Comparison of contaminant levels in American Alligators (*Alligator mississippiensis*) on an On-Refuge (Lake Woodruff NWR) and an Off-Refuge (Lake Griffin) site in Central Florida.

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I ABSTRACT

Between 1998 and 2000 Alligators in Lake Griffin, Florida, demonstrated unusually high mortality that was thought to be associated with that lakes hyper eutrophic and possibly contaminated condition. We have conducted preliminary analysis of Organo-chloride and heavy metal levels in tissues of American alligators and also examined several other factors that may have affected alligators in Lake Griffin and Lake Woodruff. The results of these investigations suggest that the levels of Organochloride and toxic metal contaminants on both Lake Griffin and Lake Woodruff are at background levels and are insufficient to explain the observed mortality of alligators. Alligators in each lake demonstrate different patterns of low OC residue that are thought to reflect the different history and locations of the lakes. We have also examined and eliminated disease (Botulism, viral infection including West Nile Virus, bacterial infection) and direct effects of toxic blue green algae as causes of alligator mortality. We have also made a comparison of the diet of alligators in the two lakes and conducted a detailed pathology investigation.

Separate studies published elsewhere suggest that a dietary deficiency of thiamin induced by a diet of thiaminase rich Gizzard shad (*Dorosoma cepedianum*) contributes to the observed mortality of alligators in Lake Griffin. However additional factors including blue green algal toxins and contaminants are still under investigation.

II Background and Justification.

The national refuge system provides a series of large area sanctuaries for wild organisms, habitats and ecosystems that are protected from most obvious and disruptive human activities. However, refuges are subject to influences of historical useage predating their protection and from influences originating outside their borders. The integrity of the national refuge system requires understanding of these external influences. One external influence of current concern is contaminants that affect wildlife.

Contaminants in aquatic systems are well known to cause wildlife mortality when present at toxic concentrations, however, in recent studies, the effects of contaminants at lower non-lethal concentrations has been implicated in developmental disruption and reproductive failure of organisms. Contaminants enter freshwater aquatic systems by run-off from surrounding agricultural and urban sources and may persist for many years. Ecological processes within these systems affect contaminant distribution, storage, binding and toxicity. The effects of contaminants may also interact with other influences in the system and its organisms, such as climate change, nutrient levels, eutrophication, succession and changes in biodiversity and species composition at every level in the food chain. Although major inputs of contaminants (such as chemical spills) may cause immediate wildlife die-offs and be obvious, he complexity and timing of ecological interactions makes recognition of sub-lethal contaminant effects difficult to recognize.

This study was designed to evaluate levels of common and persistent contaminants in organisms at different levels in the food chain and exhibiting different dietary habits in an on-refuge (Lake Woodruff) and off-refuge (Lake Griffin) site in Florida. The focus of the study was the American alligator, which was undergoing unexplained mortality and reproductive failure in Lake Griffin and is the top predator in these systems. Alligators are long lived (decades), have large body mass (1-350 kg) and can maintain large stores of body fat, and therefore might be expected to bioaccumulate contaminants. By examining contaminants in alligators and their prey we hoped to elucidate pathways and amounts of contaminants in the system, provide comparative data for more contaminated and less contaminated locations and explain the alligator mortality.

Table 1. Target organisms, Alligator prey items at different levels of the food chain and different dietary habits.

Alligators	(*Alligator mississippiensis*)	The top predator,
Comorants	(*Phalacrocorax auritus.*)	a fish eating bird
Common moorhen	(*Gallinula chloropus*)	a vegetarian bird.
Softshell turtles	(*Apalone ferox*)	a predatory turtle
Redbellied turtles	(*Psuedemys nelsonii*)	a vegetarian turtle
Racoon	(*Procyon lotor*),	an omnivourous mammal
Brown/Yellow bullhead	(*Ameiurus nebulosus/A. natalis)*	predatory/scavenging fish
Apple snails	(*Pomacea paludosa*),	detritivore browser

Lake Griffin was chosen as the off refuge site because of a documented history of wildlife mortality and reproductive failure since 1997. The contaminant study was part of a larger and more comprehensive examination of ecosystem functions and effects on wildlife in these systems. In addition to contaminants, a series of other possible factors affecting wildlife health and alligator mortality were examined.

The comprehensive project was designed as a continuing, multidisciplinary, cooperative project among several agencies and institutions, supported by various funding sources. The Florida Museum of Natural History, University of Florida (FLMNH) served as the main coordinating agent and the USGS Cooperative Wildlife Unit served as the main administrative and logistic agent. Other participants are listed in Appendix I

The project was conducted in three phases:

1) Collection, preparation and curation of specimens and samples (1999-2000).
2) transfer for analysis to the FWS-Patuxent Wildlife center for analysis of contaminants.(2001)
3) Interpretation of data and preparation of reports. 2002-2003

The first phase was completed on schedule and a diverse set of animal tissues obtained and stored (table 2).

Table 2. Specimens collected for the study.

Species	**1999**		**2000**		**2001**	
	Lake Griffin	**L. Woodruff**	**L. Griffin**	**L. Woodruff**	**L. Griffin**	**L. Woodruff**
Alligator	9	6	7	4	38	5
Racoon	1	-	5	4	-	-
Oppossum *	2	-	-	-	-	-
Cormorant	9	0	8	8	-	-
Moorhen	12	8	8	8	-	-
Red-bellied turtle	-	1	5	-		-
Florida cooter *	-	-	4	-	-	-
Softshell turtle	-	1	-	-	-	-
Tilapia	3	-	2	-	-	-
Brown Bullhead	20	2	8	-	7	-
Yellow catfish	10	4	-	2	-	-
Other catfish ***	1	-	-	1	3	-
Shad	5	-	-	-	23	-
Apple snails	-	-	20	9	-	-
Mussels	-	-	3	-	-	-

* *Didelphis virginiana*

** *Pseudemys floridana*

The project and additional results have been reported in several published and grey literature outlets (Ross 2000 a, Ross 2000 b, Ross et al. 2000, Ross et al. 2001, Ross et al. 2002a, Ross et al. 2002 b).

III Methods

A sample of organisms representing the alligator food chain were collected from the field between February and October of 1999 and 2000. Specimens were collected under permits issued by FWCC and Animal care and handling approval of the University of Florida. Collection techniques were tailored to each organism as described in the original proposal. Alligators were caught by noose and capture dart, mammals were trapped in baited live traps, birds were shot, fish were caught by net and by trot line, turtles were caught by hand and snails were trapped in unbaited 'crayfish' traps.

Each specimen collected was immediately tagged, a data sheet recording capture details (locations, date, organisms characteristics etc), placed in a plastic bag and held on ice in a cooler. Specimens were returned to facilities in Gainesville for necropsy where the internal organs and tissues required for analysis were removed using clean techniques placed into whirl-pacs or vials and immediately frozen and held in a low temperature freezer at -70^{0}C. A data base recording all the details of specimen capture, body data (weight, size , sex etc.) and the tissue samples obtained from it was prepared in MS Access. Paper data sheets and chain of custody forms were established and maintained for each specimen. Specimens collection and preparation was accomplished in 1999 and 2000 and additional specimens were collected in 2001 and through 2002 to support other elements of the comprehensive study.

Surveys were conducted every two weeks on Lake Griffin. The circumference of the lake was traveled by airboat in a period of 5-8 hours and an experienced observer (D. Carbonneau, FWC) located dead alligators. Dead alligators were marked with spray paint to avoid counting them more than once and their size and gender recorded. Alligator size was determined in the field by measuring the Total Length (TL) with a flexible tape measure. TL is the length from the tip of the snout to the end of the tail measured along the length of the body from either dorsal or ventral aspect.

We collected alligators on Lake Griffin each week from February to May and intermittently through November from 1998 to 2001. Lake field trips were conducted by airboat. Alligators were located during the day and those assessed as clearly impaired were captured by hand lasso or capture dart, brought to the boat, secured, sexed and measured. Additional specimens of unimpaired alligators were located at night with a spotlight and captured in the same way. The size of captured alligators was measured by both Total length (TL previously defined) and Snout-Vent length (SVL). SVL is the distance from the tip of the snout to the anterior edge of the cloaca measured on the ventral aspect of the animal. SVL is a more precise measure of alligator size because it is less influenced by the animals mass or condition (stoutness) and because many alligators are missing the tip of their tail. TL is approximately 2 X SVL.

A blood sample of approx. 20 ml was drawn by syringe from the vertebral sinus. Blood samples were decanted to lithium heparin (2 tubes) and plain (1 tube) vacutainer tubes and held on ice. Within 2-6 hours the chilled tubes were centrifuged at 8,000 rpm for 10 minutes and serum or plasma decanted to 2 ml cryovials and frozen.

Captured, sick alligators were lavaged for stomach contents held restrained overnight and euthanized the next morning by cervical section and exsanguination. Alligators were transported to a necropsy facility and a full veterinary necropsy and tissue collection were undertaken. Twenty six euthanized alligators were examined by a qualified veterinary pathologist (Dr. S. Terrell) using a standardized necropsy protocol (Table 3). Additional specimens were necropsied by other personnel (Ross, Finger, Owen) using the same protocol. Major organs were examined for gross pathology and samples obtained for additional pathology analysis.

