Flambeau River Monitoring
at the Flambeau Mine
Rusk County, Wisconsin

4. WALLEYE TISSUE MONITORING
Analysis, Comments and Recommendations

prepared for
Wisconsin Resources Protection Council

Dr. Ken Parejko
Prof. Emeritus
Biology Department,
University of Wisconsin-Stout

2501 Fifth St.
Menomonie, WI 54751
715-595-4846
April 10, 2009

INTRODUCTION

Potentially toxic substances including various metals found in the surface waters and sediments of lakes and rivers can make their way into the vertebrate community inhabiting or making use of the aquatic ecosystem. This vertebrate community includes a variety of fish species, and of especial interest to humans, the edible or recreationally-important fish such as walleye. Most of us are familiar with warnings about consuming fish due to mercury accumulation in their bodies. Other metals can accumulate in fish as well due to natural or anthropogenic causes.

Because of the importance of fish to the riverine community and human populations, and because fish sampling is one way to measure human impacts on river ecosystems, industries located along riverways are sometimes required to monitor fish for bioaccumulation of potential toxins. 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.

In 1991-2000 and 2005-2008 FMC sampled walleye (*Sander vitreus*) on an annual basis at two different sampling sites in the Flambeau River. This was part of a broader monitoring program designed to ascertain any effects the company's Flambeau Mine might have on the biota in the river. 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.

Locations chosen for walleye analysis in the Flambeau River are shown in the map included in Appendix I. They included the Ladysmith flowage (Site F-1; about 3.8 miles upstream of the mine) and the Thornapple flowage (Site F-2; about 7.6 miles downstream of the mine). Electroshocking was utilized to collect nine walleye specimens annually at each location. Walleye in specified size ranges, with the smallest being 10-12 inches in length and the largest 22 inches or greater, were targeted for collection. Specimens were handled, processed and analyzed as follows, as described in FMC's 1991 Annual Report:

> *Fillets (with skin left on) are to be tested for total mercury. The livers of the fish collected at each of the two sampling stations are to be composited into one upstream and one downstream sample. Each is to be analyzed for the metal parameters included on the list of analytical parameters for sediments. Each organism is to be measured for total length, sexed, and the stomach contents noted. The age of each individual fish is to be determined using commonly-accepted techniques.*

The focus of the present report is on the results of the liver analyses, and to a lesser degree, the fillet analyses.

In terms of the parameters tested, the composite walleye liver samples collected between 1991 and 2006 were analyzed for a suite of trace elements including aluminum [Al], silver [Ag], arsenic [As], cadmium [Cd], chromium [Cr], copper [Cu], iron [Fe], manganese [Mn], mercury [Hg], nickel [Ni], lead [Pb], selenium [Se], and zinc [Zn]. Beginning in 2007 samples were analyzed only for Cu, Fe, Mn, and Zn. The individual walleye fillets were only analyzed for total mercury (1991 – 2006). All trace element data are presented on a wet wt. basis.

Issues concerning the collection of baseline data, the selection of sampling sites, appropriate replication and toxicity assessment are discussed below.

SAMPLING AND REPORTING ISSUES

1. Adequate baseline data for the present study is lacking. According to Table 3.8-3 of *Volume 2, Environmental Impact Report for the Kennecott Flambeau Project, April 1989*, only two walleye specimens were collected for background analysis, a 20-inch fish caught at Thornapple Dam on 8/24/88 and a 14-inch specimen caught “north of Meadowbrook” on 6/20/88. This is problematic for several reasons:
    a. Two fish cannot be considered representative of the general walleye population in the Flambeau River upstream and downstream from the mine site. To establish reliable baseline conditions, several years of background monitoring data involving larger sample sizes should have been gathered.
    b. Since both Thornapple Dam and Meadowbrook Creek are downstream from the mine site, it appears that no upstream walleye specimen was collected as part of the baseline study. In addition, “north of Meadowbrook” is not a specific enough term to truly determine the site where the second fish was caught.
    c. Metal analysis performed on the two walleye specimens did not include aluminum, iron or manganese, three metals present in measurable quantities in walleye collected in later studies.
    d. Even though a more comprehensive monitoring program for walleye was put in place in 1991-1992, by that time significant pre-mining activity had already commenced at the site (see previous reports).

2. The upstream sampling site selected for the walleye study, effective 1991, was the Ladysmith Flowage, located about 3.8 miles upstream from the mine site. The downstream sampling site at Thornapple Dam is about 7.6 miles downstream from the project area. Fish collected as far upstream and downstream as this are subject to environmental variability which may readily not be related to the mining activity.

