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DO ALTERED COASTAL HABITATS PROMOTE NON-NATIVE FISH INVASIONS INTO THE CHARLOTTE HARBOR ESTUARY?

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ABSTRACT
Non-native species and habitat alteration were the top causes of species extinction in North America in the 20th century. As coastal urban development spreads, the ecological and economic impacts of non-native fish species is expected to increase, and documenting their spread is the first step in determining their potential impacts. Native to Central America and first documented in Florida in 1983 in Everglades National Park, Mayan cichlids (*Cichlasoma urophthalmus*) are now established in freshwater and some estuarine habitats of South Florida. Mayan cichlids were first recorded in July 2003 in southwest Florida's Charlotte Harbor mangrove creeks, and have been captured in 12 of 13 sample months since. Fish were captured in the same salinity and temperature ranges as in their native range; fish size was related to salinity and temperature, with small fish (< 40 mm SL) most common in < 7ppt, and fish of all sizes mostly in < 25ppt. Highest abundances occurred in creeks with altered upland habitats. With no spawning behavior observed in the creeks, and small fish present only in high freshwater flow periods, spawning likely occurs in upland freshwater habitats. Tag-recapture data indicate limited movement and only moderate residence time in creek habitats by adults, further supporting colonization from upland locations.

INTRODUCTION

There are more than 50,000 non-native species in the United States which cause major environmental damage totaling approximately $137 billion annually (Pimentel et al. 2000), and the proliferation of these species is enhanced by habitat alterations (Ross 1991, Courtenay and Williams 1992). Invasions of the Gulf of Mexico by non-native species are increasingly common, and potentially problematic. The mild temperatures, low salinities and altered habitats make Gulf of Mexico ecosystems more vulnerable to invasion by non-native fishes than many other parts of the nation (Fuller et al. 1999, Peterson et al. in revision a, Shafland 1996).

Non-native species and habitat alteration were the top causes of species extinction in North America in the 20th century (Vitousek et al. 1997). Since habitat alteration enhances the proliferation of non-native species (Courtenay and Williams 1992, Shafland 1996), we can expect that as human population increases and coastal urban development spreads, the ecological and economic impacts of non-native fish species in estuarine habitats will also increase. Thus far, the rate of invasions by non-native species has increased concurrent with coastal development, heightening the threat to native species and native fishery resources. Controlling the spread of non-native species and limiting their effect on native species and fisheries productivity are now national priorities (GAO 2000, NISC 2001).

Fishes in the family Cichlidae (a nominally freshwater family) contribute more invasive species to North America than any other fish family, especially in Florida, where 13 of 18 invasive fishes are cichlids (Shafland 1996). A recent alarming development across the Gulf of Mexico is the occurrence of cichlid species in commercially and recreationally valuable estuarine habitats (O'Connell et al. 2002, Peterson et al. in revision a, in revision b, Loftus 1987, Faunce et al.2002, Bergmann and Motta 2004). The environmental plasticity of cichlids makes them especially able-bodied invaders. This paper reports the discovery and continuing occurrence of the exotic Mayan cichlid, *Cichlasoma urophthalmus* in mangrove creeks of Charlotte Harbor, Florida, with estimates of size structure and persistence.

MATERIALS AND METHODS

Study System

Charlotte Harbor is a sub-tropical coastal plain estuary on the southwest coast of Florida (Figure 1), and is one of the largest estuaries in the state. Three rivers and numerous small creeks transport large amounts of freshwater into the estuary, primarily during the wet season (June – September). The estuary is connected to the Gulf of Mexico through numerous natural passes. The climate is subtropical, mean water temperature ranges from 12^{o} to 36^{o} C, and freezes are infrequent (Poulakis et al. 2003). Approximately 80% of Charlotte Harbor's mangrove fringe shoreline (red mangrove, *Rhizophora mangle*; black mangrove, *Avicennia germinans*) remains under protection (R. Repenning, Punta Gorda, FL, Department of Environmental Protection, pers. comm.; Hammett 1990).

Despite shoreline protections, Charlotte Harbor's drainage has been drastically altered for development, water management, and mosquito control, as has occurred along much of Florida's southern coastline. The historical hydrologic conditions were characteristic of coastal uplands surrounding Southwest Florida estuarine systems: gentle, low, flat slopes provided for slow overland sheet flow of surface waters, which were retained for extended periods in open palustrine wetland depressions within wet and mesic slash pine flatwoods during wet season, but were dry during dry season. These isolated wetlands were connected during periods of high water by wet prairies, hydric hammocks, and sloughs that conveyed surficial flows to oligohaline and mesohaline marshes at the upper reaches of the tidal estuarine system. In areas with upstream drainage basins, these surficial flows were concentrated into tidal creek systems that provided an important salinity and water chemistry gradient to the estuary. These conditions were significantly altered in the early 1950's by agriculture and mosquito ditches, and even more so by recent increases in residential development.

