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Data on the Reproductive Biology and Temperature-dependent Patterns of Henkel's Leaf-tailed Gecko, *Uroplatus henkeli*, at the BION Terrarium Center

The island of Madagascar has a remarkably diverse and unique biota due to its long isolation from other landmasses. The Malagasy herpetofauna is no exception, as 99% of the amphibians and 90% of the reptiles are endemic (Glaw and Vences 2007). Much of this diversity is still being uncovered, and consequently, very little is known about the conservation status of these species. Unfortunately, rates of forest loss in Madagascar are nearly as high as the levels of endemism. High levels of deforestation in Madagascar were already observed by the end of the twentieth century (Jolly et al. 1984). Some studies reported that only 10–20% of the original forests remained intact in 2010 (Schwitzer et al. 2014). Other sources estimate a decrease of forest cover by 40% in the period from the 1950s to the 2000s, with a reduction of "core forest" (i.e., forests >1 km from deforested edges) of almost 80% (Harper et al. 2007; Clark 2015). In short, there is a great need for scientific information to guide the conservation of this unique biota.

A number of studies have examined impacts that amphibian and reptile populations might experience on the edges of their natural distributions. For example, due to global climate change, variations in temperature regimes near habitat edges would be expected to alter the ranges of many reptiles and amphibians because of their reliance on the environment to provide optimal body temperature (Green and Sussman 1990). Changes in seasonal temperature regimes may also result in the rise of isolated populations that are too small to stay genetically viable. In such marginal populations, overall reproductive success may be reduced as well (Bobyn and Brooks 1994). Shorter periods of winter rest can negatively affect recovery from previous breeding seasons and disrupt hormonal cycles and production of gametes (Licht et al. 1969; Fitch 1970; Pearson et al. 1976). Though there are currently no reports of temperature-dependent sex determination in *Uroplatus*, in chelonians, it was found that even a 1°C increase in temperature could lead to rapid increase of females (up to 75%) hatched in a clutch of eggs (Lawson and Rollinson 2021), as females tend to develop at extreme incubation temperatures.

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The appearance of invasive species can also negatively affect populations of local biota through predation or spread of pathogens or parasites, leading to a decrease in the effective population size (Kliman et al. 2008; Schierenbeck 2017). Another possible negative consequence of introduction of invasive species is hybridization (Capezzone 1995). Physical destruction of suitable habitats for local biota due to mass deforestation (Harper et al. 2007), agricultural transformation (Rakotomanana et al. 2013), and the impacts of tourism, i.e., when animals are moved from their natural habitats to popular touristic routes (Stephenson 1993; Harper et al. 2007) can result in threats of extinction (Jenkins et al. 2014; Nekrasova et al. 2021). Reptiles tend to be one of the most sensitive groups of vertebrates. They, along with amphibians, are among the first vertebrates to experience decreasing numbers, extinction events, and morphological anomalies because of biotic and abiotic environmental changes (Henle et al. 2017). Consequently, they require specific actions for their conservation both in situ and ex situ situations (D'Cruze et al. 2008; Gardner et al. 2015; Marushchak et al. 2019). With 21 species currently described within *Uroplatus*, this genus is no exception (Ratsoavina et al. 2020).

This work is dedicated to the study of the reproductive cycles of Henkel's Leaf-tailed Gecko, *Uroplatus henkeli*, in controlled laboratory conditions with the goal of establishing this species in the herpetoculture sphere for conservation purposes (Marushchak et al. 2020). Like the other representatives of the genus, *U. henkeli* is listed in Appendix II of CITES ("Convention" 2022) species that are not necessarily threatened with extinction at present but may become so unless trade is closely controlled.

Fig. 1. Standard laboratory terrarium for one pair of *Uroplatus henkeli* at BION Terrarium Center, Kyiv, Ukraine.

Table 1. Approximate temperature and photoperiod provided to captive *Uroplatus henkeli* throughout the study.

