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Experimentally derived salinity tolerance of hatchling Burmese pythons (*Python molurus bivittatus*) from the Everglades, Florida (USA)

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ABSTRACT

In a laboratory setting, we tested the ability of 24 non-native, wild-caught hatchling Burmese pythons (*Python molurus bivittatus*) collected in the Florida Everglades to survive when given water containing salt to drink. After a one-month acclimation period in the laboratory, we grouped snakes into three treatments, giving them access to water that was fresh (salinity of 0, control), brackish (salinity of 10), or full-strength sea water (salinity of 35). Hatchlings survived about one month at the highest marine salinity and about five months at the brackish-water salinity; no control animals perished during the experiment. These results are indicative of a "worst-case scenario", as in the laboratory we denied access to alternate fresh-water sources that may be accessible in the wild (e.g., through rainfall). Therefore, our results may underestimate the potential of hatchling pythons to persist in saline habitats in the wild. Because of the effect of different salinity regimes on survival, predictions of ultimate geographic expansion by non-native Burmese pythons that consider salt water as barriers to dispersal for pythons may warrant re-evaluation, especially under global climate change and associated sea-level-rise scenarios.

1. Introduction

Recently established non-native Burmese pythons (*Python molurus bivittatus*) are successfully breeding in the Everglades (Florida, USA; Krysko et al., 2008; Snow et al., 2007a). They occur primarily in fresh-water sites, but have also been found in brackish-water habitats such as mangroves. There is uncertainty about the current and predicted geographic extent of their invasion (Reed and Rodda, 2009), as well as the routes by which the population is expanding. Thus, in the laboratory we tested the ability of pythons to survive when given only water containing salt to drink. This information may assist in determining whether pythons can inhabit salt water habitats for long periods of time.

Geographic spread of an invasive species such as the Burmese python is curtailed where the climates of the native and invaded range are dissimilar (Bomford et al., 2008; Reed and Rodda, 2009; Rodda et al., 2008). However, other factors such as availability of microhabitats, availability of prey and lack of predators may also allow for survival of non-native vertebrate species in their newly established sites. Recently, Fujisaki et al. (2009) performed a risk assessment to predict establishment success of non-native reptiles imported into Florida, and found that in addition to climate match, several human-related factors were important predictors of species' establishment success. Thus, the presence or absence of a species in a given habitat is related to the combined effects of both biotic (e.g., competition, predation) and abiotic factors (e.g., temperature, salinity, humidity, oxygen, pH; Connell, 1975). Moreover, Dunson and Mazzotti (1989) suggested that salinity was the limiting factor affecting the distribution of reptiles in coastal habitats such as Florida Bay in south Florida, particularly because of a generally poor ability of reptiles to tolerate high salinities.

Previous tests of the ability of native reptiles to tolerate salinities found in south Florida estuarine areas include blood sampling and collection of cloacal fluids of American Crocodiles, *Crocodylus acutus* (Ellis, 1981) and a study of sodium and water balance in the water snake *Nerodia fasciata* (Dunson, 1980). To our knowledge, no previous descriptions or tests of the ability of Burmese pythons to tolerate salt water conditions in their native range in Southeast Asia are available for comparison. However, pythons have been found in brackish-water locations along the Everglades margin (e.g., Biscayne Bay, Shark River estuary), indicating at least a temporary tolerance of salt water for this established, non-native species.

In the USA, the native mangrove snake (*Nerodia* spp) lives in high-salinity habitats, thus it may be instructive to compare the salt-water tolerance of this species to that of introduced pythons. In addition, the mangrove snake has no salt gland, but instead has a great degree of resistance to dehydration due to a low net uptake of salts and a low net loss of water (Dunson, 1980). In laboratory tests of salt-water tolerance and sodium and water balance of estuarine and freshwater races of the snakes *N. fasciata*, *N. sipedon*, and *N. valida*, Dunson

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(1980) found that all freshwater snakes died in less than two weeks when immersed in salt water. In contrast, *N. f. compressicauda* and *N. f. clarki* lived for long periods (e.g., two weeks to 99 days) in sea water. Currently, mechanisms of osmoregulatory biology are unknown for Burmese pythons or other large constrictors now present in south Florida (e.g., African rock python *Python sebae*, Reed et al., 2011).

