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**Differences in urbanization and degree of marine influence are reflected in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of producers and consumers in seagrass habitats of Puerto Rico**

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ABSTRACT

Couplings between land use and marine food webs in tropical systems are poorly understood. We compared land-sea coupling in seven sites around Puerto Rico, differing in the degree of precipitation and urbanization, by measuring  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in producers and consumers.  $\delta^{15}\text{N}$  values were influenced by human activity: the food web from sites near urbanized centers was on average 1‰ heavier in  $\delta^{15}\text{N}$  compared to undeveloped sites. This is most likely due to wastewater inputs from septic systems relatively near the shoreline. Changes in  $\delta^{13}\text{C}$  were best explained by differences in the degree of marine influence. Where terrestrial inputs from a major river dominated,  $\delta^{13}\text{C}$  values were lighter, whereas sites further from land and in locations exposed to oceanic currents had

heavier  $\delta^{13}\text{C}$  values, characteristic of a marine source of dissolved organic carbon. We found no significant effect of precipitation on connectivity in spite of a two-fold difference in annual average rainfall between the north and south coast. The results suggest there is some connectivity between land and sea in Puerto Rico, despite high rates of evaporation relative to precipitation.

Keywords: *Thalassia testudinum*; stable isotopes; wastewater; trophic level; land sea coupling

## 1. Introduction

Coupling between watershed land use and coastal waters determines the degree to which land-derived nutrients enter coasts and estuaries (Bowen et al 2007; Bowen and Valiela 2008). In coupled systems, groundwater flow or stream runoff transports nutrients derived on land to coasts and estuaries. With increased human development in the coastal zone, including agriculture and urbanization, nutrient loads to estuaries may increase and lead to eutrophication (Nixon et al. 1995; Valiela et al. 1992, 1997). In systems where precipitation is low or potential evapotranspiration is high, rates of water transport through the watershed may be low enough to limit the export of solutes to receiving waters thereby uncoupling estuaries from the watershed (Caraco and Cole 2001; Bowen and Valiela 2008). The couplings between land use and estuarine food webs have been well described in temperate latitudes (Valiela et al. 1992; McClelland et al. 1997; Valiela et al. 1997; McClelland and Valiela 1998; Deegan et al. 2002; Carmichael and Valiela

2005), but tropical estuaries have received less attention (but see Bowen and Valiela 2008), even though urbanization and alteration of natural land cover are proceeding rapidly in the tropics (Galloway et al. 1994).

Stable nitrogen isotopes can be used to assess the extent of coupling between watersheds and estuaries. Nutrients transported from the watershed to the estuary may carry isotopic signatures that are characteristic of land uses in the watershed (Cole et al. 2004; McClelland and Valiela 1998). Nitrate from atmospheric sources typically have  $\delta^{15}\text{N}$  signatures between 0.5 and 8 ‰ (Kreitler and Jones 1975; Kellman and Hilliare-Marcel 2003), nitrate from synthetic fertilizers ranges from -3 to 3 ‰ (Macko and Ostrom 1994; Kellman and Hilliare-Marcel 2003), and nitrate from human and animal wastes typically have a  $\delta^{15}\text{N}$  signature that is enriched relative to other sources ranging from 9 to 25 ‰ (Kreitler et al. 1978; Kreitler and Browning 1983; Rolston et al. 1996). When there is coupling between the watershed and the receiving estuary, the isotope signature of land-derived nitrogen can be reflected in vegetation in coastal fringes such as salt marshes (Cole et al. 2004; Wigand et al. 2007; Bannon and Roman 2008) and mangroves (Fry et al. 2000), aquatic macrophytes (McClelland and Valiela 1998; Castro et al. 2007; Bannon and Roman 2008), and benthic and pelagic consumers (McClelland and Valiela 1998; Martinetto et al. 2006). Several studies have for example shown an overall shift in  $\delta^{15}\text{N}$  signatures of consumers and producers towards heavier values as a result of increasing urbanization of watersheds leading to increases in the N loads and relative contribution by wastewater, in both temperate (McClelland et al. 1997; McClelland and Valiela 1998; Martinetto et al. 2006) and tropical (Yamamuro et al. 2003; Abreu et al. 2006; Tefwik et al. 2007) estuaries. Stable isotopic analyses of fauna

and macrophytes can be therefore be used as a tool to infer coupling between the watershed and estuary.

In addition to tracing nutrient sources, stable isotope techniques can be used to resolve food web relationships.  $\delta^{15}\text{N}$  signatures can be used to determine the trophic position of consumers (Peterson and Fry 1987) due to predictable  $^{15}\text{N}$  enrichment with each trophic level (Wada et al 1991; Michener and Schell 1994; Post 2002; Vanderklift and Ponsard 2003), whereas  $\delta^{13}\text{C}$  can be used to determine consumer diets (Fry and Sherr 1984) due to consumers having  $\delta^{13}\text{C}$  signatures similar to those of their food with an enrichment of around 1‰ or less (Fry and Sherr 1984; DeNiro and Epstein 1978; Post 2002). Producer  $\delta^{13}\text{C}$  depends largely on the degree of fractionation during carbon assimilation (Smith and Epstein 1971; Lajtha and Marshall 1994) and the signatures of the available carbon sources (Raven et al. 2002; Beer et al. 2002) resulting in different ranges of  $\delta^{13}\text{C}$  values for different producer types (Hemminga and Mateo 1996; Fry and Sherr 1984). The  $\delta^{13}\text{C}$  of primary producers may also be strongly influenced by the  $\delta^{13}\text{C}$  of dissolved inorganic carbon (DIC), which varies in different environments. Remineralized DIC tends to be lighter in  $\delta^{13}\text{C}$  and may be a significant source of DIC in the coastal zone, creating a gradient in DIC  $\delta^{13}\text{C}$  from relatively light in estuarine waters (-29 to +2‰), to heavier in marine waters (0 to +3‰) (Coffin et al. 1994). These contrasting end members create gradients in  $\delta^{13}\text{C}$  of producers (Zieman et al. 1984) and consumers (Marguillier et al. 1997; Hemminga et al. 1994; Lin et al. 1991) from land to offshore.

