U.S. Fish and Wildlife Service

Region 2

Contaminants Program

CONTAMINANTS IN SONORAN MUD TURTLES FROM QUITOBAQUITO SPRINGS, ORGAN PIPE CACTUS NATIONAL MONUMENT, ARIZONA

by

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ABSTRACT: This opportunistic investigation explored levels and potential effects of organochlorine pesticides and heavy metals as factors limiting Sonoran mud turtles (*Kinosternon sonoriense*) at Quitobaquito Springs. Turtles found dead between 1989 and 1993 and pond sediments from Quitobaquito Springs were analyzed for pesticides and metals. Current levels of organochlorines are low and do not pose a threat to turtle survival and reproduction. Fat reserves in Sonoran mud turtles appeared relatively low suggesting an inadequate diet and possible dietary stress. Mean concentrations of boron, chromium, selenium, strontium, and zinc were significantly higher in turtles from Quitobaquito Springs than in softshell turtles from the highly contaminated Gila River. High concentrations of several elements in combination with a protein restricted diet may be a factor limiting turtle survival.

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The population of Sonoran mud turtles (*Kinosternon sonoriense*) at Quitobaquito Springs, Organ Pipe Cactus National Monument, Pima County, Arizona, has declined drastically since the 1950s when the turtles probably numbered in the hundreds (Rosen and Lowe 1996). By 1970, the population declined to about 143 individuals and by the early 1980s, Rosen and Lowe (1996) estimated that only about 100 individuals were present at Quitobaquito Springs. The reason for the decline was largely attributed to an inadequate food base, but organochlorine pesticides and heavy metals may also have played a role (Rosen and Lowe 1996). Between 1989 and 1993, eight turtles were recovered dead from Quitobaquito Springs by the authors (PCR) and cooperators during radiotelemetry studies to determine survival, movements, and other population dynamics. An informal cooperative U.S. Fish and Wildlife Service/University of Arizona arrangement was completed in 1994 whereby the Service would chemically analyze the turtles found dead. This report summarizes organochlorine compound and heavy metal concentrations detected in the eight Sonoran mud turtles from Quitobaquito Springs.

METHODS

Turtles found dead were necropsied and results discussed by Rosen and Lowe (1996). After necropsy, turtle carcasses were stored frozen for chemical analysis. Whole body turtles were analyzed for organochlorine compounds and heavy metals.

To supplement the turtle analytical data, the authors (KAK and CTM) collected four sediment samples from Quitobaquito Springs October 12, 1994. Sediment samples were taken at relatively equidistant intervals along a north-south transect from the spring entrance to the pond's south shore. Sediment samples were weighed then placed on wet ice for about 12 hours before transfer to a commercial freezer. Sediments were analyzed for element content only (not pesticides).

Turtles were analyzed for organochlorine compounds and metalloids at Hazleton Environmental Services, Inc., Madison, Wisconsin. Samples were analyzed for p,p'-DDE, p,p'-DDD, p,p'-DDT, dieldrin, heptachlor epoxide, hexachlorobenzene (HCB), oxychlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, endrin, toxaphene, mirex, and polychlorinated biphenyls (PCB). For each organochlorine analysis, the sample was homogenized and a portion mixed with anhydrous sodium sulfate and extracted with hexane in a Soxhlet apparatus for 7 hours. Lipids were removed by Florisil column chromatography (Cromartie et al. 1975). Sep-pak Florisil cartridges were used for removal of lipids (Clark et al. 1983). The organochlorine compounds were separated into four fractions on a SilicAR column to ensure the separation of dieldrin or endrin into an individual fraction (Kaiser et al. 1980). The individual fractions were analyzed with a gas-liquid chromatograph equipped with an electron-capture detector and a 1.5/1.95% SP-2250/SP-2401 column. Residues in 10% of the samples were confirmed by gas chromatography/mass spectrometry. The lower limit of quantification was 0.1 Fg/g for all organochlorine pesticides and 0.5 Fg/g for PCB. Organochlorine compounds are expressed in parts per million (ppm) wet weight.

