Environment Canada Environnement Canada

Fifth National Assessment of Environmental Effects Monitoring Data from Pulp and Paper Mills Subject to the *Pulp and Paper Effluent Regulations*

National Environmental Effects Monitoring Office, Industrial Sector Directorate,
Environment Canada

December 2012

Canada

Cat. No.: En14-84/2013E-PDF
ISBN 978-1-100-21715-4

Aussi disponible en français

Acknowledgement

We would like to thank the members of the Environmental Effects Monitoring National Team and Science Committee for providing comments and scientific expertise during the preparation of this report.

Executive Summary

The purpose of this document is to report the results of the environmental effects monitoring (EEM) studies conducted by pulp and paper mills in Canada during Cycle 5 (2007 to 2010). It also summarizes the studies conducted to determine the causes of environmental effects and to identify solutions for environmental effects caused by mill effluent. For contextual purposes, this document also compares EEM results from cycles 1 to 5.

The *Pulp and Paper Effluent Regulations* (PPER) under the *Fisheries Act* direct pulp and paper mills to conduct EEM as a condition governing the authority to deposit effluent. Through an iterative system of monitoring and interpretation, the potential effects of effluents on aquatic receiving environments are evaluated. EEM studies required by the PPER consist of biological monitoring studies to assess and investigate effluent effects on fish, fish habitat and the use of fisheries resources; and sublethal toxicity testing of effluent to monitor effluent quality. Information obtained through EEM requirements is used to help assess the effectiveness of the Regulations in protecting these aquatic resources.

During Cycle 5, 32 mills conducted fish population studies, and 38% of these mills reported at least one effect with a magnitude equal to or greater than the critical effect size (CES).[1] Twenty-seven mills conducted benthic invertebrate community studies to evaluate fish habitat; of these, 48% reported at least one effect with a magnitude equal to or greater than the CES.

The number, magnitude and type of effects observed in Cycle 5 were similar to those observed in previous cycles and illustrated the same common patterns of effects, namely nutrient enrichment in fish and benthic invertebrate communities (eutrophication), co-occurring with metabolic disruption demonstrated as gonad reduction in fish.

Previous national analyses of EEM biological monitoring study results showed that many mill effluents had effects on fish and fish habitat but very rarely had effects on the use of fisheries resources.

When effects observed in cycles 2 to 5 on the fish population, benthic invertebrate communities and use of fisheries resources are considered together, 54 of the 81 mills (67%) in production and subject to the PPER during Cycle 5 reported the same effect in consecutive studies (known as a confirmed effect) in at least one of the EEM effect indicators. Of these 54 mills, 11 reported at least one confirmed effect with a magnitude less than the CES and 43 reported at least one confirmed effect with a magnitude equal to or greater than the CES. Three mills confirmed through biological monitoring studies that their effluent did not have any effect on fish populations and benthic invertebrate communities. Six mills (7%) had unconfirmed effects (the same effects or absence of effects were not observed in consecutive cycles), and 18 mills were not required to conduct biological monitoring studies as per the Regulations due to low effluent concentration in the receiving environment.

[1] A CES is a threshold above which an effect may be indicative of a higher risk to the environment.

Since 1996, when all facilities became subject to the full requirements of the PPER, the sublethal toxicity of mill effluent on a national basis has remained constant, with 50% of tests showing no sublethal toxicity. The freshwater and marine reproductive and fertilization tests showed the greatest responsiveness (most inhibition) to mill effluent.

Two common patterns of effects observed across the country have been investigated: eutrophication and gonad size reduction in fish. A third effect, isolated to one location, involving fish tissue exceedances of dioxin/furan concentrations has also been investigated.

In Cycle 5, there was an increase in the number of mills conducting investigation of cause and solution studies. During Cycle 5, 62 mills conducted 50 biological monitoring studies (some mills conduct studies jointly). The 50 studies consisted of 33 studies to assess effects (35 mills) and 17 studies (27 mills) to investigate effects (either investigation of cause or investigation of solutions studies).

The mills conducting investigation of cause (IOC) studies for eutrophication found that the observed effects were related to organic enrichment of sediments, biosolids in mill effluent (both current and historical), nutrients (especially phosphorus) in final effluent, and changes to water quality due to mill effluent. Investigations to identify solutions for eutrophication included nutrient management and nutrient source assessment studies. Common solutions identified included minimizing the amount or changing the type of supplemental phosphorus used, reducing the organic material entering the treatment system and improving operator training.

The mills conducting IOC studies for reduced gonad size in fish found that egg production in laboratory tests appeared to have the greatest potential for assessing an effluent's ability to affect fish reproduction. While the relationship between egg production in the laboratory and gonad size in wild fish is unclear at this time, the laboratory egg production test was identified as having the most potential for future investigation of cause and investigation of solution studies. The IOC studies identified options for future investigation of solution studies that involve minimizing losses of organic matter and upsets to biological treatment.

As mills have progressed through the PPER EEM requirements, their EEM activities have focused on investigating the causes of observed effects and on finding solutions for these effects. Action toward determining causes and identifying solutions for all confirmed effects is expected to be accelerated in Cycle 6 (2010 to 2013) by applying a level of effort commensurate with the level of risk. By focusing more effort on the highest risks and reducing effort directed toward lower risks, it may be possible to determine the causes of—and identify solutions for—most effects in an accelerated time frame.

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1. Introduction

The 1992 *Pulp and Paper Effluent Regulations* (PPER) under the *Fisheries Act* include the requirement[2] for environmental effects monitoring (EEM) to evaluate the potential effects of effluents on fish, fish habitat and the use of fisheries resources. Information obtained through EEM requirements is used to help assess the effectiveness of the Regulations in protecting these aquatic resources.

EEM studies are designed to detect and measure changes in aquatic ecosystems (i.e., receiving environments). EEM goes beyond the end-of-pipe measurement of chemicals in effluent; it examines the effectiveness of environmental protection measures directly in aquatic ecosystems. Long-term effects are assessed using regular cyclical monitoring and interpretation phases designed to assess and investigate the impacts on the same parameters and locations. In this way, both a spatial characterization of potential effects and a record through time to assess changes in receiving environments are obtained.

This document reports the results of the EEM studies conducted in Cycle 5 and compares some EEM results from cycles 1 to 5. It also summarizes the studies conducted to determine the causes of environmental effects and to identify solutions for environmental effects caused by pulp and paper mill effluent.

1.1 Environmental Effects Monitoring Studies

EEM studies required by the PPER consist of biological monitoring studies to assess and investigate effluent effects on fish, fish habitat and the use of fisheries resources, as well as sublethal toxicity testing of effluent to monitor effluent quality.[3]

Biological monitoring studies are usually conducted in three-year cycles. The requirements for each study are dependent on the results of the previous cycle. To assess effects, biological monitoring studies are conducted for three components:

- a **fish population** study to assess effects on fish health;
- a **benthic invertebrate community** study to assess effects on fish habitat (fish food); and
- a **fish tissue** study for dioxins and furans to assess the human usability of the fisheries resources.

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[2] For the regulatory EEM requirements, refer to sections 28 to 31 and Schedule IV.1 of the PPER, located on the following website: http://laws.justice.gc.ca/eng/SOR-92-269/index.html.
[3] For more information on pulp and paper EEM studies, refer to the *2010 Pulp and Paper Environmental Effects Monitoring (EEM) Technical Guidance Document*.

To investigate[4] observed effects, biological monitoring studies are conducted for the purpose of:

- describing the magnitude and geographic extent of effects;
- determining the causes of effects; and
- identifying possible solutions to eliminate effects.

In addition to the cyclical biological monitoring, mills conduct sublethal toxicity (SLT) testing twice a year on effluent collected from the discharge point that has potentially the most adverse environmental impact. This testing monitors effluent quality by measuring survival, growth and/or reproduction inhibition in marine or freshwater plant and invertebrate organisms in a controlled laboratory environment.[5] The requirement to also test a fish species[6] was removed in the 2008 PPER amendments because experience had shown that the required fish species tests were often not responsive to pulp and paper effluent.

1.2 Defining and Confirming Effects

The studies for the fish population and benthic invertebrate community components are conducted in both exposure and reference areas or along gradients of exposure. The exposure area means all fish habitat and waters frequented by fish that are exposed to effluent, and the reference area means water frequented by fish that is not exposed to effluent and that has fish habitat that, as far as is practical, is most similar to that of the exposure area.

