

Annual Review

THE HAZARD ASSESSMENT OF PULP AND PAPER EFFLUENTS IN THE AQUATIC ENVIRONMENT: A REVIEW

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Abstract—The hazard assessment of pulp and paper effluents in the aquatic environment is a complex task. Hundreds of individual compounds in pulping effluents and site-specific differences in processes, effluent treatment, and receiving ecosystems hinder hazard assessment. As a result, it is difficult to relate environmental effects with specific contaminants. Conventional parameters such as organic and nutrient loadings, solids deposition, and color complicate efforts to define chemical toxicants by causing environmental impacts at community and population levels. Reproduction is the most sensitive, consistent, and relevant end point tested to date in the laboratory, in mesocosms and experimental streams, and in field situations near some pulping discharges. Despite the application of a wide range of within-organism measurements, only the induction of mixed-function oxidase activities has been associated with exposure to particular effluent compounds in field situations. No complete mechanism of toxic action has been demonstrated that connects contaminant exposure, within-organism responses, whole-organism effects, and effects at the population and the community levels. Hazard assessments of pulping effluents require multidisciplinary efforts that integrate chemical, toxicological, and biological data at several organizational levels. Tiered or stepwise assessments are recommended that first clarify what adverse effects have occurred and then attempt to identify the responsible contaminants.

Keywords—Hazard assessment Organochlorines Paper Pulp

INTRODUCTION

The industrial conversion of wood into fiber and paper occurs primarily in the Northern Hemisphere, and environmental research on the aquatic impacts of these industrial effluents has been concentrated in Scandinavia and North America. Regulatory intervention before the 1980s focused on conventional aquatic pollutants such as oxygen depletion, suspended solids, and both organic and nutrient loading. Current regulatory concerns have shifted to chemical toxicity. Toxicity control strategies vary from single-species chronic toxicity tests in the United States to discharge limits for specific classes of compounds such as organochlorines in Sweden [1].

The major objectives of a hazard assessment are to identify which agents cause environmental impacts and to define a no-observed-adverse-effect level (NOAEL). At issue for ecotoxicologists are the appropriate experimental designs and measurement systems to accomplish these objectives. Pulp and paper discharges represent difficult candidates for hazard assessment due to the interaction of

complex effluents with site-specific factors. The following baseline data are needed at each site: (a) the chemistry and variability of the operating processes, (b) the chemistry, available dilution, and turnover of the receiving waters, and (c) the sensitivity of the local ecosystem to enrichment and toxicants. In addition, sophisticated spatial and temporal sampling is necessary to coordinate chemical and biological data for interpretation. If information for the site is unavailable or insufficient, a stepwise or tiered design might be necessary. In such cases, preliminary studies would seek (a) to identify which pollutants and chemical contaminants were present, (b) to define the zone of contamination, and (c) to discover what biological effects might have occurred. Subsequent protocols would then be designed to identify the contaminant(s) causing these effects and to establish a NOAEL for the contaminant(s) [2].

Ecological responses are measured at organizational levels that range successively downward through communities, populations, whole organisms, and then within organisms at further decreasing levels such as organ, tissue, cell, subcellular,

and molecular. Ecologically relevant end points should be defined regardless of the organizational level [3]. Traditional field measurements have used the structure and functions in communities to determine the following relevant or meaningful effects: the diversity, richness, and abundance of species; the survival and reproduction of species; and the presence or absence of species that respond to polluted conditions. Changes in these parameters have provided evidence for either adverse impacts or a presumption of ecological health. However, in complex mixtures such as pulping effluents, field measures cannot identify which contaminants have caused toxic effects. Therefore, the development of new measurement systems is necessary.

Sublethal measurements, particularly at the within-organism level, may address exposure, assess individual health, or diagnose stress on key end points such as reproduction. Ideal measurements respond to chemical toxicity with the following attributes:

1. a response linked to meaningful effects at community and population levels
2. a dose-dependent or threshold response at environmental levels of contaminants
3. a response to only certain identified classes of compounds
4. a response that precedes environmental effects at higher organizational levels [4].

In theory, successive states of health may be envisioned, such as homeostasis with the environment, successful adaptation or compensation leading to an altered homeostasis, and a detrimental effect lowering reproductive success or increasing mortality. In practice, detrimental effects must be identified in organisms that display a highly adaptive physiology to different environmental conditions. Stated another way, detrimental effects must be distinguished from the potentially large range or universe of values reflecting homeostasis in varied habitats. At present, data are limited on those factors that may influence within-organism values such as age, gender, reproductive cycle, season, and so on.

Background for the environmental hazard assessment of pulping discharges

Pulping discharges are generated by processes falling into two basic categories: mechanical and chemical. The mechanical process is based on applying mechanical force with a minimum of chem-

ical treatment to liberate usable wood fiber such as newsprint. The chemical processes depolymerize and dissolve lignin, producing a purified cellulose fiber.

There are two basic chemical processes: kraft or sulfate and sulfite. The modern kraft process dominates the current industry due to the recovery and recycling of chemicals and organics to minimize discharges. Fibers from both kraft and sulfite processes may be treated further to remove or to decolorize residual lignin. Referred to as *bleaching*, this subsequent treatment discharges a range of organic materials. For example, if chlorine agents are used to remove lignin, then a range of organochlorines are discharged. Total discharge load is related to the residual lignin, measured as *kappa number*, which enters the bleaching process (see Fig. 1). The processes and discharge loads have been reviewed [5–11], including the variables of organochlorine formation [11–23].

The biology of wood and the pulping process result in a complex discharge with numerous compounds: extractives, organic acids, a range of sulfur-containing compounds, chlorinated phenolics, chlorinated neutral compounds, and chlorinated organic acids [24,25]. Therefore, sum parameters such as chemical oxygen demand (COD), total organic carbon (TOC), and biological oxygen demand (BOD) are not adequate to characterize the effluent. Several classes are difficult to characterize due to their chemical heterogeneity and their molecular size, especially high molecular-weight degradation products of lignin.

One class of compounds of current concern is organochlorines. Related compounds such as polychlorinated biphenyls (PCBs) have demonstrated environmental persistence, long-range environmental transport, and either bioaccumulation from receiving waters or biomagnification in the food web. Sum parameters for organochlorines have been developed on the basis of elemental chlorine content analogous to the nitrogen Kjeldahl assay: total organic chlorine (TOCl) and adsorbable organic halogens (AOX). The AOX test is now preferred [1,26–28]. About 70% of the AOX under 1,000 Da have been identified as individual compounds [29]. However, a substantial portion of AOX (50–70%) has a molecular weight (mol. wt.) exceeding 1,000 Da [25]. Process conditions, primarily the quantity of molecular chlorine, affect the AOX quantity and the nature of formed individual compounds [12,13,19,20, 30–35]. For example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzo-*p*-furan (TCDF)

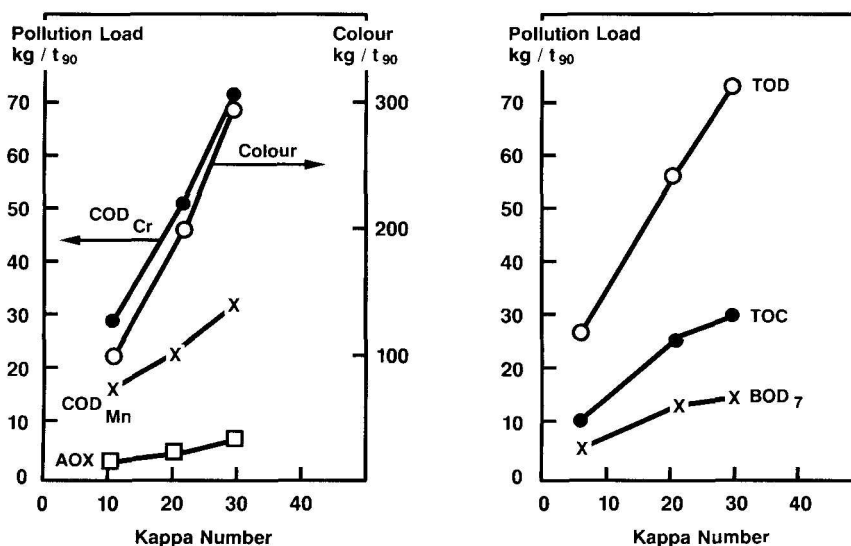


Fig. 1. Relationship between the quantity of lignin entering the bleach plant and pollution discharge loads. Lignin is measured by permanganate oxidation and is reported as a kappa number (x -axis). The discharge parameters have been normalized to the quantity of pulp produced: t_{90} = a metric ton of pulp at 10% moisture. Abbreviations: COD_{Cr}—chemical oxygen demand using chromate; COD_{Mn}—chemical oxygen demand using manganese; AOX—adsorbable organic halogens; TOD—total oxygen demand; TOC—total organic carbon; and BOD₇—biological oxygen demand over a 7-day test period. From Mattinen and Wartiovaara [268] reprinted with permission.

are inadvertently produced by high application rates of molecular chlorine [36–40] in the presence of certain precursors [38]. Despite the aromatic nature of lignin, significant chemical oxidation results in only 1 to 4% of the total AOX partitioning into organic solvents as hydrophobic material [13,41]. The high mol. wt. AOX material has been partially characterized [42–47], and again few aromatic rings escape oxidative cleavage [43,44,47].

The acute toxicity of pulping effluents is primarily due to resin and fatty acids, which are substantially removed by biological treatment [1,24,48,49]. Standardized chronic protocols for effluents are in place for several taxonomic levels [50]. However, these protocols presently address only direct bioaccumulation, not biomagnification or sediment contact and ingestion. Sediment toxicity strategies and protocols are still undergoing development [51] and have not been widely applied to pulping situations. Mutagenic activity has been found in untreated wastewaters from molecular chlorine processes [52–54]. The mutagenic activity rapidly decreases with time, neutral or alkaline pH, or biological treatment [55–57]. Mutagenic activity has been found in receiving waters near pulping discharges, and this activity has been removed by the installation of biological effluent treatment (I.

