

## Tissue damage and parasite frequency in flounders, *Platichthys flesus* (L.) chronically exposed to bleached kraft pulp mill effluents

Karl-Johan Lehtinen, Mats Notini & Lars Landner

Lehtinen, K.-J.; Notini, M & Landner, L. 1984: Tissue damage and parasite frequency in fish chronically exposed to bleached kraft pulp mill effluents. — Ann. Zool. Fennici 21: 23-28.

One-year-old flounders, *Platichthys flesus* (L.), were exposed to kraft pulp mill effluent during a two-month period in an artificial ecosystem corresponding to the littoral zone of the Baltic Sea.

The fish were exposed to 1 or 2.5 % concentrations (v/v) of different types of total effluents from mills producing bleached and unbleached pulp, respectively. Fish caught in the vicinity of the discharge pipe of a pulp mill were used as reference field material.

Structural differences in gill and liver tissue were detected in the exposed fish. These seemed to be positively correlated with the chlorine bleaching in the process of pulp production.

The reference fish were most heavily infested with a tricotinid ciliate, followed by the fish exposed to effluents from mills using oxygen and chlorine in the bleaching process.

*Karl-Johan Lehtinen, Swedish Environmental Research Institute (IVL); Baltic Sea Laboratory, Utövågen 5, S-371 37 Karlskrona, Sweden.*

*Mats Notini, Swedish Environmental Research Institute (IVL), Baltic Sea Laboratory, Utövågen 5, S-371 37 Karlskrona, Sweden.*

*Lars Landner, Swedish Environmental Research Institute (IVL), Box 21060, S-100 31 Stockholm, Sweden.*

### 1. Introduction

Within the framework of a Swedish project concerning environmental effects of bleached kraft pulp mill effluents, experimental studies were carried out in model ecosystems simulating the littoral (e.g. coastal) zone of the Baltic (Notini & Lehtinen 1982). These controlled experiments, performed in 7.5 m<sup>3</sup> outdoor model ecosystem tanks including most of the floral and faunal components of the natural *Fucus* zone, have served as an important link between pure laboratory experiments and field studies. As such they facilitate the understanding of the emanating disturbances due to discharge of complex industrial effluents into a recipient waterway.

In natural ecosystem fish are always (to some extent) carriers of potentially pathogenic microorganisms, which normally do not harm the host. If the host is confronted with an increased environmental stress such as toxic substances in the surrounding water, however, it is probable that the immunological defence of the host is affected and diseases may break out (Sniezko 1974).

One objective of the present studies in the

model ecosystem, was to investigate whether histological changes in fish tissue could be detected after exposure to low concentrations of kraft pulp mill effluents. Another objective (of this particular study) was to elucidate possible correlations between environmental stress, tissue damage and parasite frequency in fish and compare experimentally obtained results with field data to obtain a preliminary estimation of the size of the problem.

### 2. Material and Methods

Twentyfive one-year old flounders, *Platichthys flesus* (L.), belonging to the same population (mean total length 103.5 ± 10.5 mm, captured with a dip net in a clean brackish water bay outside the laboratory), were evenly distributed into five tanks with a water volume of 7.5 m<sup>3</sup> each. Brackish water (7 ‰) was pumped into each tank at a rate of 2.4 l/min from 40 m depth from Tvären, a brackish water bay outside the laboratory at Studsvik (80 km SW of Stockholm). The flounders served as predators in the model ecosystem simulating the littoral (*Fucus*) zone of

the Baltic. The methodology of the model ecosystem has been described by Notini *et al.* (1977).

The fish were exposed for two months (September to October, 1980) to total effluents from three different pulp mills, two of which (A, B) produced bleached (A: conventional chlorine bleaching with the sequence C + DEHDED; B: pre-bleaching with oxygen with the sequence O(CD)EDED), and one (C) unbleached pine pulp. The meaning of the letters given in the bleaching sequences is as follows: C = acid chlorine step, D = chlorine dioxide, E = alkaline step with sodium hydroxide, H = hypochlorite, and O = oxygen. The total effluents were sampled at specific sampling sites within the mills before reaching the recipient. The test waters were transported from each mill in 2 m<sup>3</sup> polyethylene tanks and continuously aerated during the experiment. Two concentrations (1 and 2.5 % v/v) of the total effluent from mill A were used, whereas only one concentration (2.5 % v/v) of the effluents from B and C was used. In addition to the test tanks, one tank was used as a control receiving pure brackish water only. The water temperature in the test tanks was +1 to +20°C and oxygen saturation varied between 80 and 120 per cent over the test period.

