BOREAL ENVIRONMENT RESEARCH 5: 53–65 ISSN 1239-6095
Helsinki 28 March 2000

Monitoring the recovery of a polluted lake with biomarkers: Responses of whitefish (*Coregonus lavaretus* L. *s.l.*) experimentally exposed to pulp and paper mill effluents

Aarno Karels[1)], Markus Soimasuo[1)], Riku Suutari[2)] and Aimo Oikari[1)]

[1)] *Department of Biological and Environmental Sciences, University of Jyväskylä, P.O. Box 35, FIN-40351 Jyväskylä, Finland*
[2)] *Finnish Environment Institute, P.O. Box 140, FIN-00251 Helsinki, Finland*

Karels, A., Soimasuo, M., Suutari, R. & Oikari, A. 2000. Monitoring the recovery of a polluted lake with biomarkers: Responses of whitefish (*Coregonus lavaretus* L. *s.l.*) experimentally exposed to pulp and paper mill effluents. *Boreal Env. Res.* 5: 53–65. ISSN 1239-6095

A kriging interpolation of fish biomarker data was utilized as a novel tool to describe the ecotoxicological status of a lake polluted by pulp and paper mill effluents. Liver ethoxyresorufin *O*-deethylase (EROD) activity, hematological and immunological biomarkers were studied in juvenile whitefish (*Coregonus lavaretus* L. *s.l.*) experimentally exposed for one month to effluents from three pulp and paper mills at southern Lake Saimaa. In comparison with the reference areas, liver EROD activitiy was 40%–80% higher in whitefish 3–6 km downstream of one of the mills. Liver EROD was positively correlated with the effluent tracers, lake-water sodium and conductivity. No remarkable differences between whitefish from mill and reference sites in their plasma immunoglobulin M, blood hematocrit, hemoglobin, glucose and lactate levels were observed. In this study, liver EROD activity in whitefish 3–6 km downstream of one of the mills in 1996 was 14%–15% of that recorded in 1991, i.e. before introduction of ECF bleaching and the activated sludge treatment of effluents at the mill in 1992. Our results are comparable with, and confirm the findings of a related caging experiment in 1995, indicating a decreased but still continued exposure of the fish to pulp mill effluent compounds.

Introduction

While investigating the impact of pulp mill effluents on fish, the liver monooxygenase (MO) activity, also known as the mixed function oxygenase (MFO) system, has proved to be the most consistent biochemical marker of exposure (Owens 1991, Sandström 1996). However, the main compounds in pulp mill effluents responsible for induction of the MO activity in fish are largely unknown, and may vary from case to case, particularly in cases of modernized mills with elemental chlorine free (ECF) and total chlorine free (TCF) bleaching (Munkittrick 1994, Hodson 1996). The liver MO activity is often measured as ethoxyresorufin *O*-deethylase (EROD) activity. Measurement of EROD activity is a common method to examine the catalytic activity of cytochrome P450 1A, an important MO isoenzyme catalyzing phase 1 reactions of xenobiotic compounds.

In the southern part of Lake Saimaa (southern Lake Saimaa), liver EROD activities and chlorophenolics (CPs) concentrations in fish bile have been used since the 1980s as biomarkers of exposure to pulp mill effluents (Lindström-Seppä and Oikari 1989, 1990, Oikari & Holmbom 1996, Soimasuo *et al.* 1995, 1998, Petänen *et al.* 1996, Karels *et al.* 1998). Southern Lake Saimaa receives effluents from three pulp, paper and cardboard mills, located in the towns of Lappeenranta, Joutseno and Imatra. To monitor the ecotoxicological status of the lake we measured biomarker responses of whitefish caged for one month upstream and downstream from the pulp and paper mills. Caging experiments with whitefish, a native species in the study area suffering from pulp mill effluents, offer a good assay tool for monitoring effects of pulp mill effluents on a local fish species.

The aim of this second large scale lake study was to compare the biomarker responses in caged whitefish with those reported in the earlier studies at southern Lake Saimaa, and to verify the results of a related study in 1995 (Soimasuo *et al.* 1998). Results of the 1995 study showed a decreased exposure of fish to the mill effluents after the implementation of ECF bleaching and activated sludge treatment of effluents in the early 1990s (Oikari and Holmbom 1996, Kaplin *et al.* 1997, Soimasuo *et al.* 1998). Results, however, also revealed large site specific and temporal inconsistency in the mixing and dispersion of effluents downstream of two of the mills.

As a new tool to describe the ecotoxicological status of the lake we conducted a spatial kriging interpolation of EROD and bile CPs. The kriging interpolation is a method of estimating the value of a spatially distributed variable at a given point from known nearby values while applying the interdependence expressed in a variogram (Cressie 1993). The EROD activity and bile CPs in fish at additional water sampling sites were calculated with a help of the equation of their relationship with the effluent tracer sodium (Na^+). For this purpose, we made a detailed dilution model of the pulp mill effluents using sodium as the (inert) effluent tracer.

Material and methods

Characteristics of the study area, pulp and paper mills and effluents

Southern Lake Saimaa (609 km^2), is a large oligotrophic lake in SE Finland. It has received effluents from the forest industry for decades. At the present time, three pulp, paper and cardboard mills discharge their effluents into the lake (Fig. 1). For this study, the lake area was divided into five areas: reference area I (sites R1 and R2), reference area II (sites R3, R4 and R5), and the mill effluent receiving areas A (sites A1, A2, A3, A4 and A5), B (sites B1, B2, B3, B4 and B5) and C (sites C1, C2, C3 and C4) and the common outfall (site D), at the River Vuoksi (Fig. 1). The main charge of water flows into the lake at Rastinvirta, being practically the same as the outflow through the River Vuoksi into the Lake Ladoga, about 550 $m^3\ s^{-1}$. The retention time of water in the lake is about 60 days. A pump station, which displaces water from the clean area of Lake Saimaa to the watercourse upstream of mill A, causes a net water flow in the subarea A from southwest to northeast. As a result, lake water passes the outlet point of mill A with a flow of about 40 $m^3\ s^{-1}$, diluting its effluents. The water flow downstream of mill B is more multidirectional, whereas the water flow in the area close to mill C is strong towards the River Vuoksi (Fig. 1). The three mills studied are re-

