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## **PORTADA**

Foto de estudiantes de Wesleyan University muestrando hojas del yagrumo hembra (*Cecropia schreberiana*) en el Bosque Experimental de Luquillo. Para detalles de este estudio consulte a Doria et al. en este volumen. La foto se tomó en enero del 2008 por Timothy Ku.

# ACTA CIENTÍFICA

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## **EDITORIAL**

Este número de Acta Científica contiene una sección especial basada en trabajos de campo de estudiantes del Departamento de Earth and Environmental Sciences, Wesleyan University, en Connecticut, Estados Unidos de Norte América. Estos estudiantes sub-graduados participaron en un curso mandatorio para obtener su grado que se componía de un Seminario y un Proyecto de Investigación en el Campo. Durante el curso de otoño los estudiantes estudian la evolución de la Tierra en su totalidad y proponen proyectos de investigación originales compuestos por pequeños grupos con aproximadamente medio día dedicado a la recopilación de muestras o data en el campo. En enero, los estudiantes y maestros viajaron a Puerto Rico para llevar a cabo el trabajo de campo y después terminar el análisis e interpretación de data en la primavera. Miembros de la facultad de la Universidad dirigieron los estudios de campo, supervisaron el análisis de datos y revisaron los manuscritos. Los profesores principales en este proyecto a quienes estamos agradecidos por esta colaboración son: Timothy Ku, Dana Royer y Phillip Resor.

No es la primera vez que las páginas de Acta se utilizan para la publicación de investigaciones llevadas a cabo por estudiantes. Varios volúmenes pasados contienen trabajos de estudiantes de escuelas públicas y universidades en Puerto Rico. Estamos colaborando con otros profesores para continuar esta práctica, la cual consideramos de importancia para el desarrollo en los estudiantes de una cultura científica que enfatice la importancia de llevar los trabajos científicos a su justa conclusión: la publicación del trabajo en una revista dedicada a la ciencia.

Ariel E. Lugo

*Editor*

## CHEMICAL ANALYSES OF *THALASSIA TESTUDINUM* - DETERMINING THE ENVIRONMENTAL CONDITION OF LAGUNA GRANDE AND THE SEVEN SEAS, PUERTO RICO

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**Key words:** *Nutrients, isotopic composition, seagrasses, metal element concentration, Redfield ratio*

### ABSTRACT

The isotopic and elemental compositions of seagrasses can be used as *in situ* indicators of ecosystem health because seagrasses continually exchange solutes with the surrounding water column and sediments. This study assesses the nutrient (C:N:P) and metal (Cu, Cd, Fe, Pb) availability within two marine ecosystems. One is Laguna Grande, a restricted bioluminescent bay, and the other is the Seven Seas area, an adjacent open ocean coastal sea, located on the northeast tip of Puerto Rico. The seagrass *Thalassia testudinum* is the dominant seagrass species in tropical regions, and was sampled at the selected sites as an indicator of environmental change. Seagrass C, N, and P concentrations between the two ecosystems were more similar than expected, but both had relatively high N concentrations and N:P ratios above 30, suggesting phosphorus-limited growth. In both ecosystems,  $\delta^{15}\text{N}$  seagrass values ranged from -4.6 to +2.9 ‰ AIR, indicating that these environments are not currently affected by anthropogenic activities, and that the natural source of nitrogen to both systems is similar. The bioluminescent bay seagrass rhizomes and sediments had higher concentrations of Fe, Cu, Cd, and Pb than those of the open ocean environment. These elevated metal concentrations are attributed to natural sediment processes and restricted water fluxes rather than anthropogenic sources.

### RESUMEN

Las composiciones elementales e isotópicas de los pastos marinos pueden ser utilizados como indicadores locales de la salud de los ecosistemas marinos ya que los pastos marinos continuamente intercambian solutos con los sedimentos y la columna de agua circundante. Esta investigación evalúa la disponibilidad de nutrientes y metales dentro de dos ecosistemas marinos, uno es la Laguna Grande, una bahía bioluminiscente restringida y el otro es la bahía Las Cabezas, un área costanera adyacente al mar abierto, situados ambos en la punta noreste de Puerto Rico. Como indicador de cambio ambiental, *Thalassia testudinum*, la especie de hierba marina dominante en el trópico, se muestreó en sitios escogidos. Las concentraciones de carbono, nitrógeno y fósforo en las praderas marinas entre los dos ecosistemas eran más parecidas de lo esperado, sin embargo ambos tenían una

concentración relativamente alta de nitrógeno y proporciones de nitrógeno a fósforo sobre 30, lo que sugiere que el fósforo limita el crecimiento biológico. En ambos ecosistemas el  $\delta^{15}\text{N}$  varió de -4.6 a +2.9 ‰ AIR, lo que indica que actualmente estos ecosistemas no están siendo afectados por actividades antropogénicas y que la fuente natural de nitrógeno es similar en ambos. Los sedimentos y rizomas de las praderas marinas en la bahía bioluminiscente mostraron concentraciones más altas de hierro, cobre, cadmio y plomo que esos en un ambiente marino abierto. Estas concentraciones elevadas de metales se atribuyen más a los procesos naturales de sedimentación y los flujos de aguas restringidas que a fuentes antropogénicas.

## INTRODUCTION

In the past 50 years, there has been a great deal of interest in seagrass ecology. Seagrasses are increasingly being used in tropical and subtropical estuarine habitats as *in situ* biological indicators of ecological health. The balance between nutrient supply and nutrient demand within seagrass ecosystems determines the concentration of nutrients present in seagrass tissue. As such, the nutrient concentration in seagrass reflects nutrient levels in the water column and can provide insight into the health of the broader environment (Fourqurean et al. 2005). Seagrasses also track anthropogenic pollution because proxies of pollution are recorded in the plant tissues (Bortone 2000).

Seagrass C:N:P ratios are the primary indicator of nutrient dynamics within a marine ecosystem. The nutrient composition of marine phytoplankton is generally close to that of the Redfield ratio, which is a molar C:N:P ratio of 106:16:1. The Redfield ratio for macrophytes (such as seagrasses) is closer to 550:30:1 (Atkinson and Smith 1983, Fourqurean and Zieman 1992). This Redfield ratio can be used to determine the limiting nutrient factor, since N or P or both can be the limiting nutrient in coastal ecosystems (Howarth and Marino 2006). Near-shore areas of the surrounding Caribbean, such as inner Florida Bay, are known to be phosphorus-limited, as found by studies like the 2002 Fourqurean et al. nutrient-addition study of the Florida Bay seagrass *Thalassia testudinum*, which measured an average seagrass N:P ratio of 40.2 (Fourqurean et al. 2002).

The nitrogen isotope composition of seagrasses ( $\delta^{15}\text{N}$ ) can identify anthropogenic N sources

because fertilizer and anthropogenic sewage have higher  $\delta^{15}\text{N}$  values ( $> 10$  ‰ AIR) than seagrass populations in unpolluted environments, which typically have  $\delta^{15}\text{N}$  values of 0 to +4 ‰ AIR (Clark and Fritz 1997, Fourqurean et al. 2005). In Florida Bay, there is a gradient in  $\delta^{15}\text{N}$  values from a populated coast out to an open ocean area, with populated areas having  $\delta^{15}\text{N}$  seagrass values around +10‰, and seagrass beds further from anthropogenic sources having values of 0 to +4‰ (Fourqurean et al. 2005). The high  $\delta^{15}\text{N}$  seagrass values are attributed to anthropogenic pollution of the coastal water system (Fourqurean et al. 2005).

Seagrass tissue metal concentrations also reflect anthropogenic impacts. Seagrasses accumulate metals in their leaves and rhizomes through two pathways. Metals are either taken up directly from the water column or from interstitial water in the sediments (Roméo et al. 1995). While bulk and tissue chemistry analysis may reveal spatial variation in anthropogenic inputs of metals, the relative change in local seawater metal chemistry over time can be reconstructed by analyzing individual rhizome internodes, because the internodes are remnants of past leaves and record the chemistry of the plant during their time of growth (Roméo et al. 1995). This retroactive dating technique is termed lepidochronology and has been successfully used to determine concentrations of Cd, Cu, Fe, Pb, and Zn within the seagrass scales of *Posidonia oceanica* by Roméo et al. 1995. Their study found that levels of Cu had increased over time within areas frequented by pleasure boats, and also found overall higher metals concentrations in seagrasses near on-shore centers of industrial pollution (Roméo et al. 1995).

This study analyzed nutrient and metal concentrations of *Thalassia testudinum*, more commonly known as turtle grass, from two different, adjacent ecosystems in northeastern Puerto Rico (Fig. 1). One study location is the Seven Seas area, an open-ocean, CaCO<sub>3</sub> reef-containing, oligotrophic bay, known as Bahía las Cabezas. The second, separated from the first by a brush-covered sandbar only 10 meters wide, is Laguna Grande, a bioluminescent lagoon. The bioluminescence is most likely caused by the dinoflagellate *Pyrodinium bahamense* var. *bahamense*, as has been reported for Puerto Mosquito in Vieques, Puerto Rico and Bahía Fosforescente near La Parguera, Puerto Rico (Seliger et al. 1971). This bioluminescent bay has one outlet, a twisted 900-meter long mangrove inlet that provides limited exchange with the open ocean environment. The aim of the study is to determine the nutrient and metal concentrations of seagrasses within these two ecosystems and to use this data with measurements of seagrass  $\delta^{15}\text{N}$  to evaluate the extent to which both ecosystems are currently impacted by anthropogenic pollution.

## METHODOLOGY

*Thalassia testudinum* was collected at four locations in Bahía Las Cabezas and three locations in Laguna Grande (Fig. 1). Within each study area, field groups dispersed to identified beds of *Thalassia testudinum*. At each site, five seagrass plants were collected and care was taken to obtain samples with complete vertical rhizomes. After collection, samples were cleaned with tap water and razor blades to remove epiphytes. Each plant was then rinsed in a vinegar solution (5 percent acetic acid) to remove CaCO<sub>3</sub> particles and frozen.

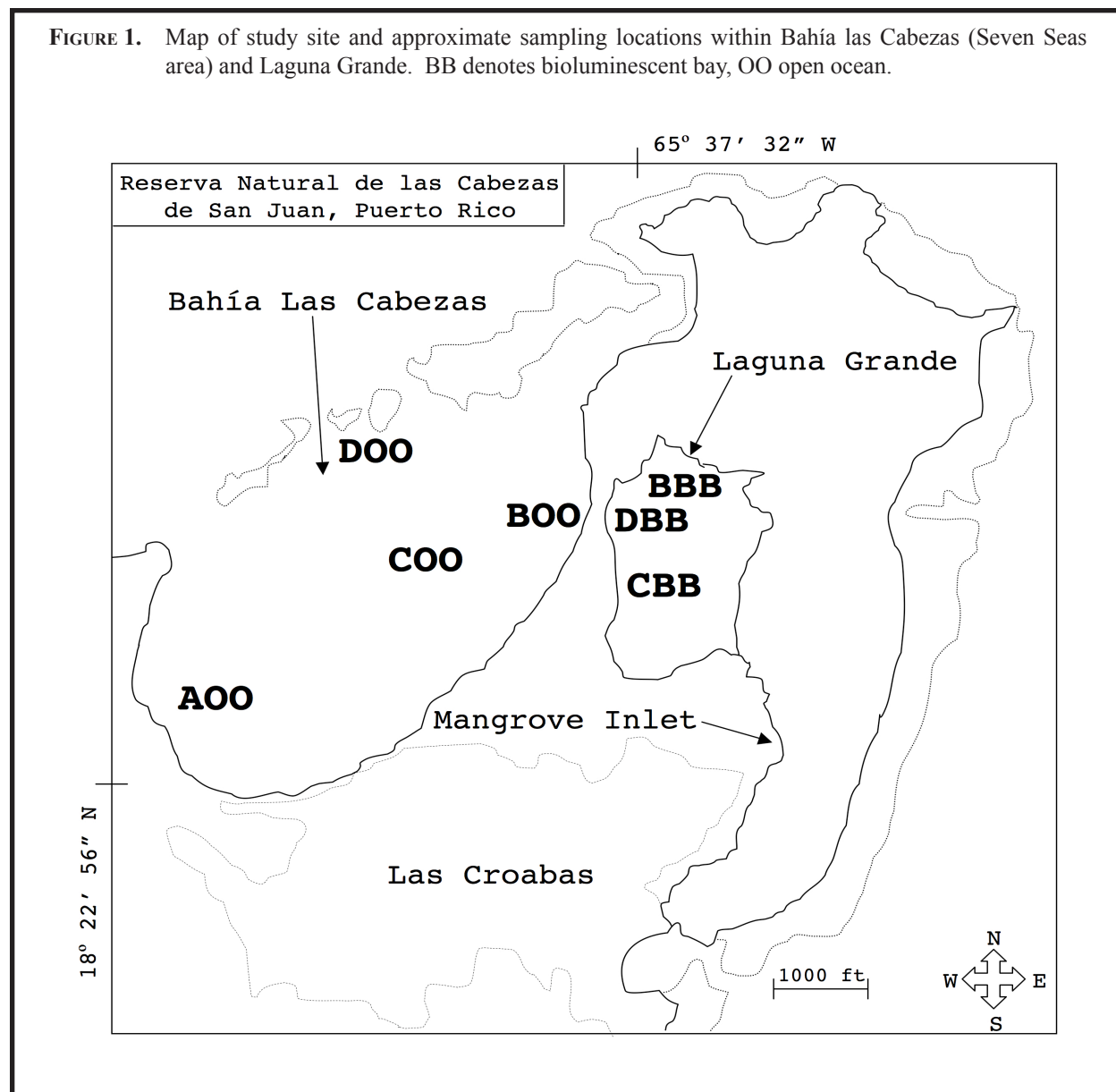
Seagrass leaf chemistries were determined by sub-sampling the midsection of included seagrass leaves to avoid variation in nutrient concentration along the blades. The C and N concentrations were determined on a Thermo Flash 1112 Elemental Analyzer. Phosphorus concentrations were determined using a modified method of Solórzano and Sharp (1980). Briefly, 5 to 20 mg of sample was heated at 90°C with 0.5 mL of 0.17 M Na<sub>2</sub>SO<sub>4</sub> and 2.0 mL of 0.017 M MgSO<sub>4</sub>, then ashed for three hours at 500°C. Before phosphorous concentrations were measured, 5.0 mL of 0.2 N HCl and 10 mL

of de-ionized water were added to the samples. Total phosphorous concentrations were measured on a Beckman-Coulter DU 530 spectrophotometer at a wavelength of 885 nm using the method of Murphy and Riley (1962). For the  $\delta^{15}\text{N}$  analysis, the midsections of the seagrass leaves were sub-sampled, as they were for the C and N analysis. Nitrogen isotope compositions were determined by CF-IRMS at the Indiana University Stable Isotope Laboratory and are reported in standard delta notation relative to the AIR standard.

Metal analysis of seagrass rhizomes followed the method of Roméo et al. (1995) and Alfonso et al. (2008). Leaf sheaths were too small for metals analysis, so sections of the seagrass vertical rhizome were cut every five scars to be analyzed. The sample location closest to the emerging leaves represents the youngest portion of the vertical rhizome, while successive sections lower on the stem were considered to be progressively greater in age. One vertical rhizome was analyzed for two sites in both Laguna Grande and in the Seven Seas area. Samples were digested with concentrated nitric acid at 70 °C for 8 hours followed by a second digestion with concentrated hydrogen peroxide at 70 °C for 4 hours. Metal concentrations of the resulting solutions were determined by Inductively coupled plasma-mass spectrometry at Lehigh University, and are held to be estimates, as the chosen method was not a total digest.

## RESULTS

Overall, the seagrass leaves for the two sites, Laguna Grande and the open ocean, had similar nutrient concentrations (Fig. 2). The wt. % C ranged from 36.97 to 39.9. The wt. % N ranged from 1.41 to 3.65 and wt. % P ranged from 0.08 to 0.23. Both the average wt. % P and wt. % N were slightly higher in the bioluminescent bay (0.16, 3.02 wt. %) than in the open ocean (0.15, 2.55 wt. %). An exception is the bioluminescent bay site CBB, which had lower wt. % N than all of the open ocean sites (2.06 wt. %). Site COO had the lowest wt. % P overall (0.10 wt. %). The average C:P and C:N was slightly higher in the bioluminescent bay (670, 16.1) than in the open ocean (700, 17.6). Overall, the C:P ratio ranged from 435 to 1,163, the C:N ratio ranged from 12.01 to 22.43, and the average



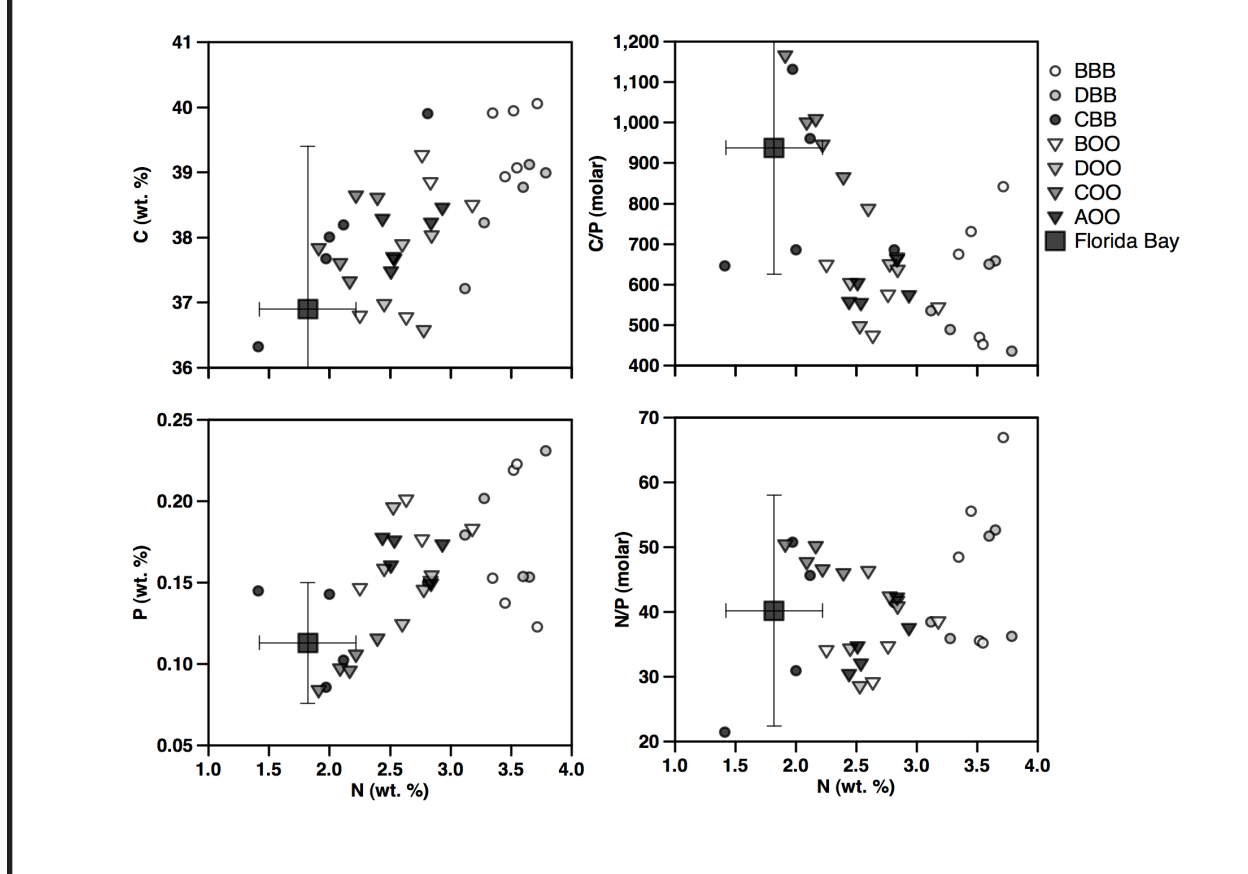
N:P ratio for the different sites varied between 35 and 48.

Measured values of  $\delta^{15}\text{N}$  in the bioluminescent bay and open ocean ranged from -4.6 to +2.9 ‰ AIR (Fig. 3). There was little variation in seagrass  $\delta^{15}\text{N}$  for the two study areas, as seagrasses at nearly all sites registered  $\delta^{15}\text{N}$  values between 0 and 3 ‰ AIR. The exception was site COO, where seagrass  $\delta^{15}\text{N}$  values were -4.55 and -1.49 ‰ AIR. There was no evident correlation between seagrass  $\delta^{15}\text{N}$  values and measured seagrass nitrogen concentrations.

Overall, Cu, Fe, Pb, and Cd concentrations within sediments and seagrass internodes are higher in the bioluminescent bay than in the open Seven Seas area (Fig. 4). In the Seven Seas seagrasses, rhizome metal concentrations remain fairly constant over time (Fig. 5). For sampled bioluminescent bay seagrasses, internode metal concentrations varied considerably, with internodes 1 and 6 at site DBB and internode 2 at site CBB having particularly elevated concentrations of all four metals (Fig. 5). For all sites, Cu, Cd, and Pb were higher in the seagrass tissues than in the respective



**FIGURE 2.** Carbon and P concentrations and C:P and N:P ratios versus N concentrations at all study sites. BB denotes bioluminescent bay, and OO open ocean. Refer to Figure 1 for sampling locations. Values from Florida Bay were taken from Fourqurean et al. (2002) and error bars show standard deviations.



surrounding sediments, except for Cu at site AOO (Fig. 4).

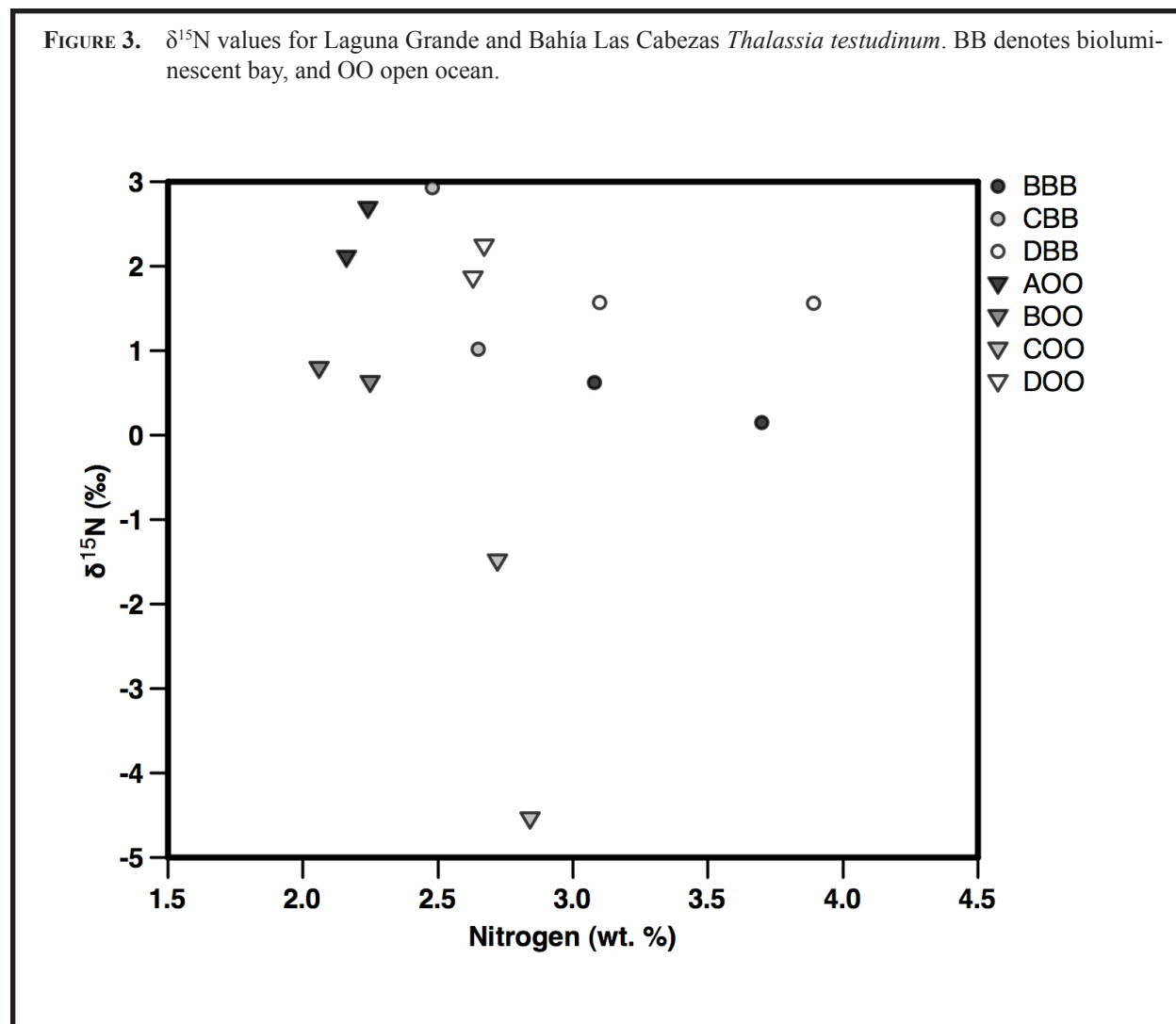
## DISCUSSION

Puerto Rican bioluminescent bays support large populations of *Pyrodinium bahamense*, which are typically associated with eutrophic or nutrient-rich waters (Phlips et al. 2006). In contrast, coral reefs are associated with oligotrophic or nutrient-poor waters. That Laguna Grande had only slightly higher C, N, and P concentrations (36.3 - 40.1, 1.41 - 3.79, 0.09 - 0.23 wt. %) than the Seven Seas area (36.6 - 39.3, 1.91 - 3.18, 0.08 - 0.20 wt. %) is remarkable given that these two environments are so different. It was expected that nutrient concentrations would

be much lower in the open ocean ecosystem than in the largely closed, highly biologically productive, bioluminescent bay environment.

Average N:P ratios of seagrass tissues at both sites are above 35. Given that the seagrass Redfield ratio is 30, these elevated values indicate that both systems are phosphorus limited, like the near-shore Florida Bay locations (Fourqurean et al. 2002). These high N:P ratios are the result of high nitrogen concentrations within seagrasses in both systems. For the open ocean sites, the average nitrogen concentration was 2.5 wt. %, while the tissue of bioluminescent bay seagrass was on average 3 wt. % N. For comparison, a large study of Florida Bay *Thalassia testudinum* found

FIGURE 3.  $\delta^{15}\text{N}$  values for Laguna Grande and Bahía Las Cabezas *Thalassia testudinum*. BB denotes bioluminescent bay, and OO open ocean.

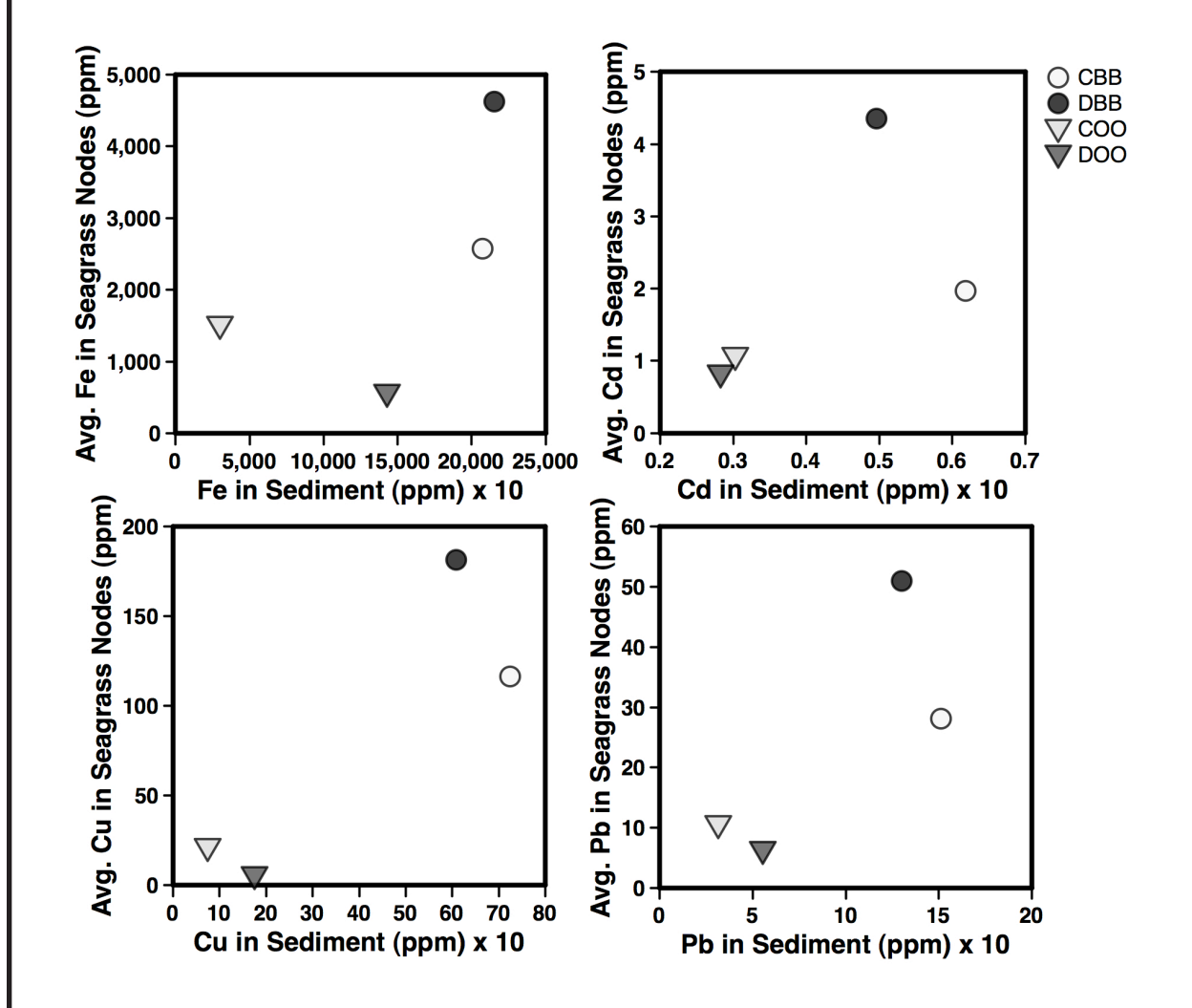


an average nitrogen concentration of  $1.82 \pm 0.40$  wt. %, showing that this study's research area is particularly enriched in nitrogen (Fourqurean et al. 2002). Given high N concentrations, the  $\delta^{15}\text{N}$  of seagrass leaves can be used to help evaluate the source of the nitrogen. Normal seagrasses have  $\delta^{15}\text{N}$  between 0 and +4 ‰ and anthropogenically polluted seagrasses have  $\delta^{15}\text{N} > 6$  ‰ (Fourqurean et al. 2005). The  $\delta^{15}\text{N}$  values of open ocean and bioluminescent bay seagrasses were largely similar, with the  $\delta^{15}\text{N}$  of all but two samples falling between 0 and 4 ‰ (Fig. 3). This indicates that the source of nitrogen to Laguna Grande and the Seven Seas area is not only the same, but also natural. Outlying  $\delta^{15}\text{N}$  values of -1.49 and -4.55 ‰ were measured in seagrasses located at the open ocean site COO.

