30,222

Evaluation of copper concentrations in crayfish (Pacifastacus trowbridgi) from various segments of the Clark Fork River drainage, Montana

Prepared by:

Glenn Phillips
Montana Department of Fish, Wildlife and Parks
Capitol Station
Helena, MT 59620

Funding Source:

Champion International Corporation
Frenchtown Mill
Drawer D
Missoula, MT 59806

INTRODUCTION

During the evaluation of Champion International Corporation's request for a year around discharge permit, it was argued that oxygen demanding materials could cause reducing conditions to develop at the sediment-water interface which in turn could increase the mobility and subsequent bioavailability of metals present in the sediments. It was further argued that these conditions might exist only in or on the surface of the substrate and would therefore not be apparent from measurements of dissolved oxygen in the water column.

To evaluate the above, we collected pacific crayfish (<u>Pacifastacus trowbridgi</u>) from several locations in the Clark Fork River drainage including locations both upstream of and downstream from the pulp mill; copper concentrations were determined in selected tissues including exoskeleton, hepatopancreas, and gill.

We reasoned that crayfish were an appropriate subject for this evaluation because they have a relatively small home range (hence migration is not a problem) and they tend to inhabit areas of low current velocity where deposition of both organic material and metals rich suspended solids would be expected.

Copper was chosen over other metals because previous work (Phillips 985) has demonstrated that copper is present in the Clark Fork River at higher concentrations than other metals relative to its toxicity. Data collected in the Clark Fork River upstream of its confluence with Rock Creek suggest the copper is limiting fish populations in that portion of the river.

METHODS

Sampling

Crayfish were collected from several locations in the mainstem of the Clark Fork River and from one location in the Bitterroot River (Fig. 1); the latter served as a control. Collection occurred from September 10-14, 1984. Sampling sites included downstream of Milltown Dam, downstream of Missoula but upstream of the confluence with the Bitterroot River, downstream of the pulp mill outfall, near Superior, downstream of the confluence with the Flathead River, downstream of Thompson Falls Dam, and downstream of Noxon Dam. Late summer sampling was preferred because we wanted to sample crayfish following the season of higher water temperatures and lower dissolved oxygen concentrations and after the first year of summer discharge by the Champion International Mill. We also wanted to collect crayfish well after their spring molting but prior to the time when they molt in the fall.

Crayfish were captured in baited traps constructed with two inch funnel openings at each end. Traps were submerged in likely crayfish habitat, fished overnight, and retrieved the following day. Sample sizes ranged from seven to ten crayfish per site. So as not to introduce bias, we discarded crayfish that had just completed molting.

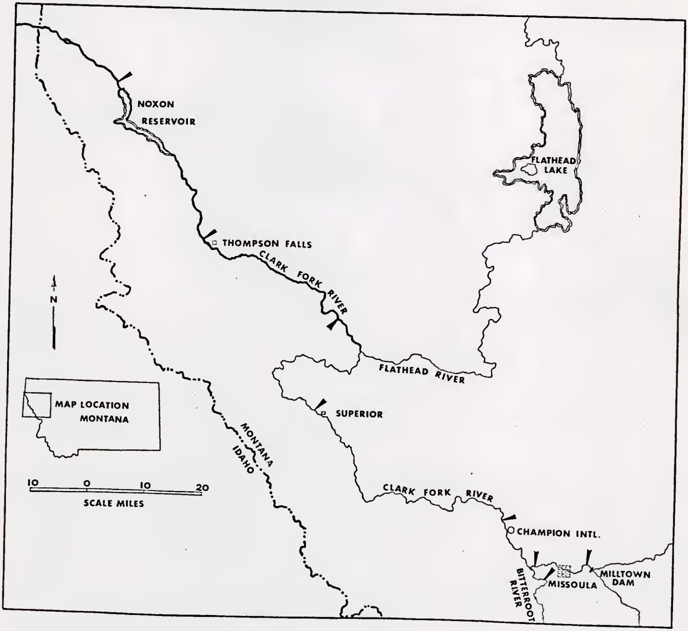

Figure 1. Map of the Clark Fork River showing locations where crayfish were sampled (indicated by arrows).

