

# A Test for a Trade-Off in Salinity Tolerance in Early Life-History Stages in *Lucania goodei* and *L. parva*

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***Lucania goodei* and *L. parva* are close phylogenetic relatives that differ in abundance along a salinity gradient where *L. goodei* is found predominantly in freshwater sites, and *L. parva* is found most often in brackish water sites. It has been suggested that these taxa diverged along a salinity gradient. In order for selection to cause divergence, there must be local adaptation where each species does best in the environment in which it is most often found. In this paper, I test for local adaptation in *L. goodei* and *L. parva* during early life-history stages by raising eggs and larvae of each species at 0, 10, 20, and 30 ppt salinity, covering the gradient between fresh water and sea water found along the coast of the Gulf of Mexico. Salinity tolerance differed between the two species. *Lucania parva* had high survival at all salinities. *Lucania goodei* had high survival at 0 and 10 ppt, but low survival at 20 and 30 ppt. Hence, there is no evidence for a trade-off in the early life-history stages between these two species. Current research is investigating whether trade-offs occur at later life-history stages.**

THE ability to tolerate salinity has had profound effects on the evolution of teleost fishes (Nelson, 2006; Barton, 2007). The challenge to animals in fresh water is to retain salts while getting rid of excess water; the challenge to animals in sea water is to retain water while extruding excess salt (Evans et al., 2005). The transition from fresh water to sea water at the coast is marked by a rapid change in fish assemblage structure, suggesting that most species cannot tolerate both habitat types (Gunter, 1945, 1950). At a larger scale, some entire groups are restricted to fresh water (e.g., Cypriniformes, Characiformes), some groups are restricted to marine habitats (e.g., Acanthuridae, Labridae), and some groups readily transition between fresh and marine habitats (e.g., Gasterosteiformes, Fundulidae, Atherinopsidae; Nelson, 2006). Hence, understanding the extent to which adaptation to salinity limits niche space is critical to understand divergence and diversification of teleosts.

*Lucania goodei* and *L. parva* are an excellent pair of species for studying evolution of salt tolerance and its role in speciation. There are three species in the genus *Lucania*: *L. goodei*, *L. parva*, and *L. interioralis* (Hubbs and Miller, 1965). *Lucania interioralis* is an endangered species found in a few populations (reported as slightly to highly saline) in the Cuatro Ciénegas system located in the state of Coahuila (Hubbs and Miller, 1965; Miller et al., 2005). While there has been no modern phylogenetic work on the relationships within *Lucania*, allozyme data suggest a genetic distance (D) of 0.16 between *L. goodei* and *L. parva* which is consistent with the two species being closely related (Duggins et al., 1983). Many studies have suggested that *L. goodei* and *L. parva* diverged along a salinity gradient as Florida underwent multiple changes in sea-level (Relyea et al., 1976; Burgess and Franz, 1978).

*Lucania goodei* is found primarily in freshwater sites in Florida with a few additional populations found in Alabama, Georgia, and South Carolina (Lee et al., 1980; Page and Burr, 1991; Boschung and Mayden, 2004). *Lucania parva* is widespread along the Atlantic coast from Massachusetts to southern Florida and along the entire coast of the Gulf of

Mexico. It is typically found in brackish water, but there are exceptions to this general pattern. Kilby (1955) found four *L. goodei* individuals at a site with 10.3 ppt. In the same study, *L. parva* was found at Cedar Key, FL in salinities ranging from 15 to 30 ppt. However, at Bayport, FL, *L. parva* was found in salinities ranging from 0 to 25 ppt, with 80% of the individuals found between 0 to 10 ppt (Kilby, 1955). Burgess et al. (1977) indicate the presence of 12 freshwater populations of *L. parva* within the St. John's River Drainage in Florida. There are also several published reports of sites where the two species are sympatric in freshwater springs (Hubbs and Allen, 1943; Herald and Strickland, 1949). In Mississippi, *L. parva* is found along the coast in salinities ranging from 0.7 to 24.2 ppt, but is most common at 4–8 ppt (Peterson and Ross, 1991; Ross, 2001). Hence, while *L. parva* is found more often in brackish water than *L. goodei*, there is evidence that *L. parva* can tolerate fresh water at some sites.

