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Effects of interspecific competition, salinity, and hurricanes on the success of an invasive fish, the Rio Grande cichlid (*Herichthys cyanoguttatus*)

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirement for the degree of

Doctor of Philosophy
in
Conservation Biology

by

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Table of Contents

List of Figures iii
List of Tables iii
Abstract iv
Introduction 1
Chapter 1 8
Introduction 9
Materials and Methods 12
Results 17
Discussion 23
Acknowledgements 29

Chapter 2 30
Introduction 31
Materials and Methods 32
Results 36
Discussion 40
Acknowledgements 44

Chapter 3 45
Introduction 46
Materials and Methods 47
Results 49
Discussion 52
Acknowledgements 56
Summary 57
Literature Cited 59

Vita 73

List of Figures

Chapter 1

Figure 1. Sampling locations for the three different surveys.....10
Figure 2. Sampling sites from the seawall. 13
Figure 3. Map of six sampling sites in Bayou St. John and City Park.....16
Figure 4. Box-plots of monthly salinity (ppt) measured at the seawall.....18
Figure 5. Box-plots of monthly temperature (ºC) measured at the seawall.....19
Figure 6. Bayou Saint John and City Park survey results.....22

Chapter 2

Figure 7. Aggressive acts of *H. cyanoguttatus* and *L. macrochirus*. 35
Figure 8. Specific behaviors of *H. cyanoguttatus.*.....37
Figure 9. Specific behaviors of *L. macrochirus.*.....38
Figure 10. Aggressive acts of resident *L. miniatus* with invading *H. cyanoguttatus.* 39

Chapter 3

Figure 11. Growth of *H. cyanoguttatus* at different salinities 50
Figure 12. Growth of *H. cyanoguttatus* and *L. macrochirus* intra- and interspecifically..51
Figure 13. Growth rates with *L. macrochirus* as resident.....52

List of Tables

Chapter 1

Table 1. Fishes collected in the surveys before and after the hurricanes.....20
Table 2. Abiotic data collected from the surveys before and after the hurricanes.....21

ABSTRACT

The Rio Grande cichlid (*Herichthys cyanoguttatus*) has been established in the Greater New Orleans Metropolitan Area (GNOMA) for at least 20 years. It is often the most common fish species in urban canals and has also been found in natural waterways outside of the GNOMA. The effects and potential for further spread of *H. cyanoguttatus* is uncertain. My research addressed how extensive the cichlids spread in the GNOMA, how *H. cyanoguttatus* interacted with *L. macrochirus,* a native fish, and what salinity tolerance this species has. Surveys on Lake Pontchartrain and in the GNOMA indicated that *H. cyanoguttatus* is well established in urban habitats. These surveys also indicate that *H. cyanoguttatus* has spread rapidly into Bayou Saint John and City Park in recent years and that *H. cyanoguttatus* populations were relatively unaffected by Hurricanes Katrina and Rita. There is little evidence that *H. cyanoguttatus* has become established outside of the GNOMA, but this lack of persistence cannot be explained by abiotic variables I measured. Salinity may be a factor and this was measured in growth trials of *H. cyanoguttatus*. Salinities up to 16 ppt, however, had no significant effect on *H. cyanoguttatus* growth. Interspecific behavioral experiments were conducted to examine potential biotic interactions with native fish species. Prior resident trials indicated that *H. cyanoguttatus* was aggressive whether holding territory or not, and that native bluegill (*Lepomis macrochirus*) was only aggressive while holding territory. Feeding experiments were performed to examine biotic interactions between *H. cyanoguttatus* and *L. macrochirus*. *Lepomis macrochirus* grew faster than *H. cyanoguttatus* when inter- and intraspecific trials were compared; however, no significant growth differences were seen when trials were structured with *L. macrochirus* as prior residents. The major findings of my research are a high salinity tolerance of *H. cyanoguttatus,* a potential mechanism for *H. cyanoguttatus*

affecting native fishes through aggression as residents and invaders, and the presence of *H. cyanoguttatus* throughout the GNOMA, before and after the hurricanes,

Keywords: cichlid, cichlidae, *Herichthys cyanoguttatus*, *Lepomis macrochirus*, invasive species, dispersal, survey, aggression, behavior, salinity tolerance, interspecific aggression

Introduction

Invasive species affect native fauna in multiple ways, and are often considered the largest threat to native species, second only to habitat destruction (Vitousek et al. 1997). The number of introduced fish species has been steadily increasing in the United States (Crossman 1991). At least 536 non-indigenous fish species have been recorded in all 50 states, with 71% of taxa being recorded from the 11 states crossing or below the 35th parallel (Fuller et al. 1999). Fuller et al. (1999) also showed that fishes of the Family Cichlidae have been found in 30 states. Louisiana has had individual records of introduced Oscars (*Astronotus ocellatus*), Guapote tigre (*Parachromis managuensis*), unidentified tilapia (Fuller et al. 1999), and only one established species of introduced cichlid, the Rio Grande cichlid (*Herichthys cyanoguttatus*). This species has become established in natural and degraded habitats of the Greater New Orleans Metropolitan Area (GNOMA). Its presence has been confirmed for at least ten years, though anecdotal evidence suggests it may have been in these canals for twenty to thirty years (Fuentes and Cashner 2002, O'Connell et al. 2002). This species is native to the southern drainages of the Rio Grande River with its type locality being Brownsville, Texas, at the mouth of the Rio Grande River (Baird and Girard 1854).

The genus for this species has gone through considerable taxonomic revision. The group *Herichthys* was originally described as cichlids "with a compressed body, sub-elliptical outline, with small sub-conical teeth" (Baird and Girard 1854). For most of the 20th century it was part of the large genus *Cichlasoma* (Regan 1905), until Kullander (1983) restricted *Cichlasoma* to a few South American cichlid species. The genus *Herichthys* defined several Central American cichlids, including *cyanoguttatus* until it was restricted to the cichlids occurring in northeastern Mexico (Kullander 1998). This current grouping appears to be monophyletic (Hulsey et al.

2004) and is now roughly defined as “sharing a color pattern of short vertical bars or black spots posteriorly from the middle of the side, and a unique breeding color pattern in which the dorsal half of the entire head and anterior flank region turns a pale grayish in contrast to black or dark gray adjacent areas, or the entire body turns pale” (Kullander 1998).

As with other members of the Family Cichlidae, *H. cyanoguttatus* exhibits strong parental care, though it shows a markedly seasonal reproductive pattern for a cichlid. In Texas, spawning has been observed from March through August, with a distinct peak in April (Buchanan, 1972). Pairs of *H. cyanoguttatus* always include a larger male and a smaller female (Itzkowitz and Draud 1992). *H. cyanoguttatus* has an omnivorous diet that usually consists of vegetable matter or detritus (Buchanan 1972). For example, in the GNOMA canals, the major diet item appeared to be filamentous algae (O’Connell, pers. comm.). This generalist diet combined with strong parental care of juveniles may help explain the success of this invader in non-native habitats.

Once restricted to the southern half of the Rio Grande River Basin (in the United States part of the range), *H. cyanoguttatus* has now been introduced into many parts of Texas, including the Guadalupe River basin, the San Antonio area, and the Edwards Plateau region (Brown 1953, Hubbs et al. 1978, Fuller et al. 1999, Nico and Fuller 2005). Outside of Texas, the only established populations are in Florida and Louisiana (Fuller et al. 1999, Nico and Fuller 2005). In Florida, their success has been confined to artificial habitats such as canals (Fuller et al. 1999). The source of Florida populations was likely from fish farms or aquarium releases; the suspected source of Louisiana populations is multiple aquarium releases (O’Connell, pers. comm.).

The first *H. cyanoguttatus* known from the New Orleans area was caught in southeastern Lake Pontchartrain on 17 June 1996 (Fuentes and Cashner 2002). From 1996 to 1998, individual

specimens were caught (less than 5 fish per site) at various sites within GNOMA and even to the West of GNOMA in the La Branche Wetlands (O'Connell et al. 2002). Large numbers of *H. cyanoguttatus* were caught beginning in 30 May 1998 with a fish kill of 23 *H. cyanoguttatus* in a Jefferson Parish canal. Reproducing populations were found yearly within the GNOMA from this point forward, and some larger numbers of *H. cyanoguttatus* were occasionally caught in the La Branche wetlands up until 2004 (Cheek, A.O., Broch, S., Henry, B. pers. comm.). A diffusion model was created to examine where *H. cyanoguttatus* originated and how it was dispersing (O'Connell et al. 2002). The proposed point of origin is central Jefferson Parish in 1989, with pump stations and Lake Pontchartrain aiding in the spread to other canals and lake sites.

The potential threat of *H. cyanoguttatus* is unclear, though it appears to compete with native centrarchids such as bluegill (*Lepomis macrochirus*) for breeding sites (Courtenay et al. 1974, O'Connell pers. comm., pers. obs.). It is aggressive towards other fishes (e.g., largemouth bass, *Micropterus salmoides*; western mosquitofish, *Gambusia affinis*; sailfin molly, *Poecilia latipinna*) in natural settings (pers. obs.). Cichlids, in general, are aggressive fishes (Breau and Grant 2002, Grant et al. 2002, Draud et al. 2004). Aggression from invasive species can inhibit growth and reproduction as well as shift habitat use of native species (Marchetti 1999, Schrank et al. 2003). With habitat and diet shifts, effects can be far-reaching, possibly even extending to terrestrial systems, as seen with terrestrial spiders affected by invasive fish in Japan (Baxter et al. 2004).

When *H. cyanoguttatus* interacted with native sheepshead minnows (*Cyprinodon variegatus*) in experimental pools, the native fish did not reproduce successfully in the presence of the aggressive non-native (Mire, unpublished abstract). Competition for food by *H.*

cyanoguttatus may also affect native fishes. The cichlid may disrupt the food web in any number of ways with their varied diet, which is thought to shift in the presence of centrarchid fishes (Birkhead 1980). As with other invasive generalist fishes, *H. cyanoguttatus* may also be able to alter their diet as certain foods become scarce, a tactic few native fishes can achieve. Competition for breeding sites may be more significant, depending on the availability of suitable sites. Competition for cover from predation, however, may be the most important. Many species of birds, fish, mammals, and reptiles feed on centrarchids, so cover is a valuable resource. Bluegill are often found forced into open water in areas heavily populated with *H. cyanoguttatus* (pers. obs.). Risk of predation is an important indirect consequence of competition for the wading bird, *Tringa totanus* (Minderman et al. 2006). Interference competition affected behavior to the point where activity increased and susceptibility to predation by sparrow hawks (*Accipiter nisus*) increased (Minderman et al. 2006). In California streams, an invasive crayfish (*Pacifastacus leniusculus*) was shown to displace native Paiute sculpin (*Cottus beldingi*) from limited habitats that were best suited for cover (Theo 2005). There could be a similar fate for species competing with *H. cyanoguttatus* for cover in the GNOMA. The occurrence of *H. cyanoguttatus* in non-native ecosystems has been followed by subsequent invasions by other species. This has occurred both in Six Mile Creek, Florida (Courtenay et al. 1974) and in the upper San Antonio River, Texas (Edwards 2001). *Herichthys cyanoguttatus* may be a pioneer species that initiates subsequent invasional meltdowns. Unfortunately, the effects involved with invasions and invasional meltdowns are most often observed after-the-fact (Schofield and Chapman 1999, Ricciardi 2001).

Most invasive species do not establish themselves in their new habitat (Ricciardi and Mottiar 2006). Native species, disturbed habitats, and abiotic conditions may either resist or facilitate invasions. Leprieur et al. (2008) examined river systems of the world to see what factors facilitate invasive species. They found no evidence that species-rich communities impede the establishment of non-natives ("biotic resistance" hypothesis). They also found no evidence for "biotic acceptance", where environmentally suitable habitats determined success of introduced species. The most important factor appeared to be the "human activity" hypothesis. This points out the important role of disturbed habitats for invasive riverine fishes, which would include *H. cyanoguttatus*.

Even though the "biotic acceptance" theory has little support, it is important to determine how *H. cyanoguttatus* fits into the physiogeography of southeastern Louisiana. Abiotic factors such as temperature and salinity can influence the spread of invasive species and may also affect the continued dispersal of *H. cyanoguttatus*. Cichlids are a tropical family of fishes (Keenleyside 1991) with clear boundaries of thermal tolerance that preclude their spread into temperate regions (Shafland and Pestrak 1982). Brackish water may also negatively affect *H. cyanoguttatus*, though cichlids are secondary division freshwater fish with a wide variety of tolerances to salinity (Dial and Wainwright 1983, Courtenay et al. 1984, Martinez-Palacios et al. 1990, Keenleyside 1991). For example, the Mayan cichlid (*Nandopsis urophthalmus*) tolerates and even prefers higher salinities (Martinez-Palacios et al. 1990, Stauffer and Boltz 1994) while the blue tilapia (*Oreochromis aureus*) has been shown to survive under marine conditions (Courtenay et al. 1984, Nugan 2003). High salinity levels may either inhibit or enhance establishment of *H. cyanoguttatus* in the slightly brackish Pontchartrain and the relatively brackish La Branche Wetlands. The Lake Pontchartrain Estuary intermittently has fresh and

brackish water fish and salinities near 10 ppt (Hastings et al. 1987, Cashner et al. 1994, O'Connell et al. 2004).

The salinity tolerance of *H. cyanoguttatus* in New Orleans could depend on whether or not the fish is a hybrid with *H. carpintis*. Such hybrids are commonplace in the aquarium hobby, largely because of the similar appearance of these two species. *H. carpintis* is a species whose native range is south of *H. cyanoguttatus* (Miller 2005). This species is found in habitats more downstream and brackish when compared to *H. cyanoguttatus* (Miller 2005, Don Conkel pers. comm.). The variation in spot patterns of introduced cichlids in GNOMA resembles the natural hybrids of these species found in Rio Josefina in the Soto La Marina basin of Mexico (Miller 2005, Juan Artigas, pers. comm.). An introduced hybrid of these species could have the cold tolerance of *H. cyanoguttatus* and the salinity tolerance of *H. carpintis*, making it an ideal invader for the subtropical and brackish conditions of southeastern Louisiana. Whether or not this invasive fish is a hybrid, however, was not studied here.

For my dissertation, I examined three topics relevant to the spread of this aquatic invasive species. First, I surveyed *H. cyanoguttatus* along the perimeter of Lake Pontchartrain, before and after the hurricanes of 2005, and during their rapid spread into Bayou Saint John and City Park in order to examine the spread and survivorship of this invasive species. Second, I studied aggressive interactions involving territory defense by both *H. cyanoguttatus* and a common native centrarchid, *Lepomis macrochirus* to examine the nature of this invasive-native interaction. Lastly, I examined feeding trials between *Lepomis macrochirus* and *H. cyanoguttatus* and observed growth rates of *H. cyanoguttatus* across a range of salinities. Understanding the spread, survival, and salinity tolerance of *H. cyanoguttatus* can help us understand the potential threat and barriers of this invasive species. Understanding the

interspecific interactions can provide a mechanism by which this species may affect natives. The working hypotheses here are high salinity tolerances, high rates of aggression between *H. cyanoguttatus* and native species, and persistence and spreading of the range of *H. cyanoguttatus.*

CHAPTER 1

Establishment and post-hurricane survival of the non-native Rio Grande cichlid (*Herichthys cyanoguttatus*) in the Greater New Orleans Metropolitan Area.

