

Rapid Communication

First record of a reproducing population of the African clawed frog *Xenopus laevis* Daudin, 1802 in Florida (USA)

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Abstract

The African clawed frog *Xenopus laevis* Daudin, 1802 is a global invader with established non-native populations on at least four continents. While Florida, USA has the largest established non-native herpetofauna in the world, there has been no evidence of *X. laevis* establishment in the state. Surveys during July 2016 in the Tampa Bay region of west-central Florida revealed an active breeding site of this species in an urban detention pond. The pond (~458 m²) is located adjacent to a small tributary of the Alafia River, which receives discharge from the pond. Two historic *X. laevis* collection locations were sampled but no individuals were detected. An additional 15 detention and retention ponds and 5 stream crossings near the breeding pond were also surveyed but no *X. laevis* specimens were collected at any location except the one active breeding site. No eggs were found in the breeding pond and early stage tadpoles were rare, but middle and late stage tadpoles, froglets, and juvenile frogs were common. At least 13,000 tadpoles and froglets were removed during four sampling dates. Climate modeling suggests that west-central Florida is not suitable for establishment and is warmer than the native range of *X. laevis*. However, daytime temperatures in the pond were relatively cool (25–26°C) for summertime in west-central Florida due to shading provided by an extensive tree canopy. We suspect that the availability of relatively cool refuge habitat in shaded streams and temporary pools, augmented by frequent cooling summer rains, strongly influences *X. laevis* dynamics in the region. The breeding site was unique among surveyed locations due to its combination of ephemeral nature but with adequate hydroperiod for tadpole development and metamorphosis, lack of fishes and low abundance of other potential predators, and heavy shading. Surveys during other seasons, across a broader geographic range, and using multiple sampling gears might detect additional breeding sites or increase the known range of *X. laevis* in Florida.

Key words: invasive, breeding site, tadpole, established

Introduction

The African clawed frog *Xenopus laevis* Daudin, 1802 is somewhat unique as a non-native species because a major source of introductions has been the release of laboratory animals (Measey et al. 2012). This species has historically been an important amphibian model organism (Cannatella and De Sá 1993; Gurdon and Hopwood 2000). Human pregnancy testing using *X. laevis* was developed in the late 1930s (Gurdon and Hopwood 2000) which caused a high demand for frog exportation from South Africa to laboratories all over the world (Measey et al. 2012; van Sittert and Measey 2016). The species has been widely introduced into the environment,

particularly since it was replaced by different methods in pregnancy testing (Gurdon and Hopwood 2000; Measey et al. 2012). Measey et al. (2012) expressed concern over another possible surge in releases from laboratory facilities because of the potential replacement of *X. laevis* in some disciplines as indicated by a general decline in publication rate of studies using *X. laevis* and an increasing use of *Silurana* (*Xenopus*) *tropicalis* Gray, 1864 as a research model organism. Other sources of introduction include escapes from culture facilities and release of pet animals (Measey et al. 2012; Somma 2016).

Records of introductions are common worldwide and non-native populations of *X. laevis* are established on multiple continents including Asia (Japan),

