Effects of increased salinity on tadpoles of two anurans from a Caribbean coastal wetland in relation to their natural abundance

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Abstract. Many amphibians depend on wetland ecosystems for reproduction and survival, and coastal wetlands are not the exception. Recent advances on climate change research predict a reduction in land cover of coastal wetlands due to sea-level rise in response to global warming. Although this scenario will contribute to further amphibian population declines worldwide, the impacts of sea-level rise and its related salt water intrusion on anuran assemblages in coastal wetlands remain largely unknown. I documented patterns of abundance of the native Caribbean white-lipped frog (Leptodactylus albilabris) and the introduced marine toad (Bufo marinus) along an inland-to-coastal salinity gradient in Puerto Rico. In addition, I investigated the effects of increasing salinity on larval growth and survival to metamorphosis in L. albilabris and B. marinus in laboratory experiments. In the field, relative abundance of adults of L. albilabris decreased with increasing salinity, while B. marinus showed the opposite pattern. Laboratory experiments with L. albilabris and B. marinus revealed that percentage of larvae surviving to metamorphosis in both species was greatly reduced in 22-25% seawater (8 ppt), which is within salinity levels found in their natural distribution. In this salinity level, the native L. albilabris showed ~100% metamorphosis failure while the introduced B. marinus showed ~60% metamorphosis failure. The reduction in metamorphosis was due to high mortality in L. albilabris and was accompanied with morphological abnormalities in B. marinus. Tadpoles of only L. albilabris reared for four weeks showed significant weight loss at 8 ppt, but showed no difference in length. These results suggest that anuran tadpoles may be living near their physiological limit for salinity in the studied wetland. Conservation implications are profound, however, as salt water intrusion and urban encroaching inland may result in anuran population replacement, from native species to introduced species in this wetland.

Keywords: anuran metamorphosis, cane toad, coastal wetlands, salinity response, salinization, sea-level rise, tadpole development, white-lipped frog.

Introduction

Coastal wetlands are critical habitat for many animal species worldwide, but are threatened by global warming and anthropogenic disturbances (Mitsch and Gosselink, 2000). For example, the 0.74°C increase in the mean global temperature during the 20th century has increased mean sea level between 0.12 and 0.22 m (mean 0.17 m) during the same period (IPCC, 2007). In addition, best estimates of global warming range between 0.6 and 4.0°C with corresponding increases in mean sea level between 0.3 and 6.4 m by year 2100 (IPCC, 2007). Sea-level rise due to thermal expansion of warmer seawaters will lead to salt-water intrusion in coastal areas (IPCC, 2007), which will change hydope-
non-saline, moist environments (Boutilier et al., 1992; Duellman and Trueb, 1994). For example, increased salinity limits growth and causes low metamorphic success and increased mortality in adults and tadpoles of many anurans that depend on water for reproduction in areas affected by secondary (anthropogenic) salinization of soils (Viertel, 1999; Christy and Dickman, 2002).

In tropical coastal wetlands, there is a high abundance of anurans, which are also among the most important components of these wetlands. On Caribbean islands like Puerto Rico, for example, the estimated mean abundance of adult anurans (4 leptodactylid frogs [3 Eleutherodactylus sp. and Leptodactylus albilabris], 1 ranid [Rana grylio], 1 hylid [Scinax rubra], 1 bufonid [Bufo marinus]) among four coastal wetlands is 1983 frogs ha$^{-1}$ (range: 828-7930; N. Rios-López, unpubl. data). Considering an estimated biomass of 0.83 kg ha$^{-1}$ and a prey consumption rate of 10 987 items ha$^{-1}$ day$^{-1}$, respectively (N. Rios-López, unpubl. data), these wetland anurans can be highly important for the regulation of local ecosystem nutrient and energy flows through predator-prey interactions. Thus, salt-water intrusion may be a critical issue for tropical anurans and their role in the maintenance of ecosystem function in coastal wetlands because the combined effect of sea-level rise in the seaward side and anthropogenic disturbances in the landward side may result in local populations of anurans subsisting in physiologically detrimental conditions. It is important to investigate the ways in which increased salinity may affect survival of these and other amphibians to develop future species conservation plans in light of predicted sea-level rise and salt-water intrusion due to global warming.

