USGS

WATER RESOURCES RESEARCH GRANT PROPOSAL

Title: Effect of toxic algae on alligators and alligator egg development

Focus categories: TS, COV, ECL

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Congressional District: 5th

Statement of critical regional water problems

Increased human density in the S.E. region, and particularly in Florida, has led to detrimental changes in wetland ecology. Diking, draining, channelization, and water level stabilization to meet flood control, agricultural, housing, recreational and navigational needs have caused profound changes in water quality. The influence of runoff from agriculture and urbanization has been recognized as an additional factor in wetland degradation. In the shallow lakes of subtropical and temperate Florida a general pattern of eutrophication has been recognized. Increased dissolved nutrient levels, warm temperatures and a year round growing season promote extensive micro-algal blooms. This results in a transition from clear water lakes supporting extensive floating and emergent macrophyte communities to turbid lakes where the predominant flora is Phytoplankton. Concomitant with this transition is the development of extensive layers of flocculent organic sediments, the disappearance of the macrophyte flora and changes in the biota of the lakes, including the loss of valuable sport fisheries. Some algae produce toxins that can affect wildlife. Harmful algal blooms have become the focus of a statewide task force to assess the impacts of this problem. While a major emphasis has been marine algae such as Gymnodinium breve (Red tide), Pfiesteria sp. and Gambierdiscus toxicus (Ciguatera), the potential effects of algal blooms in fresh water on wildlife mortality, water supply quality and human health is widely recognized. A blue green alga (Cyanobacteria) Cylindrospermopsis raciborskii is a newly described component of the algal blooms in several Florida lakes (Chapman & Schelske 1997). C. raciborskii is known to produce toxins (Carmichael 1997) and has been implicated in wildlife mortality in many areas of the world. Preliminary investigations of unusual numbers of dead and moribund alligators during 1997-1998 in Lake Griffin, central

Florida, suggest a temporal association with heavy blooms of C. raciborskii, which has become the dominant blooming algae in Lake Griffin.

Alligators provide a valuable bio-indicator or sentinel species for ecosystem perturbations. They are large, long-lived predators and feed at the top of food chains. They accumulate contaminants and can carry large body loads in their fat but their robust physiology reduces lethal effects on adults. However, their developing eggs are particularly prone to embryological disruption by exogenous compounds of either maternal or environmental origin. The presence of contaminants in run-off waters, as well as the direct introduction of herbicides and pesticides into lakes, has introduced anthropogenic compounds capable of exerting profound disruptive effects on the embryonic development of organisms at very low doses by endocrine disruption. The effects of hormone disrupting contaminants in alligators has become a model for examining this process and its physiological and ecological effects. The presumptive association of algal blooms and alligator mortality and impaired reproduction opens the possibility that phytotoxins may have similar effects. The effects of algal toxins in alligators need to be evaluated both by the direct effect on alligator physiology (including sublethal and reproductive effects) and by examining pathways of toxin transfer through the ecosystem to these top predators. Elucidating these effects in the Oklawaha system will provide valuable information for the control and mitigation of algal toxicity at the State and regional level.

Statement of results, benefits, and information

Information developed in this study will be used by the SJD and GFC to develop additional studies and mitigation strategies for this problem. Information will also be provided to the Florida Harmful Algal Blooms Task Force and municipal water managers. The results will also be of interest to the public who reside near Lake Griffin and use it for recreation and to commercial alligator farmers in Florida who collect wild alligator eggs for commercial production. The proposed study will allow the analyses of materials (alligator tissue, egg yolk, water samples) to establish presence of algal toxins. Associated routine monitoring of alligators and water quality will establish preliminary assessment of the seasonality, climatic, hydro regime and physico-chemical associations of algal toxicity. Parallel studies will be looking at heavy metal and chlorinated hydro-carbon contaminants in the same test system and a proposed study will examine in detail the embryological effects of these factors in alligators and turtles. From this information we expect to elucidate the relative importance of algal toxin and contaminant effects and lay the ground work for detailed investigations into their mechanisms, ecological pathways of transmission and physiological effects. With this information we approach the knowledge necessary to manage and mitigate these environmentally disruptive impacts on Florida wetlands.

