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BIOMARKER RESPONSES IN RIVER OTTERS EXPERIMENTALLY EXPOSED TO OIL CONTAMINATION

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ABSTRACT: Investigations in Prince William Sound (Alaska, USA) following the *Exxon Valdez* oil spill (*EVOS*) revealed that river otters (*Lontra canadensis*) on oiled shores had lower body mass and elevated values of biomarkers, than did otters living on nonoiled shores. In addition, otters from oiled areas selected different habitats, had larger home ranges, and less diverse diets than animals living in nonoiled areas. These differences between river otters from oiled shores and those from nonoiled areas strongly suggested that oil contamination had an effect on physiological and behavioral responses of otters. In this study, we explored the effects of crude oil contamination on river otters experimentally. We hypothesized that exposure to oil would result in elevated values of biomarkers, indicating induced physiological stress. Fifteen wild-caught male river otters were exposed to two levels of weathered crude oil (i.e., control, 5 ppm/day/kg body mass, and 50 ppm/day/kg body mass) under controlled conditions in captivity at the Alaska Sealife Center in Seward (Alaska, USA). Responses of captive river otters to oil ingestion provided mixed results in relation to our hypotheses. Although hemoglobin (Hb, and associated red blood cells) and white blood cells, and possibly interleukin-6 immunoreactive responded in the expected manner, other parameters did not. Aspartate aminotransferase, alanine aminotransferase, and haptoglobin (Hp), did not increase in response to oiling or decreased during rehabilitation. Conversely, principle-component analysis identified values of alkaline phosphatase as responding to oil ingestion in river otters. Our results suggested that opposing processes were concurring in the oiled otters. Elevated production of Hp in response to tissue damage by hydrocarbons likely occurred at the same time with increased removal of Hp-Hb complex from the serum, producing an undetermined pattern in the secretion of Hp. Thus, the use of individual biomarkers as indicators of exposure to pollutants may lead to erroneous conclusions because interactions in vivo can be complicated and act in opposite directions. Additionally, the biomarkers used in investigating effects of oiling on live animals usually are related to the heme molecule. Because of the opposing processes that may occur within an animal, data from a suite of heme-related biomarkers may produce results that are difficult to interpret. Therefore, we advocate the exploration and development of other biomarkers that will be independent from the heme cycle and provide additional information to the effect of oiling on live mammals.

Key words: Captivity, crude oil, hemoglobin, haptoglobin, hydrocarbons, liver enzymes, *Lontra canadensis,* oil spill, season.

INTRODUCTION

Investigations in Prince William Sound (PWS; Alaska, USA) following the *Exxon Valdez* oil spill (*EVOS*), revealed that coastal river otters (*Lontra canadensis*) on oiled shores had lower body mass and elevated levels of biomarkers than those living in nonoiled areas (Duffy et al., 1993, 1994a, b, 1996; Blajeski et al., 1996; Taylor et al., 2000; R. T. Bowyer et al., unpubl. data). In addition, otters from oiled areas selected different habitats, had larger home ranges, and less diverse diets than those living in nonoiled areas (Bowyer et al., 1994, 1995). These observed differences between river otters from oiled shores and those from nonoiled areas indicated that oil contamination had an effect on physiological and behavioral processes in river otters. Moreover, these effects had the potential to become chronic and may have impeded recovery of populations of river otter if the exposure to hydrocarbons continued. Between 8 to 16% of the 39,000 metric tons of crude oil spilled by the super tanker *Exxon Valdez* remains

buried in marine sediments (O'Clair et al., 1996). Moreover, microbial analyses indicated that oil in sediments along some shorelines was still several orders of magnitude more common than in nonoiled areas (Braddock et al., 1996). Oil buried in sediments, which may be re-suspended during storms and tidal action, is not subject to degradation by aerobic bacteria and therefore remains in a form that is toxic to many vertebrates (Braddock et al., 1996). Thus, oil may still be available for biological transport from benthic invertebrates through the food web.

Recent studies exploring effects of pollution on wild animals sought nonlethal methods to replace the traditional extraction of tissues from carcasses. Such traditional studies involve killing a sufficient number of animals to allow for proper statistical evaluations (for examples see Evens et al., 1998; Gutleb et al., 1998). This approach may lead to extirpation of subpopulations or cause genetic bottlenecks when populations are small (R. T. Bowyer et al., unpubl. data) and add to the damage already created by the pollution event. Thus, researchers embraced the use of biomarkers to assess effects of pollution, including exposure to and damage from hydrocarbons in living vertebrates (Miranda et al., 1987; Stegeman et al., 1992; Akins et al., 1993; Duffy et al., 1996; Zentano-Savin et al., 1997).

Several specific biomarkers as well as general blood panels have been used in studies related to hydrocarbon exposure (R. T. Bowyer et al., unpubl. data). Haptoglobin (Hp) and interleukin-6 immunoreactive (IL-6*ir*) were used to indicate increased liver activity. These acute-phase proteins are synthesized in response to trauma, toxicological damage, or infection (Duffy et al., 1993, 1994a, b; Prichard et al., 1997). Additionally, researchers assayed porphyrins extracted from fecal samples (Akins et al., 1993; Blajeski et al., 1996; Taylor et al., 2000). Chemically induced changes in patterns of porphyrins have been observed in several avian species following exposure to aromatic hydrocarbons (Miranda et al., 1987). Cytochrome P450-1A was added to the suite of biomarkers used for assessing exposure to hydrocarbons. Cytochrome P450s are a group of enzymes that metabolize a wide variety of xenobiotic compounds. P450-1A is induced by planar aromatic or chlorinated hydrocarbons, and thus its presence serves as a bioindicator of hydrocarbon exposure (Woodin et al., 1997).

Studies initiated following *EVOS* indicated that several vertebrate predators displayed physiological stress related to oil toxicity, in addition to data collected on river otters (Collier et al., 1996; Laur and Haldorson, 1996; Loughlin et al., 1996; Piatt and Ford, 1996; Ballachey and Kloecker, 1997; Woodin et al., 1997). Oiled sea otters (*Enhydra lutris*), collected for rehabilitation, suffered from emphysema, ulcers, anemia, lesions and organ congestion (Williams et al., 1995). Similarly, free-ranging sea otters from oiled regions had greater antigenic stimulation than animals from nonoiled areas, and young sampled in those regions had lower hemoglobin (Hb) levels than young from nonoiled areas (Rebar et al., 1994). Pigeon guillemots (*Cepphus columba*) had elevated levels of Hp and blood proteins in specific locations and years, although dosing experiments in the field failed to demonstrate the connection between oiling and those parameters (Prichard et al., 1997; Seiser et al., 2000). Changes in plasma proteins and white blood cells (leukocytes), reduction in the number of red blood cells (erythrocytes), and electrolyte imbalance have also been observed in mink (*Mustela vison*) and polar bears (*Ursus maritimus*) following exposure to hydrocarbons (Øristland et al., 1981; Mazet et al., 2000).

Although previously collected data strongly indicated a relation between oil contamination and physiological stress in river otters and other vertebrates in Prince William Sound, interpretation of that evidence will be aided by controlled experi-

ments. In this study, we investigated the effects of exposure to oil on physiological processes in captive river otters and hypothesized that exposure to oil would result in changes in levels of biomarkers, indicating physiological stress.

We adopted two approaches to data evaluation. First, we tested whether exposure to known doses of oil resulted in significant changes in levels of biomarkers for those markers that were previously used in the field studies on river otters (R. T. Bowyer et al., unpubl. data), or were reported for other species (Mohn and Nordstoga, 1975; Øritsland et al., 1981; Prichard et al., 1997; Zentano-Savin et al., 1997). Second, we used a data-exploration approach to detect other measures that may have responded to oil exposure in hopes to provide additional tools for future studies of biomarkers in vertebrates.

