Herpetological Review, 2008, 39(1), 58–63.

Laboratory Protocols for Husbandry and Embryo Collection of *Anolis* Lizards

THOMAS J. SANGER*
PAUL M. HIME
MICHELE A. JOHNSON
JACK DIANI
Washington University in St. Louis, Department of Biology
Campus Box 1229, St. Louis, Missouri 63130, USA

and
JONATHAN B. LOSOS
Department of Organismic and Evolutionary Biology
Harvard University, Cambridge, Massachusetts 02138, USA

**Corresponding author; present address:*
Department of Organismic and Evolutionary Biology
Biological Laboratories, 16 Divinity Avenue, Office 4110
Harvard University, Cambridge, Massachusetts 02138, USA
e-mail: TSanger@oeb.harvard.edu

Anolis lizards, or anoles, are a model system for evolutionary biology (e.g., Emerson 2002; Losos 1994), behavioral and physiological ecology (e.g ., Huey et al. 2003; Irschick and Garland 2001; Lovern et al. 2004; Stamps 1983), community ecology (e.g., Pacala and Roughgarden 1985; Schoener 1968), toxicology (e.g., Burger et al. 2004), and physiology and neuroendocrinology (e.g., Greenberg 2003; Wade 2005). Because the genome of *Anolis carolinensis* has recently been sequenced (http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/GreenAnoleLizardAmericanAlligatorSeq.pdf), work on this genus is expected to expand as it becomes more accessible to new disciplines such as developmental and statistical genetics, comparative genomics, and the biomedical sciences. For this reason it is imperative that methods for working with *Anolis* be developed to facilitate comparisons between studies and assure the ethical treatment of these animals as they are used by researchers not accustomed to working with reptiles.

The goal of this manuscript is to describe methods for the maintenance of captive breeding colonies of *Anolis* species. The general characteristics of a species that must be considered to maintain healthy breeding populations include its availability (in the wild or the pet trade), physiological needs (e.g., temperature, humidity, nutrition), and sociality (density of housed individuals) (Greenberg 1992). These factors also must be independently considered for the care of juveniles to assure their proper growth and development. In addition to species-specific factors, practical limitations such as available space, number of species to be housed together, and available resources (incubators, cage washers, climate control systems, etc.) must be considered when developing protocols for the maintenance of animals in captivity. These likely vary greatly from institution to institution. A broad perspective on the general use of reptiles for research can be found in Greenberg (1992) and Pough (1991) and on reptilian egg incubation in Deming (2004).

Here we describe the detailed methods we have found to be successful for the care and maintenance of 13 *Anolis* species from southern Florida and four Caribbean islands that inhabit a wide

range of microhabitats. While we have worked primarily with *A. sagrei*, *A. carolinensis*, and *A. cristatellus*, we also have experienced success breeding *A. chlorocyanus, A. coelestinus, A. cybotes, A. distichus, A. evermanni, A. grahami, A. gundlachi, A. krugi, A. lineatopus*, and *A. valencienni*. Lovern et al. (2004) provided protocols for *A. carolinensis*, the northernmost species of the genus. We have modified these protocols to fit the needs of an additional 12 species. The methods we describe here are readily amenable to larger-scale laboratory settings where large numbers of animals from different species are maintained. We successfully raised all species through the F1 generation except for *A. cristatellus*, which we raised through the F2 generation. We developed these methods under the guidance of Washington University's animal facilities management (J. Diani), veterinary services, and the Institutional Animal Care and Use Committee. Based on explicit calculations of the effort and expense needed to care for these lizards (space, lighting, climate control, basic daily care, etc.), we were charged $0.16 per lizard per day to maintain these lizards in our animal care facility.

