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**PIGEON GUILLEMOTS AS A SENTINEL SPECIES: A DOSE-RESPONSE
EXPERIMENT WITH WEATHERED OIL IN THE FIELD****Alexander K. Prichard¹, Daniel D. Roby², R. Terry Bowyer¹,
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ABSTRACT

Nests of pigeon guillemots (*Cephus columba*) were located along the south shore of Kachemak Bay, Alaska, and monitored during the breeding seasons of 1994 and 1995. Rates of nestling growth were measured and blood samples were collected for measurements of serum biomarkers. Haptoglobin, total protein, alanine aminotransferase, aspartate aminotransferase, and sodium in sera were measured as potential biomarkers of oil ingestion. Differences in mean levels of biomarkers were observed between years, and between nestlings and adults, as well as among locations within Kachemak Bay. During summer 1995, a controlled dose-response experiment was conducted with weathered Prudhoe Bay Crude Oil. Fifty-one nestlings were divided into three groups: controls, nestlings fed 0.05 ml of oil, and nestlings fed 0.20 ml of oil. Each experimental nestling was fed the dose of weathered oil twice: once at approximately day 20, and again 5 days later at approximately day 25 post-hatching. Blood samples were collected immediately before dosing on days 20, 25, and again on day 30 post-hatching. Site-specific differences in some blood variables were observed among treatments. These results suggest that the doses of weathered oil administered to guillemot nestlings were not sufficient to induce a persistent inflammatory response.

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1. INTRODUCTION

The grounding of the *Exxon Valdez* in Prince William Sound, Alaska in March 1989, demonstrated the potential for ecosystem-wide damage by a major oil spill in the subarctic. The spill also illustrated the inherent difficulties in attempting to quantify the biological significance of a large influx of oil into a marine ecosystem. Immediate lethal effects of the spill on seabirds and mammals could be estimated with some reliability from carcass counts; Piatt et al. (1990) Ford et al. (1996) and Piatt and Ford (1996) estimated that 100,000 - 645,000 birds of 90 species were killed outright. But sublethal, chronic effects on individuals or populations of seabirds have been more elusive and difficult to determine.

Young seabirds are likely to encounter oil in one of three ways: by external contact with the egg during incubation; external oiling of the nestling from contact with the adult; or consumption of food contaminated with oil. Physiological effects of oiling may vary for these different types of exposure. In the past, crude oil has been demonstrated to have a variety of deleterious effects on birds, including external physical effects, physiological responses to ingestion, and effects on survival and reproduction. External physical application has caused loss of buoyancy, inability to fly, inflammation, and increased basal metabolic rate (Hartung and Hunt, 1966, Lee et al., 1985, Lambert et al. 1982). In addition, oil ingestion often occurs when birds preen oiled plumage (Hartung, 1963). Short-term effects of oil ingestion included reduced rate of growth (Szaro et al. 1978, Peakall et al. 1982) and numerous other physiological changes (Fry and Lowenstine, 1985). Crude oil affects reproductive success through lowered hatchability and altered yolk structure of eggs (Grau et al. 1977), and through slower development and reduced survival of chicks (Trivelpiece et al. 1984). Long-term effects include extensive damage to the liver, kidneys, and intestine (Khan and Ryan 1991, Patton and Dieter 1979, Fry and Lowenstine 1985). Leighton et al. (1983) reported that herring gulls (*Larus argentatus*) and Atlantic puffins (*Fratercula arctica*) fed large daily doses of oil (up to 20 ml/kg) showed Heinz-body hemolytic anemia and up to a 50% decrease in packed-cell volume.

Possible biomarkers of oil exposure in free-ranging species are molecules that are induced in response to a contaminant and presumably can be related to the health or fitness of the birds (Hugget et al. 1992, Peakall and Shugart, 1993). Biomarkers can provide evidence of exposure to chemicals that do not bioaccumulate or are rapidly metabolized as well as integrate the interactions from exposure to a complex mixture of contaminants. Acute-phase proteins may be useful as nondestructive biomarkers of oil exposure in vertebrates, and measure early responses of organisms to toxicant exposure. The acute-phase response is a series of reactions to bacterial or viral infection, physical trauma, or exogenous poisons (Silverman and LeGrys 1987). This response includes a local inflammatory reaction, as well as neurological, endocrine, and metabolic alterations (Koj and Gordon 1985). An acute-phase protein, haptoglobin, has been shown to increase in response to infection in sheep (Skinner and Roberts 1994), in swine (Hall et al. 1992), and in birds (Johnson et al. 1993).

Building on this approach, we proposed the pigeon guillemot (*Cephus columba*) as an upper-trophic level sentinel species for contaminants in nearshore marine ecosystems. Pigeon guillemots were chosen as sentinels for several reasons: guillemots nest semi-colonially throughout Southcentral Alaska, and along the Pacific Coast of North America as far south as California (Sowls et al. 1978); guillemots usually feed within 7 km of the nest (Kuletz 1983, Nelson 1987) on benthic fishes from the intertidal and subtidal zones, as well as pelagic schooling fishes (Kuletz 1983); breeding guillemots appear to forage fairly opportunistically in the environment local to the nest, so changes in diet may be a good indicator of local changes in fish populations (Drent and Daan, 1980; Lehnhausen 1980). Because 13% of the oil from the *Exxon Valdez* oil spill was deposited in subtidal sediments (Wolfe et al. 1994), most species consumed by guillemots are likely to be exposed to oil contamination.

