CLINICAL AND NECROPSY FINDINGS ASSOCIATED WITH INCREASED MORTALITY AMONG AMERICAN ALLIGATORS OF LAKE GRIFFIN, FLORIDA

Authors: Schoeb, Trenton R., Heaton-Jones, Terrell G., Clemmons, Roger M., Carbonneau, Dwayne A., Woodward, Allan R., et al.

Source: Journal of Wildlife Diseases, 38(2) : 320-337

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-38.2.320

Journal of Wildlife Diseases, 38(2), 2002, pp. 320–337

CLINICAL AND NECROPSY FINDINGS ASSOCIATED WITH INCREASED MORTALITY AMONG AMERICAN ALLIGATORS OF LAKE GRIFFIN, FLORIDA

Trenton R. Schoeb,[1,6,7] Terrell G. Heaton-Jones,[1] Roger M. Clemmons,[2] Dwayne A. Carbonneau,[3] Allan R. Woodward,[3] Diane Shelton,[4] and Robert H. Poppenga[5]

[1] Division of Comparative Medicine, Department of Pathobiology, University of Florida College of Veterinary Medicine, Gainesville, Florida 32611, USA
[2] Department of Small Animal Clinical Sciences, University of Florida College of Veterinary Medicine, Gainesville, Florida 32611, USA
[3] Florida Fish and Wildlife Conservation Commission, 4005 S. Main St., Gainesville, Florida 32601, USA
[4] Comparative Neuromuscular Laboratory, University of California San Diego, La Jolla, California 92093, USA
[5] Laboratory of Pathology and Toxicology, New Bolton Center, University of Pennsylvania, Kennett Square, Pennsylvania 19348, USA
[6] Current address: Department of Genomics and Pathobiology, University of Alabama at Birmingham, 402 Volker Hall, 1670 University Blvd, Birmingham, Alabama 35294-0019, USA
[7] Corresponding author (e-mail: trs@uab.edu)

ABSTRACT: From December, 1997, through November, 2000, 306 deaths were documented among adult and subadult American alligators (*Alligator mississippiensis*) of Lake Griffin, Florida (USA). Some live alligators were lethargic and unresponsive to approach. To determine the cause, we examined ten alligators captured from Lake Griffin between December 1997 and June 1999. Initially, four alligators, three of which were clinically unresponsive, were sacrificed for routine diagnostic necropsy. The other six Lake Griffin alligators, and five control alligators captured from Lake Woodruff National Wildlife Refuge, Florida, where mortality was negligible, were studied extensively by clinical neurologic examination, electromyography, hematology, serum chemical analyses, and blood culture, then sacrificed and necropsied. Samples of brain, spinal cord, peripheral nerves, skeletal muscle, and major internal organs were examined by light microscopy for abnormalities. Samples of nervous tissue also were examined by electron microscopy, and samples of various tissues were collected for toxicologic analyses. Clinical signs included swimming in circles, inability to submerge, lethargy, weakness, unresponsiveness, slow reflexes, dragging the dorsal surfaces of the hind feet, head tilt, and anisocoria. Lake Griffin alligators had significantly lower distal sciatic nerve conduction velocities than Lake Woodruff alligators, and the most severely affected alligators had the lowest velocities; but morphologic abnormalities in peripheral nerves were not evident in most cases. Three severely affected alligators had acute focal necrosis of the torus semicircularis in the midbrain, two had skeletal myofiber atrophy, another had diffuse nonsuppurative encephalomyelitis, and one mildly affected alligator had skeletal myodegeneration. The cause or causes have not yet been identified.

Key words: *Alligator mississippiensis*, American alligator, electromyography, encephalopathy, Florida, neuropathy.

INTRODUCTION

In the spring of 1997, Florida Fish and Wildlife Conservation Commission (FWC) personnel conducting annual night spotlight surveys for population monitoring observed unusual numbers of dead subadult and adult American alligators (*Alligator mississippiensis*) on Lake Griffin, (28°54′N, 81°50′W), Florida (USA). Subsequent twice monthly daylight surveys of Lake Griffin from December 1997 through November 2000 detected 306 alligators that had died of undetermined causes. Furthermore, some live Lake Griffin alligators were observed to be lethargic and unresponsive to approach by humans. Mortality on other monitored lakes remained negligible, suggesting that the problem was unique to Lake Griffin. These observations prompted concerns about possible toxicant exposure, such as that associated with a spill of the pesticide dicofol near Lake Apopka (Woodward et al., 1993). An outbreak of infectious disease also seemed possible, inasmuch as a mycoplasmal disease was reported to cause episodes of

high morbidity and mortality among captive alligators at a commercial facility in northeast Florida (Brown et al., 1996).

The objective of this study was to determine the cause or causes of the observed illness and mortality among Lake Griffin alligators.

MATERIALS AND METHODS

Alligators

Nighttime spotlight population surveys and daytime mortality surveys were conducted from airboats by FWC personnel. Daytime mortality surveys of Lake Griffin were conducted every 2 wk. Limited resources prevented regular daylight mortality surveys of lakes other than Lake Griffin. Dead alligators for which a probable cause of death was evident, such as gunshot wound or boat propeller trauma, were excluded from the study. Alligators were captured by FWC personnel from Lake Griffin or Lake Woodruff National Wildlife Refuge (29°05′N, 81°25′W) using a noose or snare. Lake Woodruff is used by FWC as a reference site for alligator studies because it is considered to be less affected by human activity than other large Florida lakes and because FWC survey results indicate healthy alligator populations with no evidence of possible health problems, such as decreased egg hatch rates. The animals were restrained by taping the jaws closed and the limbs against the body with duct tape. The eyes were also covered with tape to calm the animals and reduce struggling during transport. In cases in which alligators were captured at night, they were held at FWC facilities until the next day.

The first four Lake Griffin alligators (numbers 40574, 40575, 40665, and 40713) were given brief clinical examinations, then humanely killed by intravenous injection of 1 ml to 2 ml per 2.5 kg body weight of pentobarbital euthanasia solution (Beuthanasia-D Special,® Schering-Plough Animal Health, Kenilworth, New Jersey, USA) for routine diagnostic necropsy. Based on clinical signs and absence of evidence for other causes, a protocol was adopted for examination of subsequent alligators that included clinical and post mortem procedures to evaluate the nervous system and musculature and collection of tissue samples for toxicologic analysis. Due to its poor condition (see below), Lake Griffin alligator 40523 was killed immediately after delivery to the Veterinary Medical Teaching Hospital (VMTH; University of Florida, Gainesville) and was not clinically evaluated.

Alligators held for clinical examination were maintained in University of Florida (UF) animal facilities and housed outdoors in dog runs that had concrete sides and sloped concrete floors modified to allow flooding, with provision for draining and cleaning. Tap water from the Gainesville (Florida) city water system, flowing continuously, was used to flood the runs to a depth of about 40 cm, allowing the animals to submerge. Water temperatures were checked daily and remained at 24 C. The deep ends of the runs were shaded and the shallow ends unshaded to allow the animals areas for basking and for protection from excessive heat as needed.

This study was conducted under permit from FWC. All animal-related procedures were approved by the UF Institutional Animal Care and Use Committee.

Neurologic examination

Except as noted, neuromuscular function of each alligator was assessed by electromyography (EMG) and electroencephalography (EEG). When possible, each alligator was observed and evaluated over a period of 2 wk to try to determine whether the condition of affected Lake Griffin alligators improved or deteriorated after removal from the lake, and two neurologic examinations were conducted to assess reproducibility within subjects and to assess improvement or deterioration in neuromuscular function. The alligators were allowed 5–7 days after capture for acclimation and observation before the first examination. Five to 7 days later, a second examination was conducted. Immediately thereafter, the animals were humanely killed as described above and necropsied. Alligators 36904, 40553, 40577, 40693, and 40698 were examined twice; alligators 40552, 40578, 40579, 40672, and 40675 were examined once. Two severely affected Lake Griffin alligators were examined only once; one (40552) died spontaneously and the other (40579) was killed when it became moribund.

Each alligator was observed daily for responses to approach by personnel. Reflex examinations were conducted immediately before anesthesia (see below) for each electrophysiologic evaluation. Righting, biting, corneal, and front and rear limb toe pinch withdrawal reflexes were scored from 1 (slow, clearly abnormal responses) to 4 (rapid responses typical of Lake Woodruff alligators). Lack of responses to stimulation also were recorded. Righting reflexes of Lake Woodruff alligators were difficult to evaluate and were considered normal if the alligator struggled and fought against restraint by thrashing, biting at the catch pole, and rolling.