The key factor in developing protocols for the maintenance of captive breeding colonies is an understanding of a taxon's natural history. Anoles are remarkably variable in their natural history, ecology, behavior, and physiology. They inhabit a broad variety of habitats from hot xeric semi-deserts to cool montane rainforests, ranging from the equatorial forests of South America throughout the Caribbean Islands and into southeastern North America. Caribbean anoles, including populations found in the southern United States, breed from early spring through late-summer depending on the temperature and humidity of a given year (Licht and Gorman 1970). Mean field body temperatures range from 21.0–33.0°C (Clark and Kroll 1974). Some species narrowly regulate their body temperature by basking, whereas others are thermoconformers; furthermore, within a species, mean body temperature changes with altitude and season (Hertz 1981; Hertz and Huey 1981; Huey and Webster 1976; Ruibal 1961). Although fewer field data are available, similar observations are made regarding interspecific variation among anoles in their hydric environments (Hertz 1980, 1992), size at reproductive maturity, hatchling size, and growth rate (Kobayashi et al. 1983; Michaud and Echternacht 1995). In some cases, physiological differences observed among populations or even species may represent phenotypic plasticity, rather than genetic differences (e.g., Wilson and Echternacht 1987, 1990). Given these many and varied differences, there is no one-size-fits-all prescription for caring for and raising anoles; rather, to the extent possible, husbandry needs to be tailored to the biology of each species.

For the following discussion, it is important to note that many of the species we have maintained in captivity occur primarily in open, sunny habitats (but see Interspecific Variation below). Such species often thermoregulate actively and maintain relatively high body temperatures; moreover, they tend to be less sensitive to water loss than many other anoles (Hertz 1981, 1980, 1992). Because the species we have used in our research naturally occur in the Caribbean and southeastern United States, we chose mean conditions for humidity, moisture, and light based on climatic data in those regions for the months of peak breeding, April to June (for Florida and southeastern United States: Hamlett 1952, Lee et al. 1989, TJS pers. obs.; for the Caribbean: Jenssen and Nunez 1994).

Husbandry and Egg Collection

We have collected adults of each species from the wild or, for species found within the United States, purchased them from professional reptile vendors. Select species are also available from the pet trade; however the history and genetics of these individuals are not typically known. Occasionally, new animals in the lab do not breed or deposit eggs at first, but after two weeks, individuals generally show no visible signs of distress (as described in Greenberg 2002, 2003) and breed regularly. Wild-caught anoles commonly host parasitic mites, often found on the dewlap, groin, base of the tail, or axillae. Although specific host-parasite relationships likely exist between mite and lizard species from the same island, lizards may be more severely affected by novel parasites introduced from other locations. Because we do not have space to house each lizard species in its own room, lizards from different islands must be kept together. Consequently, upon arrival to the animal care facility, we remove all mites using forceps and treat the lizards with commercial de-miting solution as described by the manufacturer (Reptile Relief, Natural Chemistry). Endoparasites may be problematic as well. For example, we have discovered infestations of endoparasitic worms as tumor-like growths on the joints and jaws in wild-caught *A. cybotes* and *A. cristatellus*. We immediately cull individuals from our population as soon as a growth is detected to limit the spread of this parasite between individuals. Sarcophagic fly infestations are also common in some populations of wild-caught anoles (Irschick et al. 2006). We do not prophylactically treat for viral or bacterial infections.

To establish breeding groups, we kept three or four adult females (depending on size) and one adult male together in a standard rat cage (42 cm length × 27.5 cm width × 21 cm height, Ancare Corp.) with oak sticks collected from the outdoors to provide perches (Fig. 1). Before using the sticks as perches, we sterilize them with one cycle of a standard cage washing machine at 180°C. These sex ratios are within the range of those found in the wild for *A. carolinensis* (Jenssen and Nunez 1998) and in many species, several female territories occur within the territory of an adult male. This creates a breeding regime in which juveniles from each cage are half-sibs in genetic analyses, with known father and unknown mother. However, anoles are known to store sperm (Fox 1963; Licht 1973) for many months, so if females were reproductively mature when captured, one cannot assume that the male in the cage fathered the offspring; molecular testing is needed to verify that conclusion.

