Journal of Aquatic Ecosystem Stress and Recovery **7:** 247–257, 2000.

Secondary and tertiary stress responses to BKME exposure in the St. Maurice River fish populations

M.M. Gagnon[1,*], D. Bussières[2], P.V. Hodson[3] & J.J. Dodson[2]
[1]*School of Environmental Biology, Curtin University of Technology, Perth 6845, Australia;*
[2]*Département de Biologie, Université Laval, Ste-Foy, Québec, Canada G1K 7P4;*
[3]*Director, School of Environmental Studies, Queens University, Kingston, Ontario, Canada K7L 3N6*
*(*Corresponding author: Phone: +61 8 9266 3723; Fax: +61 8 9266 2495; E-mail: m.gagnon@curtin.edu.au)*

Received 14 September 1999; accepted in revised form 19 April 2000

Key words: BKME, *Catostomus commersoni*, fish lipids, river enrichment, pulp & paper, river continuum, white sucker

Abstract

In the St-Maurice River, Canada, white suckers (*Catostomus commersoni*) collected at two stations located downstream of a bleached kraft mill effluent (BKME) outfall exhibited increased muscle lipid concentrations relative to upstream reference fish. White suckers collected in a physicochemically comparable but uncontaminated river, also demonstrated an increasing trend of lipid accumulation with distance downstream. The similar general trends in both rivers may result from a river continuum in which increasing loads of nutrients with distance downstream increase the energy available to fish.

However, the general trends differed in two respects. The gradient of lipid accumulation in the St. Maurice R. was irregular, suggesting a sharply increased nutrient input by BKME. Secondly, in the reference Gatineau river, increased lipid reserves were accompanied by increased growth and reproductive effort relative to body size. In contrast, increased growth rates in the contaminated St. Maurice River were accompanied by a reduced reproductive effort relative to body size. The failure to translate increased energy intake, as indicated by increased muscle lipids, into increased reproductive effort suggests one or more components of BKME have a toxic effect. Accelerated river enrichment contributed by the discharge of the bleached kraft effluent combined with the natural river continuum made it difficult to isolate the reproductive effects related to effluent exposure.

1. Introduction

Stressors acting upon organisms can be biological, physical, or chemical in nature. A stress response, defined by Selye (1950) as the physiological compensation that an organism attempts to make in order to cope with the imposed pressure, can be measured at different levels of biological organization. Responses measured at lower (molecular to organism) levels of biological organization generally provides sensitive and specific responses to particular toxicant, and often represent a measure of exposure; on the other side, responses measured at higher levels of biological organization (bioenergetics, populations and communities) represent a measure of induced ecological effects (McCarthy & Shugart, 1990). Populations and communities will respond to environmental stressors by two general pathways: where the limits of compensation are exceeded, or when there is inappropriate forms of compensation that are deleterious to the population (Adams et al., 1998). Under field situations, the interaction of complex effluents with site-specific factors along with environmental and biological variations makes the establishment of causal links between any one specific stress and measured biological response difficult.

Exposure of animals to environmental stressors initiates a primary stress response which is expressed as biochemical and physiological changes. Biological responses such as mixed function oxygenase

(MFO) enzyme induction following exposure to certain classes of contaminants, or the release of corticotropin in the bloodstream after a temperature shock (Mazeaud & Mazeaud, 1981) represent a primary stress response. Sustained primary stress reactions such as biochemical alterations will, in the long term, induce a secondary stress response in which changes in energy allocation and metabolism take place. In this case, alterations in lipid utilization and storage may be observed in chronic exposures and in field-collected specimens (Mazeaud & Mazeaud, 1981). Animal populations exposed to a stress of sufficient magnitude and duration may also develop a tertiary stress response expressed as changes in growth, reproduction, or even survival of individuals and/or populations (Wedemeyer et al., 1984).

Energy reserves accumulated in lipid storage are important in regulating and maintaining stability and overall fitness of fish populations (Dey et al., 1983). Natural or anthropogenic induced stresses are energy-demanding processes that can deplete the fish's lipid reserves over time (Adams, 1999). Reduced lipid reserves may result in increased susceptibility of fish to diseases, overwinter mortality, reduced growth, and lowered reproductive success (Stearns, 1992).

Fish growth and reproduction are key parameters used to evaluate the impacts of chemical inputs to the environment. These measures of secondary stress response are known to change in fish populations chronically exposed to environmental stressors (Dehn & Schirf, 1986). However, population parameters may also change in response to a natural river continuum created by an increasing load of nutrients downstream which results in greater transfer of energy to fish (Vannote et al., 1980). Ecosystems do not have definite boundaries, and ecological gradients are a normal part of their assemblage (Chapman, 1992). The possibility that growth and reproductive responses may be induced by natural gradients and not by chemicals must always be considered.

On different sampling occasions, higher (Adams et al., 1992) or lower (Adams et al., 1996) visceral lipid storage were reported in redbreast sunfish (*Lepomis auritus*) at river sites 60 km downstream of a BKME discharge. In lentic environments, Sandström et al. (1988) observed increased energy storage expressed as the condition factor in pulp mill effluent-exposed perch (*Perca fluviatilis*), but McMaster et al. (1991) described reduced visceral lipid reserves accompanied by an increased condition factor in BKME-exposed white suckers (*Catostomus commersoni*). It has been established that pulp and paper mills can discharge significant organic carbon and nutrient loads, which have the potential to alter or degrade aquatic habitats (Owens, 1991). Reported trend differences in biological responses may be due to nutrient enrichment caused by pulp mill effluents, but may also be due to dissimilarities in the environment where fish are collected, differences in fish species and/or in the chemical composition of the effluent to which they are exposed.

