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Population Biology Of A Coastal Dune-Dwelling Spider (Arctosa Sanctaerosae) Along The Hurricane Disturbed Northern Gulf Of Mexico Coast

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POPULATION BIOLOGY OF A COASTAL DUNE-DWELLING SPIDER (ARCTOSA

SANCTAEROSAE) ALONG THE HURRICANE DISTURBED NORTHERN GULF OF

MEXICO COAST

A dissertation

presented in partial fulfillment of requirements

for the degree of Doctor of Philosophy

in the Department of Biology

The University of Mississippi

by

ROBERT ANDREW HATAWAY

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ABSTRACT

With the continued increase in the number of tourists visiting the Northern Gulf coast in the last century and the resulting development of this coastline the habitat of *Arctosa sanctaerosae* has become fragmented; and the sprawl of large cities along the coast has further degraded available habitat. In addition to anthropogenic disturbance to this coastal region, hurricanes are an additional and natural perturbation to the ecosystem. This habitat has seen a number of major tropical storms over the last decade and I have sought to explore the impact of habitat destruction and storm-induced disturbance on a species of spider endemic to the coastal dunes of the Northern Gulf Coast. *Arctosa sanctaerosae*, family Lycosidae, is a wolf spider endemic to the secondary dunes of the white sandy beaches of the Northern Gulf of Mexico. The data presented here explore the status of populations of this species spanning the entire known range and the factors influencing population demography. These findings demonstrate the significant impact of storms on both disturbed and undisturbed habitat and reveal factors influencing the recovery of the spiders, relating this to ecological factors including the height of the dunes and density of vegetation before and following hurricanes Ivan and Katrina. These results reveal habitat characteristics that appear to play a large role in population persistence and components of human disturbance of habitat that have the greatest impact on populations of spiders. Using microsatellite markers I characterize the current structure of the subpopulations of *Arctosa sanctaerosae,* and current and historical patterns of interpopulation migration. Contemporary modeling methods compare current and historical levels of gene flow and document the decline in migration due to habitat fragmentation. Since the introduction of dense

human development along the Northern Gulf of Mexico Coast, *Arctosa sanctaerosae* has seen what appears to have been a single, contiguous population subdivided and the isolates reduced in size. These results point to the need for further exploration of the status and continued monitoring of the species.

I would like to dedicate my dissertation work to my family.

My wife and parent's unwavering confidence in my abilities

has pushed me to complete this goal and for that I am thankful.

My sister and brother-in-law have been there for me

throughout the entire doctorate program.

I also dedicate this dissertation work to Dr. Ronald Jenkins

whose support and guidance made it possible.

LIST OF ABBREVIATIONS AND SYMBOLS

NGC	Northern Gulf Coast
A. sanctaerosae	*Arctosa sanctaerosae*
A. littoralis	*Arctosa littoralis*
m	Meter
mm	Millimeter
m^2	Square meter
St.	Saint
ABM	Alabama Beach Mouse
et al.	Latin *et alia*, and others
AL	Alabama
FL	Florida
MS	Mississippi
Ft.	Fort
ANOVA	Analysis of variance, a statistical method
R	Growth rate
ln	Natural logarithm
log	Logarithm
N_0	Size at time step zero
N_1	Size after one time step

dh	Dune height
gps	Global positioning system
N	Sample size
p	P-value, a statistical term
S.E.	Standard error
adj	Adjusted
AIC	Akaike Information Criterion
w	Akaike Information Criterion model weight
km	Kilometers
R^2	Regression statistic
e.g.	Latin *exemli grati*, for example
DNA	Deoxyribonucleic acid
bp	Base pairs
SDS	Sodium dodecyl sulfate
SSC	saline-sodium citrate
μL	Microliter
TLE	Tris low Ethylenediamine tetra-acetic acid
dNTP	Deoxyribonucleotide triphosphate
$MgCl_2$	Magnesium chloride
mg	Milligram

°C	Degrees Centigrade
No.	Number
F_{is}	Inbreeding coefficient
He	Expected heterozygosity
Ho	Observed heterozygosity
$\bar{x}$	Mean
PCR	Polymerase chain reaction
v.	Version
ABI	Applied Biosystems Incorporated
STR	Short tandem repeat
HWE	Hardy-Weinberg equilibrium
WSR/PK	West Santa Rosa Island/ Perdido Key
HSP	Henderson State Park
sec	Seconds
min	Minutes
MCMC	Markov chain Monte Carlo, a statistical method
K	Number of genetically unique subpopulations
F_{st}	Fixation index
A_r	Allelic richness
H_s	Gene diversity

M	Molar
Ne	Effective migration rate
C.I.	Confidence interval
ID	Identification
etc.	Latin *Et cetera*, and so on

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INTRODUCTION

With the continued increase in the number of tourists visiting the Northern Gulf coast in the last century and the resulting development of this coastline the coastal dune ecosystem has become fragmented; and the sprawl of large cities along the coast has further degraded the available habitat. In addition to anthropogenic disturbance to this coastal region, hurricanes are an additional perturbation to the ecosystem. This habitat has seen a number of major tropical storms over the last decade and I have sought to explore the impact of habitat destruction and storm induced disturbance on a species of spider endemic to the coastal dunes of the Northern Gulf Coast. *Arctosa sanctaerosae*, family Lycosidae, is a wolf spider endemic to the secondary dunes of the white sandy beaches of the Northern Gulf of Mexico. The data presented here explore the status of populations of this species spanning the entire known range and the factors influencing population demography.

This study provides a clearer picture of the status and distribution of *A. sanctaerosae* along the beaches of the Northern Gulf of Mexico. *A. sanctaerosae* was found in all three states surveyed, with highly variable densities (Figure 2). This study suggests that when dune systems are compromised by commercialization, however slightly, the habitat of *A. sanctaerosae* is compromised, causing a significant decrease in density. The most severe reductions in population density are in areas where the density of human commercial development is highest.

The impact of hurricanes on the density of *A. sanctaerosae* is both direct and density independent. Distance from landfall of a hurricane predicts 64 % of the density reduction seen after the storm while ecological factors appear less useful as a predictor of effects of storms. Dune height after storms was the most significant single factor in determining the rate of recovery in the year following a major tropical storm.

Analyses of population structure recovered five distinct subpopulations along the NGC. The observed genetic variation follows a distinct pattern, decreasing from east to west. Initially, the distribution of sites into the 5 clusters appears not to be driven by biologically significant factors. For example, sites that were found on the same island (Navarre and Ft. Pickens) did not cluster together, but separately with sites found on the mainland. It is not until commercial development is overlaid with the geography that the clusters recovered demonstrate an intuitive pattern. For example, Gulf Shores and Orange Beach AL separate cluster number 1 from 2.

The genetic isolation seen in the subpopulations of *Arctosa sanctaerosae* is due to reduced gene flow and subdivision of the previously panmictic population. Allelic richness and gene diversity are highest in the east and decrease as you move westward. The three most likely explanations for the higher genetic diversity seen in the eastern clusters are the relatively few numbers of hurricanes to make landfall in the east in recent times, significantly fewer large scale commercial developments, or the possibility that *A. sanctaerosae* has expanded its distribution westward over time from some site in the east where it could have possible evolved.

There has been a significant decrease in the amount of migration between the five subpopulations within the last 100 years. This lack of migration in the recent past combined with the correlation of the patterns of genetic structure and human encroachment found across clusters

supports placing the timing of the subdivision to within the last 100 years. This corresponds to the time frame in which the commercialization of the NGC began.

The effective population sizes of the clusters of *A. sanctaerosae* along the NGC were significantly smaller than the census sizes. This is to be expected given the high levels of inbreeding seen in the clusters, though this may be an artifact of non-random sampling.

There is a significant difference in historic effective population sizes compared to estimates of recent N_e. The test of M ratios demonstrated recent severe reductions in population size in clusters 1, 2 and 3. The clusters have been reduced from some previous larger size to their current effective sizes. This timing appears to correlate with increases in commercial development of the NGC.

Each of the clusters has experienced a decline in effective population size and migration within the last 100-500 generations. *A. sanctaerosae* maintains high levels of inbreeding and low levels of dispersal normally. These clusters experience severe reduction in size due to hurricanes or other catastrophic events. Hurricanes remove genetic diversity but historically this effect was ameliorated thru migration across the NGC to fill in vacant patches. Recently, these populations have been reduced in size and diversity as a direct result of subdivision of the NGC. Once subdivided, the migration between clusters was reduced and genetic drift, direct removal of diversity due to severe tropical storms and commercial development has lead to declining population sizes and reduced diversity.

CHAPTER 1. POPULATION BIOLOGY OF A COASTAL DUNE-DWELLING SPIDER, *ARCTOSA SANCTAEROSAE*.

Introduction

The subdivision of large populations into smaller subpopulations is a common occurrence and can be caused by behavioral, geographic or ecological factors. Once subdivided, persistence of these subpopulations is directly related to their size and connectivity to one another. The persistence of populations is determined by random genetic drift, directional selection, and mutation. High levels of effective migration between these subpopulations homogenize the genetic diversity across them. This migration can provide a rescue effect for smaller subpopulations on the brink of local extinction. Metapopulations are a type of subdivision of a larger population in which the subpopulations are distinct and there is an availability of unoccupied habitat. Species occupying habitat patches that fluctuate in size, extent, or condition temporally can experience corresponding changes in spatial distribution and density (Carlsson and Kindvall 2001; Van Horne et al. 1997).

Human degradation of habitat can interrupt migration between subpopulations through fragmentation and can influence population viability (e.g., Reed et al. 2003; Reed 2004). This human degradation represents a novel disturbance to species that evolved in the absence of anthropogenic factors (Pickett & White 1985). Organisms that occupy habitats frequently subjected to natural disturbance may evolve specific dispersal mechanisms or produce resistant stages of growth that ameliorate the possible effects of the disturbance. The high rate at which human encroachment occurs may prevent the evolution of behaviors of life history traits to avoid

extinction or extirpation (Boulding & Hay 2001; Stockwell et al. 2003). For example, specific dispersal mechanisms in habitats frequently subjected to the same type of environmental disturbance with the effect of efficiently re-colonizing habitat patches where local extinction has occurred. The inclusion of greater fragmentation at such a high pace may interact with the natural disturbance to reduce the effectiveness of the dispersal mechanisms making the organism unable recolonize the vacated habitat patches. Naturally occurring extreme environmental perturbations (catastrophes) have a profound the persistence time of populations/species (Lande 1993; Mangel & Tier 1993; Young 1994; Spiller et al. 1998; Brooks & Smith 2001; Schoener et al. 2001; Reed et al. 2003; Reed 2007). Gaining insight into the effects of environmental perturbations, anthropogenic encroachment, and genetic forces would be invaluable for setting up and achieving the long-term conservation goals of the species in affected areas.

The burrow-dwelling wolf spider, *Arctosa sanctaerosae* (Araneae: Lycosidae) was chosen as an example organism. Using *A. sanctaerosae*, I was able investigate the effects of habitat fragmentation (human encroachment) and a catastrophe regime (severe tropical storms) as well as the interaction of these natural and anthropogenic disturbances. This species is an ideal subject to explore these questions, as it is entirely restricted to the secondary dunes in the coastal dune system of the Northern Gulf of Mexico Coast (NGC). Furthermore the beach, and by extension its dune systems, is a simplified ecological system where critical habitat features can be correlated with population demography. Given the general lack of invertebrate conservation work (Skerl & Gillespie 1999) and the discrete generation length that spiders have, this taxon, interesting in its own right, will also provide insight into threats faced by other invertebrates and small vertebrate species of interest in the region (e.g. several species of the beach mouse *Peromyscus polionotus*).

A known endangered species of beach mouse, *Peromyscus polionotus ammobates*, is endemic to the costal dune system of the Alabama Gulf Coast. This species is under the same pressures as the model system chosen, including habitat fragmentation, and is presented with the same amounts of perturbation by hurricanes. The Conservation Breeding Specialist Group of the International Conservation Union was invited to conduct a population habitat viability analysis workshop for the Alabama Beach Mouse (ABM). The model suggests that population dynamics, given current habitat restrictions, are driven by the frequency and severity of the hurricanes (Traylor-Holzer et al. 2005). This illustrates the need for further studies of NGC endemics and the interaction of hurricanes and habitat fragmentation

Arctosa sanctaerosae is found on secondary dunes of the NGC (McNatt et al., 2000)), which are found inland from primary dunes and the high tide mark. The coastal dune system is one of the most dynamic and fragile ecosystems (Stalter & Odum 1993). Dunes are formed through sediment being deposited on the active beach by wave action. Once dry, wind blows this sediment inland and deposits it on the plants and debris that slow the wind. Once small mounds form, the wind deposits more sediment on them until finally an entire system of dunes covered in habitat-specific species of plants evolves. These plants help to hold and stabilize the dune system. A healthy dune system is made of a primary dune located near the water, followed by secondary and tertiary dunes. Secondary dunes can be recognized by their vegetation such as sea oats, *Uniola paniculata*, which primary dunes lack. The tertiary dunes, located further inland, have a shrub community made of yaupon (*Ilex vomitoria),* myrtle (*Myrica cerifera)*, and other characteristic flora (Figure 1). The maintenance of beaches is dependent upon a well-preserved and contiguous dune system as the dunes are the first line of defense against erosion (Stalter & Odum 1993).

