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Differences in Blood Haptoglobin and Length–Mass Relationships in River Otters (*Lutra canadensis*) from Oiled and Nonoiled Areas of Prince William Sound, Alaska

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ABSTRACT: Significant differences in levels of blood haptoglobin occurred between river otters (*Lutra canadensis*) inhabiting oiled ($\bar{x}$ = 361 mg/100 ml, SD = 38, n = 6) and nonoiled ($\bar{x}$ = 306 mg/100 ml, SD = 87, n = 8) areas of Prince William Sound, Alaska (USA) following the *Exxon Valdez* oil spill in 1989. Additionally, male river otters from oiled areas had significantly lower body mass (1.13 kg) than male otters from nonoiled areas. We propose oil-related causes for these differences.

Key words: Haptoglobin, body mass, oil spill, hydrocarbons, river otter, *Lutra canadensis*, Prince William Sound, Alaska.

Subtidal and intertidal zones are critical habitats for river otters (*Lutra canadensis*) living in marine environments because these mustelids rely on such areas for fish and invertebrates that compose much of their diet (Larsen, 1984; Woolington, 1984). Consequently, contamination of extensive sections of shore line in Prince William Sound, Alaska (USA) by oil spilled from the *Exxon Valdez* in late March 1989 may have had serious effects on river otters inhabiting this area. Indeed, river otters are especially sensitive to pollutants in aquatic systems (Wren et al., 1980; Clark et al., 1981; Halbrook et al., 1981; Henny et al., 1981; O'Conner and Nielson, 1981; Sheffy and St. Amant, 1982; Wren, 1984, 1985). European otters (*L. lutra*) were killed by ingesting fuel oil following a spill along the coast of Scotland (Baker et al., 1981). Sublethal and indirect effects of residual oil also may have adverse consequences for otters. For instance, bivalve molluscs, a prey of otters, are directly damaged by oiling, and may accumulate hydrocarbons (Neff et al., 1980). Further, long-term exposure of marine fish to crude oil results in lowering of lipid reserves, which in turn affects energy balance and thereby survivorship (Thomas et al., 1980; Dey et al., 1983). Thus, chronic effects on river otters living in oiled areas of Prince William Sound might include factors related to a reduced food supply, and immunological and physiological changes brought about by ingestion of oil-contaminated foods.

We reasoned that if oil affected river otters, then differences in mass–length relationships and blood protein levels related to chronic affects of oil exposure should differ among otters between oiled and nonoiled study areas in Prince William Sound. Our objective was to test for such differences.

Research was conducted in an area that received heavy oiling (Herring Bay, Knight Island; 60°30′N, 147°40′W) and an area free from obvious exposure to crude oil (Esther Passage; 60°53′N, 147°55′W), Prince William Sound, Alaska. These study areas are about 40 km apart; telemetered otters did not move between areas. Study sites were selected because otter density, based on number of latrine sites (Jenkins and Burrows, 1980), appeared similar prior to the spill, and the areas were ecologically similar.

Prince William Sound is typified by steep slopes that drop abruptly to broken, rocky shorelines, although numerous bays and inlets are present. Lower elevations are dominated by old-growth forest (mostly *Tsuga heterophyla* and *Picea sitchensis*) and areas higher in elevation are characterized by alpine tundra (Eck, 1983). The climate is cool and maritime with an annual precipitation of about 220 cm; snowpack frequently is >100 cm.