Since 1911, the St. Maurice River, Canada, has received the effluents of a bleached kraft mill located at La Tuque, Quebec. At the time of this study, the waste waters from the mill were treated by a 4-minute passage through a primary settling tank to reduce suspended solids. The mill discharged about 10 T of chlorinated compounds per day into the St. Maurice River (Hodson et al., 1992). The effluent was diluted in the river to about 1% (v/v), and mixing was complete within 2 km of the discharge due to hydroelectric dam and rapids downstream. At this rate of dilution, there were no measurable temperature differences among sites (Hodson et al., 1992). The bleaching process used elemental chlorine until 1992, when the conversion to chlorine dioxide was implemented. Previous field investigations conducted in the St. Maurice River provided evidence of chronic exposure via the bioaccumulation of chlorophenolic compounds, which resulted in a variety of effects, including induction of the MFO system, lowered plasma steroid hormone concentrations, disrupted fecundity-weight relationships, delayed age at maturity, increased size at maturity, and increased growth rates for fish aged from 2 to 10 years old (Gagnon et al., 1994, 1995). Fish from this river also exhibited increased visceral lipid accumulation in BKME-exposed white sucker populations 95 km downstream of the effluent discharge (Hodson et al., 1992). Both primary and tertiary responses have been described in detail using sophisticated molecular, biochemical, and statistical techniques, but the nature of the secondary stress responses remain more speculative.

Lipid reserves are a dynamic balance of nutritional status and physiological energy demands. Examples include the overall quantity and quality of the food supply, the ability of the fish to avail itself of that food supply, reproductive effort, overwintering demands, and biological stresses induced by competition or by exposure to chemicals. Lipid reserves may reflect modifications in any or all of these. Investigations of food supply at the initiation of the program in 1990 lead to inconclusive results as the river bed was covered with up to 5 meters of bark, making the sampling of bottom invertebrates practically impossible (J. Carey, CCIW, pers. comm.). Although lipid reserves are not considered direct indicators of contaminant stress, they integrate the combined effects of metabolic stress and nutritional status (Adams, 1999) and can therefore indicate a biologically significant secondary stress response of chronic exposure of fish to BKME.

The objective of the present study was to relate lipid reserves of fish to trends in growth and reproduction in the BKME-contaminated and reference rivers, in order to improve understanding of relationships among biomarkers at the organism and population levels. Energy reserves were evaluated by extracting lipids from the homogenized carcasses which consisted of whole body minus gonad and viscera. We believe this results in a more consistent measure than visual estimation or weighing of visceral lipids for two reasons: firstly, lipid extraction is a quantitative method in which extractions are performed in duplicate, while visual evaluation of the visceral lipid index is a rather subjective measure based on a team-specific scale (McMaster et al., 1991; Swanson et al., 1994). Secondly, because liver lobes are intertwined with the intestine and visceral lipid is not discrete in *Catostomus* species, errors in the weight of the lipid-intestine complex may be introduced by manipulating the intestines to collect the lipids.

2. Materials and methods

2.1 *Study sites*

The St. Maurice and the Gatineau Rivers, both located in the province of Québec, exhibit similar watershed characteristics in term of vegetation, soil types, weather conditions, water temperature, and fish communities (Gagnon et al., 1995). The town of La Tuque discharges its municipal effluents to the St. Maurice River; the town of Maniwaki (with approximately half the population of La Tuque) discharges its municipal effluents to the Gatineau River. Because the St. Maurice R. discharge is twice that of the Gatineau R., the nutrient input from municipal effluents is proportionally similar for the two rivers. The St. Maurice River differs from the Gatineau River primarily in the daily discharge of 240,000 m^3 of effluent and a greater average discharge associated with a greater watershed area.

Three sites were sampled on each river (Figure 1). The reference site 1 of the St. Maurice River was located 10 km upstream of the mill and was isolated from downstream sites by a hydroelectric dam situated beside the mill. This site was characterized by slow currents flowing through a shallow reservoir. Site 2 was located immediately downstream of the mill next to the town of La Tuque, but upstream of its municipal effluent. Site 3 was located 95 km downstream of the mill in a wider section of the river, with slow currents. Log floating was present at all three sites of the St. Maurice River at the time of the study. Similarly, three sites were sampled on the Gatineau River, which also had log floating and hydroelectric dams, but received no industrial effluents. The upstream site 1 was located in the Reservoir Baskatong, 5 km upstream of a hydroelectric dam. A river section neighboring the town of Maniwaki was selected as site 2, located 35 km downstream of site 1, while site 3 was located in a second reservoir 105 km downstream of site 1.

2.2 *Sample collection and analyses*

Fish were collected with gill nets composed of three 50-m panels of 7.7-, 8.9-, and 10.1-cm stretched mesh. Although the design of the larger research project included several other sampling times (Gagnon et al., 1994, 1995), fish used in the present study on lipids were sampled from the St. Maurice River in May, August and November 1990, and from the Gatineau River in August 1991. Supporting data on age-length relationships and body-mass adjusted gonad weight are extracted from analyses performed on fish captured in 1991 and 1992, and described in Gagnon et al. (1995). The use of fish sampled in different years does not confound interpretation; Bussières et al. (1998) have shown that despite some yearly variations in responses, inter-site variations in fish population dynamics (age- and size-at-maturity, age structure, fecundity, etc.) remained the same between years.

For all fish captured alive, measurements were made of fork length, weight of the gonad, viscera (liver, intestine, spleen, etc.) and carcass (whole body minus gonad and viscera), fecundity, age, external and internal deformities and parasites, liver MFO activity, and tissue concentrations of chlorophenols. Details of findings on biochemical (primary) and demographic (tertiary) responses of over 3 600 white suckers collected over 4 years (1990–1993) in the St-

