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Mercury contamination in Foothill Yellow-legged Frogs (*Rana boylii*) and Invertebrates from Harley Gulch, California, 2007

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ABSTRACT.—Fish and wildlife may bioaccumulate mercury (Hg) to concentrations that adversely affect their reproduction, growth, and survival. In May 2007, we collected aquatic invertebrates and foothill yellow-legged frogs (*Rana boylii*) from sites within the Harley Gulch watershed for comparison with those collected from Harley Gulch in earlier years and from reference sites. Aquatic invertebrates were analyzed for both total Hg and MeHg. Methylmercury concentrations in water striders and larval dragonflies collected in 2007 were higher from below the confluence of the west and east forks of Harley Gulch (lower Harley Gulch) than from the East Fork. Dragonflies from the West Fork wetland pond were also higher than the East Fork. All samples had higher MeHg concentrations than references collected from the Bear River at the Highway 20 Bridge in 1999-2002. The 2007 samples, collected at Harley Gulch in the spring (May) had lower concentrations of Hg than the samples collected in the fall (October) of 2002. All frogs were analyzed for total Hg at a contract laboratory; selected frogs were also analyzed for methylmercury (MeHg). Mercury concentrations in frogs from lower Harley Gulch in 2007 were similar to frogs collected in 1997 and 1998 from lower Harley Gulch and from upstream in the Turkey Run and Abbot Mine drains. Mercury concentrations in foothill yellow-legged frogs collected from lower Harley Gulch were significantly higher than both frogs collected from the east branch of Harley Gulch in 2007 and those from three reference sites sampled in 1997. In 31% of the frogs collected from lower Harley Gulch in 2007, the concentration of total Hg exceeded the FDA criterion (1.0 μg/g) for regulation of commercial fish, and all frogs exceeded the EPA criterion (0.3 μg/g) for issuance of human health advisories for fish consumption. The Hg concentrations in frogs collected from lower Harley Gulch and the mine drains in 1997-1998 and from lower Harley Gulch in 2007 all exceeded the MeHg criterion for the protection of piscivorous wildlife (0.077 μg/g). Mercury bioaccumulation in frogs and invertebrates corroborated previous findings that identified the presence of significant sources of Hg within the Harley Gulch subwatershed.

INTRODUCTION

Amphibians and other aquatic wildlife may be adversely affected by exposure to environmental Hg, especially in its more bioavailable form, MeHg. Excessive Hg may adversely affect amphibians, causing reduced survival, growth inhibition, behavioral modification, impaired

reproduction, and various sublethal effects (Zillioux et al., 1993), including malformations in larvae (Unrine et al., 2004).

The Cache Creek watershed, which lies within the North Coast Range of California, is an area with abundant geologic sources of Hg and a long history of Hg contamination (Rytuba, 2000). In addition to lower Cache Creek, Clear Lake, Davis Creek Reservoir, Bear Creek, and Sulfur Creek in the Cache Creek watershed, Harley Gulch is listed as impaired by Hg contamination by Section 303(d) of the Clean Water Act (Central Valley Regional Water Quality Control Board, 2003). 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 mercury mining operations in the upper watershed (Foe and Croyle, 1998). 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).

Reasons for amphibian decline are unclear (Jennings, 1988), but certain contaminants may be affecting species in specific areas (Davidson et al., 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, a California species of special concern (Jennings and Hayes, 1994).

Because they tend to bioaccumulate metals and are sensitive to their effects, amphibians may serve as good bioindicators of metals contamination (Cooke, 1981). In addition, amphibians are potentially good biomonitors of Hg contamination because they have obligate aquatic larval stages, and they are often able to persist in aquatic systems unsuitable for fish. They are also normally less mobile than fish, sometimes spending their entire life cycle in a given pond or reach of a stream.

Information on the concentrations of Hg in water, sediments (Foe and Croyle, 1998; Domagalski, 2001; Domagalski et al., 2004), invertebrates (Slotton et al., 1997, 2004), and fish (Slotton et al., 1995) from the Cache Creek watershed have helped define the sources and magnitude of Hg

contamination in the watershed. However, more information on Hg concentrations in the higher trophic levels, especially amphibians, is needed. In many cases, particularly where fish are not available, as in upper Harley Gulch, amphibians occupy a higher trophic level and may be good biomonitors of Hg bioaccumulation in the ecosystem.

The objectives of this study were to quantify Hg accumulation in foothill yellow-legged frogs and their potential invertebrate prey in Harley Gulch and to evaluate the significance of these concentrations by comparing them with reference values.

MATERIALS AND METHODS

Study Area and Field Methods.—Harley Gulch, a subwatershed of the 2950-km^2 Cache Creek watershed, is located in the North Coast Range, Lake County, California, about 130 km north of San Francisco (Fig. 1). The six study sites sampled in 2007 were all within Harley Gulch. One site (H-8) was a pond within the West Fork of Harley Gulch wetlands, one was on the East Fork of Harley Gulch (H-3), and the other four (H-4 – H-7) were downstream of the two forks of Harley Gulch (lower Harley Gulch) (Fig. 1). These sites were selected to assist with the evaluation of historical mercury contamination emanating from the Abbott and Turkey Run Mines.

As part of a study in 1997, foothill yellow-legged frogs were collected from three reference sites: Bear Creek at Brim Road (BRIM), Spanish Creek (SPCR), and East Fork of Middle Creek (EFMC) (Fig. 1). Data on frogs from these sites, located in the upper reaches of the Cache Creek watershed, presumably above sources of both anthropogenic and natural Hg (R. L. Hothem, unpublished data), are presented for comparison purposes. A site on the Bear River at the Highway 20 bridge in Nevada County (BR20) (Fig. 1), sampled as part of an evaluation of mercury contamination within the Bear River and South Yuba River watersheds (Alpers et al. 2005) during 1999-2002, was used as a reference site for invertebrates. No amphibians were collected at that site.

