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Copper Contamination and Cadmium, Silver, and Zinc Concentrations in the Digestive Glands of American Lobster (*Homarus americanus*) from the Inner Bay of Fundy, Atlantic Canada

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The Bay of Fundy, Atlantic Canada is the habitat for important commercial stocks of the American lobster, *Homarus americanus,* as well as a number of other species. In order to protect these valued resources, and the consumers, information regarding the contaminants and the associated environment is essential. The Bay of Fundy is one of the better studied tidal estuaries in the world in the field of sedimentology (Amos 1984), various biological components (Gordon and Dadswell 1984), and since 1976, the chemistry of the water column and sediments, including inorganic nutrients, plant pigments, organic carbon and nitrogen, and inorganic elements, has been examined (Keizer *et al.* 1984). With the exception of work conducted in Saint John, New Brunswick (Dadswell 1979), however, data for metal contaminants in the marine biota are largely unavailable for the Inner Bay of Fundy.

It has been shown that lobsters can accumulate high concentrations of heavy metals (Cu, Cd, Zn and Ag) in the digestive gland and thus are good indicators for monitoring changes in environmental metal levels (Chou *et al.* 1987; Chou and Uthe 1978; Conn. Dept. Env. Prot. 1987; NOAA 1996). In addition, metal levels in lobsters can exceed levels acceptable for human consumption (NOAA 1994); for example unsafe Cd concentrations, as high as 223 µg/g wet weight, were reported in lobsters from Belledune, New Brunswick at the site of a lead smelter plant (Uthe and Chou 1985). In a prior study of baseline metal levels in lobsters from the eastern and southern shores and the Annapolis Basin of Nova Scotia, unusually high levels of Cu (70.5 µg/g wet weight) were found in Annapolis Basin lobsters, compared to 10.4 µg/g wet weight in animals from Pubnico, the control site (Chou *et al.* 1998). Although Cu is a micronutrient in fish, it can also be toxic at elevated levels (Van Aggelen and Moore 1986). It is a required element in human nutrition, however, excessive consumption can negatively affect people, in particular in those suffering from Wilson's disease (CDA-UK 1992).

Accordingly, this research was undertaken to compile baseline data to assess the distribution of metals in Bay of Fundy lobsters. It is part of an overall program to map the concentrations of heavy metals in the digestive glands of lobsters

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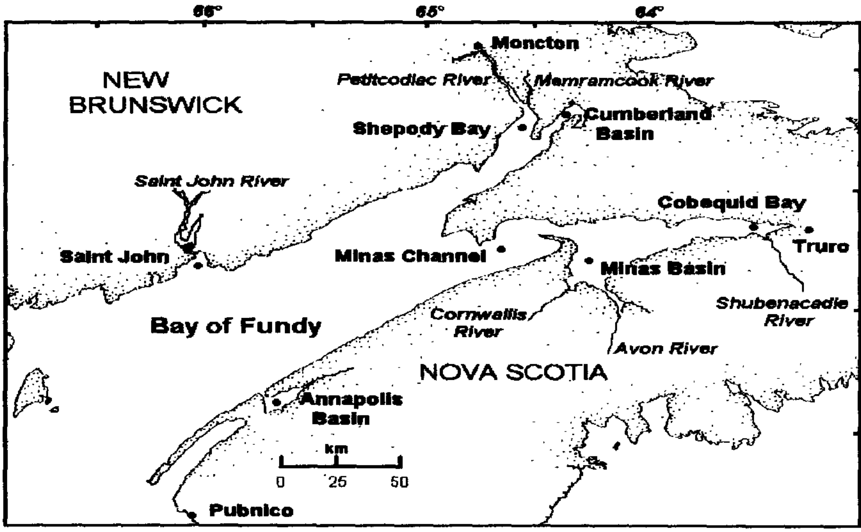

Figure 1. Sample locations.

captured from around Nova Scotia to gauge the well-being of lobster stocks and to use them as a general index of marine environmental quality as required by the Department of Fisheries and Oceans Canada.

