Ecotoxicology
https://doi.org/10.1007/s10646-022-02608-5

High mercury concentrations in steelhead/rainbow trout, sculpin, and terrestrial invertebrates in a stream-riparian food web in coastal California

David E. Rundio[1] · Roberto Rivera[2,3] · Peter S. Weiss-Penzias[2]

Accepted: 20 November 2022

Abstract

Stream and riparian food webs are connected by cross-habitat exchanges of invertebrate prey that can transfer contaminants including mercury. Marine fog has been identified as a source of methylmercury (MeHg) to some terrestrial food webs in coastal California, suggesting that terrestrial invertebrates might have elevated MeHg relative to stream invertebrates and might lead to higher mercury exposure in fish that consume terrestrial subsidies. As an initial step to examine this possibility, we analyzed mercury concentrations in terrestrial and aquatic invertebrates and two fish species, steelhead/rainbow trout (*Oncorhynchus mykiss*) and coastrange sculpin (*Cottus aleuticus*), in a small watershed. Mean MeHg and total mercury (THg) concentrations in terrestrial invertebrates were three to four times higher than in aquatic invertebrates of the same trophic level. MeHg was >1000 ng/g dw in some individual centipede and scorpion samples, and also relatively high (100–300 ng/g dw) in some terrestrial detritivores, including non-native isopods. Mean THg in age 0 trout was 400 ng/g dw compared to 1200–1300 ng/g dw in age 1+ trout and sculpin, and the largest trout sampled had THg >3500 ng/g dw. However, the similar mercury concentrations between age 1+ trout and sculpin, despite different diet types, indicated that Hg concentrations in fish were not related simply to differences in consumption of terrestrial invertebrates. The high mercury concentrations we found in terrestrial invertebrates and fish suggest that further research on the sources and bioaccumulation of mercury is warranted in this region where *O. mykiss* populations are threatened.

Keywords Mercury · Steelhead/rainbow trout · Sculpin · Invertebrates · Terrestrial-aquatic subsidies · Non-native species

Introduction

Mercury is a global contaminant of concern for many fish and wildlife populations due to its persistence in the environment and potentially serious effects as a toxin (Driscoll et al. 2013; Eagles-Smith et al. 2016a; Chételat et al. 2020). Human activities have increased mercury levels in the atmosphere by more than 3-fold in the past 150 years (Streets et al. 2017). Most mercury in the environment is from atmospheric deposition of inorganic mercury, while methylmercury (MeHg) is the biologically available organic form that bioaccumulates in organisms and biomagnifies with increasing trophic level within food webs (Driscoll et al. 2013; Eagles-Smith et al. 2016a; Chételat et al. 2020). Methylation of inorganic mercury to MeHg primarily occurs through microbial activity under reducing conditions typically present in aquatic environments such as wetlands, sediments in freshwater and coastal habitats, and in the mid and upper ocean (e.g., MeHg maxima occur around 300 m

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10646-022-02608-5.

✉ David E. Rundio
dave.rundio@noaa.gov

[1] Fisheries Ecology Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 110 McAllister Way, Santa Cruz, CA 95060, USA

[2] Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, USA

[3] Present address: Department of Materials Science and Engineering, University of California, Irvine, 544 Engineering Tower, Irvine, CA 92697, USA

Published online: 30 November 2022

depth off California; Coale et al. 2018), leading to high MeHg concentrations in many aquatic food webs and consumers of aquatic organisms (Driscoll et al. 2013; Eagles-Smith et al. 2016a; Chételat et al. 2020).

Aquatic and terrestrial food webs are connected by reciprocal fluxes of materials and organisms across habitat boundaries (Polis et al. 1997; Nakano and Murakami 2001; Baxter et al. 2005), which can also result in transfer of mercury and other contaminants between ecosystems (Walters et al. 2008; Kraus et al. 2020). Because of high concentrations of MeHg and other contaminants in aquatic habitats, most attention regarding transfers between ecosystems has focused on the export of contaminants in aquatic organisms to consumers in adjacent terrestrial food webs (Sullivan and Rodewald 2012; Kraus et al. 2020). For example, stream and riparian food webs are closely linked by reciprocal subsidies of invertebrate prey (Nakano and Murakami 2001; Baxter et al. 2005), and emerging adult aquatic insects transfer MeHg to terrestrial predators including birds, bats, and spiders (Cristol et al. 2008; Tsui et al. 2012; Becker et al. 2018; Jackson et al. 2021). However, terrestrial invertebrate subsidies are a major energy source to fish and some aquatic invertebrate predators in many freshwater habitats, especially small streams and rivers in forest ecosystems (Nakano and Murakami 2001; Baxter et al. 2005), and may influence mercury dynamics in stream-riparian food webs depending on the relative concentrations of mercury in aquatic and terrestrial prey taxa. For example, subsidies of terrestrial invertebrates reduced the mercury burdens of fishes in streams in northeastern North America where aquatic insects had high mercury concentrations (Jardine et al. 2012; Ward et al. 2012). In contrast, terrestrial subsidies transferred MeHg to aquatic predators (water striders and juvenile steelhead trout) in small headwater streams in northern California, although concentrations in both fish and invertebrates were relatively low (Tsui et al. 2012, 2014).

Marine fog has recently been identified as an important source of MeHg to terrestrial food webs in coastal California, through a pathway where inorganic mercury is methylated in the midwater zone of the ocean and then brought by strong seasonal upwelling to the surface boundary layer where it enters the atmosphere through air-sea mixing and bubble burst and is subsequently transported to land as MeHg in fog and marine aerosols (Weiss-Penzias et al. 2012; Coale et al. 2018). This ocean-derived MeHg in fog appears to be the source of elevated mercury concentrations in coastal mountain lion populations, where fog MeHg enters the food web through lichen and bioaccumulates in deer and ultimately mountain lions, leading to mercury concentrations three times higher in coastal than inland populations in California (Weiss-Penzias et al. 2019). Mercury concentrations in several terrestrial invertebrate taxa also were higher along the coast of central California (Ortiz et al. 2015) than in an interior-facing, fog-sheltered basin in the coastal mountains of northern California (Tsui et al. 2019), suggesting that predators that consume terrestrial invertebrates in areas with fog might have increased exposure to mercury.

Terrestrial invertebrates are a major prey source for stream-dwelling steelhead/rainbow trout (*Oncorhynchus mykiss*) in watersheds on the Big Sur coast of central California, providing 15–20% of the annual energy in the diet of age 0 (young-of-year) trout and up to 60% of the energy in age 1+ trout (Rundio and Lindley 2008, 2019, 2021). Steelhead populations in this region are listed as threatened under the U.S. Endangered Species Act (U.S. Office of the Federal Register 2014), however the level of exposure to mercury and potential for harm have not been assessed for these populations in coastal streams. The recent studies on fog transfer of marine-derived MeHg in coastal California suggest that terrestrial invertebrates might have elevated MeHg concentrations relative to aquatic invertebrates in streams in this region and might expose trout that consume terrestrial subsidies to elevated mercury levels as well. As an initial step to assess this possibility, we analyzed mercury concentrations of terrestrial and aquatic invertebrate prey and fish predators, *O. mykiss* and coastrange sculpin (*Cottus aleuticus*), in a riparian-stream food web in a small basin on the Big Sur coast. Our objectives were to determine the range of mercury concentrations among consumers within the food web and to test two basic hypotheses: (1) that concentrations would be higher in terrestrial invertebrates than aquatic invertebrates of the same trophic level; and (2) that, based on differences in diet, concentrations would be higher in age 1+ *O. mykiss* than in age 0 trout or sculpin, which consume primarily aquatic insects (Rundio and Lindley 2019; Moyle 2002). Among terrestrial invertebrates, we were particularly interested in mercury concentrations in non-native isopods, as they are a major prey item for trout in Big Creek (accounting for 20–30% of the annual energy in the diet, more than any other prey taxon; Rundio and Lindley 2008) and other Big Sur streams (Rundio and Lindley 2021) and are known to bioaccumulate heavy metals including mercury (Hopkin et al. 1986; Dallinger et al. 1992 Pedrini-Martha et al. 2012).