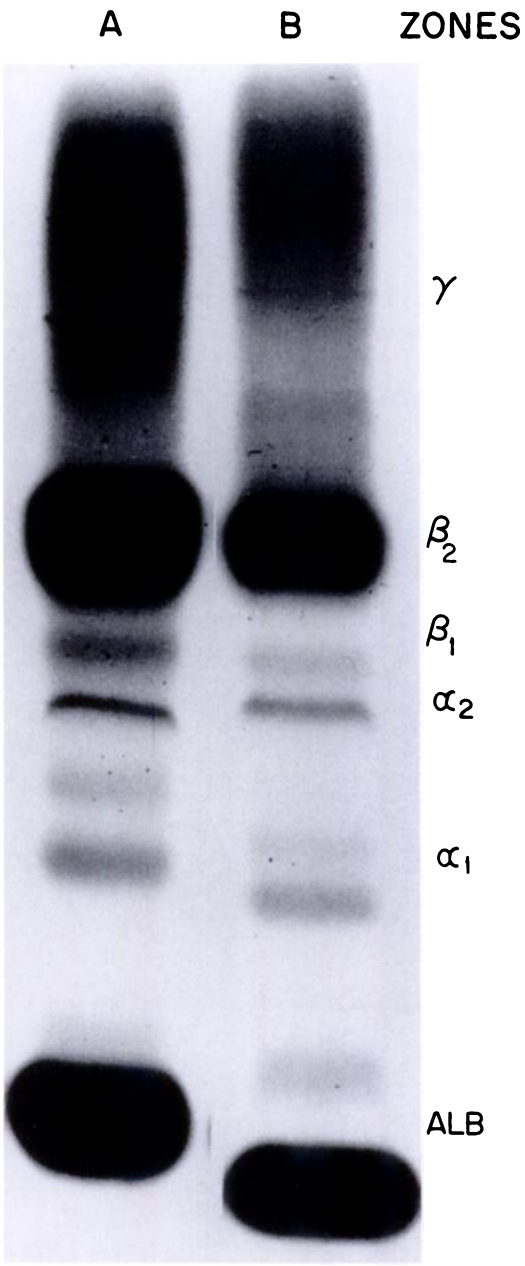

FIGURE 1. Representative electrophoresis patterns of plasma protein for river otters from Prince William Sound, Alaska. Sample A, HBM12, is from the oiled area while sample B, EPM01 is from the nonoiled area. Note the prominent bands in the β_2 globulin zone.

Despite extensive efforts to clean oil from contaminated areas around Herring Bay, numerous signs of weathered oil remained throughout our study. On many rocky shores a zone of about 3 m was covered by a thin layer of asphalt pavement concentrated near the high tide line. Moreover, an oil sheen was observed at the water's surface on incoming and outgoing tides.

Otters were captured from oiled (Herring Bay) and nonoiled (Esther Passage) areas of Prince William Sound using Hancock live traps (Melquist and Dronkert, 1987). Traps were placed on trails at latrine sites and monitored by means of a trap transmitter (Telonics®, Mesa, Arizona, USA) that signaled when a trap was sprung. The otter initially was immobilized in the trap with a hand injection of ketamine hydrochloride (11 mg/kg estimated body weight, Sigma®, St. Louis, Missouri, USA) and placed in a drugging box (Melquist and Hornocker, 1983). The animal was then transported to the vessel where surgery was performed by a licensed veterinarian. Telazol® (11 mg/kg; A. H. Robins, Richmond, Virginia, USA) was used for immobilization during surgery to implant radio transmitters for other aspects of this study. Surgery lasted approximately 1 hr and otters were released when judged to have fully recovered from effects of the drugs (5 to 13 hr after surgery). Weights and measurements were taken prior to surgery and the blood sample drawn from the jugular vein at its completion. Otters from both oiled and nonoiled areas were treated in the same manner. Sexes were distinguished by the relative position of urogenital openings and palpitation of the baculum (Larson and Taber, 1980). Age determinations were based on tooth wear and overall size of otters (Stephenson, 1977). All procedures used in this study were approved by an Institutional Animal Care and Use Committee at the University of Alaska Fairbanks, Fairbanks, Alaska.

