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ASSESSMENT OF EFFECTS OF DIET AND THIAMIN ON LAKE GRIFFIN ALLIGATOR MORTALITY:

Final report to St. Johns River Water Management District
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EXECUTIVE SUMMARY

Mortality and low egg hatch rate was demonstrated in alligators in Lake Griffin between 1998 and 2001. Previous studies have shown the alligator mortality is due to neurological impairment. Mortality appears not to be the direct result of contaminants (organochlorines, organophosphates or heavy metals), toxic algae or disease. We have examined the hypothesis that the mortality is due to a syndrome of thiamin deficiency induced by eating a diet of fish (gizzard shad, *Dorosoma cepedianum*) that may be high in thiaminase.

We have previously shown that Lake Griffin alligators showing a lack of coordination in the field and neurological pathology have lower thiamin levels than apparently healthy alligators or alligators from other lakes. In this study during 2002-2003 we continued to monitor alligator mortality; attempted to experimentally induce thiamin deficiency in captive alligators with a diet of shad; examined the diet of alligators in Lake Griffin compared to the diet of alligators in Lake Woodruff and Lake Apopka; and assessed gizzard shad in Lake Griffin and Lake Apopka for thiaminase activity.

Mortality of alligators in Lake Griffin during 2002 and 2003 was greatly reduced from previous years with just 5 dead alligators located in 2003. Several simultaneous changes in the Lake Griffin system occurred in this period including increases in water level, improvements in water quality, reduction in blue-green algal blooms and removal of large numbers of shad by commercial fishing.

Seven alligators were captured in the wild from Lake Griffin and Lake Woodruff, held in captivity and fed gizzard shad. Over a period of 6-12 months they maintained weight but blood, muscle and liver thiamin levels were low and three animals died demonstrating symptoms similar to those seen in Lake Griffin. The individuals with the greatest reduction in thiamin died. We also treated two alligators by feeding them thiamin rich food and providing thiamin supplements. Thiamin levels in the treated alligators returned to higher levels comparable to healthy wild alligators.

By examining the biomass of fresh prey items eaten we demonstrate differences in the proportion of different prey eaten by alligators among the three lakes and show that the quantity of shad eaten by Lake Griffin alligators declined in 2002-03 compared to previous years. However, the diets of alligators in Lake Woodruff and Lake Apopka have a large proportion of gizzard shad.

We show for the first time that gizzard shad in Lake Griffin and Lake Apopka have very high levels of thiaminase, higher than those reported to cause thiamin deficiency in salmon in the Great Lakes. These high thiaminase levels are present in most months and across a wide range of sizes of shad.

These results support the hypothesis that a diet of gizzard shad causes thiamin deficiency and neurological pathology leading to death in alligators in Lake Griffin. However, the absence of the syndrome in other lakes (e.g. Lake Apopka) where conditions are similar, suggests other factors are involved in Lake Griffin. While the shad do have thiaminase, and a diet of shad can cause thiamin deficiency, other factors may affect the vulnerability of alligators and the final effect. Review of results to date and expert discussion is recommended to direct further research on this topic.

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The following files are attached as Excel files and provide raw data of the work summarized and presented in the report.

ACKNOWLEDGEMENTS

In addition to this contract from St. Johns River Water Management District, funding for this project was provided by the Lake County Water Authority and the Wildlife Foundation of Florida (Fl. Fish and Wildlife Conservation Commission). We are grateful to Walt Godwin, Carol Lippincott, Roxanne Conrow and Mike Coveney at the District for continual support for this work. Mike Perry, Joe Hill and the board members of LCWA have also been consistently supportive. Frank Montalbano, Tim O Meara and Harry Dutton at FWC have provided valuable support from their staff. Dwayne Carbonneau (FWC) has been integral to the project's success and Allan Woodward has provided much logistic and intellectual support. Logistic assistance with vehicles and airboats was obtained from Franklin Percival at the Fl. Wildlife Coop Unit-USGS. Harold Morrow and his staff provided assistance and permission for the work on Lake Woodruff National Wildlife Refuge.

Thiamin analyses and much valuable discussion was provided by Dale Honeyfield, Joy Hinterkopf and Emily Eggler of the Biological Research Division, USGS, Leetown Science Center, Northern Appalachian Research Laboratory, Wellsboro PA. Thiaminase analyses were conducted by Scott Brown, Environment Canada. Paul Zajicek provided additional thiaminase data. Dr. Roger Kline of North American Nutrition Companies Inc. kindly supplied us with thiamin to treat our alligators. We are particularly grateful to Sylvia Scudder and Irvy Quitmyer, collection managers of the FLMNH Zooarcheology collection, for access to their collections and allometric data bases and extensive assistance identifying small animal fragments. Trenton Schoeb, University of Alabama, Birmingham and Scott Terrell, Disney Animal World and University of Florida provided expert diagnosis of alligator brain histology and lesions.

Michael Welker managed the captive alligator facility with great diligence and sincere concern for his captive alligators. We are grateful to Don Morgan who provided alligator housing facilities at CST alligator farm. Assistance in the field was provided to A. Rice by many members of the FWC alligator management team including Allan Woodward, John White, Arnold Brunnell and Chris Visscher. Additional field assistance was given by student assistants Jeremy Olsen, Chad Rieschard and Esther Langhan. Matt Milnes, U.F. Department of Zoology, provided alligators from Lake Woodruff. Esther Langhan, and Pat Gomez sorted stomach samples. We are grateful for additional assistance in sample analysis from Henri van Rinsvelt, Gene Dunnam, Ivan Kravchenko and students Ralf Kelly and Brian Wingert, Dept of Physics, University of Florida. Skip Goerner of Fisherman's Wharf provided logistic support and field facilities on Lake Griffin. Dave Mimms (Lake Griffin) and Frank Dean (Lake Apopka) provided us with shad.

INTRODUCTION

Unknown factors are affecting alligators (*Alligator mississippiensis*) in Lake Griffin, Florida (Ross et al. 2000a, b). Between 1994 and 1997 the hatch rate of alligator eggs collected on Lake Griffin was less than 10% compared to 40%-80% normally (Woodward et al. 1999). Egg hatch rate has since recovered to 30%-40% but remains low relative to pristine Florida lakes. Since November 1997, over 420 dead adult alligators have been recorded in Lake Griffin (D. Carbonneau Florida Fish and Wildlife Conservation Commission (FWC) pers. comm.). The deaths were observed in a seasonal spring peak that reached over 100 specimens in some years. Postulated causes include contaminants, disease, nutrition, and toxins produced by blooms of blue green algae. Studies through 2001 showed that organochlorine and organophosphate pesticides, heavy metals, algal toxins, botulism and infectious disease do not seem to be the direct cause (Ross et al. 2002a). We observed alligators demonstrating uncoordinated behavior in Lake Griffin, that subsequently die, often by drowning. Pathology examination has revealed that they are affected by severe neurological pathology of unknown cause (Schoeb et al. 2002). This neuropathology includes slowed nerve conduction velocity, histological changes to the nerves and myelin sheaths and a distinctive lesion of the torus semicircularis of the midbrain.

Similar pathology is reported for salmonid fish which eat prey fish rich in thiaminase (McDonald, et al. 1998) resulting in a thiamin deficiency, adult impairment and hatchling mortality, termed Embryonic Mortality Syndrome (EMS). Our attention was first drawn to thiamin by the similarity between brain lesions reported for thiamin deficient fish and the lesions we observed in alligators (T. Schoeb pers. comm.).

The primary prey species that contain thiaminase are the alewife (*Alosa pseudoharengus*) and smelt (*Osmerus mordax*) in the Great Lakes and herring (clupeids) in the Baltic Sea, all filter feeders. In the Great Lakes and New York Finger Lakes alewives, thiaminase levels are highly variable both seasonally, within lakes and among lakes. The cause of this variability in thiaminase in these filter feeding fish is unknown, but thiaminase-positive algae have been isolated from alewife. (Honeyfield et al., in press). There is also a positive correlation (r = 0.84) between summer abundance of blue green algae, particularly *Microcystis sp*. and incidence of EMS in coho (Hinterkopf et al. 1999). In Florida lakes, gizzard shad (*Dorosoma cepedianum*) is an abundant clupeid filter feeding fish that is eaten by alligators.

Prompted by the similarity to salmonid pathology, we analyzed thiamin levels in alligator tissues collected from Lake Griffin and Lake Woodruff in 1999 and 2000. We have established that alligators in Lake Griffin have lower thiamin levels than alligators in Lake Woodruff and that more seriously impaired alligators have lower thiamin than less seriously impaired specimens (Ross et al. 2002 a, b). The similarity between Florida alligators in Lake Griffin and EMS in salmonids is striking: low thiamin, reproductive failure due to embryo mortality, adult neural impairment and mortality, brain lesions and the association with abundant filter-feeding clupeid forage fish.

This project has addressed four elements of the proposed hypothesis that alligator mortality in Lake Griffin is caused by thiamin deficiency induced by a diet of gizzard shad. We have continued to monitor alligator mortality in Lake Griffin to determine the continuing progress of the phenomenon. We have examined the diet of alligators in three Florida lakes, including Lake Griffin to determine if the proportion of gizzard shad in alligator diets differ among central Florida lakes and whether it changes over time. We have also evaluated the presence of thiaminase in gizzard shad and analyzed seasonal variations in thiaminase levels. We have attempted to induce thiamin deficiency and mortality in captive alligators by feeding them a diet of shad and also investigated the effects of thiamin supplements on thiamin status and condition.

METHODS

ALLIGATOR MORTALITY SURVEY.

Every two weeks an experienced observer (Dwayne Carbonneau of the FL Fish and Wildlife Conservation Commission) patrolled the whole shoreline of Lake Griffin and adjacent waters by airboat. Dead alligators were located visually and sometimes by smell and occasionally reported by lakeside residents. Dead alligators were marked with spray paint to avoid counting animals more than once and, if their condition allowed, their total length and sex determined.

ALLIGATOR CAPTURE.

Alligator capture and handling was conducted under Special Use Permit WXO1261b issued by the Florida Fish and Wildlife Conservation Commission and with Animal Care and Handling Approval D005 issued by the University of Florida. Alligators were located from an airboat at night with a spotlight and captured by pole snare or capture dart, brought to the boat, secured, sexed and measured. The size of captured alligators was measured by both total length (TL tip of snout to end of tail) and snout-vent length (SVL tip of snout to the posterior border of the cloaca). The circumference of the base of the tail was also measured as an additional measure of body condition. Alligators were weighed by suspending them in a canvas sling from a Salter Spring scale accurate to 1 kg.

INDUCTION OF THIAMIN DEFICIENCY IN CAPTIVE ALLIGATORS

Seven wild alligators were captured in July 2002 and moved to an unused alligator farm at Keystone Heights FL made available for this purpose by the owner. The facility consisted of an enclosed building containing four concrete pens either side of a central walkway (8 pens total). Each pen was constructed of concrete walls approximately 110cm high enclosing a concrete floor area of 4m x 4m. Half of the area was set 40 cm below the level of the remainder and held water. Each pen had an independent water supply and drainage system. Water was supplied directly from a deep well at a constant 24°C. On the dry portion of each pen we glued a 2 ft by 8 ft piece of ¾ -inch plywood painted matte black over which were suspended 2 heat lamps of 120 watts at a height of 90 cm that provided a radiant heat source for the alligators. Commercial electric timers were set to turn the heat lamps on and off on a daily cycle that was set 12:12 except from November to March when it was set 8 on:16 off. One alligator was housed in each pen.

Temperature in the facility was monitored with two Onset Corp. 'Hobo-Temp' electronic temperature recorders housed in water proof containers. One unit was deployed centrally in the facility at waist-height in the air and the other was placed in the water of one of the pens. Hobo temps were calibrated against a mercury-in- glass thermometer and programmed to read at 30 minute intervals, giving temperature monitoring coverage for 37 days at a time. Hobos were read on Onset Corp Boxcar for Windows 3.7.2 software and transferred to Excel worksheets for manipulation.

An approximate maintenance diet for alligators was calculated from literature and commercial sources at 7% of body weight of fresh (wet) food weekly. Each alligator was weighed when placed in the facility and then fed approximately 1/3 of the calculated weekly ration three times each week, usually Monday, Wednesday, Friday. Food was weighed to the nearest gram before being placed into the pens and any uneaten food remaining at the next feeding was either weighed or the proportion of the original estimated and the difference recorded as food consumed. A grating prevented all except the smallest particles of uneaten food from being lost into the pen drain. In general, alligators ate either all the food offered or none of it.

Alligators were offered and ate gizzard shad for most of the experimental period. Each animal ate 1 or 2 shad weighing a total of 200- 600 g per meal. Because gizzard shad from Lake Griffin were not available we obtained shad from commercial sources on Lake Apopka in June and September 2002 and fed these to alligators June 2002- March 2003. In February 2003 we were able to obtain gizzard shad from Lake Griffin and alligators were fed Griffin shad for the remainder of the experiment unless otherwise noted. Samples of the shad fed to alligators were submitted for thiaminase analysis as detailed below.

On 24 April 2003 two animals, 50826-50827 and 50828-50829, were randomly selected to change their food to a thiamin rich diet of pork and chicken (thiamin rich foods USDA 1991). Thiamin hydrochloride (Roche product code 04 1303 8) was obtained in powdered form from North American Nutrition Co. A stock solution was prepared of 5g ThHCl dissolved in 200 ml injectable normal saline solution. On 6 June 2003 the two thiamin-supplemented alligators received approximately 250 mg of thiamin as an injection of 10 ml of stock solution. Quantities of between 274 and 384 mg of powdered thiamin (mean 344 mg, S.D. 36 mg) were placed in gelcap capsules. One of these capsules was placed into the food of each of the supplemented alligators on six occasions between 16 June and 14 July 2003 (total possible thiamin dose 2064 mg). However, we were unable to determine if the thiamin capsules were actually eaten or if they dissolved before being eaten.

At monthly intervals each alligator was captured, weighed to the nearest ¼ kg on a spring balance and a blood sample (7-10 ml) taken from the post occipital venous sinus. The reflex and neurological status of each animal was evaluated from a series of observations and tests:

- General behavior prior to capture- movement, basking, orientation, coordination
- Vigor of resistance to capture (graded as vigorous, OK, weak)
- Toe pinch test- with the animal restrained and quiet, a sharp pinch to a rear toe was administered with a pair of hemostats. Response was graded as normal (rapid withdrawal of the pinched toe and that foot) or weak (flaccid, slight or no withdrawal).
- Righting response- under restraint (mouth bound, eyes covered) the animal was rolled over onto its dorsal surface. Response was graded as vigorous (immediate energetic

writhing and return to normal vent down position) or weak (weak, ineffective or delayed response).

On three occasions in November 2002, March and June 2003 we also collected small muscle biopsy samples from each animal's tail muscle. Muscle, brain and liver samples were also obtained from each animal after it died or was euthanized at the end of the experiment in August 2003.

Blood and tissue samples were held on ice. Blood samples were removed to the laboratory where they were centrifuged at 6000 rpm for 5 minutes, and supernatant plasma was removed and stored in 2 ml cryovials. The remaining hematocrit was resuspended in normal saline (8 g NaCl dissolved in 1000 ml distilled water), centrifuged again at 6000 rpm for 5 min. The resultant mass of saline-washed red blood cells (RBC) was removed and stored in 2 ml cryovials. Tissue, plasma and RBC were kept in a freezer at -70°C until shipped on dry ice for analysis.

Alligators that died during captivity were observed for symptoms prior to death, removed as soon as possible to a chiller and then necropsied. We examined the animals externally and internally for any indication of cause of death and removed samples of liver, tail muscle and the whole brain. The brain was bisected sagitally and half fixed in 10% formalin for histology and half stored frozen. Blood and tissue samples were shipped on dry ice to the USGS Appalachian Research Center, Wellsboro PA for analysis of thiamin.

