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National Assessment of Cycle 4 Data from the Pulp and Paper Environmental Effects Monitoring Program

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Executive Summary

Under the *Fisheries Act*, the *Pulp and Paper Effluent Regulations* (PPER) require Canadian pulp and paper mills discharging to aquatic environments to conduct Environmental Effects Monitoring (EEM) to assess effects potentially caused by their effluent. These studies include two key components: a fish population field survey to assess effects on fish and a benthic invertebrate community field survey to assess effects on fish habitat. Some mills are also required to assess effects on the usability of fisheries resources, including a study of dioxins and furans in fish tissue. In addition, sublethal toxicity testing is conducted to aid in assessing effluent quality.

The Pulp and Paper EEM Program is structured into "cycles", whereby a mill conducts an EEM study every three to six years. Studies carried out in Cycles 1 through 3 were aimed at characterizing and assessing the condition of the receiving environment and determining the extent and magnitude of effects in cases where effects were detected and confirmed. Investigation of cause (IOC) studies were introduced in May 2004 PPER amendments (during Cycle 4) for those mills that have detected and confirmed effects and determined their extent and magnitude. The IOC study is undertaken to gain a better understanding of the cause of the observed effects. Investigation of solutions (IOS) is a new component of the EEM Program included in the most recent PPER amendments (August 2008) which now apply to Cycle 5.

The purpose of this report is to present and discuss the results of the national assessment of EEM data collected from the receiving environments of pulp and paper mills across Canada for Cycle 4, and to compare these findings to those from Cycles 2 and 3.

Two complementary quantitative approaches were used to provide an assessment of the overall effects of pulp and paper mill effluents on aquatic biota: 1) tabulation of the results of individual mill comparisons and 2) meta-analyses to investigate national patterns of effects. In addition, a qualitative review of the IOC studies undertaken by mills that found repeatable, large effects in previous cycles is also presented in this report.

The national average response patterns in Cycle 4 were quite similar to those observed in Cycles 2 and 3, for both the fish survey and the benthic invertebrate community survey. For fish, they were characterized by increased condition, growth rate, and relative liver size, together with decreased relative gonad size. This response is typical of nutrient enrichment conditions, co-occurring with disruption in resource allocation to gonadal growth.

The benthic invertebrates' national average response was typical of various degrees of nutrient enrichment. This response was characterized by increased density and changes in community structure (Bray-Curtis endpoint), together with a variety of responses for taxon richness. It is noteworthy that the pulp and paper industry has recently proactively developed Best Management Practices (BMP) for nutrient management (FPAC, 2008). As these BMP will be implemented in the future, one would expect to see improvements at sites with eutrophication responses.

A quick look at the fish results might suggest significant reductions in effects in Cycle 4, relative to the earlier cycles. This, however, was mostly a result of absence of data from many of the large-effect mills pursuing IOC studies in Cycle 4; that is, they did not submit standard survey data that could be included in the analyses. After correcting for this bias, this apparent reduction in effects mostly disappeared. The notable exception was relative gonad size, which showed a significant lessening of the national average effect, even after correcting for the bias. It remains to be seen whether this trend in the gonad reduction effect will continue into future cycles.

An interesting insight gained by looking at national response patterns for fish in Cycle 4, without correcting for large-effect mills going to IOC, is that it shows what the pulp and paper industry as a whole could achieve if mills having large effects are successful in their investigation of solution (IOS) efforts and in mitigating their effects. In the future, this kind of analysis would provide a means of measuring how successful the industry as a whole has been in ameliorating effects.

In contrast to the findings for fish, the influence of some large-effect mills pursuing eutrophication IOC studies was much smaller when looking at national response patterns for benthic invertebrates. As larger numbers of mills pursue IOC and IOS for nutrient enrichment studies and introduce BMP for nutrient management in Cycle 5 and beyond, future meta-analyses would provide a means for measuring industry-wide improvements in benthic invertebrate effects that are expected to occur.

It should be noted that further analyses (Barrett and Munkittrick, unpublished) have shown that some of the cycle-to-cycle variability in national response patterns for fish is likely due to using different species of fish (from cycle to cycle) for monitoring. Other likely causes for cycle-to-cycle variability in national response patterns are changes in study design and changes in reference and exposure sampling locations, as well as environmental variability due to factors other than effluent exposure.

The qualitative review of the IOC interpretive reports on eutrophication (17 studies), reduced gonad size (10 studies), and elevated levels of dioxins and furans in fish tissue (1 study), has shown a wide variety in the study designs implemented, recommendations, and paths forward even within the same category of IOC. Mill recommendations stated in reports vary from suggesting reduced monitoring to recommending that study methods be refined and applied once again in Cycle 5. Other approaches put forward by the mills were limited to a continuation of the monitoring effort implemented or pursued at the IOC stage.

The IOC studies conducted in Cycle 4 focused on identifying the causes responsible for the major response patterns seen so far through the Pulp and Paper EEM Program. It should be recognized however that other potential effects of pulp and paper effluents may exist and that these may warrant some attention.

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1.0 Introduction

1.1 The National EEM Program for Pulp and Paper

Under the *Fisheries Act*, the 1992 *Pulp and Paper Effluent Regulations* (PPER) prescribe discharge limits for total suspended solids and biochemical oxygen demand, and require effluent to be non-acutely lethal to fish. While introduction of secondary treatment of effluents allowed mills to meet these limits they also provide a national baseline standard that is intended to protect fish, fish habitat and the use of fisheries resources. At the time the regulations were developed, it was acknowledged that there was uncertainty about the effectiveness of the new limits for protecting the diverse variety of aquatic environments receiving pulp and paper mill effluents in Canada. To assess the adequacy of the effluent regulations in protecting the aquatic environment, Environmental Effects Monitoring (EEM) was included as a requirement in the 1992 PPER. In May 2004, the *Regulations Amending the Pulp and Paper Effluent Regulations* (RAPPER) came into force and further clarified the requirements of the 1992 PPER.

In January 2005, the Smart Regulation Project on Improving the Effectiveness and Efficiency of Pulp and Paper Environmental Effects Monitoring was launched in response to stakeholder feedback on the EEM program. This project brought together a group of policy experts from the federal government, industry and the aboriginal and environmental communities who, together, made a number of recommendations for changes to the structure of the program to improve its efficiency (Environment Canada 2005). As a result, further amendments to the regulations have been introduced and the newest version of the *Regulations Amending the Pulp and Paper Effluent Regulations* was published on August 6, 2008. The amendments will be reflected in studies to be conducted in Cycle 5. Changes to the EEM study requirements introduced in the recent set of amendments are described at the following link: *http://gazetteducanada.gc.ca/partII/2008/20080806/html/sor239-e.html*.

Canadian pulp and paper mills that are subject to the PPER are required to conduct studies on their receiving environments in order to assess and monitor effects potentially caused by their effluent. These studies may include the following components:

- a fish population survey to assess fish health;
- a benthic invertebrate community survey to assess effects on fish habitat; and
- studies to assess effects on the usability of fisheries resources, including a study of dioxins and furans in fish tissues.

The sublethal toxicity testing and measurements of supporting water and sediment quality variables are conducted to contribute to the program in areas such as interpretation of biological data and assessing effluent quality. Unlike the biological field data, these supporting variables are not used to determine whether environmental "effects" are occurring at mill sites. Instead, they are meant to provide further information that may help to evaluate effects on a site-specific basis. This national assessment report is not intended to cover all data submitted to fulfill the requirements of the program nor to cover all analyses conducted. Rather, it focuses on the core components of the EEM Program used for decision making and interpreting major response patterns (i.e., fish population survey and benthic invertebrate community survey). Further site-specific information on the supporting variables is included in the interpretive reports produced by each mill.

The Pulp and Paper EEM Program is structured into "cycles", whereby a mill conducts an EEM study every three or six years with both monitoring and interpretation phases. Within each cycle, a mill develops a site-specific study design in consultation with Environment Canada regional staff. At the end of each cycle, each mill is required to submit an interpretive report that summarizes its monitoring results. The EEM Program has evolved to use a tiered approach to monitoring. Initial studies that characterize and assess the condition of the receiving environment are followed by targeted or focused studies to determine if effects occur. Extent and magnitude of effects are determined where effects are detected and confirmed. Monitoring is reduced where effects are not found. Mills that have detected and confirmed effects and determined their extent and magnitude move beyond monitoring and focus on finding the causes of the observed effects through an investigation of cause (IOC) study. In the Pulp and Paper EEM Program, IOC studies were first conducted on a national scale in Cycle 4 following introduction of this requirement in the 2004 PPER amendments. Chapter 7.0 of this report is entirely dedicated to IOC. Mills that have identified the cause in IOC studies can now move towards investigation of solutions (IOS), the new component for Cycle 5. Technical guidance is developed by Environment Canada on all aspects of EEM studies, including study design, analyses and interpretation of data. Additional information on the EEM program is available at http://www.ec.gc.ca/eem/.

Currently, mills have completed their fourth cycle of monitoring and reporting. Cycle 1 which was completed in 1996, was used primarily as a baseline to gain better understanding of the variability of the field measurements, to identify problems and to make appropriate changes and adjustments for future cycles. Many Canadian mills upgraded their effluent treatment systems during Cycle 1 to meet the requirements of the 1992 PPER. This upgrade substantially reduced toxicity of mill effluents and improved effluent quality. It should be noted that effluent quality at individual mills can also vary from year to year due factors such as changes in furnish, product, production rates, etc. The national assessments of biological monitoring data for Cycles 2 and 3 (Lowell *et al.*, 2003, 2004, 2005) showed that pulp and paper mill effluents continued to affect fish health and habitat. The effects measured in the fish and benthic invertebrate communities were, for the most part, very similar between Cycles 2 and 3. Most notably, both cycles have shown 1) evidence of overall nutrient enrichment and eutrophication (increased invertebrate abundance and Bray-Curtis dissimilarity index as well as fatter, faster growing fish with larger livers) and 2) a reduction in fish gonad size indicative of metabolic disruption. The nature of the Cycle 4 studies conducted by each mill is dependent upon their results of previous cycles. Mills that found repeatable, large effects in either the invertebrate or fish surveys in Cycles 1 through 3, attempted to determine the extent and magnitude of the effects or to investigate the cause of the effects (Chapter 7.0) in their Cycle 4 studies. The remaining mills were required to conduct standard biological monitoring.

1.2 Objectives of the Report

The purpose of this report is to present and discuss the results of the national assessment of standard biological monitoring data for Cycle 4 collected in the 2005 and 2006 seasons. Some summary results on the investigation of cause studies are also presented. For the standard surveys, the data analyses focused on the following questions:

1) What are the types and magnitudes of effects of pulp and paper mill effluents on adult fish and benthic invertebrate communities?
2) How does Cycle 4 compare with Cycles 2 and 3 in terms of magnitudes of effects and types of effects?
3) How do measured effects compare with predefined critical effect sizes established to help determine which mills are having the most serious impacts?

In addition to the above analyses, the results of two mills that have looked at the effects of their effluents on the use of fisheries resources (levels of dioxins and furans in fish tissue) are also presented.

A qualitative review of the IOC studies is presented in order to describe the methodological approaches implemented and to summarize and compare the paths forward as they are outlined in the reports submitted by facilities involved in IOC studies.

1.3 Effect Indicators and Critical Effect Sizes

In EEM, an effect is defined as a statistically significant difference in one of the measurements taken in fish or benthic invertebrates from the exposure area relative to those from a reference area or along a gradient of effluent exposure (Environment Canada 2004a). Environment Canada has developed critical effect sizes (CES) for fish and benthos (Environment Canada 2004b) to help identify differences considered to be having significant environmental impacts and where more information is needed in order to better understand the ecological significance of these differences (e.g., extent and magnitude of effects). For fish, the CES are based on 1) the magnitude of observed pulp mill effluent effects that were previously demonstrated in Canada and Sweden, 2) natural variations typically observed and 3) the magnitude of effects observed at mills with the greatest impacts in Cycle 2 (Environment Canada 2004b, Lowell *et al.* 2003). For benthic communities, the CES are also based on the magnitude of effects measured during Cycle 2, as well as the concept that effects exceeding the "normal range" of variability in reference areas are important (Lowell 1997, Kilgour *et al.* 1998, Lowell *et* *al.* 2003). The effect endpoints and CES used to determine effects on fish and benthic invertebrates are presented in Table 1 whereas background information on these endpoints including calculation methods, is available in Environment Canada (2005).

Table 1. Fish population and benthic invertebrate community survey endpoints and their respective critical effect sizes (CES) for the Pulp and Paper EEM Program.