3. Individual walleye fillets from the 18 fish collected each year were analyzed for mercury content, allowing variations among individual fish to be assessed. The same procedure, however, was not followed for walleye liver analysis, for which composited samples were used. The availability of only one composite liver sample/site/year (1991-2008) limited the ability to do statistical analyses and draw meaningful conclusions regarding the level of potential risk to walleye. This is especially true for any given year’s data. While it was possible, using data gathered over a number of years, to make statistical inferences concerning metal concentrations in walleye livers, without in-year replication, this is not possible

for any given year. E.g. in 1995 copper concentrations in liver tissue from walleyes collected upstream (Ladysmith flowage) were higher than in those from fish collected downstream at the Thornapple flowage (13 mg/kg vs. 3.6 mg/kg.) But in 1996 those differences had reversed themselves (26 mg/kg vs. 45 mg/kg.) This nearly double copper concentration downstream vs. upstream is quite striking; but without replication we can't know anything about the statistical significance of that difference. In other words, without in-year replication, we have to wait for a number of years' data to make statistical inferences about the differences observed. In the above example, copper levels measured annually in composite liver samples from downstream walleye were higher than in the upstream fish not only in 1996, but remained so for the next six years, thereby allowing one to make reliable statistical inferences *in retrospect*.

An important goal of monitoring is to provide current information about the status of an ecosystem, so management decisions can be made in a timely fashion, based on reliable statistical analyses. As it is, without in-year replication, these decisions require waiting for multi-year sampling results which only allow statements such as "Yes, there *was* a difference in parameter X between sampling sites," rather than, Yes there *is* a difference in parameter X between sampling sites."

Additional in-year replication will naturally also increase the reliability of statistical inferences when comparing data over a number of years.

4. Yet another limitation imposed by the lack of in-year replication in the FMC study design relates to toxicity assessment. As mentioned above, 9 walleye livers/site/year were composited for analysis. As a result, the variation in walleye livers among individual fish is not known. This makes it much harder to make reliable inferences, from a toxicological viewpoint, about the effects of the measured metal concentrations on individual fish. The theory behind compositing is that the concentration in a composite of fish is roughly equal to the mean for those fish had individual samples been analyzed. Compositing is often done to save money. In the present instance where 9 walleye have been collected at an upstream site and 9 at a downstream site, only 2 samples are chemically analyzed rather than 18 samples. What is lacking with a composite sample, however, is any idea of the variation that is present. For instance a mean of 20 can be arrived at with 2 different scenarios: (1) if the values for the 9 individual fish are 35, 47, 42, 20, 5, 8, 10, 6 and 7 or (2) if the values for 9 individual fish are 21, 19, 17, 22, 21, 20, 18, 19, and 23. Those 2 distributions tell us different things about the flowages they came from even though the means are identical in the two groups. There are more fish that have elevated concentrations in the first compared to the second scenario. If, for instance, there is a hazardous threshold of 25 then 1/3 of the fish are at risk in the first scenario whereas none are at risk in the second scenario. There is no way to adequately assess the toxicological risk without having data from individual fish rather than from a single composite sample.

5. The measured levels of metal concentrations in biota and sediments during monitoring are to an important degree affected by surface water metal concentrations. FMC, however, did not include surface water testing at either of the two walleye sampling sites as part of the study protocol.

6. It appears, from information provided by FMC, that the sampling sites for walleyes for 2007 were moved somewhat upstream from sampling sites used for previous years (see Appendix I and II.) Whether the sites were intentionally changed or if the labeling on the maps provided by FMC was simply not accurately done is unclear. Again, it is important to maintain across-year consistency in both sampling and reporting.

RESULTS

Table 1 shows that for walleyes, as for crayfish, for most years most metals were below detection limit. For that reason the further discussion of walleye liver metals involves only copper, zinc, iron, manganese, and aluminum which showed measurable levels.

Table 1. Number of years in which metals were below the detection limit in composite specimens, 1991-2006[1]
(n=13 years for crayfish and n=12 years for walleye)[2]

	Al	As	Ag	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
Walleye													
Ladysmith	3	11	10	2	9	0	0	4	0	8	11	7	0
Thornapple	3	11	10	7	10	0	0	4	0	8	11	8	0
Crayfish													
Blackberry Lane	0	10	11	9	8	0	NT	11	NT	9	11	11	0
Meadowbrook Creek	0	10	11	9	8	0	NT	11	NT	9	11	11	0
Port Arthur Dam	0	10	11	9	7	0	NT	10	NT	8	11	11	0

NT = Not Tested

[1] Crayfish and walleye were also tested in 2007 and 2008, but for only copper, iron, zinc and manganese, all of which were above the detection limits.
[2] Excluding studies conducted in 2007 and 2008, crayfish were sampled in 1991-2001, 2004 and 2006; walleye were sampled in 1991-2000 and 2005-2006.

Concentrations of copper, zinc, iron, manganese and aluminum in composite liver samples from walleye collected upstream and downstream from the mine site were plotted by year with the vertical dashed lines indicating the period of mine operation (Fig. 1-5, below). Figures 6 and 7 show mercury concentrations in individual (not composite) fillets.

