OIL TOXICITY IN A EURYHALINE TELEOST: IMPAIRED OSMOREGULATION IN KILLIFISH, *Fundulus heteroclitus*

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Although aqueous extracts of both crude and refined oils are acutely toxic to teleost fish (Pollution and Physiology of Marine Organisms, pp. 285-310, 1974), little is known about mechanisms of oil toxicity. To determine if oil, like other organic pollutants, such as DDT, affects osmoregulation in teleosts (Environ. Hlth. Perspect. 1:169-173, 1972), we exposed sea water (SW) and fresh water (FW) adapted killifish to an aqueous extract (AE) of #2 fuel oil (oil obtained from American Petroleum Institute and AE prepared as described in Figure 1, legend). Experimental and control fish were maintained in separate static systems at 15°C as previously described (Environ. Hlth. Perspect. 1:169-173, 1972). Typically, SW killifish exposed to #2AE exhibited concentration dependent changes in behavioral patterns ranging from hyper-excitability to loss of both righting ability and buoyancy; after 15h exposure to undiluted (100%) #2AE, 10-30% of the experimentals were dead. We observed no mortalities in SW controls or experimentals exposed to #2AE diluted 1/1 with SW (50% #2AE). As shown in Figure 1, exposure to 100% #2AE caused transient impairment of osmoregulatory ability in both SW (elevated plasma Na) and FW (depressed plasma Na) killifish

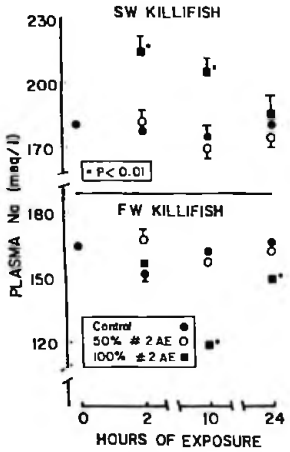

Figure 1. Effect of exposure to #2 fuel oil AE on plasma Na levels in SW and FW adapted killifish. AE was prepared by mixing oil with FW or SW (1/9) for 20h at 15°C. Fish were exposed to undiluted (100%) AE or AE diluted 1/1 with either FW or SW (50% AE). Each point represents the mean value derived from 8-18 (SW) or 3-7 (FW) fish; when large enough, variability is given by SE bars.

In SW fish, when 100% #2AE was replaced with fresh extract after 10h, plasma Na levels remained elevated for at least 14 additional h. Finally, initial experiments with killifish exposed to an AE from a South Louisiana crude show similar evidence of osmoregulatory impairment. Studies are in progress to identify target organs and mechanisms of oil toxicity in killifish. Preliminary data suggest that Na,K-ATPase activities in intestine and gill are not reduced in SW fish exposed to 100% #2AE for 10h.

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EFFECTS OF INGESTION OF A WEATHERED CRUDE OIL ON IMMATURE BLACK GUILLEMOTS, *Cepphus grylle*, AND HERRING GULLS, *Larus argentatus*

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Our recent studies have shown that crude oil ingestion inhibits growth and impairs plasma osmoregulation in immature herring gulls (Science, in press, 1977). Since oil spilled at sea rapidly changes composition

because of evaporation, oxidation and extraction of water soluble components, we decided to test the effects of a weathered South Louisiana crude (WSLC; American Petroleum Institute reference oil weathered over sea water according to Environ. Physiol. Biochem. 5:92) on immatures of two species of marine birds. Single 0.1-1.0 ml doses of WSLC were administered by stomach tube to herring gulls and black guillemots. Controls received either corn oil or nothing at all. Gulls were maintained as before at MDIBL, but guillemots were dosed and sampled in their nests on Old Man Island, off Cutler, Maine (Miller, Peakall and Kinter, MS in preparation). Birds were weighed and blood samples were drawn daily (gulls) or at 3-5d intervals (guillemots). At various times, guillemots were brought to the Laboratory. At the end of the experiment, birds were decapitated and tissue samples processed for histology, transport experiments, enzyme analyses and oil residue determinations (much of this data is not yet available).

Experimental guillemots and gulls exhibited a dose dependent chronic inhibition of growth and a transient elevation of plasma Na levels (Figures 1-3); this elevation of plasma Na was not observed when experiments were given a second dose of WSLC. Nasal gland, but not intestinal, Na,K-ATPase specific activities were reduced 20-30% in experimentals of both species, but total gland activity was generally not reduced because of hypertrophy of gland tissue. We also found dose and time dependent hypertrophy of adrenal tissue, but no increase in liver mass, except for gulls at the highest dose level tested (1.0 ml).

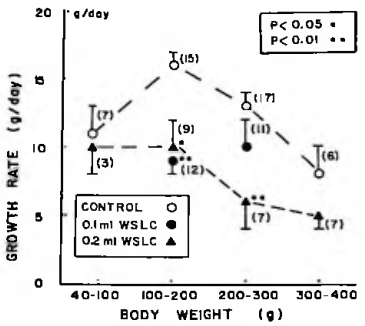

Figure 1. Effect of a single oral dose of WSLC on growth rates in immature black guillemots. Experimentals and controls were weighed at 3-5 day intervals and average daily changes were grouped according to the bird's BW at the beginning of the 3-5 day period. Experimentals were weighed 3-17 days after dosing. Date expressed as mean ± SE with the number of measurements in parentheses.

