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Comparison of Metal Concentrations in the Fore and Hindguts of the Crayfish *Cambarus bartoni* and *Orconectes virilis* and Implications Regarding Metal Absorption Efficiencies

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The anthropogenic perturbation of trace metal cycle has resulted in increased emission of trace metals into the atmosphere (Nriagu 1988). This in turn has resulted in the elevation of trace metals in recently deposited sediments of lakes far removed from the original source of emissions (Wong et al. 1984). Benthic invertebrates, such as crayfish, live and feed directly on recently deposited sediments and, therefore, are in direct contact with metals both of natural and anthropogenic origin. As a result of this intimate association, recent studies have suggested that crayfish may be good indicators of sediment-metal levels as they appear to retain tissue-metal concentrations that are correlated to environmental levels (e.g., for Cu, Zn and Fe, Bagatto and Alikhan 1987a, 1987b; Zia and Alikhan 1989; for Cd, Mirenda 1986a; Naqvi and Howell 1993). However, several other studies have suggested that as crayfish homeostatically control tissue elemental concentrations they, in fact, cannot be used as indicators of environmental metal levels (e.g., for Cu, Evans 1978; Anderson and Brower 1978; for Zn, Bryan 1968).

One aspect that is often missing in studies relating crayfish elemental tissue concentrations to that of environmental levels is a comparison of how efficiently crayfish can absorb the element from its food under different environmental conditions. Specifically, the amount of element that is absorbed may depend on whether the element is in excess or whether amounts in the environment are meeting metabolic requirements. For example, Bryan (1968) suggested homeostatic control of Zn tissue levels by crayfish is achieved through fecal production while the animal is feeding. As the amount of Zn increases in the food, a smaller percentage is absorbed by the hepatopancreas and more is lost via the feces. It is possible that for essential elements that are not in excess (i.e., uncontaminated sites), the assimilation from the food may be highly efficient, whereas for non-essential trace elements, or for essential trace elements which are in excess in the surrounding environment (i.e., contaminated sites), absorption from food may be extremely low. Differences in the ability of the crayfish to absorb elements from food will in turn influence ultimate tissue concentrations.

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One possible way to assess the importance of food as a source of elemental contamination is through gut content analysis; an estimate of the amount of element absorbed from the food can be made by comparing foregut elemental concentrations to the concentrations of elements in the hindgut, i.e., the difference being the amount absorbed. This approach assumes that excretion of the element via fecal production is the primary route of excess elemental elimination. (Bryan (1968) has shown that this does indeed occur for Zn). For essential elements not in excess (i.e., uncontaminated sites), foregut concentrations should either be equal to or greater than hindgut concentrations. Alternatively, essential elements that are in excess (i.e., from contaminated sites) or non-essential elements such as Cd, foregut elemental concentrations should be less than hindgut (i.e., greater concentrations in hindgut as crayfish are actively excreting excess element). Hence, the objective of this study was to determine the importance of food (foregut contents) as a source of essential (Zn, Cu, Mn, Fe, Mg and Ca) and non-essential elements (Al, Cd) in four populations of crayfish sampled from two metal and acid stressed-sites (herein referred to "stressed-sites") versus two non-contaminated reference sites. A previous study by Bendell-Young and Harvey (1991) determined that concentrations of gill Cd and Mn, muscle Mn and carapace Mn were elevated in crayfish populations sampled from the stressed versus reference sites.

