Larval Growth and Natural History Traits
of *Lithobates warszewitschii*

**M. Delia Basanta[1,8], Heidi Ross[2], Roberto Brenes[3], Mirna G. García-Castillo[4],
Carolina Lambertini[5], Sara L. Anaya-Morales[6], Sam Lapp[7], Michel E. B. Ohmer[6],
Justin Kitzes[7], and Jamie Voyles[1]**

[1]*Department of Biology, University of Nevada, Reno, 1664 North Virginia Street, Reno, Nevada, USA*
[2]*El Valle Amphibian Conservation Center Foundation, Calle El Hato, El Valle de Antón, Panamá*
[3]*Biology Department, Carroll University, 100 North East Avenue, Waukesha, Wisconsin, USA*
[4]*Facultad de Ciencias, Universidad Nacional Autónoma de México,*
External Circuit of Ciudad Universitaria, Mexico City, Mexico
[5]*Department of Biology, The Pennsylvania State University, 606 Mueller Laboratory,*
University Park, Pennsylvania, USA
[6]*Department of Biology, University of Mississippi, 214 Shoemaker Hall, University, Mississippi, USA*
[7]*Department of Biological Sciences, University of Pittsburgh, Fifth and Ruskin Avenues, Pittsburgh, Pennsylvania, USA*
[8]*Corresponding author, e-mail: delibasanta@gmail.com*

Abstract.—Understanding amphibian life-history and reproductive traits is essential for developing effective conservation strategies. Central America is a region with a high amphibian diversity, but we have a limited understanding of the life history of many species. One compelling example is the Warszewitsch's Frog *(Lithobates warszewitschii = Rana warszewitschii)*, a species that declined following the emergence of infectious disease in Panama, and for which we lack key details on important life-history characteristics. Here, we provide information on *L. warszewitschii* life-history and reproductive traits, including descriptions of oviposition habitat, egg masses, tadpole growth rates, and vocalization. We recorded egg masses of *L. warszewitschii* attached to rocks in shallow stream pools, with a mean of 455 eggs per mass, and documented tadpole growth in captivity at a rate of 3.4 mm in total length per week, with metamorphosis (Gosner stage 42) beginning after 14 weeks. Additionally, we analyzed *L. warszewitschii* vocalizations, which consisted of eight or nine two-note groups, and showed considerable variation in repetition rate, inflection, and dominant frequency within a single calling bout. The non-pulsatile calls that we recorded may represent an undescribed call from the known trill. The differences in traits between previously published studies and our findings may arise from previously undescribed life-history characteristics and/or intraspecific variation. The information that we provide for *L. warszewitschii* can inform future conservation management planning for this and other threatened amphibian species in the Neotropics.

Key Words.—amphibians; *Batrachochytrium dendrobatidis*, chytridiomycosis; conservation; development; life-history traits; recovery

Introduction

A complete understanding of amphibian life-history characteristics is a cornerstone of conservation programs (Michaels et al. 2014), especially in regions of high species diversity but finite conservation resources. The Neotropics of Central America is a region of high amphibian richness, yet there is a dearth of natural history information (Michaels et al. 2014). For example, for many Neotropical amphibian species, we currently lack basic reproductive information such as general breeding locations (e.g., terrestrial, aquatic, or arboreal) and microhabitat conditions, the numbers of eggs per egg mass, tadpoles per clutch, and clutches per reproductive cycle, as well as the time to the beginning of metamorphosis, adult lifespan, and types of parental care.

Life-history information is critical for a wide range of conservation efforts (Michaels et al. 2014; Gagliardo et al. 2008). Amphibians are among the most imperiled of all vertebrate taxa, with more than 435 species declining rapidly (Stuart et al. 2004; Scheele et al. 2019; International Union for Conservation of Nature [IUCN] 2025). The causes of amphibian declines include habitat destruction, introduced species, over-exploitation, climate change, contaminants, and infectious diseases (Scheele et al. 2019; IUCN 2025). As such, researchers and conservation programs are working to optimize conservation actions, including establishing captive

breeding programs (Michaels et al. 2014), identifying potentially vulnerable species that need conservation intervention (Lips et al. 2003; Howard and Bickford 2014), and understanding the prospects for species recoveries following declines (or lack thereof; Knapp et al. 2016; Voyles et al. 2018; Vega-Yáñez et al. 2024). As such, conservation programs in regions that are grappling with multi-faceted challenges would be bolstered by addressing fundamental gaps in understanding the natural history of a species (Gagliardo et al. 2008).

One species that has exhibited significant declines in recent years is the Warszewitsch's Frog (*Lithobates warszewitschii = Rana warszewitschii*; Schmidt 1857; Crawford et al. 2010; Longo et al. 2023). This species is considered part of the *Rana palmipes* species group, which consists of eight taxa and is restricted to Central and South America (Yuan et al. 2016). The distribution of *L. warszewitschii* ranges from Honduras through Central America, along the Pacific coast, to Panama, and across an elevational range from 0–1,740 m (Cryer et al. 2019). Despite its widespread distribution, the available natural history information is limited (Hillis and De Sá 1988; Villa 1990). This species has been regarded as a terrestrial species, principally associated with the forest floor (Abarca 2012). Previous reports note that this species can be seen during the day by roadside ditches, streams, and ponds (Savage 2002). Furthermore, previous studies have documented that breeding occurs at the beginning of the dry season and that tadpoles inhabit streams, but there are no available reports with information about egg masses (e.g., where they are deposited, number of eggs per mass) or developmental rates (Villa 1990; Savage 2002). Regarding tadpoles, Starrett (1960) provided a brief description of tadpole morphology (based on 15 tadpoles collected in one locality in Costa Rica), reporting a maximum total length of 115 mm. Adult *L. warszewitschii* reportedly have a maximum snout-vent length (SVL) of about 63 mm in females and about 48 mm in males (Villa 1990). Additionally, previous studies noted that *L. warszewitschii* vocalizes without vocal sacs or slits (Hillis and De Sa 1988). Greding (1972) described a short, trilled vocalization (0.12–0.35 s duration consisting of 4–9 pulses), with a frequency range of 160–2,080 Hz and pulse rate of 16.6–32.0 pulses per second. This report is based on recordings of five individuals from Costa Rica and, to the best of our knowledge, there are no other recordings of *L. warszewitschii* vocalizations available.

This species is intriguing, and potentially informative for conservation, due to its decline following emergence of a lethal infectious disease (chytridiomycosis; Crawford et al. 2010) in Central America. In Panama, before the early 2000s, this species had a low to moderate abundance of adults and high tadpole densities in streams (Lips et al. 2006; Ranvestel et al. 2004; Brem and Lips 2008), whereas following the emergence of chytridiomycosis, it was reported as extirpated (Crawford et al. 2010). Recent studies, however, suggested recovery in areas where the species previously declined in Panama and Costa Rica (Barquero et al. 2010; Hilje and Aide 2012; Perez et al. 2014; Voyles et al. 2018; Acosta-Chaves et al. 2019). Yet, we currently lack important life-history data to fully understand these patterns.