MATERIALS AND METHODS

Trapping and handling the experimental animals

River otters were live-captured in northwestern PWS (60°40′N 147°50′W) from late April to late May 1998, with No. 11 Sleepy Creek® leg-hold traps (Blundell et al., 1999). Traps were placed on trails at latrine sites and monitored by trap transmitters (Telonics, Mesa, Arizona, USA) that signal when a trap is sprung (Blundell et al., 1999; R. T. Bowyer et al., unpubl. data). Processing of otters began within 1 to 2 hr from capture. Otters were anesthetized with Telazol (9 mg/kg; A. H. Robins, Richmond, Virginia, USA) administered using Telinject® darts (Telinject, Saugus, California, USA) and a blowgun. Data on morphometrics, collected from anesthetized otters, included body mass (nearest 0.1 kg); body length, tail length, and total length (nearest 1 mm); total skull length and width of zygomatic arch (nearest 1 mm). Age of otters (juvenile, young adult, adult, and old adult) was estimated based on body size, tooth wear and staining. Blood and tissues were sampled from each individual otter at that time. Of the 51 individuals captured (R. T. Bowyer et al., unpubl. data), 15 young adult males (estimated age 1–5 yr) were selected for the controlled experiments and transferred under sedation via air to the Alaska Sealife Center (ASLC) in Seward (Alaska, USA).

Wild-caught river otters were held in captivity at ASLC from May 1998 to March 1999. Animals were housed as one large group in an area of 90 m^2 surrounding six pools (one large salt-water pool of 4.5 m diameter by 3 m depth; four small salt-water pools of 2 by 1.5 by 1.5 m; and one small fresh-water tote of 1 by 1 by 1 m). Fences were covered with clear acrylic panels to prevent the otters from climbing, and an electric fence was mounted at a height of 1.6 m to further deter the otters from escaping the pen. The gate to the enclosure was locked at all times, and only trained personnel were allowed access to the pen. Thirteen plywood sleeping boxes (1.3 × 0.6 × 0.6 m) were equipped with polyester fleece blankets and stationed throughout the enclosure. Otters were fed twice daily ad libitum on a diet of frozen fish of the following species: pink salmon (*Oncorhynchus gorbuscha*), capelin (*Mallotus villosus*), Pacific herring (*Clupea pallasii*), walleye pollock (*Theragra chalcogramma*), prawns (*Pandalus platyceros*), and eulachon (*Thaleichthys pacificus*). These fishes were purchased from commercial harvest and approved for human consumption. Two to three times per week, the diet was supplemented with live prey (pink salmon, kelp greenling (*Hexagrammos decagrammus*), and rockfish (*Sebastes* sp.)) captured in Resurrection Bay (Alaska; ADFG permit CF 98-024; Ben-David et al., 2000). Initially, vitamins (Hi Vite™, EVSCO Pharmaceuticals, IGI, Inc., Buena, New Jersey, USA) were provided with food, but because otters seemed reluctant to consume those fishes, we injected B complex vitamins (0.5 ml Phoenix Scientific, St. Louis, Missouri, USA) intramuscularly during the blood-sampling sessions every 3 weeks. Minerals were provided as a mineral block and otters had continuous access to that resource.

Administration of crude oil

Experiments began in August after allowing the animals 2.5 mo to acclimate to the enclosure, feeding regimes, and handling procedures. During that time, the average daily food intake of otters was monitored to quantify the amount of oil required for achieving each oiling level. Daily food intake per animal averaged 1,000 g/day over the acclimation period. At the end of that period, otters were randomly assigned to three experimental groups of five individuals each: a control group that received no oil; a low-dose group that received 5 ppm of oil/day/kg body mass (i.e., 0.1 g every other day or 100 ppm/kg of food every other day); and a high-dose group that received 50 ppm of oil/day/kg body mass (i.e., 1.0 g every other day or 1,000 ppm/kg of food every other day). Individuals were assigned to treatment groups with a randomized complete-block design. Original assignment of otter identification numbers

were based on body mass at capture to control for potential differences in age and size. For the complete-block design otter identification numbers were randomly permutated within blocks (performed by T. L. McDonald, West Ecosystems Technology Inc. Cheyenne, Wyoming, USA).

Prudhoe Bay crude oil (obtained from Williams Petroleum Inc. Fairbanks, Alaska, USA) was mixed in seawater and stirred continuously for 10 days at 25 C. Two batches of oil were weathered separately and a sample from each batch was sent for analysis at Auke Bay Laboratory (J. Short, National Oceanographic and Atmospheric Administration, Juneau, Alaska). The two batches differed slightly in composition, but both were comparable to the oil profile of *EVOS* shortly after landfall in 1989 (Table 1; Short and Heintz, 1997). Weathered oil was separated from water and administered to otters in gel capsules (500 mg) hidden in fishes every other day. Quantity of oil was measured with a micropipette (Rainin Instruments Co., Emeryville, California, USA) and weighed on an analytical microbalance (nearest 0.01 g; Mettler Toledo, Columbus, Ohio, USA). Occasionally the otters bit into the capsule when feeding and subsequently dropped the fish. On such occasions, oil was administered again during the following feeding to ensure that each otter ingested the amount of oil required for the experiment. Careful notes on feeding of oil were kept throughout the experiment. Feeding of oil lasted 100 days from 21 August to 28 November 1998. The first batch of oil was fed to the otters between August 21 and October 19, as well as between November 19 and November 28. The second batch was fed to the otters between October 20 and November 18 (Table 1). Data collection continued for additional 100 days of rehabilitation. Animals were then fitted with radiotransmitters and released at the site of their capture in PWS. Animals are currently being monitored to determine post-release survival.

Sampling of blood

Prior to exposure to oil, a series of blood and tissue samples were collected (29–30 June and 15–16 August 1998) from each individual otter for analysis of biomarkers (Table 2). Blood and tissue sampling continued from 15 August 1998 until 12 January 1999 every three weeks. An additional sampling session occurred on 22–24 February 1999 in conjunction with implanting of radiotransmitters.

Otters were anesthetized with a combination of ketamine hydrochloride (100 mg/ml, Ketaset®, Aveco Co., Fort Dodge, Iowa, USA) at a dosage of 10 mg/kg, and midazolam hydrochloride (5 mg/ml, Versed®, Hoffman-LaRoche, Nutley, New Jersey, USA) at a dosage of 0.25 mg/kg (Spelman et al., 1993). The dosage was mixed in the same syringe and administered intramuscularly with Telinject® darts and a blowgun or hand injected while the otter was immobilized in a squeeze-box. Before darting the otter, each individual received one fish containing 0.5 cc Versed®. This dosage calmed the animals and reduced the stress associated with handling.

We drew blood from the jugular vein of each otter with care to keep samples sterile. A portion of the sample was preserved with EDTA (purple top Vacutainer®; Becton Dickinson Labware, Franklin Lakes, New Jersey, USA) for complete blood counts (CBC) and refrigerated until analysis (within 48 hr; Table 2). The remaining blood (approximately 10 ml) was collected in a red top Vacutainer® and allowed to clot; serum was removed (within 8 hr) following centrifugation at 3,000 rpm for 10 min, and refrigerated until analyses of serum chemistry (Table 2). Samples for haptoglobin (Hp) and IL-6*ir* analyses were frozen at -20 C. In addition, four blood smears were made for each river otter at the time the blood was drawn and samples were investigated later for cell abnormalities (Duncan et al., 1994).

Biomarker analysis

Serum-chemistry profiles were assayed with an Olympus 7000 analyzer (Olympus, Melville, New York, USA) and complete blood counts were performed with a Stack-S whole blood analyzer (Coulter, Miami, Florida, USA). Samples were analyzed at Quest Diagnostics Incorporated (Portland, Oregon, USA).

