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Cadmium, Copper, Manganese, Silver, and Zinc in Rock Crab (*Cancer irroratus*) from Highly Copper Contaminated Sites in the Inner Bay of Fundy, Atlantic Canada

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In 1999, American lobsters (*Homarus americanus*) from the Inner Bay of Fundy, Atlantic Canada, were found to contain extremely elevated levels of Cu in the digestive gland, plus high levels of Cd and Zn (Chou *et al.*, 2000). The trend in geographic distribution of Cu in lobsters illustrated that the most elevated levels were found in lobsters from the more sheltered, inner parts of the Bay, and less elevated levels were in lobsters from the more open waters of the Minas Channel. Sediments did not show high Cd, Cu, or Zn levels in the area, compared with normal sediment background data (Loring, 1979), and there were no relationships between sediment and lobster metals (Chou *et al.*, 2000). Prior to the 1999 lobster study, with the exception of work conducted in Saint John, New Brunswick (Dadswell 1979), and the Mussel Watch (Chase *et al.* 1998), data for metal contaminants in marine biota have been largely unavailable for the Inner Bay of Fundy. Blue mussels were monitored as part of Mussel Watch, but the observed metal levels did not detect the high copper contamination problems in the Inner Bay of Fundy sites. As this finding suggests the selection of proper bioindicators for monitoring marine environmental contamination is crucial.

The Bay of Fundy is an area of high biodiversity, and an important site for commercial and non-commercial fisheries resources including marine mammals and their habitat (Buzeta, 2000). In order to investigate the degree and extent of metal contamination in the Bay of Fundy ecosystem, other species, such as crabs, and other shellfish must be studied for toxic metal levels. Crustaceans are known to accumulate metals from the environment and have been used as bioindicator species (Chou and Uthe, 1978). In this paper, heavy metal levels in the digestive glands of rock crab, a lobster dietary species collected from lobster sampling sites in the Inner Bay of Fundy, and from Dalhousie, New Brunswick, are reported. Crab range widely in their distribution, are easily catchable at lobster capture sites, and are a good bioindicator species for monitoring the quality of the marine environment.

MATERIALS AND METHODS

Rock crabs (*Cancer irroratus*) were captured in lobster traps by fishermen during the fishing season, in June 2000, at sample sites in the Inner Bay of Fundy:

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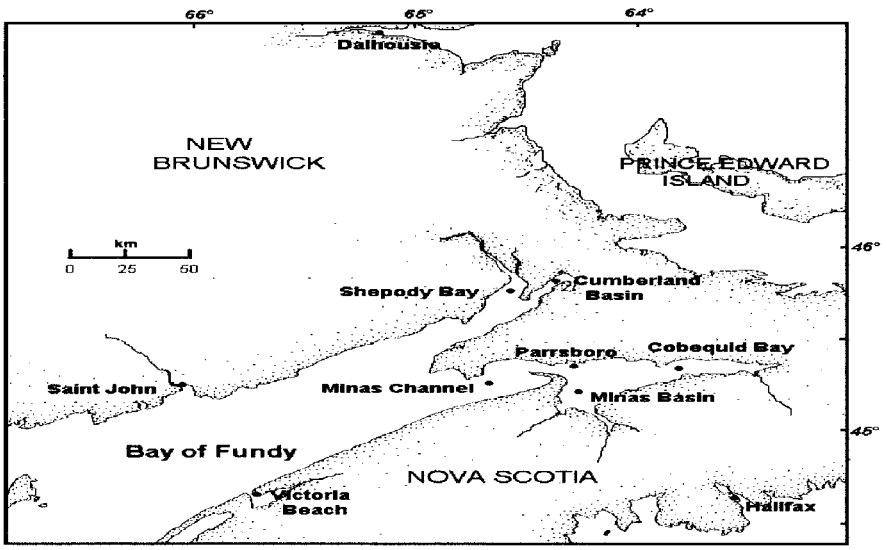

Figure 1. Crab sampling sites.

