Effects of bleached kraft mill effluent on reproduction of brown trout (*Salmo trutta* L.) on a restricted diet

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A shortened life-cycle test was carried out to investigate the influence of bleached kraft mill effluent (BKME) on brown trout. Parent fish were exposed to the effluent at nominal concentrations of 0, 0.2, and 0.5 % (v/v) for 3 months before spawning time. All the trout in the effluent concentration of 2.0 % were dead after 15 days of exposure. Effluent-exposed trout in other concentrations lost weight more than did the control fish, and had lower Hb concentrations and Hct readings. Effluent exposure diminished the egg numbers of trout. Moreover, the eggs of the trout exposed to 0.5 % effluent were smaller and fertilized less well than the eggs of the control trout. Fertilization in effluent dilutions and the fertilizing ability of milt from effluent-exposed males were not affected. The sac fry from female trout exposed to 0.5 % effluent were smaller and abnormal, their metabolism was retarded, they could not absorb their yolk and finally they died, regardless of whether they were incubated in effluent of the same concentration or in clean water. The sac fry incubated in 0.2 % effluent were smaller and their heart rate lower, compared to sac fry incubated in clean water.

The maximum acceptable toxicant concentration (MATC) of BKME was < 0.2 % for brown trout.

I. Introduction

Pulp and paper mill effluents contain e.g. resin and fatty acids, as well as chlorinated phenols, catechols and guaiacols, many of which are even in small concentrations (< 2 mg l^{-1}) acutely lethal to fish (SERVIZI et al. 1968, LEACH & THAKORE 1973, 1975). Some of the chlorophenols and resin acids are quite persistent and have been shown to accumulate in fish tissues (LANDER et al. 1977, PAASIVIRTA et al. 1980, OIKARI et al. 1980, 1982). Effluents, especially from the bleaching stage, contain a large number of compounds that are still unidentified (HOLMBOM & LEHTINEN 1980).

A number of studies have been made concerning the sublethal effects of pulp and paper mill effluents on fish. Effects have been observed in cough frequency (HOWARD & WALDEN 1974), in oxygen consumption (MACLEOD & SMITH 1966), in histology (FUJIYA 1961), in carbohydrate metabolism and in blood variables (MCLEAY 1973, MCLEAY & BROWN 1974, 1979). However, the changes observed have not been related to the growth or reproduction of fish. Experiments on fish growth have led to conflicting results: in some growth is evidently

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inhibited (WEBB & BRETT 1972, WARREN et al. 1974), but in others it is apparently stimulated (MCLEAY & BROWN 1974, 1979).

MOUNT & STEPHAN (1967) proposed that laboratory experiments should be carried out over one generation, collecting data on the effects on fish growth, reproduction, and spawning behaviour, as well as the viability of eggs and growth of fry. "Maximum acceptable toxicant concentration" (MATC) could then be established on the basis of such chronic exposures. Life-cycle toxicity tests have provided the most reliable data on the long-term effects of toxic chemicals on fish (MCKIM 1977). According to GERKING (1979), reproduction (defined as egg maturation in the ovary, egg laying, and egg viability) is the most sensitive part of the entire life cycle. Life-cycle experiments have been done for example with copper (MCKIM & BENOIT 1971), cadmium (BENOIT et al. 1976), lead (HOLCOMBE et al. 1976), and pesticides (e.g. CARLSON 1971, MAYER et al. 1975). The accumulation of xenobiotics from the marine environment has been found to diminish the hatchability of flounder eggs (VON WESTERNHAGEN et al. 1981). Only recently the effects of bleached kraft mill effluent (BKME) on fish (zebra fish, *Brachydanio rerio*) reproduction have been studied (VIKTOR et al. 1980).

In this work, which is part of a larger experimental investigation of the effects of BKME on fish, we have studied its effects on the reproduction of brown trout. Brown trout parent fish were exposed to BKME for 3 months, their eggs were fertilized and incubated, and development of offspring was observed until the end of yolk absorption. This series of observations forms a type of shortened life-cycle test. Tissue and blood variables in samples taken after exposure were measured to clarify the significance of these factors in long-term exposures and to illustrate modes of action.

II. Material and methods

1. Mill

The kraft pulp mill, whose effluent was used in the study, has two product lines. One (Line I) uses only pine as the raw material (production about 100 tons per day) and the other (Line II) uses pine and birch (production 200—250 tons per day). The combined effluent from these two lines is composed of the effluents from pulp washing, cooking liquor preparation, evaporation, drying machines, bleaching, and cooking condensates. The bleaching sequence of line I is: C-E-H-H-D and that of line II: C-E-H-H-D-E-D (C = chlorination step, E = alkali step, D = chlorine dioxide step, H = hypochlorite step). The mill produces annually about 120 000 tons of bleached pulp. The amount of effluent per ton of bleached pulp in 1980 was 172 m^3. The effluents containing fibres are cleaned mechanically before being discharged.

2. Exposure conditions

Exposure tanks were in a shed with plastic walls located near the main effluent drain of the kraft mill. The roof of the shed was impervious to light to provide shelter from direct sun; but the walls were transparent to allow a natural photoperiod. The diluent water was led to its constant level tank from the cooling water circulation of the mill, into which water is taken from Lake Keitele upstream from the mill. The scheme of the exposure system is presented in Figure 1. First the effluent was pumped from the main effluent drain into a constant level tank, from where it was led to a small constant level tank to make a 10 % dilution with lake water. The flows of diluent water, effluent, and diluted effluent were adjusted making use of the hydrostatic pressure of the constant level tanks, and by choosing suitable diameters for the nozzles at the ends of the pipes (GRANMO & KOLLBERG 1972). The nozzles were cleaned every day to prevent clogging. The replacement time of effluent in its constant level tank was about three hours. Due to pump failures, there were occasional interruptions in effluent flow that lasted from about one hour to a few days. Water temperature in the fish tanks varied seasonally. The temperature, pH, and dissolved oxygen (DO) content of samples taken from the removal pipes of the tanks were

measured daily for every tank. Chemical oxygen demand (COD_{Mn}) was determined once a week. During exposure, the pH of the diluent water was 6.97 ± 0.01 (mean ± SE, N = 84), and COD_{Mn} was 4.5 ± 0.1 mg l^{-1} (N = 28).