MATERIALS AND METHODS

The four study lakes are located in south-central Ontario, two in the La Cloche Mountain area and two in the Muskoka-Haliburton area of Ontario, Canada. The two lakes in the LaCloche Mountain region have been recently acidified by anthropogenic acids. One of these lakes (George) contains elevated levels of Cu in the recently deposited sediments. Both have elevated concentrations of Zn and Mn in the overlying water column relative to the two reference lakes (Bendell-Young and Harvey 1991). The two reference lakes are circumneutral and are remote from point sources of element emission. Relevant water and sediment chemistry are outlined in Table 1. Crayfish were retrieved from the four lakes by either modified minnow trap or hand-picked by SCUBA divers. Captured crayfish were placed in plastic bags and frozen until analysis. Prior to tissue removal, crayfish length, sex and weight were recorded (see Bendell-Young and Harvey 1991). Species were identified by carapace and cheliped characteristics after Crocker and Barr (1968). A total of 56 crayfish were sampled for gut contents; 16 and 18 *Cambarus bartoni* from lakes George and Lumsden, respectively, and 16 and 5 *Orconectes virilis* from lakes Blue Chalk and Red Chalk, respectively. Complete foregut (stomach contents) and hindguts were removed from crayfish using glass knives and plastic forceps. Gut contents were dried to a constant weight and analyzed for Zn, Cu, Cd, Mn, Fe, Al, Ca, and Mg via Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES) at the University of Toronto Analytical Services. Limits of detection for the tissue analysis were 0.008, 0.004, 0.006, 0.0012, 0.002, 0.033, 0.003 and 0.027 mgL^{-1} for Zn, Cu, Cd, Mn, Fe, Al, Ca and Mg respectively. Quality control of the

Table 1. Relevant water* and sediment chemistry (±1 S.D.) in the four study lakes (Alk = alkalinity, DOC = Dissolved Organic Carbon).

Water Chemistry

Lake	pH	Alk μeqL^{-1}	DOC mgL^{-1}	Ca mgL^{-1}	Mn μgL^{-1}	Fe μgL^{-1}	Al μgL^{-1}	Zn μgL^{-1}
George	5.0	-3.2	1.5	2.5	108	17	26	13
Lumsden	5.3	-3.0	0.5	1.7	171	50	95	17
Red Chalk	6.3	64.0	2.0	2.8	24	180	15	<10
Blue Chalk	6.4	64.0	1.8	2.9	24	15	50	<10

Sediment Chemistry#

Lake	Mn mgg^{-1}	Al mgg^{-1}	Fe mgg^{-1}	Zn μgg^{-1}	Cu μgg^{-1}	Cd μgg^{-1}
George	3.3 (0.7)	27 (4)	48 (22)	256 (102)	106 (24)	3.5 (2)
Lumsden	0.8 (0.5)	28 (2)	33 (12)	55 (15)	40 (13)	4.0 (2)
Red Chalk	2.3 (1.5)	15 (5)	45 (26)	210 (37)	64 (4)	2.5 (0.4)
Blue Chalk	0.8 (0.4)	12 (1)	26 (6)	192 (19)	49 (14)	2.4 (0.5)

*. n=3-5. # Total acid extractable sediment chemistry (±1 S.D.). Means of the upper-most cm of sediments at 3-5 locations/lake. Sediment metal concentrations for Lumsden Lake is the sum of inorganic + organically bound metal.

elemental analysis was ensured through the inclusion of bovine and oyster tissue standards of the National Bureau of Standards. Analytically determined values were always within acceptable levels (i.e., 10-20%) of certified values. Statistical analysis was done through Statistical Analysis Systems (SAS Institute Inc. 1988) on $\log_{10}$ transformed values. A two-way analysis of variance with LAKE and TISSUE as the two factors was performed to determine variation in elemental concentrations of gut contents among crayfish populations and variation in fore and hindgut elemental concentrations within crayfish populations. Significance was accepted at $P<0.05$.

