



Published by the British Herpetological Society

Sharon Pratt Anzaldúa & Javier Goldberg

Hotspot of tadpole abnormality in suburban south-west Florida

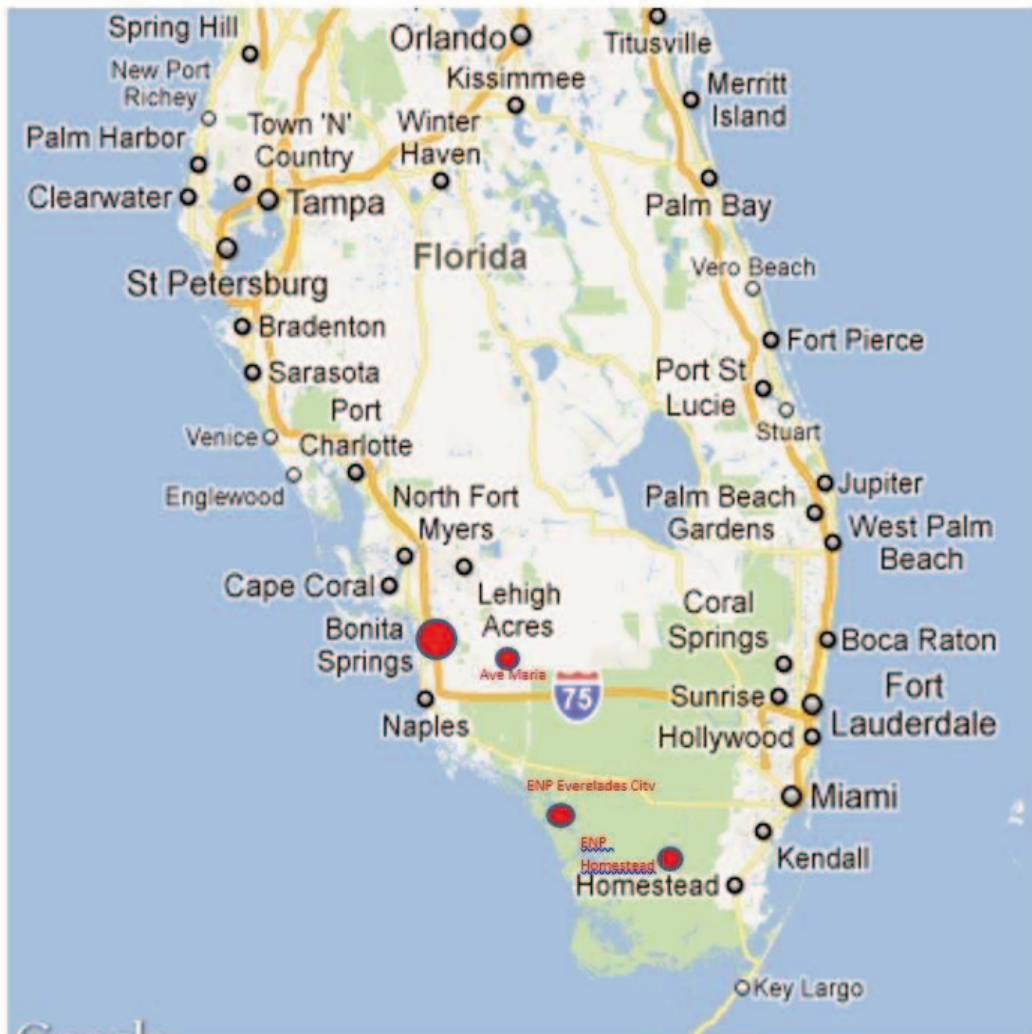


Figure S1. Map of Florida. The red circles indicate field study site locations.

