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# Prolonged Exposure to Mine Tailings and Survival and Reproductive Success of Ovigerous Tanner Crabs (*Chionoecetes bairdi*)

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Recently, plans to reopen the Alaska-Juneau (AJ) gold mine near Juneau, Alaska included submarine tailings disposal (STD) as an option for disposal of the finely crushed rock that remains after milling and mineral separation. Because information is scarce on the effects of STD on marine life, numerous studies were initiated to assess the potential biological consequences of STD (Johnson et al. 1998, US EPA 1996a). In addition to smothering the sea floor, STD may introduce elevated metal concentrations to the benthic environment and pose a toxicological hazard to marine life because non-target metals would remain as part of the tailings. Tanner crabs (*Chionoecetes bairdi*), a commercially important resource in Alaska, are intimately associated with benthic sediment; females completely or partially bury in sediment while brooding eggs up to one year and may need to oviposit in a soft substratum to allow for complete cementation of the eggs to the setae (Wickham 1979). Sublethal effects of exposure to heavy metals on crustacean embryos or larvae include inhibition of egg sac development, prolonged larval development, inhibition of larval molting, and reduced growth (D' Agostino and Finney 1974, Mortimer and Miller 1994). Tanner crabs may initially avoid areas affected by STD (Johnson et al. in press) but later recolonize the altered sea floor and incorporate harmful levels of contaminants into their tissues.

Objectives of this study were to determine 1) effect of prolonged exposure to mine tailings on survival and reproductive success (e.g., egg and larval survival) of ovigerous Tanner crabs, and 2) bioavailability of heavy metals to adult crabs. The study was conducted in a laboratory using tailings from the proposed AJ mine. Although plans to reopen the AJ mine were canceled in January 1997, STD remains a future option for some coastal mines in Alaska (Coldwell and Gensler 1993). Results from this study will provide resource managers with valuable information on the potential effects of STD on a dominant benthic invertebrate which supports important commercial and recreational fisheries.

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## MATERIALS AND METHODS

Ovigerous Tanner crabs were collected with baited commercial pots on 30 January 1997 in Young Bay near Juneau, Alaska. Sixty-three multiparous females (i.e.; have previously reproduced and are anecdysial) with no missing appendages and free of Bitter Crab Disease (Love et al. 1993) were selected for use. Mean carapace width (CW) was 103.7 mm (range 91.0 to 116.7 mm) and all had full egg clutches.

The experimental design consisted of three aquaria containing natural marine sediment (control) and three aquaria containing mine tailings. Sixty crabs were randomly assigned among the six aquaria (148 X 55 cm); the remaining three crabs were frozen whole at -20°C for determination of background metal levels. Tailings were prepared for Echo Bay Mines, Ltd., by Lakeland Research in Ontario, Canada, as part of an evaluation to determine the effects of STD from the AJ mine; a complete characterization of the tailings is provided in US EPA (1996b). Prior to the present study, tailings were aged about 100 d in flow-through seawater tanks. Control sediment was collected with plastic utensils just below mean lower low water from a relatively pristine area near Juneau, frozen at -20°C for 72 hr, and sieved through a 500 µm screen to approximate the grain-size composition of the tailings and to remove macrofaunal prey items. Depth of sediment in each aquarium was 3 cm and water on top of sediments was 35 cm. Seawater (30-32‰) flow rate was maintained at 3L/m and water temperature ranged from 3.2°C to 4.2°C during adult exposure. Particle size analysis, compressive strength (internal friction), and metal concentration of test sediments were determined at the end of the study. Compressive strength was determined by hand-held penetrometer. Triplicate sediment samples were collected from each treatment (control and tailings) for metal analyses.

The study was initiated on 31 January 1997. Crabs were monitored daily for mortality and larval hatch. Crabs were fed 60 g of chopped squid mantle and blue mussels (*Mytilus trossulus*) twice a week. Unconsumed food and feces were removed daily with a glass pipette. A sample of 300+ eggs was collected from each crab at the beginning of the study (0 d), near the mid-point (50 d), and at the end of the study (90 d). Eggs were collected from the anterior margin of the outer-most left pleopod and preserved in Stockard's solution. All samples were collected from the same location within the clutch to eliminate possible error due to differential mortality within the egg mass. Preserved egg samples were observed under light microscopy at 12 or 25 X; setae were placed on a petri dish and the first 300 eggs examined for mortality and presence of symbionts. Mortality estimates were made without knowledge of treatment and expressed as percent dead eggs in the sample. Dead eggs included discolored eggs, and partially empty or empty egg cases with intact funiculi attached to the setae. At 90 d, empty egg cases due to hatching were not included; these eggs could be distinguished from dead eggs by the relatively straight membrane rupture and the lack of sediment or microbial fouling of the egg case.

