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Salinity tolerance of the Seminole killifish, *Fundulus seminolis*, a candidate species for marine baitfish aquaculture

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ABSTRACT

Aquaculture of marine baitfish species is still in its relative infancy and the increasing value of coastal property is forcing marine aquaculture inland. *Fundulus seminolis*, a freshwater killifish species endemic to Florida, has shown economic potential for use as a marine baitfish, with a small number of commercial operations currently in production. The objectives of this study were to determine both acute and gradual salinity tolerances as well as an upper lethal salinity tolerance for the species. Two separate acute acclimation experiments, natural seawater and sodium chloride, were carried out to determine if survival was influenced by the salinity source. *F. seminolis* were able to tolerate acute transfer to 0, 8, and 16 g/L using both salinity sources but only those in natural seawater were able to survive in 24 g/L. No survival was observed regardless of salinity source after acute transfer to 32 g/L. A gradual acclimation using natural seawater was also investigated to examine survival at various acclimation rates. A survival rate of 100% was achieved regardless of acclimation rate when salinity was changed from 0 to 32 g/L in 24, 48, 72, and 96 h treatment groups. An upper lethal salinity determination yielded an LC-50 of 60 g/L with a maximum salinity tolerance of 78 g/L. Results of these experiments provide information pertinent to the successful culture of this rarely studied species. Additionally, experimental outcomes will help to shape the marketing and distribution strategies for *F. seminolis* as a marine baitfish.

1. Introduction

The Seminole killifish, *Fundulus seminolis* (Girard), is an endemic Florida killifish with a geographic range within peninsular Florida from the St. Johns and New River drainage basins to just south of Lake Okeechobee (Page and Burr, 1991). Relatively little is known regarding the life history of *F. seminolis*, with only one publication by DuRant et al. (1979) devoted entirely to the species. It has been referenced anecdotally or as a component in a larger study or survey in several publications (McLane, 1955; Phillips and Springer, 1960; Tabb and Manning, 1961; Gunter and Hall, 1963; Gunter and Hall, 1965; Foster, 1967; Griffith, 1974a; Nordlie, 2006). This species, as well as several other members of the *Fundulus* genus are commonly referred to as a "bullminnow" or "mudminnow". The Seminole killifish is one of the largest members of the genus, reaching total lengths of 20 cm (Hoyer and Canfield, 1994). Its popularity as a local freshwater baitfish for largemouth bass, *Micropterus salmoides*, and other piscivorous game fish has generated interest in this species as a potential candidate for aquaculture.

With previous data placing the upper salinity tolerance of *F. seminolis* at 23.4 g/L (Griffith, 1974a), culture of this species for use as a saltwater baitfish warrants further investigation. If the species is able to acclimate to full strength seawater, it could be produced exclusively in freshwater ponds or recirculation systems only needing to be acclimated to saline water prior to marketing and distribution. Additionally, with coastal property values at a premium and limited access to seawater, baitfish producers would be able to utilize inland resources for the culture of a marine baitfish.

Determination of salinity tolerance following gradual and acute transfer is necessary to evaluate the species' physiological limitations which will influence culture and marketing practices. Similar studies have been conducted by Lotan (1971), Griffith (1974a), Stanley and Fleming (1977), Chervinski (1983), Nordlie (1987), Crego and Peterson (1997), Nordlie (2000) and Fuller (2008) on salinity tolerance of various members of the order cyprinodontiformes. For

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the most recent review of cyprinidontoid salinity tolerance consult Nordlie (2006). The previous studies examined multiple salinity ranges utilizing various salt sources (seawater and synthetic sea salts) and freshwater dilutions to achieve experimental salinities both hypo and hypersaline to natural seawater (32–35 g/L). Distinct salinity thresholds defined by survival and select hematological indices were then characterized for the species in question. Experimental salinity determinations such as these may be a more accurate representation of the organism's true salinity tolerance than maximum reported field salinities (Kefford et al., 2004). This can be seen in Phillips and Springers' (1960) record of *F. seminolis* inhabiting waters with a salinity of 13.5 g/L, the highest recorded salinity from published field observations. Griffith's (1974a) subsequent experimental salinity determination for this species placed the upper salinity tolerance much higher, with an experimental salinity range of 19.3–33.4 g/L. However, Griffith (1974a) reported high mortalities in maintaining this species in captivity and his sample size for salinity tolerance determinations was only four individuals. Although the lower lethal temperature has yet to be established for this species, the experimental water temperature of 15 °C might have exacerbated the physiological stress caused by the acclimation if this temperature impaired the functionality of essential enzymes. Taken together, these circumstances call into question the accuracy of the salinity range reported by Griffith (1974a).

Investigations into the salinity tolerance of freshwater species have shared a predominant ecological motivation. Investigations by Bringolf et al. (2005) and Schofield et al. (2006) assessed salinity tolerances as barriers to invasion for the flathead catfish, *Pylodictis olivaris*, and goldfish, *Carassius auratus*, respectively. While the impetus for the current study is the evaluation of *F. seminolis* as an aquaculture candidate; investigations into its salinity tolerance should provide valuable information about the species' ability to handle osmoregulatory stressors and could further be extrapolated for ecological applications.