Fig. 1: Walleye liver copper concentrations, ug/g, wet weight (one composite sample/site/year)

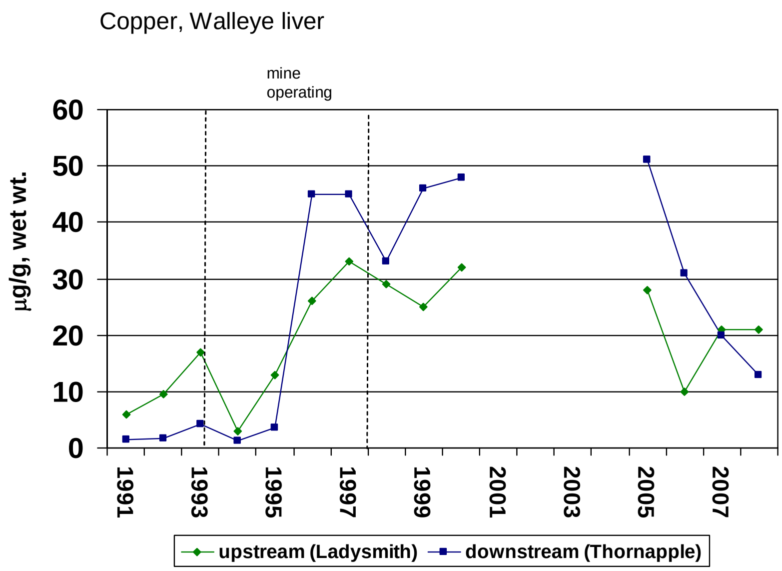


Figure 1.

Fig. 2: Walleye liver zinc concentrations, ug/g, wet weight (one composite sample/site/year)

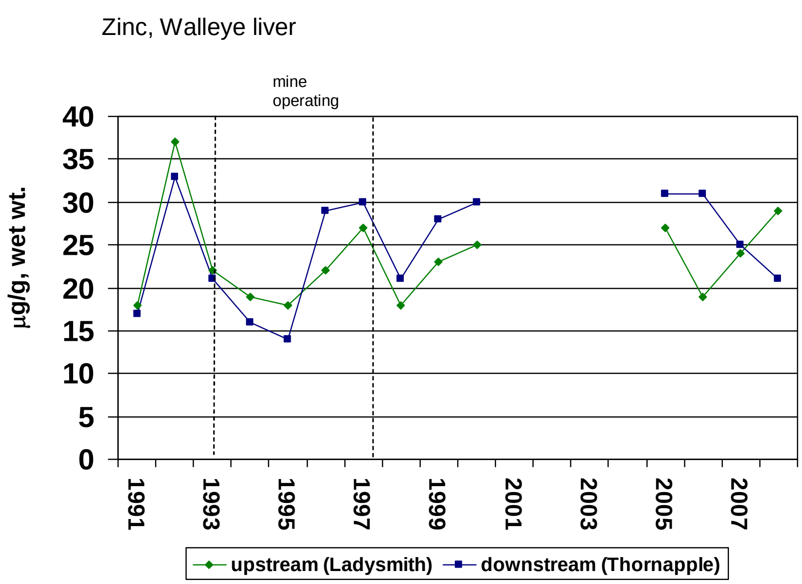


Figure 2.

Fig. 3: Walleye liver iron concentrations, ug/g, wet weight (one composite sample/site/year)

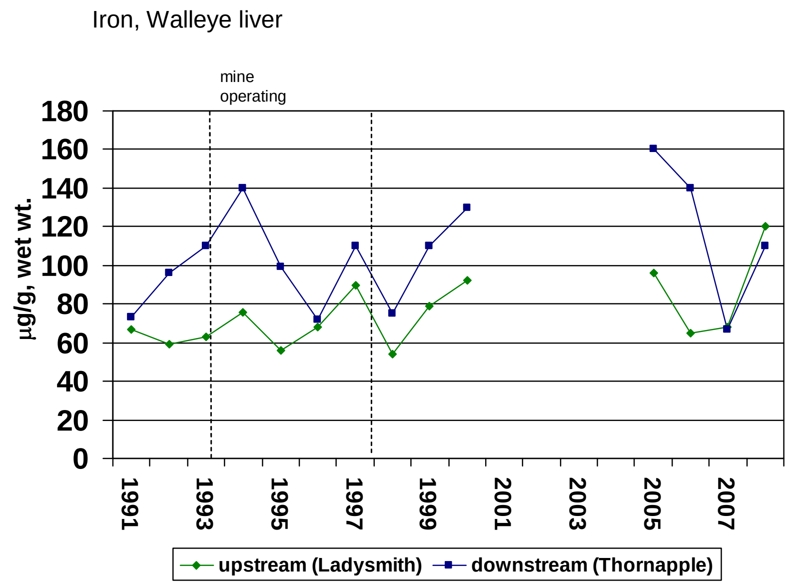


Figure 3.

Fig.4: Walleye liver manganese concentrations, ug/g, wet weight (one composite sample/site/year)