Figure 1 Complete Dune System including primary secondary and tertiary dunes
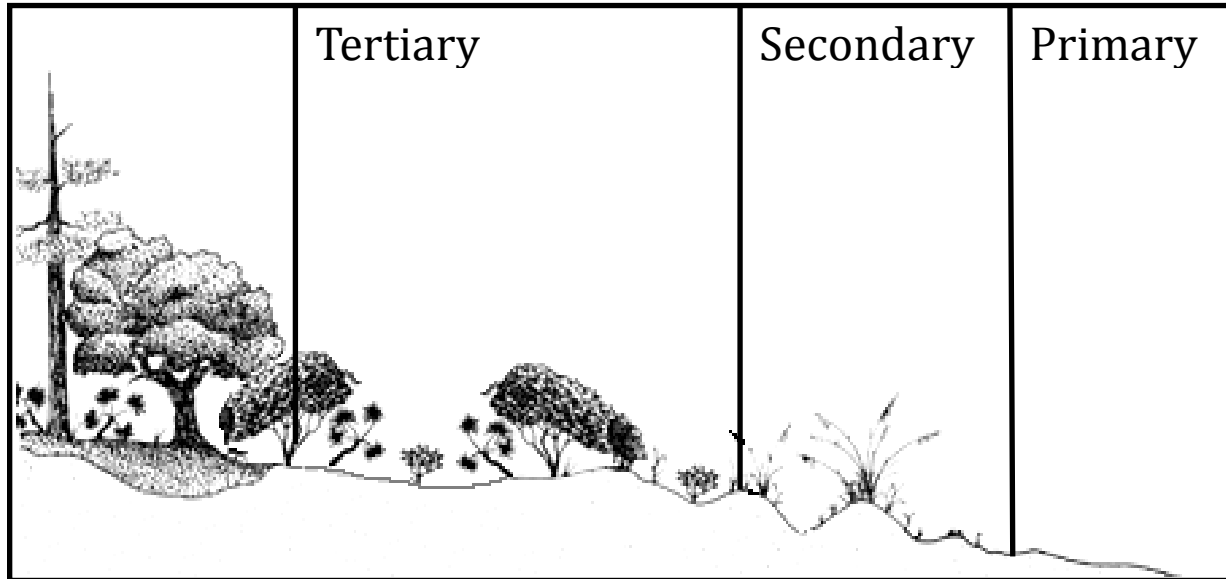
The same wind and water that create this habitat cause it to be extremely dynamic. Erosion is the major factor that affects the evolving shape of coastal dune systems. Vegetation and the continuity of the dune system are its only defenses against the powerful forces of erosion. Once interrupted, the dunes can shrink and erode away causing the beach to recede and disappear (Watson 2003). In the Northern Gulf of Mexico Coast (NGC) ecosystem hurricanes can, through erosion, amplify the small breaches in the continuous dune system caused by human encroachment in the form of walking paths, vehicle paths, and construction, ultimately degrading beaches and threatening the existence of a number of animal and plant species. When a hurricane makes landfall, water is washed onto the dunes through these small breaches causing severe erosion between the dunes. This process can create what is called a "blow out". A blow out is defined as an area of the dune system that has been breached and washed out to sea with the receding storm surge leaving the area flat and removing the vegetation in the process. If severe enough a blow out can result in an island being cut in half if the dunes and beach are not

replenished (United States 2001). It is this interaction between anthropogenic and natural perturbations that threaten the continuity and safety of this dune community.

Human population growth in coastal areas is placing pressure on coastal species at an alarming rate globally. Douglass et al. outlined the most recent trends concerning the dune systems in Alabama (1999). From 1970 to 1996, the percentage of undeveloped Alabama Beaches declined from 72% to 39% as residential and commercial development increased. Unencumbered by anthropogenic influence, beaches are dynamic, changing shape and extent as storms; wave action and wind patterns change. Human modifications severely limit this ability to vary naturally (Nordstrom, 2000) or can amplify the impacts of naturally occurring stochastic disturbances (Jonzen et al. 2004 and Schrott et al. 2005). These modifications include alteration to the supply and transport of the sand as well as climate change-induced sea level and surface temperature rise (Komar, 1998). This surface temperature rise is predicted to increase the severity of tropical storms (Slott et al., 2006).

There is now a global trend towards coastline erosion making way for the inland migration of beaches. This migration inland is blocked by human infrastructure. Current management of coastal erosion does not allow for this migration of the beaches but rather attempts to reverse it through engineered solutions of artificial re-nourishment or by fortifying the shoreline with hard structures to prevent erosion. These management strategies restore the geology through dredging and bulldozing to replace or retain the sandy substrate. Beach re-nourishment has proved too slow or reverse the effects of erosion (Peterson et al. 2000). However, these habitat conservation measures can have substantial consequences on the endemic biota including loss of biodiversity, productivity of the ecosystem, and loss of critical habitat including the sub-tidal zone, which, is critical for recruitment for beach animals (Peterson &

Bishop, 2005; Dugan & Hubbard, 2006; Peterson et al., 2006; Speybroeck et al., 2006). Re-nourishment at Perdido Key, Florida, USA, led to sustained (2 years) negative impacts on the macrobenthic community (Rakocinski et al. 1996).

The role of hurricanes and other severe tropical storms as catastrophic disturbances and their effect on population dynamics is a concept that has been of theoretical interest, but there is little empirical data. These natural disturbances are stochastic events that have the ability to restructure habitats and whole ecosystems through the movement of wind, water, and substrate materials. Disturbance regimes in ecosystems are important aspects of organisms' ecology. Severe perturbations (catastrophes) are thought to have profound impacts on extinction risk but there have been few studies that look at the role of hurricanes as a form of disturbance regime and their role at the individual as well as population levels (Lande 1993, Reed et al. 2003).

The frequency and the wide geographic reach of hurricanes suggest that endemics to storm affected areas should evolve life histories that enable them to ameliorate the effects of these disturbances. The distance from the point of landfall of the hurricane should explain the intensity of effects, with the most severe effects experienced closest to the point of landfall and decreasing with increased distance from the storm. The spatial heterogeneity in hurricane impacts suggests that spatial autocorrelations in population fluctuations (Burgman et al. 1993; McCarthy & Lindennayer 2000; Reed 2004) might be especially important to metapopulation persistence in this system. Because hurricanes reduce population size via the direct destruction of habitat, results from one habitat-specific species with similarities in vulnerability to storm-driven mortality should be relevant to the persistence of all species limited to that habitat.

Few studies have examined the direct and indirect effects of hurricanes on populations. Avian studies generally consider the effects on residential and migratory species separately.

Studies have demonstrated that within 18 months of a hurricane, resident bird populations have fully or nearly recovered (Askins and Ewert 1991, Waide 1991, Wunderle et al. 1992, Lynch 1991, Willig 1991).

Woolbright found that the number of forest frog, *Eleutherodactylus coqui*, increased fourfold one year after Hurricane Hugo, a category 5 hurricane, in Puerto Rico. The factor leading to such a large increase in population size was the creation of critical habitat through the deposition of debris on the forest floor due to the destruction of the canopy (Woolbright 1991).

The effect of Hurricane Hugo on the canopy dwelling Anoline lizards of Puerto Rico was that of a compressed habitat. During the period just after the hurricane these three species, which previously were vertically stratified in their niches, were forced to cohabitate on the forest floor and the two species best adapted for this habitat thrived. However, by the end of the first year after the storm populations were returning to their normal distributions and sizes (Reagan 1991).

Spiller et al. studied the effects of hurricanes on island populations of Anoline lizards as well as web spiders following Hurricane Lili in 1996 (a category 4 storm) on 19 islands near Great Exuma, Bahamas. This study re-surveyed populations of spiders and lizards following Hurricane Lili (both days later and one year later). After surveying, the lizards were assumed to be completely removed (with the exception of the offspring still in their eggs) from the islands. The spider populations were decimated to the point of local extinction on the islands but re-colonized these habitat patches quickly compared to the lizards in a period of one year. This rapid recolonization of the empty habitat patches by the web spiders was attributed to over-water dispersal while the lizards were assumed to repopulate the patches using the surviving eggs (Spiller et al. 1998 and Schoener et al. 2001).

In each of the studies, there were adaptations in survivorship of young in eggs, dispersal techniques, and flexibility in niche that allowed them to recover from severe tropical storms quickly with the speed of recovery often similar to the unaided recovery of the habitat.

Records have been kept of the intensity and location of hurricane landfall for over a hundred years. In that same period, the human population of the major cities of the coastal region has increased approximately 15 fold (from 10,000 individuals to 150,000 according to United States Census data). The NGC has had 15 hurricanes of category 3 or higher make landfall in the last 100 years. Major tropical storms that have made landfall in the study area region include: Audrey (1957), Hilda (1964), Betsy (1965), Camille (1969), Carmen (1974), Eloise (1975), Fredrick (1979), Elena (1985), Andrew (1992), Opal (1995), Ivan (2004), Dennis (2005), Katrina (2005) and Rita (2005). All of these storms with the exception of hurricane Camille (a category 5) were category 3 storms when they made landfall along the NGC. This study focused on the most recent major hurricanes (3+ on Saffir-Simpson scale) that had the most direct impact on the sites region, Ivan and Katrina.

Arctosa sanctaerosae (Gertsch 1935, Gertsch and Wallace 1935) is a wolf spider of the family Lycosidae that inhabits only the white sandy beaches of the NGC. Lycosidae are defined by the presence of an anterior row of four small eyes, as well as a recurved posterior row of four larger eyes, and the presence of three claws on the last segment of the leg (Kaston, 1978). They are wandering hunters with excellent eyesight. Their prey capture methods vary from opportunistic hunting to waiting for prey to pass by the opening of the burrows that several species of this family make. Lycosid females are known to carry their egg sac with their spinnerets until fully developed. At this point, the spider allows the young to live on her back

until they disperse. The Lycosid family is made up primarily of nocturnal hunters that do not use entrapments (i.e. webs).

Arctosa sanctaerosae is off-white to tan in hue, thereby camouflaging it on the sandy substrate where it lives, and possesses a darker anterodorsal abdominal mark. Dondale and Redner (1983) defined the characteristics specific to this species, including the presence of three teeth on the promargin of the cheliceral fangs and a single dorsal macro seta on Tibia I. It can be easily distinguished from its closest relative, *A. littoralis* (Hentz 1884), by its lack of color, leg banding, and dorsal abdominal pattern. Males range in size from 8.2-13.2 mm while females are 10.9-12.0mm. *A. sanctaerosae* is unique in its narrow habitat preference and range. They are found primarily on the secondary dunes in burrows they create in the sand. This species' range was described roughly as being from the panhandle of Florida to the western border of Mississippi (Dondale & Redner 1983)(Figure 2).

Figure 2 County map showing geographic range of *A. sanctaerosae*. All counties in which *A. sanctaerosae* was found are shaded however, individuals were only found on the coastal secondary dunes of said counties.
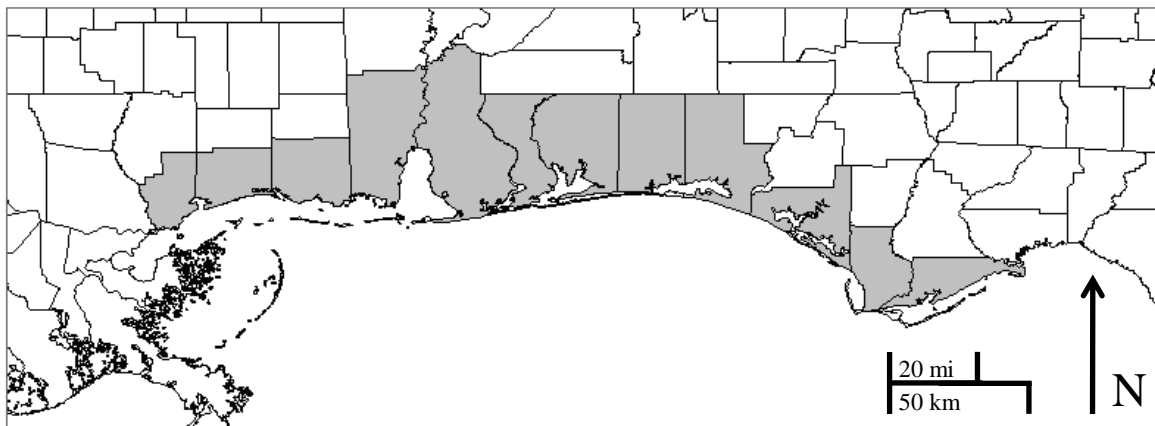
The purpose of this study was threefold: (1) document the current range and densities of the wolf spider (2) examine the effects of beach development and beach stability with population persistence and density of *A. sanctaerosae* (3) explore the effects of severe tropical storms on spider density, and relate these to the physical attributes of the dune system, extent of disturbance and the distance from the point of landfall of the tropical storm.

Methods

Density measures

Density measures of *A. sanctaerosae* were made one hour after nightfall on clear nights inside 12m by 12m quadrants ($144m^2$) between June 4th and 14th, 2004. Quadrat perimeters were marked during the day with four stakes and string. Based on the findings of McNatt et al. (2000), which described basic life history, and habitat preferences of this species, counts of individual spiders were made on the secondary dunes found at each of the sites. Locating the wolf spiders was easily done at night due to the green reflectance of the tapetum of their eyes making them stand out in the darkness. Once a spider was spotted using a headlamp the species identity was verified. The time, date, total number of individuals counted, and coordinates of the quadrat were recorded. A single voucher specimen (1 female) was deposited at the Mississippi Entomological Museum.

