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Food Habits and Selective Foraging by the Texas River Cooter (*Pseudemys texana*) in Spring Lake, Hays County, Texas

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ABSTRACT.—**The fall-winter food habits of the Texas river cooter (*Pseudemys texana*) were investigated at Spring Lake, Hays County, Texas, by examination of stomach contents. Samples were collected from 33 live turtles by flushing stomach contents with water (Fields et al., 2000). Four aquatic macrophytes composed 91.6% of the diet. Aquatic macrophyte availability at Spring Lake was estimated using the Daubenmire technique (Daubenmire and Daubenmire, 1968) and compared to occurrence in the diet to assess selectivity in foraging. Cooters actively selected Carolina fanwort (*Cabomba caroliniana*), a plant low in fiber and high in total digestible nutrients. Hydrilla (*Hydrilla verticilata*), an invasive exotic aquatic plant, was an important food item due to the large amount present in the lake.**

The Texas river cooter, *Pseudemys texana* (Ward 1984), is a freshwater basking turtle of the family Emydidae. It is endemic to the Brazos, Colorado, Guadalupe, and San Antonio river basins of central and south Texas. Rivers, streams, and nearby ponds, especially those with abundant aquatic vegetation and basking sites, are preferred habitats ((??)Vermersch, 1992). It also is found in man-made impoundments, irrigation ditches, canals, and cattle tanks (Carr, 1952).

Prior to Seidel and Smith (1986), some species of *Pseudemys* were included in the genus *Chrysemys* (Carr, 1952; McDowell, 1964; Zug, 1966; Weaver and Rose, 1967). In addition, *P. texana* was included within other species of *Pseudemys*, until it was recognized as a distinct species by Ward (1984).

An early study of the food habits of *P. texana* (Strecker, 1927) found only mollusks (*Sphaerium* sp., *Planorbis* sp., and *Lymnaea* sp.) in the digestive tracts. Young *P. texana* have been observed to pursue insects, crayfish, and other invertebrates ((??)Vermersch, 1992). Other members of the genus are presumed to consume more invertebrates when young and become less carnivorous with maturation (Ernst and Barbour, 1989; Vermersch, 1992; Ernst et al., 1994). In general, *P. texana* is considered to be herbivorous when mature, although quantitative analysis of the diet is lacking.

Analysis of stomach contents can provide information about which food items are palatable, nutritious, or abundant in the environment (Ward and Keith, 1962). Many herbivores have been shown to select plants based on their nutrient content (Gwynne and Bell, 1968). Consumption of a dietary component disproportionate to its availability in the environment would suggest selectivity in foraging (Johnson, 1980). Although, the nutritional quality of plants is important to herbivores, important food plants also may be eaten in proportion to their availability without selection.

Knowledge of food habits and foraging selectivity of *P. texana* is essential to the thorough understanding of how this species functions within its habitat and how it may influence the structure of the ecosystem. The objectives of this study were to describe the fall-winter food habits of *P. texana* at Spring Lake, Hays County, Texas; to determine if *P. texana* foraged selectively by comparing usage to availability in the environment; and to compare the nutritional quality of major components of the diet.

MATERIALS AND METHODS

Study Site.—This study was conducted at Spring Lake, a 7.9-ha reservoir at the headwaters of the San Marcos River in Hays County, Texas (29°53′N, 97°55′W). Initially dammed in 1849, the lake is fed by some 200 artesian springs that issue from the Edwards Aquifer along the San Marcos portion of the Balcones Fault. San Marcos Springs is the second largest spring system in Texas (Brune, 2002), and historically is one of the most stable and reliable spring systems in the southwestern United States. San Marcos Springs has an annual discharge of 169cfs (Gandara et al., 2000). The mean annual water temperature at the springs source is 21°C, and the temperature in headwaters varies annually by less than 3°C (Groeger et al., 1997). The headwaters of the San Marcos River, which is characterized by a relatively constant physicochemical environment, supports a diverse and abundant aquatic macrophyte community that provides forage and cover for aquatic biota (Lemke, 1989; Longley, 1991). Watkins (1930) suggested that, given the stability of the physicochemical environment at Spring Lake, light was the principle factor affecting seasonal changes in the macrophyte community.

Individual *P. texana* were collected at Spring Lake by two methods from 3 October 1996 to 14 March 1997. A floating basking trap (2.5 × 1.3 × 0.7 m) was placed in shallow, open water to capitalize on this species' gregarious basking habits. Turtles also were collected with a dipnet from a canoe at night.

Stomach contents were obtained from subadult and mature turtles (carapace length 127–307 mm; plastron length 114–275 mm) using a stomach flushing technique described by Legler (1977) and refined during the course of this study (Seaman, 1997; Fields et al., 2000). Stomach contents were preserved in 10% formalin. The percent composition of each food item was determined using a point frame technique (Chamrad and Box, 1964) modified by Seaman (1997). One hundred hits were

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TABLE 1. Percent composition of aquatic macrophytes in the diet of *Pseudemys texana* (proportion used) and in Spring Lake (proportion available), Hays County, Texas, with Manly's alpha preference index scores.

Aquatic plant	% Composition in diet	% Composition in Spring lake	Manly's alpha[1]
Hydrilla	33.5	34.7	0.086
Carolina fanwort	24.5	4.0	0.551
Parrot's feather	20.2	13.5	0.130
Common coontail	13.4	11.0	0.110
Delta arrowhead	0.7	5.2	0.013
Cone-spur bladderwort	0.2	8.0	0.002
Filamentous algae	4.4	12.3	0.035
Other	3.1	11.3	—

[1] Scores >1/m (0.125) indicate preference.

recorded for each stomach and each food item was identified to the lowest possible taxon.

Percent coverage of aquatic plant species in Spring Lake was estimated using Daubenmire estimates (Daubenmire and Daubenmire, 1968) from 274 quadrats placed each 5 m along 22 line transects spanning Spring Lake.

Dietary selectivity was measured by comparing the proportion of food items found in stomach contents to the proportion found in the macrophyte community in Spring Lake. Log-likelihood chi-square analysis (Manly et al., 1993) was used to test the hypothesis that *P. texana* consumes aquatic macrophytes in proportion to availability at Spring Lake. To determine expected consumption, confidence intervals were calculated on consumed foods. The constant prey formula for Manly's alpha preference index (Manly, 1974) was used to estimate selectivity in foraging.

Six plant species commonly found in the diet of *P. texana* were collected from Spring Lake for nutritional analysis: Carolina fanwort (*Cabomba caroliniana*), cone-spur bladderwort (*Utricularia gibba*), common coontail (*Ceratophyllum demersum*), delta arrowhead (*Sagittaria platyphylla*), hydrilla (*Hydrilla verticillata*), and parrot's feather (*Myriophyllum* sp.). Samples of these species were sent to Texas A&M University Soil, Water and Forage Testing Laboratory, College Station, Texas, for analysis of crude protein (CP), digestible crude protein (DCP), total digestible nutrients (TDN), digestible energy (DE), and acid detergent fiber (ADF) (Stokes and Prostko, 1998). The CP and DCP of a plant are measures of total protein and available protein, respectively. Protein is a measure of food plant quality because it is typically not abundant in the vegetative parts of a plant (Bolin and Robinson, 2002). The energy value (Mcal/kg.) of a plant is described by TDN and DE. Less soluble plant compounds such as cellulose, lignin, silica, insoluble CP, and ash are expressed by ADF. Plants low in ADF provide greater net energy to herbivores.

RESULTS

Analysis of stomach samples from 33 turtles showed that the diet of *P. texana* was dominated (91.6%) by four aquatic macrophytes: hydrilla (33.5%), Carolina fanwort (24.5%), parrot's feather (20.2%), and coontail (13.4%) (Table 1). Hydrilla was present in 87.9% of the stomach samples, Carolina fanwort in 78.8%, parrot's feather in 90.9% and common coontail in 57.6%.

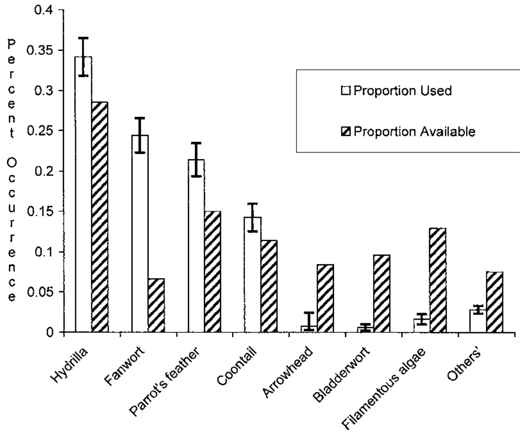

FIG. 1. Comparison of availability of aquatic plants to use by the Texas river cooter at Spring Lake, Hays County, Texas. Error bars represent 95% confidence intervals with Bonferroni correction to the *z*-statistic.

Two other aquatic macrophytes and filamentous algae were present in the diet in much smaller amounts. Delta arrowhead constituted 0.7% of the diet. It was found in 6.0% of the stomach samples, with one sample containing 75.1% of the total amount. Cone-spur bladderwort comprised 0.2% of the diet. It occurred in 9.1% of the stomachs, with 60.0% of the total amount found in one stomach. Filamentous algae comprised 4.4% of the diet, with 51.4% of the total amount in one stomach. Filamentous algae were found in 30.3% of the stomachs.

Remaining stomach contents were categorized as "other." This category included identified species occurring in trace amounts and unknown vegetation. Unknown vegetation accounted for 2.1% of the diet, found in one third of the stomachs.

Leaves were the most common plant part found in the stomach samples. Leaf buds from Carolina fanwort, common coontail, hydrilla, and parrot's feather also were consumed. Stems of hydrilla and parrot's feather accounted for only a small part of the diet.

Eighteen species of aquatic plants were encountered in Spring Lake. Seven of these species accounted for 88.7% of the plants available in the habitat: 34.7% hydrilla, 13.5% parrot's feather, 11.0% coontail, 8% bladderwort , 5.2% delta arrowhead, 4.0% Carolina fanwort, and 12.3% filamentous algae. The six macrophytes with the highest representation in the habitat also were those comprising the greatest proportion of the diet.

The log-likelihood goodness of fit comparisons showed that the proportion of plants in the stomach samples deviated significantly ($P < 0.05$, $\chi^2 = 319.691$; df = 8) from the expected proportion occurring in the habitat (Fig. 1). Comparing the availability of plants in the habitat with the 95% confidence intervals for occurrence of these plants in the diet show that Carolina

TABLE 2. Nutrient analysis of common food plants of *Pseudemys texana* at Spring Lake, Hays Country, Texas.

Aquatic macrophyte	Crude protein (CP) (%)	Digestible crude protein (DCP) (%)	Total digestible nutrients (TDN) (%)	Digestible energy (DE) Mcal/Ib	Acid detergent fiber (ADF) (%)
Hydrilla	16.3	12.6	46.4	0.93	45.4
Carolina fanwort	16.8	13.2	57.0	1.14	26.5
Parrot's feather	14.8	11.2	−2.2	−0.04	62.8
Common coontail	16.6	12.9	9.18	0.18	59.7
Delta arrowhead	19.9	16.1	56.1	1.12	25.1
Cone-spur bladderwort	14.6	11.0	37.7	0.76	49.7

fanwort, coontail, hydrilla, and parrot's feather were consumed significantly more than expected. Cone-spur bladderwort, delta arrowhead, and filamentous algae were consumed less than expected.

According to Manly's alpha preference index (Table 1), Carolina fanwort and parrot's feather were selected foods, while hydrilla, coontail, delta arrowhead, cone-spur bladderwort, and filamentous algae were consumed less than expected based on availability.

The 95% confidence interval comparison and Manly's alpha preference index were in agreement for four of these plants. The ambiguous results for hydrilla and coontail probably should be interpreted as use being in proportion to availability.

Results from nutrient analysis of selected food items (Table 2) showed that Carolina fanwort ranked second in CP and DCP. It contained the highest DE (Mcal/kg), TDN, and one of the lowest ADF values compared to other plants analyzed. The remaining plants, except delta arrowhead, were lower in quality in all categories.

DISCUSSION

Pseudemys texana is a herbivorous turtle species that forages selectively among available food plants. In Spring Lake, with 18 species of aquatic plants available, this species confines most of its foraging to leaves and leaf buds of four species of aquatic plants, hydrilla, Carolina fanwort, parrot's feather, and common coontail.

Based on the amount consumed and the high frequency of occurrence among the turtles sampled, hydrilla appears to be the most important turtle food plant in this aquatic system. However, it was not a selected species according to Manly's alpha preference index. Although Carolina fanwort contributed less to the diet than hydrilla, its presence in the habitat was substantially smaller. As indicated by Manly's alpha preference index and the 95% confidence interval, Carolina fanwort was a highly selected species.

Hydrilla is an introduced species, which grows invasively, often supplanting native aquatic plants (Langeland, 1996). From a nutritional standpoint, the displacement of native species may represent a negative impact on the river cooter's habitat, since hydrilla is nutritionally inferior to Carolina fanwort (Table 2). Information of this type may be valuable in assessing the impact of invasive plants on food quality and availability for turtles and other aquatic species. Such data are applicable to developing models for protection, recovery, and restoration of habitats for aquatic turtle populations.

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Ontogenetic Changes in the Coloration of the Northern Watersnake, *Nerodia sipedon sipedon*

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ABSTRACT.—**Juvenile *Nerodia sipedon sipedon* possess a striking dorsal pattern, whereas adults often have a more uniform dorsal coloration. Past researchers have hypothesized that this color shift occurs through a darkening of the ground color, but this hypothesis has never been tested quantitatively. Analysis of digital images of the dorsum and venter of *N. s. sipedon* individuals of all sizes from islands in Lake Michigan indicated that as snakes increase in length, the dorsal ground color darkens, but the coloration of dorsal blotches and ventral pattern does not change. Potential costs and benefits of a change in dorsal coloration while ventral pattern remains relatively constant are discussed in relation to variation in size.**

Coloration in snakes is often arranged into distinct patterns, presumably to provide protection and concealment. Brightly colored patterns may serve to warn potential predators (Brodie, 1993), while both bright and dark colors may provide crypsis (Pough, 1976). Striped and blotched patterns can serve to break up the outline of a stationary snake and to create a visual image that is confusing to predators when the snake is in motion (Pough, 1976; Bechtel, 1978). Color pattern may also provide thermoregulatory benefits (King, 1988; Bittner et al., 2002).

Despite its potential importance, there are relatively few quantitative studies of color pattern in snakes. One exception is the Lake Erie Watersnake, *Nerodia sipedon insularum*, which is variable in color pattern (King and Lawson, 1997). Although not as intensively studied as *N. s. insularum*, the closely related Northern Watersnake (*Nerodia sipedon sipedon*) also has a variable color pattern (Conant, 1938). For example, most *N. s. sipedon* individuals exhibit a color pattern shift in which juveniles with strikingly blotched dorsal patterns develop into adults that are uniformly colored (Conant, 1938; Beatson, 1976; Pough, 1976; Conant and Collins, 1998). It is unknown whether the ventral pattern of *N. s. sipedon* also changes as snakes grow in size.

How does a change in dorsal color pattern take place? Pattern elements do not change in position or relative size during the life of the snake (King, 1993). Perhaps for this reason, Conant (1938) stated that the proximate cause of the change in dorsal pattern of *N. s. sipedon* is a darkening of ground coloration, whereas

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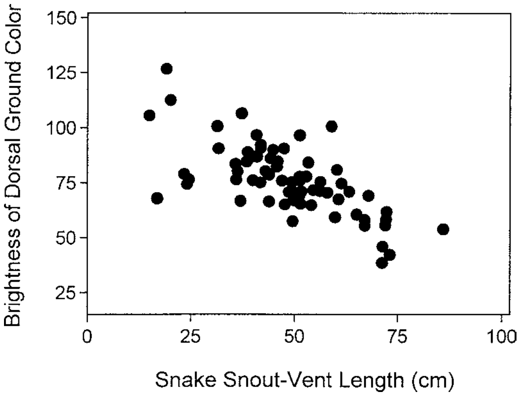

FIG. 1. The relationship between the brightness of dorsal ground coloration and the snout–vent length of *Nerodia sipedon sipedon* individuals from the Beaver Archipelago of Lake Michigan (Spearman's Rho correlation $r = -0.6524$, $P < 0.0001$, $N = 70$). See text for a definition of brightness.

blotch color remains constant. Alternatively, both aspects of dorsal pattern might change color and "meet in the middle." The present study attempted to quantitatively test Conant's hypothesis by determining which components of dorsal pattern change as snakes increase in size. The ventral coloration of *N. s. sipedon* individuals was also examined to determine whether it changes ontogentically.

MATERIALS AND METHODS

To determine how color pattern changes with snake size, I sampled populations of *N. s. sipedon* on islands in the Beaver Archipelago of Lake Michigan, specifically Beaver Island, Garden Island, and High Island. After transporting snakes to the laboratory, I measured the SVL of each animal to the nearest millimeter using the "squeeze-box" method of Quinn and Jones (1974) and determined the sex of each animal by probing for hemipenes. Snakes were marked by clipping ventral scales (Brown and Parker, 1976) and released as soon as possible after capture.

