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Mercury contamination in three species of anuran amphibians from the Cache Creek Watershed, California, USA

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Abstract Fish and wildlife may bioaccumulate mercury (Hg) to levels that adversely affect reproduction, growth, and survival. Sources of Hg within the Cache Creek Watershed in northern California have been identified, and concentrations of Hg in invertebrates and fish have been documented. However, bioaccumulation of Hg by amphibians has not been evaluated. In this study, adult and juvenile American bullfrogs (*Lithobates catesbeianus*) and foothill yellow-legged frogs (*Rana boylii*), adult Northern Pacific treefrogs (*Pseudacris regilla*), and larval bullfrogs were collected and analyzed for total Hg. One or more species of amphibians from 40% of the 35 sites had mean Hg concentrations greater than the US Environmental Protection Agency's tissue residue criterion for fish (0.3 μg/g). Of the bullfrog tissues analyzed, the liver had the highest concentrations of both total Hg and methyl mercury. Total Hg in carcasses of bullfrogs was highly correlated with total Hg in leg muscle, the tissue most often consumed by humans.

Keywords Amphibians · Bioaccumulation · American bullfrog · Cache Creek · California · Foothill yellow-legged frog · *Lithobates catesbeianus* · Mercury · Northern Pacific treefrog · *Pseudacris regilla* · *Rana boylii*

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Introduction

Amphibians may be adversely affected by exposure to environmental mercury (Hg), especially in its more bioavailable form, methylmercury (MeHg). As shown in laboratory studies with southern leopard frog (*Rana sphenocephala*) larvae, amphibian development may be adversely affected and survival through metamorphosis may be decreased by dietary Hg (Unrine et al. 2004). Other effects may include impaired reproduction, growth inhibition, behavioral modification, and various sublethal effects (Zillioux et al. 1993). Of Hg, cadmium, copper, manganese, and zinc, Jayaprakash and Madhyastha (1987) found that Hg was the most toxic to larval ornate narrow-mouthed toads (*Microhyla ornata*). Teratogenic and lethal effects of Hg have also been documented for other larval amphibians (Dial 1976; Chang et al. 1974; Punzo 1993). In addition to being at risk for Hg toxicity themselves, amphibians may play a role in the transport of Hg from the aquatic to the terrestrial environment as well as the conversion of elemental Hg to the more bioavailable MeHg (Unrine et al. 2007).

There is growing evidence that some amphibians are declining or have disappeared from significant parts of their historical ranges in the western USA (Blaustein et al. 1994; Jennings 1995; Fisher and Shaffer 1996). The role of contaminants in these declines is unclear (Cory et al. 1970; Hayes and Jennings 1986; Jennings 1988), but contaminants may be affecting species in specific areas (Davidson et al. 2001; Davidson 2002; Sparling et al. 2001). Of most concern in the Cache Creek Watershed is the effect of Hg on the native foothill yellow-legged frog (*Rana boylii*), a California species of special concern (Jennings and Hayes 1994).

The Cache Creek Watershed, located within the North Coast Range of California (Fig. 1), is an area with abundant geologic sources of Hg and a long history of Hg contamination (Rytuba 2000). Waterways in the Cache Creek Watershed listed as impaired by Hg contamination by Section 303(d) of the Clean Water Act include: lower Cache Creek, Clear Lake, Davis Creek Reservoir, Harley Gulch, Bear Creek, and Sulphur Creek (Central Valley Regional Water Quality Control Board 2003). Domagalski (2001) reported high concentrations of Hg in both water and streambed sediments in the Cache Creek Watershed. Studies conducted by the California Regional Water Quality Control Board during 1996–1998 confirmed that Cache Creek was a major source of Hg to the Sacramento–San Joaquin River Delta and San Francisco Bay Estuary (Foe and Croyle 1998). Sources of Hg in the Cache Creek Watershed include geothermal springs, agricultural runoff, erosion of naturally Hg-enriched soils, and atmospheric deposition, but the majority of the Hg exported from the watershed originates from historic Hg mining operations in the upper watershed (Foe and Croyle 1998).

Information on the concentrations of Hg in water, sediments (Foe and Croyle 1998; Domagalski 2001; Domagalski et al. 2004), invertebrates (Slotton et al. 1997), and fish (Slotton et al. 1995) from the Cache Creek Watershed have helped define the sources of Hg in the watershed and the magnitude of its contamination. However, more information on Hg concentrations in the upper trophic levels, especially amphibians, is needed. Amphibians may be useful indicators of metal contamination (Cooke 1981), especially where fish cannot survive. They bioaccumulate and are particularly sensitive to metals, have obligate aquatic larval stages, and sometimes spend their entire life cycle in a given pond or reach of a stream.

This study of Hg bioaccumulation in amphibians was part of a larger study conducted in cooperation with the US Fish and Wildlife Service that evaluated Hg bioaccumulation by macroinvertebrates, amphibians, fish, and insectivorous birds from the Cache Creek Watershed. In this paper, we focus on an evaluation of Hg in three species of amphibians. The objectives of this part of the study were to: (1) quantify Hg bioaccumulation in larval, juvenile, and adult amphibians inhabiting the watershed, (2) relate Hg bioaccumulation by these amphibians to sources of Hg within the watershed, and (3) evaluate Hg and MeHg concentrations in various tissues of American bullfrogs (*Lithobates catesbeianus*) collected from the watershed.