Generally, a thorough understanding of life-history traits can provide essential data for developing population models used in extinction risk assessments and endangered species management programs (Lindenmayer and Burgman 2005; IUCN Species Survival Commission 2012). In this study, we focused on documenting a variety of life-history traits, including oviposition habitat, clutch size, tadpole growth rates, timing of the emergence of froglets after metamorphosis, and call descriptions of *L. warszewitschii* from Panama. These traits may prove to be essential in detecting *L. warszewitschii*, assessing their status in regions where they previously declined, and evaluating their potential for recovery (Capdevila et al. 2022; Longo et al. 2023). This information can serve as a foundation for future research on the ecology and conservation of *L. warszewitschii* as well as other amphibian species.

MATERIALS AND METHODS

We conducted this study on *L. warszewitschii* using two approaches. First, we collected *L. warszewitschii* egg masses and reared tadpoles in a laboratory setting. By maintaining the tadpoles in the laboratory, we were able to accurately measure daily growth to determine their growth rates. Second, we collected basic morphology information from tadpoles in two wild populations. These measurements allowed us to compare laboratory-reared animals to their counterparts in the wild. In addition, we opportunistically collected audio recordings of *L. warszewitschii* calls in Panama to describe a previously undocumented vocalization. We selected two populations of *L. warszewitschii* in Panama: one in the town of El Valle de Antón
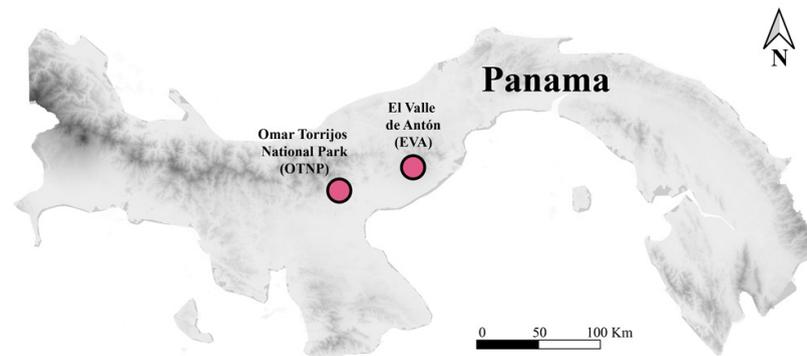
FIGURE 1. Locations of study sites for the Warszewitsch's Frog (*Lithobates warszewitschii*) in Panama. We sampled egg masses and tadpoles in El Valle de Antón (EVA) and wild tadpoles in EVA and Omar Torrijos National Park (OTNP). We recorded *L. warszewitschii* calls in EVA. Light-to-dark shading represents low-to-high elevation from elevation model derived from Japan Aerospace Exploration Agency. (Image taken from https://www.eorc.jaxa.jp/ALOS/en/dataset/aw3d30/aw3d30_e.htm).

(639 m elevation; Fig. 1) and one in Omar Torrijos National Park (710 m elevation; Fig. 1). These areas are described as tropical moist forests with two annual rainfall patterns: a dry season that ranges from December to mid-April, and a wet season that ranges from May to November (https://www.imhpa.gob.pa/es/regimen-pluviometrico-panama).

Collection of egg masses.—For the first part of this study, we collected egg masses on 12 December 2022 of *L. warszewitschii* found along a small stream of El Valle de Antón, where observers had anecdotally reported high densities of *L. warszewitschii* adults (Heidi Ross, pers. comm.). The stream site we focused on is situated in the eastern aspect of the Cordillera Central of Panama. During field collection, four observers walked along 200 m of the stream between 1000 and 2000 on 12 December 2022. We counted the total number of egg masses, recorded their location and habitat information, and collected a variety of water quality measurements, specifically water temperature, conductivity, total dissolved solids, salinity, and pH using a multiparametric water meter (PCTS 50, OAKTON Instruments, Vernon Hills, Illinois, USA). We collected six egg masses using new vinyl gloves and carefully transferred them to plastic bags, using one fresh bag for each mass (McDiarmid 1994). For each egg mass, we took a photo using a white background to accurately count the total number of eggs (Paton and Harris 2009). We did not include egg masses with hatched tadpoles attached to the mass (i.e., with tadpoles that had emerged from the egg jelly) in our egg counts.

Tadpole rearing in laboratory.—We placed six egg masses, one per tank, in 75-L outdoor tanks located in the shade near the collection site. Afterward, we transported them to the Smithsonian Tropical Research Institute, Panama, according to the Institutional Animal Care and Use Committee transportation requirements. We collected 3 L of stream stones and sand and boiled them (to sterilize) to provide a substrate for the tadpoles (Mantellato et al. 2013). We placed the sterilized substrate in tanks containing 30 L of dechlorinated carbon-filtered tap water, filling the tanks to approximately 50% of their capacity. We covered tanks with screen lids to prevent the entrance of external animals, debris, and rain. To maintain water quality, we removed about 20% of the water 3–4 times a week and replaced it with dechlorinated carbon-filtered tap water (Camperio Ciani et al. 2018).

We closely monitored the hatching time for all egg masses by visually inspecting them daily. After hatching, we fed the tadpoles daily using Tetramin® fish flakes (Tetra, Melle, Osnabrück, Germany). Because *L. warszewitschii* tadpoles are grazers, we moistened the food, spread the paste > 1 mm thick on acrylic plates, and dried it to a film (Camperio Ciani et al. 2018). We haphazardly selected 10 individuals from each tank weekly to collect data on mass as well as snout-to-vent length (SVL) and total length to determine growth rates through time. After measuring them, we returned the individuals to their respective tanks. We collected the two length measures because they provide complementary but different information on body sizes and developmental variation, helping to account for potential differences

in growth patterns and morphological traits among populations (Altig 2007; Hector et al. 2012). In addition, some historical studies report SVL whereas others calculate total length (Altig 2007; Biancardi and Cerbo 2010).

Seven weeks after hatching, we removed 200 tadpoles at Gosner (1960) stage 25 from the outdoor tanks to rear indoors in a temperature-controlled laboratory. In the laboratory, we placed the tadpoles in two 75-L tanks. Each tank contained 100 tadpoles with 30 L of dechlorinated carbon-filtered tap water. We maintained tadpoles under natural light conditions (about 12/12) and at a constant temperature of about 24° C (mean = 24.29° $\pm$ 0.61° C standard deviation), and a mean pH of 7.58 ($\pm$ 0.17), which are similar to the temperature and pH conditions of the stream water where we found the eggs masses. We recorded water temperature every hour using a Pendant HOBO recorder (Onset, Bourne, Massachusetts, USA), attached to the bottom of each tank.

