Organochlorine Pesticides and Thiamine in Eggs of Largemouth Bass and American Alligators and Their Relationship with Early Life-stage Mortality

Authors: Sepúlveda, Maria S., Wiebe, Jon J., Honeyfield, Dale C., Rauschenberger, Heath R., Hinterkopf, Joy P., et al.

Source: Journal of Wildlife Diseases, 40(4) : 782-786

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-40.4.782

Journal of Wildlife Diseases, 40(4), 2004, pp. 782–786

Organochlorine Pesticides and Thiamine in Eggs of Largemouth Bass and American Alligators and Their Relationship with Early Life-stage Mortality

Maria S. Sepúlveda,[1,5] **Jon J. Wiebe,**[1,2] **Dale C. Honeyfield,**[3] **Heath R. Rauschenberger,**[1] **Joy P. Hinterkopf,**[3] **William E. Johnson,**[4] **and Timothy S. Gross**[1,2] [1] Center for Environmental and Human Toxicology, Department of Physiological Sciences, University of Florida, Gainesville, Florida 32611, USA; [2] USGS/Florida Integrated Science Center/Center for Aquatic Resource Studies, Gainesville, Florida 32653, USA; [3] USGS Northern Appalachian Research Laboratory, Wellsboro, Pennsylvania 16901, USA; [4] Florida Fish and Wildlife Conservation Commission, Eustis Fisheries Research, Laboratory, Eustis, Florida 32726, USA; [5] Corresponding author (email: marisep@ufl.edu)

ABSTRACT: Thiamine deficiency has been linked to early mortality syndrome in salmonids in the Great Lakes. This study was conducted to compare thiamine concentrations in American alligators (*Alligator mississippiensis*) and Florida largemouth bass (*Micropterus salmoides floridanus*) eggs from sites with high embryo mortality and high exposure to organochlorine pesticides (OCPs) (Lakes Apopka and Griffin, and Emeralda Marsh, Florida, USA) to those from sites that have historically exhibited low embryo mortality and low OCPs (Lakes Woodruff and Orange, Florida). During June–July 2000, 20 alligator clutches were collected from these sites, artificially incubated, and monitored for embryo mortality. Thiamine and OCPs were measured in one egg/clutch. During February 2002, 10 adult female bass were collected from Emeralda Marsh and Lake Woodruff and mature ovaries analyzed for thiamine and OCP concentrations. Although ovaries from the Emeralda Marsh bass contained almost 1,000-fold more OCPs compared with the reference site, Lake Woodruff, there were no differences in thiamine concentrations between sites (11,710 vs. 11,857 pmol/g). In contrast, alligator eggs from the reference site had five times the amount of thiamine compared with the contaminated sites (3,123 vs. 617 pmol/g). Similarly, clutches with >55% hatch rates had significantly higher concentrations of thiamine compared with clutches with <54% hatch rates (1,119 vs. 201 pmol/g). These results suggest that thiamine deficiency might be playing an important role in alligator embryo survival but not in reproductive failure and recruitment of largemouth bass. The cause(s) of this thiamine deficiency are unknown but might be related to differences in the nutritional value of prey items across the sites studied and/or to the presence of high concentration of contaminants in eggs.

Key words: Alligators, early life stage mortality syndrome, Florida, largemouth bass, thiamine.

