Biological Services Program

FWS/OBS-79/23

JULY 1979

ECOLOGICAL AND PHYSIOLOGICAL/ TOXICOLOGICAL EFFECTS OF PETROLEUM ON AQUATIC BIRDS

A Summary of Research Activities FY76 through FY78

Interagency Energy-Environment Research and Development Program

OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY

and Wildlife Service

U.S. Department of the Interior

The Biological Services Program was established within the U.S. Fish and Wildlife Service to supply scientific information and methodologies on key environmental issues that impact fish and wildlife resources and their supporting ecosystems. The mission of the program is as follows:

- To strengthen the Fish and Wildlife Service in its role as a primary source of information on national fish and wildlife resources, particularly in respect to environmental impact assessment.
- To gather, analyze, and present information that will aid decisionmakers in the identification and resolution of problems associated with major changes in land and water use.
- To provide better ecological information and evaluation for Department of the Interior development programs, such as those relating to energy development.

Information developed by the Biological Services Program is intended for use in the planning and decisionmaking process to prevent or minimize the impact of development on fish and wildlife. Research activities and technical assistance services are based on anaysis of the issues, a determination of the decisionmakers involved and their information needs, and an evaluation of the state of the art to identify information gaps and determine priorities. This is a strategy that will ensure that the products produced and disseminated are timely and useful.

Projects have been initiated in the following areas: coal extraction and conversion; power plants; geothermal, mineral, and oil-shale development; water resource analysis, including stream alterations and western water allocation; coastal ecosystems and Outer Continental Shelf development; and systems inventory, including National Wetland Inventory, habitat classification and analysis, and information transfer.

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PREFACE

This report summarizes the research conducted from 1 July 1975 to 30 September 1978 by the U.S. Fish and Wildlife Service (FWS) about the effects of petroleum on aquatic birds. The following assessments were made:

1. Effects of oiling on hatchability of eggs.
2. Effects of oil ingestion on physiological condition and survival of birds.
3. Effects of oil ingestion on reproduction in birds.
4. Accumulation and loss of oil by birds.
5. Development of analytical methods for identification and quantification of oil breakdown products in tissues and eggs of ducks.

Research was funded by the Environmental Protection Agency (EPA) Federal Interagency Energy-Environment Research and Development Program and the FWS Environmental Contaminants Evaluation Program, with Dr. Allan Hirsch, Chief, Office of Biological Services, serving as FWS coordinator. Coastal Ecosystems Project contacts were Drs. William Palmisano and Howard Tait. Mr. Clinton Hall served as EPA's coordinator. The research was conducted at the Patuxent Wildlife Research Center, under the direction of Dr. Lucille F. Stickel and Dr. Michael P. Dieter.

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ECOLOGICAL AND PHYSIOLOGICAL/TOXICOLOGICAL EFFECTS OF PETROLEUM ON AQUATIC BIRDS

INTRODUCTION

Oil and gas development and exploration in marine coastal areas and the Great Lakes will result in unavoidable spills of polluting oil. Although large oil spills may kill thousands of birds and stimulate much public concern, the bulk of oil that reaches aquatic environments is released in the course of normal operations, with a total input into the world's oceans estimated at 6 million metric tons per year. The effects of sublethal low-level oil pollution may be more deleterious to bird populations over the long term than the spectacular bird kills resulting from oil spills.

The physiological and ecological effects of oil on waterbirds were examined in a series of laboratory and field experiments, including studies of the effects of oiling on hatchability of eggs; the effects of an oil-contaminated diet on physiological condition, reproduction, and survival; and the accumulation of oil in body tissues. Chemical methodology was developed in support of these studies.

EFFECTS OF OILING ON HATCHABILITY OF EGGS

Very small quantities of oil applied to aquatic bird eggs in the laboratory caused embryo mortality (fig. 1). As little as 5 μl (about one drop) of South Louisiana crude oil, No. 2 fuel oil, Kuwait crude oil, Bunker C fuel oil, or Prudhoe Bay crude oil applied to the shell surface on the eighth day of incubation reduced hatching of mallard eggs by 90%, 70%, 68%, 62%, and 26%, respectively. No embryos survived application of 50 μl of any of the tested oils. Fifty microliters of oil represented approximately 1 ppm of the egg.