Abstract

The non-native Rio Grande cichlid (*Herichthys cyanoguttatus*) is established in canals of the Greater New Orleans Metropolitan Area (GNOMA) and a dispersion model predicts that this freshwater species is expanding its range using estuarine corridors in Lake Pontchartrain. I tested this prediction by trapping along the Lake's south shore in 2003-2004. To determine *H. cyanoguttatus* responses to 2005 hurricane effects, I compared pre- and post hurricane trapping data collected within the GNOMA. To better understand possible post-hurricane expansion of *H. cyanoguttatus*, I monitored *H. cyanoguttatus* abundance monthly over two years (2006-2007) in Bayou St. John and other City Park water bodies. I confirmed that *H. cyanoguttatus*: 1) occurs in Lake Pontchartrain at salinities up to 8 ppt, 2) was not noticeably affected by hurricane-related levee failures, and 3) significantly increased in number from 2006 to 2007 at three sites in City Park.

Introduction

The Rio Grande cichlid (*Herichthys cyanoguttatus*) is native to the southern drainages of the Rio Grande River with its type locality being Brownsville, Texas, at the mouth of the Rio Grande River (Baird and Girard 1854). Once restricted to the southern half of the Rio Grande River Basin, *H. cyanoguttatus* has been introduced into multiple drainages of Texas, including the Guadalupe River basin, the San Antonio area, and the Edwards Plateau region (Brown 1953, Hubbs et al. 1978, Fuller et al. 1999, Nico and Fuller 2005). Other established populations have been observed in Florida where their success has been confined to artificial habitats such as urban canals (Fuller et al. 1999, Nico and Fuller 2005). *Herichthys cyanoguttatus* has become established in natural and man-made waterbodies of the Greater New Orleans Metropolitan Area (GNOMA). Introduced populations in the GNOMA occur mostly in freshwater urban canals and bayous that are bounded by the Mississippi River to the south and the oligohaline Lake Pontchartrain estuary to the north. Natural swamps and marshes are present to the east (e.g., Bayou Sauvage National Wildlife Refuge) and west (La Branche Wetlands) of these habitats (Fig. 5). The presence of *H. cyanoguttatus* in the GNOMA has been confirmed for at least ten years, though anecdotal evidence suggests it may have been in the region for twenty to thirty years (Fuentes and Cashner 2002, O'Connell et al. 2002).

To better understand the dynamics of the early stages of *H. cyanoguttatus* expansion, a dispersion model was constructed based on known occurrence data (O'Connell et al. 2002). One model predicts that *H. cyanoguttatus*, nominally a freshwater species, dispersed into new areas by traveling through estuarine corridors of Lake Pontchartrain (O'Connell et al. 2002). If this can be verified, then one important implication is that estuarine habitats may not serve as a barrier to continued *H. cyanoguttatus* expansion beyond the GNOMA and into

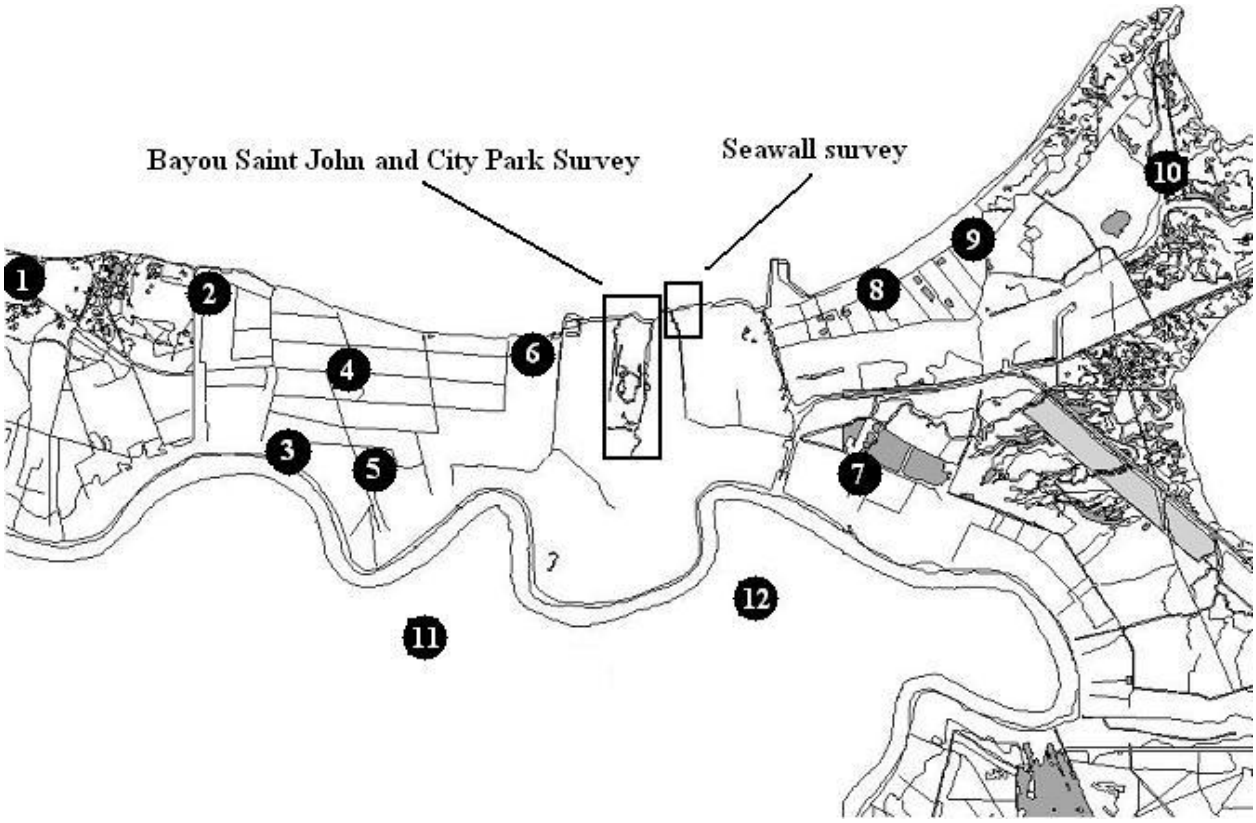

Figure 1. Sampling locations for three different surveys of *H. cyanoguttatus* in the GNOMA. Black circles represent sites sampled for the pre- and post Hurricane survey. Sites numbered are: 1. Bayou Trepagnier, 2. West Kenner, 3. Mississippi River, 4. Veterans Canal, 5. Harahan, 6. Bonnabel Canal, 7. St. Bernard Parish, 8. St. Charles Canal, 9. Gannon Canal, 10. Bayou Sauvage, 11. Bayou Segnette, 12. Algiers. Three of the other sites are found within the other surveys, the Ruddock site is farther west and north and is not shown.

valuable nearby fishery habitat. It is possible, though, that the more natural swamp and marsh habitats outside of the GNOMA may offer some form of biotic resistance to *H. cyanoguttatus*. Darwin (1859) felt that invasive species could either be outcompeted by similar native congeners (biotic resistance) or that they might be 'preadapted' to be successful where similar niches already existed (naturalization hypothesis). Either scenario may be true for *H. cyanoguttatus*; however, tests of positive and negative effects of native congeners on invasive fishes have so far been inconclusive (Ricciardi and Mottiar 2006). *Herichthys cyanoguttatus* has been periodically collected in natural water bodies of the La Branche Wetlands west of the GNOMA, (C. Schieble, A. Cheek, B. Henry, S. Temple, pers. comm., pers. obs.). Although *H. cyanoguttatus* has

occurred in considerable numbers in these wetlands, their occurrence is never as consistent as it is in the more urban habitats. To examine the possible roles of salinity and biotic resistance on *H. cyanoguttatus*, I conducted surveys in Lake Pontchartrain in 2003-2004 and the GNOMA and adjacent natural areas in 2005.

The levee failures that followed hurricanes Katrina and Rita inundated large portions of the GNOMA with estuarine waters and interrupted my 2005 survey for *H. cyanoguttatus*. I used this opportunity to conduct a pre/post hurricane study to determine if the non-native fishes were affected by the disasters. A second survey was conducted in 2006 at the same sampling locations and approximately during the same time of year. To further explore possible post-hurricane effects, I conducted monthly samples at six sites in Bayou St. John and other City Park water bodies from January 2006 to December 2007. Bayou St. John and City Park are located in the north-central portion of Orleans Parish and both were inundated with floodwaters for approximately three weeks in 2005. While *H. cyanoguttatus* occurred in Bayou St. John prior to the levee failures, the non-native species had only just begun to disperse into City Park water bodies in 2005.

Using these multiple surveys, I attempted to answer the following questions: 1) Does *H. cyanoguttatus* occur in Lake Pontchartrain estuarine habitats as predicted by a dispersion model? 2) Did populations of *H. cyanoguttatus* survive the effects of the 2005 levee failures and hurricanes? 3) Has *H. cyanoguttatus* increased in numbers in Bayou St. John and other City Park water bodies since 2005?

Materials and Methods

Potential Estuarine Corridors Survey (2003-2004)

To test the prediction that *H. cyanoguttatus* use estuarine habitats as dispersion corridors, I placed traps along the armored seawall that dominates the southern shore of Lake Pontchartrain. This artificial habitat represents a challenge to most conventional fish collecting methods because it consists of 12-15 concrete steps (height = 30 cm) that extend from land to below the water surface. The area of high-energy turbulence associated with the edge of the seawall precludes safe collection of fishes with gillnets, seines, or hoopnets. This high-energy area is also not likely to be conducive to *H. cyanoguttatus* movement for the same reason. Electrofishing along the seawall is also impractical because of periodically elevated salinities in this estuarine habitat. If active or passive *H. cyanoguttatus* movement along the seawall occurs, then it will be along the submerged step portion of the seawall where the most laminar current flow occurs (O'Connell, personal observation).

Trapping was conducted from February 2003 to January 2004 at three sampling sites near the mouth of London Avenue Canal, which flows into Lake Pontchartrain near the University of New Orleans in the north-central region of Orleans Parish (Fig. 6).

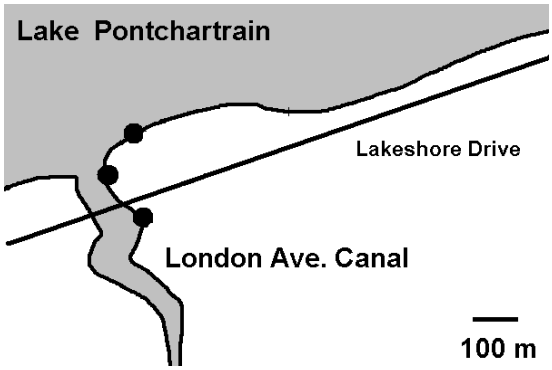

Fig 2. Sampling sites from the seawall. Traps were positioned to capture *H. cyanoguttatus* as it dispersed into Lake Pontchartrain.

I constructed 9 double funnel traps (90 cm X 40 cm X 20 cm) that were custom designed to fit on the seawall and block or capture fishes moving along the inundated steps. Traps were constructed of plastic-treated mesh (3 cm X 1 cm mesh) of the type used for crab traps. Similar traps have been used to capture *H. cyanoguttatus* at sites in Texas (Buchanan 1971). Both adult and juvenile *H. cyanoguttatus* have been collected from London Avenue Canal, making it a potential source for individuals moving into or through the Lake. The double funnel traps allowed me to determine the direction of fish movement along the seawall, with funnels facing either towards or away from the mouth of London Avenue Canal. The three sampling sites were located approximately 200 m apart along the seawall (Fig. 6) and at each site three traps were fished along the three uppermost inundated steps. Sampling consisted of setting funnel traps once monthly for a year with all traps being simultaneously fished from sunrise to sunset (to match the diurnal activity of *H. cyanoguttatus*). Funnel traps were set for 24 hours, baited with raw chicken necks, and they were checked every 12 hours. During high wave conditions funnel traps were weighted down with cinder blocks. Salinity (ppt) and temperature (ºC) were measured five times throughout the day at each site during each sampling period using a YSI meter to determine under which conditions *H. cyanoguttatus* were collected. All fishes collected were anesthetized with sodium bicarbonate, fixed in 10% formalin, and preserved in 70% ethanol. Fishes were later identified, counted, weighed to the nearest gram, measured to the nearest mm for standard length, and added to the University of New Orleans Vertebrate Collection.

Pre- and Post Hurricane Surveys (2005-2006)

Since an early survey of sites in Orleans and Jefferson parishes in 1998 (Fuentes and Cashner 2002), there has been no assessment of the geographical extent of *H. cyanoguttatus* dispersion within and outside of the GNOMA. I surveyed 16 sites in and around the GNOMA to determine which regions contained *H. cyanoguttatus* populations (Fig. 5). Sampling consisted of trapping with the funnel traps used in the 2003 seawall survey. Site selection was based on my attempt to find the extent of expansion for *H. cyanoguttatus,* so two sites were in Lake Pontchartrain, one site was in the Mississippi River, and two sites (Bayou Segnette and Algiers) were across the River on the West Bank (one of which, Bayou Segnette, is a natural waterway). Other sites included two natural waterways to the west of the GNOMA (Ruddock and Bayou Trepagnier) and one natural site to the East (Bayou Sauvage). Finally, the remaining seven sites were located within the more urbanized sections of the GNOMA but included Bayou St. John, a semi-natural water body that is part of the City Park lagoon system (Fig. 7).

Trapping was conducted over a six-week period from June to mid-July 2005 and consisted of 12 h night sets of each trap. Traps were baited with canned catfood. Water temperature, general hardness, carbonate hardness, salinity, pH, and dissolved oxygen were measured using a standard YSI meter at each sampling location. All trapped *H. cyanoguttatus* and native sunfishes (Family Centrarchidae) were counted. I focused solely on sunfishes because these are the most common fishes found in association with *H. cyanoguttatus* and are the most likely to be interacting with the non-native fish over spawning sites (pers. obs.). To determine if the 2005 hurricanes impacted the abundance or distribution of *H. cyanoguttatus* at these sites, I repeated these survey methods the following summer from June to mid-July in 2006. The number of fishes collected between these two periods was compared using a paired,

non-parametric sign test (SPSS v. 15.0) because the data was not normal enough for Gaussian stats. Whether more fishes were caught in urban or natural environments was tested with a multivariate general linear model (Wilks' Lambda test). Environmental data were not replicated and could not be compared statistically.

Bayou S. John and City Park Survey (2006-2007)

As part of a survey to assess post-hurricane fishery recovery in Bayou St. John and other water bodies located in City Park, I sampled six sites monthly for two years from January 2006 to December 2007. The three Bayou St. John sites (Bayou St. John North, Central, and South) are considered estuarine, receiving intermittent water from Lake Pontchartrain to the north (Fig. 3). The Bayou St. John North site is closest to Lake Pontchartrain and tends to have higher salinities (2006 salinity range = 7.0 - 8.1 ppt) than the other five sites. Salinities are slightly lower at the central (2006 salinity range = 6.4 – 7.7 ppt) and south Bayou St. John sites (2006 salinity range = 6.9 – 7.8 ppt), but these two sites are also closer to more urbanized portions of New Orleans (Fig. 3). Prior to 2005, *H. cyanoguttatus* had been observed nesting at all three of these sites in oligohaline conditions. The three City Park sites are less saline, though indirectly connected to Bayou St. John though various pump systems (Fig. 3).