MATERIALS AND METHODS

Market size lobsters, preferably weighing about 450 g, were purchased from lobster fishermen during the fishing season, May to July, 1999, at each of the sample sites in the Inner Bay of Fundy: Shepody Bay (SHB), Cumberland Basin (CMB), Saint John (SAJ), Cobequid Bay (CBY), Minas Basin (MIB), and Minas Channel (MIC) (Fig. 1). Fishermen harvested lobsters later in the season at the CMB site. These animals were ready to molt, which was reflected by significantly lower meat weights than were available from the other 5 sites. Thirty lobsters (15 males and 15 females) were collected from each site. The lobsters 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. Total body weight, carapace length and sex were recorded for each lobster. Upon dissection, digestive glands were weighed and placed in labelled Whirl-Pak© bags, and frozen at -27°C to await chemical analysis. In preparation for sample digestion, thawed lobster digestive glands were homogenized by hand-kneading the Whirl-Pak© bags, and 1.00 g portions from each sample were pooled, to yield 1 pooled sample per sex, per sampling site. For each site, 1.00 g of homogenized pooled sample was weighed into a 50 mL plastic centrifuge tube (triplicate analysis), 5.0 mL concentrated HNO_3 (Fisher Optima) was added, and all 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 prevent a violent reaction between HNO_3 and tissue, and to avoid tube damage. After each stage, 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. After digestion, samples were diluted to 25 mL with de-ionized water for analysis.

Six to eight sediments were collected at each of the lobster capture sites, using a VanVeen grab sampler (approximate capacity of 0.015 m^3). Sediments were removed from the grab using a stainless steel scoop, transferred to clean plastic Whirl-Pak© bags and frozen. Thawed samples were dried at 60°C for 24 hours, sieved to remove coarse material > 2mm, and ground using an agate mortar and pestle. Sub-samples of approximately 0.2 g were weighed into 50 mL plastic centrifuge tubes and digested using 6 mL concentrated HF (Fisher Trace Metal Grade) and 1 mL concentrated HNO_3; microwave digestion conditions were the same as for lobster tissues (in triplicate). Following digestion, the samples were diluted to 50 mL with de-ionized water.

Ag, Cd, Cu, and Zn were determined in the lobster 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). Cu and Zn were also determined in the sediments by flame AAS. Cd and Ag were analysed in the sediments by a high temperature graphite furnace atomic absorption spectrophotometry method using a Perkin-Elmer Model 403 atomic absorption spectrophotometer equipped with HGA-2000 controller (Chou and Uthe 1978). Concentrations determined for the metals are listed as µg/g wet wt. for digestive glands and µg/g dry wt. for the sediment samples.

RESULTS AND DISCUSSION

A summary of the biological parameters measured, sample weights and length of carapace, for lobster collected from the 6 sites can be found in Table 1. Although the aim was to obtain lobster of approximately 450 g, those collected from CBY, MIB and MIC were about 20% heavier than this. Also their average weights showed a higher standard deviation (between 13 and 17%) than lobster collected from the other 3 sites whose average weights were close to target and only exhibited less than 10% difference in weight. Results for the metal analyses can be found in Tables 2 and 3 for the digestive glands and sediment, respectively.

Uniquely, copper values ranged widely over the different sites and were conspicuously elevated (Table 2). Lobsters highly contaminated with Cu, were

Table 1. Mean total weight (g), digestive gland weight (g), meat weight (g), and carapace length (mm) for lobsters from the Inner Bay of Fundy, Atlantic Canada.

Biological Parameter	Sex	Location SHB	SAJ	CMB	CBY	MIB	MIC
Total Wt. (g)	Male	437±31.6	455±47.8	456±28.3	521±54.9	529±58.1	519±64.5
	Female	458±34.3	461±34.4	449±31.9	544±117	614±131	562±67.6
	Mean	447±34.2	458±42.2	453±29.8	532±90.3	568±106	540±68.6
D.G. Wt.(g)	Male	18.8±3.3	24.8±3.3	15.5±3.2	25.8±3.7	28.1±3.8	26.7±4.3
	Female	20.5±4.0	24.3±4.0	14.5±3.9	25.3±4.8	30.6±5.6	27.4±3.8
	Mean	19.7±3.7	24.6±3.5	15.0±3.5	25.5±4.2	29.3±4.8	27.1±4.0
Meat Wt. (g)	Male	115±19.7	128±22.3	72.0±10.1	127±21.0	130±21.8	149±21.4
	Female	113±20.7	133±21.6	46.5±44.7	136±34.6	151±36.4	163±20.6
	Mean	114±19.8	130±21.7	59.7±33.4	132±28.5	140±30.9	156±21.8
Length (mm)	Male	82.5±1.0	81.9±1.7	83.8±1.6	86.9±3.7	87.6±4.0	84.7±3.6
	Female	83.5±1.2	81.4±0.8	82.8±0.8	87.5±5.4	90.2±6.7	86.5±2.5
	Mean	83.0±1.2	81.7±1.4	83.3±1.4	87.2±4.6	88.8±5.5	85.6±3.2