Nature, scope and objective of the research

Background: Between 1994 and 1998 the hatch rate of alligator (Alligator mississippiensis) eggs collected on Lake Griffin and hatched in commercial facilities

dropped from a range of 40%-60% to less than 10%. Since the spring of 1997 observations of dead adult alligators in Lake Griffin have increased to 2-4/week. In other lakes in Florida 'normal' alligator egg hatch rates fall in the range of 75%-90% and it is rare to see dead alligators at all. Studies of sportfish in Lake Griffin (large-mouth bass, Micropterus salmoides, and speckled perch, Pomoxis nigromaculatus) indicate missing year classes and reduced reproduction in these species are causing a virtual collapse of the formerly lucrative sport fishery. Whether other organisms in the system such as waterbirds, amphibians or invertebrates are affected is not yet known.

From the mid 1980's to present, Lake Griffin has undergone some profound changes in ecology similar to those observed in many highly eutrophic Florida lakes. The lake has changed from a clear-water, sand bottom lake dominated by rooted aquatic macrophytes and supporting extensive sportfish populations to a turbid water system characterized by extensive flocculent organic sediments, dominated by unicellular algal blooms and planktoniverous fish (gizzard shad Dorosoma cepedianum). Increased loading of sediments, nutrients and contaminants from human activity in the surrounding watershed is thought to be driving these changes. Occasional die-off of fish (shad and Gar Lepisosteus sp.) and freshwater turtles have been noted but not adequately quantified to evaluate.

The lakes of central Florida are highly manipulated environments subject to multiple anthropogenic inputs such as fertilizer and pesticide run-off, sewage treatment effluent, aquatic plant and insect control, and water level manipulation. "Muck farming" on these lakes involved diking of previous sawgrass marshes, intense pesticide use, and repetitive draining and flooding, transporting contaminants and nutrients into the lakes. The causal linkage among these events and observed wildlife effects is not yet clear, but probably involves ecological interactions requiring a multi disciplinary approach.

American alligators on several large Florida lakes within the Ocklawaha River basin, have exhibited poor clutch viability (the proportion of total eggs from a clutch that successfully hatch), abnormal reproductive hormone levels, and unexplained adult mortality. Clutches from alligators on Lake Apopka experienced severe declines from 50% to 4% in clutch viability during the mid-1980s (Rice 1996). Clutches on other Ocklawaha lakes: Griffin Jessup, George, and Monroe, had only moderate (40-60%) viability (Jennings et al. 1988; Woodward et al. 1989, 1993; Masson 1995, Rice 1996). These rates are well below clutch viability rates observed on other Florida lakes: Lake Woodruff National Wildlife Refuge (79%), Orange Lake (82%), and the Everglades Water Conservation Areas (65-75%), as well as other alligator populations in the US.

Endocrine disruption by contaminants was implicated in the reproductive failure in alligators and turtles in Lake Apopka in the 1980's. Recent reports have indicated altered reproductive hormone levels for juvenile alligators on Lake Apopka as well as alterations in sexual differentiation, lower hatching success and increased neonatal mortality. Additionally, alterations in secondary sexual characteristics for Lake Apopka juvenile alligators have been suggested (Guillette et al. 1994 and 1996). Overall, these findings

suggest that unknown influences are disrupting the normal reproductive and health processes of alligators and other wildlife.

Most recently, similar declines in alligator egg hatchability has been reported for Lake Griffin, in the upper Ocklawaha system. Egg collected for commercial alligator production have shown a declining hatch rate from a mean of 54% (1996) to less than 8% (1997) and 21% (1998). Additionally, abnormally high levels of adult alligator mortality have been reported throughout 1997-1998.

Pesticides (including herbicides), blue-green algae toxins, nutritional changes, density-related stress, and diseases have been suggested as possible causal factors for the observed wildlife health declines in the Ocklawaha River basin. A pesticide spill from a chemical manufacturing plant in 1980 near Lake Apopka was temporally associated with the decline in reproductive success and consequent alligator population decline on Lake Apopka during the 1980s. However, the recent sharp and relatively sudden drop in clutch viability on Lake Griffin was not accompanied by any notable event other than changes in water chemistry and Phytoplankton communities. Poor reproductive success of alligators under captive conditions has been attributed to poor nutrition (Noble et al. 1993), diseases, and high social stress (Cardeilhac 1990). Poor reproductive success of non-flooded and non-disturbed wild alligator eggs/nests has also been attributed to exposure to pesticides (Woodward et al. 1993, Guillette et al. 1994) and poor quality nesting media (Rice 1996)

Blue-green algae (Cyanobacteria) are often the dominant Phytoplankton in warm fresh waters and some of these can produce toxic secondary compounds which affect animals. In several Florida lakes there appears to have been a recent shift in dominance from Anabaena sp. and Microcystis sp. to Cylindrospermopsis raciborskii (Chapman and Schelske 1997). These blue-green algae are known to produce hepatotoxins and neurotoxins under some conditions and these can be concentrated in aquatic food chains and have been implicated in wildlife mortality (Carmichael 1992). C. raciborskii is a widespread tropical species not reported in Florida until recently and has been implicated in severe toxic episodes in several countries. Alligator mortality appears to be contemporaneous with the known appearance and dense blooms of C. raciborskii and may be among the causes of the observed wildlife effects.