Methods

Sampling design

For this study, we collected invertebrate samples in summer 2020 and used muscle tissue from fish specimens collected in 2005–2019 from previous sampling in the watershed that

were archived in a −20 °C freezer. We used these existing fish specimens to avoid needing to sacrifice new fish, particularly for threatened *O. mykiss*. Trout samples were incidental mortalities from backpack electrofishing surveys from a long-term study of population dynamics in Big Creek, which limited the number and sizes of archived specimens available. Sculpin were collected in 2017 as a proxy for threatened *O. mykiss* for some analyses in a study involving otolith (ear stone) chemistry. Workplace restrictions during the covid-19 pandemic restricted both field and laboratory activities and limited the number of samples we could collect and analyze. In addition, a large wildfire burned the study basin in August 2020, preventing additional invertebrate sampling and all fish sampling for the long-term population study that year. In January 2021, a post-wildfire debris flow caused massive disturbance to the stream and riparian zone, preventing replication of sampling across years. Despite these limitations on our dataset, the samples allowed for a general assessment of the range of mercury concentrations in fish and invertebrate consumers in the stream-riparian food web in this system and testing for basic differences between groups of consumers.

Study area

We conducted the study at the University of California (UC) Landels-Hill Big Creek Reserve (36.0708°, −121.5991°) within the Santa Lucia Mountains on the Big Sur coast of central California (Supplementary Information [SI] Fig. 1). The region is characterized by steep mountains, which reach 1600 m within 5 km of the coast, and a coastal Mediterranean climate, with a warm but foggy dry season and a cool and rainy wet season. The coastal marine layer and fog typically occur from sea level to 400–500 m elevation. Big Creek (watershed area 58 km^2) is typical of the many small coastal basins that drain the Santa Lucia Mountains, with narrow stream channels and riparian zones confined in steep hillsides. The basin is in relatively natural condition. Limited farming, livestock grazing, and logging occurred in some areas 70–130 years ago, but since the late 1970s the basin has been protected with the lower portion within the UC Reserve and the upper portion within the Los Padres National Forest Ventana Wilderness Area.

Our study area was the lower portion of Big Creek within 1.8 stream km from the ocean and elevation < 100 m, and included the mainstem of Big Creek (0–1200 m from the mouth) and the lower 600 m of the two main branches, upper Big Creek and Devils Creek (SI Fig. 1), overlapping with previous fish and food web studies in the basin. This area was within the zone with highest fog exposure in the basin, and fog penetrated up through the study reaches in both tributaries. Stream habitat was a mixture of pools and rapids and the channel gradient was 3–8%. Stream width averaged 5–6 m during summer base flow, with mean water depth of about 35 cm and maximum depth of about 1.75 m in pools. The streams had perennial flow, cool water temperatures (daily mean typically 8–10 °C during the winter wet season and 15–17 °C during the summer dry season), and were alkaline (pH typically 8.3–8.6). The riparian forest was primarily coast redwood (*Sequoia sempervirens*), white alder (*Alnus rhombifolia*), and bigleaf maple (*Acer macrophyllum*), with an understory of shrubs, and the canopy over the stream channel was almost fully closed from spring through fall. Riparian habitat on the streambanks included narrow gravel and cobble bars, boulders, soil, logs, and litter from leaves and redwood needles.

Steelhead/rainbow trout (*O. mykiss*) and coastrange sculpin (*C. aleuticus*) were the only fish species present in the stream. The *O. mykiss* population in Big Creek is partially migratory, consisting of both anadromous (steelhead) and nonanadromous (resident rainbow trout) individuals (Rundio et al. 2012). Adult *O. mykiss* spawn in the stream during winter and spring. After hatching, juveniles rear for 2–3 years before either migrating to the ocean (steelhead) or maturing in the stream (resident rainbow trout). Adult steelhead typically return to spawn after 1–2 years in the ocean, while resident trout may reach 6–7 years total age in the stream. Thus, *O. mykiss* present in the stream year-round include juveniles of both life-history types and mature resident trout (typically > 150 mm in length), but hereafter we will refer to all as 'trout' for simplicity. Adult coastrange sculpin also spawn in the stream during winter and spring, and after hatching larvae drift downstream for a brief (3–5 week) marine planktonic stage before returning to freshwater as juveniles to rear for 2–3 years until maturing (Moyle 2002; Rundio, unpublished data). Coastrange sculpin in California appear to reach 35–45 mm in length after their first year and can live up to 8 years and reach 145 mm (Moyle 2002).

Sample collection

We collected aquatic and terrestrial invertebrates using hand searches on two dates in June and August 2020, targeting larger taxa from a range of taxonomic groups and from both primary (detritivore/herbivore) and secondary/tertiary (predator) consumer levels. The sampling method and target taxa were chosen under the logistical constraints imposed by covid-19 workplace restrictions, where our goal was to sample representative taxa from the two trophic levels (including important trout prey such as terrestrial isopods) likely to span the range of mercury concentrations in the food web and that could be collected efficiently by a single person during limited visits. Invertebrates were collected throughout the study area in the lower basin, including mainstem Big Creek and both major branches, to overlap

with collection locations of the archived fish samples; given the similar habitat conditions and close proximity within this area, no differences in mercury concentrations were expected among stream reaches. Aquatic insects were captured by searching on and under large gravel and cobble and through accumulations of leaf/needle litter. We included large specimens of the largest-bodied and longest-lived predatory aquatic insects present in the stream (Perlidae stoneflies, Corydalidae dobsonflies, and Aeshnidae dragonflies), which we expected would reflect maximum mercury concentrations in aquatic invertebrates. Terrestrial invertebrates were captured by searching on and under gravel, cobbles, boulders, logs, and leaf/needle litter within 2 m of the stream. Samples were collected following clean techniques (gloved hands and clean forceps), placed into polypropylene vials, and stored on ice until being placed in a −20 °C freezer within 8 h. The number of specimens per sample ranged from one to 28 (mean = 6) depending on size of taxa and availability during searches, and we collected replicate samples for most taxa. Sample information is summarized in SI Table 1. With respect to the overlap between invertebrate samples and fish diets, all invertebrate orders sampled except scorpions were found in *O. mykiss* diets in previous studies (Rundio and Lindley 2008, 2019). The main prey taxa not included in this study were a few families of small-bodied aquatic insects (Baetidae mayflies and Chironomidae and Simuliidae true flies) and terrestrial hymenoptera, but we assume that mercury concentrations in these taxa were likely to fall within the range of values of sampled taxa.

