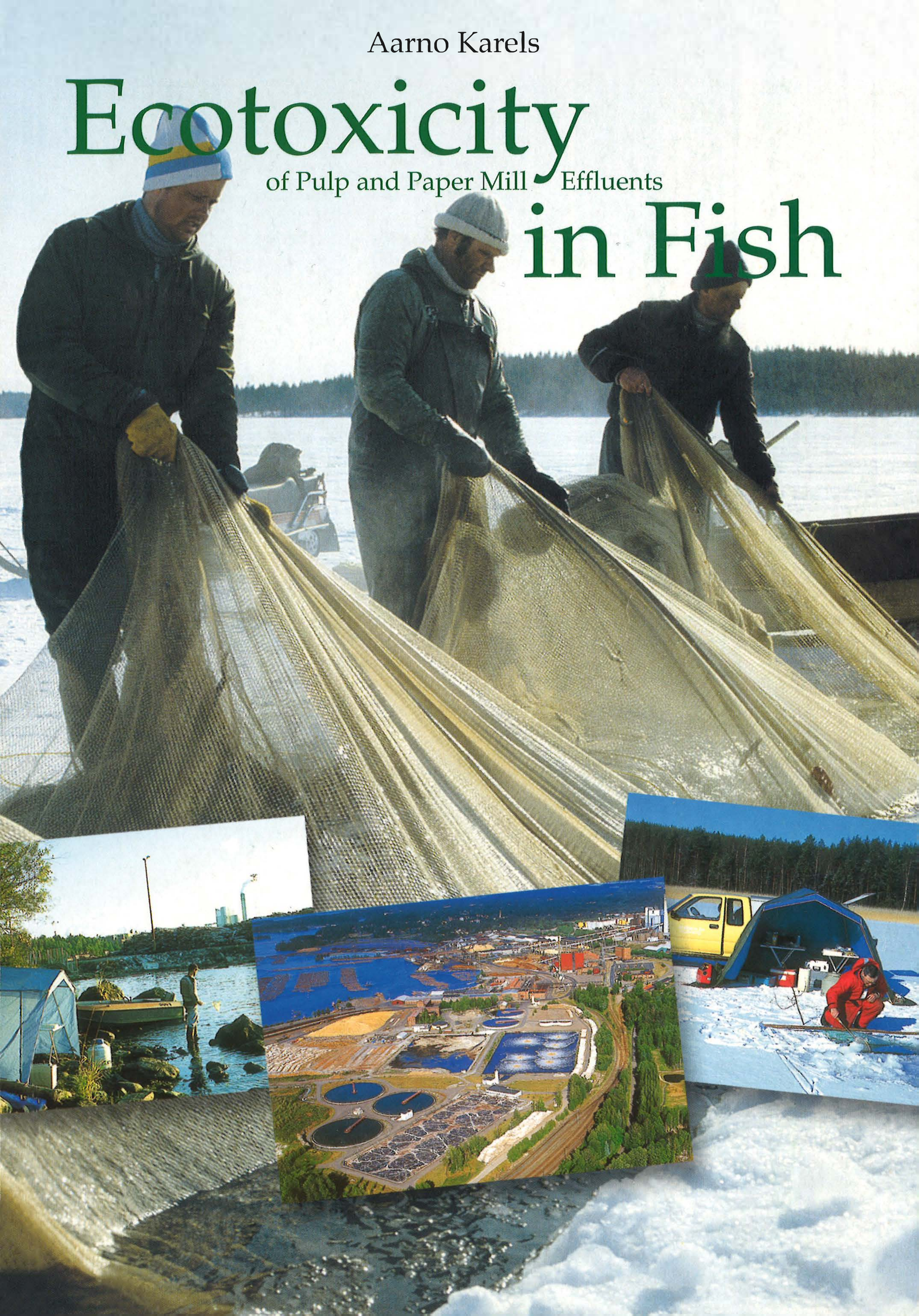
Aarno Karels

Ecotoxicity of Pulp and Paper Mill Effluents in Fish



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Aarno Karels

Ecotoxicity of Pulp and Paper Mill Effluents in Fish

Responses at Biochemical, Individual, Population and Community Levels

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UNIVERSITY OF JYVÄSKYLÄ

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ABSTRACT

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Ecotoxicity of pulp and paper mill effluents in fish. Responses at biochemical, individual, population and community levels.
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Feral perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) populations, the fish community and experimentally exposed juvenile whitefish (*Coregonus lavaretus* L. s.l.) were studied in the recipient areas of three pulp and paper mills and at reference areas in the Southern Lake Saimaa, Finland. The mills used elemental chlorine free (ECF) bleaching and activated sludge effluent treatment technologies. The exposure of feral and caged fish to pulp mill effluents, as measured by concentrations of chlorophenolics in the bile and liver EROD activity, was low and almost the same as the reference levels. Nevertheless, resin acid concentrations in the bile of fish near one of the mills were 10-90 times higher compared to the reference points. Reproductive steroid hormones, measured at different reproductive stages, showed that plasma estradiol-17ß and testosterone concentrations were significantly lower in exposed perch and roach during periods of gonadal development. This coincided with a lower gonad size and fecundity in female perch. The gonad size in male perch, as well as the gonad size, egg size, fecundity and plasma and liver cytosol vitellogenin (VTG) in roach, however, remained unchanged. A higher liver size in exposed perch and roach suggests alterations in the metabolic and nutritional status of the fish. However, the body condition and hematological and immunological parameters of exposed fish were not affected. The perch population in the recipient of one of the mills exhibited an abnormal size and age distribution. Spawning behavior, growth and age at maturity of perch and roach was similar between mill and reference areas. The fish communities in the different study areas in the Southern Lake Saimaa were dominated (> 60%) by perch and roach. Biomass and fish densities were highest in the polluted area (5-15 km from the mills) and lowest in the reference area and close (2-5 km) to the mills. The number of species was similar among the areas. Species like bleak and ruffe were typical to the polluted area, while vendace, whitefish and minnow seemed to avoid polluted waters. The results indicate that, despite decreased exposure of fish to pulp mill effluents during the 1990s, the reproductive status, measured by serum steroid hormone levels, gonad size and fecundity, was affected in perch, and to a lesser extent in roach, living in the recipients of the mills. Although there are clear signs of recovery, it is evident that the fish communities and populations in the Southern Lake Saimaa still vary in relation to loading by pulp and paper mill effluents.

Key words: Biomarkers; fish; population; community; pulp and paper mill effluents; reproduction.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals.

I 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.

II Karels, A.E. & Oikari, A.O.J. 2000: Effects of pulp and paper mill effluents on the reproductive and physiological status of perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) during the spawning period. Annales Zoologici Fennici 37: 65-77.

III Karels, A.E., Markkula, E. & Oikari, A.O.J. 2000: Reproductive responses, bile metabolites and liver EROD activity in prespawning perch and roach exposed to pulp and paper mill effluents. Environmental Toxicology and Chemistry (submitted).

IV Karels, A., Soimasuo, M. & Oikari, A. 2000: Biomarker responses in experimentally exposed fish and feral fish in a lake receiving pulp mill effluents. In: Stuthridge, T.R., Marvin, N.M., Slade, A.H. & Gifford, J.S. (eds). Aquatic Impacts of Pulp and Paper Effluents. Proceedings of the 3rd International Conference on Environmental Fate and Effects of Pulp and Paper Mill Effluents. SETAC press, Lewis Publishers, Boca Raton (in press).

V Karels, A., Soimasuo, M., Suutari, R. & Oikari, A. 2000: Monitoring the recovery of a lake with aid of fish biomarkers: Responses of whitefish (*Coregonus lavaretus* L. s.l.) experimentally exposed in a large lake receiving pulp and paper mill effluents. Boreal Environment Research 5 (1): 53-65.

VI Karels, A. & Niemi, A. 2000: Fish community responses to pulp and paper mill effluents at the Southern Lake Saimaa, Finland. Environmental Pollution (submitted).

Additionally the thesis includes original data on research unpublished:

- Karels, A.E., unpublished. Population characteristics of perch and roach exposed to pulp and paper mill effluents in the Southern Lake Saimaa, Finland.

- Karels, A.E., Jokinen, I., Leppänen, H., Paranko, J., Soimasuo, M. & Oikari, A., unpublished. Sex-specific steroid hormone and vitellogenin levels in juvenile whitefish experimentally exposed to pulp and paper mill effluents.

Responsibilities of Aarno Karels in the articles of this thesis

Paper I. The study was planned together with Aimo Oikari. I conducted the field work alone. The biochemical analyses were mainly carried out by myself, with support from Markus Soimasuo and Jarmo Lappivaara, GC with Harri Leppänen; IgM was analyzed by Tuula Aaltonen and VTG gene expression by Pirkko Mellanen. I analyzed the data and wrote the draft of the article, which was then completed with Aimo Oikari.

Paper II. The study was planned together with Aimo Oikari. I coordinated and conducted the field work. Fish were caught with the help of the personnel of the Southern Karelia Fisheries Centre, Lappeenranta. I coordinated the laboratory analysis and conducted the analysis by myself and with support from some colleagues and technicians. I analyzed the data and wrote the draft of the article, which was then completed together with Aimo Oikari.

Paper III. The study was planned by myself. I coordinated and conducted the field work. Fish were caught with the help of the personnel of the Southern Karelia Fisheries Centre, Lappeenranta. I coordinated the laboratory analysis and conducted the analysis by myself and with support from technicians. Eveliina Markkula analyzed VTG and IgM. I analyzed the data and wrote the draft of the article, which we then completed together.

Paper IV. The studies were planned together with Aimo Oikari. I coordinated and conducted the field sampling of feral fish and took part in the field and laboratory work of the study with experimentally exposed fish. I analyzed the data and wrote the draft of the article, which we then completed together.

Paper V. The study was planned by Aimo Oikari and I organized the field and laboratory work together with Markus Soimasuo. Riku Suutari conducted the Kriging interpolations. I wrote the draft of the article, which we then completed together.

Paper VI. The study was initiated by Aimo Oikari and planned together with Asko Niemi. The field surveys were conducted with the help of the personnel of the Rural Business District of Kymi, Fisheries Unit, Kouvola. I analyzed the data and wrote the draft of the article, which we then completed together.

Jyväskylä, March 15, 2000

Aarno Karels

Abbreviations

ANOVA	analysis of variance
ANCOVA	analysis of covariance
AOX	adsorbable organic halogen
BKME	bleached kraft mill effluent
BOD	biological oxygen demand
CF	condition factor
COD	chemical oxygen demand
CP	chlorophenol
CPUE	catch per unit of effort
CYP 1A	cytochrome P450 1A
E_2	17ß-estradiol
ECF	elemental chlorine free
ELISA	enzyme-linked immunoassay
EROD	7-ethoxyresorufin *O*-deethylase
FA	fatty acid
GC-MS	gas chromatography - mass spectrometry
GSI	gonadosomatic index
Hb	hemoglobin
Hct	hematocrit
i.p.	intra peritoneal
IgM	immunoglobulin M
LSI	liver somatic index
MFO	mixed function oxygenase
MO	mono-oxygenase
mRNA	messenger RNA
NADPH	nicotinamide adenine dinucleotide phosphate, reduced form
PAH	polycyclic aromatic hydrocarbon
PROD	pentoxyresorufin *O*-dealkylase
RA	resin acid
SS	suspended solids
T	testosterone
TCF	total chlorine free
VTG	vitellogenin

1 INTRODUCTION

1.1 Ecotoxicity and environmental pollution

The technical development and increased productivity of industries during the 20th century have contributed immensely to the standard of living in most of Western Europe and North America. On the other hand, increased consumption in our developing world has led to a concomitant increase in industrial wastes. It is estimated that more than 100,000 commercial chemical compounds are in use, of which 2,000 are high production volume chemicals (HPVCs; van Leeuwen 1993). However, for the majority of these HPVCs adequate information on ecotoxicological effects is not available. Each year, in addition several hundred new chemicals are introduced. The final sink of many of these chemicals is the aquatic environment, and it is there that the first harmful effects of these compounds are likely to be found. To assess the ecotoxicity of xenobiotic compounds, knowledge about the fate, exposure and effects in the environment is needed, this being the main objective of ecotoxicology (Truhaut 1977). Ecotoxicology as a science covers a broad interdisciplinary field, essentially based on toxicology, environmental chemistry and ecology (Koeman 1984).

In Finland, aquatic environmental problems are generally of a local scale. The major activity affecting Finnish inland and coastal aquatic systems is the pulp and paper industry. In 1998, the Finnish pulp and paper industry produced 6.7 billion tons of cellulose and 12.7 million tons of cardboard and paper, while discharging 21.060 t/y of suspended solids, 19.070 t/y BOD, 217.080 t/y COD and 0.20 kg/ton AOX into recipient waters (Forest industry yearbook 1998). The introduction of elemental chlorine free (ECF) bleaching and modern activated sludge wastewater treatment technologies during the early 1990s, however, have reduced the acute toxicity of effluents. Nevertheless, in the same period several studies, mainly in North America and Sweden, reported effects on fish populations and fish reproduction in waters polluted by pulp mill effluents (McMaster et al. 1991, 1992; Munkittrick et al. 1991, 1992, 1994; Adams et al. 1992; Hakkari 1992; Sandström 1996; Van Der Kraak et al. 1998), however, conclusive cause-effect relationships were not established.

Causal relationships between contaminants and biological responses can be identified using integrated studies at different biological levels (e.g., biochemical, individual, population, community; Adams 1992). Integrated studies also identify where the main effects of contaminants operate on fish populations. In Finnish inland waters polluted by pulp mill effluents, however, an integrated study at different monitoring levels in fish has thus far not been established. Thus, the main aim of this study was to assess the exposure and health of several fish species exposed to modern pulp mill effluents and to integrate responses at biochemical, individual, population and community levels.

1.2 Aquatic pollution by pulp and paper mill effluents

Pulp and paper mill effluents are complex mixtures of inorganic and high and low molecular weight organic compounds, including natural wood compounds, as well as compounds formed during the pulping and bleaching process (LaFleur 1996). The chemical composition of the effluent is dependent on many factors including the wood species processed, cooking, washing, and bleaching technologies, as well as final effluent treatment.

Natural wood composition
Wood consists of cellulose (40-55%), hemicellulose (15-30%), lignin (15-30%) and wood extractives (0.2-7.0%). The major wood extractives are monoterpenes, diterpene resin acids, fatty acids and plant sterols (LaFleur 1996; Sjöström 1993).

Compounds formed in pulping
The main objective of kraft pulping is to remove the bulk of lignin while minimizing the degradation of cellulose. The chemicals formed from the reaction of lignin with the pulping liquors are generally polar compounds (LaFleur 1996). These chemicals often contain ionizable functional groups, which aid in their dissolution in the highly caustic cooking liquor. The compounds include simple phenols, aromatic and aliphatic carboxylic acids and diacids, and reduced sulfur compounds. After the washing process, most of these compounds have been removed, while some are carried-over.

Compounds formed in bleaching
Products formed in bleaching can result from reaction of bleaching chemicals with the residual lignin, with cellulose or with chemicals carried over from the pulping process (LaFleur 1996). In the early 1990s the most widely used bleaching chemicals were elemental chlorine and, somewhat later, chlorine dioxide, which leads to the formation of numerous chlorinated organic by-products. Unlike chlorine bleaching, the formation of multi-chlorinated

compounds in chlorine dioxide bleaching is much lower. During the 1990s, however, the use of total chlorine free (TCF) bleaching, with chelating agents such as ethylenediaminetetraacetic acid (EDTA), has increased in the Scandinavian pulp and paper industry (Strömberg et al. 1996). While there exists more extensive knowledge about the chemistry of ECF bleaching effluents, the chemistry of TCF bleaching effluents is less characterized (Bright et al. 2000).

1.3 Toxicity of pulp and paper mill effluents to fish

Lethal and sublethal effects

The toxicity of pulp and paper mill effluent, being a mixture of chemical compounds, is dependent on the pulping, bleaching and effluent treatment processes used. Exposure to pulp and paper mill effluents is known to cause a variety of toxic responses in fish, including biochemical and physiological responses (Owens 1991; Sandström 1996). Up until the 1970s and even the 1980s, conventional pulp and paper mill effluents still exhibited acute lethal toxicity to fish (McLeay et al. 1986). Resin and fatty acids as well as chlorophenolic compounds, were the major compounds responsible for the toxicity during this period (Owens 1991).

With the introduction of elemental and total chlorine free (ECF or TCF) bleaching and activated sludge effluent treatment processes during the last decade, the toxicity of effluents was drastically reduced and acute lethal effects were no longer observed (Priha 1996; Verta et al. 1996). Exposure to current effluents, however, is known to cause biochemical and reproductive responses in fish (Sandström 1996; Soimasuo 1997; Van der Kraak et al. 1998), which can lead to population changes.

Reproductive effects

Exposure to pulp and paper mill effluents can lead to alterations in endocrine and reproductive functions in fish (Sandström 1996; Van der Kraak et al. 1998). Reproductive responses of exposed fish included decreased steroid hormone levels, reduced gonad size and fecundity, delayed sexual maturation and reduced expression of sexual characteristics (Lindström-Seppä & Oikari 1989a; Munkittrick et al. 1991, 1992, 1994; McMaster et al. 1991, 1992; Gagnon et al. 1994; Sandström 1996; Van Der Kraak et al. 1998). The effects of pulp and paper mill effluents on steroid hormone levels and other reproductive parameters varies between mills based on differences in pulping, bleaching and effluent treatment methods (Munkittrick et al. 1994; Van der Kraak et al. 1998). However, the compounds responsible for the reproductive effects have not yet been identified. At first it was suspected that persistent organochlorines caused these reproductive effects, but now it seems that wood derived compounds such as sterols, lignans, stilbenes and resin acids are responsible for the observed effects (Mellanen et al. 1996; Van der Kraak et al. 1998; Lehtinen et al. 1999). However, some studies have not shown any critical reproductive effects in feral fish exposed to pulp and paper mill effluents (Hodson et al. 1992; Kloepper-Sams et al. 1994; Landner et al. 1994; Swanson et al. 1994).