The PPER defines an effect for indicators in each of the three biological monitoring components and further prescribes the data assessment required for specific indicators. Generally, an effect on the fish population or benthic invertebrate community means that there is a statistical difference between data collected in an exposure area and data collected in a reference area from a study on the fish population or benthic invertebrate community (tables 1 and 2). An effect on fish tissue means that there is a concentration of chlorinated dioxins and furans, expressed as toxic equivalents of 2,3,7,8-tetrachlorodibenzo-para-dioxin, exceeding 15 picograms per gram (pg/g) wet weight in muscle or 30 pg/g wet weight in liver or hepatopancreas of fish taken in the exposure area.

[4] These investigation studies are referred to as investigation of cause (IOC) and investigation of solutions (IOS) studies.
[5] SLT testing methods required by the PPER specify the use of the following species: *Pseudokirchneriella subcapitata* and *Ceriodaphnia dubia* (freshwater plant and invertebrate, respectively); *Champia parvula* and echinoderm species (marine plant and invertebrate, respectively).
[6] Fish species specified prior to the 2008 amendment included *Pimephales promelas* and *Oncorhynchus mykiss* (freshwater); *Menidia beryllina* and *Atherinops affinis* (marine).

Table 1. Fish population survey – effect indicators and endpoints

Effect Indicators	**Effect Endpoints**
Survival	Age
Growth (energy use)	Size-at-age (body weight relative to age)
Reproduction (energy use)	Relative gonad size (gonad weight to body weight)
Condition (energy storage)	Condition (body weight to length) Relative liver size (liver weight to body weight)

Table 2. Benthic invertebrate community survey – effect indicators and endpoints

Effect Indicators	**Effect Endpoints**
Total benthic invertebrate density	Number of animals per unit area
Taxa richness	Number of taxa
Evenness index	Simpson's Evenness
Similarity index	Bray-Curtis Index

In order to establish if there are any effects on the indicators, data collected on specific effect endpoints are assessed to determine whether statistical differences exist between exposure and reference areas. To confirm that observed effects are not simply the result of a one-time study, biological monitoring studies to assess effects are repeated in a subsequent three-year cycle. If the same effect on the fish population, benthic invertebrate community or fish tissue occurs in studies from consecutive cycles, the effect is considered confirmed.

1.3 Critical Effect Size

Critical effect sizes (CESs) were developed for the pulp and paper EEM program after EEM data showed that most mills observed an effect in at least one of the indicators. A CES is a threshold above which an effect may be indicative of a higher risk to the environment. A risk-based approach was developed using CESs to identify and focus efforts on the highest risks at the present time.

The values for the fish CESs were derived from the magnitude of pulp and paper mill effluent effects reported in scientific literature, natural variability typically observed, and the magnitude of effects observed in Cycle 2 of the pulp and paper EEM (Munkittrick et al. 2009). The values for benthic invertebrate CESs were derived from the scientific literature, the magnitude of effects observed in Cycle 2, and what was considered to exceed the "normal range" of variability in reference areas. With the exception of age and weight-at-age, which were added to the list in 2009, the CESs listed in Table 3 have been used to focus effort on higher risk since Cycle 4 (2004). A CES was not developed for the fish tissue effect, since this effect is defined by the level of dioxins and furans in fish tissue from fish exposed to effluent and is not a comparison between exposure and reference area fish.

Table 3. Critical effect sizes for pulp and paper environmental effects monitoring

Fish Effect Endpoints	CES	Benthic Effect Endpoints	CES
Relative gonad size	± 25%	Density	± 2SD
Relative liver size	± 25%	Taxa richness	± 2SD
Condition	± 10%	Simpson's Evenness	± 2SD
Weight-at-age	± 25%	Bray-Curtis Index	+ 2SD
Age	± 25%		

Note: Differences in fish population effect endpoints are expressed as percent (%) of reference mean, while differences in benthic effect endpoints are expressed as multiples of within-reference-area standard deviations (SDs).

2. Cycle 5 Study Results

Cycle 5 started on April 1, 2007, and ended on March 31, 2010. The 81 pulp and paper mills in production and subject to the PPER during this time period conducted SLT testing on their final effluent and, if required, conducted biological monitoring studies to either assess effects or investigate confirmed effects.

Of the 81 mills, 19 were not required to conduct biological monitoring studies as per the Regulations—18 due to low effluent concentration in the receiving environment and 1 due to the confirmation in previous studies of the absence of effects.[7] The remaining mills (62) conducted 50 biological monitoring studies: 33 studies[8] to assess effects (standard or alternative fish, fish tissue and/or benthic invertebrate community surveys) and 17 studies[9] to investigate confirmed effects (Figure 2.1). The results from the studies to assess effects and from SLT testing are summarized below.

Two common patterns of confirmed effects observed across Canada were investigated in Cycle 5: eutrophication and gonad size reduction in fish. An overview of all investigation studies conducted to date (cycles 4 and 5) to determine causes of confirmed effects and to identify solutions is provided in section 4.

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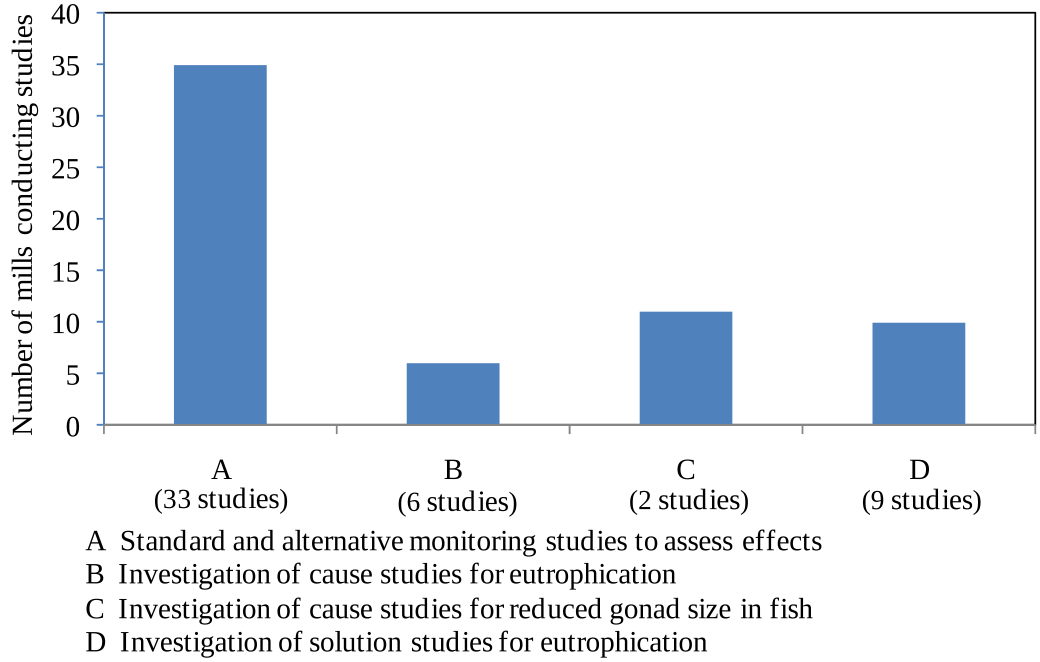
[7] Biological monitoring studies are conducted once per cycle by all mills in production and subject to the PPER that have a concentration of effluent greater than 1% in the area beyond 100 m from the point of deposit. Prior to the 2008 amendment, all mills in production conducted benthic invertebrate community biological monitoring studies, and those mills with a concentration of effluent greater than 1% in the area located beyond 250 m of a point of deposit of effluent also conducted fish biological monitoring studies. Mills confirming an absence of effects in a component monitor that component on a six-year time frame.
[8] Includes two joint studies involving two mills each.
[9] Includes one joint IOS study involving two mills and two joint studies to investigate reduced gonad size in fish (one study with two mills and the National Study with nine mills).

Figure 2.1. Biological monitoring studies conducted in Cycle 5



Note: Some mills conducted studies jointly.

