Human Recreation and the Nesting Ecology of a Freshwater Turtle (Chrysemys picta)

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The Status of *Apalone atra* Populations in Cuatro Ciénegas, Coahuila, México: Preliminary Data

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ABSTRACT. – **The species-level designation of the Mexican softshell turtle, *Apalone atra*, has been repeatedly challenged, yet no DNA evidence has been collected. We conducted field studies of all the drainages of the Cuatro Ciénegas basin, and the only pure morphological population of *A. atra* found was in Tío Candido, the type locality for the species. One nuclear intron known to show species-level divergence in the family Trionychidae, 2 nuclear genes, and a mitochondrial gene revealed little molecular divergence for *A. atra* when compared with *A. spinifera emoryi* from the Rio Grande. Further, no reciprocal monophyly of the mitochondrial gene tree was seen between *A. atra* and *A. s. emoryi* morphotypes.**

For many species, hybridization and high migration rates are important components of an organism's long-term evolutionary trajectory (Maddison 1997), but in a short time scale, these behaviors create difficulty in the delimitation of these organisms as evolutionarily significant units (ESU) for conservation protection (Moritz 1994). ESUs are defined in 2 ways: 1) populations that have reciprocal monophyly at mitochondrial loci with significant divergence in nuclear loci (Moritz 1994) and 2) populations that are substantially reproductively isolated and represent distinct evolutionary legacies (i.e., genetic variability; Waples 1991, 1995). Here we investigate DNA evidence to determine if the turtle species *Apalone atra*, the endemic Cuatro Ciénegas black softshell, meets either of these criteria.

The taxonomic and conservation standing of *A. atra* is controversial. This turtle is currently listed on the CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) Appendix I endangered species list, but it has also been reported to be extinct (Smith and Smith 1979). Hybridization between *A. atra* and a south Texas species, *Apalone spinifera emoryi*, is thought to have begun in the 1880s when irrigation canals were constructed, opening the Cuatro Ciénegas basin hydrologically (D. Hendrickson, *pers. comm.*; Webb 1973). Winokur (1968) could not determine the rate of hybridization, concluded the identification of some specimens as hybrids to be uncertain, and verified pure *A. atra*. By 1979, Smith and Smith considered *A. atra* to be an extinct lineage due to hybridization with *A. spinifera emoryi.* In 1983, a softshell resembling pure *A. atra* was noted in a field trip to the Cuatro Ciénegas basin (Ernst and Barbour 1992).

In this study, we sought to illuminate the taxonomic status of the endemic Cuatro Ciénegas black softshell, *A. atra*, by assessing its molecular distinction from the invasive congener, *A. s. emoryi*. We sampled all 5 drainages in the basin (Evans 2005) for turtles with *A. atra* morphological characteristics and used mitochondrial DNA sequence data from Cuatro Ciénegas softshell turtles and *A. spinifera* subspecies to reconstruct a haplotype network and a phylogenetic tree. In addition, we built distance matrices with the mitochondrial DNA data and 3 nuclear loci.

Methods. — Morphological surveys were extensive; we inspected all currently defined and flowing drainages in El Área de Protección de Flora y Fauna Cuatro Ciénegas, Coahuila, México, for *A. atra* (Fig. 1; Minckley 1969). This sampling included the type locality, Tío Candido, and spanned 62 days of trapping from 16 May to 16 June 2003 and from 4 June to 5 July 2004 (Webb and Legler 1960). Turtles were captured in lobster or hoop traps that were baited with sardines and checked every 12–14 hours. Each turtle was tattooed in a unique pattern on the plastron.

Morphologically, *A. atra* is characterized by 5 main traits: 1) "blackish" pigmentation of dorsal surface, 2) speckled pigmentation on ventral surfaces, 3) faint marginal bands and posterior white tubercles on the carapace of males only, 4) longitudinal corrugations on the posterior of the carapace, and 5) ovoid adult carapaces (Webb and Legler 1960; Winokur 1968). *Apalone spinifera emoryi* is defined by 1) tan to olive-brown carapace; 2) no ventral pigmentation; 3) defined pale marginal band; 4) white, raised tubercles on the back third of the carapace; and 4) a clear triangular facial pattern (Ernst et al. 1994). In this study, *A. atra* were distinguished from *A. s. emoryi* by dark pigmentation on the carapace, speckled pigmentation on the plastron, and corrugations on the posterior carapace. These traits showed no sexual dimorphism and little to no change through adult life and were exclusive to *A. atra*. See Appendix I for morphological characteristics and habitat types and Appendix II for photo voucher accession numbers for each specimen.

Less than 0.5 ml of blood were drawn from the caudal vein of the 26 field-collected animals, stored in buffer (0.01 M Tris, 10 mM EDTA, 0.01 M NaCl, and 1% SDS), and frozen. DNA was extracted using Roche High Pure Template Preparation Kit (Cat. 1796828). DNA sequence data were obtained using an ABI 3730 DNA Analyzer.

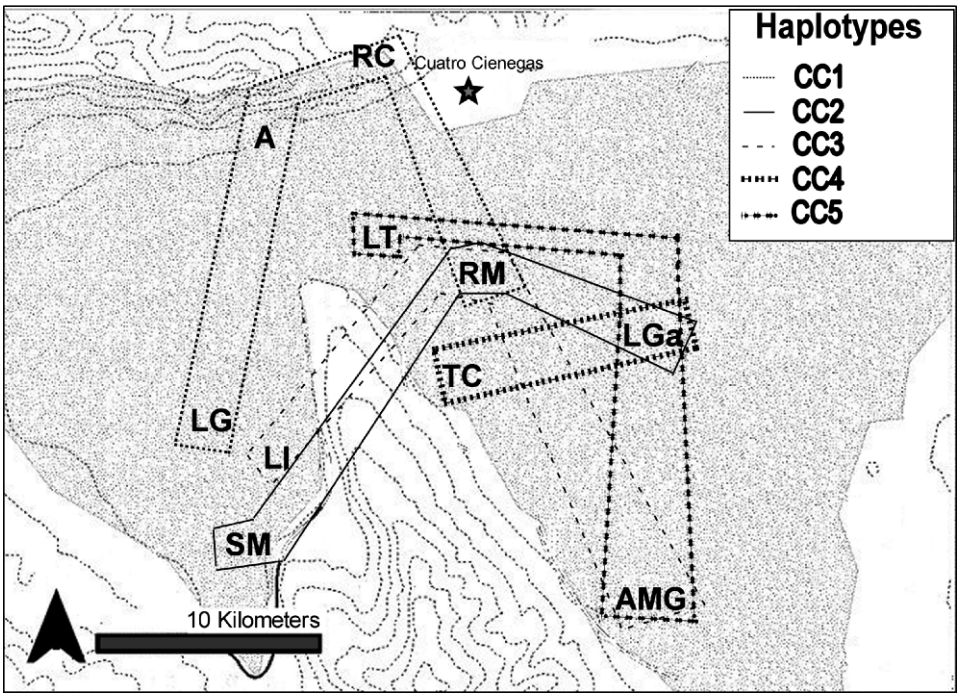

Figure 1. Approximate localities sampled in the Cuatro Ciénegas basin. Map adapted from Moline et al. (2004). Populations include AM: Anteojo; AMG: Antiguos Mineros Grande; LG: Laguna Grande; LGa: Los Gatos; LI: Laguna Intermedia; LT: Posa de las Tortugas; TC: Tio Candido; RC: Rio Cañón; RM: Rio Mesquites at Las Salinas; SM: San Marcos. Laguna Intermedia and Laguna Grande are connected by a short canal and considered 1 site. Rio Cañón is located north of the town of Cuatro Ciénegas. Morphological but not molecular data were taken from drainage 4.

This included 725 base pairs (bp) of cytochrome *b* mitochondrial gene (CytbF: 5′ ACAGGCGTAATCC-TACTAC 3′; DW1594; see Weisrock and Janzen 2000) from putative *A. atra* in the type locality ($n = 6$), 8 other localities in the basin ($n = 19$), and the pallid softshell (*A. s. pallida*; MidCon63). Additionally, 1638 bp of recombination activating gene-1 (RAG-1; see Krenz et al. 2005 for primers), 886 bp of RNA fingerprint protein 35 nuclear intron (R35; see Fujita et al. 2004 for primers), and 538 bp of the oncogene C-*mos* (see Saint et al. 1998 for primers) were used to construct distance matrices to compared the type locality specimens ($n = 2$) to *A. s. pallida* (CME63 from Irion County, Texas), *A. s. emoryi* (TXsc from Valverde County, Texas, and NMrg from Socorro County, New Mexico; Weisrock and Janzen 2000), and *A. mutica* (LAcrm1 from East Baton Rouge Parish, Baker, Louisiana; Weisrock and Janzen 2000). The nuclear intron R35 has been successful in resolving species-level phylogenies in the family Trionychidae (Engstrom et al. 2004), and RAG-1 has been successful in resolving species-level phylogenies in other Testudines (Krenz et al. 2005). The third nuclear marker, C-*mos*, has not been extensively used for phylogenetics in turtles. All PCR products were gel purified for the appropriate band size using Qiagen's QIAquick Gel Extraction Kit (Cat. 28706). Some heterozygosity was observed in the RAG-1 sequences. As needed, we cloned separate alleles using pGEM-T Easy Vector System I (Promega A1360) and One Shot Mach I Competent Cells (Invitrogen C8620–03). GenBank accession numbers are located in Appendix II.

Eight additional samples from a previous study were used for the total phylogenetic analysis (total $n = 34$; Weisrock and Janzen 2000; LAcr1m, NMrg, ONtr1, FLer1, GAsr, TXcc, TXki, and TXsc). Alignments were performed in CLUSTAL W (Thompson et al. 1994), and sequences were visually reviewed and corrected in BioEdit 7.0.0 (Hall 1999). Modeltest 3.7 (Posada and Crandall 1998) was used to estimate parameters of sequence evolution for all genes using Akaike's information criterion (Posada and Buckley 2004). No model of sequence evolution was proposed or tested in other studies of *Apalone* phylogeography (e.g., Weisrock and Janzen 2000), but the models for cytochrome *b* in this study were tested with representatives from major clades of Weisrock and Janzen (2000). These parameters were taken into account when constructing distance matrices in PHYLIP 3.62 (Felsenstein 2005). A maximum likelihood tree using cytochrome *b* was constructed using parameters estimated in Modeltest 3.7 and bootstrapped for 100 replicates using Paup 4.0b. Parsimony and neighbor-joining trees carried the same signature of weakly supported nodes and can be obtained from the first author. Nuclear DNA sequences showed little to no divergence, and therefore, no trees were constructed using those data. Haplotype groups were defined with DNASP (Rozas et al. 2003). Haplotype networks were built using statistical parsimony in TCS with gaps set as missing data (Clement et al. 2000).

Results. — Animals that were morphologically concordant with *A. atra* were found predominantly in the type locality, Tío Candido (Fig. 2). Here, no *A. s. emoryi*

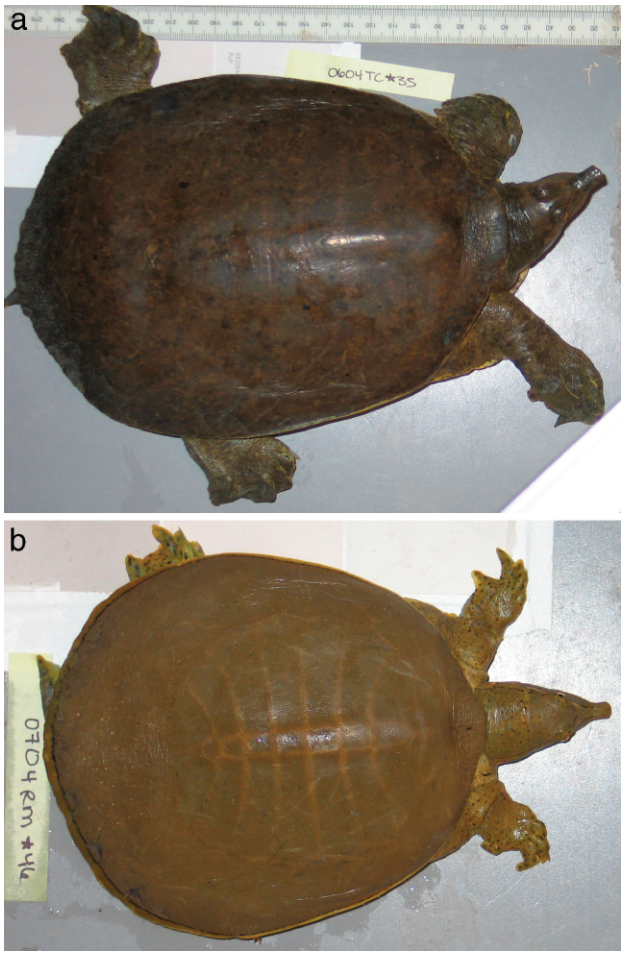

Figure 2. Putative *Apalone atra* from Tio Candido (TC35, female, 2a) and *Apalone spinifera emoryi* from the Rio Mesquites at Las Salinas (RM25, male, 2b). *Apalone atra* is characterized by dark, "blackish" pigmentation of dorsal and ventral surfaces and longitudinal corrugations on the posterior part of the carapace. Defining characteristics for *A. s. emoryi* are a tan or olive-brown carapace with the back third covered in white tubercles. Photos by S. McGaugh.

morphs were found in 11 days of trapping (Webb and Legler 1960). Turtles trapped in Antiguos Mineros Grande, and 3 turtles trapped in Posa de las Tortugas (e.g., LT104; also known as Mojarral Este) also were morphologically concordant to the description of *A. atra*, but *A. s. emoryi* morphs and potential hybrids were also present. Within the other 3 sites, Antejeo, Rio Mesquites, and Rio Cañón, *A. atra*–like traits were found in conjunction with *A. s.* emoryi–like traits in the same animal. Hybrids and backcrosses were difficult to accurately define; although, 9 turtles presented both *A. s. emoryi* and *A. atra* characteristics (Appendix I). Only morphological characteristics of *A. s. emoryi* were found at San Marcos, Los Gatos, and Laguna Grande.

Results from distance matrices are illustrated in Table 1. Cytochrome *b* divergence of *A. atra* (TC38 and TC36, Table 1) with unequivocal species and subspecies, *A. mutica* (~ 9.24%) and *A. s. pallida* (1.24%), was substantial. Cytochrome *b* divergence of *A. atra* with *A. s. emoryi* was nearly an order of magnitude lower (0.14%–0.28%). Likewise, the nuclear loci showed divergence between *A. mutica* and *A. atra* (RAG-1: 0.93%, R35: 1.14%, C-*mos*: 0.58%) but very little divergence between *A. s. emoryi* and *A. atra* (RAG-1 < 0.01%, R35: 0.10%, C-mos: 0.0.09%). *Apalone atra* did not show substantial divergence with *A. s. pallida* at these nuclear loci either (RAG-1 < 0.01%, R35 < 0.01%, C-mos: 0.14). This combined genetic information, with special emphasis on the cytochrome *b* data, suggests that sequence divergence between the Rio Grande *A. s. emoryi* (Txsc and NMrg; Weisrock and Janzen 2000) and individuals from the type locality for *A. atra*, Tío Candido, where *A. s. emoryi* morphs were never caught, is insufficient for strong ESU delimitation through Waples's (1991, 1995) requirement of reproductive isolation.

Results from the phylogenetic analysis strengthened the hypothesis that *A. atra* does not represent a unique species. The maximum likelihood tree is illustrative of all trees constructed (Fig. 3). The lack of reciprocal monophyly of *A. atra* morphs in comparison to *A. s. emoryi* morphs suggests that *A. atra* does not qualify as an ESU through Mortiz's (1994) definition (Fig. 3).

Nine mitochondrial haplotypes were recorded among 25 individuals from the 9 sites in the Cuatro Ciénegas basin, 2 Rio Grande *A. s. emoryi* samples (TXsc and

Table 1. DNA pairwise distances of *Apalone spinifera emoryi* (TXsc and NMrg), *A. atra* (Tío Candido, Cuatro Ciénegas, México; TC38 and TC36), *A. s. pallida* (Irion County, TX; CME63), and *A. mutica*. Values are given in percentages for 725 base pairs (bp) of cytochrome *b*, 1638 bp of *recombination activase gene-1*, 886 bp of RNA fingerprint protein 35 nuclear intron, and 538 bp of C-*mos*, respectively.

	A. atra (TC36)	*A. atra* (TC38)	*A. s. emoryi* (TXsc)	*A. s. emoryi* (NMrg)	*A. s. pallida* (CME63)
A. atra (TC36)	0				
A. atra (TC38)	0.001, 0.001, 0.001, 0.100				
A. s. emoryi (TXsc)	0.276, 0.001, 0.001, 0.092	0.276, 0.001, 0.001, 0.092			
A. s. emoryi (NMrg)	0.139, 0.001 0.103, 0.0911	0.139, 0.001 0.103, 0.091	0.139, 0.001, 0.103, 0.061		
A. s. pallida (CME63)	1.261, 0.001, 0.001, 0.141	1.261, 0.001, 0.001, 0.141	1.261, 0.001, 0.001, 0.071	1.120, 0.001, 0.103, 0.071	
A. mutica	9.243, 0.934, 1.144, 0.582	9.243, 0.939, 1.144, 0.582	9.249, 0.939, 1.144, 0.550	9.085, 0.939, 1.2492, 0.551	8.447, 0.939, 1.144, 0.176

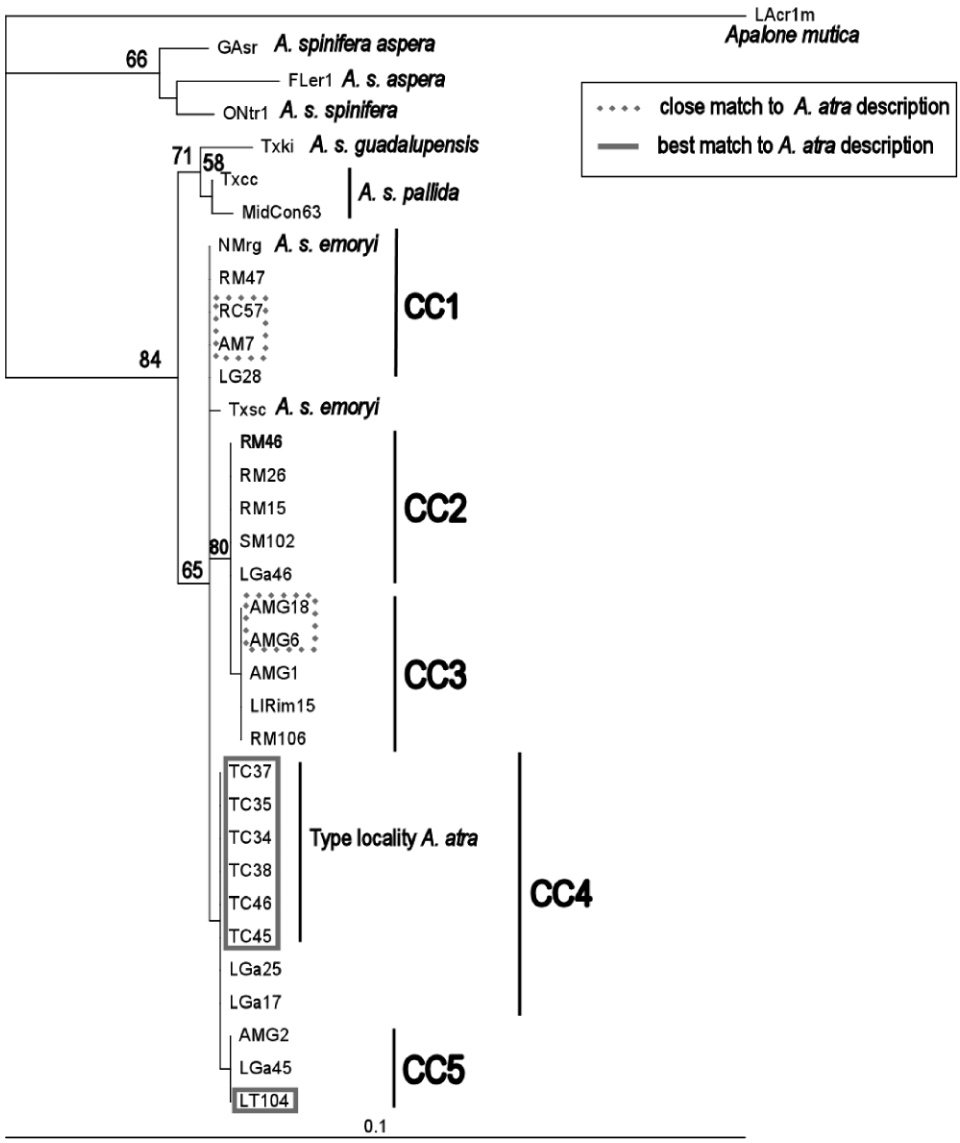

Figure 3. Maximum likelihood tree for cytochrome *b* samples within the Cuatro Ciénegas basin. Bootstrap values are given. *Apalone spinifera* subspecies are given in the tree, including *A. s. emoryi* from the Rio Grande (NMrg: Socorro County, New Mexico; TXsc: Valverde County, Texas; see Appendix I for full descriptions). The out-group is *A. mutica* (Lacr1m) from East Baton Rouge Parish, Baker, Louisiana. Haplotypes are defined only within the Cuatro Ciénegas basin. Solid gray boxes indicate those specimens that were most concordant with species descriptions of *A. atra.* Dotted gray boxes indicate specimens that are very similar but not perfectly concordant to species descriptions of *A. atra*.

NMrg), 2 south Texas *A. s. pallida* samples (TXcc and MidCon63), and 1 southeast Texas *A. s. guadalupensis* sample (TXki; Fig. 4). Although for a within-population analysis including south Texas *Apalone* may not be accurate, including these samples provided perspective on the close relationship of *A. s. emoryi* and *A. atra* haplotypes. Five of the 9 mitochondrial haplotypes (CC1–CC5) occurred in the Cuatro Ciénegas basin, with 1 of those 5 (CC1) containing a Rio Grande sample (NMrg; Fig. 4). One mitochondrial haplotype (CC4) was shared among all Tío Candido turtles, and 2 other turtles from another eastern locality in the basin (Los Gatos; Fig. 4). This preliminary sampling was not sufficient to perform statistical evaluations of haplotype genealogy and diversity, but examination of the geographic distribution of haplotypes (Fig. 1) suggests that some microgeographic genetic structuring may potentially exist.

Discussion. — Weisrock and Janzen (2000) reported that *A. spinifera* and *A. mutica* exhibit relatively large amounts of intraspecific mitochondrial cytochrome *b* DNA variation. This marker is sufficiently variable to reveal sequence divergences between legitimate species (e.g., *A. spinifera* and *A. mutica* are 8.0%–8.8% divergent from one another; Weisrock and Janzen 2000). Two Rio Grande populations of *A. s. emoryi*, Socorro County, New Mexico, and Valverde County, Texas, have low sequence divergence for cytochrome *b* (0.1%), and these samples served as primary references for evaluating the genetic divergence between *A. atra* and *A. s. emoryi* morphotypes in the Cuatro Ciénegas basin. We found that sequence diver-

gences between the Rio Grande *A. s. emoryi* and the type locality for *A. atra* in the Tio Candido drainage of Cuatro Ciénegas (0.14%–0.28%) are comparable to the within–Rio Grande levels of divergence for *A. s. emoryi*, providing no mitochondrial evidence of previous speciation between these taxa. In the case of sexually symmetrical hybridization, one would expect to find a distinct, remnant mitochondrial signature if the populations were separate in the recent (~ 130 years) past. No evidence for asymmetrical gene flow (from *A. s. emoryi* females to *A. atra* males) is apparent, and so the homogeneous nature of haplotypes reported here supports the idea that these putative species were probably not historically separate in gene flow.

The phylogenetic and haplotype analyses of the mitochondrial data indicate that there is nonreciprocal monophyly between morphologically putative *A. atra*, the *A. s. emoryi* morphotypes in the basin, or Rio Grande individuals (Figs. 3, 4). With such weak divergences and low bootstrap support for within-basin comparisons (Fig. 3), important insight can be drawn from a haplotype network (Fig. 4) in conjunction with the phylogenetic analysis. Network examination reveals greater diversity is present between haplotypes within the Cuatro Ciénegas basin than between the *A. s. emoryi* of the Rio Grande and *A. atra*. Although type locality individuals (Tío Candido) are all of the same haplotype (CC4), Los Gatos individuals are from the same haplotype group and are morphologically *A. s. emoryi*.

The haplotype analysis is probably unaffected by the analysis of only 2 samples of *A. s. emoryi*. These samples are separated by greater than 1100 km of river distance; still, the haplotypes were only 0.1% divergent from one another (Weisrock and Janzen 2000). Thus, it could be safely assumed that the total divergence across the entire range of *A. s. emoryi* is probably similarly low. Even with more extensive *A. s. emoryi* sampling, haplotypes will most likely continue to be very similar to those found in the Cuatro Ciénegas basin.

At least 2 factors must be considered before conservation management recommendations are made. First, morphological variation within the basin is abundant and could be indicative of real, locally adapted morphs. Winokur (1968) hypothesized that this morphological variability may be related to habitat type. Overall, darker, *A. atra*–like individuals were found in dark-bottomed lagoons, while lighter, *A. s. emoryi*–like individuals were found in light-bottomed playa lakes and rivers (Winokur 1968; Webb 1973). This view is supported, at least, for coloration, a trait that is hypothesized here to be a result of background matching (Appendix I; McGaugh 2008). Background matching is a physiological color change in response to light or dark surroundings and is known to happen over several weeks to months in *A. spinifera* (Bartley 1971; Ernst et al. 1994). Second, even if the morphological variability can be potentially explained by habitat parameters, the limited geographic distribution of

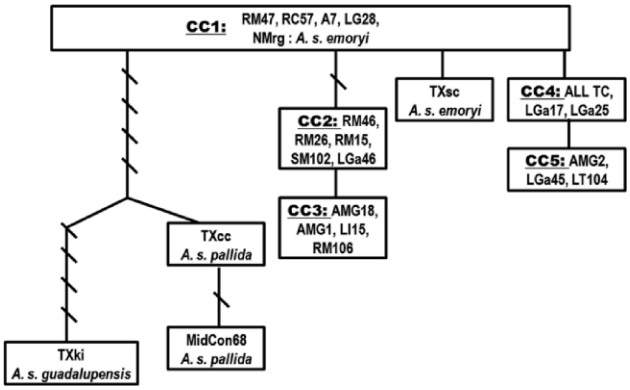

Figure 4. Haplotype network of cytochrome *b* generated by statistical parsimony. Each vertical bar represents 1 mutation, and diagonal bars separate mutation events. Haplotypes are named only within the Cuatro Ciénegas basin. It is clear from the network that *Apalone* from the Cuatro Ciénegas basin share or are very close to *A. s. emoryi* (NMrg and TXsc) haplotypes. More variability occurs within the Cuatro Ciénegas basin than between the 2 putative taxa (*A. atra* and *A. s. emoryi*). Haplotypes from other Texas *A. spinifera* subspecies (TXki: *A. s. guadalupensis*; TXcc and MidCon63: *A. s. pallida*) are more distant to each other and to the *A. s. emoryi*-Cuatro Ciénegas haplotypes than the *A. s. emoryi* and Cuatro Ciénegas haplotypes are to each other.

mitochondrial haplotype CC4 suggests that some genetic substructuring may potentially exist. An analysis of mitochondrial haplotypes containing more individuals and incorporating population-level nuclear markers, such as microsatellites or amplified fragment length polymorphism, could help delimit management units within the Cuatro Ciénegas basin (Moline et al. 2004; Carson and Dowling 2006).

Our analysis exemplifies a notable problem of current biology and taxonomy: delimiting species and ESUs. In the last thorough examination, Winokur (1968) maintained the status of *A. atra* as a distinct species based on the assumption that gene flow was prezygotically restricted between *A. atra* and *A. s. emoryi* by ecological preferences. Our analysis reiterates that morphological variation is associated with habitat type (*A. atra*–like animals in lagoons and *A. s. emoryi*–like individuals in rivers and playa lakes) but demonstrates little to no molecular distinctions and no reciprocal monophyly between the animals in the Cuatro Ciénegas basin and those in the Rio Grande. These data provide strong evidence that *Apalone atra* is not a separate species from *Apalone spinifera emoryi*.

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Appendix I. Sample names are given along with locality, habitat type, and morphological identification.

Sample	Locality and morphological information
LAcr1m*	Comite River, East Baton Rouge Parish Baker, Louisiana, *A. mutica*
TXsc*	Sycamore Creek, Valverde County, Texas, *A. s. emoryi*
NMrg*	North Elephant Butte Reservoir, Socorro County, New Mexico, *A. s. emoryi*
FLer1*	Escambia River, Escambia County, Florida, *A. s. aspera*
Gasr*	Suwanee River, Lanier County, Georgia, *A. s. aspera*
ONtr1*	Thames River, north of London, Ontario, Canada, *A. s. spinifera*
TXki*	Kingsville, Kleber County, Texas, *A. s. guadalupensis*
TXcc*	Coleto Creek, Goliad County, Texas, *A. s. pallida*
MidCon63	Middle Concho River, Irion County, Texas, *A. s. pallida*
TC34mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC35mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC37mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC38mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC36mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
TC45mt	Tio Candido, *A. atra* type locality: Lagoon, *Rugose margin, dark coloration on carapace and dark markings on plastron*
AM7mt	Antejeo: Lagoon, *Slightly rugose margin, medium dark carapace pigmentation, few markings on plastron*
AMG18	Antiguos Mineros Grande: Lagoon, *Slightly rugose margin, dark pigmentation on carapace, some dark markings on plastron*
AMGst6	Antiguos Mineros Grande: Lagoon, *Slightly rugose margin, dark pigmentation on carapace, some dark markings on plastron*
AMGst1	Antiguos Mineros Grande: Lagoon, *Margin very slightly rugose, medium dark pigmentation on carapace, few dark markings on plastron*
AMGst2	Antiguos Mineros Grande: Lagoon, *Margins not rugose, medium dark pigmentation on carapace, no dark markings on plastron*
LT104	Morjarral Este: Lagoon, *Rugose margins, dark pigmentation, dark markings on plastron*
SM102	Eijido San Marcos: Waste water pond, *Margins not rugose, no dark pigmentation*
RM46mt	Rio Mesquites: River, *Margin not rugose, medium dark carapace pigmentation, no markings on plastron*
RM26mt	Rio Mesquites: River, *Slightly rugose margin, dark carapace pigmentation, few markings on plastron*
RM47mt	Rio Mesquites: River, *Slightly rugose margin, medium dark carapace pigmentation, few dark markings on plastron*
RM106	Rio Mesquites: River, *Slightly rugose margin, medium dark pigmentation, few dark markings on plastron*
RM15mt	Rio Mesquites: River, *Margin not rugose, medium dark pigmentation, no markings on plastron*
RC57mt	Rio Cañón: River, *Very slightly rugose margin, dark pigmentation on carapace, some dark markings on plastron*
LGst28	Laguna Grande: Playa lake, *Margin not rugose, no dark pigmentation*
LIRim15	Laguna Intermedia: Playa lake, *Slightly rugose margin, no dark pigmentation*
LGa46	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*
LGa45	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*
LGa25	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*
LGaRIM17	Los Gatos: Playa lake, *Margins not rugose, no dark pigmentation*

Appendix II. GenBank and photo voucher accession numbers. An asterisk denotes sequences obtained from GenBank; all but R35 for LAcrm were obtained from Weisrock and Janzen (2000). Photo vouchers are not available for these GenBank specimens, and this is indicated as NA. GenBank accession numbers are given for cytochrome *b*, RAG-1, C-*mos*, and R35, respectively, and separated by semicolons for each gene. Alleles for each gene are separated by commas.

