

Flambeau River Monitoring  
at the Flambeau Mine  
Rusk County, Wisconsin

**3. CRAYFISH**  
**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  
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## INTRODUCTION

Crayfish are crustacean decapods common in Wisconsin lakes and streams. Though they show some ability to manipulate bodily loads of some metals, they can be impacted by potential toxins in both sediments and the surface waters. Crayfish are also regularly eaten by vertebrates such as fish, birds, and mammals, and as such metals or other toxins in their bodies can make their way into those organisms. Because they are common and easily-captured invertebrate inhabitants of rivers such as the Flambeau River in northern Wisconsin, crayfish have been used in studies designed to measure the impact of human activities on the riverine community. One such activity is mining.

Flambeau Mining Company (FMC), a subsidiary of Kennecott Minerals of Salt Lake City, Utah, 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 came 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. Due to the proximity of its mine to the Flambeau River, FMC was required to institute a Flambeau River monitoring program as a condition for approval of its Mine Permit.

In 1991-2001, 2004 and 2006-2008 FMC collected 25-30 crayfish (Cambaridae family) on an annual basis at each of three 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 crayfish analysis in the Flambeau River are shown in the map included in Appendix I. They included Blackberry Lane (Site M-1; about 0.7 mile upstream from the open pit site), Meadowbrook Creek (Site M-2; about 0.3 mile downstream of the project area, immediately above the creek's outfall) and Port Arthur (Site M-3; about 3.1 miles downstream). Crayfish were collected by kick-seining and pooled into a single composite whole-body sample which was chemically analyzed by companies under contract to FMC. The collection and analysis procedures appear to be appropriate in methodology and similar from year-to-year and site-to-site.

The composite samples were analyzed for a suite of trace elements (aluminum [Al], silver [Ag], arsenic [As], cadmium [Cd], chromium [Cr], copper [Cu], mercury [Hg], nickel [Ni], lead [Pb], selenium [Se], and zinc [Zn]) from 1991 to 2006. In 2007 and 2008 samples were only analyzed for copper, zinc, iron [Fe] and manganese [Mn].

Issues concerning the collection of baseline data, appropriate replication and co-location are discussed below.

## SAMPLING & REPORTING ISSUES

1. Adequate baseline data for the present study is lacking. While Table 3.8-3 of *Volume 2, Environmental Impact Report for the Kennecott Flambeau Project, April 1989*, shows whole-body metal analyses of crayfish collected in August of 1988, the sampling locations are not recorded in the table. Without knowing which results are for upstream vs. downstream specimens, it is impossible to utilize the information with any degree of confidence. In addition, the report states that "There are no significant differences in the background metals from

the two sites.” But since only two composite samples of approximately 12 crayfish each (later samples used 25-30 crayfish each) were tested, such a claim is anecdotal and not statistically defensible. It is also important to point out here that though considered “background” monitoring, results for 1991 and 1992 reported in later studies may have been affected by preliminary work on the mine site done in 1991 (see previous reports).

2. Iron and manganese were not added to the crayfish test panel until 2007. This appears to have been an oversight on the part of FMC, since both of these metals were tested in walleye and river sediment from the very beginning of the river monitoring program in 1991. Now that iron and manganese are being tested in crayfish, measurable levels have been detected. Interpretation of the data, however, is impeded by the lack of a reference point.
3. The availability of only one composite sample/site/year (1991-2008) limited the ability to do statistical analyses and draw meaningful conclusions regarding the level of potential risk to crayfish or organisms feeding on the crayfish. 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 crayfish, without in-year replication, this is not possible for any given year. E.g. in 1993 copper concentrations in crayfish collected upstream (at the Blackberry Lane site) were higher than in those collected at the Port Arthur site (15 mg/kg vs. 12 mg/kg.) But in 1994 those differences had reversed themselves (9.9 mg/kg vs. 18 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. 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 relates to toxicity assessment. As mentioned above, 25-30 crayfish/site/year were composited for analysis. As a result, variations among individual crayfish are 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 crayfish, or the likelihood of a predator of the crayfish consuming prey abnormally or dangerously high in certain metals. The theory behind compositing is that the concentration in a composite of crayfish is roughly equal to the mean for those crayfish had individual samples been analyzed. Compositing is often done to save money and may sometimes be necessary if individual samples do not provide enough tissue for analysis. In the present instance where say 27 crayfish have been collected at an upstream site and 27 at