Peakall et al. (1980) dosed nestling black guillemots (*C. grylle*) with 0.1 to 0.5 ml of South Louisiana Crude Oil that was weathered for 36 hours. These authors were able to detect a transient rise in plasma sodium, a decrease in growth rate, and hypertrophy of adrenal glands, but no aliphatic or aromatic hydrocarbons were detected in samples of liver or fat. More recent studies of pigeon guillemots in Prince William Sound showed that reproductive rates in 1989, immediately after the

Exxon Valdez oil spill, were similar to prespill years. Population numbers in oiled areas, however, showed a greater decline than in nonoiled areas (Oakley, 1990), and this decline was more pronounced along oiled shore lines (Oakley and Kuletz, 1996). Pigeon guillemots have been classified as one of the few avian species that have failed to recover from the damage caused by the oil spill (Exxon Valdez Oil Spill Trustee Council, 1995). The population of pigeon guillemots in Prince William Sound was estimated to be 15,000 in the early 1970's (Islieb and Kessel, 1973), but was less than 4,900 after the spill (Sanger and Cody, 1993).

The *Exxon Valdez* oil spill had significant effects on physiology and growth of certain mammals (Duffy et al. 1993, 1994) and fish populations (Jewett et al. 1995). This study examined the effects of ingestion of weathered Prudhoe Bay Crude Oil (PBCO) on nestling pigeon guillemots. We tested whether small doses of weathered PBCO caused changes in blood biomarkers, such as acute-phase proteins, sodium, and liver enzymes. By studying guillemots nesting throughout Kachemak Bay, we were able to examine the influence of interannual variation on baseline levels of potential biomarkers.

2. MATERIALS AND METHODS

2.1 Study Area:

Kachemak Bay (59° 35'N, 151° 19'W) is located in lower Cook Inlet, Southcentral Alaska, and is about 62 km long and 38 km wide at the mouth. This area has been the site of thriving commercial and sport fisheries, as well as a large tourism industry. In addition, offshore production of oil has become increasingly important in Lower Cook Inlet. On the north shore of the bay are sandy bluffs (maximum elevation 230 m) and the town of Homer. The south shore is rocky and bordered by the mountains, glaciers, and fjords of Kachemak Bay State Park. Homer Spit projects 7km from the town of Homer, dividing the bay into inner and outer sections. In addition to the town of Homer (human population 4,000) there are smaller settlements in Halibut Cove and Seldovia Bay. Contamination of Kachemak Bay from the *Exxon Valdez* spill was probably negligible; the oil that washed ashore was highly weathered and easily removed (Wolfe et al. 1994, Gilfillan et al. 1995). Over 16 families and 100 species of birds have been identified as full or part-time residents of Kachemak Bay (Trasky et al. 1977). As the only ice-free bay in Lower Cook Inlet, this area is used by approximately 90% of the overwintering populations of birds in Lower Cook Inlet (Trasky et al. 1977).

2.2 Sampling and Biochemical Assays:

Field work was conducted from mid-May to mid-August in both 1994 and 1995 on the south shore of Kachemak Bay, Alaska (Fig. 1). We monitored guillemot aggregations on the water along the south shore of Kachemak Bay to locate active nests. Most nests were located in crevices in cliffs and could only be accessed using ropes. New nests were located throughout the field season, especially once chicks had hatched and parents began provisioning young with food. In 1995, nests that were previously inaccessible were accessed using improved climbing equipment and rappelling-ascending techniques.

We examined nests about every 4 days. During the incubation period, nests were examined for presence or absence of eggs and signs of hatching. Nestlings were weighed to the nearest gram using Pesola spring scales and wing length and length of the fifth and outer (10th) primaries were measured to the nearest 1 mm with a ruler. We identified fish brought to nestlings by adults to lowest possible taxon using either binoculars from a boat or a spotting scope from shore.

Blood samples were collected from nestlings on approximately day 20, 25, and 30 post-hatching (hatching was considered day 0). About 1 cc of blood was collected in heparinized vials by puncturing the brachial vein. Blood samples were kept cool and transported to the field camp at Halibut Cove, where samples were centrifuged at 3,000 rpm for 20 minutes. Serum was then removed with a pipette and stored in snap-top plastic vials at -20°C for laboratory analysis at the University of Alaska Fairbanks. In addition, five adult guillemots were captured in the nest during either the late stages of incubation or while brooding young nestlings. Blood samples from adults were collected as described for nestlings.

Haptoglobins were quantified by measuring the hemoglobin-haptoglobin complex. After sample preparation (Duffy et al. 1993), the sample mixture was electrophoresed on agarose gels at 100 volts for 1 h, fixed with 7.5% trichloroacetic acid, and the gels were stained for hemoglobin using o-dianisidine. The Hp-Hb complex, which migrates in a different region from hemoglobin, was quantified by densitometry and results are expressed as mg of hemoglobin binding capacity per 100 ml of serum

For measurement of cytokines, duplicate samples were added to a microliter plate coated with a monoclonal antibody for IL-6. After washing away any unbound proteins, an enzyme-linked polyclonal antibody for IL-6 was added to the wells and incubated to allow for any IL-6 binding. After a final wash, a substrate solution was added to the wells. After color developed, sample concentrations for IL-6 immunoreactivity (IL-6ir) were determined from a standard curve (Duffy et al. 1994).

To assay for P450 1A1 analytical SDS-PAGE was done using standard procedures with prepared mini gels. Sample buffer (2.35% [w/v] SDS, 10% [v/v] glycerol, 5% [v/v] β -mercaptoethanol, and 62.5 mM Tris-HCl; pH 6.8) was added to microsomal preparations. Samples were heated to 90°C for several minutes and equal volumes loaded on the gel and run at 20 mA constant current per gel for approximately 2 h to resolve individual bands. These SDS-gels were electroblotted on to nitrocellulose membranes using 25 mM Tris base, 192 mM glycine, and 20% (v/v) methanol for 1 h at 100 V. The membranes were then blocked in 5% nonfat dried milk in PBS, washed four times in PBS, and incubated with antibody to 1A1, which recognizes a conserved epitope present in several mammals. The blots were washed as before and incubated with an HRP-conjugated secondary antibody. For color development, the blot was washed as before and stained using a chemoluminescence system (Strasburger and Kohen, 1990).

