ABSTRACT

PEREZ-GONZALEZ, BEATRIZ DE LOS A. Density and Diversity of Migratory Shorebirds and Their Prey in Hypersaline Lagoons of the Caribbean. (Under the direction of Dr. David B. Eggleston).

Hypersaline salt-flats and coastal lagoons throughout the Caribbean serve as important habitat for seasonal migrations of shorebirds from North America. During both, southbound and northbound migrations shorebirds stop to feed, rest and gain the necessary energy for their next flight. The distribution and abundance of hypersaline lagoons in the Caribbean, as well as climate, can vary widely, with distinct wet and dry seasons that determine the areal extent and salinity of lagoons. The salinity of lagoons likely determines the composition of shorebird prey and, together with the distribution and abundance of lagoons, likely determines the density and diversity of shorebirds throughout the Caribbean. The main goal of this study was to describe how the relative density and number of shorebird species vary between the Greater and Lesser Antilles regions in the Caribbean, and as a function of wet versus dry seasons. We also assessed if variables that could influence salinity and areal extent of lagoons, such as mean high water from tidal intrusion of ocean water or decreased in salinity from rainfall, influences bird density and number of species. We also determined if the density and number of shorebird species varied among years (2009-13) and between specific islands: Puerto Rico (Greater Antilles) and USVI (Lesser Antilles). Lastly, we characterize in detail the concentrations of shorebird prey in two hypersaline lagoons in Puerto Rico, as well as the factors influencing these prey concentrations. We found significantly higher densities of shorebirds in the Lesser versus Greater Antilles regions of
the Caribbean, and a negative relationship between the density and number of bird species and mean high water. In addition, we found that bird density was ~ three times higher in the U.S. Virgin Islands than in Puerto Rico, with a peak in bird density in both locations during 2012. Regarding prey, the concentration of *Artemia spp.* was higher in the *Jungle* lagoon in Puerto Rico, whereas concentration of other prey species (mostly *Ephydra spp.*) was higher in the *Providencia* lagoon. Concentration of *Trichocorixa spp.* prey decreased with salinity and depth, and biomass of *Artemia spp.* decreased with temperature. The information obtained from this project will help better manage hypersaline lagoons in support of populations of migratory shorebirds and their prey through an increased understanding of regional differences in the density and number of bird species using these lagoons in the Caribbean, as well as factors influencing shorebird prey concentrations. This study also contributes to a growing literature on the ecology and restoration of hypersaline lagoons in tropical and arid ecosystems.
Density and Diversity of Migratory Shorebirds and Their Prey in Hypersaline Lagoons of the Caribbean.

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Master of Science Forestry and Environmental Resources

Raleigh, North Carolina
2014

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ACKNOWLEDGMENTS

Special thanks to my committee members, who never said no, for their support and guidance at NC State: Drs. Dave Eggleston, Stacy Nelson, Jaime Collazo and Robert Mayer. To my family that was always there for me unconditionally, thank you. This research would not have been possible without the financial support of the US Forest Service, Puerto Rico Department of Natural and Environmental Resources and US Fish and Wildlife Service. Thank you to the research technicians for their field and laboratory work: Loreli Sepulveda, Raúl Rivera, Amarilys Irizarry and Katsí Ramos. Last but not least, thanks to my friends and lab partners for their support and help throughout these past three years.
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1.0 INTRODUCTION

The majority of shorebirds in the northern hemisphere are migratory, breeding in the Arctic during summer and over-wintering in temperate and tropical regions (including Caribbean salt flats) (Myers et al. 1987). Salt flat habitats and coastal, hypersaline lagoons are among the most important ecosystems for migratory shorebirds in the Caribbean (Collazo et al. 1995). Salt flats or salt ponds (lagoons) are coastal enclosed or semi-enclosed water bodies within mangrove wetlands and are typically hypersaline, with salinities exceeding 50 parts per thousand (ppt) (Jarecki and Walkey, 2006). Mangrove wetlands and their associated lagoons are the predominant type of coastal wetland in the Caribbean and are quickly being replaced by coastal developments, making them some of the most threatened habitats on Earth (Jarecki and Walkey 2006). These unique ecosystems, where salinities can reach 60 to >200 parts per thousand (ppt), support high concentrations of shorebird prey such as brine shrimp (*Artemia* spp.) and water boatmen (*Trichocorixa* spp.) (Vargas 2011). These prey organisms are well-adapted to survive in hypersaline lagoons where salinity, temperature and dissolved oxygen are at extreme levels and fluctuate greatly. Although both prey species provide similar caloric value to shorebirds, there is a great energetic expense for shorebirds to process brine shrimp from very high salinities (> 65 ppt) compared to water boatmen in relatively low salinities (<65 parts per thousand, ppt) (Tripp and Collazo 2003). When shorebirds feed on high salinity prey, hyperosmotic stress may be induced followed by weight loss, which could end with death (Tripp and Collazo 2003).

Salinity is the main abiotic factor limiting the diversity and density of shorebirds and their prey within these ecosystems (Grear 1992, Tripp and Collazo 2003, Mayer 2004).
Temperature, dissolved oxygen and food availability are the main factors that affect brine shrimp concentrations in hypersaline lagoons (Mayer 2004). Brine shrimp exhibit both ovoviviparous and oviparous reproductive modes depending on environmental conditions. When food supplies, salinity and temperature are favorable, females hatch naupliar larvae. Conversely, when conditions are unfavorable, such as salinities >200ppt, low dissolved oxygen concentrations, low food availability and high temperatures, females develop highly resistant protective shells (cysts) that enclose dormant embryos, that eventually float and accumulate on the shore of the lagoon. The cysts accumulate on a thin freshwater lens when they are washed back into the lagoon from shore during rain events, leading to a metamorphosis from the cyst stage to a swimming-stage. Shorebirds are not the only predators of brine shrimp--water boatmen also prey on brine shrimp, however, when conditions are not favorable for water boatmen, brine shrimp practically live in a predator-free environment (Mayer 2004).