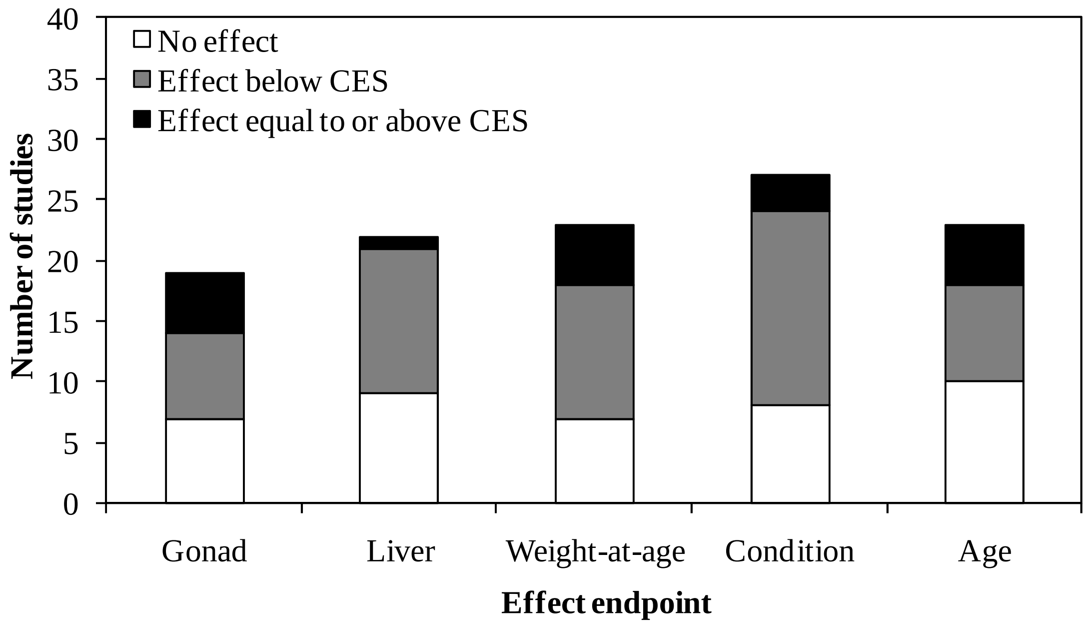
2.1 Effects on Fish

In Cycle 5, 32 mills conducted 30 biological monitoring studies[10] to assess effects on fish. These studies consisted of 16 lethal fish surveys, 3 lethal wild bivalve surveys, 4 fish surveys using non-lethal sampling and 7 alternative monitoring studies. Of the 32 mills, 24 reported an effect in at least one of the EEM fish effect indicators, and of these 24 mills, 12 reported at least one effect with a magnitude equal to or greater than the CES. Four mills reported an absence of effects, and the studies for 4 mills did not provide any effect endpoint data due to complications in conducting the study or to the type of alternative study conducted.

Figure 2.2 shows the number of non-significant (absence of effect) and significant (presence of effect) differences observed in each endpoint for all studies conducted. The number of significant differences with magnitudes equal to or greater than each endpoint's CES is also shown. The number, magnitude and type of effects observed in Cycle 5 were similar to those observed in previous cycles (Lowell et al. 2003, 2005; Tessier et al. 2009) and illustrated the same common patterns of effects—namely nutrient enrichment, co-occurring with metabolic disruption, demonstrated as gonad reduction in fish.

[10] Included two joint studies involving two mills each.

Figure 2.2. Results from 30 Cycle 5 fish population studies

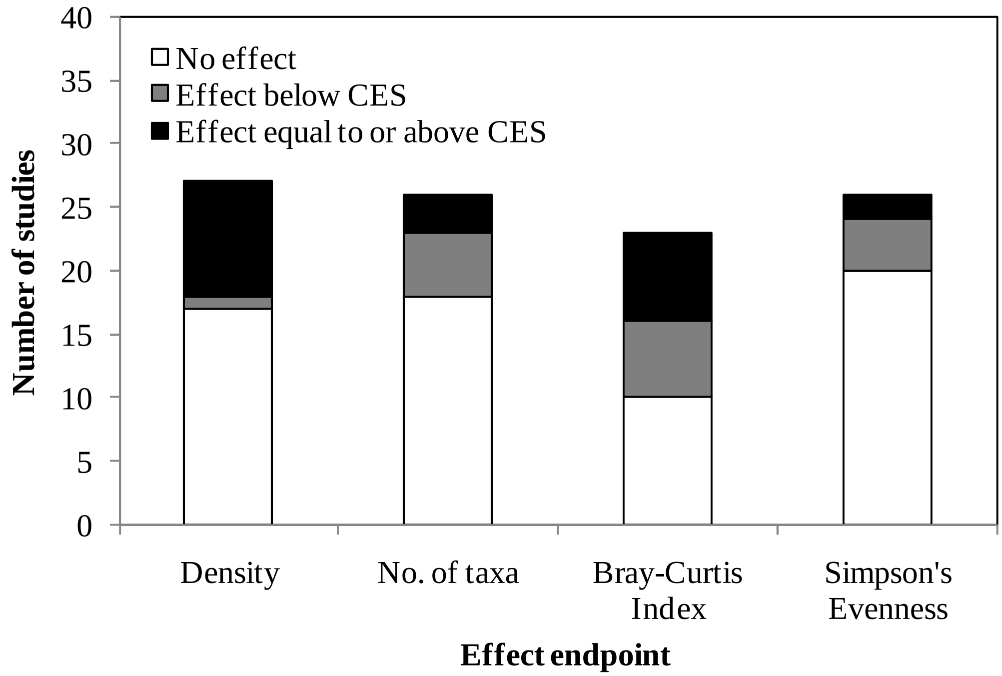


2.2 Effects on Benthic Invertebrate Communities

In Cycle 5, 27 mills conducted EEM biological monitoring studies to assess effects on benthic invertebrate communities. These studies included 22 control-impact and 4 gradient design studies, plus one alternative study employing artificial substrates. Of the 27 mills, 23 reported an effect in at least one of the EEM benthic effect indicators, and of these 23 mills, 13 reported at least one effect with a magnitude equal to or greater than the CES. Four mills reported an absence of effects in all four benthic endpoints.

Figure 2.3 shows the number of non-significant (absence of effect) and significant (presence of effect) differences observed in each endpoint for all studies conducted. The number of significant differences with magnitudes equal to or greater than each endpoint's CES is also shown. The number, magnitude and type of effects observed in Cycle 5 were similar to those observed in previous cycles (Lowell et al. 2003, 2005; Tessier et al. 2009) and illustrated the same common pattern of effects—namely various degrees of eutrophication (i.e., nutrient enrichment conditions).

Figure 2.3. Results from 27 Cycle 5 benthic invertebrate community studies



2.3 **Effects on the Use of Fisheries Resources**

In Cycle 5, two mills conducted the fish tissue survey to assess effects on human usability of fisheries. Both studies reported that at some stations, fish tissue concentrations of dioxins/ furans exceeded the level defined in the PPER as an effect (see section 1.2). Although effects on fish tissue were observed, the Cycle 5 concentrations of dioxin and furan in fish tissue were lower than concentrations measured in the previous cycle and have greatly decreased since the early to mid-1990s reporting.

2.4 **Sublethal Toxicity Testing Results**

Mills are required to conduct SLT testing twice each calendar year. Tests are conducted to look at effects on reproduction, growth and fertilization. The endpoint used to measure SLT of effluent is the inhibiting concentration that produces a 25% effect (IC_{25}), i.e., the effluent concentration that causes a reduction in performance (e.g., lower growth or reproduction) of 25% relative to the performance of control organisms. If a 100% concentration of effluent does not cause at least a 25% inhibition, the effluent is reported as showing no SLT for that test (i.e., reported as an $IC_{25} > 100\%$).

In Cycle 5, all 81 operating mills subject to the PPER conducted SLT testing of their final effluent, and test results were similar to the results observed in cycles 2, 3 and 4. For Cycle 5, the total number of tests conducted on fish is lower than those conducted on other species, since the requirement to conduct a fish test was removed in the 2008 PPER amendments.

More than 80% of the SLT tests conducted on fish species reported no SLT. Growth inhibition tests on the freshwater plant species reported no SLT in 70% of tests. Tests on invertebrate reproduction or macroalgal fertilization inhibition reported no SLT in less than 30% of the tests (figures 2.4 and 2.5). The Cycle 5 fish species *Oncorhynchus mykiss* (freshwater) and *Atherinops affinis* (marine) results are not shown, since very few tests were conducted (23 and 17 results, respectively).