Smith, personal communication). Mutagenic activity can be prevented by reducing the use of the molecular chlorine or by replacement with chlorine dioxide [52,58].

BIOLOGICAL STUDIES OF PULPING IMPACTS

Poole et al. [59], Pearson [60], and Pearson and Rosenberg [61] concluded that environmental effects occurred in chemical and biological gradients from a discharge, that impacts varied with the quantity and the composition of the effluent, and that conventional pollutants were the primary causes of a characteristic pattern of biological impacts. This review first expands these observations to freshwater conditions and to situations with lower conventional loadings. The application of sublethal measurements at whole-organism and within-organism levels to pulping effluent exposures is then reviewed. In conclusion, a singular case study is reviewed in which effects at several organizational levels were simultaneously measured to gain insight on the mechanisms of effects connecting organizational levels.

Ecosystem field studies

Field studies with community and population end points define whether meaningful effects have

occurred. A substantial body of previous work implicates three conventional pollutant factors in pulping discharges as causing adverse environmental impacts: (a) fiber and suspended solids, (b) color and turbidity, and (c) organic and nutrient enrichment loads. The pattern of impact depends on the total discharge load and the so-called assimilative capacity of the ecosystem.

Fiber and suspended solids. Environmental impacts from the release of fibers and suspended solids are (a) the loss of stationary or poorly mobile benthic organisms and (b) the depletion of feeding and reproductive habitat for mobile organisms. Bacterial activity within fiber deposits can lead to oxygen depletion, particularly in sediments, and generation of hazardous compounds such as hydrogen sulfide [62]. Fiber mats have been observed in riverine [63,64] and marine situations [65,66]. McLeay [24] has reviewed fiber impacts and remediation at British Columbia mills. Other noteworthy studies are the temporal sequences of fiber accumulation at Loch Eil [67,68] and fiber removal in the Saltkällefjord [69–72]. The pattern of impacts was confirmed at paper mills that released converted fiber but not pulping or bleaching chemicals [73–75].

Color and turbidity. Color in pulping effluents absorbs light in a concentration-dependent manner, and may (a) reduce primary production by restricting photosynthesis and (b) diminish visual clues necessary for organisms to feed or to reproduce. Impacts may be further magnified whenever discharges stratify near the surface. Reduced light-dependent ^{14}C assimilation has been correlated with periods of mill operation, distance from the mill discharges, and available dilution [76]. In studies reviewed by McLeay [24], distinct zones of primary productivity losses varied with discharge intensity, season, depth, and distance from the discharge. At Baltic sites, plankton biomass and chlorophyll concentrations did not decrease as primary productivity fell, and utilization of added organic matter was proposed to replace photosynthetic losses [6,77].

Primary production losses have also been measured in freshwater systems [78,79]. In a riverine study, primary production by stationary periphyton adapted to color, but primary production in phytoplankton was affected when unadapted organisms entered the plume [80]. Controlled exposures in experimental streams have demonstrated a dose response to color in both cold and warm water systems. Chlorophyll levels fell with depth and in-stream color concentration, and species on ar-

tificial substrates shifted from autotrophs to heterotrophs. These results indicated other nutrients replaced photosynthesis [81–83] and supported the hypothesis that organic carbon inputs may compensate for lost photosynthesis.

Organic and nutrient enrichment loads. Pulp and paper mills can discharge significant organic carbon and nutrient loads, which readily alter or degrade local habitats when unchecked [48]. A community and population gradient due to organic and nutrient enrichment has been proposed (Fig. 2) [61]. Field surveys demonstrating a spectrum of sites and discharge loads have been listed showing both chemical (Table 1A) and biological (Table 1B) gradients. The differences in discharge loads result in sharp contrasts: (a) Lakes Keurusselkä and Vanajavesi in Finland, where discharges overwhelmed local ecosystems; (b) Nipigon Bay in Canada, Lake Päijänne in Finland, and Loch Eil in Scotland, having local impacts; (c) recoveries from impacts at the Saltkällefjord in Sweden and the Fox and the Wisconsin rivers in the United States; (d) where no community or population effects have been found at the Sacramento and the Flint rivers in the United States.

These examples demonstrate several inadequacies in study design: the absence of baseline biological characterization prior to mill operation, absent or limited effluent and receiving water chemistry, and absent or limited effluent toxicity characterization. The value of baseline studies is shown by the preoperation benthic characterization at Loch Eil [84,85]. From the baseline conditions, a temporal sequence of impacts was established:

1. stimulation of native species
2. shifts to pollution-tolerant native species
3. colonization by new, previously nonresident species
4. a diminution of total species with high densities of particular pollution-tolerant species
5. a zone depleted to a few individuals tolerating anoxia [86,87].

At other sites, baselines have been reconstructed by using chemical and fossil analysis of undisturbed sediments [88,89].

Many studies omitted chemical and toxicological end points and the biological end points used were not standardized between studies. Effluent characterization rarely extended beyond sum parameters, except at Nipigon Bay [90–92] and Flint River [93]. Toxicity testing was performed only at

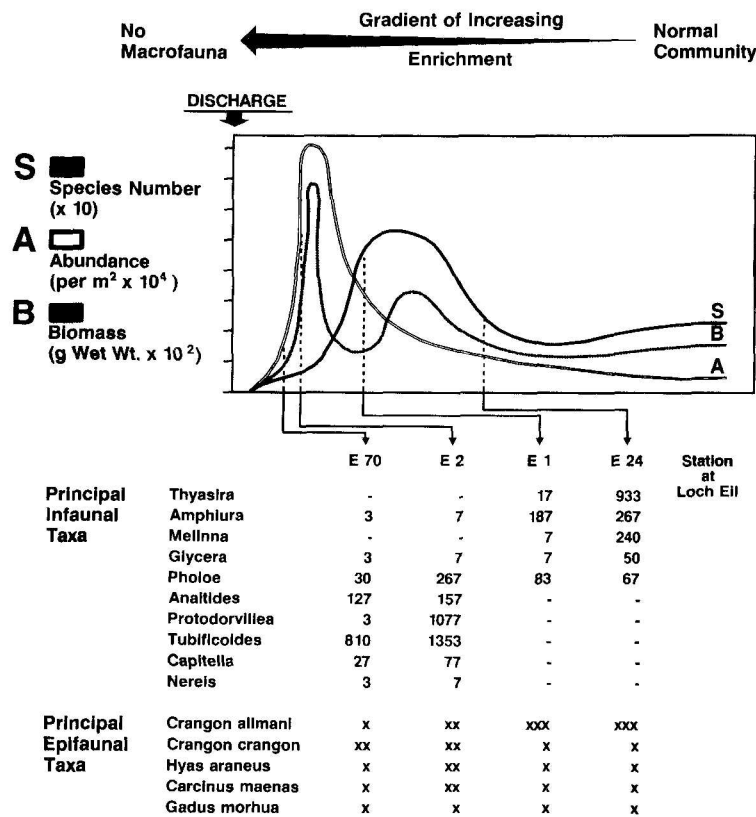


Fig. 2. Model of biological response of benthic infauna to a gradient of organic enrichment by a pulp mill at Loch Eil, Scotland. Response of principal population parameters with distance from an enrichment source. The extremes are the absence of organisms near the discharge and a normal community at some distance from the discharge. The parameters are number of species (S), abundance per square meter (A), and biomass in grams wet weight per square meter (B). The numbers for representative species from each taxon at the stations are given below the figure. Adapted from Feder and Peason [112], reprinted with permission.

Nipigon Bay [94,95], Sacramento River [96], and Flint River [93,97]. A spectrum of population end points were utilized, such as flora [98], benthos [99–102], and zooplankton [103,104] (see Table 1B). In certain studies, several end points were assessed simultaneously: at Lake Päijänne benthos, zooplankton, and fish [79]; at Nipigon Bay bacteria, benthos, and fish [78,105–107]; at Loch Eil bacteria, protozoa, benthos, and fish [108–112]; and at Flint River bacteria, protozoa, diatoms, benthos, and fish [97,113].

The field studies suggest that structural and functional impacts are most evident in the benthos and in primary producers and consumers. A clear interrelationship existed at Nipigon Bay between the discharge gradient, sediment chemistry, benthic impacts, and fish distribution impacts [106,114,115]. Bacterial populations in the sediments were

supported by mill nutrients, including heterotrophic and sulfur cycle organisms [106], and bacterial shifts in composition and densities were considered to be the most sensitive indicators of the mill's spatial influence [116]. At Loch Eil, changes in the sediment chemistry were based on bacterial degradation of cellulose from the discharge [117,118]. The bacterial biomass correlated with the discharge load [108,110], and in turn, the bacteria supported protozoan populations as a food source [109]. At other sites, distinct functional shifts occurred: Zooplankton near the mills were bacterial and detritus feeders versus phytoplankton predators at unpolluted sites in Lake Keurusselkä [103]; fish population distributions were affected in Lake Päijänne [79] and Lake Nipigon [107]; at Flint River the conclusions of no environmental impact were based on the absence of

Table 1A. Summary of process and treatment types, effluent discharge loads, and receiving water analyses and sediment analyses performed in selected environmental studies of pulping effluents

Site	Mill type	Effluent treatment	Discharge (t/d)		Receiving environment	Effluent dilution	Receiving water assays				Sediment assays
			BOD ^a	TSS			Color	O ₂	pH	Other	
Keuruselkä, Finland	BS ^b	None	28	7	FW lake ^c	5–10×	X	X	X	B, L, C, K, N, P ^d	
Vanajavesi, Finland	BS	None	21	13	FW lake	4–50×	X	X	X	C, N, P, COD	
Fox River, WI	BS, BK	None Secondary	226 15	180 17	FW river	Variable		X			
Wisconsin River, WI	BS, BK	None Secondary	400 25	240 55	FW river	Variable					
1516 Saltkällefjord, Sweden	BS	None	30	5	BR to SW fiord	100–300×		X	X	B, L, C, K, N, P	B, K, OC
Lake Päijänne, Finland	BS	None	60	26	FW lake	18–>500×	X	X	X	B, L, C, K, N, P	
Loch Eil, Scotland	BS	None	20–35	15–20	SW fiord	NK		X		B, COD	N, P, OC, S, M
Nipigon Bay, Ontario	UK	Primary	20.6	5.9	FW lake	6–>1,000×		X	X	B, Na, C, R, Ph, N, P, S, COD	R, OC, S, M
Sacramento River, CA	BK	Secondary	1.5	1.7	FW river	130–300×					
Flint River, GA	BK	Secondary	1.1		FW river	120–300×		X	X	N, P, M	

^aBOD – biological oxygen demand; TSS – total suspended solids.