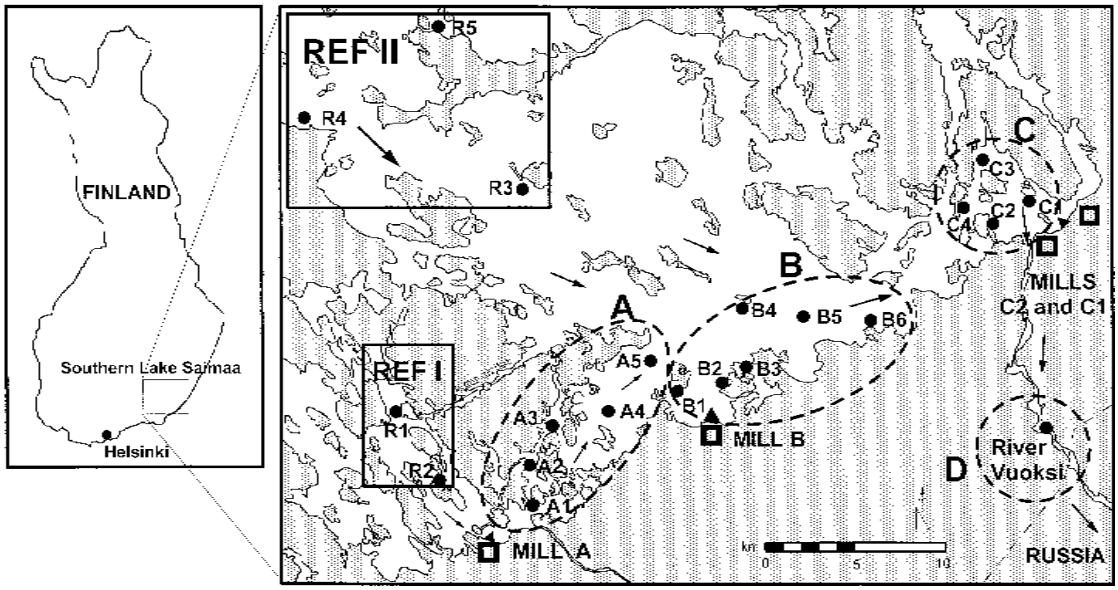

Fig. 1. The research area at the southern Lake Saimaa, SE. Finland with the different subareas: Reference areas I and II, the effluent receiving areas A, B and C, and the common outflow D (River Vuoksi). The experimental sites are indicated by black filled dots. Arrows indicate the direction of the water flow in the area.

ferred to as follows: the Kaukas Inc. pulp and paper mill in Lappeenranta (mill A), the Joutseno Pulp pulp mill in Joutseno (mill B), and the Stora-Enso pulp, paper and cardboard mill (mill units CI + CII) in Imatra. Mill C is regarded as one entity, since the separate mill units have a joint wastewater treatment plant for their effluents. Characteristics of the mill processes and effluents are given in Tables 1 and 2. During the caging experiment in May–June 1996, all the mills were under normal operating conditions, i.e. without prominent (max. 1 day) shutdowns in the production of pulp, paper or cardboard.

Lake water quality

In the beginning of the field experiments in May, the mean water temperature was around 6.9 °C, whereas at the end the temperatures close to the cages (5 m) varied from 10.8 °C at site R4 to 15.1 °C at site C4. Dissolved oxygen within the experimental sites remained > 8 mg l^{-1}, only at site A1 a somewhat lower concentration (6.3 mg l^{-1}) was recorded at the end of the caging period.

Concentrations of chlorophenolics (CPs), and resin acids (RAs) in the lake water at the study sites were presented earlier in Leppänen *et al.* (1998). The concentrations of CPs in the lake water at the study site were all close to the detection limit (0.1–1.0 μg l^{-1}). RAs concentrations varied between 0.6 and 4.6 μg l^{-1}, with highest concentrations on locations R2, A3 and B1. There was no clear distance related decrease in CPs and RAs concentrations in the lake water downstream of the mills.

Effluent tracer

During the time of the study, the lake water and effluents were assumed to be properly mixed downstream of the mills because the spring overturn (with no thermal stratification) occurred during the experimental period. We used sodium as an effluent tracer because it is inert and the pulp mill effluents contain, as compared with the lake water, high concentrations of it (Table 2 and Fig. 2). Southern Lake Saimaa water sodium contents in spring and autumn in 1990, 1991, 1992, 1993, 1994, 1995 and 1996 were all strongly correlated with one another (Spearman correlation coefficient; $0.65 < r < 0.95$, $p < 0.001$, $17 < n < 34$). This indicates that dilution of effluents in the lake

Table 1. Characteristics of the pulp and paper mills in the study area at the southern Lake Saimaa.

Mill type/year/production (t y^{-1})			Effluent treatment/volume (m^3 d^{-1})	
Mill A	(pulp, paper and sawmill)		Biological:	
1996	ECF bleached kraft pulp		activated sludge/110 000	
	Softwood	226 000 (D(Eop)DED)		
	Hardwood	190 000 (OD(Eo)DepD)		
	Groundwood	135 000		
	Paper	391 000		
	Sawn goods	364 000		
1991	Bleached kraft pulp		aerated lagoons/135 000	
	Softwood	115 000 (C_{43}/D_{57}(Eop)DED)		
	Hardwood	240 000 (C_5/D_{95}(Eo)D(Ep)D)		
	Paper	380 700		
	Sawn goods	355 800		
Mill B (pulp)			Biological:	
1996	Bleached kraft pulp	306 000 (DoO/OD(Ep) D)	activated sludge/70 000	Softwood
	Hardwood	0		
Mill CI + CII (pulp, paper and cardboard)			Biological:	
1996	Bleached kraft pulp	175 000 (DE(Eo)D(Ep)D)	activated sludge for pulp	Softwood
	Hardwood	364 000 (DE(Eop)D(Ep)D)	effluents/148 000	
	Chemi-thermo mechanical pulp	43 000	Chemical: for paper and cardboard	
	Kraft pulp	133 000 (unbleached)	effluents/50 000	
	Paper	220 000		
	Cardboard	688 000		

Abbreviations for bleaching sequences are: C = chlorine, D = chlorine dioxide, E = caustic extraction, Eo= caustic extraction with addition of oxygen, Ep= caustic extraction with addition of peroxide, O = oxygen delignification, O/O = two-stage oxygen delignification, P = peroxide.