LAcr1m*	NA; AF168766*; DQ529173; DQ529206; AY259581.1*
FLer1*	NA; AF168751*
GAsr*	NA; AF168752*
ONtr1*	NA; AF168757*
TXki*	NA; AF168759*
TXcc*	NA; AF168758*
TXsc*	NA; AF168760*; DQ529147, DQ529148; DQ529185, DQ529186; DQ529125
NMrg*	NA; AF168756*; DQ529151; DQ529187; DQ529127
MidCon63	ISUA200614; DQ529103; DQ529157, DQ529158; DQ529192; DQ529122
TC34	ISUA200620; DQ529113
TC35	ISUA200621; DQ529112
TC37	ISUA200622; DQ529111
TC38	ISUA200623; DQ529114; DQ529132, DQ529133; DQ529174; DQ529118
TC36	ISUA200625; EU040193; EU040200; EU040201, EU040202; DQ529119
TC45	ISUA200624; EU040194; DQ529134, DQ529135; DQ529175; no data R35
AM7	ISUA200717; EU040181
AMGst18	ISUA200716; EU040186
AMGst6	ISUA200714; EU040187
AMGst1	ISUA200713; EU040188
AMGst2	ISUA200712; EU040195
LT104	ISUA20073; EU040199
SM102	ISUA20075; EU040189
RM46	ISUA20077; EU040193
RM26	ISUA20079; ; EU040184
RM47	ISUA20076; EU040179
RM106	ISUA20074; EU040192
RM15	ISUA200715; EU040185
RC57	ISUA20078; EU040180
LG28	ISUA200718; EU040182
LIRim15	ISUA200710; EU040191
LGa46	ISUA20072; EU040190
LGa45	ISUA20071; EU040196
LGa25	ISUA200711; EU040197
LGaRim17	ISUA200719; EU040198

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Human Recreation and the Nesting Ecology of a Freshwater Turtle (*Chrysemys picta*)

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ABSTRACT. – **Over a 3-year period, we studied the relationship between the intensity of human recreation and the nesting ecology of the painted turtle (*Chrysemys picta*) at a major nesting beach. Our results suggest that the intensity of human recreation at this site had no effect on the decision of turtles to emerge from the water and nest, or on habitat selection by nesting turtles. This apparent lack of effect of human recreation is contrary to the results of many previously published studies on other taxa and underscores the variability in wildlife responses to human recreation and the need for species-specific and population-specific studies.**

The effects of human recreation on wildlife populations have recently received a great deal of scientific attention, in part because of a rapid increase in outdoor recreation activities over the last several decades (Flather and Cordell 1995). To date, most reported effects of recreation and human disturbance on wildlife have been negative (Boyle and Samson 1985; Carney and Sydeman 1999). However, some investigators have suggested that the effects of human disturbance on wildlife populations may be overestimated (Boyle and Samson 1985; Nisbet 2000), and the impact of human recreation on groups such as reptiles is not well studied (Boyle and Samson 1985).

Although declines in populations of organisms such as amphibians (Wake 1991) have been well publicized, concordant declines in turtle populations have received comparatively little attention (Gibbons et al. 2000; Klemens 2000). Although habitat alteration is a major factor in turtle population declines (Mitchell and Klemens 2000), human recreation can also be detrimental (Garber and Burger 1995; Bury and Luckenbach 2002). The potential effects of human disturbance on the nesting ecology of freshwater turtles are significant because females may alter nest-site selection based on the risk that they themselves will be depredated (Spencer 2002; Spencer and Thompson 2003).

If females perceive humans as a predation risk and alter their nesting behavior, maternal and offspring fitness can be altered through combinations of a variety of factors. For example, the site where a female chooses to deposit eggs can affect the probability of nest depredation through nest density (Valenzuela and Janzen 2001; Marchand et al. 2002; but see Burke et al. 1998) and edge effects (Temple 1987; Kolbe and Janzen 2002a). Nest-site selection may also affect offspring survival through temperature-related incubation success (Schwarzkopf and Brooks 1987; Wilson 1998) and overwintering success (Weisrock and Janzen 1999), as well as offspring sex ratio in turtles with temperature-dependent sex determination (reviewed in Bull 1983; Ewert and Nelson 1991; Janzen and Paukstis 1991; Shine 1999).

The purpose of this study was to determine the effects of human recreation on the nesting behavior of the painted turtle (*Chrysemys picta*). In particular, we evaluated how different levels of human recreation on a major nesting

beach affected the decision of turtles to emerge from the water and nest, as well as components of habitat selection of nesting turtles. We hypothesized that an increased number of humans on the nesting beach would decrease the number of turtles emerging to nest and cause turtles to choose low-quality nesting sites.

Methods. — This research represents a portion of a long-term study of painted turtle nesting ecology (Janzen 1994) at the Thomson Causeway Recreation Area (TCRA) near Thomson, Illinois. The Thomson Causeway is an ~450- × 900-m island on the eastern bank of the Mississippi River, and it contains an ~1.5-ha nesting area that is bordered on the east side by a 200-m-wide slough from which most turtles emerge to nest. The site is managed and maintained by the United States Army Corp of Engineers (USACE; see Kolbe and Janzen 2002a for a more complete site description). The TCRA is a popular destination for recreationists with motor homes (recreational vehicles [RV]) during the spring and summer months. Use of the area is variable, with most activity occurring on weekends and holidays (Bowen and Janzen, *pers. obs.*). The nesting area is interspersed with concrete pads for parking RVs and has a paved road running through it. This setting provides an opportunity to investigate the nesting responses of painted turtles to different levels of human recreation throughout the nesting season.

Chrysemys picta is a small- to medium-sized freshwater turtle that ranges from southern Canada to New Mexico and from the Atlantic to the Pacific Oceans (Ernst et al. 1994; Starkey et al. 2003). At TCRA, the mean clutch size is 10.5 ± 2.0 standard deviation (SD) eggs, and individual females may lay up to 3 clutches in a nesting season from late May to early July (Morjan 2003). *Chrysemys picta* has temperature-dependent sex determination, with cooler temperatures producing males (Ewert and Nelson 1991). Hatchlings remain in the nest during the winter and emerge from the nest and enter water in the spring (Ernst et al. 1994; Weisrock and Janzen 1999).

The nesting beach at TCRA was monitored for nesting turtles from early to late June in 2001 and from late May to early July in 2002 and 2003. The turtles were individually marked by using a series of notches in the marginal scutes of the carapace (Cagle 1939). Each year we observed females leave the water, construct nests, and lay eggs. Once a female had finished nesting, we temporarily mapped the location of the nest by using nearby landmarks (e.g., trees, posts, and RV sites), and determined the amount of overstory vegetation (% shaded) in all 4 cardinal directions over the nest by using a spherical densiometer (see Janzen 1994; Weisrock and Janzen 1999). Once the nesting season was complete, we returned to the location and established precise Cartesian coordinates for each nest by using the program INTERPNT (Boose et al. 1998). Geographic information system coverages were created by using these coordinates in ArcView® (ESRI Inc., Redlands, CA). We used these location data to determine the distance of each nest from the water. Error in this mapping and measuring process ranges from 0 to 15 cm (see Kolbe and Janzen 2002a for more detail). We also followed the fate of each nest through the third week of September (i.e., until all hatching was completed) by noting which nests had been depredated and which remained intact.

As an indicator of the level of human disturbance at TCRA during the nesting season, we used data on the number of RVs present daily at the nesting beach and nearby sites for the months of May through July in the years 2001–2003 obtained from the USACE. We used nested analysis of variance (ANOVA) to determine the effect that varying numbers of RVs had on the decision of turtles to nest. We used the number of nests constructed on a given day as an indicator of the decision of turtles to emerge from the water and nest. Effects in the nested ANOVA were the number of RVs, the Julian date, and the interaction between these two (all nested within year). The Julian date was included in an attempt to control for seasonal changes in nesting behavior that might be independent of human activity.

We hypothesized that the number of turtles emerging to nest would decrease as the number of RVs increased. This relationship was considered important because mass nesting events in response to decreased human presence might increase nest density on small scales and serve as cues for nest predators. Higher nest densities are known to result in higher probabilities of nest depredation at TCRA (Valenzuela and Janzen 2001).

We used nested ANOVAs to examine the effect of human recreation on habitat selection of nesting turtles by comparing the number of RVs present on a given day to the distance from water of nests laid on that day and by comparing the number of RVs present on a given day to the south and west overstory vegetation cover of nests laid on that day. Effects for each nested ANOVA were the number of RVs, Julian date, maximum air temperature on the day of nesting, water temperature on the day of nesting, and the interactions between these variables (all nested within year).

Weather variables were included in an attempt to account for the effects of weather on habitat selection. Maximum air temperature and water temperature were chosen because these variables appear to affect the decision of turtles to emerge from the water and nest at TCRA (Bowen et al. 2005). We obtained weather data for the nesting seasons from the USACE Lock and Dam 13, ca. 12 km south of the study site.

We hypothesized that as the level of human activity increased females would perceive a greater risk to themselves, and the distance of nests from the water would decrease (Spencer 2002). This relationship was considered important because rates of nest depredation are higher along the water edge in most years at TCRA (Kolbe and Janzen 2002a). Sites near the water are less shaded at TCRA; therefore, we hypothesized that nest overstory

Table 1. Descriptive statistics (mean ± 1 SD) and sample sizes for human recreational activity and nest variables of the painted turtle (*Chrysemys picta*) at a nesting beach in northwestern Illinois.

Y (nests)	No. nests/d	Nest distance to water (m)	Nest southwest overstory vegetation (%)	RV/d[a]
2001 (147)	7.1 ± 7.6 (23 d)	34.3 ± 24.6	39.2 ± 21.0	5.6 ± 9.1
2002 (158)	7.7 ± 9.3 (23 d)	28.6 ± 23.6	42.8 ± 22.6	18.3 ± 13.8
2003 (218)	6.6 ± 7.3 (35 d)	24.7 ± 23.0	41.7 ± 22.4	23.6 ± 19.0

[a] recreational vehicle.

vegetation cover would decrease with increasing levels of human recreation (Kolbe and Janzen 2002a). This relationship was considered important because south and west overstory vegetation cover is a predictor of nest temperature and offspring sex ratio at this site in most years (Janzen 1994; Morjan and Janzen 2003). Nesting turtles are known to choose nesting sites nonrandomly with respect to overstory vegetation at TCRA (Janzen and Morjan 2001).

Although some females at TCRA lay multiple clutches within a nesting season (Morjan 2003), we used only the first nest for each female within years in our analyses. We did so to ensure that each nesting event was independent within years (i.e., females may exhibit different nesting behavior during their second attempt, based on what they experience during their first attempt). Adding data from second and third nests did not change our findings (results not shown). All statistical analyses were performed by using JMP (SAS Institute Inc).

Results. — Nesting began by 7 June in each year and the nesting beach was monitored until at least 1 July. Either 23 (2001 and 2003) or 35 (2002) nesting days for each year were included in the analyses. The number of nesting days varied, in part, as a result of weather conditions that inhibited nesting on some days. Data were analyzed from 147 nests in 2001, 158 nests in 2002, and 218 nests in 2003. Nest parameters were highly variable during the nesting season in all years studied, as was the number of RVs present at TCRA (ranging from 0 to 64 RVs per day; Table 1). Overall, substantial within- and among-year variation existed in the data set for the response and predictor variables of interest in this study.

The number of nests laid on a given day was not affected by any of the predictor variables (overall $p = 0.7993$, $r^2 = 0.09$; Table 2). A similar pattern was observed for overstory vegetation (overall $p = 0.4360$, $r^2 = 0.06$; Table 3). The nested ANOVA for the distance of the nests from water was statistically significant (overall $p = 0.0009$), but none of the individual effects approached statistical significance and the r^2 value was small ($r^2 = 0.12$; Table 4). The biological significance, therefore, is likely to be negligible.

Table 2. Results for individual effects from a nested analysis of variance to determine the effect of the number of recreational vehicles (RV) on the number of painted turtle (*Chrysemys picta*) nests constructed on a nesting beach in northwestern Illinois during the years 2001–2003.[a] An asterisk (*) signifies an interaction between terms.

Source of variation	df	Sum of squares	*F*-ratio	*p* value
RVs	3	100.49657	0.5084	0.6778
Julian date	3	29.93952	0.1515	0.9284
RVs*Julian date	3	80.81181	0.4088	0.7471
Y	2	20.06618	0.1523	0.8590

[a] All effects were nested within year. The *p* value and r^2 value for the overall test were 0.7993 and 0.09, respectively.

Discussion. — Human recreational activity, as measured by the number of RVs on and near a major nesting beach, did not appear to affect large-scale patterns in the nesting ecology of the population of painted turtles studied here. Variables that represented both the decision to emerge from the water to nest and habitat selection had no biologically significant relationship with the number of RVs present.

Based on the results of previous studies, the lack of effect found here was unexpected. For example, breeding and nest survival of colonial waterbirds may be negatively affected by human recreation (Yorio et al. 2001; reviewed in Carney and Sydeman 1999). Garber and Burger (1995) documented that 2 populations of the wood turtle (*Glyptemys insculpta*) declined 100% within 10 years after the opening of habitat to human recreation (foot traffic leading to opportunistic removal of turtles). Bury and Luckenbach (2002) found that a population of desert tortoises (*Gopherus agassizii*) that was subjected to human recreation in the form of off-road vehicles appeared to be less dense and less healthy than a population that was protected. Given that human recreation and disturbance can have adverse effects on breeding organisms in general and on turtle populations in particular, combined with the likelihood for some turtles to alter nesting behavior when disturbed or when they perceive danger (Iverson and Smith 1993; Spencer 2002; Spencer and Thompson 2003), one might assume that nesting turtles would suffer from human activity. It is important to note, however, that we studied behavioral responses and not population dynamics.

Our results give tentative support to the assertion of a number of investigators (Whittaker and Knight 1998; Miller and Hobbs 2000; Nisbet 2000) that wildlife responses to human recreation are difficult to generalize. Wildlife may respond to human recreation in many ways, thus studies of these effects should be done on a species-specific level (Miller and Hobbs 2000). A population-specific level may be necessary if some species are capable of habituating to disturbance by humans (i.e., they no

Table 3. Results for individual effects from a nested analysis of variance to determine the effect of the number of recreational vehicles (RV) on the south and west overstory vegetation of painted turtle (*Chrysemys picta*) nests constructed on a nesting beach in northwestern Illinois during the years 2001–2003.[a]

Source of variation	df	Sum of squares	*F*-ratio	*p* value
RVs	3	0.18924736	1.2962	0.2750
Julian date	3	0.08643256	0.5920	0.6205
Maximum air temperature	3	0.10417674	0.7135	0.5442
Water temperature	3	0.05369669	0.3678	0.7763
Y	2	0.02290302	0.2353	0.7904
RVs*Julian date	3	0.04975900	0.3408	0.7958
Maximum air temperature*water temperature	3	0.3947058	0.2703	0.8468
Julian date*water temperature	3	0.02635718	0.1805	0.9096
RVs*water temperature	3	0.05441639	0.3727	0.7727
Julian date*maximum air temperature	3	0.03492956	0.2392	0.8690
RVs*maximum air temperature	3	0.01753739	0.1201	0.9483

[a] All effects were nested within year. The *p* value and r^2 value for the overall test were 0.4360 and 0.06, respectively. An asterisk (*) signifies an interaction between terms.

longer respond to human disturbance; Whittaker and Knight 1998). In the future, we plan to test the hypothesis that our turtles have habituated to human presence by using comparative experimental studies with nearby populations.

There is an important caveat to consider in interpreting our results: the analytical methods used are capable of detecting only large-scale changes in turtle nesting behavior as a result of human recreation. We did not evaluate the responses of individual turtles per se, nor did we directly track population dynamics. We have observed turtles abandon nesting attempts as the result of direct human intrusion, adults and hatchlings killed by automobiles, and removal of turtles from the study area by recreationists. These types of situations are not accounted for in our analysis. Examining the response of an individual turtle under different conditions might be more instructive in determining the effects of human recreation. However, this experimental approach would be difficult to implement given that we cannot control when a turtle emerges to nest nor can we directly manipulate the intensity of human recreation.

What do our results mean for managers? Human recreation at TCRA does not appear to have effects on large-scale patterns of painted turtle nesting behavior, and the needs of these turtles and human recreationists may be reconcilable. However, we emphasize that these results should not be taken to suggest that human recreation does not affect freshwater turtles. Generalizations to other species and other forms of recreation should be avoided. Furthermore, even if painted turtles are unaffected by large-scale human activity, the actions of individual humans (removing or disturbing nesting turtles, road kills) should still be taken into account. Education of the public (Klein 1993; Taylor and Knight 2003) concerning the plight and sensitivity of turtles is a good first step. At TCRA, education over the past 15 years by both our research team and USACE park rangers has minimized, but not eliminated, individual human disturbance of painted turtles. Monitoring and enforcement of applicable laws will still be necessary in most cases.

We generally agree with other investigators (Boyle and Samson 1985; Nisbet 2000) that the large number of studies that suggest a negative relationship between human recreation and wildlife should not be applied to all species and all situations. Furthermore, studies that test explicit hypotheses and attempt to determine the fitness effects of recreation on wildlife (Boyle and Samson 1985) should be

Table 4. Results for individual effects from a nested analysis of variance to determine the effect of the number of recreational vehicles (RV) on the distance from water of painted turtle (*Chrysemys picta*) nests constructed on a nesting beach in northwestern Illinois during the years 2001–2003.[a]

Source of variation	df	Sum of squares	*F*-ratio	*p* value
RVs	3	1910.2945	1.1771	0.3179
Julian date	3	1285.4191	0.7921	0.4987
Maximum air temperature	3	462.8087	0.2852	0.8361
Water temperature	3	1089.7052	0.6715	0.5699
Y	2	216.1942	0.1998	0.8189
RVs*Julian date	3	2443.1515	1.5055	0.2123
Maximum air temperature*water temperature	3	778.8275	0.4799	0.6964
Julian date*water temperature	3	777.8249	0.4793	0.6968
RVs*water temperature	3	756.6510	0.4662	0.7060
Julian date*maximum air temperature	3	405.0941	0.2496	0.8616
RVs*maximum air temperature	3	545.6739	0.3362	0.7991

[a] All effects were nested within year. The *p* value and r^2 value for the overall test were 0.0009 and 0.12, respectively. An asterisk (*) signifies an interaction between terms.

designed where feasible. Finally, although research on "secure" populations of freshwater turtles is important (Congdon et al. 2003), ecological and evolutionary studies on human-influenced populations (Kolbe and Janzen 2002b; Feinberg and Burke 2003) are equally crucial given the current rate of habitat alteration and the conservation status of most turtle species. Future studies should focus on the area around individual nesting turtles that must be kept inviolate, if they are to remain undisturbed (area of influence; Miller et al. 2001), and on the effects of recreation-related deaths and adult removals on population dynamics (Garber and Burger 1995).

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Population Structure of the Alligator Snapping Turtle, *Macrochelys temminckii*, on the Western Edge of its Distribution

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ABSTRACT. – A mark-recapture project on *Macrochelys temminckii* was conducted between 1997 and 2000 at Sequoyah National Wildlife Refuge, Muskogee and Sequoyah counties, in eastern Oklahoma. Turtles were captured in all streams and exhibited equal sex ratios, marked sexual-size dimorphism, and population densities between 28 and 34 animals per km stretch of stream. There was evidence of past population perturbations, with very few large adults captured, and a cohort of subadults highly underrepresented.

Turtles have long been recognized as an integral part of aquatic communities, and all relevant literature on river turtle diversity, ecological roles, and community structure was recently reviewed in Moll and Moll (2004). Within this synopsis though, it is clear that outside of common species, such as the slider turtle, *Trachemys scripta* (Cagle 1950; Gibbons 1990), in-depth life history studies of individual species are noticeably absent. Detailed life-history strategies have been constructed only for a handful of species, most notably the Blanding's turtle, *Emydoidea blandingii* (Congdon et al. 1993), and the common snapping turtle, *Chelydra serpentina* (Congdon et al. 1994). The data collected on *C. serpentina* were representative only of populations at the northern reaches of the species' distribution and so did not demonstrate geographic variation in life-history strategies for that species. With many species of turtles facing various threats, a better understanding of these life-history strategies is much needed for developing sound management strategies.

The alligator snapping turtle, *Macrochelys temminckii*, is a large, riverine, bottom-dwelling species that occupies a predator-scavenger role in the southeastern United States (Moll and Moll 2000). Shipman and Riedle (1994) and Shipman and Neeley (1998) surveyed 2 populations in southeastern Missouri. In each, turtles were 2–24 kg in body mass; the sex ratio for the 2 populations was 1 male to 1.09 females. Trauth et al. (1998) surveyed 2 sites in Arkansas with a population sex ratio of 1:1 and reported that males were significantly larger than females. Males were also significantly larger than females from examination of specimens at a commercial meat-processing facility in Louisiana (Tucker and Sloan 1997). Based on growth curves, *M. temminckii* reached sexual maturity when the straight carapace length (CL) was 370 mm in males and 330 mm in females (Dobie 1971; Tucker and Sloan 1997).

Because of the apparent decline of the species throughout its range (Pritchard 1989; Ernst et al. 1994),

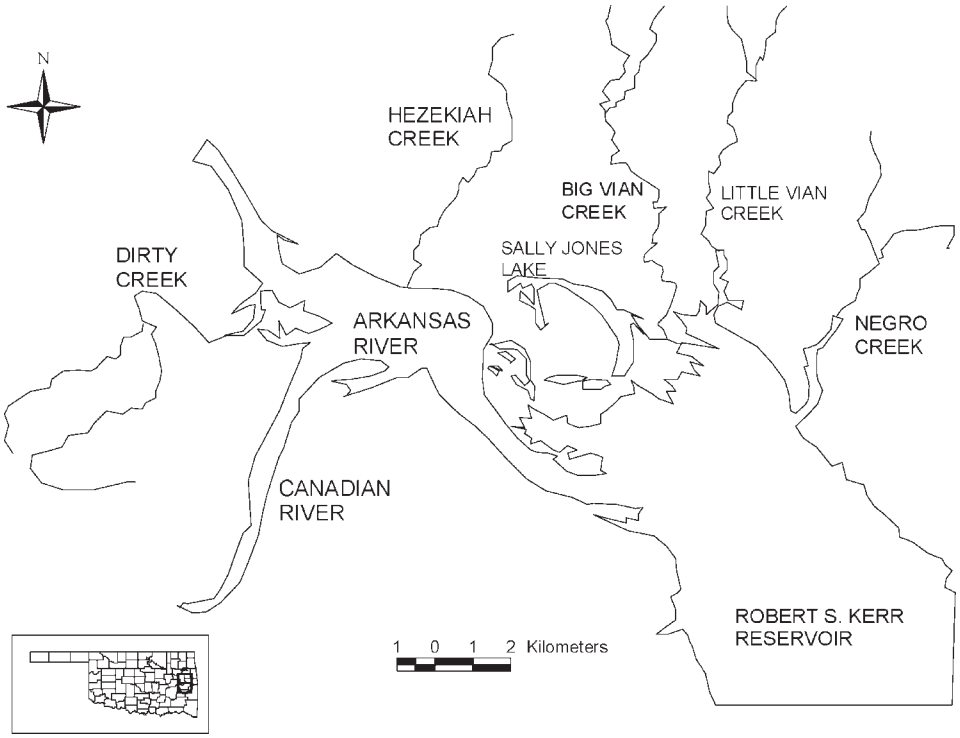

Figure 1. Sequoyah National Wildlife Refuge, Muskogee and Sequoyah counties, Oklahoma.

large unimpacted populations are difficult to find. Despite the need for aggressive conservation measures throughout its range, information on ecology and demography of unimpacted populations is largely nonexistent but obviously necessary for the management or restoration of impacted populations. The eastern third of Oklahoma was surveyed in 1997–1999 specifically for *M. temminckii*, which has experienced drastic declines throughout its range, and only 2 seemingly healthy populations were discovered (Riedle et al. 2005). Our objective was to describe the demographic structure of a population of *M. temminckii* by quantifying population size and density, sex ratio, sexual dimorphism, and size-class distribution, which are needed to develop conservation strategies.

Methods. — Sequoyah National Wildlife Refuge (SNWR) is a 51,376-ha area in Oklahoma that encompasses parts of the Canadian and Arkansas rivers and their confluence. Primary habitat was bottomland flood plain with many small tributaries that drained into both rivers. We sampled SNWR sporadically in 1997 and 1998, and more intensively in 1999 and 2000. Several small streams were surveyed, including Dirty Creek, Hezekiah Creek, Big Vian Creek, Little Vian Creek, and Negro Creek. Sally Jones Lake, a shallow lake connected to Big Vian Creek, also was surveyed (Fig. 1). Surveying was conducted again in 2001 to capture *M. temminckii* at sites where it was not previously captured. Throughout the study, Big Vian Creek and Little Vian Creek were sampled more intensively because of their easy access and were used to estimate population size and density. Both streams are tributaries of the Arkansas River, and their mouths were about 0.5 km apart. The navigable (by a 4.2-m flat-bottomed boat) stretches of both streams were surveyed. The navigable stretch of Little Vian Creek was 2 km in length, reaching from its mouth until the stream became shallow and predominated by riffles. Big Vian Creek was 4.5 km in length from the mouth to where the stream became very shallow and clogged with fallen logs.

All streams were sampled by using commercial hoop nets that were 2.1 m in length and constructed of four 1.05-m hoops covered with 2.5-cm mesh. Nets were set upstream from submerged structures, such as fallen trees. Nets were baited with fresh fish suspended by a piece of twine on the hoop furthest from the opening of the net. Bait fish were procured with gill nets or incidental capture in the turtle nets. Turtle nets were set late in the afternoon or evening and were checked the following morning.

We recorded basic morphometric data on each *M. temminckii* captured, including mass (to the nearest 0.1 kg), sex, and maximum CL and plastron length (PL) to the nearest millimeter. All individuals of *M. temminckii* captured were uniquely marked and fitted with numbered tags. The marking was done by using a hole drilled into specific marginal scutes along the carapace. We placed short plastic cable ties in all holes to ensure that they did not prematurely close. Numbered plastic cattle ear tags also were attached to one of the holes by a plastic cable tie.

Each *M. temminckii* was assigned to 1 of 3 groups based on sex and size. Sex was determined by the presence or absence of a penis. The penis, if present, can be felt by

Table 1. Number of individuals and composition of turtle species captured at Sequoyah National Wildlife Refuge, Oklahoma, between 1997–2000.

Species	*n*	% Total captures
Trachemys scripta	2287	82.8
Macrochelys temminckii	197	7.1
Graptemys ouachitensis	103	3.7
Chelydra serpentina	64	2.3
Apalone spinifera	40	1.4
Pseudemys concinna	32	1.1
Sternotherus odoratus	18	0.6
Graptemys pseudogeographica	17	0.6
Kinosternon subrubrum	1	<0.1

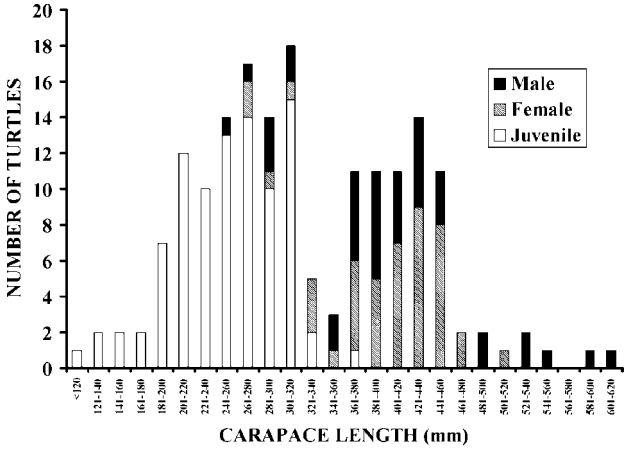

Figure 2. Size classes, based on carapace length in millimeters, of *Macrochelys temminckii* captured at Sequoyah National Wildlife Refuge, Oklahoma.

inserting a finger into the turtle's cloaca. Turtles that were too small to examine for a penis were classified as juveniles (sex unknown). Morphologic measurements were compared between males and females by using 2 sample *t*-tests. A χ^2 analysis of sexes by size class was used to compare number of males to females in 2 size classes: medium (361–480 mm) and large (481–620 mm).

Results. — Population Size and Density. — We surveyed for 565 net nights (1 net night = 1 net/night) between 1997 and 2000 on Dirty Creek, Hezekiah Creek, Big Vian Creek, Little Vian Creek, Sally Jones Lake, and Negro Creek and made 197 captures of *M. temminckii*. *Macrochelys temminckii* was not captured in Sally Jones Lake or Negro Creek between 1997 and 2000. We marked and released 157 *M. temminckii,* with a recapture rate of 21%. An additional 26 captures (22 new individuals, 4 recaptures) of *M. temminckii* were made in 2001, 4 of those on Negro Creek. Nine species of aquatic turtles were captured, and *M. temminckii* was the second most abundant, which represented 7% of all captures (Table 1).

We used a Lincoln-Peterson estimator of population size based on capture-mark-recapture data in 1997–2000. Estimated population sizes were 127.5 ± 24.5 standard error (SE) individuals, with a density of 28.3 turtles/km in Big Vian Creek, and 68.4 ± 18.2 SE individuals, with a density of 34.2 turtles/km in Little Vian Creek.

Size Distribution. — Mean sizes of turtles were 8.71 kg (range 0.22–46.4 kg), 330 mm CL (110–614 mm), and 240 mm PL (72–470 mm). We captured few small juveniles and large adults, and turtles with CL between 321 and 360 mm were noticeably underrepresented (Fig. 2).

Sex Ratio and Sexual-Size Dimorphism. — We captured 41 males, 47 females, and 91 juveniles. The male:female ratio (1:1.1) did not differ from 1:1 ($\chi^2 = 0.0036$, df = 1, $p = 0.952$). We were able to determine sex of males ≥240 mm CL and females ≥260 mm CL in most cases. We were able to determine sex of all individuals (except one) at CL > 360 mm (Fig. 2).

We evaluated sexual-size dimorphism of adult turtles based on the upper end of the range of size at sexual maturity (400 mm; Dobie 1971; Tucker and Sloan 1997). Males were significantly larger than females in CL ($t = 2.45$, df = 53, $p = 0.017$) and mass ($t = 2.84$, df = 56, $p = 0.006$). The number of males and females differed between the medium and large size classes ($\chi^2 = 4.76$, df = 1, $p = 0.029$); males were more abundant in the large adult cohort (Fig. 2).

Discussion. — Macrochelys temminckii was the second-most abundant species captured at SNWR, which occurred in high densities relative to most other populations in the state (Riedle et al. 2005). Because the Lincoln-Peterson estimator assumes no emigration or immigration, it may have overestimated population size, but *M. temminckii* was captured frequently and still exhibited a low recapture rate. Unfortunately, there is a paucity of information on absolute population densities of *M. temminckii* to compare with SNWR densities.

The adult sex ratio of 1:1.1 is similar to values from populations in Missouri and Arkansas (Shipman and Riedle 1994; Shipman and Neeley 1998; Trauth et al. 1998). Among the larger turtles (CL > 480 mm), males significantly outnumbered females (7:1). Although we were able to sex some individuals at relatively small sizes, our ability to sex small turtles was inconsistent from individual to individual (limited by the inability to insert a finger far enough into the cloaca to feel for the presence or absence of a penis). We also were not able to determine the size of sexual maturity, because we did no internal analysis of follicular or testicular maturation. We were, however, able to accurately sex all individuals (except one) with CL ≥ 380 mm, which was within the size range for sexually mature turtles in Louisiana (Tucker and Sloan 1997). Populations of *M. temminckii* in Louisiana reached sexual maturity at 13–21 years for females and 11–21 years for males, but estimates of age to maturity are not available for Oklahoma populations. A wide range of size classes was captured, which provided evidence for a stable population with good recruitment (Fig. 2), with some

exceptions. Three cohorts were rare or absent from our sample: hatchling-size turtles, turtles in the size class 321–360 mm CL, and large adults.

Although the population of *M. temminckii* at SNWR appeared stable, there was evidence of current and historic population perturbations. Hatchling-size turtles were absent from our sample, and turtles <180-mm CL were rare. There may have been a trap bias toward larger turtles because the 2.5-cm mesh was small enough to contain hatchling turtles, but the throat, designed to capture larger turtles, may have been large enough to allow the escape of small turtles. Juvenile common snapping turtles, *Chelydra serpentina*, occupy small streams after hatching and disperse from those streams as they reach sexual maturity (Graves and Anderson 1987). This could also be occurring with hatchling *M. temminckii* at SNWR, with smaller turtles occupying smaller streams where we did not trap at all. Lastly, the absence of small turtles could be attributed to mammalian predation, principally raccoons, *Procyon lotor*. Raccoons have been identified as one of the most important predators on North American turtles (Stancyk 1982; Ernst et al. 1994), with raccoon related egg and hatchling mortality rates that reach 100% in many populations (Mitchell and Klemens 2000).

Individuals with 321–360-mm CL were underrepresented in our sample. One hypothesis may be that the refuge population experienced some past disturbance that may have had a negative impact on nest success, and that the underrepresented size-age class is the lingering "footprint" of such relative nest failure. Such a past disturbance, known to affect other species of turtles (Moll and Moll 2000), was the flooding of upstream areas after construction of the dam to make the Robert S. Kerr Reservoir downstream from SNWR. Construction of the dam began in April 1964, and the closure occurred in October 1970. Subsequently, stream levels rose significantly based on anecdotal information from SNWR personnel and the presence of remnant hardwood structure in the current streambed. This dramatic rise in water level may have temporarily destroyed nest sites along the streams. The time elapsed since this post-1970 flooding some 25 years ago may correspond to the age of the 321–360-mm CL underrepresented size class, although, based on Tucker and Sloan (1997), these turtles seem too young. Nevertheless, the growth rate of Oklahoma *M. temminckii* is unknown, and the time course of habitat disruption because of upstream flooding after the creation of a large reservoir is also unknown.

Large adults (25–55 kg) also were scarce in our sample; although, many such large individuals were captured throughout eastern Oklahoma in the past (Webb 1970; Carpenter and Krupa 1989). We captured 7 large males (25.0, 28.1, 34.5, 36.3, 41.8, 42.3, and 46.4 kg) and one large female (26.8 kg) while sampling at SNWR. One hypothesis for the scarcity of large turtles in our study is that historic harvest of large turtles may have occurred at SNWR. Shipman and Riedle (1994) and Trauth et al. (1998) reported differences in body size between harvested and unharvested populations, with the absence of larger turtles from exploited populations.