RESULTS AND DISCUSSION

A comparison of foregut versus hindgut concentrations of essential and non-essential elements demonstrated the following four trends; **Zn and Cd:** Foregut concentrations < hindgut concentrations (ANOVA, $P=0.0004$ and $P=0.0038$ for Zn and Cd, respectively, Table 2), with the ratio of foregut elemental concentrations/hindgut elemental concentrations < 1 (Fig. 1). **Cu, Mn, and Mg:** Foregut concentrations = hindgut concentrations (2-way ANOVA, $P=0.55$, 0.57, and 0.88 for differences between tissues for Cu, Mn, and Mg, respectively) with the ratio of foregut to hindgut elemental concentrations = 1, (Fig. 1). **Ca:** Foregut concentrations > hindgut concentrations (2-way ANOVA, $P=0.0001$, Table 2) with the ratio of Ca foregut concentrations to hindgut concentrations being > 1 (Fig. 1). **Fe and Al:** For the two crayfish populations from the stressed sites, foregut < hindgut, with the ratio of foregut to hindgut < 1 versus crayfish from the reference sites where foregut > hindgut concentrations with the ratio of foregut to hindgut ≥ 1 (2-way ANOVA $P=0.015$ for variation between

Table 2. Concentrations in μgg^{-1} for Zn, Cu, and Cd and in mgg^{-1} for Mn, Fe, Al, Ca and Mg dry weight (±1 S.D.) for foregut and hindgut tissue of crayfish from four south-central Ontario lakes. Results of the 2-way ANOVA are given at the end of each column.

	Foregut			Hindgut		
Lake	Zn	Cu	Cd	Zn	Cu	Cd
George	174 (53)	43(19)	13.5 (2.0)	378 (260)	61 (27)	11.7 (8.0)
Lumsden	120 (53)	80(55)	1.6 (1.4)	222 (96)	56 (20)	3.8 (2.4)
Red Chalk	358(191)	31(25)	0.6 (0.3)	459 (246)	33 (19)	2.0 (1.8)
Blue Chalk	229 (85)	71(50)	1.5 (1.4)	307 (165)	50 (23)	2.3 (0.5)

Results of two-way ANOVA:

	Zn	Cu	Cd
Tissue	0.0004	0.55	0.0038
Lake	0.0005	0.06	0.0001
Tiss*Lake	0.37	0.11	0.68

Lake	Mn	Fe	Al	Mn	Fe	Al
George	1.4 (0.8)	1.6(1.1)	1.4 (1.0)	2.3 (1.6)	3.4 (2.6)	3.0 (2.3)
Lumsden	1.4 (1.2)	1.4(1.2)	0.7 (0.6)	1.0 (1.0)	4.4 (4.1)	2.5 (2.1)
Red Chalk	0.5 (0.1)	1.9(1.4)	0.6 (0.4)	0.6 (0.4)	2.0 (0.9)	0.6 (0.2)
Blue Chalk	0.5 (0.3)	1.8(0.1)	0.4 (0.4)	0.4 (0.4)	2.0 (2.1)	1.3 (1.0)

Results of two-way ANOVA:

	Mn	Fe	Al
Tissue	0.57	0.0015	0.0015
Lake	0.0001	0.06	0.0054
Tiss*Lake	0.06	0.53	0.45

Lake	Ca	Mg	Ca	Mg
George	27 (14)	1.7 (0.4)	15 (5.4)	1.8 (0.8)
Lumsden	39 (21)	1.5 (1.0)	13 (10.0)	1.0 (0.8)
Red Chalk	20 (10)	1.3 (0.4)	18 (9.0)	1.7 (0.5)
Blue Chalk	34 (9)	1.7 (0.5)	15 (7.4)	1.8 (0.6)

Results of two-way ANOVA:

	Ca	Mg
Tissue	0.0001	0.88
Lake	0.24	0.043
Tiss*Lake	0.09	0.34

tissues for both Fe and Al Table 2, Fig. 1).

Zinc is an essential element required by crayfish for the production of carbonic anhydrase and alkaline phosphatase enzymes (reviewed by Rainbow 1988). Accumulation and toxicity studies of Bryan (1968) and Mirenda (1986b) have demonstrated that Zn concentrations in crayfish tissue are well regulated.

