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Inorganic Analytes in Light-Footed Clapper Rail Eggs, in Their Primary Prey, and in Sediment From Two California Salt Marsh Habitats

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The salt marshes of California have undergone significant changes in the last century. The increased human population in California has reduced the viability of salt marshes by elimination or reduction of fresh water input, reduction of tidal flush, introduction of exotic predators, increased anthropogenic contamination, and a reduction in the total number of marshes (Goodbred *et al.*,1996).

The light-footed clapper rail (*Rallus longirostris levipes*) is believed to have been common in salt marshes and has an historical range extending from Santa Barbara County, California, to northern Baja California, Mexico (Grinnell *et al.*, 1918). Its diet is known to consist primarily of invertebrates (various crab species, California horn snail, *Cerithidea californica*), but softer material like vegetation, spiders, and insects that are not detectable in its regurgitated pellets may also be part of its diet (Jorgensen, 1975). They also take small (2-4 cm) intertidal fish (Ledig, pers. obs). The light-footed clapper rail is listed as an endangered species by both the federal and the California State governments (US Fish and Wildlife Service, 2001; California Department of Fish and Game, 2001).

Because of concern about contamination impacts on rail populations, we collected samples of addled rail eggs, crabs, and sediment from the salt marshes at Seal Beach National Wildlife Refuge in Orange County and Tijuana Slough National Wildlife Refuge in San Diego County, both in southern California. Previous analyses for organic contaminants showed no evidence that either organochlorine pesticides (primarily DDT metabolites) or polychlorinated biphenyls were affecting reproductive output of the light-footed clapper rail at Seal Beach or Tijuana Slough marshes (Goodbred *et al.,* 1996). Our tests for inorganic analytes, however, show potential threats from Cr and Cu, both from possible anthropogenic sources.

MATERIALS AND METHODS

Samples were collected during 1991 at Seal Beach and Tijuana Slough marshes. Light-footed clapper rail eggs that failed to hatch, composite samples of live shore crabs (*Pachygrapsus crassipes*), and composite samples of surficial sediments (< 5 cm deep) were collected in active rail breeding territories to assess contaminant

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levels within each salt marsh. Details of the collection process and the QA and QC are presented in Goodbred *et al.* (1996).

The eggs, crabs, and sediment were tested for a suite of 19 inorganic analytes (Al, As, B, Ba, Be, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Se, Sr, V, Zn) soon after collection in 1991. The Geochemical and Environmental Research Group (GERG) in College Station, Texas, performed the analytical tests under contract to the Patuxent Analytical Control Facility (PACF), part of the U.S. Fish and Wildlife Service. Samples were digested with acid and analyzed using cold vapor atomic absorption spectrometry (Hg), graphite furnace atomic absorption spectrometry (As, Se, Cd, Pb), or inductively coupled plasma emission spectroscopy. For each analytical sequence, blanks, duplicates, standard reference materials and analyte spikes (to assure accuracy) were used. The PACF also spot-checks results in their own laboratory. The blanks had a mean value (all analytes) of 0.86 μg (n=76) recovered, duplicates had a mean error of 14% (n=152) of the mean value, standard reference materials had a mean measurement of 99.4% (n=24) of the known value, and the mean spike recoveries were 99.5% (n=28) of the known value. All means are arithmetic means unless otherwise noted.

Comparisons of analyte concentrations among data sets were performed using analysis of variance (ANOVA). For data sets in which one or both of the distributions were not normal (Kolmogorov-Smirnov test) or had unequal variances (Levene Median test), the comparisons were performed using ANOVA on ranks. The differences between data sets were considered significant if $P \leq 0.05$. If half or more of the samples from a given data set were below the lower limit of detection (LLOD) for a given analyte, that analyte was considered below detection and no further statistical tests were performed on those samples. When less than half the samples were below the LLOD, the individual samples below detection were assigned values of one-half the detection limit and included in the data set for statistical treatment, a technique which minimizes nominal type I error rate (Clarke, 1998).

