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Analyzing Invasion Success of the Mayan Cichlid (Cichlasoma urophthalmus; Günther) in Southern Florida

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ANALYZING INVASION SUCCESS OF THE MAYAN CICHLID (*CICHLASOMA UROPHTHALMUS GÜNTHER*) IN SOUTHERN FLORIDA

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Elizabeth Harrison

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To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Elizabeth Harrison, and entitled, Analyzing Invasion Success of the Mayan Cichlid (*Cichlasoma urophthalmus* Günther*)* in Southern Florida, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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DEDICATION

This work is dedicated to all the friends, labmates, and family members who gave of their time, energy, and love to help me collect fish from Florida, Mexico, and Central America. I also dedicate it to the fish that valiantly gave their lives in the pursuit of scientific knowledge.

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ABSTRACT OF THE DISSERTATION

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Invasive species have caused billions of dollars in damages to their introduced environment through direct effects on wildlife and by altering their introduced habitats. For a species to be considered invasive, it must successfully navigate the stages of invasion: it must be introduced, become established, spread, and have a quantifiable impact on its introduced environment. The numbers of introductions and individuals released affects the genetic diversity of nonnative populations which, in turn, can affect their invasion success.

The Mayan Cichlid (*Cichlasoma urophthalmus*) is endemic to the Atlantic coast of Mexico and Central America. It was first detected in the United States in 1983 in Everglades National Park. Since then, it has spread across more than 70,000 hectares throughout southern and central Florida. I have established the Mayan Cichlid to be a successful invader in Florida by quantifying per capita negative impacts of Mayan Cichlids on densities of Sheepshead Minnow (*Cyprinodon variegatus*), Marsh Killifish (*Fundulus confluentus*), and Eastern Mosquitofish (*Gambusia holbrooki)* over a 15-year

period. I also analyzed the role of genetics in the invasion success of the Mayan Cichlid. I used a mitochondrial gene, cytochrome b, and 17 microsatellite loci to identify the sources for the Mayan Cichlid introduction into Florida. Cytochrome b data supported an introduction from Guatemala; microsatellite data suggested movement of Mayan Cichlids from the upper Yucatán Peninsula to Guatemala and introductions from Guatemala and Belize to Florida. I also found evidence of cytonuclear disequilibrium together with low genetic diversity within the Florida population which indicate a population bottleneck and admixture between two distinct lineages upon introduction, followed by rapid spread resulting in a panmictic population genetically distinct from the native range populations. I found much less genetic structure and a weaker correlation between genetic diversity and geographic distance within Florida compared with Mexico and Central America. Low number of effective alleles, heterozygosities, and F_{ST} values and the genetic similarity of Florida sites also indicate an admixed population or one that has rapidly expanded from a small initial group.

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CHAPTER 1

INTRODUCTION

More than 50,000 species have been introduced into the United States resulting in billions of dollars in damages to the introduced environments, including impacts on resident species, as well as in management costs (Pimental et al. 2005). Invasive species can impact native species through predation, competition, parasitism, hybridization, and habitat destruction (Lockwood et al. 2007).

What makes a species invasive?

Not all species introduced outside of their native ranges become invasive. For a species to be considered invasive, it must be successfully transported to the new area, become established, spread, and have a quantifiable impact on its new environment (Kolar and Lodge 2002; Lockwood et al. 2007; Fig 1.1).

Species can occupy new ranges either by natural dispersal and colonization, which can be aided by natural events such as hurricanes or floods, or through human-mediated transport. Human-mediated introductions can be intentional such as for food and game (Lever 1996; Moyle 2002; Mack 2003; Cowie and Robinson 2004; Weigle et al. 2005), stocking environments with aesthetically or culturally desirable species including ornamental plants, pets, and the aquarium trade (Fuller 1999; Kraus 2004), biocontrol of unwanted pest species (Lever 1996; Simberloff et al. 2000; Hoddle 2004), protection and conservation of endangered species in zoos, botanical gardens, and wildlife preserves (Sigler and Sigler 1987), and for scientific research (Meinesz 1999; Cowie and Robinson 2004). Some species are introduced accidentally as the byproduct of movement of other goods; they can be hitchhikers on, within, or alongside other

nonnative species, for example, the importation of bait species for catching nonnative game fish (summarized in Fuller 2004). Individuals can also be transported within cargo holds or packing material, within ship ballast (Carlton 2000; Vazquez and Simberloff 2001; Mack 2004) and within ship and airplane holds as in the case of the brown tree snake (*Boiga irregularis*) in Guam (Fritts and Rodda 1998). The most common transportation vectors for introduced species within the United States are for food and game, the plant nursery trade, and for the pet trade including fish, amphibians, birds, and reptiles (Lockwood et al. 2007). The kind of introduction pathway can influence the number of introduction events and the number of individuals released. The more individuals released, the more locations individuals are released to, and the healthier the individuals, the higher the propagule pressure and the greater the probability of successful establishment (D'Antonio et al. 2001; Lockwood et al. 2007).

For a species to become established, it must be able to quickly adapt to its new, introduced environment by finding resources to exploit and by founding a self-sustaining, viable population (Sakai et al. 2001). Spread is facilitated by population growth and the ability to disperse to new environments and exploit resources therein (Lockwood et al. 2007). While many studies of introduced species focus on establishment and spread, few quantify impacts of nonnative species (Parker 1999). There is some debate in the literature about impacts of nonnative species (Gozlan, 2008; Leprieur et al., 2009; Vitule et al., 2009) and there is a need for more research about quantifiable impacts (Casal 2006).

The mild climate of Florida facilitates establishment of nonnative tropical species, including fishes (Wilcove et al., 1998; Pimental et al., 2005). The majority of nonnative

fishes in Florida are members of the family Cichlidae; at least 13 species are established in Florida (Fuller et al. 1999; Shafland et al. 2008). The Mayan Cichlid (*Cichlasoma urophthalmus* Günther) is found along the Atlantic versant of Mexico, Belize, Guatemala, Honduras, and Nicaragua (Miller 1966; Miller et al. 2005). It was first recorded in Everglades National Park in 1983 (Loftus 1987); since then, it has spread across approximately 70,000 hectares and is found throughout southern and parts of Central Florida (Adams and Wolfe, 2007; Paperno et al. 2008; USGS 2013). In Chapter 2, I analyzed 15 years of fish assemblage data to evaluate the impacts of this nonnative species on native fishes in the southern Florida Everglades. The Mayan Cichlid species has become established and spread; the elucidation of quantifiable impacts over time would classify this species as invasive.

The role of genetics in invasions

By definition, nonnative populations are founded by a set of individuals transplanted out of their native range into a new area. Therefore, introductions usually involve a small portion of the original population that would carry only a fraction of total genetic diversity of the donor population (Lee 2002). The degree to which genetic diversity of a species decreases upon introduction depends on the number of individuals released and the number of release events. High levels of genetic variation are thought to be necessary for rapid adaptation to the new environment so genetic diversity within the founding population will affect success of the nonnative species (Allendorf and Lundquist 2003). Studies have shown that multiple introductions of an invasive species are correlated with establishment and success because the founding population exhibits

more genetic variation than one with a single release, especially if the multiple introductions are from different source populations (Gillis et al. 2009; Kolbe et al. 2004; Sakai et al. 2001). Introductions from multiple sources can produce novel genetic combinations that increase fitness and enhance invasion success (Crawford and Whitney 2010; Ellstrand and Schierenbeck 2000; Keller and Taylor 2010). However, there have also been cases of small introductions and subsequent low genetic diversity in founding populations that resulted in nonnative establishment (Dybdhal and Drown 2011; Grapputo et al. 2005). Whether a species undergoes single or multiple introductions determines the genetic variability of the founding population which in turn will affect its ability to adapt to its new range, become established, spread and impact its environment. It seems that successful invasions can result from both population and genetic bottlenecks and decreased genetic diversity, and elevated diversity from intermixing genetically distinct propagules. In Chapter 3, I identified the spatial sources of introduction of Mayan Cichlids using mitochondrial and nuclear loci and thus determined whether a limited introduction or multiple releases led to the establishment and spread of a successful invader. Reconstructing and identifying introduction pathways and vectors are also necessary to prevent further introductions of the same species or of other species along similar routes.

In Chapter 4, I used microsatellite loci to examine the effects of introduction pathway on genetic variation within the Mayan Cichlid population in Florida by comparing population genetic structure within the introduced and native ranges. I determined whether introduction decreased or elevated genetic diversity in the founding population over time. Small, limited releases are expected to lower genetic variation (Nei

et al. 1975) while introductions from multiple sources that mix previously separate lineages should increase genetic diversity when compared to similar areas in the native range (e.g. Kolbe et al. 2004). I also analyzed genetic sub-structure and proposed movement pathways of the species within its native range. Multiple introductions from a source population that is highly genetically structured across its native range tend to result in founding populations with high genetic diversity (e.g., Novak et al. 1993; Martel et al. 2004).

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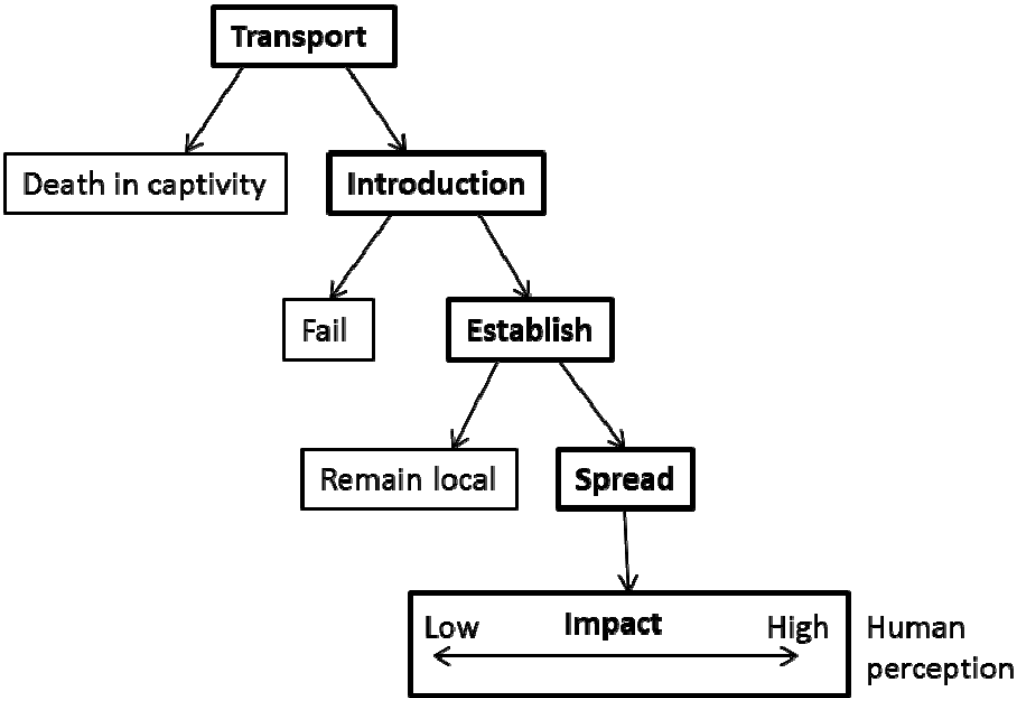

Figure 1.1. Model showing the steps in the invasion process. Successful invaders are able to navigate the stages outlined in bold; alternative outcomes are also included. This model was adapted from Figure 1.2 in Lockwood et al. 2007.

CHAPTER 2

PER CAPITA EFFECTS OF NON-NATIVE MAYAN CICHLIDS (*CICHLASOMA UROPHTHALMUS* GÜNTHER) ON NATIVE FISH IN THE ESTUARINE SOUTHERN EVERGLADES

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ABSTRACT

The Mayan Cichlid (*Cichlasoma urophthalmus)* is an omnivorous fish endemic to Central America that was first recorded in South Florida in 1983. We examined their effects on native fishes in estuarine mangrove habitats between 1991 and 2006. Four major cold fronts passed during the study period and each killed many Mayan Cichlids, providing multiple opportunities to observe native fish responses to fluctuation in cichlid densities. Fish assemblage data were collected using drop traps placed at three estuarine sites and one impounded site. Analysis of similarity indicated that differences in assemblage structure among the four sites correlated with the presence of Mayan Cichlids. At two sites with high Mayan Cichlid density, SIMPER analysis revealed that relative densities of Sheepshead Minnows (*Cyprinodon variegatus)*, killifish species, Clown Gobies (*Microgobius gulosus)*, Eastern Mosquitofish (*Gambusia holbrooki),* Sailfin Molly (*Poecilia latipinna*), Tidewater Silverside (*Menidia peninsulae*), and *Lepomis* species were correlated with Mayan Cichlid relative density. Time series analysis of data from the two sites with high Mayan cichlid density indicated negative relationships between their density and density of Sheepshead Minnow, Marsh killifish (*Fundulus confluentus)*, and Eastern Mosquitofish after controlling for salinity. When present, the per capita impacts on Sheepshead Minnows were 40% to 60% greater than on the other taxa. Partial regression slopes of native fish density on Mayan Cichlid density were negative with unpatterned residuals across a broad range of cichlid densities, providing no indication of predator saturation or interference at high density. This may have resulted because of immigration of native fish to these sites during the South Florida dry season.

INTRODUCTION

There is an ongoing debate about the relative impacts of non-native fishes introduced to freshwater and estuarine ecosystems (Gozlan, 2008; Leprieur et al., 2009; Vitule et al., 2009). A universal theme in this debate is the need for more research on impacts to provide a stronger basis for management recommendations. Though many accounts indicate that most introduced fish species appear to be relatively benign (Moyle and Light, 1996), some introductions have been shown to have major negative effects on native biota (Pope et al., 2008; Koel et al., 2005). Unfortunately, scientists have limited ability to predict the degree of impact at the initial appearance of a species (Lockwood et al., 2007). The inability to detect an impact after introduction does not preclude such impacts (Simberloff, 1997), but the apparent lack of impact may result from limitations in sampling or experimental methods. Also, it is possible for an introduced population to remain small and locally restricted for many years before expanding markedly and becoming a management problem (Richardson et al., 2008).

Semantics of what constitutes an impact hinders progress in invasion biology. Parker et al. (1999) proposed that the total impact of an invasive species is determined by its range, abundance, and per capita effect. While many authors attempt to quantify impacts of an introduced species, not all aspects of total impact are examined, especially per capita effects (Britton et al., 2010; Pilger et al., 2010; Kulhanek et al., 2011). Furthermore, impacts are seldom quantified over multi-year time scales in nature, making evaluation of long-term effects difficult (Arthur et al., 2010; Sellheim et al., 2010; Young et al., 2010; Brewer, 2011). We believe that more analysis of per capita impacts is needed to overcome our limited ability to predict the effects of biological invasions (e.g.,

Gherardi, 2007). In this paper, we analyze the density and per capita impact of a nonnative cichlid species, *Cichlasoma urophthalmus* (Mayan Cichlid), on native fishes over a fifteen year period from a portion of its introduced range.

The mild climate of Florida facilitates establishment of nonnative tropical species, including fishes (Wilcove et al., 1998; Pimental et al., 2000). The majority of exotic fishes in Florida are members of the family Cichlidae, a family with no species indigenous to the state but at least 13 established within the past 40 years (Fuller et al., 1999; Shafland et al., 2008). The Mayan Cichlid is found along the Atlantic slope of Mexico, Belize, Guatemala, Honduras, and Nicaragua (Miller, 1966). In its native ranges, it is economically important to artesanal fisheries and aquaculture (Martinez-Palacios et al., 1990; Chavez-Lopez et al., 2005). It was first recorded in the Everglades National Park in 1983 (Loftus 1987); by 1999, it had spread to the north over 200 miles on both coasts, and was found throughout southern Florida (Adams and Wolfe, 2007; Paperno et al., 2008; USGS 2012). Gut content data have shown that Mayan Cichlids feed on native fish in their introduced range, including Eastern Mosquitofish , Bluefin Killifish and Sailfin Mollies (Howard, 1995; Loftus, 2000; Rehage et al., 2009; the scientific names for all fishes referenced are provided in Table A1 of the Appendix). However, it is not known whether Mayan Cichlid density influences the community structure and population dynamics of native fish in a way that is ecologically significant. This study allowed us to investigate the potential for Mayan Cichlids to alter native fish assemblages.

We used 15 years of fish assemblage data, collected at four sites, to analyze the impacts of Mayan Cichlids on native fishes within estuarine habitats of the southern

Everglades. We defined impacts as interspecific relationships quantifiable as a per capita effect by the nonnative species within species assemblages (Parker et al., 1999). We first compared the fish assemblages between two sites where Mayan Cichlids were rare with two sites where Mayan Cichlids were abundant. Given that Mayan Cichlids are known to prey on native fish (Howard, 1995; Loftus, 2000; Rehage et al., 2009), we hypothesized that differences in densities of native fishes between the two groups of sites resulted from the effects of Mayan Cichlids because no other non-native species are present in numbers in this area. We further examined the fish assemblages within the two sites where Mayan Cichlids were more abundant to explore the temporal relationships between Mayan Cichlids and specific fish species. Because Mayan Cichlids are, at times, very abundant, we also hypothesized that their per capita impacts on native taxa will diminish as a function of cichlid density because of predator interference as prey are depleted (a ratio-dependent type II functional response; Skalski and Gilliam, 2001).

MATERIALS AND METHODS

Study sites and sampling methods **–** We sampled four estuarine sites within the southern Everglades (Fig. 2.1). Taylor River (TR), Joe Bay (JB), and Highway Creek (HC) are located in the Taylor Slough/C-111 drainage area of Everglades National Park; Barnes Sound (BS) is an impounded coastal wetland site. At each site, two habitats were sampled, continuously inundated creeks and surrounding, seasonally inundated flats. All four sites experienced wind-driven tides; only Barnes Sound was subject to small diurnal tides of 9 – 15 cm on calm days. Wind-driven tides were capable of changing surface water levels by more than 40 cm within a 24 h period (Lorenz and Serafy, 2006).

Vegetation at all sites consisted mainly of widely-spaced dwarf red mangroves (*Rhizophora mangle)* (0.5 – 5.0 m between trees; 0.5 – 2.0 m tall). There was seasonal growth of emergent spikerush, *Eleocharis cellulosa,* and the submerged macrophytes *Utricularia* spp. and *Chara* spp. (Lorenz and Serafy, 2006). The substrate for all sites was flocculent, unconsolidated carbonate marl (Browder et al., 1994).

Sites were sampled with 9-m^2 drop traps that were fixed at permanent locations (Lorenz et al., 1997). Six traps were deployed per site with three in each sub-habitat, creeks and flats. Each trap was placed to surround a dwarf mangrove tree so that both prop root habitats and the open areas between trees were sampled. Trees were selected for sampling such that at each site, equivalent prop root density was sampled; the stationary habitat was consistently sampled from site to site (Lorenz et al., 1997). These sites were sampled each year in June and September, then monthly from November to April (eight sampling months), from 1991 to 2006. The drop trap surrounded an area of 9 m^2, was approximately 70 cm high and was made of light-weight cloth that prevented the movement of water from inside to outside of the trap to prevent unintended mortality outside the trap when a toxicant was applied inside (Lorenz et al., 1997). Species densities were recorded at each drop trap. The traps were set, left in place overnight, and deployed within 2 hours of sunrise. All fishes were cleared from the trap following application of rotenone. The traps were re-checked the next day for missed fish which were added to the sample. Specimens were taken fresh from the field, frozen, and then processed in the laboratory; specimens were identified and counted so that species densities could be recorded for each drop trap. Salinity, water temperature and depth

measurements were taken on days that the traps were deployed. Water depth was recorded at each trap while salinity and water temperature were measured by a gauge at each site.

Analysis of fish communities across sites – We analyzed data from the four sites to compare their fish assemblages. At each site, the creek and flats were analyzed separately. Species that comprised less than 0.1% of total fishes captured were removed from the data to minimize complications in analysis due to excessive zeros. For each sample, we calculated the density for each species by averaging their densities from the three traps in each of the two habitat types (creeks and flats) and then we calculated the relative density of each species for that sample. Those data were fourth-root transformed to balance the impact of rare and abundant taxa within each sample for subsequent analyses. We produced Bray-Curtis dissimilarity matrices using Primer 5 software (Clarke and Warwick, 2001). The four sites were divided into two groups: two sites where Mayan Cichlid density was low (Barnes Sound and Highway Creek) and two sites where Mayan Cichlid density was high (Joe Bay and Taylor River); mean Mayan Cichlid density differed between the two groups of sites ($t = 33.13$, $p < 0.01$). An analysis of similarity (ANOSIM) was performed and non-metric multi-dimensional scaling (NMDS) plots (McCune and Grace, 2002) were used to illustrate patterns of native fish assemblage composition (excluding Mayan Cichlids) at the two site groups. Mayan Cichlids were excluded from the ANOSIM and NMDS plots to illustrate distributions of other species between the two-site groups since it had been previously shown that Mayan Cichlid densities differed significantly between them. NMDS is an ordination method that produces multi-dimensional visual representations of dissimilarities among sample

points; points that are close together in ordination space display higher levels of similarity than points farther apart. Similarity percentage (SIMPER) analysis examines the contribution of each species to the mean Bray-Curtis dissimilarity between and within groups of samples (Clarke and Warwick, 2001). SIMPER analysis was used to identify the fish species that contributed most to the dissimilarities in assemblage composition between the two site groups. The densities of the species identified by SIMPER analysis differed between the two-site groups and were hypothesized to be affected by Mayan Cichlid density. To evaluate this hypothesis, the densities of these species were used in regression analyses to determine the per capita effect of Mayan Cichlids.

Analysis of fish density within two sites – To examine the effects of Mayan Cichlids on specific resident fish species, we analyzed data collected from Joe Bay (JB) and Taylor River (TR). These sites were selected because Mayan Cichlid density varied greatly over time, permitting us to analyze impacts of Mayan Cichlids when their densities were very low and very high (Fig. 2.2). We also analyzed two habitats, creeks and flats; both habitats had the same species pool, but Mayan Cichlid density was consistently lower in flats than creeks. Sharp declines in Mayan Cichlid density followed cold fronts when water temperatures were below 20 ^{o}C for several days (dotted lines in Fig. 2.2). The temporal fluctuations in Mayan Cichlid density at Joe Bay and Taylor River were used to evaluate the impacts of Mayan Cichlids on resident fish species.

For the regression models, fish densities were log-transformed and every sample was included in the analyses (i.e. there was no averaging across samples or sites). Seven candidate models were tested for each fish species to determine the effects of Mayan

Cichlid density on native fishes and to evaluate alternative hypotheses affecting native species densities (Table 2.1). Because we were interested in the specific effects of Mayan Cichlids, the only biotic parameter included within our models was Mayan Cichlid density. We also incorporated environmental variables such as salinity, water temperature, water depth, sampling month and hydrological year, which were log-transformed. Data regarding changes in vegetation were not available, but Lorenz et al. (1997) indicated that vegetation, other than the mangroves was sparse. Sampling months were labeled as 1 to 8 denoting the beginning of the wet season, June, (month 1) to the end of the dry season, April, of the following year (month 8). To incorporate a temporal variable of biological significance into our models, we calculated the days since the depth at each net was 13 cm. Previous studies have shown that fish at our sites avoided depths of 13 cm or less (Lorenz, 2000).

The second-order bias correction of Akaike's Information Criteria (AICc) and Likelihood R^2 were used to select the best models from our seven candidate models (Burnham and Anderson, 1998; Anderson, 2007). At some sites, there were species whose models had likelihood R^2 values that were less than 0.1, indicating that none of our candidate models adequately explained variation in the densities of these fishes. As a result, those species were excluded from further analysis. Relative AICc values were used to determine which models contained the most information about the dependent variables. Akaike weights (Δ_i) were calculated from the differences between the AICc value of each model and the minimum AICc and are directly proportional to the likelihood of each model (Burnham and Anderson, 1998). The model with an Akaike weight greater than or equal to 0.9 or with the lowest AICc value by more than 2 units

was considered the most suitable model given our data (Anderson, 2007). Models with AICc values within 2 of the minimum AICc value were considered functionally equivalent (Burnham and Anderson, 1998; Anderson, 2007). Of the models with AICc values within 2 of the minimum AICc value, we present the model with the highest likelihood R^2. We also calculated 95% confidence intervals for Mayan Cichlid parameters and we considered models whose intervals did not include zero as biologically important. Descriptive statistics for our seven candidate models are presented in the Appendix (Tables A2 and A3). Our main objective was to determine the impact of Mayan cichlids while accounting for abiotic variables that may have also affected native fish density, so we compared model fit with and without Mayan cichlid density to determine if model fit improved and presented all tested models instead of averaging models.

Ratio-dependent type II functional response models, such as the Beddington-DeAngelis model (Skalski and Gilliam, 2001), predict a log-linear decrease in prey per capita mortality as a function of predator density. Thus, prey density should decrease at a decreasing rate as predator density increases because of predator interference, yielding a non-linear slope on a log-log scale. We evaluated the potential of such simple saturating predator-prey models to explain our data by examining partial regression plots (Gunst and Mason, 1980) for evidence of non-linearity (a backwards J-shape is predicted). Partial regression plots were also used to separate the impacts of Mayan Cichlids on native fishes from effects of abiotic factors.

RESULTS

Analysis of fish communities across sites – The four sites displayed similar salinity and temperature regimes throughout the study (mean salinities and temperature of 4.8 – 7 psu and 23 – 24 ^{o}C, respectively) except for Barnes Sound, which experienced a higher mean salinity (20.7 psu) than the other sites (Table 2.2). Barnes Sound is an impounded site that receives freshwater inflow solely from rainfall and, unlike the other sites, does not receive sheetflow from the Everglades. Mayan Cichlid density fluctuated from 1991 to 2006 in creeks and flats at the four sites. However, their average density was up to 900% greater per trap in creeks at Joe Bay and Taylor River than at Barnes Sound and Highway Creek (Fig. 2.2; Table 2.2). More Mayan Cichlids were collected in the continuously inundated creeks than in the seasonally inundated flats (Fig. 2.2; Table 2.2). For both creeks and flats, across all hydrological years sampled, Mayan Cichlids were least abundant at Barnes Sound.

We included 22 fish species in the assemblage analyses (Appendix Table A1). One-way ANOSIMs indicated that fish assemblage composition (excluding Mayan Cichlids) differed in creeks between the sites with low cichlid abundance (Barnes Sound and Highway Creek) and the sites with higher cichlid abundance (Joe Bay and Taylor River) (Global $R = 0.254$; $p < 0.01$). The same was also true for assemblage composition, excluding Mayan Cichlids, of the flats between the two site groups (ANOSIM: Global $R = 0.169$; $p < 0.01$). NMDS plots of creeks and flats from the two site groups showed that the assemblage compositions were relatively dissimilar and formed clusters (Stress values < 0.2; Fig. 2.3) (Clark and Warwick 2001). The NMDS plot of flats from the two-site groups contained one extreme outlier; this was the only sample that contained one fish, a

Goldspotted Killifish. Low R-values and some overlapping of points are expected for long-term datasets with repeated sampling and similar species among sites (Clark and Warwick, 2001), especially if some species are affected by Mayan Cichlid density more than others.

