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## Evidence for Recovery of Body Mass and Haptoglobin Values of River Otters Following the Exxon Valdez Oil Spill

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**ABSTRACT:** Levels of blood haptoglobin (Hp) and interleukin-6 immunoreactive protein (IL-6 ir) were significantly elevated in river otters (*Lutra canadensis*) inhabiting oiled areas of Prince William Sound, Alaska (USA) following the Exxon Valdez oil spill in 1989. By May and June 1992, however, such differences were not apparent. Mean body mass of otters, adjusted for sex, age-class, and total length with analysis of covariance, differed between oiled and non-oiled areas from 1990 to 1992, but were nearly identical by May and June 1992. We propose that river otters may be recovering from chronic effects that we observed in 1990 and 1991 following the 1989 Exxon Valdez oil spill, but further research is necessary to test this hypothesis.

**Key words:** Haptoglobin, interleukin-6, oil spill, river otter, *Lutra canadensis*, body mass, hydrocarbons, Exxon Valdez, Prince William Sound, Alaska.

Extensive sections of shoreline in Prince William Sound, Alaska (USA) were contaminated by oil spilled from the tanker Exxon Valdez in late March 1989. River otters (*Lutra canadensis*) are sensitive to hydrocarbon pollution in the marine ecosystem (Duffy et al., 1993), in part because of their diet of subtidal and intertidal fishes and molluscs (Larsen, 1984). Indeed, damage from hydrocarbon accumulation in mussels and damaged populations of fish in oiled areas of Prince William Sound have been noted (S. C. Jewett, pers. comm.). Otters also may be exposed to crude oil from grooming their pelage (Duffy et al., 1993). We reported increases in blood haptoglobin (Hp) and lower body mass among river otters associated with oiled areas of Prince William Sound in 1990 (Duffy et al., 1993), and elevated Hp, and interleukin-6 immunoreactive protein (IL-6 ir) for otters living in oiled areas in 1991 (Duffy et al., 1994). We reasoned that

if the elevated levels of Hp and IL-6 ir and the reduction in body mass were evidence of chronic, oil-related effects on the health of river otters, differences in blood values and mass-length relations should decline as the oil available decreased. Moreover, otters in oiled areas were living in close proximity to mussel beds (*Mytilus edulis*) where oil was known to be trapped in substrates, which may provide a source of continued oil contamination. Our objective was to determine if physiological and morphological differences previously detected in river otters (Duffy et al., 1993, 1994) would occur among various sex and age classes from oiled and nonoiled areas, and to test whether such differences could be detected across broad areas of Prince William Sound.

In May to September 1991 and May to June 1992, river otters were captured with Hancock live traps (Hancock Trap Co., Buffalo Gap, South Dakota, USA), with closed dimensions of 95 cm by 59 cm by 40 cm, from areas throughout Prince William Sound (Fig. 1) that either received heavy oiling or were not oiled by the Exxon Valdez spill in March 1989. Site selection was based on Alaska Department of Environmental Conservation maps, using procedures described by Duffy et al. (1993). River otters were immobilized with ketamine hydrochloride (11 mg/kg estimated body mass; Sigma, St. Louis, Missouri, USA), transported to a ship, weighed, measured, and had a blood sample drawn from their jugular vein; otters were released near their site of capture as soon as they fully recovered. Sex and age-class (adult, juvenile, pup) of each animal was determined by using the procedures of