These negative values suggest an anomalous source of nitrogen for this specific site, or a different process of fractionation for these seagrasses. Site COO sediment is markedly Fe-poor, suggesting a limited input of terrestrial material that could influence nitrogen cycling at this location.

No single consistent time-trend of metal accumulation or depletion within seagrass internodes was observed, which may be due to inconsistencies in rates of seagrass growth and internode production. However, metal concentrations within seagrass nodes and sediments from the bioluminescent bay were greater than in those from the open ocean environment (Fig. 5). Given that the  $\delta^{15}\text{N}$  analysis provided no evidence

FIGURE 4. Average Fe, Cu, Cd, and Pb concentrations of nodes vs. Fe, Cu, Cd, and Pb concentrations in sediments. BB denotes bioluminescent bay, and OO open ocean.

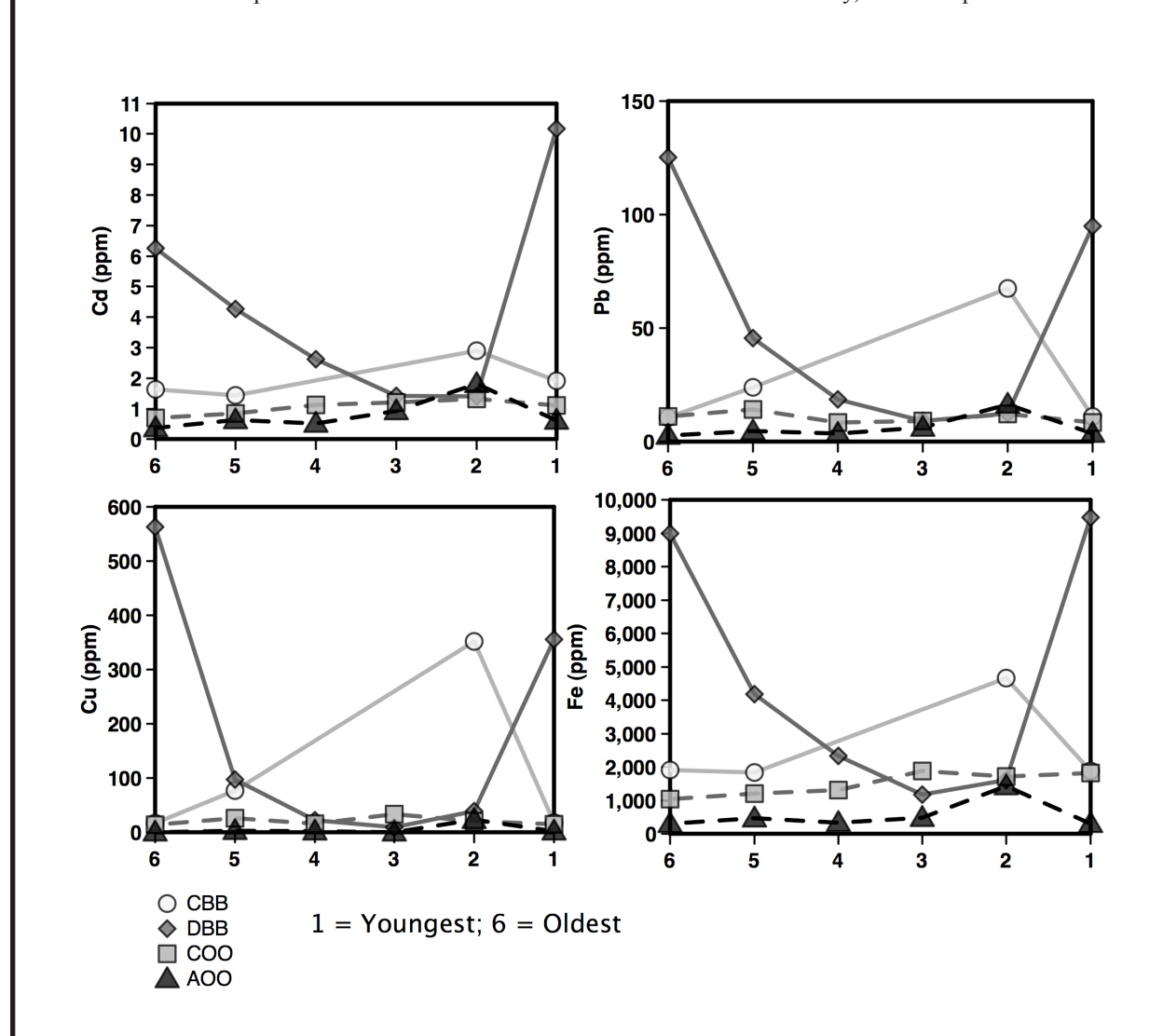


of anthropogenic input of nitrogen to these ecosystems, it is hypothesized that these elevated concentrations of metals within the bioluminescent bay are not due to anthropogenic metals pollution. It is likely that lower concentrations of  $\text{CaCO}_3$  within the bioluminescent bay (though not tested) would explain a greater concentration of metals within these sediments. Alternatively, it is suggested that either greater pore water mobility of metals within bioluminescent bay sediments, sediment diagenesis, or limited rates of exchange may have lead to higher metal concentrations within this environment (Roméo et al. 1995).

## CONCLUSION

Overall, C/N/P concentrations and ratios show little difference between the closed bioluminescent bay seagrasses and those within the open ocean environment. In both the Laguna Grande and Bahía las Cabezas ecosystems, seagrass nitrogen concentrations are on average 0.93 wt. % higher than in Florida Bay, and both ecosystems are phosphorus limited. Despite the relatively high level of nitrogen within both environments,  $\delta^{15}\text{N}$  values show that the nitrogen source is not derived from anthropogenic pollution. In addition,  $\delta^{15}\text{N}$  values suggest that the source of nitrogen to both

FIGURE 5. Variation in Cd, Pb, Cu, and Fe concentrations over time. 1 = youngest portion of vertical rhizome, and 6 = oldest portion of vertical rhizome. BB denotes bioluminescent bay, and OO open ocean.



ecosystems is likely the same except at one  $\text{CaCO}_3$  rich site.

Metal concentrations of *Thalassia testudinum* rhizomes and sediment showed that metal concentrations overall are higher within the bioluminescent bay environment than in the open ocean area, but no single chronosequence is displayed. Given the lack of evidence for anthropogenic inputs to either system, it is concluded that the elevated metal concentrations within the bioluminescent bay seagrasses and sediments are due to sediment diagenesis, the mobility of metals

within sediment pore waters, or the limited extent to which the lagoon is flushed. Future studies should seek to analyze seagrass sheaths in place of rhizomes.

#### ACKNOWLEDGMENTS

This study would like to thank Pure Adventure for logistical assistance and the Harold T. Stearns and Joe Webb Peoples endowments for providing funding. Timothy Ku and Phillip Resor are also thanked for their support and guidance in both the field and lab.

APPENDIX A. Seagrass, C, N, and P concentrations and  $\delta^{15}\text{N}$  results.

Sample Site	Carbon (wt. %)	Nitrogen (wt. %)	Phosphorus (wt. %)	C/N (molar)	C/P (molar)	N/P (molar)	$\delta^{15}\text{N}$ (‰)
Bio-Bay B	40.1	3.72	0.12	12.6	842	66.9	0.62
Bio-Bay B	39.9	3.52	0.22	13.2	470	35.5	0.15
Bio-Bay B	39.9	3.35	0.15	13.9	674	48.5	-----
Bio-Bay B	39.1	3.55	0.22	12.8	452	35.2	-----
Bio-Bay B	38.9	3.45	0.14	13.2	731	55.5	-----
Avg. Bio-Bay B	39.6	3.52	0.17	13.1	634	48.3	0.39
Bio-Bay C	36.3	1.41	0.14	30.1	646	21.5	2.93
Bio-Bay C	38.0	2.00	0.14	22.2	686	30.9	1.02
Bio-Bay C	37.7	1.97	0.09	22.3	1132	50.8	-----
Bio-Bay C	38.2	2.12	0.10	21.0	960	45.6	-----
Bio-Bay C	39.9	2.81	0.15	16.6	686	41.4	-----
Avg. Bio-Bay C	38.0	2.06	0.13	22.4	822	38.1	1.98
Bio-Bay D	39.0	3.79	0.23	12.0	435	36.3	1.57
Bio-Bay D	39.1	3.65	0.15	12.5	658	52.6	1.56
Bio-Bay D	38.8	3.60	0.15	12.6	650	51.7	-----
Bio-Bay D	37.2	3.12	0.18	13.9	535	38.4	-----
Bio-Bay D	38.2	3.28	0.20	13.6	489	35.9	-----
Avg. Bio-Bay D	38.5	3.49	0.18	12.9	554	43.0	1.56
Open Ocean A	38.3	2.44	0.18	18.3	557	30.4	2.11
Open Ocean A	38.4	2.93	0.17	15.3	572	37.4	2.69
Open Ocean A	38.2	2.84	0.15	15.7	662	42.1	-----
Open Ocean A	37.7	2.54	0.18	17.3	554	31.9	-----
Open Ocean A	37.5	2.51	0.16	17.4	602	34.6	-----
Avg. Open Ocean A	38.0	2.65	0.17	16.8	589	35.3	2.40
Open Ocean B	36.8	2.25	0.15	19.0	648	34.0	0.79
Open Ocean B	36.8	2.63	0.20	16.3	473	29.0	0.62
Open Ocean B	39.3	2.76	0.18	16.6	574	34.7	-----
Open Ocean B	38.8	2.83	0.15	16.0	665	41.6	-----
Open Ocean B	38.5	3.18	0.18	14.1	543	38.5	-----
Avg. Open Ocean B	38.0	2.73	0.17	16.4	581	35.6	0.70
Open Ocean C	38.6	2.22	0.11	20.3	944	46.5	- 4.55
Open Ocean C	37.6	2.09	0.10	21.0	1000	47.6	- 1.49
Open Ocean C	37.3	2.17	0.10	20.1	1007	50.1	-----
Open Ocean C	37.8	1.91	0.08	23.1	1164	50.4	-----
Open Ocean C	38.6	2.39	0.12	18.8	864	45.9	-----
Avg. Open Ocean C	38.0	2.16	0.10	20.7	996	48.1	- 3.02
Open Ocean D	36.6	2.78	0.15	15.4	649	42.3	2.24
Open Ocean D	38.0	2.84	0.15	15.6	636	40.8	1.86
Open Ocean D	37.9	2.60	0.12	17.0	786	46.3	-----
Open Ocean D	37.0	2.45	0.16	17.6	603	34.3	-----
Open Ocean D	37.7	2.53	0.20	17.4	496	28.5	-----
Avg. Open Ocean D	37.4	2.64	0.16	16.6	634	38.4	2.05

**APPENDIX B.** Seagrass and sediment metal concentrations.

<b>Sample Site</b>	<b>Fe (ug/g)</b>	<b>Cu (ug/g)</b>	<b>Cd (ug/g)</b>	<b>Pb (ug/g)</b>
Bio-Bay C	1883	17.96	1.91	10.92
Bio-Bay C	4662	351.96	2.90	67.37
Bio-Bay C	1831	77.48	1.43	23.78
Bio-Bay C	1906	17.28	1.63	10.52
Avg. Bio-Bay C	2571	116.17	1.97	28.15
Bio-Bay C Sed (10X)	20742	72.49	0.62	15.13
Bio-Bay D	9472	355.24	10.17	95.01
Bio-Bay D	1607	39.11	1.40	12.30
Bio-Bay D	1167	9.86	1.42	9.01
Bio-Bay D	2330	22.46	2.62	18.53
Bio-Bay D	4177	97.60	4.27	45.47
Bio-Bay D	8985	562.87	6.25	125.26
Avg. Bio-Bay D	4623	181.19	4.36	50.93
Bio-Bay D Sed (10X)	21529	60.91	0.50	13.00
Open Ocean A	304	2.35	0.61	3.49
Open Ocean A	1421	22.93	1.81	16.41
Open Ocean A	488	0.48	0.93	6.21
Open Ocean A	330	1.73	0.52	3.50
Open Ocean A	466	2.92	0.63	4.53
Open Ocean A	300	0.14	0.37	2.62
Avg. Open Ocean A	551	5.09	0.81	6.13
Open Ocean A Sed (10X)	14298	17.48	0.28	5.53
Open Ocean C	1826	14.73	1.11	8.32
Open Ocean C	1712	20.66	1.32	12.10
Open Ocean C	1876	33.29	1.21	8.97
Open Ocean C	1310	15.59	1.12	8.21
Open Ocean C	1202	25.97	0.84	14.01
Open Ocean C	1043	13.95	0.70	10.98
Avg. Open Ocean C	1495	20.70	1.05	10.43
Open Ocean C Sed (10X)	2979	7.40	0.30	3.15

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## NUTRIENT LIMITATION IN BAHIA FOSFORESCENTE, PUERTO RICO: EVIDENCE FROM SEAGRASS C:N:P RATIOS IN *THALASSIA TESTUDINUM*

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**Key words:** *N:P ratios, Redfield ratios, C:N ratios, seagrasses*

### ABSTRACT

Bahía Fosforescente, located in southwestern Puerto Rico, contains high concentrations of the bioluminescent dinoflagellate species *Pyrodinium bahamense* var. *bahamense*. Because high concentrations of *Pyrodinium bahamense* are commonly found in phosphorus-limited environments, phosphorus sources and availability may be determining factors in controlling the population dynamics of *Pyrodinium bahamense* var. *bahamense*. In Bahía Fosforescente, leaf concentrations of nitrogen and phosphorus in the seagrass species *Thalassia testudinum* convey high overall concentrations of nutrients across the bay compared to other *Thalassia testudinum* studies, with mean N concentrations at  $2.3 \pm 0.3$  wt % and mean P concentrations at  $0.10 \pm 0.03$  wt %. Despite relatively high nutrient concentrations, C:N:P ratios reveal phosphorus limitation on primary production throughout the bay. High overall nutrient concentrations indicate that this bay ecosystem is P-limited due to a relative overabundance of N, rather than low P concentrations. Spatial distribution patterns of N:P ratios display decreasing relative P content and increasing relative N content with distance from the mouth of the bay. The high relative N content toward the back of the bay suggests that a terrestrial source of nitrogen is possibly driving the bay to P limitation.

### RESUMEN

La bahía Fosforescente, localizada al sudoeste de Puerto Rico, contiene altas concentraciones del dinoflagelado *Pyrodinium bahamense* var. *bahamense*. Debido a que las altas concentraciones de dinoflagelados generalmente coinciden con ambientes limitados en fósforo, las fuentes y disponibilidad de este elemento pueden ser factores determinantes en el control de la dinámica poblacional de *Pyrodinium bahamense* var. *bahamense*. En la bahía Fosforescente, los valores de porcentaje en peso (% p/p) de nitrógeno (N) y fósforo (P) en la especie de pradera marina *Thalassia testudinum* presentan, en general, altas concentraciones de nutrientes a lo largo de la bahía, con concentraciones promedias de N de  $2.3 \pm 0.3\%$  p/p y  $0.10 \pm 0.03\%$  p/p de P. A pesar de las altas concentraciones



de nutrientes, la relación C:N:P revela una limitación de P en la producción primaria a lo largo de la bahía. Altas concentraciones de nutrientes en general indican que este ecosistema tiene una limitación de P debido a una mayor abundancia de N, y no por bajas concentraciones de P. Los patrones de distribución espacial de la relación N:P demuestran que los valores de P disminuyen, mientras que los de N aumentan con la distancia a la boca de la bahía. Los altos valores de N encontrados hacia el fondo de la bahía sugieren que una fuente terrestre de N está llevando la bahía a una limitación de P.

## INTRODUCTION

Bahía Fosforescente, on the southwestern coast of Puerto Rico, fosters a very high concentration of the bioluminescent dinoflagellate species *Pyrodinium bahamense* var. *bahamense* with concentrations as high as 100 individuals/ml (Seliger et al. 1971). Interest in bioluminescent bays is high not only because of their importance as an ecological wonder and tourist attraction, but also as a possible environmental health concern. Until recently, *Pyrodinium bahamense* var. *bahamense* was thought to be nontoxic, though a closely related Pacific Ocean species, *Pyrodinium bahamense* var. *compressum*, has been known to produce neurotoxins that cause fatal in paralytic shellfish poisoning (Landsberg et al. 2006). However, cases of saxitoxin pufferfish poisoning associated with *Pyrodinium bahamense* var. *bahamense* suggest that it is also capable of producing dangerous toxins (Phlips et al. 2004). The existence of high concentrations of this potentially toxic dinoflagellate raises concern about the health of the local community and prompts further investigation into the conditions that allow for blooms to occur. Salinity, shallow water depth, temperature and high nutrient concentrations have been noted to contribute to the occurrence of dinoflagellate blooms around the world (Phlips et al. 2004, Phlips et al. 2006).

Dinoflagellate population growth is often limited by nutrient availability (Hodgkiss and Ho 1997, Phlips et al. 2004, Phlips et al. 2006). While nitrogen limitation is more common in open ocean environments, dinoflagellate blooms often coincide with phosphorus limitation (Hodgkiss and Ho 1997, Phlips et al. 2006). Nutrient limitation for

*Pyrodinium bahamense* var. *bahamense* has been observed in the Indian River Lagoon, Florida, where large rainfall events increased nutrient-rich runoff and stimulated dinoflagellate blooms. The largest blooms were closely associated with large increases in dissolved phosphorus, indicating probable phosphorus limitation for dinoflagellates in this study location (Phlips et al. 2004).

This study seeks to investigate the role of nutrient limitation in controlling dinoflagellate populations in Bahía Fosforescente. To assess nutrient status, carbon, nitrogen, and phosphorus concentrations, C:N:P ratios are compared between seagrasses in Bahía Fosforescente, the open ocean (with a low concentration of dinoflagellates), and a nearby inlet with lower concentrations of *Pyrodinium bahamense* var. *bahamense*, and a published *Thalassia* dataset from Floriday Bay (Fourqurean and Zieman 2002).

Analysis of seagrass nutrient content has been employed to assess nutrient availability and limitation in many coastal ecosystems (Fourqurean et al. 1992, Fourqurean et al. 1997, Fourqurean and Zieman 2002, Fourqurean et al. 2005). The use of seagrasses as proxies for nutrient limitation has several advantages over other methods such as seawater analysis or sampling of phytoplankton. Compared to direct analysis of dinoflagellates, which are mobile and transported in and out of the bay through tidal exchange, seagrasses are fixed to the substratum and continually sample seawater throughout their lifetimes, providing a record of the average nutrient availability in a particular location. In this way, seagrasses act as “long-term integrators” of seawater nutrient availability (Burkholder et al. 2007). Moreover, seagrasses

have also been shown to be particularly sensitive to environmental changes (Fourqurean et al. 1997). The common seagrass species examined in this study, *Thalassia testudinum*, can have a lifespan on the order of 10 years (Duarte 1990).

A comparison of the nutrient content of *Thalassia testudinum* in Puerto Rico to the Redfield ratio analog for seagrasses (550 mol C:30 mol N:1 mol P; Atkinson and Smith, 1983) gives a first-level indication of nutrient availability for seagrasses in these different environments. However, the amount of natural variation in C:N:P in *Thalassia testudinum* in any given location can be relatively large and can render assessments of nutrient limitation status statistically insignificant (Duarte 1990). Therefore, comparisons of C:N:P content of seagrasses to the Redfield ratio should be strengthened by comparing the C:N:P ratios in a particular study location. Moreover, spatial differences in C:N : P ratios can be useful in identifying different nutrient sources (Burkholder et al. 2007).

## METHODS

In January of 2008, *Thalassia* samples were collected from a total of 15 sites between the three study locations. 12 samples were taken from Bahía Fosforescente (17.972° N, 67.015° W), 1 sample was taken from an open ocean environment (17.938° N, 66.881° W), and 2 samples were taken from the small inlet with intermediate concentrations of dinoflagellates (17.973° N, 67.066° W). Benthic biogeography maps provided by the National Oceanic and Atmospheric Administration were used to identify regions within the study site where seagrasses were most likely to be found (NOAA Biogeography Branch 2007). At least 3 stalks of seagrass were collected from each site. Distance from the mouth of the bay for each site was determined as the distance from the site to a transect across the mouth of the bay.

Seagrass samples were cleaned of adhering sediments and epibionts, rinsed with distilled water, and dried at 60° C. Once dry, a subsample was collected from the midsections of each seagrass leaf

to account for differences in nutrient concentrations along the length of a stalk of seagrass.

Total carbon and nitrogen concentrations were determined on a Thermo Flash 1112 Elemental Analyzer. P content was measured with a modification of the method presented by Solorzano and Sharp (1980) for particulate total P determination. Duplicate subsamples of each sample were weighed (5-20 mg) into glass scintillation vials. Then, 0.5 ml of 0.17 M Na<sub>2</sub>SO<sub>4</sub> and 2.0 ml of 0.017 M MgSO<sub>4</sub> were added and samples were dried at 90°C. Blanks were treated identically. Dry vials were ashed at 500°C for 3 h. After cooling, 5.0 ml of 0.2 N HCl was added before heating samples at 80°C for 30 min. Each vial was diluted with 10.0 mL of deionized water, shaken, and allowed to stand overnight to allow the ash to settle. Samples were analyzed for phosphorus concentrations on a Beckman-Coulter DU 530 spectrophotometer using the ascorbic acid reduction method (U.S. EPA 1983). Briefly, 500 µl of sample was diluted with 5.5 ml of ultrapure water and 960 µl of a mixed reagent composed of ammonium molybdate, sulfuric acid, L-ascorbic acid, and potassium antimony tartrate. Phosphate concentrations were determined by quantifying the 885 nm peak relative to standard solutions.

## RESULTS

### *Nutrient Concentrations*

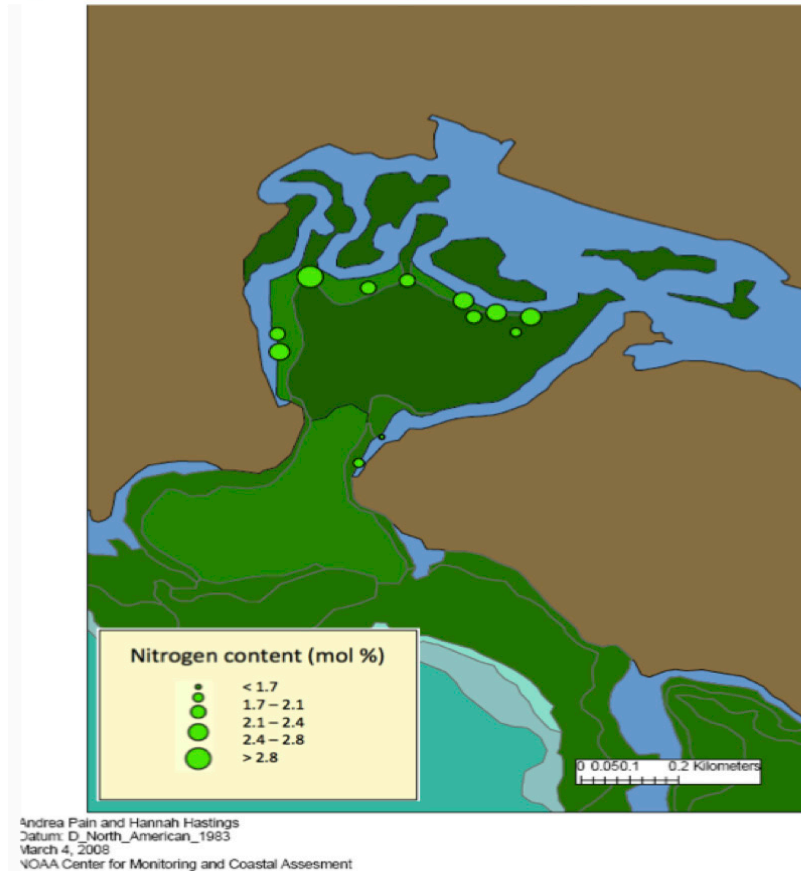
Nutrient concentrations in seagrass samples are compared to a much larger study of *Thalassia testudinum* in Florida Bay, as these data may be more representative of the global average (Fourqurean and Zieman 2002). Nitrogen concentrations in Florida Bay seagrasses range from 0.9 – 4.0 wt.% with a mean of  $1.8 \pm 0.4$  wt. % while phosphorus concentrations range from 0.05 – 0.24 wt. % with a mean of  $0.13 \pm 0.04$  wt. %. Nutrient concentrations in seagrass samples from Bahía Fosforescente are relatively high in nitrogen and comparable in phosphorus (N=  $2.3 \pm 0.3$  wt. %; P =  $0.10 \pm 0.03$  wt. %) compared to seagrasses from Florida Bay (Table 1).

**TABLE 1.** Summary statistics of nutrient concentrations and ratios of *Thalassia testudinum* samples by sampling location. Nutrient ratios were calculated on a mol-mol basis. \*Data taken from Fourqurean and Zieman, 2002 (=504).

Element		C wt. %	P wt. %	C:N	C:P	N:P
Bahía	Mean	2.3	0.10	20.3	870	43.2
Fosforescente	St. Dev.	0.3	0.03	2.8	207	10.4
(n = 12)	Min – Max	1.7 – 2.9	0.08 – 0.18	16.3 – 26.7	551-1266	26.3 -56.6
Inlet site	Mean	2.1	0.14	22.8	717	32.3
(n = 2)	St. Dev.	0.5	0.00	5.1	22	8.1
	Min – Max	1.8 – 2.1	0.14 – 0.14	19.2 – 26.4	702 – 732	26.6 – 38.1
Open ocean	Mean	2.1	0.15	21.8	668	30.7
(n = 1)	St. Dev.	--	--	--	--	--
	Min – Max	--	--	--	--	--
Florida Bay*	Mean	1.8	0.11	24.6	937	40.2
	St. Dev.	0.4	0.04	5.2	312	17.8
	Min – Max	0.9 – 4.0	0.05 – 0.24	11.1 – 47.1	373 – 1901	15.4 – 107.1

**FIGURE 1.** Distribution of nitrogen concentrations in seagrass in Bahía Fosforescente. Larger circles indicate higher concentrations.

### Spatial Distribution of Seagrass Nitrogen Concentrations



Compared to other sampling locations, samples taken within Bahía Fosforescente have higher nitrogen concentrations than all other samples (open ocean, inlet, and Florida Bay samples), with lower phosphorus concentrations (Table 1). The spatial distribution of nutrient concentrations within Bahía Fosforescente reveals that nitrogen concentrations are higher toward the back of the bay, increasing with distance from the mouth (Fig. 1). The opposite trend is observed for phosphorus concentrations, which are highest near

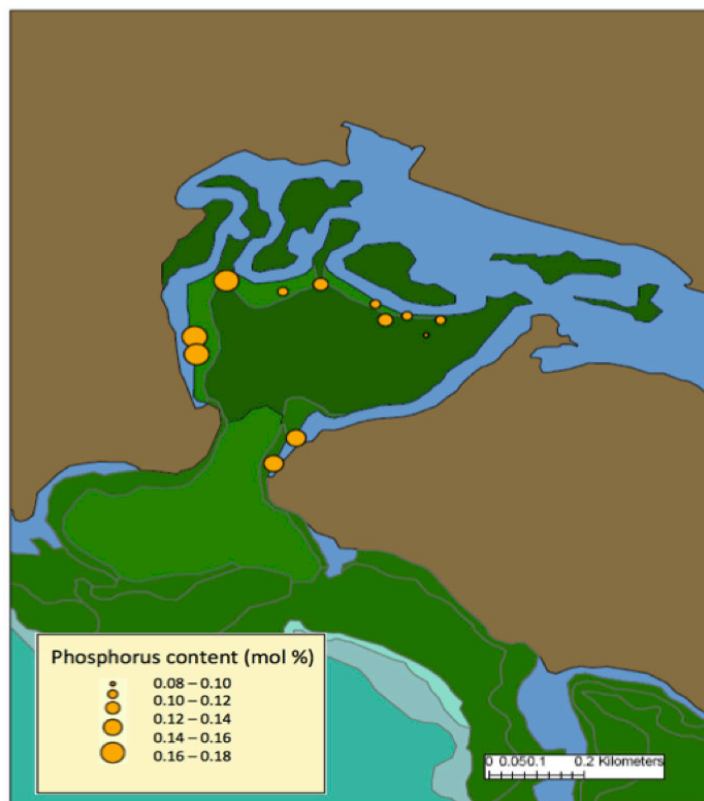
the mouth of the bay and decrease with distance (Fig. 2).

### C:N:P Ratios

Nutrient ratios in seagrasses from this study location are somewhat different from those measured in the Florida Bay study (Table 1). C:N ratios in Bahía Fosforescente range from 16.3-26.7 with a mean of  $20.3 \pm 2.8$ , which is slightly lower compared to Florida Bay seagrass C:N ratios (mean

**FIGURE 2.** Distribution of phosphorus concentrations in seagrasses in Bahía Fosforescente. Larger circles indicate higher concentrations.

### Spatial Distribution of Seagrass Phosphorus Concentrations



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 Datum: D\_North\_American\_1983  
 March 4, 2008  
 NOAA Center for Monitoring and Coastal Assessment

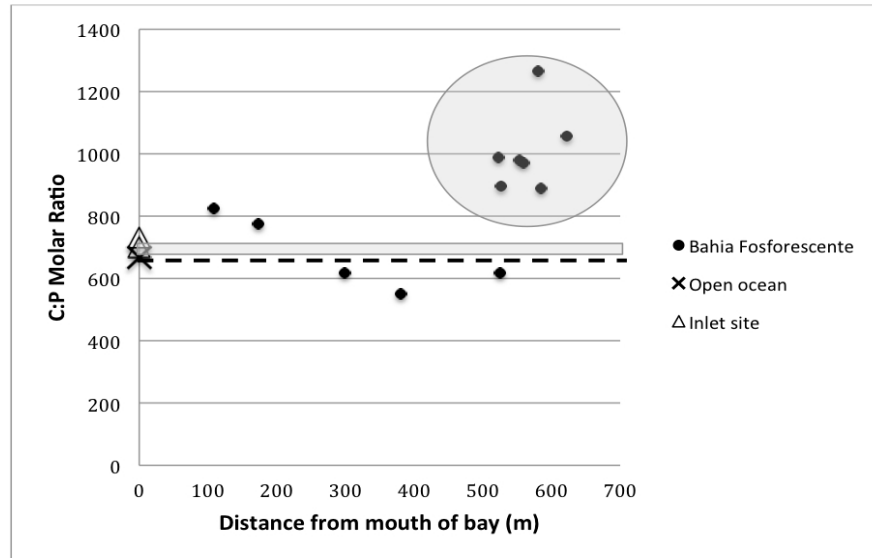
=  $24.6 \pm 5.2$ ). Likewise, C:P ratios (551 – 1266; mean =  $870 \pm 207$ ) are somewhat low compared to Florida Bay seagrass C:P ratios (mean =  $937 \pm 312$ ). However, N:P ratios (26.3 – 56.6; mean =  $43.2 \pm 10.4$ ) are slightly higher than Florida Bay seagrasses (mean =  $40.2 \pm 17.8$ ) (Fourqurean and Zieman 2002).

There is also considerable variation in nutrient ratios between study sites. Compared to the open ocean and inlet site, C:P ratios and N:P ratios in Bahía Fosforescente are relatively high, while C:N ratios from all three sample locations fall within the same range (Table 1).