Stable isotope values of coastal consumers and producers may be altered by a variety of sources and influences, including land-based human, marine, and climatic

factors. In this study we examined how the stable isotope ratios in species of coastal communities varied in relation to differences in nearby human influences and different precipitation regimes in a tropical setting. We selected seven sites on the coast of Puerto Rico that differed in their degree of urbanization and average annual precipitation, and investigated whether these differences were reflected in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of producers and consumers.

## 2. Materials and methods

### 2.1. Sample sites

Samples of producers and consumers were collected from seven sites around Puerto Rico in May of 2007 (Fig. 1). The sites differed in the 2 factors that we were examining (precipitation regime and urbanization of land). The precipitation regime differed dramatically among the sites sampled in this study (Table 1). Puerto Rico lies in the path of northeasterly trade winds (Fig. 1) that bring large amounts of moisture from the Atlantic Ocean. The center of the island is dominated by the east-west trending Cordillera Central mountain range and most of the precipitation falls on the upwind sides of the mountains creating large differences in annual rainfall over relatively short distances. The wettest part of the island is the northeast surrounding El Yunque National Forest and has a mean annual rainfall of 1900 to >4000 mm per year (calculated from precipitation records 1971-2000; NOAA National Weather Service 2000). The south coast of the island is warmer and drier, owing to the rain shadow effect of the central mountain range, and receives between 890 and 1300 mm of rain per year (NOAA National Weather Service 2000). Evapotranspiration is one of the most important factors

in the water cycle in Puerto Rico, since it reduces the available runoff by on average 60% (DNER 2005). Rates of evapotranspiration vary spatially and long-term average monthly reference evapotranspiration ( $ET_o$ , which is calculated from pan evaporation data assuming a hypothetical grass reference crop and taking into account climatic variables) is higher on the south coast ( $ET_o = 3.5\text{-}5.75$  inches) compared to the north east of the island ( $ET_o = 3.0\text{-}5.0$  inches) (Harmsen et al. 2003). As a result of higher precipitation and lower evapotranspiration, the northeast part of Puerto Rico has higher runoff rates compared to the south coast (Gerbert et al 1987; Larsen and Webb 2009). Of the sites sampled in this study (Fig. 1), Paradisus and Punta Picua in the NE corner of the island had higher precipitation and runoff compared to the remaining five sites (Salinas, Las Mareas, Cayos Caribes, Jobos Bay Inner, and Middle) that were located on the drier south coast (Table 1).

The sample sites differed in the amount of development and urbanization. Two of the sites, Salinas and Paradisus, were heavily altered by human use and had the highest concentrations of  $NO_3$  (Table 1). Playa de Salinas is a coastal community where human disturbance and development go right up to the edge of the water and there is no mangrove fringe as is typically found along unaltered coastline. The main town of Salinas located further inland is partially sewered (around 50%), however Playa de Salinas is entirely reliant on septic systems for waste water disposal (Rodriguez 1996). Paradisus is the location of a large hotel complex on a peninsula largely cleared of natural vegetation to make way for buildings, roads, and golf courses. Adjacent to this site is the outfall of Rio Espiritu Santo, which travels through several heavily urbanized centers where approximately 50% of households relying on septic systems (Molina-Rivera 1996).



Riverine inputs are likely a significant source of human-derived nitrogen to this site. The remaining sites were largely undeveloped with coastline mostly covered in natural vegetation and DIN concentrations between 0.99 and 1.4  $\mu\text{M}$  (Table 1).

## 2.2. Site categorization

To answer questions about land-sea coupling we characterized the sites based on two different categories; precipitation and degree of urbanization (Table 1). For each of these two classifications we split the sites into two contrasting groups.

First, we grouped sites by precipitation. There was a clear division into two categories; sites on the south coast that had an average annual precipitation of  $< 1205$  mm (“low”) and sites on the north coast where average annual precipitation was around twice as high  $> 2258$  mm (“high”).

Second, using aerial photographs we assessed the degree of urbanization by estimating the % of urbanized cover on the shoreline adjacent to the sample location in an area 0.5 km along the coast and 0.5 km inland. There were two reasons for this approach. First, we did not have watershed delineations or land cover characteristics and nitrogen loads have not been estimated for these sites. Second, we assumed based on the limited connectivity between land and sea in Puerto Rico (Bowen and Valiela 2008) meant that only sources very close to the sample site would have an influence. Five of the sites had largely undeveloped coastlines ( $< 20\%$  developed) and were classified as having a “low” degree of urbanization. The remaining two sites had  $> 70\%$  development on the adjacent coastline, which was considered a “high” degree of urbanization (Table 1).

### 2.3. Producers and consumers

To examine the effects of precipitation and degree of urbanization on benthic food webs and stable isotope signatures of producers and consumers we collected all producers and consumers we could find at each site. Where mangroves were present, leaves were collected from several specimens along the shore. Seagrass and macroalgae were collected by snorkeling. Seagrass, macroalgae and mangroves were cleaned of any epiphytes or sediment by lightly rubbing under water or, if necessary, with a razor blade. Invertebrates were collected by hand or using a net while snorkeling. Samples were kept on ice and taken to the laboratory for processing. All invertebrate fauna were held in filtered seawater for at least 12h to allow evacuation of their guts. Large specimens with calcified shells were dissected and their shell material discarded. Smaller specimens were processed whole. We used a 5 m seine (1cm mesh size) to collect fish, crabs, and large shrimp. Samples were kept on ice and taken to the laboratory for processing. Fish were immediately dissected to remove their digestive tract, bones, and scales.

At least 3-7 specimens of the same species were pooled for each site. Species where fewer than 3 individuals had been collected from the same site were not included in the subsequent analysis. Producer and consumer samples were rinsed with deionized water before drying in an oven at 60°C. Samples were then ground to fine powder, weighed, and loaded into tin capsules. Subsamples of algae and invertebrates that had  $\text{CaCO}_3$  structures left after dissection were decalcified (Nieuwenhuize et al. 1994) before  $\delta^{13}\text{C}$  analysis, while subsamples for  $\delta^{15}\text{N}$  measurements were not decalcified because of potential problems associated with this process (Ng et al. 2007).

