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HEALTH ASSESSMENT OF FREE-RANGING ALLIGATOR SNAPPING TURTLES (*MACROCHELYS TEMMINCKII*) IN GEORGIA AND FLORIDA

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ABSTRACT: The Alligator Snapping Turtle (*Macrochelys temminckii*) is a large freshwater turtle endemic to river systems that drain into the Gulf of Mexico. Turtle populations were sharply reduced by commercial harvest in the 1970s and 1980s; however, the species has yet to be protected under the Endangered Species Act. While anthropogenic stressors such as habitat fragmentation and degradation and illegal capture continue to threaten populations, the degree to which disease may be contributing to any decline of the Alligator Snapping Turtle is unknown. Data were collected from 97 free-ranging Alligator Snapping Turtles in nine waterways in Florida and Georgia from 2001 to 2006. Eleven turtles were captured more than once, resulting in a total sample pool of 123. Reference ranges were established for complete blood count, plasma biochemistry values, trace metals (mercury, zinc, copper, lead, and arsenic), and nutrient parameters (vitamins A, E, D, and selenium). Variations by capture location, sex, and season were detected and likely resulted from external factors such as habitat and diet. Turtles sampled in one location were positive for tortoise herpesviral antibodies. Blood mercury values also differed among populations. This study provides justification for the use of these long-lived aquatic turtles as biologic monitors of the health of local freshwater ecosystems.

Key words: Alligator Snapping Turtle, hematology, *Macrochelys temminckii*, mercury, plasma biochemistry, wildlife health.

INTRODUCTION

The Alligator Snapping Turtle (*Macrochelys temminckii*), a species easily identified by its sharp claws, spiked carapacial ridges, and hooked beak, is North America's largest freshwater turtle. It is nocturnal and aquatic, and its range is restricted to Gulf of Mexico drainages from the Florida Panhandle to eastern Texas (Pritchard, 2006). Individual turtles have a very limited range, and drainages define genetically distinct populations (Roman et al., 1999). Previous studies have shown that the life-history strategy of Alligator Snapping Turtles requires high adult survivorship to compensate for predation of nests and low hatchling survival and that no level of commercial harvest from the wild is sustainable (Reed et al., 2002; Schlaepfer et al., 2005).

The Alligator Snapping Turtle has survived decades of predation by humans. In the 1970–80s, Alligator Snapping Turtle populations declined over much of their range due to local and international demand for turtle meat and turtle soup (Pritchard, 2006). Subsequently, state laws put an end to legal, large-scale commercial harvest. However, thousands of Alligator

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Snapping Turtles, both farmed and illegally wild-caught, have continued to be exported outside the USA for commercial use (Schlaepfer et al., 2005). In June 2006, the species was listed in Appendix III of the Convention on International Trade in Endangered Species (CITES). This listing status strengthens regulations related to the trade in Alligator Snapping Turtles; however, responsibility for protecting the species currently remains at the state rather than the federal level (US Fish and Wildlife Service and Department of the Interior, 2005). In addition to ongoing exploitation for food and the pet trade, habitat degradation and destruction pose serious threats to this species (Dickerson et al., 1999; US Fish and Wildlife Service and Endangered Species Office, 1999; Gibbons et al., 2000).

Because they are highly aquatic, nonmigratory, top-trophic-level predators, Alligator Snapping Turtles could serve as a good bioindicator species for the health of freshwater stream ecosystems in the southeastern USA. Their entire life span, which can rival that of humans (Gibbons, 1987), is spent in one stream drainage. Other turtle species, such as the Diamondback Terrapin (*Malaclemys terrapin*) and the Common Snapping Turtle (*Chelydra serpentina*), have previously been identified as sentinel species in particular for mercury levels in Atlantic coast estuaries (Golden and Rattner, 2003). In order for the Alligator Snapping Turtle to be used in this capacity, periodic population health assessments will be needed. However, published data on the health of Alligator Snapping Turtles, especially information on infectious disease exposure and nutritional and baseline blood parameters, do not exist in the primary literature. This absence of information on the health of Alligator Snapping Turtles in particular and wild chelonians in general is hindering efforts to fully assess the status of these species and the ecosystems in which they live.

In order to establish a physiologic database and help determine the roles that disease and environmental contamination may play in the conservation of Alligator Snapping Turtles, we conducted an epidemiologic assessment of the health of free-ranging Alligator Snapping Turtles with a focus on populations in Florida and Georgia. We determined reference ranges for the complete blood count, plasma biochemistry, and nutrient parameters (vitamins A, E, and D) and investigated exposure to environmental contaminants (organochlorines, organophosphates, and polychlorinated biphenyls), trace elements (mercury, copper, zinc, lead, arsenic, and selenium), and infectious diseases (herpesvirus, iridovirus, mycoplasma, West Nile virus) important in threatened reptile populations.

MATERIALS AND METHODS

Data collection

Samples were collected from 97 free-ranging Alligator Snapping Turtles at nine sites in northwest Florida and southwest Georgia over the course of 5 yr (2001–06; Fig. 1). Of the 97 individuals, eleven animals were captured more than once, resulting in 123 total captures. Study sites in Georgia included: Spring Creek (30°94′71.9″N, 84°74′54.1″W; $n=58$), Pataula Creek (31°46′60.3″N, 85°1′22.9″W; $n=23$), Ichawaynochaway Creek (31°17′38.4″N, 84°29′55.6″W; $n=5$), and the Chattahoochee River (georeference data were not collected; $n=1$). In Florida, turtles were trapped in the Santa Fe River (29°55′11.5″N, 82°25′19.4″W; $n=14$), Econfina Creek (30°45′16.3″N, 85°53′23″W; $n=9$), Yellow River (30°41′08.4″N, 86°38′51.6″W; $n=6$), Escambia River (30°35′11.0″N, 87°7′5.1″W; $n=5$), and the Shoal River (30°25′2.2″N, 86°20′42.7″W; $n=2$). Trapping occurred in June, July, August, and October 2001; August and September 2002; August and September 2003; April, May, and August 2004; May and August 2005; and April 2006. All sites were not trapped in each time period. Turtles were captured using fish-baited 1.2-m-diameter single-opening hoop traps (Champlin Net Company, Jonesville, Louisiana, USA). Traps were placed upstream from microhabitats favored by turtles, such as log jams or undercut banks. Because Alligator Snapping Turtles are predominantly nocturnal, all traps were set in the late