Corneal reflexes were tested by touching a 20-gauge soft plastic catheter to the cornea at the medial canthus and observing nictitating membrane closure or eyelid closure. Toe pinch withdrawal reflexes were assessed with a hemostat.

Electrophysiologic testing was done at the VMTH Clinical Neurology Laboratory, using a TECA Sapphire II ME® electromyography instrument (Oxford Instruments, Inc., Pleasantville, New York, USA) and a TECA Discovery® electroencephalograph (Oxford). Segmental needle EMG assessments were made for major limb and head muscles and for spinal epaxial and neck muscles. Proximal (hip to knee) and distal (knee to ankle) segment sciatic nerve conduction velocities (NCV's), repetitive stimulus responses, and spinal cord evoked potentials were evaluated. During testing, heart rates and respiratory rates were monitored, and corneal reflexes were periodically assessed. Electrocardiographic monitoring was conducted continuously with a Hewlett Packard Page Writer 200 Electrocardiograph (Ledford Medical Electronics, Inc., High Point, North Carolina, USA). Respiratory rates were manually counted at 15–30 min intervals during the procedures, which required an average of 175 min. Body temperatures also were recorded during the procedures.

Anesthesia

Alligators subjected to electrophysiologic testing were anesthetized by injection into the triceps muscle a combination of 160 μg/kg medetomidine (Domitor®, Pfizer Animal Health, West Chester, Pennsylvania, USA) and 10 mg/kg ketamine (Ketaset®, Fort Dodge Animal Health, Overland Park, Kansas, USA) and securely taped to a board. Injections were administered in the front limbs to avoid possible reduction of efficacy due to clearance via the renal and hepatic portal systems and to avoid possible interference with measurement of sciatic nerve conduction velocities. Body weights were estimated prior to injection for the first examination, and one-half of the calculated dose was administered, resulting in a state of sedation or light anesthesia. Actual weights were then determined and the remaining dose administered only if required to immobilize the animal during testing. A modification of this protocol was necessary in one case. Due to its size, alligator 40578 could not be manually controlled and was administered tiletamine and zolazepam (Telazol®, Fort Dodge Animal Health) in the pterygoideus muscle via pole syringe before administration of medetomidine and ketamine. Dosage was 5 mg/kg based on an estimated body weight of 150 kg.

After examination, 800 μg/kg atipamezole (Antisedan®, Pfizer Animal Health) was administered for medetomidine reversal by injection into the triceps muscle of the front limb opposite that used for medetomidine-ketamine injection. Reversal was indicated by return of corneal reflexes and typical threat behavior (gaping and hissing).

Hematology and serum chemical analyses

Blood samples were collected from the vertebral vein by venipuncture at the dorsal midline of the junction of the head and neck (Olson et al., 1975). Complete blood counts were done by technicians of the VMTH Clinical Pathology Laboratory. Values determined were erythrocyte count; mean corpuscular volume, hemoglobin, and hemoglobin concentration; blood hemoglobin concentration, packed cell volume, thrombocyte count, and total leukocyte count (WBC); and counts of neutrophils, heterophils, azurophils, lymphocytes, monocytes, eosinophils, and basophils. Icteric indices, fibrinogen, and total protein values also were determined. Erythrocyte counts were determined with a Coulter Zbi® counter (Coulter, Hialeah, Florida). Total leukocyte counts were done manually using Natt-Herricks staining (Pierson, 2000) and a hemocytometer. Differential counts were done using blood films stained with Wright's and Giemsa stains.

Serum chemical analyses were done either in the VMTH Clinical Pathology Laboratory, using an Alcyon 3000i® analyzer (Abbott, Chicago, Illinois, USA), or in the clinical laboratory of Shands Hospital at the University of Florida, using a Vitros 950® analyzer (Johnson & Johnson, Rochester, New York). In some cases, samples were analyzed in both laboratories to verify results. Values determined included activities of the enzymes alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase, creatine kinase (CK), and lactate dehydrogenase; concentrations of sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, calcium, phosphorus, glucose, total bilirubin, cholesterol, uric acid, total protein, albumin, and globulin; anion gap; and albumin/globulin ratio. Serum cholinesterase determinations were done by the VMTH Clinical Pathology Laboratory, using a Vitros 250® analyzer (Johnson & Johnson).

Microbiology

Blood samples for culture were collected either from the occipital sinus, in the case of live animals, or from the exposed heart at necropsy. Occipital sinus samples were collected after vigorous, repeated scrubbing of the skin with

gauze pads soaked in 70% ethanol. For collection of heart blood samples, the pericardium was exposed, sprayed with 70% ethanol, and opened with flamed scissors and forceps. A sterile syringe and needle were then used to collect the sample through the myocardium.

Cultures were done in the UF Division of Comparative Medicine Clinical Laboratory. Two blood culture bottles (SeptiChek®, Becton-Dickinson, Cockeysville, Maryland, USA) were incubated at 37 C or 32 C, and samples were Gram stained 1, 7, and 21 days after inoculation. Samples of bottles showing growth were plated onto 5% sheep blood agar plates and incubated at 37 C or 32 C. Colonies were identified by staining, morphology, and biochemical testing using MiniTek® aerobic system kits (Becton-Dickinson). Samples of blood and swabs of joints were cultured for mycoplasmas in SP4 medium (Tully et al., 1977).

Necropsy

Alligators were euthanized by intravenous injection of euthanasia solution as described above. Samples routinely collected after gross examination included trachea, lung, myocardium, stomach, large and small intestine, liver, pancreas, gonad, kidneys, thyroid, adrenals, spleen, and brain, which were fixed in buffered 10% formalin. Samples of blood were collected for culture for bacteria and mycoplasmas, and swabs of large joints such as knee and elbow joints were cultured for mycoplasmas. Because there was no evidence of significant parasitism a systematic effort to identify and count parasites was not made. Parasites were collected for identification according to standard parasitologic keys (E. Greiner, Department of Pathobiology, College of Veterinary Medicine, UF).

Alligators subjected to neurological examination and one Lake Griffin alligator sacrificed immediately on presentation due to poor condition (alligator 40523) were studied similarly, with additional procedures for evaluation of central and peripheral nervous system tissues and skeletal muscle. Both brain and spinal cord (cervical through sacral regions) were removed. The brain was hemisectioned and a small sample was collected from the left half of the telencephalon and frozen at −80 C in tissue culture medium for future virus isolation attempts. The remainder of the left half of the brain was frozen separately at −80 C. From the right half, samples were collected for electron microscopy from the telencephalon, mesencephalon, cerebellum, and medulla and fixed in buffered 4% glutaraldehyde or Trump's fixative (McDowell and Trump, 1976). The remainder of the right hemisection was fixed in buffered 10% formalin for histologic examination. Samples of spinal cord were taken from the approximate middle of the cervical, thoracic, lumbar, and sacral segments. The total length of the cord and the distance from the cervical end at which each sample was collected were recorded. Approximately 2 cm segments were fixed in formalin for histologic examination, and adjacent samples were fixed in glutaraldehyde or Trump's fixative for electron microscopy. The remainder of the cord was frozen at −80 C. Left and right sciatic and brachial nerves were removed, dissected free of vessels, tied to wooden applicator sticks with silk suture material, and fixed by immersion in formalin, and additional samples were fixed in glutaraldehyde or Trump's fixative. Samples of left and right pterygoideus, biceps, quadriceps, gastrocnemius, and lateral tail muscle near the vent were similarly tied to applicator sticks and fixed in buffered formalin and glutaraldehyde or Trump's fixative.

Histopathology and electron microscopy

Fixed, trimmed tissues were processed routinely for histology. After paraffin embedding, tissues were sectioned at 5–6 μm and stained with hematoxylin and eosin (HE). Sections of brain and spinal cord also were stained with Luxol fast blue (Sheehan and Hrapchak, 1980) for evaluation of myelin, and sections of peripheral nerves were stained with Luxol fast blue-periodic acid-Schiff (Sheehan and Hrapchak, 1980) for examination of myelin and with Bodian's stain (Prophet et al., 1994) for examination of axons.

Samples of brain of alligator 40523 and sciatic nerve of alligators 40552, 40579, 40675, 40693, and 40698 were processed for ultrastructural examination. Fixed trimmed tissues were embedded in EmBed® (Electron Microscopy Sciences, Ft. Washington, Pennsylvania, USA), sectioned at 1 μm, and stained with toluidine blue. Based on examination of toluidine blue stained sections, blocks were trimmed for ultramicrotomy, and sections of desired areas were cut, mounted on copper grids, stained with lead acetate and uranyl acetate, and examined by routine transmission electron microscopy.