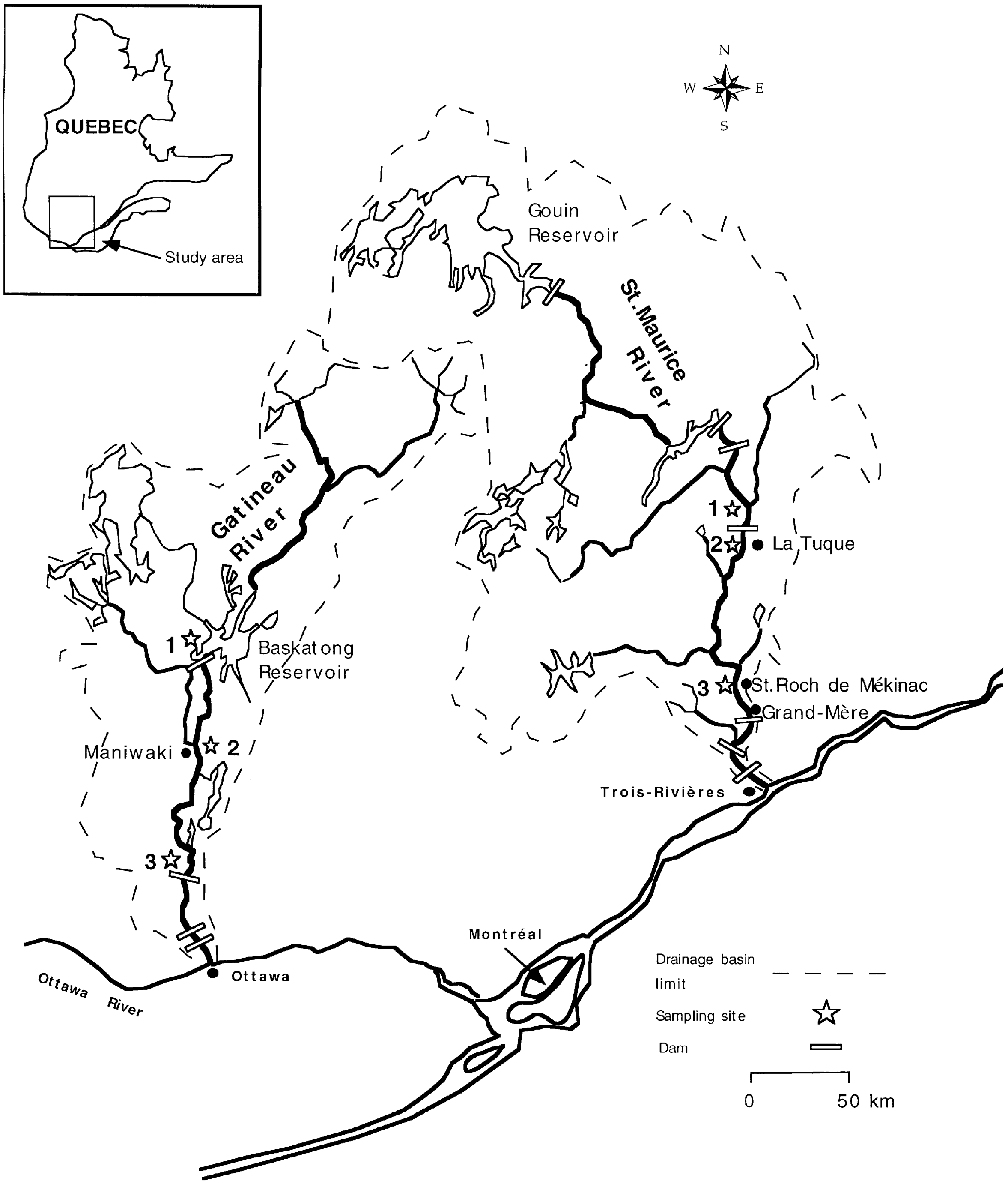

Figure 1. Sampling stations on the St. Maurice and Gatineau rivers. On the St. Maurice River, white sucker populations are isolated by hydroelectric dams at La Tuque and Grand-Mère. A pulp mill located at La Tuque is the major industry discharging effluent into the river. On the Gatineau River, dams were located immediately downstream of stations 1 and 3, but no major industry occurs in the study area. Adapted from Gagnon et al., 1995.

Maurice and Gatineau Rivers can be found in Gagnon et al. (1994, 1995), in Couillard et al. (1995) and in Bussières et al. (1998).

Power analyses based on results from the 1989 St. Maurice River preliminary study established that it was necessary to collect 12 fish of each sex to allow detection of differences in biological (length, weight) parameters with 80% chance to detect a 20% difference (M. Gagnon, unpublished). Therefore, for detailed chemical and biochemical analyses, it was decided to sample the first 30 fish collected at each station, optimizing chances to obtain at least 12 individuals of each sex. The carcasses of the first 30 live fish captured at each site were immediately frozen on dry ice, and kept at −20°C or colder until homogenization. On some occasions, less than 30 live fish were captured per site, resulting in a lower number of carcasses available for lipid extraction. In addition, some of the carcasses were used for purposes other than homogenization and lipid extraction. The age, weight, and length data used in the present report are issued from these first 30 fish sampled during the year 1990 on the St-Maurice R., and 1991 on the Gatineau R. Details of population parameters measured on a greater number of fish can be found in the above-mentioned references. Some of the population parameters are restated in the present paper to facilitate understanding of links between individual and population responses.

In the laboratory, each semi-thawed carcass was passed through a commercial Hobart meat grinder three times to ensure a homogenous mix, and the homogenate was kept at −20°C in sealed and solvent-cleaned glass containers. Lipids were extracted according to Randall (1974) from 273 homogenates of adult fish of relatively uniform sizes (X ± SE: 808 ± 14.3 g, n = 265) [note: carcass weight was not recorded for 8 fish captured in May 1990]. A 1-g subsample of the homogenate was oven-dried, immersed in hot ethyl ether for 10 minutes, raised out of the solvent and further extracted and rinsed with the solvent condensate for 20 minutes. The ethyl ether was removed by evaporation and the residue containing the lipids was weighed. All lipid analyses were performed in duplicates.

2.3 *Statistical analyses*

Because the St. Maurice and Gatineau Rivers are two independent ecosystems with many different environmental variables characterizing them, they can not be directly compared statistically. Therefore, downstream trends in stress indicators are compared between the two systems. The following statistical analyses were performed using only the data related to the fish on which lipids were extracted, while links to population parameters (growth, reproductive parameters) are derived from other studies using greater numbers of fish (Gagnon et al., 1995; Bussières et al., 1998).

Normality of data was verified by examining graphs of residuals versus fitted values, and homogeneity of variance was tested with a Levene test (SPSS, 1994). Lipid data as well as age and fork length did not require transformation, and variances were homogenous amongst groups. Carcass weights were log transformed to achieve normality and homogeneity of variances. Within a river, differences in carcass weight, fish length, and age among sampling sites were tested with a one-way ANOVA. For the purpose of this study, it is assumed that lipid content of the fish carcass is proportional to total body lipids including visceral lipids. In addition, we assumed that total body lipids are reliable indicators of energy reserves for the fish, along with the inputs of food supply and the outputs of reproductive efforts, growth, and maintenance. To verify this last assumption, regression analyses have been performed between carcass lipid content and body dimensions, specifically length, carcass or total weight, condition factor, and gonado-somatic index of the first 30 fish captured at each site. For a given station, when two or more regressions were significant ($P \leq 0.05$) for one sex at one sampling time, an analysis of covariance (ANCOVA) was applied to determine parallelism in slopes. Confirmation of parallelism lead to a comparison of adjusted means to determine significant ($P \leq 0.05$) differences among stations.