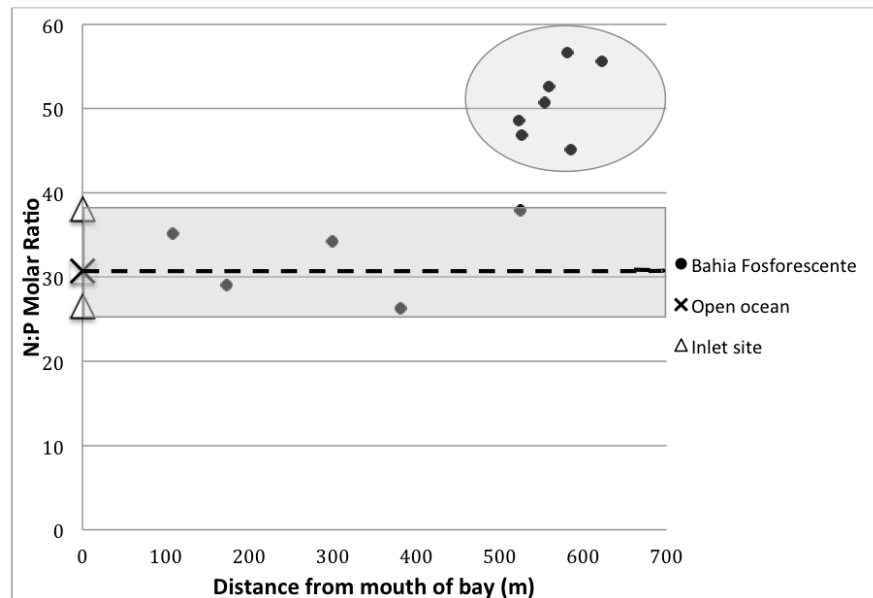
The spatial dependence in nutrient ratios within Bahía Fosforescente is observed by plotting nutrient ratios for each sample site against the distance of the site from the mouth of the bay. Both C:P and N:P ratios show similar spatial distributions within the bay, with lower values measured in samples near the mouth (Figs. 3 and 4). C:N ratios show less of a spatial trend, but decrease slightly with distance from the mouth of the bay (Fig. 5).

Both C:P and N:P ratios of seagrasses near the mouth of the bay are around nutrient ratios measured in the open ocean site. For sites less than 500 meters from the mouth of the bay, C:P ratios

**FIGURE 3.** Spatial pattern of molar C:P ratios of seagrasses with distance from the mouth of Bahía Fosforescente. The horizontal dashed line represents “open ocean” concentrations, and the shaded rectangle represents the variation measured in seagrasses from the inlet site. The shaded circle includes points near the back of the bay with considerably different C:P ratios.



**FIGURE 4.** Spatial pattern of molar N:P ratios of seagrasses with distance from the mouth of Bahía Fosforescente. The horizontal dashed line represents “open ocean” concentrations, and the shaded rectangle represents the variation measured in seagrasses from the inlet site. The shaded circle includes points near the back of the bay with considerably different molar N:P ratios.

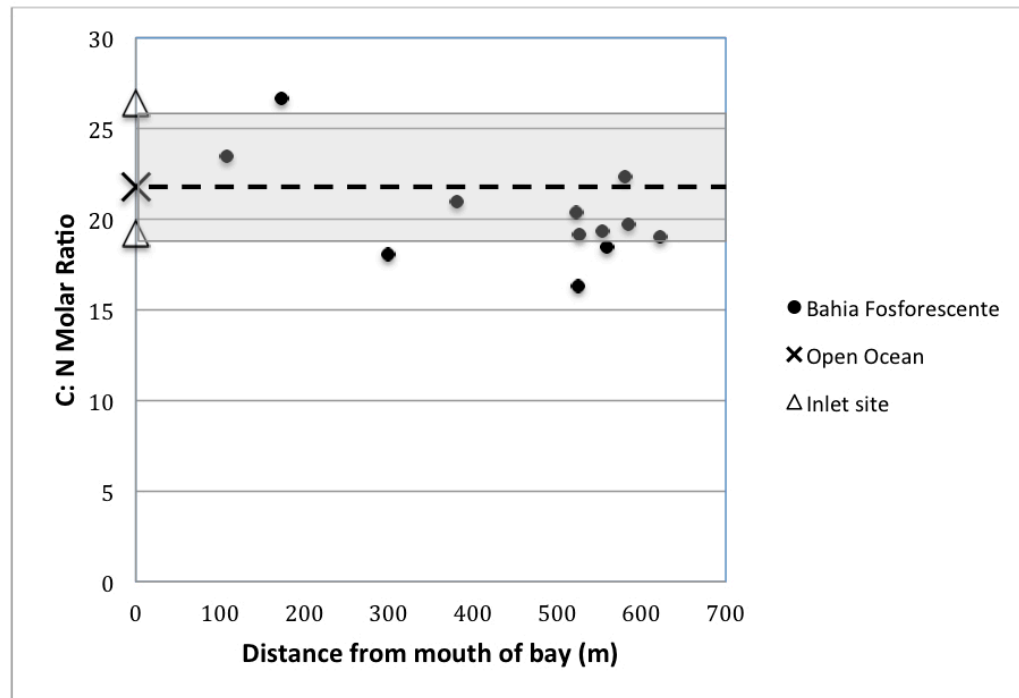


remain close to the open ocean ratio of 668, varying from 825 to 551. At over 500 m from the mouth, ratios increase dramatically to values of 1266 at 581 m from the bay mouth (Fig. 3). A similar trend is observed in N:P ratios, where sites close to the bay remain close to the open ocean N : P ratio of 30.7 (ranging from 26.3 – 35.1) until a distance from the mouth of the bay of approximately 500 m. At distances greater than 500 m, N:P ratios increase dramatically to a maximum of 56.6 at 581 m from the bay mouth (Fig. 4). C:N ratios remain consistently close to the C:N ratio of the open ocean site of 21.8, though C:N ratios near the mouth of the bay (maximum C:N = 26.7 at 173 m from bay mouth) are somewhat higher than those toward the back of the bay (minimum C:N = 16.3 at 525 m from the bay mouth; Fig. 5).

## DISCUSSION

### Nutrient Concentrations

Overall nutrient concentrations in seagrasses in this study location are higher than those in Florida Bay. If nutrient content in Florida Bay seagrasses are considered to be representative of global *Thalassia testudinum*, seagrasses around Bahía Fosforescente are relatively nutrient-rich compared to average *Thalassia testudinum*. While both carbon and phosphorus concentrations in Bahía Fosforescente seagrasses are higher than the mean carbon and phosphorus concentrations in Florida Bay seagrasses, the most striking difference between Florida Bay seagrasses and those in this study is in the nitrogen concentrations, with a mean of 2.3 wt.%  $\pm$  0.3 in Bahía Fosforescente seagrasses



**FIGURE 5.** Spatial pattern of molar C:N ratios of seagrasses with distance from the mouth of Bahía Fosforescente. The horizontal dashed line represents “open ocean” concentrations, and the shaded rectangle represents the variation measured in seagrasses from the inlet site.

APPENDIX 1. Data for nutrient content for all samples.

Sample location	Distance from Mouth (m)	N (% dry weight)	C (% dry weight)	P (% dry weight)
Bahía Fosforescente	108	1.9	38.5	0.12
Bahía Fosforescente	622	2.5	40.9	0.10
Bahía Fosforescente	581	2.1	39.4	0.08
Bahía Fosforescente	526	2.4	38.6	0.11
Bahía Fosforescente	173	1.7	38.0	0.13
Bahía Fosforescente	559	2.6	40.8	0.11
Bahía Fosforescente	554	2.5	41.1	0.11
Bahía Fosforescente	523	2.3	40.1	0.10
Bahía Fosforescente	585	2.4	40.3	0.12
Bahía Fosforescente	525	2.9	39.8	0.17
Bahía Fosforescente	381	2.2	39.3	0.18
Bahía Fosforescente	299	2.5	39.3	0.16
Open Ocean				
	0	2.1	38.9	0.15
Inlet Site				
	0	2.5	40.6	0.14
Inlet Site				
	0	1.7	38.9	0.14

versus  $1.8 \text{ wt.\%} \pm 0.4$  in Florida Bay seagrasses (Table 1). The slightly elevated concentrations of phosphorus and the high concentrations of nitrogen in seagrasses from this study location indicate that overall nutrient availability in Bahía Fosforescente is high.

### Nutrient Limitation

A qualification of seagrasses as phosphorus or nitrogen-limited can be made by comparing measured nutrient ratios in seagrasses to the Redfield ratio analogue for seagrasses. While the suggested analog for the Redfield ratio for seagrass is 550 mol C:30 mol N:1 mol P, seagrass nutrient content is highly variable (Atkinson and Smith 1983). Fourqurean et al. (1992) compiled C:N:P ratios in field studies assessing seagrass nutrient content and found the mean and median N:P ratio to be 25:1 rather than 30:1, and therefore used a ratio of 25:1

to indicate phosphorus limitation. This value will be used to assess phosphorus limitation here due to the proximity in study locations. Using this value, the C:P and N:P ratios of all samples analyzed are higher than the Redfield ratio for seagrasses. As the Redfield ratio predicts the nutrient content of seagrasses experiencing no nutrient limitation, the seagrasses analyzed here show signs of phosphorus limitation as they contain proportionally less phosphorus than would be expected in non nutrient-limiting conditions.

In contrast, comparison of C:N ratios in Bahía Fosforescente to the Redfield C:N ratio of 22: 1 indicates that seagrasses near the mouth of the bay may be nitrogen limited. The seagrasses nearest the mouth have C:N ratios of 23.5 and 26.7 (108 and 173 m from the mouth, respectively). As C:N ratios decrease with distance from the mouth, seagrasses at the back of the bay are enriched in nitrogen relative to carbon (Fig. 5).



APPENDIX 2. Data for nutrient ratios for all samples.

Sample location	Distance from Mouth (m)	C:P (mol/mol)	N:P (mol/mol)	C:N (mol/mol)
Bahía Fosforescente	108	825	35.1	23.5
Bahía Fosforescente	622	1058	55.6	19.0
Bahía Fosforescente	581	1266	56.6	22.4
Bahía Fosforescente	526	897	46.9	19.2
Bahía Fosforescente	173	775	29.1	26.7
Bahía Fosforescente	559	972	52.6	18.5
Bahía Fosforescente	554	980	50.7	19.3
Bahía Fosforescente	523	988	48.6	20.4
Bahía Fosforescente	585	890	45.1	19.7
Bahía Fosforescente	525	618	37.9	16.3
Bahía Fosforescente	381	551	26.3	21.0
Bahía Fosforescente	299	618	34.2	18.1
Open Ocean	0	668	30.7	21.8
Inlet Site	0	732	38.1	19.2
Inlet Site	0	702	26.6	26.4

While comparison to the Redfield ratio indicates relative scarcity of nitrogen near the mouth of the bay and phosphorus near the back of the bay, the high concentration of nutrients in these seagrasses relative to other studies indicates that the high incidence of phosphorus limitation in Bahía Fosforescente is likely due to excess nitrogen availability rather than low phosphorus availability.

### Nutrient Sources

Spatial trends in nutrient concentrations and ratios in seagrasses can be used to make implications about the sources of nutrients for this bay. Overall, P concentrations are higher toward the mouth of the bay (Fig. 2), as is the proportional concentration of P relative to C and N (lower C:P and N:P ratios) (Figs. 3 and 4). This indicates that the main source of P in this bay may be tidal exchange from the open ocean. A visual, qualitative analysis of

seagrass standing crop distribution within Bahía Fosforescente indicates that highest amounts of standing crop also occurs nearest the mouth of the bay, supporting an oceanic source of phosphorus as well as phosphorus limitation for seagrasses within the bay (Duarte 1990). Higher phosphorus availability near the mouth, potentially resulting from increased oceanic exchange, leads to higher amounts of primary production able to take place, suggesting that phosphorus is limiting the amount of seagrass growth within Bahía Fosforescente.

Though tidal exchange may be a source of phosphorus for seagrasses in Bahía Fosforescente, comparison between the N:P ratio in standard ocean water and in seagrass samples indicates that tidal exchange cannot be the only source of nutrients in this bay. The N:P ratio in standard seawater is 16:1 (Redfield 1958). The optimal N:P ratios for seagrass growth defined by the Redfield ratio (25:1)

are much higher than the 16:1 N:P ratio in seawater, so if seawater were the only source of nutrients, it would be delivering proportionally much more P relative to N than is needed to meet seagrass nutrient requirements. Since these seagrasses are most likely not N-limited, there must be an additional source of nitrogen in this bay.

Higher N concentrations, coupled with lower C:N ratios toward the back of the bay, suggest a terrestrial source of N. While there is not a direct river or stream input into Bahía Fosforescente, terrestrial runoff is a potential source of nitrogen. A terrestrial source of N could be linked to anthropogenic sources, as nitrogen is a component of many fertilizers. High N:P ratios of anthropogenic waste has been linked to phosphorus limitation in Florida Bay, and could also be a mechanism for inducing P-limitation here (Fourqurean and Zieman 2002).

### CONCLUSIONS

Seagrasses in Bahía Fosforescente have high concentrations of nitrogen and slightly elevated concentrations of phosphorus compared to mean nutrient concentrations in Florida Bay seagrasses. C:N ratios are generally low compared to the Redfield ratio, indicating that nitrogen limitation is unlikely in these seagrasses. C:P and N:P ratios, however, both indicate phosphorus limitation in all seagrass samples in this study. Spatial trends of nitrogen and phosphorus concentrations and C:P and N:P ratios indicate that phosphorus availability decreases and nitrogen availability increases with distance from the mouth of the bay. Seagrasses toward the mouth of the bay reflect the nutrient concentrations and ratios measured in seagrasses collected from the open ocean, seagrasses from the back of Bahía Fosforescente, however, are considerably different, with higher nitrogen concentrations, lower phosphorus concentrations, and higher N:P and C:P ratios. This increase in nitrogen content toward the back of the bay is suggestive of a terrestrial source of N, and that this source of nitrogen is potentially driving the

bay towards phosphorus limitation. The source of excess nitrogen in Bahía Fosforescente could be constrained by comparing  $\delta^{15}\text{N}$  ratios in seagrasses with  $\delta^{15}\text{N}$  ratios in potential nutrient sources in this bay system, including streams and runoff, anthropogenic waste, and other sources of N like petroleum byproducts (Burkholder et al. 2007).

The evidence of phosphorus limitation in this bioluminescent bay is consistent with that observed in other high *Pyrodinium bahamense var. bahamense* populations, providing further support for the notion that large dinoflagellate blooms are associated with high nutrient environments and phosphorus limitation.

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## ISOTOPIC TRENDS IN BAT GUANO AS A PROXY FOR CLIMATE AND ECOLOGY IN PUERTO RICO

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**Key Words:** *guano, carbon enrichment, nitrogen enrichment, Puerto Rico*

### ABSTRACT

Two regions of Puerto Rico were investigated for climate and trophic trends through the carbon and nitrogen isotopic analyses of bat guano, plants, and insects. Carbon isotope enrichment with warmer and drier climates was observed in the guano and a subset of the plant and insect samples. Nitrogen isotope enrichment with trophic level was seen between plants and bats, with most insects intermediate between the plant and bat values. Depth profiles of historic guano indicate that carbon isotopes are stable with decomposition, but nitrogen is enriched as guano decomposes. Overall, the study demonstrates the strength of guano as a proxy for climate and trophic level, and points to the value of establishing an isotopic baseline from local vegetation if results are to be compared to other regions.

### RESUMEN

A partir de análisis de isótopos de carbono y nitrógeno de guano, plantas e insectos, se investigaron el clima y las tendencias alimenticias de los murciélagos en dos regiones de Puerto Rico. En el guano y en una sección de las muestras de plantas e insectos se observó enriquecimiento isotópico de carbono con climas más cálidos y secos. Además, se detectó enriquecimiento isotópico de nitrógeno con el aumento de nivel trófico desde plantas a murciélagos, con la mayoría de insectos presentando valores isotópicos intermedios. Perfiles de profundidad de guano histórico indicaron que los isótopos de carbono son estables con la descomposición, pero que el nitrógeno se enriquece a medida que el guano se descompone. En general, este estudio demuestra la importancia del guano como estimador del clima y nivel trófico, y apunta hacia el valor de establecer una línea isotópica base a partir de la vegetación local en caso que los resultados vayan a ser comparados con los de otras regiones.

### INTRODUCTION

Stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic analyses are useful proxies for reconstructing climate and trophic-level patterns (Stewart et al. 1995, Post 2002). This is because

these isotopic values often correlate with temperature, precipitation, and diet. For example,  $\delta^{15}\text{N}$  values become more enriched as one moves up a trophic system (Vander Zanden 1996, McCutchan 2003). This enrichment occurs because biologic metabolism preferentially consumes the lighter

isotopes and because of the differing dietary habits of organisms at different trophic levels (Scrimgeour 1995, Vander Zanden 1996). As a result, species lower in trophic positioning (i.e., prey) are more likely to be isotopically depleted in  $\delta^{15}\text{N}$  compared to species higher up in the trophic system (i.e. predators). Typically, the enrichment is 2.6-3.4‰ per step in trophic level (standard deviation = 1.0‰) (Vander Zanden 1996, Post 2002). For example, Herrera et al. (2001) observed in southeast Mexico a  $\delta^{15}\text{N}$  enrichment of 3.45‰ between fruit and insects, 3.34‰ between fruit and frugivorous bats, and 1.03‰ between insects and insectivorous bats.

Stable carbon isotope values typically do not correlate strongly with trophic level: the mean enrichment is 0.4‰ per level (standard deviation = 1.3‰) (Post 2002). Instead,  $\delta^{13}\text{C}$  often relates inversely with mean annual precipitation (MAP) and mean annual temperature (MAT) (Post 2002). The isotopic enrichment under droughted conditions is largely driven by stomatal closure (Read and Farquhar 1991, Stewart et al. 1995, Bowling et al. 2008).

Consumer tissues are not always available for collection. In this case, it is possible to infer stable isotopic ratios from consumer feces because little fractionation occurs between the two pools (Wurster et al. 2007). The guano may also be collected at varying depth, which will give historic values once isotopic enrichment by bacterial decomposition of organic matter (decreased %C & %N) is accounted for (Nadelhoffer and Fry 1994). The current study investigates the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of bat guano and neighboring insects and plants at two cave sites in wet and semi-dry regions of Puerto Rico. The aim of the study is to assess the robustness of bat guano for capturing trophic and climatic information. We hypothesize that a progressive enrichment in  $\delta^{15}\text{N}$  from plants to insects to bats will be present, and that the  $\delta^{13}\text{C}$  of materials from the climatically-drier cave will be more enriched. We note that because Puerto Rico is dominated by  $\text{C}_3$  vegetation, the effect of variable mixing of  $\text{C}_3$  and  $\text{C}_4$  vegetation on carbon isotopic values is largely avoided (e.g., Wurster et al. 2007).

## METHODS

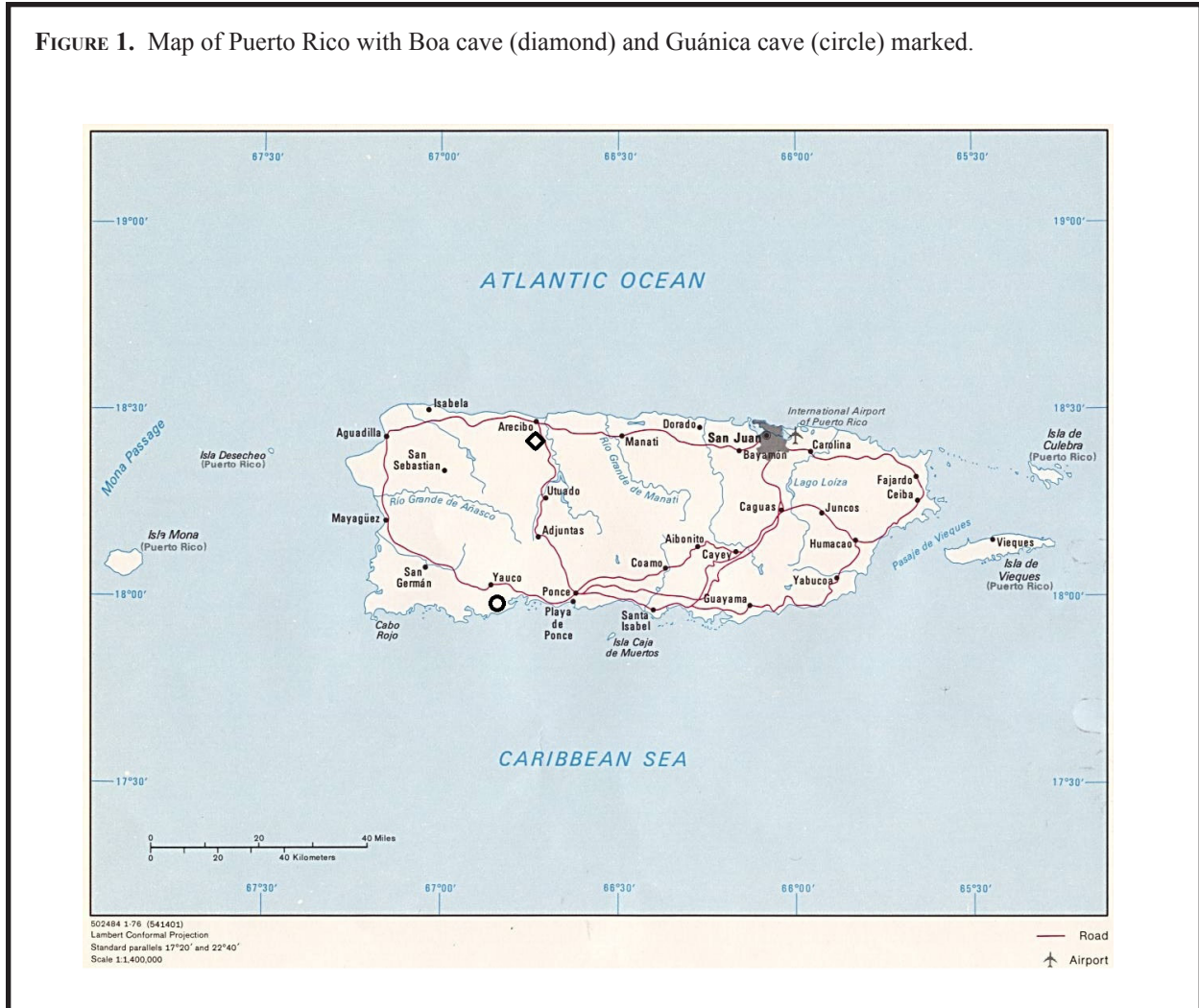
### Study Areas

Two regions (Fig.1) in Puerto Rico were selected based partly on the availability of published isotopic and biological data (Costas and Gonzáles 1990, Soto Centeno 2004). Each region has a large cave with large bat populations. The Boa cave (La Cueva de los Culebrones), located 8 km north of Arecibo, is part of the Mata de Plátano field station and is surrounded by subtropical wet deciduous vegetation. The MAT is 25.3 °C and MAP is 151 cm (Fig. 2, Table 1). The ratio of summer to winter precipitation near the Boa Cave is 0.94, indicating a near absence of winter drought; there is only a 2 percent increase in rainfall between the winter and summer (Table 1).

The second cave, Guánica cave, is located in the Guánica Commonwealth Forest, a protected subtropical dry forest. The MAT here is 26.2 °C and the MAP is 90 cm (Fig. 2, Table 1). The ratio of summer to winter precipitation is 2.66, with a 16 percent difference between summer and winter months (Table 1). This indicates water stress on local vegetation during the winter months.

Five species of bats are native to the Boa cave: *Erophylla sezekorni* (Brown Flower Bat), *Monophyllus redmani* (Greater Antillean Long-Tongued Bat), *Mormoops blainvillii* (Antillean Ghost-Faced Bat), *Pteronotus parnelli* (Parnell's Mustached Bat), and *Pteronotus quadridens* (Sooty Mustached Bat) (Gannon et al. 2005). Approximately 300,000 individuals roost at this site at any time (Soto Centeno 2004). *E. sezekorni* consumes equivalent amounts of fruit, insects, and pollen, with a high proportion of Coleoptera beetle. *M. redmani* has a diet with little fruit and large amounts of pollen and insects, especially Diptera flies (Soto Centeno 2004). Both species consume *Muntingia calabura* (Strawberry tree). The other three major bat species at the Boa cave consume mostly insects, including moths, beetles, flying ants, and flies (Gannon 2005).

FIGURE 1. Map of Puerto Rico with Boa cave (diamond) and Guánica cave (circle) marked.



The Guánica cave contains five bat species and a great number of individual bats, but three species predominate: *Monophyllus redmani* (Greater Antillean long-tongued bat), *Mormoops blainvilli* (Antillean ghost-faced bat), and *Brachyphylla cavernarum* (Antillean fruit bat) (Costas and Gonzáles 1990). *B. cavernarum* eat mainly fruit and pollen, including mango, papaya, sapodilla, and angelin, but has also been known to consume beetles (Gannon 2005). The other two species have the same diets as their conspecifics in the Boa cave.

### Field Collection

Guano was collected with coring and surface sampling techniques. Guánica cave has a deep guano lake near the back of the main chamber that

allowed coring. Three meter-long plastic tubes were pounded into the guano, the top marked, and distilled water was slowly poured into the remaining length to create suction. The exhumed cores were sealed and processed on the same day. The extruded and cut cores did not have yearly layers; instead, the cores were sampled at 2 cm intervals. Boa cave has shallow guano ponds that precluded coring; here, fresh surface guano was collected in four areas of the cave: at the back, at the middle, at the highest temperature (largest roost), as well as under the nectar-eating bat roost (Appendix).

Plants and insects were collected near the caves (Appendix). Leaves, fruit and seeds were matched to a field guide of bat diet. The leaves were pressed and the samples were photographed.

**TABLE 1.** Climate information for the Boa and Guánica caves. Mean annual temperature (MAT), mean annual precipitation (MAP), and summer (S; Jun-Aug) to winter (W; Dec-Feb), summer to annual, and winter to annual precipitation ratios were all calculated from four nearby climate stations (NOAA 2007). Annual and seasonal temperatures were calculated from the Arecibo (7.1 km N of Boa cave) and Ponce (59.9 km E of Guánica cave) stations. Annual and seasonal precipitation calculations were based on the Arecibo station for Boa cave, the Yauco station (39.6 km N) for historic data of Guánica cave, and the Boca station (40.7 km E) for present-day data of Guánica cave. The calculations exclude years without twelve months of data. See Figure 2 for temporal patterns in MAT and MAP.

	MAT ( C)	MAP (cm)	S/W	S/MAP	W/MAP
Boa	25.3	151.3	0.94	0.22	0.24
Guánica	26.2	90.2	2.66	0.26	0.10

The photographs were used to confirm the identity of the plants. Insects were collected at night with fluorescent lights on a white sheet. The insects attracted to the sheet were placed in jars with ethanol and transferred to individual cases on the same day.

### Sample Processing

Guano was air-dried for 1 week and treated to remove carbonate. First, 2N HCl was added dropwise until visible reaction stopped. Then 20 mL of HCl was added and the samples were allowed to sit overnight. The samples were then centrifuged, decanted, and diluted with deionized water three times to neutralize the acid. The samples were then oven-dried, ground with a mortar and pestle, and placed in labeled sample vials.

Insects were identified and, due to samples size requirements, grouped into predacious beetles, herbivorous beetles, moths, leafhoppers, and flies. The samples were allowed to air dry. They were crushed with mortar and pestle and acetone was used to rinse the ground powder into samples vials. The labeled vials were oven dried.

Plants were identified and placed in paper towels to oven dry. Seeds and fruit matter were sampled using tweezers and leaf matter was sampled using a hole-punch on multiple sections of

several leaves. The samples were placed in paper envelopes to prevent bacterial decomposition.

Samples were analyzed on an elemental analyzer for %C and %N (by weight). Approximately 0.5-5 mg of insects, 5-20 mg of plant matter, and 30-70 mg of guano was wrapped in foil and placed in a tray for the elemental analyzer. A subset of samples that allowed comparison between caves was chosen for isotopic analysis, measured at the isotope facilities at Indiana University (Department of Geological Sciences).

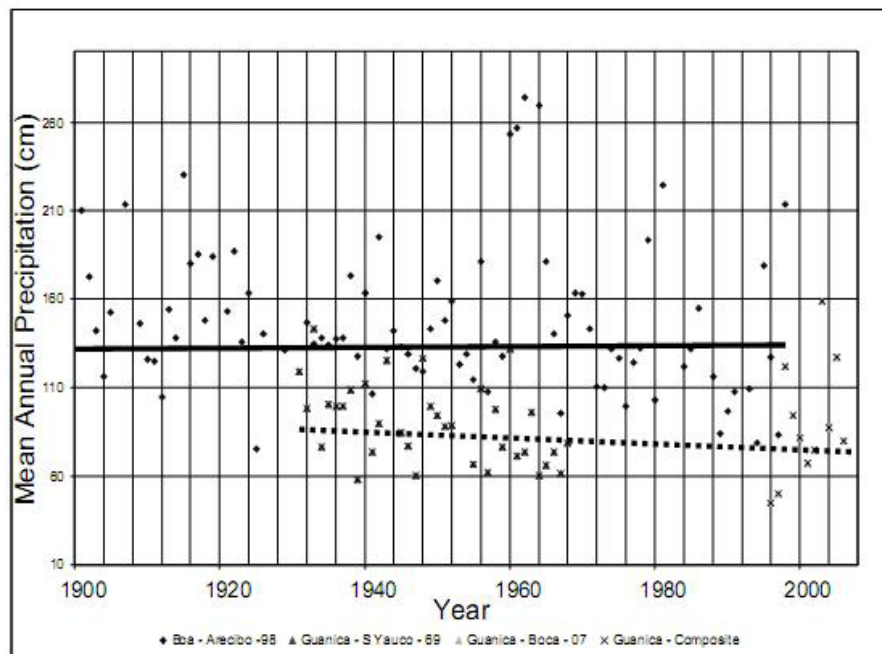
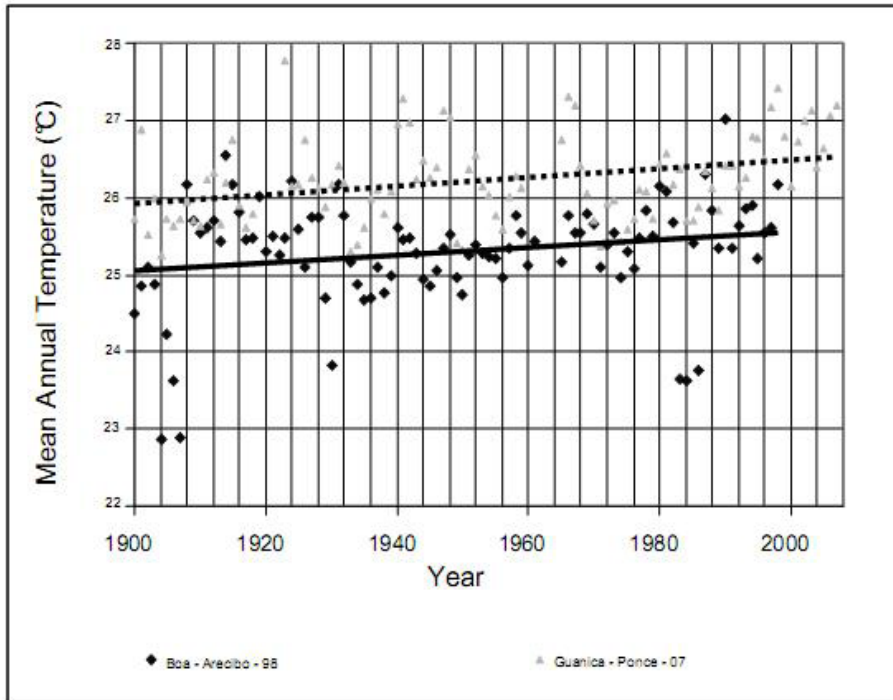
## RESULTS

### Carbon and Nitrogen Data

At both caves, the  $\delta^{15}\text{N}$  of guano is enriched by  $\sim 3\text{‰}$  relative to the local vegetation (Fig.3, Appendix). Based on limited sampling ( $n = 2$  at each cave), the  $\delta^{15}\text{N}$  of insects are intermediate between their respective vegetation and guano, with one exception (BC B sample in Fig. 3). A similar enrichment in  $\delta^{13}\text{C}$  is seen between vegetation and guano (1-3‰). The  $\delta^{13}\text{C}$  of two insect samples (BC M and GC M) fall between their respective guano-vegetation endpoints; however, the two beetle samples (GC B and BC B) are isotopically-enriched in  $^{13}\text{C}$  relative to both guano and vegetation (Fig. 3).

