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# Sensitivity of Ten Aquatic Species to Long-Term Crude Oil Exposure

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When assessing the potential effects of crude oil on an ecosystem, it is necessary to know whether some groups of animals are more sensitive than others and what factors affect this sensitivity. Much of the literature on the toxicity of oil to aquatic organisms is based on 96-hr static bioassays, and resource managers must base decisions about the potential effects of oil to aquatic ecosystems on the LC50's from these tests. Static oil bioassays are excellent for comparing relative toxicities of chemicals; however, they are not the best method for comparing animal sensitivities to oil because hydrocarbon concentrations decline to 20% of the initial level during the 96 hr of the test (Rice et al. 1984). Although attempting to simulate oil spills, static oil bioassays do not address sensitivities of animals that may require more than a day or two before they respond to the crude oil. Managers using results from laboratory studies on oil toxicity have had to be cautious about comparing the results of bioassays derived from different studies because of the differences in analytical methods.

A few researchers have used long-term exposures to crude oil water-soluble fraction (WSF) to assess the effects of petroleum on such processes as bioenergetics (Stickle et al. 1984, 1987), growth (Moles and Rice 1983), reproduction (Rice et al. 1987), stamina (Thomas and Rice 1987), and feeding rate (O'Clair and Rice 1985). This is the first paper, however, to compare long-term toxicity of oil to a wide variety of phyla, using the same stable oil generation and analytical methods for comparison. We compare the 4- and 28-d sensitivities of 10 marine animals to the WSF of Cook Inlet crude oil. We also compare our results to published 4-d static bioassay values for the same species. These data are intended to provide resource managers with information on the comparative vulnerability of different species in a variety of habitats.

## MATERIALS AND METHODS

The test animals used in this study are listed in Table 1. Animals were chosen from a wide variety of habitat and phyla to ensure the widest range of responses.

We chose two intertidal mollusks because the periwinkle was motile and the mussel was sessile. Because the adults were too large to assay, the salmon,

Table 1. Test organisms used in flow-through oil toxicity tests.

| Species | Collection habitat | Length (mm) |
|---|---|---|
| *Oncorhynchus gorbuscha* (pink salmon) | pelagic fish | 32 |
| *Platichthys stellatus* (starry flounder) | demersal fish | 65 |
| *Boeckosimus nanseni* (amphipod) | planktonic crustacean | 20 |
| *Pandalus hypsinotus* (coonstripe shrimp) | subtidal crustacean | 170 |
| *Paralithodes camtschaticus* (king crab) | benthic crustacean | 40 |
| *Hemigrapsus nudus* (shore crab) | intertidal crustacean | 20 |
| *Evasterias troschelii* (ocher starfish) | intertidal echinoderm | 300 |
| *Chlamys hericus* (pink scallop) | subtidal mollusk | 50 |
| *Nucella lima* (file periwinkle) | intertidal mollusk | 25 |
| *Mytilus trossulus* (blue mussel) | intertidal mollusk | 71 |

flounder, shrimp, and king crab were tested as juveniles. These four species were chosen because of their economic importance. The remaining test species were adults. The amphipod *Boeckosimus* was collected near Point Barrow, Alaska, in the Arctic in May at 4°C and acclimated in the laboratory to 7°C. All other species were collected in southeastern Alaska by beachcombing, pots, diving, or beach seining as appropriate. Collections and testing were done between June and August to minimize effects of reproductive condition on test results. Test animals were collected and tested in ambient flowing seawater at 7-10°C and 28-30‰ salinity.

Animals were exposed to flow-through dilutions of Cook Inlet crude oil WSF using the method of Moles et al. (1985). This involves dripping water through a column of crude oil. The resulting WSF was saturated with mono- and di-aromatic hydrocarbons, which remain in solution at a stable concentration, but did not contain dispersed or emulsified oil. A detailed description of the chemical composition of the resulting WSF is given in Moles et al. (1985). Following preliminary tests to establish a tight range of concentrations for exposure, a series of doses were prepared by dilution. Twenty animals were exposed to each concentration, with 5-7 concentrations per test. Flow rates were set to maintain fully saturated oxygen levels in each exposure container. Exposure containers varied with the size of the animals.

Median lethal concentration LC50 (the concentration that killed half the animals) and associated 95% confidence bounds were calculated after 4 and 28 d of exposure, using logit analysis (Silverstone 1957); 28 d was chosen as the endpoint because all LC50's stabilized before 28 d. Daily concentrations of aromatic hydrocarbons in the WSF were measured by gas chromatography (details in Moles and Rice 1983). Concentrations in the exposure containers were not allowed to deviate more than 2% from the target concentration. LC50 values are reported as mg/L total aromatic hydrocarbons.