1.4 Fish biomonitoring

1.4.1 Fish as biomonitors of aquatic pollution

The suitability of a habitat for fish is determined by a large number of biotic and abiotic factors. The physical and chemical quality of water and sediment cannot be used exclusively to determine the acceptability of the environment as a habitat. It is therefore necessary to monitor the biological components (e.g. fish, benthos, periphyton) of aquatic ecosystems to ensure that there have been no adverse changes. Fish species represent a variety of trophic levels and are sensitive to a variety of direct and indirect stressors (Munkittrick & Dixon 1989a,b; Gibbons & Munkittrick 1994). Some species are relatively long-lived and therefore good indicators of long-term effects. Furthermore, fish are also recognized for their socioeconomic and recreational value and fish are relatively easy to collect and identify to species. As a consequence, fish have been, and continue to be, regularly used for evaluating environmental impacts on aquatic systems.

1.4.2 Monitoring levels

Effects of aquatic pollution can be monitored from lower levels of biological organization (bottom-up or reductionistic approach) to higher levels (top-down or holistic approach). Effects of pollutants at lower levels (e.g. molecular, biochemical and physiological alterations) are usually the first detectable responses to environmental changes and usually precede effects at higher levels of organization (Andersson et al. 1988; Adams 1992). Effects of pollutants at higher levels (individual, population and community alterations) usually tend to be manifest only after longer periods of time. The advantage of the top-down approach is that responses are more likely to be ecologically significant, the weakness is that it provides little diagnostic and preventive information.

The advantage of the bottom-up approach is that it allows for rapid detection of responses, although alterations are not necessarily indicative of significant changes at the individual, population or community level. Bottom-up monitoring is also insensitive to habitat structure changes associated with industrial activities.

Nevertheless, the use of 'early warning' signals, or biomarkers, serving as indicators of exposure or the effect of chemicals, can demonstrate that toxicants have entered organisms and are exhibiting toxic effects (McCarthy and Shugart 1990). Biomarkers are measurable in body fluids, cells or tissues, indicating biochemical or cellular modifications due to the presence and magnitude of toxicants, or host response (NRC 1987). The term 'biomarker' can be used in a broad sense to include almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical or biological (WHO 1993). A biomarker may also be defined as a change in a biological response (ranging from molecular through cellular and physiological responses to behavioural changes) which can be related to exposure to or the toxic effects of environmental chemicals (Peakall 1994). In the next paragraph, a short introduction to the fish biomarkers used in this study will be given.

1.5 Fish biomarkers

1.5.1 Biotransformation enzymes

Biotransformation can be defined as an enzyme-catalyzed conversion of a xenobiotic compound into its metabolite, generally a more water-soluble form, which can be excreted from the body more easily than the parent compound (Lech & Vodicnik 1985). Alterations in levels and activities of biotransformation enzymes are in general the most sensitive biomarkers. In fish, the activity of these enzymes may be induced or inhibited upon exposure to xenobiotics. While investigating the impact of pulp mill effluents on fish, biotransformation systems like the liver mono-oxygenase (MO), also known as the mixed function oxygenase (MFO) system, has proved to be one of the most consistent biomarkers of exposure (Owens 1991; Sandström 1996). The activity of the liver MO system is often measured as 7-ethoxyresorufin O-deethylase (EROD) activity. Measurement of EROD activity constitutes a common method of examining the catalytic activity of cytochrome P450 1A (CYP 1A), an important MO isoenzyme catalyzing phase 1 biotransformation reactions of xenobiotic compounds. Inducers of CYP 1A isolated from ECF bleached pulp mill effluents exhibit properties of PAHs, but their full identity has remained unclear (Hodson 1996). Tentative candidates, however, are retene and other related microbial PAH-type metabolites derived from resin acids, which are present in and bioavailable from sediments contaminated by pulp and paper mill effluents (Billiard 1999; Leppänen & Oikari 1999).

1.5.2 Biotransformation products

The exposure of an organism to xenobiotic compounds which are rapidly degraded in general cannot be assessed by simply measuring their tissue levels. In such instances the measurement of metabolites may provide evidence of exposure to these chemicals. The metabolites of xenobiotic chemicals may accumulate to high levels in certain tissues or body fluids or become bound to specific macromolecules in a manner that facilitates detection of exposure and indicates potential harm to the organism (Melancon et al. 1992). Previous studies have shown e.g. that fish bile metabolites of chlorophenolics and resin acids serve as the most sensitive biomarkers of exposure to pulp and paper mill effluents (Oikari & Ånäs 1985; Lindström-Seppä & Oikari 1989b; Söderström & Wachtmeister 1991).

1.5.3 Reproductive parameters

Xenobiotics can directly or indirectly affect all aspects of fish reproduction, from gonadal development through to spawning. Pollutants can have a direct effect on reproduction by interacting directly with the gonad cells, hatching and the number of viable larvae and having an indirect effect by disturbing the reproductive endocrine system (Kime 1998). Possible target organs are the hypothalamus, the pituitary, the gonads and the liver. Altered steroid hormone levels in fish may result from changes in hypothalamic gonadotrope releasing (GnRH) or pituitary gonadotrope hormone (GTH) secretion, altered activity of any of the enzymes in steroidogenesis, or altered hepatic catabolism and excretion (Thomas 1990).

The fish ovary undergoes a seasonal reproductive cycle which may be divided into four main phases: (1) Vitellogenesis, the major growth phase of the ovary during which ovarian secretion of estradiol-17ß (E2) stimulates hepatic synthesis of the egg yolk precursor protein vitellogenin (VTG), which is transported in the blood to the ovary and incorporated into the developing oocyte. (2) Oocyte maturation, during which the germinal vesicle migrates to the perifery of the oocyte and breaks down under control of pituitary gonadotrophins and ovarian progestogens. (3) Ovulation and spawning. (4) Postspawning in which the gonads regress in preparation for the next reproductive cycle (Matty 1985; Kime 1998). Commonly used gauges of pollutants effects on reproduction are the gonadosomatic index (GSI), oocyte stage, egg size and fecundity, or for examination of the specific mechanisms involved, plasma levels of VTG and sex steroid hormones (Kime 1998). Although male fish have less well defined stages of maturation and the hormonal regulation is less clear than it is in females, the GSI, and histological studies of the stage in spermatogenesis provide useful information.

1.5.4 Hematological parameters

Hematological parameters are often non-specific in their responses to chemical stressors, but can provide an indication of the general physiology and health status of the organism. Utilization of these biomarkers is optimized when the entire body energy reserves are also evaluated (Mayer et al. 1992, Van der Oost 1997). The blood is the major system for transporting energy-related biomolecules between storage and utilization sites in fish. The hematological parameters used in this study, hemoglobin (Hb), hematocrit (Hct), plasma glucose and lactate are generally less specific than serum enzymes. However, they may still be usefull as biomarkers of toxicant effects (Van der Oost 1997). Plasma glucose or lactate and Hb or Hct may indicate possible effects on carbohydrate metabolism and oxygen transport capacity in fish.

1.5.5 Immunological parameters

Nonspecific and specific immunological parameters like humoral antibody assays have been used in various laboratory and field experiments to analyze effects of toxicants on the immune response and disease resistance of invertebrates (Weeks et al. 1992). The major role of the antibodies is to protect the host from infectious diseases. Although the immunology of fish species is less well characterized than that of mammals, immune system biomarkers in fish are considered to have considerable potential for application in pollution biomonitoring (Wester et al 1994; Van der Oost 1997; Soimasuo 1997). Humorally mediated immunity can be assessed in fish by quantifying levels of circulating antibodies in fish like, for instance, plasma immunoglobulin M (IgM; Aaltonen et al. 1994; Jokinen et al. 1995).

1.5.6 Gross indices

Gross indices used in the present study are the condition factor (CF), the liver somatic index (LSI) and the GSI. The CF and LSI provide information on energy allocation to storage and reflect the nutritional status and health of the fish, while the GSI provides information on the reproductive status. Changes in CF and LSI have also been associated with exposure to contaminants (Hodson 1992; Mayer et al. 1992; Sandström 1996). The CF may be affected if food assimilation is limited or if food consumption is impaired due to stressors or by persistent osmoregulatory disturbances. An increased LSI can be associated with elaboration of cellular structures such as the endoplasmatic reticulum for protein synthesis (Andersson et al. 1988), including induction of the MO system, or with a high carbohydrate diet resulting in carbohydrate storage (Dixon & Hilton 1985). Although measurements of CF and LSI are crude and prone to the effects of non-pollutant factors (e.g., season, disease, biological rhythms), they seem to serve as a first tier screen indicative of effect (Mayer et al. 1992).

1.6 Fish population and community parameters

Methods for assessing the health status of the environment with the use of fish populations and communities are described, among others, by Colby (1984), Munkittrick & Dixon (1989a,b) and Gibbons & Munkittrick (1994). Colby (1984) proposed that the response of fish to environmental stressors was distinct and predictable, and could be classified and used to define the status of the fish population. Responses to stressors were characterized by comparing fisheries data (e.g., mean age, size at age, age at maturity, growth rate, condition, gonad size, fecundity, etc.) to data from previous samplings or data from reference sites. Colby (1984) classified the responses of fish to environmental stressors into five generalized response patterns: characteristics of exploitation, recruitment failure, multiple stressors, food limitation and niche shift.

Munkittrick & Dixon (1989a,b) revised Colby's original work and incorporated three other response patterns: metabolic redistribution, chronic recruitment failure and no response. Gibbons & Munkittrick (1994) further reorganized this monitoring framework.

In general, measurements from individual fish can be used to assess the response of fish populations to environmental stressors. From standard measurements like length, weight, age, sex, liver and gonad weights, egg size and fecundity it is possible to calculate population characteristics such as growth, reproductive investment (gonad weight, fecundity, age at maturity), age structure (age distribution, mean age) and energy stores (condition and liver weight).

Assessing responses of the fish community to environmental stressors starts with assessing the characteristics and presence of fish populations. Parameters describing the fish community are species composition and diversity, species abundance and biomass.

2 OBJECTIVES

After the introduction of ECF bleaching and activated sludge treatment technologies at one of the mills at the Southern Lake Saimaa in 1992, the exposure of fish to pulp and paper mill effluent compounds was dramatically decreased (Oikari & Holmbom 1996; Soimasuo 1997). However, in the early 1990s several studies elsewhere in the world reported the effects of pulp mill effluents on fish reproduction at physiological levels. Reproductive and biochemical markers, nevertheless, are closely associated with many seasonal, site and population specific factors. We suggested that in order to ascertain possible effects of pulp mill effluents on fish reproduction and populations in the Southern Lake Saimaa, more information on natural levels and seasonal variations of these markers need to be collected and effects at higher levels of biological organization need to be studied. From 1995 to 1997 we therefore conducted a series of field studies on perch and roach populations and the fish community in the Southern Lake Saimaa. In order to study the pollution gradient in the lake and to combine the newer studies to earlier ones, studies were conducted with experimentally exposed whitefish.

The objectives of this study have been:

- To study the exposure, health and possible recovery of fish populations in the Southern Lake Saimaa, after the introduction of ECF bleaching and activated sludge treatment technologies at the pulp and paper mills discharging into the lake (I-VI).
- To study natural levels and seasonal variations of common reproductive and biochemical characteristics in feral perch and roach during different stages of the reproductive cycle (I-IV).
- To study the possible effects of modern ECF pulp and paper mill effluents on these reproductive and biochemical markers in feral perch, roach and experimentally exposed whitefish (I-V).
- To examine whether biomarker responses in whitefish experimentally exposed for one month by caging equal biomarker responses in exposed feral fish (IV).
- To integrate responses of fish to pulp and paper mill effluents at different biological levels and to identify potential causal relationships between responses and contaminants (I-VI).

3 MATERIAL AND METHODS

3.1 Study area

3.1.1 Description of the lake and study sites

Description of the lake

The study area, the southern part of Lake Saimaa - the Southern Lake Saimaa - is a large oligotrophic and oligo-mesohumic lake in South-East Finland (Fig. 1). Three pulp, paper and cardboard mill units, located in the cities of Lappeenranta (mill A), Joutseno (mill B) and Imatra (mill C), discharge their effluents into the lake. The Southern Lake Saimaa has a surface area of approximately 610 km^2, a water volume of 5.2 km^3 and a mean depth of 8.4 m. There are more islands in the western and eastern part than at other parts of the lake which are more open. Its shores are usually barren, stony, or sandy. Areas with aquatic vegetation are small and situated in sheltered bays. The main discharge of water into the lake is at Rastinvirta, being about 93% of the mean outflow through the River Vuoksi into Lake Ladoga (596 m^3/s). The estimated retention times of water in different subareas in the lake are relatively short. The retention time in the large middle part is about 76 days, in the western part, upstream of mill A, 32 days and in the area 0-16 km downstream of mill A, 57 days (Pertti Laine, personal communication).

As the treated municipal effluents of the cities of Lappeenranta and Joutseno are not discharged into the lake, the pulp and paper mills are the primary source of contamination (chemicals, nutrients and log-floating). An important hydrological factor in the western part of the lake is the pump station at Vehkataipale, pumping water from the clean area of the lake to the watercourse upstream of mill A, causing a net water flow in the study area of mill A from west to north-east. As a result, the lake water passes the outlet point of mill A with a flow of about 40 m^3/s, thus diluting the mill's effluent.

Study sites

The sampling sites for perch and roach were located 1-2 km and 6 km downstream of pulp and paper mill A and 1-2 km downstream of pulp mill B. The reference sites were located upstream of the mills and were considered not to be influenced by the pulp and paper mill effluents (Fig. 1 upper). In the field caging experiments with whitefish, in May-June 1996 and 1997, the study area included five subareas with a total of 21-22 different study sites (Fig. 1). For the purpose of the fish community study, the lake was divided in 3 subareas, a 'polluted' area (0.5-4.0% effluent), an 'intermediate' area (0.1-0.5%) and a 'clean' reference area (Fig. 1 lower). Fish were sampled by gill nets at sublittoral and profundal waters, and by electrofishing and beach seine at stony shores.

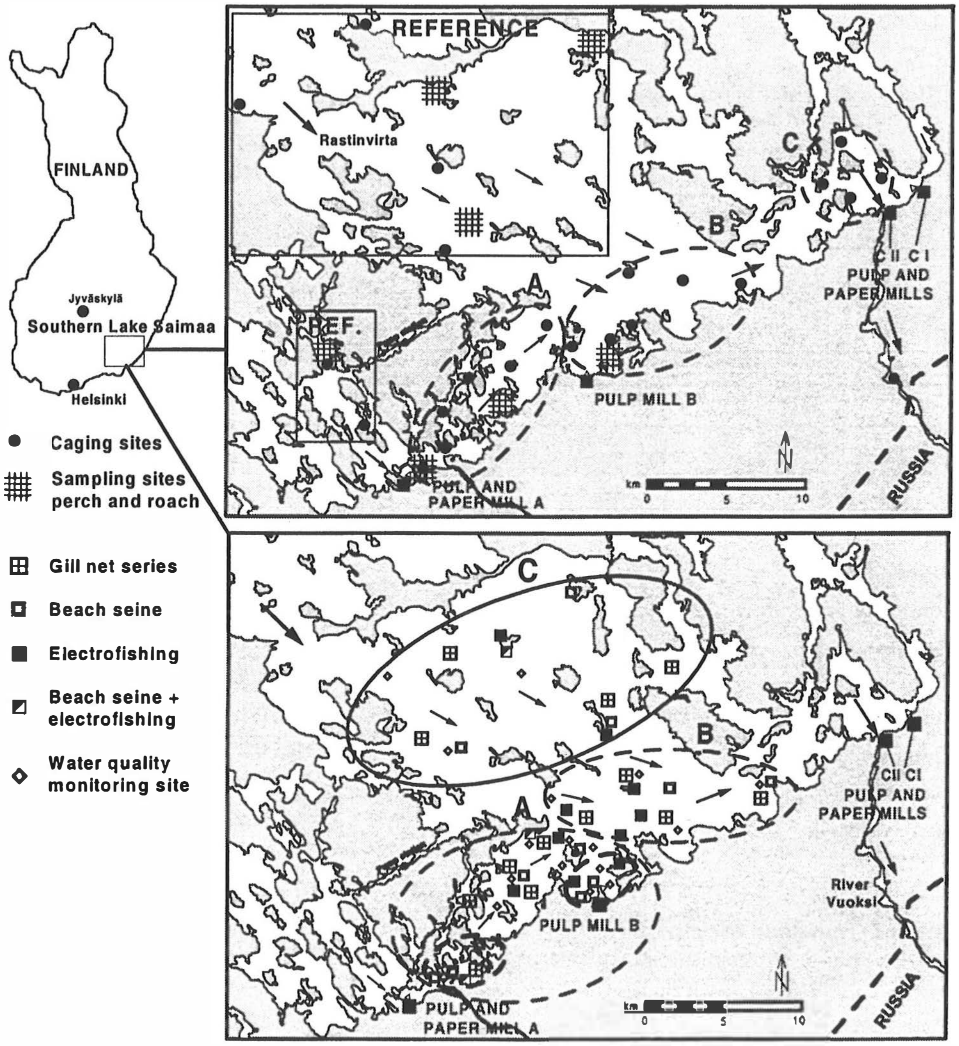

FIGURE 1 The study areas in the Southern Lake Saimaa, S-E Finland. Upper map: Sampling sites of the feral perch and roach studies (1995, 1996 and 1997) located upstream and downstream of mills A and B. The experimental caging sites of whitefish (1996-1997) located in the recipient subareas A, B and C and in reference areas. Lower map: Fish community study areas with sampling sites of gillnet, electrofishing and beach seine surveys and water quality sampling sites. For the purpose of the fish community survey the lake was divided into 3 subareas, area A (termed 'polluted'), B ('intermediate') and C ('clean' reference). Arrows indicate the direction of the water flow in the lake.

