Reports

Ingestion of Petroleum by Seabirds Can Serve as a Monitor of Water Quality

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The ingestion by seabirds of fossil fuel hydrocarbons and other pollutants has been of great interest. This paper reports that storm-petrels ingest petroleum at sea and that residues can be detected in their stomach oil. The incidence of gut samples containing fossil fuel hydrocarbons (dirty samples) increased significantly after oil spills, and significantly more birds regurgitated dirty samples after large nearby spills than small distant ones. This appears to be one of the first reported instances where individuals of a natural population of marine birds have been shown to ingest sublethal doses of oil from sources of low-level, long-term pollution or from oil spills. Because of certain natural traits, Procellariiformes could serve as monitors of pollutants in the marine environment.

Petroleum products are, and will probably continue to be, the most common toxic substances released into the world's oceans (*1*). In addition to trace metals contained in crude oils, nonaromatic components of petroleum hydrocarbons can have long residence times in seawater (*2*), resulting in long-term exposure for marine organisms. Since many organisms are likely to be ingesting oil, it is essential to know what quantities of petroleum and other toxic compounds exist in the marine environment and to understand how ambient and fluctuating levels of these substances affect marine ecosystems.

Reproductive patterns and traits of seabirds are known to reflect environmental conditions (*3*). Foraging seabirds continually sample the ocean and have been shown to be useful indicators of the presence of pollutants such as DDT, trace metals, and polychlorinated biphenyls (PCB's) (*4*). In 1980 I began a study to determine whether Procellariiformes, an order of seabirds, could be used to monitor fossil fuel hydrocarbons in the marine environment. The results of this study confirm that storm-petrels do ingest petroleum at sea and that the residues can be detected in their stomach oil. Furthermore, the number of birds regurgitating stomach contents containing fossil fuel hydrocarbons (dirty samples) correlated significantly with pollution events ($P = 0.035$), and the increase in the number of dirty samples was greater after large nearby spills than small distant ones ($P = 0.05$). These findings show that marine birds in natural populations are ingesting sublethal doses of oil from sources of low-level, long-term pollution or small spills. The findings also suggest that Procellariiformes could be used to monitor changes in water quality that result from oil development and transport.

I used Procellariiformes for this study because several of their natural history traits suggest that they would be suitable for monitoring oceanic conditions. (i) They forage at the surface of the ocean, where pollutants such as petroleum, pesticides, and heavy metals concentrate; hence, they are likely to ingest these substances with their food (*4, 5*). (ii) They forage over large areas, feeding intermittently (*6*); as a result their foraging pattern is similar to spot sampling of a large area. (iii) Oil from their food accumulates and is digested slowly (*7*). (iv) Because storm-petrels readily regurgitate oil at predators, and hence at humans when captured in nets or burrows, samples of stomach contents can be collected for analysis of pollutants without harm to the bird. (v) Procellariiformes are widely distributed throughout all the major oceans of the world.

The Barren Islands, Alaska (58° 55′N, 152° 10′W), were chosen as the primary study site because they are at the mouth of Cook Inlet, where an offshore oil field has been in production for more than a decade and where more development may occur (*8*). The Barren Islands also support one of the largest seabird colonies in the region, including hundreds of thousands of fork-tailed storm-petrels (*Oceanodroma furcata*). Between 1980 and 1983 I collected 1848 gut samples from four species of Procellariiformes: 1727 samples from fork-tailed storm-petrels, 100 from leach's storm-petrels (*O. leucorhoa*), 7 from northern fulmars (*Fulmarus glacialis*), and 14 from sooty storm-petrels (*O. tristrami*). Approximately 85 percent of the samples came from the Barren Islands. Other samples came from St. Lazaria Island, Alaska; Emerald and Egg Islands in the Aleutian Islands; Tatoosh Island, Washington; and Laysan and Nihoa Islands in the Hawaiian Islands.