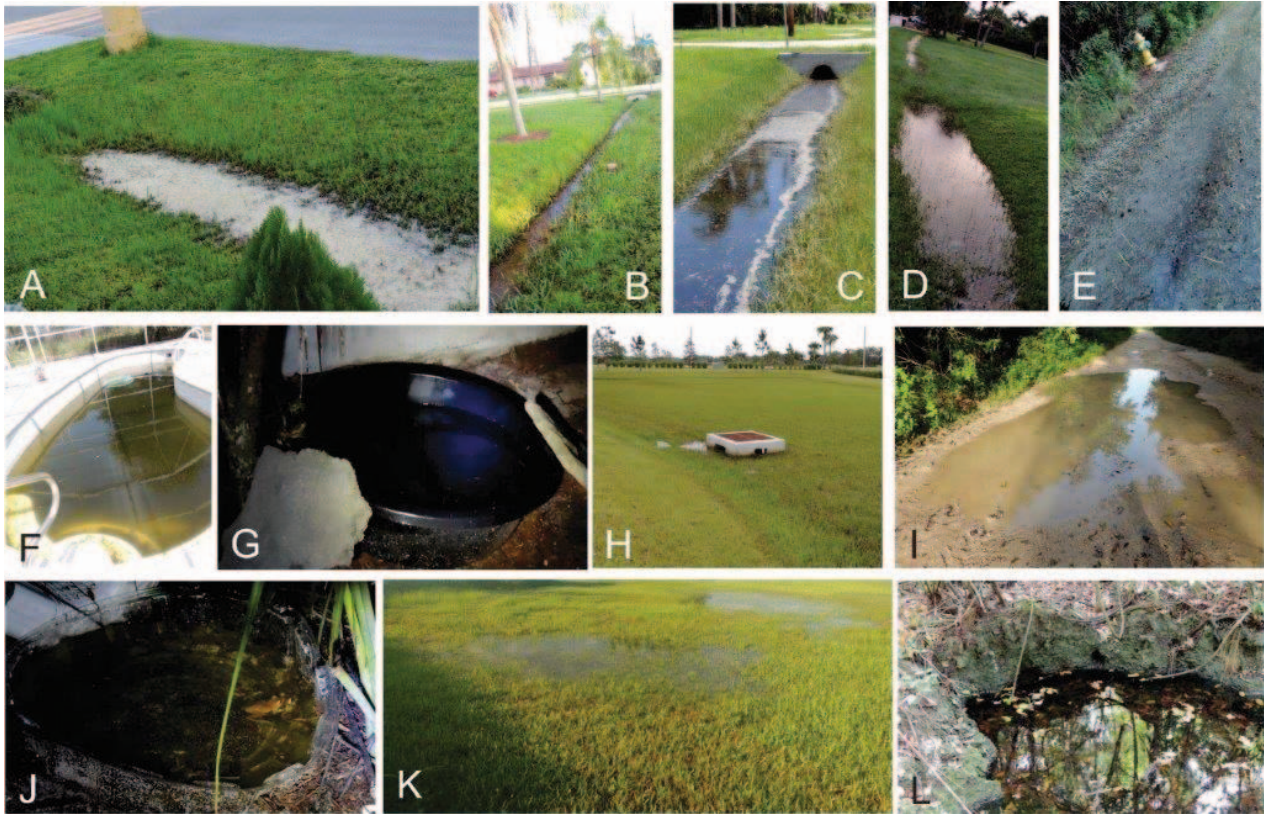


Figure S2. Photographs of each field study site. (A) H roadside drainage ditch, treated. (B) HC roadside drainage ditch, treated. (C) RF roadside drainage ditch, treated. (D) RP roadside drainage ditch, treated. (E) CL roadside drainage ditch, treated. (F) H artificial pond, untreated. (G) H yard container pool, untreated. (H) AM yard pool control, untreated. (I) AM rural road ditch pool control site, untreated. (J) H yard container pool, untreated. (K) ENP yard pool control, untreated. (L) ENP trail pool control, untreated.

RESULTS

Normal Pre-Hatching to Larval stages based on External Morphology

Stages 1-25 (Fig S3): The size of the oviposited egg was approximately 2 mm for a large 60 mm-80 mm SVL *Osteopilus septentrionalis* female. Females smaller than 55 mm laid smaller eggs, of around 1 mm (Fig. S3A). All eggs in a single clutch laid by one female were equal in size and developed at equal or very close developmental rates. In just 3 hrs after fertilisation the eggs could be found at GS 4 (Fig. S3B). Others continued development to GS 8 in which the animal pole of the egg turned more diffuse (Fig. S3C). The pigmentation of the animal hemisphere extended beyond the equatorial plane.