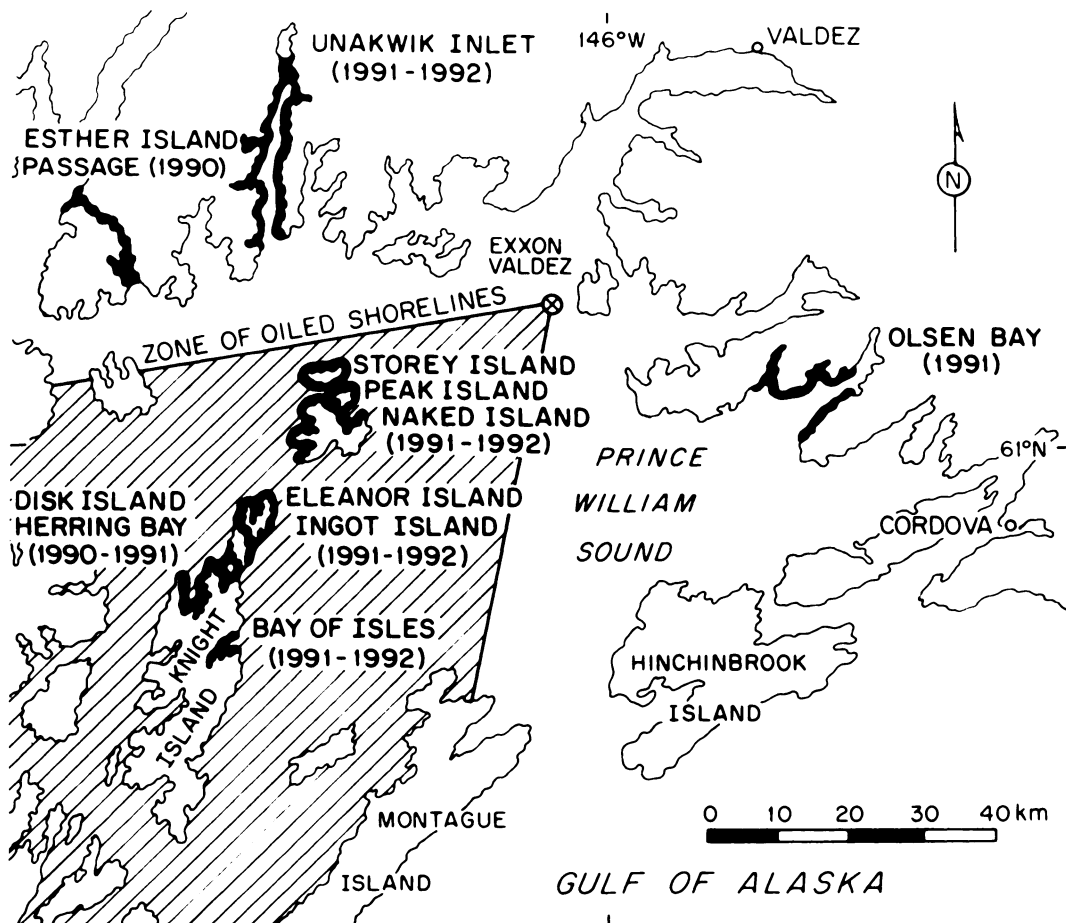


FIGURE 1. Prince William Sound, Alaska, showing oiled and nonoiled areas where we live-captured river otters (darkened areas) from 1990 to 1992.

Duffy et al. (1993). All methods used in this research were approved by an independent Animal Care and Use Committee at the University of Alaska Fairbanks, Fairbanks, Alaska.

Blood samples collected in the field were stored in vacutainers, and sera were separated later by low-speed centrifugation. Gel electrophoresis of Hp was performed by the methods of Duffy et al. (1993). IL-6 ir was measured using immunochemical assay (Quantakine Elisa, R & D, Inc., Minneapolis, Minnesota, USA) following the methods of Duffy et al. (1994).

We used analysis of covariance (ANCOVA), with body mass as the dependent variable, and oiling as a treatment effect, with age-class, sex, and total length as co-

variates (Dixon, 1985). This approach reduces error variability and allows pooling of samples to examine changes in adjusted body mass of otters (Neter et al., 1985); we met assumptions of homogeneous slopes.

When effects of sex, age-class, and total length (Table 1) were adjusted with a one-tailed ANCOVA, otters from oiled areas had significantly lower body mass than otters inhabiting nonoiled areas ( $F = 3.15$ ,  $P = 0.04$ ). Nonetheless an apparent trend of increasing body mass through time occurred for otters living in areas exposed to crude oil, whereas mass of otters on nonoiled areas declined from 1990 to 1991, and then stabilized from 1991 to 1992 (Fig. 2). Variability in body mass of otters inhabiting oiled areas also increased with time (Fig. 2). Because there were no pre-

TABLE 1. Body mass (kg) and total length (cm) of river otters from oiled and nonoiled areas of Prince William Sound, Alaska.

Otter sex and age class by area	1991			1992		
	Sample size	Body mass mean $\pm$ SD	Total length mean $\pm$ SD	Sample size	Body mass mean $\pm$ SD	Total length mean $\pm$ SD
<b>Oiled areas</b>						
Adult male	3	9.0 $\pm$ 0.5	119.7 $\pm$ 3.0	9	9.3 $\pm$ 0.8	119.7 $\pm$ 2.5
Pup male	1	4.5	92.2	0	—	—
Adult female	6	7.9 $\pm$ 0.6	113.9 $\pm$ 5.3	1	8.9	114.6
Pup female	2	3.8	87.2 $\pm$ 3.3	0	—	—
<b>Nonoiled areas</b>						
Adult male	2	9.4 $\pm$ 1.4	119.2 $\pm$ 3.8	1	10.0	122.0
Adult female	6	8.4 $\pm$ 0.9	117.3 $\pm$ 3.9	1	7.1	106.1
Yearling female	2	6.8	112.0 $\pm$ 2.8	0	—	—
Pup female	1	2.4	67.2	0	—	—

\* No yearling males were collected; no yearling females were collected in oiled areas and no male pups were collected in nonoiled areas.

spill data for otters in Prince William Sound, we are unsure whether weight gains in oiled otters to levels observed for nonoiled ones in 1991 and 1992 represent recovery. Body mass for oiled otters in 1992 still was below that observed for nonoiled otters in 1990 (Fig. 2). Likewise, caution should be used in suggesting complete recovery has occurred because of small sample sizes on nonoiled areas in 1992 (Fig. 2). Another interpretation is that otters living within the area of the spill and exhibiting lower body mass and elevated levels

of Hp and IL-6 ir may have succumbed to the effects of oil and no longer were available for us to capture. Indeed, Duffy et al. (1994) reported that river otters abandoned latrine sites about three times more often on oiled than nonoiled areas of Prince William Sound; thus the otter population on oiled areas may have declined. Kruuk et al. (1989) demonstrated a strong positive relationship between number of resident females and number of active holts (latrine sites) for European otters (*Lutra lutra*).