Focal Species

Cichlasoma urophthalmus is native to the Atlantic continental slope of Central America (Miller 1966). The species is euryhaline (0 – 37ppt), omnivorous (fishes, invertebrates, algae, detritus), tolerant of temperatures from 15 - 37°C, and capable of spawning in low and high salinities (Morales-Gomez et al. In Review, Loftus 1987, Faunce et al 2002, Bergmann and Motta 2004). Like many cichlids, *C. urophthalmus* shows considerable plasticity in ecological, biological, and physiological characteristics, allowing exotic populations to depart from characteristics considered standard in the native range (e.g., Morales-Gomez et al. In Review, Loftus 1987, Faunce et al. 2002, Bergmann and Motta 2004, Martinez-Palacios et al. 1990, Martinez-Palacios and Ross 1992), thus increasing their invasive potential. In Florida, *C. urophthalmus* was first documented in 1983, in Everglades National Park (Loftus 1987), and is now considered established in freshwater and some estuarine habitats of South Florida (Shafland 1996). In July 2003, and every month thereafter, *C. urophthalmus* have been captured in mangrove creeks in Charlotte Harbor – the farthest north and west they have been recorded, substantially expanding the USGS-documented species range.

Field Methods

Seine Sampling: Four mangrove-fringed estuarine creeks, each approximately 2 km long, on the eastern shoreline of Charlotte Harbor (Figure 1), have been sampled since February 2003, with a 21 x 1.2 m center bag seine (1.2 x 1.2 x 1.2 m bag dimensions) with 3.2 mm mesh. For the purpose of this study, the creeks were categorized within a habitat degradation continuum: the two northernmost creeks were defined as 'lightly degraded' and the two southern creeks as 'highly degraded'. The morphology of the lightly degraded creeks remains largely intact (compared to 1953 aerial photos), and their upland drainages are undeveloped but contain remnant mosquito ditches. Creek widths range from 2 m in narrow passes to > 60 m in wider bays, depth is shallow (< 0.5 m except in narrow passes where depths reach 1 m), shorelines are lined entirely by *R. mangle*, and bottom is mixed mud and sand. The submerged aquatic vegetation in the upper stratum is entirely *Ruppia maritima,* whereas the middle and lower strata are dominated by *Halodule wrightii*. In contrast, the drainages and wetlands of the highly degraded creeks have been altered for development, creating impervious surfaces and altering freshwater flow regimes. The altered flow regimes cause short, pulsing hydroperiods limited to the immediate creek with little overland sheet flow. The bottoms are mostly hard sand, and the submerged aquatic vegetation (*R. maritima* and *H. wrightii*) is patchy and limited to the lower two-thirds of the creeks. The creeks are narrow (mean = 5 m) for the upper two-thirds of their length, and have wider bays (≥ 60 m) only in their extreme lower portions. Narrow sections are scoured to > 2 m depth with undercut banks, with greatly reduced width of the intertidal *R. mangle* prop root habitat.

From February 2003 through October 2004, creeks were sampled every other month. Sampling was less frequent from October 2004 through February 2006 (sampling occurred in October 2004; April, July, October, December 2005; January 2006). Each creek was divided into three equal-length strata (upper, middle, lower – approximately 0.7 km each), with five samples per stratum per sample period. Sample locations within each creek stratum were selected haphazardly in each sample month. For each sample, the net was set in a semi-circle against a mangrove shoreline and pursed to force fish into the bag. Seine sampling occurred mostly near low tide, because this forced fish out of the flooded mangrove prop-roots, making them more susceptible to capture. Fish were measured (standard length, SL) and released at the site of capture. Temperature, salinity, and dissolved oxygen were recorded with a handheld YSI 556 MPS. Beginning July 2005, *C. urophthalmus* > 65 mm SL were tagged before release (see below for tagging procedures).

Tag-Recapture: Beginning in July 2005, we used sub-dermal PIT (Passive Integrated Transponder) tags to tag *C. urophthalmus* to provide coarse estimates of fish occurrence in creeks. Each PIT tag has a unique 10 digit number that allows individual fish identification. Fish were captured with a 30.3 x 1.2 m center bag seine (1.2 x 1.2 x 1.2 m bag, 19 mm mesh). After capture, *C. urophthalmus* > 65mm SL were retained in floating mesh bags (25 mm mesh, 1 m^3 volume) until tagging. Pre-tagging retention time was a maximum of 30 minutes. All tagging occurred near the site of capture, with latitude and longitude recorded at the release location. Salinity, dissolved oxygen, and water temperature were also recorded for each tagging event. Fish were tagged by inserting a half-duplex PIT tag (23 x 3.4 mm) into the abdominal cavity through a 3mm incision made with a surgical scalpel. Tag retention rates in a Pilot Study and findings on other species (Baras et al. 2000, Jepsen et al. 2002; Adams et al. 2006) indicated that post-tagging sutures were not necessary.