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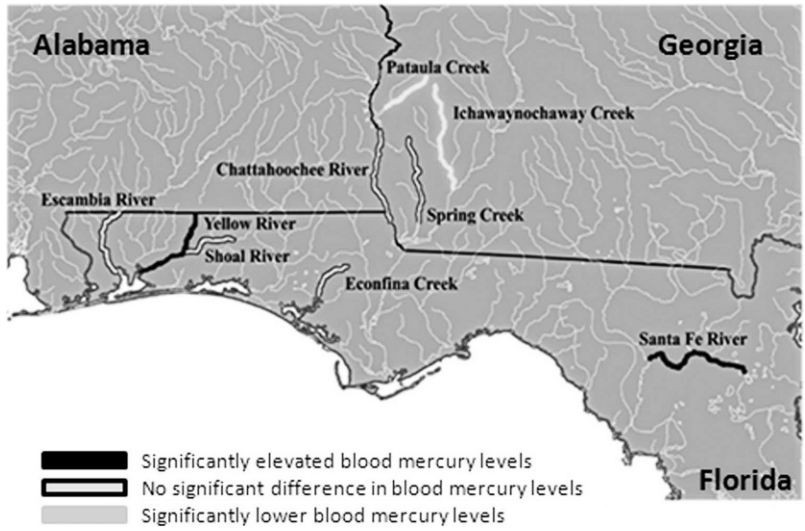


FIGURE 1. Waterways in Georgia and Florida: Blood mercury levels were tested for 74 Alligator Snapping Turtles between 2001 and 2006.

afternoon and retrieved the following morning. All trapping was conducted by personnel from the Georgia Department of Natural Resources and the Florida Fish and Wildlife Conservation Commission.

All turtles were given a complete physical examination, under manual restraint, immediately upon retrieval from traps. Body weights and morphologic measurements, including head width and carapace and plastron length and width, were determined using a 200-lb. Pelstar Viking Scale and 100-cm Haglof calipers. Body weights were measured to the nearest pound and later converted to kilograms. Methods to approximate age, including counting scute annuli, have not proven to be accurate (Powders, 1978); therefore, age classification was limited to juvenile and adult based on body weight and size at sexual maturity (Dobie, 1971). External sexually dimorphic characteristics were used to determine the sex of mature animals. Mature males have longer, thicker tails and grow to a much larger body size than females. Another reliable feature is the position of the cloacal opening, which lies beyond the carapace in males (Mader, 2006; Pritchard, 2006). At Spring Creek, where trapping occurred on multiple occasions, a permanent identification system was established, which consisted of small holes drilled in specific marginal scutes of the carapace. Additionally, passive integrated transponder (PIT) tags were placed intramuscularly in the lateral aspect of the tails of these animals.

Approximately 20–25 ml of whole blood (<5 ml/kg body weight) were drawn via 22-gauge needle and vacutainer from the dorsal coccygeal vein of each animal. The blood was collected in both lithium heparinized and trace metal–free blood-collection tubes (Muro et al., 1998; Redrobe and MacDonald, 1999). Four smears were prepared from blood collected in the lithium heparinized tubes within 10 min of collection. These were dried and fixed in methanol. Slides were later stained with Wright-Giemsa, examined microscopically for parasites, and used for differential white blood cell counts. Blood tubes were stored in a cooler until centrifuged within 4 hr of collection. Plasma was transferred to 1.8-ml cryotubes and immediately placed in liquid nitrogen until transfer into a −70 C freezer. Leeches found on the skin and shells were removed with hemostats and preserved in 70% alcohol. Leech identification was performed at the American Museum of Natural History, Division of Invertebrate Zoology (M. Siddall, New York, New York, USA).

Sample analysis

A small amount of heparinized whole blood was transferred to a microhematocrit tube and centrifuged to measure packed cell volume. Plasma total solids were measured by refractometer. Manual methods were used to obtain total leukocyte estimates using previously established techniques, because reptiles' nucleated thrombocytes and erythrocytes rule out the use of automated cell counts (Mader, 1996). White blood cell counts were calculated

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in the field using the Eosinophil Unopette™ (Becton-Dickson, Rutherford, New Jersey, USA) hemocytometer technique (Cray and Zaias, 2004). All cell counts were performed by the same veterinarian (T. Norton). White blood cell differentials and parasite examination were performed at White Oak Conservation Center (Yulee, Florida, USA) by a single veterinary technician (M. Oliva).

Biochemistry profiles were performed on plasma samples using standard dry-slide determinations with a Kodak 700XR™ chemical analyzer by the Department of Pathology, University of Miami (Miami, Florida, USA). Vitros Performance Verifiers I and II (Ortho Diagnostics, Rochester, New York, USA) were used to test the chemistry analyzer. Results from the solutions, representing high and low controls, were compared to Vitros ranges. The analyzer was also calibrated with Ortho Vitros reagents. Any "flags" or errors were fully investigated. The following blood values were measured: glucose, sodium, potassium, carbon dioxide, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, phosphorus, calcium, uric acid, creatine phosphokinase (CPK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), lipase, amylase, cholesterol, glucose, and gamma-glutamyl transferase (GGT). Protein fractions were evaluated by plasma electrophoresis (Zaias and Cray, 2002). Plasma vitamin D (25-hydroxycholecalciferol; $n=52$) was measured by radioimmunoassay at Boston University using previously described methods (Chen et al., 1990). Plasma vitamin A (retinol; $n=58$) and vitamin E (α tocopherol; $n=60$) concentrations were determined by high-performance liquid chromatography (HPLC) at the Wildlife Nutrition Laboratory, Wildlife Conservation Society (Bronx, New York, USA; Catignani and Bieri, 1983) and at Mystic Aquarium Department of Research and Veterinary Services (Mystic, Connecticut, USA) using the same methodology.