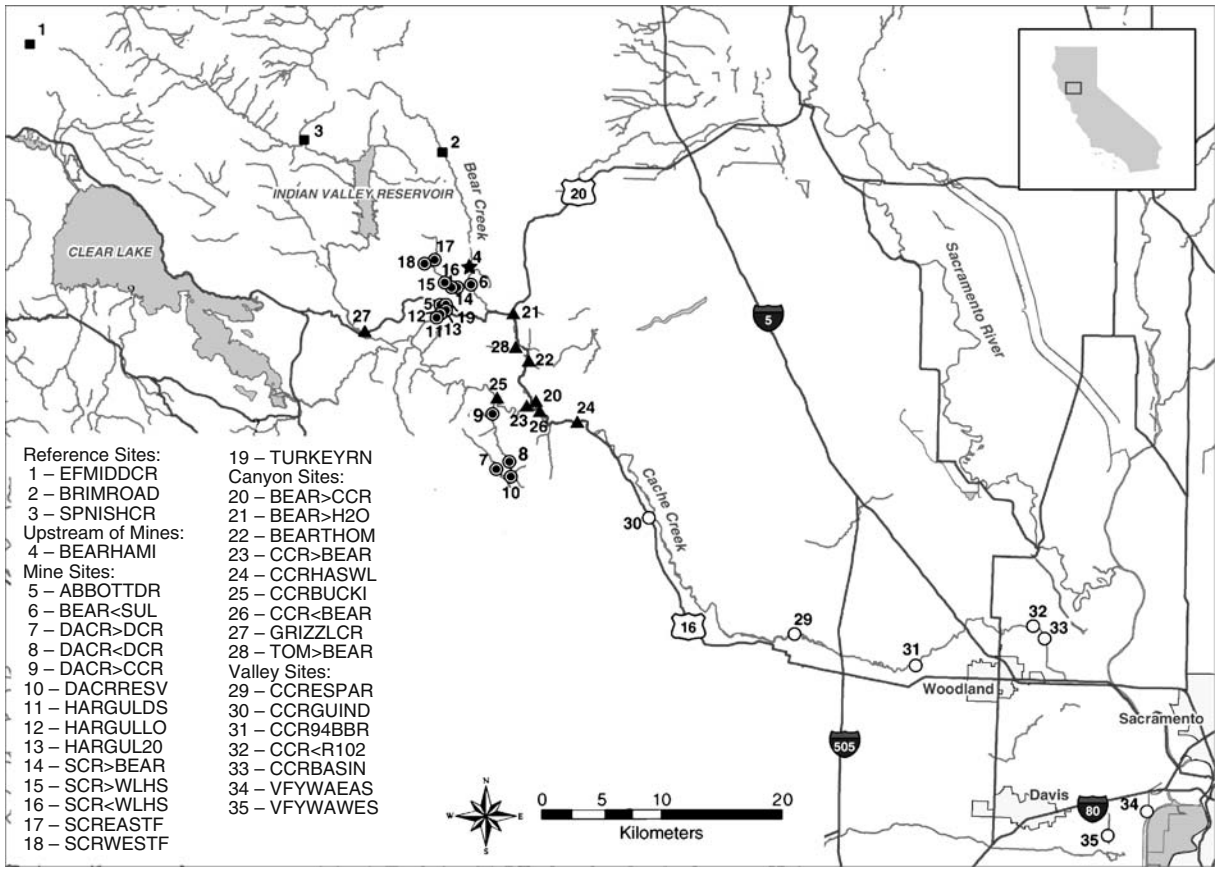

Fig. 1 Sites, by region, within the Cache Creek Watershed sampled for amphibians during 1997–1998. Reference sites indicated by a *blackened square*, Mine sites indicated by a *bulls eye*, Canyon sites indicated by a *triangle*, the site between reference and mine (BEARHAMI) indicated by a *star*, and Valley sites indicated by an *open circle*. Samples collected per site and per year are listed in Table 1

Materials and methods

Study area

The 2,950-km^2 Cache Creek Watershed is located in the North Coast Range of California, about 130 km north of San Francisco (Fig. 1). The watershed is primarily located in Lake, Colusa, and Yolo counties, but extends into parts of Napa, Mendocino, and Sonoma counties. Study sites on the main stem of Cache Creek ranged from Buck Island downstream to the Yolo Basin Wildlife Area and included sites on Bear Creek, Sulphur Creek, Harley Gulch, Davis Creek, Grizzly Creek, and three references sites: East Fork of Middle Creek, Mill Creek at Brim Road, and North Fork of Cache Creek at Spanish Creek (Fig. 1). Specific study sites within these reaches were selected based on current knowledge of Hg contamination, accessibility, and the presence of appropriate study organisms.

For geographic comparison of Hg concentrations, the study area was classified into four major regions based primarily on proximity to Hg mine sites and stream gradient (Table 1 and Fig. 1). Uniform atmospheric deposition of Hg was assumed throughout the watershed and was not considered in the analyses. The three reference sites were located from 13.6 to 50.2 km from the nearest mine site in the upper reaches of the

Table 1 Collection sites, by region, and numbers of American bullfrogs (BULL), foothill yellow-legged frogs (FYLF), and northern Pacific treefrogs (PATR) collected from the Cache Creek Watershed, 1997–1998 (map key refers to Fig. 1)

Map key	Site description	Site code	Latitude	Longitude	Samples collected (1997/1998)		
					BULL	FYLF	PATR
Reference sites							
1	East Fork Middle Creek	EFMIDDCR	39°15′09″ N	122°57′00″ W	U/N[a]	3/N	U/N
2	Mill Creek above Brim Road	BRIMROAD	39°09′45″ N	122°26′59″ W	3/5	3/U	U/U
3	Spanish Creek	SPNISHCR	39°10′17″ N	122°37′05″ W	U/N	3/U	U/N
Total samples from reference sites					3/5	9/0	0/0
Site above mines							
4	Bear Creek at Hamilton Canyon	BEARHAMI	39°03′24″ N	122°24′41″ W	6/N[b]	3/N	3/N
Total samples from above mine site					6/0	3/0	3/0
Mine sites							
5	Abbott Mine Drain	ABBOTTDR	39°00′56″ N	122°26′28″ W	N/U	N/1	N/U
6	Bear Creek below Sulphur Creek	BEAR<SUL	39°02′22″ N	122°24′28″ W	6/N[b]	3/N	3/N
7	Davis Creek above Davis Creek Res.	DACR>DCR	38°51′49″ N	122°22′13″ W	6/N[c]	3/N	3/N
8	Davis Creek below Davis Creek Res.	DACR<DCR	38°51′54″ N	122°21′20″ W	3/N	U/N	U/N
9	Davis Creek above Cache Creek	DACR>CCR	38°55′45″ N	122°22′41″ W	N/U	N/3	N/U
10	Davis Creek Reservoir	DACRRESV	38°51′29″ N	122°22′03″ W	3/N	U/N	U/N
11	Harley Gulch downstream of Fork	HARGULDS	39°00′36″ N	122°26′04″ W	U/U	3/1	1/U
12	Harley Gulch, Lower W. Fork	HARGULLO	39°00′39″ N	122°26′03″ W	N/U	N/U	N/4
13	Harley Gulch, Upper W. Fork	HARGUL20	39°00′55″ N	122°26′23″ W	N/U	N/U	N/4
14	Sulphur Creek Above Bear Creek	SCR>BEAR	39°02′13″ N	122°24′38″ W	N/U	N/3	N/U
15	Sulphur Creek Above Wilbur Hot Springs	SCR>WLHS	39°01′59″ N	122°25′47″ W	U/N	3/N	3/N
16	Sulphur Creek Below Wilbur Hot Springs	SCR<WLHS	39°02′15″ N	122°24′56″ W	N/U	N/3	N/U
17	Sulphur Creek, East Fork	SCREASTF	39°03′37″ N	122°27′27″ W	N/U	N/5	N/U
18	Sulphur Creek, West Fork	SCRWESTF	39°03′23″ N	122°27′39″ W	N/U	N/5	N/U
19	Turkey Run Drain	TURKEYRN	39°00′57″ N	122°26′26″ W	U/U	1/U	U/1
Total samples from mine sites					18/0	13/21	10/9
Canyon sites							
20	Bear Creek above Cache Creek	BEAR>CCR	38°55′42″ N	122°20′01″ W	3/N	3/N	U/N
21	Bear Creek above Hwy 20	BEAR>H20	39°00′42″ N	122°21′40″ W	3/10[b]	U/U	U/U
22	Bear Creek at Thompson Canyon	BEARTHOM	38°58′19″ N	122°20′26″ W	U/3	3/1	U/U
23	Cache Creek above Bear Creek	CCR>BEAR	38°55′34″ N	122°20′00″ W	2/N	2/N	U/N
24	Cache Creek at Camp Haswell Bridge	CCRHASWL	38°54′36″ N	122°16′43″ W	3/N	U/N	U/N
25	Cache Creek at Buck Island	CCRBUCKI	38°55′50″ N	122°22′39″ W	N/2	N/1	N/U
26	Cache Creek below Bear Creek	CCR<BEAR	38°55′29″ N	122°19′55″ W	3/N	U/N	U/N
27	Grizzly Creek	GRIZZLCR	38°59′38″ N	122°31′30″ W	N/U	N/2	N/U
28	Thompson Canyon above Bear Creek	TOM>BEAR	38°58′26″ N	122°20′41″ W	N/U	N/2	N/6