The method used for thiamin analysis was essentially that in Brown et al (1998) with the minor modifications of the elution gradient. The Shimadzu (Columbia, MD 21046) HPLC system was a 10Avp. All reagents were made with Burdick Jackson HPLC water (VWR Scientific Products, So. Plainfield, NJ 07080) 25 mM ammonium phosphate (Sigma Chemicals, St. Louis, MO 63178) buffer was used instead of potassium phosphate. The flow rate was set at 1 ml/min and the following binary gradient was used: For 0.1 min. 100% buffer (0% DMF) was pumped, during 0.1 to 1 min. DMF was increased to 0.5%, between 1 and 5 min. DMF went to 10%, from 5 to 7 min. %DMF changed to 30, at 9 min. DMF was up to 35% and it was held for 1 min. from 9 to 10 minutes. At 10.10 min. DMF dropped to 0 and buffer was maintained at 100% for the remainder of the run. 11.8 minutes were allowed for re-equilibration of the column and total run time was 22 minutes.

Modifications to sample preparation were : Tissue amount used was decreased to approximately 0.1 to 0.2 g for liver samples and 0.3 to 0.6 g for muscle samples with the larger mass used if thiamin amount was known to be low. We used 3 ml of 5% trichloroacetic acid (Fisher Scientific, Pittsburgh, PA 15275) with 2 uM HgCl added to inhibit thiaminase activity if present, then sample was homogenized with a teflon homogenizer. Tubes were boiled for exactly 5 minutes. Immediately upon removal from boiling bath, 3 ml ice cold 10% TCA was added, tubes were immersed in an ice bath where they remain until centrifuging and the samples were rehomogenized. Tubes were allowed to sit for at least 15 min prior to centrifuging for 10 min at 0º C. Three ml of supernatant were pipetted into a glass tube and refrigerated overnight to be used within 24 hours, and 3 ml were pipetted into small plastic tubes and frozen at –20º C where they are stable for several months. Samples were washed with hexanes and ethyl acetate according to Brown et al. (1998). Potassium ferricyanide and NaOH were added simultaneously to the vials. Vials were mixed thoroughly once capped.

ALLIGATOR DIET.

Stomach samples were collected using the lavage and Heimlich maneuver method described by Fitzgerald 1989. To calibrate the method on alligators, stomach samples were recovered by lavage from 38 live alligators subject to later euthanasia and necropsy. Comparison of stomach contents recovered by lavage and post mortem confirmed that 100% of contents can be reliably recovered by lavage. Incomplete removal of contents is usually obvious during lavage due to the low outflow, lack of material and palpation of the remaining stomach contents. In this study a small number of alligators for which lavage was judged to be incomplete were eliminated from the analysis.

Water for stomach pumping was provided from a domestic water supply or for field application we modified a Teel 1P985B thermoplastic pump and 3.5 hp Briggs and Stratton motor to provide water flow of 5-100 gal/minute. Lavage tubes were made of clear plastic ("Tygon") thick walled laboratory tubing of approx. 2 m length and 10 mm, 15 mm and 20 mm ID, constructed with a carefully smoothed distal insertion end and fitted to a standard hose fitting at the other. Due to the low pH and subsequently low residence time of most prey items in alligator stomachs, (Barr, 1997) special effort was made to stomach pump and collect content within 1-3 hours after capture.

Individuals selected for stomach pumping were laid on an 8 ft by 1.3 ft by 3/4 inch plywood plank, secured with straps and placed vertically- inclined 'head-up' to about 45 degrees on a wood sawhorse. The subjects' mouths were then opened by gentle manipulation and a PVC (Schedule 40, heavy duty) pipe of approximately 18 cm (7 inch) diameter was placed between the jaws and secured in place with either duct tape or multiple large rubber bands. A lavage hose was selected based on the animal's size, coated with clear mineral oil for lubrication and then inserted into subject's mouth and down the esophagus. An external indicator was identified during necropsy to locate the posterior of the stomach wall. The insertion depth needed to enter the individual's stomach can be measured from the tip of the snout to the fourth whorl of scutes anterior to the hind legs. Overcoming the resistance of the pyloric sphincter is an additional indication of correct tube placement into the stomach.

After the lavage tube was correctly in place, subjects were then tilted in a seesaw type motion over the sawhorse to a head down ~ 30-degree decline, then water applied through garden hose spigot (or pump). One person, either reaching around or straddling the alligator, then administered Heimlich maneuver-like thrusts to the stomach region of the abdomen. Simultaneously, a second person held the tubing in place and collected the expelled stomach contents and water in an 18-gallon plastic container. Approximately 15 gallons of water was flushed through the animal's stomach at a time, then water was shut off and the animal allowed to breath. The procedure was then repeated two more times and stomach contents filtered through a paint-grade, fine mesh nylon strainer. The mesh strainer was calibrated under a 20X binocular microscope against known size particles and a fine ruler and established to retain all particles of >0.5 mm diameter.

Stomach contents were then stored in 1 L screw top plastic jars, completely immersed in 10% buffered formalin. Subsequently, samples were washed through the same mesh strainer with water to remove formalin, then preserved in 70% ethanol. Samples were decanted into a tray and individual items removed by hand and similar items placed together in storage vials for identification and quantification. Identification was by standard keys and comparison to a reference collection of hard parts (bones, scales, feathers, claws, hairs etc.) in the collections of the Florida Museum of Natural History (FLMNH). The stomach content samples were identified to the lowest taxonomic level and minimum number of individuals was determined by counting unique preserved parts.

Quantitative analysis of the stomach content samples was expressed as frequency of occurrence and percent composition by estimated mass. The equation $n/t \times 100$ was used to determine frequency of occurrence, where n = the number of stomach content samples containing a given food item and t = the total number of stomach content samples in a given lake. The minimum number of individuals of each prey type were gauged from the number of unique elements (e.g. skulls, opercula etc.). The mass of the ingested prey items was determined from available allometric studies (Hoyer and Canfield 1994, Dunning 1993, Burt & Grossenheider 1998) or by morphometric relations determined from samples of organisms in the collection of the FLMNH or collected in the field. We also differentiated freshly ingested items from those represented only by persistent, undigestible hard parts that may have been in the stomach for an unknown, extended time. Alligator stomachs have a very low pH and powerful digestive enzymes that completely digest soft parts and cartilage in a few hours and decalcify and dissolve bone in 24-48 hours. We followed Barr (1997) in classifying vertebrates that had articulated spines or articulated non-eroded bones which retained flesh, snails with attached flesh and invertebrates that were more or less whole as 'fresh' meals probably ingested within 24-48 hours. Other prey were identified from single bones, feathers, keratinized scutes, scales, tests and opercula and could often be identified to species but were considered remnants of older meals. The relative abundance of different prey was expressed as the proportion of the total estimated ingested mass of fresh prey items. This measure estimates the contribution by mass of each kind of prey item to the alligator's diet. These calculations were performed for each fresh prey item in each stomach and summed for the stomachs from each lake.

THIAMINASE IN GIZZARD SHAD.

Samples of 6 to 10 shad (*Dorosoma cepedianum and D. petenense*) were collected monthly on Lake Griffin using a cast net thrown from a small boat or airboat. Shad were measured (fork length in cm) and weighed then dropped immediately onto dry ice (CO_2) for preservation of enzyme titers. To ensure minimal change of enzyme levels, fish were frozen solid immediately on capture and maintained frozen solid until analyzed. Frozen shad were stored at –70°C until they could be prepared for shipping and analysis. A sample of shad was preserved in formalin for identification.

Additional samples of shad were obtained from the fresh catch of commercial fishermen who capture shad in gill nets under permit from FWC. Fresh dead fish were selected from the catch, stored on ice for transport to Gainesville (2 hours) then frozen solid. We also analyzed thiaminase from a sample of gizzard shad caught in previous years and treated as the commercial samples above, transported on ice for 2-4 hours prior to freezing.

Whole frozen shad were macerated in a plastic bag by crushing them with a mallet against a hard surface then the pieces ground to powder in a heavy duty Waring blender. The blender container was pre-cooled in dry ice and a small quantity of dry ice ground with the fish with care taken to discard any thawed material. The mixed fish-and-dry ice powder was then stored in an open plastic bag at -70°C to allow the CO_2 to sublimate off. The bags were then closed and shipped on dry ice by courier for analysis. To provide sufficient material for analysis, several specimens of smaller fish (less than 10 g weight and 8 cm fork Length) were pooled together. All samples were received for analysis in solid frozen condition.

Thiaminase analyses were conducted by Dr. Scott Brown, Environment Canada, Burlington Ontario, Canada. Total thiaminolytic activity was determined in extracts of selected fish or their tissues, using a radiometric method (14C-thiamine) developed by Zajicek et al. (2001. The assay is based on the procedures of Edwin and Jackman (1974) and McCleary and Chick (1977) that was optimized in terms of substrate and co-substrate concentrations, incubation time, and sample dilution. To ensure assay to assay reproducibility and comparability, "in-house" control material prepared from Lake Michigan alewives was used as a positive standard for each assay.

RESULTS 1. ALLIGATOR MORTALITY

The number of alligators found dead in Lake Griffin in 2002 and 2003 was the lowest we have recorded since beginning this project in 1998. Just 26 dead alligators were noted in 2002 and five in 2003, which is comparable to natural mortality seen on other Florida lakes. It is apparent that the severity of the alligator mortality has been declining since the peak in 2000. No 'sick' alligators demonstrating poor coordination or any other symptoms were observed in 2002 or 2003.

Effort and diligence of search were identical to previous years, one full day spent every second week carefully searching the whole shoreline of the lake. Water levels were higher in 2002- 2003 than in 1999-2001 and this increased the area of inundated marginal woodland where dead alligators might not be seen. However, dead alligators are large, obvious and very smelly items that usually attract scavengers such as vultures and crows. Numbers of dead alligators were counted in the flooded woods in 1999 and 2000. Also much of the shoreline of Lake Griffin does not have flooded woods and many alligators were counted in these areas in earlier years. The reduction in mortality appears to be a real effect and not an artifact of search effort or conditions.

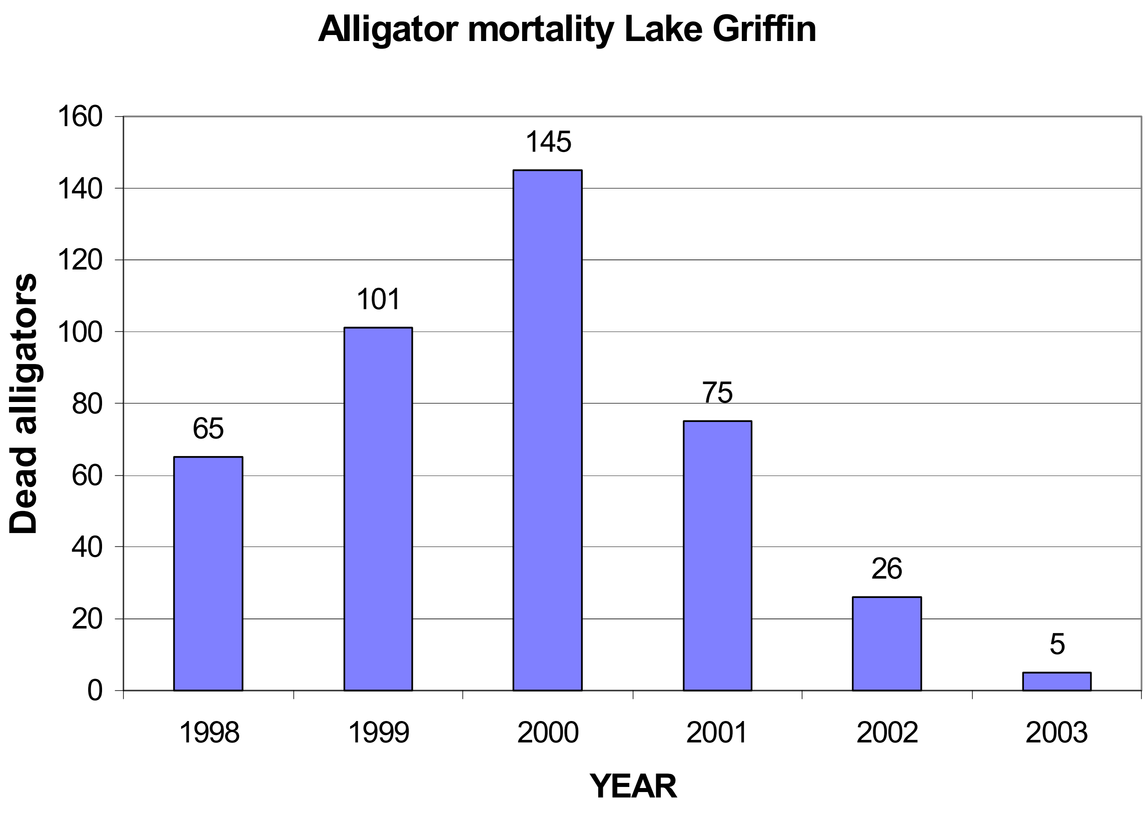

Figure 1. Annual mortality of alligators at Lake Griffin, FL, 1998-2003. Dead alligators counted in biweekly surveys. Data courtesy D. Carbonneau FWC.

RESULTS 2. EXPERIMENTAL INDUCTION OF THIAMIN DEFICIENCY

Ambient temperature is an important variable that affects alligator metabolism and health. Alligator's preferred active temperature is around 31°C. When given opportunities to regulate body temperature (Tb) by basking or moving between warmer and cooler areas they maintain Tb between about 22° and 32° in the wild (Howarter 1999). Alligators stop feeding when ambient and body temperature drops below about 20°C and become inactive at around 12°C, although they can survive periods of several days exposure to 0°C. The monthly mean temperature of air and water in our facility ranged between 16°C and 28°C and the extremes were 12°C and 35°C. Through July through November 2002 and again from March to July 2003 a range of temperatures comfortably within the alligators preferred range was always available (Figure 2). Between November and February the mean and maximum temperatures were at or just below 20°C and the alligators did not eat during this period. Throughout the period alligators could also elevate Tb by lying beneath the heat lamps provided. We established by observation and indirect means (observations of wet marks on the plywood, displacement of small amounts of sand) that the animals did regularly use the basking lamps.

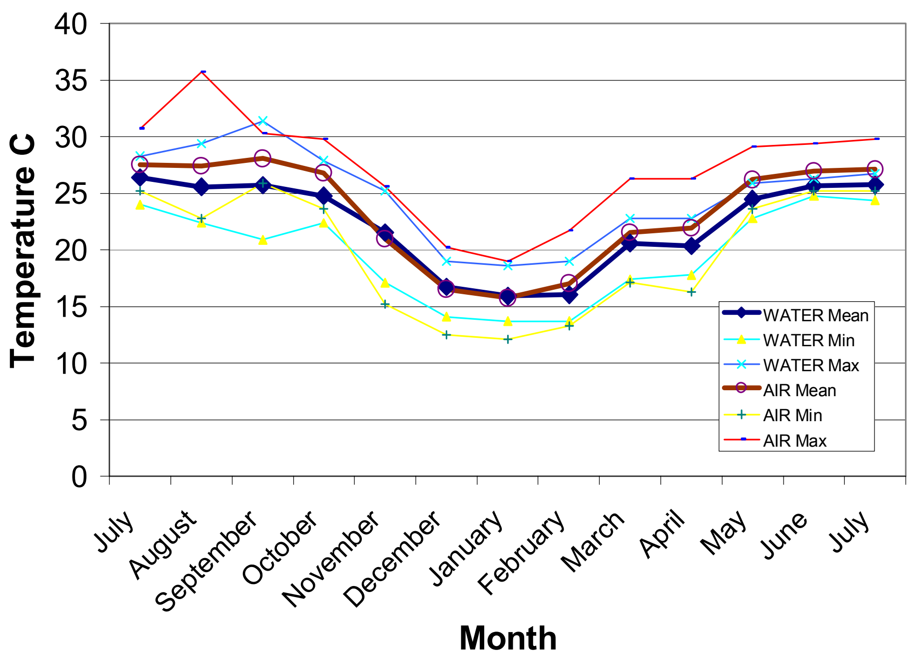

Figure 2. Monthly mean, maximum and minimum water and air temperatures in the alligator holding facility.