In this paper, I determined the distribution of two frog species in a coastal wetland, and the tolerance of anuran tadpoles to variable salinities. Specifically, the aims of this study were to: 1) estimate abundance of a Puerto Rican native species, Leptodactylus albilabris, along an inland-to-coastal salinity gradient, and 2) examine the effects of increased salinity on growth of its tadpoles under laboratory conditions. In addition, I compared field and laboratory results from L. albilabris with those of the introduced marine toad, B. marinus, which frequently occur in Puerto Rican coastal wetlands.

Materials and methods

Study sites

The study area is located in the swamp of San Pedro “Ciénaga de San Pedro”, Sabana Seca (18°27'47"N, 66°12'08"W; datum WGS84), Toa Baja municipality, northern Puerto Rico (fig. 1). The wetland at Sabana Seca is classified in the Subtropical Moist Forest life zone (Ewel and Whitmore, 1973). In Puerto Rico, similar coastal wetlands cover approximately 42% of all geographic regions (Lugo et al., 2001). However, most of these wetlands had a larger cover but were drained and deforested for agriculture, including the study site, since the arrival of Spaniards in early 1500s. The Puerto Rican economy shifted from agriculture to industry during 1930s, and much of these areas were abandoned and either became invaded by herbaceous vegetation that arrested forest succession or were transformed by urban development (Grau et al., 2003). This land-use history resulted in patches of forested and non-indigenous herbaceous wetlands surrounded by urban areas to the east, west, and south of the study site (fig. 1). In recent years, average annual rainfall and temperature was 1693 mm yr$^{-1}$ and 27°C, respectively (Eusee and Aide, 1999). Both rainfall and temperature are mildly seasonal with most of the rain occurring from May to November, which are also the warmer months. The soils are characterized by having poor drainage and are covered by a thick layer of peat. Topography ranges between <1 m a.s.l. to the coast and 2 m a.s.l. inland. Topography varies slightly resulting in an inland-to-coastal vegetation continuum, which limits the classification of the study site as either a salt swamp or a tidal freshwater swamp (fig. 1). Along this continuum, vegetation consists of mangroves dominated by the white mangrove, Laguncularia racemosa, in saline wa-

Figure 1. Aerial photograph of the study site showing the inland-to-coastal salinity gradient and the T. domingensis-P. officinalis-L. racemosa vegetation ecotone. The white circles in the photograph represent the location of the sample points with each sampling transect located between adjacent sample points. Also shown are reference numbers of each sampling point relative to the low salinity end along the 1.1 km area. Star in inset map indicates location of the study area; collection sites of tadpoles for experiments one and two are represented by filled circles and numbers for L. albilabris (one collection site in Río Piedras and two collection sites in Trujillo Alto, northern Puerto Rico) and by a filled square for B. marinus (one collection site in Quebradillas, north-western Puerto Rico).
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mers towards the sea, the blood-wood tree, *Pterocarpus officinalis*, in brackish waters inland, and herbaceous vegetation dominated by cattail, *Typha domingensis*, in fresh waters further inland (fig. 1). Along this continuum, water salinity during the wet season (May to November) is influenced mainly by fresh water runoff from inland areas, which lowers salinity towards the mangrove; water salinity during the dry season (December to April) is influenced by tides, which slightly increase salinity inland towards the *Typha domingensis* herbaceous wetland. Between February and April (e.g., mid-dry season), standing water is minimal or non-existent through most of the wetland, but soils remain muddy and never dry up completely.

Anurans in the study area include the native *Eleutherodactylus cochraneae*, *E. coqui*, and *Leptodactylus albilabris* (Leptodactylidae) and the exotic *Bufo marinus* (Bufonidae). *Rana grylio* (Ranidae), and *Scinax rubra* (Hylidae). In mangroves, *B. marinus* is frequently found; in the brackish-water *Pterocarpus* forest, *E. coqui* and *R. grylio* are frequently found along with *L. albilabris* and *B. marinus*; in the fresh water *Typha domingensis* herbaceous habitat, *E. cochraneae*, *E. coqui*, *S. rubra*, and *R. grylio* are frequently found except *B. marinus*, which is found along the margins of this habitat.