Storm-petrels were captured in mist nets at night or from their burrows during the day. A Nalgene funnel rinsed in dichloromethane was thrust in front of the captured bird to collect its regurgitate, which was then rinsed into a scintillation vial with nanograde dichloromethane. Teflon cap liners were placed in the lid of each vial to prevent contamination. As a test for contamination, blanks were collected periodically and treated as samples. Thin-layer chromatography performed in the field and laboratory showed that the chemical composition of the samples was stable during storage (*9*). Gas chromatographic analysis was performed with a chromatograph equipped for automatic sample injection and capillary analysis (Hewlett-Packard model 5880). Confirmation of hydrocarbon components in select samples was done with a chromatograph (Finnigan model 4023) equipped with a direct Nova 3 computer, Incos software, and a 30,000-entry library of mass spectra.

I analyzed a portion of each sample equivalent in residue mass to 25 mg. Samples were run in batches of ten and contained one sample spiked with petroleum. To measure both the amount of fossil fuel hydrocarbons present and my ability to detect them, I added to each sample an internal standard (2 μg) of a specific branched hydrocarbon (hexamethylbenzene, HMB) chemically similar to the compound of interest but present only in minor amounts in fossil fuels (*9*).

Spectra of petroleum products showed regular *n*-alkane peaks of similar height and frequently also had an unresolved mixture of higher weight compounds (Fig. 1). Because biological hydrocarbons did not show these patterns, I used the uniform distribution of aliphatics (that is, no odd or even *n*-alkane dimensions) coupled with the presence of an unresolved complex mixture as the criterion for distinguishing petroleum. Samples were scored as dirty, that is, as containing petroleum, when the sample showed 95 to 100 percent of the 21 *n*-alkane peaks [C-11 to C-32, excluding C-17 because this peak was frequently buried by pristane, which was present in nearly every sample (Fig. 1)] of the standard sample of Prudhoe Bay crude oil. This is a conservative measure since extended weathering of petroleum results in loss and lowered intensity of these peaks.

The specific procedures for preparing and analyzing samples were developed and refined during the course of this study (*9*).

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When final protocol for the analytic technique was accomplished, 207 analyzed samples were suitable for use in identifying the presence or absence of fossil fuel hydrocarbons and in measuring the amount of petroleum present. Nine samples (4 percent) had 95 to 100 percent matched peaks and an unresolved mixture of higher weight compounds (Table 1). These samples were considered dirty. Seventeen samples (8 percent) had 90 percent or more of the 21 *n*-alkane peaks expected. Slightly more than half the analyzed samples (106 samples or 51 percent) had 11 or more matching *n*-alkane peaks, and the remaining 101 samples (49 percent) had less than 50 percent of the *n*-alkane peak matches (Table 1).

Fossil fuel hydrocarbons were found in the gut contents of both leach's and fork-tailed storm-petrels. Comparison of the percentage of dirty samples collected from sites where both species coexist (St. Lazaria, Tatoosh, and the Aleutian Islands) showed no significant differences between the species: 3 of 49 samples (6 percent) collected from fork-tailed storm-petrels were dirty, and 2 of 30 samples (7 percent) collected from leach's storm-petrels contained petroleum. The samples from sooty storm-petrels were contaminated, possibly by the vial caps or by ingested plastic, and no samples from northern fulmars were analyzed by the final procedure used; therefore no information is available for these species.

To measure the amount of crude oil contained in gut samples, I assumed (i) that the average collected sample was 1 ml on the basis of observation, (ii) that the weight of stomach oil is approximately that of water (1 ml = 1 g), and (iii) that the average food load fed to chicks of fork-tailed storm-petrels is 2 g per night (*10*). The amount of crude oil extracted from individual samples ranged from 0.002 to 0.0004 ml. As judged from these relatively conservative assumptions, chicks of fork-tailed storm-petrels ingest between 0.004 and 0.0008 ml of fossil fuel hydrocarbons per feeding when adults encounter petroleum pollution.