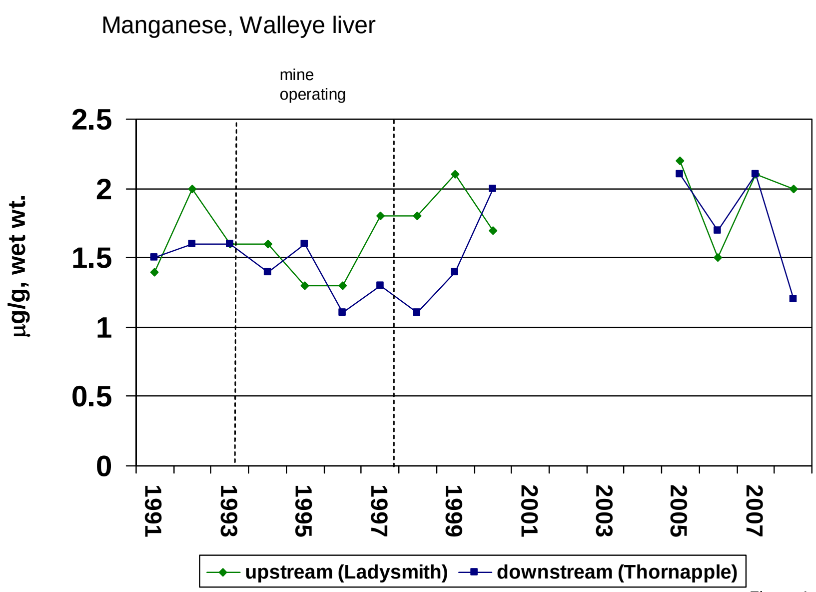


Figure 4.

Fig. 5: Walleye liver aluminum concentrations, ug/g, wet weight (one composite sample/site/year)

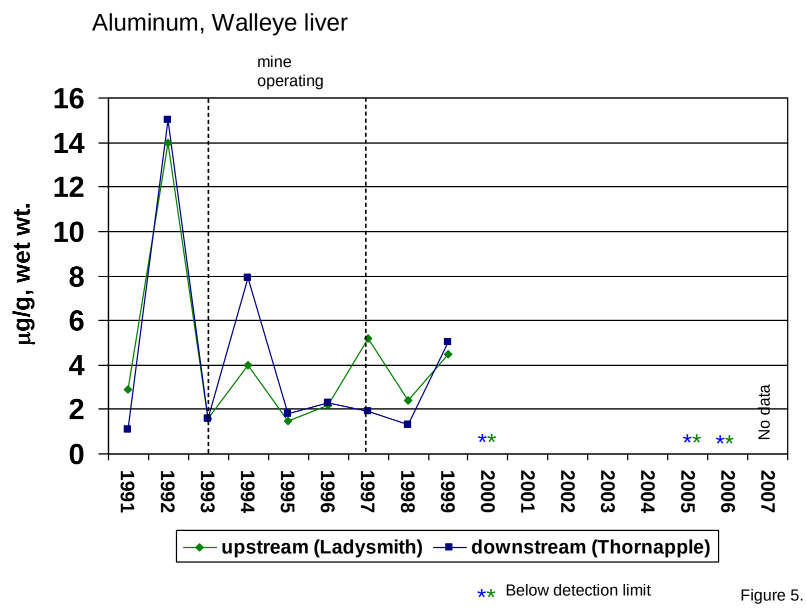


Fig. 6: Individual walleye fillet geometric mean mercury concentrations, ug/g wet wt.

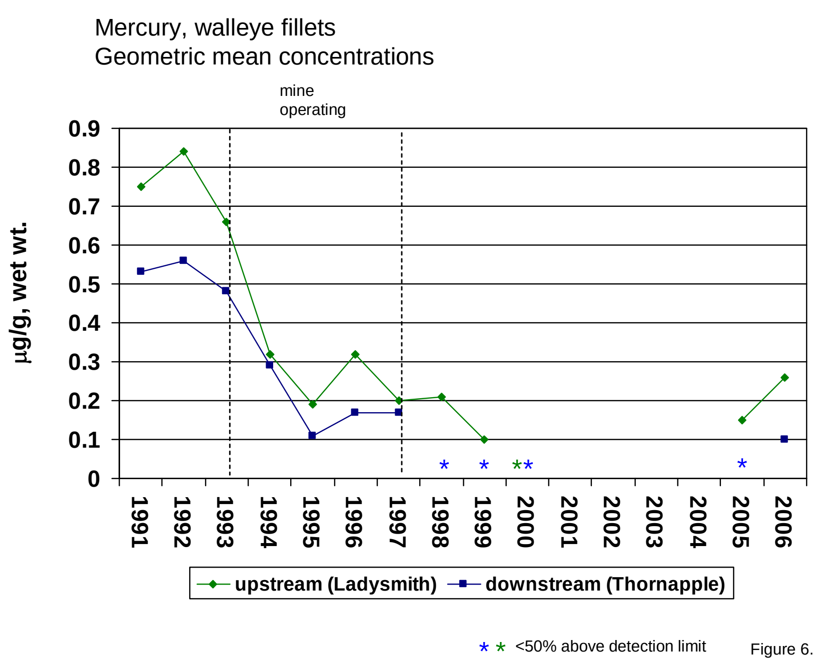


Fig. 7: Individual walleye fillet geometric mean mercury concentrations with 95% confidence intervals, ug/g wet wt.