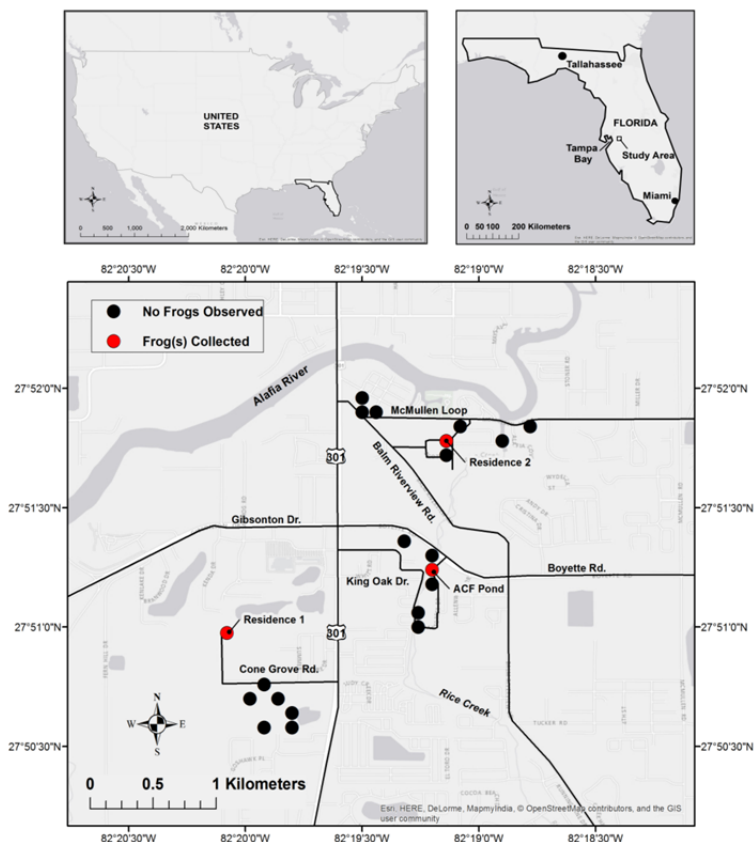


Figure 1. Map of sampling sites in the Riverview, Florida area with location relative to Florida and the United States. The red location labeled “ACF Pond” is the site described in the present study.

Europe (France, Italy, and Portugal), North America (Arizona USA, California USA), and South America (Chile) (Measey et al. 2012; Somma 2016). Other potentially established populations also occur in parts of Europe, Mexico, and the United States (Measey et al. 2012; Peralta-Garcia 2014; Somma 2016). A variety of negative effects have been attributed to non-native *X. laevis* populations, including predation on native invertebrates, fish, and amphibians, decline in native tadpoles, and introduction of parasites and disease, including *Batrachochytrium dendrobatidis* (Weldon et al. 2004; Lillo et al. 2011; Vredenburg et al. 2013; Somma 2016). In the native range this species can be a nuisance due to occasional mass overland migrations during rainy periods (Tinsley et al. 1996) and invasion of fish culture facilities (Schramm 1987).

Despite the widespread introduction of *X. laevis* worldwide and that the State of Florida (USA) has the largest established non-native herpetofauna in the world (Krysko et al. 2011), records of this species in Florida are few and there has been no evidence of establishment outside of captivity. The earliest record in Florida is the release of ca. 200 individuals

into the Hialeah Canal, Miami-Dade County (King and Krakauer 1966), though *X. laevis* failed to establish at this site (Somma 2016). A single specimen was reportedly collected in the mid-1970s in the Riverview area, Hillsborough County, of the Tampa Bay watershed in west-central Florida (Somma 2016). No voucher specimens exist for either record (Krysko et al. 2011). The first vouchered specimen from Florida was collected in 2010 in a retention pond in Titusville, Brevard County, east-central Florida (Krysko et al. 2011). An additional adult thought to be a pet release was collected in Homestead, Miami-Dade County, in 2014 (Krysko et al. 2011, 2016). Ultimately, prior to 2016, all records have been for juvenile or adult frogs.

In winter 2013–2014, a homeowner in Riverview, Hillsborough County collected 2 frogs over a 4-day period in a residential garage following heavy rains (Somma 2016; Figure 1). These records prompted sampling of nearby waterbodies by staff of the University of Florida/IFAS Tropical Aquaculture Laboratory (TAL), Ruskin, including an abandoned ornamental aquaculture facility near the collection site (see Krysko et al. 2016; Somma 2016). No

X. laevis were observed or collected. The same homeowner collected a single specimen in early summer of 2014 at the same location. All three specimens collected by the homeowner were archived in the Florida Museum of Natural History (FLMNH), Gainesville (UF Herpetology #172054, 172055, and 173050; Krysko et al. 2016). Two additional frogs (including UF Herpetology #178169) were collected during summer 2016 by the same individual but at a new location about 2.25 km northeast of the original collection site (Figure 1). The occurrence of additional specimens led TAL staff to conduct a wide-ranging survey for *X. laevis* during which a pond was found containing the first confirmed breeding population in Florida. The objectives of the present paper are to (1) report on the survey, (2) document the location and characteristics of a site supporting reproduction, and (3) describe physical attributes and life stages of captured individuals.