Turtles and sediments were also analyzed for aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, chromium, copper, iron, lead, manganese, mercury, molybdenum, nickel, selenium, strontium, vanadium, and zinc. Atomic absorption spectroscopy hydride generation was used to quantitate selenium and arsenic. Mercury concentrations were determined by cold vapor atomic absorption. All other elements were analyzed by inductively coupled plasma atomic emission spectroscopy. Blanks, duplicates, and spiked samples were used to maintain laboratory quality assurance and quality control (QA/QC). QA/QC was monitored by Patuxent Analytical Control Facility (PACF). Analytical methodology and reports met or exceeded PACF QA/QC standards. Element concentrations are reported in ppm dry weight. Mean concentrations of selected metals were compared between areas using a one-way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

<u>Organochlorines in turtles</u>: DDE was the only organochlorine compound detected in turtles (Table 1). Residues were present in one-half of the samples and concentrations ranged from not detected to 0.035 ppm. Organochlorine residues at these levels are relatively low and probably would not have any adverse effects on turtle survival or reproduction.

<u>Metals in sediment</u>: Background data for most elements commonly occurring in Arizona soils are presented in Table 2. Unfortunately, no information is available for boron and strontium. Of the remaining elements, only selenium concentrations in Quitobaquito Springs sediments were above the range of normally encountered Arizona soils (mean " 2SD). The threshold level of selenium in sediments above which effects on fish and wildlife might be expected is $4 ppm dry weight (Finley 1985, Garrett and Inman 1984). Selenium in Quitobaquito Springs sediments was 1.57 ppm or less, suggesting a low potential for selenium related problems.

<u>Metals in turtles</u>: Concentrations of seven elements designated by the Environmental Protection Agency (EPA) as priority pollutants were detected in turtles (Table 3). Apparently, ours is the first study to document contaminant levels of Sonoran mud turtles in Arizona, as we were unable to locate any data for meaningful within-species comparisons. To provide a frame of reference, we compared levels of elements in Sonoran mud turtle from Quitobaquito Springs with those detected in spiny softshell turtles (*Trionyx spiniferus*) collected from the lower Gila River (King unpub. data). Because of species differences in rates of pesticide and metal accumulation, this cross-species comparison is not entirely valid but, in the absence of Sonoran mud turtle comparative data, this cross-species comparison may aid in the interpretation of residue data.

Contamination of softshell turtles in the lower Gila River is well documented (Kepner 1987, Kepner unpub. data, King unpub. data); therefore, we expected that metal concentrations in spiny softshell turtles would be significantly higher than those in mud turtles collected from relatively pristine Quitobaquito Springs. However, this was not always the case. Only mean mercury concentrations were significantly (P= 0.0014, one-way ANOVA) higher in Gila River softshell turtles (0.362 ppm) than in Sonoran mud turtles (0.092 ppm) (Table 3). Mean arsenic and nickel residues were similar between species (P$ 0.1068, one-way ANOVA). Mean concentrations of boron, chromium, selenium, strontium, and zinc were significantly higher in turtles from Quitobaquito Springs than in those from the Gila River (P# 0.0012, one-way ANOVA).

Arsenic: Background arsenic concentrations in biota are usually less than 1 ppm wet weight (3 - 4 ppm dry weight) (Eisler 1988). None of the turtle samples contained arsenic that approached this concern level.

Boron: Boron is a naturally occurring trace element generally considered environmentally innocuous, but boron has been documented to severely impair mallard (*Anas platyrhynchos*) reproduction at levels found naturally occurring in the environment (Smith and Anders 1989, Hoffman et al. 1990a). Elevated levels of boron are often associated with agricultural drainwaters. Hatching success and duckling survival was significantly reduced in feeding studies when mallard hens were fed 1000 ppm dry weight boron, less than one-third the highest boron concentrations found in plants of California's San Joaquin Valley. Additional research is needed to determine boron levels in aquatic plants at Quitobaquito Springs and assess their potential effects on Sonoran mud turtles.

Chromium: The organs and tissues of fish and wildlife that contain >4.0 ppm total chromium dry weight should be viewed as presumptive evidence of chromium contamination (Eisler 1986). Two of eight samples exceeded this concern level. However, we did not find any data that correlate concentrations of chromium in turtles with biological effects; therefore, even though two of eight samples contained elevated concentrations of chromium, toxicity cannot be established.

Copper: Copper is an essential dietary element for both plants and animals but at sufficient concentrations, copper may also be toxic (EPA 1980). Information is lacking on whole body residues and biological effects in many species, including turtles. We were unable to interpret the biological significance of copper at levels detected in the Sonoran mud turtles.

