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Analysis of Metals in Blue Crabs, *Callinectes sapidus,* from Two Connecticut Estuaries

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Intelligent decisions involving the use and allocation of resources in the nation's coastal and estuarine regions require reliable and continuous information about the status and trends of environmental quality in those areas (US Department of Commerce 1987). In recent years much attention has been focused on assessing environmental risks resulting from the manufacture, distribution, use and disposal of chemicals. Legislation and public concern have produced numerous regulations that continue to scrutinize the introduction of chemicals into surface waters which increase the potential for human exposure. There is considerable concern about the human health aspects of metal cycling especially in coastal and inland waters that are in proximity to large populations or industrial centers. Many of these compounds tend to remain in the ecosystem and eventually move from lower to higher trophic levels within the human food chain.

Blue crabs, *Callinectes sapidus* Rathbun, are important members of the estuarine food chain due to high abundance and their multiple role as scavengers, predators and prey. Because of their omnivorous feeding characteristics and association with sediments, blue crabs are exposed and may potentially accumulate significant amount of pollutants above background seawater concentrations (Nimmo *et al.* 1975; VanderOost *et al.* 1988). This species represents one of the most valuable fishery resources in the southern states. According to Marcus and Mathews (1987), blue crabs ranked fifth in 1984 and fourth in 1985 behind shrimp, swordfish, oysters and hard clams in economic value, but first in total weight for both years in South Carolina. Although in Connecticut blue crabs are harvested mostly for personal consumption, their commercial value in other states makes the results from any environmental survey of great interest from the consideration of risk to human health.

To date, no studies have examined tissue burdens of metals in crustaceans from the tidally influenced Quinnipiac and Connecticut River estuaries, despite considerable recreational fishing for blue crabs. Blue crabs are harvested during the summer months (June - September) in an active recreational fishery. To evaluate the level of contaminants, the concentration of selected metals was measured in samples of muscle and hepatopancreas tissue.

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Muscle was chosen for analysis because it is the major tissue consumed by humans and constitutes a significant percentage of the organism's body mass, while the hepatopancreas (tomale) was selected because it is the major lipid storage organ and predominant site of accumulation and is frequently consumed together with edible tissue.

MATERIALS AND METHODS

The sampling area in the Quinnipiac River included a one-half mile stretch between the Route 91 and Ferry Street bridges, while the sampling area in the Connecticut River estuary included stations around Smith Neck, Great Island and the mouth of the Black Hall River.

Legally harvestable (> 12.5 cm carapace width) blue crabs were collected in September 1994 from eight to ten baited commercial-style traps. Three pairs of traps were deployed at each estuary and were marked with surface buoys. All traps were deployed overnight at each location for two nights. Crabs were removed daily from each trap. Blue crabs were also collected from pilings and other substrates within the sampling zone using a dip net.

The sex of collected crabs was determined and specimens with carapace width exceeding 12.5 cm were frozen on dry ice (carbon dioxide). Among blue crabs collected from the Quinnipiac River, 40% and 60% were males and females, respectively. The ratio of males to females in the Connecticut River was 46% and 54% respectively. Tissue samples were prepared by cutting thawed blue crabs along the middle of the carapace; resected muscle and hepatopancreas tissue were collected separately. Muscle tissue was removed from the crabs and was refrozen and held overnight for shipment. The muscle and hepatopancreas were pooled from 25 crabs collected from the Quinnipiac River, yielding a total of 1046 g and 296 g of muscle and hepatopancreas tissue, respectively, while tissue pooled from 15 crabs from the Connecticut River yielded a total of 603 g of muscle and 124 g and hepatopancreas tissue. Frozen tissue samples were sent to Aqua Air (A2) Analytical, Inc. for chemical analysis after removal from the carapace.

Table 1. List of metals and their detection limits.

Analyte	Detection Limit (mg/kg)
Aluminium	0.003
Arsenic	0.002
Cadmium	0.00015
Copper	0.001
Lead	0.001
Mercury	0.0001
Nickel	0.013
Selenium	0.002
Silver	0.0002
Titanium	0.001
Zinc	0.0015

A blank accompanied each batch of samples through all analytical steps. Accuracy and precision estimates for the analytical methods were made by adding known amounts of representative metals to aliquots of sample tissue from each estuary and determining the recovery of each metal. Aliquots of each tissue type were removed from the total homogenized sample collected from each estuary for analysis. Samples were analyzed for the following metals: aluminum, arsenic, cadmium, copper, lead, mercury, nickel, selenium, silver, titanium and zinc. Analysis of metals was accomplished using furnace atomic absorption spectroscopy (AAS). Sample preparation for analysis consisted of a standard acid digestion procedure to convert organic forms of metal to inorganic forms and to minimize organic interferences (SW-846 method 3050, US EPA 1986). Tissue samples were pulverized and digested in hydrogen peroxide and nitric acid and refluxed with nitric acid. Samples for each specific metal were then prepared for injection into a graphite tube furnace. The sample aliquot was evaporated to dryness, ashed and atomized. Metal concentration was quantified based on the absorption of hollow cathode radiation during atomization. The detection limits for the chemical analyses performed on muscle and hepatopancreas are summarized in Table 1.