Total mercury, lead, arsenic, and cholinesterase levels were measured in whole blood; organochlorines (OCs), polychlorinated biphenyls (PCBs), copper, and zinc were measured in plasma. The detection limit was 0.05 ppm for all metals, except mercury, which was 0.01 ppm. All toxicology assays were performed by the same toxicologist (R. Poppenga; 2001–05 samples at the College of Veterinary Medicine Toxicology Laboratory, University of Pennsylvania [UP], Philadelphia, Pennsylvania, USA; 2006 samples at the California Animal Health and Food Safety Laboratory [CAHFS], University of California, Davis, California, USA). Total lead was measured in whole blood by atomic absorption spectrophotometry (AAS). Mercury and arsenic were measured in whole blood by hydride-generation inductively coupled plasma–optical-emission spectroscopy (ICP-OES) at the CAHFS or by AAS at UP. Copper and zinc were measured in plasma either by ICP-OES at CAHFS or inductively coupled argon plasma–mass spectrometry (ICP-MS) at UP. Each metal was measured along with blanks, standards, and standard reference materials (SRMs) containing known amounts of the metals of interest. Respective spike levels and percent spike recoveries were: lead, 2 ppm and 98% recovery; mercury, 2 ppm and 96% recovery; arsenic, 2 ppm and 99% recovery; copper, 25 ppm and 101% recovery; and zinc, 25 ppm and 117% recovery. Detection of the metals within established SRM ranges by the various methodologies indicated the consistency of results irrespective of the method used for analysis.

Cholinesterase levels in whole blood were measured by an enzyme kinetic, spectrophotometric method (Tor et al., 1994). Briefly, the cholinesterase in the sample hydrolyzes acetylthiocholine to thiocholine. The thiocholine then reacts with 5,5′-dithio-bis (2-nitrobenzoic acid) to produce a yellow color, which is then measured at 405 nm by a microplate ultraviolet (UV) reader. Lyophilized human serum was run with each sample batch for quality-control purposes.

Organochlorines (OCs) and polychlorinated biphenyls (PCBs) were analyzed in plasma samples by gas chromatography with electron-capture detection (GC-ECD). Plasma samples were prepared by first extracting a sample aliquot into an organic solvent, followed by a Florisil solid-phase extraction column sample cleanup prior to analysis. Suitable blank and fortified plasma samples were run with the samples to ensure the accuracy and precision of the extraction and analytical procedures. The presence of any OC or PCB identified by GC-ECD was confirmed by gas chromatography–mass spectrometry (GC-MS). The recoveries for the OCs and PCBs ranged from 70% to 100%.

In order to pilot infectious disease testing, plasma samples from nine turtles from Pataula Creek were submitted to the Mycoplasma Research Laboratory for analysis with an enzyme-linked immunosorbent assay (ELISA) for the detection of *M. agassizii*–specific antibodies (Schumacher et al., 1993). Samples from 11 turtles, also from Pataula Creek, were submitted for evaluation of tortoise herpes virus antibodies by ELISA (Origgi et al., 2001). As a means of assessing exposure to iridovirus, an indirect ELISA was used to determine the

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presence of anti-ranavirus antibodies in samples from 17 turtles from Spring Creek and five turtles from the Santa Fe River using the technique established by Johnson et al. (2007; Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA). Additionally, samples from 39 turtles from Florida and Georgia were tested by a plaque-reduction neutralization test (PRNT) for exposure to West Nile virus (Lindsey et al., 1976). Mycoplasma, tortoise herpesvirus, and iridovirus diagnostics were performed at the College of Veterinary Medicine, University of Florida (Gainesville, Florida, USA). West Nile virus diagnostics were performed at the Southeastern Cooperative Wildlife Disease Study (Athens, Georgia, USA).

Statistical analysis

The distribution, mean, median, range, kurtosis, and skewness were determined for each hematologic and plasma biochemistry value (Minitab, version 14.0, State College, Pennsylvania, USA). Blood samples from apparently healthy turtles with both packed cell volume $<10\%$ and total solids <1.5 mg/dl were considered to be of poor quality and were discarded ($n=2$). Kruskal-Wallis one-way analysis of variance (KWANOVA) was used to evaluate differences in blood parameters by capture site (Daniel, 2005). A nonparametric procedure was chosen for comparison because there were multiple locations with small sample sizes. Multiple pair-wise comparisons for values that varied by location were calculated using Dunn's test for nonparametric procedures and Sigmastat (Systat Software, Inc., Point Richmond, California, USA; Zar, 1996). Two capture sites where fewer than five turtles were sampled (Chattahoochee River, Shoal River) were not included in the KWANOVA. The Mann-Whitney *U*-test was used to compare blood values by sex and season of capture (Daniel, 2005). Capture seasons were classified either as spring or summer based on the calendar definition. Reference values for each parameter were calculated using 95% confidence intervals (SPSS, version 14.0, Chicago, Illinois, USA), and statistical significance was defined as $P \leq 0.05$ (Daniel, 2005).

RESULTS

Physical examination

Over a period of 5 yr, 123 Alligator Snapping Turtle samples were collected in 21 trap nights. Fifty-seven animals were identified as male, and 31 animals were identified as female; sex of the remaining 35 turtles could not be determined either because the turtles were immature and did not yet display obvious dimorphic characteristics of either sex or because the external traits of young males and adult females were too similar to distinguish. All juvenile turtles in this study weighed 9.1 kg or less, with a carapace length $\leq$35 cm. Adult females weighed 15.9 ± 0.54 kg (mean $\pm$ S.E.), with a range of 8.4 to 20.4 kg. Adult males weighed 26.2 ± 1.2 kg, with a range of 8.6 to 45.4 kg. Males were significantly larger than females in all physical parameters measured, including body weight ($P<0.001$), carapace length ($P<0.001$) and width ($P<0.001$), plastron length ($P<0.001$) and width ($P<0.001$), and head width ($P<0.001$). Mean body weights did not differ significantly among sample sites ($P=0.155$).

The most frequently observed abnormal physical exam findings were: shell abnormalities, including regions of extreme wear and cracked or broken scutes ($n=19$); fresh or healed wounds consistent with trauma ($n=15$); missing portions of the tail ($n=12$), beak ($n=1$), or claw ($n=1$); and leech infestation ($n=46$). Miscellaneous abnormalities included an enlarged, asymmetrically shaped hard palate in the oral cavity of one animal and a palpable mass cranial to the front edge of the plastron in another.

Additionally, numerous turtles were found to have a yellow coloration of the head and, occasionally, the carapace ($n=18$). All of the turtles with this coloring were large, weighing $\geq$17.2 kg with carapace length $\geq$46.1 cm. Although we did not attempt to age the turtles, because the yellow individuals were larger, we suggest this is a change that occurs as the turtles become older.

