Patterns of Abundance, Distribution, and Size Composition of the Rainwater Killifish (Lucania parva) in a Subtropical Bay

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PATTERNS OF ABUNDANCE, DISTRIBUTION, AND SIZE COMPOSITION OF THE RAINWATER KILLIFISH (Lucania parva) IN A SUBTROPICAL BAY

By

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PATTERNS OF ABUNDANCE, DISTRIBUTION, AND SIZE COMPOSITION OF THE RAINWATER KILLIFISH (*Lucania parva*) IN A SUBTROPICAL BAY

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A throw-trap survey of the nearshore flora and fauna of Biscayne Bay, Florida, USA, was conducted in the shallow open-water area along the western shoreline of South Biscayne Bay (Matheson Hammock to Turkey Point) in 2005, in order to gain an understanding of fish and invertebrate species structure and assemblages of this habitat. The rainwater killifish (*Lucania parva*), was the most abundant species in our samples and was examined in relation to biotic and abiotic factors that might influence the distribution of this species. Individual fish were counted, weighed, and measured, while salinity, temperature, and depth were recorded at the site, and the benthic habitat was quantified on site using the Braun-Blanquet method. This survey yielded 1,990 individuals over the course of two sampling seasons that were designated as wet (August) and dry (February) seasons. Forty-seven sites were sampled each season. A length-weight relationship was generated, and density, biomass, and other abundance indices were generated. The density of *L. parva* at our sites was much higher than other reported densities for this species, and there was a clear seasonal trend in the abundance of rainwater killifish, with twice as many individuals in the wet season. The proportion of juveniles in the samples suggested that reproduction occurred at least twice a year, prior to both sampling periods. Salinity and density of *L. parva* varied inversely. Using a model developed by Diego Lirman (2007), segments of the mainland shoreline were identified as clusters having
similar salinity regimes. Density and size composition varied significantly between salinity clusters. More juveniles were observed in the wet season and in cluster 2, defined by its moderate salinity and relatively low salinity variability. Significantly fewer killifish were observed within cluster 3, a cluster characterized by a high amount of canal discharge and salinity variability. Canopy height of the seagrass was the most significant factor affecting the abundance of *L. parva*. Coverage of *Thalassia testudinum* and mixed algae also appeared to play a role in the abundance of this species. Together, canopy height and salinity formed the strongest relationship with *L. parva* abundance. Results suggest that salinity and certain habitat variables may be accurate predictors of the abundance and distribution of this species along the mainland shoreline of Biscayne Bay. There are a number or regulations already in place that protect the mangrove habitat in which *L. parva* resides. Fewer regulations address critical seagrass habitats. The Comprehensive Everglades Restoration Plan aims to correct decades of destructive modifications to the hydrology of South Florida by creating a more natural sheetflow and minimizing point source freshwater discharge into Biscayne Bay. These alterations are likely to have consequences for the flora and fauna of the Bay, but will ultimately have a positive impact on many species that reside in Biscayne Bay, including the rainwater killifish.
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Patterns of Abundance, Distribution and Size Composition of the Rainwater Killifish (*Lucania parva*) in a Subtropical Bay

INTRODUCTION

Nearshore systems, especially those composed of mangroves and shallow seagrass, play a vital role in the growth and development of many tropical and subtropical fish species. Some species may spend their entire lives in nearshore communities, while others will use these areas primarily as nursery areas until they mature and move to habitats offshore. Research suggests that the structure created by the complex mangrove root systems and dense seagrass beds creates quality foraging opportunities for fish, while also providing protection from predation (Downing 1991, Heck and Crowder 1991, Rozas and Odum 1988). Organisms inhabiting these nearshore communities must possess physiological and/or behavioral adaptations in order to cope with extreme conditions, especially fluctuations in temperature and salinity. These fluctuations can occur gradually over time (seasonal fluctuations), but they can also occur rapidly. In Biscayne Bay, the normal operation of freshwater canal locks can cause rapid and dramatic changes in salinity (Fatt 1986).

The Comprehensive Everglades Restoration Plan (CERP)

Centuries ago, many European settlers and entrepreneurs saw Florida’s Everglades as a useless swamp, too wet for development and agriculture. Most of south Florida was inundated by the natural flow of freshwater which began with the many lakes, springs and rivers in the present-day Orlando area and flowed south into the giant catch basin known as Lake Okeechobee. Historically, the lake would overflow during
the wet season, creating the main water source for the Everglades. As a result, coastal estuaries in South Florida (especially Biscayne Bay, Florida Bay, and Charlotte Harbor) received large influxes of freshwater on a regular and seasonal basis. Modification of this water flow started almost a century ago. Since then, the Army Corps of Engineers has built over 1400 miles of canals and levees in South Florida (Serafy et al. 1997). Originally designed to drain the Everglades, these canals now divert the natural flow of water for human use. The drainage of the Everglades watershed has altered natural salinity gradients and has greatly reduced or eliminated critical estuarine habitat that for organisms that require consistently low to moderate salinities. The normal operation of canal locks and other flood control structures greatly alters the amount of freshwater delivered into the bays, often resulting in rapid fluctuations between hypo- and hypersaline conditions. These freshwater pulses are and can be sudden and erratic, resulting in a high amount of physiological stress on the organisms in this area. Previous studies have documented the adverse effects that these hydrological modifications have had on nearshore organisms like pink shrimp (Browder et al. 1999) and seagrasses (Robblee et al. 1991) in Florida Bay. Additionally, at least half of the Everglades wetlands have succumbed to development, there has been a 90-95% reduction in the number of wading birds, 68 species of plants and animals have become endangered or threatened, declines in commercially important fish stocks have ensued, along with seagrass die-offs, saltwater intrusion, and excessively high levels of mercury (http://www.evergladesplan.org/about/why_restore_pt_04.aspx).