To prevent escape, we place screen mesh inserts in the cage tops rather than the cloth filter tops typically used when housing mice (New York Wire, mesh 16 × 84 inches). We cover cage bottoms with synthetic cage carpet (ReptiCage Carpet, ZooMed). Cage carpet is readily available, can be easily cleaned and sterilized, and is reusable. The carpet also holds moisture raising the humidity within each cage. We have avoided the use of gravel or sand as a cage substrate because we found that smaller individuals ingest these substrates and become impacted. We discovered that wood chips and sphagnum moss promoted fungal growth after a short time, needed to be changed often, and are not reusable. We clean and sterilize cage carpet and perches approximately once each

FIG. 1. A) A typical breeding cage for *Anolis*. Note the potted plant, cage carpet floor, sticks to act as perches, and several females held with one male. B) A typical rack of juvenile cages. Note the proximity of the UV lights over each row of cages.

month by soaking them in T.B.Q. disinfectant solution (Steris) while cages are washed using a cage washing machine. Anoles may exhibit "cage-novelty induced stress" when placed in unfamiliar conditions and thus perches and carpet should be exchanged separately so as not to return a lizard to a completely novel cage setting after cleaning (Greenberg 1992; Morgan and Tromborg 2007). Opaque cage dividers separate the cages to limit intraspecific male aggression. In cages not separated by cage dividers, males tend to develop open sores on their snout as they attempt to fight with males in adjacent cages. We recommend labeling each cage with the species name, locality of the collection, origin (wild caught or captive bred), date entered into the facility, number of individuals in the cage, and when applicable, age of the individuals and parentage information.

We maintain room temperature, humidity, and photoperiod using an automated climate control system (METASYS, Johnson Controls), cycled seasonally to mimic natural coastal Caribbean wet and dry season fluctuations. During the summer months room temperature cycles daily with a 28°C day, 25°C night and a constant 60–65% humidity. We mist each cage twice daily, raising the humidity within each cage to approximately 85% and supplying the animals with substantial drinking water. Relatively low humidity (less than 35%) is known to retard ovarian growth in *A. carolinensis* (Summers 1988). We also keep a small potted plant in each cage to help maintain relatively high levels of humidity between misting times. During the winter, we drop the room's humidity to approximately 50% and lower the temperatures by 1–2°C. The cages are still misted twice daily during this time. We keep light cycles at 13 h light/ 11 h dark cycles during the summer months and gradually shift to 11 h light/ 13 h dark cycles from early October though late March, approximately simulating the light cycles of the Caribbean. We light the room with both standard fluorescent ceiling lights and 15–20W fluorescent UV bulbs (ReptiSun 5.0) set directly over each cage. To simulate dawn and dusk we turn the ceiling lights on or off 30 minutes before or after the UV lights respectively. In the absence of an automated climate control system these conditions can be maintained with cost-effective, widely available alternatives such as electric timers, standalone humidifiers, and heat lamps (see Bartlett and Bartlett 2001; Hunziker 1994; Lovern 2004 for several more alternatives).

We feed adult anoles a diet of crickets twice weekly, sized appropriately for the species. Crickets are purchased from Timberline Fisheries Corp. (Marion, Illinois) sold in standard English units of 0.25," 0.5," or 0.75." To supplement the lizards' nutrition we dust crickets with a 1:1 mix of commercial vitamin supplement and a calcium additive (Rep-Cal brand Herpvitive and Cal-

cium Supplement) every other feeding. To maintain our cricket colony, we feed the crickets a diet of Laena Poultry (Purina) *ad libitum*. Be aware that some cricket suppliers regularly feed their animals diets that will be transferred to the lizards and may influence a specimen's growth or experimental results. For example, some cricket suppliers prophylactically administer antibiotics to their crickets. During our study of skeletal development (TJS), we found signs of the calcium chelator, tetracycline, in developing long bones. Tetracyclines are known to retard bone growth in many mammals, and thus we may have inadvertently compromised our experiment on bone growth by indirectly feeding it to lizards. We recommend inquiring into the treatment of lizard food when it is purchased from a commercial supplier.