To qualitatively test the heating capacity of these radiant sources we constructed a "black body" from a 1 L coffee can filled with water and painted matte black and placed on the plywood floor beneath the lamps. A Hobo temperature recorder in the can indicated a rise in temperature from ambient 21°C to 27°C in 5 hours.

Captive alligators adapted to confinement and began to eat enthusiastically after about one month with the exception of one animal GFC 50811-50812. This animal refused food for periods of up to 2 months before occasionally eating the offered ration and then again refusing food. While this animal declined in weight during its long fast, the occasional feeding was sufficient to restore weight to close to original capture weight and its maximum weight loss did not exceed 20% of original capture weight (our arbitrary threshold for eliminating an animal from the experiment) and it remained active and apparently healthy. The remaining animals gained weight steadily through November 2002.

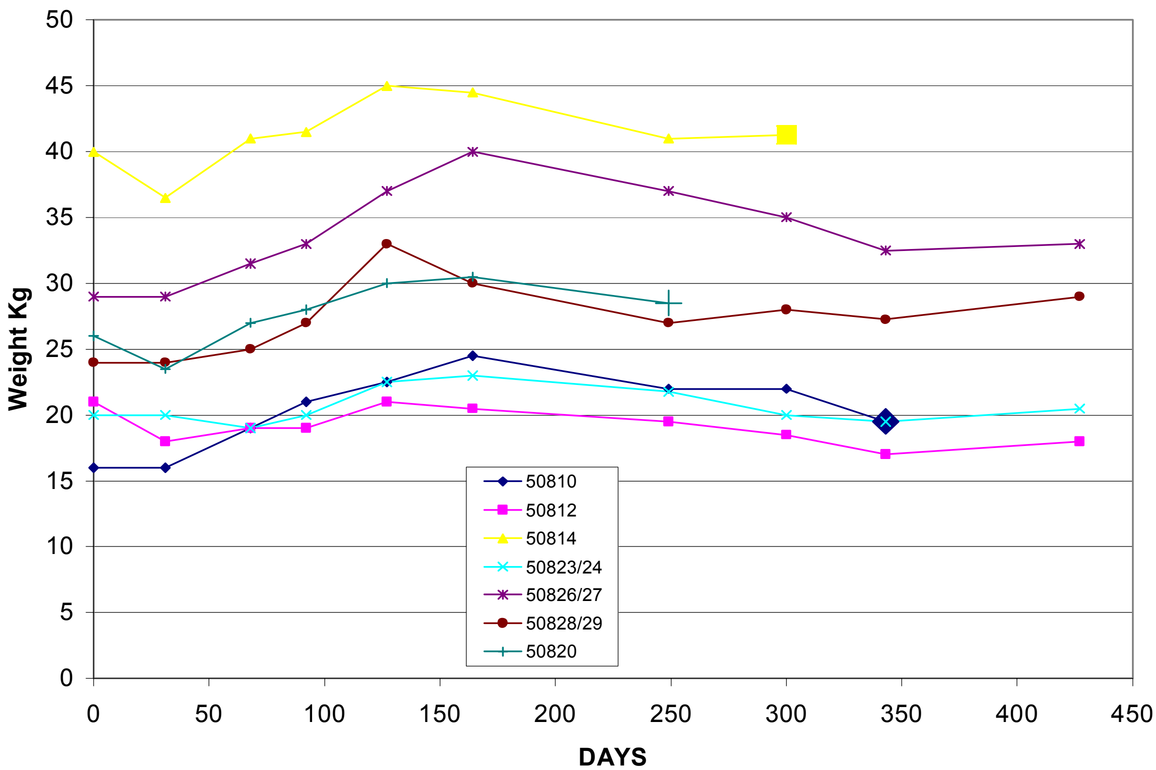

Figure 3. Weight (Kg) of seven captive alligators 1 July 2002 (Day 1) to 29 August 2003 (Day 427)

All the animals stopped feeding as ambient temperatures dropped below 20°C in mid November (Day 150) and steadily lost weight until June (Day 350). When ambient temperatures again rose above 20°C in March we began to offer the animals food but they ate intermittently until mid-June.

Three alligators died between March and July 2003, showing symptoms similar to those observed of impaired alligators in the wild. After a period of anorexia (sometimes beginning after the animals had resumed occasional feeding in March) the animals became lethargic and unresponsive and were found dead in their enclosures, apparently having drowned. One of the two animals chosen to change to a thiamin rich diet in April 2003 had resumed eating shad in March but then stopped eating and began to show symptoms of lethargy (No 50826-27). This animal received a thiamin injection 6 June but did not eat any pork or chicken with thiamin capsules. The animal resumed eating spontaneously 30 June readily taking chicken and was given one thiamin capsule (344 mg) on 14 July. The other thiamin- supplemented animal (No. 50828-29), spontaneously began eating shad on 21 March, readily ate 300-450g pork or chicken at each feeding and often ate its ration, including vitamin capsule immediately it was offered. The two surviving non-supplemented animals also began feeding spontaneously on shad between 11 and 25 June. The experiment was terminated by euthanizing the four surviving animals on 29 August 2003 when they had all stabilized or increased weight.

Table 1. Origin, capture weight, sex, shad eaten in 12 months as a proportion of initial body weight, and fate of seven experimental alligators. Thiamin- supplemented animals were euthanized 29 Aug. 2003.

GFC#	**Lake**	**Wt**	**Sex**	**Kg shad eaten /weight**	
50809/10	Woodruff	19.5	F	1.11	Died 20 June 03
50811/12	Griffin	17	F	0.41	Euthanized 29 Aug 03
50813/14	Griffin	39	F	0.68	Died 30 May 03
50819/20	Griffin	28.5	F	0.90	Died 14 Mar 03
50823/25	Woodruff	19.5	F	1.08	Euthanized 29 Aug 03
50826/27	Woodruff	32.5	M	0.98	Thiamin supplement
50828/29	Woodruff	27.5	F	0.82	Thiamin supplement

Blood thiamin levels of captive alligators were monitored monthly. Blood thiamin levels are weakly correlated with muscle thiamin levels (Figure 4).

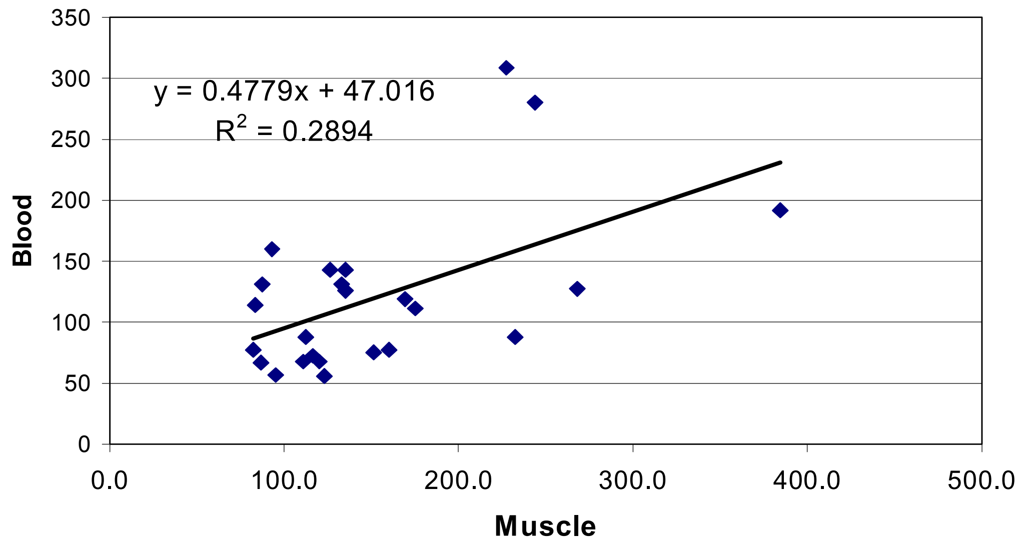

Figure 4. Relationship between blood and muscle thiamin levels (pmol/g) in captive alligators.

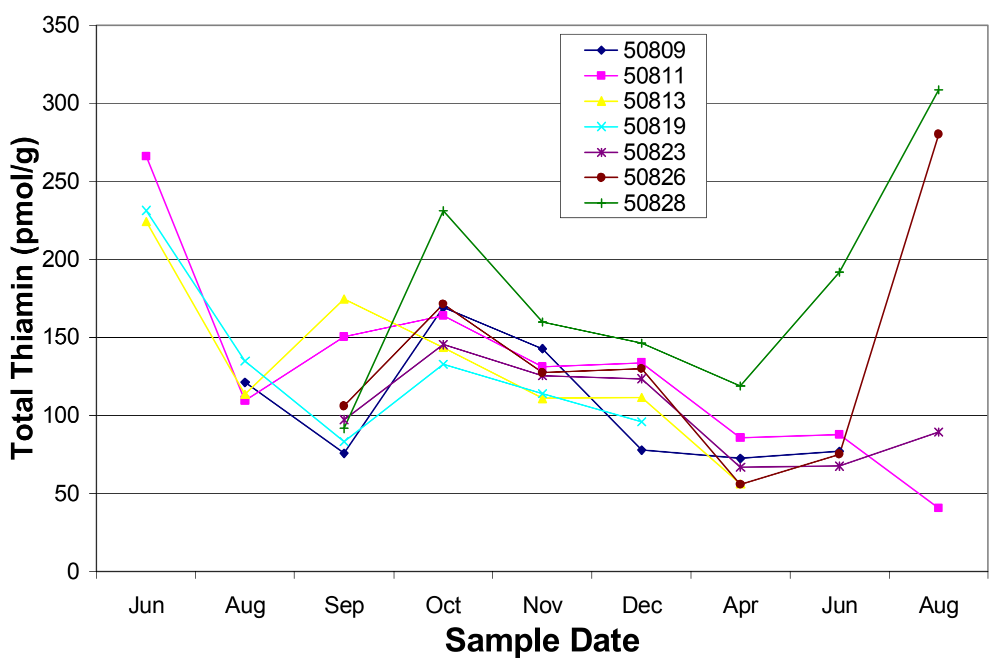

Figure 5. Blood thiamin levels of captive alligators fed gizzard shad. The two individuals 50826 and 50828 received thiamin treatment in April 2004.

Blood samples were available for three animals from Lake Griffin at capture and these all showed blood values above 200 pmol/g that correspond to muscle values of 300-400 pmol/g (from Fig. 4 above) comparable to values of healthy wild alligators. In captivity the blood values of all alligators fell to less than 150 pmol/g and except for alligator # 50828-50829 remained at low levels. Alligators 50827-50827 and 50828-29,which received extensive thiamin supplement both in their pork/chicken diet and by injection and oral capsule, showed a clear increase in blood thiamin (Fig 5).

The alligators with the lowest blood values in November and December were the animals that died the next spring. One animal 50828-29 given thiamin supplements April 24 through June markedly increased thiamin levels. Animal 50826/27 was given a 250mg thiamin injection in April but refused food and thiamin supplements until late June. This animal showed a small increase in thiamin level from April to June.

The two thiamin- supplement animals were selected by random numbers but happened to be the two with highest and lowest blood thiamin in April 2002. The prediction from the December data is that the animals with the lowest thiamin are most likely to die. It appears that thiamin injection may have 'rescued' 50825/26 from terminal thiamin deficiency.

Table 2. Thiamin values of muscle, liver and blood (saline washed red blood cells) for alligators. pmol/g Mean and 2 Standard error

	Blood	Muscle	Liver
Lake Woodruff	-	327 ± 136 N=15	2888 ± 336 N=15
Lake Griffin healthy	173 ± 144 N=5	223 ± 176 N=10	2731 ± 316 N=22
Lake Griffin Sick	124 ± 48 N=10	148 + 42 N=20	1975 ± 418 N=21
Captive untreated	123 ± 15 N=7	117 + 26 N=7	740 ± 160 N=7

Muscle and liver thiamin levels are thought to be a better indicator of the thiamin status of an animal. In November, before thiamin treatment and when they reached their lowest levels, muscle thiamin levels averaged 145 pmol/g in the captives, which is lower than, and significantly different from, values for healthy wild alligators and lower than the sick alligators from Lake Griffin.

Table 3. One Way ANOVA pairwise comparison (P Values) of thiamin levels in muscle of four groups of alligators Woodruff and Lake Griffin healthy and sick were samples from the wild taken in 2000-2001. Captive values are pooled values for untreated alligators (see table 2).

	Average Thiamin pmol/g	**Woodruff**	**Griffin Healthy**	**Griffin Sick**	**Captives Nov 02**
Woodruff	327 ± 38	--	0.83	0.005	0.001
Griffin Healthy	223 ± 68	--	--	0.061	0.105
Griffin Sick	148 ± 20	--	--	--	0.43
Captives	117 ± 23	--	--	--	--

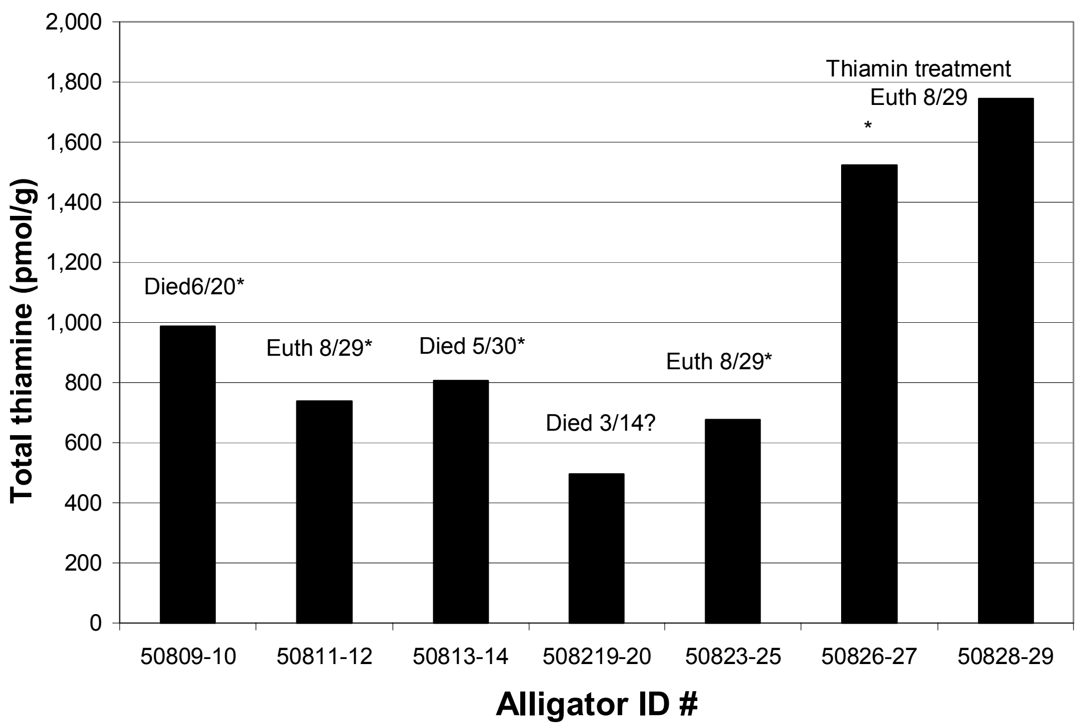

Figure 6. Total thiamin levels (pmol/g) of alligator livers post mortem. * indicates brain lesions in the midbrain present.

Liver thiamin was also at levels comparable to sick Lake Griffin alligators in the field for all the untreated alligators.

HISTOLOGY OF CAPTIVE ALLIGATORS.