Table 1 (continued)

Map key	Site description	Site code	Latitude	Longitude	Samples collected (1997/1998)		
					BULL	FYLF	PATR
Total samples from canyon sites					14/15	8/6	0/6
Valley sites							
29	Cache Creek at Esparto Bridge	CCRESPAR	38°42′48″ N	122°00′35″ W	3/N	A[a]	U/N
30	Cache Creek at Guinda Bridge	CCRGUIND	38°49′43″ N	122°10′57″ W	3/N	A	U/N
31	Cache Creek at Road 94B Bridge	CCR94BBR	38°41′19″ N	121°51′52″ W	3/N	A	U/N
32	Cache Creek below Rd. 102	CCR<R102	38°43′43″ N	121°43′26″ W	3/5	A	U/U
33	Cache Creek Settling Basin	CCRBASIN	38°42′50″ N	121°42′28″ W	3/5	A	U/U
34	Vic Fazio Yolo Wildlife Area, East Side	VFYWAEAS	38°32′27″ N	121°35′17″ W	N/10	A	U/U
35	Vic Fazio Yolo Wildlife Area, West Side	VFYWAWES	38°32′10″ N	121°37′48″ W	N/10	A	N/U
Total samples from valley sites					15/30	0/0	0/0

[a] *N* site not sampled that year, *U* species not observed that year, *A* foothill yellow-legged frog absent, outside range

[b] Three of these samples in 1997 were individual larvae

[c] Three of these samples in 1997 were composite samples of larvae (three, three, and four larvae per sample)

watershed, presumably above sources of both anthropogenic and natural sources of Hg. The mine region included those sites located just downstream of Hg mines or natural sources of Hg. Canyon sites were located 5.1–19.2 km downstream from the mine sites on high-gradient streams. The Valley region was defined as sites located in the low-gradient part of Cache Creek, from 12.2 to 85.1 km downstream from site 24 (Fig. 1). Amphibians from site 4, on Bear Creek about 2 km upstream from Sulphur Creek (BEARHAMI; Fig. 1), were not included in any of the four regions because the site was considered close enough to mine sites that dispersal of amphibians from both contaminated and reference sites was possible.

Field methods

We collected individuals of one or more anuran species from 22 sites in 1997 and 19 sites in 1998 (Table 1). The collected species included adult Northern Pacific treefrog (*Pseudacris regilla*), adult and juvenile foothill yellow-legged frog, and adult, juvenile, and larval (Gosner stage 25–35) American bullfrog. The total numbers of specimens collected per species per site and year did not necessarily relate to the population density of that species. Funding limitations precluded collection of more than three samples per site. In addition, our State of California scientific collection permit imposed limits per site for the treefrogs and yellow-legged frogs. Generally, the collection of only one or two samples per site indicated that the population density at that site was low, however. Bullfrog larvae were present at a limited number of sites, and inclusion of the larvae was designed to compare them with bullfrogs and other sympatric species.

Frog specimens were collected by hand or with a net during the day or by hand or using a gig with a spotlight after dark. Each specimen was placed in a Ziploc® plastic bag in native water, and the site, species, date, time, and collector were written on the bag. Individual frogs were held in the field on wet ice, were humanely euthanized with MS-222 the same day they were collected (American Society of Ichthyologists and Herpetologists et al. 1987), and were stored frozen (−20°C) until they could be processed within 2 days after collection.

For each specimen processed for contaminants analyses, we used chemically clean tools, weigh dishes, and disposable latex gloves to avoid cross contamination. For post-metamorphic frogs, we thawed the specimen, rinsed it with tap water to remove debris, and then rinsed it with deionized

Table 2 THg (μg/g, wet weight) in American bullfrogs (BULL), foothill yellow-legged frogs (FYLF), and northern Pacific treefrogs (PATR) from sites within the Cache Creek Watershed, 1997–1998 (see Fig. 1)

Map key	Species/ Year	Location	Region	Adult	Juvenile	Male	Female	Larvae	Geometric mean THg	Range
2	BULL/1997	BRIMROAD	Reference	3	0	2	1	0	0.032	0.02–0.05
4	BULL/1997	BEARHAMI	>Mine	3	0	2	1	0	0.135	0.12–0.14
4	BULL/1997	BEARHAMI	>Mine					3[a]	0.045	0.02–0.10
6	BULL/1997	BEAR<SUL	Mine	3	0	2	1	0	0.423	0.36–0.58
6	BULL/1997	BEAR<SUL	Mine					3[a]	0.092	0.04–0.16
7	BULL/1997	DACR>DCR	Mine	3	0	0	3	0	0.206	0.16–0.25
7	BULL/1997	DACR>DCR	Mine					3[b]	0.082	0.06–0.14
8	BULL/1997	DACR<DCR	Mine	3	0	1	2	0	0.159	0.14–0.19
10	BULL/1997	DACRRESV	Mine	2	1	1	2	0	0.153	0.11–0.19
20	BULL/1997	BEAR>CCR	Canyon	3	0	2	1	0	0.478	0.18–1.40
21	BULL/1997	BEAR>H20	Canyon					3[a]	0.212	0.18–0.26
23	BULL/1997	CCR>BEAR	Canyon	2	0	1	1	0	1.09	0.59–2.00
24	BULL/1997	CCRHASWL	Canyon	3	0	1	2	0	0.232	0.14–0.52
26	BULL/1997	CCR<BEAR	Canyon	3	0	1	2	0	0.288	0.08–1.20
29	BULL/1997	CCRESPAR	Valley	3	0	1	2	0	0.152	0.12–0.21
30	BULL/1997	CCRGUIND	Valley	3	0	1	2	0	0.141	0.06–0.33
31	BULL/1997	CCR94BBR	Valley	3	0	2	1	0	0.123	0.10–0.14
32	BULL/1997	CCR<R102	Valley	3	0	3	0	0	0.164	0.11–0.28
33	BULL/1997	CCRBASIN	Valley	3	0	3	0	0	0.110	0.10–0.12
1	FYLF/1997	EFMIDDCR	Reference	3	0	1	2	0	0.080	0.06–0.12
2	FYLF/1997	BRIMROAD	Reference	3	0	0	3	0	0.082	0.07–0.10
3	FYLF/1997	SPNISHCR	Reference	3	0	0	3	0	0.070	0.06–0.09
4	FYLF/1997	BEARHAMI	>Mine	3	0	0	3	0	0.159	0.11–0.23
6	FYLF/1997	BEAR<SUL	Mine	3	0	3	0	0	0.328	0.31–0.35
7	FYLF/1997	DACR>DCR	Mine	3	0	2	1	0	0.377	0.37–0.39
11	FYLF/1997	HARGULDS	Mine	3	0	2	1	0	0.443	0.35–0.58
15	FYLF/1997	SCR>WLHS	Mine	3	0	0	3	0	0.686	0.32–1.10
19	FYLF/1997	TURKEYRN	Mine	1	0	0	1	0	0.79	–
20	FYLF/1997	BEAR>CCR	Canyon	3	0	1	2	0	0.783	0.56–1.20
22	FYLF/1997	BEARTHOM	Canyon	3	0	0	3	0	0.405	0.12–0.74
23	FYLF/1997	CCR>BEAR	Canyon	2	0	1	1	0	0.118	0.08–0.16
4	PATR/1997	BEARHAMI	>Mine	3	0	3	0	0	0.166	0.10–0.22
6	PATR/1997	BEAR<SUL	Mine	3	0	3	0	0	0.258	0.15–0.55
7	PATR/1997	DACR>DCR	Mine	3	0	2	1	0	0.152	0.10–0.22
11	PATR/1997	HARGULDS	Mine	1	0	1	0	0	0.023	–
15	PATR/1997	SCR>WLHS	Mine	3	0	3	0	0	0.378	0.20–0.59
2	BULL/1998	BRIMROAD	Reference	0	5	4	1	0	0.043	0.03–0.08
21	BULL/1998	BEAR>H20	Canyon	3	7	3	7	0	0.390	0.23–0.87
22	BULL/1998	BEARTHOM	Canyon	1	2	1	2	0	0.561	0.07–2.80
25	BULL/1998	CCRBUCKI	Canyon	1	1	2	0	0	0.140	0.06–0.32
32	BULL/1998	CCR<R102	Valley	5	0	2	3	0	0.108	0.09–0.12
33	BULL/1998	CCRBASIN	Valley	1	4	2	3	0	0.103	0.05–0.14
34	BULL/1998	VFYWAEAS	Valley	6	4	3	7	0	0.102	0.05–0.18
35	BULL/1998	VFYWAWES	Valley	9	1	3	7	0	0.109	0.05–0.26
5	FYLF/1998	ABBOTTDR	Mine	1	0	1	0	0	1.7	–
9	FYLF/1998	DACR>CCR	Mine	3	0	0	3	0	0.065	0.04–0.11
11	FYLF/1998	HARGULDS	Mine	1	0	1	0	0	1.1	–
14	FYLF/1998	SCR>BEAR	Mine	3	0	1	2	0	0.846	0.47–1.5
16	FYLF/1998	SCR<WLHS	Mine	3	0	3	0	0	0.402	0.27–0.77
17	FYLF/1998	SCREASTF	Mine	5	0	4	1	0	0.166	0.07–0.35
18	FYLF/1998	SCRWESTF	Mine	4	1	1	4	0	0.318	0.08–0.91

