

# Endolymphatic Sac Use and Reproductive Activity in the Lesser Antilles Endemic Gecko *Gonatodes antillensis* (Gekkota: Sphaerodactylidae)

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## ABSTRACT

Geckos are among the most diverse radiations of lizards; however, the lack of baseline natural history data on the reproductive biology for many species creates a challenge for predicting their long-term persistence. This study aims to fill a gap in our understanding of the reproductive biology of an enigmatic nocturnal gecko endemic to the islands of Curaçao and Bonaire: *Gonatodes antillensis*. Using radiographs of specimens from natural history collections, we conduct the first investigation of the potential role of endolymphatic sacs in the reproduction of the species. We find that females have larger endolymphatic sacs than males, with further quantification of endolymphatic sac sizes between females with or without visible eggs supporting the hypothesis that calcium stores are built up in the early reproductive phase and depleted during the development of the egg. Additionally, we combined data on endolymphatic sacs sizes with examinations of gravidity to expand the known reproductive interval of the species into fall and winter months and suggest the possibility of year-round reproduction. Along with providing baseline data, our findings raise a new conservation concern for the species. The spread of the invasive gecko *Hemidactylus mabouia* has resulted in a notable decline in the abundance of *Gonatodes antillensis* across its native range. This decline has been attributed to *Hemidactylus mabouia* acting as both a competitor and possible predator of *Gonatodes antillensis*. However, stress can inhibit calcium uptake in endolymphatic sacs, and these findings raise the possibility that *Hemidactylus mabouia* may also be indirectly affecting the reproductive success of this species.

## KEYWORDS

*Hemidactylus mabouia*, calcium gland, stress, physiology, sexual dimorphism, natural history, nocturnal, invasive, conservation, ecology

## Introduction

With more than 1,600 species, geckos (Gekkota) represent one of the most species-rich and arguably evolutionarily successful clades of living lizards (Gamble et al. 2011). As geckos occupy

habitats that span tropical forests (Vitt et al. 2005; Raxworthy et al. 2008; Leaché and Fujita 2010), alpine mountains (McFarlane 2007; Comas et al. 2014) and arid deserts (Pianka 1986; Watkins-Colwell et al. 2010; Gamble et al. 2012; Collins et al. 2015), their ability to overcome novel repro-

ductive challenges has been a pivotal feature underlying their evolutionary radiation. For example, changes in climate (Lourdais et al. 2004; Shine 2005; Brandt and Navas 2011), fluctuating prey resources (Andrén and Nilson 1983) or the availability of key nutrients (Evans et al. 2015) represent just a few of the potential challenges to successful reproduction faced by gecko lineages colonizing new adaptive zones. Recent phylogenetic investigations suggest many changes in reproductive biology over evolutionary time that correspond to geckos adapting to environmental or ecological challenges. Such changes include transitions between viviparity and oviparity (Cree et al. 2003; King and Lee 2015), changes in the frequency of reproduction (Cree and Guillette 1995; Ibargüengoytia and Casalins 2007), sexual and asexual reproduction (Murakami et al. 2015), body size (Vitt 1986; Doughty 1996, 1997) and also the co-option of existing anatomical structures as energy or nutrient reservoirs (Dial and Fitzpatrick 1981; Bauer 1989). Unfortunately, this high level of reproductive diversity now poses a challenge for predicting the conservation needs of species facing environmental change, urbanization, or threats from nonnative species.

For many species with documented population declines, very little is known about even basic aspects of their reproductive biology. A lack of baseline natural history data impedes successful evaluation of whether factors such as changes in environmental regimes (Gibbons et al. 2000; Böhm et al. 2016; Mitchell et al. 2016), invasions by nonnative species (Case and Bolger 1991; Short and Kenneth 2012; Smith, Cogger et al. 2012) or habitat alteration (Luiselli 2006; Swihart et al. 2006; Zeng et al. 2014) are having a negative impact on the reproductive success of a species. In egg-laying geckos, investigating how such factors can potentially interfere with egg development represents a fundamental requirement for effective conservation planning or management. However, assessing the reproductive requirements for many sphaerodactylid (217 species; Uetz et al. 1995–2016) and gekkonid (1,078 species; Uetz et al. 1995–2016) species is complicated by adult females utilizing modified endolymphatic sacs for calcium storage in order to meet the demands associated with egg formation (Bauer 1989; Ineich and Gardner 1989). Although many other reptile species use endolymphatic sacs as storage