a downstream site, only 2 samples are chemically analyzed rather than 18 samples (assuming 3 crayfish are needed to provide enough tissue). 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 composite samples of 3 crayfish each are 35, 47, 42, 20, 5, 8, 10, 6 and 7 or (2) if the values for the 9 composite samples 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 crayfish that have elevated concentrations in the first compared to the second scenario. If, for instance, there is a hazardous threshold of 25 then more of the crayfish (or their potential predators) are at risk in the first scenario than in the second. In general, the smaller the number of individuals in each composite sample, the less likely compositing will mask individuals exceeding potentially hazardous levels. It is difficult to adequately assess toxicological risk without having data from individual crayfish or relatively small composite samples rather than from a single large composite sample.

5. The upstream monitoring site employed for the crayfish study (Blackberry Lane) was the same as that used for surface water, sediment and macroinvertebrate sampling throughout the duration of the study at hand. Prior to 2007, however, there was no surface water or sediment testing at the downstream monitoring site at Meadowbrook Creek, and the second downstream site at Port Arthur had only limited sediment testing (1991-1993) and no surface water testing at all. 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.

The co-location problem at Flambeau was partially corrected in 2007 as a result of a negotiated agreement reached between opposing parties at a contested case hearing. As of 2007, crayfish and surface water are both being tested at the Meadowbrook Creek site, and this testing will continue on an annual basis (crayfish) and semi-annual basis (surface water) through 2011. The agreement also called for a one-time sediment study at the Meadowbrook Creek site which was conducted in 2008. The Port Arthur site, however, continues to only be monitored for crayfish.

## RESULTS

Out of the eleven trace elements tested in crayfish samples, eight were below the level of detection or quantification in the majority of years (See Table 1, which also includes walleye data to be discussed in a separate report). The number of years in which the composite samples contained detectable concentrations was similar across locations (above, at, or below the mine). Even in the years when these eight elements were detected they tended to be at or near the detection or quantification limits, for example Cd. Although some of these elements can be toxic if concentrations are high enough, these low concentrations did not warrant further analysis. Data for these undetectable, non-quantifiable or very low concentration trace elements were not considered further.

**Table 1. Number of years in which metals were below the detection limit in composite specimens, 1991-2006<sup>1</sup>**

(n=13 years for crayfish and n=12 years for walleye)<sup>2</sup>

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

NT = Not Tested

<sup>1</sup> 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.

<sup>2</sup> 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.

Two of the metals tested were consistently detected in whole body crayfish samples between 1991 and 2008. Those two elements were copper and zinc. In addition, aluminum was consistently detected between 1991 and 2006, although this metal is no longer being monitored.

In 2007 FMC reduced the number of metals being monitored in crayfish from 11 to 4. Copper and zinc remained on the test panel, the remaining metals originally monitored were eliminated, and two new metals (iron and manganese) were added. In 2007 and 2008, all four metals on the test panel were detected in measureable quantities.

Copper, aluminum and zinc concentrations in the composite samples were plotted by year with the vertical dashed lines indicating the period of mine operation (Fig. 1 - 3). In terms of baseline data, note that copper was found at 16 and 20 ug/gm dry wt. in crayfish whole-body samples taken in 1988, but which result is upstream, which downstream is not clear from Table 3.8-3 of *Volume 2, Environmental Impact Report for the Kennecott Flambeau Project, April 1989*. Zinc is recorded in that document as 23 and 29 ug/gm dry wt., again being unclear which value is upstream vs. downstream. Note however that these values for copper and zinc are similar to those found in 1991.

Iron and manganese graphs were not constructed due to the fact that only two data points were available (2007 and 2008), not enough to establish any sort of trend.