**Study organisms**

In Puerto Rico, one of the most abundant anurans in coastal wetlands is the native Caribbean white-lipped frog, *Leptodactylus albilabris*. Males of *L. albilabris* call from burrows dug into the mud or from beneath rocks and logs, while females can be found either in a burrow along with a male or on surface of soil and leaf litter (Rivero, 1998; pers. obs.). Tadpoles of this species hatch from eggs laid in terrestrial foam nests and develop either in the nest (rarely) or in temporary ponds or channels after being washed away by wave foam nests and develop either in the nest (rarely) or in temporary ponds or channels after being washed away by wave. In Puerto Rico, one of the most abundant anurans in coastal wetlands is the native *Bufo marinus* (Bufonidae), *Rana grylio* (Ranidae), and *Scinax rubra* (Hylidae). In mangroves, *B. marinus* is frequently found; in the brackish-water *Pterocarpus* forest, *E. coqui* and *R. grylio* are frequently found along with *L. albilabris* and *B. marinus*; in the fresh water *Typha domingensis* herbaceous habitat, *E. cochraneae*, *E. coqui*, *S. rubra*, and *R. grylio* are frequently found except *B. marinus*, which is found along the margins of this habitat.

**Estimates of relative abundance of adults, tadpole richness, and environmental measures**

Adult *L. albilabris* and *B. marinus* were sampled monthly in the wetland using the visual encounter survey and audio strip transect techniques (Zimmerman, 1994; Crump and Scott, 1994). Sampling occurred between June 2005 and March 2006, which included the rainy and the dry seasons. The sampling was performed along a 1.1 km area using transect and point methodology. This area represented an inland-to-coastal gradient (fig. 1). I recorded numbers of frogs calling and observed along 22 adjacent 50 m transects located parallel to the edge of the wetland (fig. 1). Anuran abundance was sampled between 18:30 and 21:30 hr along these transects in the following manner: I walked at an even pace along the first transect in the low salinity area (fig. 1) counting the number of frogs seen and heard a few steps ahead at an estimated distance of 5 m into the wetland facing south. At the end of this transect I stopped briefly to annotate the abundance of each species and begin counting along the next transect until the final transect (22) (fig. 1). On average, the counting in each transect took 6 min to complete. Given
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the life history of each species, the risk of double counting a frog was minimal and the result of this counting was an estimate of relative abundance of frogs along a salinity gradient.

I also sampled presence of tadpoles in the wetland by sweeping with a dip net (dimensions: 40 cm wide × 40 cm long × 25 cm deep; 3 mm mesh size) along the wetland margins. I used dip netting technique over other techniques (e.g., seining, enclosure sampling, or trapping [e.g., using the funnel-trap principle]) for its advantage as a rapid assessment approach to sample tadpole richness in conditions of vegetation-choked bodies of shallow waters (see Shaffer et al., 1994, for detail explanation of each technique). The sampling of tadpoles was in the following manner: tadpole abundance was sampled in each of 22 sampling points located between sampling transects (fig. 1). At each sampling point, I went 5 m into the wetland and performed ten sweeps haphazardly and at different areas with standing water. The sampling of tadpoles was conducted between 15:00 and 18:00 hr on the same day of frog counting. In addition, water depth was measured with an aluminium pole calibrated to the nearest 2.5 cm and water quality (salinity, temperature, and dissolved oxygen at 0.2 m below water surface) was measured at each sampling point during each monthly visit using a YSI-85 salinity and temperature meter (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Salinity/metamorphosis experiment