Previous work on salinity tolerance in *Lucania* has focused on adult survival. In a lab experiment, Dunson and Travis (1991) found that adult *L. goodei* had low survival when transferred from fresh water or isotonic water (12 ppt) to water with high salinity (35 ppt). In contrast, both *L. goodei* and *L. parva* had high tolerance for fresh water provided that it was hard (i.e., high pH and high mineral content). Whereas Dunson and Travis (1991) focused on adult fish, this current study focuses on early life stages (i.e., eggs and larvae). Osmoregulation in the egg and larval stages is profoundly different because the gills are still developing. Osmoregulation in embryonic fish relies first upon skin (and ionocytes therein), then the digestive track and drinking behavior, and finally the gills and urinary system (Varsamos et al., 2005).

Is there evidence for a trade-off in *L. goodei* and *L. parva* survival across a salinity gradient? The hypothesis that differential adaptation to salinity has driven speciation requires that there be a trade-off where each species does best in the environment in which it is most frequently found (Schluter, 2000; Coyne and Orr, 2004). In other words, *L. goodei* should have higher fitness than *L. parva* in

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fresh water, and *L. parva* should have higher fitness in brackish and seawater. I tested this hypothesis by raising eggs from each species in 0, 10, 20, and 30 ppt salinity treatments and comparing survival to hatching and independent eating stage.