When 50 μl of an alkane mixture of compounds naturally occurring in crude oils (paraffins) or propylene glycol was applied to the shell surface, embryo mortality did not occur even though the shell surface was coated. This indicated that some other component of the oil was affecting the embryos, and that they were not dying because of oxygen deprivation caused by clogged shell pores.

The toxicity of various oils to embryos is not confined to mallards. Common eider, great black-backed gull, laughing gull, Louisiana heron, and Sandwich tern eggs were oiled artificially both in the field and the laboratory. Variations in toxicity from that demonstrated for mallards can be attributed to differences in embryo size, which change the dose-to-weight relationship, and differences in ages of embryos at treatment (younger embryos are more sensitive). As an example, mortality was 26% in 16 clutches of naturally incubated common eiders treated in the nest with 20 μl of No. 2 fuel oil and opened 7 days after treatment (table 1). Mortality was 60% among 25 clutches of great black-backed gulls (table 2). Embryos of both species are approximately twice the size of mallard embryos and those tested were of mixed ages at the time of treatment. Mortality of 8-day-old mallard embryos treated with 20 μl of No. 2 fuel oil and artificially incubated was 100%.

Thirty clutches of great black-backed gulls treated with 20 μl of fuel oil were incubated to term by the parents on the Isles of Shoals, Maine. Hatching success was again one-half that of controls and production of fledglings was one-third that of controls. External environmental factors did not appreciably modify the toxic effects of the oil in this or other field studies.

No. 2 fuel oil and Prudhoe Bay crude oil were weathered over water in outdoor troughs at the Patuxent Center. After 2 weeks of

