Growth of Non-Native Rio Grande Cichlids (*Herichthys cyanoguttatus*) at Different Salinities and in the Presence of Native Bluegill (*Lepomis macrochirus*)

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ABSTRACT

The Rio Grande cichlid (*Herichthys cyanoguttatus*) is a non-native fish that has become established in the greater New Orleans metropolitan area. We conducted laboratory experiments to determine if *H. cyanoguttatus* growth was impacted either by salinity or the presence of bluegill (*Lepomis macrochirus*), a native fish. There was no significant difference in growth of *H. cyanoguttatus* held at three different salinities (0, 8, and 16 psu) for 14 d. When *H. cyanoguttatus* and *L. macrochirus* were held at 0 psu for 28 d both with and without the other species present (n = 6 per trial), there was a significant difference in growth between species. *Lepomis macrochirus* held on their own grew faster than *H. cyanoguttatus* on their own and faster than *H. cyanoguttatus* held with *L. macrochirus* ($p < 0.005$). Also, *L. macrochirus* housed with *H. cyanoguttatus* grew faster than *H. cyanoguttatus* on their own and faster than *H. cyanoguttatus* with *L. macrochirus* ($p < 0.001$). 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 [n=7]; half with a smaller *H. cyanoguttatus* as invader [n=7]). This experiment showed no significant difference in growth between the species ($p = 0.064$). These results indicate that salinity levels will not limit *H. cyanoguttatus* dispersal in southeastern Louisiana, while biotic resistance in the form of native *L. macrochirus* may or may not impact growth 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 is currently at high densities throughout much of the GNOMA and has been found in lower densities in natural areas outside of the city (Fuentes and Cashner 2002, O'Connell et al. 2002). We have observed this species interacting aggressively with native species, and it is also known to compete for breeding sites in native spawning habitats (Courtenay et al. 1974). The reason for the limited number of *H. cyanoguttatus* collected outside of the GNOMA could be related to many things. This fish may prefer the disturbed habitat of urban canals, which may serve as thermal refugia during colder periods (e.g., concrete canals 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 it. However, a brackish barrier was shown to be a non-issue for another invasive cichlid in Florida; the Mayan cichlid (*Cichlasoma urophthalmus*) could tolerate salinity up to and exceeding that of seawater (Stauffer and Boltz 1994). Other invasive cichlids, especially multiple species of tilapia, have similar levels of salinity tolerance (Lemarie et al. 2004).

Some tilapia 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 between native and non-native fishes may either negatively impact 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 greater numbers of competitive species occur there. Conversely, it is also possible that the generalist diet of *H. cyanoguttatus* allows it to compete for food against a variety of species. One native generalist, the bluegill (*Lepomis macrochirus*), coexists with *H. cyanoguttatus* in the canals. This coexistence may be due to the adaptive abilities of *L. macrochirus* in disturbed habitats or because of the competitive traits *L. macrochirus* possesses. As an invasive fish in California, *L. macrochirus* that interacted with native Sacramento perch (*Archoplites interruptus*) was highly competitive (Marchetti 1999), and *L. macrochirus* was also aggressive when defending its territories against cichlids when they it was the prior resident (our unpublished observations). Determining the competitive outcome of possible *H. cyanoguttatus* - *L. macrochirus* interactions would allow us to better understand whether the non-native fish will continue to disperse beyond the urban limits of the GNOMA.

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

METHODS AND MATERIALS

H. cyanoguttatus growth: salinity trials

Juvenile *H. cyanoguttatus* (weight range: 0.500 - 1.500 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 (to the nearest 0.001 g) and placed individually in 37 L aquaria. Temperature was 23.0 ° C, and photoperiod was 12:12. All fish were fed *ad libitum* every day with Hikari brand food sticks.

Salinity was raised by 2.3 psu a day, by addition of 32 psu water, until the target treatment salinities were reached. 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. After a total time of 14 days, all fish were weighed individually and measured (standard length).