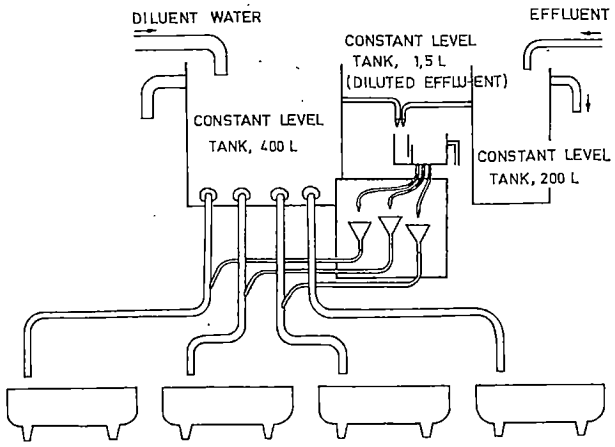

Figure 1. The test equipment used in brown trout exposures to BKME.

3. Fish and experiments

Brown trout (*Salmo trutta* L.) were five-year-old trout taken from the stock of the Rautalampi water course. The Laukaa Fish Culture Research Station of the Finnish Game and Fisheries Research Institute kindly provided the trout. The fish were caught with seines from growing ponds, and transported to the experiment site. There they were anesthesized with MS-222, weighed, measured (total length), marked with Carlin-tags, and randomly distributed into four tanks (about 28 fish per tank). The weight of the trout was 1 105 ± 19 g (mean ± SE) and length 44.6 ± 0.3 cm. The gonadosomatic indexes of the ten killed trout were 3.3 ± 0.3 % (♀♀) and 1.7 ± 0.4 % (♂♂). The fish were acclimatized for eight days before the beginning of effluent exposure.

Exposure commenced on 16 July and was ended on 15 October 1980. The test concentrations were selected on the basis of the 96-h LC50 values that were determined for brown trout fry. The nominal exposure concentrations were 0, 0.2, 0.5 and 2.0 % (v/v). The tanks were rectangular and made of fiberglass. The surface area of each tank was 4 m^2 and the water volume about 1 600 liters. Water flow was 2.4 l per g of fish per day. Figure 2 shows the variation in water temperature, DO content, and pH in the control tank during the experiment. Mean values of test waters and effluent analysis results are presented in Table 1. Water temperature, DO content, and pH were the same in all of the tanks.

Feeding was begun three days after transporting and tagging. The trout were fed with frozen herring that was

Table 1. The pH, dissolved oxygen content as percent of saturation, and chemical oxygen demand, in water of trout exposure tanks; and pH and COD_{Mn} of the effluent. Number of analyses in parenthesis.

	pH	O_2, %	COD_{Mn}, mg l^{-1}
0 %	6.70 ± 0.01 (84)	68 ± 0 (72)	25 ± 0 (29)
0.2 %	6.72 ± 0.01 (81)	70 ± 1 (70)	26 ± 1 (27)
0.5 %	6.70 ± 0.01 (81)	69 ± 1 (70)	30 ± 1 (26)
2 %	6.66 ± 0.02 (26)	68 ± 1 (25)	32 ± 1 (9)
Effluent	4.97 ± 0.17 (59)	—	1200 ± 58 (23)

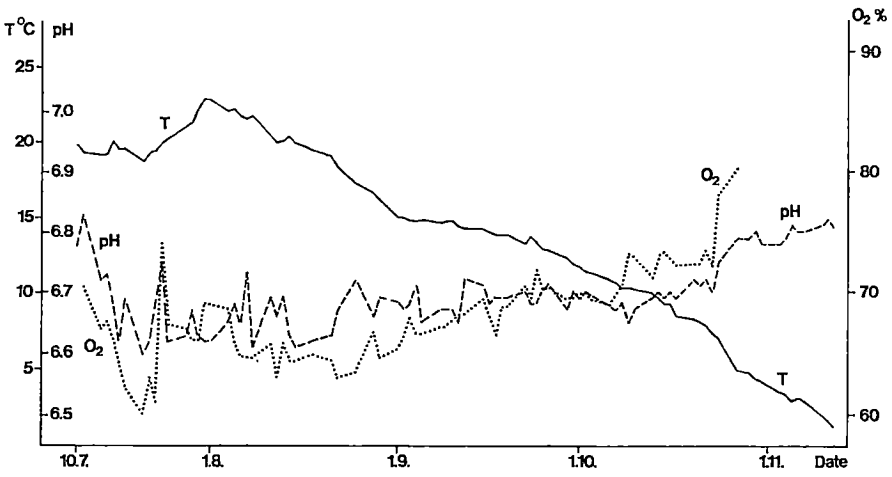

Figure 2. The fluctuation of water temperature (continuous line), pH (broken line), and dissolved oxygen (DO) content as percent of saturation (dotted line), in the control tank during brown trout exposure.

thawed just before feeding, and with dry feed (Ewos), to a total of 2 % of body weight per day. Herring was given in the morning and dry feed in the afternoon; feeding was completed in one hour on both occasions. On Saturdays and Sundays the entire 2 % portion was given at one meal, first dry feed and then herring. From the beginning of September the fish were fed only with herring, because they no longer took the dry feed: feeding was later stopped when the trout ceased to take any feed. The uneaten feed was removed from the bottom of the tanks after feeding.

The eggs were stripped from the trout in all of the exposure concentrations where there were fish left (0, 0.2 and 0.5 %) on 23—24 October 1980, at which time they were also measured and weighed. The condition factors (K) were calculated from the length and weight before stripping, using the formula: $K = (\text{weight (g)} / \text{length (cm)}^3) \times 100$. The amount of stripped eggs was also weighed. A few grams of eggs were taken for size determination; the major part of the eggs were fertilized and used as described below.