reservoirs of calcium obtained from the egg for posthatch juvenile growth (Stewart and Ecy 2010), the secondary use of these organs for calcium storage in reproductively active adult females is particularly pervasive across these two clades (Kluge 1987; Bauer 1989). Environmental stress can interfere with calcium uptake in the endolymphatic sacs of these lineages (Brown, Osborne et al. 1991; Brown, Jensen et al. 1996), thereby potentially lowering calcium content in the yolk for the developing embryo or weakening eggshell formation. Either of these factors could result in lowering the reproductive success and connectivity of populations across a landscape. Given that patterns of endolymphatic sac use for egg formation are heterogeneous between species (Kluge 1987; Bauer 1989), case studies are required to determine which species are at risk for decreased eggshell quality and hatchability.

In this study, we examined several aspects of the reproductive biology of the Antillean gecko *Gonatodes antillensis*, a nocturnal species endemic to the islands of Bonaire and Curaçao (van Buurt 2004, 2006). Using radiographs of specimens, we quantified the total projected area of the endolymphatic sacs in female and male *G. antillensis* individuals to evaluate the potential for sexual size dimorphism of mineral deposits. We further quantified area differences in the endolymphatic sacs of gravid females, females lacking a visible egg and males to investigate the role that endolymphatic sacs may play in egg formation. Finally, we used additional specimens to assess temporal patterns in reproductive activity for the species. As the past several decades have yielded a notable decline in the abundance of *G. antillensis* across many portions of its native range (Hughes et al. 2015), our study provides a new axis of baseline data critical for the planning and implementation of long-term conservation initiatives of this unique sphaerodactylid gecko.

## Materials and Methods

The projected area of endolymphatic sacs was quantified from radiographs of specimens stored at the Peabody Museum of Natural History, Yale University, New Haven, Connecticut, USA (YPM;  $n = 47$ ), and the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA (MCZ;  $n = 32$ ), using the measurement