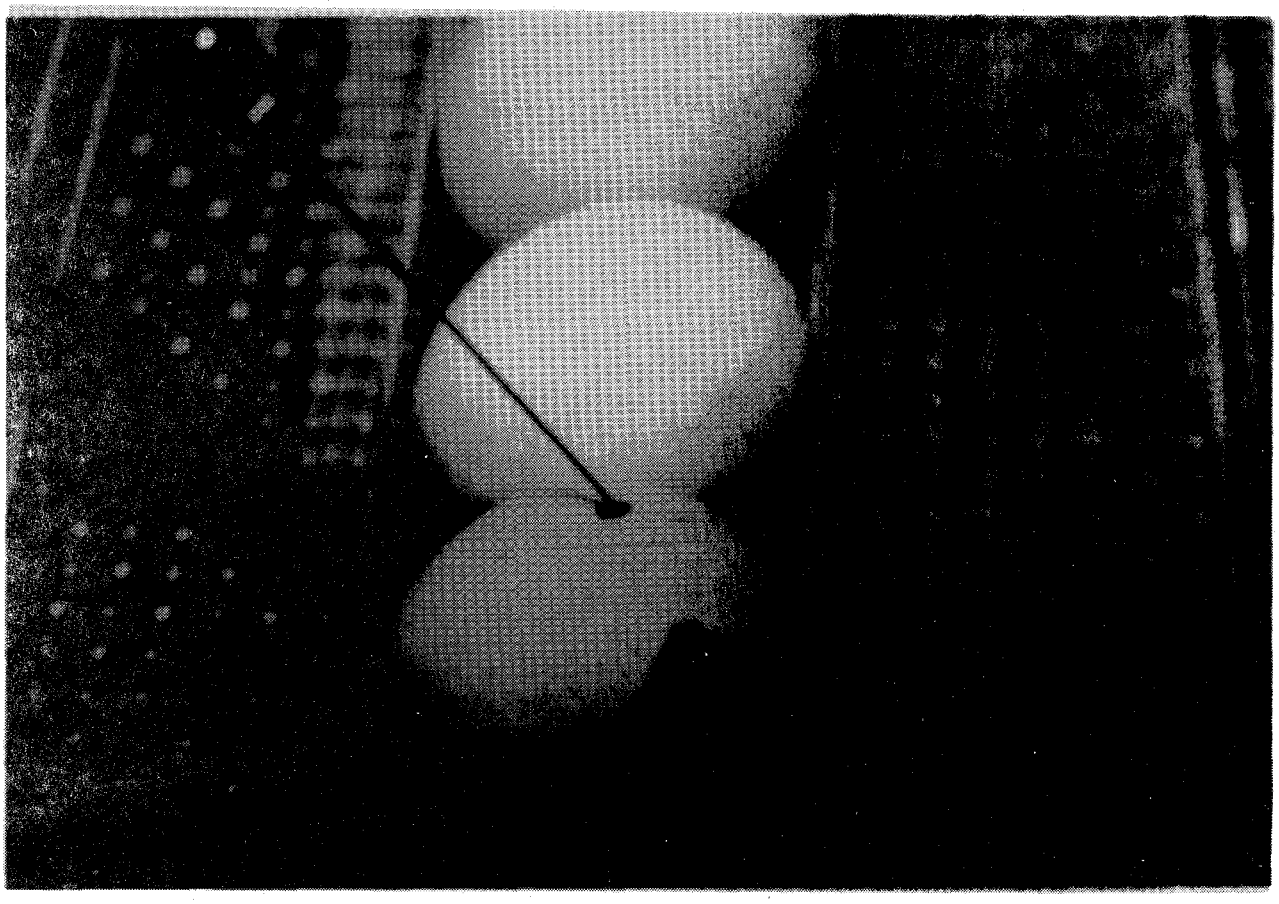

Figure 1. Very small amounts (5 to 50 μl) of oil were applied to mallard eggs with chemist's syringe, as shown.

Table 1. Effects of No. 2 fuel oil on embyos of the common eider (Albers and Szaro 1978).

Treatment	Nests[1]	No. of eggs	% alive	Mean clutch size	% of clutch alive: Mean of transformed percentages[2]	% of clutch alive: Mean % [3]
Control	13	60	98	4.6	75.42	93.7
No. 2 fuel oil (5 μl)	19	84	94	4.4	72.54	91.0
No. 2 fuel oil (20 μl)	16	72	74	4.5	59.40	74.1

[1]Three nests were found; 9 nests were abandoned or destroyed by predation.

[2]Arcsine transformation for binomial proportions; angle equals arcsine $\sqrt{}$ percentage. Significant one-way analysis of variance, $P \leq 0.05$; 20 μl group significantly different from control group, t-test, $P \leq 0.025$.

[3]Mean of transformed percentages converted back to percent.

Table 2. Embryo survival in naturally incubated great black-backed gull eggs 8 days after treatment with No. 2 fuel oil (Coon et al. 1979).

Treatment	No. of clutches	Survival Index	No. of eggs	Condition of embryo: Alive	Dead	% alive
Control	28	95.8	81	72	9	88.9
No. 2 fuel oil (5 μl)	26	90.3	72	58	14	80.6
No. 2 fuel oil (20 μl)	25	32.7*	72	29	43	40.3

Most clutches contained three eggs; however, some contained only two at the time of treatment. For each clutch, a percentage of the total embryos alive 8 days after treatment was computed. Clutch survival data were evaluated statistically after angular transformation, arcsin $\sqrt{X}$. This transformation is applicable to binomial data expressed as percentages and covering a wide range of values (Steel and Torrie 1960). The Survival Index reported can be described by the following expression: $[\sin (1/n \Sigma \arcsin \sqrt{\%})]^2$ and is a transformation back to the original scale. Statistical comparisons were made on the transformed scale, rather than on the reported values.

*Significantly different from control, $P<0.05$ (Student's t test).

weathering, No. 2 fuel oil was significantly less toxic than fresh oil when applied to mallard eggs. Prudhoe Bay crude oil was less toxic than its fresh counterpart after 3 weeks of weathering. Even after the oils weathered 4 weeks, however, 20 μl of each still caused 50% mortality when applied to mallard eggs (fig.2).

The minute quantities of oil that produce marked reductions in embryonic survival suggest that oil pollution could seriously affect marine and estuarine bird populations by transfer of oil from the plumage of incubating birds to their eggs. Laughing gulls were captured at their nest sites in Texas to examine this possibility. Forty-two were treated with 2.5 ml of No. 2 fuel oil applied with a syringe to the feathers over and surrounding their brood patches. Twenty additional birds were treated with water. All were released immediately after treatment. After 5 days, their eggs were collected and examined. Embryo mortality was 41% in the eggs incubated by the oiled gulls, but only 2% in those incubated by the water-treated gulls (table 3).

Paired mallards were kept in pens containing water troughs in which they could swim during the breeding season. Prudhoe Bay crude oil was added to the water during the first week of incubation. One-third of the troughs were treated with 100 ml of oil per square meter of surface area, another one-third were treated with 5 ml of oil per square meter, and the last group were left untreated. Hatching success was 45%, 85%, and 95% respectively.