Salinity tolerance is an important consideration in the culture of marine and freshwater organisms. It provides information about basic husbandry requirements necessary for the species to thrive in captivity as well as potential applications for the cultured organisms. Additionally, economic considerations associated with culture of marine or brackish water species make low saline or freshwater culture an attractive alternative. Research into low salinity aquaculture of marine species is common, but few studies have been conducted on acclimation of freshwater species to seawater. Experiments examining abrupt transfer of black sea bass, *Centropristis striata,* to low salinities have helped to identify a salinity threshold for the successful culture of this species (Young et al., 2006). Similarly, gradual acclimation experiments with Nile tilapia, *Oreochromis niloticus*, and blackchin tilapia, *Sarotherodon melanotheron*, (Lemarie et al., 2004) as well as larval salinity tolerance experiments with striped mullet, *Mugil cephalus*, thick-lipped grey mullet, *Chelon labrosus* (Hotos and Vlahos, 1998), and cobia, *Rachycentron canadum* (Faulk and Holt, 2006), have provided valuable evidence regarding the osmoregulatory ability of a species for use in conventional aquaculture conditions.

The purpose of this study was to characterize the salinity tolerance of *F. seminolis*, a potential candidate for marine baitfish aquaculture. Abrupt and gradual salinity acclimations were evaluated as well as salinity sources (sodium chloride vs. seawater). A maximum lethal salinity resulting in 100% mortality was also investigated by means of gradual acclimation with a corresponding determination of the salinity concentration which resulted in 50% mortality (LC-50). This investigation represents the first comprehensive study focused on the salinity tolerance of *F. seminolis*.

2. Methods

F. seminolis were collected by seine net from the eastern shore of Lake George, in Volusia County Florida and transported to the University of Florida Indian River Research and Education Center in Fort Pierce. Fish were assessed for pathogens and treated accordingly to ensure healthy research specimens for the subsequent salinity experiments. The acute salinity tolerance of *F. seminolis* to varying concentrations of sodium chloride (NaCl) and natural seawater (NSW) were investigated as well as survival following gradual NSW acclimation. A maximum lethal salinity investigation was also conducted.

2.1. Sodium chloride acute salinity tolerance

Thirty five fish were transferred from a 6900 L recirculating system to 85 L glass aquaria with one fish per aquarium during the entire acclimation and experimental periods. Specimen's weight and total length (TL) were recorded prior to transfer. Length and weight ranges of 120–145 mm and 17.0–29.4 g were recorded with means of 131.2 ± 6.3 mm and 23.2 ± 3.6 g, respectively. Aquarium systems were maintained at <1 g/L salinity well water and recirculated through biofilter media during the 96 h acclimation period. Dissolved oxygen (DO), pH, temperature, salinity, total ammonia nitrogen (TAN), and nitrite were recorded daily during the acclimation and experimental periods with total alkalinity and total hardness recorded on days one and three of acclimation and daily during the course of the experiment. DO and temperature were measured using a YSI 550A meter (YSI Inc., Yellow Springs, Ohio). Salinity was determined using a handheld refractometer, pH was measured using a Hach sensION1 portable pH meter and total alkalinity and total hardness were determined using standardized titration techniques (Hach Co., Loveland, Colorado). TAN and nitrite were evaluated spectrophotometrically using a Hach DR 4800 spectrophotometer (Hach Co., Loveland, Colorado). Aquaria were held at ambient temperature with a range of 19.9–26.5 °C and a mean temperature of 22.7 °C during the experimental period. Temperature differences among aquaria never exceeded 2 °C. An ambient photoperiod of 11 L: 13 D was used during the experiment. Fish were fed once a day to satiation on days two and three of acclimation and food was withheld on days one and four of acclimation and during the entire 96 h experimental period.

Following the 96 h freshwater acclimation, aquaria were made static and individual aquariums were randomly assigned to one of five treatments with seven replicates per treatment. Differences among treatment group's lengths and weights were not significant ($F_{4,30} = 0.68$, $p = 0.610$; $F_{4,30} = 0.20$, $p = 0.939$, respectively). Treatment salinities examined were 0 (control), 8, 16, 24, and 32 g/L. Salinities were abruptly changed by removing the appropriate amount of fresh water and adding a predetermined volume of a concentrated brine solution made by dissolving 99.5% NaCl (Morton White Crystal Solar Salt, Morton International Inc., Chicago, IL) into well water. Control aquaria had a predetermined volume of fresh water removed and subsequently replaced to maintain similar treatment of control and experimental groups. Tanks were aerated to thoroughly mix the water, then salinities were remeasured to confirm the desired concentrations were attained. Individual biofilters which had been preconditioned to treatment salinities were placed in each tank to control nitrogenous wastes. Aquaria were examined for mortalities as follows: once every hour from 0–12 h, once every 6 h from 12–48 h, and once every 12 h from 48–96 h. A final weight was recorded upon discovery of a mortality or upon the termination of the 96 h exposure period. Mortality as used throughout this investigation was defined as loss of opercular movement and no response to physical stimulus.

2.2. Natural seawater acute salinity tolerance

Methods for the NSW acute salinity toxicity trial adhere to the previous methods listed in Section 2.1. with exceptions as noted. Treatment salinities were abruptly changed by removing a predetermined volume of fresh water and adding a known volume of natural seawater collected from the Atlantic Ocean and filtered through a 1 micron cartridge filter.