We recorded the time to the beginning of metamorphosis by closely monitoring tadpoles for emergent forelimbs (Gosner 1960, stage 42), which we considered as the beginning of metamorphosis (Gosner 1960). We removed individuals from the tanks at this stage and placed them in single plastic containers (19 × 11 × 14 cm) containing half of a sterilized plastic pot as a hiding place. We positioned the containers on an incline to allow 100 ml of water to pool at one end. Upon completion of metamorphosis (Gosner 1960, stage 46), we fed the froglets with 5–10 flies or crickets with vitamin-dusted Repashy® Superfoods Vitamin A Plus (Repashy Ventures, Inc., Oceanside, California, USA) every 2 d until all tadpoles had completed metamorphosis, after which we ended the study.

Morphometrics in wild populations.—We collected morphological data from *L. warszewitschii* tadpoles from both wild populations. We captured tadpoles on 25 March 2023 in El Valle de Antón and on 7 May 2023 in Omar Torrijos National Park. We collected tadpoles using a new clean net, and we used vinyl gloves to carefully handle them to take body measurements. We measured SVL and tail length using calipers (0.01 mm error; AnyTime Tools, Los Angeles, California, USA) and body mass with an electronic scale (0.0001 error; OfficeSU, Beijing, China). We calculated the total length (SVL + tail length). After taking the measurements, we released each tadpole at the site of capture.

Analysis of tadpole size and growth rates in laboratory.—We calculated tadpole growth rate by dividing the total length over time (in weeks). We performed all statistical analyses using the software R v.3.6.1 (R Development Core Team 2019). We fitted a Generalized Linear Model (GLM) for tadpole growth using QuasiPoisson distributed errors and a logarithmic link function with week as the independent factor, and total tadpole length as the response variable. We tested for differences in SVL and total length in wild and captive populations. For this, we first tested the normality of tadpole lengths using a Shapiro-Wilk Test. Because our data were non-normal, we used a non-parametric Kruskal–Wallis Test for the comparisons.

Acoustic detection.—During our field sampling, we observed calling behaviors in adult *L. warszewitschii* on the streamside in El Valle de Antón. We opportunistically recorded the calling of two adults. We recorded the first individual on 1 December 2019 using an Iphone 7 (Apple, Inc., Cupertino, California, USA) and the second individual on 30 November 2023 using an Iphone 15 (Apple, Inc.).

We preprocessed the audio recording using the noisereduce Python package (Sainburg et al. 2020) and applying low-pass (1,700 Hz cutoff) and high-pass (900 Hz cutoff) filters (-36 dB/octave) to reduce background noise. Then, we created a log-scale linear-frequency spectrogram with a Hanning window of 1,024 samples wide with a step size of 102 samples using the Python package scipy (Virtanen et al. 2020). We measured the fundamental and dominant frequencies and call bandwidth of each note from a power spectrum and measured note duration and interval from an oscillogram according to Köhler et al. (2017). We performed inspection and measurements in Audacity® (version 3.4.2; https://www.audacityteam.org). Because our recordings have high background noise and low signal-to-noise ratios, we could not accurately measure the 90% bandwidth. Instead, we report the approximate prevalent bandwidth based on visual inspection of the power spectrum (Köhler et al. 2017).

Results

During our surveys on 12 December 2022, we found nine egg masses attached to rocks in shallow pools on each side of the stream (Fig. 2). One of the egg masses was attached to human-generated debris in the stream. The physicochemical properties of

oviposition microhabitats (n = 9) in the stream were a temperature of 22.2° (± 0.15°) C, a pH of 7.34 (± 0.36), a conductivity of 121.6 (± 15.3) μS, a salinity of 0 ppm (± 0), and a total dissolved solids of 85.9 (± 11.9) ppm. The mean number of eggs per mass was 455 (± 45, range of values, 370–496, n = 9).
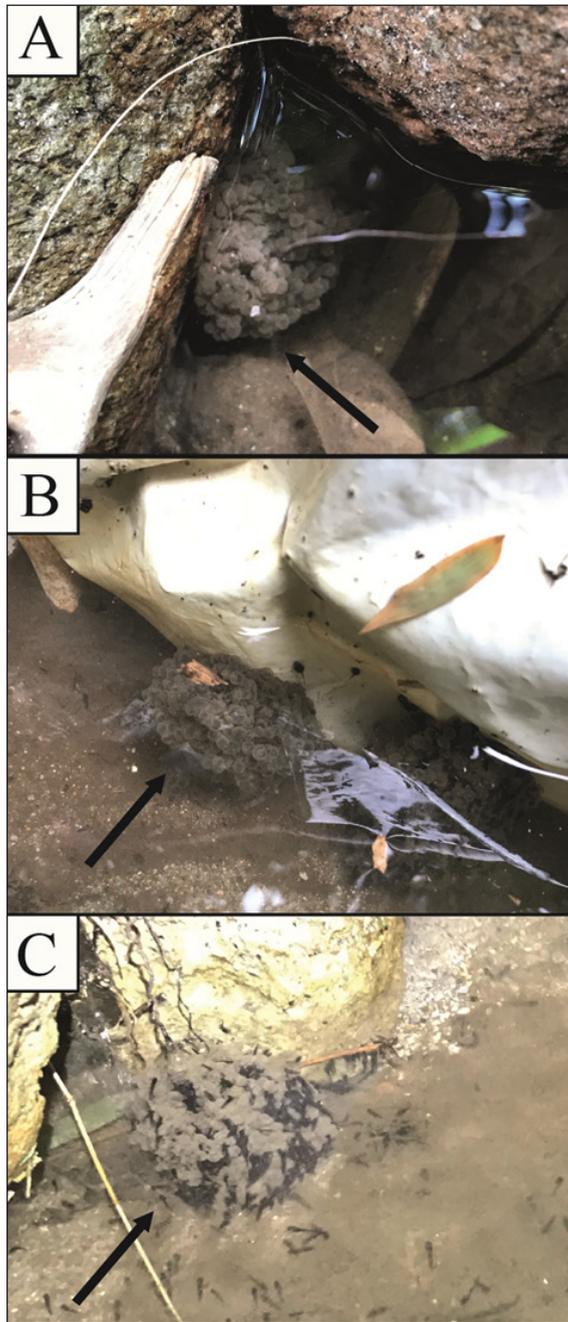

FIGURE 2. Egg masses of the Warszewitsch's Frog (*Lithobates warszewitschii*) on a stream in El Valle de Antón, Panama. (A) Egg mass attached to rocks, (B) egg mass attached to trash, and (C) egg mass with tadpoles attached. Arrows denote egg masses. (Photographed by M. Delia Basanta).

