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Hepatic metallothionein induction in fish (White Sucker, Catostomus commersoni) from the St. Lawrence River

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ABSTRACT

White sucker (*Catostomus commersoni*), a benthic fish species, were captured by electro-fishing in the St. Lawrence River near Montreal, Quebec, and put into cages placed upstream and downstream of an industrial complex (heavy metals). After exposure of about a week, the cages were retrieved and the livers of the fish were dissected out for MT determination (silver-saturation method). Three experiments were carried out: July, August, and October, 1991. Hepatic metal content was determined on some specimens from the August experiment. Results indicated that there was a direct relationship between hepatic copper and zinc concentrations and MT content, all being higher in specimens from the upstream site. Recent data on sediments from the same sites suggest that MT induction in white sucker may be more related to sediment contamination (Cu and Zn) than to the presence of point sources.

INTRODUCTION:

The complexing protein, metallothionein (MT), is a low molecular weight polypeptide with a high cysteine content, which binds metals through clusters of thiolate bonds. Its synthesis is homeostatically regulated and can be induced by metals (groups IB and IIB of the periodic table: Cu, Ag, Au, and Zn, Cd, Hg) as well as by glucosteroids and stress conditions [1]. Its role is thought to be the metabolic regulation of the essential metals Cu and Zn, and the detoxification of the non-essential metals Cd and Hg. The potential of MT induction, especially in fish, as an indicator of heavy metal pollution in the aquatic environment has generated considerable interest, as there is a strong correlation between the contamination levels and MT concentrations in fish liver, intestine and gill [2,3,4].

A study on hepatic metallothionein in white sucker, a common benthic species in the St. Lawrence River, was carried out in 1991. Specimens were taken in the Beauharnois region of Lake St. Louis (Fig. 1). This region is known for its industrial complexes, discharging metals such as Cu, Zn, Cd, and Hg to the

St. Lawrence River. The study design was based on various hypotheses, stating among others that fish living downstream would show higher MT levels than those living upstream of the industrial complex. The results given in this paper are part of a much larger project, involving various biomarkers and blood biochemistry. The details of the complete project, regarding hypotheses, design, experimental set-up, analytical techniques, results, etc., are described by Bureau *et al.* [5].

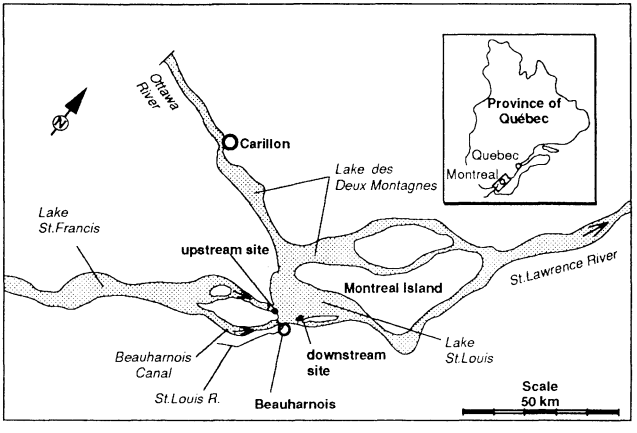


Figure 1 St. Lawrence River and study area.

MATERIALS AND METHODS

White Sucker, *Catostomus commersoni* (Lacépède), were caught by electro-fishing at one site upstream (us) and at another site downstream (ds) of the industrial complex at Beauharnois (fig 1). Conductivity, alkalinity and hardness of the ambient water were around 300 μSiemens/cm, 90 mg/L and 125 mg/L (the latter two as $CaCO_3$), respectively. Oxygen levels were at saturation values. The fish were set out in cages on the bottom at at each site (Z = 5-10 m), where 3 cages contained fish (about 8-10 individuals per cage) from the same site, while 3 other cages contained fish from the second site. The cages, of a 6 m long cylindrical fyke-net style, consisted of three metallic hoops, 1.5 m in diameter, covered with a 25 mm mesh net and held together with three metallic bars (6.5 mm ϕ).

Three experiments were carried out in 1991: exp. 1, June 27 - July 3; exp. 2, July 31 -7 August; exp. 3, September 26 - October 7. Survival of the fish during the exposure period was monitored by divers; the length exposure was partly decided by the fish conditions observed. After 6 days of exposure for experiment 1, 7 days for experiment 2 (water temperature 23 °C), and 11 days for experiment 3 (water temperature 12 °C), the cages were retrieved and the fish sacrified for liver analyses. Mortality averaged 40 % (0-75 % per cage) during the 1st experiment, and 35 % (0-50 % per cage) for the 2nd experiment. No mortality was observed during the 3rd experiment. At the time of retrieval, another set of fish (control) from each site was caught for liver exctraction on the same day. All individuals (control and experimental fish) were weighed, measured, sexed and aged; gros pathology was also noted.