I took digital images of dorsal and ventral pattern using a Nikon E800 Digital Camera (1600 by 1200 pixels). To standardize images, I used four incandescent lights as the only source of lighting, provided a uniform background (wooden board), placed the camera at a uniform distance (20 cm) from every snake, and placed a photographic gray card in every picture as a constant color reference. Manual settings on the camera were as follows: picture quality set to "normal," light metering set to "spot" with the spot on the gray card, white balance adjusted for incandescent lighting, image adjust standard, and sensitivity and focus set to "auto." The shutter speed for all photographs was one-sixtieth of a second at a lens setting of F 3.5. I did not photograph a snake if it appeared to be approaching ecdysis (opaque eye caps), although I did measure and mark all captured snakes. I assumed that cross-sectional color data were reasonable approximations of longitudinal changes in color pattern.

For each snake, I measured the brightness of a 10 pixel sample from 10 sites each on the dorsal blotches, dorsal ground color, ventral surface, and gray card. The same gray card was used in every image, so gray card measurements provided baseline color data to compare with snake color data. Sites were chosen by progressing along the image of the snake and placing sample squares within the appropriate areas of color pattern. Sample sites were not sampled repeatedly on a given snake. I then averaged each set of 10 samples, resulting in a measurement of average brightness for each aspect of pattern for each snake. Brightness is a unitless measure of how light or dark a color is. For example, the addition of black to a color decreases the brightness of that color (Maerz and Paul, 1930; Birren, 1969). I analyzed the digital images with Image Pro Express® 4.0 software. In this software package, brightness is referred to as "intensity" and is the mean of the red, green, and blue color values of the area being measured.

I used Spearman's Rho correlation tests to analyze the relationships between snake SVL and the brightness of the various aspects of color pattern. Spearman's Rho tests were also run between the brightness of gray card measurements (from dorsal and ventral images) and snake SVL to provide a control; in theory there should be no statistically significant relationship between gray card brightness and snake size. Presence of such a relationship would suggest that experimental conditions were not properly standardized and that brightness could not be reliably measured from image to image. All statistical tests were interpreted at an alpha of 0.05. I used JMP IN 4® (SAS Institute, Inc.) for statistical analyses.

RESULTS

Forty-two snakes were captured on Beaver Island, 27 on Garden Island, and one snake was from High Island. Snout–vent length ranged from 15–86 cm. Spearman's Rho correlation tests revealed no statistically significant relationship between the brightness of dorsal blotches and SVL ($r = -0.1421$, $P = 0.0652$) or between the brightness of ventral coloration and SVL ($r = 0.1312$, $P = 0.2788$). A statistically significant negative relationship was detected between the brightness of dorsal ground color and SVL ($r = -0.6524$, $P < 0.0001$; Fig. 1). The dorsal coloration of *N. s. sipedon* appears to darken ontogenetically because the ground color becomes darker, whereas the color of the blotches remains stable. Ventral coloration did not darken with snake size. Gray card correlations were nonsignificant for both dorsal ($r = 0.1617$, $P = 0.1811$) and ventral ($r = 0.0247$, $P = 0.8391$) comparisons, suggesting that the results of the pattern correlations were not spurious.

DISCUSSION

The dorsal coloration of *N. s. sipedon* darkens as snakes lengthen because the dorsal ground color gradually darkens and approaches the color of the dorsal blotches. This result supports Conant's (1938) hypothesis. The ventral coloration of *N. s. sipedon* does not appear to change as snakes increase in size. Conversely, the venter of *N. s. insularum* becomes darker overall as these snakes increase in size (King, 1993). Perhaps *N. s. insularum* differs from *N. s. sipedon* in both ventral and dorsal coloration. The methods used in this study and the *N. s. insularum* study are dissimilar, however, and could account for the difference in results.

The cryptic value of a particular pattern may change as the size of the snake changes (King, 1992). When a small *N. s. sipedon* individual is in motion, blotch color and ground color blend to give the illusion of a snake that is uniform in color. When the snake stops, it can quickly "change color" and confuse predators. As a snake increases in size and the absolute sizes of ground color cross-bands increase, higher speeds are necessary to maintain the illusion of uniform coloration (Pough, 1976). Eventually the necessary speed may be beyond the capability of the snake. Also, a large adult watersnake may no longer benefit from a cryptic pattern because it is less vulnerable to predation than a juvenile. Although a blotched pattern may be useful at a small size, it may not be useful at a larger size.

However, this does not mean that color pattern will change with snake size. In theory, some benefit should be gained from the change. It may be that large *N. s. sipedon* individuals can gain a thermoregulatory advantage from uniformly dark coloration, as found for large melanistic Garter Snakes (*Thamnophis sirtalis*; Bittner et al., 2002). Further research will be necessary to test this hypothesis. Ventral patterning may remain stable with snake size because it is a neutral trait, because it is beneficial to snakes of all sizes, or because it is neutral at some sizes and beneficial at others. Future studies with *N. s. sipedon* should attempt to identify benefits of ventral pattern, analyze the costs and benefits of different dorsal patterns, and further describe the physiological processes involved with a shift in coloration.

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Presence of Modified Serous Glands in the Caudal Integument of the Red-Backed Salamander (*Plethodon cinereus*)

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Abstract.—Terrestrial salamanders of the genus *Plethodon* live in a world dominated by chemical-mediated senses. Little is known, however, about the histochemistry of the integumentary glands that produce chemicals used for communication and predator defense. We used histochemical staining techniques to identify the caudal integumentary glands in both male and female *Plethodon cinereus*. Our research suggests that there are three types of caudal integumentary glands, including nongranular mucous glands, and two types of granular serous glands. The two serous glands are histochemically and morphologically distinct, are concentrated in different areas around the tail's circumference, and are believed to have different function.

Many amphibians are partially or completely terrestrial. During the transition from water to land, epidermal glands arose presumably to keep the skin moist and enhance cutaneous gas exchange, and some glands took on defensive and other functions (Toledo and Jared, 1995). Eastern Red-Backed Salamanders (*Plethodon cinereus*) are of particular interest because they have specialized cutaneous glands and chemical products that are implicated in the mediation of both social behavior (Sever, 1989; Jaeger and Gabor, 1993; Simons et al., 1999) and predator defense (Brodie, 1983). Granular glands found posterior to the cloaca on the midventral surface of the tail are implicated in territorial scent marking (Simons and Felgenhauer, 1992; Jaeger and Gabor, 1993; Simons et al., 1999). This species uses a territorial marking behavior described as the postcloacal press (PCP), during which the midventral portion of the tail is pressed against a substrate, and pheromones are released (Jaeger and Gabor, 1993). Other caudal glands are presumed to secrete the adhesive secretions used to defend against predation (Brodie 1983). Behavioral studies have shown that skin swabs from dorsal versus ventral tail regions cause different reactions in conspecific males (Simons and Felgenhauer, 1992).

One problem in understanding intraspecific communication and predator defense secretions in plethodontid salamanders has been the uncertainty of the specific glandular source(s) of the pheromones (Jaeger and Gabor, 1993). Although the general location of scent mark production has been resolved, no histological studies have precisely characterized differences between the presumed "scent-marking" glands and other glands in the tail, including those functioning in predator defense.

It is logical to expect that territory marking and defense secretions are produced from different glands; thus,we hypothesized that there would be three types of caudal integumentary glands in *P. cinereus*, including mucous glands that are found throughout amphibians to moisten the skin (Toledo and Jared, 1995), and two morphochemical types of granular or serous glands associated with scent marking and predator defense. We found mucous glands and two distinct granular glands in the integument of *P. cinereus* that differ in their histochemistry, morphology and distributions in the tail.

Materials and Methods

Thirty-nine adult specimens (19 male and 20 female) of *P. cinereus* were collected from the Nature Preserve of Binghamton University, Binghamton, New York, in September 2001. Orajel (dental anesthetic) was placed on their heads, and then the animals were sacrificed rapidly via decapitation. They were then fixed in 10% neutral buffered formalin for three weeks before preparation for light microscopy.

All tails were removed from the salamander 1 mm posterior to the vent. A lateral incision was made on each tail, and the vertebrae were dissected out to avoid having to decalcify the tissue for sectioning, which compromises later staining and histological analysis. The tails were then processed and embedded in paraffin wax according to standard histological procedures (Carson, 1997). All sections were made using a rotary microtome. Sagittal sections (5–8 μm thick) of the tails were taken from 17 males and 18 females in the midline region. Transverse sections (5–8 μm thick) were taken from another two males and females 10 mm posterior to the vent.

Histochemical analysis of the tail glands was performed using the following stains and reactions: eosin (for a measure of acidophilic tissue), strong Gill-type hematoxylin (HX; Hematoxylin-Extra, Anatech Ltd. Battle Creek, MI; for a measure of basophilic tissue), periodic acid and Schiff's reagent (PAS, for neutral carbohydrates), alcian blue at pH 1.0 (AB/1.0, for sulfonic glycosaminoglycans), alcian blue at pH 2.5 (AB/2.5, for any acid mucosubstances), brilliant indocyanine (BI, for proteins), Amyloid Red (AR; Anatech Ltd; for nonionic hydrophobic groups), and Luxol fast blue (LFB, for phospholipids). Harris hematoxylin (HH) was also used to view general structure. Most staining procedures were taken from Humason (1997) or from product literature from Anatech Ltd (Battle

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TABLE 1. Histochemical staining results of serous (S1) glands, serous (S2) glands, and mucous glands from the tail of *Plethodon cinereus*.

Stain	Mucous gland	S1 granules	S1 cytoplasm	S2 granules
Harris H and eosin (H&E)	(−)	Light pink	Light pink	Pink
Hematoxylin-extra (strong gill-type)	Violet	(−)	(−)	(−)
Periodic acid schiff	Magenta	Magenta	(−)	(−)
Alcian blue pH 1.0	Turquoise	(−)	(−)	(−)
Alcian blue pH 2.5	Dark turquoise	(−)	(−)	(−)
Brilliant indocyanine	(−)	Light blue	Pale blue	Blue
Amyloid red	(−)	(−)	(−)	(−)
Luxol fast blue	(−)	(−)	(−)	(−)

Creek, MI). Brilliant indocyanine is described by Kiernan (1999). Permount was used as a mounting media. All 39 specimens were used in the analysis to identify gland types and their developmental and functional cycles.

The numbers of territorial scent-marking glands in males and females were compared using the number of male and female values above and below the population median in a Chi-square 2 × 2 contingency test corrected for continuity (Siegel and Castellan, 1988).

RESULTS

Three different gland types were identified based on their structure and histochemistry (Tables 1–2). These glands, although concentrated in different areas, occupy the entire integument of the tail (Fig. 1), and appear equally abundant and developed in both sexes. Mucous glands have ducts, appear sporadically among the caudal integumentary glands and are the smallest of the three caudal gland types. They make up approximately 20% of the gland population (Figs. 1–3) but because of their small size appear less abundant. Results from staining suggest that the mucous is a richly acidic carbohydrate. Mucous glands stained positive for Hematoxylin-Extra, which indicates that they are basophilic. They also stained positive for PAS, AB/pH 2.5, and AB/pH 1.0, which indicates they contain an acidic carbohydrate (probably sulfonated and carboxylated mucopolysaccharides).

The remaining glands are granular in nature, organized as simple acini and marked by prominent secretory granules. There are two types: serous 1 (S1) and serous 2 (S2). Both S1 and S2 glands secrete onto the surface of the skin through well-defined ducts. S1 glands make up approximately 10% of all caudal glands but appear more abundant because of their large size, and are characterized by an outer layer of simple cuboidal or columnar cells around a central lumen (Figs. 1–3). S1 are most abundant along the ventral portion of the tail, making up approximately 20% of the glands (male median % = 25, range 0–95; female median % = 17.5, range 0–80), but a majority of the tissue mass, at this location. There was no difference in the number of ventral S1 glands for males and females ($\chi^2 = 0.02$; df = 1; $P > 0.80$). These glands are often clustered, enlarged (up to 5 times the size of a mucous gland), and each gland usually occupies the entire depth of the dermis, especially in the ventral region (Figs. 2, 3b). S1 glands are occasionally found in the lateral and dorsal dermis of the tail, and appear to be more similar in size to large mucous glands at these locations (Figs. 2–3A).

The cuboidal or columnar cells of the S1 glands have intact cytoplasm staining slightly positive for brilliant indocyanine, indicating a slight proteinaceous component. Both the cytoplasm and the granules within S1 gland cells stain slightly positive for eosin, indicating mildly acidophilic conditions. Distinctive granules are also found in the lumen of S1 glands, where they are stored until discharge. S1 lumen granules stain positive for PAS and negative for AB/pH 2.5, which likely indicates a neutral carbohydrate. Mild acidophilia probably comes from a proteinaceous component.

S2 glands are the most abundant glands in the tail, making up approximately 70% of all the caudal integumentary glands (Figs. 1–3). They are most profuse along the dorsal and lateral surfaces, but may also be located occasionally along the ventral dermis of the tail. S2 glands are 2–3 times the size of mucous glands and are characterized by a perimeter of squamous cells surrounding a lumen (Fig. 1). S2 cells have little or no cytoplasm and possess few if any granules. Distinctive

TABLE 2. Histochemical tests (shown in Table 1) suggest specific chemical components of serous (S1) glands, serous (S2) glands, and mucous glands from the tail of *Plethodon cinereus*.

Gland type	Component	Histochemical indications
Mucous	Mucous	Basophilic, Acid Carbohydrate (probably sulfonated and carboxylated mucopolysaccharides), protein component is not significantly demonstrated
S1	Granules	Mildly acidophilic, neutral carbohydrate with a positive charge which probably comes from aproteinaceous component
S1	Cytoplasm	Mildly acidophilic, noncarbohydrate, slight proteinaceous component
S2	Granules	Acidophilic, noncarbohydrate or nonreactive carbohydrate, strong proteinaceous component

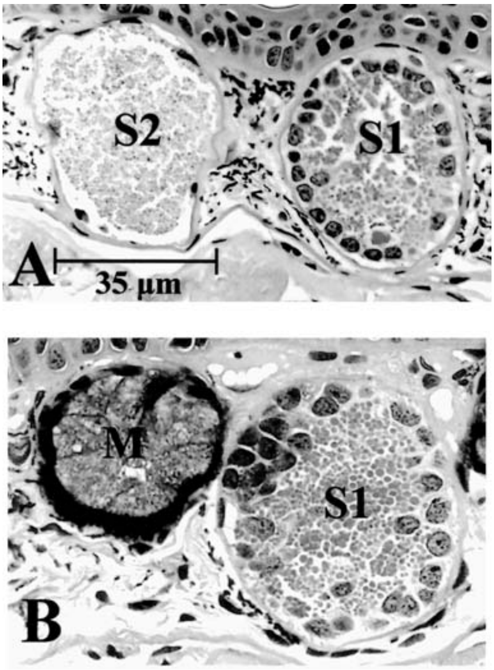

FIG. 1. Transverse sections of the dorsal tail glands in *Plethodon cinereus* stained with PAS, Alcian Blue pH 2.5, and hematoxylin at 200×. Photographs A and B show both types of serous glands (S1 and S2) and the mucous gland (M) on the dorsal surface of the tail.

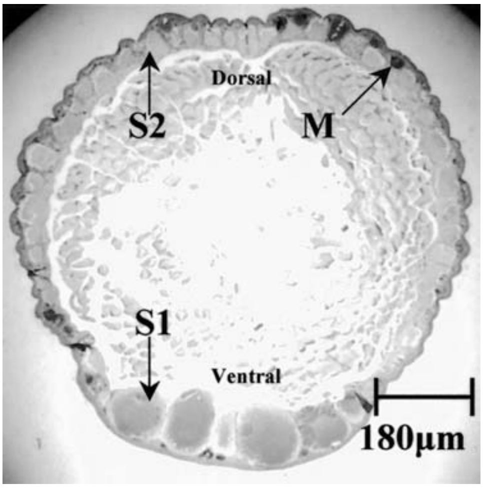

FIG. 2. Photograph of a transverse section of a *Plethodon cinereus* tail at 12×. Tail is stained with Alcian blue pH 2.5, PAS, and hematoxylin. Serous 1, serous 2, and mucous glands are indicated by S1, S2, and M, respectively.

granules, however, are found in the lumen of S2 glands and are histochemically and morphologically different from S1 lumen granules. S2 granules are 1.5–2 times larger than S1 granules, and stain positive for eosin, indicating they are acidophilic. Unlike S1 granules, S2 granules also stain positive for brilliant indocyanine, indicating a strong proteinaceous component. S2 glands are frequently seen partially emptied but only rarely completely emptied (Fig. 4).

DISCUSSION

Histochemical and morphological results suggest that there are two distinct granular serous gland types in the tail integument of *P. cinereus*. Because the function of these different gland types is still not completely understood, we named the glands based on their secretory contents: serous 1 (S1) and serous 2 (S2).

Scent-marking behavior has been described in many amphibians including *P. cinereus* (Simons et al., 1992; Jaeger and Gabor, 1993; Simons et al., 1999). In fact, several studies suggest that ventral postcloacal glands in *P. cinereus* are involved in scent-marking (Simons and Felgenhauer, 1992; Simons et al., 1994, 1999). However, the correlation between the behavior of the salamander and the production site of the pheromones is not clearly understood. Simons et al. (1999) conducted a key study on scent production in the caudal integumentary glands of *P. cinereus*. Their research proposed that ventral serous glands in the caudal integument are involved in scent-marking, and furthermore, they undergo changes in gland development after bouts of scent-marking: empty, renewing, and full. Our data support these findings, because the ventral glands were seen in all stages of development. However, Simons et al. (1999) did not distinguish between the distinct granular gland types in the tail of *P. cinereus*. Our research identifies enlarged S1 glands on the ventral surface of the tail of *P. cinereus*, and we speculate that these specific glands are responsible for scent-marking.