Recent predictions of the possible extent of invasion of Burmese pythons in the Everglades and in other parts of the USA by Pyron et al. (2008) and Rodda et al. (2008) were based on climatic suitability and incorporated climate-change scenarios. However, results of those climate-matching models were varied and even contradictory with respect to their predictions of the possible geographic extent of invasion. To accurately predict the eventual spread of a species, an understanding of the tolerance of the species to environmental factors, especially those that may serve as physiological stressors or barriers, is necessary. Given this lack of biological information, our objective was to test the ecological limitations of Burmese pythons with respect to salt water tolerance. As Pyron et al. (2008) suggested, the ecological limitations of the species in south Florida are not known, so possible effects on the predicted expansion of the species are currently unclear. Given the proximity of the Everglades to the sensitive environments of the Florida Keys, there is much concern about the ability of pythons to tolerate salt-water conditions and a need to determine possible pathways for reaching the Keys (e.g., across salt-water estuaries). Thus, we specifically aimed to test the ability of wild-caught hatchling Burmese pythons from the Everglades to survive in realistic salt-water conditions in an experimental set-up.

2. Methods

2.1. Experimental animals

We collected all snakes in July 2009 from Everglades National Park. Twenty-four hatchlings (average mass = 115.7 g ± 3.10 SE; range 49–126 g; average total length = 60.1 cm ± 0.90 SE; range 45.3–66.6 cm) and one young-of-the-year (YOY; mass = 954 g; total length = 116 cm) python were used for this study. Seventeen of the hatchlings were collected as eggs from the same nest that hatched on 23 July 2009. Immediately after collection, we weighed, measured and determined gender of the snakes. We then transported all snakes to the US Geological survey laboratory in Gainesville, FL and allowed them to acclimate for one month with access to fresh water. Once the experiment began, we fed snakes once every four weeks by giving hatchlings "hopper" sized mice (range = 5.4–15 g), and the YOY enough mice to equal approximately 60 g.

2.2. Experimental set-up

Each experimental enclosure consisted of one 75 L clear plastic storage bin (60.5 l × 37 w × 28 h cm) with small holes drilled along each side for ventilation. Each bin held one individual snake. The bin was secured with a snap-down lid and reinforced with a heavy-duty bungee cord. We lined the bottom of each bin with newspaper, and provided each snake a "hide" box constructed using pasteboard boxes (e.g., cereal containers) for shelter. Newspaper and hide boxes were replaced weekly. We also provided a separate container (4 L clear plastic box, 33 l × 22.5 w × 7 h cm) inside each large bin filled with approximately 1 L of water. This smaller container formed a pool that the snake could enter partially or fully, but could also avoid. Each pool covered about 40% of the surface area of the bottom of the enclosure.

We assigned snakes to one of three treatments: fresh-water control (salinity of 0.2), brackish (salinity of 10) or marine (salinity of 35). Fresh-water came from an onsite well in Gainesville, Florida. Water used for the salinity treatments was made by adding synthetic aquarium salts (Crystal Sea® Marine Mix) to well water. For each salt-water treatment, we mixed water in a single large container (within salinity of 0.1) and then distributed it to the pools in the enclosures. We measured salinity with a YSI® (Yellow Springs Instrument) model 556 mps meter and recorded temperature and humidity once per day, five days per week (including during the acclimatization phase) using an Extech® 44550 humidity/temperature pen. Photoperiod was kept constant (12 L: 12 D) throughout the experiment.

2.3. Experimental design

Eight hatchlings were assigned to each of three treatment salinities: control, brackish, or marine salinity. The 17 sibling snakes were dispersed roughly evenly among treatments, with approximately equivalent genders in each. The YOY was assigned to the marine salinity treatment. . Because the YOY was significantly larger/older than the hatchlings it was not included in the data analysis; however, we report its survival for qualitative comparison to the hatchlings. The experiment began one week after each snake accepted food for the first time in the laboratory. Snakes did not begin feeding at the same time, therefore the experiment start dates were staggered: 1 September 2009 (n = 11), 12 September 2009 (n = 12), 22 September 2009 (n = 1), and 13 October 2009 (n = 1). Snakes were checked once per day, five days per week. Water for all treatments was changed weekly. Total time in treatment for surviving hatchlings was about seven months (193–217 days).