^bAbbreviations for mill process types: BS – bleached sulfite; BK – bleached kraft; UK – unbleached kraft.

^cAbbreviations for receiving environments: FW – freshwater; BR – brackish water; SW – saltwater.

^dAbbreviations for receiving water and sediment analyses: B – biological oxygen demand; L – lignosulfates; Na – sodium; C – conductivity; R – resin; Ph – phenols; K – permanganate demand for lignin products; N – nitrogen; P – phosphorus; OC – organic carbon; S – sulfur; COD – chemical oxygen demand metal analyses.

NK – not known.

Table 1B. Summary of biological end points and effects investigated in selected environmental studies of pulping effluents

Site	Sediment anoxia	Benthic populations										Fish populations		Toxicity tests	References			
		B-P ^a		PC ^b		Other		Phytoplankton		Zooplankton		Periphyton				Survival	Distribution	Reproduction
		B-P ^a	PC ^b	Other	Phytoplankton	Zooplankton	Periphyton	Survival	Distribution	Reproduction								
Keuruselkä, Finland	X			X	X				X									[98,103,264]
Vanajavesi, Finland		X	X	C ₁ M ₁ I ^c														[119,259]
Fox River, WI		X	X	I														[265]
Wisconsin River, WI				I														[266]
Saitkällefjord, Sweden	X			X	X	X	C ₁ M ₁ I ₁ E											[71,72,99-101,260,267]
Lake Päijänne, Finland			X	X	X	X	C ₁ M ₁ I		X				X		X			[79,102,104,154,261]
Lock Eil, Scotland		X	X	X	X	X	C ₁ M		X				X		X			[108-112]
Nipigon Bay, Ontario		X	X	X	X	X	C ₁ M ₁ I		X				X		X			[78,94,95,105-107,267]
Sacramento River, CA					X	X	C ₁ M ₁ I											[96]
Flint River, Georgia		X	X	X	X	X	C ₁ M ₁ I		X				X		X			[93,97,113,263]

^aB-P: bacterial and protozoan populations.

^bPC: polychaete and annelid populations.

^cAbbreviations for benthic population surveys: C – crustaceans; M – molluscs; I – insect larvae; E – echinoderms.

^dAbbreviations for toxicity tests: A – acute; C – chronic.

structural and functional shifts in protozoan and diatom populations at the base of the food web [93,97].

Yearly and seasonal fluctuations have been observed in several studies. Oxygen depletion in Lake Vanajavesi was most extreme after winter ice cover. Anoxia in bottom layers invariably occurred and sporadically reached the lake surface [119]. The contribution of nonpulping discharges to the disruption of the Fox River was found to be highly seasonal; summer algal blooms originating upstream in Lake Winnebago had a major adverse impact [120]. These examples suggest (a) that data be gathered to determine the seasonal period of highest stress in the ecosystem and (b) that extended monitoring may be necessary to understand yearly fluctuations and to establish long-term trends at a site. Extended monitoring may be especially useful to assess remediation efforts.

Discussion of ecosystem field studies. The data support the conclusions that (a) organic and nutrient enrichment have in the past been primary causes of community and population effects and (b) may continue to produce environmental impacts at sites having restricted dilution and turnover, nutrient limitations, or older process facilities or those sites lacking effluent treatment. As these impacts could confound or mask chemical toxicity [48], hazard assessments should be designed to address the degree of enrichment.

Few previous field studies at pulping sites have attempted to either test for chemical toxicity or integrate data from several organizational levels. Only the Nipigon Bay and the Flint River studies approximate an integrated approach with chemical and toxicological characterization of the effluent and thorough community and population surveys. These field results were consistent with the organic and nutrient loads: local impacts at Nipigon Bay and no apparent impacts from either enrichment or chemical toxicity at Flint River.

The studies demonstrate the value of incorporating several population end points in the starting or initial tier of a hazard assessment study. Firstly, the severity of adverse impacts and the most affected populations are identified. These populations can then be targeted for sublethal studies in subsequent study tiers. Secondly, if the degree of habitat change induces a response in sublethal measurements, the community and population data provide the means to select representative control areas. Therefore, community and population end points continue to be necessary (a) to establish whether meaningful effects have occurred, (b) to

assess enrichment impacts, and (c) to guide the application of sublethal measurements.

Sublethal measurements

Sublethal measurements of pulping discharges have been reviewed previously by McLeay [24] and Kovacs [121]. This review concentrates on the measurements that have been most widely applied to pulping effluents: (1) body burdens of pulping compounds, (2) whole-organism effects on reproduction, early life stages, and morphology, and (3) within-organism effects on histopathology, physiology, and biochemistry. Additional measures such as swimming stress have been reviewed by Kovacs [121], and these are not included.

As toxic effects originate at the within-organism level, potential advantages of these measurements are less costly monitoring and an increased sensitivity to detect effects prior to impacts at higher levels [4,122]. However, extensive research has not yet connected within-organism measurements and impacts at the community and population levels. Further, the large natural ranges of within-organism values is not well understood, hindering interpretation of the significance of within-organism measures [122].

Body burdens of pulping compounds. Body burdens estimate toxicant exposure, which arises from direct bioaccumulation, food chain biomagnification, and environmental transformation or internal metabolism. Body burdens of pulping components have been reviewed by McLeay [24]. The number of studied pulping compounds has not substantially increased since the first body burden

observations were made for resin acids [123] and chlorophenolics [124]. Both classes of compounds appear to be bioaccumulated from the water phase, reaching equilibrium in fish within days and exhibiting rapid depuration [124–126]. Thus body burdens of these compounds may reflect only short-term exposure. A more persistent compound, TCDD, has been found below bleached pulp mills [127,128] with an apparent half-life in fish of up to 90 d [129,130].

Extractable organic chlorine (EOX) has been used as a sum parameter to study body burdens of pulping organochlorines in attempts to circumvent the laborious analysis of individual compounds. Extractable organic chlorine is the organically bound chlorine that partitions into nonpolar solvents. Noteworthy findings are: (a) an EOX background exists in addition to pulp sources [131–133], (b) contaminated sediments appear to be reservoirs for EOX [131], (c) in some cases a substantial portion of EOX can be identified as pulping compounds (chlorophenolics) [134], and (d) some EOX can be incorporated in lipid via ester bonds [135].

Oikari and coworkers analyzed effluent, receiving water, body burdens, and within-organism responses at Lake Saimaa, Finland. The bleached kraft mill discharged 17.5 t of BOD and 9 t of suspended solids per day [5] with effluent dilutions of 2 to 7% in the study area [136,137]. Water column chlorophenolic levels are given in Table 2. Caged rainbow trout (*Salmo gairdneri*), caged native plankton-feeding whitefish (*Coregonus muksun*), and native feral fish (perch, roach, rudd, pike, and bream) were used [126,136,138–142]. The biocon-

Table 2. Water column AOX and chlorophenolic concentrations from selected studies

	Site: Norrsundet		Saimaa	Experimental streams		
	Distance from discharge: Estimated dilution:	2 km 1.2%	4.5 km 0.8%	3 km 4.0%	Warm 12.2%	Cold 1.2%
AOX (ppb) ^a		260	—	—	1,400	670
Chlorinated phenols (ng/L) ^b						
2,4,6-Trichlorophenol		370	180	1,000	500	228
Tetrachlorophenol		84	147	600	100	46
3,4,5-Trichloroguaiacol		866	445	700	1,200	268
4,5,6-Trichloroguaiacol		118	28	300	500	168
Tetrachloroguaiacol		1,200	500	400	600	234
3,4,5-Trichlorocatechol		—	—	700	1,600	562
Tetrachlorocatechol		1,340	148	500	600	806

^aAOX data for Norrsundet from Sodergren [201] and for experimental streams from NCASI (D. Borton, personal communication).

^bChlorophenolic Norrsundet data are from Xie et al. [204], Lake Saimaa from Oikari et al. [137], experimental streams from NCASI for warm water [164] and cold water [150,165].

centration factors (BCFs) in rainbow trout plasma ranged from 80 to 800 for resin acids and from 100 to over 1,200 for chlorophenolics. An apparent relationship between bioconcentration and the degree of chlorine substitution of the phenolic ring was observed [136,139]. Both chlorophenolic and resin acid concentrations were highest in the bile and were excreted as glucuronide conjugates [126,143,144].