Other indications of toxicity include teratogenic effects (production of malformed individuals) when oil is applied to eggs during the first few days of embryo development. Treatment of mallard eggs at 24 hr of development with 5 μl of crude oil produced a significant number of abnormal survivors (table 4). The most common abnormalities included deformed bills, incomplete ossification of the wing or foot bones, reduction in size of the liver lobes, and stunting. Teratogenicity was increased when vanadium, nickel and mercury, metals normally found in petroleum, were added.

Mortality was greater when artificially formulated mixtures of aromatic compounds were applied to the egg surface than in controls

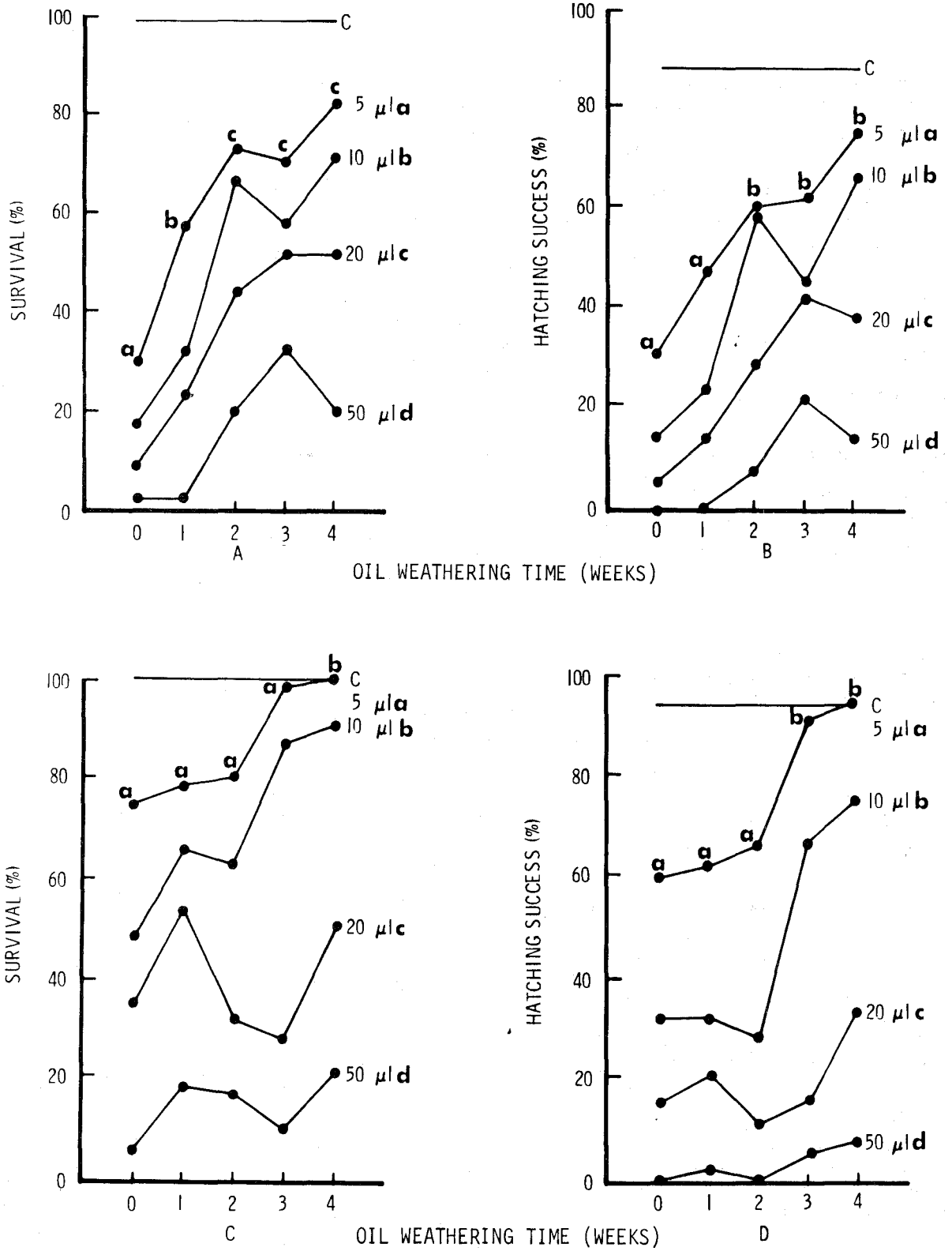

Figure 2. Survival 6 days after treatment and hatching success of mallard embryos treated (on the 8th day of incubation) with petroleum hydrocarbons (A and B, No. 2 fuel oil; C and D, Prudhoe Bay crude). Note: Treatments or weeks that do not have a letter in common were significantly different at $\alpha \leq 0.05$, using Tukey's multiple comparison procedure. Differences between controls and 5 µl of fresh oil (weathered 0 weeks) were significant at $\alpha \leq 0.05$, using a binomial test. Sample size is 47 for all groups except the controls, where $n=94$ (Szaro et al. 1979).

or those treated with the alkanes or paraffin components. Mortality was particularly enhanced by the presence of the tetracyclic aromatic chrysene even at half its normal concentration in oil (0.2% in Kuwait crude oil and 0.5% in South Louisiana crude oil).

Table3. Embryonic mortality in eggs of laughing gulls with breast feathers treated with 2.5 ml No. 2 fuel oil (King and Lefever unpubl).

		Dead		Infertile	
Group	N[a]	No.	%	No.	%
Control	53	1	2	2	4
Oil-treated	105	43	41[b]	2	2

[a]N=number of eggs recovered after 5 days of incubation.