Survey of range and impact of development

A system was created to classify the amount of ecological damage to the beach habitat by assigning beach classifications according to the extent of human impact. Twenty-one sites from Bay St Louis, Mississippi to St. George Island, Florida were surveyed. The sites were chosen to

produce equal numbers of the three beach quality categories within the total geographic area surveyed. Both mainland and barrier islands were surveyed in AL and FL while only the mainland was studied in MS due to logistical difficulties in reaching the MS barrier islands. A beach quality ranking of "1" was assigned to the least disturbed habitats that have maintained dune integrity by minimizing erosion and commercial encroachment (i.e. National Parks and National Wildlife Refuges). Healthy populations of sea oats and other vegetation stabilize the rate of natural dune erosion and maintain dune integrity at these highest quality sites. A quality ranking of "2" was assigned to moderately commercialized beaches. Dunes with breaks in their length, such as walking trails, single-family homes, or the disappearance of the vegetation characterize these beaches. In these cases, dunes have usually begun to partially erode due to the loss of the tertiary dunes or pine forests, thereby allowing wind to blow inland unimpeded. From a development aspect, these beaches can be characterized by single-family homes or heavily used state parks and recreation areas. Heavy commercialization and destruction of natural dune systems characterized the third type of habitat ("3"). In these, the berm or primary dunes are overrun with commercial features and secondary dunes were often removed. Sites and their quality ranking are listed in Table 1.

A one-way ANOVA was proposed for the analysis of the variation in density of *A. sanctaerosae* surveyed using the beach quality as an independent variable. The analysis of variance is robust to heterogeneity of variances when sample sizes are equal or nearly equal. If these assumptions are not met and the larger variances come from the samples with larger size then the probability of a type one error will be greater than alpha (Zar 1999). Levene's test (Levene 1960) was used to determine the level of homogeneity of variances. Levene's test was used in favor of the Bartlett test due to its insensitivity to departures from normality. The

assumption of equal variance between groups was violated. Thus a nonparametric, Kruskal-Wallis, test was employed to determine if there was a significant difference between the means of all categories. The nonparametric Mann-Whitney test was used to determine pairwise differences in means among beach categories.

Decline and recovery following severe tropical storms

With these data I also sought to explore the population dynamics of spiders with regard to the physical attributes of the dune system before and after tropical storms. To do this, a subset of 10 of the highest quality sites from the original the 21 sites were chosen. This was done to remove the assumed effects of human conversion of habitat. Data was collected between 2004 and 2007 quantitatively assessing the size and health of both the dune system and the spider populations. Density measures were made one hour after nightfall on three consecutive clear summer nights inside 3 independent 12m by 12m quadrants ($144m^2$) randomly placed within the secondary dunes of the site. These quadrants were relocated each year using GPS data and resampled. Growth rate (R) was calculated as R= ln (N_1/N_0) for each site, N_0 represents the mean density across nights and quadrants for a given site at time point 0 while N_1 represents the mean density measured in the same way for the same site after one year. These rates were calculated for comparison in periods before and after landfall of a major hurricane. All density estimates of zero were changed to 0.5 to ease statistical analysis based on the assumption that these population numbers were most likely not zero but too small to be detected.

Table 1 Site location and Ranking of Beach Quality

Location	State	GPS Data		Ranking of Beach Quality
		°N	°W	
American Legion Pier, Bay of St. Louis	MS	30.30641	89.3283	3
Jimmy Rutherford Fishing Pier, Bay of St. Louis	MS	30.31358	89.32397	3
Public Beach, Pass Christian	MS	30.30694	89.28545	2
Pitcher Point, Long Beach	MS	30.33581	89.17561	2
Public Beach, Biloxi	MS	30.39501	88.91071	3
Gulf Park Estates, Fontainbleau	MS	30.34696	88.74271	2
Public Beach Park, Pascagoula	MS	30.34378	88.53374	3
Dauphin Island Sea Lab, Dauphin Island	AL	30.24666	88.08306	1
Ft. Morgan, Bon Secor	AL	30.22378	88.00841	1
Pine Beach, Bon Secor	AL	30.22948	87.82775	1
Orange Beach	AL	30.27775	87.535	3
Condo next to Perdido Key area, Approx. 50m	FL	30.29776	87.41977	3
Perdido Key Area, Gulf Islands National Seashore	FL	30.29844	87.41816	1
Ft. Pickens, Pensacola Beach	FL	30.32523	87.18689	2
Gulf Islands National Seashore, Navarre Beach	FL	30.37146	86.92539	2
Destin Point, Public Beach	FL	30.39178	86.51043	2
Destin Point, Condo next to Public Beach	FL	30.39027	86.50888	3
Henderson State Park, Destin	FL	30.35619	86.26646	1
Condo next to Henderson State Park	FL	30.38224	86.43166	3
Public Beach Access, St. Joe	FL	29.88353	85.35449	1
St. George Island	FL	29.61541	84.95381	2

Physical measures of dune height and amount of vegetation were collected from all sites to test for possible correlates to population density. Dune height (dh) and the density of vegetation were also quantified within the 12 X 12 m transects from 2004-2007. These two measures were included due to their provision and maintenance of habitat for *A. sanctaerosae* as well as prey. Vegetation cover of dunes was quantified by counting the number of stems or shoots of sea oats and similar vegetation within three independent, randomly chosen one square meter quadrants within these 12 X 12 m quadrants on each of the three sampling nights per site (a total of 9 vegetation density quadrants). Dune height was measured from the height of the

apparent high tide mark on the active beach. This height was not a static measure over time due to the change in tide levels; however, this cannot be avoided. Other methods for measuring elevation (GPS, altimeters, clinometer) are not sensitive or accurate at the scale needed to capture the dynamic nature of the habitat. The methodology created for measuring dh includes running a level string from the top of the secondary dune to the high tide mark. At the high tide mark the string intersects a 3m vertically oriented dowel marked incrementally. Where the line intersects the dowel is the height of the secondary dune (Figure 2).

Figure 3 Methodology for Measuring Dune Height
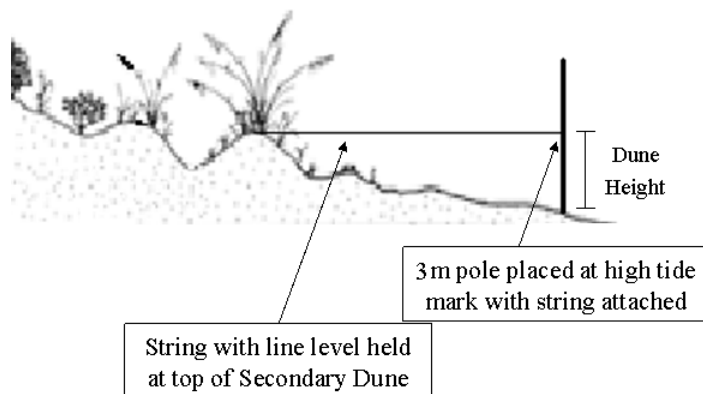
Models selection was accomplished using an information-theoretic approach. Stepwise multiple regression was subsequently used to explore the relationships among these ecological factors and changes in them, as a function of hurricane landfall, associated with each beach site in order to identify those that best explained the variation in population decline and recovery observed after each of these two hurricanes.

Results

Current Range and impact of development

The sampling of sites from the western border of Mississippi to Saint George Island, Florida showed *A. sanctaerosae* present wherever there was a dune system with secondary dunes. While this range of the species appears large, the available suitable habitat along this range is quite limited and highly fragmented. The majority of high quality habitat is limited to areas protected by governmental agencies (National and State Parks as well as a National Wildlife Refuge).

The density of individuals ranged from 0.06 to 0.36 m^2 in 2003. This year was used as the point for comparisons among habitat types and between states due to the time since the landfall of the last major tropical storm. It had been eight years since the landfall of Hurricane Opel. Comparing the densities of spiders on secondary dunes by state and moving from west to east, Mississippi's densities ranged from zero in the most impacted areas to 0.04 spiders per m^2 in areas of natural beach found in Fontainebleau, Mississippi.

The habitat found in Alabama on the mainland that includes these full healthy dune systems allows for the presence of larger populations of spiders ranging from 0.26 individuals per m^2 in the Bon Secour National Wildlife Refuge to as low as zero in the most commercialized areas such as Orange Beach. There was an inverse relationship between the density of individuals and the extent of beach development. The beaches of Florida followed similar trends identified in Alabama. The density of spiders varied from 0.36 to zero, depending on the amount and type of commercial development.

When viewed from a land use perspective, Mississippi's mainland coastal beaches were generally classified as belonging to the third, or most disturbed, category, while most of the

beaches in Alabama and Florida were classified as moderately disturbed (category 2). In all cases, the highest quality beaches were found in National Wildlife Refuges and National Parks, the majority of which were classified in the first, or least disturbed, quality category, with some light erosion and degradation of the dune system.

Statistical comparisons were made between the beach quality categories. A difference in mean number of spiders per quadrant was found to be statistically significant for all pairwise comparisons of the categories. The most significant difference was found between the sites with little or no human impact and the heavily developed beaches (Mann-Whitney, $N = 12$, $p = 0.002$). The difference between means of the low impact and moderately developed sites (Mann-Whitney, $N=13$, $p = 0.010$) as well as the difference between means of the moderately and heavily developed sites (Mann-Whitney, $N = 15$, $p = 0.014$) were both also highly significant. The density of spiders found at the sites varied greatly based on the qualitative classification formed between the first, second and third ranked beaches.

Impact of severe tropical storms

Two major storms made landfall in the study area after 2003, Hurricane Ivan in 2004 and Hurricane Katrina in 2005. These hurricanes led to severe erosion of the dunes of the NGC. Hurricane Ivan made landfall at Gulf Shores, Alabama on September 16, 2004 as a category three3 hurricane. Then, on August 25, 2005, Hurricane Katrina made its original landfall in southeast Louisiana as a category three hurricane. The effects of both Ivan and Katrina showed heterogeneity in their effects. The extreme of this variation in effects is demonstrated by the fact that sites furthest away from the landfall of Hurricane Katrina maintained positive growth rates despite the storm, while those closest to the storm experienced severe population density

reductions. It is assumed that those sites with positive growth rates were outside the reach of the effects of Katrina.

Figure 4. Map showing position of high quality sites chosen for studying the impacts of hurricanes Ivan and Katrina. The location of landfall of Ivan(1) and Katrina(2) shown
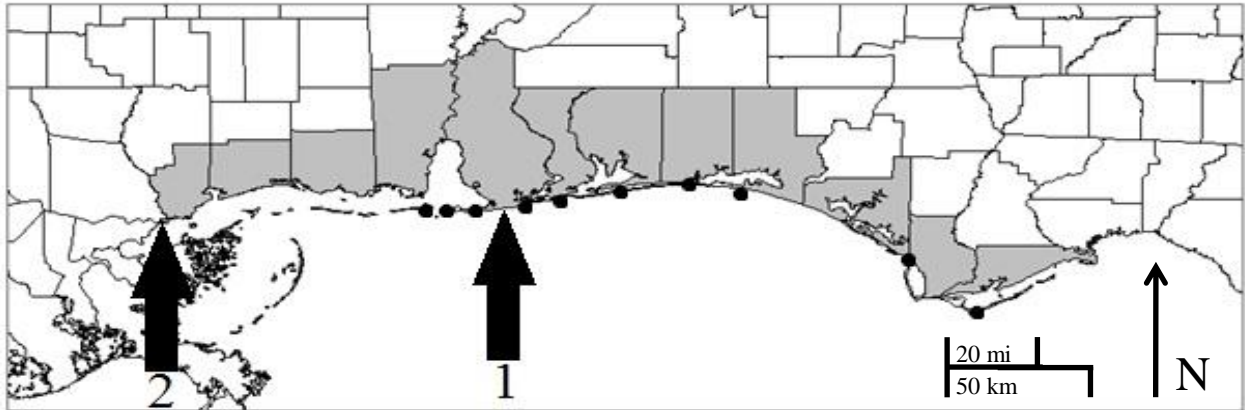
The storm surge and strong winds from Hurricane Ivan leveled the secondary dunes by depositing sand in the valleys between the dunes. Of the twenty-one sites used in 2003, 10 of the highest quality sites were assessed and density-data obtained where possible and feasible one month after landfall of the hurricane.

The mean population growth (R) across sites from 2003-2004 was -2.703 with a S.E. of 0.517. In the following year (2004-2005) the growth rate across sites was 1.738 with a S.E. of 0.416 (Table 2). This demonstrates a very clear pattern of reduction and recovery presumably as a result of the hurricane, assuming that the sites had been stable for some time before this. This assumption is based on the extended period of time (8 years) since the last major tropical storm and the protected status of the sites used.

The 2005 hurricane season was severe, seeing two major tropical storms make landfall. There was an increase in density of spiders at all sites in the period following Ivan's landfall however

population densities had not returned to the levels they were found at in 2003 prior to the landfall of Katrina.