Staub and Paladin (1997) also described enlarged PAS positive glands on the ventral surface of *Aneides lugubris*, which they termed "modified granular glands." These glands are distinct from other granular glands in the tail and their proposed function of the modified granular glands is scent-marking. These glands appear to be similar to S1 glands in *P. cinereus*. Perhaps some amphibians that are capable of scent-marking possess differential granular glands for this specific use.

Thomas et al. (1993) also identified unique granular glands in anurans, which have been termed sexually dimorphic skin glands (SDSG). Similar to S1 and S2 glands described in this paper, SDSGs are also chemically and structurally different from other granular glands in anurans. Furthermore, SDSGs also have distinctive functions, indicating that these glands are truly a separate and distinct gland type.

Behavioral evidence also supports our findings. Salamanders react differently to the substance produced by the ventral postcloacal glands of *P. cinereus* compared to the substance produced by any other region of the tail (Simons and Felgenhauer, 1992; Simons et al., 1994, 1999). Skin swabs from dorsal versus ventral tail regions cause different reactions in conspecific males (Simons and Felgenhauer, 1992). These studies support our results in that scent-marking glands located on the ventral surface of the tail are histochemically distinct from other glands in the tail.

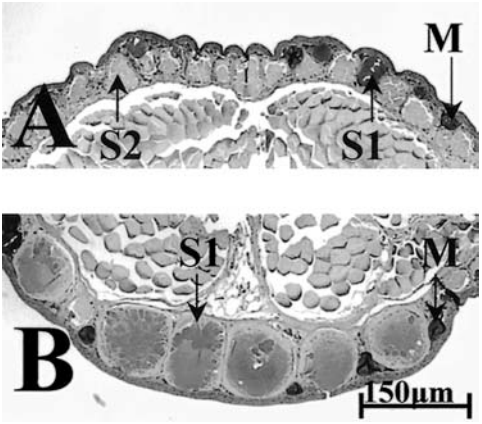

FIG. 3. Transverse-sectional detail (40×) of dorsal (A) and ventral (B) sections of the tail of a male *Plethodon cinereus* stained with alcian blue pH 2.5, PAS, hematoxylin. Serous 1, serous 2, and mucous glands are indicated by S1, S2, and M, respectively.

Granular glands containing a proteinaceous substance may serve a dual function in nutrient storage and defense (Williams and Larsen, 1986; Evans and Brodie, 1994). Considering the largely proteinaceous contents of the S2 glands and their dorsal and lateral location, we speculate that the S2 glands may play a role in both nutrient storage and defense. Granular glands are seldom completely depleted during encounters with predators (Williams and Larsen, 1986), which could compromise later energy needs. Our data support these findings in that, S2 glands are frequently seen partially emptied and only occasionally completely depleted.

Licht and Sever (1993) described seeing two types of granules in one gland type. Rarely do S1 and S2 granules appear in the same gland in *P. cinereus*; however, this has occasionally been seen in partially depleted S2 glands. We speculate that, rather than being syncytial tissue, regenerating S2 glands go through an S1 phase that only for a brief period looks syncytial. Future studies will focus on understanding this phenomenon.

We propose that S1 and S2 glands are distinct gland types, each having specialized functions: S1 glands likely play a role in ventral scent-marking; S2 glands, in defense and nutrient storage. This research may provide insight into the precise production sites of scent-marking pheromones and defensive secretions, which may then be more adequately correlated with scent-marking and defensive behavior, respectively. However, the developmental relationship between S1 and S2 glands is not understood, and both functional and developmental issues need further study.

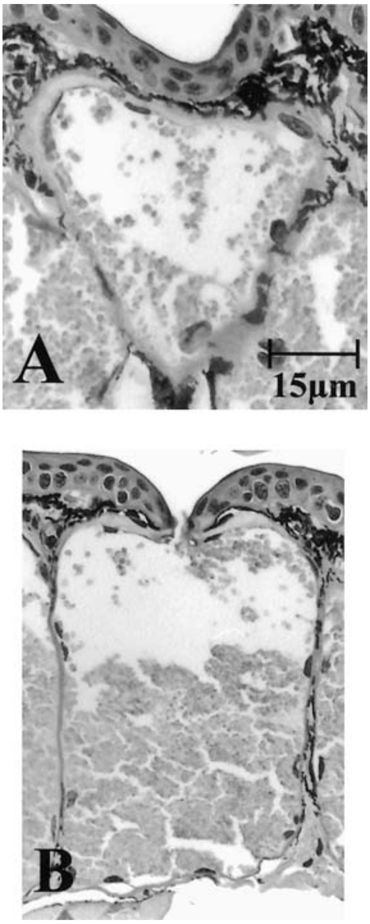

FIG. 4. Photographs of dorsal tail glands of *Plethodon cinereus* stained with alcian blue pH 2.5, PAS, and hematoxylin at 200×. Photographs show an empty S2 gland (A) and a partially emptied (renewing) S2 gland (B).

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Parasitism by Helminths in *Eurolophosaurus nanuzae* (Lacertilia: Tropiduridae) in an Area of Rocky Outcrops in Minas Gerais State, Southeastern Brazil

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ABSTRACT.—**We studied the helminth fauna of the digestive tract of the lizard, *Eurolophosaurus nanuzae*, from the Serra do Cipó, Minas Gerais State, Brazil and tested for sexual, ontogenetic, and seasonal differences in prevalence (proportion of infected individuals) and intensity of infection (number of parasites per host). We also analyzed the distribution patterns of the helminths along the digestive tract of *E. nanuzae*. We found the nematodes *Physaloptera lutzi*, *Subulura lacertilia*, *Parapharyngodon sceleratus*, and *Strongyluris oscari* and the cestode *Oochoristica vanzolinii*. Males and females differed in prevalence for *P. lutzi*, *S. lacertilian*, and *O. vanzolinii*. None of the helminth species differed in intensity of infection between sexes. Prevalence was significantly higher in adults than in juveniles for *P. lutzi* and *S. lacertila*; however, this difference was not observed for *P. sceleratus*. Only adults were infected by *S. oscari* and *O. vanzolinii*. Intensity of infection increased with lizard body size for *P. lutzi*, *S. lacertilian*, and *S. oscari* but not for *P. sceleratus* and *O. vanzolinii*. Only *P. lutzi* differed in prevalence between seasons, with lizards being more parasitized during the wet season. *Physaloptera lutzi* and *S. lacertilia* differed in infection intensity between seasons. For both species the mean intensity of infection was higher in the dry season than in the wet season. *Physaloptera lutzi* used the stomach and the other helminths used the intestines.**

Many lists of endoparasite species of amphibians and reptiles are available (e.g., Baker, 1987) and new parasite species are frequently described. However, relatively few data are available on parasite life history and their effects on the structure and dynamics of host populations (Zug et al., 2001). Most studies available on lizard helminth faunas are descriptive and/or taxonomic (e.g., Bursey and Goldberg, 1992; Vicente et al.,

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1993, 2000; Goldberg et al., 1995). In ecological studies, most available data report parasite prevalence and intensity of infection, linking the observed patterns for each lizard species with ecological features from either the host or parasite such as diet, behavior, habitat use by the host, and parasite life cycles (e.g., Rocha, 1995; Ribas et al., 1998; Vrcibradic et al., 2000, 2002). Thus, the degree that extrinsic and intrinsic factors affect nematode species associated with lizards is poorly known, limiting our understanding of the infection patterns of these parasites in lizards (Ribas et al., 1998).

The small rupiculous lizard *Eurolophosaurus nanuzae* (Tropiduridae; formerly in the genus *Tropidurus*; see Frost et al., 2001) is found in many localities of the Espinhaço mountain range, eastern Brazil, at 900 m or more above sea level (Rodrigues, 1981). Available information on this lizard's helminth fauna are limited to the description of the nematode *Subulura lacertilia* by Vicente et al. (2000) and to some epidemiological data on that species by Van Sluys et al. (2000). In the present study, we survey the helminth fauna found in a population of *E. nanuzae*, specifically addressing the following questions: (1) Which helminth species occur in the digestive tract of *E. nanuzae*? (2) What are the prevalences and intensities of infection by the parasites (overall and by species)? (3) Are there sexual, ontogenetic, and seasonal variations in prevalence and intensity of infection? and (4) Do the helminth species differ in their distribution along the host digestive tract?

MATERIALS AND METHODS

The study was carried out in an area of "campos rupestres" (rocky fields; see Eiten, 1992) habitat at the Serra do Cipó (19°12′S; 43°30′W), in Minas Gerais State, southeastern Brazil. The Serra do Cipó is located in the southern part of the Espinhaço mountain chain, which extends from south-central Bahia State to southern Minas Gerais State, encompassing altitudes above 800 m (Giulietti et al., 1987). The vegetation is rupiculous with a predominance of the families Eriocaulaceae, Xyridaceae and Velloziaceae (Giulietti et al., 1987). The cold dry season occurs from May to September and the warm wet season from October to April. Annual mean temperatures vary between 17°C and 18.5°C, with mean annual rainfall between 1450 and 1800 mm (Galdino, 2000).

We collected lizards monthly from June 1996 to June 1997, by hand or noose, and euthanized them with ether. We measured snout–vent length (SVL) with calipers (to the nearest 0.1 mm) and mass with Pesola® spring scales (to the nearest 0.2 g). Lizards were then fixed in 10% formalin and stored in 70% alcohol.

We removed the digestive tract of each lizard for helminth inspection and identification. For nematode identification, we transferred them from 70% alcohol to acetic glacial acid and later cleared them in phenol. For cestode identification, we stained them in haematoxylin and later cleared them in xylene. Finally, helminths were mounted on slides and observed under a microscope (400×) for identification, Voucher specimens of helminths are deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC 35109-35118) and in the U.S. National Parasite Collection (USNPC 91942-91943).

The total number of individual helminths of each species was counted and their location (stomach, small intestine, or large intestine) was recorded. To test for differences between sexes and between seasons, we used adult lizards to avoid any effects of ontogenetic variation. Prevalence was calculated as the percentage of infected hosts (Bush et al., 1997). To calculate the mean intensity of infection, we used the arithmetic mean number of worms in infected lizards (Bush et al., 1997). To test for ontogenetic variation in prevalence, we categorized lizards as juveniles or adults based on the smallest size of reproductive individuals (males: 32.0 mm SVL; females: 44.7 mm SVL; Galdino, 2000). We tested for differences in prevalence between males and females, between juveniles and adults, and between seasons using *Z*-tests for proportions (Zar, 1999). To test for differences between males and females and between seasons in the intensity of infection, we used Mann-Whitney *U*-tests. We analyzed the effect of ontogenetic variation on intensity of infection using simple linear regression analysis. Means are given ±1 SD.

RESULTS

We found 15,280 helminths of five species in the digestive tract of *E. nanuzae*: four nematodes (*P. lutzi* [Physalopteridae], *S. lacertilia* [Subuluridae], *P. sceleratus* [Pharyngodonidae], *S. oscari* [Heterakidae]) and one cestode (*O. vanzolinii* [Linstowiidae]). Among analyzed individuals ($N = 262$), 90.5% were infected by at least one helminth species with an overall mean intensity of infection 64.4 ± 62.4. Prevalence and mean intensity of infection for each helminth species is presented in Table 1; prevalence by sex and season is presented in Table 2.

The vast majority of *P. lutzi* ($N = 12946$) were found in the stomach (90.0%), with a few found in the small and large intestine (8.0% and 2.0%, respectively). *Subulura lacertilia* ($N = 2164$) and *Parapharyngodon sceleratus* ($N = 23$) occurred mainly in the large intestine (92.2% and 87.0%, respectively) but were also found in the small intestine (7.5 and 13.0, respectively). *Strongyluris oscari* ($N = 77$) occurred in both the small and large intestines (46.8% and 51.9%, respectively). The cestode *Oochoristica vanzolinii* ($N = 70$) was found exclusively in the small intestine.

The prevalence of *P. lutzi* was significantly greater in females (98.9%; $N = 90$) than in males (89.7%; $N = 146$; $Z = -2.72$; $P = 0.003$). Prevalence of *S. lacertilia* in males (63.7%) was significantly greater than in females (47.8%; $Z = 2.40$; $P = 0.008$). Females (23.3%) had a higher prevalence of *O. vanzolinii* than males (12.3%; $Z = -2.21$; $P = 0.01$). We did not observe intersexual differences in the prevalence of *P. sceleratus* ($Z = -0.15$; $P = 0.44$) or *S. oscari* ($Z = 1.02$; $P = 0.15$). No species differed significantly in intensity of infection between the sexes of *E. nanuzae* (all $P \geq 0.26$).

Prevalence in adults was significantly greater than in juveniles for *P. lutzi* ($Z = -8.78$; $P < 0.0001$) and *S. lacertilia* ($Z = -4.75$; $P < 0.0001$; Table 2). *Oochoristica vanzolinii* and *S. oscari* parasitized only adult lizards (Table 2). However, we found no significant differences between juveniles and adults in the prevalence of *P. sceleratus* ($Z = -0.46$; $P = 0.32$; Table 2).

Intensity of infection significantly increased with lizard body size for *P. lutzi* ($R^2 = 0.32$; $F_{1,227} = 107.8$; $P < 0.0001$; Fig. 1A), *S. lacertilia* ($R^2 = 0.04$; $F_{1,137} = 4.2$; $P = 0.04$; Fig. 1B), and *S. oscari* ($R^2 = 0.13$; $F_{1,34} = 5.11$; $P = 0.03$; Fig. 1C). There was no effect of host size

TABLE 1. Prevalence (in percent) and mean intensity of infection (arithmetic mean ± SD) of the helminth species found along the *Eurolophosaurus nanuzae* digestive tract (N = 262), at Serra do Cipó, Brazil.

Helminth species	Prevalence (%)	Mean intensity of infection (range)
Nematoda		
Physaloptera lutzi	87.4	56.5 ± 56.5 (1–310)
Subulura lacertilia	53.1	15.6 ± 24.1 (1–127)
Strongyluris oscari	13.7	2.1 ± 1.7 (1–9)
Parapharyngodon sceleratus	6.1	1.4 ± 0.8 (1–4)
Cestoda		
Oochoristica vanzolinii	14.9	1.8 ± 1.3 (1–5)

on intensity of infection by *P. sceleratus* (R^2 = 0.001; $F_{1,14}$ = 0.015; P = 0.90) and *O. vanzolinii* (R^2 = 0.000; $F_{1,37}$ = 0.014; P = 0.91).

Lizards were more heavily parasitized by *P. lutzi* during the wet season than during the dry season (Table 2; Z = –2.11; P = 0.02). For the other helminth species there was no seasonal variation in prevalence (Table 2; all $P > 0.23$).

The intensity of infection was higher in the dry season than in the wet season for *P. lutzi* (U = 1242.5; $P < 0.0001$) and *S. lacertilia* (U = 371.5; P = 0.02; Table 2). There were no differences in intensity of infection between seasons for the other species (Table 2; all $P \geq$ 0.21).

DISCUSSION

All helminth species (with the exception of *S. lacertilian*) found in the digestive tract of *E. nanuzae* commonly occur in lizards, and have already been reported for other tropidurids (e.g., Vicente et al., 1993; Van Sluys et al., 1994, 1997; Ribas et al., 1998). *Subulura lacertilia* is known only for *E. nanuzae* (Van Sluys et al., 2000; Vicente et al., 2000). Vrcibradic et al. (2000) also reported the occurrence of an unidentified *Subulura* species in *Tropidurus torquatus* in a restinga habitat in Rio de Janeiro State. They concluded that the presence of *Subulura* was accidental, because only one individual harbored these worms. However, the occurrence of *Subulura* in *T. torquatus* may suggest that the occurrence of this nematode genus in lizards may be more frequent.

The high prevalence and intensity of infection found for *P. lutzi* and *S. lacertilia* in *E. nanuzae* may be explained by the lizard's feeding habits. These parasite species require an intermediate host (Goldberg and Bursey, 1989; Anderson, 2000). Lizards presumably feed on the intermediate host of these species. Similarly, Bursey and Goldberg (1992) reported that *Oochoristica* also have intermediate hosts. However, the lower prevalence and intensity of infection by *O. vanzolinii* in *E. nanuzae* suggests that the intermediate hosts of this helminth may not be an important item in this lizard's diet, or this helminth species may not be abundant in the area studied.

Parapharyngodon sceleratus and *S. oscari* (Anderson, 2000) infect their definitive host via direct ingestion of its eggs, and thus they do not require an intermediate host (Van Sluys et al., 1994; Ribas et al., 1998). The low

TABLE 2. Prevalence (in percent) and mean intensity of infection (arithmetic mean ± SD) values for each helminth species found in the digestive tract of *Eurolophosaurus nanuzae* (Tropiduridae) at Serra do Cipó, Brazil. N is given parenthesis.