To facilitate the collection of eggs, we supply each cage with a small leafy plant potted in a disposable plastic container (*Ziplock Snap and Seal*™*,* medium bowl or equivalent). Anoles lay one egg from alternating oviducts every one to four weeks (Andrews 1985; Andrews and Rand 1974; Hamlett 1952). We collected eggs every one to three days by removing the plant from the cage, removing the plant from the pot and thoroughly sifting through the root ball and potting substrate. Female anoles lay eggs at all depths in the pot. To ensure that eggs are not missed during collections, we completely remove the potting substrate from the container and search it by hand. Because female anoles can retain their eggs during times of drought (Socci et al. 2005; Stamps 1976), we remoisten the soil after each egg collection. When females are first brought into captivity, they may take some time to begin laying eggs in the potted plant. Initially, eggs may be laid on the cage bottom where they rapidly desiccate.

Viable eggs are generally white and range 0.5–1.5 cm in length. We clean eggs of excess soil using forceps or by hand and transfer them to a standard tissue-culture dish (100 mm × 20 mm) packed with moist, coarse-grained vermiculite, 1:1 vermiculite to de-ionized water by weight. We then cover the dish with its lid, secure it with *Fisherbrand* labeling tape and incubate at 27°C and ~75% humidity for 10–14 days. At this time, we remove the lid and cover the dishes with fiberglass screening to facilitate gas exchange (New York Wire, mesh 16" × 84"). We cut the screening into 15 cm squares and secure it around the circumference of the dish with rubber bands. We restack the dishes in the incubator in such a way that each dish acts as the cover for the dish below it. The top dish is loosely covered with its original lid and the weight of each dish is recorded. We then reweigh the dishes every one to two days and rehydrate them with drops of de-ionized water until the dish is returned to its initial weight. Additionally, to limit the evaporation from the culture dishes, we keep a pan of de-ionized water in the incubator.

Eggs of many squamates are highly permeable and actively exchange water and gas throughout embryonic development. Eggs not relatively free of debris will often desiccate or the embryo will die leading to fungal growth on the egg's surface (PMH, pers. obs). A fine balance also exists between the water within the egg and that in the surrounding substrate (Ackerman and Lott 2004). If the substrate is drier than the egg, the egg will lose water and desiccate. Inversely, if the egg is drier than the substrate, water will flood the egg, possibly damaging the embryo due to increased pressure. While these scenarios represent the extremes of a vast continuum, water balance in reptilian eggs is known to influence many developmental processes (e.g., Packard et al. 1999, 2000) and care should be taken to keep these conditions stable between species and throughout the period of development. Anole species living in different environments probably have adaptively differentiated in their ability to withstand humidity and desiccation, but this has been little studied (Andrews and Sexton 1981).

Care for Juveniles

For the 13 *Anolis* species we have bred in the laboratory, eggs hatch between 25 and 42 days from the date of laying. Upon hatching, we remove juvenile lizards from the culture dish and place them in a standard mouse cage (26 × 20.5 × 15.5 cm, Ancare Corp.) with conditions as described above. We immediately mist hatchlings with water (for drinking and to remove vermiculite stuck to their bodies) and feed them flightless *Drosophila* maintained in a stock culture. As individuals mature, we gradually transfer them to a standard cricket diet. Crickets are chosen by size: smaller lizards, ~2.5cm to 4.0cm SVL, are fed smaller crickets, "pinhead" to 0.25". We later transfer juvenile anoles larger than ~4.0 cm to a diet of larger crickets, 0.5", by adding crickets of both sizes to the cage for approximately one month. We only feed the largest crickets, 0.75", to cages with adult males greater than 5.0 cm SVL. Because we occasionally observed crickets feeding on juvenile lizards, we remove uneaten crickets from the cages within several hours of feeding.