Table 2 Mean site densities per quadrant and growth rates across study sites and years

Location	2003 Mean Den	2003-2004 Growth Rate	2004 Mean Den	2004-2005 Growth Rate	2005 Mean Den	2005-2006 Growth Rate	2006 Mean Den	2006-2007 Growth Rate	2007 Mean Den
Dauphin Island Sea Lab, Dauphin Island	11.00	-1.89	1.67	2.03	12.67	-1.44	3.00	-0.20	2.44
Ft. Morgan, Bon Secor	23.00	-2.15	2.67	1.01	7.33	-0.69	3.67	0.98	9.78
Pine Beach, Bon Secor	38.00	-4.33	0.50	2.69	7.33	-0.38	5.00	0.89	12.22
Perdido Key Area, Gulf Islands National Seashore	52.00	-4.64	0.50	3.58	18.00	-0.30	13.33	0.66	25.78
Ft. Pickens, Pensacola Beach	24.00	-3.87	0.50	0.00	0.50	0.00	0.50	1.59	2.44
Gulf Islands National Seashore, Navarre Beach	10.00	-3.00	0.50	2.48	6.00	0.20	7.33	-0.16	6.22
Destin Point, Public Beach	9.00	-2.89	0.50	2.77	8.00	0.85	18.67	-0.12	16.56
Henderson State Park, Destin	28.00	-4.03	0.50	2.59	6.67	1.47	29.00	-0.04	27.89
Public Beach Access, St. Joe	50.00	-0.08	46.00	0.00	46.00	-0.07	43.00	0.05	45.11
St. George Island	15.00	-0.14	13.00	0.23	16.33	0.02	16.67	0.02	17.00

Immediate impact from hurricanes

Multiple regression models were created to explore what ecological factors varied with

population reductions for each of the two storms independently. The ecological factors included

were: distance from landfall, dune height and vegetation before landfall, and proportional loss of

dune height and vegetation after landfall. The model that best describes the effects seen from the

storms only includes the distance from the point of landfall in both the case of Hurricane Ivan

(R^2 adjusted = 0.638, p = 0.002, AIC adjusted = 5.046, w= 0.880)(Table 3) and Katrina (R^2

adjusted = 0.0369, p = 0.014, AIC adjusted = 39.561, w= 0.922)(Table 4).

Table 3 Comparison of models describing the proposed factors contributing to proportional

population reduction as a result of Hurricane Ivan

Model	AICc	w
Distance from Landfall of Eyewall?		
Eye center? Ivan	5.406	0.880
+ Dune Height before	9.425	0.118
+ Vegetation before	17.443	0.002
+ loss of Vegetation	32.005	$1.474E^{-06}$
+ loss of Dune Height	59.770	$1.379E^{-12}$

Table 4 Comparison of models describing the proposed factors contributing to proportional

population reduction as a result of Hurricane Katrina

Model	AICc	w
Distance from Landfall of Katrina	39.5611	0.922
+Loss of Dune Height	44.5507	0.076
+Loss of veg	52.2326	0.002
+2005-2006 veg	63.7523	$5.150E^{-06}$
+2005 Dh	93.7519	$1.576E^{-12}$

The average distance of study sites from the landfall of Hurricane Ivan was 106 km,

while for Hurricane Katrina it was 288 km. The furthest sites from Katrina were over 400 km

away and experienced little or no population reduction and in most cases had positive population

growth rates despite the landfall of the hurricane. When the density of *A. sanctaerosae* prior to

Hurricane Ivan is regressed against the proportional reductions of density of *A. sanctaerosae* at

each site (density before storm divided by density after storm) the results are found to be non-

significant (p=0.67, R^2 =0.031). The same is true for Hurricane Katrina (p= 0.7127, R^2 =0.018).

This confirms what had long been assumed, the effects of major hurricanes are density

independent which in this case means the density of spiders prior to landfall of a major hurricane

has no apparent impact on the magnitude of the loss of density after the landfall of the storm.

Recovery in year immediately following storms

The multiple regression models for the recovery of density included the possible

explanatory variables: distance from landfall, dune height and vegetation minimums after storms,

and proportional recovery of dune height and vegetation in the year following the storms

landfall. The model that best predicted the recovery of population density of *A. sanctaerosa*e

after Hurricane Ivan suggests that variation in the recovery of dune height in the year after the

storm best explains the variation in the recovery of the population (R^2 adjusted = 0.416, p = 012,

AIC adjusted = 36.261, w= 0.887)(Table 5). In the recovery from Hurricane Katrina, the best

model incorporated both the recovery of dune height as well as the distance from the place of

landfall (R^2 adjusted = 0.595, p = 0.009AIC adjusted = 22.214, w= 0.806)(Table 6).

Table 5 Comparison of models describing the proposed factors contributing to population growth rate in the year immediately following Hurricane Ivan

Model	AICc	w
Recovery of Dune Height	36.261	0.887
+ Distance from Landfall Ivan	40.649	0.099
+Dune Height minimum after storm	44.474	0.015
+Vegetation minimum after storm	58.645	$1.222E^{-05}$
+Recovery of Vegetation	88.460	$4.099E^{-12}$

Table 6 Comparison of models describing the proposed factors contributing to population growth rate in the year immediately following Hurricane Katrina

Model	AICc	w
Recovery of Dune Height	25.510	0.155
+ Distance from Landfall Katrina	22.214	0.806
+Vegetation minimum after storm	28.269	0.039
+Dune Height minimum after storm	41.979	$4.115E^{-05}$
+ Recovery of vegetation	71.957	$1.272E^{-11}$

The recovery of the sites after Hurricane Ivan did appear to display a level of density dependent growth. The proportional reduction explained 45% of the rate of growth in the year following the storm ($R^2 = 0.452$, p = 0.0332): the larger the proportion of reduction, the faster the rate of recovery. This result is logical considering the increased habitat availability at sites whose densities were impacted more severely leaving open habitat patches.

Discussion

The goals of this study were three fold: (1) document the known range and density of the wolf spider (2) examine the interaction between trends in development impacting beach stability and site densities of *A. sanctaerosae*, and (3) explore the effects of severe tropical storms on

spider density to the physical attributes of the dune system and the distance from the point of landfall of the tropical storm.

Documentation of range and impact of development

This study provides a clearer picture of the status and distribution of *A. sanctaerosae* along the beaches of the Northern Gulf of Mexico. *A. sanctaerosae* was found in all three states surveyed, with highly variable densities (Figure 2). Mainland Mississippi is protected by a series of barrier islands, resulting in few dune ecosystems, thus relatively few sites were found to harbor the target species. Mainland western Alabama is situated behind Dauphin Island, similar to the majority of mainland MS, and as such does not have a coastal sand dune system. Though the Mississippi barrier islands were not sampled due to lack of access, they should be surveyed for *A. sanctaerosae* in the future and could show high densities due to the lack of development and continuity of the dune systems. *A. sanctaerosae* was found in varying density at all of the beaches surveyed that had a healthy dune system in place. Areas directly adjacent to healthy dune systems with a high density of spiders that had high-density human developments built on them had low to zero individuals per quadrant.

Human population growth in coastal areas and the ensuing commercial development halt the dynamic nature of the coastal dune ecosystem. The natural course of deposition and erosion of sand onto the dune system is altered through direct removal of dunes for building projects and management strategies that work to stop the erosion that shapes and maintains the dynamics of this ecosystem.

This study suggests that when dune systems are compromised by commercialization, however slightly, the habitat of *A. sanctaerosae* is compromised, causing a significant decrease

in density. The most severe reductions in population density are in areas where dense human commercial development of the NGC is highest.

When these data are coupled with the trends in commercialization and studies concerning the natural erosion of NGC beaches, the future of *A. sanctaerosae* looks bleak. The sites with the highest densities were those receiving some form of protection (wildlife refuges, national and state parks). It would appear that in this commercially valuable area, the only refuges for an endemic are the areas purposely set aside by government and that without said protection all populations would be severely impacted.

The impact of hurricanes on the density of *A. sanctaerosae* is both direct and density independent. Distance from landfall of a hurricane predicts 64 % of the density reduction seen after the storm while ecological factors appear less useful as a predictor of effects of storms. Hurricane Katrina shows a gradient of severity ranging from the most severe effects in the western sites to no measurable effect in the most easterly sites. Hurricane Ivan, by contrast, was centered directly over the western sites and was severe enough that all of the sites were affected. The effects of the storms were density independent but not independent of the effects of distance. The distance at which sites maintained positive population growth rates following Hurricane Katrina was approximately 283 km. This appears to be the limit of the broad impact of Katrina. There is a limit to the effects of hurricanes along the NGC and the best predictor of the magnitude of that impact is the distance from the point of landfall

There was spectrum of effects on the density of *A. sanctaerosae* after the storms from zero in the most distal sites to severe at sites such as Fort Pickens, FL. The density of individuals at Fort Pickens experienced the most severe effects from Hurricane Ivan. Extensive flooding and over-washing of the western portion of Santa Rosa Island occurred where this collection site is

located. It was sampled in 2005-2007 (it was not sampled in 2004. Access to the site was restricted) with zero individuals being located in the first 3 years after Ivan. It was not until 2007 that individuals were found at this site. This suggests either a local extinction or such a severe reduction that individuals were not detectable with our sample sizes. Other sites saw depressions in population density but not as severe or sustained as what was seen at Ft. Pickens, FL.

Neither the ecological factors measured (dune height or more vegetation density) nor pre-storm population density explains the proportional loss of individuals at the study sites due to hurricanes. While apparently not contributing significantly to the decrease in population density after a storm, dune height does explain the rate of increase in population density in the year after the storm. Despite the variation in the distance from landfall and the corresponding variation in population density reduction the recovery of the height of the dune in the year following after the storms was the most significant single factor in determining the rate of recovery in the year following a major tropical storm.

Implications for management

Beach erosion is a serious problem along the Atlantic and Gulf coasts (Douglas & Pickel, 1999; Anderson & Wellner, 2001). In part, beach erosion is a natural process involving a complicated interaction of wind, wave action, topography of off shore barriers, the contour and vegetation of the coastline and severe weather. McNatt et al. (2000) verified a significant correlation between the native forests inland of the beaches of Dauphin Island to the dune health and related this to the density of *A. sanctaerosae*. Beyond these natural variables, the findings presented here demonstrate the strong relationship between the population density of *A. sanctaerosae*, the extent of commercial beach development and the history of severe tropical

storms. These data suggest that, in addition to inland forests, dune health as measured by height is a significant factor in the persistence and recovery of a NGC endemic in the face of development and severe tropical storms. Also, the maintenance of habitat without commercial development will be critical to the persistence of the endemic, *Arctosa sanctaerosae*. Viable alternatives to removal of the secondary dunes for commercial development exist. Single family homes and high density housing complexes (condominiums, hotels, etc.) built behind the tertiary dunes that include an elevated boardwalk for access to the beach are an option for maintaining contiguous healthy dune systems as development of the NGC moves forward. This type of system is already in use in areas including portions of the Saint Joe, FL region of the coast. A redevelopment plan which included moving the current "no build line" (beyond which development isn't allowed) inland, beyond the dune system, for all future development and reconstruction following major storms that destroy the current development would benefit the area. While controversial and difficult to implement, this type of withdrawal from the dune system would have fiscal, as well as, ecological benefits. The protection afforded the developments by a healthy dune system would reduce devastation by major storms and save property owners across the NGC millions of dollars.

I also suggest using *A. sanctaerosae* as an indicator species of Gulf beach health. The most important reason is that it is highly selective in its preferred habitat: secondary dunes of the northern Gulf beaches with limited commercial impact. Other Gulf beach inhabiting species have been studied and protected by the U.S. Fish and Wildlife Agency, however many of these actions have been criticized because of the flexibility in critical habitat of the protected species, meaning that they are not restricted to the coastal dunes of the NGC (Brennan et al., 2003). The Alabama Beach Mouse, *Peromyscus polionotus ammobates* (Bowen, 1968), and the Perdido Key

Beach Mouse, *Peromyscus polionotus trissylepsis* (Bowen, 1968), are examples of protected species that have since been found to reside beyond the critical habitat of the coastal beaches (Brennan et al., 2003). For *A. sanctaerosae* this is not possible due to niche rigidity caused by competitive disadvantage with conspecifics and increased predation outside secondary dunes due to increased contrast between body and substrate color. I propose that *A. sanctaerosae* should be continuously monitored as a secondary measure of NGC coastal ecosystem deterioration.

CHAPTER 2. DEVELOPMENT OF 10 MICROSATELLITE LOCI IN THE WOLF SPIDER ARCTOSA SANCTAEROSAE

The white beach spider, *Arctosa sanctaerosae*, is a burrowing wolf spider endemic to the dune ecosystem of the northern coast of the Gulf of Mexico (NGC). Species endemic to this ecosystem are ideal for examining the effects of disturbance (e.g. hurricanes, habitat fragmentation/degradation) on population persistence. It is widely recognized that the primary threat to these populations is habitat fragmentation, but with the reduced gene flow associated with anthropogenic habitat modification and a predicted increase in the intensity of tropical storms (Goldenberg et al. 2001), the outlook for this dynamic, fragile system is bleak. *Arctosa sanctaerosae* shares this habitat with several species of endangered beach mouse for which small population size has limited the inference of population structure and dynamics. I have suggested developing *A. sanctaerosae* as a complimentary, sister system to aid in conservation planning for the entire dune ecosystem that is itself in an advanced stage of degradation (Martinez and Pstusy 2004).