RESULTS AND DISCUSSION

The chemical analyses of muscle tissue and hepatopancreas collected from the Quinnipiac and Connecticut Rivers indicated that, in general, concentrations of aluminum, cadmium, copper and silver were higher in hepatopancreas tissue than muscle tissue in crabs sampled from both estuaries. On the contrary, concentrations of mercury, zinc and nickel were found to be somewhat higher in muscle tissue than in the tomale of sampled crabs from both areas. With the exception of lead and nickel, the mean concentration of metals in edible muscle tissue was similar in crabs from the Quinnipiac and Connecticut Rivers.

Concentrations of lead were 10 to 100X higher in the muscle and tomale of organisms removed from the Quinnipiac River than in crabs sampled from the Connecticut River. Concentrations of nickel in crabs sampled from the Quinnipiac River were relatively high in comparison to crabs from the Connecticut River, in which concentrations of this metal were below the established detection limit. On the average, concentrations of aluminum, arsenic, cadmium, copper, mercury, selenium, silver and zinc were 36% higher in the hepatopancreas of crabs sampled from the Quinnipiac River than in the same tissue of crabs from the Connecticut River.

The mean concentrations of metals were calculated for values above the limit of detection. Mean values are based on a total of eighteen muscle tissue and nine hepatopancreas tissue aliquots for the Quinnipiac River estuary and on six muscle tissue and three hepatopancreas tissue aliquots for the Connecticut River. A summary of the mean concentrations of metals (mg/kg) is presented in Table 2.

To determine the risk level or potential impact of human consumption of these crabs on public health, reference dose values (Table 3) were compared to analytical data for each metal. The risk assessment for aluminum, cadmium, copper, lead, mercury, nickel, selenium, silver, and zinc were based on FDA levels of concern. An initial risk assessment shows that the concentrations of aluminum, lead, mercury, nickel, selenium, silver, and zinc in crab muscle or hepatopancreas from both the Quinnipiac and Connecticut Rivers are below human consumption risk

levels. Cadmium concentrations in crabs hepatopancreas from both sites and copper from the Quinnipiac River exceed the EPA risk level by less than a factor of two. Since the EPA risk level is based on a subsistence diet (54 g/day, for 350 days/year, over 30 years), and a blue crab hepatopancreas subsistence diet is not likely, the risk from cadmium and copper are also mitigated for shellfish (FDA 1993 a,b,c,d) and EPA non-carcinogenic risk-based concentrations (EPA 1995).

Table 2. Concentration of metals in the muscle and hepatopancreas of blue crabs. All values are reported as mg/kg. Values in parentheses represent standard deviations.

	Quinnipiac River		Connecticut River	
Metal	Muscle	Hepatopancreas	Muscle	Hepatopancreas
Aluminum	3.35 (2.31)	6.99 (8.98)	3.01 (1.87)	4.39 (1.15)
Arsenic	0.76 (0.44)	0.84 (0.18)	0.62 (0.18)	0.60 (0.06)
Cadmium	0.05 (0.03)	1.18(0.10)	0.40 (0.79)	0.93 (0.02)
Copper	15.95 (3.44)	94.82 (11.46)	16.20 (11.55)	20.73 (30.92)
Lead	0.12 (0.21)	0.30 (0.12)	0.01 (0.02)	0.003 (0.002)
Mercury	0.06 (0.01)	0.04 (0.04)	0.11 (0.02)	0.02 (0.01)
Nickel	2.07 (0.72)	1.73 (0.75)	BDL	BDL
Selenium	0.15 (0.04)	0.32 (0.07)	0.16 (0.04)	0.13 (0.02)
Silver	0.33 (0.08)	1.57 (0.88)	0.27 (0.21)	1.20 (0.05)
Titanium	BDL	BDL	BDL	BDL
Zinc	32.76 (6.29)	28.31 (2.12)	31.25 (4.37)	27.20 (0.14)

BDL - below detectable limits.