Haptoglobins (Hp) are alpha 2 glycoproteins that stoichiometrically bind free hemoglobin (Hb) in a haptoglobin-hemoglobin (Hb-Hp) complex. Using the standard protocol described by Duffy et al. (1993, 1994a, b) the Hp-Hb complex, was quantified by densitometry and results were expressed as mg Hb-bound/100 ml serum.

Values of interleukin-6 immunoreactive (IL-6*ir*) were determined with an immunochemical assay. Replicates of each sample were added to a microtiter plate coated with a monoclonal antibody for IL-6 (Quantikine, R&D systems, Minneapolis, Minnesota, USA). After washing away any unbound proteins, an enzyme-linked polyclonal antibody for IL-6 was added to the wells and incubated to allow for binding. After a final wash, a substrate solution was added to the wells. Following color development, sample concentrations were determined from a standard curve (Duffy et al., 1994a, b).

Statistical analyses

Of 165 samples collected through the experiment, 6 samples clotted thus providing no data

TABLE 1. Concentrations of hydrocarbons in the two batches of weathered Prudhoe Bay Crude oil fed to river otters at the Alaska Sealife Center in Seward Alaska from 21 August to 28 November 1998. The first batch of oil was fed to the otters between August 21 and October 19, as well as between November 19 and November 28. The second batch was fed to the otters between October 20 and November 18.

Compound	Analyte concentrations (μg/g)	
	Batch 1	Batch 2
Naphthalene	283.14	60.16
2-methylnaphthalene	904.43	616.81
1-methylnaphthalene	753.80	531.44
2,6-dimethylnaphthalene	641.37	577.26
C-2 naphthalenes	2,601.33	2,359.91
2,3,5-trimethylnaphthalene	268.37	278.51
C-3 naphthalenes	2,284.48	2,305.84
C-4 naphthalenes	609.96	593.84
Biphenyl	155.55	120.85
Acenaphthylene	0.00	0.00
Acenaphthene	13.42	12.43
Fluorene	97.31	100.48
C-1 fluorenes	228.37	247.95
C-2 fluorenes	273.45	309.82
C-3 fluorenes	169.23	183.75
Dibenzothiophene	177.90	188.40
C-1 dibenzothiophenes	359.69	378.64
C-2 dibenzothiophenes	473.14	511.84
C-3 dibenzothiophenes	371.25	387.41
Phenanthrene	244.29	263.38
1-methylphenanthrene	182.57	196.85
C-1 phenanthrenes/anthracenes	759.29	820.86
C-2 phenanthrenes/anthracenes	874.31	940.14
C-3 phenanthrenes/anthracenes	485.21	540.15
C-4 phenanthrenes/anthracenes	80.23	85.26
Anthracene	7.72	3.41
Fluoranthene	4.34	4.46
Pyrene	11.70	12.34
C-1 fluoranthenes/pyrenes	66.05	644.75
Benz-a-anthracene	6.64	4.28
Chrysene	44.48	45.27
C-1 chrysenes	66.13	67.56
C-2 chrysenes	66.78	71.22
C-3 chrysenes	15.75	33.08
C-4 chrysenes	2.76	3.25
Benzo-b-fluoranthene	14.73	15.66
Benzo-k-fluoranthene	0.00	0.00
Benzo-e-pyrene	9.89	11.76
Benzo-a-pyrene	2.90	2.21
Perylene	1.03	11.09
Indeno-123-cd-pyrene	1.03	0.58
Dibenzo-a,h-anthracene	1.31	1.07
Benzo-g,h,i-perylene	2.50	2.97

for CBC. Missing values for those samples were replaced by a means of near points approach (Johnson and Wichern, 1992). To determine effects of oiling on values of biomarkers (dependent variable) in river otters, we used repeated measures ANOVA with oiling group (i.e., control, low dose, and high dose) and bleeding session as factors (Johnson and Wichern, 1992; GLM procedure, SPSS 7.0 for Windows, SPSS Inc., Chicago, Illinois, USA). The first bleeding session, that occurring at capture, and the 2 following sessions (June and August) provided information on the acclimation period. The oiling period included five sessions (3 wk interval;

TABLE 2. List of blood variables, abbreviations, units of measurement, and values for 15 young adult male river otters live captured in PWS and held in captivity at the Alaska Sealife Center in Seward (Alaska, USA), from May 1998 to March 1999. For comparison, published values for these variables in river otters live-trapped in nonoiled areas in PWS 1998 are presented (adapted from R. T. Bowyer et al., unpubl. data), as well as values for zoo animals (adapted from Reed-Smith, 1995).

		Values for river otters in this study			Values for river otters from nonoiled areas in PWS			Values for zoo otters (from literature)		
Variable name	Abbreviation	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE
Alanine Aminotransferase (U/l)	ALT	15	—[a]		24	418.92	74.21	31	91.1	6.3
Albumin (g/dl)	ALB	15	—[a]		24	3.09	0.05	37	3.0	0.05
Alkaline Phosphatase (U/l)	ALK PHOS	15	—[a]		24	139.79	9.21	40	95.7	6.15
Aspartate Aminotransferase (U/l)	AST	15	—[a]		24	176.88	13.68	39	89.9	6.5
Blood Urea Nitrogen (mg/dl)	BUN	15	—[a]		24	43.50	3.26	40	27.6	1.2
Calcium (mg/dl)	Ca	15	8.86	0.02	24	8.86	0.08	40	9.1	0.1
Chloride (mEq/l)	Cl	15	110.13	5.03	24	112.25	0.76	36	113	4.0
Cholesterol (mg/dl)	CHOL	15	276.33	0.2	24	166.04	10.91	35	254	12.0
Cholesterol/High Density Lipid Ratio	CHOL/HDL	15	2.43	0.04	24	1.83	0.07			
Direct Bilirubin (mg/dl)	Dir Bili	15	0.03	0.05	24	0.08	0.01			
Gamma Glutamyl Transpeptidase (U/l)	GGT	15	17.33	0.74	24	27.79	5.55			
Globulin (g/dl)	GLOB	15	—[a]		24	3.83	0.10	23	3.2[b]	0.1
Glucose (mg/dl)	GLU	15	—[a]		24	136.79	10.14	40	88.2	3.5
Haptoglobin mg hb-bound/100 ml	Hp	15	—[a]		24	22.38	8.28			
Hemoglobin (g/dl)	Hb	15	—[a]		21	15.72	0.24	51	14.2	0.3
High Density Lipids mg/dl	HDL	15	120.18	6.33	24	89.13	3.78			
Interleukin-6 immunoreactive (pg/ml)	IL-6*ir*	15	—[a]		24	0.75	0.31			
Lactate Dehydrogenase (U/l)	LDH	15	—[a]		24	221.33	27.30	34	324	76.9
Low Density Lipids (mg/dl)	LDL	15	146.85	4.04	24	65.92	6.74			

TABLE 2. Continued.