Samples of sciatic nerve and skeletal muscle from the two Lake Griffin alligators having the lowest NCV's (40552 and 40579) and from the two Lake Woodruff alligators having the highest NCV's (40693 and 40698) were plastic embedded, sectioned at 1 μm, and stained with toluidine blue. Nerve fiber diameter frequency distribution was evaluated by computerized morphometry. Non-overlapping fields repre-

sentative of an entire sciatic nerve cross-section were digitized and captured with a video camera and an LX-450 system digitizer (Optronics Engineering, Goleta, California, USA). Final magnification of the digitized image was equivalent to 2.625 pixels per micrometer. Morphometric analyses of myelinated nerve fiber axonal perimeters were performed on a Macintosh Quadra 900 computer with NIH Image v1.43 software. All axons in the field of view were analyzed unless the profiles were classified as longitudinal, paranodal, or artifactual. Diameter for each axon was estimated by calculating from a circle of equivalent perimeter. A mean axonal diameter was calculated for each animal.

Toxicology

Samples of liver, kidney, skeletal muscle, fat, brain, spinal cord, plasma, and, when present, stomach contents, were collected and stored at −80 C. Samples of liver and kidney of two Lake Griffin alligators (40552 and 40579) and two Lake Woodruff alligators (40553 and 40693) were analyzed for arsenic, calcium, cadmium, cobalt, chromium, copper, iron, mercury, magnesium, manganese, molybdenum, lead, selenium, tin, thallium, and zinc with an inductively coupled plasma optical emission spectrometer (GBC Scientific Equipment, Inc., Arlington Heights, Illinois, USA). Samples of adipose tissue of Lake Griffin alligators 40523, 40552, 40577, 40578, and 40579 and Lake Woodruff alligators 36904, 40553, 40693, and 40698 were analyzed for organochlorines by electron capture gas chromatography (Varian 3400 and 3600 gas chromatographs; Varian, Inc., Walnut Creek, California).

Statistical analysis

Monthly mortality results were analyzed using two-way analysis of variance (Snedecor and Cochran, 1980), with supplemental testing of main effect means for months and years with Tukey's test (Snedecor and Cochran, 1980). Numerical clinical data were compared between Lake Griffin and Lake Woodruff alligators and between alligators judged to be clinically ill and those not apparently ill using Student's *t*-test for unpaired observations (Snedecor and Cochran, 1980). Where repeated measurements were made, the means of the values for each alligator were analyzed. Ordinal data (reflex scores) were analyzed with the rank sum test (Snedecor and Cochran, 1980). Comparisons with $p \leq 0.05$ were considered significant. Statistical software was Statistix (Windows® version 7.0, NH Analytical, Tallahassee, Florida).

RESULTS

Mortality surveys

Daylight surveys detected 306 adult and subadult (≥218 cm) Lake Griffin alligators that died of unknown causes from December 1997 through November 2000 (Table 1). Dead alligators were found out of the water on the lake shore as well as in the water. Numbers of dead alligators encountered during night spotlight population surveys gave no evidence of increased mortality on other monitored lakes. For example, totals for the 1997 through 2000 seasons were three for Lake Woodruff, two for Lake Orange (29°27′N, 82°10′W), and two for Lake Apopka (28°40′N, 80°39′W), but 26 for Lake Griffin (not including results of additional daytime mortality surveys of Lake Griffin). In previous years, the numbers of dead alligators encountered in night surveys of Lake Griffin were small and comparable to those of other lakes. Alligator mortality at Lake Griffin differed significantly among the years of the study ($P = 0.034$), with the highest annual mortality recorded for 2000. Mortality also differed significantly among months ($P = 0.0003$) and was highest in the spring; the mean for April was significantly greater than that for all other months except May.

Case histories

Alligators examined in this study are listed in Table 2. Alligator 40523 was found floating on its left side with the head and tail hanging limply below the surface and was unable to submerge for more than a few seconds. The next day, the animal was again located but it apparently had been struck by a boat, resulting in multiple fractures of the mandible and skull. The alligator was killed and necropsied immediately. Alligator 40552 was found at the water's edge and was very passive when lifted into the boat. Alligator 40575 was lethargic. Alligator 40577 was found basking on a mud flat and it resisted capture with normal vigor. Alligator 40578 was found floating at the surface and apparently having

TABLE 1. Results of daylight surveys of Lake Griffin for alligator mortality.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Mean ±SD[a]
1997												2[b]	
1998	6	5	8	15	9	2	5	2	1	0	8	7	5.7[c] ± 4.2
1999	4	5	6	26	18	10	5	3	6	5	10	4	8.5[c,d] ± 6.9
2000	14	14	17	46	16	4	7	0	4	6	6		12.2[d] ± 12.5
Mean ± SD[2]	8.0[e] ± 5.3	8.0[e] ± 5.2	10.3[e] ± 5.9	29.0[f] ± 15.7	14.3[e,f] ± 4.7	5.3[e] ± 4.2	5.7[e] ± 1.2	1.7[e] ± 1.5	3.7[e] ± 2.5	3.7[e] ± 3.2	8.0[e] ± 2.0	4.3[e] ± 2.5	

[a] SD: standard deviation.
[b] Number of dead alligators (≥218 cm).
[c,d] Yearly means having the same superscripts are not significantly different ($P > 0.05$; Tukey's test).
[e,f] Monthly means having the same superscripts are not significantly different ($P > 0.05$; Tukey's test).

difficulty remaining upright and when captured was uncoordinated. Alligator 40579 was found on the shore, moved into the water, and weakly resisted capture. Alligator 40665 was found floating in the water and struggled briefly and weakly after being lifted into the boat. Alligator 40675 was readily snared but actively resisted capture. Alligator 40713 was found basking and sluggish. When noosed it attempted to escape for about 10 min.

Alligators 36904, 40553, 40672, 40693, and 40698 were captured at night from Lake Woodruff. All actively resisted capture.

Clinical findings

Alligator 40675 appeared alert and readily responded to personnel by gaping and hissing. However, it dragged the dorsal surfaces of its hind feet when attempting to avoid handling and on one occasion was unable to use its rear limbs for locomotion. Alligator 40552 readily responded to personnel by gaping and hissing, but its limb movements were weak and it became progressively sluggish and unresponsive. Alligator 40577 tended to remain submerged and quiet but when prodded responded with typical threat behavior. Alligator 40578 responded only when approached closely by gaping and hissing, but when prodded had a rapid reflexive head turn and tail slap response. The clinical behavior of Alligator 40579 was similar to alligator 40578. It had a left head tilt and anisocoria.

The alligators from Lake Woodruff remained submerged most of the time, but usually responded immediately and vigorously to an observer's presence by rising up on the front limbs and gaping and hissing. If the observer remained close by, they would either retreat into the water or continue with threat displays. Alligator 40553 was somewhat aggressive, lunging at personnel and biting at the gate.

There were no significant differences in reflex responses between Lake Griffin and Lake Woodruff alligators or between alli-

TABLE 2. Source and date, sex, size, and clinical condition of alligators examined December 1997–May 1999.

Alligator		Lake of origin	Capture date	Sex	Total length (cm)	Weight (kg)	Clinical condition
1	40574	Griffin	17 Dec 97	M	178	not done	normal
2	40575	Griffin	12 Feb 98	F	193	23.9	abnormal
3	40665	Griffin	19 Feb 98	F	245	79.1	abnormal
4	40713	Griffin	21 Apr 98	F	248	54	abnormal
5	40675[a]	Griffin	23 Jun 98	M	180	21	abnormal
6	40523	Griffin	11 Feb 99	M	295	116	abnormal
7	40552[a]	Griffin	18 Mar 99	M	185	23.6	abnormal
8	40577[a]	Griffin	14 Apr 99	M	173	15	normal
9	40578[a]	Griffin	21 Apr 99	M	298	155	abnormal
10	40579[a]	Griffin	5 May 99	M	178	18	abnormal
11	40672[a]	Woodruff	16 Jun 98	M	206	30	normal
12	40698[a]	Woodruff	25 Mar 99	M	193	25.4	normal
13	40693[a]	Woodruff	14 Apr 99	M	173	13	normal
14	40553[a]	Woodruff	15 Apr 99	M	189	19	normal
15	36904[a]	Woodruff	29 May 99	M	189	19.5	normal

[a] Alligators examined by electromyography.

gators judged to have normal or abnormal clinical behavior (P = 0.31–0.43). Responses considered abnormal (scored 1 or 2) were seen only in rear limb toe pinch responses in two Lake Griffin alligators (40552 and 40577).