Eggs were fertilized in each experiment as follows: The eggs and plenty of milt were first mixed in a wash pot, and water was added to just cover the eggs. The wash pot was swirled gently for five minutes, after which the eggs were rinsed with a few aliquots of water. The eggs were left to swell overnight in wash pots in a water bath at the temperature of the test tanks. The following day the eggs were transported to the fish culture station and laid on incubation trays.

The eggs of each female were incubated on separate trays that were made by soldering stainless steel net (AISI 316, mesh 3 mm) to two cylinders of polyethylene pipe (diameter 100 mm). The cylinders were set upright on the bottoms of separate incubation boxes and a few layers of eggs were laid on the net. A water flow (0.1 l min^{-1}) was led under the net and through the egg layers. The DO content of water was on the average 81 % of saturation (range 73—98 %).

Experiments I and II. The eggs of four or five females from each concentration (0, 0.2 and 0.5 %) were fertilized with milt from three control males, and divided into two equal portions. One-half of the eggs from each female was incubated in the same effluent concentrations in which the parent fish were exposed (Experiment I) and the other half in clean water from Lake Peurunka (Experiment II). The effluent used in the week-long incubation was pumped from the main effluent drain for about half an hour and then stored at —20 to +4 °C until used (maximally *c.* one week). The dosing equipment used was in principle the same as that used for the parent fish exposure (Fig. 1). The results of the water analyses of the different incubation concentrations and the undiluted effluent are given in Table 2. The temperature range during the incubation period was 1.8—2.4 °C and during the sac-fry stage 2.4—4.0 °C. The eggs were incubated until hatching, when the numbers hatched were counted. The experiments were terminated when the fry either swam freely and began to eat, or died. In this study we have used the term sac fry as a synonym for the term eleutheroembryo proposed by BALON (1975).

When all the sac fry had hatched, being then on the average two weeks old, the sac fry of each female were photographed; and heart rates were measured with the aid of a microscope, while the sac fry was kept in a water bath at the temperature of the experiment. The weight, length, and water content (gravimetrically after 24 hours drying at 105 °C) of sac fry were measured. Sac fry were also homogenized with a Potter-Elvehjelm glass-teflon homogenizer in 10 volumes of distilled water. The homogenate was centrifuged and the supernatant used for determinations of total protein (LOWRY et al. 1951) and total

Table 2. Water quality parameters of different effluent concentrations and effluent during the incubation of the eggs of brown trout. Number of analyses in parenthesis.

	0 %	0.2 %	0.5 %	Effluent
pH_{25}	6.83 ± 0.03 (28)	6.81 ± 0.03 (29)	6.76 ± 0.03 (29)	5.21 ± 0.36 (41)
Conductivity, mS m^{-1}	5.0 ± 0.0 (29)	5.3 ± 0.1 (29)	5.6 ± 0.1 (29)	140 ± 10 (39)
COD_{Mn}, mg l^{-1}	17 ± 0 (29)	19 ± 0 (29)	22 ± 1 (29)	1212 ± 95 (39)
Suspended matter, mg l^{-1}	0.3 ± 0.1 (28)	0.4 ± 0.0 (28)	0.4 ± 0.1 (28)	38.2 ± 5.4 (39)
Color, mg Pt l^{-1}	11 ± 1 (29)	13 ± 1 (29)	16 ± 1 (29)	752 ± 67 (22)
Hardness, $CaCO_3$ mmol l^{-1}	0.17 ± 0.00 (29)	0.17 ± 0.00 (29)	0.17 ± 0.00 (28)	0.47 ± 0.04 (32)

lipid concentrations (test kit No 124 303, Boehringer Mannheim, GmbH). For each of these determinations 5—12 sac fry per female were used.

Experiment III. The eggs from five females of the control group were divided into four equal portions which were fertilized in 0, 0.2, 0.5, and 2.0 % of effluent with milt from three males of the control group.

Experiment IV. The eggs from five females of the control group were divided in two equal portions and fertilized in clean water with milt from three males of the exposure concentrations of 0.2 and 0.5 %. The control group of Experiment V served as control.

Experiment V. The eggs from five females from each of the effluent concentrations of 0, 0.2, and 0.5 % were fertilized in clean water with milt from three males from the corresponding group.

The eggs in Experiments III—V were incubated in clean water until the eyed stage when the proportions of live and dead embryos were counted. Figure 3 presents the scheme of all the trout experiments.

Three weeks after stripping, *i.e.* on 13 November 1980, blood samples were taken from the males left in each concentration (0 %: N = 11, 0.2 %: N = 5, 0.5 %: N = 4). The trout had then been in clean water for four weeks after the end of effluent exposure. For sampling, the fish were left to calm down for two days in individual restrainers (Soivio et al. 1975). Each fish sampled was stunned by a blow on the head, and the blood sample taken by heart puncture into a heparinized 5 ml syringe. A hematocrit tube was immediately filled and centrifuged for the hematocrit (Hct) reading, and 25 µl was pipetted for determination of the hemoglobin (Hb) concentration. 0.1 ml of blood was precipitated with 0.5 ml of 0.6 mol l^{-1} perchloric acid for determination of glucose and lactate concentrations. The rest of the blood was centrifuged to separate plasma, which was deep-frozen (—20 °C) for determination of chloride (Cl^-) and total protein concentrations. Tissue samples were taken for histological examinations (not presented here). The Hb concentration was determined as cyanmethemoglobin, the total protein concentration by the biuret-method, glucose and lactate concentrations with test kits (No 124 028 and 124 842, Boehringer Mannheim, GmbH), and the Cl^- concentration with chloride titrator (Radiometer CMT 10). The mean corpuscular hemoglobin concentration (MCHC) was calculated from the Hb concentration and the Hct reading.

Statistical significances were tested with Student's t-test.