To determine the effect of increased salinity on larval survival to metamorphosis, tadpoles of L. albilabris and B. marinus were collected in June 2004 from four non-wetland locations throughout Puerto Rico: one collection site in Río Piedras (18°24′26″N, 66°02′31″W, elevation 15 m a.s.l.) and two collection sites in Trujillo Alto (18°20′32″N, 66°00′32″W, elevation 43 m a.s.l.; 18°20′57″N, 66°00′14″W, elevation 29 m a.s.l.) for L. albilabris; one collection site in Quebradillas (18°25′28″N, 66°55′32″W, elevation 208 m a.s.l.) for B. marinus. These locations represent inland populations of both species. Tadpoles were transported to the laboratory and were placed in aquaria, mixing all tadpoles by species from each sampled area in each aquarium. Aquaria contained dechlorinated and aged tap water, a 2.5 cm gravel layer, and an immersed water filter with activated carbon and a foam-filter media. Tadpoles were maintained in aquaria for two weeks and tadpoles were feed commercially available flake food (Nutrafin®Max, Rolf C. Hagen Inc., Montreal, Canada) ad libitum on a daily basis. During the two-week period of larval acclimatization, 30 19-L buckets were filled with 15 L of water at laboratory temperature (22-23°C) and the initial water depth was 29 cm. Each bucket was also supplied with an air filter and a foam media as physical and biological filter, and one 6-inch foam plate was added to each bucket as a floating surface for emerging metamorphs. Buckets were randomly assigned to a control (0 ppt) and five salinity treatments: 1 ppt (2.7-3.1% seawater or sw; 1.12 g/L), 2 ppt (5.4-6.3% sw; 2.34 g/L), 4 ppt (10.8-12.5% sw; 4.76 g/L), 8 ppt (21.6-25.0% sw; 9.64 g/L), and 12 ppt (32.4-37.5% sw; 14.53 g/L). These salinity treatments reflected the salinity levels found along the inland-to-coastal salinity gradient, which ranged between 0.2 ppt (0.54-0.63% sw) and 20.5 ppt (55.4-64.1% sw). Each salinity treatment was prepared by diluting sea salt commercially available for aquarium use (Instant Ocean® Synthetic Sea Salt, Aquarium Systems Inc., Mentor, Ohio; see table 1 in Christy and Dickman [2002] for major ion composition of this product) in 15 L of dechlorinated and aged tap water at 22-23°C in the following approximate amounts: 17 g (1 ppt), 35.5 g (2 ppt), 72 g (4 ppt), 146 g (6 ppt), and 222 g (12 ppt). Final salinity per treatment was measured with the salinity and temperature meter (model YSI-85) and saltwater or freshwater was added to achieve the required salinity and initial water depth.

I randomly selected 30 groups of 12 tadpoles that were between Gosner stages 23 and 25 (tadpoles which lacked limb buds, but which had developed opercula) (Gosner, 1960) from each aquarium. Groups of tadpoles were distributed among 19-L buckets such that each salinity treatment and the control had five replicates. Buckets were randomly distributed within the experimental area and they were checked every one to four days to count tadpoles and metamorphs. A 12:12 h photoperiod was set in the laboratory and additional lights for heat (120W, Dura Max® Indoor BR40 Flood, Philips Lighting Co., New Jersey) were installed on top of buckets. These heating lights were turned on for 6 hours during the day (between 09:30 and 15:30 hr) to raise water temperature to 24.6 ± 1.4°C, which is within the range of daytime water temperatures in the wetland (see Results). Water temperature and salinity were measured in all buckets (n = 50) and recorded every four to six days during the first two months, the period in which approximately 50% of larvae underwent metamorphosis. After each reading, dechlorinated tap water was added to each bucket to maintain its target salinity level. Afterwards, salinity was measured every four to six days as to maintain target salinity levels until completion of the experiment. Water quality variables such as pH (n = 39), ammonia (NH₃/NH₄⁺) (n = 39), nitrite (NO₂⁻) (n = 39), nitrate (NO₃⁻) (n = 34), and dissolved oxygen (mg/L) (n = 39) were measured in fewer buckets, but also during the first two months. Based on ammonia, nitrite, and nitrate readings, denitrifying bacteria (Aquarium Pharmaceutical®, Aquarium Pharmaceuticals Inc., Chalfont, Pennsylvania; Marbel®, Virbac Animal Health Inc., Fort Worth, Texas) were added to all buckets as needed to maintain levels of these variables low according to recommended safe standards for fish aquaria. Except for temperature, salinity, and dissolved oxygen, water quality variables were measured with commercially available test kits for fish aquaria (Wardley®, Hartz Mountain Co., Secaucus, New Jersey). pH was measured qualitatively with dipstick paper (Hydroid, Micro Essential Laboratory Inc., Brooklyn, New York). Tadpoles were maintained in buckets until all had metamorphosed or died. Emerging morphs were preserved in 70% ethyl alcohol, weighed, and measured (snout-vent length).

Salinity/growth experiment

Based on information gained from the first experiment, I performed a second experiment with only tadpoles of the
native *L. albilabris* to examine the influence of salinity on growth. Tadpoles of this species were collected in November 2005 from two localities in Trujillo Alto (see above), northern Puerto Rico. Tadpoles were brought to the laboratory and acclimatized as before. The experimental setting followed the one described above except that a treatment of 6 ppt (16.2-18.8% sw; 6.74 g/L) was added and the treatment of 12 ppt was eliminated. Water temperature, salinity, and tadpoles were maintained and monitored as before over a four-week period. One week after initiation of the experiment, each tadpole was taken from its bucket, excess water dried with a towel, weighed on a digital analytical balance, photographed along with a reference scale, and measured (snout-vent length, tail length, and total length) using a computer software for image analyses (Scion Image, Scion Corporation, Maryland). This procedure was repeated during the third and fourth week. The experiment ended by the fourth week in which all tadpoles were preserved in 70% ethyl alcohol. Tadpoles from this experiment and metamorphs from the previous experiment were all deposited in the Biology Museum of the University of Puerto Rico Río Piedras Campus.