Lake Griffin alligators had significantly lower distal (knee-to-ankle) sciatic nerve conduction velocities (NCV's) than Lake Woodruff alligators (Table 3). Comparison of NCV's in alligators considered depressed or lethargic vs. those considered clinically normal also was significantly different. Also, NCV's were lowest in alligators that were the most severely affected clinically. Needle EMG responses, repetitive stimulus responses, spinal cord evoked potentials, and electrocardiograms (ECG's) were considered normal in both Lake Griffin and Lake Woodruff alligators. There was no apparent difference in EEG wave patterns between Lake Griffin and Lake Woodruff alligators. Brainstem auditory evoked potentials in Lake Woodruff alligator 36904 were bilaterally similar and were considered normal; however, Lake Griffin alligator 40579, which had among the lowest NCV's recorded, and which had head tilt and anisocoria, had no auditory evoked potential on the left side, indicating reduced or absent hearing. There was no evidence of a relationship between either proximal or distal NCV and length, weight, or weight/length ratio (r^2 = 0.008–0.084; P = 0.42–0.94).

Induction of anesthesia sufficient to allow electrophysiologic testing required an average of 41 min. Corneal blink reflexes

TABLE 3. Sciatic nerve conduction velocities in alligators from Lake Griffin and Lake Woodruff, Florida.

	Velocity (m/sec)					
	Lake of origin			Clinical condition		
	Griffin (n = 5)	Woodruff (n = 5)	P[a]	Abnormal (n = 4)	Normal (n = 6)	P[a]
Proximal	47.5 ± 14.9[b]	62.0 ± 12.7	0.14	47.2 ± 17.2	59.8 ± 12.6	0.21
Distal	40.3 ± 7.9	53.7 ± 5.6	0.015	37.9 ± 6.6	53.1 ± 5.2	0.0036

[a] P = probability for each comparison (t-test).
[b] Means ± standard deviations.

were greatly reduced or absent during testing. Recovery was uneventful except for two alligators. Recovery was prolonged in alligator 40552, which was severely depressed clinically; after 54 min, recovery was only partial. Additional atipamezole was administered, but the animal never fully recovered and was found dead the following morning. Alligator 40578 also had a prolonged recovery (94 min).

Hematology, serum chemical analyses, and blood cultures

Hematology results are summarized in Table 4. Most alligators had hemogregarine parasites in the peripheral blood, which were considered an incidental finding. Alligator 40579 had intracellular bacteria in peripheral blood leukocytes. Results of serum chemical analyses are summarized in Table 5. Creatine kinase values were higher than expected in both Lake Griffin and Lake Woodruff alligators although the mean for Lake Griffin alligators was not significantly greater than that for Lake Woodruff alligators ($P = 0.33$). Serum cholinesterase values were not significantly different between Lake Griffin and Lake Woodruff alligators or between clinically normal and abnormal alligators ($P = 0.59$ and 0.77, respectively). Results of blood bacterial cultures are shown in Table 6. Mycoplasmas were not isolated from any sample.

Gross necropsy

There were no gross lesions considered likely to be related to the observed clinical signs. Most of the alligators had at least a few nematode parasites in the stomach; in some cases these were numerous and were associated with multiple, up to 2 cm deep ulcers. One alligator (40575) had a 3 cm circular ulcer in the gastric mucosa.

Nematodes and trematodes such as *Dujardinascaris* sp., *Polyctyle ornata,* and *Acanthostomum* sp. were identified in some gastrointestinal tracts. Most alligators also had at least a few pentastomes (*Sebekia mississippiensis*) in vessels of the liver and lungs.

Histopathology

Significant findings were limited to the nervous system and skeletal musculature. Three Lake Griffin alligators (40575, 40578, and 40579) had acute necrosis of the torus semicircularis of the midbrain (Figs. 1, 2). These areas were characterized by rarefaction of the neuropil, acute necrosis of neuron cell bodies, and capillary endothelial swelling. No glial reaction was evident. Alligator 40523 had diffuse nonsuppurative encephalomyelitis (Fig. 3). Lesions were characterized by perivascular cuffing with lymphocytes in both gray and white matter. These were most severe in the brain and they became progressively less severe in distal segments of the spinal cord. All other organs and tissues, including liver and kidney, were considered to be normal or to have only incidental findings.

Sciatic nerve sections of Lake Griffin alligators 40578 and 40675 had sparse, scattered axonal swellings or spheroid formation (Fig. 4). Plastic sections of sciatic nerve of Lake Griffin alligators 40552 and 40579 gave the impression of having increased proportions of small diameter fibers, which could indicate loss of large diameter fibers; however, morphometric analysis, using sections from the two Lake Woodruff alligators having the highest NCV's (40693 and 40698) as controls, did not show statistically significant differences in the distribution of nerve fiber diameters. Skeletal muscle samples from the two affected Lake Griffin alligators (40552 and 40579) had diffusely decreased myofiber diameters, indicating myofiber atrophy consistent with atrophy secondary to loss of motor innervation (Fig. 5). Some sections of sciatic nerve from alligator 40523 had increased amounts of collagenous connective tissue (endoneurial fibrosis), which also could indicate chronic degeneration and loss of nerve fibers. There was no evidence of myelin degeneration in either central or peripheral nervous systems.

TABLE 4. Hematology results from Lake Griffin and Lake Woodruff alligators.

Variable	Lake of origin			Clinical condition		
	Griffin (n = 5–8)[a]	Woodruff (n = 5–6)[a]	P[b]	Abnormal(n = 3–6)[a]	Normal (n = 6–8)[a]	P[b]
RBC/mm^3	613,143 ± 163,251[c]	536,667 ± 103,859	0.35	659,400 ± 166,168	526,875 ± 98,740	0.095
Hb (g/dl)	8.39 ± 2.16	7.73 ± 0.57	0.54	9.37 ± 1.67	7.53 ± 1.11	0.058
PCV (%)	26.95 ± 7.30	25.02 ± 2.80	0.57	28.60 ± 7.89	24.68 ± 3.57	0.40
MCH (pg)	136 ± 16.2	1,512 ± 32.6	0.38	136 ± 18.4	147 ± 29	0.53
MCHC (g/dl)	31.4 ± 3.93	31.3 ± 2.40	0.98	30.7 ± 2.48	31.6 ± 3.43	0.69
MCV (fl)	483 ± 27.2	476 ± 68.3	0.84	490 ± 26.3	475 ± 57.6	0.70
WBC/mm^3	16,843 ± 3,065	10,401 ± 3,737	0.0082	16,059 ± 2,471	12,259 ± 5,647	0.18
Neutrophils/mm^3	9,314 ± 4,001	4,132 ± 1,638	0.028	11,213 ± 2,715	4,799 ± 2,606	0.0077
Lymphocytes/mm^3	4,007 ± 3,610	2,985 ± 1,355	0.51	2,715 ± 1,265	4,448 ± 3,784	0.33
Monocytes/mm^3	1,845 ± 1,698	1,017 ± 694	0.33	1,412 ± 1,762	1,624 ± 1,193	0.80
Eosinophils/mm^3	986 ± 959	1,386 ± 1,192	0.52	538 ± 518	1,656 ± 1,098	0.040
Basophils/mm^3	1,155 ± 686	883 ± 977	0.56	941 ± 517	1,144 ± 989	0.66
Protein (g/dl)	7.20 ± 0.95	6.52 ± 0.89	0.25	7.44 ± 1.12	6.58 ± 0.74	0.16
Fibrinogen (mg/dl)	250 ± 147	180 ± 130	0.47	283 ± 161	175 ± 117	0.28

[a] Numbers of observations vary because not all values were determined for every alligator.
[b] P = probability for each comparison (t-test).
[c] Means ± standard deviations.

TABLE 5. Serum chemical analysis results from Lake Griffin and Lake Woodruff alligators.