Clinical pathology

The biochemistry and hematologic reference ranges (95% CI) presented in Tables 1 and 2 were established based

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TABLE 1. Plasma biochemistry values for free-ranging Alligator Snapping Turtles (*Macrochelys temminckii*) from eight waterways in Georgia and Florida, 2001–06 (male and female, adult and juvenile).

Measurement[a]	*n*	Mean±SE	Reference range (95% confidence interval)
Glucose (mg/dl)	103	48.0±2.3	43.5–52.6
Sodium (mmol/l)	104	128.2±0.53	127.2–129.2
Potassium (mmol/l)	103	3.7±0.05	3.6–3.8
Phosphorous (mg/dl)	103	3.7±0.10	3.5–3.9[b]
Uric acid (mg/dl)	104	0.74±0.046	0.65–0.83
Calcium (mg/dl)	103	9.3±0.18	8.9–9.7[c]
Total protein (g/dl)	106	4.2±0.13	3.9–4.4
Albumin (g/dl)	106	0.75±0.028	0.70–0.81
Globulins			
Alpha 1 (g/dl)	105	0.13±0.0077	0.12–0.15
Alpha 2 (g/dl)	106	0.76±0.035	0.69–0.83
Beta (g/dl)	106	1.6±0.056	1.5–1.8
Gamma (g/dl)	106	0.91±0.049	0.81–1.0
A:G ratio	106	0.22±0.0050	0.21–0.23
AST (U/l)	103	167.7±8.3	151.2–184.2
ALT (U/l)	102	23.0±1.5	20.0–25.9
LDH (U/l)	102	5,716.9±280.0	5,161.5–6,272.2
GGT (U/l)	104	7.5±0.24	7.1–8.0
CPK (U/l)	104	398.5±93.0	213.9–583.0
Cholesterol (mg/dl)	104	57.2±3.4	50.4–64.0[d]
Triglycerides (mg/dl)	103	48.7±7.0	34.8–62.6[e]
Amylase (U/l)	102	299.1±8.6	282.0–316.1
Lipase (U/l)	103	10.0±1.3	7.4–12.6
Bile acid (μmol/l)	106	0.19±0.032	0.13–0.25
BUN (mg/dl)	104	24.8±1.8	21.3–28.3
Creatinine (mg/dl)	104	0.30±0.012	0.27–0.32
BUN:creatinine ratio	104	96.1±7.6	81.0–111.2
Total CO_2 (mmol/l)	104	32.5±1.1	30.4–34.5

[a] AST=aspartate aminotransferase, ALT=alanine aminotransferase, LDH=lactate dehydrogenase, GGT=gamma-glutamyl transferase, CPK=creatine phosphokinase, CO_2=carbon dioxide, BUN=blood urea nitrogen.

[b] Reference ranges are given for all sex/ages combined; specific female 95% CI: 3.7–4.7.

[c] Reference ranges are given for all sex/ages combined; specific female 95% CI: 10.2–12.1.

[d] Reference ranges are given for all sex/ages combined; specific female 95% CI: 59.8–96.2.

[e] Reference ranges are given for all sex/ages combined; specific female 95% CI: 70.9–158.3.

on plasma and heparinized whole-blood samples from apparently healthy Alligator Snapping Turtles, of all ages, from eight different watersheds ($n \leq 106$). Reference ranges were not separated by age because no significant differences in blood values were detected between juvenile and adult turtles. The data were not broken down by sex for reference ranges because they did not vary for most parameters and did not appear to be clinically relevant, except for those values indicated.

Statistical analysis revealed differences by capture location, sex, and season for several blood values. Biochemistry variables that significantly differed by capture location included: glucose ($P=0.009$), sodium ($P<0.001$), phosphorus ($P=0.010$), uric acid ($P<0.001$), albumin ($P<0.001$), triglycerides ($P=0.032$), AST ($P<0.001$), ALT ($P=0.031$), CPK ($P<0.001$), GGT ($P<0.001$), amylase ($P=0.007$), lipase ($P<0.001$), BUN ($P=0.002$), and creatinine ($P=0.006$). A pattern was not detected for most locations, although turtles sampled from Pataula Creek had the highest levels of glucose, total protein, albumin, bile acids, and triglycerides, as well as the highest levels of the enzymes AST, ALT, and LDH. Blood values from

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TABLE 2. Hematology values for free-ranging Alligator Snapping Turtles (*Macrochelys temminckii*) from eight waterways in Georgia and Florida, 2001–06 (male and female, adult and subadult).

Measurement[a]	*n*	Mean±SE	Reference range (95% confidence interval)	*n*[b]	Absolute number (Mean±SE)	Reference range (95% CI)
PCV (%)	101	23.2±0.6	22.1–24.4	–	–	–
Total solids (mg/dl)	–	–	–	102	3.69±0.10	3.49–3.89
Total WBC ($\times 10^3$/μl)	–	–	–	90	12.3±0.57	11.2–13.4
Heterophil count ($\times 10^3$/μl)	106	40.2±1.6	37.0–43.3	90	4.716±0.322	4.076–5.355
Lymphocyte count ($\times 10^3$/μl)	106	23.3±1.3	20.7–25.9	90	3.101±0.230	2.645–3.556
Azurophil count ($\times 10^3$/μl)	106	5.6±0.3	5.0–6.2	90	0.660±0.050	0.560–0.760
Eosinophil count ($\times 10^3$/μl)	106	22.3±1.1	20.2–24.5	90	2.556±0.141	2.276–2.836
Basophil count ($\times 10^3$/μl)	106	8.6±1.1	6.5–10.8	90	1.273±0.232	0.813–1.733

[a] WBC=white blood cell, PCV=packed cell volume.

[b] Absolute WBC count was not available for 16/103 turtles sampled; therefore, absolute cell number was not calculated for all individuals.

turtles sampled from the Santa Fe River were among the lowest in numerous parameters, including glucose, total protein, albumin, bile acids, potassium, and phosphorus.

Compared with males, female Alligator Snapping Turtles had significantly higher calcium levels ($P<0.001$); the mean value was 11.1 mg/dl for females and 8.4 mg/dl for males. Phosphorus ($P=0.010$), cholesterol ($P=0.018$), and triglycerides ($P=0.001$) were also significantly higher in females than males.