Today, the Florida Everglades is no longer viewed as a useless swamp, but as a unique and priceless ecosystem that is rapidly wasting away. In 2000, the U.S. Congress
passed the largest environmental restoration project ever attempted, the Comprehensive Everglades Restoration Plan (CERP). The main goal of the CERP is to capture freshwater that now flows unused into the ocean and redistribute the water to areas that need it most, reviving a desiccated ecosystem. The Biscayne Bay Coastal Wetlands Project (BBCWP) is a large component of the CERP, and aims to restore the natural hydrology and increase freshwater flow into the coastal wetlands and estuaries while minimizing the point source discharge of freshwater into Biscayne Bay. Spreader canals will be built in order to simulate the more natural “sheetflow” of water that used to occur in this region, rehydrating coastal wetlands and more evenly distributing freshwater across the Bay. This should create a better nursery environment, and present less physiological stress on the fish in the mangrove and seagrass habitats. This project will likely cause changes in salinity regimes, volume, timing, and location of freshwater flow, nutrient dynamics, and contaminant loads, all of which may have important consequences for the nearshore flora and fauna of Biscayne Bay. It is very important that current research focuses on the potential impacts on the fishes in this area, before, during, and after the implementation of the BBCWP and other CERP projects.

**The Rainwater Killifish**

One of the fishes that utilizes the nearshore mangrove-seagrass complex is the rainwater killifish (*Lucania parva*). A small, but abundant Cyprinodontid in Biscayne Bay, the maximum recorded length for *L. parva* is 6.2 cm total length (McEachran and Fechhelm 1998). *Lucania parva* preys on larval crustaceans and is a major predator of saltmarsh mosquito larvae (Harrington and Harrington 1961). The rainwater killifish
plays an important ecological role as a major prey item in nearshore habitats. The young of many economically important predatory fish that utilize these nearshore nurseries consume a variety of small Cyprinodontiform (killifishes) and Atheriniform (silversides) fishes, including *L. parva*. Schmidt (1989) demonstrated that in Florida Bay, the rainwater killfish and the goldspotted killifish were the two most common prey items found in the gut of juvenile great barracuda (*Sphyraena barracuda*). Rainwater killfish have also been shown to be an important prey item in brackish and freshwater ecosystems where they are consumed by largemouth bass (*Micropterus salmoides*) (Bartolini 1998).

Based on previous studies, *L. parva* appears to be one of the more abundant species in salt marsh and estuarine ecosystems in the Southeastern United States. Research has shown that *L. parva* can survive in normal seawater (Breder 1948), but typically prefers brackish waters that receive a considerable amount of freshwater on a regular basis (Hubbs and Miller 1965, Springer and Woodburn 1960, Kilby 1955). Despite the apparent preference for less saline environments, *L. parva* is rarely encountered in freshwater (Foster 1967) but may occasionally move into coastal rivers and creeks in peninsular Florida (Hubbs and Miller 1965, Loftus and Kushlan 1987). *Lucania parva* has been shown to be one of the most abundant fishes in Biscayne Bay, Florida (Barimo and Serafy 2003, Serafy et al. 1997). Studies in Florida Bay and Charlotte Harbor have also noted the abundance of *L. parva* in seagrass beds (Poulakis et al. 2003, Sogard et al. 1987). *Lucania parva* also appears to associate with submerged aquatic vegetation (SAV) in the Southeastern United States (Sogard et al. 1987, Rogers et al. 1992, Duffy and Baltz 1998). Previous research has suggested that the rainwater killfish may be able
to resist the effects of some abiotic stressors such as fluctuations in salinity (Dunson et al. 1993).

Although the presence of the rainwater killifish has been well documented in the Southeast, most of the data obtained is qualitative or minimally quantitative. Despite the abundance of the rainwater killifish in coastal bays and estuaries, relatively little is known about the population structure and habitats of these often overlooked fish. Few studies have focused on the relationships and trends of the rainwater killifish population over such a large and important area as Biscayne Bay. Alterations in water quality, the proximity of Biscayne Bay to a major metropolitan area, and the effects that these factors may be having on the rainwater killifish also makes this an interesting and worthwhile study. Additionally, data from this study can be compared with data from similar future studies, especially ones that take place after the Comprehensive Everglades Restoration Plan.

**Current Laws and Regulations**

At this time, there are no express regulations that directly protect the diminutive rainwater killifish from take or harvest. Considering the gross abundance of this species, and the fact that it is not consumed or used by humans, providing no direct economic benefit, it seems that regulations specific to this species of killifish are hardly warranted. However, the rainwater killifish is a valuable component of estuarine ecosystems where it serves as a principal prey item for a variety of piscivorous fishes that are highly regulated and very important from an economical and ecological standpoint. Though the harvest of
this species is not regulated, laws protecting mangroves and seagrasses, the principal
habitat of the rainwater killifish, do exist.

In the State of Florida, the trimming and alteration of mangrove trees is highly
regulated. The Mangrove Trimming and Preservation Act (F.S. 403.9321-403.9333) was
instituted on July 1, 1996 under Title XXIX (Public Health) and Chapter 403
(Environmental Control), and set forth regulations regarding the trimming and alteration
of mangroves within the state. The Florida Department of Environmental Protection
(FDEP) is charged with the responsibility of enforcing these regulations. This Act
prohibits the removal or alteration (alteration is defined as anything other than trimming)
of mangroves without a permit. Trimming of mangroves is highly regulated and may
also require a permit. A permit is not required for a property owner to trim mangroves
that are less than 10 ft tall, but they can only be trimmed to a minimum height of 6 ft. If
the property owner wishes to trim mangroves that are taller than 10 ft, a professional
mangrove trimmer must be used, but mangroves taller than 24 ft may not be trimmed at
all. Mangroves from 16-24 ft in height must be trimmed in stages so that no more than
25% of the foliage is removed in a single year. If the shoreline of the property exceeds
150 ft in length, no more than 65% of the mangroves on that property may be trimmed.
The use of herbicides or other chemicals on mangroves is also prohibited. Any trimming
or alteration activities that do not fall into the above categories require a state issued
permit. A violation of this act may result in a penalty of $5,000 per violation. For
subsequent violations, a charge of up to $100 per mangrove illegally trimmed, or up to
$250 per mangrove illegally altered may be assessed. In cases where more than 5% of
mangroves are destroyed or trimmed below a height of 6 ft, the property owner is usually
required to perform some manner of mangrove restoration or mitigation as laid out by section 403.9332.

