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# Survival, Growth, and Bioaccumulation of Heavy Metals by Juvenile Tanner Crabs *(Chionoecetes bairdi)* Held on Weathered Mine Tailings

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Between 1891 and 1944, over 80 million metric tons of tailings from three gold mines (Alaska-Gastineau, Alaska-Juneau, and Treadwell) were deposited in Gastineau Channel near Juneau, Alaska (Stone and Stone 1980). Tailings are rock materials that have been subjected to some form of milling and mineral separation process. After at least 50 years of weathering (e.g., rain, tidal flushing) or continuous submergence in seawater, elevated levels of some metals have been found in these tailings (Ecology and Environment, Inc. 1988), but their present availability to biota are unknown. With renewed interest to reopen one of these mines and marine disposal of tailings a possible option, examination of the impact historic tailings have on the environment may reflect future environmental conditions. Thus, further study is needed to document and identify metal concentrations in these weathered tailings and to determine availability and possible impacts to marine life.

Tanner crabs *(Chionoecetes bairdi)* are seasonally abundant in nearshore waters of southeast Alaska to a depth of about 500 m and are harvested by commercial and personal use fisheries. We have observed juvenile crabs on submerged mine tailings in Gastineau Channel. Tanner crabs often bury in and frequently ingest sediment while feeding. The routes of metal absorption in decapod crustaceans are from food in the digestive tract and across the permeable gill membranes (Rainbow 1988).

Objectives of this study were to determine the bioavailability of heavy metals to juvenile Tanner crabs held for 500+ days on weathered mine tailings and to examine possible effects on survival, growth, and health of the animals. In addition, baseline information is scarce on metal concentrations in juvenile Tanner crabs.

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## MATERIALS AND METHODS

Tailings were collected from a large disposal area (20 ha) near the mouth of Sheep Creek in Gastineau Channel where the A1aska-Gastineau Mine deposited 10 million metric tons of tailings between 1912 and 1920 (Ecology and Environment, Inc. 1988). A transect was established perpendicular to the shoreline from mean high water to mean lower low water (MLLW). Approximately 2 kg of sediment was collected from the upper 3 cm every 10 m along the transect. Sediment was collected during low tide with plastic buckets and composite in 125-L plastic containers. Control sediment was collected similarly from a relatively pristine area, approximately 35 km north of Juneau. Tailings and control sediment were homogenized thoroughly and placed in three 500-L flow-through aquaria (2 control, 1 tailings) to a depth of 5 cm. A sediment sample was collected from each aquarium at the beginning of the study for particle size and metal analyses.

Juvenile Tanner crabs were collected between 19 and 21 May 1993 at Amalga Harbor, approximately 31 km north of Juneau. Crabs were collected with SCUBA at depths between -10 and -21 m MLLW. Crabs were allowed to depurate in sand-filtered seawater for 1 wk prior to the start of the study. Twenty-four crabs were sacrificed and frozen whole at -20 °C for determination of background metal levels. Forty crabs were randomly assigned to each aquarium and placed in separate 15 by 17 cm compartments to prevent cannibalization during ecdysis. Because all crabs were immature, gender was not considered a factor in metal uptake. Carapace width (CW) was measured for each crab. All crabs were approximately the same size (33.8 -47.6 mm CW) and in good health (i.e., no missing appendages or gross evidence of disease).

The study was initiated on 26 May 1993. Crabs were monitored daily for molting and mortality. Growth was calculated by subtracting pre-molt CW from post-molt CW. Post-molt CW was measured a minimum of 21 d after ecdysis to allow for complete calcification and hardening (i.e., growth). Mortalities were removed within 24 hr. Each crab was fed a diet of squid mantel rinsed in seawater as follows; 1) day 0-23, 1 g once per wk, 2) day 24-250, 1 g twice per wk, and 3) after day 250, 3 g twice per wk. Unconsumed food was removed after 24 hr. Sand-filtered seawater was maintained at a flow rate of 6 L /min. Water temperature was monitored daily and ranged from 2.9-9.0 °C. Salinity ranged from 30.0 to 32.1‰ during the study.

