were present on grids from mid-November through late-February. Presence of eggs on the egg grid was the sole or primary source of identification in 49 of the 69 ponds. Egg identification confirmation was made by the examination of larvae later in the season.

A note of caution: considerable care should be taken when examining habitat within the range of the introduced tiger salamander (*A. tigrinum*; Bean 1999). Under these circumstances neither eggs nor larvae should be used for species identification. Genetic testing should be used to discriminate between these species.

The addition of this technique to the survey protocol may increase detection of *A. californiense*, particularly in turbid water bodies. Additionally, it can reduce or eliminate the need for Federal 10(a)(1)(A) permit for take of California red-legged frog (*Rana aurora draytonii*) larvae that are sympatric with *A. californiense*. Further, many ponds can be surveyed simultaneously with relatively little time expended and a marked reduction in potential mortality.

Acknowledgments.—I thank W. K. Weber for her contributions to the design of the egg grids and for her helpful comments on the manuscript; M. van Hattem for his insightful observation and comments; and J. L. Alvarez for her helpful comments on the manuscript. I thank the Contra Costa Water District for support.

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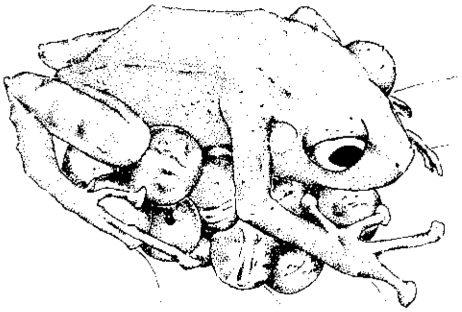

Eleutherodactylus verecundus. Female, 30 mm SVL, with clutch of 18 eggs. Colombia: Nariño, Reserva Natural La Planada. Illustration (from a photograph) by Fernando Vargas Salinas.

HERPETOLOGICAL HUSBANDRY

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Proximate Cues for Ovarian Recrudescence and Ovulation in the Brown Treesnake (*Boiga irregularis*) Under Laboratory Conditions

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The reproductive biology of the brown treesnake (*Boiga irregularis*), an invasive tropical species known primarily for the extensive ecological damage it has caused on the island of Guam (Savidge 1987), is poorly understood. This is not for lack of sampling effort by researchers—it is simply because reproduction apparently occurs in all months of the year (Rodda et al. 1999) and periodic sampling in such systems cannot detect the phenology of major reproductive events (e.g., ovarian recrudescence, ovulation, oviposition) and other important life history attributes (e.g., frequency of reproduction). In such systems, repeated observations of individuals may be the only recourse for obtaining such information. Accordingly, we recently established a captive colony of brown treesnakes at our facilities and were able to successfully induce reproduction in a number of females (Mathies and Miller 2003). Here, in our second effort to induce reproduction in this colony, we report on the phenology of ovarian recrudescence, the conditions that initiate this process, and the possibility that copulation may be necessary to induce ovulation.

Details on origin, husbandry, and procedures for mating of the snakes in this study are the same, or similar, to those given in Mathies and Miller (2003). Snakes in our colony (10 males, 15 females) were collected as adults on Guam and are the same individuals used in a previous breeding study (Mathies and Miller 2003). Because of space limitations, snakes were housed in two adjacent rooms. Temperatures in the rooms were thermostatically controlled and no other heat sources were available to snakes. Differences in air temperatures between the two rooms were unintended and due to equipment malfunction (Fig. 1). Overall mean temperatures during the study period, however, were similar (Room A: 22.8°C; Room B: 22.9°C). Relative humidity in both rooms was maintained at about 80%. Room lighting was provided by fluorescent bulbs and the photoperiod was 12L:12D. The time of year this study was conducted was chosen out of convenience. Dates provided herein should not necessarily be taken to imply that the brown treesnake is more likely to become reproductive at this time of year than any other.

Mating trials were conducted by placing a female in the cage of a male and then observing the pair for at least 30 minutes for courtship behaviors. Trials were conducted in near darkness and pairs were observed using Sony CCD-TRV58 video cameras equipped with infrared light emitters (Sony Electronics Inc., New Jersey, USA.). Trials were conducted at approximately 14-day intervals beginning on 7 November 2001 and ending on 3 March 2002. Each female was placed with a male once during each group of trials and with a different male each time. In the majority of trials, males showed little or no interest in females. Males performed dorsal advances followed by tail searching behavior in only four trials with no one male or female participating more than once. These four trials were distributed fairly evenly throughout the period of mating trials. There were no successful intromissions. We assume the males were in adequate reproductive condition because these same males were highly attracted to females when kept under similar environmental conditions to those used here (Mathies and Miller 2003).

