Bull. Environ. Contam. Toxicol. (2000) 64:873-879

DOI: 10.1007/s001280000084

Bulletin of Environmental Contamination and Toxicology

Impact of Mechanical Pulp Mill Effluent on Egg Hatchability of Brown Trout

K. Johnsen,[1] C. Grotell,[2] J. Tana,[3] G. E. Carlberg[4]

[1] The Norwegian Pulp and Paper Research Institute, N-7034 Trondheim, Norway
[2] Environmental Research Group-MFG, Sjöleden 9, SE-995 91 Kil, Sweden
[3] Environmental Research Group-MFG, Tekniikantie 12, FIN-021 50 Espoo, Finland
[4] Norske Skog Research, Post Office Box 329, N-1326 Lysaker, Norway

Received: 15 December 1999/Accepted: 6 April 2000

Egg hatchability and fry development are considered to be the sensitive stages of fish to different chemical toxicants and effluents. Effects of chemical pulp mill effluents on reproduction and early life stages have been studied in laboratory (Tana & Nikunen 1986, Vuorinen & Vuorinen 1987) and in these studies a negative impact on egg or sperm viability and embryo and fry quality were demonstrated. On the other hand, in laboratory studies with secondary treated thermomechanical pulp mill effluent, no effects on hatching of the eggs or hatchability of the second generation eggs of fathead minnow were observed (Kovacs et al, 1995). The succes of reproduction and survival of fry can, however, depend on other factors than direct toxic effects on egg and fry. Pre-exposure of the parent fish may be an important initiator to effects on reproduction. Under laboratory conditions, a standard egg and fry test with zebrafish *(Brachydanio rerio)* is often used when testing the toxicity of a substance or an effluent. Feral fish species, however, have been used to a limited extent in reproduction studies.

The objective of this study was to determine the effects of both untreated and treated effluent from a mill producing magazine paper from mechanical pulp on the early life stages from pre-exposed sexually mature brown trout (*Salmo trutta*) under laboratory conditions. Brown trout is a cold water species and is therefore adapted to conditions found in the receiving waters in boreal regions. The parental fish exposure included the last four months prior to spawning when gametes reach their final size and maturation.

MATERIALS AND METHODS

The mill investigated is an integrated magazine paper mill. Thermomechanical and stone ground wood pulp are produced from spurce (*Picea abies*), and hydrogen peroxide is used as the bleaching agent. The effluent treatment plant includes secondary treatment, followed by flotation. The secondary treatment comprises an anaerobic stage followed by an aerobic stage with activated sludge plant. The tested effluents were sampled daily before and after the biological treatment system and mixed to week samples and stored in cold (+ 4 C°). The chemical oxygen demand (COD_{Cr}) in the untreated effluent was 1500 mg/l and in the treated effluent

Correspondence to: J. Tana

150 mg/l. Total resin acid concentration in the untreated effluent was 16 mg/l and in the treated effluent 0.05 mg/l.

The parental fish were placed in 1 m^3 polyethylene pools receiving fresh water with a continuous flow of 500 l/h. Effluents tested were flowed directly from the mill pipes before and after the secondary treatment plant and mixed with the fresh water by dosing it into the inflow tubes of each pool. Six females and three males were placed in each pool, and allowed to acclimate for two weeks prior to the exposure start. The fish were exposed to clean fresh water (control=A), untreated effluent in dilution 1:800 (B) and treated effluent in dilution 1:40 (C).

The dilutions were chosen according to previous work with sublethal effects of mechanical pulp mill effluents (Johnsen et al. 1995) and medium water flow in the effluent receiving river. The exposure of parental fish lasted for four months from September 1995 to January 1996 while water temperature decreased from 10 C° to 4 C°. A light:dark regime of 12: 12 hrs was maintained during the first three months and thereafter changed to 8: 16 hrs, to simulate the daylight.