Endpoints	**Indicator**	**Critical effect size**[a]
Fish population survey		
Gonad weight relative to body weight	Reproduction	± 25%
Liver weight relative to body weight	Condition	± 25%
Body weight relative to body length	Condition	± 10%
Age	Survival	± 25%
Body weight relative to age	Growth	± 25%
Benthic invertebrate survey		
Total density	Number of animals	± 2 SD
Taxon richness	Number of taxa or kinds of animals	± 2 SD
Simpson's evenness	Measure of how evenly the animals are distributed among the taxa	± 2 SD
Bray-Curtis index	Measure of dissimilarity in community composition among sites	± 2 SD[b]

[a] CES for fish endpoints are expressed as percent (%) difference of exposure relative to reference data. CES for the benthic invertebrate surveys apply only to control/impact designs and are expressed as exceeding ±2 standard deviations (SD) of the mean of the reference data. For gradient designs, a significant correlation in two consecutive cycles is used in place of the CES. More information on CES can be obtained from Lowell (1997) and Lowell *et al.* (2002, 2003), Environment Canada 2004b, 2005.
[b] CES for Bray-Curtis index is under review.

The CES presented in Table1 are currently being used to make decisions in the EEM program. However, following the Smart Regulation review in which the recommendation to "strengthen the role of CES in focussing and accelerating action towards identification of cause and solution and in improving efficient targeting of resources by identifying mills that could go to reduced monitoring" was made, a review of the CES has been initiated. This review is being conducted to ensure adequacy in identifying effects of most importance.

In this national assessment the standard survey distributions of measured effect sizes are presented and the measured effects are compared with the CES in Table 1. At the individual mill level, the EEM program uses exceedance of CES (when statistically significant effects are confirmed in two consecutive cycles) to help identify those mills expected to conduct more focused monitoring to assess the extent and magnitude of effects or to investigate the cause of the observed effects at a site.

2.0 Overview of Cycle 4 Studies

In Cycle 4, 98 mills conducted EEM studies. Table 2 presents a regional summary of the number and type of standard field surveys conducted by mills for Cycle 4 as well as the number of mills that only conducted sublethal toxicity tests. A total of 48 fish surveys were conducted, of which most were lethal surveys. Four non-lethal fish surveys were conducted, a method introduced to the program in Cycle 4. Six surveys used alternative methods including the use of caged and wild bivalves and fish mesocosms.

There were 60 mills that conducted benthic invertebrate surveys, which included 46 control/impact designs, 12 gradient designs and 2 studies that followed the reference condition approach (RCA). As some mills conducted joint studies, the total number of mills is not equal to the number of performed surveys. A total of 28 mills were exempted from conducting a fish survey for various reasons, including an effluent concentration in the exposure area that was less than 1% within 250 m of the point of discharge (20 mills), lack of effects in previous cycles (7 mills), and prohibition by Transport Canada for safety reason with regards to commercial navigation (1 mill). In addition, 10 mills were exempted from undertaking a benthic invertebrate survey for reasons including the lack of effects in previous cycles (6 mills), the absence of appropriate methods for sampling in some portions of the St. Lawrence ecosystem, combined with the lack of adequate alternative methods (2 mills), the presence of confounding factors (1 mill), and the prohibition by Transport Canada for security reasons (1 mill).

Table 2: General summary of Cycle 4 pulp and paper EEM studies (excluding magnitude and extend and IOC studies).

Region	**No. of mills that conducted EEM studies**	**Fish surveys**			**Benthic invertebrate surveys**			**No. of mills that conducted only sublethal toxicity tests**
		Lethal	**Non-lethal**	**Alternatives**	**Control / Impact**	**Gradient**	**RCA**	
Atlantic	18	4	1	3[a]	11	3	1	0
Quebec	37	18	0	2[b]	23	4	0	3
Ontario	17	9[c]	1[c]	1[d]	5	2	0	2
Prairie and Northern	8	4[c]	1[c]	0	5	0	0	2
Pacific and Yukon	18	3	1[e]	0	2	3	1[f]	4
Subtotal	98	38	4	6	46	12	2	11
Total	**98**	**48**			**60**			**11**

[a] One caged bivalves, two wild bivalves
[b] Two caged bivalves

[c] One mill conducted both a lethal and non-lethal fish survey
[d] One fish mesocosm study
e includes one joint survey of four mills
f includes one joint survey of four mills

In addition to the mills that conducted standard fish and benthic surveys, some mills did a magnitude and extent study or a study investigating the cause of effects found in previous cycles (Table 3). One mill did a magnitude and extent study on both fish and benthos, while three other mills conducted this study on benthos only. A total of 28 investigation of cause studies were conducted; 17 on eutrophication, 10 on reduced gonad size and 1 on dioxins and furans in fish tissue.

Table 3: General summary of magnitude and extent and investigation of cause studies conducted in the Cycle 4 Pulp and Paper EEM Program.

Region	No. of mills that conducted EEM studies	Magnitude and Extent	Investigation of Cause		
			Eutrophication	Fish Gonad	Fis Tissue (D/F)
Atlantic	18	0	0	4	0
Quebec	37	0	2	6	0
Ontario	17	3[a]	5	0	0
Prairie and Northern	8	1[b]	2	0	0
Pacific and Yukon	18	0	8	0	1
Subtotal	98	4	17	10	1
Total	**98**	**4**	**28**		

[a] One mill did extent and magnitude for both fish and benthos; two mills did extent and magnitude for benthos only
[b] Extent and magnitude for benthos only

3.0 General Methods

3.1 Assessment of Standard Fish and Invertebrate Survey Data

3.1.1 Data Preparation and Analysis

The methodologies used to carry out the national assessment of data from the standard fish and benthic invertebrate community surveys conducted in Cycle 4 were similar to the previous national assessments (see Lowell *et al.* 2003, 2005 for further details). Two complementary quantitative approaches were used to synthesize the large number of independent studies to provide a national overview of the effects of pulp mill effluents: 1) tabulation of the results of individual mill comparisons and 2) meta-analyses. The tabulations are presented in this study as frequency distributions of magnitudes of effects (exposure vs. reference percent differences) and as histograms of the number of significant or non significant differences at the level of the individual mill. Interpretation of the histograms was limited by the fact that the significance level is dependent not only on the magnitude of difference between an exposure and reference area, but also on the sample size. Meta-analysis does not have the same limitations as individual study tabulations since it is a technique used to statistically examine the magnitude of effects in a way that loses less information due to the constraints of individual study sample sizes and scale of measurement (Hedges and Olkin 1985, Rosenberg *et al.* 2000, Gurevitch and Hedges 2001). In this meta-analysis approach, the separate studies are treated as replicates; as such, it is possible to look at questions that are difficult to examine at the individual mill level (e.g., the relation of sampling Cycle to national-level effluent effects in the field). A full description of how meta-analysis was used for the national assessment can be found in Lowell *et al.* (2003).

The standard fish surveys were based on the control/impact approach where sampling stations were located in reference and exposure areas. Most standard benthic invertebrate community surveys were conducted following a control/impact design, although some followed a gradient design where sampling stations are located along an exposure gradient. Five mills used the reference condition approach for their benthic invertebrate community surveys but these were not included in the national assessment (see explanation below). Further information on EEM study designs and respective analyses for the fish and benthic invertebrate surveys is provided in Glozier *et al.* (2002), Lowell *et al.* (2002, 2003) and the Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring (Environment Canada 2005).

The national assessment focused on near-field effects to investigate the more pronounced effects that are occurring nationally for the control/impact fish and benthic invertebrate surveys. Some mills collected data from multiple areas (e.g., multiple near-field areas). Data from more than one area were pooled if warranted based on inspection of the interpretive reports. A Statistical Assessment Tool (SAT) developed initially by the National Water Research Institute of Environment Canada was used to calculate the magnitude and statistical significance of effects for the five core fish and four core benthic invertebrate endpoints shown in Table 1 for control/impact study design only. For the benthic invertebrate surveys following a gradient design, regression analysis was used to analyze changes in the benthic invertebrate endpoints with increasing distance from the effluent outfall.

The first step common to both the fish and invertebrate analyses is the screening of the electronic data for obvious errors (missing data fields, obvious data entry errors, etc.). SAT then aided with the selection of the appropriate data for analysis, including removal of outliers. Finally, SAT or a spreadsheet were used to analyze the data via ANOVA (Analysis of Variance), ANCOVA (Analysis of Covariance), or regression analysis, depending on the study design, to determine the relationship between exposure and reference areas (or correlation coefficient for gradient designs) for each of the endpoint values for each mill. The ANOVA and ANCOVA analyses provided area means, standard deviations and the magnitudes of effects, which were required for subsequent tabulations and meta-analyses of measured effects for the control/impact designs. For gradient benthic invertebrate designs regression analyses provided correlation coefficients for tabulations of results. For the purposes of the tabulations presented here, the significance level (α) was set at 0.1 for the ANOVA and ANCOVA and at 0.05 for the regression analyses.

The fish data were log-transformed and analyzed using ANCOVA (all endpoints except age); fish age data were non-transformed and were analyzed using ANOVA. The invertebrate data were non-transformed, except density (which was log-transformed), and analyzed using ANOVA. Further discussion regarding data transformation and methods of analysis can be found in the Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring (Environment Canada 2005).

The frequency distributions of the magnitudes of effects are presented for data from Cycles 2, 3 and 4. Cycle 1 data were not used in the assessment as they primarily were used to understand the variability of the field measurements, to identify problems, and to improve the program for future cycles. In order to have a valid comparison of data among cycles, the Cycle 2 and 3 data from mills conducting Cycle 4 IOC studies were excluded. However, if Cycle 4 data from an IOC study was submitted electronically and could be analysed as a standard survey, all cycles of data were included. The histograms of the number of significant or non significant differences at the level of the individual mill are presented for Cycle 4 data alone.

3.1.2 Meta-Analysis Procedures for Determining National Response Patterns

Meta-analysis is a set of statistical procedures used to quantitatively synthesize the results of a large number of independent studies. Further, it permits overall response patterns to be determined. The meta-analyses required determination of a standardized magnitude of effect, the Hedges' d effect size, which was calculated as the difference between the exposure and reference means, divided by the pooled standard deviation (this value is multiplied by a correction factor that accounts for the effect of small sample sizes) (Rosenberg *et al.* 2000).

The main meta-analytical results are presented in the format illustrated in Figure 1. The standardized effect size is on the x-axis, with the vertical line representing a zero effect. The result for each mill grouping is presented as a horizontal 95% confidence interval about a vertical tick mark indicating the average effect size for that grouping of mills. Mill distributions to the right of the zero effect line indicate that the average effect associated with effluent exposure was an increase in the measured endpoint. Similarly, mill distributions to the left of the zero effect line indicate an effluent-associated decrease in the measured endpoint. The increase or decrease is statistically significant for the group as a whole if the 95% confidence interval does not overlap the zero effect line. Larger mill groupings (that are non-significant as a whole) can be composed of smaller subgroups, some or all of which may be significantly different from zero.

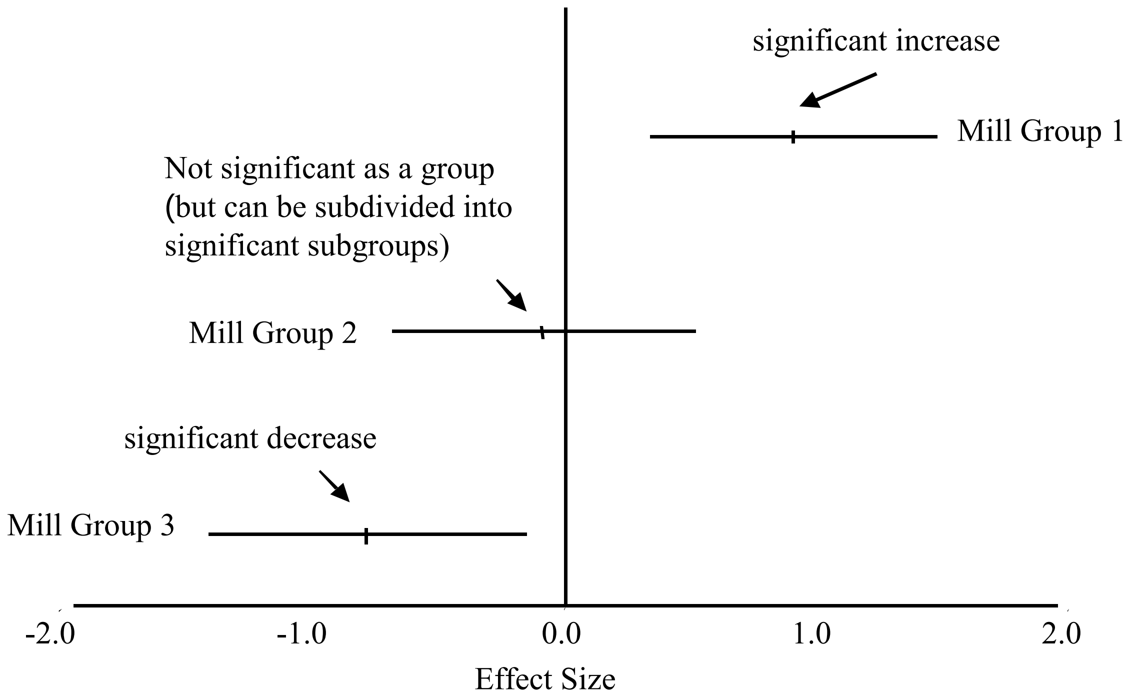

Figure 1: Example of a meta-analysis summary figure. The effect size was measured as Hedges' d (see text).