Water boatmen can be found in salinities less than 65ppt, but they need salinities of 45ppt or less for successful hatching of eggs (Davis, 1966). Adults, however, can survive in salinities up to 170ppt (Balling and Resh, 1984, personal observation). These organisms breed year round in the tropics and can disperse from one lagoon to another by flying (as adults) or within the high tides (as nymphs) that inundate ponds (Balling and Resh, 1984, Grear 1992). An increase of only 8.5±2.1ppt beyond a salinity of 40ppt can result in 50% of mortality of water boatmen (Tripp and Collazo 2003).

Salinity within shallow water bodies can fluctuate widely due to the relatively large surface area of lagoons compared to their shallow depth, resulting in high evaporation rates.
In addition to fluctuating evaporation rates, short-term and seasonal rainfall patterns can influence salinity (Grear 1992, Jarecki and Walkey 2006). Moreover, annual rainfall patterns in the Caribbean can vary widely (Gamble and Curtis 2008). Nevertheless, there are noticeable wet and dry periods, with the wet season occurring from May to November (Taylor et al. 2011). Within the wet period, however, there can be a noticeable mid-summer drought, with decreased rainfall during July and August. Moreover, storms and strong winds can force birds away from their typical straight-line flights (Burger et al. 2012, Downs and Horner 2008). For example, along the East Asian-Australasian Flyway, Ma et al. (2011) found that wind conditions during fall affect stopover decisions, with more birds departing and arriving with tailwinds than with headwinds. Other than weather, detours during migration can also occur due to temporal availability of stopovers (e.g., completely dry lagoons), habitat quality, physical barriers or predisposition to established routes (Downs and Horner 2008). Also, if stopovers are not well arranged spatially, birds are forced to travel greater distances, which can increase mortality rates, cause birds to arrive at breeding grounds in poor condition, or cause delayed nesting or reproductive success (Grear 1992, Downs and Horner 2008, Piersma 2007).

Although coastal tidal ranges throughout the Caribbean are relatively small, tidal ranges increase during the wet season which can affect prey availability and shorebird distribution (Kjerfve 1981, Collazo et al. 1995). Increasing tidal range and rainfall can impact lagoons in two ways: (i) decreasing the salinity via introduction of ocean water or rainfall into hyper-saline lagoon waters, and (ii) increasing the area extent of the lagoon by adding more water (Jarecki and Walkey 2006). For instance, the intrusion of ocean water (35ppt, as
high tides) into a lagoon will decrease salinity, which could increase energetically favorable prey such as water boatmen which, in turn, could enhance bird density and diversity. Conversely, intrusion of ocean water could increase the surface area and depth of a lagoon. Depth is another important factor for many shorebird species that have short legs or short bills because they need very shallow water to feed. Thus, an increase in water depth with influx of ocean water via high tides, or via rainfall, could have a negative effect on shorebirds, whereas reduced salinities in hypersaline lagoons could have a positive effect. These hypotheses remain to be tested.

Migration distance of shorebirds varies widely. For example, some species might fly directly from breeding to over-wintering areas, a distance of up to 12,000 km (Myers et al. 1987). Others may stop once or twice if stopover areas are limited. This “hopping” migration strategy is used by shorebirds which fly short distances and then refuel for short periods of time (Skagen and Knopf 1994). There is also variation in migration strategy within and between shorebird families. Using plovers (Charadriidae) and sandpipers (Scolopacidae) as examples, some species within these two families may prefer to stay near their natal area (no more than 100m away), or fly thousands of kilometers to cross the ocean (Piersma 2007). Similarly, there are some species that rely on freshwater wetlands during their nonbreeding season. Whichever the case, shorebirds that breed farther north often winter as far south as possible.

Both southbound and northbound flyways occur over the Caribbean region. The southbound migrations occur from early July to late October, with a first peak dominated by adults during July and early August, and a second peak by young birds from late August
through October (Hunter et al. 2002). The northbound migration of shorebirds is usually from mid-March to late May (Hunter et al. 2002). Shorebirds do not take the same route for both seasonal flyways: (i) southbound shorebirds usually follow an overseas or trans-Atlantic route, and (ii) northbound shorebirds typically fly over the western Caribbean (Richardson 1974, Hunter et al. 2002). Recent research has focused on stopover sites throughout the Caribbean, with Puerto Rico in particular receiving more attention than any other island (Wunderle et al. 1989, Lee 1989, Grear 1992, Collazo et al. 1995, Grear and Collazo 1999, Rice et al. 2007). Stopover sites provide opportunities for shorebirds to rest and feed, as well as provide protection from predators (Downs and Horner 2008) and human disturbances (Burton et al. 2002, Myers et al. 1987). For example, McGowan et al. (2007) found the only breeding site for the greater flamingo (*Phoenicopterus ruber*) within the territory of Anegada, British Virgin Islands, and Lyons et al. (2008) in their study of refueling rates within five stopover sites found low refueling rate at their site in the Dominican Republic due to predators (e.g., merlins) attacking and hunting shorebirds.