## RESULTS AND DISCUSSION

After 28 d, the LC50's for the 10 species tested were very similar (mean = 1.21 ± 0.29 mg/L). Sensitivities (LC50's) ranged from 0.62 mg/L for shrimp to 3.8 mg/L for amphipods (Figure 1). The nine temperate species had 28-d LC50's between 0.6 and 1.4 mg/L, a difference that was not statistically significant (as measured by non-overlap of 95% confidence bounds). In contrast, the arctic species *B. nanseni* was twice as tolerant as the most tolerant temperate species tested (*Mytilus trossulus*). Carls and Korn (1985) did not find arctic species to be unusually resistant to crude oil exposure but found *B. nanseni* more tolerant of short term oil exposure than other arctic amphipods. Thus, these tests probably represent the range of sensitivity to water-borne crude oil exposures.

Length of exposure was a major factor in the comparative sensitivities of the test species (Figure 1). Pink salmon fry, a pelagic fish, responded quickly to oil exposure, and the 4-d and 28-d values were the same (1.2 mg/L). Subtidal animals (flounder, shrimp, king crab, and scallops) continued to respond to oil exposure for several days; 28-d LC50's were 52-58% lower than 4-d LC50's. Intertidal animals (shore crab, starfish, snail, and mussel) responded very slowly to oil; none died during the first week, but their 28-d LC50's were lower than those of juvenile fishes.

Rice et al. (1979) previously noted that pelagic fishes were the most sensitive animal to oil exposure and intertidal invertebrates were the most resistant. Intertidal animals have adapted to withstand the rigorous stresses of their environment in lieu of rapid mobility and can also resist the stress of hydrocarbon exposure. Intertidal animals, and to some extent subtidal animals, minimize stress by temporarily isolating themselves from the environmental stress by burrowing, decreasing metabolism, closing their shells, or shifting to anaerobic metabolism. If their intake of pollutants is reduced, such animals may require much longer to accumulate sufficient amounts of hydrocarbons in their tissues to be affected adversely by oil exposure. We recommend 28 d as the minimum time needed in oil bioassays to establish a stable LC50 value for invertebrates and demersal fishes. Four-day (96-hr) flow-through bioassays, which are standard for assessing aquatic toxicity, appear to be adequate for pelagic fishes.

These 28-d results contrast with the previously published short-term tests, which show intertidal and subtidal invertebrates to tolerate oil exposure (Rice et al. 1979). Animals such as starfish, snails, and flounders, which did not die in 4-d tests, were at least as sensitive to long-term (28-d) oil exposure as pelagic fishes. The most resistant animals were the planktonic amphipods, which are adapted for the rigorous variations associated with arctic habitat. Amphipods, which must also survive a neustonic habitat, were actually more resistant to oil after 28 d than the mussel, which can isolate itself from environmental stress for several days.