Invertebrates— The target macroinvertebrates for this study were predatory insects, depending on their abundance and availability at each sample site. Taxa collected in 2007 were larval

dragonflies (Order Odonata, families Libellulidae and Aeshnidae) and adult water striders (Order Hemiptera, family Gerridae). These taxa were also collected at Harley Gulch on October 16, 2002. Water striders were collected from the BR20 reference site in October 1999, September 2000 and 2001, and August 2002. Larval dragonflies (family Aeshnidae) were collected at BR20 in September 2001 and August 2002.

Invertebrates were collected from all sites using dip nets and by hand and placed in zip-lock plastic bags with native water. Samples were kept in a cooler and allowed to depurate in native water on wet ice for 4-24 hours before they were processed at the end of each collection day. Individuals were sorted by family and placed in disposable dishes using Teflon-coated forceps or by hand while wearing disposable latex gloves. Organisms were thoroughly rinsed with deionized water, patted dry with a clean paper towel, and composited by family, with the goal of obtaining a minimum of one gram wet biomass. Each sample consisted of 1-30 individuals of the same family (1.0-5.0 g total mass). Samples were weighed on an electronic balance (± 0.01 g), placed into chemically cleaned glass jars with Teflon-lined lids, and stored frozen for 5 months until they could be shipped to the Brooks Rand Laboratory in Seattle, WA for analysis for Hg and MeHg. Invertebrates collected in 1999-2002 were kept frozen until they could be sent to the Trace Element Research Laboratory (TERL) in College Station, TX for analysis for Hg and MeHg.

Frogs—In 2007, foothill yellow-legged frogs were collected by hand or with a net during the day from Harley Gulch. For each specimen, we recorded the site, species, date, time, and collector, and attached this information to the specimen or its container. Individual frogs were held in the field in their own plastic zip-lock bag on wet ice. Frogs were humanely euthanized the same day they were collected and kept frozen until they could be processed within 2 days after collection. Foothill yellow-legged frogs were collected from Harley Gulch and the three reference sites in 1997 and 1998 using the same collection techniques.

For each specimen processed for Hg analysis (1997-1998 and 2007), we used chemically clean tools, weigh dishes, and disposable latex gloves to avoid cross contamination. We thawed the specimen, rinsed it with tap water to remove debris, and then thoroughly rinsed it with deionized

water. Excess moisture was removed by patting the specimen dry with a clean paper towel. We determined the total mass (± 0.01 g) for each specimen using an electronic balance. We measured the length from the tip of the snout to the cloaca (snout-vent length (SVL)) (± 0.1 mm) using calipers, and we examined each specimen for gross abnormalities. The digestive tract was removed, and the stomach contents were identified and discarded. 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 –20º C pending chemical analysis. The carcasses of all frogs collected in 2007 were analyzed for total Hg at Brooks Rand Laboratory in Seattle, WA within 3 months of collection. In addition, one individual from each site was also analyzed for MeHg. Carcasses of frogs collected in 1997-1998 were kept frozen until they could be sent to the Trace Element Research Laboratory (TERL) in College Station, TX for analysis for total Hg.

Chemical Analyses at Brooks Rand

Frogs and Invertebrates, 2007

Dry Weight Correction (% Solids) EPA Method 160.3 (SOP BR-1501)

A solid sample is homogenized and an aliquot is measured into a pre-weighed vessel, dried in an oven overnight, weighed again and the percent of dried solid material is calculated. This standard operating procedure (SOP) is analogous with EPA method 160.3 (Residue, total).

Sample Homogenization (SOP BR-0106)

Once thawed, the samples were homogenized using pre-cleaned commercial grade homogenization equipment. A homogenization blank was collected after cleaning the equipment and prior to homogenization of the samples. The blank was digested as a tissue sample and analyzed along with the associated homogenates. The result for the homogenization blank was less than 10 times the lowest sample result, indicating that no significant contamination occurred during homogenization.

Total Mercury by the Appendix to the EPA Method 1631 (BRL SOP BR-0002)

Samples were digested in nitric acid (HNO_3) and sulfuric acid (H_2SO_4), and then further oxidized with bromine monochloride (BrCl). Samples were analyzed with stannous chloride ($SnCl_2$)

reduction, single gold amalgamation, and cold vapor atomic fluorescence spectroscopy (CVAFS) detection using a BRL Model III CVAFS Mercury Analyzer. All sample results for low-level mercury analysis were blank corrected, as outlined in the calculations section of EPA Method 1631.

Monomethyl Mercury, EPA Draft 1630 Modified (SOP BR-0011)

All samples were prepared by potassium hydroxide (KOH) methanol (CH_3OH) digestion. Samples were analyzed by aqueous phase ethylation, Tenax trap collection, GC separation, isothermal decomposition and atomic fluorescence detection (CVAFS) using a BRL Model III CVAFS Mercury Analyzer. All sample results for low-level mercury analysis were blank corrected, as outlined in the calculations section of Brooks Rand SOP BR-0011.

Chemical Analyses at Trace Element Research Laboratory (TERL)

Frogs and Invertebrates, 1997-2002

Chemical analyses of invertebrates in 1999-2002 and frogs in 1997 and 1998 were conducted by the Trace Element Research Laboratory (TERL) in College Station, Texas. Before samples were analyzed by the cold-vapor atomic absorption spectroscopy (CVAAS) method, the Hg was converted to the Hg^{++} form. Mercury was digested by a modified version of EPA method 245.5 and 245.6. Tissue samples were homogenized in the original sample containers either after freeze-drying or with a Tekmar Tissumizer and sub sampled. Samples were digested with nitric acid, sulfuric acid, potassium permanganate, and potassium persulfate in polypropylene tubes in a water bath at 90-95° C. Before analysis, hydroxylamine hydrochloride was added to reduce excess permanganate, and the samples were brought to volume with distilled-deionized water. In the CVAAS procedure, Hg^{++} was reduced to the elemental state (Hg^0) by a strong reducing agent (stannous chloride). Gaseous Hg^0 entered the sweep gas and was introduced into an atomic absorption cell, where light produced by a mercury vapor lamp was absorbed by the free Hg atoms. Mercury in the sample was determined by comparing light absorption of the sample with that of external calibration standards.