Cobequid Bay (CBY), Minas Basin (MIB), Parrsborro (PARS) and Minas Channel (MIC) (Fig. 1). Twenty crabs were collected from each site. Crabs were also collected at Dalhousie, on the northeast shore of New Brunswick, for a metal analysis requested by the Fisheries Protection Branch, Tracadie, Gulf Region of the Department of Fisheries and Oceans, for the purpose of licensing crab fishing. With the exception of crabs from Dalhousie, all were transported live to the Bedford Institute of Oceanography, Dartmouth, N.S., and held in tanks of running ambient seawater for 24 hours before dissection. For Bay of Fundy crabs, total body weight, carapace width, and sex were recorded for each crab. Upon dissection, digestive glands were weighed and placed in labeled Whirl-Pak® bags, and frozen at -27°C to await chemical analysis. Dalhousie crabs were dissected by staff at the Gulf Fisheries Centre, New Brunswick and digestive gland pooled samples, based on a total of 20 crabs per pool, were prepared. In preparation for sample digestion, thawed crab digestive glands were homogenized by hand-kneading the Whirl-Pak® bags. For each site, 1.00 g of homogenized sample was weighed into a 50 mL plastic centrifuge tube, 5.0 mL concentrated HNO_3 (Fisher Optima®) was added, and samples were digested using a domestic microwave oven (900 Watts). To prevent most fumes from entering the chamber, sample tubes were placed in a sealed Nordicware® microwave cooker. The sample digestion protocol consisted of 3 stages: (1) 1 minute at 40% power (2) 2 minutes at 40% power (3) 5 minutes at 40% power. These 3 stages were necessary to control reaction between HNO_3 and tissue, and to avoid tube damage. After each stage, the Nordicware® cooker was opened to vent off fumes. The 3-stage procedure improved dissolution of the analyte and reduced organic matrices in the digest. A Teflon© beaker containing 50 mL water was placed in the oven at each stage to protect the magnetron. For QA/QC, certified reference

Table 1. Carapace width (mm), mean total weight (g), and digestive gland (D.G.) weight (g), for rock crabs from the Inner Bay of Fundy, Atlantic Canada.

Biological Parameters	Location			
	MIC	CBY	MIB	PARS
Carapace Width (mm)	89.6±10.2	102±4.61	88.2±12.8	95.2±9.12
Total Wt. (g)	113±41.2	172±26.4	116±58.8	145±40.1
D.G. Wt. (g)	5.85±4.08	6.78±2.36	4.56±2.82	5.49±2.75

materials, TORT-1 and LUTS-1 (lobster digestive gland), obtained from the National Research Council of Canada, were also prepared according to the sample procedure. After digestion, digests were diluted to 25 mL with de-ionized water for analysis, as well as reagent blanks. Ag, Cd, Cu, Mn and Zn were determined in the crab digestive gland by flame atomic absorption (AAS) using a Perkin-Elmer Model 403 flame atomic absorption spectrophotometer equipped with deuterium arc background correction (Chou *et al.* 1987). Concentrations determined for the metals are listed as μg/g wet wt. for digestive glands.

RESULTS AND DISCUSSION

Table 1 summarizes the biological parameters for the rock crabs. Carapace widths ranged from 88.2-102 mm, with the highest at CBY and lowest at MIB. Total weight ranged from 113-172 g, with the highest at CBY and lowest at MIC. Digestive glands ranged from 4.56-6.78g, with highest at CBY and lowest at MIB. There are slight variations in crab weights and lengths between sites in the Inner Bay of Fundy indicating site specific differences.

For QA/QC purposes, the analytical procedure was verified for accuracy with certified reference material, TORT-1, lobster digestive gland. Metal results for TORT-1 were as follows: Cd was 26.7±1.1 μg/g (101% of certified 26.3±2.1 μg/g), Cu was 447±29.5 μg/g (102% of certified 439±22 μg/g), Mn was 23.6±1.27 μg/g (101% of certified 23.4±1.0 μg/g), and Zn was 177±7.8 μg/g (100% of certified 177±10 μg/g). No certified value was available for Ag in TORT-1, thus Ag was determined in LUTS-1, non-defatted lobster digestive gland, and the result was 0.63±0.07 μg/g (109% certified value 0.58±0.05 μg/g). Overall, the metal results for TORT-1 and LUTS-1 were in good agreement with certified values. Table 2 shows the concentrations of Ag, Cd, Cu, Mn, and Zn (wet weight), in the digestive glands of rock crabs from 4 sites in the Inner Bay of Fundy. The crab metal results for the Inner Bay were compared with both Victoria Beach (Chou and Uthe, 1978), and Dalhousie Harbour results

Table 2. Ag, Cd, Cu, Mn and Zn concentrations (μg/g wet weight) in the digestive glands of New Brunswick, Atlantic rock crabs (*Cancer irroratus)* from the Inner Bay of Fundy, Annapolis Basin, and Dalhousie, Canada.