Our initial goal was to terminate the experiment after each crab molted twice. The study was terminated after 502 d at which time all crabs but one had molted at least twice (two crabs molted 3 times). Crabs were starved during the last three days of the study to allow for complete gut evacuation and transferred to aquaria with no sediment to depurate for 48 hr. Three replicates of 10 crabs were randomly selected from each treatment (tailings and both controls) for metal

analysis. Gill and muscle tissue from the merus of the first 3 periopods were resected from each crab. Tissues were resected with corrosion-resistant stainless steel instruments and composite by tissue and treatment into certified metal-free 118-g glass jars. Samples were kept frozen at -20 °C for about one wk before metal analyses.

Sediment and tissue samples were analyzed for As, Cd, Cr, Cu, Ni, Pb, and Zn. The extraction procedure for sediments included multi-acid digestion ($HF$-$HNO_3$-$HClO_4$-$HCl$) and analysis by atomic absorption (Bondar-Clegg, 130 Pemberton Ave., N. Vancouver, B.C., V7P 2R5). Tissue analysis of metals followed the methods of the NOAA National Status and Trends Program (Crecelius et al. 1993). The extraction procedure for tissues included digestion in nitric acid and analysis with inductively-coupled plasma mass spectrophotometry. For tissue samples, a NIST certified reference sample (oyster tissue) was analyzed for quality control and mean detection limits were determined using three times the standard deviation of the blanks. Mean detection limits ranged from 0.02 µg/g for Pb and Cd to 0.3 µg/g for Cr and Zn. Percent recoveries were 88 to 115% at a 5µg/g matrix spike (one outlier - As at 137%) and 88 to 108% at a 25µg/g matrix spike for all metals.

Survival, growth, disease, condition index, and branchial histopathological changes were examined at the end of the study to determine health of the crabs. Differences in survival between treatments was tested with Chi-Square analysis. Total growth and growth at each molt (2 per crab) were compared between treatments using a one-way ANOVA. Intermolt period (the number of days between molts) was calculated for each crab and differences tested with a one-way ANOVA. Hemolymph was examined from each crab sampled for the presence of the parasitic dinoflagellate which causes Bitter Crab Disease (Love et al. 1993) and infection rate was calculated as no. infected/n. Incidence of Bitter Crab Disease between treatments was tested with Chi-Square analysis. One gill from 10 crabs of each treatment was dissected and prepared on a microscope slide using standard histological techniques and analyzed for histopathological abnormalities.

We hypothesized that stressed crabs would have atrophied muscles and developed a condition index to test this. For each crab sampled, the merus of the third periopod was broken at the autotomy plane and sliced with a scalpel at the joint between the merus and carpus. Each merus was dried to a constant weight at 65 °C for 72 hr. The ratio of muscle dry weight of the merus versus carapace weight for each sample was analyzed with one-way ANOVA. Because of potential stress associated with limb loss, crabs with missing appendages were excluded from analysis. Crabs which had molted within 60 d of final sampling were also excluded from analysis because of incomplete muscle regeneration. Significance for all tests was accepted at $P \leq 0.05$.

## RESULTS AND DISCUSSION

Survival was high for both treatments during the 502 d study. Only 12 crabs (11.3%) died, and there was no significant difference in survival between treatments ( $\chi^2 = 0.814$; df = 1, P = 0.367). Although not necropsied, no mortalities showed any obvious signs of disease.

Growth of crabs did not differ significantly between treatments for the first growth period (day 0 to first molt; P = 0.19) or the second growth period (first to second molt; P = 0.07) (Table 1). The average growth increment to first molt was about 12 mm, whereas growth between the first and second molt was about 15 mm. Intermolt period between the first and second molt did not differ significantly (P = 0.97) between control ($\bar{x}$ = 275.4 d, range = 165-376 d, n = 62) and tailings crabs ($\bar{x}$= 275,7 d, range= 178-351 d, n = 35).