Compared to mangroves, seagrasses are afforded little protection from the state government. Section 403.813 of the Florida Statute states that floating vessel platforms and boat lifts must be constructed so as to minimize the impact on adjacent seagrass beds. The Florida Fish and Wildlife Conservation Commission (FWCC) has proposed a penalty system for the damage of seagrass beds. The FWCC has recognized that propeller scarring has caused some serious damage to seagrass beds in Florida, and currently, there is little incentive for boaters to avoid these “prop-scars.” Boat sales continue to rise in Florida, especially sales of “flats boats” that are designed to travel at high speeds through very shallow water where seagrass is usually present. With the increasing population and watercraft sales, this problem is likely to become more serious without some form of regulation or management. The FWCC has proposed a system in which a monetary fine may be assessed to someone who has caused damage to the seagrass. Repeat offenders would be subject to increasing fines depending on the frequency of their offense.

The State of Florida also has adopted laws against damage to a natural resource via pollution. Currently, compensatory damages are set at $1 per square foot of mangrove or seagrass impacted by the pollution. Section 376.121 of Florida Statutes Title XXVIII (Natural Resources, Conservation, Reclamation, and Use), Chapter 376 (Pollutant Discharge Prevention and Removal), deals with liability for damage to a natural resource. Additionally, local regulations are set forth by Miami-Dade County’s Department of Environmental Resource Management (DERM) regarding the ecological
protection of the Biscayne Bay waters and habitat. Specific county regulations also set
guidelines for responsible shoreline development (F.S. § 380.19).

The Federal Government also provides for the protection of mangroves and
seagrasses. The U.S. Army Corps of Engineers (ACE) is a federal agency that has
statutory authority over the Rivers and Harbors (1899), the Clean Water Act, and the
Marine Protection Research and Sanctuaries Act (1972). There are also many ACE
regulations regarding dumping and dredging in navigable waters [33 CFR 320-331]. In
1996, the U.S. Congress amended the Magnuson-Stevens Fishery Conservation and
Management Act with the Sustainable Fisheries Act (SFA) [P.L. 104.297, Oct. 11, 1996]
to include the need to protect fish habitat. This Act requires fishery management
councils to identify areas necessary for a fish species to perform basic life functions as
Essential Fish Habitat (EFH) [16 U.S.C. §1855(b)]. In the event that an agency action
might adversely impact EFH, the agency must consult with the National Marine Fisheries
Service (NMFS) that subsequently issues conservation recommendations to the agency in
an attempt to remedy the situation [16 U.S.C. §1855(b)(2)]. South Florida mangroves
and seagrass beds constitute Essential Fish Habitat for a variety of economically
important species and are therefore protected under the Sustainable Fisheries Act (Final
Habitat Plan for the South Atlantic Region: Essential Fish Habitat Requirements for

A large portion of Biscayne Bay, and the majority of the study site for this thesis
report, is located within Biscayne National Park. The National Park Service, as a
government agency, has the power and duty to provide additional protection to the
organisms and habitats that reside within the park. Biscayne National Park must abide by
and enforce a variety of laws including the Endangered Species Act (ESA), the National Environmental Policy Act (NEPA), and the Clean Water Act. Title 36 of the Code of Federal Regulation (CFR) provides a listing of the rules within all national parks.

Fishing within Biscayne National Park is regulated by Florida State Law [16 U.S.C. §410gg-2(a)]. A fisheries management plan has been developed for the park with the combined efforts of Biscayne National Park, the FWC, and members of other government agencies, universities, and the public.

This study is designed to identify the major determinants of distribution patterns and trends of the *L. parva* population along the western shoreline of Biscayne Bay. The major objectives of this study are to determine the abundance, distribution, and size composition of *L. parva* in the nearshore environment of Biscayne Bay in relations to salinity regime and habitat variables. The null hypothesis is that there will be no change in the density or size composition of *L. parva* across seasons, among locations within the Bay, across salinity gradients, or between habitat variables.

**METHODS**

**Site Selection**

The study area is western shoreline of Biscayne Bay from around Matheson Hammock Marina to Turkey Point (Fig. 1). This is about a 16-mile stretch of shoreline that is dominated by red mangrove (*Rhizophora mangle*) and to a lesser extent, black mangrove (*Avicennia germinans*). The nearshore benthos is composed of mostly sand or mud and variously covered with a variety of algae and seagrass species. A stratified random sampling approach was used to locate 47 sampling sites along the shoreline in
Shallow water that is less than 1m deep and near the mangrove-seagrass interface. Most of the study area is located within Biscayne National Park, which has few restrictions in terms of permitting human use.

**Sampling Gear and Specimen Collection in the Field**

The 2005 calendar year was broken into a dry season and a wet season. Dry season sampling took place in February, while wet season sampling took place in August. During each of these two seasons, sampling was done over a 2-week period. The throw-trap was the gear of choice for specimen collection. Throw-traps have been found to be an effective means of catching small nekton and benthic organisms and can generate reliable quantitative data, particularly in shallow estuarine environments (Robblee et al. 1991, Rozas and Minello 1997, Jordan et al. 1997). Our throw-trap samples a 1-m² area and can be used effectively in depths of a few centimeters up to 3 meters. The throw-trap consists of an open-ended aluminum box measuring 1-m² by 45cm deep, with two curtains of nylon netting (3-mm stretch mesh) attached on parallel edges that are designed to cover the top of the trap. At each predetermined sampling site the throw-trap was deployed randomly three times per site. The heavy trap sinks into the soft sediment, preventing any animals from escaping out the bottom while the mesh curtain nets prevent escape out the top of the trap. Sampling was done on or near the high tide. Each time the trap was thrown it was swept by a series of four bar seines (3mm mesh) in an attempt to catch all the living animals within the trap. Browder et al. (2005) determined that four sweeps of the bar seine was sufficient to catch 95% of the animals in the trap. Samples were rinsed through a sieve on the boat and then tagged, bagged and placed in a large
freezer upon return to the laboratory. Depth, temperature, and salinity were also recorded at each site using a YSI multi-probe instrument.

