellae densities between LP and HP sites (nested ANOVA, $F_{8,125} = 8.0$, $P < 0.001$; Fig. 1) in which the mean zooxanthellae density from LP sites was $2.3 \times 10^6 \pm 1.6$ (cells g⁻¹ ww), while the mean zooxanthellae density from HP sites was $3.3 \times 10^6 \pm 1.90$ (cells g⁻¹ ww). Cherokee, an HP site, had the highest zooxanthellae density (9.64×10^6 cell g⁻¹ ww) and North Bight (an LP site) had the lowest zooxanthellae density (3.54×10^5 cell g⁻¹ ww). Zooxanthellae densities were found to be negatively correlated to %P concentrations in seagrass tissue (Pearson bivariate correlation, $r = -0.40$, $P = 0.003$; Fig. 2C), but were not correlated to water column nutrient concentrations (Figs. 2A, B).

Mean dry gut content weight was not different between HP and LP sites (nested ANOVA, $F_{8,125} = 1.4$, $P = 0.19$; Fig. 1); however, the highest gut content weight (0.02 g per jellyfish)

Table 1. Comparison of reported zooxanthellae density values (cells g⁻¹ ww) from *Cassiopea xamachana* medusae sampled in the Florida Keys and The Bahamas / Comparación de las densidades de zooxantelas (células g⁻¹ ww) de *Cassiopea xamachana* muestreada en Florida Keys y Las Bahamas

Source	Zooxanthellae densities (cells g ⁻¹ ww)	Bell diameter size range (cm)	Collection site
Present Study- HP sites	3.2x10 ⁶	8-10	Abaco, Island Bahamas
Present Study- LP sites	2.2x10 ⁶	8-10	Abaco, Island Bahamas
Verde & McCloskey (1998)	9.6x10 ⁶	1-10	Key Largo, FL
Vodenichar (1995)	4.5x10 ⁶	4-13	Marathon, FL
Estes <i>et al.</i> (2003) (oral arms only)	~4.9x10 ⁶	10-14	Marathon, FL