Lake water quality characteristics

Average lake water quality characteristics of May-June 1995, 1996 and 1997, during the spring overturn, at the fish community survey study areas are given in Table 1.

TABLE 1 Lake water quality characteristics in the fish community study areas (Fig. 1, lower map). Data are the ranges or average (± SEM) of 1995, 1996 and 1997. Water samples were collected during the spring overturn in May – June, and are means of water column values from 1 m to near bottom (data from the Saimaa Water Protection Association Inc., Lappeenranta).

	AREA			
	A		B	C
	Polluted		Intermediate	Reference
	0-5 km	*5-15 km*		
Na (mg l^{-1})	15 – 20	10 – 15	5 – 10	3 – 5
Estimated effluent %	3.0 – 4.0	0.5 – 3.0	0.1 – 0.5	<< 0.1
pH	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	6.9 ± 0.1
Oxygen (mg l^{-1})	11.2 ± 0.3	11.9 ± 0.2	12.4 ± 0.2	12.0 ± 0.4
Conductivity (mS m^{-1})	11.6 ± 1.2	9.1 ± 0.8	5.8 ± 0.2	5.8 ± 0.5
Secchi-dept (m)	2.2 ± 0.1	2.5 ± 0.2	3.6 ± 0.1	4.7 ± 0.4
Colour mg Pt l^{-1})	48.8 ± 4.4	46.0 ± 8.0	34.6 ± 3.9	31.9 ± 6.0
COD, Mn (mg l^{-1})	9.7 ± 0.3	8.6 ± 0.2	6.8 ± 0.1	6.7 ± 0.1
Chlorophyll (µg l^{-1})	7.3 ± 0.4	8.0 ± 0.6	3.5 ± 0.2	1.9 ± 0.1
Total P (µg l^{-1})	18.0 ± 1.2	15.3 ± 1.9	7.1 ± 0.7	7.5 ± 1.2
Total N (µg l^{-1})	523.5 ± 20.1	450.3 ± 19.5	415.9 ± 6.8	423.4 ± 10.0

TABLE 2 Total load of suspended solids (SS), BOD_7, COD_{Cr}, AOX, P and N of the pulp and paper mills in the study area in 1995, 1996 and 1997 (Forest industry yearbooks 1995, 1996 and 1997).

		SS t yr^{-1}	BOD_7 t yr^{-1}	COD_{Cr} t yr^{-1}	N t yr^{-1}	P t yr^{-1}	AOX kg t^{-1}
Mill A	1995	1 059	548	14 746	129	8	0.32
	1996	1 848	715	16 642	136	8	0.35
	1997	2 117	1643	20 696	190	7.5	0.20
Mill B	1995	558	1142	16 301	109	18	0.25
	1996	442	913	15 637	119	18.7	0.28
	1997	320	305	12 595	75	11.9	0.25
Mill C	1995	1 741	2026	22 182	232	11	0.47
	1996	2 665	2365	21 397	217	15	0.30
	1997	2 804	2355	22 985	213	19	0.30

Lake water characteristics during the different study periods on feral perch and roach and field caging experiments with whitefish are described in detail in the papers (I-V).

3.1.2 Pulp and paper mills

Mill characteristics

The three mills discharging into the lake are referred to as follows: a bleached kraft pulp and paper mill in Lappeenranta (mill A), a bleached kraft pulp mill in Joutseno (mill B), a bleached kraft pulp, paper and cardboard mill (mill CI) and an unbleached pulp and cardboard mill (mill CII), both in Imatra (Fig. 1). Mills CI and CII were regarded as one entity (mill C), since they discharge from the same effluent pipe. During 1995, 1996 and 1997, the three mills together discharged on average about 330.000 m^3 day^{-1} biologically and 55.000 m^3 day^{-1} chemically treated effluent into the lake area. During the study period, all three mills used elemental chlorine free bleaching processes and effluents were biologically treated in activated sludge wastewater treatment plants. A more detailed description of the bleaching processes the production and effluent characteristics of the mills during the different study periods is presented in papers I-V. The total load of suspended solids (SS), BOD_7, COD_{Cr}, AOX, P and N of each mill in 1995, 1996 and 1997 is presented in Table 2.

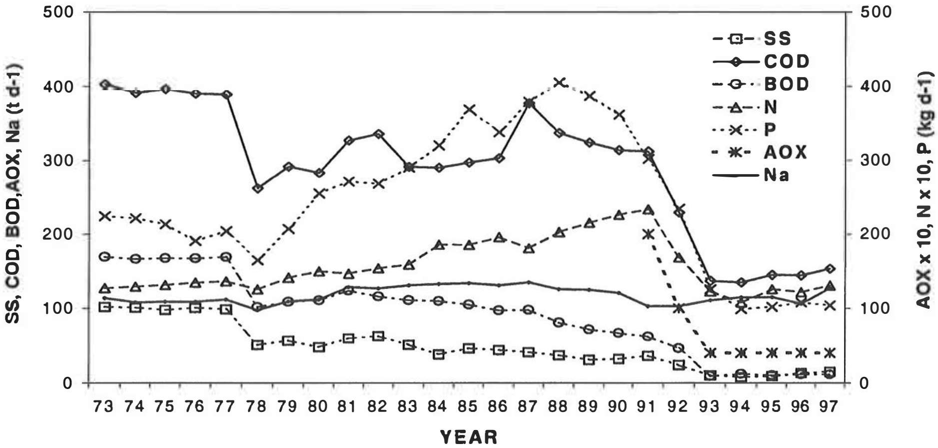

FIGURE 2 Total loading of suspended solids (SS), chemical oxygen demand (COD_{Cr}), biological oxygen demand (BOD_7), nitrogen (N), phosphorus (P), adsorbable organic halogens (AOX) and sodium (Na) of the pulp and paper mill factories in Lappeenranta, Joutseno and Imatra from 1973 to 1997 (from Laine & Minkkinen 1998).

History of pollution by the pulp and paper mill industry

The Southern Lake Saimaa has been affected by the pulp and paper industry for one century: pulping began in Lappeenranta (Mill A) in 1897, in Joutseno (Mill B) in 1908 and in Imatra (mill C) in 1935 (Laine & Minkkinen 1998). The discharging of wastewater into the lake reached its maximum in the 1960s. In the same period, the mills employed elemental chlorine for bleaching purposes. The first improvements in the effluent quality were achieved in the 1970s and 1980s, with developments in the process technology (black liquor recovery and burning) and the start of mechanical purification and the first generation of biological effluent treatment. Despite the increasing production, a clear decrease in the amount of suspended solids, BOD and COD in the effluent took place at the mills (Fig. 2). However, AOX, phosphorus and nitrogen concentrations remained high until the early 1990s, when modern activated sludge wastewater treatment plants and ECF bleaching was introduced at the mills (Laine and Minkkinen 1998). By the end of 1992 all the mills were using ECF bleaching processes. Since 1992, effluents of mill A have been treated in a modern activated sludge wastewater treatment plant, and by 1997 all the mills were using similar systems.

3.2 Feral and experimentally exposed fish

Feral perch and roach
Eurasian perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) are both abundant in the research area. Perch and roach are relatively stationary and so they are assumed to reflect the quality of their environment. Perch is strictly carnivorous and eats larger invertebrates and fish. Roach is omnivorous and feeds on detritus, plants, attached algae and benthic invertebrates (Persson 1983 a, b; Craig 1987; Koli 1990; Horppila 1994). Both perch and roach spawn in spring; the spawning of perch can last several weeks whereas the spawning of roach takes a few days only. For both species the sexual resting period lasts until the end of August, and the development of gonads starts at the beginning of September (Craig 1987; Koli 1990; Rask 1990; Jamet and Desmolles 1994).

Experimentally exposed whitefish
In the field caging experiments, hatchery reared immature (1+ year old) whitefish (*Coregonus lavaretus* L. s.l.) from the Central Fish Culture and Fisheries Research Station for Eastern Finland, Enonkoski, were used. The type of whitefish used in the present study is a plankton and seston feeder, an authentic species which is well able to manage in cages during a one-month exposure (Oikari and Sillanpää 1993), allowing subchronic experiments to be made.

3.3 Sampling and caging methods

3.3.1 Lake water and mill effluents

Lake water samples were collected in glass bottles at reference and mill sites as composites from the water column from 1 m to near the bottom. The lake water was sampled at each caging site, four times during the course of the caging periods, in May-June 1995 (Soimasuo et al. 1998a), 1996 (Leppänen et al. 1998) and 1997 (Karels et al. unpublished) and during the winter sampling of feral fish in 1997 (III). All samples were kept frozen (-20°C) for a maximum of eight weeks prior to the analysis.

Composite effluent samples were collected daily with an autosampler and combined into one-week samples for each mill during May-June 1995 (Soimasuo et al. 1998a), 1996 (Leppänen et al. 1998) and 1997 (Karels et al. unpublished). The one-week samples were combined in the laboratory into one sample, which represented the average quality of the mill effluent during the 1-month experimental period.

3.3.2 Perch and roach populations

Perch and roach were caught at the mill and reference sites in the spring of 1995 and 1996 (spawning period), in the summer of 1995 (resting period), in the autumn of 1995 (early vitellogenesis) and in the winter of 1997 (advanced vitellogenesis). During the spawning period fish were caught with wire traps; in the other periods they were caught with a line and hook. Captured fish were kept in the water, and placed in cages at a depth of about 3-4 m to recover for 24-48 hours prior to sampling. The sampling procedure was similar in all studies and is described in detail in the papers (I-III). Length, total, gonad and liver weight were measured. Plasma, bile and liver tissue samples were placed in liquid nitrogen, transferred to the laboratory and kept in a deep freeze (-80 °C) for later analyses. Ovaries were preserved in 10% buffered formalin and sub-sampled for egg counts and egg size measurements. Opercular bones were taken for age determination.

For the purpose of the population study, a total of 3,196 (the spring of 1996) and 731 (the winter of 1997) perch and 662 (1996) and 450 (1997) roach were sampled. Fish were caught with wire traps in 1996 and with a line and hook in 1997. For each fish, the total weight, fork length, sex and the reproductive stage were determined and opercular bones were taken for age determination; part of the fish was sampled for egg size, fecundity, liver and gonad weight. Male and female fish were divided in 0.5 cm-groups and length-frequency diagrams were developed for each site. Mean age, age at maturity, length at age diagrams, condition, relative liver size, relative gonad size and sex ratios were determined.

3.3.3 Caging and sampling of whitefish

In May 1996 and 1997, immature (1+ year old) whitefish were transported (max. 4 h) from the hatchery in polyethylene bags filled with oxygenated water at a temperature of about 5 °C to the experimental area in the Southern Lake Saimaa. The study area included five subareas, upstream and downstream of the mills, with a total of 21-22 research sites (Fig. 1, upper map). Twelve to fifteen fish (mean weight 39 g range 10 g) were exposed in oval shaped 250-litre cages submerged on the bottom, at a depth of about 4-5 m. After about 30 days of exposure, fish were sampled in a laboratory on the research vessel Muikku. Fish were sampled as described in detail in paper V.

3.3.4. Fish community survey

Species composition, relative abundance and biomass data of the fish community in the study areas were gathered by fishing with a gillnet series in sublittoral and profundal waters (1995 and 1996), by backpack electrofishing at stony shores (1996) and by a beach seine survey on vendace larvae (1995-1998). The relative abundance and biomass of each species were expressed as catch-per-unit-effort (CPUE). The CPUE was calculated by taking the total catch and dividing it by the total effort. Sampling sites, methods and materials are described in detail in paper VI.

3.4 Analytical methods

3.4.1 Chlorophenolics, resin acids and sterols in water and bile

Chlorophenolics, resin acids and sterols were quantified by gas chromatography, compounds were identified by their retention time and with mass spectrometry (I-IV). Total (free and bound) chlorophenolics (phenols, guaiacols, catecols and vanillins) in effluent and lake water samples were analysed according to Voss et al. (1981) and Paasivirta et al. (1992). Total resin acids and ß-sitosterol were analysed according to Hemming & Holmbom (1992) and Örså et al. (1992). Total chlorophenolics, resin acids and sterols in fish bile were analysed according to Oikari & Ånäs (1985) and Hemming & Holmbom (1992).

3.4.2 Biotransformation enzyme assays

The 7-ethoxyresorufin O-deethylase (EROD) (I-V) and pentoxyresorufin O-dealkylase (PROD) activity (I,V) of liver microsomes of feral and caged fish was measured fluorometrically according to the method of Burke et al. (1985), adapted for microplate format (Soimasuo et al. 1998a), as described in detail in papers (I-V). Microsomal fractions were prepared as described in papers I-V. Positive controls were liver samples from rainbow trout (*Oncorhynchus mykiss*) dosed by i.p. injection with 100 mg/kg ß-naphtoflavone (BNF) in corn oil. The protein concentration of the microsomes was measured with a Bio-Rad DC Protein Assay Kit, using bovine serum albumin as a standard.

3.4.3 Plasma and blood measurements

Plasma concentrations of estradiol-17ß and testosterone were measured using Fenzia Enzyme Immunoassay test kits (EIA, Orion Diagnostica, Finland) and read with a platereader (Labsystems EMS Reader MF V2.7-0, Finland) at 405 nm (I-IV). The method used for the measurement of plasma vitellogenin in roach is described in detail in paper III. The vitellogenin gene expression of perch (I), was detected with rainbow trout vitellogenin cDNA and Northern blot analysis of total RNA, as described in Mellanen et al. (1996). Plasma calcium (Ca^{2+}) concentration (II, III) was measured using a Boehringer Mannheim GmbH test kit No 1553 593. Plasma immunoglobulin M (IgM) in roach (I-IV) and whitefish (V) was measured by enzyme-linked immuno sorbent assay (ELISA) as described in Aaltonen et al. (1994). Hemoglobin (Hb) was measured spectrophotometrically using the cyanmethemoglobin method (V). Glucose and lactate (V) were determined using Boehringer Mannheim test kits, (GOD-Perid method 124036 and the L-lactic acid 256 773 UV-method).

3.4.4 Gross indices

The condition factor (CF) was calculated as:

$$CF = 10^5 \times \frac{\text{total weight (g) - gonad weight (g)}}{\text{fork length}^3\text{ (mm)}}$$

Liver (LSI) and gonadosomatic indices (GSI) were calculated as:

$$\text{LSI or GSI} = 100 \times \frac{\text{tissue weight (g)}}{\text{total weight (g) - gonad weight (g)}}$$

Thus, in calculations of CF, LSI and GSI the total body weight was adjusted for gonad weight to avoid the bias due to variations in sexual maturation.

3.4.5 Statistics

All the data were first assessed for normality and homogeneity of variance and log-transformed where appropriate. Length and weight were compared by one-way analysis of variance (ANOVA). Age, plasma steroid hormones, VTG, calcium, IgM, liver EROD and PROD, bile chlorophenolics, resin acids and sterols were compared using the non-parametric Kruskal-Wallis test. Estimates of condition, liver size, gonad size, fecundity and egg size were compared using analysis of covariance (ANCOVA), with adjusted body weight (body weight – gonad weight) as covariate to eliminate possible effects of altered gonad weight. The catch per unit of effort (CPUE) in the fish community survey, was compared using the Kruskal-Wallis test. The significance of all tests was set at $p < 0.05$. Correlations with two-tailed significance were determined using the non-parametric Spearman Rank Correlation Coefficient. Statistics were performed using SPSS ® software (Statistical Product Service Solutions, Chicago, IL, US). The Kriging interpolations (Cressie 1993; Suutari et al. 1999) of the spatially distributed variables, including liver EROD and bile CPs, in the lake were performed by Variowin (2.01) and Surfer (6.03) software, as described in detail in paper V.

4 RESULTS

4.1 Dilution and dispersion of effluents in the lake

The dilution and dispersion of mill effluents in the lake area was assessed with concentrations of the (inert) effluent tracer sodium in the lake water and mill effluents (Fig. 3). A spatial Kriging interpolation was utilized to assess the geographic area impacted by the effluents (V). Results show large differences in areal dispersion and dilution of effluents between the recipient areas of mill A, B and C. Downstream of mill A effluent concentrations are highest and there is a distinct dispersion and effluent dilution gradient up to about 15 km downstream of the point source of the mill. In the mixing zone of mill B, however, relatively low effluent concentrations and large temporal variations in the dilution and dispersion of the effluent are observed. The effluent mixing zone of mill C is small and the dilution of effluent in the recipient area is high, because of the large water volumes flowing into the river Vuoksi, the outflow of the whole Lake Saimaa area.