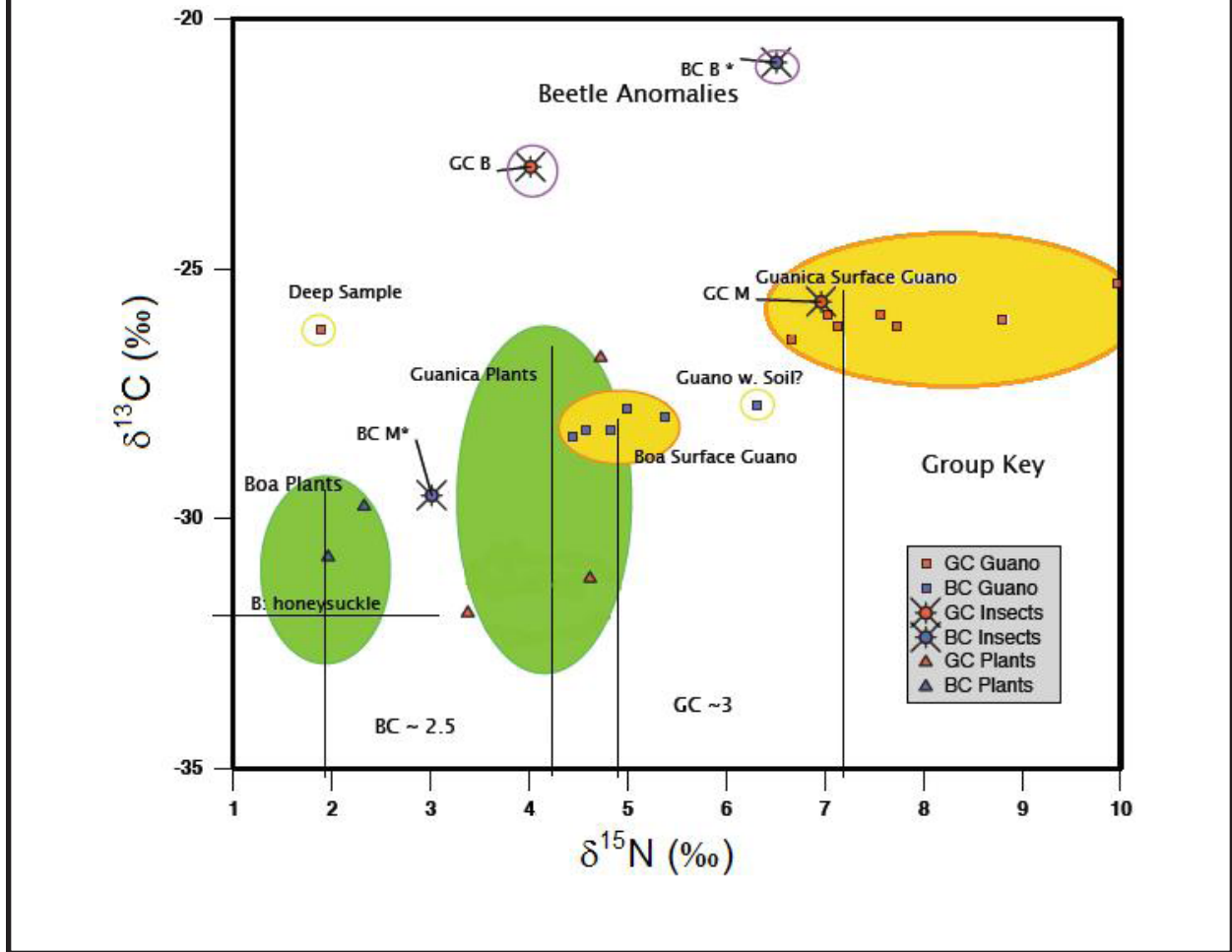
For both guano and vegetation,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values at Guánica are enriched by  $\sim 2\text{‰}$  relative to values at the Boa cave (Figure 3). However, the

**FIGURES 2A AND 2B.** Mean annual temperature (2A) and precipitation (2B) data for the past 108 years for the Boa and Guánica caves from four nearby climate stations. The least-squares trendlines indicate relative differences between years and between caves. See Table 1 for climate means and for details about the climate stations.





**FIGURE 3.** Stable isotope trends for the Guánica Cave (GC) and Boa Cave (BC): guano (square), insects (star), and plant (triangle) isotopic values for Guánica (red) and Boa (blue). Circles are drawn around each grouping of Guánica and Boa samples. Each circle has a vertical line through the average value to approximate trophic fractionation between plants and guano. Guánica has approx. 2.5‰ difference and Boa has approx. 3‰ difference. Insect data could not be grouped due to very small number of samples. Moth (M) and beetle (B) are labeled separately and the beetle samples are treated as outliers. Honeysuckle has no  $\delta^{15}\text{N}$  data; its  $\delta^{13}\text{C}$  value is indicated by the horizontal line. The “Guano w. Soil” and “Deep Sample” labels indicate guano samples suspected to contain a large fraction of soil.



$\delta^{13}\text{C}$  spread in plants is much greater than that in guano (~5‰ vs. ~1‰), largely obscuring any potential differences.

The depth profiles from Guánica cave demonstrate a progressive shift in C:N ratios from ~8-12 for fresh guano to ~14 for deeper, older guano (Fig. 4, Appendix). Similarly, we observe an enrichment in  $\delta^{15}\text{N}$  with depth (~2‰); no strong patterns in  $\delta^{13}\text{C}$  with depth are apparent (Fig. 4,

Appendix). One deep sample (at 37 cm) and one surface sample contain a large fraction of soil and consequently have considerably lower C:N ratios; these two samples are omitted from Fig. 4.

### DISCUSSION

The current study attempts to track nitrogen isotopic trends in a trophic system (from plants to insects to bat guano) as well as to correlate carbon

isotopic ratios to climate in two different regions of Puerto Rico. We observe an enrichment in  $\delta^{15}\text{N}$  moving up trophic levels and more enriched  $\delta^{13}\text{C}$  values at the drier Guánica site (Fig. 3), confirming our original expectations, although outliers are present. The guano outliers are likely due to soil contamination. This contamination is clearly seen in the %C (3.86) and %N (0.50) data, which are considerably lower than the ranges observed in the other guano samples (17.9-47 %C; 1.7-6.2 %N). The beetle outliers probably result from the chitinous exoskeleton (Merzendorfer and Zimoch. 2003).

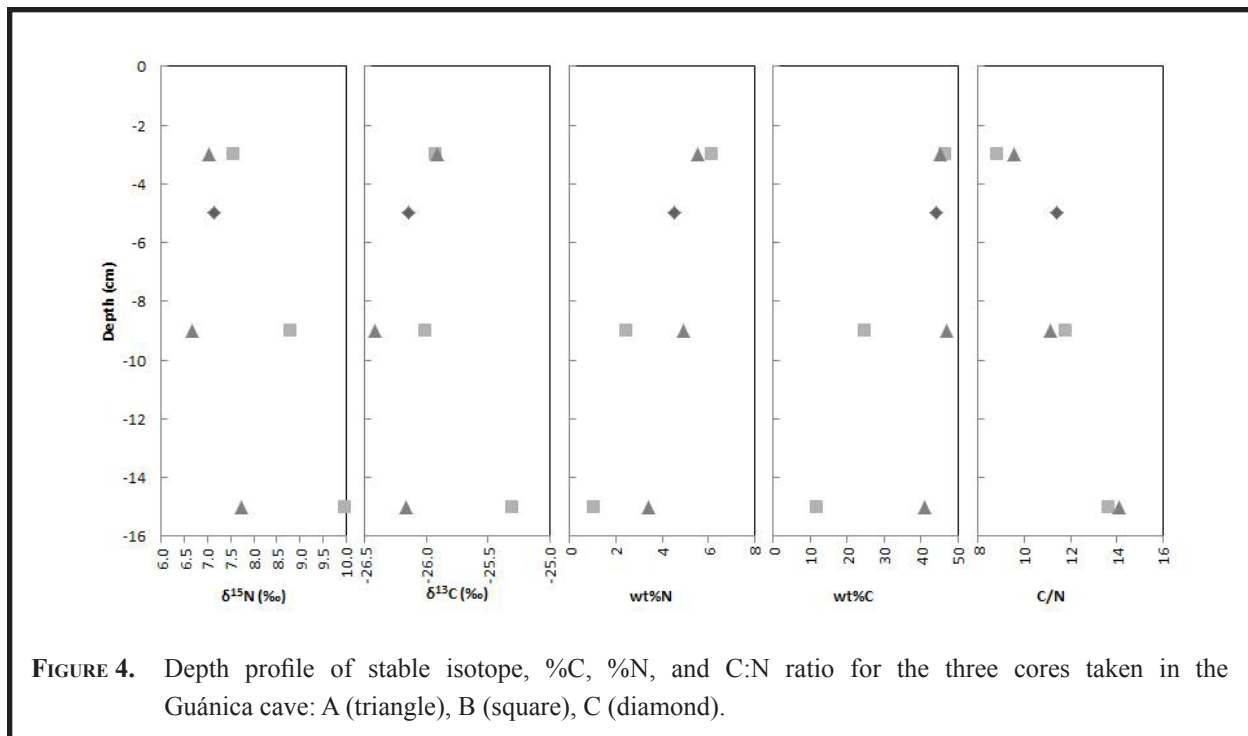
This study confirms the isotopic trend of  $\delta^{15}\text{N}$  enrichment with each successive trophic level in the ecosystem (McCutchan 2003, Scrimgeour 1995, Vander Zanden 1996). Our results reveal that guano samples were more enriched in  $\delta^{15}\text{N}$  than plants at both caves (Figure 3, Appendix). Insects, represented by the moth samples (BC M and GC M in Fig. 3), lie between their respective plants and guano in  $\delta^{15}\text{N}$ , although at Guánica insects are not distinguishable from the guano samples. This may indicate that the bats roosting in the sampled area of the cave are primarily frugivorous. Also, in

insects factors such as insect fasting, dietary habit, and age can greatly affect  $\delta^{15}\text{N}$  values (Scrimgeour 1995). Therefore, the study's small insect sample size may not adequately represent the entire insect population, and a larger sample size may help to clarify patterns.

One unexplained pattern is the  $\sim 2\text{‰}$  difference in  $\delta^{15}\text{N}$  between caves; this was observed both in the vegetation and guano (Fig. 3). This difference underscores the importance of establishing an isotopic baseline with vegetation before using guano to reconstruct attributes of diet.

Carbon isotope values are more enriched at Guánica (Fig. 3). These findings are consistent with the stronger presence of drought in the Guánica region. Interestingly, the difference in  $\delta^{13}\text{C}$  between caves is more clearly seen in the guano than in the vegetation. This may be due to the more limited sampling of vegetation, but it is probably also related to guano more efficiently capturing a well-mixed, whole-ecosystem signal.

Historic guano samples from Guánica show an increase with depth in both C:N ratios and



## APPENDIX A1. Carbon and nitrogen analyses of guano, insects, and plants.

	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	Total N (wt %)	Total C (wt %)	C:N (molar)
<b>Guánica Cave (Guano)</b>					
GC:B2	7.57	-25.92	6.14	46.59	8.84
GC:A2	7.02	-25.92	5.52	45.30	9.57
GC:C3	7.13	-26.15	4.52	44.25	11.42
GC:A5	6.67	-26.42	4.93	46.95	11.11
GC:B5	8.80	-26.01	2.43	24.67	11.82
GC:B8	9.96	-25.31	1.02	11.90	13.66
GC:A8	7.73	-26.16	3.39	41.03	14.12
GC:C19 deep w/soil	1.88	-26.21	3.69	44.34	14.00
<b>Boa Cave (Guano)</b>					
BC Nectar 1 w/ soil	6.31	-27.72	0.50	3.86	9.05
BC High Temp	5.00	-27.81	2.15	21.11	11.43
BC mix high temp	5.38	-27.98	1.78	17.90	11.72
BC Middle Mix A	4.59	-28.22	2.37	23.33	11.47
BC Back Mix B	4.82	-28.24	3.44	35.67	12.09
BC Middle Mix B	4.45	-28.35	2.56	26.29	11.98
<b>Guánica Cave (Insects)</b>					
GC Moth – all species	6.96	-25.67	12.01	48.20	4.68
GC Predaceous Beetle – all species	4.01	-22.96	11.73	49.39	4.91
<b>Boa Cave (Insects)</b>					
BC Moth – all species	3.01				
BC Predaceous Beetle – all species	6.51				
<b>Guánica Cave (Plants)</b>					
G42 <i>Albizia lebeck</i> Seeds	4.73	-26.77	5.12	47.41	10.81
G1 <i>Cajoba arborea</i> Leaves	3.38	-31.88	3.07	41.29	15.69
G43 <i>Muntingia calabura</i> L. Leaves	4.62	-31.19	2.92	41.90	16.75
<b>Boa Cave (Plants)</b>					
B17 <i>Thespesia grandiflora</i> Flower	2.33	-29.74	2.67	47.08	20.58
B8 <i>Lonicera japonica</i> Leaves		-31.97	2.28	49.03	25.04
B13 <i>Acacia farnesiana</i> Fruit	1.96	-30.75	4.08	45.32	12.96

$\delta^{15}\text{N}$  values. These patterns are consistent with an increase in bacterial decomposition with depth (Nadelhoffer and Fry 1994). No trend in isotopic carbon can be seen from the historic samples.

This study demonstrates that stable carbon and nitrogen isotopic ratios in bat guano can serve as proxies for climate and trophic scaling. However, establishment of a nitrogen isotopic baseline

through the analysis of other materials (plants, insects, soil) is critical before inferring trophic information from guano.

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## ALTITUDINAL LEAF VARIATION IN *CECROPIA SCHREBERIANA* (URTICACEAE) IN THE LUQUILLO EXPERIMENTAL FOREST, PUERTO RICO

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**Key words:** leaf morphology, elevation gradient, leaf chemistry Luquillo Mountains, leaf traits

### ABSTRACT

In many plants leaf morphology and chemical composition varies with altitude. This variation is thought to be in response to environmental factors. We measured variations in leaf area, leaf mass per area, nitrogen content and carbon-nitrogen ratios within *Cecropia schreberiana* (Urticaceae) along an elevational gradient in the Luquillo Mountains of northeastern Puerto Rico. We found that leaf area increases with elevation, probably in response to a parallel increase of precipitation. We also detected a decrease in mass-based nitrogen content and an increase in leaf mass per area and carbon:nitrogen ratios with elevation, which can be explained in the context of the leaf economics spectrum. In these mountainous tropical wet forests, plants growing at higher elevations are investing more resources in producing sturdier leaves better adapted to harsher environmental conditions. Qualitative and quantitative analyses of leaf epidermal features with structural and/or protective functions (trichomes and idioblasts) also support this idea. By characterizing the variation of leaf traits of this tropical species and considering our findings in terms of the leaf economics spectrum, we gain insight into vegetational shifts that will accompany our changing climate.

### RESUMEN

En muchas plantas la morfología foliar y la composición química de las hojas varía con la altitud. Se cree que esta variación ocurre en respuesta a cambios ambientales. En este trabajo medimos la variación en área foliar, masa de hoja por área foliar, contenido de nitrógeno y la razón carbono-nitrógeno en *Cecropia schreberiana* (Urticaceae) a lo largo de un gradiente altitudinal en las montañas de Luquillo, al noreste de Puerto Rico. Encontramos que el área foliar aumenta con la altura probablemente en respuesta a un aumento paralelo en la precipitación. Además, detectamos disminución en el contenido de nitrógeno por unidad de masa y aumento en masa por unidad de área y razón carbono:

nitrógeno con altitud que pueden ser explicados en el contexto del espectro de la economía foliar. En estos bosques húmedos tropicales de montaña, las plantas que crecen a mayores altitudes invierten más recursos en producir hojas más robustas y mejor adaptadas para resistir condiciones ambientales más difíciles. Análisis cualitativos y cuantitativos de excrecencias epidérmicas con funciones estructurales y/o protectoras (tricomos e idioblastos) dan soporte a esta idea. Caracterizar la variación de aspectos foliares de esta especie tropical y considerar nuestros hallazgos en términos del espectro de la economía foliar, nos ayuda a entender las alteraciones de la vegetación que acompañarán a un clima cambiante.

## INTRODUCTION

*Cecropia schreberiana* Miq. (Urticaceae) is a neotropical pioneer tree native to the Antilles and northern South America and is strongly associated with post-hurricane, or other disturbance, colonization (Brokaw 1998). For species with a wide ecological distribution as *C. schreberiana*, variation in physiological, morphological, and anatomical characteristics may be achieved by a combination of genotypic differentiation and phenotypic plasticity (Cordell et al. 1998). Plasticity can be expressed at various biological levels within an organism from behavioral to biochemical and physiological to developmental (Pigliucci et al. 2006). Phenotypic level observation lends itself to the study of the complex interplay between genetics and environmental conditions in determining ecosystem structure and evolutionary process. In this study we explore morphological and chemical variation in leaves of *Cecropia schreberiana* growing at four different altitudes in the Luquillo Experimental Forest, also known as El Yunque National Forest, Puerto Rico, with an emphasis on placing our findings in the context of leaf resource budgeting (Wright et al. 2004).

*Cecropia schreberiana* is characterized by abundant seed production, wide seed dispersal, seed dormancy, disturbance-cued germination, shade intolerance, fast height-growth --typically reaching 20 m in height and 60 cm of diameter at breast height (DBH)--, early maturity, and short life (Brokaw 1998). The hurricane-driven dynamics in the Luquillo mountains allows enough disturbed area to promote sufficient regeneration to support

*C. schreberiana* as one of the most important tree species (Brokaw 1998). Evidence to support intercanopy phenotypic plasticity, and not genetic variability, as the source of leaf trait variability in Luquillo *Cecropia* populations is presented by Fetcher et al. (2000). They found a distinct lack of differentiation on several growth and physiological traits (e.g., plant size, leaf anatomy, photosynthetic rate, stomatal density) in three common angiosperm species of the Luquillo forest (*Clibadium erosum* –Asteraceae, *Psychotria berteriana* –Rubiaceae, *Prestoea Montana* also reported as *Prestoea acuminata* var. *montana* –Arecaceae) at different altitudes, signaling high gene flow between forest types facilitated by effective dispersal, a robust seed bank, and human disturbance. Moreover, a population study on the genetic structure of *Cecropia obtusifolia* in a Mexican rainforest also found high levels of gene flow within the pioneer populations in different forest areas (Álvarez Buyllia and Garay 1994). While these studies lack explicit genetic mapping, we infer that variation in our measured traits is driven more by phenotypic plasticity than by genotype differences.

The worldwide leaf economics spectrum developed by Wright et al. (2004) finds correlations (for ~2,500 species at 175 sites) among chemical, structural and physiological traits of leaves that are largely independent of specific plant functional type, biome, or climate. These correlations support the theory that vascular plants invest in minerals and photosynthates according to economic principles. These investments are constrained by environmental conditions and determine the photosynthetic return over the leaf's lifespan. For example, plants with high photosynthetic rates

tend to have leaves with a short lifespan, low mass per area, and high nutrient concentration. These coordinated relationships reflect fundamental tradeoffs in resource acquisition, and can be described as a spectrum running from ‘fast-return’ to ‘slow-return’ specialists (Wright et al. 2004).

Leaf area (LA) and leaf dry mass per area (LMA) are traits that commonly vary within species (Bostrack 1993a). Environmental triggers directly or indirectly related to elevation can interact in complex ways. For instance, larger leaves are commonly associated with increased precipitation (Wilf et al. 1998), yet a “regular and abundant” water supply (*i.e.*, when moisture stress is not a factor) may reduce the importance of this general trend regionally. Leaf dry mass per area measures the dry-mass investment per unit of light intercepting leaf area deployed (Wright et al. 2004). An increase in LMA and a decrease in LA with elevation is commonly observed (Brokaw 1998, Cordell et al. 1998). Smaller leaves with denser or thicker parenchyma material may be often more capable of withstanding a physical onslaught of precipitation, wind, or scorching sun because they are heftier, more solid, and compact than their flimsy counterparts (Velázquez Rosas et al. 2002). Also, small leaves with high LMA are able to capture more sun in spotty exposure by having more leaf material per area exposed (Velázquez Rosas et al. 2002). Leaf traits associated with high LMA (for example, thick leaf blade; small, thick-walled cells) have been interpreted as adaptations that allow continued leaf function (or at least postpone leaf death) (Wright et al. 2004). Leaves at higher altitudes should be “expensive” leaves with high LMA, which means, requiring a high initial energetic investment but compensating the plant with a higher residence time.

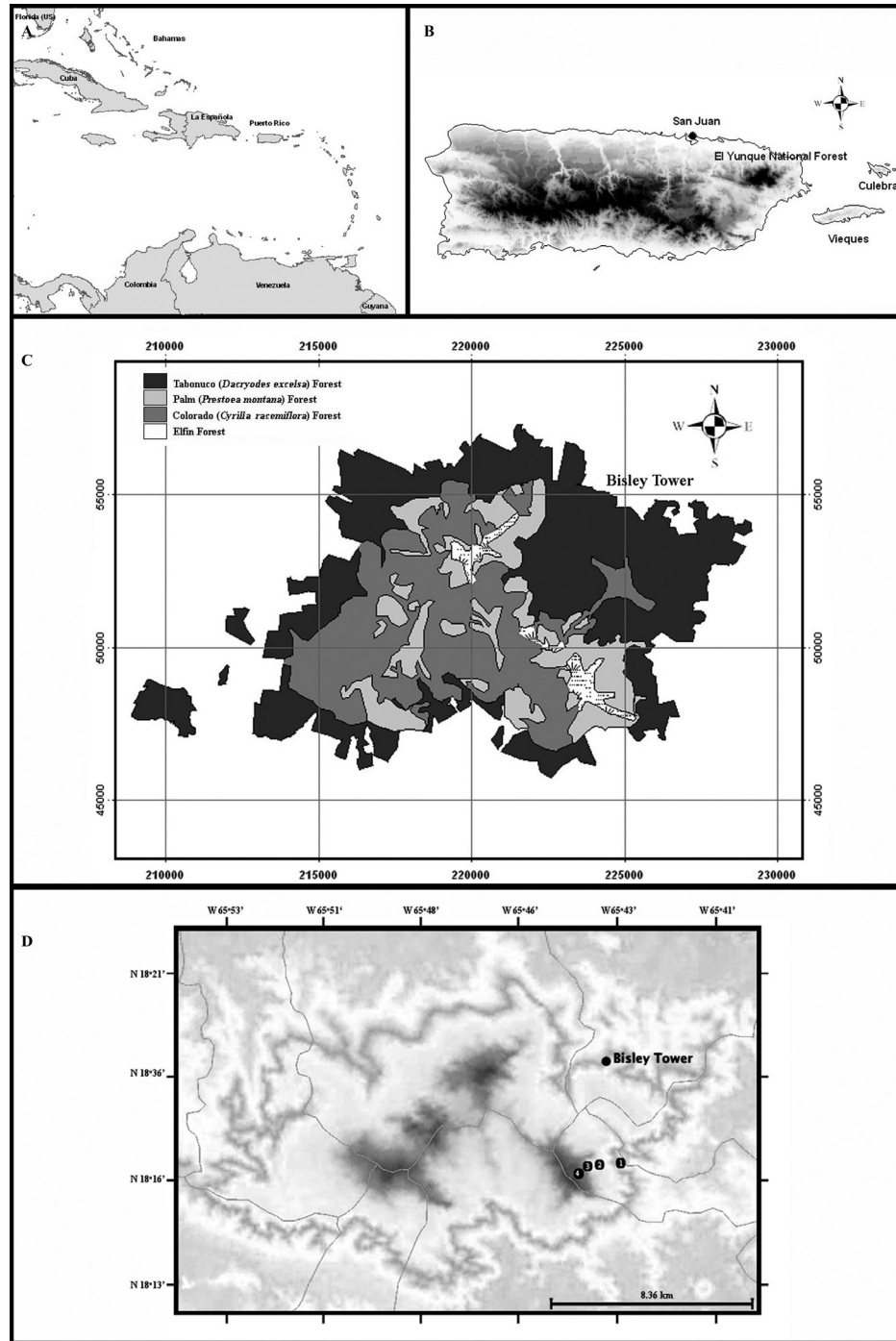
Nitrogen is especially integral to the proteins of photosynthetic enzyme rubisco (Wright et al. 2004). Using the leaf economics spectrum as a framework, we expect that an increase in elevation would demand less N investment because high LMA leaves, as expected in higher elevations,

have low protein (N-rich molecules) content, and tend to have high concentrations of other expensive compounds such as lipids or lignin.

Leaf epidermal features such as trichomes and idioblasts may also be related to the leaf economics spectrum. The whitish silvery finishes of the lower surface of many *Cecropia* species are produced by trichomes. Trichomes are epidermal outgrowths of diverse form, structure, and function (Dickison 2000). These leaf hairs (pubescence) function in defense, but also affect gas exchange and temperature (Gutschick 1999). In particular, trichomes can attenuate the absorption of shortwave radiation by leaves and thus keep them cooler (Baldochi et al. 1983, Bongers and Popma 1990). Pubescence facilitates gas exchange by keeping water droplets away from the leaf surface and stomata (Smith and McClean 1989, Brewer and Smith 1995). In tropical areas cloud formation associated with trade winds often occurs as a result of orographic effects. In the Antilles, mountains represent a barrier for the humid trade winds from the Caribbean Sea and prevailing winds undergo orographic uplift and condensation can occur forming orographic clouds. The eastern mountains of Puerto Rico receive cloud water input at their highest altitudes, between 900-1100 m (Scholl and Giambelluca, 2010). We would expect trichomes to be more abundant in leaves of *C. schreberiana* growing at the higher elevations, since plants growing at higher altitudes in tropical mountains facing trade winds (*i.e.*, cloud forest) may be exposed to greater exposure to cloud water than at lower altitudes.

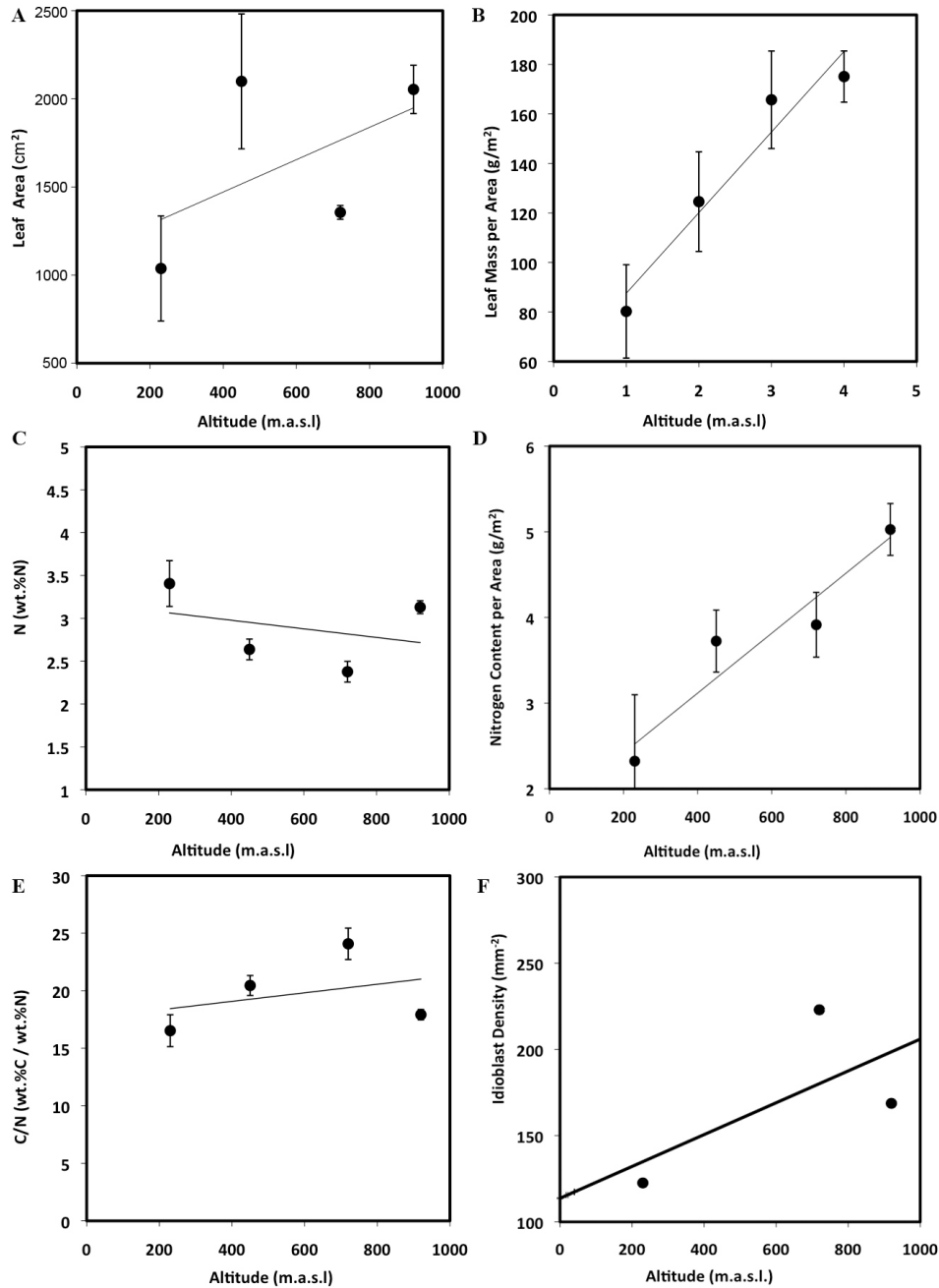
Idioblasts are enlarged epidermal cells accumulating minerals such as silicon, calcium and magnesium. Their function is not clear, but it has been proposed that they influence optical properties and the light environment within the leaf (Setoguchi et al. 1993). Silicon, for instance, has been reported to benefit plants in a number of ways: (1) increase canopy photosynthesis as a result of keeping leaf blades erect; (2) increase resistance to certain insects and disease-causing fungi and bacteria; (3) reduce accumulation of toxic concentrations

**FIGURE 1.** A. Location of Puerto Rico in Central America and Caribbean Region; B. Location of Luquillo Forest in Puerto Rico; C. Vegetation of the Luquillo Experimental Forest classified into four forest types, described by dominant species or physiognomy. Modified from LUQ LTER (2001). D. Location of *Cecropia schereberiana* trees included in this work: (1) 230 m.a.s.l.; (2) 450 m.a.s.l.; (3) 720 m.a.s.l.; (4) 920 m.a.s.l. Darker shades in D correspond to higher altitudes, elevation map based on SRTM data V1 (2004).