Metal	MIC	CBY	MIB	PARS	Victoria Beach[a]	Dalhousie[b]
[Ag]	3.76±1.88 (0.60-8.42)	3.59±1.47 (1.99-6.61)	4.09±1.24 (0.57-6.50)	5.09±1.54 (2.29-8.24)	1.51 (0.47-2.47)	0.29
[Cd]	19.3±22.7 (2.27-99.5)	48.8±41.4 (8.76-187)	53.7±30.2 (13.1-112)	61.1±36.8 (8.37-143)	2.02 (0.54-20.6)	7.53
[Cu]	79.9±58.9 (8.8-286)	165±95.3 (17.8-421)	150±62.8 (20.6-294)	215±126 (56.9-528)	69.7 (21.8-192)	7.98
[Mn]	2.72±0.50 (1.8-3.77)	2.78±0.52 (1.8-3.52)	2.50±0.49 (1.70-3.77)	2.90±0.56 (1.91-3.70)	1.68 (1.25-2.28)	2.6
[Zn]	30.0±6.14 (23.2-46.4)	27.9±6.33 (19.7-46.4)	38.9±12.1 (21.7-69.6)	42.4±14.1 (23.7-70.4)	24.2 (13.0-41.0)	24.6

[a]Data originally published in Chou and Uthe (1978) [b]3 Pooled samples, total = 80 Range of concentrations shown in brackets

(unpublished data). Copper concentrations in rock crab digestive glands vary between sites and can be divided into 2 groups: 1) higher Cu concentrations are inside the Inner Bay at CBY (165±95.3 μg/g), MIB (150±62.8 μg/g) and PARS (215±126 μg/g), and 2) lower concentrations are in more open waters, away from the Inner Bay, at MIC (79.9±58.9 μg/g) and Victoria Beach (69.7μg/g). These values are highly elevated compared with 7.53 μg Cu/g observed in crabs sampled from Dalhousie, and compared with crabs from other areas reported in the literature. In a study of Cu in crabs during the intermolt cycle, Martin (1975) reported 27 μg Cu/g (wet wt.) in digestive glands. He also reported 19.9±11.8 μg Cu/g (wet wt.) in whole crab samples from Terence Bay, Nova Scotia (1974). In rock crabs collected from near a sewage disposal site in the New York Bight Apex, Greig *et al.* (1982), reported Cu gill concentrations ranging from 10 to 50 μg Cu/g (wet wt.) in intermolt crabs; generally gills have lower metal concentrations than the hepatopancreas (Overnell and Trewhella, 1979). In an exposure study using the green crab, *Carcinus maenas* (Rainbow, 1985), the regulated level was 122 ± 57.6μg Cu/l concentration, and reached ~320 μg Cu/g (dry wt.) in the digestive gland when the crabs were exposed to 1,000 μg Cu/l conditions. In another study, the highest Cu concentrations, 174 μg Cu/g (dry wt.), were observed in crab digestive glands from mining sites in Norton Sound, Alaska, U.S.A. (Jewett and Naidu, 2000). Note that the reported values from those studies are given in dry weight terms, which are 3-5 times higher than those for wet weights. The Cu values for Inner Bay of Fundy crab are still far higher than the high Cu results reported in the literature for crabs from laboratory exposure studies and sites contaminated by mining. The source(s) of elevated Cu in the Inner Bay of Fundy are unknown. Crab metal results, however, indicate that the more sheltered inner bays of the Bay of Fundy (i.e. Cobequid Bay and Minas

Basin) are more elevated in copper than the more open, deeper waters (i.e. Minas Channel). Victoria Beach, which is the furthest away from the Inner Bay, has the lowest Cu (69 μg Cu/g) among the four study sites in the Bay of Fundy, but is still elevated. The pattern in geographic distribution of Cu in rock crab is consistent with the results for American lobster *Homarus americanus* collected from the same study sites in the Inner Bay of Fundy (Chou *et al.*, 2000).

Crab zinc concentrations were 27.9±6.33 μg/g at CBY, 42.4±14.1 μg/g at PARS, 38.9±12.1 μg/g at MIB, and 30.0±6.14 μg/g MIC. In comparison with Victoria Beach (24.2 μg/g) and Dalhousie (24.6 μg/g), zinc is elevated in Inner Bay crabs. This is noteworthy as Zn is normally regulated in decapod crustaceans (Bryan, 1968). In exposure studies under the conditions of 2.5-3,162 μg Zn/l, Rainbow (1985) reported a regulated level of 126±60.9 μg Zn/g dry wt. in the digestive gland of the green crab (*Carcinus maenas*) and a threshold metal concentration of 354 μg Zn/l beyond which a net accumulation in the digestive gland began. A concentration of 500 μg Zn/g (dry wt.) in the digestive gland was reached at a high exposure of 3,162 μg Zn/l. Zinc in Inner Bay of Fundy crab is not very high when compared with *Carcinus maenas* results at high exposure conditions obtained by Rainbow, but in comparison to Martin's results (1975) of a maximum ~27 μg Zn/g (wet wt.) in *Cancer irroratus* digestive glands during intermolt, MIB and PARS site crabs (located between MIC and CBY sites) have elevated Zn. This supports the finding of highest Zn in lobster from MIB (129 μg/g), although Zn accumulation in crab is one-third that found in lobsters (Chou *et al.*, 2000).