Extraction of organo-mercury compounds followed the method of Uthe et al. (1972), and measured the sum of all organo-mercury species extracted into the solvent. This determination

was essentially equivalent to the gas chromatography method for analyzing MeHg in fish muscle tissue. Homogenized aliquots were extracted into an organic solvent, with potassium bromide and copper sulfate added to improve partitioning between phases. The organic phase was digested in combusted glass vials, using nitric and sulfuric acids and potassium permanganate, to convert all Hg species to ionic Hg and to remove traces of organic solvent that would otherwise affect the measurement.

Moisture content was determined by weight loss upon freeze-drying and was expressed as a percent of the original wet sample weight. Mercury and MeHg concentrations are reported on a wet-weight basis.

Quality Assurance/Quality Control

TERL

The U.S. Fish and Wildlife Service's Patuxent Analytical Control Facility (PACF) was responsible for assuring the quality of the chemical analyses provided by TERL. Their reports indicated that the limits of detection for Hg were less than 0.20 μg/g, dry weight. Spiked sample recoveries were between 80.4 and 110% for Hg 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 required numbers of duplicate sample analyses were performed, and the average relative percent difference (RPD) between duplicates were within normal limits, except that the variability of Hg was slightly high in the duplicate analysis of one frog. It was concluded that this abnormal result would not affect the interpretation of the data.

Brooks Rand

At Brooks Rand, duplicate samples were analyzed for Hg at a rate of 5%, with at least one duplicate per matrix per analytical run to estimate the precision of the methods. Two frog Hg duplicates were run with relative percentage differences (RPD) of 9 and 17%; one invertebrate duplicate sample had an RPD of 3%. The RPD for the frog MeHg duplicate was 1%, and for the invertebrates, the RPD ranged from 4 to 11%. All were within the acceptable criterion of an RPD < 30%.

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. For the frogs, both the average for the Hg and MeHg blanks were 0.00 ng/g. For the invertebrates, the results of the two Hg blanks were 0.04 and 0.30 ng/g, and the MeHg blank was 0.00 ng/g. All blank results were less than the acceptable criterion of twice the method detection limits (MDL).

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. The spike recovery for frogs for Hg was 110%, with a duplicate spike RPD of 13%. For frog MeHg, the recovery was 126%, with a duplicate RPD of 13%. For the invertebrates, the Hg spike recovery was 107% with an RPD of 3%, while the MeHg recovery was 93-112%, with an RPD of 3-5%. Both taxa met the criterion of 70-130% recovery and an RPD $\leq$ 30%.

Standard Reference Materials (DORM-2) were analyzed at a rate of 5% to insure that the method worked with naturally incorporated mercury. For frogs, the recovery was 103% for Hg and 115% for MeHg. For invertebrates, the recovery was 89-104% for Hg and 113% for MeHg. All results were within the criterion of 75-125%.

Statistical Analyses

Since we collected only one composite sample of each invertebrate taxon per site, statistical comparisons were not made; qualitative comparisons with previous data and with results from a reference site, however, were made. To compare Hg concentrations in frogs from different sites, we used One-way analysis of variance (ANOVA). When there were significant differences among sites, we used the Tukey pairwise multiple comparison procedure. Mercury concentrations in frogs were compared using $\log_{10}$-transformed Hg concentrations (wet weight basis), and where more than one sample was collected per site, we calculated geometric means. We compared the body mass of the frogs by sex using one-way ANOVA, and we evaluated the

relationship between both snout-vent length and body mass and Hg concentration using linear regression. The significance level for all tests was $\alpha = 0.05$.

RESULTS AND DISCUSSION

INVERTEBRATES

In conjunction with the sampling of frogs in Harley Gulch, we collected 13 composite samples of aquatic invertebrates for Hg and MeHg analyses from the same sites as the amphibians and one additional site (H8-07) located within the West Fork Harley Gulch Wetland area near Highway 20 (Fig. 1, Table 1). We attempted to collect water striders and dragonfly nymphs from each of the sites for comparison with previous years at Harley Gulch and with a reference site. We also wanted to collect invertebrates that might be eaten by the frogs. Unfortunately, only two frogs had water striders in their stomachs, while none had dragonfly larvae. We collected water striders from four of the six sites and dragonflies from all six sites (Aeshnidae: 3 sites; Libellulidae: 6 sites), and all 13 samples were analyzed for Hg and MeHg (Table 1).

Comparisons were made with samples collected in 2002 from one site on lower Harley Gulch (composites by taxon of water striders and both families of dragonflies). In addition, we compared water striders and Aeshnidae with the same taxa collected from the BR20 reference site (Alpers et al. 2005). The reference samples, collected during 1999-2002, had total Hg concentrations ranging from 0.02 to 0.07 µg/g and MeHg concentrations ranging between 0.01 and 0.05 µg/g. All Hg concentrations were far lower than Hg concentrations observed from Harley Gulch in 2002 and 2007 (Table 1; Fig. 2). Mean MeHg concentrations in water striders (0.493 µg/g) and larval dragonflies (0.650 µg/g in Aeshnidae and 0.846 µg/g in Libellulidae) collected in 2007 were 3-4 times higher from lower Harley Gulch than from the East Fork (0.15 µg/g in Gerridae and 0.22 µg/g in Libellulidae) (Fig. 2). Dragonflies from the West Fork wetland pond (0.44 µg/g in Libellulidae and 0.50 in Aeshnidae) were also higher than the East Fork. Concentrations of MeHg in samples from all Harley Gulch sites, including the East fork, were higher than the reference samples collected from the BR20 reference site in 1999-2002. Although invertebrate MeHg concentrations were elevated at Harley Gulch in 2007, they were lower than what was observed in October 2002. The MeHg concentrations in the fall (October) 2002 samples from Harley Gulch ranged from were 2.7 to 4.9 times higher in Gerridae and

Aeshnidae, respectively, than the samples collected in the spring (May) of 2007 (Table 1; Fig. 2). A potential seasonal or combination year-seasonal effect may have accounted for this difference.