A 3-way ANOVA with sampling season, site and sex as classification variables was performed to establish if there were differences in lipid concentrations on the St. Maurice River. A 2-way ANOVA was used for the Gatineau River as data were available from only one sampling season. Fisher's protected LSD was applied to perform post-hoc tests identifying which sites were different from the reference site at $P \leq 0.05$ (SPSS, 1994).

3. Results

One-way ANOVAs on carcass weight, fork length and age revealed that in August 1990, fish captured at site 2 of the St. Maurice River were larger and older relative to fish captured at the reference upstream station. In addition, fish captured at site 3 were heavier in May and August 1990 relative to the upstream site (Table 1). At all other sampling times, and within both rivers, fish were of similar size and age at all sites (Table 1).

Carcass weight to lipid content relationships indicated that for female fish collected in the Gatineau River, the amount of lipid in the carcass increased with the size of the fish (Figure 2). ANCOVA results for these fish demonstrated that fish at site 1 had significantly (P = 0.017) lower lipid reserves for a given body mass relative to fish collected at site 3. Female fish collected at site 2 were not different (P = 0.592) from fish collected at the upstream or downstream sites. At other sampling seasons in the St. Maurice River, and for male fish of the Gatineau River, the carcass weight-to-lipid relationships were occasionally but not consistently significant, most likely due to lower numbers of fish collected at these times.

For lipid concentrations, the 3-way ANOVA demonstrated that the interaction "season$*$site$*$sex" was non-significant (P = 0.71). The analysis was then repeated excluding this interaction term. Of all possible combination of factors, only the interaction "season$*$site" was significant (P = 0.0003) (Table 2). Lipid concentrations in white sucker carcasses are therefore presented per sampling season, per site.

In May and August 1990, BKME-exposed fish had significantly higher carcass lipid content relative to fish collected at the reference site ($P \leq 0.0001$, Figure 3). In November 1990, however, this difference was true only for fish captured at site 2 (P = 0.0005), with fish from site 3 being similar to the reference fish in their carcass lipid concentrations. In the Gatineau River, fish collected at the three sites were all different from each other in their muscle lipid content ($P \leq 0.0001$), with lipid concentrations increasing with distance downstream.

4. Discussion

The findings of the present study confirm that fish accumulated greater muscle lipid reserves at downstream sites of both the St. Maurice and the Gatineau Rivers (Figures 2 and 3). However, the pattern of response differed between the two rivers, with faster nutrient enrichment observed in the St. Maurice R. In both rivers, repeated investigations on white sucker populations revealed that these increasing downstream lipid trends are accompanied by consistently higher visceral lipid accumulation at all downstream sites (Hodson et al., 1992), faster growth (Figure 4), but reduced size-adjusted gonad weight (Figure 5) and delayed age at first maturity in female fish, and disrupted fecundity-weight relationships for the BKME-exposed fish (Gagnon et al., 1995; Bussières et al., 1998).

Lipid stores accumulated by fish are the result of nutritional status and physiological energy demands. Data from multiple field studies investigating BKME-exposed ecosystems concluded that organic and nutrient enrichment have in the past been primarily causes of effects at the population level (Owens, 1991). It is possible that food supply provided by the effluent-induced river enrichment, and by the natural river continuum (Vannote et al., 1980) may counteract the chemical stress related to effluent exposure, resulting in greater lipid accumulation at exposed sites. This pattern of effluent-induced enrichment resulting in increased food availability to fish has been reviewed in Owens (1991) and others (Hall et al., 1991; Lowell et al., 1995). In freshwater ecosystems receiving BKME, field-caught redbreast sunfish (*Lepomis auritus*) exhibited a higher visceral lipid somatic index (Adams et al., 1992). Similarly, Swanson et al. (1994) reported higher mesenteric fat stores in BKME-exposed longnose suckers (*Catostomus catostomus*). However, higher mesenteric lipid storage was not accompanied by greater lipid stores in the fish fillets which appeared to be similar for both exposed and reference longnose suckers (Swanson et al., 1994). In the St. Maurice River, higher concentrations of visceral lipids in exposed fish were obvious during the 1989 preliminary study (Hodson et al., 1992), as well as most other sampling occasions from 1990 to 1993 (P. Hodson, unpublished results). Only in November 1990 were the carcass lipid reserves at the most downstream site of the St. Maurice River similar to the ones measured at the reference site 95 km upstream (Figure 3). Adams et al. (1996) reported that in redbreast sunfish, visceral lipid reserves returned to reference levels in fish captured 75 km downstream of the BKME discharge. The November 1990 results may not indicate a recovery at the most downstream site, because visual observations during several other sampling seasons, as well as the quantitative results of May and August 1990, indicated that downstream fish of the St. Maurice River are fattier than upstream fish. Lower lipid concentrations at site 3 may have

Table 1. Carcass weight (g), fork lengths (cm) and ages (years) ± SEM (N) of white suckers on which total lipids were extracted. Sample sizes vary due to missing data for carcass weights or age of some fish. SM: St. Maurice River, GAT: Gatineau River (adapted from Gagnon et al., 1994).