Date	T (°)	Photoperiod (h)
6 March–18 November	23–26	12–14
19–26 November	23–25	12
27 November–3 December	23–25	10
4–10 December	23–25	8
11–17 December	22–24	6
18 December–10 February	18–24	4
11–16 February	20–24	6
17–23 February	22–25	8
24 February–5 March	23–25	10

Uroplatus henkeli is endemic to Madagascar, inhabiting the northern and northwestern forests in addition to the coastal island of Nosy Be. The species' distribution range stretches from the Sambirano region in the south to Tsingy de Bemaraha. Records originate from Ankarafantsika, Benavony, Berara Forest, Manongarivo, Nosy Comba, Tsarakibany, Sahamalaza, Ambohimarina, Kelifely, and Ankara. Despite this large area of distribution, populations from Nosy Be and the southernmost locality are genetically similar (Hyde-Roberts and Daly 2014; Gehring 2020). These geckos inhabit monsoon rainforests and xerophytic forests of the west and northwestern coast of Madagascar. They live on rainforest tree trunks at heights from 1–4 m and typically sleep in plain sight during the day at heights from 70–130 cm (Gehring 2020). Tree trunks with diameters of 2–6 cm are most commonly used for perching because the animals are able to get a safe grip around them (Svatek and van Duin 2001). Separate specimens have been discovered in patches of bamboo forests as well (Bora et al. 2010). The region's climate can be characterized as seasonally wet tropical, without any extreme annual fluctuations of temperature. Occasionally, temperatures below 24°C occur during the winter months, but these are the exception (Svatek and van Duin 2001; Gehring 2020). Over the summer, when most precipitation occurs, temperatures average 27°C (Svatek and van Duin 2001; Gehring 2020). The relative humidity averages ca. 83% over the year and fluctuates from 75–88% over the same period (Svatek and van Duin 2001). *U. henkeli* demonstrate crepuscular and nocturnal activity, while resting during the daytime on branches. It can camouflage with the help of dermal flaps, which, if pressed against the bark, break up the outline of the body and eliminate the shadow (Böhme and Ibisch 1990).

Uroplatus henkeli is a large gecko with overall lengths up to 29 cm (12–19 cm SVL, 7–10 cm tail length). A dermal fringe measuring several millimeters wide runs laterally along the whole body, including the head and legs. The large head is rather flat and terminates in a long, pointed snout (Svatek and van Duin 2001). Males are easy to distinguish due to pronounced hemipenial bulges. Both sexes have pinkish-brown or beige eyes with red spots (Bauer and Russell 1989; Glaw and Vences 2007). The dorsal coloration of this species is light brown with a faint pattern of speckling. Some geckos have more striking patterns that include more melanin or patches of brown and yellow. These geckos can change their coloration depending on the time of day. During the day, they imitate pieces of bark on tree trunks. But they show extremely contrasting patterns at night. Even their eyes become orange or red at night (Allen 1996; Svatek and van Duin 2001; Gehring 2020). There are also many individuals that display aberrant patterns that are sought after among herpetoculture hobbyists. These include geckos with exaggerated white or black speckles, especially on the head, and specimens that demonstrate wide white crossbands along the body vernacularly referred to as "piebalds" or "piebs."

Due to its appearance, *U. henkeli* has become a popular exotic pet and a part of many reptile collections worldwide (Gehring 2020), and the genus is included under Appendix II by CITES (CITES 2021). Legal animals are occasionally available for official export from breeders worldwide. However, smuggled animals do appear in the illegal pet trade (Gehring 2020). Breeding the species under controlled conditions, including for commercial purposes from legal founder stock, is a possible way of contributing to the ex-situ conservation of this species (Mattioli et al. 2006; Smith et al. 2011; Robinson et al. 2015; Marushchak et al. 2022).