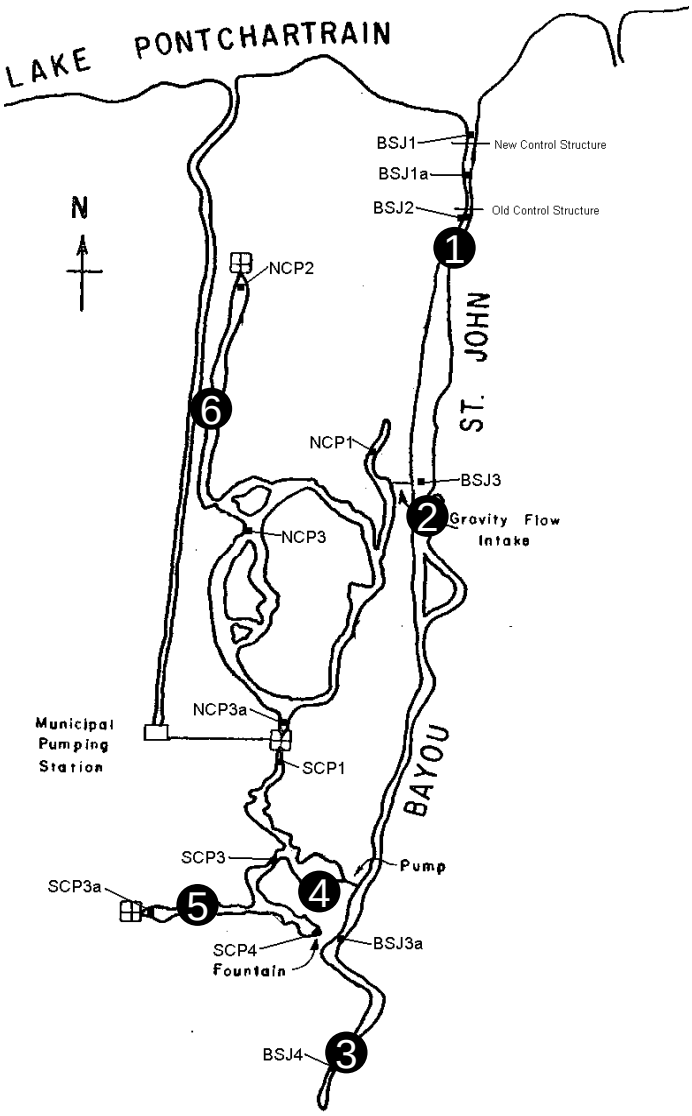

Figure 3. Map of six sampling sites in Bayou St. John and City Park in north-central Orleans Parish, Louisiana. Sites are: 1. Bayou St. John, North, 2. Bayou St. John, Central, 3. Bayou St. John, South, 4. Pontchartrain Lagoon City Park, 5. Metairie Bayou City Park, and 6. Marconi Lagoon City Park.

Pontchartrain Lagoon (2006 salinity range = 3.0 – 5.4 ppt) is the City Park site closest to Bayou St. John and is a large open pond with little riparian vegetation. Metairie Bayou (2006 salinity range = 2.8 – 5.4 ppt) is a formerly natural bayou that has been contained within the City Park

system. Both sites regularly receive water from Bayou St. John from pumps (Fig. 3) but are mostly influenced by rain run-off from the park itself. Prior to 2005 I observed only a few *H. cyanoguttatus* at these two park sites and no nesting was observed, though there was anecdotal evidence of recent expansion into the park. The Marconi Lagoon site (2006 salinity range = 2.3 – 3.8 ppt) has the freshest water conditions and is the most isolated of the six sites. Habitat conditions in Marconi Lagoon are more natural than the other sites and prior to the current surveys, no *H. cyanoguttatus* had been observed in the area.

To better understand the survival and possible expansion of *H. cyanoguttatus* populations at these sites, I counted all *H. cyanoguttatus* collected during this time period. Sampling consisted of three replicate seine (5 m X 1.5 m) hauls at each site each month with a standard area of approximately 25 m^2 sampled for each seine haul. I compared the number of *H. cyanoguttatus* collected at each site between 2006 and 2007 (72 total seine pulls) to test for significant increases using a paired, non-parametric sign test (SPSS v. 15.0). Salinity (ppt) and water temperature (ºC) were measured for each seine haul using a YSI meter. These variables were also compared between 2006 and 2007 to assess any relationship between them and *H. cyanoguttatus* numbers.

Results

Potential Estuarine Corridors Survey (2003-2004)

From February 2003 to January 2004 (with no sampling in September 2003 due to turbulence), traps were fished along the seawall for a total of 1,073.07 hours (9 traps X 119.23 trap hours). Specimens of *H. cyanoguttatus* were collected in May (n = 5), June (n = 1), and October (n = 2), confirming the occurrence of this species in estuarine habitats of Lake

Pontchartrain. Salinities for these collections ranged from 0.4 ppt in June to 5.1 ppt in October (Fig.4), while collection temperatures ranged from 21.8 ºC in October to 30.5 ºC in June (Fig. 5).

Only 14 fishes were collected in traps and *H. cyanoguttatus* (n = 8) was the most common species. The remaining fishes included three Gulf killifish (*Fundulus grandis*), one of which was collected in February and the other two were collected in October. Also collected were one sailfin molly (*Poecilia latipinna*) in December, one naked goby (*Gobiosoma bosc*) in March, and one southern flounder (*Paralichthys lethostigma*) in July.

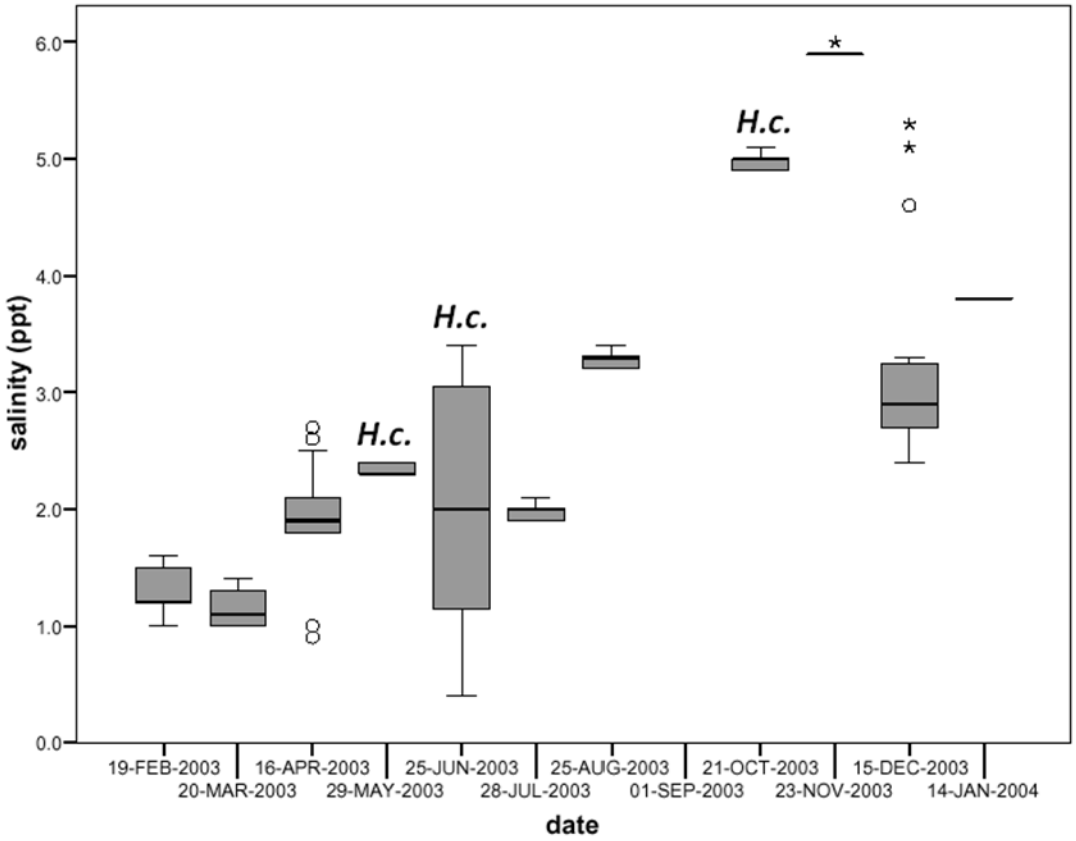

Figure 4. Box-plots of monthly salinity (ppt) measured at seawall trapping sites located along the south shore of Lake Pontchartrain. Data are combined from five monthly readings taken at each of the three trapping sites (n = 15). Sampling periods in which *H. cyanoguttatus* were collected are denoted with *H.c.* No samples were taken in September 2003 due to turbulence.

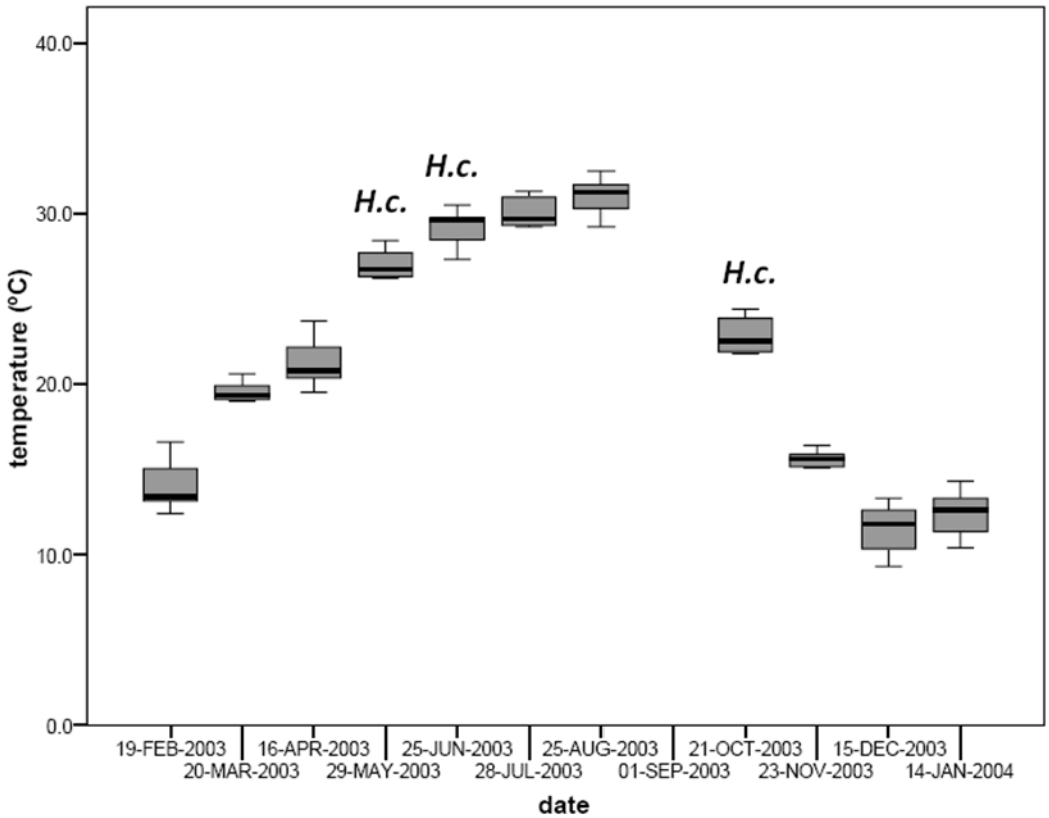

Figure 5. Box-plots of monthly temperature (ºC) measured at seawall trapping sites located along the south shore of Lake Pontchartrain. Data are combined from five monthly readings taken at each of the three trapping sites (n = 15). Sampling periods in which *H. cyanoguttatus* were collected are denoted with *H.c.* No samples were taken in September 2003 due to turbulence.

Pre and Post Hurricane Surveys (2005-2006)

A total of 81 fishes representing nine species were collected during two surveys (51 fishes in 2005 and 30 fishes in 2006). Only *L. macrochirus* exhibited a significant (Sign Test, $p = 0.039$) change in abundance (decrease) from 2005 to 2006. There was no significant change in abundance over between years for any other species, including *H.cyanoguttatus* and there was no significant decrease in centrarchid species when they were found sympatrically with *H.*

cyanoguttatus. There were significantly more Rio Grande cichlids in urban than in non-urban habitats (GLM, Wilk's Lambda Test, $F_{(22,146)} = 2.523$, $p < 0.001$), whereas there was no difference for centrarchids. In 2005, *H. cyanoguttatus* was collected at 9 of the 16 sampled sites, most of which were urban sites within the GNOMA except for the Lake Pontchartrain 2 site (Table 1).

Table 1. Fishes collected in the surveys before and after Hurricanes Katrina and Rita.

	2005							2006						
	H. cyanoguttatus	*L. macrochirus*	*M. salmoides*	*L. miniatus*	*L. gulosus*	*L. cyanellus*	*L. microlophus*	*H. cyanoguttatus*	*L. macrochirus*	*M. salmoides*	*L. miniatus*	*L. gulosus*	*L. cyanellus*	*L. microlophus*
Urban sites														
Veterans Canal	3	1	0	0	0	0	0	1	0	0	0	0	0	0
Bonnabel Canal	5	2	2	0	0	0	0	2	0	0	0	0	0	0
Harahan	3	1	0	0	0	1	0	4	0	0	0	1	0	0
Western Kenner	1	1	0	0	0	0	0	6	0	0	0	0	0	0
St Charles Canal	6	0	0	0	0	0	0	3	0	0	0	0	0	0
Gannon Canal	5	0	0	0	1	0	0	0	0	0	0	0	0	0
Bayou St. John	1	1	1	0	0	0	0	1	0	1	0	0	0	0
St. Bernard Parish	2	1	1	0	0	0	0	0	0	1	0	0	0	0
Algiers	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Natural waterways														
Bayou Segnette	0	0	0	1	0	0	0	0	1	0	0	4	0	0
Ruddock	0	1	1	0	1	0	0	0	0	0	0	1	0	0
Sauvage	0	1	0	1	0	0	1	0	0	0	0	0	0	0
Bayou Trepagnier	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lake/River sites														
Mississippi River	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lake Pontchartrain 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lake Pontchartrain 2	1	1	1	0	0	0	0	1	3	0	0	0	0	0

In 2006, *H. cyanoguttatus* was collected at 7 of the 9 sites where the species was collected in 2005. The two sites that had *H. cyanoguttatus* in 2005 and not in 2006 were the St. Bernard (new Parish record) and Gannon Canal sites (Table 1). The remaining 7 sampling stations (the

Algiers site, Bayou Segnette, Ruddock, Bayou Sauvage, Bayou Trepagnier, the Mississippi River site, and the Lake Pontchartrain 1 site) produced no *H. cyanoguttatus* in either year (Table 1). These sites include the four natural localities (Bayou Segnette, Ruddock, Bayou Sauvage, and Bayou Trepagnier) located outside of the GNOMA.