After four months exposure one parental fish at a time was caught from the pool. The abdomen of the fish was dried and the eggs and milt were stripped into two saparate pots. Eggs from control parental fish were fertilized in clean water, and in different dilutions of untreated and treated effluent. Eggs from parental fish exposed to untreated effluent were fertilized in clean water and untreated effluent (dilutions 1:400 and 1: 1000) and eggs from fish exposed to treated effluent were fertilized in clean water and treated effluent (dilutions 1:40 and 1:60). The fertilized eggs were placed in net baskets (15x15x15 cm) and incubated in the same type of effluent or clean water as they were fertilized. The different exposure groups are presented in Table 1.

Table 1. Explanation for the symbols used in the description of the experiment.

Adult exposure	*Egg exposure*	*Group*
Clean water	Clean water	A1
Clean water	Untreated 1:400	A2
Clean water	Untreated 1:1000	A3
Clean water	Treated 1:40	A4
Clean water	Treated 1:60	A5
Untreated 1:800	Clean water	B1
Untreated 1:800	Untreated 1:400	B2
Untreated 1:800	Untreated 1:1000	B3
Treated 1:40	Clean water	C1
Treated 1:40	Treated 1:40	C2
Treated 1:40	Treated 1:60	C3

Bile was sampled from females and males in each group, respectively, pooled, and frozen and stored on dry ice for later analysis of resin acids and cholesterol. Blood was collected from each fish from caudal vessels. The blood sample (l-2 ml) was centrifuged, and about 500 µl plasma from each fish was pooled together and frozen with dry ice until analysis of testosterone and 17-β-estradiol, which were analyzed by standard methods.

During the incubation, dead eggs were counted and removed. Hatching was followed daily by counting the hatched fry and unsuccesful hatching registered. The weight and lenght of fry were measured twice, three weeks and seven weeks after hatching. The results from the two measurements from the exposed fry groups were compared statistically (Student's t-test) with the control (Al).

RESULTS AND DISCUSSION

In fish exposed to treated effluent the bile concentration of resin acids (females 13 800 µg/g and males 7 700 µg/g d.w.) were less than half of the concentrations in parental fish exposed to untreated effluent (females 31 000 µg/g and males 30 500 µg/g d.w.) The bile concentrations of resin acids were of the same order of magnitude as previously observed in studies with primary treated (Johnsen et al. 1995) and chemically treated (Johnsen et al. 1998) mechanical pulp mill effluents. Resin acids were not detected in the bile of reference fish. The cholesterol levels in fish exposed to untreated effluent (females 17 200 µg/g and males 18 500 µg/g d.w.) were strongly elevated (6-10 times) as compared to the reference fish (females 3 100 µg/g and males 1 800 µg/g d.w.). The exposure to biologically treated effluent resulted in doubling of the cholesterol levels (females 8 000 µg/g and males 3 700 µg/g d.w.) as compared to reference fish.

The testosterone levels were lower especially in male parental fish exposed to untreated effluent as compared to control fish (Table 2). This finding is in accordance with results obtained for feral fish caught outside mills with bleached chemical pulp production (Munkittrick et al. 1992). The 17-β-estradiol levels showed no differences between exposed and reference fish.

Table 2. Plasma hormone concentrations of the parental fish and the concentration of resin acids in roe.

Exposure	*Testosterone nmol/l*	*17-β-estrdiol nmol/l*	*Resin acids in roe µg/g d.w.*
Clean water F[1]	34.7	>0.18	-
Clean water M[2]	35.9	0.051	
Untreated F	16.0	>0.18	40
Untreated M	4.0	0.052	
Treated F	25.9	>0.18	6
Treated M	23.1	0.065	