Variable	Lake of origin			Clinical condition		
	Griffin (n = 8–10)[a]	Woodruff (n = 6)[a]	P[b]	Abnormal (n = 8)[a]	Normal (n = 7)[a]	P[b]
ALT (U/l)	203 ± 281[c]	62.8 ± 31.8	0.18	201 ± 301	84.7 ± 64.7	0.32
AST (U/l)	777 ± 905	343 ± 106	0.19	807 ± 962	370 ± 121	0.24
SAP (U/l)	39.4 ± 10.7	31.2 ± 15.4	0.24	40.4 ± 11.0	31.3 ± 14.1	0.19
CK (U/l)	126,849 ± 295,336	29,267 ± 33,158	0.33	137,964 ± 331,149	42,548 ± 56,287	0.45
Na (mEq/l)	136 ± 17.1	148 ± 5.4	0.067	134 ± 17.7	148 ± 5.0	0.066
K (mEq/l)	4.1 ± 0.86	4.0 ± 0.80	0.91	4.0 ± 0.89	4.1 ± 0.77	0.80
Cl (mEq/l)	97 ± 15	110 ± 4.8	0.030	95 ± 15	110 ± 4.4	0.028
CO_2 (mEq/l)	17.2 ± 4.5	13.5 ± 4.2	0.13	16.9 ± 4.7	14.4 ± 4.6	0.33
BUN (mg/dl)	1.3 ± 0.4	1.2 ± 0.7	0.54	1.3 ± 0.4	1.3 ± 0.7	0.92
Creatinine (mg/dl)	0.46 ± 0.17	0.36 ± 0.10	0.19	0.49 ± 0.16	0.34 ± 0.10	0.052
Ca (mg/dl)	12.6 ± 4.1	11.2 ± 0.5	0.35	12.7 ± 4.4	11.3 ± 0.5	0.41
P (mg/dl)	5.4 ± 2.2	8.4 ± 2.4	0.029	5.1 ± 2.2	8.2 ± 2.2	0.017
Glucose (mg/dl)	204 ± 198	104 ± 28	0.17	214 ± 209	107 ± 27	0.20
Total bilirubin (mg/dl)	0.2 ± 0.1	0.3 ± 0.3	0.31	0.2 ± 0.2	0.3 ± 0.3	0.30
Cholesterol (mg/dl)	84 ± 32	75 ± 13	0.59	80 ± 33	81 ± 18	0.99
Urates (mg/dl)	3.5 ± 1.6	1.5 ± 0.4	0.0098	3.6 ± 1.6	1.6 ± 0.5	0.017
Total protein (g/dl)	6.6 ± 0.7	6.2 ± 1.1	0.37	6.6 ± 0.8	6.2 ± 1.1	0.49
Albumin (g/dl)	2.3 ± 0.4	1.8 ± 0.3	0.021	2.3 ± 0.4	1.8 ± 0.3	0.024
Globulin (g/dl)	4.3 ± 0.6	4.4 ± 0.9	0.88	4.3 ± 0.6	4.4 ± 0.9	0.70
A/G ratio	0.54 ± 0.130	0.42 ± 0.089	0.083	0.55 ± 0.133	0.42 ± 0.081	0.052
Cholinesterase (U/l)	1,588 ± 982 (n = 4)	1,284 ± 283 (n = 5)	0.59	1,573 ± 1202 (n = 3)	1,342 ± 290 (n = 6)	0.77

[a] Numbers of observations vary because not all values were determined for every alligator.
[b] P = probability for each comparison (t-test).
[c] Means ± standard deviations.

TABLE 6. Blood culture results from Lake Griffin and Lake Woodruff alligators.

Alligator	Lake	Clinical condition	Organisms isolated
40574	Griffin	normal	Not done
40575	Griffin	abnormal	Negative
40665	Griffin	abnormal	Negative
40713	Griffin	abnormal	*Pseudomonas paucimobilis*
40675	Griffin	abnormal	Negative
40523	Griffin	abnormal	Not done
40552	Griffin	abnormal	*Aeromonas hydrophila*
40577	Griffin	normal	*Enterobacter sakazakii, Aeromonas sobria,* unidentified Gram neg rod
40578	Griffin	abnormal	*Citrobacter amaloniticus*
40579	Griffin	abnormal	*Aeromonas hydrophila, Citrobacter diversus, Klebsiella oxytoca*
40672	Woodruff	normal	Not done
40698	Woodruff	normal	*Serratia liquefaciens, Klebsiella oxytoca*
40693	Woodruff	normal	*Serratia marcesens*
40553	Woodruff	normal	*Aeromonas hydrophila, Streptococcus intermedius, Hafnia alvei*
36904	Woodruff	normal	*Vibrio parahemolyticus, Staphylococcus sciuri, Proteus vulgaris*

Lake Griffin alligator 40675 had multifocal to diffuse, moderate myodegeneration in tail, limb, and jaw muscles (Fig. 6). This process appeared to be subacute, inasmuch as there was sarcolemmal nuclear proliferation in addition to the acute changes of myofiber swelling, loss of striations, and fragmentation.

Electron microscopic findings provided little additional information, other than to confirm light microscopic findings. There was no evidence of myelin degeneration, nor were any virus particle or other possible etiologic agent found in sections from the alligator (40523) with encephalomyelitis.

Toxicology

Concentrations of arsenic, cadmium, mercury, lead, selenium, and thallium in liver and kidney were at or below detection limits (0.5 mg/kg to 2 mg/kg wet weight basis), or, where measurable amounts were present, were not significantly different between Lake Griffin and Lake Woodruff alligators, and in no case exceeded 2 mg/kg. Liver copper concentrations for Lake Griffin alligators were lower, but not significantly so, than for those of Lake Woodruff alligators (3.77 mg/kg and 3.39 mg/kg vs. 10.1 mg/kg and 15.7 mg/kg, respectively; $P = 0.08$). Concentrations of organochlorines in adipose tissue also were below detection limits or very low, in nearly all cases less than 1 mg/kg and in most cases less than 200 μg/kg. Exceptions were *p,p′*-DDE, which was found at up to 1.5 mg/kg in samples from all alligators and at 5.6 mg/kg in alligator 40553 (Lake Woodruff), and for toxaphene, which was found at 1.1 mg/kg, 2.2 mg/kg, and 0.72 mg/kg in Lake Griffin alligators 40578, 40577, and 40579, respectively (mean = 0.8 mg/kg vs. below detection limits in all samples from Lake Woodruff alligators, $P = 0.12$).

DISCUSSION

Mortality among adult and subadult Lake Griffin alligators during the period of the study clearly was higher than in previous years and greater than in other Florida lakes, based on numbers of dead alligators encountered in night population surveys and taking into account differences in lake areas and alligator population densities. The degree to which mortality among Lake Griffin alligators was in-