Blood samples were collected in the field in vacutainers, and sera were separated later by low speed centrifugation. Agarose gel electrophoresis of total serum proteins was performed as described by the man-

ufacturer using a high resolution electrophoresis kit (Helena Laboratories,® Beaumont, Texas, USA). Electrophoresis was used to resolve the protein pattern into multiple zones (Fig. 1). Two microliters of serum were applied to the agarose gel, which was subjected to electrophoresis in a cooled chamber at 100 volts for 1 hr. The agarose gels were stained with Coomassie blue and individual zones were quantitated using a Beckman® Model R-112 densitometer (Beckman, Palo Alto, California, USA) (Jeppson et al., 1979; Tilley et al., 1989). Serum protein levels were determined using the Bio-Rad® protein assay with bovine serum albumin as a standard (Bradford, 1976).

Haptoglobins (Hp) are alpha$_2$ glycoproteins that stoichiometrically bind free hemoglobin (Hb) in a haptoglobin-hemoglobin complex (Gordan and Koj, 1986). Excess hemoglobin was added to the serum sample in a 1 part of a 10% hemoglobin suspension to 20 parts of undiluted serum, and allowed to mix for 5 min. Two microliters of the sample mixture were then electrophoresed on agarose gels at 100 volts for 1 hr. After fixing the protein complex with 7.5% trichloroacetic acid, gels were stained for hemoglobin using o-dianisidine, as described by the manufacturer (Helena Laboratories Technical Bulletin Number 5445). The Hp-Hb complex, which migrates in a different region from hemoglobin (Fig. 2), is quantitated by densitometry and results are expressed as mg of hemoglobin binding capacity per 100 ml of serum as described by the manufacturer (Helena Laboratories Technical Bulletin Number 5445; Valeri et al., 1965). Two samples from the nonoiled Esther Passage with exceptionally low haptoglobin levels were not included in our analysis because we suspected these samples may not have been representative; this results in our statistical comparison being conservative.

Differences in haptoglobin levels in otters from oiled and nonoiled areas of Prince William Sound were tested with multi-response permutation procedures on Euclidean distances (Melke et al., 1981; Zimmerman et al., 1985; Biondini et al., 1988; Cade and Hoffman, 1990) using BLOSSOM statistical software (Slauson et al., 1991). For this analysis, blood samples from Esther Passage were treated as the excess group (Mielke et al., 1983). Differences in otter lengths and body mass between seasons were evaluated with the Mann-Whitney test (Zar, 1984). Linear regressions of length–mass relationships were compared according to Neter et al. (1985); curvelinear procedures did not significantly improve fits of lines.

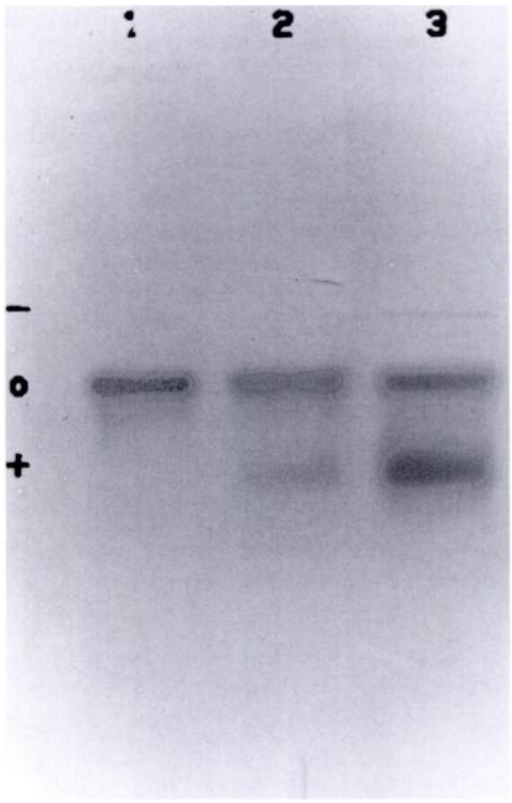

FIGURE 2. Representative haptoglobin analyses of oiled and nonoiled river otters from Prince William Sound, Alaska. Haptoglobin–hemoglobin complexes were separated from nonbound hemoglobin by electrophoresis. Lane 1 is a blank (hemoglobin alone), lane 2 is specimen EPM01 from the nonoiled area, and lane 3 is specimen HBM12 from the oiled area. Note the prominent haptoglobin-hemoglobin complex band at the lower end of Lane 3. O is the origin, + is the anode, and − is the cathode.