Researchers from the Great Lakes and the Baltic Sea have linked mortalities of salmonids during early-life stages with lower than normal concentrations of vitamin B_1 (thiamine). Clinical signs of this early mortality syndrome (EMS, also known as M74 and Cayuga syndrome) include loss of equilibrium, anorexia, reduced yolk-sac utilization, and death (Fitzsimons et al., 1999). The ability of thiamine to reverse and prevent this syndrome has led to the speculation of a cause-effect relationship between low egg thiamine levels and EMS in salmonids. Recent studies, however, support the possibility of a multifactor etiology, as not all progeny of salmonids having low thiamine exhibit this syndrome. Indeed, it has been hypothesized that the presence of high concentrations of contaminants (such as polychlorinated biphenyls and organochlorine pesticides, OCPs) can affect thiamine status in animals. The first set of proposed mechanisms involve a direct enzymatic inhibition of the biochemical pathway by which thiamine and transketolase are involved in the production of metabolic energy through the pentose phosphate pathway (Roode et al., 2002). Transketolase catalyses the transition of xylulose and ribose into heptulose and glyceraldehydes, respectively, for their later use in the glycolytic pathway. Transketolase is dependent on thiamine pyrophosphate, which is formed from ATP and thiamine in a reaction catalyzed by the enzyme thiamine-pyrophosphotransferase. Contaminants may affect enzyme activity directly via a molecular interaction with transketolase, thiamine pyrophosphate, or with thiamine-pyrophosphotransferase (Roode et al., 2002). Alternatively, contaminants might also deplete total thiamine concentrations indirectly by increasing the demand for nicotinamide adenine dinucleotide phosphate (final energy yield from the pentose phosphate pathway) due to induced synthesis and activity of detoxifying enzymes (e.g., CYP450) or redox cycling (Yagi et al., 1979; Pélissier et al., 1992; Kerman et al., 1998).

Between 1955 and 1990, extensive sawgrass marshes were drained for farming in central Florida (USA). In an effort to restore them to their original condition, these agricultural sites (now referred to as Emeralda Marsh Wildlife Management Area) were reclaimed by the state in the early 1990s, flooded, and stocked with Florida largemouth bass (*Micropterus salmoides floridanus*). However, sediments from these sites still contain high concentrations of OCPs (e.g., mean of 39 and 7 ppm of toxaphene and sum of dichlorodiphenyltrichloroethane (DDT) and derivatives, respectively; Marburger et al., 2002). In addition, little to no recruitment of bass has been observed in these reflooded lands (Benton and Douglas, 1996). Populations of American alligators (*Alligator mississippiensis*) in Florida could also be affected by exposure to OCPs, as embryo mortality is higher in contaminated lakes (such as Apopka) compared with reference lakes (Woodruff) (Heinz et al., 1990). The objective of this study was to determine whether populations of Florida largemouth bass and American alligators from sites with low recruitments, high egg/embryo mortality, and high exposure to OCPs also exhibit lower than normal concentrations of thiamine.

During June–July 2000, 20 alligator clutches were collected from Lakes Apopka (28°37.11′N, 81°37.46′W), Griffin (28°50.32′N, 81°51.13′W), and Emeralda Marsh (a reclaimed/flooded agricultural marsh adjacent to Lake Griffin, 28°57.01′N, 81°48.22′W). Orange Lake (29°27.41′N, 82°10.26′W) served as the reference site. From each nest, all eggs were collected prior to being marked on the surface with a pencil and placed in a plastic tub filled with nest material, which was later covered with a lid that had holes for ventilation. Because alligator embryos attach to the surface of the egg very early during development, marking them prevents positioning the embryo on the bottom surface of the egg and causing asphyxiation. After eggs were collected, two to three snare traps were set around each nest with the objective of capturing adult females. Among other parameters, females were measured (snout-vent length) as an indirect estimate of age. Upon arrival to the laboratory (<8 hr after collection), eggs were candled and, if viable, placed with the pencil markings facing up in plastic tubs filled with sphagnum moss, and artificially incubated in a temperature/humidity-controlled incubator (31–33 C and 88–92%, respectively). Clutches were monitored for embryo mortality (by candling eggs four times during the ~65-day incubation period). In addition, on arrival, one egg was randomly selected from each clutch and yolks collected and saved in −80 C for later thiamine and OCPs analyses. Thiamine concentrations were determined as described in Brown et al. (1998). Briefly, a known amount of the frozen yolk sample was first placed in 2% trichloroacetic acid (TCA, Sigma, St. Louis, Missouri, USA) homogenization solution. The extract was then washed with ethyl acetate: hexane (3/2, vol/vol, Sigma) to remove excess TCA. An aliquot of the washed solution was reacted with potassium ferricyanide (Sigma) to produce thiochrome derivatives. The resulting derivatives were separated on a Hamilton PRP-1 column (Alltech, Deerfield, Illinois, USA) and detected with a spectrofluorometer set at 375 nm excitation wavelength and 433 nm emission (Shimadzu, Columbia, Maryland, USA). Authentic standards of thiamine pyrophosphate, thiamine monophosphate, and thiamine-HCL (ICN Biomedicals, Montreal, Quebec, Canada) were used to quantify the amount of thiamine in each sample. For the pesticide analyses, yolks were first homogenized and a 2–5-g sample extracted into ethyl acetate. Samples were purified using C18 and NH2 solid-phase extraction cartridges, and pesticide concentration was determined by gas chromatography-mass spectroscopy, according to US Environmental Protection Agency (EPA) method 8270C (USEPA, 1996). Samples were analyzed at least three times in full scan mode for analyte identification and in selected ion mode for quantitation to improve sensitivity. Percent recovery ranged between 75% and 100%, with a limit of detection of 0.75–1.5 ng/g. Concentrations in this study are given as non-lipid normalized wet weight values.

