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# Behavioral Reactions of Fishes Exposed to Unbleached Kraft Mill Effluent

G. Jeffords Campbell and Phillip W. Bettoli

Tennessee Cooperative Fishery Research Unit, Tennessee Technological University, Cookeville, Tennessee 38505, USA

Behavioral changes induced in fish by the liquid wastes of various industries may be as great a threat to survival as the acute lethal effects of these wastes. The pulp and paper industry has been of historic concern because the amount of wastewater involved is often large and the potential alteration of ambient waters is great.

The Tennessee River Pulp and Paper Company (TRPP) in Counce, Tennessee, produces unbleached kraft linerboard and discharges its effluent at Tennessee River Kilometer 330. The effluent outfall is 2 km below Pickwick Dam, which serves as the upstream boundary of Kentucky Lake, Tennessee's largest impoundment. Permit requirements set the minimum dilution of TRPP effluent with Tennessee River water at 100:1.

The tailwater below Pickwick Dam has been a point of concentration for sauger (Stizostedion canadense) during annual spawning migrations. In the late 1980's public concern was expressed that declines in the sauger population might be attributed to the effluent discharged by TRPP. The specific concern was that migrating sauger were avoiding the effluent plume, which would disrupt the movement of sauger in the upper reaches of the lake.

Numerous studies have examined the behavioral response of fish exposed to kraft mill and sulfite mill effluent (e.g., Jones et al. 1956; Kelso 1977; Wildish et al. 1977; Myllyvirta and Vuorinen 1989); these studies have usually examined the response of fish exposed to bleached kraft mill effluent. Unbleached kraft mill effluent lacks the chlorinated compounds that render bleached kraft mill effluent acutely toxic and relatively few studies have examined the response of fish to unbleached effluent. However, unbleached effluents do contain toxic constituents, notably salts of resin acids and fatty acids (Leach and Thakore 1973). Therefore, our objective was to determine if species of fish common to the Pickwick Dam tailwater area of Kentucky Lake displayed avoidance of unbleached kraft mill effluent produced by TRPP. There are few accounts of avoidance studies using fish species common to Kentucky Lake. In one laboratory study, Hall et al. (1984) detected avoidance of sulfite by striped bass (Morone saxatilis).

## MATERIALS AND METHODS

We used a modified counter-current avoidance trough to evaluate the response of fish exposed to TRPP effluent. Our avoidance apparatus was a modification of the rectangular counter-current trough designed by Scherer and Nowak (1973). Our trough (18 cm X 18 cm X 120 cm) was constructed of 12.7 mm clear acrylic and had a capacity of 38 L. Water was pumped into each end of the tank at a rate of 5 L/min. Inflow deflector paddles and perforated styrene baffles located at each tank end reduced turbulence and provided an effluent/water mixing zone. Water drainage through nine ports in the bottom and sides at the middle of the trough was controlled by clamping tubes connected to the acrylic ports. The effluent could be injected into either end of the tank using a peristaltic pump. Initial testing with the trough gave an unreliable, indistinct mid-trough boundary. We used plexiglass sheets to constrict the center of the tank to a width of 9 cm (the tank resembled an hourglass when viewed from above) to achieve a more consistent boundary. Dye injection tests in our modified trough showed a distinct mid-trough boundary formed quickly and remained indefinitely.

Sauger fry were reared for 30 d on a progressive diet of Bioproducts moist feeds (0.6 - 1.5 mm). At the time of testing, the sauger had attained a mean total length of 75 mm (N=40; SD=1.5). Two surrogate species were also chosen for testing. Channel catfish (Ictalurus punctatus) and striped bass x white bass (M. chrysops) hybrid fingerlings were obtained from commercial hatcheries and reared for 1 wk before behavioral testing; total length at testing averaged 105 mm (SD=5.0) for catfish and 101 mm (SD=2.2) for hybrid striped bass. Holding water temperatures ranged from 16°C to 22°C and pH of the water was slightly basic. Catfish are abundant in Kentucky Lake and support a large recreational and commercial fishery. Hybrid striped bass are less common, but both parental species support a substantial fishery.

The avoidance trough was in a room draped with black cloth. A closed-circuit television camera was positioned behind a curtain to the side of the tank such that the entire tank could be seen. All trials were videotaped.

The testing protocol for hybrid striped bass and catfish was derived from procedures described by Sprague (1964) and Hara (1981). A single fish was put into the tank and allowed 10-20 min for habituation. A fish had to cross the center a minimum of 5 times during the habituation period for the test to proceed. After habituation, the fish's activity was monitored and recorded for a 10-min control period. After the control period, effluent was randomly introduced into either end for another 10-15 min period. At the termination of the effluent period, the flow of effluent was stopped and the fish removed. The tank was allowed to clear for $\geq$ 10 min betwen trials.

Hybrid striped bass and channel catfish were tested at four concentrations (0.01, 0.1, 1.0, 10.0% v/v). Sauger were tested in a similar manner, but the 0.01% concentration was replaced by a 5.0% concentration. Fish were tested once at only one concentration.