Morphometrics at different developmental stages.—We found that the tadpoles in the laboratory grew at an overall rate of 3.4 mm total length per week for 16 weeks. More specifically, tadpoles increased in total length from week 1 to 16 (GLM, $t = 55.43$, df = 409, $P < 0.001$; Fig. 3). We found a tadpole maximum total length of 59.5 mm. Of the 200 tadpoles that we reared, 96% survived the 16 weeks in captivity.

We observed forelimb emergence in tadpoles after 98 d, marking the beginning of metamorphosis (Gosner stage 42). At this stage, they had a mean SVL of 22.68 mm (± 0.91 mm, n = 20) and a mean total length of 55.81 mm (± 2.33 mm, n = 20). We found that the duration of metamorphosis, which is the time period when they absorbed their tails (i.e., transition from Gosner stage 42 to 46), occurred on an average of 3 d (± 1.4 d, n = 20). Following metamorphosis, the froglets had a mean SVL of 23.95 mm (± 0.96 mm, n = 20).

Morphometrics in wild populations.—In the wild populations, we collected tadpoles between Gosner stages 25 and 38. We subselected the tadpoles at Gosner stage 25 for measurements and comparisons between wild and captive populations to standardize the stage for comparisons. The stage 25 tadpoles from El Valle de Antón had a mean SVL of 11.74 mm and a total length of 28.97 mm (n = 22; Fig. 4), whereas the tadpoles at this stage from Omar Torrijos National Park had a mean SVL of 12.27 mm and a total length of 30.1 mm (n = 24; Fig. 4). There were no significant differences in tadpole SVL and total length among captive (n = 40) and the two wild populations at stage 25 (SVL: $H = 0.376$, df = 2, $P = 0.829$; total length: $H = 0.601$, df = 2, $P = 0.741$).
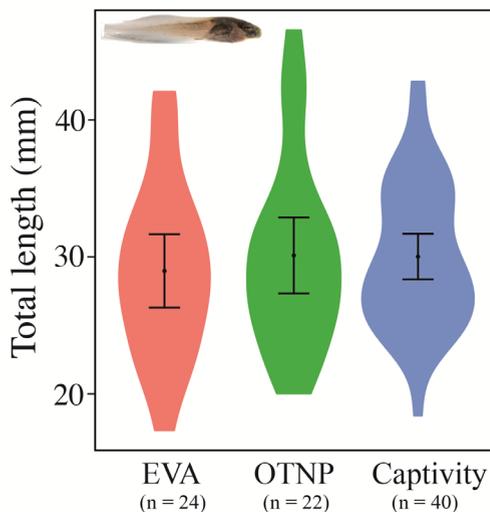
Acoustic detection.—We recorded individual vocalizations of two *L. warszewitschii* adults, the first one on 1 December 2019 at 1200, and the second one on 30 November 2023 at 2211. Both individuals were calling during the beginning of the dry season, along the side of a stream in El Valle de Antón (Supplemental Information). The ambient air temperature on 30 November 2023 was 24° C. We heard similar vocalizations by males multiple times during day and night surveys at close distances (about 2–3 m), and we observed two pairs in amplexus along the side of the stream. We did not capture the individuals whose vocalizations we recorded and, therefore, we do not have body length measurements.
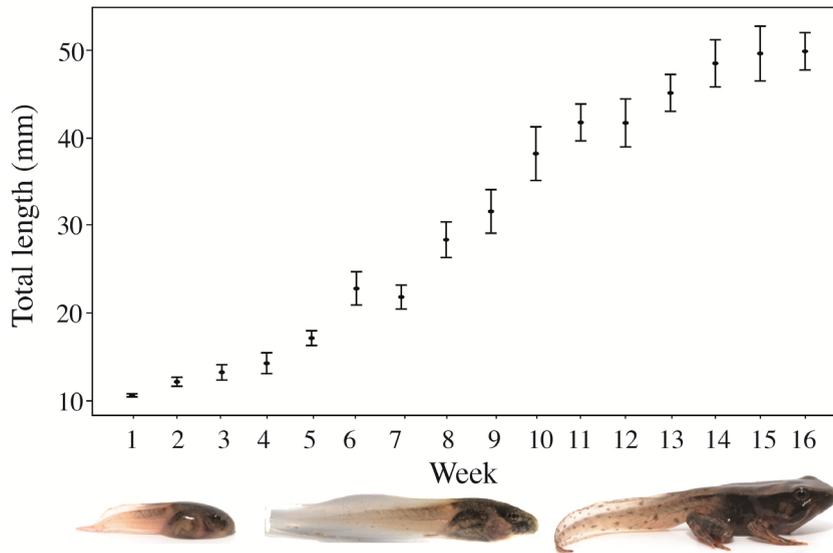
Figure 3. Mean total length (± standard error) of 20 Warszewitsch's Frog (*Lithobathes warszewitschii*) tadpoles in Panama by week in captivity. Week 1 represents one week after hatching. (Photographs by M. Delia Basanta).

The vocalizations of *L. warszewitschii* were bouts of tonal frequency-modulated notes (i.e., energy is concentrated in a narrow frequency band, and fundamental frequency changed over time within the note; Köhler et al. 2017). Each of the two recorded calls consisted of eight or nine two-note groups (Table 1). The vocalizations were not pulsed or trilled, meaning that they lacked rapid amplitude modulation.

Figure 4. Tadpole total length from wild and captive Warszewitsch's Frog (*Lithobates warszewitschii*) populations in Panama at Gosner (1960) stage 25. The vertical line represents the mean ± standard error. The colored violin polygons represent the data variation, with the width of the polygon representing the likelihood of data points along the vertical axis. Abbreviations are EVA = El Valle de Antón and OTNP = Omar Torrijos National Park. (Tadpole photograph by M. Delia Basanta).

The note groups were composed of a softer, higher frequency note type 1 (mean fundamental frequency about 1.6 kHz, e.g., at 0.4 s in the spectrogram; Fig. 5) followed by a louder, lower frequency note type 2 (mean fundamental frequency = 1.2 kHz, e.g., at 0.5 s in the spectrogram). In this recording, the note groups were repeated at an interval of 0.4 to 0.6 s. The dominant frequency (i.e., the loudest frequency) was always equivalent to the fundamental frequency (i.e., the lowest frequency). For the more distant individual, the notes were obscured by background noise, and we were only able to measure the fundamental frequency of note type 2. The note groups and individual notes showed considerable variation in repetition rate, inflection, and dominant frequency within a single calling bout.