In recent years, several new species of *Uroplatus* have been described (Ratsoavina et al. 2012; Ratsoavina et al. 2019; Ratsoaviana et al. 2020). Many aspects of the reproductive biology of this genus are poorly known and empirical data based on significant numbers of animals are missing, although there are some data available based on breeding experience in captivity (Gehring 2020). Such data are usually incidental and generally based on separate reports from different hobbyists. There is still a lack of data concerning the actual duration of gestation in

Fig. 2. *Uroplatus henkeli* female after oviposition while hiding eggs inside the substrate of a moist chamber.

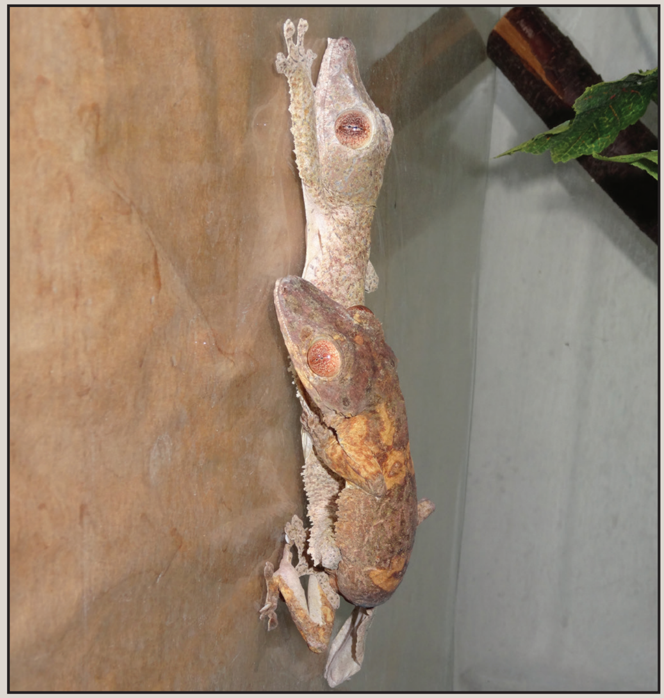

Fig. 3. Copulation of *Uroplatus henkeli.*

females and egg incubation at specific temperatures, and there is no consensus concerning breeding seasons. Research based on numerous representative samples with careful recording and analysis of significant amounts of data in captivity can provide insight into the reproductive biology, systematics, genetics, and the possibility of establishing reserve populations of rare species (Mattioli et al. 2006; Marushchak et al. 2022). In this regard, cooperation between field herpetologists and herpetoculturists has great potential.

In this study with *U. henkeli*, we provide data on reproduction cycles, maximum number of clutches produced by one female per season, and egg incubation at different temperature regimes in order to maximize reproductive success. We present data from the 2020 and 2021 breeding seasons, using the initial legally obtained parental stock that is kept at BION Terrarium Center, Kyiv, Ukraine. The methods used to determine the breeding season, winter dormancy, and egg incubation are reported for laboratory conditions.

Materials and methods

The study took place in a laboratory complex at the BION Terrarium Center, Kyiv, Ukraine. The *U. henkeli* parental stock consisted of 20 mature animals (10 males and 10 females). From the beginning of this experiment, all animals had achieved sexual maturity and were introduced for active breeding beginning in 2014–2015. The animals were legally imported as unsexed yearlings from Madagascar according to valid CITES quotas and relevant export permits. Additionally, nine more captive-bred *U. henkeli* were imported from the Czech Republic. Following an established protocol (Miller 1996), all geckos were quarantined for 30 d. They were treated for parasites with 10% Fenbendazole or Panacur solution at 20–25 mg/kg orally every 7 d, totaling 3 doses, and 0.5% metronidazole at 20 mg/kg orally once. Both solutions were offered to each gecko at ca. two drops and one drop respectively, placing the medicine on the tip of the snout so that the lizard could reflexively lick the medicine from the dropper. The animals were kept individually in conditions identical to those used for the breeding stock, but with minimal decoration (paper towel as substrate, shallow water dish, 1–2 cm thick branches, and an artificial plant as a visual barrier).