Many organizations and agencies have made progress in managing shorebirds in the Caribbean region. For instance, the Southeastern Coastal Plains-Caribbean Regional Shorebird Plan (Hunter et al. 2002) and The Caribbean Waterbird Census 2014 Report (Sorenson and Gerbracht 2014) describe in detail the distribution of many shorebirds species, as well as the necessary steps for an effective management and conservation plan in the Caribbean region. Our contribution to this effort and the specific objectives of this thesis were to determine if (1) mean migratory shorebird density and diversity within hypersaline lagoons varied according to region (Greater versus Lesser Antilles) and season (wet versus
dry), and as a function of related variables that could influence salinity and areal extent of lagoons, such as mean high water from tidal intrusion of ocean water or from rainfall. We also determined (2) whether or not mean bird density and number of species within lagoons from a single island in the Greater and Lesser Antilles varied among years. We then evaluated how prey resources are affected by water quality in Providencia and Jungle salt flat ecosystems in southwestern Puerto Rico to determine (1) whether or not prey concentration and biomass vary between lagoons and among months, and (2) if prey concentration and biomass vary with changes in water quality.

2.0 METHODS

The key response variables in this study, bird abundance and species for the period 2009-13, were obtained for islands in the Greater versus Lesser Antilles regions of the Caribbean Sea, and for wet versus dry seasons (see below), via an on-line data-base populated by bird observations from researchers and hobbyists (eBird.org). The area of a given lagoon was then determined by GIS, and bird abundance and number of species converted to densities by dividing by the area of a given lagoon. Related explanatory variables that might influence salinity in lagoons which, in turn, could influence prey species for shorebirds, included mean high water from tides, as well as rainfall—the data for both was obtained from on-line data bases (see below).

2.1 Study Areas in the Caribbean:

The study area included most of the major islands within the Caribbean, with the exception of The Bahamas (Fig. 1). We divided the Caribbean into two regions: Greater and Lesser Antilles. Islands in the Greater Antilles included Cuba, Jamaica, Haiti, Dominican
Republic and Puerto Rico. Islands in the Lesser Antilles included U.S. Virgin Islands, British Virgin Islands, Saint Kitts, Saint Lucia, Barbados, Grenada, Aruba, Bonaire and Curacao (among others in the Eastern Caribbean). This division partitioned the influence of distance to and from North America (the source of migratory shorebirds), size of islands and, most importantly, the extent and influence of seasonality of coastal and tidal wetlands for migratory shorebirds. For instance, the extent of tidal wetlands in the Lesser Antilles contracts with lower tidal amplitude during spring compared with greater tidal amplitude during autumn. Precipitation also differs between the Greater and Lesser Antilles due to variations in topography, different island orientation to prevailing winds, and atmospheric circulation. The Greater Antilles tend to be wetter than the Lesser Antilles during the dry season (Giannini et al. 2000). The reduction in coastal habitats in the Lesser Antilles during spring is not observed in the Greater Antilles because these islands contain large and permanent lagoonal systems that provide habitat for migratory shorebirds prior to their northbound migration (around early April).

The Caribbean experiences seasonal precipitation with a wet and dry period, the latter of which falls within the time period when migratory shorebirds pass through the region. The dry season occurs during December-April, and the wet season during May-November, with a slight decrease in rainfall in June and July. To determine the effect of wet versus dry seasons on shorebird density and number of species, we analyzed data from the three driest and three wettest months of the year (January, February, March and, August, September, October, respectively) for 2009-2013.
2.2 Data: Shorebirds, Tides and Rainfall

Bird counts were obtained from the eBird database (eBird.org), an online database launched in 2002 by Cornell University’s Ornithology Program. Participants in eBird.org who are recreational or professional bird watchers provide information on when, where and how they went birding, as well as provide data on the number of birds and species seen. eBird constantly updates the database. Bird count data for the years 2009-13 was retrieved from the eBird database and imported into GIS ArcMap 10.2.1 for spatial analysis of bird abundance and lagoon area. We began by plotting bird observations on a map. Only points within coastal lagoons at the islands being considered in this study were used. We then chose those points with bird sightings from only the six months that reflected dry versus wet portions of the year. After evaluating all islands, the resultant islands with bird sightings within the periods stated above were: Cuba, Dominican Republic, Puerto Rico, Barbados, British Virgin Islands, Grenada, U. S. Virgin Islands, Aruba, Bonaire, Jamaica, Saint Kitts and Saint Lucia. The last five islands were removed from further analyses due to observations from only one lagoon, or from only one year in our time series. The area of each lagoon within a given island was estimated using GIS ArcMap. Bird counts were then divided by the area of a given lagoon to estimate bird density per m². Thus, the response variables in this study were the mean density and number of species per m² of migratory shorebirds per season per island. The means were calculated by averaging the numbers of bird counts within the wet or dry months per island. For instance, Barbados during the dry season in 2013 had bird counts in 3 lagoons: Chancery Lane lagoon =19 species, Greenland Pond lagoon = 2 species, and Long Pond lagoon = 8 species. The mean number of species
was calculated by averaging bird counts from the three lagoons (e.g., mean number of species for the dry season in 2013 for Barbados was 9.67 species). In addition, we selected two specific species of migratory shorebirds of similar size to assess any potential differences in mean bird densities between Lesser and Greater Antilles, and wet and dry seasons. The shorebird species were: semipalmated sandpipers (*Calidris pusilla*, 13-15 length) and Wilson plovers (*Charadrius wilsonia*, 16-20cm length).