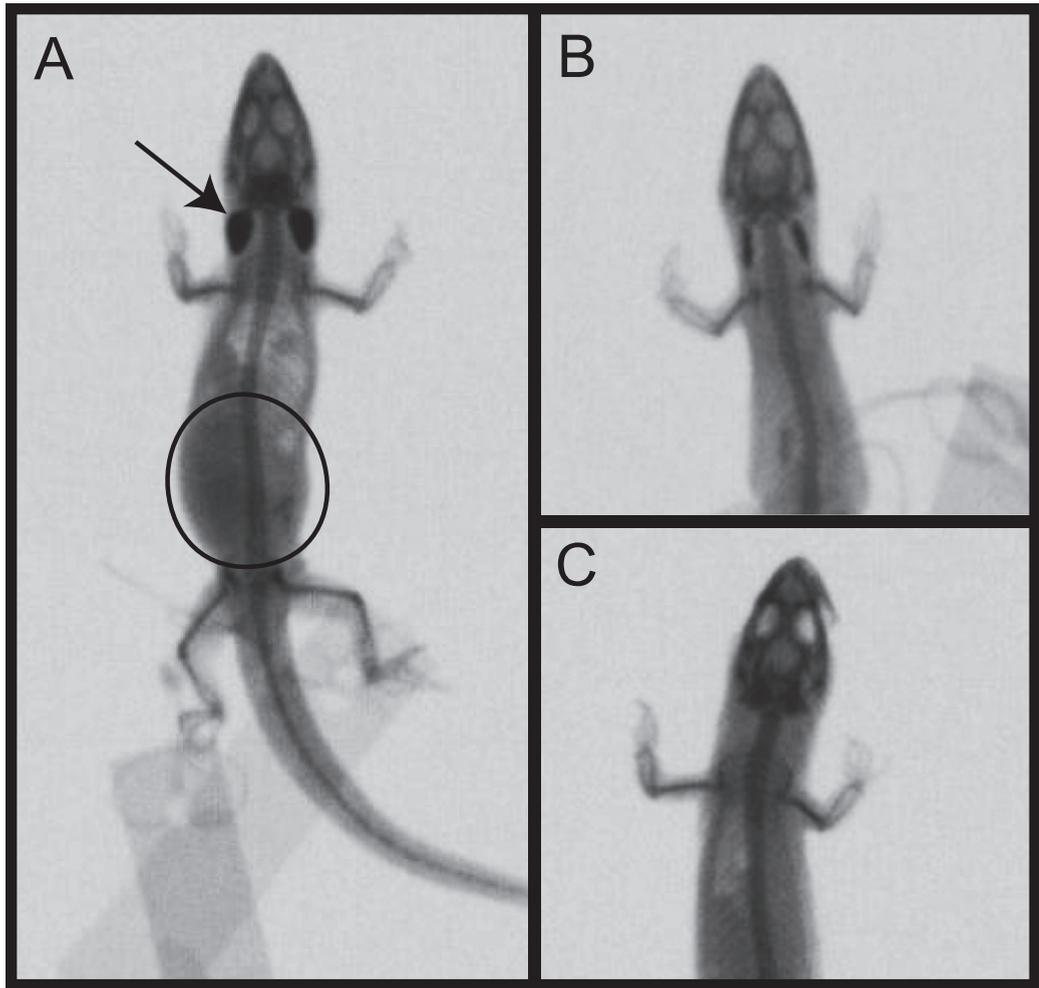


FIGURE 1. Radiographs of *Gonatodes antillensis* specimens. **A**, Gravid female with a visible egg (circle) and large endolymphatic sacs indicated by arrow. **B**, Female with small endolymphatic sacs. **C**, Male with no visible endolymphatic sacs.

software ImageJ (Broeke et al. 2015). Both the left and right endolymphatic sac projected areas were measured from the radiographically dense portions of the sac and then averaged for each specimen when visible. A projected area measurement does not account for changes in endolymphatic sac depth and, therefore, the total volume of calcium stored. However, endolymphatic sacs are filled with a calcium-rich fluid that should accumulate in a similar manner across individuals. Differences in area are thus expected to be reflective of relative changes in the amount of calcium stored. Individuals lacking visible endolymphatic sacs received a projected area score of zero.

Although it is possible that small amounts of calcium could accumulate within the intracranial portions of the endolymphatic ducts (Bauer 1989), the extracranial endolymphatic sacs can exceed the volume of the entire skull in some gecko species (Bauer 1989), making an assessment of endolymphatic sac projected area an appropriate metric.

Sphaerodactylids lay only a single egg per clutch (Fitch 1970; Vitt 1986; Pike et al. 2012; Winck and Rocha 2012), and gravidity was determined by the visible presence or absence of an egg in female specimens (Figure 1). Although the resolution of obtained radiographs prohibited detec-

tion of early stages of development prior to eggshell formation, the use of calcium stored in the endolymphatic sac should be minimal at this stage. This strategy, therefore, allowed us to nondestructively sample specimens while still assessing general trends of endolymphatic sac usage between males and females. To quantitatively assess differences in the total endolymphatic projected area between gravid females (GF), females with no visible eggs (NVE) and males (M), violin plots were constructed using the package *vioplot* (Adler 2005) in the R programming language. Violin plots add a rotated kernel density plot to a traditional box plot, allowing for simultaneous visualization of quartiles and the underlying probability distribution of the data (Hintze and Nelson 1998). To test for differences between groups, we conducted an analysis of variance (ANOVA) with a Tukey honest significance difference (HSD) using M, GF, and NVE as groups. We additionally quantified male and female cranial sizes using violin plots and tested for differences between the two groups using ANOVA to account for the possibility that endolymphatic sac area differences were driven by sexual size dimorphism. Analyses were repeated using a Mann-Whitney-Wilcoxon test to account for deviations from normality in the data. Because of the contortion of some preserved specimens, head length was measured from the tip of the snout to the base of the occipital condyle and was used as a representative measure for body size.

Box plots depicting the median, first and third quartiles and 95% confidence interval of female endolymphatic sac areas were visualized using R in order to assess monthly trends in reproduction. To predict the upper limit of endolymphatic sac area in months with no data, we quantified both a 95% confidence interval and a 90% prediction interval based on a linear regression of maximum endolymphatic size against month. Given the limited number of data points (6 months), a comparison of both types of intervals provides a means of assessing the possibility of annual endolymphatic sac projected areas in female individuals that exceed those observed in males. Further, this approach provides a baseline model of possible endolymphatic sac projected areas that can be refined by future sampling efforts.

Data from radiographed female specimens were augmented by dissections of *Gonatodes antillensis* individuals that were field collected from the

island of Curaçao between June and July of 2016. Collected females were dissected and visually inspected for the presence of an egg. Egg presence or absence based on dissected and radiographed specimens was plotted by month against mean temperature and precipitation for Curaçao from 1978 to the present day to assess the potential for reproductive seasonality (Lawrimore et al. 2011). After dissection, collected specimens were deposited at the North Carolina Museum of Natural Sciences, Raleigh, North Carolina, USA (NCSM;  $N = 38$ ; Appendix). All reproductive data collected from specimens were archived on Zenodo (<http://doi.org/10.5281/zenodo.242688>).