	Prevalence						Mean intensity of infection (range)			
Helminth species	Males (146)	Females (90)	Juveniles (25)	Adults (237)	Dry season (68)	Wet season (80)	Males (146)	Females (90)	Dry season (68)	Wet season (80)
Physaloptera lutzi	89.7	98.9	32.0	93.3	86.8	96.3	57.7 ± 52.9 (1–221)	59.1 ± 62.6 (1–310)	71.7 ± 59.1 (1–222)	29.3± 26.5 (1–115)
Subulura lacertilia	63.7	47.8	8.0	57.8	42.7	48.8	15.6 ± 23.0 (1–127)	16.2 ± 27.1 (1–92)	12.4 ± 14.9 (1–58)	10.5 ± 24.0 (1–127)
Oochoristica vanzolinii	12.3	23.3	0	16.5	13.2	16.3	1.9 ± 1.3 (1–5)	1.7 ± 1.3 (1–5)	1.9 ± 0.9 (1–3)	1.5 ± 1.1 (1–5)
Strongyluris oscari	17.1	12.2	0	15.2	17.7	16.3	2.3 ± 1.9 (1–9)	1.8 ± 1.1 (1–4)	1.9 ± 1.3 (1–4)	2.8 ± 2.4 (1–9)
Parapharyngodon sceleratus	6.2	6.7	4.0	6.3	4.4	3.8	1.7 ± 1.0 (1–4)	1.2 ± 0.4 (1–2)	2.0 ± 1.7 (1–4)	1.0

prevalences and intensities of infection found may result from the low frequency of encounters between host and parasite eggs, since *E. nanuzae* spends most of its life on rocky surfaces. In addition, there may also be an abundance effect of these nematode species in the studied habitat; *P. sceleratus* and *S. oscari* may occur at low densities. To evaluate this hypothesis, comparative studies in the same area with other sympatric and syntopic species such as *Tropidurus montanus* and *Cnemidophorus ocellifer* are necessary.

The distribution of parasites within host populations tend to have an aggregated pattern (Begon et al., 1996). Moreover, when nematode species coexist in the same host they tend to use different portions of the digestive tract (Ribas et al., 1998). Considering each portion of the digestive tract (stomach, small and large intestine) as a distinct microhabitat, our results suggest the helminth fauna of *E. nanuzae* differentially distribute along digestive tract. Shad (1963) suggested competitive interactions play a large role in determining the structure of parasite communities through selection for resource partitioning and niche diversification. However, host characteristics, such as physiology, body size, immunological components, and feeding habits, may also influence parasite communities (Aho, 1990; Simberloff and Moore, 1997). In individuals of *E. nanuzae* parasitized by only one helminth species, we observed the preferential occupation of specific portions of the digestive tract, suggesting that these helminths may simply occur where they are physiologically capable to survive.

Differences in helminth prevalence and intensity of infection in males, females, and juveniles may result from differences in their diets (Goldberg and Bursey, 1989). However, male and female *E. nanuzae* did not differ in diet, foraging mode or microhabitat use (Kiefer, 1998), yet there were significant differences in the prevalence of *P. lutzi*, *S. lacertilia* and *O. vanzolinii* between the sexes in our study. There may be ecological differences between male and female *E. nanuzae* unobserved by Kiefer (1998) since the author did not use all months of the year in her analysis. There was no intersexual difference in intensity of infection for any of the helminth species. Presumably, males and females of *E. nanuzae* ingest intermediate hosts of these parasites in similar proportions.

Ontogenetic variation in prevalence and intensity of infection by *P. lutzi* and *S. lacertilia* in *E. nanuzae* may result from the fact that adults, when compared to juveniles, ingest more coleopterans and orthopterans (Kiefer, 1998), probable intermediate hosts of those parasites (e.g., Goldberg and Bursey 1989; Anderson, 2000). However, the low R^2-value (0.04) found for *S. lacertilia* may characterize a Type I error. In the same way, the fact that only adults were parasitized by *O. vanzolinii* may indicate that the intermediate host of this helminth is not a common prey of juveniles. Moreover, adults have more time for contact with their parasites, increasing infections (Aho, 1990). In addition, when lizard body size increases, the length of the digestive tract increases: as in island biogeography, a larger host (islands) harbors more parasites (e.g., Ribas et al., 1998).

Seasonal variation in parasitism by *P. lutzi* and *S. lacertilia* may be caused by seasonal variation in the feeding habits of *E. nanuzae* if the availability of their intermediate hosts vary between seasons. Another hypothesis is that the life cycles of these parasites are seasonal. Conversely, *O. vanzolinii* did not vary seasonally in prevalence and intensity of infection, which may indicate that the intermediate host of

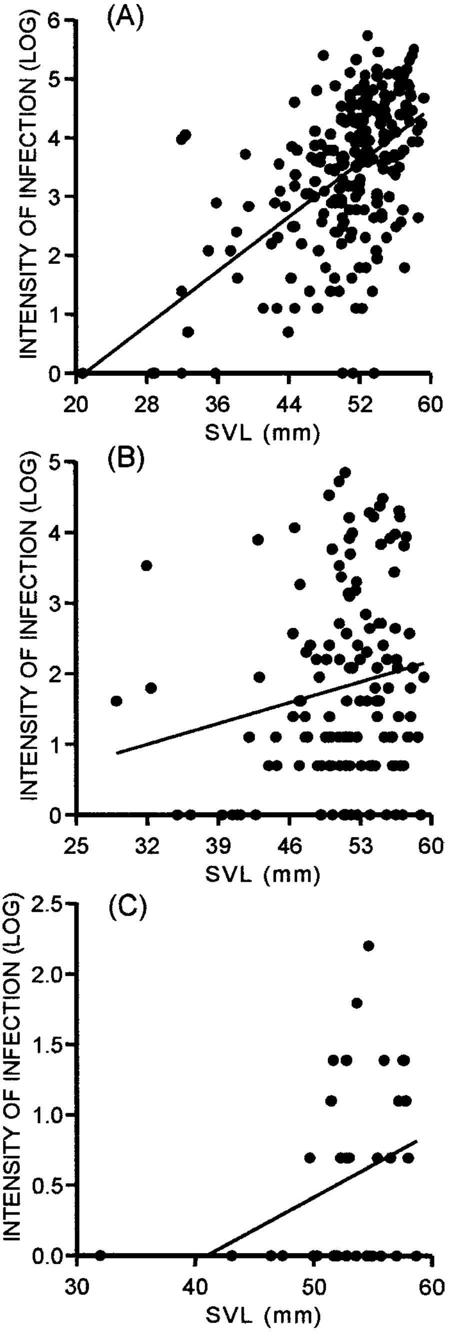

FIG. 1. Relationship between snout–vent length (SVL, in mm) of *Eurolophosaurus nanuzae* and intensity of infection by (A) *Physaloptera lutzi*, (B) *Subulura lacertilia*, and (C) *Strongyluris oscari*.

this parasite is not an important item in the diet of *E. nanuzae*, resulting in low prevalences and intensities of infection during the entire year.

The prevalences and intensities of infection of *P. sceleratus* and *S. oscari* did not vary between seasons, which can be caused by the parasite life cycle associated with the microhabitat used by *E. nanuzae*. Because the eggs of these parasites are found on the ground, and because *E.nanuzae* is a saxicolous lizard, the frequency of encounter between host and parasites is low. Probably, the host's microhabitat use may be more important than seasonal variation in the infection rates by these parasites. However, the lack of information on parasite life cycles prevents further conclusions.

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Fleshy Fruits in the Diet of Canarian Lizards *Gallotia galloti* (Lacertidae) in a Xeric Habitat of the Island of Tenerife

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Abstract.—We analyzed the frugivorous diet of the lizard *Gallotia galloti*, a Canary Island endemic, in relation to season and fruit availability in a xeric habitat from Tenerife. *Gallotia galloti* was omnivorous throughout the year (including >59% of plant material by volume in fecal pellets); only in the winter months (December and January) did invertebrates dominate the diet (>73% in volume). The most remarkable aspect of the diet was the high level of consumption of fleshy fruits, showing pronounced seasonal variation (from 30% in January to 95% in April in frequency of occurrence). Variation in fleshy fruit consumption was associated with seasonal patterns of fruits availability. A total of 4710 seeds were found in 1120 droppings analyzed during the entire year corresponding the majority to *Rubia fruticosa* and *Plocama pendula* (Rubiaceae). The extensive consumption of fleshy fruits during seven months of the year (>50% by volume) suggests *G. galloti* is an important seed disperser in the Canary Islands. These results contrast with the majority of continental lacertids that are insectivorous.

Lizards living on islands generally tend to be more herbivorous compared to lizards inhabiting mainland areas (e.g., van Damme, 1999; Cooper and Vitt, 2002 and references therein). This may be related to lower arthropod densities (Janzen, 1973), lower predation risk (Szarski, 1962), and larger body size (Szarski, 1962; Ostrom, 1963; Pough, 1973; Sokol, 1967; Cooper and Vitt, 2002) and is often associated with extremely high lizard abundance (Olesen and Valido, 2003).

Although fleshy fruits have low levels of nitrogen and protein (but high levels of easily digested sugars) and are seasonal in availability (Jordano, 2000), they have been cited as an important component of the diet of some herbivorous/omnivorous lizards on islands (see reviews in Whitaker, 1987; van Damme, 1999; Cooper and Vitt, 2002; Olesen and Valido, 2003). Omnivorous island lizards that feed on fleshy fruits must vary their diets according to seasonal availability of fruit. Therefore, it is of interest to study their diets for an entire annual cycle to assess how the degree of frugivory varies among seasons, especially because the contribution of fruit to the annual diet could be underestimated by short-term studies.

Numerous studies of island lizard diets have been completed (see reviews in van Damme, 1999; Cooper and Vitt, 2002), but they were generally restricted to short periods (usually the wet and/or the dry season), and fruits (when present) were generally lumped into a general plant matter category so that their contribution to the overall diet was not emphasized. The Canarian lizards (genus *Gallotia*, Lacertidae) are not an exception, and currently, only incomplete information about the importance of fruits in their diet has been reported (e.g., Bischoff et al., 1979; Naranjo et al., 1991; Mateo and López-Jurado, 1992; Valido and Nogales, 1994; Nogales et al., 1998).

The genus *Gallotia* Boulenger is endemic to the Canarian archipelago, and it is represented by seven extant species (Hernández et al., 2001). *Gallotia galloti* is a medium-sized lizard (maximum SVL: 145 mm) that inhabits the islands of Tenerife and La Palma. This lizard occupies a wide variety of habitats, often occurring at high densities (population densities are lower in the laurel forest), and fruits are an important item of their diet (Valido, 1999).

In this paper, we document an extensive frugivorous diet of *G. galloti* during the entire year, from a xeric zone

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TABLE 1. Composition of lizard (*Gallotia galloti*) diet during the year (September 1993 through August 1994) in Teno Bajo (Tenerife). FO: frequency of occurrence (percentage of droppings with the taxon present), and P: percentage of the total fruits or invertebrates counted in the droppings. r_s: indicates the Spearman correlation between the parameters FO and P. The highest values of each component/month appear in **bold** face. # indicates values lower than 0.006%. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: no significant. The percentage of plant material is given in volume (V). In Figure 2 the values obtained are indicated for each plant component: fruits remains, foliage and flowers.

	Sept		Oct		Nov		Dec		Jan	
	FO	P	FO	P	FO	P	FO	P	FO	P
Frugivory component										
Atriplex semibaccata	1	0.3	-	-	-	-	-	-	-	-
Lycium intricatum	-	-	-	-	-	-	-	-	-	-
Neochamaelea pulverulenta	-	-	1	0.7	-	-	1.1	2.4	-	-
Opuntia dillenii	18	0.1	11	0.1	14	0.4	11.5	0.4	-	-
Plocama pendula	**44**	**78.2**	**37**	**89.9**	**18**	**70.9**	**16.1**	**75.4**	-	-
Rubia fruticosa	29	20.8	11	9.4	12	26.3	6.9	20.9	1.9	100
Withania aristata	4	0.6	-	-	2	2.3	2.3	1.2	-	-
unidentified seeds	6	-	3	-	2	-	-	-	-	-
Animal component										
Coleoptera	9	14.5	6	5.3	5	2.9	12.6	13.5	17.3	7.9
Hemiptera	4	6.4	2	1.8	-	-	3.4	3.1	1.9	0.9
Diplopoda	2	-	-	-	1	-	-	-	1.9	-
Diptera	-	-	-	-	-	-	1.1	1.0	3.8	1.8
Formicidae	**18**	**67.7**	**20**	**88.6**	**18**	**90.9**	**33.3**	**80.2**	**30.8**	**85.0**
Others Hymenoptera	-	-	2	1.8	4	5.1	-	-	-	-
Orthoptera	2	3.2	1	0.9	2	1.1	1.1	1.0	-	-
Araneae	-	-	-	-	-	-	-	-	-	-
Gasteropoda	-	-	1	-	2	-	-	-	1.9	-
Unidentified remains	4	6.4	-	-	-	-	1.1	1.0	5.8	2.6
No. droppings analyzed	100		100		100		87		52	
Mean droppings diameter (±SD)	5.2 ± 1.1		5.1 ± 1.1		4.5 ± 1.3		3.9 ± 1.3		3.2 ± 1.1	
% (V) plant material (±SD)	66.5 (22.1)		56.1 (31.8)		49.1 (32.5)		26.1 (31.8)		23.1 (33.3)	
No. species fruits/month	5		4		4		5		1	
% droppings with:										
plant material	99		97		92		60.9		44.2	
plant material only	8		8		8		4.6		3.8	
leaves and flowers	22		31		53		35.6		42.3	
fruit remains	79		54		40		29.9		1.9	
Arthropods	92		92		92		95.4		96.2	
arthropods only	1		3		8		39.1		55.8	
r_s	0.65*		0.69*		0.60 ns		0.68*		0.29 ns	

in Tenerife (Canary Islands). We also quantify the contribution of fruits in the diet during different seasons and its relationship with fruit availability of the plant species consumed. We conclude that this species is an important seed disperser of Canarian vascular plants species (Valido and Nogales, 1994), comparable to other well-known frugivorous species (e.g., Mediterranean fall migrants and overwintering birds and mammals).

MATERIALS AND METHODS

Study Site.—Fieldwork was carried out in Teno Bajo (Barranco de Las Cuevas, northwest of Tenerife), which is one of the oldest geological sectors of the island (4.5–6.7 MYA, Ancochea et al., 1990). The climate is xeric, typical of the Canarian lowland, with an annual mean temperature of about 21°C and a mean annual rainfall of 204 mm, mainly between November and February. May through September is the driest time of the year (<3 mm of precipitation). The study site (150 m a.s.l.) encompasses some of the most characteristic xerophytic shrub land of the Canaries.

This xeric vegetation consists of a low and sparse shrub cover. Species such *Rubia fruticosa* and *Plocama pendula* (Rubiaceae), *Euphorbia obtusifolia* and *E. canariensis* (Euphorbiaceae), *Withania aristata* (Solanaceae), *Periploca laevigata* (Asclepiadaceae) and the invasive *Opuntia dillenii* (Cactaceae) are the predominant plant species (for more information about the habitat of this area, see Valido and Nogales, 1994).

Diet Analysis.—We selected five linear transects in an area of approximately 1.3 ha, which were systematically visited every 15 days from September 1993 to August 1994. These transects were chosen to ensure that the main types of vegetation in the study area were present. Along each transect (2-m width), we collected only

TABLE 1. Extended.

Feb		March		April		May		June		July		Aug	
FO	P	FO	P	FO	P	FO	P	FO	P	FO	P	FO	P
-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.5	15.1	1	3.4	-	-	5	12.1	-	-	-	-	-	-
-	-	1	0.3	-	-	-	-	2	1.3	12	6.3	3	1.2
1.2	0.01	-	-	1	#	1	#	19	0.2	23	0.2	27	0.2
-	-	1	0.9	-	-	-	-	1	0.2	9	8.3	**40**	**58.4**
23.5	**84.7**	**72**	**94.8**	**94**	**98.4**	**84**	**86.9**	**68**	**95.3**	**52**	**84.1**	33	38.8
2.5	0.2	8	0.5	10	1.5	9	1.2	12	2.9	5	0.9	7	1.3
27.2	-	15	-	4	-	11	-	5	-	3	-	1	-
14.8	42.8	9	**34.6**	11	17.2	15	12.8	12	11.3	16	7.6	18	14.4
1.2	3.6	-	-	5	9.4	9	8.0	5	5.2	4	1.9	6	4.0
1.2	-	1	-	4	-	-	-	1	-	-	-	-	-
-	-	-	-	1	1.6	1	0.8	-	-	-	-	-	-
11.1	**50.0**	**9**	**53.8**	**20**	**62.5**	**27**	**71.2**	**30**	**74.8**	**51**	**86.6**	**37**	**81.1**
1.2	3.6	1	3.8	2	3.1	3	2.4	7	6.1	3	0.8	-	-
-	-	2	7.7	3	4.7	4	3.2	1	0.9	4	1.9	1	0.6
-	-	-	-	1	1.6	1	0.8	1	0.9	1	1.9	-	-
1.2	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	1	0.8	1	0.9	-	-	-	-
81		100		100		100		100		100		100	
3.9 ± 1.6		4.6 ± 1.1		4.9 ± 1.1		5 ± 1		5.2 ± 1.3		5.9 ± 1.4		5.7 ± 0.9	
54.8 (37.6)		74.3 (23.8)		79.3 (19.1)		72.2 (23.3)		74.8 (23.8)		60.3 (30.2)		72.3 (22.5)	
4		5		3		4		5		5		5	
81.5		99		100		100		100		98		99	
7.4		11		22		14		16		8		6	
54.3		24		7		12		23		18		17	
27.2		67		95		89		79		82		78	
92.6		89		78		86		84		92		94	
18.5		1		0		0		0		2		1	
0.83*		0.75*		0.79*		0.92**		0.66*		0.54 ns		0.72*	

recent fecal pellets from *G. galloti* (the only lacertid). Prior to the first census, we removed all fecal pellets found along our transects to ensure that we would collect recent pellets in subsequent census. We tried to find at least 50 pellets per month, but in some months (December through February) it was impossible to collect this quantity because lizards were less active as a consequence of weather conditions. Each pellet was collected separately, and its diameter and length were measured using a digital calliper. Each pellet was then dissected in water with a few drops of alcohol, the content was inspected under magnification (16×), and plant and animal components were identified. Seeds were identified to species level, whereas invertebrates were identified to order (except the Formicidae).