Substantial uncertainty is associated with this hypothesis. The algae does not produce toxins under all conditions and the mechanisms and pathways by which toxins are concentrated and passed up the food chain are unknown. Algal toxin assay is complex and expensive, which makes exhaustive examination of the problem difficult. It is also unclear why, if algal toxins are the cause of alligator mortality and/or reproductive impairment, why other organisms have not been affected. However, alligators may be particularly prone to accumulating large body loads and particularly sensitive to their developmental effects (see below). The apparent absence of C. raciborskii before about 1990 may also be due to inadequate recognition and taxonomic confusion in this difficult group. It is also uncertain whether any effect of algal toxins on developing eggs is mediated via direct contact with toxins in the environment (e.g. nesting material, water)

on the egg or via concentrated toxins passing from maternal tissues to the egg yolk during vitellogenesis. For all these reasons we believe that a preliminary investigation is justified to establish the probability that algal toxins are involved in the observed wildlife effects.

Poor reproductive success threatens the long-term conservation of alligators, can alter the ecological role of alligators in affected ecosystems, and can substantially reduce the aesthetic and economic values of alligators on affected areas. Lake Griffin supports an alligator population in excess of several hundred non-hatchling individuals and until recently was considered one of the healthy and more productive populations in Florida. The current reproductive problems and mortality have a direct effect (loss) on the commercial alligator industry. However, the current mortality does not immediately threaten the population survival. Lake Apopka sustained equivalent mortality and almost total reproductive failure during the 1980 s and remains a viable population. The significance of the alligator mortality on Lake Griffin lies more in its unexplained nature and the possibility that alligators are providing an early warning of severe ecosystem disruption which may affect other wildlife and possibly people (Ross 1998). Alligators are uniquely suitable to providing such indications (Brisbin et al. 1998). They live for decades and are general predators at the top of the food chain, consuming fish, turtles, birds and mammals, and concentrating contaminants in their tissues. Their large body size, very active immune system and, possibly, their thermoregulatory capacity, appears to protect adults from lethal contaminant effects. For example, alligators show the highest levels of mercury recorded in free living wild animals (Brisbin et al. 1998). However, alligator eggs have proven to be a specially sensitive indicator of environmental disruption. The gender of alligators is determined by incubation temperature at a crucial stage of embryological development. This change is mediated by a complex cascade of neurological, hormonal and developmental events and very low concentrations of anthropogenic compounds disrupt this cascade resulting in developmental abnormalities including distorted hormone levels, gender determination, morphology and mortality (Crews and Ross 1998). Studies of alligators on Lake Apopka have demonstrated profound consequences for contaminant presence in a wildlife population and the investigation of the similar phenomena on Lake Griffin will be additional valuable information for understanding environmental perturbation in Florida lakes.

In addition to ecosystem level effects for alligators, there is also evidence of impacts on fish populations on Lake Griffin. Recent trawl samples on Lake Griffin indicated record low densities of age-0 and age-1 speckled perch (Pomoxis nigromaculatus) compared to data collected since 1977. Fall 1997 angler surveys resulted in the lowest harvest estimate in ten years. Speckled perch have dominated the fishery on Lake Griffin since the early 1970's. These surveys suggest that the speckled perch fishery will collapse in the near future, causing considerable economic loss, if low recruitment continues. Recent electrofishing samples for largemouth bass (Micropterus salmoides) in Lake Griffin were the smallest since standard sampling was begun in 1983 (W. Johnson and J. Benton GFC pers comm.). Largemouth bass, which were stocked into re-flooded muck farms of the Emeralda Marsh Wildlife Management Area (EMWMA) on Lake Griffin have shown superior growth characteristics but have had poor reproductive success. Studies of these bass in the past three years indicated low blood plasma concentrations of estrogen and

11-keto testosterone, and altered ratios of these hormones. Relatively high concentrations of Toxaphene, DDE, and Chlordane have been documented in both sediments and fish, including largemouth bass, in some water bodies of the EMWMA (T. Gross pers. comm.). Water pumped through a "marsh flowway" on one of these reflooded farms has loaded in excess of 19.4 metric tons of phosphorus to Lake Griffin since November 1995 (Walt Godwin SJRWMD pers. comm.). However, possible export of pesticides from the EMWMA to Lake Griffin has not been documented.

