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Mercury and Trace Elements in Crayfish from Northern California

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Abstract We collected two species of crayfish, *Pacifastacus leniusculus* and *Procambarus clarkii*, from Cache and Putah Creeks, California, and analyzed them for mercury and trace elements. Trace elements were higher in carcasses in 40 cases, higher in tails in 5 cases, and not different in 35 cases; no concentration exceeded levels considered harmful. Mercury concentrations were similar among sites, with no overall sex or species effect in tails. Mercury and methylmercury concentrations were higher in tails at all sites. Methylmercury concentrations in crayfish tails (0.156–0.256 μg/g) exceeded concentrations reported in health advisories for consumption of fish and crayfish from these watersheds.

Keywords Mercury · Crayfish · California · Trace elements

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The Cache Creek and Putah Creek watersheds, located within California's North Coast Range, include areas with abundant geologic sources of mercury (Hg) and a long history of Hg contamination (Rytuba 2000). Waterways in the two watersheds that have been listed as impaired by Hg contamination under Section 303(d) of the Clean Water Act include: Cache Creek from Clear Lake Dam to the Cache Creek Settling Basin and Putah Creek from Solano Lake to the Putah Creek sinks (State Water Resources Control Board 2006). Sources of Hg in the Coast Range include geothermal springs, agricultural runoff, erosion of naturally Hg-enriched soils, and atmospheric deposition, but most of the Hg exported from both the Cache Creek and Putah Creek watersheds originates from historic Hg mining operations (Foe and Croyle 1998).

Crayfish have been used as bioindicators of Hg in the environment because they accumulate Hg, primarily as methylmercury (MeHg) (Scheuhammer and Graham 1999; Simon and Boudou 2001). The omnivorous diet of the crayfish commonly includes algae and other plant material, aquatic insects, snails, and detritus. Crayfish are eaten by fish, mammals, birds, and humans. Recent health advisories for Putah and Cache Creeks provide guidelines for consumption of crayfish potentially contaminated with Hg (Gassel et al. 2005, 2006).

We evaluated the bioaccumulation of Hg and other elements in signal crayfish (*Pacifastacus leniusculus*) and red swamp crayfish (*Procambarus clarkii*) to estimate the potential hazard of this intermediate component of the food web. Prior to this study, data were not available on Hg or other toxic elements in crayfish in Cache or Putah creeks. Focusing on mercury, we sought to characterize trends in crayfish tissue concentrations by site, species, crayfish size, or sex (Heit and Fingerman 1977). The signal crayfish, native to the Pacific Northwest, including a small portion

of northern California, has been introduced throughout California. The red swamp crayfish, native to northeastern Mexico and south-central United States, is an introduced species to California.

Materials and Methods

The Cache Creek watershed is located about 130 km north of San Francisco, primarily in Lake, Colusa, and Yolo counties, but also Napa, Mendocino, and Sonoma counties. Cache Creek flows into the Cache Creek Settling Basin and then the Yolo Bypass east of the city of Woodland; the bypass eventually empties into the Sacramento River south of the city of Sacramento. The Putah Creek watershed is located south of and adjacent to the Cache Creek watershed in Napa, Lake, Yolo, and Solano counties. Putah Creek originates in Lake County and flows into Lake Berryessa, a reservoir created by construction of the Monticello Dam. Putah Creek empties into the Yolo Bypass east of the city of Davis.

Ten crayfish were collected at each of four sites from late July to early October in 1998. Signal crayfish were collected from Cache Creek at Buck Island, in Lake County, about 34 km downstream of the Clear Lake Dam (38°55′37″N, 122°22′11″W) and from Putah Creek in Yolo County, about 6.5 km downstream of the Monticello Dam (38°30′15″N, 122°02′26″W). Red swamp crayfish were collected at the Cache Creek Settling Basin in Yolo County east of the city of Woodland (38°41′10″N, 121°40′36″W) and from the west-side toe drain of the Yolo Bypass, located within the Yolo Basin Wildlife Area in Yolo County, just east of the city of Davis (38°32′10″N, 121°37′48″W).