Carbon and nitrogen isotope analyses were performed by mass spectrometer by

the Stable Isotope Facilities at University of California Davis, Boston University, and The Ecosystems Center at The Marine Biological Laboratory. Results are calculated based on comparisons with atmospheric nitrogen (for N) and Vienna Pee Dee Belemnite (for C) calculated as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 10^3,$$

where  $R$  is ( $^{15}\text{N}/^{14}\text{N}$ ) or ( $^{13}\text{C}/^{12}\text{C}$ ). Duplicate determinations on the same sample typically differed by <0.2‰.

We pooled taxa into broad trophic groups by assigning feeding habits to each species based on a review of literature (Appendix A). Fish and invertebrates were assigned to trophic guilds and producers grouped into seagrass, macroalgae, and mangroves. We used these data for two separate analyses. First, to examine food web links, we pooled the data for each trophic guild within a site, but separating fish and invertebrates, and compared mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures between sites. Second, to investigate the effects of the potentially driving variables (coastline urbanization and precipitation regime), we compared isotopic signatures of producers and consumers between “high” vs. “low” categories. To eliminate taxonomic differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  we made these comparisons between individual species. Differences in stable isotope signatures between producers and consumers from “high” vs. “low” categories were tested by means of the Wilcoxon’s signed ranks test (Systat 11 for Windows).

### 3. Results

### 3.1. Stable isotope signatures

Overall, the trophic structure of the benthic food web was similar among sites (Fig. 2) even though the assemblage of taxa sampled in each site was different. In all sites stable carbon isotope signatures differed among the three types of producers (Fig. 2). Mangroves were lightest in  $\delta^{13}\text{C}$  (-28.8 to -27.84 ‰), macroalgae intermediate (-18.09 to -11.71 ‰), and seagrasses heaviest (-10.1 to -6.06 ‰). Consumers were overall similar in  $\delta^{13}\text{C}$  to macroalgae and seagrasses. Detritivores and herbivorous fish were typically heavier in  $\delta^{13}\text{C}$  compared to other consumers reflecting diets incorporating seagrass. The fractionation in  $\delta^{13}\text{C}$  appeared low throughout the food web since there was little difference in  $\delta^{13}\text{C}$  among trophic levels (Fig. 2). Within each site  $\delta^{15}\text{N}$  values were similar for macroalgae, seagrass, and mangroves. In contrast to  $\delta^{13}\text{C}$ , there was an overall increase in  $\delta^{15}\text{N}$  with trophic level at all sites reflecting fractionation with each trophic step (Fig. 2).

Some consistent site-specific trends were found for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Fig. 2). For nearly all trophic guilds Paradisus had the lightest  $\delta^{13}\text{C}$  signatures and Cayos Caribes and Punta Picua the heaviest. On average the difference between the lightest and heaviest  $\delta^{13}\text{C}$  signatures was 5‰. Within a trophic guild the range of  $\delta^{15}\text{N}$  values spanned 1.5 to 3.3‰ for fish and 1.2 to 2.5‰ for invertebrates and signatures were typically heaviest for organisms from Paradisus and Salinas.

### 3.2. Effects of precipitation and urbanization

The values we measured for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  had considerable variation, which could have derived from the influence of precipitation and urbanization among other

variables. The sampling sites we chose were not completely orthogonal for these variables, but we could carry out a rough assay of the relative effects by plots showing isotopic signatures of the same taxa collected in sites with high and low precipitation and degree of urbanization (Fig. 3). Human, and precipitation influences had contrasting effects on stable isotopes (Fig. 3). Despite the very large differences in precipitation between the sites on the North and the South coasts, precipitation did not significantly affect  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Fig. 3 left). Urbanization produced a significant shift in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of all trophic levels (Fig. 3 right). Species from sites with high degree of urbanization were significantly lighter in  $\delta^{13}\text{C}$  ( $p < 0.001$ ) by 1.7‰ and significantly heavier in  $\delta^{15}\text{N}$  ( $p < 0.001$ ) by 1.0‰.

#### 4. Discussion

The observed  $\delta^{13}\text{C}$  values of consumers suggest that seagrass and algae were major food sources at all sites with little or no incorporation of mangroves in the food web. The limited fractionation of  $\delta^{13}\text{C}$  observed here is in agreement with previous studies typically reporting  $\Delta^{13}\text{C}$  of around 0-1 ‰ or only a slight enrichment from prey to predator (Fry and Sherr 1984; Peterson and Fry 1987; Vander Zanden and Rasmussen 2001; Post 2002).

$\delta^{15}\text{N}$  values of producers and consumers were low and consistent with what we find in systems with low nutrient inputs (Anderson and Fourqurean 2003; Wooller et al. 2003; Yamamuro et al. 2003; Fourqurean et al. 2005). These low values reflect an isotopically light DIN pool, which may get its low  $\delta^{15}\text{N}$  from biogeochemical processes such as nitrogen fixation. The very low  $\delta^{15}\text{N}$  of producers found here differ from many

other systems where incorporation of nitrogen from wastewater inputs elevated  $\delta^{15}\text{N}$  signatures, e.g. Waquoit Bay, northeastern USA ( $\delta^{15}\text{N} < 7.9\text{‰}$ ; McClelland et al. 1997) and Patos Lagoon, southern Brazil ( $\delta^{15}\text{N} < 11.43\text{‰}$ ; Abreu et al. 2006).

Based on the importance of precipitation and evapotranspiration in establishing the degree of coupling between land and sea (Caraco and Cole 2001; Bowen and Valiela 2008), we might have expected land-sea coupling to be stronger on the north coast of Puerto Rico. The results of this study, however, did not show any variation in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  based on precipitation regime, so there was no evidence that increased precipitation and runoff rates enhanced land-sea coupling at the sites sampled. Samples were collected in May, which is at the beginning of the wet season. Precipitation is highly variable in Puerto Rico (NOAA National Weather Service 2000): The dry season runs from December to March or April followed by a period of intense rainfall in April and May. June and July typically experiences diminished rainfall and the wet season stretches from August through November. The degree of coupling may vary seasonally and it is possible that stronger coupling exists during another time of the year.