Slotton et al. (2004) collected invertebrates from the Cache Creek watershed and found that MeHg was present at higher concentrations in aquatic invertebrates from Harley Gulch than any other site sampled in the watershed. Unlike our study, samples of damselflies (Coenagrionidae), dobsonflies (Corydalidae), net-spinning caddisflies (Hydropsychidae), and creeping water bugs (Naucoridae) were collected in February and April of 2000 and 2001. Those samples had concentrations of MeHg ranging from 0.274 µg/g to 1.656 µg/g, with average concentrations of 0.780, 0.617. 0.556, and 0.937 µg/g, respectively, for the four taxa. The percentage MeHg in these samples ranged from 18.1% to 53.8%, with an average of 34.8% for all samples (Slotton et al., 2004). The percentage MeHg in samples collected in 2007 was higher, ranging from 37 to 114%, with an average of 83.2%. Although the taxa were not identical, the mean concentrations of MeHg in the invertebrates we collected were similar to those collected by Slotton et al. (2004).

FROGS

Fifteen foothill yellow-legged frogs were collected on May 16, 2007 from the Harley Gulch subwatershed. Thirteen were from lower Harley Gulch, and two were from the East Fork of Harley Gulch (Table 2, Fig. 1).

Similar numbers of male and female frogs (6 females and 7 males) were collected from lower Harley Gulch (H-4 – H-7). Although the mean mass of the females (13.96 g) was greater than the males (7.50 g), the difference was not significant ($F = 2.231$; $P = 0.163$). The correlation between SVL and body mass ($r^2 = 0.956$) for the 15 Harley Gulch frogs was significant ($F = 284.2$; $P < 0.001$). Neither the correlation between SVL and Hg ($F = 1.84$; $P = 0.202$) nor between body mass and Hg ($F = 1.33$; $P = 0.274$) was significant for the lower Harley Gulch frogs. There was no significant difference between geometric mean Hg concentrations in males (0.800 µg/g) and females (0.830 µg/g) ($F = 0.034$; $P = 0.856$) from lower Harley Gulch.

Concentrations of total Hg were low from the East Fork (geometric mean = 0.051 μg/g); the geometric mean Hg concentration of the other 13 frogs (0.814 μg/g) was 16 times greater than that for the East Fork. This mean concentration was similar to that observed for leg muscle in pig frogs (*Rana grylio*) (0.911 μg/g) from a highly contaminated site in the Everglades (Ugarte et al. 2005). A One-Way ANOVA revealed no significant differences among the Hg concentrations in frogs from the lower Harley Gulch sites ($F = 0.238$; $P = 0.868$). The geometric mean of the 13 lower Harley Gulch frogs was significantly higher than that of the two frogs from the East Fork (H 3) ($F = 115.6$; $P < 0.001$).

Geometric mean concentrations of Hg in foothill yellow-legged frogs collected in 1997 from three reference sites (Fig. 1, Table 3) from the upper reaches of the Cache Creek watershed ranged from 0.072 to 0.085 μg/g. Based on a One-Way ANOVA, geometric mean concentrations of Hg did not differ among these three sites ($F = 0.250$; $P = 0.786$).

Based on a one-way ANOVA, geometric mean concentrations of mercury in frogs collected in 2007 from lower Harley Gulch (Table 2) were not significantly different from one another or from those collected from Harley Gulch in 1997 (including one Turkey Run Drain frog) and 1998 (including one Abbott Mine Drain frog) (Table 3 and Fig. 3). All Harley Gulch frogs had significantly higher geometric mean concentrations of Hg than the three references and the East Fork of Harley Gulch site (Fig. 3). The Abbott and Turkey Run mercury mines are upstream of the West Fork of Harley Gulch (Fig. 1) and appear to be important sources of mercury to the West Fork and further downstream in Harley Gulch (Slotton et al. 2004). The Hg concentration in the frog from the Abbott Drain in 1998 was similar to that in one of the frogs from lower Harley Gulch (H 5) in 2007, but it was higher than all the other frogs collected in 2007. Based on Hg concentrations in the frogs form East Fork of Harley Gulch, there do not appear to be significant sources of Hg contamination east of the confluence of the two forks.

Previous laboratory studies have shown that Hg may adversely affect amphibians. Teratogenic and lethal effects of Hg have been documented for larval amphibians (Chang et al., 1974; Dial, 1976; Punzo, 1993a); sublethal effects have also been demonstrated (Kanamadi and Saidapur, 1991, 1992; Punzo, 1993b). The toxic effects of Hg and MeHg to amphibians in the field,

however, have not been well-documented (Wolfe et al., 1998). Although we did not observe any gross abnormalities in any of the frogs we collected, we did not attempt to evaluate amphibian toxicity in this study.

Mercury concentrations in foothill yellow-legged frogs were high enough to pose a potential hazard to their predators. The concentration of total Hg exceeded the FDA criterion (1.0 µg/g) for regulation of commercial fish (USFDA 2001) in 31 % of the 13 frogs collected from lower Harley Gulch in 2007. In addition, the Hg concentrations in 100% of the frogs exceeded the EPA Hg criterion (0.3 µg/g) for issuance of health advisories for human fish consumption (USEPA 2001). The 13 frogs collected in 2007 and those collected from Harley Gulch in 1997-1998 had Hg concentrations that exceeded both the methylmercury criterion for the protection of piscivorous wildlife (0.077 µg/g: the no-effect level) and the methylmercury effect level (0.3 µg/g) (USEPA 1997). All five frogs analyzed for MeHg had concentrations > 0.35 µg/g. Based on a probabilistic risk assessment in the Everglades (Duvall and Barron 2000), it is likely that wildlife that feed on foothill yellow-legged frogs from lower Harley Gulch are at risk for Hg toxicity.