**Benthic Habitat Quantification (Braun-Blanquet method) in the Field**

In addition to the throw-trap sampling, a visual survey of benthic habitat was conducted at each site. Benthic flora was quantitatively assessed using the Braun-Blanquet method as outlined by Fourquean et al. (2002). This method involved throwing a 0.25-m² quadrat in close proximity to the throw-trap while trained observers snorkeled or used a glass-bottom bucket to examine the quadrat. The quadrat was thrown three times per site and numerical values were assigned that quantified the percent coverage of each taxon of submerged aquatic vegetation present within the quadrat. Each species received a numerical value of 0.1 (solitary individual), 0.5 (sparse), 1 (0-5% cover), 2 (5-25% cover), 3 (25-50% cover), 4 (50-75% cover), or 5 (75-100% cover). In addition, the bottom composition and the canopy height of the seagrass were measured. Using these assigned values, relative abundance, distribution, and percent cover are all accurately estimated.

**Specimen Processing and Data Entry**

In the laboratory, each sample was removed from the freezer and thawed overnight. The samples were then meticulously sorted with tweezers to insure that all fish, shrimp, carideans, and crabs were removed. A bulk weight was taken and then individual fish were identified to the species level, weighed (to the nearest 0.01g) and measured (standard length) to the nearest millimeter. Data was recorded by hand in a
data book as well as our computerized database. The animals were fixed in formalin (10% buffered) for 3 days, and then placed in ethyl alcohol for preservation. Though 23 different species of fish were caught using the shallow water throw-trap technique, the rainwater killifish (*Lucania parva*) was the most abundant species in our samples.

**Study Design and Data Analysis**

In order to provide more useful abundance and spatial distribution data, the sampling sites were grouped into clusters by salinity regime. Using 2005 data and the Biscayne Bay salinity and hydrodynamic model (Wang et al. 2003), Lirman et al. (in press) grouped the mainland shoreline of Biscayne Bay into 4 clusters that that represented distinct salinity regimes (Figure 1). Grouping by Lirman’s salinity regime clusters provides a logical framework for correlation analyses in Biscayne Bay, especially when salinity is a key variable. This technique may prove more useful than assessments by more arbitrary means (i.e. north-south gradients). All of our sites fell within Lirman’s 4 salinity clusters. In our study, we will use 3 salinity clusters called Cluster 1, Cluster 2 and Cluster 3. These Clusters correspond to Lirman’s Cluster 2, Cluster 3, and Cluster 4 accordingly. Clusters 1, 2, and 3 each contained 15 sampling sites (Table 1). Cluster 1 encompasses the northern section of our sites (Matheson Hammock to just north of Black Point) and is characterized by relatively high mean salinity (dry: 32.4, wet: 26.6). Cluster 2 consists of a small area immediately north of Black Point jetty but also includes the southern most section of our sites, from Convoy Point to Turkey Point. Cluster 2 is characterized by an intermediate mean salinity (dry: 30.0, wet: 22.9). Cluster 3 represents the area between the two segments of cluster 2, from Black Point jetty south to
Convoy Point. Cluster 3 is an area that is greatly influenced by canal discharge and therefore, has the lowest mean salinity of all the clusters (dry: 27.0, wet: 17.1) but also the highest salinity variability. Overall, analyses were done on 45 sites spread out over clusters 1, 2, and 3.

The data was natural-log-transformed and specific patterns of abundance, distribution, and size composition were examined between seasons and between salinity clusters. Significance of abundance and biomass relationships with salinity and habitat were tested by linear regression. Multiple regression analysis was used to determine the correlation of rainwater killifish density with several variables. Density differences between salinity clusters were determined using an analysis of variance. Size-frequency plots were constructed to compare the size composition between seasons and between salinity clusters. The Kolmogorov-Smirnov test was used to determine significant differences in size composition between clusters and between seasons. For all statistical tests, significance was declared at the $\alpha = 0.1$ level. Alpha values of 0.05 were considered even more significant, and $\alpha = 0.01$ was considered highly significant.

RESULTS

The total number of rainwater killifish caught in 2005 was 1,990 individuals, with a marked difference in gross abundance between the two seasons. The dry season consisted of 654 individuals or 33% of the total catch, while the wet season was represented by 1,336 individuals consisting of 67% of the total catch. The total biomass of rainwater killifish also differed between seasons, but to a lesser degree, with 146.73g (44%) of fish being caught in the dry season and 189.06g (56%) in the wet season.
Rainwater killifish were found at 83 (88%) of the 94 total sites sampled in 2005. The frequency of occurrence was higher in the wet season where 44 of 47 sites (94%) were positive for rainwater killifish, than in the dry season, where 39 of 47 sites (83%) were positive for this species (Fig. 1). The density of *L. parva* was high throughout the year, but was much higher in the wet season (9.48 fish/m²) than the dry season (4.64 fish/m²). A length-weight plot for *L. parva* resulted in a tight fit to an exponentially increasing curve with $R^2=0.76$ (Fig. 2).