Variable name	Abbreviation	Values for river otters in this study			Values for river otters from nonoiled areas in PWS			Values for zoo otters (from literature)		
		n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE
Phosphorus (mg/dl)	P	15	5.21	0.06	24	4.62	0.27	41	5.9	0.3
Platelet Count (Th/cmm)	PLAT	15	—[a]		21	357.24	11.06			
Potassium (mEq/l)	K	15	3.97	0.02	24	3.95	0.07	39	4.3	0.06
Red Blood Cell Count m/cmm (million)	RBC	15	—[a]		21	9.14	0.15	26	10.2	0.5
Serum Creatinine (mg/dl)	SCREAT	15	0.3	0.01	24	0.26	0.01	39	0.6	0.02
Sodium (mEq/l)	Na	15	148.93	0.15	24	149.50	0.63	39	150	0.7
Total Bilirubin (mg/dl)	T. Bili	15	0.22	0.02	24	0.33	0.01	40	0.2	0.02
Total Protein (g/dl)	TP	15	6.75	0.02	24	6.92	0.09	40	6.5	0.1
Triglycerides (mg/dl)	TRIG	15	—[a]		24	55.29	10.50			
Uric Acid (mg/dl)	UA	15	1.55	0.06	24	1.83	0.20	33	2.1	0.4
Very Low Density Lipids (mg/dl)	VLDL	15	15.53	0.75	24	11.00	2.00			
White Blood Cells Th/cmm (thousand)	WBC	15	—[a]		21	10.33	1.11	55	8.8	0.4

[a] Differences due to oil ingestion or conditions of captivity (see Figs. 1–4).
[b] Gamma globulins only.

depicted as Sept I to Nov II), and rehabilitation period was represented by three sessions (Dec–Feb II). We opted to present data in this manner to better illustrate response curves for the different biomarkers. This approach, however, is conservative as significant differences may be obscured by the slow changes over time in the values of biomarkers.

Analysis was conducted separately, as hypothesis tests, for each of the following variables: Aspartate aminotransferase and alanine aminotransferase (AST and ALT respectively; Duffy et al., 1994a) hemoglobin (Øritsland et al., 1981; Rebar et al., 1994; Williams et al., 1995), haptoglobin (Duffy et al., 1993, 1994a, b; Prichard et al., 1997), IL-6*ir* (Duffy et al., 1994a), white cell count (Øritsland et al., 1981; Rebar et al., 1994). Analysis was followed by Tukey's multiple comparisons to establish where significant differences occurred for those models in which either group or session effects were significant. α level was set at 0.05.

For our data-exploration approach, we used principal components analysis (PCA, SPSS for Windows) to reduce the dimensionality of data while considering effects of all variables simultaneously for each bleeding session (Johnson and Wichern 1992; R. T. Bowyer et al., unpubl. data), because the number of blood variables that were analyzed was greater than the number of individual otters in this experiment. Under such conditions employing a series of univariate tests on the individual blood values would have been incorrect. We developed the PCA from a correlation matrix rather than the variance-covariance matrix to avoid the bias in results when original data vary markedly in scale (a common occurrence in blood values). A well-known difficulty with PCA is in determining the meaning of a particular axis (Johnson and Wichern, 1992). Therefore, we followed the PCA with repeated measures ANOVA with oiling group (i.e., control, low dose, and high dose) and bleed session as factors, for those dependent variables that had the highest contribution to the variability in the data in at least five of the 11 bleeding sessions. Analysis was followed by Tukey's multiple comparisons to establish where significant differences occurred for those significant models in which either group or session effects were significant (Johnson and Wichern, 1992; GLM procedure, SPSS for Windows). We also used correlation analysis (Peasrson's ρ; Zar, 1984, SPSS for Windows) to determine the relation between each two variables that were identified as important with PCA. A significant relation at the $\alpha = 0.05$ level was reported following a sequential Bonferroni correction when multiple comparisons were conducted (Rice, 1989).

RESULTS

Hypothesis tests

Values of AST significantly declined between capture and all other bleeding sessions (Fig. 1a; repeated measures 2-way ANOVA; $P < 0.001$) for all 15 river otters. Although session effects were highly significant ($P < 0.001$), group had no effect on AST values ($P = 0.124$) suggesting that oil ingestion did not influence values of this variable. This observation is further supported by lack of increases in AST values in either the low-dose or the high-dose groups during the oiling period (21 August to 28 November; Fig. 1a). Values of AST for all otters were well below the values observed in the same individuals at capture (Fig. 1a). On 9 November (Nov I session; Fig. 1a) mean values of AST were elevated in the high-dose group although that increase was not statistically significant (Tukey's multiple comparisons; $P > 0.05$). These elevated values of AST resulted from an increase in one individual (908 U/L; EP06) that suffered from a toe with gangrene (which was amputated immediately by a veterinarian). Data from that individual were excluded from subsequent analyses.

Values of ALT did not change significantly for any of the groups throughout the experiment (Fig. 1b; repeated measures 2-way ANOVA; $P = 0.271$). For that analysis we excluded the one individual that suffered from a toe with gangrene and that had exceedingly high values of this enzyme throughout the experimental period (up to 2,523 U/L; EP06). Group association dictated the values of ALT ($P = 0.012$), with the high-dose animals having higher values than the low-dose or control animals (Fig. 1b) during the oiling period (Tukey's multiple comparisons; $P < 0.05$); no difference was detected between the low-dose and the control groups ($P > 0.7$). In addition, within each group, no significant difference in values of ALT was detected between the different sessions ($P > 0.14$), suggesting that oil ingestion did not