2.3 Dose-Response Experiment:

In 1995, a dose-response experiment was conducted. Fifty-one chicks were classified as either first-hatched (alphas), second-hatched (betas), or singletons. Each chick was then randomly assigned to one of three groups: control, 0.05 ml dose, or 0.2 ml dose of weathered crude oil (PBCO). When chicks were 20 and 25 days post-hatching, blood samples were collected from

nestlings and each was then force-fed a Pacific sand lance (*Ammodytes hexapterus*) containing a number 2 gelcap with the assigned dose of weathered PBCO that had been inserted into the abdominal cavity of the fish. Sandlance were captured by hand in the sand during low tide at Moosehead Point, and frozen until the day of use. Control birds were fed a thawed sandlance containing a gelcap with 0.2 ml of corn oil. When nestlings were 30 days post-hatching, a third blood sample was collected. PBCO was weathered by layering oil on top of water containing 3.5% NaCl and mixing for 6 days under a hood (Fry and Lowenstine, 1985). Peakall et al. (1982) reported compositional analysis of PCBO; and Stubblefield et al. (1995a) reported effects of weathering on its composition.

2.4 Data Analysis:

We analyzed data using multivariate analysis of covariance (MANCOVA) to detect relationships between serum parameters and year, location, year*location, Julian date, nestling mass, wing lengths of nestlings, and growth performance. Growth performance was measured as the residual of a regression of body mass (g) on wing length (mm), after transforming the variables to meet the assumptions of a linear fit and homogeneity of variance. Variables were transformed using the square root of body mass and the square root of the natural logarithm of wing length. Multivariate ANOVA was used to compare values of blood biomarkers between nestlings and adults. Significance levels were adjusted for multiple comparisons. We used repeated-measures MANOVA on data from 1995 to test for differences among treatments, whereas stepwise logistic regression was used on the following variables: serum haptoglobin (Hp), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total protein, sodium, and location. Nestlings from both treatment groups that were fed crude oil were pooled and compared with nestlings from the control group.

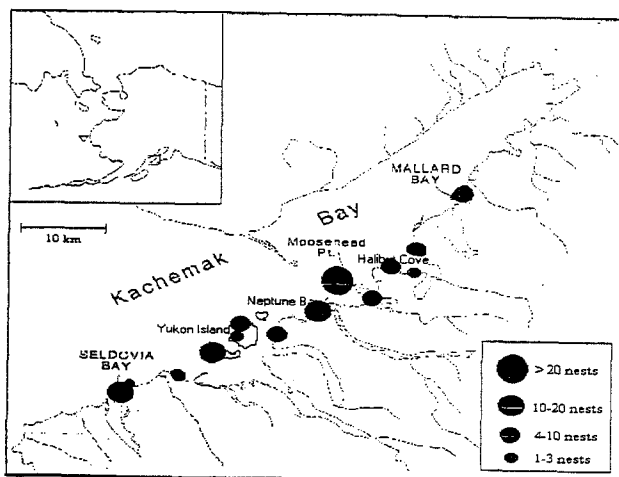


Figure 1. Locations of Pigeon Guillemot nests studied in Kachemak Bay, Alaska during the breeding seasons of 1994 and 1995.

3. RESULTS

Most active nests of guillemots were located in aggregations, with a few nests scattered throughout the study area (Fig. 1). Sixty-two and 87 accessible nests were monitored in 1994 and 1995, respectively. Nests were located along the south shore of Kachemak Bay from Mallard Bay in the northeast to Seldovia Bay in the southwest. The main nesting aggregations were located at Halibut Cove, Moosehead Point, Neptune Bay, Yukon Island, and Seldovia Bay. Colonies varied considerably in number and density of nests (Fig. 1), and in diets of chicks. The diet of chicks in the inner bay consisted of a large proportion of sand-lance, whereas the diet of nestlings in the outer bay was composed largely of epibenthic species such as gunnels (*Pholis*) and sculpins (*Oligocottus*). This overall pattern of diet was similar for the 2 years of the study.

Table 1: Annual variation in mean levels of selected biomarkers in Pigeon Guillemots from Kachemak Bay Alaska.

	1994			1995		
	n	MEAN	SE	n	MEAN	SE
HAPTOGLOBIN*	30	98.6	10.5	54	103.4	6.5
ln ALT (IU)	30	2.9	0.1	50	3.8	0.1
ln AST (IU)	29	4.3	0.1	49	5.2	0.2
PROTEIN (g/dL)	30	2.8	0.1	50	2.8	0.1
SODIUM (meg/L)	30	140.1	1.2	49	147.5	1.3

* (Hb binding /dL)

3.1 Haptoglobin:

Annual variation in the biomarkers was pronounced (Table 1). Likewise, considerable variation in biomarkers was observed in our dose-response experiment (Table 2). Levels of serum Hp for control and undosed nestlings were marginally correlated with wing length ($P = 0.067$), body mass ($P = 0.074$), and growth performance ($P = 0.062$). Hp levels varied among locations*years within Kachemak Bay ($P = 0.026$). Based on Bonferroni multiple comparisons, nestlings from Neptune Bay had a significantly lower mean level of Hp than nestlings from Halibut Cove in 1995 ($P = 0.001$; Fig. 2). Yearly variation in mean levels of Hp was quite high ($P = 0.041$; Fig. 2). Hp levels were higher in adults than nestlings ($P = 0.047$) but were similar between alpha and beta chicks (Fig. 3).