Livers were immediately conserved on site in liquid nitrogen until laboratory analysis. After thawing, the tissue was homogenized, and then centrifuged at 10 000 g for 30 min at 4°C, yielding the supernatant, S10 or the postmitochondrial fraction [5]. MT concentrations in the S10 fraction were determined in duplicate using the Ag-saturation method [6], which was modified for use in our laboratory [7]. MT concentrations are expressed as nmoles.mg^{-1} protein, the latter having been determined in the S10 fraction by a colorimetric method [8]. Heavy metals (Cu, Zn, Cd, Hg) were analysed in a selected number of subsamples of the liver tissue from experiment 2 [9,10]. Metal results are expressed as μg.g^{-1} wet weight.

RESULTS AND DISCUSSION

No differences between sites, nor between control and caged fish, were observed for experiment 3 (Table 1). No explanation has yet been found, except that there may be some seasonal effect or that colder water temperatures depressed the metabolic rate. However, for experiments 1 and 2 (Fig. 2), MT induction was in general higher at the upstream (C.us and usE.us) than at the downstream site (C.ds and dsE.ds). The pattern is somewhat different with caged fish that were transferred (dsE.us and usE.ds; Fig. 2). In experiment 1, MT induction was more or less at levels intermediate of those for the upstream and downstream sites. In experiment 2 the destination site seemed to be more important than the site of origin: fish from the downstream site transferred to the upstream site (dsE.us) showed significantly higher MT levels than those transferred vice versa (usE.ds).

Table 1 Metallothionein concentrations - nmole/mg prot.

	Exp. 1		Exp. 2		Exp.3	
Treatment	N	Mean (s.d.)	N	Mean (s.d.)	N	Mean (s.d.)
C.us	13	0.58 (0.23)	8	0.97 (0.37)	10	0.66 (0.19)
C.ds	9	0.42 (0.11)	10	0.67 (0.21)	8	0.64 (0.24)
usE.us	10	0.90 (0.25)	18	0.79 (0.26)	22	0.65 (0.35)
dsE.ds	19	0.45 (0.17)	17	0.73 (0.29)	23	0.60 (0.29)
dsE.us	14	0.53 (0.16)	14	0.85 (0.31)	22	0.63 (0.31)
usE.ds	6	0.60 (0.28)	17	0.67 (0.18)	23	0.65 (0.29)

Exp. - experiment; s.d. - standard deviation; C = control fish; E = experimental (caged) fish; us = upstream site; ds = downstream site; usE.us and dsEds = caged fish without transfer; dsE.us and usE.ds = caged fish with transfer.

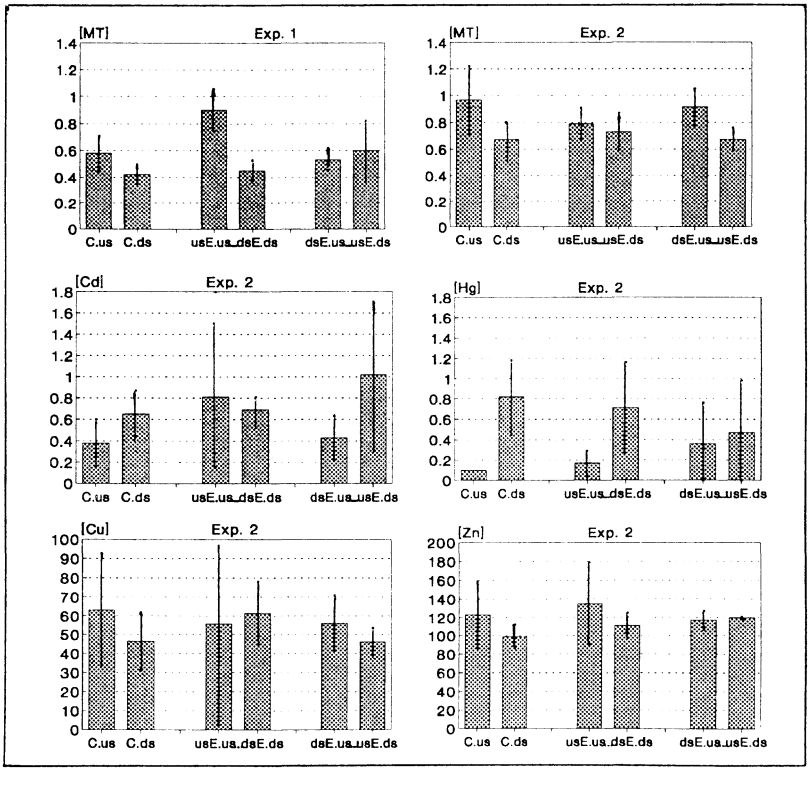


Figure 2 Means and confidence limits (95%) of metallothionein and heavy metal concentrations (nmoles/mg prot. and µg/g wet weight, resp.).