Analysis of fish communities within two sites with high Mayan Cichlid abundance – Over 88% of the 7,908 Mayan Cichlids collected were from Joe Bay and Taylor River, from which 3,236 and 3,775 individuals were taken, respectively (Appendix Table A1). SIMPER analyses indicated that the differences in assemblage composition between the two site groups (in both creeks and flats) were caused by the densities of Rainwater Killifish, Sheepshead Minnow, Goldspotted Killifish, Clown Goby, Sailfin Molly, Marsh Killifish, Eastern Mosquitofish, Gulf Killifish, Crested Goby, Bluefin Killifish, Tidewater Silverside and *Lepomis* species (Table 2.3). We hypothesized that these species differed in density across sites because of the density of Mayan Cichlids and we used these species for regression analyses.

Regression analysis for Joe Bay showed negative relationships between Mayan Cichlid density and density of Marsh Killifish and Eastern Mosquitofish ($p < 0.01$) in creeks and Sheepshead Minnows in both creeks and flats after accounting for other independent variables (Table 2.4; Fig. 2.4). For Bluefin Killifish in creeks and Marsh Killifish and Eastern Mosquitofish in flats, the best regression model did not include Mayan Cichlid density and is not reported. There was a positive relationship between density of Mayan Cichlids and *Lepomis* species ($p < 0.01$; Table 2.4; Fig. 2.4) in creeks. Environmental variables such as temperature, depth, sampling month and hydrological

year also affected native fish density and the direction of the relationships differed by species (Table 2.4).

Regression analysis for Taylor River indicated negative relationships between density of Mayan Cichlids, and density of Eastern Mosquitofish in creeks and Sheepshead Minnows in flats ($p < 0.01$; Table 2.5; Fig. 2.5). A positive relationship was observed between Mayan Cichlid and Sailfin Molly densities in creeks ($p < 0.05$; Table 2.5; Fig. 2.5). Environmental variables such as temperature, depth, sampling month and hydrological year also affected native fish species and the direction of the relationships differed by species (Table 2.5).

Overview **–**Mayan Cichlid density was negatively correlated to densities of Sheepshead Minnows, Marsh Killifish, and Eastern Mosquitofish after controlling for environmental variables such as salinity. Sheepshead Minnows experienced the greatest relative per capita impact of Mayan Cichlids (partial regression slopes -0.26 to -0.34) in Joe Bay creeks and flats of Taylor River. In these habitats, Marsh Killifish and Eastern Mosquitofish accounted for the next highest impacts, which were 40 to 60% less than those noted for Sheepshead Minnows. There was no evidence of non-linearity in any partial regression plots (Figs. 2.4 and 2.5). We evaluated this by fitting a two-term polynomial regression to these data, and in no case was the squared term significant.

DISCUSSION

Assemblage structure of small fishes differed between estuarine sites with abundant Mayan Cichlids and sites with few Mayan Cichlids. These differences were

mirrored by temporal changes in native fishes at the two sites with abundant Mayan Cichlids; as the density of Mayan Cichlids increased between winters with strong cold fronts, the density of several non-native species declined, only to resurge when the cold fronts depleted the number of cichlids. This pattern repeated several times during the course of the study, and independently at two widely separated study sites. We believe this combination of information provides strong support for the hypothesis that Mayan Cichlids were responsible for these changes. Furthermore, the per capita impact of Mayan Cichlids varied among species of small-bodied native fish, but in all cases was well described by a simple linear model with slope of less than 0 but greater than -1.0 (Tables 2.4 and 2.5). This suggests that the per capita effect on native fishes of adding Mayan Cichlids did not diminish as predicted by simple predator-prey models.

We observed negative relationships between Mayan Cichlid density and densities of Sheepshead Minnow, Marsh Killifish and Eastern Mosquitofish in creeks and flats of Joe Bay and Taylor River. Although we were unable to obtain gut content data from our samples, studies have shown that those fish species and other species of similar size ranges have been found in the gut contents of Mayan Cichlids (Howard, 1995; Loftus, 2000; Bergmann and Motta, 2005). The objective of this study was not to show that Mayan Cichlids consume native fish, this has already been shown (e.g., Loftus, 2000); instead, our goal was to quantify the impacts of Mayan Cichlids on native fish over a multi-year period, presumably resulting from predation. Mayan Cichlids were the most abundant large (up to 21cm SL) piscivore at our sites (Lorenz and Serafy, 2006); it is unlikely that other fish species would cause the observed declines in densities of smaller fishes. Mayan Cichlids may also compete with other fish for food resources since they

have broad diets, feeding on vegetation, detritus, crustaceans, insects, and gastropods, as well as fishes (Howard, 1995; Bergmann and Motta, 2005); it is very likely that Mayan Cichlids overlap with the diet of other fishes at our study sites.

We also observed some positive relationships between Mayan Cichlids and native fishes. In Joe Bay creeks, there was a positive relationship between the density of Mayan Cichlids and *Lepomis* species. In our data, *Lepomis* species include warmouth (*L. gulosus)*, redear (*L. microlophus)* and bluegill sunfish (*L. macrochirus),* with warmouth being most common. The relatively large terminal sizes of these sunfish species permit them to outgrow Mayan Cichlid predation relatively quickly and then track similar prey resources to Mayan Cichlids. There was also a positive relationship between Mayan Cichlid and Sailfin Molly density in Taylor River creeks. Sailfin Mollies are mainly herbivorous (Harrington and Harrington, 1982; Belicka et al., 2012), which would limit direct competition for food with Mayan Cichlids. They may benefit by competitive release, however, as Mayan Cichlids consume other herbivores such as Sheepshead Minnows. The low salinity range at Taylor River is optimal for Sailfin Mollies and Mayan Cichlids (Lorenz and Serafy, 2006), which may also account for their higher numbers at Taylor River and mask negative effects of predation by Mayan Cichlids. However, the specific mechanics by which Sailfin Mollies could escape Mayan Cichlid predation that is detrimental to populations of other similar fishes remain unclear and more research is required in this area.

It is important to note that at the sites where we observed negative effects of Mayan Cichlids on native fishes, Mayan Cichlids were at times very abundant. However, Mayan Cichlids are not equally abundant throughout the Everglades; their density is

lower within the Everglades freshwater marshes than in the mangrove ecotone (Trexler et al., 2000). Therefore, we cannot assume that the impacts of Mayan Cichlids on specific fish species at our sites would be observed at a regional scale throughout the ecosystem. We fit simple linear models to our data and all negative relationships had slopes between 0 and -1.0. Thus, our hypothesis of diminishing impact of Mayan Cichlids at high density was not supported (Skalski and Gilliam, 2001). This could be because these sites are not closed systems and freshwater fishes move from upstream marshes as the dry-season progresses. This increase in freshwater fishes may compensate for high-density impacts.

Our results showed that a nonnative fish can negatively impact native fish species in its introduced environment. Given the ongoing debate concerning the effects of nonnative species, particularly fishes (Gozlan, 2008; Leprieur et al., 2009; Vitule et al., 2009), and the call for more research (Casal, 2006), our study provides evidence of the adverse effects of an introduced fish on native species. Our paper also quantifies per-capita effects of Mayan Cichlids on native fishes over time, a crucial and rarely assessed component of determining impact of an introduced species (Parker et al., 1999). The effects of Mayan Cichlids on native fish assemblages may have a cascading effect on other piscivores, such as wading birds, but more research is needed to evaluate this. For example, if a cascading impact on wading birds were detected, management concerns about this species would be elevated because of the central role of wading birds in Everglades restoration (Trexler and Goss, 2009). It is important to understand the impacts of Mayan Cichlids on other species in order to determine the urgency of control or eradication programs. For example, filling canals, already under consideration for ecosystem restoration, could diminish the availability of thermal refuges and limit further

expansion. The Mayan Cichlid is a tropical species that is vulnerable to low temperatures, so it seems unlikely that they will disperse to northern states without access to thermal refuges such as canals or ditches (Schofield et al., 2010). However, if global temperatures continue to increase (Ramanathan and Feng, 2008), their potential range and impact may also expand (Rahel and Olden, 2008).

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Figure Legends

Fig. 2.1 Location of the four sampling sites in southern Florida: Taylor River, Joe Bay (JB), Highway Creek (HC), and Barnes Sound (BS).

Fig. 2.2 The mean density (per net or 9 m^2) of Mayan Cichlids in creek and flat habitats at the two site groups: BS and HC, and JB and TR from June 1991 – May 2006. The densities were averaged by season in each hydrological year, which spanned from the beginning of the wet season (June 1) of one year to the end of the dry season (May 31) of the following year. X-axis labels denote hydrological year with the year labels marking the beginning of the wet season of that year. Dotted lines indicate when the sites experienced cold fronts.

Fig. 2.3 NMDS plot of creek habitats (a) and flat habitats (b) at the two site groups: BS and HC (low Mayan Cichlid densities), and JB and TR (high Mayan Cichlid densities). Assemblage compositions were averaged by season and hydrological year for simplicity. For clarity, a two-dimensional plot is shown but the stress values for two-dimensional and three-dimensional plots are included.

Fig. 2.4 Partial regression plots for Mayan Cichlid density against densities of Sheepshead Minnow (S.M.), Marsh Killifish (M.K.), Eastern Mosquitofish (E.M.), and *Lepomis* sp (Lep.) in creeks, and densities of Sheepshead Minnow in flat habitats of Joe Bay. These species had significant ($p < 0.05$) parameter values for Mayan Cichlid density.

Fig. 2.5 Partial regression plots for Mayan Cichlid density against density of Rainwater Killifish (R.K.), Sailfin Molly (S. Mo.), and Eastern Mosquitofish (E.M.) in creeks, and density of Sheepshead Minnow (S.M.) in flat habitats of Taylor River.

These species had significant ($p < 0.05$) parameter values for Mayan Cichlid density.

Fig. 2.1

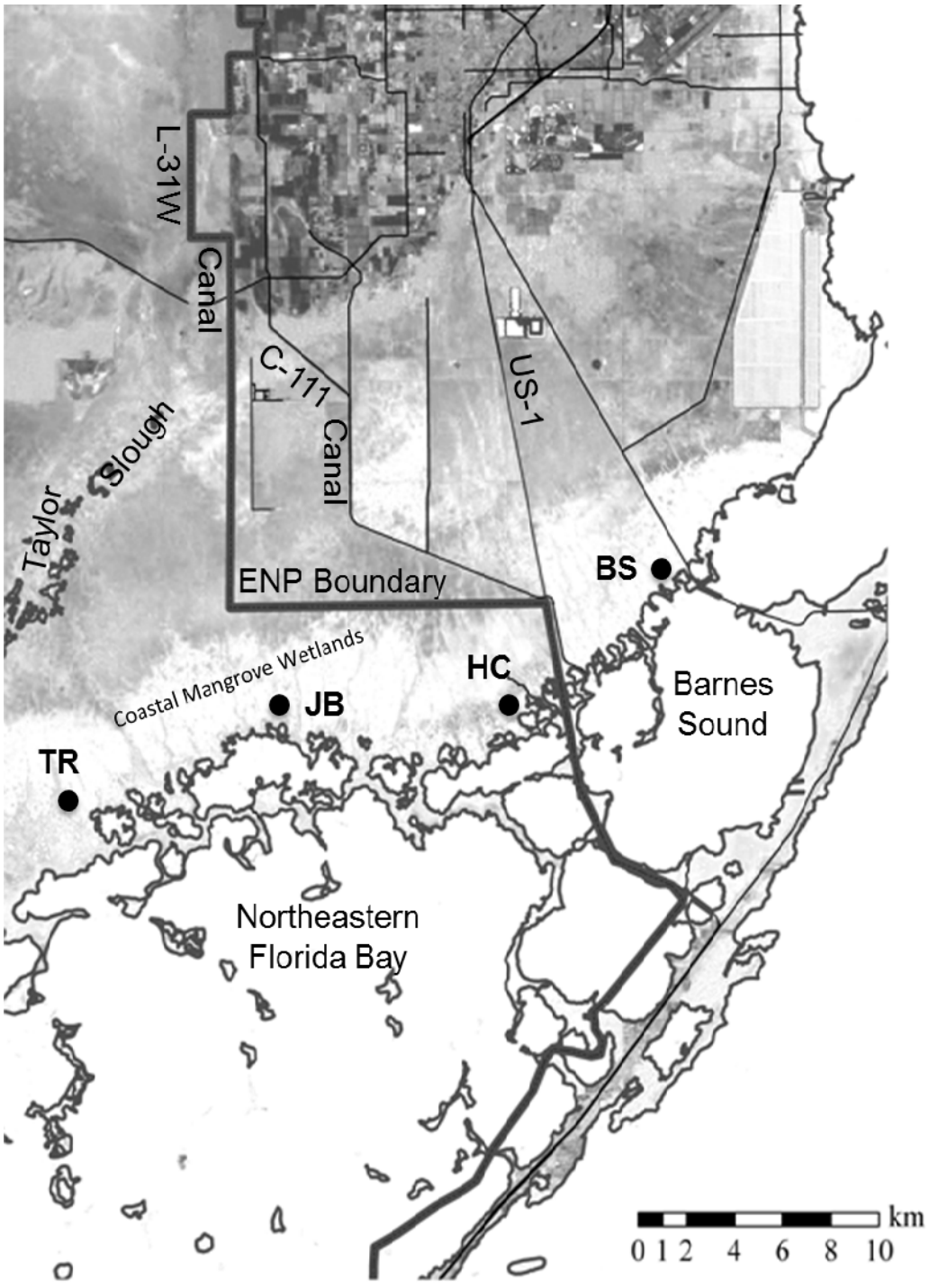
L-31W
Canal
C-111 Canal
US-1
Taylor Slough
ENP Boundary
BS
HC
JB
TR
Coastal Mangrove Wetlands
Barnes Sound
Northeastern Florida Bay
km
0 1 2 4 6 8 10

Fig. 2.2

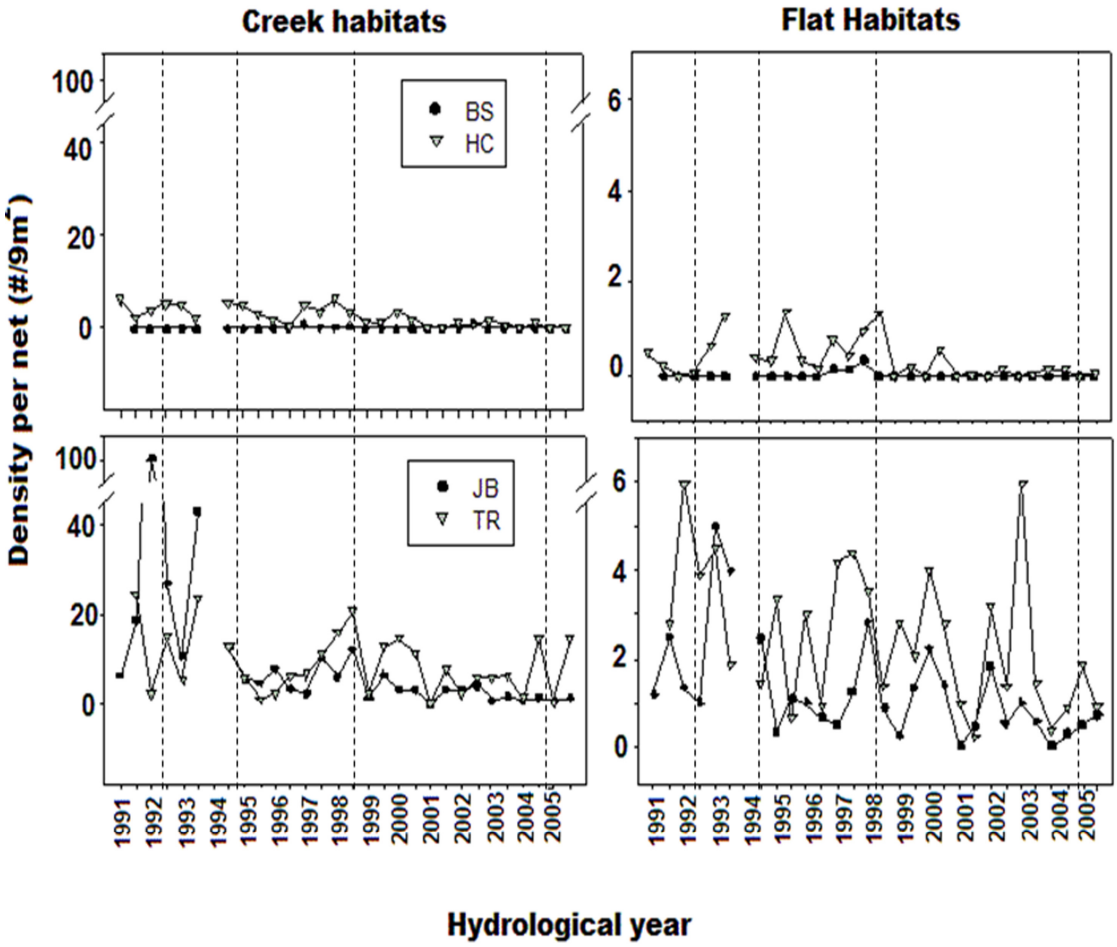
Creek habitats
Flat Habitats
Density per net (#/9m²)
BS
HC
JB
TR
100
40
20
0
6
4
2
0
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
Hydrological year

Fig. 2.3

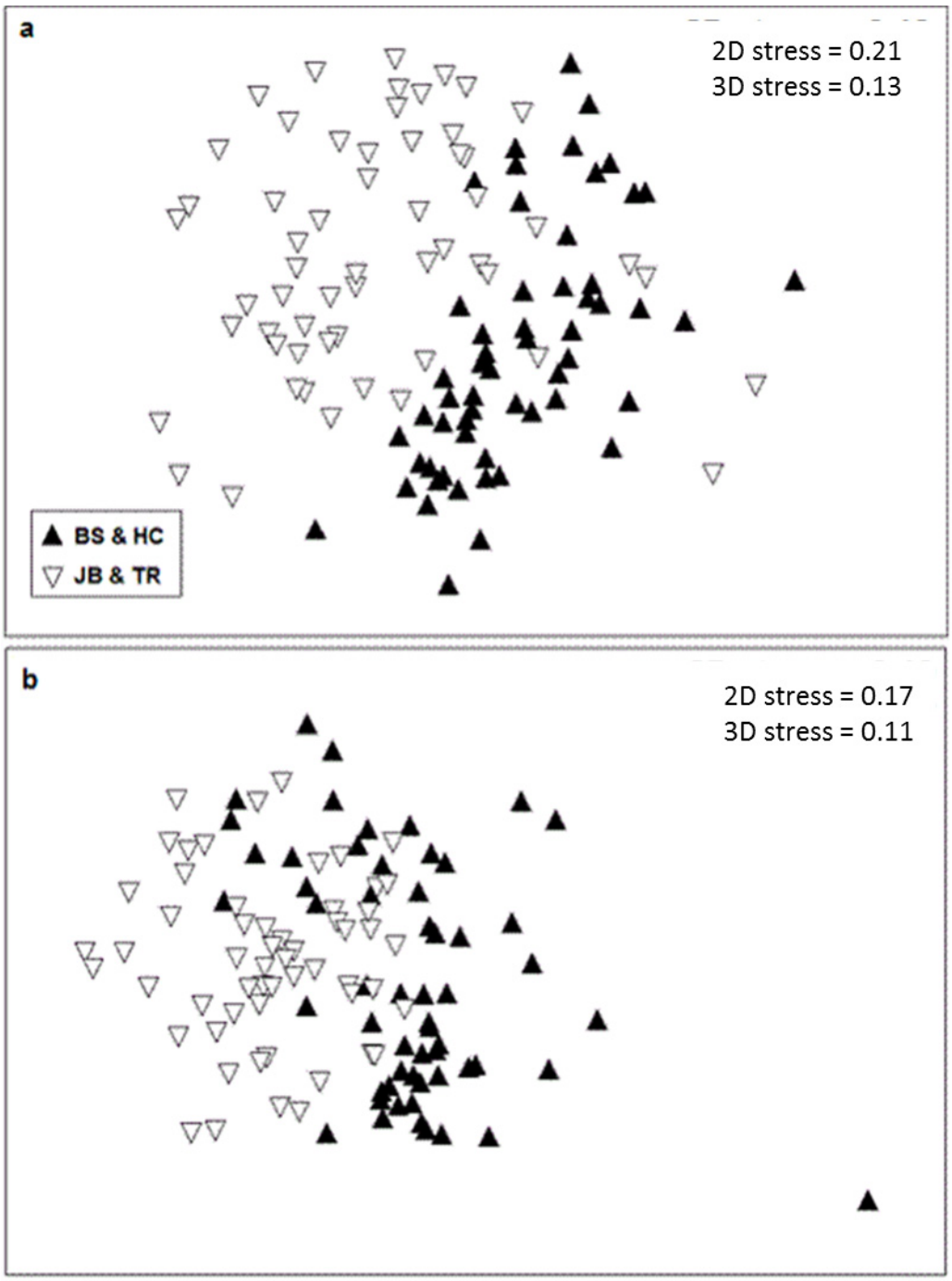
a
2D stress = 0.21
3D stress = 0.13
BS & HC
JB & TR
b
2D stress = 0.17
3D stress = 0.11

Fig. 2.4

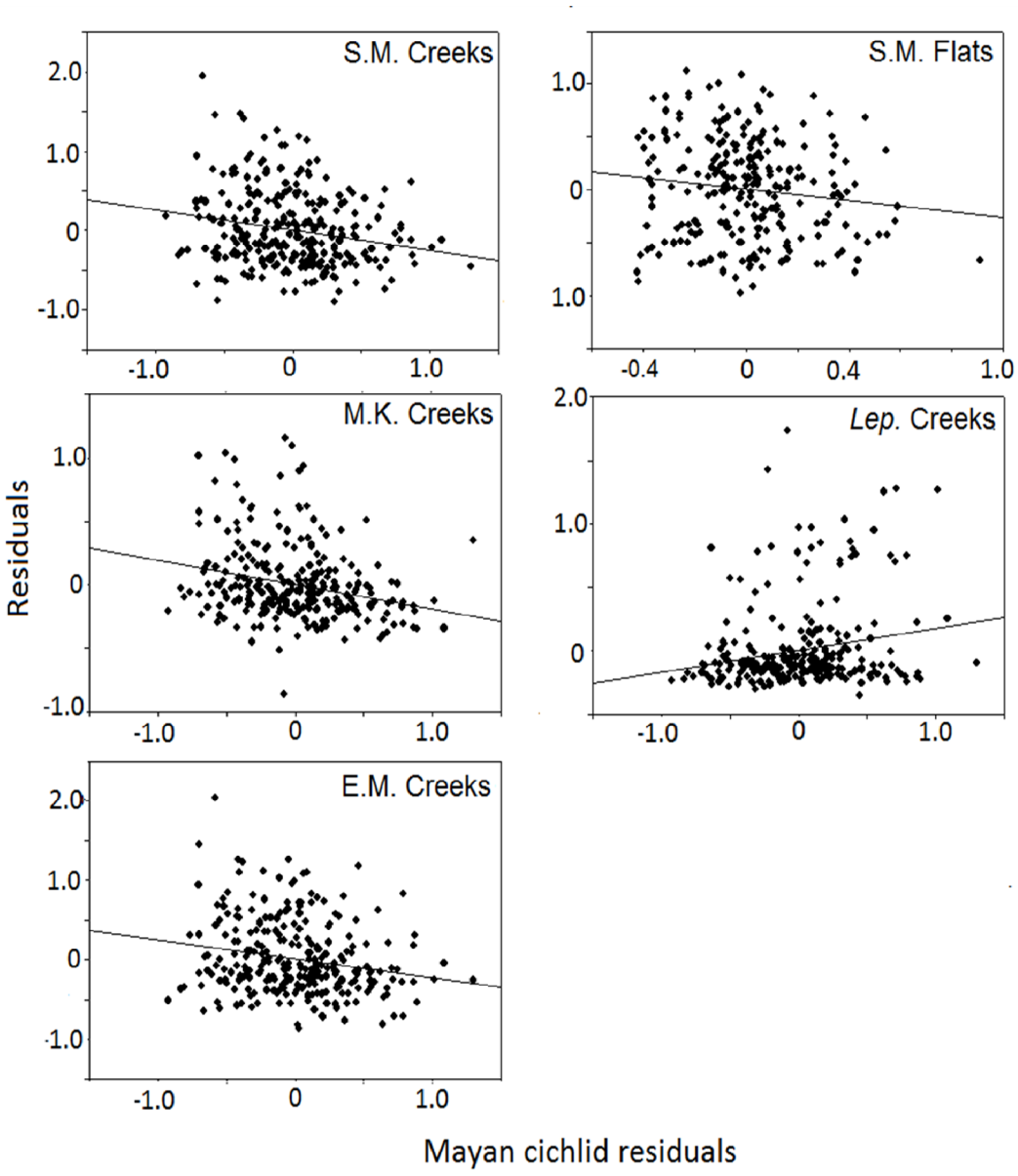
S.M. Creeks
S.M. Flats
M.K. Creeks
Lep. Creeks
E.M. Creeks
Residuals
Mayan cichlid residuals

Fig. 2.5

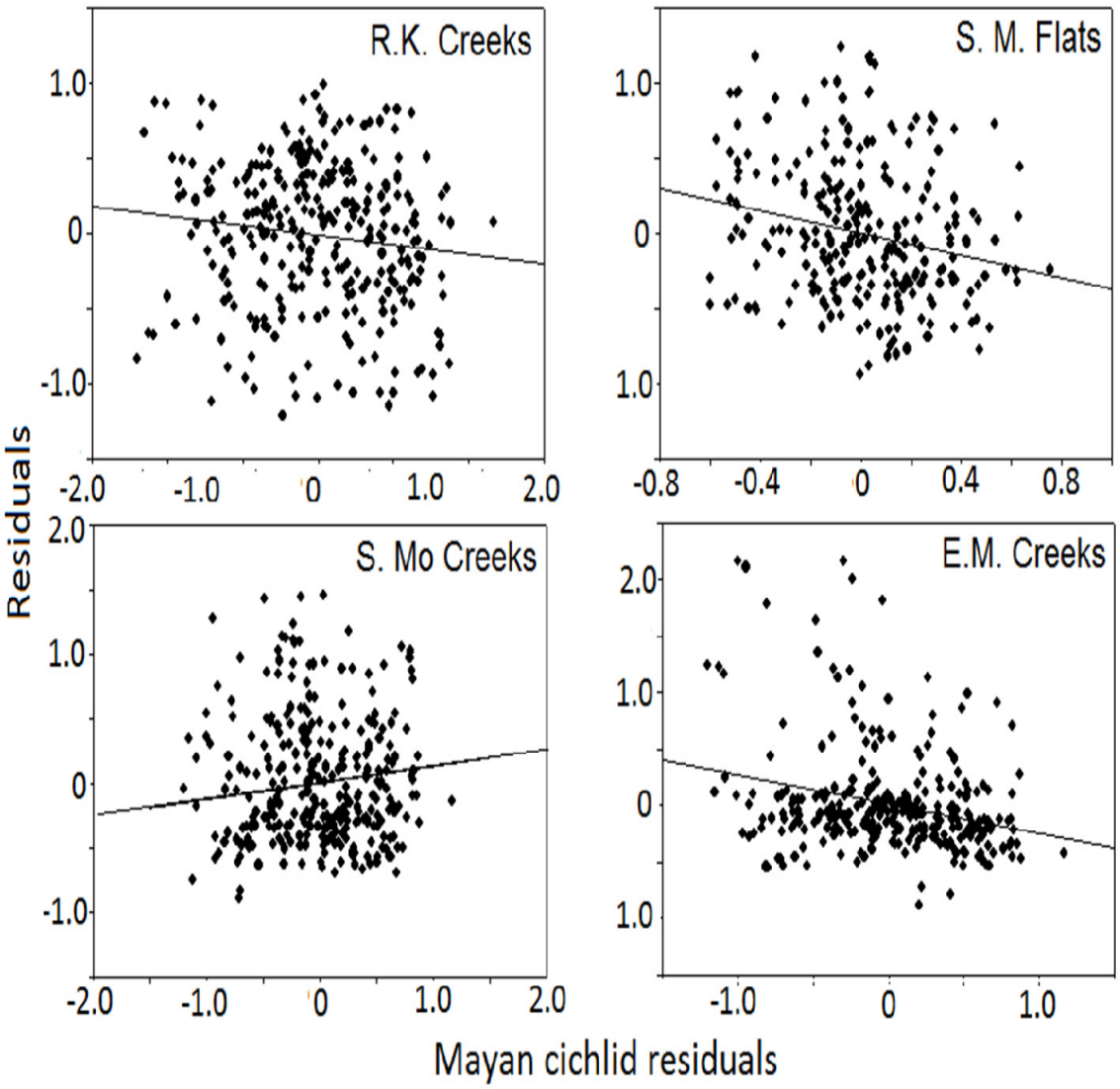
R.K. Creeks
S. M. Flats
S. Mo Creeks
E.M. Creeks
Residuals
Mayan cichlid residuals

Table 2.1. The hypotheses and models used for regression analyses to examine effects of Mayan Cichlid density on native fish density.

