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Effects of Petroleum Exposure on Predatory Behavior of Coho Salmon (*Oncorhynchus kisutch*)

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Evaluations of the effects of petroleum on the indigenous marine fauna of Alaska's continental shelf regions are of continuing importance because of existing and proposed oil exploration activities in these areas. In reviews of the acute toxicity and sublethal biological effects of petroleum on arctic and subarctic marine organisms, CRADDOCK (1977) and PATTEN (1977) presented evidence of lethargy, loss of appetite, and alterations in schooling behavior associated with exposure to various seawater-soluble fractions of petroleum. However, there were no studies reported on the influence of petroleum on predator-prey behavior of fishes, which has been described as a sensitive indicator of perturbed environmental conditions (FARR 1978, GOODYEAR 1972, HATFIELD & ANDERSON 1972, SYLVESTER 1972, 1973, COUTANT et al. 1974, YOCUM & EDSALL 1974, SULLIVAN et al. 1978, WOLTERING et al. 1978). The purpose of the present study was to determine the influence of crude oil in seawater on salmonid predatory behavior. Coho salmon (Oncorhynchus kisutch) were chosen as predators. This species has been identified as a primary predator of juvenile salmonids in seawater (PARKER 1971).

METHODS AND MATERIALS

Fish were obtained from the following sources: adult coho salmon from a stock reared at the Northwest and Alaska Fisheries Center, National Marine Fisheries Service, Seattle, WA. and rainbow trout (Salmo gairdneri) fry from Trout Lodge, Tacoma, WA. The trout fry were 4.6 ± 0.5 cm (X ± S.D.), while the coho predators were 35.2 ± 5.0 cm in fork length. The trout fry were maintained (fed to satiation) on a diet of Oregon Moist pellets (OMP). The coho predators were maintained on OMP until four months prior to the experiments, from which time they were fed exclusively on live chum salmon (O. keta) or rainbow trout fry. Rainbow trout fry, a species which may be anadromous as steelhead trout, were selected as prey primarily as a result of their availability. Although natural predation by coho salmon on S. gairdneri in seawater is unlikely, we concluded after preliminary trials that this combination provided a viable experimental design for a laboratory evaluation of the effects of petroleum on the predatory behavior of coho salmon.

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The coho predators were exposed to the seawater-soluble fraction (SWSF) of Cook Inlet crude oil which was prepared using a solubilizer similar to the one described by NUNES & BENVILLE (1978). Crude oil was pumped into the apparatus at the rate of 1 mL/min. A flow of 4 L/min of seawater was directed onto the dispersion plate and dripped through the oil layer to extract the soluble fractions of the crude oil. The SWSF was then diluted with an equal volume of seawater and siphoned into the exposure tank. Water samples were collected twice weekly during the experimental period. These samples were analyzed for total hydrocarbon content by gas chromatography (MACLEOD et al. 1976) at the National Oceanic and Atmospheric Administration, National Analytical Facility, Seattle, WA. Dissolved oxygen concentrations, pH, and temperature were also determined twice weekly for water in the exposure and control tanks (APHA STANDARD METHODS 1975).

Two rectangular fiberglass tanks (118 x 42 x 44 cm, capacity of 170 L) were used as the exposure and control tanks. Circular fiberglass tanks (1.2 m diameter, 55 cm deep, capacity of 950 l) were used for the predation tests. Seawater inflow rates to the circular tanks provided for a 95% exchange within 24 h.

The experiments were conducted during September, 1980. The coho salmon predators were randomly divided into two groups of 21 fish each. One group was designated as the control while the other 21 fish were exposed to the SWSF of Cook Inlet crude oil. All test fish were exposed (together) to the oil for a period of 17 days, with predatory evaluations occurring after 3, 10, and 17 days of exposure. Each of the test groups was further divided into subgroups of 3 fish each for the predator evaluations, so that there were 7 replicates for each group at each of the 3 evaluation periods. Each fish was freezebranded (FUJIHARA & NAKATANI 1967) for identification and maintained in the same subgroup throughout the study, with the following exceptions. Three mortalities occurred in the control group (one fish each from Subgroups 1, 3 and 6), just prior to the final testing period. The 2 surviving fish from Subgroup 1 were redistributed to Subgroups 3 and 6 to maintain the Subgroup sizes of 3, which were essential to stimulate feeding behavior. The predators were not fed between evaluation periods.