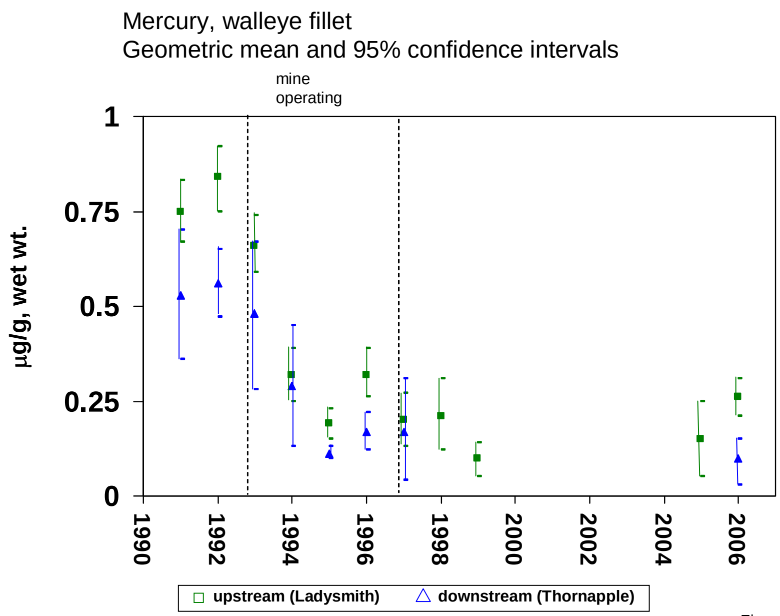


Figure 7.

DISCUSSION OF RESULTS

Based on visual inspections of the data, it appears that prior to the commencement of ore production at the Flambeau Mine in 1993 (Figure 1), copper concentrations in liver samples from walleye caught upstream from the mine site were higher than in downstream fish. A similar view was expressed by FMC's consultant in its 1996 fish sampling report: "A review of historical information (data from 1991 to 1995) suggests that relative values for copper in walleye liver from the Thornapple Flowage are fairly consistent. Walleye liver values from the Ladysmith Flowage are more variable and, in general, much higher than in the Thornapple Flowage." This trend, however, appeared to reverse during the operational phase of the Flambeau Mine, when higher copper levels began to be measured in downstream fish. This shift, first detected in walleye tested in 1996, prompted FMC's consultant to repeat the copper test done on the 1996 liver samples in an effort to confirm the results, shown in Table 2 (see *Report on Activities Associated with 1996 Fish Sampling, Appendix J, FMC 1996 Annual Report*). Similar results prompted a repeat of the same tests for 1997 (see discussion below.)

Table 2: Copper concentrations in composite walleye liver samples, ug/gm, wet weight, 1995-1997

Sampling Site	Copper Concentration in Walleye Liver (ug/gm)				
	1995	1996		1997	
	Single analysis	Original analysis (9/19/96)	Repeat analysis (10/24/96)	Original analysis (9/16/97)	Repeat analysis (12/11/97)
Upstream (Ladysmith Fl.)	13	26	45	33	33
Downstream (Thornapple Fl.)	3.6	45	40	45	43

As shown in Table 2, the increased copper levels between 1995 and 1996 were indeed confirmed. From 1995 to 1996 walleye liver copper concentrations upstream from the mine increased on the order of 2 to 3-fold. Downstream, however that increase was on the order of 11 to 12-fold. (Also see Figure 1, in which the original analyses were used). Upon reviewing this and related data, FMC's consultant suggested in its 1996 fish sampling report that the 1996 copper results "be flagged as suspicious and that monitoring data for the 1997 field season be used to evaluate possible trends and/or further explain the 1996 data set."

In 1997 the upstream composite liver sample registered a copper level of 33 mg/kg, and the downstream sample registered 45 mg/kg – both similar to the 1996 results (see *Report on Activities Associated with 1997 Fish Sampling, Appendix E, FMC 1997 Annual Report*). About three months later the company re-ran the test, and the resultant values were similar to the original ones (upstream came back at 33 mg/kg and downstream 43 mg/kg). Upon reviewing the data, FMC's consultant concluded the following in its 1997 fish sampling report:

> *"A review of the historical information (data from 1991-1997) suggests that relative values for copper in walleye liver from the Thornapple Flowage and from the Ladysmith Flowage are consistent. Moreover, it is observed that year-to-year increases and decreases in concentrations of copper in the liver of walleye are comparable from the upstream flowage to the downstream flowage. [We have] reviewed other data for the Flambeau River for this time period including crayfish tissue analysis, surface water data and sediment deposition data. None of these data sets show other than consistent copper or other metals concentrations in the ecosystem for the time period of 1991 to 1997. It is concluded that the operation of the mine has had no impact on the concentrations of metals which are observed in the liver of walleye."*

In light of the data presented above, one is naturally led to question the company's conclusion that "*None of these data sets show other than consistent copper or other metals concentrations in the ecosystem for the time period of 1991 to 1997."* And while the observed trends in metal concentrations do not prove causation, neither do the data provide support for FMC's further statement that "*...the operation of the mine has* *had no impact on the concentrations of metals which are observed in the liver of walleye."*

All walleye liver studies conducted between 1996 and 2006 showed higher copper concentrations in the livers of downstream fish compared to upstream fish (see discussion following Table 3.) In 2006, copper levels in the livers of both upstream and downstream fish began to decrease toward pre-mine levels, with the latest data (2008) showing higher copper levels in the upstream fish, as was reported prior to mining.

There is no obvious trend in zinc in walleye livers, other than perhaps somewhat of an increase during mining activities, though never to concentrations exceeding values previous to mining. Iron likewise shows no clear trend, though downstream concentrations were always at or above upstream concentrations. There were no clear trends in manganese levels. For this metal, upstream concentrations tended to be higher than downstream. Aluminum concentrations varied greatly over time, and seem in general to have been decreasing.