Table 2 (continued)

Map key	Species/ Year	Location	Region	Adult	Juvenile	Male	Female	Larvae	Geometric mean THg	Range
22	FYLF/1998	BEARTHOM	Canyon	1	0	0	1	0	0.31	–
25	FYLF/1998	CCRBUCKI	Canyon	1	0	0	1	0	0.11	–
27	FYLF/1998	GRIZZLCR	Canyon	2	0	2	0	0	0.123	0.06–0.23
28	FYLF/1998	TOM>BEAR	Canyon	2	0	2	0	0	0.320	0.21–0.49
12	PATR/1998	HARGULLO	Mine	4	0	4	0	0	0.105	0.04–0.23
13	PATR/1998	HARGUL20	Mine	4	0	3	1	0	0.082	0.03–0.12
19	PATR/1998	TURKEYRN	Mine	1	0	1	0	0	0.14	–
28	PATR/1998	TOM>BEAR	Canyon	6	0	3	3	0	0.049	0.03–0.11

[a]Three individual larvae analyzed separately

[b]Three composite samples of larvae (three, three, and four larvae per sample) analyzed

water. Excess moisture was removed by patting the specimen dry with a clean paper towel. We determined the total mass (±0.1 g) for each specimen using an electronic balance. We measured the snout-vent length (SVL; ±0.1 mm) using calipers and examined each specimen for gross abnormalities. The digestive tract was removed and the stomach contents preserved in 70% ethyl alcohol for later identification. Gender was determined where possible by examining gonadal development. Specimens with immature gonads were classified as juveniles regardless of SVL or mass; those with mature gonads were classified as adults. The carcass, including the stripped and rinsed digestive tract, was placed in a labeled, chemically clean jar (VWR TraceClean™), which was then sealed with Parafilm® and frozen at −20°C pending chemical analysis. The protocol for larval amphibians was similar to that for the adults, except that the intestinal coil and contents were removed and discarded. In 1998, the liver and right rear leg muscle (skinless) were removed from each of ten bullfrogs collected in Bear Creek between its confluence with Sulphur Creek downstream to the Highway 20 bridge (BEAR>H20; Fig. 1).

In 1997 and 1998, the total carcass, less gut contents, for all adult and juvenile amphibians was analyzed for total Hg. In 1997, nine individual and three composite samples of bullfrog larvae were analyzed for total Hg (Table 2). In 1998, livers, leg muscles, and the remaining carcasses from ten bullfrogs were analyzed for both total Hg and MeHg (Table 3).

Table 3 THg and MeHg (μg/g, wet weight) in bullfrog tissues from Bear Creek upstream of Highway 20, Cache Creek Watershed, 1998

Catalog number	Age	Sex	Carcass mass (g)	Carcass THg /MeHg	Liver THg /MeHg	Muscle THg /MeHg	Total Body THg /MeHg
1356	Juv	F	95.6	0.25/0.30	0.74/0.59	0.22/0.25	0.26/0.30
1357	Adult	F	152.2	0.39/0.41	0.57/0.55	0.40/0.37	0.40/0.41
1358	Juv	M	140.1	0.33/0.30	0.54/0.59	0.35/0.31	0.33/0.31
1359	Adult	M	148.2	0.600.74	0.81/0.97	0.61/0.65	0.60/0.74
1374	Adult	F	235.7	0.21/0.23	0.61/0.47	0.34/0.36	0.23/0.25
1433	Juv	F	61.8	0.72/0.82	5.87/1.48	1.07/1.26	0.87/0.87
1434	Juv	F	80.9	0.31/0.29	0.64/0.79	0.43/0.43	0.33/0.32
1435	Juv	M	80.0	0.63/0.64	0.81/0.72	0.40/0.36	0.61/0.62
1436	Juv	F	57.4	0.35/0.40	0.83/1.01	0.40/0.40	0.37/0.41
1437	Juv	F	110.3	0.25/0.28	0.46/0.53	0.29/0.32	0.26/0.29
Means			116.2	0.369/0.400	0.817/0.724	0.409/0.419	0.390/0.413

The mean for carcass mass is arithmetic; means for THg and MeHg are geometric

Laboratory methods

As previously reported for cliff swallows (*Petrochelidon pyrrhonota*) from Cache Creek (Hothem et al. 2008), all Hg analyses were conducted by the Trace Element Research Laboratory (TERL) in College Station, Texas. Samples were analyzed for total Hg (THg) by the cold-vapor atomic absorption spectroscopy method. Extraction of organo-mercury compounds followed the method of Uthe et al. (1972), essentially equivalent to the gas chromatography method for analyzing MeHg in fish muscle tissue. Moisture content was determined by weight loss upon freeze drying and was expressed as a percent of the original wet sample weight. Total Hg and MeHg concentrations are reported on a wet weight basis.