Females were palpated for the presence of enlarged ovarian follicles on one to two occasions within 30-day intervals beginning on 8 January 2002 and ending on 3 July 2002. The method for palpating females was quick and relatively unobtrusive; it simply involved lifting an individual from its cage and then feeling for follicles as it crawled back into its cage. We were first able to detect enlarged follicles in a few females approximately three months after the end of the cool period (Fig. 1). Within two weeks of that examination, all eight females in Room A had enlarged follicles and six of the seven females in Room B had enlarged follicles. At this time all such females contained follicles judged to be at least 30 mm in length, which is about the size of brown treesnake eggs (Mathies and Miller 2003; Rodda et al 1999). Thus, follicles were presumably at, or close to, ovulatory size. The number of follicles per female was not related to female body mass (Linear regression: $R^2 = 0.08$, $P = 0.35$), which was somewhat unexpected, but we did not measure snout–vent length, which is generally a better predictor of reproductive investment. There was no difference between rooms in the number of follicles per female (Mann-Whitney U-test: $P = 0.85$, $U = 22.50$). The modal (and maximum) number of follicles per female was nine. The frequency of females with respect to their follicle count is shown in Fig. 2. The numbers of follicles per female we observed are in line with clutch sizes reported for females from the Guam population (Mathies and Miller 2003; Rodda et al 1999) suggesting that these numbers are translatable to clutch sizes. The first examination where we noted females with either no palpable follicles, or flaccid-feeling follicles, occurred approximately two months from the date follicles were first detected. At about 12 weeks past the date follicles were first detected, no females had palpable follicles. That is, all follicles had apparently become atretic and had been, or were being, resorbed. The timing and durations of the events reported above would have been temperature dependent, thus, we do not know how representative they are of those on Guam.

The cue that initiates ovarian recrudescence in the brown treesnake has not been specifically identified. In the present, as well as a previous study (Mathies and Miller 2003), follicles became enlarged following a period of cool temperatures. However, in both studies, females were placed together with males following the cool periods and thus we cannot rule out the possibility that contact with a male was the cue. Studies on female red-sided garter snakes revealed that in some years, at least, copulation ini-