[b]Significantly different from controls, $P<0.01$, χ^2 test.

EFFECTS OF OIL INGESTION ON PHYSIOLOGICAL CONDITION AND SURVIVAL OF BIRDS

In two 6-month studies, adult mallards were fed South Louisiana crude oil (SLC) and an artificial reconstituted aromatic mixture (RAM) composed of 10 aromatic and 9 aliphatic compounds typical of those found in South Louisiana crude oil. SLC was fed at 0.25% and 2.5% of the total diet and the RAM was fed at 0.04% and 0.4% of the diet (levels that represent the aromatic content of diets containing 0.25% and 2.5% oil). Adult mallards could realistically ingest these concentrations of oil at spill sites. All birds maintained their body weights and ingested contaminated food at rates comparable to ingestion of clean food by control animals. There was no mortality in either study. Indocyanine green dye, a compound that is metabolized by the liver, was used

Table 4. Effects of South Louisiana crude oil on mallard duck embryos (Hoffman 1978).

			Crude oil	
	Control	Paraffin	1 µl	5 µl
Number treated	65	65	65	65
Percentage survival (days 3-18)	97	94	65[a]	9[a]
Sex ratio, M:F	45:55	44:56	53:57	50:50
Embryonic weight (g)				
Male	15.72±1.78[b]	16.34±1.49	16.29±1.89	15.83±0.49
Female	15.75±2.03	15.98±1.58	15.33±1.77	14.90±3.15[c]
Combined	15.74±1.90	16.14±1.54	15.74±1.82	15.73±1.84
Crown-rump length (mm)				
Male	84.1±3.7	84.9±4.0	84.9±3.8	86.7±5.1
Female	82.7±3.7	83.4±3.0	80.1±4.4	70.2±3.8[c]
Combined	82.8±3.7	84.1±3.5	82.2±4.1	77.3±4.5[c]
Bill length (mm)				
Male	13.2±0.9	13.3±0.5	13.0±0.6	13.0±0.6
Female	13.1±0.6	12.9±0.6	12.7±0.7[c]	10.8±0.9[c]
Combined	13.1±0.7	13.1±0.6	12.8±0.7[c]	11.9±0.8[c]
Percentage that were abnormal survivors[d]	4.8	3.3	4.8	66.7[a]

[a]Significantly different from control and paraffin-treated groups by χ^2, $p<0.01$.

[b]Mean ±SD.

[c]Significantly different by one-way analysis of variance ($p<0.01$) and the Duncan multiple range test ($p<0.05$).

