Flambeau River Monitoring at the Flambeau Mine Rusk County, Wisconsin

2. MACROINVERTEBRATES -- Analysis, Comments and Recommendations

prepared for
Wisconsin Resources Protection Council

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INTRODUCTION

Biodiversity is considered an important ecosystem parameter. A number of agencies administer and monitor regulations concerning biodiversity. The goal of these efforts is to protect endangered or threatened species (see below), and when possible to maintain the overall biodiversity of our natural communities. One measure of biodiversity, the simplest and most intuitive, is how many species are present in that community. In most cases determining the actual number present is not an easy thing, but using appropriate sampling protocols it is possible if sampling efforts are identical between sites and across years, to at least look for trends in biodiversity. In rivers, these biotic communities can be affected by the temperature of the water, the amount of oxygen in the water or sediments, the presence of predators or toxic substances in the water or sediments, occasional flooding events ("spates") which scour the substrate and carry downstream those individuals unable to hold on or bury themselves, and various other factors.

The bottom sediments of lakes, streams and ponds are inhabited by a complex community of organisms without backbones called macroinvertebrates. While immature stages of insects are the most commonly encountered taxa and individuals, macroinvertebrates include many other kinds of organisms, such as oligochaetes (aquatic relatives of earthworms), flatworms, leeches, clams, etc. These are all the larger members of that community, and do not include the smaller bacteria, protozoa, or rotifers, the microinvertebrates. While lakes and ponds usually contain a complex planktonic (floating or swimming) community, rivers (e.g. the Flambeau River in northern Wisconsin) do not, because these organisms by and large cannot maintain their location by swimming against the currents. A small planktonic macroinvertebrate community may be found in backwaters or bays which do not have such currents, but none would be expected in reaches of the Flambeau River considered in the present report.

Potentially toxic materials in the surface water or sediment of rivers, whether natural or anthropogenic, not only affect the macroinvertebrate population, but since many of these species are food for organisms higher on the trophic pyramid, by bioaccumulating these potential toxins the macroinvertebrates directly affect species perhaps of more interest to humans, such as the fish community, or other vertebrates such as birds which prey on the invertebrates.

Because of the importance of macroinvertebrates to the riverine community, and because macroinvertebrate sampling is one way to measure human impacts on the river, industries located along riverways are sometimes required to conduct macroinvertebrate surveys. Such was the case with Flambeau Mining Company (FMC), a subsidiary of Kennecott Minerals of Salt Lake City, Utah that constructed an open pit copper sulfide mine alongside the Flambeau River in the mid 1990s. The river formed the western boundary of the project area, and the pit itself was constructed to within 150 feet of the river. The Flambeau Mine was operational for four years. It ceased production in 1997 and has since been reclaimed.

The macroinvertebrate sampling program instituted by FMC was part of a broader monitoring program designed to ascertain any effects the Flambeau Mine might have on the Flambeau River ecosystem, including surface water, sediment and aquatic life. These effects could occur during excavation of the mine, during its operation, and beyond the date of its operation if substances such as metals or other potential toxins or erosional runoff might be making their way through surface or groundwater into the river or its tributaries. While a spectrum of organic pollutants, or oxygen-depleting substances have been shown to affect river biotic communities, those anthropogenic impacts were not expected to affect the river from the Flambeau Mine. There has been concern, however, about possible effects from metals such as copper, zinc and manganese possibly making their way into the river. A large literature exists showing the sensitivity of either total macroinvertebrate diversity or individual macroinvertebrate taxa to metal concentration in sediments or water. [e.g. 1, 2, 3, 4, 5, 6, 7]

MACROINVERTEBRATE STUDY DESIGN UTILIZED BY FMC

FMC conducted baseline macroinvertebrate studies on the Flambeau River between 1987 and 1988. In 1991 the company launched an annual sampling regime that continued through 1998. Additional studies were conducted in 2004 and 2006. During this time period a number of activities took place at the Flambeau Mine site which had the potential to impact Flambeau River macroinvertebrates. These included:

1991-1993:	Pre-production stripping and preparation of the site for excavation
1993-1997:	Blasting and ore production
1997-2001:	Partial reclamation of the site (backfilling the pit, recontouring the surface, and revegetation)
2003+:	Sporadic and ongoing reclamation/remediation activities (e.g., removal of contaminated soils in the Industrial Outlot portion of the mine site)

Over the years at least six different sites on the Flambeau River were utilized by FMC at one time or another for macroinvertebrate surveys. Local landmarks associated with the sampling sites include Tiews Road, Blackberry Lane, the Flambeau Mine pit, Meadowbrook Creek and the site of the former Port Arthur Dam (the dam was removed in 1968), as described below and shown on the maps included in Appendices I and II:

1. Tiews Road (Site M-1 on the Appendix I map; about 1.8 miles upstream of the open pit site and 1.1 miles upstream of Blackberry Lane; sampled 1987-1988 only)
2. Blackberry Lane (Site M-2 on the Appendix I map and Site M-1 on the Appendix II map; about 0.7 mile upstream of the open pit site; sampled 1987-1988, 1991-1998, 2004 and 2006)
3. Flambeau Mine Pit (Site M-3 on the Appendix I map; sampled 1987-1988 only)
4. North of Meadowbrook Creek (Site M-5 on the Appendix I map and Site M-2 on the Appendix II map; actual sampling site is about 150 feet north of the mouth of Meadowbrook Creek and about 0.3 mile downstream of the project area; sampled 1987-1988, 1991-1998, 2004 and 2006)
5. South of Meadowbrook Creek (Site M-4 on the Appendix I map; actual sampling site is about 150 feet south of the mouth of Meadowbrook Creek and about 0.3 mile downstream of the project area; sampled 1987-1988 only)
6. Port Arthur (Site M-3 on the Appendix II map; about 3.1 miles downstream of the project area, in the vicinity of the former Port Arthur Dam; sampled 1991-1998, 2004 and 2006)

The predominant procedure used by consultants hired by the Flambeau Mining Company (FMC) to conduct macroinvertebrate surveys in the Flambeau River between 1991 and 2006 was kick-sampling, a standard limnological procedure used for sampling the bottom of rivers and streams for the macroinvertebrates found there. Additional methodologies employed by FMC at one time or another included Surber sampling, hand-picking, dip netting, dredge and drift net sampling, sweep netting and kick-seining. Specimens were preserved with formalin or alcohol and sent to the laboratory for identification and enumeration. As will be discussed below, sampling site locations and procedures were not always consistent from year to year.

SAMPLING & REPORTING ISSUES

Before presenting and analyzing the macroinvertebrate data reported by FMC between 1987 and 2006, it would be prudent to point out any perceived flaws in the study design implemented by FMC or inconsistencies in how data has been reported. This includes an examination of both baseline and follow-up studies. Following are the most significant issues of concern:

1. Inconsistency in sampling site locations when transitioning from baseline to follow-up studies

Changes in sampling site location, as well as changes in sampling methodology (discussed below) seriously detract from the usefulness of baseline data. In terms of the former, Flambeau Mining Company eliminated and/or changed the locations of some of its macroinvertebrate sampling sites when transitioning from baseline to follow-up studies. The 1987-1988 baseline studies utilized five different sampling sites on the Flambeau River, referred to as M-1, M-2, M-3, M-4 and M-5 in the 1989 *Environmental Impact Report* for the mine project (see Appendix I for a map showing site locations). Starting in 1991, however, only two of those sites, one upstream (Blackberry Lane) and one downstream (north of Meadowbrook Creek) of the mine site were utilized. In addition, a new macroinvertebrate sampling site was established at Port Arthur. See Table-1 for a side-by-side comparison of where the baseline sampling sites were located relative to the sites used in later years. The issue of concern is not the reduction in the number of sampling sites but that no baseline data exists for the Port Arthur site.

Besides eliminating and changing some of the sampling site locations when transitioning from baseline to follow-up studies, FMC also changed their code numbers. In particular, the sites labeled as M-1 (Tiews Road), M-2 (Blackberry Lane) and M-3 (Flambeau Mine Pit) in the company's 1987-88 baseline study are not the same as the sites referred to as M-1 (Blackberry Lane), M-2 (Meadowbrook Creek) and M-3 (Port Arthur) in later reports. So as to avoid confusion, I will hereafter refer to sampling sites by name (e.g., Blackberry Lane) rather than code number whenever possible.

Table 1. Codes for Flambeau Mine Macroinvertebrate Sampling Sites:

Sampling Location	**Code utilized in FMC Baseline Surveys (1987-1988)** **(See Appendix I Map)**	**Code utilized in FMC Follow-Up Surveys (1991+)** **(See Appendix II Map)**
Tiews Road (Approximately 9700 feet upstream from the open pit site[1])	M-1	Site not surveyed
Blackberry Lane (Approximately 3800 feet upstream from the open pit site[1] - at the end of Blackberry Lane [2])	M-2	M-1
Flambeau Mine Pit (Opposite the open pit site[1])	M-3	Site not surveyed
Meadowbrook Creek, North (Approximately 150 feet north of the mouth of Meadowbrook Creek[1] - immediately above the confluence of the Flambeau River with Meadowbrook Creek [2])	M-5	M-2
Meadowbrook Creek, South (Approximately 150 feet south of the mouth of Meadowbrook Creek[1])	M-4	Site not surveyed
Port Arthur (Site of the former Port Arthur Dam[2])	Site not surveyed	M-3

1. Location of sampling site, as described in *Environmental Impact Report for the Kennecott Flambeau Project V. II (Report Narrative),* 1989, pp. 3.8-1 – 3.8-2.
2. Location of sampling site, as described in *2004 Macroinvertebrate Memorandum, Appendix D, FMC 2004 Annual Report*, p. D-1.

<u>2. Inconsistency in sampling methodology when transitioning from baseline to follow-up macroinvertebrate studies</u>

Macroinvertebrate sampling done in the Flambeau River from 1991 onward used a different sampling procedure than that used for baseline studies conducted in 1987-1988. This throws the baseline data into question as useful background information.

Collection techniques utilized in the 1987-1988 baseline studies included Surber sampling, handpicking and dip-netting. Semi-quantitative sampling was also undertaken, being a composite of three square-meter Surber samples from each location. FMC reported collecting some seventy-two taxa using these techniques. Relative abundances (abundant, common or rare) were also recorded. (As an aside, macroinvertebrate sampling was also conducted in the vicinity of the mine site in 1969 and 1973, when FMC first attempted to secure and was subsequently denied a mine permit. The results of these surveys were utilized in an April 1992 *Supplement to the Environmental Impact Statement* for the project that was eventually permitted. Some eighty different macroinvertebrate species were encountered in these surveys, which included not only the Surber sampling used in subsequent monitoring, but also dredge and drift-net sampling.)