Whole-organism effects Reproductive and early life stage responses to pulp mill discharges have been studied under both controlled and field conditions. In chronic laboratory studies, sheepshead minnow (*Cyprinodon variegatus*) embryo and fry mortality increased sharply at low dissolved oxygen levels [145], suggesting toxicity testing also be performed with oxygen levels reflecting field conditions. Biologically treated bleached kraft mill effluent (BKME) had no effect on egg development or hatching [146] or larval survival, growth, and condition factor [147] of striped bass (*Morone saxatilis*) at effluent concentrations under 12%. When Tana and Nikunen [148] tested egg mortality in pike (*Esox lucius*) against incompletely treated effluent, the effect concentration (EC50) was only 3.5% effluent. Egg numbers and larval growth of the blenny (*Zoarces viviparus*) were affected by untreated effluent at 0.5% concentrations [149]. These results suggest large site-to-site variabilities in the potential for effluent impacts and an inverse relationship between effect and effluent treatment. In the future, reproductive and early life stage toxicity testing with standardized protocols is suggested for each effluent.

Extensive reproductive studies are available from laboratory and experimental stream exposures to biologically treated BKME. The no observed adverse effect levels (NOAELs) for reproductive success for rainbow trout and largemouth bass (*Micropterus salmoides*) were 2 and 4% BKME concentrations, respectively, after nearly four years of continuous exposure [150] (D. Borton, personal communication). In parallel laboratory studies, fathead minnows (*Pimephales promelas*) were continuously exposed to BKME through four life cycles. The reproductive and early life stage NOAELs were 6% effluent for egg production, 10% for larval growth, 32% for larval survival, and 56% for egg hatchability [151]. In additional experiments, adult golden shiners (*Notemigonus crysoleucas*) were exposed to BKME, and their spawn were tested in the laboratory. Even with continued exposure of the eggs, hatchability and larval survival were unaffected versus hatchability and larval survival of

controls at BKME concentrations up to 32% [151]. Thus under controlled conditions and low dilutions, biologically treated BKME does not appear to affect reproduction or increase early life stage mortality.

Different BKMEs from processes using softwood trees have been compared in mesocosms. Aside from sum parameters, detailed chemical and toxicity analyses of the effluents were not performed. The early life stages of three-spined stickleback (*Gasterosteus aculeatus*) were assessed during five-month exposures to the BKMEs. Untreated BKME (1.0% effluent) from a molecular chlorine bleachery was lethal. At lower doses (0.25%) negative effects were found on growth, tissue histology, and parasite infestation of larvae. Oxygen delignification or effluent treatment partially alleviated these effects when tested at the same effluent concentrations. The most effective process modification was 50% substitution of chlorine dioxide for molecular chlorine, where larval responses to BKME exposure were equivalent to those of controls [152]. The authors did not see a correlation between biological effects and organochlorines measured as TOCl but did note positive trends with organic loads in the bleach plant and discharged resin acids [152]. In subsequent mesocosm experiments, hardwood BKMEs and unbleached kraft effluents were tested. The mortality and growth responses could not be distinguished from the softwood BKMEs using chlorine dioxide (L. Stromberg, personal communication). These results further emphasize the necessity to document process conditions, effluent chemistry, and effluent toxicity accurately to clarify potential causes for toxic effects.

Field data on reproductive effects are limited. At Loch Eil, crustacean and fish egg production were not impaired [112]. At Nipigon Bay, high densities of larval smelt (*Osmerus mordax*) were present in the untreated effluent plume, but sucker and perch larvae numbers were low versus numbers in control areas [153]. At Lake Pajanne an inverse relationship was observed between BOD levels and the presence of juvenile perch and bream in the areas receiving untreated effluents [154]. At Jackfish Bay in Canada, although no population level consequences were observed, white suckers (*Catostomus commersoni*) exposed to BKME had reductions in egg weights and serum levels of sex steroids but not gonad weights [155]. Subsequent surveys show similar effects in longnose suckers (*Castostomus castostomus*) and lake whitefish (*Coregonus clupeaformis*). Field and in vitro work indicates potential

dysfunctions in steroid synthesis, liver steroid metabolism, and pituitary–gonad feedback control (K.R. Munkittrick, personal communication).

Within-organism effects on histopathology. Organisms in contaminated habitats may demonstrate pathological effects in either gross morphology or histology. Vertebral and spinal deformities in the Baltic Sea fourhorn sculpin (*Myoxocephalus quadricornis*) have been intensively studied. Like many fish abnormalities in natural settings, these deformities are common with background rates of about 20%. Near Baltic Sea bleached kraft mills, the rates of these deformities ranged from 60% near the Husum mill to near background at the Norrsundet (16%) and the Jakobstad (9%) mills. Water column, sediment, or body burden analyses were not available from field studies, and deformity rates at control sites increased with water depth and low temperature [156]. In controlled laboratory exposures, fourhorn sculpin and bleak (*Alburnus alburnus*) have been exposed to several pulping effluents (unbleached, untreated BKME, and treated BKME), and the impact of exposure on spinal deformities has been measured. Laboratory deformities were less severe, and no overall differences were observed between the effects of unbleached effluents and those of BKMEs [157, 158]. The EOX body burdens in sculpin did not correlate with effects [157]. Further, total chlorophenolic body burdens did not show a correlation with the spinal deformities in the laboratory [159].

Histological studies of organisms exposed to pulping effluents include laboratory-exposed perch [160], mesocosm-exposed flounder [161], and laboratory-exposed rainbow trout [162,163]. Lehtinen and Oikari [160] observed apical gill swelling and mucous production in the gills at 4% BKME concentration. At lower concentrations, gill parasites and changes in liver such as pyknotic nuclei and increased cytoplasmic granulae were observed [160]. Lehtinen et al. [161] observed similar changes in gills and liver during exposures to untreated effluents from unbleached kraft, oxygen delignification, and molecular chlorine processes. In rainbow trout, a bacteria-induced fin rot has been observed at 2 and 4% BKME after 60 d [162]. Only Lehtinen and Oikari [160] chemically characterized the tested effluents, and no toxicological tests were performed.

The broadest comparison of different processes occurred during mesocosm studies of three-spined stickleback. Liver effects were noted after exposures to molecular chlorine–bleached softwood effluents: pyknotic nuclei and vacuolization. A lesser

degree of vacuolization was seen after exposure to oxygen delignification effluents. The authors suggested that vacuolization was due to liver accumulation of lipid. Normal histology was found in fish exposed to effluents with 50% substitution of chlorine dioxide [152].

Histopathology conducted in experimental streams showed no relevant effects from extended effluent exposure. Data were gathered for three fish species: largemouth bass, bluegill sunfish (*Lepomis macrochirus*), and rainbow trout. Exposure times ranged from one to four years, and effluent exposure concentrations were as high as 10%. Effluent chemistry was characterized at nearly weekly intervals, and effects in chronic toxicity tests with fathead minnow larvae occurred only at 10% and greater concentrations. No tissue damage or tumors were observed in fish exposed to 5 to 10% concentrations, and infestation rates by parasites were not increased due to effluent exposure [150, 164–166]. Although in-stream exposures lasted up to four years, this may not be sufficient to assess weak tumorigenic effects fully. Hepatic and biliary tumors have been observed in white suckers exposed to untreated BKME in fish older than seven years. However, these fish may be predisposed to tumorigenesis by viral infection (I. Smith, personal communication). These concerns appear to have been addressed by four-generation life cycle exposures in which no effects were observed in fathead minnows [164].

Within-organism effects on physiology and biochemistry. Physiological and biochemical measurements have been widely referred to as *biomarkers*, and their applications to organisms exposed to pulping effluents have been reviewed by McLeay [24] and Kovacs [121]. Initially, clinical measurements from mammalian research were utilized, such as during the exposure of salmonids to primary-treated BKME [167–170]. In certain cases recovery (adaptation) appeared to occur during prolonged exposure during which decreases in liver and muscle glycogen after 30 d returned to control values by 200 d [167].

Physiological and biochemical responses to pulping discharges have been investigated primarily in Scandinavia and Canada. Lake Saimaa in Finland has been the site of several studies. Tana and Nikunen [171] analyzed biochemical parameters in caged rainbow trout in all four seasons. Several parameters were significantly altered in a gradient fashion from the discharge. The greatest impact was seen in March, immediately after the stress of winter ice cover. However, the cumulative range of control values was equal to or greater than the sta-

tistically significant differences between exposed and control individuals. The authors suggested that to obtain more meaningful interpretation, future experiments be conducted with added stressors such as exercise [171].

Oikari and coworkers, in an initial study, found liver uridine diphosphate glucuronosyl transferase (UDPGT) decreased and serum sodium concentrations elevated in parallel with the water column sodium concentrations. Other clinical parameters showed statistical differences, but the results did not correlate with either water column or body burden analyses [137]. In a subsequent study, the effort shifted from clinical measures to detoxification and hormonal measures. Rainbow trout and two species of whitefish were caged for 21 d, while feral fish were captured near the caging stations. The UDPGT activity was not affected in caged whitefish but rose slightly in rainbow trout [141, 142]. Cortisol concentrations were measured to assess the degree of stress in exposed rainbow trout, but levels were similar to those of the controls [142]. Glutathione levels were measured, as free glutathione is necessary to detoxify many substrates. In all exposed feral species, glutathione levels were not depleted but were increased. This was interpreted as an adaptive response to exposure [143]. Mixed-function oxidase (MFO) activities were measured because these activities initiate several detoxification pathways. Significant elevations of liver MFO activities such as 7-ethoxyresorufin deethylase (EROD) were seen in whitefish [141] and rainbow trout [142]. The EROD activity was induced in feral perch, roach, and bream with species differences in the degree of MFO induction: bream > perch = roach. At unpolluted sites, MFO levels were variable in natural populations, and the authors cautioned that control sites be assessed for background range and representativeness [143].

In parallel laboratory investigations, rainbow trout were exposed to purified chlorinated phenols and resin acids for 80 d [172]. No substantive differences were observed between these chemical classes, except for UDPGT values rising with chlorophenol exposure and decreasing with resin acid exposures [172]. Several parameters were significantly changed from control values at 10 and 20 d but recovered to control values after 80 d. These data suggest that clinical measures are nonspecific and cannot distinguish between different chemicals. In addition, adaptive responses may result in statistical differences without leading to long-term detrimental consequences.