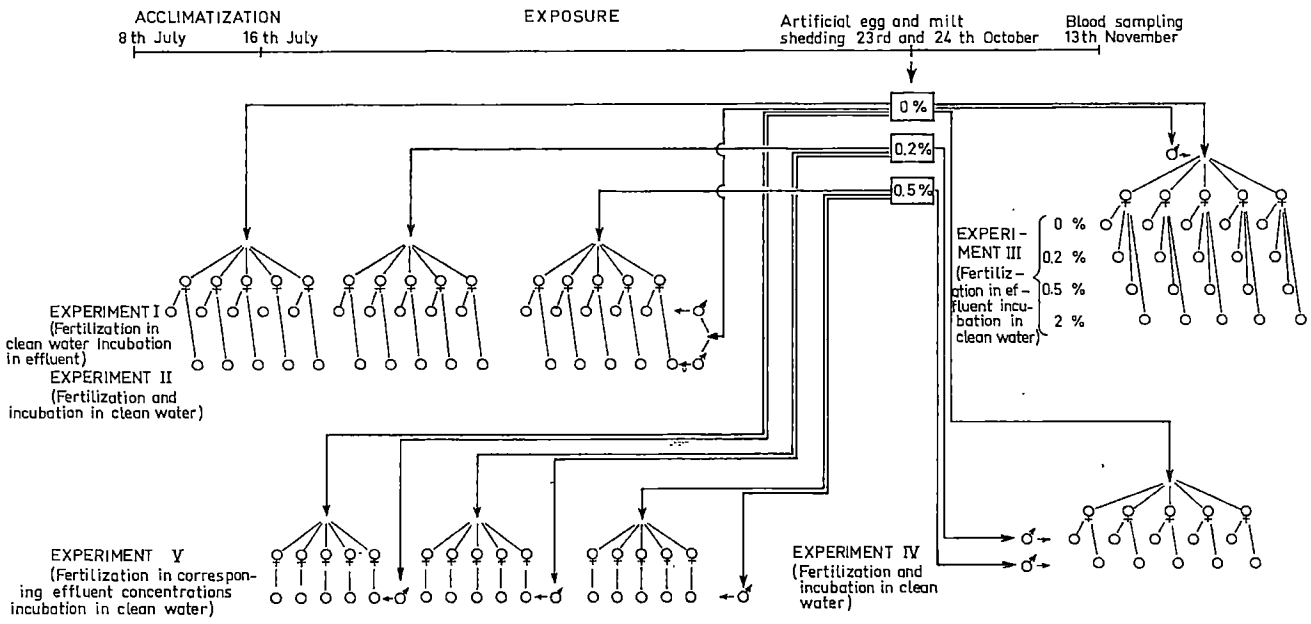

Figure 3. The scheme of the brown trout experiments. Squares represent test concentrations (0, 0.2, and 0.5 %, v/v) and circles represent egg portions.

III. Results

In the exposure to an effluent concentration of 2 %, two fish died on 23 July, one fish on 29 July, five fish on 30 July, and the rest of the fish on 31 July, after 15 days of exposure. In the 0.2 % group, one fish died on 28 July, and one on 12 September; and in the control group one fish died on 21 August. None of the fish exposed to 0.5 % effluent died.

The weight of the males in the control group slightly increased. The females of the 0.5 % group, and the males of the 0.2 and 0.5 % groups, lost significantly more weight than did the controls (Fig. 4). The condition factors of both females and males of the 0.5 % group were significantly smaller than in the control (Fig. 5). It was observed that the fish in the concentration of 0.5 % ate less than the control fish during August and September.

The percentage weight of the eggs per body weight was smaller in effluent exposed trout than in the control, but the difference was significant only in the 0.2 % group (Fig. 6). The unfertilized eggs from the females of the 0.5 % group were smaller and darker (brownish) than in other groups (dry weights and number of females in parenthesis): 0 % group, 70 ± 7 mg (22 ± 3 mg, N = 2); 0.2 % group, 73 ± 3 mg (24 ± 1 mg, N = 5); and 0.5 % group, 66 ± 2 mg (21 ± 1 mg, N = 5). Differences between the 0.2 and 0.5 % groups bordered on being significant ($P < 0.1$) in wet weight, and were significant ($P < 0.05$) in dry weight. There were no differences between the effluent concentrations in numbers of eggs per unit weight of female fish, but the standard deviation of this variable of the 0.5 % group was nearly two times greater than in the other groups (mean ± SD, eggs kg^{-1}): 0 % group, 2 494 ± 256 (N = 2); 0.2 % group, 2 330 ± 262 (N = 5); and 0.5 % group, 2 416 ± 453 (N = 5). When the egg numbers were calculated on the basis of the unit of length, a negative linear relationship with

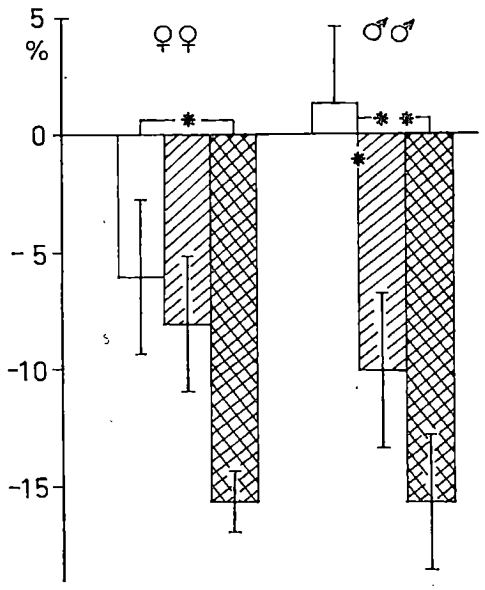

Figure 4. The percentage change in weight of female and male brown trout during effluent exposure (blank column represents control, diagonal-lined 0.2 % group and crosswise-lined 0.5 % group). The vertical bars represent ± SE. Significances are given as: ★ = $P < 0.05$, ★★ = $P < 0.01$ and ★★★ = $P < 0.001$.