Tissue specimens were collected as follows:

Sterile collection of live tissue, chilled for virus screening and cultured for bacteria.
Collection of representative tissues into 10% buffered Formalin solution for histology.
Collection of representative tissues into whirlpacks, frozen for toxicology and other analysis.

For analysis, the tissues were subdivided and shipped in their original wrapping materials on dried ice by express courier to the analysis laboratory. Specimens were kept frozen solid and carefully labeled and inventoried to maintain specimen identification. Complete data sheets, specimen inventories and chain-of-control documentation is available. At MSU the specimens were subject to standard analysis of a screen of 27 persistent organochlorines normally seen or suspected in animal tissue. Technical details of the analysis, technique, standards and quality control, are available from the laboratory.

Table 3. Abbreviated necropsy/veterinary pathology protocol.

At capture

- Tag and record FWC tag number that becomes identifier on all tissues, specimens.
- Record date, time, collectors, location (lake and GPS), sex, snout vent length.
- Collect 10-30ml blood from occipital sinus.
- 1-2 x red top tube, 2-4 x green top lithium heparin tube
- chill-hold on ice, centrifuge, separate plasma/serum to 2ml cryovials, freeze

After euthanasia (All personnel must use protective gloves, aprons, boots and bacteriostatic wash following procedure.)

- Verify specimen number, date, location of origin, sex,
- Re-measure and record, Snout vent length, total length
- Measure and record specimen weight (note units lb. or kg)
- Examine and record specimen condition and external injuries, trauma

Open thoracic-abdominal cavity (lateral incision) and reflect ventral wall.

- Aseptically collect 2-4 ml heart blood to bacteria culture bottle
- Aseptically collect 2-4 g spleen, lung to 50ml centrifuge tube, chill/ice for viral screen.

Remove head, cut (striker saw) to remove brain intact.

- Aseptically collect section cerebral cortex to 50ml centrifuge tube, chill/ice for viral screen.
- Collect 2 mm sections cerebral cortex, medulla, cerebellum, optic lobe into Trump's solution for electron microscopy. Remainder of brain into formalin.
- Remove eyes, one in formalin, one chill/ice

Locate, dissect, examine and remove thymus, thyroid, adrenal glands, gonads

- One piece into formalin
- Remainder whirlpac chill/ice.

Examine major organs and tissues Abdominal fat, ventral tail fat, Liver, Right and Left Lung, Heart, Spleen, R and L Kidney and tail muscle for appearance, lesions, malformation, parasites and collect from each as possible.

- 0.5 cm piece in formalin
- 10-15 g in aluminum foil and whirlpac, chill/ice.
- Three x 30 g in whirlpac, chill/ice
- Three x 100 g approx. in whirlpac, chill/ice

Dissect and examine Oesophagus, stomach, small and large intestine.

- Examine for lesions and parasites
- Preserve 1 cm each in formalin
- Collect and preserve stomach contents into formalin

Dissect out following nerves and muscles, adhere to applicator stick and preserve in formalin; Brachial nerve, biceps brachi, sciatic nerve, quadriceps muscle, jaw muscle, lateral tail muscle

Dissect and collect right femur, ziplock bag, chill/ice.

Collect sections of spinal cord from cervical, thoracic, and lumbar sections

- <1cm of each into cryovial, chill/ice
- 1 cm into cassette in formalin for histology

Check all labels, transfer chilled/iced samples to –70°F freezer, check and file necropsy record, dispose of carcass, trim and mount all formalin samples for histology.

LABORATORY ANALYSIS OF TISSUES

ORGANOPHOSPHATE

Samples of serum, brain and spinal cord tissue from 18 alligators collected prior to 2001 were analyzed by The Institute of Environmental and Human Health at Texas Tech University for Cholinesterase activity and reactivation to evaluate exposure to organophosphates (OP).

Brain and spinal cord tissue samples of 0.2 g – 0.4 g received frozen from Florida were macerated and homogenized in 1:9 weight/volume Tris0.05 M pH 7.4 buffer. Cholinesterase in two forms (AchE and BchE) was released with Triton-X 100 (1%). Cholinesterase activity was measured using the method of Ellman et al. 1961 as modified by Gard and Hooper 1993 for use on a SPECTROmax 96 well spectrophotometer plate reader. Reactivation analysis was performed with 2-PAM to displace ChE inhibiting organophosphates (Hooper and Schmidt, 2001).

HEAVY METALS

ADD U PHILIdalephia Methods

Analysis of heavy metals was conducted by Department of Physics, University of Florida using Proton Induced X-ray Emissions (PIXE). PIXE is a proven spectroscopic technique which has been widely used for several decades in non-destructive simultaneous trace multi-elemental analysis in a variety of research fields such as biology, medicine, botany, zoology, geology, archeology, metallurgy, and environmental sciences (Johansson and Johansson 1976). PIXE X-ray spectra are generated by bombarding the sample atoms with a beam of energetic (1-2.5 MeV) protons. Emitted X-ray quanta include 'characteristic' X-rays that possess energies uniquely identifying the atom from which each originates. The number of emitted X-rays or 'intensity' is proportional to the number of corresponding elemental atoms in the sample being analyzed. The minimum detection limit is at the ppm level or below (1 ppm=10^{-6} g/g) with the highest sensitivity obtained for the atomic numbers $20<Z<40$ and $Z>75$ (Johansson and Campbell 1988).

Tissue samples averaging a few grams each were taken from frozen and fresh material (using nonmetallic, disposable scalpels to minimize contamination) digested with nitric acid in teflon lined bombs, pipetted onto polycarbonate film backings and vacuum dried. These were individually mounted in nylon target holders for irradiation in the accelerator. Average irradiation charge was 50 micro-Coulombs, at proton beam currents averaging 30 nanoamperes, during which period the X-ray spectra were collected with a Kevex Si(Li) detector. Peaks in the X-ray spectra were calibrated against standards of known concentration and metals present calculated to parts per million dry weight in the original sample.

ORGANOCHLORINE

Thirteen alligator muscle samples were sent to EnChem Laboratories, Wisconsin, for organochlorine analysis in April 2000. An additional 12 alligator fat samples and 10 alligator eggs were analyzed for organochlorines by Mississippi State Chemical laboratory in May 2000. Fat and liver from 7 alligators and 39 samples from birds, catfish, possum and raccoon were sent to USFWS Patuxent Analytical Laboratory, March 2000 for organochlorines and metals analysis. Serum and nerve tissue samples from twelve alligators have been sent to Texas Technical University for cholinesterase re-activation analysis to reveal exposure to organophosphates. An additional XX alligator tissue samples were collected during 2000- 2003 and sent to the USFWS Patuxent Analytical Laboratory in 2003 for Organochloride analysis. Full details of the analytic methods used can be obtained from each laboratory.

IV RESULTS

Organophosphates

Nine brain, nine spinal cord and 18 serum samples were assessed for ChE activity and reactivation. These represent samples from 9 Lake Woodruff (normal), 4 Lake Griffin (normal) and 5 Lake Griffin (impaired) alligators. ChE levels are expressed in standard 'units'/g = umoles acetylthiocholine hydrolysed/minute/g

Table 8. Brain, spinal cord and serum cholinesterase activity of alligators from Lake Griffin and Lake Woodruff. (Hooper and Schmidt 2001)

	Lake Griffin			Lake Woodruff		
	Total	AchE	BchE	Total	AchE	BChE
Brain						
Mean	1.09	1.04	0.05	1.78	1.68	0.10
SD	0.41	0.44	0.05	1.42	1.28	0.17
Range	0.6 – 1.52	0.49-1.47	0 – 0.1	0.37-3.89	0.36-3.49	0 – 0.4
N	4	4	4	5	5	5
Spinal cord						
Mean	0.47	0.42	0.06	0.64	0.60	0.04
SD	0.20	0.17	0.03	0.04	0.07	0.04
Range	0.23-0.78	0.18-0.68	0.03-0.10	0.59-0.68	0.54-0.68	0 –0.07
N	6	6	6	3	3	3
Serum						
Mean	0.61	0.03	0.58	0.68	0.03	0.65
SD	0.22	0.01	0.21	0.15	0.01	0.15
Range	0.33-1.01	0.01-0.05	0.30-0.95	0.53-0.93	0.02-0.05	0.50-0.89
N	8	8	8	10	10	10

Reactivation analysis.

Cholinesterase enzymes treated with 2-PAM will increase in measurable ChE activity if the sample has previously been inhibited with organophosphate (OP). One of nine alligators from Lake Woodruff demonstrated exposure to a low level of OP. The Serum sample from Alligator G99-31 showed 8.7% inhibition of Cholinesterase activity consistent with exposure to OP although brain and serum samples from this animal showed no reactivation. The presence of carbamate-inhibited ChE was determined by comparing ChE activity before and after incubation for 1 hour at 25°C with and without 2-PAM. Spontaneous reactivation above 20% of the pre-incubation sample was taken as an indication of carbamate inhibited ChE. Alligator G99-023 from Lake Griffin showed 22.7% spontaneous reactivation indicating carbamate exposure. This animal was previously evaluated as impaired but did not show brain lesions (Schoeb et al. 1999). The remaining samples, including samples recorded as neurally impaired and demonstrating brain lesions had no indication of OP exposure.