The degree of urbanization of the adjoining coastline created a shift in  $\delta^{15}\text{N}$  of the overall food web and there was a significant correlation between urbanization and  $\delta^{15}\text{N}$ . The shift observed is similar in magnitude to what has been shown in other locations (Martinetto et al. 2006), and is most likely attributable to wastewater inputs. Despite the predicted low degree of coupling between land and sea sewage-derived nutrients from urban areas appear to reach the adjacent coast. The very close proximity of human dwellings to the shoreline combined with the lack of any intercepting vegetation, e.g. a mangrove fringe, is likely a factor here. Nitrogen originating from wastewater in septic

tanks does not have to travel far to enter coastal waters and there is no riparian zone to intercept and remove nutrients. If the natural mangrove fringe had been left intact the anthropogenic nitrogen may not have reached coastal waters, but despite the inputs from the nearby urban centers, the nutrient concentrations at the Salinas and Paradisus sites are relatively low and there are no obvious signs of eutrophication.

There was no discernable reason to expect that urbanization itself would cause lighter  $\delta^{13}\text{C}$  signature of coastal producers and consumers (Fig. 3 right). The results were most likely affected by a difference in marine forcings and terrestrial carbon inputs between the sites. A light  $\delta^{13}\text{C}$  signature may be a reflection of lighter DIC from terrestrial sources or DIC regenerated from respiration in the water column and benthos (Lin et al. 1991; Coffin et al. 1994; Hemminga et al. 1994; Marguillier et al. 1997). We expect that at more exposed sites this pool of lighter DIC would rapidly be diluted by water with heavier DIC of marine origin (Spiker and Schemel 1979; Coffin et al. 1994). In the highly urbanized category, Paradisus was likely to have dissolved and particulate carbon mainly from a terrestrial source. This site had a significant amount of riverine input from Rio Espiritu Santo, which was clearly dominant at the sample location as shown by lower salinity (Table 1) and high turbidity of the water, both indications of the large inputs of freshwater and terrestrial particulate matter. In contrast, the sites likely to have the most influence from marine carbon sources were both in the low urbanization category. Cayos Caribes was around 2 km offshore, adjacent to a series of mangrove barrier islands. Punta Picua was situated at the tip of a peninsula jutting a few km out into the ocean, directly in the path of the prevailing currents and prevailing wave fronts nearing the NE coast of Puerto Rico. These sites were directly exposed to the dominant

ocean currents, which flow east to west (Fig. 1). We expect marine inputs to be dominant at these sites. The remaining five sites were in more sheltered locations and may have had significant carbon sources both from land and ocean. We therefore suggest that the significant difference in  $\delta^{13}\text{C}$  signatures is caused by differences in the relative contributions of marine versus terrestrial carbon rather than there being a direct relationship to urbanization.

## 5. Conclusion

The coastal waters of Puerto Rico appear to have some degree of coupling to land-uses both on the arid southern coast and the rainier northern coast, despite high rates of high evaporation and low precipitation. Wastewater signatures from adjacent urban areas were detected in the  $\delta^{15}\text{N}$  signatures of coastal producers and consumers. Changes in  $\delta^{13}\text{C}$  were best explained by differences in the degree of marine influence. Samples from a site dominated by terrestrial inputs from a major river, had lighter  $\delta^{13}\text{C}$  values, whereas sites further from land and in locations exposed to oceanic currents had heavier  $\delta^{13}\text{C}$  values, characteristic of a marine source of dissolved organic carbon. We found no evidence for precipitation influencing the strength of land-sea coupling. The degree of coupling may however vary seasonally and differences between sites of differing precipitation regimes may develop at some times during the year.

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*Appendix.* Table listing species collected and their mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N} \pm$  standard error. Where no standard error is indicated the species was only present in one site and only one value was obtained. Also listed are the trophic level (Herb = herbivore, If = predator feeding mainly on invertebrates, Omn = omnivore, Pisc = piscivore, Dep f = deposit feeder, Susp f = suspension feeder, Pred = predator) and diet of each species of consumer, the types of producers (B alg = brown macroalgae, G alg = green macroalgae, R alg = red macroalgae, Mang = mangrove, Seag = seagrass) and the source of this information.

Species	Common name	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	Trophic level	Diet	Source
<b>Teleostei</b>						
<i>Sparisoma viride</i>	Stoplight parrotfish	4.95	-10.39	Herb	97% algae, 3% seagrass	Randall 1967
<i>Sparisoma radians</i>	Bucktooth parrotfish	$5.87 \pm 0.3$	$-10.80 \pm 0.81$	Herb	88% seagrass, 12% algae	Randall 1967
<i>Acanthurus bahianus</i>	Ocean surgeon	5.11	-12.36	Herb	92% algae and organic detritus, 8% seagrass	Randall 1967
<i>Scarus iseris/taneopterus</i>	Princess / Painted tail parrotfish	$4.77 \pm 0.41$	$-10.34 \pm 0.11$	Herb	80% algae, 20% seagrass	Randall 1967
<i>Sparisoma rubripinne</i>	Redfin parrotfish	$6.15 \pm 0.79$	$-9.95 \pm 1.39$	Herb	92% algae, 7% seagrass	Randall 1967
<i>Anchoa lyolepis</i>	Anchovies	$9.79 \pm 0.54$	$-16.05 \pm 0.62$	If	Invertebrate zooplankton	Whitehead et al. 1988
<i>Sphoeroides spengleri</i>	Bandtail puffer	$5.42 \pm 0.18$	$-10.83 \pm 0.47$	If	20% crabs, 16% pelecypods	Randall 1967
<i>Halichoeres poeyi</i>	Blackear wrasse	8.70	-11.00	If	25% crabs, 20% gastropods	Randall 1967
<i>Sphoeroides testudineus</i>	Checkered puffer	$6.73 \pm 0.57$	$-12.07 \pm 0.78$	If	Crabs, gastropods	Turingan 1994, Pauly 1991
<i>Chaetodon capistratus</i>	Foureye butterflyfish	$7.94 \pm 0.14$	$-13.98 \pm 0.42$	If	40% zonatharians, 30% polychaetes	Randall 1967
<i>Anguilla rostrata</i>	Common eel	5.11	-23.24	If	crustaceans	Greenfield and Thomerson 1997
<i>Haemulon</i>	French grunt	$7.49 \pm 0.36$	$-12.21 \pm 0.46$	If	40% polychaetes, 15%	Randall 1967