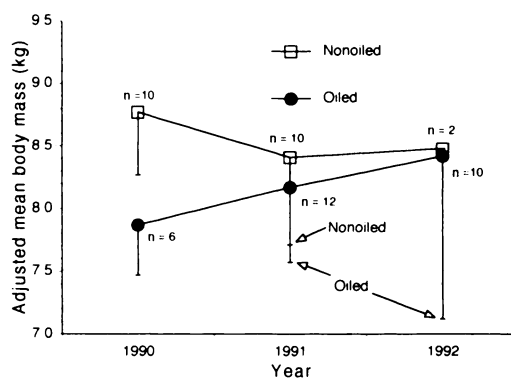


FIGURE 2. Yearly trends in mean body mass of river otters from oiled and nonoiled areas adjusted for sex, age-class (pup, juvenile, adult), and total length using a one-tailed analysis of covariance (ANCOVA), Prince William Sound, Alaska. Data for 1990 are from Duffy et al. (1993). Bars are SE.

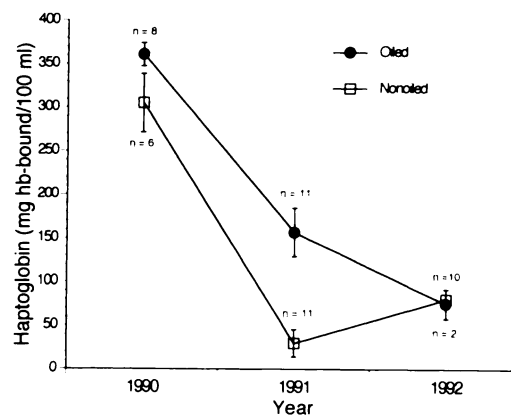


FIGURE 3. Yearly trends in blood haptoglobin for river otters from oiled and nonoiled areas of Prince William Sound, Alaska. Data for 1990 are from Duffy et al. (1993), and data for 1991 are from Duffy et al. (1994). Bars are SE.

In 1991, we were able to predict (>86%) whether otters inhabited oiled or nonoiled areas of Prince William Sound based on only three blood values: Hp, IL-6 ir, and aspartate aminotransferase (ASAT); sex and age-class failed to improve the fit of this logistic model (Duffy et al., 1994). Mean ( $\pm$ SE) levels of IL-6 ir in 1991 for otters were  $48.3 \pm 13.8$  pg/ml ( $n = 11$ ) on oiled areas and  $17.3 \pm 11.3$  pg/ml ( $n = 11$ ) on nonoiled sites (Duffy et al., 1994). In 1992, IL-6 ir was below detectable levels for otters inhabiting both oiled ( $n = 10$ ) and nonoiled ( $n = 2$ ) shorelines. Likewise, Hp values nearly were identical for otters inhabiting oiled and nonoiled areas in 1992 (Fig. 3).

Haptoglobin is an acute-phase response protein that is induced by IL-6 following tissue injury from hydrocarbons (Heinrich et al., 1990). In 1991, we noted increases in ASAT, which was positively correlated with blood levels of aspartate aminotransferase (ALAT) and creatine kinase (Duffy et al., 1994). Similarly, cell damage has caused elevated levels of ALAT in mink (*Mustela vison*) (Edqvist et al., 1992). We initially thought that high levels of haptoglobin in nonoiled otters that occurred in 1990 (Duffy et al., 1993) might have resulted from sampling otters during their mating season (Duffy et al., 1994); this seems unlikely because data from 1992 were collected at a similar time to those gathered in 1990. Another possibility is that fish contaminated by crude oil migrated the 40 km to our nonoiled study site, where they were eaten by otters. Sand lances (*Ammodytes hexapterus*), which are preyed upon by otters (Bowyer et al., 1994), are an example of such a migratory fish. We previously cautioned that interactions with environmental factors may cause variation in Hp values (Duffy et al., 1994). Whatever the cause of this variation for otters on nonoiled areas, there was an apparent reduction in both Hp (Fig. 3) and IL-6 ir values for otters from nonoiled sites in 1992.

River otters were present in areas with

mussel beds that still possessed substantial amounts of crude oil in 1992 (M. Babcock, pers. comm.). Although otters consume mussels, blood of otters in 1992 did not have elevated levels of Hp and IL-6 ir observed in previous years. This raises questions about the importance of mussel beds as a source of oil contamination for otters in 1990 and 1991 when haptoglobin and IL-6 ir values were significantly higher than on nonoiled areas (Duffy et al., 1993, 1994).

Based on our data, river otters had lower body mass (Fig. 2) and elevated levels of Hp and IL-6 ir two years after the Exxon Valdez oil spill and following a major effort to clean oil from Prince William Sound. Such chronic effects were unexpected, and we believe that research should focus on longer-term consequences of oil spills. The apparent recovery of otters observed in 1992 is encouraging, but more data are required to conclude that there are no lingering effects from the oil spill.

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