Analytical

Tissues analyzed for copper included exoskeleton, hepatopancreas and gill. A few samples were also analyzed for cadmium; however, accuracy checks using National Bureau of Standards bovine liver indicated that the level of error was unacceptable; cadmium was therefore dropped from further consideration.

Tissues were dissected, freeze dried, ground, homogenized and digested in nitric acid prior to analysis by atomic absorption spectrophotometry (Van Meter 1974). The instrument used was a Varian AA 275 BD unit and the detection limit for copper was approximately 0.7 ug/g. Analytical work was contracted to the Gordon Environmental Studies Laboratory, University of Montana.

Quality control measures included recovery of spiked samples, analysis of National Bureau of Standards reference materials, and analysis of duplicate samples (both from separate digestions of the same tissue and replicate analysis of material from the same digestion). The poorest result for any of the quality control measures for copper in tissue was 18% variation between duplicate digestions of one of the reference materials. In the majority of cases, however, checks on accuracy indicated that the error was inside of 10%. We judged, therefore, that the results for copper were acceptable.

Statistics

Duncan's multiple range test was used to compare sample means for both crayfish length and copper in exoskeleton for each of the various locations (Steel and Torrie 1960). Linear regression analysis was employed to determine, at a given location, the relationship between copper in exoskeleton and crayfish length.

RESULTS

From two of the locations, below Noxon and Milltown Dams, copper was determined in gill and hepatopancreas as well as in exoskeleton (Table 1). Copper concentrations were more than an order of magnitude higher in gill and hepatopancreas than in exoskeleton. These high concentrations are probably owing to the fact that copper is a normal constituent in the hemolymph of crayfish, playing a role similar to iron in mammalian hemoglobin. Copper may be internally regulated by highly vascularized organs. Consequently, gill and hepatopancreas of crayfish may not be appropriate tissues for gauging ambient copper concentrations in the environment. Exoskeleton was judged to be the tissue that best fit the needs of this assessment.

Crayfish collected below Milltown Dam were significantly smaller ($p < 0.05$) than those collected from several other locations including near Superior, downstream of the pulp mill, and below the confluence with the Flathead River (Table 2). We were concerned that size differences could bias comparisons between locations because many bioaccumulative chemicals tend to increase in concentration with size and age of the organisms.

Accordingly, we evaluated the relationship between crayfish length and copper in exoskeleton for each of the sampling locations using linear regression analysis (Table 3).

In all instances the correlation between copper in exoskeleton and crayfish length was poor; at five of the eight locations the coefficient of determination

Table 1. A comparison of copper concentrations (dry weight) in various tissues of crayfish collected from the Clark Fork River.

Location	Tissue	n	Copper ug/g (dry basis) Mean	Range	SD
Clark Fork, below Noxon Dam	Gill	5	424	284-560	115
	Hepatopancreas	5	495	214-734	201
	Exoskeleton	10	26	12-53	14
Clark Fork, below Milltown Dam	Gill	5	388	351-421	25
	Hepatopancreas	5	484	372-652	85
	Exoskeleton	10	34	23-46	8

Table 2. Lengths of crayfish collected from the Clark Fork drainage, September 10-14, 1984.

Location and (sample size)	Crayfish total length (in.)		Grouping[a]
	Mean ± SD	Range	
Clark Fork, near Superior (9)	4.3 ± 0.4	3.6-4.9	┐
Clark Fork, below Champion (10)	4.2 ± 0.3	3.8-4.7	│
Clark Fork, below Flathead River (7)	4.2 ± 0.5	3.6-4.8	│
Bitterroot River, control (10)	4.1 ± 0.3	3.5-4.4	┐ │
Clark Fork River, near Missoula (8)	4.0 ± 0.5	3.5-4.9	│ │
Clark Fork, below Noxon (8)	4.0 ± 0.6	2.8-4.7	│ │
Clark Fork, below Thompson Falls (8)	3.8 ± 0.6	3.0-4.9	│ ┘
Clark Fork, below Milltown (10)	3.6 ± 0.5	3.1-4.5	┘

---

[a] Means within the same line are not significantly different from each other (95% confidence level) according to Duncan's Multiple Range Test.