There was no significant correlation of physical parameters and the presence/absence of *H. cyanoguttatus*, however many sites were higher in salinity the second year (Table 2).

Table 2. Abiotic data collected from the surveys before and after Hurricanes Katrina and Rita. Note the higher salinities after the hurricanes, in particular Bayou Saint John and the second Lake Pontchartrain site, both of which contained *H. cyanoguttatus* that year.

	2005					2006				
Urban sites	Salinity	Temp	pH	GH	KH	Salinity	Temp	pH	GH	KH
Veterans Canal	0.4	32.6	8.44	140	150	0.4	28	8.08	180	130
Bonnabel Canal	0.5	29.7	8.88	220	150	0.5	30.5	8.13	220	160
Harahan	0.2	29	7.7	140	100	0.7	27.4	7.66	300	330
Western Kenner	0.5	31.8	8.15	200	140	0.4	31.6	8.67	180	110
St Charles Canal	0.4	28.2	7.46	180	90	1.9	31.6	7.34	500	120
Gannon Canal	0.5	30	8.61	160	90	1	33	6.94	300	140
Bayou St. John	3.25	30.7	8.36	560	40	7.6	30.2	6.99	560	30
St. Bernard Parish	0.3	28.2	7.69	260	180	0.5	28.7	7.08	280	120
Algiers	0.5	31.7	7.71	220	130	0.3	30.1	7.11	140	80
Natural waterways										
Bayou Segnette	0.7	30.7	8.46	220	160	1	33	8.6	240	240
Ruddock	0.7	29	7.71	200	120	2.4	29.9	7.635	580	160
Sauvage	1.1	28.7	7.34	220	70	11.3	34	8.84	560	50
Bayou Trepagnier	2.7	31.1	7.08	560	150	7.8	29.8	8.08	1600	80
Lake/River sites										
Mississippi River										
Lake Pontchartrain 1						9	30	7.6	560	30
Lake Pontchartrain 2						8	32.2	7.57	560	40

This included the Lake Pontchartrain 2 site, which had *H. cyanoguttatus* present at a salinity of 8.0 ppt. Bluegill sunfish (*L. macrochirus*) and largemouth bass (*Micropterus salmoides*) were most commonly caught with *H. cyanoguttatus*. Red-spotted sunfish (*Lepomis miniatus*) were only caught in two sites where *H. cyanoguttatus* was not found.

Bayou S. John and City Park Survey (2006-2007)

From 2006 to 2007 the number of *H. cyanoguttatus* increased significantly (Sign Test, $p < 0.05$) at all three City Park sampling sites while there was no increase in abundance at the three Bayou St. John sites (Fig. 9).

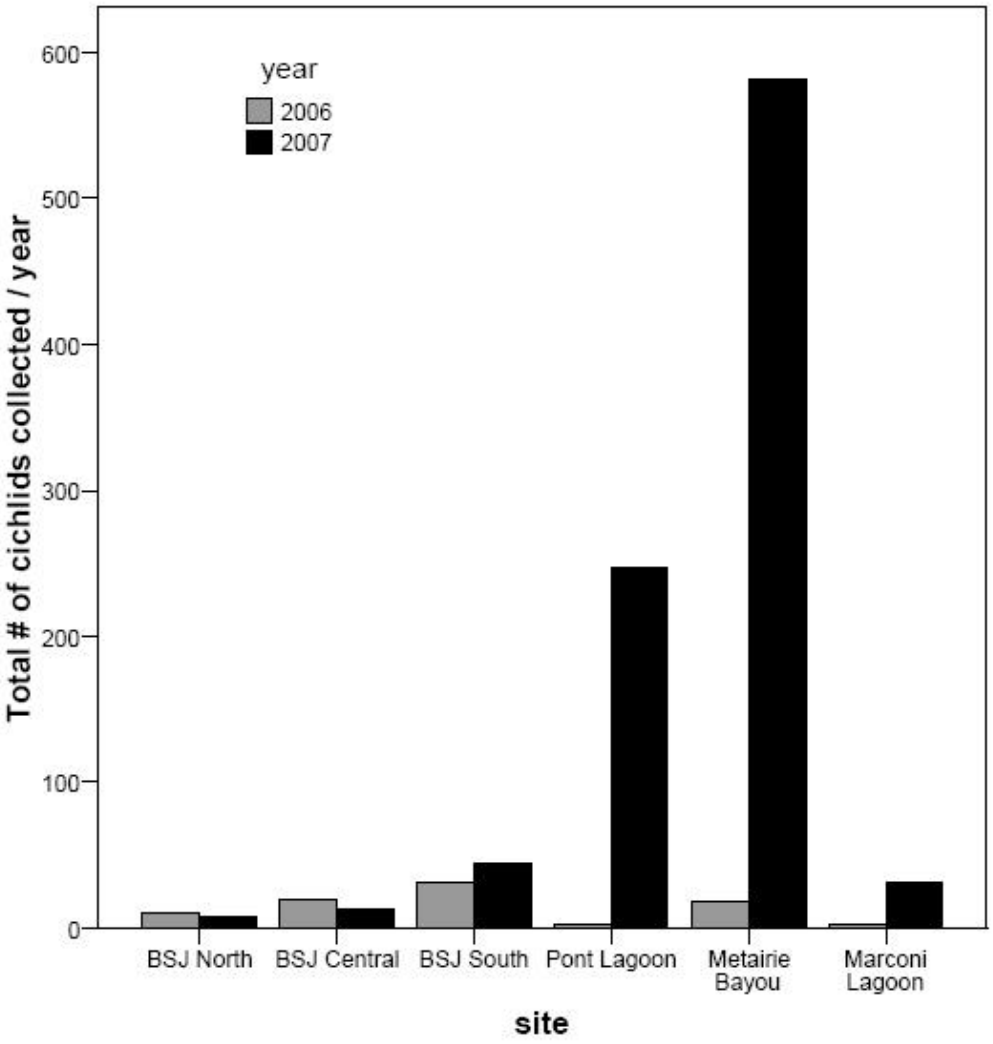

Figure 6. Survey data for Bayou Saint John and City Park sites. Salinity decreased significantly between years for Bayou Saint John sites (Sign Test, $p < 0.01$) and temperature decreased for Bayou Saint John South (Sign Test, p=0.03). Increase in numbers of *H. cyanoguttatus* was significant from 2006 to 2007 for all City Park sites (Sign Test, $p < 0.01$). Salinity decreased significant only for Marconi Lagoon in City Park (Sign Test, $p < 0.01$).

Pontchartrain Lagoon had significantly more *H. cyanoguttatus* in 2007 than in 2006 (Sign Test, $p < 0.01$) as did Metairie Bayou (Sign Test, $p < 0.01$). While these sites had similar densities to the Bayou St. John sites in 2006, in 2007 these sites consistently had the most *H. cyanoguttatus* (Figs. 6). Marconi Lagoon also exhibited a significant (Sign Test, $p < 0.01$) increase in *H. cyanoguttatus* from 2006 to 2007, but the numbers were more similar to those seen at the Bayou St. John sites (Fig. 6). This significant increase is likely due to the fact that *H. cyanoguttatus* were only first collected in Marconi Lagoon in October 2006.

Salinity decreased significantly in ppt from 2006 to 2007 at four sampling sites: Bayou St. John North (7.472 to 6.283, Sign Test; $p < 0.01$), Central (7.336 to 6.100, Sign Test; $p < 0.01$), South (7.247 to 5.892, Sign Test; $p < 0.01$), and Marconi Lagoon (2.857 to 1.594, Sign Test; $p < 0.01$). Salinity did not change between the years at Pontchartrain Lagoon (Sign Test, $p = 1.00$) and Metairie Bayou (Sign Test, $p = 0.24$). Bayou St. John, South was the only sampling site to exhibit a significant change in temperature (23.38 to 22.57 degrees Celsius, Sign Test; $p = 0.03$), with 2007 being significantly colder than 2006.

Discussion

My sampling confirmed that *H. cyanoguttatus* occurs in Lake Pontchartrain in estuarine conditions, supporting the results of an earlier dispersal model that proposed this nominally freshwater fish species was capable of expanding via estuarine corridors (O'Connell et al. 2002). I also verified that populations of *H. cyanoguttatus* survived the 2005 hurricane-related levee failures and now continue to increase their numbers in water bodies in City Park such as Pontchartrain Lagoon, Metairie Bayou, and Marconi Lagoon. During the course of conducting these surveys I received important information regarding *H. cyanoguttatus* dispersal that was not

generated by my work but complements its results. The presence of *H. cyanoguttatus* in estuarine habitats was confirmed separately by underwater photography of pairs of *H. cyanoguttatus* in Lake Pontchartrain off of Pontchartrain Beach (pers. obs., J. Carroll, Jr., J. Van Vrancken). Similarly, in 2005 just prior to the hurricanes, I observed nesting pairs of *H. cyanoguttatus* in Lake Pontchartrain at salinities of 8.0 ppt, which is higher than previously observed in GNOMA. Although my 2005-2006 pre- and post hurricane surveys did not collect *H. cyanoguttatus* from the West Bank, these fish have since been collected from there regularly. These surveys provide a recent baseline for the establishment and expansion of *H. cyanoguttatus* in the GNOMA, although much data may have been missed. There was a low abundance of fishes caught in the seawall and the pre- and post-hurricane surveys. Because of the nature of the hurricanes as a 'natural experiment', the trapping methods were not changed, so that an accurate comparison could be made between 2005 and 2006. The actual abundance and distribution of *H. cyanoguttatus* and native species may not be accurately represented because of these low abundances.

The most common fish collected in seawall traps placed along the south shore of Lake Pontchartrain was *H. cyanoguttatus*. Monthly trapping over a year caught very few fishes overall, but this result is a combination of the targeted nature of the traps themselves and the low quality of the estuarine habitats on the artificial concrete seawall. My main goal was to determine if *H. cyanoguttatus* was in Lake Pontchartrain, so I designed the traps based on previous research targeting this species (Buchanan 1971). My goal was achieved but at the possible cost of collecting other fish species that occur in these habitats. The funnel traps are not appropriate for collecting native estuarine species such as spotted gar (*Lepisosteus oculatus*), bay anchovy (*Anchoa mitchilli*), and striped mullet (*Mugil cephalus*). These species and others were

consistently observed along the seawall during trapping yet were never collected by the traps. The low number of fishes trapped may also reflect how harsh this artificial habitat is. High-energy wave events, pollution from outfall canals, and a general lack of suitable substrates likely preclude most native fish from occupying these habitats for very long. It is interesting that the non-native *H. cyanoguttatus* is present in these degraded conditions, perhaps exploiting disturbed habitat that is unsuitable for native species. In the Colorado River in Texas, non-native tilapiine cichlids utilized "niche opportunities" created by human disturbance (Olden et al. 2006). Gonzales and Moran (2005) noted that *H. cyanoguttatus* was "observed to displace native Centrarchidae and dominate when aquatic conditions are less than optimal" in the San Antonio River. The significantly higher abundance of *H. cyanoguttatus* in urban habitats versus natural habitats may indicate this as a future concern regarding centrarchids in New Orleans.

Brackish waters are not barriers for cichlids, as was demonstrated by the presence of eight native and two non-native species of cichlids living sympatrically with marine species in an estuarine lagoon in Mexico (Chavez-Lopez et al. 2005). The Mayan cichlid (*Cichlasoma urophthalmus*), an invasive in South Florida, can tolerate salinity up to and exceeding that of seawater (Stauffer and Boltz 1994). Other invasive cichlids have similar levels of salinity tolerance (Lemarie et al. 2004, Schofield et al. 2007). Some tilapia species can even survive at lower temperatures when in saline conditions than they could survive in freshwater conditions. This could partially explain the success of Nile tilapia (*Oreochromis niloticus*) in Mississippi (Peterson et al. 2005). Brackish barriers can be barriers to invasive fishes (Dial and Wainright 1983, Scott et al. 2007), but certain species (notably cichlids) have a higher tolerance of brackish conditions than previously thought. In Florida, black acara (*Cichlasoma bimaculatum*) live in conditions previously thought to be too brackish for their survival (Kushlan 1986).

The 2005-2006 pre- and post hurricane surveys showed that range of *H. cyanoguttatus* extends throughout the GNOMA and specimens are occasionally collected in more natural habitats outside of the urbanized regions. While no *H. cyanoguttatus* were collected at either of the West Bank sampling sites (Algiers and Bayou Segnette) it should be noted that since these survey efforts there have been confirmed collections of multiple *H. cyanoguttatus* from urban localities on the West Bank (Algiers, Harvey Canal). The persistence of significant numbers of *H. cyanoguttatus* in urban habitats indicates that this species will likely continue to have the population numbers necessary to disperse beyond the GNOMA into more natural areas. It is not certain why the species has been found inconsistently in more natural areas, but there are a few possible explanations. Biotic resistance is one possibility (Olden et al. 2006). This could include predators and competitors, and there are species such as *L. miniatus*, which occurs where *H. cyanoguttatus* does not occur. Behavioral experiments suggest that *L. miniatus* is more aggressive toward *H. cyanoguttatus* than its more common centrarchid relative *L. macrochirus* (Lorenz and O'Connell, Chapter 2).

The 2005-2006 pre- and post hurricane survey results also suggest that abiotic factors seem to vary little or to have little effect on *H. cyanoguttatus* in both urban and natural habitats. Measures of hardness and pH did not vary consistently between city and urban sites. *Herichthys cyanoguttatus* does not slow its growth in salinities that exceed that of surrounding wetlands and Lake Pontchartrain (Lorenz and O'Connell, Chapter 3). Temperature limits the spread of cichlid species because of the tropical nature of this family of fishes (Hubbs 1951, Shafland and Pestrak 1982, Siemien and Stauffer 1989). Some of my personal observations suggest that *H. cyanoguttatus* congregate in thermal refuges within the GNOMA during colder months, although in their native range they survive under ice during freezing temperatures (Clark Hubbs, pers.

obs.). Preliminary results with temperature measuring data loggers have indicated that temperatures are slightly lower both in the summer and winter at the more natural Bayou Trepagnier site versus the less natural Bayou St. John site (Lorenz, unpub. data). Consistent sampling in Bayou Trepagnier has yielded few *H. cyanoguttatus* and it is only found there periodically. The lack of *H. cyanoguttatus* success in natural habitats like these may be due to lower temperatures or, quite possibly, the amount of variation in temperature is more important in affecting the species. Rapid increases or decreases in temperature may have detrimental effects on the health of *H. cyanoguttatus*. Port Acara cichlids (*Aequidens portalegrensis*) have difficulty adjusting to sudden increases in temperature when young and decreases in temperature when mature adults (Morris 1962). Because there have been years when *H. cyanoguttatus* has been found in some numbers in the wetlands to the west of the GNOMA, it could only be a small obstacle for them to overcome before it becomes more widespread. Actively managing to decrease GNOMA populations of *H. cyanoguttatus* may help reduce the number of propagules available for future dispersal into more natural habitats. Future research on what current barriers prevent establishment of *H. cyanoguttatus* could be investigated.

