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Metals in the Green Sea Urchin (*Strongylocentrotus droebrachiensis*) as an Indicator for the Near-Field Effects of Chemical Wastes from Salmon Aquaculture Sites in New Brunswick, Canada

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The salmon aquaculture industry in Atlantic Canada has rapidly expanded in Southwest New Brunswick over the last 20 years. Waste from this industry is now a major anthropogenic input in this area. Many chemicals are used in aquaculture operations, such as metals in feed, antibiotics, pesticides and anti-foulants (Heinig 2001). There is little information available regarding the amounts of these chemicals released to the environment and their effects on the biota in the vicinity of salmon aquaculture cage sites. In a 1998 study, lobster, a well-known accumulator of contaminants, had elevated concentrations of Cu in hepatopancreas that were associated with sediments sampled near salmon aquaculture sites in Passamoquoddy Bay, New Brunswick, Canada (Chou *et al.* 2002a). Copper is toxic to some marine species (Chapman and Stevens 1978), and is an ingredient in antifoulants and in salmon feed used by the salmon aquaculture industry. Sea urchins have been used to monitor the trace metals in the environment (Fallis 1982; Sheppard and Bellamy 1974; Warnau *et al.* 1995). Advantages of using the green sea urchin, *Strongylocentrotus droebrachiensis*, are that it is widely distributed, abundant in many locations, and easily accessible (Himmelman 1978). The purpose of this study was to investigate metal concentrations in green sea urchins for assessing and detecting impacts of produced chemicals from salmon aquaculture activity. Specifically, sea urchins collected from areas with sediments classed as normal (A), hypoxic (B), and anoxic (C) from intensive salmon farming areas in Passamoquoddy Bay, New Brunswick, Atlantic Canada, were investigated (DELG 2001).

MATERIALS AND METHODS

In February of 2001, sea urchins in pre-spawning, were collected by diver from three salmon aquaculture sites in Passamoquoddy Bay, southwestern New Brunswick, Atlantic Canada (Figure 1) that were assigned environmental monitoring program (EMP) ratings. Sediments were rated as: (A) (normal) redox potential (Eh) > +100 mV_{NHE} (normal hydrogen electrode), sulfide <300 µM; (B) (hypoxic) (Eh) 0 to –100 mV_{NHE}, sulfide 1300-6000 µM; and (C) (anoxic) (Eh)< -1000 mV_{NHE}, sulfide >6000 µM, based on the sediment criteria for the depositional zone defined by the Department of Environment and Local Government of New Brunswick, Canada environmental management guideline

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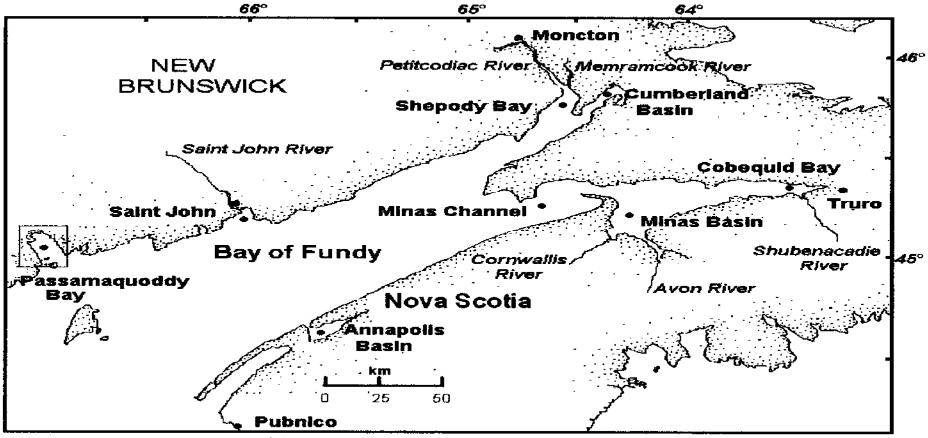

Figure 1. Passamoquoddy Bay, Bay of Fundy, Atlantic Canada.