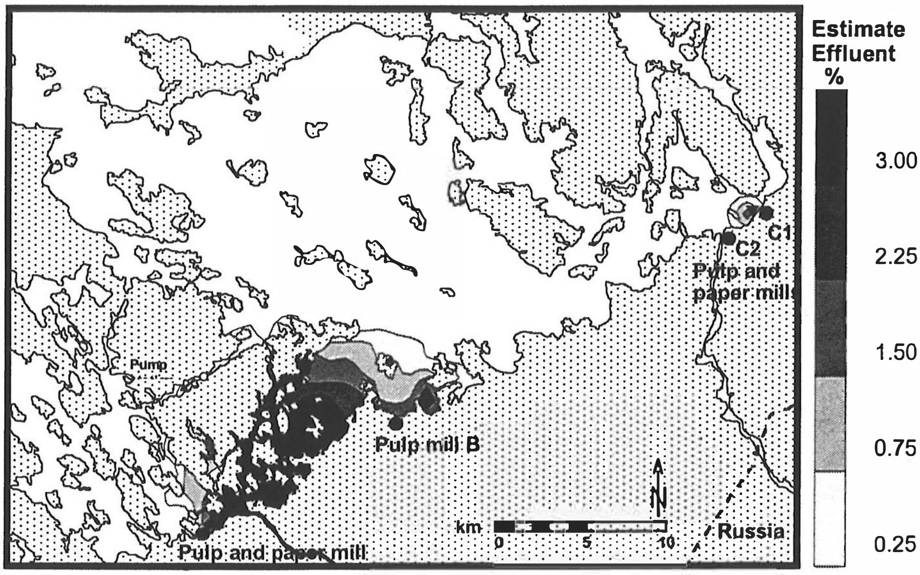

FIGURE 3 Estimated dilution and dispersion of mill effluents in the Southern Lake Saimaa based on measurements of the effluent tracer sodium at 89 different lake water sampling sites in the autumn of 1998 (V).

4.2 Chlorophenolics, resin acids and sterols in water and bile

Effluent

Concentrations of chlorophenolics, resin acids, fatty acids and sterols in treated final effluent of the pulp and paper mills in May-June 1995 (Soimasuo et al. 1998a), 1996 (Leppänen et al. 1998) and 1997 (III) are given in Table 3. The results show that chlorophenolic concentrations were relatively low in all effluents. The concentrations of resin acids, fatty acids and sterols, however, were higher and more variable among the mill effluents. Lower concentrations of resin acids, fatty acids and sterols in the effluent of mill B in 1997 show the result of the modernisation of the activated sludge treatment plant in the end of 1996.

TABLE 3 Concentrations of chlorophenolics, resin acids, fatty acids and sterols in discharged effluent of the pulp and paper mills to the study area in the Southern Lake Saimaa. Samples were taken daily during the caging experiments in May-June 1995 (Soimasuo et al. 1998), 1996 (Leppänen et al. 1998) and 1997 (concentrations are in µg l^{-1}; n.a. = not analysed; * = ß-sitosterol).

		Chloro-Phenolics	Resin Acids	Fatty Acids	Sterols
Mill A	1995	8	105	601	72
	1996	7.7	94	383	214
	1997	4.2	47	n.a.	69*
Mill B	1995	13.8	1156	1831	1058
	1996	7.6	559	727	875
	1997	5.2	12	425	40*
Mill C	1995	32	179	405	214
	1996	1.3	38	264	68
	1997	4.0	194	n.a.	49*

Lake water

During the study period, concentrations of chlorophenolics, resin acids and sterols in the waters collected from the study sites were very low, often approaching the analytical detection limits (I-IV). The detection limit for chlorophenolics was approximately 0.1 µg l^{-1} and resin acids and sterols approximately 0.5 µg l^{-1}. During the study period, no significant differences were observed in concentrations of chlorophenolics, resin acids and sterols in the lake water among sites (I,II,IV). With the exception of the winter of 1997 (III), when lake water concentrations of resin acids and ß-sitosterol 1 km downstream of mill A were 0.53 and 11.9 µg l^{-1}, respectively, concentrations at the other study sites were below the detection limit.

Fish bile

During the study period, concentrations of free and conjugated chlorophenolics (chlorophenols, chloroguaiacols and chlorocatechols) in the bile of feral perch and roach and experimentally exposed whitefish at the study sites were low (I-IV). In perch, roach and whitefish at the reference sites, concentrations of chlorophenolics in the bile ranged between 0.1-0.9 µg ml^{-1} and at the mill sites between 0.4-1.3 µg ml^{-1}. During this study, concentrations of chlorophenolics in the bile of fish exposed downstream of the mills, however, were often higher than the reference sites (Fig. 4), indicating some exposure to effluent related chlorophenolic compounds.

The concentrations of resin acids in the bile of perch, roach and experimentally exposed whitefish varied considerably between study periods and mills. In the 1996 spring (II), concentrations of resin acids in the bile of perch and roach downstream of mill A and B were low (0.2-2.0 µg ml^{-1}), although significantly higher compared to the reference sites (Fig. 5). In the 1997 winter (III), 1-2 km downstream of mill A, resin acids concentration in the bile of perch (260 µg ml^{-1}) and roach (320 µg ml^{-1}) were 10-30 x higher than the reference sites. Downstream of mill B, however, bile resin acids concentrations of perch and roach were the same as the reference sites. Isopimaric (84%), pimaric (8%) and dehydroabietic (DHAA) (8%) acids were the dominating resin acids in exposed perch, while abietic (35%), dehydroabietic (25%), isopimaric (25%) and sandaropimaric (6%) acids were the dominating resin acids in exposed roach.

In the caging experiment in May-June 1996 (V), resin acids concentrations in the bile of whitefish downstream of the mills were similar to the reference sites (Fig. 5). However, in the 1997 spring (Karels et al. unpublished), concentrations of resin acids in the bile of whitefish (140 µg ml^{-1}) 1-2 km downstream of mill A was about 55 x higher than the reference points, while bile resin acids of whitefish downstream of mill B were similar. DHAA was the predominant resin acid in the bile of exposed whitefish.

In the 1997 winter (III), ß-sitosterol was the only sterol detected in the bile of roach. Compared to the reference sites, concentrations were 2-5 times higher in roach downstream of the mills (1.5-3.5 µg ml^{-1}). Sterols were not detected in the bile of perch at either mill or reference sites.

4.3 Liver mono-oxygenase activity

EROD activity

The EROD activity of fish at the mill sites in 1995 (I) and 1996 (II,V) were often higher than at the reference sites (Fig. 6), except for female roach in 1996 (II), which exhibited a significantly lower EROD activity. In 1997, however, EROD activity in perch and roach (III) and experimentally exposed whitefish (Karels et al. unpublished) was not significantly different from the reference points (Fig. 6).

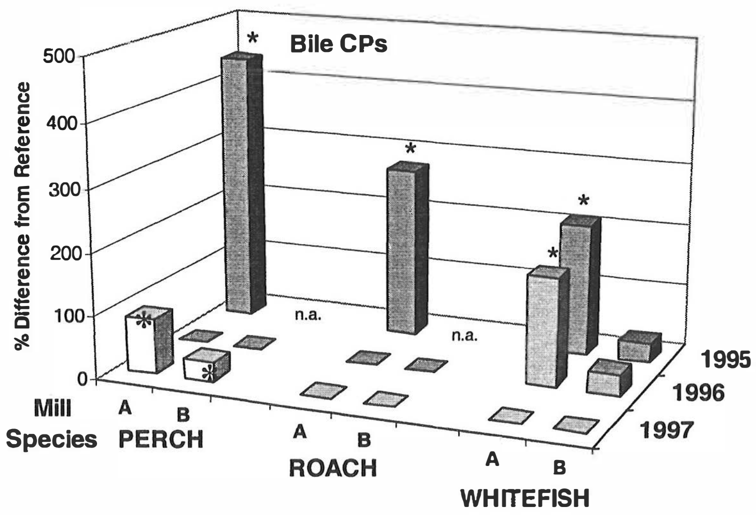

FIGURE 4 Relative differences in bile chlorophenolics concentrations of perch, roach and caged whitefish 1-2 km downstream of mills A and B compared with fish at the upstream reference sites. Perch and roach were sampled in the 1995 autumn (I), the 1996 spring (II) and the 1997 winter (III). Whitefish were caged in May-June 1995 (Soimasuo et al. 1998a), 1996 (V) and 1997 (Karels et al. unpublished). Asterisks (*) denote significant differences from the reference sites at P < 0.05.

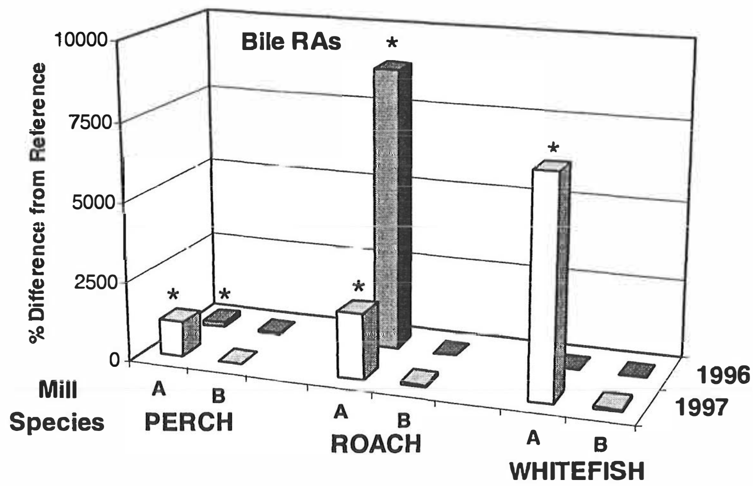

FIGURE 5 Relative differences in bile resin acids concentrations of perch, roach and caged whitefish 1-2 km downstream of mills A and B compared with fish at the upstream references. Perch and roach were sampled in the 1996 spring (II) and in the 1997 winter (III). Whitefish were caged in May-June 1996 (V) and 1997 (Karels et al. unpublished). Asterisks (*) denote significant differences from the references at P < 0.05.

The liver EROD activity exhibited seasonal, sex, species and site dependent differences (I-V). Among the seasons (at reference sites), EROD activity of perch and roach was lowest in winter (III) and highest during the spawning period (I; Fig. 7-8). As regards sex, the EROD activity of male roach was 2-3 times that of female roach, in all study periods (I-IV). By contrast, EROD activity of male perch in the 1997 winter (III) was lower than that of female perch, while in the 1995-1996 spring (II, IV) and the 1995 autumn (I), EROD activity in female and male perch was similar. Among species, EROD activity in perch was about 20-30 times higher compared to roach, and 10-20 times higher compared to juvenile whitefish.

PROD activity

PROD activity was measured in perch and roach in the 1995 autumn (I), and in whitefish in May-June 1996 (V). In 1995, the PROD activity in male roach downstream of mill A (0.45 pmol min^{-1} mg $protein^{-1}$) was 2-fold compared to the reference point. PROD activity in female roach, as well as in female and male perch, were similar between mill and reference sites (I). In 1996, PROD activity in experimentally exposed whitefish downstream of mill A and B (2.7 and 2.6 pmol min^{-1} mg $protein^{-1}$) was 2-3 fold compared to the reference fish (V).

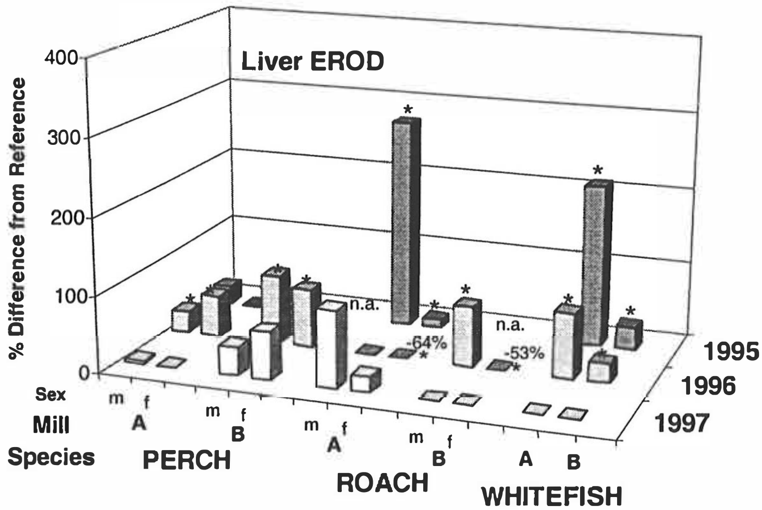

FIGURE 6 Relative differences in the liver EROD activity of male (m) and female (f) perch and roach and caged whitefish 1-2 km downstream of mills A and B compared with fish at the upstream reference sites. Perch and roach were sampled in the 1995 autumn (I), the 1996 spring (II) and the 1997 winter (III). Whitefish were caged in May-June 1995 (Soimasuo et al. 1998), 1996 (V) and 1997 (Karels et al. unpublished). Asterisks (*) denote significant differences from the reference sites at $P < 0.05$.

4.4 Reproductive parameters

4.4.1 Reproductive steroid hormones

Annual patterns female perch and roach (I-IV)
Annual patterns of estradiol, testosterone and gonadosomatic index (GSI) in female perch at upstream reference sites and 1-2 km downstream of mill A are shown in Fig. 7 and Fig. 8. Plasma estradiol-17ß and testosterone concentrations in female perch and roach are lowest during the regression period of the gonads (June-August) and increase during the period of vitellogenesis, the major growth phase of the gonads (September-March). Just before and during the spawning period (May), estradiol-17ß and testosterone concentrations decrease rapidly towards the regression period levels.

Annual patterns male perch and roach (I-IV)
Annual patterns of plasma testosterone in male perch and roach are less distinct than in female fish. At reference sites, no large differences were measured in testosterone levels of male perch between the study periods. In roach, however, testosterone levels in the 1995 autumn were below the detection limit of the assay, while in the 1996 spring and the 1997 winter testosterone levels ranged between 4-16 nmol l^{-1}.

Comparison of plasma steroids between mill and reference sites (I-IV)
The relative differences in plasma estradiol-17ß and testosterone concentrations of female and male perch and roach at 1-2 km downstream of mills A and B, compared with the reference points, is shown in Fig. 9 and Fig. 10. A comparison of steroid hormone concentrations during the spawning periods in the spring of 1995 (IV) and 1996 (II) is not shown because the levels decreased rapidly during this period, making comparisons unreliable.

In the 1995 autumn (I), plasma estradiol-17ß concentrations in female perch and roach downstream of mill A were significantly lower (37-48%) compared to the reference site. Similarly, in the winter of 1997 (III), plasma estradiol-17ß concentrations in female perch downstream of mill A were significantly lower (48%), but not in female roach. Similarly, plasma testosterone concentrations in female and male perch downstream of mills A and B in 1995 (I) and 1997 (III) tended to be lower compared to the reference sites. In experimentally exposed juvenile whitefish in May-June 1997 (Karels et al. unpublished), plasma estradiol-17ß and testosterone were similar between fish at mill and reference sites.

4.4.2 Gonadosomatic index, egg size and fecundity

Annual GSI patterns in female perch and roach at reference site(s) and 1-2 km downstream of mill A are shown in figures 7 and 8. The GSI of perch and roach increased during the period of vitellogenesis and peaked in April-May just

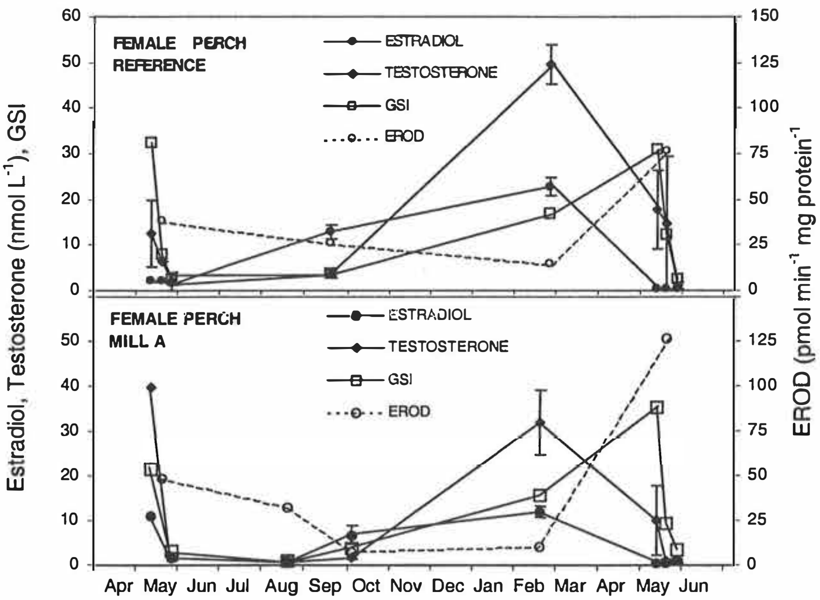

FIGURE 7 Annual patterns of estradiol, testosterone, gonadosomatic index (GSI) and liver EROD activity in female perch at upstream reference sites (upper) and 1-2 km downstream of mill A (lower). Fish were sampled in the spring of 1995 (IV) and 1996 (II), in the summer and autumn of 1995 (I) and in the winter of 1997 (III). Error bars represent the standard error.