Protocols adopted by FMC for macroinvertebrate sampling from 1991 onward differed from earlier surveys in that they primarily relied on Surber sampling and/or kick-seining. As the company states in its 1991 report, “It should be noted that previous studies included truly benthic analysis (substrate analysis), while collection techniques reported herein rely on the disturbance of substrates but not necessarily collecting and picking substrates. For this reason, species encountered may not be identical.” Since sampling methods varied in sampling procedure and intensity up to the October 1991 sample, the earlier results cannot be used for the purposes of this report.

The first macroinvertebrate surveys using the sampling protocol adopted for subsequent years were conducted in late October 1991. Since the three sampling locations utilized from 1991 forward were standardized (and sampling techniques to a lesser extent), the 1991 data may be a more appropriate baseline to use for comparative purposes than the 1987-88 study. Utilizing this data as baseline, however, presents its own set of problems. Specifically, by the time the 1991 study was conducted in late October, nearly 90 acres of land had already been cleared of vegetation and topsoil during the pre-production stripping phase of the mine project. In addition, the company’s erosion control system had washed out at three different control points in early September 1991 after a rainfall of 5.2 inches over several days. WDNR officials issued a report confirming that “water laden with fine sediments” had entered the Flambeau River after the erosion control system failed and that “existing sediment basins and bail dikes did not provide nearly enough retention time to settle out clay size particles.” It is therefore possible that the mine project may have impacted the macroinvertebrate community of the river prior to the first round of sampling in 1991. In other words, even though the data was collected prior to the onset of actual ore production in 1993, substantial work had already been done at the mine site. For this reason I place quotation marks around the word “baseline” when referring to the 1991-1992 data sets in the present report.

3. Inconsistency in sampling methodology during follow-up macroinvertebrate studies

Besides inconsistencies in sampling methodology when transitioning from baseline to follow-up macroinvertebrate surveys, there were also inconsistencies from year to year in the follow-up surveys conducted by FMC between 1991 and 2006. For example, some sweep netting was utilized in limited areas along the shore between 1993 and 1996 but is not documented to have occurred in other years. Variations also existed in terms of the time window utilized for collection. For example, in 1993 in-stream sampling was conducted for a period of two hours with periods of kicking lasting about 12 minutes per effort. In 1994, in-stream sampling was conducted for a “minimum of one hour” with periods of kicking lasting “about five minutes per kick (longer if few organisms were observed to be collected).” The reports generated for 1991 and 1992 do not indicate the time windows utilized for sampling or kicking at all, while most other reports (with the exception of 1994 and 1995) indicate a sampling window of 2 hours. To assess long-term trends in macroinvertebrate populations, sampling methods must be the same from year to year. FMC, however, failed to do this, making interpretation of the resultant data difficult.

4. Insufficient spatial and temporal co-location of sampling sites

FMC’s Flambeau River monitoring program included not only macroinvertebrate surveys, but collecting sediment, crayfish, walleye and surface water samples upstream and downstream of the mine site for metals analysis. The sampling sites utilized by FMC for these various studies were not all the same; whenever possible, they should be. When sites are not co-located, trends from individual sites may be due to differing confounding factors, which decreases the reliability of inferences visavis mining effects.

In 1991 and 1992, one of the downstream sampling sites utilized by FMC for macroinvertebrates and crayfish (M-3; Appendix II) was co-located with the Port Arthur downstream sampling site for sediments (S-2; Appendix II). This is appropriate, because sediment chemistry is likely to have an important effect on both macroinvertebrates and crayfish. But from 1993 on, the downstream sampling site for sediments was moved by FMC to the Sister's Farm site (S-3; Appendix II), about 1.6 miles upstream of the sampling site for macroinvertebrates and crayfish at Port Arthur. In addition, the downstream sampling site for surface water (SW-2; Appendix II) was about a quarter mile upstream of where macroinvertebrates and crayfish were sampled at Meadowbrook Creek (M-2; Appendix II). As a result of a negotiated agreement reached between opposing parties at a contested case hearing in 2007, a new surface water sampling site (SW-3; Appendix III) and sediment sampling site (S-4; Appendix IV), co-located with the macroinvertebrate sampling site above Meadowbrook Creek (M-2; Appendix II) were added to the study regime, but no such reference points exist for biological data collected prior to that time.

Temporal co-location (performing different types of sampling on the same day or being consistent from year to year in terms of when that sampling is performed) is also important to decrease the likelihood of potentially confounding factors occurring. FMC, however, sometimes failed to do this. For example, in 1994 crayfish were collected for analysis on August 8, the macroinvertebrate survey was conducted on October 3 and walleye were sampled on October 16-17. In addition, the dates for the annual macroinvertebrate survey ranged from mid-August (2004) to late October (1991).

5. Inconsistencies in Reporting Protocols

Reporting protocols utilized by FMC for macroinvertebrate data were not always consistent or transparent. The company's macroinvertebrate reports for 2004 and 2006 contained two data tables: Table 1, which contained results from the most recent survey; and Table 2, which was a compilation of results from the most recent survey alongside data from previous years. It appears that Table 1 is the rougher data as reported back from FMC's consultant, which is then interpreted and placed into Table 2 of each report. While this facilitates comparing results from one year to the next, inconsistencies in reporting were sometimes noted between the two tables. It was noticed, e.g. that no platyhelminthes, hydrocarina, hemiptera, hirudinae or isopoda data at all are in Table 1 for 2006, though for those taxa absences are recorded in Table 2. As it is, Table 1 makes it appear these taxa were not even looked for during the survey, even though they had been observed in previous years. There is also an inconsistency in this: Table 1 does have a row for Pelecypoda, though none were found in 2006.

There are also inconsistencies in the reporting of data within Table 2 itself. The table consists of three separate sections, one for each of the three sampling sites utilized for the collection of macroinvertebrate data (i.e. Blackberry Lane, Meadowbrook Creek and Port Arthur Dam). Yet, the same basic list of taxa and species is not utilized for reporting the presence (or absence) of species at each sample location. For example, the absence of *Helobdella stagnalis* and *Placobdella ornata* (two types of leeches) was noted in the Meadowbrook Creek and Port Arthur Dam sections of Table 2, but the Blackberry Lane section of the table had no row to record the presence (or absence) of any species within the hirudinae (leech) taxon.

When a taxon or particular species within a taxon is encountered in a previous sampling at any site, a row for recording the presence or absence of that species should appear in Table 1 and all three sections of Table 2 in all subsequent years. FMC's submissions, however, deviated from this common standard of practice. Interpretation of sampling results would be facilitated by consistency across sampling sites, i.e. listing the same taxa in all tables for all sites. These tables may change, naturally, as new taxa are encountered, but this kind of across-year and across-site consistency would be very helpful to insure that data is interpreted correctly.

Another concern regarding transparency in reporting of information is that FMC did not always include the complete assemblage of data in hard copy and electronic versions of documents that were supposedly identical (i.e., reports with the same title and from the same year). This became an issue, e.g., when for the 2006 macroinvertebrate sampling, Table 1 of the report was posted on the company's website, but Table 2, the compilation of data over years, was not. Table 2 was eventually made available to me scanned off a hard copy of the document.

6. Inconsistencies in Levels of Taxonomic Specificity Utilized for Reporting

Just as we have a taxonomy of our human relations – brother, sister, cousin, second cousin, uncle, aunt, etc. – biologists have created a taxonomy of organismal relationships. Members of the same species can, and do, interbreed. Species which are closely related to one another belong to the same genus. And so we climb up the taxonomic tree – species/genus/family/order/class/phylum/kingdom/domain. When measuring biodiversity in an ecosystem, or tracking the effect of natural or human disturbance on an ecosystem, biologists most commonly count the number of species of different organisms – frogs, insects, trees, birds, etc. Though there exist subspecies, races, and ecotypes, in many ways the species is the fundamental biological and taxonomic unit. So, in comparing two ecosystems or an ecosystem at different times, one common method is to record the number of species present – the *species* diversity.

It is very important when examining data for trend analyses or in comparing different sites that sampling and reporting methodology be unchanging and consistent. E.g. taxa should be identified to the same degree of specificity from year to year, whether family, genus or species. If this is not done, it is not possible to compare the number of taxa from year to year, since a number of different species might in some years be combined into a single genus. It is also not possible to do community similarity comparisons – an important biotic community descriptor -- across sites when different levels of taxonomic specificity are employed.

For whatever reason, this fundamental principle was not followed by FMC in the 2006 sampling year and/or data presentation, as demonstrated by the following examples listed below (Unless otherwise noted, reference in this section to macroinvertebrate data for 2006 is from Table 1 of the *2006 Macroinvertebrate Memorandum, Appendix D, FMC 2006 Annual Report.*):

a. Oligocheates in 2006 were not ID'd to genus, as previous years' were, but only to the order level, so for this taxon, the 2006 data cannot be used. This is especially problematic, because in previous years a total of ten different genera or families were encountered, in some years (e.g. 1993) up to four taxa. Consequently there is no way to infer actual number of total taxa for 2006.

b. Gastropods were only ID'd to family level in 2006. In previous years they were ID'd to genera. As with oligochaetes, this detracts from the overall usefulness of the data. In Table 2 of FMC's *2006 Macroinvertebrate Memorandum* a new taxon is added, the Ancylidae. But the genus *Ferrissia,* previously found, belong to the Ancylidae. It is likely the snails found in 2006 ID'd as Ancylidae were also *Ferrissia*. But we don't know that, and adding an entire new family-level taxa due to ID'ing only to family level in 2006 is inappropriate and, for some purposes, fatally confounds the data.

c. One mayfly in 2006 sampling was ID'd as an *Acanthopotamus*. There is no genus of mayfly with that name (*pers. comm*., Dr. Patrick McCafferty, Purdue Univ.) It is probably meant to be *Anthopotamus,* which is a common genus encountered in previous years. In fact the genus to which it was entered into Table 2 of FMC's *2006 Macroinvertebrate Memorandum* was *Anthopotamus.*

d. For this year only (2006), totals in Table 1 were given as number of individuals rather than total taxa encountered. This was corrected in Table 2.

e. Because of these inconsistencies in how taxa were identified over years (especially a & b above), it is not possible to determine a reliable actual number of total taxa encountered for 2006, to compare with previous years. Because of these identification/recording problems, 2006 total taxa of macroinvertebrates was considered fatally flawed and not included in analyses of total taxa below.