<i>flavolineatum</i>					crabs	
<i>Haemulon</i>	Smallmouth grunt	8.34 ± 0.04	-13.09 ± 0.11	If	35% crustaceans, 19% polychaetes	Randall 1967
<i>chrysargyreum</i>						
<i>Harengula sp.</i>	Herring	6.44	-16.28	If	80% Zooplankton	Randall 1967
<i>Halichoeres bivittatus</i>	Slippery dick	8.99 ± 0.51	-12.44 ± 1.36	If	22% crabs, 18% echinoids, 17% polychaetes, 13% gastropods	Randall 1967
<i>Eucinostomus argenteus</i>	Spotfin mojarra	7.66 ± 0.53	-12.43 ± 0.46	If	42% amphipods, 20% polychaetes	Randall 1967
<i>Holocentrus sp.</i>	Squirrelfish	8.91	-14.70	If	70% Crabs, shrimps	Randall 1967
<i>Canthigaster rostrata</i>	Sharpnose puffer	6.40	-9.70	Omn	16% seagrass, 15% sponges, 10% crabs, 10% gastropods	Randall 1967
<i>Stegastes leucostictus</i>	Beaugregory damselfish	8.06	-13.84	Omn	23% algae, 20% mollusk eggs, 12% polychaetes	Randall 1967
<i>Stegastes variabilis</i>	Cocoa damselfish	7.33	-13.74	Omn	50% algae, 50% invertebrates	Randall 1967
<i>Atherinomorus stipes</i>	Hardhead silverside	9.71	-14.77	Omn	crustaceans, algae, polychaetes	Brook 1977
<i>Sphyraena barracuda</i>	Great barracuda	8.83 ± 0.41	-13.03 ± 0.96	Pisc	96% fish	Randall 1967
<i>Lutjanus griseus</i>	Gray snapper	8.01 ± 0.59	-12.6 ± 0.13	Pisc/If	40% fish and 40% crabs	Randall 1967
<i>Caranx sp.</i>	Jack	8.36	-16.79	Pisc	85-100% fish	Randall 1967
<i>Platybelone argalus</i>	Keeled needlefish	8.46	-16.91	Pisc	97% Small fish esp clupeoids	Randall 1967
<i>Lutjanus synagris</i>	Lane snapper	7.85 ± 0.76	-12.27 ± 0.47	If	50% crabs	Randall 1967
<i>Lutjanus analis</i>	Mutton snapper	7.67	-15.89	Pisc/If	40% Crabs and 30% Fishes	Randall 1967
<i>Lutjanus campechanus</i>	Northern red snapper	8.37	-16.54	Pisc/If	Fish and invertebrates	Sierra et al. 1994
<i>Lutjanus apodus</i>	Schoolmaster	7.22 ± 0.52	-12.80 ± 0.29	Pisc	60% Fishes	Randall 1967
<i>Ocyurus chrysurus</i>	Yellowtail snapper	8.43 ± 0.31	-12.62 ± 0.12	If	40% crustaceans, 15% fish	Randall 1967
<b>Echinodermata</b>						
<i>Isostichopus badionotus</i>	Four-sided sea cucumber	4.09 ± 0.21	-9.75 ± 0.50	Dep f	Sediment and organic detritus	Hammond 1982
<i>Holothuria mexicana</i>	Donkey dung sea	3.43 ± 0.28	-9.32 ± 0.44	Dep f	Sediment and organic	Hammond 1982

<i>Oreaster reticulatus</i>	cucumber	4.73	-14.88	Omn	detritus	Thomas 1960
<i>Lytechinus variegatus</i>	Cushion star	0.15 ± 1.52	-13.49 ± 0.76	Herb	Sponges, sediment, algae	Moore et al 1963
	Variegated urchin				Seagrasses, macroalgae, occasionally animals	
<b>Mollusca</b>						
<i>Pinna carnea</i>	Amber penshell clam	4.78	-13.49	Susp f		Kaplan 1998
<i>Hormomya exusta</i>	Scorched mussel	4.45	-19.15	Susp f		Kaplan 1998
<i>Tellin</i> sp.	Tellin bivalve	4.02	-13.37	Susp f		Kaplan 1998
<i>Chione</i> sp.	Venus bivalve	5.38	-17.04	Susp f		Kaplan 1998
<i>Cerithium</i> sp.	Cerith snails	3.96 ± 0.49	-11.62 ± 1.82	Omn		McClanahan 2002
<i>Littorina ziczac</i>	Zebra periwinkle	3.15	-13.96	Herb		Meirelles and Matthews-Cascon 2003
<i>Modulus modulus</i>	Atlantic modulus	2.97 ± 0.40	-10.68 ± 0.71	Herb		Bologna and Heck 1999
<b>Crustacea</b>						
<i>Cynadusa</i> sp.	Gammaridean amphipods	3.77 ± 1.25	-12.90 ± 0.96	Herb	Macroalgae, detritus	Zimmerman et al. 1979
<i>Gammarus</i> sp.	Gammaridean amphipods	1.55 ± 0.27	-18.17 ± 0.69	Herb	Macroalgae, detritus	Zimmerman et al. 1979
<i>Pitho anisodon</i>	Pitho crabs	1.66	-10.90	Herb	Not specified	Livingston 2003
<i>Mithrax sculptus</i>	Green reef crab	4.16	-13.85	Herb	Macroalgae	Coen 1988
<i>Calcinus tibicen</i>	Smooth-clawed hermit crab	2.71 ± 0.22	-12.95 ± 0.94	Herb	Macroalgae	Smith 1991
<i>Clibanarius vittatus</i>	Green-striped hermit crab	3.04 ± 0.34	-13.39 ± 0.98	Omn		
<i>Pachygrapsus transversus</i>	Common shore crab	3.97 ± 0.38	-14.28 ± 0.84	Omn	Macroalgae, fish, invertebrates	Abele et al. 1986
<i>Callinectes sapidus</i>	Blue crabs	5.74 ± 0.66	-13.30 ± 1.17	Pred		Hsuseh et al. 1992
<i>Penaeus</i> sp.	Penaeid shrimp	5.10	-11.35	Pred	Gastropods, bivalves, crustaceans	Linan-Cabello 2002
<i>Hippolyte pleuracanthus</i>	Broken back shrimp	4.04 ± 0.21	-13.28 ± 1.06	Omn		Howard 1984
<i>Palaemon</i> sp.	Shore shrimp	5.18 ± 0.46	-12.27 ± 0.91	Pred		Chambers 1981