RESULTS AND DISCUSSION

Our inorganic analyses have not been previously reported. Of the 19 analytes for which the Seal Beach and Tijuana eggs were tested, eleven analytes had concentrations below detection in more than half of the samples (Al, As, B, Ba, Be Cd, Hg, Ni, Pb, Se, V) while the others (Cr, Cu, Fe, Mg, Mn, Mo, Sr, Zn) did not differ between the two sites (Table 1). In the crab samples, three were below detection (Be, Cd, Hg) and 14 did not differ between sites (Al, As, B, Ba, Cu, Fe, Mg, Mn, Ni, Pb, Se, Sr, V, Zn). Only Cr and Mo differed between sites due to concentrations below the detection limit in the Seal Beach samples (Table 2). In the sediment samples, three were below detection limits at both sites (Be, Hg, Se), one (Mo) was below detection at Tijuana Slough only, four (Cr, Cu, Ni, Pb) were higher at Seal Beach, one (Al) was higher at Tijuana Slough, and the rest (Al, As, B, Ba, Cd, Fe, Mg, Mn, Pb, Sr, V, Zn) did not differ between sites (Table 3).

Table 1. Concentrations (ppm, dry weight) of inorganic analytes in contents of failed-to-hatch eggs of the light-footed clapper rail from two marshes in southern California. Arithmetic means, geometric means (in parentheses), and ranges are indicated.

Analyte	Seal Beach (n = 3)	Tijuana Slough (n = 5)
Cr	1.81 (1.73) 0.95 - 3.85	2.17 (1.94) 0.95 - 3.85
Cu	2.94 (2.93) 2.63 - 3.37	3.34 (3.30) 2.80 - 4.03
Fe	63.33 (63.33) 62.00 - 64.80	78.12 (73.54) 42.90 - 112.0
Mg	367.0 (364.8) 317.0 - 415.0	644 (614.0) 411 - 1020
Mn	1.120 (1.12) 1.08 - 1.16	1.42 (1.22) 0.50 - 2.28
Mo	2.80 (2.75) 2.30 - 3.63	3.35 (2.81) 1.000 - 5.76
Sr	40.13 (39.98) 35.20 - 43.00	53.88 (49.89) 33.00 - 99.80
Zn	42.77 (42.74) 40.60 - 44.20	42.90 (42.58) 35.50 - 51.30

More than half the measurements were below the detection limit (in parentheses) for the following samples collected at both marshes: Al (<5), As (<0.5), B (<2), Ba (<1), Be (<0.1), Cd (<0.1), Hg (<0.1), Ni (<0.5), Pb (<0.5), Se (<0.5), V (<0.4)

Table 2. Concentrations (ppm, dry weight) of inorganic analytes in crabs from two marshes in California. Arithmetic means, geometric means (in parentheses), and ranges are indicated.

Analyte	Seal Beach (n = 5)	Tijuana Slough (n = 3)
Al	850.4 (786.1) 453 - 148	625.3 (615.5) 494 - 765
As	5.30 (5.24) 3.98 - 6.36	6.95 (6.92) 6.11 - 7.71
B	13.65 (13.03) 8.17 - 20.2	9.04 (8.80) 6.34 - 11.0
Ba	15.42 (14.75) 8.39 - 19.7	12.90 (12.76) 10.4 - 14.9
Cr	< 0.1	0.43 (0.41) 0.25 - 0.55
Cu	89.76 (86.16) 52.2 - 120	51.57 (47.50) 26.5 - 72.2
Fe	255.7 (220.2) 97.3 - 437	629.2 (379.1) 65.70 - 929
Mg	12600 (12240) 8280.0 - 15600	8293 (8232) 7420 - 9740
Mn	51.60 (45.76) 27.5 - 107	62.63 (59.70) 43.3 - 90.0
Mo	< 2	3.37 (2.55) 1.00 - 6.63
Ni	1.50 (1.13) 0.25 - 2.63	1.54 (1.31) 0.83 - 2.82
Pb	1.06 (1.03) 0.75 - 1.36	0.80 (0.61) 0.27 - 1.59
Se	0.95 (0.92) 0.68 - 1.42	1.14 (1.07) 0.74 - 1.73
Sr	1952 (1865) 1190 - 2640	2390 (2011) 1170 - 4430
V	1.16 (1.06) 0.57 - 1.68	1.06 (0.79) 0.25 - 1.90
Zn	63.06 (62.66) 53.8 - 74.1	63.53 (63.18) 54.4 - 68.8

More than half the measurements were below the detection limit (in parentheses) for the following samples collected at both marshes: Be (<0.1), Cd (<0.1), Hg (<0.1).
< indicates more than half of measurements were below detection limit indicated.

