An exploratory assessment of Cuban Treefrog (*Osteopilus septentrionalis*) tadpoles as predators of native and nonindigenous tadpoles in Florida

Kevin G. Smith[1]

The introduction and establishment of nonindigenous species is a significant threat to native biodiversity worldwide (Vitousek et al., 1996; Mack et al., 2000). The adverse ecological impacts of such species on native taxa are generally attributed to several basic mechanisms, perhaps the most obvious of which is the direct predation of native organisms by a nonindigenous species (Simberloff, 1997). In the most extreme cases, predation by nonindigenous species has caused the extinction of native and endemic faunas (e.g., Honegger, 1981; Savidge, 1987; Atkinson, 1989). Although the impacts of nonindigenous predators are not always this severe, they are nonetheless worrisome if native community structure is significantly altered. For these reasons the study of the impacts of potentially injurious invasive predators is an essential component of biodiversity conservation.

The nonindigenous Cuban Treefrog, *Osteopilus septentrionalis*, has been established in mainland Florida, USA since at least 1951 (Schwartz, 1952) and has since spread to occupy much of peninsular Florida where it occurs syntopically with several native anurans (Meshaka, 2001). Anecdotal reports of local declines of these native species coincided roughly with the appearance of *O. septentrionalis* (Bartlett, 1967), leading several researchers to consider the effect of predation on native anurans by adult *O. septentrionalis* (Bartlett, 1967; Lee, 1968; Wilson and Porras, 1983; Meshaka, 2001). In contrast, the interactions among larval *O. septentrionalis* and native tadpoles have been essentially neglected. Consequently, one important topic that has yet to be addressed is the role of larval *O. septentrionalis* as a predator of native tadpoles. There are no published reports of predation by larval *O. septentrionalis* on heterospecific anuran larvae, but Crump (1986) observed that the larvae of *O. septentrionalis* readily consume conspecific larvae ("cannibalism") when crowded. Because cannibalism is merely a special case of predation, a reasonable prediction is that *O. septentrionalis* larvae will prey on heterospecific anuran larvae, as well.

The primary objective of this study was to conduct exploratory research (sensu Jaeger and Halliday, 1998) to determine if larval *O. septentrionalis* will prey on native (Squirrel Treefrog, *Hyla squirella*) and nonindigenous (Cane Toad, *Bufo marinus*) tadpoles. The eggs and/or larvae of *B. marinus* are toxic to many aquatic predators (Crossland and Azevedo-Ramos, 1999; Crossland, 2000) including *O. septentrionalis* (Punzo and Lindstrom, 2001), so a secondary objective of this study was to determine if the consumption of *B. marinus* larvae resulted in mortality of *O. septentrionalis* tadpoles.

1 - Department of Ecology and Evolutionary Biology, 569 Dabney Hall, The University of Tennessee, Knoxville, TN 37996-1610, USA (present address)
e-mail: kgs@utk.edu
U.S. Geological Survey, Florida Integrated Science Center, 7920 NW 71st Gainesville, FL 32653, USA

Also available online - www.brill.nl

Table 1. Approximate age, range of Gosner stage and mean (±1 SE) of body length of tadpoles on 5 June. Exact age of *Hyla squirella* larvae was unknown. For all species $n = 15$.

Species	Age (d)	Gosner stage	Body length (mm)
Hyla squirella	<10	25	2.3 ± 0.03
Osteopilus septentrionalis	10	25-26	4.2 ± 0.12
Bufo marinus	8	26-29	4.0 ± 0.10

I collected freshly-laid eggs of *B. marinus* and *O. septentrionalis* from a flooded field in Dade Co., Florida on the morning of 25 May 2003. I collected eggs from several clutches of *O. septentrionalis* and from one clutch of *B. marinus*. The eggs of both species were hatched and larvae reared at the U.S. Geological Survey Florida Integrated Science Center in Gainesville, Florida (hereafter, FISC). On 5 June I collected *H. squirella* larvae from vinyl tanks outside of FISC. These tanks were used as breeding habitat by several pairs of *H. squirella* during recent rainstorms. Tadpoles of all species were fed supplementary food *ad libitum* (3:1 ratio by mass of finely ground rabbit chow pellets (primary ingredient = alfalfa) and TetraFin™ goldfish food) until 24 h prior to the beginning of the experimental trials on 5 June (*B. marinus* trial) and 8 June (*H. squirella* trial). On 5 June a subsample of tadpoles was haphazardly collected for developmental stage determination (Gosner, 1960) and body length measurement (as described in Altig and McDiarmid, 1999) (table 1).