During February 2002, 10 adult largemouth bass females were collected from Emeralda Marsh and the reference site, Lake Woodruff. Age was determined by otolith examination, and ovaries were analyzed for thiamine and OCP concentrations as already described. Ovaries were classified as fully developed and in prespawning condition through histologic evaluations as described in Sepúlveda et al. (2001).

Means and standard errors were calculated for animal size, concentrations of thiamine and OCPs, and alligator hatch rates using PROC UNIVARIATE, SAS Version 9.0. For alligators and bass, means across sites were compared by analysis of variance (ANOVA) (followed by a Tukey's multiple comparison test) or Student's *t*-tests, respectively. Statistical significance was assessed at $P \leq 0.05$.

Total yolk thiamine concentrations were fivefold higher in American alligators from Orange Lake compared with the other sites (Table 1). Eggs from Orange Lake also had low embryo mortality and low concentrations of OCPs in yolk (Table 1). In addition, there was a positive relationship between total thiamine in yolk of alligator eggs and their hatch rates

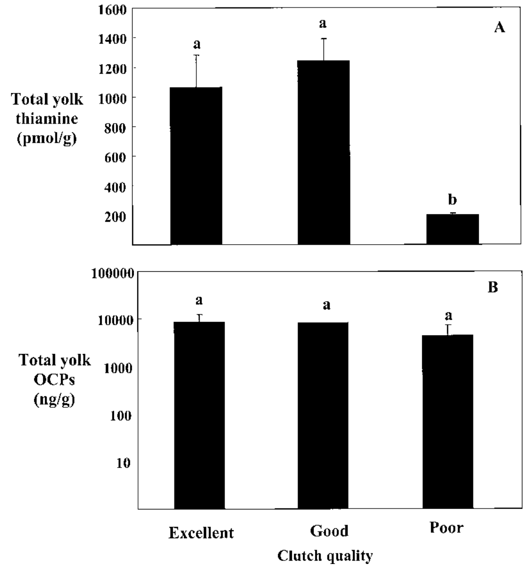

FIGURE 1. Total thiamine (A) and organochlorine pesticide (OCPs) (B) concentrations (mean±SEM) in egg yolks from American alligators sampled in north-central Florida in relation to degree of hatchability (excellent: >80%, $n = 9$; good: 79–55%, $n = 5$; and poor: <54%, $n = 6$, hatch rates). Different letters above bars denote statistically significant differences (analysis of variance, Tukey multiple comparison test, $P<0.05$).

($P<0.0001$; $R^2=0.4$). Clutches with >55% hatch rates had significantly higher concentrations of thiamine compared with clutches with <54% hatch rates (1,119 vs. 201 pmol/g; Fig. 1). Pesticides in yolk, however, did not seem to be related to overall hatch rates (Fig. 1).