1) F=Female; 2) M=Male

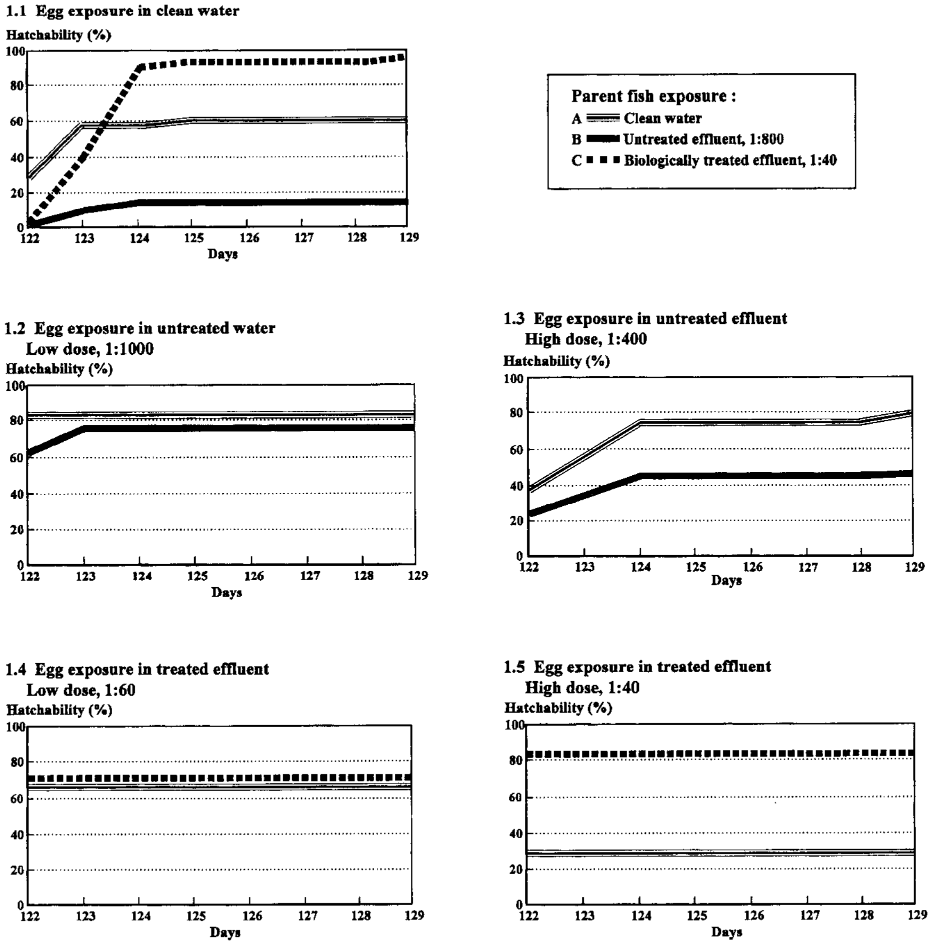

Figure 1. The succes of hatching (%) of the fry.

Resin acid analyses were also undertaken on a pooled sample of roe (Table 2) to determine if there was a direct correlation between resin acid bioconcentration and any observed reproductive effects. The bioconcentration factor for roe [(µg/g d.w.)/(µg/ml water) based upon 1:800 dilution data] was in this experiment 2000 (log BCF 3.3). In clean water exposed fish no resin acids were detected in the roe.

The eggs exposed to treated effluent were the first to hatch, and all the fry in this group had hatched before day 122 (Fig 1.4 and 1.5) In general the eggs incubated in clean water and also eggs incubated in untreated effluent hatched a couple of days later than eggs exposed to treated effluent. This might be explained by a slightly higher water temperature (+ 0.5 C°) in the treated mill effluents.

The main reference system, i.e. the group where the parent fish pool, the fertilization pots and the incubation baskets all had clean water addition only (Fig. 1.1), resulted in a final hatching succes of 60%. In comparison to this, the hatchability of other eggs derived from parents pre-exposed to clean water only, was not affected by the exposure to treated effluent in dilution 1:60 (Fig. 1.4) or untreated effluent (Fig. 1.2, 1.3). However, fertilization of eggs from clean water parents in treated effluent diluted 1:40 times gave for an unknown reason lower hatchability. The results illustrated in Figure 1 show, by comparing the levels of corresponding curves, that pre-exposure of parental fish to treated effluent and fertilization in the same effluent dilutions 1:40 and 1:60, respectively, did not affect the hatchability of eggs (Fig. 1.4, 1.5). The succes of hatching was in general lower for eggs stripped from parental fish pre-exposed to untreated effluent at 1800 dilution (Fig. 1.1, 1.3).