**Data analyses**

Relationships between mean relative abundance of each species and each water quality variable (salinity and temperature) were assessed with Spearman’s correlation. In addition, partial correlations were used to assess relationships between mean relative abundance of each species and each water quality variable controlled for geographic location along the inland-to-coastal gradient. In the first salinity experiment (*L. albilabris* and *B. marinus*), data were not normally distributed. Therefore, I used a non-parametric ANOVA (Kruskal-Wallis) to examine differences by salinity treatment in the number of larvae surviving to metamorphosis, body weight, and snout-vent length. In the second experiment (*L. albilabris*) only, data were not normally distributed even after log-transformation of the data. The small sample size within treatments and the non-linear relationship between tadpole size variables and time required Kruskal-Wallis tests with log-transformed data. This approach was adopted to assess differences among salinity treatments in tadpole size (head length, tail length, and total length) and weight within each weekly data set and to assess differences in tadpole size and weight among weekly data sets within each salinity treatment. For each experiment, non-parametric ANOVA was used to determine if differences in water quality variables were attributable to salinity treatments. All data are shown as mean ± SD. An alpha = 0.05 was adopted for significance.

**Results**

**Abiotic factors along a land-to-sea gradient**

Mean water salinity and its variation increased non-linearly along the inland-to-coastal transect (fig. 2a). Span of mean water salinity was from 0.8 ppt (range 0.2-0.9 ppt) towards sampling point one in the inland-to-coastal transect to 5.8 ppt (range 0.4-20.5 ppt) towards sampling point 22 at the end of transect (fig. 2a and fig. 1). In contrast, mean water temperature showed no pattern with distance from coast (fig. 2b). Water temperature was higher (mean 30.1°C; range 23.9-34.6°C) towards sampling point one (low salinity area), decreased in the middle of the inland-to-coastal transect (mean 26.0°C; range 22.3-27.8°C), and increased towards the end of transect (high salinity area; mean 28.2°C; range 24.7-30.6°C) (fig. 2b and fig. 1). I measured dissolved oxygen on two occasions in April 2006 along the inland-to-coastal gradient. Dissolved oxygen ranged between 0.7 mg/L and 4.5 mg/L (mean ± SD: 2.0 ± 0.9 mg/L, n = 37) when mean water temperature on these occasions was 24.0 ± 1.1°C. On these visits, there were no correlations between dissolved oxygen and water salinity ($r_s = 0.071, P = 0.68$) or between dissolved oxygen and water temperature ($r_s = 0.1604, P = 0.34$).

**Relative abundance and tadpole richness along a land-to-sea gradient**

Adult *L. albilabris* were frequently found on each sampling transect along the inland-to-coastal gradient. However, mean relative abundance of adult *L. albilabris* decreased ($r_s = -0.697, P < 0.01$) linearly with increasing salinity along the inland-to-coastal gradient, while mean relative abundance decreased with increasing water temperature ($r_s = -0.4593, P = 0.03$) in a non-linear fashion along this gradient (fig. 2c). In contrast, adult *B. marinus* were only found in 16 of the 22 sampling transects. Mean relative abundance of adult *B. marinus* showed no correlation with salinity ($r_s = -0.1249, P = 0.58$) or temperature ($r_s = 0.2882, P = 0.19$) (fig. 2d). However, when controlled for geographic location in the inland-to-coastal gradient (i.e. sampling point position along the gradient in fig. 1) using partial correlation, mean relative abundance of *B. marinus*...
increased with salinity \((r = 0.6353, P < 0.01)\). In contrast, controlling for geographic location in the gradient revealed no correlation between mean relative abundance of adult \(B. marinus\) and water temperature \((r = 0.2670, P = 0.23)\.

Dip net sampling yielded \(L. albilabris\) tadpoles only at sampling points 9 and 13 (towards the middle region of the gradient in fig. 1) and no \(B. marinus\) tadpoles.

