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Acute and Genotoxic Effects of Baku Harbor Sediment on Russian Sturgeon, *Acipenser guildensteidti*

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Oil and caviar are the two most economically valuable natural resources for the Republic of Azerbaijan. While the collapse of the Soviet Union has brought about many beneficial changes in the society of this newly independant Republic, it has also revealed a history of ecological abuse under the Soviet system. The oil industry caused some of the most extensively polluted aquatic and terrestrial habitats in the world, at least with respect to petrochemical contamination (Dumont 1995). Because many of the oil fields developed by the soviets are located in shallow water or in the coastal zone of the Caspian Sea, the extensive contamination of the nearshore environment is considered to be potentially devastating to both human health and the productivity of the fishing industry.

There are six species of sturgeon that inhabit the Caspian Sea and that use the major rivers which drain into this basin as spawning habitat. The most economically important of these are the beluga (*Huso huso*), the stellate sturgeon (*Acipenser stellatus*), and the Russian sturgeon (*Acipenser guildensteidti*). Historically, the Caspian Sea has produced up to 90% of the worlds caviar, but a combination of factors has led to a precipitous decline in production. According to data from the World Wildlife Fund, a decline across species of adult sturgeon from 142 million to 43.5 million occurred between 1978 and 1994 (De Meulenaer and Raymakers 1996). This can be explained in part by the collapse of the Soviet Union which has resulted in increased poaching of sturgeon and the use of formerly illegal fishing methods. In addition, the damming of the Volga River in Russia and the Kura River in Azerbaijan has led to the destruction of an estimated 85% of sturgeon spawning habitat. To mitigate the loss of these spawning grounds, hatcheries were constructed to supplement the natural reproduction of the Volga and Kura River sturgeon populations. However, the efficacy of the hatchery system to maintain an economically viable fishery as well as conserve the natural stocks of these fish is questionable.

The potential sensitivity of sturgeon to environmental contamination compounds these pressures on the fishery. There are two known "dead zones" in Azerbaijani waters, one off the northern coast of the Apsheron Peninsula, thought to be caused by contamination emanating from the chemical industrial plants at Sumgayit, and the other in Baku Harbor thought to be the result of crude oil contamination. In

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both of these cases, the heavily contaminated bottom sediments have caused the complete extirpation of the benthic flora and fauna (Kasumov 1994). It has been reported that in places oil lies as much as 2 meters deep on the the bottom of Baku Harbor (Rowe 1996). The rate of contamination of the Caspian Sea could increase with continued sea level rise because contaminated coastal habitats and oil wells will likely be flooded, pouring considerably more contaminants into already stressed aquatic habitats. Previous studies have shown that bottom-dwelling fish, including sturgeon (Kocan et al. 1996), are particularly vulnerable to chronic exposure to sediment contaminated with petrochemicals (Malins et al. 1988).

The exposure of marine organisms to crude oil has been shown to cause mortality due to acute toxicity (Ballachey et al. 1994) or sublethal effects which are difficult to detect and assess (McBee and Bickham 1990). Recent concern over the potential transgenerational effects of contaminant exposure, as well as sublethal effects on somatic tissues, has necessitated the development of biomarkers useful to detect subtle, long-term effects (Bickham and Smolen 1995; Colborn et al. 1996). However, there are relatively few biomarkers readily applicable to natural populations that are useful in the study of sublethal or chronic effects of hydrocarbons. This study employed the use of the micronucleus test. Micronuclei, which are formed as a result of acentric chromosomal fragments, are visible as small pieces of chromatin not incorporated into the nucleus in interphase cells. This method is well suited to detect sublethal, genetic damage from oil because polycyclic aromatic hydrocarbons (PAHs), which make up the primary mutagenic components of crude oil, are known to cause chromosomal breaks (a process called clastogenesis; Custer et al. 1994). In this study we document contaminant levels, including PAHs, heavy metals, and organochlorines, in the sediment of Baku Harbor. The acute and genotoxic effects of sediment are tested on the Russian sturgeon, a bottom dwelling species, and on the red shiner (*Cyprinella lutrensis*) which is found in the water column.