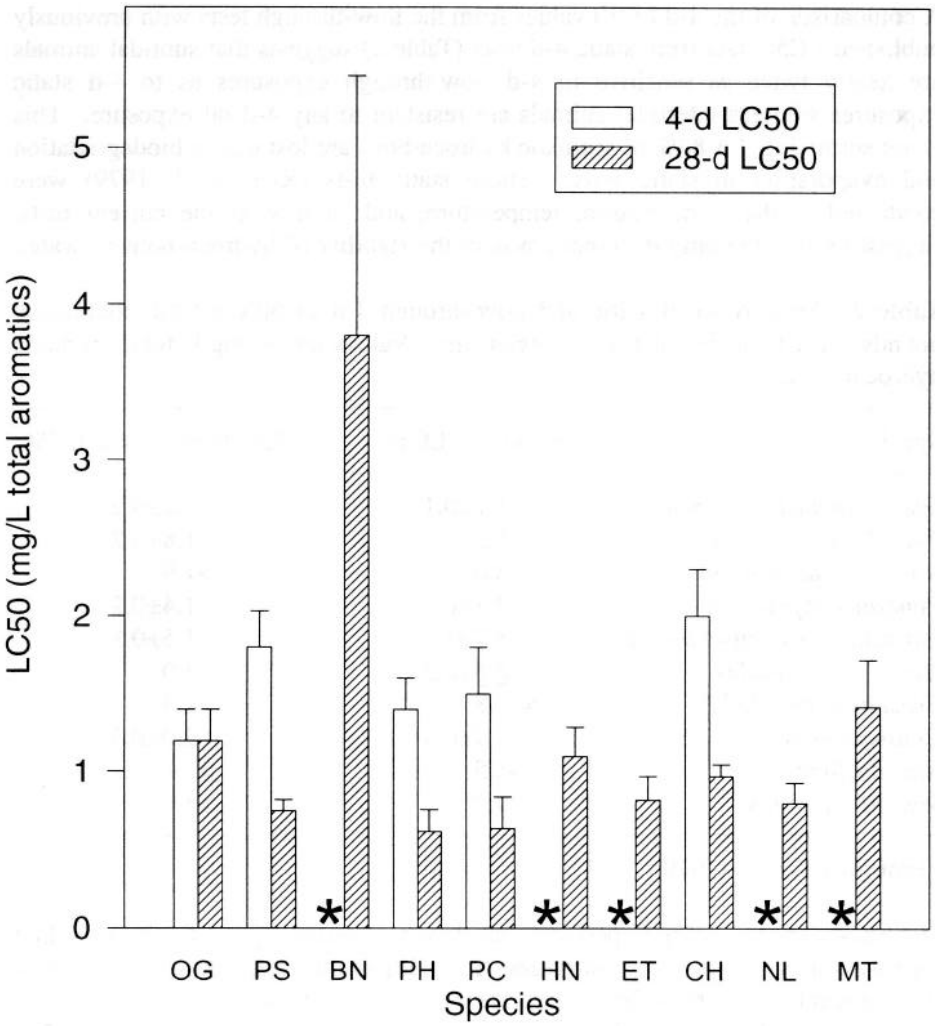


**Figure 1.** Flow-through LC50's for 10 species of marine animals OG=*O. gorbuscha,* PS=P. *stellatus,* BN=*B. nanseni,* PH=*P. hypsinotus* PC=*P. camtschaticus,* HN= *H. nudus,* ET= *E. troschelii,* CH=*C. hericus* NL=*N. lima,* MT= *M. frossulus.* * = no mortality at 3 mg/L

A comparison of the 4-d LC50 values from the flow-through tests with previously published LC50 data from static 4-d tests (Table 2) suggests that subtidal animals are nearly twice as sensitive to 4-d flow-through exposures as to 4-d static exposures whereas intertidal animals are resistent to any 4-d oil exposure. This is not surprising, for 80% of aromatic hydrocarbons are lost due to biodegradation and evaporation in static tests. These static tests (Rice et al. 1979) were conducted at the same season, temperature, and salinity as the current tests, suggesting that the only difference was in the stability of hydrocarbons in water.

**Table 2.** Comparison of static and flow-through 4-d LC50's ± 95% confidence bounds for 10 species of marine organisms. Values are in mg/L total aromatic hydrocarbons.

| Species | Static 4-d LC50[a] | Flow-through 4-d LC50 |
|---|---|---|
| *Oncorhynchus gorbuscha* | 1.7±0.1 | 1.2±0.2 |
| *Platichthys stellatus* | >5.3 | 1.8±0.2 |
| *Boeckosimus nanseni* | >8.0 | >1.9 |
| *Pandalus hypsinotus* | 2.7±0.2 | 1.4±0.2 |
| *Paralithodes camtschaticus* | 3.7±1.1 | 1.5±0.3 |
| *Hemigrapsus nudus* | 8.5±0.3 | >3.0 |
| *Evasterias troschelii* | >10.8 | >1.3 |
| *Chlamys hericus* | 3.9±0.4 | 2.0±0.3 |
| *Nucella lima* | >8.5 | >3.0 |
| *Mytilus trossulus* | >9.0 | >3.0 |

[a] From Rice et al. (1979)

Static tests are far easier to perform, but they are apparently not the best method for assessing comparative sensitivities of species to oil. Even the LC50 for pink salmon was lower in flow-through tests, suggesting that enough volatiles evaporate in the first day to give a higher estimate of sensitivity to oil with static tests than with flow-through tests. We recommend 4-d flow-through tests rather than static tests to determine relative sensitivity of pelagic fishes to short-term oil exposure.

Chronic oil exposure appears to minimize the differences in hydrocarbon sensitivity between species. Factors such as habitat and phylum are less important in assessing the relative effect of chronic oil pollution to various species than they are in acute exposures. Intertidal species, which are particularly vulnerable to chronic pollution through oiling of beaches, are also sensitive to chronic oiling. Such coated habitat may retain oil for long periods. The assumed resistance of these forms based on acute tests may not have any basis in fact during periods of chronic pollution.

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