Presence of *C. urophthalmus* in creeks was estimated from 'recaptures' by a remote PIT-tag antenna system that detected all tagged fish that swam through it (see Adams et al. (2006) for a detailed description of the PIT-tag antenna system). (No tagged fish were recaptured by seine.) One antenna each was constructed in the Upper stratum of one lightly degraded (South Silcox) and one highly degraded (Yucca Pen) creek (Figure 1). Tagging occurred in the creeks containing antennae and in adjacent water bodies.

Data Analysis

To determine whether the occurrence of *C. urophthalmus* was related to temperature or salinity, (i.e., did *C. urophthalmus* show temperature and salinity preferences (Chavez-Morales et al 2005)), catch data were classified as presence (1) or absence (0), and were examined with Mann-Whitney U tests for all samples and creeks combined. Backward stepwise regression was used to examine effects of salinity and temperature on log-transformed standard length (SL), with an $\alpha = 0.15$ to enter or remove. Backward stepwise regression was used because the model contained only two independent variables, and backward stepwise regression is better for simple models (Neter et al., 1990). To test the hypothesis that *C. urophthalmus* undergo ontogenetic shifts in salinity preference (e.g., Chavez-Lopez et al. 2005), salinity at time of capture was examined with a one-way ANOVA by size (age) class, with Tukey post-hoc comparisons of means. Size (age) classes were defined by Faunce et al. (2002) for *C. urophthalmus* in south Florida.

RESULTS

The first *C. urophthalmus* was captured in the study creeks in June 2003, with 295 individuals captured since: 163 in Culvert Creek, 25 in Yucca Pen, 93 in South Silcox, and 14 in North Silcox. With the exception of Yucca Pen, the majority of *C. urophthalmus* were captured in the upper zones of the study creeks (Table 1). Since the initial capture, *C. urophthalmus* have been captured in 12 of 13 months sampled. Similarly, the Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, Fisheries Independent Monitoring Program (FIM), which completes approximately 918 samples (seine and trawl) per year in non-creek Charlotte Harbor estuarine habitats, captured only one *C. urophthalmus* between 2000 and 2005. In 2004, FIM began sampling in the Caloosahatchee River estuarine system (Figure 1), which is heavily impacted by altered (managed) freshwater flows and urban development, and captured 166 *C. urophthalmus* in 2004 and 2005 (160 seine samples) (Patrick Casey, FWRI, Port Charlotte, FL, pers. comm.). In this study (Table 2) and in FIM samples, *C. urophthalmus* were captured in environmental conditions similar to those of their native geographical range.

There was no significant relationship between occurrence of *C. urophthalmus* and salinity ($U = 19783.5$, $p>0.1$, $\chi^2 = 2.05$, $df = 1$). Temperature, however, was a factor, with fish present in slightly cooler water (fish present, mean = 25.97°C, se = 0.54; fish absent, mean = 28.69°C, se = 0.99; $U = 30846.5$, $p<0.001$, $\chi^2 = 23.27$, $df = 1$), but . Backward stepwide regression revealed that standard length (SL) was somewhat dependent upon salinity and temperature ($R^2 = 0.25$, F =, $p<0.001$), with both independent variables contributing to the relationship ($p<0.001$ for each) (Figure 2). The relationship between length and salinity was further supported by a significant difference in salinity at time of capture between juvenile (<40mm SL, < 1 year) and larger (> 41mm SL, > 1 year) individuals (Table 3). Within each year, two distinct cohorts were captured early in the year, with the cohort of smaller fish persisting and growing through the year (Figure 3). Average standard lengths (all samples combined) were greatest in Culvert Creek and lowest in North and South Silcox creeks (Figure 4).

One-hundred-one *C. urophthalmus* were tagged with PIT tags: two in North Silcox, 73 in South Silcox, two in ponds upland of South Silcox, and 24 in Yucca Pen (Table 4). Thirty-one of these fish were recaptured by the antennae, with overall recapture rates similar in South Silcox (30% - total number tagged includes North and South Silcox and the upland pond) and Yucca Pen (33%). Of the 23 fish recaptured in South Silcox, 22 were tagged in South Silcox, and one was tagged in an upland pond. When examined by zone, the greatest percentage of recaptures occurred for fish tagged in the upper zone of South Silcox. All fish recaptured in Yucca Pen were tagged in Yucca Pen, the greatest proportion were tagged in the middle zone. No tagged fish were recaptured by seine.