Mercury in walleye fillets decreased over the period of testing, with some evidence of an increase between 2006 and 2007. (Analyses on walleye fillets collected in August of 1988 gave 0.24-0.26 ug/gm wet weight, somewhat below the 1991 values. Additional background data would likely help very much in explaining the trends in mercury noted.) Figure 7, showing the 95% confidence intervals for the mercury determinations of individual fish, indicates a sizable between-individual variance for the walleyes analyzed for mercury. The amount of variation also changed greatly from year to year, without any definite pattern. The fact that upstream and downstream walleye fillet mercury levels vary widely, together, suggests some non-mining-related cause.

Statistical analyses done on the walleye copper, zinc, iron, manganese, aluminum and mercury data, using Minitab – Release 15, are summarized in Table 3.

Table 3: Results of Statistical Tests for Metal Concentrations in Walleye Livers (1991-2008)

Metal	Test	Significant? (p)
Copper	Two-Way ANOVA Upstream*, Downstream**	Year p = 0.284 Site p < .001; Mean(Up) = 19.52 mg/kg, Mean(Down) = 24.59 mg/kg
Zinc	Two-Way ANOVA Upstream* vs. Downstream*	Year p = .009 Site p = .352
Iron	Two-Way ANOVA Upstream*, Downstream*	Year p = .062 Site p <.001; Mean(Up) = 75.2 mg/kg Mean(Down) = 106.6
Manganese	Two-Way ANOVA Upstream*, Downstream*	Year p = 0.147 Site p = 0.128
Aluminum (1991-2006)	Two-Way ANOVA Upstream**, Downstream**	Year p = 0.045 Site p = 0.81
Walleye fillet Mercury (1991-1997)	Two-Way ANOVA Upstream*, Downstream*	Year p = 0.001 Site p = 0.011; Mean(Up) = 0.47 ug/g, Mean(Down) = 0.33 ug/g

* Untransformed data considered normal by Minitab
** Data flagged as non-normal, normalized by Johnson Transformation

Table 3 indicates that there is a statistically significant difference ($p<.05$) in walleye liver metal concentrations upstream vs. downstream for copper and iron, with the downstream sites showing higher concentrations. Aluminum concentrations show a barely significant difference over years, but not between sites. ANOVA results suggest mercury in walleye fillets varied significantly from year to year, and were higher upstream than downstream.

Comparing the figures showing these elemental compositions in crayfish with those in walleye livers suggests similar year-to-year patterns. In particular, copper concentrations increased in both crayfish and walleye tissue compared to pre-mining levels beginning in the mid-1990s. The increase was more noticeable in walleye compared to crayfish. That is not surprising, since the walleye are likely eating some crayfish. It is also likely their other prey would mimic these swings in metal composition. These similar trends in elemental composition between crayfish and walleye suggests that the walleye sampled farther upstream represented to a degree, at least, what the biota was doing closer to the mine.

Copper, and to a lesser degree iron, seemed to be the only elements that may have been elevated because of mining, but other unknown factors common to the entire drainage were also operating. It is difficult, however, to infer true background levels of these metals in biological tissue, since there was some pre-mining activity at the mine site beginning in 1991 (see previous reports). Walleye liver analyses were done on two fish collected in 1988, both apparently downstream from the mine site. Copper concentrations in these analyses varied from 2-5 ug/gm, and zinc 6-13 ug/gm, similar to results from 1991, but iron was not tested at all. With such a limited sample size, no upstream specimens, and a limited test panel, reliable conclusions cannot be drawn with regard to baseline concentrations. Additional pre-activity background data would be very useful.

A limited literature search was made to compare copper concentrations in Flambeau River walleye with those found in other ecosystems. In an Ontario study involving northern pike, a close relative of the walleye, copper concentrations in liver tissues were approximately 11 μg/g wet wt.[1] Beginning in 1996, and continuing through 2005, Flambeau River walleye liver tissues consistently exceeded this value, in both upstream and downstream fish. Downstream concentrations, however, appeared to have increased more than the upstream concentrations. The analysis of individual rather than composite walleye liver samples would increase one's ability to infer differences, or the lack thereof, between upstream and downstream locations.

Concentrations of copper in walleye liver tissue appear to be moving downward, but in 2008 were still approximately nine times the 1991 "baseline" level in downstream fish (13 mg/kg vs. 1.5 mg) and three and a half times the "baseline" in upstream fish (21 mg/kg vs. 6.0 mg/kg.). The 2007-2008 results also, for the first time in more than ten years, provide downstream walleye liver copper concentrations which are less than those upstream. The sampling which will be done over the next few years will help determine whether this declining trend is real or not. While the wide variation and differing patterns of metal concentrations in walleye liver – and fillets – suggests that other environmental factors in the river other than those connected with mining had an important influence on these values, the data presented and the lack of replication make it impossible to conclude that FMC's activities had no effect on metal concentrations in walleye. Therefore the conclusion FMC drew in their 2006 annual report that "Based on review of the data, it is concluded that the operation of the mine, including the time window when reclamation and habitat restoration activities are being conducted, has had no impact on the concentrations of metals which are observed in the liver or tissue of walleye" is not warranted.