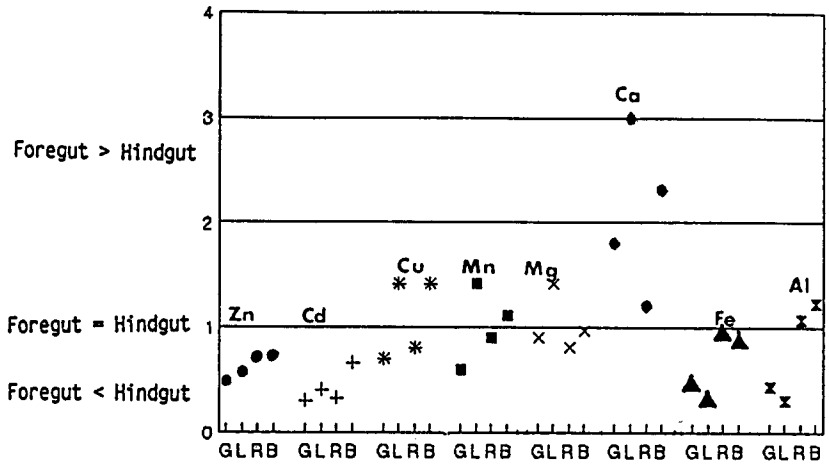

Figure. 1. Ratio of Foregut/Hindgut (F/G) elemental concentrations for the four crayfish populations. Locations are as follows: G; George, L; Lumsden, R; Red Chalk, B; Blue Chalk.

Comparison of crayfish foregut and hindgut concentrations supports the important role of fecal excretion in the homeostatic regulation of Zn by crayfish (Bryan 1968). For all four crayfish populations, Zn hindgut concentrations were significantly greater than foregut concentrations, suggesting that crayfish were effectively regulating tissue levels of Zn. Concentrations of Zn in abdominal muscle, gill and carapace of these crayfish populations were not significantly different from each other (Bendell-Young 1990; Bendell-Young and Harvey 1991), despite higher water-column levels of Zn in the stressed lakes (Table 1). The ability of crayfish to effectively regulate Zn tissue levels precludes their use as a biological indicator of environmental levels of this metal.

Cadmium, like Zn levels, was also lower in the foregut than the hindgut in all four crayfish populations. Unlike Zn, however, Cd is non-essential and is highly toxic to freshwater organisms. The significantly greater hindgut concentrations of Cd suggests that these animals are actively excreting this element. However, there is no evidence that any decapod regulates body Cd concentrations to a constant level by balancing uptake and excretion (review of Rainbow 1988). Fore and hindgut Cd concentrations were also significantly different among crayfish sampled from different lakes with crayfish from the two stressed sites containing greater concentrations in the hindgut than animals from the two reference populations. Crayfish from the stressed sites contained greatly elevated gill Cd levels versus the reference lakes (Bendell-Young 1990; Bendell-Young and Harvey 1991). As the gill is a site of Cd uptake (Mirenda 1986a), it is possible that greater hindgut Cd concentrations in crayfish from George and Lumsden

lakes were a result of these animals attempting to eliminate this harmful metal through excretion. It is probable that this excess only represented a portion of Cd that the animal was able to effectively remove via the feces as Cd is known to accumulate in some tissues, such as the gill and midgut gland (Geisy et al. 1989; Mirenda 1986a).

Copper is required for the respiratory pigment, hemocyanin. Crayfish have been shown to accumulate Cu in proportion to increased external levels to an upper limit at which point active homeostatic control begins (Evans 1978). As with Zn, this active maintenance of tissue concentrations confounds the use of crayfish as an indicator of environmental Cu levels (e.g., Anderson and Brower 1978; Stinson and Eaton 1983). The balance of foregut concentrations with hindgut concentrations suggests that all four crayfish populations met their metabolic demand for this element and did not have to excrete excess amounts of Cu via feces. This is despite the three fold higher levels of Cu in sediments of George Lake (Table 1).