Results of the first sampling of fry are presented in Figure 2. For group B1, parents in untreated water and egg exposure in clear water, the hatching was too low to continue with growth studies. A comparison of the fry within the same exposure pool shows significant growth differences. The sac fry in each control group(parents in clean water) were in general longer and weighed more than fry from pre-exposed parents, despite later hatching. Fry from parental fish exposed to untreated effluent, in general weighed more than fry from parental fish exposed to treated effluent. A comparison of fry from different exposure pools, all descending from the clean water parental group, indicates a positive growth effect of increased effluent dilution. At the second sampling a similar trend was observed: the fry from parental fish in clean water had grown better than the other groups.

The fry exposed to untreated effluent (dilution 1:400) had a considerably higher mortality (6888%) than fry exposed to treated effluent (10-45%) during the first seven weeks of development. The mortality in clean water was below 15%. The higher mortality could, however, be an indirect effect of the effluent, caused by slime growth in egg and fry baskets exposed to untreated effluent. There was no significant occurence of deformed fry in any group.

Vuorinen & Vuorinen (1987) found that hatching of brown trout in 2% bleached kraft mill effluent (BKME) was lower, and that sac fry were shorter than the control group and died within three weeks after hatching. Burton et al. (1983) did not observe any changed mortality or delayed hatching of stripped bas egg exposed to BKME. Egg from unexposed rainbow trout fertilized and incubated in BKME showed no changes in hatching frequency, and the growth rate of sac fry was higher (Grotell, pers: comm.). A Canadian team (Kovacs et al. 1995) studied the effects of a thermomechanical pulp mill (TMP) effluent on life cycle of fathead minnow. They found no effects of biologically treated TMP mill effluent on the hatching of eggs, reproduction of hatched fish and hatchability of the first generation eggs, even at dilution 1:5.

