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Behavioral Responses of Juvenile Coho Salmon (*Oncorhynchus kisutch*) Exposed to Pulp Mill Effluents

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Coho salmon (*Oncorhynchus kisutch*) smolts from the Chehalis River may encounter large discharges (>1.7×10^8 L/d) of pulp mill effluent during their downstream migration through Grays Harbor, Washington. Smolt survival is believed to be reduced by the impaired water quality resulting from effluent releases (Seiler 1989), and it is possible that the orientation behavior of young salmon could be disrupted by exposure to sublethal concentrations of these effluents (Sutterlin 1974).

The purpose of our study was to assess behavioral responses of young coho salmon to acute exposures of effluent from two Grays Harbor pulp mills. The first objective was to determine the avoidance/preference behavior of smolts to several effluent dilutions. Assays were designed to determine effluent concentrations that are nondisruptive to the smolts' migration. A second objective was to determine if the effluents could mask perception or alter behavior to a biologically significant odorant. We did this by testing whether the effluents masked the response to L-serine, an amino acid found in mammalian skin and known to elicit strong avoidance behavior in salmonids (Brett and MacKinnon 1954; Rehnberg and Schreck 1986; Rehnberg and Schreck 1987). The idea was to test the ability of the salmon to avoid the amino acid in the presence of effluent at concentrations found to be unavoided when no amino acid was present. Rehnberg and Schreck (1986) found that toxicants such as heavy metals can eliminate recognition of other odorants. During smolt migration, such a masking effect by effluent could have deleterious consequences for processes such as predator avoidance, foraging, and imprinting.

MATERIALS AND METHODS

Coho salmon smolts (mean weight = 24 g) were transported from Washington's Humptulips Hatchery to the testing site at the Aberdeen Sewage Treatment Plant in Aberdeen, Washington. Fish were held outdoors in 750 L, flow-through circular tanks supplied with water from a

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nearby tributary [Wishka River water (WRW)] and were given at least six days to adjust to these conditions before testing.

We tested effluent routinely discharged by the Weyerhaeuser Company (WeyCo) and ITT Rayonier (ITT) pulp mills. Since WeyCo effluent is acidified to a pH = 3.0 prior to release (to retard growth of fecal bacteria), this effluent was neutralized with NaOH prior to testing. This treatment was deemed appropriate because WeyCo effluent is probably neutralized quickly upon discharge into the saline waters of the estuary. Wishka River water (13-15°C, pH = 7.1) was used in control trials and served as the diluent for effluent solutions. For each effluent , we first tested 3-4 concentrations likely to be encountered by smolts in the harbor: 30%, 3%, 0.3%, or 0.03% (v:v). Then the effluent concentration that did not elicit significant avoidance was subsequently mixed with a 10^{-4} M L-serine solution (which was avoided by smolts in a preliminary positive control test) to determine if effluent masked detection of the amino acid . All tests were conducted < 24 hr after collecting effluent from each mill and allowing it to reach temperatures equivalent to WRW.

The avoidance/preference behavior of smolts was assessed using two identical Y-mazes, each illuminated with one 100 watt incandescent light fixture (Rehnberg et al. 1985). All tests were conducted between 0600 and 1800 hr. Test solutions were supplied via a constant-head delivery system capable of delivering test solution to one arm of the maze and WRW to the other arm; flow rates in each arm were 3.6 L/min. The mazes and the delivery system were drained and thoroughly rinsed with WRW between trials. To begin a trial, a single naive fish was placed in the downstream end of the maze. After a 12 min adjustment period, a gate was lifted and the fish was allowed 5 min to choose between entering either the control or effluent-treated arm, or remaining in the fork area. After this 5 min choice period, arm gates were dropped and fish choice recorded. Fish were then removed, and the mazes were drained, rinsed thoroughly, and refilled with WRW for the next trial. Test solutions were alternated between the two arms of each maze after each trial.

The numbers of fish choosing each arm were compared with a random arm selection model (1:1) using a chi-square goodness of fit test with the Yates correction for continuity (Zar 1984). Avoidance or preference was defined as significant departure from this 1:1 ratio using a $\chi^2 > 3.8$, ($P < 0.05$). Additionally, in tests where L-serine was mixed with unavoided concentrations of each mill's effluent, arm selection frequencies were compared with those obtained in the positive control test using Cochran's corrected chi-square statistic for a 2x2 contingency table (Zar 1984).