Study species

Xenopus laevis is native to southern Africa and is characterized by a narrow, pointed head, lack of a tongue, smooth skin, heavy hind legs, and the presence of claws on three toes of the rear legs (Figure 2). Uncertainty in the taxonomy of *Xenopus* and *Silurana* exists, making species determinations and native range delineations difficult (Measey and Channing 2003; Evans et al. 2011; Furman et al. 2015). However, introduced individuals worldwide have been referable to *X. laevis* and are traceable to the southwestern portion of the native range in the Cape of South Africa (Measey et al. 2012). This information is important because assumed thermal tolerances and other biological characteristics might vary across the range of *X. laevis* or among other members of the family. These characteristics may play a large role in the invasion dynamics of populations potentially limited by physiological tolerances such as *X. laevis* in west-central Florida.

The *X. laevis* life cycle is closely tied to water, often ponds, ditches, and small streams. Eggs are laid in small masses within aquatic vegetation or on bottom substrates. Eggs hatch into tadpoles which develop unique features including two long tentacles and a somewhat translucent body ending in an extended filament (Figure 3). Complete metamorphosis from egg to frog takes about 58 days at 23 °C (Xenbase 2016). Froglets and adults may be observed surfacing periodically to breathe (Ihmied and Taylor 1995). Although largely aquatic as adults, *X. laevis* migrates overland, especially in rainy weather (Tinsley et al. 1996; Measey and Tinsley 1998).



Figure 2. African clawed frog *Xenopus laevis* collected on 17 July 2016 in Riverview, Florida, Alafia River basin. Photograph by Jeffrey E. Hill.

Methods

A review of recent records of *X. laevis* from Hillsborough County in the U.S. Geological Survey's (USGS) Nonindigenous Aquatic Species database (United States Geological Survey 2016) and FLMNH (K. Krysko, FLMNH, personal communication) was done to inform the general area for field surveys. These records are located in and around the Rice Creek basin of the Alafia River drainage near Tampa, Florida (Figure 1). The Alafia River has a drainage area of > 1,060 km² and flows about 55 km from Polk County through Hillsborough County, where discharge flows into Tampa Bay (Florida Department of Environmental Protection 2002). The basin is over 91% impacted with urban development, agriculture, and phosphate mining (Florida Department of Environmental Protection 2002). The Rice Creek sub-basin flows about 6.5 km through primarily suburban and urban areas of Riverview and empties into the Alafia River in the freshwater, tidally influenced, zone about 1.3 river km upstream of the U.S. Highway 301 bridge (27°86.4620'N; 82°31.4946'W).

We examined Google Earth (Google Inc. 2015) for potential collection locations including detention/retention ponds, stream culvert/bridge crossings, county ditches, and wetlands. Field surveys were done on 15 and 20 July 2016. Collecting gear included dip net and seine (6 m long × 2.4 m high, 6 mm mesh). Visual surveys were done when water clarity allowed observation of frogs or tadpoles and also for surfacing frogs (e.g., Lillo et al. 2011). Detection or non-detection of *X. laevis* was noted at each location.

Additional data were collected at the single site where *X. laevis* individuals were found. Length and width of the pond were measured using a measuring tape reel and depth measurements were taken at several points using a meter stick. Coverage and species of aquatic vegetation were also determined. Water clarity was measured using a 120 cm turbidity tube (Carolina Biological Supply Co., Burlington, NC, USA). Temperature, dissolved oxygen, conductivity, and salinity were measured at four sites around the pond using a handheld unit (YSI Model 85, YSI Inc. Yellow Springs, OH, USA). Total alkalinity, hardness, pH, ammonia, and nitrite were measured at three sites around the pond using a HACH kit (Hach Co., Loveland, CO, USA). Qualitative dip net and seine sampling and a single quantitative seine haul (seine length \times 10 m pull) were used to capture *X. laevis*. Contents of the quantitative seine haul (leaf litter and *X. laevis* individuals) were brought to the laboratory for sorting and enumeration. Captured frogs, froglets, and tadpoles were retained for study and for voucher specimens. *X. laevis* was then sorted from the detritus and a random sample was taken to determine length frequency and staging (Xenbase 2015). The number of individuals collected in the seine haul was estimated volumetrically using five subsamples of 100 individuals to obtain an estimate of displacement. That displacement estimate was then applied to the volume of remaining individuals.