**Other**

<i>Chondrilla nucula</i>	Chicken liver sponge	2.03	-17.81	Susp f	Kaplan 1998
<i>Tethya diploderma</i>	Golf ball sponge	3.83	-16.62	Susp f	Kaplan 1998
Unid. Blue sponge	Blue sponge	4.31	-16.22	Susp f	Kaplan 1998
Unid. tunicate	Pink tunicate	4.22	-16.34	Susp f	Kaplan 1998

**Producers**

<i>Padina gymnospora</i>		4.44	-15.24	B alg	Littler et al. 1989
<i>Dictyota cervicornis</i>		2.57 ± 0.37	-15.68 ± 0.97	B alg	Littler et al. 1989
<i>Calulterpa</i> sp.		2.71 ± 0.33	-13.99 ± 1.37	G alg	Littler et al. 1989
<i>Enteromorpha</i> sp.		2.52	-20.85	G alg	Littler et al. 1989
<i>Ulva</i> sp.		5.30	-16.90	G alg	Littler et al. 1989
<i>Halimeda</i> sp.		-1.53 ± 0.75	-14.71 ± 0.58	G alg	Littler et al. 1989
<i>Penicillus</i> sp.		0.37 ± 0.41	-14.19 ± 0.63	G alg	Littler et al. 1989
<i>Udotea flabellum</i>		1.84	-11.89	G alg	Littler et al. 1989
<i>Jania adherens</i>		1.52	-10.49	R alg	Littler et al. 1989
<i>Gracilaria tikvahiae</i>		6.09	-16.14	R alg	Littler et al. 1989
<i>Acanthophora spicifera</i>		4.03	-17.75	R alg	Littler et al. 1989
<i>Rhizophora mangle</i>	Red mangrove	1.72 ± 0.75	-28.28 ± 0.21	Mang	Voss 1980
<i>Halodule wrightii</i>	Shoal grass	0.17 ± 0.24	-11.44 ± 0.30	Seag	Littler et al. 1989
<i>Syringodium filiforme</i>	Manatee grass	-0.61 ± 0.47	-9.70 ± 0.28	Seag	Littler et al. 1989
<i>Thalassia testudinum</i>	Turtle grass	1.82 ± 0.30	-8.51 ± 2.00	Seag	Littler et al. 1989

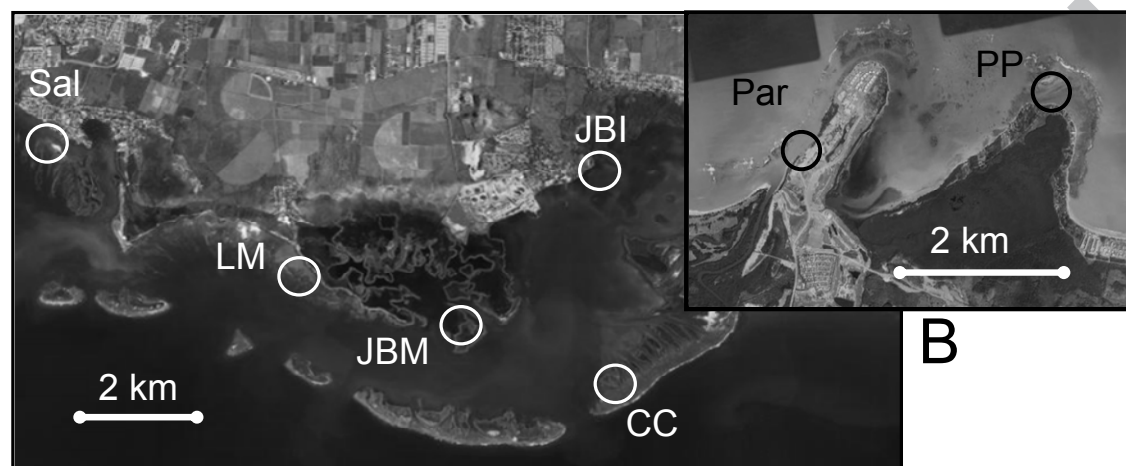
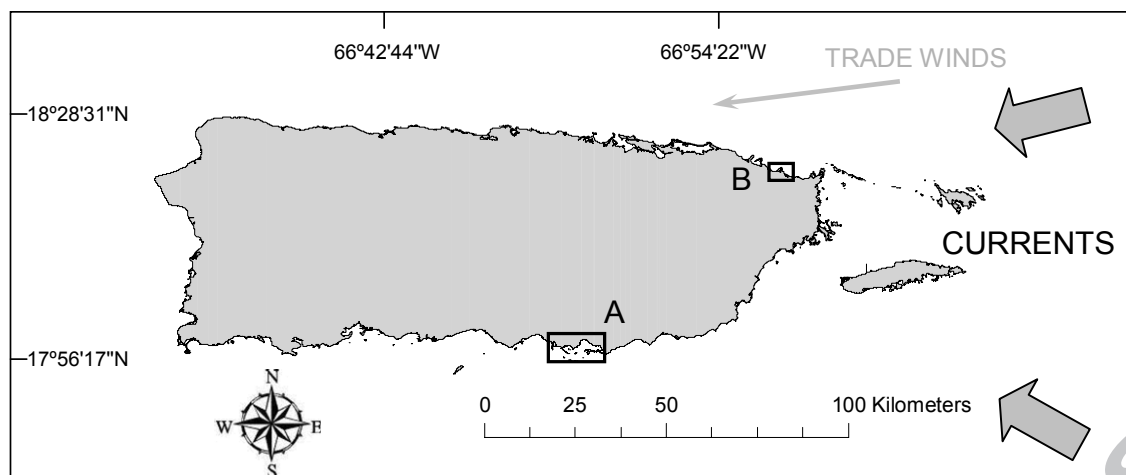
### FIGURE LEGENDS

