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Effects of Bleached Kraft Mill Effluent (BKME) on the Schooling Behavior of Vendace (*Coregonus albula* L.)

Tero P. Myllyvirta and Pekka J. Vuorinen

Finnish Game and Fisheries Research Institute, Fisheries Division, P.O. Box 202, SF-00151 Helsinki, Finland

According to Coble (1973), the behavior of prey fish is more important than their physical appearance in influencing predator choice and thus prey survival. Changes occurring in prey-fish behavior as a result of exposure to physiologically sublethal concentrations of toxicants may alter their vulnerability to predation (Coble 1973). Therefore, sublethal exposure levels will be indirectly lethal to prey fish, if they lead to selective predation. There is a general rule that predators can more easily capture abnormal, disabled or disoriented prey and there is considerable evidence that such prey is preferred (Curio 1976). Changed spawning behavior caused by the injurious compounds in waste waters may also prevent successful spawning (Mount 1968). One of the most striking qualities of a school of fish is its polarization, the parallel arrangement of the members. When the school is in motion the polarization is strong, but when the fish feed, they often form a loose group with the members facing in several directions. When the school is threatened, the members approach each other and the polarization becomes stronger. Polarization is possibly a factor which increases survival in the school (Partridge 1982).

Factors disturbing schooling behavior may be indirectly lethal to fish (Coble 1973). Moreover, the identification of behavioral changes caused by chemical agents may provide an early warning system allowing the detection of toxicity before irreversible structural and biochemical damage has occurred (Thomson & Shuster 1968). So far, however, the effects of toxicants on schooling behavior appear to have received little attention (Sullivan & Atchison 1978). The aim of this study was to discover whether low, sublethal concentrations of BKME disturb the behavior of a fish school. The reactions of fish to the effluents of the forest industry is of great interest in Finland, since this industry is the most important water pollutor in the country (Vesihallitus 1983). The vendace (*Coregonus albula* L.) was selected as the test fish because of its commercial value and its strong schooling behavior.

Send reprint requests to P. J. Vuorinen at the above address.

MATERIAL AND METHODS

The test fish, 4-mon-old vendace, were reared for the experiments at the Laukaa Fish Culture Research Station in Laukaa of the Finnish Game and Fisheries Research Institute. The experiments were also carried out at Laukaa. The water used in the laboratory was from Lake Peurunka, which supplies water for the station. Before the vendace were used in the experiments, they were acclimated in holding tanks in laboratory conditions for about 3 wk. The light rhythm during that period and the experiments was 15 h (0700 - 2200)/9 h (light/dark), and the intensity of illumination was ca. 40 lux. The fish were fed with dry feed (Ewos Ltd, Finland) twice a day, except

Table 1. Concentrations of resin acids (mg/l) and chlorophenols (µg/l) in BKME samples. Resin acid analyses with effluent 1 and 2 failed.

Substance	No. of BKME sample 1	2	3	4	5	6
Resin acids						
Pimaric acid	-	-	0.04	0.70	0.16	0.15
Isopimaric acid	-	-	0.01	0.19	0.09	0.14
Palustric acid	-	-	-	-	0.15	-
Abietic acid	-	-	0.10	0.57	0.25	0.27
Dehydroabietic acid	-	-	0.62	0.97	0.38	0.35
Neoabietic acid	-	-	-	-	-	0.12
Sandracopimaric acid	-	-	-	-	0.10	0.01
sum	-	-	0.77	2.39	1.13	1.04
Chlorophenols						
2,6-Dichlorophenol	-	-	-	2.7	47.1	28.0
2,3,4,6-Tetrachlorophenol	12.0	2.6	1.0	3.2	2.6	2.8
4,5,6-Trichloroguaiacol	44.3	15.9	26.4	14.0	16.1	20.1
Tetrachloroguaiacol	6.0	4.4	11.0	7.5	8.7	11.7
3,4,5-Trichlorocatechol	17.5	13.3	63.9	26.2	37.2	41.2
Trichlorovanillin	-	-	-	5.1	11.7	6.5
Pentachlorophenol	13.9	4.7	-	-	5.3	3.8
Tetrachlorocatechol	9.5	11.0	32.0	13.8	23.0	30.0
sum	103.2	51.9	134.3	72.5	151.7	144.4

during the time (27 h) they spent in the main test aquaria, when the feed was withdrawn. At the beginning of the 5 wk test period, the mean length of the vendace was 7.3 cm (SD = 0.3 cm) and the mean weight was 2.2 g (SD = 0.2 g). After the test period the corresponding values were 8.1 cm (SD = 0.6 cm) and 3.2 g (SD = 0.7 g) (N = 30). The test fish were taken at random from the holding tanks and were kept in water during the transfer.