The relative abundance of each component in the fecal pellets was quantified in several ways: (1) frequency of occurrence of each component (FO); (2) percentage of fruits or invertebrates in relation to the total number of these two items counted each month (P); and (3) percentage by volume (V), estimated to the nearest 10%, for fleshy fruit, foliage, flowers, and animal items. To calculate the minimum number of invertebrates, we did not take into account Diplopoda and Gastropoda because of their high degree of fragmentation. However, these components were less common in the diet, and the percentage we estimated is probably close to the actual value. The number of morphologically damaged seeds was also noted.

To assess the relationship between content in digestive systems of lizards and content in fecal pellets, we captured 60 lizards and sampled fecal pellets at the same site on two consecutive days in August 1994. To avoid sampling bias, these captured lizards had no access to bait inside the traps. We introduced a canula (coated with vaseline) into the esophagus and administered a saline solution. After a few minutes, the lizards regurgitated their stomach contents and often ejected feces. We analyzed these samples in the same way as described above. Once the lizard ejected the content of its digestive tract, it was immediately released into environment (less than 24 h).

Fruits Seasonal Availability.—We counted the total number of fruits in ten branches (cladodes in the case of *O. dillenii*) in about 20 plants of each of the fleshy-fruited species present in the study area. On these randomly selected branches, we made a follow-up count 15 days later to obtain data on the number of green, ripe and dry fruits, and how this varied during the year.

Statistical Analysis.—The temporal variation of animal and plant components in the diet was analyzed by ANCOVA (using the fecal pellet diameter as covariate, logarithmically transformed). This calculation was used to control for the effect of lizard body size. There is a significant relationship between the amount of plant items and lizard size (e.g., Mateo and López-Jurado, 1992; López-Jurado and Mateo, 1995, for data on *Gallotia* spp.). In this analysis, we used fecal pellet size as a predictor of lizard body size. There was a significant correlation between pellet diameter and lizard size (SVL; logarithmical transformation; Pearson correlation: $r_p = 0.79$; $P = 0.036$; $N = 24$ mean size of droppings obtained from 24 captive lizards a fed with omnivorous diet; Valido and Nogales, in press.).

Statistical analyses were done with the SPSS computer program (vers. 9.0), following the protocols given by Sokal and Rohlf (1979) and Siegel (1990). Means ± SD are provided, but standard errors are reported in the figures.

RESULTS

Temporal Variation in Diet and Frugivory.—Lizards from Barranco de Las Cuevas were omnivorous throughout the year (Table 1). The total plant components of the diet (average = 59.1%, $N = 1120$) varied strongly among months (from 23.1% in January to 79.3% in April); fecal pellets collected in December and January included mostly invertebrates (>73% in volume). Ants were the most common element throughout the year, although Coleoptera were more frequent in February (42.8%) and were equal to ants in March. Ants and beetles were present in the diet every month of the year; these two insect groups constituted a total of 91.2% of all invertebrates in the fecal pellets (Table 1).

Pellet diameters suggested that those collected during winter came from smaller lizards (Table 1), and these smaller pellets included a high fraction of insects. There was a significant negative correlation between fecal pellet diameter and percentage (in volume) of invertebrate remains (Pearson correlation, $r_p = -0.27$, $P < 0.001$). Elimination of the variation of plant material based on the dropping diameter (using a covariate analysis), revealed significant differences among months (angular transformation, ANCOVA, $F_{11,1019} = 30.39$, $P < 0.001$). This indicates that although fecal pellet size varied among months (logarithmic transformation; ANCOVA, $F_{11,1019} = 36.3$, $P < 0.001$), a pronounced variation existed in the importance of plant material throughout the year. This seasonal variation was probably associated with seasonal changes in availability of fleshy-fruits (Fig. 1). For example, fecal pellets collected in December and January had the lowest levels of plant components (<60%, FO), whereas in the other months (with the exception of February), fruits were present in >90% of the samples, reaching the maximum 100% (FO) in April, May and June (Table 1). Fecal pellet samples from November to February had a high frequency of leaves and flowers in contrast to fleshy-fruits, whereas fruits were the most abundant plant component during the rest of the year (Fig. 2).

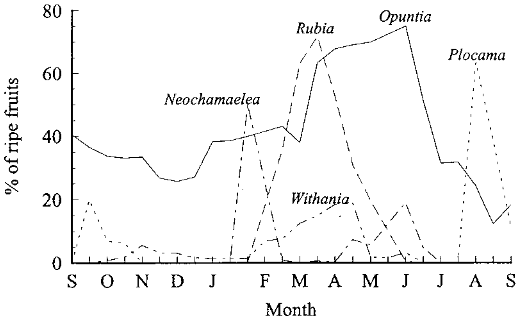

FIG 1. Availability of ripe fruits (% of the crop size) during a year (September 1993 through August 1994) in Teno Bajo (Barranco de Las Cuevas, Tenerife).

In the 1120 fecal pellets analyzed throughout the year, a total of 4710 seeds were from fleshy fruits; 49.8% *R. fruticosa*, 28.3% *P. pendula*, 9.7% *L. intricatum*, 5.6% *W. aristata*, 4.7% *O. dillenii*, and 0.5% *N. pulverulenta* (their correspondence in fruit's number consumed are 71.2%, 23.6%, 2.9%, 1.2%, 0.1%, and 0.9%, respectively). The number of different fleshy-fruited plant species in the fecal pellets ranged from one in January to five in September, December, March, and June through August. This indicates that lizards consume a greater variety of fruits at the same time when they are available. Despite the temporal overlap in fruit availability of the different plant species (see Fig. 1), most fecal pellets (83.7%) had seeds from only one plant species; 16.2% of the droppings contained seeds from two plant species and 1.9% of the droppings presented seeds from three species (Fig. 3).

Mean number of seeds per fecal pellets ranged between 0.02 ± 0.1 (January) and 6.6 ± 9.8 (September). The highest value was recorded in one fecal pellet collected in May ($N = 154$ seeds of *L. intricatum*). Only 2.7% ($N = 126$ seeds) of the 4710 inspected seeds of all species appeared damaged externally (3.4% for *P. pendula*, 1.8% for *R. fruticosa*, 9.9% for *O. dillenii*, 4.9% for *W. aristata* and 0.4% for *L. intricatum*).

The high positive correlations between frequency of appearance and number of prey items of a particular species which were statistically significant, in nine months of the year, indicates that components consumed frequently (i.e., by many individual lizards) also tend to make up the highest proportion of diet volume (Table 1).

Validation of Fecal Pellets.—To assess whether fecal pellet analysis accurately reflected the make-up of the diet, we compared these results with those from

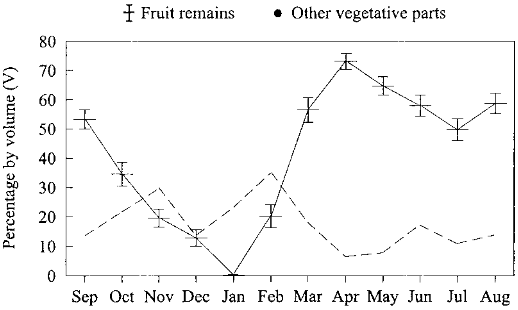

FIG 2. Volume percentage of fleshy-fruit remains and other plant parts (flowers and foliage) in the droppings of *Gallotia galloti* (September 1993 through August 1994). In the case of fruit remains, figures indicate mean (±SE). Only mean values of other plant remains are shown to simplify the figure. See Table 1 for sample sizes and total percentage of plant matter (V) in each month.

analyses of the digestive tract content (stomach and intestine) obtained in August. This showed no differences either in percentage of plant material (angular transformation, ANOVA, $F_{1,118} = 0.016$, $P = 0.89$) or number of seeds (primarily *P. pendula*; logarithmic transformation, $F_{1,115} = 0.44$, $P = 0.51$) between fecal pellets and digestive tract contents. Furthermore, the relative consumption of plant material measured did not increase significantly with lizard size ($r_p = 0.22$, $P = 0.09$, $N = 60$), although number of seeds recorded in the digestive tract increase with lizard size ($r_p = 0.28$, $P = 0.036$, $N = 60$; Valido and Nogales, 2003).

DISCUSSION

The results of this study indicate that the diet of *G. galloti* in Teno Bajo includes a large proportions of plant material, similar to diets reported for other populations of *G. galloti* in Tenerife (Barquín and Wildpret, 1975; Díaz, 1980; Valido and Nogales, 1994; Valido, 1999), and other *Gallotia* species from the Canary Islands (Naranjo et al., 1991; Mateo and López-Jurado, 1992; López-Jurado and Mateo, 1995; Nogales et. al, 1998; Pérez-Mellado et al., 1999; Valido, 1999). The overall data for *Gallotia* contrast with studies of the great majority of lacertids for which an insectivorous diet has been described (see review in van Damme, 1999).

One of the most striking features of *G. galloti* in Teno Bajo is the frequent use of fleshy fruits through the year. Fruit remains were recorded as an important percentage of the whole sample (60.2% and 41.8% in mean values in frequency of occurrence and percentage by volume, respectively), and peaking in April and May with volume values over 64%. In January, fruits were less important (only one seed from fleshy-fruit was detected), probably because in this month only juvenile lizards (with a more insectivorous diet; Valido, 1999) were active (pers. obs., and see also the lowest value in mean diameter of droppings, Table 1). Furthermore, one important consideration in this study is that fecal pellets collected in seven months had values of more than 50% of fleshy-fruit remains, both in FO and percentage by volume (V).

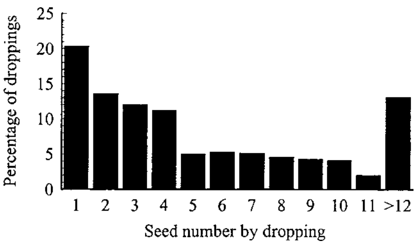

FIG 3. Frequency distribution of number of seeds found in the droppings of *Gallotia galloti* in Teno Bajo (Barranco de Las Cuevas, Tenerife). Percentages calculated from a total of 1120 fecal pellets analyzed during a year.

Presence of fruit in the diet varied with fruit availability in the study area. Fruits of *R. fruticosa* and *P. pendula* had the highest values of FO: 98.4% (*R. fruticosa* in March) and 89.9% (*P. pendula* in September). Moreover these fruits were also consumed after the period when they were first available, since lizards ate these dried-fruits directly from branches (*R. fruticosa*) or from the ground (*P. pendula*).

Although all Canarian lacertid species feed regularly on fleshy-fruits, only three previous studies have reported (in FO) the quantitative importance of fleshy-fruits in their diet: *G. atlantica* from Alegranza islet (32%; Nogales et al., 1998), and Fuerteventura (12%; Valido and Nogales, in 2003), and in an introduced population of *G. stehlini* in Fuerteventura (49%; Naranjo et al., 1991). In other lacertids some authors have reported separate values for fruits, showing the quantitative importance of this component, but especially in insular species and populations (Sadek, 1981; Salvador, 1986a,b; Hernández, 1990; Castilla and Bawens, 1991; Pérez-Mellado and Corti, 1993; Hódar et al., 1996). This island phenomenon in lacertids is also in accordance with other island species of other lizard families (e.g., Auffenberg, 1979; Schoener et al., 1982; Whitaker, 1987; Sylber, 1988; Shea, 1989; Fellers and Drost, 1991; Dearing and Schall, 1992; Willson et al., 1996; Mitchell, 1999).

In conclusion, the population of *G. galloti* we studied showed extensively, and seasonally variable, frugivorous food habits, including primarily fleshy fruits most of the year. These results are comparable to those obtained from studies of other well-known and classical frugivorous birds and mammals (Heithaus, 1982; Wheelwright, 1986; Herrera, 1989, 1995; Charles-Dominique, 1991; Willson, 1993). These omnivorous insular species may be fulfilling similar to roles birds and mammals in the seed dispersal process (Valido and Nogales, 1994; Traveset, 1995; Willson et al., 1996; Wotton, 2002). For these reasons, we emphasize the need for more exhaustive studies to demonstrate the importance of this vertebrate group as seed disperser on islands, where this mutualistic phenomenon may occur more frequently (Olesen and Valido, 2003).

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Effects of Dehydration on the Critical Thermal Maximum of the Desert Box Turtle (*Terrapene ornata luteola*)

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ABSTRACT.—**At the extreme southwestern part of its range in southeastern Arizona, annual surface activity of the Desert Box Turtle (*Terrapene ornata luteola*) is limited primarily to the three-month summer monsoon. Turtles emerging at the beginning of the monsoon are severely dehydrated. We examined the effects of dehydration on the critical thermal maximum (CTMax) of *T. o. luteola*. Compared to normally hydrated turtles, the CTMax of dehydrated turtles was depressed about two degrees from 41.0–39.0°C.**

Daily or seasonal periods of dehydration may impact ecophysiological characteristics such as oxygen transport, metabolism, and muscular and locomotor performance in various terrestrial animals (e.g., Moore and Gatten, 1989; Stefanski et al., 1989). In addition, dehydration may affect the thermal biology of both ectotherms and endotherms in various ways (Baker, 1989). For example, body water depletion is known to result in greater body temperature fluctuations, decreased heat loss, and higher core body temperatures in birds and mammals (Baker, 1987; Zhou et al., 1999), behavioral hypothermia in frogs and lizards (Crowley, 1987; Shoemaker et al., 1989; Williams and Wygoda, 1993), and decreased panting threshold at high body temperatures in lizards (Parmenter and Heatwole, 1975; Dupre and Crawford, 1986).

Ornate Box Turtles at the southwestern limit of the species' geographic range in Arizona (*Terrapene ornata luteola*) spend about nine months of the year (October through June) in underground retreats, often in burrows of the Kangaroo Rat, *Dipodomys spectabilis* (Nieuwolt, 1993, 1996; Plummer, 2003). At this locality, surface activity is associated with precipitation; most of the annual activity is restricted to the summer monsoon from July through September. Presumably, dormant subterranean *T. o. luteola* drink little as newly emerged turtles in July appear desiccated and drink extensively when given the opportunity (MVP, pers. obs.).

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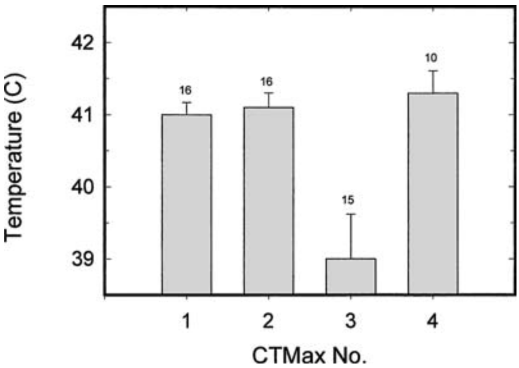

FIG. 1. Sequential CTMaxs of *Terrapene ornata luteola*. Plotted are mean ± SE. Sample sizes are indicated above each bar.

The extended inactivity period and associated desiccation in dormant southwestern *T. o. luteola* led us to examine the effects of dehydration on the species' critical thermal maximum (CTMax). To our knowledge, the effect of dehydration on CTMax has not been reported for any ectotherm or endotherm species.

MATERIALS AND METHODS

We collected seven adult male and nine adult female *T. o. luteola* from roads in the Sulphur Springs Valley in Cochise County, Arizona, in July and August 1999. Subjects were transported to Arkansas where they were provided food and water ad libitum and housed in a 7 × 15 m natural environment outdoor enclosure until moved into the laboratory for testing. In the laboratory, turtles were acclimatized for two weeks in cages at 20°C on a 16:8 photoperiod and provided with food, water, and opportunity for raising body temperature by basking. Values of acclimatization period, temperature, and photoperiod were chosen to facilitate direct comparison with previous studies on turtles (Hutchison et al., 1966). Turtles were then subjected to four sequential CTMax measurements from 11 November 1999 to 5 March 2000: (1) CTMax1 immediately followed the 14-day acclimatization period; (2) CTMax2 occurred approximately 2.5 weeks later to assess individual repeatability; (3) CTMax3 was measured approximately 90 days following CTMax2 after turtles had lost approximately 18–20% of body mass by desiccation; and (4) CTMax4 was measured after turtles were given the opportunity to rehydrate by providing them with water ad libitum for approximately one week following CTMax3.

To determine CTMax (reviewed in Lutterschmidt and Hutchison, 1997a), we used the dynamic heating/behavioral endpoint procedure as developed for turtles by Hutchison et al. (1966) and Hutchison (1979). We inserted a small thermocouple 2.5 cm into the cloaca and taped the thermocouple wire to the tail of individual turtles. Turtles were then placed individually in a 35L × 35W × 25H cm box with a 1-cm layer of sand in the bottom. Two 250-watt infrared floodlights were placed approximately 40 cm above the turtle and the height adjusted so that the body temperature of the turtle increased approximately 1°C per minute after the lights were turned on (Hutchison et al., 1966; Hutchison, 1979). We recorded the body temperature (CTMax) at a distinctive endpoint that included abrupt cessation of locomotor movements, head withdrawal, and onset of muscle spasms (Hutchison et al., 1966; Hutchison, 1979; Lutterschmidt and Hutchison, 1997b). After CTMax measurement, turtles were quickly removed from the heating box and placed under running water for 30 min after which they were either (1) returned to their laboratory cage, (2) placed in a desiccator, or (3) returned to the outdoor enclosure, depending on which sequential CTMax measurement was just completed.