Minnow traps baited with salmon heads were set in the afternoon and checked within about 4 h after dark. Crayfish were removed from the traps, kept in Ziploc® plastic bags on wet ice in a cooler for about 2 h, and then were placed in a plastic tub of deionized (DI) water for 24 h. Afterwards, each crayfish was thoroughly rinsed in DI water, placed in a labeled Ziploc® plastic bag, and frozen for a minimum of 24 h. Each crayfish was partially thawed, and the total mass (±0.1 g) was determined using an electronic balance. We used calipers to measure the length of the carapace (±0.1 mm) from the rostral tip to the posterio-median end of the cephalothorax. The sex of each crayfish was determined based on external characteristics. Each crayfish was dissected in a semi-frozen state to reduce contamination of adjoining body parts and loss of fluids. After the exoskeleton and intestine were removed, the abdominal muscle (tail) was stored frozen in a VWR TraceClean™ jar. The remainder of each crayfish (the carcass) was frozen in a separate VWR TraceClean™ jar.

Crayfish tissues were submitted to the Trace Element Research Laboratory in College Station, Texas, for chemical analyses in December 1998. Tissue samples were homogenized either after freeze-drying or with a Tekmar Tissumizer (Tekmar Co., Cincinnati, OH, USA) and subsampled. Samples were digested with nitric acid, sulfuric acid, potassium permanganate, and potassium persulfate in polypropylene tubes in a water bath at 90–95°C. Arsenic (As), cadmium (Cd), and lead (Pb) were analyzed by graphite furnace atomic absorption spectroscopy, and selenium (Se) was analyzed by atomic fluorescence spectroscopy. Cold vapor atomic absorption spectroscopy was used for Hg. The analytical procedure used to extract MeHg followed the method of Uthe et al. (1972). Other elements were measured by inductively coupled plasma optical emission spectroscopy. Concentrations of Hg and MeHg are presented as μg/g on a wet-weight basis unless otherwise specified; for all other elements, results are on a dry-weight basis. Mean values for elements are geometric means.

Mean detection limits for analyzed elements (μg/g dry weight) were: aluminum (Al), boron (B), copper (Cu), and manganese (Mn) = 0.48, arsenic (As) = 0.39, barium (Ba), lead (Pb), and selenium (Se) = 0.19, beryllium (Be) = 0.10, calcium (Ca) = 96.7, cadmium (Cd) = 0.02, chromium (Cr) and nickel (Ni) = 0.29, iron (Fe) = 2.42, magnesium (Mg) = 1.94, molybdenum (Mo) and vanadium (V) = 0.24, phosphorus (P) = 9.7, sulfur (S) = 19.4, strontium (Sr) = 0.02, and zinc (Zn) = 0.73. The mean detection limits (wet weight) for Hg and MeHg were 0.007 and 0.020 μg/g, respectively. Procedural blanks, spiked samples, standard reference materials (NRCC DOLT 2, DORM 2), and duplicate samples were analyzed for quality control. Recoveries from spikes and reference materials ranged from 80.8% to 112% and 85.9% to 104%, respectively. Although duplicate spike analyses were well within acceptable ranges for Ni, recovery from reference material was 165%. Results for Ni were not adjusted. Duplicate analyses and blank recoveries were within an acceptable range of ±15%.

Data analyses were performed using SigmaStat statistical software (Ver. 3.1, Systat Software Inc, Point Richmond, CA, USA) on log-transformed concentrations to achieve homogeneity of variance. We used two-way ANOVA on Hg and MeHg in tails, carcasses, and whole body (tail and carcass combined) concentrations to detect any sex or species effect. We used one-way ANOVA to compare results between sites and within sites by sex to detect any trends in Hg or MeHg in the various combinations for each tissue; the Bonferroni method for pairwise multiple comparisons was used. In cases where tests for normality or equal variance failed ($p > 0.05$), we performed a Kruskal–Wallis one-way ANOVA on ranks and

conducted pairwise multiple comparisons using the Holm–Sidak method. We used Student's *t*-test to compare contaminant concentrations between tails and carcasses across all sites and by site and also to compare size parameters within species. We used regression analysis to determine correlations between Hg or MeHg concentrations and crayfish carapace length or total body mass. The significance level for all tests was $\alpha = 0.05$.

Results and Discussion

Methylmercury accounted for nearly 100% of total Hg burdens from crayfish at all four sites (Table 1). As found by Pennuto et al. (2005), concentrations of both Hg and MeHg were higher ($p < 0.01$) in the tail than in the remainder of the carcass. Mercury in the tails comprised from 80.6% (Putah Creek) to 87.5% (Cache Creek Settling Basin) of the Hg in the total body. This is important because the tail of the crayfish is most commonly consumed by humans.