I designed two separate experimental trials to assess larval *O. septentrionalis* as a predator of *H. squirella* and *B. marinus*. I randomly assigned *H. squirella* or *B. marinus* to one of two treatments in each trial: a control treatment (five *H. squirella* or five *B. marinus* larvae alone) and an *Osteopilus* treatment (five *H. squirella* or five *B. marinus* larvae plus one *O. septentrionalis* larva). A third treatment, an *Osteopilus* control (one *O. septentrionalis* larva alone), was also included in the *B. marinus* trial to test for the effects of *B. marinus* toxin on *O. septentrionalis*. Treatments in the *H. squirella* trial were replicated 10 times and treatments in the *B. marinus* trial were replicated five times.

Experimental microcosms for both trials were small, hexagonal plastic weigh boats (ca. 75 mm wide × 17 mm deep) filled with 100 ml well water. All microcosms were arranged in a systematic design (2 × 10 for the *H. squirella* trial and 3 × 5 for the *O. septentrionalis* trial) on a laboratory table in the North Wet Lab of FISC. Artificial light was provided by overhead fluorescent lights on a 14 h light : 10 h dark schedule and ambient temperatures during the trials fluctuated between 23.9 and 29.3°C. The *B. marinus* trial began on 5 June and I collected data on survivorship of tadpoles on 6 June, while the *H. squirella* trial began on 8 June and I collected data on survivorship on 9 June. No food was provided during the trials. Behavioral observations were made every 2-8 h during the experimental period. At the end of the *B. marinus* trial on 6 June I transferred all *O. septentrionalis* larvae to new microcosms with fresh well water and food and observed them for an additional 24 h.

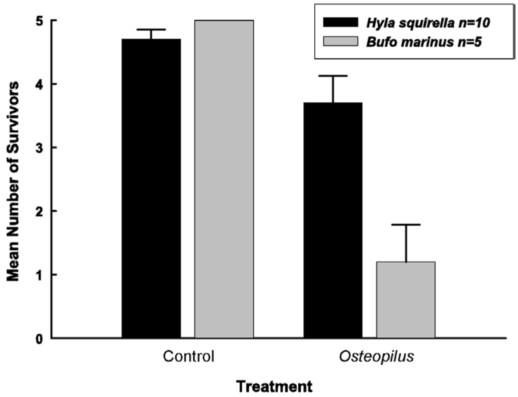

Figure 1. Effects of predation by larval *Osteopilus septentrionalis* on the mean number of surviving tadpoles. Error bars show 1 SE.

I used the nonparametric Mann-Whitney U-Test to test for the effects of the presence of *O. septentrionalis* on number of surviving *H. squirella* and *B. marinus* after each experimental trial. I used NCSS statistical software (Hintze, 2001) and a significance level of $\alpha = 0.05$ for analyses. I conducted no statistical analysis on the effects of the toxicity of *B. marinus* on *O. septentrionalis* survival because all *O. septentrionalis* in both trials survived the entire experimental period.

The presence of *O. septentrionalis* tadpoles significantly affected the number of surviving *H. squirella* (Mann-Whitney U-test, $Z = 1.99$, $P = 0.047$, $n = 10$) and *B. marinus* ($Z = 2.68$, $P = 0.007$, $n = 5$). Significantly fewer *H. squirella* and *B. marinus* larvae survived the experimental trial when they were combined with one *O. septentrionalis* tadpole than when they were housed with conspecifics only (fig. 1). Although the cause of mortality was not observed for all missing *H. squirella* and *B. marinus* tadpoles, on several occasions I observed *O. septentrionalis* consuming struggling live tadpoles of both species. In one instance I observed an *O. septentrionalis* tadpole catch a live *H. squirella* tadpole by the tail and consume it. Surviving tadpoles of both *B. marinus* and *H. squirella* appeared healthy at the end of the trial and no uneaten, dead tadpoles of any species were observed. Although adult *O. septentrionalis* are distasteful to some predators (Meshaka, 2001), the larvae of this species are not known to be toxic or allelopathic to heterospecific anuran larvae. These observations are consistent with the conclusion that all deaths were the result of predation by *O. septentrionalis* and were not caused by some other factor.

Based on the conclusion that all missing or dead tadpoles were preyed upon by *O. septentrionalis*, most individuals of *O. septentrionalis* preyed on heterospecific tadpoles. Four of five *O. septentrionalis* housed with *B. marinus* and nine of 10 *O. septentrionalis* housed with *H. squirella* consumed at least one tadpole. *Osteopilus septentrionalis* tadpoles consumed significantly more *B. marinus* tadpoles than *H. squirella* tadpoles (Mann-Whitney *U*-test, $Z = 2.49$, $P = 0.013$). The mean number of tadpoles eaten by *O. septentrionalis* (± 1 SE) was 3.8 (± 0.58) *B. marinus* tadpoles and 1.3 (± 0.42) *H. squirella* tadpoles.