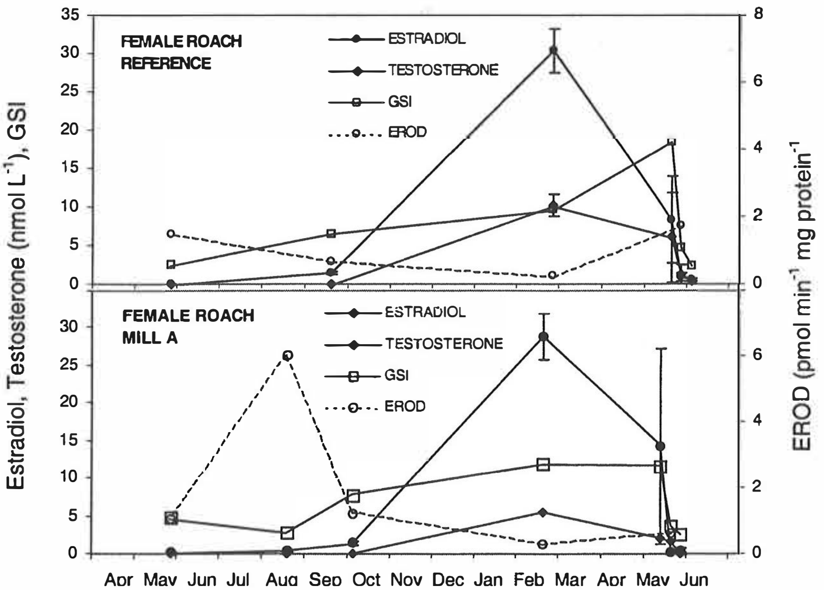

FIGURE 8 Annual patterns of estradiol, testosterone, gonadosomatic index (GSI) and liver EROD activity in female roach at upstream reference sites (upper) and 1-2 km downstream of mill A (lower). Fish were sampled in the spring of 1995 (IV) and 1996 (II), in the summer and autumn of 1995 (I) and in the winter of 1997 (III). Error bars represent the standard error.

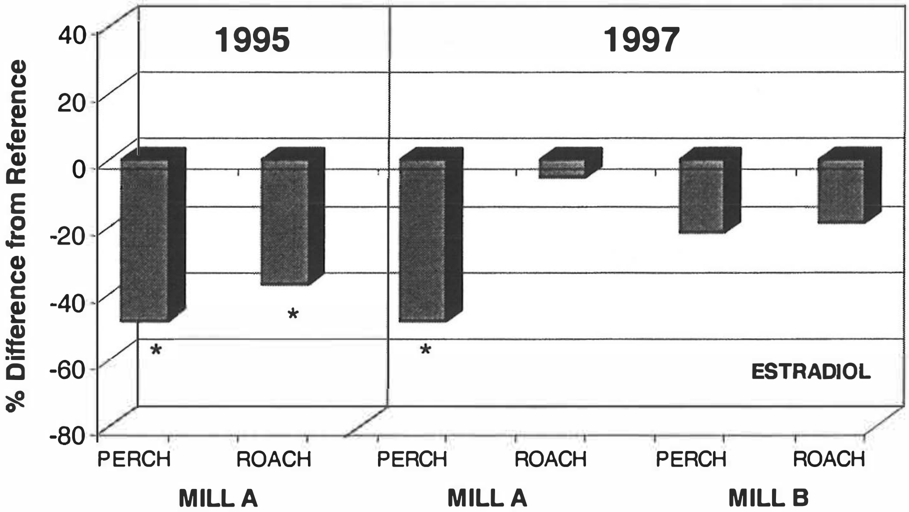

FIGURE 9 Relative differences in plasma estradiol concentrations of female perch and roach 1-2 km downstream of mills A and B compared with fish at the upstream reference sites. Perch and roach were sampled during early vitellogenesis (autumn 1995; I) and advanced vitellogenesis (the winter of 1997; III). Asterisks (*) denote significant differences from the reference points at P < 0.05.

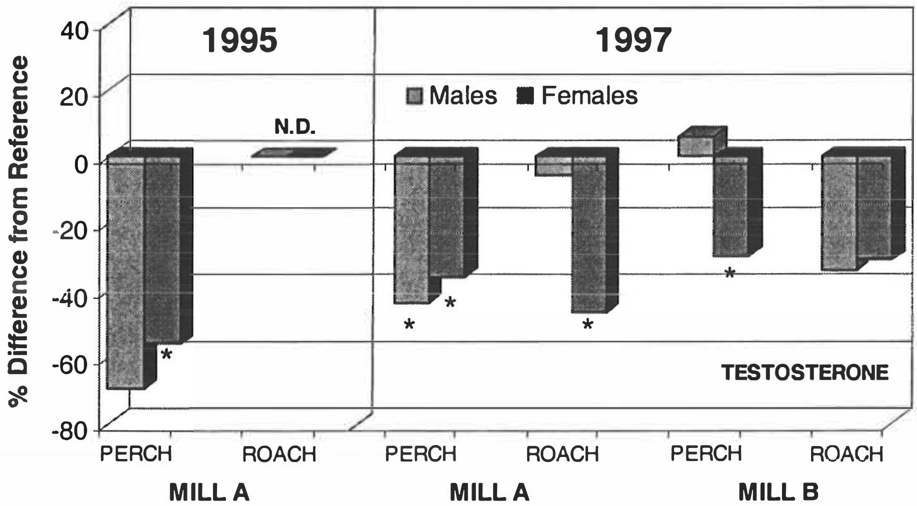

FIGURE 10 Relative differences in plasma testosterone concentrations of male and female perch and roach 1-2 km downstream of mills A and B compared with fish at the upstream reference sites. Perch and roach were sampled during early vitellogenesis (autumn 1995; I) and advanced vitellogenesis (the winter of 1997; III). Asterisks (*) denote significant differences from the reference sites at P < 0.05; N.D. = not detected.

before spawning (I-IV). The GSI dropped to the baseline in May-June, indicating that the fish had spawned. The GSI of male perch and roach exhibited a similar annual profile to that of females (I-IV). Compared to the reference sites, a lower GSI was measured in the winter of 1997 in female perch downstream of mill A and B (III), but not in roach. The GSI of male perch downstream of mill B was also lower in 1997, whereas no differences were observed in the autumn of 1995 (I). No comparisons of GSI values in the spring of 1995 (IV) and 1996 (II) were made because individual fish were at highly variable spawning stages, making stage-related comparisons unreliable.

Egg size and fecundity in perch and roach were measured in the 1997 winter (III). Compared to the reference fish, perch downstream of mill B revealed lower values, whereas roach and fish caught downstream of mill A did not.

4.4.3 Vitellogenin and calcium

Plasma vitellogenin in roach and whitefish

Concentrations of VTG in the plasma and cytosol of female and male roach during advanced vitellogenesis (III) were the same at the mill and reference sites. Interestingly, however, during early vitellogenesis (I) the vitellogenin gene expression in female perch downstream of mill A was reduced by 64% compared to the reference, but this was due to a low sample number, not statistically significant (Kruskall-Wallis, $p=0.09$, $n=5$).

In experimentally exposed juvenile whitefish in May-June 1997 (Karels et al. unpublished), plasma VTGs were similar in the fish at the mill and reference sites.

Plasma calcium

Plasma calcium was measured as an indirect marker of VTG, a calcium binding lipophosphoprotein (Matty, 1985). The strong and significant positive correlations between plasma calcium and plasma VTG ($r=0.87$, $p<0.001$, $n=42$) and plasma calcium and liver cytosolic VTG ($r=0.46$, $p<0.01$, $n=41$) in female roach at the reference sites in winter 1997 (III) confirmed that calcium can be used as an indirect marker of VTG.

In the 1997 winter (III), plasma calcium in male perch and roach was 1-2 times higher than that of female fish. Compared to the reference sites, plasma calcium in female perch downstream of mill B was lower, but not, as expected, in fish downstream of mill A.

4.5 Hematological and immunological parameters

In the spring of 1996 (V) and 1997 (Karels et al. unpublished), no significant differences in blood hemoglobin, hematocrit, glucose and lactate of caged whitefish were observed between mill and reference sites.

Between mill and reference sites, no significant differences were measured in the plasma IgM concentrations of roach in 1995 and 1997 (I,III,IV) or in caged whitefish in 1996 and 1997 (V, Karels et al. unpublished).

4.6 Gross indices

In 1995 (I), 1996 (II), and 1997 (III), the gonad free body condition factor (CF) of female and male perch and roach and experimentally exposed whitefish (V, Karels et al. unpublished) downstream of the mills were all similar to those at the reference sites.

A higher liver somatic index (LSI) was frequently measured in fish downstream of mill A and B, compared to the reference sites (I,II,III,IV). A higher LSI was measured downstream of mill A in female and male roach in 1995, 1996 and 1997 and in female perch (1995) and male perch (1996). Downstream of mill B, a higher LSI was observed in female roach and male perch in the 1996 spring. The results of GSI in perch and roach are given in chapter 4.4.2.

4.7 Perch and roach population characteristics

Densities of perch and roach in the study areas

The CPUE (ind. net series^{-1} night^{-1}) of perch and roach gillnet catches in study areas with different effluent dilutions in the lake is given in Fig. 11 (Karels, unpublished). Fish densities are highest in the polluted area 5-15 km downstream of mill A (effluent % 1.5-3.0) and lowest in the reference area and close to mill A (2-5 km, effluent % 3.0-4.0 %)

Growth, length and age distribution

The growth (Fig. 12) and age at maturity of female and male perch and roach was not different between mill and reference areas (Karels, unpublished).The length-frequency and age distribution of perch and roach at mill and reference sites in spring 1996 is shown in Fig. 13 (Karels, unpublished). The perch population in the recipient of mill A in the spring of 1996 as well as in the winter of 1997 (Karels, unpublished) was dominated by smaller and younger individuals and exhibits a smaller range of age classes than populations at the other study sites.

Spawning of perch and roach

Perch and roach were actively spawning in May 1996, and fish densities were high at the study sites. The spawning peak period for perch was between 7 and 15 May and for roach between 20 and 27 May. The majority (>97 %) of male perch and roach caught were in the spawning stage and entered the spawning grounds 1 to 2 weeks before the majority of female fish.

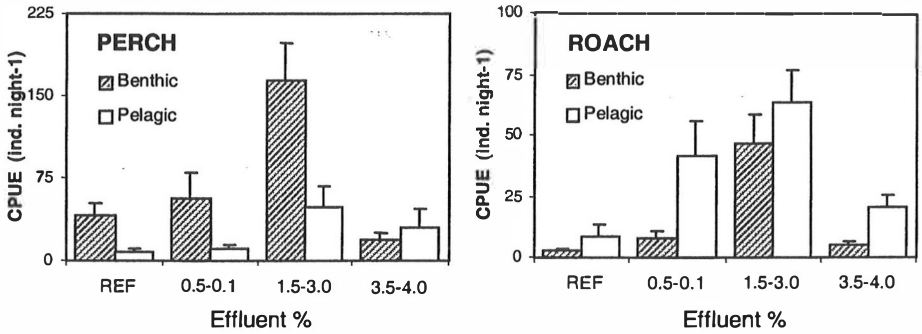

FIGURE 11 Gillnet CPUE (ind. series night^{-1} ± SEM) of perch and roach of benthic and pelagic catches in areas with different pulp mill effluent dilutions in the Southern Lake Saimaa in 1995 and 1996 (Karels, unpublished).

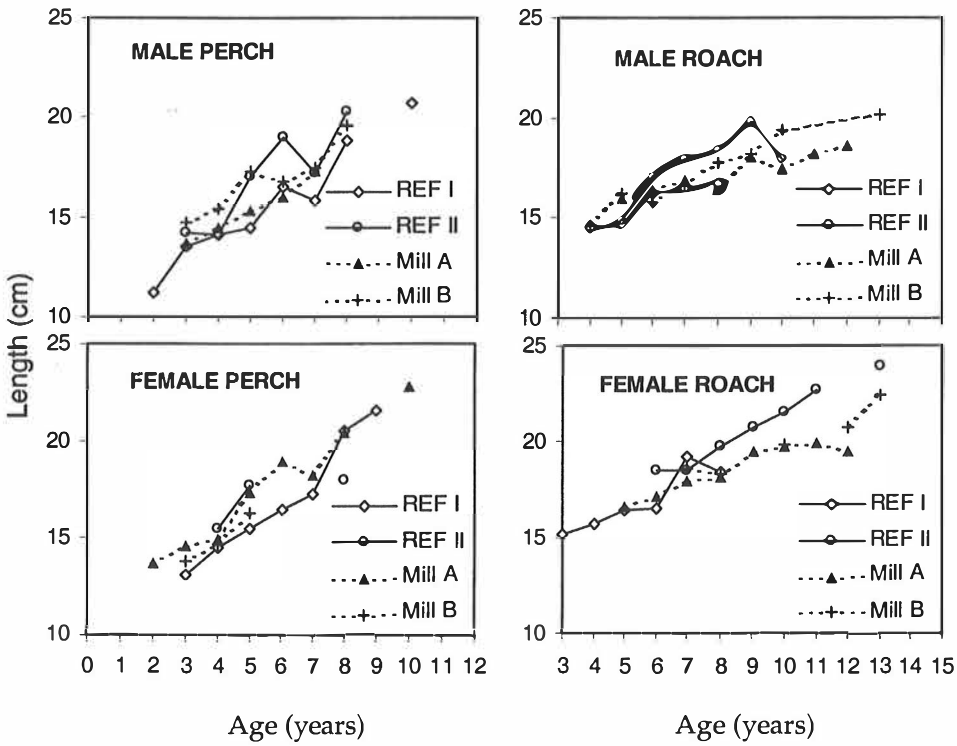

FIGURE 12 Length at age of female and male perch and roach in the spring of 1996 at reference and mill sites in the Southern Lake Saimaa (Karels, unpublished).

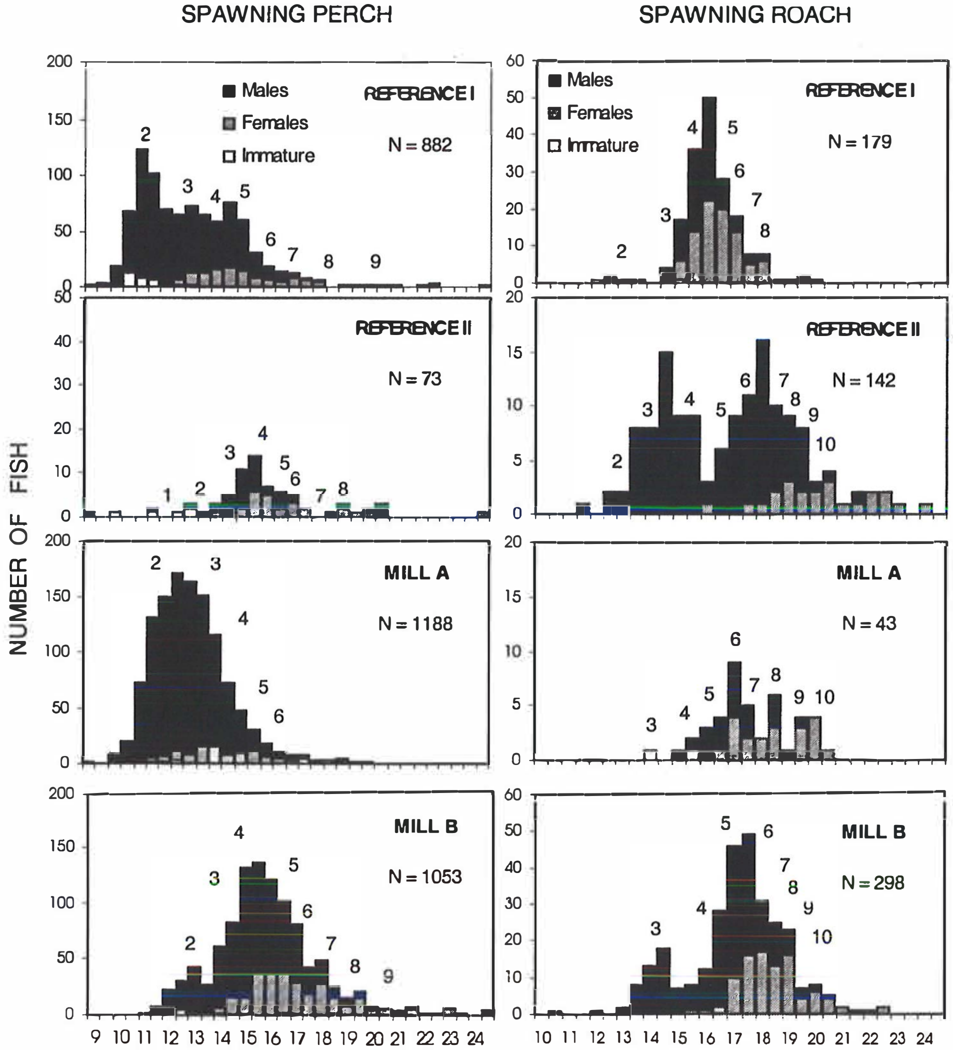

FIGURE 13 Length-frequency distributions of perch and roach collected by wire traps during the spawning period at reference and mill sites in the Southern Lake Saimaa in the spring of 1996 (Karels, unpublished). The numbers above bars indicate age groups based on operculum readings.

The duration of the spawning period of perch lasted several weeks, whereas that of roach peaked for a few days only. The first spawned egg strands of perch were observed on 5 May near mill A, on 10 and 11 May near mill B and at reference site 1, and on 23 May at reference site 2. Water temperatures at a 1 meter depth on these days at these sites were 5.0, 5.0, 5.1 and 5.9 °C, respectively. The first spawning female roach were observed on 19 May near mill A, on 24 and 25 May near mill B and at reference site 1, and on 27 May at reference site 2. Water temperatures at a 1 meter depth on these days at these sites were 8.7, 10.9, 10.0, and 9.0 °C, respectively.