Model number	Model name	Hypothesis	Model
1	Global	Species density is dependent on Mayan Cichlid density and all abiotic factors	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro
2	Abiotic	Species density is dependent on abiotic factors	days since 13cm + depth + salinity + temperature + month + hydro
3	Depth	Species density is dependent on water depth parameters and time of year	days since 13cm + depth + month
4	Salinity	Species density is dependent on salinity	salinity
5	Temper-ature	Species density is dependent on water temperature	temperature
6	Mayan Cichlid	Species density is dependent on Mayan Cichlid density only	Mayan Cichlid
7	Null	Species density is dependent on none of the measured parameters	

Table 2.2. Table describing the four sites included in the study: Barnes Sound, Highway Creek, Joe Bay and Taylor River.

Site	Barnes Sound		Highway Creek		Joe Bay		Taylor River	
Average water depth (cm)	17.24		13.59		17.4		20.45	
Range of water depth (cm)	0-55		0-42		0-48		0-60	
95% CI	2.12		2		1.96		2.28	
Average salinity (psu)	20.31		7.44		7.34		4.73	
Range of salinity (psu)	1-46		0-49		0-46		0-43	
95% CI	2.2		2.11		2.04		1.81	
Average water temperature (°C)	23.73		23.78		23.72		23.92	
Range of water temperature (°C)	15-32		13-32		14-34		15-31	
95% CI	0.74		0.77		0.08		0.67	
Average Mayan Cichlid density (per trap)	Creeks	Flats	Creeks	Flats	Creeks	Flats	Creeks	Flats
	0.11	0.02	2.4	0.36	9.23	1.2	10.26	2.13
Range of Mayan Cichlid density	0-9	0-1	0-27	0-12	0-298	0-21	0-105	0-15
95% CI	0.07	0.02	0.46	0.14	2.58	0.28	1.52	0.34

Table 2.3. SIMPER results for creek and flat habitats in the two site groups. Mayan Cichlid densities were significantly lower at BS (Barnes Sound) and HC (Highway Creek) than at JB (Joe Bay) and TR (Taylor River). Shown are the average relative densities (Avg. rel. density) and percentage contributions of each species to the dissimilarities in community composition (% contrib).

Species	Creek Habitats			Flat Habitats		
	Avg. rel. density			Avg. rel. density		
	BS & HC	JB & TR	% Contrib.	BS & HC	JB & TR	% Contrib.
Rainwater Killifish	0.32	0.22	19.42	0.24	0.13	15.23
Sheepshead Minnow	0.07	0.21	16.06	0.3	0.39	23.44
Goldspotted Killifish	0	0.12	9.87	0.01	0.2	14.71
Clown Goby	0.06	0.1	9.61	0.05	0.08	7.73
Sailfin Molly	0.1	0.09	9.22	0.08	0.03	6.59
Marsh Killifish	0.01	0.06	5.39	0.08	0.06	7.93
Eastern Mosquitofish	0.05	0.03	5.01	0.04	0.01	3.24
Gulf Killifish	0.01	0.05	4.67	0.01	0.03	2.42
Crested Goby	0.04	0	3.55	-	-	-
Bluefin Killifish	0.04	0	3.13	-	-	-
Tidewater Silverside	0.02	0.01	2.37	-	-	-
Lepomis spp.	0.03	0	2.22	-	-	-

Table 2.4. Selected models for each focal fish species with model parameters from the creeks and flats of Joe Bay. Hydro year refers to hydrological year. ^ indicates species for which there were several models with AICc values that were within 2 of the lowest; we presented the models with the highest likelihood R2 values. ‡ denotes 95% confidence intervals for Mayan cichlid density that do not include 0. Parameters that are in bold type were significant at α = 0.05. **denotes parameters that were significant at α = 0.01

Joe Bay											
Creeks					**Beta values for model paramters**						
Species	**Model#**	**AICc**	**W_i**	**Likelihood of R^2**	Mayan cichlid density	Days since 13cm	Salinity	Temp	Depth	Month	Hydro year
Rainwater Killifish ^	1	313.4	0.57	0.28	-0.109	0.017	0.105	1.003	-0.591	-0.021	**-0.033
Sheepshead Minnow	1	391.6	0.99	0.4	** -0.259‡	-0.104	0.032	**2.533	-0.543	0.026	-0.022
Sailfin Molly ^	1	408.1	0.39	0.45	0.092	0.102	0.056	0.699	**-2.337	**0.049	-0.008
Marsh Killifish	1	113	1.00	0.63	** -0.179‡	0.015	-0.012	0.397	** -1.709	-0.003	-0.015
E. Mosquitofish	1	360.2	0.90	0.37	** -0.186‡	0.059	0.004	0.979	** -1.720	** 0.046	-0.021
Gulf Killifish ^	1	110.9	0.39	0.72	-0.048	** 0.100	0.073	-0.167	** -1.417	-0.015	0.009
Bluefin Killifish	3	133.9	0.82	0.11	-	0.055	-	-	-0.204	0.019	-
Tidewater Silverside^	1	180.7	0.59	0.32	-0.085	0.077	0.078	0.513	-0.290	0.016	*-0.019
Lepomis spp ^	1	172.5	0.25	0.16	** 0.152‡	0.066	-0.020	-0.314	-0.414	0.012	0.011

Flats					**Beta values for model paramters**						
Species	**Model #**	**AICc**	**W_i**	**Likelihood of R^2**	Mayan cichlid density	Days since 13cm	Salinity	Temp	Depth	Month	Hydro year
Rainwater Killifish ^	1	242.9	0.48	0.41	0.147	0.023	-0.093	-0.082	**0.539	-0.01	-0.014
Sheepshead Minnow ^	1	342	0.58	0.32	-0.280‡	0.053	0.073	**2.640	0.019	0.023	-0.008
Sailfin Molly ^	1	85.5	0.36	0.31	0.081	0.017	0.011	**0.900	0.004	0.015	**-0.018
Marsh Killifish ^	2	116.9	0.51	0.39	-	**-0.101	-0.001	**0.952	-0.080	0.026	0.003
E. Mosquitofish	3	88.5	0.97	0.26	-	-0.013	-	-	0.028	*0.034	-

Table 2.5. Selected models for each focal fish species with model parameters from the creeks and flats of Taylor River. Hydro year refers to hydrological year. ^ indicates species for which there were several models with AICc values that were within 2 of the lowest; we presented the models with the highest likelihood R^2 values. ‡ denotes 95% confidence intervals for Mayan cichlid density that do not include 0. Parameters that are in bold type were significant at $\alpha = 0.05$. ** denotes parameters that were significant at $\alpha = 0.01$.

Taylor River											
Creeks					**Beta values for model paramters**						
Species	**Model #**	**AICc**	**Wi**	**Likelihood of R^2**	Mayan cichlid density	Days since 13cm	Salinity	Temp	Depth	Month	Hydro year
Rainwater Killifish ^	1	404.8	0.70	0.2	**-0.118**	-0.083	** **0.241**	0.871	0.436	0.009	**-0.034**
Sheepshead Minnow	5	364.8	0.66	0.09	-	-	-	**1.519	-	-	-
Clown Goby ^	1	281.7	0.63	0.44	-0.0898	-0.063	** **0.232**	** **2.471**	**0.399**	** **-0.032**	-0.014
Sailfin Molly	1	365.1	0.80	0.29	**0.130‡**	0.078	-0.001	0.260	** **-1.434**	-0.010	-0.005
E. Mosquitofish	1	295.8	0.98	0.37	** **-0.157‡**	0.237	** **0.182**	0.225	** **-1.28**	** **0.030**	**-0.036**
Crested Goby ^	2	323.3	0.73	0.19	-	0.050	**-0.128**	**0.834**	**0.660**	0.008	0.007
Bluefin Killifish ^	1	281.1	0.40	0.2	-0.058	0.148	-0.039	**-1.090	**-0.546**	0.012	**-0.041**
Tidewater Silverside ^	1	121	32.00	0.4	0.026	0.018	**0.137	**1.075**	-0.053	-0.011	*-0.022

Flats					**Beta values for model paramters**						
Species	**Model#**	**AICc**	**Wi**	**Likelihood of R^2**	Mayan cichlid density	Days since 13cm	Salinity	Temp	Depth	Month	Hydro year
Rainwater Killifish ^	1	262.5	0.47	0.22	0.13	-0.003	0.063	0.08	**0.484	-0.01	0.007
Sheepshead Minnow	1	340.6	0.99	0.31	** -0.343‡	** -0.145	-0.003	** 1.899	** 0.632	0.005	** 0.024
Clown Goby ^	1	119.7	0.65	0.55	-0.124	-0.039	-0.083	0.666	**0.675	**-0.032	0.005
Sailfin Molly	5	300.1	0.76	0.07	-	-	-	1.034	-	-	-
Marsh Killifish	2	29.7	0.7	0.86	-	**-0.086	**0.114	0.474	0.11	**0.030	-0.003
E. Mosquitofish^	2	24	0.54	0.88	-	-0.021	**0.111	0.458	0.133	**0.042	-0.007

APPENDIX

Table A1. The number of fishes caught at each site within each habitat from 1991-2006. The species are shown in descending order from the most abundant species over all the samples to the least abundant.

Site	**BS**		**HC**		**JB**		**TR**	
Habitat	**creeks**	**flats**	**creeks**	**flats**	**creeks**	**flats**	**creeks**	**flats**
No. of samples	297	282	320	258	316	282	310	280
Sheepshead Minnow *Cyprinodon variegatus*	1805	2092	5243	4310	2236	2602	1289	1924
Rainwater Killifish *Lucania parva*	2195	798	1495	241	5434	1764	4946	1159
Sailfin Molly *Poecilia latipinna*	935	254	1234	438	3029	284	2205	949
Mayan Cichlid *Cichlasoma urophthalmus*	**33**	**5**	**767**	**92**	**2899**	**337**	**3179**	**596**
Eastern Mosquitofish *Gambusia affinis*	89	20	672	140	1943	418	3166	258
Marsh Killifish *Fundulus confluentus*	333	77	1604	1183	647	518	311	279
Goldspotted Killifish *Floridichthys carpio*	1860	2112	456	311	30	121	30	21
Clown Goby *Microgobius gulosus*	1376	833	73	40	141	101	1777	506
Gulf Killifish *Fundulus grandis*	442	77	780	275	568	69	6	11
Bluefin Killifish *Lucania goodei*	0	0	67	31	421	15	1510	182
Tidewater Silverside *Menidia peninsulae*	273	211	101	73	569	56	359	213
Crested Goby *Lophogobius cyprinoides*	0	0	2	1	2	2	1296	27

Diamond Killifish *Adinia xenica*	140	37	433	537	4	27	0	0
Lepomis species	0	0	5	0	525	10	225	4
Spotted Tilapia *Tilapia mariae*	0	0	6	1	65	4	434	131
Least Killifish *Heterandria formosa*	0	0	1	0	160	20	241	74
Flagfish *Jordanella floridae*	0	0	11	11	309	16	53	83
Spotted Sunfish *Lepomis punctatus*	0	0	16	0	212	2	151	2
Golden Topminnow *Fundulus chrysotus*	0	0	4	2	77	8	134	21
Longnose Killifish *Fundulus similis*	70	8	6	26	4	31	3	4
Pike Killifish *Belonesox belizanus*	2	0	15	4	59	19	15	32
Dollar Sunfish *Lepomis marginatus*	0	0	1	0	41	0	87	7
Total fish	**9553**	**6524**	**12992**	**7716**	**19375**	**6424**	**21417**	**6483**

Table A2. Models for each focal fish species with descriptive statistics from the creek and flat habitats within Joe Bay. Hydro year refers to hydrological year. Models were ordered by increasing AICc values.

Creeks							
Species	Model #	Model	AICc	Δ AICc	W_i	Likelihood	R^2 BIC
Rainwater killifish	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	313.4	0	0.566	0.28	303.6
	2	days since 13cm + depth + salinity + temperature + month + hydro	314	0.6	0.420	0.27	305.2
	3	days since 13cm + depth + month	363	49.6	0.000	0.02	357.3
	4	salinity	358	44.6	0.000	0.02	354.3
	5	temperature	320.8	7.4	0.014	0.19	317
	6	Mayan cichlid	358.1	44.7	0.000	0.02	354.4
	7	Null	360.6	47.2	0.000	0.00	357.8
Sheepshead minnow	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	391.6	0	0.995	0.40	381.8
	2	days since 13cm + depth + salinity + temperature + month + hydro	402	10.4	0.005	0.36	393.2
	3	days since 13cm + depth + month	482.5	90.9	0.000	0.04	476.8
	4	salinity	471.5	79.9	0.000	0.07	467.8
	5	temperature	420.2	28.6	0.000	0.26	416.4
	6	Mayan cichlid	476	84.4	0.000	0.05	472.3
	7	Null	486.8	95.2	0.000	0.00	484
Sailfin molly	2	days since 13cm + depth + salinity + temperature + month + hydro	428.1	0	0.611	0.45	419.3
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	429	0.9	0.389	0.45	419.1
	3	days since 13cm + depth + month	472.8	44.7	0.000	0.30	467.1
	5	temperature	497.3	69.2	0.000	0.21	493.5
	4	salinity	545.4	117.3	0.000	0.03	541.7
	6	Mayan cichlid	551.1	123	0.000	0.01	547.4
	7	Null	551.4	123.3	0.000	0.00	548.6

Marsh killifish	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	113	0	0.998	0.63	103.2
	2	days since 13cm + depth + salinity + temperature + month + hydro	125.8	12.8	0.002	0.56	117
	3	days since 13cm + depth + month	128	15	0.001	0.52	122.3
	4	salinity	195.6	82.6	0.000	0.10	191.9
	6	Mayan cichlid	202.5	89.5	0.000	0.05	198.8
	5	temperature	203.4	90.4	0.000	0.05	199.6
	7	Null	208.4	95.4	0.000	0.00	205.6
Eastern mosquitofish	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	360.2	0	0.900	0.37	350.3
	2	days since 13cm + depth + salinity + temperature + month + hydro	364.6	4.4	0.100	0.35	355.8
	3	days since 13cm + depth + month	388.3	28.1	0.000	0.24	382.6
	5	temperature	419.9	59.7	0.000	0.10	416.2
	4	salinity	430.9	70.7	0.000	0.06	427.2
	6	Mayan cichlid	436.1	75.9	0.000	0.04	432.4
	7	Null	442	81.8	0.000	0.00	439.2
Gulf killifish	2	days since 13cm + depth + salinity + temperature + month + hydro	110	0	0.611	0.71	101.2
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	110.9	0.9	0.389	0.72	101
	3	days since 13cm + depth + month	172	62	0.000	0.43	166.3
	5	temperature	181.6	71.6	0.000	0.36	177.9
	4	salinity	224	114	0.000	0.14	220.3
	7	Null	244.8	134.8	0.000	0.00	242
	6	Mayan cichlid	245.4	135.4	0.000	0.01	241.6

Bluefin killifish	3	days since 13cm + depth + month	133.9	0	0.819	0.11	128.2
	7	Null	139.4	5.5	0.052	0.00	136.7
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	139.5	5.6	0.050	0.13	129.7
	2	days since 13cm + depth + salinity + temperature + month + hydro	140.3	6.4	0.033	0.11	131.5
	4	salinity	141	7.1	0.024	0.01	137.2
	6	Mayan cichlid	141.2	7.3	0.021	0.00	137.5
	5	temperature	148.6	14.7	0.001	-0.07	144.9
Tidewater silverside	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	180.7	0	0.587	0.32	170.9
	2	days since 13cm + depth + salinity + temperature + month + hydro	181.4	0.7	0.413	0.30	172.6
	5	temperature	197.8	17.1	0.000	0.14	194.1
	4	salinity	204.4	23.7	0.000	0.10	200.7
	3	days since 13cm + depth + month	210.1	29.4	0.000	0.09	204.4
	6	Mayan cichlid	215.6	34.9	0.000	0.03	211.9
	7	Null	217.4	36.7	0.000	0.00	214.7
Lepomis spp.	6	Mayan cichlid	170.7	0	0.617	0.08	167
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	172.5	1.8	0.251	0.16	162.7
	3	days since 13cm + depth + month	174.5	3.8	0.092	0.09	168.8
	5	temperature	177.7	7	0.019	0.03	174
	2	days since 13cm + depth + salinity + temperature + month + hydro	179	8.3	0.010	0.10	170.2
	7	Null	179.7	9	0.007	0.00	176.9
	4	salinity	180.6	9.9	0.004	0.01	176.9

Flat habitats							
Species	Model #	Model	AICc	Δ AICc	W_i	Likelihood R^2	BIC
Rainwater killifish	2	days since 13cm + depth + salinity + temperature + month + hydro	242.7	0	0.525	0.40	233.8
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	242.9	0.2	0.475	0.41	232.9
	3	days since 13cm + depth + month	273.4	30.7	0.000	0.24	267.6
	5	temperature	288.7	46	0.000	0.15	284.9
	4	salinity	301	58.3	0.000	0.08	297.3
	6	Mayan cichlid	304.2	61.5	0.000	0.06	300.4
	7	Null	314	71.3	0.000	0.00	311.2
Sheepshead minnow	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	342	0	0.577	0.32	332.1
	5	temperature	343.5	1.5	0.273	0.26	339.7
	2	days since 13cm + depth + salinity + temperature + month + hydro	344.7	2.7	0.150	0.30	335.8
	6	Mayan cichlid	388.7	46.7	0.000	0.06	385
	4	salinity	392.3	50.3	0.000	0.05	388.5
	7	Null	399.9	57.9	0.000	0.00	397.1
	3	days since 13cm + depth + month	403.6	61.6	0.000	0.01	397.9
Sailfin molly	2	days since 13cm + depth + salinity + temperature + month + hydro	84.4	0	0.620	0.29	75.6
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	85.5	1.1	0.358	0.31	75.6
	6	Mayan cichlid	92.7	8.3	0.010	0.05	89
	5	temperature	93.7	9.3	0.006	0.04	89.9
	7	Null	94.7	10.3	0.004	0.00	91.9
	4	salinity	96.6	12.2	0.001	0.00	92.9
	3	days since 13cm + depth + month	97.1	12.7	0.001	0.05	91.4

Marsh killifish	2	days since 13cm + depth + salinity + temperature + month + hydro	116.9	0	0.506	0.39	108
	3	days since 13cm + depth + month	117.9	1	0.307	0.33	112.2
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	118.9	2	0.186	0.39	108.9
	6	Mayan cichlid	140.1	23.2	0.000	0.12	136.3
	5	temperature	145.3	28.4	0.000	0.08	141.5
	4	salinity	147.2	30.3	0.000	0.06	143.4
	7	Null	151.7	34.8	0.000	0.00	148.9
Eastern mosquitofish	3	days since 13cm + depth + month	88.5	0	0.973	0.26	82.8
	4	salinity	95.8	7.3	0.025	0.13	92.1
	2	days since 13cm + depth + salinity + temperature + month + hydro	103.5	15	0.001	0.17	94.7
	7	Null	104.8	16.3	0.000	0.00	102
	6	Mayan cichlid	105	16.5	0.000	0.02	101.3
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	105.6	17.1	0.000	0.17	95.7
	5	temperature	120.6	32.1	0.000	0.00	116.9

Table A3. Models for each focal fish species with descriptive statistics from the creek and flat habitats within Taylor River. Hydro year refers to hydrological year. Models were ordered by increasing AICc values.

Creeks							
Species	Model #	Model	AICc	Δ AICc	W_i	Likelihood	R^2 BIC
Rainwater killifish	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	404.8	0	0.700	0.20	395
	2	days since 13cm + depth + salinity + temperature + month + hydro	406.5	1.7	0.299	0.19	397.8
	5	temperature	422.1	17.3	0.000	0.09	418.3
	4	salinity	430.5	25.7	0.000	0.05	426.8
	6	Mayan cichlid	437	32.2	0.000	0.02	433.3
	3	days since 13cm + depth + month	438.1	33.3	0.000	0.04	432.4
	7	Null	440.7	35.9	0.000	0.00	437.9
Sheepshead minnow	5	temperature	364.8	0	0.658	0.09	361.1
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	367.1	2.3	0.208	0.14	357.3
	2	days since 13cm + depth + salinity + temperature + month + hydro	368	3.2	0.133	0.12	359.3
	4	salinity	381.7	16.9	0.000	0.01	378
	6	Mayan cichlid	382.5	17.7	0.000	0.01	378.8
	7	Null	382.8	18	0.000	0.00	380
	3	days since 13cm + depth + month	386.2	21.4	0.000	0.01	380.5
Clown goby	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	281.7	0	0.634	0.44	271.9
	2	days since 13cm + depth + salinity + temperature + month + hydro	282.8	1.1	0.366	0.43	274.1
	5	temperature	322.5	40.8	0.000	0.25	318.8
	4	salinity	357.5	75.8	0.000	0.11	353.8
	3	days since 13cm + depth + month	362.3	80.6	0.000	0.11	356.6
	6	Mayan cichlid	367.4	85.7	0.000	0.07	363.6
	7	Null	380.5	98.8	0.000	0.00	377.7

Sailfin molly	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	365.1	0	0.798	0.29	355.4
	2	days since 13cm + depth + salinity + temperature + month + hydro	367.9	2.8	0.197	0.28	359.1
	3	days since 13cm + depth + month	375	9.9	0.006	0.23	339.3
	6	Mayan cichlid	409.1	44	0.000	0.08	405.3
	5	temperature	409.5	44.4	0.000	0.08	405.8
	4	salinity	420	54.9	0.000	0.03	416.2
	7	Null	425.1	60	0.000	0.00	422.6
Eastern mosquitofish	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	295.8	0	0.979	0.37	286
	2	days since 13cm + depth + salinity + temperature + month + hydro	303.5	7.7	0.021	0.33	294.7
	3	days since 13cm + depth + month	322.8	27	0.000	0.24	317.2
	4	salinity	348.6	52.8	0.000	0.11	344.9
	5	temperature	367.2	71.4	0.000	0.03	363.5
	7	Null	370.9	75.1	0.000	0.00	368.1
	6	Mayan cichlid	372.9	77.1	0.000	0.00	369.2
Crested goby	2	days since 13cm + depth + salinity + temperature + month + hydro	323.3	0	0.730	0.19	314.6
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	325.3	2	0.269	0.19	315.6
	5	temperature	336.5	13.2	0.001	0.09	332.8
	3	days since 13cm + depth + month	340.9	17.6	0.000	0.09	335.3
	4	salinity	344.1	20.8	0.000	0.05	340.4
	7	Null	353.1	29.8	0.000	0.00	350.3
	6	Mayan cichlid	354.2	30.9	0.000	0.00	350.5

Bluefin killifish	2	days since 13cm + depth + salinity + temperature + month + hydro	277.4	0	0.572	0.20	268.7
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	278.1	0.7	0.403	0.20	268.3
	5	temperature	283.6	6.2	0.026	0.12	279.9
	3	days since 13cm + depth + month	301.3	23.9	0.000	0.05	295.6
	7	Null	304.5	27.1	0.000	0.00	301.7
	4	salinity	305.8	28.4	0.000	0.00	302.1
	6	Mayan cichlid	306.5	29.1	0.000	0.00	302.8
Tidewater silverside	2	days since 13cm + depth + salinity + temperature + month + hydro	119.5	0	0.679	0.40	110.7
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	121	1.5	0.321	0.40	111.3
	4	salinity	135.7	16.2	0.000	0.20	132
	5	temperature	142.1	22.6	0.000	0.15	138.4
	7	Null	158.1	38.6	0.000	0.00	155.4
	3	days since 13cm + depth + month	158.3	38.8	0.000	0.05	152.6
	6	Mayan cichlid	160.2	40.7	0.000	0.00	156.4

Flat habitats

Species	Model #	Model	AICc	Δ AICc	W_i	Likelihood R^2	BIC
Rainwater killifish	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	265.2	0	0.474	0.22	255.3
	2	days since 13cm + depth + salinity + temperature + month + hydro	265.2	0	0.474	0.21	256.4
	3	days since 13cm + depth + month	269.7	4.5	0.050	0.16	263.9
	5	temperature	277.6	12.4	0.001	0.09	273.9
	6	Mayan cichlid	281.6	16.4	0.000	0.07	277.8
	7	Null	292.7	27.5	0.000	0.00	289.9
	4	salinity	294.1	28.9	0.000	0.00	290.3
Sheepshead minnow	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	340.6	0	0.990	0.31	330.8
	2	days since 13cm + depth + salinity + temperature + month + hydro	349.7	9.1	0.010	0.27	340.9
	5	temperature	364.8	24.2	0.000	0.17	361
	3	days since 13cm + depth + month	378.5	37.9	0.000	0.12	372.8
	6	Mayan cichlid	389.4	48.8	0.000	0.06	385.7
	4	salinity	392.5	51.9	0.000	0.04	388.8
	7	Null	399.5	58.9	0.000	0.00	396.7
Clown goby	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	119.7	0	0.650	0.55	109.9
	2	days since 13cm + depth + salinity + temperature + month + hydro	121	1.3	0.339	0.53	112.2
	3	days since 13cm + depth + month	127.9	8.2	0.011	0.46	122.1
	5	temperature	176.9	57.2	0.000	0.12	173.9
	4	salinity	186.4	66.7	0.000	0.05	182.6
	6	Mayan cichlid	186.8	67.1	0.000	0.05	183
	7	Null	191.3	71.6	0.000	0.00	188.6

Sailfin molly	5	temperature	300.1	0	0.761	0.07	296.3
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	304	3.9	0.108	0.11	294.2
	2	days since 13cm + depth + salinity + temperature + month + hydro	305.5	5.4	0.051	0.09	296.7
	6	Mayan cichlid	305.6	5.5	0.049	0.04	301.9
	3	days since 13cm + depth + month	306.9	6.8	0.025	0.05	301.2
	7	Null	310.5	10.4	0.004	0.00	307.7
	4	salinity	312.5	12.4	0.002	0.00	308.8
Marsh killifish	2	days since 13cm + depth + salinity + temperature + month + hydro	29.7	0	0.698	0.86	20.9
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	31.8	2.1	0.244	0.86	22
	3	days since 13cm + depth + month	34.7	5	0.057	0.72	29
	4	salinity	46.4	16.7	0.000	0.50	42.7
	6	Mayan cichlid	68.4	38.7	0.000	0.18	64.6
	7	Null	78.1	48.4	0.000	0.00	75.3
	5	temperature	78.4	48.7	0.000	0.03	74.7
Eastern mosquitofish	2	days since 13cm + depth + salinity + temperature + month + hydro	24	0	0.535	0.88	15.2
	3	days since 13cm + depth + month	25.4	1.4	0.266	0.71	19.7
	1	Mayan cichlid + days since 13cm + depth + salinity + temperature + month + hydro	26	2	0.197	0.89	16.1
	4	salinity	34.9	10.9	0.002	0.40	31.1
	6	Mayan cichlid	48.1	24.1	0.000	0.07	44.4
	7	Null	49	25	0.000	0.00	46.2
	5	temperature	56.4	32.4	0.000	-0.14	52.7

CHAPTER 3

GENETIC EVIDENCE FOR MULTIPLE SOURCES OF THE NON-NATIVE FISH *CICHLASOMA UROPHTHALMUS* (GÜNTHER; MAYAN CICHLID) IN SOUTHERN FLORIDA

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Abstract

The number and diversity of source populations may influence the genetic diversity of newly introduced populations and affect the likelihood that these populations will become established and spread. I used the cytochrome b mitochondrial gene and nuclear microsatellite loci to identify the sources of a successful invader in southern Florida, USA, *Cichlasoma urophthalmus* (Mayan cichlid). The cytochrome b data supported an introduction from Guatemala; microsatellite data suggested movement of Mayan Cichlids from the upper Yucatán Peninsula to Guatemala and introductions from Guatemala and Belize to Florida. Mayan Cichlids present a unique example of admixture between two distinct lineages upon introduction that resulted in cytonuclear disequilibrium and reduced genetic diversity in the introduced population that persists more than 30 years (7-8 generations) after introduction. The mismatch between mitochondrial and nuclear genomes suggests admixture of a female lineage from Guatemala, where all individuals were fixed for the mitochondrial haplotype found in the introduced population, and a more diverse but also relatively small introduction from Belize. The Florida cytochrome b haplotype appears to be absent from Belize (0 out of 136 fish screened from Belize had this haplotype). Genetic structure within the Florida population was minimal, indicating a panmictic population, while Mexican and Central American samples displayed more genetic subdivision. Individuals from the Upper Yucatán Peninsula and the Petén region of Guatemala were more genetically similar to each other than to fish from nearby sites and movement of Mayan Cichlids between these regions occurred thousands of generations ago, suggestive of pre-Columbian human transportation of Mayan Cichlids through this region.