**FIGURE 2.** Intercanopy variation of leaf features in *Cecropia schreberiana* along an altitudinal gradient. A. Leaf area (least-squares regression:  $y = 0.9176x + 1104.2$ ;  $R^2 = 0.28$ ), B. Leaf mass per area ( $y = 0.14x + 55.182$ ;  $R^2 = 0.95$ ). C. Nitrogen content by mass ( $y = -0.0005x + 3.1786$ ;  $R^2 = 0.11$ ). D. Nitrogen content per area ( $y = 0.003496x + 1.7196$ ,  $R^2 = 0.91$ ). E. C: N ( $y = -0.1365x + 5.5834$ ;  $R^2 = 0.94$ ). Data points in A- E: mean of 3 to 9 measurements. Error bars  $\pm 1$  s.e.m. F. Density of idioblasts, line represents linear regression ( $y = 0.092x + 113.66$ ;  $r = 0.48$ ). Circles correspond to experimental results at 230, 720 and 920 m.a.s.l., mean of 24 measurements. Error bars  $\pm 1$  s.e.m.



of  $Mn^{2+}$  and other heavy metals; (4) increase the oxidizing power of roots; and (5) reduce cuticular transpiration and improves water use efficiency (Setoguchi et al. 1993). According to (5), we expect leaves exposed to higher irradiation at higher elevations to be tougher and have higher idioblast densities. On the other hand, given the structural improvement conferred by idioblasts (1), we expect larger leaves to have higher idioblast densities. Increased investment of resources is expected in building leaves as idioblast density increases.

## MATERIALS AND METHODS

The Luquillo Experimental Forest experiences a warm and humid subtropical maritime climate with mean annual temperatures ranging from 23-27°C at lower altitudes to 17-20°C in the upper regions (Körner et al. 1986). Much of the forest's precipitation is derived from easterly trade winds carrying moisture from the ocean and releasing it upon contact with Luquillo's peaks. The presence of these moisture-laden winds creates a moisture gradient along which mean annual precipitation increases with elevation, ranging from ~3500 mm at low elevations to almost 5000 mm at higher elevations (Körner et al. 1986). Upper elevations experience persistent contact with low-lying clouds, subjecting plants to frequent contact with condensation that can form a liquid film on leaf surfaces. Due to increased precipitation, reduced average temperature, greater fluctuations in temperature, cloud contact, and increased exposure to wind, plants at higher elevations in the forest generally experience harsher conditions than those at lower latitudes (Bostrack 1993b). Four forest types have been distinguished in Luquillo, each defined by a dominant vegetative type, with a range in elevation. These forest types include the tabonuco (*Dacryodes excelsa* dominated, 200-600 m), palo colorado (*Cyrilla racemiflora* dominated, 600-900 m), and elfin forests (>900 m), as well as a palm (*Prestoea montana*) forest in steep areas where the larger tree species are less dominant (Abramoff et al. 2004). *Cecropia schreberiana* is one of the few tree species that is found in all forest types.

Collection sites were at ~230 m, ~450 m, ~720 m and ~920 m within the Luquillo Experimental Forest, with each site representing a different forest type (Fig. 1). We collected and pressed one to three *C. schreberiana* leaves from each of one to three mature trees at each site. Mature trees were identified using a minimum DBH cutoff of 10 cm. Leaves were dried in a lab oven at 50 °C for one week, then weighed and photographed digitally at 3264 x 2448 pixel resolution (Nikon E8800). Image-J (Abramoff et al. 2004) was used to determine leaf area. For nutrient analyses, one to three leaves from each altitude were included. After leaves were dried, at least 3 mg of non-veined leaf matter was hole-punched and weight percent nitrogen and carbon were determined by combustion using a CE Elantech Elemental Analyzer. Nitrogen per area ( $N_{\text{area}}$ ) was calculated as  $N_{\text{mass}} \times \text{LMA}$  (Wright et al. 2004).

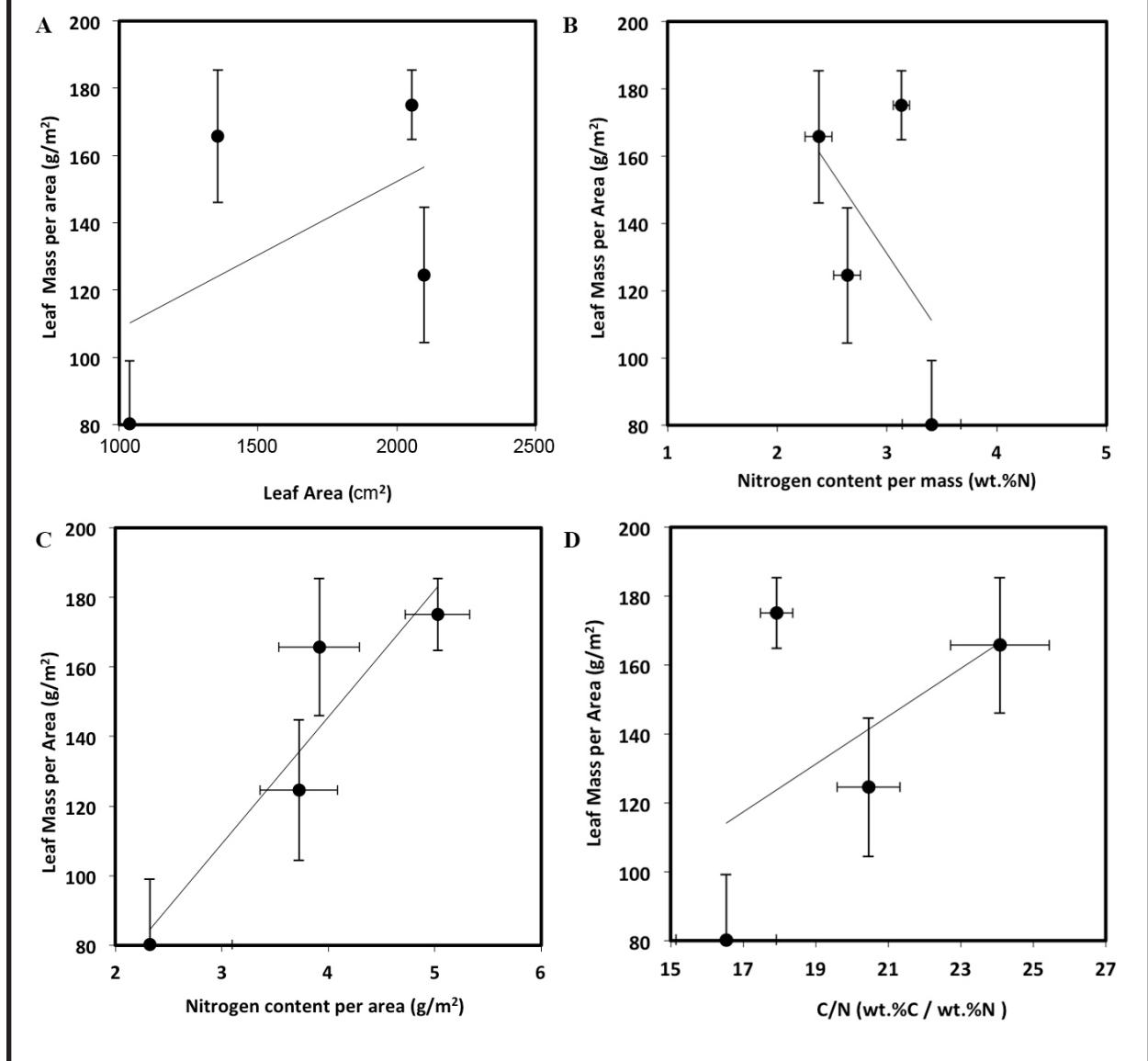
Epifluorescence microscopy (Leica DMBL; Leica Microsystems) was used for the observation of epidermal features. One leaf from each of two trees growing at 230, 723 and 924 m was selected. Four discs per leaf were obtained from an area near the base. For each disc, three different fields of view (0.1336 mm<sup>2</sup> each) were randomly selected. The number of idioblasts per field of view area was counted from photographs. A minimum of ten idioblasts per field of view was required for statistical analyses. In total 24 measurements per altitude were estimated. Additionally, scanning electron microscopy (SEM- JEOL 6390LV/LGS system) photographs were taken of leaves from the lowest (230 m) and highest (924 m) sites to document idioblasts and other epidermic leaf features such as trichomes.

## RESULTS AND DISCUSSION

### Leaf Morphology Variation

ANOVA for leaf area shows significant differences ( $P < 0.01$ ) in the variance added due to altitude, with LA highest at high elevations (Fig. 2A), although there is considerable scatter. Our two hypotheses concerning LA is that it should decline with elevation due to increasing environmental

**FIGURE 3.** Cross-plots between leaf features in *Cecropia schreberiana*. A. Leaf area vs. leaf mass per area ( $y = 0.0437x + 64.893$ ;  $R^2 = 0.28$ ). B. Nitrogen content by mass vs. leaf mass per area ( $y = -48.762x + 277.2$ ,  $R^2 = 0.27$ ). C. Nitrogen content per area vs. leaf mass per area ( $y = 0.003645x - 0.001976$ ;  $R^2 = 0.87$ ). D. C : N vs leaf mass per area ( $y = 6.9423x - 0.6831$ ;  $R^2 = 0.28$ ). Error bars  $\pm 1$  s.e.m.

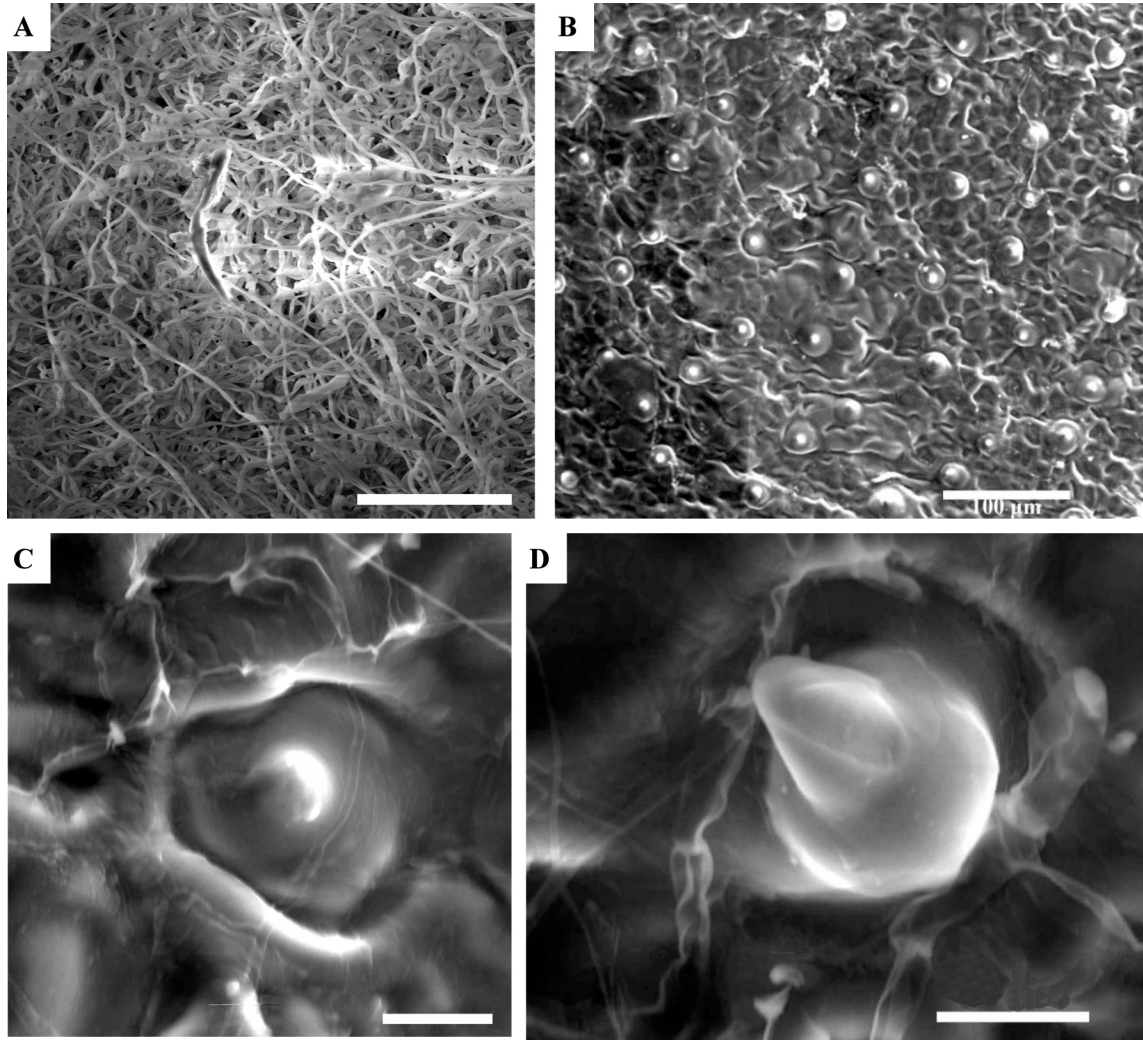


stress or that it should increase due to increasing rainfall. Our results therefore support the latter hypothesis.

ANOVA analysis for LMA also shows significant differences ( $P < 0.01$ ) due to altitude, with LMA increasing with elevation (Fig. 2B). High LMA reflects a large investment in photosynthate

per unit of leaf area (dense and/or thick leaves). According to the principles underlying the leaf economic spectrum (Wright et al. 2004), this type of investment is more worthwhile for trees at higher elevation because of their harsher growth conditions, including high winds, cooler and more variable temperatures, and high irradiance. Leaves from the dwarf forest, the highest elevation site,

**FIGURE 4.** Variation of epidermal structures of *Cecropia schreberiana* leaves along an altitudinal gradient. A. SEM photograph of the trichomes on the abaxial surface of the leaf. B-D. SEM photographs of the idioblasts on the adaxial surface of the leaves. B. Distribution in the epidermis. C-D. Two different developmental stages. Scale bars: A-B= 100 $\mu$ m, C-D: 10 $\mu$ m.

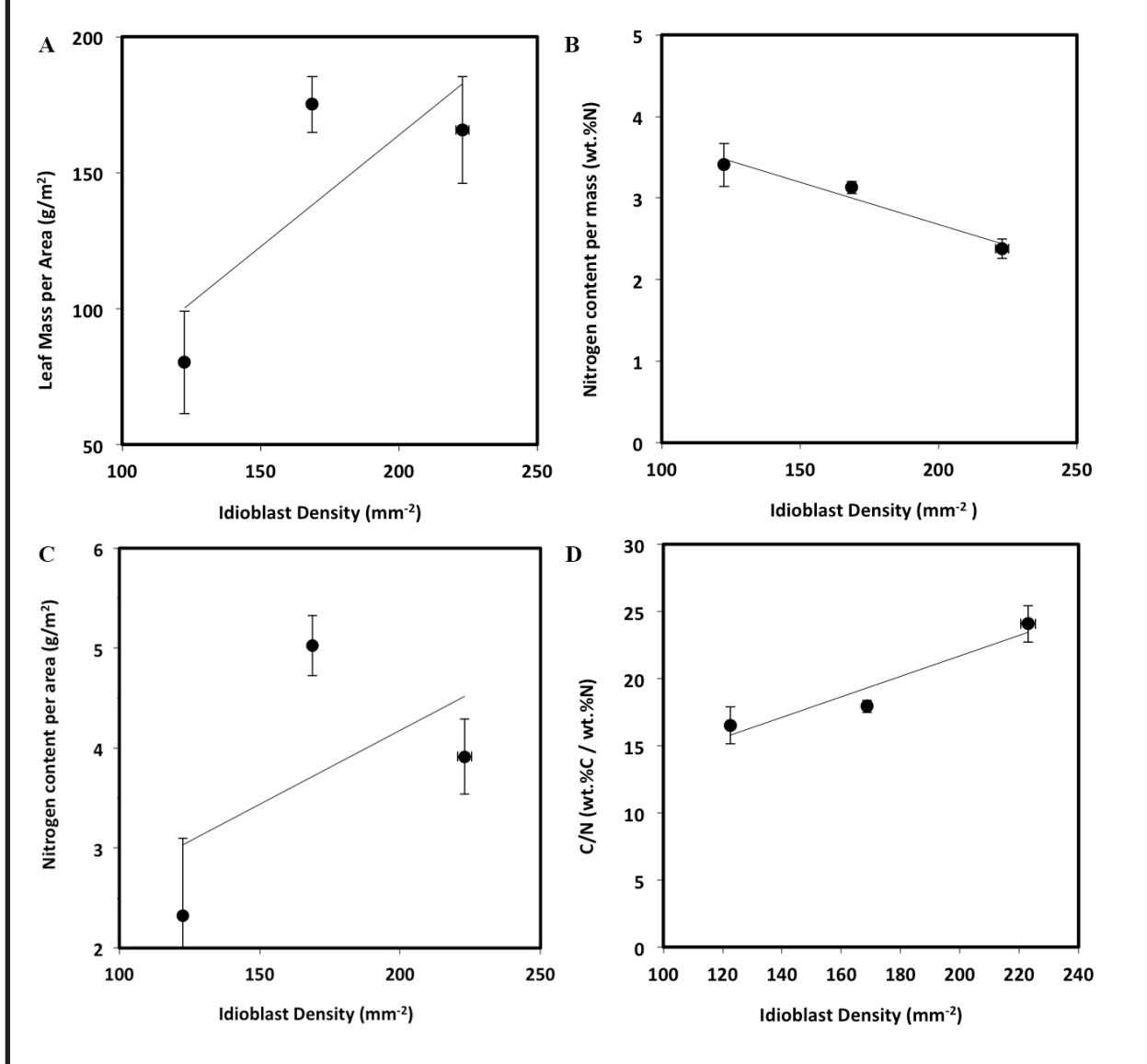


also contend with persistent liquid water films. It is likely that a combination of these factors is driving the higher-elevation *C. schreberiana* to produce more expensive, high LMA leaves.

Percent of nitrogen on a mass basis ( $N_{\text{mass}}$ ) drops slightly at higher elevations (Fig. 2C) and ANOVA shows statistically significant differences ( $P < 0.01$ ) between the treatments. A weak negative

association between  $N_{\text{mass}}$  and LMA ( $r = -0.39$ ) was detected (Fig. 3B). However, expressed in terms of area, nitrogen increases with altitude ( $P < 0.01$ ; Fig. 2C) and is positively associated with LMA (Fig. 3C;  $r = 0.7$ ).  $N_{\text{area}}$  reflects fluxes in terms of surfaces as opposed to mass for  $N_{\text{mass}}$  (Wright et al. 2004).  $N_{\text{area}}$  and  $N_{\text{mass}}$  therefore can reflect different leaf economic tradeoffs (Chabot and Chabot 1977). For example,  $N_{\text{area}}$  can result from low  $N_{\text{mass}}$  combined

**FIGURE 5.** Linear trends between idioblast density and other leaf features in *Cecropia schreberiana* at three altitudes. A. Leaf mass per area ( $y = 0.7666x - 114.09$ ;  $R^2 = 0.78$ ). B. Nitrogen content by mass ( $y = -0.0103x + 4.7448$ ;  $R^2 = 0.95$ ). C. Nitrogen content per area ( $y = 0.0148x + 1.218$ ;  $R^2 = 0.30$ ). D. C : N ( $y = 0.0763x + 6.4319$ ;  $R^2 = 0.91$ ). Error bars + 1 s.e.m.



with high LMA, high  $N_{mass}$  combined with low LMA, or some combination in between. As a result, mass-based metrics of nutrient concentration and gas exchange tend to coordinate better than equivalent area-based metrics with other leaf economic variables (Wright et al. 2004). Our data support this view:  $N_{mass}$  correlates inversely with

LMA, as expected, while  $N_{area}$  shows the opposite relation (Fig. 3B-C).

Leaf C:N ratio climbs slightly with increasing altitude (Fig. 1D) but an ANOVA was not significant for differences in the variance due to altitude ( $P = 0.157$ ). A weak positive association was detected

between LMA and C:N ratios ( $r = -0.40$ ) (Fig. 2D). Reasons for this could be an evolutionary trade-off founded on limited leaf interior space, where in high LMA leaves the proportion of carbon to nitrogen increases to accommodate structural leaf tissue like sclerenchyma (Westoby et al. 2002, Reich and Oleksyn 2004).

### Epidermal Features Variation

We made a qualitative estimation, using SEM photographs, of the trichome density of *C. schreberiana* at two sites. Trichomes in this species occur only on the lower surface of the leaves (Fig. 4A). As in very wet environments, trichomes contribute to keep water droplets off the leaf surface (Smith and McClean 1989, Brewer and Smith 1995), we expected the 924 m sample, corresponding to the cloud forest, to have more hairs protecting stomata from liquid water films than the 230 m sample, but no qualitative differences were detected. A quantitative method is required to further investigate whether altitude effects trichome expression.

Idioblasts are found in the upper surface of the leaves. This special type of trichome is regularly spaced, conical-shaped, attenuated at the tip and enlarged at the base (Fig. 4B-D). In *C. schreberiana*, idioblasts primarily accumulate silicon (Setoguchi et al. 1993). Idioblasts per unit area were counted only for leaves from three sites (230, 723 and 924 m). ANOVA for idioblast density shows significant differences in the variance added due to altitude ( $P < 0.01$ ), with values increasing with altitude (Fig. 1F). A strong positive association was calculated between number of idioblasts per area and LMA (Fig. 5A;  $r = 0.80$ ). Idioblast density was negatively associated with percent nitrogen (Fig. 5B;  $r = -0.65$ ). There was a weak positive association of idioblast density with  $N_{\text{area}}$  (Fig. 5C;  $r = 0.37$ ) and C:N values (Fig. 5D;  $r = 0.64$ ). The reason for the increase in idioblast density with altitude is not clear. Idioblasts may reduce cuticular transpiration and improve water use efficiency, and thus may be related to the exposure characteristics of the sites. They may

also be important for leaf rigidity, an important aspect regarding these very broad, palmately-lobed leaves. The role of both trichomes and idioblasts as a defense against herbivory is interesting given that many *Cecropia* species share symbiotic associations with *Azteca* ants (Brokaw 1998). Regardless of the underlying reason(s) controlling idioblast density, high density correlates with high LMA, low mass-based nitrogen concentration, and high C:N ratios. As such, idioblasts may be related to the leaf economics spectrum. Idioblasts represent a relatively expensive epidermal structure, but their structural and protective benefits may be worthwhile for plants pursuing a 'slow-return' strategy.

### CONCLUSIONS

Study of leaf plasticity along an altitudinal gradient in the Luquillo mountains shows a tendency of increasing leaf area with altitude that could be a response to higher rainfall at higher altitudes. The pattern of increasing leaf mass per area, lower mass-based content of nitrogen and higher C:N ratios at higher altitudes can be explained in terms of the investment needed to build leaves more suitable to resist harsher environmental conditions at higher altitudes. Epidermal leaf structures such as trichomes and mineral-storing idioblasts are probably playing an important role as structural and protective constituents of the leaves, and their altitudinal variation may also be related to the leaf economics spectrum.

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## CASE HARDENING IN PUERTO RICAN MOGOTES: A QUANTITATIVE ASSESSMENT

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***Key words: limestone, isotopes, recrystallization, karst, carbonates, Aymamón Formation***

### ABSTRACT

Case hardening has been proposed as a process controlling the formation of mogotes—a type of residual limestone formation found in Puerto Rico and other parts of the Caribbean. Case hardening involves the alteration of a primary limestone formation through the dissolution and reprecipitation of  $\text{CaCO}_3$ , resulting in the formation of a hard, low porosity cap over the relatively unaltered rock below. In order to quantitatively assess the presence of case hardening in mogotes, limestone from the interior and outermost section of a partially excavated mogote near Vega Baja, Puerto Rico, is analyzed for rock hardness, porosity, and  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values, which are all believed to be altered by the case hardening process. The relatively low  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values, low porosities, and recrystallization textures observed in many of these samples indicate that the mogote has been significantly altered in a manner consistent with case hardening. However, there is no systematic trend in these variables between limestone from the outermost section and interior of the mogote. This is likely due to compositional heterogeneity in the original formation and/or variability in the alteration of the mogote on small spatial scales.

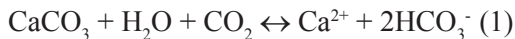
### RESUMEN

El endurecimiento superficial ha sido propuesto como un proceso que controla la formación de mogotes—un tipo de formación de caliza residual que se encuentra en Puerto Rico y otras partes del Caribe. El endurecimiento superficial implica la alteración de una formación primaria de caliza por medio de la disolución y la reprecipitación de  $\text{CaCO}_3$ , que termina en la formación de una capa dura de baja porosidad encima de la roca relativamente inalterada. Para evaluar cuantitativamente la presencia de endurecimiento superficial en mogotes, se analizó la caliza interior y exterior de una sección de un mogote parcialmente excavado cerca de Vega Baja, Puerto Rico para determinar la dureza, porosidad y valores de  $\delta^{13}\text{C}$  y  $\delta^{18}\text{O}$ , los cuales se cree sufren alteración por el proceso de endurecimiento. Los valores relativamente bajos de  $\delta^{13}\text{C}$  y  $\delta^{18}\text{O}$ , las porosidades bajas, y las texturas de recristalización observados en muchas de estas muestras indican que el mogote ha sido alterado significativamente de una manera consistente con el endurecimiento superficial. Sin embargo, no hay una tendencia sistemática en estas variables entre la caliza de la sección más al exterior y la del interior del mogote. Probablemente esto se deba a la heterogeneidad de composición en la formación original y/o variabilidad en la alteración del mogote a pequeñas escalas espaciales.



## INTRODUCTION

Mogotes are a distinctive type of karst topography generally characterized by steep-sided, 30–60 meter high limestone hills, although their size can vary widely. Mogotes are residual features, formed by the differential dissolution of carbonate rock exposed at the surface through tectonic uplift and/or dropping sea levels (Monroe 1976). The limestone that originally encompassed these hills has been eroded away through the process of carbonate solution, in which carbonic acid ( $\text{H}_2\text{CO}_3$ ) formed by the reaction of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  in soil water dissociates and dissolves limestone ( $\text{CaCO}_3$ ) into the soluble  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$  components. This results in the following net reaction:



Some areas in the limestone formation are preferentially eroded, leaving the mogotes as residual topographic highs (Easterbrook 1999).

In Puerto Rico, karst topography is concentrated in the northwestern region of the main island where a series of large, northward-sloping limestone formations outcrop at the surface. Nearly all of the island's mogotes have formed in the Aymamón Formation, a 190–200 m thick limestone layer deposited during the mid-Miocene. The Aymamón is 98–99 percent  $\text{CaCO}_3$ , and its purity likely facilitates carbonate dissolution and the formation of karst topography (Monroe 1976; Easterbrook 1999).

Dissolved limestone can reprecipitate in response to an increase in temperature, a drop in  $\text{CO}_2$  gas pressure, evaporation of water, or some combination of these factors. Reprecipitation of  $\text{CaCO}_3$  is believed to create a shell of indurated rock at the surface of limestone formations in a process known as case hardening (Monroe 1976). While still dissolved, the  $\text{CaCO}_3$  solution collects in shallow pores and subsequent reprecipitation fills in much of the pore space, thus reducing the rock's permeability. The pattern of rainfall in Puerto Rico

is ideal for the case hardening process: rain showers tend to be brief and torrential, resulting in limestone dissolution followed by rapid reprecipitation due to changing temperatures and evaporative effects (Eq. 1).

The process of carbonate solution is greatly enhanced by the presence of overlying vegetation. Addition of biogenic gas can increase the partial pressure of  $\text{CO}_2$  in soil to as high as 20 percent, compared with typical atmospheric values of 0.03–0.08 percent (Monroe 1976). This increases the acidity of meteoric water percolating through soil cover, which intensifies the dissolution of underlying limestone.

The dissolution of limestone by meteoric water with dissolved soil  $\text{CO}_2$  imparts carbon and oxygen isotope signatures to the  $\text{CaCO}_3$  that is reprecipitated during the case hardening process. Typical marine carbonates have  $\delta^{13}\text{C}$  values within a few permil of 0‰ (Friedman et al. 1969). By contrast,  $\text{CO}_2$  in soil supporting  $\text{C}_3$  plants tends to have  $\delta^{13}\text{C}$  values around -23‰ due to a carbon fractionation effect during the fixation of  $\text{CO}_2$ . A  $\delta^{13}\text{C}$  value of -15.7‰ is expected if calcite is precipitated in isotopic equilibrium with  $\text{C}_3$  plant-derived biogenic  $\text{CO}_2$  at 25°C (Deines et al. 1974; Clark and Fritz 1997). Similarly, original Cenozoic limestone typically has  $\delta^{18}\text{O}$  values of around  $0 \pm 1\%$  Vienna PeeDee Belemnite (VPDB), while mean annual precipitation near Vega Baja has a value of -37.1‰ VPDB (-7.3‰ Vienna Standard Mean Ocean Water (VSMOW) (Morse and Mackenzie 1990; Bowen and Revenaugh 2003; Bowen 2011). Carbonate reprecipitated in equilibrium with this meteoric water is expected to have  $\delta^{18}\text{O}$  values of around -9.4‰ VPDB (O'Neil et al. 1969).

Several qualitative studies have been made of Puerto Rican karst topography, most notably Monroe (1966, 1976). Monroe (1966) first suggests that the process of case hardening at least partially controls mogote formation and morphology. He estimates that the hardened layer is generally 5–10 m thick in Puerto Rican mogotes, but gives

no quantitative data describing the presence of indurated, reprecipitated limestone. Friedman et al. (1969) make carbon isotope measurements of an altered limestone group from Puerto Rico and find values significantly more negative (-5.8‰ to -14.9‰) than typical marine carbonate, which the authors attribute to limestone replacement in the presence of biogenic CO<sub>2</sub>. Ireland (1979) uses a manual rebound hammer to determine the relative hardness of limestone at the surface and at depth within mogotes located southeast of Arecibo, Puerto Rico. Based on these hardness measurements, he identifies outer case hardened layers with very consistent thicknesses of 1–2 m in these mogotes.

With this study I seek to contribute to our understanding of the role of case hardening in the formation of Puerto Rican mogotes. I apply several quantitative analytical methods to a single formation, so that variations in limestone alteration can be quantified within an individual karst feature. Unlike the studies discussed above, this study incorporates both geochemical and structural data. My goal in integrating these methods is to establish a more rigorous connection between limestone alteration and induration at the surface versus the interior of a mogote. Three sets of data were collected for limestone at various depths within a recently excavated mogote, including C- and O-isotope measurements, hardness, and porosity, all of which are affected by the case hardening process. These data sets are correlated and trends consistent with case hardening are investigated.