In other laboratory exposures, resin acids caused significant increases in blood bilirubin lev-

els, liver UDPGT activity, and vacuolization of liver tissue [160,173,174]. Chronic exposures to a mixture of resin acids and chlorophenolics reduced the growth of juvenile rainbow trout, changed erythrocyte size and hemoglobin content, but induced EROD only slight EROD induction [175]. In contrast, strong EROD induction was seen with TCDD and whole BKME, suggesting that EROD induction from BKME is related to highly chlorinated planar hydrocarbons [176]. The proposal is consistent with EROD induction by coplanar and noncoplanar PCBs [177] and TCDF [178] but inconsistent with equivalent levels of EROD induction in caged fish at an unbleached Finnish kraft mill (P. Lindstrom-Seppa, personal communication).

In mesocosms, physiological and biochemical measurements were made on rainbow trout exposed for 7 wk to the softwood BKMEs noted in previous sections. Untreated BKME from molecular chlorine processes showed several statistically significant effects, and the intensity of these effects was reduced by either biological effluent treatment or addition of oxygen delignification to the process. However, EROD induction was observed in all BKME exposures, except from processes substituting chlorine dioxide for molecular chlorine [179]. Controls were included in each set of mesocosms, and significant variations between yearly control groups were observed. At times, control variations exceeded the significant intraexperimental differences between control and exposed groups. Therefore, the authors cautioned that statistical significance alone was not sufficient to prove that an effect was deleterious [179]. Subsequently, mesocosms with bleached hardwood and unbleached kraft effluents showed statistically significant responses from controls (L. Stromberg, personal communication), which leaves unresolved the identity of components or conditions altering the biochemical responses.

Intense EROD inductions concurrent with body burdens of TCDD and other effluent compounds have been observed in feral chinook salmon (*Oncorhynchus tshawytscha*) from the Fraser River. Health and exposure indicators such as condition factor, liver somatic index (LSI), and UDPGT activity were not changed by BKME exposure. Therefore, juveniles were exposed to the same BKME under laboratory conditions—4% effluent for 60 d, 0.8% effluent for 84 d—then transferred to saline, effluent-free conditions. Mortality, growth, tolerance of hypoxia, and other tested whole-organism factors were not affected. Histological analyses were normal with two exceptions: (a) an increase in anterior-kidney nuclear diameter indicative of stress

and (b) lesions from a bacterial kidney infection possibly indicative of immunotoxicity. Chlorophenolic body burdens from laboratory individuals were similar to field values, but TCDD burdens were not detected. In contrast to field results, laboratory MFO induction was only two- to threefold [180,181] (J.A. Servizi, personal communication). These data support a route other than direct water column bioaccumulation for TCDD and potentially link TCDD and related compounds to liver EROD induction.

White suckers exposed to untreated BKME at Jackfish Bay have induced liver arylhydrocarbon hydroxylase activity (AHH), which appears to be synonymous with EROD activity [182,183]. AHH activity levels were inversely related to the sexual cycle in both sexes [155] as found in other species [184–186]. Although contaminant levels were not measured in this study, TCDD body burdens have been found in white suckers at the Jackfish Bay site by others [187]. MFO induction in white suckers has continued after the installation of secondary treatment, and MFO induction has also been observed in longnose suckers and lake whitefish (K.R. Munkittrick, personal communication).

Discussion of sublethal measurements and effects. The above studies were not designed as integrated hazard assessments: (a) chemical analyses of the receiving waters and body burdens ranged from none to several pulping components; (b) toxicity tests were often absent; (c) sediments were not tested for either contamination or toxicity; and (d) parallel community or population data were not gathered to assess meaningful impacts. The experimental stream studies were an exception with chemical analyses, effluent toxicity testing, periphyton and macrobenthic parameters, fish survival and growth tests, reproductive and histological end points in several species, and limited body burden data in several species [81–83,150,151,164–166,188]. However, physiological and biochemical measurements were sparse; only LSI, hematocrit, and leucocrit tests were performed, and these were unchanged by BKME exposure [150,164] (D. Borton, personal communication).

Despite the wide variation in effects and NOAELs, the data support tentative conclusions. Mesocosm experiments suggested that high levels of molecular chlorine use, excess organic material introduction into the bleach plant, and the absence of effluent treatment correlated with toxicity [152,179]. Reproductive end points under laboratory conditions showed a decreasing trend with effluent treatment, and the experimental stream

studies show no toxic effects at moderate concentrations with several species and end points after biological treatment [150,151,164–166] (D. Borton, personal communication). Other reviewers also have noted reduced toxicity and environmental effects with effluent treatment [1,24,48,121]. However, at Jackfish Bay effluent treatment has not immediately alleviated within-organism reproductive effects (K.R. Munkittrick, personal communication). These comparisons would be better defined if standardized chronic toxicity data were available, particularly on reproductive end points.

The data further support previous conclusions made by Sprague [189] and Kovacs [121] that reproduction and early life stages are highly sensitive end points. Most importantly, these effects can be tested and potentially integrated at several levels: (a) in laboratory, mesocosm, and experimental stream conditions; (b) in the field with larval and juvenile distributions and densities; and (c) within organisms at the organ, tissue, and biochemical levels. This suggests that reproduction and early life stage impacts are ideal end points to assess toxicity and to discover toxic mechanisms. One candidate for investigation is the reported masculinization of female fish from the natural plant sterols in pulping effluents [190,191].

Physiological and biochemical measurements require additional development beyond clinical parameters and cautious interpretation in the interim. The present evidence suggests each measurement will have a species-specific natural range varying with season, habitat, and other conditions. Therefore, statistical treatments alone may lead to over-interpretation as detrimental effects. A primary difficulty is a nonselective response to a wide range of conditions. The envisioned hazard assessment process is, first, to identify detrimental effects and, second, to identify the responsible condition or contaminant. Therefore, more specific measurements are needed to diagnose particular end points.

Environmental monitoring based on MFO induction has been proposed elsewhere [192,193]. EROD induction is a response to a broad spectrum of hydrophobic compounds, so induction is not exclusive evidence for the presence of TCDD or other organochlorines. Although PCB body burdens have been correlated with both reproductive effects and EROD induction [194], sex steroids are not apparently metabolized by the enzyme that catalyzes EROD activity [183,195–197]. As EROD induction has not been mechanistically linked to effects at higher organizational levels, the biological consequences of EROD induction are not clear.

Case study at Norrsundet, Sweden

The case study at the bleached kraft mill at Norrsundet, Sweden, on the Gulf of Bothnia is unique. Simultaneous measurements were made at three organizational levels: (a) the littoral benthic community; (b) several fish populations, including extensive reproductive and mortality studies; and (c) extensive physiological biochemical measurements in perch. Thus this study is the best existing effort with pulping effluents to test for connections between organizational levels. Community measurements found adverse effects related to eutrophication and nutrient enrichment [198]. The fish population measurements found adverse effects from both eutrophication and chemical toxicity [199]. The physiological and biochemical measurements found consistent differences between exposed and distant control areas, which were interpreted as evidence for chemical toxicity [200–203].

The Norrsundet effluent was discharged untreated into a shallow and sheltered inlet (Fig. 3). For purposes of discussion, the study area will be broken into zones: zone 1, the inlet area within 2 km of the discharge; zone 2, the inlet area from 2 to 4 km; zone 3, the open coastal area outside the inlet; and zone 4, the local control area outside the bay at Kusön. The discharge loadings during the studies are given in Table 3. The mill had been rebuilt just before the studies, and operational difficulties during the process start-up led to excessive conventional discharges during the study period

[201] (L. Landner, personal communication). An unbleached kraft mill at Sandarne was used as a control (35 km to the north). However, Sandarne is more open and better flushed than zones 1 and 2, and the discharge loads were lower (see Table 3) [201,202]. In addition to the local Kusön site (station 7, Fig. 3), Forsmark (85 km to the south) served as a control site for some study segments.

Chemical analyses. Most analyses of effluent, water column, and sediments at Norrsundet were performed before mill reconstruction and study efforts began [204]. At one kilometer the effluent concentration at 5-m depths was 1.2‰ (81-fold) and at 3 km 0.8‰ (125-fold) [204]. As the Norrsundet effluent was at the water surface [204], actual surface concentrations might have been higher. These water column data were modeled by EXAMS and FUGAMS software [205], indicating low turnover in zones 1 and 2. The chlorophenolic analyses are shown in Table 2 for comparison with experimental stream and Lake Saimaa studies. Older data show that BOD₇ values in zone 1 ranged from 10 to 17 mg/L in winter to 5 to 7 mg/L in summer [65]. The sediments in zones 1 and 2 were contaminated with chlorophenolics, and organic contents were as high as 30 to 40% [204]. Water column and sediment analyses have not been published for zone 4 (Kusön), Sandarne, or Forsmark.

Norrsundet community studies. Benthic community studies showed that Norrsundet was a severely affected site. Previous work had shown that the benthic populations in zones 1 and 2 were

Table 3. Summary of EOX bioaccumulation, chlorine use and effluent characterization at the Norrsundet mill

Year	EOX in perch ^a (ppm in lipid) Distance from outfall			Chlorine ^b used (kg/t)	BOD ₇ (t/d)	COD (t/d)	TSS (t/d)	TOCI (kg/t)
	1 km	3 km	4.5 km					
Norrsundet								
1982	—	—	—	88.2	19.2	53	2.2	7.5
1983	423	215	—	58.7	18.6	69	2.2	5.9
1984	330	295	151	37.4	14.1	51	1.8	3.8
1985	180	130	91	30.5	9.9	36	1.8	—
1988	—	—	—	20.1	9.7	36	1.0	1.7
Sandarne								
1984	120	120	—	None	4.7	12	1.6	None

History of Norrsundet mill modification during 1980s: 1982—Norrsundet had both bleached and unbleached mills. The bleached mill was without oxygen delignification and used molecular chlorine. 1983—Mill conversion was begun and oxygen delignification system was installed. 1987—Molecular chlorine use was reduced with increased chlorine dioxide substitution (40%) in the bleach plant.