6. Unacceptable Levels of Reporting Errors

Examination of data tables showing macroinvertebrate results (Table 2 of FMC's *2004 Macroinvertebrate Memorandum* and Table 2 of the *2006 Macroinvertebrate Memorandum*) for 1991-1998 and 2004 indicate an unacceptable number of reporting errors. Total number of taxa shown at the end of each table often did not correspond with the actual number of taxa collected, by summing the taxa for which individuals were counted for each year. This is summarized in Table 2.

Table 2: FMC Macroinvertebrate Reports in which the Numbers of Observed Taxa Were Incorrectly Totaled

Survey Year	Reported Sum of Observed Taxa[1] / Actual Sum of Observed Taxa[2]		
	Blackberry Lane	Meadowbrook Creek	Port Arthur
1991	31/30	37/34	42/39
1992	39/38		28/46
1993			
1994	25/24		
1995			25/24
1996	26/25	43/44	
1997		48/49	34/33
1998	38/37		
2004			30/29

1. Reported sums of observed taxa are taken from: *Table 2. Macroinvertebrates Collected from Flambeau River, Ladysmith, WI 1991-1998, 2004* as it appeared in the *2004 Macroinvertebrate Memorandum, Appendix D, FMC 2004 Annual Report.*
2. I determined the actual sums of observed taxa by manually counting the individual taxa listed in *Table 2. Macroinvertebrates Collected from Flambeau River, Ladysmith, WI 1991-1998, 2004* as it appeared in the *2004 Macroinvertebrate Memorandum, Appendix D, FMC 2004 Annual Report.*

Note that 13 of a possible 27, or nearly 50%, of cells show different actual sums of observed taxa than those reported by FMC. While in almost all cases the numbers differ by only one taxon, the 1992 Port Arthur dam total is widely different. This level of reporting inaccuracy is unacceptable, and along with the other reporting issues mentioned above casts a shadow of doubt over the overall reliability of the macroinvertebrate data and therefore our ability to make reliable inferences about the status of the macroinvertebrates in the Flambeau River.

ENDANGERED AND THREATENED SPECIES IN THE FLAMBEAU RIVER

Several species of Wisconsin endangered or threatened species of invertebrates were found in the Flambeau River in the vicinity of the mine site in May/June 1991, after mine permits had been issued by Hearing Examiner David Schwarz but prior to the commencement of mining. The subsequent discovery of endangered species by WDNR divers who were working on an unrelated project resulted in a lawsuit filed by the Lac Courte Oreilles Ojibwe and Sierra Club in July 1991. The issue was deemed serious enough by the courts that a temporary injunction on mine construction was handed down by Judge George Northrup (Dane County Circuit Court, Madison, WI) in August 1991. As the Judge wrote:

> *All permits issued [to FMC] which relate to either site preparation or mining operations and activities shall be suspended pending completion of a Supplemental Environmental Impact Study by the Department of Natural Resources.*

As a result of survey work completed during the supplemental EIS process, a number of Wisconsin endangered or threatened species were confirmed to exist in the vicinity of the mine site, including the following: the purple wartyback mussel, the bullhead mussel, and three species of dragonflies (the pygmy snaketail, extra-striped snaketail, and St. Croix snaketail.)

In their *Supplement to the Environmental Impact Statement for the Flambeau Mine Project, April 1992* the Wisconsin DNR describes their survey of the river for endangered and threatened species and evaluates the potential for the mining activity to impact these species. An attempt to further delay construction activity at the mine to afford additional protection to these species was dismissed in a June 12, 1992 court ruling which declared there was not sufficient evidence these activities would harm these species. It appears that beyond the DNR survey of the Flambeau River, FMC was not asked to, nor did they, undertake additional monitoring to ascertain the location and/or populations trends of these species near the mine. Since these species were not encountered again during regular sampling protocols, they are not discussed further in this report. The lack of appropriate close monitoring of any endangered or threatened species in ecosystems potentially impacted by mining activities should be viewed as a significant shortcoming of efforts to protect these ecosystems.

RESULTS: TAXON RICHNESS

Keeping in mind the above-mentioned caveats about the data, *corrected* total taxa data were used to examine several indices which are used to describe a biotic community. It is not possible to call this analysis “species richness,” since not every group of organisms was identified to the species level, as mentioned above. But for those organisms which were consistently identified to the same taxonomic level – species in some cases, genus or family in others – we can create what might be called “taxon richness.” These richness numbers – the total taxa encountered at a site for a given year -- represent the biodiversity present, though not always identified to the species level. A decrease in biodiversity is shown as a negative number, an increase as a positive number.

Table 3: Macroinvertebrate diversity as measured by total taxa encountered, 1991-2004

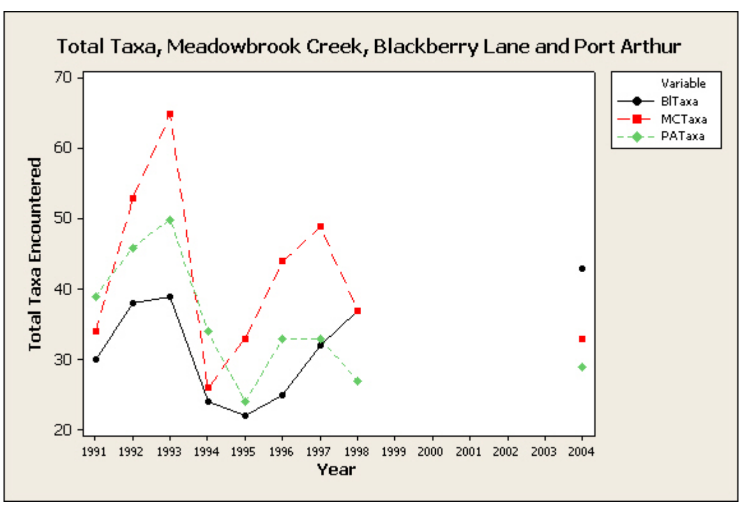
Parameter	**Trends in Number of Taxa**		
	Blackberry Lane	**Meadowbrook Creek**	**Port Arthur**
Total taxa average 1991-04	32.2	41.6	35.0
Taxa richness 91-94 vs. 95+	- 1.0	- 5.3	-12.5
Taxa richness 91-95 vs. 96+	+ 3.7	- 1.5	- 8.1
Means 91-92 vs. 97-98, % change	+ 1.5%	- 1.1%	- 29%
Means 91-92 vs. 98-04, % change	+ 17.6%	- 22%	- 34%
Regression of trend, slope and significance	slope = +.74 p = .31	slope = -0.89, p = .46	slope = -1.3 p= 0.09

Because sampling effort has a large influence on results, when comparing periods of years' sampling, it is important that equal sampling efforts (e.g. number of years) be compared. Because there were 9 reliable years' effort (1991-1998 and 2004), it was not possible to divide the years into two equal halves. So in calculating means for total taxa, 1991-1994 vs. 1995+ data, which provides 4 years' data for first category, and 5 years' for second were used, then 1991-1995 and 1996+. In other words, because there were an odd number of years' good data, both analyses were done (see Table 3.) Note though the 1991-1992 years are the closest to what might be considered "baseline" data, it would not be appropriate to use 1991-92 vs. all later years because there is less total sampling in 2 years than in the coming eight, so those total sampling efforts would not be comparable. Instead, those 2 years' sampling was compared with two separate later sampling periods,1997-98 and 1998 plus 2004.

Minitab- release 15 was used for statistical analyses.

Figure 1 summarizes the trends in total taxa encountered at the three sampling sites.

Figure 1: Trends in total number of taxa encountered (1991-2004)



Figures 2-4 below show the results of Minitab Trend Analysis for total taxa for each site. Minitab Trend Analyses do not provide significance tests for the trends. In the figures, "MAPE" is the average amount each point is away from the trend line, in percent. "MAD" is the mean absolute deviation and "MSD" the mean squared deviation, both accuracy measures based on standard deviation of the data.

Figure 2: Trend analysis, Blackberry Lane Taxa (1991-2004)

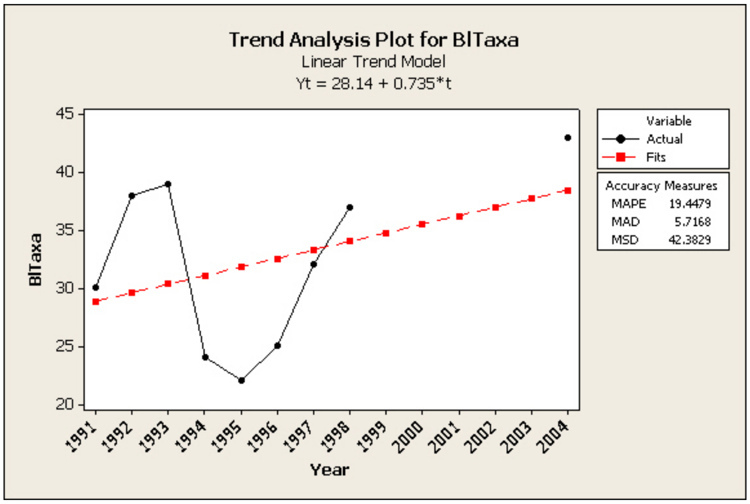


Figure 3: Trend analysis, Meadowbrook Creek Taxa (1991-2004)