Table 2. Characteristics of effluents from the pulp and paper mills discharging to the southern Lake Saimaa. The values are averages for May and June 1996, measured daily by the monitoring laboratories of the companies involved. Chlorophenols, resin and fatty acids and phytosterol results are from Leppänen *et al.* (1998).

Parameter	Mill A		Mill B	Mills CI and CII
	1991	1996	1996	1996
Flow (m^3 d^{-1})	124 000	93 500	57 300	146 700[a)] 49 000[b)]
Suspended solids t d^{-1}	9.6	12.7	1.3	3.0
COD t d^{-1}	99.2	56.7	36.3	8.5
BOD_7 t d^{-1}	14.3	2.5	1.7	6.2
AOX t d^{-1}	1.7	0.3	0.2	0.5
Chlorophenolics (µg l^{-1})	360	8.3	7.6	1.3
Resin acids (µg l^{-1})	220	94	559	38
Fatty acids (µg l^{-1})	520	383	727	264
Phytosterols (µg l^{-1})	n.d.	214	875	68
Na^+ (mg l^{-1})	230	360	470	326
N kg d^{-1}	1 272	337	320	399
P kg d^{-1}	111	24.8	65.7	23.0

[a)] pulp effluent; [b)] paper and cardboard effluent; n.d.= not determined

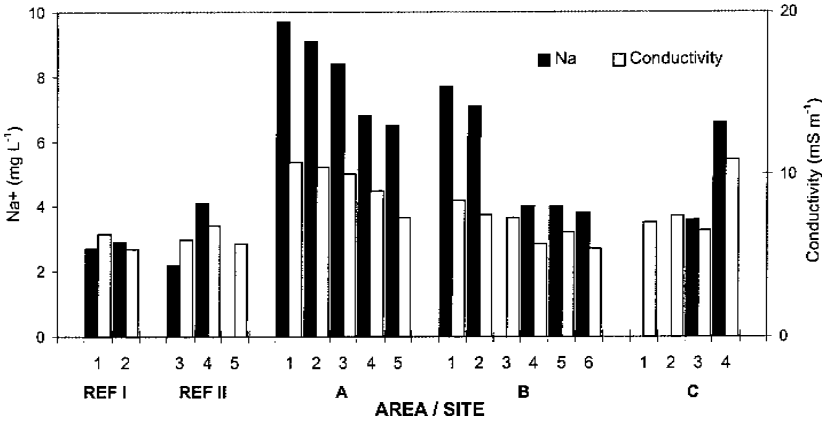

Fig. 2. The concentration of sodium (Na^+) and the conductivity of the lake water at the experimental sites in May–June 1996.

has remained similar over these years, implying a relatively stable dilution history over the 1990s. First, we assumed that sodium data of the lake water could be used for making a general model for the dilution of pulp mill effluents. In order to make a more detailed dilution model, we conducted an intensive lake water sampling in autumn 1998 in which lake water sodium at 89 different sampling sites were measured. The sodium data are means of water column values from 1 m to near bottom. Lake water sodium between autumn 1998 and spring 1991, and between autumn 1998 and spring 1996 were also strongly correlated with one another. The Spearman correlations were as follows: 1998 vs. 1991, $r = 0.81$, $p < 0.001$, $n = 28$; 1998 vs. 1996, $r = 0.80$, $p < 0.001$, $n = 30$.

Caging and sampling of whitefish

On 15 May 1996, immature (1+ year old) hatchery reared whitefish (*Coregonus lavaretus* L. *s.l.*) from the Central Fish Culture and Fisheries Research Station for Eastern Finland in Enonkoski, were transported (max. 4 h) from the hatchery to the experimental area in southern Lake Saimaa. Fish were transported in polyethene bags filled with water and gaseous O_2 at ca. 5 °C. During transportation time the bags were chilled with ice. Twelve fish (mean ± S.D. = 40.4 ± 10.7 and 16.5 ± 1.3) were placed in each oval-shaped 250-litre cage (diameters 50 and 70 cm, height 70 cm) of steel wire and polyester net construction. The cages were submerged at the bottom, at a depth of 4–5 m, i.e. into the lower epilimnion layer of the water column. Two cages per site were used, except at sites R1 and B6 (1 cage) and at site R2 (3 cages). The type of coregonid whitefish used in the present study is a plankton and seston feeder, which is well able to survive in cages for several weeks, allowing subchronic experiments to be made (Oikari and Sillanpää 1993).

After one month's exposure, the cages were relocated and raised to just beneath the water surface, and floated into a 400 l tank. The tank with a cage inside was moved gently by the side of an operating boat, kept fully immersed, and transferred and winched on board of the research vessel ca. 10–20 min. later. Fish were held in the immersed cage and sampled one by one. Animals were immobilized with a blow to the head and weighed to the nearest 0.1 g. Blood (200–500 µl) was drawn from the caudal vessels into 1 ml heparinized syringes and subsamples were taken for hemoglobin (Hb) and hematocrit (Hct, centrifuged immediately for 3 min. at 10 000 rpm) determinations. The remaining blood was centrifuged for 2 min (12 000 rpm) for plasma separation, and the liver was dissected free, and bile collected into Eppendorf tubes. Samples were stored in liquid nitrogen until analysed. Finally, the fork length of the fish was measured (1 mm). The condition factor (CF) was calculated with the formula: W (g)/ L^3 (cm) × 100. The entire sampling procedure for each fish required approximately 6 min.

Analytical methods

For the preparation of microsomes liver samples of three fish were combined for one analysis (to-

