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Effects of Temperature on the Median Tolerance Limit of Pink Salmon and Shrimp Exposed to Toluene, Naphthalene, and Cook Inlet Crude Oil

Sid Korn, D. Adam Moles, and Stanley D. Rice

Northwest and Alaska Fisheries Center Auke Bay Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 155, Auke Bay, Ala. 99821

Marine animals may be more susceptible to oil spills in Alaskan and other cold waters than they would be in warmer waters because of direct and indirect effects of low temperatures on the physical behavior of oil and the sensitivity of animals. Oil-water solutions probably remain at toxic concentrations for longer periods of time at lower temperatures because of reduced volatility and biodegradation of oil in seawater. Sensitivity of animals to oil should differ at different temperatures, but the direction (increased or decreased sensitivity) and magnitude may vary by species. We do not know how reduced temperatures change uptake, metabolism, and excretion of petroleum hydrocarbons by invertebrates or fishes.

Information on the influence of temperatures on survival of animals exposed to oil is sparse and inconclusive. RICE et al. (1977a) made general comparisons of the median tolerance limits (TLm's) to oil reported for several species of shrimp and fishes from the Gulf of Mexico and Alaska and found the Alaskan species had consistently lower TLm's. However, differences in species and oils tested render direct comparisons based on temperature differences inconclusive.

We measured the effects of temperature on the TLm's of pink salmon (Oncorhynchus gorbuscha) fry and shrimp (Eualus spp. and Pandalus goniurus) exposed to toluene, naphthalene, and the water-soluble fraction (WSF) of Cook Inlet crude oil. We used static exposures in which the initial concentration of the toxicant declines with time, a situation that would also occur with an oil spill. Both the persistence of oil and the physiology of animals are affected by temperature, and the measured TLm's are the net result of both variables operating simultaneously. Toluene, a mononuclear, and naphthalene, a dinuclear aromatic hydrocarbon, were tested because they represent aromatic compounds implicated as major contributors to oil toxicity (ANDERSON et al. 1974, RICE et al. 1976).

METHODS

Test animals were shrimp (Eualus spp. and P. goniurus) and pink salmon fry (O. gorbuscha). The shrimp were collected in pots

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in Auke Bay, Alaska, and the pink salmon fry from eggs collected at Auke Creek, Alaska. The fry were acclimated to seawater to simulate their normal migration to seawater. The shrimp (0.8 g, 6 cm long) and pink salmon fry (0.35 g, 3.5 cm long) were held in seawater with ambient temperatures of 6-8°C and salinities of 26-28°/$_{oo}$. Animals were gradually acclimated to the test temperatures over a 4-day period, with less than 2°C change per day.

Bioassays were standard, 96-h static tests conducted with one toxicant per test at temperatures of 4, 8, and 12°C. Tissue loading was less than 1g/L. Stock solutions of toluene and the WSF of Cook Inlet crude oil were prepared by the method of RICE et al. (1977b). The naphthalene stock solution was prepared by pumping water through cartridges containing naphthalene crystals.

Each stock solution was divided into three portions which were individually adjusted to the test temperatures. Test solutions were diluted with water of desired temperature and analyzed to confirm the concentration; then 10-15 pink salmon or shrimp were added to each test container. A control group of animals (no toxicant) was maintained at each temperature. The water in the naphthalene and crude oil tests was slowly aerated, while the toluene test water was not aerated until after the first 48 h of the bioassay in order to reduce evaporative loss. Oxygen concentrations never dropped below 80% saturation in any test container.

Concentrations of toluene and naphthalene were determined by uv spectrophotometry at appropriate absorbance maxima (260 nm for toluene, 219 nm for naphthalene) using 1-cm cuvettes. Optical densities were converted to concentrations in parts per million (µL/L for toluene, mg/L for naphthalene) by comparing the optical densities to appropriate standards.

Concentrations of the WSF of Cook Inlet crude oil in seawater were analyzed by gas chromatography (CHEATHAM et al.). We measured benzene; toluene; o-, m-, and p-xylenes; ethylbenzene; naphthalene; 1- and 2-methylnaphthalenes; and dimethylnaphthalenes. The sum of the concentrations of these 10 substances was taken as the total concentration of aromatic hydrocarbons in the stock solution (Table 1).

The 96-h TLm's and 95% fiducial limits for each temperature-toxicant combination were determined using probit analysis (FINNEY 1971). The 96-h TLm is the concentration of toxicant in which 50% of the exposed animals survived for 96 h.

RESULTS

The concentration of toxicants in the test containers declined with time, from either evaporation losses or biodegradation, with more rapid losses occurring at higher temperatures. Toluene declined to nondetectable levels by 72 h at 12°C and by 96 h at 8°C, and to 25% of the initial concentration by 96 h at 4°C. Naphthalene concentrations declined to nondetectable levels by 48 h at 12°C, 72 h at 8°C, and 96 h at 4°C. In 96 h the concentration of naphthalenes in the WSF of Cook Inlet crude oil declined to nondetectable levels at 12°C, 20% of the initial concentration at 8°C, and 40% at 4°C.

TABLE 1. Concentrations of individual aromatic hydrocarbons, as determined by gas chromatography, in undiluted solutions of the WSF of Cook Inlet crude oil used in bioassays with shrimp (Eualus spp.) and pink salmon (Oncorhynchus gorbuscha).