Introduction

Biological invasions have resulted in species declines, extinction of native biota, and extensive financial costs [1, 2]. Some of the largest impacts of nonnative species have been recorded in aquatic habitats [3, 4]. Since European colonization, southern Florida has experienced major habitat transformation and invasion by nonnative species. Florida's mild subtropical climate fosters the establishment of tropical fish species [2, 5] and the aquarium trade enhances the probability that introductions from multiple sources occur, especially in a shipping and transportation hub such as southern Florida. Identifying the route of invasion and the source populations of invaded areas can improve the quality of management strategies for the invader either within the source range, the pathway of invasion or the method and point of entry into the invaded regions [6].

Identification of sources and pathways of invasions has traditionally been accomplished by examining historical data such as dates of first discovery in introduced areas and importation records, or by molecular analyses of native and introduced populations [7]. Historical data alone are not usually enough to infer introduction pathways as they may be incomplete or insufficient to distinguish successful and unsuccessful establishment and spread. Molecular methods facilitate the comparison of genetic diversity of native and introduced populations to narrow the viable hypotheses of origin and spread. However, these methods are limited to *post hoc* assumptions about the genetic effects of introductions and demographic stochasticity; the challenge that unsampled populations might be the true source should also be considered [8]. Approximate Bayesian Computation and coalescent theory allows for the statistical

comparison of complex introduction pathways that incorporate changes in population size, admixture before or during introduction, and historical and biogeographical data [9] thus alleviating some of the limitations of molecular analysis.

Non-native species are typically assumed to be under strong adaptive pressure in their new environment but introduced populations often have low genetic diversity from founder effects and population bottlenecks that may limit their ability to respond to environmental challenges (the 'invasive species paradox')[10]). One resolution of this paradox is that multiple introductions of an invasive species are correlated with successful establishment, especially if the introductions arose from two or more genetically distinct sources [11-13]. Introductions from multiple sources may produce novel genetic combinations that increase fitness and facilitate invasion success [14-19]. On the other hand, limited introductions and subsequent genetic bottlenecks do not necessarily decrease genetic diversity [20] and genetic bottlenecks can result in successful establishment [21-23]. Studies have documented establishment of nonnative species resulting from multiple introductions, or introduction from multiple sources [12, 24,25] as well as from single introductions or extreme bottlenecks [26,27].

Cytonuclear disequilibrium, the nonrandom association of organellar haplotypes and nuclear alleles, has been documented for interspecific hybrids [28-32] and in host-parasite interactions [33, 34]. Cytonuclear disequilibrium may result from several demographic phenomena, including nuclear-organellar genotypic interactions affecting fitness, genetic drift in small populations, founder effects preceding rapid population expansion, and nonrandom mating from patterned admixture, migration, and hybridization [35,36]. A correlation between organellar and nuclear genes is expected as

a result of species introductions from multiple sites, which are accompanied by population bottlenecks and admixture of distinct genomes [35,37,38]. A reconstruction of invasion pathways is necessary to understand the effects of diversity of introductions, the number of founder individuals, and the combination of historically separate genotypes on introduced populations.

At least 13 species of cichlids have become established in Florida, which possesses no native members of the family Cichlidae [39]. *Cichlasoma urophthalmus* (Mayan Cichlid) is found in freshwater and salt water along the Atlantic slope of Central America including southern Mexico, Belize, Guatemala, Honduras and Nicaragua [40]. Mayan Cichlids are economically important to artesanal fisheries and aquaculture in their native range [41, 42]. They were first recorded in southern Florida in the Everglades National Park in 1983 [43]. Since then, Mayan Cichlids have spread over approximately 70,000 hectares from southern to central Florida during the 30 years since they were introduced (at least 7 generations [44-47]). Mayan Cichlids have successfully adapted to the southern Florida environment, becoming well-established throughout a range of salinities from freshwater marshes to 40 PSU in the mangrove zone, where they may dominate the fish communities [48,49]. They have been shown to alter the relative abundance of native fish populations, most likely by predation [49-51]. I used mitochondrial and nuclear molecular markers to identify the source(s) of Mayan Cichlids in Florida to determine whether this successful invader resulted from single or multiple introductions.

Materials and Methods

Ethics Statement

This study was carried out in strict accordance with the recommendations in the Guidelines for The Use of Fishes in Research of The American Fisheries Society, the American Institute of Fisheries Research Biologists, and the American Society of Ichthyologists and Herpetologists [52]. The protocol was approved by the Institutional Animal Care and Use Committee of Florida International University (Protocol approval number 08-014). Fin clippings were obtained from some fish by nonlethal means. Some fish were euthanized in a solution of 0.02% MS-222 (Tricaine methanesulfonate) and preserved for collections at Florida International University. All efforts were made to minimize suffering.

Sample Collection

I collected DNA from 670 individual Mayan Cichlids from 23 sites in Florida and 53 sites within Mexico and Central America, including sites in Belize, Honduras, Guatemala and Nicaragua (Table S1; Figure 3.1). Fish were captured using a combination of methods: hook-and-line, cast net, throw trap, seine and minnow trap in habitats that ranged from freshwater ponds to estuarine canals and mangrove habitats. In some regions of Mexico and Belize, fish were purchased from local fishermen. Some fin clippings were also obtained from sample collections at the Universidad Nacional Autónoma de México (UNAM). I also acquired two specimens from a pet store in North Miami, which had obtained them from a local fish farm, and included these specimens in mitochondrial analyses. Samples were either frozen or fixed in 90% ethanol. Total genomic DNA was

isolated from either muscle or fin tissue using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol.

Molecular Analyses

Mitochondrial gene. A portion of the cytochrome b mitochondrial gene was amplified using CytbFor5'-TGATGAAACTTCGGCTCCC-3' and CytbRev5'-CTGTTAGTCCGGCGATAGG-3'. These primers were designed specifically for this study using primers designed by [53]. The PCR reactions were carried out in a 50 μL volume using 10 μL of 5X reaction buffer, 3 μL of 25mM magnesium chloride, 2.5 μL each of 10mM forward and reverse primers, 1 μL of 10mM dNTP's, 0.5 μL of Taq DNA Polymerase (5μ /μL), 2 μL of the DNA sample (approximately 10-200 ng) and 28.5 μL of Sigma® sterilized water. Amplifications were conducted for cytochrome b with a MJ Research thermal cycler using standard methods. Thermal cycling conditions for cytochrome b consisted of an initial hot start of 55^{o} C (10 min), then 36 cycles of 95^{o} C (30 seconds), 55^{o} C (45 seconds, 72^{o} C (45 seconds), followed by 49^{o} C (1 minute). A final incubation of 72^{o} C for 4 minutes was added to ensure complete extension of amplified products. Subsequently, PCR products were subjected to gel electrophoresis in a 1.4% agarose gel run in Tris-Borate-EDTA (TBE) buffer followed by staining with ethidium bromide and visualization with UV light. For sequencing, positively amplified DNA was then purified using 2 μL of ExoSap per 5 μL of PCR product. Samples were then sequenced using Big Dye Terminator version 3.1 on a 3130XL Genetic Analyzer (Applied Biosystems). For sequencing, the internal primers designed were: CytbIntF5'-CACCAACCTCCTCTCCGC-3' and CytbIntR5'-TGGAAGGCAAAGAATCGGG-3'.

Initially, 47 fish from four sites in Florida, four sites in Mexico, two sites in Belize and one site in Honduras were sequenced for a portion of the cytochrome b gene (851 bp). These sequences revealed six haplotypes, two of which were found in 43 individuals. The two haplotypes were able to differentiate between fish from Mexico and Central America and fish from Florida, hereafter referred to as the CA haplotype and the Fl haplotype respectively; on the basis of those results, I screened my remaining samples for those two haplotypes using restriction endonucleases. Cytochrome b was first amplified using Polymerase Chain Reaction (PCR). Positively amplified DNA was then digested with EcoRV at 37^{o} C for one hour. EcoRV digestion resulted in: two fragments if an individual displayed the Fl haplotype and one fragment if the CA haplotype was present. DNA fragments were then separated electrophoretically, stained with ethidium bromide and viewed under UV light. The remaining 620 samples were screened for the CA and Fl haplotypes.

Nuclear markers. Specimens from 29 sites in Florida, Mexico, Belize, Guatemala, Honduras and Nicaragua were analyzed using 17 recently developed microsatellite nuclear markers (see [54] for primer information). I amplified DNA from fish for sites at which I had collected at least 10 specimens. The PCR reactions were carried out in 10 μL using 1 μL of 5X reaction buffer, 1 μL of 25mM magnesium chloride, 0.5 μL each of 10mM forward and reverse primers, 0.2 μL of 10mM dNTP's, 0.2 μL of Taq DNA Polymerase (5μ /μL), 1 μL of DNA sample (approximately 10-200 ng) and 5.6 μL of Sigma® sterilized water. Two panels of twelve and five primer pairs, respectively, were run for each specimen. Touchdown PCR cycling parameters were run on an MJ Research thermal cycler; see [54] for complete protocol. Thermal cycling conditions consisted of:

95° C (5 minutes), then 20 cycles of 95° C (30 seconds), a temperature of 58° C, 60° C, 66° C or 67° C depending on the locus that decreased by 0.5° C per cycle (30 seconds), and 72° C (30 seconds), followed by 20 cycles of: 95° C (30 seconds) 48° C, 50° C, 56° C or 57° C depending on the locus (30 seconds), 72° C (30 seconds), then 72° C for 5 minutes. The PCR products were run on 1.4% agarose gel and prepared for GeneScan using 9.75 μL of Hi Di™ formamide solution (Applied Biosystems), 0.25 μL of GeneScan™ LIZ-500 size standard (Applied Biosystems) and 1 μL of PCR product. The PCR products were run on a 3130XL Genetic Analyzer (Applied Biosystems) to determine DNA sizes (DNA Core Facility, Florida International University). Peak Scanner 2 (Applied Biosystems) was used to determine fragment sizes of alleles.

Data Analyses

Mitochondrial data. Sequences were aligned using Sequencer v.4.8 and checked manually. Cytochrome b haplotypes were analyzed using MRMODELTEST 2.3 [55] and MRBAYES 3.2. [56]. I conducted hierarchical hypothesis tests to select the appropriate evolutionary model for subsequent Bayesian phylogenetic analysis. The program MRMODELTEST calculated base frequencies, which were used to model the prior probability distribution; likelihood ratio tests selected the TrN model (equal transversion rates but two different transition rates) for the Bayesian analysis [12]. Bayesian phylogenetic analysis was run for 1,000,000 generations, sampling every 100 generations. I discarded the initial 10% of trees during the 'burn-in period' and made a 50% majority consensus rule from the remaining Bayesian trees. The analysis was repeated twice to avoid searching within local optima. The phylogenetic tree was used to identify distinct

clades where haplotypes were shared among Mayan Cichlids from southern Florida and from the native range. Unlike typical phylogenetic trees that include taxa on their branches, I replaced the taxa with sampling locations to examine the phylogenetic relationships among sites resulting in ageneral area cladogram [57].

To investigate the relationships between clades, haplotype networks were built using Network v. 4.6.11 and Network Publisher (http://www.fluxus-engineering.com/). The maximal pairwise difference between sequences was 6 and the tranversion:transition ratio was weighted as 2:1; I therefore specified the weighted genetic distance (epsilon) as 120 and conducted a median-joining analysis [58] using the greedy distance calculation method [59].

Nuclear data. The number of different alleles, the number of effective alleles, observed and expected heterozygosities, inbreeding coefficient (FIS) and percentages of polymorphic loci were calculated for Florida, Upper Yucatán Peninsula, South of Yucatán Peninsula, Belize, Guatemala, Honduras, and Nicaragua using GenAlEx v.6.5 [60,61]. To detect evidence of a recent bottleneck or reduction in population size of Mayan Cichlids in Florida, I used the software Bottleneck v.1.2.02 [62]. I performed the Wilcoxon signed rank test to test for heterozygosity excess. When a bottleneck occurs, it is expected that both allele frequencies and heterozygosities decrease, however, allele frequency is expected to decrease faster than heterozygosity. Thus, Bottleneck tests for heterozygosity excess by comparing expected heterozygosity under Hardy-Weinberg equilibrium to heterozygosity expected under mutation-drift equilibrium determined by the number of alleles [63]. I tested for heterozygosity excess under the Stepwise Mutation Model.

Genetic relatedness of populations was assessed using Bayesian clustering in STRUCTURE v.2.3.4 [64]. STRUCTURE was used to estimate the number of populations (K) most likely present in the samples. The parameters were set using an admixture model with independent allele frequencies and sampling locations were used as priors; values for the level of admixture (alpha) were inferred from the dataset. STRUCTURE analyses were performed using the freely available Bioportal server (http://www.bioportal.uio.no) [65]. The burn-in length was set to 50,000 and the simulation to 500,000 repetitions. Each run was iterated 20 times. I evaluated results for K = 1 to K = 35. To determine the most probable clustering of the data, K was selected using the ΔK approach [66] as implemented by Structure Harvester [67]. The variable ΔK is calculated from the rate of change of the log likelihood of the data between runs with successive values of K [66]. CLUMPP v.1.1.2 [68] was used to summarize parameters across 20 iterations and the corresponding graphical output was visualized using DISTRUCT v. 1. 1 [69].

Approximate Bayesian Computation was used to test different introduction pathways of Mayan Cichlids into Florida using the microsatellite data. Approximate Bayesian Computation uses summary genetic statistics (such as genetic distance and the number of alleles) to compare observed and simulated datasets given hypothesized scenarios. Posterior distributions of parameters for the proposed models – possible introduction pathways in my case – are calculated from the differences between the observed and simulated datasets [70,71]. Hypotheses and scenarios were generated on the basis of the results of phylogenetic analyses of cytochrome b, population assignment by cluster analysis, as well as on historical biogeography and hydrology of the native range.

Cytochrome b phylogeny indicated that samples from Belize, Honduras and Nicaragua were within the same clade and cluster analysis also grouped samples from those regions (see Results), although there appeared to be some overlap among individuals from Belize and Florida. Cytochrome b data also showed that samples from both the eastern and western coasts of Florida were within the same clade and also part of the same cluster (see Results). I tested two groups of scenarios using the software DIYABC v. 2.0 [72] wherein the scenarios increased in complexity by changing the grouping of samples into populations to improve model fit. The results from the first group of scenarios informed the second group. The first group contained 15 scenarios that used five distinct populations from Florida, Mexico, Guatemala, a possible unsampled source population, and a grouping of Belize, Honduras and Nicaraguan sites (hereafter referred to as BHN); Belize, Honduras and Nicaragua were grouped together because they shared the same cytochrome b haplotype and were assigned to the same population by Bayesian cluster analysis. Samples from East and West Florida were combined into one population because both phylogenetic analysis and cluster analysis grouped them together. In the first grouping of scenarios, I tested whether Mayan Cichlids were introduced into Florida from BHN, Mexico, Guatemala, from both Mexico and Guatemala, or from an unsampled population in Central America. I also included a possible unsampled, 'ghost' population of Mayan Cichlids in Central America which, in some scenarios, was the source for populations in Mexico and Guatemala. The second group contained nine scenarios that merged cytochrome b results and hydrology of the region; I separated the Mexican samples into two populations, Upper Yucatán Peninsula (YP) and south of the Yucatán Peninsula, and categorized Belizean samples as a distinct group because the

Belizean sites are within the Usumacinta Province [73] unlike the Honduras and Nicaraguan sites, which were grouped together. The cenote-rich Upper Yucatán Peninsula does not contain any major rivers or drainages that connect it to the regions south of the Peninsula [73,74] thus I treated those areas as separate populations for the second group of scenarios. The second group of nine scenarios used the population from south of the Yucatán Peninsula as the most recent common ancestor (MRCA) and tested whether Mayan Cichlids in Florida were introduced from Mexico, Guatemala, or Belize, or whether there were multiple introductions from those regions.

For both sets of scenario analyses in DIYABC, I deliberately broadly defined priors as I did not know true values for the parameters (Table 3.1). I used the Generalized Stepwise Mutation Model [75] with a uniform prior distribution for the mean mutation rate ($1E^4 – 1E^3$). The 'one sample summary statistics' used for each population were the mean number of alleles, the mean genetic diversity, mean size variance and, mean Garza-Williamson's M. The 'two sample summary statistics' used were compared between two populations, and included Fst, mean index of classification (the mean individual assignment likelihood of individuals collected in one population and assigned to another population), and $(\delta\mu)^2$ genetic distance [76]. For each scenario, 1,000,000 simulated datasets were created. Prior-scenario combinations were evaluated using Principal Components Analysis (PCA) as implemented by the software. Posterior probabilities of scenarios were compared with logistic regression using 1% of the closest simulated datasets, as implemented by DIYABC v. 2.0. Estimations of parameters were also computed and performance of parameter estimates was evaluated by assessing confidence and bias as implemented by the software.

Results

Mitochondrial cytochrome b

Six haplotypes were recovered from sequencing cytochrome b for 47 individuals; the remaining specimens were screened for the CA and Fl haplotypes. The CA and Fl haplotypes differed at six sites within cytochrome b. The phylogenetic tree of cytochrome b haplotypes displayed two distinct clades; one clade contained only individuals from the native range. The second clade contained all the sampled individuals from Florida, some of the individuals from five Mexican sites (Xtoloc, Ya Bal Ha, Zaci, Ria Celestun and Ria Lagartos) and all sampled individuals from two sites in Guatemala (Lago Petén Itza and Laguna Macanche) (Figure 3.2). Network analyses indicated that the CA haplotype was shared among individuals from Mexico, Belize, Honduras, and Nicaragua while the Fl haplotype was shared among specimens from the eastern and western coasts of Florida, Guatemala and some individuals from Mexico (Figure 3.3).

Nuclear microsatellite loci

Seventeen loci were analyzed for 357 specimens from 27 sites in Florida, the upper Yucatán Peninsula and south of the Yucatán Peninsula in Mexico, Belize, Honduras, Nicaragua and the Petén region of Guatemala. The Belize population exhibited the highest number of effective alleles (6.56) while Florida had the lowest (2.42) (Table 3.2). Observed and expected heterozygosities were highest in Belize; expected heterozygosity was lowest in Florida and observed heterozygosity was lowest in the upper Yucatán Peninsula (Table 3.2). Florida specimens exhibited 142 alleles, 42 of which were found in specimens from both Belize and Guatemala, 45 from Belize alone,

11 from Guatemala alone, 11 from sites in Mexico, and 33 were private alleles. The Stepwise Mutation Model did not yield significant levels of heterozygosity excess for Florida sites (Wilcoxon signed-rank one-tail test: $p = 1$). Structure analysis using the Evanno method [66] indicated that the uppermost levels of differentiation in population structure were for $K = 2$ ($\Delta K = 1395.23$) and $K = 3$ ($\Delta K = 272.83$). I presented results for both K values because they were both biologically important and reflected regional hydrology (Figure 3.4). The uppermost level of differentiation divided all of the samples into two possible populations, the first contained individuals from Florida and the second contained individuals from Mexico and Central America. When the number of possible populations was three, individuals from Florida remained within a single cluster while individuals from Belize, Honduras and Nicaragua formed a second cluster and individuals from Mexico and Guatemala formed a third grouping (Figure 3.4).

The two clusters from Florida and Mexico and Central America were analyzed separately by running additional structure analyses. Within Florida, the uppermost level of differentiation divided the data into two clusters ($\Delta K = 22.74$), with individuals from Miami Springs and the L31W canal appearing most similar (Figure 3.4). However, examination of clusters for larger K values did not reveal any distinct population structure in Florida. Within the other grouping, the data were also divided into two clusters ($\Delta K = 1908.25$); the first cluster contained individuals from Mexico and Guatemala while the second contained individuals from Belize, Honduras and Guatemala (Figure 3.4).

Scenario testing analysis of the first group of scenarios showed the highest support for scenario 10, in which fish from an unsampled source were introduced to

Mexico, then to both Guatemala and BHN, and then from Guatemala to Florida (Figure 3.5); posterior probability = 0.662, 95% confidence interval (0.617, 0.707). Scenario 10 supported the introduction of Mayan Cichlids from Mexico to Guatemala and BHN (Belize, Honduras and Nicaragua), which was incorporated into the modeled scenarios for the second grouping. Scenario 4 was the most supported from the second grouping of scenarios. In Scenario 4, fish were introduced from southern YP (Yucatán Peninsula) to upper YP, Belize, and the Honduras-Nicaragua group, followed by introductions from Upper YP to Guatemala and from Belize to Florida (Figure 3.5); posterior probability = 0.623, 95% confidence interval (0.514,0.733).

Discussion

I observed that the nuclear genetic markers, microsatellites, supported a different route for introduction of Mayan Cichlids into Florida than the mitochondrial gene (cytochrome b). The nonrandom association of mitochondrial and nuclear alleles, cytonuclear disequilibrium, is strong evidence for introductions of Mayan Cichlids to South Florida through fish from multiple origins [35,37,38]. The Mayan Cichlid is only the second example in animals of which I am aware where cytonuclear disequilibrium provided evidence for multiple introductions [38]. Mayan Cichlids displayed markedly diminished genetic variation in Florida compared to their native range, consistent with a small initial introduction followed by a rapid expansion to their current approximate 70,000 hectare range over at least 7 to 8 generations. The proposed pattern of establishment and expansion is consistent with mechanisms creating cytonuclear disequilibrium. I also found evidence of movements within Mexico and Central America

suggestive of human-assisted dispersal, possibly in pre-European times when anthropological evidence supports the presence of large and highly organized pre-Columbian societies. I shall now discuss each of these results.

Phylogenetic analysis and haplotype distribution of cytochrome b indicated an introduction of Mayan Cichlids into Florida from the Petén region of Guatemala or the upper Yucatán Peninsula of Mexico. All fish in Florida carried the same cytochrome b haplotype suggesting that either a small number of founders, or low female effective population size carrying the Fl haplotype were introduced and quickly spread [e.g. 77]. Alternatively, the Fl haplotype was fixed in the population after introduction, perhaps through selection or genetic drift acting on a small founder population [78]. The distribution of cytochrome b haplotypes which I found was consistent with research by Razo-Mendivil et al. [79], who sequenced cytochrome b for Mayan Cichlids throughout southern Mexico and Central America and found high genetic structuring corresponding with two highly divergent groups. Unlike their study, I used restriction endonuclease enzyme digestion in lieu of sequencing cytochrome b and thus found lower genetic diversity of cytochrome b within Mexico and Central America than their study. However, their most common haplotypes, Cu1 and Cu12, reflected the distributions of my CA and Fl haplotypes within Mexico and Central America, confirming the efficacy of my screening methods for phylogenetically useful cytochrome b haplotypes.