Quality assurance/quality control

Duplicate samples were analyzed at a rate of 5%, with at least one duplicate per matrix per analytical run to estimate the precision of the methods. To assure the accuracy of the methods, procedural blanks, spiked samples, and standard reference materials were analyzed. To assure that no analyte was added during the processing of the sample, procedural blanks were analyzed at a rate of 5% of the total samples, with at least one per matrix per analytical run. Spiked samples were analyzed at a rate of 5%, with at least one spike per matrix per analytical run. Spikes were samples fortified with a known quantity of analyte and analyzed as part of the run. Standard reference materials (dogfish liver and muscle) were analyzed at a rate of 5% to insure that the method worked with naturally incorporated Hg.

The limits of detection for THg were all within the minimum acceptable value of the contract (0.20 μg/g, dry weight) as described by the US Environmental Protection Agency in 40 CFR Part 136, Appendix B. Spiked sample recoveries were between 80.4% and 110% for THg and between 79.3% and 104% for MeHg, with at least 95% of the points within 2 standard deviations of the mean. The percentage recovery from standard reference materials ranged from 85.1% to 102%, and analyses of procedural blanks were within normal limits. The average relative percent difference between duplicates was within normal limits.

Statistical analyses

All analyses were done on $\log_e$-transformed THg or MeHg (wet weight basis), and the significance level for all tests was $\alpha = 0.05$. We analyzed each species separately using analysis of variance models (ANOVA) to analyze mixtures of fixed effects (region, age, year, and sex) and random effect (site nested within region) variables and interactions among fixed effects using PROC MIXED SAS version 8 (SAS Institute, 1999, Cary, NC, USA). We used backward selection to determine which of these effects were most associated with THg level. Any significant fixed and random effects were reported with F statistics and likelihood ratio chi-square statistics.

To test for differences between each pair of species (bullfrog vs. yellow-legged frog, bullfrog vs. treefrog, and yellow-legged frog vs. treefrog), we restricted the data to post-metamorphic frogs at sites where both species in the pair were sampled, and we repeated the backward selection analysis without age and with species included as a variable.

We estimated mean THg using models based on significant effects determined by the preceding methods. Data are presented as geometric means. We tested assumptions of normality by calculating Shapiro–Wilk's W statistic for the residuals from the mixed effects ANOVA.

We used linear regression to evaluate relationships between mass and SVL; we then used linear regression to evaluate relationships between those two measurements and THg concentrations. We also used linear regression to evaluate correlations among tissues from ten bullfrogs from BEAR>H20 to determine if individual tissues could be used to predict concentrations in other tissues or whole bodies. We used t tests to compare THg and MeHg concentrations in tissues between sexes. Linear regression was also used to compare THg and MeHg in individual tissues, and because the data were not normally distributed, we used Kruskal–Wallis one-way ANOVA on

ranks to compare THg/MeHg ratios in tissues. We also used Kruskal–Wallis one-way ANOVA on ranks to compare THg and MeHg concentrations in the various tissues from the same ten bullfrogs and conducted a pairwise multiple comparison using the Student–Newman–Keuls procedure.

Results

Mercury was detected in each of the 194 samples of amphibians collected in 1997–1998 (Tables 2 and 3).

During 1997, we collected amphibians from 22 sites within the Cache Creek ecosystem, including bullfrogs from 16 sites, foothill yellow-legged frogs from 12 sites, and treefrogs from five sites (Table 1). In 1998, we collected amphibians from 19 sites, including bullfrogs from eight sites, foothill yellow-legged frogs from 11 sites, and treefrogs from four sites. In all, we collected 194 samples from 35 sites, including 69 adult, 25 juvenile, and nine individual and three composite samples of larval bullfrogs, 59 adult and one juvenile yellow-legged frog, and 28 adult treefrogs. Bullfrogs were common in the lower reaches of Cache Creek, present at all seven of the Valley sites and at seven of the nine Canyon sites. However, where the streams were more intermittent, bullfrogs were often absent, being collected at only four of the 15 mine sites, not including any sites on Sulfur Creek or in the Harley Gulch area, both highly contaminated with Hg. Bullfrogs were collected at one of the reference sites and at BEARHAMI. Yellow-legged frogs were found in the upper reaches of Cache Creek, but not at any of the sites located below the confluence of Cache Creek and Bear Creek (site 20 in Fig. 1). Yellow-legged frogs were abundant in the Canyon region (seven of nine sites), the Mine region (11 of 15 sites), at all three reference sites, and at BEARHAMI. Yellow-legged frogs and bullfrogs were sympatric at eight sites, including all the sites on Bear Creek. Although treefrogs were likely present at most sites within the watershed, they were opportunistically collected only in upper Cache Creek at seven of the 15 Mine sites, at one of the nine Canyon sites, and at BEARHAMI.

Since sex information was unavailable for frogs in the larval stage, we first restricted the age classes to adults and juveniles. For bullfrogs, THg varied statistically with region ($F_{4,88} = 26.66$, $P < 0.0001$) and age ($F_{1,88} = 4.06$, $P = 0.047$), but year, sex, and site within region were removed from the model because of lack of significance. For yellow-legged frogs, THg varied among regions ($F_{3,15.7} = 3.78$, $P = 0.032$) and among sites within the same region ($\chi^2 = 15.75$, $df = 1$, $P < 0.0001$), but year and sex were removed from the model because of lack of significance. Specimens of treefrogs were collected from different sites during the 2 years, which made it difficult to discern any site effects without assuming that year effects were absent. Further, we only collected adult treefrogs. We conducted backwards selection for treefrogs, including region, sex, and site within region. No sex or region effects were found, but the random site effect was significant ($\chi^2 = 8.26$, $df = 1$, $P = 0.004$).

Age comparisons

Since no sex effects were detected in bullfrogs, we repeated the backward selection analysis after expanding the age groups to include larvae. The results were similar to the preceding results, except the age effect increased in significance ($F_{2,99} = 9.84$, $P = 0.0001$) related to lower mean concentrations of THg in larvae. The mean THg concentration in adults was 142% greater (95% CI = 58–271%) than that in larvae, and the mean THg concentration for juveniles was 76% greater (95% CI = 7–187%) than larvae. The mean THg concentration in adults was 38% greater than the mean THg concentration (95% CI = 2–86%) in juveniles.

Species comparisons

Bullfrogs and yellow-legged frogs were compared at eight sites (BEAR<SUL, BEAR>CCR, BEARHAMI, BEARTHOM, BRIMROAD, CCR>BEAR, CCRBUCKI, and DACR>DCR), and bullfrogs were compared to treefrogs at three sites (BEAR<SUL, BEARHAMI, and DACR>DCR). No significant differences were found in

either comparison ($F_{1,36} = 0.01$, $P = 0.929$ and $F_{1,15} = 0.15$, $P = 0.705$, respectively). However, the species effect between yellow-legged frogs and treefrogs significantly interacted with region over the six collection sites (BEAR<SUL, BEARHAMI, DACR>DCR, HARGULDS, SCR>WLHS, TOM>BEAR). Yellow-legged frogs had higher concentrations of THg than did treefrogs in the Canyon ($F_{1,6} = 17.09$; $P = 0.0061$) and Mine regions ($F_{1,16.3} = 13.17$; $P = 0.0022$), but not at the site (BEARHAMI) located between the Canyon and Mine regions ($F_{1,4} = 0.01$, $P = 0.917$).