The fish samples used in this study were taken from specimens captured during prior studies and stored in a −20 °C freezer. We selected 10 samples each for age 0 trout, age 1+ trout, and sculpin. The ten age 0 trout (72–90 mm) were selected from mortalities from 2010 when samples were available from all three stream reaches in the study area. For age 1+ trout, a lower incidental mortality rate and a desire to include specimens across the full range of sizes/ages present in the stream necessitated taking fish across a range of years from 2005 to 2019. The 10 age 1+ trout were 125 to 300 mm and ranged in age from probably 1.5 years (based on size) to one individual that appeared to be 7 years old (based on otolith annuli). Sculpin were collected in 2017 and were 86 to 128 mm, indicating that they were probably at least 2+ years old and likely included multiple ages (Moyle 2002). All fish were captured by backpack electrofishing, placed into individual plastic bags, and stored on ice before being frozen within 12 h. To obtain muscle samples for mercury analysis, fish were partially thawed to the point where tissues still contained ice crystals but 1–3 g (wet) skinless samples could be dissected from the dorsal muscle using a stainless steel scalpel. Fish were dissected using new gloves and scalpel blades between fish, and samples were placed in polypropylene vials and refrozen until analysis. Sample information is summarized in SI Table 2.

Laboratory analysis

Laboratory analyses were conducted at the University of California Santa Cruz (UCSC). Frozen fish and invertebrate samples were homogenized with 10% HCl-cleaned mortar and pestle and liquid nitrogen and then transferred to a 20 mL glass scintillation vial and lyophilized overnight to obtain dried sample for analysis of mercury concentration as ng/g dry weight. In fish, it generally appears that nearly all mercury (> 90–95%) is in the form of MeHg (Bloom 1992; but see Lescord et al. 2018 for exceptions), so we followed previous studies and guidelines and analyzed fish muscle samples only for total Hg (THg) under the assumption that this represented MeHg (U.S. EPA 2000; Peterson et al. 2007). To facilitate comparison with other studies and health benchmarks in which mercury concentrations in fish are often reported on the basis of wet weight in muscle or whole-body samples (see Discussion below), we assumed dry weight was 25% of wet weight (Reinitz 1983; Ciancio et al 2007) and used the muscle to whole-body regression for mercury concentration in Peterson et al. (2007). In invertebrates, the fraction of total mercury as MeHg can be more variable among taxa and feeding groups (Tsui et al. 2019; Riva-Murray et al. 2020), so we analyzed invertebrate samples for both THg and MeHg.

For THg analysis, 0.1–0.5 g of dried sample was weighed into quartz boats and analyzed in a Milestone® DMA 80 direct mercury analyzer according to EPA Method 7473 (U.S. EPA 2007). The instrument was calibrated with a liquid standard according to the manufacturer's instructions, and certified reference materials (CRMs) were run alongside the samples daily. Recoveries of CRMs (mean ± SD) were 107.0 ± 11.6% for BCR-320R ($n = 9$), 105.8 ± 13.2% for DOLT-3 ($n = 4$), 114.4 ± 8.5% for DORM-3 ($n = 4$), 98.0 ± 1.5% for DORM-4 ($n = 3$), and 96.7 ± 9.4% for IAEA-407 ($n = 14$); the mean recovery across all CRMs was 102.7 ± 11.4% ($n = 34$).

For MeHg analysis of invertebrate samples, 0.1–0.5 g of dried sample was weighed into 50 mL glass centrifuge tubes. Two mL of a 20% KOH solution in methanol was then added and heated to 60 °C for 4 h (Bloom 1989). After heating, deionized water (18.2 MΩ) was added, giving a final volume of 10 mL, and the sample was centrifuged at 1200 rpm for 10 min. The supernatant was withdrawn into a 20 mL glass scintillation vial. To convert dissolved MeHg to a volatile form so it can be detected, 0.1 mL of the digested sample was added to a purge vessel containing acetate buffer, to which sodium tetraethylborate was added according to EPA Method 1630 (U.S. EPA 1998). MeHg

was analyzed with gas chromatography separation (DB-1 capillary column, 60–120 °C temperature ramp), 800 °C pyrolysis on quartz wool, and detection with cold-vapor atomic fluorescence spectroscopy (Tekran® 2500). A control standard sample was run between every 4th sample, which consisted of 100 µL of a 1.0 ppb standard, diluted from a 1.0 ppm stock solution of CH_3HgCl (Brooks Rand Laboratories). The CRM used for MeHg analysis was DORM-4, and mean recovery was 90.3 ± 14.5% ($n = 6$).

For both the THg and MeHg analyses, we initially ran two replicate aliquots per sample. The relative percent difference (RPD) of these initial sample replicates was 12.3 ± 11.7% (mean ± SD) for THg and 23.2 ± 22.2% for MeHg. While this variability was comparable to some previous studies (e.g., Jardine et al. 2012), it was higher than others (e.g., RPD < 10%: Riva-Murray et al. 2011, Ward et al. 2012; Lescord et al 2018; Eagles-Smith et al. 2020). We suspect that this variability was due to imperfect homogenization of samples for THg and to error inherent to the manual MeHg extraction and analysis method. Therefore, we decided to run an additional aliquot for samples where the RPD was ≥ 40% to improve the accuracy of estimates of mean concentration per sample (based on all replicates); this applied to 27% of fish THg samples, 25% of invertebrate THg samples, and 28% of invertebrate MeHg samples. For a small number of invertebrate MeHg samples, a fourth ($n = 3$) or fifth ($n = 3$) aliquot was run if the RPD between the new aliquot(s) and both of the original replicates still was ≥40% or if the percent MeHg based on the means of the THg and MeHg replicates for the sample was >115%. These criteria for running additional aliquots were selected ad hoc based on inspection of the data to increase the number of replicates for samples where high variability among runs indicated possible analytical error. To formally screen the data for outliers, we calculated the RPD for all pairwise combinations of replicates per sample for both THg and MeHg. These RPD values were highly left-skewed, so to test for outliers we used the function *LocScaleB* in the R package *univOut* (D'Orazio 2021), as this method applies robust estimates of location and scale that are suitable for skewed data. Based on the outlier tests, we dropped a MeHg replicate from four invertebrate samples and a THg replicate from one invertebrate sample (out of 61 total invertebrate samples) that had extreme (and likely erroneous) values; excluding these outliers, the mean (± SD) relative standard deviation (RSD) of sample replicates was 9.4 ± 7.8% for THg and 16.2 ± 11.9% for MeHg. We took the mean of the remaining replicates per sample as our estimates of mercury concentrations for analysis (invertebrates, Table 1; fish, SI Table 2). Removing outlier replicates from the few invertebrate samples did not affect results of the statistical analyses below, and sample means with and without outliers are shown in SI Table 1.

As an additional quality assurance step, we had samples from five age 1+ trout independently analyzed for THg concentration by the Marine Pollution Studies Laboratory (MPSL) at Moss Landing Marine Laboratory. We dissected new dorsal muscle tissue samples (3–5 g wet) from the five fish, and provided the frozen samples to MPSL for analysis following their standard protocol. The mean (± SD) RPD between our original measurements of THg concentrations and those from MPSL was 14 ± 6%, and the slope of the linear regression between measurements did not differ from one (slope = 0.9997, $R^2 = 0.99$, $p = 0.99$) and the intercept did not differ from zero ($p = 0.09$) (SI Figure 2).

Data analysis

For invertebrates, we used linear mixed models (LMM) to determine whether mercury concentrations differed with respect to source (aquatic versus terrestrial) and trophic level (detritivore/herbivore versus predator). Models were fit with THg or MeHg concentration as the response, with source, trophic level, and their interaction as fixed-effect factors, and with taxon as a random effect to account for correlations among samples from the same taxon. THg and MeHg concentrations were log-transformed to meet assumptions of normality. Models were fit in the R package *nlme* (Pinheiro et al. 2021). Examination of residuals from simple linear models indicated heterogeneity of variances, so we used the *varIdent* function to allow variance to differ by the combination of trophic level and source. The significance of fixed-effect factors was determined by likelihood ratio tests between nested models, fit by maximum likelihood, with and without a specific factor. Models were re-fit by restricted maximum likelihood for estimation of mean mercury concentrations by source and trophic level.