	Carcass weight (g)			Length (cm)			Age (years)		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
SM May 90	735 ± 43 (20)	868 ± 39 (18) *P* = 0.004	988 ± 76* (10)	39.1 ± 0.88 (23)	39.9 ± 0.75 (18) *P* = 0.43	40.8 ± 1.12 (15)	8.35 ± 0.49 (23)	7.88 ± 0.53 (16) *P* = 0.80	8.21 ± 0.53 (14)
SM Aug. 90	649 ± 35 (28)	999 ± 21* (30) $P < 0.001$	774 ± 45* (38)	36.5 ± 0.68 (28)	42.6 ± 0.35* (30) $P < 0.001$	38.0 ± 0.85 (38)	6.56 ± 0.39 (27)	9.21 ± 0.56* (29) $P < 0.001$	6.39 ± 0.42 (38)
SM Nov. 90	735 ± 71 (10)	968 ± 71 (8) *P* = 0.10	735 ± 77 (15)	39.1 ± 1.38 (10)	43.6 ± 1.18 (8) *P* = 0.06	38.9 ± 1.55 (15)	6.33 ± 0.60 (9)	8.37 ± 1.03 (8) *P* = 0.18	6.15 ± 0.87 (13)
GAT Aug. 91	762 ± 33 (30)	834 ± 34 (30) *P* = 0.46	788 ± 52 (30)	38.0 ± 0.59 (30)	39.7 ± 0.65 (30) *P* = 0.26	38.3 ± 0.99 (30)	6.40 ± 0.33 (30)	6.73 ± 0.58 (30) *P* = 0.14	5.47 ± 0.42 (30)

* Significant at $P < 0.05$ (one-way ANOVA).

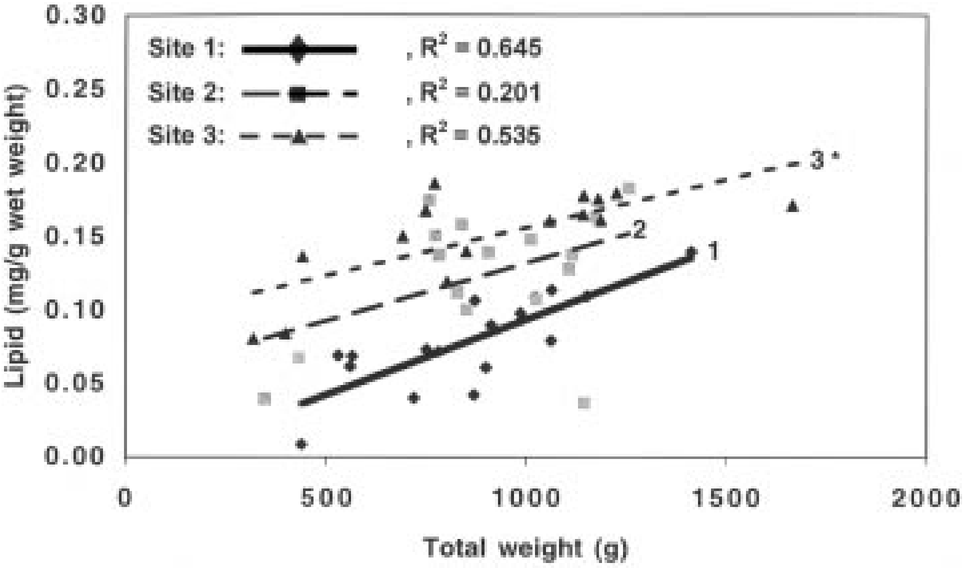

Figure 2. Lipid – (mg/g wet weight) to total body weight (g) relationships measured in carcass of female white suckers collected in the uncontaminated Gatineau River in August 1991. Fish collected at site 3 had significantly (*P* = 0.017) higher carcass lipid reserve relative to fish collected at upstream site 1. Ns = 18, 16 and 15 for sites 1, 2 and 3 respectively.

been an artifact related to lower numbers of fish captured during November. Increased downstream growth (Figure 4) and greater lipid storage in the St. Maurice and Gatineau Rivers strongly support the river continuum hypothesis whereby nutrient enrichment increases downstream (Vannote et al., 1980).

The eutrophication gradient in both rivers may result not only from naturally increasing nutrient loads related to an expanding drainage basin downstream, but also from anthropogenic sources such as the pulp mill and the small towns located along the waterways. The positive effects of nutrient enrichment in the St. Maurice River seems to counteract the negative effects of chemical stress related to continuous exposure to BKME, resulting in increasing gradients in lipid accumulation in both contaminated and reference rivers. This lipid gradient in the contaminated river reflects, however, the complex integration of factors such as chemical stress, and intra- and inter-specific competition (Adams et al., 1996). In addition, food chain effects possibly related to reduced invertebrate diversity (Kemp & Stewart, 1966) but enhanced invertebrate density (Owens, 1991) and/or larger prey size (Lowell et al., 1995) in BKME rivers, and habitat modifications (Davis et al., 1988) may result in differential lipid accumulation in fish.

While lipid concentrations increased with distance downstream in the Gatineau R., the nutrient enrich-

Table 2. Statistical results of ANOVAs performed on lipid content of the St. Maurice and Gatineau R. white suckers. Sample sizes as follow for sites 1, 2, 3 respectively (males (females)): St. Maurice R.: 28(28), 35(24), 25(43); Gatineau R.: 12(18), 14(16), 15(15)

	St. Maurice River[a]	Gatineau River[b]
F-values		
Season	3.77	
Site	45.86	41.72
Sex	0.09	2.95
Season∗site	5.64	
Season∗sex	0.81	
Site∗sex	0.88	0.69
P-values		
Season	0.03	
Site	0.00	0.00
Sex	0.77	0.09
Season∗site	0.00	
Season∗sex	0.44	
Site∗sex	0.42	0.51

[a] The interaction season∗sex∗site was non-significant (P = 0.71). Therefore, results presented are derived from the three-way ANOVA repeated excluding this interaction term.

[b] Two-way ANOVA as lipid data are derived from only one sampling season.