Region comparisons

Estimates of mean THg in adult frogs were compared among regions and are displayed separately by species in Table 4. Significant differences between adults and juveniles precluded pooling of the two age classes, and juveniles were too few to be analyzed separately. The mean THg concentration in bullfrogs was lower ($P < 0.05$) at the one reference site (BRIMROAD) than in the same species collected from the other three regions. The mean THg concentration in yellow-legged frogs from the reference sites was significantly lower ($P < 0.05$) than that in the same species from the Mine region. There was no difference between the reference and the Canyon regions. Yellow-legged frogs were not collected from the Valley region.

Treefrogs were collected from seven sites in the Mine region, but at only one site in the Canyon region, and differences between regions were not significant. Therefore, we combined the two regions to produce a common mean and confidence interval (Table 4).

Site comparisons

Mercury concentrations were generally higher at sites just downstream of known Hg contamination sources (Tables 2, 3, and 4). Areas with known sources of Hg contamination, from either abandoned Hg mines or geothermal features or both, included Sulphur Creek, Harley Gulch, and Davis Creek.

The geometric mean THg concentration in frogs was greater than the US Environmental Protection Agency's (USEPA) tissue residue criterion for fish (0.3 μg/g) at 40% of the 35 sample sites, including five sites for bullfrogs, 12 sites for yellow-legged frogs, and one site for treefrogs (Tables 2 and 3; Fig. 2). At each of 19 sites, at least one individual frog exceeded 0.3 μg/g (Tables 1 and 2; Fig. 2). The geometric mean THg concentration was greater than the FDA's criterion for commercial fish consumption (1.0 μg/g) for yellow-legged frogs at two sites and for bullfrogs at one site. At least one frog at eight of the sites had a THg concentration greater than 1.0 μg/g, with the highest (2.78 μg/g) being a bullfrog collected from BEARTHOM in 1998.

Four adult bullfrogs with THg concentrations >1.0 μg/g were collected from the Canyon region (Table 2). Six adult and six juvenile bullfrogs from the Canyon region, three adults from the Mine region, and one adult from the Valley region exceeded 0.30 μg/g THg. Five individual

Table 4 Estimated geometric mean THg (μg/g, wet weight) and 95% confidence intervals for adult frogs in four regions of the Cache Creek Watershed, based on ANOVA mixed effects model

Region	Bullfrogs	Foothill yellow-legged frogs	Pacific treefrogs[a]
Mine	0.23 (0.16–0.33)B	0.40 (0.24–0.65)A	0.12 (0.063–0.22)A
Canyon	0.44 (0.32–0.60)A	0.24 (0.12–0.48)AB	0.12 (0.063–0.22)A
Valley	0.12 (0.10–0.15)C	No data	No data
Reference	0.032 (0.016–0.064)D	0.078 (0.030–0.20)B	No data

Within species, regions in the same column not sharing upper case letters were significantly different at the 0.05 significance level

[a]The estimates for treefrogs in the Canyon and Mine regions are identical because the final model contained no differences between regions. Actual range of THg concentrations for treefrogs from the Canyon Region was 0.03–0.11 μg/g

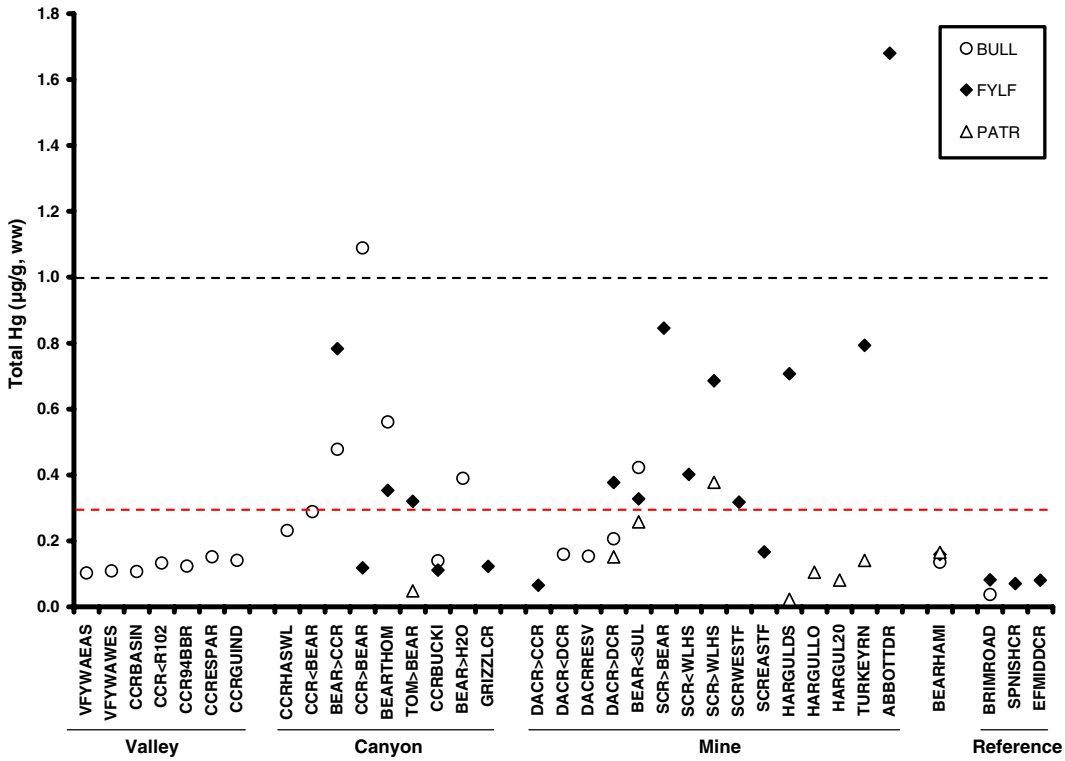

Fig. 2 Total mercury (µg/g, wet weight) in amphibians by collection site and region from the Cache Creek Watershed, 1997–1998. Values are geometric means for sites with 1 year of data and average of geometric means for sites with 2 years of data. Sites within region are arranged *left to right* based on increasing distance from mouth of Cache Creek. BEARHAMI is the site between mines and a reference site on Bear Creek about 2 km upstream of Sulphur Creek. The FDA criterion for Hg in commercial fish (1.0 µg/g, wet weight) and the USEPA tissue residue criterion for Hg in fish (0.30 µg/g, wet weight) are shown as *dashed lines*. Sample sizes are shown in Table 1 and means and ranges for THg data are presented in Table 2

yellow-legged frogs had concentrations >1.0 µg/g THg (one from the Canyon region and four from the Mine region), while 20 others from the Mine and six from the Canyon had THg concentrations >0.30 µg/g. Only three individual treefrogs, all from the Mine region, exceeded 0.30 µg/g THg.

Prediction of Hg concentration from size

We evaluated the relationship between length (SVL) and mass of adult and juvenile frogs using linear regression. SVL and mass were highly correlated for bullfrogs ($n = 94$; $r^2 = 0.922$; $P < 0.001$), yellow-legged frogs ($n = 60$; $r^2 = 0.944$; $P < 0.001$), and treefrogs ($n = 28$; $r^2 = 0.720$; $P < 0.001$). Based on these regressions, we concluded that mass and length were sufficiently related to allow us to use either parameter to evaluate relationships between size and THg concentration.

We evaluated the relationships between size (SVL) and THg concentration using linear regression.