MATERIALS AND METHODS

Sediment was obtained by use of a van Veen grab at a location called Station 2, adjacent to a dock in Baku Harbor (40° 22’ 44” N, 49° 52’ 30” S), in approximately 7 m of water. Aliquots of the sediment were stored in acid-washed glass jars and returned to Texas A&M University for chemical analyses and for toxicity studies on red shiners. Part of the sediment was taken to the Institute of Physiology in Baku for toxicity studies on Russian sturgeon. Organic and inorganic carbon, aromatic hydrocarbons, and pesticides and PCBs were analyzed by the Geochemical and Environmental Research Group (GERG) at Texas A&M University. Procedures and QA criteria for extraction, total organics, PAHs, and PCBs and pesticides are given in GERG SOP 8902 Rev 4, 9316 Rev 1, 9406 Rev 1, and 9302 Rev 4, respectively (available upon request from the authors). Metals were analyzed at the Trace Element Research Laboratory, Texas A&M University. Samples were freeze-dried and, following moisture determination, ground to a powder and leached with concentrated nitric and hydrochloric acid. Mercury concentrations were estimated by cold vapor atomic absorption. Other metals were determined using graphite furnace atomic absorption.

Fingerling Russian sturgeon were obtained from the hatchery near Neftchala, on the Kura River. Sex of all individuals was unknown and they ranged in size from 78-123 mm. Red shiners were obtained from a natural population by seine (Little Brazos River at Hwy 21, Brazos County, Texas). Only healthy appearing adult female red shiners (40-60 mm) were used in the experiments.

At the Institute of Physiology, sturgeon were placed in five porcelain containers each holding 42 liters of water. Each tank was aerated with a single air stone. Station 2 sediment was introduced to each container for a final concentration of 2.4, 1.2, 0.6, 0.3, and 0 parts per thousand (ppt) sediment obtained the same day from the harbor. Ten sturgeon, randomly selected, were placed into each container and the number of dead fish were counted and removed each 24 hrs. After 72 hrs, all fish were removed and killed. Blood was taken from the caudal vein of each individual that survived to the last day of the experiment using a 1 cc syringe with a 27 gauge needle coated with EDTA crystals. Blood smears were made on clean microscope slides for micronucleus studies and the fish were stored frozen in a conventional freezer. Slides were stained with Miles hematological stain and examined microscopically for the presence of micronuclei. A total of 1,000 red blood cells were scored for each individual in a blind analysis. At Texas A&M, a similar experiment used 10 gallon aquaria containing 2.4, 1.2, 0.6, and 0 ppt sediment. The sediment was stored frozen (-20 C; consistent with EPA SW-846 methods for handling organic analytes). Ten randomly selected female red shiners were introduced into each aquarium and the number of dead fish were counted and removed each day. Exposure time in this experiment was 14 days, after which the fish were killed and blood smears were made. Staining of the blood smears and micronucleus counts were made as described in the previous experiment. We used the PROC REG procedure in SAS ver. 6.08 (Cary, NC) to test for a dose response. The program uses the least-squares method to generate the line fit and parameter estimates.

RESULTS AND DISCUSSION

Chemical analyses revealed low levels of contamination by pesticides and PCBs in the Station 2 sediment from Baku Harbor. Values in ng/g dry weight were total chlordanes = 2.78, total DDTs = 21.35, total HCHs = 57.82, and total PCBs = 46.01. Likewise, metals were relatively low. Values in mg/kg dry weight were As = 10.3, Cd = 0.2, Pb = 23, Se = 0.7, Al = 1880, Ba = 235, Be=0.2, Cr = 11, B= 10, Mo < 5, Sr = 735, Cu = 108, Fe = 7760, Mg = 4580, Mn = 514, Ni = 9, V = 7, Zn = 110, and Hg = 0.04. However, contamination by PAHs was very high, with total PAHs = 301, 187 ng/g dry weight (Table 1).

Russian sturgeon were very sensitive to the acute effects of exposure to Station 2 sediment (Fig. 1). After three days exposure, 40% and 10% of the fish in the two

Table 1. Total Polycyclic Aromatic Hydrocarbons and values for selected species from Baku Harbor sediment (Station 2). Values are in ng/g..