To compare areas, I ranked each site according to the percentage of dirty samples collected. I assumed that the sampling of each species in different areas was similar (this assumption was not necessary for a single site such as the Barren Islands). In descending order of dirtiness, the rank for areas and years was as follows: the Barren Islands, 1980 (13 percent, $n = 8$); St. Lazaria Island, July 1981 (11 percent, $n = 28$); Tatoosh Island, 1981 (5 percent, $n = 19$); the Barren Islands, 1981 (4 percent, $n = 70$); and the Aleutian Islands, 1981 (3 percent, $n = 32$). The Barren Islands in 1982 was the cleanest site; none of the samples collected there was scored as dirty ($n = 50$).

These ranks are consistent with information available about each area, although sample sizes were inadequate for definitive ranking of water quality by region. In 1980 five oil spills occurred near the Barren Islands, and the rankings by year for this site

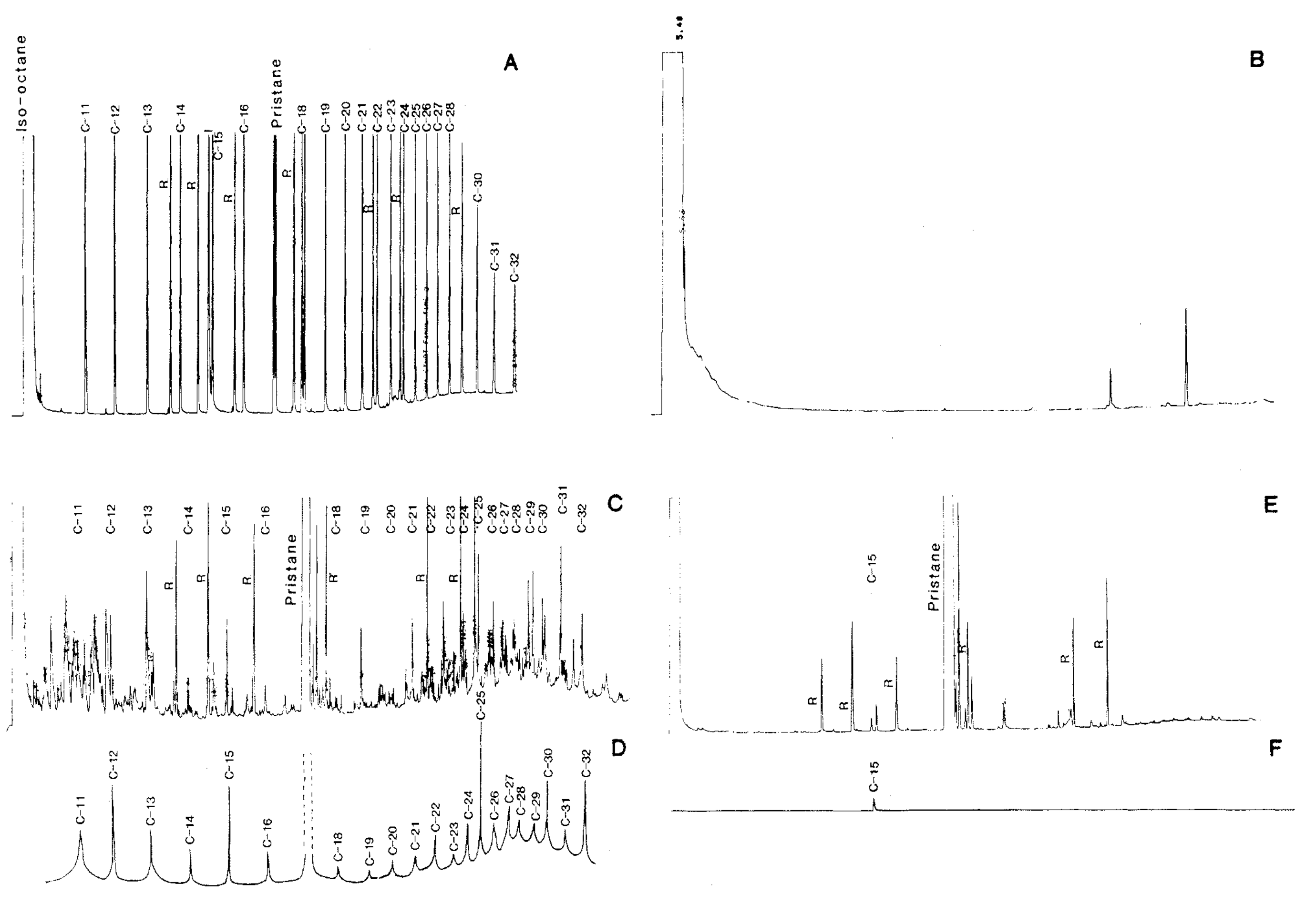

Fig. 1. Spectra of oil samples from Prudhoe Bay crude oil and guts of birds. (A) Prudhoe Bay crude oil standard showing regular *n*-alkane peaks of carbon chains and the internal (I) and reference (R) standards. (B) Field blank. (C) Gut sample from a fork-tailed storm-petrel containing fossil fuel hydrocarbons. (D) A tracing of (C) showing 21 *n*-alkane peaks matching those of the crude oil standard. The regular pattern, matching *n*-alkane peaks, and unresolved high molecular weight mixture distinguish this sample as containing petroleum. (E) Gut sample from a fork-tailed storm-petrel not containing fossil fuel hydrocarbons. (F) Tracing of (E) showing only one matched *n*-alkane peak. The absence of a regular pattern, few matched *n*-alkane peaks, and the lack of the unresolved high molecular weight mixture distinguish this sample as not containing petroleum.

correlated positively with the number of oil spills in the vicinity reported to the U.S. Coast Guard. Fishing and recreational boats are common around St. Lazaria Island. Tatoosh Island, situated near the Strait of Juan de Fuca, is also passed frequently by boats and tankers. The Aleutian Islands are the most remote of these sites from boat traffic.