As described above, salinity is a key driver of the prey assemblages for migratory shorebirds that forage in hypersaline lagoons. We assessed the relative importance of two related variables that could influence salinity within lagoons: (1) mean tidal height and (2) rainfall. We used predicted tidal height data obtained from the National Oceanic and Atmospheric Administration (tidesandcurrents.noaa.gov) instead of observed data because NOAA only provides observational data for Puerto Rico and the U.S. Virgin Islands. We assessed the accuracy of these predicted mean tidal heights by comparing predicted versus observed tidal heights for Puerto Rico and the U.S. Virgin Islands. We focused on predicted Mean High Water (MHW, the average of all high tides per month) because it can serve as a general representation of tides for the Caribbean (Kjerfve 1981).

We used two separate t-tests to determine whether or not predicted versus observed MHW was significantly different for both Puerto Rico and the U.S. Virgin Islands. For both islands, predicted MHW was significantly lower (both P<0.03) than observed means (Puerto Rico: predicted MHW = 0.084m, observed MHW = 0.130m; U.S. Virgin Islands: predicted MHW = 0.087m, observed MHW = 0.138m). These results suggest that use of predicted MHW as a proxy for potential inundation of lagoons in the wider Caribbean may provide a
A conservative estimate of inundation. We proceeded with testing whether or not mean bird density and number of species varied with predicted MHW since any relationship between birds and MHW would likely be stronger than detected in our analyses given the conservative nature of predicted MHW.

Rainfall data for our study sites during 2009-13 was obtained via the Tropical Rainfall Measuring Mission (TRMM), a joint operation between NASA and the Japanese Space Agency, which provides precipitation data within the general coordinates of 35 degrees North and South Latitudes (e.g., http://neo.sci.gsfc.nasa.gov/view.php?datasetId=TRMM_3B43M&year=2009). For the purpose of this study, we focused on data from latitudes 23° and 11° N, and longitudes 85° and 60° W. Monthly rainfall data (measured in millimeters) was downloaded in GeoTiff format with 0.25 degrees cells. These raster files were then imported to GIS ArcMap to extract precipitation data for our study area. After extraction, several small islands were not included because they were initially too small. Therefore, to calculate precipitation of the very small islands, we re-sampled the database for reduced cell sizes (from 0.25 degrees to 0.00025 degrees). The explanatory variable in this case was calculated by averaging the rainfall for a given cell within a given island per month per year. For example, in January 2009 Puerto Rico had 12 cells, each cell with a centimeter per month rainfall value (e.g., 13.6, 12.5, 11.8, 12.5, 12.6, 13.7, 13.8, 11.8, 11.7, 10.9, 11.2, 13.9 cm/month). The amount of rain that fell in Puerto Rico during January of 2009 was calculated by averaging those 12 values, which was 12.5 cm/month.
2.3 Shorebird prey in southwest Puerto Rico

The Cabo Rojo salt flats, located in the southwest tip of Puerto Rico and currently used for salt extraction, are the most studied sites for migratory shorebirds in Puerto Rico (Grear 1992, Tripp & Collazo 1997, Collazo et al. 1995, Tripp & Collazo 2003, Grear & Collazo 1999, Rice et al. 2007). There are, however, other shallow, hypersaline systems in southwestern Puerto Rico that were used for the creation of salt, and the Puerto Rico Department of Natural and Environmental Resources is interested in restoring these salt flats to better serve as foraging areas for migratory shorebirds.

We sampled shorebird prey in one of these potential restoration lagoons and an adjacent coastal lagoon located less than 500m away that could serve as a non-restored control should restoration take place in the future. These lagoons are located in Guánica, Puerto Rico: (1) Providencia (17°56’35.12”N, 66°57’22.05”W) and (2) Jungle (17°56’46.32”N, 66°57’53.11”W), both of which are located within the La Jungla sector of the western portion of the Guánica Commonwealth Forest (Fig. 3). The Providencia lagoon is targeted for future restoration. The Jungle lagoon is a relatively undisturbed site that has not been used for commercial salt extraction and is surrounded by mangrove forest and upland vegetation, with little freshwater input other than rainfall, and no connection to the ocean except during extreme storm events. Conversely, the eastern portion of the Providencia lagoon was previously used for salt extraction starting in the 14th century, and used to be connected to the ocean by a small channel. Providencia lagoon is surrounded by upland vegetation, however, the eastern portion can receive runoff from rainfall events that can lower salinity (see results below).
Concentrations of shorebird prey were quantified in the Providencia and Jungle lagoons (mean depth ~ 1m) from a kayak using either a dip-net that sampled a standard volume of water, or suction-pump that sampled a known volume of water. After sampling of prey, water quality characteristics were quantified with a YSI-85 water quality instrument, and water depth was sampled with a meter-stick. Sampling of shorebird prey occurred monthly during March, May, June, July and August 2013. Sample size for prey was allocated according to the area of each lagoon (Jungle = 40,544.8 m$^2$; and Providencia = 226,558.1 m$^2$). Thus, we randomly selected 24 sampling points in Providencia and 6 random points in Jungle using GIS ArcMap 10.2.1. A minimum distance of 10m between points was used to maintain independence of each sampling location. Each set of sampling point coordinates was reached by kayak using a hand-held gps unit.