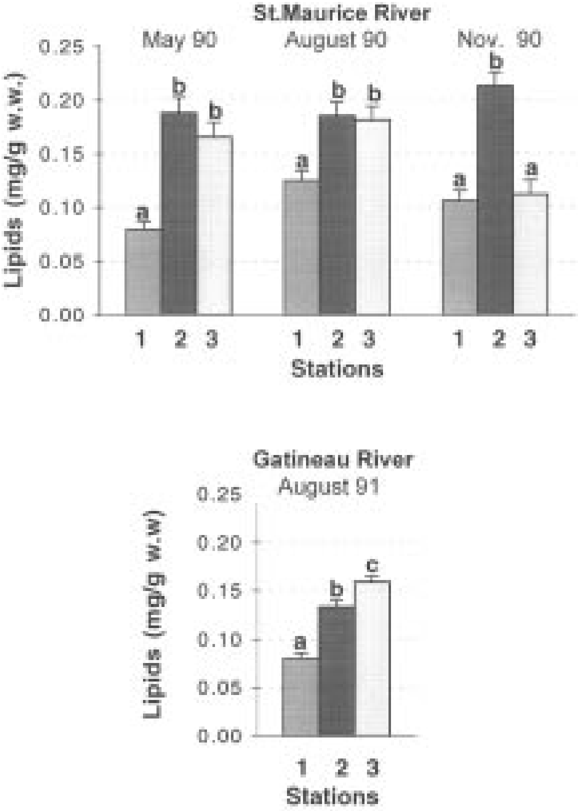

Figure 3. Carcass lipid content (mg lipid/g wet weight) ± SEM of white suckers captured in 1990 in the St. Maurice River and in 1991 in the Gatineau River. Only fish from stations 2 and 3 of the St. Maurice River are chronically exposed to BKME. Sample sizes are as indicated in Table 1 for lengths. Different letters indicate significant difference among stations ($P \leq 0.05$).

ment in the St. Maurice R. caused by the release of BKME seem to accelerate the river continuum, as demonstrated by the largest lipid reserves in fish captured immediately downstream of the effluent outfall. In the Gatineau River, a gradual increase in muscle lipid reserves demonstrates the progressive enrichment of the waterway. In the St. Maurice River however, a sharp increase occurred at sampling station 2 at all sampling occasions, despite the fact that this site is located at less than 10 km from the reference upstream site. Fish captured in August 1990 at site 2 were larger than at other sampling locations, perhaps due to stronger currents at this site in August 1990, favoring larger fish. However, at the two other sampling occasions on this river, fish captured at this station and used for the lipid extraction were of similar size to those from the upstream reference site. The sharp increase in lipid concentrations at site 2 suggests nutrient enrichment related to BKME effluents released to the St. Maurice River which would be additional to, and most likely amplify, the natural gradient already present in this system. Fish captured at site 2 on the St. Maurice R. could also have frequented the area 1 km downstream of the capture site, where the La Tuque municipal effluent is released. This would possibly enhance productivity of this river section and provide the fish with additional food; it is not possible to isolate the effect of the municipal effluent from the BKME effluent. Nevertheless, a municipal effluent release was present in both rivers, supporting the notion that the difference in lipid accumulation between the rivers was due to BKME.

In female fish, lipids stored in various body compartments during summer months will be mobilized for gonad development (Love, 1980). In the reference Gatineau River, increased available energy was reflected in faster growth, greater lipid reserves, and larger gonad size for a given body size in female fish. In the St. Maurice River, however, the increased productivity at downstream sites did not result in a greater reproductive effort in females (Figure 5). In female fish, gonad size as a function of body size was reduced downstream of the BKME discharge, and slopes of fecundity-to-carcass-weight relation-

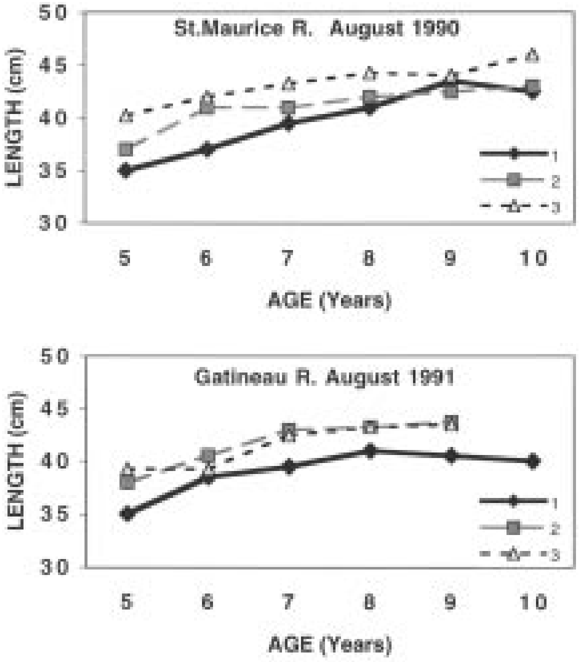

Figure 4. Age–length relationships of female white sucker aged between 5 and 10 years old captured in the St. Maurice (August 1990) and Gatineau (August 1991) Rivers. * indicates a significant difference ($P \leq 0.05$) between the upstream station (thicker line) and at least one downstream station. Each data point represents at least 5 adult fish of each age, at each station. Adapted from Gagnon et al. (1995).

ships were more variable at exposed sites of the St. Maurice River in May 1990 (Gagnon et al., 1994). Under similar river environments, Adams et al. (1996) observed that, despite reduced visceral lipid reserves, enhanced growth rates and reduced fecundity as a function of body weight occurred in BKME-exposed redbreast sunfish. McMaster et al. (1991) also reported reduced visceral lipid reserves and lower ‘total fecundity’ (total number of eggs per fish, independent of fish size) in a BKME-exposed white sucker population. Reduced lipid storage in exposed fish may be the result of chemical stress draining more energy from the fish than the extra energy provided by enhanced ecosystem productivity. In the St. Maurice River, there is either insufficient chemical stress to affect lipid accumulation, or this effect is masked by the sum of natural enrichment and anthropogenic nutrient inputs. Nevertheless, it is evident in altered reproductive parameters where increased lipid reserves did not result in higher reproductive outputs. Transfer of available energy to reproductive organs may be impaired by exposure to contaminants causing lowered steroid titers (Gagnon et al., 1994) and other biochemical dysfunctions such as reduced capacity of the liver to produce egg yolk (Adams et al., 1992).

Gonad size and fecundity generally increase with age and size of the female within a fish species (Bone et al., 1995), and failure to do so in BKME-exposed fish represent a tertiary stress response at the population level. However, fish are larger and abundant at the exposed sites, indicating that their limits to compensate for the imposed chemical stress is not exceeded despite their reduced reproductive output. Consequences of BKME exposure in the St. Maurice River appear to result in several metabolic imbalances that do not, however, reduce the fish’s fitness to a level that threatens their survival.