Total serum protein of river otters ranged from 4.6 g/100 ml to 9.1 g/100 ml, and was similar for oiled ($\bar{x}$ = 6.8 g/100 ml, SD = 1.7 g/100 ml, n = 8) and nonoiled ($\bar{x}$ = 6.6 g/100 ml, SD = 1.1 g/100 ml, n = 6) areas. There are few data on hematological indices in the blood and serum of wild mammals such as river otters, although some information is available for mink (*Mustela vison*) (Rotenberg and Jorgensen, 1971, Mohn and Nordstoga 1975). For comparison, mink had an average of 7.2 g/100 ml (SD = 0.73 g/100 ml, n = 18) (Rotenberg and Jorgensen, 1971). For serum proteins, the most notable differences from mink were the lower relative percent of the serum albumin among river otters, and higher protein levels in the β_2 globulin zone. River otter albumins ranged from 9.8% to 34.7%, whereas five samples of albumin from our laboratory mink ranged from 34% to 50.8% (L. K. Duffy, unpubl.). Rotenberg and Jorgensen (1971) reported an average of 54.7% (SD = 4.3) for 10 mink. The higher range of β_2 globulin, when compared to laboratory mink, may be related to seasonal differences in diet or nutritional status of the wild populations because certain lipoproteins are β_2 globulins. Future assessment of health in wild populations would be aided by obtaining normal hematological values for these animals in various environments. For 14 river otters from Prince William Sound, $\bar{x}$ (SD) relative concentrations present in different protein zones were: albumin, 22.0% (4.3); α_1, 2.4 (1.6); α_2, 6.3% (1.4); β_1, 6.6% (5.4), β_2, 37.2% (17.6); and γ, 23.4% (8.9).

Haptoglobin values from river otter blood serum were higher ($\bar{x}$ = 360.7 mg Hb-bound/100 ml, SD = 38.1 mg Hb-bound/100 ml, n = 8) from oiled areas (Herring Bay, Knight Island) than from areas that were free of oil (Esther Passage; $\bar{x}$ = 305.5 mg Hb-bound/100 ml, SD = 87.2, n = 6). Moreover, otters from oiled areas exhibited a substantially lower coefficient of variation in haptoglobin levels (CV = 10.6%) than otters from areas without oil (CV = 28.5%). Using multi-response permutation procedures with samples from Esther Passage as an excess group, it was evident that samples from the oiled area would not have been obtained in a random draw from the samples from unoiled areas (observed delta = 45.52, expected delta = 75.41, delta S^2 = 257.21, delta skewness = −0.4623, standardized test statistic = −1.86, P = 0.042).

The acute-phase response in protein synthesis occurs in animals following tissue damage, which can be caused by inflammation, infection or trauma (Gordon and Koj, 1985). The synthesis of a specific set of protective proteins is controlled by a group of factors called interleukins (Arai et al., 1990). Based on post-mortem examination of dogs and cattle, haptoglobin level is related to the extent of tissue damage (Echersall et al., 1989). Endotoxin treatment of mink, including hydrocarbons, also has shown changes in plasma protein (Mohn and Nordstoga, 1975). Because the Hp-Hb complex is rapidly removed by the kidney, an increase in haptoglobin levels often is interpreted to indicate that the liver is synthesizing acute-phase proteins to respond to tissue injury (Silverman and LeGrys, 1987). Additional studies of river otter blood characteristics, however, are necessary to test this hypothesis. The haptoglobin response can last up to 2 wk on one acute injury (Gordon and Koj, 1985). Levels reported herein could indicate chronic levels of inflammation and liver injury (Silverman and LeGrys, 1987), or infection. Increased haptoglobin levels are not likely the result of surgery because of the delay in development of this response (Gordan and Koj, 1985; Silverman and LeGrys, 1987), and because otters in oiled and nonoiled areas were treated in the same manner. The veterinarian noted no overt signs of disease in otters from either study site. We observed no other single factor except oil that was likely to affect otters along the 80 km of shoreline where they were sampled.