[d]These included abnormal conjoined (1.6%) and stunted (3.2%) in the control group; hydrocephaly with microphthalmia and beak defect (1.6%) and incomplete ossification of ribs and vertebrae (1.6%) in the paraffin group; cervical vertebrae missing (2.4%) and incomplete ossification of sacral vertebrae (2.4%) in the 1-µl of crude oil group; incomplete ossification of the phalanges (16.7%); incomplete ossification of the ischium (16.7%), severe edema with blisters (16.7%). and abnormal feather formation (16.7%) in the 5-µl of crude oil group.

to measure liver function in the mallard drakes fed the RAM. Plasma clearance rates were enhanced in the drakes fed the RAM at 0.4% of the diet, suggesting that adult waterfowl may be able to increase liver function to eliminate high concentrations of petroleum hydrocarbons. Although several plasma enzymes (enzymes that appear in the blood because of damage to specific organs) and electrolytes were monitored monthly in both studies, none were elevated above control levels. Adult waterfowl are apparently able to adapt to and tolerate high concentrations of petroleum hydrocarbons in their diet when not otherwise stressed. However, other adult mallards, given seawater as drinking water and subjected to mild cold stress (3° C) while being fed food contaminated with 3% Kuwait or South Louisiana crude oil, died (table 5).

Mallard ducklings survived from hatching to 8 weeks of age on diets containing 0.025% to 5% South Louisiana crude oil or No. 2 fuel oil. Those fed 2.5% and 5% oil, however, were stunted and failed to develop flight feathers (fig. 3). Subtle biochemical and behavioral changes were detected in ducklings fed as little as 0.25% oil. It is not surprising that young birds would be more seriously affected than adults because they are in the critical rapid growth phase.

Table 5. The effects of petroleum-contaminated diets on the mortalities of seawater-adapted ducks maintained for 50 days at 27° C followed by a 50-day period of continuous mild cold stress at 3° C (adapted from Holmes et al. 1978).

	No. of birds	Mortality (% of original population) Before cold stress	During cold stress	Total
Freshwater-maintained controls	10	0	20	20
Seawater-maintained controls	10	0	60	60
South Louisiana crude oil	9	22.2	66.7	88.9
Kuwait crude oil	9	0	66.7	66.7
No. 2 fuel oil	14	35.7	42.7	78.4

EFFECTS OF OIL INGESTION ON REPRODUCTION IN BIRDS

Mallard hens fed diets containing 2.5% South Louisiana crude oil (25,000 ppm) for a 6-month period produced 50% as many eggs as controls. Over a 90-day period, the oil-dosed birds laid an average of 35 eggs per hen, compared with 69 for controls (table 6). Although fewer eggs were laid by the oil-dosed birds, those that were laid hatched as well as control eggs when artificially incubated, and the hatchlings weighed as much as control hatchlings. In contrast, hens on diets containing 0.25% (2,500 ppm) of oil performed nearly as well as controls.

Dr. W. Holmes (under contract to the Patuxent Wildlife Research Center) reported similar results in studies with South Louisiana and Kuwait crude oils. Egg laying was not affected by 1% of either oil in the diet. Egg laying decreased by 75% on a diet containing 3% South Louisiana crude oils and completely ceased on a diet containing 3% Kuwait crude oils.

Paired mallards were fed vanadium, a metal contaminant of crude oils. The vanadium accumulated to higher concentrations in the bone and liver than in other tissues. Concentrations in the bones of hens were five times those in the bones of drakes, suggesting an interaction between vanadium and calcium mobilization in laying hens. Lipid metabolism was altered within 3 weeks in laying hens fed 100 ppm vanadium and within 12 weeks in hens fed 10 ppm vanadium. Vanadium concentrations in crude oil can range up to 1,400 ppm.

Table 6. Mean egg production of mallards fed diets containing South Louisiana crude oil. Total numbers of eggs laid in parentheses (Coon and Dieter unpubl).

Treatment	Sampling period Days 1-30	Days 1-60	Days 1-90
Control	24.6^{a} * (197)	49.0^{a} (392)	69.0^{a} (552)
10,000 ppm paraffin	23.6^{a} (189)	$47.1^{a,b}$ (377)	68.6^{a} (549)
2,500 ppm SLC	$19.3^{a,b}$ (154)	$37.1^{a,b}$ (297)	$52.1^{a,b}$ (417)
25,000 ppm SLC	11.0^{b} (88)	27.3^{b} (218)	34.9^{b} (279)

*Means followed by different letters are significantly different for a given sampling period, Scheffe's test, $P<0.05$.