Using all the data separately by species, there were no significant correlations for any of the three species, with r^2 ranging from 0.004 for treefrogs to 0.039 for bullfrogs ($P > 0.05$ for all species). We ran similar linear regressions for subsets of the data to determine if larger samples ($n \geq 10$) of frogs from the same site or from sites in the same area might demonstrate a closer relationship between size and THg concentration. Bullfrogs from BEAR>H2O had a higher r^2 (0.197), but the relationship was not significant ($P = 0.20$). The correlation between

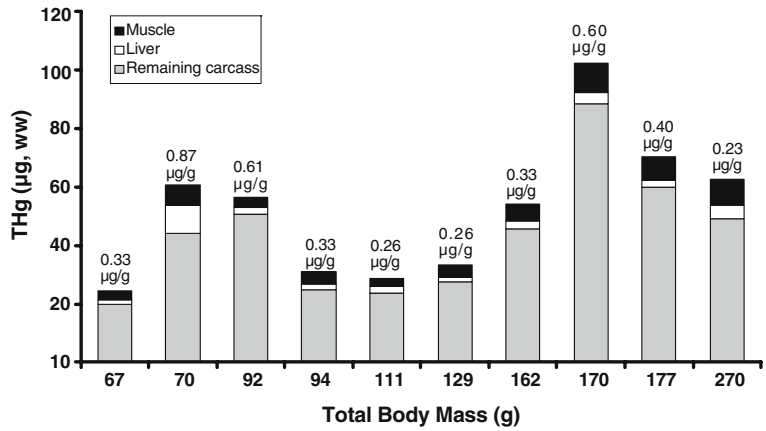

Fig. 3 Total mercury (*THg*; µg, wet weight) in tissues and THg concentration (µg/g, wet weight) in whole bodies of ten individual bullfrogs collected from Bear Creek, upstream from Highway 20, Cache Creek Watershed, 1998

size and THg concentration for yellow-legged frog from the Mine region was not significant ($n = 34$; $r^2 = 0.006$; $P = 0.67$). One pair of sites with a significant correlation was the two Yolo Wildlife Area sites (VFYWAEAS and VFYWAWES). Although the 20 bullfrogs collected from these

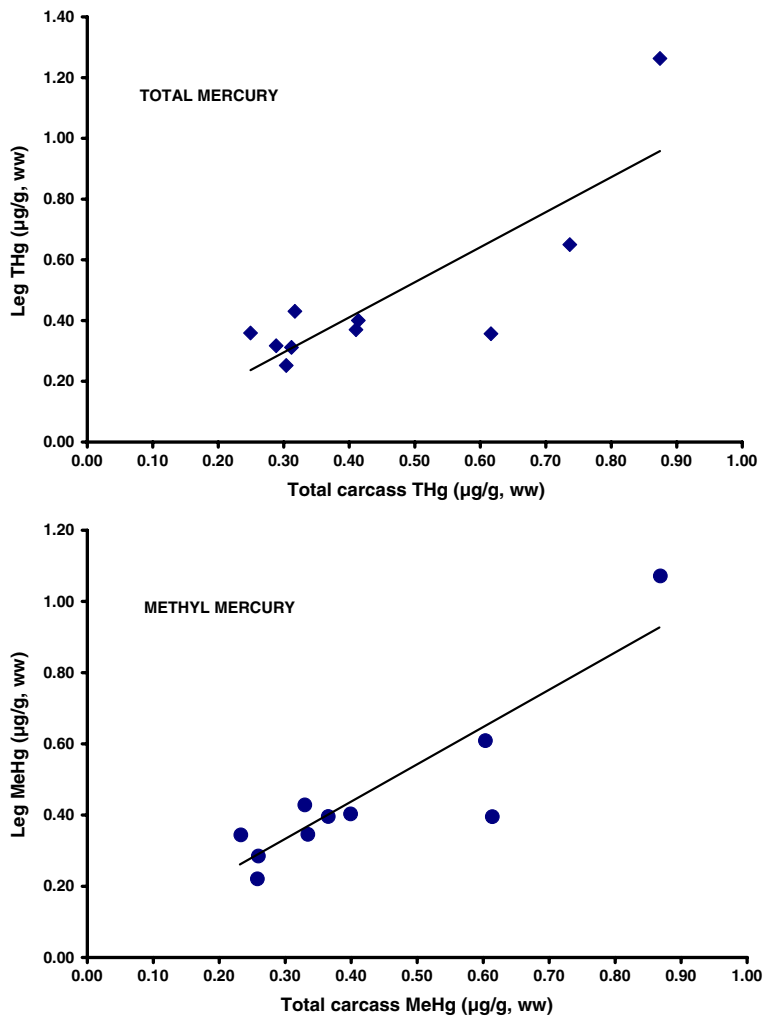

Fig. 4 Based on linear regression, correlations between THg in leg muscles and in the total bodies of bullfrogs from Bear Creek were significant for both THg ($r^2 = 0.77$; $P < 0.001$) and MeHg ($r^2 = 0.66$; $P = 0.003$)

sites did not have especially elevated THg concentrations (range, 0.05–0.26 μg/g THg), the correlation between SVL and $\log_e$ THg concentration was significant ($r^2 = 0.30$; $P = 0.007$).

Mercury in frog tissues

There were no significant differences between genders for SVL ($P = 0.83$) or THg and MeHg concentrations in tissues ($P > 0.20$) for the ten bullfrogs from BEAR>H20 (Table 3). In addition, there were no differences between ages for either THg or MeHg concentrations ($P > 0.86$). Therefore, we combined sexes and ages for subsequent analyses. Both the median THg and MeHg concentrations (Table 3) were different among tissues ($P = 0.006$ and $P = 0.019$, respectively). Based on the Student–Newman–Keuls multiple comparison procedure, concentrations of THg and MeHg were both higher in liver ($P < 0.05$) than leg muscle, remaining carcass, and total body, while concentrations in leg muscle, remaining carcass, and total body did not differ.

The mean THg/MeHg ratios in the various tissues of individual bullfrogs ranged from 0.956 in livers to 1.09 in remaining carcasses, and the medians of the four ratios were not different ($P = 0.63$). Based on linear regressions, MeHg and THg were highly correlated in all tissues, with r^2 ranging from 0.66 for liver ($P = 0.003$) to 0.95 for both remaining carcass and total body ($P < 0.001$) and 0.98 for muscle ($P < 0.001$).

The total amounts of THg and MeHg were calculated for each tissue and for the total body for each specimen (Fig. 3). The Hg in the livers, although higher in concentration, only made up an average of 6.4% of the THg and 5.1% of the MeHg in the collected bullfrogs. The Hg in the leg muscle comprised an average of 11.2% of the THg and 10.9% of the MeHg. The remainder of the carcass contained the majority of the Hg (82.4% of the total and 84.0% of the MeHg).

Linear regressions of THg and MeHg in total bodies were run separately against THg and MeHg in leg muscles (Fig. 4). The relationship between Hg concentration in leg muscle and in the total body was significant for both THg ($r^2 = 0.77$; $P < 0.001$) and for MeHg ($r^2 = 0.66$; $P = 0.003$). Correlations between Hg concentrations in liver and total body were also significant for both THg ($r^2 = 0.57$; $P = 0.007$) and MeHg ($r^2 = 0.59$; $P = 0.006$).