We desiccated turtles by placing them on a wire mesh shelf in a 40L × 40W × 90H cm desiccation chamber through which air at 28–32°C and 45–55% RH was continuously drawn. Turtles were not fed or watered while in the desiccator. Turtles were weighed every three days until they had lost approximately 18–20% of their original body mass (about 90 days).

Six turtles were excluded from the CTMax4 measurement because four were unusually lethargic and two suffered paralysis of their hind limbs following measurement of CTMax3 (desiccation). After completion of all CTMax measurements, turtles were returned to the outdoor enclosure where they were observed daily. All turtles appeared to feed and behave normally (except for abnormal locomotion in the two turtles with paralyzed hind legs), and all 16 turtles survived and maintained body mass for at least 10 months after CTMax measurements.

Statistical analyses were conducted with SYSTAT 10.2 (SYSTAT Software, Inc., Richmond, CA, 2002). All CTMaxs were normally distributed. Variances among CTMaxs were compared with *F*-tests. Variances were homogeneous among the three hydrated turtle groups, and we compared CTMax1, CTMax2, and CTMax4 with paired-samples *t*-tests and Bonferroni probabilities. We generated a single predehydration estimate of CTMax by calculating the mean of CTMax1 and CTMax2 for each turtle (CTMax1,2). Because variances were heterogeneous among all CTMax groups, we compared mean CTMaxs of pre-dehydrated (CTMax1,2), dehydrated (CTMax3), and rehydrated (CTMax4) turtles in a paired-samples design with Wilcoxon Signed-Rank tests and Bonferroni probabilities.

RESULTS

Following rehydration after the CTMax4 measurement, body mass of turtles decreased an average of 1.6% during the approximate 3.5-month period from the measurements of CTMax1 to CTMax4 (SD = 3.3%, range –7.2% to +2.2%), presumably due to starvation.

CTMaxs of hydrated turtles increased slightly, but not significantly, from CTMax1 to CTMax2 (~0.3%; t = 0.86, $P > 0.95$) and from CTMax2 to CTMax4 (~0.5%; t = 0.13; $P > 0.95$; Fig. 1). Mean CTMax1 (41.0 C, SE = 0.17) represented our best estimate of CTMax for *T. o. luteola* because it was not subject to being affected by possible heat-hardening effects of previous CTMax measurements.

Compared to the means of CTMax1 and CTMax2, individual CTMax3 values decreased in 13 of 15 turtles. Compared to CTMax3 values, individual CTMax4 values increased in nine of 10 turtles. Mean CTMax of dehydrated turtles (39.0°C, SE = 0.62) days was approximately two degrees lower than that of hydrated groups (CTMax1,2, $Z = 2.92$; $P < 0.01$; CTMax4, $Z = 2.55$; $P < 0.05$; Fig. 1). Variance was significantly greater in CTMax3 than in the other CTMaxs ($F = 11.9$; $P < 0.5$). Although unusually low CTMax values of two turtles (each > 3 SE from the mean) contributed to the low mean and high variance in CTMax3, exclusion of these two outliers from the analysis did not alter the conclusion of a significant reduction in CTMax caused by dehydration (CTMax1,2, $Z = 2.59$, $P < 0.05$; CTMax4, $Z = 2.37$, $P < 0.05$).

Discussion

Our estimate of CTMax in *T. o. luteola* was similar to that in several other turtle species with different phylogenetic histories (Hutchison et al., 1966; Hutchison, 1979). Except for extreme northern populations, the thermal biology (i.e., selected body temperatures in laboratory thermal gradients, field activity temperatures, and field maximum temperatures) of southwestern *T. ornata* did not differ fundamentally from that of populations in more eastern and northern parts of the range (Plummer, 2003). It is known that lethal temperatures (e.g., CTMax) of reptiles may vary geographically independent of activity temperatures (Schwarzkopf, 1998), but our measurement of the CTMax of southwestern *T. ornata* (41C) did not differ appreciably from the CTMax of Kansas populations of *T. ornata* (~40°C; Legler, 1960). Compared to more northern populations, the similarity of selected laboratory temperatures and field activity temperatures (Plummer, 2003) and lethal temperatures such as CTMax (this study) suggests that the thermal biology of *T. o. luteola* in extreme southwestern populations has not responded to elevated environmental temperatures as hypothesized by Legler (1960).

The slight, but nonsignificant, increase in CTMax of hydrated turtles across sequential treatments suggests a possible heat-hardening response to repeated CTMax exposures (Linquist, 1986). We would not necessarily anticipate significant heat-hardening effects because of the extended time (1–12 weeks) between CTMax measurements. In the desert lizard, *Phrynocephalus interscapularis*, heat shock protein synthesis responds to varying exposure to heat throughout a diel period (Ulmasov et al., 1999).

Because our "dehydrated" turtles were both dehydrated and starved, we cannot separate the effects of the two treatments. However, a decrease in the CTMax of dehydrated turtles followed by an increase to predehydration levels after turtles were permitted to drink suggests that hydration level alone affected the thermal biology of *T. o. luteola*. Such an effect might especially be important in environments that are extreme in terms of heat and water availability such as at the species' southwest range margin. Unknown is whether desiccation affects other thermal biology characteristics of *T. ornata* such as preferred and activity temperatures, as it does in some lizards (Crowley, 1987).

Future studies of thermal extremes in dehydrated turtles should consider modifications of standard techniques to avoid the unanticipated injuries sustained by our animals. The injuries were an unfortunate, but likely biologically relevant, occurrence that suggests possible synergistic effects that multiple stressors may have on free-living animals. For example, in the field we have observed highly agitated behavior in turtles with rapidly increasing body temperatures at maximum voluntarily tolerated levels (35–36°C; Plummer, 2003). The body temperatures of these thermally stressed turtles were 5–6 degrees from the CTMax of hydrated turtles but only 3–4 degrees from the CTMax of dehydrated turtles. Thus, dehydration might bear on survival by rendering turtles more susceptible to thermal stresses in natural environments, possibly leading to lethargy, paralysis, and eventual death either directly or indirectly (e.g., by increasing susceptibility to predation).

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Radiotelemetry Reveals Terrestrial Estivation in Sonoran Mud Turtles (*Kinosternon sonoriense*)

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Abstract.—**Drought-related survival strategies were examined in a population of Sonoran Mud Turtles (*Kinosternon sonoriense*) inhabiting an ephemeral stream in the Peloncillo Mountains, New Mexico. Behavioral responses to seasonal drying of the habitat were investigated via radiotelemetry and repeated sampling of the resident population. All transmittered turtles were observed engaging in terrestrial dormancy for periods of 11–34 days. Two nontransmittered turtles remained in a small puddle through the drought, and no turtles were observed migrating to permanent bodies of water. Our results indicate that estivation is the dominant drought-survival strategy employed by *K. sonoriense* in our study population.**

Many species of freshwater turtles inhabit environments in which water availability varies seasonally (Mahmoud, 1969; Bennett et al., 1970; Wygoda, 1979; Christiansen et al., 1985; Grigg et al., 1986; Morales-Verdeja and Vogt, 1997). When subjected to decreasing water levels in a local pond or stream, turtles have three options: migrate to more permanent bodies of water, congregate in available local aquatic habitat, or estivate terrestrially until water supplies are replenished.

Terrestrial estivation, defined here as a period of terrestrial inactivity that coincides with decreasing water availability in the local habitat, is widely used by mud turtles in the genus *Kinosternon* (Wygoda, 1979; Iverson, 1990; Morales-Verdeja and Vogt, 1997; Wilson et al., 1999). *Kinosternon sonoriense* (Sonoran Mud Turtle), however, is generally regarded as highly aquatic and confined to permanent aquatic habitats (Ernst et al., 1994). However, it has been suggested recently that terrestrial activity may be common in at least some populations of *K. sonoriense* (Stone, 2001).

Populations of *K. sonoriense* in Arizona and New Mexico appear to differ in proclivity for terrestrial activity (Ligon and Peterson, 2002). In Arizona, *K. sonoriense* are reported to be highly aquatic, inhabiting permanent creeks, streams, and ponds. Terrestrial activity is limited to periods of heavy rain and nesting. During periods of drought, individuals converge in available aquatic habitats (Hulse, 1974; Ernst et al., 1994; van Loben Sels et al., 1997). In New Mexico, Sonoran Mud Turtles inhabit farm ponds as far as five miles from permanent water (Degenhardt and Christiansen, 1974), and there are large, stable populations in canyons that dry completely in some years (Stone, 2001). Sonoran Mud Turtles in New Mexico apparently do not congregate in available water during periods of drought, and extensive terrestrial movements, some of which exceeded 1000 m, have been documented (Stone, 2001).

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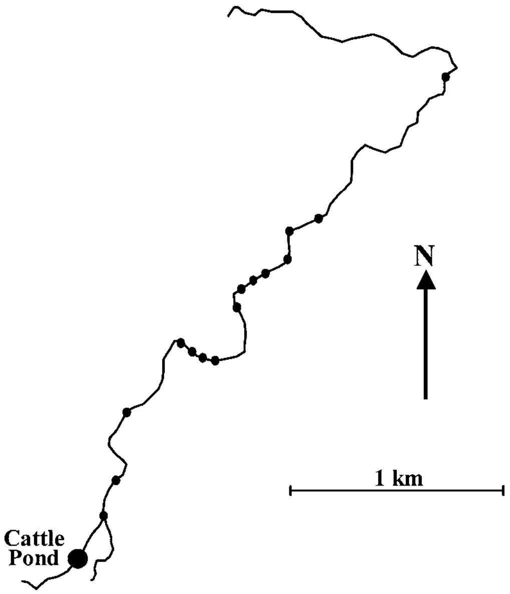

FIG. 1. Map of the study canyon in the Peloncillo Mountains of southwestern New Mexico. The large point near the south end of the canyon marks the location of the cattle pond, and smaller points along the canyon's length indicate the locations of small pools.

Using recapture patterns and movement data, Stone (2001) hypothesized that individual *K. sonoriense* frequently engaged in terrestrial activity, even when water was available in the study area. Because water is periodically unavailable during periods of drought, Stone (2001) also hypothesized that *K. sonoriense* engaged in terrestrial estivation to survive drought. Simulated estivation trials in the laboratory revealed that *K. sonoriense* is capable of surviving many weeks without food and water (Peterson and Stone, 2000; Ligon and Peterson, 2002). The response of *K. sonoriense* to such conditions was similar to *K. flavescens*, a well-documented terrestrial estivator (Seidel 1978; Iverson, 1990), treated identically (Peterson and Stone, 2000; Ligon and Peterson, 2002).

Despite these studies, there has been no direct evidence supporting the hypothesis that *K. sonoriense* engage in terrestrial estivation. In this study, we used radiotelemetry and mark-recapture techniques to examine the occurrence of such behavior in the same population of Sonoran Mud Turtles studied by Peterson and Stone (2000) and Stone (2001).

MATERIALS AND METHODS

The study area was at the eastern edge of the geographic range of *K. sonoriense*, in the Peloncillo Mountains, Coronado National Forest, Hidalgo County, New Mexico. The main study area was a gently sloping canyon approximately 3.75 km long. An ephemeral cattle pond was located approximately 0.75 km from the top of the canyon, where the elevation was approximately 1700 m. A series of discrete ephemeral pools persisted 3 km downstream from the cattle pond when streamflow subsided or became primarily subterranean (Fig. 1). However, these pools dried completely during periods of extended drought. The cattle pond was located in a narrow part of the canyon contained by a concrete dam. When full, the cattle pond was 1.9 m deep, 15 m wide and 25 m long (approximately 375 m^2 surface area). The canyon was steeper and rockier above the pond, and pools were scarce. Two permanent impounded ponds, a small permanent spring, and at least four additional ephemeral ponds were located within 4 km of the cattle pond.

The canyon was mapped previously using a 50-m tape and a compass (Stone, 2001). Sixty-seven numbered points were painted at 50-m intervals (with a few exceptions) along 3.2 km of the canyon bed. In 1999, the map was extended to include a total of 5.3 km along the main canyon bed, as well as 375 m along a small branch entering the main canyon (Fig. 1). Turtle locations were mapped according to their distance and direction from the nearest point. Mapping of turtle locations to within ±5 m was possible, as turtles were always within 25 m of a numbered point.

We measured maximum water depth in the cattle pond daily from 22 May to 4 August 1999. After the canyon pools filled in late July, water depths of 15 pools were measured at their maximum 1999 levels. Two rain gauges were placed near the lower end of the canyon and were used to measure rainfall. Rain events that were too small to be measured with the gauges (<0.1 cm) were recorded as traces.

We used hoop nets baited with sardines, chicken livers, or corned beef to capture turtles when water levels in the cattle pond permitted. Intensive hand sampling of the primary cattle pond was performed on 10 June after water levels had dropped to levels too low to set nets. We hand sampled the canyon pools regularly when the pools contained water. Four ponds outside of the study area were also sampled by hand and with hoop nets to identify possible migrants from the main study canyon. Trapping effort in the primary cattle pond was 3240 net-hours over the course of the field season and from 528–600 net-hours in ponds outside of the study canyon. We measured and marked turtles (Cagle, 1939) upon capture and mapped their locations using methods described by Stone (2001).

We attached radiotransmitters (L. L. Electronics, Mahomet, Illinois) to six male and four female turtles in the primary study canyon for periods ranging from 20–68 days. Transmitters weighed 7.8–8.0 g, which was 3.5–7.9% of the mass of the subject turtles. Transmitters were attached to the rear margin of the carapace with silicone sealant and the 25-cm antennae dragged freely behind the turtles. The sealant was allowed to dry overnight prior to release at the site of capture, after which locations were recorded at least once daily. Whenever possible, visual or tactile confirmation of each location was made. We plotted all locations onto maps of the study area. Distances to estivation sites were measured as the minimum distance from the edge of the streambed to the estivation site.

RESULTS

The cattle pond was just over half of its maximum depth on 22 May 1999 (Fig. 2). Despite a number of trace rain events in late May, the water level dropped 0.5–4.0 cm per day until mid-July. At its lowest level, the cattle pond consisted of a puddle 10 cm deep with a surface

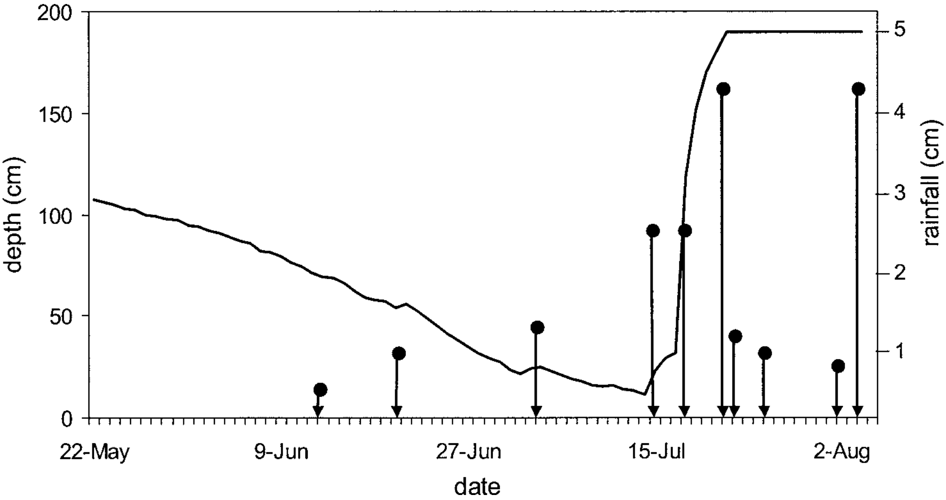

FIG. 2. Water depth in the cattle pond located in the primary study canyon in the Peloncillo Mountains, New Mexico. Depths are based on daily measurements. Rain events (right axis) during summer, 1999 are indicated by arrows. Trace showers are excluded from this figure.

area of about 2 m^2. On 15 July, 2.5 cm of rain fell, causing the cattle pond to refill to a depth of 32 cm (17% of maximum depth). An additional 9.0 cm fell during 18–23 July, filling the cattle pond and causing water to flow over the dam and down the canyon. The cattle pond was still full at the conclusion of field research on 4 August 1999.

The canyon above and below the cattle pond was dry on 22 May 1999, except for one small, algae-filled pool (appproximately 1.0 m^2 surface area) under a rock overhang. There was no water in the canyon pools until 1.0 cm of rain fell on 20 June. This shower was sufficient to fill small basins in areas where bedrock was exposed. Thirteen turtles were captured by hand from these pools within 12 h of the rain. A storm on 3 July produced 1.2 cm of rain, enough to fill all the main pools along the length of the canyon (Fig. 1). Rainfall during 18–23 July turned the canyon into a contiguous flowing stream. Stream levels were at their maximum observed depth at the conclusion of field research on 4 August 1999.

Capture success was associated positively with water availability. There was no water in the canyon pools between 22 May and 20 June, and no turtles were captured there. Water levels fell in the cattle pond during this same period. Thirty-two individuals were captured 55 times in the cattle pond, including four individuals that were subsequently outfitted with radiotransmitters. Canyon pools contained water between 20 June and 15 July. Twenty-six individuals were captured 33 times in canyon pools, including six individuals that were outfitted with radiotransmitters. Water levels continued to fall in the cattle pond from 20 June to 15 July, and only one turtle was captured in the cattle pond. Water was available in both the canyon pools and the cattle pond between 16 July and 4 August, and 83 individuals were captured a total of 119 times. Of these captures, 50 turtles were captured in the cattle pond, 29 turtles were captured in the canyon pools, and four turtles were captured on separate occasions in both the cattle pond and the canyon pools.