The patterns of presence/absence of *H. cyanoguttatus* in the GNOMA before and after the hurricanes can be interpreted in a couple of ways. Similar to other invasive species, *H. cyanoguttatus* appears to be very tolerant of disturbances and disturbed habitats. Invasive trees in Puerto Rico have lower mortality than native trees following hurricanes (Thompson et al. 2007). Bluegill (*L. macrochirus*), a native species, was the only species observed to significantly decline after the hurricanes and the invasive *H. cyanoguttatus* spread through City Park. Other sympatric species could have declined as well, but few individuals of any species other than *L. macrochirus* and *H. cyanoguttatus* were caught. Because *L. macrochirus* and *H. cyanoguttatus*

fight over territory in the field and under laboratory conditions (Courtenay et al. 1974, Lorenz and O'Connell, Chapter 2), this may be an indication of a negative impact of *H. cyanoguttatus* on native species. The fact that *H. cyanoguttatus* were not found in sites in 2006 that were occupied by them in 2005 may be relevant. The only conspicuous disappearance was in the heavily flooded Saint Bernard Parish. In Spain, invasive pumpkinseed sunfish (*Lepomis gibbosus*) increased in numbers during non-flooding years and decreased significantly with heavy flood regimes (Bernardo et al. 2003).

The survival and subsequent significant increase of *H. cyanoguttatus* populations at our City Park sampling sites and elsewhere in the GNOMA further show the persistent and resilient nature of this non-native fish species. The inundation of these urban habitats and subsequent flood waters likely provided *H. cyanoguttatus* direct routes for dispersal (Caillouet et al. in press). Their success in habitats where they had been either absent or in low numbers before 2005 suggests an ability to rapidly colonize available areas. Although I measured no significant increase in *H. cyanoguttatus* numbers from our three Bayou St. John sites in 2007, populations persist at all of these sites. A possible explanation for why *H. cyanoguttatus* did not increase over that time is that Bayou St. John experienced a marked increase in populations of *M. salmoides* in 2006 that significantly impacted other resident species (O'Connell and O'Connell, unpublished abstract). Regardless of whether the population increase of this native predator species was caused by over stocking or a natural post storm increase in numbers, it suggests the possible use of native piscivores to reduce non-native fish populations.

My results also suggest the limited role that salinity and temperature play in determining the success or failure of *H. cyanoguttatus* within these urban habitats. While all three of the Bayou St. John sites became significantly fresher from 2006 to 2007, the numbers of *H.*

cyanoguttatus did not change significantly. These sites experienced maximum salinities of 7.7 to 8.1 ppt during 2006 yet *H. cyanoguttatus* are still present and nesting at all of these sites, just as they do with low salinities. Temperatures in the three City Park sites remained similar in the two years, yet *H. cyanoguttatus* numbers increased significantly. Based on these results, the results from my other surveys where *H. cyanoguttatus* was collected at similar salinities and temperature, and the results from other laboratory experiments on abiotic influences (Lorenz and O'Connell, in review), I feel that these two factors are not strongly restrictive to the future expansion of this non-native species. As an aside, the significantly colder temperatures at Bayou St. John south in 2007 are possibly due to shade-giving emergent vegetation that was planted at the sampling site as part of habitat restoration efforts.

Acknowledgments

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CHAPTER 2

Aggressive interactions between the invasive Rio Grande cichlid (*Herichthys cyanoguttatus*) and two native centrarchids: bluegill (*Lepomis macrochirus*) and redspotted sunfish (*Lepomis minatus*)

Abstract

The Rio Grande cichlid (*Herichthys cyanoguttatus*) has been established in the Greater New Orleans Metropolitan area for at least 20 years and the effect of this species on native fishes is unknown. Behavioral trials were performed to determine if aggressive interactions occur between invasive *H. cyanoguttatus* and native bluegill (*Lepomis macrochirus*). When defending a territory on which it had established prior residence, *L. macrochirus* were markedly aggressive, averaging 11.625 aggressive actions per 10-minute behavioral trial. In contrast, *L. macrochirus* were extremely passive as invaders, with 0.525 aggressive actions per trial. *Herichthys cyanoguttatus* were equally aggressive as prior residents and as invaders, averaging 4.875 and 6.025 aggressive actions per trial, respectively. *Herichthys cyanoguttatus* interacted aggressively with native species whether they held territory or not, indicating that this invasive species may have fundamentally different strategies of aggression than native *L. macrochirus*. Aggressive actions of prior resident *L. miniatus* were higher than cichlids or bluegill in any situation, indicating possible variation in aggression within *Lepomis.* These behavioral differences may explain the continued success of *H. cyanoguttatus* as an invasive fish in southeastern Louisiana.

Introduction

Invasive species pose a significant threat to populations of native organisms (Vitousek et al. 1997) and the number of non-native species that are discovered increases steadily (Crossman 1991, Fuller et al. 1999). For example, the Rio Grande cichlid (*Herichthys cyanoguttatus*) has been established in natural and degraded habitats of the Greater New Orleans Metropolitan Area (GNOMA) for twenty to thirty years (Fuentes and Cashner 2002, O'Connell et al. 2002). This species is native to the southern drainages of the Rio Grande River with its type locality being Brownsville, Texas (Baird and Girard 1854). Populations in the United States were once restricted to the southern half of the Rio Grande River Basin but now, due to planned introductions, *H. cyanoguttatus* occurs in many parts of Texas, including the Guadalupe River basin, the San Antonio area, and the Edwards Plateau region (Brown 1953, Hubbs et al. 1978, Fuller et al. 1999). Outside of Texas, the only established populations in the United States are in Florida and Louisiana (Fuller et al. 1999).

The potential threat of *H. cyanoguttatus* is unclear. It appears to compete for breeding sites with native centrarchids such as bluegill (*Lepomis macrochirus*) in Florida (Courtenay et al. 1974), and I have observed aggressive interactions between these species in Louisiana. My unpublished field observations have revealed that *H. cyanoguttatus* also acts aggressively toward native largemouth bass (*Micropterus salmoides*), western mosquitofish (*Gambusia affinis*), sailfin mollies (*Poecilia latipinna*), and blue crabs (*Callinectes sapidus*). This type of aggression by invasive species can inhibit growth and reproduction as well as shift habitat use of native species (Marchetti 1999, Schrank et al. 2003). For example, aggression of *H. cyanoguttatus* appears to cause reproductive failure of native sheepshead minnows (*Cyprinodon variegatus*) in experimental pools (Mire, 2001).

I observed interactions between invasive *H. cyanoguttatus* and native *L. macrochirus* under laboratory conditions to determine what levels of aggression were exhibited by different species in different contexts (invader or resident). I also conducted similar observations (though with fewer trials) on interactions between *H. cyanoguttatus* and native redspotted sunfish (*L. miniatus*), a particularly aggressive native centrarchid. In general, most species of the families Cichlidae and Centrarchidae are considered aggressive freshwater fishes with members of both groups employing aggression in interspecific and intraspecific interactions. Intraspecific behavioral studies have shown that *H. cyanoguttatus* are aggressive as invaders and as residents (Turner 1994, Draud et al. 2004, Leiser et al. 2004) and *L. macrochirus* are aggressive as territory-holding adult males (Colgan et al. 1978). Relatively few studies have examined interspecific aggression within these families (Colgan and Gross 1977, Matsumoto and Kohda 2004), and cichlid-centrarchid interactions have never been studied. Such a study could demonstrate behavioral effects of invasive species and better explain success of non-native fishes in the wild. Therefore, I conducted behavioral trials in aquaria to determine what aggressive strategies are exhibited by *H. cyanoguttatus*, *L. macrochirus*, and *L. miniatus* during interspecific interactions.

Materials and Methods

Most fishes used in the trials (86 *H. cyanoguttatus*, 80 *L. macrochirus*, and 6 *L. miniatus*) were collected from canals and bayous in the GNOMA from January to August 2007 using electrofishing, seining, and trapping. Additional *L. macrochirus* were obtained from a local hatchery to supplement the number of wild-caught fish. All *H. cyanoguttatus* and *L. macrochirus* were juveniles (range = 40 to 100 mm SL) and were given at least a week to

acclimate to laboratory conditions. The six *L. miniatus* (range = 90 to 130 mm SL) may have been large enough to be adults (Ross 2001). Species were maintained separately in 400-liter aquaria prior to trials. All fishes were fed *ad libitum* with commercial fish food and aquaria were filtered by air-driven sponge filters. Water conditions (temperature = 24.5º C, pH = 7.5) were held constant throughout the trials and no mortalities occurred during these trials. Fishes were either kept for other experiments or sacrificed by freezing after the trials. All of the *L. miniatus* were donated to a local aquarium for a native species display.

Behavioral trials were conducted in 75-l aquaria containing gravel substrate and an air-driven sponge filter. Based on similar behavioral trials (Wazlavek and Figler 1989, Ratnasabapathi et al. 1992, Glova 2003, Metcalf et al. 2003), fishes were introduced to aquaria and allowed 24 h to acclimate to either a clay pot or a portion of PVC pipe (the "territory"). After 24 h of residence, an opaque divider was inserted into the middle of the aquarium and a fish of similar size but different species was introduced to the opposite side. This "invader" was also provided a temporary territory upon introduction to the aquarium (to reduce handling stress). After 10 minutes, the territory and divider were removed and all interactions were videotaped for 10 minutes. To minimize observer effects, no humans were in the room during videotaping. After each trial, both fishes were removed with a net, measured to the nearest mm standard length (SL), and weighed to the nearest g. Individual fish were used only once during these behavioral trials. Forty (40) trials were conducted with *L. macrochirus* as the prior resident and *H. cyanoguttatus* as the invader, with 20 trials having a *H. cyanoguttatus* larger than the prior resident and 20 trials having a *H. cyanoguttatus* smaller than the prior resident. No invading fish were more than twice or less than half the size of the resident. This approach was repeated for

another 40 trials with a *H. cyanoguttatus* as the prior resident and a *L. macrochirus* as the invader.

A similar approach was taken to observe *H. cyanoguttatus*-*L. miniatus* interactions, though only six total trials were conducted due to a lack of available *L. miniatus*. All trials involved *L. miniatus* as the prior resident. As with the other trials, half (3) of the trials involved a *H. cyanoguttatus* smaller than the resident and half involved a *H. cyanoguttatus* larger than the resident. For these six trials, both sets of *H. cyanoguttatus* and *L. miniatus* were larger, adult fish (range = 90 to 130 mm SL).

From the videotape, I recorded the number and type of aggressive behaviors exhibited by the fishes. Specifically, I counted the number of:

1. lunges (a fish moving toward its opponent with flared opercula);

2. lateral displays (a fish turning its body sideways to its opponent with fins spread and usually undulating); and

3. chase/bites (an escalated behavior when a fish either intends injury by chasing or actually inflicts injury with a bite).

This is a rough order of how cichlids sequentially assess one another in contests (Enquist et al. 1990). These behaviors were then summed together to obtain 'total aggressive behavior' counts for each fish. If a fish repeated the same behavior multiple times without any behavioral

interaction from the opponent (e.g., several consecutive lunges), the behavior was only counted once. Differences in the number of each type of aggressive behaviors and the total number of aggressive behaviors were analyzed using a two-way ANOVA (SPECIES effect and RESIDENCE effect) and Bonferroni Multiple Comparison Tests were conducted to make post hoc comparisons. The same analyses were used to determine if size affected number of aggressive actions by residents or invaders of each species. Analyses were carried out using SYSTAT v. 10.2.01.

Results

The two-way ANOVA indicated a significant effect of RESIDENCE ($F_{(1,156)} = 31.361$, $p < 0.001$, Figure 7) on the total number of aggressive acts of *H. cyanoguttatus* and *L. macrochirus*. The effect of RESIDENCE X SPECIES was also significant ($F_{(1,156)} = 47.535$, $p < 0.001$). The effect of SPECIES was nonsignificant.

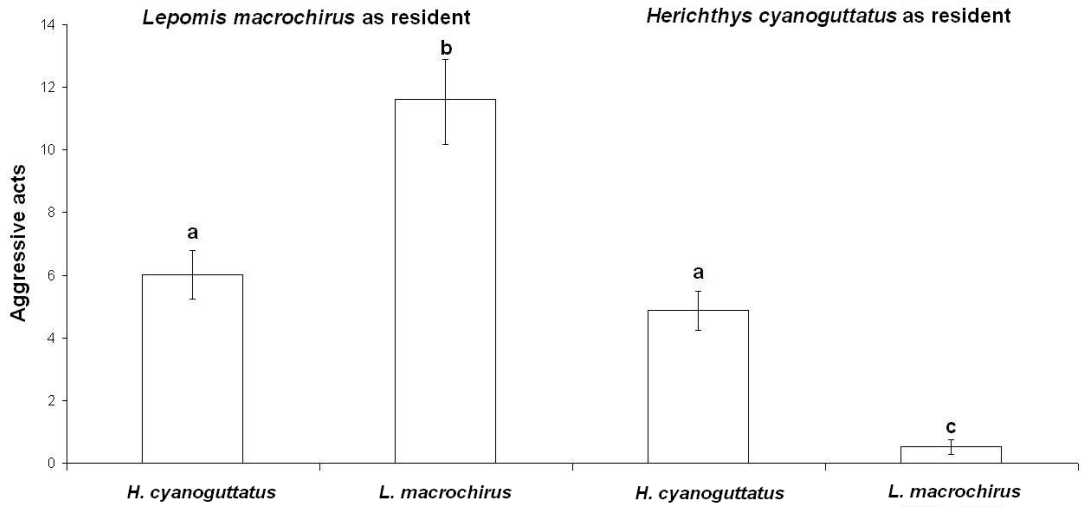

Figure 7. Mean (± 1 S.E.) number of aggressive acts of *H. cyanoguttatus* and *L. macrochirus*. *L. macrochirus* aggression as residents was significantly higher than when they were prior residents (bonferroni post-hoc, $p < 0.001$). *H. cyanoguttatus* aggression as invaders did not differ significantly when they were residents.

As prior residents, *L. macrochirus* were significantly more aggressive than invading *H. cyanoguttatus*, prior resident *H. cyanoguttatus*, and invading *L. macrochirus* (Bonferroni post-hoc, $p < 0.001$). The number of aggressive acts exhibited by *H. cyanoguttatus* either as prior residents or invaders was not significantly different (Bonferroni post-hoc, $p = 0.864$). Invading *L. macrochirus* had the least number of aggressive behaviors and the number was significantly less than that exhibited by prior resident *L. macrochirus* (Bonferroni post-hoc, $p < 0.001$) and invading *H. cyanoguttatus* (Bonferroni post-hoc, $p < 0.001$). Invading *L. macrochirus* were also significantly less aggressive than resident *H. cyanoguttatus* (Bonferroni post-hoc, $p < 0.005$). The number of *L. macrochirus* aggressive behaviors as prior residents was similar to total the number of aggressive behaviors taken by *H. cyanoguttatus* as invaders and defenders combined.