An additional site visit on 4 August 2016 was conducted in an attempt to capture larger individuals which may be less detectable by seine and dip-net surveys. Wire minnow traps ($9 \times 16''$, 1/4 inch mesh) baited with a commercial cat food were deployed to capture frogs (3 traps \times 2 hrs = 6 trap-hrs).

Results

In two days of sampling, 16 detention/retention ponds and 5 stream crossings/culverts/ditches were investigated (Figure 1; Supplementary material Table S1). Only 1 site contained *X. laevis*, a 458-m² detention pond (mean depth = 41 cm) adjacent to Rice Creek (Kingswood Mobile Home Park, west of King Oak Drive, Riverview, Florida; 27°51.227'N; 82°19.204'W; Figure 4). The site was discovered on 15 July 2016. The detention pond captures runoff from the road and mobile home community via a swale from the south and a culvert from the east and discharges to Rice Creek through a concrete control structure on the west shoreline. Water was not flowing in or out of the pond at the time of sampling on any visit date. On 15 July 2016, 1 adult *X. laevis* and an estimated 7,000 tadpoles and froglets were removed in one seine haul and many more were



Figure 3. *Xenopus laevis* at three developmental stages—tadpole, transforming to froglet, and juvenile. All specimens were collected 20 July 2016 in a Riverview, Florida detention pond, Alafia River basin. Photographs by Kevin Barden.

removed by dip net. A subsample was vouchered at the FLMNH (UF Herpetology #178650, 506 tadpoles; #178651, 28 tailed metamorphs; #178652, 13 juveniles; and #178653, 1 adult). The density estimate from the single seine haul (90 m²) was 78.6 tadpoles/m². This estimate, while imprecise and lacking replication, would extrapolate to approximately 36,000 tadpoles



Figure 4. Detention pond in Riverview, Florida (Alafia River basin) containing a breeding population of non-native African clawed frog *Xenopus laevis*. View looking south. Primary emergent vegetation included knotweed *Polygonum* sp. and Mexican petunia *Ruellia tweediana*. Photograph taken 20 July 2016 by Jeffrey E. Hill.

and froglets in the pond. An additional adult was observed but not captured. A large number of tadpoles remained in the pond after sampling was concluded.

The site was again sampled on 20 July 2016 when habitat and water parameters were measured and additional specimens were captured. Tree canopy cover over the pond was 100%. Sediments were firm with extensive leaf litter and small woody debris. A stand of knotweed *Polygonum* sp. and Mexican petunia *Ruellia tweediana* occupied about 41 m² (Figure 4). Pennywort, *Hydrocotyle* sp., was present within this stand but was sparse. Red ludwigia, *Ludwigia repens*, occurred in small clumps scattered throughout the open water area (estimated percent areal coverage <5%). Water clarity was high and exceeded the maximum 120 cm of visibility measurable using a turbidity tube. Temperature averaged 25.8 °C, dissolved oxygen averaged 0.80 mg/L, conductivity averaged 154 mS/cm, salinity was undetectable, pH averaged 6.75, alkalinity was 34.2 mg/L CaCO₃, hardness was 68.4 mg/L CaCO₃, ammonia was 0.4 mg/L NH₃-N, and nitrite was undetectable. No adult frogs were observed during this visit. An additional ~400 tadpoles and froglets were removed though large numbers remained in the pond after sampling concluded. A random sample of 250 individuals ranged from 12 to 43 mm in length (Figure 5). On 4 August approximately 5,000 tadpoles, froglets, and juvenile frogs were removed. On 11 August an additional ~600 tadpoles were removed.