The first group of scenarios I tested using Approximate Bayesian Computation supported a pathway whereby Mayan Cichlids were introduced from an unsampled source to Mexico, then to both Guatemala and the cluster of Belize-Honduras-Nicaragua, and then from Guatemala to Florida. Cytochrome b results also supported Guatemala as

the introduction source of Mayan Cichlids in Florida because they shared the Fl haplotype. I grouped Belize with Honduras and Nicaragua for the first group of scenarios because of their genetic similarity indicated by the cluster analysis. However, because Belize is within the Usumacinta drainage, unlike Honduras and Nicaragua, and because there was some genetic similarity of individuals between Florida and Belize, I grouped Belize separately for the second set of scenario testing. I investigated whether the 'unsampled population' indicated by the most supported scenario from group 1 was representative of a population near the Ria Grijalva basin where the sister species of Mayan Cichlids (*Peténia splendida;* [80,81], and perhaps Mayan Cichlids themselves, arose [82]. Thus, I used my samples from south of the Yucatán Peninsula as the most recent common ancestral population for the second group of scenarios to improve model fit. Both of the most highly supported scenarios corroborated an introduction from Mexico to Guatemala suggesting that the Fl haplotype spread from Upper Yucatán Peninsula to Guatemala, which was a likely introduction source for Florida (group 1, scenario 10). The most supported scenario from the second group and shared alleles indicated an introduction to Florida from Belize; however, a Belizean introduction is not supported by cytochrome b data because I did not find the Fl haplotype at any Belize sites.

My results showed that the Florida population contained a mitochondrial lineage from Guatemala and a nuclear lineage most similar to Belize resulting in a form of cytonuclear disequilibrum that is expected when small founding populations that are genetically differentiated at nuclear and mitochondrial loci are admixed [36-38]. There was also some genetic similarity in microsatellites between fish from Florida and

Guatemala, which is expected if Guatemala was also an introduction source. I was not able to test for cytonuclear disequilibrium within Florida populations using standard methods [37,83] because I identified only one effective haplotype within Florida (the only other haplotype I found in Florida was in a single individual). I propose that an introduction from Petén occurred, as a result of the aquarium trade [84,85], where all the females were fixed for the Fl cytochrome b haplotype followed by an introduction from Belize. Cichlid hobbyists and aquarists imported many neotropical cichlid species into the United States starting in the 1970s (Loftus *pers. comm*.). The founding population from Belize likely contained mostly males, though I cannot rule out mutation and subsequent selection for the Fl haplotype after introduction resulting in an introduced population that is genetically similar to two distinct populations. Another possibility is that the Fl haplotype was present in the Belize population, at such low frequencies that I could not identify it within Belize specimens. The breeding of Mayan Cichlids by aquarists and cichlid hobbyists prior to its release in Florida could have facilitated the hybridization of Mayan Cichlids from Guatemala and Belize or the nonrandom mating of females from Guatemala with males from Belize, which could have led to the cytonuclear disequilibrium I observed.

Mayan Cichlids within Florida formed two clusters that were not very distinct, indicating low levels of population differentiation among sites in Florida. The relatively high inbreeding coefficient and the low genetic diversity within Florida supports the hypothesis of introduction of a small number of individuals that subsequently spread throughout southern and central Florida [47] at an approximate rate of 2,300 hectares per year (total range of approximately 70,000 hectares). I used the test for heterozygosity

excess to determine the occurrence of a bottleneck because it was more robust to assumptions about mutation models than other bottleneck testing methods [86]. Although my test for a bottleneck in Florida populations did not yield significant results, this does not preclude the occurrence of a historic bottleneck. As effective population size increases after a bottleneck occurs, statistical power to detect the bottleneck decreases even with large sample sizes [86-88]. Therefore, if Mayan Cichlids suffered a bottleneck and a subsequent rapid population expansion, the populations would rapidly obtain mutation-drift equilibrium making heterozygosity excess difficult to detect.

Cytochrome b within Central America

The Fl haplotype was found in all fish from Lago Petén, Laguna Macanche, Cenote Ya-Bal-Ha, and Cenote Xtoloc, and some fish from Ría Lagartos, Cenote Zaci, and Ría Celestun. Although these areas are all part of the Yucatán Division of the Usumacinta Drainage [73], Cenote Ya-Bal-Ha, Cenote Xtoloc, Cenote Zaci, and Ría Celestun are all located in the upper Yucatán Peninsula, which has no major drainages that connect them to the rest of the Usumacinta basin [72,78] where Mayan Cichlids are believed to have arisen [73,74,90]. Dispersal between the Petén region of Guatemala and Upper Yucatán through freshwater channels is possible; a similar pattern was also found for *Gambusia yucatana* where individuals from northern Yucatán Peninsula and Petén were morphometrically more similar than with nearby sites [91]. However, I did not observe the Fl haplotype at any sampling location between Petén and the Upper Yucatán as expected with dispersal. Mayan Cichlids are tolerant of salt water [41,48,92,93] and could have arrived via marine corridors along the coast or during sea level changes

during the Pleistocene and early Holocene [79,90] although the hypothesis of strict marine dispersal by Cichlids is disputed [94-97]. It is also possible that Mayan Cichlids were transported between the Upper Yuctán and Guatemala by humans since they have been purposely introduced to many water bodies in Mexico for mosquito control and as a food source [41,42, ,82,98,99] The first description of Mayan Cichlids, using specimens from Lago Petén Itza, was published in 1862 [100] suggesting that movement of Mayan Cichlids to Guatemala occurred within or before the 1800s. The sites where the Fl cytochrome b haplotype were found are also near to Maya sites; Lago Petén Itza is surrounded by at least 27 Maya sites, Zaci and Xtoloc cenotes are both close to Chichen Itza, Reserva de la Biosfera de Ria De Celestun was part of a large Mayan province [101], and Cenote Ya-Bal-Ha is near to Ría Lagartos which was a port for exchange of goods, such as salt, between Chichen Itza and Central Mexico, Guatemala and other parts of Central America [102,103] thus the introduction of this species into cenotes by humans is likely to have occurred . Pre-Columbian peoples cultivated freshwater snails as a food source [104], developed artificial fisheries [105] and stocked their reservoirs with fish [106] so it is probable they used Mayan Cichlids as a food source. As they do today, the Maya would have used this species for food and likely introduced them along their trade routes to water bodies from which they were absent.

Conclusion

Mayan Cichlids have become established in southern Florida; they have spread and impacted their introduced environment, representing a case of a successful invader that resulted from multiple introductions. Unlike other studies, the introductions from

distinct sources did not increase overall genetic diversity compared to the native range. Instead, it resulted in a genetic bottleneck which decreased overall genetic diversity and produced novel combinations of mitochondrial haplotypes and nuclear alleles. Introduction was followed by rapid population growth and dispersal throughout south Florida. This admixture between distinct Belize and Guatemala lineages could have improved fitness and facilitated establishment and spread in Florida.

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Figure Legend.

Figure 3.1. Map of sampling sites for Mayan Cichlids in Mexico and Central America (A) and Florida (B). Numbers on the map correspond to site numbers in Table S1. "Mexico" denotes samples from Mexico that are not within the Yucatán Peninsula (states of Yucatán, Campeche and Quintana Roo). YP = Yucatán Peninsula; FL = Florida.

Figure 3.2. Consensus tree generated by Bayesian phylogenetic analysis using the sister species, *Peténia splendida,* as an outgroup. Clade credibility for branches are shown. Samples that exhibited the same haplotype from East and West Florida, Honduras and Nicaragua were each collapsed into a single branch for clarity. Branches are color-coded by region. * denotes sites where specimens were also analyzed at microsatellite loci.

Figure 3.3. Haplotype network of cytochrome b in Mexico, Central America and Florida. Circles represent different haplotypes; sizes of partitions within circles are proportional to the number of specimens per haplotype. Colors correspond to localities as indicated.

Figure 3.4. Box plots showing STRUCTURE analysis of Mexico, Central America and Florida for K = 2 (A) and K = 3 (B). Box plots of cluster analysis of sites within Central America for K = 2 (C) and within Florida for K = 2 (D).

Figure 3.5. Model (A), scenario (B), and logistic regression of posterior probabilities for scenario 10 (C) from group 1, and model (D), scenario (E) and logistic regression of posterior probabilities for scenario 4 from group 2. Population numbers are

indicated with the population names in the flow chart. YP refers to Yucatán Peninsula.

Figure 3.1

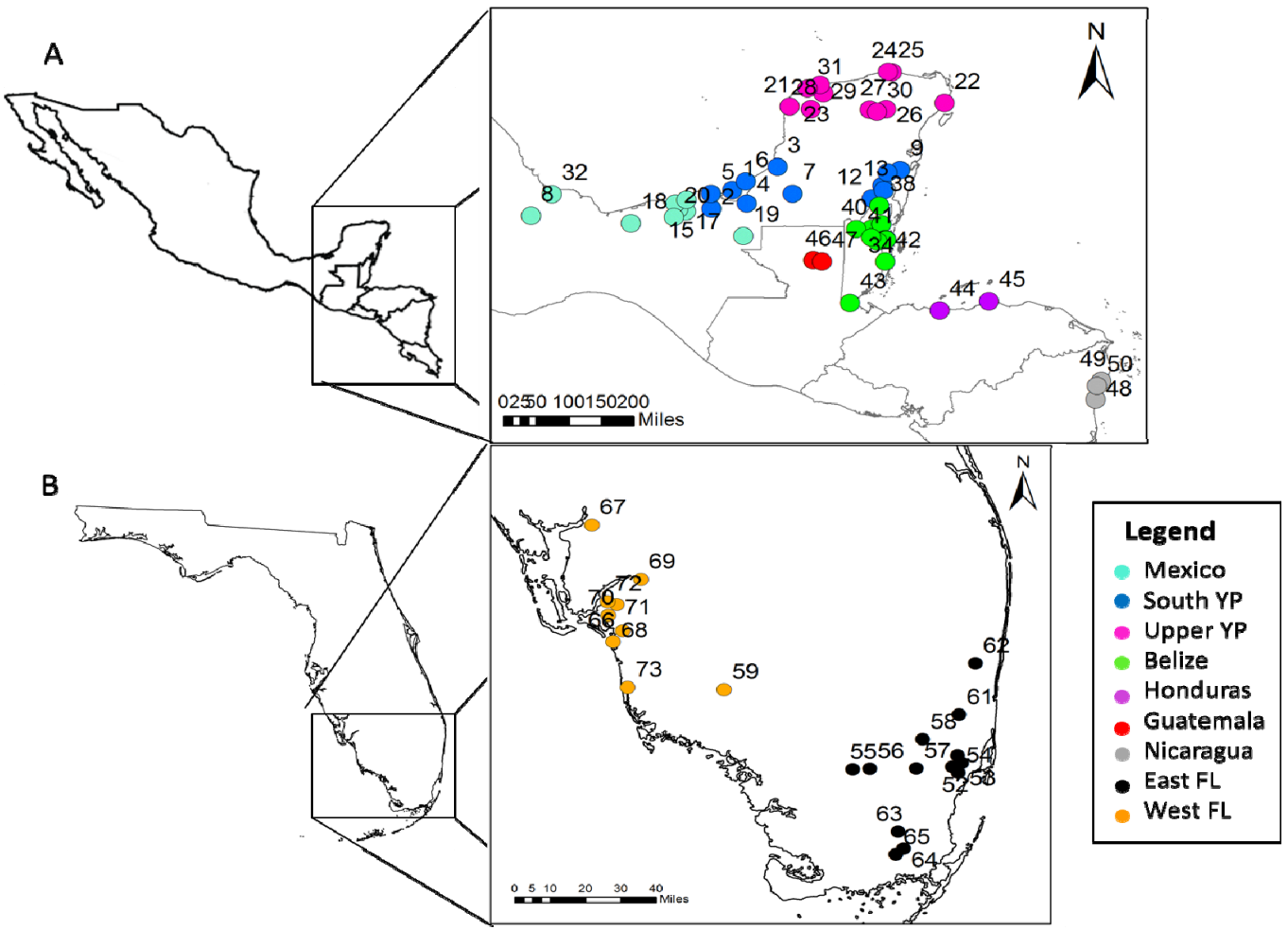

A
B
N
Legend
Mexico
South YP
Upper YP
Belize
Honduras
Guatemala
Nicaragua
East FL
West FL
0 250 100 150 200
Miles
0 5 10 20 30 40
Miles

Figure 3.2

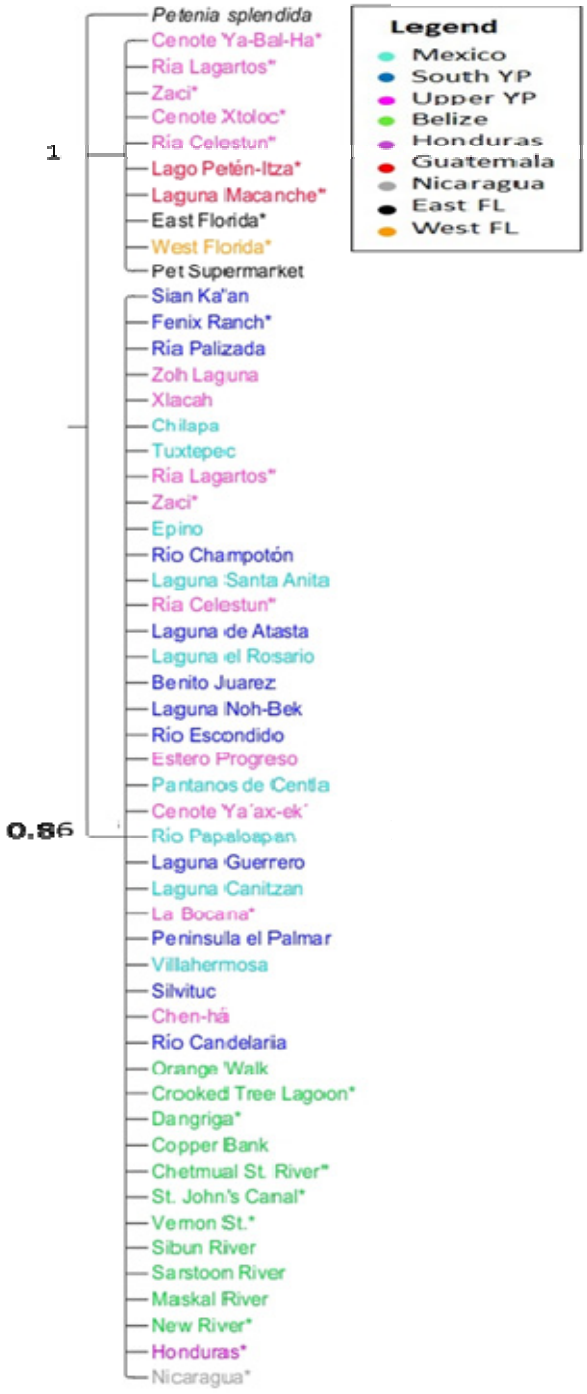

Petenia splendida
Cenote Ya-Bal-Ha*
Ria Lagartos*
Zaci*
Cenote Xtoloc*
Ria Celestun*
Lago Petén-Itza*
Laguna Macanche*
East Florida*
West Florida*
Pet Supermarket
Sian Ka'an
Fenix Ranch*
Ria Palizada
Zoh Laguna
Xlacah
Chilapa
Tuxtepec
Ria Lagartos*
Zaci*
Epino
Rio Champotón
Laguna Santa Anita
Ria Celestun*
Laguna de Atasta
Laguna el Rosario
Benito Juarez
Laguna Noh-Bek
Rio Escondido
Estero Progreso
Pantanos de Centla
Cenote Ya'ax-ek'
Rio Papaloapan
Laguna Guerrero
Laguna Canitzan
La Bocana*
Peninsula el Palmar
Villahermosa
Silvituc
Chen-há
Rio Candelaria
Orange Walk
Crooked Tree Lagoon*
Dangriga*
Copper Bank
Chetmual St. River*
St. John's Canal*
Vernon St.*
Sibun River
Sarstoon River
Maskal River
New River*
Honduras*
Nicaragua*
1
0.86
Legend
Mexico
South YP
Upper YP
Belize
Honduras
Guatemala
Nicaragua
East FL
West FL

Figure 3.3

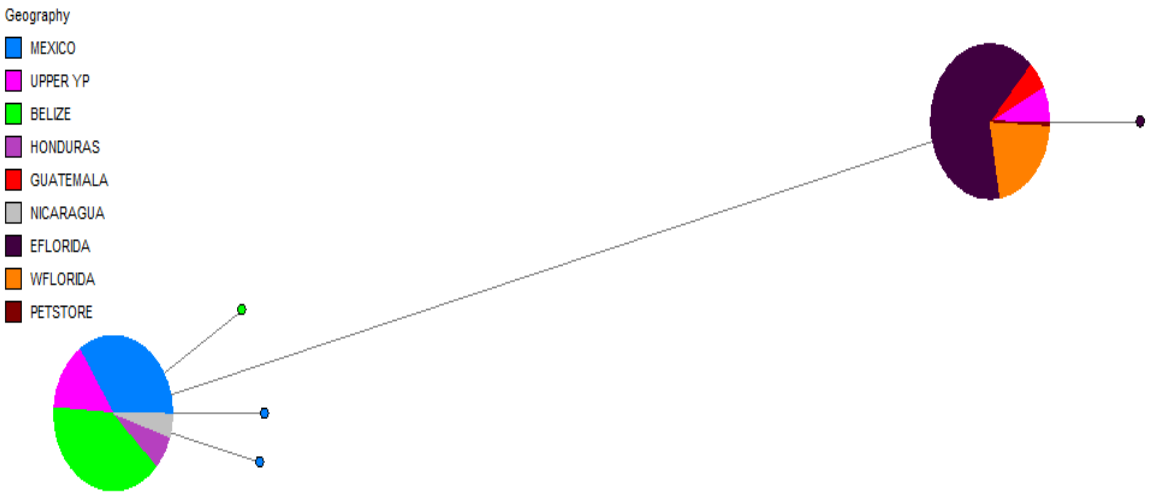

Geography
MEXICO
UPPER YP
BELIZE
HONDURAS
GUATEMALA
NICARAGUA
EFLORIDA
WFLORIDA
PETSTORE

Figure 3.4

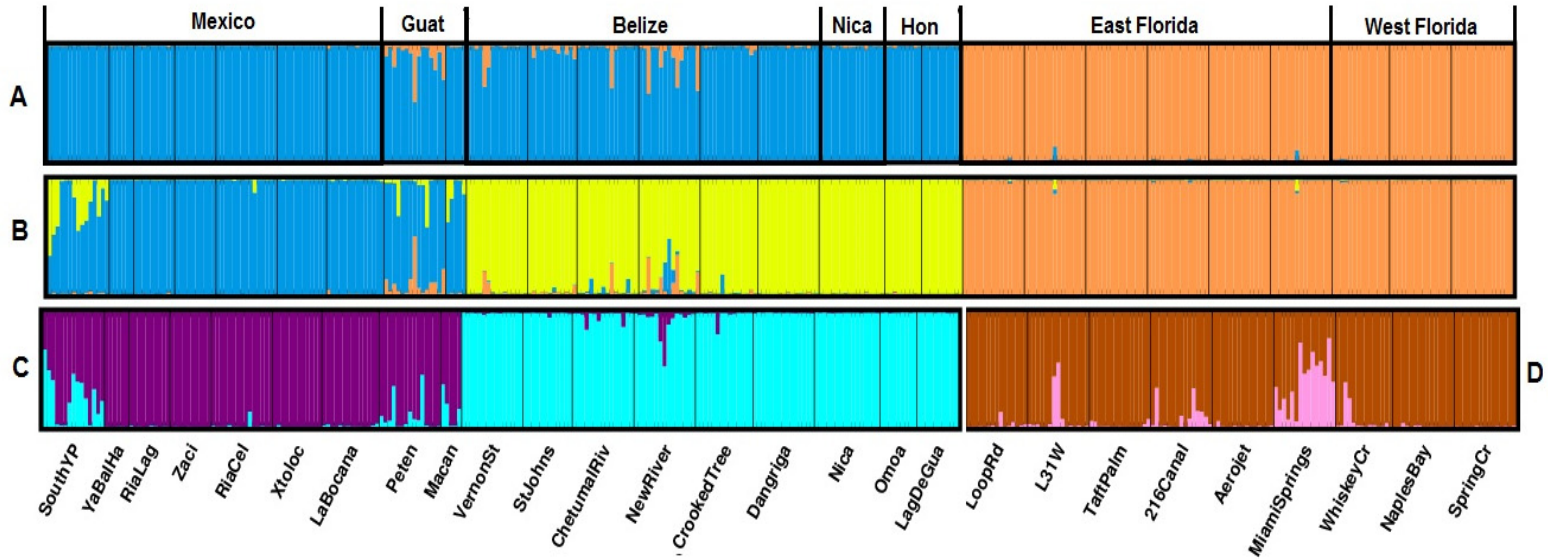

Mexico
Guat
Belize
Nica
Hon
East Florida
West Florida
A
B
C
D
SouthYP
YaBalHa
RiaLag
Zaci
RiaCel
Xtoloc
LaBocana
Peten
Macan
VernonSt
StJohns
ChetumalRiv
NewRiver
CrookedTree
Dangriga
Nica
Omoa
LagDeGua
LoopRd
L31W
TaftPalm
216Canal
Aerojet
MiamiSprings
WhiskeyCr
NaplesBay
SpringCr

Figure 3.5

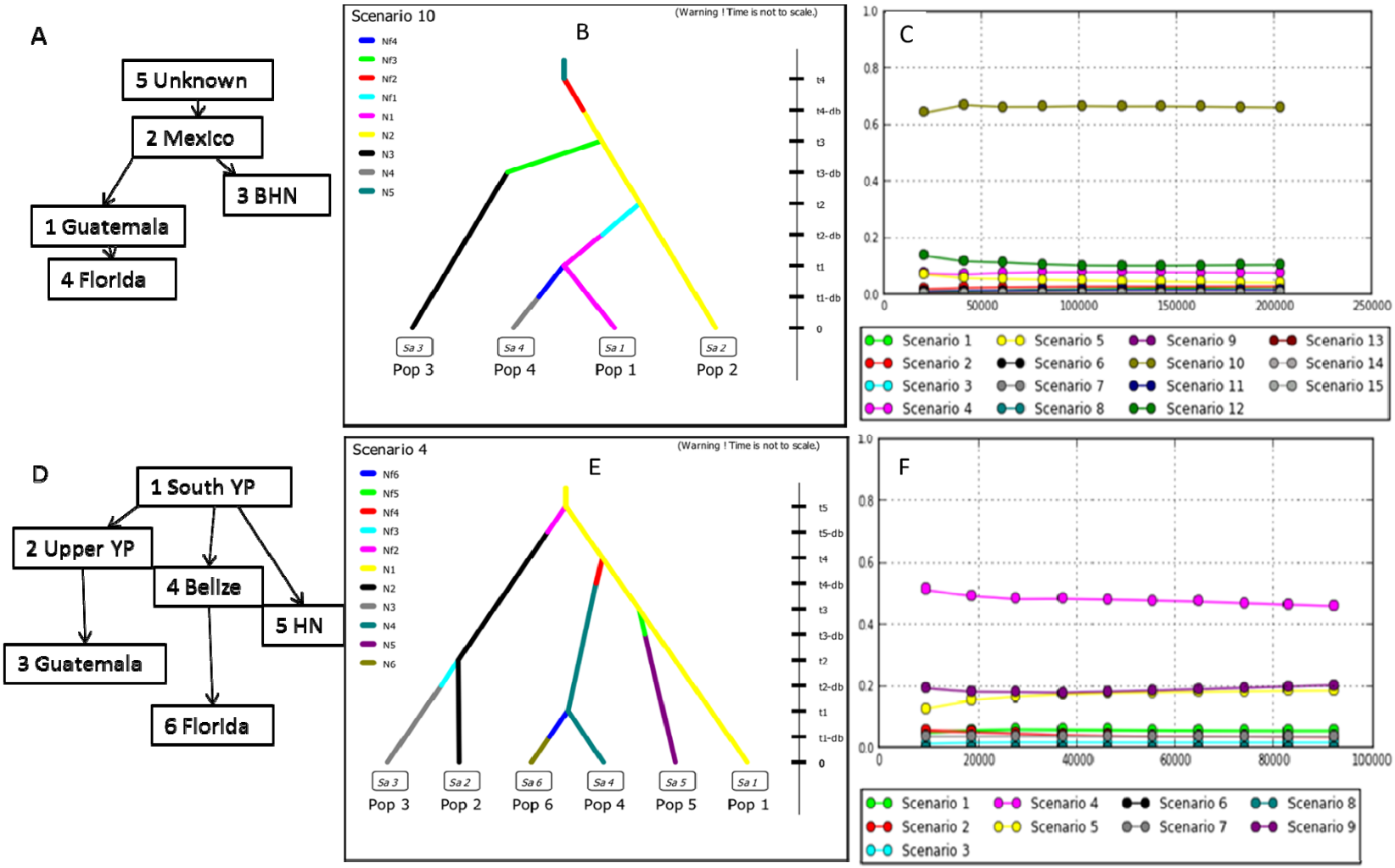

A
5 Unknown
2 Mexico
3 BHN
1 Guatemala
4 Florida
Scenario 10
(Warning ! Time is not to scale.)
B
Nf4
Nf3
Nf2
Nf1
N1
N2
N3
N4
N5
t4
t4-db
t3
t3-db
t2
t2-db
t1
t1-db
0
Sa 3
Sa 4
Sa 1
Sa 2
Pop 3
Pop 4
Pop 1
Pop 2
C
1.0
0.8
0.6
0.4
0.2
0.0
0
50000
100000
150000
200000
250000
Scenario 1
Scenario 2
Scenario 3
Scenario 4
Scenario 5
Scenario 6
Scenario 7
Scenario 8
Scenario 9
Scenario 10
Scenario 11
Scenario 12
Scenario 13
Scenario 14
Scenario 15
D
1 South YP
2 Upper YP
4 Belize
5 HN
3 Guatemala
6 Florida
Scenario 4
(Warning ! Time is not to scale.)
E
Nf6
Nf5
Nf4
Nf3
Nf2
N1
N2
N3
N4
N5
N6
t5
t5-db
t4
t4-db
t3
t3-db
t2
t2-db
t1
t1-db
0
Sa 3
Sa 2
Sa 6
Sa 4
Sa 5
Sa 1
Pop 3
Pop 2
Pop 6
Pop 4
Pop 5
Pop 1
F
1.0
0.8
0.6
0.4
0.2
0.0
0
20000
40000
60000
80000
100000
Scenario 1
Scenario 2
Scenario 3
Scenario 4
Scenario 5
Scenario 6
Scenario 7
Scenario 8
Scenario 9

Table 3.1. Prior distribution of parameters used in ABC analyses.

Parameter	Interpretation	Distribution	Minimum	Maximum
N	Effective population size	Uniform	10	100000
Nf	Number of founders for each population	Uniform	2	10000
t1, t2, t3, t4, t5 (Condition: t1<t2<t3<t4<t5)	Time of events in generations (backwards in time)	Log-uniform	1	10000
db	Duration of bottleneck in generations	Log-uniform	1	10000
r	Admixture rate	Uniform	0.001	0.999

Table 3.2. Summary statistics calculated for microsatellite markers.