protocol (DELG, 2000). Sea urchins were collected at 25m intervals along a transect in the direction of the main tidal flow from salmon aquaculture cage sites. Thus, 20 urchins were collected at each of 0-25m, 25-50m, 50-75m and 75-100m from the aquaculture site. Thirty urchins were also collected from a reference site, remote from all aquaculture activities. Sea urchins were transported live to the Bedford Institute of Oceanography and stored in tanks of running seawater overnight. For each sea urchin, total weight, width (diameter), and height were recorded. Urchin intestinal tracts (including contents) and gonads were removed, placed separately in labeled Whirl-Pak© bags and stored at –27°C. Sea urchin intestines were homogenized by hand-kneading. 1.00g of homogenate was weighed into a 50 mL plastic centrifuge tube, and digested with 5.0 mL concentrated HNO_3 acid (Fisher Optima) in a domestic microwave (900 Watts). To prevent most fumes from entering the microwave chamber, sample tubes were placed in a sealed Nordicware microwave cooker. Digestion consisted of 3 stages: (1) 1 minute (2) 2 minutes and (3) 5 minutes, at 40% power. These 3 stages were necessary to control the reaction between HNO_3 and tissue, and to avoid tube damage. After each stage, the Nordicware cooker was opened to vent the fumes. The 3-stage procedure yielded good dissolution of the analyte and reduced organic matrices in the digest. Chemical analyses (Ca, Cd, Cu, Fe, Mg, Mn, Zn) were carried out using a Perkin-Elmer Model 403 flame atomic absorption spectrophotometer following the procedure of Chou *et al.* (2002b). For quality assurance and quality control (QA/QC), certified reference materials (TORT-1) were analyzed with recoveries ranging from 100-109%. Concentrations determined for the metals are μg/g wet wt. for urchin intestines.

Principal components analysis (PCA) was applied to differentiate the distances of impact using sea urchin intestine metal concentrations, consisting of 7 metal variables (Ca, Cd, Cu, Fe, Mg, Mn, Zn). All data were normalised to 100% to give equal weight to each variable. Data manipulation including ANOVA and Bonferroni multiple comparison tests were performed using Systat 9 (SPSS Inc. 1999).

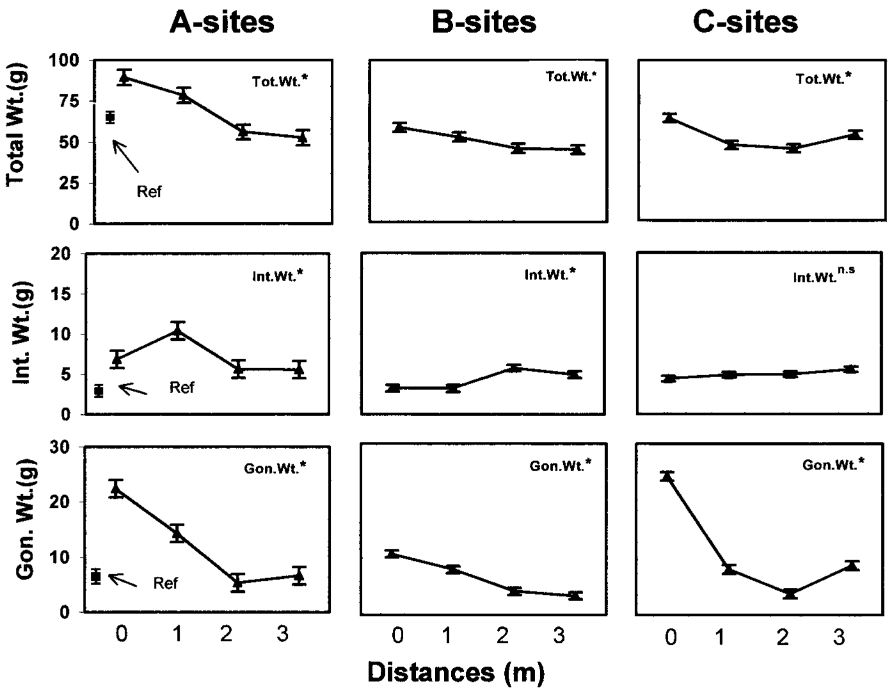

Figure 2. Means (± std error) for total weight (g), intestine weight (Int. Wt.), gonad weight (Gon. Wt.) (g), over distance where 0=0-25m, 1=25-50m, 2=50-75m, 3=75-100m for sea urchins at A, B, and C-sites (* is p>99%; n.s. is not significant).