Later, the embryo extended craniocaudally and flattened dorsally. The neural plate formed on the dorsal surface at GS 13 (Fig. S3D) and by 8 hrs (+/- 2 hrs) the embryo elongated to achieve GS 15 (Fig. S3E). The neural folds then fused to form a neural tube while the embryo (GS 16) was still contained within its egg (Fig. S3F, Fig. S3G). At 9 hrs GS 18- 19 (Fig. S3H-L) the gills and tail bud had formed and a recognisable head and tail was discernible with stomadeum and olfactory pits (Fig. S3H, Fig. S3I). At GS 19 (Fig. S3J, K), the embryo muscular response had developed, and hatching had occurred: the embryo wriggles out of its transparent jelly-like shell, and had sunk to the bottom (Fig. S3L). At 22 hrs (GS 19-

20), a progressive development commenced of external gill filaments. By these stages the embryo became mobile and swam using its recently developed tail with a transparent tail fin (Fig. S3L). Optic vesicles were also evident at this stage.

By 25 hrs, the hatch developed to GS 21 (Fig. S3M) with pronounced blood flow circulating in its branching gill filaments. This enabled the tadpole to swim in open water to feed. The intestine began to coil and the mouth cavity opened. At 35 to 63 hrs, lateral eyes and nostrils had developed (GS 23; Fig. S3N), the oral disc of the mouth continued to develop upper and lower labial teeth, the long intestine continued to coil, and the dextral vent tube formed. The development of the operculum occurred in GS 23-25 (Fig. S3N-R). The opercular fold became visible in late GS 23 and covered the external gills on the right in GS 24 between 35 to 63 hrs (Fig. S3O).

The operculum was completely formed by GS 25 (Fig. S3P 48hrs, S3Q 72hrs, S3R older 72 hrs) and the spiracle became visible on the left (Fig. S3O). At GS 25, by 72 hrs, both external gills had internalised, and the tadpole had grown to a total body length of 10 mm (Fig. S3P, Q), having successfully completed development of all external morphological traits used for species identification: Lateral eyes, dextral vent tube opening (Fig. S3R older than 72 hrs), and mouthparts with labial tooth row formulas 2/4, 2/3 (Fig. S3R), 2/2 (Fig. S3P). The intestine appeared to be completely coiled (Fig. S3P). The dorsal colour of the tadpole by 72 hrs was dark to a



Figure S3. GS1-GS25 *Osteopilus septentrionalis* normal morphology. (A) GS 1, 0 hrs, (B) GS 4, 3 hrs, (C) GS 8, 3 hrs. (D) GS 13, 10 hrs dorsal view. (E) GS 15, 8 hrs lateral view. (F) GS 16, 10 hrs dorsal view. (G) GS 16, 10 hrs dorsal view. (H) GS 18, 9 hrs lateral view. (I) GS 18, 9 hrs, lateral view inside egg. (J) GS 19, 13 hrs, inside egg. (K) GS 19, 23 hrs outside shell. (L) GS 19, 22 hrs, lateral view. (M) GS 21, 25 hrs, ventral view. (N) GS 23, 35 hrs, both gills ventral view. (O) GS 24, 58 hrs one gill. (P) GS 25, 48 hrs normal mouthpart 2/3 configuration, ventral view. (Q) GS 25, 72 hrs. (R) GS 25, lateral view of an older than 7r2 hs tadpole. The inset shows the normal older than 72 hrs GS 25, mouthpart 2/3 configuration.

light brownish to sand colour, matching the colour of the substrate. The organ systems differentiated and became functional by 72 hrs. The ventral skin of the GS 25 tadpole at 72 hrs had darkened to blackish-brown colour.