Figure 2.4. Sublethal toxicity of pulp and paper final effluent for freshwater species at 62 mills during Cycle 5

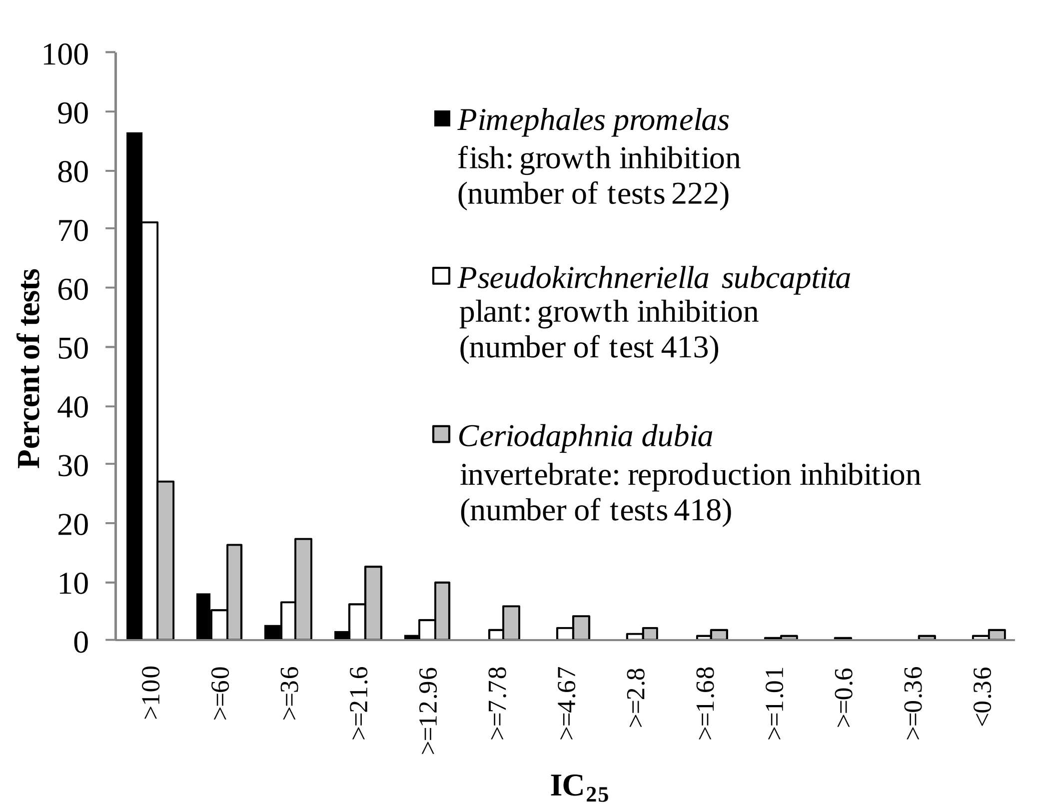
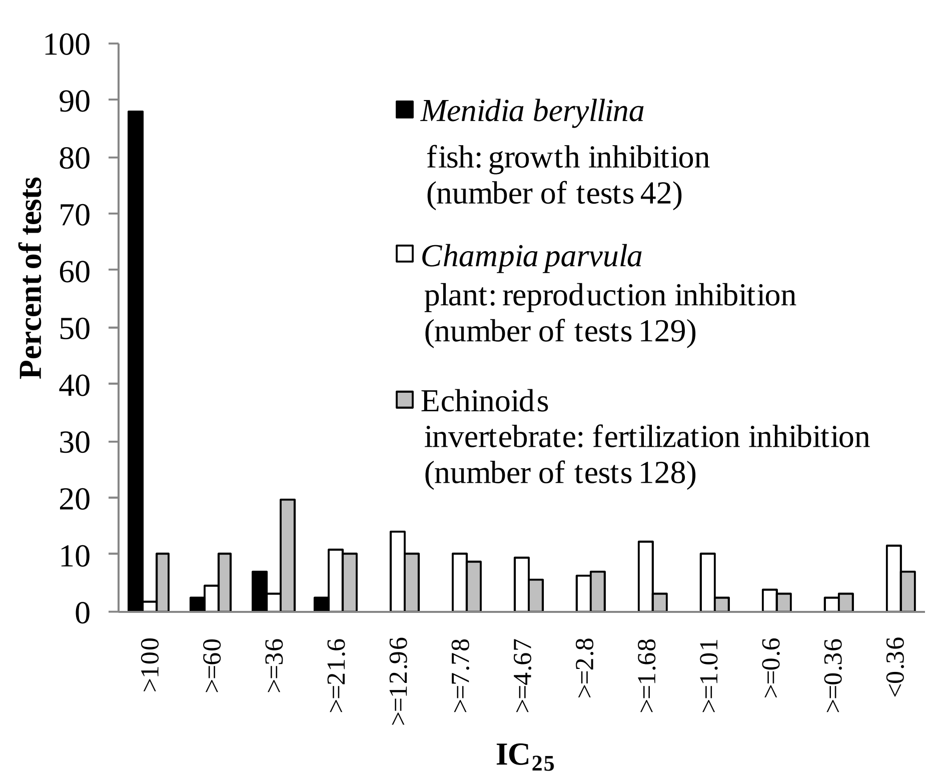


Figure 2.5. Sublethal toxicity of pulp and paper final effluent for marine species at 19 mills during Cycle 5



3. Comparing Results from Cycles 1 to 5

Currently, mills have completed their fifth cycle of EEM monitoring and reporting. The sections below describe the EEM activity that has occurred in each cycle, provide a snapshot of confirmed effects as of Cycle 5, describe the fish tissue analysis that has occurred up to Cycle 5, and compare the SLT results from each cycle.

3.1 EEM Activity

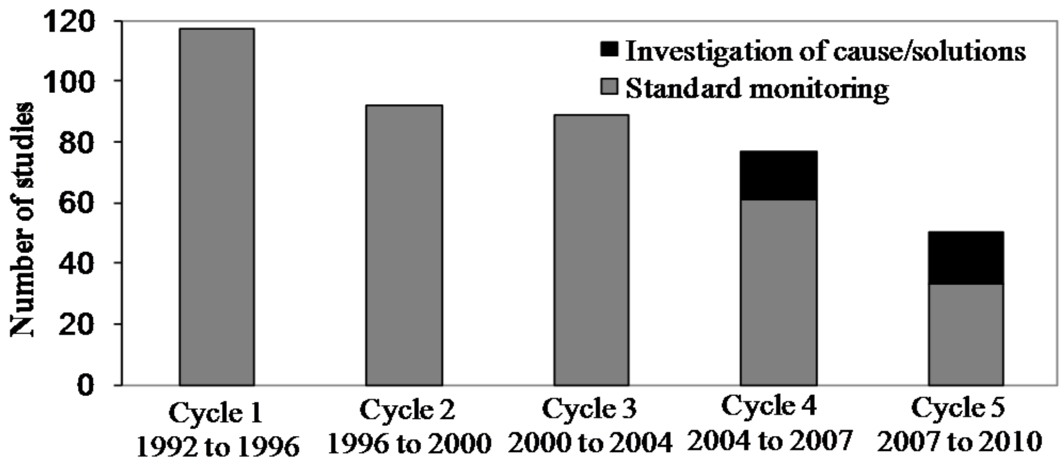
Cycle 1:	**Cycle 2:**	**Cycle 3:**	**Cycle 4:**	**Cycle 5:**
1992–1996	1996–2000	2000–2004	2004–2007	2007–2010
EEM study results used as initial data but not used to assess effects.	EEM studies to assess effects.	EEM studies to assess and confirm effects, and assess magnitude and extent of effects.	EEM studies to assess and confirm effects, and assess magnitude and extent of effects. IOC studies to investigate effects.	EEM studies to assess and confirm effects, and assess magnitude and extent of effects. IOC and IOS studies to investigate effects.

Biological monitoring studies are conducted once per cycle[11] by all mills in production that have a concentration of effluent greater than 1% in the area beyond 100 m from the point of deposit.[12] The studies conducted in Cycle 1 were not sufficiently comprehensive to assess effects, but were used to establish initial results and refine monitoring guidance to improve future studies. Studies conducted in cycles 2 and 3 were used to assess and confirm effects. In Cycle 4, the majority of mills continued to conduct biological monitoring to assess and confirm effects, but those that had confirmed effects in Cycle 3 conducted studies to determine the magnitude and the extent of those effects. Some mills conducted biological monitoring studies to investigate the causes of confirmed effects. In Cycle 5, still fewer mills continued to conduct biological monitoring studies to assess and confirm effects. Other mills continued to investigate causes, while some mills—having determined the causes of confirmed effects in Cycle 4—conducted investigations to identify solutions to eliminate those effects.