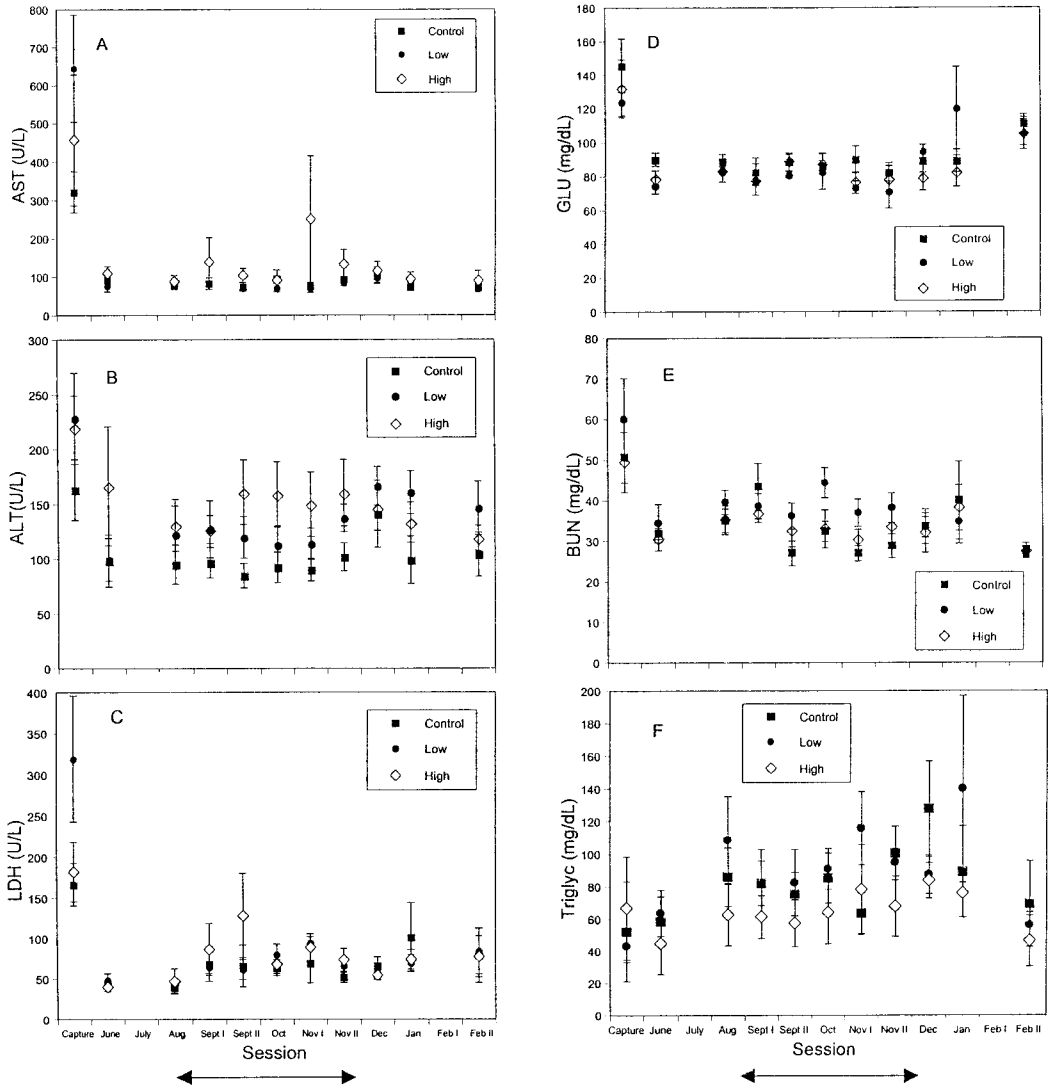

FIGURE 1. Values (mean ± SE) of AST (a), ALT (b), LDH (c), glucose (d) BUN (e) and triglycerides (f), for control, low-dose and high-dose groups (n = 15; 5 in each group) of river otters held in captivity at the Alaska Sealife Center in Seward, Alaska, USA. The period of oil administration occurred between 21 August and 28 November 1998 as indicated by arrow. For description of abbreviations and differences see text. This group of variables exhibited a response to captivity.

influence values of this variable. Values of AST and ALT were correlated for the entire data set (r = 0.52; $P < 0.001$; n = 165).

Values of haptoglobin (Hp) significantly changed throughout the experimental period (Fig. 2a; repeated measures 2-way ANOVA; P = 0.002). Although we were unable to detect a group effect (P = 0.48), session seemed to influence the values of Hp in our otters ($P < 0.001$). Values significantly increased between capture and

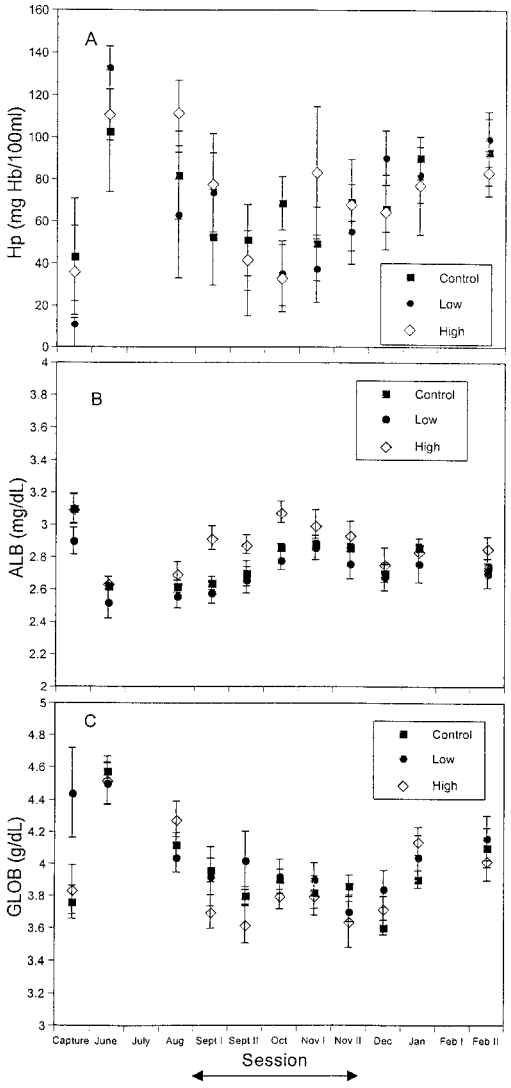

FIGURE 2. Values (mean ± SE) of haptoglobin (Hp, a), albumin (b), and globulins (c), for control, low-dose, and high-dose groups (n = 15; 5 in each group) of river otters held in captivity at the Alaska Sealife Center in Seward (Alaska, USA). The period of oil administration occurred between 21 August and 28 November 1998 as indicated by arrow. For description of differences see text. This group of variables exhibited a response to seasonal factors.

the first bleeding session at the end of June for all otters (Tukey's multiple comparisons; $P < 0.05$). Values then declined slowly for all otters until early November, after which values began to increase again (Fig. 2a). Values of Hp for both low-dose and high-dose animals were at the lowest values at the height of the oiling period (September to November), and in one session (October) significantly lower than those of the control group (Tukey's multiple comparisons; $P < 0.05$; Fig. 2a).

Values of IL-6*ir* did not change significantly throughout the experimental period (Fig. 3a; repeated measures 2-way ANOVA; $P = 0.32$). No group or session effects were detected ($P = 0.12$ and $P = 0.74$, respectively). In addition, no pattern related to the oiling period could be deciphered (Fig. 3a).

We observed a significant reduction in values of hemoglobin (Hb) from oiling in our experimental otters (Fig. 4a; repeated measures 2-way ANOVA; $P < 0.01$; group effect $P = 0.001$; session effect $P < 0.001$), and values of Hb were highly correlated with values of red blood-cell counts for the entire data set (RBC; $r = 0.875$; $P < 0.001$). Hb values decreased in a similar fashion for all animals over the 3 months from capture to sampling in August. No significant differences ($P > 0.8$) were detected between groups for that period (Fig. 4a). During the oiling period, values of Hb stabilized for the control group, while values continued to significantly decline for the oiled animals ($P < 0.05$). No significant differences ($P > 0.2$) were detected between the low and high dose during that period (Fig. 4a) and the lowest values of Hb were observed in the oiled otters in October. After administration of oil ended (28 November 1998), Hb values increased and no significant differences were detected between oiled and control animals in the December sampling (Tukey's multiple comparisons; $P > 0.05$). Similarly, no differences in Hb values were detected between the high dose and the control groups in January and February (Tukey's multiple comparisons; $P > 0.05$), but the low-dose group experienced a significant decline in Hb values during that time (Fig. 4a; Tukey's multiple compari-

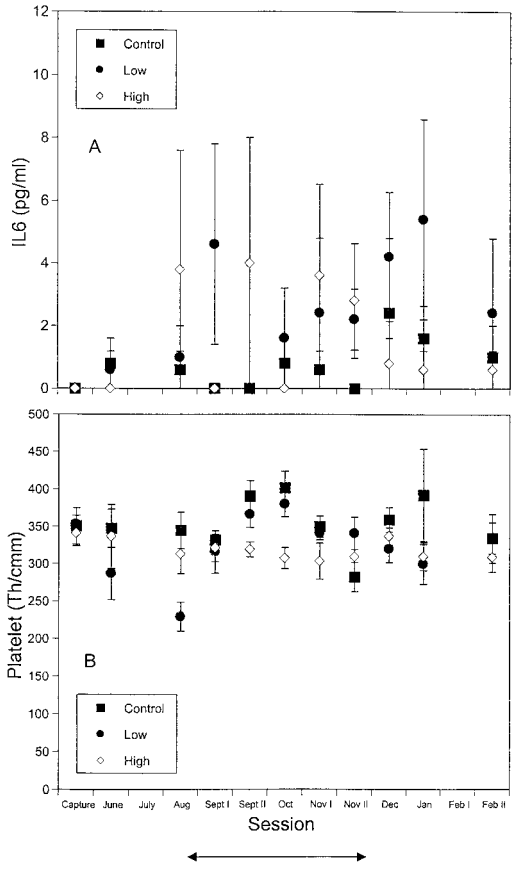

FIGURE 3. Values (mean ± SE) of IL-6*ir* (a), and platelet counts (b) for control, low-dose and high-dose groups (n = 15; 5 in each group) of river otters held in captivity at the Alaska Sealife Center in Seward (Alaska, USA). The period of oil administration occurred between 21 August and 28 November 1998 as indicated by arrow. For description of differences see text. Both variables were identified as important in explaining the high variability in the data by PCA analysis. While IL-6*ir* exhibited a potential response to oil ingestion, no relation to oil ingestion, season or captivity could be determined for platelet counts.