Discussion

Mercury deposits are present throughout the California Coast Range (Rytuba 2000), and as expected, Hg was detected in all anurans collected from the Cache Creek Watershed, including those from reference sites. Sample sizes, however, were limited by available funding, restrictions on take by the California Department of Fish and Game, and availability at specific sites. As a result, THg concentrations within sites were often highly variable, making among-site comparisons difficult. An extreme example of high variability occurred at one Bear Creek site (BEARTHOM) where THg concentrations in bullfrogs ranged from a low of 0.07 μg/g to 2.78 μg/g, which was the highest concentration observed in any frog in this study.

In 1997, bullfrog adults were consistently more contaminated with Hg than were the larvae. This difference was attributed to differences in food habits, with the larvae feeding on a wide variety of items, including algae, senesced vegetation, bacteria, fungi, zooplankton, and animal flesh (Skelly and Golon 2003), and the adults feeding at a higher trophic level. There were also interspecific differences among the adult amphibians. Based on analyses of stomach contents, adult bullfrogs should be better biomonitors of Hg contamination because their foods are more closely tied to the aquatic ecosystem (74% of the prey items were aquatic) than either the yellow-legged frogs (28% aquatic) or treefrogs (5% aquatic; Hothem et al. 2009).

Few Hg data exist for post-metamorphic anurans in the western USA, but the geometric means of THg in bullfrog carcasses from the Brim Road reference site in 1997 and 1998 (0.032 and 0.043 μg/g, respectively) were similar to the mean for THg in bullfrog leg muscle (0.056 μg/g) from southern Nevada (Gerstenberger and Pearson 2002). Foothill yellow-legged frog carcasses from HARGULDS and SCR>BEAR, sites in the MINE region, had THg concentrations (1.1 and 0.846 μg/g, respectively) that were similar to those

values (mean ± SE) found in legs of pig frogs collected from Florida's Everglades National Park (0.912 ± 0.464 μg/g; Ugarte et al. 2005). Other literature values for THg in muscle (or carcass) included <0.10–0.18 μg/g THg in three individual Northern Leopard Frogs (*Rana pipiens*) and <0.10 μg/g in a bullfrog from Lake St. Clair, Michigan (Dustman et al. 1972).

The highest values for individual adult anurans in the Cache Creek Watershed (0.59 μg/g for treefrogs, 1.7 μg/g for yellow-legged frogs, and 2.8 μg/g for bullfrogs) were three to 15 times greater than the Northern Leopard Frogs from Michigan (Dustman et al. 1972). The most contaminated amphibians noted in the literature were individual common frogs (*Rana temporaria*) and common toads (*Bufo bufo*) from the Idrija Hg mine in Yugoslavia, which had an average of 2.61 μg/g THg in muscle (Byrne et al. 1975), similar to the values observed in Cache Creek.

The geometric mean THg concentration in the livers (0.817 μg/g) of ten bullfrogs from BEAR>H20 was more than six times higher than that of livers in bullfrogs (0.125 μg/g) from southern Nevada (Gerstenberger and Pearson 2002). The mean THg concentrations in livers from common frogs from four sites and common toads from one site in Finland (0.05–0.19 μg/g) were lower than that found in Cache Creek (Terhivuo et al. 1984). However, THg concentrations in livers from individual common toads and common frogs from the Idrija Hg mine in Yugoslavia (range, 22.2–25.9 μg/g; Byrne et al. 1975) were about 25 times higher than the Bear Creek bullfrog liver concentrations.

The high THg concentrations found in amphibians from Sulphur Creek, Harley Gulch, Davis Creek, and other sites close to Hg sources (Mine region), as well as sites not far downstream from Hg sources (Canyon region), confirm the findings of Slotton et al. (1997) who sampled invertebrates from the same sites. Concentrations were generally lower in the Valley region and were lowest in the reference regions. Mercury concentrations in anurans collected from downstream sites depended on both their proximity to Hg sources and on the presence of conditions that favored methylation. For example, anurans from BEARTHOM (see Fig. 1) had mean and maximum values for both ranid species that were higher than at BEAR<SUL, a site located on Bear Creek about 11.5 km upstream at the confluence with the highly contaminated Sulphur Creek. The numerous pools in bedrock in the relatively low-gradient stream at BEARTHOM likely accumulated sediments contaminated with Hg, which was later methylated and ultimately bioaccumulated by amphibians and their prey.

In bullfrogs collected from BEAR>H20 in 1998, the concentration of MeHg actually exceeded THg in carcasses, total bodies, and muscle tissue. In livers, MeHg comprised an average of 96% of the THg, but five of the ten livers had higher concentrations of MeHg than THg. If our samples were similar to the fish samples evaluated by Bloom (1992), it is likely that most of the variability in percentage MeHg can be explained by the variability of the analyses of THg and MeHg. Difficulty in obtaining uniformly homogenized aliquots from the same carcass may result in differences in THg concentration and may not reflect differences in speciation (Bloom 1992). Bloom (1992) concluded that for all species of fish that he studied, virtually all (>95%) of the THg present was MeHg. Based on our results, it is likely that THg in bullfrogs is also nearly all MeHg. The THg in the muscle from Yugoslavian anurans was also nearly 100% MeHg (Byrne et al. 1975). However, in that study, anuran livers from the highly contaminated Idrija mine area averaged 22.4% MeHg, while the percentage in the background samples averaged 50.0%, both far lower than the 95.6% found in this study.

If frogs tend to bioaccumulate Hg with age, and assuming frogs increase in size as they get older, we anticipated that there would be positive correlations between both SVL and THg concentration and mass and THg concentration. However, we collected amphibians with a wide range in size and exposures to Hg. As a result, neither SVL nor mass was significantly correlated with THg concentration for any species. In fact, even the ten bullfrogs collected from the same site (BEAR>H20) failed to show a correlation between size and THg concentration for any tissue ($P > 0.25$). Similarly, Ugarte et al. (2005) found no significant relationship between SVL and THg concentrations in legs of pig frogs (*Rana grylio*)

collected from contaminated marshes in South Florida's Everglades.

The toxicity of Hg and MeHg to amphibians is not well documented (Wolfe et al. 1998), and we did not evaluate the toxicity to anurans in this study. However, THg concentrations in certain anurans were high enough to pose a potential hazard to human or wildlife consumption, with the total Hg concentration exceeding the FDA criterion (1.0 μg/g) for regulation of commercial fish in at least one sample at 20% of the bullfrog sites and 24% of the yellow-legged frog sites. In addition, the mean THg concentrations in bullfrogs at five sites (25% of the total) and in yellow-legged frogs at 13 of the sites (62%) exceeded the EPA Hg criterion (0.3 μg/g) for issuance of health advisories for fish consumption.

In summary, anurans from throughout the Cache Creek Watershed bioaccumulated Hg and MeHg, with concentrations dependent on proximity to Hg sources. Mercury concentrations were commonly higher than the EPA criterion for issuance of health advisories for fish consumption and were often higher than the FDA's criterion for commercial fish. The bullfrog is a sport species with no limits on when, where, or how many can be collected under a sport fishing license (California Department of Fish and Game 2003). The amount of THg in bullfrog carcasses is important when evaluating impacts to predators and the food web, and Hg concentration in the whole body was a reliable predictor of THg concentrations in leg muscle. Since the legs of bullfrogs are frequently consumed by humans, the elevated concentrations found in bullfrogs may pose a risk to human health. A health advisory for consuming bullfrogs within the Cache Creek Watershed, therefore, would seem appropriate.

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