The total effluent waters were dosed using four membrane pumps (Prominent Electronic, type A). At the end of the exposure period, the fish were caught and placed in 60 l glass aquaria with clean, running (1 l/min) brackish water (O<sub>2</sub>: 9–10 mg/l, temperature: 5–6°C) for one week to avoid possible morphological changes in the gills due to capture, whereupon they were killed. The 2nd right gill arch and a peripheral part of the liver were removed and fixed in Bouin's solution (24 hrs) (Romeis 1968), embedded in paraffin and cut into 5 µm sections. The sections were stained with hematoxylin eosin and Alcian blue-PAS-hematoxylin (Pearse 1968).

As a polluted control, seven flounders were caught with gill nets outside a mill producing bleached pulp and resembling mill B, and located on the Swedish east coast. These fish were of bigger size than the experimental fishes. The length of the fishes was not measured. However, based upon the size of the gill arches they obviously belonged to a two-year old population with sizes ranging between 150 and 180 mm. The fishing was performed at a distance with roughly 100–500 times the dilution of the total waste water (unpublished results). This material was prepared as described above.

The gills of the fish were analysed for their histology and the frequency of parasites on the 2nd right gill arch. From each fish the parasites of

four sections were counted, each consisting of 8–9 primary lamellae. To obtain a measurement comparable with that of the bigger fish caught in the recipient, the frequency of parasites in these was calculated from two sections only.

The livers were investigated with regard to structural differences such as cytoplasmic vacuolization, nuclear condensation and necrosis. To obtain a quantitative measure of structural differences, the number of condensed hepatocytic nuclei was counted in a light microscope equipped with a field grid. The mean number from eight fields, each containing ~ 250 cells, was then calculated and pooled in a group mean.

Statistical differences from the control group were calculated with Student's *t*-test.

### 3. Results

#### 3.1. Gill

Compared to the smooth epithelium of the control fish (Fig. 1) the cell surface of the exposed fish appeared diffuse (Fig. 2). This was most prominent in the fish exposed to the bleached effluents of mill B. In most of the fish, especially in those exposed to 2.5 % v/v effluent from mill B and in the fish caught in the recipient, the gill epithelium was detached from the basal membrane (Fig. 3) forming an oedematous deformation similar to that induced by heavy metals (Baker 1969, Skidmore & Towell 1972). The result is not unambiguous, however, since also one of the control fish exhibited the same effect, although to a lesser degree.

In the fish caught in the recipient and in those exposed to effluent from mill C, changes in the pillar cells resulting in local dilation of the marginal channels of the secondary lamellae, were observed (Fig. 4). Increased mucus production observed as intense dots between the secondary lamellae and frequently associated with parasites occurred in the fish from the 2.5 % v/v concentrations and in the field material (Fig. 3).

The examination for parasites revealed that all of the exposed fish, including the field material, were infested by a ciliate, *Tricodina sp.* (Figs 5 and 6), whereas only one fish in the control tank was found to be infested by this organism (Fig. 7). The tricodinids were either already present in the fish on capture, or were introduced with incoming water into the test tanks. As such the variation between doses and control tanks can thus be regarded as an effect due to the prevailing conditions in the systems. Fish exposed to mill C's waste water (unbleached pulp) were less infested