The number of oil spills near the Barren Islands decreased from 1980 to 1982: five spills were reported within 200 km of this site in 1980, two in 1981, and none within 400 km in 1982. To determine whether the incidence of dirty samples correlated with pollution events, I divided each year into 2-week intervals ($n = 8$) surrounding the dates for which I had analyzed samples; I selected 2-week periods because hydrocarbon concentrations in the ocean return to baseline levels within days or weeks after an oil spill (*11*). Within each 2-week interval I noted whether a spill had occurred and whether the frequency of gut samples containing petroleum increased (Fig. 2). If an increase in the number of contaminated samples occurred within 2 weeks after a spill, I considered this a positive correlation. If no increase occurred during 2-week periods when no spills were reported, I also considered this a positive correlation.

For the 1980 comparison I used 166 samples collected in 1980 that were prepared and analyzed with the gas chromatograph without the internal standard (HMB) added. Therefore they could not be used to measure the amount of fossil fuel hydrocarbons present (and are not included in the 207 sample figure), but the number of *n*-alkane peaks could be matched to the Prudhoe Bay crude oil standard to determine whether petroleum was present. The 95 to 100 percent matching peak criterion was used to identify dirty samples.

The frequency of petroleum in gut samples of fork-tailed storm-petrels correlated significantly with known pollution events (Sign test; $P = 0.035$; $n = 8$); seven of eight times the frequency either increased after reported spills ($n = 4$) or did not increase during intervals with no spills ($n = 3$). Only once after a spill was no increase evident; this spill was the most distant of the seven spills and covered 1.82 km. I ranked spills by their size and distance from the Barren Islands and found that more fork-tailed storm-petrels had ingested petroleum after large close spills than after small distant ones (Spearman rank = 0.71; $P = 0.05$; $n = 7$).