After 90 d, three females began hatching larvae; at that time isolated treatment groups were transferred to sterile aquaria without sediment. Eclosion occurred over a 13 d period. As each crab began hatching larvae, they were placed in 4-L plastic containers until several hundred prezoeae were present. Three replicates of 20 prezoeae were haphazardly collected and then transferred to 1-L glass containers with a large-bore glass pipette. Only actively swimming larvae near the surface of the container were selected. Larvae were held for 72 hr and monitored daily for mortality. Jars were kept in aquaria surrounded by flowing seawater. Determination of mortality was facilitated by the positive phototactic response of zoeae. Water was changed every 24 hr and larvae were not fed.

Sediment and tissue samples were analyzed for As, Cd, Cr, Cu, Ni, Pb, and Zn. The extraction procedure for sediments included total hydrofluoric acid digestion and analysis by inductively-coupled plasma mass spectrophotometry (ICP-MS). After all crabs extruded new clutches, fresh ova were resected from six randomly selected crabs from each treatment. Periopod muscle was similarly sampled. Tissue analysis of metals followed the methods of the NOAA National Status and Trends Program (Stone and Johnson 1997). The extraction procedure for tissues included digestion in nitric acid and analysis with ICP-MS. A standard reference material (SRM) for sediment and tissue was analyzed for quality control and mean detection limits were determined using three times the SD of the blanks. The concentrations of metals in the procedural blank were near or below the detection limits. The results for the SRMs were within acceptable limits (US EPA 1989).

Differences in egg mortality among treatments were examined by multiple regression; a backward elimination procedure was used to test the relevance of other potential sources of egg mortality. Treatment effect on larval survival was examined with Chi-Square analysis. After 90 d, difference in tissue burdens of metals among treatments was analyzed by Student's t-test or by Mann-Whitney Rank Sum test if normality or equal variance tests failed. All proportion data were arcsine transformed before analysis. An experiment-wise value of $P \leq 0.05$ was considered significant.

## RESULTS AND DISCUSSION

Tailings produced in a manner similar to those we examined may not be toxicologically hazardous to ovigerous Tanner crabs or their progeny if deposited on the sea floor. In our study, survival of adults was 100% after 90-d exposure to tailings and all females extruded a full clutch of ova within 36 h of complete zoeae hatch. In addition, no crabs exhibited any external signs of stress or disease (e.g., loss of limbs, lethargy). In a similar study, survival of juvenile yellowfin sole (*Pleuronectes asper*) was also not adversely affected after 60-d exposure to “fresh” (aged 0 d) AJ mine tailings (Johnson et al. 1998). Although submarine disposal of these tailings may not pose a toxicological hazard to Tanner crabs, smothering of the sea floor could reduce food availability or displace crabs from essential habitats and indirectly affect growth and survival.

Egg mortality within treatments was highly variable at 0, 50, and 90 d (range 0.3 to 50%). Mean egg mortality ranged from 4.8% to 7.8% for crabs held on control sediment and from 5.1% to 12.1% for crabs held on tailings. Egg mortality increased between 0 and 50 d, but remained relatively constant between 50 and 90 d (Fig. 1). Although crab egg mortality data are scarce, Kon (1974) estimated egg loss (mortality) from oviposition to eclosion as high as 50% for *C. opilio.*

Egg mortality among treatments was similar at 0 and 50 d but was significantly ($P = 0.026$) greater for crabs held on tailings than on control sediment after 90 d (Fig. 1). The difference at 90 d was attributable to the high egg mortality of two crabs (39% and 50%) held on tailings (Fig. 1); removal of these outliers (egg mortality was nearly twice as high as any other crab held on tailings) resulted in no significant difference among treatments. Other potential sources of egg mortality considered were the presence of 1) the nemertean *Carcinonemertes* spp. and its eggs, a known predator of crab eggs, 2) nematodes, whose role in mortality is unknown, and 3) tank effects within treatment. Symbiotic turbellarians, although present as adults or eggs in about 17% of the samples we examined, were not included in the analysis since they are non-predaceous on crab eggs (Kuris 199 1). Although Hilsinger (1976) detected a significant increase in egg mortality with an increase in female size (CW), we detected no linear relationship between these two variables for either treatment at 0, 50, or 90 d. In addition, *Carcinonemertes* spp. or their egg clusters were found in only 5 egg samples (3%) while nematodes were found in 43% of all samples. These sources of mortality (including tank effect) were insignificant through backward elimination however, and treatment could be considered the sole source of mortality.