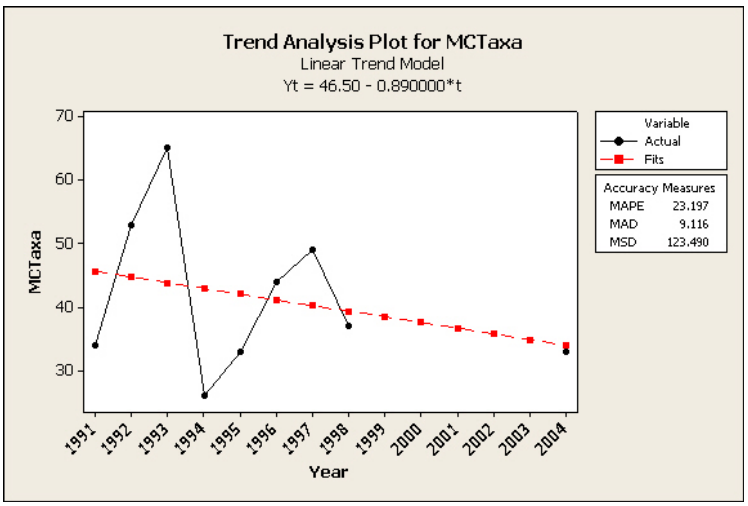
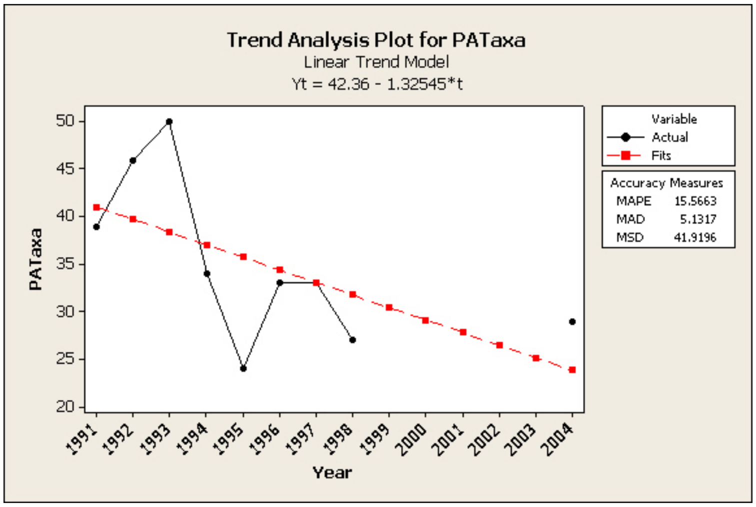


Figure 4: Trend Analysis, Port Arthur Dam Taxa (1991-2004)



Two-Way ANOVA Analysis of total taxa encountered over the years for all three sites indicated that the trend in years was significant at p= 0.008, and site was significant at p= 0.03. This means both the trends over the years and the actual sample site itself had a strong influence (significant at p =.05) on how many taxa one would expect to find in a sample at a particular sample site, with time apparently having a somewhat stronger effect than site.

DISCUSSION OF OVERALL TAXA RICHNESS

There is an apparent trend of a slight increase in macroinvertebrate diversity for Blackberry Lane (upstream from the mine) but a decrease in macroinvertebrate diversity for Meadowbrook Creek and an even more pronounced decrease at the Port Arthur Dam site (both downstream from the mine site), shown by several ways of examining the data. The Port Arthur Dam regression analysis suggests a decrease of about 30% in taxa richness, significant at p = 0.10, when comparing “baseline" to post-mining data.

In September of 1994 an unusually heavy rain caused flooding in the Flambeau River and a breach in the Ladysmith (Peavey Mill) dam, upstream of all sampling sites. This event likely affected the riverbed and macroinvertebrate community via the rapid spate and churning of the waters, scouring the bottom and washing some species but not others downstream. Macroinvertebrate sampling for 1994 took place on Oct. 4, after that breach, and the decrease in taxa diversity for 1994 and 1995 could in part at least be explained by this event. The subsequent increases in taxa richness would represent recolonization of those sites (see Figure 1). The Blackberry Lane site (upstream) however shows a more robust rebound to previous biodiversity levels than the downstream sites, suggesting the possibility of some other factor affecting these downstream sites. This is discussed in more detail below.

RESULTS FOR EPHEMEROPTERA, TRICOPTERA AND PLECOPTERA

The Hilsenhoff Biotic Index (HBI), often used to estimate the degree of human impact on streams, was not considered appropriate in this case. The HBI was developed primarily as an index based on the sensitivity of taxa to a low-oxygen environment, when the waters are impacted by organic pollutants such as sewage, manure, pulp mill wastes, etc., the decomposition of which cause a low-oxygen environment. There was little expectation of this kind of impact to the Flambeau River. In addition, the Flambeau over the reach of this study is a rapidly-flowing stream with primarily gravel and pebble substrate, and is not expected to be oxygen-limited.

A number of studies have shown that the insect orders Ephemeroptera, Plecoptera, and Tricoptera (EPT) are especially sensitive to metal pollution.[8, 9, 10] An index, the EPT index, has been developed and to some degree tested which estimates the possible effects of anthropogenic pollutants such as metals on the macroinvertebrate community.[11] The EPT index is variably reported as the total number of Ephemeroptera (mayflies), Plecoptera (stoneflies) and Tricoptera (caddisflies) taxa encountered, or the total number of these taxa (EPT) encountered vs. total taxa, in which case I express it as % EPT. The reporting issues noted above for total taxa did not appear to confound these three orders of insects, so data from the 2006 sampling were included. Figures 5 – 7 show the trends in these three common taxa of insects encountered.

I note here that Clements, Cherry and VanHassel[12] question the applicability of the EPT index to all streams. They caution that some species of Tricoptera are metal-tolerant, and that while some authors use an EPT divided by Chironomidae index (the chironomids being generally pollution-tolerant), their results suggest some species of chironomids are actually more sensitive to copper pollution (25 mcg/L for 10 days) than some tricopterans or ephemeropterans. They develop their own index, the ICS, Index of Community Sensitivity, which however requires laboratory toxicity studies. These authors make clear the danger in over-applying a particular Index to a particular macroinvertebrate community.

Figure 5: Trends for Ephemeroptera, Plecoptera, and Tricoptera (EPT) at Blackberry Lane (1991-2006)

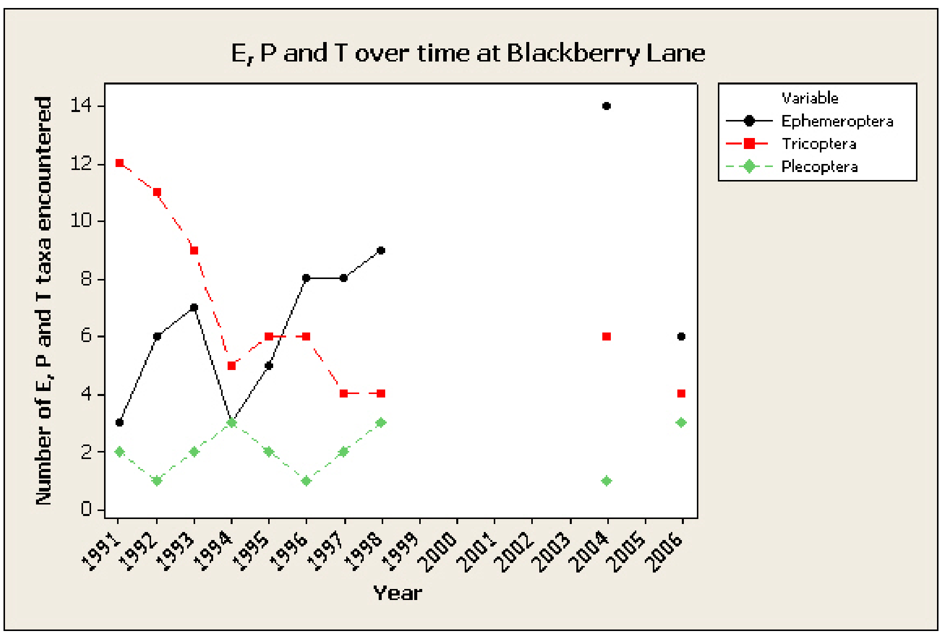


Figure 6: Trends for Ephemeroptera, Plecoptera, and Tricoptera (EPT) at Meadowbrook Creek (1991-2006)

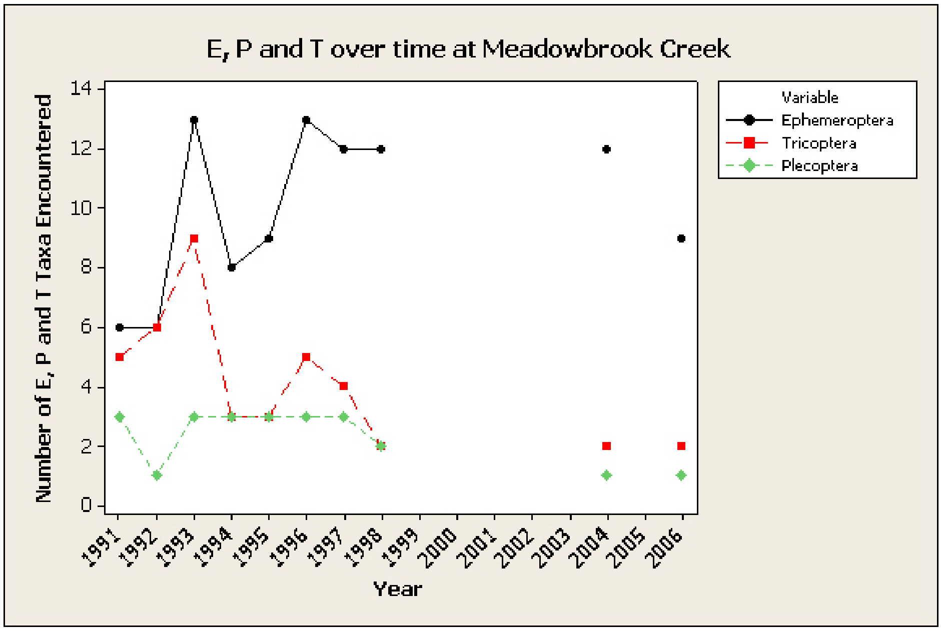
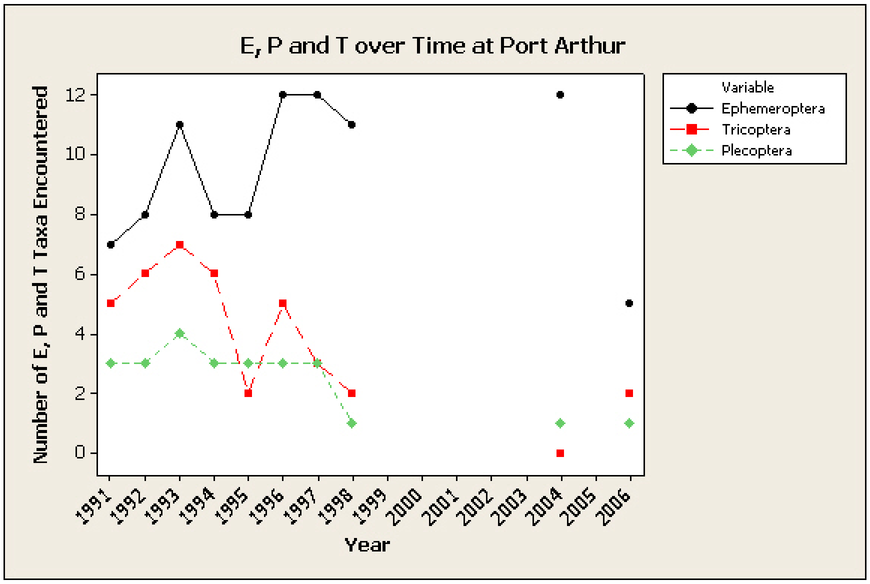