There is some evidence for an historical take of *M. temminckii* in Oklahoma (Carpenter and Krupa 1989; Pritchard 1989). Before SNWR was established in 1970, there may have been a significant harvest of *M. temminckii,* especially large ones, from that area, and not enough time has elapsed to allow for the current adult cohort to grow to larger body sizes. The current paucity of large adults supports this conclusion. If breeding adults were seriously depleted before the refuge was established, then there would have been very little recruitment of turtles (now represented by the missing size-age class). However, smaller turtles would have been commercially unimportant and left unharvested. These turtles have now grown into the small adult age class at SNWR (Fig. 2). Their offspring are the current subadults. A few turtles big enough to be commercially important before the refuge was established somehow escaped harvest and currently represent the largest size-age class at SNWR (Fig. 2).

Although the type and severity of historical impacts on populations of *M. temminckii* at SNWR are largely speculative, there is some evidence that total protection of the adult cohorts will allow populations of *M. temminckii* to recover over time. A large number of adults reaching sexual maturity and even more subadult turtles following behind, which suggests that the species can recover after historic disturbances, albeit slowly, are shown in Fig. 2. What remains unknown is what is happening with the hatchling cohorts and how that may affect the future stability of the population. Much work is needed in the future to more fully understand the life history of *M. temminckii* and its role in the aquatic turtle community.

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Potential Bacterial Pathogens Carried by Nesting Leatherback Turtles (*Dermochelys coriacea*) in Costa Rica

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ABSTRACT. – Between March and May 2003, the leatherback turtle (*Dermochelys coriacea*) nesting population of the Pacuare Nature Reserve (northern Caribbean coast of Costa Rica) was surveyed for potential bacterial pathogens associated with the cloaca and upper respiratory tracts. A total of 189 isolates that belong to 15 genera, including 113 gram-negative and 76 gram-positive bacteria, were identified from samples of 70 nesting females. The majority of the bacterial species recovered in this study, including 5 *Salmonella* isolates, may be considered as potential pathogens for sea turtles, as well as for humans.

The leatherback sea turtle (*Dermochelys coriacea*) is the only surviving member of the Dermochelyidae and the largest of living chelonians. It is highly migratory and is distributed in temperate and tropical pelagic waters (Plotkin 2003). The leatherback turtle rookery of Caribbean Central America represents 1 of the 4 largest remaining rookeries worldwide, together with French Guiana/Suriname, Gabon, and Trinidad (Troëng et al. 2004).

Incidental capture in gillnet and longline fisheries, together with egg harvest, have been indicated as the principal factors involved in the decline of many leatherback populations (Kar and Bhaskar 1982; Spotila et al. 2000; Lewison et al. 2004; Sarti-Martinez et al. 2007; Alfaro-Shigueto et al. 2007). Marine pollution by several anthropogenic contaminants, primarily plastic debris, may also have an important impact on the survival of this species (Wehle and Coleman 1983; Barreiros and Barcelos 2001). However, there is insufficient information on the impact of marine debris throughout most of this species range, nor is there adequate knowledge on the health status of leatherbacks in the wild.

No information exists on the cloacal and nasal microflora of apparently healthy leatherback turtles. Only 1 report exists on the isolation of *Vibrio damsela* that causes lethal valvular endocarditis and septicemia in a

Table 1. Number of aerobic isolates recovered from the cloacal samples of nesting leatherback turtles (*Dermochelys coriacea*) ($n = 70$), at the Pacuare Nature Reserve, Mar–May 2003.

Cloacal isolates	March ($n = 10$)	April ($n = 21$)	May ($n = 39$)	PT (%)[a]
Gram-positive bacteria				
Bacillus firmus	0	0	2	2 (2.8)
Enterococcus faecalis	0	0	6	6 (8.5)
Staphylococcus cromogenes	0	2	0	2 (2.8)
Gram-negative bacteria				
Aeromonas salmonicida	7	5	4	16 (22.8)
Bordetella avium	0	1	2	3 (4.2)
Enterobacter agglomerans	2	1	4	7 (10)
Escherichia coli	3	1	0	4 (5.7)
Klebsiella oxytoca	0	0	5	5 (7.1)
Klebsiella pneumoniae	0	0	5	5 (7.1)
Proteus mirabilis	0	0	6	6 (8.5)
Proteus vulgaris	0	1	0	1 (1.4)
Pseudomonas aeruginosa	2	5	2	9 (12.8)
Pseudomonas fluorescens	0	2	2	4 (5.7)
Salmonella spp.	2	3	0	5 (7.1)
Vibrio alginolyticus	0	0	3	3 (4.2)
Vibrio fluvialis	0	0	2	2 (2.8)
Total isolates	16	21	43	

[a] PT (%) = Positive turtles (%).

stranded female from the eastern coast of Tasmania (Obendorf et al. 1987). However, in free-ranging sea turtles, *Corynebacterium* encephalitis (George 1997), *Aerococcus viridans* esophageal infection (Torrent et al. 2002), and mixed generalized infections (Oros et al. 2005) in loggerheads (*Caretta caretta*), and *Mycobacterium chelonae* osteoarthritis in a Kemp's ridley (*Lepidochelys kempii*; Greer et al. 2003) have been recorded. This study

Table 2. Number of aerobic isolates recovered from the nasal samples of nesting leatherback turtles (*Dermochelys coriacea*) ($n = 70$), at the Pacuare Nature Reserve, Mar–May 2003.

Upper respiratory tract isolates	March ($n = 10$)	April ($n = 21$)	May ($n = 39$)	PT (%)[a]
Gram-positive bacteria				
Arcanobacterium pyogenes	1	1	0	2
Bacillus spp.	2	4	13	19
Bacillus cereus	0	1	0	1
Bacillus coagulans	0	1	0	1
Bacillus firmus	0	9	18	27
Bacillus lentus	1	1	0	2
Bacillus subtilis	0	1	1	2
Corynebacterium spp.	3	1	0	4
Enterococcus faecalis	0	0	3	3
Paenibacillus alvei	0	0	1	1
Bacillus cromogenes	0	0	1	1
Bacillus intermedius	0	3	0	3
Gram-negative bacteria				
Aeromonas salmonicida	2	1	0	3
Bordetella avium	0	1	0	1
Enterobacter agglomerans	1	3	8	12
Escherichia coli	0	0	2	2
Klebsiella oxytoca	0	0	2	2
Klebsiella pneumoniae	0	0	8	8
Proteus mirabilis	0	0	1	1
Proteus vulgaris	0	0	2	2
Pseudomonas aeruginosa	0	1	2	3
Pseudomonas putida	0	0	1	1
Vibrio alginolyticus	0	2	3	5
Vibrio fluvialis	0	0	1	1
Vibrio hollisae	0	0	2	2
Total isolates	10	30	69	

[a] PT (%) = Positive turtles (%).

Table 3. Most-probable numbers of total and fecal coliforms, enterococci from seawater samples (100 mL), and monthly precipitation at the Pacuare Nature Reserve, Mar–May 2003.

Site	Indicator trait	Months of sampling, 2003		
		Mar	Apr	May
Site 1	Total coliforms	< 2/100 mL	< 2/100 mL	> 1600/100 mL
	Fecal coliforms	< 2/100 mL	< 2/100 mL	> 1600/100 mL
	Enterococci	< 3/mL	< 3/mL	> 1600/100 mL
Site 2	Total coliforms	< 2/100 mL	< 2/100 mL	> 1600/100 mL
	Fecal coliforms	< 2/100 mL	< 2/100 mL	1600/100 mL
	Enterococci	< 3/mL	< 3/mL	> 1600/100 mL
Site 3	Total coliforms	< 2/100 mL	< 2/100 mL	1600/100 mL
	Fecal coliforms	< 2/100 mL	< 2/100 mL	22/100mL
	Enterococci	< 3/mL	< 3/mL	1600/100 mL
Precipitation (mm)[a]		148.3	99.1	500.8

[a] Precipitation data are from the National Weather Institute of Costa Rica.

documents the cloacal and nasal aerobic bacteria from apparently healthy nesting leatherbacks at the Pacuare Nature Reserve (PNR), Costa Rica.

Study Site. — The PNR is located along the northeast Caribbean coast of Costa Rica (from lat 10°07′N, long 083°11′W to lat 10°13′N, long 083°16′W), near Matina, Limón Province, and includes approximately 5.7 km of protected beach. It is bordered to the north by the Pacuare River and to the south by Mondonguillo Lagoon and is surrounded by a complex network of canals and rivers, along which several villages, farms, and banana plantations are located. Between 490 and 1286 leatherback nests are recorded annually during the nesting season, which runs from March to July (Troëng et al. 2004).

Methods. — Between March and May 2003, samples were obtained from 70 female leatherbacks, with a mean curved-carapace length of 152.5 cm. Sampling consisted of introducing sterile swabs in the cloaca (ca. 10-cm internal depth) and in 1 nasal duct (ca. 8-cm internal depth) while the turtles were completing nesting activities in the PNR. We recorded flipper tag numbers so as to not resample individuals, and we only collected samples from apparently healthy nesting leatherbacks with no external lesions or signs of diseases.

Samples were placed in Amies agar gel transport medium (Oxoid Ltd., Basingstoke, Hampshire, UK), kept on ice, and cultured within 24 hours on blood agar, MacConkey agar, manitol salt agar, thiosulfate-citrate-bile-salts-sucrose agar, and xylose-lysine-desoxycholate (XLD) agar (Oxoid). The latter 2 agars are selective for *Vibrio* spp. and *Shigella*/*Salmonella* spp., respectively. Plates were incubated aerobically at 25–27°C and were examined after 24 hours. To aid in the recovery of *Salmonella* spp., cloacal samples were also inoculated into a Rappaport-Vassiliadis-Soya peptone broth (Oxoid), incubated at 37°C for 18 hours, and subcultured on XLD agar. Bacterial isolates were identified by standard protocols described by Murray et al. (1999). Identification of gram-negative organisms was confirmed by Api System 20E and 20NE (BioMérieux, Marcy l'Etoile, France).

During the same period, March to May 2003, sea water samples from the PNR were also obtained off the nesting beach. Three samples per month corresponding to 1 sample per 1.9 km were collected in 100-mL sterile plastic bags and processed by conventional methods (American Public Health Association 1995). Total and fecal coliforms, and enterococci were enumerated by most-probable-number estimates by using standard techniques (American Public Health Association 1995).

Results and Discussion. — This study represents the only report of cloacal and nasal bacteria in leatherbacks. A total of 189 bacterial isolates that belong to 15 genera, including 113 (60%) gram-negative and 76 (40%) gram-positive bacteria, was identified (Tables 1 and 2). Eighty isolates were recovered from the cloaca and 109 from the nasal cavities. Isolates of the genus *Bacillus* were predominant among gram-positive isolates (54 of 76 isolates [71.1%]) and were recovered mostly from nasal samples (52 of 54 isolates [96.3%]). Only 10 gram-positive isolates were recovered from cloacal samples, including 6 *Enterococcus faecalis*. Enterobacteriaceae isolates, including *Enterobacter agglomerans*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, and *Salmonella* spp. were predominant among gram-negative isolates (60 of 113 isolates [53%]); whereas, *Aeromonas*, *Pseudomonas,* and *Vibrio* represented only 16.8%, 15.0%, and 11.5% of the gram-negative isolates, respectively. *Aeromonas* and *Pseudomonas* were recovered more often from cloacal samples (84.2% and 76.4%, respectively) rather than from nasal samples (15.8% and 23.6%, respectively). Such differences were not observed for Enterobacteriaceae and *Vibrio*. The majority of the bacterial species recovered in this study may be considered as potential pathogens for sea turtles, as well as for humans.

During March and April, no water bacterial pollution was detected, but a high degree of total and fecal coliforms and enterococci was found in seawater samples from May (Table 3). In May, the National Weather Institute of Costa Rica registered a strong precipitation increase in the study area (Table 3), which reflects the beginning of the rainy

season in Costa Rica. Heavy rainfall raised the level of the rivers, which caused serious flooding of the nearby villages, farms, and plantations. Alongside the high bacterial counts of sea water, some bacterial species, namely *E. faecalis*, *K. oxytoca*, *K. pneumoniae*, *P. mirabilis*, *Pseudomonas putida*, *Vibrio fluvialis*, and *Vibrio hollisae*, were recovered from the turtle samples only during May. Whether the elevated levels of coliforms and enterococci found from seawater collected in May were induced by altered environmental conditions because of heavy rainfall or were influenced by other conditions is not known. Many bacteria here recovered were microorganisms commonly found in the environment (Murray et al. 1999), and the possibility that they were acquired from seawater or beach sand contamination must be also considered.

Coliforms and enterococci are common inhabitants of intestinal tracts of larger endo- and ectothermic animals (Aguirre et al. 1994; American Public Health Association 1995; Santoro et al. 2006a, b). However, bacteriological analyses performed on fresh feces from green sea turtles (*Chelonia mydas*) yielded very low concentrations of coliforms and enterococci compared with mammal and avian faeces (Balazs et al. 1993). Most of the microbes found in this study were associated with pathologies in captive and free-ranging sea turtles (Glazebrook and Campbell 1990; Glazebrook et al. 1993; Orós et al. 2005).

Within sea turtles, *Salmonella* spp. were previously found in hawksbill (*Eretmochelys imbricata*) (Keymer et al. 1968), green (Raidal et al. 1998; O'Grady and Krause 1999), and olive ridley (*Lepidochelys olivacea*) turtles (Santoro et al. 2006a). Salmonellosis is the most recognized reptilian zoonosis. We recommend caution, particularly concerning volunteers and biologists handling chelonians or working on nesting beaches and handling eggs from natural nests.

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Relative Abundance and Morphometrics of the Travancore Tortoise, *Indotestudo travancorica*, in the Indira Gandhi Wildlife Sanctuary, Southern Western Ghats, India

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ABSTRACT. – **Relative abundance of the Travancore tortoise, *Indotestudo travancorica*, was assessed in 5 localities within the Indira Gandhi Wildlife Sanctuary, India, from December 2002 to March 2003. Relative abundance varied with site and season, but the overall mean search effort for this study was only 3.4 man-hours/tortoise. Morphometric data were collected from 49 adults and 3 juveniles; sexual differences in carapace shape (elongated in males, ovoid in females) and variations in carapace color (darker in mesic habitats) were also observed.**

First described by Boulenger (1907), the Travancore tortoise, *Indotestudo travancorica* (Fig. 1), is endemic to the forested hill ranges of the Western Ghats of India (Boulenger 1907; Vijaya 1983; Pritchard 2000; Iverson et al. 2001). It is a medium-sized brown tortoise, often with dark blotches on the plastron and the carapace (Boulenger 1907; Pritchard 2000).

Studies on wild *I. travancorica* have mainly consisted of rapid surveys and anecdotal notes (Groombridge et al. 1983; Vijaya 1983; Frazier 1989; Moll 1989; Das 1991; Bhupathy and Choudhury 1995); the only field study on the species was conducted by J. Vijaya more than 20 years ago, but the results were not published (H. Andrews, *pers. comm.* in Ramesh 2004). Population estimates are not available (Molur and Walker 1998; Choudhury et al. 2000; IUCN 2004), but *I. travancorica* is considered a vulnerable species by the International Union for Conservation of Nature (IUCN 2004). Information on its natural history is sparse; it is known to inhabit forests up to 750 m and is found near streams and marshes (Bhupathy and Choudhury 1995; Ramesh, *in press*). It uses leaf litter, tree buttresses, and rock clefts for shelter (Vijaya 1983; Das 1991; Bhupathy and Choudhury 1995), as well as dense thickets of weeds such as *Lantana camara*. Though considered a crepuscular species, during a 2-month-long survey in the Indira Gandhi Wildlife Sanctuary (IGWLS), most tortoises were encountered during the evening hours (1700–1830 hours; Ramesh, *in press*). *Indotestudo travancorica* is sexually dimorphic—in males the plastron is concave; in females, flat. The tail terminates in a claw (a horny tubercle) which is large and hooked in males, small and conical in females (Auffenberg 1964; Vijaya 1983; Das 1991).

Figure 1. The Travancore tortoise, *Indotestudo travancorica*, in the Indira Gandhi Wildlife Sanctuary, India. Photo by S.U. Saravanakumar/Ecotone.

The first intensive survey of *I. travancorica* was conducted in 2002, in the IGWLS, and because this resulted in the largest number of sightings to date ($n = 27$, Ramesh, *in press*), a more detailed study was conducted to assess the relative abundance of *I. travancorica* in different localities within the sanctuary and to collect morphometric data.

Study Area. — The IGWLS (lat 10°12′–10°35′N, long 76°49′–77°24′E) in the southern Western Ghats, is one of the largest protected areas in India, with an area of 958 km^2. Several Forest Department posts are located within its boundaries, Topslip (lat 10°28.561′N, long 76°49.992′E, 750 msl) and Varagaliar (lat 10°25.070′N, long 76°51.940′E, 600 msl) being 2 such sites. There also exist tribal settlements within the sanctuary—the Kadar settlement of Erumaparai is located 1.5 km from Topslip, and the elephant camp at Varagaliar is inhabited mainly by Malasars and Pulayars. The 2 sites are 23 km apart by road

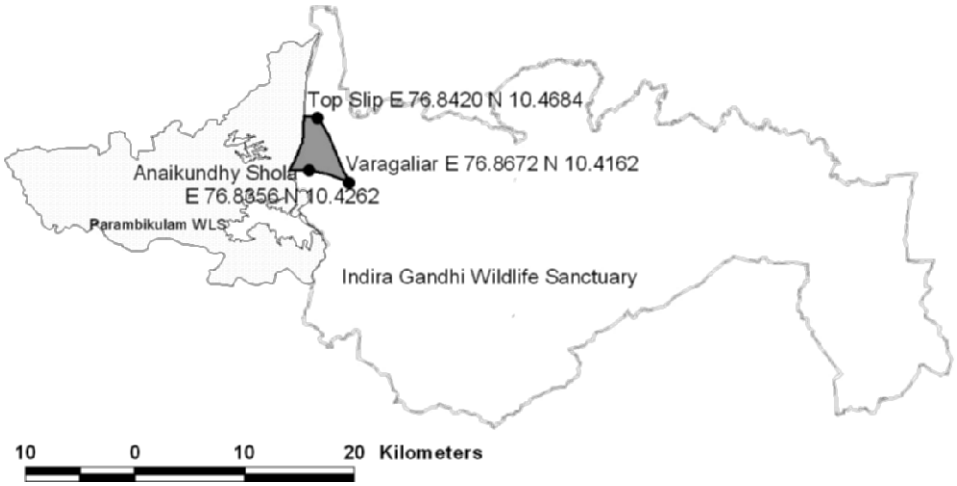

Figure 2. Map of the study area within the Indira Gandhi Wildlife Sanctuary, south India. This sanctuary lies in the state of Tamil Nadu and is contiguous with the Parambikulam Wildlife Sanctuary of Kerala.

(straight-line distance about 7 km, No.58/B/15/4, 1:15,000 Tamil Nadu Coimbatore Forest Circle, Government of India). The study area comprised the region between Topslip and Varagaliar in the east and the Kerala–Tamil Nadu interstate border in the west (Fig. 2). This region receives rainfall from both the southwest and northeast monsoons; June and July are the wettest months of the year, and December to April, the driest.

Five localities within the study area were searched intensively for tortoises. They included the Karian Shola National Park at Topslip, which has tropical wet evergreen forests of the *Drypetes–Calophyllum–Polyalthia–Vateria* type (Champion and Seth 1968; Pascal 1988); Kozhikamuthy, which has tropical moist deciduous forest of the *Terminalia–Anogeissus–Tectona* type (Champion and Seth 1968); the teak (*Tectona grandis*) plantations of Sichali and Varagaliar; and the Anaikundhy Shola—a small wet evergreen forest fragment. Small, seasonal streams run through all 5 localities, and the teak plantations also have stretches of marshy ground covered with grasses. The latter are locally known as *vayal* and are often the only source of water in the plantations in summer.

Methods. — Based on the results of the preliminary survey (Ramesh, *in press*), I conducted a more intensive study from 14 December 2002 to 31 March 2003. During this period, the area-constrained visual encounter survey method was used (Crump and Scott 1994), in which 2 persons searched the banks of streams and *vayal*s for tortoises from 1400 to 1900 hours, except for 2 days in March when 3 searchers were used. In the case of large *vayal*s, wherever possible, the middle section was also searched for tortoises that could be resting in clumps of tall grasses. Each area was searched only once daily. Apart from direct sightings, cues indicative of tortoise presence, such as trails and foraging signs, were used to locate individuals. Search effort was used as a measure of relative abundance (Klemens and Moll 1995; Platt et al. 2003). But, unlike other studies in which search effort is presented as number of tortoises observed per man-hour of searching, i.e., sighting frequency, I converted this into its reciprocal (number of man-hours of searching required to find one tortoise) to facilitate comparisons (Freilich et al. 2000).

All tortoises found were marked with a unique combination of notches on marginal scutes (Cagle 1939) and checked for evidence of external injury and ectoparasites. Straight carapace length (SCL), straight carapace width, shell height (HT), plastron length, and plastron width were measured using Mitutoyo steel calipers (300 mm); anal fork (AF) and anal notch (AN) by dial calipers (150 mm); and body mass (WT) by Zebco Deliar weighing scales (Smith et al. 2001). Tortoises were sexed on the basis of plastral and tail-claw characteristics (Auffenberg 1964; Vijaya 1983; Das 1991), and individuals smaller than 160 mm SCL were considered to be juveniles (Ramesh, *in press*). Morphometric differences between

Table 1. Search effort for *Indotestudo travancorica* across localities in the Indira Gandhi Wildlife Sanctuary (December 2001–March 2003).

Locality	Vegetation type	Man-hours of search	No. of tortoises	Hours of effort per tortoise
Anaikundhy Shola	Wet evergreen	43.6	21	2.1
Karian Shola	Wet evergreen	64.5	20	3.3
Sichali	Teak/*vayal*	47.4	12	4.0
Kozhikamuthy	Moist deciduous	14.0	2	7.0
Varagaliar	Teak/*vayal*	21.4	2	10.7

Table 2. Search effort for *Indotestudo travancorica* in the Indira Gandhi Wildlife Sanctuary from December 2002 to March 2003.

Month	Man-hours of search	No. of tortoises	Hours of effort per tortoise
Dec 2002	32.9	10	3.3
Jan 2003	53.2	6	8.9
Feb 2003	62.4	16	3.9
Mar 2003	42.5	25	1.7

male and female tortoises were tested using a 2-tailed Mann-Whitney U test.

Results. — In total, 57 individuals were found. Adults of both sexes were sighted in equal numbers (27 each), but only 3 juveniles were recorded. There were no recaptures despite repeated searches of some localities (Karian Shola, Sichali, and Anaikundhy). Apart from signs such as trails and bitten leaves, the rustling sound produced when a tortoise moves on dry leaf litter also proved useful in locating individuals. In one instance, 3 tortoises were located by another auditory cue—a tortoise (male, SCL 251mm, WT 2.3 kg) was heard grunting loudly and shell-ramming another individual (male, SCL 209.5mm, WT 1.4 kg) while a third individual remained nearby (female, SCL 266 mm, WT 3.2kg). This is the first record of male combat observed in the wild. Overall, the mean search effort was 3.4 man-hours/tortoise. Search effort was lowest in Anaikundhy Shola and highest in Varagaliar (Table 1). It also varied across the 4 months, with search effort being highest in January and lowest in March (Table 2). All tortoises were found singly, except during the month of March when after convectional summer showers, 2 or more individuals were found in close spatial proximity and therefore only 1 hour of search effort was required to locate each tortoise (Table 3).

Morphometric data were collected from 49 adults and 3 juveniles (Table 4); 5 tortoises could not be measured because fieldwork was interrupted by elephants. There were no significant differences among sexes in SCL ($z = 1.34$, $p = 0.18$), WT ($z = 0.74$, $p = 0.45$) and AN ($z = -0.46$, $p = 0.65$), but males had significantly lower HT ($z = -2.12$, $p = 0.034$) and larger AF ($z = 2.22$, $p = 0.026$). The largest tortoise (a male) measured 307 mm (SCL) and weighed 3.6 kg.

In addition, carapacial color appeared to vary according to the locality: 35% of tortoises found in Karian Shola ($n = 20$) and 33% in Anaikundhy ($n = 21$) were dark brown and/or had large black blotches on the scutes; whereas, tortoises from Sichali, Kozhikamuthy, and Varagaliar were a lighter, duller brown.

Most of the tortoises (51 of 57 found, 90%) had ticks (average = 3 ticks/tortoise) attached to the marginal scutes of the carapace. Occasionally, ticks were also found near the base of the tail. Some tortoises were found to shelter in thickets of weeds (*Lantana camara* and *Cromolarium glandulosum*) (Ramesh, *in press*; 6 of 57 found, 10.5%).

Discussion. — Because of its cryptic coloration the Travancore tortoise is considered difficult to find in the wild (Frazier 1989). Even local people such as the Kadars (a hunter-gatherer tribe), who are knowledgeable about the area and familiar with this species, consider it an arduous task to find tortoises, and in surveys conducted by other biologists, the largest number observed was 7 (Moll 1989). However, the overall mean search effort reported in this study is much lower than those reported elsewhere (Table 5). Therefore, factors that are thought to have resulted in greater capture success are discussed here. Suitable localities and microhabitats (near streams and *vayal*s) were identified and search effort was concentrated here. Signs of tortoise presence such as trails, bitten leaves, and fresh scat were used to locate individuals—trails proved to be the most reliable indicators, since they are clearly visible in wet and dry seasons and in different microhabitats. Searches were conducted only during the period when tortoises are most active, thereby improving capture probabilities (Freilich et al. 2000; Ramesh, *in press*).

Further, confining the number of searchers to 2 instead of a group improved search efficiency as tortoises were less likely to be disturbed and hide under dense cover (Ramesh, *in press*), and auditory cues (rustling of dry leaves caused by moving tortoises, grunting and shell-ramming by males) were more noticeable because the noise made by the movement of searchers was also reduced. Moreover, because the same 2 searchers worked in the 5 localities, it also reduced interobserver bias, which can be significant in locating tortoises and causing variations in capture probability (Freilich and LaRue 1998; Platt et al. 2003).

Environmental factors such as the availability of suitable microhabitats and forage need to be investigated

Table 3. Search effort for *Indotestudo travancorica* in the Indira Gandhi Wildlife Sanctuary from 10 March 2003 to 25 March 2003.

Date	Locality	No. of searchers	Man-hours of searching	Time interval[a] (minutes)	No. of tortoises		Hours of effort per tortoise
					Males	Females	
10 Mar	Anaikundhy Shola	3	7.5	90	4	2	1.3
11 Mar	Anaikundhy Shola	3	7.5	115	4	4	0.9
17 Mar	Karian Shola	2	5	70	4	3	0.7
25 Mar	Anaikundhy Shola	2	3	0	2	0	1.5

[a] Time interval was calculated as the interval between first and last capture of the day.

Table 4. Morphometric data (in mm and kg) of *Indotestudo travancorica* from the Indira Gandhi Wildlife Sanctuary, from December 2002 to March 2003.

	Males ($n = 24$)		Females ($n = 25$)		Juveniles ($n = 3$)	
	Mean ± 1 SD	Range	Mean ± 1 SD	Range	Mean ± 1 SD	Range
Straight carapace length	249.7 ± 24.2	(206–307)	238.4 ± 26.4	(192.5–295)	140.7 ± 21.9	(122.5–165)
Straight carapace width	154.9 ± 13.4	(130–190)	153.2 ± 15.5	(127.5–190)	104.2 ± 8.2	(95.7–112)
Plastron length	179.5 ± 16.3	(153.5–224)	176.4 ± 17.6	(149–219)	113.1 ± 17.5	(100.2–133)
Plastron width	145.9 ± 16.9	(121–198)	144 ± 19.1	(115–204)	94.2 ± 9.1	(86.1–104)
Shell height	91.7 ± 6.5	(81–104)	97.7 ± 9	(77–113)	69.3 ± 8.2	(60–75)
Anal fork	47.4 ± 6.6	(34.6–57.6)	43.2 ± 6.1	(32.1–52.9)	23.5 ± 2.6	(21.6–26.5)
Anal notch	48.3 ± 6.3	(37.6–57.1)	48.5 ± 9.4	(30–61.6)	21.9 ± 4.9	(17.1–26.8)
Weight	2.1 ± 0.6	(2.5–3.6)	2 ± 0.7	(1.1–3.9)	0.46 ± 0.4	(0.2–0.9)

further to explain variation in relative abundance across localities within IGWLS (Table 1). However, although Sichali and Varagaliar are very similar in terms of habitat type, the hours of search effort required to find a single tortoise at the latter site was almost 3 times as much as that required in the former. A probable reason is that Varagaliar has fairly heavy levels of disturbance caused by activities such as collection of fuel wood and forage for camp elephants. But more importantly, the inhabitants have dogs that are often used for hunting small animals, including tortoises. Kozhikamuthy is also used as a seasonal elephant camp, but by a smaller number of inhabitants, and this could contribute to the low encounter rates at that locality. The presence of dogs and increased movement of people may result in a greater number of tortoises being hunted and, therefore, affect relative abundance more significantly than habitat disturbance per se because tortoises are known to shelter even in thickets of weeds such as *Lantana camara* and *Cromolarium glandulosum*, which grow in disturbed areas.

The high relative abundance of *I. travancorica* in Anaikundhy and the variation in search effort across the 4-month study period could have been caused by seasonal changes in the microhabitat. For example, after the first 2 days of summer showers that occurred in March, 3 searchers found 14 tortoises in 15 man-hours (Table 3): individuals were found clustered together; therefore, a larger number of tortoises were found per man-hour of searching. Some chelonians are known to drink rainwater (Peterson 1996), so the sudden availability of water at the end of the dry season could have resulted in tortoises aggregating near streams and *vayal*s. Fresh forage may also have been available in these microhabitats. Such seasonal changes can alter tortoise activity thereby affecting encounter rates (Rose and Judd 1975; Berry and Turner 1986; Geffen and Mendelssohn 1989; Freilich et al. 2000). This clustering of tortoises could also be indicative of reproductive behavior because male combat reported herein is known to occur alongside courtship in captive tortoises (Das 1991; Ramesh, *pers. obs.*), and in 4 instances (10 March to 17 March), a female was found with 1 or 2 males. However, no courtship behavior was observed. Further investigation is required because there is some uncertainty regarding the breeding season of *I. travancorica*. Auffenberg (1964) reported it to be from November to January; whereas, according to another report, it coincides with the monsoons (Das 1991) beginning in June, but only single individuals were encountered in an earlier survey conducted from May to June (Ramesh, *in press*). In addition, the Kadars too believe that the summer showers (called the *Elavan pumari*) signal the onset of the breeding season of this species.

It is interesting to note that of the 57 individuals found during this study, juveniles comprised only 5% of all captures; whereas, during the earlier survey, they comprised 33% of all captures ($n = 27$). With the onset of the monsoons in June, water and forage may have been easily available, and this in turn could have increased the activity levels and capture probabilities of juveniles during the earlier study. However, this needs to be verified as in general, neonates and juvenile tortoises are very difficult to

Table 5. Comparison of search effort for *Indotestudo travancorica* in the southern Western Ghats, India.

Study	Area	Month	Man-hours of search	No. of tortoises	Hours of effort per tortoise
Groombridge et al. 1983	Anamalai Hills	Oct–Nov	37.5	6	6.3
Moll 1989	Anamalai Hills	Nov	40	7	5.7
Das 1991	—	Feb	22	4	5.5
Bhupathy and Choudhury 1995	Parambikulam Wildlife Sanctuary	Oct–Dec	60	3	20.0
Bhupathy and Choudhury 1995	Indira Gandhi Wildlife Sanctuary	Oct–Dec	60	0	—
Present study	Indira Gandhi Wildlife Sanctuary	Dec–Mar	191	57	3.4

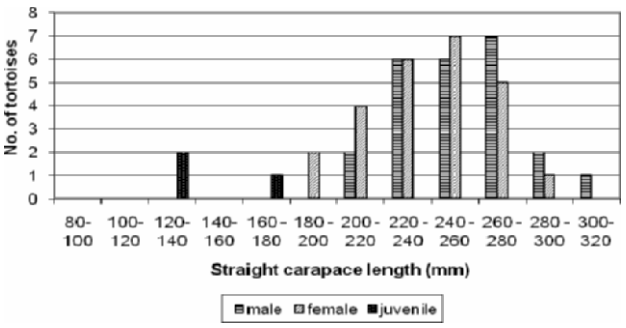

Figure 3. Straight carapace length (mm) of *Indotestudo travancorica* from the Indira Gandhi Wildlife Sanctuary.

find and, as a result their ecological requirements, are poorly understood (Berry and Turner 1986; Mason et al. 2000; Freilich et al. 2000).