Because larvae are generally more susceptible to toxic heavy metals than adults of the same species (Connor 1972) knowledge of tolerance levels for these life history stages need to be considered when developing pollution control guidelines. Larval mortality in our study was low during the 72 hr survival tests. Only 20 of 3600 larvae held for 72 hr died (0.6%) and there was no significant difference in larval mortality among females reared on control sediment and tailings ($\chi^2 = 0.74$; df = 1, $P = 0.39$). Of the 20 mortalities, 35% were failures to successfully molt from prezoea to zoea stage I.

Most metal concentrations in muscle and ova were similar among treatments after 90 d (Table 1); Pb was the only metal that was significantly greater in tissues of crabs held on tailings than on control sediment ($P = 0.002$ for muscle, $P = 0.009$ for ova) (Table 1).

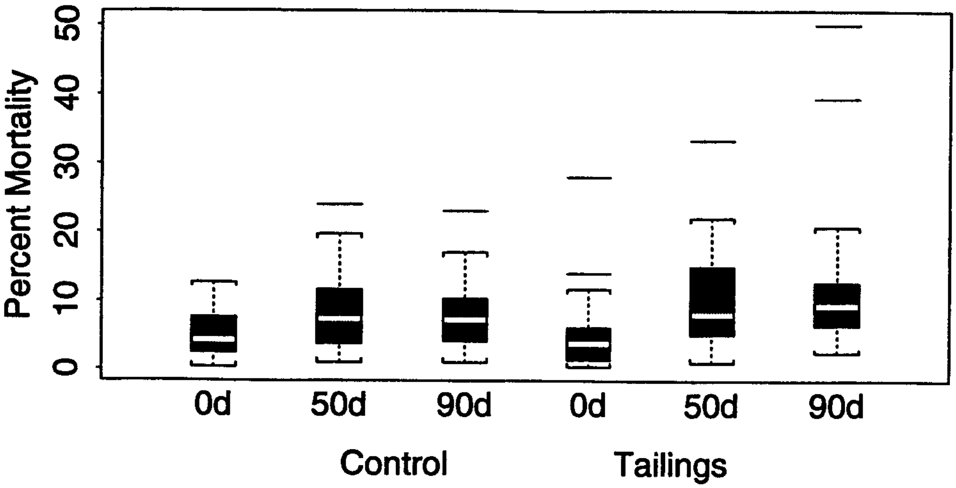


**Figure 1.** Boxplots of egg mortality from female Tanner crabs held on control sediments and mine tailings for 90 d. Egg samples were taken at 0, 50, and 90 d. Plots show percent mortality. The dark box shows limits of the middle half of the data (interquartile range); the center line represents the median. The whiskers are drawn to a point 1.5 X the interquartile range; data points beyond that are outliers.

Although Pb concentrations were at least eight times greater in muscle (0.57 µg $g^{-1}$) and ova (0.12 µg $g^{-1}$) of crabs held on tailings, concentrations were similar to those values reported for *C. bairdi* from other areas in Alaska (muscle, 0.36-0.48 µg $g^{-1}$; Hall et al. 1978). Similar concentrations of most metals in ova between treatments indicated that prolonged exposure to tailings produced no adverse effects on early development. Lead (Pb) may have been available to Tanner crabs after leaching from tailings into the water and crossing body surfaces (e.g., gills). Sediment concentrations of Cr, Pb, and Zn declined by only 10% to 14% from AJ mine tailings aged 60 d in seawater (Johnson et al. 1998). Ingestion of sediment while feeding is also a likely route of Pb uptake; of 55 Tanner crab stomachs collected near Juneau for diet analysis, 54% contained sediment (*R. P. Stone, unpublished data*).

Background concentrations of Cd, Cr, Cu, and Ni were significantly higher in muscle from crabs at the beginning of the study than observed in control crabs after 90 d (Table 1). These differences may have resulted from the controlled diet crabs were fed in the laboratory or from high natural concentrations in sediment where crabs were initially collected. Most background metal concentrations of adult crabs in this study were similar, however, to those values reported from juvenile Tanner crabs collected near Juneau (Stone and Johnson 1997).

With the exception of mercury, trace metal concentration guidelines for the safe

**Table 1.** Metal concentrations (μg $g^{-1}$ dry weight) in muscle and new ova of ovigerous Tanner crabs. Tissues were collected after crabs were held on clean marine sediment (control) and mine tailings for 90 d; values are means (±1 SE). * = P < 0.01. t-test. Baseline samples were taken at 0 d.

