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Impact of Pulp and Paper Mill Effluent on Egg Hatchability of Pike (*Esox lucius* L.)

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Toxicants affect reproduction often at concentrations well below lethal levels. Also, the acidification of fresh waters has been shown to cause extinction of teleost fish populations usually through a failure of recruitment from younger generations. Both field and laboratory studies have documented that the greatest susceptibility to low pH occurs in the early developmental stages (e.g. Lee et al. 1983; Peterson 1984). Also low concetrations of metals (Horning & Neisel 1979; Ozoh 1979, 1980) and environmental accumulation of xenobiotics (Ozoh 1979; von Westerhagen et al. 1981) diminish egg hatchability. In numerous toxicological studies egg hatchability has been a more sensitive indicator of toxic action than the other parameters studied (e.g. Henderson et al. 1981; DeGraeve et al. 1982; Lewis & Wee 1983). However, only relatively recently have the effects of bleached kraft pulp mill effluent (BKME) on fish reproduction (zebrafish Branchydanio rerio) been studied (Viktor et al. 1980; Viktor 1982) and no recipient species have been used.

The aim of the present work was to study the egg survival and malformation frequencies of a pike population living in a pulp and paper discharge area and the corresponding values of a reference group from a clean area. The effects of different pulp and paper mill effluent concentrations on survival and malformation frequencies were examined and the impact of waste water concentration at the fertilization moment was studied.

MATERIALS AND METHODS

The combined kraft pulp and paper mill used for the experiments produced in 1982 839 t/d kraft pulp, 696 t/d newsprint, 164 m^3/d timber and 1,171 m^3/d plywood. At the same period of time the recipient (Lake Saimaa) received 198,600 m^3/d wastewater, 17.9 t/d BOD_7, 0.12 tP/d, 0.56 tN/d and 10.6 t/d suspended solids. The

effluent was treated in an aerated lagoon with a retention time of 30 hours before releasing. The LC 50 (96h) of the effluent for yearling rainbow trout (Salmo gairdneri) has earlier been determined with semi-static method as 90 % (vol/vol) (Nikunen, unpublished) and its sublethal impact on the fish physiology have been studied in the recipient (Tana & Nikunen, manuscript). The wastewater samples were taken once a week in 30 L polyethylene barrels and were stored at environmental temperature with air excluded as recommended by Walden and McLeay (1974).

Two different parent pike groups were used in the experiment. The reference group was caught from an area with good water quality five km upstream from the wastewater discharge. The other parent group was caught from the heavily polluted discharge site (BKME concentration about 2 %), not generally used for fishing.

The live parent fish were transported to the laboratory in oxygenated plastic containers filled with water from the catching site. Eggs were fertilized by mixing with milt in a wash pot. Afterwards the eggs were divided into four groups. In one group the eggs were covered with clean water from lake Päijänne. The three other groups were fertilized in 0.5, 2.5 and 10 % BKME. All resultant egg groups were then divided within 30 min into four groups (about 50 eggs in each) which were then incubated in BKME concentrations 0.0, 0.5, 2.5 and 10 %. A similar experiment was conducted simultaneously in another laboratory to which the eggs were transported (0.5 hr) in the incubation containers.

The eggs were incubated in boxes which were made by melting plastic net in place of the bottom of polythene boxes. The boxes were placed in four 50 L pexiglas aquaria in which the test solution (10 L) was exchanged by flow-through method (0.5 L/h).

The significances of the differencies in the viability frequences between the groups were detected by the Wilcoxon matched-pairs signed-ranks test. The EC 50's were determined with probit analyses.

RESULTS AND DISCUSSION

When the results of the two pike populations used in the experiments were compared, a very significant difference was obtained. The eggs of pikes caught from the discharge site of the mill were more resistant to the effluent than the eggs of fish from clean area; a higher percent of eggs stayed alive in all the effluent concentrations used. Quite a different result

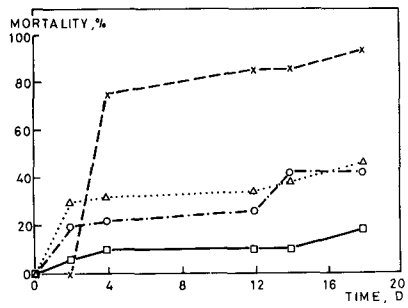

Fig. 1a.