Table 3. Regression equations and coefficients of determination showing the poor correlation between crayfish length and copper in exoskeleton for several locations in the Clark Fork River drainage.

Location and (sample size)	Regression (Copper = a length -b)	Coefficient of determination (r^2)
Bitterroot River - control (10)	$y = -22x+104$	0.47
Clark Fork, near Missoula (10)	$y = -4x+40$	0.08
Clark Fork, near Superior (10)	$y = 10x-21$	0.10
Clark Fork, below Flathead River (8)	$y = -31x+173$	0.36
Clark Fork, below Milltown Dam (10)	$y = -3x+46$	0.04
Clark Fork, below Champion (10)	$y = -3x+35$	0.03
Clark Fork, below Thompson Falls (9)	$y = 6x+18$	0.02
Clark Fork, below Noxon Dam (8)	$y = -15x+86$	0.45

was 0.10 or less. We therefore conclude that differences in lengths of crayfish collected from the various locations did not bias our comparison between sites of copper in exoskeletons.

Crayfish from the Bitterroot River, the control station, had the lowest copper concentrations in exoskeleton of all the locations sampled (Table 4). They were, however, statistically different ($p < 0.05$) only from the three locations where crayfish contained the highest concentrations of copper. Diffuse tailings deposits located throughout the upper Clark Fork watershed frequently cause high copper concentrations in Clark Fork River water, particularly during periods of high river discharge (Phillips 1985). Exceedences of criteria for the protection of freshwater aquatic life have been documented in the Clark Fork River as far downstream as Thompson Falls (Water Quality Bureau, unpublished data). Comparatively, copper is seldom present in Bitterroot River Water at concentrations above the analytical detection limit for copper (Water Quality Bureau, unpublished data). Lower copper concentrations in crayfish from the Bitterroot River compared to the Clark Fork River is consistent with water quality data.

Highest copper concentrations were in crayfish collected downstream of: (1) Milltown Dam, (2) the confluence with the Flathead River, and (3) Thompson Falls Dam. In the case of the latter two locations, copper concentrations were statistically higher ($p < 0.05$) than from all other locations sampled (Table 4),

Higher concentrations at locations downstream of the Flathead River may be related to higher water temperatures and lower oxygen concentrations that occur over that river reach. These conditions tend to increase the solubility of

Table 4. Copper concentrations in exoskeletons of crayfish collected from the Clark Fork River drainage, September 10-14, 1984.

Location and (sample size)	Copper ug/g (dry basis)		Groupings[a]
	Mean ±SD	Range	
Bitterroot River - control (10)	16±9	9-39	⎤
Clark Fork, below Champion (10)	22±6	14-34	⎥ ⎤
Clark Fork, near Superior (9)	23±13	8-54	⎥ ⎥
Clark Fork, near Missoula (8)	24±6	14-33	⎥ ⎥
Clark Fork, below Noxon Dam (8)	26±14	12-53	⎦ ⎥
Clark Fork, below Milltown Dam (10)	34±8	23-46	⎦ ⎤
Clark Fork, below Flathead River (7)	41±25	14-72	⎥
Clark Fork, near Thompson Falls (8)	42±24	17-85	⎦

[a] Means within the same line are not significantly different from each other (95% confidence level) according to Duncan's Multiple Range Test.

metals and also the metabolic rates, and hence, exposure to metals by organisms living in water. Although higher water temperatures and low oxygen concentrations persist downstream of Noxon Reservoir, copper concentrations tend to be lower below the dam than upstream (Water Quality Bureau, unpublished data). This may explain the lower concentrations of copper in crayfish from below Noxon Dam and suggests that the reservoir is acting as a sink for copper.