H. cyanoguttatus growth: intra- and interspecific group trials

Juvenile *H. cyanoguttatus* and *L. macrochirus* used in the group trials were of similar sizes (weight range: 10 - 20 g). All fishes were individually weighed and measured at the onset. As with the salinity trials, *H. cyanoguttatus* were collected locally, while *L. macrochirus* were acquired from a fish hatchery where they likely never had contact with cichlids. Group trials were conducted in 15 hard rubber tubs (400 L), each fitted with an air-driven sponge filter. Temperature was 22.0 ° C, and photoperiod was 12:12. The three group treatments were: six *H. cyanoguttatus* alone (five replicates); six *L. macrochirus* alone (five replicates); and three *H. cyanoguttatus* held with three *L. macrochirus* (five replicates). Fishes were fed Hikari food sticks, and the daily ration was calculated as 2% of the total weight of all fishes in any given tub. This ration was continued for 28 days, and all fishes were individually weighed and measured (standard length) at that time.

<u>*H. cyanoguttatus* growth: interspecific one-on-one trials</u>

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 ° C. Each trial was performed in one of 14 different tubs. For each trial, a single weighed and measured *L. macrochirus* was introduced to a tub containing a 7.5 cm diameter PVC elbow pipe-territory that provided these fish a sense of prior residence. A single weighed and measured *H. cyanoguttatus* was introduced to the tub a day later. During this one-day period, the *L. macrochirus* were not fed to avoid an artificial increase in growth from an extra day of feeding, and the treatment *H. cyanoguttatus* were also not fed. 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 days, and all fishes were weighed and measured (standard length) at the end of the trials.

RESULTS

Salinities of 8 and 16 psu had no effect on *H. cyanoguttatus* growth (ANOVA, $p = 0.980$, $F = 0.021$). The average mass increase for cichlids was 0.116 g at 0 psu, 0.123 g at 8 psu, and 0.118 g at 16 psu (Fig. 1). One *H. cyanoguttatus* in the 16 psu 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, $p < 0.001$, $F = 20.019$). 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. 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 2.820 g for bluegill and 1.310 g for cichlids (Fig. 2).

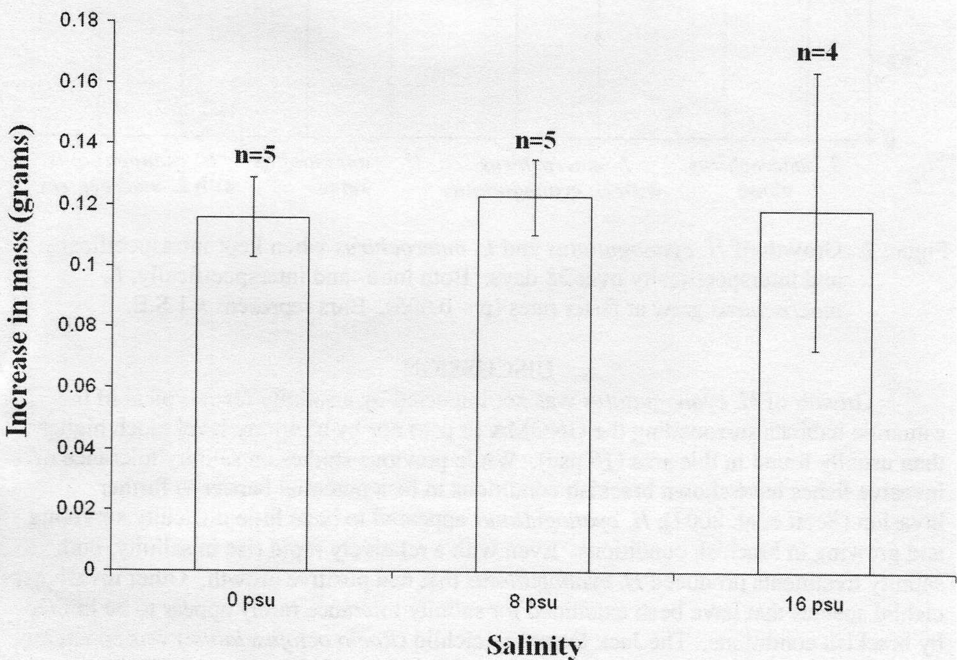

Figure 1. Growth of *H. cyanoguttatus* fed ad libitum at different salinities for 14 days. No significant differences between treatments were observed ($p = 0.980$). Bars respresent ± 1 S.E.