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Table 1. Total mercury (HgT) and methyl mercury (MeHg) (µg/g, wet wt) in individual composites of adult water striders (Gerridae) and larval dragonflies (Aeshnidae and Libellulidae) collected at Harley Gulch on May 16, 2007 (H 3-8) and October 16, 2002 (HGDS-02) and at a reference site, Bear River at Highway 20 (BR 20), during 1999-2002.

Site-Year	Date collected	Sample number	Order	Family	Age	N	Mass (g)	Ave. Mass (g)	Moisture (%)	HgT (µg/g, wet wt)	MeHg (µg/g, wet wt)	% MeHg
BR20-01	9/15/2001	BY-BH20-091501-009	Odonata	Aeshnidae	Larva	7	3.89	0.556	81.9	0.0219	0.0141	64.2%
BR20-02	8/23/2002	BY-BR20-082302-005	Odonata	Aeshnidae	Larva	8	3.63	0.454	79.9	0.0239	0.0257	107.6%
BR20-99	10/1/1999	BY-BH20-100199-001	Hemiptera	Gerridae	adult	21	1.07	0.051	57.2	NA[1]	0.0271	NA
BR20-00	9/12/2000	BY-BH20-091200-001	Hemiptera	Gerridae	adult	26	1.30	0.050	76.1	0.0284	0.0270	95.0%
BR20-01	9/15/2001	BY-BH20-091501-003	Hemiptera	Gerridae	adult	25	1.25	0.050	64.7	0.0695	0.0498	71.6%
BR20-02	8/23/2002	BY-BR20-082302-001	Hemiptera	Gerridae	adult	25	1.37	0.055	63.0	0.0451	0.0414	91.8%
HGDS-02	10/16/2002	CA02G001	Hemiptera	Gerridae	adult	25	1.34	0.054	56.4	1.308	1.443	110.3%
HGDS-02	10/16/2002	CA02A001	Odonata	Aeshnidae	larva	9	2.30	0.256	80.6	3.996	3.162	79.1%
HGDS-02	10/16/2002	CA02A002	Odonata	Libellulidae	larva	9	4.56	0.507	80.7	3.783	2.548	67.3%
H 3-07	5/16/2007	HAR-SITE3-51607-001	Hemiptera	Gerridae	adult	25	1.56	0.062	75.3	0.159	0.146	91.8%
H 3-07	5/16/2007	HAR-SITE3-51607-002	Odonata	Libellulidae	larva	5	2.29	0.458	82.9	0.191	0.218	114.1%
H 4-07	5/16/2007	HAR-SITE4-51607-001	Hemiptera	Gerridae	adult	17	1.13	0.066	65.2	0.302	0.241	79.8%
H 4-07	5/16/2007	HAR-SITE4-51607-002	Odonata	Aeshnidae	larva	3	4.1	1.367	79.3	1.180	0.855	72.5%
H 4-07	5/16/2007	HAR-SITE4-51607-003	Odonata	Libellulidae	larva	9	2.41	0.268	82.2	0.961	0.357	37.1%
H 5-07	5/16/2007	HAR-SITE5-51607-001	Odonata	Libellulidae	larva	4	1.69	0.423	83.9	0.581	0.540	92.9%
H 6-07	5/16/2007	HAR-SITE6-51607-001	Hemiptera	Gerridae	adult	25	1.71	0.068	67.7	0.701	0.690	98.4%
H 6-07	5/16/2007	HAR-SITE6-51607-002	Odonata	Libellulidae	larva	5	3.03	0.606	79.7	1.920	1.570	81.8%
H 6-07	5/16/2007	HAR-SITE6-51607-004	Odonata	Aeshnidae	larva	4	2.83	0.708	83.4	0.492	0.445	90.4%
H 7-07	5/16/2007	HAR-SITE7-51607-001	Hemiptera	Gerridae	adult	25	1.46	0.058	73.7	0.546	0.547	100.2%
H 7-07	5/16/2007	HAR-SITE7-51607-002	Odonata	Libellulidae	larva	5	1.8	0.360	87.7	0.961	0.915	95.2%
H 8-07	5/16/2007	HAR-SITE8-51607-001	Odonata	Aeshnidae	larva	5	3.61	0.722	81.9	0.863	0.498	57.7%
H 8-07	5/16/2007	HAR-SITE8-51607-002	Odonata	Libellulidae	larva	2	0.82	0.410	89.8	0.640	0.443	69.2%

[1] NA = not analyzed.

Table 2. Total mercury (Hg) and methylmercury (MeHg) (μg/g, wet wt) in foothill yellow-legged frogs from Harley Gulch, May 16, 2007.