Heavy metals

A total of 16 samples of alligator liver were sent to the University of Pennsylvania Toxicology laboratory for analysis of heavy metals (Table 5.). Liver and kidney samples from these alligators do not generally show detectable or elevated levels of toxic metals except one individual from Lake Griffin demonstrates an elevated level of lead (13.2 ppm). Mercury levels were undetectable except in one Lake Griffin (2.05 ppm) and three

Lake Woodruff alligator livers (1.48, 3.07, 5.06 ppm). These levels are comparable to those previously reported for alligator livers and fish from Florida lakes (Brim et al. 1994, Heaton Jones et al 1997, Burger et al. 2000).

Samples of kidney, liver and spinal cord were analyzed by PIXE from 12 sick Lake Griffin alligators, five apparently healthy Lake Griffin alligators and five Lake Woodruff alligators. Initially 5-8 duplicate runs were conducted on two samples to establish sample variance and calibrate the equipment. Subsequently, 2-3 runs per sample were adequate to give repeatable results and the values were pooled for the different alligator groups. Each spectrum demonstrated peaks of x-ray absorption corresponding to 16 metals but most of these showed no significant patterns. For simplicity, only values of copper, iron, rubidium, selenium and zinc are considered here (Table 9).

The values obtained for Cu, Fe and Zn are in the same range as previously measured by different techniques in a small sample of Lake Griffin and Lake Woodruff alligator livers and kidneys (Ross 2000b). In that study selenium was below the detectable limit in most samples but fell in the same range of values 1 – 2.5 ppm wet weight (calculated assuming water content of the 2001 samples is similar to that of earlier samples, around 75%). One published value for selenium in alligator livers is also comparable, 0.641 ± 0.09 ppm wet weight (Burger et al. 2000). We therefore feel confident that the values obtained in our current study are accurate.

Iron values are highly variable and we suspect that contamination from steel equipment (scalpels, knives) during necropsy may be detected by this very sensitive technique. Alligators from Lake Griffin appear to have depleted levels of selenium in their liver and kidney. We propose to expand these data to determine if this effect is statistically significant.

Table 9. Metal concentrations (ppb dry weight) for selected alligator tissues from Lake Griffin and Lake Woodruff (Pooled mean ±1 SE). (Note, dry weight concentrations are approximately 4 X wet weight values for alligator tissues).

Kidney	(N)	Cu	Fe	Rb	Se	Zn
Griffin sick	(23)	7.6±0.5	324±48	13.0±0.7	2.5±0.2	67.3+3.4
Griffin healthy	(6)	7.0±0.4	166±23	17.4±1.1	2.3±0.4	77.2+6.6
Woodruff	(24)	8.3±0.3	388±23	24.4±4.9	10.0±0.3	69.3+1.5
Liver						
Griffin sick	(10)	36.0±5.9	2139±484	10.2±2.2	1.3±0.8	81.5+6.1
Griffin healthy	(6)	31.7±6.8	1844±707	15.3±4.1	3.7±1.4	84.3+12.1

Woodruff (7)	8.2±0.8	14059±2695	17.8±2.0	9.4±1.2	55.1+3.6

Alligators from Lake Griffin have significantly lower levels of selenium than alligators from Lake Woodruff for both Liver ($t = 2.9$ $P<0.05$, 23 df) and Kidney ($t= 4.5, P<0.01$ $df=67$). Impaired alligators have significantly lower selenium in the kidney than unimpaired alligators ($t= 4.2, P<<0.01$, $df=58$). However the difference in selenium in the liver (one tailed $t= 1.5$, $P= 0.06$, $df =66$) just fails to be significant at alpha=0.05. None of the other metals show significant differences.

Organochloride analyses

Results are available from the En-Chem and MSU analyses summarized in tables 1-3. Original data sheets are included in Appendix I. For the purposes of easy comparison, and to accentuate any differences that might be present, the average values presented omit those results that were below the limit of detection. This causes the presented averages to be the average of those samples in which organochlorides were detectable and are clearly a maximum estimate of levels that might be present. The raw data are also presented and can be scrutinised.

The sensitivity of these analyses were reported as a lower limit of detection of 0.010 parts per million or approximately 25 times more sensitive than previous analyses. Routine analysis of the water and fat content of each sample indicate that one sample (No 101 Lake Woodruff) showed abnormally low fat and high water content. The sample is apparently not fat, but possibly connective tissue or muscle. This sample has been eliminated from consideration in the results.

ADD COMMENTARY SECOND ALLIGATOR SAMPLES.

Thirteen organochlorines (OC's) occurred in detectable quantities in at least some of the samples. OC levels in alligators from the same lake were quite variable and the small sample sizes and the non-normal distribution of the values make a formal statistical analysis difficult. The analyses conducted by U. Miss (Tables 2,3,4) were approximately 10 times more sensitive than those conducted by En-Chem and a greater number of OC's were detected, although the general patterns and conclusions from the two analyses are similar.

Table 1. Alligator fat from Lake Griffin, Florida. Organochlorine analyses, Mississippi State University. Values ppm , ND = Not detected. Lowest detectable limit 0.010 ppm.

	Griffin	Woodruff
COMPOUND	Average	Average
p,p'-DDE	0.918	1.884
PCB Total	0.527	1.320
Dieldrin	0.019	0.083
Mirex	ND	0.021

p,p'-DDT	ND	0.015
Toxaphene	3.105	ND
t-nonachlor	0.203	0.039
cis-nonachlor	0.099	0.029
alpha Chlordane	0.083	0.016
pp-DDD olefin	0.062	0.052
Oxychlordane	0.028	0.037
Hept. Epox.	0.024	0.025
p,p'-DDD	0.018	ND
HCB	ND	ND
alpha BHC	ND	ND
gamma BHC	ND	ND
beta BHC	ND	ND
delta BHC	ND	ND
gamma Chlordane	ND	ND
o,p'-DDE	ND	ND
o,p'-DDD	ND	ND
o,p'-DDT	ND	ND
Endrin	ND	ND
Endosulphan 1	ND	ND
Endosulphan II	ND	ND
Endosulphan sulphate	ND	ND
Methoxychlor	ND	ND
Weight (g)	48.37	24.02
Moisture %	10.24	25.82
Lipid %	84.57	68.85

Concentrations of detected OC's were in all cases relatively low (between 0.01 and 8.40 parts per million) and fall below levels normally associated with abnormal exposure or mortality of alligators or other wildlife (Heinz et al. 1991). Prominent among the OC's detected and measurable in every fat sample were p.p'-DDE, trans and cis nonachlor and oxychlordane. These probably represent persistent breakdown products of agricultural pesticides no longer in use and are common or even ubiquitous in biological samples.

A group of OC's were present in more samples and at higher concentration in the Woodruff samples. These are p,p'-DDE, PCB's Dieldrin Mirex and p,p'-DDT. The DDE result may be biased by a single high reading (5.6 ppm) from one Woodruff animal and the remaining values are very similar in the two lakes (0.6- 1.5 ppm). Mirex and p,p'DDT are present in Woodruff samples and absent from all Griffin samples.

A larger number of OC's are present in more samples, and at generally higher levels in alligator fat from Lake Griffin than Lake Woodruff. Among these is toxaphene which is absent from Woodruff samples but present, and in one case at a significant concentration

(8.4 ppm) in Lake Griffin. Also higher in Lake Griffin are trans and cis nonachlor, alpha Chlordane, Oxychlordane and p,p'-DDD. These probably reflect the greater exposure to agricultural pesticides of older and more persistent forms in Lake Griffin. The appears to be no difference in OC levels between sick and normal alligators.

All the levels of even the highest OC concentrations measured, (1 Griffin sample 8.4 ppm toxaphene and 1 Woodruff sample of 5.6 ppm p,p'-DDE) fall below levels thought to cause acute toxicity in vertebrate animals and are comparable to levels recorded for similar organisms in other Florida lakes (Heinz et al 1991, Matter et al. 1998, Rogers 1997) . There is no indication from these data that exposure to organochlorine pesticides or their breakdown products is responsible for the observed neural impairment or mortality of alligators. Alligator tissues from Lake Apopka, including one sample from this study, have organochlorine levels 5-10X higher and these animals do not currently demonstrate mortality. It therefore appears unlikely that organochlorines alone are the proximal cause of alligator mortality in Lake Griffin.

Alligator eggs from Lake Griffin also show elevated levels of organochlorines compared to Lake Woodruff (Table 4), and the reported levels are similar to those reported on Lake Apopka where they are implicated in reproductive and developmental anomalies . The levels are also similar to those reported from Lake Griffin eggs in 1982 (Woodward 1995) and 1985 (Hienz et al 1991). There is no evidence to support a significant increase in organochlorine levels over the last 15 years. The reported values are also comparable to levels reported for the eggs of other species of crocodilians e.g. *C. acutus* DDE = 1.84ppm (Matter et al. 1998), Morelet's crocodile DDE = 0.66 ppm (Wu et al 2000) and are much less than levels reported for *C. niloticus* eggs in Zimbabwe (DDE 14-16 ppm Matter et al. 1998). The levels reported in Lake Griffin appear insufficient to cause egg mortality.