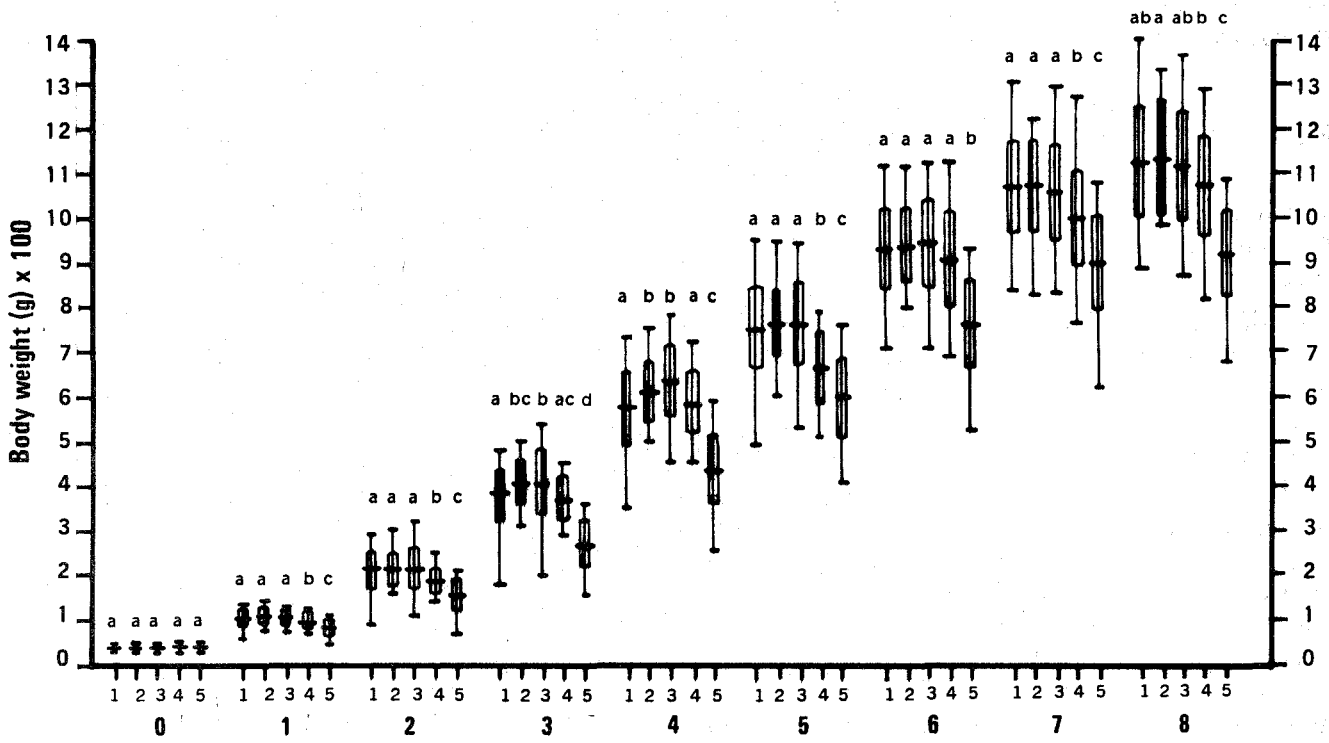

Figure 3. Growth in mallard ducklings fed (1) control diet; (2) 0.025% South Louisiana crude oil (SLC); (3) 0.250% SLC; (4) 2.5% SLC; and (5) 5.0% SLC. Treatments within a given age group that do not have a letter in common were significantly different at $P \leq 0.05$ by Scheffe's (1959) procedure for pairwise comparisons (Szaro et al. 1978).

ACCUMULATION AND LOSS OF OIL BY BIRDS

After consideration of several invertebrate and waterfowl species, the freshwater crayfish and mallard were selected for food-chain studies. Both can be successfully maintained in sufficient numbers in the laboratory and are available from commercial suppliers. Crayfish are accepted by mallards as a food item.

Food-chain studies were performed to estimate the uptake of aromatic compounds and their distribution in mallard tissue after ingestion of contaminated crayfish. Crayfish were exposed to the water-soluble fraction of No. 2 fuel oil containing known quantities of [^{14}C] naphthalene. (The water-soluble fraction was prepared by gently stirring a mixture of the oil and water for 20 hr and siphoning off the aqueous layer.) After exposure, the live crayfish were fed to ducks. After an appropriate time interval, the birds were sacrificed and their tissues examined for radioactivity.

Radioactive naphthalene was readily taken up and underwent biomagnification in the crayfish. Accumulation of radioactivity in ducks fed the crayfish was greatest in the gall bladder, followed by fat, kidney, liver and blood (table 7).