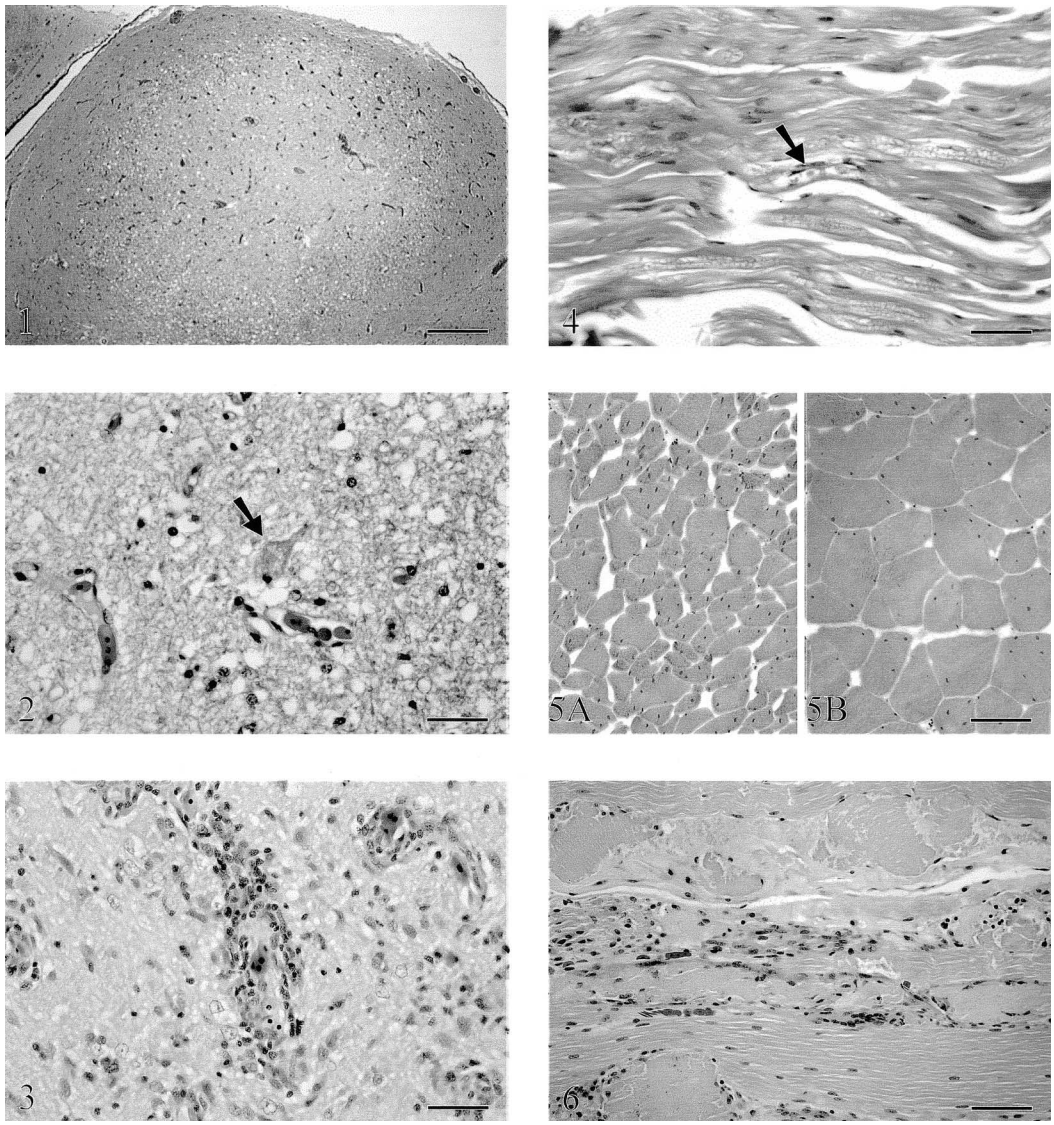

FIGURE 1. Alligator 40578 (Lake Griffin). Focal necrosis of torus semicircularis. HE. Bar = 320 μm.

FIGURE 2. Alligator 40578 (Lake Griffin). Necrotic neuron in the torus semicircularis (arrow). HE. Bar = 40 μm.

FIGURE 3. Alligator 40523 (Lake Griffin). Lymphocytic encephalomyelitis, optic tectum. HE. Bar = 40 μm.

FIGURE 4. Alligator 40578 (Lake Griffin). Longitudinal section of sciatic nerve showing degenerating axon (arrow). HE. Bar = 40 μm.

FIGURE 5. A. Alligator 40579 (Lake Griffin). Cross section of lateral tail muscle showing decreased myofiber diameters. B. Alligator 40693 (Lake Woodruff). Cross section of lateral tail muscle showing normal myofiber diameters HE. Bar = 80 μm.

FIGURE 6. Alligator 40675 (Lake Griffin). Skeletal muscle necrosis with loss of striations and fragmentation of myofibers and sarcolemmal nuclear reaction. HE. Bar = 80 μm.

creased cannot be determined, because resources have not been available to conduct regular daylight mortality surveys of lakes other than Lake Griffin. Based on negligible mortality among adult Lake Griffin alligators in years prior to this study, we estimate that the increase could be as much as ten fold. The results also indicated that the yearly average mortality increased significantly during the study. The peak in mortality in the spring is unexplained, but it would be reasonable for it to be related to resumption of feeding, or, in the case of a nutritional deficiency, to depletion of tissue reserves during the winter.

Although the cause or causes of mortality were not determined, clinical and necropsy findings indicated that the nervous system was the organ system primarily affected in the Lake Griffin alligators we examined; six of eight alligators had morphologic and/or electromyographic abnormalities in the central and/or peripheral nervous system. Findings in five of six cases were similar suggesting the cause and pathogenesis were likely to be the same.

Three of the most severely affected alligators had necrosis in the midbrain centered primarily on the torus semicircularis, which is homologous with the mammalian inferior colliculus. Its major function is in integrating auditory input and motor activities (Butler and Hodos, 1996). The presence of head tilt, anisocoria, and unilateral lack of brainstem auditory evoked potentials suggested that CNS abnormalities probably were not limited solely to the torus semicircularis. Samples for toxicologic analyses, virus isolation, as well as morphologic studies were collected for this study, thus, the entire brain was not examined histologically. As a result, it was not possible to examine all parts of the brain thoroughly enough to be certain of locating small focal lesions in other areas. Thus, it is possible that a complete histologic examination of all parts of the brain would reveal similar lesions in a larger proportion of affected alligators or in additional parts of the brain. An important question for future studies is whether such lesions result from a toxicant that targets specific areas of the brain, or from less specific effects, such as those of hypoxia or a nutritional deficiency.

Histologic and electron microscopic examination of the peripheral nervous system provided no evidence of myelin degeneration, and morphologic evidence of axonal degeneration was too rare and subtle to justify a diagnosis of neuropathy in the absence of other findings, even though such evidence was found only in Lake Griffin alligators. Nerve conduction velocities were somewhat more variable than is typical of domestic mammals examined in the VMTH Clinical Neurology Laboratory, but differences between mean distal NCV's were statistically significant whether Lake Griffin alligators were compared with Lake Woodruff alligators, or clinically impaired alligators with clinically normal ones. It is possible that NCV's in alligators change with age or size. The effect of age could not be evaluated, because the age of wild alligators can be determined only approximately, but there was no relationship between NCV's and length, weight, or weight/length ratio. Therefore, there was no evidence that the results were influenced by such factors. Furthermore, the alligators that were most severely impaired clinically had the lowest NCV's. We believe that the EMG results provide strong evidence of peripheral nervous system dysfunction in these cases, but the relationship between the peripheral neuropathy and the encephalopathy remains to be determined.

The significance of encephalomyelitis in one alligator and skeletal myopathy in another alligator remains to be determined. However, we think it unlikely that the diffuse encephalomyelitis in alligator 40523 is causally related to either the focal midbrain encephalopathy or to the peripheral neuropathy. The nonsuppurative character of the encephalomyelitis suggests a nonbacterial infectious etiology but no evi-

dence of an etiologic agent was found by electron microscopy. Degenerative myopathy in alligator 40675 also may not be related to the nervous system abnormalities. Such lesions most commonly result from vitamin E-selenium deficiency or exertional myolysis, and only rarely are due to toxicants.

Hematology, serum chemical analysis, and blood culture results were uninformative. Affected alligators had significantly higher total white blood cells and neutrophil counts and lower eosinophil counts than apparently healthy alligators. These probably were nonspecific stress responses. Clinically abnormal alligators had significantly lower serum chloride and phosphorus concentrations than normal animals. The cause and significance of these findings is unclear. Increased serum urates and albumin in affected alligators is likely the result of hemoconcentration. Both Lake Griffin and Lake Woodruff alligators had increased serum ALT, AST, and CK enzyme activities (Coulson and Hernandez, 1983; Duncan et al., 1994; Campbell, 1996), which in the case of CK values were dramatic in some alligators. This pattern suggests muscle injury. Although there was a trend toward higher values in Lake Griffin alligators, increases occurred in both Lake Griffin and Lake Woodruff alligators, and the difference between mean values was not statistically significant. Only one alligator had histologically evident myodegeneration.

Encephalopathy and neuropathy can be caused by numerous toxicants, but a plausible route of exposure for alligators is not evident in most cases. Such toxicants include several metals and organometals such as lead, selenium, thallium, methyl mercury, trimethyl tin, and arsenilic acid (Jubb and Huxtable, 1992; Anthony et al., 1996; Goyer, 1996). However, results of analyses of liver and kidney samples from two severely affected Lake Griffin alligators and two Lake Woodruff alligators provided no evidence of exposure to such substances, and concentrations were similar to those reported by others (Burger et al., 2000). Organophosphorus-induced delayed neuropathy is a well recognized upper motor neuron syndrome characteristic of poisoning by certain non-insecticide organoposphorus esters used as defoliants or as components of industrial lubricants, such as tri-*ortho*-tolyl phosphate (Jubb and Huxtable, 1992; Ecobichon, 1996), but there is no known route of exposure to such chemicals for Lake Griffin alligators. Exposure to residues of persistent organochlorine pesticides is a concern because of known contamination of aquatic ecosystems such as at Lake Apopka (Heinz et al., 1991; Matter et al., 1998). We think it extremely unlikely that such substances are responsible for neurologic disease among Lake Griffin alligators. Analytical results gave no evidence of unusual exposure of Lake Griffin alligators to such substances, nor of accumulation of amounts likely to lead to overt toxicosis; we consider the small amounts of a few organochlorine residues found in some samples to be typical of alligators in central Florida due to past agricultural usage. Furthermore, findings similar to those in affected Lake Griffin alligators are not reported even for organochlorine pesticides that are relatively neurotoxic for vertebrates, such as toxaphene. We suggest that future studies should investigate other possibilities more consistent with the findings reported here, such as exposure to methyl bromide, a soil fumigant that is widely used in Florida (USDA, 1995) and that is reported to cause peripheral neuropathy and degeneration of the inferior colliculus in humans (Cavanagh, 1992; Squier et al., 1992).

