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Short communication

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A comparative analysis of the organic and inorganic carbon content of *Halimeda* and *Penicillus* (Chlorophyta, Bryopsidales) in a coastal subtropical lagoon

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Abstract: Standing stocks of the calcifying algae, *Halimeda* and *Penicillus*, have remained stable over the 10 years surveyed (2007–2017) in Florida Bay (USA), a subtropical lagoon. The maximum contribution of calcium carbonate ($CaCO_3$; 779.75 g m^{-2}) was lower compared to tropical lagoons. *Halimeda* was more abundant and had higher inorganic:organic carbon ratios compared to *Penicillus*. The abundance of *Penicillus* varied across the surveyed sites, Sprigger Bank, Bob Allen Keys, and Duck Key, while its inorganic:organic carbon ratios did not vary significantly. Our long-term study provides a critical baseline that can help understand fluctuations in carbonate sediment production by calcareous algae in subtropical coastal waters.

Keywords: carbonate content; *Halimeda*; loss on ignition; *Penicillus*; standing stock.

Calcareous green macroalgae (CGA) such as species of the Bryopsidales (*Udotea*, *Rhipocephalus*, *Penicillus*, and *Halimeda*) and Dasycladales (*Acetabularia*, *Cymopolia*, and *Neomeris*) play an important role as engineering species producing calcareous sediments that facilitate the development of large seagrass beds in subtropical and tropical ecosystems (Hillis-Colinvaux 1980, Williams 1990, van Tussenbroek and van Dijk 2007). These algae produce inorganic carbon through the biologically mediated process of calcification. This process is affected by the anatomy and structure of each alga; different species have different proportions of organic and inorganic carbon (Wefer 1980, Lee and Carpenter 2001). *Halimeda* uses a semi-compartmentalized intercellular space, which incorporates carbonate ions to produce calcium carbonate ($CaCO_3$) in the form of needles (Borowitzka and Larkum 1976). *Penicillus* precipitates granules of $CaCO_3$ as a sheath within capitular filaments, and this extracellular calcification is 3–5× smaller than the intercellular calcification in *Halimeda* (Lee and Carpenter 2001). Therefore, a higher production of $CaCO_3$ is expected from species of the genus *Halimeda* compared with species of the genus *Penicillus*.

Florida Bay, USA (25°00′0.60″N, 80°44′35.39″W; Figure 1), a subtropical coastal lagoon sustaining one of the largest seagrass beds in the world (Fourqurean et al. 1992), has a conspicuous presence of CGA that contribute to the production of carbonate sediments (Bosence et al. 1985, Zieman et al. 1989). *Halimeda* and *Penicillus* are the two most abundant calcareous genera in the bay, with *Halimeda* being dominant towards the southwest and *Penicillus* towards the northeast region of the bay (Zieman et al. 1989). Here, we present results from a long-term (10 years; 2007–2017) study that provided information on the standing stock of species of the genera *Halimeda* and *Penicillus*, and analyzed the differential $CaCO_3$ and organic carbon proportions by genera across three sites (three quadrats per site, for at least four times a year), from Duck Key and Bob Allen Keys in the northeast region to Sprigger Bank in the southwest region (Figure 1).

We used the Loss on Ignition method (Fourqurean et al. 2014) to estimate inorganic and organic carbon production of *Halimeda* and *Penicillus*. The mass of ash after ignition was recorded as a proxy of inorganic carbon or $CaCO_3$ production, and the mass of ash after ignition subtracted from the total dry weight (before ignition) was calculated as organic carbon mass.

Our long-term data from Florida Bay suggest that total mass standing stocks of CGA are site dependent. A slight but significant ($p < 0.001$) decrease in total mass over time was observed at Sprigger Bank, but this was not detected at Bob Allen Keys or Duck Key (Figure 2). Long-term surveys by Collado-Vides et al. (2005) showed that CGA abundance from 1996 to 2003 in the Florida Keys had, in general, a positive trend in most sites; those trends were genus- and site-dependent, suggesting that genus-specific and small scale heterogeneity of site-specific conditions

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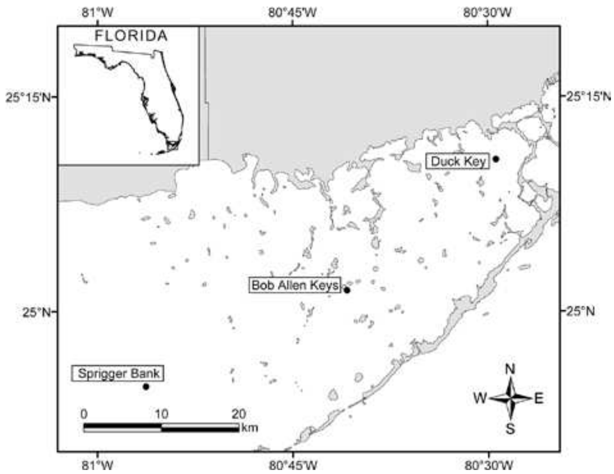

Figure 1: Map of Florida Bay showing study sites.

might play a role in determining the abundance of CGA through time.