Figure 7: Trends for Ephemeroptera, Plecoptera, and Tricoptera (EPT) at Port Arthur (1991-2006)



The overall trends in Ephemeroptera, Tricoptera and Plecoptera shown in Figures 5-7 are summarized in Table 4:

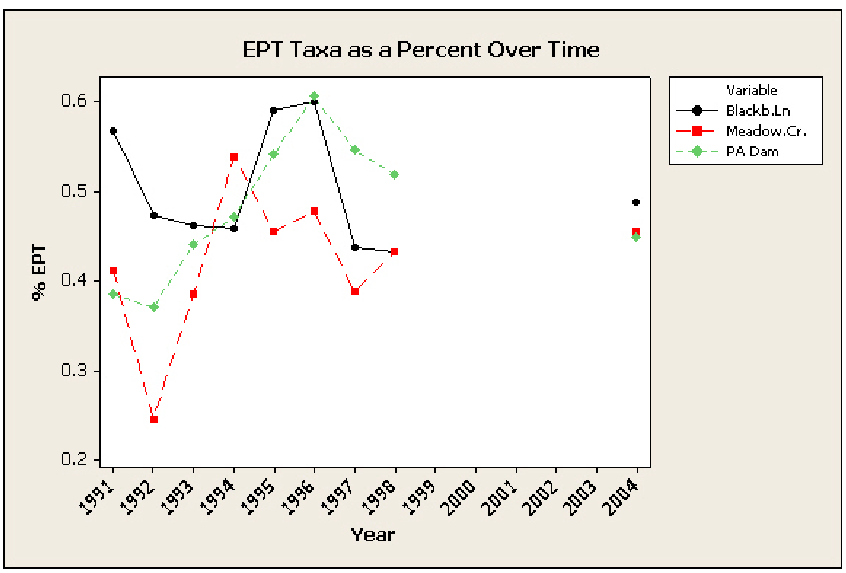
Table 4: Summary of overall trends in Ephemeroptera, Tricoptera and Plecoptera at three sampling sites in the Flambeau River (1991-2006)

Taxon	**Trend**		
	Blackberry Lane	**Meadowbrook Creek**	**Port Arthur**
Ephemeroptera	increase to 2004	slight increase	increase to 2004
Tricoptera	decrease	decrease	decrease
Plecoptera	little change	slight decrease	slight decrease

A % EPT Index was calculated by dividing the total taxa of Ephemeroptera, Plecoptera and Tricoptera encountered by total number of taxa encountered. It was not possible to calculate this index for the 2006 sample because of uncertainties about the total number of taxa, noted above.

Figure 8 below shows the % EPT Index over time at all three sites. There appears to be an increase in the % EPT to approximately 1995, then a falling off and leveling off.

Figure 8 EPT Index for Blackberry Lane, Meadowbrook Creek and Port Arthur Sites (1991-2004)



There was also no evidence of a significant difference in the number of individuals of E, P plus T sampled in the years 1991-1995 vs. 1996-2006 (data not shown, all $p>0.50$).

According to the EPT index, if there is a negative effect of metals in the river water or sediment downstream from the mine, everything else being equal, one would expect all of Ephemeroptera, Plecoptera and Tricoptera to decline downstream. How then can one explain the absence of a decrease (at least until the 2006 sampling) of Ephemeroptera at all three Flambeau River sampling sites, despite the fact that Tricoptera have decreased at each site and Plecoptera have either stayed basically the same (Blackberry Lane) or decreased (Meadowbrook Creek and Port Arthur Dam)?

One possible explanation has to do with community interactions at the sampling sites. Plecoptera (stoneflies) are considered top invertebrate predators, and are known to prey efficiently on Ephemeroptera (mayflies) and can have significant effects on prey populations.[13, 14, 15] Wooster[16] concludes based on a meta-analysis of a large number of predation studies in the macroinvertebrate community that "prey density is significantly lower in the presence of predators than in predator-free enclosures." This is not surprising. It is a well-known principle in ecology that predator populations can reduce prey populations, and prey populations often increase subsequent to predator removal.

Table 5 shows total number of Plecoptera individuals counted in the first five sampling years of the study (1991-1995) versus the last four years (1996-2006.) As can be seen, there is a decline in Plecoptera populations at all three sampling sites, with a greater decline at the downstream sites.

Table 5: Number of Plecoptera individuals – not taxa – encountered (1991-2006)

Sampling Site	# of Plecoptera 1991-1995	# of Plecoptera 1996-2006	% change	Statistical significance*
Blackberry Lane	227	171	- 25%	p= 0.41
Meadowbrook Creek	233	36	- 85%	p= 0.16
Port Arthur Dam	219	60	- 73%	p= 0.10

* Actual numbers of individual Plecopterans counted for each year's sampling (raw data) were used for the t-test rather than the totals for year-classes shown in this table. Though some sites showed a large decrease, high variance in the numbers collected and limited number of years' data decreased the power of the statistical tests.

Another way of looking at the data in Table 5 is not through t-tests but through ANOVA analysis. A Two-Way ANOVA analysis of number of Plecoptera individuals sampled (using each year's data as a data-point, not just the summed data, e.g. 1991-95, as shown in Table 5) of 1991-95 vs. 1996-2006 year classes and site, indicated that year class was significant at p=.013, though site was not (p=0.516.) This indicates, as suggested by the t-test results shown in Table 5, that there was a significant change in how many Plecopterans were encountered over time, and this change over time explains more of the difference in numbers than the sites do.

The commonest species of stoneflies found in the Flambeau River (*Agnetina capitata, Acroneuria abnormis,* and *Neoperla clymene*) are all known to be effective predators of mayflies.[17, 18, 19] Removal of the pressure of stonefly predation on the mayfly community downstream from the mine, whatever its cause, could have allowed the Ephemeroptera to rebound from the decrease in taxa after the 1994 flooding, even in the presence of somewhat higher metal concentrations or other stressors, natural or human-caused. I can find no clear explanation for the apparent decrease in the Ephemeroptera in 2006. Without further monitoring it is not possible to know whether that apparent downturn is the beginning of a trend.

POTENTIAL IMPACT OF METALS ON MACROINVERTEBRATE POPULATIONS

The potential toxicity of metals such as copper, zinc, manganese, aluminum, etc. to the macroinvertebrates in the Flambeau River depends on a combination of concentration of specific toxin and time of exposure. The exposure can come through either surface water or sediments. High doses of metals in short periods can lead to acute responses, while low doses over longer periods of time can have chronic effects. Little is known about the possible synergistic effects of small increases in individual metals when other metal concentrations also increase.

Between July 1991 and July 1998 FMC tested Flambeau River surface water on a quarterly basis, in 1999 three times, and since then apparently twice a year only, and at two different locations in the river - one upstream (SW-1; Appendix III) and one downstream (SW-2; Appendix III) from the project area. It is important to point out, however, that while the surface water sampling site historically labeled by FMC as its "downstream" site is about 100 yards downstream of the open pit site, it is about a quarter mile upstream of where runoff from the mine site enters the Flambeau River via an intermittent stream known as Stream C. This study design deficiency was exposed at a contested case hearing over the issuance of a Certificate of Completion for site reclamation in May 2007. As a result, a third sampling site (SW-3; Appendix III) was added to the monitoring regimen immediately downstream of the confluence of Stream C with the Flambeau River. Even with the additional monitoring site, however, the infrequency of sampling and the overall limited number of sites makes it difficult to ascertain potential mine impacts to surface water. It is possible – though of course there exists no evidence for this – that between samplings higher concentrations of metals may be making their way into the Flambeau River, perhaps especially downstream of Stream C.

It is difficult to calculate mean values for many of the metals in the surface waters of the Flambeau River, because of a significant number of analyses being below detection limit. There is no agreed-upon protocol for dealing with those values. However it is safe to say that sampling results revealed no obvious pulses of high concentrations of metals, nor did average individual metal concentrations exceed toxicity standards. An exception, however, occurred in January 1998 when a copper measurement of 12 mcg/L was recorded at the downstream sampling site (SW-2; Appendix II), exceeding the acute and chronic toxicity standards for Cu of 8.1 and 5.7 mcg/L, respectively (*NR 105, Wisconsin Administrative Code, Nov. 2008*). The upstream (SW-1; Appendix II) measurement, at the same time, of 7.6 mcg/L exceeded the chronic but not the acute limit.

Hickey and Clements [8] found virtual elimination of Ephemeroptera and Plecoptera at sites in New Zealand waters high in metals. These sites had copper concentrations in the water of 1.2 mcg/L to 30.6 mcg/L plus Zn concentrations of 120 to 8200 mcg/L, in combination with relatively high Cd concentrations (7-63 mcg/L.) Flambeau River surface water concentrations did at times exceed 1.2 mcg/L Cu, but Zn and Cd did not reach those concentrations. Clements, Cherry and VanHassel, cited above, also found complete elimination of some species of Ephemeroptera, and significant (>50%) reduction in all Ephemeroptera studied (6 species), and some Tricoptera and Dipterans by exposing these populations to 25 mcg/L copper for 10 days.

Nehring [20] determined TL_{50} (the concentration lethal to 50% of individuals in a study population during, in this case, 14 days of exposure) of about 200 mcg/L for the mayfly *Ephemerella grandis*, and 10-14 mg/L for the stonefly *Pteronarcys californica* for Cu. Zn TL_{50} was greater than 9 mg/L for the mayfly and even higher for the stonefly. All these concentrations are well above levels found in the Flambeau River during this study. (Nehring reported TL_{50} concentrations, but did not report lowest levels of metal exposure which showed some effect on survivability or reproduction.) Nehring found that these insects did bioaccumulate the metals considerably, so fish eating them would be exposed to even higher levels. The mayfly, depending on level of exposure, accumulated copper to almost 10 mg/gm. Stoneflies accumulated less. Zinc was also accumulated less in both insects. In general, these metals were accumulated in proportion to their concentration in the water. The average bioconcentration factor for both species & all metals was about 200-fold.