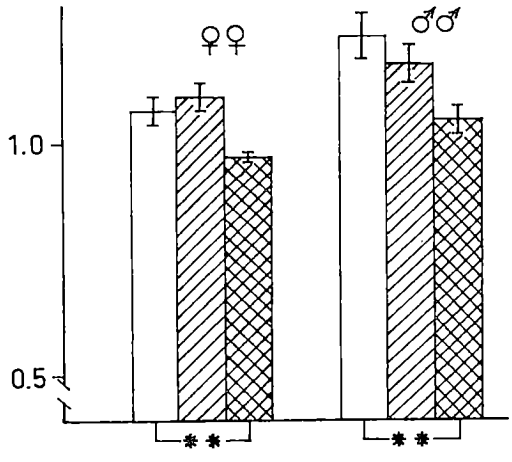

Figure 5. The condition factors of female and male brown trout at the end of effluent exposure. Key same as in Figure 4.

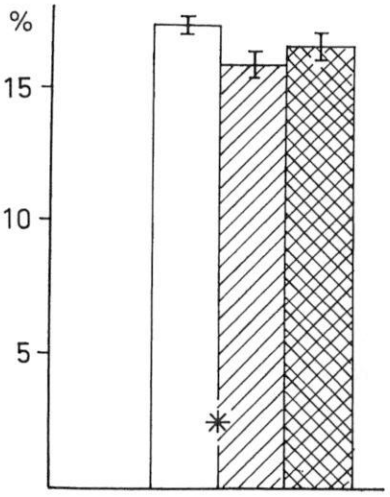

Figure 6. The percentage weight of eggs per body weight of brown trout at stripping. Key same as in Figure 4.

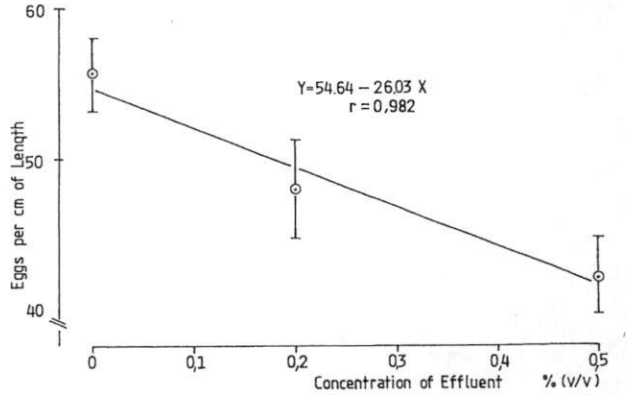

Figure 7. Egg numbers per unit of length of brown trout at stripping. The vertical bars represent ± SE.

increasing effluent concentration was found: $y = 54.6 - 26.0x$ ($r = 0.982$) (Fig. 7).

The Hct reading, the Hb concentration, and the MCHC of the male trout in the concentration of 0.5 % were smaller than in the control. There were no significant differences between the groups in blood glucose, blood lactate, plasma Cl^- and plasma protein concentrations (Fig. 8).

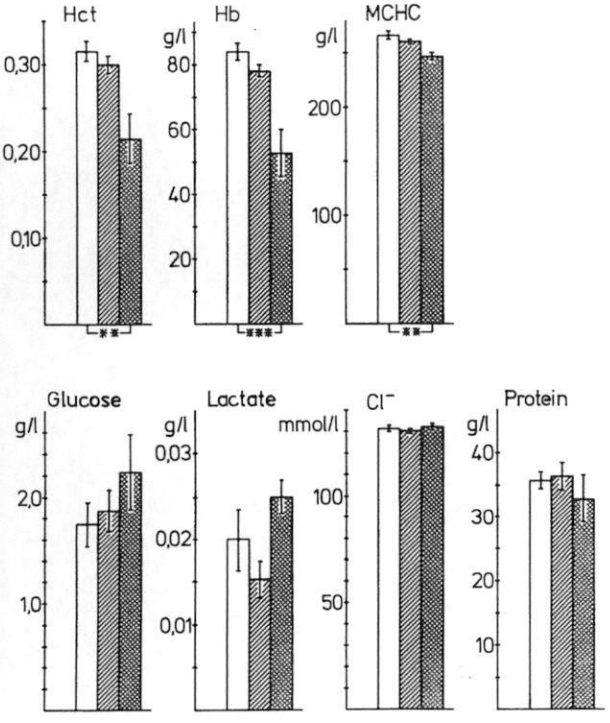

Figure 8. The blood and plasma variables of the male brown trout at the end of the experiment. Key same as in Figure 4.

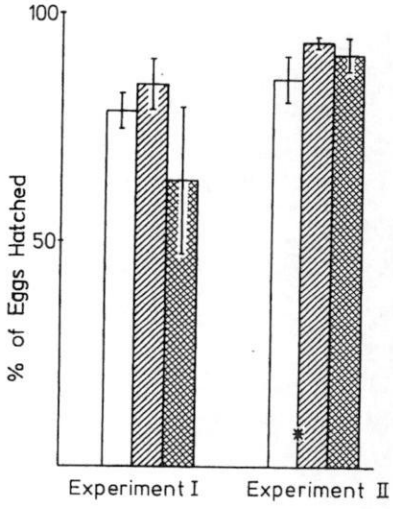

Figure 9. The percentage hatch of brown trout sac fry incubated at the same concentrations of BKME in which the parent fish had been exposed (Experiment I) and from clean water incubation (Experiment II). Key same as in Figure 4.

The sac fry in Experiments I and II hatched at the same time, during about four weeks (17 March — 15 April). The percentage of eggs hatched was greatest in both 0.2 % groups, and smallest in the 0.5 % group incubated in effluent (Experiment I). However, this difference was not significant, because of the large variation in the 0.5 % group (Fig. 9).