Tagged *C. urophthalmus* were at large in the creek systems since 25 July 2005 (Silcox Creek) and 9 September 2005 (Yucca Pen), for a tag-recapture period of 280 and 234 days, respectively, ending 1 May 2006. The average and maximum number of days at large between date of tagging and date of most recent recapture was higher in Yucca Pen (mean = 38.7, se = 11.1, min = 1, max = 123), even though more fish were at large for a longer period in South Silcox (mean = 25.7, se = 4.4, min = 0 (recaptured same day tagged), max = 104). The mean size of fish tagged was also larger in Yucca Pen (151.9mm SL, se = 4.2) than South Silcox (114.4mm SL, se = 6.7). Tagged fish were recaptured in all months sampled in both creeks.

DISCUSSION

Building upon previous documentation of the establishment of *Cichlasoma urophthalmus* in south Florida freshwater and estuarine habitats (Shafland 1996, Trexler et al. 2000), this study demonstrates the year-round occurrence of *C. urophthalmus* in estuarine habitats of Charlotte Harbor, thus greatly expanding the geographic range of this exotic species in Florida. This study has provided the baseline data necessary to frame additional research to examine the ecological effects of this invasive species in this portion of Florida.

Multiple size classes in each study year indicate that reproduction is occurring in the Charlotte Harbor watershed. This pattern was also observed within native habitats in Mexico, where bimodal size distribution was observed in all seasons (Chavez-Lopez et al 2005). In Mexico, reproduction occurs in the rainy season (May – July), with a small secondary peak in gonadal activity in December (Chavez-Lopez et al. 2005). In this study, bimodal size distribution was also observed throughout the year, with the smallest fish occurring in July through October, during the rainy season, after inland rainfall drains into the creeks and lowers salinities. In 2003, the smallest fish occurred in February, which coincided with an unusually rainy period during the dry season, and would correspond to spawning activity in December. These findings contrast with Faunce et al (2002), however, who found that the smallest fish occurred in June.

Although *C. urophthalmus* had a full range of salinities and habitats available, they were captured primarily in salinities below 25ppt. *C. urophthalmus* are able to tolerate a wide range of temperature and salinity (Stauffer and Boltz 1994), and can tolerate rapid changes in salinity (Martinez-Palacios et al. 1990), but in their native range they appear to prefer lower salinities. For example, juveniles can tolerate salinities > 37ppt, but are usually found in low salinity waters (Martinez-Palacios et al. 1990), and the mean salinity of locations where *C. urophthalmus* was captured in Mexico was 7.13ppt (Chavez-Lopez et al. 2005). In this study, small fish were most common in low salinity (mean = 5ppt), and most larger individuals were captured in mesohaline conditions despite easy access to higher salinity areas. The preference for meso- and oligohaline conditions in this study is further supported by the absence of *C. urophthalmus* in higher salinity locations with submerged aquatic vegetation, which is the preferred habitat in Mexico (Chavez-Lopez et al. 2005). Given their affinity for low salinities, preferable habitat for *C. urophthalmus* in Charlotte Harbor is likely limited to upland freshwater drainages through the upper portions of less-degraded creeks and the entire lengths of creeks with altered freshwater flows during the wet season.

Although *C. urophthalmus* are able to spawn in a wide range of salinities (Martinez-Palacios et al. 1990), it is unlikely spawning is occurring in the creeks. During four years of sampling, no spawning beds or spawning activity was observed in the creeks. Moreover, the low salinities preferred by juveniles are not present in the less degraded creeks until after the likely spawning season. In the highly degraded creeks, the currents associated with altered freshwater flows, and the resulting channelization of much of the creek beds, likely prevent building of spawning beds. Therefore, we suggest that spawning occurs within the upland drainages of the creeks, with colonization downstream during wet season freshwater flows.

Although we used Faunce et al (2002) data for age class delineation, the size frequency pattern observed in this study suggests two cohorts per year. This resembles the findings of Chavez-Lopez et al (2005) and Martinez-Palacios and Ross (1988) in the species' native range, where *C. urophthalmus* has a much faster growth rate and shorter life span. Since no age data are available in this study, and Faunce et al (2002) did not verify that otolith rings were annual, additional work is needed to determine age and growth of *C. urophthalmus* in Florida.

The use of PIT tags to monitor use of estuarine creek habitats by *C. urophthalmus* suggests small scale movements and short residence times. In South Silcox, most antenna recaptures were of fish tagged in the Upper stratum in which the antenna is located, suggesting limited movement within the creek. In addition, there was no among-creek movement of *C. urophthalmus*; this contrasts with PIT tagged juvenile snook, which have moved among creeks and between creek and open estuary habitats (AJA and RKW, unpub. data). *C. urophthalmus* also have fewer days at large (days between tagging and most recent recapture) than juvenile snook: some juvenile snook have been detected nearly two years after tagging, whereas no *C. urophthalmus* was at large for more than 123 days of a 280 day tag-recapture period.