**Effects on larval growth and survival**

Over the course of the first salinity experiment, variation in water salinity (mean ± SD) was negligible within treatments 0, 1, 2, and 4 ppt \((0.24 \pm 0.03 \text{ ppt}; 1.13 \pm 0.01 \text{ ppt}; 2.13 \pm 0.02 \text{ ppt}; 4.09 \pm 0.03 \text{ ppt}; \text{ respectively})\), but varied slightly in the 8 ppt treatment \((7.80 \pm 0.44 \text{ ppt}; n = 5 \text{ bucket/treatment/species})\). pH ranged between six and seven in all treatments throughout the experiment and no adjustments of pH were required. There were no statistical differences in water temperature \((\bar{x} \pm SD = 25.2 \pm 0.8^\circ C; F_{4,45} = 0.25, P = 0.91)\), dissolved oxygen \((6.0 \pm 0.9 \text{ mg/L}; F_{4,34} = 2.01, P = 0.11)\), ammonia \((1.2 \pm 0.8 \text{ ppm}; F_{4,34} = 0.71, P = 0.59)\), nitrite \((1.3 \pm 2.2 \text{ ppm}; F_{4,34} = 0.73, P = 0.58)\), and nitrate \((4.6 \pm 9.7 \text{ ppm}; F_{4,29} = 2.45, P = 0.07)\) among treatments. Negative relationships were found between water salinity and dissolved oxygen \((r_s = -0.4134, P < 0.02)\), ammonia and nitrite \((r_s = -0.6669, P < 0.01)\), and ammonia and nitrate \((r_s = -0.7000, P < 0.01)\). Nitrite and nitrate were positively related \((r_s = 0.7888, P < 0.01)\).

In the first experiment, all tadpoles of \(L. albilabris\) died after 3 hours in the 12 ppt treatment. Eliminating the 12 ppt treatment from statistical analysis revealed a significant effect of salinity on survival to metamorphosis \((F_{4,24} = 6.92, P < 0.01; \text{ fig. 3a})\). The proportion of \(L. albilabris\) surviving to metamorphosis decreased from a high of 41.1% in 2 ppt salinity to 1.7% in 8 ppt (fig. 3a). However, body weight \((F_{4,20} = 1.54, P = 0.24; \text{ fig. 3b})\) and length \((F_{4,20} = 1.10, P = 0.39; \text{ fig. 3c})\) of \(L. albi-
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labris metamorphs did not differ among treatments. Similar to L. albilabris, all B. marinus tadpoles died after 3 hours in the 12 ppt treatment. Eliminating the 12 ppt treatment from statistical analysis revealed, however, that there was no effect of salinity on survival to metamorphosis (F_{4,24} = 1.67, P = 0.20; fig. 3a). The proportion of B. marinus surviving to metamorphosis decreased from 67.0% in 1 ppt to 38.2% in 8 ppt (fig. 3a). Similar to L. albilabris, body weight (F_{4,24} = 2.64, P = 0.06; fig. 3b) and length of metamorphs (F_{4,24} = 1.56, P = 0.22; fig. 3c) in B. marinus did not differ among salinity treatments. However, in the 8 ppt treatment, body weight in B. marinus was lowest (mean = 0.081 ± 0.016 g) compared with the rest of the treatments and 21% (4/19) of metamorphs emerged without having developed one of their forelimbs. Finally, percent survival to metamorphosis was higher in B. marinus than in L. albilabris in the 0 ppt (F_{1,8} = 5.23, P = 0.05), 1 ppt (F_{1,8} = 33.33, P < 0.01), and 8 ppt treatments (F_{1,8} = 35.71, P < 0.01) (fig. 3a).

In the second experiment with L. albilabris only, salinity did not affect tadpole weight in the 8 ppt treatment through time (F_{2,12} = 2.09, P = 0.17) (fig. 4). In contrast, the proportions of tadpole head, tail, and total length were different between weeks one, two, and three in the 8 ppt treatment (F_{2,12} = 9.05, P < 0.01; F_{2,12} = 9.38, P < 0.01; and F_{2,12} = 8.43, P < 0.01, respectively) (fig. 4). On average, tadpoles grew longer, but weighed less through time in the 8 ppt treatment.