The brain was removed post-mortem from the three alligators that died and the surviving four after euthanasia in August 2004. Brains were divided sagitally and one half preserved in 70% buffered formalin and the other frozen. Formalin fixed brains were trimmed into blocks, embedded, sectioned and stained with hemotoxin-eosin and examined for cell structure and organization. Tissue slides were examined by Veterinary pathologists Scott Terrell, who conducted alligator pathology analysis on the project in 2001, and Trenton Schoeb who made the first observations of brain lesions in alligators from Lake Griffin. Two of the three alligators that died during the experiment demonstrated focal necrosis and lesions in torus semicircularis of the mid-brain identical to those previously observed in Lake Griffin alligators. The brain of the third dead alligator deteriorated before preservation and could not be evaluated. The two surviving, untreated alligators and one of the treated alligators 50826-27 also showed these distinctive lesions. The other treated alligator, 50828-29 did not have lesions.

Table 4. Histology of captive alligators. * Brain and liver tissue from 50819-20 deteriorated prior to collection and histology was inconclusive and thiamin value may be a low artifact.

Alligator	Fate	Lowest Muscle Thiamin pmol/g	Terminal liver thiamin pmol/g	Brain Lesions
50819-50820	Died 3/14/03	83.3	494*	Unknown *
50813-50814	Died 5/30/03	93.9	805	++
50809-50810	Died 6/20/03	82.6	988	++
50823-50825		86.9	676	++
50811-50812		87.4	738	++
50826-50827	Treated	123	1524	++
50828-50829	Treated	93.1	1745	-ve

These results support the contention that the experimental treatment resulted in a decline of thiamin levels to levels comparable to sick alligators from Lake Griffin. Thiamin levels in blood, muscle and liver were restored after treatment with thiamin. The alligators that experienced very low thiamin levels developed lesions of torus semicircularis typical of thiamin deficiency in salmonids and previously reported in Lake Griffin alligators. One of the thiamin treated alligators developed lesions. However, this animal was the one that was reluctant to feed during April-May 2003 and probably received lower thiamin amounts. While its muscle and liver thiamin eventually returned to high levels, it did experience a prolonged period of low thiamin (see blood thiamin Figure 5).

RESULTS 3. ALLIGATOR DIET

Data in this section form part of the MSc Thesis of Amanda Rice. Field work was conducted between March and October of 2001, 2002 and March-August 2003 on Lake Griffin, Lake Apopka and Lake Woodruff. An additional 27 stomachs were obtained post-mortem from alligators captured and sacrificed for other studies. Distribution of samples among the three lakes is shown in Table 5.

Table 5. Alligators caught and sampled for stomach contents 2001-2003.

	Griffin	**Woodruff**	**Apopka**
2001	39	5	5
2002	43	42	25
2003	19	0	19
Total	101	47	49

For various reasons some of the alligators have been eliminated from the following general analysis or treated separately. Fifteen alligators sampled in 2001 on Lake Griffin were judged to be suffering from neural impairment and were treated separately, most of these had empty stomachs. Lavage was unsuccessful in five alligators, one died during capture and one was missing its upper jaw and these seven individuals were not analyzed. Samples taken from alligators previously caught and lavaged (recaptures) were eliminated and 4 stomachs were completely empty.

Table 6. Summary table of useable and unusable stomach samples.

	Griffin	**Woodruff**	**Apopka**
Empty	1	3	0
Discarded	16	1	4
No fresh material	19	10	11
Some fresh material	65	33	33

Our target size for this study were alligators between 6 ft and 10 ft total length to correspond to those found dead in earlier years. The Lake Woodruff alligators had a slightly, but not significantly smaller, average length and weight due to the higher proportion of smaller individuals in this sample.

Table 7. Snout to vent length (SVL) and mass of alligators sampled for diet in this study.

	Griffin		**Apopka**		**Woodruff**	
	SVL cm	**Mass kg**	**SVL cm**	**Mass kg**	**SVL cm**	**Mass kg**
Mean	114	45	116	49	112	39
Minimum	78	14	88	22	88	16
Maximum	151	96	156	108	166	112
Std. Dev.	17	20	16	21	20	24

The overall effort and the number of samples collected from the three lakes was not identical (Table 5 above). Therefore we plotted the cumulative number of species recorded against cumulative stomach samples to evaluate whether we had fully sampled the diversity of available prey in each lake sample (Figure 7). As expected, the rate of recording new species from our samples was initially high and then became lower. The slope of all three lines is similar and samples from all three lakes appear to be approaching an asymptote. It appears as if a large proportion of prey species are identified after the first 40 samples and additional species were added slowly thereafter. However the rate of accumulation of species to our sample was different between Lake Griffin and the other two lakes. Lake Apopka and Lake Woodruff samples approach an asymptote of between 30 and 35 species while Lake Griffin levels off at more than 45 species after 60 samples. The number of species recorded for each lake is therefore partially a product of the different number of samples collected but also seems to reflect inherent differences in the number of species eaten by alligators among the lakes. These possibly also reflect different diversity of available prey among the lakes.

Most alligator stomachs (77-100%) contained some non-food items, shown in table 8. These included small amounts of fresh plant material, pieces of wood, nematode parasites and a variable amount of mineral material in the form of sand and rocks. The quantity of mineral material in Lake Woodruff stomachs was significantly less than the other two lakes Artificial or anthropogenic materials included golf balls, shotgun shell wads, fishing lures and hooks, six spark plugs and an 'action figure' plastic doll.

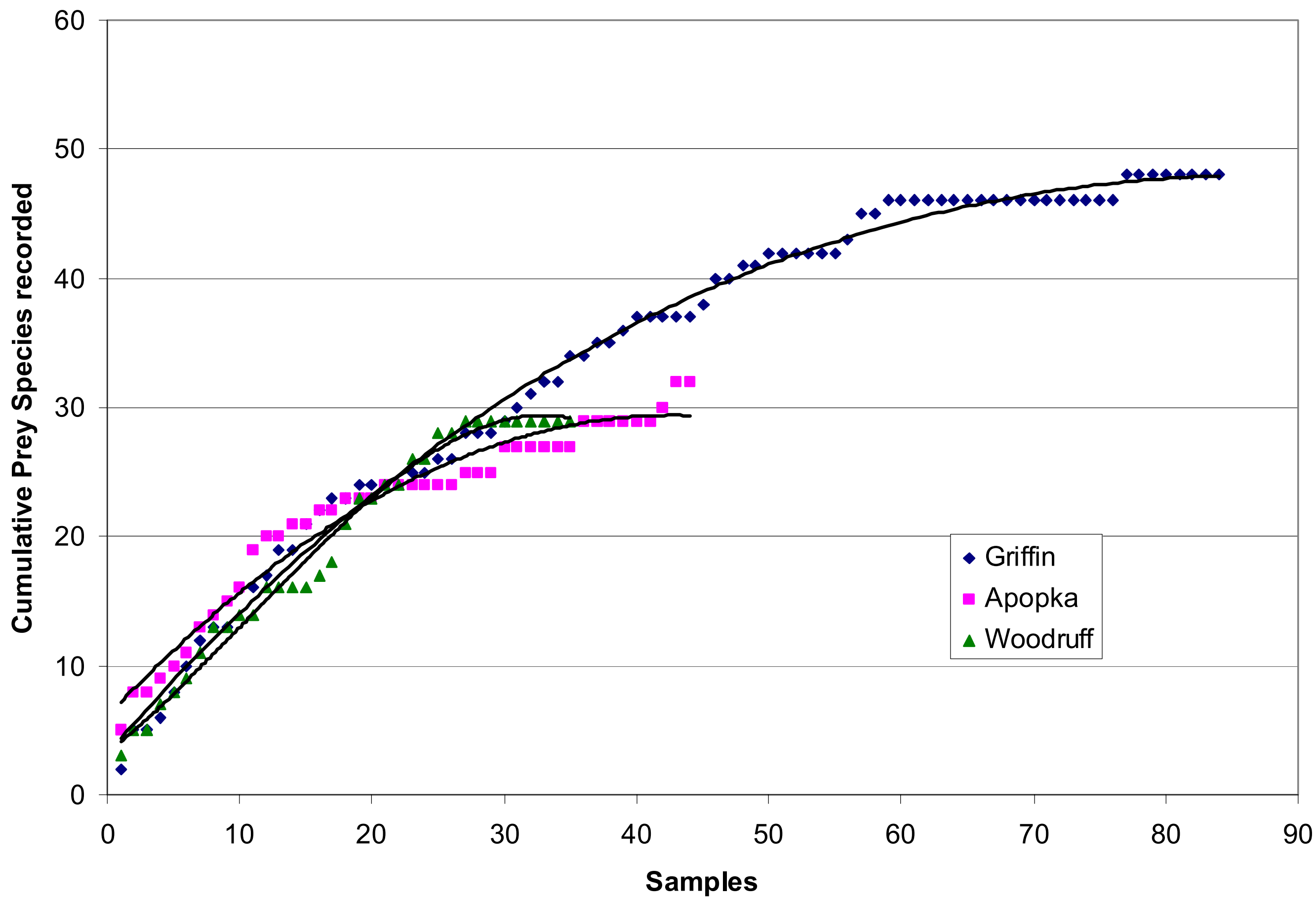

Figure 7. Cumulative number of species recorded from alligator stomachs plotted against cumulative number of samples

Table 8. Frequency of occurrence (percentage of alligator stomachs from each lake that contained non-food items) of non-food items (all samples),

	Griffin	Woodruff	Apopka
Plant	86%	95%	86%
Nematode parasites	85%	95%	98%
Wood	79%	81%	84%
Sand	26%	0%	43%
Rocks	22%	5%	41%
Artificial	17%	19%	11%

A first approximation of the diet of alligators was derived from the frequency of occurrence, that is the percentage of the stomach samples from each lake that contained a given item. Initially we calculated frequency of occurrence including both fresh and older material.

Table 9. Frequency of occurrence (percentage of stomachs from each lake in which an item occurred) of food items in a sample of 171 alligator stomachs March 2001- June 2003.

	Griffin	Woodruff	Apopka
Mollusks	74%	90%	46%
Fish	58%	60%	84%
Insects	31%	35%	61%
Reptiles	42%	16%	36%
Crustaceans	19%	27%	21%
Mammals	11%	11%	11%
Birds	12%	5%	7%
Amphibians	7%	5%	0%

Mollusks, fish and insects were present in the majority of stomachs. The mollusks were mostly apple snails (*Pomacea paludosa*) although 16% of Woodruff stomachs and 4% of Griffin stomachs also contained the freshwater mussel *Utterbachia sp.* Reptiles (turtles but also occasional snakes) were the next most common items found. Crustaceans (crayfish and grass shrimp), birds, mammals and amphibians (mostly siren and amphiuma, but also occasional frogs) completed the diet. Alligators, usually recorded as hatchling tags, were present in 13 stomachs. A full list of species identified from stomachs in each lake is given in Appendix 6. There appear to be differences among the three lakes that require additional analysis.

Frequency of occurrence data are distorted by the relative size and number of the prey items and by the different time that identifiable fragments remain in the stomach. For example we recovered 1,326 opercula of apple snails. However, alligators do not digest keratin and so the opercula remain for an unknown but prolonged time after ingestion. To partially offset this bias we reanalyzed the frequency of occurrence data using only those items that still had undigested flesh and were identifiable as more or less whole organisms. These are presumed to be recently ingested items representing recent meals. We calculated the Shannon-Weaver diversity index (H= Σ^nj pi ln pi) using both total items and again using only freshly ingested items. We also calculated biomass of the freshly ingested material.

Table 10. Species diversity of alligator diets in three Florida Lakes 2000-2003 data combined. 'Species' are the highest category of identification achieved for the sample (see Appendix 6).

	Stomach Samples	Total 'Species' recorded	Shannon Weaver Diversity index all	Shannon Weaver Diversity index fresh only
Lake Griffin	84	48	1.17	2.25
Lake Woodruff	43	29	1.28	2.52
Lake Apopka	44	32	2.50	2.45

The Shannon Weaver index indicates both number of species and the proportion of individuals of each species. Therefore, the very large numbers of undigested apple snail opercula distort the apparent diversity of the diet. The proportion of apple snails that were freshly ingested was 44% in Lake Griffin, 9% in Lake Woodruff and 3% in Lake Apopka. Considering only recently ingested material, there does not appear to be a difference in the diversity of diet among the three lakes. Considered by frequency of occurrence, fish and invertebrates (apple snails) appear to be the most important prey in all lakes.

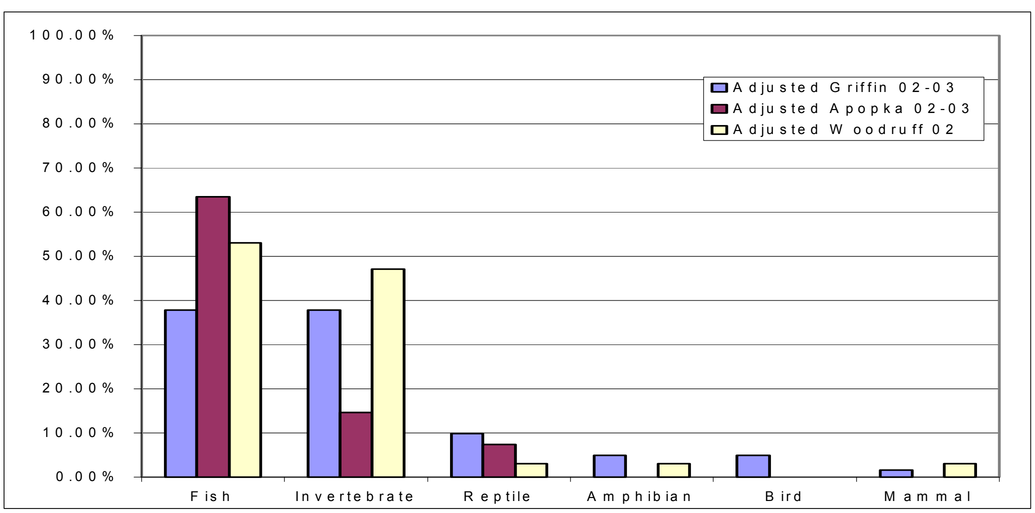

Figure 8. Frequency of occurrence of prey items in alligator stomachs adjusted to include only recently ingested prey.

The importance of fish in the diet is even more evident if the size of dietary items is considered. Shad and catfish found in alligator stomachs fall into a size range of approximately 15 cm-30 cm length and 200 g-400 g weight. In contrast, the invertebrate components although abundant, were relatively small. Apple snails weigh approximately 4-14 g (including the indigestible shell). Larger items, such as softshell turtles, raccoons, and cormorants, were represented by just one or two individuals in our sample.

We estimated the approximate biomass of different prey items in our sample by multiplying the minimum number of individuals we recovered from stomachs by the estimate of weight for each specimen recovered. Size (mass) of individual specimens were calculated from measurements of the specimen and allometric relations derived from standard sources (see methods) and expressed as a percentage of the total biomass of all the items ingested for each lake. These data were calculated for each species (from Table 12) then re-aggregated into broad class categories to provide an estimate of the quantity of different prey ingested or roughly the contribution to alligator nutrition of different classes of prey. We also divided the sum of all the biomass estimates by the number of stomachs samples for each lake to provide an estimate of the average quantity of material in a stomach.

Table 11. Estimated contribution by biomass of prey recovered from alligator stomachs 2001-2003. Fresh items only.

	Griffin	Woodruff	Apopka
Shad	12%	14%	40%
Other Fish	41%	70%	44%
Mollusks	4%	4%	<1%
Birds	15%	0%	7%
Mammals	13%	2%	2%
Turtles	7%	1%	1%
Snakes	3%	0	<1%
Alligator	<1%	<1%	<1%
Amphibians	4%	8%	0%
Insects	<1%	<1%	<1%
Crustaceans	<1%	<1%	<1%
Biomass/stomach	594 g	468 g	537 g

Some unexpected differences among lakes emerged from this analysis. The diets of alligators in the different lakes were dominated by different prey. Fish were prominent in all lakes. However gizzard shad represented a major proportion of the total in only Lake Apopka. Lake Woodruff alligators appear to eat mostly fish but also ate more mollusks (apple snails) and fewer birds and mammals. Lake Griffin alligators ate more birds, mammals and turtles than alligators in the other lakes during 2001-2003.