As discussed earlier, Plecopterans (stoneflies) and Tricopterans (caddisflies) show evidence of declines in the Flambeau River downstream from the Flambeau Mine site over the course of sampling, although Ticopterans appear to have declined upstream as well. The number of Gastropoda taxa (mollusks) also decreased at both downstream sampling sites from 1991 + 92 pooled vs. 1998 + 04 pooled samples (complete data not shown.) At Meadowbrook Creek it decreased from 4 taxa to 1, and at

Port Arthur Dam from 3 to 1, while remaining at 1 taxon at the upstream site. Gastropods are known to be sensitive to metals. For example, the gastropod *Amnicola limosa* (*Aminicola* sp. were found in the Flambeau River) has been recorded as having an LC_{50} for aluminum of 400 mcg/L.[21] The LC_{50} is the concentration lethal to 50% of a study population. Baseline (1987-1988) aluminum levels reported by FMC in the Flambeau River ranged from 42-111 mcg/L. In 1992 aluminum levels both upstream and downstream from the mine were reported variously at 420-750 mcg/L. These levels, considerably higher than others reported during the monitoring period, may be analytic or sampling outliers. Levels fluctuated considerably during the years of ore production, at times exceeding 200 mcg/L at both the upstream and downstream monitoring sites. The last time aluminum levels were tested (June 2000), the upstream value was 42 mcg/L and the downstream 160 mcg/L. No additional data is available.

One study of copper toxicity in Gastropods reported an LC_{50} for the snail *Campeloma decisum* treated with 1.7 mg/L for 96 hours, and an LC_{50} for *Physa integra* of only 39 mcg/L when treated for 96 hours, and *Thiara tuberculata* an LC_{50} of 2.2 mg/L for 72 hours,[22] all considerably higher than copper concentrations reported in the Flambeau River.

In addition to exposure to potential toxins in the surface waters, most of the macroinvertebrates (including all the immature insects) are also exposed to metals in the sediments of the river. Sediment metal toxicity dynamics are very complicated. E.g. burrowing into the sediments by the insects themselves can release copper into the water.[23] Median copper levels in Flambeau River sediments downstream from the mine from 1993-2008 were 7.0 mg/kg, slightly higher than upstream (5.8 mg/kg.) Median Zn concentrations for the same period are 30 mg/kg downstream and 23 mg/kg upstream. But the levels of copper in the sediments which appeared to show an effect on macroinvertebrate numbers in the Keweenaw Canal of Upper Michigan (reference 11, below) were 140-930 mg/kg, many times those encountered in the Flambeau River.

Concentrations of aluminum, copper and zinc in Flambeau River sediments were found to be higher downstream than upstream of the Flambeau Mine (see Sediments Report.) Because sediment metal toxicity is dependent on many factors such as the chemical form of the element (free ion, complexed or precipitated), pH, redox potential, type of sediment, water hardness, etc., and because individual organisms, ecotypes and species can differ significantly in their toxicity to different forms of these elements, even at different life-history stages, it is difficult to assign a causal relationship between higher downstream sediment metal concentrations and trends in macroinvertebrates. However, it is in my opinion equally difficult to argue that these causal relationships do not exist.

RECOMMENDATIONS

Because some of the suggested improvements to FMC's Flambeau River macroinvertebrate monitoring program that were mentioned earlier cannot be implemented retroactively but could be useful in the design of monitoring programs in the case of future mining activity, recommendations are listed in two different categories: (1) General recommendations, based on perceived shortcomings of monitoring in the present case, to improve the utility of similar monitoring programs undertaken by others in the future; and (2) Recommendations for how to continue and augment the present study to better track potential impacts of the Flambeau Mine on the associated ecosystem.

1) Flambeau Mining Company failed to gather adequate baseline data regarding macroinvertebrate populations upstream and downstream from the mine site prior to commencement of the mine project. Although some macroinvertebrate sampling occurred previous to October, 1991, the procedures used varied and were not those later adopted, disallowing comparisons with later data. Standardized sampling of the kind later used ought to have occurred previous to October 1991, since nearly 90 acres of land had already been cleared of vegetation and topsoil by that time during the pre-production stripping phase of

the mine project and the mine's erosion control system had failed six weeks earlier. Given the natural variability of populations due to intrinsic and extrinsic non-human factors, making reliable inferences about the pre-mining populations requires several years' sampling – the more, the better. The paucity of baseline data in this case makes questionable any statements about whether the mine either has or has not impacted the aquatic biota.

Recommendation for similar monitoring programs in the future: *Sampling protocol should specify that baseline studies should be conducted using the same sampling methodology employed in follow-up studies. In addition, baseline studies should entail several years' sampling and must be completed before any significant pre-mining activity such as pre-production stripping takes place.*

2) FMC changed one of its two downstream macroinvertebrate sampling sites in the Flambeau River when transitioning from baseline to follow-up studies. Hence, no baseline data exists for that site (Port Arthur).

Recommendation for similar monitoring programs in the future: *More thought should be put into carefully choosing sampling sites BEFORE the annual sampling regime is begun (also see point # 3 and point # 4 below). Once those sites are chosen, sampling protocol should specify that the same sampling locations be utilized for the duration of the study (baseline and follow-up).*

3) Throughout most of the study at hand, FMC failed to appropriately co-locate its downstream macroinvertebrate sampling sites (Port Arthur and Meadowbrook Creek) with sites being utilized for sediment testing. With regard to the Port Arthur site, FMC tested sediment there for three years (1991-1993) and then moved the sediment sampling site to Sister's Farm (see Sediments Report). At that time the collection site for macroinvertebrates (including crayfish) was not moved to the same location, despite the fact that the monitoring plan referenced in the Flambeau Mine Permit specified that the downstream monitoring site for macroinvertebrates was to "*coincide* with the sediment sampling location near the old Port Arthur Dam"[24] (emphasis added).

With regard to the Meadowbrook Creek macroinvertebrate sampling site, sediment was not tested there until 2008, seventeen years after the macroinvertebrate study commenced.

Having different sampling sites for sediment chemistry and macroinvertebrates makes it difficult to draw inferences about organismal metal concentrations. The sediment microhabitat is an environmental matrix whose chemistry and potential toxicity have a profound influence on these organisms. Events, whether anthropogenic or natural, affecting the sediment chemistry and mineral dynamics, can occur at one location while not at another. The sediments are a notoriously heterogeneous matrix, even at small scales. It is therefore difficult to make reasonable inferences about putative effects of mining activities on the macroinvertebrate and crayfish communities when the sediment metal concentrations are not being monitored in situ, but at a site distant from where the organisms are collected.

Recommendation to augment FMC's macroinvertebrate monitoring program: *One of the two historic downstream sampling sites for macroinvertebrates and crayfish (Meadowbrook Creek) coincides with Site S-4 in the Flambeau River, where sediment was sampled as a one-time event in 2008 (See Sediments Report). It is recommended that sediments at Site S-4 continue to be sampled for at least ten years in conjunction with additional macroinvertebrate and crayfish studies as recommended under point # 8 below. Sediment sampling should also continue at Site S-1 (Blackberry Lane), which coincides with the upstream macroinvertebrate and crayfish sampling location, and Site S-3 at Sister's Farm.*

An additional five years sediment sampling beyond the ten years recommended above should be required if significant changes are detected in the continuing monitoring of the biota or the sediment. These changes could be triggered statistically (the precautionary principle suggests using p = 0.10) by the biotic or sediment monitoring results, or even if not exactly statistically significant, by apparent unexplained spikes in metal concentrations in biota or sediment or notable declines in biota toward the end of the ten-year monitoring period.

Recommendation for similar monitoring programs in the future: *In the future, choice of sampling sites should be done more carefully. In particular, sampling sites for macroinvertebrates and sediment need to be co-located physically and temporally whenever possible to reduce the influence of potentially confounding factors*.

4) Throughout most of the study at hand, FMC failed to appropriately co-locate its downstream macroinvertebrate sampling sites (Meadowbrook Creek and Port Arthur) with sites being utilized for surface water testing. With regard to the Meadowbrook Creek site, surface water was not tested there until 2007, sixteen years after the macroinvertebrate study commenced. Surface water has never been tested at the Port Arthur site.

An additional problem with FMC's surface water quality monitoring program is that the historic surface water sampling site utilized by FMC for "downstream" testing (SW-2) is actually upstream of the river's confluence with Stream C, which originates at the mine site and may be conveying potential toxins to the river. This problem was corrected in 2007 with the addition of a new surface water sampling site to the study regime (SW-3), located immediately below the mouth of Stream C. But it is not possible to determine if, historically, there was a causal relationship between metal levels in the river's surface water and the observed trends in macroinvertebrate populations in the Flambeau River.

The measured level of metal concentrations in biota and sediments during monitoring are to an important degree affected by surface water metal concentrations. The interplay of sediment and surface water toxins on the biotic community is complex and differs for particular metals, species, and ecotypes. In case continued monitoring of the biota and sediments discloses unforeseen changes in the community structure or metal concentrations, it would be useful in attempting to explain those changes to have as much information on hand as possible visavis all possible causal mechanisms. It would therefore be amiss to not continue surface water monitoring of the Flambeau River.

Recommendation to augment FMC's macroinvertebrate monitoring program: *Surface water monitoring of the Flambeau River should: (1) continue for as long as sediment and biota are being monitored in the river (at least ten years); and (2) due to concerns over spatial co-location, be expanded to include not only the surface water sampling sites identified in the December 2007 Stipulation Monitoring Plan (SW-1, SW-2 and SW-3), but also the Port Arthur biota sampling site. In addition, due to concerns over temporal co-location, surface water sampling should be timed so that samples are collected on the same days as biota are sampled.*

Recommendation for similar monitoring programs in the future: *In the future, choice of sampling sites should be done more carefully. In particular, sampling sites for macroinvertebrates and surface water need to be co-located physically and temporally whenever possible to reduce the influence of potentially confounding factors.*

5) Since number of individuals and taxa encountered in biotic sampling depends so intimately on sampling effort, it is critical that each year's and site's sampling be as identical as possible. The standard methodology of sampling utilized by FMC subsequent to 1991 is not clear. It is described (e.g. in the *FMC 2004 Annual Report: Appendix D*) as "using a net with an 8 by 18-inch opening and a 800 to 900 micron mesh size." To the present author this sounds like an aquatic kick-net. The methodology is further described as "Instream sampling methods consisted of kick-seining." But kick-seining, again to the present author, involves a much larger net than 8 by 18-inch opening – usually about 4 by 4 feet – and stirring up a meter square of river-bottom at each sampling effort. The area sampled by disturbing the river-bottom in front of each kick-net or kick-seine sampling, an important sampling parameter, is not described. Sampling effort for 2004 is described as: "At each of the three sites, instream sampling was conducted for approximately two man-hours." The *FMC 1992 Annual Report* says only that "Aquatic invertebrate collections were conducted using kick sampling techniques with both Surber sampler and D-frame nets."