The number of specific aggressive behaviors (i.e., lunges, lateral-displays, and chase/bites) differed between *H. cyanoguttatus* and *L. macrochirus* (Two-way ANOVA, $F_{(11,468)} = 26.102$, $p < 0.001$). The most common behavior for *H. cyanoguttatus* was lunging, both when invading and defending (Bonferroni post-hoc, $p < 0.001$) (Figure 8).

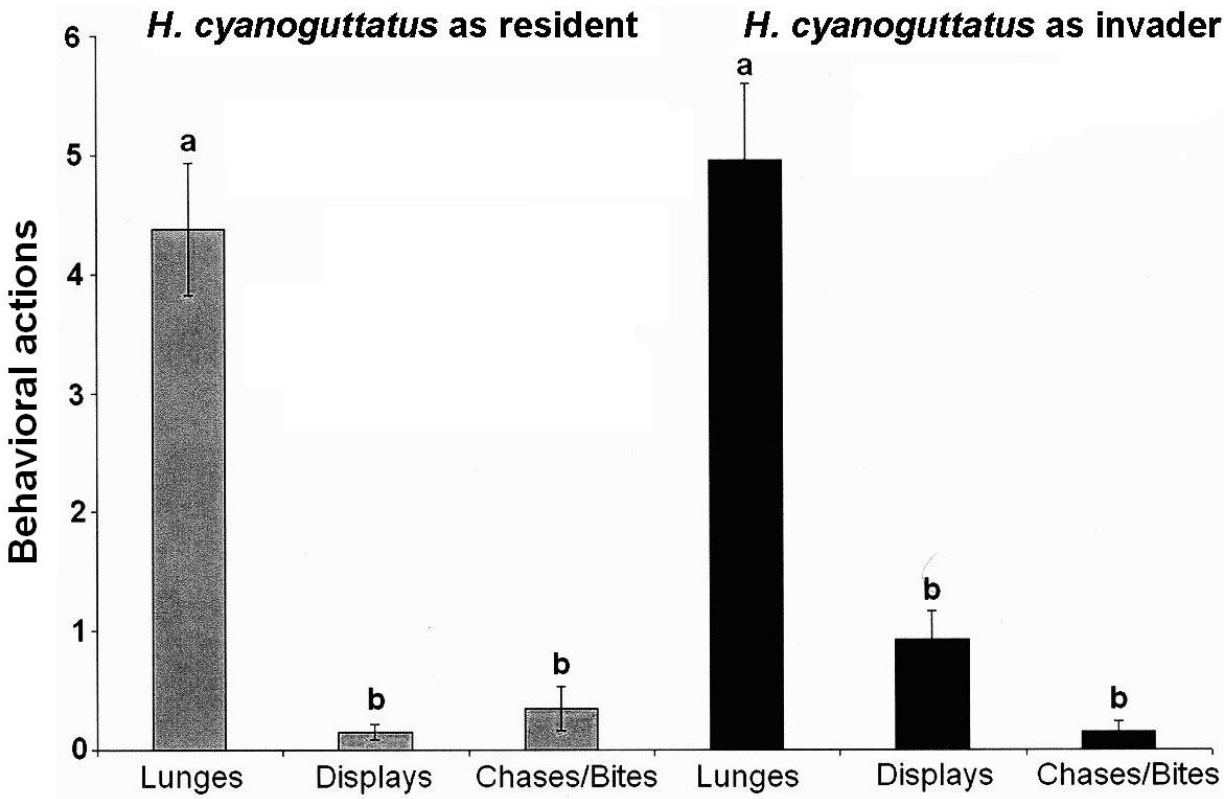

Figure 8. Mean (± 1 S.E.) number of specific behaviors of *H. cyanoguttatus*. Lunges, as residents and as invaders, were significantly more common than all other behaviors (bonferroni post-hoc, $p < 0.001$).

Prior resident *L. macrochirus* exhibited significantly more lateral displays than either lunges or chase/bites (Bonferroni post-hoc, $p < 0.001$, Figure 9). There was an effect of size on number of aggressive actions (Two-way ANOVA, $F_{(3,152)} = 2.856$, $p = 0.039$). Invading cichlids had significantly more aggressive behaviors when larger than when smaller than the resident bluegill (Bonferroni post-hoc, $p < 0.05$). Size was shown to not affect number of aggressive behaviors of invading bluegill, resident bluegill, or resident cichlids.

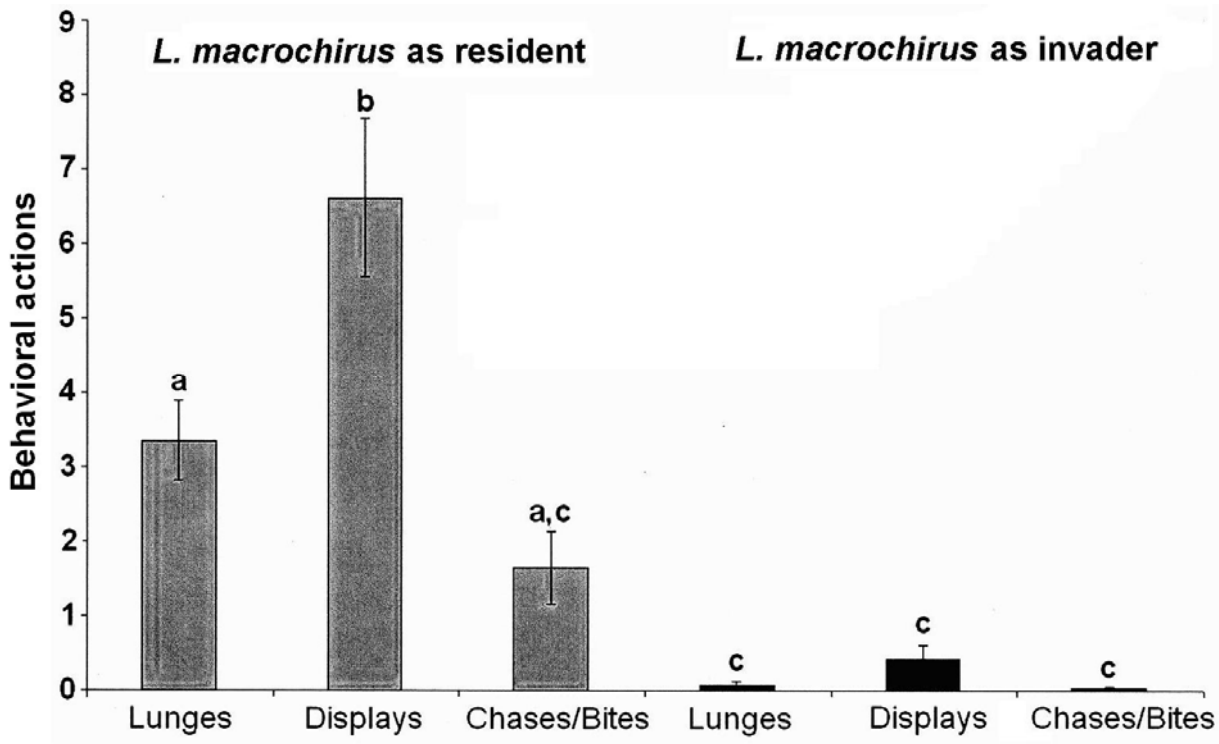

Figure 9. Mean (± 1 S.E.) number of specific behaviors of *L. macrochirus*. Lateral display, as residents, was the most common behavior (bonferroni post-hoc, $p < 0.001$).

The six *H. cyanoguttatus*-*L. miniatus* trials included three with larger *H. cyanoguttatus* invaders and three with smaller *H. cyanoguttatus* invaders. Resident *L. miniatus* were significantly more aggressive than *H. cyanoguttatus* and when compared to aggressive acts of resident *L. macrochirus* ($F_{(5,166)} = 25.123$, $p < 0.001$, Figure 4).

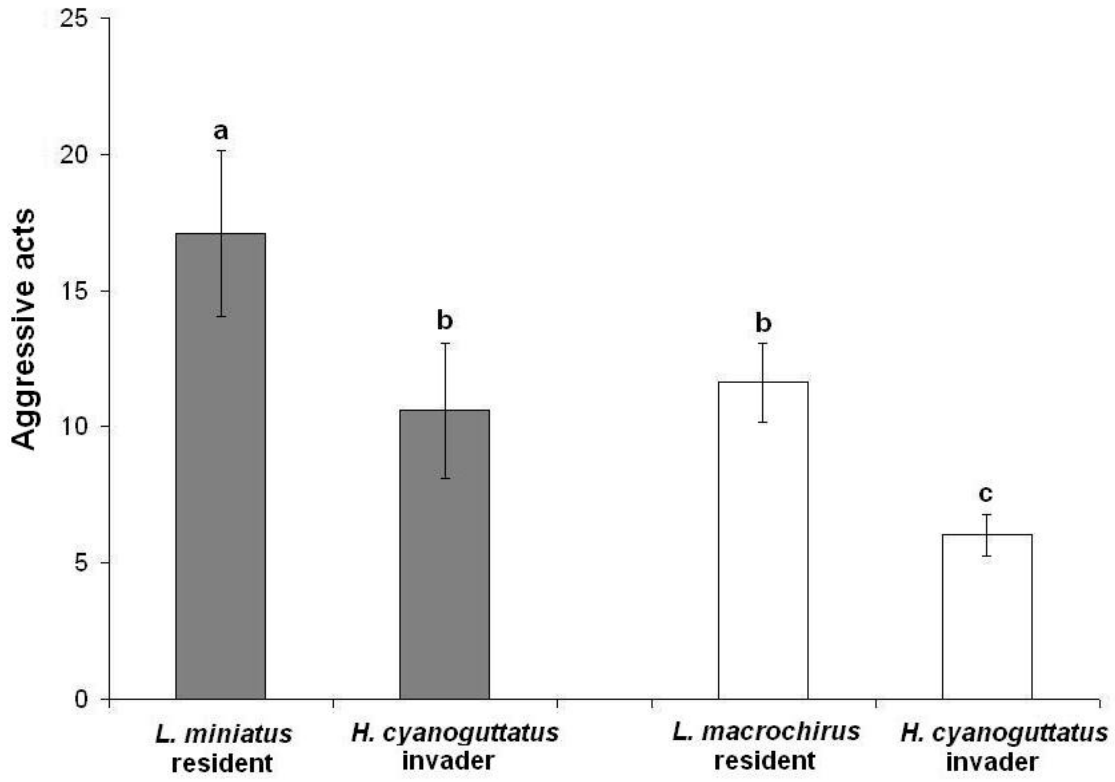

Figure 10. Mean (± 1 S.E.) number of aggressive acts of resident *L. miniatus* (redspotted sunfish) with invading *H. cyanoguttatus*. When compared to resident *L. macrochirus*, there are significantly more aggressive actions by *L. miniatus* (bonferroni post-hoc, $p<0.05$). Invading cichlids are also more aggressive in *L. miniatus* resident trials (bonferroni post-hoc, $p<0.05$).

Lepomis miniatus exhibited more aggressive acts than prior resident *L. macrochirus* (Bonferroni post-hoc, $p < 0.05$) and more aggressive acts than the *H. cyanoguttatus* that were invading the *L. miniatus* (Bonferroni post-hoc, $p < 0.01$). *H. cyanoguttatus* invading resident *L. miniatus* exhibited more aggressive actions than *H. cyanoguttatus* invading *L. macrochirus* (Bonferroni post-hoc, $p < 0.05$).

Discussion

Non-native *H. cyanoguttatus* showed consistent levels of aggression as intruders and as residents, whereas native *L. macrochirus* were only aggressive as prior residents. Native *L. miniatus* were also aggressive prior residents, even more so than *L. macrochirus*. These data show *H. cyanoguttatus* to be aggressive towards native fishes in two different contexts (as resident and invader). The difference in behaviors between the species could indicate distinct game theory strategies. Non-native *H. cyanoguttatus* appear 'hawk-like' (*sensu* Maynard-Smith 1974) because it is aggressive in all encounters, even considering the asymmetry of residence. Native *L. macrochirus* appear to be 'hawk-like' while defending territory, but 'dove-like' while invading territory. This would categorize them as 'bourgeois-like' (Maynard-Smith 1974), and note that as residents they are significantly more aggressive than the "hawk-like" *H. cyanoguttatus*. The actual game theory strategies of these species can only be discovered by examining the costs of fighting and the benefits of holding these territories. Further study of these aspects would be very important to discover if the aggressive strategies displayed here have a context in evolutionary strategies.

Species with a "hawk-like" strategy (such as we have shown here in *H. cyanoguttatus*) typically do best in situations that lack other hawks (Maynard-Smith 1982). It may be worthwhile relative to predicting future effects of *H. cyanoguttatus* to determine if other native fishes in southeast Louisiana exhibit "hawk-like" behavior and could offer some biotic resistance to the non-native. It is possible that *H. cyanoguttatus* limit their success by persisting at low frequencies, but losing their advantage at high frequencies. The original "hawk-dove" game showed persistence of both strategies due to frequency-dependent selection.

For most fishes, sequential assessment is a valuable strategy involving evaluation of opponents with displays before causing damage to each other (Enquist et al. 1990). This strategy was employed by both species in this study. Lateral displays and lunges were used before chasing and biting. There was a nonsignificant increase of lateral displays for larger *H. cyanoguttatus*. This may indicate the desire of *H. cyanoguttatus* to communicate its advantage in size to prevent any damaging combat. However, lateral displays are the most common display for *L. macrochirus* and lunges are the most common display for *H. cyanoguttatus*. Escalated behavior, chasing and biting, was most common with *L. macrochirus* that were defending territory. This indicates the perceived value of the territory for prior resident *L. macrochirus*, which were willing to risk injury by engaging in costly behavior. The interspecies communication of sequential assessment only seemed to break down at the occasional attempt by *H. cyanoguttatus* to 'mouth wrestle', which is a common aggressive behavior in cichlids (Draud and Lynch 2002). The native *L. macrochirus* did not engage in this behavior and turned away from such challenges. Interestingly, *L. miniatus* chose to bite *H. cyanoguttatus* that attempted to mouth wrestle. The higher level of aggression of *H. cyanoguttatus* when matched with *L. miniatus* indicates that *H. cyanoguttatus* may be able to adjust its level of aggression depending on species. Such communication differences between species are likely relevant to the success and effect of an invading fish species.

Many aspects of natural interactions in the field are absent in laboratory aquarium experiments. For territoriality to be observed, the habitat should be a limited and valuable resource for both species. Size of territories is also relevant. Convict cichlids (*Archocentrus nigrofasciatus*) have a cost associated with increasing territory size (Breau and Grant 2002). In conjunction with territory size, fish density will also be important. In the GNOMA canals, *H.*

cyanoguttatus live in very high densities and the combination of this with their 'hawk-like' behavior may be significant. Either this could follow game theory and cause the cichlids to be self-limiting, or the sometimes-gregarious nature of *H. cyanoguttatus* may help prevent such self-regulation. There is also the issue of multiple species interactions, which has rarely been examined. There are costs to different game theory strategies that are dependent on the resources but also on the strategies of other individuals and other species. This is relevant because new strategies, such as a 'sneaker' stealing the resource (Dubois et al. 2004) could exploit the "aggressive neglect" of hawk strategies (Wilson 1975). Large schools of fish can also overwhelm territorial fish as seen in the "St. Ignatius effect". This is seen when there are too many fish to defend a territory against (Barlow 1974). With an invasive species, it is important to examine how its strategy interacts with native strategies. This all must be viewed as speculative, however, until costs and payoffs for these strategies have been elucidated.