Cadmium concentrations in crab were 48.8±41.4 μg/g at CBY, 53.7±30.2 μg/g at MIB, 61.1±36.8 μg/g at PARS, and 19.3±22.7 μg/g at MIC. These concentrations are highly elevated compared with 7.53 μg Cd/g in Dalhousie crab digestive glands and are ~10-30 times the 2.02 μg Cd/g found in Victoria Beach crabs. Cadmium, however, is known to accumulate in proportion to exposure levels in decapod crustaceans (Rainbow, 1985; Vernberg *et al.*, 1974; White and Rainbow, 1982). In an exposure study using the green crab, *Carcinus maenas*, Rainbow (1985) reported that Cd accumulated in crab digestive gland at all exposures (0.5-1000 μg Cd/l) with no evidence for regulation of body cadmium concentrations. Jewett and Naidu (2000) reported 42.1±3.26 μg Cd/g (dry wt.) in the digestive glands of king crabs from gold mining sites at Norton Sound, U.S.A. Falconer *et al.* (1986) surveyed crab (*Cancer pagurus*) from Scottish coastal waters and reported a maximum concentration of 18.7±12.5 μg Cd/g (wet wt.) in the digestive glands from Kinlochbervie site, and the elevated Cd was attributed to regional contamination of the environment by Cd of geological origin. Ray *et al.* (1980) reported extremely high Cd, 394.3 μg Cd/g (dry wt.) in the digestive glands of rock crab from Belledune Harbour, New Brunswick, a site which was highly contaminated with Cd, associated with the Brunswick Smelting lead smelter. Inner Bay of Fundy Cd concentrations in crab are lower than the excessively high Cd levels in Belledune Harbour crabs, but are still very high compared with the crab species from the other studies. In addition, Cd is much higher (2-3 times) than the levels in lobster from the same sites (16.0-22.9 μg/g)

(Chou *et al.*, 2000), the pattern of distribution, however, is consistent with the lobster results, and indicates contamination, in particular at PARS, MIB, and CBY sites.

Manganese in crabs was 2.78±0.52 μg/g at CBY, 2.50±0.49μg/g at MIB, 2.90±0.56 μg/g at PARS, and 2.72±0.50 μg/g at MIC. Manganese concentrations for the Bay of Fundy were elevated compared with 1.68 μg/g from Victoria Beach, but are comparable to the 2.6 μg/g, in crabs from Dalhousie. Martin (1976) reported Mn values between 2-3 μg/g for intermolt crabs. Manganese exists in small quantities in the organism and is the least involved in the enzymatic reactions (Bowen, 1966).

Silver concentrations were 3.59±1.47 μg/g at CBY, 4.09±1.24 μg/g at MIB, 5.09±1.54 μg/g at PARS, and 3.76±1.88 μg/g at MIC. These values are more elevated than Ag in crab digestive glands from Victoria Beach (1.51 μg/g) and Dalhousie (0.29 μg/g), which have low digestive gland Cu levels. Chou *et al.* (1987) reported a Ag-Cu inter-relationship, in which, Ag increases as Cu increases, in lobster and crab (Chou *et al.*, 1978). In crab from the Inner Bay of Fundy sites, the elevation of Ag may be related to high Cu. The uptake of Ag in invertebrates is part of a detoxification mechanism for coping with high Cu concentrations in the environment (Chou *et al.*, 1987).

These results demonstrated high Cu, Cd, and Zn levels in crab from the Inner Bay of Fundy, which pose a serious concern requiring identification of the unknown source of discharge to the marine environment. Maine Council Mussel Watch Program did not detect elevated metal levels in these areas (Chase *et al.*, 1998) which suggests that better indicator species, such as crab and lobster are needed for monitoring contaminant discharges in the area. High metals have been shown to affect the survival, molting rate, growth and morphogenetic changes during crab larval development (Lopez Greco *et al.*, 2001). Studies of the fate, transport and distributions in other species including commercially valuable species such as scallops, fish and other marine biota are imperative. Surveys of the spatial distribution of contaminants in the Bay of Fundy to date have been limited to lobster and crab. High metal concentrations in crabs eaten by humans, are of concern, especially Cd in the edible tissue, since it accumulates in food species. In 1980, Department of Fisheries and Oceans and Health Canada approved 20 μg Cd/g in lobster digestive glands, but there is no tolerance limit for Cd in crab digestive gland.

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