[11] The EEM monitoring periods for cycles 1, 2 and 3 were extended from three to four years to allow industry and government more time to assess study results and refine future monitoring studies.
[12] Prior to the 2008 amendment, all mills in production conducted benthic biological monitoring surveys, and those mills with a concentration of effluent greater than 1% in the area located beyond 250 m of a point of deposit of effluent also conducted fish biological monitoring studies. A small number of mills were not required to conduct biological monitoring studies because hazardous conditions in the effluent receiving environment prevented sampling.

As mills have progressed through the cycles, EEM activities have become focused on investigating the causes of—and solutions for—confirmed effects (Figure 3.1). The number of studies conducted in each cycle depends on several factors, including the number of mills that are in operation, that conduct studies jointly, that have rapidly diluting effluent, that confirm the absence of effects, and that have sampling hazards in the receiving environment.

Figure 3.1. Biological monitoring studies conducted per cycle



SLT testing has been conducted throughout all cycles, twice a year by all mills in production or once a year if the mill deposited effluent on fewer than 120 days in a calendar year.

3.2 Confirmed Effects on Fish and Benthic Invertebrate Community

Previous national analyses of EEM biological monitoring studies (Lowell et al. 2003, 2005; Tessier et al. 2009) showed that many mill effluents were having effects on fish and fish habitat but were very rarely having effects on the use of fisheries resources (determined by elevated levels of dioxins and furans in fish tissue).

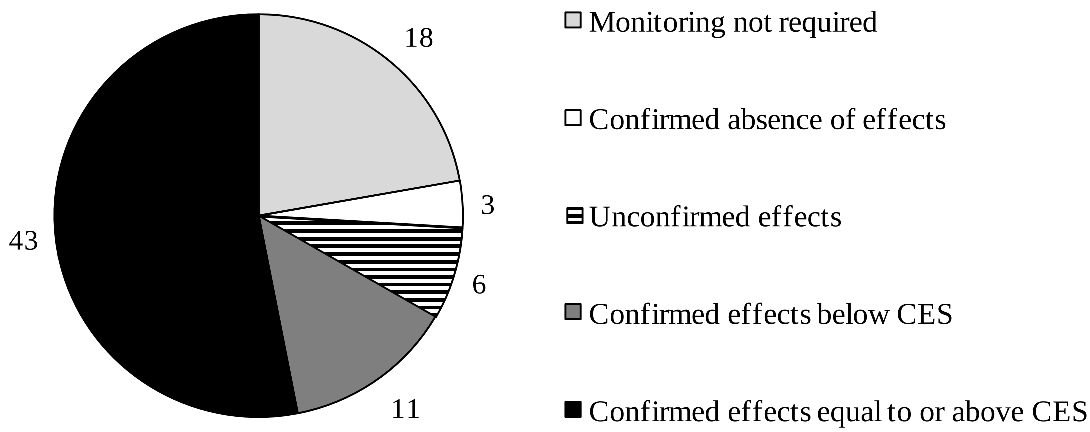
As of Cycle 3 (the first cycle in which effects could be confirmed), approximately 70% of mills confirmed an effect for at least one of the effect indicators, with approximately 45% of mills confirming effects related to eutrophication and 20% confirming a reduced gonad size effect.

In cycles 2 to 5, the national average response pattern for fish was typical of those conditions related to nutrient enrichment, co-occurring with metabolic disruption. Specifically, fish exposed to mill effluent were fatter, grew faster and had greater relative liver size but lower relative gonad size than unexposed fish.

The national average response pattern for benthic invertebrate communities (fish habitat) was typical of various degrees of eutrophication (i.e., nutrient enrichment conditions). Specifically, predominant effects were increased invertebrate density, and changes in community structure and taxon richness (i.e., species diversity or number of species).

When effects observed in cycles 2 to 5 on fish populations, benthic invertebrate communities and fisheries resources use are considered together, 54 of the 81 mills (67%) in production and subject to the PPER during Cycle 5 reported a confirmed effect in at least one of the EEM effect indicators (Figure 3.2). Of these 54 mills, 11 (14% of 81 mills) reported at least one confirmed effect with a magnitude less than the CES and 43 (53% of 81 mills) reported at least one confirmed effect with a magnitude equal to or greater than the CES. Three mills confirmed through biological monitoring studies that their effluent did not have any effect on fish populations and benthic invertebrate communities. Six mills (7%) had unconfirmed effects (the same effects or absence of effects were not observed in consecutive cycles), and 18 mills (22%) were not required to conduct biological monitoring studies for fish and benthic invertebrate communities as per the Regulations, due to low effluent concentration in the receiving environment.

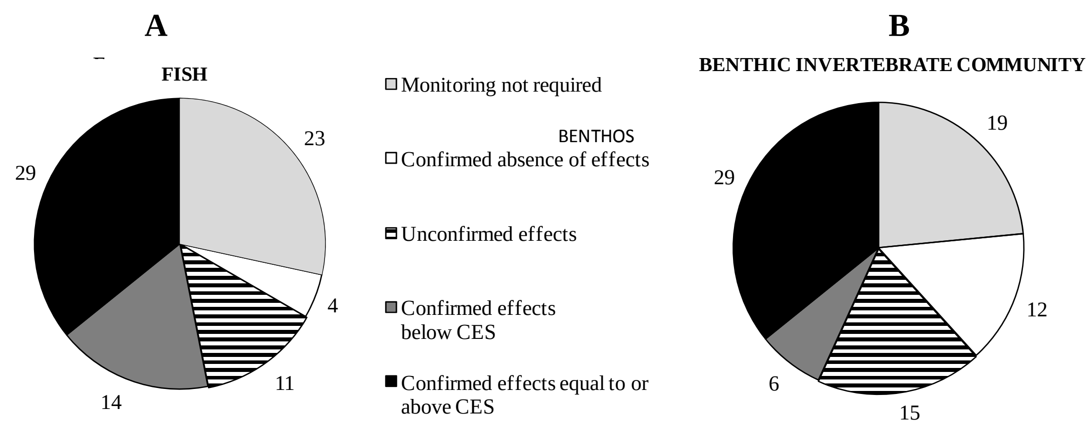
Figure 3.2. Confirmed effects of pulp and paper effluent for the 81 mills in production and subject to the PPER throughout Cycle 5



Note: Includes confirmed effects on fish, benthic invertebrate communities and fish tissue.

Of the mills in production and subject to the PPER throughout Cycle 5, 43 mills (53%) reported a confirmed effect in at least one of the EEM fish effect indicators. Of these 43 mills, 29 reported at least one confirmed effect with a magnitude equal to or greater than the CES (Figure 3.3A). Four mills confirmed through biological monitoring studies that their effluent was having no effect on fish populations, and 11 mills (14%) had unconfirmed effects on fish (the same effects or absence of effects were not observed in consecutive cycles). Twenty-three mills (28%) were not required to conduct biological monitoring studies to assess effects on fish as per the Regulations, due to low effluent concentration in the receiving environment.

Figure 3.3. Confirmed effects of pulp and paper effluent for the 81 mills in production and subject to the PPER throughout Cycle 5