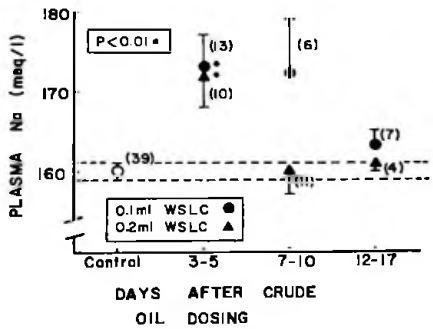

Figure 2. Effect of WSLC dosing on plasma Na levels in immature black guillemots. Control data was collected over a 5-week period. Data expressed as the mean ± SE with the number of birds in parentheses.

These preliminary findings for a weathered crude oil agree well with those reported for gulls dosed with either of two unweathered crudes (Miller et al. Science, in press, 1977), thus indicating that short-term weathering of oil does not greatly alter its toxicity to marine birds. Our data also indicate that the effects of oil ingestion in the truly pelagic guillemot (maintained in its natural environment) are similar to those in the more coastal gull (maintained in captivity). Finally, with regard to the environmental significance of our findings, one might expect that sea birds with impaired ability to regulate body weight or salt and water balance would be more vulnerable during periods of stress, e.g., storms or food shortages.

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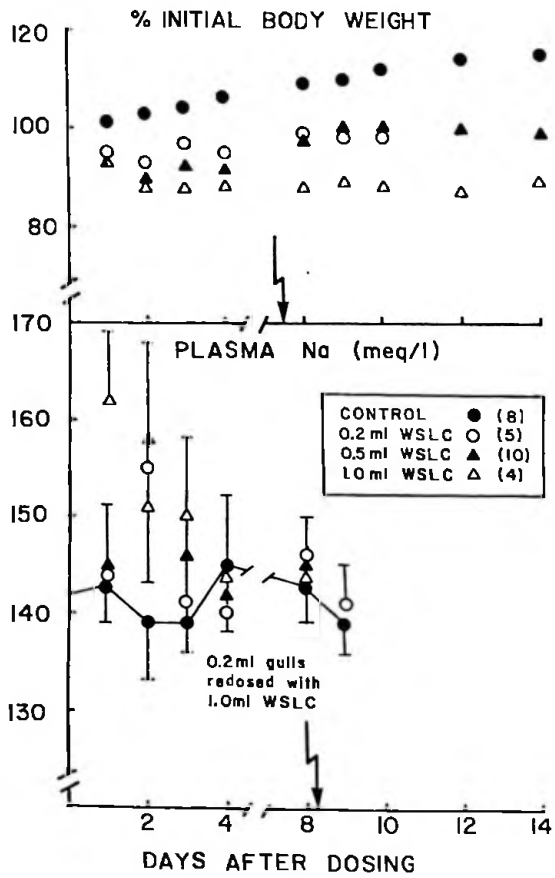

Figure 3. Effect of a single oral dose of WSLC on body weight and plasma Na concentrations in immature herring gulls maintained on sea water. Data expressed as mean ± SE with the number of birds in parentheses. Experimental body weights were significantly lower than controls ($P < 0.05$) from day 1 on. Plasma Na levels for birds dosed with 1.0 WSLC were significantly higher than controls on day 1.

INHIBITION OF CHLORIDE TRANSPORT IN FLOUNDER INTESTINE BY FUROSEMIDE AND CYCLIC AMP

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We previously showed that net Na and Cl fluxed across short-circuited flounder intestine are coupled, net Cl absorption ceasing in the absence of Na and vice versa (Field, Karnaky, Smith, Bolton and Kinter, J. Membrane Biol., in press; Field et al. MDIBL Bull. 1975). Furthermore, net Cl absorption is inhibited by ouabain suggesting that Na,K-activated ATPase provides the driving force for this transport. Similar observations have been made with rabbit ileum (Nellans et al. Am. J. Physiol. 226:1131, 1974) and gallbladder (Frizzell et al. J. Gen. Physiol. 65:79, 1975). In those two tissues, the coupling of net transepithelial fluxes of Na and Cl has been shown to originate with a 1:1 coupled influx across the brush border (luminal) membrane. Elsewhere in this bulletin Frizzell, Smith and Field show that Cl influx across the brush border of flounder intestine is also coupled to that of Na and that the coupled flux can be inhibited by increasing intracellular 3',5'-AMP (cAMP) or by adding furosemide to the mucosal medium. To establish the role of this brush border mechanism in transepithelial ion transport, changes in Cl and Na influxes must be related to changes in their transepithelial fluxes. The present study examines the effec of furosemide on transepithelial Cl fluxes and extends our prior observations on the effects of cAMP.