Rainwater Killifish abundances and biomass were pooled across seasons and shown to vary inversely with salinity ($p<0.05$ for abundance, $p<0.1$ for biomass) (Fig. 3). Analysis of variance indicated that significant differences in mean density per site and mean total biomass per site existed between clusters (Fig.4A-D). In the dry season, the mean density of *L. parva* was significantly greater in cluster 2 than cluster 3 ($p<0.001$) and cluster 1 ($p<0.01$). There was no significant difference in the density between clusters 1 and 3. The mean biomass was also significantly greater in cluster 2 than in cluster 3 ($p<0.01$) and cluster 1 ($p<0.1$) in the dry season. Biomass was also not found to be significantly different between clusters 1 and 3. During the wet season, the mean density in cluster 2 was again significantly greater than cluster 3 ($p<0.05$), but there was no significant difference between clusters 1 and 2 or 1 and 3. The mean biomass during the wet season followed the same trend, with cluster 2 being significantly greater than cluster 3 ($p<0.05$) with no other significant difference between the remaining clusters.

Length-frequency plots were constructed to evaluate potential differences in size composition. There was a highly significant difference in size composition between the wet and dry seasons. Within the dry season, the difference in size composition was
highly significant between clusters 1 and 2, and between clusters 2 and 3 ($\alpha = 0.01$, Fig. 5A). There was no significant difference between clusters 1 and 3. In the wet season, the exact same pattern was observed, with highly significant results between clusters 1 and 2, and clusters 2 and 3, and no significant difference between clusters 1 and 3 ($\alpha = 0.01$, Fig. 5B).

Four microhabitat variables (canopy height, *Thalassia testudinum* coverage, *Halodule wrightii* coverage, and mixed algal coverage) were tested for correlation with rainwater killifish abundance metrics (Fig. 6A-D) over both seasons. Of these four habitat components, canopy height represented the most significant positive correlation with abundance based on linear regression ($r^2=0.19$, $p<0.00001$). *Thalassia* coverage and mixed algal coverage also demonstrated significant, although weaker, positive correlations with abundance ($p<0.1$). There appeared to be no significant correlation between *Halodule* coverage and the abundance of *L. parva*. The habitat data was then broken down by season, and showed no obvious relationships during the dry season. During the wet season, the strongest correlation with a single variable was also with canopy height ($r^2=.24$, $p<0.05$). The addition of salinity to the equation improved this relationship so that nearly 30% ($r^2=0.29$) of the variation in *L. parva* density could be explained by the combined effect of canopy height and salinity ($p<0.1$). The following equation was generated for this relationship ($\ln(RK) = \log$-transformed density values, $CH$ = canopy height, $SAL$ = salinity):

$$\ln(RK) = 1.81 + (0.10)CH + (-0.04)SAL$$

$$r^2 = 0.29$$
DISCUSSION

The rainwater killifish (*Lucania parva*) is clearly a very abundant fish along the western shoreline of Biscayne Bay. Despite the relatively high densities of this species, very little is known about the distribution, habitat preference, and impacts of biotic and abiotic variables on *L. parva* over such a large and important area as Biscayne Bay. Salinity regime plays an important role in shaping the community structure of South Florida’s bays and estuaries, and determining its effect on the abundance and distribution patterns of rainwater killifish was one of the principal goals of this investigation.

The western shoreline of Biscayne Bay has particularly high densities of rainwater killifish. Previous studies in different locations reported densities ranging from 0.37-0.97 fish/m² in Charlotte Harbor, Florida (Poulakis et al. 2003), 1.3 fish/m² in St. Johns River Estuary, Florida (Jordan 2002), 0.117-2.519 fish/m² in Lake Pontchartrain Estuary, Louisiana (Duffy and Baltz 1998), 0.008-0.053 fish/m² in Florida Bay (Thayer et al. 1999), 0.31-2.11 fish/m² in another Florida Bay study (Sogard et al. 1987), and 0.5-69.0 fish/m² in Matagorda Bay, Texas (Gelwick et al. 2001). With the exception of the last study, the densities of *L. parva* in various estuaries of the Southeastern United States have ranged from 0-2.5 fish/m². Our study reports some of the highest densities ever recorded for *Lucania parva*, ranging from 4.64-9.48 fish/m². The large difference in rainwater killifish densities between our study and previous studies could be due, in part, to sampling method. For example, throw-trapping is a much more effective method of catching all the small, epibenthic fish in a given area than seining or trawling. However, both the Sogard et al. study (1987) and the Jordan study (2002) took place in other Floridian estuaries using a nearly identical throw trapping technique in similar habitat,
and only obtained a maximum of 2.11 fish/m². Our catch densities of *L. parva* are 2-3 times higher than those using the same method of capture. This suggests that Biscayne Bay may possess an unusually high number of rainwater killifish. It is plausible that the high density of rainwater killifish in Biscayne Bay may be a result of a particularly abundant food source or preferred habitat, or the result of decreasing abundances of fish that prey on *L. parva*. With the close proximity of Biscayne Bay to the megalopolis of Miami and a huge fishing industry, a reduction in the amount of predators in this estuary seems to be one likely explanation for the exceptionally high densities of *Lucania parva*. Future studies should attempt to determine which of these factors is likely to be playing a role in the high abundance of rainwater killifish in Biscayne Bay. An investigation to determine if the abundances of other forage fish (i.e. Atheriniformes) are exceptionally high may provide further insight into the decreased predation theory. Additional studies investigating the predation on *L. parva*, perhaps more gut content analysis studies done on other predatory mangrove fishes, would further confirm the importance of the rainwater killifish as a principal prey item in estuarine food webs.

In addition to the very high densities of *L. parva* in Biscayne Bay, this study also demonstrates a clear seasonal trend in abundance. The same amount of sampling effort in roughly the same locations resulted in a wet season catch per unit effort (CPUE) of more than double the CPUE of the dry season. Also, the total overall catch, total biomass, and frequency of occurrence were all greater in the wet season. This result is consistent with the aforementioned species abundance study in Charlotte Harbor, a similar estuarine environment on the West Coast of South Florida. In this study, Poulakis et al. (2003) also observed *L. parva* to be more abundant in the mangroves and on
seagrass flats during the wet season. Though our results are supported by this and other studies, the comparison of seasonal abundances across several years would help to build a stronger case for the seasonality of this species.