The predator-prey interactions were evaluated in the following manner: Predators were transferred from the control or exposure tanks to the circular tanks at 4 p.m. on the day prior to testing. At 9 a.m. on the day of testing 10 rainbow trout fry were transferred without acclimation into a tank containing one of the 7 predator subgroups (3 adult coho salmon), and then the number of prey surviving after 10 minutes were recorded. Surviving prey were discarded. Predators were returned to their respective control or oil-exposure tanks immediately after the predator-prey evaluations. At the termination of the experiment, 3 control (Subgroup 4 in Fig. 1) and 6 oil-exposed predators (Subgroups 1 and 6 in Fig. 1) were sacrificed and the livers and the brains excised for chemical analysis. Subgroups 1 and 6 of the oil-exposed fish were selected because of their opposing behavioral responses in the predator-prey evaluations.

Water quality for both the control and exposure tanks was as follows ($X \pm S.D.$): salinity, 26.5 ± 1.3 o/oo; temperature, 13.4 ± 0.7 °C; pH, 7.6 ± 0.1; and dissolved oxygen, 6.8 ± 1.1 mg/L. In the exposure tanks the SWSF concentrations ranged from 230-530 µg/L ($X \pm S.D. = 343 \pm 93$).

RESULTS

Many of the coho predators exposed to the SWSF of Cook Inlet crude oil began to show behavioral modifications by the tenth day of exposure. In general, the oil-exposed predators appeared lethargic and showed little or no interest in the prey presented to them (these predators were designated as noneaters). However, one of the oil-exposed subgroups (number 6, designated as eaters) demonstrated none of these behavioral modifications and continued to feed at rates comparable with those of the unexposed predators. We have also observed similar behavioral responses with eater and noneater groups in coho predators exposed to No. 2 fuel oil.

Figure 1 depicts the numbers of rainbow trout fry consumed during 10 minutes of exposure to the control or oil-exposed predators at three time intervals. An initial Yates X^2 evaluation showed a significant difference in prey consumption between the control and oil-exposed predator subgroups ($X^2 = 45.0$, $P \leq 0.005$). To determine within- and between-group differences with the small sample sizes, the data were subjected to predictive sample reuse analysis (GEISSER & EDDY 1979). With a sample size of seven, this test is designed to select the correct model >95% of the time. The data were used to select one of two models; Model 1 (test populations were equivalent) or Model 2 (test populations were unequal). Model 1 (=) was selected on the basis of this test for the control observations at 3, 10, and 17 days, as it was for the comparison tests of control and oil-exposed fish after 3 days of exposure. However, Model 2 (≠) was selected for for the control and oil-exposed fish comparison tests after 10 and 17 days of exposure. Model 1 (=) was also selected for the comparison of the 10 and 17 day oil-exposed predators. Even with the bias of Subgroup 6 toward the control values, our results clearly indicate that there was reduced predation by the adult coho salmon exposed to the SWSF of Cook Inlet crude oil for periods of 10 days or longer.

Concentrations of all hydrocarbons detected by gas chromatography in both liver and brain of the oil-exposed fish were higher in the eater subgroup than in the noneater subgroup. All three fish from the eater subgroup (number 6) had higher levels of the detected hydrocarbons in both liver and brain than did any of the three fish from the noneater subgroup (number 1). The highest concentrations detected were those of naphthalenic compounds (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene). Biphenyl and 1,2,3,4-tetramethylbenzene concentrations were also in excess of 100 ng/g in both livers and brains. All of the above hydrocarbons showed a high degree of parallelism between concentrations in the brains and livers of the oil-exposed fish. These results are similar to those found for naphthalenic compounds in rainbow trout by COLLIER et al. (1980). Benzothiophene and n-propylbenzene were