Five composite samples of bleached kraft mill effluent (BKME) were collected from the main effluent channel of the mill by an automatic continuous flow sampler, each of which represented one week's waste water production of the mill in an unbroken succession. For the experiments the effluent was neutralized with 0.1 M NaOH to pH 7 and filtered through a plankton net (mesh size 20 µm). The BKME samples were analysed according to Holmbom (1980) to determine the concentrations of resin acids and chlorophenols (Table 1).

The acute toxicity of the composite BKME samples was determined by a semistatic method (80% renewal of test solution per day) for rainbow trout (*Salmo gairdneri* Richardson) (samples 2 - 5) and vendace (samples 4 and 5). The test with sample number 1 failed (Table 2). The 96-h LC50 values were calculated by a computer program that uses probit analysis (Stephan 1977).

Before the observation period in the test aquarium, vendace were pre-exposed for 1 wk to three concentrations of each of the composite BKME samples given in Table 3. The exposure conditions (Fig. 1) accorded with the recommendations of Sprague (1969); the water

Table 2. The 96-h LC50 values (% v/v) of the composite BKME samples for rainbow trout and vendace.

BKME sample	96-h LC50 (% v/v)	
	rainbow trout	vendace
1	-	-
2	13.7	-
3	14.9	-
4	18.5	14.4
5	30.9	26.0

Table 3. Concentrations (% v/v) of the composite BKME samples used in the tests with vendace.

BKME sample	control	BKME conc. % v/v						
		0.13	0.25	0.38	0.75	1.50	2.25	4.50
1	+		+		+	+		
2	+		+		+	+		
3	+	+		+	+			
4	+				+		+	+
5	+		+		+	+		

flow was 3 l/g of fish per day. The control fish were kept in a quadrangular holding tank with a surface area of 1 m2. During the whole 5 wk test period the water temperature followed the natural temperature variations of Lake Peurunka (14.7 -19.2 °C), and the temperature was always the same for the control and test fish. During pre-exposure pH varied between 6.83 and 6.98, and the dissolved oxygen content between 87 and 93 % of saturation. The effluent concentrations deviated from the nominal values by 3 % at most.

By fixing a mirror at an angle of 45° above two aquaria (65 x 30 x 30 cm), it was possible to take photographs from the front and record the fish positions in all the three dimensions (Fig. 2). Grids of 2-cm squares formed the photographic backgrounds, thus facilitating the recording of fish positions and dimensions. The camera was placed 2.3 m from the front of the aquaria and the control and test aquaria appeared on the same photograph. A cloth (with a hole for the objective) was arranged in front of the camera to eliminate visual contact with the photographer. The pictures were lit by two flashlights placed at an angle of 45° in front of the aquaria.

Twelve hours before the first observation period, 10 vendace were placed in an aquarium with the same concentration of BKME to which they had been pre-exposed. Ten control vendace were placed in the adjacent aquarium with clean water. Thirty minutes before each photography session, a light was so adjusted above the camera that the intensity of illumination in front of the aquaria was 40 lux and the aquaria were uniformly lit; 16 - 18 photographs were then taken at 60-s intervals. Thirteen hours later, the adjustment of the lighting and the photography were repeated. The first photography period began at 0730 h and the next at 2030 h. This whole procedure was run through with all the 28 fish groups. Every other time the test fish were put in the aquarium to the right and every other time in the aquarium to the left. The dissolved oxygen content varied from 89 to 91 % of saturation in the tests (both aquaria were aerated by aquarium pumps) and the temperature was always the same in the two aquaria. Each frame was examined "blind" by a microfilm reader to eliminate the possible effect of expectation.