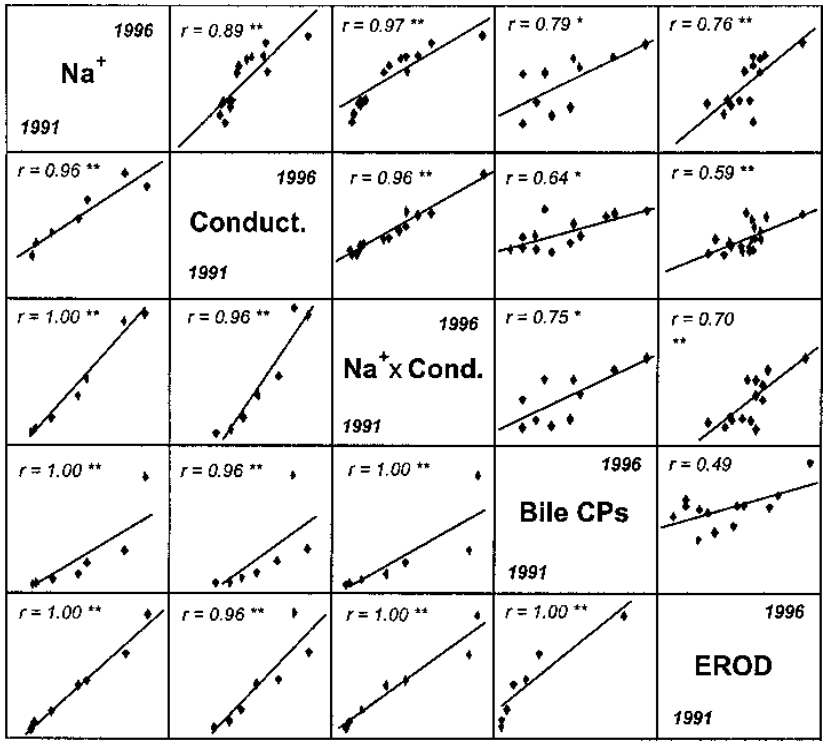

Fig. 3. Relationships between lake water sodium, conductivity, EROD activity and bile chlorophenolics (CPs) in 1991 (area A; lower triangle; data from Soimasuo *et al.* 1995) and 1996 (whole Southern Lake Saimaa; upper triangle; CP data from Leppänen *et al.* 1998). *r*: Spearman's rank correlation coefficient; *= $p < 0.05$; ** = significant correlation $p < 0.01$.

tal weight about 0.5 g). Microsome preparation, measurement of the liver 7-ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin *O*-dealkylase (PROD) activities and the determination of the protein concentration of the microsomal fraction were performed as described earlier (Lowry *et al.* 1951, Burke *et al.* 1985, Soimasuo *et al.* 1998). Positive controls for EROD and PROD analysis were liver samples from rainbow trout (*Oncorhynchus mykiss*) dosed by i.p. injecting with 50 mg kg^{-1} β-naphtoflavone (BNF) in corn oil. The detection limits for EROD and PROD assays were 0.1 and 0.01 pmol min^{-1} mg $prot.^{-1}$, respectively. The concentration of whitefish plasma immunoglobulin M (IgM) was determined with the enzyme-linked immunosorbent assay (ELISA). Antibodies against trout IgM were used as trapping and detecting agents as described earlier by Aaltonen *et al.* (1994). The assay was standardized with a known concentration of purified whitefish IgM. Samples for blood glucose and lactate were immediately mixed with 0.6 M $HClO_4$ and centrifuged for 2 min. at 12 000 rpm before freezing. Glucose and lactate were determined using Boehringer Mannheim test kits, (GOD-Perid method 124036 and the L-lactic acid 256 773 UV-method). Hemoglobin (Hb) was measured spectrophotometrically using the cyanmethemoglobin method.

Statistics

All data were first assessed for normality and homogeneity of variance. To test which sites were significantly different ($p < 0.05$) from the combined reference sites a non-parametric Kruskal-Wallis test was used for the monooxygenase activities. Log-transformed hematological parameters were compared with one-way ANOVA followed by Tukey's HTD test. Correlations with two-tailed significance were determined with the non-parametric Spearman rank correlation coefficient. All statistics were performed with SPSS® software.

Kriging interpolation

For a comparison between 1996 and 1991, liver EROD and bile CPs in fish at the additional water sampling sites were calculated with the equation of their relationship with the lake water sodium. The calculated liver EROD activities and bile CPs in 1991 and 1996 were interpolated with a kriging interpolation (Cressie 1993, Suutari *et al.* 1999). Kriging interpolation is a method estimating the value of a spatially distributed variable at a given point from known nearby values while applying the interdependence expressed in a variogram. The interpolation involves the construction of a weighted moving average equation including knowledge of the covariance between the estimation point and sample points within the range of interaction. While other lineair unbiased estimators exist, the kriging method, as a minimum variance estimator minimizes the variance of the estimation errors. It is possible to allow to alter the original measurements (microscale variation or measurement error) for better smoothing (Suutari *et al.* 1999). However, in this study the interpolations were forced to pass through the measured values at the data points, not just near them. It should be noticed that kriging, or any other interpolation method, does not produce reliable estimates at distant areas with no data points. Additionally, in these estimations, the effect of islands was ignored. Kriging interpolations of the spatially distributed variables EROD and bile CPs of southern Lake Saimaa were performed with Variowin (2.01) and Surfer (6.03) softwares.

Results

Dilution of effluents at the study sites

Although lake water sodium concentration and conductivity were highly correlated (Fig. 3), we used only sodium as effluent tracer, indicating theoretical dilutions at the study sites (Fig. 2). When compared with levels of sodium in undiluted mill effluents and at the reference sites, the calculated volume percentages of pulp mill effluent at the study areas were respectively: area A: 1.0–1.9 vol.-%; area B: 0.2–1.0 vol.-%; area C: 0.2–1.0.

Liver EROD and PROD activities

The liver PROD activity was measured in order to get a broader view on the CYP 450 system. PROD activity is considered as an indicator of CYP 2B isoenzymes in vertebrates (Stegeman *et al.* 1994). Liver EROD as well as PROD activities of reference areas I and II were combined for increased statistical power, because reference areas were not significantly different. In comparison to the combined reference areas, significant differences ($p < 0.05$) were measured at 5 mill sites (Fig. 4). The highest EROD and PROD activities were found in fish at sites nearest to the mills A and B and at the common outfall, site D. In comparison with fish in the combined reference areas, liver EROD and PROD activities were 40%–80% and 100%–210% higher, respectively, in whitefish at these sites (Fig. 4).

Plasma IgM, hematological biomarkers and condition of the fish

Data on plasma IgM, blood glucose, hemoglobin and hematocrit were combined for increased statistical power, because reference areas were not significantly different (Table 3). Plasma IgM was significantly lower in fish at site A2. Plasma lactate in fish at reference sites R1 and R5, however, were significantly lower (32% in average) than that in fish at the reference sites R3 and R4 ($p < 0.05$). As compared with the fish in the combined reference areas, blood glucose was decreased in fish at sites A3 and A4, and elevated in those at site B1. Plasma lactate was decreased fish at sites A3 and A4 and increased in those at sites B5, C2, C3 and C4 as compared with the combined references. Blood hemoglobin and hematocrit values were similar.