The biomass analysis indicates the importance of occasional large prey items to alligators. Although only four large birds (cormorants/anhingas/ibis), and one raccoon were present in the alligator stomach contents (Appendix 6) together these items provided 17% of the quantity of all material eaten (10% and 7% of the total respectively). The average estimated biomass/stomach was slightly lower in Lake Woodruff alligators, possibly reflecting their smaller size.

Biomass of shad as a proportion of total diet was not calculated in 2000 but was 24% of the biomass of fish eaten. The decline of shad from stomachs of Lake Griffin alligators after 2001 is striking. This is not due to an overall change in diet away from fish because catfish (*Ameiurus sp.*) remain the fish most often eaten and a significant part of alligator diet in all lakes and years.

To examine the shad component of the diet in more detail over time we combined data from this study and previously reported studies, Ross et al. 2002a (Table 11).

Table 12. Changes in shad consumption by alligators in Lake Griffin (data this study and from Ross et al. 2002a).

	2000	**2001**	**2002**	**2003**
Stomachs	31	39	43	16
Number of shad	19	7	0	1
Frequency of occurrence of shad	26%	16%	0%	6%
Contribution by mass	--	8%	0%	<1%

RESULTS 4- THIAMINASE IN GIZZARD SHAD

Shad samples were collected from Lake Griffin in May 1999, March, April and June 2001 and July-November 2002. Shad were very rare in the lake following commercial fishing in early 2003 and we were unable to capture any shad after February 2003. Examination of the material collected in 2002 revealed that two species had been confused in the field samples, gizzard shad, *Dorosoma cepedianum* and threadfin shad, *D. petenense*. The two can be distinguished by jaw shape and some color differences. Threadfin shad are reported to reach a typical size of 7.5-12.6 cm (Jenkins and Burkhead 1993, Pflieger 1975) and all the specimens we identified were less than 10 cm. Unfortunately some of the smaller specimens in our sample had been combined and macerated to powder prior to identification. These mixed species samples of small fish were therefore assumed to be of uncertain identification, but most probably threadfin shad. All the specimens of 15 cm or greater fork length were confidently assigned to *D. cepedianum* and among the smaller specimens some were actually identified as *D. cepedianum* and others as *D. petenense.* In the following results only fish correctly identified to species are discussed.

Thiaminase activity expressed as pmol/g/minute were very high in all the gizzard shad measured. Mean value ($\pm$ SE) for all samples, all sources and years was 16,409 $\pm$ 4.9 pmol/g/min. This can be compared with samples of alewife (*Alosa psuedoharengus*) from the Great Lakes analyzed with our samples as a control (D. Honeyfield pers comm.) of 9,918 pmol/g/min. Published values of thiaminase activity in alewives and other clupeid fishes range from 2,600 to 22,000 and are shown in table 13.

Table 13. Values of thiaminase activity reported for fish.

Location/Species	**Thiaminase activity pmol/g/min**	**Source**
Bloater chub	20	Zajicek et al. 1999
Alewife	6,600	Zajicek et al. 1999
Alewife Michigan	9,918	Honeyfield pers comm.
Rainbow smelt	2,600	Zajicek et al. 1999
Blueback herring WI	11,098	Honeyfield pers comm.
Threadfin shad L. Griffin FL	3,313	This study
Gizzard shad WI	19,724	Honeyfield pers comm.
Lake Griffin FL	16,408	This study
Lake Apopka FL	21,965	This study

The mean value for thiaminase of five samples each comprising pooled ground whole fish of 5-11 identified threadfin shad was 3,313 pmol/g/min and the value for all the mixed samples of uncertain identity was 2,984 pmol/g/min. In contrast, the values of our two smallest identified gizzard shad were 17,320 and 19,879 pmol/g/min. We are therefore confident that for the purposes of analysis we have correctly assigned fish to species and that gizzard shad have very high levels of thiaminase compared to other species.

The level of thiaminase in gizzard shad showed a weak relationship with size in the range of our sample, 7.5cm – 30.0 cm fork length and 55-400 g weight. Figure 9. Nearly all our samples showed thiaminase levels above 10,000 pmol/g/min that is higher than the levels in alewife known to induce thiamin deficiency in salmonids.

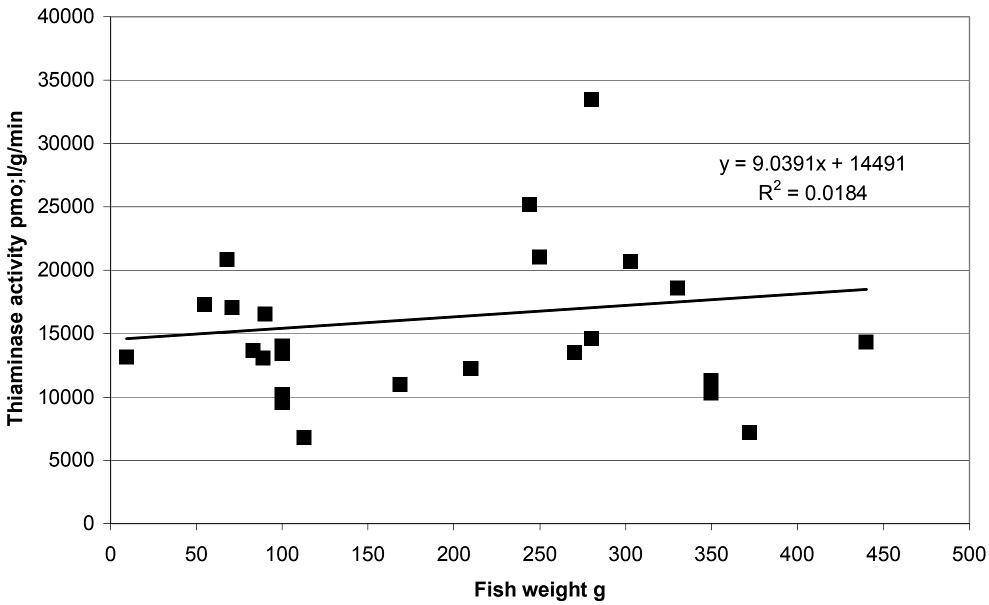

Figure 9. Thiaminase levels pmol/g/minute in samples of gizzard shad, (*Dorosoma cepedianum*) of different sizes collected in Lake Griffin, FL.

To examine the effect of season we pooled the available samples from 1999, 2001 and 2002. There does not appear to be any strong effect of month of collection (Figure 10). Bearing in mind that the values shown in Figure 10 represent samples from different years, there is no obvious trend either month to month or year to year, but the small number of samples in any treatment precludes rigorous analysis.

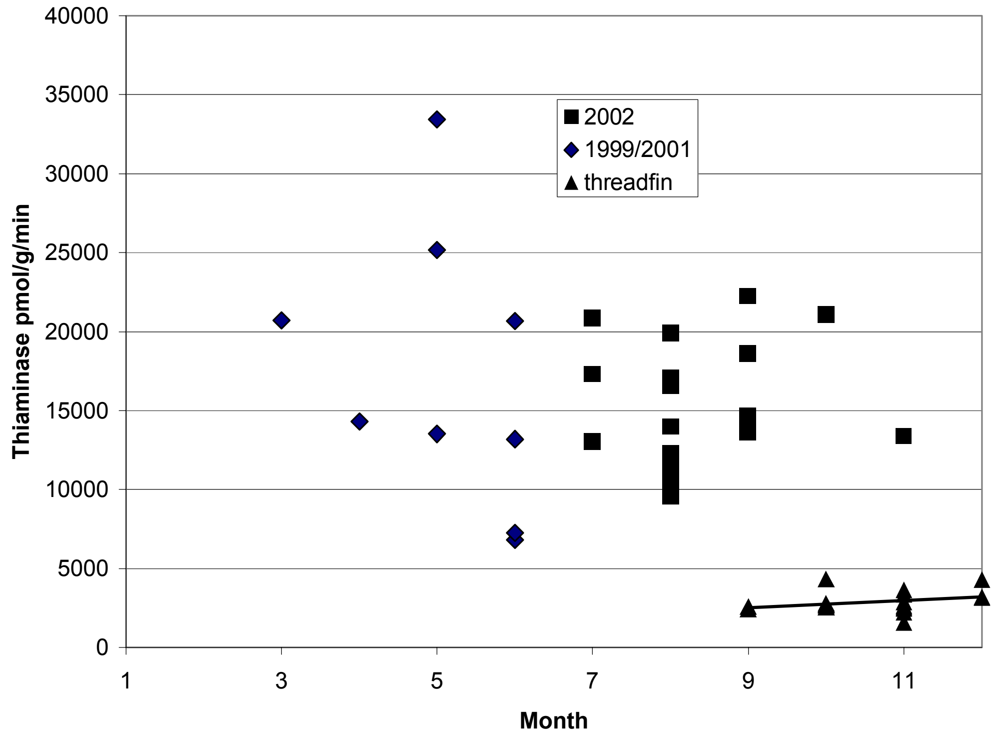

Figure 10. Levels of thiaminase pmol/g/min in gizzard shad (*Dorosoma cepedianum*) and threadfin shad (*D. petenense*) collected in different months and different years on Lake Griffin FL. (additional samples for December 2002 and February 2003 are being analyzed)

The only data we have to compare thiaminase levels between lakes is from samples obtained from commercial fisheries from Lake Apopka in July and September of 2002. The Apopka samples have a mean value of 21,965 + 37.59 pmol/g/min which is higher than, but not significantly different from, Lake Griffin (t= 1.84, 4 df). Additional commercial samples from Lake Griffin in February 2003 have been sent for analysis.

DISCUSSION

Mortality of alligators.

It is evident that the mortality syndrome observed between 1998 and 2000 began to wane in 2001 and dropped to a level indistinguishable from natural mortality levels during 2003. This is not due to any change of effort or method and represents a real amelioration of the syndrome. Several factors were observed during this period and it is impossible to assign any of them as the 'cause' of changed alligator mortality. Water levels, which had been 0.5- 1 m low for the preceding several years returned to normal-high levels after strong winter rains in 2001. Coincident with this change, water quality, and particularly chlorophyll levels as an index of phytoplankton blooms, improved. The marsh restoration-flow through system designed to transfer particulates and phosphorus from Lake Griffin to the adjacent restored Emeralda Marsh was reactivated between October 30, 2002 and February 28, 2003 . Phosphorus levels in Lake Griffin decreased to the lowest levels reported for several years (about 40 to 50 *u*g/TP/l, W, Godwin pers. comm.). Starting in early spring (February 26) of 2002, the District authorized commercial removal of gizzard shad, tilapia and garfish from Lake Griffin as part of planned phosphorus reduction activities. Fishing continued at high intensity for several weeks until fish harvest per effort fell to levels that fishermen felt were uneconomical. More than 1- million pounds of fish were removed from Lake Griffin in these periods in 2002 and 2003. Following this fairly intensive removal of larger size classes of shad (the fishery uses 4-inch mesh nets and takes shad in the size range approximately 25 cm and up) our sampling with a cast net became ineffective, apparently due to low density of shad. However the presence of a small number of shad in our alligator stomach samples indicates that low numbers were still present.

Induction of thiamin deficiency.

We believe we induced a reduction in blood, muscle and liver thiamin in our experimental group and restored thiamin levels with thiamin dietary supplements in two controls. The conditions of captivity of our alligators appeared not to be stressful as evidenced by their rapid adaptation, weight gain and general vigor through most of the period. The specimens that died, did so showing symptoms approximating those seen in impaired alligators in the wild and died in the order of lowest thiamin levels measured in November –December and post mortem in the livers. We were unable to measure muscle thiamin levels at the beginning of the experiment, but extrapolation from the measured blood levels suggests that they entered the experiment with muscle thiamin in the order of 300 – 400 pmol/g that is equivalent to levels measured in healthy alligators in the wild. Reduction occurred after 5-6 months of a diet exclusively of gizzard shad with measured high levels of thiaminase. Because our shad were frozen for storage and thawed before feeding, this may also have reduced available thiamin in this diet.

It remains possible that the changes in thiamin levels we observed are the result either of other influences of captivity or of a natural cycling of thiamin levels in alligators (about which nothing is known or published). To test this idea we are currently analyzing additional blood samples taken from wild alligators when they were captured for stomach lavage. Results of these analyses should provide a clearer picture of natural fluctuations in both Lake Griffin and other lakes unaffected by the mortality syndrome.

The presence of brain lesions in the torus semicircularis of most of our experimental group identical with those seen in Lake Griffin alligators strongly suggests that we have induced a syndrome similar to that observed in the wild. To ensure that our evaluation of lesions was accurate we cut and evaluated sections in a stepwise fashion through the relevant midbrain part of all our embedded blocks. The presence of lesions in one of the thiamin treated alligators indicates that even though we were able to restore this animal's thiamin levels with an initial thiamin injection and chicken-pork diet, apparently the exposure to low thiamin prior to treatment induced lesions. It is also noteworthy that we apparently induced lesions in four Lake Woodruff alligators. Lesions have not been reported in Lake Woodruff alligators in any of our previous work.

Diet.

The lavage technique for recovery of stomach contents of alligators is quite effective. Fitzgerald (1989) confirmed by necropsy that all food items in 100% of 24 *Caiman crocodilus* (ranging from 0.135 m – 1.086 m SVL) were successfully removed. Our own calibration exercise also indicated effective recovery of all the contents. Stomach content samples obtained from live animals represent a snapshot of the recent meal an animal has ingested but the analysis is complicated because different prey items are digested and disappear from alligator stomachs at different rates. In addition, the way that the stomach contents are quantified and expressed affects the apparent result. For example, many small persistent items (e.g. the indigestible opercula of apple snails) may cause the importance of these items to be overestimated. In this study we have corrected for both persistence and size of dietary items and expressed the results in several different ways. Our analysis is restricted to alligators in the 5 foot to 10 foot size classes, i.e. adult animals similar in size to those subject to mortality in Lake Griffin.

Previous studies indicate that alligators, like most other crocodilians, appear to be fairly non-selective carnivores, taking a wide range of prey (Webb et al. 1982, Da Silveira and Magnusson 1999). Our results are similar to those of Delaney & Abercrombie 1986, Barr 1997, Bondavilli &Ulanowicz 1998 and Delaney et al. 1999 indicating that Florida alligators consume a wide selection of prey with common fish, turtles and apple snails being the most abundant items. Louisiana alligators have a similarly diverse diet (Valentine et al. 1972). In this study our primary interest was to examine whether the diet of alligators in Lake Griffin differed from other lakes and whether it had changed since previous studies in 1999-2000 in a way that might be related to changes in mortality observed over the period.

The proportion of empty stomachs is not different among the three lakes and indicates that the frequency of feeding is similar for the three locations. Plant and some mineral items are thought to be incidentally ingested during feeding and have no nutritional function. Recent observations of other crocodilians deliberately eating leaves and fruits suggest that there may be a small nutritional value to this material but the quantities we observed were in all cases very small, just a few grams. The function of rocks and pieces of wood in crocodilian stomachs has been widely debated but remains unresolved.

Alligators and other crocodilians are reported to pick up a variety of human artifacts among which we recorded shotgun shell cases and wads, sparkplugs, golf balls, fishing lures and a plastic ‘mutant ninja turtle’ action doll.

Lake Woodruff alligators have less mineral material than either Griffin or Apopka. This seems counter intuitive as Lake Woodruff has extensive sandy bottom and several exposures of Indian shell mounds where shells and rocks might be found. A large proportion of the ‘rocks’ found in Lake Griffin and Lake Apopka appear to be material not native to the region or to Florida (e.g. coal fragments). We therefore propose that the more disturbed habitat, including extensive dikes, dredging and farm activity has made hard material of exotic origin more available to alligators.