V. Discussion

The clutch viability (proportion of all eggs laid that hatch) of alligator eggs from Lake Griffin improved from less than 10% in 1996-97 to 28% in 1998 and 32% in 1999 (Woodward et al. 1999). While these values remain significantly lower than any other Florida lake (normal range 40%-90%) this slow improvement has occurred at the same time as algal blooms and adult alligator mortality have increased in intensity.

Our examination to date of the alligator mortality in Lake Griffin indicates that the only observable pathology involves the nervous system of these animals. The indications from preliminary analyses of tissues suggest that neither organochlorides or heavy metals can be directly implicated. We are aware that there may not be a single cause for the observed mortality. Instead, a synergistic interaction of several causes may be involved. However, without a preliminary indication of a specific cause, we feel the most productive way to proceed is to continue to examine, and if possible, to eliminate, different potential causes. We also wish to strengthen the results to date indicating neural impairment, and to examine this impairment in more detail.

Pathology examination has revealed that the alligators we observe demonstrating uncoordinated behavior in Lake Griffin, that subsequently die, often by drowning, are affected by severe neurological pathology of unknown cause. 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 (Schoeb et al. 2002).

Similar pathology is reported for salmonid fish subject to diets of prey fish rich in thiaminase (G. McDonald, J. Fitzsimons and D.C. Honeyfield (1998) resulting in a thiamin deficiency, adult impairment and hatchling mortality. 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.

Prompted by this similarity we analyzed thiamin levels in alligator tissues collected from Lake Griffin and Lake Woodruff. 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.
The similarity between Florida alligators in Lake Griffin and EMS in salmonids is striking; reproductive failure due to embryo mortality, adult neural impairment and mortality, brain lesions and the association with abundant filter feeding forage fish. Prompted by these similarities we have established contact and a cooperative research program with Dale Honeyfield, U. S. Geological Survey, Wellsboro, PA.

Thiamin deficiencies are often reversible by treatment with thiamin and this is demonstrated for salmonid fish (Brown S. & D. Honeyfield. 1999). Therefore we are currently testing whether the neural impairment observed in Lake Griffin alligators can be induced by a thiaminase rich diet and reduced by thiamin treatment in captive wild alligators.

Sepearate (unpublished) studies by our colleagues at the Caribbean Science Center (USGS-BRD have indicated that alligator eggs that show low hatch rates also have depressed levels of thiamine. Their preliminary results (2002) suggest that alligator eggs with total organochloride residues at the higher end of the observed range appear more susceptible to thiamine deficiency sydnrome and mortality (T. Gross and M. Sepulveda pers. Comm. Reported in Ross et al 2002). Although overt mortality from environmental contaminants has not been found in either the Great Lakes or Baltic salmonids, the role of contaminants has not been completely excluded. Synergistic interaction between low thiamin and exposure to pollutants (dioxin like compounds) have been reported (Brown and Honeyfield 1999). Apoptosis in lake trout fry exposed to dioxin-like compounds was found to be greater in thiamin deficient fish (Whyte et al. 2001).

The source of thiaminase in Florida lakes is thought to be the Gizzard shad (*Dorosoma cepedianum*) a very abundant, filter feeding clupied that has been reported to have high thiaminase levels eslewhere. We have conducted a study of the diet of alligators in Lake

Griffin and Lake Woodruff and confirm that gizzard shad (not originally a target species of this study) are a prominent component of alligator diets. We have initiated studies of gizzard shad in Lake Griffin to establish their thiaminase levels. We have confirmed that gizzard shad do filter toxic blue green algae and can ingest, concentrate and carry significant quantities (up to millions of cells). The role of gizzard shad in alligator diets, and the interrelationship of algae, shad, thiamine and the alligator mortality syndrome is currently under investigation.

REFERENCES

Bengtsson B-E, S. Brown and D. Honeyfield. 1999. International Council for the Exploration of the Seas, Annual Science Conference Sept 29-Oct 6, 1999. Stockholm Sweden. Session report: M74 syndrome and similar reproductive disturbances in marine animals. 5 pp.

Brim, M. S., D. Bateman, R. Jarvis & G. Carmody. 1994. Mercury in Largemouth bass of the Lake Woodruff national Wildlife Refuge. US Fish and Wildlife Service. Pub. No. PCFO-EC 94-05:28 p.

Brown S. and D. Honeyfield. 1999. Report on Early Mortality Syndrome Workshop. Great Lakes Fishery Commission, Board of Technical Experts: 33 pp.

Brown, S., D. C. Honeyfield, and L. Vandenbyllaardt. 1998. Thiamin analysis in fish egg and tissues by high pressure liquid chromatography. In G. McDonald, J. Fitzsimons, and D.C. Honeyfield (Eds). Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. Symposium 21, p 73-81. American Fisheries Society, Bethesda, MD 20814.

Burger, J., M. Gochfeld, A. A. Rooney, E. F. Orlando, A. R. Woodward & L. J. Guillette. 2000. Metals and metaloids in tissues of American alligators in three Florida lakes. Arch. Environ. Contam. Toxicol. 38:501-508.

Carmichael W. W. et al. (in prep.) Isolation and characterization of the potent alkaloid hepatotoxin cylindrospermopsin from a Florida strain of the cyanobacterium Cylindrospermopsis raciborskii.

Carmichael, W. W. 1997. The Cyanotoxins. Adv. Bot. Res. 7:211-256.

Chapman, A. D. and C. L. Schleske. 1997. Recent appearance of *Cylindrospermopsis* (Cyanobacteria) in five hypereutrophic Florida lakes. J. Phycol. 33:191-195.

Crain D. A. & L. J. Guillette. 1997. Endocrine disrupting contaminats and reproduction in vertebrate wildlife. Reviews in Toxicology 1(1997):47-70.

Crain D. A. & L. J. Guillette. 1998. Reptiles as models of contaminant induced endocrine disruption. Animal Reproduction Science 53:77-86.

Crews, D. and J. P. Ross. 1998. Consequences of hormone disruption of sexual development for crocodilian conservation. Pp. 174-190 In Crocodiles, Proceedings of the 14th Working Meeting of the Crocodile Specialist Group, IUCN, Gland Switzerland.

Gross, T.S. , L.J. Guillette, H F. Percival, G. R. Masson, J. Matter, and A. Woodward (1994b) Contaminant-induced reproductive anomalies in Florida. Comparative Pathology Bull. XXVI(4):2-8.

Gross, T.S., L.J. Guillette, H.F. Percival, and A. Woodward. (1994a) Pesticide induction of developmental abnormalities of the reproductive system of alligators (*Alligator mississippiensis*) and turtles (*Trachemys scripta*). NIEHS:Estrogens and the Environment Conf..

Guillette, L. J., D. B. Pickford, D.A. Crane, A.A. Rooney and H. F. Percival. 1996. Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. General and Comparative Endocrinology 101:32-42.

Guillette, L.J., T.S. Gross, G.R. Masson, J.M. Matter, H.F. Percival and A.R. Woodward. (1994) Developmental abnormalities of the gonad of juvenile alligators from contaminated and control lakes in Florida. Environmental Health Perspectives,. 102:680-688.

Heaton-Jones, T. G., B. L. Homer, D. L. Heaton-Jones & S. F. Sundlof. 1997. Mercury distribution in American alligators (Alligator mississipiensis) in Florida. J. Zoo and Wildlf. Med. 28(1):62-70.

Heinz, G.H., Percival, H.F. and Jennings, M.L. (1991). Contaminants in American alligator eggs from lakes Apopka, Griffin and Okeechobee, Florida. Environ. Monit. Assess. 16: 277-285.

Hinterkopf, J. P., D. C. Honeyfield, J. Markarewicz and T. Lewis. 1999. Abundance of plankton species in Lake Michigan and the incidence of early mortality syndrome from 1983 to 1992. Board of Technical Experts Briefing Book, October 1999, Great Lakes Fishery Commission. p 206-207.

Honeyfield, D.C. J. P. Hinterkopf and S.B. Brown. In press. Isolation of thiaminase-positive bacteria from alewife. Transactions of the American Fisheries Society.

Hooper and Schmidt 2001. Cholinesterase activity determinations for American alligator tissues. Final report TIEHH project No. T0111. Unpub report:1-14.

Howater S. 1999. Thermal ecology of the American alligator in the Everglades. Masters dissertation. University of Florida: 70p.

Johansson S. A. E and T. B. Johansson, 1976. Analytical Application of Particle Induced x-ray Emission, Nucl.Instr. Meth. 137 p. 473 - 516.

Marburger J. E. & W. F. Goodwin. 1996. Emerelda marsh conservation area: conceptual retroation plan. Technical memorandum No. 20. Div. Environmental Scineces. St. John's River Water Management District. Palatka, Florida.

Masson, G.R. (1993). Environmental influences on reproductive potential, clutch viability and embryonic mortality of the American alligator in Florida. Ph.D., Dissertation, Univ. of Florida.

