

The Effects of Petroleum of Different Stages of Incubation in Bird Eggs

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Breeding birds with petroleum on their feet or plumage may transfer the petroleum to incubated eggs (RITTINGHAUS 1956; HARTUNG 1965; BIRKHEAD et al. 1973). Recent work with artificially incubated waterfowl eggs has shown that external applications of petroleum are very toxic to embryos. ALBERS (1977) and SZARO et al. (in preparation) applied microliter quantities of No. 2 fuel oil and Southern Louisiana crude oil to the surface of artificially incubated eggs of mallard ducks (*Anas platyrhynchos*) after 8 days of incubation. Both oils caused severe embryonic mortality. SZARO and ALBERS (1977) performed a similar experiment with artificially incubated eggs of common eider ducks (*Somateria mollissima*) that were at different stages of incubation. The mean age at treatment of eider embryos that died following application of 20 μ l of No. 2 fuel oil was much less than those that survived the application (4.3 days versus 16.1 days). Although the results of the eider study suggest that embryos are most sensitive to oil early in incubation, the evidence was not conclusive. The present study was designed to better evaluate the relationship between the stage of incubation and the toxic effects of petroleum.

METHODS

Two experiments were conducted, one with No. 2 fuel oil¹, and one with Southern Louisiana crude oil¹. The methods were identical. For each oil, domestic mallard eggs were randomly divided into 6 treatment groups and a control group of 40 eggs each. All eggs were placed in a commercial incubator. Each group was assigned for treatment on day 2, 6, 10, 14, 18, or 22 of the 26 day incubation period. On the designated day each group was candled to remove infertile eggs or eggs with dead embryos. Five microliters of oil was then applied by microliter syringe to the surface of each egg according to the method of ALBERS (1977). The control group was candled on the day of each treatment to remove eggs with dead embryos.

The treated eggs and control eggs were candled every 3-4 days after treatment. Eggs with dead embryos were opened and the embryos were aged and examined for gross malformations. Ducklings

¹Obtained from the American Petroleum Institute

were weighed 24 hours after hatching and examined for gross malformations. A sample of ducklings from each experimental group was frozen for future petroleum residue analysis.

RESULTS

Hatchability of fertile mallard eggs exposed to 5 μ l of No. 2 fuel oil or 5 μ l of Southern Louisiana crude oil decreased as the age of the embryo at treatment decreased (Tables 1 and 2). The embryos were very sensitive to both petroleum substances during the first 10 days of incubation, although Southern Louisiana crude oil killed considerably more embryos than No. 2 fuel oil during this period.

TABLE 1

Hatching success of fertile mallard eggs treated with 5 μ l of No. 2 fuel oil after 2, 6, 10, 14, 18, or 22 days of incubation

Age at treatment	Number of eggs	Hatched	Failed to hatch	Percent hatching success
Control	40	32	8	80
2-day	40	5	35	13*
Control	38	32	6	84
6-day	40	13	27	33*
Control	38	32	6	84
10-day	40	27	13	68**
Control	38	32	6	84
14-day	40	33	7	83
Control	38	32	6	84
18-day	40	32	8	80
Control	37	32	5	86
22-day	40	27	3	93

* Significantly different ($P \leq 0.01$) from the control, chi-square test, $df = 1$

** $0.01 < P \leq 0.05$, chi-square test, $df = 1$

TABLE 2

Hatching success of fertile mallard eggs treated with 5 μ l of Southern Louisiana crude oil after 2, 6, 10, 14, 18, or 22 days of incubation

Age at treatment	Number of eggs	Hatched	Failed to hatch	Percent hatching success
Control	40	40	0	100
2-day	40	0	40	0*
Control	40	40	0	100
6-day	40	1	39	3*
Control	40	40	0	100
10-day	40	3	37	8*
Control	40	40	0	100
14-day	40	31	9	78*
Control	40	40	0	100
18-day	40	35	5	88*
Control	40	40	0	100
22-day	40	38	2	95

* Significantly different ($P \leq 0.01$) from the control, Binomial test (chi-square not applicable; expected frequency in 1 of 2 categories is less than 5)

Except for eggs treated after 22 days of incubation, Southern Louisiana crude oil killed embryos quicker than No. 2 fuel oil (Table 3). Because exposure to petroleum may retard embryonic development, determination of the exact age at death is difficult. However, the age estimates do indicate the stage of development at the time of death (ALBERS 1977).

Hatching weights of survivors from No. 2 fuel oil exposure were significantly different ($P \leq 0.01$) from each other (Table 4). Pair-wise comparisons indicated that the weights of ducklings from the 6-day group were significantly different ($P \leq 0.01$) from

the weights of ducklings from the control group and the 22-day group. Hatching weights of survivors from Southern Louisiana crude oil exposure were not significantly different from each other (Table 4).