The river otters had a sexual dimor-

phism in body size with males generally heavier than females (Table 1). Additionally, individuals tended to be heavier during pre-winter (December) than post-winter (May and June) sampling periods (Table 1). When sex, age, and season were controlled by considering only adult males during May and June, a significant positive relationship ($r^2 = 0.58$, $P = 0.03$, $n = 11$) occurred between body mass (kg) and length (cm). The regression line predicting body mass for otters in the oiled area ($\hat{y} = 19.78 + 0.236x$) was depressed 1.13 kg below that of animals from oil-free zones ($\hat{y} = -18.65 + 0.236x$; $t = 2.5$, $P < 0.04$).

We propose that significant differences in mass-length relationships of male river otters between oiled and unoiled areas of Prince William Sound have an oil-related cause. Changes in prey availability through oil contamination of molluscs (Neff et al., 1980) and fishes (Dey et al., 1983) offer one possible explanation. Further, hydrocarbons in forage might affect the ability of otters to properly assimilate food, but there is no published research on this subject. Even otters from Herring Bay that selected foods free from hydrocarbon contamination might experience problems because of oil consumed while grooming their fur (Baker et al., 1981).

The increase in haptoglobin levels may be related to hemolytic anemia caused by an acute exposure to oil. Fry and Lowenstine (1985) observed hemosiderosis in oil-exposed birds, and Leighton et al. (1983) reported that hemolytic anemia developed in birds after oil ingestion. The anemia was followed by a strong regenerative response in which reduced glutathione levels as well as percentage of retulocytes increased above normal. At oil doses above 10 ml/kg, red-cell lesions such as Heinz bodies and cell surface anomalities also were observed (Leighton et al., 1983; Leighton, 1985), indicating destructive oxidative reactions. Although Heinz bodies, which are dense granular precipitates of oxidized hemoglobin, were not observed in the river otters, increased circulating haptoglobin

TABLE 1. Pre-winter (December) and post-winter (May to June) lengths and weights of river otters from oiled (Herring Bay) and nonoiled (Esther Passage) areas of Prince William Sound, Alaska, 1989 to 1990.

	Oiled								Nonoiled					
	Pre-winter					Post-winter			Post-winter					
Sex and age class	Number of animals sampled	Length (cm)		Weight (kg)		Number of animals sampled	Length (cm)		Weight (kg)		Number of animals sampled	Length (cm)		Weight (kg)	
		$\bar{x}$	SD	$\bar{x}$	SD		$\bar{x}$	SD	$\bar{x}$	SD		$\bar{x}$	SD	$\bar{x}$	SD
Adult males	5	120.1	0.85[a]	9.8	0.71	6[b]	122.8	1.54[a]	9.1	0.54	7	121.6	2.82	10.2	1.02
Juvenile males	1	102.5		8.5		2[c]	117.8	0.35	7.7		0	—		—	
Adult females	3	119.4	1.62	8.1	0.49	1	120.5		7.2		1	122.0		8.6	
Juvenile females	1	109.0		7.0		0	—		—		2	113.5	1.41	7.2	0.48

[a] $P < 0.02$ (Mann-Whitney Test) for difference between pre- and post-winter lengths; weights did not differ significantly ($P = 0.14$).
[b] Available for only four animals.
[c] Available for only one animal.

would be an important adaptive response to future episodes of hemolytic anemia. In long term-studies of chronic oil exposure, both circulating hemoglobins and haptoglobins should be monitored.

We propose that significantly elevated haptoglobin levels, and a significant reduction in body mass are the first evidence of chronic, oil-related effects on river otters in Prince William Sound. That these effects were detected more than one year after the oil spill, and following a major attempt to clean up oil, may have important consequences for other vertebrates similarly exposed to oil.

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