Naphthalene	577	C2-Diben	4106
C1-Napthalenes	1326	C3-Diben	3678
C2 Napthalenes	6993	Fluoranthene	6949
C3-Naphthalenes	23006	Pyrene	19222
C4 Naphthalenes	19796	C1-Fluoran_Pyr	28956
Biphenyl	152	Benzo (a) Anthracene	4781
Acenaphthylene	423	Chrysene	13198
Acenaphthene	1996	C1-Chrysenes	11097
Fluorene	567	C2-Chrysenes	5767
C1-Fluorenes	7618	C3-Chrysenes	242
C2-Fluorenes	15864	C4-Chrysenes	94
C3-Fluorenes	14373	Benzo (b) Fluoran	3612
Phenanthrene	6174	Benzo (k) Fluoran	992
Anthracene	2124	Benzo (e) Pyrene	3494
C1-Phenanthrenes	25462	Benzo (a) Pyrene	3675
C2-Phenanthrenes	30347	Perylene	530
C3-Phenanthrenes	20102	I123cdPyrene	1155
C4-Phenanthrenes	8371	DbahAnthra	509
Dibenzothio	324	BghiPerylene	1637
C1-Diben	1884	Total PAH's	301, 187

highest exposure groups, 2.4 and 1.2 ppt respectively, had died. No deaths were observed in the control group and in the two lowest exposure groups (0.3 and 0.6 ppt). Data for frequency of red blood cells containing micronuclei were obtained from most of the surviving fish (control N = 7; 0.3 ppt N = 8; 0.6 ppt N = 10; 1.2 ppt N = 8; and 2.4 ppt N = 5). Positive dose responses were observed for the mortality data (% mortality = 0.175 [sediment concentration] - 0.0575; $r^2 = 0.919$; $p < 0.01$) and the micronucleus data (for the control and the first three dosage groups, mean micronucleus count = 5.81 [sediment concentration] +3; $r^2 = 0.988$; $p < 0.006$). However, the frequency of cells with micronuclei declined in the highest dosage group (Fig. 1).

Red shiners were less sensitive than sturgeon to the acute and genotoxic effects of the sediment. After 14 d exposure, only 10% of the fish had died in the two highest exposure groups (1.2 and 2.4 ppt). No significant increase ($p > 0.05$) in micronucleus counts was observed in any exposure group relative to the control group and no dose response was evident in the micronucleus data set (Fig. 2).

Crude oil is known to contain mutagenic and toxic PAHs, and this class of chemicals was observed to be present in high concentration in the Baku harbor sediment. PAHs include over 300 compounds which vary in their toxicity to aquatic organisms. In general toxicity increases with molecular weight and with

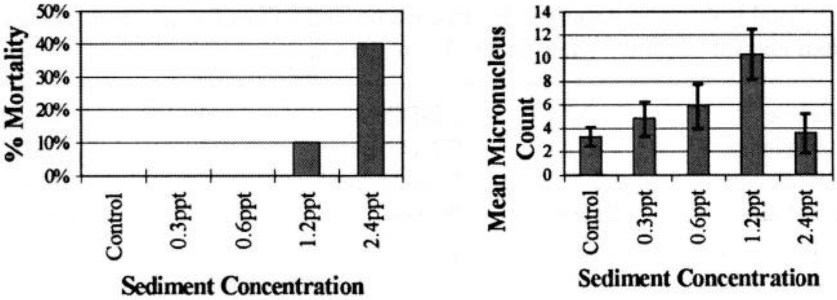

Figure 1.—Percent mortality and micronucleus counts (per 1000 cells; mean ± one standard error) from Russian sturgeon following three-days exposure to varying concentrations of Baku Harbor sediment.

increasing substitution on the aromatic ring, although acute toxicity may be reduced somewhat due to decreasing water solubility. Interaction effects of PAHs with inorganic and other organic compounds are poorly understood, and aquatic organisms vary in sensitivity to PAHs. For example, lethal water column concentrations of napthalene range from 0.050 mg/L in the copepod *Eurytemora affinis* to 150 mg/L. in the mosquitofish *Gambusia affinis* (Eisler 1987). Benthic and bottom dwelling organisms have been shown to accumulate the majority of their PAH burden by mobilizing the PAHs from the sediment/pore water matrix. This is an important consideration for understanding the toxic effects of PAHs because water column analysis will usually show low concentrations due to their low solubility, whereas significant concentrations will be found in the sediments. Evaluations of species found only in the water column may show little or no effect, whereas benthic species may accumulate high contaminant loads and show acute or sublethal effects due to the sediment/pore water contact. Numerous studies have shown that fish, especially bottom dwelling species, are vulnerable to both lethal and sublethal effects of chronic exposure to PAH contaminated sediment (Malins et al., 1988). In this study, we show that Russian sturgeon are highly sensitive to the PAH contaminated sediment of Baku harbor, whereas red shiners are much less sensitive. We interpret this to be the result of the sturgeon being exposed at a greater rate than the shiners because they fed and spent most of the time in close association with the sediments on the bottom of their tanks. The red shiners, however, are not primarily bottom feeders and spend most of the time in the water column above the sediment. Therefore, in this experiment it is likely that the route of exposure was through contact with, and possibly ingestion of, the sediment, not through exposure to dissolved PAHs.