**Table 1.** Mean carapace width (CW) ± 1 S.D. at day 0 and mean growth increment ±1 S.D. at two subsequent molts of juvenile Tanner crabs held on control and tailings sediment for 502 d. All measurements are in mm.

| CW | Control | Tailings | ANOVA |
|---|---|---|---|
| Day 0 | 41.2±3.2(n=80) | 41.1±2.8(n=40) | P=0.88 |
| Molt 1 | 11.9±l.l(n=68) | 11.6±l.0(n=39) | P=0.19 |
| Molt 2 | 14.8±1.5(n=61) | 15.5±1.8(n=35) | P=0.07 |

Crabs in this study showed no external signs of stress; condition indices were also similar (P = 0.60) between treatments. Sublethal concentrations of contaminants can stress crustaceans (Aiken and Waddy 1986). Stressed Tanner crabs are more susceptible to Bitter Crab Disease. A significantly ( $\chi^2 = 4.80$; df = 1, P = 0.029) greater number of crabs held on tailings were infected with Bitter Crab Disease. Whether this difference was due to stress from exposure to tailings is unclear, because the mechanism of infection and source of parasitism are unknown.

Histologic examination of crab gills revealed no tissue alteration due to tailings exposure. An increased prevalence of *Hematodinium,* the causative organism for Bitter Crab Disease, in gills of crabs held on tailings supported our hemolymph observations. There was an increased incidence of leucocytic infiltration in gill sections of some crabs exposed to mine tailings. Exposure to Cd and Cu can cause gill alterations with both acute and chronic effects in crustaceans (Gardner 1993).

Percent particle size distribution was similar for both treatments and consisted mostly of several gradations of sand (63-2000 μm; control = 99.5%, tailings =

97.3%). The clay-silt fraction (<63 µm) was very small for each treatment (control = 0.3%, tailings = 0.7%), possibly due to regular flushing by tides.

Metal concentrations were 1.5 (Cr) to 12 times (As) greater in the tailings than in the control sediment at day 0 (Table 2). Only Cr, Pb, and Zn in the tailings exceeded the effects-range low (ER-L) concentration; the concentration of a particular contaminant in sediment above which adverse biological effects are thought to occur (Long and Morgan 1990). The tailings originated from sulphide ores which contain As, Ni, Pb, and Zn (Ecology and Environment, Inc. 1988). No metals in the control sediment exceeded ER-L concentrations.

**Table 2.** Metal concentrations (µg/g dry weight) of control sediment and tailings (n=2) at the beginning of the study. Effects Range-Low (ER-L) values are provided for comparison (Long and Morgan 1990).

| Metal | Control | Tailings | ER-L Value |
|---|---|---|---|
| As | 2.1,2.9 | 27.9, 31.4 | 33.0 |
| Cd | <0.20, <0.20 | 1.10, 1.30 | 5.00 |
| Cr | 60.0, 61.0 | 92.0, 94.0 | 80.0 |
| Cu | 13.0, 14.0 | 32.0, 33.0 | 70.0 |
| Ni | 12.0, 14.0 | 21.0, 21.0 | 30.0 |
| Pb | 10.0, 10.0 | 60.0, 62.0 | 35.0 |
| Zn | 67.0, 67.0 | 196.0, 210.0 | 120.0 |

Some metals (Cu and Zn) are essential for normal growth and development in crustaceans, whereas others (Cd and Pb) are non-essential. Non-essential metals may be regulated, detoxified and stored in an inert form, or may accumulate and cause toxic effects (Rainbow 1988). Most baseline metal levels in muscle tissue of Tanner crabs in this study (Table 3) were below or comparable to levels measured for Tanner crabs at five other sites in Alaska (Hall et al. 1978). Only Cr was notably higher in our crab muscle samples than the above study, possibly due to the mineralogical composition of local sediments.