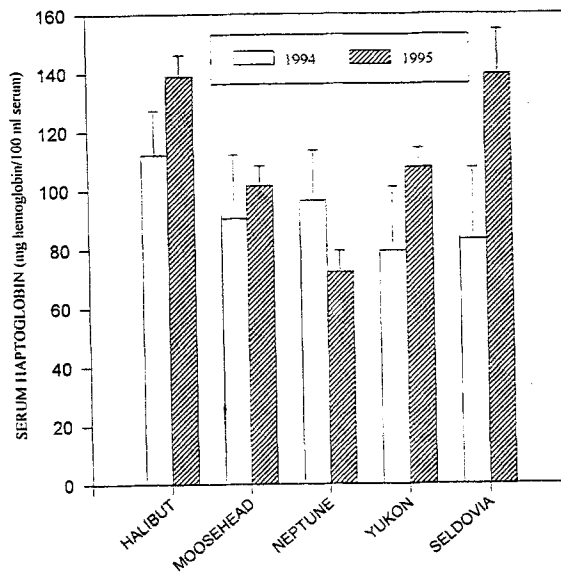


Figure 2. Serum haptoglobin levels of nestling pigeon guillemots nesting in Kachemak Bay, Alaska, during the breeding seasons of 1994 and 1995.

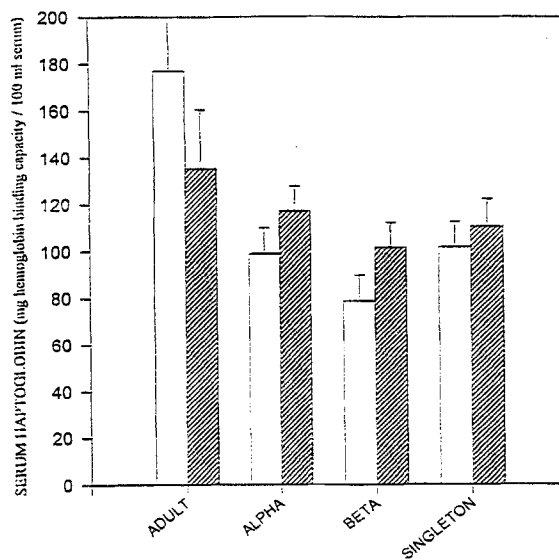


Figure 3. Serum haptoglobin levels of nestling and adult pigeon guillemots in Kachemak Bay, Alaska, during the breeding seasons of 1994 and 1995.

When Hp levels were measured 5 days after dosing with oil, nestlings from the outer bay showed a very different effect of treatment compared with nestlings from the inner bay and Neptune Bay. Changes in biomarkers can be noted in blood parameters, but the standard error overlaps in many instances (Table 2). Repeated measures MANOVA indicated that the treatment effect on haptoglobin levels ($P = 0.035$) varied with location. This difference was caused largely by an increase in Hp levels by day 30 in control nestlings that did not occur in nestlings dosed with oil (Fig. 4). Hp levels also were affected by rate of meal delivery to nests ($P = 0.0001$), and rate of meal delivery was highly dependent on location (ANCOVA, $P = 0.0002$).

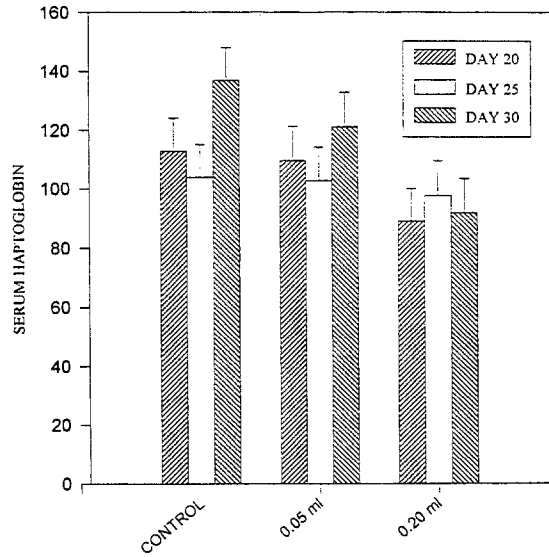


Figure 4. Serum haptoglobin levels of nestling pigeon guillemots dosed with weathered Prudhoe Bay Crude Oil compared with controls, Kachemak Bay, Alaska, 1995. Dosing occurred immediately after collection of blood samples on day 20 and day 25.

3.2 Total Serum Protein:

A marginal difference occurred in levels of serum protein among locations*years ($P = 0.062$); and this pattern was similar to that for serum haptoglobin. Adults had higher levels of serum protein than nestlings during both years ($P = 0.006$, $n = 152$).

Table 2. Dose response of pigeon guillemot selected biomarkers to PBCO in 1995.

Blood Parameter	CONTROL			0.05 ML PBCO			0.20 ML PBCO		
	n	MEAN	SE	n	MEAN	SE	n	MEAN	SE
HAPTOGLOBIN*	54	117.8	6.7	53	110.9	6.7	53	92.3	6.5
ALT (IU)	49	3.6	0.1	50	3.9	0.1	50	3.7	0.1
AST (IU)	48	4.6	0.2	48	5.0	0.2	48	4.8	0.2
PROTEIN g/dL	50	2.7	0.1	50	2.8	0.1	50	2.6	0.1
SODIUM (meg/L)	50	152.7	1.3	50	151.8	1.3	50	149.6	1.3

*(Hb binding/dL)

In the dosing experiment, we observed differences among locations in serum-protein levels over the first 5 days post-dosing ($P = 0.04$) and a Bonferroni multiple comparison between Seldovia Bay and Moosehead Point ($P = 0.049$) showed a location effect. Backwards stepwise regression indicated that 43% of the variation in the change in serum-protein levels over the first 5 days was related to location, not dose. Means for serum protein 10 days after the first dosing (day 30 post hatching) was similar to that at 5 days ($P = 0.031$), with a difference (Bonferroni) between controls and subjects in the 0.05 ml dose group ($P = 0.026$). The treatment effect (ANCOVA; $P = 0.002$) consisted of serum protein increasing or remaining constant in the 0.05-ml dose group, whereas serum protein decreased in the control group and the 0.20-ml dose group (Fig. 5).