2.4. Data analysis

We used one-way ANOVA (separately for mass, SVL and TL) to determine that neither mass nor length varied by treatment before we began the experiment and Levene's test to check for homogeneity of variances. We assessed survival of the 24 hatchlings using a Kaplan–Meier product-limit estimator (Kaplan and Meier, 1958). Hatchlings were not assigned to their treatment salinities on the same date because we waited until each individual fed before starting it in the experiment (including controls, see Section 2). Therefore, data were collected in a staggered (time-wise) fashion. We designated the day on which each hatchling was transferred to its treatment salinity as day "0". End time was scored the number of days each individual had survived when the experiment was terminated. Survival estimates for treatments that included hatchlings that were still alive at the end of the experiment are slightly biased, as survival beyond the termination of the experiment cannot be predicted. However, the occurrence of these censored data is rare within the data set. When survival estimates are provided in Section 3 for treatments that included censored cases, these are noted. We used the log-rank test to compare survivorship in treatment salinities to the control (Chambers and Leggett, 1989; Cox and Oakes, 1984; Savage, 1956; Schultz et al., 1998). We performed all calculations with SPSS version 12.0 (SPSS Inc, Chicago).

3. Results

Neither python mass nor length varied by treatment at the beginning of the experiment (mass mean = 115.7 g ± 3.1 SE, range 49.0–126.0 g; SVL mean = 52.3 cm ± 0.78 SE, range 39.6–58.3 cm; TL mean = 60.1 cm ± 0.9 SE, range 45.3–67.4 cm). Mean laboratory temperature was 26.7 °C (± 0.09 SE, range 22.0–29.7 °C and mean humidity was 55% (± 1.3 SE, range 20–87%).

Salinity strongly impacted survival of hatchlings in the laboratory. Log-rank tests comparing the survivorship of the hatchlings at the three salinities indicated each treatment was statistically different from the others ($P < 0.01$ for all comparisons; Fig. 1). Hatchlings that

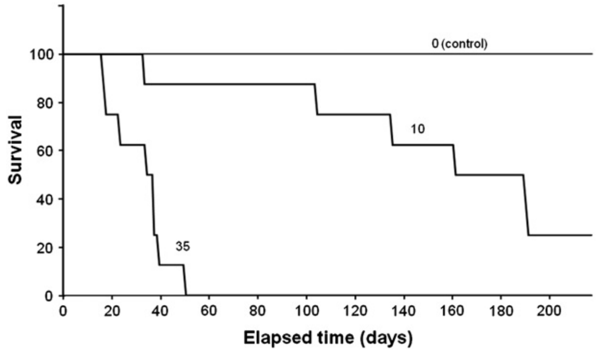

Fig. 1. Survival of wild-caught hatchling Burmese pythons over time when exposed to three different salinities (freshwater control [salinity of 0]; brackish [salinity of 10]; and marine [salinity of 35]). Each treatment is statistically distinct from the others (log-rank test; $P<0.01$ for all comparisons). See text for details of analysis.

were assigned to the marine salinity (35) treatment began dying after 17 days, with the last remaining hatchling expiring on the 50th day of the experiment (mean estimated survival time = 32 days; 95% confidence interval [CI] 23–40 days). Six of the eight hatchlings assigned to the brackish water (10) treatment perished over the course of the experiment. The first of these died on the 33rd day of the experiment. Two hatchlings (25%) survived the brackish water treatment, and were therefore censored from the dataset. These two survivors persisted for ≥200 days at 10 ppt (200 and 217 days, n = 1 each). Mean survival time for hatchlings in brackish water was estimated at 156 days (95% CI = 115–197 days). No pythons in the fresh–water control treatment died during the experiment (survival varied from 193 to 217 days, depending on each individual's start date). The YOY snake (not included in the data analyses) survived until the experiment was terminated (200 days) in full marine water (salinity of 35). Finally, we observed significant bloating in some of the hatchlings, yet we caution that this increased water-weight should not be mistaken for growth.