Clearly, our interpretation of the data is limited by relatively small sample sizes and a somewhat cursory understanding of water quality and physical habitat parameters at each of the locations where crayfish were sampled. Nonetheless, no statistically significant differences ($p < 0.05$) were seen in crayfish collected immediately upstream from and downstream of the pulp mill. The data do not support the hypothesis that the Champion International discharge is an important factor in the mobilization of copper and its subsequent accumulation by crayfish. Silverman and Gordon (1983) were also unable to find evidence that the pulp mill affects metals accumulation by crayfish.

SUMMARY

1. Copper concentrations were much higher in the gills and hepatopancreas of crayfish than in exoskeleton. Copper is a normal constituent of the hemolymph of crayfish, playing a role similar to iron in mammalian hemoglobin. Because of this, copper is naturally elevated in highly vascularized tissues such as gill and hepatopancreas. Consequently, these tissues are probably not appropriate for this type of monitoring.

2. Crayfish collected below Milltown Dam were significantly shorter than crayfish from three downstream sampling locations (near Superior and below Champion and the Flathead River). However, crayfish length was poorly correlated with copper in exoskeleton for any given location, consequently differences in crayfish lengths between locations should not bias comparison of mean copper concentrations in exoskeleton.

3. Exoskeletons of crayfish collected below Milltown Dam contained higher copper concentrations than crayfish from all locations except below the Flathead River and near Thompson Falls. However, the difference was statistically significant only in the case of the Bitterroot. The lowest average copper concentrations were in crayfish from the Bitterroot River (the control); however, these were not significantly lower than in crayfish from other locations including below Champion and Noxon Dam, and near Missoula and Superior.

4. Copper concentrations were significantly higher in exoskeletons of crayfish collected in the Clark Fork downstream of the Flathead River and near Thompson Falls than at all other locations sampled except below Milltown Dam. Several factors may be involved. Copper concentrations in Clark Fork River water tend to gradually decrease as you move downstream from Milltown Dam. However, the Flathead River is warmer than the Clark Fork hence the Clark Fork is warmer downstream of its confluence with the Flathead. Higher water temperatures increase the solubility of metals and the metabolic rates of organisms living in water. Additionally, the Flathead River experiences lower oxygen sags than the Clark Fork, hence minimum oxygen concentrations are lower in the Clark Fork downstream of the Flathead River than upstream. Lowered oxygen concentrations increase the respiration rates or organisms living in water and thereby increase their exposure to chemicals present in water.

5. Below Noxon Dam, copper concentrations in crayfish return to the lower concentrations found in crayfish upstream from the confluence with the Flathead River. Water temperatures continue to be higher and dissolved oxygen concentrations lower downstream of Noxon Dam but Water Quality Bureau data show that copper was never present at detectable levels below Noxon. Noxon Reservoir is probably a sink for copper.

6. No statistically significant differences were seen in copper present in crayfish collected immediately upstream from and downstream of the pulp mill. The data do not support the hypothesis that the Champion International discharge is an important factor in mobilization of copper and its subsequent accumulation by crayfish.

REFERENCES

Phillips, G.R. 1985. Relationships among fish populations, metals concentrations and stream discharge in the upper Clark Fork River. Proceedings Clark Fork River Symposium, Montana Acad. Soc (in press).

Van Meter, W. P. 1974. Heavy metals concentrations in fish tissue of the upper Clark Fork River. Proj. Completion Rep., Mont. Water Resour. Res. Center, Montana State University, Bozeman. 38 p.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York, NY. 481 p.

Silverman, A.J. and C.C. Gordon. 1983. A preliminary investigation into the use of crayfish as a sensitive species to pollutants in the Clark Fork River near Missoula, Montana. Proj. Completion Rep., Mont. Wat. Resour. Res. Center, Montana State University, Bozeman. 27 p.

GP/war-5b