This field study demonstrates that densities of *L. parva* in Biscayne Bay appear to increase with decreasing salinity. It is important to note that this correlation with salinity was done over pooled seasons. This may not be the best way to interpret these results because a significant difference in *L. parva* abundance exists between seasons. However, when the relationship between density and salinity was examined during each season, the sample size for the higher salinity bins was too small (n = 2 sites per salinity bin) to provide conclusive results. Despite this assertion, gross abundances across the salinity bins seem to indicate that more rainwater killifish can be found at lower salinities. This inverse relationship between density and salinity confirms the claims of several early studies (Hubbs and Miller 1965, Springer and Woodburn 1960, Kilby 1955) that suggested that *L. parva* prefers brackish waters with a considerable amount of freshwater influx. Interestingly, recent data from Browder et al. (2007) showed a positive relationship between density and salinity for a Florida Bay population of rainwater killifish. This result might be attributed to the different salinity regimes between Biscayne Bay and Johnson Key Basin in western Florida Bay, where the samples were collected (by M. Robblee, USGS, Center for Water and Restoration Studies, Ft. Lauderdale, FL). Perhaps in such an area where salinity is seldom less than 30 ppt and is less variable than along the shoreline of South Biscayne Bay, habitat indices might serve as better predictors of rainwater killifish densities.
Grouping our sampling sites based on Lirman et al.’s (in press) salinity cluster analysis of this exact same region allowed us to examine *L. parva* densities in reference to overall salinity trends within the Bay. The results showed that the greatest mean density (per site) of rainwater killifish can be found in cluster 2, which lies in the southern portion of the Bay and is characterized by a somewhat intermediate mean salinity and variability. Despite the rainwater killifish’s apparent preference for freshwater, the lowest densities of *L. parva* were seen in cluster 3, the area that is most heavily influenced by freshwater canal discharge, resulting in low mean salinities and very high salinity variability. This suggests that while *L. parva* may be well adapted to life in hyposaline and brackish waters, extreme and rapid fluctuations in salinity, such as those that occur with the normal operation of canal locks in South Biscayne Bay, may present a significant amount of stress on the osmoregulatory capabilities of the rainwater killifish. These drastic man-made fluctuations might be contributing to the consistently low densities of rainwater killifish in the cluster 3 area. Further evidence for this theory can be found in a laboratory experiment designed by Serafy et al. (1997). In this study, various fishes from the nearshore waters of Biscayne Bay were collected, placed in holding tanks, and after a period of acclimation (30-35 ppt), they were subjected to a 2-hour pulse of freshwater to simulate the effects of a canal discharge event. Serafy found that when exposed to a freshwater pulse causing a rapid and dramatic salinity change, *Lucania parva* exhibited 50% mortality. A field study that paralleled Serafy’s salinity challenge experiment also showed that significantly fewer rainwater killifish were present at canal-influenced sites (Serafy et al. 1997). It is important to note that based on the current study, salinity appears to be an accurate predictor or indicator of *L. parva*
densities in western Biscayne Bay. This does not necessarily suggest that changing salinities are the proximate cause of the variable densities of rainwater killifish. Future research might attempt to confirm the results of this study, and Serafy’s salinity challenge experiment, by observing fish abundances in a canal discharge zone before and after a large freshwater discharge event. Other studies on *Lucania parva* in Biscayne Bay should focus on the physiological constraints and the osmoregulatory capabilities of rainwater killifish. Controlled laboratory experiments designed to test an optimal salinity range, and the results of a gradual salinity change, would also be useful. Future field studies should include other abiotic parameters such as additional water quality measures (dissolved oxygen levels, turbidity, pH, etc.).

Size composition was shown to vary by season, where the wet season was represented by a significantly larger amount of juveniles. This seasonal variation in size could represent a peak in breeding activity during, or just prior to the wet season. Significant differences were also observed between salinity clusters (with the exception of clusters 1 and 3). The data suggest that clusters 1 and 3 are composed of similar sized killifish, while the size composition of cluster 2 differs from that of the other two clusters. By looking at Figure 5, it is apparent that cluster 2 consists of a large number of juvenile fish. Perhaps there is some component (biotic or abiotic) of the water quality or habitat type in cluster 2 that makes it a more desirable nursery area. The underlying reason for the difference in size composition across these salinity clusters is not entirely clear. Future studies should address other potential causes (i.e. quality of habitat, presence/absence of predation) of the marked differences in size composition within specific regions of Biscayne Bay.
Benthic habitat composition is often a key variable when attempting to determine the various factors associated with fish densities. The Braun-Blanquet technique used in this study allowed us to quantitatively assess the benthic vegetation on the microhabitat level. There was a highly significant correlation between *L. parva* density and the canopy height of the seagrass. This result is probably due to the fact that a higher seagrass canopy is likely to provide more protection from predation and a greater surface area, allowing for the attachment of more epiphytic prey items. In addition to the potential increase in prey items, the greater surface area provided by a thick seagrass bed with a high canopy may also allow for the protection and adhesion of the sticky demersal eggs that are released by spawning females. A moderate correlation between density and *Thalassia* coverage (as well as mixed algal coverage) could also be a result of these predation and protection benefits provided by the grass and algae. Using a totally different method of capture, Robblee (1988) was also able to show that canopy height and topographic relief were very important factors affecting fish abundance in the shallow seagrass beds of Florida Bay. Similar habitat-influenced abundance metrics can be observed in other marine communities, such as coral reefs, where high measures of topographic relief and rugosity often result in higher fish abundances and species richness (Wilson 2007). Presumably, *Halodule* would also provide similar protection and opportunities for predation; however, there was no correlation between *L. parva* density and *Halodule* coverage. The lack of correlation with *Halodule* might be attributed to the location of this seagrass within the Bay. *Halodule wrightii* is a freshwater tolerant grass that is usually observed in close proximity to a freshwater source, like the canal mouths along the mainland shoreline of Biscayne Bay. The extreme fluctuations in salinity
caused by the normal operation of canal locks may override any benefit afforded to the killifish by the *Halodule*, possibly resulting in lower densities of rainwater killifish in *Halodule* beds. Further research is needed to determine the validity of this theory, and to address why *Halodule* is not as important to *Lucania parva* as *Thalassia* or mixed algae.