Although higher concentrations of Mn occurred in both fore and hindgut of crayfish from the stressed sites, no differences in concentrations for all populations between the two tissues was observed. Manganese may also be under homoeostatic control with excess amounts that enter the crayfish via food being excreted via feces. Further, Mn was greater in the carapace of crayfish from the stressed versus the reference sites (Bendell-Young 1990; Bendell-Young and Harvey 1991). It is possible therefore that tissue Mn concentrations are in part regulated by the elimination of excess amounts of this element during ecdysis. Higher concentrations of Mn in guts of the crayfish from the stressed sites are most likely due to higher concentrations of Mn both in the water-column and, as in the case of George Lake, in the sediments as well.

Magnesium is the second most abundant mineral in the crayfish exoskeleton. However, it is thought to play only a minor role in the hardening of crustacean carapaces (Fieber and Lutz 1985). Hence, in contrast to Ca, where foregut concentrations greatly exceeded hindgut concentrations, foregut Mg was balanced by that in the hindgut. The lower metabolic demand for this element in comparison to Ca possibly accounts for Mg being less efficiently absorbed from gut contents by the crayfish as compared to Ca.

Calcium is of obvious importance to crayfish and it has been suggested that the range of crayfish in freshwater lakes is restricted simply by the concentrations of dissolved Ca in the water (Holdich and Lowery 1988). Greenaway (1974) suggested that these concentrations need to be at least 5 mgL^{-1} to meet crayfish needs. At ecdysis, crayfish resorb some of the carapace. However, the majority of their Ca requirements must be met through uptake from their surrounding medium (Holdich and Lowery 1988). Of the various elements analyzed, only Ca was significantly higher in the foregut versus the hindgut, emphasizing the high metabolic demand crayfish have for this element. As the freshwater lakes of the

current study are low alkalinity lakes with Ca concentrations of $< 3 mgL^{-1}$ (Table 1), and as evidenced by the significantly lower hindgut Ca concentrations noted in the four crayfish populations, it is probable that food constituted the major source of this element to these crayfish.

Iron and Al showed separate patterns that were dependent on whether crayfish were sampled from stressed verses the reference lakes. Iron was significantly greater in the hindgut of crayfish sampled from the stressed sites versus crayfish from the reference sites with hindgut concentrations being greater than foregut concentrations for the stressed crayfish whereas hindgut equalled foregut Fe concentrations for the reference populations. Crayfish from the stressed lakes also had significantly greater Al concentrations in both fore and hindguts versus the two reference populations and as with Fe, contained significantly greater amounts of Al within the hindguts versus the foregut. In the reference crayfish, hindgut and foregut concentrations were the same.

Iron is an essential element required for various enzymatic functions (reviewed in Rainbow 1988). Hence, for the reference populations, that foregut concentrations equalled hindgut concentrations suggests that the requirements are being met and are not in excess. In contrast, for crayfish from the two stressed sites, the greater hindgut Fe concentrations versus foregut concentrations suggest that these animals were actively excreting excess amounts of this element. However, neither water-column or surface-sediment Fe concentrations were higher in the stressed versus the reference lakes (Table 1).

It is possible that for Fe (although not apparent for any of the other elements), that different crayfish species have different Fe metabolic requirements. Specifically, those crayfish sampled from stressed sites (*Cambarus bartoni*) may have less of a metabolic demand as compared to crayfish from the reference populations (*Orconectes virilis*).

Aluminum is not required by crayfish for any known physiological function (Havas and Jawarski 1986). Greater foregut and hindgut Al concentrations in the stressed crayfish populations may be attributable to higher concentrations of Al in the surface-sediments of these lakes. In contrast, for crayfish sampled from the two reference sites, concentrations of Al in the foregut were not different from those of the hindgut suggesting that Al obtained via food was balanced by that Al being excreted.

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