## Results

Radiographs of *Gonatodes antillensis* demonstrated that males possess less variance in endolymphatic sac area than both gravid females and females lacking visible eggs (Figure 2A). This reduced variance corresponded with a decrease in total endolymphatic sac projected area, with M having a mean area of 1.106 mm<sup>2</sup> (Table 1). In contrast, GF and NVE had much higher total projected area means, measuring 5.427 mm<sup>2</sup> and 5.204 mm<sup>2</sup>, respectively. Total projected area of GF and NVE endolymphatic sacs broadly overlapped, with both one GF and several NVE specimens showing no visible calcium sacs. An ANOVA supported significant between-group variation ( $F = 24.87$ ,  $p \leq 0.001$ ) with a Tukey HSD on total endolymphatic sac area strongly supporting a significant difference between males and both GF ( $p \leq 0.001$ ) and NVE ( $p \leq 0.001$ ). Results from a Mann-Whitney-Wilcoxon test supported significant differences between males and females ( $p \leq 0.001$ ). In contrast, GF and NVE showed no significant differences ( $p = 0.966$ ). Differences in projected endolymphatic sac areas between males and females were likely not driven by sexual size dimorphism in cranial size, as males and females overlapped in measured head lengths (Figure 2B, Table 1) with an ANOVA not supporting any significant mean differences ( $F = 0.14$ ,  $p = 0.79$ ). Regressions of endolymphatic area against head size also did not support endolymphatic sac areas to be correlated with head length when sexes were pooled ( $r$ -squared = 0.032,  $p = 0.11$ ). Combined, these results correspond to the expectations of the hypothesis that female *G. antillensis* uses modified