Mature ovaries from largemouth bass captured at Emeralda Marsh contained almost 1,000-fold more OCPs compared with the reference Lake Woodruff, but there were no differences in thiamine concentrations between sites (11,710 vs. 11,857 pmol/g; Table 1).

All combined, these results suggest that thiamine deficiency might be playing a role in the alligator embryo mortalities observed in some Florida Lakes. In contrast, the low recruitment observed in largemouth bass at the highly contaminated Emeralda Marsh Site is probably not related to thiamine deficiency because concentrations of this vitamin were similar to

TABLE 1. Summary of animal measurements and thiamine and organochlorine pesticide concentrations (OPCs) from American alligators and largemouth bass from north central Florida. Values presented are means±SEM. Sample sizes are indicated in parentheses. Different capital letters denote statistically significant differences among sites (analysis of variance, Tukey's multiple comparison test for alligators, and Student's *t*-test for large mouth bass; $P<0.05$).

	Snout-vent length (cm)[a]	Age (yr)	Total thiamine (pmol/g)		Total OCPs[b] (ng/g)		Hatch/survival rates[d] (%)
			Egg yolk	Mature ovary	Egg yolk	Mature ovary	
American alligator							
Orange (3)	129 ± 3^{A}	—[c]	$3,123\pm477^{A}$	—	183 ± 4^{A}	—	92 ± 5^{A}
Griffin (6)	135 ± 2^{A}	—	490 ± 107^{B}	—	$1,088\pm339^{B}$	—	34 ± 11^{B}
Emeralda (7)	125 ± 4^{A}	—	774 ± 137^{B}	—	$15,798\pm2,306^{C}$	—	59 ± 10^{A}
Apopka (4)	128 ± 15^{A}	—	532 ± 132^{B}	—	$10,162\pm2,814^{C}$	—	64 ± 11^{A}
Largemouth bass							
Woodruff (10)	—	3.5 ± 0.4 $(10)^{A}$	—	$11,857\pm1,466$ $(10)^{A}$	—	47 ± 6 $(10)^{A}$	—
Emeralda Marsh (10)	—	6.0 ± 0.5 $(10)^{B}$	—	$11,710\pm1,032$ $(10)^{A}$	—	$39,283\pm8,000$ $(10)^{B}$	—

[a] For Orange Lake measurements reported were taken from females caught during the breeding season of 2002.

[b] Total concentration of organochlorine pesticides (gas chromatography-mass spectroscopy screen of 30 pesticides). Mixture composed of 94% p,p′ -2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE) and toxaphene in alligators and of 95% p,p′ -2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD) and p,p′-DDE in largemouth bass.

[c] — = not determined.

[d] Determined by dividing the number of hatchlings surviving 24 hr post hatch by the number of eggs set for incubation, times 100.

those observed in the low-OCP and highly productive Woodruff site.

In salmonid eggs, total thiamine concentrations below 3,000 pmol/g are likely to develop EMS, and concentrations below 1,000 pmol/g almost always result in high fry mortality (Fitzsimons et al., 1999). In the present study, alligator eggs from all sites except Orange Lake had low thiamine concentrations. Causes for this decline are unknown but might be related to differences in dietary thiamine concentrations in prey items or the presence of thiaminase, an enzyme that inactivates thiamine (Ji and Adelman, 1998). As already discussed, environmental contaminants might also affect thiamine status by either causing depletion of the vitamin (Yagi et al., 1979) and/or through an interaction with transketolase or its cofactor thiamine pyrophosphate (Roode et al., 2002).

We thank C. Wieser, S. Ruessler, N. Kernaghan, J. Noggle, J. Basto, A. Harvey, and B. Arnold (US Geological Services-Center for Aquatic Research Studies) and W. Wooward and W. Porak (Florida Fish and Wildlife Conservation Commission) for their assistance in the field collections. This study was supported by NIEHS Superfund Basic Research Program Grant P42-07375 to T. S. Gross.