Discussion

Our study provides new information on life-history traits for *Lithobates warszewitschii* in Panama. During our study, we found several differences between previous studies and our observations. First, *L. warszewitschii* was previously described as a primarily diurnal species that was most commonly found on trails, and adjacent ditches, streams, and ponds (Villa 1990; Savage 2002). In contrast, from our recent diurnal and nocturnal sampling, encounters with this species predominantly occurred at night, with adult *L. warszewitschii* found along the sides of streams. While this is anecdotal evidence,

TABLE 1. Acoustic measurements from notes in the recorded calls of Warszewitsch's Frog (*Lithobates warszewitschii*) in Panama. We took measurements on each note of a single call for each of two individuals. Dominant frequencies were equivalent to fundamental frequencies. For fundamental frequency, duration, and note interval, we provide the mean across all notes and the range of values in parentheses. The abbreviation n.a. denotes that the measurement was not possible due to a low signal-to-noise ratio. Heading abbreviations are NT1-FF = note type 1 fundamental frequency (kHz), NT2-FF = note type 2 fundamental frequency (kHz), NT1-D = note type 1 duration (seconds), NT2-D = note type 2 duration (seconds), NTI = note interval (seconds), APB = approximate prevalent band width (kHz).

Individual	NT1-FF (n = 9)	NT2-FF (n = 9)	NT1-D	NT2-D	NI	APB
1	n.a.	1.2 (1.11–1.41)	n.a	n.a	n.a	n.a
2	1.62 (1.48–1.78)	1.17 (1.04–1.29)	0.07 (0.06–0.08)	0.14 (0.11–0.18)	0.54 (0.49–0.61)	1.18–1.74

we suggest that this species may be better described as cathemeral (sporadic activity day and night) than diurnal (Onorati and Vignoli 2017). Second, there is currently a lack of full information about the phenology of breeding for this species. We found egg masses in December, which generally corresponds to the dry season in Panama, suggesting that at least part of the breeding season likely occurs during the transition from wet to dry seasons. Leenders (2016), however, suggested that *L. warszewitschii* may reproduce year-round, based on preliminary observations in Costa Rica. As such, it will be important to conduct more comprehensive surveys for breeding activity (including amplexing pairs and egg masses) at other times of year to fully understand the breeding phenology in this species throughout its range. Third, Greding (1972) documented a single *L. warszewitschii* egg mass with 167 eggs, which is

a lower number of eggs per mass than we found in Panama (mean = 455 eggs). Additionally, Starrett (1960) reported a larger maximum total length (115 mm) for *L. warszewitschii* tadpoles than that found in our study (59.5 mm). Overall, our findings indicate that *L. warszewitschii* may exhibit greater variability than previously documented, particularly in terms of its cathemeral activity, breeding season timing, egg mass size, and tadpole body size.

Previous reports included little to no information on egg mass locations (e.g., densities, conditions, water quality characteristics), time to the beginning of metamorphosis, tadpole growth rates, and body size (SVL and/or total length). We found that in El Valle de Antón, Panama, egg masses of *L. warszewitschii* usually adhered to rock surfaces in pools on the sides of a stream, similar to that reported by Leenders (2016). This oviposition microhabitat is similar to
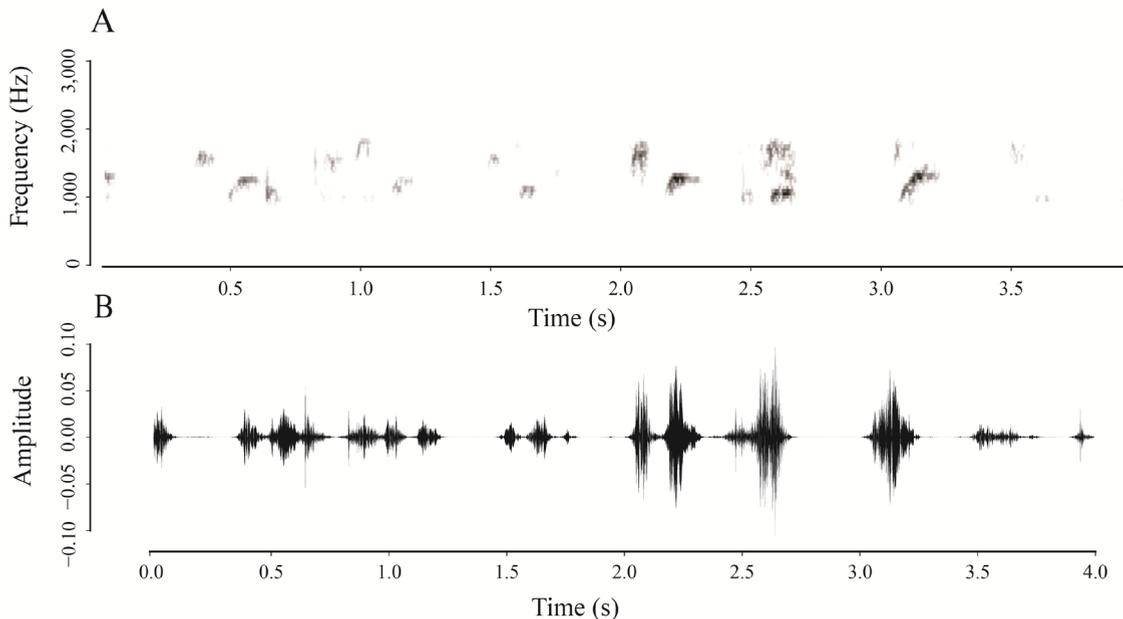
FIGURE 5. Spectrogram (A) and oscillogram (B) of the calls of a single Warszewitsch's Frog (*Lithobates warszewitschii*) recorded in El Valle de Antón, Panama. We created the linear frequency spectrogram with a Hanning window of 1,024 samples wide and a step size of 102 samples.

that of other *Lithobates* species that breed in streams, where egg masses are generally attached to rocks in areas with low currents, possibly reducing the likelihood of the eggs being swept away (Dodd 2009; Brown et al. 2019). In addition, we observed that some egg masses were located in areas containing human-generated debris (trash) and in urban areas (streams in the neighborhoods of El Valle de Antón). Finally, despite presumed differences in a variety of abiotic factors between captivity and wild conditions (e.g., water physicochemical characteristics, tadpole density, availability of food *ad libitum*, and absence of predators in the laboratory; Chivers et al. 1999; Alvarez and Nicieza 2002; Vaissi and Sharifi 2016), we found that the total lengths of tadpoles in captivity were similar to that for tadpoles from two wild populations in Panama at an early developmental stage (Gosner stage 25).

We observed intriguing differences between our description of the acoustic vocalizations in *L. warszewitschii* and previous reports. *Lithobates warszewitschii* is the only species, aside from *R. vibicaria*, that is known to vocalize without vocal sacs or slits within the *R. palmipes* group (Hillis and De Sá 1988). The only previous information about vocalizations of *L. warszewitschii* is a pulsed advertisement observed in Costa Rica (Greding 1972). The calls we observed were not pulsatile, suggesting they may represent an undescribed call type and may serve a different biological function from the previously described trill. We provide an audio clip and spectrogram, reflecting a *L. warszewitschii* call consisting of a series of repeated two-note phrases with a softer, higher frequency note and a louder, lower frequency note. The calls are presumably too quiet to act as a long-range signal in a noisy stream environment, and we suggest that they may function as a courtship call, a type of call given when males and females are in close proximity (Köhler et al. 2017).