Invertebrate percent MeHg values failed to meet the assumption of normality even after transformation, so we used the nonparametric Brunner-Munzel test (Brunner and Munzel 2000) to determine whether %MeHg differed with respect to source and trophic level. The Brunner-Munzel test is a rank order test that is robust to unequal variances between groups, which was true of our data. To avoid lack of independence of samples from the same taxon, we calculated mean %MeHg by taxon and then ran permuted Brunner-Munzel tests to determine whether %MeHg differed between aquatic versus terrestrial invertebrates of the same trophic level (e.g., aquatic predators vs. terrestrial predators) or between trophic levels within the same source (e.g., aquatic detritivores/herbivores vs. aquatic predators). We ran Brunner-Munzel tests in the R package *rankFD* (Konietschke et al 2021), with *p*-values determined by a studentized permutation test appropriate for small samples (Neubert and Brunner 2007).

Table 1 Taxonomic information and mean THg, MeHg, and %MeHg for aquatic and terrestrial invertebrate samples from Big Creek, California

Class	Order	Family	Genus/species	Common Name	Consumer Level	Consumer Type	*n*	THg (ng/g)	MeHg (ng/g)	%MeHg
Aquatic										
Insecta	Ephemeroptera	Heptageniidae	*Epeorus, Ironodes*	Mayfly	1°	H	1	69.70	55.30	79
Insecta	Plecoptera	Pteronarcyidae	*Pteronarcys californica*	Stonefly	1°	D	4	17.94	15.97	89
Insecta	Trichoptera	Limnephilidae	*Dicosmoecus gilvipes*	Caddisfly	1°	H	5	35.00	28.63	82
Insecta	Trichoptera	Limnephilidae	*Psychoglypha bella*	Caddisfly	1°	H	2	43.69	30.10	69
Insecta	Trichoptera	Thremmatidae	*Neophylax rickeri*	Caddisfly	1°	H	4	13.25	12.22	92
Insecta	Ephemeroptera	Ephemerellidae	*Drunella coloradensis*[a]	Mayfly	2°/3°	P	1	120.03	123.16	103
Insecta	Megaloptera	Corydalidae		Dobsonfly	2°/3°	P	1	140.57	108.26	77
Insecta	Odonata	Aeshnidae	*Aeshna*	Dragonfly	2°/3°	P	2	82.18	79.62	97
Insecta	Plecoptera	Perlidae	*Calineuria californica*	Stonefly	2°/3°	P	4	97.57	96.66	99
Terrestrial										
Diplopoda	Callipodida	Tynommatidae		Millipede	1°	D	2	363.88	299.51	82
Diplopoda	Polydesmida	Xystodesmidae	*Harpaphe haydeniana*	Millipede	1°	D	3	512.55	41.00	8
Gastropoda	Stylommatophora	Arionidae	*Prophysaon andersoni*	Slug	1°	H/D	1	86.13	46.76	54
Gastropoda	Stylommatophora	Helminthoglyptidae	*Helminthoglypta*	Snail	1°	H/D	1	56.08	26.75	48
Gastropoda	Stylommatophora	Polygyridae	*Vespericola*	Snail	1°	H/D	3	65.07	29.10	45
Malacostraca	Isopoda	Armadillidiidae	*Armadillidium vulgare*	Pillbug	1°	D	4	139.18	112.52	81
Malacostraca	Isopoda	Porcellionidae	*Porcellio scaber*	Sowbug	1°	D	5	329.65	285.56	87
Arachnida	Araneae	Zoropsidae	*Anachemmis*	Spider	2°/3°	P	3	777.94	678.10	87
Arachnida	Scorpiones	Vaejovidae	*Vaejovis*	Scorpion	2°/3°	P	3	777.90	757.27	97
Chilopoda	Scolopendromorpha	Scolopocryptopidae	*Scolopocryptops*	Centipede	2°/3°	P	4	685.77	609.50	89
Insecta	Coleoptera	Carabidae	*Pterostichus*	Beetle	2°/3°	P	4	128.90	117.43	91
Insecta	Coleoptera	Carabidae	*Scaphinotus*	Beetle	2°/3°	P	4	97.77	78.83	81

Primary consumers (1°) are detritivores (D) and herbivores (H), and secondary/tertiary consumers (2°/3°) are predators (P). The number of samples per taxon is indicated by n. THg and MeHg concentrations are ng/g dry weight

[a]Large, late instar nymphs are predatory (Hawkins 1985, 1990; Merritt and Cummins 1996)

Table 2 Results of significance tests from linear mixed models of THg and MeHg as a function of source (aquatic versus terrestrial) and trophic level (detritivore/herbivore versus predator) as fixed effects

	THg		MeHg	
Factor	L-ratio	*p*-value	L-ratio	*p*-value
Source	13.767	<0.001	8.473	0.004
Trophic Level	6.873	0.009	11.304	<0.001
Source*Trophic Level	0.715	0.398	0.001	0.976

Models were fit on log-transformed data, and included a random effect for taxon. Significance was determined from likelihood ratio tests between nested models with and without a particular factor. All tests have 1 degree of freedom

Table 3 Parameter estimates from linear mixed models of THg and MeHg as a function of source and trophic level as fixed effects and taxon as a random effect

Factor	THg	MeHg
Fixed Effects		
(Intercept)	3.519 ± 0.309	3.188 ± 0.327
Source (Terrestrial)	1.459 ± 0.355	1.133 ± 0.378
Trophic level (Predator)	0.940 ± 0.357	1.352 ± 0.380
Random effect		
Taxon (Intercept)	0.779	0.822
Residual	0.349	0.476

Reference levels for the fixed effects are source = aquatic and trophic level = detritivore/herbivore. For the fixed effect parameters, values are estimates ± one standard error (SE). For the random effect parameter, values are estimates of variance expressed as standard deviations (SD). Models were fit on log-transformed data

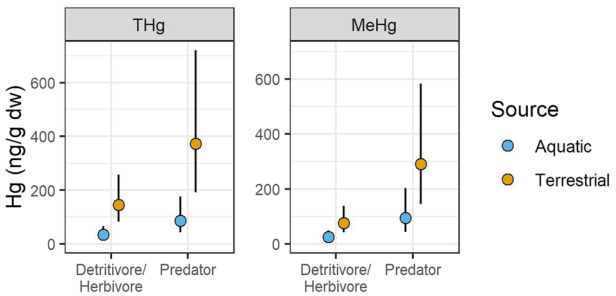

Fig. 1 Mean invertebrate THg and MeHg concentrations (with 95% confidence intervals) by source and trophic level. Estimates are back-transformed means from linear mixed models on log-transformed data, which are equivalent to geometric means on the original scale

For fish, our primary interest was whether THg concentrations differed among age 0 trout, age 1+ trout, and sculpin (age 1 +) as hypothesized based on differences in diet. Mercury concentrations also often increase with fish age and size due to bioaccumulation; however, we were unable to assess size and age class relationships for both species simultaneously (i.e., in a single model) due to the lack of samples from age 0 sculpin and the very different size ranges and size-at-age relationships between species. Similarly, samples for the different groups were collected in different years but we could not test for a year effect in a single model because only age 1+ trout were collected over multiple years. We therefore analyzed THg in fish in three steps. First, we tested whether THg concentrations differed among the three groups using one-way analysis of variance (ANOVA) followed by post-hoc pairwise tests using the package *emmeans* (Lenth 2021), recognizing that any differences between groups were confounded with size and age and collection year. Second, we used linear regression to determine whether THg concentration was related to fish length, with separate models for trout and sculpin. For trout, we included age class in the model to determine whether age classes differed after accounting for length. Finally, we used age 1+ trout samples to test whether THg concentrations were related to collection year, after accounting for fish length. We fit a linear regression model to test for a trend with year as a continuous variable, and fit LMMs with and without year as a categorical random effect to test for variation among years without a trend using a likelihood ratio test between models; both types of models included length as a fixed effect. THg values were log-transformed for all three analyses to meet assumptions of normality and equal variance.