**Fig. 1: Crayfish whole-body copper concentration, ug/g wet wt.**

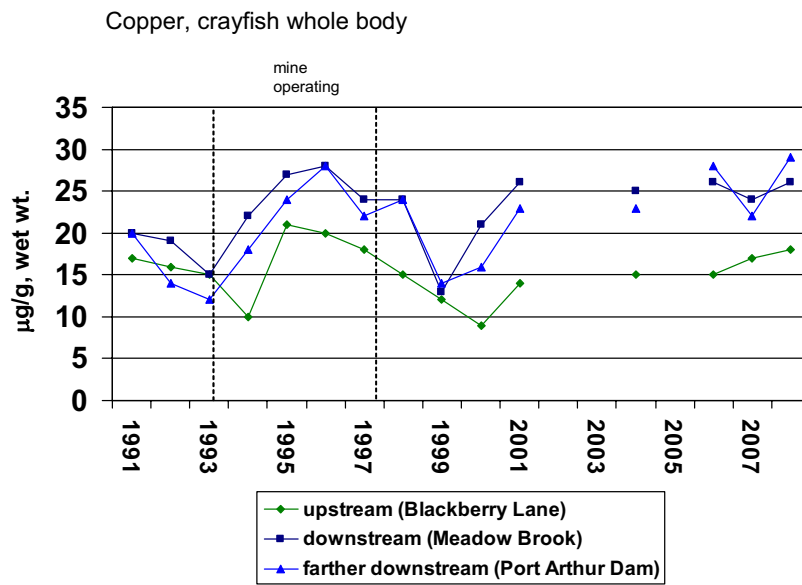


Figure 1.

**Fig. 2: Crayfish whole-body aluminum concentration, ug/g wet wt.**

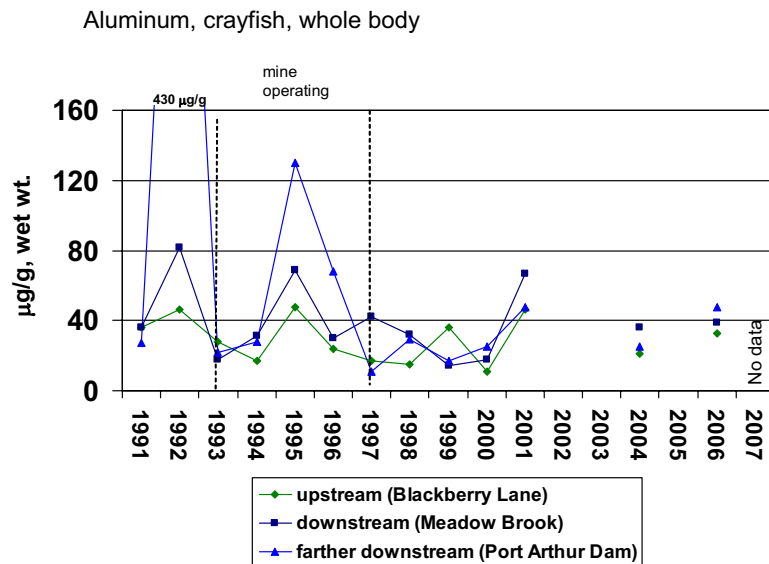


Figure 2.

**Fig. 3: Crayfish whole-body zinc concentration, ug/g wet wt.**

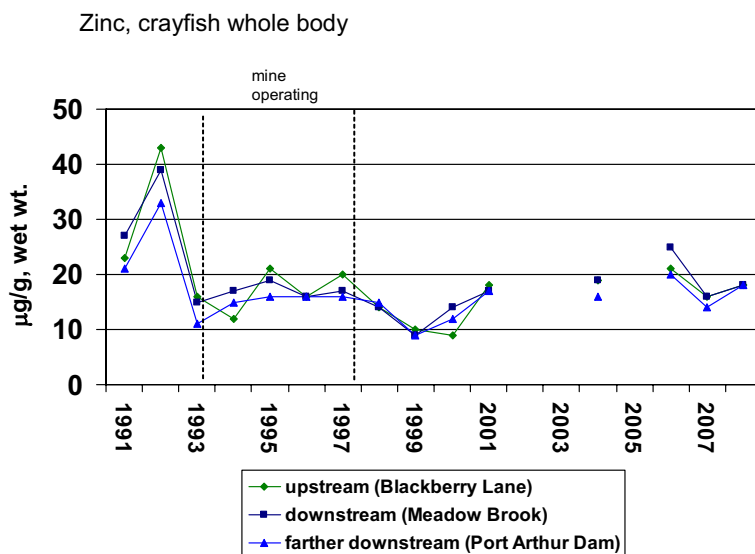


Figure 3.

## DISCUSSION OF RESULTS

Figures 1-3 suggest that there is considerable year-to-year variability in the elemental concentrations in the whole-body crayfish. That variability, however, seems to track across locations, i.e. if a year had higher or lower concentrations then those concentrations tended to be higher or lower at all three locations. Zinc, for instance, was elevated in 1992 compared to 1991 or 1993 and it was elevated at all three locations.