Discussion

Adults of both species were found naturally in areas where salinity exceeded 60% seawater (e.g. >20 ppt) in Sabana Seca. The distribution pattern of adult L. albilabris, however, showed a decrease in mean relative abundance with increasing water salinity, which contrasted with the distribution pattern of adult B. marinus. Nonetheless, the virtual lack of tadpoles of L. albilabris and B. marinus from dip net samplings may have two plausible explanations. These species may not reproduce in these waters or this technique failed to detect tadpoles from both species during the sampling period. The fact that tadpoles of a third anuran, the aquatic species R. grylio (Thorson and Svihla, 1943; Lamb, 1984), were frequently sampled along the inland-to-coast gradient with this technique.
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Figure 4. Effects of different salinity concentrations on mean head length (A), tail length (B), total length (C), and mean weight (D) of *L. albilabris* tadpoles on a weekly basis. Ellipses highlight lack of difference, at $\alpha \leq 0.05$, in pairs of means in the 8 ppt treatment between week 1 and week 2 (A, B, and C) and among weeks (D). All variables were log-transformed. $n = 12$ tadpoles for each treatment and control.

may suggest an interaction between sampling adequacy and breeding ecology of *L. albilabris* and *B. marinus* (e.g., Smith et al., 2007). I observed and heard calling males and found nests in borrow in hummocks of *Pterocarpus* trees towards the low salinity area, which does not preclude the occurrence of reproduction of *L. albilabris* in the study area. Probably, tadpoles may stay and metamorphose mostly in microhabitats (e.g., small, water-filled depressions in hummocks towards the base of *Pterocarpus*’ trees) without getting into larger bodies of water, which may explain the scarcity of tadpoles capture by dip netting along the inland-to-coast salinity gradient. *Bufo marinus* is considered a euryhaline amphibian (Liggins and Grigg, 1985) and commonly breed in sites frequently used by the crab-eating frog, *Fejervarya cancrivora* (Anura: Ranidae), the most euryhaline frog that occurs throughout Southeast Asia (Hogarth, 1999; D.S. Rabor, 1952 cited by Lever, 2003). However, previous studies have shown that selection of breeding sites of lower salinity is a strategy adopted by adult anurans adapted to brackish water and moderately saline conditions (e.g., *R. temporaria* [Viertel, 1999], *Litoria aurea* [Christy and Dickman, 2002], *B. calamita* [Gómez-Mestre and Tejedo, 2003]). *Bufo marinus* prefers open areas ( unvegetated) in lentic, isolated water bodies in its natural and invaded habitats (Evans et al., 1996; Hagman and Shine, 2006), and I found calling males and hatchlings of *B. marinus* in the *Typha domingensis* herbaceous wetland (low salinity area along the inland-to-coast gradient). Possibly, the lack of *B. marinus* tadpoles sampled throughout the sampling area could result from adults of this species using this wetland for activities other than reproduction, which is unlikely because metamorphs have been found in this wetland (see Materials and Methods, *Study organisms*) or I missed the species’ sporadic breeding events during and between sampling periods. Clearly, both species breed in this wetland, but future studies should modify this technique, its sampling regime, and/or combined it
with other approaches to estimate richness and abundance of tadpoles from all species in this and similar Caribbean coastal wetlands.

The salinity experiments showed that increased salinity had different effects on tadpoles of *L. albilabris* compared with those of *B. marinus*. Percent metamorphosis of *L. albilabris*, for example, decreased drastically in 25% seawater (e.g., 8 ppt). In contrast, tadpoles of *B. marinus* had a higher percentage surviving to metamorphosis in 25% seawater. In addition, this species showed a higher percentage surviving to metamorphosis at low (0 ppt, 1 ppt) and high (8 ppt) salinity levels, compared with *L. albilabris*. Food used in the experiments was identical among salinity treatment, both in quantity and composition, reducing the possibility of food as the main variable affecting tadpole development and survival to metamorphosis. The differences in metamorphic success are difficult to explain, however, because nothing is known about *L. albilabris* tadpoles’ (or adults’) ability to tolerate salinity (and possibly in any *Leptodactylus* species; e.g., Balinsky, 1981). These experiments did show that tadpoles of both species died after 3 hours in 37.5% seawater treatment (e.g., 12 ppt). Other studies have shown that *B. marinus* tadpoles frequently die after three days of exposure in salinities greater than 15% seawater (e.g., 5 ppt; Ely, 1944) despite that adult *B. marinus* survive in salinities up to 40% seawater (Liggins and Grigg, 1985). In addition, deformities of *B. marinus* tadpoles were found and reached 21% (4/19 metamorphs) in 25% seawater (e.g., 8 ppt). Chinathamby et al. (2007) documented deformities in the Australian brown tree frog, *Litoria ewingii*, in 12% and 16% seawater (e.g., >3.8 ppt) although the reasons are not known. Nonetheless, results from these experiments partly support earlier studies that show that amphibian tadpoles can be more strongly affected by salinity than are adults (Boutilier et al., 1992).