RESULTS AND DISCUSSION

Figure 2 shows the plots of sea urchin total, intestinal, and gonadal weights against the sampling distances of 0-25m, 25-50m, 50-75m, and 75-100m from the cages at each of A (normal), B (hypoxic), and C (anoxic) sites. For each of the A, B, and C sites, the significance of differences in biological parameters between sampling distances were determined using the Bonferroni multiple comparisons test (* is p>99%; ** is 99%>p>95%; n.s. is not significant). Results showed that all of the biological parameters, total, intestinal, and gonadal weights differed significantly over distance for A, B, and C sites, with the exception of the intestinal weights at C sites. Urchin total weight decreased with increased sampling distance from the salmon cages for A, B, and C sites. Sea urchins collected at close proximity to the cages, 0-25m, compared to the reference were in the following order: A>C>B=Reference for total weight; A>Reference>B=C for intestinal weight; C≥A>B>Reference for gonadal weights. The results indicate 1) aquaculture site urchins were bigger than the reference urchins 2) weight variations between A, B, and C may be site specific. It has been reported that urchins suspended in cages at aquaculture sites benefit from food availability and tend to have much larger gonads and total weight compared to urchins suspended in cages at non-aquaculture sites, confirming the results of the study

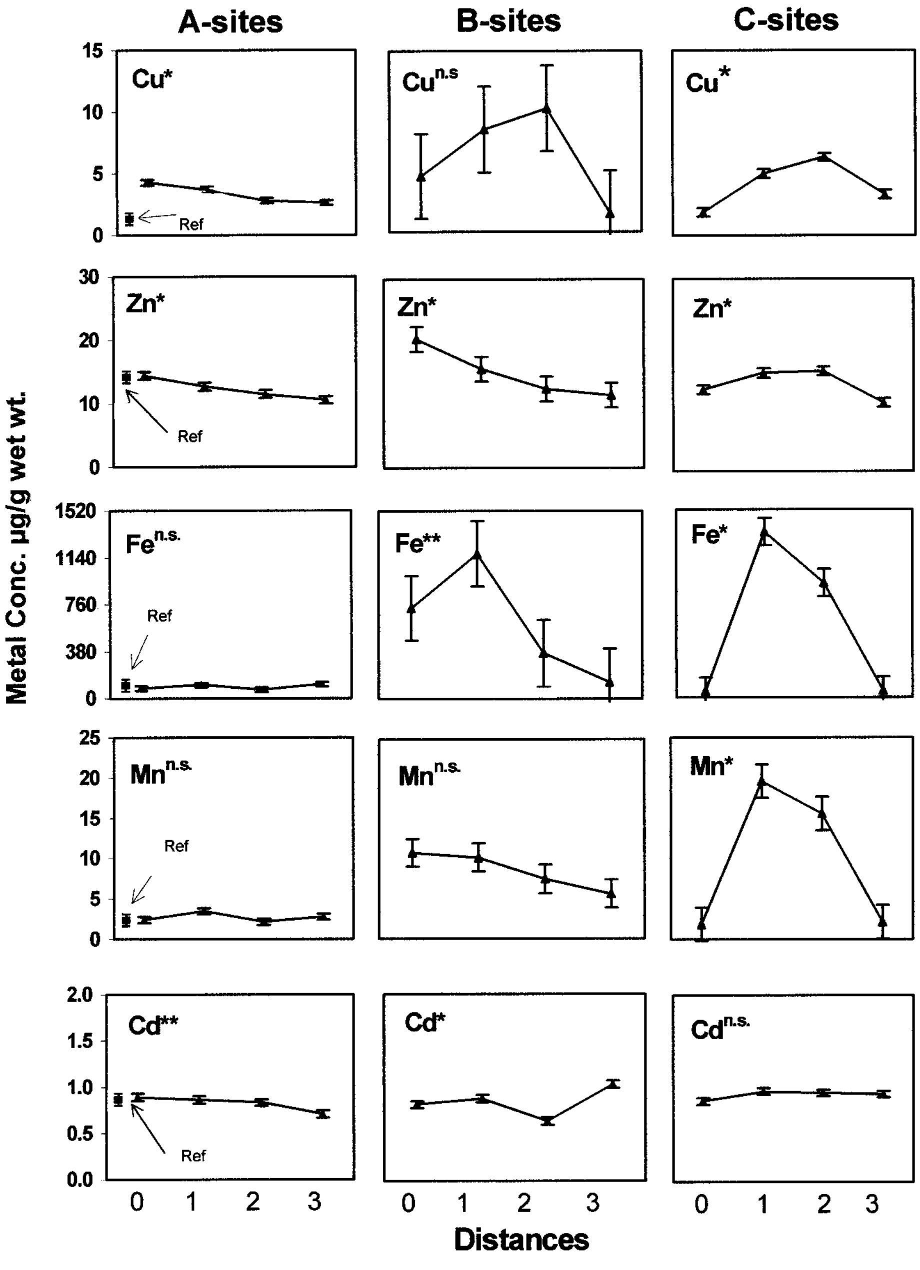

Figure 3. Means (± std errors) for Cu, Zn, Fe, Mn, and Cd concentrations in intestine, over distance where 0=0-25m, 1=25-50m, 2=50-75m, 3=75-100m for sea urchins at A, B, and C-sites (* is p>99%; ** is 99%>p>95%; n.s. is not significant).

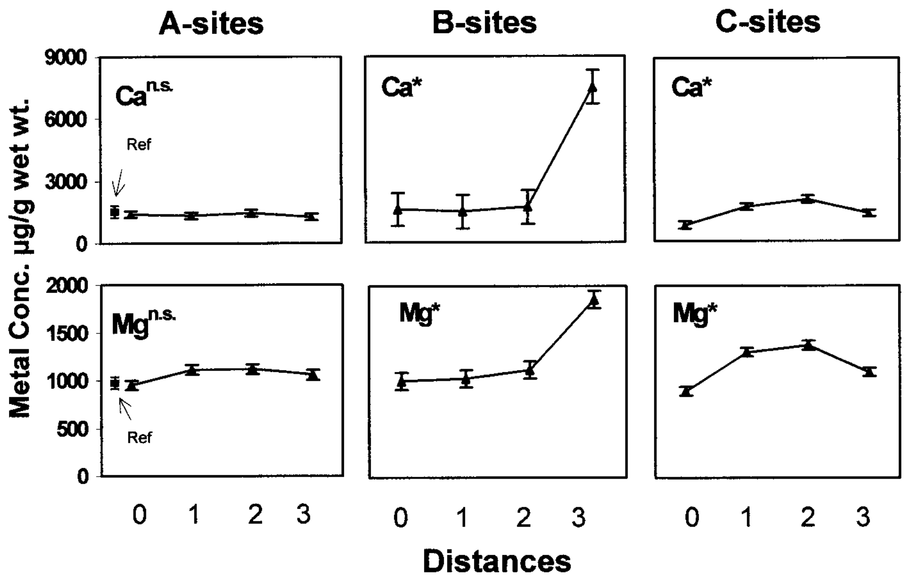

Figure 3 (continued). Means (± std errors) for Ca and Mg concentrations in intestine, over distance where 0=0-25m, 1=25-50m, 2=50-75m, 3=75-100m for sea urchins at A, B, and C-sites (* is p>99%; ** is 99%>p>95%; n.s. is not significant).

with *Psammechinus* (Kelly *et al.* 1998).