Because of their sedentary nature, sauger were allowed a longer habituation period. At the end of the habituation period, effluent was injected immediately into the side of the tank where the fish were. Response information such as movement during the habituation and effluent periods was noted.

The traditional method of analyzing avoidance trough data is to compare percent time a fish spends in both sides of the trough (Sprague 1964; Scherer 1975; Hadjinicolaou 1985). de March and Scherer (1980) described shortcomings of percent-time data and suggested several alternatives, including the use of time statistics such as mean-trip-time. We chose to analyze mean-trip-time data because they were more amenable to statistical analysis and model building. Avoidance behavior is signified by a reduction in mean trip times in the half of the tank receiving the effluent. For each fish, Trip-Times-in-effluent-side-during-Control-period (TTC) and Trip-Times-in-effluent-side-during-Test-period (TTT) were recorded and means were calculated. Values for TTT and TTC were $\log_{10}$-transformed to stabilize variances. The effluent concentration and TTC variables were then used in a step-wise multiple regression analysis to explain variability in TTT. Percent time spent by hybrid striped bass and catfish in each half of the trough during control periods was calculated to detect end bias; Student's t-test was used to test for differences.

Analysis of sauger data was limited to testing whether a relationship existed between the proportion of sauger moving and effluent concentration. The chi-square statistic evaluated the significance of the association between those two variables.

For the avoidance tests, grab samples of unbleached kraft mill effluent were collected in Nalgene containers from the flow metering station at the effluent outfall. The effluent was refrigerated and used within 10 d.

Grab samples of effluent were collected for analysis from the effluent outfall flow metering station. Samples for the analysis of volatile organic compounds (VOC's) were collected in triplicate on 4 December 1989. These samples were stored at 4°C and analyzed according to EPA Method 624 (EPA 1983) using a Hewlett-Packard 5987 GC/MS and NIH/EPA/NBS Data Base. Samples for analysis of acid and base-neutral (A/BN) extractables were collected on 12 December 1988 and 4 December 1989; extractables were quantified in the latter sample. Samples were collected in 1-L amber glass bottles, stored at 4°C, extracted within 7 d of collection, and analyzed within 30 d after extraction; samples were prepared and analyzed according to EPA Method 625 (EPA 1983). Quantification of fatty acids and resin acids was done by methylation of the acid extract with diazomethane reagent (Aldrich Technical Information Bulletin No. 180); the methyl esters were analyzed using a Hewlett-Packard 5880 GC. Samples for analysis of inorganic compounds were collected on 13 June 1989. A single determination was made for the following parameters: total nitrogen (persulfate method), total phosphorus (ascorbic acid method), sulfur (inductively coupled plasma spectrophotometer method).

## RESULTS AND DISCUSSION

Our rectangular counter-current trough with the center constriction modification was an improvement over previous designs. The well-defined boundary between the two bodies of water improved our ability to precisely repeat experimental conditions in each trial. As used in this study, the test tank functioned as a hybrid between a shuttle box and a typical counter-current trough and retained the desirable characteristics of each design. A good boundary was created (as in a shuttle-box), yet fish could easily traverse the length of the tank without relying on learned behavior.

Concentrations of the most toxic constituents of unbleached kraft mill effluent (i.e., fatty and resin acids) were low in the TRPP effluent (Table 1). Concentrations of soaps of resin acids and soaps of fatty acids $\geq$ 5 mg/L are considered acutely toxic (Leach and Thakore 1973); concentrations $\leq$ 1 mg/L are considered safe. Concentrations of all fatty acids and resin acids combined in the TRPP effluent did not exceed 0.4 mg/L. Concentrations of fatty and resin acids in TRPP effluent were much lower than concentrations reported for other pulp mills producing bleached or unbleached kraft mill

Table 1. Presence (X) or concentrations (mg/L) of volatile organic compounds (VOC's), acid and base/neutral extractables, and inorganic compounds in effluent samples of TRPP unbleached kraft mill effluent. All concentrations were measured in a single sample.

| Substance | Concentration |
|---|---|
| Volatile Organic Compounds | |
| dimethylsulfide | X |
| dimethyldisulfide | X |
| dimethyltrisulfide | X |
| bicyclo [3.1.1] hept-2-ene, 3, 6, 6-trimethyl | X |
| bicyclo [3.1.0] hexane, 4-methylene-1-(1-methyethyl) | X |
| bicyclo [2.2.1] hept-2-ene, 1, 7, 7-trimethyl | X |
| benzene, 1-methoxy,4-(1-propenyl) | X |
| pinene isomer | X |
| limonene | X |
| Acid Extractables | |
| palmitic acid | 0.081 |
| stearic acid | 0.081 |
| oleic acid | 0.027 |
| linoleic acid | 0.030 |
| linolenic acid | 0.014 |
| tetracosanoic acid | X |
| pimaric acid | 0.016 |
| abietic acid | X |
| dehydroabietic acid | 0.077 |
| Base/Neutral Extractables | |
| pinene isomer | X |
| 2-cyclopentenone | X |
| Inorganics/Physical | |
| total nitrogen | 4.89 |
| total phosphorus | 0.56 |
| sulfur | 129 |
| total dissolved oxygen | 898 |
| chemical oxygen demand | 194 |

effluent (Keith 1975; Myllyvirta and Vuorinen 1989). As expected, the TRPP effluent was high in dissolved solids, sulfur, and nutrients (Table 1) and was highly colored.