RESULTS AND DISCUSSION

Both WeyCo and ITT effluents at 30% were significantly avoided (Figure 1). Further testing revealed that effluent concentrations had to be reduced to 0.03% WeyCo and 0.3% ITT before they were no longer

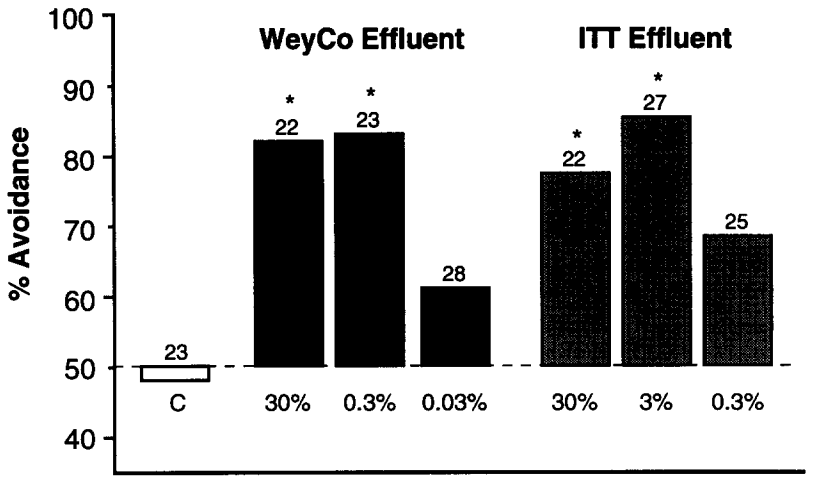

Figure 1. Avoidance of various dilutions of pulp mill effluents (percentages below bars) by juvenile coho salmon. "C" represents control test. Numbers above the bars indicate the number of fish entering either arm of the Y-maze. Asterisks refer to significant differences from a χ^2 test ($P < 0.05$).

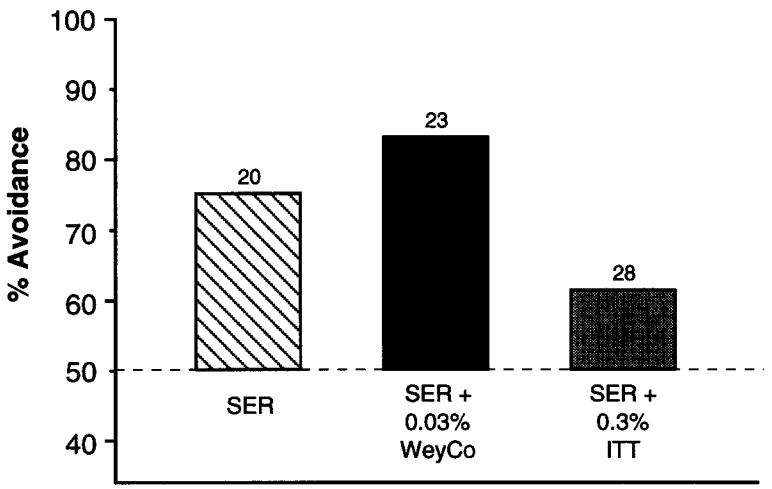

Figure 2. Avoidance of 10^{-4} M L-serine (SER) in control water only and when mixed with two previously unavoided effluents (percentages below bars). Numerals above the bars indicate the number of fish entering either arm of the Y-maze. Arm selection in the effluent tests was not significantly different from the test with SER only (χ^2 test, $P > 0.05$).

avoided. For each test, the number of fish not choosing either arm was variable (2-8 fish) for each effluent (Figure 1). Fish in the control test selected each arm with equal propensity (i.e., no significant difference from a 1:1 ratio). These results indicate that coho smolts are sensitive to minute amounts of both pulp mill effluents and, given the choice, generally prefer to avoid such waters. Interestingly, a recent telemetry study of salmon migration behavior in Grays Harbor suggests that pollutants (including pulp mill effluent) may have interfered with smolt migration (Moser et al. 1991). Effluent concentrations were not measured in this telemetry study, however fish reportedly slowed their migration in a region less than two miles from the WeyCo and ITT outfalls.

Previously unavoided concentrations of WeyCo effluent (0.03%) did not appear to inhibit the detection of 10^{-4} M L-serine. Arm selection behavior was significantly different than a 1:1 ratio and did not differ significantly from the behavior of fish in the positive control test (Figure 2). Similarly, the avoidance/preference response of fish exposed to a mixture of 0.3% ITT and 10^{-4} M L-serine was not significantly different from that of fish in the control test. However, it is interesting to note that fish avoided the ITT/L-serine mixture less strongly than the 0.3% ITT solution (Figures 1 and 2). And when compared to a 1:1 ratio, the results indicate neither avoidance nor preference for the ITT/L-serine mixture. Therefore, it is possible that low concentrations of ITT effluent could mask the detection of a generally repulsive constituent of mammalian (i.e., predator) skin odor.

Effluent concentrations avoided by smolts in our study are comparable to (or lower than) those reported as either repulsive or toxic in other studies with salmonids (Jones et al. 1956; Sprague and Drury 1969; Walden 1976; Fisher 1982). Ancillary experiments (*Stone and Schreck unpubl.*) revealed that an acute exposure (3-4 hr) to 30% WeyCo resulted in a significant increase in plasma cortisol titers in coho salmon, indicating a stress-related physiological correlate to the acute behavioral reactions in the present study. Other studies have demonstrated that acute effluent exposures can elicit secondary stress responses from salmonids (McLeay and Brown 1975; McLeay 1977). Thus, while the actual effluent concentrations encountered by emigrating coho salmon in the inner harbor are not known, it appears that smolts perceive at least higher effluent concentrations as noxious and respond accordingly.

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