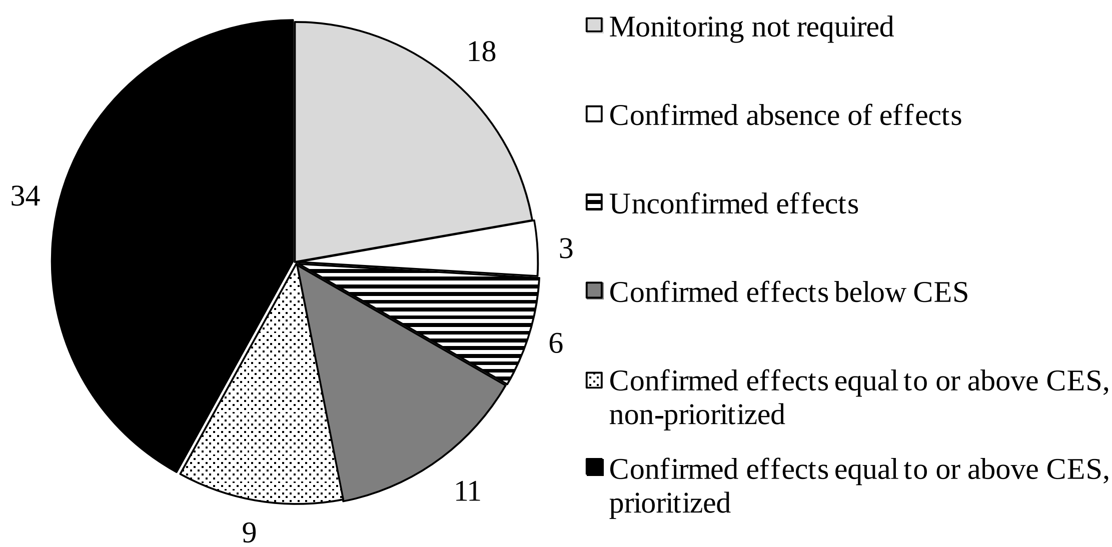


Of the mills in production and subject to the PPER throughout Cycle 5, 35 mills (43%) reported a confirmed effect in at least one of the EEM benthic effect indicators; of these 35 mills, 29 (36% of 81 mills) reported at least one confirmed effect with a magnitude equal to or greater than the CES (Figure 3.3B). Twelve mills confirmed through biological monitoring studies that effluent was having no effect on benthic invertebrate communities (fish habitat), and 15 mills (19%) had unconfirmed effects on benthic invertebrates (the same effects or absence of effects were not observed in consecutive cycles). Nineteen mills (23%) were not required to conduct biological monitoring studies to assess effects on benthic invertebrate communities as per the Regulations, due to low effluent concentration in the receiving environment.

In response to the recommendation in the 2005 Smart Regulation Initiative Report (Environment Canada 2005) that priority be given to the investigation of two predominant patterns of effects—eutrophication (nutrient enrichment) and reduced fish gonad size—a national investigation study into the causes of reduced fish gonad size was initiated in Cycle 4. In addition, the Forest Products Association of Canada (FPAC) developed the *Best Management Practices Guide for Nutrient Management in Effluent Treatment* (FPAC 2008) to assist mills in reducing eutrophication effects.

In response to the recommendation in the 2005 Smart Regulation Initiative Report that overall action toward causes and solutions be accelerated, a risk-based approach for the investigation of all confirmed effects was developed during Cycle 5 (2007 to 2010). This approach uses CES values to identify large (above CES) prioritized effects (associated with eutrophication or reduced fish gonad size) considered to be of highest risk at the present time. Action toward the determination of causes and identification of solutions for all confirmed effects is expected to be accelerated in Cycle 6 (2010 to 2013) by applying a level of effort commensurate with the level of risk.

Figure 3.4. Prioritized and non-prioritized confirmed effects of pulp and paper effluent for the 81 mills in production and subject to the PPER throughout Cycle 5



Note: Includes confirmed effects on fish, benthic invertebrate community and fish tissue.

3.3 Effects on Fisheries Resources and Usability

The effects on fisheries resources and usability are assessed by the results of a fish tissue survey. This survey determines the levels of chlorinated dioxins and furans in tissue from fish in the exposure areas. A fish tissue survey must be undertaken if, since submission of the most recent interpretive report, the mill's effluent contains a measurable concentration of 2,3,7,8-T4CDD (dioxin) or 2,3,7,8-T4CDF (furan), within the meaning of the *Pulp and Paper Mill Effluent Chlorinated Dioxins and Furans Regulations* pursuant to the *Canadian Environmental Protection Act, 1999*, or if an effect on fish tissue was reported in the most recent interpretive report.

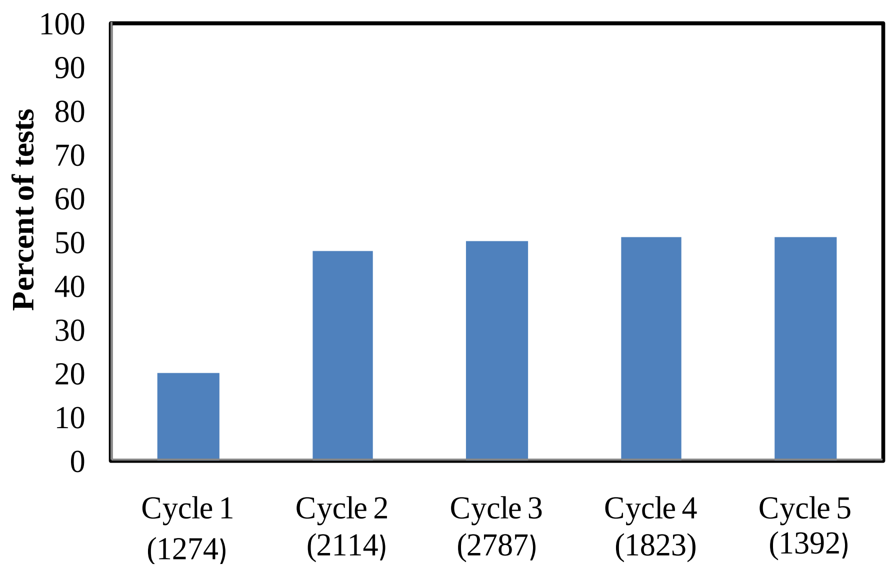
Overall, 14 mills conducted 26 fish tissue surveys at some point during cycles 2 to 5. Half of the 14 mills conducting fish tissue surveys did so over consecutive cycles, and 1 mill confirmed an effect on fisheries resources as defined by the PPER and thus conducted an investigation of cause study. The majority of mills conducting fish tissue surveys were in the Pacific and Yukon region, with the remainder in the Ontario, Quebec, and Prairie and Northern regions.

The majority of the tissue analyses found that dioxin and furan concentrations were not detectable or were below the level defined in the PPER for an effect (see section 1.2). The number of mills conducting fish tissue surveys in cycles 2 to 5 was 10, 8, 6 and 2, respectively. The progressive decline in the number of mills conducting fish tissue surveys was due to low or undetectable tissue concentrations of dioxins and furans, and to mill closures.

3.4 Sublethal Toxicity Testing Results

SLT results showed that mill effluent toxicity declined between Cycle 1 and Cycle 2 (Figure 3.5). Since 1996, when the industry reached full compliance with the PPER, mill effluent SLT, although variable for individual mills, has remained constant on a national basis, with 50% of tests showing no SLT. The improvement in effluent quality following Cycle 1 has been attributed to upgrades in effluent treatment in response to the 1992 PPER (Lowell et al. 2003). Mill effluents continue to elicit SLT responses in half of all effluent tests.

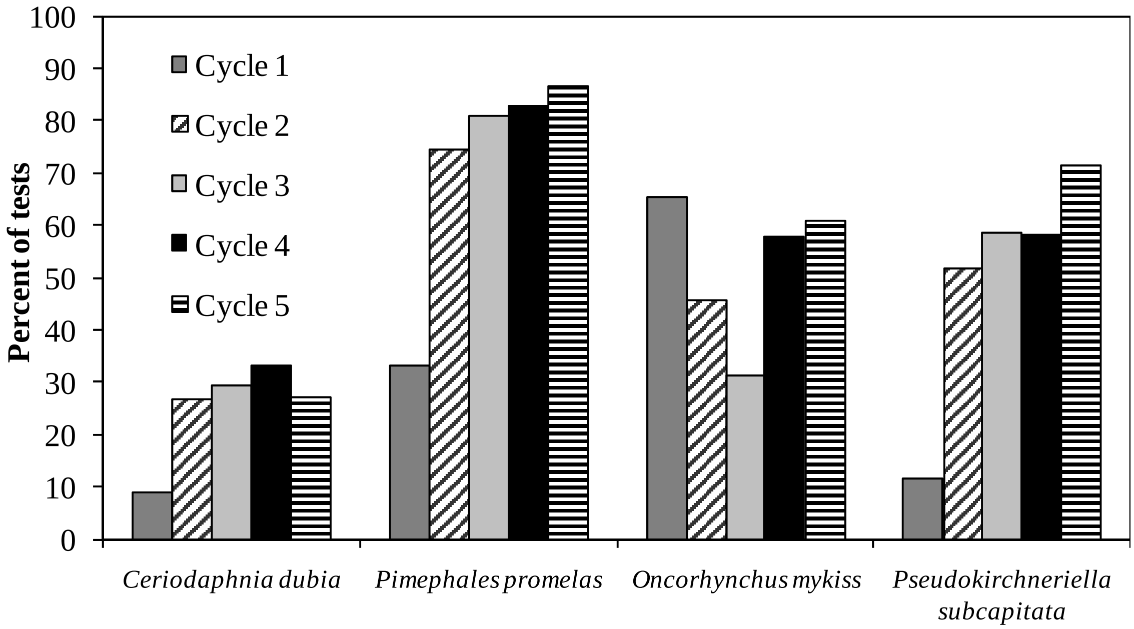
Figure 3.5. Percent of tests showing no sublethal toxicity at 100% effluent concentration per cycle



Note: Total number of tests per cycle shown in parentheses. Chart represents all SLT testing conducted on plants, invertebrates and fish.