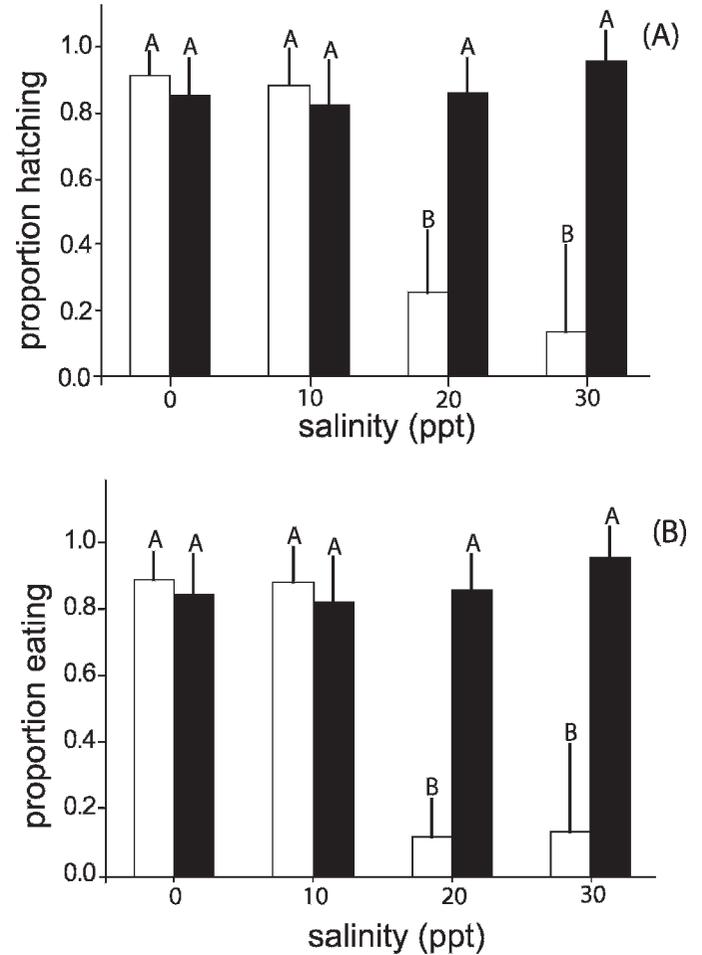
## MATERIALS AND METHODS

*Lucania goodei* were collected at 26-Mile Bend, Broward Co., FL (26°14'N, 80°28'W). *Lucania parva* were collected at Lighthouse Pond (30°4'N, 84°11'W) in the St. Mark's National Wildlife Refuge, Wakulla Co., FL. Animals were transported back to the University of Illinois and were maintained at 2 ppt salinity as measured with a YSI salinity meter. Stock animals were maintained in 76- and 113-liter aquaria with both biological and mechanical filters. Lights were maintained on a 14:10 light:dark ratio. Adults were fed frozen brine shrimp and bloodworms.

To obtain eggs from both species, breeding tanks were established in 37.8-liter aquaria containing conditioned water at 2 ppt salinity. Due to limited lab space, I set up nine 37.8-liter aquaria. I established four breeding tanks for *L. goodei* and five breeding tanks for *L. parva*. Each tank housed a single male and one or two conspecific females and also contained four yarn mops that served as spawning material. Eggs were obtained from the mops once every 2–3 days and placed in small tubs containing water at either 0, 10, 20, or 30 ppt salinity. Salinity treatments were created by adding Instant Ocean Sea Salts® to conditioned fresh water. A small amount of methylene blue was added to each tub to prevent fungal infection. The number of eggs surviving to hatching and the number of larvae surviving until independent eating were recorded. Larvae were fed with *Artemia nauplii* which allowed us to easily diagnose when larvae had eaten, as *Artemia* results in a visible pink bolus in the gut. One breeding tank of *L. parva* produced inviable eggs (100% mortality) and was replaced with another set of fish.

Eggs were retrieved from mops from 22 February 2006 through 5 April 2006. My goal was to attain a minimum of ten eggs for each of the four salinity treatments (0, 10, 20, and 30 ppt). One *L. parva* breeding tank only produced enough eggs for three treatments (0, 10, and 20 ppt). Hence, there were 35 replicates (9 breeding tanks × 4 salt treatments minus one treatment for one breeding tank). Of the 35 replicates, all but four had ten or more eggs (average egg number per salinity treatment = 14.1, minimum = 4, maximum = 50). Clutches with fewer eggs obviously introduced more sampling error into the study, but this did not create a problem. Excluding the four replicates with low egg numbers did not alter the results of the study.

Hatching success was calculated as the proportion of eggs hatching. Survival to eating was calculated as the proportion of eggs that survived until larvae had successfully eaten. General linear models were used to determine the effects of species identity, salinity, and their interaction. Both species and salinity were treated as categorical variables. Residuals were examined to ascertain that they did not differ from a normal distribution. Tukey HSD multiple comparisons were used for *post hoc* tests. Furthermore, the analysis was robust to assumptions about the underlying distribution of the data as identical results were obtained with a binomial model. Analyses were conducted using Systat V. 11 (Systat Inc., 2004) and SAS V. 9.1 (SAS Institute, Cary, NC, USA).



**Fig. 1.** (A) Hatching success and (B) survival to independent feeding stage in *L. goodei* (open bars) and *L. parva* (black bars) in four salinity treatments. Means plus 1 standard deviation are shown. Letters indicate statistically significant differences,  $P < 0.001$ . For *L. goodei*,  $n = 4$  for all salinity treatments. For *L. parva*,  $n = 5$  for 0, 10, and 20 ppt, and  $n = 4$  for 30 ppt.

## RESULTS

Salinity tolerance differed between *L. goodei* and *L. parva* (Fig. 1; hatching success: salinity\*species  $F_{3,27} = 22.10$ ,  $P < 0.0001$ ; survival to eating: salinity\*species  $F_{3,27} = 27.79$ ,  $P < 0.0001$ ). *Lucania parva* hatching success and survival to eating did not differ between salinity treatments. In contrast, *L. goodei* hatching success and survival to eating was significantly lower in 20 and 30 ppt treatments than in the 0 and 10 ppt treatment. Hatching success and survival to eating did not differ between *L. goodei* and *L. parva* in the 0 and 10 ppt treatments, but did differ in the 20 and 30 ppt treatments.