^aData on EOX from Södergren [202] and Södergren et al. [203].

^bData on Norrsundet discharge parameters from Kautsky et al. [198] and Neuman and Karás [199].

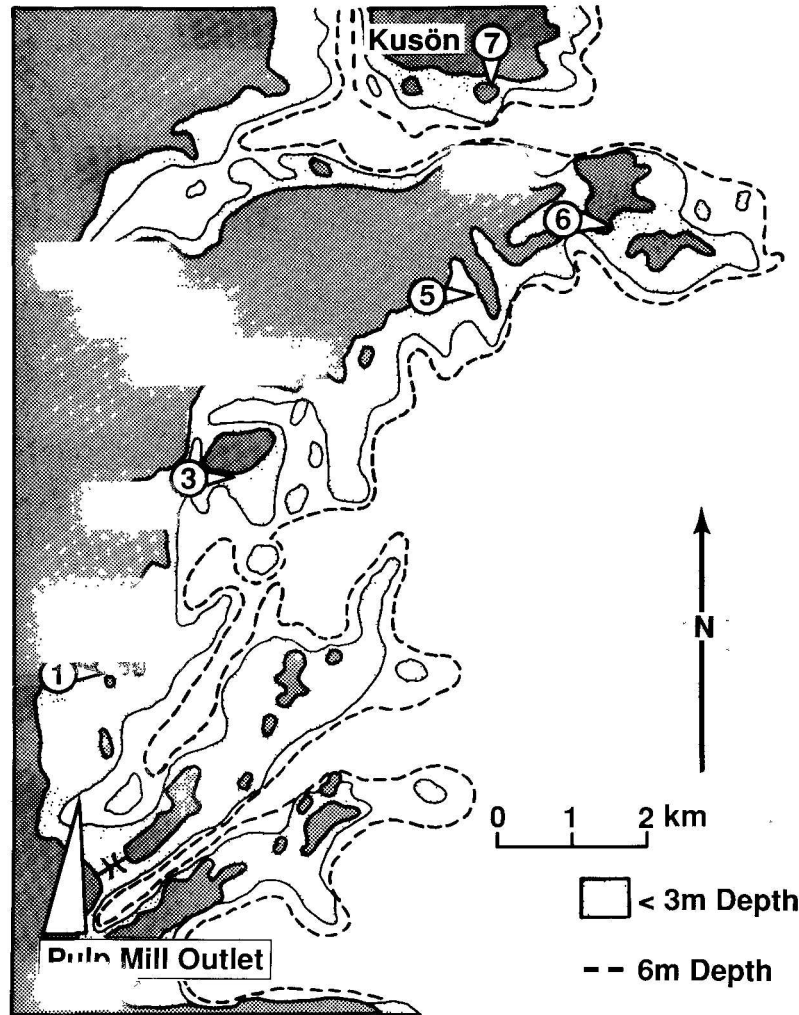


Fig. 3. Map of the Norrsundet discharge area. The sampling stations (1-7) for the community studies are shown. Depth contours are at 3 m (—) and 6 m (----). The mill discharge point is indicated with an arrow at the lower left. Sampling stations for fish populations were not precisely the same except for the Kusön site. From Kautsky et al. [198], reprinted with permission.

smothered by a fiber mat with decreased oxygen levels in the water column [65]. In 1985, the benthic community was still altered by a limited fiber mat in zone 1 and anoxia [198]. Dense biomasses of filamentous algae were present on the surface in zone 1, and the fauna was dominated by a bacterial feeding hydrozoan, *Cordylophora caspia*. A succession of communities was found in zones 2 and 3 as biomass increased and colonization occurred at greater depths. This is shown in Figures 4 and 5 for taxa and biomass, respectively. A normal

benthic community was found in zone 4 (Kusön). Kautsky et al. [198] concluded that eutrophication and enrichment caused the disruptions, noting the similarity between their observations and the model gradient proposed by Pearson and Rosenberg [61] (Fig. 2).

Norrsundet fish population studies. Fish populations were severely affected by the effluent discharge. The investigations included several species, but focused on roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*). Roach, perch, and other species

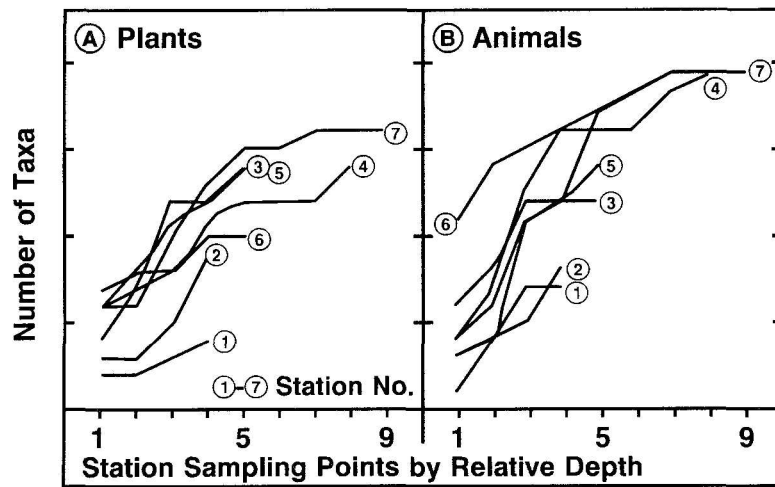


Fig. 4. Number of taxa at Norrsundet sampling stations. The total number of floral (A) and faunal (B) taxa found at the sampling stations as numbered in Figure 3 are plotted by relative depth at each station. The data are from Kautsky et al. [198], reprinted with permission.

were distributed in distinguishable zones at Norrsundet and Sandarne [199,206]. Zone 1 at Norrsundet discharge was almost devoid of fish. In zone 2 adult, fry, and larval numbers of roach were high. However, perch adults were few, and juveniles were essentially absent. In zone 3 adult perch increased, but fry numbers were still re-

duced. At Sandarne with its smaller discharge, there was no zone devoid of fish; adults, fry, and larvae of both species were more common and closer to that discharge [206]. The authors concluded that (a) eutrophication was the principal cause for the observed effects and (b) both avoidance responses to contaminants and chemical toxicity

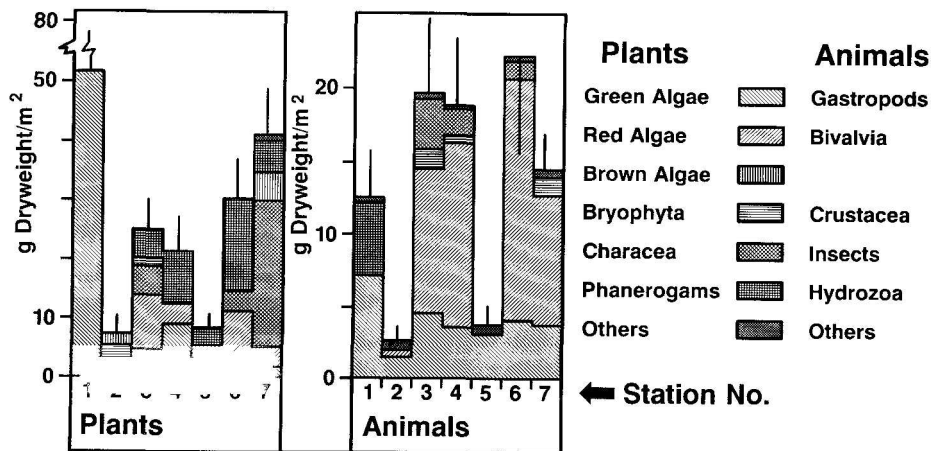


Fig. 5. Biomass of flora and fauna at Norrsundet sampling stations. The average biomasses of major plant and animal taxa are summarized for each sampling station as numbered in Figure 3. The standard deviation of the total is shown by the height of the vertical line above the stacked bar. The major groups are shaded, with the legend for plant and animal groups on the right. The data are from Kautsky et al. [198], reprinted with permission.

were found in zone 1 [199,206]. These conclusions agree with other observations in the Baltic Sea, including both bleached and unbleached processes [207–210]. Neuman [211] has reviewed fish population surveys near pulp mills, concluding that (a) eutrophication was responsible for altered fish distributions and (b) no effects unique to organochlorines could be observed. However, Neuman cautions that the experiments were not properly designed to determine toxic effects from the discharges [211]. Similar changes in roach and perch distributions have been observed at nonpulping sites [212–219] and these characteristics have been noted in reviews on percids [219,220].

Fish reproduction, growth, and mortality studies The recruitment of several fish species was impaired at Norrsundet, leading to intensive studies on perch [206]. These studies began by measuring the reproductive maturity of adult perch by gonad classification. At both control sites and at Sandarne, about 75% of the female perch had maturing gonads and were entering the spawning cycle [221], consistent with a 25-year continuous study at another site [222]. However, only 45 to 60% of the females were judged ready to spawn at the edge of zone 1, 65 to 70% in zone 2, and at control levels in zone 3. Gonad weights and gonad somatic index (GSI) values in both sexes followed a similar trend: lowest in zone 1 and rising to control values in zone 3 [221].

Subsequent studies identified perch larval mortality as the primary cause of impaired recruitment. Adult perch migrated to zones 1 and 2 and spawned sufficient eggs to support the local population [223]. Embryo abnormalities were higher in exposed (10%) than control areas (1%) with decreased embryo size and growth rates [223], but hatching rates appeared normal [206]. Larval mortality was 100% in zone 1, and the investigators concluded that acute toxicity and chronic effects on the parents were the causative factors [223]. In zone 2, roach larvae were abundant, but perch larval mortality was still high. In this zone, the difference between perch and roach mortality may include effects on larval feeding behavior by color and turbidity [199,210]. Roach larvae feed under low light conditions, whereas perch require more light for successful feeding [224]. In laboratory experiments, perch larval survival fell from 44% in high light conditions to only 1% in low light [225]. In zone 2, surviving perch larvae consumed only large zooplankton despite abundant smaller prey [223]. Therefore, several factors may be acting in

zone 2 to produce a cumulative effect on mortality rates.