In the growth experiment with only *L. albilabris*, weight loss was observed in 25% seawater treatment without a corresponding decrease in tadpole length. Previous studies have documented similar results in *B. calamita* (Gómez-Mestre et al., 2004) and in *L. ewingii* tadpoles (Chinathamby et al., 2007). In adults, one way in which amphibians deal with salinity is through the accumulation of urea to reduce internal osmolality relative to the external environment (Gordon and Tucker, 1965; Gómez-Mestre et al., 2004). However, tadpoles do not accumulate urea in saline environments and osmoregulation may occur through other means (Balinsky, 1981). One possible explanation for a reduction in tadpole weight with increased salinity includes the reallocation of energy from growth to increased osmoregulation by tadpoles that experience osmotic stress with increased salinity (Gómez-Mestre et al., 2004; Chinathamby et al., 2007). Although weight loss may possibly occur through reduced feeding, e.g. by osmotically-stressed tadpoles that are sluggish and inactive at the bottom of containers (Chinathamby et al., 2007; pers. obs. in 8 ppt treatments), dehydration with increased salinity may also result in weight loss in osmotically-stressed tadpoles (Balinsky, 1981; Gómez-Mestre et al., 2004). More detailed laboratory and field studies may be needed to determine the mechanisms (e.g., hormone-mediated; Gómez-Mestre et al., 2004) by which increased salinity reduces tadpole weight and metamorphic success of a broad range of anuran tadpoles, given that different factors may be synergistic in osmotically-stressful environments.

Results from this study showed a negative relationship between increased salinity and anuran abundance along an inland-to-coastal salinity gradient. They also demonstrated negative effects of increased salinity and tadpole growth and metamorphic success at naturally occurring salinity levels. I must emphasize, however, that these results should be taken with caution because they may represent an underestimate of tadpole tolerance to salinity at the species level, as animals of both species were collected only from inland areas. Other studies have shown geographic variation in salinity tolerance in the
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anuran *B. calamita*, which suggest local adaptation to salinity in a brackish water breeding population compared with fresh water breeding populations (Gómez-Mestre and Tejedo, 2003). The lack of information on genetic variation and genetic structure of both species in Puerto Rico, however, limit a broader discussion and understanding of the relative contribution of migrants to possible local adaptation to salinity in the studied populations, but this may not invalidate results given herein.

One important implication of these results includes wetland conservation applications, especially in light of current predictions of increased sea levels and salt-water intrusions (Melloul and Goldenberg, 1997; Michener et al., 1997; Ellison, 2004; e.g., IPCC, 2007). In Puerto Rico, land-use history in coastal wetlands resulted in a drastic reduction of their original land cover (Cintrón, 1983; Lugo and Brown, 1988), which may only decrease further with expected sea-level rises. Given the importance of this coastal wetland for anuran abundance, a plausible consequence of wetland destruction inland and sea-level rise to the coast may be limitations in the ability of anurans to maintain viable populations, despite possible local adaptation to salinity, as this ecosystem is predicted to become osmotically more stressful in the short term (IPCC, 2007). Anuran conservation efforts are thus greatly challenged for two main reasons. First, the expected degradation of environmental conditions in coastal wetlands will contribute to anuran population declines worldwide as tadpoles may live near their physiological limit for salinity. Second, salt water intrusion and urban encroaching inland may result in anuran population replacement, from native species to introduced species in this wetland. These salient points justify future salinity-anuran response research to understand broadly impacts of sea-level rise and salt-water intrusion on amphibian assemblages in different coastal wetlands, and to evaluate the generality of results from this study.

Acknowledgements. I thank S.D. Mitchell and O. Ortiz for assistance in the laboratory and data gathering, C. Beachy, S.A. Sloan, and two anonymous reviewers for improving earlier versions of this manuscript, and to the Department of Natural and Environmental Resources-Puerto Rico Commonwealth, for issuing collection permit (#06-IC-048). This research was partially funded by NSF-EPSCoR (NRL) and NASA-IRA (SDM).

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Received: February 5, 2007. Accepted: June 8, 2007.