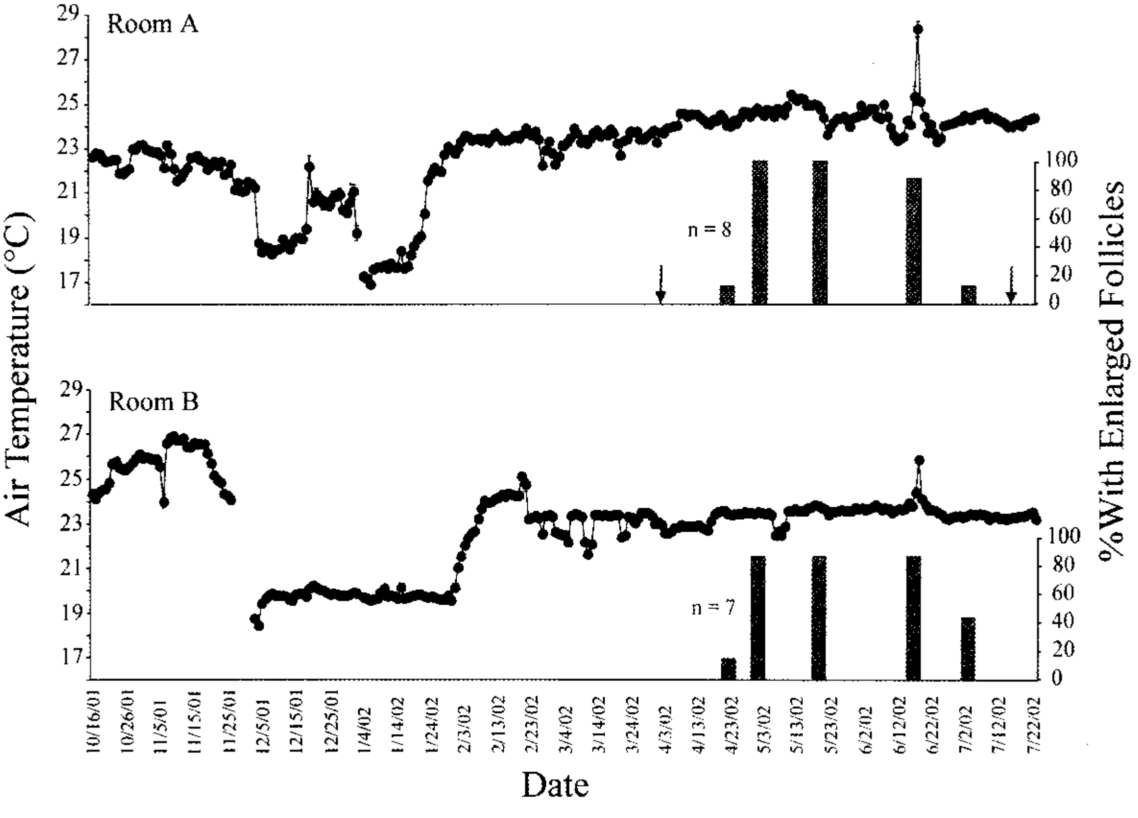

FIG. 1. Development of enlarged ovarian follicles by female brown treesnakes (*Boiga irregularis*) with respect to air temperature and date. The number of females (N) in each room is given. Palpating for the presence of enlarged follicles began 8 January 2001. Fourteen of the 15 females developed enlarged follicles. Arrows bounding the period when follicles were detected indicate the nearest examination dates (same for both rooms) where no enlarged follicles were found. Air temperatures are presented as daily means ± 1 SE. Periods of missing data were due to malfunction of data loggers.

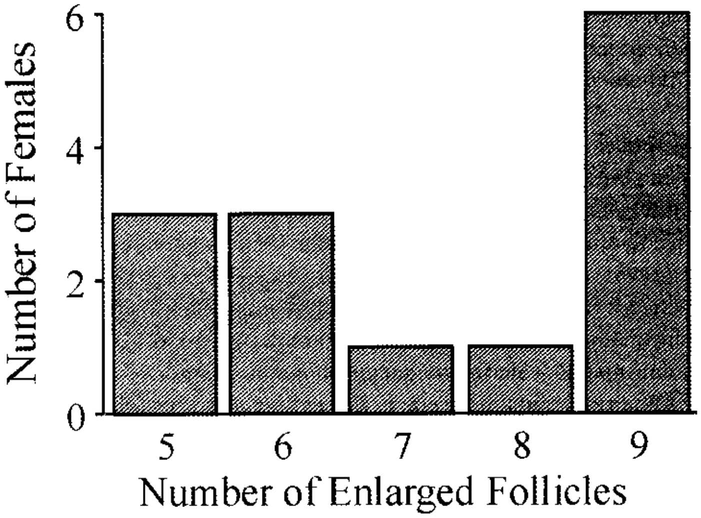

FIG. 2. Frequency distribution of the number of brown treesnake females (*Boiga irregularis*) with respect to number of enlarged ovarian follicles.

tiates a neuroendocrine reflex that is necessary for the initiation and maintenance of vitellogenesis, but this mechanism has not yet been documented in any other vertebrate species (Mendonça and Crews 1990). In the present study, it seems unlikely that the brief presence of mostly-uninterested males would induce ovarian recrudescence. In the majority of reptiles and other vertebrates, both temperate and tropical, the primary cues for ovarian recrudescence are environmental.

Why did none of our females ovulate? It is possible that palpating for follicles caused stress that resulted in resorption of follicles. Chronic exposure to stressors disrupted ovarian recrudescence in the lizard, *Mabuya carinata* (Ganesh and Yajurvedi 2002) and brown treesnakes exhibit a substantial increase in plasma levels of the stress hormone, corticosterone, in response to acute stress (Mathies et al. 2001). However, stress was an unlikely cause in the present study because females were long-term captives that appeared to be used to routine handling and cage maintenance and have previously produced viable eggs at our facilities (Mathies and Miller 2003). Thus, the stress that females experienced, if any, was presumably not appreciable or chronic. Second, if palpation was stressful, then it would have to have had a differential effect on follicular growth versus ovulation because it had no apparent effect on the former.