All *O. septentrionalis* larvae survived the experimental period; consumption of *B. marinus* and *H. squirella* larvae did not affect the survival of *O. septentrionalis*.

These results provide the first evidence that larval *O. septentrionalis* will prey on heterospecific anuran larvae. Fewer *H. squirella* and *B. marinus* survived the experimental period when housed with one *O. septentrionalis* than when housed in the control treatment. Since little mortality occurred in the *H. squirella* control treatment and no mortality occurred in the *B. marinus* control treatment (fig. 1), it is unlikely that the disappearance of tadpoles in the *O. septentrionalis* treatments was due to cannibalism by *H. squirella* or *B. marinus*. Furthermore, observations of predation by *O. septentrionalis* on both *H. squirella* and *B. marinus* suggest that the disappearance of tadpoles in this study was caused by direct predation and not by the scavenging of dead individuals.

The degree of predation documented in this study suggests that predation by *O. septentrionalis* tadpoles may reduce the survivorship of heterospecific tadpoles in natural ponds. However, the very high density and controlled nature of this study (e.g., no alternative food available), while necessary for the documentation of predation, make the extension of these results to natural systems problematic. For example, predatory behavior in this species may be conditional based on tadpole density and resource availability. In other, longer-term studies (unpubl. data) I have observed *O. septentrionalis* preying on heterospecific tadpoles (*B. marinus*, *Bufo terrestris*, and *Gastrophryne carolinensis*) at various overall densities (between 0.5 and 2 tadpole / l). In another laboratory study specifically designed to assess interspecific competition, however, the survivorship of *B. terrestris*, *Hyla cinerea*, and *B. marinus* was not affected by the presence of *O. septentrionalis*. In this latter study, alternative food was available and tadpole densities were moderate (2 tadpoles / l vs. 60 tadpoles / l, in this study). This comparison suggests that predatory behavior by *O. septentrionalis* larvae is facultative and either profitable or possible only when resources are limited and/or when tadpole densities are very high. High tadpole densities and low resource levels are conditions that are frequently met during the drying of ephemeral pools favored by *O. septentrionalis* (Crump, 1986; Meshaka, 2001), so predation of heterospecific anuran larvae by *O. septentrionalis* in natural water bodies may occur under some circumstances. The impact of such predation on the survivorship of natural populations of native tadpoles in Florida is unknown, however. Other factors (e.g., the presence of competitors or other predators) may also modify the effect of *O. septentrionalis* predation. More naturalistic confirmatory studies on the effects of predation by *O. septentrionalis* larvae on native tadpoles are needed, particularly to determine the significance of this phenomenon in natural populations.

Surprisingly, *O. septentrionalis* consumed significantly more *B. marinus* larvae than *H. squirella* larvae in this study. This result is difficult to explain since *B. marinus* larvae are distasteful, toxic, or both to many vertebrate predators in the introduced range of *B. marinus* (Crossland and Alford, 1998; Crossland and

Azevedo-Ramos, 1999; Crossland, 2000, 2001). *Osteopilus septentrionalis* larvae are susceptible to the toxin contained in *B. marinus* eggs and consumption of eggs resulted in 30% mortality of larval *O. septentrionalis* in one study (Punzo and Lindstrom, 2001). In the present study, *O. septentrionalis* did show symptoms of what is assumed to be *B. marinus* intoxication (general lethargy and lack of response to movement), although these symptoms subsided completely within 24 h. Even if the small sample size in this study (five *O. septentrionalis*, of which only four consumed *B. marinus* larvae) is responsible for the absence of observations of mortality, this does not explain why *O. septentrionalis* larvae consumed more of the larger *B. marinus* than the smaller *H. squirella* larvae, which are not known to be significantly toxic or distasteful. There was a noticeable, though not quantified, difference in microhabiat choice between the two prey species, however. *Bufo marinus* tadpoles generally remained on the bottom of the containers while *H. squirella* stayed near the surface or were more pelagic. Whether this difference in microhabitat can explain the difference in rates of predation is unknown, however.