Lizards raised without substantial social interaction throughout juvenile development rarely breed during adulthood (MAJ, pers. obs.); however, housing juveniles individually may be desirable for some studies (e.g., tracking individual growth). In this case, juvenile lizards can be allowed limited social interaction by removing cage dividers to provide visual access to the neighboring cages. Preferably, juvenile lizards would be housed in groups of three to five individuals per rat cage when individual records are not necessary or when marking individuals (e.g., Ferner 1979; Fisher and Muth 1989; Johnson 2005) will not interfere with an experiment. In this case however, the more dominant lizards tend to capture more food, leading to size disparity within each cage (PMH, pers. obs). As animals become dominant, especially males, they can be removed from the group cage and housed separately.

Captive-bred juvenile lizards often face problems during shedding. Lizards often encounter the most difficulty shedding the skin around the head, legs, and cloacal regions. On the head, unshed skin can block eyes, cover nostrils and impede feeding. Around the legs, excess skin can constrict and reduce circulation leading to the occasional loss of limb elements. Unshed skin around the cloaca can cause complications during excretion and promote infection. To prevent these complications we often removed unshed skin on juvenile and adult lizards by moistening the skin with warm water and then gently removing it with fine forceps. Care should be taken not to injure sensitive areas while removing unshed skin.

Embryo Collection

Anolis embryos of all stages can be dissected from eggs under a dissecting microscope. To do so, we remove eggs from their culture dish and submerge them in phosphate buffered saline solution (PBS; Sambrook and Russell 2001). While illuminating the

egg from the side, we then position the egg with the embryo upwards by locating its shadow beneath the shell. Using #5 watchmaker's forceps, we make a small incision outside of the darkened area and extend it, with shallow incisions, across the top of the embryo. Folding the shell away, the embryo is then visible lying on its left side within the yolk and can be easily removed from the yolk and surrounding amnionic membrane. For young embryos, adding a drop of 10% bovine serum albumin to the Petri dish may help in the removal of extra-embryonic membranes. We preserve young embryos by immediately submerging them in 4% paraformaldehyde PBS in a plastic centrifuge tube, incubating overnight at room temperature, and finally transferring them to 70% ethanol. This method of fixation is useful for many basic histological techniques such as gross examination of both soft and hard tissue. Additional measures may be needed for analysis of gene expression or other molecular or immunochemical assays. To assure the humane treatment of embryos approaching hatching, we euthanize these animals with an IP injection of xylazine (20 mg/ml) prior to fixing. Xylazine (Rompun**,** Bayer) is a non-narcotic anesthetic commonly used on mammals. We fix late-stage embryos in 10% buffered formalin for 24 h, and then move them to 70% ethanol. We have observed that *Anolis* embryos tend to adhere to "soft" polystyrene tubes during fixation and cannot be removed without damage. We prefer to use common polypropylene 15 ml conical tubes (i.e., Corning, FisherBrand) when preserving embryos for use in histological preparations or 2 ml centrifuge tubes when preserving embryos for long term storage.

Interspecific Variation

Although the methods described above have been successful for the majority of *Anolis* species we have examined, interspecific differences have required minor adjustments in our protocol. For example, most well-studied species of Caribbean *Anolis*, including the majority of species for which these methods have been utilized, occur in sunny, open areas. The temperature and humidity simulated in our animal care facilities reflect these microclimatic conditions. However, *A. gundlachi* and *A. krugi* are two species that occur in the montane rainforest of Puerto Rico. *Anolis gundlachi* occurs in the cool, deep shade of the forest, and *A. krugi* is typically found in shaded habitats, moving into the sun at higher elevations (Hertz 1992; Rand 1964; Schoener and Schoener 1971; Williams 1972). These habitats offer a very different climate than sunny, open habitats. In our work, after a number of *A. gundlachi* eggs died before hatching when incubated at the standard 27°C (i.e., no eggs from this species survived to hatching), we placed 4 *A. gundlachi* eggs in a 25°C room immediately after being laid. Three of these eggs then produced live hatchlings one month later. In addition, only 17 of 64 *A. krugi* eggs (25%) survived to hatching, but incubating these eggs at a lower temperature may have increased survivorship substantially. For this reason, we recommend matching the cage and egg incubation temperatures and humidity as closely as possible to the natural microclimates of the species for optimal breeding results. Moreover, we note that a comparative study on the thermal biology of anole eggs could prove very interesting.