3.2 Investigation of Cause

Each IOC study was summarized, taking into account the following questions: 1) was the IOC process initiated solely on the basis of the data and conclusions from previous EEM studies, or were there other studies or evidence taken into account?; 2) what was the rationale supporting the study design?; 3) what are the main results and conclusions of the IOC study?; 4) what are the next steps and timeline indicated in the report?, and; 5) was work related more with Investigation of Solutions (IOS) presented in the study document?

Chapter 7.0 outlines the general observations related to the study methods employed by the mills involved in IOC studies as well as their proposed path forward in the program process.

4.0 Fish Survey Analysis

The adult fish survey is used to determine if the effluent is affecting fish populations by comparing effluent-exposed fish with those from reference areas. The survey uses fish growth, reproduction, condition and survival to assess the overall health of exposed fish. These are assessed via measurements of five core endpoints: weight at age, relative gonad and liver weights, condition (body weight relative to length), and age (Table 1). It is recommended that mills sample adults of two sentinel fish species and conduct analyses of the five core endpoints on both species.

4.1 Data Processing and Study Designs

In Cycle 4, a total of 38 lethal fish surveys were conducted in full or partially by mills across the country (Table 2). Prior to analysis, the fish data that had been submitted electronically were screened for errors and incompleteness. Four of the 38 lethal fish surveys were omitted entirely from the analyses because of insufficient number of fish captured or the predominance of immature fish. Other mills encountered problems with their fish survey, but only part of the data had to be excluded (e.g., insufficient number of fish or immature fish for one species or gender only). Table 4 presents a summary of the problems encountered by mills while conducting their Cycle 4 standard lethal fish survey. Overall, the majority of the submitted data were of good quality and, in comparison with previous cycles, mills have had more success in completing the fish surveys (Lowell *et al.* 2005).

Table 4: Problems encountered with standard lethal fish surveys in Cycle 4 of the Pulp and Paper EEM Program.

Problems	Number of Mills
Too few fish (<12) in exposure area	6
Too few fish (<12) in reference area	2
Too few fish (<12) in exposure and reference area	1
Fish are all or mostly immature	12
Only one sentinel species due to absence of a second species found adequate for EEM purposes	2
Problems aging fish	2[a]
Other problem with electronic data	1

[a] in both cases, the fish species is three-spine stickleback (*Gasterosteus aculeatus*).

Non-lethal fish surveys were conducted by 4 mills while 6 used alternative methods such as fish mesocosms and caged and wild bivalves. Due to the different nature of their endpoints, the non-lethal and alternative studies were not included in these summary analyses.

Twenty-nine fish species were used as sentinel species by mills that conducted or attempted a lethal fish survey in Cycle 4. Of these, 25 were included in the national assessment. The frequencies of species used in lethal surveys are presented in Table 5. Only 3 sentinel species used in Cycle 4 had not been used in previous cycle's national assessments (creek chub, northern red belly dace and Pacific staghorn sculpin). The other 26 species were used at least once from Cycles 1 through 3. The complete list of sentinel species used in previous cycles is provided in Lowell *et al.* (2005).

Table 5: List and frequencies of sentinel species used in standard lethal fish surveys in Cycle 4 of the Pulp and Paper EEM Program.

Species	**Scientific name**	**Number of studies[a]**	**Number of studies in national assessment[b]**
Freshwater species			
Large-bodied fish			
White sucker	*Catostomus commersonii*	14	11
Fallfish	*Semotilus corporalis*	6	2
Longnose sucker	*Catostomus catostomus*	5	3
Rock bass	*Ambloplites rupestris*	5	4
Walleye	*Sander vitreus*	4	3
Shorthead redhorse	*Moxostoma macrolepidotum*	3	2
Brown bullhead	*Ameiurus nebulosus*	2	2
Yellow perch	*Perca flavescens*	2	2
Common carp	*Cyprinus carpio*	1	1
Goldeye	*Hiodon alosoides*	1	1
Total number of studies that used freshwater large-bodied fish		**43**	**31**
Small-bodied fish			
Emerald shiner	*Notropis atherinoides*	3	0
Pumpkinseed	*Lepomis gibbosus*	3	2
Johnny darter	*Etheostoma nigrum*	2	2
Mottled sculpin	*Cottus bairdii*	2	2
Three-spine stickleback	*Gasterosteus aculeatus*	2	2
Common shiner	*Luxilus cornutus*	1	1
Creek chub	*Semotilus atromaculatus*	1	1
Golden shiner	*Notemigonus crysoleucas*	1	1
Iowa darter	*Etheostoma exile*	1	0
Longnose dace	*Rhinichthys cataractae*	1	0
Mimic Shiner	*Notropis volucellus*	1	1
Northern red belly dace	*Phoxinus eos*	1	1
Prickly sculpin	*Cottus asper*	1	1
Spottail shiner	*Notropis hudsonius*	1	0
Torrent sculpin	*Cottus rhotheus*	1	1
Trout-perch	*Percopsis omiscomaycus*	1	1
Total number of studies that used freshwater small-bodied fish		**22**	**15**
Marine/estuarine species			
Small-bodied fish			
Mummichog	*Fundulus heteroclitus*	4	4
Atlantic silverside	*Menidia menidia*	1	1
Pacific staghorn sculpin	*Leptocottus armatus*	1	1
Total number of studies that used marine/estuarine small-bodied fish		**6**	**6**

[a] Includes both successful and unsuccessful studies.

[b] Includes only those studies for which sufficient electronic data were available (e.g., excludes studies that did not capture sufficient numbers of adult fish).

4.2 Summary of Effect Sizes

The key fish comparisons summarized in this section focus on the endpoints for which Environment Canada has had Critical Effect Sizes for the last two cycles - i.e., condition and relative gonad and liver weight (CES for age and weight at age have just been developed and their exact usage is still being evaluated). Measured differences were calculated as exposure area minus reference area adjusted means, expressed as a percentage of the reference area adjusted mean. Figure 2 provides a complete summary of the measured differences (i.e., includes both statistically significant and non-significant differences) for comparisons where exposure versus reference ANCOVA slopes are parallel, which represents the majority of the comparisons (Environment Canada 2004a). Data from standard surveys and IOC studies were included in the tabulations when the study followed a control/impact design. Where the Cycle 4 IOC data were excluded due to the different nature of its design or lack of electronic data, the Cycle 2 and 3 data for that mill were excluded as well. This exclusion of some Cycle 2 and 3 data allows for a valid comparison among all three cycles but has the disadvantage of showing mainly the mills having lesser impacts.

Similar to Cycles 2 and 3, condition factor in Cycle 4 showed the smallest range in exposure versus reference area percent differences (-15% to 45%) in which only two mills were above 20% (Fig. 2). Cycle 4 gonad weight percent differences ranged from approximately -45% to 55%, a distribution somewhat different from Cycles 2 (-95% to 75%) and 3 (-65% to 35%). In Cycle 4, liver weight percent differences ranged from -25% to 65%, which was a smaller range than in previous cycles (-35% to 75% and -55% to 65% in Cycles 2 and 3 respectively).

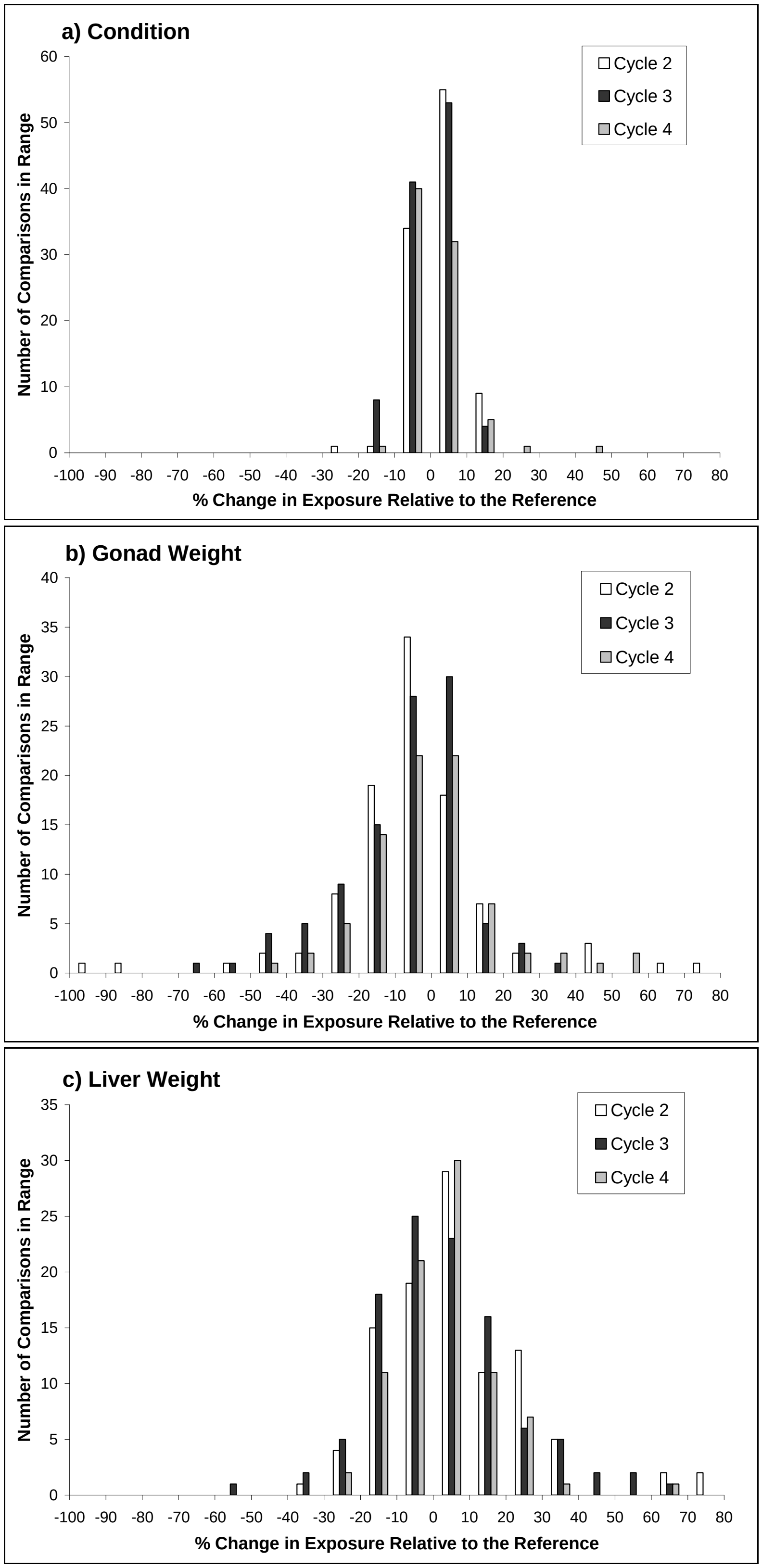

Figure 2: Distribution of measured percent differences between exposure and reference area fish in Cycles 2, 3 and 4 for a) condition, b) gonad weight and c) liver weight.

Figure 3 shows the number of comparisons that showed no significant differences, significant interactions or significant differences in adjusted means for condition, gonad weight and liver weight in Cycle 4. Significant interaction occurs when the exposure versus reference area slopes are statistically different in the ANCOVA analysis; that is, when the slopes can be considered to be non-parallel. For example, non-parallel exposure versus reference slopes for an ANCOVA regression of gonad weight against body weight could indicate that fish exposed to effluent allocate resources to gonad weight differently for fish of different size, relative to fish in the reference area. Significant interactions and significant difference in adjusted means are both considered significant effects. When the exposure and reference area slopes are considered to be parallel, the adjusted means are then compared to determine whether they are statistically different (no significant interaction, so effect of effluent exposure is approximately the same for fish of different sizes). In Figure 3, there are two categories of comparisons where the exposure versus reference area ANCOVA adjusted means were significantly different. The first of these two categories consist of a significant difference in adjusted means, but with an effect magnitude smaller than the CES. The second category consists of comparisons where the exposure versus reference area adjusted means were significantly different with an effect magnitude that exceeded the CES. A methodology has been developed to allow the calculation of effect sizes for cases where there are significant interactions (National EEM Office, in preparation). Effect sizes calculations for significant interactions were underway but not completed at the time this national assessment was undertaken. Environment Canada is currently considering the possibility of incorporating these into future assessments. See Environment Canada (2005) for further information on ANCOVA procedures and interpretation.