*Figure 1.* Map of Puerto Rico showing the location of sample sites. Details of areas A and B are shown on satellite images (source: [www.googlemaps.com](http://www.googlemaps.com)). The location of each sampling station is marked by a circle and the site abbreviation. (Par = Paradisus; PP = Punta Picua; Sal = Salinas; LM = Las Mareas; JBM = Jobos Bay Middle; JBI = Jobos Bay Inner; CC = Cayos Caribes). The directions of prevailing winds and currents are indicated.

*Figure 2.* Mean ( $\pm$ s.e.) carbon (left panel of each pair) and nitrogen (right panel) stable isotope ratios for producers and consumers from each sampling station (for station abbreviations see Fig. 1) arranged by trophic guild (P = predators, O = omnivores, S = suspension feeders, D = detritivores, H = herbivores, PP = primary producers). Producers are shown as triangles (white = mangrove, gray = macroalgae, black = seagrass) and consumers as circles (white = invertebrates, black = fish).

*Figure 3.* Species comparisons of means and ranges of  $\delta^{15}\text{N}$  (top) and  $\delta^{13}\text{C}$  (bottom) measured in ‰ of primary producers (open circles), herbivores (includes herbivores, detritivores, and suspension feeders) (gray circles), and predators (includes predators and omnivores) (black circles). Panels compare species from sites with different degrees of precipitation (left) and urbanization (right) with ‘low’ degrees of each of the variables plotted along the x-axis and ‘high’ degrees on the y-axis. The results of a Wilcoxon’s signed ranks test are shown in the bottom right hand corner of each panel. The asterisks indicate if the samples were significantly

heavier or lighter compared to a 1:1 relationship; three indicate  $p < 0.001$  and n.s. means the data were not significantly different from the 1:1 line.



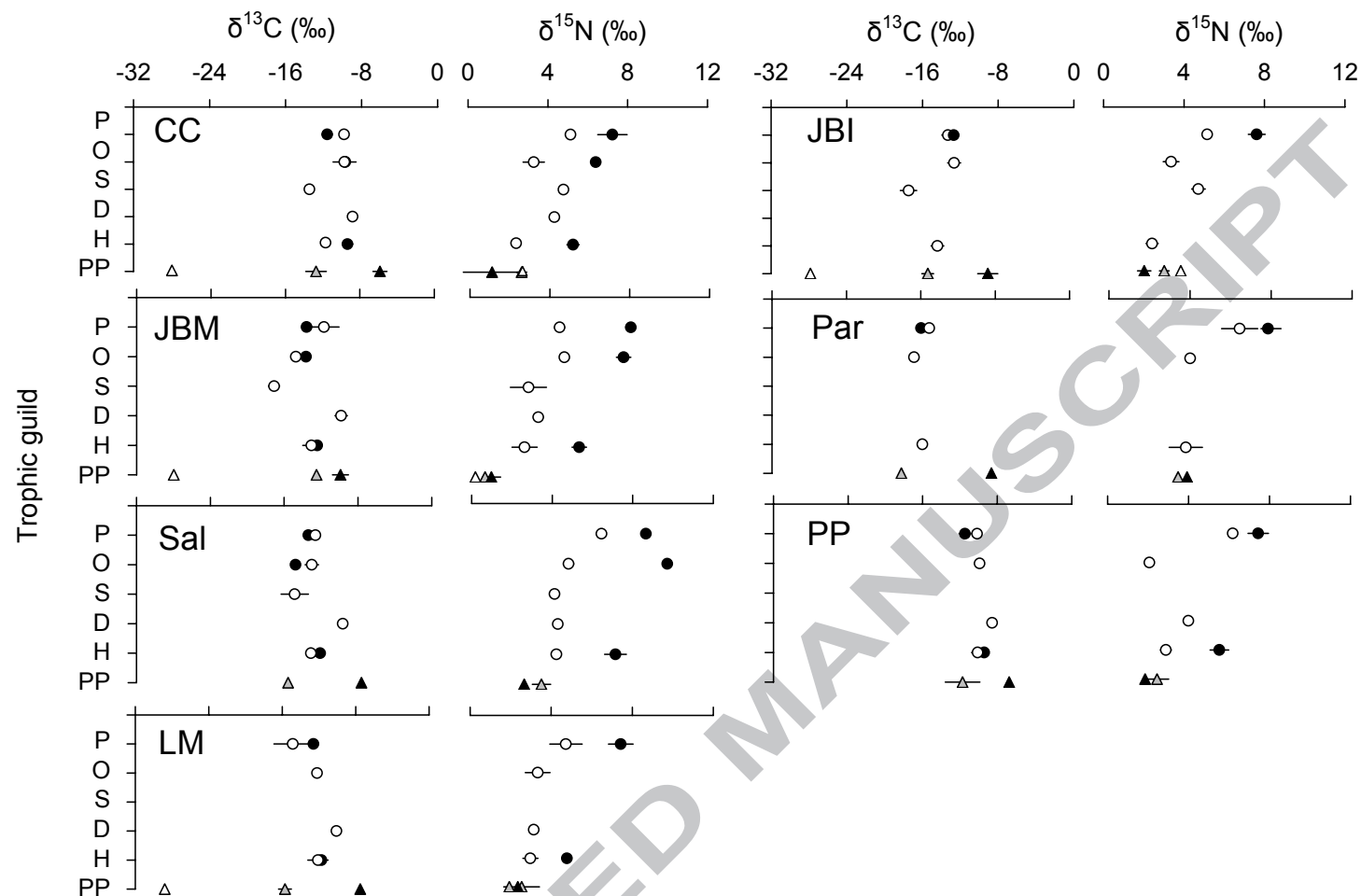


Fig 2.