Additionally, not all species of *Anolis* lizards exhibit the same egg-laying behavior. *Anolis valencienni* is a species that, at least occasionally, lays eggs communally in sites such as tree holes or bromeliads well above the ground (Rand 1967). We have observed that almost all female *A. valencienni* held in captivity do not bury their eggs in the potted plants initially, but instead deposit them on the cage carpet, adhere them to perches, or leave them on the soil surface. To collect viable eggs from this species, we found it necessary to check for eggs every day, particularly in the late evening. However, individuals that have been in captivity for longer times, typically greater than nine months, do begin to deposit their eggs in the pots; after two years in captivity, all eggs are found buried in pots. In the future, we plan to examine whether this species will preferentially lay eggs in an artificial cavity if one is provided in the cage. Whereas the other *Anolis* species we have studied have a mean incubation time of about 29 days, *A. valencienni* also appears to have a considerably longer mean incubation time of about 42 days when incubated under similar conditions.

In conclusion, basic knowledge of the reproductive biology and ecology of a species is necessary to establish successful breeding colonies of lizards. Further consideration of variation among species' ecology may allow for optimization of these methods for species from different habitats. Fortunately for anoles, a substantial body of literature exists on the natural history of many species in this genus, further supporting the utility of these lizards as a model system for a great diversity of research questions.

We have successfully maintained breeding colonies and collected embryos from a diverse collection of *Anolis* species. The above protocols should allow researchers with even a basic animal care facility to breed *Anolis* and collect their embryos for use in standard analyses.

Acknowledgments.—We would like to thank the animal care technicians C. Deggendorf, M. Kwiatkowski, G. McMurray and D. Piatchek for their dedicated attention to these lizards. S. Echternacht, M. Lovern, and J. Stamps gave valuable recommendations for our protocols when beginning our research. J. Kolbe, M. Edgar, and P. Taylor also provided useful feedback on these protocols. Special thanks go to L. Revell and for his careful attention to the animals' well-being and many useful discussions regarding these protocols. We would also like to thank the many undergraduates who have helped optimize these protocols and have ensured the daily care of our lizards, especially S. Barrett, J. Bayers, C. Fresquez, P. Garner, J. Tarsi, and S. Wang. The Washington University Institutional Animal Care and Use Committee formally approved all of the methods described in this paper. Funding for the collection and maintenance of these colonies came from a generous grant from the David and Lucile Packard Foundation and NSF DEB0519777.

Literature Cited

Ackerman, R. A., and D. B. Lott. 2004. Thermal, hydric, and respiratory climate of nests. *In* D. C. Deming (ed.), Reptilian Incubation: Environment, Evolution and Behavior, pp. 15–43. Nottingham University Press, Nottingham UK.

Andrews, R. M. 1985. Oviposition frequency of *Anolis carolinensis*. Copeia 1985:259–262.

———, and S. Rand. 1974. Reproductive efforts in anoline lizards. Ecology 55:317–327.

———, and O. J. Sexton. 1981. Water relations of *Anolis auratus* and *Anolis limifrous*. Ecology 62:556–562.

Bartlett, R., and P. Bartlett. 2001. Anoles: Facts & Advice on Care and Breeding. Barron's Educational Series, Hauppauge, New York.

Burger, J., K. R. Campbell, and T. S. Campbell. 2004. Gender and spa-

tial patterns in metal concentrations in brown anoles (*Anolis sagrei*) in southern Florida, USA. Environ. Tox. Chem. 23:712–718.