Potential vertebrate frog or tadpole predators observed included a juvenile Florida softshell turtle, *Apalone ferox*, Schneider, 1783 and a Florida water snake, *Nerodia fasciata pictiventris* Cope, 1895.

Fish were not present in the pond. Potential invertebrate predators were not specifically sampled but at least five individuals of the predaceous diving beetle, *Cybister fimbriolatus* Say, 1823, were collected while netting tadpoles. Other amphibians were not observed.

Discussion

The occurrence of several adults across spatial (1–2.25 km apart) and temporal (2.5 years) scales, as well as the presence of a high-density breeding site suggest that *X. laevis* is at least locally established in west-central Florida in the Alafia Basin. This area is highly developed by humans, a factor that may facilitate *X. laevis* establishment (McCoid and Fritts 1993; Tinsley et al. 1996; Peralta-Garcia et al. 2014). This area also contains numerous detention/retention ponds which may serve as breeding and adult habitat (Picker 1985; Schoonbee et al. 1992; McCoid and Fritts 1993), is crossed by small streams and ditches, and is subject to periodic heavy rainfall which may aid dispersal (Measey et al. 2012).

While the geographic extent of its spread is unknown, *X. laevis* has the ability to disperse through water connections and adult frogs will frequently move overland (Measey and Tinsley 1998; Measey et al. 2012), potentially overcoming dispersal barriers. Density of *X. laevis* within the introduced range is not known, nor is the detection probability if the species is present. The patchy distribution could be due to potential but unoccupied habitats containing predatory fishes, presumably making these habitats unsuitable for *X. laevis*. We speculate that overall

density is likely low in the area but that periodically, local densities may be high at suitable sites, especially breeding sites. Surveys during other seasons, across a broader geographic range, and using multiple sampling gears might detect additional breeding sites or increase the known range of *X. laevis* in Florida.

The earliest collection record of *X. laevis* in the Tampa Bay area of Florida is uncertain, and speculation that a population has occurred in the Tampa Bay region since the 1970s (Krysko et al. 2016; Somma 2016) is unconfirmed. If *X. laevis* was established in the mid-1970s, about 40 years have passed prior to detection of the species again in late 2013. Detection can be difficult and several populations of *X. laevis* in other regions have gone undetected for long time periods, 2–25 years in some cases (Measey et al. 2012). The potential challenge in detecting a non-native cryptic or rare species is highlighted by a small non-native fish, the croaking gourami *Trichopsis vittata* Cuvier, 1831. This fish was found in south Florida in 1978, considered extirpated by the 1990s, and ultimately re-discovered in 2012 (Schofield and Pecora 2013). Its highly localized range and the lack of thorough surveys may have allowed the croaking gourami to go undetected for several decades (Schofield and Shulte 2016). Similarly, *X. laevis* may have also gone undetected, despite thorough non-native fish surveys recently conducted near the locations where *X. laevis* was collected (Tuckett et al. 2016b).

The source of *X. laevis* in the Tampa Bay region is unknown. Three main sources of introduction are commonly described in the literature—laboratory release, aquaculture escape, and pet release (Measey et al. 2012). Any of these could be the source of introduction in the region we studied. Krysko et al. (2016) states that the breeding population discovered in Riverview was found in abandoned aquaculture ponds. However, the actual location was a neighborhood detention pond and was not associated with aquaculture (present study). Some recent literature focuses on an abandoned fish farm as the source for three specimens from Cone Grove Road and ultimately the individuals collected farther away on McMullin Loop Road and the Kingswood neighborhood (Krysko et al. 2016; Somma 2016). Although non-native species have escaped from aquaculture facilities in the Tampa Bay region (Hill 2002; Tuckett et al. 2016a, 2016b) and *X. laevis* has been and continues to be cultured on some facilities broadly within the Tampa Bay region (authors, personal observations), the pond-based aquaculture facility suggested as the putative origin of introduction is an unlikely source. Sampling of the abandoned ponds