RECOMMENDATIONS

Because some of the suggested improvements to FMC's Flambeau River walleye 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. Though some preliminary walleye monitoring was undertaken in 1988, data collection was insufficient to draw meaningful conclusions regarding baseline metal levels in walleye tissue. "Background" data from 1991 and 1992 may have been affected by preliminary work at the mine-site already underway in 1991. Several years' true background monitoring – before any on-site human disturbance – should always be gathered, and the procedures and protocols of that background sampling should be the same as subsequent procedures.

Recommendation for similar monitoring programs in the future: *It is recommended that adequate baseline studies be completed before initiating pre-mining or mining activity, and the protocols used for these baseline studies, including sampling locations, should be continued into the period of monitoring during pre-mining, mining or post-mining activity.*

2. It may not physically be realistic to require fish sampling directly above and directly below activities such as those undertaken by the FMC along the Flambeau River. However, fish collected as far upstream as the Ladysmith Flowage (3.8 miles) and downstream as the Thornapple Flowage (7.6 miles) are subject to environmental variability which may readily not be related to the mining activity. Whether walleyes collected nearer the mine, upstream or downstream, would have elemental compositions regularly differing from those collected farther afield is difficult to say. They might, depending on random, sporadic or regular events, or they might not. However, whenever possible – and this might require choosing a different species of fish as biomonitor – samples should be collected as near to upstream and as near to downstream of the potentially impacting human activity as possible.

Recommendation for similar monitoring programs in the future: *It is recommended that sampling locations for fish species being monitored be located as near to upstream and downstream of the potentially-impacting human activity as possible.*

3. Because the majority of the data were from composited samples (one composite sample/year/location), there was no measure of variability. This lack of replication and data on variability among individual samples makes it difficult to interpret what the metal concentrations measured in liver tissue mean from a toxicological viewpoint. Although earlier sampling can not be redone at this location, having information on current levels of variability for each of the trace elements would allow for a fuller assessment of potential risk to fish. Therefore it is recommended that walleye livers be analyzed individually, especially for copper but for the other elements as well, for some portion of the monitoring period. That will provide approximately 9 replicates per location upon which to calculate variability. It would be desirable to have a measure of variability for two or more years of data given the level of inter-annual variation seen in the Hg fillet data set. All of the other caveats for sampling would need to be considered, such as collecting the same species, same size or range of sizes, same timeframe, same habitat, etc. In addition, it would be helpful for FMC to include in its reporting a current literature assessment of toxicological thresholds for metals of concern (copper, iron, zinc, manganese and aluminum), in order to facilitate interpretation of the data.

Statistical reliability of comparisons of upstream and downstream walleye liver metal concentrations would be greatly enhanced if samples were tested individually. This is yet one more reason that individual as opposed to composite testing of walleye liver specimens is recommended for at least two years of the monitoring period.

Recommendation to augment FMC's walleye monitoring program: *It is recommended that walleye livers be analyzed individually, especially for copper but for the other elements as well, for two or more years of the monitoring period. FMC should also include a current assessment of toxicological thresholds for metals of concern in its report.*

Recommendation for similar monitoring programs in the future: *It is recommended that all specimen tissues extracted for metals analysis – fillet, liver or other – be analyzed on an individual rather than composite basis, for at least some portion (two or more years) of the monitoring period, in order to establish an estimate of variation among individuals. The entity initiating mining activity should include a current assessment of toxicological thresholds for metals of concern in its report.*

4. To strengthen inferences about the possible effect of mining on the metal concentrations in Flambeau River walleye, and to clarify if the recent declining trend in copper levels in downstream fish is real or not, it is recommended the monitoring of metals in walleye liver tissue continue on a regular basis for at least 10 years. These analyses could be limited to the five elements historically present at regularly detectable levels, i.e. copper, zinc, iron, manganese and aluminum.

Recommendation to augment FMC's walleye monitoring program: *It is recommended that walleye liver tissue analysis, using protocols discussed above, continue for an additional 10 years. If significant changes are detected during the expanded monitoring period, an additional five years sampling beyond the ten years recommended should be required. These changes could be triggered statistically (the precautionary principle suggests using* $p = 0.10$*) by the biotic monitoring results, or even if not exactly statistically significant, by apparent unexplained spikes in metal concentrations in the walleye liver tissue.*

5. The measured level of metal concentrations in biota and sediments during monitoring is to an important degree affected by surface water metal concentrations. In case continued monitoring of walleye discloses unforeseen changes in 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 per existing protocols.

Recommendation to augment FMC's walleye monitoring program: *Surface water monitoring of the Flambeau River should: (1) continue for as long as walleye 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 the walleye sampling sites at the Ladysmith Flowage and Thornapple Flowage. Due to concerns over* *temporal co-location, surface water sampling should be timed so that samples are collected on the same days as walleye are sampled, in addition to other scheduled dates.*

CONCLUSIONS

There was considerable among-year variation in metal concentrations in the walleye livers and fillets, which is typical for trace element concentrations in aquatic biota. Based on both visual inspection of the data and statistical analyses, there appears to have been an increase in walleye liver copper concentrations subsequent to mining, with downstream concentrations being significantly higher than upstream concentrations. This suggests a possible mining effect. The same can be said for crayfish whole-body specimens, as discussed in a separate report, although the elevation in copper levels appeared to be less pronounced in crayfish.