Stages 26-42 (Fig. S4): These stages corresponded to the appearance of the hind limbs at the body-tail junction, followed by the protrusion of the forelimbs. The appearance of the hind limbs on the larval body occurred by the 12th day when the tadpole had reached at least 38 mm in size (Fig. S4A). By GS 32, on the 13th day, the hind limb buds had elongated and defined pointed autopodia with a total size increase to 43 mm (Fig. S4B). By GS 35, GS 36 toes were all differentiated (Fig. S4C, D). At GS 37, interdigital tissues reached 2/3 of toes: digit tips were flattened and rounded. By GS 38-39, the inner metatarsal tubercle differentiated preaxially to toe I, and subarticular tubercles began to appear. In GS 40 both forelimbs were visible beneath the transparent skin bulge of the operculum (Fig. S4E). By day 17, GS 41, hind limbs were fully developed providing locomotion (Fig. S4F), and the vent tube had disappeared. In GS 42 (Fig. S4G) the forelimbs protrusion was asynchronous. The developed left forelimb became external first. The left forelimb finally pushed through the spiracle, quickly followed by the right forelimb breaking through the operculum (Fig. S4G). The tadpole continued to grow in size reaching its maximum size in GS 42.

Stages 43-46: These stages corresponded to mouth transformations, and tail resorption taking place within 2 days. At GS 41, the angle of the jaws still containing larval mouthparts, was situated between the nostril and eyes, with a body to tail ratio of roughly 1:3. At GS 42, the mouth shed the labial teeth and the tail shortened. At GS 43, the jaw continued to shorten and the tail continued to shorten by 8 %. By GS 44, the angle of the jaws was located between the margins of the eye in lateral view. The tail had shortened considerably, by 80 % of its maximum length. At GS 45, the tail became a stub, at 88 % loss of length, and the mouth continued widening posterior to the eyes on the 19th day. Finally, on day 19, in the metamorph by GS 46, the tail was absorbed 100 % with mouthparts and jaws completely formed.

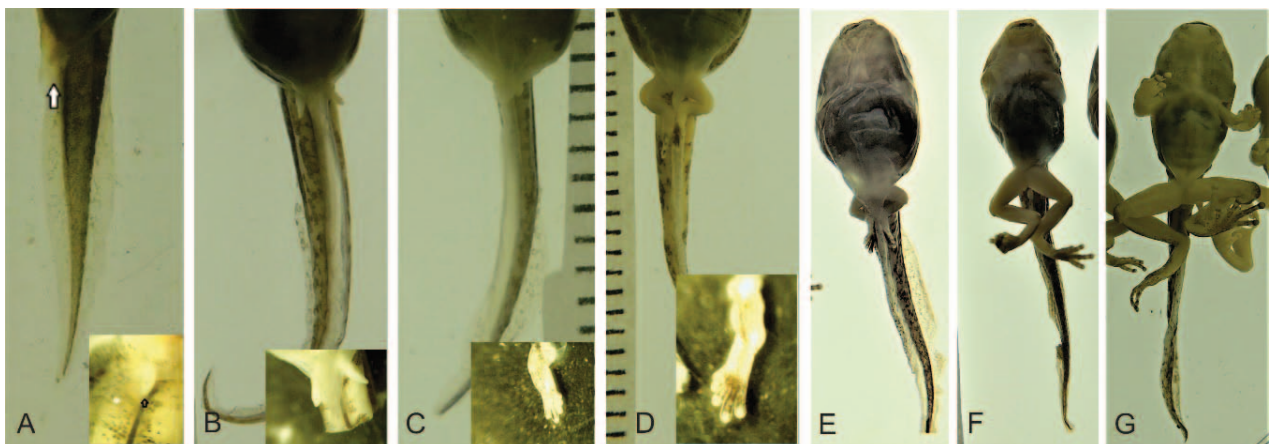


Figure S4. GS 30-GS 42 normal morphology. (A) GS 30, 12 day tail with foot paddle. (B) GS 32 13 day tail with foot indentation. (C) GS 35, 13 day tail with foot toes. (D) GS 36, 13 day. (E) GS 40, 13 day tail with hind limb pair. (F) GS 41, 17 day tail with hind limb pair complete development. (G) GS 42, 17 day forelimbs.