Table 7. Radioactivity in tissues of male and female mallard ducks fed ^{14}C-naphthalene-No. 2 fuel oil contaminated crayfish (Tarshis unpubl.).

	Naphthalene (ppm)	
Tissues	♂	♀
Gall bladder	1.040	0.496
Fat	0.149	0.114
Kidney	0.045	0.058
Liver	0.045	0.032
Blood	0.037	0.057
Testes	0.011	-----
Egg	----	0.020
Oviduct	----	0.013

DEVELOPMENT OF ANALYTICAL METHODS FOR IDENTIFICATION AND QUANTIFICATION OF OIL-BREAKDOWN PRODUCTS IN TISSUES AND EGGS OF DUCKS

Efforts of chemists involved in this project have been directed toward several goals:

- The development of methods for the detection and analysis of hydrocarbons in avian tissue
- The development of methods of quantification of individual compounds and complex mixtures in avian tissues
- Provision of analytical support for biological studies

A number of problems are encountered in the analysis of petroleum hydrocarbons in avian tissue. By their ubiquitous nature, these hydrocarbons pose continuing contamination problems for sample analysis. The heterogeneity of the mixtures necessitates the use of complex extraction and cleanup procedures. The presence of hundreds of compounds creates a need for high-resolution instruments.

During our early attempts to analyze samples, very high levels of contamination were detected. To reduce contamination, glass extraction thimbles cleaned in chromic acid were substituted for paper thimbles. Later, in simplifying our procedures, we eliminated thimbles altogether, substituting a Polytron tissue homogenizer/extractor for Soxhlet extraction. The use of nitrogen gas to blow down samples was abandoned in favor of a flash evaporator. Glassware was at first soaked in chromic acid; this was an effective but hazardous procedure. Chromic acid was replaced by an equally effective but safer technique of soaking the glass in an ultrasonic bath with 2% 'Micro' solution.

Briefly, the method of analysis that is being used in our laboratory is as follows.

The sample is cut into small pieces and mechanically extracted with a homogenizer. Bases are partitioned into acid from pentane and analyzed by gas chromatography/mass spectrometry. After the extraction of bases, pentane extracts from fat samples are saponified. The saponified mixture is partitioned into hexane.

The hexane or pentane layer from samples of fat, liver, and kidney (<0.5 g lipid) are cleaned up on Florisil; the aliphatic and aromatic hydrocarbons are separated on a silicic acid column; and the residues are screened and analyzed by gas chromatography and gas chromatography/mass spectrometry/data system.

Livers, kidneys, and fat from untreated mallard drakes and drakes fed 0.4% of the reconstituted aromatic mixture for 6 months were analyzed in our laboratory. Significantly higher residues were found in the tissues of ducks fed the aromatic mixture than in tissues from controls.

Researchers at the University of New Orleans developed a hydrocarbon analysis procedure similar to the one in use at our laboratory while under contract to the Patuxent Wildlife Research Center. Livers and kidneys from mallard ducklings and adult birds fed South Louisiana crude oil were analyzed. There were no significant differences in hydrocarbon accumulation in tissues between adult males and females. Hydrocarbon concentrations in both ducklings and adults increased from the control group through those fed 2.5% oil in the diet.

The research group at the University of New Orleans also analyzed tissues from four birds killed at the *Amoco Cadiz* oil spill site off the coast of France. One, a shag (cormorant), was heavily contaminated with hydrocarbons; the other three, a herring gull, a razorbill, and a guillemot, contained smaller amounts.

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APPENDIX A

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APPENDIX B

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APPENDIX C
MILESTONE SCHEDULE

Milestone / Fiscal year	FY75	FY76	FY77	FY78
1. Acquired staffing	•	•	•	
2. Development of analytical methods for identification and quantification of oil breakdown products in tissues and eggs of ducks.				
Preliminary report		•		
Final report				•
3. Effects of oil ingestion on reproduction in birds				
Annual report		•	•	
Final report				•
4. Effects of oil ingestion on physiological condition and survival of birds				
Annual report		•	•	
Summary report of work on ducks with report of pilot comparisons with seabirds				•
5. Effects of oiling on hatchability of eggs				
Annual report		•	•	
Final report				•
6. Accumulation of oiled birds				
Annual report			•	•
Summary report of work on ducks				•
7. Assessment of joint action of oil and toxic chemicals on survival and reproduction				
Report on experiments			•	•