We suggest that follicles were not ovulated because the appropriate cue was lacking. The most likely cue would be the stimuli attendant with the physical act of mating. Induced ovulators, where coitus is thought to produce a reflex discharge of gonadotropin-releasing hormone and hence luteinizing hormone, occurs in a variety of vertebrates. Such a mechanism has not been reported in reptiles, but its presence/absence has not been well investigated. One reason mating did not occur in this study is that females may not have been in the appropriate stage of the reproductive process and thus may not have been attractive to males when we attempted to mate them. Observations from this and our other breeding studies collectively suggest that female brown treesnakes are at the height of sexually receptivity when they contain large, pre-ovulatory follicles. Recall that in the present study, mating trials were discontinued about 50 days before the first follicles were detected. Thus, females had not yet developed mature follicles when we attempted to mate them. In the first breeding attempt at our facilities, the three females that did produce eggs did so at about 35–40 days following a period when repeated successful intromissions for each female were observed (Mathies and Miller 2003), and in a third and most recent breeding attempt, we observed that ovulation (N = 6 females) occurred approximately 30 days prior to egg laying (Mathies and Miller, unpubl. data). Thus, the three females in the first study that laid eggs likely contained mature pre-ovulatory follicles when they copulated with males. Further support for the contention that male contact is necessary for ovulation comes from studies on captive reproduction of insular species of *Epicrates*. Tolson et al. (1985) demonstrated that female *Epicrates angulifer* initiate ovarian recrudescence in response to a period of cool temperatures but do not ovulate (i.e., follicles are resorbed) unless they experience extensive male courtship. This same phenomena have apparently also been observed in other species of *Epicrates* (Tolson 1994).

Induced ovulation might be expected in a system where males are common and continuously reproductive and where females do not store sperm. The Guam population of brown treesnakes meets at least two, and possibly all, of these criteria. Snakes occur at unusually high densities (Rodda et al. 1992) and no degree of seasonality of reproduction has been detected (Rodda et al. 1999). Although oviducts of Guam females have not been examined for sperm storage areas, a study on female brown treesnakes from southeastern Queensland detected no such structures (Bull et al. 1997). However, sperm storage has been inferred (but not demonstrated) in *Boiga dendrophila* (Groves 1973) and *Boiga multomaculata* (Kopstein 1938).

Induced ovulation in brown treesnake females would be adaptive in that vitellogenic individuals that go unmated would presumably be able to recoup much of their reproductive investment through follicular resorption. In our colony, females that have resorbed their follicles maintain body masses and abdominal fat body sizes similar to those when reproductive whereas females that have recently laid eggs have relatively low body masses and small abdominal fat bodies (Mathies and Miller, unpubl. data).

The hypothesis that female brown treesnakes are induced ovulators can be easily tested; one would only need to induce folliculogenesis in females and mate one group but not the other. Our findings suggest that the appropriate time to mate females (i.e., when females are most attractive to males) is when females have large, preovulatory follicles.

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Venomous Reptile Bites in Academic Research

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Many people work with venomous reptiles, both in the field and in captivity. Researchers generally have some understanding of the risks involved in working with these animals but might not have a full appreciation of the consequences of a venomous bite. Most venomous snakes have the ability to inflict temporary injury, permanent disfigurement, or death.

Card and Roberts (1996) surveyed the incidence of bites from venomous reptiles in North American Zoos. Thirty institutions maintaining venomous animals responded, 21 of which reported having staff that had been bitten by venomous reptiles, for a total of 31 bites during a 26-year period. Over 37% of the victims were between 20 and 25 years of age and had less than five years of professional experience working with venomous reptiles.

After discussions with several researchers affiliated with colleges and universities in the United States, we wondered if similar results would be found among academic institutions. In July of 2000, a questionnaire was distributed to 130 institutions across the United States. Data were gathered regarding bite frequency, bite circumstances, handling methods and protocols, bite emergency protocols, and knowledge about the availability of antivenom.

Seventy-four institutions responded, with 40 reporting research with live venomous reptiles. Eighteen reported 42 envenomations and six dry bites from a total of 20 reptile species, with two bites attributed to unnamed species (Table 1). Thirty-three of the bites were inflicted by vipers, 25 from pit vipers, while four came from elapids, 10 from colubrids, and one from an unnamed snake. Eighteen bites occurred in the laboratory and 30 in the field. Ten institutions conducted inquiries on the circumstances of the bites, which resulted in seven changing their handling protocols. A large majority of these bites (87.5%) were the result of using dubious capture and/or restraint methods, including deliberately picking up or free handling snakes, pinning snakes, handling snakes with gloves, and improper use of restraint tubes. Sixty-one percent of the bites occurred at institutions without training protocols.