Crayfish whole-body copper appears to have been consistently higher at both downstream locations even prior to mining and to have risen at all three locations (including the upstream sampling site) during the mine operation. Concentrations fell off after the shutdown of the mine, but appear to have risen again, at all locations, around the year 2000. Due to a paucity of data it is difficult to make any statements about trends in these concentrations since 2005. Figure 1 seems to almost suggest a kind of cycle to copper concentrations. Though they are slightly higher downstream from the mine, the fact that crayfish sampled above the mine show a similar rising and falling pattern suggests some other factor is driving these cycles.

Table 2 shows the differences in the course of time between the upstream site and the two downstream sites, in crayfish copper concentrations. A one-sample t-test of the hypothesis that the differences between those sites (M-2 minus M-1 and M-3 minus M-1) are equal to zero resulted in  $p < .001$  in both cases. In other words, there is less than a 0.1% chance that there was no difference between the sampling sites. Linear regression analysis of the differences (M-2 minus M-1 and M-3 minus M-1) resulted in positive slopes indicating increasing differences in crayfish copper levels, at  $p = .07$  for Meadowbrook Creek (M-2) and  $p = .02$  at the Port Arthur Site (M-3). This indicates that the gap between upstream and downstream copper concentrations appears to have increased during operation of the mine, and has been sustained in the post-mining years

with significantly higher copper levels reported in the downstream crayfish. This suggests a possible mining effect.

**Table 2: Differences in crayfish total body copper levels between upstream and downstream sites in the Flambeau River, 1991-2008**

Year	Crayfish Total Body Copper Levels (mg/kg) <sup>1</sup>				
	Sampling Site			Difference between Downstream and Upstream Values	
	Upstream at Blackberry Lane (M-1)	Downstream at Meadowbrook Creek (M-2)	Downstream at Port Arthur (M-3)	(M-2) - (M-1)	(M-3) - (M-1)
1991 (“Baseline”)	17	20	20	+3	+3
1992 (“Baseline”)	16	19	14	+3	-2
1993	15	15	12	0	-3
1994	9.9	22	18	+12.1	+8.1
1995	21	27	24	+6	+3
1996	20	28	28	+8	+8
1997	18	24	22	+6	+4
1998	15	24	24	+9	+9
1999	12	13	14	+1	+2
2000	8.8	21	16	+12.2	+7.2
2001	14	26	23	+12	+9
2004	15	25	23	+10	+8
2006	15	26	28	+11	+13
2007	17	24	22	+7	+5
2008	18	26	29	+8	+11

<sup>1</sup> Data obtained from: *FMC 2006 Annual Report - Appendix C*; *FMC 2007 Annual Report - Appendix D*; and *FMC 2008 Annual Report - Appendix C*.

The 1992 crayfish aluminum result at Port Arthur (430 ug/gm) appears to be an outlier. Since aluminum is used as a biomarker of sediment ingestion when GI tracts of animals are analyzed, the highly variable aluminum concentrations in crayfish may result from more or less sediment being collected and analyzed with the crayfish whole bodies. There is no clear pattern to the crayfish whole-body aluminum analyses, other than that they do seem to rise and fall together at the three sites, with generally somewhat higher concentrations downstream from the mine. These higher downstream concentrations, however, appear to also occur before the mine began operating.

Crayfish zinc concentrations at upstream and downstream sampling sites appear to be highest before the period of mine operation, and track one another closely across the three sites.

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

**Table 3: Results of statistical tests for crayfish elemental analyses for years in which there is data, 1991-2008.**

<b>Metal</b>	<b>Test</b>	<b>Significance</b>
<b>Copper</b>	Two-Way Anova*	Site & Year $p < .001$
	Two-sample t-test, Blackb.L. vs. Meadow.Cr.	$p < .001$ ; Mean(Bl.) = 15.45 mg/kg Mean(MbCr.) = 22.67 mg/kg
	Two-sample t-test, Blackb. L. vs. Port Arthur	$p = .002$ ; Mean (Bl.) = 15.45 mg/kg Mean (PA) = 21.13 mg/kg
<b>Zinc</b>	Two-way Anova**	Site $p = 0.567$ , Year $p < .001$
	Two-sample t-test** Blackb.L.vs. Meadow.Cr.	$p = 0.772$
	Two-sample t-test** Blackb.L.vs. Port Arthur	$p = 0.749$
<b>Aluminum</b>	Two-way Anova**; value of 430 for PA dam left in.	Site $p = 0.149$ , Year $p = .001$
	Two-sample t-test* Blackb. Ln. vs. Meadow. Cr.	$p = 0.140$
	Two-way Anova**, value of 430 changed to 40 = mean of Port Arthur w/o that value	Site $p < 0.001$ , Year $p = 0.026$
	Two-sample t-test**, Blackb.L. vs. PA dam, value of 430 left or changed to 40	$p < .001$ ; Mean(Bl) = 29.08 mg/kg, Mean(PA) = 39.85 mg/kg