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* ($F= 3.731$, $p= 0.064$). The average change in mass for individual *H. cyanoguttatus* was actually negative (- 0.014 g), while individual *L. macrochirus* grew an average of 0.582 g (Fig. 3). There was also no significant difference in growth within or between larger or smaller introduced *H. cyanoguttatus* and larger or smaller resident *L. macrochirus* ($F = 2.298$, $P=0.103$). 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).

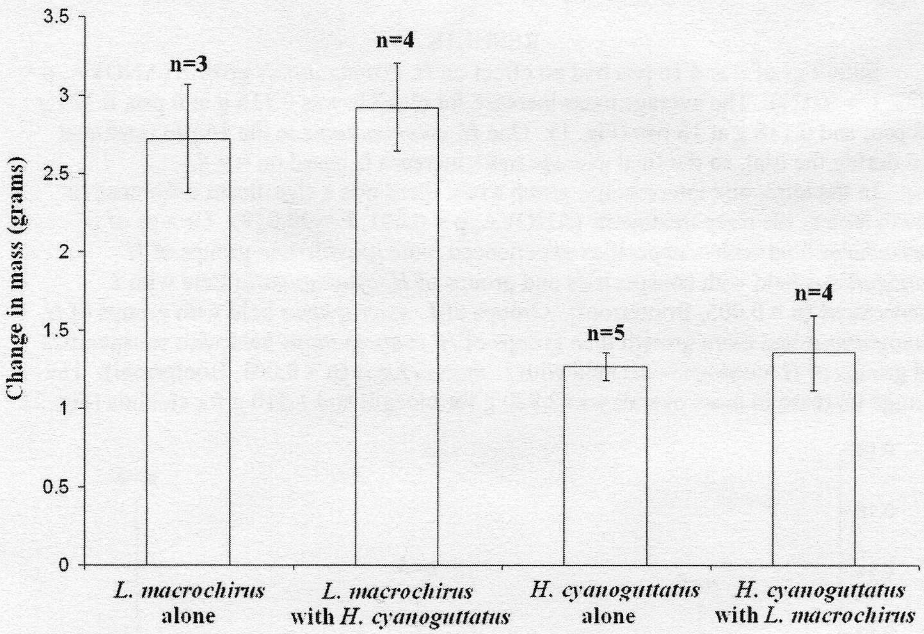

Figure 2. Growth of *H. cyanoguttatus* and *L. macrochirus* when kept intraspecifically and interspecifically over 28 days. Both intra- and interspecifically, *L. macrochirus* grew at faster rates ($p < 0.005$). Bars represent ± 1 S.E.

DISCUSSION

Growth of *H. cyanoguttatus* was not impacted by a salinity level typical of the estuarine habitats surrounding the GNOMA (8 psu) nor by a salinity level much higher than usually found in this area (16 psu). While previous studies on salinity tolerance of invasive fishes have shown brackish conditions to be a potential barrier to further invasion (Scott et al. 2007), *H. cyanoguttatus* appeared to have little difficulty surviving and growing in brackish conditions. Even with a relatively rapid rise in salinity, both salinity treatments produced *H. cyanoguttatus* that had positive growth. 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 apparently limited to conditions less than 8 psu (Dial and Wainright 1983). Other cichlids such as the black acara cichlid (*Cichlasoma bimaculatum*), however, were shown to be tolerant of brackish conditions when they had previously been thought to be salt intolerant (Kushlan 1986). Tilapias, such as the *O. niloticus* in Mississippi, have also been shown to be invasives with salinity tolerance (Peterson et al. 2005). A close relative of *H. cyanoguttatus* is *H. carpintis*, which is a species known to inhabit brackish conditions on the eastern coast of Mexico (Miller 2005). We have noted a variation in the pattern of spots and color of supposed *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). Whether or not these invasive populations include multiple species or hybrids, it appears unlikely that salinity will limit their dispersion farther into southeastern Louisiana. It should also be noted, though, that 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 influence 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. In general, though, *L. macrochirus* grew more than *H. cyanoguttatus* in both sets of trials. One simple explanation is that the native *L. macrochirus* grows better at this temperature than the non-native *H. cyanoguttatus*. It could also be suggested that *L. macrochirus* grew more because it is a better scramble competitors than *H. cyanoguttatus*, but when results from the intra- and interspecific group trials are compared, both species grew the same regardless of whether they were held with conspecifics or the other species. This suggests that interspecific competition for food had a minimal effect on how much either species grew during the trials.