observed in 3 inner bays: Cobequid Bay (CBY, 856 µg/g), Cumberland Basin (CMB, 836 µg/g), and Shepody Bay (SHB, 637µg/g), followed by Minas Basin (MIB, 405 µg/g), Saint John Harbour (SAJ, 317 µg/g), and finally, Minas Channel (MIC, 110 µg/g). These values are extremely high compared with Cu levels reported to date for lobsters in the Maritimes Region, Canada (Uthe and Chou 1978). These results also exceed mean Cu concentrations reported for Long Island Sound samples, 393.0 µg/g (Conn. Dept. Env. Prot. 1987); a maximum value of 1489.9 µg/g has also been observed for the same area (Greig and Pereira 1993). The Cu determined for lobsters from the Inner Bay of Fundy is 10-80 times higher than that found in lobsters from non-industrialized sites such as Pubnico, N.S. (Chou *et al.* 1998).

There is an obvious trend in the geographic distribution of Cu in lobsters. The highest levels were found in the inner bays whereas Cu levels were less in lobsters from deeper, more open areas as is evidenced by results for Minas Channel lobsters. Saint John Harbour lobsters were collected at a dumpsite for dredge-spoils associated with industrial activities within the harbour. Shepody Bay is the receptacle for the Petitcodiac River, which receives waste from domestic activity in Moncton, N.B. Cumberland Basin, however, is a sparsely populated rural area with no known industrial activity. Cobequid Bay has the domestic output from the town of Truro, and agricultural activity from surrounding areas.

For most sites, Zn in the digestive gland varied from 28.0-40.9 µg/g, with the exception of Minas Basin which had 129 µg/g wet weight. MIB lobsters surpassed values of up to 50.8 µg/g reported previously for Maritimes Region lobsters (Chou *et al.* 1998; Chou and Uthe 1978). Prior studies indicated that Zn

Table 2. Ag, Cd, Cu and Zn concentrations (μg/g wet wt.) in the digestive glands (pooled samples) in each of the males, females, and total sample means for lobsters from the Inner Bay of Fundy, Atlantic Canada.

Metal		Location						Range
	Sex	SHB	SAJ	CMB	CBY	MIB	MIC	μg/g
Ag	Male	12.3±0.4	6.3±0.3	2.2±0.01	11.1±0.3	9.2±0.1	3.0±0.2	2.2-12.3
	Female	9.3±0.1	7.7±0.3	2.8±0.2	11.8±0.4	10.8±0.1	2.7±0.2	2.7-11.8
	Mean	10.8	7.0	2.5	11.5	10.0	2.9	2.5-11.5
Cd	Male	16.0±0.5	10.3±0.5	14.7±0.3	20.9±1.2	22.8±0.8	17.1±1.0	10.3-22.8
	Female	14.8±0.1	12.8±0.5	15.7±0.6	20.1±0.4	23.0±0.8	15.0±0.3	12.8-23.0
	Mean	15.4	11.6	15.2	20.5	22.9	16.0	11.6-22.9
Cu	Male	673±32	303±6.8	818±12.0	816±20.1	426±8.3	136±6.7	136-818
	Female	600±2.6	332±8.4	853±14.7	896±7.7	385±2.4	85±4.3	85-896
	Mean	637	317	836	856	405	110	110-856
Zn	Male	33.3±1.7	31.2±0.8	24.7±0.9	40.4±0.5	115±2.4	36.8±0.4	24.7-115
	Female	36.8±1.1	38.9±0.6	31.3±2.1	41.4±0.7	142±2.8	33.4±0.8	31.3-142
	Mean	35.1	35.1	28.0	40.9	129	35.1	28.0-129

remained relatively constant at ~25-35 μg/g (Cooper *et al.* 1987; Pecci 1987) in lobsters from the New England coast. Slightly higher levels, 44.8 and 50.8 μg/g were found in Annapolis Basin and Country Harbour, respectively (Chou *et al.* 1998). NOAA reported a mean value of 62.0 μg/g in lobsters from Long Island Sound, a receptacle for large quantities of barged sludge, dredge-spoils, and waste introduced via the estuarine system (Conn. Dept. Env. Prot. 1987). Chou *et al.* (1987) reported that digestive gland Zn level was under biological control and independent of dietary Cd levels in juvenile lobster. At MIB, however, Zn may exceed a threshold, beyond which it can no longer be regulated as in other decapods (Nugegoda and Rainbow 1989). Further study on this point is required.