The condition factor (CF) and weight of fish at reference area I (R1 and R2) were lower as

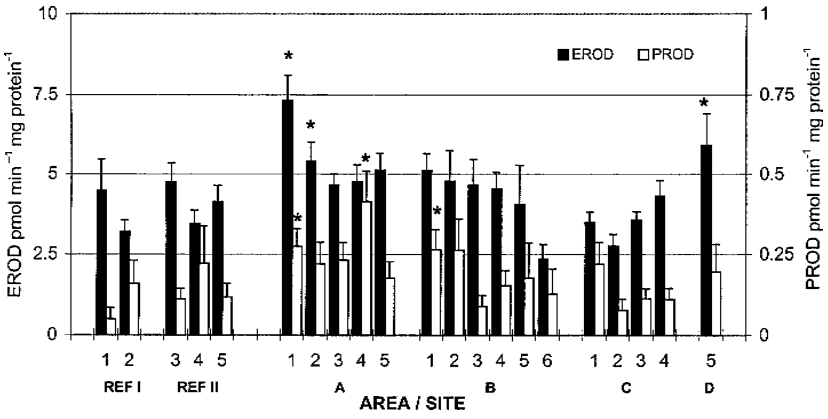

Fig. 4. Liver monooxygenase (MO) activity (pmol/min/mg prot) measured as ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin O-dealkylase (PROD) at the different experimental sites after 30-days exposure. The values are expressed as means (± SEM). A significant difference ($p < 0.05$) compared with the mean value of the combined reference areas I and II is indicated with an asterisk (Kruskal-Wallis test).

compared with all the other sites. On the other hand, length, weight and CF did not vary among the other study sites (n = 9–32 per site). Overall, no distinct pulp mill effluent related fish mortality developed during the experiment.

Correlations between biomarkers and effluent trace markers

The Spearman correlation coefficients (r) of liver EROD activities, bile CPs, lake water sodium and

Table 3. Plasma IgM, blood chemistry and hematological parameters in whitefish exposed for 30 days to pulp and paper mill effluents in southern Lake Saimaa.

Parameter/ Area		IgM[a)]		Glucose[a)]		Lactate[b)]		Hemoglobin[b)]		Hematocrit (%)	
	Site	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Ref. I	R1	1.63	0.42	23.2	5.6	12.3	1.1	50.2	5.2	33.2	3.5
	R2	1.21	0.43	NA		NA		48.0	9.2	34.2	4.9
Ref. II	R3	1.43	0.50	26.8	7.6	20.3	1.4	51.9	6.1	34.1	3.1
	R4	1.25	0.44	25.1	5.7	21.6	1.5	49.8	11.1	33.9	4.8
	R5	1.31	0.33	24.4	7.7	16.4	1.1	52.8	6.1	34.9	3.9
Area A	A1	1.25	0.16	21.5	4.9	17.4	1.1	54.8	5.3	35.2	4.7
	A2	1.13*	0.38	NA		NA		53.2	7.5	34.4	2.9
	A3	1.43	0.41	17.1*	1.6	13.9*	1.4	57.8	6.9	35.4	2.9
	A4	1.46	0.39	20.0*	1.3	15.5*	1.4	54.9	5.0	34.7	4.1
	A5	1.66	0.71	24.0	1.5	20.7	1.6	53.9	4.9	36.9	4.0
Area B	B1	1.28	0.43	31.7*	1.2	16.9	1.4	52.0	5.6	34.4	3.3
	B2	1.49	0.58	24.6	1.5	16.0	1.2	51.2	5.0	33.2	3.8
	B3	1.54	0.45	22.0	1.4	17.9	1.1	52.4	5.3	34.8	2.6
	B4	1.24	0.44	23.5	1.3	20.7	1.4	51.5	5.2	34.3	3.5
	B5	1.35	0.33	27.1	2.5	27.1*	2.5	54.7	3.2	36.1	2.5
	B6	1.45	0.44	23.4	1.8	22.9	2.0	50.2	6.8	33.6	4.7
Area C	C1	1.17	0.32	NA		NA		54.1	5.0	35.6	3.2
	C2	1.38	0.59	20.6	1.7	25.6*	2.1	53.9	5.9	35.7	3.7
	C3	1.20	0.37	27.0	1.7	24.3*	1.6	53.0	5.8	35.2	4.2
	C4	1.40	0.45	27.5	1.4	24.2*	1.1	54.4	7.2	36.7	3.4
Area D	D	1.65	0.60	NA		NA		54.5	8.1	NA	NA

[a)] g l^{-1}; [b)] mg l^{-1} ; NA = not available ; *= $p < 0.05$, compared with combined references I and II.