The fish tests with *Pimephales promelas*, *Atherinops affinis* and *Medinia beryllina* shown in figures 3.6 and 3.7 indicated that a high proportion were unresponsive to mill effluent (i.e., IC_{25} > 100% or no effect at the highest concentration). The fish tests with *Oncorhynchus mykiss* shown in Figure 3.6 indicated that approximately half were unresponsive to mill effluent. In both freshwater and marine SLT testing over all five cycles, the tests measuring reproductive endpoints (*Ceriodaphnia dubia*, Echinoids, *Champia parvula*) showed the greatest responsiveness to mill effluent.

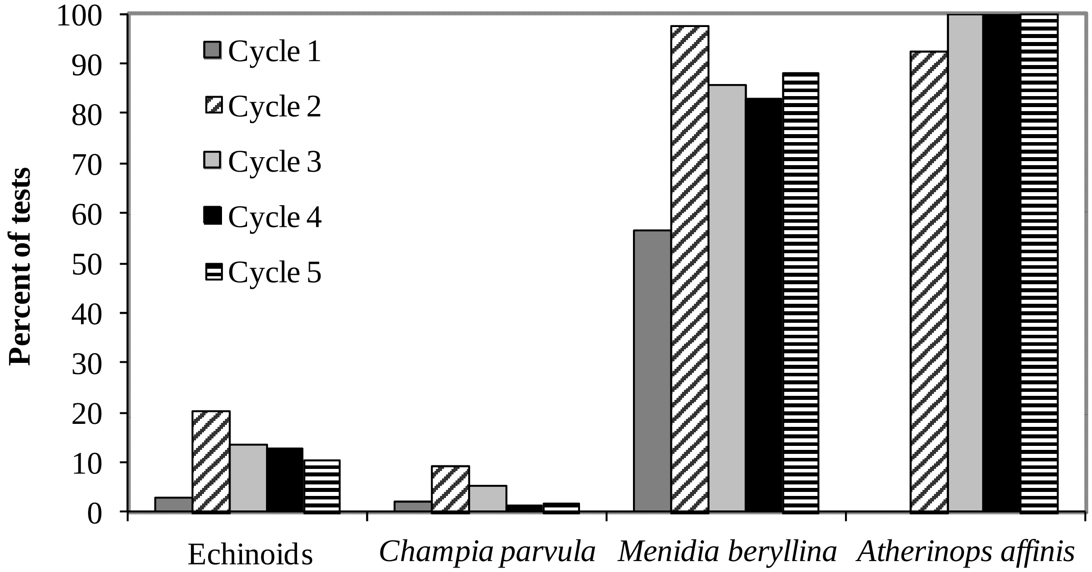
Figure 3.6. Percent of freshwater tests showing no sublethal toxicity at 100% effluent concentration in cycles 1 through 5



Species	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**
Ceriodaphnia dubia	361	514	707	471	418
Pimephales promelas	305	468	614	409	222
Oncorhynchus mykiss	52	70	93	64	23
Pseudokirchneriella subcapitata	236	532	703	481	413

Note: Number of tests conducted per species per cycle shown in table.

Figure 3.7. Percent of marine tests showing no sublethal toxicity at 100%[13] effluent concentration in cycles 1 through 5



Species	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Echinoids	108	175	215	150	128
Champia parvula	106	175	227	158	129
Menidia beryllina	106	124	163	45	42
Atherinops affinis	0	51	65	45	17

Note: Number of tests conducted per species per cycle shown in table.

[13] Due to the standard procedure used during cycles 1 to 3 of adding hypersaline brine to the effluent for salinity adjustments, 60% effluent concentration was considered full strength. The method of adjusting salinity with dry salts used in cycles 4 and 5 made it possible to test with 100% effluent. Test results reported as IC_{25} at greater than 60% and 100% effluent are included together as showing no SLT in the chart.

4. Studies to Investigate Confirmed Effects

To investigate confirmed effects, mills describe the magnitude and geographical extent of the effects, investigate their causes and identify possible solutions to eliminate them.

To describe the magnitude and extent of confirmed effects, mills conduct additional sampling downstream in the exposure area. Some mills included this additional sampling while monitoring to assess effects. As a result, these mills had the information needed to describe the magnitude and geographic extent of confirmed effects and could proceed with investigation of cause studies. Mills that do not have this information need to describe the magnitude and extent of their confirmed effects before beginning their investigation of cause studies. The goal of an investigation of cause study is to determine the cause of the observed effects and progress to investigate possible solutions.

During Cycle 4, there were 16 mills involved in 14[14] studies to investigate the causes of confirmed effects associated with eutrophication. In Cycle 5, 6 mills conducted studies to investigate the causes of eutrophication. Also in Cycle 5, 10 mills were involved in 9[15] studies to investigate solutions for confirmed effects associated with eutrophication.

Studies to investigate reduced gonad size in fish involved 12 mills in 3[16] studies in Cycle 4 and 11 mills in 2[17] studies in Cycle 5. One other confirmed effect, fish tissue exceedances in dioxin/furan concentrations, has also been investigated. The following sections provide overviews of the investigation studies conducted in cycles 4 and 5.

4.1 Studies to Investigate Causes of and Solutions for Effects Associated with Eutrophication

A wide variety of studies have been conducted to investigate confirmed effects associated with eutrophication. Investigations to determine cause included studies on sediment, benthic invertebrate communities, fish, water quality, nutrient modelling and effluent characterization. Sediment studies included tracking the fate of organic matter, measuring depositional rates and timelines, and studies to separate current from historical depositions. A variety of sediment parameters have been used, including total organic carbon, total nitrogen, carbon:nitrogen ratio and sulphides. Studies on benthic invertebrates included the tracking of the fate of organic matter in benthic invertebrates, mesocosms using periphyton or epibenthic invertebrates, and artificial substrates. Studies on fish included the tracking of the fate of organic matter in fish, and caged bivalve studies. Water quality studies included measuring several parameters, such as dissolved oxygen, temperature, nitrogen, phosphorus and chlorophyll *a*. Some investigations included the characterization of effluent and/or nutrient sources, and others involved the modelling of nutrient levels in the receiving environment.

[14] Included two joint studies involving two mills each.
[15] Included one joint study involving two mills.
[16] Included two joint studies: one involving two mills and one involving nine mills.
[17] Included two joint studies: one involving two mills and one involving nine mills.

The investigation of cause studies, on most occasions, found that the observed effects were mill-related. Some specific causes included organic enrichment of sediments, biosolids in mill effluent (both current and historical), nutrients (especially phosphorus) in final effluent, and changes to water quality due to mill effluent. Modelling studies suggested that phosphorus is a controlling nutrient in some receiving environments and that it is possible to predict the phosphorus levels that would keep algal density below nuisance levels. The reductions in mill effluent nutrient loadings to eliminate or reduce the observed eutrophication effects could also be predicted. A few investigation studies found that the observed effects were not mill-related, but were due to differences in habitat or confounding factors, such as other nearby facilities.

Investigations to identify solutions for observed effects associated with eutrophication included nutrient management and nutrient source assessment studies. Many facilities identified gaps in their existing nutrient management processes and procedures by comparing their processes to the FPAC's *Best Management Practices Guide for Nutrient Management in Effluent Treatment* (FPAC 2008). Common solutions identified included minimizing the use of supplemental phosphorus through increased monitoring of the treatment system and automated phosphorus addition, changing the type of supplemental phosphorus used, reducing the organic material entering the treatment system, and improving operator training.

4.2 Studies to Investigate Causes and Solutions for Effect of Reduced Gonad Size in Fish

Two joint studies have been conducted to investigate the confirmed effect of reduced gonad size in fish. One of the joint studies, the National Investigation of Cause Project[18] (National Study) involving as many as nine mills, focused initially on selecting the most appropriate laboratory tests for conducting investigations into this effect (Kovacs et al. 2007). The work included an assessment of wild fish and a series of laboratory tests on fish, coupled with extensive chemical analyses of the effluents tested. Laboratory tests ranged in duration from a few days to over six months and covered an assortment of reproductive indicators in fish, from the biochemical level to egg production. Once the most appropriate test had been chosen, long-term effluent monitoring studies were conducted to discern temporal effluent variability with respect to fish reproduction (Martel et al. 2010).