The differences in egg mass size, tadpole size, breeding calls, and other characteristics between our study and previous reports likely have multiple, non-mutually exclusive explanations. Most of the existing reports are from populations of *L. warszewitschii* in Costa Rica, and there is no information from populations from other parts of its range in Central America. As such, it is likely that there are numerous life-history traits that have not yet been fully characterized for this species. In addition, these differences may reflect a great genetic variation such that the group that is currently identified as *L. warszewitschii* may actually represent a cryptic species complex across its currently described distribution (Cryer et al. 2019). Overall, understanding the underlying reasons for this variation is important because while species complexes occupy broad geographical ranges, potential biological species within these complexes may have more limited distributions, making each one vulnerable to extinction (Bickford et al. 2007). Thus, more information about life-history traits, the variation in these traits, as well as systematic and taxonomic studies within the range of *L. warszewitschii*, may be warranted (Cryer et al. 2019).

Life-history traits are used to predict species responses to disturbances (Hutchings et al. 2012). For example, clutch size and tadpole growth data can help estimate the potential recovery of tadpole populations and assess their contribution to adult recruitment following disease outbreaks (Berven 1990; DiRenzo et al. 2017). Additionally, species call information could assist in the detection and monitoring of rare, cryptic, or declining species (e.g., Lapp et al. 2021), and in the estimation of changes in relative abundances before, during, and after declines (Laiolo 2010). Several declines have been reported for *L. warszewitschii* along its distribution (Puschendorf et al. 2006; Whitfield et al. 2007; Santos-Barrera et al. 2008; Crawford et al. 2010), but in recent years, this species has been reported to be recolonizing sites with higher abundances than in the past (Barquero et al. 2010; Hilje and Aide 2012; IUCN 2024). Future research is needed to understand how the species is recovering and recolonizing in these areas. We expect that life-history traits are related to (and may even underpin) their recoveries. Understanding how this species is recovering may inform shifts in amphibian communities throughout its full range.

Our study provides basic but new information on *L. warszewitschii* populations and highlights variation within this species. This information is useful for developing a comprehensive understanding of the life-history strategies and conservation requirements of *L. warszewitschii*. Furthermore, it provides natural history information that may be invaluable for assessing the recoveries of this and other species following disease-induced declines.

Research Institute; and Partners in Amphibian and Reptile Conservation (PARC). This research was approved under the Institutional Animal Care and Use Committee (IACUC) of the University of Nevada, Reno (protocol 20–08–1063) and the IACUC of the Smithsonian Tropical Research Institute (protocol 2012–0901–2015 and SI-22059–6).

LITERATURE CITED

Abarca Alvarado, J.G. 2012. Historia natural de la herpetofauna de El Rodeo y el oeste del Valle Central, Costa Rica. Brenesia 77:229–250.

Acosta-Chaves, V.J., V. Madrigal-Elizondo, G. Chaves, B. Morera-Chacón, A. García-Rodríguez, and F. Bolaños. 2019. Cambios en la diversidad de una comunidad de anfibios en un bosque premontano de San Ramon, Costa Rica. Revista de Biología Tropical 67:259–274.

Altig, R. 2007. A primer for the morphology of anuran tadpoles. Herpetological Conservation and Biology 2:71–74.

Alvarez, D., and A.G. Nicieza. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. Functional Ecology 16:640–648.

Barquero, M.D., M. Salazar-Saavedra, L. Sandoval, D. Brenes, F. Martínez, and A. Figueroa. 2010. Composition and species richness of herpetofauna in two isolated regions of southern Nicaragua. Herpetology Notes 3:341–352.

Berven, K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the Wood Frog (*Rana sylvatica*). Ecology 71:1599–1608.

Biancardi, C.M., and A.R. Di Cerbo. 2010. Morphometric study on tadpoles of *Bombina variegata* (Linnaeus, 1758) (Anura; Bombinatoridae). Acta Herpetologica 5:223–231.

Bickford, D., D.J. Lohman, N.S Sodhi, P.K.L. Ng, R. Meier, K. Winker, K.K. Ingram, and I. Das. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22:148–155.

Brannelly, L.A., H.I. McCallum, L.F. Grogan, C.J. Briggs, M.P. Ribas, M. Hollanders, T. Sasso, M.F. López, D.A. Newell, and A.M. Kilpatrick. 2021. Mechanisms underlying host persistence following amphibian disease emergence determine appropriate management strategies. Ecology Letters 24:130–148.

Brem, F.M., and K.R. Lips. 2008. *Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages. Diseases of Aquatic Organisms 81:189–202.

Brown, C., L.R. Wilkinson, K.K. Wilkinson, T. Tunstall, R. Foote, B.D. Todd, and V.T. Vredenburg. 2019. Demography, habitat, and movements of the Sierra Nevada Yellow-legged Frog (*Rana sierrae*) in streams. Copeia 107:661–675.

Camperio Ciani, J.F., J. Guerrel, E. Baitchman, R. Diaz, M. Evans, R. Ibáñez, H. Ross, E. Klaphake, B. Nissen, A.P. Pessier, et al. 2018. The relationship between spindly leg syndrome incidence and water composition, overfeeding, and diet in newly metamorphosed harlequin frogs (*Atelopus* spp.). PloS ONE 13(10):e0204314. https://doi.org/10.1371/journal.pone.0204314.

Capdevila, P., I. Stott, J. Cant, M. Beger, G. Rowlands, M. Grace, and R. Salguero-Gómez. 2022. Life history mediates the trade-offs among different components of demographic resilience. Ecology Letters 25:1566–1579.

Chivers, D.P., J.M. Kiesecker, A. Marco, E.L. Wildy, and A.R. Blaustein. 1999. Shifts in life history as a response to predation in Western Toads (*Bufo boreas*). Journal of Chemical Ecology 25:2455–2463.

Crawford A.J., K.R. Lips, and E. Bermingham. 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. Proceedings of the National Academy of Sciences 107:13777–13782.

Cryer, J., F. Wynne, S.J. Price, and R. Puschendorf. 2019. Cryptic diversity in *Lithobates warszewitschii* (Amphibia, Anura, Ranidae). ZooKeys 838:49–69.

DiRenzo, G.V., C. Che-Castaldo, A. Rugenski, R. Brenes, M.R. Whiles, C.M. Pringle, S.S. Kilham, and K.R. Lips. 2017. Disassembly of a tadpole community by a multi-host fungal pathogen with limited evidence of recovery. Ecological Applications 27:309–320.