Matter, J. M., D. A. Crain, C Sills-McMurray, D. B. Pickford, T. R. Rainwater, K. D. Reynolds, A. A. Rooney R. L. Dickerson & L. J. Guillette. 1998. In R. Kendal, R. Dickerson, J. Giesy and W. Suk (Editors) Principles and Processes for Evaluation Endocrine disruption in Wildlifepp. 267-287 SETAC Pr., Pensacola, FL.

McDonald G. , J. Fitzsimons and D.C. Honeyfield (1998) Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. Symposium 21, p 1-177. American Fisheries Society, Bethesda, MD 20814.

Millstein 1994. Report to the Florida Freshwater Fish abd Wildlife Commssion.

Noble R.C., D.C. Deeming, M. Ferguson & R. McCartney. 1991. The utilisation of yolk phophoglycerides by the alligator during embryonic debvelopment. J. Comp. Physiol. 161B:383-386.

Noble R.C., D.C. Deeming, M. Ferguson & R. McCartney. 1990. Changes in the lipid and fatty acid composition of the yolk during embryonic development of the alligator (*Alligator mississippiensis*). Comp. Biochem. Physiol (B)96:183-187

Noble R.C., R. McCartney & M. Ferguson. 1993. Lipid and fatty acid compositional differences between eggs of wild and captive-breeding alligators (Alligator mississippiensis): an association with reduced hatchability. J. Zool. Lond. 230:639-649.

Rice, K. G. 1996. Dynamics of exploitation on the American alligator: environmental contaminants and harvest. Ph.D., Dissertation, Univ. of Florida.

Rogers, J. A. 1997. Pesticide and heavy metal levels of waterbirds in the Everglades Agricultural Area of South Florida. Fl. Naturalist 25(2):33-84.

Ross, J. P. 2000 b. Effect of toxic algae on alligators and alligator egg development. Tech Research Report Project FL 03 to Water Resources Research Center USGS-University of Florida: 23pp.

Ross, J. P., , D. Carbonneau, S. Terrell, T. Schoeb, D. Honeyfield, J. Hinterkopf, A. Finger and R. Owen 2002 b . Continuing Studies of mortality of alligators on central Florida lakes: pathology and nutrition. Final report to St. Johns River Water Management District, Contract #SE122AA, 31 March 2002 (and In press, SJWMD research report series)

Ross, J. P., H. F. Percival, A. Woodward, D. Carbonneau, T. R. Schoeb, J. Burns, C. Williams & W. Carmichael. 2000. Alligator mortality and algal toxins: a preliminary report. American Fisheries Society, Florida chapter 20th Annual meeting March 2000. Abstract.

Ross, J. P., H.F. Percival , D. Carbonneau , A. Woodward and T. Schoeb. 2001. Investigations of mortality and reproductive failure of alligators in Lake Griffin, Central Florida. Florida Lake Management Association, Annual Meeting Tallahassee May 2001. Abstract.

Ross, J. Perran, Joy P. Hinterkopf, Dale C. Honeyfield, , Dwayne Carbonneau, Allan Woodward, Marisol Sepulveda & Tim Gross 2002 a . Thiamine Status and Mortality of Adult American Alligators (*Alligator mississippiensis)* in Lakes Griffin and Woodruff in Central Florida during 2000 and 2001. Proceedings of the Symposium of the Great Lakes Fisheries Commision, Ann Arbor Michigan June 2002.

Ross, J.P. 2000a. Central Florida Lakes Wildlife Initiative, Analyses of tissue samples of alligators from Lake Griffin to examine causes of unexplained mortality. Report to Lake County Water Authority, Contract No. #00032702, 30 September 2000:

Schoeb, T. R., R. M. Clemmons & T. G. Heaton-Jones. 1999. Encephalopathy and neuropathy in Florida Alligators. Report to Florida Fish and Wildlife Conservation Commission and in preparation for publication.

Schoeb, T. R., T. G. Heaton-Jones, R. M. Clemmons, D. A. Carbonneau, A. Woodward, D. Shelton and R.. Poppenga. 2002. Clinical and necropsy findings associated with incr4ased mortality among American alligators of Lake Griffin, Florida. J. Wildl. Diseases 38(2):320-337.

Temsiripong, Yosapong 1999. Growth and Survival rates of wild and repatriated hatchling American alligators (*Alligator mississippiensis*) in Central Florida Lakes. MSc. Thesis, Dept. Wildlife Ecology and Conservation, University of Florida, 55 pages.

Thomas, N. J., C. U. Meteyer & L. Sileo. 1998. Epizootic Vacuolar Myelinopathy of the Central nervous system of Bald Eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica americana*). Vet. Pathol 35:479-487.

Williams, C. A. Chapman, M. Pawlowicz, W. Carmichael & J. Burns. 2000. An assessment of toxigenic Cyanobacteria in Florida's surface water. American Fisheries Society, Florida chapter 20th Annual meeting March 2000. Abstract.

Woodward, A. R., D.S. Bermudez & D. A. Carbonneau. 1999. A Preliminary report on Alligator Clutch Viability in Florida. Florida Fish and Wildlife Conservation Commission internal report.

Woodward, A.R., Jennings, M.L. and Percival, H.F. (1989). Egg collecting and hatch rates of American alligator eggs in Florida. Wildlife Soc. Bull. 17: 124-130.

Woodward, A.R., Jennings, M.L., Percival, H.F. and Moore, C.T. (1993). Low clutch viability of American alligators on Lake Apopka. Fl. Sci. 56: 52-63.

Woodward. A. R. 1995. DDE, DDD and DDT concentration in alligator eggs from Lakes Apopka, Griffin and Orange in 1992. Unpublished ms Florida fish and Wildlief conservation Commission, Gainesville Fl.

Wu, T. H., T. R. Rainwater, S. G. Platt, S. T. McMurry & T. A. Anderson. 2000. Organochlorine contaminants in Morlet's crocodile (*Crocodylus moreletii*) eggs from Belize. Chemosphere 40:671-678. March 2000. Abstract.

Whyte J. J. and D. C. Honeyfield. 2000. Lake Trout Restoration in the Great Lakes: Early Life Stage Survival. Int. Assoc. for Great Lake Research, 43rd Conference on Great Lakes and St. Lawrence River research. May 2000. Abstracts

Whyte, J.J., H.A. Allert, C.C. Edsall, D.C. Honeyfield and D.E. Tillitt. 2001. Apoptosis in embryonic lake trout developing under nutrient and contaminant stress. SETAC Annual meeting, Nov 11-15, 2001. Baltimore, MD.: 35.

Figure 1. Alligator mortality recorded on Lake Griffin, Florida 1997 – 2003. Inspection for dead alligators is conducted every 2 weeks by personnel of the Florida Fish and Wildlife Conservation Commission. Data courtesy of D. Carboneau, FWC.

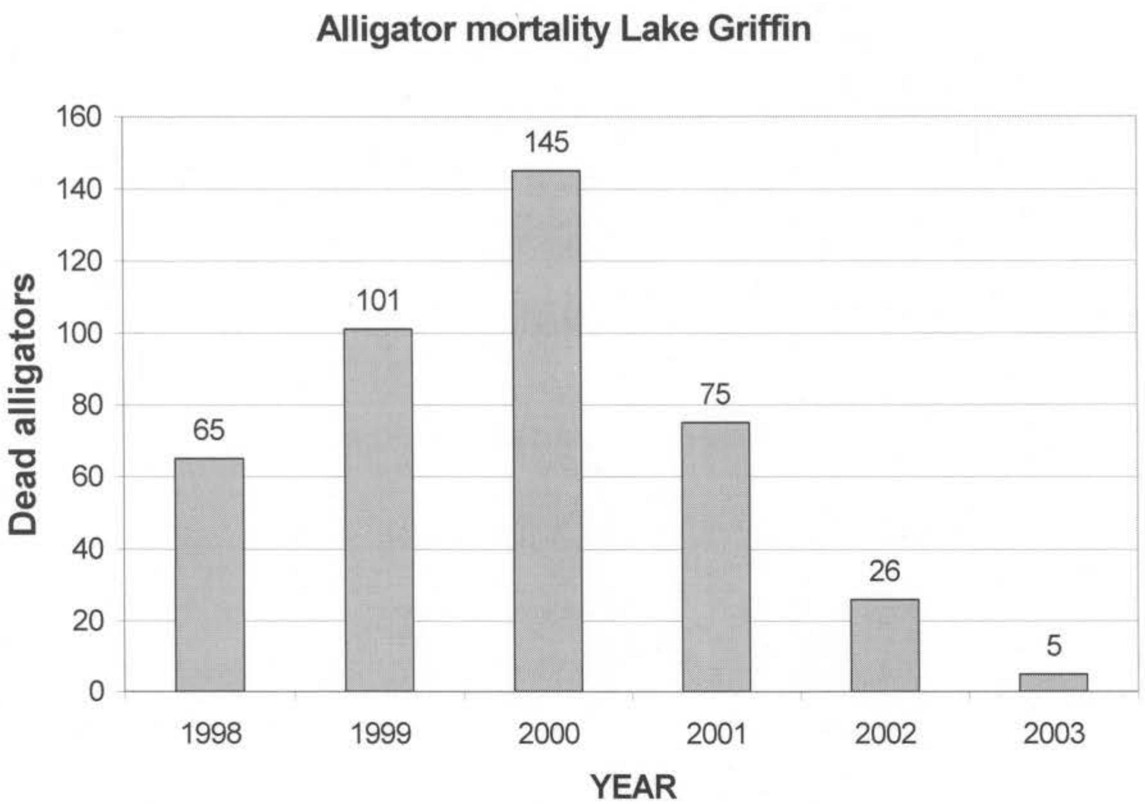

Figure 2. Algae cell volumes and species in Lake Griffin, Florida, showing the dominance of *Cylindrospermopsis*. Data courtesy of W. Goodwin, St. John's River Water Management District.