In the eggs, 17 analytes (Al, As, B, Ba, Be, Cd, Cr, Fe, Hg, Mg, Mn, Mo, Ni, Pb, Se, V, Zn) had concentrations similar to those in randomly-selected eggs of snow geese (*Anser c. caerulescens*; Hui *et al.,* 1998) and are not considered remarkable. However, these analyte burdens also provide some insight to more general aspects of the relationship of light-footed clapper rails to their environment and exposures.

Table 3. Concentrations (ppm, dry weight) of inorganic analytes in sediment from two marshes in California. Arithmetic means, geometric means (in parentheses), and ranges are indicated.

Analyte	Seal Beach (n = 5)	Tijuana Slough (n = 3)
Al*	26,600 (26,650) 25700 - 27800	31,500 (31,350) 27300 - 34100
As	6.912 (6.747) 4.61 - 8.81	15.45 (13.60) 7.94 - 26.4
B	69.580 (64.83) 46.7 - 125	83.53 (80.80) 55.4 - 99.7
Ba	26.38 (25.00) 14.3 - 39.4	75.67 (47.87) 22.7 - 177
Cd	1.652 (0.4873) 0.10 - 7.13	0.238 (0.2106) 0.10 - 0.335
Cr*	46.58 (46.39) 40.80 - 51.90	37.167 (36.92) 31.3 - 40.1
Cu*	45.56 (45.14) 39.2 - 56.5	32.03 (31.78) 26.6 - 35.6
Fe	47,520 (47,120) 41400 - 59800	66,333 (64,730) 4.69 - 7.71
Mg	9702 (9685) 8990 - 10700	38800 (22,080) 1010 - 9510
Mn	562.2 (547.5) 419 - 817	398.7 (393.2) 313 - 469
Mo	7.086 (6.405) 2.50 - 10.8	< 5
Ni*	35.94 (35.71) 30.7 - 40.4	23.83 (23.66) 21.0 - 27.9
Pb	69.66 (65.45) 49.7 - 124	42.60 (42.25) 35.9 - 49.3
Sr	83.10 (82.39) 71.2 - 102	108.9 (104.9) 70.6 - 134
V	96.88 (96.69) 90.4 - 106	118.0 (117.7) 108 - 128
Zn	193.6 (191.8) 153 - 230	154.0 (153.2) 133 - 171

More than half the measurements were below the detection limit (in parentheses) for the following samples collected at both marshes: Be (<0.1), Hg (<0.1), Se (<0.5).
< indicates more than half of measurements were below detection limit indicated.
* indicates significant differences ($P < 0.05$; ANOVA or ANOVA on ranks).

The highest mean concentration of Cr in the sediments (46 ppm at Seal Beach) is below the mean concentration of 125 ppm in the earth's crust (Langard & Norseth, 1986). All Cr concentrations in the crabs of this study were within the 0.4 - 4.2 ppm (dry weight) range found in crustaceans from unpolluted sites in the Gulf of Mexico (Horowitz & Presley, 1977).

The addled eggs at the Seal Beach and Tijuana Slough marshes had Cr concentrations similar to randomly-selected eggs of herring gulls (*Larus argentatus*; 2.7 ppm dry weight; Burger, 1994), and roseate terns (*Sterna dougallii*; 2.8 ppm dry weight; Burger, 1994) after those waterbirds had a significant exposure to Cr from anthropogenic sources (Gochfeld & Burger, 1998). However, common terns (*Sterna hirundo*) with normal fledging success had similar Cr concentrations (2.15 ppm; Connors *et al.*, 1975). Thus, it is not clear if the rails at Seal Beach and Tijuana Slough marshes had accumulated high levels of Cr in their eggs despite normal concentrations in the crabs and sediment or if they have normal concentrations. We were not able to find any reports of Cr in the eggs of other species or populations of rails.

The maximum Cr level in crabs in either of the marshes was well below 10 ppm. In contrast, Cr levels in sediment at both Seal Beach and Tijuana Slough marshes, although below the mean level in the earth's crust, are well above 30 ppm. Direct (possibly incidental) ingestion of soil can provide greater exposure to contaminants than the rest of the diet (Arthur & Gates, 1988) and the sediment in these marshes had much higher concentration of Cr than the crabs. Although sediment ingestion behavior of the light-footed clapper rail is unknown, dietary exposure is possible. Black bellied plovers (*Pluvialis squatarola*) ingest enough sediment to account for 29% of their diet (Hui & Beyer, 1998).