Among adult *I. travancorica*, it has been reported that males are larger than females (Das 1991), but despite the relatively large sample size of this study (24 males, 25 females), there was no significant difference in carapace length (Fig. 3). The difference in shell height and anal notch has been recorded earlier, and the latter is believed to provide the tail of the male greater freedom of movement during copulation (Vijaya 1983). There is a marked difference in the shape of the carapace as well: males tended to have a more uniformly elongated carapace; whereas, females have an ovoid shape, with a broader anterior end. In addition to the characters mentioned earlier, this may also serve as useful guide to differentiate between sexes in the field. In view of the fact that both the male-to-female ratio (1:1 December–March; 2.6:1 May–June) and representation of juveniles varies according to season, sampling during both wet and dry periods may be necessary to determine size distribution of the population.

The carapacial color variation seen in tortoises is believed to provide effective camouflage (Pritchard 1979), with tortoises in wetter habitats being darker than those in dry habitats (Kabigumila 2000). Therefore, the color variation observed in *I. travancorica* may serve a similar purpose. It should be noted that *I. travancorica* may have fairly large home ranges; there is a recorded instance of a tortoise travelling at least 20 km in as many years (Ramesh 2004), and given the mosaic landscape of the Western Ghats, habitat variables can vary considerably between sites.

To conclude, the search methodology described here can be used to effectively sample this rare species and assess the relative abundance of *I. travancorica* in different types of habitats. If surveys are conducted during March–April, the sound of shell-ramming and grunting can be heard about 50 m away and, therefore, can serve as an important auditory cue and further reduce search effort. At the same time, it could yield valuable data on the reproductive behavior of this species. Although further studies are necessary in order to estimate the size and structure of the population, *I. travancorica* may not be as rare as believed earlier because it has been sighted even in moderately disturbed areas during this study and others (Bhupathy and Choudhury 1995); it is known to occur in several protected areas totaling about 3900 km^2 (Das 1991; Bhupathy and Choudhury 1995); and so far, large-scale trade or exploitation of this species has not been recorded (Das 1991; Choudhury et al. 2000). However, it is necessary to continue with the enforcement of wildlife protection laws applicable to the species because tortoises are very vulnerable to exploitation by humans (Moll 1989) and to large-scale habitat destruction such as those caused by wildfires, conversion of forests to plantations, and construction of hydroelectric dams (Groombridge et al. 1983; Das 1991; Choudhury et al. 2000).

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Terrestrial Movements of Hatchling Wood Turtles (*Glyptemys insculpta*) in Agricultural Fields in New Jersey

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ABSTRACT. – Hatchling *Glyptemys insculpta* did not travel to water directly upon emergence from their nests but remained in agricultural fields for several days to weeks, during which time they fed and grew. Hatchlings were less active and occupied sites with significantly lower air and substrate temperatures than adult turtles. A management scheme to delay agricultural harvesting until turtles have entered aquatic habitats for hibernation is advised.

Little is known of the behavior, ecology, and physiology of hatchling turtles in cold-temperate climates that emerge in autumn and do not overwinter in or below the nest cavity. Kolbe and Janzen (2002) posited that some hatchling turtles move quickly from the nest to water, minimizing exposure to predators but increasing water loss, while others delay the journey, increasing the chances of predation but minimizing the risk of desiccation. Overall, the migration rates of hatchling turtles are influenced by body size (Janzen et al. 2000a; Tucker 2000), water loss (Finkler et al. 2000; Kolbe and Janzen 2002), predation risk (Janzen et al. 2000b), weather conditions (Filoramo and Janzen 2002), and nest-site characteristics (Kolbe and Janzen 2001).

Figure 1. Hatchling *Glyptemys insculpta* [CL = 37 mm] fitted with radiotransmitter.

Despite the trade-offs considered by Kolbe and Janzen (2002), a simple assumption is that, upon emergence, hatchlings of aquatic turtles from moderate climates move to water in as direct a manner as practical to begin feeding, which presumably will occur provided that water temperatures are not prohibitive (Ultsch 1989). However, some aquatic species are known to feed terrestrially, such as bog turtles (*Glyptemys muhlenbergii*; Barton and Price 1955), Blanding's turtles (*Emydoidea blandingii*; Cahn 1937 in Ernst et al. 1994), and wood turtles (*Glyptemys insculpta*; Farrell and Graham 1991). Furthermore, hatchling Blanding's and wood turtles spend time in terrestrial environments where they feed prior to entering aquatic hibernacula (Butler and Graham 1995; Tuttle and Carroll 2005).

The wood turtle is a semi-aquatic freshwater turtle that is widely distributed throughout the northeastern United States and southeastern Canada (Ernst et al. 1994). In New Jersey, *G. insculpta* is primarily terrestrial from late May to mid-September but returns to aquatic overwintering sites in October and November (Farrell and Graham 1991). Hatchling *G. insculpta* are not known to overwinter in the nest chamber (Buech et al. 2004; but see Parren and Rice 2004), as do hatchlings of some other sympatric species (e.g., *Chrysemys picta*; Gibbons and Nelson 1978). Recent research using fluorescent powder showed that neonate *G. insculpta* in New Hampshire spent several days on land before entering an aquatic habitat (Tuttle and Carroll 2005).

Much of the natural history of hatchling turtles, including *G. insculpta*, is unknown because of the difficulty of tracking such small animals. However, with the advent of radiotransmitters small enough to attach to turtle hatchlings, it is now possible to reliably track hatchlings as soon as they emerge from their nests. We used radiotelemetry to describe the movements and behavior of hatchling *G. insculpta* in New Jersey during their journey to aquatic habitats for hibernation. Our objectives were to document hatchling behavior with a different tracking method than used in previous studies and to gather information on hatchlings in this geographic region.

Methods. — Our study was conducted in 2002 along a 3.8-km stretch of stream that terminates at the Delaware River in Warren County, New Jersey. The stream was bordered by deciduous forest composed primarily of silver maple (*Acer saccharinum*), black walnut (*Juglans nigra*), oaks (*Quercus* spp.), and agricultural fields. The agricultural fields were used to grow corn (*Zea mays*) and included 42% (66 ha) of the total site area. The farming schedule practiced here was soil tillage in April, planting in May, fertilizing and herbicide application in June, and harvesting in October. Turtles used the cornfields for nesting during the last week of May and first 2 weeks of June. The majority of hatchlings emerged in mid to late August.

We installed nest protectors constructed of 2.5-cm wire fencing over 4 nests at the time of egg deposition (Graham 1997). The mean straight-line distance between the nests and the nearest aquatic habitat was 67 ± 21 m (mean ± 1 standard deviation; range 49–90 m). We monitored the nests throughout the incubation period and later weighed and measured hatchlings within 24 hours of their exiting the nest chamber during emergence. Ten hatchlings from the 4 nests were fitted with BD-2A transmitters (Holohil Systems Ltd, Carp, Ontario, Canada) with an expected battery life of 2–3 weeks and signal range of approximately 500 m (Fig. 1). Transmitters were glued to the carapace using Devcon 5-min epoxy (ITW Brands, Wood Dale, IL) and removed or replaced after 15–19 days, near the anticipated end of battery life. The total package mass of the transmitter and epoxy averaged 0.8 g and was 8–10% of neonate weight. We did not have enough transmitters to replace all of them on all hatchlings until they entered water; on 3 hatchlings the transmitters were replaced once, and on one it was replaced twice. These 4 individuals were weighed and measured when the original transmitters were removed and before the replacement transmitters were attached. The site was visited between 0700 and 1900 hours (EST) from 1 August to 15 October 2002. The mean time interval between tracking locations was 1.5 ± 0.1 day (range 1–9). The position change of hatchlings relative to their last location was determined by Universal Transmercator coordinates at each capture location using a Garmin III Plus handheld Global Positioning System unit with an error of approximately 3 m (Garmin International Inc., Olathe, KS).

To compare behavior between age classes, we fitted 10 adult *G. insculpta* (5 males and 5 females) with R1535 transmitters (Advanced Telemetry Systems, Isanti, MN) and tracked them simultaneously with hatchlings. We recorded turtle behavior at each capture location as resting (beneath vegetation or in forms), basking (fully or partially exposed), foraging (prey on beak or in mouth), or traveling (moving between locations). We measured air temperature at 1 m above ground for each capture location using a rapid register thermometer (Miller & Weber Inc, Ridgewood, NY). Substrate temperature was measured at the

Table 1. Movement data for 10 *G. insculpta* hatchlings following emergence from nests in New Jersey.

Turtle no.	Emergence date	Incubation period (d)	Nest to water distance (m)	Tracking period (d)	Times located	Total straight-line distance moved (m)	Straight-line distance moved per day (m)[a]
1	19 Aug	70	49	29	19	425	24 ± 43 (0–141)
2	19 Aug	70	49	29	19	163	7 ± 21 (0–81)
3	20 Aug	73	90	15	13	298	23 ± 59 (0–210)
4	18 Aug	69	50	14	13	334	26 ± 44 (0–147)
5	18 Aug	69	50	23	22	756	34 ± 64 (0–257)
6	13 Aug	76	80	63	33	1052	27 ± 59 (0–190)
7	20 Aug	73	90	37	20	423	24 ± 41 (0–146)
8	19 Aug	70	49	0	0	—	—
9	13 Aug	76	80	0	0	—	—
10	13 Aug	76	80	0	0	—	—

[a] Mean ± SD (range); based only on observations separated by 1 day. Sample sizes: 1 (14), 2 (16), 3 (13), 4 (13), 5 (22), 6 (26), and 7 (16).

surface of the ground beside each individual. We used Student *t*-test to determine if air and substrate temperatures differed between hatchling and adult *G. insculpta* locations; significance was accepted when $p < 0.05$. We report the results as means ± 1 standard deviation.

Results. — Hatchlings emerged from the nests from 13 to 20 August after incubation periods of 69–76 days (Table 1). The average mass and straight-line carapace length of the 10 hatchlings prior to being fitted with transmitters were 8.9 ± 0.6 g (range 8.0–10.1 g) and 37.9 ± 1.1 mm (range 36.0–39.2 mm), respectively. Three hatchlings were lost within 24 hours. The mean tracking period for the remaining 7 was 30 ± 17 days (range 13–62 days), and the mean total straight-line distance traveled was 493 ± 306 m (range 163-1052 m) (Table 1). The average minimum daily movement (straight-line distance between location sites) was 24 ± 8 m (range 7–34 m), expressed as the mean of the means for each hatchling for observations separated by 1 day; if mean daily movement is calculated as (total straight-line distance moved)/(total days tracked), this figure is 18 m/d. Movements were greatest during the first 24 hours, when they averaged 131 ± 84 m (range 2–257 m), which could be an artifact due to the handling associated with transmitter attachment. Movements varied greatly among and within individuals. For example, turtle 2 made an initial movement of 81 m and then stayed in the same area for 14 days. Four hatchlings remained in the agricultural fields during the tracking period, while 3 reached water (Delaware River or parental home stream). The mean travel time and total distance traveled by these latter 3 were 43 ± 17 days (range 29–62) and 546 ± 457 m (range 163–1052). This distance was 8 times greater than the mean straight-line distance between the nests from which they emerged and the nearest water.

When located during the day, the hatchlings were found resting 57.6% of the time, basking 36.8%, foraging 4.0%, and traveling 1.6%. Resting hatchlings were located primarily beneath dead corn leaves and stalks (from the previous season) but also beneath flattened patches of green corn, yellow wood sorrel (*Oxalis europaea*), woodland horsetail (*Equisetum sylvaticum*), dewberry (*Rubus flagellaris*), carpetweed (*Mollugo verticillata*), yellow nutsedge (*Cyperus esculentus*), black nightshade (*Solanum nigrum*), and poison ivy (*Toxicodendron radicans*). Basking hatchlings were found partially covered by vegetation or adjacent to the prop roots of standing corn. Traveling hatchlings were observed walking between cornrows.

Hatchlings were observed foraging 7 times on slugs (*Arion subfuscus*); 6 of these events occurred on overcast days with light to heavy rain. After 12 to 18 days from release, 4 hatchlings increased their carapace length an average of 2.6 ± 0.8 mm (range 1.7–3.4 mm) and mass 1.1 ± 0.3 g (range 0.9–1.5 g). Hatchlings that reached water were found submerged beneath branches, duckweed (*Lemna* sp.), and floating mats of *Elodea*, at depths of 0.1–0.5 m. Average water temperature for these observations was 22.3° ± 2.8°C (range 19.5°–25.0°C).

The average mass and straight-line carapace length for the 10 adults radiotracked simultaneously with the hatchlings were 892.9 ± 149.3 g (range 735.0–1190.0 g) and 192.9 ± 14.5 mm (range 168.8–220.0 g), respectively. The total number of tracking locations for adults was 202. The percentages of observations made on the diurnal activity of adults were 49.1% resting, 22.7% basking, 21.1% traveling, and 7.1% foraging. Air temperatures at the capture sites for adults were significantly ($t = 4.01$, $df = 15$, $p = 0.001$) higher (28.1° ± 1.7°C, range 24.7–31.5°C) than those for hatchlings (25.3° ± 0.8°C, range 23.9°–26.3°C). Likewise, substrate temperatures at the capture sites for adults were significantly ($t = 5.62$, $df = 15$, $p < 0.001$) higher (26.4° ± 1.1°C; range = 24.4°–28.0°C) than those for hatchlings (23.5° ± 1.0°C; range = 22.5°–25.1°C).

Discussion. — Some species of turtles that typically live in or near water can feed on land as well as in the water, including *G. insculpta*, *E. blandingii*, and *G. muhlenbergii*. For these species, moving directly to water after emergence from the nest may not be as crucial as it is for species that

can only feed in water (e.g., *Apalone mutica* and *A. spinifera*; Doody 1995). Perhaps for hatchlings of the more terrestrial species, it is only necessary to eventually move to water for hibernation. Wood turtle hatchlings in New Jersey spent 4–12 weeks on land where they fed and grew. Our findings are consistent with those of Tuttle and Carroll (1997, 2005). They found that *G. insculpta* hatchlings in New Hampshire could take as long as 26 days to enter a brook, and would sometimes move away from the nearest brook rather than toward it. They also found that of 12 turtles that were tracked using fluorescent powder from their nest to water, transit times averaged 6.5 days (range 1–24 days). Similarly, *E. blandingii* hatchlings in Nova Scotia have also been found to tarry on their nest to water journey and even to actively avoid large bodies of water adjacent to their nests in favor of more distant, shallower waters (Standing et al. 1997; McNeil et al. 2000). In Massachusetts, *E. blandingii* hatchlings moved more directly to water, but the nests were near wetlands and transit time was as long as 9 days (mean 2.9 days; Butler and Graham 1995).

For the few aquatic species that can and do feed on land, there is no immediate need to enter water for feeding; although, desiccation and predation are still potential threats. At least 7 of the 10 neonate *G. insculpta* that we radiotracked remained primarily terrestrial following emergence, and they fed and grew during the period they spent on land. While an increase in mass might be attributed to water gain, the increase in carapace length indicates that growth was occurring prior to hibernation. Our study and those of Tuttle and Carroll (1997, 2005) found that hatchlings spent the night in forms. All 3 studies found most hatchling activity to occur in the morning, and activity to be stimulated by rainfall. These observations are consistent with avoidance of potential desiccation associated with higher midday temperatures, as is our finding that adult turtles are more active at higher temperatures, which would not pose as great a threat of desiccation to them because of their larger size. Ernst (1968) noted that young turtles, including *G. insculpta*, experienced greater water loss than older individuals in laboratory experiments and suggested that this difference was due to the greater surface to mass ratio of smaller individuals. In addition, Tuttle and Carroll (2005) reported that at least one hatchling made a "beeline" for a brook, indicating that remaining on land for protracted periods after emergence is not an inviolable rule. Three of the 10 hatchlings that we released were not located again, and it is possible that they were either taken by predators, were lost due to transmitter failure, and/or they moved directly to water and then moved far enough downstream to be out of receiver range.

The average time spent on land by 12 *G. insculpta* hatchlings in New Hampshire that were followed to water was 6.5 days, with a maximum of 24 days; another was found still on land after 26 days (Tuttle and Carroll 1997, 2005). At our New Jersey site, the mean time spent on land for the 3 hatchlings followed to water was substantially longer (mean = 43 days, maximum = 62 days). Since the climate is more moderate in New Jersey than New Hampshire, we assume that the ultimate factor determining movement to water is the necessity of aquatic hibernation, and the cue to leave the terrestrial environment is falling temperature. It has been demonstrated that extrinsic loads may impair locomotion in turtles (Zani and Claussen 1995). Thus, transmitters could have affected the travel rate of turtles in our study; however, the ratio of transmitter weight to body weight did not exceed the recommended maximum for reptiles (10%; Anonymous 1987). Furthermore, the average daily movement of turtles in New Jersey (24 m) was the same for turtles in New Hampshire (Tuttle and Carroll 2005).

While it is well documented that hatchlings of several species of turtles overwinter terrestrially in or below the nest cavity without emerging above ground, there are no data to suggest that hatchling turtles in cold-temperate climates that do not overwinter in the nest routinely overwinter on land. However, terrestrial overwintering may be possible under certain conditions for a few individuals that do not reach aquatic hibernacula before the advent of cold weather (Congdon et al. 1983, 2000; Standing et al. 1997, 1999; McNeil et al. 2000; Pappas et al. 2000; Dinkelacker et al. 2004). Now that radio-transmitters are manufactured small enough to place on neonatal turtles, it is possible to study the behavior of hatchlings between the time of emergence from the nest and their first hibernation period. Moreover, researchers now have the capability to identify the locations and characteristics of aquatic hibernacula, which are currently undescribed but likely to be different from those of adults (Reese et al. 2004; Ultsch 2006).

The terrestrial behavior of hatchling *G. insculpta* following emergence from nests in agricultural fields has implications for the conservation and management of this species. The prolonged stay of hatchlings in this habitat following emergence might put them at risk of injury or death inflicted by farm machinery if crop harvest occurs before they have migrated to aquatic hibernacula. Injuries and deaths caused by farm machinery have been reported for adult *G. insculpta* at this location (Castellano 2007) and elsewhere (Saumure and Bider 1998; Saumure et al. 2007). At our New Jersey site, late-maturing varieties of corn are grown in order to delay harvest until mid-October when both adult and neonate turtles have exited the fields. A management scheme to delay harvesting until turtles have entered aquatic habitat for hibernation is advised for other locations.

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Observations on Terrestrial Feeding Behavior and Growth in Diamondback Terrapin (*Malaclemys*) and Snapping Turtle (*Chelydra*) Hatchlings

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ABSTRACT. – **Food acquisition, feeding, and growth in moist terrestrial laboratory conditions were compared in 2 species of turtles that inhabit brackish water environments. *Malaclemys* hatchlings showed normal growth, but *Chelydra* hatchlings became severely stressed. The results suggest that the extended use of terrestrial habitats as a means of osmoregulation in high salinity habitats is a viable adaptive strategy for diamondback terrapin but not for snapping turtle hatchlings.**

Osmotic stress, particularly on hatchlings, is an important factor that determines the distribution of turtle species in estuarine environments. Dunson (1985) found that hatchling diamondback terrapins are unable to grow in the high salinities found in the vicinity of many nesting sites without periodic access to freshwater for drinking. Kinneary (1993) reported that water salinity is a dominant factor that limits snapping turtle distribution in estuaries and that the turtles will, on occasion, use nesting sites at which the nearest water is not suitable for hatchlings because of high salinity (Kinneary 1996). Laboratory studies conducted by Dunson (1985) and Dunson and Mazzotti (1989) identified incidental swallowing of saline water during feeding as the major source of sodium influx in hatchling diamondback terrapins and a primary source of salinity toxicity in hatchling snapping turtles kept in 50% sea water. Hatchlings of both species appear to make use of terrestrial refugia (Pitler 1985; Lovich et al. 1991; Buhlmann and Gibbons 2001), and adults have been observed to feed on land (Pearse et al. 1925; Ernst et al. 1994). However, the ability of hatchlings to grow under terrestrial conditions has not been considered. The objective of this study was to observe and compare feeding and growth of snapping turtle and diamondback terrapin hatchlings under moist terrestrial conditions in the laboratory.

Table 1. Monthly growth of diamondback terrapin hatchlings ($n = 4$) and snapping turtle hatchlings ($n = 4$; in parenthesis) kept in the laboratory under moist terrestrial conditions. Mass in grams. Values are means ± SD.

Month	Wet mass	% Change wet mass
0 (hatching)	5.39 ± 0.22 (7.63 ± 0.20)	—
2	5.09 ± 0.40 (7.08 ± 0.23)	–5.57 (–7.21)
3	7.24 ± 1.51 (6.03)[a]	42.24 (–14.83)[a,b]
4	12.06 ± 4.24	66.57
5	20.45 ± 7.78	69.57
6	39.30 ± 11.53	92.18
7	51.17 ± 10.35	30.20

[a] $n = 2$.
[b] By month 3.5, 3 of 4 snapping turtle hatchlings had died after losing more than 20% hatching wet mass.

Methods. — Turtle eggs were incubated at room temperature in a sealed plastic container that contained 50% vermiculite and 50% distilled water by mass. Upon hatching, 4 turtles of each species were placed in a communal, covered, clear plastic container (57 × 24 × 18 cm) that contained 1 part sphagnum moss to 10 parts tap water by mass. Additional water was subsequently added periodically during the course of the experiment. An effort was made to saturate the sphagnum moss without providing any free water other than that formed from condensation on the top and sides of the container. This was done to minimize desiccation, yet, at the same time not provide any free water to aid in the capture and swallowing of food items. The sample size was limited because of the difficulty in obtaining a variety of live-food items small enough for newly hatched turtles to easily handle. Food items were offered 5 to 7 times per week and included a wide variety of live annelids, mollusks, isopods, and insects, as well as chopped fish (*Menidia*) and commercial pet food. Drinking water was not overtly provided, however, the hatchlings did have access to water droplets that continually formed on the sides of the container. The animals were kept at room temperature (21.6 ± 1.3°C). A light (Gro-Light, 75 W) was placed at one end of the container. The photoperiod was 12L:12D. Wet mass (WM) was recorded upon hatching and at subsequent monthly intervals.

Results and Discussion. — All 4 diamondback terrapin hatchlings (WM = 5.4 ± 0.2 g SD) began accepting live food approximately 1 month after hatching, and, during the third month, other foods, such as chopped fish and commercial pet food, were readily accepted. They ate voraciously whenever food was offered and appeared to have no difficulty or hesitation in obtaining and swallowing food in the absence of water. The turtles grew at an average rate of 3.7% of their hatching WM/d over the

230-day test period (Table 1). Straight-line carapace length increased to 6.4 ± 0.05 cm SD by the end of this period. These data show that hatchling terrapins are capable of normal growth (Ernst and Barbour 1972) when kept in the laboratory under terrestrial conditions. It is suggested that, in addition to their complex suite of behavioral and physiological responses to high salinity environments (summarized in Davenport et al. 2005), extended use of terrestrial habitats by hatchlings and juveniles may be a viable strategy for dealing with osmotic stress in the field.

As a group, the snapping turtle hatchlings lost 7.2% WM after 60 days, compared with a WM loss of 5.57% for the diamondback terrapin hatchlings over the same period. By contrast, however, the snapping turtles showed little interest in the variety of foods offered and were never observed feeding. Three of 4 died after losing more than 20% of their initial WM during the first 3.5 months of the experiment. The remaining animal, which appeared severely stressed, was placed in enough tap water to cover the carapace on day 113. This specimen immediately accepted food after being placed in water and continued to feed underwater, eventually regaining its vigor. Although snapping turtle hatchlings can be induced to accept and swallow food in the absence of water (*pers. obs.*), the results of this study suggest they are maladapted in terms of food acquisition, feeding, and growth in terrestrial habitats. When considering their high degree of water permeability and evaporative water loss (Dunson 1986; Finkler 2001), the extended use of even very moist terrestrial habitats in high salinity environments is probably not a realistic alternative for these animals.

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Ecology of the Bushmanland Tent Tortoise (*Psammobates tentorius verroxii*) in Southern Namibia

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ABSTRACT. – Tortoises are the group of reptiles most under threat in Namibia and are poorly researched, with little understanding of their basic ecology. This paper investigates some aspects of the ecology of female Bushmanland tent tortoises (*Psammobates tentorius verroxii*) in southern Namibia, specifically their movement, home range, activity pattern, diet, resting site selection, and orientation.

The ecology of *Psammobates tentorius verroxii* is poorly known (Boycott and Bourquin 2000), and despite

its wide range, it is not easily found and often occurs at low densities (Branch 1989; Branch 1998). Virtually nothing is known of the feeding habits of *P. tentorius verroxii*; although, Branch (1998) stated that they are known to feed on certain small succulents, and Boycott and Bourquin (2000) included *Mesembryanthemums* and *Crassulas* in their diet. Although the diet of a similar species, *P. oculiferus*, has been published (Rall and Fairall 1993), this study on *P. tentorius verroxii* contributes to the understanding of a generally poorly known southern African tortoise and will assist with its protection.

Study Site. — This study was conducted between February 2003 and August 2004 on the farm Velloor approximately 80 km south of Karasburg and 20 km north of the Orange River (28°34′S, 19°11′E, elevation 800 m) in southern Namibia. Although the farm is 40,000 ha in size, the tortoises were located and studied close to the farm house in an area approximately 500 ha in size. The general vegetation type is classified as Dwarf Shrub Savanna (Giess 1971) or Nama Karoo biome (Lovegrove 1999) and is dominated by *Stipagrostis* species grasses, *Rhigozum trichotomum* and *Eriocephalus* spp. shrubs, and *Acacia erioloba* trees along the ephemeral drainage lines. Annual precipitation occurs primarily as thundershowers during summer months with an annual average rainfall of 50–100 mm (Van der Merwe 1983; Mendelsohn et al. 2002). Average daily minimum and maximum temperatures for the coldest and hottest months range between 3°–4°C and 36°–37°C, respectively (Van der Merwe 1983; Mendelsohn et al. 2002). The dominant soils in the area are eutric Leptosols with a low water holding capacity and often subject to droughts (Mendelsohn et al. 2002).

Methods. — Four female tortoises were located on 28 February 2003 by scouring the general area specifically targeting likely resting places. Radiotransmitters each weighing 10 g (2% of average body weight) were attached using glue and clear silicon to the side of the carapace so as not to affect any mating attempts. Tortoises were visited monthly (dependent on authors' availability) to determine home range with foraging observations being conducted for a few days each month during the warmer months. These transmitters were left on until 31 August 2004, when they were removed.

Tortoise movement was estimated in 2 ways. The first was during direct observations while conducting foraging observations—i.e., the total distance traveled was measured with a measuring tape (m) after the tortoise had become active until assuming a resting position. The second was by plotting the positions of the resting positions and then estimating the direct distance between resting positions with the aid of a GPS.

The home range was determined by plotting the positions of the resting places (♀1, $n = 14$; ♀2, $n = 28$; ♀3, $n = 26$; and ♀4, $n = 16$) with the aid of a GPS and then using the Minimum Convex Polygon option in the animal movement analysis ARCVIEW extension to determine the home range.

Direct observations were conducted during summer (i.e., when tortoises were most active—March, April, September, October, November, and December 2003 and January to April 2004) to determine their activity pattern. They were also visited during the winter months, but very little movement and activity was evident during this time. Tortoise activity was categorized into active (moving and foraging) versus passive (resting). The temperature was measured at the start and end of a particular activity period (during morning and afternoon observations) with a thermometer at 30-cm height in the shade.

Daily foraging data (during morning and afternoon observations) were obtained from one study animal at a time after first locating the individual before it became active and then following it throughout the day at a distance of approximately 5 m. When a tortoise was observed feeding, the plant species and number of bites per species was recorded. Binoculars (8×40) were used to facilitate observations. Plants utilized were immediately identified where possible or samples collected for later identification or verification. A total of 590 minutes (♀1 = 10 days, ♀2 = 22 days, ♀3 = 28 days, and ♀4 = 14 days) were spent on foraging observations.

Data on resting places (i.e., plant species or terrain features used as shelter) and resting orientation were collected throughout the year. The latter was included to determine if tortoises were selecting for a certain orientation for thermoregulatory purposes. A resting place was classified as an "open area" if the tortoise was not physically in contact with or underneath vegetation (i.e., $>$ 10 cm away).

A rapid survey of the vegetation (species composition) was conducted within the general area utilized by the tortoises by identifying plant species at 1-m intervals along 2×200 m transects in the area.

Results. — Four female *P. tentorius verroxii* were followed from sunrise to sunset on 5 consecutive days during 1–5 March 2003. During this period they moved an average distance of 81.2 m (SD = 71.57 m, range 6.5–190 m) and 91.1 m (SD = 76.44 m, range 12–169 m) during morning and afternoon observations, respectively. There was a significant difference between morning and afternoon foraging distances (one-way ANOVA, $df = 9$, $F = 0.05$, $p = 0.84$). The longest distances covered in a session were 190 m (morning) and 169 m (afternoon). The mean average distance (straight line) that the 4 females had moved away from their respective release sites (i.e., after fitting the transmitters) after a period of 18 months was 855 m (SD = 642 m, range 350-1750 m). The mean ($n = 82$) average distance that the 4 females moved between resting places was 100 m (SD = 178 m, range 0–1117 m) with no significant difference observed between individuals (one-way ANOVA, $df = 2$, $F = 2.18$, $p = 0.096$); although, the sample size was low.

The mean average home range as determined throughout the study period (i.e., summer months, as tortoises did not move much during the winter months) for

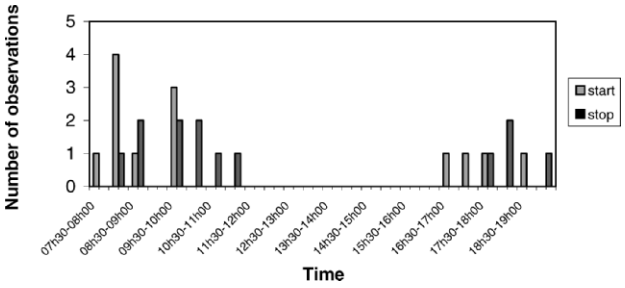

Figure 1. Activity pattern for 5 female and 4 male *P. tentorius verroxii* during summer (i.e., warmer months: March, April, September, October, November, December 2003 and January to April 2004) in southern Namibia ($n = 9$).

the 4 females with radiotransmitters ($n = 84$ resting places) was 31.97 ha. It is certain that some movements were missed, but it was clear that the tortoises stayed within a certain area and did not venture far from this area. As a result of male tortoises not initially being found, no formal study was conducted on them, but subsequent limited data indicated a home range of 6.65 ha (SD: 4.5 ha) ($n = 4$) for males.

Nine tortoises observed (5 female and 4 male—including the 4 radiotracked females originally located) had a distinctive bimodal activity pattern during summer with peak foraging activity between 0730 and 0900 hours (Fig. 1). Foraging started and stopped during the morning with mean ambient temperatures of 26.2°C (SD = 2.77°C, range 22°–29°C) and 29.4°C (SD = 2.41°C, range 26°–32°C), respectively. A lesser mode of activity was observed in the afternoon between 1630 and 1900 hours (Fig. 1). Foraging started and stopped during the afternoon with mean ambient temperatures of 33.4°C (SD = 1.52°C, range 32°–36°C) and 30.2°C (SD = 1.48°C, range 28°–32°C), respectively.

The 4 female tortoises spent an average 72 minutes (SD = 38.9 minutes) and 51 minutes (SD = 24.7 minutes) active during morning and afternoon observations during summer, respectively. No significant difference was observed for time spent active between morning and afternoon activity periods (one-way ANOVA, df = 2, $F = 1.05$, $p = 0.36$). Average activity levels during summer over 24 hours were 8.5% active (AM = 5% and PM = 3.5%) and 91.5% inactive. Winter activity levels were almost nonexistent except during occasional fog when tortoises became active.

A total of 18 different food items, of which 17 were plant matter (the other item eaten included weathered bone), were included in the diet of the 4 female tortoises during summer observations.

Grasses were the most utilized food item as determined by frequency of visit (51.2%) followed by herbs, bulbs, and succulents (41.9%), trees and shrubs (2.3%), other plant matter (2.3%), and bone (2.3%). However, herbs, bulbs, and succulents were the most utilized food item as determined by frequency of bite (56.9%) followed by grasses (35.2%), other plant matter (7.1%), bone (0.6%), and trees and shrubs (0.2%). The most utilized plant species as determined by frequency of visit were the grasses *Stipagrostis obtusa* and *S. uniplumis*, followed by unidentifiable herb seedlings (Table 1). The most sought after plant species determined by frequency of bite were *S. obtusa*, *Anacampseros albissima*, unidentifiable herb seedlings, and *Trachyandra saltii* (Table 1). The top 4 food items selected by the 4 female tortoises in terms of frequency of visit/plant and frequency of bite/plant were responsible for 74.4% and 71.3% of all the observations, respectively (Table 1).

Eight tortoises observed (5 female and 3 male—including the 4 females originally located) used a variety of resting sites (Table 2) and included 15 shrub species, 3 tree species, and 2 grass species. Shrubs as resting places were favored, followed by holes under trees and shrubs, and thirdly under trees alone (Table 2). Resting places used during this study indicated that 90.5% of the trees and 36.4% of the shrubs had thorns. Holes used as resting places were almost exclusively (97%) located under vegetation with thorns.