When comparing biochemistry values by capture season, differences were detected in plasma total protein ($P=0.001$) and albumin ($P=0.003$) levels, and levels were significantly higher in summer than in spring. The enzymes AST ($P=0.007$), ALT ($P=0.019$), LDH ($P=0.021$), and GGT ($P<0.001$) also followed this pattern and were higher in late summer than early spring. Creatinine ($P=0.006$), BUN ($P=0.014$), and potassium ($P=0.042$) significantly differed by season. All were elevated in the late summer but remained at the high end of reference ranges established here. Among the metabolites included in the chemistry panel, only uric acid ($P=0.033$), amylase ($P=0.013$), and lipase ($P=0.003$) were significantly higher in the spring than in the summer.

Hematologic values were also compared among capture season and location. Seasonal variation was detected in the basophil ($P=0.001$) count in Alligator Snapping Turtles, as well as in PCV ($P=0.043$) and total solids ($P=0.040$); again, values were higher in summer than in spring. Location differences were also detected in PCV ($P=0.001$), total solids ($P=0.006$), basophils ($P<0.001$), and eosinophils ($P=0.012$). There was no apparent pattern among these location differences. Thrombocytes were determined to be adequate in all turtles evaluated.

The mean value for plasma vitamin A (retinol) in this study was 0.19±0.03 μg/ml, and values ranged from 0.0040 to 1.039 μg/ml (Table 3). The mean concentration of vitamin E (α tocopherol) was 4.14 μg/ml, and values ranged from 0.80 to 13.49. Turtles sampled from the Escambia and Yellow Rivers in Florida had the lowest plasma concentrations of vitamin A, with mean values of 0.019±0.004 μg/ml and 0.076±0.04 μg/ml, and the lowest concentrations of vitamin E, with means of 1.17±0.23 μg/ml and 2.55±0.66 μg/ml, respectively. A mean value of 5.192±0.60 ng/ml was measured for vitamin D (25-hydroxycholecalciferol). Vitamin levels did not differ significantly between males and females.

Arsenic was detected at low levels consistent with background concentra-

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TABLE 3. Selected metals and vitamins detected in whole blood, serum, or plasma of free-ranging Alligator Snapping Turtles (*Macrochelys temminckii*) from eight waterways in Georgia and Florida, 2001–06.

Measurement	*n*	Mean±SE	Reference range (95% CI)
Zinc[a] (ppm)	54	6.56±0.28	6.01–7.12
Copper[a] (ppm)	55	0.342±0.02	0.302–0.381
Arsenic[b] (ppm)	47	0.086±0.008	0.070–0.101
Selenium[c] (ppm)	47	0.454±0.02	0.406–0.499
Vitamin A[a] (retinol; μg/ml)	58	0.192±0.03	0.138–0.246
Vitamin E[a] (α tocopherol; μg/ml)	60	4.14±0.39	3.36–4.92
Vitamin D[a] (25-hydroxycholecalciferol; ng/ml)	52	5.192±0.60	3.99–6.40

[a] Selected metal or vitamin measured in plasma.

[b] Selected metal or vitamin measured in whole blood.

[c] Selected metal or vitamin measured in serum.

tions in freshwater biota (Table 3; Eisler, 1988). Copper, zinc, and selenium were also detected at what appeared to be low levels; however, information on acceptable levels of metal exposure in turtles is scarce (King et al., 1996).

Pathogen exposure

West Nile virus antibody titers were negative in all 39 turtles assayed. In addition, all blood samples ($n=9$) tested for antibodies to *Mycoplasma agassizii* were negative. All 22 plasma samples evaluated for antibodies to iridovirus via ELISA were below the positive cutoff value, indicating a lack of exposure to ranavirus. The prevalence of antibodies to herpes viral antigens (HV1976, HV4295/7R/95) was 63.6% (7/11) in turtles captured at Pataula Creek, Georgia, in 2001. Because this assay was not available after initial testing, samples from other areas were not tested for herpesvirus exposure.

Mercury

Mercury was detected (≥0.010 ppm) in 93% of the blood samples ($n=74$), and concentrations ranged from 0.010 ppm to 1.840 ppm. Males exhibited significantly higher levels of blood mercury than females ($P=0.03$). Juveniles did not significantly differ in mercury level when compared with adults ($P=0.12$). Geographic variation was found in mean blood mercury concentrations ($P<0.001$; Figs. 1, 2). The highest mean values were found in turtles from Florida rivers, particularly the Santa Fe River (0.603 ppm) and Yellow River (0.625 ppm), and the lowest mean values were detected in Georgia at Pataula Creek (0.092 ppm) and Spring Creek (0.133 ppm). A significant difference ($P=0.002$) was also detected in blood mercury concentration by season: higher levels were found in turtles in September than in April. Serial blood mercury concentrations were measured for two turtles from Spring Creek, Georgia. The first turtle weighed 18.1 kg when captured in August 2001 and 17.2 kg at capture in August 2002. At first sampling, the turtle's blood mercury level was 0.20 ppm, and at recapture, it was 0.13 ppm. It weighed 22.0 kg when trapped in May 2004; however, mercury measurements were not available from this capture. The second turtle, a male, displayed mercury values of 0.19 ppm in August 2001 (weight = 29.0 kg), 0.12 ppm in August 2002 (26.8 kg), and 0.13 ppm in April 2006 (27.2 kg). Some variation in weight measurements likely results from fluctuations in bladder or gastrointestinal tract volume.