**Predicted Changes in the Rainwater Killifish Population and Habitat as a Result of CERP**

The Comprehensive Everglades Restoration Project is likely to have a positive impact on *Lucania parva*. The rainwater killifish already appears to be doing very well in Biscayne Bay, and the proposed modifications involved with the CERP may result in even better conditions for this species. Restoring the sheetflow of freshwater should result in a more stable estuarine system that lacks the high salinity variability that is currently seen in the Bay. The rainwater killifish is an estuarine fish that prefers brackish waters which experience some regular freshwater influx (Hubbs and Miller 1965, Springer and Woodburn 1960, Kilby 1955). The current challenge for *L. parva* in Biscayne Bay is the physiological stress that results from rapid salinity fluctuations as a result of canal discharge. Serafy et al. (1997) demonstrated this in his salinity challenge experiment by showing 50% mortality in *L. parva* when they were subjected to a rapid freshwater pulse. A concurrent study revealed a relative absence of this species around canal mouths, providing further evidence for this theory (Serafy et al. 1997). This thesis study confirms lower densities of *L. parva* in a portion of the Bay that is most heavily influenced by freshwater discharge and experiences large and rapid salinity fluctuations. Reducing the high salinity variability and creating a more uniform sheetflow of freshwater into the Bay is likely to positively affect the abundance and distribution of the
rainwater killifish by resulting in a more uniform distribution throughout the Bay, instead of the somewhat patchy distribution that is currently seen.

There is also the potential for habitat alteration as a result of the proposed changes in the CERP. The seafloor of Biscayne Bay is currently dominated by *Thalassia testudinum*, a seagrass that does very well in saline waters. *Halodule wrightii* is a freshwater tolerant seagrass that is seen in isolated patches in Biscayne Bay, usually in the vicinity of canal mouths (Lirman and Cropper 2003). A more natural sheetflow of freshwater should result in an increase in the abundance and distribution of freshwater tolerant seagrasses like *Halodule wrightii* and *Ruppia maritima* and a decrease in *Thalassia testudinum* (Fourqurean et al. 2003). Lirman and Cropper (2003) also suggest that a hypothetical restoration scenario could lower mean salinity values enough to allow *Halodule* to outcompete *Thalassia*. This thesis study demonstrates no correlation between the *Lucania parva* abundance and *Halodule wrightii*. It was hypothesized that the apparent lack of rainwater killifish in beds of *Halodule* may be a result of the proximity of these seagrass beds to canal mouths, or point sources for rapid freshwater discharge, rather than the quality of the *Halodule* as habitat for *L. parva*. With the more natural sheetflow of freshwater, these *Halodule* beds may become a more desirable habitat for a variety of fishes because they will no longer be subject to the poor water quality and high salinity variability that they currently experience in the Bay. A less common species of seagrass in Biscayne Bay, *Syringodium filiforme*, appears to do well at intermediate salinities (25 ppt) (Lirman and Cropper 2003). After the restoration of freshwater sheetflow, theses intermediate salinities may become more prevalent at certain
locations within the bay, encouraging the growth of *Syringodium filiforme*, thus allowing for greater habitat heterogeneity, which usually results in higher species diversity. Mangrove forests may also benefit from a more uniform freshwater flow into the Bay. Though species such as the red mangrove (*Rhizophora mangle*) are well adapted to life in seawater, faster growth is exhibited in less saline waters. One particular study collected *Rhizophora mangle* propagules from 2 sites in Biscayne Bay and grew them in high salinity (36 ppt) and low salinity (5 ppt) treatments. The results of this study suggest that regardless of the salinity where the parent plant was located, the mangrove seedlings demonstrated much faster growth in the low salinity treatments (Smith and Snedaker 1995). Restoring the natural sheetflow aims to evenly distribute freshwater across the Bay, insuring that the waters surrounding mangroves maintain a more stable, lower salinity, resulting in faster growth of red mangroves, which should result in better nursery habitat for fish. This restoration plan is also designed to create conditions that are favorable for the re-establishment of oysters and oyster reef communities. The return of these filter feeders to Biscayne Bay should contribute to an increase in water quality in these critical nursery areas. The effects of the CERP on the nearshore habitat and water quality in these nursery areas are likely to benefit animals in the entire estuarine food web, not just *Lucania parva*.

**Recommendations for the Management and Continued Protection of the Fishes and Nearshore Habitat of Biscayne Bay**

Preserving a community by protecting the rainwater killifish population follows a “grass-roots” approach to ecosystem management. This means that the protection of prey items near the bottom of the food web will contribute to the growth and abundance of
economically important species near the top of the trophic guild by providing a constant and plentiful source of food. Unlike many other fish species in South Florida, rainwater killifish populations are not threatened by overfishing. The real threat to this species comes from habitat degradation, pollution, and the altered hydrology of South Florida. For these reasons, direct regulations governing the take of rainwater killifish are not appropriate and will result in little to no benefit to this species. Instead, the management of the rainwater killifish should be taken into consideration along with the management of other fishes in the nearshore areas of Biscayne Bay in a community-based or ecosystem-based management plan. Protection and preservation of nearshore mangrove and seagrass habitats will benefit a variety of species that use these areas, including the rainwater killifish.

Adequate measures protecting mangroves in Florida are already in place. Seagrass communities receive less protection than mangroves, but still constitute Essential Fish Habitat. A penalty system for damage to seagrass beds, such as the one proposed by the FWCC, may provide an additional measure of protection for this habitat. The major factor affecting the nearshore habitat of Biscayne Bay is the altered freshwater flow and nutrient loads that result from canal discharge into the Bay. The flow of water shapes and controls all of South Florida’s ecosystems. In order for smaller scale management measures to be effective, the primary concern – the flow of freshwater into Biscayne Bay – must be addressed first.