Figure 3 shows the results of the multiple comparison tests for each intestine metal over distance (0-100m) at A, B, and C sites. At normal (A) sites, metals (Fe, Mn, Ca, Mg) were constant and not different from 0-100m, but Zn, Cu and Cd differed significantly between some sampling distances. Metal concentrations (Cd, Zn, Mg, Ca, Fe) in intestines for sea urchins from B sites and (Ca, Cu, Fe, Mg, Mn, Zn) from C sites had a large number of differences in metal accumulation with sampling distances. Fewer differences (mostly n.s.) for metal concentrations with sampling distances (0-100m) and metal concentrations at A sites similar to the reference site (Figure 3) suggest that the environments were not yet impacted with feed and chemical wastes from the aquaculture operations. Urchins from the B and C site over 25-75m sampling locations had higher Cu, Fe and Mn concentrations than those from the reference site. There were sharp increases in concentrations of Ca and Mg at 100m in sea urchins from the B site. At the C sites inflection points were also observed for the metals (Fe and Mn at 50m and Cu, Mg, Ca, and Zn at 75m). These inflection points, the sharp increases, and higher metal concentrations present at B or C sites indicated that urchins inhabited impacted environments. In sediments from these sites, the sulfide levels were more elevated; hypoxic sites >1300µM, anoxic sites >6000µM, compared with normal sites <300µM (DELG 2001). Boothman *et al.* (2001) reported elevated metal uptake by organisms related to the binding of metals to sulfide. In sea urchins at the 100m sampling distance, most metal levels sharply dropped at B and C sites, suggesting that urchins were exposed to sediment less contaminated than the urchins nearby the cage and less excess salmon feed is available.

The pattern of total, gonadal, and intestinal weights (Figure 2) and of intestinal metal concentrations (Figure 3) over sampling distances (0-100m) revealed that differences in metals (Zn, Cu, and Cd) at A sites were related to total weight, as was Zn at B sites. For C sites, a concave shaped curve for metals (Cu, Zn, Fe, Mn, Ca, and Mg) and a convex shaped curve for total and gonadal weights over distance, were observed, whereas the Cd did not differ, but was similar to intestinal weight. It is also noted that urchins proximate to the C site cages, had low intestinal metal concentrations (Ca, Cd, Cu, Fe, Mg, Zn, and Mn) and high total and gonadal weights. This is probably related to the abundant availability of nutrients associated with aquaculture activity. Walker (1982) reported that urchins are capable of relatively rapid growth rates with readily available food, and of storage of excess energy in gonadal vacuoles. Sea urchins can exist for long periods on barren grounds, resulting in small animals with little excess energy stored in the gonads (Lang and Mann 1976; Johnson and Mann 1982). There is a lack of information on urchin metal accumulation related to gonad development in the literature. Observations of low metal accumulation at good nutrient sites have been reported for scallops (*Placopecten magellanicus*) from the highly Cd-contaminated Belledune Harbour, New Brunswick, the site of a lead smelter (Uthe and Chou 1987), in comparison with George's Bank scallops that had high metal uptake with poor nutrient availability.

By principal components analysis (PCA, Figure 4), it was possible to explore the contaminant variables thereby revealing different patterns of PCA components for normal and impacted collection sites based on sea urchin uptake of aquaculture produced chemicals. For A sites, sea urchins had no definite grouping patterns by distance, suggesting they were indistinguishable from the 4 locations of 0, 1, 2, 3 (0 to 100m sampling distances). For B sites, the urchins from the 0 and 1 locations (0-25m, 25-50m) were influenced by elevated Fe concentrations, and were to the upper right of the plot. Urchins from location 2 (50-75m) were near the origin on a 45° angle, and location 3 (75-100m) urchins formed a band extending between the lower and upper left quadrants, and were affected by Ca and Mg. For the C sites, locations 0 and 3 (0-25m, 75-100m) urchins were found in a band at a 45° angle extending between the upper and lower right quadrants and were affected by Ca, Mg, Cu, and Zn. Locations 1 and 2 (25-50m and 50-75m) urchins were in the upper left corner quadrant, and were especially influenced by elevated Fe and Mn. The PCA shows that urchin bio-accumulation data identifies zones of "impact', supporting urchin use in monitoring programs near aquaculture sites.