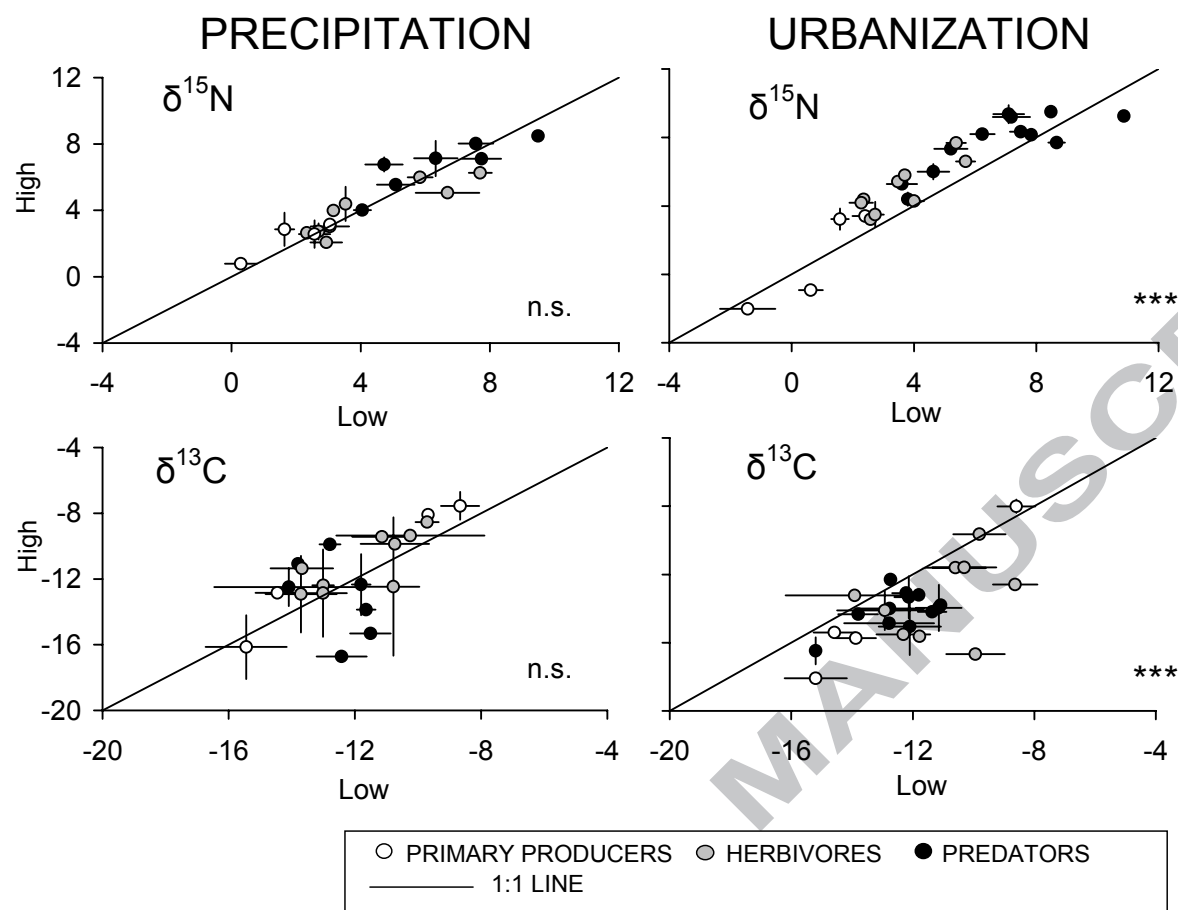


Fig. 3.

*Table 1.* Characteristics of sample sites. Water column nutrients and salinity were measured in May 2007. Coastline development was estimated from examination of a 500 m x 500 m area adjacent to each site using aerial photographs. Average annual precipitation values were obtained from NOAA National Weather Service (2000) based on data collected 1971-2000.

<b>Characteristics</b>	<b>Jobos Bay Inner</b>	<b>Jobos Bay Middle</b>	<b>Las Mareas</b>	<b>Salinas</b>	<b>Cayos Caribes</b>	<b>Paradisus</b>	<b>Punta Picua</b>
Abbreviation	JB I	JB M	LM	Sal	CC	Par	PP
<b>Water column nutrients</b>							
Phosphate ( $\mu\text{M}$ )	0.66	0.53	0.65	0.62	0.62	0.62	0.53
Nitrate ( $\mu\text{M}$ )	0.58	0.90	0.81	2.61	0.70	1.33	0.67
Ammonium ( $\mu\text{M}$ )	0.41	0.40	0.60	0.50	0.37	0.53	0.40
DIN ( $\mu\text{M}$ )	0.99	1.30	1.40	3.11	1.07	1.86	1.07
N:P	1.50	2.45	2.15	5.02	1.73	3.01	2.02
Salinity (‰)	30.3	31.0	32.6	32.8	34.8	28.4	31.7
Depth (m)	0.3-0.5	0.5-1.3	0.3-0.5	0.5-1.4	0.4-1.2	0.2-0.7	0.2-0.7
<b>Classification</b>							
<b>Degree of urbanization</b>							
Coastline development	<5%	0%	<3%	>80%	0%	>70%	<15%
Rank Urbanization	Low	Low	Low	High	Low	High	Low
<b>Precipitation</b>							
Avg. annual precipitation (mm)	990	1020	1205	1205	1020	2285	2285
Rank Precipitation	Low	Low	Low	Low	Low	High	High