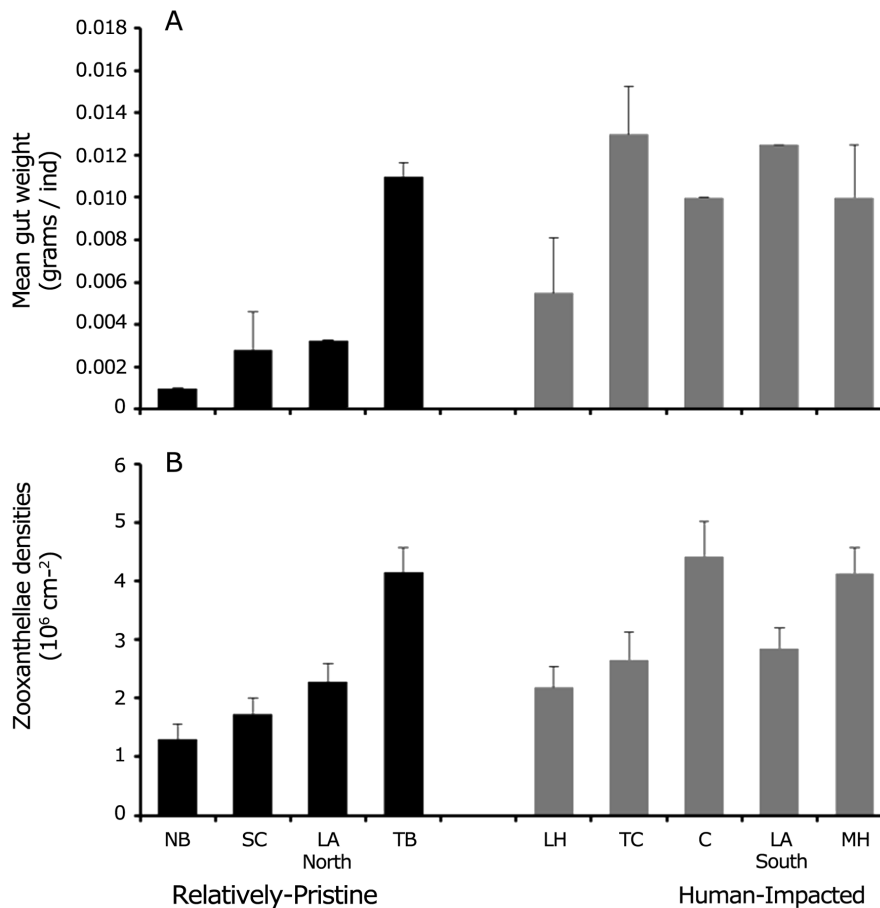


Figure 1. Mean (\pm SE) gut content dry weight values from *Cassiopea xamachana* medusae (A) and mean (\pm SE) zooxanthellae density values (B) collected from relatively-pristine sites (black bars) and human-impacted sites (grey bars) on Abaco, The Bahamas / La media (\pm EE) para el peso seco del contenido del tracto digestivo en la medusa *Cassiopea xamachana* (A) y la densidad media (\pm EE) de zooxantelas (B) recogidas de los sitios relativamente prístinos (barras negras) y los sitios impactados por actividades humanas (barras grises) en Abaco, Las Bahamas