Fifty-six turtles were captured in three ponds outside of primary study canyon. A fourth pond outside of the study canyon was sampled, but no turtles were captured. No within-season migration between the study canyon and other ponds was detected by our sampling efforts. However, two turtles that had been marked previously in the primary study canyon were found in an ephemeral pond 1.5 km south of the primary cattle pond. The first was a male originally marked in August 1997 and caught a second time in May 1998. The second was a male originally marked in May 1998. These migrations must have included extensive overland movements, as the two watersheds are separated by steep, rocky terrain.

Four turtles captured originally in the cattle pond were outfitted with radiotransmitters between 25 and 31 May. All four turtles remained in the cattle pond until 14–15 June, when all entered terrestrial estivation. All four returned to the cattle pond when it filled with water on 18 July. Three turtles were captured in canyon pools and outfitted with radiotransmitters on 22 June, when rain filled several small basins in the canyon. Three other turtles were captured in canyon pools and outfitted with radiotransmitters on 3 July, when additional rain filled all the major pools in the canyon. All six of these turtles commenced terrestrial estivation within 24 h after they were released. All six turtles returned to water during 15–16 July, when the canyon became a flowing stream.

Thus, all 10 turtles wearing radiotransmitters engaged in bouts of terrestrial estivation (Table 1). The duration of observed estivation ranged from 11–34 days. All turtles wearing radiotransmitters emerged from estivation and returned to water during 15–18 July.

Turtles estivated in shallow depressions in which the turtle's posterior was sometimes visible. Estivation sites were located under clumps of bear grass (*Nolina microcarpa*), sotol (*Dasylirion wheeleri*), pointleaf manzanita (*Arctostaphylos pungens*), or under large rocks. Estivation sites were 1–79 m (19 ± 23.8; Table 1) from

TABLE 1. Activity ranges and estivation movements of 10 radiotransmittered turtles in the Peloncillo Mountains, New Mexico. "Days" is the total number of days for which each turtle carried a radiotransmitter. "Estivation Movement" is the distance from the streambed to the estivation form. **Bold** font represents animals originally released into the cattle pond.

I.D.	Sex	Days	Estivation movement (m)
62	m	29	1
18	f	35	29
227	**f**	**67**	**79**
2300	m	42	1
172	m	26	8
179	m	20	1
250	f	41	5
161	**m**	**65**	**15**
7	**m**	**67**	**27**
104	f	68	24

the dry streambed, and were located in a variety of habitats that included dense pine canopy with a thick layer of litter and the top of a sparsely vegetated rocky ridge with full exposure to the afternoon sun.

DISCUSSION

Kinosternon sonoriense in this study population exhibit terrestrial estivation in response to extended drought. Thus, they are confirmed to make behavioral use of the physiological capacity for estivation that has been demonstrated in the laboratory (Peterson and Stone, 2000; Ligon and Peterson, 2002). All animals equipped with radiotransmitters engaged in terrestrial estivation during the study, all were estivating at the onset of the rainy season, and all returned to the aquatic environment within a few days of the onset of the rainy season.

Little evidence supports the two alternatives to estivation for surviving droughts (i.e., migrating between bodies of water and/or converging in remaining water holes). Two turtles originally marked in the study area were found to have migrated over rough terrain to a pond in a watershed 1.5 km to the south. However, these two turtles represent a small proportion of the turtles captured. Similarly, a small puddle of water was present in the main study canyon in mid-July prior to its refilling with rain water, and two turtles remained in this puddle, apparently never going onto land to estivate. Again, the behavior exhibited by these two turtles was atypical of the population. We are confident that no other small bodies of water persisted through the summer in the canyon, suggesting that less than 1% of the population, estimated at 212 individuals (Stone, 2001), remained entirely aquatic throughout the study.

Our findings differ from descriptions of the terrestrial habits of *K. sonoriense* in Arizona. In particular, Hulse (1974:16) stated that, "*Kinosternon sonoriense* is totally aquatic, seldom venturing onto land except to lay eggs. In several years of collecting I observed only one specimen, a basking male, out of the water." Evidence suggests that behavioral differences affect the capacity for estivation among populations (Ligon and Peterson, 2002). However, it remains unclear if the lack of published data on estivation in Arizona *K. sonoriense* accurately reflects the degree to which estivation is used by this species. Future research aimed at investigating terrestrial activity in Arizona populations of *K. sonoriense* and in other *Kinosternon* that are putatively confined to permanent aquatic habitat (e.g., *Kinosternon hirtipes*) is clearly warranted.

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Reproductive Characteristics of *Colostethus marchesianus* from Its Type Locality in Amazonas, Brazil

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Abstract.—**We assessed the reproductive characteristics of the type population of *Colostethus marchesianus* on the banks of the Uaupés River (upper Rio Negro basin, Amazonas, Brazil). *Colostethus marchesianus* occupied floodplain forest areas on the river margin, and the peak of the reproductive season coincided with the peak of the rainy season. Sexual size dimorphism was not detected and adult size ranged from 15.0–17.0 mm snout–vent length. Males were territorial and employed three types of vocalization, two high-intensity advertisement calls and a lower intensity courtship call. Duration of courtship and mating behavior was less than 30 min and involved 2–3 sequential cephalic amplexi. From 1–5 egg clutches were found within each of several oviposition sites within male territories. Number of eggs in 63 clutches ranged from 10–29. Only males were observed to carry tadpoles (stage 25) to water on their backs. The mean number of transported tadpoles was similar to the mean clutch size. Additional descriptive information on reproduction and general behavior, which is highly variable among *Colostethus* species, is needed to confirm the specific status of other populations identified as *C. marchesianus* throughout the Amazonian basin.**

The Neotropical dendrobatid genus *Colostethus* has more than 100 described species (Frost, 2000). The few species that have been studied show great interspecific variability in reproductive behavior. For example, *Colostethus beebei* exhibit biparental care and feeding of trophic eggs to tadpoles (Bourne et al., 2001), *Colostethus stepheni* have two periods of amplexus that occur about 5 h (Juncá, 1998) apart, and *Colostethus caeruleodactylus*, has prolonged and complex courtship, with vocal, visual, and tactile components but no amplexus (Lima et al., 2002a). At least four *Colostethus* species have endotrophic tadpoles, three of them are nidicolous (*C. stepheni*, Juncá et al., 1994; *Colostethus chalcopis*, Kaiser and Altig, 1994; and *Colostethus nidicola*, Caldwell and Lima, 2003), and one is exoviviparous (Lescure, 1984). Territorial behaviour of males and/or females also varies among species. In *Colostethus palmatus* and *Colostethus inguinalis*, both males and females are aggressive (Lüddecke, 1999; Wells, 1980a), whereas in *Colostethus collaris* and *Colostethus trinitatis*, females are more aggressive than males (Dole and Durant, 1974; Wells, 1980b). In *C. stepheni* and *Colostethus caeruleodactylus*, only the males are aggressive (Juncá, 1998; Lima et al., 2002). Thus, aspects of reproductive behavior can separate morphologically similar species of *Colostethus*.

Colostethus marchesianus, as currently recognized, is a widespread species that occurs throughout much of Amazonian Brazil, Colombia, Ecuador, Peru, and Bolivia (Coloma, 1995; Caldwell et al., 2002a,b). The original description of *C. marchesianus* provided only a short description based on the morphology of two females collected during an expedition to Amazonas, Brazil (Melin, 1941). Subsequent redescriptions of *C. marchesianus* based on individuals from the type locality provided information on live coloration, vocalization, and tadpoles (Caldwell et al., 2002a,b). Information about behavior is available only for populations identified as *C. marchesianus* from Santa Cecilia, Ecuador (Crump, 1974) and a locality near Manaus, Brazil (Juncá, 1998). Here, we describe territoriality, courtship, parental care, and clutch and oviposition site characteristics of *C. marchesianus* from the type locality.

Materials and Methods

The type locality of *C. marchesianus* is located in the state of Amazonas, Brazil, at the village of Missão Taracuá, on the upper Uaupés River, within the present limits of the Alto Rio Negro Indian Reservation (Melin, 1941). We visited the area from 30 April through 8 May 2000. Our visit coincided with the peak of the rainy season but before the maximum seasonal flood of the Uaupés River (Fig. 1). We found no *Colostethus* in deforested areas around the village or inland from the

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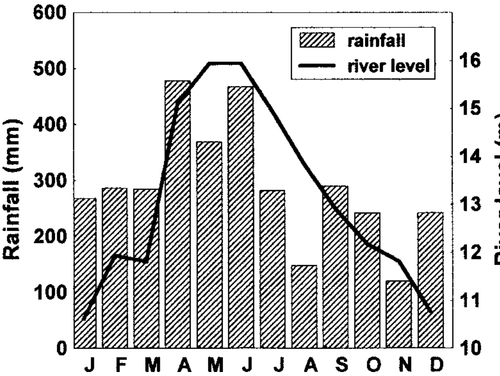

FIG. 1. Monthly mean values of rainfall and the water level of the Uaupés River at Taracuá village for the period 1998–2000. Data were obtained from a local weather station kept by CPRM (Compania de Pesquisas de Recursos Minerais).

river. Of 17 sites searched on the margins of the Uaupés River and a smaller, nearby tributary called Igarapé da Chuva, only one species of *Colostethus* was found. The subsequent comparison of the specimens we collected with the two syntypes on which the original description of the species is based (Melin, 1941) confirmed that the population studied by us belongs to the same species collected in 1924, and described as *C. marchesianus* in 1941 by Melin (for more details, see Caldwell et al., 2002b).

The rainfall and water level of the Uaupés River were collected daily (rain gauge and stage gauge) from a local weather station at Taracuá village. We calculated the monthly average of these data for the period 1998–2000 (Fig. 1).

We made field observations of reproductive behavior at six sites, but most observations were made during six days of fieldwork at two sites within 1 km north and 1 km south of Missão Taracuá. Most males were located early in the morning when they were vocalizing. No males were heard vocalizing at midday. Only males that had been located during the morning were followed when vocalization started again in the afternoon. Calling males, pairs in courtship, and males tending eggs and tadpoles were observed through focal animal observation. Males and females were caught by hand, and SVL was measured to the nearest 0.1 mm. The areas around calling perches where males vocalized were searched for eggs. Leaves on which clutches occurred were measured, and eggs and tadpoles of each clutch were counted. A random sample of clutches was taken to the laboratory, and eggs were measured with a stereoscopic microscope. Males carrying tadpoles were captured near the water or after tadpoles climbed onto their backs at oviposition sites, and the number of tadpoles was counted. We used *t*-tests to assess differences in SVL between males and females and Pearson correlations to analyze the relationship between clutch variables and parental SVL.

RESULTS

Reproductive Habitat and Spatial Distribution of Males.—Breeding *C. marchesianus* were found only in areas of igapó (forest on the river margins that is flooded by up to several meters of water in the rainy season). Individuals occupied areas of dense leaf-litter in the immediate vicinity of the water's edge. The 29 males that were observed at irregular intervals during a full daily cycle used sticks or leaves as calling perches that were usually <30 cm above ground, and <1 m from their oviposition sites. No agonistic interaction between males was observed, but no male was observed to call <2 m from another, and our observations indicate that males always vocalized within the same small (<2 m^2) area.

Courtship and Mating Behavior.—The following generalized description is based on the observation of three mating pairs. The female moved directly toward the vocalizing male. Upon detecting the female, the male proceeded toward the egg-laying site. If the female stopped, the male resumed vocalizations, alternating typical advertisement calls with softer and shorter syllables, which we defined as "courtship calls." Once inside the oviposition site, the male produced alternating courtship calls and low-intensity advertisement calls until the female followed him into the oviposition site. Inside the oviposition site, the male continued to use occasional courtship calls. Within 15 min, the first of 2–3 cephalic amplexi occurred, which consisted of the male clasping the female with his front limbs pressed against her chin (also described by Juncá, 1998). The interval between each amplexus varied from 1–5 min, and during those intervals, the male emitted low-intensity advertisement calls. The male left the oviposition site first, whereas the female stayed in the oviposition site for about 10 min longer and circled around the clutch. The male went back to the oviposition site about 10 min later and sat on top of the eggs. In two cases, we could see that the male urinated on the clutch. The male resumed advertisement calls shortly after leaving the oviposition site. One male mated with another female earlier in the same day. Courtship and mating lasted less than 1 h. In addition to the three mating pairs, three pairs were found soon after egg laying. All egg laying that we observed occurred before 1000 h.

Oviposition Site Selection.—Leaves used as nest sites were of variable size and shape but were always curved or bent to form a roofed chamber for the egg clutches. Most oviposition sites were in leaves of *Araceae* sp., but leaves of *Bromeliaceae* and *Marantaceae* were also used. From one to four oviposition sites were found in areas where males vocalized (*N* = 29). Most oviposition sites contained 2–3 clutches (86%), with a range of 1–5 clutches per oviposition site. Clutches in a single oviposition site were usually in various developmental stages.

Adult and Clutch Characteristics.—Mean SVL was 15.7 ± 0.5 mm for reproductive adults (range 15.0–17.0, *N* = 36). Mean SVL of males (mean = 15.6 ± 0.5 SD, range 15.0–16.6 mm) was not significantly different from that of females (15.9 ± 0.4 SD, range 15.1–17.0 mm) in our sample of 20 males and 16 females ($t = 1.9$, $P = 0.06$). Mean clutch size was 18.6 eggs (range 10–29, *N* = 63) and was not significantly correlated with female body size ($r^2 = 0.16$, $P = 0.43$, $N = 6$). The mean diameter of recently laid eggs was 1.5 mm (range 1.2–1.7, *N* = 42).

Parental Care.—Only males were observed to urinate on the eggs, attend clutches, and carry tadpoles. On three occasions, we observed tadpoles climb onto the backs of males inside the nest. In each case, the male spent 20–30 min sitting on the jelly and shifting its body laterally while tadpoles made "swimming" movements in the direction of the male body. One male was observed to repeat this operation twice in the same oviposition site during the same day, carrying 19 tadpoles from 1115–1135 h and 24 tadpoles from 1145–1210 h. Another male carried 26 tadpoles and left behind 20 at the same stage of development. We captured eight males carrying stage 25 (Gosner, 1960) tadpoles on their backs. The mean number of tadpoles carried by a male at one time was 20.6 ± 9.7 (median = 18.5, range 8–37) and was not correlated with male body size ($r^2 = 0.001$, $P = 0.93$, $N = 8$).

Discussion

At the beginning of May in Taracuá, we found many calling males and oviposition sites of *C. marchesianus*. Most oviposition sites contained >2 clutches, many of them with tadpoles ready to be carried to the water. These observations indicate that the peak of reproductive activity in this population coincides with the peak of rainfall, which occurs from April to June (Fig. 1). Crump (1974) found gravid females of a *Colostethus* species (identified as *C. marchesianus*) throughout the year at a locality in Amazonian Ecuador. Hence, she classified the species as a continuous breeder. This is not likely the case at Taracuá, because the breeding grounds of *C. marchesianus* were about to be flooded. We found no individuals calling further inland or in deforested areas around the village. During the low water season, water pools in the forest dry out, and the border of the river is separated from the forest edge by wide sand beaches. We believe these circumstances prevent *C. marchesianus* from reproducing in the dry season because of a lack of adequate water bodies to which they could carry their tadpoles. The reproductive season of most *Colostethus* species coincides with the rainy season (e.g., *C. inguinalis*, Wells, 1981; *C.* cf. *marchesianus* and *C. stepheni*, Juncá, 1998; *C. subpunctatus*, Fandiño et al., 1997; *C. caeruleodactylus*, Lima et al., 2002a).

Colostethus marchesianus displayed three types of calls at Taracuá: two advertisement calls (Caldwell et al., 2002b) and a much lower intensity courtship call (this study), that might aid in stimulating oviposition after a female has been led to the oviposition site. Two advertisement calls have also been recorded for a population identified as *C. marchesianus* near Manaus (central Amazon), but one of these calls was also used as a courtship call inside the oviposition site (Juncá, 1998). Only one advertisement call and one distinctive courtship call have been recorded for *C. inguinalis* (Wells, 1981). A three-call vocalization repertoire has been recorded for several other *Colostethus* (e.g., *C. stepheni*, Juncá, 1998; *C. palmatus*, Lüddecke, 1999; *C. beebei*, Bourne et al. 2001; *C. caeruleodactylus*, Lima et al., 2002a) that included a call to attract females, a courtship call, and a call to express aggressiveness toward male territory intruders. Because we observed no aggressive interactions between males at Taracuá, we do not know whether this population displays a fourth call for territorial defense.

Courtship and mating behavior of *C. marchesianus* from the type locality was characterized by short duration (15–20 min), repeated bouts of cephalic amplexus and females leaving the nest 10–15 min after the male. Intermittent amplexi and the female remaining in the nest after the male had left were also observed for *C. marchesianus* near Manaus (Juncá, 1998). Courtship and mating sequences are generally more elaborate and last more than 4 h in other *Colostethus* (e.g., *C. collaris*, Dole and Durant, 1974; *C. stepheni*, Juncá, 1998; *C. beebei*, Bourne et al., 2001; *C. caeruleodactylus*, Lima et al., 2002a). The longest recorded courtship for dendrobatids was for *Epipedobates femoralis* in Peru, in which the female stays for over two days in the male's territory, and is courted daily (Roithmair, 1992).

Most clutches of *C. marchesianus* were laid in oviposition sites where at least one other clutch had already been deposited. Multiple clutches in the same nest have also been observed in *C. caeruleodactylus* (Lima et al., 2002a), *C. stepheni* and *C. marchesianus* near Manaus (Juncá, 1998). These records may reflect active female choice for oviposition sites that have already been "approved" by other females or a shortage of adequate nesting sites.