Similar to Cycle 3, the analyses revealed that about half of the comparisons in Cycle 4 were not statistically significant. Significant interactions, where the slopes were considered to be non-parallel were found for 14 to 20% of the comparisons. Depending on the endpoint, 27 to 35 % of the comparisons were significantly different with parallel slopes (last two categories in Figure 3). Cycle 4 showed a lower proportion of significant effect above CES (7%) compared to Cycle 3 (16%), which may be due to the mills with greater impacts now being in IOC (Lowell *et al.* 2005).

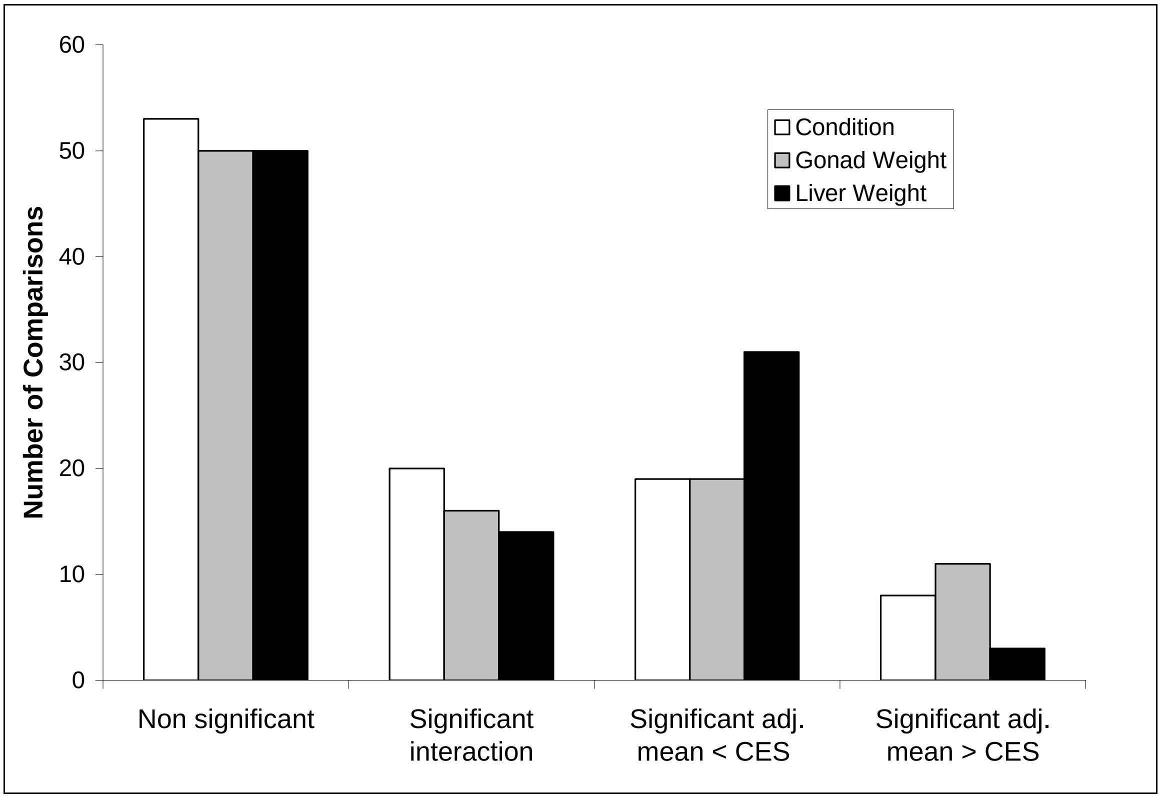

Figure 3: Number of exposure versus reference Cycle 4 fish comparisons showing no significant difference, a significant interaction, a significant difference in adjusted means less than CES and a significant difference in adjusted means greater than CES for condition, gonad weight and liver weight.

4.3 Response Patterns and Meta-analyses

Three main response patterns were observed in the Cycle 2 and 3 EEM fish surveys: nutrient enrichment, nutrient limitation and metabolic disruption (Lowell *et al.* 2003, 2005). These responses, and the changes in the EEM endpoints associated with them, have been widely described in the literature (see Munkittrick *et al.* 1991, 1994, 2000 for reviews). It should be noted, however, that other response patterns may also occur at some mills. The first of the three main patterns, nutrient enrichment, was generally associated with increases in gonad and liver weight, as well as increases in condition and often growth rate (weight at age). The second main pattern, nutrient limitation together with chemical toxicity or other inhibitory effects, was associated with decreases in these endpoints. Nutrient limitation is defined broadly here to include some combination of limited availability of food, appetite suppression and/or internal alteration of food absorption (leading to decreases in several endpoints). Factors other than nutrient availability (such as chemical toxicity) may also contribute to decreases in several of the endpoints. However, chemical toxicity may occasionally lead to increased liver size (as part of the detoxification mechanism), together with decreases in the other endpoints.

The third, and most prominent, response pattern seen in Cycles 2 and 3 was associated with increases in condition and liver weight, and decreases in gonad weight. This was the national average response pattern (Fig. 4) and is generally indicative of nutrient enrichment coupled with metabolic disruption (Munkittrick *et al.* 2000). Thus, the national averages (grand means) in Figure 4 show that fish in effluent-exposed areas were significantly faster growing (increased weight at age), were significantly fatter (increased condition), and had significantly larger livers than fish in reference areas, but exposed fish also had significantly smaller gonads. This response pattern can occur when effluent exposure disrupts normal allocation of resources to gonadal development and may include some element of endocrine disruption associated with difficulties in producing sufficient sex steroid hormones (Munkittrick *et al.* 1991, Van Der Kraak *et al.* 1992, Damstra *et al.* 2002) and is an area of active research (Hewitt *et al.* 2005, McMaster *et al.* 2005, Parrott 2005). Note that the age and weight at age endpoints are included here for informational purposes, although they should be interpreted with care due to difficulties in aging fish at some mills during earlier cycles of the program.

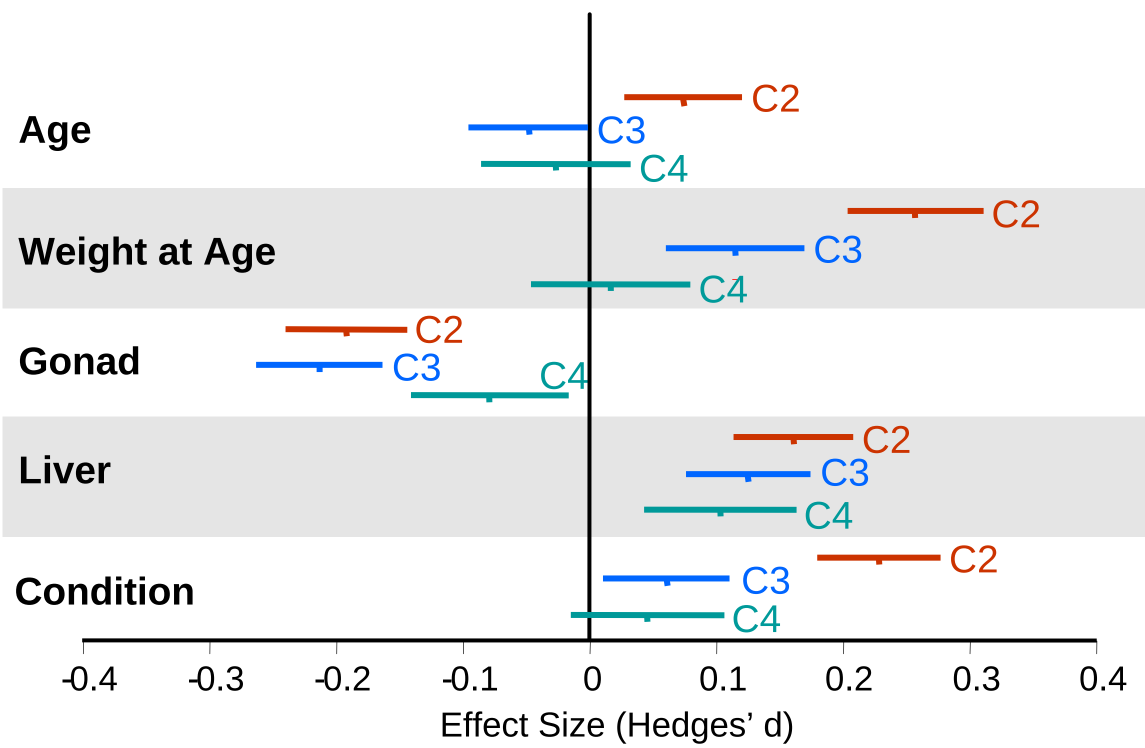

Figure 4: Pulp and Paper National Average Fish Effects for Cycles 2-4 (C4 not corrected for missing IOC mills).

The national average response pattern in Cycle 4 was quite similar to that seen in the earlier cycles (Figs. 4 through 6). Nationally, the trend was toward increases in condition, liver size, and growth rate, together with a significant decrease in gonad size. At first glance, the uncorrected data in Figure 4 would suggest a lessening of effects for all of the endpoints in Cycle 4 compared to previous cycles. With the exception of the gonad endpoint, however, this was primarily due to many of the mills having large effects pursuing IOC in Cycle 4.

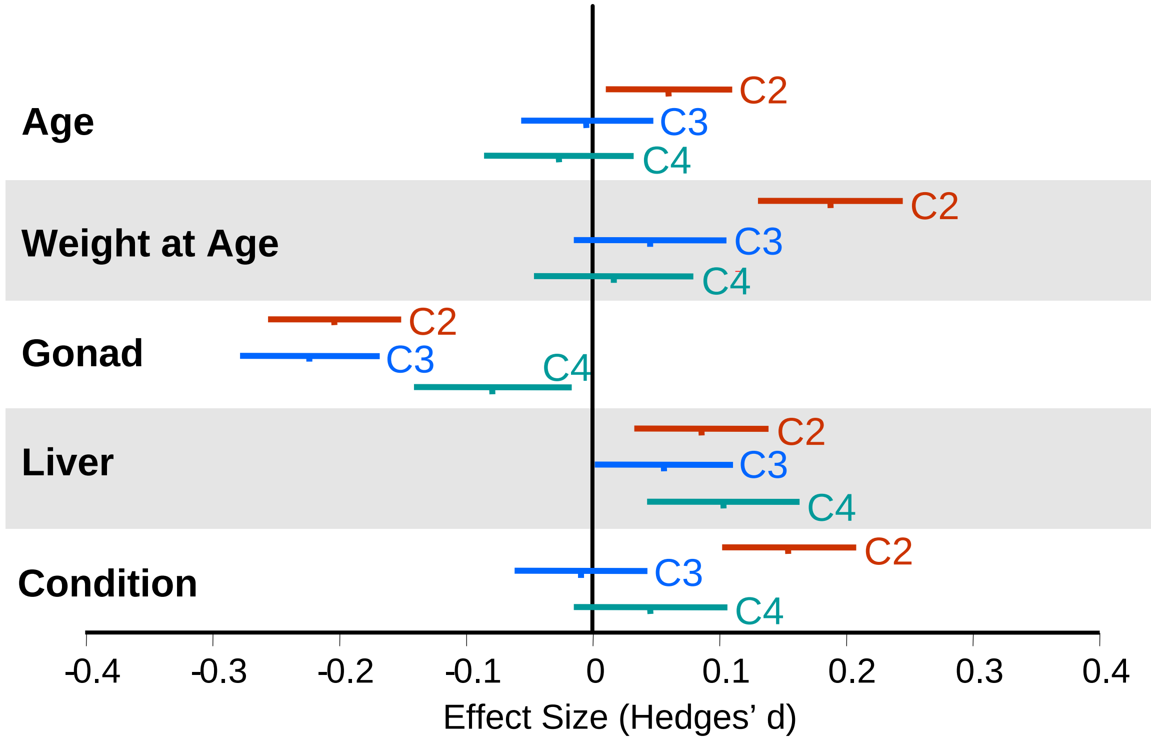

Figure 5: Pulp and Paper National Average Fish Effects - Cycles 2-4 (IOC mills removed for all cycles).