The data were collected between 10 March 2020 (moment of forming the first pairs) and 9 March 2022 (hatching of the last gecko). All females that laid at least one egg, whether it was fertile or infertile ("slug"), were considered as entering the reproductive cycle—a period starting from development of oocytes to the very last clutch in the season and hatching of the first babies (Duvall et al. 1982; Williams 2018). After pairs had been formed, the geckos were observed for the first two nights to observe and document copulation.

We observed 48 gestation periods, counted exclusively between clutches of fertile eggs. This counting method was used because when introducing geckos for breeding after wintering, we did not observe immediate copulation in every pair. We did observe that after laying eggs, females often copulated again later the same day. We therefore assume that when breeding pairs are left together, the date of copulation and date of previous clutch are close enough to be considered the same day for the purposes of measuring gestation. We found that females usually laid a slug before fertile eggs were produced. Cases when a female laid a slug after a fertile clutch were not taken into account. Laying a slug means that the female was not gravid with fertilized eggs, therefore the period between first fertile eggs and then slugs was not counted as a period of gestation. At the same time, cases when a slug was followed by a fertile clutch were included into the count. The cases when it was impossible to tell the exact date of laying (slugs looked dried, or a daily check was missed) were not included into the total count. Duration of incubation was determined, based on 61 successful hatching events, as the number of days between oviposition and hatching (Marushchak et al. 2022). The gestation data were recorded and charted using Microsoft Excel 2010 and Statistica version 10.0 (Weiß 2007).

Initial maintenance of the geckos was based on established methods in the literature (Burger 1993; Svatek and van Duin 2001; Meister 2002; Gehring 2020), but upgraded according to the laboratory conditions at BION Terrarium Center (Dubyna et al. 2019) with the aim of maximizing productivity of the available breeding stock. All mature animals were kept in one laboratory with climate control systems to establish a temperature regime that correlated with data from the wild (Svatek and van Duin

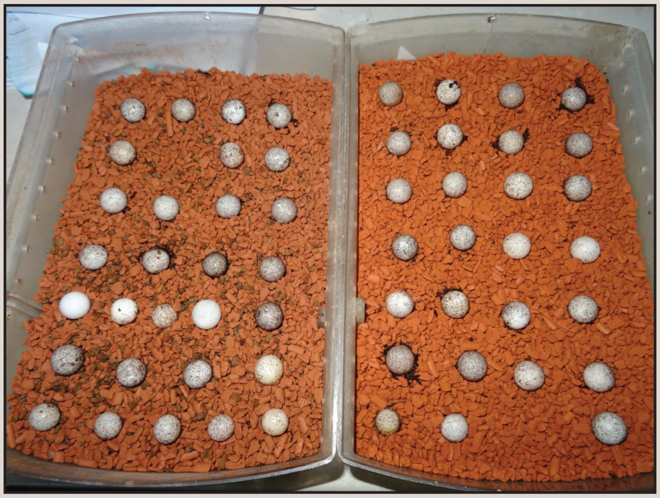

Fig. 4. A sample of fertile eggs laid by *Uroplatus henkeli* females during the 2021 breeding season that were incubated on Seramis substrate.

2001). The animals were kept in glass terrariums that measured 75 × 60 × 70 cm (L × W × H; Fig. 1). Air flow was provided by a screen wire top and a wide band of screen on the lower section of the front of the terrarium. The enclosure was pre-disinfected with Agri'Germ 1000 water solution (Laboratoires Ceetal, France).