Total Hg and MeHg did not significantly differ between any of the four sites in either tails or carcasses. With data from all four sites pooled, two-way ANOVA detected no significant sex or species effect in total Hg or MeHg in the tail. Although total Hg in the carcass was higher in signal crayfish (0.046 vs. 0.034 μg/g, $p = 0.004$), carcass MeHg did not differ by sex or species. Signal crayfish were larger than red swamp crayfish ($p < 0.01$) in both mean carapace length (58.1 vs. 49.8 mm, respectively) and mean body mass (59.2 vs. 30.1 g, respectively). Neither measure of crayfish size, however, was strongly correlated with tissue concentrations of total Hg or MeHg. The overall correlation of carapace length to total body Hg ($r^2 = 0.01$) and MeHg ($r^2 = 0.02$), and total crayfish mass to total body Hg ($r^2 = 0.001$) and MeHg ($r^2 = 0.06$) were low.

Within sex, the only difference was for red swamp crayfish. The mean MeHg concentration in the tails of males from the Cache Creek Settling Basin (0.25 μg/g) was higher ($p = 0.038$) than that in males from the Yolo Basin Wildlife Area (0.11 μg/g). Only two sex effects were detected within sites. At the Yolo Basin Wildlife Area, the mean MeHg concentration in the tail of female red swamp crayfish (0.22 μg/g) was significantly higher ($p = 0.032$) than in males (0.11 μg/g). This difference was expected because the females were larger (mean carapace length = 50.1 vs. 45.4 mm) and heavier (mean mass = 30.3 vs. 22.9 g) than the males at this site. At the Putah Creek site, however, the mean total Hg in carcasses of female signal crayfish was higher ($p = 0.043$) than in the males (mean = 0.061 vs. 0.035 μg/g), even though mean length (62.5 vs. 57.9 mm) and mean mass (66.7 vs. 67.9 g) were not different between the sexes.

Mean MeHg concentrations in crayfish tails at all four sites (Table 1) were higher than the average reported in crayfish for the January 2005 Cache Creek health advisory for fish consumption (Gassel et al. 2005). The Cache Creek Settling Basin (0.241 μg/g) and Putah Creek (0.256 μg/g) sites had the highest MeHg concentrations in crayfish tails, 1.3–1.8 times higher than the concentrations reported in the Cache Creek (0.14 μg/g) and Mokelumne River (0.18 μg/g) health advisories, and similar to those in health advisories for Putah Creek (0.21 μg/g) (Gassel et al. 2006) and the Consumnes River (0.29 μg/g) (Klasing et al. 2006). Mean MeHg concentrations found in crayfish at Buck Island (0.171 μg/g) and the Yolo Basin Wildlife Area (0.156 μg/g) were similar to the Cache Creek and Mokelumne River advisory concentrations.

The highest total Hg concentrations in crayfish have been from river systems contaminated by chloralkali plants and pulp mills. Mercury in crayfish tails (*Orconectes virilis*) sampled from the stomachs of hooded mergansers (*Lophodytes cucullatus*) from the Wabigoon-English River system in Ontario, Canada, ranged from 4.7 to 9.6 μg/g (Vermeer et al. 1973). Wren and Stokes (1986) sampled crayfish (*Cambarus* spp.) from the same system and found

Table 1 Geometric mean total mercury (THg) and methylmercury (MeHg) (μg/g, wet weight) in crayfish collected from Cache Creek and Putah Creek watersheds, California, 1998

	Red swamp crayfish						Signal crayfish					
	Cache Creek Settling Basin			Yolo Basin Wildlife Area			Buck Island			Putah Creek		
	Carcass	Tail	Total Body	Carcass	Tail	Total Body	Carcass	Tail	Total Body	Carcass	Tail	Total Body
THg	0.037	0.260	0.080	0.031	0.178	0.059	0.045	0.190	0.068	0.046	0.254	0.066
n	10	10	10	10	10	10	10	10	10	10	10	10
SD	0.014	0.140	0.023	0.018	0.099	0.028	0.015	0.130	0.018	0.024	0.054	0.025
MeHg	0.034	0.24	0.074	0.031	0.156	0.054	0.043	0.170	0.066	0.055	0.256	0.076
n	6	6	6	6	6	6	5	5	5	3	3	3
SD	0.014	0.090	0.020	0.025	0.071	0.031	0.010	0.130	0.028	0.014	0.026	0.014