Undoing more than a century worth of destructive modifications to the natural hydrology of South Florida is a massive task that some might consider to be prohibitively expensive and time consuming. Despite the difficulties associated with a project of this
scale, the Comprehensive Everglades Restoration Plan has laid the foundation for this ambitious, precedent-setting, ecosystem restoration effort. The CERP is the ultimate ecosystem-based management plan. If the BBCWP and the CERP are successful at restoring the freshwater input to a more natural sheetflow and minimizing point source freshwater discharge, we can expect to see improved water quality, the return of oyster reefs, enhanced nursery habitat for a variety of fishes, and improved shrimp and finfish fisheries in Biscayne Bay. The benefits of small scale management measures (i.e. species-specific fisheries management plans, seagrass and mangrove regulations, etc.) will only become apparent once the natural freshwater flow to Biscayne Bay is restored. Without the CERP, the benefits of other management measures will be marginal and temporary at best.

The Comprehensive Everglades Restoration Plan is a huge leap in the right direction for the environmental planning and ecosystem management of South Florida. While it is a promising solution to a major environmental problem, it is certainly not a quick fix. There is a need for multi-agency, long term monitoring studies to determine the impact that these changes might have on the flora and fauna of Biscayne Bay, and to evaluate the success of the CERP. This study has provided evidence that rainwater killifish distributions are affected by freshwater flow and salinity variation in Biscayne Bay. For this reason, *Lucania parva* is an excellent model species for monitoring changes in water quality along the western shoreline of Biscayne Bay, during and after the implementation of the CERP. The recommendations outlined by the Biscayne Bay Coastal Wetlands Project and the Comprehensive Everglades Restoration Plan, with support from multiple government agencies, should re-establish Biscayne Bay as a
productive estuary, critical to the growth, survival, and reproduction of hundreds of ecologically and economically important species.

Biscayne Bay is an ever changing estuarine ecosystem that is subjected to a variety of anthropogenic impacts. The Comprehensive Everglades Restoration Plan (CERP) will alter the hydrology of South Florida and Biscayne Bay in the next decade. Based on the results of this study, restored freshwater flow to Biscayne Bay is likely to have little negative effect on *Lucania parva*. In fact, a return to a state where salinity fluctuations are gradual and seasonal, with less variability, is probably going to contribute to a robust population with a more uniform distribution along the mainland shoreline of the Bay. As a small forage fish in a volatile nearshore community, the rainwater killifish population may serve as an indicator of the health of this estuarine system. Continued monitoring of water quality, habitat, and abundances of nearshore fishes like *Lucania parva* is an important way to measure the impact that CERP and other anthropogenic changes may have on the flora and fauna of Biscayne Bay.
Figure 1. Study area, sampling sites, salinity clusters, and occurrence of *Lucania parva* in the Dry (A) and Wet (B).
<table>
<thead>
<tr>
<th>Salinity Cluster</th>
<th>Dry 2005</th>
<th>Wet 2005</th>
<th>Total</th>
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<tr>
<td>1</td>
<td>15</td>
<td>15</td>
<td>30</td>
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<tr>
<td>2</td>
<td>15</td>
<td>15</td>
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<tr>
<td>3</td>
<td>15</td>
<td>15</td>
<td>30</td>
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<tr>
<td>Total</td>
<td>47*</td>
<td>47*</td>
<td>94*</td>
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*Table 1*: Distribution of sampling effort within salinity clusters. *2 sites in each season fell outside of the clustered areas and were therefore omitted from the cluster analysis.
Length-Weight Relationship for *Lucania parva*

\[ y = 0.0093x^{3.7391} \]

\[ R^2 = 0.76 \]

**Figure 2**: Length-Weight Relationship for *Lucania parva* based on the measurements of 1,990 individuals.
Figure 3: Abundance (Solid squares, solid line) and biomass (open squares, broken line) of *Lucania parva* as they relate to mean salinity. Abundances and biomass are ln-transformed and pooled across seasons. Vertical lines on either side of the points indicate 1 SE.
Figure 4: Mean density of *L. parva* per site in the dry season (A) and the wet season (C) and the mean total biomass per site during the dry season (B) and wet season (D). Means sharing the same lower case letter are not statistically different. Vertical lines above the bars indicate 1 SE.
Figure 5: Size composition by salinity cluster in the dry season (A) and the wet season (B). Cluster 1 is represented by the broken line, cluster 2 by the thick line, and cluster 3 by the thin line. In both plots, the D- and P-values indicate Kolmogorov-Smirnov two sample test results: $D_{x,y}$ = maximum difference between cumulative size frequency distributions of clusters $x$ and $y$; $P$ = probability of obtaining the observed difference by chance alone. The vertical line (with arrows) represents the minimum size at which *Lucania parva* reaches sexual maturity, obtained from the literature.
Figure 6. Habitat correlations with abundance of *Lucania parva* (ln transformed). Panels represent the correlation between abundance and canopy height (A), *Thalassia* coverage (B), *Halodule* coverage (C), and Mixed algal coverage (D).


VITA

Joseph Armindo Tomoleoni was born in Chicago, Illinois on August 29, 1983. His parents are Richard Tomoleoni and Linda Tomoleoni. He received his elementary education at Culver Elementary School and his secondary education at Niles West High School. In August 2001 he entered the College of Arts and Sciences at the University of Miami from which he graduated with a BS degree (Marine Science and Biology) in May 2005. In August 2005 he was admitted to the Rosenstiel School of Marine and Atmospheric Science at the University of Miami, where he was granted a MS degree in December 2007. While attending graduate school, he worked as a research assistant for the Southeast Fisheries Science Center of the National Marine Fisheries Service.

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