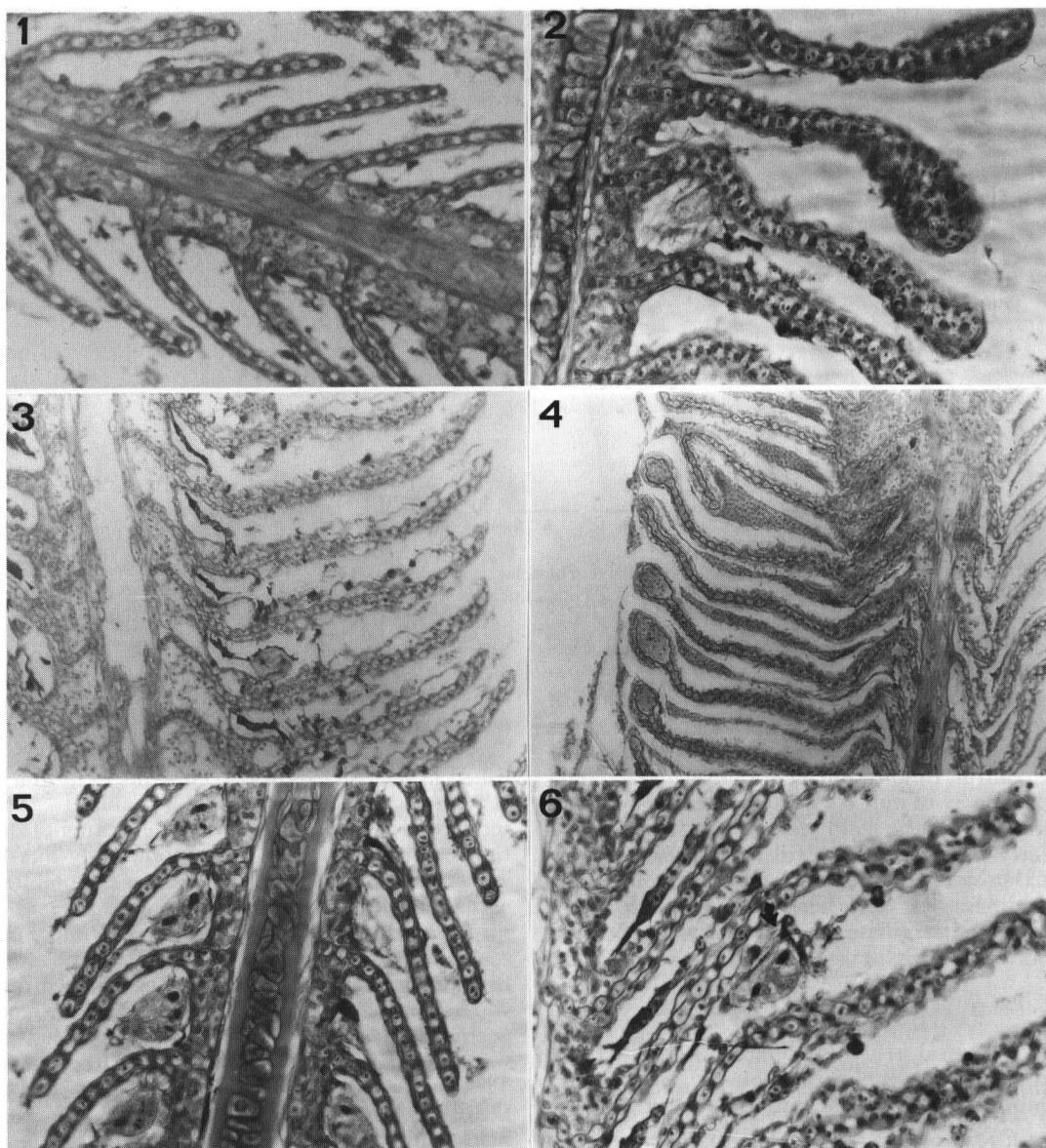


Fig. 1. Gill arch of an unexposed fish (control tank). The epithelium of the secondary lamellae is smooth and even. Alcian blue-PAS-Htx (x 140).

Fig. 2. Secondary lamellae of an exposed fish (mill B). The epithelium surface appears to be in a proliferative stage. Also note the parasites basally between the secondary lamellae. Alcian blue-PAS-Htx (x 280).

Fig. 3. Extensive primary lamellae oedema and separation of the epithelial lining of the secondary lamellae in a fish exposed to effluent from mill B. Alcian blue-PAS-Htx (x 140).