To simulate seasonal temperature and humidity fluctuations needed for stimulation of mating behavior and proper development of germ cells, changes to the photoperiod and temperature regimes were made according to the scheme shown in Table 1. Temperatures ranged from 23.0°C at night to 26.0°C during the day. The temperature never exceeded 26.0°C or dropped lower than 23.0°C during the breeding season. A 12–14-h photoperiod was provided using Zoomed T5 fixtures with fluorescent 10.0 UVB tubes connected to a timer. Heating with a 40W incandescent lamp was used only during the winter months or cool weather to maintain daytime temperatures. Humidity levels were maintained at 75–90% with double misting in the morning (0930–1030 h) and evening (1700–1800 h) each day. Enclosure furniture included thick branches, sheets of paper as substrate, a moist chamber (8.0 L plastic box with humid coconut-fiber substrate), and a shallow Petri dish regularly filled with fresh water. The diet consisted of insects, including Two-spotted Crickets (*Gryllus bimaculatus*), House Crickets (*Acheta domesticus*), Jamaican Field Crickets (*Gryllus assimilis*), and Turkestan Cockroaches (*Shelfordella tartara*) of appropriate size. Each gecko was offered 3–5 insects 2–3 times a week, depending on the animals' appetite and physical state. Juvenile Garden Snails (*Cornu aspersum*; 7–10 mm) were offered as a source of calcium for eggs development in *Uroplatus* (Gehring 2020). The snails were available on a regular basis inside the terrarium (Marushchak et al. 2022).

Males and females were placed together in pairs one week before increasing the photoperiod and temperature. Only animals of similar size were paired together since size difference may lead to suppression of smaller individuals (pers. obs.). The minimum weight for females to be introduced into a breeding situation was 60 g. Animals were kept in pairs throughout the breeding season. The geckos were carefully monitored for signs of aggression with subsequent temporary separation of aggressive pairs and selection of appropriate partners.

Eggs were transferred to incubation containers without changing their orientation. If the eggs were found immediately after laying, keepers waited 2–3 h before moving them in order to allow the shells to harden (Dubyna et al. 2019; Marushchak et al. 2022). The eggs were placed in Seramis incubation substrate (Westland Deutschland GmbH, Germany) no deeper than 2/3 the diameter of the egg (Köhler 2005). Air exchange for the eggs was provided by ventilation holes in the side of the container as well as opening the lid every 2 d. The containers were placed in the laboratory with the following incubation temperatures: 26°C during the day with a nighttime drop to 24°C in the 2020 breeding season and 24°C during the day with a nighttime drop to 22°C in the 2021 breeding season. The change in temperatures were made in accordance with the reports published in literature (Gehring 2020) and several relevant Facebook groups.

Results

All females studied entered reproductive cycles, i.e., laid at least one fertile (Fig. 2) or infertile clutch. There were cases when females first laid one unfertilized egg followed by a fertile one in a relatively short period of time. Four cases of such laying were documented in 2020 and one in 2021. The average gap between laying fertile and infertile eggs was 9 d (min = 6, max = 13). On three occasions, females ate infertile eggs soon after oviposition. This is potentially a way of recovering valuable resources (calcium and nutrients from the yolk) for further egg development. This behavior has also been observed in female *U. phantasticus* (Marushchak et al. 2022).

All observed copulations (N = 18) during the two breeding seasons were registered during the first 3–4 h after switching off the lights for the night (Fig. 3). The courtship lasted ca. 20–40 min and included several behaviors from the male: licking the female's body, intensive tail waving, and gentle biting of the female's neck and dermal flaps. The average copulation lasted 27 min (min = 20, max = 36).

A total of 119 eggs were produced from the parental stock (66 in 2020; 53 in 2021), of which 76 (63.87%) were classified as fertile (33 in 2020; 43 in 2021) and incubated (Fig. 4). Remaining eggs were classified as decalcified slugs.

The gestation period for females housed continuously with males, based on 48 exact counts of inter-clutch periods, ranged from 8–57 d, with a mean of 29 d (SD = 10.9). Females can lay more than one clutch during the breeding season, so this figure was counted for each female as well. Average number of clutches per female in 2020 was three (min = 1, max = 4) while in 2021 it was six (min = 2, max = 8).