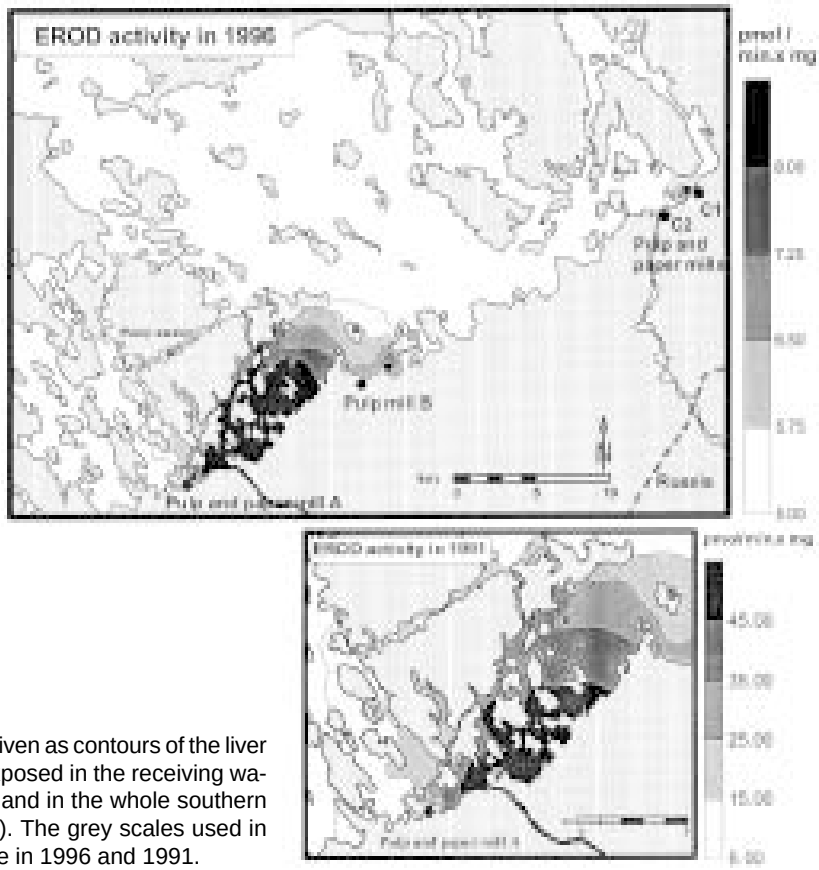

Fig. 5. Kriging interpolation, given as contours of the liver EROD activity of whitefish exposed in the receiving waters of mill A in 1991 (lower) and in the whole southern Lake Saimaa in 1996 (upper). The grey scales used in the graph are not comparable in 1996 and 1991.

conductivity between sites were determined. The studied parameters correlated significantly with one another ($p < 0.05$), only with exception of EROD vs. bile CPs in 1996 ($p = 0.09$) (Fig. 3). Liver EROD activity, bile CPs, the lake water sodium and conductivity in the subarea A correlated stronger with one another in 1991 than 1996 (Fig. 3). As compared with conductivity, the lake water sodium correlated better with liver EROD activities and bile CPs concentrations. The factorial product of the lake water sodium with conductivity did not improve the strength of the correlation with liver EROD nor with bile CPs.

Kriging interpolation of EROD activity and bile CPs in 1991 and 1996

In order to estimate the area affected toxicologically by the pulp mill effluents we conducted a kriging interpolation of EROD activity and bile CPs. EROD activities and bile CPs at 89 sites were calculated with the equations of their relationship with lake water sodium. The equations of these relationships in 1991 were: EROD = 3.066[Na] – 5.804; CPs = $6.914e^{0.2463[Na]}$; and in 1996: EROD = 0.387[Na] – 2.338; CPs = 0.067[Na] + 0.003. The estimated areal distribution of liver EROD activity and bile CPs of caged whitefish in 1991 (area A) and 1996 (whole Southern Lake Saimaa) are shown by a kriging interpolation in Figs. 5 and 6.

Discussion

Dilution of effluents in the different subareas

Because of the large study area (this is the largest field experiment of this kind thus far known for

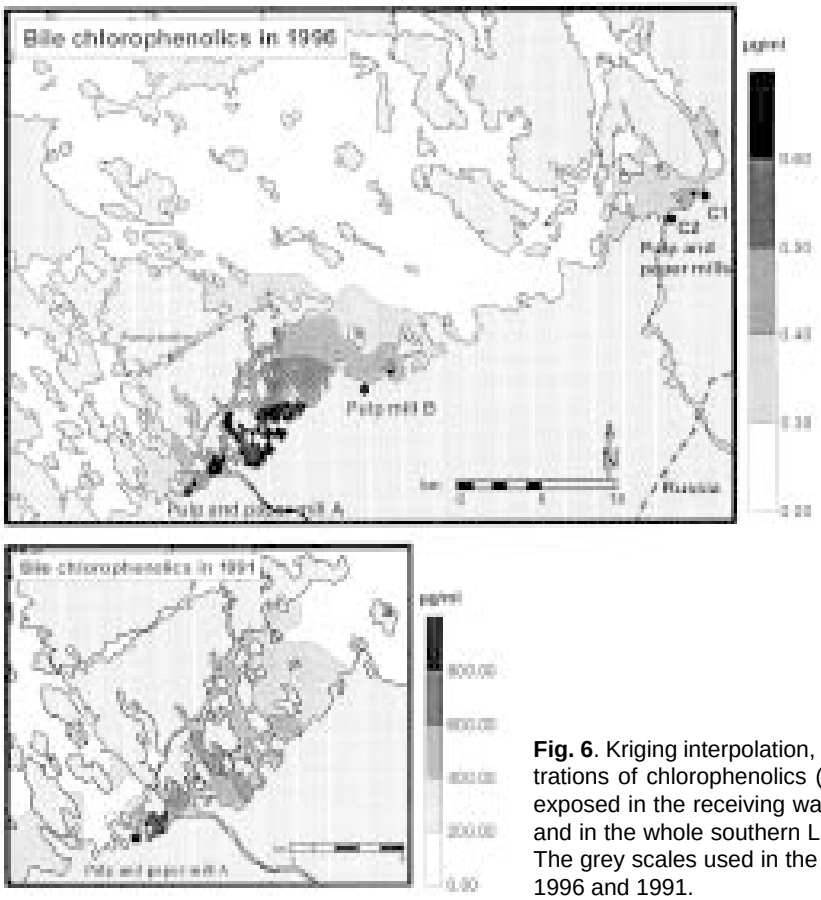

Fig. 6. Kriging interpolation, given as contours of concentrations of chlorophenolics (CPs) in the bile of whitefish exposed in the receiving waters of mill A in 1991 (lower) and in the whole southern Lake Saimaa in 1996 (upper). The grey scales used in the graph are not comparable in 1996 and 1991.

biomarker studies), it is important to estimate the exposure of the caged fish to the mill effluents. Both ambient water parameters as well as internal exposure values and responses can be used to increase the realism of exposure under natural conditions. According to the effluent trace marker sodium, a considerably higher dilution of effluents was measured at areas B and C than in area A. This indicates a substantially lower potential exposure of fish to pulp mill effluents discharged from mills B and C. On the other hand, temporal variations in effluent dilution and dispersion in areas B and C make estimates of the real long-term exposure of the fish less accurate. This emphasizes the importance of the experimental cage location in relation to the effluent plume. The sites downstream of mill A, however, show a more consistent pattern in this respect, with effluent dilutions theoretically ranging, from 1.9% at 3.3 km to 1.0% at 16 km from the effluent source.