Tortoise orientation while in a resting place varied between morning and afternoon during summer. Tortoise orientation was mostly towards the north (41%, including northeast and northwest) and east (32%, including

Table 1. Food selection as determined by frequency of visit/plant and frequency of bite/plant for 4 female *P. tentorius verroxii* in southern Namibia. All food items were available throughout the summer.[a]

Species	Frequency of visit/plant %	Rank	Frequency of bites/plant %	Rank
Stipagrostis ciliate	4.7	6	1.2	8
S. obtuse	25.6	**1**	22.9	**1**
S. uniplumis	20.9	**2**	11.2	**4**
Anacampseros albissima	2.3	7	12.9	**2**
Bone	2.3	7	0.5	9
Euphorbia sp.	9.3	**4**	11	5
Rhigozum trichotomum	2.3	7	0.2	10
Tribulus terrestris	7	5	8.8	6
Trachyandra saltii	4.7	6	11.4	**3**
Unidentifiable herb seedlings	18.6	**3**	12.9	**2**
Unidentifiable windblown leaves and seeds	2.3	7	7	7

[a] Top 4 food items are bolded.

Table 2. Resting sites during summer for 5 female and 3 male *P. tentorius verroxii* individuals in southern Namibia.

Species	Observations	%
Trees		
Acacia erioloba	4	
Acacia mellifera	17	
Boscia foetida	4	
Total	25	18.7
Shrubs		
Adenolobus gariepensis	4	
Eriocephalus sp.	3	
Galenia africana	3	
Kleinia longiflora	4	
Lycium sp.	6	
Malvacaea sp.	2	
Pentzia sp.	7	
Phyoptelum spinosum	2	
Protasparagus ssp.	1	
Rhigozum trichotomum	10	
Rhus undulata	1	
Salsola sp.	1	
Zygophylum rigidum	5	
Zygophylum rectofractum	3	
Aizoon schellenbergii	1	
Total	53	39.6
Grasses		
Stipagrostis obtusa	3	
S. uniplumis	4	
Total	7	5.2
Open space	18	13.4
In hole		
Under *Acacia mellifera*	1	
Under *Boscia foetida*	1	
Under *Lycium* sp.	15	
Under *Rhigozum trichotomum*	14	
Total	31	23.1
	134	100

northeast and southeast) during morning observations (Table 3 and Figure 2). Orientation during the heat of the day (i.e. after the morning foraging period) was mainly in an easterly and southerly direction (50%—including northeast and southeast). These positions were usually left during the afternoon foraging period with new resting

Table 3. Orientation of the posterior part of the carapace of 5 female and 4 male *P. tentorius verroxii* individuals in southern Namibia while resting.

Orientation	AM	PM
Summer		
North	22	11
South	5	7
East	17	12
West	11	12
Northeast	1	3
Northwest	1	0
Southeast	1	1
Southwest	1	0
Winter		
North	1	1[a]
South	3	3[a]
East	3	3[a]
Northeast	1	1[a]
Southeast	2	2[a]

[a] Tortoises were inactive on the days that their orientations were recorded.

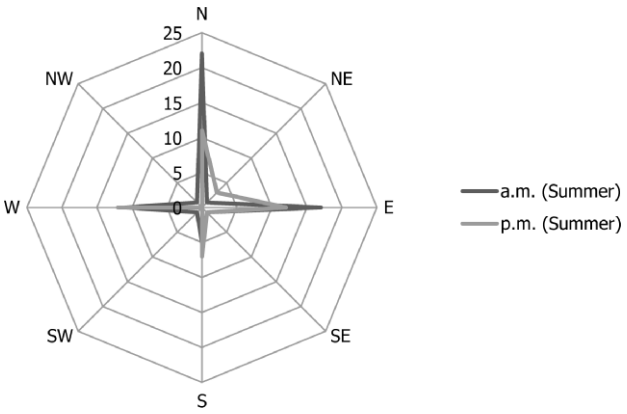

Figure 2. Orientation of the posterior part of the carapace of 5 female and 4 male *P. tentorius verroxii* individuals during summer (AM and PM) in southern Namibia while resting ($n = 9$).

places located during the late afternoons. Orientation during winter did not change during the day due to the inactivity of the individuals during this period, with most tortoises (60%) orientated towards the east (including northeast and southeast) and north (Table 3).

The vegetation in the general area utilized by the tortoises is dominated by perennial *Stipagrostis* grasses (*S. obtusa*, 30% and *S. uniplumis*, 12%) with only 6% of the vegetation comprised of thorny species (*Rhigozum trichotomum*, 3.5%; *Acacia mellifera*, 2%, and *Protasparagus* ssp., 0.5%).

Discussion. — Female *P. tentorius verroxii* were sedentary, as indicated by the distances covered while foraging, distance from release site after 18 months, distance moved between resting places, and home range size. Foraging also does not occur on a daily basis during the summer and is possibly influenced by food availability, food consumed the previous day, and local environmental conditions.

The activity pattern of *P. tentorius verroxii* was typically bimodal and affected by ambient temperature. Ramsay et al. (2002) indicated that *Chersina angulata*, a tortoise common in the southwestern Cape in South Africa, followed a bimodal activity pattern as an adaptation to high ambient temperatures, an activity that would assist the animal in maintaining its water balance. Lambert (1981) stated that the activity mode of *Testudo graeca* became bimodal when ambient temperatures rose above 28°C. Similarly, *Kinixys spekii* is inactive when ambient temperatures exceed 29°C (Hailey and Coulson 1996). This bimodal activity would thus be even more of an advantage to *P. tentorius verroxii* from southern Namibia due to the general higher average ambient temperatures experienced there. Female *P. tentorius verroxii* stop foraging and seek shade when mean ambient temperatures reach 29.4°C (SD = 2.41°C) during the morning. This is slightly higher than documented for *C. angulata* from the southwestern Cape (28.7°C) (Ramsay et al. 2002) and in the eastern Cape (28.5°C) (Els 1989).

Afternoon foraging usually ceased around sunset; although, some individuals were observed still being active after sunset. This probably occurs when extreme daytime temperatures prevent tortoises from foraging before sunset.

During winter the 4 female *P. tentorius verroxii* were not very active with the exception being during fog. On one occasion when fog occurred during winter (9 August 2003) all 4 individuals were observed away from cover in the open with a lot of local activity observed as indicated by their tracks. This was possibly due to the tortoises "fog collecting" as described by Branch (1998) and Boycott and Bourquin (2000). Although fog incidences are relatively infrequent in southern Namibia (5–10 days annually) (Mendelsohn et al. 2002) the above-mentioned activity indicates that *P. tentorius verroxii* may actively utilize moisture from fog when available.

Holes were not utilized as hiding places by the 4 females under observation during winter as compared to 23.1% ($n = 31$) of the total observations as resting places during summer. Holes are possibly not as important for thermoregulation during winter as during hot summer days.

This study indicates a variety of plants being utilized by female tortoises, including the importance of grasses in their diet during periods of below-average rainfall. Rall and Fairall (1993) confirmed the tendency to utilize grasses during unfavorable periods for *P. oculifer*, a similar-sized species also inhabiting dry areas in southern Africa. *Stipagrostis obtusa* is the most important plant species included in the diet of the 4 female *P. tentorius verroxii* as observed by frequency/visit and frequency/bite. This small perennial climax grass is one of the best grazing grasses for livestock in the arid regions of southern Africa (Van Oudtshoorn 1999) and has a relatively high nutritive value. It remains relatively palatable and well utilized by animals even when dry (Müller 1984). Herbs, bulbs, and succulents are sought after as observed by frequency/bite. The succulent *Anacampseros albissima* (known as "skilpadkos"—tortoise food in Afrikaans) and *Trachyandra saltii* were the second and third most sought after plant species as observed by frequency/bite, although not very common throughout the area. These plants are usually consumed entirely when encountered. It is suggested that *P. tentorius verroxii* may feed more on annuals (e.g., forbs) when encountered, but due to low rainfall throughout the study area/period, annuals were sparse, and they thus had to utilize the available perennial grasses more.

Tortoises utilized a variety of plants as shelters when resting; although, thorny plants were used more often (36% of shrubs and 84% of trees used as resting places are thorny), which probably assists with defense and or thermoregulation as most thorny plants also present better shade (denser growth form, bigger leaves) during summer. Furthermore, thorny plants make up a small percentage (6%) of the vegetation in the general area utilized by the tortoises, suggesting that these plants are selected. Holes under bushes (23%) were also used, which would possibly further assist with defense and thermoregulation. The use of holes during this study was only observed during summer during an exceptionally warm period with daytime ambient temperatures reaching 40°C. Branch (1998) stated that tortoises are known to burrow into sandy soil at the base of low shrubs emerging after the onset of rains. The use of resting places (i.e., site selection) and holes as resting places (i.e., to what extent are these utilized, do they dig these themselves or utilize existing holes) should be investigated further.

Orientation of the carapace during summer was mainly in a northerly and easterly direction during morning observations, which could suggest that *P. tentorius verroxii* position themselves when going into a resting site the previous evening. This is probably to enable them to reach an optimum temperature quickly before the heat of the day to commence foraging while still relatively cool in the morning. Orientation away from the sun (i.e., easterly and southerly direction) during the heat of the day is probably to avoid overheating during this period. Orientation of the carapace during winter towards the north and east (70%) is probably similar to that as suggested during summer.

Although *P. tentorius verroxii* inhabits an extremely marginal habitat in southern Namibia, the species seem well adapted to handle the adverse environmental conditions; although, the effect that climate change (aridification) may have is unknown. However, the relatively sedentary lifestyle, slow reproductive rate (Branch 1998; Boycott and Bourquin 2000; Cunningham et al. 2004), and anthropogenic factors (e.g., road kills and habitat alteration by livestock overgrazing) makes the species susceptible to local extinctions. Illegal collecting and/or indiscriminate utilization by humans for food exacerbates its fate and are thus condemned.

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Nesting Behavior in Three-Toed Box Turtles (*Terrapene carolina triunguis*) Following Oxytocin-Induced Oviposition

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ABSTRACT. – Between 1997 and 2001, we observed nesting behavior following oxytocin-induced oviposition in 10 three-toed box turtles. Oviposition preceded nesting behavior by 0.5–17 days. Three turtles oviposited subsequent clutches (without oxytocin induction) a mean of 30 days after the oxytocin injections but only 22 days after they constructed empty nests. The mean internesting interval was 21 days between consecutive ovipositions without oxytocin induction by these 3 females in the same and subsequent seasons.

Oxytocin is a hormone used to induce muscular contractions of the oviduct in many reptiles. Oxytocin is apparently not present in reptilian pituitaries, but the presence of the hormones arginine vasotocin (AVT) and mesotocin has been confirmed (see Ewert and Legler [1978] and references therein). Oxytocin, AVT, and mesotocin are all neurohypophysial polypeptides (La Pointe 1977; Ewert and Legler 1978). Although AVT and mesotocin appear to be the primary source of oxytocic activity in turtles, oxytocin can be used to induce oviposition in turtles, where it has been used extensively in field and laboratory-based reproductive studies (Ewert and Legler 1978; Congdon and Gibbons 1985; Feldman 2007) and in veterinary applications to relieve dystocia (Highfield 1990, 1996; Frye 1991; Plumb 2002).

Nesting behavior has been observed in several turtle species when gravid females were allowed access to a suitable substrate following oxytocin injection (Ewert and Legler 1978; Ewert 1979). Ewert and Legler (1978) suggested oxytocin injection as a potentially useful means of studying nesting behavior; although, they noted it was erratic in occurrence, even with the same individuals. They also noted one instance in which the injection of a nongravid, female *Rhinoclemmys pulcherrima* led to digging behavior. Subsequently, several reports of nesting behavior occurring after oviposition was induced with oxytocin have appeared, including in emydids, *Chrysemys picta* (Iverson and Smith 1993) and *Trachemys scripta elegans* (Tucker et al. 1995; Tucker 2000; Feldman 2007), and Australian chelids (*Chelodina expansa* and *Emydura signata*; McCosker 2002). This behavior has been reported as occurring up to 2 weeks after induction with oxytocin in *T. scripta elegans* (Feldman 2007), or 15 to 21 days in the chelids (McCosker 2002).

During the course of reproductive studies of the three-toed box turtle, *Terrapene carolina triunguis*, we were able to make careful observations of nesting behavior after oxytocin-induced oviposition. These observations, the first for this species, were made in a relatively controlled situation, as were those of Feldman (2007), rather than in the field (Tucker et al. 1995; McCosker 2002).

Materials and Methods. — Between 1997 and 2001, 8 female *T. carolina triunguis* were collected from roads in Louisiana and Arkansas. When found, the turtles were returned to the lab in Monroe where they were palpated or radiographed and determined to be gravid. Veterinary-grade oxytocin (20 IU/mL) was administered intramuscularly (thigh) at approximately 1–2 IU/50 g body weight as

Table 1. Timing of events related to oxytocin injections and nesting behavior in *T. carolina triunguis*.

Female no.	Injection date	Injection time (h)	Date moved to study site	Date of empty nest construction	Time when covering empty nest (h)	Elapsed time from injection to covering (h)
Wild-caught group						
1	10 Jun 1997	1555	12 Jun	16 Jun 1997	2200	150.5
2	18 Jun 1997	0950	22 Jun	26 Jun 1997	2330	206.0
3	3 Jun 1998	1430	5 Jun	8 Jun 1998	0130	131.0
4	13 Jun 1999	1350	16 Jun	20 Jun 1999	2130	176.0
5	30 Apr 2000	1230	4 May	12 May 2000	2300	295.0
6	25 Jun 2001	1530	1 Jul	6 Jul 2001	2300	271.5
7	18 Jun 2001	1025	20 Jun	5 Jul 2001	1930	417.0
8	10 Jul 2001	1400	12 Jul	19 Jul 2001	2300	225.0
Dystocia group						
3	9 Aug 1997	1155	1989	9 Aug 1997	2330	12.0
9	29 Jun 2001	1300	1998	5 Jul 2001	1000	141.0
10	24 Jul 2001	1330	2000	27 Jul 2001	2330	75.0

recommended by Ewert and Legler (1978). The time of injection was recorded to the nearest 5 minutes, after which the turtle was placed in a bucket of water on a wire mesh platform, which allowed us to collect the eggs undamaged. Eggs were usually oviposited within 3–4 hours, but occasionally it was necessary to administer a second dose of oxytocin (turtles 1 and 10). After egg laying, females were maintained in the lab in a dry, uncluttered plastic or cardboard container at room temperature. Turtles 1–8 were released at our study site 2–6 days after oxytocin injection. The outdoor study site in Bastrop, Louisiana is a 31 × 34 m semi-natural habitat described in Messinger and Patton (1995). All females at the study site received the same treatment in food, water, and shelter allocation. Handling was kept to a minimum except in case of illness or injury. Turtles were identified by individual head, leg, and shell characteristics.

Also, 3 females residing at our Bastrop study site developed dystocia (egg binding), providing the opportunity for additional observations (one of these 3 was also one of the original 8 wild-caught specimens—turtle 3). When we suspected dystocia based on atypical behavior, the turtle was confirmed to be gravid by radiograph and administered oxytocin. The turtle then was returned immediately to the outdoor enclosure where oviposition took place.

During the nesting season, we made repeated trips throughout the day, evening, and early hours of darkness looking for signs of nesting. Most nesting takes place beginning in late afternoon and evening, with completion often occurring after dark. When signs of nesting were observed, we made frequent visits to follow its progress. The time when the female was noticed covering the nest was recorded to the nearest 0.5 hour. Because of variation in the duration of nest covering and since the entire nesting sequence was not observed, the time to nest completion may be short by as many as 2 hours. Elapsed time between the oxytocin injection and nest covering was rounded to the nearest half hour; this measure is used as an indication of the degree of dissociation between the time of oviposition and nest construction. Nest sites were marked and excavated later. We excavated nest cavities to ascertain the physical structure of the cavities, and to document the presence of eggs. Dates of subsequent nesting by these same females also were recorded.

Results. — Injection with oxytocin lead to oviposition in all 11 cases involving 10 individual females, and this was followed by typical nesting behavior, i.e., oviposition and nesting behavior were not associated. Other than the cases detailed here, nesting behavior and oviposition always occur in association with one another. Data on timing of injection and nesting are found in Table 1. The range of values for elapsed time was from less than one day (12 hours) to more than 17 days (417 hours) (mean = 191 hours [8 days]).

For the 8 wild-caught females, the elapsed time between oxytocin injection and nest covering ranged from 131 to 417 hours (mean = 234.5 hours [9.8 days]). This elapsed time includes a mean of 3.1 days spent in the lab before transfer to the study site during which time a suitable nesting substrate was not available. The 3 dystocia-affected turtles (Table 1) had been residents of the study site for at least a year. Although a suitable nesting substrate was available immediately following their oxytocin injection, none of the 3 dystocia-affected females dug a nest in response to oxytocin induction; eggs were deposited on the surface of the ground. The mean elapsed time for these 3 was 76.0 hours (3.2 days) between oxytocin administration and nest covering. The elapsed time was significantly greater for the wild-caught group than the dystocia group ($t = 3.011$, $p = 0.024$, df = 6).

The nests of all oxytocin-injected females were typical of nests constructed by gravid *T. carolina triunguis*, including the duration of behaviors and the physical dimensions of the nests. All nests were closed using surface litter and soil from the excavated nest cavity (Messinger and Patton 1995), but there were no eggs in the nest. From the time of their arrival at the study site until after nests were completed, the behavior of the 8 wild-caught females was typical of gravid *T. carolina triunguis*,

Table 2. Data on internesting intervals for wild-caught female *T. carolina triunguis* that oviposited a subsequent clutch in the same season as the oxytocin-induced clutch.

Female no.	Date of next oviposition	Interval since oxytocin-induced oviposition (d)	Interval since empty nest construction (d)	Subsequent intervals			
				Mean (d)	SD	*n*	range (d)
2	13 Jul 1997	24	17	19.7	3.2	15	16–26
4	8 Jul 1999	25	18	23.0	6.3	5	18–34
6	5 Aug 2001	41	30	20.0	4.2	2	17–23
Total		30.0	21.7	20.9			16–34

as was that of the 3 dystocia-group females following oxytocin-induced oviposition.

There were two other circumstances when dissociation between oviposition and nesting was observed. Turtle 9 excavated and covered a complete nest that had no eggs while she was suffering from dystocia 4 days before she received the oxytocin injection detailed in Table 1. One other female (11) constructed and covered 7 empty nests sequentially over a 2-year period in 1998 and 1999. She was never observed to oviposit a clutch; although, in all other respects, her nesting behavior appeared normal.

Nesting interval data for 3 of the wild-caught females that oviposited a subsequent clutch in the same nesting season as the oxytocin-induced oviposition are presented in Table 2. The time between oxytocin-induced oviposition and subsequent oviposition averaged 30.0 days. Internesting intervals calculated from the date of empty nest construction (following the oxytocin-induced oviposition) to the subsequent nesting closely coincided with all subsequent internesting intervals recorded for the 3 individuals (21.7 vs. 20.9 days).

Discussion. — Oviposition coinciding with nesting behavior is well established in turtles, except for a few species that oviposit without construction of a nest (Kuchling 1999). The typical behavioral sequence includes nest-site selection, nest-site preparation, egg-cavity construction, oviposition, and nest covering (Ehrenfeld 1979; Kuchling 1999). This behavioral sequence is associated with a complex set of physiological events involving gonadal hormones, neurohypophysial hormones, and peripheral neuronal regulation (Feldman 2007). Sea turtles are known to exhibit consecutive spikes of 2 neurohypophysial hormones, AVT and neurophysin, preceding two prostaglandin peaks (PGF and PGE_2) during oviposition (Owens 1997; Kuchling 1999). The generality of this specific series of physiological events with respect to turtle oviposition is not established; although, it seems likely to play a role in the highly stereotypical nesting behavior of chelonians (Ehrenfeld 1979). For sea turtles that oviposit multiple clutches in one season, ovulation of subsequent clutches occurs within approximately 48 hours after nesting and is correlated with surges of luteinizing hormone and progesterone (Licht 1982; Owens 1997).

Administration of oxytocin to gravid *T. carolina triunguis* led to atypical behavior in which oviposition and nesting behaviors were decoupled, a phenomenon termed "false nesting" by Tucker et al. (1995). Rather than coinciding, oviposition preceded nesting behavior by anywhere from 12 to 417 hours, and the elapsed time between these 2 events could be extended by withholding a suitable nesting substrate (i.e., time spent in the lab). These observations are in accord with previous reports for deirochelyine emydids (Iverson and Smith 1993; Tucker et al. 1995; Feldman 2007) and chelids (McCosker 2002), and may indicate a susceptibility of many chelonians after the administration of oxytocin. Tucker et al. (1995) suggested that oxytocin induced the post-ovipositional nesting behavior. We agree with McCosker (2002), however, that this is not likely a cause and effect relationship since the oxytocin concentration in the blood should drop continuously post-injection as it is metabolized by the liver and kidneys (Plumb 2002). In mammals, the half-life of oxytocin is measured in minutes (Plumb 2002), yet we recorded elapsed times between oxytocin injection and nesting behavior in hours and days.

The dissociation between oviposition and nesting behavior following oxytocin injection indicates that a complete sequence of nesting behavior is not dependent on the presence of eggs in the oviduct(s), nor the actual oviposition of eggs. Other than the 11 nesting events by 10 individuals reported here, 2 additional sets of observations support this hypothesis (this is out of a total of 1060 nesting events by 79 females over an 11-year period, 1996–2006). In one case, a female with dystocia went through an entire nesting behavioral sequence to produce an empty nest before receiving oxytocin. Another female maintained at the study site for several years appeared physically and behaviorally normal and was never administered oxytocin, yet she was observed on 7 occasions to exhibit a complete set of nesting behaviors that lead to typical nest cavities but without eggs. She did this at regular intervals typical of the interclutch interval for *T. carolina triunguis* (Messinger and Patton 1995). These observations support the hypothesis of McCosker (2002) and Feldman (2007) that the "normal nesting-related hormonal sequence" does not occur when oxytocin is administered to obtain eggs, and thus the female returns to the behavioral task of nesting at a later time when that hormonal sequence can play out. Extension of this idea suggests that subsequent clutches in the same nesting season will be delayed by the elapsed time between oxytocin administration and completion of the nesting

behavior (the "false" nest), which would account for prolonged internesting intervals mentioned by Iverson and Smith (1993).

The mean internesting interval we observed was 21.7 days between empty nest construction and subsequent nesting versus 30.0 days between oxytocin-induced oviposition and subsequent nesting. This compares with a mean internesting interval of 20.9 days for intervals between consecutive nests for these females in the same and subsequent seasons. This mean is near the 19.4-day mean of 105 internesting intervals in *T. carolina triunguis* (Messinger and Patton 1995), and the 21.3 days of a more recent calculation for 2001 and 2002 internesting intervals ($n = 206$, Messinger and Patton, *pers. obs.*). Our data suggest that subsequent clutches in the same season will be delayed until completion of the nesting behavior and its associated hormonal sequence that occurs at some point after the oxytocin-induced oviposition. Thus, ovulation of a subsequent clutch depends on completion of nesting behavior and not oviposition.

We found a significant difference between prior residents (dystocia group) and new residents (wild-caught group) at the study site in the time elapsed from oxytocin-induced oviposition to nest covering (3.2 vs. 9.8 days). It is possible that stress from the initial handling and unfamiliar environment may suppress reproductive function (Kuchling 1999; Feldman 2007), leading to the longer elapsed times in the new study site residents. Additionally, the period of days post-injection (2–6) in the lab without access to a nesting substrate may have influenced our results.

The use of exogenous oxytocin has proven useful in gathering reproductive data from turtles (Ewert 1985) and to relieve dystocia (Frye 1991; Highfield 1996). Oxytocin differs from AVT in structure by only a single amino acid but is capable of binding to receptors in the oviduct and stimulating smooth muscle contractions that will expel eggs. Oxytocin appears to override the normal sequence of hormonal events related to nesting, and may not enter into the regulatory feedback of the hypothalamic–pituitary–gonadal axis. As a consequence, a turtle in which oviposition has been induced with oxytocin may still undergo a hormonal cascade and associated nesting behavior at a later time in spite of lacking oviductal eggs.

Tucker et al. (1995:138) suggested "that turtles be held at least 24 h post-induction to reduce nesting efforts that expose turtles to unnecessary risk of mortality." Tucker (2000) later held *T. scripta elegans* for 48 hours after oxytocin-induced oviposition in order to "prevent" nesting behavior. Our findings indicate that in *T. carolina triunguis*, nesting after oxytocin-induced oviposition may be delayed for a much longer period of up to 17 days. Although taxon-specific differences are possible, the variability we have observed in *T. carolina triunguis* suggests that a 24- to 48-hour holding period is insufficient (also supported by McCosker [2002] and Feldman [2007]), and it is not clear that it is possible to prevent nesting behavior subsequent to oxytocin-induced oviposition. When oxytocin is used, in order to shorten the period between induced oviposition and nesting, we suggest that females be released back into the environment from which they came as quickly as possible in order to alleviate stress associated with a lab/captive stay and exposure to an unfamiliar environment. Alternatively, we recommend that a suitable nesting substrate be made available if the female is not immediately released.

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Abundance of Juvenile Eastern Box Turtles Relative to Canopy Cover in Managed Forest Stands in Alabama

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ABSTRACT. – Between 2002 and 2005, we used drift fences and artificial pools to sample juvenile eastern box turtles (*Terrapene carolina*) in northeastern Alabama in forest stands experimentally treated to retain various amounts of overstory trees—clear-cuts and those with 25%–50% and 75%–100% of trees retained. We captured juvenile turtles only in clear-cut and 25%–50% retention plots; microhabitats in these plots are characterized by a combination of abundant vegetative ground cover and leaf litter.

Knowledge of habitat use by each life history stage and sex within animal populations is necessary to understand a species' habitat requirements. Because they are rarely observed, little is known about the habits of juvenile eastern box turtles, *Terrapene carolina carolina* (Ernst et al. 1994), especially in landscapes modified by humans. Lack of detailed knowledge of habitat requirements, especially of juveniles, is a common hindrance in an understanding of species of terrestrial turtles in North America (Morafka 1994), including box turtles (Legler 1960; Jennings 2007). The objectives of this study were to examine the impact of forest canopy removal and the associated changes in understory habitat on the use of these areas by juvenile box turtles. Because juvenile box turtles prefer habitats with open canopy and abundant vegetative ground cover (Forsythe, et al. 2004; Jennings 2007), we predicted that juvenile box turtles would be more abundant in early successional habitat created by canopy removal.

Methods. — Study Site. — The study took place on the Cumberland Plateau of Jackson County, northern Alabama. Sites were located on the escarpment of the Plateau with slopes between 12% and 20% and stony to gravelly loam soils. These slopes were covered by second-growth forests (80–100 years old) and contained mostly oaks (*Quercus* spp.) and hickories (*Carya* spp.) with a strong component of yellow poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandifolia*).

The results presented here are part of a larger study concerning the potential of several shelterwood cuts for regenerating oak forests and the impacts these silvicultural techniques have on wildlife. Treatments involved 3 levels of basal area retention including clear-cuts (0% retention), 25%–50% retention, and 75%–100% retention of subcanopy and canopy trees. Trees were felled with a chainsaw and grapple skidded in the clear-cut and 25%–50% retention treatments. The midstory was killed with an herbicide (Imazypr), thus creating small gaps in the subcanopy on half of 75%–100% retention treatment plots. Treatments were applied in the fall of 2001 and followed a randomized complete block design, with 1 block at Miller Mountain (lat 34°58′11″N, long 86°12′21″W) on south and southwest facing slopes and 2 blocks at Jack Gap (lat 34°56′30″N, long 86°04′00″W) on north-facing slopes. Each experimental block had 5 4-ha plots that were adjacent to one another: 2 each of 25%–50% and 75%–100% basal area retention treatments and 1 of clear-cut treatment. Basal area was uniform across plots prior to treatment (Schweitzer 2003).

Data Collection. — Turtles were captured in drift fences and small artificial pools. Drift fences were 15 m long with terminal 19-L buckets and 2-sided funnel traps at their midpoint. Three fences were installed in each of the 15 plots. Fences were opened intermittently for periods of 5 continuous days and checked daily between July and August 2002 and March and September 2003–2005. Drift fences were opened for a total of 1455 trap nights on blocks 1 and 3 and 1575 trap nights on block 2. A trap night was 1 drift fence opened for 24 hours. Plastic pools were 91 × 61 × 46 cm and held 60 L of water. A group of 3 pools was buried flush with the ground near the center of each plot and filled with rainwater. Several juvenile box turtles were found floating in the pools. Turtles were measured for carapace length and width to the nearest tenth of a millimeter using calipers and mass to the nearest

Table 1. Relative abundance and size (average ± standard error) of juvenile eastern box turtles captured in Jackson County Alabama, 2003–2005. None were captured in the 75%–100% canopy retention treatment.

Treatment	Relative abundance (turtles/trap night)	Carapace length (mm)	Carapace width (mm)	Mass (g)
Clear-cut	0.002 ± 0.0008	48.2 ± 5.0	42.2 ± 4.1	24.4 ± 9.8
25%–50% retention	0.001 ± 0.0004	57.7 ± 6.8	43.9 ± 2.5	55.1 ± 25.5

tenth of a gram using Pesola spring scales, given a unique notch on marginal scutes (Cagle 1939), and immediately released at the site of capture.

The microhabitat features measured along 20-m line transects at each drift fence location during late summer in 2002–2005 included percent cover of leaf litter, vegetative ground cover, slash, and coarse woody debris and percent canopy cover above 3 m. Microhabitat features were averaged across sampling locations within each of the 15 plots and across years. We used analysis of variance (ANOVA) to compare number of juvenile turtles captured per trap night across treatment levels and Tukey tests to separate means, with $\alpha < 0.10$ accepted for significance. We chose this relaxed α because of the low replication and high levels of variation usually found in large-scale ecological studies such as this one (deMaynadier and Hunter 1995). Turtle captures were square-root transformed to better meet assumptions of ANOVA (Zar 1999).

Results. — A total of 20 juvenile box turtles were captured. Five of these were found in pools and 15 in drift fences. No juvenile turtles were recaptured. Turtles ranged between 31.5- and 112.9-mm carapace length and were captured between April and September (Table 1). The largest turtle included in these analyses approached the carapace length of sexually mature males (Dodd 2004) but showed no secondary sexual characteristics.

Relative abundance of juvenile box turtles differed among treatments ($F_{2,10} = 14.08$, $p = 0.001$) and was highest in clear-cut and 25%–50% retention treatments (Fig. 1). No turtles were captured in the high-retention treatment (75%–100% retention). Tree removal treatments created a gradient from closed-canopy stands with low coverage of vegetation, coarse woody debris, and slash to open-canopy stands with dense understory vegetation, slash, and woody debris at the ground level (Fig. 1). All juvenile turtles were captured in open-canopy stands.

Discussion. — It appears that the relative abundance of juvenile eastern box turtles is affected by canopy tree removal. Juveniles were never captured or observed in closed-canopy stands. The removal of more than 75% overhead canopy resulted in the growth of a dense ground covering (> 80%) of herbaceous and woody vegetation and an increase in slash and coarse woody debris associated with the cutting operations. At our sites, the stand was thinned to at least 16 m^2/ha (70 $feet^2$/acre) of basal area to achieve this level of canopy cover.

The relative abundance of juvenile turtles was related to the increase in the habitat features described previously. Although possibly a correlation, we hypothesize that the treatments created suitable habitat for juvenile box turtles by opening the forest canopy. Presence of juvenile box turtles could be related to food resources: increased production of vegetative growth is likely accompanied by increased biomass of dietary items such as fruits and leaves (Greenberg et al. 2007). In 2002, air temperatures were on average 1.5°C higher on clear-cuts than controls, soil temperatures were 3°C higher, and both showed a positive gradient associated with increasing tree removal (Felix et al. 2003). Increased heat and food availability may be required to meet metabolic demands during the rapid growth of early life stages (Dodd 2004). Alternatively, increased numbers of juvenile turtles could be related to cover requirements. Although juvenile box turtles could find cover in the plentiful, deep leaf litter in closed canopy, they may prefer a combination of litter and the abundant vegetative ground cover, slash, and coarse woody debris in cut plots. Juvenile Florida box turtles (*T. c. bauri*) used areas with thick understory vegetation and leaf litter during movements (Jennings 2003) and were found most frequently beneath dense understory vegetation under leaf litter or other organic debris (Jennings 2007). Hatchling eastern box turtles in Illinois were found in an open-canopy grassy field with abundant herbaceous and woody vegetation. Within these habitats, turtles preferred microhabitats with less canopy closure and herbaceous vegetation and more leaf litter than random locations (Forsythe et al. 2004). Based on these observations, it appears that important features of juvenile box turtle habitat include open canopy, abundant vegetative ground cover, and leaf litter.