\* Untransformed data considered normal by Minitab

\*\* Non-normal data normalized by Johnson Transformation

Table 3 indicates that zinc concentrations changed significantly over the years, but inter-site difference was not significant at  $p = .05$ .

Aluminum concentrations also varied significantly from year to year. Mean aluminum in crayfish collected at Port Arthur was significantly greater than Blackberry Lane when considering the entire period of sampling, whether the apparent outlier of 430 mg/kg was left in, or changed to the mean value for that site, when the 430 value is removed.

Table 3 also indicates that copper concentrations in the crayfish changed significantly over the years of testing, and specimens collected at the two sampling sites located downstream from the mine had significantly higher levels of copper than the upstream crayfish (also see Table 2, above). While it is not possible to prove a mining effect on crayfish copper concentrations, the FMC 2006 annual report statement that: "Based on all data collected, including that which was collected in 2006, there are no impacts to crayfish relative to metal uptake whether we are looking at upstream/downstream effects or effects due to time (active mining phase, mine site reclamation, or post-reclamation" should be considered over-reaching.

A brief survey of the literature suggests that absorption and release of metal ions by crayfish and related organisms is both metal and species-specific. Whole-body metal concentrations in crayfish and other aquatic species often do track ambient (sediment or

water-column) concentrations, but there is also evidence the individual organism can regulate some metal ions under broad ranges of exposure.<sup>1, 2, 3, 4</sup> Toxicity caused by the metals monitored by FMC is complicated by water hardness, disturbance of the sediments, the sensitivity of individual organisms and their varying ability to dump (depurate) excess ions, etc. Though it is unlikely metal concentrations found in the bodies of crayfish in the Flambeau River reached toxic or physiologically stressful levels, or levels which might endanger predators consuming the crayfish, the use of composite samples without proper replication prevents us from making that conclusion with a statistical level of confidence otherwise attainable.

## RECOMMENDATIONS

Because some of the suggested improvements to FMC's Flambeau River crayfish 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 crayfish monitoring was undertaken by FMC in 1988, ambiguous recording made the results uninterpretable. In addition, "background" data from 1991 and 1992 may have been affected by preliminary work at the mine site already underway in 1991 (see previous reports).

***Recommendation for similar monitoring programs in the future:*** *It is recommended that several years' true background monitoring be gathered before initiating pre-mining or mining activity and that care be taken to avoid ambiguous recording of data. It is also recommended that the protocols used for these baseline studies, including sampling locations, remain constant during the pre-mining, mining and post-mining period.*

2. Iron and manganese were not added to the crayfish test panel until 2007. As a result, interpretation of current test results showing measureable concentrations of these metals in crayfish specimens has been impeded.

***Recommendation for similar monitoring programs in the future:*** *Test panels should be thoroughly reviewed at the onset of any monitoring program such as that undertaken by FMC so that important data sets are not overlooked.*

3. The availability of only one composite sample/site/year limited the ability to do statistical analysis and draw meaningful conclusions on a timely basis for a given year. It was only after a number of years' data was collected that it became possible to make statistically-significant inferences visavis metal concentrations in the crayfish.

An additional problem with composite samples is that they mask any individual organisms which might, because of their particular physiology, microhabitats, or diet have accumulated metals to potentially toxic or otherwise harmful levels. The

uncertainties around potential hazards to individual crayfish from copper in the Flambeau River are succinctly summarized in a December 12, 2001 memo written by Elisabeth Harrahy, an environmental toxicologist with the Wisconsin Department of Natural Resources (DNR). In that memo Ms. Harrahy, in an analysis of metals in Flambeau River crayfish reported by FMC up to that date, states that “Without more in-depth monitoring, it is difficult to draw any conclusions on the effects of this Cu on these crayfish.”