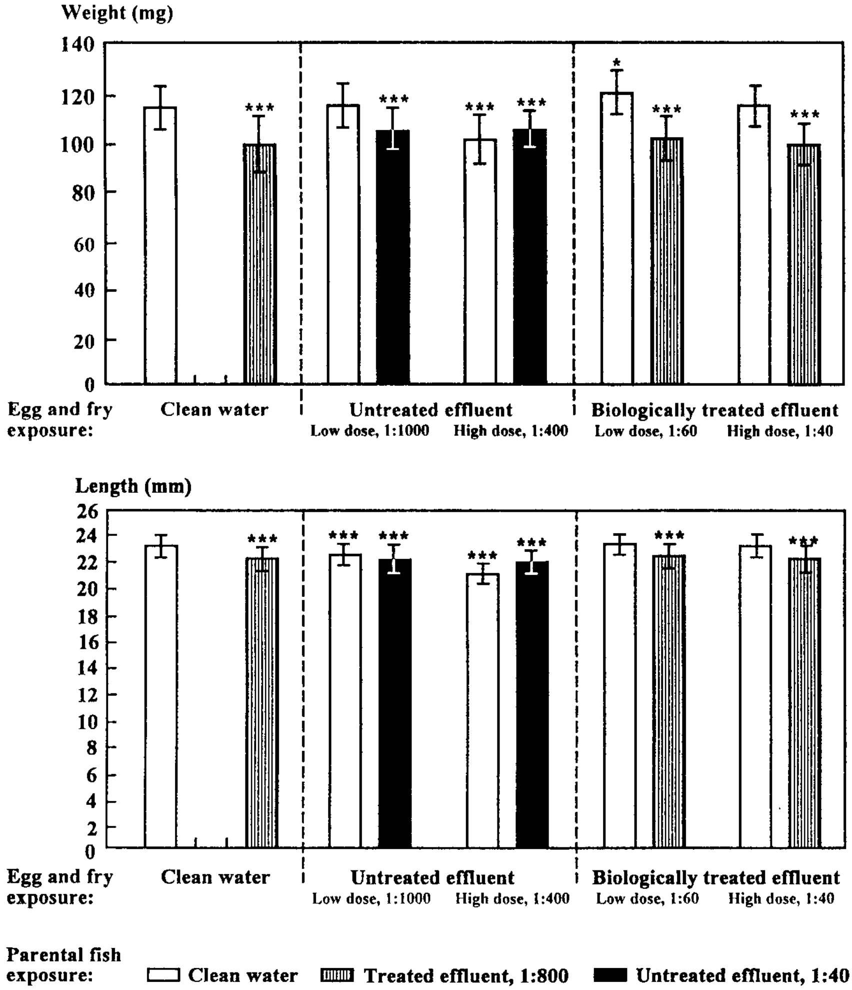

Figure 2. The lenght (mm) and weight (mg) of unexposed and exposed sac fry at the first sampling, i.e. three weeks after hatching. All the fry groups are statistically compared to the main reference group Al, where parental fish, as well as eggs and fry, have been exposed to clean water only. The levels of significance are noted as 95%(*), 99%(**), 99,9%(***).

As a conclusion, the present study gives indications that pre-exposure of parental fish can have effects on the quality of eggs and milt and therefore have negative impact on egg hatchability and fry growth. When eggs from unexposed parents were fertilized in clean water or in untreated or treated effluent, no significant differences were observed in the hatchability. The succes of hatching on the other hand was lower for eggs stripped from fish pre-exposed to untreated effluent and the quality of fry was lower as compared to fry from parents in clean water. Pre-exposure to treated effluent, though in lower dilution, did not have the same kind of effect on egg hatchability but affected fry growth and mortality. The results therefore indicate that exposure of parental fish can have more effects on egg hatchability and fry growth than the conditions during actual fertilization and incubation. According to the results in this study some correlation between egg hatchability and the roe resin acid concentration was observed.

Acknowledgments. The financial support of The Research Council of Norway is acknowledged. Thanks are due to Ole Stensrud and Olav Berg for technical assistance.

REFERENCES

Burton DT, Hall LW, Kaluda Jr RJ, Margrey SL (1983) Effects of treated bleached kraft mill effluent on roes and prolarvae of stripped bass (*Morone saxatilis*). Wat Res Bull 19:869-879.

Johnsen K, Mattsson K, Tana J, Stuthridge TR, Hemming J, Lehtinen K-J (1995). Uptake and elimination of resin acids and physiological responses in rainbow trout exposed to total mill effluent from an integrated newsprint mill. Environ Toxicol Chem 14:1561-1568.

Johnsen K, Tana J, Lehtinen K-J, Stuthridge TR, Mattsson K, Hemming J, Carlberg GE (1998) Experimental field exposure of brown trout to river water receiving effluent from an integrated newsprint mill. Ecotoxicol Environ Safety 40:184-198.

Kovacs, TG, Gibbons JS, Martel PH, O'Connor BI, Voss RH (1995) Effects of a secondary-treated thermomechanical pulp mill effluent on aquatic organisms as assessed by short- and long-term laboratory tests. J Toxicol Environ Health 44:485-502.

Munkittrick KR, Van Der Kraak GJ, McMaster ME; Portt CB (1992) Response of hepatic MFO-activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effluent and mill shutdown. Environ Toxicol Chem 11: 1427-1439.

Tana J, Nikunen E (1986) Impact of pulp mill effluent on egg hatchability of pike *(Esox lucius L).* Bull Environ Contam Toxicol 36:738-743.

Vuorinen P, Vuorinen M (1987) Effects of bleached kraft mill effluent on early life stages of brown trout (Salmo trutta L). Ecotox Environ Safety 14: 117-128.