5. Conclusions

The muscle lipid analyses reported in the present study complement biochemical, physiological, and population response data previously reported (Hodson et al., 1992; Gagnon et al., 1994, 1995; Bussières et al., 1998). BKME-exposed white suckers demonstrated increased lipid accumulation compared to upstream reference fish, similar to the lipid gradient observed in fish collected from a reference river. However, the pattern of distribution amongst downstream sites was different. The release of the BKME effluent appears to accelerate the natural gradient of enrichment, as shown by a sharp rather than a gradual increase in lipid reserves in fish collected immediately downstream of the pulp and paper mill.

Modified energy allocation in white suckers in the St. Maurice River may represent a secondary stress response induced by chronic exposure to BKME. In exposed fish, a greater lipid accumulation was reflected in increased growth rates indicating increased energy intake. However, increased intake did not translate into higher reproductive output, as demonstrated by reduced gonad size in female fish. It is concluded that in the St. Maurice River, nutrient enrichment by the effluent results in an energy gain for the fish, rather than a net energy expenditure related to chemical stress. The resulting demographic gradients along the contaminated river represents the integration of all directly and indirectly acting natural and anthropogenic pressures, and appears to be consistent through years of sampling (Bussières et al., 1998). The results of the present study, along with those of other studies, reinforce the notion that the impact of

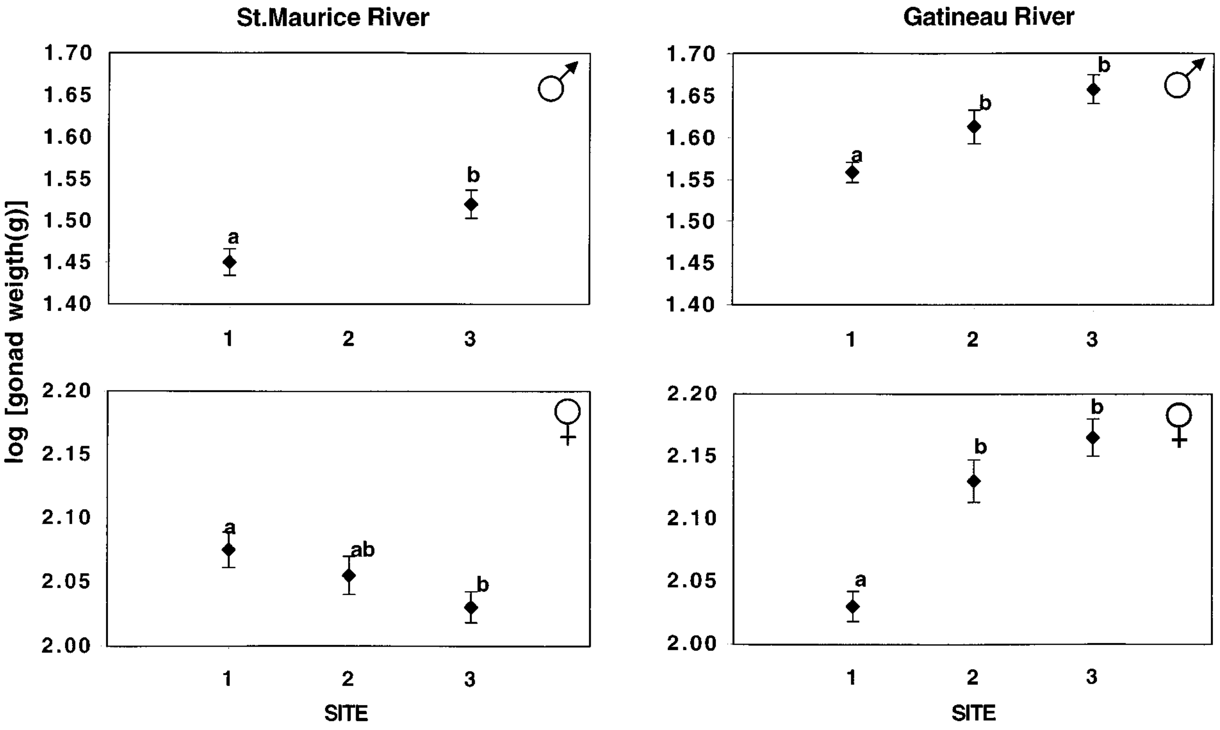

Figure 5. Site to site comparisons of body mass-adjusted mean weight of gonads from male and female white suckers captured during the spawning runs in the St. Maurice River in May 1990 and Gatineau River in May 1992. Different letters indicate significant difference among stations ($P \leq 0.05$). Bars indicate 95% confidence limit. Adapted from Gagnon et al. (1995).

pulp mill effluent on fish populations has to be evaluated on a case-by-case basis, and emphasize the need for repeated sampling and appropriate experimental design involving uncontaminated, reference rivers.

The effluent-induced river enrichment has the potential to complicate efforts to define chemically-induced biological responses, especially when measured responses such as physiological indices may be responsive to a wide range of environmental conditions. Thus studies investigating induced effects of pulp and paper effluents on ecosystems should measure biological responses at several levels of biological organization in order to establish connection between organizational levels and enhance understanding of relationships between primary, secondary and tertiary biological responses.

Acknowledgements

The authors are grateful to Catherine Couillard, Marie-Helène Michaud, Denis Thivierge, Andrée Tremblay, and Paul Robichaud for their technical assistance during field sampling. We also thank Serge Higgins for performing the lipid extractions. Financial assistance of the Groupe Interuniversitaire de Recherche Océanographique du Québec (GIROQ), the Department of Fisheries and Oceans (Institut Maurice-Lamontagne), and the Natural Sciences and Engineering Research Council of Canada (NSERC) is gratefully acknowledged.

References

Adams, S. M., W. D. Crumby, M. S. Greeley Jr., L. R. Shugart & C. F. Saylor, 1992. Responses of fish populations and communities to pulp mill effluents: A holistic assessment. Ecotoxicol. Environ. Saf. 24: 347–360.

Adams, S. M., K. D. Ham, M. S. Greely, R. F. LeHew, D. E. Hinton & C. F. Saylor, 1996. Downstream gradients in bioindicator responses: Point source contaminant effects on fish health. Can. J. Fish. Aquat. Sci. 53: 2177–2187.

Adams, S. M., K. D. Ham & R. F. LeHew, 1998. A framework for evaluating organism response to multiple stressors: Mechanisms of effect ad importance of modifying ecological factors. In: J. J. Cech, B. W. Wilson & D. G. Crosby (eds), *Multiple Stresses in Ecosystems*, pp. 13–33. Lewis, Boca Raton FL.