Laying chicken hens (*Gallus domesticus*) exposed to chromium transfer enough chromium to the embryo and eggshell to consider the egg a means of chromium excretion (Burger, 1994). Avian embryos exposed to excess Cr-III or Cr-VI suffer teratogenic effects (Asmatullah & Shakoori, 1998) and have damaged livers and red blood cells (Misra *et al.,* 1994).

Because the Cr concentrations in the sediments at Seal Beach and Tijuana Slough marshes are well within normal ranges, we suspect that there may be localized areas of high concentration at which the rails may be exposed. The Tijuana River, which drains into the Tijuana Slough, receives uncontrolled dumping and run-off from the Mexican city of Tijuana (Price, 1973). Its meandering course through the marsh may result in locations more conducive than others to the deposition of Cr. Further studies are needed to determine if this is the case. Another possibility is that Cr may be concentrated in food items other than the crabs. We did not test other food items.

The Seal Beach marsh is surrounded on three sides by the Naval Weapons Station Seal Beach (WPNSTA). Before the creation of the Seal Beach National Wildlife Refuge (SBNWR) from part of the WPNSTA, the Navy utilized numerous land-fill sites for disposal of a variety of refuse. Some of these sites are within current SBNWR boundaries and others are near it (Southwest Division Naval Facilities Engineering Command, 1995). Some landfills are deeper than the local water table that flows into the marsh. In addition, these landfills include chromates (Southwest Division Naval Facilities Engineering Command, 1995). Locations of high Cr concentration have not been documented.

Copper is absorbed by crabs from the water column but not from the sediment (Berge & Brevik, 1996). Crabs maintain a constant level within their body unless their regulatory systems are overwhelmed by high concentrations in the water (Rainbow, 1985). The crab *Carcinus maenas*, although not the crab we studied, is capable of regulating body copper concentrations at about 40 ppm (Rainbow, 1985). The crabs at Seal Beach and Tijuana Slough have pooled copper concentrations that vary over the range 26.5 - 120.0 ppm, indicating that some may be exposed to concentrations of copper in the water high enough to overwhelm their regulatory systems.

Water at Tijuana Slough and at Seal Beach marshes may contain elevated levels of copper for different reasons. Tijuana Slough receives water from the Tijuana River, which flows into the United States after passing the city of Tijuana in Mexico. The river is subject to uncontrolled dumping and runoff (Price, 1973). In contrast, Seal Beach marsh has no fresh water input and shares tidal flow with Anaheim Bay, which has a marina in the part closest to the slough at Seal Beach. Although no measurements were taken, copper from antifouling paint on boats in the marina may be carried into Seal Beach by tidal flow.

The mean concentrations of Cu in the addled eggs from Seal Beach (2.94 ppm) and Tijuana Slough (3.34 ppm) did not differ significantly. They are slightly higher than those reported for the healthy eggs of Audouin's gull (*Larus audouinii*; range = 2.38 – 2.95 ppm; Morera *et al.,* 1997), slightly lower than those reported for a healthy population of common terns (4.89 ppm; Connors *et al.*, 1975), and essentially the same as those reported for the randomly-selected eggs of lesser snow geese (3.0 ppm; Hui *et al.,* 1998). We were not able to find any reports of Cu in the eggs of other species or populations of rails. The high levels of Cu in crabs are not reflected in the Cu concentrations of the rail eggs.

The mean concentration of Sr in the rail eggs was about one order of magnitude greater than in the eggs of snow geese (Hui *et al.,* 1998). Because Sr is similar to calcium in its chemistry and marine animals are high in Sr (Venugopal & Luckey, 1978), we infer that the incidence of high Sr in the addled eggs is from the Sr in the shells and carapaces of tidal or marine prey items. We have no Ca measurements that would allow a comparison of Sr:Ca ratios in crabs and rail eggs. Such ratios may increase or decrease the strength of our inference. Because strontium is of very low toxicity to mammals and so ubiquitous in the marine environment (Venugopal & Luckey, 1978), we infer that the levels of Sr we report do not indicate a health hazard.

If the exposures of rails to Cr at Seal Beach and Tijuana Slough is excessive, those exposures may be contributing to the "struggling" aspect of these rail populations. In the nine years after the collection of samples (1991-1999) the rail population at Seal Beach rose from 6 pairs in 1989 to a peak of 66 pairs in 1994 (after predator control efforts) and then declined to 15 pairs in 1999; and at the Tijuana Slough the population was at 14-17 pairs during 1988 - 1990 and then rose to 80 pairs in 1999 (Zembal and Hoffman 1999). Concentrations of Cr in eggs at Tijuana are unknown after 1991.