***Recommendation to augment FMC’s crayfish monitoring program:*** *To allow more timely management decisions to be made, it is recommended that the total composite crayfish sample be divided into replicate subsamples of say 5 each, and analyses done on these subsamples. If only 2 or 3 crayfish provide enough tissue for the analyses, then smaller composites should be used, the principle being to provide as many subsamples per site/year as possible, to improve the ability to do statistical analyses comparing sites and years. In addition, FMC should include in its report a current literature assessment of toxicological thresholds for the metals being monitored in order to facilitate interpretation of the data.*

***Recommendation for similar monitoring programs in the future:*** *Crayfish or other chosen macroinvertebrates sampled for metal analyses should be done in such a way as to provide as many subsamples per site as possible, and include a current literature assessment of toxicological thresholds for the metals being monitored.*

4. To strengthen inferences about the possible effect of mining on the metal concentrations in Flambeau River invertebrates, it is recommended the monitoring of crayfish metals continue on a regular basis for at least 10 years. These analyses could be limited to only the five elements historically present at regularly detectable levels, i.e. zinc, aluminum, manganese, iron and copper.

The need for continued monitoring of the crayfish receives further support from a statement in the memo mentioned in #3 above, written by the Wisconsin DNR’s Elisabeth Harrahy. In that letter Ms. Harrahy states: “However, because metals are expected to continue moving from the mine pit to the river, and because metals can build up in sediments over time and bioaccumulate in organisms (with potential for cascading up the food chain), continued monitoring could yield much important information.”

***Recommendation to augment FMC’s crayfish monitoring program:*** *It is recommended that crayfish 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 crayfish.*

5. Chemical analyses of macroinvertebrates in addition to crayfish were not done by FMC. As mentioned in the macroinvertebrate report, because of the ability of macroinvertebrates to bioaccumulate metals, regular analysis of a select set of macroinvertebrates (in addition to crayfish) for total body metal concentrations could

provide much useful information with regard to tracking potential toxins that might be making their way into the higher food chain.

***Recommendation for similar monitoring programs in the future:*** *It is recommended that monitoring programs such as those undertaken by FMC include whole-body elemental analyses of invertebrates in addition to crayfish – e.g. mayflies, stoneflies, mussels, etc.*

6. Inferences regarding the possible effects of human activities on river or stream ecosystems are strengthened when sampling sites for specimens such as crayfish, macroinvertebrates, sediment and surface water are spatially and temporally co-located. In particular, 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 crayfish by FMC 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 crayfish monitoring program:*** *Surface water monitoring of the Flambeau River should: (1) continue for as long as crayfish 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 crayfish sampling site at Port Arthur. Due to concerns over temporal co-location, surface water sampling should be timed so that samples are collected on the same days as crayfish are sampled, in addition to other scheduled dates.*

***Recommendation for similar monitoring programs in the future:*** *Whenever possible, the various studies (e.g., metals analyses of crayfish, walleye, sediment and surface waters and/or biota surveys) implemented by an industry or agency to assess potential impacts of human activity on the riverine community should be spatially and temporally co-located.*

## CONCLUSIONS

Copper was the element of interest that showed the clearest pattern during the period of monitoring crayfish whole-body metal concentrations. While levels of copper in the crayfish showed an overall increase both upstream and downstream from the mining activity, it was significantly higher at both downstream sites than upstream, and the gap between downstream and upstream sites widened over time, suggesting a possible mine effect. Copper levels did not appear to reach toxic or otherwise harmful levels in this organism during the time period in question (1991-2008), although one's confidence in that inference is lessened by the monitoring protocols used. Monitoring should continue

and procedures be improved to strengthen any inferences made regarding the effect, if any, of mining activities on the benthic invertebrates such as crayfish.

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<sup>1</sup> Bryan, G.W. (1967) *J. Exptl. Biol.*;46(2):281-96

<sup>2</sup> Guner, U. (2007) *Environ Monit Assess* 133:365–369

<sup>3</sup> López FJ, et al (2004) *Environ Monit Assess.* Apr-May;93 (1-3):17-29.

<sup>4</sup> Bagatto, G. and Alikhan, M.A. (1987), *Bull. Environ. Contain. Toxicol.* 38:1076-1081.

## Appendix I

### Crayfish Sampling Locations in the Flambeau River (1991-2008)

(Source: Flambeau Stipulation Monitoring Plan, August 2007)