Dodd, C.K., Jr. 2009. Amphibian Ecology and Conservation: A Handbook of Techniques. Oxford University Press, New York, New York, USA.

Gagliardo, R., P. Crump, E. Griffith, J. Mendelson, H. Ross, and K. Zippel. 2008. The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. International Zoo Yearbook 42:125–135.

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

Greding, E.J. 1972. Call specificity and hybrid compatibility between *Rana pipiens* and three other *Rana* species in Central America. Copeia 1972:383–385.

Hector, K.L., P.J. Bishop, and S. Nakagawa. 2012. Consequences of compensatory growth in an amphibian. Journal of Zoology 286:93–101.

Hilje, B., and T.M. Aide. 2012. Recovery of amphibian species richness and composition in a chronosequence of secondary forests, northeastern Costa Rica. Biological Conservation 146:170–176.

Hillis, D.M., and R. De Sá. 1988. Phylogeny and taxonomy of the *Rana palmipes* group (Salientia: Ranidae). Herpetological Monographs 2:1–26.

Howard, S.D., and D.P. Bickford. 2014. Amphibians over the edge: silent extinction risk of data deficient species. Diversity and Distributions 20:837–846.

Hutchings, J.A., R.A. Myers, V.B. García, L.O. Lucifora, and A. Kuparinen. 2012. Life-history correlates of extinction risk and recovery potential. Ecological Applications 22:1061–1067.

International Union for Conservation of Nature and Natural Resources and Species Survival Commission (IUCNSSC). 2012. IUCN Red List categories and criteria. Version 3.1. 2nd Edition. Gland, Vaud, Switzerland.

International Union for the Conservation of Nature (IUCN). 2024. The IUCN Red List of Threatened Species. Version 2024–1. https://www.iucnredlist.org.

International Union for the Conservation of Nature (IUCN). 2025. The IUCN Red List of Threatened Species. Version 2025-1. https://www.iucnredlist.org.

Knapp, R.A., G.M. Fellers, P.M. Kleeman, D.A. Miller, V.T. Vredenburg, E.B. Rosenblum, and C.J. Briggs. 2016. Large-scale recovery of an endangered amphibian despite ongoing exposure to multiple stressors. Proceedings of the National Academy of Sciences 113:11889–11894.

Köhler, J., M. Jansen, A. Rodriguez, P.J. Kok, L.F. Toledo, M. Emmrich, F. Glaw, C.F.B. Haddad, M.O. Rödel, and M. Vences. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. Zootaxa 4251:1–124.

Laiolo, P. 2010. The emerging significance of bioacoustics in animal species conservation. Biological Conservation 143:1635–1645.

Lapp, S., T. Wu, C. Richards-Zawacki, J. Voyles, K.M. Rodriguez, H. Shamon, and J. Kitzes. 2021. Automated detection of frog calls and choruses by pulse repetition rate. Conservation Biology 35:1659–1668.

Leenders, T. 2016. Amphibians of Costa Rica: A Field Guide. Cornell University Press, Ithaca, New York, USA.

Lindenmayer, D., and M.A. Burgman. 2005. Practical Conservation Biology. CSIRO Publishing, Collingwood, Australia.

Lips, K.R., F. Brem, R. Brenes, J.D. Reeve, A.R. Alford, J. Voyles, C. Carey, L. Livo, A.P. Pessier, and J.P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences 103:3165–3170.

Lips, K.R., J.D. Reeve, and L.R. Witters. 2003. Ecological traits predicting amphibian population declines in Central America. Conservation Biology 17:1078–1088.

Longo, A.V., K.R. Lips, and K.R. Zamudio. 2023. Evolutionary ecology of host competence after a chytrid outbreak in a naive amphibian community. Philosophical Transactions of the Royal Society B 378:20220130. https://doi.org/10.1098/rstb.2022.0130.

Mantellato, L., G. Gaikhorst, R. Kruger, S. Vitali, and H. Robertson. 2013. Growth and development of captive *Geocrinia rosea* (Myobatrachidae): a rare species analogue. Zoo Biology 32:374–380.

McDiarmid, R.W. 1994. Preparing amphibians as scientific specimens. Pp. 289–297 *In* Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek and M.S. Foster (Eds.). Smithsonian Institution Press, Washington, D.C., USA.

Michaels, C.J., B.F. Gini, and R.F. Preziosi. 2014. The importance of natural history and species-specific approaches in amphibian ex-situ conservation. Herpetological Journal 24:135–145.

Onorati, M., and L. Vignoli. 2017. The darker the night, the brighter the stars: consequences of nocturnal brightness on amphibian reproduction. Biological Journal of the Linnean Society 120:961–976.

Paton, P.W., and R.N. Harris. 2009. Egg mass and nest counts. Pp. 143–165 *In* Amphibian Ecology and Conservation: A Handbook of Techniques. Dodd, C.K., Jr. (Ed.). Oxford University Press, New York, New York, USA.

Perez, R., C. Richards-Zawacki, A.R. Krohn, M. Robak, E.J. Griffith, H. Ross, B. Gratwicke, R. Ibañez, and J. Voyles. 2014. Field surveys in

Western Panama indicate populations of *Atelopus varius* frogs are persisting in regions where *Batrachochytrium dendrobatidis* is now enzootic. Amphibian & Reptile Conservation 8:30–35.

Puschendorf, R., F. Bolaños, and G. Chaves. 2006. The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. Biological Conservation 132:136–142.

R Development Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org.

Ranvestel, A.W., K.R. Lips, C.M. Pringle, M.R. Whiles, and R.J. Bixby. 2004. Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. Freshwater Biology 49:274–285.

Sainburg, T., M. Thielk, and T.Q. Gentner. 2020. Finding, visualizing, and quantifying latent structure across diverse animal vocal repertoires. PLoS Computational Biology 16:e1008228. https://doi.org/10.1371/journal.pcbi.1008228.

Santos-Barrera, G., J. Pacheco, F. Mendoza-Quijano, F. Bolaños, G. Cháves, G.C. Daily, P.R. Ehrlich, and G. Ceballos. 2008. Diversity, natural history and conservation of amphibians and reptiles from the San Vito Region, southwestern Costa Rica. Revista de Biología Tropical 56:755–778.

Savage, J.M. 2002. The Amphibians and Reptiles of Costa Rica: a Herpetofauna Between Two Continents, Between Two Seas. University of Chicago Press, Chicago, Illinois, USA.

Scheele, B.C., F. Pasmans, L.F. Skerratt, L. Berger, A.N. Martel, W. Beukema, A.A. Acevedo, P.A. Burrowes, T. Carvalho, A. Catenazzi, et al. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459–1463.