Site/ sample no.	Latitude/ Longitude	Age	Sex	Length (mm)	Mass (g)	Site Description	Hg	MeHg
H 3/2056	39° N 00' 37"/ 122° W 26' 00"	Adult	Female	30.2	3.31	E. Fork Harley Gulch	0.045	0.059
H 3/2057	39° N 00' 37"/ 122° W 26' 00"	Juvenile	Female	29.7	2.65	E. Fork Harley Gulch	0.059	
H 4/2052	39° N 00' 36"/ 122° W 26' 04"	Adult	Male	42.2	9.42	Harley Gulch, just below confluence of W. and E Forks	0.525	
H 4/2053	39° N 00' 36"/ 122° W 26' 04"	Adult	Female	32.4	4.61	Harley Gulch, just below confluence of W. and E Forks	0.785	0.403
H 4/2054	39° N 00' 36"/ 122° W 26' 04"	Adult	Male	36.6	6.11	Harley Gulch, just below confluence of W. and E Forks	0.795	
H 4/2055	39° N 00' 36"/ 122° W 26' 04"	Adult	Female	63.3	28.03	Harley Gulch, just below confluence of W. and E Forks	1.130	
H 5/2049	39° N 00' 33"/ 122° W 26' 08"	Adult	Male	49.9	14.86	Harley Gulch, 200 m below confluence of W. and E Forks	1.660	
H 5/2050	39° N 00' 33"/ 122° W 26' 08"	Adult	Male	36.3	7.18	Harley Gulch, 200 m below confluence of W. and E Forks	0.733	0.351
H 5/2051	39° N 00' 33"/ 122° W 26' 08"	Adult	Male	32.4	5.04	Harley Gulch, 200 m below confluence of W. and E Forks	0.525	
H 6/2043	39° N 00' 30"/ 122° W 26' 11"	Adult	Female	58.9	27.12	Harley Gulch, 320 m below confluence of W. and E Forks	0.895	
H 6/2044	39° N 00' 30"/ 122° W 26' 11"	Adult	Male	33.2	4.26	Harley Gulch, 320 m below confluence of W. and E Forks	0.734	0.400
H 6/2045	39° N 00' 30"/ 122° W 26' 11"	Adult	Female	42.6	8.91	Harley Gulch, 320 m below confluence of W. and E Forks	0.568	
H 7/2046	39° N 00' 27"/ 122° W 26' 22"	Adult	Female	44.1	10.54	Harley Gulch, 600 m below confluence of W. and E Forks	0.616	
H 7/2047	39° N 00' 27"/ 122° W 26' 22"	Adult	Male	37.1	5.65	Harley Gulch, 600 m below confluence of W. and E Forks	1.070	0.523
H 7/2048	39° N 00' 27"/ 122° W 26' 22"	Adult	Female	35.1	4.55	Harley Gulch, 600 m below confluence of W. and E Forks	1.180	

Table 3. Total mercury (Hg) (µg/g, wet wt) in foothill yellow-legged frogs from Harley Gulch and reference sites, 1997-1998.

Site/ sample no.	Collection date	Latitude/ Longitude	Age	Sex	Length (mm)	Mass (g)	Site Description	Hg
EFMC/1005	5/14/97	39° N 15' 09"/ 122° W 57' 00"	Adult	Female	74.7	47.1	East Fork Middle Creek	0.120
EFMC/1004	5/14/97	39° N 15' 09"/ 122° W 57' 00"	Adult	Female	60.9	30.3	East Fork Middle Creek	0.079
EFMC/1003	5/14/97	39° N 15' 09"/ 122° W 57' 00"	Adult	Male	53.7	19.3	East Fork Middle Creek	0.055
BRIM/927	4/11/97	39° N 09' 45"/ 122° W 26' 59"	Adult	Female	61.7	33.8	Mill Creek at Brim Road	0.103
BRIM/929	4/11/97	39° N 09' 45"/ 122° W 26' 59"	Adult	Female	50.7	17.0	Mill Creek at Brim Road	0.081
BRIM/928	4/11/97	39° N 09' 45"/ 122° W 26' 59"	Adult	Female	54.2	18.1	Mill Creek at Brim Road	0.066
SPCR/1001	5/12/97	39° N 10' 17"/ 122° W 37' 05"	Adult	Female	56.4	20.7	Spanish Creek	0.089
SPCR/1002	5/12/97	39° N 10' 17"/ 122° W 37' 05"	Adult	Female	57.1	26.7	Spanish Creek	0.068
SPCR/1000	5/12/97	39° N 10' 17"/ 122° W 37' 05"	Adult	Female	43.2	7.6	Spanish Creek	0.057
TRKY/926	3/27/97	39° N 00' 57"/ 122° W 26' 26"	Adult	Female	47.8	13.4	Turkey Run Mine	0.793
HGDS/963	4/25/97	39° N 00' 34"/ 122° W 26' 05"	Adult	Female	47.2	11.8	Lower Harley Gulch	0.583
HGDS/961	4/25/97	39° N 00' 34"/ 122° W 26' 05"	Adult	Male	41.9	9.4	Lower Harley Gulch	0.419
HGDS/962	4/25/97	39° N 00' 34"/ 122° W 26' 05"	Adult	Male	36.4	6.2	Lower Harley Gulch	0.355
ABBT/1201	3/16/98	39° N 00' 56"/ 122° W 26' 29"	Adult	Male	56.0	23.4	Abbott Drain	1.680
HGDS/1190	3/11/98	39° N 00' 34"/ 122° W 26' 05"	Adult	Male	54.3	23.1	Lower Harley Gulch	1.130