For each tissue type, metal concentrations were similar between treatments at the end of the study (Table 3). A major exception was Cr; for unknown reasons, the concentration of Cr was about six times greater in the muscle of control crabs than in tailings crabs. The overall similarity in tissue burdens of metals between treatments may indicate either low bioavailability of metals from tailings or, if metals were bioavailable, that crabs were able to effectively depurate metals

through normal physiological processes or store them in other tissues (i.e., hepatopancreas). Red king crab *(Paralithodes camtschaticus)* may purge Ni through the exoskeleton during ecdysis (Rusanowski et al. 1989).

**Table 3.** Metal concentrations (µg/g dry weight) in gill and muscle tissue of juvenile Tanner crabs. Baseline samples (n=2) were composites of 12 crabs each. Tissues for treatment samples were collected after crabs were held on control and tailings sediment for 502 d; values are means (±1 SE). Treatment samples were composites of 10 crabs each (n=3).

| | Gill | | | Muscle | | |
|---|---|---|---|---|---|---|
| Metal | Baseline | Control | Tailings | Baseline | Control | Tailings |
| As | 17.70 | 9.79 | 8.95 | 18.90 | 8.90 | 8.13 |
| | | (0.29) | (0.60) | | (0.43) | (0.17) |
| Cd | 2.39 | 9.09 | 8.60 | 0.87 | 0.23 | 0.24 |
| | | (0.07) | (0.80) | | (0.02) | (0.02) |
| Cr | 3.96 | 28.30 | 22.30 | 5.44 | 6.09 | 1.28 |
| | | (2.10) | (1.67) | | (0.33) | (0.09) |
| Cu | 144.00 | 220 | 209 | 63.55 | 37.5 | 48.6 |
| | | (2.60) | (10.7) | | (2.35) | (4.10) |
| Ni | 2.93 | 2.31 | 2.55 | 1.40 | 0.20 | 0.25 |
| | | (0.17) | (O. 16) | | (0.03) | (0.06) |
| Pb | 0.16 | 2.37 | 3.42 | 0.03 | 0.04 | 0.05 |
| | | (0.32) | (0.37) | | (0.003) | (0.004) |
| Zn | 101.75 | 72.80 | 72.50 | 93.10 | 97.8 | 91.6 |
| | | (1.08) | (2.63) | | (2.83) | (1.49) |

Regardless of treatment, the concentrations of most metals (Cd, Cr, Cu, Ni, and Pb) were greater in gill than in muscle (Table 3). Studies with other crustaceans have also reported similar results (Anderson and Brewer 1978). The only metal that was greater in muscle than in gill was Zn. Arsenic concentrations were about equal in gill and muscle (Table 3).

In both treatments, most metal concentrations changed over the course of the

study. For example, arsenic concentrations decreased by about 50% in tissues of study crabs compared to background concentrations (Table 3). This may have resulted from the strict diet of squid mantle crabs were fed. Low concentrations of arsenic have been measured in squid mantle (Hall et al. 1978). These changes also could have been due to differences in sediment chemistry; arsenic levels may have been naturally high where crabs were initially captured.

After decades of weathering, tailings deposited into Gastineau Channel from the Alaska-Gastineau Mine do not appear to be deleterious to juvenile Tanner crab. We found no significant differences in survival and growth of crabs held on tailings and control sediment for 500+ days and through two molts. Tissue burdens of metals were also similar between treatments. Whether or not there was some leaching and increased bioavailability of metals the first few years after tailings disposal ceased is unknown. Some leaching may have occurred, especially in intertidal deposits exposed to air, tides, and storms. Waste rock deposited intertidally from a lead-zinc mine in Greenland resulted in severe pollution of surface seawater and marine organisms (Asmund 1992). With renewed interest to reopen one of the mines adjacent to Gastineau Channel and submarine tailings disposal a preferred option, future studies should focus on metal leaching and potential impacts to biota that may occur within the first few years after tailings are deposited on the sea floor.

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