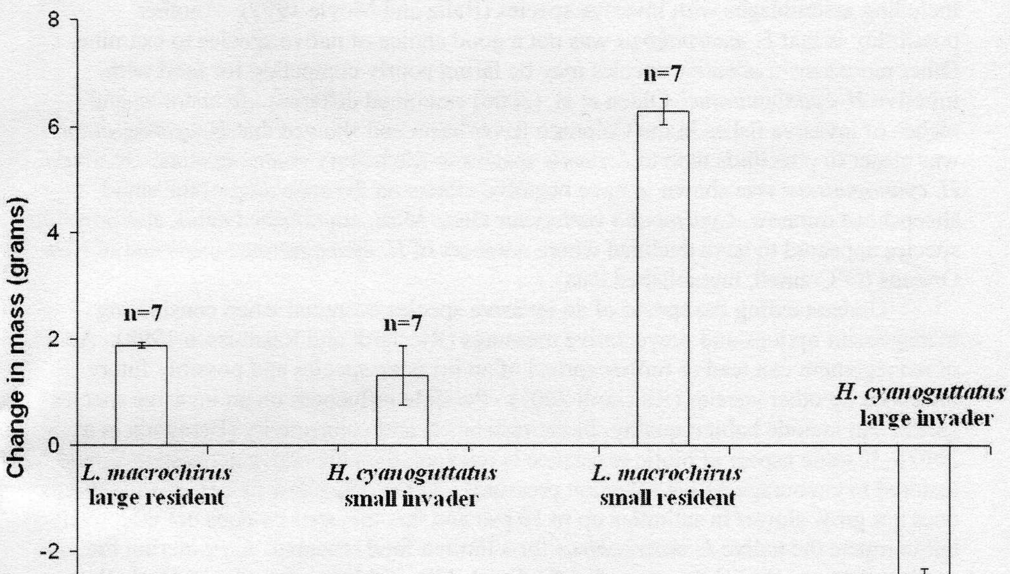

Figure 3. Growth of *H. cyanoguttatus* and *L. macrochirus* kept together (one fish from each species) for 28 days when *L. macrochirus* was the prior resident. There was no significant difference in growth ($p = 0.062$). Bars represent ± 1 S.E.

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 ° C versus 22.0 ° 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, including as high as 31 ° 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. In other work, we have found that *L. macrochirus* was aggressive as a prior resident and that size determined territory dominance for both *L. macrochirus* and *H. cyanoguttatus* (unpublished data). 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, consistently grew slower, although not enough to produce significant results. It is possible that there was a cost incurred by defending the territories in the experimental tubs.

Feeding competition may be irrelevant for both of these species because of their generalist diets (Ross 2001, Buchanan 1971). 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. For example, predation has been shown to be an important factor shaping assemblages, including assemblages with invasive species (Baltz and Moyle 1993). 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 competing for food with invasive *H. cyanoguttatus*. Olden et al. (2006) examined different life histories and niches of invasive fishes in the Colorado River basin and showed that *H. cyanoguttatus* was closer to poeciliids than to *Lepomis* species in life history characteristics. Similarly, *H. cyanoguttatus* was shown to have negative effects on the spawning of the small sheepshead minnow, *Cyprinodon variegatus* (June Mire, unpublished data), and poeciliid species appeared to have declined where numbers of *H. cyanoguttatus* increased 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 (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 psu and that this species does not effectively out-compete the native *L. macrochirus* for a limited food resource. Considering the results of this work 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 impact *L. macrochirus* through feeding competition; however, competition for cover from predators should be investigated.

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