In this study a school is defined as a group of mutually attracted fish, irrespective of polarized orientation or state of movement. For the fish group to be identified as an undivided school, at least 8 of the 10 fish must form an aggregation in which none of the fish is further than 10 cm from its neighbor. With the help of the three dimensions of the group of 10 fish (X: side to side; Y: top to bottom; and Z: front to back), the smallest prism was calculated which could contain all the 10 fish. This has been assumed to give a good estimate of the attraction existing among the fish.

Student's t-test (Alder & Roessler 1964) was used to examine possible differences between the control and test groups in the estimates of a) the water volumes that could contain all the 10 fish and b) the proportion of divided and undivided schools.

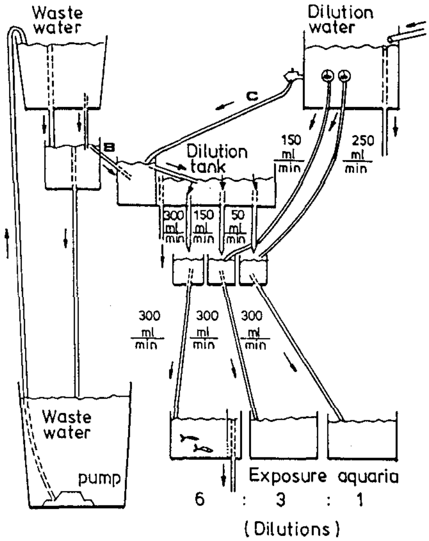

Figure 1. The exposure equipment. The maximum concentration in the dilution tank is obtained by mixing the waste water from tube C. The concentrations in the other aquaria are obtained by mixing the water from the dilution tank with the dilution water.

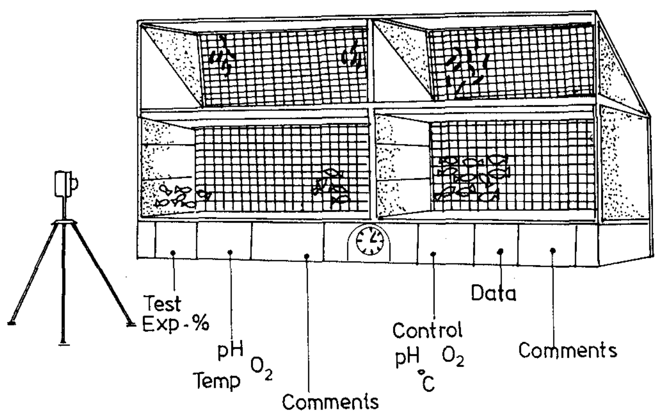

Figure 2. Test aquaria and the photographic equipment.

RESULTS AND DISCUSSION

Vendace proved to be ideal fish for this kind of study. Their way of swimming was calm and they swam constantly. There were no sudden rushes and the fish occupied only a small part of the total water volume of the aquarium. This indicates strong attraction between the fish and schooling behavior. The fish mostly swam to and fro in the aquarium. The polarization between the fish was strongest when they were in the middle of the aquarium. Closer to the wall of the aquarium, the swimming speed slowed down and the degree of polarization decreased, increasing again with faster swimming speeds. Polarization was observed to decrease with increasing school volumes. No differences in swimming activity could be noted between the control and test groups.

As the chemical analysis and toxicity tests showed that the quality of the effluent samples varied widely, the results of the experiments were treated separately for each sample. The dilation of the schools of test fish was most consistent ($p < 0.001$) with BKME sample 5, and with sample 4 it appeared only in the greatest (4.5 %) exposure concentration (Fig. 3). When the same five effluent samples were used to study avoidance reactions in vendace (Myllyvirta & Vuorinen, unpubl.), the fish showed a weaker avoidance reaction in a low concentration gradient of BKME sample 4 than in sample 5.