Sixty-one eggs were incubated successfully (Fig. 6) while fifteen eggs (19.73%) did not hatch as the embryos died inside the eggs with no obvious cause. The average incubation periods in two seasons at different temperature regimes were compared and counted separately (Fig. 5). In the 2020 breeding season, the incubation temperature was 26°C during the day and 24°C at night, resulting in an average incubation period of 117.2 d (min = 107 d, max = 133 d, SD = 6.13). In the 2021 breeding season, the incubation temperature was 24°C during the day and 22°C at night. This resulted in a considerably longer incubation period with an average length of 158.4 d (min = 148 d, max = 174 d, SD = 8.15). According to the results of a two-sample t-test, there was a statistically significant difference between the mean incubation time of eggs incubated at higher temperatures and those incubated at lower temperatures (P = 0.00001; t-value = -21.1493; df = 59).

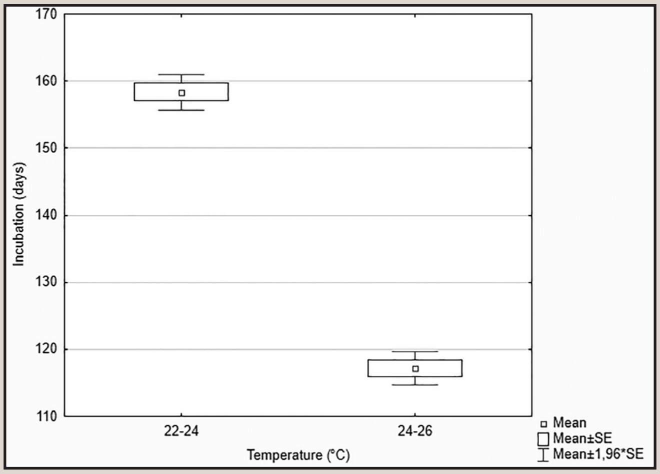

Fig. 5. Duration of incubation periods of *Uroplatus henkeli* at two different incubation regimes.

Fig. 6. *Uroplatus henkeli* hatchlings, 2021.

Discussion

After studying the aspects of *U. henkeli* reproduction in the 2020 and 2021 breeding seasons, we found that maintaining this species in minimalistic laboratory conditions was sufficient for significant breeding success. Providing the correct husbandry parameters (light cycle, UV-light, temperatures, humidity level, terrarium decoration, winter dormancy) enables the animals to breed successfully and in significant quantities (N = 76 fertile eggs obtained from ten females over two breeding seasons). After adding snails to the diet in 2021, the average number of fertile clutches per female doubled (from three clutches in 2020 to six in 2021) as well as the maximum number of clutches produced by a female (from four in 2020 to eight in 2021). The maximum number of clutches previously known from literature was six (Burger 1993; Svatek and van Duin 2001; Gehring 2020). In 2020, the only source of calcium was calcium carbonate dusted on the feeder insects. Snails are a better source of calcium, which is stored in the female's endolymphatic sacs to be used for the proper calcification of eggs. However, these results are preliminary and more detailed studying of the role of snails for female fertility is needed.

Based on the observation that one clutch may consist of an infertile egg (laid earlier) and a fertile egg, we conclude that the development of eggs in *U. henkeli* may not always be simultaneous. While an unfertilized egg is moving through the oviduct the second egg could be fertilized in case of timely mating. The same observation was made earlier for *U. phantasticus* (Marushchak et al. 2022). No cases of sperm storage were observed during the time of observation; however, such cases are known for other species of the genus (Gehring 2020; Marushchak et al. 2022).

Extremely short intervals between fertile clutches consisting of two fully developed eggs each (shortest observed = 8 d) were observed for one female. These cases were considered separate laying sessions as both of them consisted of two fertile eggs. If we observed 1 slug/fertile egg and after 8 d another slug/fertile egg, this was considered a single egg laying session. Similar cases of short intervals between fertile clutches have also been documented for species including *U. phantasticus* (Marushchak et al. 2022), *Uroplatus ebenaui* (Gehring 2020), *Norops sagrei* (Cox and Calsbeek 2010), and *Paroedura picta* (Kubička and Kratochvil 2009). Theoretically, shortening inter-clutch intervals compensates for small clutch size, therefore increasing the summed effort on reproduction as was demonstrated for *P. picta* (Weiser et al. 2012). When kept under optimal conditions, *P. picta* females can have a temperature dependent duration of gravidity of 9, 10, and 15 d at temperatures of 30°C, 27°C, and 24°C, respectively (Kubička et al. 2012). Occasional documentation of such cases can be explained by spontaneous ambient temperature increases induced by outer temperatures in summer that could have resulted in shortening the periods of gravidity. However, this effect should be studied more thoroughly.