Sac fry in the 0.2 and 0.5 % groups in effluent incubation were shorter than the controls, measured on the average two weeks after hatching (Experiment I). However, also in clean water incubation (Experiment II) the sac fry of the females exposed to the 0.5 % effluent were smaller than the control sac fry (Fig. 10A). The sac fry from one female exposed to 0.5 % effluent were badly curved in both clean water and effluent incubations, and were not included in length measurements. The sac fry of the effluent exposed females in Experiment I were shorter (0.2 %: $P < 0.01$; 0.5 %: $P < 0.05$) than the sac fry of the respective females in clean water (Experiment II). The wet weight of the sac fry from the females exposed to 0.5 % effluent was significantly smaller than that of the control in both Experiments I and II (Fig. 10B). The sac fry of the respective females in effluent and clean water incubations did not differ in wet weight from each other.

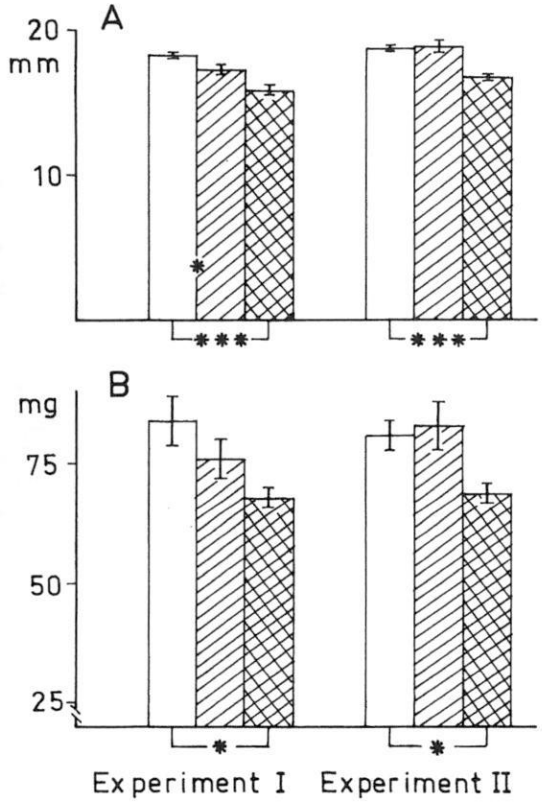

Figure 10. The length (A) and weight (B) of the brown trout sac fry. See key to Figure 4 and the text of Figure 9.

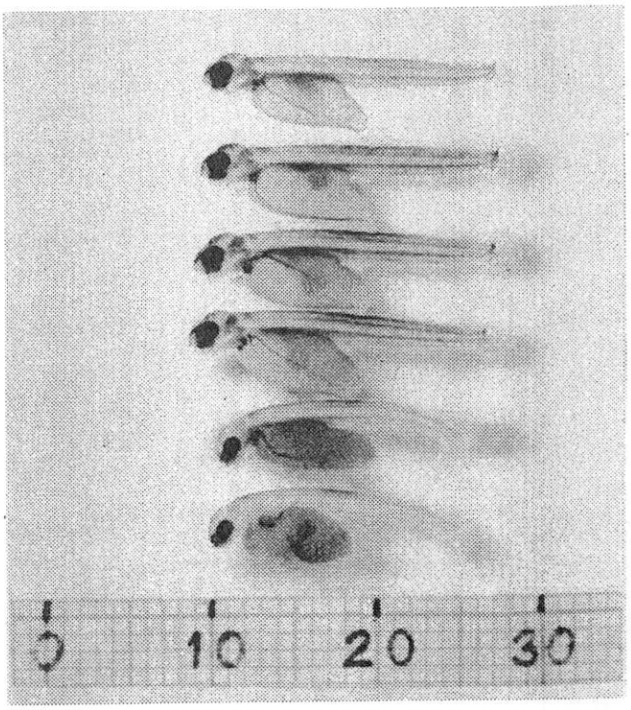

Figure 11. Brown trout sac fry of the same age from the clean water incubation (Experiment II). The two sac fry at the top are from a control female, the two in the middle are from a 0.2 % effluent exposed female, and the two at the bottom from a 0.5 % effluent exposed female. The scale is in millimetres.

The sac fry of the females exposed to 0.5 % effluent (in both Experiments I and II) were less developed than the other sac fry, based on observations of morphology (Fig. 11). Their yolk sacs were proportionally larger and more spherical in shape. There were sac fry whose yolk had constricted into two parts (Fig. 11, bottom-most sac fry). The network of the blood vessels of the yolk sac of these fry was abnormal, due to these strictures; and there were disturbances in the blood circulation. Some of the sac fry from the females of the 0.2 %

effluent concentration also had strictures of yolk. The total protein concentration per dry weight of sac fry from the females exposed to 0.5 % effluent was significantly greater, but the differences in the total lipid concentration were not significant (Fig. 12). The heart rate of these sac fry was significantly lower than in the control. The heart rate of the 0.2 % sac fry in the effluent incubation (Experiment I) was also lower than in the control (Fig. 13); and was lower ($P < 0.001$) than in the clean water incubation (Experiment II). In the concentration of 0.5 % there were no difference between Experiments I and II.

By the end of the experiment all the sac fry from the females exposed to 0.5 % effluent had died, except for 14 % of the fry of one female, which survived in Experiment II. The fry could not absorb the yolk. The yolk appeared to harden, and white spots appeared in it; the yolk coagulated. The yolk sac swelled and greenish-yellow fluid accumulated inside it.