Additional contaminants

Lead was not detected in blood sampled from turtles ($n=75$). Plasma samples ($n=44$) were negative for organochlorine contaminants, including alpha chlordane

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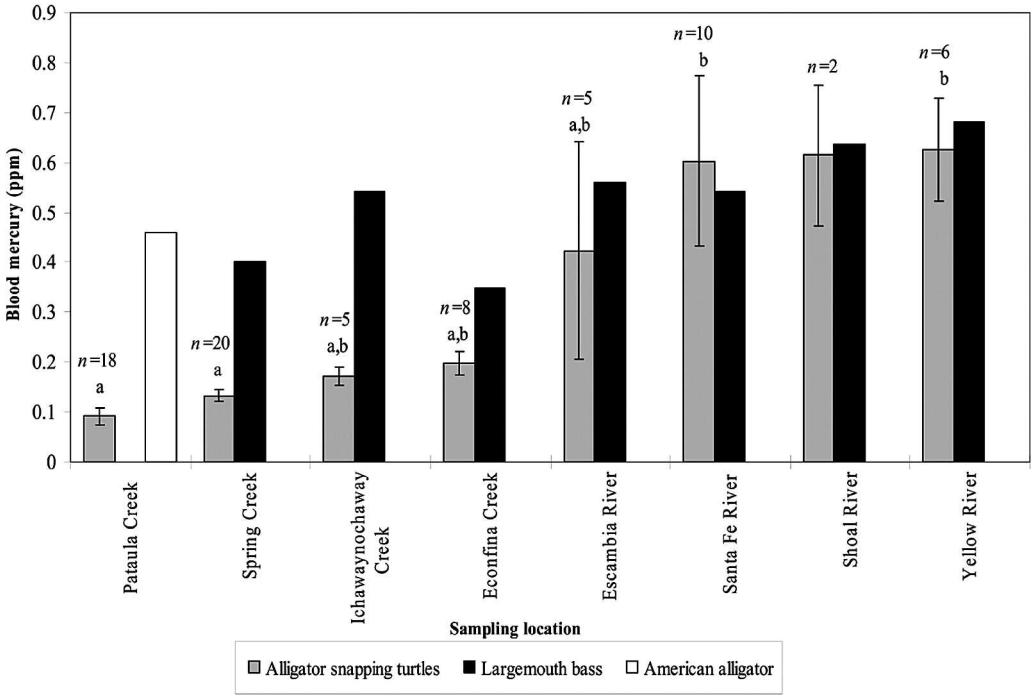


FIGURE 2. Comparison of total mercury levels within sampling sites. Sites with subscripts in common are not significantly different for Alligator Snapping Turtle (*Macrochelys temminckii*) blood mercury levels. Shoal River was excluded from analysis because of small sample size ($n=2$). Largemouth bass (*Micropterus salmoides*) and American alligator (*Alligator mississippiensis*) mercury values were supplied by the Florida Fish and Wildlife Commission and the Georgia Environmental Protection Division.

at the 0.025 ppm detection limit; aldrin, BHC, dieldrin, endosulfan, endosulfan II, endrin, gamma chlordane, HCB, heptachlor, heptachlor epox, lindane, methoxychlor, and mirex at the 0.05 ppm detection limit; p,p'DDD, o,p'DDD, o,p'DDE, o,p'DDT, and dicofol at the 0.1 ppm detection limit; chlordane at the 0.25 ppm detection limit; p,p'DDE and p,p'DDT at the 0.5 ppm detection limit; toxaphene at the 2.0 ppm detection limit; and PCBs (as arochlor#1260) at the 1.0 ppm detection limit.

Whole-blood cholinesterase was also measured as a biomarker of organophosphate and carbamate exposure, since cholinesterase levels are depressed in animals exposed to these pesticides (Pauli and Money, 2000). Cholinesterase values were lower in females than in males ($P=0.006$), and they were also lower in females than in immature individuals ($P=0.025$); they did not differ significantly between males and immature turtles ($P=0.45$). The mean value was 0.233 ± 0.29 μM/ml/min for females, 0.363 ± 0.05 μM/ml/min for males, and 0.436 ± 0.24 μM/ml/min for juveniles (detection limit=0.1 μM/ml/min). A significant difference was also detected among locations ($P=0.001$), where turtles from Pataula Creek and Ichawaynochaway Creek exhibited the lowest cholinesterase values.

Parasites

An unidentified *Haemogregarina* species was found in the erythrocytes of all but one of the turtles evaluated during the course of this study. *Haemogregarina* parasitemias varied from 0.1% to 6.9%. An investigation into this parasite and the Alligator Snapping Turtle's role in its life cycle is ongoing (S. Telford, pers. comm.).

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In the Alligator Snapping Turtle, these intracellular protozoan parasites are transmitted by leeches, the presumed invertebrate host (Mader, 1996). Not surprisingly, leeches were found on 40% of the turtles examined ($n=46$). The predominant attachment sites were the carapace, neck, and head, especially around the eyes, and inside the oral cavity. Four different species of leech were identified: *Placobdella multilineata*, an atypical *Placobdella ornata*, *Placobdella parasitica*, and an atypical *Placobdella parasitica* that has not been described previously (M. Siddall, pers. comm.).

DISCUSSION

Reptile biochemistry and hematologic data are influenced by environmental conditions, including water chemistry, contaminant exposure, and temperature (Anderson et al., 1997; Mazet et al., 2000; Keller et al., 2004). Nutritional status also affects the evaluation of health parameters (Mader, 2006). Alligator Snapping Turtles are opportunistic predators and scavengers. Although fish are the staple of their diet, they are known to eat everything from smaller turtles and mussels to acorns and hickory nuts, and prey choice varies by location and availability (Elsey, 2006; Pritchard, 2006). In a survey of the stomach and intestinal contents of 109 Alligator Snapping Turtles in Arkansas and Louisiana, mammals such as hogs (*Sus scrofa*) and nutria (*Myocastor coypus*) appear to have been consumed as carrion, while at other locations, prey was mostly limited to fish, vegetation, and invertebrates (Elsey, 2006). As the protein content of the diet increases, these changes are reflected in numerous biochemistry values. For example, uric acid levels commonly increase up to twice the normal value on the day following a high-protein meal (Mader, 2006). Blood urea nitrogen is also likely to fluctuate post-prandially. In turtles, BUN can be an indicator of hydration status and nitrogen balance, but it has low clinical importance for monitoring renal disease (Mader, 1996).

Alternatively, decreases in biochemistry values such as phosphorous, potassium, albumin, and total protein have been noted in chelonians during periods of decreased dietary intake (Christopher et al., 1999; Mader, 2006). The low glucose levels found in Alligator Snapping Turtles are likely normal for this species as a result of their high protein diet (Redrobe and MacDonald, 1999). At room temperature, prolonged exposure of serum to erthyrocytes leads to a loss of glucose through continued uptake and metabolism of glucose by red blood cells (Kaneko, 1997). However, we stored blood in vacutainer tubes, which attenuates this process (Sacks, 2006), and samples were placed in a chilled cooler until separation within 4 hr. Therefore, we believe any loss through glycolysis was minimal. Many of the differences we detected among locations were in values such as uric acid, sodium, phosphorus, glucose, and albumin levels. Therefore, we conclude that physiologic processes associated with variations in diet are reflected in the normal fluctuations in clinical pathology and nutrient parameters of Alligator Snapping Turtles. Other intrinsic processes, such as seasonal hormonal changes, could also account for differences detected in this study (Mader, 2006).