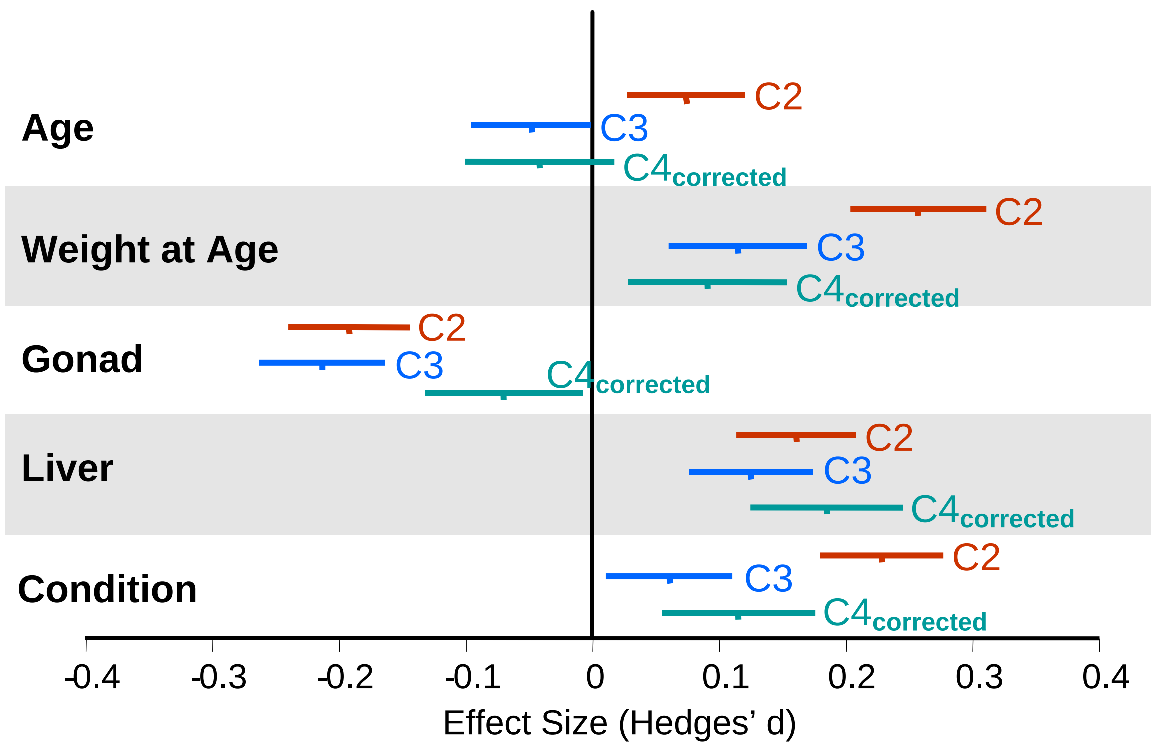

Figure 6: Pulp and Paper National Average Fish Effects for Cycles 2 to 4 (C4 corrected for missing IOC mills).

Most mills conducting IOC studies in Cycle 4 did not conduct standard monitoring and, thus, are not included in Figure 4. This biased the national patterns toward smaller effects in the meta-analyses. This biasing effect can be seen in Figure 5, where the IOC mills from Cycle 4 have been removed from the data sets for Cycles 2 and 3, as well. Put another way, Figure 5 shows what the national patterns would have looked like across all cycles if the mills having biggest effects did not exist. Comparing Figure 5 to Figure 4 shows (for both Cycle 2 and 3) an apparent lessening of national average effects for all of the endpoints except gonad size. This shows the bias introduced by not including the IOC (biggest effect) mills in the analyses. The bias was likely not as apparent for the gonad endpoint because mills went to IOC for both gonad reduction effects and for nutrient enrichment effects (which are sometimes associated with increased gonad size). Therefore, removal of these two extremes in gonad effects may have cancelled each other out, resulting in no net bias in the national average pattern for the gonad endpoint.

For illustrative purposes, the bias (evident for all the endpoints except gonad size) in the Cycle 4 data is corrected for in Figure 6. This was done by measuring the magnitude of bias introduced by removing the IOC mills from Cycles 2 and 3 (i.e., measuring the magnitude of shift for the Cycle 2 and 3 meta-analysis bars in Figure 5 vs. Figure 4), and then using the averaged Cycle 2 and 3 shift for each endpoint to apply a correction factor to the Cycle 4 data. Thus, Figure 6 provides a better picture of current national patterns of effects than does Figure 4. As can be seen in Figure 6, no improvement was seen in effects in Cycle 4 for the age, weight at age, liver, or condition endpoints. Even after correcting for IOC bias, however, a significant lessening of gonad reduction effects was seen in Cycle 4.

5.0 Fisheries Resources and Usability

In Cycle 4, only two freshwater mills have undertaken fish tissue analyses for dioxins and furans to evaluate the impact of effluent on the usability of fisheries resources by humans. Results for both mills indicate that dioxins and furans in fish tissue were not exceeding Health Canada fish consumption guidelines for dioxins and furans that are set at 15pg/g of wet weight in muscle tissue or 30pg/g of wet weight in liver or hepatopancreas (Environment Canada 2004a).

6.0 Benthic Invertebrate Community Survey

The other key component of the EEM program is the benthic invertebrate community survey, which assesses the impacts of mill effluent on fish habitat. The benthic invertebrate survey helps to provide information on the availability of aquatic food resources to fish and on the degree of habitat degradation due to organic enrichment or other forms of physical and chemical contamination. The four endpoints used to assess the effects of pulp and paper effluents on benthic invertebrate communities are total density, taxon richness (number of taxa), Bray-Curtis index of dissimilarity and Simpson's evenness. Taxa were analysed at the family level or above family level when data were reported only at a higher level. For further discussion on the rationale for the level of taxonomic resolution, see Bowman and Bailey (1997), Bailey *et al.* (2001), Lenat and Resh (2001) and Culp *et al.* (2003).

6.1 Data Processing and Study Designs

The standard benthic invertebrate community surveys conducted by mills in Cycle 4 included 46 control/impact designs and 12 gradient designs. There were 5 mills that followed a RCA and an additional 8 mills used artificial substrates; these were excluded from the national assessment. To be consistent with the analyses conducted for the Cycle 3 national assessment, 2 gradient designs were analysed following a control/impact design. In addition, data from 4 control/impact studies conducted as part of IOC studies, and for which data were available for inclusion in this national assessment, were analysed as gradient designs for consistency with the analyses conducted in previous cycles. Similar to the fish survey, the benthic invertebrate data were initially screened for errors. Overall, most data submitted were of good quality and data from only two surveys had to be excluded; in one case because of the different type of habitat between the exposure and the reference area and the other due to errors found in the dataset.

6.2 Summary of Effect Sizes

Figure 7 presents the Cycle 2, 3 and 4 distributions and ranges in measured exposure versus reference area percent differences for total density, taxon richness, Simpson's evenness and the Bray-Curtis index of dissimilarity. The measured differences were calculated as the exposure area mean minus the reference area mean, expressed as a percentage of the reference area mean. As for fish, data from standard surveys and IOC studies were included in the tabulations when the study followed a control/impact design (Fig. 7). Where the Cycle 4 IOC data were excluded due to the different nature of its design or lack of electronic data, the Cycle 2 and 3 data for that mill were excluded as well. These exclusions of some Cycle 2 and 3 data allows for a valid comparison among all three cycles. Figure 7 includes both statistically significant and non-significant differences. Gradient designs are excluded, since percent differences, as presented here, cannot be calculated for this type of study design without a greater degree of site-specific information than was available for these national-level analyses.

For all three cycles of data, total density is the endpoint showing the most extreme range (-100% to 2000%). The distribution is in general similar among cycles with the exception that there is a larger number of mills in the -100% to -80% range for Cycle 2. For taxon richness, Cycle 2 data show a wider range than for subsequent cycles. However, the overall distributions are similar with 81% (Cycle 2) to 87% (Cycle 3) of the mills falling between -40% and 40% differences. In contrast, the distribution of the Bray-Curtis index data has somewhat changed in Cycle 4 where 76% of the mills are in the -40% to 200% range compared to 57% and 49% of the mills in Cycles 2 and 3 respectively. In Cycle 4, the Simpson's evenness index shows a greater peak at -20% to 0% which comprises 42% of the mills in comparison to less than 25% of the mills in previous cycles.

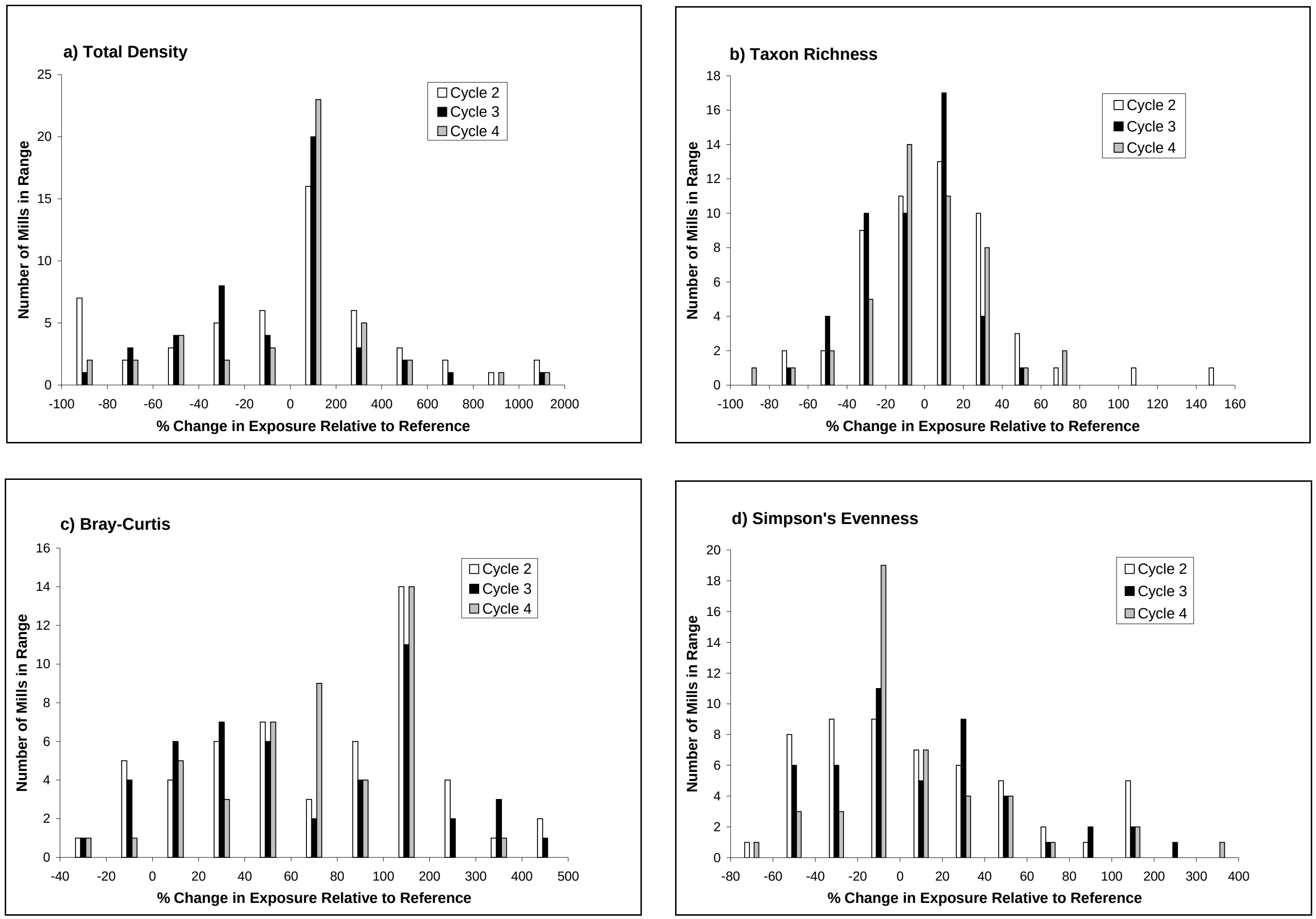

Figure 7: Distribution of measured percent differences between exposure and reference areas for the benthic invertebrate survey (control/impact designs only) for a) total density, b) taxon richness, C) Bray-Curtis and d) Simpson's evenness.

Figure 8 was developed using all of the Cycle 4 results (control/impact and gradient designs) and shows the number of mills that had no significant effect versus a significant effect for the four endpoints. Mills that had a significant effect are divided into those where the effect was less than the CES of ± 2 standard deviations (± 2SD) from the reference mean and those where the effect exceeded the CES of ± 2SD. For total density, taxon richness and Simpson's evenness, a higher number of mills showed no significant difference whereas significant differences above the CES was the most common result for the Bray-Curtis index. These patterns observed in Cycle 4 were also seen in previous cycles (Lowell *et al.* 2005). Similar percentages of non significant effects were observed between Cycle 4 (56%) and Cycle 3 (62%). In both Cycle 3 and 4, approximately half of the mills that found at least one significant effect exceeded the CES for total density or taxon richness.