CLARK, D. R., AND J. C. KROLL. 1974. Thermal ecology of anoline lizards: temperate vs. tropical strategies. Southwest. Nat. 19:9–19.

DEMING, D. C. (ed.). 2004. Reptilian Incubation: Environment, Evolution and Behavior. Nottingham University Press, Nottingham UK.

EMERSON, B. C. 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Mol. Ecol. 11:951–966.

FERNER, J. W. 1979. A review of marking techniques for amphibians and reptiles. SSAR Hepetol. Circ. No. 9:41.

FISHER, M., AND A. MUTH. 1989. A technique for permanently marking lizards. Herpetol. Rev. 20:45–46.

FOX, W. 1963. Special tubules for sperm storage in female lizards. Nature 198:500–501.

GREENBERG, N. 1992. The saurian psyche revisited: Lizards in research. *In* D. O. Schaeffer, K. M. Kleinow, and L. Krulish (eds.), The Care and Use of Amphibians, Reptiles, and Fish in Research. Scientists Center for Animal Welfare, Bethesda, Maryland.

———. 2002. Ethological aspects of stress in a model lizard, *Anolis carolinensis*. Integ. Comp. Biol. 42:526–540.

———. 2003. Sociality, stress, and the corpus striatum of the green *Anolis* lizard. Physiol. Behav. 79:429–440

HAMLETT, G. W. D. 1952. Notes on the breeding and reproduction in the lizard in the lizard *Anolis carolinensis*. Copeia 1952:183–185.

HERTZ, P. E. 1980. Responses to dehydration in *Anolis* lizards sampled along altitudinal transects. Copeia 1980:440–446.

———. 1981. Adaptation to altitude in two West Indian anoles (Reptilia: Iguanidae): field thermal biology and physiological ecology. J. Zool., London 195:25–37.

———. 1992. Temperature regulation in Puerto-Rican *Anolis* lizards—a field-test using null hypotheses. Ecology 73:1405–1417.

HUEY, R. B., P. E. HERTZ, AND B. SINERVO. 2003. Behavioral drive versus behavioral inertia in evolution: A null model approach. Am. Nat. 161:357–366.

———, AND T. P. WEBSTER. 1976. Thermal biology of *Anolis* lizards in a complex fauna: the *cristatellus* group on Puerto Rico. Copeia 1976:496–504.

HUNZIKER, R. 1994. Green Anole: Selection, Care and Breeding. TFH Publications, Neptune City, New Jersey.

IRSCHICK, D. J., AND T. GARLAND. 2001. Integrating function and ecology in studies of adaptation: Investigations of locomotor capacity as a model system. Ann. Rev. Ecol. Syst. 32:367–396.

———, G. GENTRY, A. HERREL, AND B. VANHOOYDONCK. 2006. Effects of sarcophagid fly infestations on green anole lizards (*Anolis carolinensis*): An analysis across seasons and age/sex classes. J. Herpetol. 40:107–112.

JENSSEN, T. A. AND S. C. NUNEZ. 1994. Male and female reproductive cycles of the Jamaican lizard, *Anolis opalinus*. Copeia 1994:767–780.

———, AND ———. 1998. Spatial and breeding relationships of the lizard *Anolis carolinensis*: Evidence of intrasexual selection. Behavior 135:981–1003.

JOHNSON, M. A. 2005. A new method of temporarily marking lizards. Herpetol. Rev. 36:277–279.

KOBAYASHI, D., W. J. MAUTZ, AND K. A. NAGY. 1983. Evaporative water loss: humidity acclimation in *Anolis carolinensis* lizards. Copeia 1983:701–704.

LEE, J. C., D. CLAYTON, S. EISENSTEIN, AND I. PEREZ. 1989. The reproductive cycle of *Anolis sagrei* in southern Florida. Copeia 1989:930–937.

LICHT, P. 1973. Influence of temperature and photoperiod on the annual ovarian cycle in the lizard *Anolis carolinenesis*. Copeia 1973:465–472.