Fig. 4. Apical dilation (telangiectasis) of the secondary lamellae in a fish exposed to effluent from mill C. Alcian blue-PAS-Htx (x 140).

Fig. 5. Gill regions heavily infested by tricodinid ciliates. Alcian blue-PAS-Htx (x 280).

Fig. 6. A parasite attached to the separating gill epithelium of an exposed fish (arrow). Alcian blue-PAS-Htx (x 310).

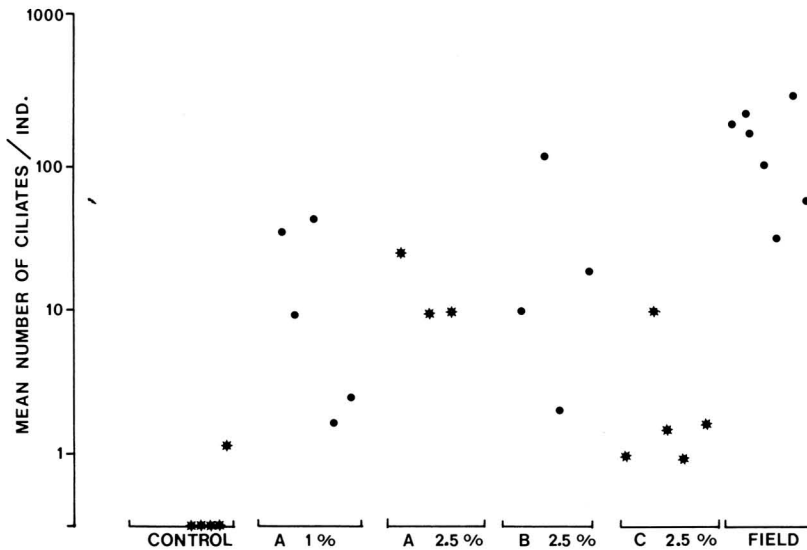


Fig. 7. Mean number of ciliates/ind. of flounders exposed to bleached and unbleached kraft pulp mill effluents (BKME) in model ecosystems and of flounders from a BKME polluted recipient. Numbers refer to the specific gill volume counted from the sections.

than the fish in the other polluted tanks. The fish from the recipient displayed a roughly 200 times higher frequency of trichodinids in the gills than the control. In some gill sections from fish exposed to 2.5 % v/v of mill B's waster water, as well as in some fish in the field material, parasites were clearly attached to the gill epithelium (Fig. 6). Furthermore, mucus and emptying goblet cells were observed underneath the parasites, indicating that the ciliate was parasitizing on the host.

### 3.2. Liver

The general structural differences consisted of a cytoplasmic vacuolization of the hepatocytes combined with a varying degree of nuclear

condensation (Figs 8 and 9). As the frequency of condensation was calculated, statistically significant differences from the control were established in the fish exposed to effluents from mill A (1% v/v), mill B and mill C (Table 1). The individual variation in the flounders caught from the recipient was very high, apparently due to great variations in residence time in the polluted area. The fish exposed to effluent from mill B displayed the most heterogeneous reaction of the test tanks, however. The mean value of one of the fish was drastically higher than the group mean.

The degree of vacuolization varied from a focal occurrence, often in connection with central veins, to massive distribution in the liver tissue. The most prominent changes were prevalent in the fish exposed to the effluent of mill B and in the field material. Another characteristic feature of the exposed fish and the field material, which deviated from the control group, was a membranous disintegration of the hepatocytes (Fig. 9).

Moreover, aggregations of PAS-positive material were observed in the exposed organisms, both extracellularly and intracellularly (Fig. 10). Extracellularly these aggregations were of macrophage size, whereas the intracellular aggregations were mainly of lysosome size (Lehtinen & Oikari 1980). It must be pointed out that the control fish also displayed similar intracellular structures, but not of the same size or frequency.

Table 1. Number of condensed nuclei of liver cells in *P. flesus* (L.) exposed to BKM effluents in model ecosystems and in liver cells of flounders from a BKME polluted recipient. Numbers are mean values  $\pm$  SD for each group,  $n$  = number of individuals per group. Asterisk indicates statistically significant difference from the control group. (\*\*\*) =  $P < 0.001$ , (\*\*) =  $P < 0.01$ , (\*) =  $P < 0.05$ .