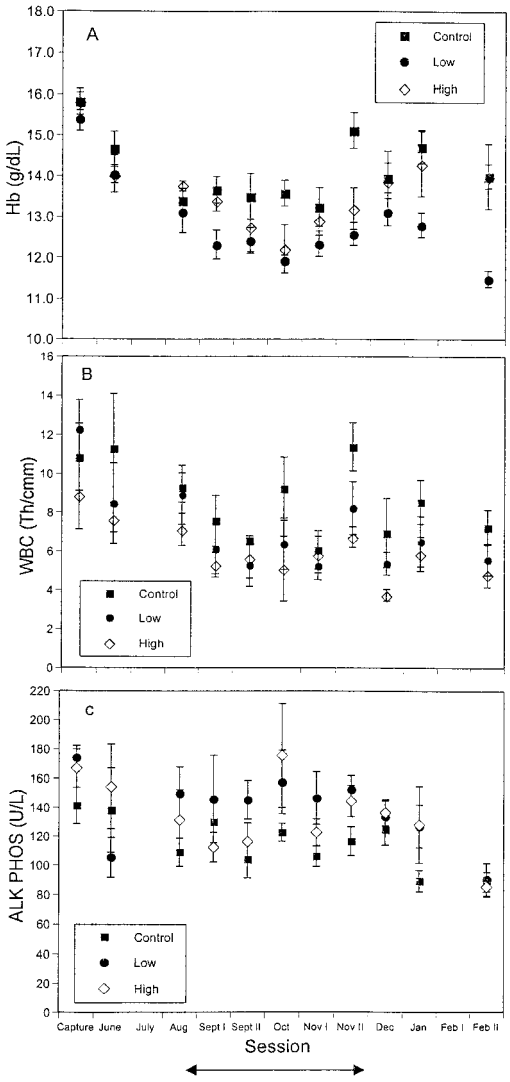

FIGURE 4. Values (mean ± SE) of hemoglobin (Hb, a), white blood cells (WBC, b), and alkalaine phosphatase (ALK PHOS, c) for control, low-dose and high-dose groups (n = 15; 5 in each group) of river otters held in captivity at the Alaska Sealife Center in Seward (Alaska, USA). The period of oil administration occurred between 21 August and 28 November 1998 as indicated by arrow. For description of differences see text. This group of variables exhibited a response to oil ingestion.

sons; $P < 0.05$). We were able to determine that this decline was caused by an iron deficiency (62 ± 17 μg/L; mean ± SE; reported normal values 127.9 ± 40.5 μg/L; Davis et al., 1992). We reversed the deficiency with weekly iron injections before the animals were released. Scanning of blood smears for cell abnormalities revealed nucleated erythrocytes in the ane-

mic animals but no Heinz bodies were apparent.

Hb and Hp were slightly negatively correlated ($r = -0.18$; $P = 0.02$) as were Hb and IL-6*ir* ($r = -0.22$; $P = 0.005$). In addition, AST was positively correlated with Hb ($r = 0.4$; $P < 0.001$). A repeated measures ANOVA model with Hp as the dependent variable, cumulative dose of oil and session as the factors, and Hb as a covariate ($P = 0.007$) indicated that values of Hb had a significant influence on values of Hp ($P = 0.013$).

Individual variation in reduction in Hb was high among the oiled animals. Although several individuals lost only 15–20% of their Hb during the experiment, others lost up to 32%. This is further supported by the large influence of animals ID on the result of the ANOVA model ($P < 0.001$). We were unable to correlate the reduction in Hb with the cumulative amount of oil ingested by each animal ($r = 0.08$; $P = 0.26$).

White blood-cell count (WBC) differed significantly between groups and sessions (Fig. 4b; repeated measures 2-way ANOVA; $P < 0.001$; group effect $P < 0.001$; session effect $P < 0.001$). Large variations in WBC occurred throughout the experiment for all groups but a trend of continuous decline was more evident for the oiled groups (Fig. 4b; Tukey's multiple comparisons; $P < 0.05$). The pattern of recovery we observed in Hb at the end of the oiling period was not evident in WBC (Fig. 4b; Tukey's multiple comparisons; $P > 0.05$). Nonetheless, WBC exhibited a weak but significant correlation with Hb ($r = 0.185$, $P = 0.017$) and a weak negative correlation with IL-6*ir* ($r = -0.177$, $P = 0.023$).

Data exploration

The first three principle components in the PCA analyses identified eight variables as explaining most of the variation in these data and accounted for 51 to 81% of the variation in each session. Those variables included albumin (ALB; Fig. 2b), alkaline phosphatase (ALK PHOS; Fig. 4c), ALT (Fig. 1b), globulin (GLOB; Fig. 2c), Hb (Fig. 4a), lactate dehydrogenase (LDH; Fig. 1c), platelet counts (PLAT; Fig. 3b), and triglycerides (TRIG; Fig. 1f). All those variables, excluding LDH, differed significantly between groups and sessions.

Values of ALK PHOS differed significantly between groups and sessions (repeated measures 2-way ANOVA; $P = 0.001$; group effects $P = 0.013$, session effect $P < 0.001$). Values of ALK PHOS significantly declined for the control group through the experiment (Tukey's multiple comparisons; $P < 0.05$), but did not for the low and high dose animals (Tukey's multiple comparisons; $P > 0.05$). Significant differences between controls and treatment animals were only evident during October to November II sessions (Fig. 4c), as well as the January session. That pattern may indicate effects of oiling although the process is difficult to interpret. ALK PHOS was not correlated with either Hb or ALT ($r = 0.115$ and $r = 0.142$ respectively), and only slightly correlated with WBC ($r = 0.152$, $P < 0.05$). In contrast, ALK PHOS was negatively correlated with Hp ($r = -0.21$, $P < 0.05$) and positively with ALB and AST ($r = 0.191$ and $r = 0.27$ respectively, $P < 0.05$).

ALB (Fig. 2b) and GLOB (Fig. 2c) exhibited opposite patterns to each other, although both had a significant group and session effects (ALB: repeated measures 2-way ANOVA; $P < 0.001$; group effects $P < 0.001$, session effect $P < 0.001$; GLOB: repeated measures 2-way ANOVA; $P < 0.001$; group effects $P = 0.001$, session effect $P < 0.001$). Although a significant group effect occurred for both variables, no relation to oiling could be detected because the control group had values in between those of the high-dose and the low-dose animals (Fig. 2b and Fig. 2c). Both ALB and GLOB were significantly correlated with Hp (ALB: $r = -0.36$, $P < 0.001$; GLOB: $r = 0.29$, $P < 0.001$), but only ALB was correlated with Hb ($r = 0.36$, $P < 0.001$). ALB and GLOB were

also negatively correlated ($r = -0.4$, $P < 0.001$).

LDH significantly decreased from the capture session, but no difference was detected between the treatment groups (Fig. 1c; repeated measures 2-way ANOVA; $P < 0.001$; group effects $P = 0.08$, session effect $P < 0.001$). The pattern in values of LDH was similar to that of AST (Fig. 1a), and indeed those two variables were significantly correlated ($r = 0.73$, $P < 0.001$). In addition, a similar pattern was observed in values of glucose and BUN (Fig. 1d and 1e), which were positively correlated with LDH ($r = 0.263$ and $r = 0.39$ respectively, $P < 0.05$).