Region	Geographic Location	# of samples	# of different alleles	# of effective alleles	Observed heterozygo-sity	Expected heterozygo-sity	% of polymorphic loci	Inbreeding coefficient (FIS)
Mexico	South of Yucatán Peninsula	15	6	4	0.54	0.65	100%	0.13
Mexico	Upper Yucatán	67	11	5	0.33	0.61	94.12%	0.45
Central America	Guatemala	20	8	5	0.42	0.73	100%	0.41
Central America	Belize	86	16	7	0.57	0.74	100%	0.22
Central America	Nicaragua	16	6	4	0.54	0.57	94.12%	0.03
Central America	Honduras	18	6	3	0.42	0.58	94.12%	0.29
Florida	Florida	134	8	2	0.35	0.48	100%	0.34

Table 3.3. Median estimates of parameters from group 1, scenario 10 and from group 2, scenario 4.

Parameter	Group 1 Scenario 10	Group 2 Scenario 4
N1	2.02E+03	3.50E+03
N2	8.31E+03	7.70E+04
N3	8.23E+03	5.64E+04
N4	3.09E+03	3.31E+04
N5	2.45E+02	3.54E+04
N6		7.18E+03
Nf2	2.35E+03	2.37E+03
Nf3	4.65E+03	8.90E+03
Nf4	1.64E+03	2.27E+03
Nf5		4.27E+03
Nf6		5.57E+01
t1	2.48E+03	2.69E+03
t2	4.17E+03	6.67E+03
t3	5.30E+03	8.10E+03
t4	5.35E+03	7.55E+03
t5		9.44E+03
db1	4.65E+02	7.37E+03
db2	9.25E+03	6.38E+03
db3	5.35E+03	7.86E+03
db4	7.65E+03	1.17E+03
db5		7.10E+03

The parameter values correspond to those in Figure 3.6.

SUPPLEMENTARY INFORMATION

Table S1. Location and number of Mayan cichlid samples collected at each site.

* denotes the sites where specimens were also analyzed at microsatellite loci.

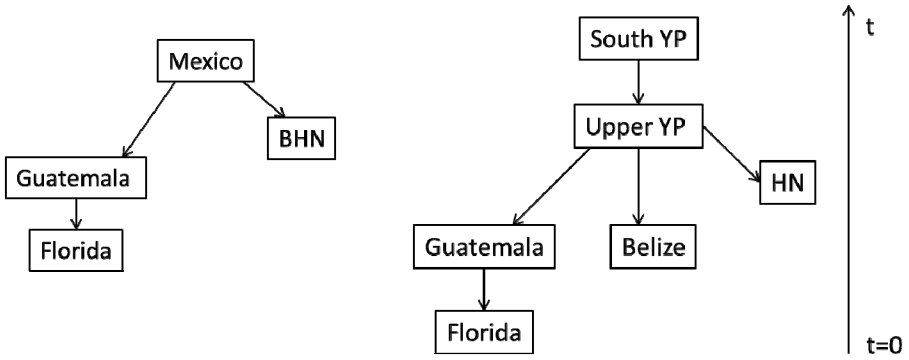
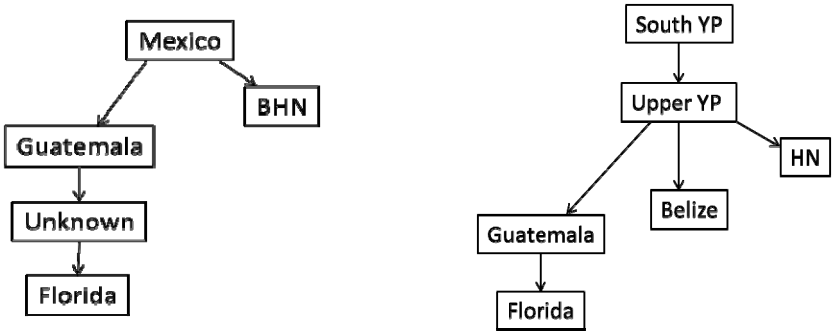
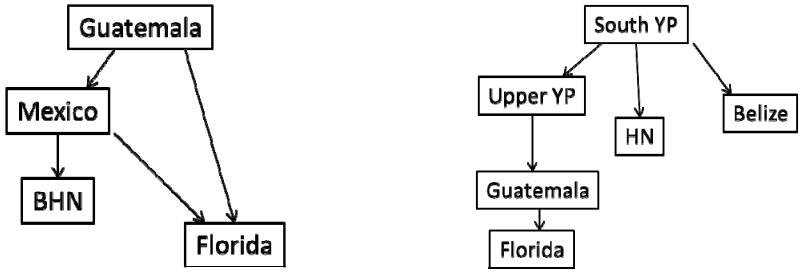
Site #	Geographical Location	Region	Collection Site/Population	Number of samples
1	Mexico	Campeche	Fenix Ranch*	19
2		Campeche	Ría Palizada	1
3		Campeche	Río Champotón	9
4		Campeche	Río Candelaria at Zaragoza	4
5		Campeche	Laguna de Atasta	3
6		Campeche	Peninsula el Palmar	9
7		Campeche	Silvituc	1
8		Oaxaca	Tuxtepec	3
9		Quintana Roo	Sian Ka'an	4
10		Quintana Roo	Benito Juarez	2
11		Quintana Roo	Laguna Noh-Bek	4
12		Quintana Roo	Río Escondido at Ucum	4
13		Quintana Roo	Laguna Guerrero	10
14		Tabasco	Chilapa	6
15		Tabasco	Epino	8
16		Tabasco	Laguna Santa Anita	3
17		Tabasco	Pantanos de Centla	5
18		Tabasco	Laguna El Rosario	4
19		Tabasco	Laguna Canitzan	10
20		Tabasco	Villahermosa	1
21		Upper YP	Ría Celestun*	7
22		Upper YP	Zoh Laguna	1
23		Upper YP	Xlacah	4
24		Upper YP	Cenote Ya-	6

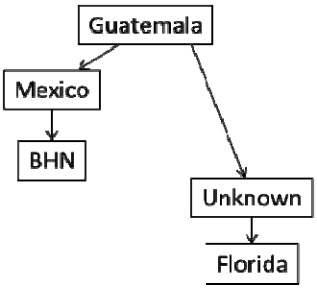
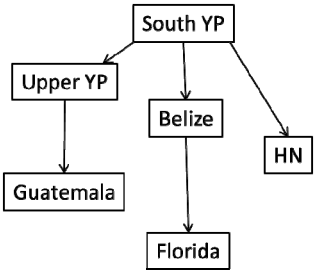
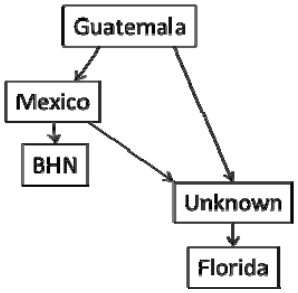
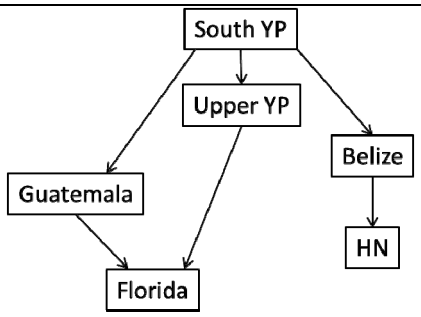
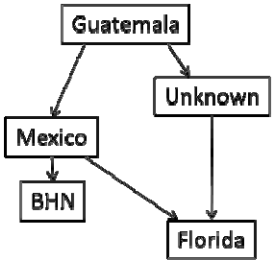
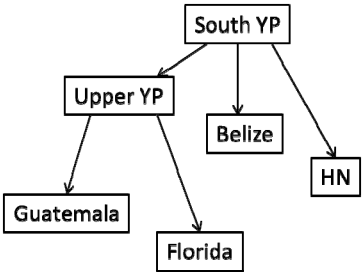
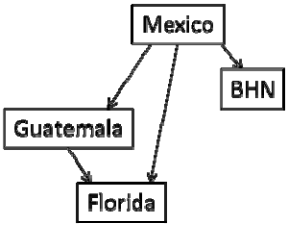
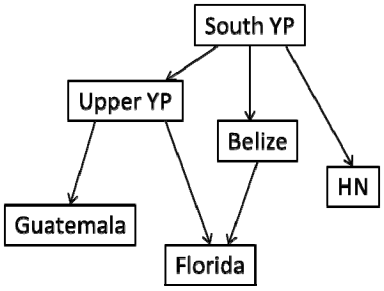
			Bal-Ha*	
25		Upper YP	Ría Lagartos*	6
26		Upper YP	Cenote Zaci*	10
27		Upper YP	Cenote Xtoloc*	12
28		Upper YP	Cenote Chen-há	4
29		Upper YP	Estero Progreso	6
30		Upper YP	Cenote Ya´ax-ek´	4
31		Upper YP	La Bocana*	14
32		Veracruz	Río Papaloapan	10
33	Belize	Belize	Crooked Tree Lagoon*	16
34		Belize	Maskal River	6
35		Belize City	Chetumal River*	15
36		Belize City	St. John's College Canal*	13
37		Belize City	Vernon Street River*	26
38		Corozal	Copper Bank	6
39		Hattieville	Sibun River	8
40		Orange Walk	Orange Walk	8
41		Orange Walk	New River*	17
42		Stann Creek	Dangriga*	13
43		Toledo	Sarstoon River	8
44	Honduras	Cortés	Omoa*	8
45		Colón	Laguna de Guaymoreto*	10
46	Guatemala	Petén	Lago Petén-Itza*	14
47		Petén	Laguna Macanche*	5
48	Nicaragua	RAAN	Laguna de Wouhnta*	8
49		RAAN	Puerto Cabezas*	5
50		RAAN	Laguna de	3

			Karata*	
51	East Florida	Miami Urban Canal	Miami Springs*	27
52		Miami Urban Canal	216 Canal*	19
53		Miami Urban Canal	Airport Lakes	9
54		Miami Urban Canal	57th Avenue	6
55		Tamiami Trail	Loop Road*	21
56		Tamiami Trail	Water Conservation Area 3A	13
57		Tamiami Trail	Everglades Gun Range canal	8
58		North of Tamiami Trail	Krome Avenue	4
59		I-75	Marker 50	6
60		North Miami	Pet Store	2
61		Broward Urban Canal	Taft Palm Ave*	26
62		Palm Beach Urban Canal	441 W	6
63		ENP	L31W*	32
64		ENP	Aerojet Canal*	18
65		ENP	C-111 Canal	8
66	West Florida	Corkscrew Swamp	Corkscrew	10
67		Charlotte Harbor	Punta Gorda Ditch	1
68		Estero Bay	Spring Creek*	15
69		Fort Myers	Montego Bay Condominiums	1
70		Fort Myers	Henry Creek	11
71		Fort Myers	Coral Waters	10
72		Fort Myers	Whiskey Creek*	19
73		Naples	Naples Bay*	15

RAAN represents Región Autonoma Atlántico Norte. * denotes samples that were included in microsatellite analysis.

Table S2. Scenarios 1-15 for group 1 and scenarios 1-9 for group 2 in DIYABC analyses showing the hypothesized movement pathways for Mayan Cichlids (indicated by downward arrows). For all models, time (t) increases upward.

Scenario Number	Group 1	Group 2
1		
2		
3		

4	 Guatemala Mexico BHN Unknown Florida	 South YP Upper YP Belize HN Guatemala Florida
5	 Guatemala Mexico BHN Unknown Florida	 South YP Upper YP Belize Guatemala HN Florida
6	 Guatemala Unknown Mexico BHN Florida	 South YP Upper YP Belize HN Guatemala Florida
7	 Mexico BHN Guatemala Florida	 South YP Upper YP Belize HN Guatemala Florida

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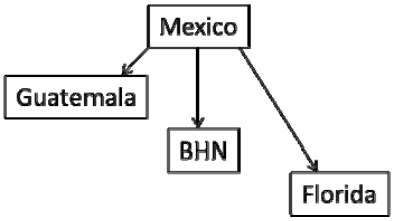
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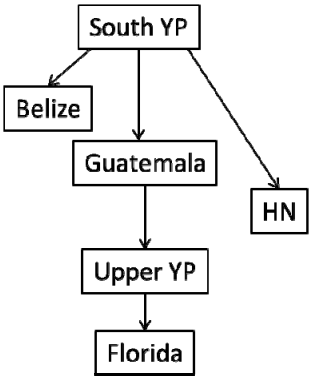
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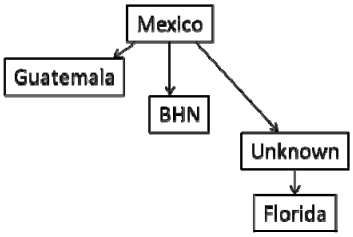
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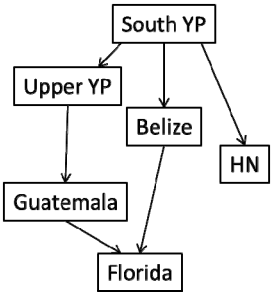
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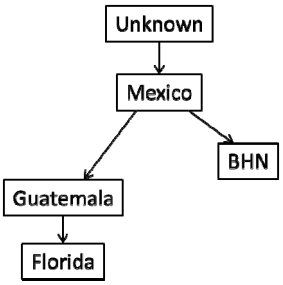
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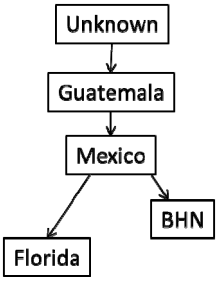
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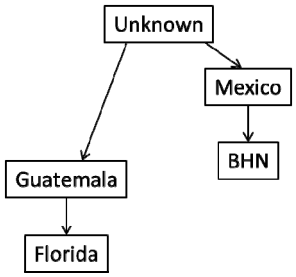
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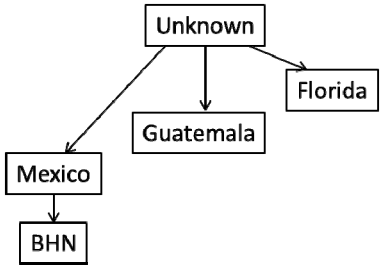
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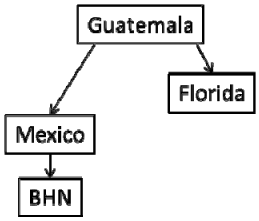
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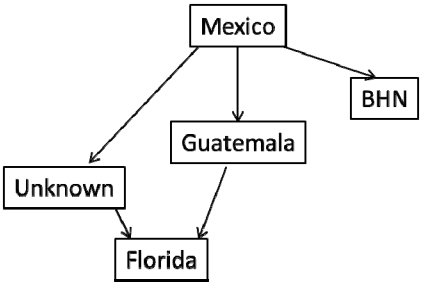
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CHAPTER 4

POPULATION GENETICS OF THE MAYAN CICHLID (*CICHLASOMA UROPHTHALMUS* GÜNTHER) IN ITS NATIVE AND INTRODUCED RANGES

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Abstract

The Mayan Cichlid (*Cichlasoma urophthalmus* Günther) is endemic to the Atlantic coast of Central America, but has been introduced into Florida, the first population reported in Everglades National Park in 1983. I used 17 polymorphic microsatellite loci to document the population genetic consequences of introduction within south Florida by comparing population genetic structure among sites within and between the native (Mexico and Central America) and introduced (Florida) ranges. I sampled from 18 sites in Mexico and Central America and 9 sites in Florida, with 3 to 15 fish per site. The number of effective alleles and observed heterozygosities were lowest in Florida and highest in Belize. Bayesian clustering analyses indicated that Florida was genetically distinct from Mexico and Central America, but Principal Coordinates Analysis, that used Nei's Genetic Distance, indicated some genetic similarity between sites from East Florida and Belize. Within Florida, Mexico, and Central America, gene flow generally decreased with increasing distance; however, Honduras and Nicaragua were very similar to each other genetically, while fish from cenotes within the relatively isolated Upper Yucatán Peninsula were the most differentiated genetically. Bayesian clustering analysis identified six distinct sub-populations within Central America, with distinct sub-populations in Honduras, Nicaragua, Belize, Guatemala, and Laguna de Términos; within the Upper Yucatán Peninsula, the sacrificial cenotes at Zaci and Xtoloc clustered together. Florida exhibited much less genetic structure than Mexico and Central America with a maximum of two population clusters that were not clearly defined; low F_{ST} values and the genetic similarity of East and West Florida indicated a panmictic population or one that has rapidly expanded from a small initial group. The lower genetic diversity of Mayan

cichlids in Florida compared with Mexico and Central America supports a bottleneck upon introduction followed by rapid population expansion.

Introduction

More than 700 fish species have been accidentally or deliberately introduced into the United States, resulting in economic losses of approximately $1 billion per year (Pimental et al. 2005; USGS 2013). Most nonnative fish species have become established in areas with mild climates such as Florida (Pimental et al. 2005; Wilcove et al. 1998; USGS 2013). Introduced fish can negatively impact native species indirectly by altering the environment or directly through hybridization, competition and predation. Successful invasion of a new environment by a nonnative species is a process that involves three steps: establishment, spread, and impact (Kolar & Lodge 2002). During the establishment phase, the colonists must be able to cope with new environmental conditions and found a self-sustaining, viable population (Sakai et al. 2001). The ability of the organism to disperse to new environments also has a strong influence on invasion success (Lockwood et al. 2007).

Species invasions can originate from limited introductions that result in lower genetic variation, small effective population size and high genetic drift (Allendorf & Lundquist 2003; Zachos et al. 2007; Tsutsui et al. 2000); or from multiple introductions that result in higher genetic variation than in the native range, larger effective population size, and higher adaptive potential (Crawford & Whitney 2010; Facon et al. 2008; Lavergne & Molofsky 2007). Genetic variation within the founding population will affect success of the nonnative species because high levels of genetic variation are thought to be

necessary for rapid adaptation to the new environment (Allendorf & Lundquist 2003). Studies have found that multiple introductions of an invasive species are correlated with establishment and success because the founding population exhibits more genetic variation than a species with a single release, especially if the multiple introductions are from different source populations (Gillis et al. 2009; Kolbe et al. 2004; Sakai et al. 2001). Introductions from multiple sources can produce novel genetic combinations that increase fitness and enhance invasion success (Crawford and Whitney 2010; Ellstrand and Schierenbeck 2000; Keller & Taylor 2010). However, there have also been cases of small introductions that have resulted in successful establishment (Dybdhal and Drown 2011; Grapputo et al. 2005) and even successful invaders that reproduce asexually (Huotori et al. 2011; Sakai et al. 2001). Low genetic diversity of invaders is usually attributed to founder effects or bottlenecks, but responses to selection and rapid evolution *in situ* may also decrease genetic diversity (Dlugosch and Parker 2008). If conditions that decrease genetic variation are followed by rapid population expansion, the invader can be successful (Carew et al. 2013; Zachos et al. 2007). Population genetic structure of a nonnative species can provide information about its dispersal and establishment history (Sakai et al. 2001) and provide insight into the genetic consequences of invasion as well as information on movement pathways, invasion routes, and overall invasion potential (Anderson and Congden 2013).

Since European colonization, southern Florida has experienced major habitat transformation and invasion by nonnative species; some invasions have resulted in species declines and extinction of native biota (Forys and Allen 2002). The mild climate of Florida facilitates establishment of tropical species including fish (Wilcove et al. 1998;

Pimental et al. 2000). The majority of exotic fishes in Florida are members of the family Cichlidae; a family with no species indigenous to the state, but more than 30 established within the past 40 years (Fuller et al. 1999; Shafland et al. 2008). The Mayan Cichlid (*Cichlasoma urophthalmus* Günther) is found along the Atlantic slope of Mexico, Belize, Guatemala, Honduras and Nicaragua (Miller 1966). In its native range, it is economically important to artesanal fisheries and aquaculture (Martinez-Palacios et al. 1990; Chavez-Lopez et al. 2005). Mayan Cichlids were first recorded from Everglades National Park in 1983 (Loftus 1987), and by 1999, they had spread through most of southern Florida ranging as far as 200 miles north along the west and east coasts (Adams & Wolfe 2007; Paperno et al. 2008; USGS2013). They are hypothesized to have been introduced as the result of the aquarium trade and impact native fish populations most likely through predation (Howard 1995; Trexler et al. 2000; Harrison et al. 2013a). Work by Harrison et al. (2013b) suggested that Mayan cichlids were introduced into Florida from Guatemala and Belize. Here I investigated how introduction affected genetic structure of Mayan cichlids in Florida. Microsatellite markers have high mutation rates and enable fine-scale analyses of population genetic structures (Goldstein and Schlötterer 2001; Hedrick 1999). I used microsatellite loci to analyze population genetic differentiation across different spatial scales both within and between the native and introduced ranges of the Mayan Cichlid.

Materials and Methods

Sample collection and DNA extraction

I collected 356 samples from seven sites in Mexico, six in Belize, two each in Guatemala and Honduras, three in Nicaragua, and nine in Florida. Three to fifteen fish were collected per site ($\bar{X}$ = 12.3; Table 4.1). Sites were grouped into eight locations for analyses: Upper Yucatán, Laguna de Términos, Guatemala, Belize, Honduras, Nicaragua, East Florida and West Florida. Collection habitats ranged from freshwater ponds to estuarine canals and mangrove habitats using a combination of angling, cast netting, throw traps, and minnow traps. Some samples were also obtained from collections from Universidad Nacional Autónoma de México (UNAM). In some regions of Mexico and Belize, fish were purchased from local fishermen. Samples were either frozen or fixed in 90% ethanol.

Total genomic DNA was isolated from either muscle or fin tissue using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. Specimens were analyzed using 17 recently developed microsatellite markers (Harrison et al. 2013c). The PCR reactions were carried out in 10 µL using 1 µL of 5X reaction buffer, 1 µL of 25mM magnesium chloride, 0.5 µL each of 10mM forward and reverse primers, 0.2 µL of 10mM dNTP's, 0.2 µL of Taq DNA Polymerase (5µ/µL, 1 µL of DNA sample (approximately 10-200 ng) and 5.6 µL of Sigma® sterilized water. Two panels of twelve and five primer pairs, respectively, were run for each specimen. Touchdown PCR cycling parameters were run on an MJ Research thermal cycler; see Harrison et al. (2013c) for complete protocol. Thermal cycling conditions consisted of: 95^{o} C (5 minutes), then 20 cycles of 95^{o} C (30 seconds), a temperature of 58^{o} C, 60^{o} C, 66^{o} C or

67° C depending on the locus that decreased by 0.5° C per cycle (30 seconds), and 72° C (30 seconds), followed by 20 cycles of: 95° C (30 seconds) 48° C, 50° C, 56° C or 57° C depending on the locus (30 seconds), 72° C (30 seconds), then 72° C for 5 minutes. PCR products were run on 1.4% agarose gel and prepared for GeneScan using 9.75 µL of Hi Di™ formamide solution (Applied Biosystems), 0.25 µL of GeneScan™ LIZ-500 size standard (Applied Biosystems) and 1 µL of PCR product. The PCR products were run on a 3130XL Genetic Analyzer (Applied Biosystems) to determine DNA sizes (DNA Core Facility, Florida International University). Peak Scanner 2 (Applied Biosystems) was used to determine fragment sizes of alleles.

Variation between native and introduced ranges

I calculated the effective number of alleles, observed and expected heterozygosities, and the percentage of polymorphic loci using GenAlEx v.6.5 (Peakall & Smouse 2006, 2012) for all fish at 17 microsatellite loci. I also calculated linkage disequilibrium for all loci at each site using POPGENE v. 1. 3. 2. To examine the relationship between genetic isolation and distance, a Mantel test was conducted among sites within Florida and within Mexico and Central America using pairwise Slatkin's linearized F_{ST} [$F_{ST} - (1 - F_{ST})$] values and geographic distances that were log-transformed (Rousset 1997). Significance was assessed with 9999 permutations using GenAlEx v. 6.5.

To compare genetic variation in the introduced and native ranges, I pooled regions into a Florida group and a Mexican and Central American group and conducted an Analysis of Molecular Variance (AMOVA) to determine whether the genetic variation

in the dataset could be attributed by geographic region (GenAlEx v. 6.5). Significance was assessed with 999 permutations using GenAlEx v. 6.5. Principal Coordinates Analysis was performed (GenAlEx v. 6. 5) using Nei's Genetic Distance (Nei 1972) among sites within Florida and Central America. Nei's genetic distance assumes that genetic differences arise from mutation and genetic drift and is appropriate for microsatellite data (Takezaki & Nei 1996).Genetic relatedness of populations both within the native and introduced ranges and between them was assessed using Bayesian clustering in STRUCTURE v.2.3.4 (Pritchard et al. 2000a). STRUCTURE was used to estimate the number of populations (K) most likely present in the samples. The parameters were set using an admixture model with independent allele frequencies and sampling locations were used as priors; values for the level of admixture (alpha) were inferred from the dataset. STRUCTURE analyses were performed using the freely available Bioportal server (http://www.bioportal.uio.no) (Kumar et al. 2009). The burn-in length was set to 50,000 and the simulation to 500,000 repetitions. Each run was iterated 20 times. I evaluated results for K = 1 to K = 35. To determine the most probable clustering of the data, K was determined using the ΔK approach (Evanno et al. 2005) as implemented by Structure Harvester (Earl and vonHoldt 2012). CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007) was used to summarize parameters across 20 iterations and the corresponding graphical output was visualized using DISTRUCT v. 1. 1 (Rosenberg 2004).

Within-range variation

Populations were grouped as following for within-range analyses: (i) Mexico and Central America (Upper Yucatán Peninsula, Laguna de Términos, Belize, Guatemala, Honduras, and Nicaragua) and (ii) Florida (eastern and western coasts and Everglades National Park). The AMOVA and population pairwise F_{ST} were calculated for both groupings to examine the partitioning of genetic variation within and among sites in the introduced and native ranges. STRUCTURE analysis was also applied to each grouping to calculate the number of possible populations within the native and introduced ranges. I evaluated results for K = 1 to K = 25 for the Mexican and Central American grouping and K = 1 to K = 20 for the Florida grouping. I considered population structure for values of K that were most supported (Evanno et al. 2005) and hydrologically relevant for Mayan Cichlids.

Results

Variation between native and introduced ranges

I used 356 Mayan cichlids for analysis at 17 microsatellite loci from locations in south Florida and the Atlantic slope of Mexico and Central America (Table 4.1; Fig. 4.1). All 17 loci were polymorphic with a total of 164 effective alleles. Some loci showed significant deviations from Hardy –Weinberg Equilibrium within sites but no loci showed consistent deviations across sites, so I used all 17 loci for analyses. Some loci showed significant pairwise correlations ($p \leq 0.01$) indicating linkage disequilibrium within sites but no loci displayed significant pairwise correlations at multiple sites so all loci were included in analyses. Average observed heterozygosity was consistently lower than the

expected heterozygosity (Table 4.2). Observed heterozygosity of populations ranged from 0.321 (West Florida) – 0.567 (Belize); number of effective alleles was also highest in Belize (6.56) and lowest in East and West Florida (2.61 and 2. 04 respectively; Table 4.2). The Mantel test indicated a significant positive correlation between geographic distance and Slatkin's linearized F_{ST} values among sites in Central America and Mexico ($r = 0.52$; $p < 0.01$ Fig. 4.2). There was a weak positive correlation between geographic distance and Slatkin's linearized F_{ST} values among sites in Florida ($r = 0.2$; $p = 0.09$; Fig. 4.2).