Effluent	Number of nuclei	$n$
Control	1.1 $\pm$ 0.56	4
Mill A 1 % v/v	4.4 $\pm$ 0.38***	5
Mill B 2.5 % v/v	7.8 $\pm$ 4.9*	5
Mill C 2.5 % v/v	6.7 $\pm$ 2.7**	5
Field material	8.0 $\pm$ 5.7	7

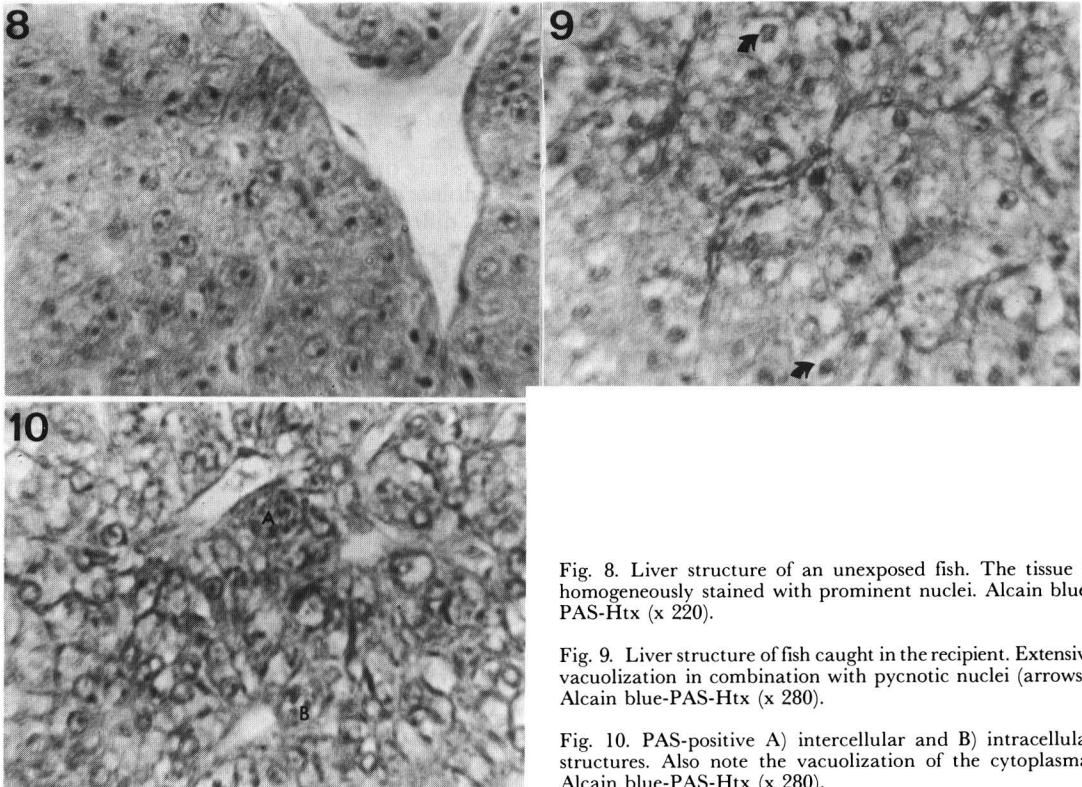


Fig. 8. Liver structure of an unexposed fish. The tissue is homogeneously stained with prominent nuclei. Alcain blue-PAS-Htx (x 220).

Fig. 9. Liver structure of fish caught in the recipient. Extensive vacuolization in combination with pycnotic nuclei (arrows). Alcain blue-PAS-Htx (x 280).

Fig. 10. PAS-positive A) intercellular and B) intracellular structures. Also note the vacuolization of the cytoplasm. Alcain blue-PAS-Htx (x 280).