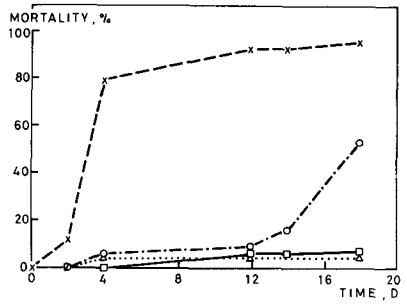

Fig. 1b.

Figure 1. The impact of BKME concentrations on egg mortality during hatching. Eggs fertilized in clean water. Fig. 1a = reference population. Fig 1b = population from the discharge site. □ = control; △ = 0.5 % BKME; O = 2.5 %; and x = 10 %.

was obtained when some of the eggs of both parent groups were transported to an other laboratory after one week of incubation. Hauling did not affect the viability frequency of the reference group but the additional stress sharply increased the mortality of the exposed population. Consequently, when the eggs were incubated in clean Lake Päijänne water the mortality of the eggs of the population from the discharge site exceeded the corresponding values of the reference group. Viktor et al. (1980) obtained comparable results when the impact of parent zebrafish exposure on egg hatchability and effluent resistance was studied. In the mosquitofish (Gambusia affinis), local populations more resistant than others have been found in streams adjacent to pesticide factories (Chambers & Yarbrough 1973, 1974; Watkins 1975).

All the waste water concentrations used caused significant increases of egg mortality. The impact of BKME concentrations used on egg hatchability is expressed in Figure 1. The highest concentrations used raised the egg mortality quickly whereas the eggs died later in the more dilute solutions. Similar results have earlier been obtained by Rask (1983) when the effect of low pH on perch (Perca fluviatilis) egg development was studied.

The difference between the two pike populations is most distinct in lowest waste water concentrations (0.5 and 2.5 %). In the 10 percent concentration the mortality of both groups was large. The difference between pike populations was especially distinct when

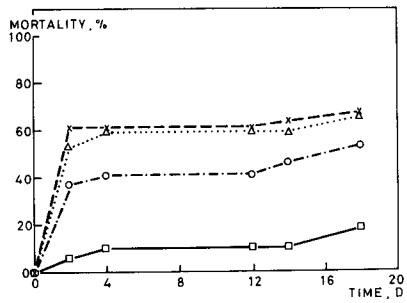

Fig. 2a.

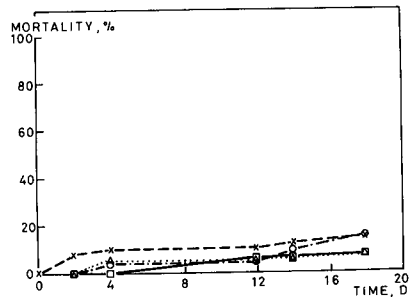

Fig. 2b.

Figure 2. The effects of BKME concentrations at the moment of fertilization on the egg mortality. Fig. 2a = reference population. Fig. 2b = population from discharge area. For other legend see Fig. 1.

the impact of BKME concentration at the moment of fertilization was studied (Fig. 2). The pike population from the discharge area was more resistant so that even 10 percent BKME concentration did not significantly influence the egg viability.

In the regression analysis a significant positive linear correlation ($r = 0.789^{***}$) between the effluent concentration and mortality was obtained. The EC 50 for the waste water was 4.0 % when determined at the moment of hatching. Due to the greater mortality of newborn pikes hatched in the largest waste water concentrations the EC 50 determined four days later was 3.5 percent.

No significant alterations in malformation frequencies could be detected. This may partly be due to the relatively small egg number in the experiment.

The bioassay method used proved useful in estimating the impact of BKME on the recipient fish. Even the lowest BKME concentration of 0.5 % which exists at large recipient areas had significant deleterious effects on the reproduction of pike population. However, the EC 50's were approximately twice as large as the BKME concentration present in the recipient.

Accordingly, the pike population that lived in the recipient site showed indices of acclimatization to the strongly altered water quality. The difference between pike populations was especially large when

the impact of BKME at the fertilization moment was studied. Comparable results have been obtained by Rask (1983) who noticed improved lethal resistance to low pH of perch eggs and alevins from acidified lakes. Trojnar (1977) had earlier obtained similar results after sublethal low pH exposure.

Hauling, however, strongly increased the mortality of the eggs of the exposed pike population. As the weakened capacity to survive in stress situations is of ecological importance, it has earlier also been recommended that ecological experiments should more often be conducted in combination with additional stressors (McLeay & Brown 1975; Wedemayer & McLeay 1981). In this case the intolerance to hauling may indicate that the acclimatization to pulp and paper wastewater had diminished the capacity to survive in simultaneous stress. Consequently, the advantage of the adaptation may in practice be questionable.

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