LITERATURE CITED

BENTON, J., AND D. DOUGLAS. 1996. Ocklawaha Fisheries investigations: 1 July 1994 through 30 June 1995: Study XIII assessment of fisheries restoration potential for reclaimed agricultural lands in the Upper Ocklawaha Basin. State of Florida Game and Fresh Water Commission, Eustis, Florida, 78 pp.

BROWN, S., D. C. HONEYFIELD, AND L. VANDENBYLLAARDT. 1998. Thiamine analysis in fish tissues. *In* Proceedings: Early mortality syndrome: Disruptions in fish of the Great Lakes, New York Finger Lakes, and the Baltic Region, G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield (eds.). American Fisheries Society Symposium 21, Bethesda, Maryland, pp. 73–81.

FITZSIMONS, J. D., S. B. BROWN, D. C. HONEYFIELD, AND J. G. HNATH. 1999. A review of early mortality syndrome (EMS) in Great Lakes salmonids: Relationship with thiamine deficiency. Ambio 28: 9–15.

HEINZ, G. H., H. F. PERCIVAL, AND M. L. JENNINGS. 1990. Contaminants in American alligator eggs from Lake Apopka, Lake Griffin, and Lake Okeechobee, Florida. Environmental Monitoring and Assessment 16: 277–285.

JI, Y. Q., AND I. R. ADELMAN. 1998. Thiaminase activity in alewives and smelt in Lakes Huron, Michigan, and Superior. *In* Proceedings: Early mortality syndrome: Disruptions in fish of the Great Lakes, New York Finger Lakes, and the Baltic Region, G. McDonald, J. D. Fitzsimons, and D. C. Honeyfield (eds.). American Fisheries Society Symposium 21, Bethesda, Maryland, pp. 154–159.

KERMAN, G., U. TJÄRNLUND, AND L. BALK. 1998. Evidence for anthropogenic substances causing thiamine deficiency in fish larvae when using model substances. 8th Annual Society of Environmental Toxicology and Chemistry-Europe Congress, Bordeaux, France, p. 265.

MARBURGER, J. E., W. E. JOHNSON, T. S. GROSS, D. R. DOUGLAS, AND J. DI. 2002. Residual organochlorine pesticides in soils and fish from wetland restoration areas in central Florida, USA. Wetlands 22: 705–711.

PÉLISSER, M. A., M. H. SIESS, M. LHUISSIER, P. GROLIER, M. SUSCHETET, J. F. NARNONNE, R. ALBRECHT, AND L. W. ROBERTSON. 1992. Effects of prototypic polychlorinated biphenyls on hepatic and renal vitamin contents and on drug-metabolizing enzymes in rats fed diets containing low or high levels of retinyl palmitate. Food Chemistry and Toxicology 30:723–729.

ROODE, D. F., P. J. VUORINEN, AND A. T. C. BOSVELD. 2002. Effects of furazolidone, PCB77, PCB126, arochlor 1248, paraquat and p,p′-DDE on transketolase activity in embryonal chicken brain. Toxicology 173: 203–210.

SEPÚLVEDA, M. S., D. S. RUESSLER, N. D. DENSLOW, S. E. HOLM, T. R. SCHOEB, AND T. S. GROSS. 2001. Assessment of reproductive effects in largemouth bass (*Micropterus salmoides*) exposed to bleached/unbleached kraft mill effluents. Archives of Environmental Contamination and Toxicology 41: 475–82.

US ENVIRONMENTAL PROTECTION AGENCY SW−846. 1996. US EPA Method 8270C, Semivolatile organic compounds by gas chromatography/mass spectrometry. *In* Test methods for evaluating solid wastes physical/chemical methods. Office of Solid Waste, Department of Commerce, National Technical Service, Springfield, Virginia, 54 pp.

YAGI, M., K. KAMOHARA, AND Y. ITOWAKA. 1979. Thiamine deficiency induced by polychlorinated biphenyls (PCB) and dichlorodiphenyltrichloroethane (DDT) administration to rats. Journal of Environmental Pathology and Toxicology 2: 1119–1125.

Received for publication 15 August 2003.