Adult mortality rates were increased in zones 1 and 2 (53%) versus those of both zone 4 (44%) and a remote area (42%) [226]. The control values agreed with long term observations at another well-studied site (42%) [222]. The mortality rates were supported by following perch scarred by fin erosion, a condition associated with degraded and contaminated habitats [227] and seen in chronic laboratory exposures to BKME [162]. Fin erosion was observed in zones 1 and 2 during the mill start-up [206,228], marking these individuals. The mortality rate for scarred individuals was 59% [226], confirming the overall observations.

Despite the increased mortality rates, juvenile and adult growth rates and condition factors in perch increased in a gradient toward the discharge [201,221,229]. Again, several factors might have been involved: (a) growth is inversely related to low population densities [222,230,231], (b) growth accelerates under eutrophic conditions [215,216,232], and (c) reduced reproductive effort in some individuals could accelerate adult growth.

Body burden studies Body burden analyses included 3,4,5- and 4,5,6-trichloroguaiacols in bile, TCDD and TCDF in fillet, and EOX levels in lipid. The 4,5,6-trichloroguaiacol levels at Norrsundet [201] were lower than those at Lake Saimaa [139,140]. Elsewhere, only fillet levels have been reported [181,188,233], emphasizing the need for standardized protocols. TCDD levels ranged from 2.6 to 19 pg/g lipid, implicating the mill as a TCDD source [128,201]. The EOX levels indicated high background levels of 50 to 120 $\mu\text{g/g}$ at Kuson, Sandarne, and other sites [134,202,203]. Those EOX levels beyond zone 2 were near this background range throughout the study period. Within zones 1 and 2, EOX levels declined parallel to chlorine use (see Table 3), EOX levels were not analyzed at Forsmark.

Physiological and biochemical measurements The physiological and biochemical efforts at Norrsundet represent a potential bridge between meaningful effects at the field level and chemical contaminants. The field study focused on perch as a target species, in part due to the extensive physiological and biochemical measurements made on perch under both freshwater and brackish field conditions and in the laboratory [234–236] and also due to efforts to develop protocols minimizing capture variables [237]. At unpolluted sites, the spans of physiological and biochemical values (a) were

measurement-specific with C.V.s ranging from 9.2% for serum magnesium to 83.5% for serum lactate [235] and (b) fluctuated with season, age, and size [235,238]. Many of these same measurements were applied to feral perch at the Norrsundet mill [200,239–241] and to fourhorn sculpin in the laboratory, using untreated BKME from the Husum mill [157,159,242,243].

Over 20 physiological and biochemical measurements were performed on Norrsundet perch on four separate occasions. These data for zone 2 perch have been assembled in Table 4. There were four reference data sets to evaluate the Norrsundet

responses: the remote control site at Forsmark, the local control site at Kusön (zone 4), the parallel laboratory exposures, and the background of data at other unpolluted sites and laboratory exposures [234–236]. Comparison of zone 2 with Forsmark data (see Table 4) shows that several measurements were not statistically different (blood sodium levels and white blood cell numbers) or had inconsistent patterns (blood glucose levels and thrombocyte numbers). However, 12 measurements showed consistent statistical differences on at least three sampling occasions. Most importantly, these within-organism data exhibited an overall gradient pattern and

Table 4. Summary of biochemical and physiological measurements on perch at Norrsundet Zone 2, Kusön, and Forsmark with laboratory studies on sculpin

Sample date:	Zone 2 ^a				Kusön 9/85	Laboratory ^b	
	5/84	8/84	10/84	9/85		Pine	Birch
EOX in lipid (ppm)	nd	300	nd	170	nd	320	nd
Assay							
Liver somatic index	+ ^c	+	+	+	NS	+	NS
Gonad somatic index	nd	–	–	–	NS	nd	nd
Blood sodium	NS	NS	NS	NS	– ^d	NS ^e	NS
Blood chloride	–	– ^d	– ^d	– ^d	– ^d	– ^c	NS
Blood potassium	+	NS	NS	NS	–	NS ^e	–
Blood calcium	NS	– ^d	– ^d	+ ^d	NS	NS ^e	NS
Blood magnesium	+	– ^d	– ^d	+ ^d	–	NS ^e	NS
Blood glucose	–	NS	NS	+ ^d	+ ^d	NS ^e	nd
Blood lactate	+	NS	+	+ ^d	NS	NS ^e	nd
Liver glycogen	NS	+	+ ^d	NS	–	nd	nd
Blood bilirubin	+	+	NS	+	NS	nd	nd
UDP-glucuronyltransferase	+	NS	+	NS	NS	NS	NS
Muscle glycogen	NS	+ ^d	+	NS	NS	+	NS
Hematocrit	+	+	+ ^d	+ ^d	NS	NS	–
Hemoglobin	+	NS	NS	+ ^d	+ ^d	NS	–
RBC no.	+	+	+	+	+ ^d	–	NS
Mean cell hemoglobin	–	– ^d	– ^d	–	NS	NS	–
Mean cell volume	–	–	–	NS	NS	+	NS
Met-hemoglobin	+	nd	+	+	NS	–	NS
WBC no.	NS	NS	NS	–	– ^d	NS	NS
Lymphocyte no.	NS	– ^d	– ^d	–	– ^d	NS	NS
Thrombocyte no.	NS	NS	+	– ^d	NS	NS	NS
Granulocyte no.	+	NS	NS	NS	NS	NS	NS
EROD	+	+	+	+	NS	+	NS
Ascorbic acid	+	+	+	+	NS	nd	nd
ALA-D	NS	+	–	+	+ ^d	nd	nd

EOX data from [202], field data from [200], laboratory data from [242,243], and background data for biochemical and physiological measurements from [234–237]. nd: not done, NS: not significant, +: significantly higher than control ($p < 0.01$) by Duncan's new multiple-range test, –: significantly lower than control by same statistical measure.

^aZone 2 Norrsundet sampling site at two kilometers; Kusön is Zone 4.

^bHusum mill BKME, pine and birch bleach line effluents diluted to 0.6%.

^cNo variation data available for May sampling times.

^dSignificantly different from Forsmark, but within variation of other studies at other sites in same species, sex, and season.

^eData available only for lower dose (0.12%) of pine effluent.

spatial influence equal to the community and the population observations

However, when the Norrsundet values are compared against the Kuson site data, the laboratory data, and the values at unpolluted sites during the same season, the responses appear somewhat less severe. As the control area for benthic and fish population studies, the Kuson site is situated outside and north of the affected area. Mill contamination was unlikely as the current in this region flows from north to south [201,204]. Further, this site did not show the intense EROD induction characteristic of exposure to BKME (see Table 4). Strong differences between Kuson and the affected zone 2 include LSI, GSI, white blood cell numbers (immunotoxicity?), and EROD induction. The Kuson site also showed statistical differences with Forsmark (see Table 4), further indicating the inherent variability of these measurement systems. In the laboratory studies, only EROD induction, mean cell hemoglobin content, and serum chloride levels had similar responses to field exposures.

Discussion of the case study Both enrichment and chemical toxicity would be expected at the Norrsundet site. Discharge loads equivalent to Norrsundet's have caused enrichment and eutrophication impacts (see Table 1A and Table 3), particularly given the limited turnover in zones 1 and 2. The mesocosm studies [152,179] and observations with untreated effluents [148,149] indicate that sublethal chemical toxicity may be expected in zone 1 and possibly zone 2. In addition, avoidance responses and acute toxicity have been observed with untreated effluents like those at Norrsundet at similar or slightly higher effluent concentrations [24,48,141,244–246].

Eutrophication and enrichment effects on both

the benthos [65,198] and the fish population effects [199,206] were found at Norrsundet. This habitat degradation complicates efforts to define chemical toxicity, because physiological and biochemical measurements show gradients and spatial influence similar to those of the habitat disruption. The biochemical measurements along with fish avoidance responses, extensive field data on larval mortality, and adult mortality data support the presence of chemical toxicity but do not define the responsible contaminants. At unbleached discharges at Piteå, Sweden, with effluent loads similar to those in Norrsundet [5], increased growth and decreased gonad indices have also been reported (see Table 5) [247,248]. Improved effluent, water column, and body burden analyses, along with toxicity tests, were needed to clarify which contaminants might have been related to the observed effects.

The biological studies at Norrsundet were designed to connect tests at several organizational levels by using a single, representative target species—perch. Perch were a major member of the fish community and common throughout Scandinavia, so they were relevant to study and were available in sufficient numbers. In addition, perch were relatively stationary, their reproductive behavior and diet were known, and the adult body size was sufficient for biological sampling. Further, data bases on physiological and biochemical variations of perch in the field were available in the late summer/early fall time frame used for three Norrsundet samplings. Both the strategy of using target species at all organizational levels and the selection criteria for the target species are highly recommended for future investigations.

The field studies at Norrsundet provided clear evidence for impacts on the reproduction and the

Table 5 Growth and reproductive effects in perch near Pitea unbleached kraft mills in the Gulf of Bothnia

	Reference site	At unbleached discharges	
		Mill 1 0 2 ^a	Mill 2 1 2 ^a
Length (five-year-old males—mm) ^b	158	169	164
Length (five year old females—mm)	162	180	175
GSI (male) ^b (%)	5.98	4.79	2.41
GSI (female) ^b (%)	1.90	0.96	1.23
Sexually mature (female) (%)	59	sites combined 43	

Data are from Bergelin et al. [248].