All analyses were conducted in the program R version 4.1.1 (R Core Team 2021), and all plotting was done using the package *ggplot2* (Wickham 2016). For reporting results, estimated means and effect sizes (i.e., parameter estimates for fixed-effects factors) from models on log-transformed Hg concentrations were back-transformed to the original scale where they are equivalent to geometric means and multiplicative differences of geometric means, respectively.

Results

For invertebrates, concentrations of both THg and MeHg differed significantly by source and by trophic level (Table 2). Mean THg was estimated to be 4.3 (95% CI: 2.0–9.1) times higher in terrestrial invertebrates than in aquatic invertebrates, and mean MeHg 3.1 (95% CI: 1.4– 6.9) times higher (Table 3, Fig. 1), and these differences were the same regardless of trophic level (i.e., no source by trophic level interaction; Table 2). Mean THg in predators was estimated to be 2.6 (95% CI: 1.2–5.4) times higher than in detritivores/herbivores, and MeHg 3.9 (95% CI: 1.7–8.6) times higher, for both terrestrial and aquatic invertebrates (Table 3, Fig. 1). There also was very high variability in mercury concentrations among taxa, with the variance associated with the random effect for taxa of nearly the same magnitude as the parameter estimates for trophic level and source

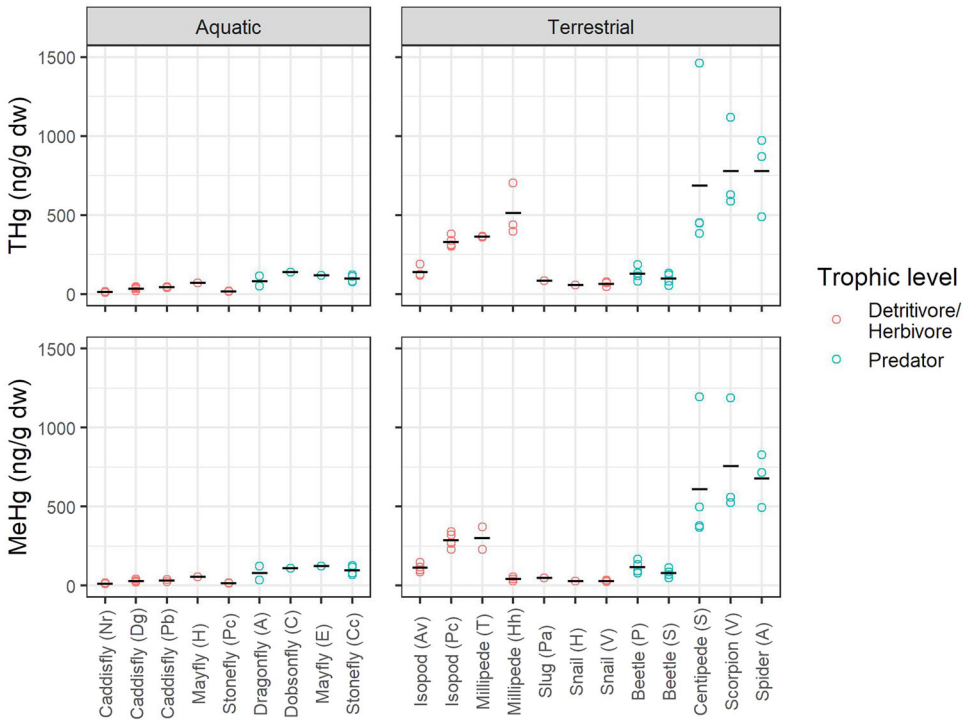

Fig. 2 Invertebrate THg and MeHg concentrations by individual taxa. Dots are individual samples, and bars are means by taxon. Taxa are listed by common name, with genus/species or family indicated in parentheses (see Table 1)

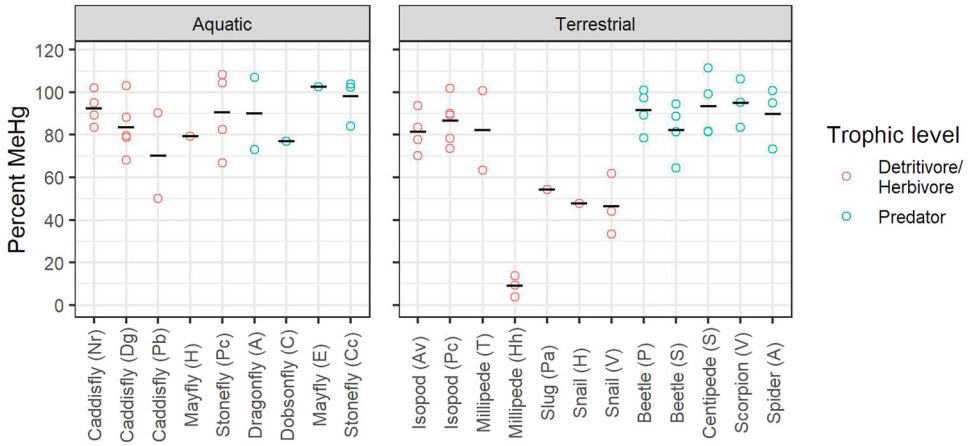

Fig. 3 Invertebrate % MeHg by individual taxa. Dots are individual samples, and bars are means by taxon. Taxa are listed by common name, with genus/species or family indicated in parentheses (see Table 1)

(Table 3). Terrestrial centipedes, scorpions, and spiders had the highest mercury levels measured, with mean THg and MeHg of 600–775 ng/g dw and with individual samples having values up to 1000–1500 ng/g dw (Table 1, Fig. 2). In contrast, predatory ground beetles (Carabidae) had much lower concentrations (mean THg and MeHg of 80–130 ng/g dw) that were more similar to predatory aquatic insects (Table 1, Fig. 2). Terrestrial isopods and millipedes had high mercury concentrations relative to other terrestrial detritivores/herbivores (gastropods) and most aquatic taxa, although there also was considerable variability among isopod and millipede taxa (Table 1, Fig. 2). For non-native isopods, mean THg and MeHg were 110–140 ng/g dw for *Armadillidium vulgare* compared with 285–330 ng/g dw for *Porcellio scaber*. For millipedes, mean THg was about 510 ng/g dw for *H. haydeniana* and 365 ng/g dw for Tynommatidae, but mean MeHg was only about 40 ng/g dw for *H. haydeniana* compared to 300 ng/g dw in Tynommatidae (Table 1, Fig. 2).