4.8 Fish community

4.8.1 Gillnet survey of sublittoral and pelagic waters

The results of the gillnet survey in sublittoral and pelagic waters are shown in Fig. 14. The fish community in the study areas was dominated (> 60%) by perch and roach. Biomass and densities of fish were highest in the polluted area, and lowest in the reference area.

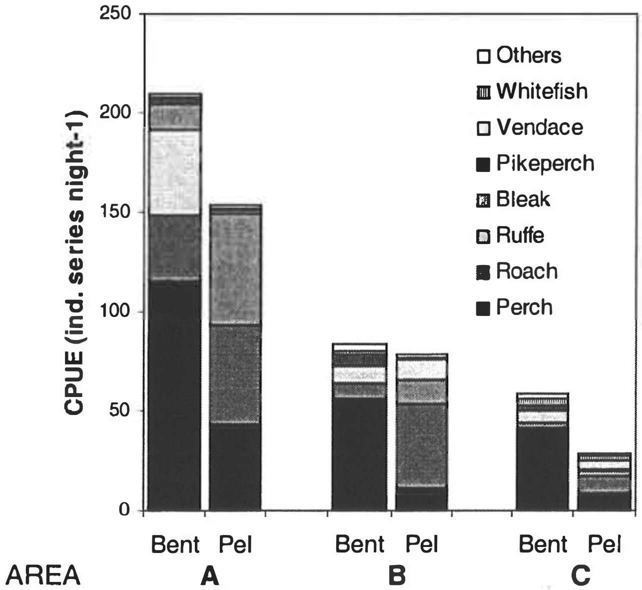

FIGURE 14 The CPUE (ind./net series/night) of benthic (Bent) and pelagic (Pel) gillnet catches of fish species in the most polluted study area A, the intermediate area B and the reference area C in the Southern Lake Saimaa in 1995 and 1996 (VI).

The number of species caught was similar among areas. Bleak and ruffe are typical species for the polluted area, while vendace and whitefish are typical for the reference and intermediate area. The results are described in detail in paper VI.

4.8.2 Electrofishing at stony shores

The fish community at stony shores, assessed by electrofishing, was dominated by bullhead, stone loach and minnow, the densities being lowest in the polluted area and highest in the intermediate area (Fig. 15). Minnow, apparently a more sensitive species to pulp mill effluents, was not caught in the most polluted area (VI).

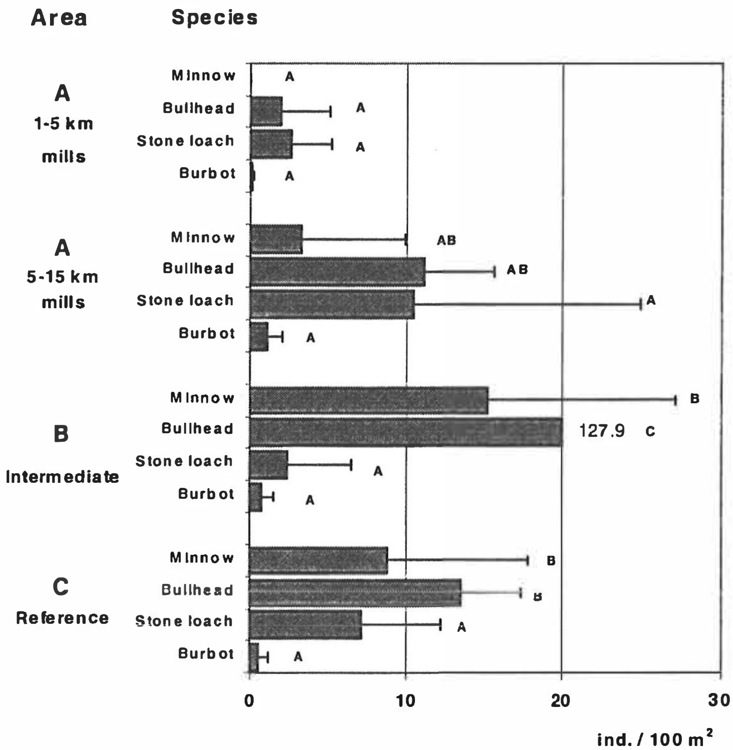

FIGURE 15 Mean densities (ind. / 100 m^2 ± S.D.) of the most common fish species caught by electrofishing at stony shores in the study areas in the Southern Lake Saimaa in 1996 (VI). Within a species, differences between areas ($p<0.05$) are denoted by different alphabetical letters.

4.8.3 Vendace larvae beach seine survey

The catch of vendace larvae was significantly different among years, within the study areas. Within years, the catch of vendace larvae was not statistically different between polluted and reference areas. In 1998, larvae were regularly caught in the polluted area, an emerging sign of recovery as indicating the tolerance of larvae to the water and sediment quality in this area (VI).

5 DISCUSSION

5.1 Effects of new process technologies on the effluent quality

Modernized pulp bleaching technologies
The use of chlorine dioxide (ClO_2) for bleaching wood pulp (residual delignification and brightning) has largely replaced the earlier use of elemental chlorine (Cl_2) in most Scandinavian and North American pulp mills during the last decade. It is obvious that the replacement of Cl_2 by ClO_2 in the bleaching results in more oxidation processes than halogen substitutions when reacting with lignin (Robinson et al. 1994). As a consequence the total quantity of chlorinated material in ECF bleaching effluent is only 10-20% of that found in chlorine-based bleaching effluent and the release of chlorinated compounds has decreased dramatically in effluents. Nearly 60% of the chlorinated compounds in effluents of ECF bleaching are chlorophenolics and chlorinated hydrocarbons. The remainder include a variety of classes such as acids, furanones, aldehydes and ketones (Bright et al. 2000). As a result of ECF bleaching processes, levels of polychlorinated dibenzo-p-dioxins and –furans (PCDD/PCDF) and polychlorophenols in effluents regularly are below current detection limits (Bright et al. 2000). Less knowledge, however, is available about the compounds present in effluent from mills using total chlorine free (TCF) bleaching (i.e. bleaching without chlorine or chlorine dioxide). TCF bleaching is currently made possible by treating the pulp with chelating agents like ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA) prior to the hydrogen peroxide stage (Sillanpää 1997).

During the present study from 1995 to 1997, all mills in the Southern Lake Saimaa produced kraft pulp and used ECF bleaching methods. The replacement of Cl_2 by CLO_2 at mill A in 1992, efficiently eliminated chlorinated phenolic compounds (reduction 98%) and reduced AOX levels by 87% in the final effluent (Oikari & Holmbom 1996; Kaplin et al. 1997). After the introduction of ECF bleaching, similar reductions in AOX and CPs levels in the final effluent were also observed at mill B and mill C in the Southern lake Saimaa (Table 3). As a result of this, chlorophenolics concentrations in the lake waters downstream of the mills were low (often approaching detection limits) and of the same order as the reference sites. When comparing water chlorophenolic levels with natural background levels, it should be recognized that many chlorinated organic compounds, identical, or similar to those formed in ClO_2 bleaching, are formed as a result of natural processes. Consequently, the pulp mill effluent compounds of major concern are non-chlorinated, originating from pulping rather than from bleaching. These compounds may be biodegraded in activated sludge effluent treatment plants, but some (e.g. retene) may be produced by the treatment itself (Leppänen 1999).

Biological treatment of effluents
At present, secondary biological effluent treatment is regularly used in the pulp and paper industry in Finland. Its introduction has significantly reduced the amount of organic carbon, measured as BOD_7, COD_{Cr} and suspended solids in the wastewaters. As a consequence, well designed and operated biological treatment systems in combination with ECF bleaching have substantially reduced the amount of chlorinated organic matter and wood extractives such as resin acids, fatty acids, lignans and monoterpenes in pulp mill effluents. Phytosterols, however, are removed less efficiently and chelating agents such as EDTA and DTPA are largely not removed at all by biological treatment (Strömberg et al. 1996; La Fleur & Barton 2000). For instance, the use of EDTA by mill A in the bleaching was also detected in the receiving waters of mill A (Sillanpää & Oikari 1996).

Of the mills in this study, mill A employed the activated sludge process for effluents in 1992. The yearly average removal efficiency of the activated sludge plant of mill A during the study period (1995-1997) was between 72-78% for COD_{Cr}, 98-99% for BOD_7 and 58-66% for AOX (Ilkka Westergren, personal communication). Compared to the times before the changes, reductions caused by the activated sludge treatment have been 94-99.6% for resin acids, 85% for fatty acids and 60-72% for chlorophenolic concentrations (Kaplin et al. 1997; Soimasuo et al. 1998). However, concentrations of sterols, like the main sterol, ß-sitosterol, have remained almost unchanged. Mill B employed an older type activated sludge treatment facility in 1986. Compared to mill A and C, discharges of resin acids, fatty acids and sterols in the final effluent of mill B were substantially higher until 1996 (Table 3). However, after the employment of the new activated sludge treatment facilities in November 1996, concentrations of resin acids, fatty acids and sterols in the final effluent were reduced to levels comparable with those of mills A and C. The yearly average removal efficiency of the activated sludge plant of mill B during the study period was between 40-61% for COD_{Cr}, 85-96% for BOD_7 and 0(1995)-41% for AOX (Mauno Ruhanen, personal communication). Mill C employed the activated sludge process for effluents in 1992, effluents from paper and cardboard production being chemically treated. The yearly average removal efficiency of the activated sludge plant of mill C during the study period was between 64-68% for COD_{Cr}, 96-97% for BOD_7 and 43-50% for AOX (Hilkka Hännikainen, personal communication).

5.2 Assessment of exposure of fish to pulp mill effluent

Dilution of effluents in the recipient areas
Due to the large study area and recipient areas with large differences in hydrology, it is important to estimate the exposure of fish to each mill effluent. Both ambient water quality parameters, effluent tracers, as well as internal exposure values of fish and their responses, can be used to assess the exposure of fish to mill effluent. According to the effluent trace marker sodium, the

dilution of effluents was considerably higher in the recipients of mills B and C than in that of mill A. This suggests a substantially lower potential exposure of fish to effluents in the recipients of mills B and C. Temporal variations in effluent dilution and dispersion in the recipient areas of mills B and C make an assessment of the real long-term exposure less accurate. The recipient area downstream of mill A, however, exhibits a more consistent pattern in this respect, with effluent dilutions theoretically ranging from about 4.0 % at 1 km to 1.0 % at 16 km, from the effluent source. The more precise delineation of the effluent dilution and dispersion zone downstream of mill A, resulting in more stable exposure conditions, make this area desirable - almost ideal - for ecotoxicological field studies.

Bile metabolites as markers of exposure

Earlier studies in the Southern Lake Saimaa revealed that the exposure of fish to chlorophenolics, as measured in effluents, the lake water and fish bile, was dramatically reduced after introduction of ECF bleaching and activated sludge effluent treatment processes in the early 1990s (Oikari & Holmbom 1996; Petänen et al. 1996; Soimasuo et al. 1998a). Accordingly, in the present study, concentrations of chlorophenolics in the lake water and fish bile demonstrated that the exposure of fish to chlorophenolic compounds appeared to be low in the recipients of the mills and was of the same order as at the reference sites. There were no great differences in bile chlorophenolic concentrations between whitefish, perch and roach. Being different in their feeding patterns, this may indicate that these fish receive most of their chlorophenolics burden directly from the water and not from their food.

Compared to the situation before the introduction of ECF and activated sludge technologies, bile chlorophenolic levels in perch and roach, 1 km downstream of mill A were only 0.1-0.2% of those in 1983 (Oikari 1986). Similarly, bile chlorophenolic levels in experimentally exposed whitefish in 1996 (Leppänen et al. 1998) and 1997 (Karels et al. unpublished) were 0.1-5% of those in 1991 (Soimasuo et al. 1995). However, the concentrations of bile resin acids in feral and caged fish 1 km downstream of mill A were 1100-8900% of that at the reference sites, indicating that fish are still exposed to resin acids derived from pulp mill effluents. Bile resin acids in perch and roach in the present study were 30-50% lower compared to 1983 (Oikari 1986).

The detection of ß-sitosterol in the bile of roach in recipient areas, confirm that this compound, suspected as having reproductive effects, is bioavailable to feral fish. The presence of ß-sitosterol was also reported in the bile of Crucian carp (Carrasius carassius) exposed to pulp mill effluents (Kukkonen et al. 1999).

Liver mono-oxygenase activity as marker of exposure

Liver MO activity, measured as EROD activity, is commonly used as an indication of exposure and sometimes also as an indication of effect of pulp mill effluents (Owens 1991; Stegeman et al. 1992; Munkittrick et al. 1994; Sandström 1996; Hodson 1996).

Liver EROD activity also proved to be the most prominent response to pulp mill effluents in earlier studies in the Southern Lake Saimaa (Lindström-Seppä &

Oikari 1989b, 1990; Oikari & Holmbom 1996; Petänen et al. 1996; Soimasuo et al. 1995, 1998a). Earlier observations on feral perch and roach 1 km downstream of mill A in 1987, before the shift to ECF technology, exhibited 3-7 times higher EROD activity compared to the reference (Lindström-Seppä & Oikari 1990), while a study in 1993, after the changes at mill A, exhibited about twice as high liver EROD activities in exposed perch and roach (Kantoniemi et al. 1997).

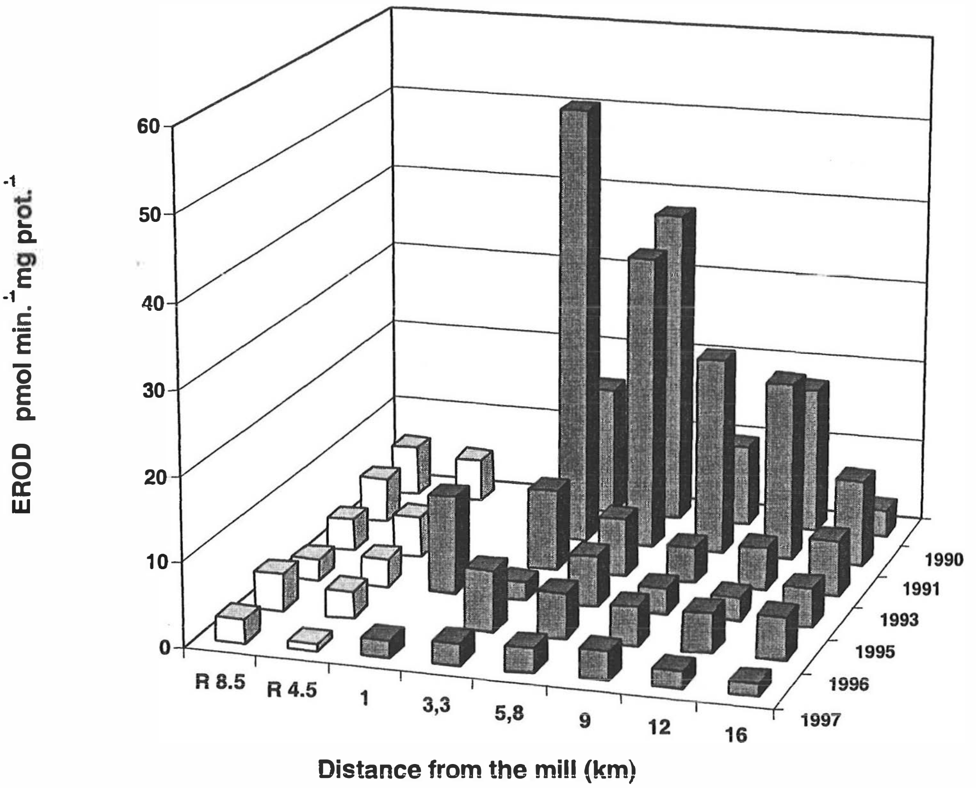

FIGURE 16 Liver EROD activity (mean, pmol min.$^{-1}$ mg prot^{-1}) of whitefish experimentally exposed at different distances from pulp and paper mill A in the Southern lake Saimaa in 1990, 1991 (Soimasuo et al. 1992, 1995), 1993 (Petänen et al. 1996), 1995 (Soimasuo et al. 1998a), 1996 (V) and 1997 (Karels et al. unpublished). The nearest site (1 km) was only used in 1995 and 1997. In 1990, all exposed fish died at site 3.3 km from the mill.

Studies using experimentally exposed whitefish in the Southern Lake Saimaa in 1993 and 1995 also showed that EROD induction in exposed whitefish was substantially less after the modernization's at mill A in 1992, but still higher than at the reference sites (Petänen et al. 1996; Soimasuo et al. 1998a). Over a distance of about 16 km from the effluent source, the liver EROD activity in whitefish in 1996 (this study) amounted to only 14–49% of those measured in 1991 (Soimasuo et al. 1995). The mean level of EROD activity in whitefish at the reference sites (about 4 pmol min.$^{-1}$ mg prot^{-1}), however, has remained the same over the years (Soimasuo et al. 1992, 1995, 1998a; Petänen et al. 1996, V; Karels et al. unpublished).

In terms of the liver EROD activities of fish in the present study, the EROD activity in exposed fish has decreased during the time of the study from significantly higher levels to almost reference levels (Fig. 16). This confirmed that the exposure to CYP 1A inducing compounds has been further reduced after the introduction of ECF bleaching and activated sludge effluent treatment at the mills at the Southern Lake Saimaa.