Schmidt, O. 1857. Diagnosen neuer Frösche des zoologischen Cabinets zu Krakau. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Classe 24:10–15.

Starrett, P. 1960. Descriptions of tadpoles of Middle American frogs. Miscellaneous Publications, Museum of Zoology, University of Michigan 110:1–39.

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Vaissi, S., and M. Sharifi. 2016. Variation in food availability mediate the impact of density on cannibalism, growth, and survival in larval Yellow Spotted Mountain Newts (*Neurergus microspilotus*): implications for captive breeding programs. Zoo Biology 35:513–521.

Vega-Yánez, M.A., A.B. Quezada-Riera, B. Rios-Touma, M.C. Vizcaíno-Barba, W. Millingalli, W., O. Ganzino, L.A. Coloma, E.E. Tapia, N. Dupérré, M. Páez-Vacas, et al. 2024. Path for recovery: an ecological overview of the Jambato Harlequin Toad (Bufonidae: *Atelopus ignescens*) in its last known locality, Angamarca Valley, Ecuador. PeerJ 12:e17344. https://doi.org/10.7717/peerj.17344.

Villa, J.D. 1990. *Rana warszewitschii* (Schmidt). Catalogue of American Amphibians and Reptiles 459:1–2.

Virtanen, P., R. Gommers, T.E. Oliphant, M. Haberland, T. Reddy, D. Cournapeau, E. Burovski, P. Peterson, W. Weckesser, J. Bright, et al. 2020. SciPy 1.0: fundamental algorithms for scientific computing in Python. Nature Methods 17:261–272.

Voyles, J., D.C. Woodhams, V. Saenz, A.Q. Byrne, R. Perez, G. Rios-Sotelo, G., M.J. Ryan, M.C. Bletz, F.A. Sobell, and C.L. Richards-Zawacki. 2018. Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation. Science 359:1517–1519.

Whitfield, S.M., K.E. Bell, T. Philippi, M. Sasa, F. Bolanos, G. Chaves, J.M. Savage, and M.A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. Proceedings of the National Academy of Sciences 104:8352–8356.

Yuan, Z.Y., W.W. Zhou, X. Chen, Jr., N.A. Poyarkov, H.M. Chen, N.H. Jang-Liaw, W.H. Chou, N.J. Matzke, K. Iizuka, M.S. Min, et al. 2016. Spatiotemporal diversification of the true frogs (genus *Rana*): a historical framework for a widely studied group of model organisms. Systematic Biology 65:824–842.

Supplemental Information; video: https://vimeo.com/1011489104?share=copy#t=0.

 M. Delia Basanta is currently a Postdoctoral Researcher at the University of Nevada, Reno (UNR), USA. She earned her Ph.D. from the Universidad Nacional Autónoma de México, Mexico City, Mexico. Her research focuses on understanding how environmental factors, pathogens, and hosts interact in the dynamics of infectious diseases. At UNR, Delia is currently investigating the mechanisms of recovery in amphibian species following declines caused by chytridiomycosis in Panama. (Photographed by M. Delia Basanta).

 Heidi Ross is the Project Director at the El Valle Amphibian Conservation Foundation (EVACC), El Valle de Antón, Coclé, Panama. She has 20 y of experience breeding and raising endangered amphibians of Panama. She also has extensive field work experience in neotropical streams and forests. Her research interests focus mostly on successfully introducing captive-bred amphibians back into their natural habitat. (Photographed by Evelyn Ortiz).

 Roberto Brenes is an Associate Professor of Biology at Carroll University in Waukesha, Wisconsin, USA. He received a Ph.D. in wildlife diseases from the University of Tennessee, Knoxville, USA. His research mainly focuses on the effects of emerging infectious diseases in amphibian larvae, particularly the ecosystem effects of species extirpations in tropical streams. (Photographed by Roberto Brenes).

 Mirna G. Garcia-Castillo is a Professor at the Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM), in Mexico City. Her research focuses on molecular systematics, taxonomy, morphology, biogeography, and the evolution of the salamander genus *Chiropterotriton*. She has also collaborated on projects related to amphibian diseases, natural history, and ecology. Currently, Mirna teaches Systematics, Biogeography, and Evolution to undergraduate students at UNAM. She combines teaching with research through the coordination of a workshop aimed at developing scientific skills in undergraduates, with a focus on Mexican amphibians. (Photographed by Mirna G. García-Castillo).

 Carolina Lambertini is a Postdoctoral Researcher at Pennsylvania State University, University Park, USA. She earned her Ph.D. at the State University of Campinas (UNICAMP), Brazil. Passionate about amphibians and conservation, her research focuses on the ecology of infectious diseases, particularly chytridiomycosis. Carolina investigates how amphibian skin properties confer resistance among tropical and temperate species recovering from disease. She is also involved in the Atelopus Survival Initiative, helping define conservation priorities and actions for *Atelopus*, the amphibian genus most severely impacted by chytridiomycosis. (Photographed by Michelle A. Werdann).

 Lucia Anaya is a third-year Ph.D. student in Biology at the University of Mississippi, University, USA, studying amphibian thermal physiology, immune function, and disease ecology. Her research focuses on how antimicrobial peptides, the skin microbiome, and infection by the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) interact to shape host responses. She integrates field and lab work to understand how amphibians respond to environmental stressors. (Photograph courtesy of the University of Mississippi).

 Sam Labb is a Ph.D. student in the Kitzes Lab at the University of Pittsburgh, Pennsylvania, USA, where he is developing analyical methods for passive acoustic monitoring data. His research interests include conservation technology, acoustic communication, and acoustic signal analysis with machine learning. (Photographed by Sam Lapp).

 Michel Ohmer is an Assistant Professor of Biology at the University of Mississippi, University, USA. She received her Ph.D. from the University of Queensland in Brisbane, Australia. She is an Ecophysiologist and Disease Ecologist who studies the impact of global change on host-pathogen interactions, primarily focusing on amphibians and a widespread fungal pathogen, the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*). (Photographed by Melissa Sung.).

 Justin Kitzes is a Quantitative Ecologist and Conservation Biologist with specific expertise in the use of automated acoustic methods for large scale biodiversity surveys. He is an Associate Professor in the Department of Biological Sciences at the University of Pittsburgh, Pennsylvania, USA. (Photograph courtesy of by the University of Pittsburgh).

 Jamie Voyles is a Professor in the Department of Biology and the Ecology, Evolution, and Conservation Biology (EECB) Program at the University of Nevada, Reno (UNR), USA. She is also the current appointee of the Trevor J. McMinn Endowed Research Professor in Science at UNR. As a disease ecologist, her research program is highly interdisciplinary, bridging multiple biological subdisciplines to resolve key questions concerning emerging infectious diseases in amphibians and other host species. (Photographed by Jennifer Kent).