In addition to prior residence there are other asymmetries. Different genders may place different value on territory or asymmetries (Draud et al. 2004). Size is important in determining the outcome of contests in cichlids (Barlow et al. 1986, Wazlavek and Figler 1989, Keeley and Grant 1993, Turner 1994). Considering the large amount of *L. macrochirus* aggressive behavior regardless of *H. cyanoguttatus* invader size, *L. macrochirus* may not consider size as important as residence. An independent study of *L. macrochirus*-*L. macrochirus* interactions, using size and residence, could demonstrate potential intraspecific differences in *L. macrochirus* strategies. *Herichthys cyanoguttatus* was significantly more aggressive as a large invader than as a small invader, possibly indicating that *H. cyanoguttatus* recognized their size advantage. Temperature would also be a potential factor, especially because the family Cichlidae is a tropical family of fishes that shows more aggression at higher temperatures (Ratnasabapathi et al. 1992).

Introduced Eastern mosquitofish (*Gambusia holbrooki*) showed less aggression towards native Spanish toothcarps (*Aphanius iberus* and *Valencia hispanica*) when temperatures were lower (Rincon et al. 2002). Breeding condition also can create an asymmetry. Male contests were determined by testes size more than fish weight in another cichlid species, redbelly tilapia (*Tilapia zillii*) (Neat 1998).

The aggression of juvenile fish in the present study suggests that territoriality is not just restricted to breeding adults, though strategies are known to change in fishes when they become larger and more capable of inflicting damage (Leiser et al. 2004). It also suggests that the territory being defended is more likely associated with increased food resources or protection from predators. The convict cichlid, *Archocentrus nigrofasciatus*, will defend predictable resources of food (Grand and Grant 1994). However, both *H. cyanoguttatus* and *L. macrochirus* have generalist feeding strategies and *L. macrochirus* leaves a food patch when food resources are plentiful (Wilhaber et al. 1994), indicating that food patches are not a critical item for them to defend. Studies of aggressive behavior under different levels of predation pressure and cover could demonstrate the relative importance of these variables to individual species.

Aggression of *H. cyanoguttatus* in these trials may play a role in affecting native fishes, especially since similar aggression has been observed in the field (Courtenay et al. 1974, pers.obs.). My results suggest a more specific explanation as to why *H. cyanoguttatus* continues to expand its range in Louisiana. There is an energetic cost to aggression and these trials show that there is typically a contest of long duration between *H. cyanoguttatus* and *L. macrochirus* if the sizes are close, especially if *L. macrochirus* are the residents. This is a serious concern, because there are high densities of *H. cyanoguttatus* in the areas where it is found, and prior residence will not dissuade *H. cyanoguttatus* from aggressively interacting with native species.

Invasive species that initiate aggressive encounters can displace native species, as has been seen with the invasive Argentine ant (*Linepithema humile*; Human and Gordon 1999). Other studies show effects of invasive species on natives via aggressive behavior (Gunckel et al. 2002, Warburton and Madden 2003, Klocker and Strayer 2004, Dame and Petren 2006). Further examination of behavioral interactions between native and non-native species can help resource managers identify species that pose the greatest threat to native communities.

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CHAPTER 3

Effects of salinity and interspecific competition with native bluegill (*Lepomis macrochirus*) **on growth of non-native Rio Grande cichlids (*Herichthys cyanoguttatus*)**.

Abstract

The Rio Grande cichlid (*Herichthys cyanoguttatus*) is a non-native fish that has become established in freshwater and estuarine habitats in the Greater New Orleans Metropolitan Area, where it may be competing with native species. I conducted laboratory experiments to determine if *H. cyanoguttatus* growth was affected either by salinity or the presence of native bluegill (*Lepomis macrochirus*). There was no significant difference in growth of *H. cyanoguttatus* held at 0, 8, or 16 ppt for 14 d. When *H. cyanoguttatus* and *L. macrochirus* were held at 0 ppt for 28 d both with and without the other species present, there was a significant difference in growth between species. *Lepomis macrochirus* grew about twice as fast as *H. cyanoguttatus*, regardless of whether fish were held alone or with a competitor of the other species. A second 28 d trial compared growth of individual *H. cyanoguttatus* and *L. macrochirus* held together with different relative sizes (half with a larger *H. cyanoguttatus* as invader, half with a smaller *H. cyanoguttatus* as invader). This experiment showed no significant difference in growth between the species. These results indicate that moderate salinity levels will not limit dispersal of *H. cyanoguttatus* in southeastern Louisiana, whereas biotic resistance from (or competition with) native *L. macrochirus* may affect the success of this non-native species.

Introduction

The Rio Grande cichlid (*Herichthys cyanoguttatus*) has been established in canals of the Greater New Orleans Metropolitan Area (GNOMA) for over 20 years (Fuentes and Cashner 2002, O'Connell et al. 2002). This non-native species occurs at high densities throughout much of the urbanized portions of GNOMA and has been found in lower densities in natural areas outside of the city (Fuentes and Cashner 2002, O'Connell et al. 2002, Lorenz and O'Connell, Chapter 1). I have observed this species interacting aggressively with native species and they compete for breeding sites with bluegill (*Lepomis macrochirus*) in Florida (Courtenay et al. 1974). There are many possible reasons for the limited number of *H. cyanoguttatus* collected outside of the GNOMA. These fish may prefer the disturbed habitat of urban canals, which may serve as thermal refugia during colder periods (e.g., concrete canals may retain heat longer than natural bayous). It is also possible that the brackish habitats of surrounding swamps and estuarine habitats of Lake Pontchartrain are uninhabitable to them. Salinity appears to have little effect on the Mayan cichlid (*Cichlasoma urophthalmus*), which can tolerate salinity up to and exceeding that of seawater (Stauffer and Boltz 1994). Other invasive cichlids, especially tilapiine cichlids, have similar levels of salinity tolerance (Lemarie et al. 2004). Some tilapiine species can even survive at lower temperatures when in saline conditions, which could partially explain the success of Nile tilapia (*Oreochromis niloticus*) in Mississippi (Peterson et al. 2005).

Competition for food or other resources between native and non-native fishes may either negatively affect native species or serve as a form of biotic resistance against the dispersal of *H. cyanoguttatus*. For example, *H. cyanoguttatus* may not fare well outside of the GNOMA because there are more species to compete with for food. Conversely, it is also possible that the generalist diet of *H. cyanoguttatus* allows them to compete for food against a variety of species.

One native generalist, the bluegill (*Lepomis macrochirus*), survives alongside *H. cyanoguttatus* in the canals. The coexistence of native and non-native fishes may be due to the adaptive abilities of *L. macrochirus* in disturbed habitats which may include the aggressive traits of *L. macrochirus*. As an invasive fish in California, *L. macrochirus* that interacted with native Sacramento perch (*Archoplites interruptus*) were highly competitive (Marchetti 1999) and *L. macrochirus* were also aggressive when defending territories against *H. cyanoguttatus* when they were the prior resident in territorial trials (Lorenz and O'Connell, Chapter 2). Determining the competitive outcome of possible *H. cyanoguttatus* - *L. macrochirus* interactions would allow us to better predict whether the non-native fish will continue to disperse beyond the urban limits of the GNOMA.

I conducted laboratory experiments to determine if *H. cyanoguttatus* growth was affected either by salinity (an abiotic factor) or the presence of *L. macrochirus* (a biotic factor). More specifically I asked the following questions: 1) Are there differences in *H. cyanoguttatus* growth among three levels of salinity (0, 8, and 16 ppt)? 2) Does *H. cyanoguttatus* growth differ when held with a group of conspecifics versus a group of *L. macrochirus*? 3) Is *H. cyanoguttatus* growth affected when interacting one-on-one with *L. macrochirus* of different sizes?

Materials and methods

Salinity growth study

Juvenile *H. cyanoguttatus* (weight range: 0.5 - 1.5 g) were collected from local GNOMA populations by seining and trapping. When returned to the laboratory, these fish were observed for 24 h to ensure their health and suitability for testing. Fifteen individuals were then weighed and placed individually in fifteen separate 37 l aquaria. The temperature was kept at 23.0 º C

and lighting was controlled for 12-hour days. All fish were fed ad libitum every day with Hikari brand food sticks.

Salinity was raised by 2.3 ppt a day, allowing the 8 ppt and 16 ppt treatments to be completed within a week. The brackish treatments had the necessary amount of 32 ppt water added daily for this week. If necessary, water was removed from aquaria to allow for this increase in salinity. The aquaria with no salinity treatments were stirred to stimulate the same amount of disturbance. Fish were then allowed to grow for a second week without salinity increase. All fish were weighed and measured (standard length) at the end of the trials (total time 14 days).

Competition study

Juvenile *H. cyanoguttatus* and *L. macrochirus* used in the group trials were of similar sizes to each other (weight range: 10 - 20 g). As with the salinity trials, *H. cyanoguttatus* were collected locally whereas *L. macrochirus* were acquired from a fish hatchery where they likely had never had contact with *H. cyanoguttatus*. Group trials were conducted in 15 hard rubber tubs (400 l), each fitted with an air-driven sponge filter. Temperature was kept at 22.0 º C and lighting was controlled for 12-hour days. The three group treatments were: 1. six *H. cyanoguttatus* alone (5 replicates); 2. six *L. macrochirus* alone (5 replicates); and 3. three *H. cyanoguttatus* held with three *L. macrochirus* (5 replicates). Fishes were fed Hikari food sticks and the daily ration was calculated as 2% of the total weight all fishes in any given tub. Aquaculture has used similar percentages of food for fish growth (Davis 1991). This ration was continued for the entire 28 d and all fishes were weighed and measured (standard length) at the end of the trials.

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H. cyanoguttatus growth: interspecific one-on-one trials

For one-on-one trials, all conditions (e.g., tubs, light cycle, feeding proportions, etc.) were identical to the group trials except that temperature was held slightly higher at 23.5 º C. Each trial was performed in one of 14 different tubs. For each trial, a single *L. macrochirus* was introduced to a tub containing a 7.5 cm diameter PVC elbow pipe territory that provided these fish refugia. A single *H. cyanoguttatus* was introduced to the tub a day later. During this one-day period, *L. macrochirus* were not fed to avoid an artificial increase in growth from an extra day of feeding. Introduced *H. cyanoguttatus* were either larger or smaller than the resident *L. macrochirus*. Again, fish were fed 2% of the total weight of fish in each tub for 28 d and all fishes were weighed and measured (standard length) at the end of the trials.

Results

Salinity had no effect on *H. cyanoguttatus* growth (ANOVA, $F_{(2,11)} = 1.866$, $p = 0.201$; Fig. 11). One *H. cyanoguttatus* in the 16 ppt treatment died during the trial, so the final average mass increase is based on n = 4. In the intra- and interspecific group trials, there was a significant difference in growth among the three treatments (ANOVA, $F_{(3,12)} = 17.230$, $p < 0.001$). Groups of *L. macrochirus* held with conspecifics experienced more growth than groups of *H. cyanoguttatus* held with conspecifics and groups of *H. cyanoguttatus* held with *L. macrochirus* ($p < 0.005$, Bonferroni). Groups of *L. macrochirus* held with groups of *H.*

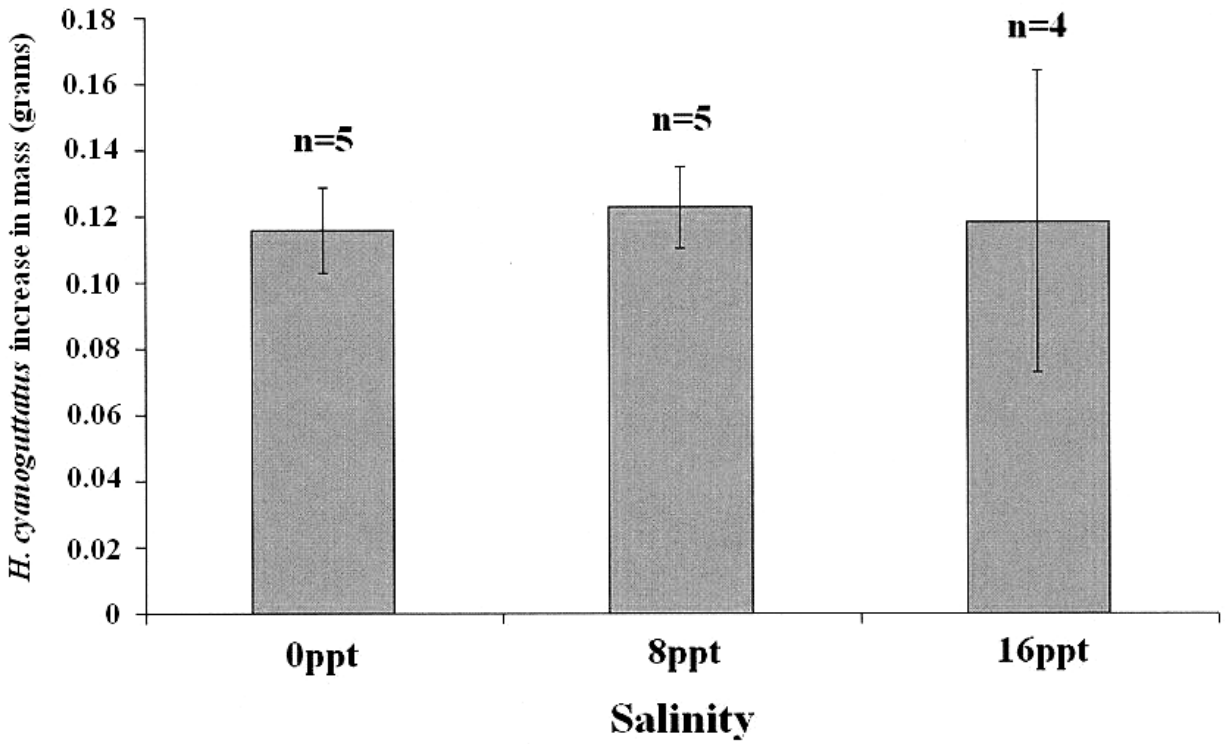

Figure 11. Mean (± 1 S.E) growth of *H. cyanoguttatus* fed ad libitum at different salinities for 14 days. No significant differences among treatments were observed ($p = 0.210$).

cyanoguttatus had more growth than groups of *H. cyanoguttatus* held with conspecifics and groups of *H. cyanoguttatus* held with *L. macrochirus* ($p < 0.001$, Bonferroni). The average increase in mass overall was 2.82 g for *L. macrochirus* and 1.31 g for *H. cyanoguttatus* (Fig. 12).

In the interspecific one-on-one trials, there was no significant difference in growth between *H. cyanoguttatus* and *L. macrochirus* regardless of the size (bigger or smaller than the resident *L. macrochirus*) of the introduced *H. cyanoguttatus* ($F_{(1,26)} = 3.731$, $p = 0.064$). The average change in mass for individual *H. cyanoguttatus* was negative (- 0.014 g) whereas individual *L. macrochirus* grew an average of 0.582 g (Fig. 13).