Minimal responses in both wild and laboratory studies were observed in the National Study. Chemical fingerprinting and fish test results suggested that effluent quality may be somewhat variable. Egg production in laboratory tests appeared to have the greatest potential for assessing an effluent's ability to affect fish reproduction and was identified as having the most potential for future investigation of cause and solution studies. It was acknowledged that the exact relationship between effluent effects on egg production in short-term tests using laboratory fish species and gonad size in different species of wild fish is presently

[18] The National Study is a collaborative initiative undertaken by industry, academic and government researchers to determine the cause of reduced gonad size in fish. It involved as many as nine mills.

unclear. However, effects on egg production were considered important, and directing investigation of cause and solution studies using such a test would have environmental benefits. Leads for future investigation of solution studies were identified that involve minimizing losses of organic matter and upsets to biological treatment. The National Study continues into Cycle 6, and plans are to include longer-term tests incorporating egg production and gonad size measurements as part of a validation process.

The other joint study, involving two mills, refined a bioassay based on the production of eggs in adult Mummichog to test effects of final effluent and effluent streams on fish reproduction. Toxicity source and identification evaluations were applied to an effluent to isolate the waste stream(s) within the mill potentially causing the effect. Methods for identifying the class of chemicals that might cause depression of plasma sex steroids in fish were developed and refined by iteratively applying them to biologically active waste streams. Chemical profiling or fingerprinting of effluent was conducted using gas and mass spectrometry; in laboratory bioassays, separated compounds demonstrated effects. The joint study concluded that androgen-receptor binding experiments could prove promising for isolating specific compounds with a potential role in the depression of fish hormones related to reproduction.

Mummichog exposed to one of the participating mill's final effluent in laboratory tests did have an impact on condition factors (body weight and length), but there was no significant effect on egg production. The study concluded that the mill's effluent now has low potential to cause reproductive effects in the laboratory test species as well as in fish in the wild, possibly due to improved effluent quality. The mill had changed production from newsprint to high-quality super-calendared paper and had upgraded its waste treatment processes. The other mill's final effluent did have a significant effect on Mummichog egg production in the laboratory, but the effect persisted only for the first week of a three-week test.

4.3 Studies to Investigate Causes and Solutions for Fish Tissue Effects

One mill conducted studies to investigate a confirmed effect on fish tissue.[19] The mill's study traced sources of organic matter and contaminants in crab habitat (sediment survey) and in crabs (crab tissue survey) and established a sedimentation timeline (sediment coring survey). Recently deposited sediments showed relatively low dioxin/furan concentrations and had similar stable isotope signatures to sediments deposited prior to the opening of the mill. Sediments deposited after the mill opened, but prior to the implementation of secondary treatment of effluent, exhibited different isotope signatures and contained higher concentrations of dioxins and furans. Concentrations in hepatopancreas tissues of crabs exceeded the 30 pg/g wet weight level defined in the PPER as an effect. The age of adult crabs ranged between 4 and 10 years, increasing the likelihood that the crabs fed on historical contaminants at some point in their life cycle. Slow sedimentation rates have

[19] An effect on fish tissue means that there is a concentration of chlorinated dioxins and furans, expressed as toxic equivalents of 2,3,7,8-tetrachlorodibenzo-para-dioxin, exceeding 15 picograms per gram (pg/g) wet weight in muscle or 30 pg/g wet weight in liver or hepatopancreas of fish taken in the exposure area. If the same effect on the fish tissue occurs in studies from consecutive cycles, the effect is considered confirmed.

resulted in slow burial of fibre and other contaminants related to historical effluent discharges. Historical deposits are therefore still accessible to potential crab prey (e.g., clams), and crabs may take up contaminants from organisms feeding on these historical deposits.

In terms of solutions, it was suggested that crab tissue dioxin/furan concentrations will likely continue to decline as contaminated sediments become buried, although low levels of dioxins and furans may persist beyond this time due to bioturbation[20] and decomposition of deceased contaminated biota. Contaminated strata are likely already buried below biologically active layers in sheltered or depositional areas; however, in exposed or erosional areas, highly contaminated strata will not be buried for at least 10 years, and moderately contaminated strata will not be buried for at least 20 years.

These investigation studies were conducted in Cycle 4. In Cycle 5, the mill conducted a fish tissue survey and found that total toxic equivalent concentrations in crabs at four stations were now below the effect level and concentrations at the remaining three stations had declined relative to Cycle 4.

[20] Bioturbation is the stirring or mixing of sediment or soil by organisms, especially by burrowing or boring.

5. Conclusions

Cycle 5 Study Results
In Cycle 5, there was an increase in the number of mills conducting investigation of cause and solution studies. The number, magnitude and type of effects observed in Cycle 5 for mills that conducted biological monitoring studies were similar to those observed in previous cycles and illustrated the same common patterns of effects, namely nutrient enrichment in fish and benthic invertebrate communities (eutrophication), co-occurring with metabolic disruption demonstrated as gonad reduction in fish. SLT testing results in Cycle 5 were similar to the results observed in cycles 2, 3 and 4. Tests on reproduction or fertilization inhibition showed the most toxicity.

Comparing Results from Cycles 1 to 5
Previous national assessments showed that some mill effluents were causing effects on fish and fish habitat but rarely had an effect on the use of fisheries resources.

In cycles 2 to 5, the national average response pattern for fish was typical of those conditions related to nutrient enrichment, co-occurring with metabolic disruption. Specifically, fish exposed to mill effluent were fatter, grew faster and had greater relative liver size but lower relative gonad size than unexposed fish. The national average response pattern for benthic invertebrate communities (fish habitat) was typical of various degrees of eutrophication (i.e., nutrient enrichment conditions). Specifically, predominant effects were increased invertebrate density and changes in community structure and taxon richness (i.e., species diversity or number of species).

When effects observed in cycles 2 to 5 on the fish population, benthic invertebrate communities and use of fisheries resources are considered together, 67% of mills reported a confirmed effect in at least one of the EEM effect indicators, and 53% reported at least one confirmed effect with a magnitude equal to or greater than the critical effect size.

SLT testing results showed that mill effluent quality improved between Cycle 1 and Cycle 2. Since 1996, when all facilities became subject to the full requirements of the PPER, mill effluent SLT on a national basis has remained constant, with mill effluents eliciting SLT responses in half of all effluent tests. The freshwater and marine reproductive and fertilization tests showed the greatest responsiveness (most inhibition) to mill effluent.

Studies to Investigate Confirmed Effects
Two common response patterns observed nationally, based on confirmed effects observed in previous cycles, have been investigated during cycles 4 and 5: eutrophication and gonad size reduction in fish. One other confirmed effect, fish tissue exceedances in dioxin/furan concentrations, has also been investigated.

Common solutions identified for eutrophication included minimizing the use of or changing the type of supplemental phosphorus used, reducing the organic material entering the treatment system and improving operator training.

Potential investigation of solution studies regarding reduced gonad size in fish were identified that involve minimizing losses of organic matter and upsets to biological treatment. Investigations of cause and solutions for reduced gonad size in fish will continue in Cycle 6.

The mill conducting an investigation of cause for fish tissue effects found that historical deposits are still accessible to potential prey, so predators (fish and crabs) may accumulate dioxin/furan contaminants from organisms feeding on these historical deposits. The burial of contaminated sediments over time by natural processes has already led to a reduction of dioxin/furan levels in prey species, and it is expected that this reduction will continue with time.

As mills have progressed through the PPER EEM requirements, EEM activities have focused on investigating the causes of—and solutions for—observed effects. Action toward the determination of causes and identification of solutions for all confirmed effects is expected to be accelerated in Cycle 6 (2010 to 2013) by applying a level of effort commensurate with the level of risk. By focusing more effort on the highest risks and reducing effort directed toward lower risks, it may be possible to determine causes and identify solutions for most effects in an accelerated time frame.

6. 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 environmental effects monitoring 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.

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 toward 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 that must 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 determine the cause of the effect.

Investigation of solution – Once an effect on fish, fish tissue or fish habitat is confirmed and the cause is determined, a mill is required to conduct studies to identify possible solutions 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.

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.

Sublethal toxicity – In the context of environmental effects monitoring, sublethal toxicity tests 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 (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 lifespan of a fish, the rate of increase in size may decline as the fish ages.

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