Numerous biological toxins from plankton, bacteria, fungi, and plants are known to affect either the peripheral nervous system or specific areas of the central nervous system, although a plausible route of exposure of alligators also does not exist for most of these. Examples include kainic and domoic acids from marine diatoms and algae; botulinum and other clostridial toxins; mycotoxins, such as that of *Fusar-*

ium moniliforme, which causes leukoencephalomalacia in horses; and a variety of plant toxins, such as that of *Centaurea solstitialis,* or yellow star thistle, the cause of nigropallidal encephalomalacia in horses (Jubb and Huxtable, 1992). The possibility of exposure to cyanobacterial toxins has become a concern in Florida because of the proliferation in eutrophic Florida lakes of *Cylindrospermopsis raciborskii,* a species of nitrogen-fixing, potentially toxic cyanobacteria that has become the dominant phytoplankton in Lake Griffin (Chapman and Schelske, 1997). Cyanobacteria can produce a variety of neurotoxic and hepatotoxic metabolites (Carmichael, 1994; Sivonen, 1996); however, the results of this study do not support the hypothesis that Lake Griffin alligators are affected by known cyanobacterial toxins. Hepatotoxic cyanobacterial toxins, such as cylindrospermopsin and microcystin, are unlikely to be involved, inasmuch there was no histologic evidence of liver injury in any of the affected alligators. Cyanobacteria also can produce neurotoxic substances, but these toxins act at the neuromuscular junction (Carmichael, 1994). The EMG results gave no evidence of impaired neuromuscular junction activity, inasmuch as repetitive stimulus responses were normal in all alligators. These results, and the lack evidence of differences in serum cholinesterase activities between Lake Griffin and Lake Woodruff alligators, also provide evidence against acute organophosphate poisoning, which is similarly characterized by neuromuscular junction blockage. Furthermore, some affected alligators had CNS lesions that are not reported to be caused by such agents.

Also to be considered are oxygen deprivation and agents that interfere with cellular respiration, such as nitrate or nitrite, carbon monoxide, cyanide, and fluoroacetate, although none of these seems likely. It is possible that the brain lesions are simply due to hypoxia from drowning, inasmuch as some of the most severely affected alligators were observed swimming in circles or struggling to get their heads above water to breathe. However, in some cases drowning clearly was not the cause of death. Dead alligators were found by FWC personnel on the lake shore at distances from the water unlikely to have resulted from wave action, and in some cases sick alligators were observed by members of the public to have crawled out of the water and died.

Nutritional deficiencies should be considered as possible causal or contributory factors. Chief among these is thiamine deficiency, which is a recognized cause of both neuropathy and encephalopathy in several species (Jubb and Huxtable, 1992), and which can be due either to primary dietary deficiency or to diets containing thiaminase. Thiamine deficiency has been observed in farmed alligators fed shrimp bycatch (J. C. Newton, pers. comm.). Affected alligators had acute necrotizing brain lesions similar to those we found in severely affected Lake Griffin alligators, but clinical signs were considerably different. Alligators with thiamine deficiency appeared to be blind and showed increased aggression, whereas affected Lake Griffin alligators were lethargic and unresponsive, and did not appear to be blind. Deficiencies of vitamin E, selenium, riboflavin, and copper all are associated with degenerative nervous system lesions in at least one species (Jubb and Huxtable, 1992). Analytical results suggested that Lake Griffin alligators had lower liver concentrations of copper than Lake Woodruff alligators, but analysis of additional samples will be required before it can be determined whether this actually is the case. If nutritional factors are involved, considerable effort is likely to be required to understand their origin, inasmuch as presently there is no evidence that the diet of Lake Griffin alligators differs greatly from that of alligators in other eutrophic lakes in central Florida.

Preliminary results of continuing studies of Lake Griffin alligators indicate that the animals continue to be affected, and that

nutritional factors may be involved. To date, 11 additional cases of encephalopathy have been identified, and results of analyses in progress indicate that tissues of Lake Griffin alligators have significantly lower concentrations of thiamine than those of Lake Woodruff alligators (J. P. Ross, pers. comm.). Future studies are now being planned to evaluate the relationships among erythrocyte transketolase activity (Bayoumi and Rosalki, 1976), tissue thiamine concentrations, nervous system function, and presence of encephalopathy.

The lack of unusual morbidity and mortality among alligator populations of other Florida lakes suggests that the cause or causes are related to a factor or factors unique to the Lake Griffin environment. Lake Griffin has a history of pollution from agricultural, industrial, and municipal wastes. Approximately 2,600 ha of deep muck soils on the east side of the lake were converted to vegetable farms during the 1940's and 1950's. To maintain optimum moisture conditions on these farms, they were periodically flooded, and excess water, laden with nutrients and pesticides, was pumped back into the lake. Major citrus groves were planted during this same time period and, until 1985, were present on over one-third of the surrounding uplands. Lake Griffin has received waste water from a citrus processing plant in Leesburg and municipal storm water runoff, both of which have been important sources of nutrients and chemicals. These sources of pollution are generally thought to have contributed to the gradual degradation of water quality of Lake Griffin. However, nutrient and chemical inputs from these sources have been substantially reduced. The St. Johns River Water Management District purchased the vegetable farms for reclamation during 1990–1992, and the citrus groves were destroyed by severe freezes during 1983 and 1985.

Although other Florida lakes have been similarly affected by nutrients and contaminants without observed increases in alligator mortality, the decline in the population of alligators of Lake Apopka in the 1980s was associated with increased numbers of dead adult alligators as well as severely decreased egg hatch rates and increased mortality of hatchlings among Lake Apopka alligators (Woodward et al., 1993; Masson, 1995; Rice, 1996). The causes of increased adult and hatchling mortality were not investigated. Investigations of the decline have focused almost exclusively on possible reproductive toxicity of organochlorine pesticide residues (Matter et al., 1998), but have not provided convincing evidence of a causal relationship between the decline and exposure to such residues. In the mid- and late 1990's, hatch rates for Lake Griffin alligator eggs also decreased below that of previous years (Cardeilhac et al., 1998). These observations raise concerns that Lake Griffin alligators may be at risk of undergoing a population decline similar to that which occurred among Lake Apopka alligators. Further investigation clearly is needed to evaluate risks to the populations of alligators and other wildlife species of Lake Griffin. Although currently there is no evidence of significant risks to human health, such risks also need to be determined, even if only to allay the public concern that has arisen as a result of publicity regarding the alligator deaths.

Future studies must take into account several important factors. First, a better understanding of the nature of the condition is needed, both to help reduce the number of possible causes to consider and to provide the basis for determining whether putative causes that might be tested in future experimental studies produce disease that is consistent with that in naturally affected alligators. Second, although the most likely causes are among the numerous toxicants known to affect the nervous system, few individual toxicants are known that could produce all observed abnormalities. The possibility should be considered that there are multiple, interacting causes, each of which is

necessary, but none alone is sufficient, to result in the observed condition or conditions. Third, any conclusions as to the role of toxicants would have to account for effects on other species, or lack thereof. To date, some dead longnose gar (*Lepisosteus osseus*) and Florida softshell turtles (*Apalone ferox*) have been observed on Lake Griffin, but systematic efforts to document illness or mortality among species other than alligators have not been possible. A fourth problem is that so little is known of naturally occurring diseases, especially potential toxicoses or the relative susceptibility of wild alligators to potential toxicants. For example, methyl mercury is highly neurotoxic to mammals and birds, but alligators appear to be much more resistant. Alligators in south Florida bioaccumulate mercury and were found to have significant amounts of mercury in nervous and other tissues, but there was no clinical evidence of neurologic impairment or histologic evidence of nervous system damage (Heaton-Jones et al., 1997). Alligators also could respond in unanticipated ways to known toxicants, or they might be unusually susceptible to substances not heretofore determined to be neurotoxic in other species.