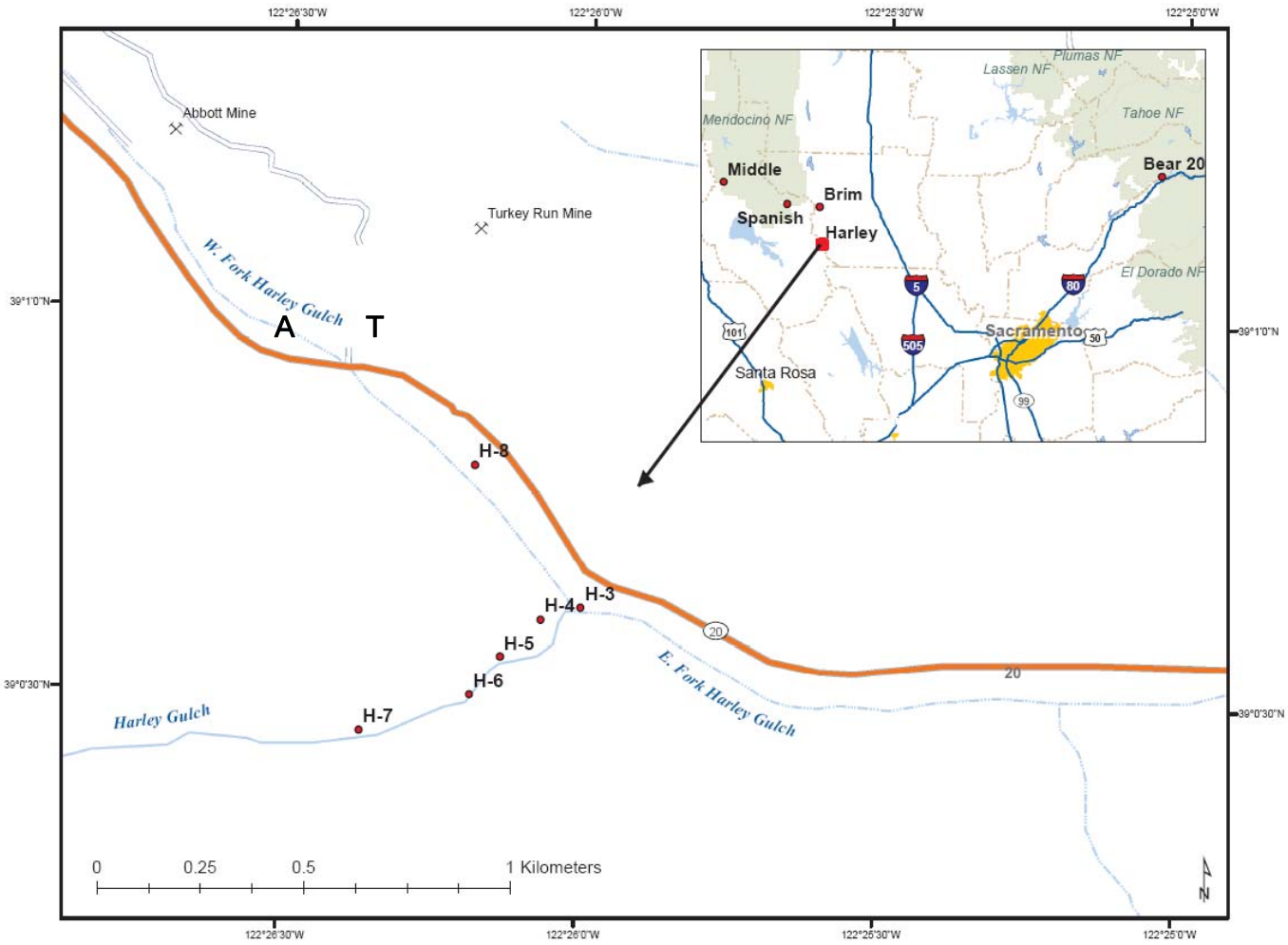

Figure 1. Locations of study sites at Harley Gulch (H-3 – H-8) in 2007, foothill yellow-legged frog reference sites in 1997 (East Fork of Middle Creek [Middle], Spanish Creek [Spanish], and Bear Creek at Brim Road [Brim]), and the invertebrate reference site, the Bear River at Highway 20 (Bear 20). Approximate collection sites for the frog from the Abbott Mine Drain in 1998 and the Turkey Run Mine Drain in 1997 are shown on the map as A and T, respectively. Other frogs collected from Harley Gulch in 1997 and 1998 were collected in the reach between H-4 and H-5.