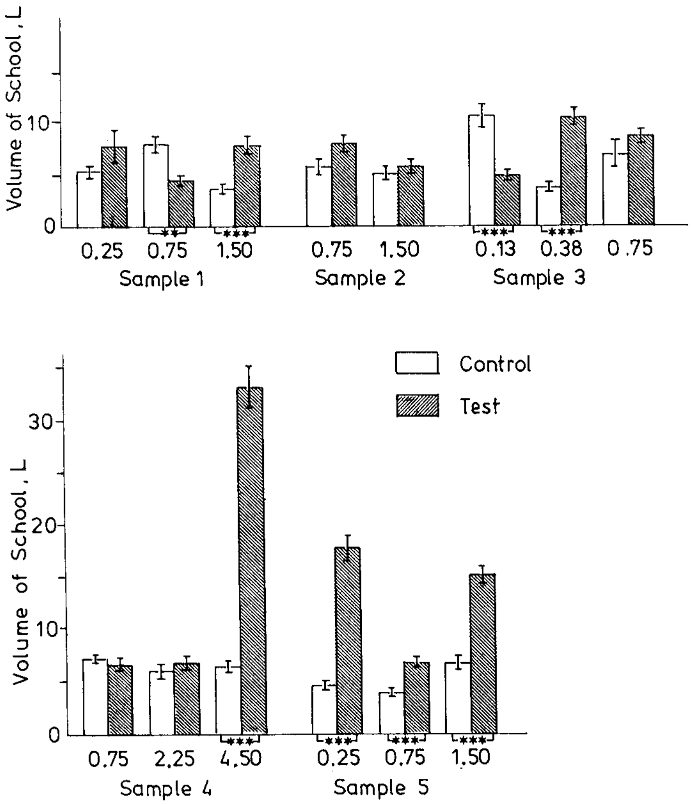

Figure 3. Comparison between the control and test fish of the smallest prism that contains all the 10 fish. The test was made with five different BKME samples. Significant differences (Student's t-test; ** = $p < 0.01$, *** = $p < 0.001$) and the SE are given.

With BKME samples 1-3, the effects of exposure on school volumes were somewhat obscure, though some significant differences existed.

When the proportion of divided schools (Table 4) is compared with the water volume occupied by the schools, it appears that they are often significantly greater in the same tests. This is to be expected, because a fish which is separated from other fish increases the water volume that can accommodate the 10 fish. In both cases it is a question of weakened attraction between the fish. According to van Olst & Hunter (1970), similarities in "headings" between two fish decrease in a school with increasing distance between the fish. The same observation was made in the present study. The more heterogeneous behavior occurring with increased inter-fish distance may make it easier for predators to pick out an individual from the group. The school benefits each of its members by diminishing the predator's chance of finding prey and confusing the predator once the prey is found (Partridge 1982). The weakened schooling behavior among the test fish should diminish both of these advantages. Sullivan & Atchison (1978) demonstrated increased vulnerability to predation of fathead minnows (Pimephales promelas) by largemouth bass (Micropterus salmoides) after the fathead minnows were subjected to acute (24 h) and subacute (21 d) cadmium treatment. The fish exposed to cadmium displayed altered behavior patterns, including abnormal schooling behavior. Both test and control fish schooled, but cadmium-treated members often changed their direction within the school. The same behavior was evident among the vendace exposed to BKME at the present sublethal levels and this would possibly increase the predation pressure. Therefore, all the conceivable advantages of schooling behavior may be weakened in

Table 4. Percentages of divided schools in the control and test groups. Numbers and concentrations (v/v %) of BKME samples also given. Significances tested with Student's t-test. N = number of frames analysed.

BKME samples	Exposure conc.	Control Divided,%	Test group Divided,%	N	p<
1	0.25	28.1	28.1	32	NS
1	0.75	18.2	21.2	33	NS
1	1.50	2.9	17.7	34	0.05
2	0.75	6.5	12.9	31	NS
2	1.59	12.1	12.1	33	NS
3	0.13	43.8	21.9	32	NS
3	0.38	11.8	52.9	34	0.001
3	0.75	6.5	32.3	31	0.01
4	0.75	37.5	28.1	32	NS
4	2.25	13.3	6.7	30	NS
4	4.50	3.1	87.5	32	0.001
5	0.25	3.1	37.5	32	0.001
5	0.75	11.4	17.1	35	NS
5	1.50	6.9	17.2	29	NS

the field as well. As calculated from the water and effluent flows in 1981 (S. Yli-Karjanmaa, pers. comm.), the mean BKME concentrations in the watercourse 10 and 50 km downstream from the mill were 0.6 and 0.3 % (v/v).

The inter-fish responses in a school are of great importance, and changed behavior in one fish disturbs the whole school (Partridge 1982). Alteration of schooling behavior by exposure to effluent may be of greater importance for fisheries than accidental fish kills, because much larger numbers of fish are exposed to sublethal concentrations of effluents than to lethal concentrations.

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