Vitamin A, a nutrient acquired from the diet, is essential in many biologic processes in turtles, including growth, reproduction, and immune function (Frutchey et al., 2003). Because of these important roles, the establishment of baseline reference ranges for vitamin A, as well as for levels of vitamins E and D, in Alligator Snapping Turtles was critical. Plasma vitamin E concentrations in Alligator Snapping Turtles in this study were similar to values reported for free-ranging marine turtles and for terrestrial chelonians (Dierenfeld et al., 1999; Frutchey et al., 2004), despite differences in habitat and prey preferences among these species. In

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a study of 12 species of semi-aquatic freshwater turtles, retinol concentrations, measured as a proxy for vitamin A activity, ranged from 0.04±0.02 μg/ml to 0.35±0.1 μg/ml (Dierenfeld et al., 1999). The mean retinol concentration in Alligator Snapping Turtles (0.192±0.03 μg/ml) also fell within this range.

Deficiencies in vitamin A have been linked to organochlorine exposure and aural abscesses in box turtles (*Terrapene carolina carolina*; Brown et al., 2003). In the past, organochlorines were widely used as herbicides and pesticides (Stell et al., 1995). We suspected, due to historically heavy pesticide use near our study locations, that Alligator Snapping Turtles in these areas may have been exposed to organochlorine pesticides. These contaminants were not detected, potentially because detection limits available were not sensitive enough or because they are no longer present in our study locations. As more sensitive tests for organochlorine exposure become available, the reference ranges established by this study can be used as a tool to investigate the link between contaminants and nutrient parameters in turtles.

Although Alligator Snapping Turtles do not hibernate during the winter months, it is probable that their behavior and feeding habits change with the seasons as they enter a period of winter inactivity (Harrel et al., 1996; Boundy and Kennedy, 2006). Overall, turtles from Pataula Creek exhibited the highest biochemistry values in parameters such as glucose, total protein, albumin, bile acids, and triglycerides. In contrast, turtles sampled from the Santa Fe River were found to have some of the lowest values for these same parameters. Because all 23 turtles from Pataula Creek were sampled exclusively in the summer months, when it appears they are most active, and most of the 14 turtles from the Santa Fe River were sampled in spring, it is not possible to pinpoint location differences because of the influence that season may exert on biochemistry values. Differences that were detected in hematologic values, such as basophils and eosinophils, were also likely caused by seasonal variation. The number of circulating eosinophils in reptiles is highly variable; however, many species have normal values up to 20% (Mader, 2006).

Variation in body temperature, regardless of season, has also been shown to cause alterations in biochemistry values. New Guinea snapping turtles (*Elseya novaeguineae*) held in captive, controlled conditions at 24.5 C and 30.0 C exhibited differences in chemistry levels as a result of the water-temperature change, including higher levels of glucose, ALP, AST, ALT, and total carbon dioxide measured at the higher temperature (Anderson et al., 1997). In our study, increases in AST and ALT coincided with summer, when the highest water temperatures were measured. Additionally, plasma total protein, albumin, LDH, creatinine, BUN, and potassium were elevated in summer compared to spring.

Clinical pathology values are expected to fluctuate in female Alligator Snapping Turtles during their seasonal reproductive cycle. In this study, variation was detected in the reference ranges for calcium, phosphorus, cholesterol, and triglyceride levels between females and males. Elevations in all four values are consistent with egg production and vitellogenesis in female chelonians (Christopher et al., 1999). However, we did not have an adequate sample size of female turtles to further analyze seasonal variation in metabolites.

Enzymes such as CPK, AST, ALT, and LDH are found in numerous organ systems. Elevations in these enzymes are nonspecific but can indicate tissue damage. A large number of turtles sampled in this study had superficial or deep wounds, perhaps as a result of confrontations with American alligators (*Alligator mississippiensis*) or other Alligator Snapping Turtles; therefore, variations in tissue enzymes were not unexpected. Elevations

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in CPK and AST could indicate that animals struggled in the trap, although we rarely witnessed this behavior. Ranges for LDH established here are more than four times higher than values published for other turtles (Raphael, 2003; Mader, 2006). The mean LDH value for the painted turtle (*Chrysemys picta*) is 1702 International Units (IU)/l, and for the red-eared slider (*Trachemys scripta*), it is 724 IU/l, compared to the mean value in this study of 5717 IU/l. In birds, the freezing and thawing of plasma samples during storage has been shown to cause a moderate increase in LDH activity, although the values remained within published reference intervals (Hawkins et al., 2006). Since the LDH level in this study was consistent across all Alligator Snapping Turtles, we believe this finding is normal for the species.

Finally, some variation in our results may be attributable to lymph contamination, a problem often encountered while collecting blood from the dorsal tail vein (Redrobe and MacDonald, 1999). A recent study comparing venipuncture sites in the marginated tortoise (*Testudo marginata*) found differences in the level of packed cell volume, total protein, uric acid, calcium, phosphorus, AST, ALT, and LDH in blood collected from the brachial vein when compared to that from the dorsal coccygeal vein. Due to the reduced cellular and enzymatic components of lymph compared to blood, lower values of the metabolites were detected in samples from the tail vein (Lopez-Olvera et al., 2003). Overall, these metabolites were no lower in this study than values for other reptiles. Additionally, any alterations caused by this problem would likely lead to nondifferential bias in the study since the sampling method was standardized. Care was also taken not to include obvious lymph-contaminated samples.

The disease diagnostics in this study focused on infectious pathogens previously identified or that are emerging in reptiles. *Mycoplasma* spp. have been implicated as the cause of respiratory disease in threatened chelonian populations (Origgi and Jacobson, 2000; Brown et al., 2001). Although exposure to this pathogen was not detected in Alligator Snapping Turtles, we must emphasize that the screening assay used here has only been validated for *Gopherus* spp. and that sample size was limited. Iridovirus epizootics have been shown to be the primary cause of amphibian mortality events in the USA; however, the virus has only recently been confirmed to cause disease in chelonians (Green et al., 2002; Johnson et al., 2007). In amphibians, one mechanism of iridovirus transmission is the ingestion of infected animals or water sources (Pearman et al., 2004). Although we did not find evidence of this virus in our study group, because of their diverse diet and aquatic nature, Alligator Snapping Turtles must continue to be monitored for the introduction of iridovirus into populations.