I developed 10 novel microsatellite loci using the enrichment protocol of Glenn and Schable (2005). Whole genomic DNA was extracted from the legs of *A. sanctaerosae* using the DNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions. DNA concentration was determined using a spectrophotometer and genomic DNA was then digested with the restriction enzymes RsaI and XmnI to yield fragments between 300 bp and 1000 bp long. To the ends of these fragments I then ligated SuperSNX24 linkers (F; GTTTAAGG CCTAGCTAGCAGAATC,

R; GATTCTGCTAGCTAGGCCTTAAACAAAA) and a polymerase chain reaction was performed to ensure ligation was successful. Genomic fragments were enriched using a probe mix containing four biotinylated oligonucleotides (AAT_{10}, $AAAT_7$, AAC_6 and $AGAT_8$) and separated with streptavidin magnetic beads. This mixture was washed with a 2xSSC, 0.1% SDS solution twice and a 1X SSC, 0.1% SDS solution four times. A magnetic particle collector was used between washes to capture the magnetic beads. After the last wash, fragments were removed from the probes by denaturing at 95°C for five minutes and precipitating with 95% ethanol and 3M sodium acetate. These fragments were then air-dried and re-suspended in 25 μL of TLE. To increase the quantity of these recovered enriched DNA we amplified the enriched pool by PCR using the SuperSNX24-F primer. These amplified fragments were then transformed and cloned using a TOPO TA Cloning Kit (45-0641). Blue-white selection revealed two hundred and eighty-eight clones that were then screened for inserts suitable (large enough) for microsatellite development by PCR using M13 forward and reverse primers (Glenn and Schable 2005). After amplification, PCR products containing inserts of suitable size were cleaned using ExoSAP-IT® (USB) per the manufacturer's protocol. Sequencing reactions used the Big Dye Terminator version 3.5 (Applied Biosystems) chemistry and were cleaned using G-50 Sephadex in a MultiScreen PCR Filter Plates (Millipore). Sequences were generated using an ABI 3130 sequence analyzer. These were edited using GENEIOUS (Drummond et al. 2009) and checked for short tandem repeats (STR) using TROLL (Tandem Repeat Occurrence Locator; Castelo et al. 2002). Primers were then designed for STR containing loci using PRIMER3 (Rozen and Skaletsky 1996) in GENEIOUS (Drummond et al. 2006)(Table 8).

These loci were evaluated for primer performance, variation among individuals, and populations were assayed for allelic frequencies, yielding ten polymorphic loci that were scored

for 273 individuals. Fluorescence-labeled fragments were visualized on an ABI 3130 and allele

sizes were determined through comparison with a known size standard (GeneScan -500 Rox)

using GENEMAPPER version 3.7. Polymorphism was screened on 273 individuals from 11

sites. The number of alleles ranged from 3-11. Expected heterozygosity was computed among

and within populations using Levene (1949) in the software package POPGEN (Yeh et al.,

1999). The loci were tested and each was found to be segregating independently. Tests for

departure from HWE showed sites exhibiting evidence of inbreeding for a number of loci (Table

7, Table 8).

Table 7 Within population heterozygosity levels across loci and Fis estimates of *Arctosa*

sanctaerosae.

Population	$\bar{x}\ H_o$	$\bar{x}\ H_e^a$	Fis
Dauphin Island Sea Lab, Dauphin Island, AL	0.140	0.149	0.06
Ft. Morgan, Bon Secour, AL	0.143	0.150	0.043
Pine Beach, Bon Secour, AL	0.112	0.121	0.074
Perdido Key Area, Gulf Islands National Seashore, FL	0.206	0.216	0.047
Ft. Pickens, Pensacola Beach, FL	0.172	0.187	0.084
Gulf Islands National Seashore, Navarre Beach, FL	0.183	0.203	0.098
Destin Point, Public Beach, FL	0.213	0.244	0.124
Henderson State Park, Destin, FL	0.277	0.306	0.094
Public Beach Access, Mexico Beach, FL	0.347	0.405	0.145
Public Beach Access, St. Joe, FL	0.341	0.423	0.194
St. George Island, FL	0.354	0.444	0.203

The isolation and characterization of these 10 loci enabled me to determine

heterozygosity levels in each population and examine historical levels of gene flow between

what are now disjunct populations of *Arctosa sanctaerosae* along the NGC. I utilized this set of

markers to explore the dynamics of these populations and their responses to both anthropogenic

(development) and natural (hurricanes) disturbance. The identification of source and sink

populations and tests for genetic bottlenecks allowed me to measure relative health of these

habitat fragments and provide a new faunal model for the study of these highly endangered

ecosystems.

Table 8 Characterization and level of variability at 10 microsatellite loci in 11 populations of *Arctosa sanctaerosae*

Marker Name	Primer Sequence	Motif	No. of Alleles	Fragment Size	Total Size Range	H_O	H_E[a]
AS6	F: AATCGCAGGGTCAATAATGC R: GGCCTATTTGTTCAGCCATC	TA	11	419	416-442	0.3114	0.6288
AS7	F: TTCATGGCTGTTTGCCACTA R: TTGTCCTGCACTTGCATAATTT	TA	4	308	310-324	0.0952	0.1377
AS8	F: TGATTTCGCGAATCCCTAGT R: TGAAAGCCGTTTAATCCTTACA	TAGA	3	281	282-286	0.0842	0.1275
AS9	F: CCATTGAAACTGGGACATTCT R: GATCCGGTGGAACGAAAGTA	TA	3	398	420-428	0.1062	0.1552
AS10	F: TTTACGCGCAATCGTGTTTA R: ATGCCCTGGAACTAATGCAG	AAC	3	325	324-330	0.1941	0.3592
AS11	F:CATAAATTCGTAATTATTTTTGATGC R: AACAACTTTCAAAAGGCATGTG	TAT	3	150	168-174	0.1136	0.2206
AS13	F: CAGCGTTTCCCACACCATA R: TCTACCCTGCCACGTGATATT	GAG	4	297	291-303	0.1868	0.3105
AS14	F: AGAAAACACAGCTGCACGAA R: TTCTTAACAACAACTCCCACGA	GA	5	292	292-298	0.3407	0.3759
AS15	F: TCAAGTTTGACGTCCAGTCG R: ACCCCTCATGACAGGTAAGG	TA	3	291	308-312	0.0806	0.1368
AS16	F: AGCAGAAAAATTCAAACGTGA R: CAAGTCGCCAAAAGGGATTA	TG	5	269	270-290	0.6044	0.6176

[a] Expected heterozygosity was computed using the method of Levene (1949)
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Chapter 3. HURRICANES AND HUMAN CONVERSION OF HABITAT LEADS TO POPULATION ISOLATION IN THE COASTAL WOLF SPIDER ARCTOSA SANCTAEROSAE

Introduction

Habitat fragmentation is known to shape population genetic structure. Reductions in population size, gene flow between populations and reduced availability of high quality habitat can lead to genetic isolation and eventual extinction of populations (Reed 2008). Understanding dynamics of recently fragmented and isolated populations has become and will continue to be significant for conservation biology (*e.g.*, Buza et al. 2000; Richards 2000; Reed 2004). The burrow-dwelling wolf spider, *Arctosa sanctaerosae* (Aranea: Lycosidae) is entirely restricted to the secondary dune habitat in the coastal dune system of the Northern Gulf of Mexico Coast (NGC). Loss and fragmentation of high quality habitat for this habitat specialist makes this species of possible interest for conservation management.

Arctosa sanctaerosae, family Lycosidae, is a wolf spider that inhabits only the white sandy beaches of the NGC. The geographic range of this species stretches from the panhandle of Florida to the western border of Mississippi (Dondale & Redner 1983). *A. sanctaerosae* is found primarily on secondary dunes, inland from the high tide mark (McNatt et al., 2000). The health of this critical habitat is dependent upon the continuity of coastal dunes, which are the first line of defense against severe erosion (Stalter & Odum 1993). The NGC has seen large-scale change, beginning in the early 20th century, as it became a popular vacation destination. With this tourism

came increased traffic on the beaches and dune systems as well as increased commercial development. In the state of Alabama alone, the percentage of undeveloped beaches dropped from 72% to 39% between 1970 and 1996 (Douglass et al. 1999). This development is a major source of fragmentation of the NGC's dune ecosystem.

In addition to the increased conversion of beaches to lower quality habitat, the NGC has seen an increase in the number of severe tropical storms making landfall. These storms eroded the beaches of the NGC within the reach of their storm surge and high winds. Peninsulas and islands are particularly vulnerable to over-wash due to storm surge; severe storms are capable of physically altering habitat (e.g. cutting peninsulas off from the mainland). In areas of even moderate human development, the storms decimated the dunes and vegetation that hold them in place.

A metapopulation is a group of subpopulations that are interconnected by some level of dispersal. Richard Levins (1969) described it as "a population of populations". The multiple distinct subpopulations occupy available habitat patches leaving other patches unoccupied and available. These populations fluctuate in size and the patches go through periods of occupancy and availability. In classical metapopulation theory, subpopulations fluctuate independently and it is demographic stochasity that leads to local extinction times. The size of these subpopulations is the largest factor in determining their persistence time, smaller subpopulations disappearing sooner than larger subpopulations. The connectivity among subpopulations in a metapopulation is correlated to that metapopulation's persistence through time. Both commercialization of habitat and increased severity of tropical storms have the capability to decrease the persistence time of a metapopulation by making the subpopulations smaller and more isolated. By employing genetic data and modern analytical techniques, I explored the

genetic structure of *Arctosa sanctaerosae* to reveal the demography of populations (migration, population size, stability etc.).

The goals of this study are threefold: (1) define the current biologically significant clusters or subpopulations, (2) describe the genetic diversity and structure of these subpopulations, and finally (3) to investigate gene flow, past and present, among these. The comparison of historic versus recent gene flow can potentially be used to differentiate isolation of populations caused by hurricanes and other forms of pre-human isolation (historically constant) versus contemporary causes of reduced connectivity including those caused by conversion of habitat by humans (within the last 100 years). Assuming that hurricanes have occurred since the evolution of this species and that human disturbance on a large scale started only a hundred years ago, the dominant force that is shaping the current status of the species and its populations should emerge.

Methods

Sample Collection Amplification and Scoring

I collected tissue samples from at least 20 individuals from 11 sites along the NGC including, the ten sites used in Chapter 1 plus one additional site, Mexico Beach, FL (Figure 3, Table 7) between June 1st and 11th 2007. All individuals were stored at -80°C in 100% ethanol. I sampled the sites on warm clear summer nights and sampled the full length of the site. Approximately 1 mg of tissue (legs) was used for subsequent extraction of DNA.

I extracted DNA using a Qiagen DNeasy kit. I then amplified target sequences by PCR using 11 microsatellite primers. Ten of these primers were developed specifically for this species (Hataway et at. 2011, Chapter 2) while the last had been developed for another species of

Lycosidae (Reed et al. 200x). PCR reaction contained 10x reaction buffer, 50 m$_M$ MgCl$_2$, 160 μ$_M$ each dNTP, 1 μ$_M$ each fluorescent-labeled primer, and 1 U Taq polymerase. PCR reactions were cycled at 94°C for 1 min followed by 20 cycles of: 94°C for 30 sec, 60°C for 25 sec decreasing every cycle by 0.3°C (Touchdown PCR), 72°C for 40 sec; then 8 cycles of 94°C for 30 sec, 56°C for 25 sec, 72°C for 40 sec and a final extension of 72°C for 30 min. Fluorescence-labeled fragments were visualized on an ABI 3130 and allele sizes were determined through comparison with a known size standard (GeneScan -500 ROX) using GENEMAPPER version 3.7. All scores were checked manually and ambiguous fragments were reanalyzed.

Population differentiation

I used the Bayesian clustering algorithms in the programs STRUCTURE (Pritchard et al. 2000) and GENELAND ver. 3.2.4 (Guillot et al. 2005, Guillot and Santos 2009). Both of these programs have idiosyncrasies and can be used in concert to not only suggest a current number of clusters/populations and assign individuals to them, but also to find support for a suggested model. GENELAND was run and included the correlated model, which assigns individuals to geographic clusters without prior knowledge of the site where that individual was sampled. This accounts for several factors relevant to this system. Spatially, we expect areas of intense human impact to create a barrier between populations and so we add into the model the set of georeferenced coordinates, in this case the location of our collection sites (Guillot and Santos 2009). This model assumes Hardy-Weinberg equilibrium, spatially correlated genotypes, and that loci are unlinked. I ran 8 independent runs of 1 million Markov Chain Monte Carlo (MCMC) iterations allowing the number of genetically distinct clusters (K) to vary from one to eleven. The resulting clusters were then analyzed for differentiation, gametic equilibrium, and

the assumption of Hardy-Weinberg equilibrium. The second program, STRUCTURE, was then run using 500,000 replicates and 50,000 burn-in cycles for 8 independent runs. Again the number of clusters was allowed to vary from one to eleven. The "Locprior"(Hubisz et al. 2009) option was used which included the geospatial data into the model *a priori*. I used the admixture method with the correlated allele frequency option (Falush et al. 2003) that works in a similar manner to the correlated option in GENELAND.