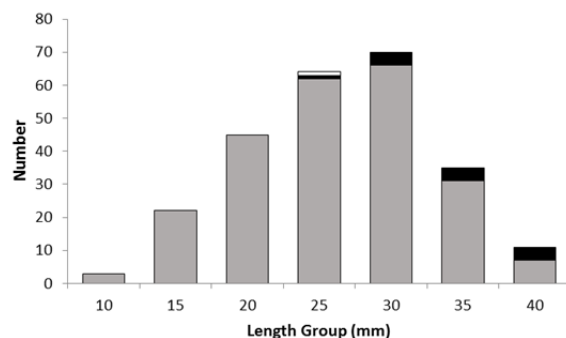


Figure 5. Distribution of length groups for a random sample ($n = 250$) of African clawed frogs *Xenopus laevis* collected from a detention pond in Riverview, Florida (Alafia River basin) on 15 July 2016. Gray indicates tadpoles (stage 46–55), black indicates tadpoles with visible legs (stage 56–63), and white indicates froglets (stage 64–66). Nieuwkoop and Faber developmental stages according to Xenbase (2016).

in 2014 revealed no *X. laevis* (QMT, unpublished data). The farm facility has been recently developed into housing and the culture ponds replaced with detention ponds; sampling at this site in July 2016 revealed no *X. laevis* (present study). In Florida, *X. laevis* is not a pond-cultured species because of its relatively poor heat tolerance (Casterlin and Reynolds 1980; Ihmied and Taylor 1995; Walsh et al. 2008) and the ability of frogs to escape culture ponds (John Skidmore, personal communication). Dispersal of frogs offsite or to other culture ponds onsite would cost producers money and allowing escape of frogs would be in violation of regulations governing containment of non-native species (Florida Department of Agriculture and Consumer Services 2015; Tuckett et al. 2016a). Recent, intensive sampling for non-native fish near aquaculture facilities in the region, some of which culture or wholesale *X. laevis*, has not detected any specimens (Tuckett et al. 2016b). Aquaculture escape is not ruled out, but we disagree that there is definitive proof of origin for any *Xenopus* records in west-central Florida.

A recent climate modeling study indicated that the Tampa Bay region is unsuitable for *X. laevis* (Measey et al. 2012). In laboratory studies, *X. laevis* has a thermal preferendum of 22 °C and an upper voluntary temperature range of 32 °C (Casterlin and Reynolds 1980). At 30 °C, mortality of metamorphosing tadpoles was elevated (23%) relative to cooler temperatures (Walsh et al. 2008). Florida has a warmer climate than its native range in South Africa and summertime pond temperatures regularly

exceed these higher temperatures (Larry Lawson, personal communication). In some cases, daytime water temperatures in Florida ponds may approach critical thermal maxima for *X. laevis* tadpoles and froglets at temperatures of 35.5–37.6 °C (Sherman and Levitis 2003). Temperature was likely depressed in the Riverview pond due to the shading provided by the extensive canopy cover. Daytime temperature measured in the breeding pond during the study (summer in Florida) averaged 25–26 °C, a high but suitable temperature for *X. laevis* reproduction. High temperatures may play a role, at least seasonally, in the availability of suitable habitat, especially breeding sites. We suspect that the availability of relatively cool refuge habitat in shaded streams and temporary forest pools, augmented by frequent cooling summer rains, strongly influences *X. laevis* dynamics in the region.

Identifying abiotic and biotic factors influencing the success of *X. laevis* in west-central Florida would better inform management. The breeding site was unique among surveyed locations due to its combination of ephemeral nature but with adequate hydroperiod for tadpole development and metamorphosis, lack of fishes and low abundance of other potential predators, and heavy shading keeping water temperatures within acceptable limits for tadpole survival. Knowledge of the influence of these factors along with other aspects of water chemistry, food availability, and habitat connectivity would provide key insights not only into the detection and management of *X. laevis* in Florida but further into the invasion dynamics of this worldwide invader. The apparent uniqueness of the Florida site and thus its potential importance to regional recruitment suggests that directed control programs may help reduce propagule pressure into surrounding habitats.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Georeferenced locality information for sites sampled.

This material is available as part of online article from:

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