ACKNOWLEDGMENTS

This work was supported by the Florida Fish and Wildlife Conservation Commission. We thank D. Brown and M. B. Brown for culturing samples for mycoplasmas, E. Greiner for identifying parasites, M. Kalichman for peripheral nerve histomorphometry, and J. C. Newton for case materials from alligators with thiamine deficiency. We also thank those who provided valuable suggestions, consultation, or other assistance: W. Carmichael, R. Dawson, E. Gallagher, T. S. Gross, L. J. Guillette, Jr., B. Hall, D. Heaton-Jones, B. Homer, E. Jacobson, S. Jones, W. P. Knisley, H. F. Percival, D. Pollock, L. J. Richey, J. P. Ross, M. Spalding, J. Stevens, K. Vliet, and J. Yonchek.

Histologic sections were prepared in the University of Florida Interdisciplinary Center for Biotechnology Research (UF ICBR) Affiliate Research Histology and Biomechanics Core Laboratory or the histology laboratory of the Department of Pathobiology. Histomorphometry was done in the Comparative Neuromuscular Laboratory of the University of California San Diego. Electron microscopy was done by the UF ICBR Electron Microscopy Core Laboratory. Toxicologic analyses were done in the University of Pennsylvania Laboratory of Large Animal Pathology and Toxicology and the Mississippi State Chemical Laboratory at Mississippi State University.

LITERATURE CITED

ANTHONY, D. C., T. J. MONTINE, AND D. G. GRAHAM. 1996. Toxic responses of the nervous system. *In* Casarett & Doull's toxicology. The basic science of poisons. C. D. Klaassen (ed.). McGraw-Hill, New York, New York, pp. 463–486.

BAYOUMI R. A., AND S. B. ROSALKI. 1976. Evaluation of methods of coenzyme activation of erythrocyte enzymes for detection of deficiency of vitamins B1, B2, and B6. Clinical Chemistry 22: 327–335.

BROWN, D. R., T. L. CLIPPINGER, K. E. HELMICK, I. M. SCHUMACHER, R. A. BENNETT, C. M. JOHNSON, K. A. VLIET, E. R. JACOBSON, AND M. B. BROWN. 1996. Mycoplasma isolation during a fatal epizootic of captive alligators (*Alligator mississippiensis*) in Florida. International Organization of Mycoplasmology Letters 4: 42–43.

BURGER, J., M. GOCHFELD, A. A. ROONEY, E. F. ORLANDO, A. R. WOODWARD, AND L. J. GUILLETTE, JR. 2000. Metals and metalloids in tissues of American alligators in three Florida lakes. Archives of Environmental Contamination and Toxicology 38: 501–508.

BUTLER, A. B., AND W. HODOS. 1996. Comparative vertebrate neuroanatomy. Evolution and adaptation. Wiley-Liss, New York, pp. 216–234.

CAMPBELL, T. W. 1996. Clinical pathology. *In* Reptile medicine and surgery. D. R. Mader (ed.). W. B. Saunders Co., Philadelphia, Pennsylvania, p. 253.

CARDEILHAC, P. T., D. L. WINTERNITZ, AND J. D. BARNETT. 1998. Declining reproductive potential of the alligator population on Lake Griffin in central Florida. Proceedings of the International Association for Aquatic Animal Medicine 29: 30–37.

CARMICHAEL, W. W. 1994. The toxins of cyanobacteria. Scientific American 270: 78–86.

CAVANAGH, J. B. 1992. Methyl bromide intoxication and acute energy deprivation syndromes. Neuropathology and Applied Neurobiology 18: 575–578.

CHAPMAN, A. D., AND C. L. SCHELSKE. 1997. Recent appearance of *Cylindrospermopsis* (Cyanobacteria) in five hypereutrophic Florida lakes. Journal of Phycology 33: 191–195.

COULSON, R. A., AND T. HERNANDEZ. 1983. Alligator metabolism. Pergamon Press, New York, New York, p. 9.

DUNCAN, J. R., K. W. PRASSE, AND E. A. MAHAFFEY.

Veterinary laboratory medicine. Clinical pathology, 3rd Edition. Iowa State University Press, Ames, Iowa, p. 253.

ECOBICHON, D. J. 1996. Toxic effects of pesticides. *In* Casarett & Doull's toxicology. The basic science of poisons. C. D. Klaassen (ed.). McGraw-Hill, New York, New York, pp. 643–689.

GOYER, R. A. 1996. Toxic effects of metals. *In* Casarett & Doull's toxicology. The basic science of poisons. C. D. Klaassen (ed.). McGraw-Hill, New York, New York, pp. 691–736.

HEATON-JONES, T. G., B. L. HOMER, D. L. HEATON-JONES, AND S. F. SUNDLOF. 1997. Mercury distribution in American alligators (*Alligator mississippiensis*) in Florida. Journal of Zoo and Wildlife Medicine 28: 62–70.

HEINZ, G. H., H. F. PERCIVAL, AND M. L. JENNINGS. 1991. Contaminants in American alligator eggs from lakes Apopka, Griffin and Okeechobee, Florida. Environmental Monitoring and Assessment 16: 277–285.

JUBB, K. V. F., AND C. R. HUXTABLE. 1992. The nervous system. *In* Pathology of domestic animals, 4th Edition, Vol. 1. K. V. F. Jubb, P. C. Kennedy and N. Palmer (eds.). Academic Press, San Diego, California, pp. 360–362.

MASSON, G. R. 1995. Environmental influences on reproductive potential, clutch viability, and embryonic mortality of the American alligator in Florida. Ph.D. Dissertation, University of Florida, Gainesville, Florida, 123 pp.

MATTER, J. M., D. A. CRAIN, C. SILLS-MCMURRY, D. B. PICKFORD, T. R. RAINWATER, K. D. REYNOLDS, A. A. ROONEY, R. L. DICKERSON, AND L. J. GUILLETTE JR. 1998. Effects of endocrine-disrupting contaminants in reptiles: Alligators. *In* Principles and processes for evaluating endocrine disruption in wildlife. R. J. Kendall, R. L. Dickerson, J. P. Giesy and W. P. Suk (eds.). Society of Environmental Toxicology and Chemistry Press, Pensacola, Florida, pp. 267–289.

MCDOWELL, E., AND B. TRUMP. 1976. Histological fixatives for diagnostic light and electron microscopy. Archives of Pathology and Laboratory Medicine 100: 405–414.

OLSON, G. A., J. R. HESSLER, AND R. E. FAITH. 1975. Technics for blood collection and intravascular infusion of reptiles. Laboratory Animal Science 25: 783–786.

PIERSON, F. W. 2000. Laboratory techniques for avian hematology. *In* Schalm's veterinary hematology. B. F. Feldman, J. G. Zinkl and N. C. Jain (eds.). Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, p. 1145.

PROPHET, E. B., B. MILLS, J. B. ARRINGTON, AND L. H. SOBIN (eds.). 1994. Armed Forces Institute of Pathology laboratory methods in histotechnology. American Registry of Pathology, Washington, D.C., pp. 90–91.

RICE, K. G. 1996. Dynamics of exploitation of the American alligator: Environmental contaminants and harvest. Ph.D. Dissertation, University of Florida, Gainesville, Florida, 165 pp.

SHEEHAN, D. C., AND B. B. HRAPCHAK. 1980. Theory and practice of histotechnology. Battelle Press, Columbus, Ohio, pp. 263–264.

SIVONEN, K. 1996. Cyanobacterial toxins and toxin production. Phycologia 35: 12–24.

SNEDECOR, G. W., AND W. G. COCHRAN. 1980. Statistical methods. Iowa State University Press, Ames, Iowa, 507 pp.

SQUIER, M. V., J. THOMPSON, AND B. RAJGOPALAN. 1992. Case report: Neuropathology of methyl bromide intoxication. Neuropathology and Applied Neurobiology 18: 579–584.

TULLY, J. G., R. F. WHITCOMB, H. F. CLARK, AND D. L. WILLIAMSON. 1977. Pathogenic mycoplasmas: Cultivation and vertebrate pathogenicity of a new spiroplasma. Science 195: 892–894.

UNITED STATES DEPARTMENT OF AGRICULTURE. 1995. Agricultural Chemical Usage—Vegetables—1994 Summary. United States Department of Agriculture, National Agricultural Statistics Service, Economic Research Service, Washington, D. C., 287 pp.

WOODWARD, A. R., H. F. PERCIVAL, M. L. JENNINGS, AND C. T. MOORE. 1993. Low clutch viability of American alligators on Lake Apopka. Florida Scientist 56: 52–63.

Received for publication 27 December 2000.