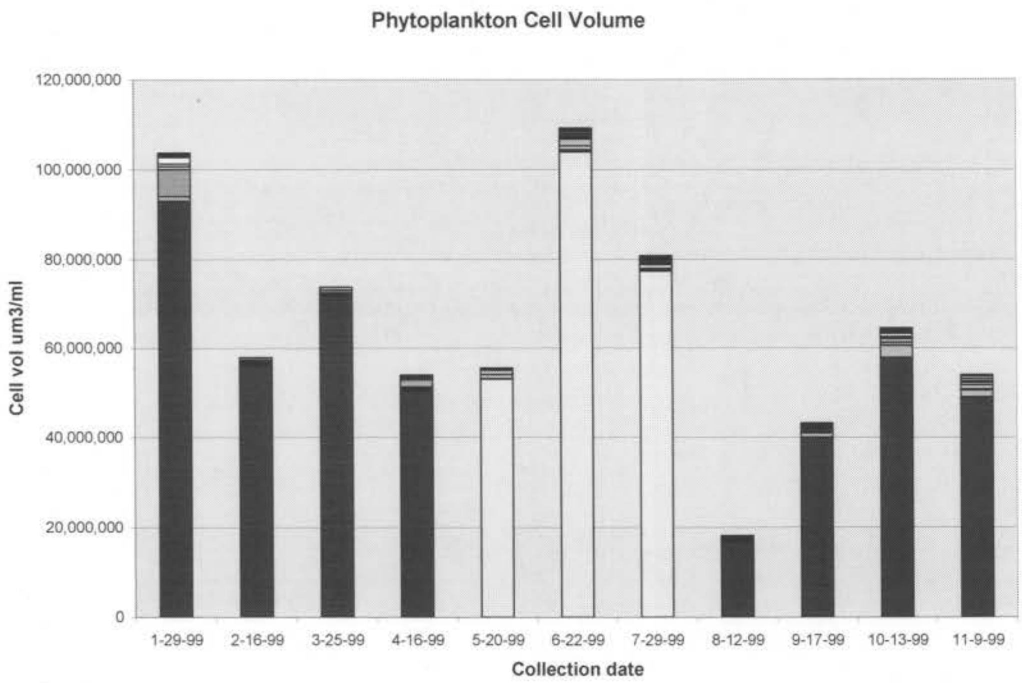

Figure 3. Regression of Volume of Cylindrospermopsis and dead alligators in Lake Griffin, Florida. Dec 1997 – Nov 1999.

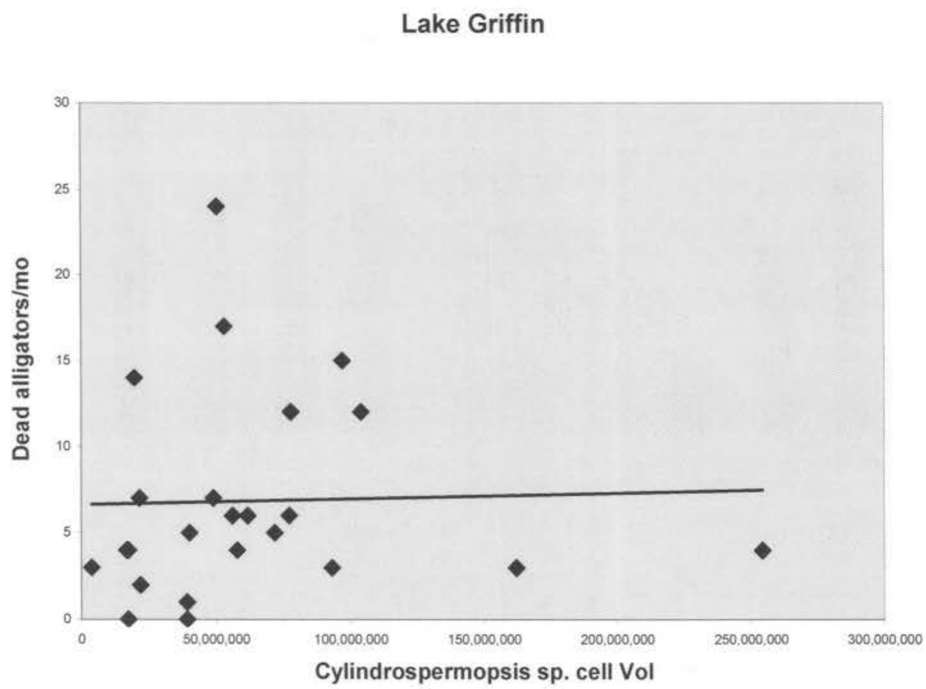

Figure 4. Regression of Volume of *Planktothrix* (*Oscilatoria*) *limnetica* and dead alligators in Lake Griffin, Florida. Dec 1997 – November 1999.

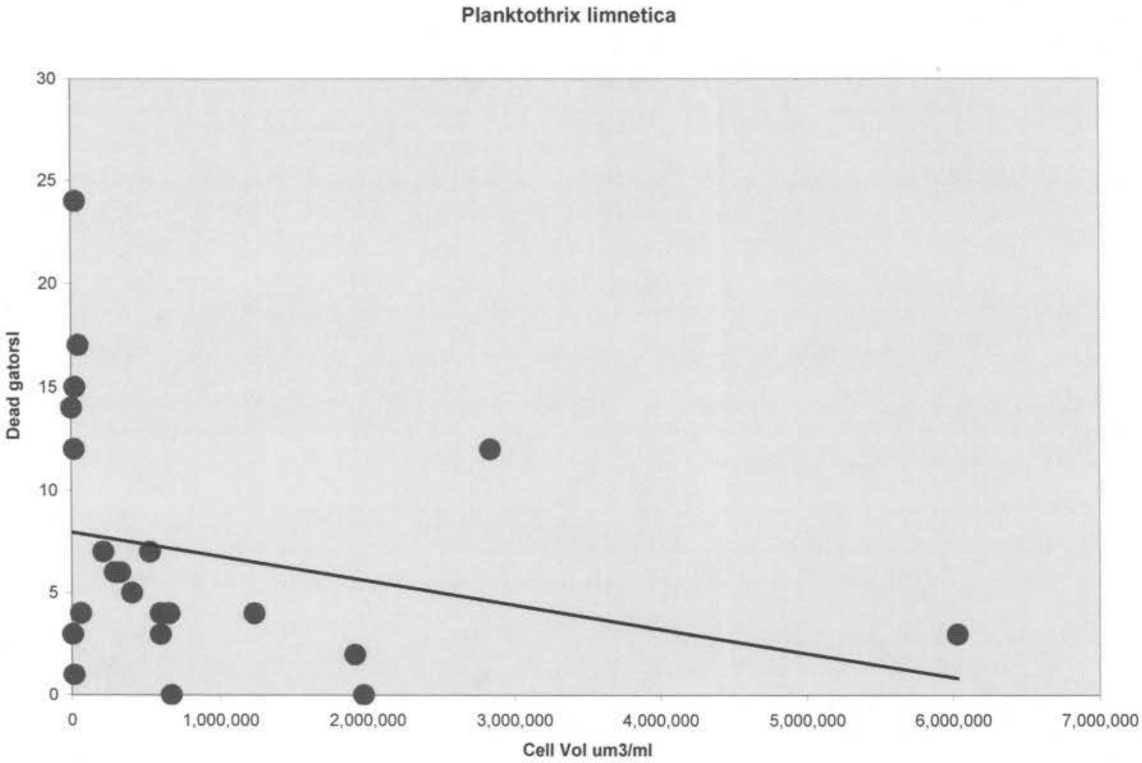

Table 1. Alligator tail muscle tissues from Lake Griifin and Lake Woodruff, Florida. Organochlorine analyses En-Chem inc. DDE was the only organochlorine detected.