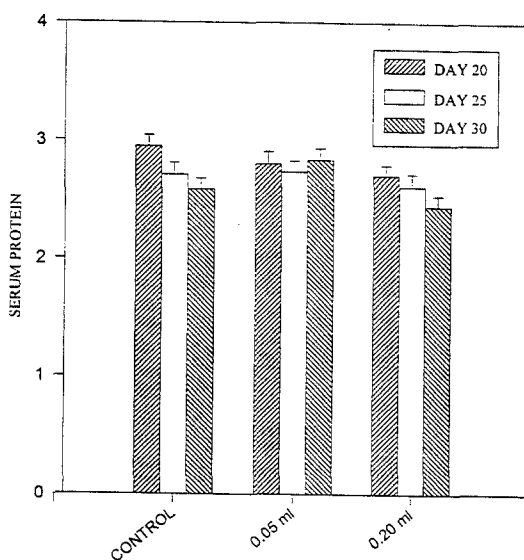


Figure 5: Serum protein levels of nestling pigeon guillemots dosed with weathered Prudhoe Bay Crude Oil compared with controls, Kachemak Bay, Alaska, 1995. Dosing occurred immediately after blood sample collection on day 20 and day 25.

3.3 Sodium:

Among undosed birds, serum Na was positively correlated with Julian date ($P = 0.039$) and serum Na levels were also higher in nestlings sampled in 1995 than in 1994 ($P < 0.004$). The change in serum Na over the first 5 days post-dosing was associated with initial levels of serum Na at day 20 ($P < 0.0005$). A repeated measures analysis of variance showed no effect of treatment or location on serum Na levels.

3.4 Liver Enzymes:

Levels of ALT at day 20 (pre-dosing) were not different among treatments and did not differ between nestlings and adults. ALT differed between years; nestlings had higher ALT in 1995 ($P < 0.035$; Table 2). No effect of treatment on levels of either ALT or AST was observed.

A P450 cross-reactive protein was detected by an antibody, which recognizes a 58 Kd molecular weight protein in rats, river otters (*Lutra canadensis*) and mink (*Mustela vison*). No immunoreactive protein at that molecular weight was detectable in pigeon guillemots; however, the antibody recognized a protein with a 90 Kd molecular weight whose levels varied between birds and oil treatments. In general, there was a higher induction of this cross-reactive protein (P450 IAI ir) in the oiled-dosed chicks than in the nondosed chicks. The mean for the nondosed (control chicks) was 0.59ug/5ug protein, whereas for 0.2 mL-dosed chicks the mean was 0.99ug/5ug protein.

3.5 Growth and Body Mass:

We analyzed body mass of nestlings, measured at 20, 25, and 30 days post-hatching, as a function of treatment using repeated-measures ANOVA. Wing length at age 20 days was used as a covariate to control for variation in development among nestlings at first dosing. Growth in body mass between age 20 and 30 days was not affected by treatment.

3.6 Logistic Regression Analysis:

We categorized nestling guillemots that received the doses of weathered crude oil as dosed and a stepwise logistic regression was used to model the probability of being dosed or not receiving crude oil. Five groups of variables were considered: the first used blood values from day 25, the second used values from day 30, the third used the change in blood values over the first 5 days after dosing, the fourth used the change in blood values 5 days after the second dosing, and the final group included the change in values between day 20 and day 30. Variables included in the model were the three general locations (inner bay, Neptune Bay, outer bay), Hp, total protein, ALT, AST, and sodium (Table 3).

Table 3. Significance levels (*P*-values) for factors influencing serum parameters for undosed pigeon guillemots in Kachemak Bay, Alaska during summer 1994 and 1995.

variable	Blood Parameter				
	haptoglobin	protein	AST	ALT	sodium
location					
nestlings vs adults	0.047	0.006	0.092		
Julian date					0.039
growth performance	0.062				
wing length	0.067				
year	0.041			0.035	0.0004
body mass	0.074				
year*location	0.026	0.062			

For the logistic regression on blood values at day 30, both Hp and the location variables entered the model. The Wald Chi-Square *P*-value was 0.0121 for haptoglobin and 0.1338 for the location variable, differentiating between Neptune Bay and the outer bay. The overall chi-square value was 9.417, with d.f. = 2, *P* = 0.009. The model had a concordance of 76.8% and the residual chi-

square indicated the model was apt ($P = 0.9879$). Growth of body mass declined with dosing ($P = 0.0997$), and the change in serum protein increased, but not significantly, ($P = 0.2479$). When the change in blood values from day 25 to day 30 was used, however, only the change in haptoglobin levels entered, but the model was not significant ($P = 0.1314$); concordance was only 64.8%.

4. DISCUSSION

With the influx of contaminants into natural systems in recent decades, there has been renewed interest in developing methods for measuring the effects of pollutants on ecosystems. Initially, toxicological studies emphasized only the quantity of toxins in the environment or in an individual animal. Although this information is important, it does not measure the biological availability of the contaminant or reflect interactions among contaminants within the organism or population. This study attempts to develop the pigeon guillemot as a bioindicator species for petroleum hydrocarbon pollution in nearshore marine habitats of Alaska. Guillemots as sentinel species can serve as indicators of composition and health of intertidal communities of fish. If suitable markers are found, biomarker levels in blood can be correlated with contaminant levels and individual health status. Using guillemots as a model sentinel species, we described the baseline variation in blood biomarkers. The sample of 30 individuals was sufficient to obtain significant annual variation in some biomarkers. In the dosing experiment, however, the unexpected variation among locations and our randomly selected controls not being equally distributed among locations prevented a complete analysis of effects of individual locations. Because of the large among-individual variation and variation among sites, when the total Kachemak Bay was considered as a single system, the treatment effect of oil was masked. The distribution of controls prevented more specific analysis at the individual sites, and future studies will be necessary to address this shortcoming.