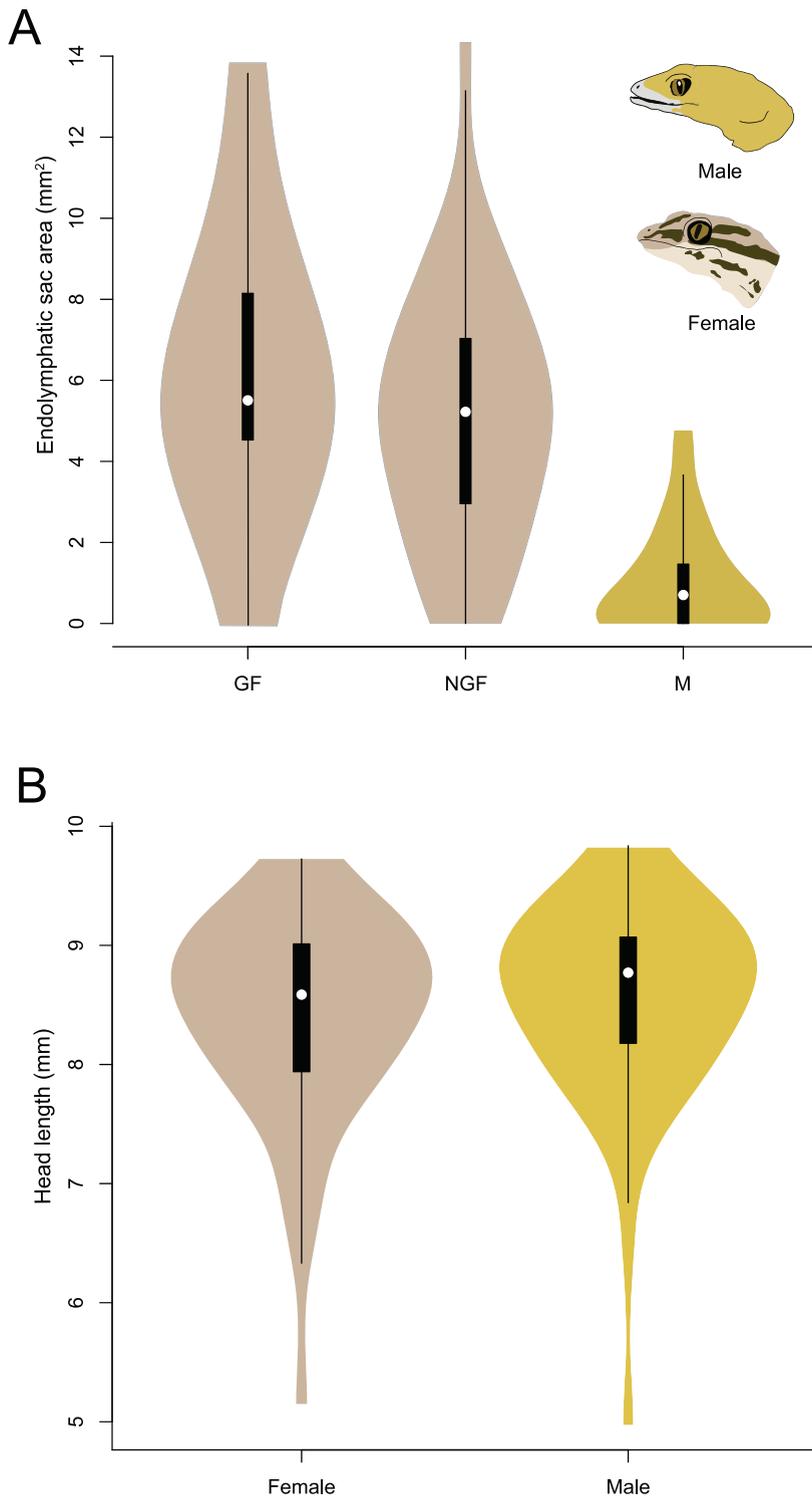


FIGURE 2. Violin plots with embedded box plots depicting the first, second (median) and third quartiles. **A**, Endolymphatic sac size area (mm<sup>2</sup>) for gravid females (GF), females with no eggs (NVE) and males (M). **B**, Head length (mm) for females and males.

TABLE 1. Summary statistics of endolymphatic sac area. *Abbreviations:* N, sample size; SD, standard deviation.

Endolymphatic sac area	N	Minimum	Maximum	Mean	SD	Quartile		
						25%	50%	75%
Gravid female (GF)	12	0.000	13.835	5.427	3.860	3.122	5.067	7.106
Female no visible egg (NVE)	34	0.000	14.337	5.204	3.060	3.056	5.239	7.094
Male	33	0.000	4.763	1.016	1.335	0.000	0.073	1.466
Cranial length (CL)								
Female	46	5.150	9.726	8.418	0.868	7.940	8.587	9.012
Male	33	4.959	9.837	8.494	0.924	8.178	8.771	9.070

endolymphatic sacs as calcium reservoirs for eggshell formation.

Visualization of mean precipitation or temperature ( $\pm 1$  standard deviation) revealed little seasonal variation (Figure 3A). Average precipitation varied slightly ( $\sim 2.5$  cm) across months, and average temperature differed by approximately  $2^\circ\text{C}$  between summer and winter months. Our survey provided evidence of gravid females in 5 of the 6 months sampled, with February and July yielding the highest number of visibly gravid females relative to overall collected specimens. Plotting endolymphatic sac projected area by month revealed large projected areas for females in all months surveyed (Figure 3B).

## Discussion

This study provides new insights into the reproductive biology of *Gonatodes antillensis* and represents the first investigation of the potential reproductive role of endolymphatic sacs of any species of *Gonatodes*. Our analyses support the use of these structures as mineral stores for egg development, revealing that adult females have a substantially larger range of endolymphatic sac sizes than males. The large endolymphatic sacs found in both gravid females and females lacking visible eggs are in line with previous research in other gecko species, and this congruence supports the hypothesis that calcium stores are built up in endolymphatic sac prior to, or in the early stages of, egg formation and depleted during the development of the egg (Kluge 1987). Additionally, our survey of reproductive activity patterns in the species expands the known reproductive interval of the species, suggesting the possibility of year-round reproduction. Together, our results contribute valuable new baseline natural history data

for the species and highlight additional areas of conservation concern and management for this and other gekkonid and sphaerodactylid species that utilize this method of calcium storage.

The role of endolymphatic sacs as mineral stores has been documented to aid supplying calcium during times of need in both embryonic and adult reptiles (Ruth 1918; Jenkins and Simkiss 1968; Packard and Packard 1988; Allen et al. 1993). Reptilian egg yolks generally contain calcium levels higher than those of other vertebrate eggs, and calcium from the yolk or egg shells that are ingested posthatch is stored in the endolymphatic sacs of hatchlings to assist in early juvenile development (Stewart and Ecaý 2010). Reproducing females belonging to several gekkonid and sphaerodactylid species are faced with an additional calcium demand, the development of highly calcareous, rigid eggshells (Kluge 1967; Bustard 1968; Werner 1972; Kratochvil and Frynta 2006; Pike et al. 2012). In these lineages, the evolution of rigid-shelled eggs from a parchment-shelled ancestor has been hypothesized to provide protection from desiccation and pathogens and has been highlighted as a major innovation facilitating diversification (Pike et al. 2012). However, this evolutionary innovation places a high calcium demand on reproducing females. Given the limited supply of calcium that can be stored within the body at one time, meeting this demand requires females of at least some species to utilize endolymphatic sacs for calcium supplementation (Bauer 1989; Russell and Bauer 2002).