Results from the heavy metal analyses and corresponding MT values are presented in Table 2. Due to high intra-station variability and, in some cases, few replicates, the observed differences were rarely significant. There was, however, a certain consistency in the pattern that strongly suggested some relationship with site location (Fig. 2). Hepatic Cd and Hg concentrations are higher at the downstream site than at the upstream site. Again, destination, i.e. the location of the transferred caged fish during exposure, seemed to be more important than the origin, i.e. the area where the fish were caught.

Table 2 Concentrations of hepatic heavy metals (µg/g wet weight) and Metallothioneins[1] (nmoles/mg prot.) - experiment 2

Treat.	N	Mean (standard deviation)				
det. lim.:		Cu (0.5)	Zn (0.75)	Cd (0.3)	Hg (0.2)	MT
C.us	2	63 (41)	123 (50)	0.10 (0.08)	0.10 (N=1)	1.53 (0.82)
C.ds	10	46 (25)	99 (21)	0.65 (0.32)	0.82 (0.56)	0.76 (0.23)
usE.us	4	56 (42)	135 (45)	0.81 (0.82)	0.24 (0.16)	0.97 (0.24)
dsE.ds	11	61 (28)	111 (24)	0.69 (0.21)	0.71 (0.75)	0.77 (0.24)
dsE.us	7	56 (18)	117 (13)	0.43 (0.21)	0.36 (0.35)	0.78 (0.26)
usE.ds	2	46 (5,2)	120 (0)	1.02 (0.50)	0.40 (0.42)	0.69 (0.05)

[1] MT resultats for individuals corresponding to heavy metals analyses
Treat. - treatment; N - number of samples; det. lim. - detection limits;

The distribution pattern of hepatic Cu and Zn concentrations with respect to site showed a similarity to what was observed for the MT concentrations (Fig. 2). To investigate this possible relationship, Pearson's correlation coefficients were calculated between the metal concentrations and MT levels in the fish livers (Table 3). The relationship is highly significant for Cu and Zn concentrations, but not in the case of Cd and Hg. Cu and Zn were also strongly correlated between themselves (r = 0.75).

Table 3 Pearson's Correlation Coefficient (N=46)

	Cu	Zn	Cd	Hg
MT	0.77*	0.76*	0.21^{NS}	0.20^{NS}

* significatif p<0,001; NS not significant p>0.05

Since both metals are highly correlated with MT, the data were subjected to a multiple step-wise regression analysis (Table 4). The independent variables Cu and Zn were tested individually and then together. The analysis indicated that the best model was a multiple regression, using both independent variables.

Table 4 Stepwise Regression Parameters

step	variable in the model	coefficient	R^2
1	Copper	0,01392	0,92
2	Zinc	0,00734	0,94
3	Copper Zinc	0,00533 0,00470	0,95

Recent data on sediments indicate that Cu and Zn concentrations are higher in the upstream site than downstream [11,12]. Mercury levels in sediments were higher downstream, which confirms our observation on hepatic Hg concentrations, while the situation for Cd is less clear. White sucker is a benthic species living at the sediment - water interphase, feeding on benthic invertebrates. It may be that sediment heavy metal concentrations are more important than water concentrations. It is not known, however, what role food, such as insect larvae (e.g. chironomids) and molluscs, plays in MT induction.

Water concentrations of Cu and Zn in the study area are about 1 - 4 µg/l and 1 - 1708 µg/l, respectively [13]. Cu concentrations seemed to be slightly higher upstream than downstream. McCarter and Roch [14] observed that at copper concentrations of 50 µg/l and higher, MT induction took place. These concentrations are higher than those cited for the St. Lawrence. Thus, it seems plausible that concentrations at the sediment - water interphase, which can be expected to be much higher than in the above-lying water column [15], are mainly responsible for MT indiction in white sucker. This may not be true for pelagic fish, which are exposed to ambient metal levels in the water column.

Klaverkamp *et al.* [16] also observed that elevated levels of metals in liver of white sucker could be explained by sediment concentrations rather than direct loading from point sources. Indirectly, heavy metals in sediment levels in the upstream site of our study likely originate from upstream sources. It is not

known, however, what these sources might be, either present or historically, since little industry is located upstream of the study area.

CONCLUSIONS

In general, MT induction in control fish and caged fish without transfer was higher at the upstream than at the downstream site. This was contrary to the basic working hypothesis, whereby we expected that, based on the presence of point sources in the Beauharnois area, fish populations from downstream would be exposed to higher levels of heavy metals than fish from upstream.

MT concentrations varied directly and significantly with hepatic Cu and Zn concentrations, but not with Cd and Hg concentrations. Based on recent sediment data, which showed higher Cu and Zn concentrations upstream than downstream from Beauharnois, we postulated that MT synthesis in white sucker, a benthic species living and feeding at the sediment-water interphase, is induced more by sediment concentrations than by concentrations in the overlying water column.

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