U= below detection limit for analysis

	4.4'-DDE ug/kg	Location	Sex	Length cm	Date collected	Neural evaluation
G99-023	150	Griffin	Male	91	Mar-99	Impaired
G99-010	120	Griffin	Male	248	Apr-99	Impaired
G99-021	64	Griffin	Male	144	Feb-99	Impaired
G99-032	18	Griffin	Male	178	May-99	Impaired
G99-129	11	Griffin	Male	185	Nov-99	Impaired
G99-028	5.7	Griffin	Male	298	Apr-99	Impaired
G99-029	U	Griffin	Female	173	Apr-99	Normal
G98-012	U	Griffin	Male	180	Jun-98	Impaired
AVG	46.088					
Std Dev	59.1					
G99-030	11	Woodruff	Male	189	Apr-99	Normal
G99-031	76	Woodruff	Male	173	Apr-99	Normal
G99-034	9.5	Woodruff	Male	189	May-99	Normal
G99-024	U	Woodruff	Male	193	Mar-99	Normal
AVG	28.5					
Std Dev	41.4					

G99-128	11000	Apopka	-	259	Sep-99	Normal
	Also had 240ppb Dieldrin					

Table 2. Alligator fat from Lake Griffin, Florida. Organochlorine analyses, Mississippi State University. Values ppm , ND = Not detected. Lowest detectable limit 0.010 ppm.

	Griffin	G	G	G normal	G	G	Griffin
COMPOUND	G99-021	G99-023	G99-028	G99-029	G99-032	#40582	Average
p,p'-DDE	1.300	0.700	0.910	0.920	0.680	1.000	0.918
PCB Total	0.610	0.530	0.560	0.490	0.590	0.380	0.527
Dieldrin	0.027	0.020	0.015	ND	0.010	0.025	0.019
Mirex	ND	ND	ND	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	ND	ND	ND	ND
Toxaphene	ND	ND	1.100	2.200	0.720	**8.400**	3.105
t-nonachlor	0.120	0.120	0.150	0.190	0.110	0.530	0.203
cis-nonachlor	0.120	0.086	0.094	0.085	0.071	0.140	0.099
alpha Chlordane	ND	0.051	0.087	ND	0.063	0.130	0.083
pp-DDD olefin	0.061	0.035	0.076	0.023	0.026	0.150	0.062
Oxychlordane	0.010	0.022	0.019	0.035	0.019	0.064	0.028
Hept. Epox.	0.022	0.025	0.025	0.048	0.026	0.000	0.024
p,p'-DDD	0.023	0.014	0.024	0.011	ND	ND	0.018
HCB	ND	ND	ND	ND	ND	ND	
alpha BHC	ND	ND	ND	ND	ND	ND	
gamma BHC	ND	ND	ND	ND	ND	ND	
beta BHC	ND	ND	ND	ND	ND	ND	
delta BHC	ND	ND	ND	ND	ND	ND	
gamma Chlordane	ND	ND	ND	ND	ND	ND	
o,p'-DDE	ND	ND	ND	ND	ND	ND	
o,p'-DDD	ND	ND	ND	ND	ND	ND	
o,p'-DDT	ND	ND	ND	ND	ND	ND	
Endrin	ND	ND	ND	ND	ND	ND	
Endosulphan 1	ND	ND	ND	ND	ND	ND	
Endosulphan II	ND	ND	ND	ND	ND	ND	
Endosulphan sulphate	ND	ND	ND	ND	ND	ND	
Methoxychlor	ND	ND	ND	ND	ND	ND	
Weight (g)	74.40	45.90	85.50	13.30	19.40	51.70	48.37
Moisture %	14.80	9.36	8.04	10.20	5.42	13.60	10.24
Lipid %	79.80	85.80	87.10	86.60	91.80	76.30	84.57
LLD 0.010ppm							

Table 3. Alligator fat from LakeWoodruff, Florida. Organochlorine analyses, Mississippi State University. Values ppm , ND = Not detected. Lowest detectable limit 0.010 ppm.

COMPOUND	Woodruff	W	W	W	W	W	Woodruff
	G99-024	G99-030	G99-031	G99-034	G99-335	#101	Average *
p,p'-DDE	1.000	**5.600**	1.500	0.700	0.620	0.016	1.884
PCB Total	1.600	0.860	2.000	0.640	1.500	ND	1.320
Dieldrin	0.014	0.210	0.037	0.070	ND	ND	0.083
Mirex	0.024	ND	0.016	ND	0.023	ND	0.021
p,p'-DDT	ND	0.015	ND	ND	ND	ND	0.015
Toxaphene	ND	ND	ND	ND	ND	ND	ND
t-nonachlor	0.065	0.037	0.033	0.024	0.036	ND	0.039
cis-nonachlor	0.044	0.024	0.029	0.020	0.030	ND	0.029
alpha Chlordane	0.016	ND	ND	ND	0.016	ND	0.016
pp-DDD olefin	ND	0.052	ND	ND	ND	ND	0.052
Oxychlordane	0.026	0.048	0.048	0.048	0.017	ND	0.037
Hept. Epox.	0.015	0.016	0.030	0.023	0.039	ND	0.025
p,p'-DDD	ND	ND	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
	ND	ND	ND	ND	ND	ND	
Weight (g)	18.50	22.90	25.40	10.10	27.90	39.30	24.02
Moisture %	7.95	17.90	7.89	22.80	16.80	81.60	25.82
Lipid %	88.00	83.30	88.50	71.50	81.80	**0.01**	68.85

* 101 omitted from average

Table 4. Alligator eggs from Lake Griffin and Lake Woodruff Florida. Analyses of organochlorine by U. Miss.

	Griffin			*		Woodruff		
	GR99-311	GR99-343	GR99-372	GR99-104	GR99 342	WO99-120	WO99-1081	WO991082
PCB Total	ND	0.067	ND	0.078	0.059	0.18	0.24	0.2
Toxaphene	0.32	0.86	1.6	ND	0.52	ND	ND	ND
p,p'-DDE	0.2	0.66	2.3	0.16	0.59	0.085	0.11	0.095
p,p'-DDD	ND	0.048	0.17	ND	ND	ND	ND	ND
pp-DDD olefin	ND	0.043	0.18	ND	0.041	ND	ND	ND
Dieldrin	0.011	0.06	0.19	ND	0.054	ND	ND	ND
t-Nonachlor	0.022	0.077	0.14	0.023	0.071	ND	ND	ND
o,p'-DDT	ND	ND	ND	ND	0.042	ND	ND	ND
alpha Chlordane	ND	0.03	0.054	ND	ND	ND	ND	ND
cis-nonachlor	ND	0.019	0.039	ND	0.012N	ND	ND	ND
Oxychlordane	ND	0.016	0.033	ND	0.014	ND	ND	ND
Hept. Epox.	ND	0.016	0.025	ND	0.012	ND	ND	ND
gamma Chlordane	ND	ND	ND	ND	ND	ND	ND	ND
HCB	ND	ND	ND	ND	ND	ND	ND	ND
alpha BHC	ND	ND	ND	ND	ND	ND	ND	ND
gamma bhc	ND	ND	ND	ND	ND	ND	ND	ND
bete BHC	ND	ND	ND	ND	ND	ND	ND	ND
delta BHC	ND	ND	ND	ND	ND	ND	ND	ND
o,p'-DDE	ND	ND	ND	ND	ND	ND	ND	ND
o,p'-DDD	ND	ND	ND	ND	ND	ND	ND	ND
Endrin	ND	ND	ND	ND	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	ND	ND	ND	ND	ND
Mirex	ND	ND	ND	ND	ND	ND	ND	ND
Endosulphan I	ND	ND	ND	ND	ND	ND	ND	ND
Endosulphan II	ND	ND	ND	ND	ND	ND	ND	ND
Endosulphan sulph	ND	ND	ND	ND	ND	ND	ND	ND
Methoxychlor	ND	ND	ND	ND	ND	ND	ND	ND
Weight(g)	69.1	60.8	70.2	83.4	68.6	67.9	67.1	72.2
Moisture (%)	77.6	75.9	75.1	79.3	75.1	75.4	72.6	75.4
Lipid (%)	8.39	10.8	11	8.12	10.1	10.1	12.7	11.1

Table 5. Alligator tissue from Lake Griffin and Lake Woodruff, Florida. Analysis of metals by University of Pennsylvania.

First batch analysed mid 1999

	Lake	Tissue	As	Cd	Cr	Hg	Pb	Se	Tl	Sn	Ca	Co	Cu	F
G99-023	Griffin	kidney	<0.50	0.107	<0.20	<1.00	<0.50	<1.00	<2.00	<1.00	57.8	<0.10	1.37	6
G99-032	Griffin	kidney	<0.50	0.103	0.838	<1.00	<0.50	<1.00	<2.00	<1.00	189	<0.10	1.73	5
G99-030	Woodruff	kidney	<0.50	0.124	<0.20	<1.00	0.774	<1.00	<2.00	<1.00	56.1	<0.10	1.33	4
G99-031	Woodruff	kidney	<0.50	0.183	0.211	1.97	<0.50	<1.00	<2.00	<1.00	88	<0.10	2.69	7
G99-023	Griffin	liver	<0.50	<0.10	<0.20	<1.00	<0.50	<1.00	<2.00	<1.00	15.9	<0.10	3.77	[illegible]
G99-032	Griffin	liver	<0.50	0.1	<0.20	<1.00	<0.50	<1.00	<2.00	<1.00	41.4	<0.10	3.39	[illegible]
G99-030	Woodruff	liver	<0.50	<0.10	<0.20	<1.00	<0.50	<1.00	<2.00	<1.00	64.5	<0.10	10.1	[illegible]
G99-031	Woodruff	liver	<0.50	<0.10	<0.20	1.48	<0.50	<1.00	<2.00	<1.00	51.6	<0.10	15.7	[illegible]