We tried several methods of simultaneously sampling brine shrimp, which tend to be concentrated throughout the water column, and water boatmen, which tend to hide within loosely consolidated algal mats just above the bottom, yet are also found throughout the water column, before choosing a combination of sweep-net and pump-sampling. Using a kayak, a sweep-net measuring 15 cm X 12 cm with 0.5 mm-mesh was swept along the bottom for 50cm and then brought to the surface at an angle of 45 degrees and pulled for another 50cm. Thus, each sweep of the net travelled a total distance of 100 cm for a total volume sampled of 18L. The contents of the sweep-net were emptied into a 0.5-mm sieve, and then transferred to a sampling jar with 70% ethanol. For water depths less than 20cm, samples were taken using a bucket with a pump. At shallow sampling sites, the bucket was dropped at the desired sampling coordinates, and then water quality was measured adjacent
to the sampling bucket, where sediment was not disturbed. The amount of water within the bucket was suctioned with the pump and then passed through a 0.5mm sieve to collect the organisms. The maximum water volume collected with the bucket was 15L (bucket radius=15.2cm, maximum water depth 20cm). Samples were brought to the laboratory where organisms were identified to species whenever feasible and enumerated with a dissecting microscope. Concentration of organisms was calculated by number of individuals per liter.

To account for potential changes in the biomass of individual prey over time, we also quantified the dry weight biomass of prey. To calculate biomass, prey samples were dried in an oven for 48 hours at 60°C and weighed to the nearest 0.0001g using an analytic balance.

Thus, the six response variables were the (i) concentrations and (ii) biomass of: (1) brine shrimp, (2) water boatmen, and (3) other species, which was predominantly *Ephydra spp*.

Water quality (temperature, dissolved oxygen and conductivity) was sampled using a hand-held YSI-85 water quality instrument. This instrument works well for salinities under 80 parts per thousand (ppt), however, salinities in both lagoons often averaged 160 ppt and sometimes exceeded 300 ppt. Thus, salinity was measured with a refractometer that was able to read salinities up to 160 ppt. Any sample more than 160 ppt was brought back to the laboratory, and the salinity was calculated by dilution (Petrucci et al. 2007), which was completed the same day to avoid crystalization of salt in the sample. The formula to calculate salinity was:

\[ Ci = \frac{Vf \cdot Cf}{Vi} \]

where \( V \)=volume of water, \( C \)=concentration of salt, \( i \)=initial, and \( f \)=final. We mixed the same quantity of distilled and salt water (1:1, \( Vi \) and \( Vf \)), read the salinity concentration with the refractometer (\( Cf \)), and then calculated the initial
concentration. For example, for a specific water sample from the Jungle lagoon, we mixed 40ml of the salt water from the lagoon and 40ml of distilled water, the refractometer reading after mixing was 145ppt. Using the formula we calculated: \( \frac{80\text{ml} \times 145\text{ppt}}{40\text{ml}} \), the salinity reading for that point in Jungle was 290ppt.

2.4 Statistical Analyses

We tested if mean bird density and number of species varied significantly according to Region or Season with a two-way ANCOVA model with Region (Greater vs Lesser Antilles) and Season (Wet vs Dry) as factors, and MHW and rainfall as covariates. We also tested if bird density and number of species varied among years and across seasons with a two-way ANOVA model for Puerto Rico and the U.S. Virgin Islands, separately. We used only Puerto Rico and the U.S. Virgin Islands in this case because they were the only two islands with bird sightings throughout the 5 year study period. We did not include island (Puerto Rico and U.S. Virgin Islands) as a factor in this 2-way ANOVA model since the previous analyses using a 2-way ANCOVA showed that mean bird densities were higher in the Lesser (e.g., U.S. Virgin Islands) versus Greater (e.g., Puerto Rico) Antilles (see results below). We tested if mean bird density of semipalmated sandpipers and Wilson plovers differed by region or season using a two-way ANOVA. We tested if the concentration or biomass of shorebird prey varied according to month (Mar., May, June, July, Aug. 2013) and between lagoons (Jungle versus Providencia), and as a function of water depth and water quality covariates (salinity, DO, temperature) with separate two-way ANCOVA models.
For data analyses using ANOVA models, data were tested for normality and homogeneity of variance using Brown-Forsythe- and Bartlett & Levene’s tests, respectively. Log-transformed (log(x+1)) or ln-transformed data were used when necessary to meet assumptions of normality and homogeneity of variance. In some instances, transformed data remained non-normal—in these circumstances transformed data was still analyzed with ANOVA due to its robustness and insensitivity to skewness (Glass et al. 1972). In instances where variances were heavy-tailed despite transformation, examination of the residual plots indicated they were close to being normal and therefore were used with transformation for data analysis (D. Dickey, NCSU Statistics Department, personal communication). When higher level interactions were present, they were dissected using lower-level ANOVA models or a two-sample independent t-test (Underwood 1981). Multiple comparisons among treatment levels were tested with Tukey’s Honestly Significant Difference (HSD) multiple comparisons test.

3.0 RESULTS

3.1 Migratory shorebirds and the Caribbean

Mean density of shorebirds observed in hypersaline lagoons in the Caribbean varied significantly as a function of Region, but not Season (Two-way ANCOVA; Region: df=1, 5, p=0.0011, Season: df=1, 5, p=0.6323), with densities higher in the Lesser than the Greater Antilles (Multiple comparison test; Fig. 4a). MHW tidal range was greater in the Lesser Antilles (One-way ANOVA, df=1, p=0.0183, Fig. 4b). Mean bird density declined significantly with MHW, but did not vary with rainfall (MHW: df=1, 5, p=0.0356, rainfall: df=1, 5, p=0.9110; Fig. 5). The mean number of bird species did not vary significantly with
either region or season (Two-way ANCOVA; all p>0.0706). The number of bird species declined significantly with MHW, but not with rainfall (MHW: df=1, 5, p=0.0488; rainfall: df=1, 5, p=0.4846), similar to the patterns observed for bird density (Fig. 5). There were no significant factor X covariate interaction effects (all p>0.06). Also, high tides range was higher within the Lesser Antilles when compare with the Greater Antilles (one-way ANOVA; MHW: df=1, p=0.0183, Fig. 4b).