Mercury: Mercury concentrations are of special concern because mercury can bioconcentrate in organisms and biomagnify through the aquatic food chain. Mercury has no known biological function. The highest concentration of mercury detected in turtles from Quitobaquito Springs, 0.14 ppm dry weight, was below the 0.5 ppm level generally accepted as the concentration in biota from unpolluted environments (Abernathy and Cumbie 1977).

Selenium: Selenium is an essential trace element in animal diets, but it is toxic at concentrations only slightly above required dietary levels. No data were located regarding normal or background concentrations of selenium in turtles. Almost all, (18 of 19) softshell turtles from the lower Gila River contained selenium concentrations below the lowest level detected in Sonoran mud turtles from Quitobaquito Springs. The significance of selenium in the 1.63-2.11 ppm dry weight range as detected in turtles from Quitobaquito Springs is yet to be determined.

Strontium: The mean level of strontium in Sonoran mud turtles from Quitobaquito Springs was 4.8-times that in spiny softshell turtles from the Gila River (Table 3). No comparable data are available to assess whether strontium concentrations reported in this study were elevated or within the normal background range.

Zinc: Zinc is another essential element which at elevated concentrations can be toxic. Although tissue residues are not reliable indicators of zinc contamination, zinc poisoning usually occurs in birds and mammals when the liver or kidney contains >210 ppm dry weight. Zinc interacts with numerous other elements and the patterns of accumulation, metabolism, and toxicity from these interactions sometimes greatly differ from those produced by zinc alone (Eisler 1993). Mixtures of zinc/copper and zinc/nickel are generally acknowledged to be additive or more-than-additive in toxicity to a wide variety of aquatic organisms but, unfortunately, no data are available for turtles.

<u>Sources of contamination</u>: We are at a loss to explain why some elements appeared to be elevated in many Sonoran mud turtles. There are no obvious point sources of contamination in Quitobaquito Springs. Elevated levels may reflect aerial transport of contaminants, but additional research is needed to confirm this hypothesis. Since Quitobaquito Springs pond has no outlet during normal flow periods, the area may act as a sump for contaminants. Water is lost from the pond through evaporation; therefore, elements in the water column should tend to concentrate over time. We would expect that element levels in sediments to be especially elevated, but this was not the case.

<u>Reduced protein and metal interactions</u>: Reduced protein intake in combination with elevated metal levels may be suppressing turtle populations at Quitobaquito Springs. The Sonoran mud turtle is an omnivore that prefers invertebrates and fish when available (Hulse 1974). Aquatic invertebrates as food for mud turtles may be in chronic short supply at Quitobaquito Springs as evidenced by digestive tract and feces examination (Rosen and Lowe 1996). Body lipid reserves also were depleted in Sonoran mud turtles (mean = 2.81%) compared to those in softshell turtles (mean = 20.66%) indicating a possible dietary deficiency. In experimental studies with birds, protein deficient diets supplemented with elevated levels of arsenic, boron and selenium resulted in decreased growth rates and mortality (Hoffman et al. 1990b). Nutritional deficiencies may enhance certain element toxicity in birds and mammals and elements such as selenium can cause immunosuppression possibly rendering individuals more susceptible to disease (Hoffman et al. 1990b) and stress. It is also possible that a protein deficient diet and high metal levels combined with the stress of capture and handling may have resulted in the unusual death of three of four radio transmitter-tagged turtles.

RECOMMENDATIONS AND SUGGESTIONS FOR FUTURE RESEARCH

This opportunistic investigation has raised more questions than it has answered. We offer the following suggestions on future research needs.

1. All turtles found dead should be salvaged and necropsied to determine which animal and plant species are being consumed. Carcasses should be stored frozen for further metal residue analysis.

2. Aquatic animals and plants most likely consumed by the Sonoran mud turtle should be collected and analyzed for selected trace metals to determine if environmentally hazardous levels are present.

3. Laboratory studies should be initiated to determine if current environmental levels of selected elements in combination with protein restrictions could be affecting adult Sonoran mud turtle physiology and reproductive success. Ideally, the test species should be the Sonoran mud turtle but, in the absence of populations large enough to withstand collections, a surrogate species such as the yellow mud turtle (*Kinosternon flavescens*) could be used.