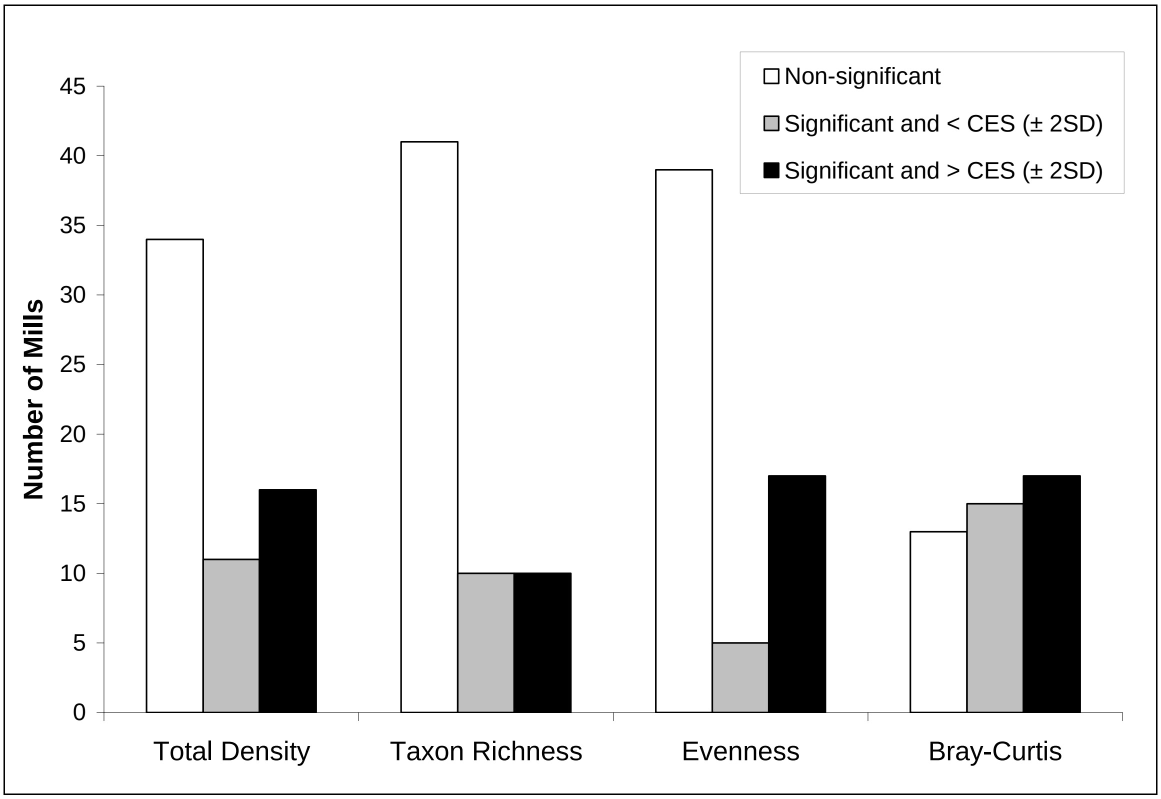

Figure 8: Number of mills in Cycle 4 showing no significant difference, a significant difference less than the CES of ± 2SD and a significant difference greater than the CES of ± 2SD. Includes both control/impact designs (n = 45) and gradient designs (n = 16). Note that CES have not yet been developed for gradient designs; therefore, mills that used a gradient design and found a statistically significant effect are included under "significant and > CES" (see Lowell *et al.* 2005 for further explanation). Note also that the Bray-Curtis index was not calculated for the gradient designs because it requires highly site-specific information that was not available at a national scale (Lowell *et al.* 2003).

For control/impact designs, a mill advances to assessing magnitude and geographic extent or to IOC study in the subsequent cycle if the CES for total density or taxon richness was exceeded (with statistical significance) in the same direction for two consecutive cycles. In the case of gradient designs, focused monitoring is conducted when a statistically significant effect (significant correlation coefficient) was measured for total density or taxon richness in the same direction for two consecutive cycles (see Lowell *et al.* 2005 for further discussion). See Chapter 7.0 for an overview on the IOC studies conducted by mills in Cycle 4.

6.3 Response Patterns and Meta-analyses

Three primary benthic invertebrate community response patterns were observed in Cycles 2 and 3 (Lowell *et al.* 2003, 2005). These are illustrated in Figure 9, which shows expected changes in total density (abundance), taxon richness and community structure with changing effluent quality (pulp mill effluent effects on invertebrates reviewed in Lowell *et al.* 1995, 2000; Chambers *et al.* 2000; Culp *et al.* 2000; Lowell and Culp 2002). In this context, "effluent quality" refers to that experienced by organisms exposed in the effluent receiving environment, as distinguished from end-of-pipe effluent effects measured under controlled conditions in the laboratory. The x-axis progresses from better quality effluent and less deleterious effects on the left to poorer quality effluent and more deleterious effects on the right. More deleterious effects may also be associated with past historical effects (such as the smothering effects of fibre mats generated over many years of mill operation).

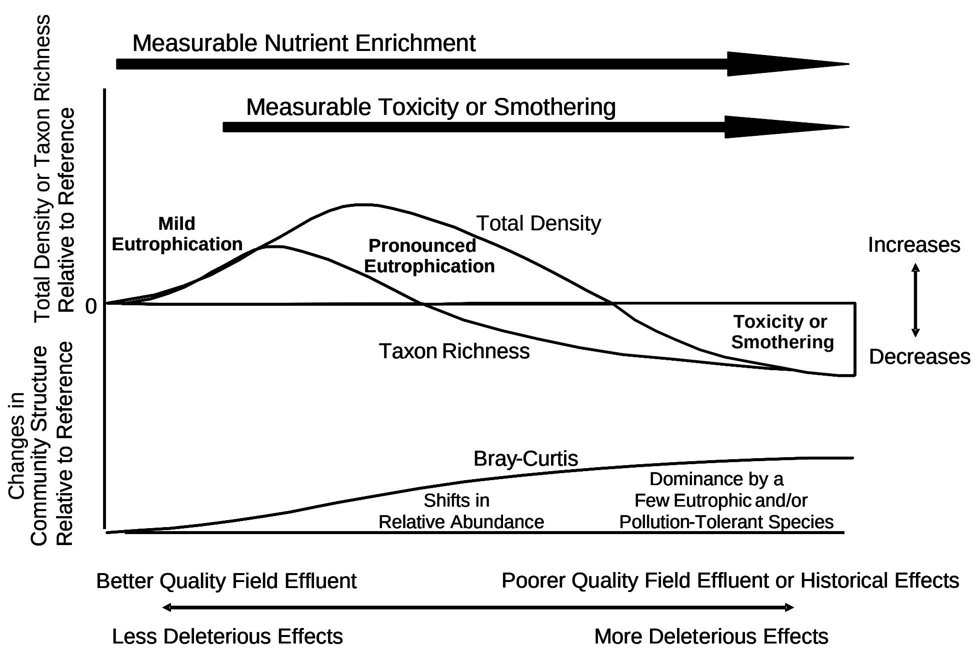

Figure 9: Predicted response patterns for benthic invertebrate communities (Lowell *et al.* 2003).

In general, nutrient enrichment (or eutrophication) increases from left to right, along with increasing toxicity or smothering effects (Fig. 9). Nutrient enrichment can often be measured at lower effluent concentrations than toxicity, and toxic effects are often masked by eutrophication at low to medium concentrations. Mild eutrophication is typified by small to moderate increases in both total density and taxon richness. Progressing to the right, more pronounced eutrophication is typically associated with lessened increases in taxon richness, together with large increases in density. Further to the right, even greater eutrophication is commonly associated with decreases in taxon richness, even while density is still greater than that found in reference areas. Finally, decreases in both taxon richness and density are typically a sign of overall inhibitory effects, such as toxicity or smothering.

In the EEM program, changes in invertebrate community composition are measured by changes in the Bray-Curtis index of dissimilarity. The value of this index usually increases with poorer quality effluent, reflecting changes in community structure (particularly community composition). This is illustrated at the bottom of Figure 9. Evenness similarly measures changes in community structure and typically decreases (or sometimes increases) with poorer quality effluent. However, it should be emphasized that changes in community composition are not always tied to changes in total density and taxon richness, as there are many different ways in which benthic communities may be affected in the field. Due to complex direct and indirect effects (e.g., substitution of more sensitive species by less sensitive species), effluent exposure may lead to pronounced effects on community composition without large effects on total density or taxon richness, and vice versa.

The national average response pattern in Cycles 2 and 3 was consistent with one of mild to pronounced eutrophication, as indicated by increases in density and either decreases in taxon richness or no national average change in taxon richness (although subgroups of mills showed either significant increases or significant decreases in taxon richness) (Fig. 10; Lowell *et al.* 2003, 2005). Note that Figures 10 through 12 focus on control/impact mill data, the most commonly used design in the EEM program. This allowed direct comparisons among all four EEM benthic endpoints (calculation of the Bray-Curtis endpoint for gradient designs requires highly site-specific information that was not readily available at a national scale; Lowell *et al.* 2003, 2005).

Similar to the findings for the fish survey, the national average response pattern observed for benthic invertebrates in Cycle 4 was quite similar to that observed in Cycles 2 and 3 (Figs. 10 through 12). The pattern was characterized by significant increases in the density and Bray-Curtis endpoints, and little change across cycles for the evenness endpoint. As for earlier cycles, this response pattern was likely related to continuing nutrient enrichment in exposure areas, together with other effects of effluent exposure at some mills. It is noteworthy that the pulp and paper industry has recently proactively developed Best Management Practices (BMP) for nutrient enrichment (FPAC, 2008). As these BMP will be implemented in the future, one would expect to see improvements at sites with eutrophication responses.

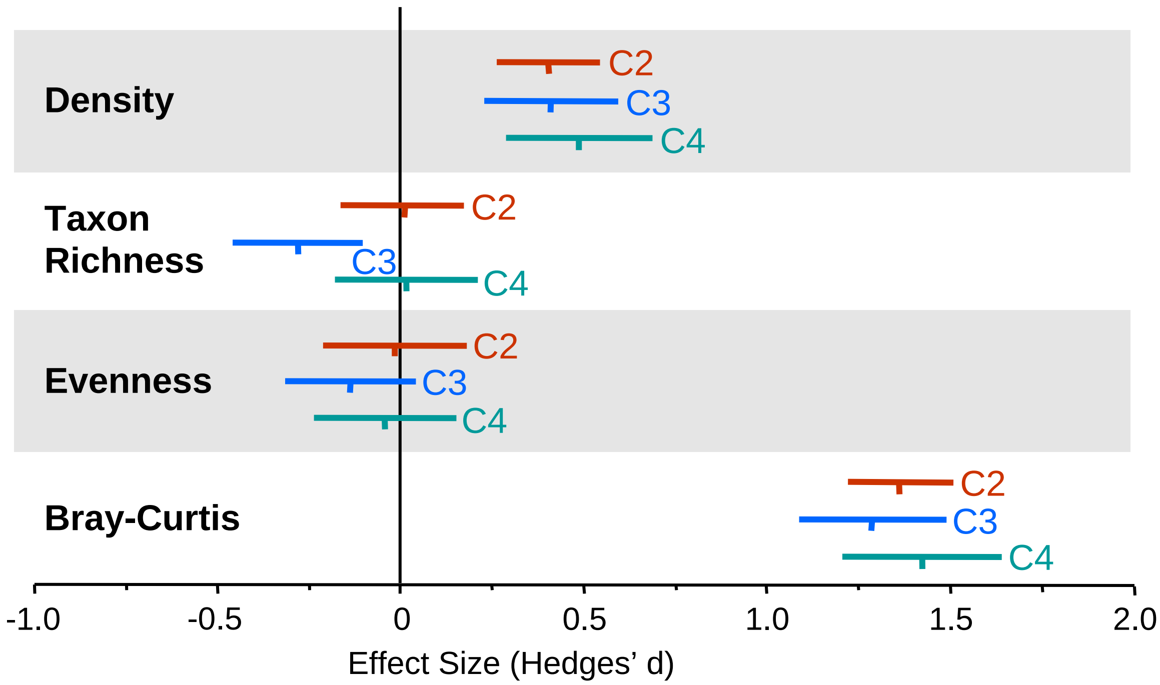

Figure 10: Pulp and Paper National Average Benthos Effects - Cycles 2-4 (C4 not corrected for missing IOC mills).