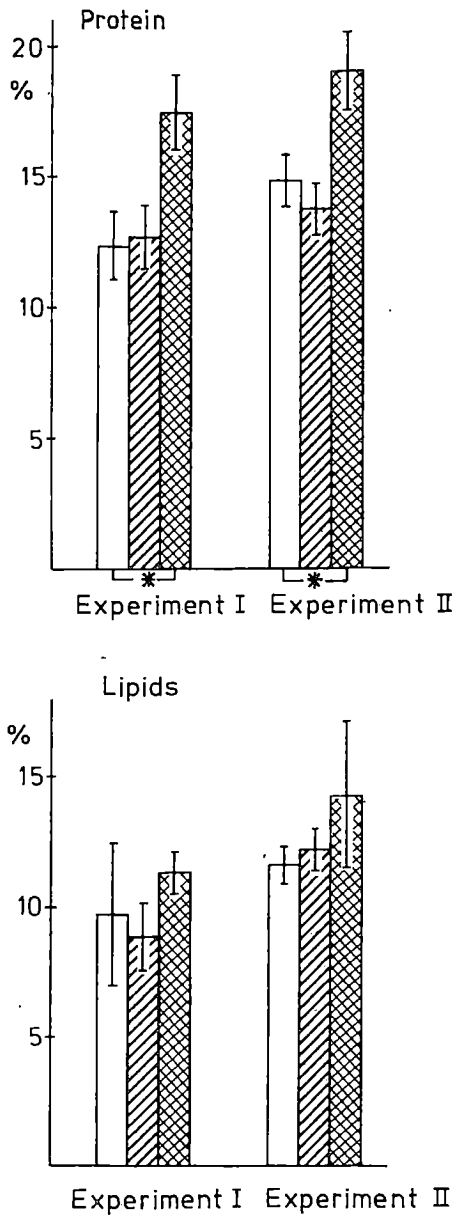

Figure 12. The total protein and total lipid contents of brown trout sac fry as a percent of dry weight. See key to Figure 4 and the text of Figure 9.

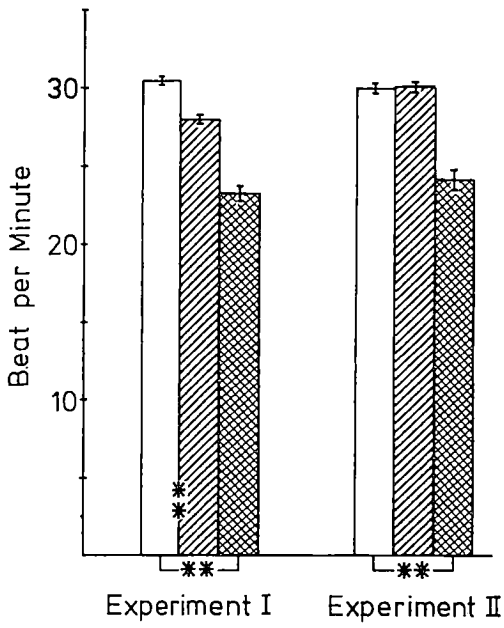

Figure 13. The heart rates of brown trout sac fry as beats per minute. See key to Figure 4 and the text of Figure 9.

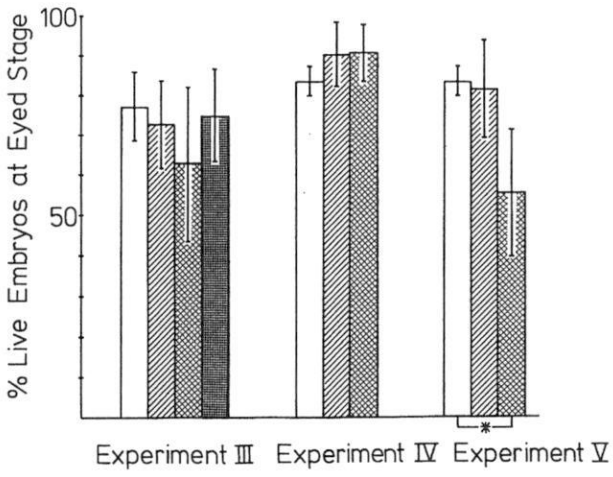

Figure 14. Live embryos of brown trout at the eyed stage in fertilization experiments. Experiment III: control group eggs fertilized with milt from control males in different effluent concentrations. Experiment IV: control group eggs fertilized with milt from males of different exposure concentrations. Experiment V: eggs from different exposure group females fertilized with milt from respective group males. Darkest column to the right in Experiment III represents 2 % effluent group. Key otherwise same as in Figure 4.

The results of the fertilization experiments are shown in Figure 14. Fertilization was not affected by effluent, when the eggs of control females were fertilized with milt from control males in different effluent concentrations (0, 0.2, 0.5, and 2.0 %; Experiment III). Effluent exposure of males had no effect whatsoever on the ability of milt to fertilize eggs (Experiment IV), but the eggs from the females exposed to 0.5 % effluent fertilized less well than did the eggs of the control females (Experiment V).

IV. Discussion

The trout in the concentration of 2 % died on the same days that the water temperature was at its highest value (22.8 °C). The incipient lethal temperature for brown trout acclimatized to a water temperature of 20—22 °C is 24.7 °C (Elliott 1981). According to Cairns et al. (1975) toxicants may alter lethal thermal limits. None of the fish in the control died at times of high water temperatures. During this time the water pH in the tanks was quite low (6.5—6.6), but not unusual. A decrease in pH increases the toxicity of kraft pulp and paper mill effluents according to McLeay et al. (1979). The most toxic components of the waste waters of a Finnish kraft pulp mill are resin acids and chlorinated phenolics (Holmbom & Lehtinen 1980). The proportion of the undissociated form of a resin acid, dehydroabietic acid, increases from 5 % at pH 7 to 83 % at pH 5 (Zanella 1983): the undissociated form is more toxic. The toxicity of the acid chlorinated phenols also increases as the pH decreases (Saarikoski & Viluksela 1981). The DO content was 65—69 % of saturation at the time of fish kill. This oxygen content is sufficient *per se*. The fish in the effluent concentration of 2 % died due to effluent exposure; they did not survive effluent exposure at high water temperatures with the prevailing DO content.