Percent MeHg in invertebrates was around 70–100% in aquatic taxa and 80–100% in terrestrial predator taxa (Table 1, Fig. 3). In contrast, terrestrial detritivores/herbivores had much greater variability in %MeHg among taxa, ranging from about 10% in *H. haydeniana* millipedes to about 50% in snails and slugs to about 80–90% in isopods and Tynommatidae millipedes (Table 1, Fig. 3). At the group level, terrestrial detritivores/herbivores had significantly lower %MeHg than terrestrial predators (Brunner-Munzel

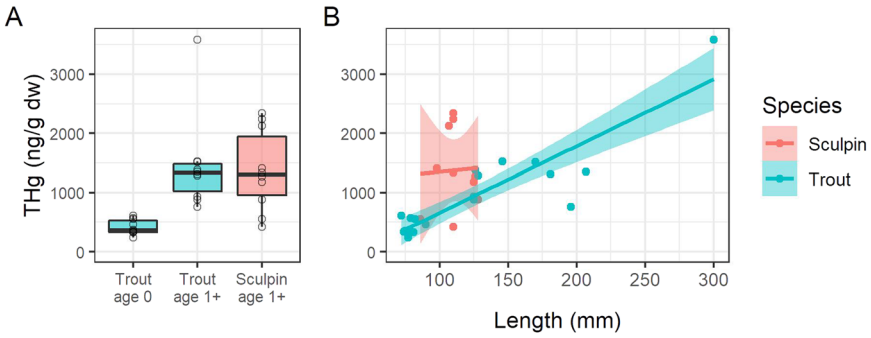

Fig. 4 Fish THg concentrations. **A** THg in age 0 trout, age 1+ trout, and sculpin. Boxplots indicate median and interquartile range (IQR), and whiskers are 1.5*IQR. Points are individual samples. **B** Relationship between THg and fish length by species. Line and shading are linear regression with 95% confidence interval

test, $t = 11.667$, $p = 0.004$) but did not differ significantly from aquatic detritivores/herbivores ($t = 2.182$, $p = 0.082$). Percent MeHg also did not differ between aquatic and terrestrial predators ($t = 0.396$, $p = 0.722$) or between aquatic detritivores/herbivores and aquatic predators ($t = 0.939$, $p = 0.407$).

For fish, mean THg was estimated to be three times lower in age 0 trout (399 ng/g dw; 95% CI: 298–534) than in age 1+ trout (1326 ng/g dw; 95% CI: 992–1772) or sculpin (1199 ng/g dw; 95% CI: 897–1620) (pairwise post-hoc tests, $t > 5.50$, $p < 0.001$), which did not differ from one another (post-hoc test, $t = 0.458$, $p = 0.89$) (Fig. 4a). For trout, a linear regression model with both length and age class indicated that THg increased significantly with length ($F = 71.569$, $p < 0.001$) but also differed between age classes ($F = 10.066$, $p = 0.006$), with THg in age 1+ trout estimated to be 2.0 times higher (95% CI: 1.3–3.2) than in age 0 trout after accounting for length. There was no relationship between length and THg for sculpin ($F = 0.182$, $p = 0.681$) (Fig. 4b). For age 1+ trout, there was no relationship between THg and collection year, either as a linear trend ($F = 0.924$, $p = 0.368$) or as random categorical effect ($L = 0.575$, $p = 0.448$), after accounting for length. Among samples from individual fish, THg was as high as 2100–2400 ng/g dw in some sculpin and nearly 3600 ng/g dw in the largest and oldest age 1+ trout (Fig. 4, SI Table 2).

Discussion

Mercury concentrations in terrestrial invertebrates and fish (steelhead/rainbow trout and coastrange sculpin) in this small coastal basin were very high for habitats without point-source contamination, with THg and MeHg > 1000 ng/g dw in some individual terrestrial invertebrate predator samples and THg > 3500 ng/g dw in the oldest trout sampled. Mercury concentrations were about 3 to 4 times higher (for MeHg and THg, respectively) in terrestrial invertebrates than aquatic invertebrates of the same trophic level, consistent with our hypothesis. However, THg concentrations in fish in Big Creek did not support our hypothesis that higher consumption of terrestrial invertebrates by age 1+ trout would lead to higher mercury levels relative to age 0 trout or sculpin that feed primarily on aquatic insects. While THg was twice as high (after accounting for length) in age 1+ trout as in age 0 trout, age 1+ trout and sculpin had similar THg, indicating that mercury concentrations in fish in this stream are not related simply to differences in diet and that other mechanisms of bioaccumulation are also important. Therefore, although the elevated levels of mercury we found in terrestrial invertebrates relative to aquatic invertebrates in this basin suggest that cross-habitat prey subsidies may potentially increase mercury exposure to threatened *O. mykiss*, additional studies using mercury stable isotopes (e.g., Tsui et al. 2012, 2014) will be needed to determine the sources of mercury to different consumers in this coastal stream-riparian food web.

Comparison of mercury concentrations in Big Creek to values reported by Tsui et al. (2012, 2014, 2019) from a fog-sheltered study area on the inland side of the coast range in northern California appears to provide some support that terrestrial invertebrates may have higher MeHg in fog-exposed basins. The studies by Tsui et al. were conducted in the University of California Angelo Coast Reserve in the forested headwaters of the South Fork Eel River. The Angelo Reserve is approximately 12–15 km from the ocean but is blocked from marine fog by a high ridge of the coastal mountains (https://angelo.berkeley.edu/about-angelo/geo-context/). Mercury concentrations of aquatic invertebrates were similar in Big Creek and the Angelo Reserve (Tsui et al. 2012). In contrast, terrestrial invertebrates in Big Creek had higher MeHg concentrations than in the Angelo: the difference was greatest for spiders, centipedes, and scorpions (about 3- to 6-fold) but occurred in other taxa (ground beetles, slugs, and millipedes) as well. MeHg concentrations reported by Ortiz et al. (2015) for spiders and ground beetles from fog-exposed sites near Monterey Bay also were higher than the Angelo Reserve and more similar to levels in Big Creek. While very limited, this apparent difference in MeHg levels in terrestrial

invertebrates between fog-exposed and fog-sheltered sites in California is similar to the pattern seen in deer and mountain lions (Weiss-Penzias et al. 2019) and consistent with observations of high MeHg levels in marine fog and aerosols (Weiss-Penzias et al. 2012; Coale et al. 2018). However, studies from additional sites varying in fog exposure are needed before conclusions about the influence of fog on mercury concentrations in terrestrial invertebrates can be reached.

With respect to broader geographic patterns, MeHg concentrations in terrestrial invertebrates also appear to be higher in Big Creek than in forests in the eastern United States, whereas concentrations in aquatic invertebrates in Big Creek appear to be lower than in eastern streams. For example, mean MeHg concentrations in terrestrial predators such as spiders, centipedes, and scorpions in Big Creek were 2–3 times higher, or more, than in studies from the eastern U.S. (Rimmer et al. 2010; Rodenhouse et al 2019; Tsui et al. 2019). Further, MeHg levels in some detritivores in Big Creek (Tynommatidae millipedes and Porcellionidae isopods) exceeded levels in predators in those studies, and were about 10 times greater than millipedes at the locations studied in Tsui et al. (2019). In addition, %MeHg in terrestrial invertebrates also was higher in Big Creek than the sites in Tsui et al. (2019). In contrast, for aquatic invertebrates, MeHg concentrations in predators (for which the most taxa are in common across studies) in Big Creek were 2–3 times lower than in eastern streams (Riva-Murray et al. 2011; Jardine et al. 2012; Broadley et al. 2019). Mercury concentrations in aquatic organisms tend to be highest in acidic streams (Ward et al. 2010; Jardine et al. 2013), so the higher pH (> 8) in Big Creek may partly explain the lower mercury concentrations in aquatic insects relative to streams in eastern North America with lower pH (Riva-Murray et al. 2011; Jardine et al. 2012; Broadley et al. 2019).