We discarded 15 of the 41 channel catfish trials conducted because the fish did not meet the _a priori_ requirement of 5 boundary crossings in the habituation period. No statistically significant difference in the percent time spent by the 26 channel catfish in the right half versus the left half of the trough occurred during the control periods (t-test; $\underline{P} \geq 0.05$). Therefore, there was no apparent tank-end bias. The step-wise multiple regression analysis using the independent variables TTC and effluent concentration yielded two significant regressions. The best one-variable model used effluent concentration to explain the most variability in the dependent variable, TTT (F = 6.6; DF = 1,24; P= 0.011). The slope of the line was slightly positive (0.1124), indicating that mean-trip-time during test periods tended to <u>increase</u> with increasing effluent concentration. That is, channel catfish displayed a slight <u>preference</u> for the effluent. Most variability in TTT remained unexplained in the one-variable model ($r^2$ = 0.3664). Incorporating the second independent variable (TTC) into the analysis did not improve the model ($r^2$ = 0.3666).

Hybrid striped bass were more active than channel catfish, suggesting that they were better suited for testing in the counter-current trough. Mean trip times in both halves of the trough during the control period averaged 23.1 sec for hybrid striped bass, compared to 48.6 sec for channel catfish. Nine of the 32 hybrid striped bass trials were discarded because of too few boundary crossings. Percent time spent by hybrid striped bass in both halves of the trough during the control period did not differ; therefore, there was no apparent tank end bias. The best one-variable model generated by the regression analysis used the variable TTC to explain the most variation in the dependent variable (F = 9.32; DF = 1,21; $\underline{P}$ = 0.006). The slope was slightly positive (0.5581), meaning that trip times during the test periods were positively correlated with trip times during the control periods. Most of the variability in TTT remained unexplained ($r^2$ = 0.31). Incorporating the variable of greatest concern, effluent concentration, yielded a model only slightly improved ($r^2$ = 0.38) and that variable did not contribute significantly to a reduction in the error sum of squares (F = 2.55; DF = 1,20; P = 0.126).

A total of 40 sauger (10 at each concentration) were tested. Sauger were extremely sedentary; fish usually remained in the tank end into which they had been placed at the beginning of the habituation period. The tank end in which the fish resided during habituation was also the tank end into which effluent was released. When effluent was released, two of ten sauger at each concentration $\geq$ 1.0% moved within 2 min of effluent injection; no sauger moved at 0.1% effluent. No relationship existed between effluent concentration and movement of sauger in the avoidance trough (chi-square = 2.36; DF = 3; P > 0.10). Fish mobility (routine boundary crossing) is necessary for counter-current, avoidance behavior testing (Sprague 1964; Scherer 1975; Hara 1981). Although sauger were reared and maintained with little difficulty, this species was clearly unsuited to testing in avoidance troughs. We also observed the same sedentary behavior in pilot experiments using adult sauger and a large (100-L) avoidance trough. Conducting trials at low light levels and experimenting with different wavelengths of light did not improve their mobility.

Channel catfish and hybrid striped bass were better test organisms than sauger, although many trials (catfish - 36%; bass - 28%) had to be discarded because the fish were too sedentary. However, individuals that met our a priori requirements for minimum number of boundary crossings appeared well suited to the avoidance trough apparatus. The added cost of conducting extra trials may be offset by the appropriateness and desirability of using test animals common to the waters under study. Ideally, behavioral toxicity testing involves several species endemic to a particular water body and species for which extensive toxicological data already exist.

Our tests failed to generate data that could be interpreted as signifying avoidance of the unbleached kraft mill effluent produced by TRPP by any of the species we tested. Effluent concentration had a weak effect on the mean trip times of channel catfish, but those data revealed a preference for the effluent side of the tank at high concentrations. We did not observe any effluent avoidance at concentrations 10 times higher than the maximum concentrations fish might experience in the Pickwick Dam tailwater region of Kentucky Lake. We were unable to generate appropriate avoidance-trough data for sauger, but the fact that two dissimilar surrogate species did not display any avoidance supported our limited observations on sauger behavior when exposed to the effluent. In the absence of additional information, we conclude that the decline in the sauger population in Kentucky Lake is not due to sauger avoiding the effluent plume. Because concentrations of nutrients and suspended solids were high, the effluent may be affecting other aspects of the biology of sauger, but these concerns remain speculative until additional research is conducted. Confirmation of our conclusion that sauger (and other fishes) do not avoid the effluent plume will be sought when we radio-tag and track Kentucky Lake sauger during their spawning migrations in 1992 and 1993.

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