TABLE 3

Time to embryonic mortality in mallard eggs treated with 5 μ l of No. 2 fuel oil or 5 μ l of Southern Louisiana crude oil

Age at treatment	No. 2 fuel oil		S. Louisiana crude oil	
	Number of embryos	Mean days to death	Number of embryos	Mean days to death
2-day	35	6.2	40	4.7
6-day	27	5.4	39	3.5
10-day	13	5.9	37	1.6
14-day	7	8.4	9	6.7
18-day	8	6.2	5	4.4
22-day	3	1.7	2	2.5

Embryos of eggs treated with either oil on or before day 10, that died between days 10 and 12, had a high incidence (about half of 55 embryos) of abnormal bills. The bills were about half the normal length and were turned up against the front of the head. There were 5 instances of gross external malformations (missing/deformed limbs, deformed head, short bill) among the survivors of the experiments: 1 control duckling, 1 duckling treated after 2 days of incubation, 1 treated after 10 days, 1 treated after 14 days, and 1 treated after 18 days. No abnormal behavior was noted among normally developed ducklings as they were weighed.

TABLE 4
Hatching weight (gm) of mallard ducklings

Age at treatment	No. 2 fuel oil		S. Louisiana crude oil	
	N	$\bar{X} \pm \text{S.D.}^*$	N	$\bar{X} \pm \text{S.D.}^{**}$
Control	32	38.1 ± 3.9	40	38.1 ± 2.8
2-day	5	35.2 ± 1.6	--	--
6-day	13	33.2 ± 3.2	--	--
10-day	27	36.5 ± 4.4	3	37.7 ± 2.3
14-day	33	36.7 ± 3.3	31	36.7 ± 4.1
18-day	32	36.3 ± 3.0	35	38.2 ± 3.9
22-day	37	38.3 ± 4.8	38	39.1 ± 4.1

* Significant ($P \leq 0.01$) one-way analysis of variance; significant pair-wise comparisons ($P \leq 0.05$), Scheffe procedure, between controls and 6-day ducklings and between 22-day and 6-day ducklings

** Non-significant ($P > 0.01$) one-way analysis of variance; no significant pair-wise comparisons ($P > 0.05$), Scheffe procedure

DISCUSSION

Mallard embryos are most sensitive to petroleum during the early stages of incubation. Although both of the oils tested caused significant reductions in hatchability, Southern Louisiana crude oil was more toxic to mallard embryos than No. 2 fuel oil.

No. 2 fuel oil significantly reduced the hatching weights of ducklings from eggs treated after 6 days of incubation. However, ALBERS (1977) and SZARO et al. (in preparation) found that hatching weights of ducklings from eggs treated with various amounts of No. 2 fuel oil after 8 days of incubation were not significantly different from each other or from the control.

These results indicate that embryonic exposure to No. 2 fuel oil generally does not affect hatching weights of mallard ducklings, but eggs exposed on day 6 may be susceptible to retardation of weight gain. The results of SZARO et al. (in preparation) support my findings that embryonic exposure to Southern Louisiana crude oil does not affect hatching weights of mallard ducklings.

Petroleum may cause bill abnormalities among embryos exposed on or before day 10 and dying between days 10 and 12. I observed this abnormality in a small number of ducklings from a similar study (ALBERS 1977) but did not report it. In that study, all eggs were treated after 8 days of incubation and fewer embryos died between days 10 and 12 than in the present study. Because malformed bills were uncommon among survivors of the oil exposure, this abnormality may only occur in conjunction with a lethal exposure to petroleum.

If the findings of the present study are true for most birds (e.g., SZARO and ALBERS 1977) then colonial aquatic birds would be in the greatest danger from oil contamination when a large portion of the breeding birds are in the early stages of incubation.

SUMMARY

Artificially incubated mallard eggs were treated externally with 5 μ l of No. 2 fuel oil or 5 μ l of Southern Louisiana crude oil at various times during the incubation period. Embryos were most sensitive to petroleum during the first 10 days of incubation. Southern Louisiana crude oil was more toxic to mallard embryos than No. 2 fuel oil. Hatching weights of ducklings from treated eggs were usually not different from hatching weights of control ducklings. Petroleum may cause bill abnormalities among embryos exposed to a lethal amount of oil early in incubation, but few external malformations of any kind were observed among survivors of the oil exposure. The breeding effort of colonial aquatic birds would be in the greatest danger from oil contamination when a large portion of the birds are in the early stages of incubation.

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