Mercury concentrations in trout and sculpin in Big Creek were higher than average levels in these species across freshwater habitats in western North America (Peterson et al. 2007; Eagles-Smith et al. 2016b). For example, mean THg in age 1+ trout in Big Creek was four times higher than the mean concentration for age 1+ rainbow trout across streams and rivers in the western United States (approximately 325 ng/g dw after converting from whole-body wet weight, Peterson et al. 2007) and 4–5 times higher than age 1+ trout in the Angelo Reserve in northern California (Tsui et al. 2014). Likewise, mean THg in age 0 trout in Big Creek was more than five times higher than age 0 trout in the Angelo Reserve, despite similar mercury concentrations in aquatic insects between the sites (Tsui et al. 2012); in fact, age 0 trout in Big Creek had higher concentrations than most age 1+ trout in Peterson et al. (2007) and Tsui et al. (2014). The high mercury concentrations in trout and sculpin in Big Creek were more similar to levels in salmonids and blacknose dace (a benthic insectivore) in acidic streams in eastern North America where aquatic invertebrates have high mercury concentrations (Riva-Murray et al. 2011; Jardine et al. 2012; Ward et al. 2012; Broadley et al. 2019). However, the relatively low mercury concentrations in aquatic invertebrates in Big Creek suggest that the sources or processes driving biomagnification and bioaccumulation in these systems differ.

Mercury concentrations in some age 1+ trout (at sizes corresponding to both juvenile steelhead prior to ocean migration and juvenile and resident trout) and sculpin in Big Creek are in the range where studies have found negative health effects in fish, although assessing the potential consequences is complicated by several uncertainties. In reviews that involved primarily laboratory experiments but also included some field studies, Beckvar et al. (2005) suggested that sublethal effects on growth, behavior, and reproduction may occur in fish with muscle concentrations greater than about 1400 ng/g dw (0.2 µg/g whole-body ww). Similarly, Sandheinrich and Weiner (2011) concluded that changes in biochemical processes, damage to cells and tissues, and reduced reproduction occur at muscle concentrations of about 2200–5400 ng/g dw (0.3–0.7 µg/g whole body ww) based on lab experiments, and that these effects were supported by correlations between mercury levels and health biomarkers (such as gene and hormone activity, tissue histology, and condition factor) in field studies. Beyond sublethal effects, Dillon et al. (2010) suggested that low rates (3–13%) of severe injuries related to lethality (survival, spawning and hatching success, and severe developmental abnormalities) may occur in juvenile and adult fish at muscle tissue concentrations of about 700–3800 ng/g dw (0.1–0.5 µg/g whole body ww) from a dose-response curve based on lab experiments, although the 95% confidence intervals for injury rates included zero at 700–1400 ng/g dw.

However, in addition to the considerable ranges in health effect thresholds in the above reviews, interpreting the potential for negative health effects is further complicated by uncertainty and debate around how lab studies apply to fish under natural conditions and how selenium may interact with mercury to influence toxicity. With respect to the applicability of lab studies, Harris et al. (2003) showed that the form of MeHg in fish tissue is methylmercury cysteine (MeHgCys) and appears to be less toxic than the form of MeHg used in most lab exposure studies (methylmercury chloride, MeHgCl), leading them and Peterson et al. (2009) to suggest that lab experiments may overestimate toxicity. However, Sandheinrich and Weiner (2011) argued that the correspondence between limited field studies or lab studies using natural diets and lab studies using MeHgCl suggested that this is not the case, and Depew et al. (2012) concluded that there is insufficient evidence to determine whether

dietary MeHgCl and MeHgCys differ in toxicity. In addition, Dillon et al. (2010) speculated that their dose-response model based on lab studies probably underestimated injury rates in wild fish due to the additional stressors, such as predation, competition, and environmental conditions, that occur in nature.

With respect to selenium, its role in mediating mercury toxicity is another potentially important but poorly understood factor that was not addressed in the above reviews that developed mercury health thresholds. Some studies, primarily in mammals and birds, have been used to suggest that selenium may have protective effects against mercury toxicity when Se:Hg molar ratios in fish tissues are > 1 (Peterson et al. 2009). However, recent reviews have argued that conclusions about the potential protective effects of selenium to fish and other aquatic organisms are still unclear due to limited and variable results among studies, the complexity of biochemical pathways and effects involving selenium and mercury within organisms, and the toxicity of selenium itself (Eagles-Smith et al. 2018; Gerson et al. 2020). In spite of this uncertainty, after finding high mercury levels in fish in Big Creek, we analyzed selenium concentrations from a small number of age 1+ trout and invertebrates to provide additional context for interpreting potential health effects. This preliminary analysis indicated that Se:Hg molar ratios were > 1 in age 1+ trout and aquatic and terrestrial invertebrates (SI Table 3), and also that selenium concentrations in trout (1.7–4.2 µg/g dw muscle; SI Table 3) were below levels associated with reproductive harm and juvenile mortality in fish due to toxicity of selenium itself (8–11 µg/g dw muscle: Lemly 2002; U.S. EPA 2021). In sum, the limitations and debates involving toxicity studies described above make it unclear whether the high mercury concentrations in trout and sculpin in Big Creek are potentially leading to negative health effects, but this topic appears to warrant further research for threatened *O. mykiss*.

Non-native species occur in many ecosystems and can have strong ecological effects, yet there has been very little research on their role in mercury dynamics in food webs or invertebrate subsidies to aquatic and terrestrial predators (Eagles-Smith et al. 2018; Rundio and Lindley 2021). In Big Creek, non-native terrestrial isopods (*A. vulgare* and *P. scaber*) appear to be a potentially important source of mercury to trout due to the combination of their abundance in the diet (Rundio and Lindley 2008, 2019) and high mercury concentration relative to most aquatic invertebrate taxa and many terrestrial taxa (Table 1, Fig. 2). Non-native isopods have become established in temperate regions around the world and often reach very high densities (summarized in Rundio and Lindley 2021), and isopods are known to bioaccumulate heavy metals including mercury (Hopkin et al. 1986; Dallinger et al. 1992; Pedrini-Martha et al. 2012), suggesting that they, and potentially other non-native species, may be important to mercury bioaccumulation and transfer in many areas.

Our study had several limitations that restricted our dataset and some of the analyses and inferences we could draw. Our sample sizes were relatively small due to constraints collecting and analyzing samples from covid-19 restrictions, the wildfire and debris flow in the study basin, and using archived specimens of threatened *O. mykiss*. We were also unable to replicate sampling across years due to the fire and debris flow, which necessitated comparing samples types collected in different years. Although concentrations in age 1+ trout (for which we had samples from multiple years) did not differ among collection years, this test likely had low power due to the small sample. Given the different size ranges and size-at-age relationships for trout and sculpin, lack of samples from age 0 sculpin, and lack of age information for age 1+ fish, we were unable to evaluate relationships between THg and fish size and age rigorously or between species. For invertebrates, we sampled large-bodied individuals and taxa due to logistical constraints and omitted some small aquatic taxa that are important in fish diets as well as canopy and aerial terrestrial taxa such as Lepidoptera, Diptera, and Hymenoptera, and also lacked replication for some sampled taxa. There also was high variability in mercury concentrations both among samples within some taxa and among taxa within the same trophic level. Despite these caveats, our dataset allowed for a general assessment of the range of mercury concentrations in fish and invertebrate consumers in the food web and testing for basic differences between groups of consumers.