GENELAND and STRUCTURE both recovered 5 clusters and these assignments were used for all subsequent demographic analyses. FSTAT ver. 2.9.3.2 (Goudet 2002) was used: (1) to test for Hardy-Weinberg equilibrium within populations (2) to estimate F_{is} (3) to calculate allelic richness (A_r) and (4) to calculate gene diversity (H_s). GENEPOP (Raymond and Rousset 1995, Rousset 2008) was used to test population differentiation and to estimate the number of null alleles. ARELEQUIN ver. 3.5.1.2 (Excoffier et al. 2005) was used to calculate expected (H_e) and observed (H_o) heterozygosities and pairwise F_{st} values between populations. Other statistics have been suggested for estimating gene flow in microsatellites (Goldstein et al. 1995, Goldstein and Pollock 1997, Zhivovtovsky 1999, Weir and Cockerham 1984) but F_{st} is the most commonly used metric and I employed it here. Expected heterozygosity was computed among and within populations using Levene's method (1949) in the software package POPGEN (Yeh et al., 1999). Isolation by distance was tested using a Mantel test of log transformed linearized F_{st} and geographic distance values was conducted among the clusters with 10,000 randomized matrices based on permutations with 10000 permutations.

Historical and Recent Geneflow Estimation

Historical mutation scaled migration rates (M) were calculated using MIGRATE ver. 3.2.1 (Beerli 2010). The estimates given are long-term averages heavily influenced by the recent past and are calculated over the last $4N_e$ generations. These analyses were run using maximum likelihood, the Brownian motion mutation model, and the matrix migration model. After a burn in of 30,000 genealogies, I ran 15 short chains, until 500 genealogies were recorded from 50,000 sampled, and then ran 10 long chains until 500 genealogies were sampled from $8x\ 10^7$.full matrix migration model that allows for migration from a cluster to any of the other populations. MIGRATE does so using a Markov chain Monte Carlo approach (MCMC). This approach consists in starting a run (or chain) from an arbitrary value that is iteratively modified in such a way that after many iterations, the distribution of the simulated parameter approximates the posterior distribution. The first iterations that correspond to the so-called burn-in period are discarded and a subset of the simulated values is saved (thinning) to save disc space and time. Burning the first iterations of a chain is done to minimize the effect that initial starting value (or seed) has on the posterior inference. After a burn in of 30,000 genealogies, I ran 15 short chains and 10 long chains. These chains were thinned to 500 recorded genealogies from a sampled 50,000 and 8x10^7 genealogies respectively. I then summarized results across chains as recommended in the program documentation (Beerli 2010). The rate of recent gene flow was calculated using BAYESASS ver. 1.3, which estimates migration rates within the last two to three generations using a Bayesian inference framework and gametic disequilibrium among immigrants and their descendants. I ran $8x10^7$ iterations with a burn-in of 1x107 (Chiucchi and Gibbs 2010). I grouped individuals from each of the five clusters suggested by GENELAND for these tests.

Results

Cluster analyses

GENELAND and STRUCTURE identified five population clusters that were consistent across all independent runs. The three sites of western Alabama were clustered as a single population (cluster 1) despite Dauphin Island and Fort Morgan being separated by a 5 km stretch of water. The pairs of sites in clusters 2 (Perdido Key / Fort Pickens) and 3 (Navarre Beach / Destin Point), are also separated by a stretches of water. Geographically isolated by human development, Henderson Park (cluster 4) did not cluster with any other sites. Finally the three most easterly sites (Mexico Beach, St. Joe, and St. George Island) were grouped into a single cluster (cluster 5) (Table 9, Figure 4).

Table 9 Population cluster assignments and gps data

Site	Cluster Assignment ID	GPS Data	
		°N	°W
Dauphin Island Sea Lab, Dauphin Island	1	30.24666	88.08306
Ft. Morgan, Bon Secor	1	30.22378	88.00841
Pine Beach, Bon Secor	1	30.22948	87.82775
Perdido Key Area, Gulf Islands National Seashore	2	30.29844	87.41816
Ft. Pickens, Pensacola Beach	2	30.32523	87.18689
Gulf Islands National Seashore, Navarre Beach	3	30.37146	86.92539
Destin Point, Public Beach	3	30.39178	86.51043
Henderson State Park, Destin	4	30.35619	86.26646
Public Beach Access, Mexico Beach	5	29.879053	85.350037
Public Beach Access, St. Joe	5	29.88353	85.35449
St. George Island	5	29.61541	84.95381

Figure 5 Grouping of sites into 5 subpopulation clusters
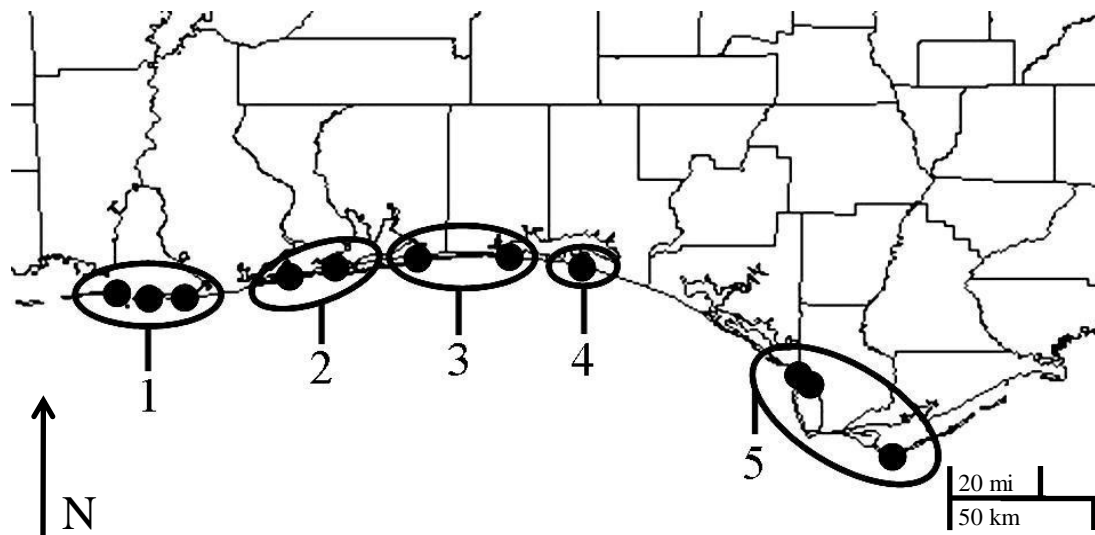
Genetic variation and structure

The number of alleles per locus varied from 3 to 11 and null alleles were estimated to be <0.16% across all loci. Each of the fifty-five pairs of loci tested was found to be in gametic equilibrium. Tests of linkage disequilibrium, within and across populations, were all insignificant, satisfying the assumption that all loci are unlinked. The measures of diversity (allelic richness and gene diversity) decreased longitudinally, with the lowest levels of observed diversity in the westernmost populations. Heterozygosity, gene diversity and allelic richness all followed this east – west pattern (Table 10). F_{is} scores for each of the clusters loosely followed this pattern and ranged from 0.18-0.06. Pairwise F_{st} scores ranged from 0.05 to 0.30 and all were significant (Table 11). The Mantel test showed a Pearson's r of 0.68 that was highly significant (p = 0.001meaning that individuals are more likely to find mates from populations geographically close to themselves rather than at random across all populations.

Table 10 Number of individuals (N), mean allelic richness (A_r), gene diversity (H_s), observed heterozygosity (H_o), expected heterozygosity (H_e) and, the inbreeding coefficient (F_{is}) for *Arctosa sanctaerosae* in five population clusters along the Northern Gulf of Mexico Coast

Cluster (ID. Area)	N	A_r	± 95% C.I.	H_s	± 95% C.I.	H_o	SD	H_e	SD	F_{is}
1. Western Alabama	92	1.84	0.73	0.14	0.14	0.13	0.21	0.14	0.21	0.06
2. West Santa Rosa Is. / Perdido Key	46	2.09	0.86	0.23	0.16	0.21	0.21	0.23	0.23	0.07
3. East Santa Rosa Is. / Destin Point	48	2.11	0.60	0.28	0.17	0.24	0.23	0.27	0.26	0.11
4. Henderson State Park	27	2.73	0.80	0.37	0.14	0.33	0.18	0.36	0.21	0.09
5. St. Joe/St George	60	3.56	1.26	0.54	0.11	0.43	0.18	0.53	0.20	0.18

Table 11 Pairwise F_{st} Scores below diagonal and pairwise geographic distances (km) above the line

	1. Western Alabama	2. West Santa Rosa Is. / Perdido Key	3. East Santa Rosa Is.	4. Henderson State Park	5. St. Joe/St George
1. Western Alabama	*	40.56	87.93	149.79	237.07
2. West Santa Rosa Is. / Perdido Key	0.0773	*	25.4000	87.6200	178.71
3. East Santa Rosa Is.	0.1459	0.0464	*	23.1800	119.35
4. Henderson State Park	0.1577	0.0421	0.0179	*	98.08
5. St. Joe/St George	0.2891	0.1696	0.1250	0.0530	*

Figure 6 Plot of isolation by distance (Pearson's r=0.64)
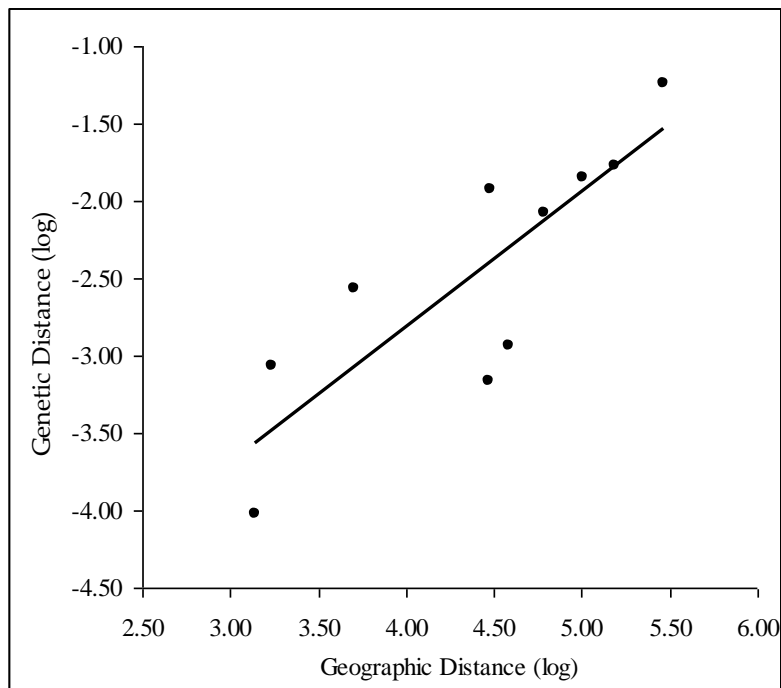
Historic and recent geneflow

Using MIGRATE, a mean historical migration rate was calculated as the number of effective migrants. These estimates demonstrated non-zero historic levels of geneflow among populations (Table 12). BAYESASS estimates of recent migration were significantly lower, suggesting these populations were more isolated than in the past. A paired t-test of the means finds the difference between historical and recent migration rates of *A. sanctaerosae* to be statistically significant (df =4, p<0.002).

Table 12 Comparison of the mean number of effective immigrants per generation reaching a population recently (as estimated by BAYESASS) versus the mean number of immigrants reaching a site historically (as estimated by MIGRATE)

| Population	Nm Recent	Nm Historic
Alabama	0.01	1.23
West Santa Rosa/Perdido Key	0.57	2.68
East Santa Rosa Area	0.14	2.75
Henderson State Park	0.69	3.34
St. Joe/St George	0.09	1.28

Discussion

The goals of this study were threefold: (1) define the current biologically significant clusters or subpopulations, (2) describe the genetic diversity and structure of these subpopulations, and finally (3) to investigate geneflow, past and present, among these.

Cluster analyses

GENELAND recovered five distinct subpopulations along the NGC. Analyses using STRUCTURE, and examination of F_{st} values and differentiation tests all confirmed this pattern. The observed genetic variation follows a distinct pattern, decreasing from east to west. Initially, the distribution of sites into the 5 clusters appears not to be driven by biologically significant factors. For example, sites that were found on the same island (Navarre and Ft. Pickens) did not cluster together, but separately with sites found on the mainland. It is not until commercial development is overlaid with the geography that the clusters recovered demonstrate an intuitive pattern (Figure 6). Cluster number 1 is separated from 2 by Gulf Shores and Orange Beach AL. Population cluster number 3 is isolated from number 2 by the Pensacola Beach FL. The genetically isolated population of Henderson State Park cluster (4) is in a habitat patch

surrounded by the development of Destin, Fl. In addition to Destin, Panama City Beach, FL lies to the east of cluster 4 and separates it geographically from cluster 5.