The frequency of occurrence data suggest some difference among the lakes with snails recorded for more Woodruff alligators and fish recorded in more Apopka alligators during this study. The diversity of species in the diet, measured by an index that calculates both the number of species and their relative proportions in the diet, suggested that Lake Apopka has a more diverse diet than Griffin or Woodruff if all the material, including older persistent parts like snail opercula, are considered. However, this is apparently due to the very large number of snail opercula recorded in Griffin and Woodruff stomachs which dominate the diet and so reduce its diversity. When this index was recalculated using only recently ingested material, the diversity of species in the diet is similar among the three lakes. We recorded more species in alligator stomachs in Lake Griffin than the other two lakes. This is in part due to the larger number of samples collected in Lake Griffin but also reflects real differences in the number of species eaten, and possibly the number of prey species available. We expected that Lake Woodruff, a more natural and less disturbed lake than Griffin or Apopka, would have a higher natural diversity of organisms that alligators could eat. However, both total species and fish species indicate this is not a strong factor (Woodruff-12 fish species, Griffin-11 fish species, Apopka- 9 fish species). Differences in the diets of alligators must therefore be due to differences in the relative abundance of different items, or possibly to physical differences that make capture of certain prey easier.

When we consider only the more recently ingested material as a less biased picture of alligator diets, and also quantify by the amount of material (biomass) rather than the number of items, then a much clearer picture emerges. Alligators in each lake show a characteristic predominance of some prey. Fish are the main component of the diet in all lakes. The larger contribution of mollusks in Lake Woodruff and Lake Griffin is evident but contributes relatively little to mass intake. In Lake Griffin, a reduced intake of gizzard shad appears to be compensated by more reptiles, mammals and birds. The very prominent position of gizzard shad in Lake Apopka, where they are very abundant, is clear. The biomass analysis also indicates the importance of the occasional large dietary item such as a cormorant, large turtle or raccoon. The average quantity of material in each stomach is least in Lake Woodruff, suggesting that on average alligators in Lake Woodruff either eat less frequently or take less prey. We cannot say if this is due to less prey or the density of alligators, but it is reflected in the less plump condition of

Woodruff alligators which we commonly report during necropsy and is reflected in their relative condition index .

Our detailed analysis of fish intake shows that Lake Griffin alligators ate almost no gizzard shad in 2002-2003 although they continued to eat other fish. We tentatively attribute this to the reduction of the density of gizzard shad in the lake from commercial fishing which caused both the fishermen and ourselves (and apparently the alligators also) to have difficulty capturing significant numbers of shad. However, shad remained present in the lake.

We conclude that the diet of alligators in the three lakes does differ and is most likely a product of the relative abundance and ease of capture of prey items. These differences are tempered by the different dietary value derived from different sizes of prey. Overall- alligators eat mostly fish. In Lake Griffin there was a measurable reduction in fish intake caused by the scarcity of gizzard shad compared to other lakes and to Lake Griffin data from previous years.

Thiaminase

Reproduction of salmonid fish in the Great Lakes, Finger Lakes (New York) and the Baltic Sea is reduced by fry mortality due to a thiamin responsive syndrome (Brown and Honeyfield 1999; Bengtsson, Brown and Honeyfield 1999; Whyte and Honeyfield 2000). This syndrome, called Early Mortality Syndrome (EMS), Cayuga Syndrome or M74, was first reported in the early 1990s and has received intensive study. The syndrome is recently reported to also affect adult lake trout, coho salmon, baltic salmon and steelhead trout (Honeyfield and Brown personal comm.). EMS in salmonids is thought to be a result of ingestion of prey species (fish) that contain the enzyme thiaminase which destroys thiamin. Thiamin deficient salmon lay eggs that are thiamin deficient and the resultant fry have been found with brain lesions and suffer high incidences of mortality. Thiamin deficiency in salmonid eggs can be reversed by injecting thiamin into gravid females three weeks before spawning or by immersing eggs (or fry) in a thiamin bath (Brown and Honeyfield 1999). The levels of thiaminase in the prey species (Alewife, Smelt) that induce this syndrome is of the order of 5,000 – 10,000 pmol/g/min (whole fish). Thiaminase levels in prey fish are reported to vary widely among northern lakes. The causes for these variations have been associated with changes in the phytoplankton-blue green algae of these lakes. However, northern lakes experience a winter period of little or no plankton productivity.

In this study we demonstrate for the first time that gizzard shad in Florida do have uniformly high thiaminase at levels well above those reported to affect thiamin levels in salmonid fish. We further show that gizzard shad of all sizes down to about 8 cm fork length have high thiaminase and that thiaminase levels can be high in most months of the year. This is consistent with the year round warm temperature and productivity of Florida lakes. Whatever factors do induce thiaminase production in fish probably operate more consistently than in northern lakes resulting in continued accumulations.

Our data have one difficult aspect in regard to the hypothesis that thiamin deficiency, induced by a diet of gizzard shad is responsible for the observed mortality in Lake Griffin during 1997-2001. That is that gizzard shad are shown to be prominent in the diet of Lake Apopka alligators and that these shad do have high levels of thiaminase, but mortality of Apopka alligators from a thiamin deficiency syndrome is not reported. This may require rejection of the hypothesis. However before doing so we should remember that Lake Apopka alligators have experienced drastic reproductive failure and mortality in the period 1980-1990 associated with chemical spills and other environmental disruption of that highly disturbed lake. The alligator population in Lake Apopka is now recovering from these effects (Rice 1996) but may have had some rigorous selection for behavior or physiology that somehow protects them from thiaminase induced deficiencies. Another possibility is that shad in Lake Apopka are feeding on very different algal mix than Lake Griffin resulting in a different thiamin-thiaminase interaction. Variability in thiaminase levels in forage fish and different expression of thiamin deficiencies in salmonids is widely reported (e.g., Brown and Honeyfield 1999).

Thiaminase is reported to be highest in the viscera of fish, which may reflect either its location of production or possibly induction by a dietary factor such as ingested algae. As an enzyme, thiaminase is probably rapidly denatured in the alligator stomach and is unlikely to pass intact into the alligator. A more likely mode of action is that the thiaminase in the viscera of shad is released into the shad's body as digestion proceeds, destroying thiamin in the shad and protected for a short time from digestive denaturing by the alligator. The alligator therefore ends up with a food item that has low thiamin by the time it is digested and absorbed. We propose that an alligator that eats shad gets little thiamin from such a diet, but can get thiamin from any other diet components. An individual alligator's response to a diet of shad must be affected by a series of factors.

- Its initial thiamin levels.
- Recent history of thiamin use and intake e.g. over-wintering without eating.
- Relative intake of shad compared to other diet items.
- Occasional sources of thiamin such as large bird or mammals in the diet.
- Activity, temperature and other factors affecting rate of thiamin depletion.
- Individual variations in thiamin metabolism
- Additional unknown complementary factors leading to critical deficiency.

From this we would predict that even in lakes where the thiaminase- thiamin syndrome is operating, the response of alligators will be quite varied. Some will become thiamin depleted but then eat a cormorant or turtle and recover. Some will balance their shad with other fish and not be come critically depleted. At any given time relatively few alligators will be affected and only some will enter irreversible thiamin deficiency and die. This is exactly what is reported for Lake Griffin, with only a small proportion of alligators affected, no more than 3% at any time and with pronounced seasonality (Ross et al. 2000a). We also consider that thiamin deficiency may be a necessary and pre-disposing factor, but not by itself a sufficient factor to cause the observed syndrome. It remains possible that thiamin deficient alligators are more susceptible to environmental toxins, contaminants or even disease. Thiamin operates at numerous biochemical locations as a co-factor in metabolic processes and increased susceptibility to other stressors is a common result of vitamin deficiencies. We also remain interested in the observations of early embryo mortality and low hatch success in Lake Griffin alligator eggs and the effects of both contaminants and thiamin which remain poorly researched or understood.

It does seem noteworthy that while earlier studies failed to establish a temporal association between alligator mortality and blue-green algae blooms on a month to month time scale (Ross 2000b), there is a clear association on a coarser time scale of seasons or year to year. The current remission of alligator mortality following water quality improvement and reduced algae blooms is an example. Re-examination of toxic algae effects using the longer data set now available and considering a wider variety of toxins and indirect actions could be fruitful.

CONCLUSIONS

We have examined the levels of mortality in wild alligators in Lake Griffin, attempted to induce thiamin deficiency in a small group of captive alligators by feeding them gizzard shad, examined alligator diets and assessed gizzard shad in Lake Griffin for thiaminase activity. We conclude that:

Alligator mortality was reduced in 2002 and 2003, approaching natural levels comparable to those seen on many Florida Lakes.

The variety of changes in the physical and biological environment in Lake Griffin, including water level, water quality, plankton blooms and fisheries activity, precludes assignment of a cause of the reduced mortality, but provides some intriguing supporting evidence for the thiamin - diet hypothesis.

We fed alligators gizzard shad and their blood, muscle and liver thiamin showed levels comparable to those seen in neurologically impaired alligators in Lake Griffin.

We also restored thiamin levels in two captive alligators by feeding them thiamin rich food and providing thiamin in the diet and by injection.

We have shown that the diet of alligators among three lakes in central Florida are characterized by different proportions of fish and other prey. However, these data do not support the hypothesis of thiamin deficiency induced by shad diets as Lake Apopka alligators eat more shad than either Lake Griffin or Lake Woodruff and do not show the syndrome.

We demonstrated for the first time that gizzard shad in Lake Griffin and Lake Apopka have very high levels of thiaminase, higher than those reported to cause thiamin deficiency in salmon in the Great lakes. These high thiaminase levels are present in most months and across a wide range of sizes of shad.

However, shad were reduced in Lake Griffin in 2002 and 2003, probably by commercial fishing, and shad became rare in alligator diets in this period compared with 1999-2000 when shad were eaten commonly and the mortality syndrome was evident.

Because of the complexity of the Lake Griffin system and the number of uncontrollable variables both natural (e.g. water levels) and human (e.g. fishing) it is very difficult to design definitive experiments or draw precise predictive conclusions.

However, it does appear that the recent improvement in water quality in Lake Griffin is contemporaneous with a reduction in alligator mortality. Careful evaluation of data collected addressing this problem is needed to further plan research activities.

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Appendix 1. Weights of captive alligators, July 2002-June 2003

Days	Date	Alligator						
		50809-10	50811-12	50813-14	50819-20	50823-25	5082-27	50828-29
0	1-Jul	16.00	21.00	40.00	26.00	20.00	29.00	24.00
31	31-Jul	16.00	18.00	36.50	23.50	20.00	29.00	24.00
68	6-Sep	19.00	19.00	41.00	27.00	19.00	31.50	25.00
92	30-Sep	21.00	19.00	41.50	28.00	20.00	33.00	27.00
127	4-Nov	22.50	21.00	45.00	30.00	22.50	37.00	33.00
164	11-Dec	24.50	20.50	44.50	30.50	23.00	40.00	30.00
249	5-Mar-03	22.00	19.50	41.00	28.50	21.75	37.00	27.00
300	24-Apr-03	22.00	18.50	41.25		20.00	35.00	28.00
343	6-Jun-03	19.50	17.00			19.50	32.50	27.25
427	29-Aug-03		18.00			20.50	33.00	29.00

Appendix 2. Food intake of captive alligators, July 2002-June 2003

alligator	**50810**	**50812**	**50814**	**50820**	**50823/25**	**50826/27**	**50828/29**
weight		21000	40000	26000	20000	29000	24000
food eaten							
20-Jun	900						
3-Jul	312	0	74	199			
5-Jul	500	0	0	0			
8-Jul	40	0	20	40			
10-Jul	163	0	0	95			
12-Jul	300	0	0	280			
15-Jul	411	0	0	0			
19-Jul	376	0	30	270			
24-Jul	476	0	112	396	0	0	0
29-Jul	660	0	346	605	0	705	868
31-Jul	698	0	704	584	554	744	646
total eatn	4836	0	1286	2469	554	1449	1514
weight	16000	18000	36500	23500			
		-3000	-3500	-2500			
%wt loss /gain		-14%	-9%	-10%			
consumed							
% Body wt	30%	0%	4%	11%			
%Bw/day	1.08%	0.00%	0.13%	0.38%			
%Bw/week	8%	0%	1%	3%	3%	5%	6%

alligator	**50809/10**	**50811/12**	**50813/14**	**50819/20**	**50823/25**	**50826/27**	**50828/29**
captureweight	16000	21000	40000	26000	20000	29000	24000
food eaten							
2-Aug	420	0	0	356	0	502	392
5-Aug	396	0	333.8	705	472	696	676
7-Aug	352	0	424	516	330	596	480
9-Aug	325	0	280	592	450	840	496
12-Aug	320	0	408	712	440	646	480
14-Aug	406	0	720	568	440	620	328
16-Aug	332	0	1020	512	448	752	0
19-Aug	382	0	1035	558	428	671	504
21-Aug	372	0	792	537	442	672	498
23-Aug	384	0	608	542	420	640	416
27-Aug	388	0	1203	516	408	712	483
29-Aug	321	0	921	564	403	703	419
total eatn	4398	0	7744.8	6678	4681	8050	5172
weight 6 sep	19000	19000	41000	27000	19000	31500	25000

gain/loss	3000	-2000	1000	1000	-1000	2500	1000
	19%	-10%	3%	4%	-5%	9%	4%
% Body wt	23%	0%	19%	25%	25%	26%	21%
%Bw/day	0.83%	0.00%	0.67%	0.88%	0.88%	0.91%	0.74%
%Bw/week	6%	0%	5%	6%	6%	6%	5%

alligator	50809/10	50811/12	50813/14	50819/20	50823/25	50826/27	50828/29
captureweight	16000	21000	40000	26000	20000	29000	24000
food eaten							
3-Sep	344	0	675	520	443	685	509
4-Sep	345	0	812	528	372	464	490
6-Sep	360	0	0	500	440	740	580
9-Sep	354	0	0	704	456	688	472
11-Sep	316	0	375	480	604	629	476
13-Sep	365	0	827	596	458	632	512
16-Sep	320	0	1039	609	444	662	413
17-Sep	340	0	852	755	479	760	684
20-Sep	276	0	593	511	384	379	369
23-Sep	299	0	864	788	452	596	483
25-Sep	347	0	510	532	495	667	484
27-Sep	378	264	828	559	667	610	473
30-Sep	360	0	962	660	548	616	484
total eatn	4404	264	8337	7742	6242	8128	6429
weight 30sep	21000	19000	41500	28000	20000	33000	27000
gain/loss	5000	-2000	1500	2000	0	4000	3000
	31%	-10%	4%	8%	0%	14%	13%
% Body wt	21%	1%	20%	28%	31%	25%	24%
%Bw/day	0.70%	0.05%	0.67%	0.92%	1.04%	0.82%	0.79%
%Bw/week	5%	0%	5%	6%	7%	6%	6%

alligator	**50809/10**	**50811/12**	**50813/14**	**50819/20**	**50823/25**	**50826/27**	**50828/29**
captureweight	16000	21000	40000	26000	20000	29000	24000
food eaten							
4-Oct	360	417	852	667	399	724	620
7-Oct	315	440	852	568	399	696	316
9-Oct	316	452	867	512	444	676	503
11-Oct	241	428	668	503	376	385	378
14-Oct	408	548	968	562	511	604	568
16-Oct	312	432	604	492	384	659	440
19-Oct	321	0	712	381	336	579	367
21-Oct	316	112	405	476	302	531	351
23-Oct	328	0	697	465	394	639	456
26-Oct	288	0	744	338	349	465	376