Combined, the data presented in this study suggest that *C. urophthalmus* are established in altered upland freshwater or oligohaline habitats (artificial ponds full year-round, and deep enough for fish to escape cold winter temperatures), and colonize estuarine creek habitats seasonally, usually associated with increased freshwater flows from upland drainage areas. When compared among creeks, greatest abundances occur in creeks with altered upland habitats, which concurs with previous correlations between habitat alteration and exotic species invasions (Courtenay and Williams 1992). With 25% of United States coastal habitats expected to be developed by 2025 (14% of coastal habitats had been developed by 2002) (Beach 2002), there is an exigent need to develop a better understanding of the ecological implications of invasive species such as *C. urophthalmus*. Future research should examine interactions between *C. urophthalmus* and juveniles of economically important fish species *Centropomus undecimalis* and *Megalops atlanticus*, which use oligohaline mangrove habitats as nurseries.

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Table 1. Number of *Cichlasoma urophthalmus* captured by Creek and Zone during sampling with the 21m seine. See Figure 1 for Zone depictions.

	Zone			
Creek	Lower	Middle	Upper	Total
Culvert Creek	13	61	89	163
Yucca Pen	17	8	0	25
South Silcox	7	18	68	93
North Silcox	1	8	5	14
Total	38	95	162	295

Table 2. Salinity and temperature at time of capture for *Cichlasoma urophthalmus* in this study, and salinity and temperature ranges of occurrence in their native range in Central America.

	Native Range*	This Study
Salinity (ppt)	0-37	0-27
Temperature (^{o}C)	15-37	18-32
Maximum Length (mm)	200	212

* Martinez-Palacios et al. 1990

Table 3. Salinity at time of capture of Cichlasoma urophthalmus by size (age) class.

A). Results of one-way ANOVA on salinity at time of capture by size (age) class[a].

Source	df	SS	MS	F	p
Size (age) Class	6	5406.93	901.16	20.73	<0.001
Error	263	11433.24	43.47		

B) Size (age) classes and mean salinity at time of capture. Size classes that share a letter (Tukey Results) were not significantly different.

		Salinity		
Size Class (mm SL)	Age[b]	Mean	Standard Error	Tukey Results
0-40	1	5.49	0.75	c
41-74	2	13.84	0.86	d
75-103	3	16.27	0.55	e
104-128	4	19.12	1.31	f
129-149	5	16.57	1.97	e
150-166	6	16.2	2.81	d, e
>166	≥7	21.68	1.08	f

[a] Size (age) classes from Faunce et al. (2002)

[b] Age at maximum length of size class.

Table 4. Number of *Cichlasoma urophthalmus* tagged, by Creek and Zone, and number and percent recaptured by remote antennae in the Upper Zones of Yucca Pen and South Silcox. No fish were tagged in Culvert Creek.

Creek/Zone	Number Tagged	Number Recaptured	Percent Recaptured
Yucca Pen	24	8	33
Lower	14	2	14
Middle	10	6	60
Upper	0	0	0
South Silcox	73	22	30
Lower	11	1	9
Middle	2	1	50
Upper	60	20	33
North Silcox	2	0	0
Lower	0	0	0
Middle	1	0	0
Upper	1	0	0
South Silcox Upland Pond	2	1	50
Total	101	31	31