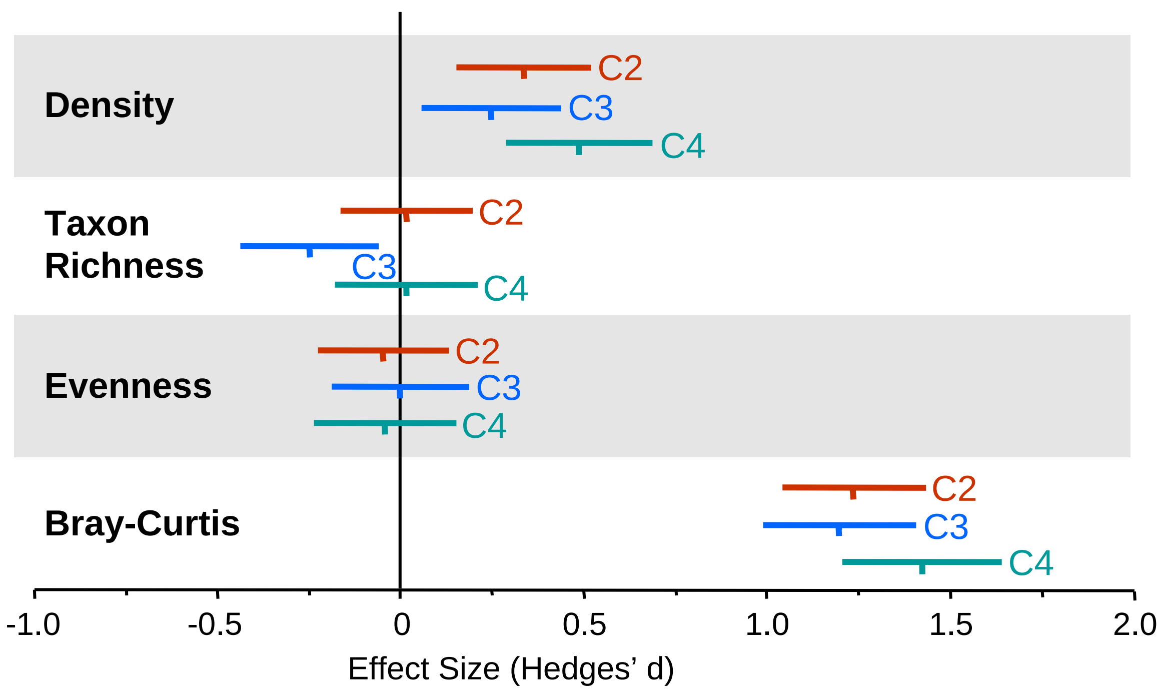

Figure 11: Pulp and Paper National Average Benthos Effects - Cycles 2-4 (IOC mills removed for all cycles).

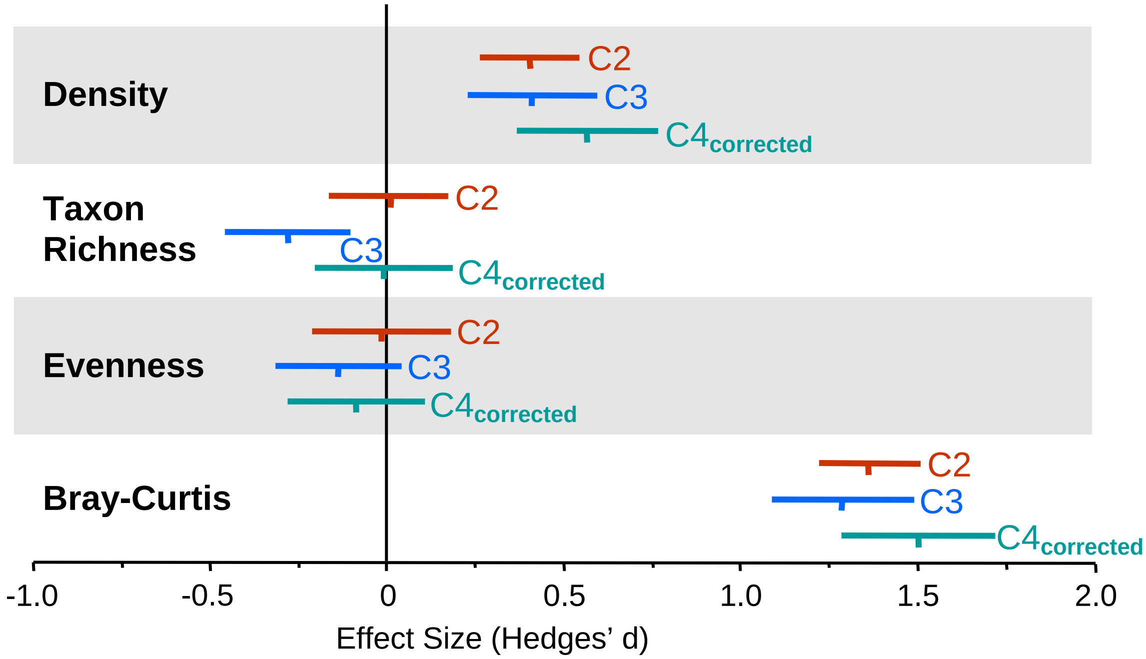

Figure 12: Pulp and Paper National Average Benthos Effects - Cycles 2-4 (C4 corrected for missing IOC mills).

Compared to Cycle 3, there appeared to be an improvement in Cycle 4 with respect to effects on taxon richness. But, given that the Cycle 4 national average was almost identical to that measured in Cycle 2, the shift relative to Cycle 3 may reflect cycle-to-cycle variability due to extrinsic (non-effluent related) factors, such as changes between cycles in study designs and/or selection of sampling locations.

The uncorrected national average effects for Cycle 4 shown in Figure 10 were shifted due to IOC (large effect) mills not performing standard field monitoring, and thus not submitting data for inclusion in these analyses. These shifts can be seen by comparing Cycle 2 and 3 data in Figure 10 to Figure 11. In Figure 11, the IOC mills from Cycle 4 were removed from Cycles 2 and 3, as well. Note, however, that the IOC-related shift in the national patterns for benthic invertebrates was not as great as the shift observed for the fish survey.

Figure 12, in turn, corrects for this bias (non-inclusion of big-effect IOC mills) by using the shifts for each endpoint (averaged over Cycles 2 and 3) to apply a correction factor to the Cycle 4 data. Thus, Figure 12 provides a better picture of current national average effects in Cycle 4 than does Figure 10.

7.0 Investigation of Cause

7.1 Investigation of Cause in the Pulp and Paper Effluent Regulations

This chapter outlines the general observations related to the study methods employed by the mills involved in IOC studies as well as their proposed path forward in the program.

In the PPER, Schedule IV.1, subsection 4(2), the requirement of investigating the cause of a confirmed effect is stated as follows:

> *"If the most recent interpretive report indicates the magnitude and geographical extent of an effect on the fish population, on fish tissue or on the benthic invertebrate community, or that the cause of the effect has not been identified, the study design shall consist of only the summary referred to in paragraph (1) (g) and a detailed description of field and laboratory studies that will be used to determine the cause of the effect."*

Effects, as defined in the PPER, must be confirmed and their extent and magnitude assessed in order to develop an IOC study most suited towards the context at hand. The IOC study is undertaken to gain a better understanding of the cause of the observed effects with the goal of implementing measures that will mitigate the effects. Flexibility, judged to be an integral part of the PPER, was used by mills to move rapidly from confirming effects to an IOC study.

Once the cause of the observed effects is found under an IOC study, the next step is to proceed with an Investigation of Solutions (IOS). Environment Canada has published guidance on how to initiate follow-up actions and what to consider when conducting IOS studies on the EEM website (http://www.ec.gc.ca/eem/English/Publications/AfterEEM.cfm). Further guidance will be published in the Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring, which is currently being updated.

Lowell *et al.*, 2005 have defined response patterns based on published works and results from Pulp and Paper EEM studies. Two main categories of IOC studies were conducted to address the two major response patterns observed in previous cycles: eutrophication (either based on benthic invertebrate community studies or fish studies) and fish gonad reduction associated with metabolic disruption. In addition, an IOC study was also implemented to address a specific case where dioxin/furan concentrations exceeded the effect concentrations defined in the PPER.

The following paragraphs outline the first overview of IOC studies produced within Cycle 4 of the Pulp and Paper EEM Program.

7.2 Overview of IOC Implementation in Cycle 4

Out of a total of 28 mills conducting IOC studies during Cycle 4, 17 mills submitted an IOC report for eutrophication, 10 mills were involved in an IOC for reduced fish gonads, and 1 mill was involved in an IOC for elevated fish tissue concentrations of dioxins and furans. Two studies in the IOC for eutrophication are joint projects each involving two mills. The reduced fish gonad IOC studies include 2 joint studies, one involving 8 mills (collaborative Paprican-Environment Canada-academia project), another composed of 2 mills.

All mills conducting IOC studies have based their approach on the interpretation of data from previous EEM studies (Table 6). Among the 17 mills involved in eutrophication IOC, a total of 14 facilities have also considered information not produced by the EEM program to aid in their justification for IOC. Eight mills opting for reduced fish gonad IOC considered information not produced within the EEM program to justify their choice. The mill heading into IOC for dioxins and furans based its choice on information from outside of the EEM study process in addition to study results from previous EEM cycles. In all cases, information produced either under different regulatory requirements or published scientific research was used to define study objectives.

One mill opted for an eutrophication IOC study based on fish study results. The Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring (Environment Canada, 2004a) does make reference to a response pattern typical of eutrophication in cases where fish collected show increases in relative gonad weight, liver weight and condition in exposed samples compared to fish collected in a reference area.

There are 2 mills involved in eutrophication IOC and 1 mill conducting a reduced fish gonad IOC that included a component that could be more affiliated with IOS. These are discussed in section 7.4.

Table 6: Total number of mills within the different categories of IOC and number of mills having moved forward into IOC studies based on EEM and non-EEM studies.

IOC Category	Total number of mills	Use of previous EEM study results	Use of information from non EEM sources
Eutrophication	17	17	14
Reduced fish gonads	10	10	8
Dioxins and furans	1	1	1

7.3 Methods used to implement IOC studies

7.3.1 Eutrophication IOC

Table 7 shows the wide variety of study methods employed by mills conducting IOC studies for eutrophication. Each of the 17 mills conducting IOC studies for eutrophication employed a unique set of methods, with most mills using more than one method to implement their study. The most common methods used were standard benthic studies, stable isotopes, analysis of dioxins and furans and periphyton versus nutrient loading models.

Table 7: Methods used by mills conducting IOC studies for eutrophication in Cycle 4.

Methodology	**Number of mills**
Standard benthic study	11
Stable isotopes	5
Dioxins and furans	4
Periphyton versus nutrient loading models	4
Benthic study (artificial substrates)	2
Benthos transplants	2
Chironomid deformities	2
Fibre mat delineation	2
Relative contribution of effluent, sewer and other sources	2
Chlorophenols	1
Epibenthos	1
Sediment bacteria	1
Mesocosm (periphyton, benthos)	1
Nutrient diffusing substrates experiment	1
Optimisation of phosphoric acid in secondary treatment	1
Periphyton biomass model and community metrics	1
Sulphides	1

7.3.2 Reduced fish gonad IOC

Two joint IOC studies on reduced fish gonads were conducted, one consisting of 2 mills, the other composed of 8 mills. Table 8 presents the methods used in each joint study.

Table 8: Methods used by mills conducting IOC studies for reduced fish gonads in Cycle 4.

Methodology	**Number of mills**
Mummichog bioassay (mesocosm)	2
Characterisation of causative toxic compounds	
Assessment of wild fish using white sucker	8
Several laboratory tests with different species of fish (life-cycle test with fathead minnow, short and medium-term tests with fathead minnow and rainbow trout, short and medium-term tests with mummichog	
Determination of androgenic potency using three-spine stickleback	
Effluent chemistry and *in vitro* tests for chemical analogs	

7.3.3 IOC for dioxins and furans

The mill conducting an IOC study for dioxins and furans employed a stable isotope analysis in biota and abiotic media. The objectives of the study were to attempt to answer questions concerning the bioavailability of chlorinated contaminants (dioxins and furans) in sediments and crabs, the role of current versus historical effluent deposits, the sediment dynamics contributing to the persistence or elimination of bioavailable contaminants in sediments, and the fidelity of crabs to particular feeding areas.

7.4 Investigation of Solutions initial work

Two mills, as part of their IOC studies, also investigated solutions through the study of nutrient optimization techniques. Of these mills, one was involved in eutrophication IOC and the other conducted a reduced fish gonad IOC.

At one mill, phosphorus optimization studies focused on a fine assessment of nutrient (P) concentrations entering and exiting the mill's effluent treatment system. Based on the study results, the mill proposes to refine the analytical methods and pursue nutrient optimization.