The acute-phase response (APR) has been shown to be indicative of inflammation in birds (Cronenberger 1987, Johnson et al., 1993, Skinner and Roberts 1994, Sugii and Hirota, 1994), but whether it can be used to assess direct exposure to xenobiotics in wild populations has not been established. The biomarker model proposed by Duffy et al. (1994) to distinguish river otters from oiled and nonoiled areas of Prince William Sound was not suitable to distinguish oil-dosed vs. control nestlings. The river otter model used Hp, IL-6, and AST levels in serum to classify otters. IL-6 was not consistently detected in the blood of guillemots, suggesting the antibody did not cross-react with the bird cytokine. Also, mean levels of Hp varied among locations in the two low-dose treatments, making ecosystem-wide interpretations difficult. Predicted increases in Hp were seen in some locations, but variation in controls at the different colonies was quite high.

There are a number of possible explanations for the weak treatment effect on blood proteins and sodium. We used realistic low dosages of highly weathered Prudhoe Bay Crude Oil. These dosages were not high enough to induce the acute responses observed by other studies using larger amounts of less-weathered oil, or Prudhoe Bay Crude Oil may lack the toxic fractions of other crude oils.

Another source of variation in biomarkers was the physiological development of nestlings. For example, nestlings differed in Hp means but as they grew, the mean would change. Similarly, Boersma et al. (1988) reported that fork-tailed storm-petrels fed 0.1 ml weathered PBCO gained body mass more slowly compared with controls from 0-21 days post-hatching, but not from 22-51 days. Recently, Rattner et al. (1996) suggested that P-450 in nestling black-crowned night herons

(*Nycticorax nycticorax*), was not an accurate predictor of contaminant exposure because the nestlings were in a stage of rapid growth.

Hp levels differed among locations; diet composition and growth of young birds also varied among locations. Consequently, oil-induced changes in means for Hp varied with location and made it difficult to assess Hp as a biomarker for oil pollution in guillemots. We used observations of fish delivered to nests to reconstruct diets of nestlings. Our ability to identify fish to species accurately may have introduced some error into this analysis, but such a bias would have been the same for all locations. Thus, we believe our analysis was valid. The increase in means of serum Hp with increasing rates of meal delivery suggests that Hp levels may be sensitive to nutrition in guillemots. Also, the variable effects of oil treatment on guillemots at different locations may have been a reflection of different prior exposure to contaminants or stressors, different thermal exposure, or differences in health status. In a small survey 12% of 8 adult guillemots in Cook Inlet tested positive for polycyclic aromatic hydrocarbons on their feathers (Duffy, Blajeski and Huffman, unpublished).

Our study indicates that further development of Hp or acute phase proteins may be worthwhile because the logistic regression models consistently chose serum Hp to differentiate between oil-dosed and control nestlings. Hp values were lower in dosed nestlings; however, so the effect was the opposite of that predicted. This difference may be attributed to a sharp increase in Hp levels in control birds at day 30. More study is needed to determine the cause of the increase in nestling Hp from the control group. On the other hand, values of AST and ALT reported here are much higher than those reported for adult mallards (*Anas platyrhynchos*; Rattner 1981, Stubblefield et al. 1995b), and adult sandhill cranes (*Grus canadensis*; Fleming et al. 1982) and mallard ducklings (Szaro et al. 1978). A consistent relationship with dose, however, was not observed.

Lochmiller et al. (1993) reported that northern bobwhite (*Colinus virginianus*) chicks fed low protein diets had suppressed immune responses. Recent data from Prince William Sound suggest that changes in the species composition of guillemot diets accompanied the *Exxon Valdez* oil spill (D.L. Hayes, unpublished). Changes in herring stocks and the proportion of sand lance in the diet of guillemots in Prince William Sound may indicate that one long-term effect of oil spills on guillemots may be on their food supply and the availability of high-quality forage fishes during the breeding season. We saw no obvious relationship between Hp means and species composition of the diet.

This study noted no increase in serum levels of sodium or decrease in growth rate of body mass with ingestion of Prudhoe Bay Crude Oil. These results are contrary to those reported by Peakall et al. (1980) in their study of black guillemots following ingestion of similar quantities of South Louisiana Crude Oil. Differences in the relative amounts of toxic fractions between the two types of oil may be responsible for different toxicities (Ottway 1971, Hartung and Hunt 1966). Also, Gorman et al. (1978) observed no effect of Forties Field Oil from the North Sea on the growth of herring gull (*Larus argentatus*) chicks. Weathered crude oil is less toxic than unweathered crude oil (Stubblefield et al. 1995a). Weathering causes a loss of low molecular weight aliphatics by evaporation and of low molecular weight aromatic fractions by evaporation and dissolution (Payne et al., 1991). Peakall et al. (1982) suggested that the aromatic fraction of crude oil from Prudhoe Bay was responsible for retardation of growth and hypertrophy of adrenal and nasal glands in nestling herring gulls.

Our study on wild populations of pigeon guillemots agrees with several other studies that suggest that weathered PBCO does not have strong toxic effects on birds when ingested. Our study fed relatively small amounts of weathered Prudhoe Bay Crude Oil (~0.2 mg) to nestlings when compared with these other studies. Fleming et al. (1982) observed little effect of large quantities of unweathered Prudhoe Bay Crude Oil on serum chemistry in adult sandhill cranes, although lethargy and decreases in body mass were apparent in birds dosed with 10 ml kg⁻¹ day⁻¹, and Rattner (1981) noted only a transient decrease in body mass gain and no biochemical changes in adult mallards fed mash containing 1.5% unweathered crude oil from Prudhoe Bay for 7 days. In contrast to our study, Szaro et al. (1978) did find increased levels of ALT, decreased growth rates, and impaired avoidance behavior in mallard ducklings fed mash containing up to 5% South Louisiana Crude Oil. Stubblefield et al. (1995b) dosed adult mallards with naturally weathered Prudhoe Bay Crude Oil and reported biochemical changes only in females fed extremely high doses (100 g/kg).

This study reports the first baseline levels of biomarkers in a population of birds that can potentially serve as an upper trophic level sentinel species and has a wide distribution in the north. Further work is needed to determine if APR induction is caused by only larger, more toxic ingestion or very long term chronic exposure to contaminated food sources. Moreover, our study emphasizes the need for more information on background levels of biomarkers and how they vary with location.