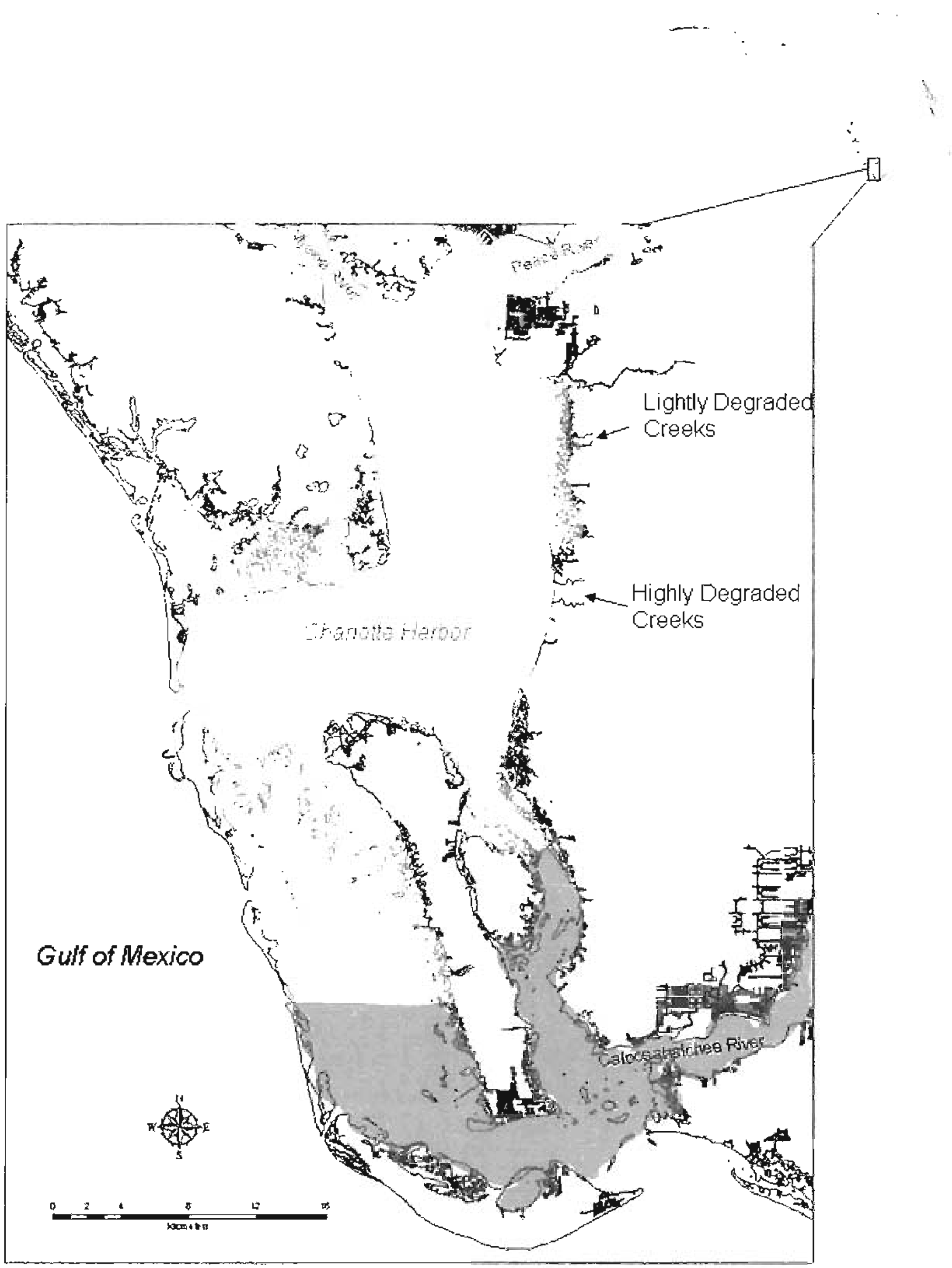
FIGURE LEGENDS

Figure 1. Location of Charlotte Harbor, FL, and study area. Stippling denotes area sampled 2001 – 2005, and dark gray sampled 2005 – 2006, by Florida Fish and Wildlife Research Institute, Fisheries Independent Monitoring Program.

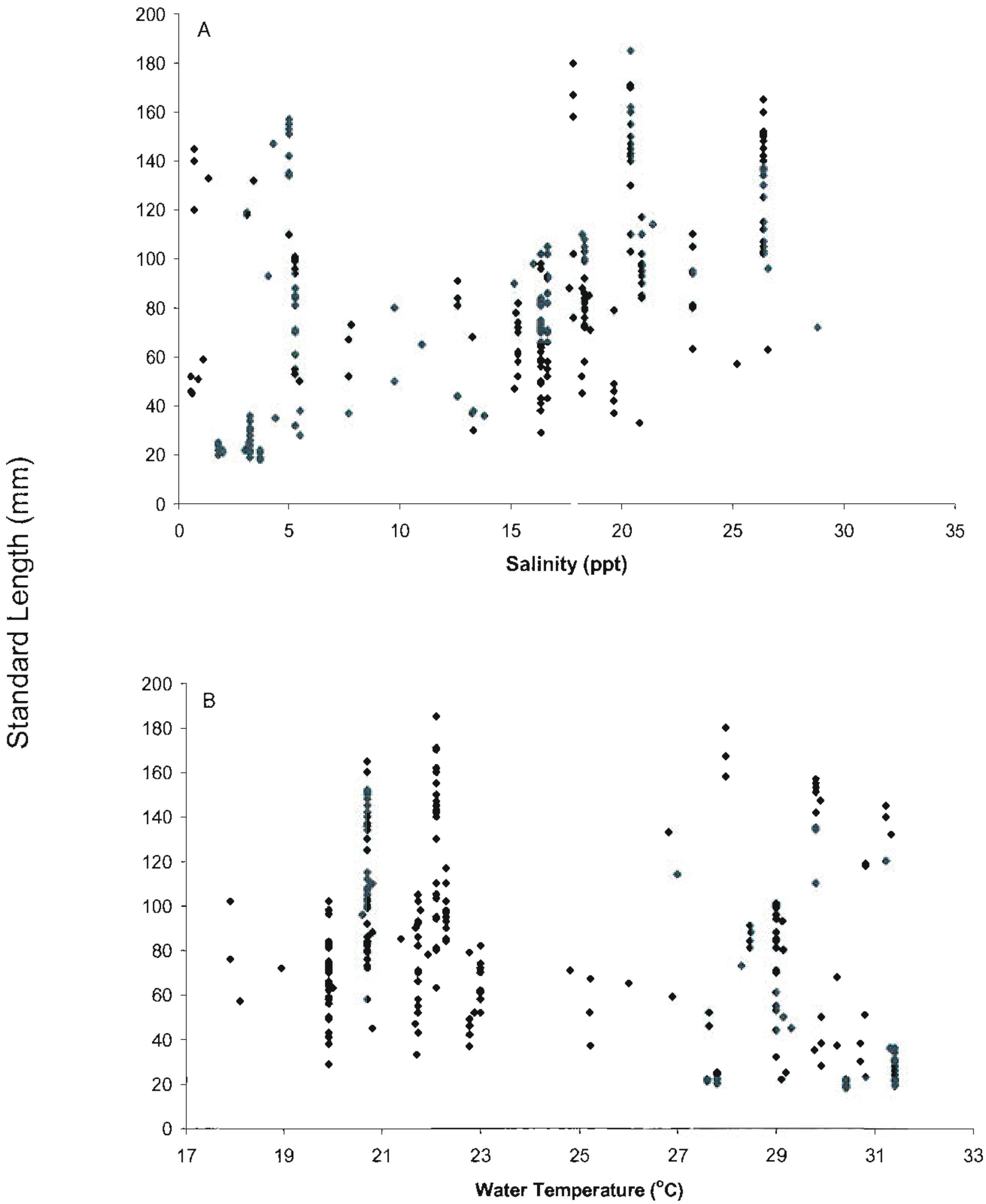
Figure 2. Relationship between standard lengths of *Cichlasoma urophthalmus* captured with the 21m seine and salinity (A) and water temperature (B). Backward stepwise regression (both dependent variables significant at α to enter or remove <0.001): Standard Length = -63.41 + 4.02(Salinity) + 3.68(Temperature); $R^2 = 0.25$; $F = 45.868$; $df = 2,67$; $p < 0.001$.