Ag concentrations were also elevated. Ag in Bay of Fundy lobster digestive gland ranged from 2.5-11.5 μg/g compared to 0.8-2.4 μg/g (Chou *et al.* 1998) and 0.44-2.22 μg/g (Chou and Uthe 1978) in previous surveys of Maritimes region harbours, Canada. Greig and Pereira (1993) reported a range of values between 4.8-23.4 μg/g and 2.3-8.8 μg/g at 2 sites in Long Island Sound. Ag is known to interact highly with Cu in lobsters (Chou *et al.* 1987; Chou *et al.* 1978); as the uptake of Cu increased, Ag concentration was also observed to increase. This relationship remained intact for wild populations from the eastern and southern shores of Nova Scotia (Chou *et al.* 1998), and for some sites in this study. At CBY, Cu was 856μg/g and Ag was 11.5 μg/g; at MIC, Cu was 110 μg/g and Ag was 2.9 μg/g. However, at CMB, the extremely high Cu value (836 μg/g) was not accompanied by proportionally elevated Ag values (only 2.5 μg/g) compared with the other sites. The low Ag concentration in CMB lobsters may be related to 1) molt stage at the time of sampling 2) disruption of the Ag-Cu relationship by

Table 3. Ag, Cd, Cu and Zn concentrations (μg/g dry wt.) in sediments from the Inner Bay of Fundy, Atlantic Canada.

Metal	Location						Range
	SHB	SAJ	CMB	CBY	MIB	MIC	μg/g
Ag	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Cd	0.03	0.11	0.03	0.02	0.03	0.02	0.02-0.11
Cu	15.0	19.2	16.8	17.0	9.3	16.2	9.3-19.2
Zn	58.6	69.4	65.5	48.9	35.1	46.1	35.1-69.4

N.D.: not detected

other metals, such as Cd, which compete for protein metal-binding sites (Chou *et al.* 1987).

Cd concentrations in Bay of Fundy lobsters (11.6-22.9 μg/g) were also more elevated than those reported for lobsters from other areas (Prince Edward Island, New Brunswick, Newfoundland, and elsewhere in Nova Scotia): 3.67-12.9 μg/g (Chou *et al.* 1998); 2.82-17.22 μg/g (Uthe and Freeman 1980); 3.67-12.9 μg/g (Chou and Uthe 1978). However, exceedingly high levels, peaking at 223 μg/g, have been previously reported in lobsters from a site near a lead smelter plant at Belledune, New Brunswick, Canada (Uthe and Chou 1985). The cause of the elevated Cd in Bay of Fundy lobsters is unknown.

Ag was generally higher in female lobsters than in males with the exception of MIC and SHB. Similar results were observed for Zn at all sites except MIC. For Cd and Cu no sex-related trends were observed. At the SAJ and CMB sites, female lobsters were consistently higher for the 4 metals studied, suggesting site specific uptake.

Table 3 shows the results for sediment Cd, Cu and Zn; Ag was not detected in sediments at any site. Cu concentrations ranged from 9.3-19.2 μg/g dry wt. and were comparable to Loring's survey results which showed an average of 15 μg/g from the Bay of Fundy (range 5-32 μg/g) (Loring 1979). Sediment Zn ranged from 35.1-69.4 μg/g which compares with the average of 51 μg/g (range 18-104 μg/g) reported by Loring (1979). Our sediment Cd values ranged from 0.02-0.11 μg/g which was also similar to Loring's range of 0.03-0.52 μg/g for the Bay of Fundy. SAJ sediments had the highest levels.

Relationships between sediment and digestive gland metals were not observed which agrees with our previous findings for biota and associated sediments (Chou *et al.* 1999; Chou *et al.* 1998). This suggests that the sediments are not the source of Cu in lobsters from the Bay of Fundy. This is consistent with reports from Francesconi *et al.* (1994) and Chou *et al.* (1987) that suggest the major intake of metals in lobsters is via the diet. The problem therefore involves bioaccumulation along the food chain and thus there is a pressing need to determine the copper source and the reason for such a dramatically increased level in the lobsters' digestive glands. As well, although lobsters appear to be able to withstand and concentrate extremely high copper levels when it is presented in the diet, there must be an upper limit beyond which there is an adverse effect with ultimately toxic and potentially negative consequences for the lobster stock. Thus there is a need to pinpoint the source of the copper and its impact on the lobsters.

There are no official Recommended Daily Allowances for Cu for humans. The amounts determined to be safe and adequate are 0.5 to 0.7 mg for infants, and 2 to 3 mg for adults and children 11 years and older (Life Well website 1999). Tolerance limits for Cu have yet to be established by the FAO/WHO (1998). They are likely to suggest that the population mean intake of Cu should not exceed 12 mg/day for adult males and 10 mg/day for adult females (CDA-UK website 1992).

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