The relationship between exposure group and response, both in terms of percent mortality and micronucleus counts, showed a clear dose-response relationship in the sturgeon (Fig. 1). The sole exception to this is that the number of micronuclei in the highest exposure group (2.4 ppt) was not different statistically from the control group. The most likely explanation for this is that the survivors of the

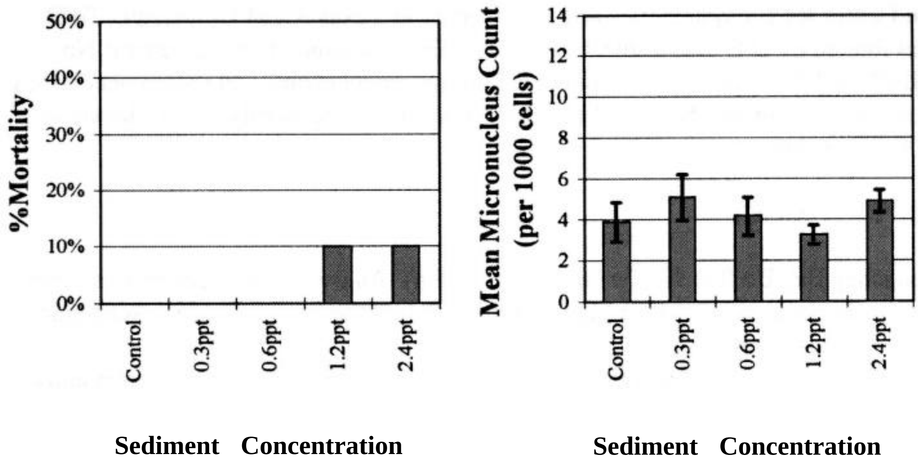

Figure 2.—Percent mortality and micronucleus counts (mean ± one standard error) from red shiners following 14-days exposure to varying concentrations of Baku Harbor sediment.

highest exposure group were less sensitive to the genotoxic effects than were the fish from which micronucleus data were obtained from the other exposure groups. In other words, the experiment had selectively removed the most sensitive fish, by way of acute toxicity, from the highest exposure group. This is consistent with the fact that although these fish were taken from a hatchery and were fairly homogeneous in age and size, they do not represent an inbred line and thus genetic variability in survivorship genes could be expected. This could indicate that the presence of oil contamination in the Caspian Sea might result in some degree of selection among wild sturgeon by favoring individuals with genotypes capable of survival in polluted habitats. The alteration of patterns of genetic diversity, termed "evolutionary toxicology" (Bickham and Smolen 1994), in fish populations exposed to environmental contaminants has been observed in brown bullheads (Murdock and Hebert 1994) and mosquitofish (Theodorakis and Shugart, 1997).

The sturgeon caviar industry is the second most important source of foreign capital for the Republic of Azerbaijan. The fact that Russian sturgeon are highly sensitive to the acute and genotoxic effects of oil contamination in the Caspian Sea is a critical issue to be addressed by an industry already threatened by the effects of poaching and the damming of two of the most important spawning rivers. The area contaminated by oil in the vicinity of Baku Harbor, on the south side of the Apsheron Peninsula, probably does not include a major portion of the habitat for any of the sturgeon of the Caspian Sea. However, this combined with the much larger dead zone emanating from the industrial center of Sumgayit and the pollution of the Kura and Volga Rivers is indicative of a large scale problem of which sturgeon might be especially sensitive. Studies are needed to determine the role environmental contamination might play in the decline of the sturgeon fishery.

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