In conclusion, *O. septentrionalis* larvae will prey on heterospecific tadpoles under certain conditions (high tadpole density, low resource availability). The effect of such predation on the survival and recruitment of native tadpoles is unknown and will depend in part on whether the mortality is compensatory or additive. Even small increases in additive mortality may significantly affect recruitment in those tadpole species with very high mortality (e.g., as high as 92-100% mortality for *Rana sylvatica* larvae (Berven, 1990)). In contrast, compensatory mortality will have no effect on recruitment. Additionally, aquatic predators can significantly alter the outcome of the competitive interactions among tadpoles (e.g., Morin, 1981; Wilbur and Fauth, 1990), so the addition of a significant nonindigenous predator to native tadpole communities may alter native tadpole community structure. Although the results of this study also suggest that the consumption of *B. marinus* larvae does not significantly affect survivorship of *O. septentrionalis* larvae as a result of the toxicity of the former species, this conclusion is weakened by low statistical power due to a small sample size. Further experimentation on the ecological interactions of *O. septentrionalis* and *B. marinus* is needed since these two species frequently occur syntopically in south Florida in combination with the larvae of native anurans.

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References

Altig, R., McDiarmid, R.W. (1999): Body plan: Development and morphology. In: Tadpoles: The Biology of Anuran Larvae, p. 24-51. McDiarmid, R.W., Altig, R., Eds, Chicago, The University of Chicago Press.

Atkinson, I. (1989): Introduced animals and extinction. In: Conservation for the Twenty-first Century, p. 54-75. Western, D. and Pearl, M.C., Eds, New York, Oxford University Press.

Bartlett, R.D. (1967): Notes on introduced herpetofauna in Dade County, Florida. Bull. Pac. NW Herp. Soc. **2**: 5-7.

Berven, K.A. (1990): Factors affecting population fluctuations in larval and adult stages in the wood frog (*Rana sylvatica*). Ecology **71**: 1599-1608.

Crossland, M.R. (2000): Direct and indirect effects of the introduced toad *Bufo marinus* (Anura: Bufonidae) on populations of native anuran larvae in Australia. Ecography **23**: 283-290.

Crossland, M.R. (2001): Ability of predatory native Australian fishes to learn to avoid toxic larvae of the introduced toad *Bufo marinus*. J. Fish Biol. **59**: 319-329.

Crossland, M.R., Alford, R.A. (1998): Evaluation of the toxicity of eggs, hatchlings and tadpoles of the introduced toad *Bufo marinus* (Anura: Bufonidae) to native Australian aquatic predators. Aust. J. Ecol. **23**: 129-137.

Crossland, M.R., Azevedo-Ramos, C. (1999): Effects of *Bufo* (Anura: Bufonidae) toxins on tadpoles from native and exotic *Bufo* habitats. Herpetologica **55**: 192-199.

Crump, M.L. (1986): Cannibalism by younger tadpoles: another hazard of metamorphosis. Copeia **1986**: 1007-1009.

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**: 183-190.

Hintze, J. (2001): Number Cruncher Statistical Systems. Kaysville, Utah. www.ncss.com

Honegger, R.E. (1981): List of amphibians and reptiles either known or thought to have become extinct since 1600. Biol. Conserv. **19**: 141-158.

Jaeger, R.G., Halliday, T.R. (1998): On confirmatory versus exploratory research. Herpetologica **54**: S64-S66.

Lee, D.S. (1968): Feeding habits of the Cuban Treefrog *Hyla septentrionalis* in South Florida. Bull. Maryland Herp. Soc. **4**: 63-64.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, F.A. (2000): Biotic invasions: Causes, epidemiology, global consequences, and control. Ecol. Appl. **10**: 689-710.

Meshaka, W.E., Jr. (2001): The Cuban Treefrog: Life History of a Successful Colonizing Species. Gainesville, Florida, University Presses of Florida.

Morin, P.J. (1981): Predatory salamanders reverse the outcome of competition among three species of anuran tadpoles. Science **212**: 1284-1286.

Punzo, F., Lindstrom, L. (2001): The toxicity of the eggs of the giant toad, *Bufo marinus* to aquatic predators in a Florida retention pond. J. Herpetol. **35**: 693-697.

Savidge, J. (1987): Extinction of an island forest avifauna by an introduced snake. Ecology **68**: 660-668.

Schwartz, A. (1952): *Hyla septentrionalis* Duméril and Bibron on the Florida mainland. Copeia **1952**: 117-118.

Simberloff, D. (1997): The biology of invasions. In: Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida, p. 3-17. Simberloff, D., Schmitz, D.C., and Brown, D.C., Eds, Washington, D.C., Island Press.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Westbrooks, R. (1996): Biological invasions as global environmental change. Am. Sci. **84**: 468-478.

Wilbur, H.M., Fauth, J.E. (1990): Experimental aquatic food webs: Interactions between two predators and two prey. Am. Nat. **135**: 176-204.

Wilson, L.D., Porras, L. (1983): The Ecological Impact of Man on the South Florida Herpetofauna. Univ. Kansas Mus. Nat. Hist. Spec. Publ. 9.

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