Iron concentrations in walleye livers were higher downstream than upstream, though this was true before mining activity began, and the trend in concentrations subsequent to the start of mining activities is not clear. Zinc, manganese, and aluminum concentrations in walleye livers do not show clear trends or between-site differences. Had the study protocol included within-year replication of liver samples instead of only one composite sample per site per year, one's ability to draw statistically defensible conclusions from the study at hand would have been significantly enhanced. Walleye fillets, which were tested individually, showed highly variable within-year mercury levels. These levels were significantly higher upstream than downstream, and declined significantly over the course of the study.

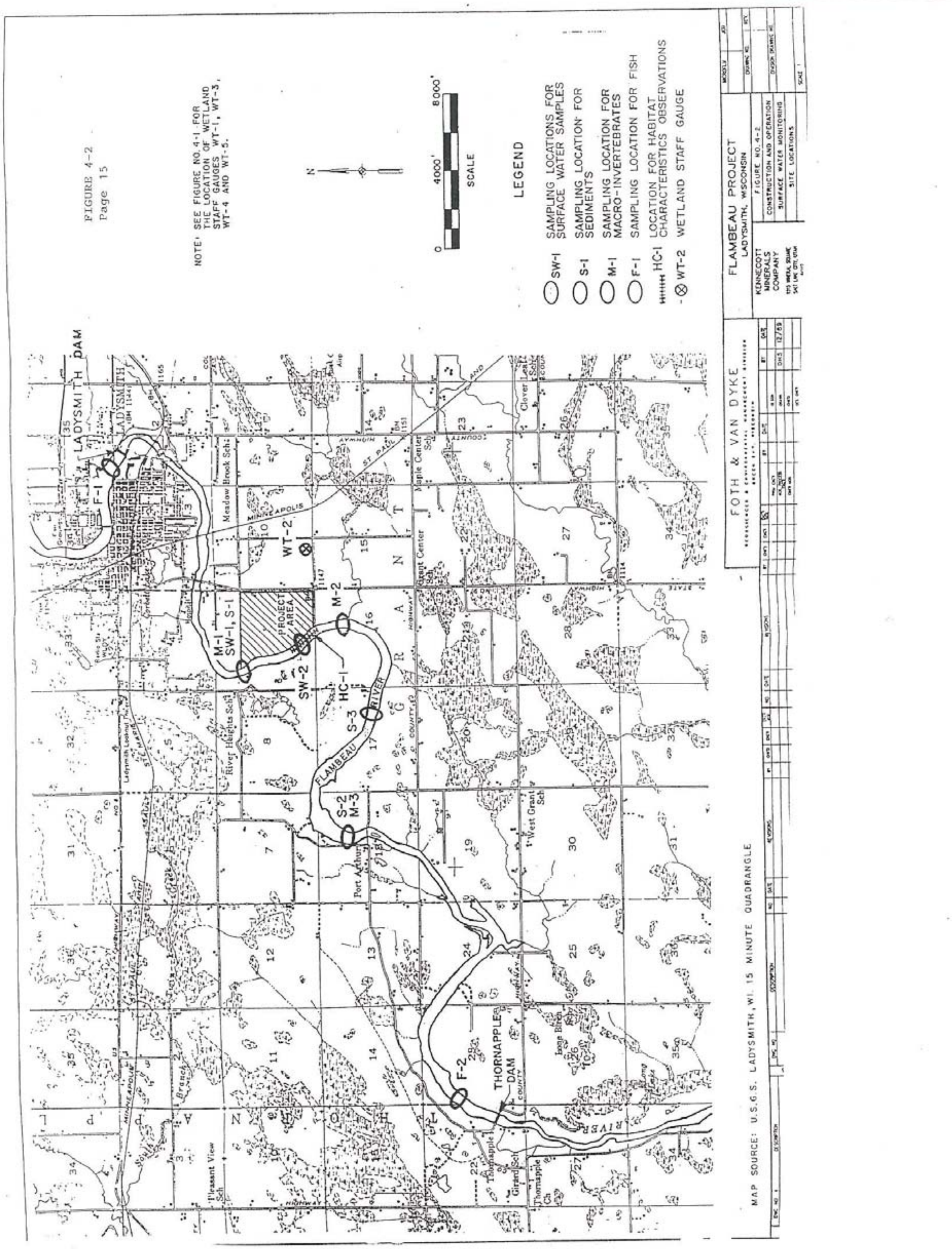
Suggested improvements in monitoring procedures would allow making stronger inferences about the effects of mining activity, if any, on walleye metal loads.

---

[1] Eisler, R. 1998. C Biol. Sci. Report USGS/BRD/BSR 1997-0002, Contaminant Hazard Reviews Report No. 33. 120 pg. Available online at http://www.pwrc.usgs.gov/

Appendix I

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 II

Flambeau River Walleye Sampling Locations (2007-2008)

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