Training of personnel varied widely in depth and detail. Although 23 institutions required that researchers demonstrate minimal competence with animal manipulation, 19 institutions performing research with venomous reptiles reported having no training program at all on venomous animal manipulation. Of the others with some type of training program, only 16 required physical practice and four provided a written text regarding this topic. Nineteen reported having no emergency response protocol, and 13 reported that antivenom was not kept at the institutions and that it was unavailable at local medical facilities.

Equally interesting to us are the methods by which venomous reptiles were manipulated. Thirty-one institutions reported using tongs, while 30 used hooks, and 22 employed tubes. Seventeen institutions reported pinning, and nine used leather gloves in the

TABLE 1. Species involved in bites (envenomations and dry bites).

Species	Number of bites
Agkistrodon contortrix	9 (8 envenomations/1 dry)
Agkistrodon piscivorus	1
Atheris nitschei	1
Atractaspis irregularis	1
Boiga irregularis	10
Bothrops asper	1
Boulengerina christyi	1
Causus maculatus	3 (1 envenomation/2 dry)
Crotalus atrox	4
Crotalus cerastes	3
Crotalus durissus terrificus	1 (dry)
Crotalus horridus	1
Crotalus lepidus	2
Crotalus sp.	1
Crotalus viridis	2
Crotalus willardi obscurus	1
Micrurus fulvius	1
Naja melanoleuca	1
Naja naja	1
Sistrurus miliarius	1
Trimeresurus borneensis	1 (dry)
Unnamed genus/species	1 (dry)
Totals	48 (6 dry)

*These numbers underestimate the actual number of bites that occurred at these institutions. We are aware of several bites that were not reported.

capture of venomous reptiles. Three reported that researchers free-handled (unrestrained) venomous reptiles.

The frequency of envenomation appears to exceed that of reptile keepers in public zoos and herpetaria, even though these latter institutions house a greater number and variety of venomous species, and keeper interaction with them is more frequent and extensive. The data suggest that the number and circumstances of bites in academia correlate with inadequate training programs at these institutions. In discussions with several researchers, we have learned that bites from venomous animals are regarded by some as an inevitable result of this type of research—a view that is both erroneous and dangerous.

The data also suggest that a cavalier attitude toward working with venomous reptiles might exist among some academic researchers and that they might not be familiar with all of the protocols currently employed to prevent envenomations. Considering that these individuals might also be responsible for training future herpetologists, we believe that most researchers would benefit from developing and imparting a more cautious attitude toward manipulating venomous wildlife. They would also profit from more knowledge and training in the areas of venomous animal manipulation, as well as greater diligence in developing and employing safety protocols.

Appropriate procedures for handling venomous reptiles and medical care procedures for bite victims should be developed by institutions involved in venomous animal research. Researchers should identify local physicians familiar with snakebite treatment, plot routes to the hospital, and contact emergency personnel beforehand so that they are aware of the type of venomous reptiles involved. In case medical advice or additional serum is needed, phone numbers for poison control centers should be listed, and antivenom stocks should be kept current. If venomous reptiles are maintained in a laboratory, consideration should be given to installation of an alarm system, and development of a snakebite protocol with contact numbers for snakebite consultants, including first aid measures to be administered by co-workers. The envenomation protocol in use at the Arizona-Sonora Desert Museum is available by contacting us.

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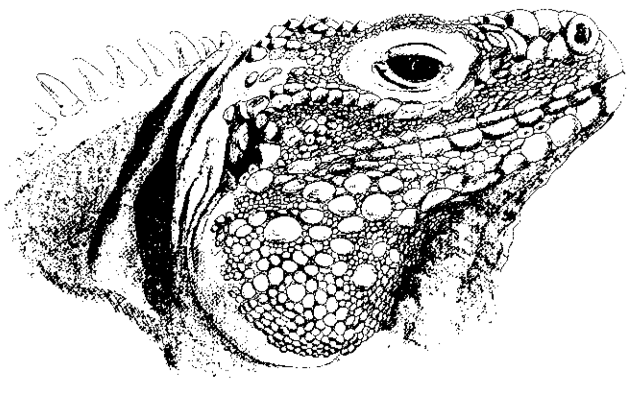
CARD, W., AND D. T. ROBERTS. 1996. Incidence of bites from venomous reptiles in North American zoos. Herpetol. Rev. 27:15–16.