West Nile virus led to epizootics and death of farm-raised alligators in Georgia in 2001 and in both Florida and Georgia in 2002 (Miller et al., 2003; Jacobson et al., 2005). In 2002 and 2003, we tested Alligator Snapping Turtles in both states, and all animals were seronegative. Unlike alligators, which are exposed to mosquitoes while basking, Alligator Snapping Turtles rarely bask, leaving the water only to nest (Powders, 1978). This lack of exposure to the vector likely protects Alligator Snapping Turtles from infection.

A high proportion of the Pataula Creek turtles that were tested, 64% (7/11), were seropositive for tortoise herpesvirus exposure. Within chelonian populations, herpesviral infections are currently one of the most significant infectious disease threats. The range of infections includes a rhinitis-stomatitis syndrome, which leads to pneumonia and systemic disease in tortoises (Harper et al., 1982; Drury et al., 1998; Origgi et al., 2004) and fibropapilloma-associated turtle herpesvirus in sea turtles

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(FPTHV; Ene et al., 2005; Greenblatt et al., 2005). The etiology of FPTHV is likely multifactorial, but researchers believe that environmental stress, resulting from pollution, increased human density, and agricultural runoff, contribute to the current syndrome (Aguirre and Lutz, 2004). None of the Alligator Snapping Turtles examined in this study displayed clinical signs associated with these syndromes; however, gross and microscopic necropsy data along with additional testing are needed to definitively rule out herpesvirus-related pathology. Unfortunately, dead Alligator Snapping Turtles are rarely found in the wild and necropsied, now or in the past. The relationship between herpesviruses and reptiles in general has not been fully characterized, but it appears that numerous serologically distinct host-adapted herpesviruses exist within closely related reptile genera (Mader, 2006). With this evidence, we conclude that, like other wildlife species, Alligator Snapping Turtles are likely to have coevolved with their own species-specific herpesvirus. Although this virus does not appear to be causing clinical signs in the population at this time, we cannot rule out the possibility that if the turtles were exposed to extreme stress, including other infectious diseases, this herpesvirus could contribute to a disease outbreak in the population. While their physiology differs from that of marine turtles, Alligator Snapping Turtles are also subject to environmental stressors such as those linked to FPTHV, which is why further classification and continued monitoring of this herpesvirus are needed.

The primary environmental contaminant found in Alligator Snapping Turtles in this study was mercury. Elevated levels of methylmercury, the organic form of mercury, cause acute and chronic toxicity in wildlife, although the threshold for these effects has not been established for reptiles (Linder and Grillitsch, 2000). Because none of the systems where we sampled has any significant industry that could be the source for the mercury detected in the turtles, it is possible that the mercury is coming from atmospheric sources that are deposited within these mostly rural watersheds. The Yellow River and the Santa Fe River, where turtles with the highest mercury levels were found, are strongly acidic black-water systems with increased levels of dissolved organic carbon (DOC). This type of system favors mercury methylation and bioavailability (Thompson-Roberts and Pick, 2000). Additionally, blood mercury levels reflect both dietary intake, as well as long-term accumulation (Day et al., 2005). This may explain why mercury values fluctuated slightly in turtles measured more than once but remained relatively stable over time.

Levels of mercury detected in blood samples from Alligator Snapping Turtles in the Santa Fe, Yellow, and Shoal Rivers exceeded the Florida health advisory level for limited consumption of fish (0.5 ppm). Previous studies focusing on common snapping turtles and sea turtles determined that blood mercury levels were similar to those found in muscle (Golet and Haines, 2001; Day et al., 2005); therefore, we assumed that blood and muscle levels in Alligator Snapping Turtles would also be equivalent. All of our Florida sample sites and two out of three of our Georgia sites (Ichawaynochaway Creek, Spring Creek) are under fish consumption advisories. Largemouth bass (*Micropterus salmoides*), one of the species sampled to establish mercury advisories, consume similar sources of mercury-laden prey as Alligator Snapping Turtles (Wainwright and Richard, 1995). Therefore, we compared total blood mercury levels measured in this study to mercury levels measured in edible tissue from largemouth bass and alligators from data collected to develop consumption advisories for Georgia and Florida waterways (Florida Fish and Wildlife Conservation Commission, Georgia Department of Nat-

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ural Resources Environmental Protection Division, unpubl. data; Fig. 2). As detected in Alligator Snapping Turtles, the highest mercury levels in largemouth bass were measured in the Yellow River and the Shoal River, indicating that Alligator Snapping Turtles display similar patterns of mercury accumulation within their habitat. However, an important difference between these species is their life span. The average life span of largemouth bass is 10 to 15 yr, depending on location (Bennett, 1937), compared with Alligator Snapping Turtles, which can live well beyond 60 yr (Gibbons, 1987). This adds further evidence to the argument against harvesting turtles for human consumption, which is neither sustainable nor advisable (Reed et al., 2002; Schlaepfer et al., 2005). More importantly, Alligator Snapping Turtles can be used as sentinels for long-term ecosystem changes.

Historically, Alligator Snapping Turtles weighing well over 75 kg have been captured in the wild, and one male reached 107 kg in captivity (Ernst and Barbour, 1972). During separate census counts in Georgia, the first in 1988 and the second from 1997 to 2001, maximum weights of the turtles captured were 39 and 49 kg, respectively (Jensen and Birkhead, 2003). Since 2001, the single largest turtle captured for this study weighed just 45 kg and had a carapace width of 49 cm and length of 64 cm. We have yet to determine if the size distribution of our captures was influenced by methodology, such as small hoop-trap diameter, or whether most of the larger turtles of the past have been harvested. However, our study now provides baseline health data. With the advent of advanced molecular techniques, including the ability to detect contaminants at nominal levels, analyze variations in hormone levels, and differentiate specific pathogen infection from cross-reactivity, these data on population health may assist researchers and wildlife managers investigating future threats to this vulnerable species.

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