### STUDY AREA

Sampling was carried out at a mogote located west of Vega Baja, Puerto Rico (18°26' N, 66°25' W) at the center of an onramp (Exit 42A) to Highway 22. The mogote was partially excavated during highway construction, and the southeast side is now incised with a series of four step-like terraces (Fig. 1). This allowed for the sampling of limestone at depth within the original (pre-excavated) formation. The mogote is composed of limestone from the Aymamón Formation and is

approximately 50–60 m tall, with dense vegetation on the unexcavated surfaces. Extensive discussions of the geology of this region can be found in Monroe (1966, 1976).

### METHODS

Hardness measurements and limestone samples were taken at four of the terraces cut into the southeast side of the mogote. Two sampling sites were selected on the wall of each terrace—one site at the edge of the terrace as close to the outer surface of the original mogote as possible, and a second site at the center of the terrace as deep into the interior of the original mogote as possible. “Edge” and “Center” samples are identified using the terrace numbering scheme shown in Figure 1, e.g., sample “2-Center” refers to the sample taken from the center of terrace 2. As an example, sample locations for terrace 1 are also indicated in Figure 1.

Limestone hardness was measured at each sampling site using a manual rebound hammer. Sites were chosen with no visible fractures and as few surface irregularities as possible. To prepare each site for sampling, a ~0.5 ft<sup>2</sup> section of the limestone surface was sanded by hand with corundum grinding stones until as flat and vertical as possible. The manual rebound hammer was then used to obtain five rebound numbers on the sampling surface. Following Poole and Farmer (1980), the highest rebound number was taken to represent a best estimate of limestone hardness. Measurements are reported as rebound numbers (R), which give relative rather than absolute hardness values.

To estimate limestone macroporosity, thin sections were made of each sample and impregnated with blue epoxy. These thin sections were then digitally scanned and the blue pore space was isolated using image processing software. Following the method of Anselmetti et al. (1998), macroporosity was then determined as:

$$(\text{Area of Pore Space}) / (\text{Total Area of Slide}) \times 100 \quad (2)$$

**FIGURE 1.** The study mogote viewed from the southeast. For scale, the yellow, semi-circular structures seen partway up the mogote are approximately 3 meters tall. The terraces cut into the side of the mogote have been numbered for identification of sample sites. ‘Center’ and ‘Edge’ sample sites for terrace 1 are indicated.



It is important to note that this technique only accounts for macroporosity, while microporosity can make up as much as 30–40 percent of the total porosity in carbonate rocks (Anselmetti et al. 1998).

For measurement of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values each limestone sample was first cut into  $\sim 0.5$  cm thick slices using a diamond saw. These slices were dried in an oven and crushed to a fine powder using an agate mortar and pestle. The samples were analyzed by mass spectroscopy methods at the Stable Isotope Laboratory of University of Massachusetts Amherst. All carbon and oxygen isotope measurements are reported in standard delta notation relative to the VPDB standard.

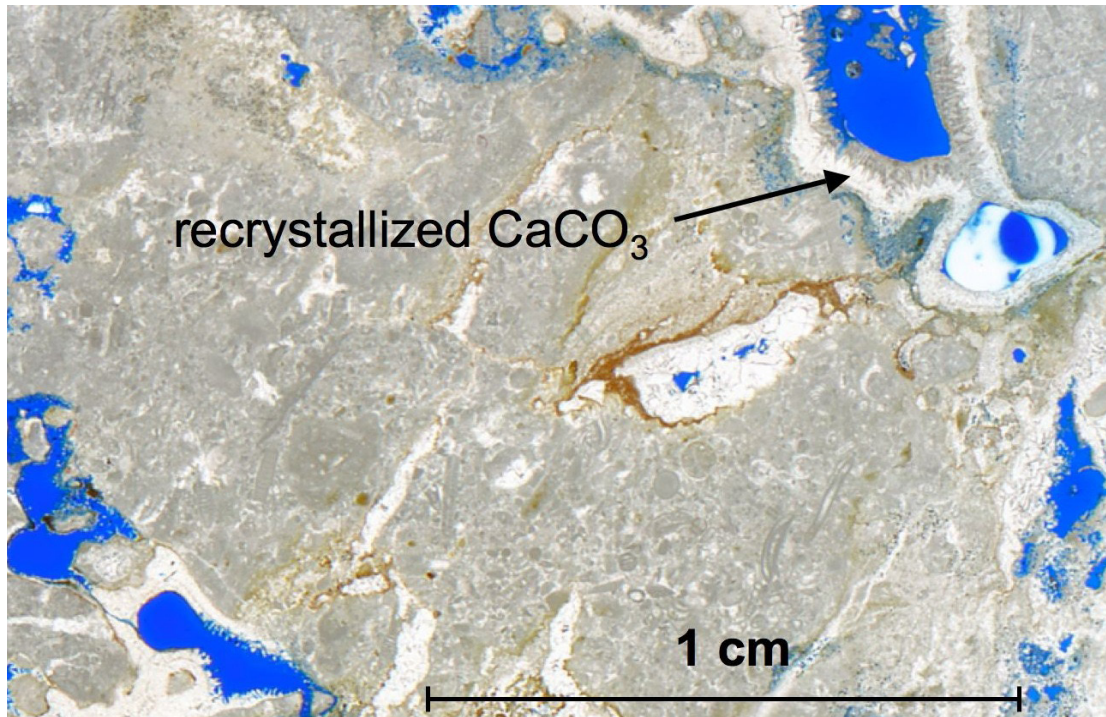
## RESULTS

Thin sections of selected samples suggest variability in limestone alteration among

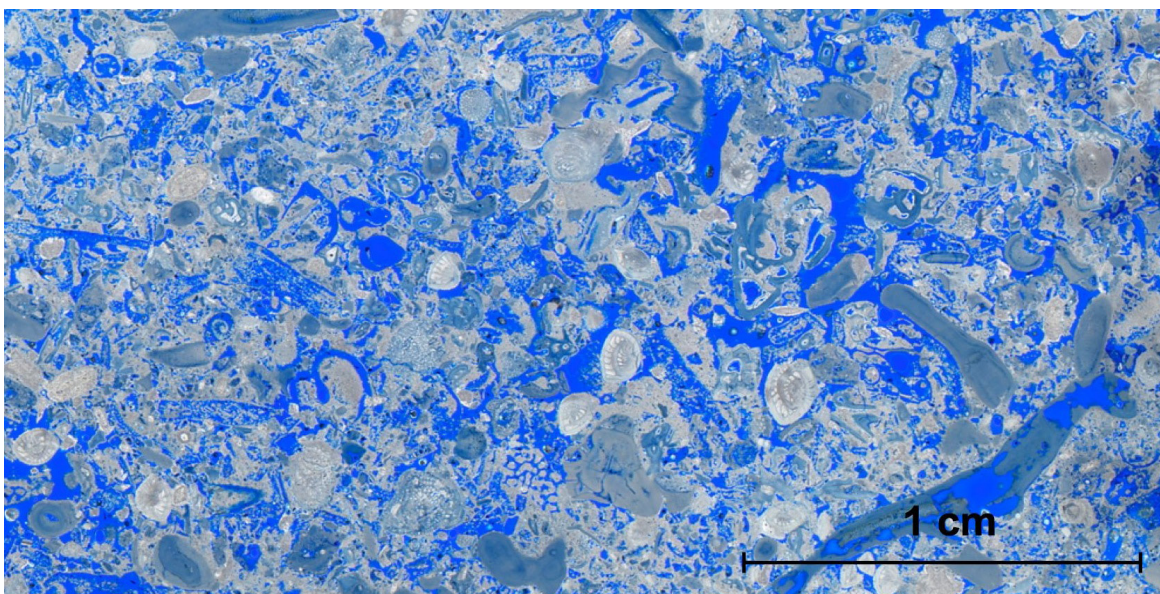
the sampling sites. Recrystallized  $\text{CaCO}_3$ —characterized by coarse, radial-fibrous crystals—is observed filling pore space in sample 1-Edge (Fig. 2). Areas of very low macroporosity are also visible in this sample, possibly as a result of carbonate solution and reprecipitation. By contrast, in sample 3-Center primary structures such as shell casings are easily identifiable, and the amount of macroporosity is much greater (Fig. 3).

Values for limestone hardness, porosity, and  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  at all eight sampling sites are shown in Table 1. Three of the terraces show an increase in limestone hardness (increase in R value) from the interior to the outer edge of the mogote (from the “Center” to the “Edge” sample sites). This increase is very large at terrace 1, and less so at terraces 3 and 4. At terrace 2 there is an opposite trend of decreasing hardness from the center site to the edge site. There is also an inconsistency in the trends

**FIGURE 2.** Photograph of sample 1-Edge in thin section (plane polarized light). Note the recrystallization textures and areas of low-to-no porosity. Blue areas represent pore space.



**FIGURE 3.** Photograph of sample 3-Center in thin section (plane polarized light). Note the abundance of primary structures and greater porosity relative to Figure 2.



**TABLE 1.** Hardness, % pore space, and  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values for mogote samples.

Sample Site:	Hardness (R)	% Porosity (VPDB)	$\delta^{13}\text{C}$ (‰) (VPDB)	$\delta^{18}\text{O}$ (‰)	% Soil $\text{CO}_2$
1-Edge	62	7.8	-7.84	-4.03	31.1
1-Center	14	3.7	-7.71	-3.55	30.5
2-Edge	44	8.3	-8.58	-3.29	34.4
2-Center	55	4.4	-7.16	-2.63	28.0
3-Edge	50	6.2	-6.84	-2.68	26.6
3-Center	30	19.8	-5.58	-2.53	20.8
4-Edge	66	2.8	-7.88	-2.97	31.3
4-Center	62	1.7	-9.24	-3.10	37.5

for porosity values between the sampling sites on each terrace (Table 1). Samples from terraces 1, 2 and 4 all have between 1.7–8.3 percent pore space, with greater porosities measured at the edge sites. Terrace 3 shows a much greater difference between the center and edge sites, with the center being significantly more porous (19.8 percent) than the edge (6.2 percent). Sample 3-Center has over twice as much macroporosity as any of the other samples collected.

Values for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  are plotted against each other in Figure 4. Isotopic compositional ranges for two end members—original (unaltered) marine  $\text{CaCO}_3$ , and replacement  $\text{CaCO}_3$  that has recrystallized in equilibrium with soil  $\text{CO}_2$  and meteoric water—are also shown. All of the mogote samples plot between these two end members, indicating that the limestone at all eight sites has undergone some degree of alteration. The isotopic composition of sample 3-Center shows the least amount of alteration, while sample 1-Edge appears to have been the most heavily altered. Overall there is no consistent trend in the relative alteration between the center and edge samples from each terrace.

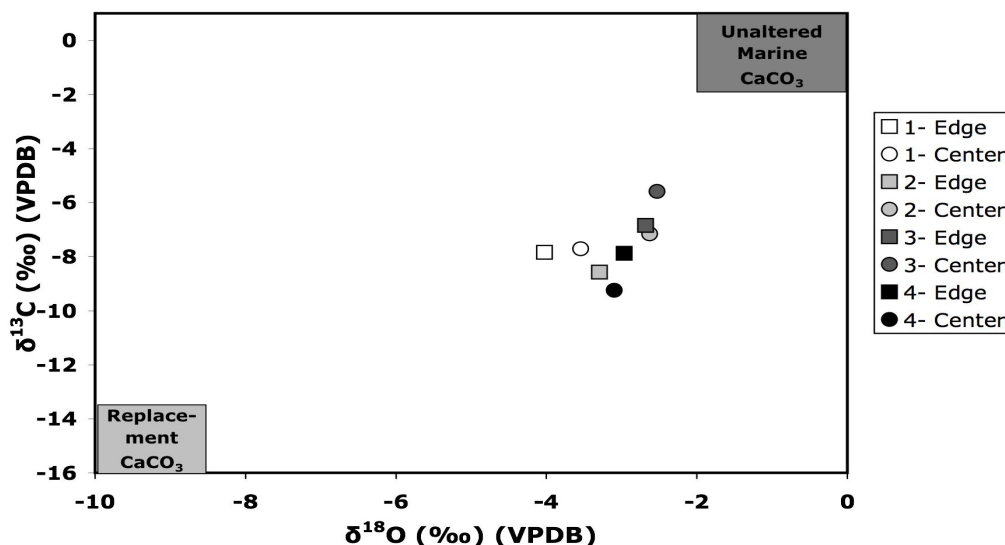
The percentage of soil  $\text{CO}_2$  making up the total carbon in each limestone sample was calculated using the following end-member mixing model:

$$\% \text{ Soil CO}_2 = \frac{\delta^{13}\text{C}_{\text{altered limestone}} - \delta^{13}\text{C}_{\text{unaltered marine limestone}}}{(\delta^{13}\text{C}_{\text{soil CO}_2} - \delta^{13}\text{C}_{\text{unaltered marine limestone}})} \times 100 \quad (3)$$

A  $\delta^{13}\text{C}$  value of -1‰ was used for the unaltered marine limestone end member, and a  $\delta^{13}\text{C}$  value of -23‰ was used for the soil  $\text{CO}_2$  end member (Friedman et al. 1969; Clark and Fritz 1997). Higher percentages of soil  $\text{CO}_2$  indicate more extensive limestone replacement. According to this model, soil  $\text{CO}_2$  accounts for between 20–38 percent of the carbon in these limestone samples (Table 1). Differences in percent soil  $\text{CO}_2$  between center and edge samples range from ~0.6 percent in terrace 1 to ~6.5 percent in terrace 2. Again there is no consistent trend in alteration between the center and edge sites among these terraces— the relative amount of soil  $\text{CO}_2$  increases toward the outer edge of the mogote in terraces 1, 2 and 3, but decreases in terrace 4.

Case hardening variables are plotted against each other in Figures 5 and 6. The arrows included in each figure indicate the direction in which values are expected to move with greater limestone alteration. In a plot of hardness versus percent pore space (Fig. 5) there is a fairly linear trend in the data along the direction of alteration, with the exception of sample 1-Center, which has relatively low values for both percent pore space and hardness. Sample 3-Center shows the least amount of alteration,

**FIGURE 4.**  $\delta^{13}\text{C}$  vs.  $\delta^{18}\text{O}$  for all mogote samples. The isotopic compositional ranges of two end members—unaltered marine  $\text{CaCO}_3$  and replacement  $\text{CaCO}_3$ —are indicated by the labeled boxes. End member compositions follow Bowen (2011), Bowen and Revenaugh (2003), Clark and Fritz (1997), Deines et al. (1974), Friedman et al. (1969), Morse and Mackenzie (1990), and O’Neil et al. (1969).



while the center and edge samples from terrace 4 are the most altered. There is no clear overall division between center and edge samples.

On a plot of  $\delta^{13}\text{C}$  versus percent pore space sample 3-Center again stands out as the least altered sample (Fig. 6). The other seven samples cluster together at the more highly altered range of the plot, with sample 4-Center being the most altered. Again there is no clear separation between the center and edge samples.

## DISCUSSION

There are multiple lines of evidence indicating that the mogote investigated in this study has undergone significant alteration. These include the recrystallization textures observed in thin section (Fig. 2), the depleted  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values compared to primary marine  $\text{CaCO}_3$  (Fig. 4), and

the generally low porosities (Table 1) found in these samples relative to unaltered carbonates, which typically range from 20–70 percent pore space (Budd 2002; Schmoker and Halley 1982). It should be noted that microporosity—which was not measured in this study—might also contribute significantly to the porosity of these samples.

While these alteration features suggest that this mogote has undergone the process of carbonate dissolution and reprecipitation that is believed to result in case hardening, there is no systematic trend in case hardening variables between the sample sites at the interior of the original mogote and those near the original surface. The samples from terrace 3 show an increase in alteration from the interior to the surface of the mogote for all case hardening variables, while the center and edge sites from the other three terraces have generally undergone comparable degrees of alteration.

FIGURE 5. Hardness vs. percent pore space. The arrow indicates the direction in which values are expected to shift as the limestone is altered during the case hardening process.

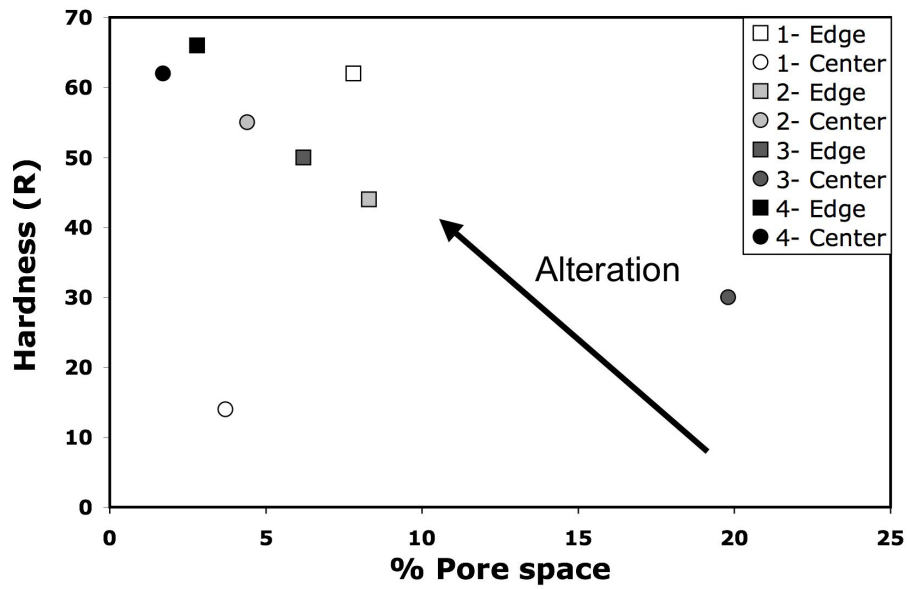
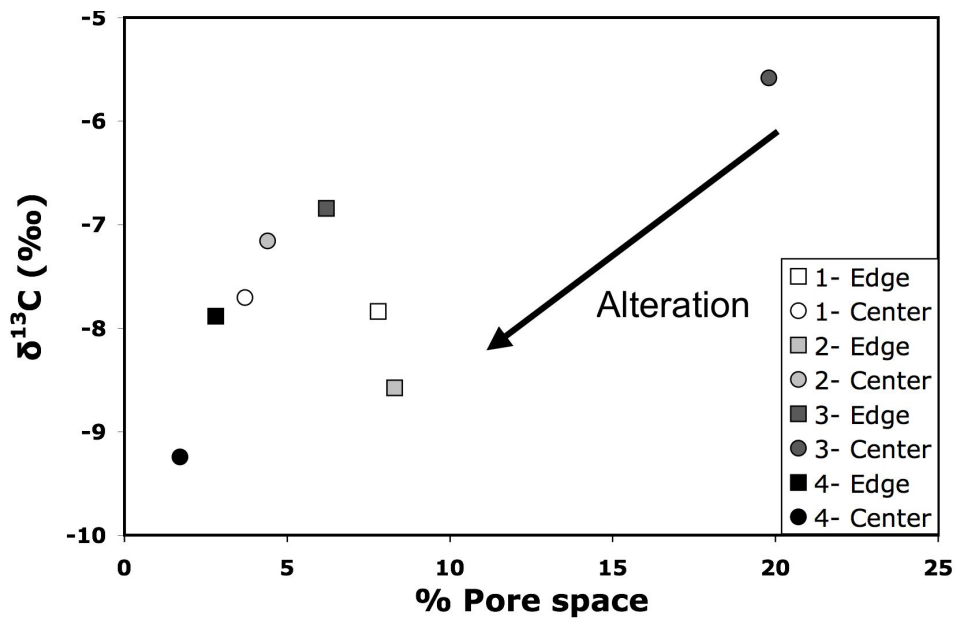


FIGURE 6.  $^{13}\text{C}$  vs. percent pore space. The arrow again indicates the direction in which values are expected to shift as the limestone is altered during the case hardening process.



The constancy of alteration at the various sample locations could indicate that the center and edge sampling sites have been similarly altered since the mogote was excavated and the surfaces were exposed during the 1980s. Alteration post-exposure would thus obscure any variability in case hardening that may have existed between the inner and outer sections. While this explanation can account for the similarities in alteration between the center and edge sites on terraces 1, 2 and 4, it does not explain why the center of terrace 3, which presumably was exposed at the same time as the other sampling sites, is relatively unaltered. Alteration post-exposure also would not account for the relatively low  $\delta^{13}\text{C}$  values observed in the center samples. Because a vegetated soil layer has not covered the limestone at these sites since the excavation of the mogote, the isotopic signature of reprecipitated limestone should not be affected by biogenic  $\text{CO}_2$ , and therefore should not be significantly lower than the  $\delta^{13}\text{C}$  of unaltered marine  $\text{CaCO}_3$ .

A second possible explanation for these data is that this study only sampled the case hardened layer of the original mogote. If only the case hardened layer was exposed during excavation then all of these samples represent case hardened limestone, and little variation should be expected between the center and edge sample sites. As with the first proposed explanation, this accounts for the data from terraces 1, 2 and 4, but does not explain the systematic variation seen between the two sampling sites on terrace 3. Sample 3-Center may be the only one to penetrate beyond the case hardened layer. The exact depths of these sampling sites within the original mogote have not been determined, but based on estimates from visual extrapolation of the unexcavated edges of the mogote, the center sampling sites are almost certainly greater than two meters within the interior of the original mogote. If this explanation is correct then it suggests that the case hardened layer in this mogote is thicker than those found by Ireland (1979;  $\leq 2$  m), and probably closer to the thicknesses estimated by Monroe (1966; 5–10 m).

A third possibility is that compositional alteration in the outer mogote is more heterogeneous than expected on relatively small spatial scales. This could result from compositional variability in the original formation or from fractures in the limestone that produce channels for water movement through the interior of the mogote. Describing mogote alteration in terms of a discrete case hardened outer layer surrounding an unaltered interior, as in Ireland (1979), may thus be an oversimplification. Instead mogotes may be compositionally analogous to a marble cake, with pockets of variably altered material occurring throughout the formation. According to this hypothesis sample 3-Center would most likely represent a pocket of relatively unaltered material, while the smaller variations between the other samples might be accounted for by compositional differences in the original limestone.

## CONCLUSION

Limestone samples taken from near the surface and from the original interior of the partially excavated mogote show evidence for significant alteration that is consistent with the case hardening process. However these samples do not show consistent trends in alteration with depth in the mogote; most samples from the outer section and interior of the original formation are similarly altered with respect to case hardening variables. This could be due to post-exposure alteration of the interior samples, a particularly thick case hardened layer, or compositional heterogeneity and variable alteration within the mogote on small spatial scales. It is also possible that none of these hypotheses can account for the data collected in this study, or that the data result from some combination of them. However, the third explanation—that alteration of the limestone varies on small spatial scales—seems to best account for the overall variability in limestone hardness, porosity, and isotopic composition observed in these samples. If this hypothesis is correct then it suggests that mogote alteration is more complicated than previously proposed, and descriptions of mogotes probably cannot be reduced to a case hardened layer surrounding unaltered limestone.



## APPENDIX 1. Manual rebound hammer data.

Sample:	Test 1	Test 2	Test 3	Test 4	Text 5	Highest	Mean
1-Center	12	10	1	14	13	14	12.2
1-Edge	22	42	32	48	62	62	41.2
2-Center	46	55	53	46	44	55	48.8
2-Edge	40	29	44	43	41	44	39.4
3-Center	29	20	24	24	30	30	25.4
4-Center	53	62	61	62	54	62	58.4
4-Edge	65	66	66	66	65	66	65.6

(All hardness measurements are expressed as rebound numbers (R))

## APPENDIX 2. Porosity data.

Sample	Number of Pores	Total Pore Area (m <sup>2</sup> )	Average Pore Size (10 <sup>-6</sup> m <sup>2</sup> )	% Pore Space
1-Edge	2394	0.082	34.1	7.8
1-Center	2046	0.020	9.96	3.7
2-Edge	17825	0.098	4.99	8.3
2-Center	8075	0.047	5.79	4.4
3-Edge	2399	0.066	27.5	6.2
3-Center	8867	0.195	22.0	19.8
4-Edge	4390	0.028	6.29	2.8
4-Center	2984	0.013	4.51	1.7

A more comprehensive sampling strategy would help to better constrain patterns of case hardening in Puerto Rican mogotes. Future work should aim at a complete, systematic characterization of limestone alteration at well-defined depths within a formation.

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## SAXITOXIN AND METALS IN OYSTERS OF BAHIA FOSFORESCENTE, PUERTO RICO

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**Key words:** *toxins, metals, oysters, food safety*

### ABSTRACT

Saxitoxin and metal (Hg, Cu, Zn) analyses of oysters and suspended particulate matter (~plankton) were carried out in Bahía Fosforescente, a bioluminescent bay in southwestern Puerto Rico. Bahía Fosforescente has high concentrations of the bioluminescent dinoflagellate, *Pyrodinium bahamense var. bahamense*, which produces saxitoxin and is associated with high nutrient waters. Oyster and plankton saxitoxin concentrations were determined using Jellett Rapid Tests and ELISA (Enzymelinked immunosorbent assay) bioassays. Saxitoxin concentrations were below the limit of detection (40 µg STX/100 g shellfish) of the Jellett Rapid Test and ranged from below detection (plankton) to 0.1-0.3 µg STX/100 g shellfish (oysters) based on ELISA bioassays. All measured saxitoxin concentrations were below the safety level for human consumption (80 µg STX/100 g shellfish). Copper and mercury concentrations in Bahía Fosforescente oysters ranged from 5-11 ppm and 0.01-0.02 ppm, respectively, which were below the Maximum Permissible Levels (MPL), but zinc concentrations were greater than the MPL of 50µg/g wet wt. for human consumption (Tobago 1998). These results demonstrate that oysters from Bahía Fosforescente contain concentrations of saxitoxin, copper, and mercury that are not a human health concern; however, the concentration of zinc in oysters exceeds MPL and should be monitored in the future.

### RESUMEN

Se llevó a cabo un análisis de saxitoxina y metales (Hg, Cu, Zn) de las ostras y las partículas (~plancton) en la Bahía Fosforescente, una bahía bioluminiscente ubicada en el suroeste de Puerto Rico. La Bahía Fosforescente tiene altas concentraciones del dinoflagelado bioluminiscente, *Pyrodinium bahamense var. bahamense*, que produce saxitoxina, asociado con aguas con altos nutrientes. Las concentraciones de saxitoxina en las ostras y el plancton se determinaron utilizando pruebas rápidas Jellett y pruebas biológicas ELISA (Enzymelinked immunosorbent assay). Las concentraciones de saxitoxina reflejaron estar por debajo del límite de detección de la prueba rápida Jellett (40 µg STX/100 g de crustáceos) con un rango por debajo de la detección (plancton) a 0.1-0.3 µg STX/100 g de crustáceos (ostras), basado en las pruebas biológicas ELISA. Todas las concentraciones de saxitoxina

medidas reflejaron estar por debajo del nivel de seguridad para el consumo humano (80 µg STX/100 g de crustáceos). Las concentraciones de cobre y mercurio en las ostras de La Bahía Fosforescente fluctúan entre 5-11 ppm y 0.01-0.02 ppm, respectivamente, lo que está por debajo de los niveles máximos permitidos (MPL, por sus siglas en inglés), pero las concentraciones de zinc son mayores que el MPL de 50µg/g de peso húmedo para el consumo humano (Tobago 1998). Estos resultados demuestran que las ostras de La Bahía Fosforescente tienen concentraciones de saxitoxina, cobre y mercurio que no representan un problema para la salud o el consumo humano. Sin embargo, la concentración de zinc en las ostras sobrepasa los niveles de seguridad para el consumo humano y debe mantenerse bajo observación.

## INTRODUCTION

Bioaccumulation is the process by which an organism acquires an increasing concentration of a substance from its intake of food and exposure to the substance in its surrounding environment. Within an ecosystem, substances can biomagnify between trophic levels as upper level feeders repeatedly acquire their prey's lifetime accumulation of a substance, thus resulting in increasingly higher concentrations of a substance at higher levels in a food chain (Chemistry 1993). Both saxitoxin and metals bioaccumulate within aquatic organisms, thus elevated concentrations of these substances create a human health concern for seafood consumers.

Here we investigate the concentrations of saxitoxin, a neurotoxin, and metals (Cu, Hg, Zn) in a bioluminescent bay with previously reported high concentrations of the dinoflagellate *Pyrodinium bahamense* var. *bahamense* (Viruet 2005). The *P. bahamense* dinoflagellate is associated with nutrient-rich waters and has recently been found to produce saxitoxin (Landsberg 2006). Metals copper (Cu), mercury (Hg), and zinc (Zn), are harmful to humans at high concentrations and may indicate anthropogenic input sources to the bay ecosystem. To examine bioaccumulation and biomagnification of saxitoxin and metals in the bay ecosystem, the dinoflagellate species *P. bahamense* var. *bahamense* was used to represent the lower trophic level while the oyster species *Crassostrea rhizophorae* was examined as a higher trophic level (Amaral et al. 2005).

## Saxitoxin Background

The saxitoxin molecule acts by binding to an organism's voltage-gated sodium channels, thereby preventing the passive influx of sodium ions into excitable cells (Etheridge et al. 2006). This blocks the propagation of action potentials along nerve and muscle membranes, resulting in symptoms such as paralysis of the extremities, nausea, vomiting, ataxia, drowsiness, difficulty in speaking, progressively decreasing ventilatory efficiency, and in extreme cases, death by asphyxiation caused by respiratory paralysis (Etheridge et al. 2006, Landsberg 2006). The USFDA has defined the Maximum Permissible Level (MPL), or safety level, for human consumption as 80 µg STX/100 g shellfish (Jellett 2002, Ltd 2008).

Historically, it was believed that the dinoflagellate *Pyrodinium bahamense* var. *compressum* produced saxitoxin while a related variety, *Pyrodinium bahamense* var. *bahamense*, was non-toxic. However, Landsberg et al. (2006) reported saxitoxin production by the *bahamense* variety in the Indian River Lagoon in Florida. Following this discovery by Landsberg, et al. others found that in the post-drought period of 2001-2002, significant increases in total phosphorus concentrations and high total nitrogen levels was associated with *P. bahamense* var. *bahamense* blooms. It is possible that human-induced nutrient loading increased *P. bahamense* populations, causing greater saxitoxin input into the environment.

## Metals Background

Copper, mercury, and zinc are harmful to humans at high concentrations and will bioaccumulate over one's lifetime. High levels of copper can increase liver disease and neurological defects (Uriu Adams and Keen 2005). Mercury is especially dangerous for human health because it is a neurotoxin and can damage the nervous, cardiovascular and immune systems (Tchounwou 2003). High levels of exposure to zinc can adversely affect the gastrointestinal system (Goyer and Clarkson 2008). Although dangerous levels depend on the frequency and amount of metals consumed over time, the Republic of Trinidad and Tobago specified the maximum permissible levels of copper and zinc for human consumption in seafood respectively as 20µg/g wet weight and 50µg/g wet weight in their 1998 Food and Drug Act (Tobago 1998). The FDA safety limit for human consumption of mercury is 1ppm (Foulke 1995).