In addition to oil, other pollutants can be monitored by seabirds to provide information about oceanic conditions. Seabirds also ingest plastic substances (*12*), and plastic particles (mean size, 0.27 by 0.13 mm;

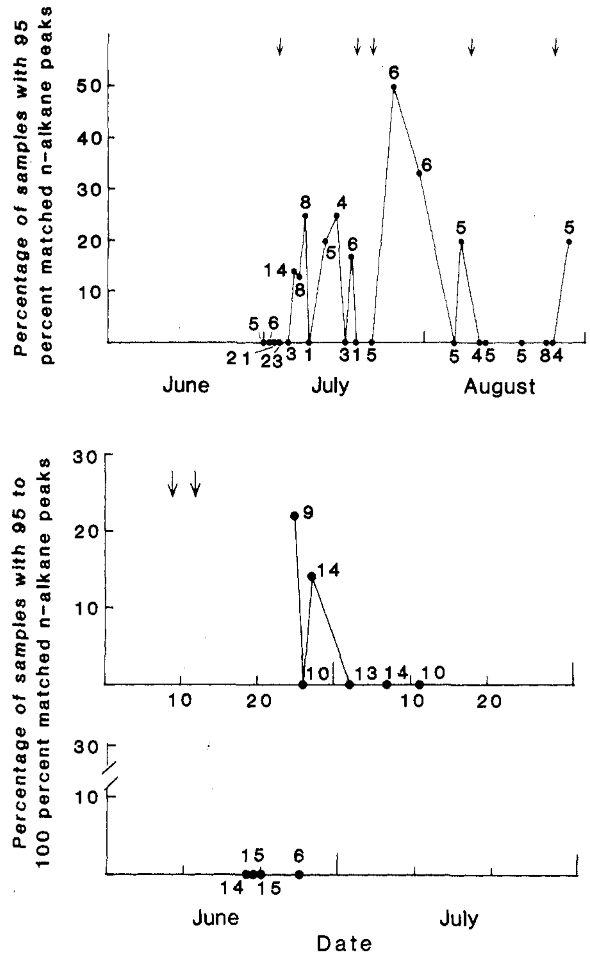

Fig. 2. The percentage of samples from guts of fork-tailed storm-petrels containing fossil fuel hydrocarbons from the Barren Islands, Alaska, for 1980 (top), 1981 (middle), and 1982 (bottom). Samples were scored for the presence or absence of fossil fuel hydrocarbons on the basis of the criterion of 95 to 100 percent *n*-alkane peaks matching a Prudhoe Bay crude oil standard. Oil spills within 400 km of the Barren Islands are indicated by an arrow (no spills were reported in 1982). The number of samples analyzed for each day is given for each data point.

Table 1. Number of spectra of gut samples from fork-tailed and leach's storm-petrels showing various numbers of *n*-alkane peaks matching a Prudhoe Bay crude oil standard. The number of samples is given in parentheses for each location.

Matching *n*-alkane peaks (number)	Spectra (number) with matching *n*-alkane peaks: Barren Islands 1980 (n = 8)	Barren Islands 1981 (n = 70)	Barren Islands 1982 (n = 50)	Aleutian Islands, 1981 (n = 32)	St. Lazaria Island, 1981 (n = 28)	Tahoosh Island, 1981 (n = 19)	Total
0							0
1		3	1		1		5
2		3	3		1	2	9
3		2	6	1	1		10
4		1	5	1			7
5	1	4	6	4	1		16
6		5	5	1			11
7		5	2	2			9
8	1	5	2	1	1		10
9		5	2	1		1	9
10		5	4	1	3		13
11		5		2		1	8
12	1	2	7	1	4	2	17
13		2	2	2	2	1	9
14	1	4	1	1	2	4	13
15		5	2	2	1	2	12
16	2	4	2	3	5	1	17
17	1	1		1		2	5
18		4		3	2	1	10
19		2		4	1	1	8
20	1	2			2	1	6
21		1		1	1		3

$n = 4$) were regurgitated with food in 1 percent (15 of 1709) of the fork-tailed storm-petrel samples; one bird regurgitated 12 particles. Sooty storm-petrels that forage in major tanker lanes near the Hawaiian Islands frequently ingest plastic; 6 of the 14 collected samples (43 percent) contained plastic. However, no samples ($n = 101$) from leach's storm-petrels contained plastic.