Platelet counts significantly differed between groups and sessions (Fig. 3b; repeated measures 2-way ANOVA; $P = 0.002$; group effects $P = 0.009$, session effect $P = 0.038$), but no pattern could be discerned from these data (Fig. 3b). Similarly, although identified by the PCA as explaining part of high variability in these data, triglycerides exhibited a trend of increase in the captive otters (Fig. 1f; repeated measures 2-way ANOVA; $P = 0.113$; group effects $P = 0.002$, session effect $P = 0.05$), but no pattern related to oiling could be deciphered. Otter ID had significant effect on values of TRIG ($P = 0.009$). None of the other variables measured in this study exhibited any noticeable patterns related to oil ingestion, captivity, or season (Table 2).

DISCUSSION

General

Responses of the captive river otters to oil ingestion provided mixed results in relation to our hypotheses. Although Hb (and associated RBC), WBC and possibly IL-6*ir* responded in the expected manner, other parameters did not. AST, ALT, and Hp did not increase in response to oiling or decreased during rehabilitation. In addition, of those variables identified in the PCA, only ALK PHOS responded to oil ingestion.

Responses to captivity

Although AST and ALT were strongly correlated with each other, they exhibited different patterns in relation to group association and bleeding session. That outcome probably occurred because values of ALT did not decrease significantly between capture and captivity. Values of AST during the acclimation, oiling, and rehabilitation periods were significantly lower than those recorded at capture, and comparable to values recorded for other captive otters (Table 2). Thus, it seems that the responses of AST to conditions in captivity may have obscured any possible responses associated with ingestion of oil.

The increase in Hp between captures and the first bleeding session (June) may be related to stress associated with captivity. Although other studies documented similar increases associated with physical, environmental and psychological stress (Aikawa et al., 1990; Kalmovarin et al., 1991; Boosalis et al., 1992; Zentano-Savin et al., 1997), we are unable to determine whether the increases we observed were related to such factors. Nonetheless, the decrease in levels of Hp by the end of the acclimation period (August) without a change in either diet, or levels of activity may support the possibility of psychological stress. In this experiment we introduced 15 adult male river otters, captured at four geographical locations in PWS, to a relatively small enclosure. These animals probably have never been in close contact with humans before and also had to adjust to new members of their own species.

A group of variables exhibited a similar pattern of a significant decrease between capture and the June sampling session, followed by stable values throughout the experiment. Values of AST, GLU, BUN, and LDH during the experiment were below the mean value recorded for 24 animals live-captured in nonoiled areas in 1998 (Table 2; R. T. Bowyer; Unpubl. data), but in agreement with those reported for other captive river otters (Table 2; Reed-Smith,

1995), suggesting that these variables are associated with trapping stress. Several studies identified these parameters as indicators of capture stress in a variety of wild animals (Seal and Hoskinson, 1978; Williams et al., 1992; Boonstra et al., 1998; Keech et al., 1998; Hartup et al., 1999). Unfortunately, this result may seem to contradict our interpretation of the initial increase in Hp in captivity. Values of Hp in Alaskan pinnipeds, however, were similar between anesthetized and non-anesthetized animals, and no increases were documented with increases in handling time suggesting that elevation in Hp is not directly related to capture (Zentano-Savin et al., 1997). Therefore, the initial increase in Hp was likely a result of stressors not directly related to handling the animals for drawing blood. That these stress-related variables were low throughout our experiment indicates that the otters acclimated well to the enclosure and our handling. Thus, we believe that interpreting the results from other variables can be accomplished without accounting for handling stress.

In contrast, the high variability within and between groups in values of triglycerides was probably a result of diet preference of individuals in captivity. Although some individuals preferred capelin, others consumed more salmon and yet others showed preference to eulachons. This outcome is particularly evident when the effect of otter ID on levels of TRIG is considered. Values of triglycerides were higher than those observed for 24 river otters live-captured in nonoiled areas in Prince William Sound (Table 2; R. T. Bowyer et al., unpubl. data). These values probably reflect the higher lipid contents of the food we offered to the captive otters (herring, salmon, and capelin) in comparison with the intertidal fishes otters usually consume in the wild (Bowyer et al., 1994). This conclusion is supported by the high values of cholesterol, and high and low density lipids observed in the captive otters compared to the ones caught in PWS (Table 2).

Responses to seasonal factors

Hp, ALB, and GLOB did not exhibit a pattern related to oiling, indicating that additional processes may have been operating in our animals. Values of ALB were lower than those reported for 24 river otters live-captured in nonoiled areas in Prince William Sound (Table 2; R. T. Bowyer, Unpubl. data) as well as for other captive otters (Table 2; Reed-Smith, 1995), except for the high-dose group. Similarly, levels of GLOB for the low-dose group were higher than those reported for 24 river otters live-captured in nonoiled areas in Prince William Sound (Table 2; R. T. Bowyer, Unpubl. data) as well as for other captive otters (Table 2; Reed-Smith, 1995) at least during the oiling period. We recorded lower values of Hp and other globulins from September to December followed by an increase in January and February. During this later period, we noted changes in testicular size as well as increased aggression among our otters that suggest the onset of the breeding season. Investigating the relations between levels of Hp, ALB, and GLOB and those of testosterone in male river otters will enhance our understanding of the potential effects of season on these parameters.

Responses to ingestion of oil

Our expectation that Hp levels would be elevated in the oiled animals was based on observations in the field immediately following *EVOS* (Duffy et al. 1993, 1994a, b). Nonetheless, previous studies demonstrated that serum concentrations of Hp are significantly reduced during hemolytic episodes because of increased removal rate of the Hp-Hb complex (Laurell and Gronwall, 1962). Indeed, Hp and Hb were negatively correlated in our experiment and levels of Hb had a significant influence on levels of Hp, supporting this interpretation. We hypothesize that two opposing processes were occurring in our oiled ot-

TABLE 3. Theoretical predictions for the direction of response (plus = high, minus = low) in several biomarkers under differing levels of exposure to hydrocarbons. Pathways are described in Figure 5. Levels of P450 are expected to be low in high-exposure with anemia because of competition for the heme molecule. Copro III is expected to be low because of increase in demand for heme.

Level of exposure	IL-6	Hp	Hb	ALB	P450	Copro III
None (without anemia)	−	−	+	+	−	−
Low (without anemia)	+	+	+	−	+	+
High (with anemia)	+	−	−	−	−	−

ters: the first, elevated production in Hp in response to tissue damage by hydrocarbons, and the second increased removal of Hp-Hb complex from the serum (Table 3). That the Hp and Hb were only slightly correlated, suggests that additional factors were interacting in these animals. Hp is known to block the exchange of heme between methemoglobin and albumin (Koj, 1974). Albumin and hemoglobin were significantly positively correlated in our animals and both were negatively correlated with Hp. The complex interactions between those parameters may mask any clear pattern originating from exposure to hydrocarbons in live animals, which may explain the inconclusive results obtained in a study of pigeon guillemots (Prichard et al., 1997). Figure 5 depicts the potential relations between WBC, IL-6, Hp, Hb, ALB and GLOB (Marks, 1985; Heinrich et al., 1990). IL-6 secreted from WBC (mostly macrophages and monocytes; Heinrich et al., 1990) induces synthesis of Hp and other α- and β-globulins in the liver. The increased levels of Hp are associated with a decrease in ALB (Duncan et al., 1994). In instances where hemolytic processes are concurring, Hp binds with free Hb resulting in an overall decrease in levels of Hp (Laurell and Gronwall, 1962). The directions of the correlation between those variables in our experiment support the idea that the complex interactions between them are responsible for the observed pattern in Hp, ALB, and GLOB. Duffy et al. (1993) suspected that river otters in oiled areas in PWS in 1990 experienced anemia, although they were limited in their analysis to examination of blood smears. If this indeed occurred, the anemia experienced by those animals likely was not severe. If the removal of Hp-Hb complex from blood can result in a significant reduction of Hp levels as was observed by Laurell and Gronwall (1962), and as indicated by our results, the high levels of Hp recorded by Duffy et al. (1993) will indicate low levels of anemia in otters exposed to hydrocarbons (Table 3). Indeed, values of 16.3 ± 0.6 g/dl of Hb were recorded in river otters captured in oiled areas in PWS in 1991 (n = 11; Duffy et al., 1994*a*), whereas levels of Hp in these same individuals were 156.9 ± 27.9 (mg Hb binding/dl), and IL-6*ir* 48.3 ± 13.8 (pg/ml). Values for both these variables are higher than those observed in our study.