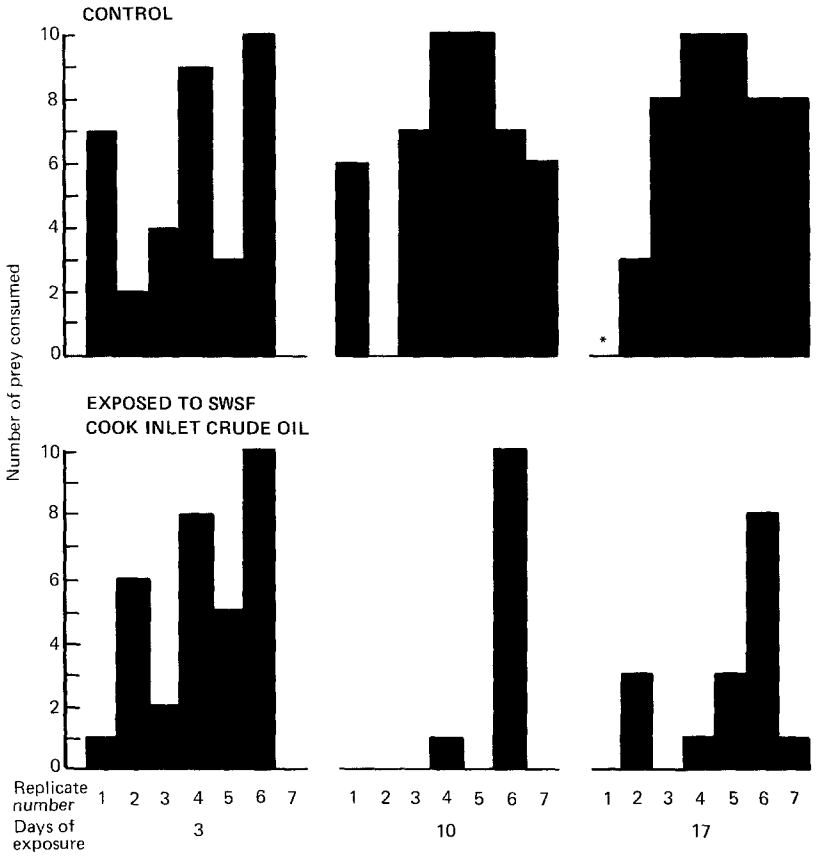

Figure 1. Ten rainbow trout fry were offered to each of the seven (3 fish) replicate control and oil-exposed coho salmon predator subgroups after 3, 10, and 17 days of oil exposure. The histograms represent the number of prey consumed by each of the control and oil-exposed subgroups at the three sampling periods. Each numbered subgroup represents the same three predators throughout the experiment. (* = no data due to mortalities. Other absences of a bar represents no prey consumed).

also detected at levels in excess of 100 ng/g in the livers, but not in the brains of the eater subgroup.

DISCUSSION

Our studies have shown that exposure to SWSF of Cook Inlet crude oil can significantly impair the capturing of prey by coho salmon predators. An interesting observation associated with that behavioral difference was that levels of the parent hydrocarbons were markedly higher in the tissues of the oil-exposed eater subgroup than in those of the noneater oil-exposed subgroup. Brain and liver hydrocarbon concentration differences between the eater and noneater subgroups suggest differential uptake, excretion and/or metabolism of these

chemicals. If the lower hydrocarbon levels in the noneaters reflect metabolism and, therefore, the activity of the mixedfunction oxidase enzymes, differential induction could be a possible explanation.

Since the eater subgroup had much higher tissue concentrations of the parent hydrocarbons in both brain and liver than the noneaters, it appears unlikely that the parent hydrocarbons were the xenobiotics primarily influencing feeding behavior. The lower concentrations of the parent hydrocarbons in the noneater subgroup suggest that the metabolic products of the crude oil may have been responsible for the cessation of feeding. These preliminary results are in apparent contrast with reported findings that acute neurotoxic effects (SAVOLAINEN 1977) and behavioral changes (DIXIT & ANDERSON 1977) were related to accumulation of the parent compounds rather than nonconjugated metabolites of the parent compounds. Further studies are in progress in which we will attempt to determine if aryl hydrocarbon hydroxylase (AHH) activity directly correlates with changes in feeding behavior, and metabolite concentration and distribution in organs.

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