Seasonal and sex differences in liver EROD activity were apparent in both perch and roach. Among seasons, EROD activity of perch and roach was lowest in winter and highest during the spawning period. Seasonal variations in the xenobiotic metabolism in the liver of fish have been studied previously and have been associated with environmental and internal, hormonal factors (Koivusaari 1981; Lindström-Seppä 1985; Stegeman & Hahn 1994). Among the sexes, male roach exhibited a higher EROD activity in spring and winter than female roach. In fact, it has been repeatedly demonstrated that EROD induction is more prominent in male than in female fish, and represented a sensitive, short-term indicator of exposure to pulp mill effluents (Ahokas et al. 1976; Lindström-Seppä 1985; Jiminez et al. 1990; Munkittrick et al. 1994). Male perch, by contrast, exhibited significantly lower EROD activity than female perch in winter, but this was similar in autumn and spring.

Species differences in EROD induction in fish near the mills were also apparent in the autumn of 1995, when EROD and PROD activities were induced in exposed roach but not in perch, while, by contrast, EROD activity in the spring of 1996 was induced in exposed perch but reduced in exposed female roach. It is a known fact that a number of variables, like health, condition, nutritional status, and reproductive and developmental status, influence the expression or function of MO systems in fish (Stegeman & Hahn 1994).

Despite many similarities in the production processes and effluent treatment at the mills in the present study, EROD activity was found to vary between mill areas, site specific factors like hydrology, effluent dilution and dispersion being most likely responsible for these differences. Significant positive correlations between EROD activity and bile chlorophenolics in whitefish and concentrations of the effluent tracer sodium in the lake water in 1996 confirmed that EROD and bile chlorophenolic levels were related to the mill effluents discharged into the lake (V). Dilution of the effluents at the study sites therefore most likely determine the level of MO induction and accumulation of chlorophenolics in the exposed fish.

5.3 Reproductive status of fish

Reproductive cycle of perch and roach

As described earlier in this thesis, the reproductive cycle of female fishes can be divided into four main phases: (1) vitellogenesis, the major growth phase of the ovary (2) oocyte maturation, (3) ovulation and spawning and (4) postspawning, regression of gonads. Male fish have less defined stages of maturation and in them hormonal regulation is less clear than it is in females. In fish, endocrine changes taking place during the reproductive cycle include variations in the levels of pituitary and plasma gonadotropin (GTH) and plasma sex steroid hormones like estradiol-17ß, testosterone and 11-ketotestosterone. Besides estradiol-17ß, testosterone too, has been identified in female fish, both hormones reaching peak levels during the pre-spawn period (Liley & Stacey 1983; Matty 1985; Kime 1998). Testosterone and 11-ketotestosterone appear to be the predominant testicular steroids in fish. While testosterone attains peak levels during the pre-spawn period in male fish, 11-ketotestosterone levels appear to reach their peak after the spawning period (Liley & Stacey 1983; Matty 1985; Kime 1998).

In the present study, plasma estradiol-17ß and testosterone concentrations in female perch and roach were lowest during the regression period (June-August), increased during the period of vitellogenesis (September-March) and decreased rapidly towards regression period levels just before and during the spawning period (May). This is in accordance with other studies on annual steroid levels in feral perch and roach (Matty 1985; Rinchard et al. 1997; Sulistyo et al. 1998). In the present study, the act and timing of spawning of perch and roach downstream of the mills was not generally affected. Fish at the mill site A, however, tended to spawn earlier than at the other sites, this being most likely due to the slightly higher water temperatures downstream of mill A (II).

Effects of pulp mill effluents on fish reproduction

In this study, plasma steroid hormone levels were reduced in perch and roach downstream of the mills. This coincided with a lower gonad size and fecundity in female perch downstream of mill A and lower plasma calcium concentrations in female perch at mill site B, indicating alterations on plasma VTG levels. On the other hand, other reproductive parameters in roach remained unchanged. Reproductive effects like decreased steroid hormone levels, reduced gonad size and fecundity, have been earlier reported in several fish species exposed to pulp and paper mill effluents (Lindström-Seppä & Oikari 1989; Munkittrick et al. 1991, 1994; McMaster et al. 1991, 1992; Gagnon et al. 1994; Sandström 1996; Van Der Kraak et al. 1998). While some studies have not revealed any reproductive effects (Hodson et al. 1992; Kloepper-Sams et al. 1994; Landner et al. 1994; Swanson et al. 1994), the consequences of e.g. alterations in steroid levels to whole animal reproductive fitness, as measured by e.g. gonad size, fecundity and egg size, varies between fish species and mills (Munkittrick et al. 1994; Van der Kraak et al. 1998).

In the ecosystem context, clear cause-effect relationships between exposure and reproductive effects in most studies are often difficult to establish and the precise compounds responsible for the reproductive effects have not yet been identified. Nevertheless, there is evidence that wood derived compounds such as, sterols, lignans, stilbenes and resin acids with their microbial metabolites (e.g. retene) are responsible for the observed effects (Mellanen et al. 1996; Van der Kraak et al. 1998; Lehtinen et al. 1999; Billiard et al. 1999). For instance, waterborne exposure to ß-sitosterol (the major sterol compound in pulp mill effluents) demonstrated decreased plasma steroid hormone levels in goldfish (MacLatchey et al. 1997). ß-sitosterol also caused a reduction in the capacity of goldfish gonadal tissues to secrete steroid hormones in vitro (MacLatchey et al. 1997; Van Der Kraak 1998) and induced the expression of VTG in juvenile rainbow trout (Mellanen et al. 1996) and VTG synthesis in male goldfish (MacLatchey et al. 1995). ß-sitosterol was found in the bile of roach in this study (III) and in the bile of Crucian carp exposed to pulp mill effluents (Kukkonen et al. 1999) indicating that ß-sitosterol is bioavailable to feral fish living in polluted waters.

In experimentally exposed juvenile whitefish in the spring of 1997 (Karels et al. unpublished), no significant changes in plasma reproductive steroids and vitellogenin were observed. On the other hand, a laboratory simulation revealed that reproductive steroid levels were decreased in juvenile whitefish exposed for 1 month to effluents from mill A in 1995 (Soimasuo et al. 1998b). Furthermore, the vitellogenin gene expression in experimentally exposed whitefish in the vicinity of mill B was significantly increased in 1995 (Mellanen et al. 1999), before installation of the novel activated sludge treatment process. Because Mill B had not yet employed the new effluent treatment facilities in 1995, it discharged higher amounts of wood-derived compounds compared to the other mills.

5.4 Hematological and other physiological responses

Hematological parameters

Hematological parameters like plasma glucose or lactate and Hb or Hct may indicate effects on carbohydrate metabolism and oxygen transport capacity in fish. Disturbances in the carbohydrate metabolism have been observed earlier in fish exposed to pulp mill effluents (Anderson 1988). Before the introduction of ECF bleaching and modern effluent treatment technologies, a reduction in the red blood cell number or hemoglobin concentration has been a common effect in fish exposed to pulp and paper mill effluents (McLeay 1973; Oikari et al. 1988; Soimasuo 1995). Resin acids appeared to cause the breakdown of red blood cells (Bushnell et al. 1985 Mattsof & Nikinmaa 1987).

In this study, similar to the case in 1995 (Soimasuo et al. 1998a), plasma glucose and lactate concentrations in experimentally exposed whitefish were significantly changed at 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. water temperature. This suggests that the changed concentrations found in the subareas B and C were not related to pulp mill effluent. Overall, in the Southern Lake Saimaa in the late 1990s, blood glucose or lactate and Hb or Hct indicated minor or no effects on carbohydrate metabolism and oxygen transport capacity in fish exposed to modern ECF type of pulp mill effluents. Moreover, the situation for these parameters is similar and has stabilized since 1993.

Immunocompetence
Several environmental factors have been found to affect the immunity system in fish, including pulp and paper mill effluents (Jokinen et al. 1995). Earlier, in 1991, decreased plasma IgM levels were observed in whitefish subchronically exposed to effluent of mill A (Soimasuo et al. 1995). Actually, however, the recovery of apparent immunodeficiency ceased within one year after the process changes at mill A in 1992 (Petänen et al. 1996). In the present study the immunocompetence of roach (I, III) and experimentally exposed whitefish (IV, V), as measured by plasma IgM, was not affected at the mill sites, verifying the continuously recovered ecosystem condition in this sense.

Gross indices
Disturbances in the condition factor (CF) have been reported earlier in fish exposed to pulp mill effluents (Sandström 1996). In the review by Sandström (1996), covering 25 reported CF estimations, a higher CF was noted in 12 studies (sometimes, however only in either sex) and a lower CF only in two studies. The CF may be affected, if food assimilation is limited or if food consumption is impaired due to stressors. In this study, the general condition of the fish was similar at the mill and reference sites.

An increased liver somatic index (LSI) has often been observed in fish exposed to pulp mill effluents (Andersson et al. 1988; Hodson et al. 1992; McMaster et al. 1991; Huuskonen & Lindström-Seppä 1995). It can be associated with the elaboration of cellular structures such as the endoplasmatic reticulum for protein synthesis (e.g. CYP) (Andersson et al. 1988), with a high carbohydrate diet resulting in carbohydrate storage (Dixon & Hilton 1985), or with alterations in the osmoregulation (Oikari & Nakari 1982). In this study, a higher LSI was measured downstream of mill A in female and male roach (I, II and III) and in female (I) and male perch (II). Downstream of mill B, a higher LSI was measured in female roach and male perch in the spring of 1996 (II). These results are in accordance with an increased capacity of biotransformation of the liver or indicate alterations in energy storage in exposed fish.

5.5 Population and community responses

Contamination by pulp and paper mill effluents has been associated with habitat alteration and subsequent effects on the population (Hansson 1987; Neuman & Karås 1988; Sandström et al. 1988, 1996; Karås et al. 1991; McMaster et al. 1991; Munkittrick et al. 1991; Adams et al. 1992; Hakkari 1992) and community levels (Neuman & Karås 1988; Adams et al. 1992; Hakkari 1992). Effects were associated with the grade of eutrofication due to nutrient enrichment, low oxygen concentrations and chemical toxicity by pulp and paper mill effluent compounds. Repelling compounds in the effluents are also expected to have an effect on fish populations (Myllyvirta & Vuorinen 1989; Hakkari 1992). Other studies, on the other hand, revealed only minor effects from pulp and paper mill effluents at the population and community levels

(Hodson et al. 1992; Swanson et al. 1994; Landner et al. 1994). The majority of the population studies mentioned were conducted in areas receiving poorly treated effluents from mills often using elemental chlorine in the bleaching of pulp.

Population responses

Sandström (1996) reviewed the impact of pulp mill effluent on life-history variables in fish. The review covered 30 North American and Swedish field studies conducted from 1983 to 1993. Although Finland has a long history of pulp mill research no similar field studies were included in that review. In the review, population indicators describing growth and reproduction, such as condition, age at maturity, gonad weight and fecundity were summarized. Growth rate was reported in 19 studies covering 15 mills and 6 species, but most studies were unable to show any differences. The Swedish studies on perch, however, often demonstrated a positive growth effect of exposure to pulp mill effluents. A stimulated energy allocation to storage became clearer when the condition of fish was observed. A higher CF was noted in 12 studies (sometimes, however only in either sex) and a lower CF only in two studies. In the present study, because there were differences between the reference areas, the growth and condition of perch and roach populations near the mills were similar to those at the reference areas.

Sandström (1996) reported a significant delay in sexual maturation or age at maturity in 8 of the 10 populations investigated. A reduced relative gonad size was observed in 14 of the 24 reported studies on gonad development. The small amount of fecundity studies (5) usually supported the GSI data. In the present study, age at maturation in perch and roach populations near the mills was no different from that at the reference areas, while gonad size was lower in perch at the mill sites but not in roach. A lower egg size and fecundity was found in perch downstream of mill B. In roach and fish downstream of mill A, however, no differences were observed.

Densities and biomass of perch and roach in the Southern Lake Saimaa were highest in the polluted area and lowest in the reference area and at a more polluted area close to mill A. The perch population in the recipient of mill A was dominated by relatively smaller and younger individuals and exhibited a smaller range of age classes than at the other study sites. This is similar to an earlier study of Swanson et al. (1996), which reported that fish populations in the vicinity of a pulp mill were younger and smaller than at the reference point. Munkittrick et al. (1991) and Adams et al. (1992) on the other hand, reported that fish populations near pulp mills were older and larger than at the referenc points. Recruitment failure was suggested by Adams et al. (1992) as the most likely alternative to size-selective mortality of younger fish as a mechanism for explaining the skewed population structure.

The year-classes of perch caught downstream of mill A in the present study (1995, 1996 and 1997) were born after the major process alterations at the mill A took place in 1992. This is indicative of prevailing recruitment failures of perch downstream of the mill before 1992. Accordingly, Karås et al. (1991) reported that the recruitment of perch was seriously affected downstream of a bleached sulphate pulp mill, without biological treatment of effluent. Hence, the high abundance of young year classes of perch downstream of mill A in the present study is a sign of recovery of the habitat quality in this area.

Community responses

Effects of pulp and paper mill effluents at the fish community level have been studied earlier in coastal waters of the Baltic Sea (Hansson, 1987; Neuman & Karås 1988; Landner 1994), in a North-American river system (Adams et al. 1992) and in Finnish inland waters (Hakkari 1992). Catches in the polluted coastal areas of the Baltic Sea were dominated by perch, roach and ruffe. Catches of roach and ruffe increased in the vicinity of the pulp and paper mills, regardless of the production process (Hansson 1987; Neuman & Karås 1988). Species such as herring, perch and sand-goby occurred in lowered densities. The study of Landner et al. (1994), however, exhibited minor effects of pulp and paper mill effluents on the composition, abundance and biomass of the fish community downstream of the studied mill. However, like many other coastal areas in the Baltic Sea, effluents are rapidly and extensively diluted and dispersed, being subject to around 1000-fold dilution within 3-4 km, so the exposure of local fish populations to effluent was assumed to be very low (Landner et al. 1994).

Adams et al. (1992) reported an imbalance in the trophic structure of the fish community and a much lower species richness and composition in a river contaminated by pulp mill effluents. The river fish community in this study was classified by an index of biotic integrity, intended to reflect the overall fish community health.

In Finnish inland waters Hakkari (1992) reported highest total catches in areas polluted by pulp mill effluents. Bream, perch, roach, blue bream, ruffe and bleak dominated gill net catches in the polluted areas, while vendace, whitefish, smelt, perch and roach dominated gill net catches in the background oligotrophic areas. Nevertheless, fish density was very low close to the mills (0-5 km) and fish fry were absent for up to 15 km downstream. After the introduction of activated sludge treatment, however, fish fry of roach and perch were seined even at a distance of 7 km from the mill, while species diversity was increased (Hakkari 1992).

Earlier fish community studies with gillnets in the Southern Lake Saimaa (Heinonen & Falck 1971; Sauvonsaari 1974) showed that the fish community in polluted waters was dominated by ruffe, roach, perch, bleak and bream. More sensitive species like vendace and whitefish avoided polluted areas. During this period, however, the mills used solely elemental chlorine bleaching processes and the mechanical purification of effluents.

In the present study, conducted in the Southern lake Saimaa about 20-25 years later, perch and roach dominated the fish communities in the study areas and the densities and biomass of fish were highest in the most loaded area. Fish densities at the reference area and close to mill A, however, were lowest. Bleak and ruffe are typical species for the polluted area, while vendace and whitefish are typical for the reference and intermediate area. Compared to the earlier studies at the lake (Heinonen & Falck 1971; Sauvonsaari 1974), the most remarkable change was the decrease in ruffe and an increase in perch and whitefish in the catches in the polluted area. The improvement of the water quality during the early 1990s possibly contributed to these changes in the fish community. It is evident, however, that the fish community and population structures still vary in relation to effluent loading by modern pulp and paper mills.

Stony shore fish community

Stony shores are one of the main shore types in the Southern Lake Saimaa. The species composition of the stony shore fish community in the present study was comparable with the results of an earlier study in clean areas of Lake Saimaa (Bagge & Hakkari 1985), stone loach and bullhead being caught regularly in all clean areas. The main difference was the absence of minnow in the polluted area. Similar studies at stony shores of Lake Päijänne also exhibited an absence of minnow in areas polluted by pulp mill effluents (Bagge & Hakkari 1992). Results from the polluted areas in the Southern Lake Saimaa confirm that minnow seems to be a more sensitive indicator species to pulp mill effluents, apparently even when ECF bleaching and activated sludge treatment processes are applied at the mills.

Vendace larvae occurrence

Annual differences in larval densities are typical to vendace in Finnish lakes (Viljanen 1988; Hakkari & Bagge 1992). Although factors affecting the recruitment are being extensively studied, the annual variations in year-class strength of vendace seems to be unpredictable. However, estimates of larval abundances after the first three weeks after hatching are a reasonably reliable predictor of the subsequent recruitment of the year (Karjalainen et al. 2000).

In an earlier study in Central Lake Päijänne, Hakkari & Bagge (1992) exhibited large spatial and annual differences in vendace larvae densities, while in an area polluted by pulp mill effluents vendace larvae were caught only occasionally. Despite improvements in the water quality in Central Lake Päijänne in the 1980s due to replacement of the sulfite by a thermomechanical process at the mill discharging into the lake, the reproductive success of vendace remained low.

In an earlier study at the Southern Lake Saimaa in 1984, before the major process changes at the mills, Viljanen (1985) reported that vendace spawned in the intermediate polluted area and clean reference areas. However, the number of eggs deposited on the bottom and larval densities were lower in the intermediate polluted area than in the clean reference area. Polluted sites close to the mills, however, were not sampled in the study of Viljanen (1988).