Mean bird densities were generally three-times higher in the USVI than Puerto Rico, and bird densities at both islands showed a trend towards highest densities in 2012 compared to other years (Fig. 6), although the Year effect was marginally non-significant in both cases (PR: p = 0.078; USVI p = 0.084). For example, mean bird densities in Puerto Rico and the USVI did not vary significantly among Years, by Season, or their interaction (Two-way ANOVA; all p > 0.078). Similarly, the mean number of bird species did not vary significantly among Years, across Seasons, nor their interaction (Two-way ANOVA; all p > 0.084). When we compared the mean density of similar sized species by region or season, there was no significant difference in mean densities of semipalmated sandpipers or Wilson plovers by region (two-way ANOVA; sandpipers, region: df=1, 2, p=0.16; plovers, region: df=1, 2, p=0.70). The mean density of Semipalmated sandpipers were higher during the wet season than the dry season (two-way ANOVA; season: df=1, 2, p=0.0211). Conversely, mean densities of Wilson plovers were higher during the dry season than the wet season (two-way ANOVA; season: df=1, 2, p=0.02).
3.2 Shorebird prey in southwest Puerto Rico

The mean concentration of brine shrimp varied significantly according to lagoon, but not month (Two-way ANCOVA; Lagoon: df=1, 9, p=0.0484; Month: df=4, 9, p=0.1035). The mean concentration of brine shrimp was significantly higher at Jungle than Providencia (t-test, Fig. 7a). Salinity and temperature were marginally not significant, with a decrease of brine shrimp concentrations within both parameters (Two-way ANCOVA; Salinity: df=1, 9, p=0.0659; Temperature: df=1, 9, p=0.07). Mean concentrations of water boatmen decreased significantly with depth and salinity (Two-way ANCOVA; Depth: df=1, 9, p=0.02; Salinity: df=1, 9, p=0.0214, Figs. 8a, b), however, the overall model was marginally non-significant (df=1, 9, p=0.06). The mean concentration of “other prey” varied significantly among lagoons and months, with higher concentrations in Providencia than Jungle, and higher concentrations during March and July than other months (Two-way ANCOVA; Lagoon: df=1, 9, p<0.01; Month: df=4, 9, p<0.01, Figs. 7b, c). Also, the mean concentration of “other prey” decreased with depth (Two-way ANCOVA; Depth: df=1, 9, p=0.02, Fig. 8c). Biomass of brine shrimp decreased significantly during March (Two-way ANCOVA; Month: df=4, 9, p=0.05, Fig. 7d). Also, biomass of brine shrimp decreased significantly with temperature (Two-way ANCOVA; Temperature: df=1, 9, p=0.02, Fig. 8d). Statistical analyses for water boatmen biomass could not be estimated due to many missing values since this prey species was not encountered in the Jungle lagoon. Mean biomass of “other prey” varied significantly among months, with the higher biomass during July than other months (Two-way ANCOVA; Month: df=4, 9, p=0.05, Fig. 7e). There were no significant factor X covariate interactions (all p > 0.05).
4.0 DISCUSSION

Numerous species of migratory shorebirds use coastal ecosystems in the Caribbean for feeding and resting during their annual spring and fall migrations from North America. Most of these stopover sites are chosen by shorebirds for their quality in terms of prey resources, and birds often return to these sites year after year (Grear 1992, Tripp and Collazo 2003). In our study, bird density was significantly higher in the Lesser than the Greater Antilles, which suggests higher quality hypersaline lagoons for shorebirds in the Lesser versus Greater Antilles or simply the termination region for their southbound migration. Another potential explanation for greater mean bird densities in the Lesser versus Greater Antilles might simply be habitat limitation. For example, the total areal footprint of hypersaline lagoons in the Lesser Antilles is three orders in magnitude lower than the Greater Antilles (e.g., LA =0.061km² vs GA = 20.81 km²). Thus, the overall abundance of birds could be similar, but when divided by area of lagoon, the densities higher in the Lesser versus Greater Antilles. Lastly, the mean density of birds could also be skewed by the number of observers between regions, however, when we divided mean bird densities by the number of observers that generated that mean, the mean birds-unit-effort was still higher in the Lesser (0.12 birds/observer) than the Greater Antilles (0.09). Thus, the relatively high densities of migratory shorebirds in the Lesser versus Greater Antilles may simply be due to low availability of lagoons in the former.

Most lagoons within the Lesser Antilles can dry completely, therefore, changes in the ecosystems are more evident than in Greater Antilles lagoons, and this can cause great variability in density and diversity of shorebirds within regions. Mean bird density and
number of species declined with MHW. Increases in MHW may inundate coastal lagoons (depending on their direct/indirect connection to the ocean) influencing water depths and therefore, bird density and number of species. There was also a difference in tidal range between the Greater and Lesser Antilles (Fig. 4b), with greater MHW ranges in the Lesser Antilles. Since we have more bird density in the Lesser Antilles, this might suggest that most lagoons are not directly connected to the ocean, and if some lagoons are directly impacted by high tides (increasing depth), birds probably move from one lagoon to another within the Lesser Antilles.