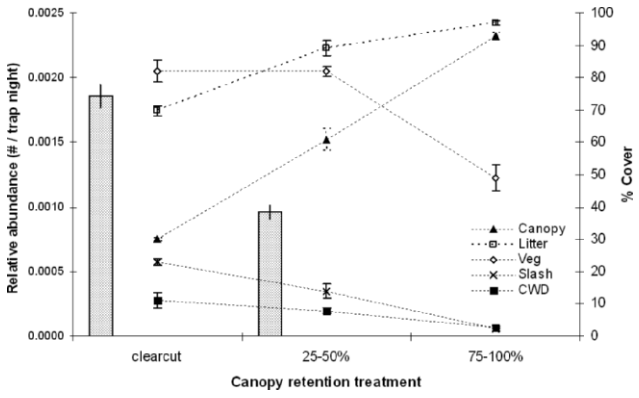

Figure 1. Relative abundance of juvenile box turtles captured and percent cover of 5 microhabitat categories in each of 3 basal area retention treatments 2002–2005, Jackson County, Alabama. Bars represent average number of juvenile turtles captured per trap night, and lines show 4-year averages for percent cover of microhabitat features. Error bars are ± 1 standard error.

This information is only 1 small puzzle piece in determining habitat requirements of eastern box turtles. For example, *T. c. bauri* show ontogenetic shifts in habitat use (Hamilton 2000), and adult turtles use different habitat than that of juveniles. Flitz and Mullin (2006) showed that adult females use different habitat types while nesting than in other seasons. At our study sites, canopy tree removal increased the relative abundance of juveniles. Increased relative abundance could be the result of differential habitat use by nesting females or juveniles. Flitz and Mullin (2006) showed that adult females tend to select relatively open habitats while nesting than at other times. If nesting habitat is limited in closed-canopy forests, tree removal might increase recruitment by increasing availability of suitable nest sites. The amount of benefit gained would depend, in part, on the balance of the amount of open- and closed-canopy habitat required by juveniles and adults and the interactions of these habitat types with other demographic features of the population. For example, edges created by forest openings support higher predator populations such as raccoons (Dijak et al. 2000), leading to greater predation on box turtle nests, juveniles, and adults (Temple 1987) than in nonecotonal areas. Optimal size and number of forest openings can be determined only with additional information on habitat requirements and home range size of adult and juvenile turtles as well as impacts of openings on survival of all life stages.

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A Mitochondrial DNA Phylogeny of Extant Species of the Genus *Trachemys* with Resulting Taxonomic Implications

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ABSTRACT. – The phylogenetic relationships among taxa within the emydid genus *Trachemys* have largely remained unresolved. A 768-basepair fragment of ND4, as well as the histidine, serine, and leucine tRNAs were sequenced from 18 of 26 of the extant species and subspecies of *Trachemys*. The aligned sequences were analyzed using maximum parsimony, maximum likelihood, and Bayesian methods. The results support the taxonomy of the genus as proposed by Seidel.

The genus *Trachemys* is a speciose group of turtles in the family Emydidae. Species of this genus are spread throughout North, Central, and South America as well as the Caribbean Islands. Most members of this genus were historically placed into the ambiguously defined *T. scripta* complex, which has been attributed to the fact that few members of this genus are sympatric (Seidel 2002). More recent studies have argued that some, if not many, of these are likely to actually be species rather than subspecies (Stephens and Wiens 2003). If the species designation of many of the subspecies is correct, then the interspecific relationships within this genus are largely unresolved, and a more comprehensive phylogenetic analysis of the genus is needed to resolve these issues (Seidel et al. 1999; Seidel 2002). This is especially true in the case of the *T. scripta* complex (Seidel et al. 1999; Stephens and Wiens 2003). We use the taxonomy proposed by Seidel (2002) to avoid confusion among historical species and subspecies.

In this study, mitochondrial DNA sequence data from the NADH 4 region and flanking tRNAs of 52 individuals of 18 of the 26 extant species and subspecies in *Trachemys* were analyzed by maximum parsimony, maximum likelihood, and Bayesian analysis methods. Our explicit goal was to provide an mtDNA phylogeny, which includes sequence data for a majority of the currently described taxa. Particular emphasis was given to the North American species group, specifically the relationship and validity of *Trachemys gaigeae*.

Methods. — Blood samples were collected from wild caught, pet trade, and zoo animals by various individuals (mainly MRJF, DES, and James Dixon; for a list of specimens, see Appendix 1). Remaining blood and/or DNA samples are in the MRJ Forstner Frozen Tissue Collection at Texas State University San Marcos.

Blood was isolated from each individual and stored in blood storage buffer (100 mM Tris pH 8.0, 100 mM Na_2EDTA, 10 mM NaCl, and 1% SDS) at −80°C until needed. DNA was extracted from blood using the proteinase K protocol of Maniatis et al. (1982), as modified by Hillis and Davis (1986). The primers used in polymerase chain reaction amplification were obtained from Arevalo et al. (1994). The primers ND4 and leucine were chosen because they show a high degree of conservation within turtle sequences. Additionally, this region has been shown to be phylogenetically informative in squamates (Arevalo et al. 1994; Forstner et al. 1995). A 992-basepair fragment of mtDNA was amplified by these primers and contained the last 768 bases of the ND4 gene and the tRNAs histidine, serine, and leucine. Sequencing reactions were performed using the Applied Bio-Systems (ABI) Dideoxy termination cycle sequencing kit in conjunction with an ABI 373A automated sequencer.

All sequences were aligned using MacClade 4 (Madison and Madison 2003). All sequences from individuals of the same species that were identical were collapsed into a single sequence, again using MacClade. This resulted in a data set of 54 individual sequences from 20 taxa. All sequences used in this analysis were accessioned into NCBI GenBank (see Appendix 1). A partition homogeneity test was conducted using PAUP* 4b10 (Swofford 2002) to determine if it would be necessary to partition the tRNAs and the protein coding fragment of ND4. Modeltest 3.5 (Posada and Crandall 1998) was used to determine the appropriate model of sequence evolution for this data set under the Akaike Information Center (AIC) criteria (Posada and Buckley 2004) with 4 different outgroup arrangements. The outgroups tested were *Testudo kleinmanni* only; *Testudo* and *Pseudemys texana*; *Testudo*, *Heosemys*, *Sacalia*, and *Callagur*; and finally *Pseudemys*, *Testudo*, *Heosemys*, *Sacalia*, and *Callagur*. Neighbor joining analyses were conducted using Maximum Likelihood Estimate (MLE) distance settings corresponding to the results of the model selection process for each outgroup arrangement, and the results were compared in order to ascertain sensitivity of the data to outgroup selection. All 4 outgroup arrangements resulted in the selection of the same model in Modeltest 3.5 (GTR + G) and produced analogous neighbor joining topologies using MLE distances. Thus, the data set was not sensitive to outgroup selection and a single outgroup arrangement was chosen (*Testudo kleinmanni* and *Pseudemys texana*), providing a distantly related taxon, as well as a proximal sister taxon within the same family.

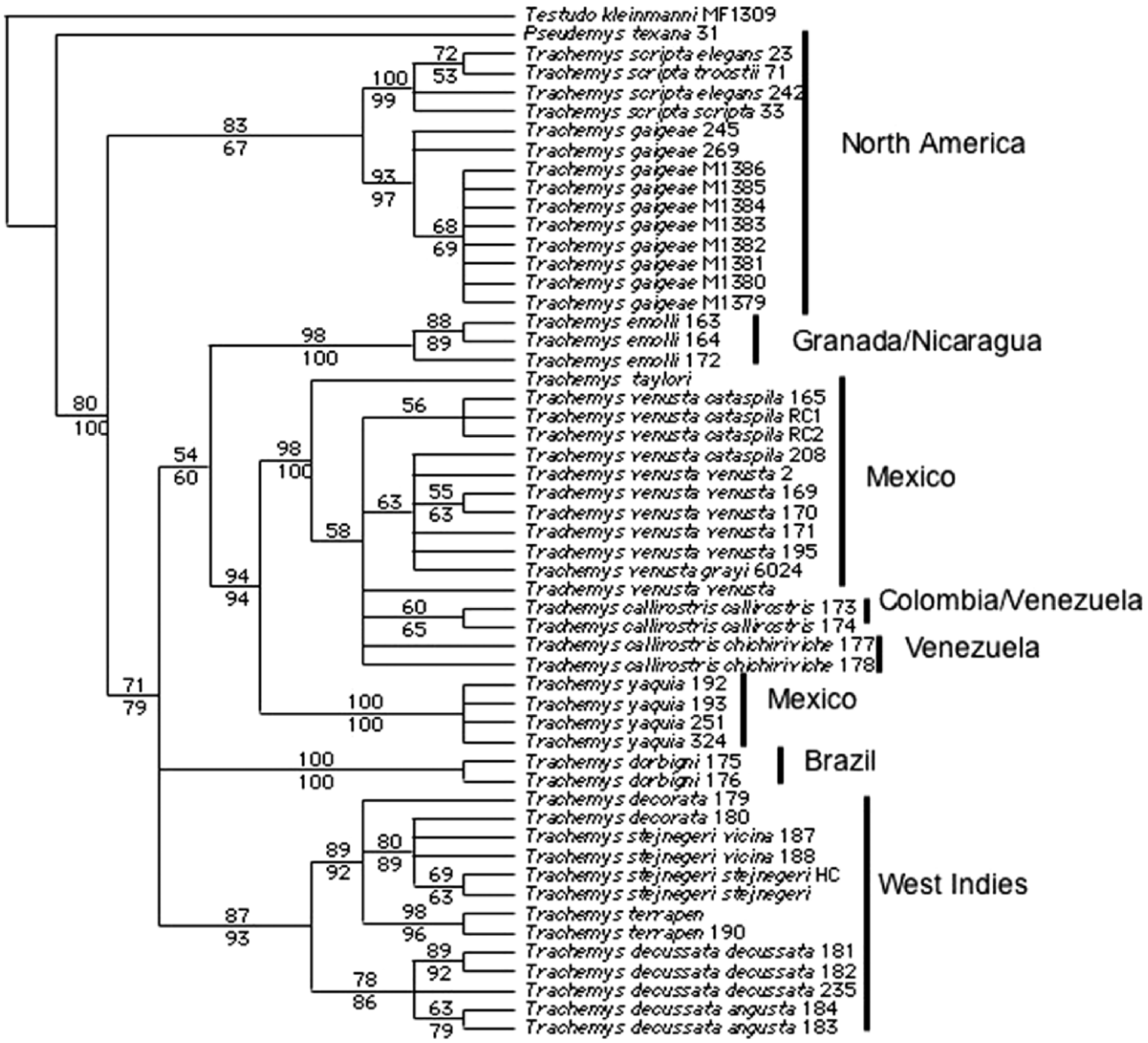

Figure 1. Bootstrap consensus of the maximum parsimony and maximum likelihood analyses of ND4-leucine tRNA region of mitochondrial DNA in *Trachemys*. ML bootstrap support values are shown above supported branches, and MP bootstrap values are shown below. Major regional clades are illustrated to the right of taxon names.

The model selected by Modeltest (GTR + G) was then used in maximum likelihood analysis of the dataset in PAUP*. The parameter estimates from Modeltest were used in this analysis. The resulting ML topology was bootstrapped (1000 replicates) to evaluate support of the relationships proposed.

MrModeltest was used to determine the most appropriate model using AIC (GTR+G) for Bayesian analysis using MrBayes (Huelsenbeck and Ronquist 2001). An MCMC analysis was conducted in MrBayes using the GTR+G model to implement a "best" model. This analysis was run for 1×10^6 generations, sampling every 100, with 1 cold and 3 hot chains. A burn in of 300 samples (sumt burnin = 300) was determined to be appropriate from stabilization of a log likelihood plot, and posterior probabilities for the resulting topology were calculated using PAUP*.

A partitioned Bayesian analysis was also conducted using MrBayes. The data set was divided into 4 partitions, one for each codon position in the protein coding ND4 portion, and the fourth partition contained the tRNAs. Each partition was independently run through MrModeltest, and the best model for each partition selected by AIC. The selected model and parameter estimates for each partition were then input in MrBayes. Six chains (5 hot, 1 cold) were run for 3×10^6 generations, sampling every 1000 generations. The first 25% of the samples were discarded, equivalent to a burn in of 750 samples. Posterior probabilities for the resulting topology were calculated using PAUP*.

Parsimony analyses were conducted using PAUP*. The most parsimonious tree for the dataset was found using a full heuristic search with simple stepwise addition and

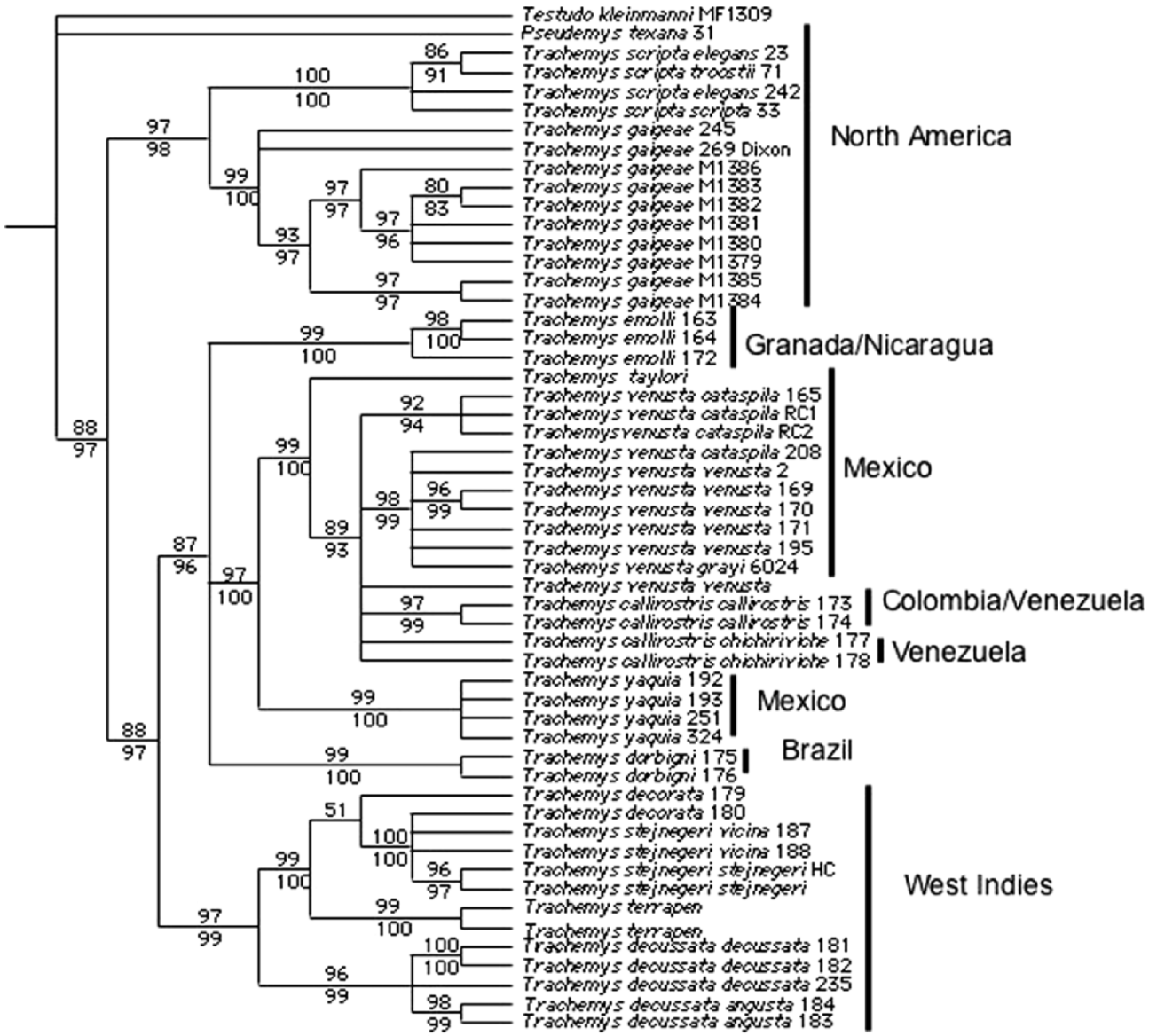

Figure 2. Results of Bayesian analyses of the ND4-leucine tRNA region of mitochondrial DNA in *Trachemys*. Posterior probabilities from analysis using a single model are shown above supported branches, and the posterior probabilities from the partitioned analysis are shown below. Regional clades are illustrated to the right of taxon names.

tree bisection-reconnection (TBR). The result was then subjected to a nonparametric bootstrap as implemented in PAUP*, for 1000 replications with 10 TBR steps each, and the resulting 50% consensus topology was retained.

Results. — The result of the partition homogeneity test was not significant ($p = 0.15$); therefore, partitioning of the data set was not required. Modeltest selected GTR+G as the most appropriate single model for the dataset. Base frequencies for A, C, G, and T were 0.3513, 0.2635, 0.1305, and 0.2547, respectively. The rate variation followed a gamma distribution with a shape parameter of 0.4655, and there were 4 rate categories and 6 substitution types. For the partitioned dataset, MrModeltest selected the GTR model for the first codon position, HKY+I for the second position, and GTR+G for the third position partition. HKY+G was selected for the tRNA partition.

The results of the ML (Fig. 1) and Bayesian (Fig. 2) analyses were generally congruent with each other and with the taxonomy of Seidel (2002). Both topologies supported the significance of *T. gaigeae*, *T. emolli*, *T. taylori*, *T. yaquia*, *T. dorbigni*, *T. terrapen*, and *T. decussata* lineages. The results of both analyses also showed clearly resolved North American (*T. scripta scripta*, *T. scripta troostii*, *T. scripta elegans*, and *T. gaigeae*), Meso-American (*T. emolli*, *T. taylori*, *T. venusta venusta*, *T. venusta cataspila*, *T. venusta grayi*, *T. callirostris callirostris*, *T. callirostris chichiriviche*, *T. yaquia*, and *T. dorbigni*), and West Indian (*T. decorata*, *T. stejnegeri stejnegeri*, *T. stejnegeri vicina*, *T. terrapen*, *T. decussata decussata*, and *T. decussata angusta*) monophyletic units.

Discussion. — While the three main monophyletic lineages (North American, Meso-American, and West

Indian) apparent in the results of these analyses are generally consistent with the results of other studies (Seidel 2002; Stephens and Wiens 2003), there are some incongruences regarding the relationships among some species.

The analysis of Stephens and Wiens (2003) placed *T. gaigeae* in a clade with species from South America and Mexico, while our analysis places this taxon as more closely related to the North American *T. scripta* complex, and as part of the monophyletic North American lineage. Our placement of *T. gaigeae* is strongly supported by both the MP and ML bootstrap values and Bayesian posterior probabilities from both partitioned and nonpartitioned analyses (Figs. 1 and 2).

Together with the concept of the evolutionarily significant unit (Ryder 1986; Moritz 1994), which in some cases is the equivalent of a "species" (Moritz 1994), our analysis supports the species status of *T. gaigeae* as proposed by several authors (Weaver and Rose 1967; Ward 1984; Seidel et al. 1999; Seidel 2002). Our intention here, however, is to recognize this lineage as unique and worthy of treatment as a unit for conservation, rather than contribute to the overabundance of literature arguing the appropriate criteria for species definition.

Our study failed to resolve the *T. venusta* and *T. callirostris* species complexes of Seidel (2002). However, the lack of phylogenetic resolution does not provide an inherent default hypothesis, and therefore Seidel's taxonomy is provisionally retained as we feel that this makes the most use of all available data. These ambiguous relationships may eventually be resolved as more data are collected and analyzed.

In conclusion, it appears that when mtDNA data are considered, the taxonomy of *Trachemys* proposed by Seidel (2002) is the most reasonable for the genus. The proposed species status of *T. gaigeae* (Weaver and Rose 1967; Ward 1984; Seidel et al. 1999; Seidel 2002) is also supported by our data. In our evaluation of the specific status for this taxon, we have sought to use historical evaluations in conjunction with supported results from our current mtDNA hypothesis. In our support for *T. gaigeae*, we explicitly acknowledge our failure to more broadly evaluate the remaining potential evolutionarily significant units within this genus (Moritz 1994). This decision was made in keeping with the recent voucher paper (Lehn et al. 2007) in which we agree that significant systematic decisions should not be completed in the absence of traditional voucher specimens. We would still suggest, however, that the proposed taxonomy of Seidel (2002) represents the best current working taxonomy of *Trachemys*. This taxonomic arrangement does the most to preserve the diversity contained within the genus by recognizing diagnosable lineages as unique.

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Appendix 1. Specimen data for all taxa. Texas samples were collected under permission of the Texas Parks and Wildlife Department SPR-0290–022. Captive samples maintained under ESC8945 (PC1) and Tennessee collection made under license 2504378. Living voucher material will be deposited into the Texas Cooperative Wildlife Collection (Texas A&M University) as available after normal mortality of the individuals.

Name	GenBank accession no.	Location	Collection	Museum no.
Pseudemys texana 31	DQ338475	Colorado R., Travis Co., TX	Texas Cooperative Wildlife Collection	TCWC 72324
Trachemys decorata 179	DQ338515	Pet trade	Private collection 1	Living voucher
T. decorata 180	DQ338516	Pet trade		Blood only
T. decussata angusta 183	DQ388521	Grand Cayman	Released	Photo voucher
T. decussata angusta 184	DQ388520	Grand Cayman	Released	Photo voucher
T.s decussata decussata 181	DQ338517	Pet trade	Private collection 1	Living voucher
T. decussata decussata 182	DQ338518	Pet trade	Private collection 1	Living voucher
T. decussata decussata 235	DQ338519	Pet trade		Blood only
T. dorbigni 175	DQ338513	Uruguay	Private collection 1	Living voucher
T. dorbigni 176	DQ338514	Uruguay	Private collection 1	Living voucher
T. gaigeae 245	DQ338480	Rio Grande R., Dona Ana Co., NM	Texas Cooperative Wildlife Collection	TCWC 72425
T. gaigeae 269	DQ338481	Rio Grande R., Dona Ana Co., NM	Texas Cooperative Wildlife Collection	TCWC 86270
T. gaigeae M1379	DQ338489	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. gaigeae M1380	DQ338488	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. gaigeae M1381	DQ338487	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. gaigeae M1382	DQ338486	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. gaigeae M1383	DQ338485	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. gaigeae M1384	DQ338484	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. gaigeae M1385	DQ338483	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. gaigeae M1386	DQ338482	Black Gap WMA, Brewster Co., TX	Released	Photo voucher
T. callirostris callirostris 173	DQ338504	Pet trade	Private collection 1	Living voucher
T. callirostris callirostris 174	DQ338505	Pet trade	Private collection 1	Living voucher
T. venusta cataspila 165	DQ338494	Northern Mexico	Private collection 1	Living voucher
T. venusta cataspila 208	DQ338495	Northern Mexico	Private collection 1	Living voucher
T. venusta cataspila RC1	DQ338496	Unknown		Blood only
T. venusta cataspila RC2	DQ338497	Unknown		Blood only
T. callirostris chichiriviche 177	DQ338506	Venezuela	Private collection 1	Living voucher
T. callirostris chichiriviche 178	DQ338507	Venezuela	Private collection 1	Living voucher
T. scripta elegans 23	DQ338476	Rio Grande R., Cameron Co., TX	Texas Cooperative Wildlife Collection	TCWC 72426
T. scripta elegans 242	DQ338477	Rio Grande R., Cameron Co., TX		Photo voucher
T. emolli 163	DQ338490	Pet trade	Private collection 1	Living voucher
T. emolli 164	DQ338491	Pet trade	Private collection 1	Living voucher
T. emolli 172	DQ338492	Panama	Private collection 1	Living voucher
T. scripta scripta 33	DQ338478	Flint R., Dougherty Co., GA	Texas Cooperative Wildlife Collection	TCWC 72278
T. scripta troostii 71	DQ338479	Tennessee R., Bradley Co., TN		Blood only
T. venusta venusta 169	DQ338500	Cozumel, Mexico	Private collection 1	Living voucher
T. venusta venusta 170	DQ338501	Lake Bacalar, Belize	Private collection 1	Living voucher
T. venusta venusta 171	DQ338502	Lake Bacalar, Belize	Private collection 1	Living voucher
T. venusta venusta 195	DQ338503	New River, Belize	Private collection 1	Living voucher
T. yaquia 192	DQ338509	Mexico	Private collection 1	Living voucher
T. yaquia 193	DQ338510	Mexico	Private collection 1	Living voucher
T. yaquia 251	DQ338511	Mexico	Private collection 2	Living voucher
T. venusta grayi 6024	DQ338508	Unknown		Blood only
T. stejnegeri stejnegeri	DQ338527	Caguas, Puerto Rico	Private collection 1	Living voucher
T. stejnegeri stejnegeri HC	DQ338526	Caguas, Puerto Rico	Private collection 2	Living voucher
T. stejnegeri vicina 187	DQ338524	San Domingo, Dominican Republic	Private collection 1	Living voucher
T. stejnegeri vicina 188	DQ338525	San Domingo, Dominican Republic	Private collection 1	Living voucher
T. taylori	DQ338493	Unknown	Private collection 2	Living voucher
T. terrapen	DQ338522	Ocho Rios, Jamaica	Private collection 1	Living voucher
T. terrapen 190	DQ338523	Ocho Rios, Jamaica	Private collection 1	Living voucher
T. venusta venusta	DQ338498	New River, Belize		Blood only
T. venusta venusta 2	DQ338499	New River, Belize		Blood only
T. yaquia 324	DQ338512	Mexico	Private collection 2	Living voucher

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Dietary Observations on the Asian Softshell Turtle (*Amyda cartilaginea*) from Sarawak, Malaysian Borneo

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ABSTRACT. – **We examined the diet of *Amyda cartilaginea* from 2 localities in Sarawak: Loagan Bunut National Park and Balai Ringin. The most commonly found items in stomach contents, when using percentage frequency of occurrence, were plant material (77%) and unknown vertebrate parts (55%). Fecal analysis indicated similar results: plant material (100%), unknown vertebrate parts (84%), fish (69%), and unknown arthropods (62%). Results indicate that *A. cartilaginea* is an opportunistic omnivore.**

The Asian softshell turtle (*Amyda cartilaginea*) is a large, locally widespread trionychid from the lowlands of tropical Southeast Asia (Fig. 1). Its currently known range is from northeast India; Myanmar; Thailand; Laos; Vietnam; Cambodia; Malaysia; Singapore; Indonesia; and the islands of Borneo, Sumatra, Java, Lombok, Sulawesi; and some of the smaller associated islands (Ernst and Barbour 1989; Cox et al. 1998; Lim and Das 1999; Pawar and Choudhury 2000). In Sarawak, on Malaysian Borneo, *A. cartilaginea* inhabits a variety of freshwater types, including clear or muddy rivers, lowland peat swamps, ponds, and irrigation canals (Lim and Das 1999).

Little is known about the natural history of *A. cartilaginea,* and information on its diet is largely anecdotal. As part of a larger ecological study of freshwater turtles of Sarawak, information on the diet of this species was obtained from stomach contents and feces. The primary study area was Loagan Bunut National Park (coordinates for the park headquarters are at lat 03°44′N, long 114°09′17″E; datum wgs84), located in the northern part of Sarawak. Field work was concentrated at the park (description of area and its herpetofauna in Das and Jensen 2006). In addition, 2 visits were made to Balai Ringin (lat 01°03′N, long 110°45′E), a fishing village ca. 2-hour drive from Kuching, Sarawak's capital city. Both sites are located within peat swamp forests. Loagan Bunut National Park contains the only freshwater floodplain lake in Sarawak (Sayer 1991), and encompasses 650 ha[2] at its maximum level. The lake is completely dry during periods of prolonged drought. The lake dries up annually, between 3 and 6 times, usually in the months of February, May, and June.

Methods. — The study described here was conducted between May 2004 and April 2005. A variety of collecting techniques, as previously described for freshwater turtles in the literature, were attempted to see which was the most effective for capturing *A. cartilaginea*. Hoop traps, commonly used to catch freshwater turtles in the Western hemisphere (Frazer et al. 1990; Legler 1960; Vogt 1980) were used. Native hoop traps, called *bubu*, were also used, in addition to another local fishing device called a *selembau*, which comprised a special system of scoops and nets attached to long poles. The device is stretched across a river or stream, scooping up whatever comes into contact with it (Fig. 2). Manual capture, otherwise known as muddling (Cagle 1942), was an effective albeit labor-intensive method of capturing softshell turtles. The technique involves wading through streams and probing areas of sand or mud and among roots with a stick, hands, or feet and could only be accomplished during times of low water level.

Dietary analysis was conducted based on both stomach and fecal samples of living specimens. Dissection of stomachs to examine contents was not possible in this study because all turtles are protected under the Sarawak Wildlife Protection Ordinance (Sarawak Government Gazette 1998). Within 1 hour after returning from the field, stomachs were flushed by using the method developed by Legler (1977), which incorporated the modifications of Fields et al. (2000). Turtles were kept in plastic basins, which were 60 cm in diameter and 30 cm in depth, with some water. They were kept under observation until they defecated, usually 24 to 48 hours, and were released afterward at the place of capture. Each sample (stomach content or fecal matter) was washed in water by using a mesh strainer of 0.2-mm mesh size. These samples were immediately preserved in 70% ethanol for study.

Contents of the stomach and fecal samples were examined separately for each turtle. Types of food were sorted under low magnification by using an Olympus SZX9 dissecting microscope. The presence of each dietary group was recorded to the lowest identifiable taxon, typically to ordinal level of classification. In addition, 2 categories were used: unknown arthropods and unknown vertebrates. Any unidentifiable part of an insect or any other arthropod, i.e., wing, claw, etc., was grouped into the first category. The unknown vertebrates category represented any part known to be from a vertebrate, i.e., bone fragments, but unclassifiable further. The volume of each kind was determined by the displacement of water in either a 5-mL or 25-mL graduated cylinder, as described by Moll and Legler (1971). Descriptive analysis of data that pertained to contents of stomachs and fecal samples were expressed in 2 ways:

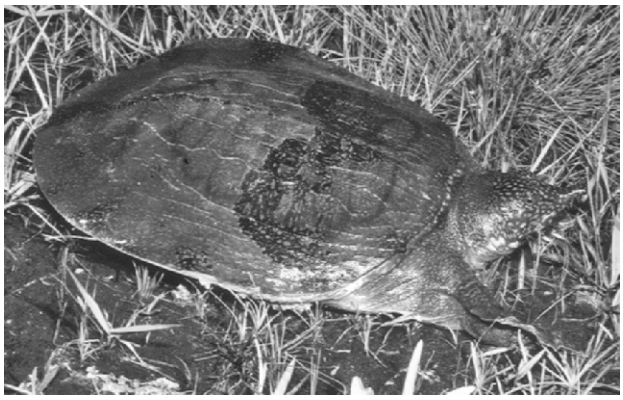

Figure 1. Adult female *Amyda cartilaginea* (field number KJ–31).

$$\text{Percentage frequency of occurrence} = \frac{\text{Number of turtles in which a given food item was found} \times 100}{\text{Number of individuals that provided samples}}$$

$$\text{Total percentage of food volume} = \frac{\text{Volume of diet category} \times \text{over all individuals}}{\text{Volume of all diet categories over all individuals}}$$

We tested for correlation between body size and the different food categories and volume found of either stomach contents or fecal matter by using the Pearson correlation test. Data were log-transformed. Analyses were performed by using SPSS for Windows ver. 10.1.

Results. — In total, 18 animals were obtained, which comprised 3 juveniles, 9 females, and 6 males. Juveniles did not provide stomach samples and yielded little in terms of fecal matter and therefore, were excluded from the dietary analysis. Only 9 adult animals (4 males and 5 females) provided stomach contents; whereas, 13 animals (5 males and 8 females) provided fecal samples. Only 7 turtles provided both stomach contents and feces; whereas, 6 animals provided feces only, and 2 animals provided only stomach contents. Parasitic nematodes were found in 1 female and 2 male turtles, all from Loagan Bunut National Park. Furthermore, sand- or gravel-type substrate was found in all fecal samples. It is assumed that these items were not intentionally ingested and consequently were excluded from further analysis.

All 15 turtles that provided either stomach samples, feces, or both were captured evenly during the different seasons. Five animals, all female, were caught during the end of the northeast monsoon (wet season), which extends from November to March. All 5 had eaten plants, 4 had also eaten unknown vertebrates, and 3 had taken various arthropods and had fish parts in either their stomach or fecal samples. Five animals (3 male, 2 female) were captured during the southwest monsoon (dry season), which occurs from June to September. Four had plants in their stomach or fecal samples; 3 had fish and unknown vertebrate material; and only 1 provided arthropod parts. In the nonmonsoonal period (April, October), 5 animals (3 male, 2 female) were also caught. All animals had plant material and fish parts in their fecal or stomach samples; 4 animals had insect parts and parts of an unidentifiable vertebrate.

Figure 2. A native trap, known as *selembau*, comprising a system of scoops and nets attached to long poles and set across a river, used for catching everything from fish to turtles.

In total, 60% ($n = 9$) of turtles obtained in this study provided stomach contents. In all, 66% ($n = 4$) of the males provided stomach contents; whereas, 55% ($n = 5$) of the females provided stomach contents. They consisted of both aquatic and terrestrial plants, invertebrates, and the remains of fish. Muscle tissues and bone fragments from unidentifiable vertebrate species also occurred in stomach contents. Seven of the 9 samples contained plant material; although, only 5 samples contained animal parts. Frequency of occurrence of animals found in the stomach contents included unknown arthropods (1 female), dragonflies (Odonata) (1 female), spiders (Arachnida) (1 female), teleost fishes (Pisces) (1 female, 1 male), and unknown vertebrate remains (2 females, 3 males).