## DISCUSSION

There is no evidence for a trade-off in salinity tolerance in early life stages between *L. goodei* and *L. parva*. Although *L. goodei* had lower survival at high salinity, there was no evidence that *L. parva* had lower survival in low salinity. These results are consistent with the distribution of both *L. goodei* and *L. parva*. Fuller and Noa (unpubl. data) reviewed museum accounts of both species and found that *L. parva* sites were commonly recorded in fresh water, brackish water, and marine water (22%, 46%, and 32% respectively).

In contrast, *L. goodei* was found predominantly in freshwater sites (92.7% fresh water, 6.9% brackish water, <1% marine). In a survey of over 20 species of *Fundulus*, Griffith (1974) found that all brackish and marine species investigated could survive in fresh water. All of the freshwater species investigated could tolerate water from 0 to approximately 20 ppt, while the brackish and marine species could generally tolerate hypersaline water (i.e., >35 ppt). These results are highly relevant to *Lucania* because *Lucania* and *Fundulus* are close phylogenetic relatives (Parenti, 1981; Wiley, 1986; Cashner et al., 1992; Bernardi, 1997).

Freshwater killifish lack the ability to tolerate brackish water and saltwater conditions suggesting that maintaining these physiological mechanisms is costly. One possibility is a trade-off whereby freshwater species increase their fitness by losing the ability to tolerate marine conditions. This current study found no evidence for such a trade-off in early life stages, but a trade-off may be manifested in later life stages such as juvenile and adult growth, survival to adulthood, female fecundity, and/or male mating success (Fuller et al., 2007).

The detection of a trade-off may also depend on the presence of additional stressors in the environment. Relyea et al. (1976) suggested that at the time of divergence, *L. goodei* faced selection for both freshwater tolerance and cold tolerance. This hypothesis suggests that multiple abiotic factors were critical in the divergence between these two groups. Kidder et al. (2006) have shown that cold temperature (4°C) does impair active transport mechanisms in *F. heteroclitus*. Whether Florida populations are ever cold enough to impair active transport mechanisms in *Lucania* remains to be demonstrated.

Biotic factors such as competition may also be critical in detecting trade-offs in salinity tolerance. Dunson and Travis (1991) found evidence for differential effects of salinity on competition between *L. goodei* and *L. parva*. *Lucania goodei* grew faster in 0 ppt than in 15 ppt and the difference between 0 and 15 ppt increased when *L. goodei* was raised in competition with *L. parva*. *Lucania parva* also grew faster in 0 ppt than in 15 ppt, but this pattern reversed when raised in competition with *L. goodei*. This suggests a strong interaction between biotic and abiotic factors in determining species niches.

The other possibility is that freshwater killifish lose their ability to tolerate marine conditions due to genetic drift (Kimura and Ohta, 1971). This hypothesis suggests that animals that live solely in fresh water never use the genes that convey high salinity tolerance and that over time these genes accumulate deleterious mutations that render them non-functional. The extent to which this scenario occurs in *L. goodei* is questionable because some *L. goodei* populations (particularly those close to the coast) occasionally face high salinity conditions due to hurricane surges or simple fluctuations in flow rates along rivers. Hence, genes conveying high salinity tolerance should be utilized occasionally in some populations, although this remains to be shown empirically.

In conclusion, *L. parva* and *L. goodei* differ in salinity tolerance, but there is no evidence for a trade-off in early life stages where each species outperforms the other in a different salinity. *Lucania parva* tolerated all salinity conditions, whereas *L. goodei* had low survival in salinities above the isotonic point (20 and 30 ppt treatments). The evolutionary forces leading to divergence in plasticity are

unknown. However, this divergence in salinity tolerance is most likely important in the evolutionary divergence in teleost fishes as it has profound implications for the distributions and abundances of species.

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