2nd batch analysed May 2000

	Lake	Tissue	As	Cd	Cr	Hg	Pb	Se	Tl	Sn	Ca	Co	Cu	F
G98-010	Griffin	liver	<0.50	0.189	0.701	**2.05**	**13.2**	1.68	<2.00	<1.00	121	<0.10	16.5	4[illegible]
G98-012	Griffin	liver	<0.50	<0.10	<0.20	<1.00	<0.50	<1.00	<2.00	<1.00	47.4	<0.10	7.07	[illegible]
G99-021	Griffin	liver	<0.50	<0.10	<0.20	<1.00	<0.50	<0.10	<2.00	<1.00	65.1	<0.10	4.88	[illegible]
G99-028	Griffin	liver	<0.50	<0.10	<0.20	<1.00	<0.50	1.04	<2.00	<1.00	48.5	<0.10	5.47	[illegible]
G99-029	Griffin	liver	<0.50	<0.10	<0.20	<0.10	<0.50	<0.10	<2.00	<1.00	37.3	<0.10	7.91	[illegible]
G99-129	Griffin	liver	<0.50	<0.10	<0.20	<0.10	<0.50	<0.10	<2.00	<1.00	47	<0.10	6.39	[illegible]
G98-011	Woodruff	liver	<0.50	<010	<0.20	<1.00	<0.50	1.08	<2.00	<1.00	50.1	<0.10	16.7	[illegible]
G98-024	Woodruff	liver	<0.50	<010	<0.20	<1.00	<0.50	<0.10	<2.00	<1.00	58.4	<0.10	8.16	[illegible]
G99-034	Woodruff	liver	<0.50	<010	<0.20	<1.00	<0.50	<0.10	<2.00	<1.00	59.7	<0.10	11.1	[illegible]
335	Woodruff	liver	<0.50	<010	<0.20	<1.00	<0.50	<0.10	<2.00	<1.00	61.6	<0.10	22	[illegible]
336	Woodruff	liver	<0.50	0.147	0.631	**5.06**	<0.50	2.26	<2.00	<1.00	46.4	<0.10	10.5	3[illegible]
337	Woodruff	liver	<0.50	0.121	0.393	**3.07**	<0.50	2.08	<2.00	<1.00	70.9	<0.10	27.8	2[illegible]

All values ppm wet weight

Table 6 (draft) Combined OC and Thiamin Values for alligators collected on Lake Griffin and Lake Woodruff. 1999-2002.

See Excel Workbook OC Thiamin Summary 2004

ID Tag	41006	41004	41007	41005	41003	40583	40723	40577	41011	41013	41002	41012	41014	41018
Lake	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin
Health Sta	1	1	1	1	1	1	1	1	3	3	3	3	3	3
Date	18-Apr-01	5-Apr-01	18-Apr-01	12-Apr-01	5-Apr-01	11-May-00	11-May-00	2000	2-May-01	3-May-01	28-Mar-01	2-May-01	9-May-01	23-May-01
weight kg	14.4	19.9	33.5	48	54.4	61	71		14	37.6	44	68	193	231
sex	M	F	F	F	M	F	M		F	F	F	F	M	M
Total Thiar	248.74	777.45	88.02	92.74	377.94	196.87	97.30	180.72	309.79	127.18	231.30	76.16	334.10	292.24
mmol/g	2.49	7.77	0.88	0.93	3.78	1.97	0.97	1.81	3.10	1.27	2.31	0.76	3.34	2.92
TOTAL OC	0.000	0.569	0.020	0.875	0.000	0.490	0.218	4.04	0.031	0.307	0.026	0.037	1.278	2.110
p.p'DDE	ND		0.020	0.083		0.280	0.133	ND	0.031	0.152	0.026	0.037	0.704	1.590
o,p' DDE	ND	ND	ND	0.0138	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB total	ND	0.478	ND	ND	ND	0.165	0.063	ND	ND	0.139	ND	ND	0.473	0.378
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
pp-DDT	ND	ND	ND	0.563	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Toxaphen	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
t-nonachlo	ND	0.078	ND	ND	ND	0.045	0.023	ND	ND	0.016	ND	ND	0.073	0.104
cis nonach	ND	0.013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.028	0.038
a Chlordar	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
g Chlordar	ND	ND	ND	0.057	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
pp DDD ol	ND	ND	ND	0.087	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxychlord	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mirex	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCB	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hept. Epox	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
o,p'DDT	ND	ND	ND	0.072	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
% Lipid	3.87	4.7	2.61	4.47	11.9	9.74	14		3.54	18.3	5.44	12.3	4.12	4.61
% water	66.7	75.4	70	73.4	69.4	46.3	65.6		52.2	61.4	74.5	69.3	74.5	74.5

ID Tag	40523	40552	40582	40585	40586	40584
Lake	Griffin	Griffin	Griffin	Griffin	Griffin	Griffin
Health Sta	3	3	3	3	4	4
Date	2000	2000	2000	1-Feb-01	7-Feb-01	18-Sep-00
weight kg					33.5	34.4
sex				M	F	M
Total Thiar	200.85	29.79	70.87	46.30	177.05	53.19
mmol/g	2.01	0.30	0.71	0.46	1.77	0.53
TOTAL OC	2.33	1.63	10.85	0.029	0.062	0.021
p.p'DDE	ND	ND	ND	0.029	0.062	0.021
o,p' DDE	ND	ND	ND	ND	ND	ND
PCB total	ND	ND	ND	ND	ND	ND
Dieldrin	ND	ND	ND	ND	ND	ND
pp-DDT	ND	ND	ND	ND	ND	ND
Toxaphen	ND	ND	ND	ND	ND	ND
t-nonachlo	ND	ND	ND	ND	ND	ND
cis nonach	ND	ND	ND	ND	ND	ND
a Chlordar	ND	ND	ND	ND	ND	ND
g Chlordar	ND	ND	ND	ND	ND	ND
pp DDD ol	ND	ND	ND	ND	ND	ND
Oxychlord	ND	ND	ND	ND	ND	ND
Mirex	ND	ND	ND	ND	ND	ND
HCB	ND	ND	ND	ND	ND	ND
Hept. Epox	ND	ND	ND	ND	ND	ND
o,p'DDT	ND	ND	ND	ND	ND	ND
% Lipid				4.29	3.06	4
% water				74.1	76.2	75

41015	41009	41008	40578	40579
Griffin	Griffin	Griffin	Griffin	Griffin
4	4	4	4	4
16-May-01	27-Apr-01	20-Apr-01	2000	2000
67	74.4	84.8		
F	M	M		
133.31	114.67	118.51	74.01	57.32
1.33	1.15	1.19	0.74	0.57
0.681	0.121	0.059	3.08	2.34
0.344	0.108	0.059		
ND	ND	ND	ND	ND
0.196	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
0.121	0.013	ND	ND	ND
0.020	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
ND	ND	ND	ND	ND
4.35	4.27	9.92		
75.1	74.8	69.1		

ID Tag	40859	50575	50587	40853	50722	40845	50703	40854	50732	40553	40573	40693
Lake	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff	Woodruff
Health Stat	1	1	1	1	1	1	1	1	1	1	1	1
Date	26-May-00	2-May-01	2-May-01	1-Jun-00	30-May-01	4-May-00	16-May-01	26-May-00	30-May-01	2000	2000	2000
weight kg	23.6	24	32	44.9	46	50	59.8	83.5	90.7			
sex	F	F	M	F	F	M	F	M	M			
Total Thiar	114.14	427.19	469.51	285.89	352.32	26.01	255.14	227.04	287.76	262.90	432.14	356.89
mmol/g	1.14	4.27	4.70	2.86	3.52	0.26	2.55	2.27	2.88	2.63	4.32	3.57
TOTAL OC	0.264	1.309	0.370	0.836	0.395	0.131	0.689	1.426	0.620	6.90	0.12	3.74
p.p'DDE	0.075	0.060	0.314		0.111	0.040	ND	0.424	0.021	ND	ND	ND
o,p' DDE	ND	0.0132	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
PCB total	0.189	ND	0.056	0.391	0.270	0.091	0.655	0.934	0.578	ND	ND	ND
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
pp-DDT	ND	0.742	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Toxaphene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
t-nonachlo	ND	0.025	ND	0.043	0.014	ND	0.034	0.051	0.022	ND	ND	ND
cis nonach	ND	0.024	ND	0.022	ND	ND	ND	0.017	ND	ND	ND	ND
a Chlordar	ND	0.042	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
g Chlordar	ND	0.187	ND	0.231	ND	ND	ND	ND	ND	ND	ND	ND
pp DDD ol	ND	0.098	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxychlord	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mirex	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCB	ND	ND	ND	0.019	ND	ND	ND	ND	ND	ND	ND	ND
Hept. Epox	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
o,p'DDT	ND	0.118	ND	0.130	ND	ND	ND	ND	ND	ND	ND	ND
% Lipid	5.94	2.87	2.89	7.11	4.1	7.17	6.81	3.42	3.57			
% water	69.6	74.8	71.6	70.8	73.8	75.9	71.4	67.5	73.9			