Our data indicated that tadpole transport in *C. marchesianus* at its type locality is carried out exclusively or predominantly by males, although females may occasionally transport tadpoles in some *Colostethus* species (Aichinger, 1991; Juncá, 1998; Lima et al., 2002b). The mean number of transported tadpoles, and our observations of males picking up tadpoles and leaving other tadpoles (25 stage) at oviposition sites, suggests that males usually do not transport more than one clutch at a time to the water.

Colostethus from Santa Cecilia, Ecuador (Crump, 1974), have reproductive characteristics that are similar to *C. marchesianus* at Taracuá in that males carry tadpoles to the water, fresh eggs are about 1.5 mm in diameter, and female size is not related to clutch size. However, mean clutch size at Santa Cecilia was smaller than at Taracuá, even though mean body size was larger. Females with mature ovarian eggs were 13% larger than males, and gravid females were 4% larger. Reproduction was apparently continuous throughout the year at Santa Cecilia, whereas reproduction is likely to be seasonal at Taracuá, and females are only about 2% larger than males. *Colostethus marchesianus* at Manaus, Brazil (Juncá, 1998) have similar reproductive characteristics to *C. marchesianus* from Taracuá in that reproduction occurred only during the rainy season, more than one amplexus per clutch occurs, more than one clutch is laid in one site, and females stay in the nest longer than the male. However, *C. marchesianus* from Manaus has no distinctive courtship call. Additional information on reproductive traits of *Colostethus* at these and other sites would aid in assessing their taxonomic status.

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Limited Genetic Heterozygosity and Status of Two Populations of the Ramsey Canyon Leopard Frog: *Rana subaquavocalis*

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Abstract.—**Using starch gel electrophoresis, we examined proteins specified by 41 presumptive loci representing two breeding populations of *Rana subaquavocalis*, a recently described species of leopard frog from Arizona. Individual and population levels of heterozygosity were low. Mean number of alleles detected (1.2) and mean levels of individual heterozygosity were higher at the Barchas Ranch location (0.042) compared with those from Ramsey Canyon (0.029). Nine loci among the 41 surveyed were polymorphic. Four of these were common to both populations. Each population was polymorphic for the remaining three loci, and each possessed one unique allele. Both populations went extinct by 1996.**

Rana subaquavocalis is a recently described species of leopard frog and member of the *Rana pipiens* complex from southeastern Arizona (Platz, 1993). In North America, there are currently seven additional species of leopard frogs, two widely distributed within the eastern half of the United States, including *R. pipiens* and *Rana sphenocephala*. Six species, *R. pipiens*, *Rana berlandieri*, *Rana blairi*, *Rana chiricahuensis*, *Rana yavapaiensis*, and *Rana onca* occur in the western half of the United States, and of these, the last five have more restricted distributions. *Rana subaquavocalis* was originally described (Platz, 1993) from a single locality in the Huachuca Mountains of southeastern Arizona. Subsequently, four additional populations were documented with breeding known to occur at two of these. No population estimate for any of the sites exceeds 50 adults, and the localities are geographically restricted to a 10 km radius within the Huachuca Mountains. As a result, the U.S. Fish and Wildlife Service (15 November 1994, Federal Register Notice) designated *R. subaquavocalis* as a category 1 candidate for federal listing as endangered or threatened. Collection and possession has been prohibited under Arizona Game and Fish Commission Order 41. Populations containing 50 or fewer individuals generally warrant concern regarding the long-term potential for loss of genetic variability (Shaffer, 1981). In this paper, we report the status of genetic variability in the two known breeding populations of *R. subaquavocalis*, identify factors that explain the low levels of heterozygosity and document the eventual fate of each population of this rare species.

Materials and Methods

A sample of 15 large tadpoles from the concrete lined pond (type locality) in Ramsey Canyon (elevation 1622 m), 7 km southwest of Sierra Vista; 31°26′59″N, 110°18′13″W, Cochise County, Arizona, were collected in July 1992 and flash frozen in liquid nitrogen, as were 17 newly metamorphosed juvenile leopard frogs from the Barchas Ranch stock tank (elevation 1528 m); 31°28′28″N, 110°17′53″W, Cochise County, Arizona. Specimens were shipped on dry ice to the laboratory for tissue preparation. Muscle, liver, and heart tissues were dissected from juveniles and homogenized using a grinding buffer (0.1 M Tris, 1mM EDTA, 0.1mM NAD, 0.1mM NADP, 0.25% v/v B Mercaptoethanol, pH 7.0). Gut contents were removed from tadpoles and entire individuals homogenized in grinding buffer. Tissue homogenates were stored at –70°C, thawed, and centrifuged at 13,000 rpm for 10 min prior to electrophoresis in 12% starch gels (60% Starch-Art starch, 40% U.S. Biochemicals starch). Two buffer systems were used: TAG 7 (Wright and White, 1992) and LiOH (Turner, 1983). The products of 41 presumptive protein-coding loci (Table 1) were resolved and genotype counts were analyzed using the BIOSYS-1 program (Swofford and Selander, 1981).

One of us (JEP) kept in close contact with personnel at the Ramsey Canyon Mile High Preserve since 1990 and resided on site in Ramsey Canyon from May 1 to 26 June of 1995 to monitor population composition and breeding activity at the type locality. Adult counts were made three to five nights per week. Counts made in 1990, 1991, and 1992 were total counts of transformed individuals. Those made after this time only included adults. Ramsey Creek was surveyed once per week during the 1995 season from the visitors center to 700 m upstream of the concrete lined pond.

Results

Seven of 41 loci were polymorphic (polymorphic criterion: more than one allele detected) in one or both of the populations (Table 2). Three loci were polymorphic in both populations: EST-1, SOD, and PK. Four were polymorphic in only one or the other of the populations: Barchas Ranch was polymorphic at the EST-3 and ACO-2 loci, and Ramsey Canyon was polymorphic at MDH-1 and ME-1 (Table 2). Significant differences in allele frequency occurred between populations at the MDH-1 locus, but none of the others was statistically different using the sequential Bonferonni correction for simultaneous tests (Rice, 1989). Despite allele frequency differences at MDH-1, the overall contingency Chi-square value was not statistically

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TABLE 1. Locus Nomenclature, E.C. numbers, and electrophoretic conditions.

Locus	Enzyme	E.C. #	Tissue and buffer system
ACO-1,-2	Aconitase	4.2.1.3	Liver, TC7
ADH-1,-2	Alcohol Dehydrogenase	1.1.1.1	Liver, TC7
AGP	Alphaglycerophosphate Dehydrogenase	1.1.1.8	Liver, TC7
ALD	Aldolase	4.1.2.13	Liver, TC7
ALP	Alkaline Phosphatase	3.1.3.1	Liver, TC7
AP	Acid Phosphatase	3.1.3.2	Muscle, TC7
CK-1,-2	Creatine Kinase	2.7.3.2	Muscle, TC7
DLR	Dihydrolipoamide Reductase	1.6.4.3	Muscle, TC7
DIP	Dipeptidase	3.4.13.11	Liver, LiOH
EST-1,-2,-3	Esterase (alpha napthyl acetate substrate)	3.1.1.1	Liver, LiOH
FBP	Fructose biphosphatase	3.1.3.11	Liver, TC7
FUM	Fumarase	4.2.1.2	Liver, TC7
G3PD	Glyceraldehyde-3-phosphate Dehydrogenase	1.2.1.12	Liver, TC7
G6PD-1,-2	Glucose-6-phosphate Dehydrogenase	1.1.1.49	Liver, TC7
GOT	Glutamate Oxaloacetate Transaminase	2.6.1.1	Liver, LiOH
HEX	Hexokinase	2.7.1.1	Muscle,LiOH
HBDH	Hydroxybuterate Dehydrogenase	1.1.1.30	Liver, LiOH
IDDH	Iditol Dehydrogenase	1.1.1.14	Liver, TC7
IDH-1,-2	Isocitrate Dehydrogenase	1.1.1.42	Liver, TC7
LDH-1,-2	Lactate Dehydrogenase	1.1.1.27	Liver, LiOH
MDH-1,-2	Malate Dehydrogenase	1.1.1.37	Liver, TC7
ME-1,-2	Malic Enzyme	1.1.1.40	Liver, TC7
MPI	Mannose Phosphate Dehydrogenase	5.3.1.8	Liver, LiOH
PGI	Phosphoglucose Isomerase	5.3.1.9	Muscle, TC7
PGM	Phosphoglucose Mutase	2.7.5.1	Muscle, TC7
6PGD	6-Phosphogluconate Dehydrogenase	1.1.1.44	Muscle, TC7
PNP	Purine-nucleoside Phosphorylase	2.4.2.1	Liver, TC7
PK	Pyruvate Kinase	2.7.1.40	Liver, TC7
SOD	Superoxide Dismutase	1.15.1.1	Liver, TC7
TPI	Triose Phosphate Isomerase	5.3.1.1	Liver, TC7
XDH	Xanthine Dehydrogenase	1.2.1.37	Liver, LiOH

greater than expected from sampling error ($F = \chi^2\text{observed}/\chi^2\text{error} = 26.560/82 = 0.324$, df = 10, n.s.). The standardized genetic variance ($F_{ST} = 0.056$) was not significantly different from zero (F_{ST} [$2N_T$] = $\chi^2 = 3.584$, df = 1, $0.05 < P < 0.10$); (Workman and Niswander, 1970). Average individual heterozygosities were 2.9 ± 1.3% for Ramsey Canyon and 4.2 ± 1.7% for Barchas Ranch. Neither of the populations deviated from Hardy-Weinberg equilibrium expectations (Fisher's exact test, $P > 0.177$). Genetic distance estimates between the two populations were low: Nei unbiased genetic distance = 0.003; Modified Rogers' distance = 0.063; Cavalli-Sforza and Edwards chord distance = 0.079.

Regular monitoring of the Ramsey Canyon population and Ramsey Creek from May 1 to 26 June 1995 revealed a fixed number of adults which could all be accounted for at each census. Of the total of 19, 16 were in residence at each survey of the concrete lined pond. Of these, 11 were adult females and five were adult males. Weekly daytime surveys of Ramsey Creek, which is the water source for the concrete pond, revealed two adult females and a subadult of unknown sex. These three were seen consistently at the same location between the visitor center and the concrete lined pond. No adult individuals were seen above the pond, and no juveniles were seen anywhere. The Duck pond on the Barchas Ranch was surveyed four times during the May/June 1995 season. Thirty eight of an estimated 50 juveniles and subadults were toe clipped.

One of us (JEP) continued to monitor the two populations after the completion of the genetic assessment presented here with the following results. In early summer of 1996, a severe drought caused the Barchas Ranch Duck pond to dry out eliminating it as suitable habitat and reducing the known number of breeding populations to one (E. Wallace, pers. comm.). Through September 1995, there were 19 individuals in the concrete pond in Ramsey Canyon (H. Riley, pers. comm.). In summer of 1996, there were no sightings of frogs at the Ramsey Canyon concrete pond. From that time until the present, no breeding activity has been documented at the type locality despite reintroduction efforts by Arizona Game and Fish Non-Game Division. In 2000, a dead frog was documented to have a chytrid fungal infection (M. Sredl, pers. comm.).

DISCUSSION

Early efforts to quantify individual heterozygosity levels in amphibians (Selander and Johnson, 1973; Nevo et al., 1984) based on databases of 11 and 61 species, respectively, produced mean *H*-values of 0.082 and 0.067, respectively. These were the highest among major vertebrate groups. More comprehensive reports based on data representing 188 species of amphibians (Nevo and Beiles, 1991) and the work of Ward et al. (1992) involving data for 116 species of amphibians, reaffirm that amphibians as vertebrates, have among the highest mean levels of heterozygosity (*H*).

TABLE 2. Genetic variability assessment of two populations of the Ramsey Canyon Leopard Frog based on 41 electrophoretic loci. Standard errors are given in parentheses.

Population	Sample size	Mean no. of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity (direct count)[a]	Mean heterozygosity Hardy-Weinberg expected[b]
Barchas Ranch	15	1.2 (0.1)	17.2	0.042 (0.017)	0.039 (0.015)
Ramsey Canyon	17	1.2 (0.1)	17.1	0.029 (0.013)	0.030 (0.013)

[a] A locus was considered polymorphic if more than one allele was detected.
[b] Unbiased estimate based on Nei, 1978.

Two studies, which are of comparative value with our results, involve several ranid species that live in the less mesic western half of the United States. A third involves a mesic situation where rates of gene flow between populations is likely to be substantially higher. Both Case (1978) and Green (1988) surveyed several Pacific Coast ranid frogs including *Rana aurora*, *Rana cascadae*, *Rana pretiosa*, *Rana boylii*, and *Rana muscosa*. Green (1988) is more comparable to our work in that he examined 31 loci, whereas Case (1978) surveyed 15 loci. Mean levels of heterozygosity were variable ranging from 0.046 in one population of *Rana aurora aurora* to 0.08 in a sample of *R. pretiosa*. Case (1978) reported a similar but somewhat lower range of values (0.025–0.06). In a more mesic location, one of us (TG) examined multiple populations of *R. sylvatica* for 50 loci, in the Shenandoah Mountains of Virginia and found mean *H*-values from 0.094–0.189 (unpubl.). Higher levels seen in *R. sylvatica* are undoubtedly influenced by the mesic nature of the area and the fact that this species is an explosive breeder, often congregating in numbers approaching 4000 individuals in parts of the Shenandoah Mountains of Virginia, according to Berven (1981). Among the studies cited here, both Ramsey Canyon ($H = 0.042$) and Barchas Ranch ($H = 0.029$) are comparable to the lower values reported by Green (1988) and Case (1978) for western ranids.

A number of well-known factors can contribute to low levels of heterozygosity in diploid bisexual species including genetic drift, founder effect, inbreeding, biased sex ratio, and mating system. Nei et al. (1975) showed that organisms with high intrinsic rates of increase rapidly regain heterozygosity if population size is reduced briefly, but if the number of breeding adults is greatly reduced and the population remains small (recruitment is low; *r* is relatively small), then erosion of genetic variability is expected to be severe. Suitable habitat at both of our study sites is quite limited; therefore both populations qualify as small. If no other factors are at work, populations lose variability each generation but allelic variation at all loci should be in Hardy-Weinberg equilibrium. Seven of the polymorphic loci in our study showed allelic frequencies within Hardy-Weinberg expectations, consistent with loss of genetic variability under genetic drift.

Inbreeding in small populations results in over representation of homozygotes at the expense of heterozygotes. The fact that six of seven polymorphic loci in our study contained heterozygotes in expected numbers suggests that losses are not the product of inbreeding. In fact, the overall population average value of F (F_{IS}) was −0.074, which indicates a slight, but not statistically significant, excess of heterozygotes ($F_{IS}^2 [N] = \chi^2 = 0.175$, df = 1, n.s.; Li, 1965).

Biased sex ratio in which adult males are under represented or a breeding system in which some do not breed, further reduce effective population size (N_e). Subsequent to our electrophoretic survey, one of us (JEP) monitored the population at Ramsey Canyon documenting a decline from 56 individuals in 1992 to 19 individuals in 1995. Of 56 individuals in 1992, 42 were used in a skeletochronology study (Platz et al., 1997). Of these, 22 were male and 20 female. By 1995, the only place that egg masses were documented was in the concrete lined pond where the 16 adults were monitored. Assuming that all five males participated in mating, the sex ratio of 1:3.2 would result in an N_e of 13.75. Therefore, biased sex ratio came in to play post-1995.

Virtually nothing was known about *R. subaquavocalis* prior to its description (Platz, 1993), but in all likelihood it dispersed into our study sites along Ramsey Creek from larger populations inhabiting the San Pedro River 8 km to the east. This is based on the notion that according to Davis (1986), large numbers of beaver occupied the river. Beaver presence creates ideal habitat for leopard frogs. About 150 years ago, the beaver were trapped out of the San Pedro, and water was subsequently diverted for irrigation, further rendering the river unsuitable for leopard frogs. *Rana subaquavocalis* is now restricted to a few human-made ponds like the ones we examined.

Whether the levels of individual heterozygosity we observed are "abnormally low" is problematic. Case's (1978) findings and those of Green (1988) are similar to or only marginally higher than those of the current study. Nevo and Beiles (1991) reported a value of 0.046 as an average mean *H*-value for 17 species of aquatic amphibians, which include those of Case (1978) and Green (1988). Our results are marginally lower but similar to those of Nevo and Beiles (1991) for aquatic species. They concluded that levels of heterozygosity in amphibians are better explained by ecological factors than demographic ones and that aquatic forms are buffered compared to those species that live in more terrestrial environments.

Some relatively widespread amphibians show low levels of heterozygosity but do not seem to be in immediate jeopardy. *Ambystoma tigrinum stebbinsi* in Arizona possess even lower levels of individual heterozygosity ($H = 0.005$; Jones et al., 1988). Nevo et al. (1984) identified factors that best explained patterns of levels of heterozygosity among the amphibia; often these were ecological. Our findings lie within the lowest 17% among the 188 species they reported on. Demographic factors, chiefly population size, best explain our findings. Low levels of observable protein polymorphisms, an indication of limited genetic variability, are an important concern if novel, harsh selection pressures arise. Although the focus on ecological (environmental factors) is important in terms of what drives variability in large populations over long time periods, small population size resulting in limited variability in the face of novel environmental influences such as a chytrid fungus may predispose them to extinction.

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