There was also no significant difference in growth within or between larger or smaller introduced *H. cyanoguttatus* and larger or smaller resident *L. macrochirus* ($F_{(1,24)} = 2.298$, $P = 0.103$).

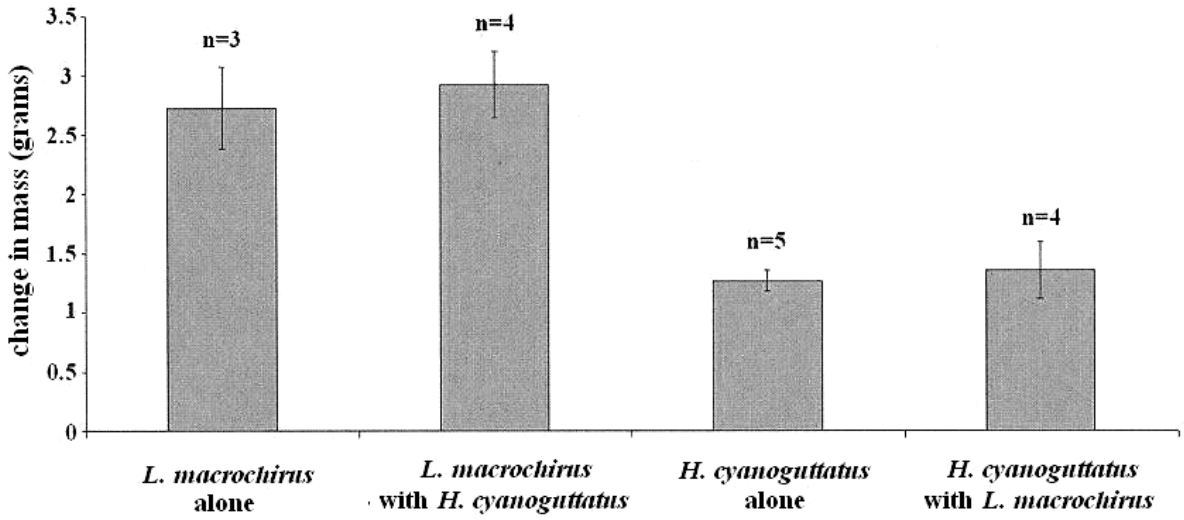

Figure 12. Mean (± 1 S.E) growth of *H. cyanoguttatus* and *L. macrochirus* when kept intraspecifically and interspecifically over 28 days. Both intra- and interspecifically, *L. macrochirus* grew at faster rates ($p < 0.005$).

There was a trend for larger *H. cyanoguttatus* to average negative growth (-3.97 g) while smaller invading *H. cyanoguttatus* had slightly positive growth (0.186 g). Both sets of individual *L. macrochirus* exhibited positive growth with larger residents showing less average growth (0.267 g) than smaller *L. macrochirus* residents (0.897 g).

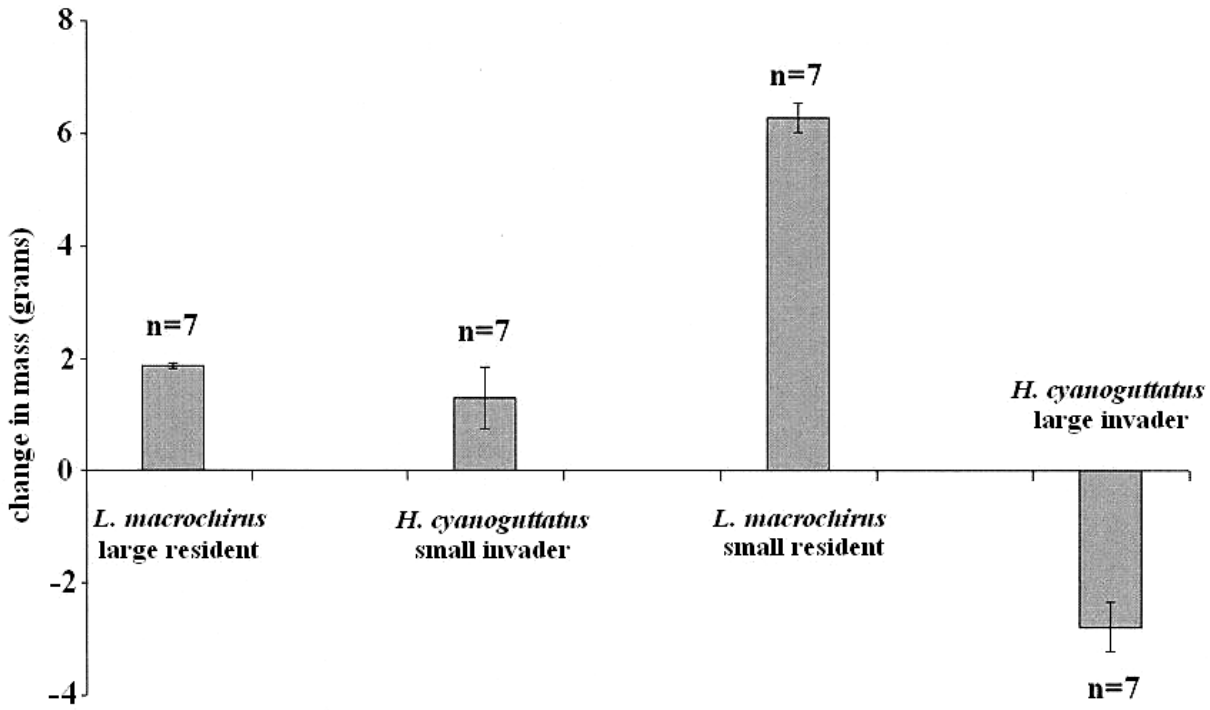

Figure 13. Mean (± 1 S.E) growth rate of *H. cyanoguttatus* and *L. macrochirus* kept together (one fish from each species) for 28 days when *L. macrochirus* was the prior resident. There was no significant difference in growth (p = 0.062).

Discussion

Growth of *H. cyanoguttatus* was not affected by a salinity level (8 ppt) typical of the estuarine habitats surrounding the GNOMA, nor by a salinity level (16 ppt) much higher than usually found in this area. Previous studies on salinity tolerance of invasive fishes implicated salinity as a barrier to further invasion (Scott et al. 2007). *Herichthys cyanoguttatus*, however, appears to have little difficulty surviving and growing in brackish conditions. Even with a

relatively rapid rise in salinity, both salinity treatments resulted in positive growth of *H. cyanoguttatus*. Other invasive cichlid species that have been examined for salinity tolerance rarely appear to be limited by brackish conditions. The Jack Dempsey cichlid (*Rocio octofasciatum*) was limited to conditions less than 8 ppt in laboratory studies (Dial and Wainright 1983). Other cichlids such as the black acara (*Cichlasoma bimaculatum*), however, tolerate brackish conditions when it previously was thought to be salt intolerant (Kushlan 1986). Tilapias, such as the *O. niloticus* in Mississippi, are invasives with pronounced salinity tolerance (Peterson et al. 2005). A close relative of *H. cyanoguttatus* is *H. carpintis*, which is a species that inhabits brackish conditions on the eastern coast of Mexico (Miller 2005, Don Conkel, pers. comm.). I have noted a variation in the pattern of spots and color of putative *H. cyanoguttatus* collected in the GNOMA. It is possible that these invasive populations may include *H. carpintis* or even *H. cyanoguttatus* X *H. carpintis* hybrids such as those that occur in a hybrid zone of these two species in the Rio Josefina in the Soto La Marina basin of Mexico (Miller 2005, Juan Artigas, pers. comm.). Whether or not these invasive populations include multiple species or hybrids, it appears unlikely that salinity will limit their dispersal further into southeastern Louisiana. Salinity could have long-term or possibly synergistic effects with other environmental conditions. Other abiotic factors such as a lack of thermal refugia in natural habitats outside of the GNOMA (Lorenz, unpublished data) may limit the continued spread of these animals.

The results of the intra- and interspecific growth trials were mixed, with *L. macrochirus* exhibiting significantly more growth than *H. cyanoguttatus* in the group trials and no significant difference in growth seen between the species in the one-on-one trials. One simple explanation is that the native *L. macrochirus* grows better at this temperature than the non-native *H.*

cyanoguttatus. *Lepomis macrochirus* may have grown grew more because it is a better scramble competitor than *H. cyanoguttatus*, but when results from the intra- and interspecific group trials are compared, the growth rate of each species was unaffected by whether they were held with conspecifics or heterospecifics (Fig. 12). This suggests that interspecific competition for food had a minimal effect on how much either species grew during the trials.

The interspecific one-on-one growth trials, which used *L. macrochirus* as prior residents, were less conclusive than the group trials with no significant differences in growth being exhibited. One possibility is that the slightly higher temperatures of these trials (23.5 º C versus 22.0 º C in the group trials) may have helped *H. cyanoguttatus* grow and compete more effectively for food. However, higher temperatures are preferred by *L. macrochirus* as well, up to 31 º C (Medvick et al. 1981). It is also possible that *H. cyanoguttatus* mobility and growth are more suited to higher temperatures in situations involving feeding competition. The other difference between these growth trials was the prior residence aspect. *Lepomis macrochirus* were aggressive as prior residents and that size determined territory dominance for both *L. macrochirus* and *H. cyanoguttatus*. Interestingly, the trends in these results are opposite of what would be expected based on territorial behavior in these species. Larger fishes, whether *H. cyanoguttatus* invaders or *L. macrochirus* residents, did not have the greatest increases in growth. It is possible that there was a cost incurred by defending the territories in the experimental tubs.

Competition for food may be irrelevant for both of these species because of their generalist diets (Buchanan 1971, Ross 2001). Paddlefish (*Polyodon spathula*) were outcompeted for a plankton food resource when raised with bighead carp (*Hypopthalmichthys nobilis*), but *P. spathula* is an obvious feeding specialist for plankton (Schrank and Guy 2003). Food resources

for *H. cyanoguttatus* and *L. macrochirus* may not be a limiting factor in natural conditions and territories may be held for other reasons besides food resources. It is possible that territories are defended for cover from predators, which would be possible to do if food was not a limiting resource. Another possibility is that *L. macrochirus* was not a good choice of native species to examine. Other more sensitive native species may be faring poorly with competing for food with invasive *H. cyanoguttatus*. Life histories and niches of invasive fishes in the Colorado River Basin showed *H. cyanoguttatus* to be closer to poeciliids than *Lepomis* species in life history characteristics (Olden et al. 2006). Similarly, *H. cyanoguttatus* have negative effects on the spawning of the small cyprinodontid,*Cyprinodon variegatus*, and poeciliid species (*Gambusia affinis*, *Poecilia latipinna*) appear to decline where numbers of *H. cyanoguttatus* increase in New Orleans (O'Connell, unpublished data).

Understanding the spread of an invasive species is crucial when considering management options and preventative measures (Ricciardi and Rasmussen 1998). A lack of management can lead to further spread of an invasive species and possibly future invasions by other species, causing an 'invasive meltdown' (Ricciardi 2001). Possible influences on an invasive species spread can include habitat quality, biotic resistance, and connectivity (Benjamin et al. 2007). If some aspect of biotic resistance is relevant, then the native ecosystem should be restored to encourage native selection pressures. These data show that *H. cyanoguttatus* does not grow slower in salinities up to 16 ppt and that this species does not effectively out-compete the native *L. macrochirus* for a limited food resource. The estuarine conditions of Lake Pontchartrain should not be a limiting factor in their spread, as salinities in the lake rarely exceed 8 ppt. Considering the results of the current laboratory trials and the generalist feeding habits of this species, it is unlikely that food competition is relevant in limiting *H. cyanoguttatus* dispersion. It

is also unlikely that this species would affect *L. macrochirus* through feeding competition, however competition for cover from predators should be investigated.

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SUMMARY

The Rio Grande cichlid, *Herichthys cyanoguttatus*, has been established in New Orleans for at least 20 years. It has spread rapidly, including dispersal into most of Bayou Saint John, City Park, the West Bank, and Saint Bernard Parish in only five years. It survives in Lake Pontchartrain and may use the lake to spread to new areas. Hurricanes Katrina and Rita did not reduce their range and may have facilitated the spread of cichlids into new areas. Salinity up to 16ppt may not be a barrier to these fish, which could indicate their potential spread south of the West Bank and anywhere in Lake Pontchartrain. Aggressive interactions with bluegill (*L. macrochirus*) showed how *H. cyanoguttatus* was aggressive whether it was an invader or a defender of a territory. In contrast, bluegill were only aggressive while holding territory. Future research could show what costs and benefits are involved with these behaviors, further demonstrating the importance of examining game theory strategies in the context of aggressive invasive species. A further examination of salinity limits, possibly up to full seawater, would also be worthwhile. One possible explanation for the observed salinity tolerance may be that the fish currently in the GNOMA are hybrids of *H. cyanoguttatus* and *H. carpintis*. Molecular studies could determine if this is the case. Comparative studies of salinity tolerance between these fish, true *H. cyanoguttatus*, and true *H. carpintis* could show if this is relevant. A more thorough study examining the differences between inter- and intraspecific interactions would also be helpful. Sampling that captures larger numbers of fishes (electrofishing, seining) in important areas would be wise. Two of the best areas to examine would be the La Branche wetlands and the extreme eastern part of the GNOMA, to determine the spread and survival of *H. cyanoguttatus* in habitats outside of the city. Perhaps more important is examination of habitats to the south, on the West Bank, where neither temperature nor salinity should be limiting for this

species. Also, a range of sampling methods should be evaluated and employed to ensure that accurate abundance and distribution data are available to natural resource managers.

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Vita

Otto Thomas Lorenz ("Tom") was born in Farmington Hills, Michigan, and had strong interests in biology from early childhood. He attended Western Michigan University and received a Bachelor's of Science degree in biology. After undergraduate coursework he spent time as a wildlife biologist in Florida. He worked on the Kissimmee River restoration and performed species inventories for South Florida Water Management District. Fieldwork with reptiles and amphibians was very inspirational, as were the inventories on invertebrates and small mammals. After further developing this hands on experience with wildlife, he decided to broaden his education and discover how he might participate in peer-reviewed research. During the late 1990s he was a graduate student at Southeastern Louisiana University in Hammond, Louisiana. Here he performed more fieldwork for graduate assistantships and continued to expand his experience with reptiles and amphibians. Fieldwork included protected species such as the dusky gopher frog (*Rana sevosa*), eastern indigo snake (*Drymarchon corais*) and yellow-blotched map turtle (*Graptemys flavimaculata*). Tom's research subject was a more common species, the southern watersnake (*Nerodia fasciata*). He studied the hormone cycles of male and female snakes kept in outdoor enclosures and defended this Master's thesis in 2001. Upon completion of this degree, Tom spent two years teaching at Southeastern Louisiana University. Classes included Comparative Anatomy and General Biology but, because of the lack of permanent options and because of further interest in research, he decided to return to school to get his doctoral degree at the University of New Orleans. He was fortunate enough to get a Board of Regents fellowship and a CREST grant and proceeded to work towards this Ph.D. in Conservation Biology under the advisors Robert Cashner and Martin O'Connell.