4. Contaminant implications are obvious for the endangered desert pupfish (*Cyprinodon macularius*) which also occupies Quitobaquito Springs. Pupfish found dead should be salvaged for trace element residue analysis. Food items for pupfish should also be collected for residue analysis.

5. If contaminant concentrations are detected at high levels in pupfish samples, a surrogate species should be selected and laboratory studies initiated to determine levels of contaminants that could result in reduced survival and reproduction.

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Table 1. Physical characteristics and organochlorine residues (ppm wet weight) in Sonoran mud turtles collected from Quitobaquito Springs, 1989-1993.[a]

Sample Number	Date collect.	Age	Sex	Wt. (g)	Prcnt moist	Prcnt lipid	ppm DDE
C2R5L	09/15/93	Ad.	F	280	59.6	2.75	0.022
C1R5L	11/01/92	$17	M	252	58.3	3.05	0.014
C1R2R11L4R	09/15/93	Ad.	F	266	60.1	0.89	<0.01
C4L8L9L	07/17/93	8.5	M	152	50.4	3.66	0.035
C8L11L	unkn./90	.14	M	190	52.8	2.37	0.011
C4R8R11L	08/15/93	5.5	F	183	63.2	3.50	<0.01
C1L9L10L	07/17/93	2.5	F	56	70.9	4.18	<0.01
None	10/06/89	2.0	Un.	33	67.6	2.08	<0.01

[a]Samples were collected by Phil Rosen and sample numbers are his. All turtles were found dead except the one listed as "None" which accidentally drowned in a trap.

Table 2. Element concentrations in sediments from Quitobaquito Springs compared to Arizona background levels.

	Concentration, ppm dry weight[1]									
Sample	Al	As	B	Cr	Cu	Hg	Ni	Se	Sr	Zn
Quito Sp.										
1	4406	21.94	40.12	76.97	6.79	0.061	3.94	1.48	1473	19.39
2	3934	9.19	25.62	27.57	5.86	0.03	3.90	1.22	1060	12.25
3	6417	12.92	30.21	45.00	7.71	0.042	4.96	0.86	946	20.58
4	9012	16.86	47.50	70.35	12.12	0.058	6.69	1.57	1186	31.51
AZ mean[2]	55213	9.8	NA	61.3	30.0	0.10	27.5	0.30	NA	62.1
std. dev.	28245	17.2	--	66.0	31.0	0.13	30.5	0.26	--	34.0

[1]All elements are EPA designated priority pollutants except aluminum, boron, and strontium.

[2]Arizona background data (mean and standard deviation) from Earth Technology Corp. (1991).

Table 3. Element concentrations in Sonoran mud turtles collected from Quitobaquito Springs compared with those in spiny softshell turtles from the lower Gila River, Arizona.

Area & Species	Year	N[2]	Mean concentration, ppm dry weight, (n) / range[1]								
			Arsenic	Boron	Chrom.	Copper[3]	Mercury	Nickel	Selenium	Strontium	Z
Q. Spgs.											
Sonoran m. turtle	1989-1993	8	0.39 (7) ND[4] - 0.60	71 (8)* 10-154	3.98 (8)* 3.10-5.15	4.58 (8) 0.86-12.23	0.09 (8)$^{+}$ 0.06-0.14	4.11 (6) ND - 15.5	1.86 (8)$^{+}$ 1.63-2.11	869 (8)$^{+}$ 643-1216	[illegible] 14
Gila R.											
Softshell turtle	1993-1994	19	0.29 (14) ND - 0.57	4 (19)* 1 - 10	1.68 (19)* 1.05-2.72	118.9 (19) 1.25-1129	0.36 (19)$^{+}$ 0.02-0.73	1.86 (13) ND - 8.11	1.08 (11)$^{+}$ ND - 2.51	181 (19)$^{+}$ 105-421	5

[1]All elements are EPA designated priority pollutants except boron and strontium. n= number of samples that contained detectibl concentrations.

[2]N= number of samples analyzed.

[3]Differences between means for copper were not determined because several Gila River turtles were collected using copper-coated .22 shells that may have biased residue analysis.

[4]ND= None detected.

*P< 0.001

$^{+}$P< 0.01