Both female and male trout in the 0.5 % exposure concentration were leaner than the controls at the end of exposure. In the 0.5 % concentration, trout showed a lack of appetite, which was not observed in the 0.2 % concentration. However, the 0.2 % group fish, especially males, also lost weight. Effluent exposure might have increased maintenance energy costs, reducing the proportion of energy available for growth, as observed by Webb & Brett (1972). Trout were offered dry feed and herring corresponding to 2 % of their weight per

day, but feeding was completed in one hour. According to ELLIOTT (1975), the satiation time of brown trout weighing over 300 grams was not achieved until after 130—150 minutes at a water temperature of 13—18 °C. By extrapolating from ELLIOTT's (1975) results, a 1 200-gram brown trout should eat about 1 % of its weight per day at a water temperature of 15 °C. However, the fish in ELLIOTT's study were juvenile, and the trout in our study were preparing to spawn. In the 10-month experiment carried out by BAGENAL (1969a) with mature brown trout, both well-fed and starved fish grew in weight and length. The calculated rations were about 0.8 and 0.4 % of body weight per day, respectively. In the present experiment, it may be that enough feed was offered, but that it was given in too short a time; after feeding there was always feed left at the bottom of the tanks.

There were no significant differences in the blood glucose and lactate concentrations of trout males, but there seems to be a slight trend towards a higher blood glucose concentration in the exposed trout. The plasma glucose and lactate concentrations of juvenile coho salmon *(Oncorhynchus kisutch)* were elevated compared to the control even after 200 days of exposure to BKME (MCLEAY & BROWN 1974, 1979). Similar results were found in raibow trout reared in the water of the Rhine River for 18 months (POELS et al. 1980). In the present study, exposure ended four weeks before sampling, so the glucose values may have partly recovered. The Hb concentration, the Hct reading, and the MCHC of male trout were significantly smaller in the 0.5 % group than in the control. These low values indicate anemia. The Hct reading of coho salmon was decreased after 25 days of exposure in BKME (MCLEAY 1973). After 18 months of exposure to Rhine River water, the Hb concentration of rainbow trout was diminished and their growth decreased (POELS et al. 1980).

Scarcity of feed usually leads to decreased fecundity, and starved trouts (rainbow and brown trout) produce less eggs but of the same size as well-fed fish (SCOTT 1962, BILLARD et al. 1981). According to SCOTT (1962) this is due to increasing follicular atresia. However, very badly starved brown trout produced smaller eggs than before, and the fecundity increased (BILLARD et al. 1981). In our study, the number of eggs per unit weight of brown trout was nearly the same in all the concentrations, although the eggs were smaller in the 0.5 % group. However, because trout were leaner in effluent concentrations of 0.2 % and especially 0.5 % than the controls, a better picture of fecundity is given by comparing egg numbers to length than to weight. This length ratio shows that the egg numbers decreased with increasing effluent concentration. The percentage weight of eggs per body weight for brown trout decreased as the effluent concentration increased. CARLSON (1971) also found reduced percentage weight of eggs of fathead minnow after nine months of exposure to Carbaryl.

The viability of eggs from the brown trout in the 0.5 % effluent concentration was decreased, according to the results of the fertilization experiment (V) and Experiments I and II. Fertilizing in effluent concentrations (Experiment III) did not affect the fertilization rate; neither was the fertilizing ability of milt affected by the effluent exposure of males (Experiment IV). The exposure of zebra fish to BKME during the later phase of oogenesis diminished the hatching success of fry, and the newly hatched fry of exposed females were more sensitive to the BKME than the control fry on the grounds of LC50 tests (VIKTOR et al. 1980). Further, a high PCB content in flounder eggs decreased hatching success (VON WESTERNHAGEN et al. 1981). The viability of eggs diminished as a function of increasing toxaphene concentration in a six months' exposure of parent brook trout *(Salvelinus fontinalis)*. It was a more sensitive variable than growth (MAYER et al. 1975). Starvation has not been shown to affect egg viability. Feeding brown trout for nine months with reduced rations led to diminished

percentage weight of eggs and reduced fecundity, but did not affect egg viability (BAGENAL 1969a); and forty days' starvation of rainbow trout also had no effect on egg viability (RIDELMAN et al. 1984).

The present exposure was performed at the time of vitellogenesis, when vitellogenin is synthesized in the liver and transported to the ovaries (DE VLAMING 1983). Vitellogenesis may have been disturbed, appearing as decreased percentage weight and numbers of eggs (atresia), diminished egg size of 0.5 % trout, and reduced viability of eggs of effluent exposed trout. Three factors, reduced feed intake (or increased maintenance energy cost), impaired liver function, and/or disturbed hormone metabolism, may have interfered with vitellogenesis.

The hatched sac fry, as well as the eggs, of the 0.5 % group of brown trout were smaller than those of the control in both the effluent and clean water incubations. It is known that small fry emerge from small eggs (BAGENAL 1969b). However, these fry were not viable: they were malformed and could not absorb their yolk. The heart rates were lowered too, which indicates retarded development (ROSENTHAL & ALDERDICE 1976). Finally nearly all of them died. The symptoms (enlarged yolk sac with greenish-yellow fluid and white spots in the yolk) that were observed in the sac fry from the 0.5 % group females, correspond to those of blue sac disease, which is caused by physiological disorder (WEDEMEYER et al. 1976).

The influence of incubation in effluent concentrations was seen in that the sac fry incubated in the 0.2 % effluent concentration were shorter than the sac fry in the clean water incubation; and their heart rate was also lower.

The MATC proposed by MOUNT & STEPHAN (1967) was found in this study to be less than 0.2 % of BKME for brown trout.

V. Conclusions

The effluent exposure of brown trout decreased tolerance to environmental changes. Exposed parent brown trout became leaner than the controls, due apparently to diminished appetite and/or increased maintenance energy costs. Increased follicular atresia produced a reduction in the percentage weight and number of eggs. This fact, coupled with smaller egg size, poorer fertilization of eggs, and decreased viability of offspring, indicates disturbances in vitellogenesis that may be due to interferences in metabolism, restricted rations, or disturbed hormone metabolism, or perhaps to accumulation of effluent toxicants within eggs. It was only the development of oocytes that was affected; fertilization in effluent concentrations or the fertilizing ability of sperm were not affected. In addition, the incubation of eggs in effluent dilutions was reflected in retarded development of sac fry. Effluent exposure lowered the Hct reading and the Hb concentration of male trout; however, reproduction was disturbed even at lower effluent concentration.

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