These descriptions of sampling effort are inappropriately vague. On the one hand it's not clear exactly what the sampling methodology for any given year was – kick-net, Surber or kick-seining – and on the other it is not possible to verify that between sites and years, sampling effort was equivalent. "Approximately two-hours sampling effort" is quite vague, especially as the amount of sampling accomplished in two hours can vary depending on the individual. The purpose of benthic macroinvertebrate sampling is to gather information adequately reflecting the range of taxa and populations sizes of the macroinvertebrates in the river. The protocol used by FMC is a time-based protocol, which includes sorting of individual organisms from sediments. In addition to depending on the expertise, etc. of the individual doing the sampling another problem with time-based protocols is that they can underestimate large populations and over-estimate small populations. This is because the actual number of subsamples taken in the river is reduced when a large number of individual organisms needs to be sorted in a given time-frame, while when populations are low, more subsamples can be taken. The number (and total area sampled) of subsamples taken therefore more accurately represents the biota in the river than a time-based protocol.

A much more clearly-described and carefully-chosen sampling protocol would assure that data across sites and years is in fact comparable, and more reliably represents populations within the river. Such a protocol might include e.g. using the same number and size of subsamples (Surber, kick-net or kick-seine samples) for each site, and a description of the method used to locate subsamples – e.g. random within a site, equally or randomly spaced along a cross-river transect, etc. Collecting subsamples (e.g. kick-net samples) into separate containers, and identifying and recording them separately would also increase the statistical usefulness of the collection.

Recommendation for similar monitoring programs in the future: *A sampling protocol needs to be defined. The protocol should include number and size of subsamples for each site, and a description of the method used to locate subsamples.*

6) Consistency in sampling and data presentation is critical. Although it is not always feasible because of lack of taxonomic knowledge or expertise and due to time constraints, whenever possible specimens should be identified to the species level. This is especially important for the Ephemeroptera, Plecoptera, and Tricoptera, which are used in the calculation of the EPT index. As mentioned above, some species of these orders are much more tolerant of pollutants than others, and without knowing the community structure to the level of species it's difficult to make inferences about possible human impacts on the macroinvertebrates. In other words, without proper species-level identification for these taxa, these indices have considerably less usefulness.

I also found it necessary to not use the results from FMC's 2006 survey because identification of specimens was not done in the same manner as in previous years. Longitudinal (cross-year) sampling consistency is essential! As an example, if you are trying to determine trends in traffic on a highway, and for eight years you count all traffic, but discover that in the last year of the study, motorcycles & buses weren't counted, that makes that year's data useless for most analyses. If taxa are ID'd to species (or genus) level in one year, they should be in all years.

Recommendation for similar monitoring programs in the future: *Specimens should be identified to the species level. If this is not possible, then the reasons for foregoing species level identification should be clearly recorded. Once a particular level of taxa identification has been established (e.g., species vs. genus), that same level of identification should be maintained throughout the survey.*

7) Changing daylengths and water temperatures affect macroinvertebrate behavior in ways which might make them less susceptible to capture. It is therefore important that the principle of temporal co-location be applied to macroinvertebrate studies. This was not always done by FMC. For example, macroinvertebrate sampling was conducted in late October 1991, late September 1992 and mid-August 2004.

Recommendation for similar monitoring programs in the future: *Sampling protocol should specify that sampling be conducted at the same date (except for extenuating circumstances, within a 2- week window each year) and day-time (again, except for extenuating circumstances, within a 2-hour time window of the day.)*

8) It is important to continue macroinvertebrate monitoring at the Flambeau Mine site to determine if the apparent trends in decreased macroinvertebrate biodiversity downstream from the mine are real. Plecopterans (stoneflies), Tricopterans (caddisflies) and Gastropods (mollusks) show evidence of downstream declines over the course of sampling, although Ticopterans appear to have declined upstream as well. Ephemeropta (mayflies) showed an apparent decrease in taxa encountered in 2006, upstream and downstream. These trends are potentially important. Problems with the 2006 data for total taxa sampled (discussed above) make inferences about future trends especially difficult.

There appears to be no good explanation visavis human activities to explain the observed decline in some macroinvertebrate fauna downstream from the mine. The observed changes may or may not be completely unrelated to the mining activity. The greatest change in the macroinvertebrates, as noted above, seems to have been at the Port Arthur Dam site, far enough downstream from the mine that other, e.g. agriculture-related impacts may have caused those changes. However, the changes may in some causal way be connected to mining activities, as suggested by the observation of similar though perhaps not as profound changes at the Meadowbrook Creek site, near the mine.

To clarify these issues and because of the sensitivity of these organisms to metallic toxins, additional monitoring of macroinvertebrates in the Flambeau River is warranted.

Recommendation to augment FMC's macroinvertebrate monitoring program: *It is recommended that an additional six to ten years of macroinvertebrate sampling be done at the Blackberry Lane, Meadowbrook Creek and Port Arthur sampling sites, perhaps done every other year. If significant changes are detected in taxon richness or the EPT (or % EPT) index during the expanded monitoring period, an additional five years sampling beyond that already recommended should be required. These changes could be triggered either statistically (the precautionary principle suggests using* $p = 0.10$*) by* *the monitoring results or even if not exactly statistically significant, by apparent unexplained declines in either taxon richness or the EPT (or % EPT) index.*

9) Because of the ability of macroinvertebrates to bioaccumulate metals, regular analysis of a select set of macroinvertebrates (instead of only crayfish) for total body metal concentrations should be done, upstream and downstream from the potentially impacting activity. Copper or other metals in the macroinvertebrates will likely make their way into the higher food chain. In some streams near Yellowstone Park copper concentrations in macroinvertebrates reached levels which killed half of trout fed food with the same copper concentration.[25] Chemical analyses of macroinvertebrates in addition to crayfish were not done by FMC.

Recommendation for similar monitoring programs in the future: *Based on macroinvertebrate taxa present and their relative abundance, it is recommended that a select set of macroinvertebrates be identified for total body metal analysis.*

10) A number of endangered and threatened species were found in potentially-impacted reaches of the Flambeau River, previous to mining activity. As far as can be ascertained, no special effort was made to determine the location and numbers of these endangered populations either during the years of ore production or after mining ceased. Without such monitoring, it is not possible to make any reasonable statements visavis the effect of FMC's mining operation on these species of concern.

Recommendation to augment FMC's macroinvertebrate monitoring program: It is recommended that FMC conduct follow-up surveys to determine the fate of the following endangered or threatened species identified in the *Supplement to the Environmental Impact Statement for the Flambeau Mine Project, April 1992*: the purple wartyback mussel, the bullhead mussel, and three species of dragonflies (the pygmy snaketail, extra-striped snaketail, and St. Croix snaketail).

Recommendation for similar monitoring programs in the future: It is recommended that specific monitoring for endangered or threatened species be undertaken whenever a new mining operation is under consideration, and that additional monitoring specifically targeting any such species identified be required if the mine is permitted.

CONCLUSIONS

Due to a lack of baseline data, flaws in FMC's study design and inconsistencies in the reporting of data, it is not possible to ascertain with any degree of certainty whether or not the Flambeau Mine has had or may presently be having an impact on macroinvertebrate biota in the Flambeau River. In addition, the lack of follow-up studies on the fate of endangered and threatened species identified in and around the Flambeau River prior to mining is unacceptable.

There is enough evidence however to suggest that there were declines in some macroinvertebrate species downstream from the mine during the course of its operation, e.g. especially the Plecoptera and the Gastropoda. While it is not possible to identify the Flambeau Mine itself as *the* cause of these changes, or *a* significant cause of several, it is also not possible to say with any reasonable certainty that the Flambeau Mine did *not* play a part, however slight or however significant, in these observed changes.

Exactly what the changes in the macroinvertebrate communities have been, and how long they might last, is difficult to say unless the river continues to be carefully monitored and study design issues are resolved. To have a better understanding of possible effects visavis any future mining projects in Wisconsin, the biomonitoring protocols should be improved with consideration of the recommendations noted herein, including especially the reliability of the data as reported and the inclusion of studies to evaluate the fate of any threatened or endangered species identified at the project site.