We found endolymphatic sacs in both sexes of *Gonatodes antillensis*, with significantly larger endolymphatic sac projected areas in adult females (Figure 2A). The variation of endolymphatic sac projected areas found across female *G. antillensis* is in line with the expectation of cal-

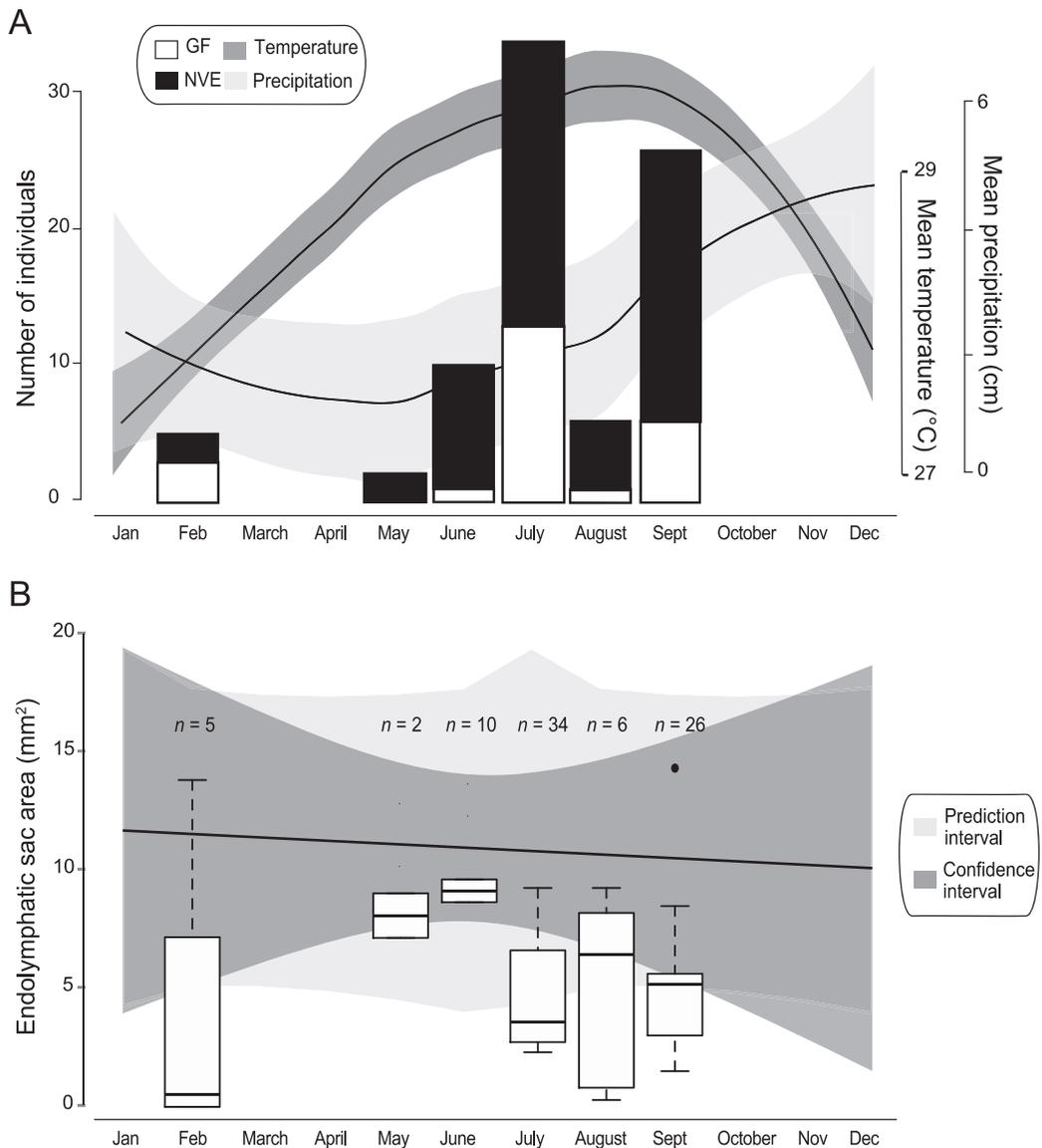


FIGURE 3. Reproductive activity patterns of *Gonatodes antillensis*. **A**, Histogram showing the total number of female specimens (both gravid [GF] and those with no visible egg [NVE]) collected per month, plotted against monthly temperature and precipitation (solid lines correspond to the mean of each climatic variable; shaded regions correspond to the standard deviation of the climatic data by month). **B**, Box-and-whisker plots showing median, first and third quartiles of female endolymphatic sac areas ( $\text{mm}^2$ ) plotted by month. Shaded regions correspond to the 95% confidence interval and 90% prediction interval of maximum female endolymphatic sac size by month.