At Sprigger Bank, CGA standing stock had an average total mass (total dry weight) of 163.29 g m^{-2} (range 0–1013 g m^{-2}), Bob Allen Keys, had an average total mass of 1.63 g m^{-2} (range 0–16 g m^{-2}) and Duck Key had an average total mass of 3.50 g m^{-2} (range 0–31 g m^{-2}). At Sprigger Bank, CGA $CaCO_3$ content (ashes after ignition) average was 126.40 g m^{-2} (range 0–778 g m^{-2}), at Bob Allen Keys, CGA $CaCO_3$ content average was 1.05 g m^{-2} (range 0–12 g m^{-2}) and at Duck Key, $CaCO_3$ content average was 2.23 g m^{-2} (range 0–20 g m^{-2}).

The content of inorganic carbon at our study sites was similar to values reported at other regional sites, such as, the backreef of the Florida Keys (Bosence et al. 1985) and the Marquesas Keys (Hudson 1985). However, when compared across the Caribbean, our results show that Florida Bay has lower values compared to neighboring tropical regions. For example, Ortegón-Aznar et al. (2017) reported an average of total mass for their sites in Yucatán, México of 1215 g m^{-2} with a maximum of 1336 g m^{-2}, which are higher values than those reported in our results for Sprigger Bank, the site with the highest total mass. Florida Bay's location at the northern limits of the tropics can be a contributing factor to the low standing stocks being reported in our study.

In our study, *Halimeda* had significantly higher total mass and inorganic carbon content than *Penicillus* at Sprigger Bank (Mann Whitney U-test, $p<0.001$), the only site where both genera were present. *Halimeda* had a significantly higher proportion of inorganic to organic carbon (80:20) than *Penicillus* (59:41) (Mann Whitney U-test, $p<0.001$). Further, both species maintained the inorganic:organic carbon ratio independent of total mass per quadrat (Figure 3), suggesting that the inorganic:organic carbon ratios among the CGA genera we analyzed are independent of abundance, but genus-specific. These results are similar to those reported by Wefer (1980) who also found differences among CGAs in Bermuda, with *Halimeda* having a higher contribution of $CaCO_3$ than *Penicillus*. Wefer (1980) suggested that the

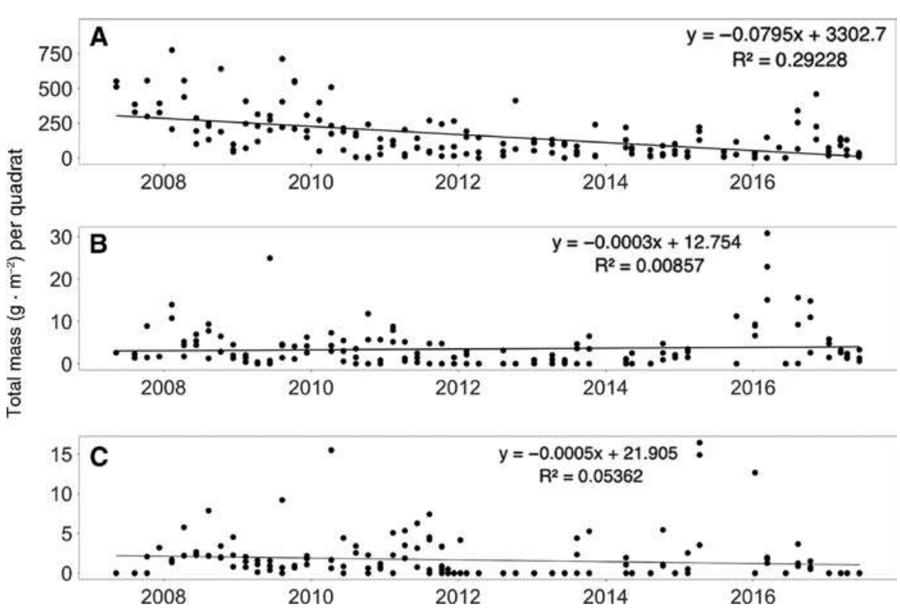

Figure 2: Linear regressions of total mass of calcareous green algae per quadrat against time at (A) Sprigger Bank ($p<0.001$), (B) Duck Key ($p=0.480$) and (C) Bob Allen Keys ($p=0.002$), from 2007 to 2017.