^aDistance from the discharge (km).

^bDifferences in male length and GSI values for both sexes were statistically significant ($p < 0.05$) by Student's *t* test.

recruitment of several species. For several species the deeper, anoxic bottoms were unsuitable reproductive habitat [199]. The reproductive behavior of perch led this species to spawn in zone 1, nearest the discharge. There, acute toxicity in larvae was 100% and directly led to low population density of perch throughout the area, requiring outside recruitment [223]. The cause of the acute toxicity was not resolved, and a host of pulping-related contaminant analyses in the effluent and the receiving waters would be needed to resolve the issue. An alternative hypothesis is toxicity due to contaminant burdens in the egg yolk resulting in symptoms similar to blue sac disease. Mortality would be expected to occur during the same larval stages when target tissue burdens rose from yolk absorption. The decreased adult gonad size and fewer individuals entering the sexual cycle [221] point to stress on the hormonal control of reproduction. Given Canadian findings of lowered sex steroid levels and the dysfunction of hormonal control [155] (K.R. Munkittrick, personal communication), a logical second tier of analyses would include sex steroids and the pituitary control of reproduction.

The within-organism measurements suggested several potential connections with environmental contaminants and with biological measurements at higher levels. Possible connections with contaminants include:

1. Increased methemoglobin has been associated with aqueous pollutants such as nitrite [122].

2. Bilirubin levels, smaller erythrocytes, and lower cell hemoglobin contents have been associated in the laboratory with resin acid toxicity [165, 176].
3. Ascorbic acid levels may reflect a response to increased detoxification [235].
4. EROD induction as previously noted has been associated with planar organochlorines and hydrocarbons.

Physiological and biochemical measurements also suggest potential connections with effects at higher organizational levels:

1. higher muscle and liver glycogen stores with decreased reproductive effort, increased growth, and higher condition factor
2. increased numbers of white blood cells with the simultaneous appearance of increased mortality and fin rot with increased effluent discharges during the loss of mill process control in early 1984
3. the simultaneous appearance of EROD induction with reproductive dysfunction.

The hematocrit values are a paradox: Exposed perch appear within the normal values [234,235,237] versus those at Forsmark (see Fig. 6).

Although the total weight of the evidence suggests that perch were strongly affected at the within-organism level, the clinical measurements remain difficult to interpret and are not definitely

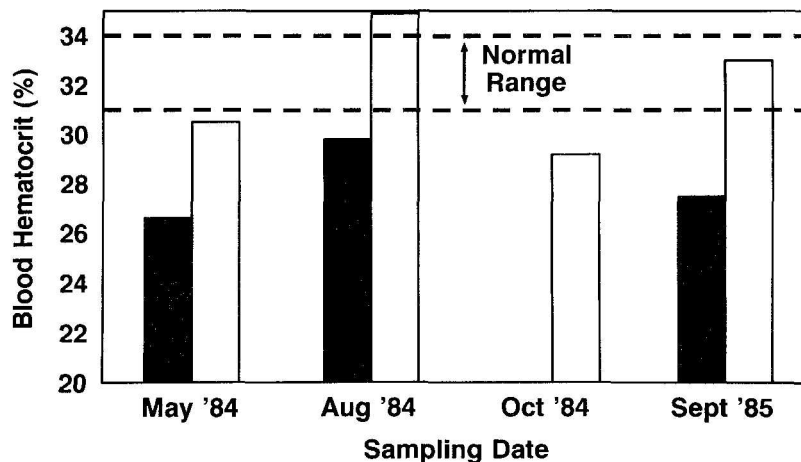


Fig. 6. Hematocrit values of Norrsundet and Forsmark perch compared to background measurement variability. Hematocrit values for female perch at each of four sampling periods at Norrsundet and Forsmark [200] are compared to hematocrit measurements of female perch in unpolluted Swedish waters or the laboratory [234–236].

linked to the observed effects at the population level. Despite this critical shortcoming, the general gradient response of these measurements is enticing and supports further efforts to develop validated and more specific physiological and biochemical measures for poikilothermic aquatic organisms.

The Norrsundet results show that primary difficulties of clinical measurements continue to be their variability and lack of specificity. A strategy to overcome these difficulties with clinical measurements would be to apply redundant measures to particular end points. For example, serum lipid levels and total body lipid would be analyzed parallel to glycogen measurements for energy reserves. However, the identification of responsible contaminants would remain highly speculative. Although one purpose of the Norrsundet study was to test for associations with organochlorines, clinical measurements did not show a dose response to organochlorine body burdens as measured by EOX. As molecular chlorine use fell and EOX body burdens decreased (see Table 3), most differences with the Forsmark site were maintained. In addition, whereas total AOX and chlorophenolic levels are similar to exposures at Lake Saimaa and in experimental streams (see Table 2), biochemical effects at Lake Saimaa appeared to have been less severe, and reproductive and whole-organism effects were not observed in experimental streams. Thus, firm linkage of biological response with chemical contaminants is still absent. This reiterates that selection criteria for within-organism measurements are needed: (a) diagnosis of specific and meaningful end points such as reproduction, detoxification, or immunocompetence or (b) response to specific classes of contaminants.

Future trends

Future hazard assessments of pulp and paper discharges to the aquatic environment face three trends that will drive the development of improved biological measurements. First, discharge loadings are decreasing for both conventional pollutants and compound classes such as organochlorines via process improvements and treatment modifications [1,249–251]. Second, environmental concerns now extend from local to potential regional impacts. For example, in the Fraser River the persistence and long-range dispersal of pulping compounds have been observed [252], and in the Gulf of Bothnia attempts have been made to assess both the regional distribution of pulping chemicals [253] and any regional impacts on the benthos [254]. However, while the pulp mill contaminants showed re-

gional gradients in Bothnian sediments [253], the benthos displayed no regional effects from the discharges [254]. Finally, national monitoring programs for pulping discharges are envisioned that would standardize protocols for chemical, toxicological, and biological measurements [255–257].

Currently, process changes in the industry are rapid. As ecosystem recoveries may take years and sediment reservoirs of persistent contaminants may require time to depurate, both the before and after chemical and toxicological characterization of effluents and long-term site monitoring will be necessary to distinguish these new discharges from the impact of residual contaminants or previous eutrophication and enrichment impacts. Benthic studies of many North American pulping sites show continued subtle habitat alterations (usually stimulation) from enrichment [48,258]. Although fish population densities and distributions sometimes have been investigated in these studies, few actually address reproduction and recruitment in receiving waters below the discharges [258]. This reinforces the extension of hazard assessment to (a) long-term site monitoring to assess remediation and (b) additional meaningful end points in the field.

The development of national monitoring programs for pulping discharges offers significant opportunities to further develop hazard assessment. The envisioned initial step at each site would include: (a) physical characterization of the site; (b) chemical analyses of effluents, receiving waters, sediments, and body burdens; (c) toxicity testing for effluents, receiving waters, and sediments; and (d) field surveys of communities and populations. In this framework, chemical, toxicological, and field tests could be integrated and interpreted to design a second tier of effort. Community and population studies would indicate whether meaningful environmental impacts had occurred and identify the affected populations. From these data, target species, end points of concern, and potential contaminants could be selected for the second tier of investigation. Then, the appropriate measurements would be selected for chemical analyses and for within-organism and whole-organism levels. Throughout the investigation, population measurements would be envisioned as a constant reference to interpret the relevance of whole- and within-organism findings.

The shortcomings in both the fate and the transport of pulping compounds and current measurement systems at lower organizational levels need to be addressed with basic research. The procedures to assess exposure to pulping effluents are weak, and the knowledge on uptake, depuration,

and metabolism of pulping compounds is very limited. The environmental fate, transport, and potential impact of many pulping compounds are poorly known, and this is particularly true for high molecular weight compounds. As previously noted, an improved focus is suggested on within-organism measurements, using detoxification, hormonal regulation, and immune function end points, rather than clinical assays. One strategy to develop the necessary biochemical measurements is to use mesocosms and experimental streams, for which doses are known and to evaluate proven and experimental measurement systems simultaneously. There is still a need to evaluate measurements under field conditions. A strategy of using established, well-characterized sites and multiple reference areas within long-term investigations is recommended to assess measurement variability and significance in target species.

SUMMARY AND CONCLUSIONS

Where impacts from pulping effluents occur at the community, the population, and even the within-organism levels, there are distinguishable gradients or zones of effects. Historically, organic and nutrient enrichment were largely responsible for adverse environmental impacts from pulping discharges. There is also evidence for chemical toxicity at sites that are simultaneously experiencing enrichment effects. Where organic and nutrient enrichment are sufficiently controlled by process design and biological effluent treatment, community and population effects have not been observed. However, these data are limited, and the possibility of chemical toxicity has not been fully explored.

Continuing impacts from conventional pollutants, the complexity of pulping effluents, the often fragmentary design of experiments, and the limitations of the measurement systems have combined to hamper progress in defining toxicity. Design and measurement shortcomings include limited chemical analyses for exposure assessment, lack of acute and chronic toxicity testing, and lack of fundamental understanding of many within-organism responses. National strategies for effluent regulation and monitoring hold promise to address several shortcomings. However, substantial and sustained research is necessary to understand and to apply within-organism measurements.

Integrated assessments are made necessary by the effluent complexity and site-specific differences, which prevent generalization and extrapolation between sites. The objective of integration is to compile the individual measurement results to yield an identified cause. A stepwise, tiered strat-

egy is recommended to methodically reduce the uncertainties associated with complex effluents. The first tier identifies meaningful adverse effects and characterizes the effluent both chemically and toxicologically. Subsequent steps or tiers then seek to clarify the effects by identifying the within-organism lesion(s) and to discover the responsible contaminant(s). The final outcome is to establish NOAELs for these contaminants with adequate safety margins for the aquatic environment.

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