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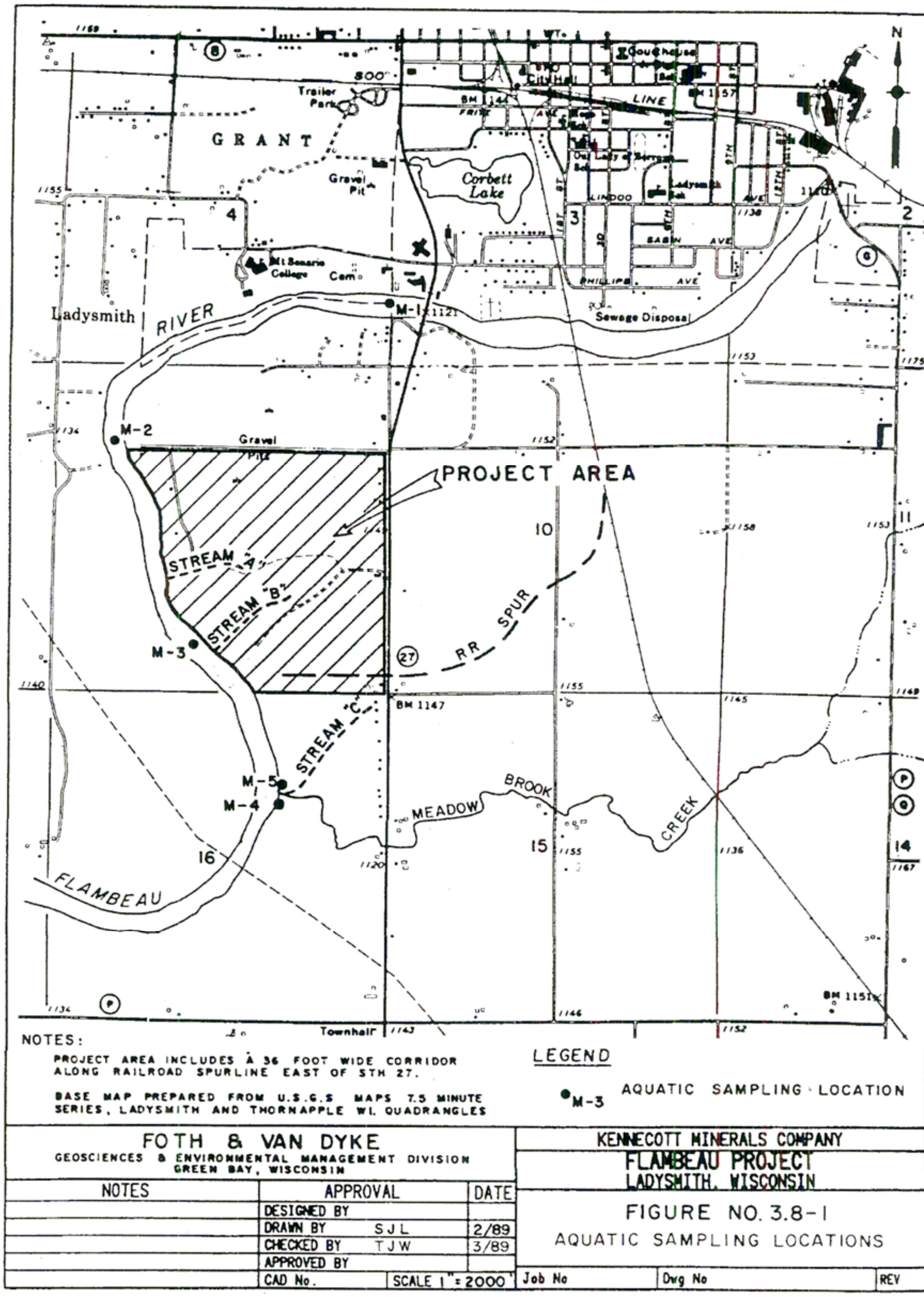
Appendix I

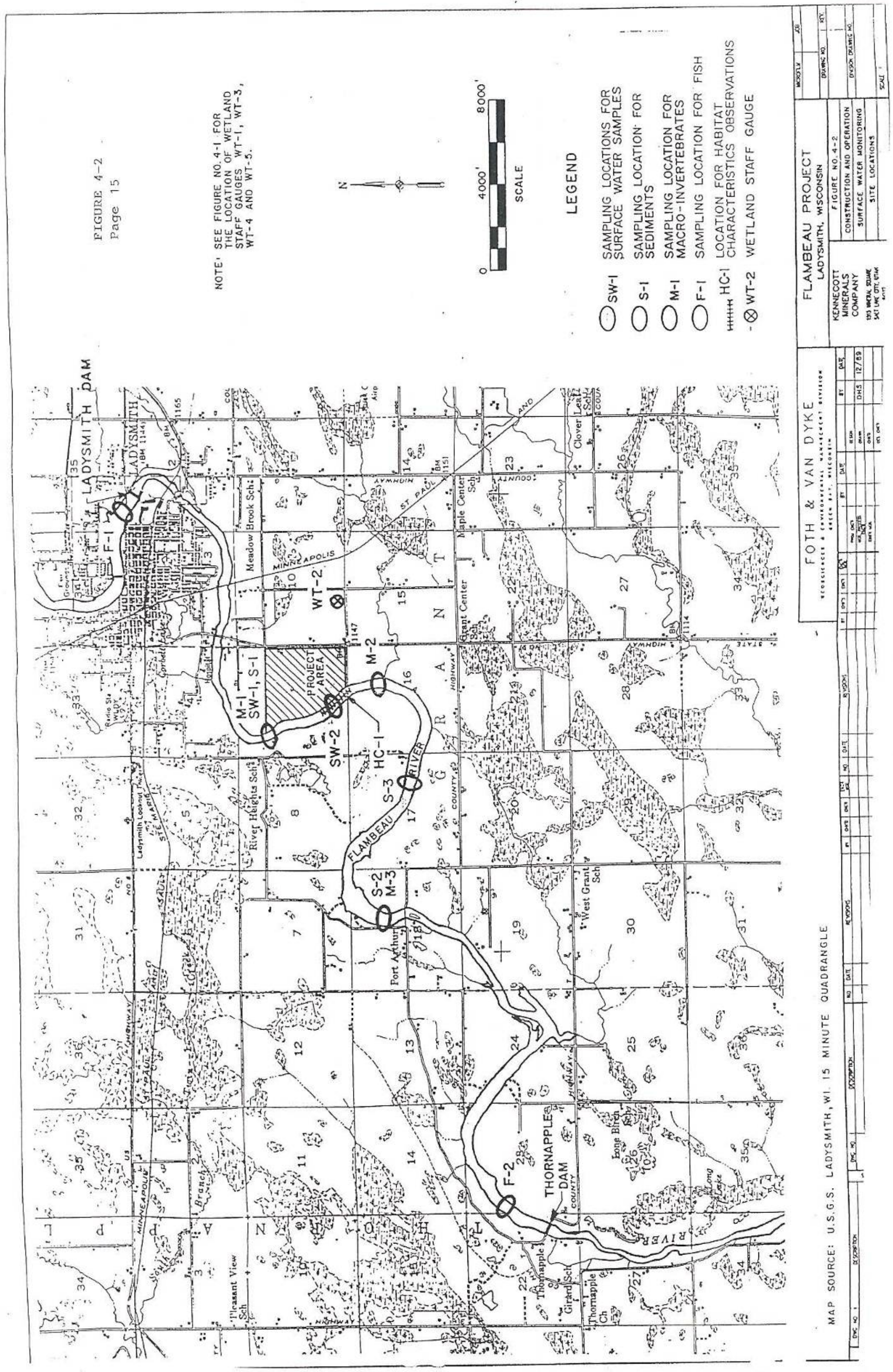
Flambeau River Macroinvertebrate Sampling Locations Utilized for Baseline Testing (1987-1988)

(Source: *Volume 2: Environmental Impact Report for the Kennecott Flambeau Project, 1989*)



Appendix II

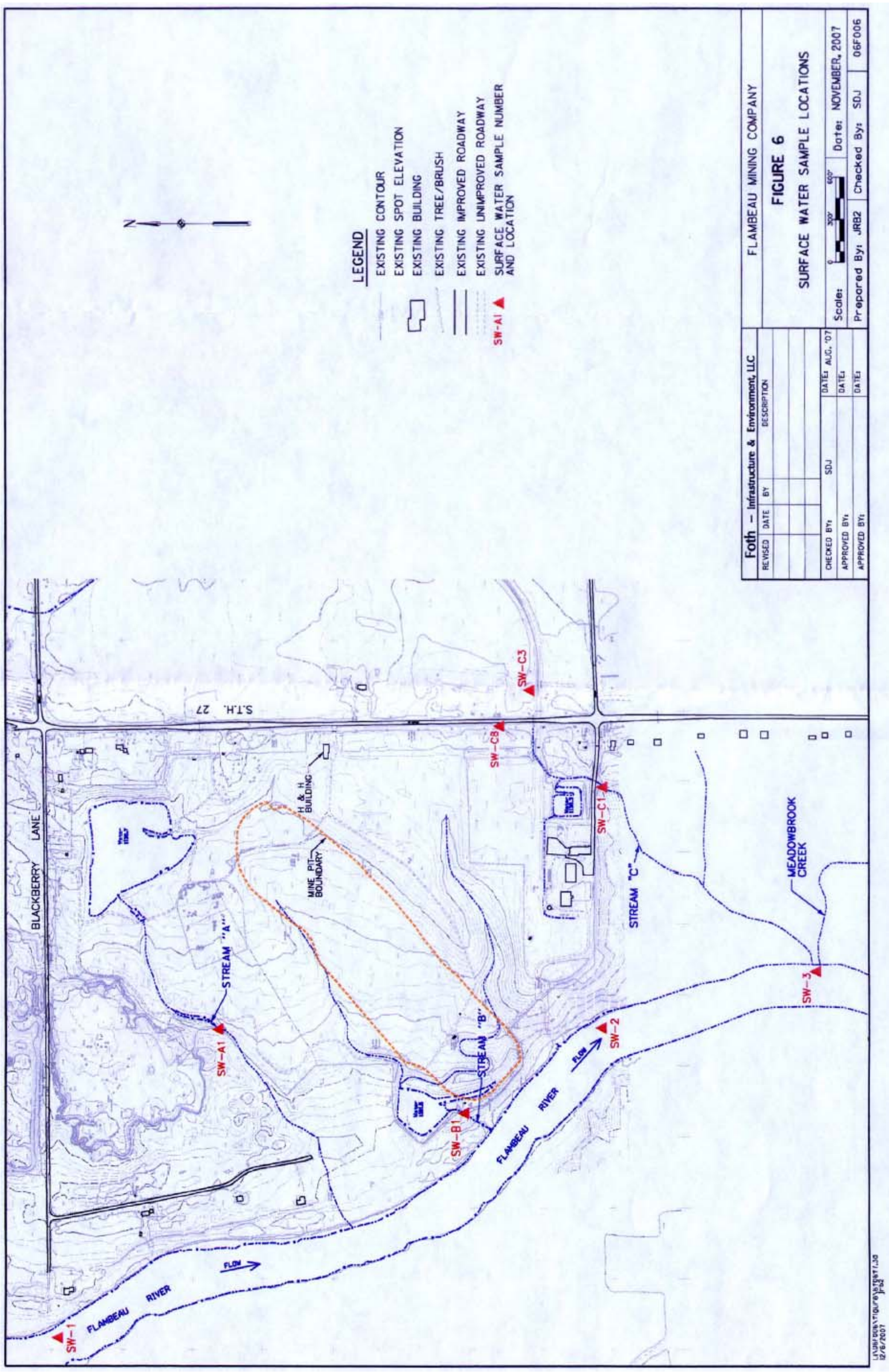
Flambeau River Surface Water, Sediment and Biota Sampling Locations Used at One Time or Another between 1991 and 2007 (Source: *Flambeau Mining Company 1993 Annual Report*)



Appendix III

Surface Water Sampling Locations at the Flambeau Mine Site (2008)

(Source: *Flambeau Stipulation Monitoring Plan, December 2007*)



Appendix IV

Sediment Sampling Locations in the Flambeau River (2008)

(Source: Flambeau Stipulation Monitoring Plan, December