Mercury and MeHg concentrations were significantly higher in tails than in carcasses at all sites ($p < 0.01$)

Table 2 Geometric mean element concentrations (μg/g, dry weight) (and sample size), arithmetic mean percent moisture, and significant differences (*) in crayfish from Cache Creek and Putah Creek watersheds, CA, 1998

Element	Cache Creek Settling Basin (4)		Yolo Basin Wildlife Area (4)		Buck Island (3)		Putah Creek (3)	
	Carcass	Tail	Carcass	Tail	Carcass	Tail	Carcass	Tail
Al	289*	8.16	291*	7.24	270*	7.20	146*	5.41
As	0.50*	0.24	1.10*	0.54	0.87	0.56	0.85	0.68
B	17.3*	4.93	9.47*	2.59	8.31*	3.13	3.58*	0.98
Ba	424*	3.46	488*	3.49	298*	3.60	263*	2.82
Ca	151,000*	2,080	153,000*	2,850	138,000	3,310	134,000*	3,090
Cd	0.10	0.03	0.08*	0.03	0.05	0.02	0.07	0.04
Cr	1.12*	0.44	1.07	0.42	1.20	0.59	0.76	0.61
Cu	65.7*	36.6	62.4	52.6	86.4*	51.9	47.8*	20.7
Fe	378*	20.6	292*	17.2	263*	19.3	134	14.2
Mg	4,300*	1,570	4,400*	1,560	3,850*	1,480	3,570*	1,360
Mn	221*	8.28	316*	8.96	106*	7.32	99.9*	7.55
Mo	NC[a]	NC	0.23	0.36	ND[b]	NC	ND	0.28
Ni	5.86*	2.14	3.78	1.47	2.70	0.98	2.86*	1.24
Pb	0.21	NC	0.26	NC	0.13	0.08	ND	ND
Se	0.56	0.85	0.61	1.73*	0.45	0.75	0.71	0.95
Sr	1,030*	11.8	1,060*	15.0	886*	19.6	689	13.5
V	1.03	NC	1.41	NC	0.74	ND	0.71	ND
Zn	80.2*	71.4	81.9	82.4	75.4	92.7*	99.0	108
P	9,500	9,670	10,800	9,430	12,000	9,500	13,600*	9,250
S	2,580	6,630*	2,900	7,240*	3,730	8,070*	4,310	8,820
% moisture	62.2	80.9	69.0	82.1	74.6	83.4	74.2	84.1

[a] NC = geometric mean not calculated because ≤50% of samples were below the limit of detection (See "Materials and methods")

[b] ND = not detected; all samples were below the limit of detection (See Materials and methods)

Hg concentrations up to 2.41 μg/g in tails. Sheffy (1978) analyzed crayfish (primarily *Orconectes* sp.) from the Wisconsin River, a system contaminated by chloralkali plants and pulp mills, and found tail Hg concentrations up to 0.56 μg/g. Signal crayfish sampled from the Columbia River in Washington showed tail Hg concentrations up to 2.18 μg/g downstream of a chloralkali plant (Buhler et al. 1973). A site in Nevada with a moderate level of Hg contamination from gold ore processing produced results in tails of signal crayfish similar to the Cache Creek sites, with total Hg concentrations ranging from 0.075 to 0.344 μg/g (estimated from dry weight concentrations and percent moisture) (Gustin et al. 2005). Mean Hg concentrations in crayfish from all four Cache Creek study sites were four to five times higher than those from the Gustin et al. (2005) reference site.

Of the 21 other elements analyzed, only Be was not detected in any sample. Forty of the 80 combinations of other elements and sites (Table 2) had significantly higher geometric mean concentrations in the carcass than in the tail muscle. In addition to Hg and MeHg at all four sites, only Se at one site, Zn at one site, and S at three sites had higher concentrations in the tail muscle; the other comparisons for these three elements were not different. In 25 element-site combinations, there was no difference between tail and carcass, while Mo, Pb, and V at 10 combinations had too few samples with detected amounts to be tested (Table 2).

Although wildlife tend to consume the whole crayfish, humans normally consume only the tail muscle. Thus, those elements that accumulate in the tail are most likely to affect humans adversely. Besides Hg, elements of concern found in the sampled crayfish, including As, Cd, Cr, Ni, Pb, Zn, and Se, were generally not concentrated in the tail, and concentrations were lower than those considered harmful to human or crayfish health (e.g., Eisler 1985, 1988).

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