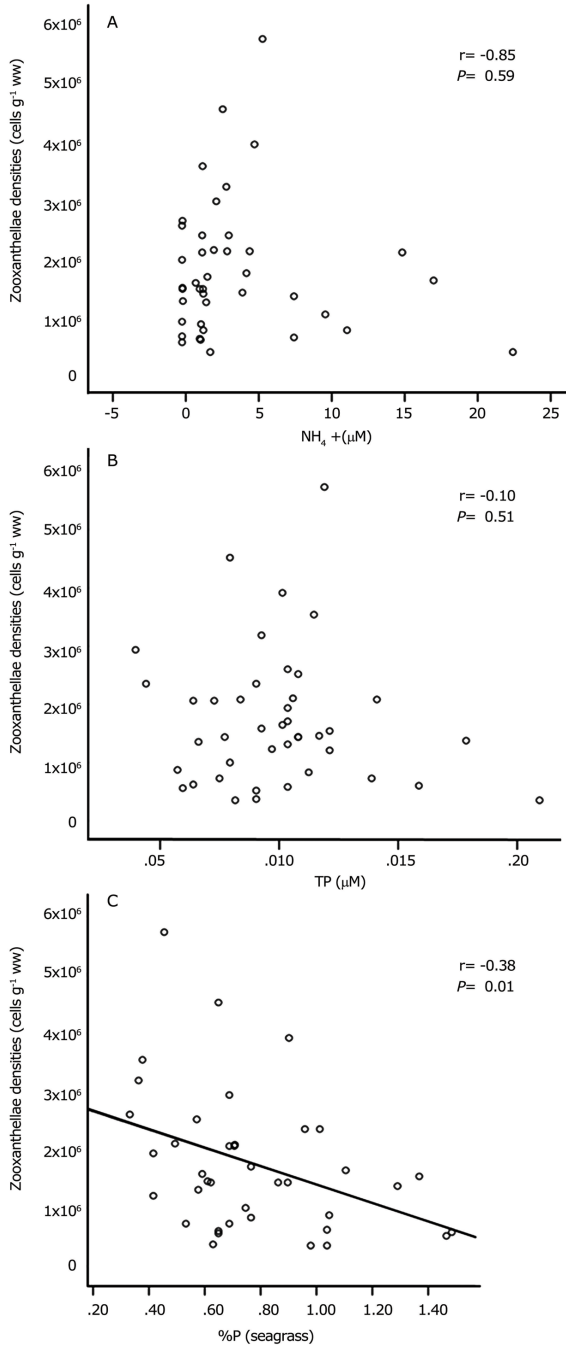


Figure 2. Correlations between zooxanthellae densities and (A) ammonium concentrations in water, (B) total phosphorus concentrations in water, and (C) percent phosphorus in *Thalassia testudinum* tissue. r = Pearson correlation coefficient and P = p -value for Pearson correlation. A trendline is included for correlations where $P < 0.05$ / Correlaciones entre las densidades de zooxantelas y (A) concentraciones de amonio en agua, (B) concentraciones de fósforo total en el agua, y (C) porcentaje de fósforo en el tejido de *Thalassia testudinum*. r = coeficiente de correlación de Pearson y P = valor p de correlación de Pearson. Una línea de tendencia se incluye para correlaciones $P < 0,05$

came from an HP site (Marsh Harbour). There was a significant positive correlation between zooxanthellae densities and gut content weight (Pearson bivariate correlation, $r = 0.26$, $P = 0.003$). Neither zooxanthellae densities nor gut weight values in jellyfish were correlated to jellyfish bell diameter (Pearson bivariate correlation, $r = -0.35$, $P = 0.39$ and $r = -0.34$, $P = 0.39$, respectively).

Our results suggest that mean zooxanthellae densities from *Cassiopea* medusae are elevated in disturbed nearshore systems compared to systems with little to no anthropogenic activity. Anthropogenic disturbances in coastal areas of The Bahamas have been shown to lead to increased densities and size of *Cassiopea* (Stoner *et al.* 2011). Herein we extend these findings by showing that some nearshore, impacted areas also may be related to higher densities of zooxanthellae in *Cassiopea* tissue, a potential mechanism driving *Cassiopea* blooms. Jellyfish tissues from Abaco had lower zooxanthellae densities than medusae collected from the Florida Keys (Table 1; Vodenichar 1995, Verde & McCloskey 1998, Estes *et al.* 2003). The Florida Keys are more heavily populated than Abaco, so it is conceivable that higher zooxanthellae densities from Florida *Cassiopea* reflect increased nutrient availability derived from human activities.

Correlations between ambient nutrient concentrations (NH_4^+ and TP) and zooxanthellae densities were not observed. We did, however, find a negative correlation between %P of seagrass and zooxanthellae densities. It is unclear what mechanism may drive this correlation, but competition between jellyfish and seagrasses is one possibility (*e.g.*, zooxanthellae rapidly uptake nutrients, reducing nutrient availability to other autotrophs). Nevertheless, because of the complex nature of coastal ecosystems in The Bahamas and the scarce knowledge of the ecological relationships between biological components, it is clear that more work is needed to elucidate the extent to which nutrients are responsible for elevated zooxanthellae densities in HP sites.

Elevated zooxanthellae densities may also be an indirect function of heterotrophically-derived sources of nutrition (*e.g.*, external food resources for jellyfish). Although we did not detect a difference in gut weight between LP and HP sites, we did find that there was a strong, positive, correlation between zooxanthellae densities and gut weight. This is consistent with the idea that increased heterotrophically-derived nutrition may stimulate zooxanthellae population growth. It is also possible that higher ambient nutrient concentrations may support greater zooplankton biomass in some human impacted coastal areas, providing a readily available food source for jellyfish.

In some parts of the world, jellyfish blooms are increasing; however, little empirical evidence exists to support specific mechanisms driving these blooms in human-impacted environments (Condon *et al.* 2013). Our results suggest that nutrient loading is possibly one of the main drivers of jellyfish blooms in nearshore regions of The Bahamas. Bloom events of *Cassiopea*, in which hundreds of individual jellyfish aggregate, may have severe consequences on ecosystem processes in seagrass beds. For example, high *Cassiopea* densities have been shown to drastically reduce seagrass densities and shift benthic community composition (Stoner *et al.* 2014a, b). Understanding the mechanisms that drive jellyfish blooms is crucial for developing effective coastal management strategies where these events are a recurring problem.

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