#### 4. Discussion

The structural changes in the gills observed in this work, a slightly increased mucus production, detached gill epithelium and disintegration of the pillar cells, are reactions which have been established previously in connection with exposure to different chemical substances (Skidmore & Towell 1972, Chliamovitch & Kühn 1977, Roberts 1978). Whether the observed vasodilation in the secondary lamellae actually affects the fish cannot be elucidated in the present study, however.

It has been shown that fish are fairly extensively able to regulate the blood flow through the gills by opening or closing blood vessels (see for instance Hughes 1976, Soivio & Hughes 1978, Hughes *et al.* 1978). By this mechanism the fish are able to regulate the blood flow after the damage mentioned above. As two fish from the tank receiving total effluent from mill C (producing unbleached pulp) also suffered from the same damage, components other than those typical of the bleaching process obviously contribute to the effects found. Resin acids might contribute to the toxicity in this respect (Holmbom & Lehtinen 1980, Lehtinen & Oikari 1980). The detaching

gill epithelium in the exposed fish and in the fish caught in the polluted recipient indicate that osmoregulatory disturbances in the gills due to toxic substances in the water or secondary effects due to parasitic infestations, might be prevalent (Sniezko 1974).

The visually estimated, slightly increased mucus production might primarily be a secondary effect induced by the parasites demonstrated on the gill surface. The prevalent tricodinic group is both parasitic and nonparasitic. Wild fish are frequently slightly infested, but these parasites do not usually harm the host. During this ectocommensal state the organism feeds on bacteria, algae and organic particles in the water. However, during the parasitic state the organism feeds on detaching epithelial cells of the host and under these circumstances it is harmful to the host since mucus production is stimulated and the epithelium damaged (Lom 1973, Calenius 1980). Although it was not possible to discover the exact degree of infestation, parasites in contact with the epithelium in both the experimental and the field material were qualitatively clearly demonstrated. This indicates that at least some of the ciliates had begun to live parasitically, which might indicate

that the condition of the test fish had deteriorated as a consequence of the exposure to pulp mill effluents. Even if the ciliates in the present study had only acted ectocommensally in great numbers on the gills, they might have harmed the host fish by a mechanical reduction of the water flow over the gills (Fig. 5, Hughes 1978): Fibres in pulp mill effluents induce physiological stress such as increased metabolism (Smith et al. 1966). "Particles" in the form of ciliates might act in the same way.

The most prominent histological effects in the liver of fish in the present study are similar to those found in fish caught from an area polluted by arsenic (Sörensen et al. 1980). In particular the reaction of the fish caught in the recipient, the extensive cytoplasmic vacuolization and nuclear condensation, are very similar to the results of the study referred to above. In a previous study by Lehtinen & Oikari (1980) intracellular PAS-positive granules were also found in hepatocytes of fish exposed to pulp mill effluents. In the present study granules were found in both controls and exposed fish, but were of greater size and higher frequency in the exposed groups. The extracellularly observed PAS-positive macrophage (Roberts 1978) aggregations support the assumption of destructive effects on the liver tissue. The function of macrophages is to eliminate dead cells and an increased frequency of macrophages might thus be an indication of an increasing number of dying hepatocytes in the present study.

The results of the histological study of the liver follow the general trend of the other parameters studied in the present work: the greatest differences between unexposed and exposed fish were found in the field material and in the fish exposed to total effluents from mill B. An interesting observation was that one fish exposed to unbleached pulp mill effluent (C) displayed an effect different from that of the effects on the other groups. In the liver of this fish focally hypertrophied hepatocytes similar to those in human patients suffering from chronic active hepatitis (CAH) were found (Patric & McGee 1980).

The effects reported in this work, above all the increased frequency of parasites on gills, appear to be sensitive biological parameters, which might be of great value in diagnosing the situation in the recipient or in indicating the extension of stress by direct investigations of wild fish populations.

The combination of the results of the present study indicates disturbances in a vital ecological correlate, the host-parasite relation. This might cause severe disturbances in fish populations within affected areas. What kind of effects this may have, e.g. on fish production, remains to be settled, however.

*Acknowledgements.* We wish to thank Ms Louise Andersson for preparing the manuscript. The project was financed by Stiftelsen Skogsindustrins Vatten- och Luftvårdsforskning (SSVL).

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