Adams, S. M., 1999. Ecological role of lipids in the health and success of fish populations. In: M. Arts & B. Wainmann (eds), *Lipids in Freshwater Ecosystems*, pp. 132–160. Springer-Verlag, New York.

Bone, Q., N. B. Marshall & J. H. S. Blaxter, 1995. *Biology of Fishes, Blackie Academics and Professional*. Glasgow U.K. 332 pp.

Buhler, D. R., M. E. Rasmusson & W. E. Shanks, 1969. Chronic oral DDT toxicity in juvenile coho and chinook salmon. Toxicol. App. Pharmacol. 14: 535–555.

Bussières, D., M. M. Gagnon, J. J. Dodson & P. V. Hodson, 1998. Does annual variation in growth and sexual maturation of white sucker (*Catostomus commersoni*) confound comparisons between pulp mill contaminated and reference rivers? Can. J. Fish. Aquat. Sci. 55: 1068–1077.

Chapman, P. M., 1992. Ecosystem health synthesis: Can we get there from here? J. Aquat. Ecosyst. Health 1: 69–79.

Couillard, C. M., P. V. Hodson, P. Gagnon & J. J. Dodson, 1995. Lesions and parasites in white suckers, *Catostomus commersoni*, in bleached-kraft pulp mill-contaminated and reference rivers. Environ. Toxicol. Chem. 6: 1051–1060.

Davis, T. M., B. D. Vance & J. H. Rodgers Jr., 1988. Productivity responses of periphyton and phytoplankton to a bleached-kraft mill effluent. Aquat. Toxicol. 12: 83–106.

Dehn, P. F. & V. R. Schirf, 1986. Energy metabolism in large-mouth bass (*Micropterus floridanus salmoides*) from stressed and non-stressed environments: Adaptation in the secondary stress response. Comp. Biochem. Physiol. 84A: 523–528.

Dey, A. C., J. W. Kiceniuk, U. P. Williams, R. A. Khan & J. F. Payne, 1983. Long term exposure of marine fish to crude petroleum I. Studies on liver lipids and fatty acids in cod (*Gadus morhua*) and winter flounder (*Pseudopleuronectes americanus*). Comp. Biochem. Physiol. 75C: 93–101.

Gagnon, M. M., D. Bussières, J. J. Dodson & P. V. Hodson, 1995. White sucker (*Catostomus commersoni*) growth and sexual maturation in pulp mill-contaminated and reference rivers. Environ. Toxicol. Chem. 14: 317–327.

Gagnon, M. M., J. J. Dodson, P. V. Hodson, G. Van der Kraak & J. H. Carey, 1994. Seasonal effects of bleached kraft mill effluent on reproductive parameters of white sucker (*Catostomus commersoni*) populations of the St. Maurice River, Québec, Canada. Can. J. Fish. Aquat. Sci. 51: 337–347.

Hall, T. J., R. K. Haley & L. E. LaFleur, 1991. Effects of biologically treated kraft mil effluent on cold water stream productivity in experimental stream channels. Environ. Toxicol. Chem. 10: 1051–1060.

Hodson, P. V., M. McWhirter, K. Ralph, B. Gray, D. Thivierge, J. H. Carey, G. Van der Kraak, D. M. Whittle & M.-C. Levesque, 1992. Effects of bleached-kraft mill effluent on fish in the St. Maurice River, Québec. Environ. Toxicol. Chem. 11: 1635–1651.

Kemp, L. E. & R. K. Stewart, 1966. *Effects of Pollutants on Biota of the Pigeon River, Cocke Country, Tennessee*. Technical Services Program, Federal Water Pollution Control Administration, Cincinnati, Ohio.

Love, R. C., 1980. *The Chemical Biology of Fishes*, Vol. 2. Advances 1968–1977. Academic Press, New York, 943 pp.

Lowell, R. B., J. M. Culp & F. J. Wrona, 1995. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environ. Toxicol. Chem. 14: 1529–1541.

Mazeaud, M. M. & F. Mazeaud, 1981. Adrenergic response to stress in fish. In: A. Pickering (ed.), *Stress and Fish*, pp. 49–76. Academic Press, New York.

McCarthy, J. F. & L. R. Shugart, 1990. Biological markers of environmental contamination. In: J. F. McCarthy & L. R. Shugart (eds), *Biomarkers of Environmental Contamination*, pp. 3–14. Lewis, Boca Raton FL.

McMaster, M. E., G. J. Van der Kraak, C. B. Portt, K. M. Munkittrick, P. K. Sibley, I. R. Smith & D. G. Dixon, 1991. Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft mill effluent. Aquat. Toxicol. 21: 199–218.

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

Randall, E. L., 1974. Improved method for fat and oil analysis by a new process of extraction. Journal of the AOAC, 57: 1165–1168.

Sandström, O., E. Neuman & P. Karås, 1988. Effects of a bleached pulp mill effluent on growth and gonad function in Baltic coastal fish. Wat. Sci. Tech. 20: 107–118.

Selye, H., 1950. Stress and the general adaptation syndrome. Br. Med. J. 1: 1383–1392.

SPSS, 1994. *SPSS Professional Statistic 6.1*. SPSS Inc., Chicago, IL.

Stearns, S. C., 1992. *The Evolution of Life Histories*. Oxford University Press, New York. 249 pp.

Swanson, S. M., R. Schryer, R. Shelast, P. J. Kloepper-Sams & J. W. Owens, 1994. Exposure of fish to biologically treated bleached-kraft mill effluent. 3. Fish habitat and population assessment. Environ. Toxicol. Chem. 13: 1497–1507.

Vannote, R. L., G. V. Minshall, K. W. Cummins, J. R. Sedell & C. E. Cushing, 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37: 130–137.

Wedemeyer, G. A., J. D. McLeay & C. P. Goodyear, 1984. Assessing the tolerance of fish and fish populations to environmental stress: The problems and methods of monitoring. In: V. W. Cairns, P. V. Hodson & J. O. Nriagu (eds), *Contaminants Effects on Fisheries*, pp. 164–195. Wiley, New York.