Percentage of volume within stomach contents is presented in Table 1. Plant material was present in 33% of the stomach samples; whereas, unknown vertebrate parts represented 48% of the total volume of stomach contents. The remaining percentages were teleost fishes (7%), unknown arthropod remains (4%), Odonata, and Arachnida, each comprised 4% of total volume. Percentage frequency of occurrence for food items found in stomachs of both male and female *A. cartilaginea* is presented in Fig. 3.

A Pearson correlation test was used to determine the relationship between body size and the number of identifiable prey types in the stomach. No correlation ($r = -0.313$, $p = 0.412$) was observed between these contents and body mass, which indicated that larger turtles would not necessarily take a greater type of dietary resources. The largest mass of an individual that provided stomach contents was 4 kg. Turtles contained food from 1 to 3 prey items in their stomachs. A Pearson correlation test was also used to check for any relationship between body size and the volume of stomach contents. There was

Table 1. Stomach contents of 9 *Amyda cartilaginea* from Sarawak, collected between May 2004 to April 2005.[a]

Food item	Males $n = 5$		Females $n = 4$		Total $n = 9$		Total % volume
	n	%	*n*	%	*n*	%	
Plants	4	100	3	60	7	77	33
Unknown Arthropoda	0	0	1	20	1	11	4
Odonata	0	0	1	20	1	11	4
Arachnida	0	0	1	20	1	11	4
Pisces	1	25	1	20	2	22	7
Unknown Vertebrata	3	75	2	40	5	55	48

[a] Also see Fig. 3.

no correlation ($r = –0.518$; $p = 0.153$) between these 2 variables.

Of the 15 adult turtles obtained, 13 (87%) provided fecal samples. Five (83%) of the males provided fecal samples; whereas, 8 (89%) of the females provided fecal samples. Items found in fecal samples expressed in frequency of occurrence are summarized in Table 2. They consisted of plant material (100%), arthropod remains (62%), flies (Diptera) (7.7%), beetles (Coleoptera) (7.7%), ants (Hymenoptera) (23%), dragonflies (Odonata) (7.7%), snails (Gastropoda) (7.7%), teleost fish (Pisces) (69%), birds (Aves) (7.7%), and unknown vertebrates (84%). The percentage frequency of occurrence of prey items found in feces is summarized for both sexes pooled in Fig. 4.

The percentage of volume of total contents in the fecal samples is presented in Table 2. Plant material had a total percentage volume of 56%, followed by teleost fish at 16%. Unknown vertebrates followed at 14% of the total volume, with birds at 6%, miscellaneous unidentifiable arthropods at 4%, Hymenoptera at 1%, and Diptera, Coleoptera, Odonata, and Gastropods each at $< 1\%$. The small sample size does not permit statistical testing, but there seems to be no difference between the dietary preferences between males and females. All males and females that provided fecal samples contained plant materials. Three of the 5 males provided had insect parts; whereas, 5 of the 8 females had the same. Four of the 5 males provided unknown vertebrate material; whereas, 6 of the 8 females did the same. Interesting items recovered were bird feathers and bones found in a single adult male (field number KJ–126).

Similar to the results of the stomach contents, no correlation ($r = 0.005$, $p = 0.987$) was found between body mass and the number of identifiable prey items, which indicated that larger animals would not necessarily harvest a greater number of prey resource types. The largest animal that provided fecal matter for analysis was a female (field number KJ–31), which weighed 18 kg. Similar to the results of the stomach contents, there was no correlation ($r = –0.101$; $p = 0.742$) between body size and the volume of fecal matter.

Discussion. — Overall, there appears to be a dearth of studies on diets of wild softshell turtles (reviewed by Moll and Moll 2004), perhaps because of their large size (hence, difficulty in capture and manipulation) and their presumed rarity or at least low densities relative to other organisms of similar size. Collection of large sets of field data on *A. cartilaginea* is difficult in Sarawak, where this species is actively hunted for food by nearly all indigenous peoples, and the species may consequently have become trap shy or otherwise not easily captured. In addition, it appears to be an extremely elusive animal. Although it may not move great distances, it cannot be seen from above or in the water, which is highly turbid (19–50 cm at Loagan Bunut National Park, 29–39 cm at Balai Ringin), and hence, with low or no visibility, the carapace color being either brown or black, apparently does not bask, at least on land, and does not enter hoop nets. Therefore, obtaining large data sets for this species is difficult and expensive.

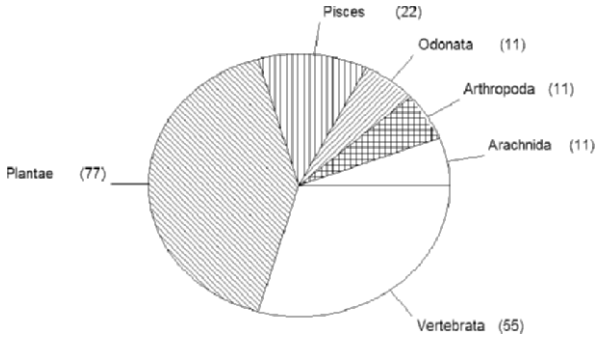

Figure 3. Total percentage frequency of occurrence food items in stomachs of both sexes of *Amyda cartilaginea.*

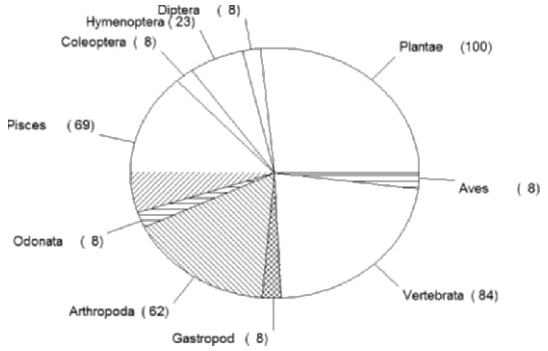

Figure 4. Total percentage frequency of occurrence food items in feces of both sexes of *Amyda cartilaginea.*

Table 2. Fecal sample of 13 *Amyda cartilaginea* from Sarawak, collected between May 2004 through April 2005.[a]

Food item	Males $n = 5$		Females $n = 8$		Total $n = 13$		Total % volume
	n	%	*n*	%	*n*	%	
Plants	5	100	8	100	13	100	56
Unknown Arthropoda	3	60	5	62.5	8	62	4
Diptera	0	0	1	12.5	1	7.7	<1
Hymenoptera	1	20	2	25	3	23	1
Coleoptera	1	20	0	0	1	7.7	<1
Odonata	0	0	1	12.5	1	7.7	<1
Gastropoda	0	0	1	12.5	1	7.7	<1
Pisces	5	100	4	50	9	69	16
Aves	1	20	0	0	1	7.7	6
Unknown Vertebrata	4	80	6	80	10	84	14

[a] Also see Fig. 4.

The dominant foods found in both stomach and fecal analysis were plant materials, followed by unknown vertebrates, which indicated that *A. cartilaginea* is an omnivore. Tendencies toward omnivory were reported for another softshell turtle, *Trionyx triunguis* (see Branch 1988; Ernst and Barbour 1989), and the tendency to feed upon carcasses is known from other softshell species (Das 1995, *Aspideretes gangeticus* and *Aspideretes hurum*; Taskavak and Atatür 1998, *Rafetus euphraticus*; Akani et al. 2001, *T. triunguis*). Identifiable terrestrial vertebrates were found in 1 fecal sample in this study. Several white feathers, along with bone and muscle tissue that belonged to an indeterminate bird were recovered from an adult male. These remains may have come from an intermediate egret (*Egretta intermedia*) because this is the only bird species found at Loagan Bunut National Park with completely white plumage (Laman et al. 2006). Turtles have been known to take birds off the water surface for food. Pryor (1996) observed a *Chelydra serpentina* taking a semi-palmated sandpiper (*Calidris pusilla*) from the water's surface. In this article, Pryor also reviewed other accounts of *C. serpentina* predation on birds: laughing gull (*Larus atricilla*), lesser yellowlegs (*Tringa flavipes*), and possibly Forster's tern (*Sterna forsteri*), all migratory sea or shore birds. Unspecified species of birds have also been recorded in the diet of *Trionyx triunguis* (Gramentz 2005).

It is possible that the our softshell turtle took the intermediate egret off the water surface as it was foraging. Intermediate egrets are migratory birds and come to the wetlands of Borneo seasonally and at Loagan Bunut when water and fish are plentiful in the lake. This may represent a larger variety of food sources for these turtles during certain periods. Whether *A. cartilaginea* is preying upon terrestrial birds, as well as animal carcasses, it could be considered a keystone species in its contribution to recycling nutrients in the peat swamp ecosystem.

Results of the Pearson correlation test indicated that there was no correlation between body size and the number and volume of identifiable prey items in the stomach. Although the sample size was small ($n = 9$), these results may be related to problems with the stomach-flushing technique. It would be a safe assumption that larger turtles consume larger amounts of food. However, this was not what we found in our study. In fact, the largest sample (0.7 mL) was provided by a turtle that weighed 1.1 kg. Stomach flushing success is often inversely proportional to the size of turtle, and the volume of water injected with a hand-operated syringe may be insufficient for large animals (Legler 1977; Demuth and Buhlmann 1997). Without dissection, it is impossible to know whether failure to dislodge food from the stomach means that the stomach is empty or the technique is ineffective.

Sexual and ontogenetic differences in diet in the species remain unknown and appear to be negligible, but the sample in this study did not permit appropriate statistical testing. In addition, data were unavailable for juvenile turtles. Such differences have been highlighted in other trionychids, such as *Apalone mutica* (Plummer and Farrar 1981). In several other freshwater turtle species, there is a well-documented shift from carnivory to herbivory with increasing body size: *Trachemys scripta* (Clark and Gibbons 1969; Hart 1983), *Emydura krefftii* (Georges 1982), *Pangshura tentoria* (Das 1985), and *Chelonia mydas* (Bjorndal 1985). Further sampling with a wide range of size classes is needed to answer these questions.

There was no correlation between body mass and the number of prey items, nor between body mass and the volume of fecal matter. The sample size did not permit quantitative analysis of any seasonal differences. Seasonal differences in rainfall and water availability may influence the diet and behavior of turtles, especially in places where streams dry out completely, and turtles may be forced to aestivate or perhaps burrow and remain underground in a quiescent state (Jensen and Das 2008). Changes in the water level may influence feeding behavior, especially in areas where many of the streams dry out completely. During these periods, turtles were found buried in mud. All 3 individuals located in mud were found at Loagan Bunut National Park in small streams, buried at 0.3- to 0.5-m depth in wet mud in previously submerged stream banks. A smaller number of turtles may have had insects in

their diet during the dry season because aquatic insects tend to breed during the wet season and, consequently, are less likely to be available during this period. Conversely, as the water levels decrease, fish are confined to smaller areas, which make them more easily harvestable prey for turtles. In addition, during the dry season, there is a large die-off of fish at Loagan Bunut; therefore, some of the fish intake may be from scavenging. Although the samples were small, these data are a noteworthy addition to the knowledge of the ecology of this turtle species.

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Hematological Parameters, Plasma Polypeptide Profiles, and Human Anti-Cancer Bioactivity of *Testudo graeca* and *Testudo horsfieldii* Plasma

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ABSTRACT. – We documented differential blood cell counts and plasma polypeptide profiles in *Testudo graeca* and *T. horsfieldii* raised in captivity in Lebanon, and investigated bioactivity of *T. graeca* plasma against a human colon cancer cell line (HCT-116). The percentage of red blood cells, thrombocytes, heterophils, basophils, lymphocytes, monocytes, and eosinophils did not differ significantly between the species during winter or spring. The total number of plasma polypeptides differed between the species, with 2 additional polypeptide bands in *T. horsfieldii*. Plasma from *T. graeca* significantly affected the percentage proliferation and percentage viability of human cancer cell line HCT-116.

Tortoises have become widely popular as pets but they are facing extinction (Behler 1997; Barzyk 1999; Altherr and Freyer 2000) because of the increased pet trade (Jenkins 1995; Yiming and Dianmo 1998) and habitat destruction, mainly by expansion of intensive agriculture (Shrestha 1997; Thirakhupt and Van Dijk 1997; Lagarde et al. 2002). Two different tortoise species (Testudinidae) were included in this study: *Testudo graeca*, the Mediterranean spur-thighed tortoise, and *Testudo horsfieldii*, the Horsfield's or steppe tortoise (Ernst and Barbour 1989).

The establishment of baseline data on hematologic parameters, such as differential blood cell count and serum polypeptides is of importance in programs that target the conservation of threatened tortoise species because serum polypeptide patterns are often used in the differentiation of various species, subspecies, and races of different animals (Lykakis 1971; Taylor and Jacobson 1981; Lawrence and Hawkey 1986). In addition, such hematologic data can help veterinarians and pathologists document any deviation from baseline data, which could help in disease diagnosis. Dangerfield et al. (1976) reported that lipoprotein patterns in serum or plasma samples taken from a wide range of mammals, birds, and reptiles differ among each other and are further greatly modified by oviparity. Moreover, establishing baseline data of the hematologic parameters and serum polypeptide profiles of tortoises facing extinction will aid in captive breeding programs and possible repatriation (Behler 1997; Barzyk 1999; Altherr and Freyer 2000).

Anticancer effects of various animal tissues were previously reported, including the shell of *T. graeca* (Bayazit 2004; Bayazit and Khan 2005). Furthermore, studies on human serum revealed the presence of the tumor necrosis factor, a class of cytokines known to induce apoptosis in transformed cells (Walczak et al. 1999). Such information led us to hypothesize a possible role of *Testudo* spp. plasma in defense against human cancer cells. In addition, traditional Chinese medicine attributes many benefits to various tissues derived from turtles and tortoises, including anticancer effects (Guynup 2005). Most of these beliefs have not been scientifically tested. This research hopefully will shed some light on this largely unexplored field.

To our knowledge, there is no previous work that established baseline data of plasma polypeptide bands and differential blood cell count in *T. graeca* and *T. horsfieldii*. This study provides the first such data. In addition, no study of bioactivity of *T. graeca* plasma against human

Table 1. Average percentage of red blood cells (RBC) and thrombocytes in 2 different tortoise species in their second year of life during winter and spring.[a]

	Average %			
	RBC		Thrombocytes	
Species	Winter	Spring	Winter	Spring
Testudo horsfieldii	93.4	91.8	4.6	6.2
Testudo graeca	93.6	91.7	3.7	6.6

[a] Number of tested individual tortoises per species = 9.

cancer cell lines has been reported; this study investigates the anticancer effect of different concentrations of *T. graeca* plasma on viability of human colon cancer cell HCT-116.

Methods. — Nine native *T. graeca* and 9 alien *T. horsfieldii*, all 1 year of age, were selected from a breeding farm in Lebanon (33.5°N, 35.5°E, 0 m altitude). Tortoises of each species were placed in a separate room (7.5 m^2), with daily monitoring of temperature and humidity. The respective average temperature and relative humidity were 17°C and 64% for the cold months (December–February), and 22.5°C and 75% for the warm months (March–April). Light and temperature were controlled through automatically timed neon lamps, providing 13 light hours a day (0500–1800 hours) throughout the experimental period, and infrared heat lamps that were kept on for 24 hours a day throughout the cold months (December–February). The room's cement floors were covered by wood shavings that were changed twice during the 6-month period of the experiment. In addition, some rocks provided a shelter for the tortoises.

Blood (1 mL) was sampled from the heart of the tortoises with a 27-gauge (0.4 mm × 13 mm) tuberculin needle that was introduced through soft tissues in the axillary region at the base of the forelimbs (Gandal 1958; Stephens and Creekmore 1983; Taylor and Jacobson 1981). Anesthesia was not used. Individual Wright-Giemsa–stained blood smears were prepared by using the method of Kolmer et al. (1959). A contact time of 1 minute between the stain and the blood cells was allowed before adding a buffer solution of monobasic potassium phosphate and dibasic sodium phosphate. The mixture was allowed to remain over the smeared cells for 4 minutes, before flooding with tap water for 30 seconds. A Giemsa stain was then poured and kept on each slide for 2 minutes, then washed with tap water and dried at room temperature. Differential cell counts of 10 randomly chosen fields of each individual smear were done at a magnification of ×400.

The remaining blood was injected into lithium heparin–containing microcapillary tubes, which were placed in an IEC MB centrifuge (DAMON/IEC Division, MA, USA) and spun at 12,700 × g for 5 minutes for separation of plasma from blood cells. The capillary tube was cut to remove the packed blood cells, and the remaining part of the capillary, which contained the plasma was sealed from both ends by crytoseal cement and preserved at −20°C. The preserved plasma was later used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to determine the banding pattern of serum polypeptides and their color intensities. In addition, a portion of the plasma was used to study the antiproliferation and cytotoxicity effect on human colon cancer cells (HCT-116).

The SDS-PAGE was used to compare the banding pattern of the plasma polypeptides of the 2 tortoise species and the intensity of each banded polypeptide (Barbour et al. 1989). A Mini PROTEAN II electrophoresis cell (Bio-Rad Laboratory, Richmond, CA, USA) was assembled according to the procedure described by the Bio-Rad Manual (Bio-Rad Laboratory, Richmond, CA, USA). A 12% separating gel was prepared and allowed to polymerize for 45 minutes, and the stacking gel polymerization for 60 minutes. Four individual plasma samples from each of *T. horsfieldii* and *T. graeca* were diluted 1:40 with the sample buffer, without the addition of SDS; whereas, the low-range marker of 14.4–97.4 kDa was diluted 1:40 with the stock sample buffer supplemented with SDS. A volume of 20 μL of each plasma sample and 5 μL of the marker were loaded on individual lanes of the stacking gel. Electrophoresis was performed at 60 mA for 45 minutes. The gel was stained with 0.1% Coomassie blue for 30 minutes with continuous shaking. The background was destained for 2 hours. Gels with banded polypeptides were photographed by using the Gel Doc system (Bio-Rad Laboratories, Inc, Hercules, CA, USA). This system allowed the determination of the density of the developed color in each band in pixel units.

A preliminary pilot study screened plasma of 5 randomly chosen individual *T. graeca* collected in winter

Table 2. Average percentage of white blood cells in 2 different tortoise species during winter and spring (see Table 1 for definition of abbreviations).[a]

	Average %									
	Heterophils		Basophils		Lymphocytes		Monocytes		Eosinophils	
Species	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter	Spring
T. horsfieldii	43.0	29.9	32.3	22.7	11.7	32.6	10.7	10.4	2.3	4.4
T. graeca	19.3	29.8	39.9	33.1	26.9	25.3	11.5	7.1	2.4	4.7

[a] Number of tested individual tortoises per species = 9.

Table 3. Average[a] intensity of different plasma polypeptide bands in 2 different tortoise species (see Table 1 for definition of abbreviations).

Banded polypeptide (kDa)	Average intensity of each polypeptide band in pixel units		SEM
	T. horsfieldii	*T. graeca*[b]	
26.1	179.0[c]	0.0[d]	—
29	200.7	208.8	8.43
42.1	130.4	92.8	14.5
44.5	126.4[c]	67.3[d]	14.22
66.3	111.2	117.5	14.56
68.3	131.3[c]	0.0[d]	—
98.4	158.2	160.6	17.14
102.8	157.7	198.5	19.83
103.8	167.6	209.4	21.96

[a] Average of 4 *T. graeca* and 4 *T. horsfieldii* plasma samples.
[b] A value of 0.0 indicates the absence of band.
[c,d] Average intensities-pixels in a row, followed by different superscripts are significantly different ($p < 0.05$).

and another 6 collected in spring for comparison of their antitumor bioactivities in human cancer cell lines. HCT-116 human colon cancer cells were grown in RPMI 1640 at 37°C in a 5% CO_2 incubator. The HCT-116 cell culture medium was supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum. The seeding of the HCT-116 cells in 6-well plates was at the level of 2.0×10^4 cells in 100-μL wells, incubated for 24 hours at 37°C before treatment with tortoise plasma. Triplicates of each tortoise plasma, diluted to 5%, were each applied in 5-μL volumes on the HCT-116 cell culture. The percentage HCT-116 cell proliferation and percentage viability were determined by using the respective Cell Titer 96 nonradioactive cell proliferation assay and the Cytotox 96 nonradioactive cytotoxicity assay, according to the instructions of the manufacturer (Promega Corp, Madison, WI, USA) (Moravec 1994). The proliferation assay is an MTT (Methyl Thiazolyl Tetrazolium)-based method that measures the ability of metabolically active cells to convert tetrazolium salt into a formazan product, and its absorbance is recorded at 570 nm. The Cytotox 96 assay quantitatively measures the lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis. Released LDH in culture supernatants is measured with a coupled enzymatic assay, which results in the conversion of a tetrazolium salt into a red formazan product, the absorbance of which is recorded at 490 nm. Control wells were included in each of the 2 assays by depriving the HCT-116 from contact with tortoise plasma, thus establishing 2 reference points of 100% proliferation and 100% viability, respectively. A second investigation compared the effect of 3 different concentrations of tortoise plasma (5%, 10%, and 15%) of 7 randomly chosen individual *T. graeca* on the average percentage viability of HCT-116 human colon cancer cells. Each concentration of each individual plasma sample was run in triplicate. Controls were included, and the viability assay was performed as described above.

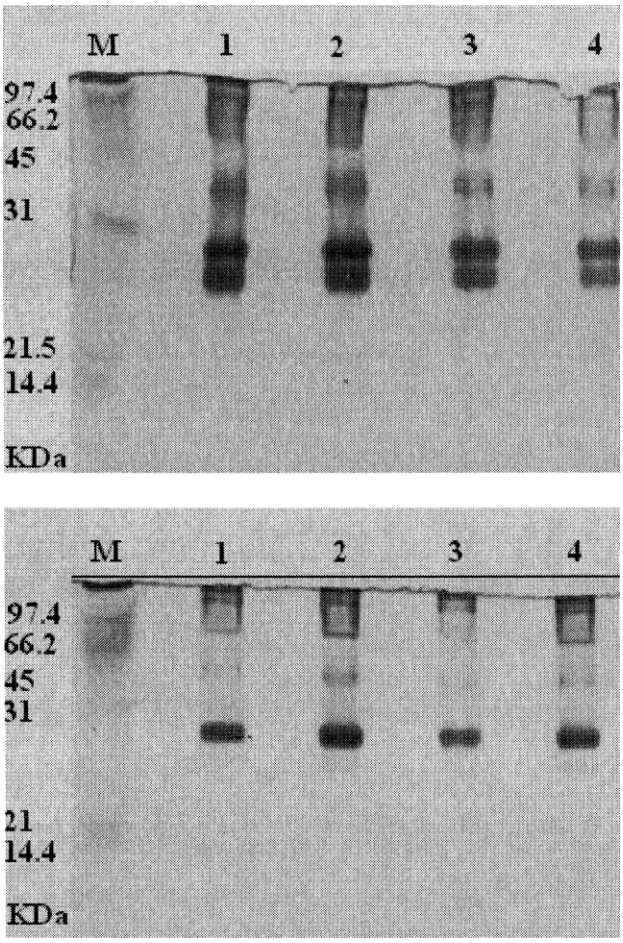

Figure 1. Comparison of plasma polypeptide profile of *Testudo horsfieldii* and *Testudo graeca*. Four individual plasma samples (lanes 1–4) of each species were run on individual lanes (20 μl/lane). The marker's protein bands and their molecular weight in KDa are on the left lane labeled "M".

Computerized statistical analysis was performed by using the MSTAT software (MSTATC, 1991, Michigan State University, MI, USA). The experimental design was of a 2-factor completely randomized structure. One-way analysis of variance of the different measured parameters was performed. Means were then separated by Duncan's multiple range test ($\alpha = 0.05$).

Results and Discussion. — Results showed no significant difference ($p > 0.05$) between *T. horsfieldii* and *T. graeca* in the percentage of red blood cells (RBC), thrombocytes, heterophils, basophils, lymphocytes, monocytes, and eosinophils in either winter or spring (Tables 1 and 2). Others (Taylor and Jacobson 1981; Lawrence and Hawkey 1986) have shown a drop in the RBC and leukocyte count at the beginning of hibernation (fall season), a period that is not included in this study. Future work should include the 4 seasons of the year and other years in the life of tortoises. The absence of a significant difference between the hematologic parameters obtained in this experiment could be related to the slight difference in the average temperature and relative humidity between winter (December–February) and spring (March–May). In

Table 4. Impact of the 5% concentration of *T. graeca* (TG) tortoise plasma collected in winter and spring on HCT-116 colon cancer cells (see Table 1 for definition of abbreviations).

		Effect of 5% plasma concentration on HCT-116 cancer cell line	
Season	Tortoise	% Proliferation[a]	% Viability[a]
Winter	TG1	46.37[c]	94.84[d]
	TG2	81.51[c,d]	98.59[b]
	TG3	95.91[c]	96.34[c]
	TG4	58.04[d,e]	96.11[c]
	TG5	99.96[c]	99.27[a]
	Average	76.36	97.03
Spring	TG1	86.05	72.68
	TG2	88.29	60.53
	TG3	92.04	64.88
	TG4	95.17	97.74
	TG5	86.49	95.16
	TG6	120.39	59.30
	Average	94.74	75.04

[a] Percentage proliferation and viability were obtained as averages of effects of triplicates of each tortoise plasma.
[b,c,d,e] Average percentages within a column that are followed by different superscripts are significantly different ($p < 0.05$).

addition, the average temperature in this study for the winter season was relatively high compared with other conditions mentioned in other studies, namely that of Lawrence and Hawkey (1986) who conducted their experiment in Britain where winter temperatures are significantly lower.

The respective intensities in pixel units of each of the 9 banded plasma polypeptides in *T. horsfieldii* vs. *T. graeca* are presented in Table 3. There were some statistical differences in the intensities of certain polypeptide bands when compared with the 2 species. The presence of 9 banded polypeptides in each of 4 plasma individual serum samples of *T. horsfieldii* (lanes 1–4) is shown in Fig. 1; however, each of the 4 individual plasma samples of *T. graeca* showed only 7 banded polypeptides (Fig.1, lanes 1–4). Plasma polypeptide profiles can be used as support for the differentiation of animal species (Lykakis 1971; Dangerfield et al. 1976). This new finding of the presence of 2 additional polypeptide bands in *T. horsfieldii,* namely, the 26.1 kDa and 68.3 kDa bands, enables us to add this character as differentiating this species from *T. graeca*. The existence of these 2 polypeptide bands in *T. horsfieldii* needs further investigation for its genetic basis, and the biological function of these 2 polypeptides.

Results of the study on the bioactivity of *T. graeca* plasma on HCT-116 human colon cancer cells showed that a concentration of 5% plasma collected in the winter induced significant differences ($p < 0.05$) in percentage proliferation and percentage viability of the targeted human colon cancer cells (Table 4). The range of cancer cell percentage proliferation induced by the 6 individuals of *T. graeca* widened to 46.37%–99.96%. A significant difference in impact of plasma on cancer cell line viability was also present in the winter; however, the range was narrower, at 94.84%–99.27%. In contrast, the plasma collected during spring did not show significant variation among the individuals regarding both the percentage proliferation and the percentage viability ($p > 0.05$) (Table 4). Moreover, the average impact of all individual plasma on percentage proliferation and percentage viability of cancer cells did not differ significantly between the winter and spring ($p > 0.05$).

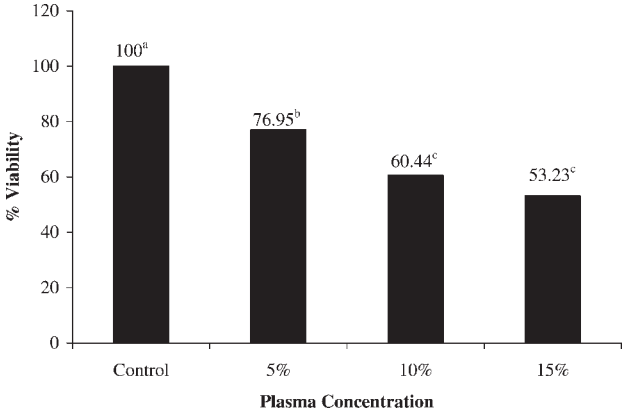

Figure 2. The average impact of 3 different plasma concentrations (Control, 5, 10 and 15%; x-axis) of 7 *T. graeca* plasma samples on the average % viability (y-axis) of HCT-116 colon cancer cell lines. Average percent viability columns with different letters, differ significantly ($p < 0.05$).

These preliminary data need further investigation to uncover the reason behind the significant differences among individuals of *T. graeca* regarding the impact of the plasma collected in the winter season on the percentage proliferation and percentage viability of human colon cancer cells. As the concentration of the plasma collected from 7 individual *T. graeca* increased from 5% to 15%, the percentage viability of the cancer cells was significantly reduced, from 76.95% to 53.23% ($p < 0.05$) (Fig. 2). This anticancer effect was clearly a plasma concentration-dependent relationship. Future investigations will target the anticancer-amplification mechanisms in tortoise plasma that could result in more significant reduction of percentage viability of HCT-116 human colon cancer cells and will also focus on identification of the anticancer factor present in certain individuals of *T. graeca.*

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Homing in the Red-Eared Slider (*Trachemys scripta elegans*) in Illinois

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ABSTRACT. – We recaptured 40 of 69 (58%) female red-eared sliders (*Trachemys scripta elegans*) that had been moved 3 km from Swan Lake to Long Lake in west-central Illinois. Twenty-one of 33 (63.6%) control turtles that were not moved from Swan Lake were recaptured. The similarity in recapture rates between control and experimental turtles suggests that statistically all translocated turtles returned to Swan Lake regardless of the actual percentage recaptured. We also found that males were less likely than females to make cross-river movements between backwaters of the Illinois River. The frequency of cross-river movements in sliders was small, only 86 recaptures (0.8%) out of a total of 10,373 recaptures made from 19,018 trapped turtles marked and released at our study areas between 1994 and 2006.

Many turtle species are known to exhibit homing behavior, i.e., return to their original site of capture after release elsewhere. Homing behavior includes natal homing during which turtles return to their natal sites from long distances (Bowen and Karl 1996; Valenzuela 2001). This type of homing behavior is an important feature in sea turtle life histories (Avens et al. 2003; Bowen et al. 2004). Sea turtles also migrate long distances between their foraging areas and nesting sites (i.e., beach fidelity) with considerable accuracy (Limpus et al. 1992; Tripathy and Pandav 2007). However, the degree to which homing ability is expressed has been shown to vary in freshwater turtles (e.g., Cagle 1944; Williams 1952; Freedberg et al. 2005; Smar and Chambers 2005). It has been suggested that homing may have important impacts on fitness and even on sex ratio (Freedberg and Wade 2001; Valenzuela and Janzen 2001). Generally turtles that demonstrate nest site fidelity must have some sort of homing ability in order

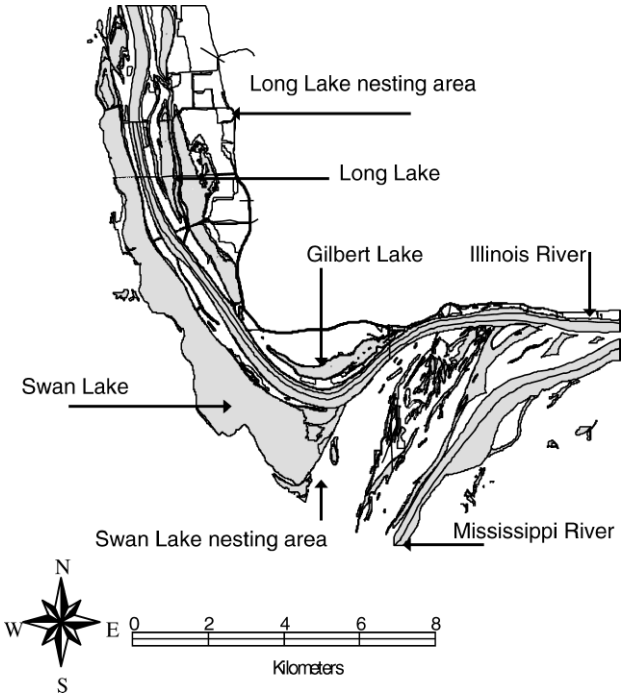

Figure 1. Map of the study area showing locations of Long Lake, Gilbert Lake, and Swan Lake.

to return to a preferred nest site. Nest site fidelity has been reported in many freshwater turtle species (e.g., Congdon et al. 1983; Jackson and Walker 1997; Freedberg et al. 2005) including the red-eared slider (*Trachemys scripta elegans*) (Tucker 2001).

Research conducted at the Great Rivers Field Station has focused on many aspects of the biology of the red-eared slider. An important finding has been that females from the western side of the Illinois River lay fewer but larger eggs than do those from habitats on the eastern side of the Illinois River (Tucker et al. 1998). The basis for this difference is unknown, but a reasonable hypothesis is that females in each area are responding to local edaphic conditions and not that they have evolved different optimal egg sizes. To test this hypothesis during 1999, we translocated females from the western side of the river to the eastern side. Female sliders from Swan Lake (western side of the river and producers of few but large eggs) were moved to Long Lake (eastern side of the river where females produce more but smaller eggs). Our goal was to expose Swan Lake turtles to Long Lake conditions and examine their reproductive output. Here we report the homing behavior of female sliders after being translocated from one side of the river to the other. The effect of turtle size on the degree of mobility among sliders is not understood. Although the influence of size on mobility is not a focus of this study, we did record and compare sizes during the course of the study.