28-Oct	356	0	691	541	419	648	495
30-Oct	267	0	977	393	351	421	361
total eatn	3828	2829	9037	5898	4664	7027	5231
weight 4 Nov	22500	21000	45000	30000	22500	37000	33000
gain/loss	6500	0	5000	4000	2500	8000	9000
	41%	0%	13%	15%	13%	28%	38%
% Body wt	17%	13%	20%	20%	21%	19%	16%
%Bw/day	0.61%	0.48%	0.72%	0.70%	0.74%	0.68%	0.57%
%Bw/week	4%	3%	5%	5%	5%	5%	4%

alligator	50809/10	50811/12	50813/14	50819/20	50823/25	50826/27	50828/29
captureweight	16000	21000	40000	26000	20000	29000	24000
food eaten							
2-Nov	0	0	0	0	0	0	0
4-Nov	258	0	0	470	307	604	432
11-Nov	396	0	0	434	457	516	363
13-Nov	288	0	0	408	376	526	410
16-Nov	315	0	0	493	364	520	352
18-Nov	0	0	0	0	0	0	0
20-Nov	252	0	0	500	360	504	0
23-Nov	0	0	0	0	0	0	0
25-Nov	304	0	0	484	416	512	0
27-Nov	0	0	0	0	0	0	0
29-Nov	0	0	0	0	0	0	0
total eatn	1813	0	0	2789	2280	3182	1557
weight 11 dec	24500	20500	44500	30500	23000	40000	30000
gain/loss	8500	-500	4500	4500	3000	11000	6000
	53%	-2%	11%	17%	15%	38%	25%
% Body wt	7%	0%	0%	9%	10%	8%	5%
%Bw/day	0.26%	0.00%	0.00%	0.33%	0.35%	0.28%	0.19%
%Bw/week	2%	0%	0%	2%	2%	2%	1%

alligator	50809/10	50811/12	50813/14	50819/20	50823/25	50826/27	50828/29
captureweight	16000	21000	40000	26000	20000	29000	24000
food eaten							
2-Dec	0	0	0	0	0		0
5-Dec	0	0	0				
7-Dec	0	0	0				
9-Dec	292	0	0			540	
		0	0				

			0	0				
			0	0				
			0	0				
			0	0				
			0	0				
			0	0			0	
total eatn		292	0	0	0	0	540	0
weight 11 dec		24500	20500	44500	30500	23000	40000	30000
gain/loss		8500	-500	4500	4500	3000	11000	6000
		53%	-2%	11%	17%	15%	38%	25%
% Body wt		1%	0%	0%	0%	0%	1%	0%
%Bw/day		0.04%	0.00%	0.00%	0.00%	0.00%	0.05%	0.00%
%Bw/week		0.28%	0.00%	0.00%	0.00%	0.00%	0.32%	0.00%

alligator		50809/10	50811/12	50813/14	50819/20	50823/25	50826/27	50828/29
captureweight		16000	21000	40000	26000	20000	29000	24000
food eaten								
	10-Dec	0	0	0	0	0	0	0
Jan		0	0	0	0	0	0	0
Feb		0	0	0	0	0	0	0
	5-Mar	0	0	0	0	0	0	0
total eatn		0	0	0	0	0	0	0
weight		24500	20500	44500	30500	23000	40000	30000
weight		22000	19500	41000	28500	21750	37000	27000
gain/loss		-2500	-1000	-3500	-2000	-1250	-3000	-3000
% loss		-10%	-5%	-8%	-7%	-5%	-8%	-10%
%loss/day		-0.12%	-0.06%	-0.09%	-0.08%	-0.06%	-0.09%	-0.12%
% capture		53%	-2%	11%	17%	15%	38%	25%
% capture		38%	-7%	3%	10%	9%	28%	13%
difference		-16%	-5%	-9%	-7%	-6%	-10%	-13%

alligator		**50809/10**	**50811/12**	**50813/14**	**50819/20**	**50823/25**	**50826/27**	**50828/29**
captureweight		16000	21000	40000	26000	20000	29000	24000
food eaten								
	5-Mar	280	0	0	0	0	576	0
	10-Mar	234	0	0	0	0	627	0
	12-Mar	484	0	0	0	0	739	0
	17-Mar	260	0	0	dead	0	623	0
	21-Mar	284	0	0		304	424	495
	24-Mar	286	0	0		302	506	370
	31-Mar	320	0	0		414	0	383

total eatn	2148	0	0	0	1020	3495	1248
weight	22000	19500	41000	28500	21750	37000	27000

Not weighed

alligator		50809/10	50811/12	50813/14	50819/20	50823/25	50826/27	50828/29
captureweight		16000	21000	40000	26000	20000	29000	24000
food eaten					dead			
	5-Apr	0	0	0		272	0	394
	7-Apr	0	0	0			0	
	9-Apr	0	0	0		452	0	0
	14-Apr	0	0	0		330	0	306
	16-Apr	0	0	0			0	
	18-Apr	0	0	0		274	0	392
	21-Apr	0	0	0		0	0	
	24-Apr	0	0	0		0	0	0
	28-Apr	0	0	0		0	0	0
total eatn		0	0	0		1328	0	1092
weight		22000	19500	41000		21750	37000	27000
		22000	18500	41250		20000	35000	28000
gain/loss		0	-1000	250		-1750	-2000	1000
% loss		0%	-5%	1%		-8%	-5%	4%
%loss/day			0.01%	0.00%		0.00%	0.00%	0.00%

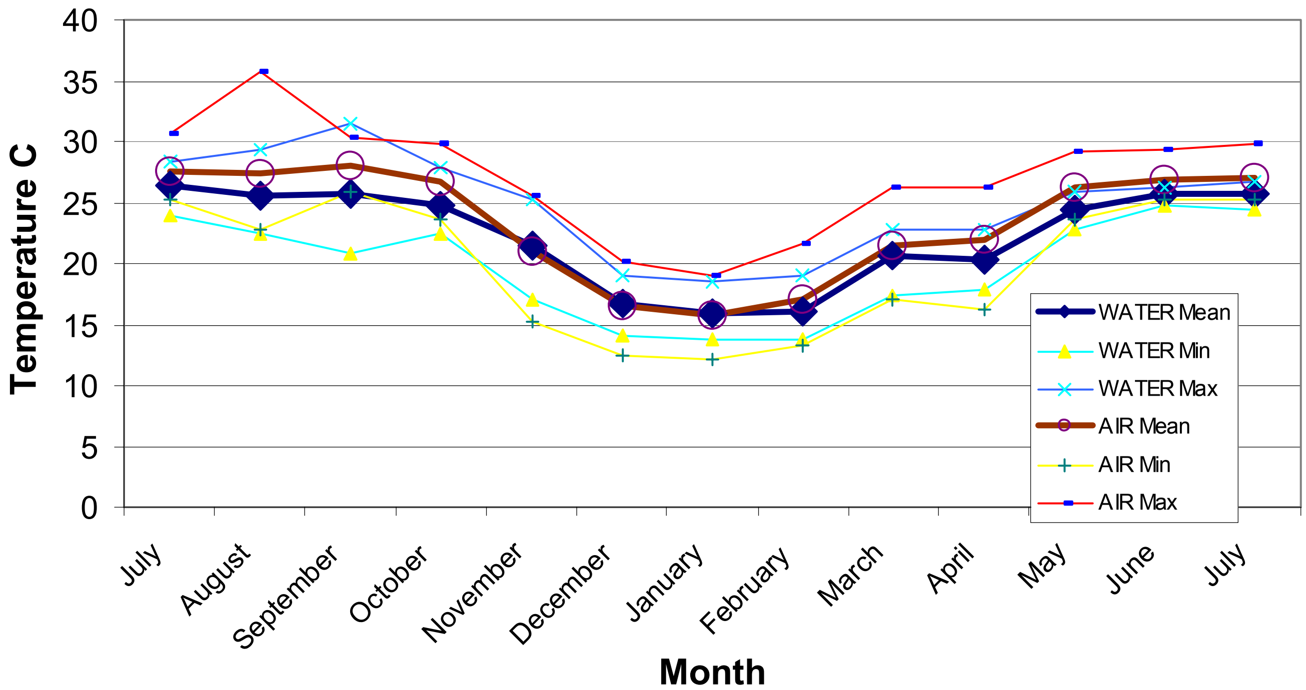
alligator		50809/10	50811/12	50813/14	50819/20	50823/25	50826/27	50828/29	
captureweight		16000	21000	40000	26000	20000	29000	24000	
food eaten					dead				
	2-May	0	0	0		0	0	428	pork
	7-May	0	0	0		0	0	415	
	12-May	0	0	0		0	0	440	
	16-May	0	0	0		0	0	476	
	21-May	0	292	0		0	0	341	chicken
	26-May	0	0	0		0	0	284	
	30-May	0	0	dead		0	0	395	
total eatn		0	292	0		0	0	2351	
		22000	18500	41250		20000	35000	28000	

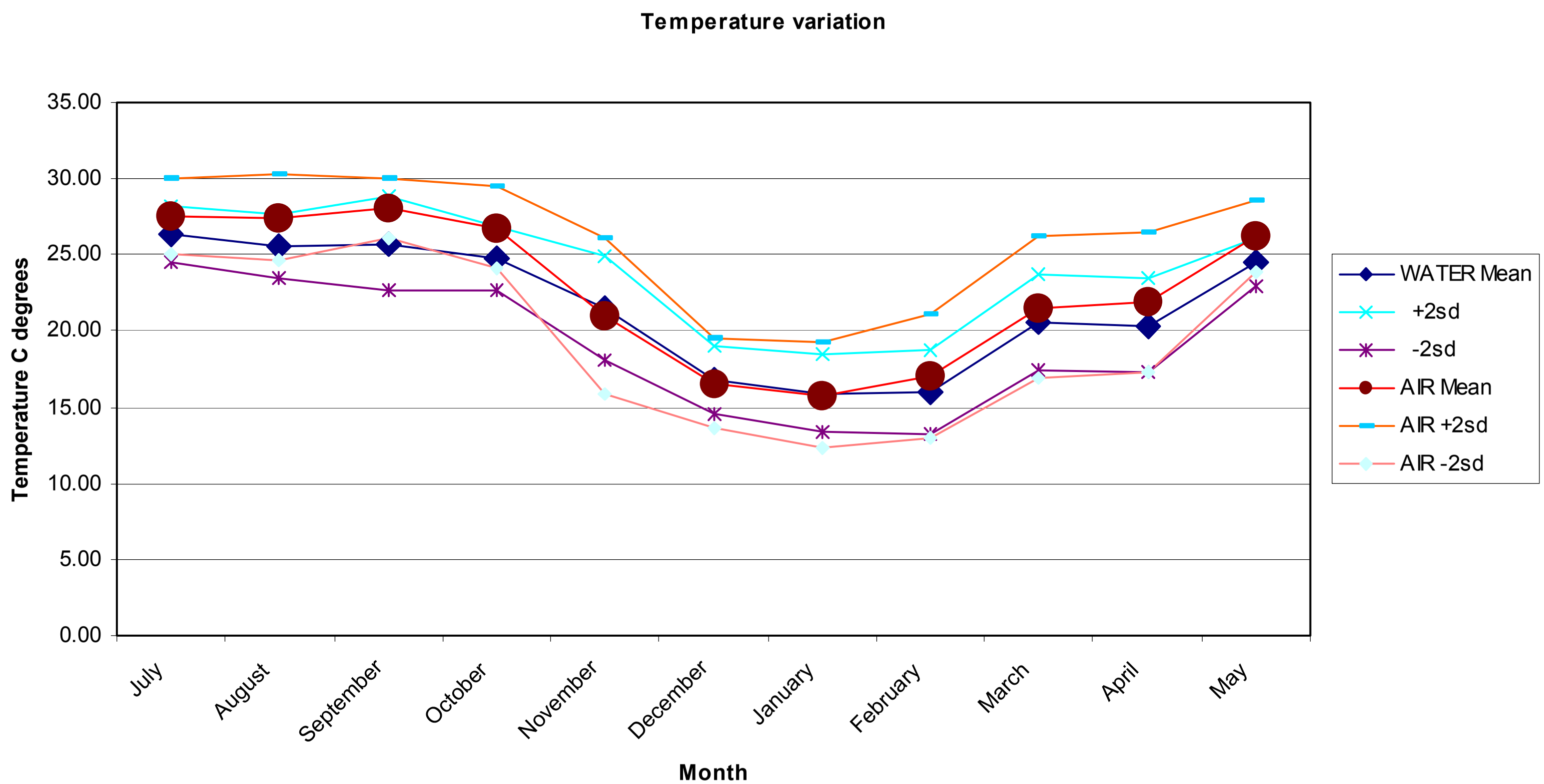
Chicken

alligator		**50809/10**	**50811/12**	**50813/14**	**50819/20**	**50823/25**	**50826/27**	**50828/29**
captureweight		16000	21000	40000	26000	20000	29000	24000
food eaten				dead	dead			
	6-Jun	0	0			0	0	0
	11-Jun	0	447			0	0	420
	16-Jun	0	449			0	0	372
	18-Jun	0				0	0	323
	20-Jun	dead	459			0	0	329
	25-Jun		282			184		369
	30-Jun		300			188	194	413
total eatn		0	1937			372	194	2226
						chicken		
weight		19500	17000			19500	32500	27250
gain/loss		3500	-4000			-500	3500	3250
		22%	-19%			-3%	12%	14%
% Body wt		0%	11%			2%	1%	8%
%Bw/day		0.00%	0.41%			0.07%	0.02%	0.29%
%Bw/week		0%	3%			0%	0%	2%

Controls* Received 250 mg thiamin in 10ml saline soln im on 6 June
Received thiamin pill 344mg with each meal eaten

Appendix 3. Air and water ambient temperatures for captive alligators July 2002-June 2003




Temperature variation
Temperature C degrees
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
July
August
September
October
November
December
January
February
March
April
May
Month
WATER Mean
+2sd
-2sd
AIR Mean
AIR +2sd
AIR -2sd

Appendix 4. Thiamin values in captive alligators

Captive Alligator Muscle Values

Values are expressed in pmol/gram.