Aromatic hydrocarbon	Concentration (ppm) in solutions used for-- Shrimp bioassays	Pink salmon bioassays
Benzene	2.00	2.71
Toluene	2.15	2.07
o-Xylene	0.377	0.322
m- and p-Xylene and ethylbenzene*	0.782	0.714
Naphthalene	0.115	0.181
1-Methylnaphthalene*	0.0706	0.0771
2-Methylnaphthalene	0.0579	0.101
Dimethylnaphthalenes*	0.0280	0.043
Total	5.58	6.22

*Concentration includes some contribution from undetermined compounds, possibly paraffins.

TABLE 2. Ninety-six-h TLm's for pink salmon (O. gorbuscha) and shrimp (P. goniurus and Eualus spp.) tested with Cook Inlet crude oil WSF (total aromatic hydrocarbons), toluene, and naphthalene at three temperatures. The 95% fiducial limits are given in parentheses.

Species and toxicant	96-h TLm (ppm) at-- 4°C	8°C	12°C
Oncorhynchus gorbuscha			
Cook Inlet WSF	1.45 (1.28-1.62)	1.69 (1.47-1.83)	1.77 (1.58-1.99)
Toluene	6.41 (5.73-7.18)	7.63 (6.86-8.48)	8.09 (7.45-8.78)
Naphthalene	1.37 (1.11-1.68)	1.84 (1.22-2.80)	1.24 (0.95-1.62)
Eualus spp.			
Cook Inlet WSF	1.68 (1.66-1.80)	1.86 (1.66-2.07)	1.58 (1.42-1.73)
Toluene	21.4 (19.5-23.5)	20.2 (17.9-22.8)	14.7 (13.1-16.6)
Pandalus goniurus			
Naphthalene	2.16 (1.76-2.64)	1.02 (0.770-1.34)	0.971 (0.780-1.22)

The 96-h TLm of pink salmon fry exposed to toluene was significantly lower (non-overlapping 95% fiducial limits) at 4° (6.41 ppm) than at 12°C (8.09 ppm) (Table 2). The trend toward lower TLm's at lower temperatures was observed for the Cook Inlet crude oil WSF, but the differences were not statistically significant. The differences between 24- and 96-h TLm's were insignificant for all three toxicants since, in each test, nearly all deaths occurred during the first 24 h.

The 96-h TLm's for shrimp exposed to toluene and naphthalene solutions were significantly higher at 4°C (toluene = 21.4 ppm, naphthalene = 2.16 ppm) than at 12° (toluene = 14.7 ppm, naphthalene = 0.97 ppm) (Table 2). Temperature did not affect the TLm of shrimp to the Cook Inlet crude oil WSF. We tested the relative sensitivities of Eualus spp. and P. goniurus with the Cook Inlet WSF at 8°C and found that they were equally sensitive, with 96-h TLm's of 1.86 (95% fiducial limits = 1.66-2.07) and 1.94 (95% fiducial limits = 1.68-2.26), respectively.

DISCUSSION

The loss of toluene, naphthalene, and other hydrocarbons from seawater is influenced by temperature. In the range of 5 to 12°C, CHEATHAM et al. observed that the higher the temperature the faster the loss of mononuclear and dinuclear aromatic hydrocarbons from Cook Inlet crude oil WSF by evaporation and biodegradation. We observed even greater losses at 12°C than did CHEATHAM et al., probably because our solutions contained the test organisms and their associated bacteria.

Because aromatic hydrocarbons persist longer at lower temperatures, the TLm's for animals exposed to oil toxicants at lower temperatures should be lower than for animals exposed at higher temperatures. This simplistic prediction of decreased survival at lower temperature because of increased hydrocarbon persistence was not uniformly confirmed in this study. Although survival of pink salmon fry exposed to toluene, and to some extent the WSF of Cook Inlet crude oil, was less at lower temperatures, there was little effect of temperature on fry exposed to naphthalene. In contrast, the TLm's of shrimp exposed to toluene and naphthalene were lower when exposures were at higher temperatures, despite the greater losses of toxicants from the water. Generalizations about temperature effects on increasing or decreasing the survival of animals exposed to oil or oil components cannot be made. Changes in temperature affect the survival of fishes exposed to a variety of pollutants in a nonuniform and unpredictable manner (SPRAGUE 1970), and we think effects of temperature on invertebrate survival could be equally unpredictable.

Temperature could affect survival of animals exposed to oil in at least three ways: (1) by altering the persistence of toxic hydrocarbons in water by changing the rate of loss through evaporation and biodegradation; (2) by altering sensitivity of the animals by changing the rates of hydrocarbon uptake, metabolism, and excretion of hydrocarbon metabolites or parent compounds (all of these processes are affected by temperature, but not necessarily at equal rates); and (3) exposure at temperatures deviating significantly from the optimum temperature for a particular species can be expected to add stress to the animal, with oil toxicity stress acting either independently or synergistically with temperature stress. The magnitude of each of the above three variables will vary considerably, depending on temperature and species. These three factors may occur simultaneously, and possibly in opposing vectors, making the prediction of toxicity-sensitivity at different

temperatures very difficult. Measured TLm's express the "net result" of all factors operating on the test animals.

The increased persistence of toxic aromatic hydrocarbons at lower temperatures could explain the lower TLm's of pink salmon fry exposed to toluene and the Cook Inlet crude oil WSF. However, the decrease in TLm's of shrimp exposed to toluene and naphthalene at higher temperatures, despite the greater loss of toxicants at these higher temperatures, can only be explained by increased sensitivity of the organisms. Similar increases in the sensitivity of aquatic animals with increases in temperature have been noted with many toxicants (CAIRNS et al. 1975). The increased metabolism at increased temperature in ectothermic organisms could result in faster accumulation of the toxicant with subsequent greater effects.

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