Decreasing the incubation temperature resulted in a prolonged incubation period of almost one month. Of 33 eggs in 2020, 24 hatched successfully (72.7%), while in 2021, this figure increased to 37 of 43 eggs (86.1%). It should be taken into consideration that adding snails to the females' diet in 2021 might also be an additional factor that, combined with the changed temperature regime, might have affected gravidity rates, number of eggs laid, and hatch rates. However, more studies are needed to figure out potential role of females' diet on different aspects of incubation.

According to the previous observations at BION Terrarium Center and breeding results from these two highlighted years, it is estimated that stable large-scale breeding of *U. henkeli* is possible with a more utilitarian approach and minimal laboratory equipment that does not appear to negatively impact the animals and still meets their biological needs. Currently, this species is fully established in herpetoculture worldwide and results obtained from BION Terrarium Center's collection have made a significant contribution to the establishment of ex situ populations of *U. henkeli* geckos. Current progress of herpetoculture in Europe, Japan and North America has created a growing trend for captive-bred specimens of reptiles to meet the needs of the pet trade (Robinson et al. 2015). Thanks to its interesting appearance, coloration, morphs, and behavioral patterns, *U. henkeli* has settled into its niche among hobbyists who are passionate about arboreal geckos. Consequently, forming stable breeding groups of genetically diverse ex situ populations is an effective way to preserve the species in captivity. Understanding the aspects of the reproductive cycles of these geckos will help to produce healthy, well-adapted animals for breeding centers, zoos, and responsible breeders. In turn, this may enable the creation of assurance populations for possible reintroduction to the wild.

Preserving wild populations of *U. henkeli*, especially in nature reserves, is the most important goal for the long-term conservation of this species. Legal, mass reproduction in captivity in certified breeding centers and zoos can be an effective way of conducting scientific research and combating smuggling. The ongoing *U. henkeli* project at BION Terrarium Center is an excellent example of an ex situ approach to creating a sustainable captive population.

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The Effects of Tetany Syndrome on the Reproductive Success of *Atelopus zeteki* at the Maryland Zoo in Baltimore

Atelopus zeteki is a montane species from the highlands of central Panama and is listed as critically endangered by the IUCN (IUCN 2019). Like many other amphibian species in Central America, wild *A. zeteki* populations were decimated by *Batrachochytrium dendrobatidis* (*Bd*), the pathogenic fungus that causes chytridiomycosis (Poole 2006; Crawford et al. 2010; IUCN 2019). By the late 1990s, *Bd* rapidly spread throughout Central America causing massive declines in other highland members of the genus *Atelopus* including *A. senex, A. chiriquiensis,* and *A. varius* (Gagliardo et al. 2008; DiRenzo et al. 2014; Lewis et al. 2019). In 1999, Project Golden Frog, a collaboration between zoological facilities and amphibian biologists, was formed as a proactive response to the potential threat of extinction to *A. zeteki* (Zippel 2002; Gagliardo et al. 2008). Project Golden Frog was established to conduct field research, in situ/ex situ captive management, and educational programs (Poole 2006). Fourteen amplexed pairs along with 12 unsexed individuals from two different localities, Mata Ahogado and Sorá, were exported from Panama to the Maryland Zoo in Baltimore (MZiB) to establish an assurance colony with the main goal of reproducing the species in human care and maintaining genetic diversity to eventually reintroduce frogs back to Panama (Poole 2006; Bronson et al. 2021). The MZiB currently houses approximately 615 individuals, which is the largest collection of breeding *A. zeteki* outside of Panama (Eustace et al. 2018).

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