SAMPLE ID	Sample date	TPP	TP	Thiamine	Total thiamine
50809 M	4-Nov-02	126.10	9.20	0.00	135.31
died June 20, 2003	5-Mar-03	74.81	29.93	11.66	116.40
	6-Jun-03	53.68	17.02	89.60	160.30
	20-Jun-03*	58.3	14.9	9.3	82.6
50811 M	4-Nov-02	111.38	15.11	6.46	132.95
euthanized August 29, 2003	5-Mar-03	61.03	19.21	7.21	87.45
	6-Jun-03	73.73	18.77	20.17	112.67
	29-Aug-03	189.4	35.7	7.4	232.5
50813 M	4-Nov-02	154.58	20.53	0.00	175.11
died May 30, 2003	5-Mar-03	64.87	22.96	6.15	93.98
	30-May-03*	68.47	11.41	15.39	95.27
50819 M	4-Nov-02	72.64	10.64	0.00	83.28
died Mar 14, 2003	5-Mar-03	40.42	19.48	9.20	69.11
	14-Mar-03	58.58	22.16	39.86	120.60
50823 M	4-Nov-02	120.24	15.01	0.00	135.24
euthanized August 29, 2003	5-Mar-03	54.81	24.80	7.34	86.95
	29-Aug-03	94.6	20.7	5.0	120.3
50826 M	4-Nov-02	238.53	29.46	0.00	267.99
diet was supplemented... but it didn't	5-Mar-03	80.03	31.77	11.38	123.18
eat for 1 month, started again late June	6-Jun-03	88.06	16.38	46.79	151.23
euthanized August 29, 2003	29-Aug-03	209.4	27.6	7.0	244.0
50828 M	4-Nov-02	84.42	8.68	0.00	93.10
diet was supplemented	5-Mar-03	123.24	31.34	14.73	169.31
euthanized August 29, 2003	6-Jun-03	258.88	48.18	77.49	384.55
	29-Aug-03	191.4	30.7	5.5	227.6

*= date on sample is diff., but has been changed according to PR date of death

Captive Blood Thiamin A Blood results
Rec'd 6/18/03
Values are expressed in pmol/g

Sample ID	TPP	TP	T	Total Thiamine
50809 RBC				
died June 20, 2003				
2-Aug-02	93.3	25.3	2.6	121.2
6-Sep-02	48.7	20.8	6.2	75.7
30-Sep-02	113.8	31.0	24.3	169.1
4-Nov-02	99.9	28.6	14.4	142.8
11-Dec-02	43.8	25.0	8.9	77.7
24-Apr-03	50.2	20.2	2.0	72.4
6-Jun-03	41.7	22.0	13.2	77.0
50811 RBC				
euthanized August 29, 2003				
25-Jun-02	215.4	47.0	3.6	266.0
2-Aug-02	80.7	25.2	3.8	109.7
6-Sep-02	111.8	31.3	7.3	150.4
30-Sep-02	113.5	26.8	23.5	163.9
4-Nov-02	91.8	32.7	6.5	131.0
11-Dec-02	87.2	38.9	7.5	133.6
24-Apr-03	68.5	15.1	2.0	85.7
6-Jun-03	54.4	22.4	10.9	87.7
29-Aug-03	22.2	11.3	7.1	40.6
50813 RBC				
died May 30, 2003				
25-Jun-02	177.7	42.3	3.9	224.0
2-Aug-02	**87.1**	**16.7**	**9.6**	**113.4**
6-Sep-02	**128.0**	**27.3**	**19.2**	**174.5**
30-Sep-02	94.9	26.9	21.6	143.5
4-Nov-02	75.0	27.4	8.5	111.0
11-Dec-02	69.2	33.6	8.6	111.4
24-Apr-03	45.8	9.4	1.1	56.3
50819 RBC				
died June 20, 2003				
25-Jun-02	185.0	42.5	3.8	231.3
2-Aug-02	105.2	26.2	3.2	134.6
6-Sep-02	55.2	19.5	8.5	83.2
30-Sep-02	91.6	20.6	20.4	132.7
4-Nov-02	83.0	24.6	6.4	114.0
11-Dec-02	61.6	25.5	9.0	96.1
50823 RBC				
euthanized August 29, 2003				
6-Sep-02	62.2	20.2	14.9	97.4

30-Sep-02	102.2	25.1	17.8	145.2
4-Nov-02	86.3	32.3	7.0	125.6
11-Dec-02	83.9	31.6	8.0	123.5
24-Apr-03	49.3	16.1	1.3	66.7
6-Jun-03	37.5	20.0	10.0	67.5
29-Aug-03	59.1	20.8	9.2	89.2
50826 RBC				
diet was supplemented... but it didn't				
eat for 1 month, started again late June				
euthanized August 29, 2003				
6-Sep-02	72.0	26.9	6.9	105.9
30-Sep-02	124.8	28.3	18.1	171.2
4-Nov-02	99.5	24.4	3.3	127.3
11-Dec-02	87.5	34.3	8.0	129.8
24-Apr-03	46.6	8.5	0.5	55.6
6-Jun-03	35.2	17.4	22.5	75.1
29-Aug-03	234.3	37.2	8.3	279.9
50828 RBC				
diet was supplemented				
euthanized August 29, 2003				
6-Sep-02	65.2	19.6	6.8	91.6
30-Sep-02	133.4	45.6	51.9	230.9
4-Nov-02	124.8	29.2	5.9	159.9
11-Dec-02	96.8	39.0	10.4	146.2
24-Apr-03	80.2	24.4	14.2	118.8
6-Jun-03	138.4	40.8	12.5	191.7
29-Aug-03	261.0	38.0	9.5	308.5

Captive Alligator Livers, post-mort.

Values are expressed in pmol/g

Sample ID	TPP	TP	T	Total Thiamine
50809-10	583.4	230.1	174.5	988.0
50811-12	511.6	139.6	87.3	738.4
50813-14	553.0	161.0	91.6	805.6
508219-20	238.5	115.5	140.4	494.4
50823-25	414.2	181.4	80.3	676.0
50826-27	1,084.7	304.4	135.0	1,524.0
50828-29	1,276.4	359.2	110.1	1,745.6

Appendix 5. Thiaminase values measured in shad from Lake Griffin and Lake Apopka

Test Run	Sample ID	Origin	Species	No. in sample	Fork length mm	Weight g	Month collection	Thiaminase Pmol/min/g
33	114	Lake Griffin	Whole gizzard shad	1	270	n/a	May-99	13502.3
34	116	Lake Griffin	Whole gizzard shad	1	280	n/a	May-99	33447.9
35	117	Lake Griffin	Whole gizzard shad	1	244	n/a	May-99	25148.4
36	214	Lake Griffin	Whole gizzard shad	1	n/a	n/a	Mar-01	20707.1
37	226	Lake Griffin	Whole gizzard shad	1	440	n/a	Apr-01	14316.1
38	247	Lake Griffin	6 shad @9.5g ea	6	9.5	n/a	Jun-01	13171.6
39	248 pkg1	Lake Griffin	Whole gizzard shad	1	113	n/a	Jun-01	6810.1
40	248 pkg2	Lake Griffin	Whole gizzard shad	1	372	n/a	Jun-01	7222.1
41	248C	Lake Griffin	Whole gizzard shad	1	303	n/a	Jun-01	20668.5
42	248Cduplicate	Lake Griffin	Whole gizzard shad	1				20981.2
74	Control	CCIW	Alewife	n/a	n/a	n/a	n/a	8145.1
75	250	Lake Griffin	Gizzard Shad(whole body)	7	55	8	Jul-02	17320.0
76	251	Lake Griffin	Gizzard Shad(whole body)	1	68	17	Jul-02	20852.3
77	252	Lake Griffin	Gizzard Shad(whole body)	1	89	17.5	Jul-02	13032.2
78	253	Lake Griffin	Gizzard Shad(whole body)	1	71	17.5	Aug-02	17029.3
79	254	Lake Griffin	Gizzard Shad(whole body)	5		7.6	Aug-02	19879.4
80	255	Lake Griffin	Gizzard Shad(whole body)	1	169	23.5	Aug-02	10972.1
81	256	Lake Griffin	Gizzard Shad(whole body)	1	100	20	Aug-02	13985.7
82	257	Lake Griffin	Gizzard Shad(whole body)	1	90	19	Aug-02	16555.8
83	258A	Lake Griffin	Gizzard Shad(whole body)	1	100	20	Aug-02	10170.8
84	258 Duplicate	Lake Griffin	Gizzard Shad(whole body)	1	100	20	Aug-02	9599.1
85	259	Lake Griffin	Gizzard Shad(whole body)	1	350	27	Aug-02	11280.5
86	259-Duplicate	Lake Griffin	Gizzard Shad(whole body)	1	350	27	Aug-02	10345.0
87	Control	CCIW	Alewife	n/a	n/a	n/a	n/a	8203.4
88	260	Lake Griffin	Gizzard Shad(whole body)	1	210	24.5	Aug-02	12277.9
89	261	Lake Griffin	Threadfin Shad?	11	34.1	6.04	Sep-02	2570.5
90	262	Lake Griffin	Gizzard Shad(whole body)	1			Sep-02	22250.2
91	263	Lake Griffin	Gizzard Shad(whole body)	1	83	18	Sep-02	13645.6
92	264	Lake Griffin	shad ?	6	28.6	7	Sep-02	2408.5
93	265	Lake Griffin	Gizzard Shad(whole body)	1	330	28	Sep-02	18583.9
94	266	Lake Griffin	Gizzard Shad(whole body)	1	280	27	Sep-02	14637.5

Test Run	Sample ID	Origin	Species	No. in sample	Fork length mm	Weight g	Month collection	Thiaminase Pmol/min/g
95	267	Lake Griffin	shad ?	6	25.4	6.61	Oct-02	2797.4
96	268	Lake Griffin	shad ?	11	26.1	5.53	Oct-02	2539.9
97	269	Lake Griffin	shad ?	9	40	6.79	Oct-02	2543.1
98	269 duplicate	Lake Griffin	shad ?	9	40	6.79	Oct-02	2706.7
99	270	Lake Griffin	shad ?	10	38.6	6.67	Oct-02	2677.2
100	Control	CCIW	Alewife	n/a	n/a	n/a	n/a	6524.0
101	271	Lake Griffin	Gizzard Shad(whole body)	1	250	25	Oct-02	21045.4
102	272	Lake Griffin	shad ?	10	44.9	7.06	Oct-02	4331.6
103	273	Lake Griffin	shad ?	5	24.5	6.86	Nov-02	2223.7
104	274	Lake Griffin	shad ?	5	23.4	6.78	Nov-02	1591.1
105	275	Lake Griffin	shad ?	5	18.4	6.52	Nov-02	2643.3
106	276	Lake Griffin	shad ?	5	25	7.18	Nov-02	3633.2
107	277	Lake Griffin	Threadfin Shad	6	23.4	6.57	Nov-02	3356.7
108	278	Lake Griffin	shad ?	5	28.8	7.52	Nov-02	2538.3
109	279	Lake Griffin	Gizzard Shad(whole body)	1	100	17.5	Nov-02	13391.3
110	280	Lake Griffin	shad ?	5	28	7.32	Nov-02	2872.8
111	290	Lake Griffin	shad ?	5	22.8	6.96	Nov-02	2448.3
112	290-duplicate	Lake Griffin	shad ?	5	22.8	6.96	Nov-02	5168.9
113	Control	CCIW	Alewife	n/a	n/a	n/a	n/a	8192.2
114	291	Apopka- feed shad	Gizzard Shad(whole body)	1	320	30	Sept.30/02	28041.9
115	292	Apopka-feed shad	Gizzard Shad(whole body)	1	400	30	Jul.25/02	14364.4
116	293	Apopka- feed shad	Gizzard Shad(whole body)	1	350	30	Sept.30/02	22842.8
117	294	Apopka- feed shad	Gizzard Shad(whole body)	1	360	30	Sept.30/02	22612.7
118	281	Lake Griffin	Threadfin Shad	5	35	7	Dec-02	3147.4
119	282	Lake Griffin	Threadfin Shad	8	44	6.5	Dec-02	4294.7
120	283	Lake Griffin	Threadfin Shad	10	52	7.2	Dec-02	3196.5
121	285	Lake Griffin	Gizzard Shad(whole body)	1	120	19	Dec.6/02	25309.8
122	285 duplicate	Lake Griffin	Gizzard Shad(whole body)	1	120	19	Dec.6/02	20526.4

Appendix 6 . Species identified from alligator stomachs in three Florida lakes. (2001-June 2003). Minimum number of individuals inferred from recorded parts and fresh material (see text).

Appendix 6 a. Species recovered from alligator stomachs, Lake Griffin (N = 101 stomachs)

Group	Species		Min Individs(all)	Min. Fresh
Fish	Gizzard Shad	*Dorosoma cepedianum*	19	17
	Catfish	*Ameiurus sp.*	32	24
	Centrarchid fish	*Centrarchidae*	5	5
	Sunfish	*Lepomis sp.*	1	1
	Black Crappie	*Pomoxis nigromaculatus*	1	1
	Florida Gar	*Lepisosteus platyrhincus*	7	6
	Mosquito Fish	*Gambusia holbrooki*	2	2
	Lake Eustis pupfish	*Cyprinodon variegatus hubbsi*	1	1
	Sailfin Molly	*Poeocilis latipinna*	1	1
		Fundulus sp.	2	2
	Atlantic Needlefish	*Strongylura marina*	1	1
	Blue Tilapia	*Oreochromis aureus*	1	1
	Bowfin	*Amia calva*	1	1
Reptiles	Stink pot turtle	*Sternotherus odoratus*	18	6
	Fl. Red belly turtle	*Pseudemys nelsoni*	5	1
	Gopher tortoise	*Gopherus polyphemus*	2	2
	Florida Softshell	*Apalone ferox*	1	1
	Loggerhead musk turtle	*Sternotherus minor*	2	2
	Striped mud turtle	*Kinosternon baurii*	1	
	Brown water snake	*Nerodia taxispilota*	2	
	Cottonmouth	*Agkistrodon piscivorous*	3	
	American alligator	*Alligator mississippiensis*	7	
Amphibians	Frog	*Rana sp.*	3	
	Siren	*Siren/Amphiuma sp*	3	
Birds	Anhinga	*Anhinga anhinga*	2	

	Common moorhen/coot	*Gallinula chloropus/Fulica americana*	2	
	Double crested cormorant	*Phalacrocorax auritus*	2	
	White ibis	*Eudocimus albus*	1	
Mammals	Hispid cotton rat	*Sigmodon hispidus*	1	
	Raccoon	*Procyon lotor*	1	
Invertebrates				
Gastropoda	Apple snails	*Pomacea paludosa*	962	70
Bivalvia	Mussel	*Utterbachia sp.*	2	
Crustacea	Crayfish	*Procambrarus sp.*	2	
	Grass shrimp	*Palaemonetes intermedius*	158	
Insecta	Grasshopper	*Romalea guttata*	12	
	Dragonflies	*Odonata*	5	
	Water bugs	*Heminoptera*	4	

Appendix 6 b. Species recovered from alligator stomachs, Lake Woodruff (N = 40 stomachs)

Group	Species		Min. Individs all	Min Fresh
Fish				
	Gizzard Shad	*Dorosoma cepedianum*	5	5
	Catfish	*Ameiurus sp*	4	3
	Florida Gar	*Lepisosteus platyrhincus*	2	1
	Centrarchid fish	*Centrarchidae*	5	3
	Redear Sunfish	*Lepomis microlophus*	1	1
	Sunfish	*Lepomis sp.*	5	5
	Black Crappie	*Pomoxis nigromaculatus*	1	1
	Largemouth bass	*Micropterus salmoides*	4	4
	Warmouth	*Lepomis gulosus*	1	1
	Mosquitofish	*Gambuis holbrooki*	1	0
	Atlantic needlefish	*Strongylura marina*	3	2
Reptiles	Stinkpot turtle	*Sternotherus odoratus*	3	1
	Loggerhead musk turtle	*Sternotherus minor*	1	0
	Alligator	*Alligator mississippiensis*	1	
Birds	Unidentifiable		1	
Mammals	Hispid cotton rat	*Sigmodon hispidus*	1	
Invertebrates				
	Apple snails	*Pomacea paludosa*	266	23
	Grass shrimp	*Palaemonetes intermedius*	8	
	Crayfish	*Procambarus sp.*	7	
	Water bugs	*Belostomatidae*	8	
	Beetles	*Elatridae*	1	
	Dragonfly	*Odonata*	2	
	Bessbug	*Passalidae*	2	

Appendix 6c. Species recovered from alligator stomachs, Lake Apopka (N= 49 stomachs)

Group	Species		Min. Individs all	Min. Fresh
Fish				
	Gizzard shad	*Dorosoma cepedianum*	66	40
	Catfish	*Ameiurus sp*	15	8
	Florida gar	*Lepisosteus platyrhincus*	3	2
	Centrarchid fish	*Centrarchidae*	3	3
	Blue gill	*Lepomis macrochius*	4	4
	Black crappie	*Pomoxis nigromaculatus*	1	1
	Blue tilapia	*Oreochromis aureus*	8	6
	Golden shiner	*Notemigonus crysoleucas*	1	1
Reptiles	Florida Softshell	*Apalone ferox*	1	
	Stinkpot	*Sternotherus odoratus*	6	1
	Florida mud turtle	*Kinosternon subrubrum*	1	
	Gopher tortoise	*Gopherus polyphemus*	1	1
	Cottonmouth	*Agkistrodon piscivorous*	1	
	Mud snake	*Farancia abacura*	1	
	Alligator	*Alligator mississippiensis*	5	
Birds	Unidentified bird		4	
Mammals	Eastern wood rat	*Neotoma floridana*	1	
	Cotton mouse	*Peromyscus gossypinus*	1	
Invertebrates				
	Apple snails	*Pomacea paludosa*	98	3
	Grass shrimp	*Palaemonetes intermedius*	17	
	Crayfish	*Procambarus sp.*	4	
	Grasshoppers	*Romalea guttata*	26	
	Beetles	*Elatridae*	1	
	Dragonfly	*Odonata*	2	
	Green june beetle	*Cotinis nitida*	11	