The second mill, rather than putting emphasis on nutrient optimization as an initial goal, focused on reducing effluent impact on receiving environment biota. This example of IOS implementation in an IOC context demonstrates that mill process or effluent system improvement may lead to reduced levels of nutrients and with *in situ* monitoring these successful technological changes will be confirmed. This mill initiated research with the objective to monitor the potential of the effluent to affect fish reproduction in laboratory tests during a period where changes in operating conditions were implemented in the multiprocess mill. Monitoring of the outcome of various technological changes on effluent quality was expected to help reduce the causative agents as well as key factors for effective mitigating strategies. Chemical profiling/fingerprinting has produced preliminary results and efforts in this area will continue. Such research objectives illustrate a flexible approach to IOC/IOS implementation which could be considered as acceptable in terms of regulatory compliance. Here again the ultimate goal is effluent quality improvement with verification of improvements in exposed aquatic biota.

7.5 Path forward

A variety of paths forward was recommended by the mills conducting IOC studies (Table 9). For eutrophication IOC studies paths included repeating monitoring, reducing monitoring, incorporating IOS and Best Management Practices (BMP Guide for Nutrient Management in Effluent Treatment, FPAC, 2008), redoing study with refinement of methods, and increasing partnership with other local water resource users. An expanded report including additional results is expected from one mill.

The two joint studies for reduced fish gonads recommended that the analytical methods be refined. The eight mills involved in the larger Paprican-Environment Canada-academia collaborative study on reduced fish gonad size put forward the recommendations drafted by the participating scientists that expressed the need to refine the study methods used and to test them at another mill and in laboratory conditions. The two mills involved in the other joint IOC study indicated further necessity to do research as well as the need to refine analytical methods and pursue the work undertaken. No mention of any path forward appears in the report of the IOC study for dioxins and furans in fish tissue.

Table 9: Paths forward stated in the IOC reports submitted for Cycle 4.

Path Forward Indicated in Report	Eutrophication	Reduced Fish Gonads	Dioxins and Furans
Incorporate IOS and BMP	3	-	-
Refine study and analytical methods and reapply	3	10	-
Increase partnership with other local water resource users	1	-	-
Expanded report to follow	1	-	-
Repeat monitoring	5	-	-
Reduce monitoring	2	-	-
No information	5	-	1

7.6 Discussion

Cycle 4 of the Pulp and Paper EEM Program was the first period during which IOC studies have been implemented on a national scale. Three major IOC study categories were observed in the following areas: eutrophication, reduced fish gonads, dioxins and furans levels.

Monitoring benthos or wild fish is still a part of the IOC process in some cases, often completed with methods that are not described in the Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring, such as sediment-associated benthic "transplant" studies or assessment of chironomid deformities. Study methods new to the EEM process are used to answer questions such as: 1) is the effluent the major contributor of organic matter? This question was studied by some mills through the use of stable isotope measurement methods, or again via the study of the relative contribution of organic material from several sources ending in the receiving environment; 2) is the effluent causing the observed effect on the biota? This question is still an issue at this stage of the EEM program for certain mills and was approached through the implementation of stable isotope studies and; 3) can we link nutrient concentrations to biotic endpoints? This question was the basis for the development of periphyton endpoint models defined as a function of nutrient concentrations.

Method refinements applied during the next IOC studies are expected where traditional methods were limited in their applicability. Mills engaged in collaboration with research institutions and academic scientists to benefit from state-of-the art tools that could be "field-proofed" in these projects, while contributing to the mill's need to comply with existing regulation.

Response patterns as the ones considered for the present IOC work are most probably the major ones seen so far in the EEM process. This does not preclude the existence of other, possibly lesser known, response patterns that also merit attention. Since implementing of solutions for one effect may not always lead to the disappearance of the second, mills that show effects attributable to eutrophication and reduced fish gonads may ultimately have to implement both types of IOC studies, given that subsequent monitoring will confirm effects still exist in the other component.

8.0 Summary and Conclusions

While studies conducted in Cycles 1 through 3 provided a good knowledge of the nature of the effects of pulp and paper mill effluents on fish and fish habitat, Cycle 4 was unique in that mills that found repeatable large effects in previous cycles were required to investigate the cause of the effects. Approximately 25 % of the mills that conducted EEM studies in Cycle 4 did an investigation of cause study while the remaining mills did standard monitoring. Ten mills were exempt from both fish and benthic invertebrate surveys and an additional 18 mills were exempt from the fish survey only.

Similar to Cycle 3, about half of the Cycle 4 standard fish survey comparisons between exposure and reference areas were not significant (Section 4.2). However, among the significant effects, Cycle 4 showed a lower proportion above Critical Effect Size (7%) compared to Cycle 3 (16%) which may be due to the mills with greater impacts now being in IOC. For benthic endpoints, a slightly higher percentage of significant effects is observed in Cycle 4 (44%) compared to Cycle 3 (38%) (Section 6.2). However, compared to Cycle 3, a greater proportion of these significant effects were below CES (10% in Cycle 3 vs. 18% in Cycle 4).

The national average response patterns in Cycle 4 were quite similar to those observed in previous cycles, for both the fish survey and the benthic invertebrate community survey. For fish, these were characterized by increased condition, growth rate, and relative liver size, together with decreased relative gonad size (Section 4.3). This response is typical of nutrient enrichment conditions, co-occurring with disruption in resource allocation to gonadal growth.

For benthic invertebrates, the national average response was one of increased density and changes in community structure (Bray-Curtis endpoint), together with various combinations of increases, decreases, or no change in taxon richness (with increases and decreases in taxon richness cancelling each other out for the national average; Section 6.3). This response is typical of various degrees of eutrophication. It is expected that the incidence of this response in the future should be reduced as the industry implements Best Management Practices. It should be noted that, for both the fish and invertebrate surveys, other less common response patterns also occurred at some mills.

A superficial look at the fish results (Fig. 4) might suggest significant reductions in effects in Cycle 4, relative to earlier cycles. This, however, was mostly a result of the bias introduced into the analyses due to many of the large-effect mills conducting IOC studies in Cycle 4, and therefore not submitting standard survey data that could be included in the analyses. After correcting for this bias (Fig. 6), this apparent reduction in effects mostly disappeared. The notable exception was relative gonad size, which showed a significant reduction of the national average effect, even after correcting for the bias. It remains to be seen whether this apparent lessening in the gonad reduction effect will continue to be measured in future cycles (see discussion of cycle-to-cycle variability below).

An interesting insight gained by looking at national response patterns for fish in Cycle 4, without correcting for large-effect mills going to IOC, is that this figure (Fig. 4) shows what the pulp and paper industry as a whole could achieve if mills having large effects are successful in their IOS efforts and in “fixing” their effects. In the future, this kind of analysis would provide a means of measuring how successful the industry as a whole has been in ameliorating effects.

In contrast to the findings for fish, the influence of some large-effect mills moving to IOC was much smaller when looking at national response patterns for benthic invertebrates. This suggests that a larger number of mills would have to lessen their effects on invertebrates before a similarly large industry-wide improvement is seen for benthic invertebrate effects.

It should be noted that further analyses (Barrett and Munkittrick, unpublished) have shown that some cycle-to-cycle variability in national response patterns for fish is likely due to changes between cycles in fish species that were used for monitoring. This is not the only cause of cycle-to-cycle variability in patterns, however, as evidenced by similar variability seen for the invertebrate response patterns, where fish species was not a factor. Other likely causes for cycle-to-cycle variability in national response patterns are changes in study design and selection of reference and exposure sampling locations, as well as environmental variability due to factors other than effluent exposure. A few more (possibly two) cycles of data (as called for in the initial design of the Pulp and Paper EEM Program) would prove invaluable in separating non-effluent related cycle-to-cycle variability from true industry-wide improvements in effluent effects.

The qualitative review of the reports of studies investigating the cause of eutrophication (17 studies), reduced gonad size (10 studies) and elevated levels of dioxins and furans in fish tissue (1 study), has shown a wide variety in the types of study designs implemented, recommendations, and paths forward and some studies included IOS components. This assessment recommends that guidance for both IOC and IOS studies incorporate the new information learned from Cycle 4 studies.

9.0 Glossary

Benthic invertebrate community – The interacting populations of small animals (excluding fish and other vertebrates), living at the bottom of a water body, on which fish may feed. Measuring changes in invertebrate communities helps to understand changes in aquatic habitats and provides an evaluation of the aquatic food resources available to fish.

Bray-Curtis index – An index that measures the degree of difference in community structure (especially community composition) between sites. This measure helps to evaluate the amount of dissimilarity between benthic invertebrate communities at different sites.

Condition – A measure of the physical condition of fish that describes the relationship between body weight and body length. Essentially, the condition factor measures how "fat" fish are at each area.

Control/impact design – A study design consisting of no less than one reference area, usually upstream from the mill or situated in a different watershed, and one or a series of exposure areas that are often downstream from the mill.

Density – The total number of individuals of all taxonomic categories collected at the sampling station, expressed per unit area (i.e., total abundance).

Effect – In the context of the EEM program, an effect is a statistically significant difference between measurements taken from the exposure area and from the reference area or measurements taken from sampling areas that have gradually decreasing effluent concentrations.

Endocrine – The endocrine system controls a number of internal body functions via hormonal secretions, which are transported throughout the body in the blood.

Endpoint – A particular measurement that is used as an indicator of potentially important effluent effects on receiving water biota. Examples of endpoints are gonad weight, liver weight, condition, age and weight at age for fish or density, taxon richness, Simpson's evenness index and Bray-Curtis index of dissimilarity for benthic invertebrates.

Eutrophication – The process of over fertilization of a body of water by nutrients that often results in excessive production of organic biomass and is typified by large numbers of organisms and, when pronounced, few species. Eutrophication can be a natural process, or it can be accelerated by an increase of nutrient loading to a water body by human activity.

Exposure area – A sampling area where fish and benthic invertebrates are exposed to pulp and paper mill effluent. This area may extend through a number of receiving environments and contain a variety of habitat types.

Gradient design – Generally, sampling is done along a gradient of decreasing effluent concentration, starting with exposure areas close to the mill and progressing towards less exposed areas farther from the mill. This study design was sometimes used in situations where rapid effluent dilution was a factor.

Investigation of cause – Study required to be conducted once an effect on fish, fish tissue or fish habitat is found and confirmed. The investigation of cause study is meant to collect information to help identify the cause and causative agent(s) of the effect.

Investigation of solution – Once an effect on fish, fish tissue or fish habitat is characterized and the cause and causative agent(s) found, a mill is encouraged to take follow-up actions to determine and implement a solution to eliminate the effect.

Metabolic disruption – Metabolism is a mechanism used by the body whereby complex substances are synthesized from simple ones or complex substances are broken down. The disruption of this system can occur from exposure to deleterious substances in the environment and can cause important imbalances in the maturation, sexual behaviour, growth, etc. of the organism.

Nutrient enrichment – The effect of adding large quantities of organic and inorganic nutrients to the environment.

Reference area – A sampling area that has no effluent exposure from the pulp and paper mill in question and natural habitat features that are similar to those of the exposure area, including anthropogenic impacts.

Reference Condition Approach – A study design that uses multiple reference sites to establish a reference condition referring to the regional range in biological benthic communities (i.e., endpoints) found at unimpaired sites. The biological community in a specific exposure area is compared to communities found at regional reference sites of similar environmental attributes.

Relative gonad weight – A measure of fish reproductive investment that describes the relationship between gonad weight and body weight.

Relative liver weight – A measure of fish energy storage and response to toxicant exposure that describes the relationship between liver weight and body weight.

Simpson's evenness index – A measure of how evenly individuals are distributed among taxa. This measure helps to evaluate changes in the relative abundance of taxa.

Smothering – The overaccumulation of organic matter derived from pulp mill effluent at the bottom of a water body, impeding the functioning of organisms and sometimes causing death.

Sublethal toxicity – In the context of EEM, sublethal toxicity tests usually measure the proportion of organisms affected by their exposure to specific concentrations of pulp mill effluent in a laboratory setting. A sublethal toxicity test measures what is detrimental to the organism (e.g., effects on growth or reproduction), but below the level that directly causes death within the test period.

Taxon – Organisms are classified into categories based on similarities and evolutionary relationships between them. Each of these categories (e.g., species, genus, family, phylum, etc.) is called a taxon (plural taxa).

Taxon richness – The total number of different taxonomic categories collected at a sampling station.

Weight at age – A measurement of the rate of growth of fish described by the relationship of size (weight) to age. Over the entire life span of a fish, the rate of increase in size may decline as the fish ages.

10.0 Acronyms / Abbreviations

ANOVA	– analysis of variance
ANCOVA	– analysis of covariance
BMP	– best management practices
C2	– cycle 2
C3	– cycle 3
C4	– cycle 4
CES	– critical effect size
EEM	– Environmental Effects Monitoring
IOC	– investigation of cause
IOS	– investigation of solution
PPER	– *Pulp and Paper Effluent Regulations*
RAPPER	– *Regulations Amending the Pulp and Paper Effluent Regulations*
RCA	– reference condition approach
SAT	– statistical assessment tool
SD	– standard deviation

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