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REFERENCES

1. Boersma, P.E., E.M. Davies, and W.V. Reid. Weathered crude oil effects on chicks of Fork-tailed Storm-Petrels (*Oceanodroma furcata*). Archives of Environmental Contamination and Toxicology 17:527-531, (1988).
2. Cronenberger, J.H. Physiological responses to infection. Journal of Medical Technology 4:150-153, (1987).
3. Drent, R.H., and S. Daan. The prudent parent: energetic adjustments in avian breeding. Ardea 68:225-252, (1980).
4. Duffy, L.K., R.T. Bowyer, J.W. Testa, and J.B. Faro. Differences in blood haptoglobin and length-mass relationships in river otters (*Lutra canadensis*) from oiled and nonoiled areas of Prince William Sound, Alaska. Journal of Wildlife Diseases 29:353-359, (1993).

5. Duffy, L.K., R.T. Bowyer, J.W. Testa, and J.B. Faro. Chronic effects of the Exxon Valdez oil spill on blood and enzyme chemistry of River Otters. *Environmental Toxicology and Chemistry* 13:643-647, (1994).
6. Exxon Valdez Oil Spill Trustee Council. 1995. Status Report. Anchorage Alaska. 23 pp.
7. Fleming, W.J., L. Sileo, and J.C. Franson. Toxicity of Prudhoe Bay Crude Oil to Sandhill Cranes. *The Journal of Wildlife Management* 46:474-478, (1982).
8. Ford, R.G., M.L. Bonnell, D.H. Varoujean, G.W. Page, and H.R. Carter. Total direct mortality of seabirds from the *Exxon Valdez* oil spill. *American Fisheries Society Symposium* 18:684-711 (1996).
9. Fry, D.M., and L.J. Lowenstine. Pathology of Common Murres and Cassin's Auklets exposed to oil. *Archives of Environmental Contamination and Toxicology* 14:725-737, (1985).
10. Gilfillan, E.S., T.H. Suchanek, P.D. Boehm, E.J. Harner, D.S. Page, and N.A. Sloan. Shoreline impacts in the Gulf of Alaska region following the Exxon Valdez oil spill. pp. 444-481. In *Exxon Valdez* oil spill: fate and effects in Alaskan waters, ASTM STP 1219, P.G. Wells, J.N. Butler, and J.S. Hughes, Eds., American Society for testing and materials, Philadelphia, (1995).
11. Gorman, M.L., and C.E. Simms. Lack of effect of ingested forties field crude oil on avian growth. *Marine Pollution Bulletin* 9:273-276, (1978).
12. Grau, C.R., T. Roudybush, J. Dobbs, and J. Wathen. Altered yolk structure and reduced hatchability of eggs from birds fed single doses of petroleum oils. *Science* 195:779-781, (1977).
13. Hall, W.F., T.E. Eurell, R.D. Hansen, and L.G. Herr. Serum haptoglobin concentrations in swing naturally or experimentally infected with *Actinobacillus pleuropneumoniae*. *Journal of American Veterinary Medical Association* 201:1730-1733, (1992).
14. Hartung, R. Ingestion of oil by waterfowl. *Papers of the Michigan Academy of Science, Arts, and Letters* 48:49-55, (1963).
15. Hartung, R., and G.S. Hunt. Toxicity of some oils to waterfowl. *Jour. Wildlife Manag.* 30: 564-570, (1966).

16. Hugget, R.J., R.A. Kimerle, P.M. Mehrle Jr., and H.L. Bergman (eds). Biomarkers; biochemical, physiological, and histological markers of anthropogenic stress. Lewis Publishers, Chelsea, MI, (1992).
17. Islieb, M.E. and B. Kessel. Birds of the North Gulf Coast-Prince William Sound Area, Alaska. Biological Papers, University of Alaska, No. 14:1-149, (1973).
18. Jewett, S.C., T.A. Dean, R.O. Smith, M. Stekoll, L.J. Haldorson, D.R. Laur, and L. McDonald. The Effects of the Exxon Valdez Oil Spill on Shallow Subtidal Communities in Prince William Sound, Alaska. Alaska Department of Fish and Game. Restoration Project 93047. Final Report, (1995).
19. Johnson, R.W., S.E. Curtis, R. Dantzer, J.M. Baker, and K.W. Kelley. Behavior in birds caused by peripheral or central injection of endotoxin. *Physiology and Behavior* 53:343-348, (1993).
20. Khan, R.A. and P. Ryan. Long term effects of crude oil on Common Murres (*Uria aalge*) following rehabilitation. *Bulletin of Environmental Contamination and Toxicology* 46:216-222, (1991).
21. Koj, A. and A.H. Gordon. Eds. The Acute-phase response to injury and infection. Elsevier, (1985).
22. Kuletz, K.J. Mechanisms and consequences of foraging behavior in a population of breeding Pigeon Guillemots. M.S. Thesis, University of California, Irvine. 79 pp., (1983).
23. Lambert, G., D.B. Peakall, B.J.R. Philogene, and F.R. Engelhardt. Effect of oil and oil dispersant mixtures on the basal metabolic rate of ducks. *Bulletin of Environmental Contamination and Toxicology* 29:520-524, (1982).
24. Lee, Y.Z., F.A. Leighton, D.B. Peakall, R.J. Norstrom, P.J. O'Brien, J.F. Payne, and A.D. Rahimtula. Effects of ingestion of Hibernia and Prudhoe Bay crude oils on hepatic and renal mixed function oxidase in nestling herring gulls (*Larus argentatus*). *Environmental Research* 36:248-255, (1985).
25. Lehnhausen, W.A. Nesting habitat relationships of four species of alcids at Fish Island, Alaska. M.S. Thesis, University of Alaska, Fairbanks, (1980).
26. Leighton, F.A., D.B. Peakall, and R.G. Butler. Heinz-body hemolytic anemia from the ingestion of crude oil: a primary toxic effect in marine birds. *Science* 220:871-873, (1983).