The difference in MHW between the Lesser and Greater Antilles is likely due to the position of the amphidromic point in the Caribbean. The amphidromic point is a point of zero amplitude of the observed or constituent tide. This point is located about 200 km south of Puerto Rico and the tidal amplitude or range increases from this point, which explains the regional tidal differences.

We recognize certain caveats in applying data collected from the eBird data base in this study. First, given that both professional and recreational bird watchers provide information to the eBird database, the (1) number of observers could have varied in space and time, thereby affecting the number of bird observations per unit time, (2) observers could have made observations for a portion of lagoon only, and (3) observers could have double-counted birds (since many shorebirds stay at sites for several days). We suggest that despite these caveats, that this type of citizen-science data base provides a very broad perspective on shorebird distribution and abundance patterns that go beyond the funding level of single research programs, and should be applied as the caveats described above are controlled for.
over time. We suggest that use of a mean birds/observer (i.e., a type of catch-per-unit-effort) would be one way to control for variation in bird counts due to varying numbers of observers, making estimates of bird densities more accurate. Although the average number of observers for the Greater Antilles (290.7 observers/year reporting) is higher than for the Lesser Antilles (138 observers/year), the finding described above of a higher mean number of birds-per-observer in the Lesser Antilles suggests that despite nearly twice as many observers recording birds to the eBird data base in the Greater versus Lesser Antilles, the pattern of higher mean bird densities in the latter held.

A previous study (Wunderle 1989) of seasonal, estuarine habitat use by shorebirds in Puerto Rico suggested greater habitat use by birds during their southbound migration in Fall (wet season) than northbound migration in spring (dry season), possibly due to limited food resources during spring or dry seasons when conditions in the habitat are extreme (e.g., increased evaporation rates and therefore salinity). Our study, however, did not demonstrate a difference in shorebird density or number of species between wet and dry seasons. The concentrations of one of the preferred shorebird prey species, *Trichocorixa* spp, increases with episodes of rainfall (Tripp and Collazo 2003), and therefore should affect bird density, but our study did not find an effect on bird density or number of species with rainfall. We may not have detected an effect of season on mean bird density and number of species because if drastic changes occurred in some lagoons, they probably had the opportunity to move to another lagoon nearby.

In this study, the most common shorebird species were: ring-necked duck (*Aythya collaris*), stilt sandpiper (*Calidris himantopus*), least sandpiper (*Calidris minutilla*),
semipalmated sandpiper (*Calidris pusilla*), black-necked stilt (*Himantopus mexicanus*),
ruddy duck (*Oxyura jamaicensis*), American flamingo (*Phoenicopterus ruber*) and lesser
yellow-legs (*Tringa flavipes*). Also, the Greater and Lesser Antilles have some unique
species observed only within each region (Table 1). For instance, bird watchers observed stilt
sandpipers (*Calidris himantopus*), Wilson snipes (*Gallinago delicata*), and northern jacanas
(*Jacana spinosa*) within the Greater Antilles only. Whereas sanderlings (*Calidris alba*),
Wilson plovers (*Charadrius wilsonia*) and the green herons (*Butorides virescens*) were only
observed within the Lesser Antilles.

We found mean bird densities that were ~ 3-times higher over a five year period in
the U.S. Virgin Islands than Puerto Rico (Fig. 6). This pattern could also have been due to
habitat limitation of lagoons in the U.S. Virgin Islands (e.g., area extent of lagoons in USVI
= 0.09km², PR = 1.31km²). Further research should determine the reasons for relatively high
bird densities in the Lesser versus Greater Antilles and, perhaps could initiate such a broad-
scale study by focusing initially on a detailed comparison and contrast between Puerto Rico
and the USVI.

Previous studies identified brine shrimp and water boatmen as major prey for
migratory shorebirds (Tripp and Collazo 2003). We found brine shrimp in both of our study
lagoons in Puerto Rico, however, they were at lower concentrations in Providencia and
Jungle (Providencia: max. concentration 11.6 brine shrimp/L; Jungle: max. concentration 5.4
brine shrimp/L) than the concentrations found by Tripp and Collazo (2003) in the Cabo Rojo
salt flats (max. concentration 23brine shrimp/L). These differences of brine shrimp
concentrations might be due to their different accumulations throughout the lagoons (some
points of the lagoon would have higher accumulations of brine shrimp than others) and more samples from these patches (personal observation). Patchiness of brine shrimp may be attributed to not only wind-driven surface currents, but also variations in sun-light, since these organisms are phototactic and avoid strong and direct sun-light (Mayer 2004). Water boatmen were only found in Providencia (0.03 water boatmen/L), and in lower concentrations than found previously in the Cabo Rojo salt flats (max. concentration ~37 water boatmen/L) (Tripp and Collazo 2003). The relatively low concentrations of water boatmen in Providencia may reflect the somewhat low number of sampling points within the low salinity areas in Providencia.

Mayer (2004) documented brine shrimp can live in predator-free environments when salinities are very high; which is the case in Jungle where the mean salinity was 244 ppt in contrast to mean salinities at Providencia of 163ppt. This difference in salinity likely explains higher concentration of brine shrimp in the Jungle lagoon than in Providencia. Mean concentrations of brine shrimp decreased with salinity and temperature. In contrast, the mean concentrations of brine shrimp did not vary with salinity in the Cabo Rojo salt flats (Tripp and Collazo 2003).