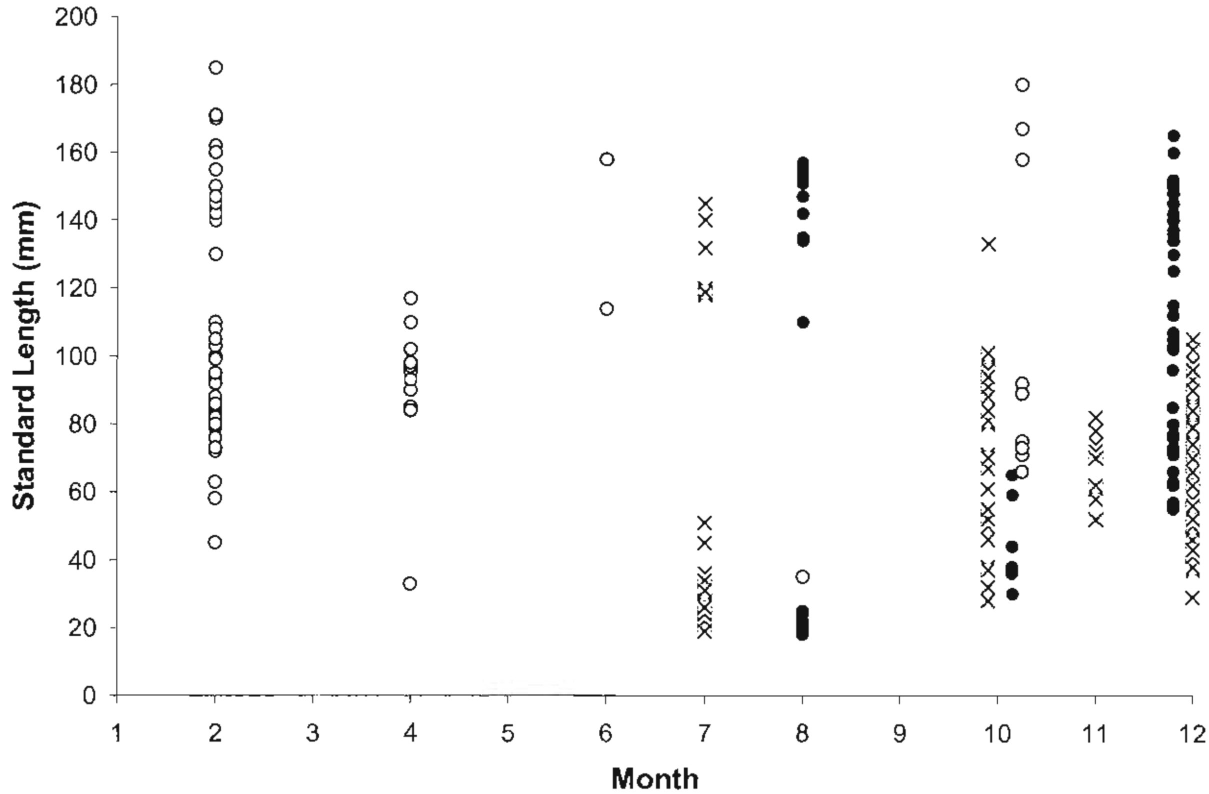
Figure 3. Standard length of *Cichlasoma urophthalmus* captured with the 21m seine by year and month. ○= 2003, ● = 2004, × = 2005.