Table 3. Summary of dietary consumption levels of metals

Metal	FDA LOC (mg/kg)	EPA RBC (mg)	RfDo/CPSo mg/kg/day
Aluminum	NA	1400	1.00
Arsenic	86	0.41	0.0003
Arsenic (carcinogen)	86	0.0018	1.75
Cadmium	3.7	0.68	0.0005
Copper	NA	50	0.0371
Lead	1.7	NA	NA
Mercury	NA	0.41	0.0003
Nickel	80	27	0.02
Selenium	NA	6.8	0.005
Silver	NA	6.8	0.005
Zinc	NA	410	0.3

FDA LOC - FDA Level of Concern - from Guidance Documents for Shellfish (FDA 1993 a,b,c,d)
EPA RBC - EPA Region Ill (1995) risk-based concentration table
RfDo - Reference dose oral (mg/kg/day)
CPSo - Carcinogenic potency slope oral (risk per mg/kg/day)
NA - Not available

The risk assessment for arsenic was based on both the carcinogenic and non-carcinogenic risk-based concentrations. The arsenic concentration in blue crab tissue is two orders of magnitude below the FDA level of concern. However, based on the more conservative EPA risk level, arsenic concentrations in crab muscle tissue suggest that the potential for both non-carcinogenic and carcinogenic exposure exists in both river systems, and that further evaluation of the arsenic risk would be warranted. This evaluation was accomplished by looking at two factors, the EPA risk-based concentration calculation assumptions and the speciation and metabolism of arsenic. The EPA carcinogenic risk-based concentration (RBC) for arsenic was calculated as:

$$RBC = \frac{TR \times Bwa \times ATc}{Efr \times Edtot \times \frac{IRF}{1000} \times CPSo}$$

while the EPA non-carcinogenic risk-based concentration for arsenic was calculated using the following equation:

$$RBC = \frac{THQ \times RfDo \times Bwa \times ATn}{Efr \times Edtot \times \frac{IRF}{1000}}$$

where,

RBC	=	Risk-based concentration for edible fish (mg/kg)
TR	=	Target cancer risk, one in a million
Bwa	=	Body weight, adult (70 kg)
Atc	=	Averaging time carcinogens (25,550 days)
Efr	=	Exposure frequency (350 days/year)
Edtot	=	Exposure duration, total (30 years)
IRF	=	Fish ingestion (54 g/day)
CPSo	=	1.75, carcinogenic potency slope oral (risk per mg/kg/day)
THQ	=	Target hazard quotient (1)
RfDo	=	Reference dose oral (3×10^{-4} mg/kg/day)
Atn	=	Averaging time non-carcinogens (ED x 365 days)

The risk-based concentration calculations are based on a worst-case-scenario using a subsistence diet of fish. Since the objective of this study was to perform tissue analysis on blue crabs from a recreational fishery, it is reasonable to perform the

risk-based concentration calculations utilizing a non-subsistence diet of blue crabs. A non-subsistence diet was used to further evaluate the risk, still assuming a consumption rate of 54 g/day (IRF) but only 17 days of consumption in a year (Efr). The number of days of consumption are based on eating blue crabs one day a week but only during a four-month period (June, July, August, and September). Using these assumptions the risk-based non-carcinogenic concentration for arsenic in blue crab muscle would be 8.3 mg/kg, which would mitigate any non-carcinogenic risk. However, using the same assumptions, the risk-based carcinogenic concentration would be 0.037 mg/kg, which would not completely mitigate the carcinogenic risk. It is common for total arsenic concentrations in marine species to contain over 80% of non-toxic arsenobetaine (Beauchemin et al. 1988). According to the US FDA (1993 a,b,c,d), only 10% of the total arsenic in shellfish may be represented by the toxic (As^{+3} and As^{+5}) inorganic form. By assuming that 10% of the arsenic in blue crab muscle is a toxic species, the carcinogenic risk would be reduced to within a factor of 2.

Blue crabs can be considered indigenous to the area from which they were collected and they represent one of the most recreationally-harvested species. The concentration of each metal found in blue crabs from both locations were similar. The difference between blue crab tissue and hepatopancreas "tomale" concentrations is not that significant in the Northeast since ordinarily only muscle tissue is consumed. In areas such as Chesapeake Bay where soft shell (whole) crabs are consumed the potential for increase metal uptake is much greater. Although the scope of this study did not include a full risk assessment, comparison of the dose level for each metal with the reference values indicate that concentrations of aluminum, lead, mercury, nickel, selenium, silver, and zinc were well below worst-case human health risk levels. Using reasonable non-subsistence diet assumptions coupled with the knowledge of metal speciation and metabolism, the concentrations of arsenic, cadmium, and copper in edible blue crab tissues from both sites also pose no risk to human health. Even with these assumptions, the odds of contracting cancer due to arsenic in blue crab muscle may increase from 1:1,000,000 to 1:500,000 for the Quinnipiac River and 1:600,000 for the Connecticut River.

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