Cyclura lewisi (Blue Iguana), adult male. Grand Cayman. Illustration by John Bendon (Lizardwizard@btinternet.com).

NATURAL HISTORY NOTES

The Natural History Notes section is analogous to Geographic Distribution. Preferred notes should 1) focus on observations with little human intrusion; 2) represent more than the isolated documentation of developmental aberrations; and 3) possess a natural history perspective. Individual notes should, with few exceptions, concern only one species, and authors are requested to choose a keyword or short phrase which best describes the nature of their note (e.g., Reproduction, Morphology, Habitat, etc.). Use of figures to illustrate any data is encouraged, but should replace words rather than embellish them. The section's intent is to convey information rather than demonstrate prose. Articles submitted to this section will be reviewed and edited prior to acceptance.

Electronic submission of manuscripts is requested (as Microsoft Word or Rich Text format [rtf] files, as e-mail attachments). Authors without the ability to send manuscripts electronically may supply hard copy instead. Figures can be submitted electronically as JPG files, although higher resolution TIFF or BMP files will be requested for publication. If figures cannot be provided in this format, you may send them to the section editor for scanning. Additional information concerning preparation and submission of graphics files is available on the SSAR web site at: http://www.ssarherps.org/HRinfo.html. Manuscripts should be sent to the appropriate section editor: **Marc P. Hayes** (amphisbaenids, crocodilians, lizards, and *Sphenodon*; mhayesrana@aol.com); **Charles W. Painter** (amphibians; cpainter@state.nm.us); **Andrew T. Holycross** (snakes; holycross@asu.edu); and **James Harding** (turtles; hardingj@pilot.msu.edu).

Standard format for this section is as follows: SCIENTIFIC NAME, COMMON NAME (for the United States and Canada as it appears in Crother [2000. *Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico, with Comments Regarding Confidence in Our Understanding*. Herpetol. Circ. 29:1–82; available online at <http://herplit.com/SSAR/circulars/HC29/Crother.html>]; for Mexico as it appears in Liner [1994, *Scientific and Common Names for the Amphibians and Reptiles of Mexico in English and Spanish*, Herpetol. Circ. 23:1–113]), KEYWORD. DATA on the animal. Place of deposition or intended deposition of specimen(s), and catalog number(s). Then skip a line and close with SUBMITTED BY (give name and address in full—spell out state names—no abbreviations). (NCN) should be used for common name where none is recognized. References may be briefly cited in text (refer to this issue for citation format).

Recommended citation for notes appearing in this section is: Lemos-Espinal, J., and R. E. Ballinger. 1994. *Rhyacosiredon leorae*. Size. Herpetol. Rev. 25:22.

CAUDATA

***AMBYSTOMA* SPP. (Mole Salamanders). LOTIC BREEDING.** Breeding in *Ambystoma* salamanders is commonly associated with lentic habitats such as forested pools, ponds, and wetlands throughout North America. However, a review of Petranka (1998. Salamanders of the United States and Canada. Smithson. Inst. Press, 587 pp.) indicates that reproduction also occurs in flowing water for numerous species. Here we report additional evidence of lotic reproduction by *Ambystoma* species collected from primary headwater streams (< 259 ha watershed size) in Ohio.

Ambystoma texanum larvae were collected 17 May 2000 from two channel modified streams with agricultural land use in Union County (Allen and Jerome townships). Total length of nine voucher specimens ranged from 20.2–29 mm for the Allen Township population; and 18–26.5 mm (N = 3) for Jerome Township. Larvae of *A. barbouri* were collected 20 July 2000 from a headwater stream with intact riparian zone in Warren County, Washington Township. Body length of three voucher specimens ranged from 34–39.0 mm. A larva with TL 36.2 mm had reduced gills, which suggests the population was close to metamorphosis. The stream substrate was dominated by cobble-gravel-boulders with pools of water connected by subsurface flow. In this stream, *A. barbouri* larvae were coexisting with larvae of the southern two-lined salamander, *Eurycea cirrigera*. A large (TL 62.3 mm) and uniformly dark *A.*