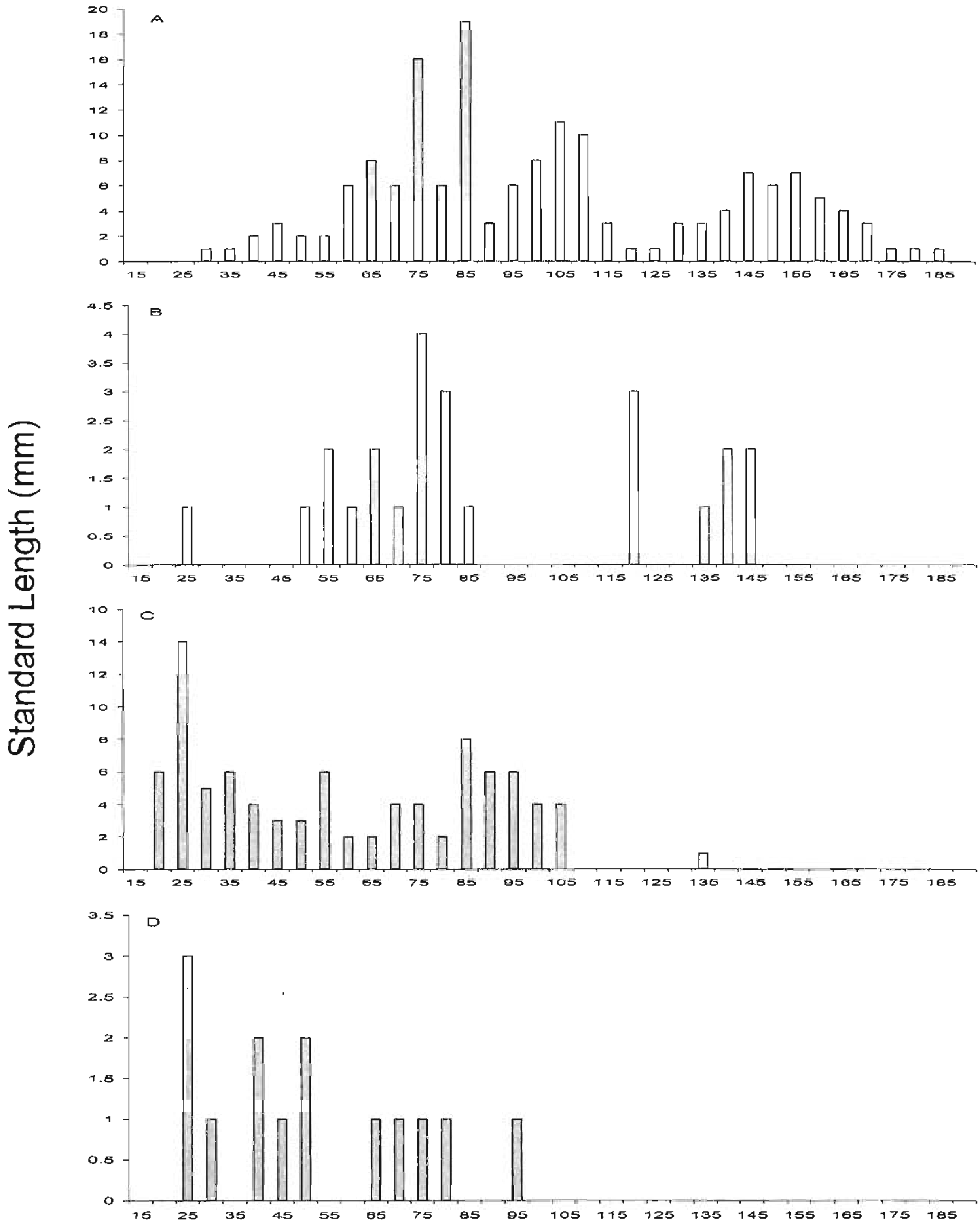
Figure 4. Standard length of *Cichlasoma urophthalmus* captured with the 21m seine by creek, all samples combined. A = Culvert Creek, B = Yucca Pen, C = South Silcox, D = North Silcox.



Peace River
Lightly Degraded
Creeks
Highly Degraded
Creeks
Charlotte Harbor
Gulf of Mexico
Caloosahatchee River
N
W
E
S
0
2
4
8
12
16


Standard Length (mm)
A
Salinity (ppt)
B
Water Temperature (°C)


Standard Length (mm)
200
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
Month


Standard Length (mm)
A
B
C
D
15 25 35 45 55 65 75 85 95 105 115 125 135 145 155 165 175 185