The highest proportion of molecular variance was found within individuals, then among individuals, among locations (Upper Yucatán, Laguna de Términos, Guatemala, Belize, Honduras, Nicaragua, East Florida and West Florida), and between regions (Florida and Mexico and Central America) respectively; genetic variation between regions, among sites and among individuals within sites (F-statistics; 999 permutations) were all significantly different from zero at $\alpha = 0.01$ (Table 4.3). Genetic differentiation among all locations (overall F_{ST}) was 0.217. All location pairwise F_{ST} values were significantly greater than zero at $\alpha = 0.01$ and ranged from 0.027 to 0.327; the lowest F_{ST} value was between east and west Florida while the highest value was between Upper Yucatán Peninsula and Honduras (Table 4.4). Principal Coordinates Analysis using Nei's genetic distance showed differentiation of East and West Florida sites, Honduras and Nicaragua sites, and Upper Yucatán Peninsula sites; however, there was some overlap of sites from Miami Springs (in East Florida) and Belize. The Laguna de Términos samples overlapped with sites from Guatemala, Upper Yucatán Peninsula, and Belize; Belize sites also grouped closely with Honduras and Nicaragua (Fig. 4.3). Nicaraguan sites were

analyzed individually and as a group for STRUCTURE analysis and because there was no difference I present results for Nicaragua sites as a single group for simplicity. Bayesian STRUCTURE analyses indicated that the most highly supported numbers of genetically distinct populations were K=2 ($\Delta K = 1395.23$) followed by K=3 ($\Delta K = 272.83$). For K=2, the populations consisted of (i) Florida and (ii) Mexico and Central America (Fig. 4.4A). For K=3, the populations consisted of (i) Florida, (ii) Belize, Honduras, and Nicaragua, and (iii) Laguna de Términos Upper Yucatán, and Guatemala (Fig. 4.4B).

Within-range variation

The Central America grouping contained 223 individuals from 20 sites, divided into six geographic locations (Upper Yucatán Peninsula, Laguna de Términos, Guatemala, Belize, Honduras, and Nicaragua). Observed heterozygosity at each site was lower than expected heterozygosity except for the three Nicaraguan sites. The number of effective alleles was highest in Belize and lowest in Honduras (averages of 6.56 and 3.4 respectively); observed heterozygosity was highest in Belize and lowest in Upper Yucatán Peninsula (0.57 and 0.33 respectively; Table 4.5).

The AMOVA for Central America sites showed that genetic variation within and among individuals accounted for 74.25% of total genetic variation, followed by variation among locations, then among sites. Genetic variation among locations, among sites and among individuals within sites (F-statistics; 999 permutations) were all significant at $\alpha = 0.01$ (Table 4.6). Genetic differentiation among all locations (overall F_{ST}) was 0.257. Pairwise F_{ST} between sites ranged from 0.591, between Cenote Xtoloc and Laguna de

Karata, and 0.016, between Crooked Tree Lagoon and Vernon Street. All pairwise location F_{ST} values were significantly greater than zero at $\alpha = 0.01$ (Table 4.7). Bayesian clustering analysis indicated that the highest average log likelihood occurred at K = 2 ($\Delta K = 1908.25$); the next highest supported value for K was K = 6 ($\Delta = 11.81$). For K = 2, the populations consisted of: (i) Laguna de Términos, Upper Yucatán Peninsula, and Guatemala, and (ii) Belize, Honduras and Nicaragua (Fig. 4.4C). For K = 6, the sub-populations consisted of: (i) Laguna de Términos, (ii) Guatemala, (iii) Belize, (iv) Honduras and Nicaragua; sites in the Upper Yucatán Peninsula were split into (v) Cenote Zaci and Cenote Xtoloc, and (vi) Cenote Ya-Bal-Ha, Ría Lagartos, La Bocana, and Ría Celestun (Fig 4.4D).

The Florida grouping contained 134 individuals from nine sites, divided into two locations, West Florida and East Florida – which included sites from Everglades National Park. Observed heterozygosity was less than expected at all sites. The average number of effective alleles, observed heterozygosity, and fixation index were higher in East Florida than West Florida, but lower than Central American sites (Table 4.5). AMOVA showed that 89.5% of molecular variance was attributed to variation within and among individuals, followed by variation among sites and then locations (Table 4.6). Genetic variation among sites and among individuals within sites (F-statistics; 999 permutations) were significantly greater than zero at $\alpha = 0.01$; while genetic variation between East and West Florida was not significant. Genetic differentiation among all locations (overall F_{ST}) was 0.105 (Table 4.6). Pairwise site F_{ST} values ranged from 0.015 between Loop Road and L31W ($p = 0.1$) and 0.281 between Spring Creek and Whiskey Creek ($p < 0.01$; Table 4.8). The most supported number of population clusters as

determined by Bayesian analysis was K = 2 (ΔK = 22.74; Fig 4.4E). Partitions between the two clusters were not strictly defined, but indicated that individuals from Miami Springs displayed higher genetic variation than other sites; the average number of effective alleles was also highest in Miami Springs (Ne = 2.77; Table 4.5). Examining box plots for higher values of K did not reveal further genetic structuring of the populations.

Discussion

Variation between native and introduced ranges

There was significant genetic variation separating Mexico, Central America, and Florida and among locations within Mexico and Central America. Bayesian clustering indicated that Mayan cichlids in Florida are genetically distinct from Mayan cichlids in Mexico and Central America. There was a greater level of population genetic structure within Mexico and Central America than within Florida, whose Mayan populations are genetically homogeneous compared to their native range. Principal Coordinates Analysis and the positive relationship between geographic distance and genetic differentiation indicated that Mayan cichlids were grouped geographically, suggesting that historical dispersal is mainly responsible for their distribution within Central America and Mexico. There is little doubt that introduction of Mayan cichlids from Central America to Florida resulted from human activity, and probably from the aquarium fish trade in the 1960s and 1970s (Matamoros et al. 2005; Wessel 2002). Analysis of cytochrome b of Mayan cichlids from many of the same sites as I sampled (Razo-Mendivil et al. 2013; Harrison et al. 2013b) showed overlapping haplotypes between fish from Florida and a small number

of individual fish from Upper Yucatán, Lago Petén-Itza, and Laguna Macanche, implying mitochondrial gene flow between those regions and the source of Florida fish (Harrison et al. 2013b). Principal Coordinates Analysis and low pairwise F_{ST} values showed that Mayan cichlids from the western and eastern regions of south Florida were very similar to each other; however, some individuals from east Florida were also genetically similar to some individuals from Belize, suggesting a possible introduction from Belize in addition to the link with Guatemala based on mitochondrial markers (Harrison et al. 2013b). Genetic similarities among Florida, Belize and Guatemala suggest introductions into Florida from Belize and Guatemala.

Variation within native and introduced ranges

The AMOVA showed that genetic differentiation among specimens from Laguna de Términos, Upper Yucatán, Belize, Guatemala, Honduras, and Nicaragua accounted for a significant percentage of molecular variance. The well-defined genetic structure between sub-populations of Belize, Guatemala, and Mexico indicated that those Mayan cichlid sub-populations had been hydrologically isolated for many generations. Both Bayesian STRUCTURE analyses and Principal Coordinates Analyses showed that Mayan cichlids from Nicaragua and Honduras genetically were very similar to each other and to some individuals from Belize City and Crooked Tree Lagoon. The similarity suggests that Mayan cichlids from Honduras and Nicaragua originated in Belize which is part of the Usumacinta drainage where Mayan cichlids are believed to have arisen (Hubbs 1936; Miller et al. 2005). Samples from Laguna de Términos, Upper Yucatán Peninsula, Belize, and Guatemala were more genetically similar to each other than to

samples from Honduras, Nicaragua, or Florida as evidenced by pairwise F_{ST} values; this is most likely because Laguna de Términos, Upper Yucatán Peninsula, Belize, and northern Petén (the location of my Guatemala sites) are all part of the Yucatán Division of the Usumacinta Drainage (Miller 2005). The genetic similarity between Laguna de Términos, Upper Yucatán Peninsula, Belize, and Petén supports the hypothesis that Mayan cichlids originated within the Río Usumacinta/ Río Grijalva basin in southern Mexico and then dispersed north and east to the Upper Yucatán Peninsula, Belize, and Petén (Hubbs 1936; Miller 2005).

Within Upper Yucatán Peninsula, gene flow did not always decrease with increasing geographic distance. Low genetic differentiation between Cenote Ya-Bal-Ha and Ría Lagartos ($F_{ST} = 0.04$) and between La Bocana and Ría Celestún ($F_{ST} = 0.027$) were expected given their close geographic proximities and the positive correlation between distance and genetic differentiation. However, F_{ST} between Cenote Zaci and Cenote Xtoloc are higher than for other sites in Upper Yucatán Peninsula despite their geographic proximity. Both cenotes are relatively isolated with little or no hydrologic connections to other areas (Hubbs 1936) although their assignment to the same genetic sub-population implies that there is either a hydrological connection between Cenote Zaci and Cenote Xtoloc or that they were colonized in the past by related individuals (Hubbs 1936). The Maya may have moved Mayan Cichlids around and stocked them in various water bodies as a food source (Hubbs 1936; Matamoros et al.2007, Miller et al. 2005; Pérez-Sánchez and Páramo-Delgadillo 2008). Low genetic differentiation between east and west Florida, low heterozygosities, and weak correlation between genetic isolation and distance among sites in Florida suggested an admixed population that resulted from a

population bottleneck or limited introduction of a few individuals near Everglades National Park followed by rapid spread. Since their introduction, Mayan cichlids have spread approximately 70,000 hectares within southern Florida over at least 7-8 generations either by migration or by human agency since Mayan Cichlids were an aquarium fish (Matamoros 2005; Wessel 2002) and are often targeted by anglers. Comparable rates of spread have been observed for nonnative fish internationally (Freshwater et al. 2009; Pinder et al. 2005) and within Florida including African Jewelfish (*Hemichromis letourneuxi*), Midas Cichlid (*Amphilophus citrinellus*), Black Acara (*Cichlasoma bimaculatum*), Oscar (*Astronotus ocellatus*), and Brown Hoplo (*Hoplosternum littorale*) (USGS 2013). Interestingly, the highest level of genetic differentiation (F_{ST} = 0.281) in Florida was between two sites on the western coast, Whiskey Creek and Spring Creek. Spring Creek is within the Estero Bay estuary which covers about 39 km^2 with connections to the Gulf of Mexico, Imperial and Estero Rivers while Whiskey Creek is farther inland, adjacent to a housing complex and more geographically isolated. Multiple introductions into Florida from other countries and/or sub-introductions from other parts of Florida may have occurred as Mayan Cichlids have been found as far north as Merritt Island, 200 miles north of Everglades National Park (Paperno et al. 2008); however, the sites analyzed for this study indicated a thoroughly mixed Florida population.

Conclusion

Even a limited introduction and resulting low genetic variation can result in a widespread, successful invader. There are lower levels of genetic variance between Belize and East Florida and between Guatemala and East Florida than between other areas of Central America and Florida, supporting the hypothesis of introduction sources from Guatemala and Belize. The lack of genetic structuring within southern Florida suggests small introductions from Belize and Guatemala followed by rapid population expansion throughout southern and central Florida of approximately 70,000 hectares Higher genetic diversity than Florida populations and genetic structure of Mexican and Central American sub-populations indicate colonization of the upper Yucatán Peninsula, Petén, and Belize from southern Mexico many generations ago and subsequent dispersal of individuals from Belize to Honduras and Nicaragua. Molecular methods allow analysis of genetic diversity of invasive populations, facilitating identification of introduction sources, verification of establishment in a new area, and determination of dispersal patterns of invaders.

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Figure Legends

Figure 4.1 Map of sampling sites in (A) Mexico and Central America (B) and Florida. Numbers represent site numbers in Table 4.1. Lag de Ter = Laguna de Terminos; YP = Yucatán Peninsula; FL = Florida.

Figure 4.2 Graph of Slatkin's linearized F_{ST} $[F_{ST} - (1 - F_{ST})]$ and natural log of geographic distance, in kilometers, for pairwise comparisons of sites in Mexico and Central America (A) and Florida (B). The best fit lines are shown, Mexico and Central America: $y = 133.79x + 0.14$ ($R^2 = 0.27$); $r = 0.52$; $p < 0.01$. Florida: $y = 67.37x + 0.09$ ($R^2 = 0.04$); $r = 0.21$; $p = 0.09$.

Figure 4.3 Principal Coordinates Analysis using Nei's Genetic Distance among 29 sites. LagTer = Laguna de Terminos, YP = Yucatán Peninsula.

Figure 4.4 Box plots showing cluster analysis of Central America and Florida for (A) K = 2 and (B) K = 3. Box plots of cluster analysis of sites within Central America for (C) K = 2 and (D) K = 6. Box plot of cluster analysis of sites within Florida for (E) K = 2.

Figure 4.1

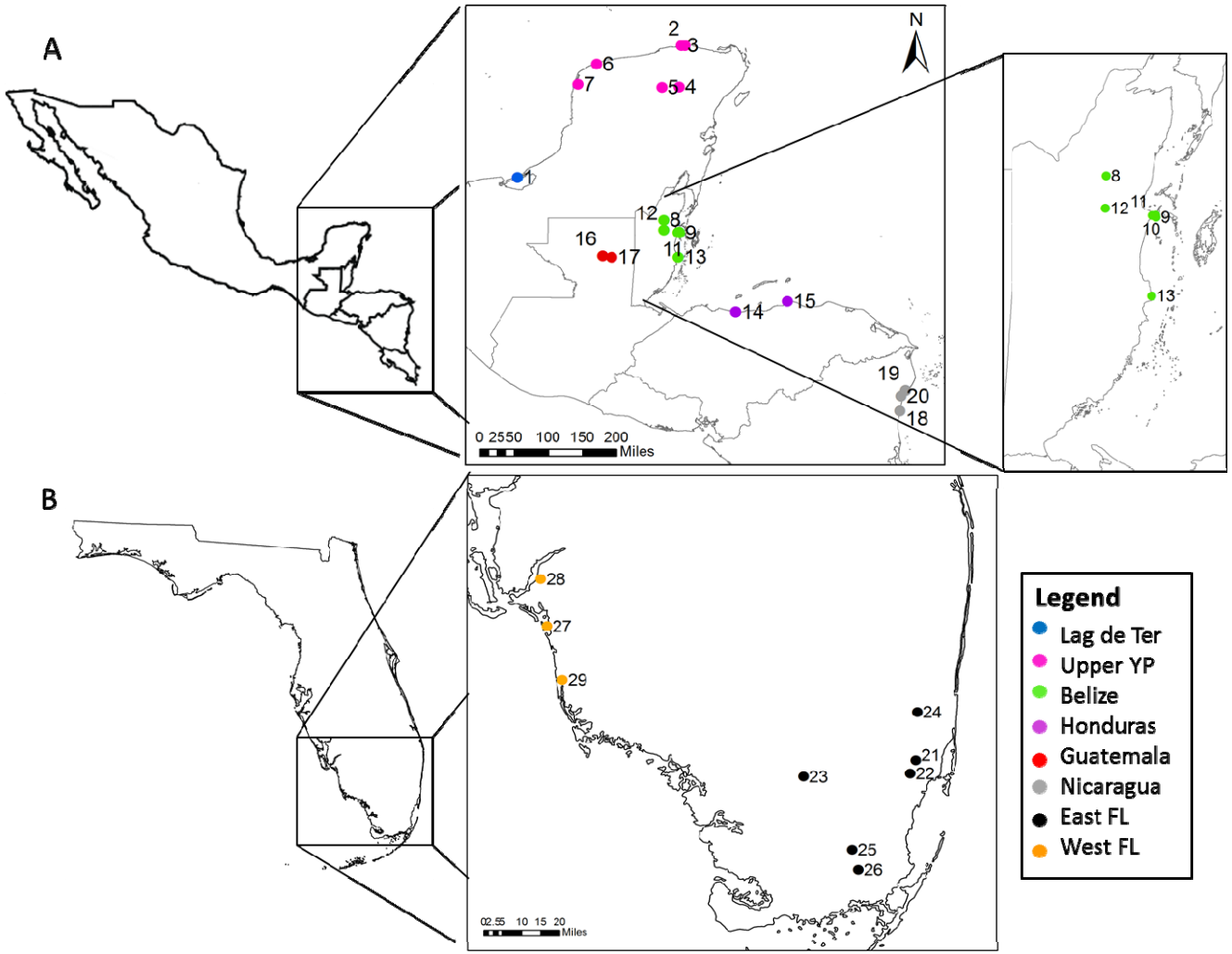
A
B
N
0 2550 100 150 200
Miles
0 2.55 10 15 20
Miles
Legend
Lag de Ter
Upper YP
Belize
Honduras
Guatemala
Nicaragua
East FL
West FL

Figure 4.2

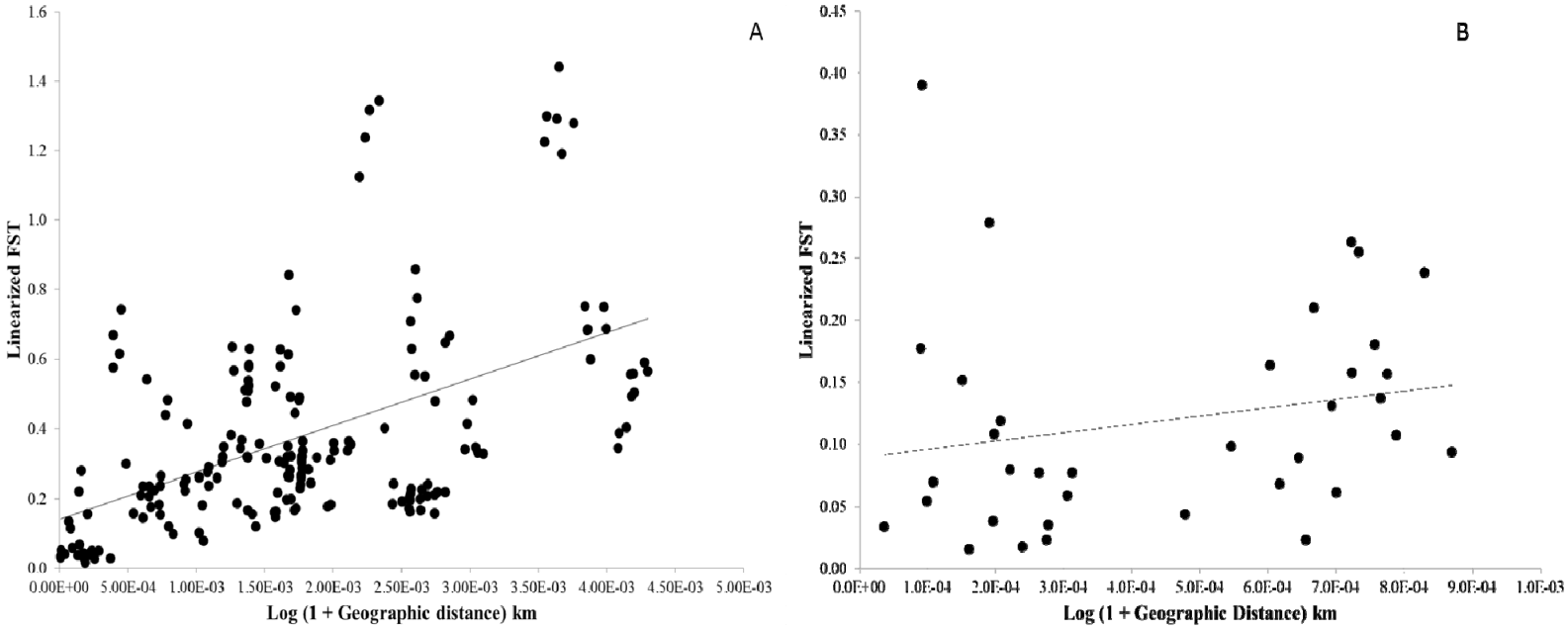
A
Linearized FST
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.00E+00 5.00E-04 1.00E-03 1.50E-03 2.00E-03 2.50E-03 3.00E-03 3.50E-03 4.00E-03 4.50E-03 5.00E-03
Log (1 + Geographic distance) km
B
Linearized FST
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.0E+00 1.0E-04 2.0E-04 3.0E-04 4.0E-04 5.0E-04 6.0E-04 7.0E-04 8.0E-04 9.0E-04 1.0E-03
Log (1 + Geographic Distance) km

Figure 4.3

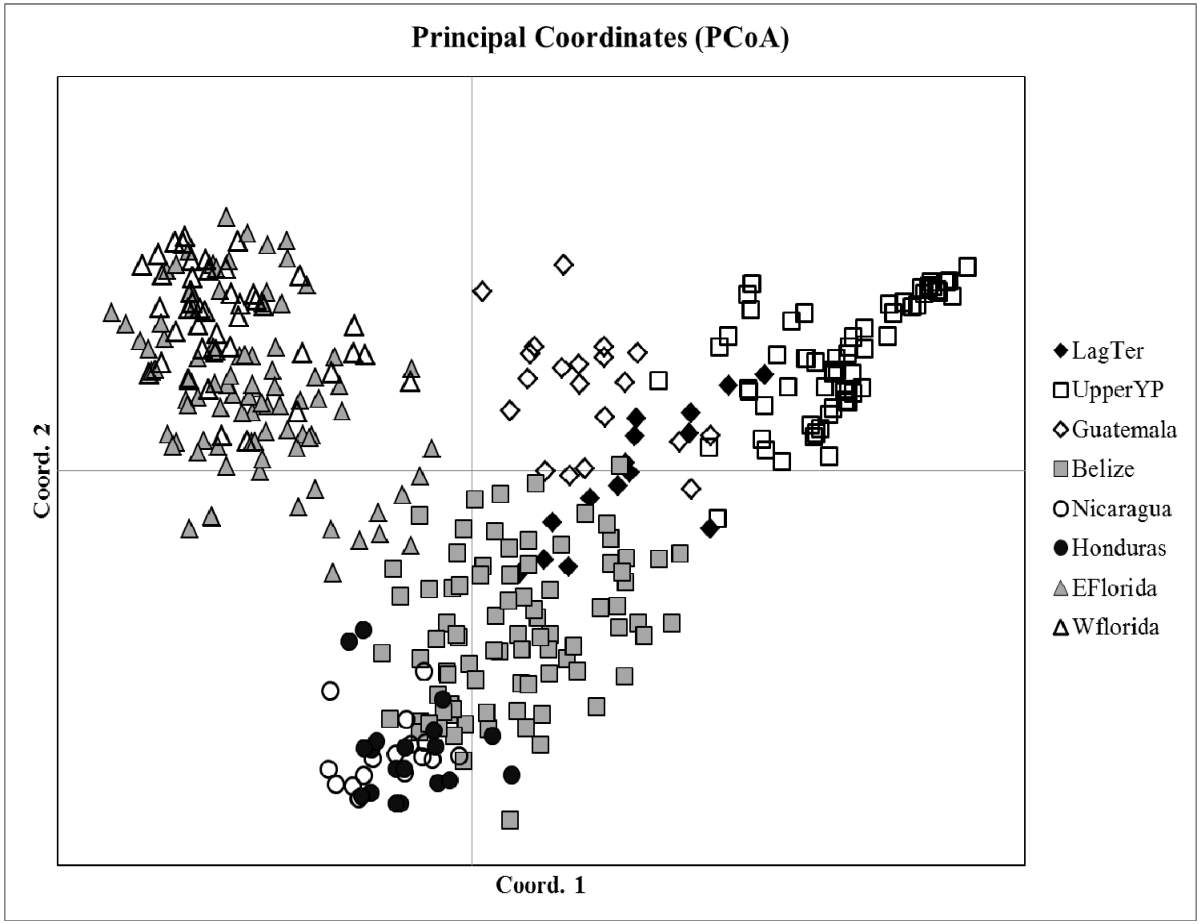
Principal Coordinates (PCoA)
Coord. 2
Coord. 1
LagTer
UpperYP
Guatemala
Belize
Nicaragua
Honduras
EFlorida
Wflorida

Figure 4.4

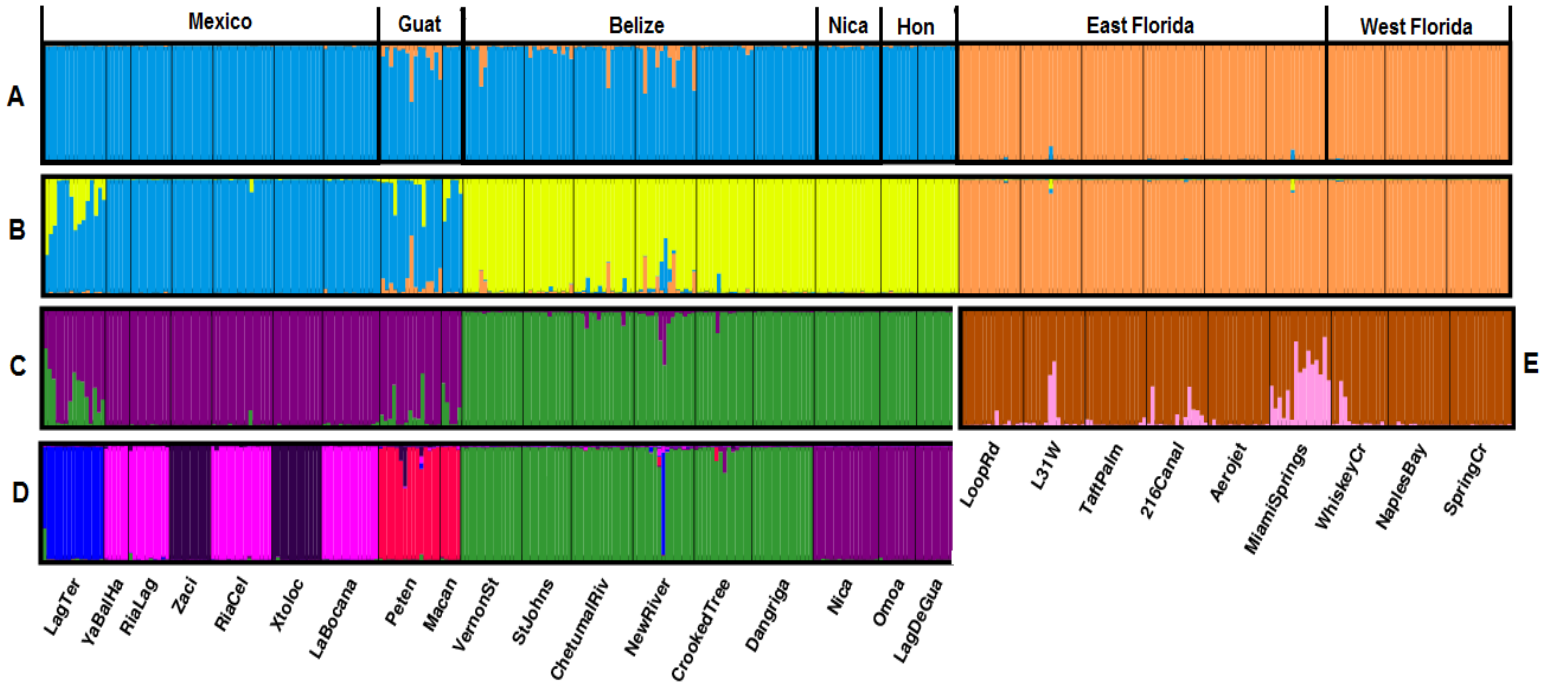
Mexico
Guat
Belize
Nica
Hon
East Florida
West Florida
A
B
C
D
E
LagTer
YaBalHa
RiaLag
Zaci
RiaCel
Xtoloc
LaBocana
Peten
Macan
VernonSt
StJohns
ChetumalRiv
NewRiver
CrookedTree
Dangriga
Nica
Omoa
LagDeGua
LoopRd
L31W
TaftPalm
216Canal
Aerojet
MiamiSprings
WhiskeyCr
NaplesBay
SpringCr