cium cycling that has been observed in lizard species utilizing endolymphatic sacs. In these species, calcium content is highest prior to the formation of the eggshell and declines variably as the shell is formed depending on the degree of individual supplementation (Ineich and Gardner 1989; Brown et al. 1996). Examining the mineral

content in the endolymphatic sacs compared with the size and mineral content of eggs in gravid females would advance the understanding of reproductive physiology of geckos; however, further analyses are needed to formalize this relationship. Our analyses suggest that that these structures are also used to a lesser degree in male

individuals. However, at this time it is unclear as to how male *G. antillensis* are using stored calcium and what importance calcium stores may serve to the physiological functions of male individuals.

Our quantification of endolymphatic sac size also provides a new perspective on patterns of reproductive activity for *Gonatodes antillensis*. To date, the study of reproductive activity patterns in this species has been limited. Based on field-collected data during the month of June, Bennett and Gorman (1979) found evidence of both recent hatching and gravid females. However, the absence of juveniles with snout-vent lengths between 21 and 32 mm led the authors to conclude that reproduction is seasonal within the species (Bennett and Gorman 1979). Our radiographs do not conform to these expectations of seasonality and instead depict a consistent trend of enlarged endolymphatic sac projected areas in most female specimens, regardless of the month collected (Figure 3B). Predicting the maximum endolymphatic sac projected area based on linear regressions further suggests that regardless of high uncertainty in area values, individual females with calcium stores exceeding the maximum levels observed in males (Figure 2A) are likely present year-round (Figure 3B). The small winter sample sizes in this study prohibit a detailed answer to the extent of annual *G. antillensis* reproductive activity patterns. However, our analyses of endolymphatic sac size demonstrate one necessary prerequisite condition for year-round reproduction. Further, our documentation of gravid females in both fall and winter (Figure 3A and B) expands the known timing of reproductive activity in this species.

Our finding of reproductively active *Gonatodes antillensis* females throughout much of the year (Figure 3A) is consistent with the observation that many gekkonid and sphaerodactylid species tend to deposit multiple clutches throughout the reproductive season (Vitt et al. 2000; López-Ortiz and Lewis 2002; Ramírez-Sandoval et al. 2006; Serrano-Cardozo et al. 2007). Although the results of our study do not rule out the possibility of bursts of seasonal reproduction, such changes in gecko reproductive activity patterns are often attributed to shifts in environmental conditions such as temperature oscillations (Bustard 1968; Parker 1972; Ota 1994; Sakai 2016)

or shifts between wet and dry seasons (Miranda and Andrade 2003; Vences et al. 2004). Given the minimal fluctuations in both temperature and precipitation in Curaçao (Figure 3A), year-round reproductive activity is not unlikely. Further studies filling the sampling gaps of our study are needed to test this hypothesis and to create a robust baseline model of reproductive activity and range of endolymphatic sac sizes in *G. antillensis*. As even small changes in climate can alter the timing or frequency of reproduction in ectotherms (McKay and Phillips 2012), climatic change predictions (Karmalkar et al. 2011) suggest such data will be essential for assessing changes in this species' reproductive behavior over the next century.