At present then, the 'cause' of wildlife mortality and impaired reproduction on Lake Griffin remains unexplained, although the combined effects of eutrophication, contamination and algal blooms may be involved. A better understanding of the limnology and biological communities of these waters is necessary for the design and implementation of restoration efforts for this important recreational and environmental resource.

Recent preliminary investigations, 1998. To address this problem several State agencies conducted routine water quality analysis and monitoring during 1998 as the preliminary activities of a multi-disciplinary and multi-agency initiative. The following summary is derived from monitoring data released by the St. John's River Water Management District and the Florida Game and Fresh Water Fish Commission and displayed at the Central Florida Lakes Wildlife Initiative web page at http://www.flmnh.ufl.edu/natsci/herpetology/lakes/lakes.htm

1997-1998 was an atypical year influenced by climatic consequences of the large El Nino event and the following 'La Nina'. Water levels were unusually high in the winter of 1998 but declined to lower regulated levels through the summer. Water temperatures were also slightly elevated by 2-3 degrees C. Secchi depths were uniformly shallow (0.2-0.4m) indicating almost continuous algal and suspended sediment turbidity, except for a brief clearing in mid June. This is also supported by chlorophyll a measures indicating levels above 250ug/L in late 1997-early 1998 and levels between 100 ug/L and 250 ug/L through 1998 except for June when levels fell below 50ug/L. Total nitrogen and phosphorus values also suggest a eutrophic/algal bloom episode in the late fall and winter of 1997-1998 and subsequent recovery through the summer of 1998, although high algal levels were maintained.

Alligator population density is routinely surveyed by GFC night light count and results from 1991-1998 show an abrupt increase in dead alligators from a baseline level of 1-2 per survey to 4 and 7 respectively in May 1997 and 1998. More intensive monitoring of dead alligators at two week intervals was initiated in November 1997 and since that time more than 80 have been recorded and marked (to prevent multiple counting of the same carcass), peaking between April and June of 1998 at 6-7 per 2 week interval. A parallel study of the appearance of decomposing carcasses allows the approximate back-dating of carcasses found at various levels of decomposition to the calculated time of death. Alligators usually sink when freshly dead and do not resurface until gasses of decomposition buoy them to the surface. However, the Lake Griffin mortality has been noteworthy for a number of alligators found dead or dying on the lakeshore. This

suggests that some alligators voluntarily emerged prior to death. All the dead alligators recorded are large sub-adults or adults (greater than 6 feet total length). It is unknown whether the event does not affect smaller alligators or whether their smaller carcasses are overlooked in the survey.

Most alligator carcasses discovered have been too decomposed for useful analysis. However four individuals were encountered between May and July 1998 in a passive, moribund condition. These were brought alive to the UF College of Veterinary Medicine for necropsy. Necropsy and veterinary pathology examination has been consistently uninformative. The alligators (6-9 feet total length) appear in good condition and show no anomalous gross pathology or histological abnormality in examination with a standard veterinary pathology screen. A single animal was subjected to clinical neurological examination and electromyography while alive and demonstrated reduced neural transmission rates (41 m/sec) compared to normal alligators and other vertebrates. Tissue specimens (liver) sent to Dr. Wayne Carmichael at Wright University for bio-assay for algal toxins demonstrated low levels (2-7 ng/g) of microcystin toxin. Results on neurotoxic analyses are not yet available.

Following alligator nesting late May-early June 1998 , all the eggs from 30 nests were collected and incubated under standard conditions at the GFC Wildlife Research Laboratory in Gainesville. Similar samples from three other Florida lakes were also tested. Clutch viability (percentage of eggs successfully hatching) was 24% for Lake Griffin, 44% for Lake Apopka, 62% for Orange Lake and 83% for Lake Woodruff. Lake Woodruff is considered a relative uncontaminated reference lake and Orange and Apopka are known to have reduced clutch viability. Lake Griffin alligator eggs continue to show abnormally low hatch success. It is unknown whether the observed adult mortality and the low hatch success are due to the same cause or causes. Reference samples of tissues (muscle, fat, liver, brain) and eggs have been stored in ultra-low temperature(-80oC) freezers awaiting funding for analysis for contaminants and algal toxins.