Liver monooxygenase activity and bile conjugates as markers of exposure

This work reports the seldom derived geographic distribution of pollutants' effects on a large lake system by means of a kriging interpolation. One of the most consistent effects of pulp mill effluents, including more modern ECF type of effluents, on fish is the induction of the liver CYP1A MO system (Hodson 1996, Sandström 1996). While known CYP1A inducers are quite toxic and may have various effects, the links between induction and its biological consequences in effluent exposed fish are not clear (Hodson 1996, Kloepper-Sams 1996). Similar to this study, liver MO activity in caged as well as in feral fish proved to be the most prominent response to pulp mill effluents in earlier studies at southern Lake Saimaa (Oikari and Holmbom 1996, Petänen *et al.* 1996, Soimasuo *et al.* 1995, 1998, Lindström-Seppä and

Oikari 1990, Karels *et al.* 1998, 1999). Despite many similarities in production processes and effluent treatment (Table 2), the liver MO activity (measured as EROD/PROD activity) was found to vary slightly between the different mill areas. As in the 1995 study, site specific factors like hydrology, effluent dilution and dispersion seemed to cause these differences. Liver EROD activity in 1996 was only 14%–49% of that measured in 1991 (Soimasuo *et al.* 1995), i.e. before the introduction of ECF bleaching and activated sludge treatment at mill A in 1992. The mean level of EROD activity in whitefish at the same reference sites in earlier studies at southern Lake Saimaa (about 4 pmol/min/mg protein) has remained the same over the years (Soimasuo *et al.* 1995, 1998, Petänen *et al.* 1996).

Previous field and laboratory studies revealed that bile conjugates of CPs and RAs serve as sensitive indicators of the exposure of fish to pulp mill effluents, displaying a good relation to the distance and dilution from the source of effluent (Oikari and Kunnamo-Ojala 1987, Lindström-Seppä and Oikari 1989, Söderström and Wachtmeister 1991). The introduction of ECF bleaching and activated sludge treatment of effluents, however, have reduced, indeed almost eliminated, these compounds in the mill effluents. This resulted in a remarkably lower exposure to CPs and RAs of fish downstream of the mills (Oikari and Holmbom 1996, Petänen *et al*. 1996, Karels *et al*. 1998, Soimasuo *et al*. 1998). In this study, total CPs in the bile of whitefish varied between 0.03 and 0.83 $\mu g\ ml^{-1}$ with a clear distance related correlation only downstream of mill A (Leppänen *et al*. 1998). The highest concentration was measured at site A2 (2.1 $\mu g\ ml^{-1}$). In contrary to bile CPs, bile concentrations of RAs and FAs did not correlate with the effluent tracers (Leppänen *et al*. 1998). Bile CPs in this study were 99.6% lower than those recorded in 1991, i.e. before introduction of the ECF bleaching processes and the secondary treatment at mill A, they still showed, however, a moderate dependence on the distance to the mill (Soimasuo *et al*. 1995, Leppänen *et al*. 1998).

Liver EROD activity, bile CPs, the lake water sodium and conductivity in area A correlated stronger with one another in 1991 than in 1996 (Fig. 4). This was probably caused by the more distinct gradient of EROD activities and bile CPs concentrations in fish downstream of the mill in 1991. EROD activity and bile CPs were, respectively, 2–7 and 160–500 times higher in 1991 than in 1996. Also the use of a non-parametric test in combination with a relatively low number of sampling sites ($n = 7$) gives a stronger correlation in 1991. PROD activity in 1996 also correlated well with the other parameters, respectively with, sodium: $r = 0.69$, $p < 0.01$; conductivity: $r = 0.46$, $p < 0.05$; EROD: $r = 0.61$, $p < 0.01$; bile CPs, $r = 0.59$, $p < 0.05$. Despite the significance of most correlations, a linear relationship seemed to be a poor model for bile CPs concentrations in 1991. An exponential relationship with bile CPs and the other parameters appears to fit better than a lineair relationship (Fig. 3).

In conclusion, the significant positive correlation of the liver MO activity and the bile CPs with the effluent tracers indicate that the liver MO induction and the accumulation of CPs in the fish bile were related to the mill effluents discharged into the lake. Dilution of the effluents at the study sites therefore most likely determined the level of MO induction and accumulation of CPs in the exposed fish.

Immunological and other physiological responses

Several environmental factors have been found to affect the immune system of fish, including pulp and paper mill effluents (Jokinen *et al.* 1995). In the present study, no effects on plasma IgM were seen in fish exposed to pulp and paper mill effluents. Earlier, in 1991, decreased plasma IgM levels were observed in whitefish similarly exposed to effluent of mill A (Soimasuo *et al.* 1995). However, the recovery of apparent immunodeficiency was abolished within one year (i.e. 1993) after the installation of the secondary treatment system (Petänen *et al.* 1996).

As in 1995 (Soimasuo *et al.* 1998), plasma glucose and lactate concentrations were significantly changed in several sites in the pulp and paper mill subareas. However, similar changes were also found at some sites in the reference areas, implying pollution-unrelated causes e.g. wa-

ter temperature. This suggests that the changed concentrations found in the subareas B and C were not related to pulp mill effluent. On the average, blood glucose or lactate and Hb or Ht indicated minor or no effects on carbohydrate metabolism and oxygen transport capacity in fish exposed to modern ECF type of pulp mill effluents. Overall, the situation for these parameters is similar and has stabilized since 1993 for the following years until 1996.

Conclusions

The results of this study are comparable with, and confirm the results of a related caging experiment in 1995. Higher liver MO activities were measured downstream of the mills. The general physiological and immunological parameters measured showed minor or negligible differences among the study sites. When compared with the situation before the modernization of the mills, the actual exposure of fish to pulp mill effluents, considering liver MO activity and bile conjugates as indicators, was low in the present study. However, liver MO activities and bile CPs in fish downstream of the mills were still 1.5–3 fold of those in the reference fish, indicating a decreased but still continued exposure to pulp mill effluent compounds. As a new tool to to monitor the ecotoxicological status of a lake, a kriging interpolation of fish biomarker data was utilized.

Acknowledgments: The authors wish to thank the crew of R.V. *Muikku*, Jarmo Lappivaara, Harri Leppänen and Jarno Mikkonen for their assistance in the field work, Dr. Ilmari Jokinen and Tuula Aaltonen for conducting the IgM assays, Anni Kekki for assistance with the EROD/PROD measurements, and the Saimaa Water Protection Association Inc., Lappeenranta, for measurement of the general water quality parameters. This work was supported by a grant from the Academy of Finland/The Research Council for the Environment and Natural Resources, and additionally financed by the UPM Kymmene Inc. Kaukas and Joutseno Pulp and the Stora-Enso Group, Imatra.