| Metal | Muscle | | | New ova | |
|---|---|---|---|---|---|
| | Baseline n = 3 | Control n = 6 | Tailings n = 6 | Control n = 6 | Tailings n = 6 |
| As | 80.77 | 80.22 | 68.82 | 19.87 | 16.25 |
| | (8.30) | (6.44) | (6.52) | (1.79) | (1.75) |
| Cd | 0.74 | 0.27 | 0.31 | 0.20 | 0.23 |
| | (0.24) | (0.03) | (0.03) | (0.02) | (0.03) |
| Cr | 0.60 | 0.35 | 0.38 | 0.34 | 0.36 |
| | (0.04) | (0.01) | (0.01) | (<0.01) | (<0.01) |
| Cu | 76.60 | 30.55 | 33.42 | 76.83 | 77.65 |
| | (4.86) | (1.64) | (3.80) | (3.46) | (3.57) |
| Ni | 0.60 | 0.16 | 0.18 | 0.47 | 0.45 |
| | (0.21) | (<0.01) | (0.02) | (0.12) | (0.08) |
| Pb | 0.06 | 0.07 | 0.57* | 0.01 | 0.12* |
| | (<0.01) | (<0.01) | (0.05) | (<0.01) | (0.02) |
| Zn | 103.67 | 106.73 | 106.50 | 85.60 | 83.28 |
| | (2.03) | (2.73) | (0.72) | (3.11) | (0.99) |

consumption of fish and shellfish have yet to be developed in the United States. Concentrations of Cd, Cu, Pb and Zn from crab muscle tissue in this study, however, were within safe guidelines developed in Europe (Brown and Balls 1997).

Metal concentrations ranged from 4 times greater (As) to 24 times greater (Pb) in tailings than in control sediment (Table 2). Only Cd and Zn, however, exceeded the effects range - median (ERM) value; that concentration of a contaminant in marine sediment above which adverse biological effects were frequently or always observed (Long et al. 1995). Similar tissue burdens of Cd and Zn in crabs held on tailings and control sediment for 90 d (Table 2) indicates either low bioavailability of these metals from tailings or, if they were bioavailable, that crabs were able to effectively depurate or store them during normal physiological processes.

Crabs were seldom observed buried in control sediment and never in tailings

**Table 2.** Metal concentrations (μg $g^{-1}$ dry weight) of control sediment and mine tailings used in a 90 d exposure study of ovigerous Tanner crabs; values are means (±1 SE), n=3. Effects range - median (ERM) values define the concentration of a particular contaminant in sediment above which adverse biological effects were frequently or always observed (Long et al. 1995).

| Metal | Control | Tailings | ERM Value |
|---|---|---|---|
| As | 4.70 (0.13) | 21.52 (1.78) | 70.0 |
| Cd | 0.54 (0.03) | 12.17 (1.09) | 9.60 |
| Cr | 103.0 (6.57) | 97.9 (0.90) | 370 |
| Cu | 11.75 (0.18) | 79.07 (2.66) | 270 |
| Ni | 47.30 (1.23) | 37.47 (0.83) | 51.6 |
| Pb | 5.54 (0.15) | 134.46 (3.26) | 218 |
| Zn | 54.36 (1.77) | 516.86 (41.70) | 410 |

during our study. From a submersible in the proposed discharge area for the AJ mine (Taku Inlet), we have observed ovigerous Tanner crabs partially buried in soft substrates of silt and very fine sand; compressive strength of these sediments was <0.10 kg $cm^{-2}$ and silt made up 65-85% of the sediment (*R. P. Stone, unpublished data*). Although grain-size composition of control sediment and tailings was similar for both treatments (<250μm; control sediment 63%, tailings 75%), tailings formed a much more compact substrate than the control sediment (compressive strength, 4.1 kg $cm^{-2}$ vs 0.13 kg $cm^{-2}$) possibly due to the high angularity of the grains (US EPA 1996c). In a laboratory study, ovigerous Tanner crabs avoided AJ mine tailings when given a choice, possibly due to the inability to bury in the substrate (Johnson et al. in press). The inability of females to bury in the substrate may promote unsuccessful oviposition and egg brooding, while increasing their risk to predation. In addition, an altered sea floor may not support an adequate prey population for foraging Tanner crabs.

We have shown that tailings similar to those that may be produced from a gold mine-mill complex and aged about 100 d in seawater were not deleterious to ovigerous Tanner crabs. Most mining operations, however, would probably not age tailings prior to STD. We believe the results of our study would have been the same had we used "fresh" tailings because leaching of metals from these tailings was minimal after 60 d.

We found no significant differences in survival of adult crabs, eggs, or larvae after exposure to tailings and control sediment for the last 90 d of the brood cycle. Tissue burdens of metals were also similar between treatments in this study and within safe consumption guidelines implemented in Europe. Although STD may not be a toxicological hazard to Tanner crabs, protection of essential habitat remains a priority. Location of STD sites in areas of low productivity and high natural sedimentation rates (e.g., large glacial river mouths) would have the least effect on this commercial resource; high natural sedimentation would accelerate recovery of the sea floor.

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