Methods and Materials. — All translocated females ($N = 69$) were initially captured in late June near the end of the nesting season in 1999 at nesting areas near Swan Lake (Tucker et al. 1998). Some ($N = 40$) were recaptures of females previously marked and others were first captured in 1999 ($N = 29$). We recorded carapace length, plastron length, carapace height, carapace width, and gravid mass of captured turtles (Tucker et al. 1998). Holes were drilled in the marginal scutes to uniquely mark each individual. All gravid females had oviposition induced using oxytocin (Tucker et al. 1998, 2007). All were then kept captive for a minimum of 72 hours to reduce the incidence of false nesting (Tucker et al. 1995). All 69 females were then released into a ditch that nesting females from Long Lake routinely traversed to reach their nesting area (see Tucker 2000 and Fig. 1). An additional 33 females first caught in 1999 in Swan Lake were returned to Swan Lake to serve as a control group.

Individuals were recaptured in 2 ways. Most were recaptured while they were attempting to nest. Nesting areas (Fig. 1) were visited daily during the nesting season from 2000 to 2006 (see Tucker and Warner [1999] for details on nesting areas). Others were recaptured in aquatic traps set between 2003 and 2006. Baited hoop traps were used and were employed a minimum of 15 days per year at Swan Lake (2003–2006), Gilbert Lake (2003–2006), and Long Lake (2001–2006). We used a minimum of 10 traps in Swan Lake, Gilbert Lake, and Long Lake during each trapping period. Traps were also placed along the channel border of the Illinois River outside Swan Lake and outside Gilbert Lake. We used a minimum of 5 traps on each side of the river. This trapping could only be done occasionally when periods of stable river stages could be predicted. Rising river water levels could submerge channel border traps and cause drowning.

Cross-river movements were recorded during mark and recapture trapping conducted between 2001 and 2006 in order to compare them to homing in females. Each turtle was uniquely marked and then measured and weighed as above. All were released at their original capture location excepting the 69 females translocated from Swan Lake to Long Lake. Cross-river movements were recorded for males and females. The distance between Gilbert Lake and Swan Lake is 1 km compared to 3 km between Long Lake and Swan Lake (Fig. 1). The frequency that each sex made such movements was compared using chi-square analysis. Recapture frequency was also compared using chi-square analysis. Means were compared with the nonparametric Kruskal–Wallis test or the Wilcoxon two-sample test, which ever was appropriate. All statistics were performed using SAS for Windows (SAS Institute 2000).

Results. — Between 2000 and 2006, we made 2005 captures of nesting females at the nesting areas associated with Long Lake and 899 captures of nesting females at nesting areas associated with Swan Lake (Fig. 1). Trapping at Swan Lake from 2003 to 2006 resulted in 7035 captures of females; whereas, trapping at Long Lake from 2001 to 2006 yielded 8086 captures of females. The nesting area captures and trap captures resulted in recapture of 40 of the 69 females (58%) that we translocated to Long Lake and in recaptures of 21 of 33

Table 1. Descriptive statistics for female red-eared sliders (*Trachemys scripta elegans*).

	Recaptured translocated females ($N = 40$)		Translocated but not recaptured ($N = 29$)		Control females ($N = 33$)	
Variable[a]	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Carapace length	231 (13.4)	196–257	231 (15.1)	191–266	229 (14.1)	205–259
Plastron length	215 (12.5)	184–244	215 (14.1)	183–244	216 (13.0)	189–245
Carapace width	170 (7.8)	151–187	171 (9.7)	145–195	168 (9.4)	153–189
Carapace height	92 (6.8)	71–112	91 (7.3)	78–105	89 (6.6)	75–104
Mass	1906 (327)	1260–2650	1889 (335)	1160–2790	1753 (308)	1260–2470

[a] Measures of length, width, and height are in millimeters and mass is given in grams.

control females (63.6%). The proportion of recaptured females among those translocated and the controls did not differ ($\chi^2 = 0.46$, df = 1, $p = 0.6060$). All of the recaptures of translocated females were made at Swan Lake nesting areas ($N = 32$) or in traps set in Swan Lake ($N = 8$). No translocated females were recaptured at Long Lake. Recaptured translocated females ($N = 40$), translocated females that were not recaptured ($N = 29$), and control females that were not translocated ($N = 33$) did not vary in any measure of size (Table 1; Kruskal–Wallis tests for each trait, $p > 0.05$). Size details for these 102 females are in Table 1. During these same years we made a total of 16,294 captures of male turtles (7932 at Long Lake and 9267 at Swan Lake). Males and females caught at Swan Lake were larger than males and females caught at Long Lake (Table 2; Wilcoxon two-sample tests for each trait, $p < 0.0001$).

Based on our trapping results, males were less likely than females to make cross-river movements between Gilbert Lake and Swan Lake. Only 31 males (out of 16,294 captures of males) vs. 55 females (out of 18,025 captures of females) were observed crossing the Illinois River between Gilbert Lake and Swan Lake. The female bias was statistically significant ($\chi^2 = 6.70$, 1 df, $p = 0.0097$) indicating that more females than males made this trip. In contrast, 22 turtles (9 males and 13 females) that were initially marked in Swan Lake between 2000 and 2006 were later recaptured in Long Lake. No turtles that were initially marked in Long Lake were subsequently recaptured in Swan Lake.

Discussion. — Homing ability is clearly well developed in adult female red-eared sliders (*Trachemys scripta elegans*). Not only did they return to their original capture sites, but statistically, essentially all of them did. The lack of difference in recapture rates between translocated females and the control group strongly suggests that nearly all translocated turtles returned to Swan Lake. We note that in previous studies of homing, no reference group or control was used. Consequently, without a control group, it is not possible to estimate the proportion of translocated turtles that homed. Instead, only the proportion of those recaptured can be determined. No inferences can be made about those turtles not recaptured without a control group. Ours is the first homing study of turtles to use a control group.

This high rate of return is likely not due to turtles including both lakes in their home ranges. During the course of the study, we found only 22 turtles that had moved from Swan Lake to Long Lake out of 19,018 captures that we made. Turtles originally marked in Long Lake were never recaptured in Swan Lake. Management practices could explain this one-way movement. In Long

Table 2. Descriptive statistics for male and female red-eared sliders (*Trachemys scripta elegans*) caught at Long Lake and Swan Lake.

	Long Lake females			Long Lake males		
Variable[a]	*N*	Mean (SD)	Range	*N*	Mean (SD)	Range
Carapace length	10,091	189 (51.3)	30–296	7931	157 (41.7)	36–261
Plastron length	10,091	177 (47.0)	28–268	7931	143 (36.0)	34–240
Carapace width	10,091	142 (34.2)	30–218	7931	118 (26.3)	34–191
Carapace height	10,091	73 (20.4)	15–123	7931	57 (14.3)	16–101
Mass	10,002	1193 (711.8)	6.2–3100	7930	637 (410.5)	10.5–2300
	Swan Lake females			Swan Lake males		
Variable[a]	*N*	Mean (SD)	Range	*N*	Mean (SD)	Range
Carapace length	7934	207 (38.9)	43–276	9266	189 (29.1)	62–260
Plastron length	7934	193 (35.5)	41–257	9266	171 (24.6)	60–231
Carapace width	7934	155 (26.3)	41–210	9266	138 (18.4)	56–187
Carapace height	7934	79 (15.8)	20–120	9266	68 (10.3)	20–120
Mass	7915	1388 (586.2)	40–3100	9264	953 (346.0)	40–2420

[a] Measures of length, width, and height are in millimeters and mass is given in grams.

Lake, water is not drawn down, but portions of Swan Lake are dewatered each year. We frequently see turtles crossing from Swan Lake to the Illinois River during such events. Apparently most of these turtles move to the nearest available backwater (Gilbert Lake, Fig. 1). Thus it seems unlikely that our Swan Lake females would have been in Long Lake before. It should also be noted that 32 of these females not only returned to Swan Lake but they also returned to the same nesting area from which they had been removed. Nest site fidelity has been previously demonstrated for slider females from Long Lake (Tucker 2001).

These same management practices may also cause the size differences between turtles from Swan Lake and Long Lake. The basis for the difference is that young turtles (2–4 years old) are much less common among turtles trapped in Swan Lake; whereas, these young turtles make up a large portion of the turtles trapped in Long Lake. Underlying differences in recruitment rates may explain the apparent difference in age structure, and we continue to study recruitment at both lakes. Habitat differences between the 2 lakes are many. Swan Lake does not have submersed aquatic vegetation, and a levee borders one side of the lake. The levee has little cover for juvenile turtles, and the lake has large numbers of predatory birds that line the banks and moist-soil units when portions of the lake are drawn down. In contrast, Long Lake has extensive cover in the form of emergent vegetation and shrubs that extend into the lake. Moreover, submersed aquatic vegetation is present in parts of the lake. Finally, Long Lake is not dewatered on a regular basis.

Similar to map turtles (Freedberg et al. 2005), female sliders have well developed homing ability. Nest site fidelity is also well developed in slider females (Tucker 2001). Well developed homing ability and nest site fidelity were used as evidence of natal homing by Freedberg et al. (2005). It could be possible that that natal homing is part of the life history strategy of the slider because sliders and map turtles have similar patterns of homing and nest site fidelity.

Our experiment concentrated only on adult females, and we did not attempt to translocate juveniles or males. Males and young turtles may or may not be as adept at homing as adult females. Smar and Chambers (2005) found that male stinkpots (*Sternotherus odoratus*) were more likely than females to home after translocation. Adult sliders are mobile, and the Illinois River is not a barrier as we recaptured turtles that moved from one side of the river to the other. Interestingly, we found females more likely to make this journey than males.

Generally, male sliders are known to be more mobile than females possibly because increased mobility may enhance mating opportunities (Morreale et al. 1984; Parker 1984). Moreover, male sliders have larger home ranges than do female sliders (Schubauer et al. 1990). Our observations do not necessarily contradict these findings. We did not determine home ranges and males may have larger home ranges in our study area as well. Nonetheless, one other study of sliders found that males make fewer long-distance moves (Bodie and Semlitsch 2000).

Moreover, the cross-river movements we observed are extrapopulational (Gibbons et al. 1990). We suspect that females move in response to low water levels. The water level in Gilbert Lake is often lowered during summer months to encourage growth of moist-soil vegetation. Females seem to respond to draw downs by seeking deeper water sites and may be more likely to leave than males. Females return each year during the nesting season due to their nest site fidelity (Tucker 2001) making them more likely to be captured on both sides of the river (Tucker 2001).

The frequency of cross-river movements in sliders should be kept in perspective. They represent only 86 recaptures (0.8%) out of a total of 10,373 recaptures made from 19,018 trapped turtles marked and released at our study areas between 1994 and 2006. Sliders seem not to be nearly as mobile as riverine turtles such as map turtles (Freedberg et al. 2005). These map turtles identified as *Graptemys kohnii* returned from as far away as 6 km within 1–2 days of translocation. The female sliders that we studied also were capable of rapid movements. One individual was found making a false nesting attempt (see Tucker et al. 1995) at the Swan Lake nesting area within 48 hours of its release some 3 km away. This recapture was not counted in our totals, but it demonstrated that females could rapidly return from Long Lake.

The ease with which sliders can cross the Illinois River stands in contrast to the barrier presented by other rivers to turtle dispersal. Bodie and Semlitsch (2000) found that turtles, including sliders, were apparently unable to cross the Missouri River. Turtles, however, regardless of species inhabiting backwater lakes or riverside lentic habitats, cannot be assumed to be limited in their mobility by lotic habitats. Only comprehensive trapping surveys on both sides of rivers can determine their impact on movements of aquatic turtles.

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Role of Trapping in Detection of a Small Bog Turtle (*Glyptemys muhlenbergii*) Population

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ABSTRACT. – We used data collected from a small but increasing population of bog turtles to evaluate the surveying methods for detecting such a small population and the level of trapping effort required to reliably detect it. Trapping with unbaited traps detected this small population more reliably and with less investigator-time investment than did widely used visual and manual search techniques. This population was most easily detected in May and June, but we still needed more than 9000 trap-hours of trapping effort to reach a 95% likelihood of capturing one of the turtles present. Based on our results, we suggest a standard of 20 traps per hectare for 20 days (a 20–20 rule) as an effort level that has a very high likelihood of detecting such small but potentially viable populations.

The bog turtle, *Glyptemys muhlenbergii,* formerly assigned to the genus *Clemmys* (Holman and Fritz 2001), is declining throughout its range as a result of habitat destruction and alteration. Bog turtles are naturally vulnerable to loss of small wetlands because they occur in discrete and isolated populations (Ernst 2001). The turtle is now protected as threatened or endangered in all 12 US states of occurrence. In 1997, the northern population was federally listed as threatened and the southern population was listed as threatened by similarity of appearance (USFWS 1997a, b).

Surveys are being conducted throughout the range of the bog turtle to assess potential sites for various reasons. Natural areas inventories are being conducted by state agencies to assess the quality of habitat and the presence of rare species. Nongovernmental entities, e.g., Project Bog Turtle, conduct surveys to identify new sites and to determine the conservation status of the turtles throughout the south. Developers also initiate surveys in sites scheduled for projects to satisfy the regulatory obligation for mitigation, to establish the need for buffer areas, or justify project modifications. Since the bog turtle was federally listed in 1997, there has been an increased demand for surveys to meet regulatory obligations.

When potential bog turtle sites are slated for development, surveyors are called upon to make a determination as to the presence or absence of these animals. In the experience of ABS, these requests come at all times of year and are often purported to be urgent. The searching-probing method, also known as the visual search method, is the most common method of detecting bog turtles (Smith 1994; Herman 1994). This method is described as a phase 2 survey in the Guidelines for Bog Turtle Surveys in the Bog Turtle Northern Population Recovery Plan (USFWS 2001). Researchers follow turtle tracks, look for basking turtles, and seek concealed animals by groping in the mud with their hands or probing with wooden sticks. Phase 2 surveys are conducted between 15 April and 15 June, and compliance with the protocol requires intensive investment of search time under defined weather conditions. Trapping is an additional method of surveying for bog turtles. Fahey (1992, 1998) first used this method in the south in the late 1980s. Most recent researchers have used trapping to some degree, usually in sites that look promising or in historic sites that have not yielded turtles by using the phase 2 survey method. Sites under permit review that are surveyed without finding evidence of bog turtles may then be destroyed.

Demographic data remain largely unavailable for most sites, but indications are that most populations are small (Table 1). In addition, bog turtles are difficult to detect because they are small, rare, cryptically colored, and behaviorally inconspicuous. It is probable that some sites that do contain turtles, especially those with small populations, are being overlooked. The question then arises: how and when should potential sites be examined to minimize the chances of overlooking a potentially viable population of bog turtles?

Under one commonly used evaluation system (Klemens 1993; USFWS 2001), sites occupied by as few as 6–10 turtles are regarded as "potentially viable" or "fair" if other habitat factors are favorable. To avoid the loss of such small populations through poorly timed or insufficiently intensive search, it is important to investigate the likelihood of detecting such small populations with commonly used search techniques. We drew upon data collected in the course of a habitat-use study of a small population of bog turtles of not less than 7 animals to explore this issue. By using data collected from 1993 to 2001, we compared the effectiveness of 2 common search techniques (searching-probing vs. trapping), determined the best season for such survey work, compared the detection success at this site with that for a larger population, and estimated the level of trapping effort required to reliably detect this small group.

Methods. — The Sites. — S3 is a complex of wetlands on a privately owned farm in the Piedmont region of North Carolina, approximately 450 m (1500 ft) above sea level. There are 3 very small and distinct wetland patches contained within a diameter of about 0.5 km and separated by a gravel-and-cobble dominated stream. Together they amount to 1.025 ha of wetlands suitable for bog turtles. Survey work at the site began in May of 1993. Based on extensive survey effort, we are confident that the population size, including both adults and hatchlings recruited during the study, was 7 turtles in 1993 and 13 in 2001. According to the habitat and population factors presented in Klemens (1993), the S3 site ranks as "possibly viable" among 3 ranks: "viable", "possibly viable", or "nonviable". These categories were later revised to "good", "fair", and "poor" (USFWS 2001).

G2 is also on a privately owned farm located in the Piedmont in North Carolina. Although configured somewhat differently, the size of the wet area suitable for bog turtles is similar to that of S3. A study conducted during an overlapping time period (Green 1994, 1995, 1996) resulted in 48 turtles being captured at the site (Herman 1999), and the actual population is undoubtedly larger. G2 ranks as "viable" or "good" on the Klemens assessment scale.

Capture Methods. — Turtles were captured by using visual searches, probing, and handmade unbaited traps. Traps were shaded, placed in shallow water, and checked every 24–48 hours. Captured bog turtles were permanently marked and released at the point of capture. Traps were used in S3 for slightly more than 175,000 trap-hours over the 8-year period (1 trap-hour = 1 trap in site for 1 hour). In 1993 and 1994, traps were distributed across the 3 patches of S3. After 1995, traps were placed in areas where turtles were expected, and areas that had been nonproductive in the past were avoided. Visual search times were estimated and recorded at the end of each visit. Time invested by the workers tending traps was also estimated.

Table 1. Most bog turtle populations are small.

Study	State	Population size
Buhlmann et al. 1997	Virginia	$<$ 20 individuals in most sites
Herman and Tryon 1997	North Carolina	$\leq$ 35 turtles per ha in most sites
Ernst 2001	Not specified	usually $<$ 50 individuals

Data Analysis. — For comparison of the phase 2 techniques with trapping, a turtle yield value was computed for each calendar month of effort at the site as the number of turtles captured per hour of investigator time. The calendar year was divided into 2-month categories to achieve a statistically useful number of yield values for each time category. Two-way analysis of variance (ANOVA) was used to examine the effect of time period and capture technique.

For a comparison of trapping success between sites, we aggregated trap hours on a monthly basis:

Monthly trap-hours = number of traps present × hours of trap effort for month

We compared effectiveness of trapping turtles in S3 (N = 7–15) and G1 (N = 48+). Because of inter-year variation in turtle activity from weather conditions, only periods of simultaneous study were compared. Trapping occurred on both sites simultaneously during 4 months (June 1993, May 1994, June 1994, and June 1995). A Mann-Whitney U-test was used to compare trapping success, expressed as turtles/trap-hour, across the 2 sites.

To evaluate the intensity of trapping effort needed to detect the small S3 population, we computed the cumulative trap-hours that led up to each successful trapping event. We began either from the initial placement of traps in the site for the first capture or from the previous trap capture in the site. Sometimes two or more turtles were captured on the same day in the same or different traps. In these cases, all were assigned cumulative trap-hours from the most recent capture, rather than treating the second and subsequent turtles as requiring zero trap-hours for their capture. We transformed these data logarithmically to achieve a normal distribution and used the transformed data to calculate a cumulative trap-hour effort that would encompass 95% of this distribution. We performed this analysis on the whole data set and on a subset of captures that occurred within May and June during the 1993–1995 time frame in case improved trap placement late in the study influenced the time required.

We also derived an alternative estimator of the trapping effort necessary to detect the S3 population through an inverse prediction procedure. By using May and June data only, we modeled the number of turtles captured during a calendar month as a function of the trap-hours of trapping effort in the site during that calendar month. We then obtained a 95% confidence interval for the trap-hours needed to capture a single turtle. Because capture of one animal would suffice to confirm presence at the site, the upper bound of the 95% confidence interval represents the trapping effort required for this probability of detecting the population.

The R system (R Development Core Team 2005) was used for all statistical operations described.

Results. — Technique and Season at S3. — Trapping was much more effective than visual searching for detecting bog turtles at S3 (Fig. 1). Locating turtles by phase 2 methods at the S3 site required an average of 27.4 search hours per turtle captured for the years 1993 to 1997 and 2000. No turtles were captured this way in 1998 or 1999. Effects of both bimonthly category ($p = 0.006$) and capture technique ($p < 0.001$) were highly significant, and the trapping proved to be a more effective investment of the investigator's time. May and June were the most rewarding times to search for turtles by any method at this site.

Locating bog turtles remained difficult at S3 during May and June compared with G2 (Fig. 2). Trapping effort per turtle capture at S3 site for the period of overlap averaged 2600 trap-hours per turtle trapped as opposed to an average of 176 trap-hours at the G2 site. Average trapping success during the 4 months of concurrent effort was more than an order of magnitude lower at S3 (3.05×10^{-4} turtles/trap-hour) than at G2 (6.38×10^{-3} turtles/trap-hour). Despite the small sample size, the intersite difference was statistically significant ($p = 0.021$).

Detection of the Small Population at S3. — The number of turtles trapped in a calendar month at S3 depended on the trapping time invested, as well as the month of the year. Turtles were not captured during every month of active work at the site. During 7 of the 23 months in which trapping was conducted, no turtles were caught; in 3 of those months $>$ 1000 trap-hours were invested without success. Analysis of the cumulative trap presence times leading up to each S3 capture indicated that the average (log-transformed) capture occurred at 1820 trap-hours overall. In the subset of May and June effort early in the study, the corresponding value was 1850 trap-hours. The upper bound of the range into which a new capture was 95% likely to fall throughout the study was 9286 trap-hours across the entire study, or 10,079 trap-hours for the May-June subset of the data from early in the study.

The inverse prediction approach applied to May and June data yielded a much larger estimate, 18,645 trap-hours (Fig. 3). Two points that represented May and June of 1999, a drought year, had a very large effect on this analysis. Eliminating these 2 points from the inverse prediction yielded an investment of 10,550 trap-hours for a

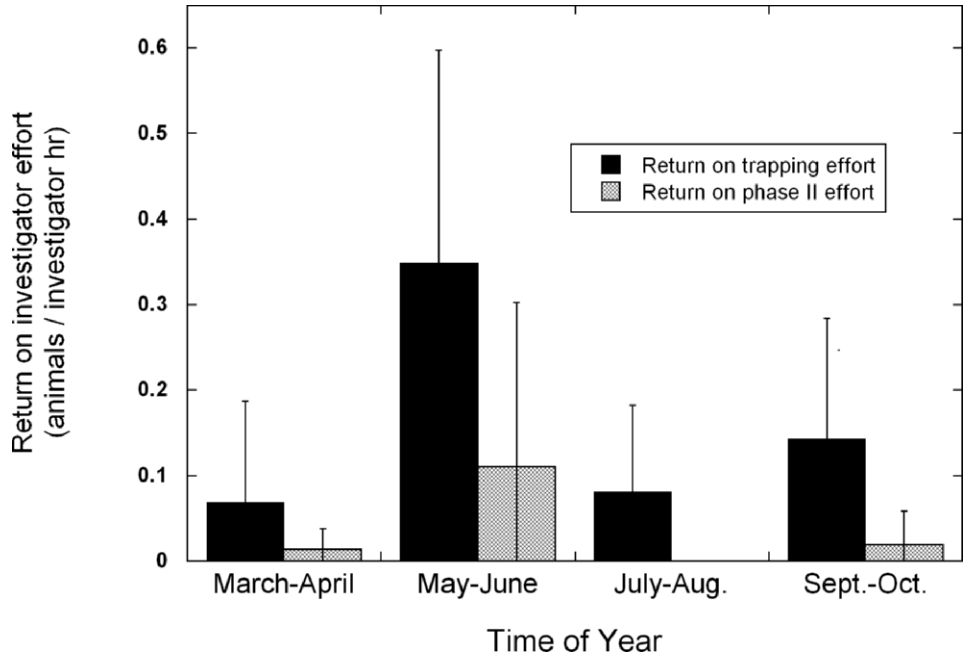

Figure 1. Monthly yield of turtles per investigator hour of effort at S3 is shown as a function of season and search method. Solid bars represent trapping results; whereas, hatched bars show results of phase 2 survey techniques. Error bar = SEM.

95% likelihood of detection: a value in reasonable accord with those derived from the cumulative trap-presence calculations.

Discussion. — Search Method and Season. — Our findings agree with earlier studies (Lovich et al. 1992; Ernst et al. 1994; Carter 1997; USFWS 2001) that the optimal time for finding turtles is in the spring. In all seasons, trapping yielded more turtles per hour of investigator time spent in the site than did searching-probing. S3 was a difficult site in which to find bog turtles by these phase 2 methods, compared with other locations where such data have been collected. Rates reported in the literature for visual search success range from 0.27 to 0.6 turtles per investigator hour, with seasonal variation in success (Table 2). The highest hand yield for any month at the S3 site was approximately 0.1 captures per investigator hour, a lower rate than that found by either Lovich et al. (1992) or Smith (1994). We are not aware of any published data on captures per hour of investigator effort for the trapping method.

Detectability: 20–20 Rule. — Turtles in small populations present a special difficulty in detectability.

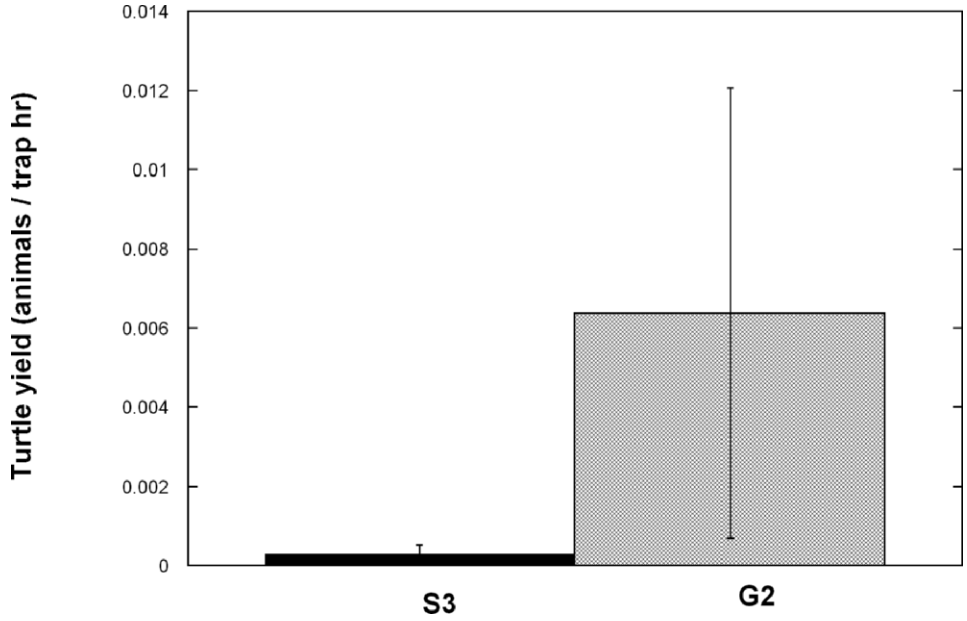

Figure 2. Monthly turtle yields in turtles per trap-hour are compared for 4 months of overlapping trapping effort at a "fair" (S3, solid) and a "good" (G2, hatched) site. Error bar = SEM.

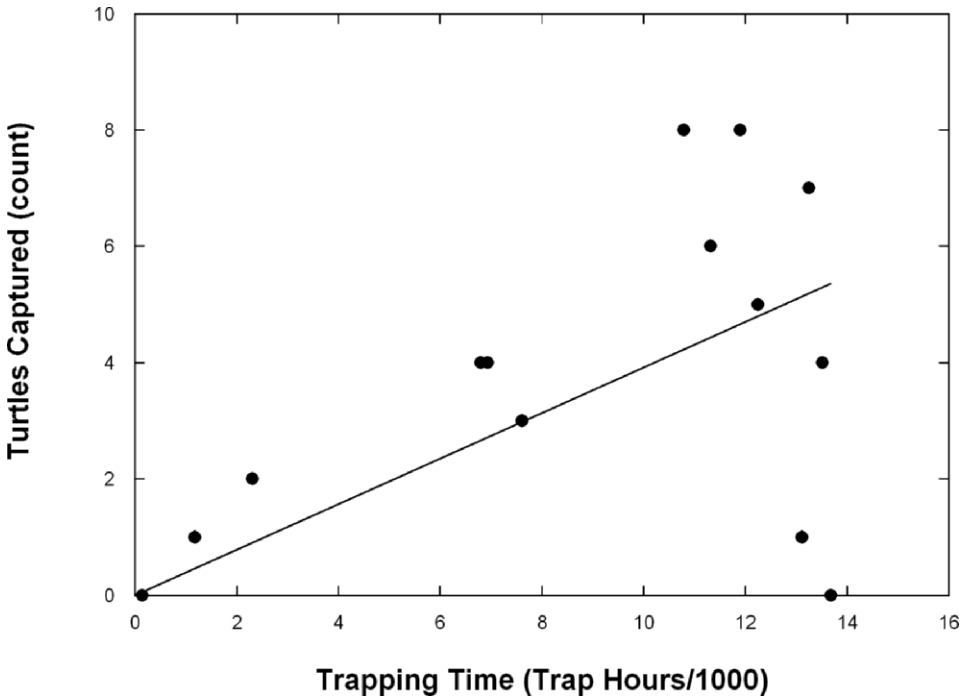

Figure 3. The number of bog turtle captures during May and June trapping attempts are plotted as a function of trapping time in thousand trap-hours invested during that calendar month. The regression line is forced through the origin.

Results of this study suggests that small populations of turtles in potential sites may easily go undetected when using only visual searches. According to our cumulative trap-hour analysis, a minimum of roughly 9000 trap-hours per ha of trapping effort in May and June is needed to have 95% confidence of detecting a population of bog turtles of the size reported in this study (N ≈10). The considerably larger value obtained from our inverse prediction approach was heavily influenced by 2 data points (Fig. 3), which represented May and June of 1999, a very dry year. Omission of these points may be justified if we assume that no responsible surveyor would attempt to evaluate an unknown potential site in a year as dry as 1999 was in the North Carolina Piedmont. The approximately 10,500 trap-hour value obtained by eliminating these outliers appears reasonable because the cumulative trap-hour analysis could not include unsuccessful efforts. Because the effort levels derived from these 2 methods are roughly equivalent to setting 20 traps for 20 days in May and June, we call this our "20–20 rule". The influence of 1999 on the data suggests that detecting the S3 population during a drought would require much more than a 20–20 effort.

Detection failures represent a serious problem for bog turtle conservation because such a large proportion of the known sites are occupied by relatively few animals. Herman and Tryon (1997) determined that only 23% of the sites in the southern range of the turtle could be considered "viable". Viability was defined in that study as a population of 30 or more individuals with sufficient core habitat and evidence of reproduction or recruitment. When using a similar suite of characteristics as outlined in Klemens (1993), USFWS (2001) combined occurrences into population assessment sites (PAS) for evaluation and concluded that only 38% of ranked sites (104 of 275) in the northern range of the turtle could be considered "good". By using the same PAS method of aggregating sites to assess the populations in the south, Herman (2003) concluded that 50% ranked as "good".

By any reckoning, the sites that can be considered "good" or "viable" deserve protection. Because the majority of the known bog turtle sites rank below this level, identification and protection will also be needed for some of the "possibly viable" or "fair" sites. Caughley and Gunn (1996) provide convincing evidence that adequate conservation plans require the preservation of

Table 2. Effectiveness of visual searching varies widely among published reports.

Study	Turtles per investigator hour	Time of year
Lovich et al. (1992) North Carolina	0.27	Mar–Dec
Smith (1994) Maryland	0.6[a]	late Apr to early Jul
Somers and Mansfield-Jones (present study)	0.04	Mar–Oct

[a] Approximate rate, calculated from data reported on time to first capture.

as many individuals as possible within the greatest possible area of high-quality, protected habitat. A wide variety of criteria have been used to evaluate bog turtle sites (Collins 1990; Klemens 1993; Herman and Tryon 1997; USFWS 1997b, 2001), and, by most such assessments, the S3 site would be ranked as "possibly viable" or "fair". Such sites may warrant consideration for conservation or site enhancement, especially if they are part of a meta-population. The population increase at S3 over the course of this study (Somers 2000) suggests that conservation efforts for populations of this size may be worthwhile.

Any question of the appropriate conservation effort for a marginally viable site is irrelevant if a turtle population that uses the site goes undetected. Detection failures at S3 present convincing evidence that sites with small populations can be and most likely are being overlooked when using only phase 2 survey techniques. Such oversights can result in irreversible habitat degradation if sites are under permit review, scheduled for alteration or project development. It will take further assessment that involves known and suspected bog turtle sites in both the northern and southern ranges, and which have the widest possible array of site characteristics, to determine if the 20–20 rule outlined above will be fully adequate to identify potentially viable populations that would otherwise have gone undetected.

Current guidelines (USFWS 2001, 2006) do not require phase 3 surveys (trapping); however, the revised guidelines (USFWS 2006) allow for the recommendation of additional surveys (phase 2 and/or phase 3 surveys) if phase 2 surveys are negative and habitat is of sufficient quality and quantity. Results of our study suggests that traps should be used to assess marginal sites within the turtle's range that show hydrology and vegetation characteristics capable of supporting bog turtles if searches fail to produce bog turtles or signs of bog turtles. Searching-probing surveys that fail to confirm the presence of bog turtles in a site slated for development should be considered inconclusive and trapping in May and June should be required.

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