———, AND G. C. GORMAN. 1970. Reproductive and fat cycles in Caribbean *Anolis* lizards. Univ. California Publ. Zool. 95:1–52.

LOSOS, J. B. 1994. Integrative approaches to evolutionary ecology: *Anolis* lizards as model systems. Ann. Rev. Ecol. Syst. 25:467–493.

LOVERN, M. B., M. M. HOLMES, AND J. WADE. 2004. The green anole (*Anolis carolinensis*): A reptilian model for laboratory studies of reproductive morphology and behavior. Ilar J. 45:54–64.

MICHAUD. E. J., AND A. C. ECHTERNACHT. 1995. Geographic variation in the life history of the lizard *Anolis carolinensis* and support of the pelvic constraint model. J. Herpetol. 29:86–97.

MORGAN, K. N., AND C. T. TROMBORG. 2007. Sources of stress in captivity. App. Anim. Behav. Sci. 102:262–304.

PACALA, S., AND J. ROUGHGARDEN. 1985. Population experiments with the *Anolis* lizards of St. Maarten and St. Eustatius. Ecology 66:129–141.

PACKARD, G. C., K. MILLER, M. J. PACKARD, AND G. F. BIRCHARD. 1999. A field study of environmentally induced variation in body size and condition in hatchling snapping turtles (*Chelydra serpentina*). Can. J. Zool. 77:278–289.

———, M. J. PACKARD, AND G. F. BIRCHARD. 2000. Availability of water affects organ growth in prenatal and neonatal snapping turtles (*Chelydra serpentina*). J. Comp. Phys. B170:69–74.

POUGH, F. H. 1991. Recommendations for the Care of Amphibians and Reptiles in Academic Institutions. National Academy Press, New York.

RAND, A. S. 1964. Ecological distribution in anoline lizards of Puerto Rico. Ecology 45:745–752.

———. 1967. Communal egg laying in anoline lizards. Herpetologica 23:227–230.

RUIBAL, R. 1961. Thermal relations of five species of tropical lizards. Evol. 15:98–111.

SAMBROOK J., AND D. W. RUSSELL. 2001. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SCHOENER, T. W. 1968. The *Anolis* lizards of Bimini: Resource partitioning in a complex fauna. Ecology 49:704–726.

———, AND A. SCHOENER. 1971. Structural habitats of West Indian *Anolis* lizards II. Puerto Rican uplands. Breviora 375:1–39.

SOCCI, A. M., M. A. SCHLAEPFER, AND T. A. GAVIN. 2005. The importance of soil moisture and leaf cover in a female lizard's (*Norops polyepis*) evaluation of potential oviposition sites. Herpetologica 61:233–240.

STAMPS, J. A. 1976. Egg retention, rainfall and egg laying in a tropical lizard *Anolis aeneus*. Copeia 1976:759–764.

———.1983. The relationship between ontogenetic habitat shifts, competition and predator avoidance in a juvenile lizard (*Anolis aeneus*). Behav. Ecol. Sociobiol. 12:19–33.

SUMMERS, C. H. 1988. Chronic low humidity-stress in the lizard *Anolis carolinensis*, effects on ovarian and oviductal recrudescence. J. Exp. Zool. 248:192–198.

WADE, J. 2005. Current research on the behavioral neuroendocrinology of reptiles. Horm. Behav. 48:451–460.

WILSON, M. A., AND A. C. ECHTERNACHT. 1987. Geographic variation in the critical thermal minimum of the green anole, *Anolis carolinensis* (Sauria: Iguanidae), along a latitudinal gradient. Comp. Biochem. Physiol. 87A:757–760.

———, AND ———. 1990. A comparison of heat and cold tolerance of two morphs of *Anolis carolinensis* (Iguanidae) from southern Florida. J. Herpetol. 24:330–333.

WILLIAMS, E. E. 1972. The origin of faunas. Evolution of lizard congeners in a complex island fauna: A trial analysis. Evol. Biol. 6:47–89.