Previous studies found that water boatmen cannot tolerate salinities >65ppt (Grear 1992, Tripp and Collazo 2003), which is consistent with results from this study in which we found that the concentration of water boatmen decreased with increasing salinity. The concentration of water boatmen in Providencia declined with water depth, which may have been because they were only found in the most easterly crystalizers of Providencia where salinities fluctuate greatly with storm-water runoff.
*Ephydra* spp. is usually present in salinities ~150ppt (Jones & Stokes Associates 1993), which probably explains why we found higher concentration of “other species” (*Ephydra* spp. larvae) in Providencia, where averaged salinity was 163ppt, than in Jungle where salinities were much higher. Temperature affects most of the life cycle of *Ephydra* spp, controlling hatching, growth, development, pupation, metamorphosis and egg laying. Probably, during March, habitat conditions were optimum (e.g., temperatures ~25°C, salinities ~150ppt) (Wirth 1971) and the production of larvae increase. This might also explained the increase in biomass during July as well.

The results of this study will help understand the ecosystem function of hypersaline lagoons in supporting migratory shorebird populations throughout the Caribbean region. We identified relatively high densities of shorebirds in hypersaline lagoons in the Lesser Antilles, however, the mechanisms underlying this pattern are unclear. Within Puerto Rico, the Providencia lagoon has the potential to support good prey resources for migratory shorebirds, especially if the salinity in this lagoon can be reduced to favor water boatmen prey, which provide a net caloric advantage to shorebirds over brine shrimp. This study will contribute to the growing literature on the ecology and restoration of hypersaline lagoons in tropical and arid ecosystems.
5.0 REFERENCES


Table 1: Unique species observed by region.

<table>
<thead>
<tr>
<th>Greater Antilles unique species</th>
<th>Lesser Antilles unique species</th>
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<tr>
<td><em>Calidris himantopus</em></td>
<td><em>Anas acuta</em></td>
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<tr>
<td><em>Dendrocygna arborea</em></td>
<td><em>Anas clypeata</em></td>
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<tr>
<td><em>Gallinago delicata</em></td>
<td><em>Ardea herodias</em></td>
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<tr>
<td><em>Pardirallus maculatus</em></td>
<td><em>Bartramia longicauda</em></td>
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<td><em>Rallus longirostris</em></td>
<td><em>Butorides virescens</em></td>
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<tr>
<td><em>Chlidonias niger</em></td>
<td><em>Calidris alba</em></td>
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<tr>
<td><em>Chordeiles gundlachii</em></td>
<td><em>Calidris fuscicollis</em></td>
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<td><em>Ixobrychus exilis</em></td>
<td><em>Calidris melanotos</em></td>
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<td><em>Jacana Spinosa</em></td>
<td><em>Calidris minutilla</em></td>
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<td><em>Leucophaeus atricilla</em></td>
<td><em>Calidris pusilla</em></td>
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<td><em>Numenius phaeopus</em></td>
<td><em>Charadrius wilsonia</em></td>
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<td><em>Plegadis falcinellus</em></td>
<td><em>Egret caerulea</em></td>
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<td><em>Porzana carolina</em></td>
<td><em>Egretta thula</em></td>
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<td><em>Sula leucogaster</em></td>
<td><em>Egretta tricolor</em></td>
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<td><em>Tringa solitaria</em></td>
<td><em>Fulica americana/caribaea</em></td>
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<tr>
<td><em>Branta bernicla</em></td>
<td><em>Nomonyx dominicus</em></td>
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<tr>
<td><em>Chordeiles sp.</em></td>
<td><em>Nyctanassa violacea</em></td>
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<tr>
<td><em>Gelochelidon nilotica</em></td>
<td><em>Phoenicopterus ruber</em></td>
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<td><em>Mergus serrator</em></td>
<td><em>Podilymbus podiceps</em></td>
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<td><em>Nycticorax nycticorax</em></td>
<td><em>Puffinus herminieri</em></td>
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<tr>
<td><em>Phalaropus tricolor</em></td>
<td><em>Tringa semipalmata</em></td>
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<tr>
<td><em>Recurvirostra americana</em></td>
<td><em>Stern hirundo</em></td>
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<tr>
<td><em>Sula sula</em></td>
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Figure 1: Study area with focal islands named.
Figure 2: Bird density relationships with rainfall and high tides within lagoons in the Caribbean.
Figure 3: Providencia and Jungle lagoon study sites in Puerto Rico.
Figure 4: A: Mean bird density between Lesser and Greater Antilles (error bars=Standard Errors). B: MHW difference between Greater and Lesser Antilles.
Figure 5: The relationship between mean bird density and MHW across regions. The black, round-data points are the mean bird density from original data. Grey diamond data points are the predicted values generated by the ANCOVA model, which takes into account all the factors within the model, as well as the linear regression line (Region: p=0.0011; MHW: p=0.0356).
Figure 6: Mean bird density (+SE) among years in Puerto Rico (A) and U.S. Virgin Islands (B).
Figure 7: Shorebird prey concentrations and biomass across lagoons and months. Least Square Means and their Standard Errors were used. A, B, C: Calculations were derived from transformed data Log(x+1) predicted values within ANCOVA model. D: Calculations were derived from Ln(x) -transformed data. E: Calculations derived from the original data.
Figure 8: Shorebird prey concentrations and biomass versus water quality parameters. A, B: Plotted points were derived from original data predicted values within ANCOVA model (Depth p=0.0230; Salinity p=0.0214). C: Plotted points were derived from Log(x+1) transformed data predicted values from the ANCOVA model (Depth= 0.0248). D: Plotted data points were derived from Ln(x) transformed data predicted values from the ANCOVA model (Temperature p=0.0199).