27. Lochmiller, R.L., M.R. Vestey, and J.C. Boren. Relationship between protein nutritional status and immunocompetence in Northern Bobwhite chicks. *Auk*: 503-510, (1993).
28. Nelson, D.A. Factors influencing colony attendance by Pigeon Guillemots on Southeast Farallon Island, California. *Condor* 89:340-348, (1987).
29. Oakley, K.L. Assessment of injury to waterbirds from the Exxon Valdez oil spill: effects on the population and reproductive success of Pigeon Guillemots in Prince William Sound. U.S.F.W.S. Bird Study Number 9, Final Report, (1990).
30. Oakley, K.L. and K. J. Kuletz. Population, reproduction, and foraging of pigeon guillemots at Naked Island, Alaska, before and after the Exxon Valdez oil spill. *American Fisheries Society Symposium* 18:759-769 (1996).
31. Ottway, S. The comparative toxicities of crude oils pp. 172-180. *In*: The ecological effects of oil pollution on littoral communities, E.B. Dowell Ed. Elsevier:172-180, (1971).
32. Patton, J.F. and M.P. Dieter. Effects of petroleum hydrocarbons on hepatic function in the duck. *Comp. Biochem. Physiol.* 65C:33-36, (1979).
33. Payne, J.R., J.R. Clayton, G.D. McNabb, and B.E. Kirstein. *Exxon Valdez* oil weathering fate and behavior: Model predictions and field observations. *Proceedings International Oil Spill Conference (Prevention, Behavior, Control, Cleanup)*, San Diego, CA, USA, March 4-7, pp. 641-654 (1991).
34. Peakall, D.B., D.S. Miller, and R.G. Butler. Effects of ingested crude oil on Black Guillemots: a combined field and laboratory study. *Ambio* 9(1):28-30, (1980).
35. Peakall, D.B., D.J. Hallett, J.R. Bend, and G.L. Foureman. Toxicity of Prudhoe Bay crude oil and its aromatic fractions to nestling Herring Gulls. *Environmental Research* 27:206-215, (1982).
36. Peakall, D.B. and L.R. Shugart. *Biomarkers; research and application in the assessment of environmental health*. Springer-Verlag, Berlin. 119 pp (1993).
37. Piatt, J.F., and R.G. Ford. How many seabirds were killed by the *Exxon Valdez* oil spill? *American Fisheries Society Symposium* 18:712-719 (1996).
38. Piatt, J.F., C.J. Lensink, W. Butler, M. Kendziorek, and D.R. Nysewander. Immediate impact of the "Exxon Valdez" oil spill on marine birds. *Auk* 107:387-397, (1990).

39. Rattner, B.A. Tolerance of adult mallards to subacute ingestion of crude petroleum oil. *Toxicology Letters* 8:337-342, (1981).
40. Rattner, B.A., M.J. Melancon, T.W. Custer, R.L. Hothems. Cytochrome P450 and contaminant concentrations in nestling Black-Crowned Night Herons and their interrelation with sibling embryos. *Environmental Toxicology and Chemistry* 15(5), 715-721, (1996).
41. Sanger, G.A., and M.B. Cody. Survey of Pigeon Guillemot colonies in Prince William Sound, Alaska. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 93034). U.S. Fish and Wildlife Service, Anchorage, Alaska. 53 pp. (1994).
42. Silverman, L.M. and V.A. LeGrys. The acute phase response to clinically significant proteins. *Journal of Medical Technology* 4(4):154-157, (1987).
43. Skinner, J.G. and L. Roberts. Haptoglobin as an indicator of infection in sheep. *The Veterinary Record* 134:33-36, (1994).
44. Sowls, A.L., S.A. Hatch, and C.J. Lensink. Catalog of Alaskan seabird colonies. Technical Report FWS/OBS/78-78. U.S. Fish and Wildlife Service, Anchorage. 252 pp., (1978).
45. Strasburger, C.J. and Kohen, F. Two-site and competitive chemiluminescent immunoassays. *Methods in Enzymology* 184:481-491 (1990).
46. Stubblefield, W.A., G.A. Hancock, W.H. Ford, and R.K. Ringer. Acute and subchronic toxicity of naturally weathered Exxon Valdez crude oil in Mallards and Ferrets. *Environmental Toxicology and Chemistry* 14:1941-1950, (1995a).
47. Stubblefield, W.A., G.A. Hancock, H.H. Prince, R.K. Ringer. Effects of weathered Exxon Valdez crude oil on Mallard reproduction. *Environmental Toxicology and Chemistry* 14:1951-1960, (1995b).
48. Sugii and Hirota. Identification of a Ca^{2+} -dependent agarose-binding protein in chicken serum. *Jour. of Vet. Med. Sci.* 56:143-145, (1994).
49. Szaro, R.C., M.P. Dieter, G.H. Heinz. Effects of chronic ingestion of South Louisiana crude oil on Mallard ducklings. *Environmental Research* 17:426-436, (1978).
50. Taylor, E.H. Ed. *Clinical Chemistry*. John Wiley and Sons. New York 291 pp. (1989).
51. Trasky, L.L., L.B. Flagg, and D.C. Burbank. Environmental studies of Kachemak Bay and Lower Cook Inlet. Volume I: impact of oil on the Kachemak Bay environment. Alaska Department of Fish and Game 133 pp. (1977).

52. Trivelpiece, W.Z., R.G. Butler, D.S. Miller, and D.B. Peakall. Reduced survival of chicks of oil dosed adult Leach's Storm Petrels. *Condor* 86:81-82, (1984).
53. Wolfe, D.A., M.J. Hameedi, J.A. Galt, G. Watabayashi, J. Short, C. O'Claire, S. Rice, J. Michel, J.R. Payne, J. Braddock, S. Hanna, and D. Dale. The fate of the oil spilled from the Exxon Valdez. *Environmental Science and Technology* 28(13):561-568, (1994).