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REFERENCES

Arthur III WJ, Gates RG (1988) Trace element intake via soil ingestion in pronghorns and in black-tailed jackrabbits. J Range Manage 41:162-166

Asmatullah SNQ, Shakoori AR (1998) Hexavalent chromium-induced congenital abnormalities in chick embryos. J Appl Toxicol 18:167-171

Berge JA, Brevik EM (1996) Uptake of metals and persistent organochlorines in crabs (*Cancer pagurus*) and flounder (*Platichthys flesus*) from contaminated sediments: mesocosm and field experiments. Mar Pollut Bull 33:46-55

Burger J (1994) Heavy metals in avian eggshells: another excretion method. J Toxicol Environ Health 41:207-220

California Department of Fish and Game (2001) website http://www.dfg.ca.gov/whdab/assets/docs/TEAnim2001_Jan.pdf. Updated January

Clarke JU (1998) Evaluation of censored data methods to allow statistical comparisons among very small samples with below detection limit observations. Environ Sci Technol 32:177-183

Connors PG, Anderlini VC, Risebrough RW, Gilbertson M, Hays H (1975) Investigations of heavy metals in common tern populations. Canandian Field-Naturalist 89:157-162.

Gochfeld M, Burger J (1998) Temporal trends in metal levels in eggs of the endangered roseate tern (*Sterna dougallii*) in New York. Environ Res A77:36-42

Goodbred SL, Ledig DB, Roberts CA (1996) Organochlorine contamination in eggs, prey and habitat of light-tooted clapper rails in three southern California marshes. US Fish and Wildlife report. Carlsbad Field Office, Carlsbad, CA

Grinnell J, Bryant HC, Storer TI (1918) The game birds of California. Univ. Calif. Press, Berkeley

Horowtiz A, Presley BJ (1977) Trace metal concentrations and partitioning in zooplankton, neuston, and benthos from the south Texas outer continental shelf. Arch Environ Contam Toxicol 5:241-255

Hui CA, Beyer WN (1998) Sediment ingestion of two sympatric shorebird species. Science Total Environ 2124:227-233

Hui CA, Takekawa JY, Baranyuk VV, Litvin KV (1998) Trace element concentrations in two subpopulations of lesser snow geese from Wrangel Island, Russia. Arch Environ Contam Toxicol 34:197-203

Jorgensen PD (1975) Habitat preference of the light-footed clapper rail in Tijuana marsh, California. MS Thesis, San Diego State University, San Diego, California

Langard S, Norseth T (1986) Chromium. In: Friberg LG, Nordberg F, Vouk VB (Eds) Handbook on the toxicology of metals, second edition, Vol. II: specific metals. Elsevier, Amsterdam. p 185-210

Misra J, Alcedo JA, Wetterhahn KE (1994) Two pathways for chromium(VI)-induced DNA damage in 14-day hick embryos: Cr-DNA binding in liver and 9-oxo-2'-deoxyguanosine in red blood cells. Carcinogenesis 15:2911-2917

Morera M, Sanpera C, Crespo S, Jover L, Ruiz X (1997) Inter- and intraclutch variability in heavy metals and selenium levels in Audouin's gull eggs from the Ebro Delta, Spain. Arch Environ Contam Toxicol 33:71-75

Price JA (1973) Tijuana: Urbanization in a border culture. U Notre Dame Press, Notre Dame, IN

Rainbow PS (1985) Accumulation of Zn, Cu and Cd by crabs and barnacles. Estaur Coast Shelf Sci 21:669-686

Southwest Division Naval Facilities Engineering Command (1995) Naval weapons station (NWS) Seal Beach, California technical memorandum stationwide background study final. 1220 Pacific Highway, San Diego, California

U. S. Fish and Wildlife Service (2001) website http://ecos.fws.gov/servlet/TESSSpeciesReport/generate. Updated 21 February

Venugopal B, Luckey TD (1978) Metal toxicity in mammals 2. Plenum Press, New York

Zembal R, Hoffman SM (1999) Light-footed clapper rail management and study, 1999. Report to US Fish and Wildlife Service, Carlsbad, California.