4. Discussion

We determined that a high proportion of wild-caught hatchling Burmese pythons can survive in a laboratory setting with access to only salt water; on average individuals in the experimental treatments survived for about a month in full-strength sea-water (salinity of 35), and about five months in brackish-water (salinity of 10). In addition, one YOY python survived 200 days in the full marine salinity treatment; this individual continued to feed until the termination of the experiment. It is unclear how much longer the YOY snake would have persisted in salt water. The data indicated that hatchling pythons can survive either dehydration (from not drinking as much salty water) or an imposed salt load (by drinking salty water). This information may be useful for evaluating the expansion capabilities of pythons and the control tools available for managing the spread of the species through both brackish- and salt-water pathways (e.g., canals, estuaries) adjacent to the core population of the species in the Everglades.

Pyron et al. (2008) showed that a predicted expansion of Burmese pythons outside of south Florida (i.e., from the sites occupied in south Florida northward throughout the State and into neighboring States in the southeast USA) was unlikely, based on what was known at that time about the basic ecology of the species and the climate of the USA. However, our results show the ability of hatchling pythons to persist under salt-water conditions and provide new information for evaluating possible pathways to expansion if our results are transferable to the wild. Survival in salt water conditions during the first year of life for this species was unknown before now, and current global warming scenarios that incorporate sea-level rise are predicted to significantly reduce the area of suitable habitat for Burmese pythons (Pyron et al., 2008; Rodda et al., 2008). However, if hatchling pythons can survive in the wild in full salt water conditions for at least a month, almost as long as native mangrove snakes (Dunson, 1980), the species may be able to expand into and occupy other suitable habitats not previously considered in modeling efforts, or those currently occupied by native species.

Rodda et al. (2008) pointed out that in Florida, 31 vertebrate species listed as threatened or endangered under the US Endangered Species Act are of a size and habit that may make them vulnerable to consumption by Burmese pythons. Whether all of these species are vulnerable to predation by Burmese pythons is not clear. However, where the risk of predation was previously thought to be low because pythons were thought to be intolerable to salt water conditions, this risk now needs to be re-evaluated. In particular, if our laboratory results are transferable to the wild and hatchling Burmese pythons could survive on average 32 days under full salt-water conditions and on average 156 days or ~5 months in brackish water conditions, it may be possible for them to reach the sensitive environments of the Florida Keys through various land and sea pathways. Certainly the survival of our YOY snake for 200 days in full marine salinity water highlights a possible cause of concern for the ability of larger snakes to survive longer durations in full salt-water conditions. Future tests of the ability of larger pythons to tolerate full salt water conditions are warranted, as are tests that incorporate access to fresh water sources during the experiment.

Our data represent the first results for a direct, simple test of the ability of Burmese pythons to survive when given only salt-water to drink. We intentionally set up a “worst-case scenario” by stressing hatchlings with salt-water treatments; we were able to determine differences in python survival over a seven-month time frame, attributable to differences in salinity concentrations. In addition to providing a time-frame for expectation of survival under these conditions, this information may be used in species-specific risk assessments that utilize realistic environmental tolerances to drive model selection. As discussed in Fujisaki et al. (2009), combining information from geographic risk assessments with that obtained through species-based assessments would allow for identification of high-risk areas for potential invaders. Thus, this information on the lab-derived ability of hatchling Burmese pythons to survive when given access to only salt water will be valuable for further investigation of mechanisms of python spread through the landscape. However, further studies are needed to evaluate whether extrapolations from our observations in the laboratory are indicative of python salinity tolerance in the wild, where free-ranging hatchling pythons may feed at intervals different from our experimental conditions, with variable access to freshwater resources. Additional longer-term laboratory studies with similar salt-water treatments should test for effects of these treatments on individual python growth rates, using ash-free mass to alleviate the effect of bloating on true mass gain. As well, studies that incorporate analysis of python blood sodium concentrations over time would be instructive for ascertaining the mechanisms behind the ability of pythons to tolerate salt water conditions. Such information will be valuable for developing further inferences about fitness of hatchling pythons under seawater conditions.

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