In the study of impacts on the benthos, Heinig (2001) reported that influences from the aquaculture operations were generally confined to within 30m of the salmon net pens. However, in this study of sea urchins, there was evidence of

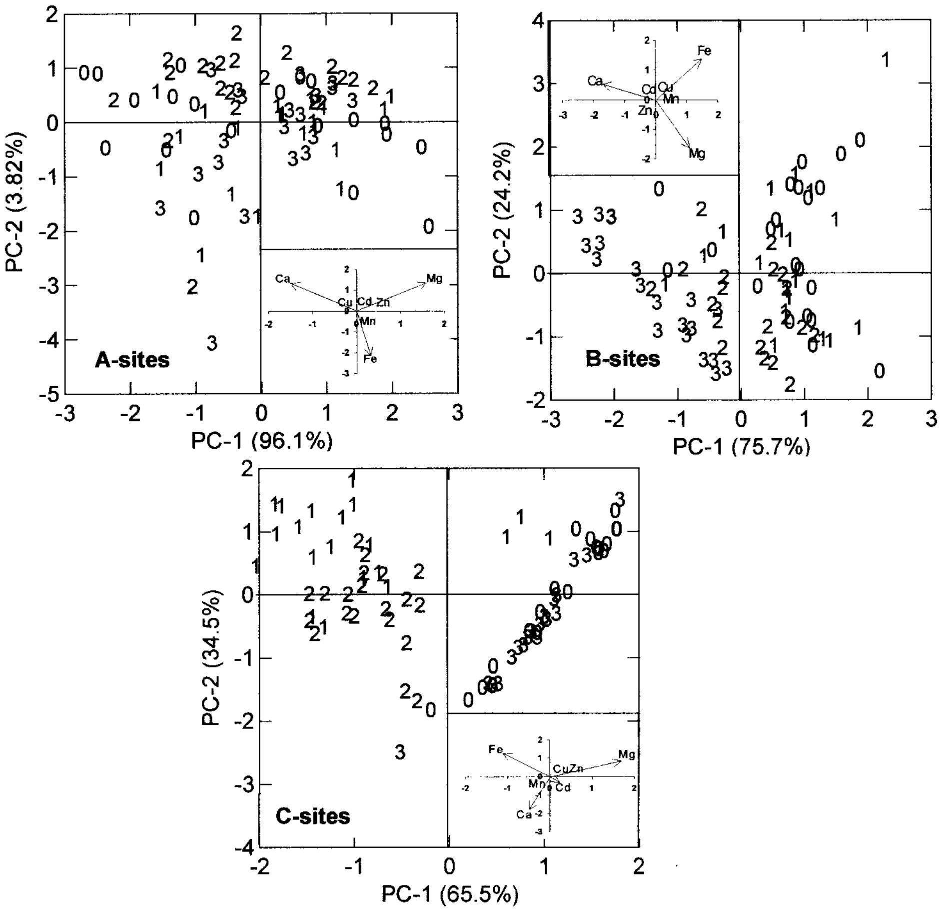

Figure 4. PCA analysis on 7 metal variables in sea urchin intestine at A, B, and C sites over the distances from 0-100m (0=0-25m, 1=25-50m, 2=50-75m, 3=75-100m).

impacts to at least 75m based on the intestine metal concentrations and that sea urchins were capable of reflecting the sediment conditions of A (normal), B (hypoxia), and C (anoxia) observed at salmon aquaculture sites. The sea urchin appears to be a sensitive species for detecting the impacts of aquaculture operations.

Based on the elevated sea urchin gonadal and total weights, and lower intestinal metal concentrations, there are benefits to this indigenous organism of being near the salmon aquaculture cages. *S. droebrachiensis* should be employed to establish acceptable environmental quality standards besides the emphasis that is placed on sediment chemistry and EMP ratings, and to refine the monitoring tools for use in the aquaculture regulatory process.

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