Copper, mercury, and zinc bioaccumulate in oysters, and are often used as biomarkers of anthropogenic pollution (Amaral et al. 2005, Ruus et al. 2005). To assess potential human effects and safety, we measured the concentrations of Cu, Hg, Zn, and saxitoxin in suspended particulate matter and oysters and compared the concentrations to the Maximum Permissible Levels.

## Study Area

Bahía Fosforescente is located in the southwest corner of Puerto Rico, near the town of La Parguera, and is about 20 ha with a maximum depth of 4.5 meters (Cintrón et al. 1970). Bahía Fosforescente is a world-renown bioluminescent bay and tourist attraction. The bioluminescence in Bahía Fosforescente is caused by high concentrations of the dinoflagellate species *Pyrodinium bahamense var. bahamense* (Hernández Becerril and Navarro 1996).

Over the last 30 years, numerous marinas, hotels and residential homes have been built in

close proximity to the bay (Hertler et al. 2009). Between 1994 and 1997, the population around Bahía Fosforescente increased by over 200%, not including the transient population associated with hotel rentals or tourist facilities (Hertler H. 2002). This has led to a large increase in pollution in the forms of sewage, boat discharge, and petroleum hydrocarbons from the shore and channels around Bahía Fosforescente (Siung Chang 1997). Large populations and tourism industries can cause increases in copper, mercury, and zinc.

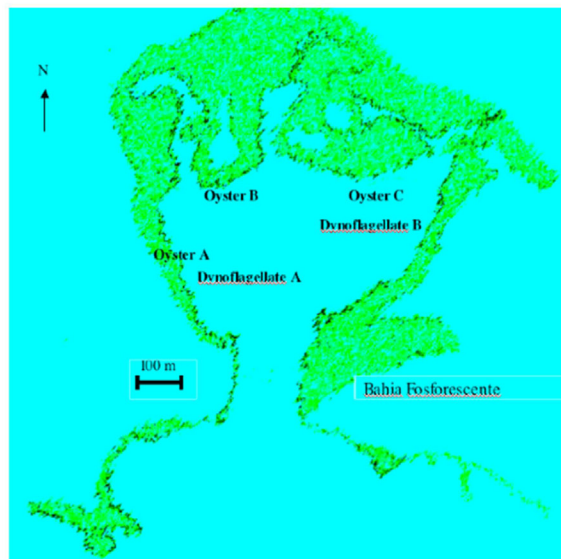
Bivalves and phytoplankton within Bahía Fosforescente have not been tested for harmful metals or saxitoxin, though the water column, bottom sediments, mangrove leaves, and surrounding ground have had some metal testing. In Bahía Fosforescente, Viruet (2005) reported sediment arsenic, lead, and mercury concentrations that were Below Detection Limits (BDL), but the concentrations of iron and copper were above BDL detection limit. Viruet (2005) attributed the higher concentrations of iron and copper to high concentrations of *Pyrodinium bahamense var. bahamense* that consume the phytoplankton with the essential iron and copper micronutrients.

## METHODS

In January 2009 two suspended particulate samples and three combined oyster samples were collected from La Paraguera. Filtered particulate samples were designed to capture dinoflagellates and were collected at two sites within Bahía Fosforescente, one near the mouth of the bay, and one within the bay (Fig. 1). At each site, 10 L of surface water was filtered and particles between 10 and 120 µm were collected and frozen for later analyses.

*Crassostrea rhizophorae* oysters were collected from three sites within the bay (Fig. 1). Approximately 50 live oysters (~120 g flesh) were collected from mangrove roots at each site. The oysters were shucked and homogenized in a blender. A comparison sample from Long Island

**FIGURE 1.** Bahía Fosforescente sample collection locations. Two dinoflagellate samples and three oyster samples were taken in the bay. Latitude: 17-58'20" N, longitude: 067-00'51" W. Map based on (Seliger et al. 1971).



Sound was used and was treated identically as the Puerto Rico oysters.

Oysters were tested in the field for the presence of saxitoxin using the commercially available Jellett Rapid Test (Oshiro et al. 2006). Homogenized samples were extracted and applied to test strips according to manufacturer instructions (Oshiro et al. 2006). The detection limit of this test is 40  $\mu\text{g}$  STX/100 g shellfish, which is below the safety level of 80  $\mu\text{g}$  STX/100 g shellfish used by the USFDA (U.S. Food and Drug Administration) (Jellett et al. 2002). The ELISA bioassay method that quantifies toxins such as saxitoxin was also used (Hattenrath et al. 2010, Naar et al. 2002).

The U.S. Environmental Protection Agency Method 7473 was used to analyze the oyster samples (EPA February 2007a). The oyster samples were freeze-dried and then analyzed for mercury using the Direct Mercury Analyzer-80 (DMA-80) at Wesleyan University. Two analyses were conducted per sample and the mercury levels were confirmed based on two Standard Reference Materials (SRMs): TH2 (from Environment

Canada) and PACS-2 (from National Research Council Canada). The percent error was within 10% of the true values of the SRMs. Oysters were then ashed at 450°C, digested with HCl and HNO<sub>3</sub>, then analyzed for copper and zinc using an ICP-MS at Lehigh University according to US EPA Method 6020A (EPA February 2007b). Each sample was analyzed twice to check for consistency.

## RESULTS

Our analyses of the shellfish and dinoflagellates in Bahía Fosforescente indicate that saxitoxin is not present in concentrations to be of concern for human health (Tables 1 and 2). The Jellett Rapid Test indicated no saxitoxin (<40  $\mu\text{g}$  STX/100g) in any of the three oyster samples. The ELISA bioassay showed that oyster Sample A had 0.3  $\mu\text{g}$  STX/100g shellfish and oyster Sample C had 0.14  $\mu\text{g}$  STX/100g shellfish (Table 1). The saxitoxin concentration in the phytoplankton sample was below the detection limit of the ELISA method.

Oyster copper concentrations were 5-11 ppm, which was below the Maximum Permissible Level

**TABLE 1. Jellett and ELISA Bioassay results.** The Jellett Rapid Test measures the presence or absence of saxitoxin to an accuracy of 40 µg saxitoxin/100 g shellfish. The ELISA bioassay quantifies saxitoxin. The FDA safety limit is 80 µg saxitoxin/100 g shellfish. These results indicate that the oysters are safe to eat with respect to saxitoxin levels.

Sample	Jellett Field Test Result	ELISA Bioassay Result
Oyster A (Puerto Rico)	Negative	0.3 µg STX/100g shellfish
Oyster B (Puerto Rico)	Negative	N/A
Oyster C (Puerto Rico)	Negative	0.14 µg STX/100g shellfish
Control ( CT)	Negative	N/A
Dinoflagellates	N/A	Below detection levels

of 20 ppm for oysters from Puerto Rico. The comparison oyster from Long Island Sound had copper concentrations above 325 ppm. Mercury concentrations for both our Puerto Rican and Long Island Sound comparison oysters were below 0.02 ppm, which was below the MPL of 1 ppm (Foulke 1995). Zinc concentrations in oysters from both Puerto Rico and Long Island Sound were above 140 ppm, which was above the MPL of 50 ppm (Table 2).

## DISCUSSION

The low levels of saxitoxin may be due to a depressed population of *Pyrodinium bahamense*, reduced bioaccumulation rates, or to the fact that the *Pyrodinium bahamense* within the bay are not producing saxitoxin. A population count, however, was not conducted. Indeed, the production of saxitoxin by *Pyrodinium bahamense* has only been documented in the Indian River Lagoon in Florida by Landsberg et al. (2006) and it is possible that different environmental conditions in Bahía Fosforescente do not support the production of saxitoxin by *Pyrodinium bahamense*. Possible explanations for a population decline include normal population variations, weather and ocean circulation changes, changing nutrient and sediment conditions, and sampling problems. Previous studies indicate that concentrations of dinoflagellates should have been approximately

40,000 dinoflagellates/10L seawater collected (Seliger et al. 1971). A long-term study of the dinoflagellate population in Bahía Fosforescente would be useful in understanding dinoflagellate population fluctuations.

The Jellett Rapid Test and the ELISA bioassay indicate saxitoxin concentrations below the FDA safety limit of 80 µg saxitoxin/100 g shellfish. Thus, the oysters and, most likely the other seafood from this area, will not cause Paralytic Shellfish Poisoning in consumers. Our results indicated that the oysters are bioaccumulating zinc and have concentrations well above the established MPL. However, it is possible that sediment may have contaminated the oyster flesh and caused the high concentrations of zinc in the oysters. It will thus be important to continue monitoring metal concentrations in oysters and eliminate possible contamination to the samples. With increasing pollution, metal bioaccumulation could pose a potential health threat to humans in the future, as well as an environmental threat to the ecosystem and the tourist industry that depends on the bioluminescence of the bay.

In this study we did not discriminate between the different sections of the oyster, for example byssus and soft tissue, although each section has been shown to bioaccumulate metals at different rates (Szefer et al. 2006). The Puerto Rican oysters

**TABLE 2.** Metal concentrations in Puerto Rican oysters and control oysters. Zinc concentrations were above Maximum Permissible Levels, while copper concentrations for oysters from Puerto Rico were below MPL. Copper concentrations for oyster from Long Island Sound were above MPL. Mercury levels were all below the Safety Level for human consumption.

Sample	Copper (ppm) (per wet sample)	Mercury (ppm) (per dry sample)	Zinc (ppm) (per wet sample)
Oyster A1	8.24	0.02	335.19
Oyster A2	5.40	0.02	277.02
Oyster B1	6.14	0.02	146.19
Oyster B2	6.27	0.01	152.61
Oyster C1	8.77	0.01	238.51
Oyster C2	10.61	0.01	381.38
Oyster control 1	332.5	0.01	3319.7
Oyster control 2	329.5	0.01	3318.8
Maximum Permissible Level or Safety Limit	20	1.0	50

were small (roughly 5 cm in length) increasing the ratio of byssus to soft tissue. Szefer et al. (2006) found that the byssus contained significantly higher concentrations of copper, iron, and lead in comparison with the soft tissue in heavily contaminated sites. This is due to the detoxification role of the byssus (Szefer et al. 2006) as well as the metal binding sites in the collagen rich byssal threads (Coombs and Keller 1981). The study also did not take into account the effects of environmental parameters on the physiology and growth of the bivalves. Environmental parameters affect metal bioaccumulation not just through the bioavailability of the chemical elements in marine waters, but also by changing the actual size and structure of the bivalve (Phillips 1980, Rainbow 1995, Rainbow and Phillips 1993, Szefer 2002). Future environmental monitoring of Bahía Fosforescente using oysters should consider differentiating between parts of the oysters and conducting a time series analyses.

### CONCLUSION

The oysters and plankton from Bahía Fosforescente had very low concentrations of

saxitoxin that were below the human consumption safety limits. The mercury and copper levels were well below regulatory safety concentrations for human consumption. Zinc concentrations in oysters from both Puerto Rico and Long Island Sound were above MPL, indicating that these areas may be impacted by anthropogenic activities. Future toxin studies will be important as the region around the bay continues to develop and the human population continues to increase.

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## INTRACANOPY LEAF PLASTICITY IN *DACRYODES EXCELSA* IN BISLEY EXPERIMENTAL WATERSHEDS, PUERTO RICO

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**Key words:** leaf attributes, stomatal density, guard cells, upper canopy interior leaves, shade leaves, sun leaves

### ABSTRACT

Charting the phenotypic plasticity of plants provides insights into plant ecology. Because leaves on a single tree share the same genetic composition, intracanopy study highlights the potential for environmentally responsive changes independent of genetic difference. Both hydraulic gradients and environmental factors such as irradiance, wind, and leaf temperature have been proposed as controls for intracanopy leaf-trait variation. To investigate the relative roles of these two factors, we report leaf area, leaf mass per area, stomatal density, and guard cell length along height and positioning (interior vs. exterior leaves along single branches) gradients for a single tree of the woody tropical species *Dacryodes excelsa* (Burseraceae) in Bisley Experimental Watersheds, Puerto Rico. We find that leaves from greater heights are smaller and have a higher leaf mass per area, higher stomatal density, and smaller guard cell length. In contrast, we find few differences between interior and exterior leaves, with the exception of stomatal density. Because upper-crown leaves experience higher irradiance, wind speed, and temperature, our results support the idea that abiotic factors are more important than hydraulic gradients for controlling plasticity in our measured traits.

### RESUMEN

El estudio de la plasticidad fenotípica en plantas aporta importante información sobre la ecología vegetal. Debido a que las hojas de un único árbol comparten la misma carga genética, la variación morfológica dentro del dosel representa el potencial de respuesta a cambios ambientales independiente de la diferencia genética. Tanto el gradiente hidráulico como factores abióticos tales como radiación, viento, y temperatura foliar se han propuesto como determinantes de la variación intradosel de características foliares. Con el fin de

investigar el papel relativo de estos dos factores, reportamos el área foliar, la razón de la masa de hoja por área, la densidad estomatal y el largo de la célula guardián a lo largo de gradientes de altura y posición (hojas interiores vs. exteriores a lo largo de las ramas) para un árbol de la especie leñosa tropical *Dacryodes excelsa* en el Bosque Experimental de Bisley, Puerto Rico. Encontramos que las hojas de alturas más altas son más pequeñas y tiene mayor masa de hoja por área y densidad estomatal y menor largo de célula guardián. Por el contrario, encontramos pocas diferencias entre hojas interiores y exteriores, a excepción de la densidad estomática. Debido a que las hojas del follaje superior están más directamente expuestas a la radiación solar, a la velocidad del viento y a la temperatura, nuestros resultados apoyan la idea de que Los factores abióticos son más importantes que los gradientes hidráulicos en el control de la plasticidad de las características foliares examinadas.

## INTRODUCTION

Phenotypic plasticity is the ability of individual genotypes to generate varying phenotypes in response to environmental gradients (Pigliucci et al. 2006). Intracanopy leaf plasticity is important for understanding the influence of light distribution and hence overall canopy structure and activity. It also helps to identify trends in the balance of water, carbon and energy among leaves (Sack et al. 2006). Because leaves on a single tree share the same genetic composition, intracanopy study analyzes the potential for environmentally responsive changes independent of genetic difference (Sack et al. 2006).

A variety of mechanisms for intracanopy leaf plasticity have been proposed. Intracanopy discrepancies in leaf morphology have traditionally been described along a “sun-leaf : shade-leaf dichotomy,” with shade leaves being larger, thinner, and having lower leaf mass per area (LMA), and usually lower stomatal densities than sun leaves (Bongers and Popma 1990, Sack et al. 2003). In a closed forest canopy, most sun leaves occur near the top of the crown. Alternatively, it has been proposed that leaf plasticity within a tree may be affected by the hydraulic gradient up a plant altering leaf characteristics due to limits in water and nutrient access to leaves most distant to the roots (Woodruff et al. 2004). Therefore, intracanopy variation is likely to be driven by some combination of intrinsic factors such as the hydraulic gradient, which defines

ontogenetic changes in morphological expression (Zwieniecki et al. 2004), and abiotic factors such as irradiance, wind speed, and leaf temperature (Sack et al. 2006). Both sets of factors work in concert to place constraints on water and nutrient use (Miyazawa et al. 2006, Rubio De Casas et al. 2007). Importantly, hydraulic conductance varies both with height and distance along a branch at a given height, while abiotic factors vary primarily by height. The very moist environment present in our study area may reduce the importance of the hydraulic path length as a driving factor for plasticity. If determined by hydraulic gradient, leaf plasticity may result from physical constraints, while plasticity due to irradiance could point to optimization as the leaves develop. Thus, one way to deconvolve the relative roles of these factors is to measure leaf traits both along a height gradient and along single branches.

Here we investigate the relative roles of hydraulic gradients and abiotic factors by quantifying leaf area (LA), leaf mass per area, stomatal density (SD), and guard cell length (GCL) throughout the canopy of a single tree of *Dacryodes excelsa* (tabonuco) in the Luquillo Mountains of Puerto Rico.

Intracanopy leaf plasticity has been studied in several temperate tree species. A study on walnut trees found that leaves covered in a screen that reduced the red/far infrared ratio had reduced LMA, photosynthetic capacity, and leaf nitrogen per unit

area (Frak et al. 2002). Additionally, leaves with greater overall irradiance exposure had increased LMA, photosynthetic capacity, and leaf nitrogen. These findings led the researchers to conclude that local light level and quality are the major factors driving photosynthetic acclimation and intracanopy distribution of leaf characteristics, whereas local transpiration rate is of less importance (Frak et al. 2002). Wyka et al. 2007 studied the variation in leaf plasticity between highly and moderately shade tolerant evergreen species and found no correlation between plasticity and shade tolerance. The authors found, however, that plasticity did vary between evergreens (less) and deciduous trees (greater), indicating that plasticity might be significantly related to the lifespan of leaves. Another study of temperate deciduous trees found that shading leaves increases leaf area while LMA decreases (Sack et al. 2006). Leaves from the tree top and more exterior leaves are less likely to be shaded by other leaves (Sack et al. 2006). Increased LMA and decreased leaf area on these upper and exterior leaves would be consistent with leaf economic principles because smaller, denser leaves with a longer lifetime would be better equipped to take advantage of the high irradiance on upper and exterior branches, while their compact form would minimize wind damage and other hazards that accompany their elevated position. Leaves from the tree top and more exterior leaves are less likely to be shaded by other leaves (Sack et al. 2006). If *D. excelsa* leaves respond similarly to microclimate, we would expect the higher leaves and the exterior leaves to have smaller leaf area and greater LMA.

For stomata, the reduction in partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ) with altitude is thought to exert a physiological limitation on plant photosynthesis, which is compensated by increasing the plant leaves' maximum conductance to gaseous diffusion, attained by increased SD (Körner et al. 1986, Woodward 1986). Stomatal diffusion resistance is also directly related to the size of stomata, and there is a general negative correlation between guard cell length and stomatal density (Franks and Beerling 2009). In many plants this relationship is almost

exactly compensatory resulting in a nearly constant stomatal conductance (Hetherington and Woodward 2003). On the other hand, stomatal conductance (which is related to the ease with which water vapor escapes from plant leaves through stomata) within tree crown is thought to be limited by light availability and by hydraulic constraints (Sellin and Kupper 2005). As leaves modify their local environment through reduced irradiance, wind speed, and vapor pressure deficit (Lambers et al. 1998), we expect for *D. excelsa* differences in the behavior of the more exposed (exterior) leaves compared with the more "protected" (interior) leaves. Because exterior leaves are more directly exposed to irradiance and wind, and because they experience greater water stress, we hypothesize that exterior leaves should have a higher SD and lower GCL.

## MATERIALS AND METHODS

*Dacryodes excelsa* Vahl. (Burseraceae) is a prominent species in forests that cover the northern and mid-altitude slopes of Puerto Rico's mountains (Brokaw 1998). In Puerto Rico, tabonuco trees are commonly found in areas where the mean annual temperature ranges from 21-25 °C and the mean annual precipitation ranges from 2000-4000 mm. Mature *D. excelsa* can reach about 35 m and have a diameter-at-breast-height of about 180 cm (Lugo and Wadsworth 2007).

From the 28 m Bisley Research Canopy Tower at Bisley Experimental Forest, we sampled inner and outer leaves along single branches from an individual *D. excelsa* tree at five different heights at 5.5 meter intervals. Leaves were pressed and dried in oven at 50 °C for one week, then weighed and photographed digitally at 3264 x 2448 pixel resolution (Nikon E8800). Image-J (Miyazawa et al. 2006) was used to measure LA.

Epifluorescence microscopy (Leica DMBL; Leica Microsystems) and Image-pro Plus image software (version 5.1.2.59) was used to measure SD and GCL. Samples were obtained from the

basal part of one of the most basal leaflets of the pinnately compound leaves, using a hole puncher. Four discs were cut from a single leaflet at each height in the canopy for both interior and exterior locations (10 leaves total). For each disc, SD and GCL were measured from three different fields of view ( $0.1336 \text{ mm}^2$  each); all measurements are based on a minimum of ten stomata.

## RESULTS AND DISCUSSION

### Leaf Area and Leaf Mass per Area

Our measurements in a *D. excelsa* tree show that leaf area decreases (Fig. 1A) and leaf mass per area increases (Fig. 1B) with height. An ANOVA for LA shows significant differences due to height ( $P < 0.01$ ), regardless of whether the interior and exterior positions are pooled or treated separately. In contrast, there are no significant differences in LA between interior and exterior leaves ( $P = 0.82$ ). An ANOVA analysis for LMA is similar to that for LA, with significant differences across heights ( $P < 0.01$ ) but no significant differences between interior and exterior leaves ( $P = 0.55$ ).

The leaf area results are consistent with the hypothesis that leaves from greater height are smaller and have a higher LMA. The compact, more robust form is more suitable for potentially more stressful conditions at increased height, including higher wind and irradiance levels.

Our prediction of smaller leaf area and greater LMA for exterior leaves relative to interior leaves along a single branch at the same height was not confirmed. This suggests that the hydraulic path length is not a driving factor in determining these leaf morphological traits for *D. excelsa*. However, this may be due to the limited horizontal span we were able to sample from the tower.

### Stomatal Variation

Stomata in *D. excelsa* are regularly spaced in the areoles on the lower surface of the leaf. An ANOVA for SD of *D. excelsa* shows significant differences ( $P < 0.01$ ) in the variance added due to

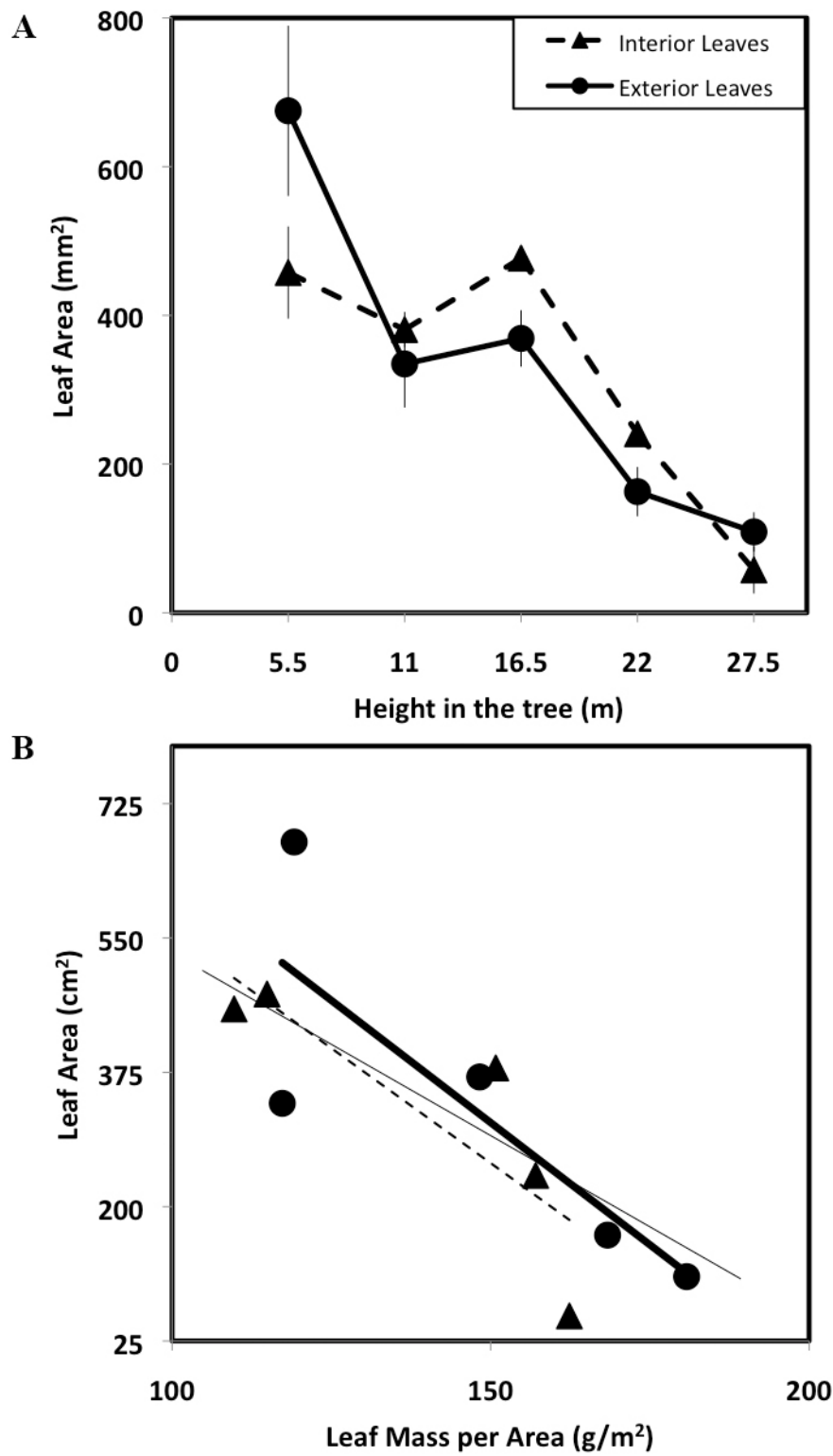
height, irrespective of whether interior and exterior leaves are pooled. We found a general tendency towards increasing SD with height in the canopy (Fig. 2A), except by height 22 m, which has higher stomatal density than height 27.5 m in both interior and exterior leaves. Planned comparisons testing differences in SD between interior and exterior leaves allowed significant differences ( $P < 0.01$ ) between the groups. As stomatal resistance affects the use of light and water, and is proportional to SD, an increase in SD optimizes the physiological control of gas diffusion and loss of water, and helps to regulate leaf temperature, which is important in the leaves that are more exposed to abiotic factors as irradiance and wind. The higher SD in exterior leaves is consistent with a higher vapor pressure deficit relative to interior leaves with a shorter path length to roots.

Guard cell length for *D. excelsa* presents significant differences ( $P < 0.01$ ) in the variance added due to height, with a general tendency towards decreasing GCL with height, but no significant differences between interior and exterior leaves ( $P = 0.59$ ) (Fig. 2B). A weak negative association was detected between SD and GCL ( $r = -0.54$ ; Fig. 2C). Significant inverse relationships are commonly observed both within and across species (Hetherington and Woodward 2003, Sack et al. 2006); it is not clear what underlies the weak trend in our data.

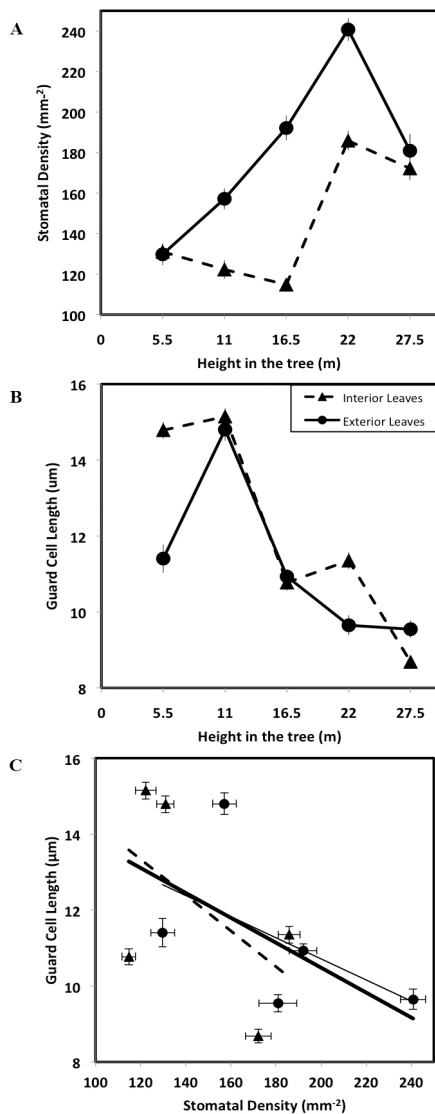
## CONCLUSIONS

The results of the intracanopy study of leaf variation and stomatal features in *D. excelsa* are consistent with the hypothesis that leaves from greater height are smaller and have a higher leaf mass per area, higher stomatal density and smaller guard cell length than lower leaves. In general, traits measured along single branches at the same height did not differ significantly. Vapor pressure deficit and abiotic factors such as irradiance, wind, and leaf temperature, both vary strongly with height within a canopy but only vapor pressure deficit varies considerably along a single branch (especially low in the canopy). As a result, we

FIGURE 1. Intracanopy variation of leaf features in *Dacryodes excelsa* at five heights in a single tree. A. Leaf area, B. Leaf mass per area. Error bars = +1 s.e.m.



**FIGURE 2.** Intracanalopy variation of stomatal features in *Dacryodes excelsa* at five heights in a single tree. A. Stomatal density; each data point represents the mean of 12 measurements. B. Guard cell length; each data point represents the mean of 40 measurements. Error bars in A and B,  $\pm 1$  s.e.m. C. Cross-plot between stomatal density and guard cell length. Least-squares regression for interior leaves (triangles, dashed line):  $y = -0.0471x + 18.99$ ,  $R^2 = 0.29$ ; exterior leaves (circles, thick line):  $y = -0.0278x + 16.275$ ,  $R^2 = 0.29$ ; interior and exterior leaves combined (thin line):  $y = -0.0328x + 17.046$ ,  $R^2 = 0.30$ .



conclude that abiotic factors are more important than hydraulic gradients for explaining our leaf trait gradients. This contrasts with some previous studies (e.g., Woodruff et al. 2004). The differences may be the result of us sampling across more modest branch lengths. Alternatively, the consistently moist environment of El Yunque leads us to believe that water limitation, even very far from the roots, may not be as important as microclimate conditions such as irradiance and wind speed.

## ACKNOWLEDGEMENTS

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## EL SAN PEDRITO: CANDIDATO IDÓNEO A AVE MUNICIPAL OFICIAL DE LOÍZA, CAPITAL DE LA TRADICIÓN

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La selección del ave municipal oficial es uno de los proyectos que es promovido por el Instituto Internacional de Dasonomía Tropical (IIDT). Dentro de esa perspectiva, las legislaturas municipales juegan un papel de destacada importancia ya que junto al alcalde crean ordenanzas que oficializan este proyecto. Uno de los parámetros muy utilizados por las legislaturas municipales lo constituyen los colores de las aves a considerar y los respectivos colores de sus banderas municipales. Ante esa consideración la Legislatura Municipal de Loíza se encamina a emular lo que ya ha acontecido para con el San Pedrito como ave municipal oficial de los municipios de Ceiba y Jayuya.

El propósito de este artículo es documentar los parámetros que fueron utilizados por la Legislatura Municipal de Loíza con el asesoramiento del IIDT para establecer el paralelismo simbólico entre los colores del San Pedrito y los respectivos de la municipalidad denominada Capital de la Tradición. Al unísono se describe que los colores gris y negro que posee esta ave guardan una relación con la negritud loiceña y de que esa consideración también fue evaluada. Una cita muy apropiada es incorporada. Su propósito hace referencia al nombre científico de esta ave.

El San Pedrito cuyo nombre científico es *Todus mexicanus* (Biaggi 1970), es el candidato idóneo a ave municipal oficial de Loíza, Capital de la Tradición. Esa distinción se escenifica de forma simbólica en el paralelismo que existe entre los colores de la bandera loiceña y los colores de un ave que su vez es autóctona de Puerto Rico.