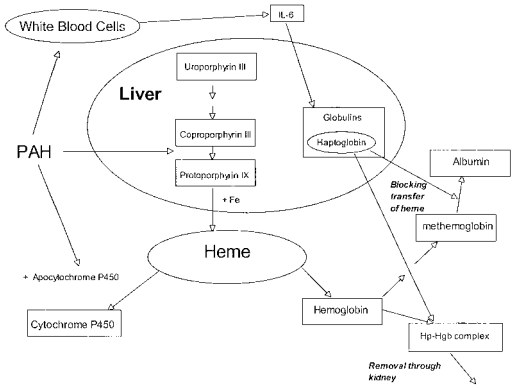

FIGURE 5. Schematic description of the potential interactions between different biomarkers related to the heme cycle (based on information from Heinrich et al., 1990; Koj, 1974; Laurell and Gronwall, 1962; Marks, 1985). Effects of PAH on heme synthesis depicted here in the liver are assumed to be similar in other tissues.

IL-6*ir* did not exhibit the expected pattern and was not correlated with either globulins or Hp. The pattern we observed in IL-6*ir* suggests pulsating secretion as is common in several hormones such as testosterone (Wong et al., 1983). Therefore, our sampling interval may have been insufficient to detect such secretions. Therefore, the individual results should be interpreted with caution. Nonetheless, overall mean values for the control group were comparable to those of 24 river otters live-captured in nonoiled areas in Prince William Sound (Table 2; R. T. Bowyer et al., unpubl. data). In comparison, those overall mean values were double for the high-dose animals (1.47 ± 0.5) and triple for the low-dose animals (2.2 ± 0.5). That the overall values were higher in the oiled animals compared with the controls indicates that oil ingestion may have influenced the secretion of this factor. Similarly, the high variability in WBC complicates the interpretation of our results, although the pattern of reduction in WBC was more evident in the oiled animals. Production and elimination of WBC is an intricate process and is affected by conditions in the bone marrow, exposure to antigens, nutrition, and immuno-suppression (Duncan et al., 1994). Nonetheless, the values of WBC we recorded in our experimental otters were lower than those reported for 24 river otters live-captured in nonoiled areas in Prince William Sound (Table 2; R. T. Bowyer et al., unpubl. data) as well as for other captive otters (Table 2; Reed-Smith, 1995). That WBC and IL-6*ir* were slightly negatively correlated supports our interpretation that oil ingestion may have influenced the secretion of IL-6.

The reduction in WBC, and the reduction in RBC and Hb we documented in the river otters agree with findings of other studies (Fry and Lowenstine, 1985; Leighton et al., 1983; Øritsland et al., 1981; Rebar et al., 1994; Williams et al., 1995). These observations point to potential damage to bone marrow tissues. Examinations of smears made from bone marrow of oiled and nonoiled animals should provide insights to the effects of hydrocarbons on production of blood cells by that tissue. The reduction in WBC potentially points to suppression of the immune system and may have profound effects for free-ranging animals challenged with other antigens.

That the reduction in Hb and RBC did not differ between the low and the high dose groups indicates that even low doses of hydrocarbons can cause significant responses in these variables, and that the response probably is threshold activated. Alternatively, ingestion of higher doses of oil may reduce the levels of hydrocarbons assimilated in the animal because the oil acts as a lubricant in the gut. Our investigation of the effect of oiling dose on passage rate and assimilation efficiency in these captive otters suggested that consumption of oil increased passage rate of food in the gut and reduced assimilation of both food and oil (Ormseth and Ben-David, 2000). This outcome may explain results for other biomarkers in our study such as those of IL-6*ir* (high dose animals had values between those of low dose and control animals).

Values of Hb in our experiment were lower than those reported for 24 river otters live-captured in nonoiled areas in Prince William Sound (Table 2; R. T. Bowyer et al., unpubl. data) as well as for other captive otters (Table 2; Reed-Smith, 1995) suggesting clinical anemia. The occurrence of nucleated erythrocytes in blood smears is indicative of regenerative anemia and abnormal release from the bone marrow (Duncan et al., 1994), and supports our interpretation of damage from hydrocarbons to bone marrow tissues. That Hb and RBC significantly declined in response to oiling could have major implications to maintenance of body condition, survival, and reproduction of coastal river otters as well as other diving mammals and birds. Ben-David et al. (2000) documented an increase in energetic costs of terrestrial locomotion, decrease in aerobic dive limit, and a potential increase in foraging time

in a companion study on these same captive river otters.

The values of ALT for the high-dose animals were elevated compared with levels reported for captive otters (Table 2; Reed-Smith, 1995), but significantly lower than the levels reported for 24 free-ranging river otters captured in Prince William Sound (Table 2; R. T. Bowyer et al., unpubl. data). In many cases, increases in both enzymes are indicative of liver or muscle necrosis, as was evident in the otter with the gangrenous toe. Nonetheless, in acute liver disease a 50% decrease or more in circulating ALT levels are common (Duncan et al., 1994). That we observed no significant differences in values of ALT between groups during the oiling period indicates that our animals did not experience an acute liver damage, although some necrosis may have occurred as can be inferred from the increased values of ALK PHOS. The values of ALK PHOS for the oiled otters were higher than those reported for 24 river otters live-captured in nonoiled areas in Prince William Sound (Table 2; R. T. Bowyer et al., unpubl. data) as well as from those of other captive otters (Table 2; Reed-Smith, 1995). Nonetheless, ALK PHOS can increase in response to several physiological processes such as bone growth, intestinal lesions and hepatic function and can not be considered a specific marker (Duncan et al., 1994). Because AST occurs in erythrocytes, hemolysis may increase serum concentrations of this variable (Duncan et al., 1994). Values of AST in our experimental animals were positively correlated with levels of hemoglobin (Hb).

Not all individuals responded to oiling in the same fashion. The reduction in Hb was doubled in several individuals compared with their counterparts. This result may point to genetic variability in the ability of animals to acclimate and compensate for chronic exposure to toxins, and may have far reaching implications from an evolutionary perspective. Zentano-Savin et al. (1997) suggested that the observed differences in Hp levels in pinnipeds in Alaska could be associated with different genetic stocks. In this study we investigated responses to oil ingestion in young adult male river otters. Therefore, inferences from our results to other age and gender categories should be made with caution. R. T. Bowyer et al., (unpubl. data) explored differences between gender and age classes in wild river otters captured in oiled and nonoiled sites in PWS from 1996–98. Only diet related parameters (i.e., cholesterol and lipid values) differed between the genders in their analysis, suggesting that age and gender classes would have little effect on individual responses to ingestion of hydrocarbons.

Conclusions

Two main conclusions can be drawn from our study. First, the use of individual biomarkers as indicators of exposure to pollutants can lead to erroneous conclusions because interactions in vivo can be complicated and act in opposite directions (Fig. 5, Table 3). Additionally, biomarkers used in investigating effects of oiling on live animals are usually related to the heme cycle (Fig. 5). Because of the opposing processes that may occur within an animal, data from the suite of heme-related biomarkers may produce results that are difficult to interpret. Therefore, we advocate the exploration and development of other biomarkers that will be independent from the heme cycle and will provide additional verification to the effect of oiling on live animals.

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