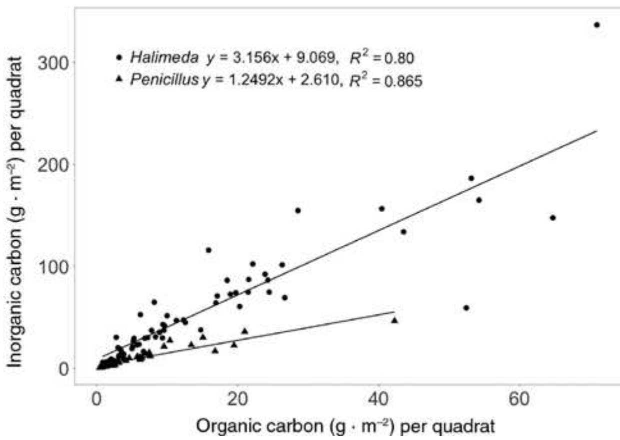

Figure 3: Linear regressions of organic carbon vs. inorganic carbon of *Halimeda* ($p < 0.001$) and *Penicillus* ($p < 0.001$) in Florida Bay.

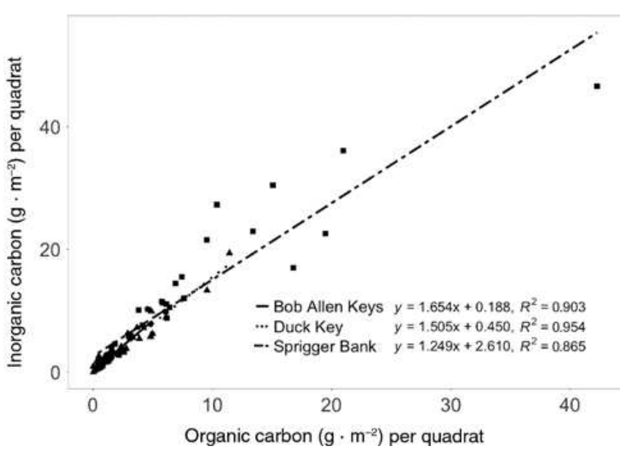

Figure 4: Linear regressions of organic carbon vs. inorganic carbon of *Penicillus* at the three sites: Sprigger Bank ($p < 0.001$), Bob Allen Keys ($p < 0.001$) and Duck Key ($p < 0.001$).

lower proportion of $CaCO_3$ in *Penicillus* compared to *Halimeda* is due to slower growth rates and longer dormant growth periods in *Penicillus* compared to *Halimeda*. The differences in inorganic carbon content can also be related to the differences in anatomy of the two genera. Higher content of inorganic carbon by *Halimeda* may be attributed to its organization of siphons and the semi-compartmentalized intercellular space, which is exclusive to this genus (Borowitzka and Larkum 1976, Multer 1988).

Penicillus was the only genus observed at all three sites, allowing us to detect any site-dependent differences at the genus level. Total *Penicillus* mass was found to be site-dependent, with significantly higher abundances at Sprigger Bank compared with Bob Allen Keys and Duck Key (Kruskal-Wallis test, $p < 0.001$), but Bob Allen Keys and Duck Key had no significant differences (ANOVA, $df = 1$, $F = 1.641$, $p = 0.204$). The proportion of organic to inorganic carbon was similar across sites (Kruskal-Wallis test, $p = 0.079$) and independent of total mass per quadrat (Figure 4).

Long-term studies help identify changes occurring due to shorter-term biological processes or longer-term ecological patterns that may not be evident from short-term studies alone (Franklin 1989). Our long-term study shows that spatial distribution and standing stock of CGA are site- and genus-dependent. *Halimeda* species dominated total mass in the southwest region, and *Penicillus* towards the northeast region of Florida Bay, consistent with previous studies of this ecosystem (Zieman et al. 1989, Fourqurean et al. 1992, Frankovich and Fourqurean 1997). The slight decreasing trend of total mass with time detected along 10 years will require further analysis to determine which environmental interactions are driving such decrease. The distribution pattern presented here provides a baseline for Florida Bay, a subtropical coastal lagoon, that will be useful to evaluate the potential negative impacts of local nutrient enrichment, ocean acidification, and global warming (Barry et al. 2013). In addition, our data provide a baseline that can be incorporated into the estimations of $CaCO_3$ production by CGAs from a subtropical coastal lagoon (Florida Bay, USA) to the global carbon cycle (Krause-Jensen and Duarte 2016).

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Bionotes

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Ligia Collado-Vides is a marine botanist with a research emphasis on subtropical and tropical marine macroalgae, including ecological and floristic approaches. Her studies are on seagrass and reef ecosystems in the Mexican Caribbean and South Florida. Her group monitors the organic and inorganic carbon contribution by *Halimeda* and *Penicillus* along a salinity and nutrient gradient, as part of the Florida Everglades Long Term Ecological Research program. She is interested in conducting research that is directly applicable to marine conservation, and management of coastal ecosystems, particularly in Marine Protected Areas. Her group has also developed a monitoring network of *Sargassum* landings in South Florida.