In the present study (1995-1998), vendace larval densities 3-5 weeks after the melting of the ice (May-June) were similar between the study areas. This indicates that vendace, an autumn spawning species, is spawning in the polluted areas and that eggs are able to develop and survive on the bottom of the polluted area during the winter period. This is an emerging sign of recovery as expressed as tolerance of eggs and larvae to the sediment and water quality in this area.

5.6 Assessment of and relationships between responses

It is obvious that the fish community in the polluted areas differed from the reference area. The densities of more sensitive species like vendace, whitefish and minnow were low in the polluted area, although there are signs of recovery of these populations. On the other hand, the densities of perch and roach populations were relatively high in polluted waters, and these species seem to be better adapted to exposure to the pulp and paper industry activities. Although the use of less tolerant species is ecologically more relevant, perch and roach were chosen as the target species because their populations were relatively easy to sample in the study areas, allowing proper upstream – downstream comparisons.

A summary of whole organism, physiological and biochemical responses to pulp and paper mill effluents in feral perch and roach and experimentally exposed juvenile whitefish is given in Table 4. Reproductive dysfunction in perch and roach near the mills is suggested by the lower levels of estradiol-17ß and testosterone, this coinciding with a lower gonad size and fecundity in female perch but not in roach. At the same time higher concentrations of resin acids and ß-sitosterol in the lake water and in the bile of exposed fish were observed. This suggests that the observed reproductive responses in perch were related to the presence of pulp and paper mill effluent compounds in the environment of the fish. The results confirms earlier studies which reported that there is evidence that wood derived compounds, such as, sterols and resin acids are responsible for reproductive effects in fish (Mellanen et al. 1996; Van der Kraak et al. 1998; Lehtinen et al. 1999). Concentrations of bile chlorophenolics and the liver EROD activity in feral and caged fish, however, were almost comparable to reference levels at the end of this study in 1997. This confirmed that the exposure to chlorophenolics and CYP 1A inducing compounds has become further reduced after the introduction of ECF bleaching and activated sludge tecnologies and that these compounds are probably not responsible for the observed reproductive effects. The observed differences in reproductive responses between perch and roach are assumed to be associated with species differences in food preference, reproduction, endocrinology and physiology. Of the two species, roach seems to be better adapted to pulp mill effluent exposure. The differences in the biochemical and physiological responses of fish between the mill sites are most likely due to the differences in the hydrology and the dilution of effluents. Near mill A, effluent concentrations were distinctly higher and the exposure of fish less variable than near mill B.

In order to assess the fish population responses to industrial discharges, more holistic methods were described by Colby (1984) with further considerations by Munkittrick & Dixon (1989a,b) and Gibbons & Munkittrick (1994). For ease of presentation, Gibbons & Munkittrick (1994) reorganized the monitoring framework by reducing the description of response characteristics to the basic groups which describe age structure, energy expenditure and energy storage. The age structure group included mean age or age distribution; energy expenditure included growth rate , gonad weight, fecundity and age at maturity wheras energy storage included body condition, liver weight and lipid levels. Fish populations from a study area are described in terms relative to reference fish and may exhibit an increase, decrease or no change at all in response characteristics (Gibbons & Munkittrick 1994).

TABLE 4 Comparison of changes in whole organism and physiological parameters in prespawning perch and roach and experimentally exposed juvenile whitefish, 1 km downstream of mill A and B (see Fig. 1) relative to fish from the upstream reference sites in the Southern Lake Saimaa, Finland (0 = no change, + = significant increase, - = significant decrease; $p<0.05$; n.a. = not analysed).

	Perch				Roach				Whitefish	
Sex	Female		Male		Female		Male		Juvenile	
Parameter / Mill	A	B	A	B	A	B	A	B	A	B
Length	-	0	-	0	0	0	0	0	n.a.	n.a.
Weight	-	0	-	0	0	0	0	0	n.a.	n.a.
Mean age	-	0	-	0	0	0	0	0		
Age at maturity	0	0	0	0	0	0	0	0		
Growth	0	0	0	0	0	0	0	0		
CF	0	0	0	0	0	0	0	0	0	0
LSI	+/0	0	+/0	+/0	+/0	+/0	+/0	0	n.a.	n.a.
GSI	0/-	-	0	-	0	0	0	0		
Fecundity	0	-			0	0				
Egg size	0	-			0	0				
Estradiol-17ß	-	0			-/0	0			0	0
Testosterone	-	-	-	0	-/0	0	0	0	0	0
VTG/Ca	0	-	0	0	0	0	0	0	0	0
Plasma IgM	n.a.	n.a.	n.a.	n.a.	0	0	0	0	0	0
Hematological par.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0	0
EROD activity	+/0	+/0	+/0	+/0	+/-	+/-	+/0	+/0	+/0	+/0
Bile CPs	+/0	+/0	+/0	+/0	+/0	0	+/0	0	+/0	0
Bile RAs	+	0	+	0	+	0	+	0	+	0
Bile ß-sitosterol	0	0	0	0	+	+	+	+	0	0

The method adopted by Gibbons & Munkittrick (1994) was further assumed to be suitable for identifying responses of perch and roach populations to the pulp and paper mill discharges in the present study. According to the method, the roach populations near mill A and B were most likely unaffected by stressors, because roach exhibited no changes in age structure, energetic expenditure or energy storage. The observed higher relative liver size in exposed roach and also perch, however, reflects a change in the ability of fish to process energy or metabolic disruptions evoked by xenobiotic chemicals.

The perch population near mill B showed a normal age structure, but the population near mill A was dominated by relatively younger fish. Growth and age at maturity were not affected. The gonad size in perch near the mills tended to be lower, while the relative liver size was often increased. This is indicative of metabolic disruption in perch near the mills. According to the method used by Gibbons & Munkittrick (1994), the observed response pattern indicates that the ability of the perch population to respond to stressors is high and that the population is in an intermediate stage and returning to the reference levels. The fact that year-classes of perch caught near mill A were born after the major process alterations at the Lappeenranta mill in 1992, indicating that the recruitment of perch was seriously affected before it, confirmed the observed response pattern. After 1992, at the same time exposure biomarker responses of feral and experimentally exposed fish downstream of the mill were decreased almost towards reference levels (Oikari & Holmbom 1996; Soimasuo et al. 1998a; I, II, III, IV, V). This verifies the usefulness of biomarkers as possible 'early warning signals' for changes at the population level.

6 CONCLUSIONS

The assesment of actual exposure of fish to pulp mill effluents in the Southern Lake saimaa, based on fish bile conjugates and liver MO activity, indicates that the exposure of feral and experimentally exposed fish to pulp mill effluent compounds has further decreased after the modernization of the mills in the 1990s. In 1997, concentrations of chlorophenolics in the bile and liver EROD activity were almost decreased to reference levels. However, resin acid concentrations in the lake water and fish bile were significantly higher downstream of one of the mills, indicating a continued exposure to resin acids derived from pulp mill effluents.

In the annual reproductive cycle of fish like perch and roach, plasma estradiol-17ß and testosterone concentrations are lowest during the regression period of the gonads and increase during the period of vitellogenesis, the major growth phase of the gonads. Just before and during spawning, estradiol-17ß and testosterone concentrations decrease rapidly towards the regression period levels. Annual patterns of plasma testosterone in male perch and roach were less distinct than in female fish.

During the period of gonadal development, plasma estradiol-17ß and testosterone concentrations in perch and roach caught in the vicinity of the mills were significantly lower compared to the reference sites. This coincided with a lower gonad size and fecundity in female perch at the most contaminated site. Gonad size, egg size, fecundity and plasma and liver cytosol vitellogenin (VTG) in roach, however, remained unchanged.

The general physiological and immunological parameters measured in the present study exhibited minor or negligible differences related to the pulp and paper mill effluent source and distribution. These differences, however, were not effluent related but approached the variability due to natural reasons such as water temperature and seasonality.

A higher liver size was frequently measured in exposed perch and roach indicating a change in the ability of fish to process energy or metabolic disruptions by xenobiotic chemicals. The condition of the fish, however was similar.

The age structure of perch populations in the recipient area with the highest effluent concentrations was shifted to younger fish compared to the other study areas. Growth and age at maturity of perch and roach populations in the recipient areas were similar to those at the reference areas.

The fish communities in the study areas at the lake were dominated (> 60%) by perch and roach. Biomass and fish densities were highest in the polluted area (5-15 km from the mills). The number of species were similar among the areas. Bleak and ruffe are typical species for the polluted area, while the densities of more sensitive species, like minnow, vendace and whitefish, are low in areas affected by pulp and paper mill effluents.

The results indicate that despite decreased exposure of fish to pulp mill effluent compounds, the reproductive status of perch, and to a lesser extent roach, was affected in the recipients of the mills studied.

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YHTEENVETO

Sellu- ja paperiteollisuuden jätevesien ekotoksisuus kaloille. Tutkimus kalojen biokemiallisista, fysiologisista sekä populaatio- ja yhteisövasteista

Väitöskirjatyössä tutkittiin Etelä-Saimaalla sijaitsevien kolmen sellu- ja paperitehtaan alapuolisten vesialueiden ja vertailualueiden ahven- ja särkipopulaatioita, alueiden kalayhteisöjä kokonaisuudessaan sekä alueilla kokeellisesti sumputettuja ei-sukukypsiä siikoja. Kaikki alueen tehtaat käyttivät tutkimuksen aikana alkuainekloorivapaata (ECF) sellun valkaisua, ja tehtaiden jätevesien käsittely suoritettiin aktiivilieteprosessin avulla. Tehtaiden jätevesialtistuksen osoittimet, kalan sapen kloorifenolisten yhdisteiden pitoisuudet sekä maksan vierasaine-entsyymin (EROD) aktiivisuudet olivat tehtaiden lähialueiden kaloissa lähes samantasoisia vertailualueiden kaloihin nähden. Toisaalta kalojen sapen hartsihappopitoisuudet kohosivat vertailualueisiin nähden tehtaiden lähialueilla 10-90 –kertaisiksi. Ahvenen ja särjen veriplasmasta lisääntymisjakson eri vaiheissa mitattujen muuttujien, 17ß-estradiolin sekä testosteronin, pitoisuudet olivat gonadien kypsymisvaiheessa tehtaiden alapuolisilla vesialueilla merkittävästi alhaisemmat kontrollialueisiin verrattuna. Alentuneiden kalojen sukupuolisteroidien lisäksi tehtaiden alapuolisten vesialueiden naarasahventen gonadien koko oli pienempi ja fekundideetti alhaisempi kuin vertailualueilla. Koirasahventen gonadien koko, samoin kuin särkien gonadien ja mätimunien koko, fekundideetti sekä veriplasman ja maksan vitellogeniinin (VTG) pitoisuudet eivät tehtaiden lähialueilla poikenneet vertailualueista. Jätevesille altistuneissa ahvenissa ja särjissä havaittu suurempi maksan kokoindeksi viittasi kuitenkin kalojen energiametabolian tai ravitsemustilan muuttuneen. Kuntoindeksin sekä hematologisten ja immunologisten muuttujien perusteella jätevesille altistuneet kalat eivät poikenneet vertailuryhmistä. Yhden tutkitun tehtaan alapuolisella vesialueella havaittiin poikkeavuuksia ahvenpopulaation koko- ja ikäjakaumassa. Tutkittujen tehtaiden alapuolisten ahven- ja särkipopulaatioiden kutukäyttäytyminen ei poikennut vertailualueista, kuten ei kasvu eikä myöskään sukukypsyysikä. Tutkimusalueiden kalastosta ahven ja särki muodostivat yli 60 % kalabiomassasta ja yksilömäärästä. Kalabiomassan sekä kalatiheyden havaittiin olevan suurimmillaan 5-15 km etäisyydellä tehtaista sekä pienimmillään vertailualueilla ja tehtaiden välittömässä läheisyydessä, 2-5 km:n etäisyydellä tehtaista. Kalalajimäärä oli jätevesien vaikutusalueilla ja vertailualueilla yhtäläinen. Salakka ja kiiski olivat tyypillisiä lajeja jätevesien vaikutusalueilla, kun taas siika ja mutu välttivät jätevesien vaikutusalueita. Huolimatta 90-luvulla tapahtuneesta alentuneesta jätevesialtistuksesta, tutkimus osoitti jätevesien vaikuttavan ahvenen ja vähemmässä määrin särjen lisääntymiskuntoon, steroidihormonisäätelyyn, gonadien kokoon ja hedelmällisyyteen. Vaikka kalalajistojen palautumista on tapahtunut, on ilmeistä, että sekä nykyinen sellu- ja paperiteollisuuden jätevesialtistus että aikaisempi järven saastekuormitus edelleen vaikuttavat alueen kalapopulaatioihin ja kokonaiseen kalayhteisöön.

SAMENVATTING

Ecotoxiciteit van afvalwater van pulp- en papier fabrieken in vis. Een onderzoek op biochemisch, fysiologisch, populatie- en gemeenschapsniveau

Dit proefschrift beschrijft een onderzoek naar effecten van afvalwater van pulp- en papier fabrieken in vis. Het onderzoek werd uitgevoerd aan baars (Perca fluviatilis L.) en blankvoorn (Rutilus rutilus L.) populaties en visgemeenschappen in vervuilde en referentie gebieden in het zuidelijke deel van het Saimaa meer in Z-O Finland. Tijdens de onderzoeksperiode (1995-1997) werden tevens kooistudies met grote marene (Coregonus lavaretus L.) uitgevoerd. De pulp en papierfabrieken in deze studie gebruikten elementair chloor voor het bleken van pulp, terwijl het afvalwater werd behandeld in actief slib installaties. De blootstelling van vis aan afvalwater, gemeten aan de hand van concentraties van chloorphenolen in de gal van vis en de EROD activiteit, was laag en vergelijkbaar met vis in de referentiegebieden. Desondanks waren concentraties van harszuren in de gal van blootgestelde vis 10-90 x hoger in vergelijking met vis uit de referentiegebieden. Voortplantings parameters zoals plasma estradiol-17ß en testosteron concentraties in baars en blankvoorn in de verontreinigde gebieden waren significant verlaagd. Ovaria-grootte en fecunditeit in vrouwelijke baars in de verontreinigde gebieden waren tevens verlaagd. De testis-grootte in mannelijke baars was echter onveranderd. In blankvoorn waren voortplantings parameters zoals ovaria en testis grootte, ei-grootte, fecunditeit en vitellogenine (VTG) in plasma en lever cytosol onveranderd. Een hoger relatief lever gewicht in baars en blankvoorn in de verontreinigde gebieden duidde op veranderingen in het metabolisme en de voedingstoestand van de vis. De conditie en hematologische en immunologische parameters in vis in de verontreinigde gebieden waren echter onveranderd. De leeftijds- en grootte verdeling van de baars populatie in de nabijheid van een van de fabrieken was verschillend in vergelijking met de andere gebieden. Paaigedrag, groei en leeftijd van volwassenheid waren gelijk tussen fabrieks- en referentiegebieden. Visgemeenschappen in de studiegebieden werden gedomineerd (>60%) door baars en blankvoorn. Biomassa en dichtheden van vis waren het hoogst in de vervuilde gebieden (5-15 km stroomafwaarts van de fabrieken) en het laagst in het referentiegebied en in de nabijheid (2-5 km) van de fabrieken. De soortendiversiteit was gelijk tussen de onderzoeksgebieden. Soorten zoals alver en pos zijn typisch voor vervuilde wateren, terwijl soorten als kleine- en grote marene en elrits de vervuilde gebieden vermeden. Resultaten van deze studie laten zien dat ondanks een verlaagde blootstelling van vis aan afvalwater van pulp en papier fabrieken gedurende de negentiger jaren, voortplantings parameters, zoals steroid hormoon concentraties, ovaria grootte en fecunditeit waren beinvloed in baars en in mindere mate in blankvoorn in wateren vervuild door afvalwater van de pulp- en papierfabrieken. Ondanks tekenen van herstel, is het duidelijk dat visgemeenschappen en vispopulaties nog steeds verschillen in relatie tot huidige en/of historische vervuiling door afvalwater van de pulp- en papierfabrieken.

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ORIGINAL PAPERS

I

Effects of ECF bleached kraft mill effluent on reproductive steroids and liver MFO activity in populations of perch and roach

Aarno Karels, Markus Soimasuo, Jarmo Lappivaara, Harri Leppänen, Tuula Aaltonen, Pirkko Mellanen & Aimo Oikari, 1998

Ecotoxicology 7: 123-132

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II

Effects of pulp and paper mill effluents on the reproductive and physiological status of perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) during the spawning period

Aarno Karels & Aimo Oikari, 2000

Annales Zoologici Fennici 37: 65-77

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III

Reproductive responses, bile metabolites and liver EROD activity in prespawning perch and roach exposed to pulp and paper mill effluents

Aarno Karels, Eveliina Markkula & Aimo Oikari

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IV

Biomarker responses in experimentally exposed fish and feral fish in a lake receiving pulp mill effluents

Aarno Karels, Markus Soimasuo & Aimo Oikari

Aquatic Impacts of Pulp and Paper Effluents
Proceedings of the 3rd International Conference on Environmental Fate and Effects of Pulp and Paper Mill Effluents

SETAC press, Lewis Publishers, Boca Raton (in press)

V

Monitoring the recovery of a lake with aid of fish biomarkers: Responses of whitefish (*Coregonus lavaretus* L. s.l.) experimentally exposed in a large lake receiving pulp and paper mill effluents

Aarno Karels, Markus Soimasuo, Riku Suutari & Aimo Oikari, 2000

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VI

Fish community responses to pulp and paper mill effluents at the Southern Lake Saimaa, Finland

Aarno Karels & Asko Niemi

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