#### *Stress, Calcium Storage, and the Conservation of Gonatodes antillensis*

Species that utilize endolymphatic sacs for reproduction face a significant challenge to their continued persistence: stress. Experimental studies have shown stress to inhibit calcium uptake in endolymphatic sacs, even when supplemented with calcium-rich diets (Brown, Osborne et al. 1991; Brown, Jensen et al. 1996). Sources of stress for geckos native to the islands of Aruba, Bonaire, and Curaçao are representative of stress factors faced by geckos worldwide, such as habitat alteration (Gibbons et al. 2000; Smith, Bull and Driscoll 2012; Dayananda et al. 2016), urbanization (McKinney 2006; Hamer and McDonnell 2010) and the introduction of exotic species (Case et al. 1994; Cole et al. 2005; Newbery and Jones 2007; Zozaya et al. 2015; Williams et al. 2016). How much impact these, and other, potential stress factors have on *Gonatodes antillensis* remains unclear. For example, strong selective pressures associated with urbanization have been found to drive rapid evolution as lineages adapt to changes in habitat composition and prey base accessibility (Donihue and Lambert 2015; Winchell et al. 2016). Whether *G. antillensis* is adapting to the urban landscape or is bearing a cost to calcium uptake in urbanized settings remains unknown. However, regardless of uncertainty in how urbanization and habitat alteration place stress on *G. antillensis* populations, there is a growing body of evidence suggesting that the presence of the nonnative predators and competitors is a significant source of stress to the species

(van Buurt 2004, 2006; Dornburg, Warren et al. 2011; Hughes et al. 2015; Dornburg, Lippi et al. 2016).

Across the islands of Aruba, Bonaire and Curaçao, population declines of native geckos have been repeatedly attributed to the presence of the nonnative tropical house gecko, *Hemidactylus mabouia* (van Buurt 2004, 2006; Dornburg, Warren et al. 2011; Hughes et al. 2015; Dornburg, Lippi et al. 2016). For *Gonatodes antillensis*, population declines have been attributed to *H. mabouia* acting as both a competitor (Hughes et al. 2015) and an opportunistic predator (Dornburg et al. 2011). Given that stress from dominance interactions alone has been shown to lower levels of calcium storage in endolymphatic sacs (Brown et al. 1991), our findings of endolymphatic sac use in *G. antillensis* suggest that *H. mabouia* may indirectly affect successful reproduction of *G. antillensis*. Investigating this as a potential factor for population declines of *G. antillensis* is important for the conservation of the species as well as the conservation of tropical geckos globally. *H. mabouia* has invaded large portions of the New World (Baldo et al. 2008; Rödder et al. 2008; Torres-Carvajal 2015), and the presence of this aggressive species has been linked to the displacement or decline of many native and nonnative gecko species throughout its invaded range (Meshaka 2000; Short and Kenneth 2012; Telles et al. 2015; Williams et al. 2016). Likewise, introductions of other species of *Hemidactylus* on Pacific islands have had similar effects on the native gecko fauna (Petren and Case 1996, 1998; Cole et al. 2005; Newbery and Jones 2007). Future laboratory and field-based studies of endolymphatic sac use in the presence of *H. mabouia* or other invasive *Hemidactylus* species represents an unexplored, yet potentially crucial axis of investigation if we are to successfully predict changes in gecko population dynamics in the wake of *Hemidactylus* invasions.

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**Appendix**  
**Museum Catalog Numbers**  
**of Specimens Used**

*Abbreviations:* MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; NCSM, North Carolina Museum of Natural Sciences, Raleigh, North Carolina, USA; YPM HERR, Division of Vertebrate Zoology Herpetology Collection (Reptiles), Peabody Museum of Natural History, Yale University, New Haven, Connecticut, USA.

Specimen type	YPM	MCZ	NCSM
Gravid female (GF)	YPM HERR 018447, 018196, 017582, 018606, 018451	R-149371, R-141593, R-82969, R-82974, R-69584, R-69592, R-69587	89405, 89424, 89427, 89433, 89436, 89438, 89441, 89442, 89445, 89446, 89447, 89452
Female no visible egg (NVE)	YPM HERR 018642, 018455, 018459, 018197, 018440, 018189, 017583, 018438, 018643, 018191, 018453, 018442, 017584, 018458, 017581, 018641, 018188, 018605, 018439, 018198, 018640, 018456, 018441, 018644, 018603, 018460	R-149369, R-82971, R-69583, R-69581, R-81514, R-27548, R-82979	89384, 89386, 89387, 89491, 89394, 89395, 89396, 89397, 89402, 89403, 89404, 89406, 89407, 89408, 89409, 89410, 89411, 89412, 89420, 89421, 89426, 89428, 89429, 89432, 89434, 89448
Male (M)	YPM HERR 018445, 018194, 018190, 018443, 018449, 018604, 017580, 018452, 018193	R-149370, R-149268, R-149367, R-141600, R-82968, R-82973, R-69586, R-69580, R-60579, R-69577, R-69596, R-69597, R-82976, R-82978, R-149349, R-149351	89452, 89385, 89390, 89392, 89393, 89398, 89399, 89400, 89401, 89422, 89423, 89425, 89430, 89431, 89435, 89437, 89419, 89439, 89440, 89443