References

Aaltonen T.M., Jokinen E.I. & Valtonen E.T. 1994. Antibody synthesis in roach (*Rutilus rutilus*); Analysis of antibody secreting cells in lymphoid organs with ELISPOT-assay. *Fish Shellfish Immunol.* 4: 129–141.

Burke M.D., Thompson S., Elcombe C.R., Halpert J., Haaparanta T. & Mayer R.T. 1985. Ethoxy-, pentoxy and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Pharmacol.* 34: 3337–3345.

Cressie N.A.C. 1993. *Statistics for spatial data*. Rev. ed. Wiley, New York.

Hodson P.V. 1996. Mixed function oxygenase induction by pulp mill effluents — advances since 1991. In: Servos M.R., Munkittrick K.R., Carey J.H. & van der Kraak G.J. (eds.), *Environmental fate and effects of pulp and paper mill effluents*, St. Lucie press, Delray Beach, Florida, pp. 349–358.

Jokinen E.I., Aaltonen T.M. & Valtonen E.T. 1995. Subchronic effects of pulp and paper mill effluents on the antibody responses of roach (*Rutilus rutilus*). *Ecotoxicol. Environ. Saf.* 32: 219–225.

Kaplin C., Hemming J. & Holmbom B. 1997. Improved water quality by process renewal in a pulp and paper mill. *Boreal Env. Res*. 2: 239–246.

Karels A.E., Soimasuo M., Lappivaara J., Leppänen H., Aaltonen T., Mellanen P. & Oikari A.O.J. 1998. Effects of ECF bleached kraft mill effluent on reproductive steroids and liver MFO activity in populations of perch and roach. *Ecotoxicology* 7: 123–132.

Kloepper-Sams P.J. 1996. Field and laboratory studies of biochemical responses associated with pulp mill effluents: Status in 1991, 1994 and beyond. In: Servos M.R., Munkittrick K.R., Carey J.H. & van der Kraak G.J. (eds.), *Environmental fate and effects of pulp and paper mill effluents*, St. Lucie press, Delray Beach, Florida, pp. 439–445.

Leppänen H., Marttinen S. & Oikari A. 1998. The use of fish metabolite analyses as exposure biomarkers to pulp and paper mill effluents. *Chemosphere* 36: 2621–2634.

Lindström-Seppä P. & Oikari A. 1989. Biotransformation and other physiological responses in whitefish caged in a lake receiving pulp and paper mill effluents. *Ecotoxicol. Environ. Saf.* 18: 191–203.

Lindström-Seppä P. & Oikari A. 1990. Biotransformation activities of feral fish in waters receiving bleached pulp mill effluents. *Environ. Toxicol. Chem.* 9: 1415–1424.

Lowry O.H., Rosebrough N.J., Farr A.L. & Randall R.J. 1951. Protein measurements with Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.

Munkittrick K.R., Van Der Kraak G.J., McMaster M.E., Portt C.B., van den Heuvel M.R. & Servos M.R. 1994. Survey of receiving-water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. *Environ. Toxicol. Chem.* 13: 1098–1101.

Oikari A. & Kunnamo-Ojala T. 1987. Tracing of xenobiotic contamination in water with the aid of fish bile metabolites: A field study with caged rainbow trout (*Salmo gairdneri*). *Aquat. Toxicol.* 9: 327–341.

Oikari A. & Sillanpää A. 1993. Density stress in whitefish

and harmonization of fish caging technique for field experiments. In: Nikinmaa M. (ed.), *Biochemistry and physiology of environmental adaptions in fish*: 230. University of Helsinki, Finland.

Oikari A. & Holmbom B. 1996. Ecotoxicological effects of process changes implemented in a pulp and paper mill: A Nordic case study. In: Servos M.R., Munkittrick K.R., Carey J.H. & van der Kraak G.J. (eds.), *Environmental fate and effects of pulp and paper mill effluents*, St. Lucie press, Delray Beach, Florida, pp. 613–625.

Owens J.W. 1991. The hazard assessment of pulp and paper effluents in the aquatic environment: A Review. *Environ. Toxicol. Chem.* 10: 1511–1540.

Petänen T., Soimasuo R. & Oikari A. 1996. Use of fish biomarkers to asses the recovery of a lake ecosystem receiving pulp and paper mill effluents. *Paperi ja Puu — Paper and Timber* 78: 299–304.

Sandström O. 1996. *In situ* assesments of the impact of pulp mill effluent on life-history variables in fish. In: Servos M.R., Munkittrick K.R., Carey J.H. & van der Kraak G.J. (eds.), *Environmental fate and effects of pulp and paper mill effluents*, St. Lucie press, Delray Beach, Florida, pp. 449–457.

Soimasuo R., Jokinen I., Kukkonen J., Petänen T., Ristola T. & Oikari A. 1995. Biomarker responses along a pollution gradient: Effects of pulp and paper mill effluents on caged whitefish. *Aquat. Toxicol.* 31: 329–345.

Soimasuo M.R., Karels A.E., Leppänen H., Santti R. & Oikari A.O.J. 1998. Biomarker responses in whitefish (*Coregonus lavaretus* L. *s.l.*) experimentally exposed in a large lake receiving effluents from pulp and paper industry. *Arch. Environ. Contam. Toxicol.* 34: 69–80.

Stegeman J.J. & Hahn M.E. 1994. Biochemistry and molecular biology of monooxygenases: Current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. In: Malins D.C. & Ostrander G.K. (eds.), *Aquatic toxicology, molecular, biochemical and cellular perspectives*, Boca Raton, US: Lewis Publishers, Boca Raton, US, pp. 87–206.

Suutari R., Johansson M. & Tarviainen T. 1999. Aineistojen alueellistaminen kriging-menetelmällä ympäristömallintamisessa. Suomen Ympäristö 268. Suomen Ympäristökeskus.

Söderström M. & Wachtmeister C.A. 1991. Fish bile as a monitoring instrument for phenolics and other substances related to bleached kraft mill effluent and a study of chlorophenolic metabolic transformation products in a periphyton community. In: Södergren A. (ed.), *Environmental fate and effects of bleached pulp mill effluents*, Swedish EPA Report 4031: 203–206.

Received 29 June 1999, accepted 19 November 1999