Figure 7 GENELAND clusters in circles with major human developments labeled: (A) Gulf Shores, AL, (B) Pensacola Beach, FL, (C & D) Destin, FL
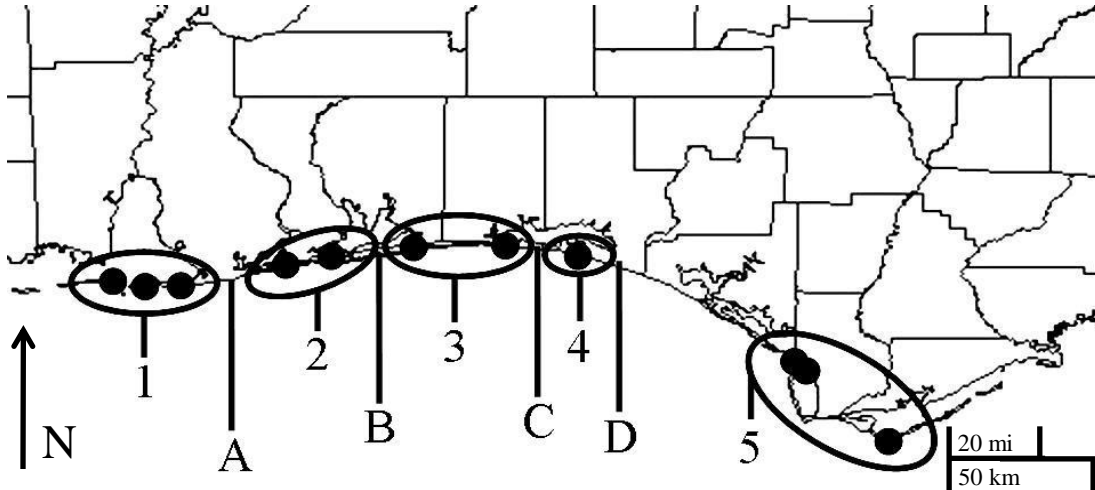
Genetic variation and structure

Habitat fragmentation can potentially shape population genetic structure by reducing gene flow. Reductions in population size, geneflow between populations and reduced availability of high quality habitat can lead to genetic isolation and eventual extinction of populations (Reed 2008). The genetic isolation seen in the subpopulations of *Arctosa sanctaerosae* is due to reduced gene flow and subdivision of the previously panmictic population. Once population size is reduced through habitat fragmentation, random genetic drift and nonrandom mating drive the changes in genetic diversity. Previous studies of spider population demography in fragmented habitats have found similarly high levels of structure and inbreeding (Reed et al. 2011). The alleles in the populations are subsets of the alleles found in the population to the east of them. Allelic richness and gene diversity are highest in the east and decrease as you move westward.

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The best three possible explanations for the higher genetic diversity seen in the eastern clusters are the relatively few numbers of hurricanes to make landfall in the east in recent times, significantly fewer large scale commercial developments, or the possibility that *A. sanctaerosae* has expanded its distribution westward over time from some site in the east where it could have possible evolved.

Historic and recent geneflow

There has been a significant decrease in the amount of migration between the five subpopulations within the last 100 years. This lack of migration in the recent past combined with the correlation of the patterns of genetic structure and human encroachment found across clusters supports placing the timing of the subdivision to within the last 100 years. This corresponds to the time frame in which the commercializationof the NGC began. It appears the major human population centers, which have interrupted the previously contiguous dune system of the NGC, are effective barriers to gene flow. Within clusters there remains enough geneflow to maintain panmixia, regardless of distance, water, or limited human impact (Single-Family homes etc.) between sites within clusters.

Clusters 2 and 4 are receiving more effective migrants than the other three clusters. This is best explained by the isolation and small size of cluster 4 in relation to the other populations, suggesting this locality may serve as a sink population that requires constant immigration to persist. The severity of effects experienced by cluster 2 by hurricanes over the recent past could explain the high number of migrants from cluster 1. The spatial heterogeneity of hurricane effects is not limited to the distance from landfall (Chapter 1). The hurricane's leading edge has higher wind speeds relative to the trailing edge and is formed on the eastern side of the storm in

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the northern hemisphere. This frequently leads to an increased number of tornados spawned by the storm and more destructive power to the east of the storms point of landfall (Williams and Sheets 2001). Cluster 2 is directly to the east of site of landfall of Hurricane Ivan and thus the severity of effects may have been higher than that at Cluster 1 allowing a rescue effect provided by immigrants from Cluster 1.

Conservation Implications

With the severity of tropical storms predicted to increase over time due to elevations in surface temperature of the Gulf of Mexico caused by climate change (Slott et al., 2006), population connectivity will become increasingly important for population and even species persistence. Historically, the distribution of *A. sanctaerosae* appears to have been contiguous. However, with the development of the ecosystem for tourism, the population has become fragmented and subdivided into distinct subpopulations existing in a metapopulation structure. Moving forward, conservation measures must recognize the need for a contiguous dune system, not only for the physical preservation of the coastal dune ecosystem, but for maintaining population structure and connectivity of this and presumably other species. Prior to the introduction of commercialization to the NGC, migration among *A. sanctaerosae* populations was facilitated by the continuous dune system. Importantly, increased continuity and protection of these dunes will also lead to a healthier dune system that prevents erosion and inland destruction in the face of tropical storms. It must be recognized that a contiguous dune system, while beneficial to the focal species, also serves the broader goals of ecological and the commercial interests along the NGC. By removing the dunes for commercial development we have increased erosion and must reclaim the beach sands regularly as well as replace structures

damaged during major tropical storms at great cost. It makes ecological as well as financial sense to restore these dune systems to their original state and maintain them. Only the halting and/or reversal of the current developmental trend, including the complete removal of barriers interrupting the corridors for inter-population migration will result in the long-term persistence of *A. sanctaerosae*.

CHAPTER 4. BOTTLENECKS IN DECLINES IN POPULATION SIZE OF THE WOLF SPIDER ARCTOSA SANCTAEROSAE

Introduction

Small populations experience reduced population viability and persistence (Saccheri et al. 1998, Reed et al. 2003a, Palstra and Ruzzante 2008, Reed 2008). This is due to the fact that small populations are more susceptible to loss of genetic diversity due to random genetic drift, they maintain lower levels of fitness (Reed and Frankham 2003, Reed 2005), and have reduced adaptive potential (Reed et al. 2003b, Blows and Hoffman 2005, Reed 2005) when compared to larger populations.

The density of individuals present in a population is useful for estimating census population size (N_c) when paired with habitat extent. However, census population size often fails to provide a clear picture of the effective population size. The rate of genetic drift, and associated reductions in fitness and loss of adaptive potential is not proportional to census population size (N_c). Rather, it is proportional to effective population size (N_e). Effective population size is the size of an ideal population that experiences genetic drift at the rate of the population in question. Populations that have stable population sizes, randomly mating, and have equal numbers of males and females will have an effective population size that equals its census size. Deviations from these assumptions can lead to inequality in N_e and$< N_c$.

Genetic techniques have been adopted over the last two decades for the detection of population bottlenecks and effective population size. Genetic bottlenecks are events in which a population has experienced a recent reduction of their effective population size. This potentially

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leads to a sudden reduction in genetic diversity. Detection of these events using modern methods works best when the events are severe, leaving tens of individuals (Luikar and Corneut 1998, Swatidipon et al. 2010). The genetic signature of a bottleneck can be erased from a population through subsequent migration or population recovery (Luikart and Corneut 1998, Busch et al. 2007, Hundertmark and Dael 2010). The current method used to establish a timeframe for when a reduction occurred is to compare historical versus contemporary effective population sizes (N_e).

The burrow-dwelling wolf spider, *Arctosa sanctaerosae* (Aranea: Lycosidae) is endemic to the coastal dune system of the Northern Gulf of Mexico Coast (NGC). The geographic range of this species stretches from the panhandle of Florida to the western border of Mississippi (Dondale & Redner 1983). *A. sanctaerosae* is found primarily on secondary dunes, inland from the high tide mark (McNatt et al., 2000). The health of this critical habitat is dependent upon the continuity of coastal dunes, which are the first line of defense against severe erosion (Stalter & Odum 1993). The NGC has seen large-scale change, beginning in the early 20th century, as it became a popular vacation destination. The once contiguous population has been subdivided and genetically isolated clusters exhibit extensive genetic structure (Chapter 3).

The goal of this study is 2 fold: (1) estimate the current census and effective population size (2) estimate historical effective population sizes to determine the timing of population declines (3) to attempt to identify genetic bottlenecks during the period of increasing rates of development.

Methods

Sample collection, Amplification, and Scoring

I collected tissue samples from a minimum of 20 individuals per site at 11 sites along the NGC between June 1st and 11th 2007 for a total of 287 individuals. All samples were stored at -80°C in 100% ethanol. I sampled the sites on warm clear summer nights using a headlamp to spot the eye shine of individuals. They were then identified as *A. sanctaerosae* before proceeding to extraction of genetic material. Approximately 1mg of tissue from the legs was used for subsequent extraction of DNA.

I extracted DNA using a Qiagen DNeasy kit. I then amplified target sequences by PCR using 11 microsatellite primers. Ten of these primers were developed specifically for this species (Hataway et at. 2011, Chapter 2), the other two had been developed for another species of Lycosid. PCR reactions contained 10x reaction buffer, 50 m_M $MgCl^2$, 160 μ_M each dNTP, 1 μ_M each primer, and 1 U Taq polymerase. The PCR Cycle was 94°C for 1 min followed by 20 cycles of: 94°C for 30 sec, 60°C for 25 sec decreasing every cycle by 0.3°C (Touchdown PCR), 72°C for 40 sec; then 8 cycles of 94°C for 30 sec, 56°C for 25 sec, 72°C for 40 sec and a final extension of 72°C for 30 min. Fluorescence-labeled fragments were visualized on an ABI 3130 genetic analyzer and allele sizes were determined through comparison with a known size standard (GeneScan -500 Rox) using GeneMapper version 3.7 (Applied Biosystems). All scores were checked manually and ambiguous fragments were reanalyzed.

Population subdivision

I used the Bayesian clustering algorithms found in the programs STRUCTURE (Pritchard et al. 2000) and GENELAND ver. 3.2.4 (Guillot et al. 2005, Guillot and Santos 2009). GENELAND was run using the spatial and correlated model, which assigns individuals to clusters without prior knowledge of where that individual was sampled. This accounts for several

factors of importance in this system. Spatially we expect areas of intense human impact to create a barrier between clusters and so we add into the model the set of georeferenced coordinates (Guillot and Santos 2009). This model does assume Hardy-Weinberg equilibrium that genotypes are spatially correlated, and loci are unlinked. I ran 8 independent runs of 1 million Markov Chainchain Monte Carlo (MCMC) iterations allowing the number of genetically distinct clusters (K) to vary from one to eleven. The resulting clusters were then analyzed for differentiation, gametic equilibrium, and the assumption of Hardy-Weinberg equilibrium. I grouped all sites into five subpopulations as determined by GENELAND for all subsequent analyses.

Bottlenecks and recent effective population size

To test for evidence of recent bottlenecks, I calculated the M ratio (Garza and Williamson 2001) in ARLEQUIN version 3.1 (Excoffier et al. 2005, Excoffier 2010) and levels of heterozygosity BOTTLENECK version 1.2.02 (Piry et al. 1999). The M ratio is a measure of the mean ratio of the number of alleles compared to the range in allele size (that is the range in number of repeats or physical length of an allele) for a given subpopulation, and values smaller than 0.68 indicate recent and severe bottlenecks (Garza and Williamson 2001). Simply put, the M ratio is predicted to go down after a bottleneck because alleles are lost to a type of sampling error. As alleles are removed, the range of allele sizes should not decrease at the same rate because the frequency of alleles is not distributed normally (Garza and Williamson 2001). The effectiveness of this method has been shown to be maximized when the bottleneck is older and lasted several generations before recovery (Williamson-Natesan 2005).

I used BOTTLENECK (Cornuet and Luikart 1996, Piry et al. 1999) for estimations of bottlenecks within the previous 4Ne generations, for this species approximately 100-500 years.

The method employed in this program has been shown to have the ability detect less severe and more recent reductions in population size. Excess heterozygosity, caused by a faster loss of alleles than total heterozygosity, Bottleneck does this through testing for heterozygosity excess, which is defined as expected heterozygosity at Hardy Weinberg (H_e) minus heterozygosity at the mutation-drift equilibrium (H_{eq}). This is not to be confused with excess heterozygosity ($H_o>H_e$). When severe declines occur alleles are lost at a faster rate than heterozygosity. BOTTLENECK compares the amount of heterozygoisty to the total number of alleles present in order to detect these declines. This was assessed using both the stepwise mutational model (SMM) and two-phased mutation model (TPM) separately for 10,000 iterations and a Wilcoxon test for significance. The suggested minimum number of individuals and polymorphic loci for performing this test are 15 and 10 respectively.

Recent effective population sizes were estimated using ONeSamp (Tallmon et al. 2008) as well as LDNe (Waples and Do 2008). LDNe uses linkage disequilibrium to estimate N_e. The major issue affecting its usefulness for this study is that linkage disequilibrium can be caused by inbreeding, substructure, or immigration. The first is known to be an issue in this species and so the results of these tests will bear careful scrutiny. ONeSAMP on the other hand uses eight different genetic parameters including: "the number of alleles divided by allele length range (Garza & Williamson 2001), the difference of the natural logarithms of variance in allele length and heterozygosity (King et al. 2000), expected heterozygosity (Nei 1987), number of alleles per locus, Wright's F_{IS} (Nei 1987), the mean and variance of multilocus homozygosity, and the square of the correlation of alleles at different loci (Hill 1981)."(Tallmon et al. 2008). This is expected to provide more accurate results, although high inbreeding levels (F_{IS}) may confound

results. I report the results for both methodologies including estimates using both 0.01 and 0.02 for the lowest allele frequencies for the analysis in LDNe.