Los colores rojo, amarillo y verde de la bandera de Loíza se ubican o localizan entre los colores que posee el ave denominada San Pedrito. Dentro de esa perspectiva, los colores amarillo y rojo provienen de los colores de la bandera de España. Dichos colores hacen alusión o referencia a la herencia española o al legado español en la jurisdicción de Loíza. En cuanto al San Pedrito, su garganta es de color roja y en Loíza es equivalente a la pasión que es innata entre los loíceños por realizar nobles hazañas para enaltecer su pueblo y por consiguiente a su país. El vientre y las cobijas internas de las alas de esta ave tan distinguida son amarillentas (Biaggi 1970) como amarillento es el sol que nace por el este del país. Por su parte la porción amarilla de las alas del San Pedrito evocan las alturas que alcanzan los pensamientos loíceños en pro del bienestar y desarrollo de su pueblo. El verde, el color más abundante de esta ave, se ubica en las partes dorsales del rabo (Biaggi 1970). En la bandera loiceña alude a la tradición de San Patricio, o sea, que posee un énfasis religioso como verde es la esperanza o la redención de la humanidad.



Foto: Iván Vicéns

Bandera del Municipio de Loíza



El San Pedrito posee otros colores que reflejan un gran simbolismo en la Capital de la Tradición: el gris y el negro. El gris, el cual se encuentra en el pecho, y el negro de forma ventral en el rabo de esta ave (Biaggi 1970) simbolizan la negritud de este pueblo tan histórico y noble.

El siguiente evento histórico relativo a esta ave y que se relaciona con su nombre científico merece ser considerado.

*“...el señor R.P. Lesson, en 1838, informaba que su hermano el Dr. Adolph Lesson, médico de la Marina, coleccionó especímenes de un San Pedrito en Vera Cruz y Tampico en Mexico, y en Puerto Rico. A la especie de Mexico se le dio el nombre de **Todus mexicanus** y al de Puerto Rico el nombre de **Todus portoricensis**. Más tarde se comprobó o se supuso que especímenes informados de Vera Cruz y Tampico aparentemente debieron haber sido coleccionados también en Puerto*

*Rico, ya que esa ave no se conoce en Mexico. Por prioridad en el nombre quedó la especie de **mexicanus** y desapareció la especie **portoricensis**. Es ésta la razón por la cual esta avecilla que no se encuentra en Mexico y que es autóctona de Puerto Rico lleva el apelativo específico de **mexicanus**”* (Biaggi 1970).

El reconocimiento en Loíza del San Pedrito como el ave municipal oficial basado en la relación de colores de esta ave y los colores de su bandera hacen causa común con lo acontecido heráldicamente en los pueblos de Ceiba y Jayuya. Dentro de este contexto, ello ilustra que en la eventualidad el San Pedrito será una de las aves a considerar como ave oficial de Puerto Rico. Hasta el momento esta ave autóctona ha escrito páginas heráldicas en el municipio costero de Ceiba así como en el ubicado entre las montañas, Jayuya. Por otro lado, lo referente a **mexicanus** no ha impedido, luego de la aclaración antes descrita, que esta ave autóctona sea reconocida como el ave oficial municipal en Ceiba y Jayuya.

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## LA ALMENDRA (*TERMINALIA CATAPPA*) Y LOS ÁRBOLES DISTINGUIDOS DEL PUEBLO DE LOÍZA

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La especie arbórea conocida como almendra y cuyo nombre científico es *Terminalia catappa* posee un conjunto de características que han motivado a la Legislatura Municipal de Loíza a reconocerle como el árbol oficial de la Capital de la Tradición. Entre las razones o motivos que han proliferado en esa dirección se ubican los siguientes: su abundancia en la jurisdicción municipal, la utilización de su fruto en la elaboración de un dulce típico loiceño, el cambio de la tonalidad de colores en el proceso de maduración de la hoja en la cual se manifiestan los colores de la bandera, la incorporación del fruto como un recurso heráldico presente en el escudo municipal y el empleo de las hojas verdes como un molde natural en la elaboración de ciertas frituras en especial las alcapurrias.



Árbol de almendra (*Terminalia catappa*)

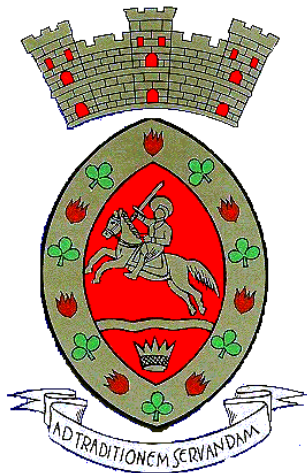
La almendra es un árbol muy conocido en Puerto Rico. Su principal utilización es como árbol de sombra, ornato y por su semilla solitaria la cual es comestible (Little, Wadsworth, y Marrero 2001). Es nativo de las Indias Occidentales y Oceanía pero se ha cultivado y naturalizado en las regiones tropicales. En Loíza, es un árbol muy común y se localiza desde las zonas costeras hasta los lugares de mayor altura en la jurisdicción municipal. Dentro de ese marco escénico se le reconoce en muchos lugares a lo largo de las carreteras loiceñas.

Una de las características más distintivas de esta especie arbórea lo constituye la disposición de sus ramas. Éstas brotan del tronco de forma horizontal a diversos niveles. Ese arreglo de las ramas de la almendra simboliza las diferentes experiencias de la vida del hombre y la mujer loiceña. Son peldaños de la vida. Es Loíza en la constante búsqueda de mejores niveles de vida.

Los frutos de la almendra, los cuales son una especie de nuez, constituyen la materia prima para la elaboración de uno de los dulces típicos de Loíza. Es en las manos loiceñas, Capital de la Tradición, donde la elaboración del dulce de almendras cobra un mayor significado. En ese proceso se conjuga la tradición oral y las manos artesanales para producir un manjar que emerge con una fuerza cultural de antaño. Por otro lado, la forma almendrada del fruto de esta especie arbórea cobra un significado heráldico único para Loíza.

La forma almendrada del fruto de esta especie arbórea está representado en el escudo municipal de Loíza (Municipio de Loíza 1998). De ahí su

exclusividad heráldica entre todos los escudos municipales oficiales de Puerto Rico. Al unísono representa las raíces de una negritud ancestral. Dentro de ese escenario heráldico también se ubican de forma significativa y destacada los colores de las hojas de esta especie arbórea y los respectivos de la bandera de Loíza.



Escudo y bandera municipal oficial de Loíza,  
Capital de la Tradición

La bandera oficial de Loíza consta de tres colores: verde, oro y rojo. Son estos los colores que va adquiriendo la hoja de almendra en el proceso de maduración de la misma, o sea, a lo largo de su existencia. Son hojas verdes como la esperanza del pueblo loiceño en busca de un mejor bienestar. Luego, en el proceso de maduración éstas se van tornando amarillentas, como si fueran entrando en la edad de oro. Ya próximas a caer se tornan rojizas para de esa forma continuar el reciclaje constante

de la naturaleza. Por otro lado, las hojas verdes de la almendra guardan una relación directa con la gastronomía loiceña.

Las hojas verdes de la almendra son moldes naturales que utilizan las manos loiceñas en la confección de diversas frituras, o sea, deleitosos manjares que dan testimonio de una gastronomía que posee unas raíces ancestrales pero que late y palpita a cada momento, a cada instante. Adjunto a lo antes expuesto, la corteza, el fruto y las hojas de la almendra producen un tinte negro que puede emplearse como tinta de escribir (Little, Wadsworth, y Marrero 2001). Esa tinta negra simboliza la expresión de las artes, como lo es la literatura, en sus múltiples manifestaciones loiceñas.

El reconocimiento de parte de la Legislatura Municipal de Loíza con la almendra para ser considerado como el árbol municipal oficial requiere de la redacción de una ordenanza. Dicha ordenanza puede ser modificada, rechazada o aprobada por el Hon. Eddie Manso Fuentes, Alcalde de Loíza. De recibir su aprobación, Loíza se une a la ciudad de Humacao como el segundo municipio en reconocer a la almendra como su árbol municipal oficial (Legislatura Municipal de Humacao 2003).

En el proceso histórico de Loíza, esta municipalidad posee una serie de árboles distinguidos los cuales ocupan un sitio de destacada importancia. Ante esa consideración la Legislatura Municipal de Loíza reconoce a tal distinción a los siguientes: la palma de cocos (*Cocos nucifera*), la maría (*Calophyllum calaba*), la uva de playa (*Coccoloba uvifera*), el mangle negro (*Avicennia germinans*), la ceiba (*Ceiba pentandra*) y el corcho (*Guapira fragans*).

Los cognomentos: Los cocoteros y Los Comecocos (Sánchez Martínez 1993) en alusión a la palma de cocos posee una dimensión que se ramifica hacia diversas facetas de la vida, la cotidianidad o la idiosincrasia loiceña. Entre esas manifestaciones se ubican los deportes a través del béisbol, la gastronómica mediante el plato típico

del arroz con coco y la artesanal con la confección de las máscaras de coco. En adición, en el paisaje costero de Loíza, la palma de cocos demuestra todo su esplendor. Ejemplo de ello lo constituye Piñones y su conexión con Vacía Talegas. Las talegas, los sacos con destino comercial entre cuyo contenido figuraban los cocos, permanece aún en la tradición oral y en la toponimia loiceña.

El árbol de maría adquiere en Loíza una destacada importancia para con el legado indígena y los estudios arqueológicos. Dentro de ese escenario la Cueva de María la Cruz representa, y es un testimonio arqueológico, de la primera evidencia de la presencia humana en Loíza y en Puerto Rico mismo (Alegría 1988).

La uva de playa, la especie arbórea considerada la más próxima al mar (Little, Wadworth, y Marrero 2001) es en Loíza un centinela que ha reducido su presencia ante el avance del desarrollo de unos intereses particulares. No obstante, ello no ha impedido, entre otras cosas, que sus frutos y sus hojas sean utilizados ampliamente. Dentro de esa perspectiva, los frutos los cuales se reconocen por su forma de collares aluden a la belleza costera. De ellos se nutren una gran cantidad de aves. Por otro lado, los frutos de la uva de playa son utilizados por las manos artesanales como la materia prima en la confección de otros productos. Al unísono, sus hojas así como las del almendro, son empleadas como moldes naturales en la confección de ciertas frituras.

El mangle negro, el cual también se conoce con los nombres de mangle prieto o mangle salado, es uno de los cuatro tipos de manglar en Puerto Rico (Archivo General de Puerto Rico 1885-1889). Históricamente ha sido utilizado como fuente de combustible. Su ubicación costera, por lo regular, luego del mangle colorado posee una relación muy estrecha con el sistema de mangles más grande de nuestro país, el Bosque Estatal de Piñones (Rivera Arbolay 1999). De hecho, Piñones, o lugar de los Piñón, hace referencia toponímica a un negro liberto, Francisco Piñón y su familia, los cuales se establecieron a inicios de la colonización española

en las inmediaciones de San Juan (López Cantos 1986). Durante siglos la leña y el carbón que producía esta especie arbórea era empleado en las chimeneas de las haciendas cañeras de la zona de Loíza y áreas aledañas así como en los hornos militares y en fogones de la zona de San Juan (Archivo General de Puerto Rico 1885-1889). Por otro lado, su negritud es un símbolo de la negritud loiceña, de una herencia africana que cada día aflora más en nuestra puertorriqueñidad.

El árbol denominado corcho guarda en Loíza una estrecha relación con las festividades de Santiago Apóstol (Gobierno Municipal de Loíza 1996). Según la tradición y la leyenda la aparición de Santiago en Loíza es una de carácter milagroso. Ante ese panorama diversas teorías versan sobre la primera aparición de una talla de madera, en representación de la imagen de Santiago. Una de ellas señala que dicha imagen apareció de forma milagrosa entre las raíces de un árbol de corcho en Medianía. La imagen fue llevada a la iglesia del pueblo en tres ocasiones y desaparecía para volver a aparecer en las raíces del árbol de corcho. Desde entonces se considera a Santiago como el patrón en Medianía.

Ceiba es voz indígena mediante el cual se reconoce y denomina a uno de los árboles más majestuosos de América (Hernández Aquino 1977). Dentro de ese contexto hace referencia al legado indígena de Loíza. La majestuosidad y belleza de este árbol es símbolo de la grandeza de espíritu y de lucha de los loiceños. Por otro lado, el nombre de este árbol figura en la toponimia de Loíza en el barrio de Medianía Alta y en la xilografía que Rafael Tufiño creó en 1963 y que tituló: La ceiba de Loíza (Hermandad de Artistas Gráficos de Puerto Rico 1998). Es ésta una expresión artística en donde la grandeza, majestuosidad y belleza quedan ampliamente manifestada.

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## **APUNTES EN TORNO A LA PERSPECTIVA HISTÓRICA EN EL DESARROLLO DE UN PLAN DE MANEJO FORESTAL SUSTENTABLE**

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El desarrollo de un plan de manejo forestal sustentable es uno de los tópicos o temas de discusión que en la actualidad ha ido escalando entre otros escenarios: en la opinión pública, en seminarios o reuniones ambientales e inclusive dentro de las esferas político gubernamentales. Por otro lado, el incremento, ascenso o advenimiento de este tema, en esos y otros escenarios, guarda una relación directa con las repercusiones o influencias que emergen en varias direcciones hacia un individuo, una familia, una comunidad, un barrio, un pueblo, un municipio, una región o zona ecológica, en fin, con todo un país. No obstante, las perspectivas de naturaleza histórica para con el desarrollo de un plan de manejo forestal sustentable requieren que sean considerados como parte integrante de los parámetros a ser evaluados.

El propósito de este artículo es el de reconocer la perspectiva histórica en el desarrollo de un plan de manejo forestal sustentable a través de los siguientes parámetros: tenencia y usos de la tierra, la influencia de la agricultura en la economía puertorriqueña sobre todo a partir de fines del siglo XVIII, la toponimia y los yacimientos arqueológicos. Por otro lado, se destacan los principales pilares de la investigación para el estudio de esa perspectiva en cualquier lugar del país: el Archivo General de Puerto Rico (AGPR), el Registro de la Propiedad del Departamento de Justicia (RPDJ), la sección de Fotogrametría del Departamento de Transportación y Obras Públicas (DTOP) y la biblioteca del Instituto Internacional de Dasonomía Tropical (IIDT).

La elaboración de cualquier proyecto a desarrollarse requiere de una planificación en la

cual sean descritos, evaluados y analizados en su justa perspectiva cada uno de los parámetros a ser considerados. Ante ese cuadro escénico emerge, por ejemplo, un área boscosa la cual anhelamos laborar dentro de un plan de manejo forestal sustentable. Por consiguiente, la perspectiva histórica adquiere una vitalidad que de una manera u otra va a ir integrándose con la diversidad de temas que van aflorando dentro del plan de manejo forestal sustentable.

En Puerto Rico el estudio de la tenencia y usos de la propiedad posee, entre otros, los siguientes recursos: el RPDJ, las fotos aéreas de la sección de Fotogrametría del DTOP, los informes estadísticos del uso de la tierra de los municipios de Puerto Rico de 1950 de la División de Economía Agrícola del Departamento de Agricultura y Comercio (DEADAC), y las fuentes documentales que están bajo la custodia del AGPR. Entre esas fuentes documentales se ubican las planillas del Negociado de Tasación de la Propiedad del Departamento de Hacienda de la primera mitad del siglo XX, los catastros municipales de las fincas rústicas y urbanas de fines del segundo lustro de la década de 1890 y los protocolos notariales.

El establecimiento en 1885 del Registro de la Propiedad en Puerto Rico constituye un recurso vital para la reconstrucción de la historia de la tenencia y usos de la tierra de cualquier lugar en Puerto Rico. Para lograr ese objetivo el primer paso lo constituye la determinación actual sobre la titularidad del área bajo consideración. La primera fuente de información en esa dirección está representada por un documento legal que se



denomina como escritura de la propiedad. Dicho documento describe, entre otras cosas, la tenencia, procedencia, los linderos o colindancias y los usos actuales de la tierra. Por otro lado, la escritura de la propiedad requiere de un plano de agrimensura lo cual le convierte en un documento adicional de gran importancia para el desarrollo de un plan de manejo forestal sustentable.

Cualquier área bajo consideración y que es descrita en la escritura de la propiedad posee un número de identificación municipal. Ese número nos indica la ruta de procedencia titular a seguir en el Registro de la Propiedad que corresponda. Ante la disponibilidad de ese dato es viable la reconstrucción del uso y tenencia de la tierra en Puerto Rico a partir del 1885. No obstante, el número de identificación municipal de una finca puede cambiar de una fecha a otra dependiendo de las variaciones en la tenencia de la tierra tales como las segregaciones, o de cualquier otro mecanismo en acorde con las leyes de la propiedad del país.

El Catastro de fincas rústicas y urbanas de los pueblos del segundo lustro de la década del 1890 y que posee el AGPR es un recurso histórico que nos ubica dentro del marco escénico de la tenencia y usos de la tierra de esa época. Sin embargo, no existe uniformidad en los datos recopilados ya que en algunos pueblos no se incluye el desglose por cuerdas del uso de la tierra. Solo se describe la cabida total de la propiedad con mención de los usos de la misma. Por otro lado, este catastro, el cual está organizado por pueblos en acorde al índice alfabético de los apellidos de los dueños de la propiedad no posee necesariamente una correlación de datos con los que para esa época poseía el Registro de la Propiedad.

El Fondo de Obras Públicas del AGPR es la fuente histórica obligada para el estudio de la tenencia y usos de los terrenos baldíos de la Corona española que fueron distribuidos con anterioridad a la creación del Registro de la Propiedad. De 1818 a 1875 la Junta Superior para el Repartimiento de los Terrenos Baldíos (JSPRTB) funcionó como

una especie de agencia gubernamental responsable de la distribución y uso de la tierra de la Corona española. La finalidad de esa distribución consistía en hacerlos productivos en términos agrícolas y al unísono el de engrosar los fondos de las arcas gubernamentales. Los informes de esa especie de agencia gubernamental poseen, entre otras cosas, datos referentes a las especies arbóreas de los terrenos que estaban en proceso de distribución.

La adquisición de los terrenos baldíos por manos privadas estaba reglamentada por una legislación que requería de una serie de responsabilidades so pena de perder éstos. Entre esas responsabilidades se ubicaba la obligación del pago de las contribuciones y el desmonte a efectuarse en un período de tiempo previamente ya reglamentado.

Los expedientes de la Inspección de Montes de Puerto Rico (IMPR), como agencia sucesora de la JSPRTB poseen una riqueza documental que abarca el último cuarto del siglo XIX. Es para esa época y mediante la IMPR que se desarrolla una política forestal en el país. Dentro de esa perspectiva, advienen entre otros eventos, la creación de un sistema de reservas forestales y la discusión sobre el mejor uso de la tierra. Ante esa circunstancia, afloran las inquietudes sobre cuales tierras debían de conservarse como bosques. Por su parte, las inquietudes forestales envolvían, entre otros temas, la influencia de la floresta en las condiciones climatológicas del país así como en la calidad y cantidad de las aguas.

Las fotos aéreas de Puerto Rico, las cuales datan en series desde el 1939, y que se localizan en la sección de Fotogrametría del DTOP representan una importante fuente documental que es necesario consultar. Ante el examen minucioso de este recurso se obtiene una radiografía de los diversos cambios que han ocurrido en un área a partir de 1939. Entre esos cambios se ubica la cobertura forestal. No obstante, las fotos aéreas de la medianía del siglo XX poseen el auxilio interpretativo de los informes estadísticos y los mapas cuadrangulares del uso de la tierra en esa época.

Los informes estadísticos del uso de la tierra en los municipios de Puerto Rico en 1950 de la DEADAC constituyen una fuente de destacada importancia en la tenencia y usos de la tierra. Los mapas de las subdivisiones de los cuadrángulos que estos informes poseen requieren de un examen detallado pues en ellos se desglosa mediante un índice gráfico el uso a la cual eran destinadas las tierras en el país en la medianía del siglo pasado. En ellos se describe, entre otros usos, las zonas de bosques, cañaverales, zonas tabacaleras o cafetaleras, construcciones o edificaciones diversas tales como escuelas, casas particulares, cementerios, iglesias, carreteras. Entre los lugares donde existen copias de tales documentos se ubica la biblioteca del IIDT.

La historia de la deforestación en Puerto Rico guarda una relación directa con los procesos, transformaciones o reorientaciones de la economía del país. Dentro de esa perspectiva la deforestación que acontece desde fines del siglo XVIII y la centuria siguiente emerge como consecuencia de una reorientación de una economía de subsistencia a una economía agrícola y de exportación basada principalmente en el cultivo de la caña de azúcar, el café y el tabaco.

La reorientación de la economía de una de naturaleza agrícola basada en la exportación del café, la caña de azúcar y el tabaco, y que acontece durante el siglo pasado, a una encaminada hacia la industrialización motivó, entre otras cosas, el abandono de las tierras agrícolas y por consiguiente, el advenimiento de zonas de bosques los cuales en su mayoría hoy día poseen una edad promedio de 60 a 80 años. Por otro lado, en términos generales, dichos cultivos se concentraban en zonas particulares de la geografía del país: la caña de azúcar en los llanos costeros y los valles del interior, el café en la zona montañosa del centro rumbo al oeste, y el tabaco en las montañas del centro en dirección al este del país así como de otras áreas comprendidas en los municipios de Arecibo, Aguadilla, Isabela, Utuado, Ciales y Morovis. Adjunto a esa particularidad de ubicación geográfica esos productos agrícolas

requerían de una deforestación que antecedió al establecimiento y desarrollo de esas zonas agrícolas.

El cultivo de la caña de azúcar, el café y el tabaco requerían de una deforestación muy particular. En las plantaciones de la caña de azúcar la deforestación era total; en la zona cafetalera se requería de sombra parcial en especial de aquellas especies arbóreas fijadoras de nitrógeno mientras que en la zona tabacalera la especie arbórea que permanecía en el escenario era la palma real. Esta especie arbórea adquirió ante la población tabacalera un lugar privilegiado debido a que ésta proporcionaba, entre otras cosas, los siguientes beneficios: pencas para los ranchos de tabaco, alimento para la ceba del ganado porcino y tablas procedentes del tronco muy útiles en la construcción de los bohíos del campesinado. Por otro lado, las pencas de la palma real poseen una afinidad religiosa ya que se utilizaban en las procesiones del Domingo de Ramos de la Iglesia Católica. Eventualmente la palma real fue distinguiéndose, entre otras cosas, como una característica del paisaje tabacalero y como símbolo del Departamento de Agricultura y Comercio.

El reconocimiento del proceso histórico que han escenificado la agricultura y la economía del país a través del estudio del paisaje ofrecen otros puntos de vista, evaluación y análisis que es necesario considerar. Al examinar el paisaje que ofrece el país en la actualidad y dependiendo de su ubicación geográfica podemos descifrar cierta información que la misma naturaleza nos provee y que es necesario conocer en el desarrollo de un plan de manejo forestal sustentable. Como parte integrante de ese análisis es necesario evaluar la toponimia del lugar bajo estudio.

La toponimia, o sea el estudio sobre el origen y evolución del nombre o de los nombres propios o comunes de un lugar, constituye un recurso de interés para el desarrollo de un plan de manejo forestal sustentable. Dentro de esa perspectiva advienen un conjunto de voces que guardan una relación directa o indirecta con el área o zona a

considerarse. Entre las variantes toponímicas se ubican las inherentes a la flora, la fauna, la antroponimia y la hidronimia. Por otro lado, emergen las respectivas voces indígenas, españolas y africanas que es necesario conocer. En ocasiones éstas se hacen presentes entre los expedientes de la JSPRTB, la IMPR e inclusive en la tradición oral del lugar. Algunas de esas voces poseen el potencial para ubicarnos o para hacer una aproximación sobre ciertos yacimientos arqueológicos.

La presencia de yacimientos arqueológicos, en especial, los correspondientes a la época pre-hispánica del país poseen la facultad de modificar los restantes parámetros que ya se habían planificado para el desarrollo de un plan de manejo

forestal sustentable. Dada esa circunstancia las recomendaciones o evaluaciones del Instituto de Cultura Puertorriqueña podrían contribuir o limitar las expectativas anticipadas.

Todos los puntos de vista que se han señalado o expuesto con anterioridad son ilustrativos de que es necesario o requerido la presencia de un historiador forestal en el desarrollo de cualquier plan de manejo forestal sustentable. Dentro de esa perspectiva, adviene una evaluación multidisciplinaria que oscilará hacia una mayor o menor consideración de los parámetros bajo estudio de un lugar. De ahí la necesidad de un modelo para el desarrollo de un plan de manejo forestal sustentable cuya finalidad sea el ajuste para cada lugar en particular.

## FORGOTTEN FORESTRY INFORMATION

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Now that the island is more than half covered with forests and we are having an unprecedented number of students searching for the ecology of nature in Puerto Rico there is an unexpected fundamental source of information about the trees and forests that appeared between 1940 and 1964. It was also a period in which the objectives were different. Reservation of abandoned farmland called for reforestation. It called for trees propagated, adapted, fast growing, and productive of useful woods.

Tree propagation in nurseries rose to 9 million trees per year, and in a 9-year period more than 29 million trees were planted. The number of tree species used was 500, 190 natives and 310 introduced species. An array of sites throughout the island was included, making the findings of interest almost everywhere. Much was learned and documented about both failures (roughly 1/3) and successes. Within the remaining natural forests the comparative growth rates of many species of trees under different conditions led to a practice for greatly accelerated tree growth.

Specific findings included:

- Seed culture and propagation of valuable tree species
- Solutions to pest problems in tree nurseries.
- Limitations of adaptation and rapid growth of native species
- Potentials of tree species rapid growing elsewhere
- Potentials of the world's most prized tropical timber species
- The most promising tree species for each of the public forests

- Land preparation for forest replanting
- Planting practice
- Weed control in young plantations
- Thinning of plantations and growth effects
- Forest density reduction and productivity effects
- Puerto Rico's woods, their properties and values
- Seasoning and preservation of local woods
- 

Results are published in the Caribbean Forester, copies indexed on a disk and copies available at the International Institute of Tropical Forestry library.

Study reports on forest areas:

- Aguirre 9                      Mona 3
- Cambalache 17                Río Abajo 2
- Carite 16                      Río Piedras 4
- El Yunque 48                St. Just 7
- Guajataca 19                Susúa 7
- Guánica 14                  Toro Negro 28
- Guilarte 9                     Virgin Islands 18
- Maricao 16
- 

Native tree species with 5 references or more in the journal

<i>Alchornea latifolia</i>	7
<i>Alchorneopsis portoricensis</i>	9
<i>Anacardium occidentale</i>	9
<i>Andira inermis</i>	28
<i>Avicennia germinans</i>	9
<i>Buchenavia tetraphylla</i>	23
<i>Bucida buceras</i>	23
<i>Bursera simaruba</i>	17
<i>Byrsonima cuneata</i>	6
<i>Byrsonima spicata</i>	20
<i>Calophyllum calaba</i>	43

<i>Cecropia schreberiana</i>	29	<i>Pouteria multiflora</i>	25
<i>Cedrela odorata</i>	44	<i>Prestoea montana</i>	11
<i>Ceiba pentandra</i>	5	<i>Prunus occidentalis</i>	7
<i>Citharexylum fruticosum</i>	18	<i>Rhizophora mangle</i>	11
<i>Coccoloba diversifolia</i>	6	<i>Sabal causiarum</i>	6
<i>Coccoloba grandiflora</i>	6	<i>Schefflera morototoni</i>	23
<i>Coccoloba uvifera</i>	6	<i>Sideroxylon foetidissimum</i>	11
<i>Colubrina arborescens</i>	13	<i>Sloanea berteriana</i>	23
<i>Cordia alliodora</i>	55	<i>Spondias mombin</i>	9
<i>Cordia nitida</i>	6	<i>Stahlia monosperma</i>	9
<i>Cordia sulcata</i>	6	<i>Tabebuia heterophylla</i>	53
<i>Cupania americana</i>	10	<i>Tabebuia rigida</i>	11
<i>Cyrilla antillana</i>	16	<i>Tetragastris balsamifera</i>	26
<i>Dacryodes excelsa</i>	45	<i>Tetrazygia eleagnoides</i>	5
<i>Dipholis salicifolia</i>	5	<i>Thespesia grandiflora</i>	28
<i>Eugenia aeruginea</i>	5	<i>Vitex divaricate</i>	23
<i>Eugenia stahlia</i>	10	<i>Zanthoxylum flavum</i>	18
<i>Ficus laevigata</i>	6	<i>Zanthoxylum martinicense</i>	6
<i>Genipa americana</i>	9		
<i>Guaiacum officinale</i>	26	Introduced tree species with 10 references or more	
<i>Guarea guidonia</i>	48	<i>Albizzia lebeck</i>	13
<i>Guazuma ulmifolia</i>	9	<i>Casuarina equisetifolia</i>	49
<i>Henriettia squamulosa</i>	13	<i>Cupressus lusitánica</i>	16
<i>Hernandia sonora</i>	13	<i>Eucalyptus alba</i>	10
<i>Hieronima clusioides</i>	17	<i>Eucalyptus resinifera</i>	15
<i>Homalium racemosum</i>	12	<i>Eucalyptus robusta</i>	31
<i>Hufelandia pendula</i>	7	<i>Eugenia jambos</i>	27
<i>Hura crepitans</i>	5	<i>Hibiscus elatus</i>	17
<i>Hymenaea courbaril</i>	29	<i>Laguncularia racemosa</i>	22
<i>Inga laurina</i>	20	<i>Pinus caribaea</i>	28
<i>Inga vera</i>	19	<i>Prosopis pallida</i>	13
<i>Linociera domingensis</i>	11	<i>Senna siamea</i>	20
<i>Lonchocarpus latifolius</i>	5	<i>Swietenia macrophylla</i>	63
<i>Magnolia portoricensis</i>	10	<i>Swietenia mahagoni</i>	83
<i>Magnolia splendens</i>	15	<i>Tectona grandis</i>	51
<i>Manilkara bidentata</i>	38		
<i>Mayapea domingensis</i>	19		
<i>Micropholis chrysophylloides</i>	26		
<i>Micropholis garcinifolia</i>	13		
<i>Ocotea leucoxylon</i>	10		
<i>Ocotea moschata</i>	21		
<i>Ocotea spathulata</i>	15		
<i>Ochroma lagopus</i>	13		
<i>Ormosia krugii</i>	12		
<i>Petitia domingensis</i>	37		
<i>Phyllanthus nobilis</i>	6		

# Acta Científica

ASOCIACIÓN DE MAESTROS DE CIENCIA DE PUERTO RICO

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**ACTA CIENTÍFICA** es la revista multidisciplinaria de la Asociación de Maestros de Ciencia de Puerto Rico. ACTA considera para su publicación, trabajos originales en cualquier área de la ciencia, a saber, física, química, bioquímica, zoología, botánica, ecología, biomédica, medicina, ciencias terrestres, ciencias atmosféricas, psicología del comportamiento, tecnología farmacéutica o matemáticas. Un artículo describe un estudio completo y definitivo. Una nota es un proyecto completo, pero más corto, que se refiere a hallazgos originales o importantes modificaciones de técnicas ya descritas. Un ensayo trata aspectos relacionados con la ciencia, pero no está basado en resultados experimentales originales. Una revisión es un artículo que comenta la literatura más reciente sobre un tema especializado.

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