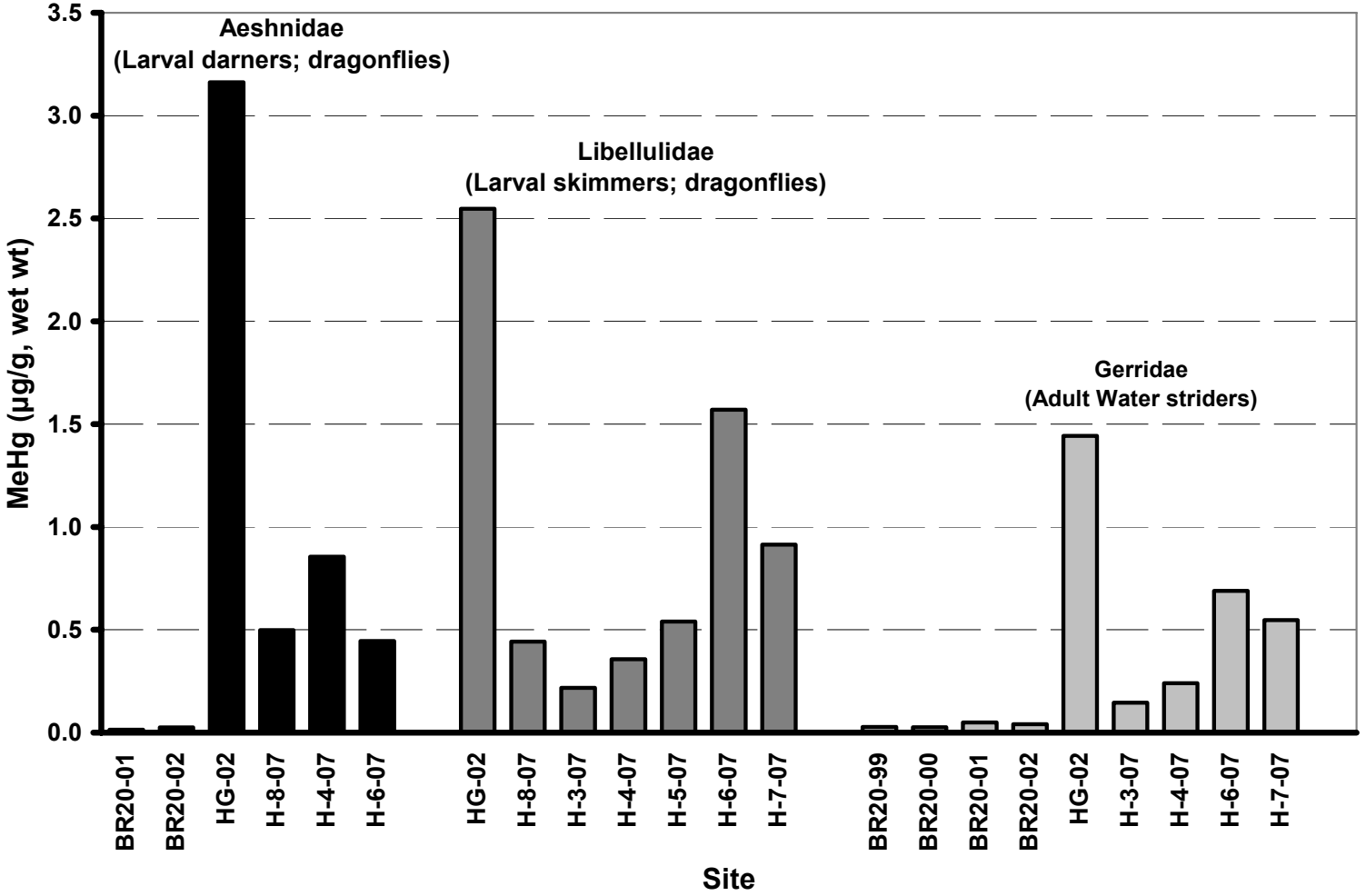

Figure 2. Methylmercury (MeHg, µg/g, wet wt) in individual composite samples of invertebrates (Aeshnidae, Libellulidae, and Gerridae) collected from Harley Gulch in October 2002 (HG-02) and May 2007 (H-4-8) and from a reference site, the Bear River at the Highway 20 bridge (BR20) during 1999-2002 (See Table 3).

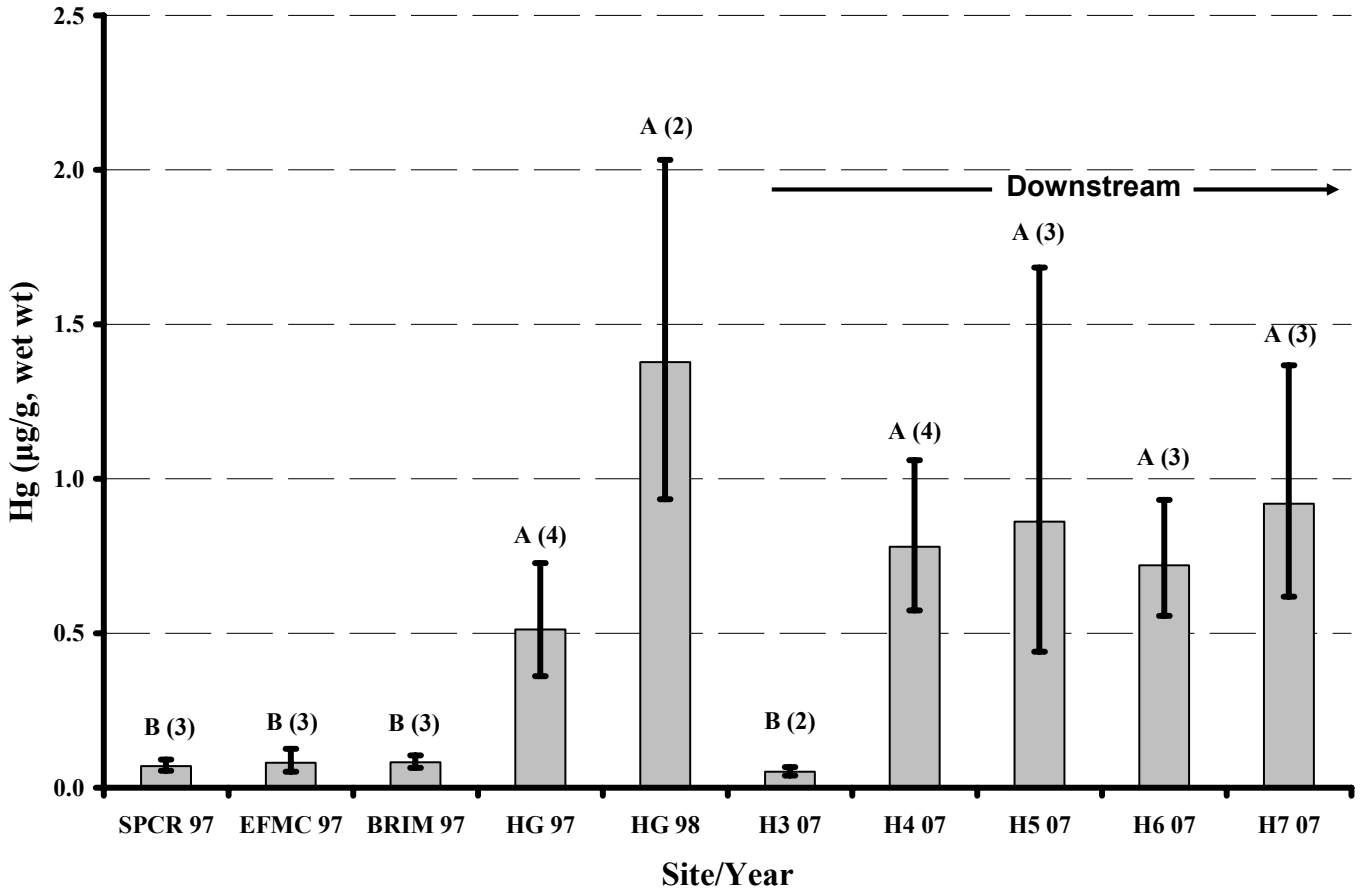

Figure 3. Geometric mean total Hg (µg/g, wet wt), 95% confidence limits, and sample sizes (n) in whole bodies of foothill yellow-legged frogs (FYLF) collected from East Fork Harley Gulch (H-3) and four sites (H 4-7) downstream of the confluence of East and West Forks Harley Gulch in May 2007 (Table 1), from three reference sites during April-May 1997 (SPCR = Spanish Creek; EFMC = East Fork Middle Creek; Brim = Bear Creek at Brim Road), and from Harley Gulch in 1997 and 1998 (Table 2). Harley Gulch included one FYLF from Turkey Run upstream of the West Fork of Harley Gulch in 1997 and one FYLF from the Abbott Mine Drain in 1998 (Fig. 1). Means not sharing a common letter were different ($P < 0.05$) by Tukey pairwise multiple comparison procedure.