Historical Effective Populations Sizes

To calculate historical effective population sizes I used three methods. First I used the equation $\Theta = 4N_e\mu$ where Θ is the mutation scaled effective population size and μ is the mutation rate (Gaggiotti and Excoffier 2000). I held mutation rate constant and at a rate of 5 x 10^{-4}. A coalescent approach was used estimate of Θ over the last $4N_e$ generations in the program MIGRATE (Beerli and Felsenstein 1999, Beerli 2010). This was run using maximum likelihood, the Brownian motion mutation model, and the matrix migration model. After a burn in of 30,000 genealogies, I ran 15 short chains, until 500 genealogies were recorded from 50,000 sampled, and then I ran 10 long chains until 500 genealogies were recorded from 8x 10^7 sampled. I then summarized across chains as recommended (Beerli 2010).

I also calculated Ne using the method of Hartl and Clark (1989) and Ohta and Kimura (1973). These methods assume an infinite allele model and a step-wise mutational model respectively. Both methods hold N_e as a function of H_e. The mutational models estimate the upper and lower extremes of mutation and so the true Ne is likely to be found between the two estimates (Busch et al. 2007). A paired-t test was then carried out between the historic and recent estimates of Ne.

Census Population Sizes

Census population sizes were calculated for each of the five clusters by multiplying population densities across the clusters by the total habitat area. Population density was

calculated one hour after nightfall on three consecutive clear summer nights inside 3 independent 12m by 12m quadrants (144m^2) randomly placed within the secondary dunes of the eleven high quality sites developed for Chapter 1. These quadrants were relocated each year using GPS data and re-sampled. Densities were averaged across sites within clusters for further analysis. The total extent of habitat was estimated by calculating the area of secondary dunes within each cluster. This was done using GOOGLE EARTH by creating polygons and using the area tool to calculate the total area. The density per 144m^2 was then multiplied against total area to estimate census population size.

RESULTS

Cluster analysis

GENELAND and STRUCTURE identified five population clusters that were consistent across all independent runs. The three sites of western Alabama were clustered as a single population (cluster 1) despite Dauphin Island and Fort Morgan being separated by a 5 km stretch of water. The pairs of sites in clusters 2 (Perdido Key / Fort Pickens) and 3 (Navarre Beach / Destin Point) are also separated by stretches of water. Geographically isolated by human development, Henderson Park (cluster 4) did not cluster with any other sites. Finally the three most easterly sites (Mexico Beach, St. Joe, and St. George Island) were grouped into a single cluster (cluster 5) (Figure 7).

Figure 8 Map showing extent and location of clusters identified by GENELAND
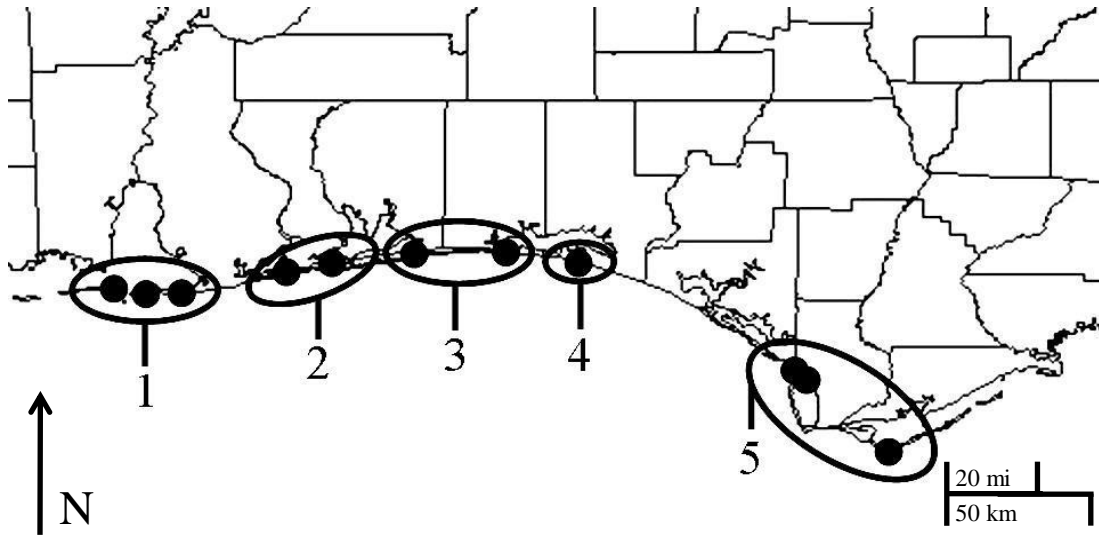
Census population size

Census population sizes were large and ranges from 71,000 to 315,000 individuals spread across the available habitat per cluster (Table 13). There was a significant difference between recent Ne and estimates of N_c for each of the clusters.

Historical and recent effective population size and bottlenecks

Tests of linkage disequilibrium, within each population as well as across populations, were all insignificant. This satisfies the assumption that all loci are unlinked. Estimates of recent effective population size from LDNe varied widely and included negative values as well as upper estimates that reached infinity. Varying the lowest allele frequency from 0.01 to 0.02 did not narrow the 95% confidence intervals (Table 13). ONeSAMP gave results that appeared to be more biologically important. Each of the clusters has a N_e of less than one hundred individuals with the exception of cluster 5. Mean estimates range from 32 to 153 individuals with less variation around the estimates compared to LDNe. Only the ONeSAMP estimates were used for comparisons against historic N_e.

Historic estimates of N_e (Table 14) across methods varied. The mean of the three

methods was used for subsequent analyses and was significantly higher than the estimate of

recent effective population size (paired-t test results here) and the lower C.I. didn't cross the

upper C.I. of the estimates of recent N_e.

Table 13 Estimates of recent effective population size (N_e) and census population size (N_c)

Cluster	LDNe 0.02= lowest allele frequency			LDNe 0.02= lowest allele frequency			ONeSAMP			Census population size
	Lower C.I.	Ne	Upper C.I.	Lower C.I.	Ne	Upper C.I.	Lower C.I.	Ne	Upper C.I.	
1	247	-	∞	64.5	-	∞	53.7	68.4	81.4	128,350.0
2	8.6	29	1059.2	16.5	62.3	∞	18.6	32.7	43.7	87,597.9
3	48.6	-	∞	50.8	-	∞	34.5	50.2	68.4	71,066.7
4	40.2	-	∞	42.1	-	∞	31.1	46.8	56.3	196,48.6
5	142.7	-	∞	123.8	-	∞	101.7	153.9	243.6	315,842.2

Table 14 Estimates of Historic effective population size (N_e)

Cluster	SMM			IAM			Migrate			Mean of three methods		
	Lower C.I.	Mean	Upper C.I.	Lower C.I.	Mean	Upper C.I.	Lower C.I.	Mean	Upper C.I.	Lower C.I.	Mean	Upper C.I.
1	58.8	88.0	117.2	71.8	90.4	111.0	98.7	112.7	125.8	88.7	94.0	160.4
2	136.5	171.6	206.7	105.4	149.4	193.3	137.7	159.6	173.2	115.5	160.2	204.9
3	175.4	219.1	262.8	140.9	184.9	229.0	159.4	176.8	211.5	103.3	193.6	283.9
4	309.8	360.3	410.8	230.6	281.3	331.9	384.6	411.3	485.2	287.6	351.0	614.3
5	841.9	881.7	921.5	524.0	563.8	603.7	789.1	873.4	942.3	544.4	773.0	1,501.5

Tests of heterozygosity in BOTTLENECK showed no significant excess in the five clusters. However, the M ratios (generated by ARLEQUIN) ranged from 0.17 to 0.72. The three western clusters all had upper 95% C.I. estimates below the critical value of 0.68 (Figure 7). These results support recent and severe bottleneck events in clusters one, two and three. Cluster four has an M ratio below the critical value but its confidence intervals overlap it suggesting an older or less severe event. Cluster five has an M ratio above the critical value and doesn't appear to have been bottlenecked.

Figure 9 M ratios (and 95% C.I.) for five clusters along the NGC and critical value (0.68)
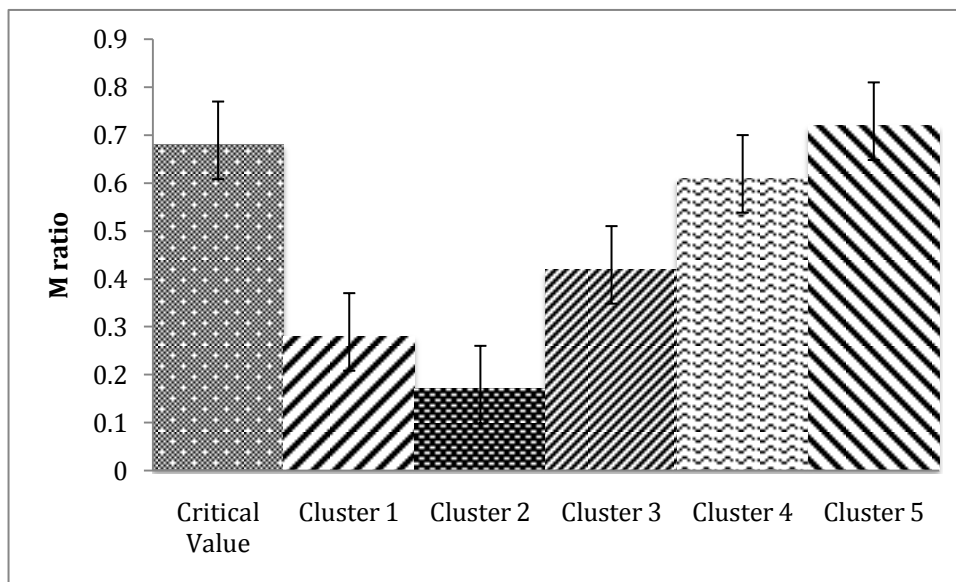
DISCUSSION

The goals of this study were 3 fold: (1) estimate the current census and effective population size (2) estimate historical effective population sizes to compare to contemporary estimates for determining the timing of expected population declines due to commercialization of the NGC and (3) to attempt to identify genetic bottlenecks during the period of increasing rates of development.

Current N_e versus N_c

The effective population sizes of the clusters of *A. sanctaerosae* along the NGC were significantly smaller than the census sizes. This is to be expected given the high levels of inbreeding seen in the clusters (Chapter 3), though this may be an artifact of non-random sampling. Individuals genotyped were from multiple discrete sites within what became a single distinct cluster. These N_e scores should be considered estimates. F_{is} scores ranged from 0.18-0.06. This suggests low amounts of dispersal across available habitat. This comparison of effectively very small populations when compared to their census size is a direct result of a lack of exchange of migrants across all clusters; however sites within clusters are still exchanging migrants. This migration appears to be elevated after major storm events (chapter 3).

Historic versus current Ne

There is a significant difference in historic effective population sizes compared to estimates of recent N_e. These estimates of N_e are subject to error and have wide 95% confidence intervals. It is difficult to estimate when this decline happened. Tests within BOTTLENECK do not have the power to recover recent events. BOTTLENECK attempts to detect declines over the last 4Ne generations (100-500 years). The test of M ratios did however demonstrate recent severe reductions in population size in clusters 1, 2 and 3. This contrast between low M ratios and no evidence of a genetic signature using BOTTLENECK does not mean that the decline is older than 500 years, as BOTTLENECK has known issues resolving recent events (Williamson-Natesan 2005). The clusters have been reduced from some previous larger size to their current effective sizes. This timing appears to correlate with increases in commercial development of the NGC.

Conservation Implications

Each of the clusters has experienced a decline in effective population size and migration (Chapter 2) within the last 100-500 generations. *A. sanctaerosae* maintains high levels of inbreeding (Chapter 2) and low levels of dispersal normally. These clusters of *A. sanctaerosae* along the NGC experience frequent density independent catastrophes in the form of severe tropical storms. These clusters experience severe reduction in size due to hurricanes or other catastrophic events; however, they have the ability to recover within one to two generations through migration to recently vacated habitat patches. This migration erases enough of the genetic signature of the reduction that it is undetectable using the current methodology. The reductions in genetic diversity is a function of the subdivision what was once a much larger single cluster along the NGC rather than the temporary reductions in population size due to hurricane activity. Hurricanes remove genetic diversity but historically this effect was ameliorated thru migration across the NGC to fill in vacant patches. Recently, these populations have been reduced in size and diversity as a direct result of subdivision of the NGC. Once subdivided, the migration between clusters was reduced and genetic drift, direct removal of diversity due to severe tropical storms and commercial development has lead to declining population sizes and reduced diversity.

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VITA

Robert Andrew Hataway

BS Biology (2004) Samford University Thesis: "Correlation of Population Density of *Arctosa sanctaerosae* (Araneae, Lycosidae) to Human Impact along the Northern Rim of the Gulf of Mexico" Advisor: Dr. Ronald L. Jenkins

BA History (2004) Thesis: "A comparison of the role and rhetoric of women involved in the progressive environmental and children's conservation movements" Advisor: Dr. Marjorie A. Walker

Academic Awards

Awarded the University of Mississippi Graduate Student Council Research Grant Award 2005
Second Prize in the Poster Competition at the annual meeting of the American Arachnological Society 2004