These results indicate that Procellariiformes can serve as effective monitors of marine environmental quality. From chemical analysis of gut samples, levels of oil pollution can be measured and compared between different geographical areas, and degradation of water quality within a particular region could be tracked. For example, plastics detected in gull and tern pellets on the eastern coast of the United States were eventually traced to several industrial sources (*13*). Because pollution from petroleum, plastics, and other contaminants is likely to be patchy and difficult or costly to sample directly, use of seabirds to monitor oceanic water quality and environmental change could contribute to protecting our marine resources.

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14. I thank E. Davies, T. Friedman, V. Louden, D. McDonald, A. Power, W. Reid, personnel from the Fish and Wildlife Service in Anchorage and Hawaii, and D. Kalman and the staff of the Trace Organic Laboratory of the University of Washington for field or laboratory assistance; J. Imm, C. Manen, R. T. Paine, and B. Peterson for technical assistance; and R. Barrick for the Prudhoe Bay crude oil standard chromatogram. Funded by the Bureau of Land Management through an interagency agreement with NOAA as part of the Outer Continental Shelf Environmental Assessment Program.

6 May 1985; accepted 4 September 1985

Retinal Dystrophy: Development Retarded by Galactose Feeding in Spontaneously Hypertensive Rats

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Retinal photoreceptor cell dystrophies have been widely observed in humans and in animals, but pathogenetic mechanisms are known in only a few such disorders, and successful therapeutic intervention has been reported in fewer still. Spontaneously hypertensive albino rats develop a retinal photoreceptor cell dystrophy with onset late in the first year or early in the second year of life. Between 60 and 70 percent of the animals are affected. A substantial reduction in the prevalence and severity of the dystrophy occurred in such animals whose diet contained 30 percent (by weight) D-galactose. Neither an inhibitor of the enzyme aldose reductase, present in the diet, nor diabetes mellitus, induced by streptozotocin, had any statistically significant influence on the dystrophy. Ambient light and systolic blood pressure levels also did not seem to influence the course of the disorder. The mechanism by which galactose exerts its effect is unknown, but a mutant enzyme with an elevated Michaelis constant (K_m) for galactose is plausible.

GENETICALLY DETERMINED RETINAL dystrophies have been described frequently in humans and in several animal species (*1*). In only a few cases, however, do we have sufficient anatomic or biochemical information to propose a hypothesis for the cause of the disorder. In the Royal College of Surgeons (RCS) rat strain, initial anatomical findings include an elongation of the outer segments of the retinal photoreceptor cells (*2*) due to a failure of the retinal pigment epithelial (RPE) cells to phagocytose the distal tips of the outer segments (*3*). Ultimately, the photoreceptors degenerate; complete degeneration and disorganization of the entire retina follows. In a study of tetraparental chimeras, Mullen and LaVail (*4*) demonstrated that the defect resides in the RPE cells and not in the photoreceptors. A specific biochemical defect has been demonstrated in the photoreceptor cells of C3H mice and of Irish setter dogs with a hereditary rod-cone dysplasia (*5*). In these animals, deficient activity of cyclic guanosine monophosphate (cGMP) phosphodiesterase in the retinal outer segment permits large and eventually toxic (*6*) concentrations of cGMP to accumulate in the photoreceptor cells. In a rare human disorder, gyrate atrophy of the choroid and retina, absence or defective functioning of the enzyme ornithine aminotransferase leads to an accumulation of the amino acid ornithine, presumably resulting in retinal and choroidal degeneration (*7*). This is the only genetic retinal dystrophy for which claims have been made of effective dietary treatments, including diets low in protein and in the basic amino acid arginine (*8*), or, in some cases, dietary supplementation with large doses of pyridoxal phosphate (*9*), an essential cofactor for the defective enzyme.

We report here the dietary modulation of another retinal dystrophy (*10*) in the spontaneously hypertensive (SHR) strain of albino rats (*11*). A photoreceptor dystrophy was initially described in SHR rats by Mizuno *et*

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