Table 4.1 List of sampling locations and numbers of specimens collected in Mexico, Central America, and Florida. YP = Yucatán Peninsula

Site #	Location	Region	Site	Number of specimens
1	Mexico	Campeche	Laguna de Términos	15
2		Upper YP	Cenote Ya-Bal-Ha	6
3		Upper YP	Ría Lagartos	10
4		Upper YP	Cenote Zaci	10
5		Upper YP	Cenote Xtoloc	12
6		Upper YP	La Bocana	14
7		Upper YP	Ría Celestún	15
8	Belize	Belize	Crooked Tree Lagoon	14
9		Belize City	Chetumal River	15
10		Belize City	St. John's College Canal	12
11		Belize City	Vernon Street River	15
12		Orange Walk	New River	15
13		Stann Creek	Dangriga	15
14	Honduras	Cortés	Omoa	8
15		Colón	Laguna de Guaymoreto	10
16	Guatemala	Petén	Lago Petén-Itza	15
17		Petén	Laguna Macanche	5

18	Nicaragua	Región Autonoma Atlántico Norte	Laguna de Wouhnta	8
19		Región Autonoma Atlántico Norte	Puerto Cabezas	5
20		Región Autonoma Atlántico Norte	Laguna de Karata	3
21	East Florida	Miami Urban Canal	Miami Springs	15
22		Miami Urban Canal	216 Canal	15
23		Tamiami Trail	Loop Road	15
24		Broward Urban Canal	Taft Palm Ave	15
25		ENP	L31W	15
26		ENP	Aerojet Canal	15
27	West Florida	Estero Bay	Spring Creek	15
28		Fort Myers	Whiskey Creek	14
29		Naples	Naples Bay	15

Table 4.2 Number of samples, average number of different alleles, average number of effective alleles, observed and expected heterozygosities and percentage of polymorphic loci for eight locations: Laguna de Términos, Upper Yucatán, Guatemala, Belize, Nicaragua, Honduras, East Florida, and West Florida within two regions: Mexico and Central America, and Florida (the native and introduced ranges)

Region	Location	Number of samples	Number of different alleles	Number of effective alleles	Observed heterozygosity	Expected heterozygosity	Polymorphic Loci (%)	Inbreeding coefficient (FIS)
Mexico	Laguna de Términos	15	6.35	3.91	0.54	0.65	100	0.135
Mexico	Upper Yucatán	67	11.47	4.58	0.33	0.61	94.1	0.449
Central America	Guatemala	20	8.12	4.63	0.42	0.73	100	0.412
Central America	Belize	86	16.41	6.56	0.57	0.74	100	0.222
Central America	Nicaragua	16	5.88	3.60	0.54	0.57	94.1	0.027
Central America	Honduras	18	5.88	3.40	0.42	0.58	94.1	0.286
Florida	East Florida	90	6.94	2.61	0.36	0.49	100	0.362
Florida	West Florida	44	5.18	2.04	0.32	0.43	100	0.270

Table 4.3 Analysis of Molecular Variance (AMOVA) showing the distribution of genetic variation between: two regions (Mexico and Central America, and Florida), among eight locations within regions (Mexico and Central America: Laguna de Términos, Upper Yucatán, Guatemala, Belize, Nicaragua, Honduras; Florida: East Florida, and West Florida), and among and within individuals within locations.

Source of Variation	df	Variation in Fst	F-statistic	P-value
Between regions	1	7.32%	0.073	< 0.001
Among locations	6	14.43%	0.156	< 0.001
Among individuals	348	33.96%	0.217	< 0.001
Within individuals	356	44.29%	0.434	< 0.001
Total	711	100.00%		

Table 4.4 Pairwise F_{ST} values (fixation indices) for eight populations: Laguna de Términos, Upper Yucatán, Guatemala, Belize, Nicaragua, Honduras, East Florida, and West Florida within two regions: Mexico and Central America, and Florida (the native and introduced ranges). F_{ST} values are below the diagonal while p-values are above.

Laguna de Términos	Upper Yucatán	Guatemala	Belize	Nicaragua	Honduras	East Florida	West Florida	
–	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Laguna de Términos
0.134	–	0.001	0.001	0.001	0.001	0.001	0.001	Upper Yucatán
0.190	0.185	–	0.001	0.001	0.001	0.001	0.001	Guatemala
0.112	0.185	0.131	–	0.001	0.001	0.001	0.001	Belize
0.261	0.323	0.222	0.125	–	0.001	0.001	0.001	Nicaragua
0.273	0.327	0.250	0.155	0.104	–	0.001	0.001	Honduras
0.225	0.284	0.170	0.124	0.204	0.245	–	0.001	East Florida
0.250	0.304	0.189	0.158	0.256	0.283	0.027	–	West Florida

Table 4.5 Number of specimens, average number of different alleles, average number of effective alleles observed and expected heterozygosities, and percentage of polymorphic loci for 29 sites within Mexico, Central America and Florida. RAAN refers to Región Autonoma Atlántico Norte in Nicaragua.

Location	Area	Site	Number of samples	Number of different alleles	Number of effective alleles	Observed heterozygosity	Expected heterozygosity	Polymorphic Loci (%)
Mexico	Campeche	Laguna de Términos	15	6.17	4.03	0.49	0.66	100
	Upper YP	Cenote Ya-Bal-Ha	6	3.83	2.68	0.50	0.50	83.3
	Upper YP	Ría Lagartos	10	4.67	3.06	0.37	0.53	83.3
	Upper YP	Cenote Zaci	10	2.08	1.56	0.19	0.28	66.7
	Upper YP	Cenote Xtoloc	12	1.83	1.20	0.10	0.16	66.7
	Upper YP	La Bocana	14	6.42	4.16	0.39	0.58	91.7
	Upper YP	Ría Celestún	15	6.33	3.89	0.43	0.58	91.7
Belize	Belize	Crooked Tree Lagoon	14	8.58	5.24	0.58	0.68	100
	Belize City	Vernon Street	15	7.00	3.85	0.56	0.65	100
	Belize City	St. John's College	12	7.25	4.93	0.48	0.69	100
	Belize City	Chetumal River	15	7.67	4.24	0.56	0.69	100
	Orange Walk	New River	15	8.50	5.28	0.55	0.75	100
	Stann Creek	Dangriga	15	6.33	3.92	0.49	0.63	100

Honduras	Cortés	Omoa	8	4.83	3.26	0.44	0.58	91.7
	Colón	Laguna de Guaymoreto	10	3.00	2.09	0.37	0.40	66.7
Guatemala	Petén	Lago Petén-Itza	15	7.25	4.64	0.36	0.73	100
	Petén	Laguna Macanche	5	4.00	3.05	0.48	0.65	100
Nicaragua	RAAN	Laguna de Wouhnta	8	4.33	3.24	0.62	0.55	83.3
	RAAN	Puerto Cabezas	5	3.17	2.66	0.52	0.47	75
	RAAN	Laguna de Karata	3	2.92	2.51	0.53	0.52	91.7
Florida	East Florida	Miami Springs	15	4.47	2.71	0.44	0.50	88.2
	East Florida	216 canal	15	3.53	2.19	0.32	0.43	88.2
	East Florida	LoopRd	15	3.35	2.15	0.30	0.40	88.2
	East Florida	TaftPalm	15	3.24	2.18	0.33	0.42	82.4
	East Florida	L31W	15	3.53	2.36	0.39	0.46	88.2
	East Florida	Aerojet Canal	15	3.06	2.26	0.40	0.45	82.4
	West Florida	Spring Creek	15	2.82	1.68	0.28	0.32	70.6
	West Florida	Whiskey Creek	14	2.94	1.90	0.35	0.38	82.4
	West Florida	Naples Bay	15	3.47	1.90	0.37	0.41	100

Table 4.6 Analysis of Molecular Variance (AMOVA) showing the distribution of genetic variation within Mexico, Central America and Florida. Mexico and Central America were comprised of six populations (Laguna de Términos, Upper Yucatán, Guatemala, Belize, Nicaragua, and Honduras) and 20 sites. Florida was comprised of two populations (East Florida and West Florida) and nine sites.

Mexico and Central America				
Source of Variation	df	Variation in Fst	F-statistic	P-value
Among locations	5	15.65%	0.157	< 0.001
Among sites	14	10.10%	0.120	< 0.001
Among individuals	203	24.84%	0.257	< 0.001
Within individuals	223	49.41%	0.335	< 0.001
Total	445	100.00%		
Florida				
Source of Variation	df	Variation in Fst	F-statistic	P-value
Among locations	1	0.26%	0.003	0.090
Among sites	7	10.20%	0.102	< 0.001
Among individuals	125	41.67%	0.105	< 0.001
Within individuals	134	47.87%	0.465	< 0.001
Total	267	100.00%		

Table 4.7 Pairwise F_{ST} values between 20 sites in Mexico and Central America. F_{ST} values are shown below the diagonal while p-values are above

Laguna de Términos	YaBalHa	Ría Lagartos	Zaci	Xtoloc	La Bocana	Ría Celestún	Crooked Tree	Vernon St	St John's College	Chetumal River	New River	Dangriga	Omoa	Lag de Gua	Lago Petén-Itza	Lag Macanche	Lag Wouhnta	Puerto Cabezas	Lag Karata	
-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Laguna de Términos
0.155	-	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	YaBalHa
0.151	0.040	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Ría Lagartos
0.308	0.401	0.366	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Zaci
0.343	0.427	0.381	0.219	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Xtoloc
0.158	0.091	0.108	0.326	0.352	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	La Bocana
0.154	0.074	0.092	0.293	0.305	0.027	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Ría Celestún
0.136	0.259	0.241	0.362	0.389	0.234	0.240	-	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Crooked Tree
0.141	0.266	0.252	0.366	0.387	0.243	0.242	0.016	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Vernon St
0.129	0.242	0.236	0.350	0.369	0.211	0.219	0.027	0.030	-	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	St John's College
0.139	0.232	0.222	0.338	0.344	0.206	0.208	0.042	0.050	0.034	-	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Chetumal River
0.108	0.195	0.188	0.323	0.338	0.167	0.178	0.055	0.064	0.037	0.036	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	New River
0.166	0.264	0.252	0.367	0.386	0.236	0.240	0.029	0.043	0.049	0.036	0.048	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Dangriga
0.286	0.415	0.386	0.529	0.553	0.355	0.357	0.192	0.196	0.182	0.203	0.207	0.210	-	0.001	0.001	0.001	0.001	0.001	0.001	Omoa
0.324	0.462	0.437	0.568	0.573	0.401	0.393	0.242	0.233	0.242	0.257	0.256	0.225	0.230	-	0.001	0.001	0.001	0.001	0.001	Lag de Gua

0.216	0.262	0.262	0.326	0.329	0.221	0.231	0.191	0.192	0.134	0.155	0.128	0.184	0.268	0.329	-	0.001	0.001	0.001	0.001	Lago Petén-Itza
0.206	0.266	0.252	0.426	0.457	0.197	0.209	0.174	0.189	0.149	0.171	0.136	0.191	0.276	0.380	0.104	-	0.001	0.001	0.001	Lag Macanche
0.287	0.429	0.408	0.543	0.561	0.361	0.371	0.180	0.167	0.144	0.184	0.180	0.162	0.221	0.263	0.247	0.325	-	0.001	0.001	Lag de Wouhnta
0.279	0.429	0.406	0.551	0.564	0.358	0.357	0.175	0.170	0.164	0.187	0.194	0.196	0.147	0.240	0.249	0.293	0.135	-	0.002	Puerto Cabezas
0.255	0.407	0.375	0.565	0.591	0.335	0.330	0.136	0.147	0.141	0.178	0.172	0.156	0.143	0.144	0.257	0.254	0.182	0.119	-	Lag de Karata

Table 4.8 Pairwise F_{ST} values between nine Florida sites. F_{ST} values are below the diagonal while p-values are above

Miami Springs	216 canal	LoopRd	TaftPalm	L31W	Aerojet Canal	Spring Creek	Whiskey Creek	Naples Bay	
-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	Miami Springs
0.065	-	0.001	0.001	0.001	0.010	0.001	0.001	0.001	216 canal
0.106	0.071	-	0.019	0.055	0.002	0.001	0.001	0.001	LoopRd
0.151	0.132	0.017	-	0.001	0.001	0.001	0.001	0.002	TaftPalm
0.097	0.071	0.015	0.034	-	0.003	0.001	0.001	0.001	L31W
0.073	0.023	0.037	0.056	0.033	-	0.001	0.001	0.001	Aerojet Canal
0.208	0.193	0.089	0.058	0.116	0.136	-	0.001	0.001	Spring Creek
0.121	0.085	0.141	0.203	0.153	0.097	0.281	-	0.001	Whiskey Creek
0.174	0.136	0.042	0.022	0.064	0.081	0.052	0.218	-	Naples Bay

CHAPTER 5

CONCLUSION

The goals of my dissertation research were to: i) determine whether Mayan Cichlids could be considered successful invaders by quantifying their *per capita* impacts on native fish, ii) identify the sources for Mayan Cichlids in Florida thus resolving whether they underwent limited or multiple introductions, and iii) understanding how the method of introduction affected population genetics of Mayan Cichlids by comparing genetic structure in their introduced and native ranges.

Can Mayan Cichlids be considered a "successful invader?"

To be considered a successful invader, an introduced species has to be transported to a new range, become established, spread, and have a quantifiable impact on its introduced environment (Lockwood et al. 2007). Mayan Cichlids are native to the Atlantic slope of Mexico and Central America (Miller et al. 2005) and are believed to have been brought to Florida through the aquarium fish trade (Matamoros et al. 2005; Socolof 1998; Wessel 2002). They were first observed in Florida in 1983 within Everglades National Park where they became established and founded multiple reproducing populations (Loftus 1987). Mayan Cichlids have spread approximately 70,000 hectares from southern to central Florida over at least 7-8 generations; they dispersed throughout the extensive Florida canal system and/or were moved by human agency because they are a popular aquarium fish (Matamoros 2005; Socolof 1998; Wessel 2002) and are targeted by anglers.

Mayan Cichlids have become established and spread within Florida. Gut content data have shown that Mayan Cichlids feed on native fish in their introduced range,

including Eastern Mosquitofish *(Gambusia holbrooki),* Bluefin Killifish (*Lucania goodie*) and Sailfin Mollies (*Poecilia latipinna*) (Howard, 1995; Loftus, 2000; Rehage et al. 2009). I wanted to quantify impacts of Mayan Cichlid predation on native fish populations and determine whether these impacts were sustained over time. In Chapter 2, I quantified impacts of Mayan Cichlids on native fish using long-term data collected from sites south of Everglades National Park. I analyzed densities of fish communities at four sites in estuarine mangrove habitats over 15 years during which four cold fronts occurred that sharply decreased Mayan Cichlid densities. I found negative relationships between Mayan Cichlid density and densities of Sheepshead Minnow (*Cyprinodon variegatus*), Marsh killifish (*Fundulus confluentus*), and Eastern Mosquitofish that were most likely because of predation. The *per capita* impact of Mayan Cichlids on Sheepshead Minnows was 40-60% greater than for other taxa. Studies have shown that those fish species and other species of similar size ranges have been found in the gut contents of Mayan Cichlids (Howard, 1995; Loftus, 2000; Bergmann and Motta, 2005). Mayan Cichlids may also compete with these fish species for food and space. Mayan Cichlids have a broad diet consisting of vegetation, detritus, crustaceans, insects, and gastropods, as well as fishes (Howard, 1995; Bergmann and Motta, 2005); it is very likely that Mayan Cichlids overlap with the diet of other fishes at my study sites. They are also aggressive and territorial, particularly during their mating season where they forcefully guard their nests (*personal observation*), which could lead to decreasing densities of other species that compete for substrate. The data were somewhat unexpected in that the *per capita* effect of Mayan Cichlids on other species did not decrease as Mayan Cichlid density increased as predicted by predator-prey models (Skalski and Gilliam, 2001). This was most likely

because freshwater fishes moved downstream from marshes into estuarine habitats during the dry-season ensuring that there is a constant influx of prey.

Associations between Mayan Cichlids and native fish were not always negative. I also found positive relationships between Mayan Cichlid densities and densities of *Lepomis* species and Sailfin Mollies. *Lepomis* species were grouped together in the dataset and consisted of several sunfish species but Warmouth was the most common. Warmouth achieve large terminal sizes that allow them to quickly outgrow Mayan Cichlid predation. They also track similar prey species to Mayan Cichlids and would therefore follow Mayan Cichlid densities. Sailfin mollies are herbivorous (Harrington and Harrington 1982; Belicka et al. 2012) and so probably do not directly compete with Mayan Cichlids for food and achieved high numbers at sites where salinity was optimal for them (Lorenz and Serafy 2006); however, how they managed to escape predation unlike other similarly sized species is unknown.

In Chapter 2, I was able to quantify *per capita* impacts of Mayan Cichlids on native fish over time, a crucial and oftentimes overlooked component of determining effects of nonnative species (Parker et al. 1999). Mayan Cichlids have become established, spread and detrimentally affect native fish in their introduced range, therefore they can be considered a successful invader.

<u>What were the source(s) for Mayan Cichlids in Florida?</u>

In Chapter 3, I used the cytochrome b gene from 670 fish and microsatellite markers from 356 fish to determine the sources for Mayan Cichlids in Florida.

Cytochrome b was able to differentiate between Mayan Cichlids caught in the native versus the introduced ranges. I found overlapping cytochrome b haplotypes in individuals from Florida, the Upper Yucatán Peninsula, and the Petén region of Guatemala which suggested introductions from both Mexico and Guatemala. However, because all of the Guatemalan fish but only some individuals from the Upper Yucatán Peninsula carried the Florida haplotype of cytochrome b, I tested the hypothesis that Mayan Cichlids moved from the Upper Yucatán to Guatemala and then to Florida. I tested this hypothesis along with other possible introduction pathways using 17 microsatellite loci. The microsatellite data showed some genetic similarity between individuals from Florida and Guatemala, but specified Belize as the source of Mayan Cichlids in Florida. The Florida cytochrome b haplotype was not found in Belize and thus does not support Belize as an introduction source. The mismatch between mitochondrial and nuclear genomes, a cytonuclear disequilibrium, suggests admixture of a female lineage from Guatemala, where all individuals were fixed for the mitochondrial haplotype found in the introduced population, and a more diverse but also relatively small introduction from Belize. Cichlid aquarists and hobbyists began importing Cichlid species to the United States in the 1970s which would have permitted the introduction of fish from disparate sources (Loftus *personal communication*; Socolof 1998; Wessel 2002).

Despite the novel combination of a mitochondrial lineage from Guatemala and nuclear lineages from Belize, all Mayan Cichlids in Florida exhibited the same cytochrome b haplotype and there was little nuclear and/or mitochondrial genetic variation. Mayan Cichlids in Florida are a panmictic population with low genetic diversity that resulted from introductions from at least two disparate sources. Low genetic

variation within and among Florida sites is also evidence for the introduction of a few individuals followed by rapid population expansion.

In Chapter 3, I showed that Mayan Cichlids were introduced to Florida from Guatemala and Belize and then rapidly spread throughout southern Florida to both eastern and western coasts. Both releases were probably small and resulted in a novel combination of alleles as fish sampled at least 30 years post-release displayed low genetic variation indicative of a bottleneck and retained a maternal lineage from Guatemala and a nuclear lineage from Belize.

How did introduction affect population genetic structure of Mayan Cichlids in Florida?

In Chapter 4, I used 17 microsatellite loci from 356 fish to compare population genetic structure within and among sites in the native and introduced ranges of Mayan Cichlids. I showed that Mayan Cichlids in Florida are genetically distinct from those in Mexico and Central America and that there was no real genetic sub-structure within Florida, even between the eastern and western coasts. There was less genetic distance and some gene flow between some fish from eastern Florida, Belize, and Guatemala; this is expected if Belize and Guatemala are introduction sources as indicated by analyses in chapter 3.

Chapter 4 supported results in Chapter 3; low genetic variation between eastern and western Florida and the weak correlation between genetic differentiation and geographic distance indicated that Mayan Cichlids in southern Florida comprise one, admixed population. Low genetic diversity throughout Florida implied that a population bottleneck or release of a few individuals occurred upon their initial introduction into

South Florida which was followed by rapid population expansion at a rate of approximately 10,000 hectares per generation. It should be noted that my data cover much, but not all, of the total Mayan Cichlid population within Florida; Mayan Cichlids are found as far north as Merrit Island on the east coast(USGS Nonindigenous Aquatic Species Database) and it is possible that additional introductions from Mexico and Central America and/or from other sites within Florida have occurred in these areas. However, data from sites I sampled indicated that Mayan Cichlids in Florida are panmictic.

Movement of Mayan Cichlids within Mexico and Central America

In chapters 3 and 4, I analyzed population structure of Mayan Cichlids within their native range; those analyses revealed new information about their origin and dispersal pattern within Mexico and Central America. Those results will be discussed here.

I found the Florida cytochrome b haplotype within the cenote-rich Upper Yucatán Peninsula and in the Petén region of Guatemala, but not at any sites between those areas. Microsatellite data supported movement of Mayan Cichlids from the Upper Yucatán Peninsula to Guatemala. Though it is possible that Mayan Cichlids dispersed to Guatemala on their own through freshwater channels or marine corridors (Hubbs 1936), I propose that Mayan Cichlids were moved by pre-Columbian people such as the Maya. The Maya may have introduced Mayan Cichlids to water bodies along their trade routes or near their settlements as a food source. The cenotes in which fish exhibited the Florida haplotype are close to major Maya sites and trading ports where salt and other goods

were historically transported between the Upper Yucatán Peninsula and Petén (Rathje 1971; Capurro 1985; Batllori et al. 1990).

There was well-defined population genetic sub-structure within Mexico and Central America indicating that sub-populations in the native range, especially in Mexico, Belize and Guatemala, have been hydrologically isolated for many generations. The genetic similarity between Laguna de Términos, Upper Yucatán Peninsula, Belize, and Petén supported the hypothesis that Mayan cichlids originated within the Río Usumacinta/ Río Grijalva basin in southern Mexico and then dispersed north and east to the Upper Yucatán Peninsula, Belize, and Petén (Hubbs 1936; Miller 2005). Approximate Bayesian Computation analyses and relatively high genetic similarity among fishes from Belize, Honduras, and Nicaragua suggested that Mayan Cichlids from Belize colonized Honduras and Nicaragua. Fish could have moved from Belize to Nicaragua or from Honduras to Nicaragua after Honduras was colonized by Belizean fish.

Chapters 3 and 4 supported my hypotheses that Mayan Cichlids originated in southern Mexico, dispersed to the Upper Yucatán Peninsula, Belize and Petén and from Belize to Honduras and Nicaragua. Mayan Cichlids also moved from the Upper Yucatán Peninsula to Petén either through long-distance dispersal through freshwater or marine corridors or by human agency. Mayan Cichlids were then moved from Guatemala and Belize to Florida facilitated by the aquarium trade (Figure 5.1).

Summary and Future Work

My dissertation research showed that the Mayan Cichlid should be classified as a successful in Florida because it has been introduced, become established, spread rapidly,

and had quantifiable *per capita* impacts on native species there. As a result, caution should be taken if this species is introduced elsewhere; it has already been introduced in Thailand (Nico et al. 2007). More research is needed to determine if impacts on native fish in turn impact piscivorous predators such as wading birds.

My research also determined that Mayan Cichlids were introduced from Guatemala and Belize through the aquarium trade and is one of few studies that found cytonuclear disequilibrium in an introduced population as evidence of multiple introductions. I also presented some of the genetic consequences of two disparate but small introductions; Mayan Cichlids in Florida exhibit novel allele combinations when compared with the native range, but with low genetic diversity within and among sites. Introduction in Florida was followed by rapid population expansion over approximately 70,000 hectares in roughly 7-8 generations resulting in a large, admixed population. Further research his needed to determine if novel allele combinations in Florida induced phenotypes that allowed Mayan Cichlids to readily adapt to their new environment. Because of the hybridization associated with their introduction, Mayan Cichlids in Florida are genetically distinct from conspecific fish remaining in their native range.

This project also examined Mayan Cichlid population structure within Mexico and Central America. I proposed that Mayan Cichlids dispersed from southern Mexico to the Upper Yucatán Peninsula, Petén, and Belize, then from Belize to Honduras and Nicaragua. Though highly speculative based on the current data, I also hypothesize that Mayan Cichlids were moved from the Upper Yucatán Peninsula to Petén by the Maya as a food source.

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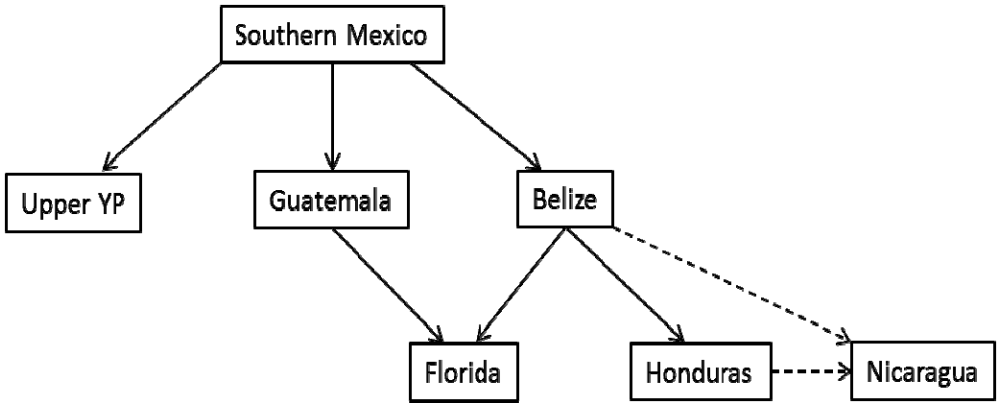

Figure 5.1. Proposed movement pathways of Mayan Cichlids within the native range and from the native range to Florida. Dotted lines represent two possible dispersal pathways from Belize to Nicaragua or from Honduras to Nicaragua.

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