In conclusion, our study documented high mercury concentrations in terrestrial invertebrates and fish in this coastal basin, but the relationship between cross-habitat subsidies of terrestrial prey and mercury concentrations in fish remains unclear. Nevertheless, concentrations in both age classes of trout and sculpin were high relative to other locations in the western U.S., reaching levels in some fish potentially associated with negative health effects, which suggests that mercury may be a possible stressor for fish populations in this region where *O. mykiss* is listed as threatened. These initial results indicate that further research is warranted to determine the sources and transport of mercury, including the possible role of fog, leading to high concentrations in coastal food webs at the intersection of marine, terrestrial, and freshwater ecosystems in this region.

Acknowledgements We thank Wes Heim (Marine Pollution Studies Laboratory at Moss Landing Marine Laboratory) for analyzing samples for quality control comparisons; Bob Wisseman (Aquatic Biology Associates), Dessie Underwood (California State University, Long Beach), William Shear (Hampden-Sydney College), and James Labonte (Oregon Department of Agriculture) for identifying voucher specimens of invertebrates; Martin Tsui (University of North Carolina,

Greensboro) for information on interference issues with sample digestions methods for MeHg; and Chris Russo (W.M. Keck Collaboratory for Plasma Spectrometry, Oregon State University) for analyzing samples for selenium. We thank Mark Readdie for support for field sampling at the University of California Landels-Hill Big Creek Reserve. We are grateful to Darren Ward (Humboldt State University) and three anonymous reviewers for helpful comments that improved the manuscript. This study was supported by NOAA Fisheries and a Keeley Coastal Scholars undergraduate scholarship at the University of California, Santa Cruz, to RR. Reference to trade names or manufacturers is for descriptive purposes only and does not imply U.S. Government endorsement of commercial products.

Author contributions DR conceived of the study, and PSW-P provided input to study design and supervised laboratory analysis. DR collected field samples, and RR, DR, and PSW-P performed laboratory analysis. DR analyzed the data and wrote the first draft of the manuscript. PSW-P and RR commented on previous versions of the manuscript, and all authors read and approved the final manuscript.

Funding This study was supported by NOAA Fisheries and by a Keeley Coastal Scholars undergraduate scholarship at the University of California, Santa Cruz, to RR.

Compliance with ethical standards

Competing interests The authors declare no competing interests.

Ethics approval Capture and handling of fish was conducted under National Marine Fisheries Service Section 10(a)(1)(A) Scientific Research Permits 1044 and 17219 and California Department of Fish and Wildlife Scientific Collection Permit 13029.

References

Baxter CV, Fausch KD, Saunders WC (2005) Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshw Biol 50:201–220. https://doi.org/10.1111/j.1365-2427.2004.01328.x

Becker DJ, Chumchal MM, Broders HG, Korstian JM, Clare EL, Rainwater TR, Platt SG, Simmons NB, Fenton MB (2018) Mercury bioaccumulation in bats reflects dietary connectivity to aquatic food webs. Environ Pollut 233:1076–1085. https://doi.org/10.1016/j.envpol.2017.10.010

Beckvar N, Dillon TM, Read LB (2005) Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Environ Toxicol Chem 24:2094–2105. https://doi.org/10.1897/04-284R.1

Bloom N (1989) Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Can J Fish Aquat Sci 46:1131–1140. https://doi.org/10.1139/f89-147

Bloom NS (1992) On the chemical form of mercury in edible fish and marine invertebrate tissue. Can J Fish Aquat Sci 49:1010–1017. https://doi.org/10.1139/f92-113

Broadley HJ, Cottingham KL, Baer NA, Weathers KC, Ewing HA, Chaves-Ulloa R, Chickering J, Wilson AM, Shrestha J, Chen CY (2019) Factors affecting MeHg bioaccumulation in stream biota: the role of dissolved organic carbon and diet. Ecotoxicology 28:949–963. https://doi.org/10.1007/s10646-019-02086-2

Brunner E, Munzel U (2000) The nonparametric Behrens-Fisher problem: asymptotic theory and a small-sample approximation. Biom J 42(1):17–25. 10.1002/(SICI)1521-4036(200001)42:1%3C17::AID-BIMJ17%3E3.0.CO;2-U

Chételat J, Ackerman JT, Eagles-Smith CA, Hebert CE (2020) Methylmercury exposure in wildlife: a review of the ecological and physiological processes affecting contaminant concentrations and their interpretation. Sci Tot Environ 711:135117. https://doi.org/10.1016/j.scitotenv.2019.135117

Ciancio JE, Pascual MA, Beauchamp DA (2007) Energy density of Patagonian aquatic organisms and empirical predictions based on water content. Trans Am Fish Soc 136:1415–1422. https://doi.org/10.1577/T06-173.1

Coale K, Heim WA, Negrey J, Weiss-Penzias P, Fernandez D, Olson A, Chiswell H, Byington A, Bonnema A, Martenuk S, Newman A, Beebe C, Till C (2018) The distribution and speciation of mercury in the California current: Implications for mercury transport via fog to land. Deep Sea Res Part II: Topical Studies Oceanogr 151:77–88. https://doi.org/10.1016/j.dsr2.2018.05.012

Cristol DA, Brasso RL, Condon AM, Fovargue RE, Friedman SL, Hallinger KK, Monroe AP, White AE (2008) The movement of aquatic mercury through terrestrial food webs. Science 320:335–335. https://doi.org/10.1126/science.1154082

Dallinger R, Berger B, Birkel S (1992) Terrestrial isopods: useful biological indicators of urban metal pollution. Oecologia 89:32–41. https://doi.org/10.1007/BF00319012

Depew DC, Basu N, Burgess NM, Campbell LM, Devlin EW, Drevnick PE, Hammerschmidt CR, Murphy CA, Sandheinrich MB, Wiener JG (2012) Toxicity of dietary methylmercury to fish: derivation of ecologically meaningful threshold concentrations. Environ Toxicol Chem 31:1536–1547. https://doi.org/10.1002/etc.1859

Dillon T, Beckvar N, Kern J (2010) Residue-based mercury dose–response in fish: An analysis using lethality-equivalent test endpoints. Environ Toxicol Chem 29:2559–2565. https://doi.org/10.1002/etc.314

D'Orazio M (2021) univOutl: detection of univariate outliers. R package version 0.3. https://CRAN.R-project.org/package=univOutl.

Driscoll CT, Mason RP, Chan HM, Jacob DJ, Pirrone N (2013) Mercury as a global pollutant: sources, pathways, and effects. Environ Sci Technol 47:4967–4983. https://doi.org/10.1021/es305071v

Eagles-Smith CA, Wiener JG, Eckley CS, Willacker JJ, Evers DC, Marvin-DiPasquale M, Obrist D, Fleck JA, Aiken GR, Lepak JM, Jackson AK, Webster JP, Stewart AR, Davis JA, Alpers CN, Ackerman JT (2016a) Mercury in western North America: A synthesis of environmental contamination, fluxes, bioaccumulation, and risk to fish and wildlife. Sci Tot Environ 568:1213–1226. https://doi.org/10.1016/j.scitotenv.2016.05.094

Eagles-Smith CA, Ackerman JT, Willacker JJ, Tate MT, Lutz MA, Fleck JA, Stewart AR, Wiener JG, Evers DC, Lepak JM, Davis JA, Pritz CF (2016b) Spatial and temporal patterns of mercury concentrations in freshwater fish across the Western United States and Canada. Sci Tot Environ 568:1171–1184. https://doi.org/10.1016/j.scitotenv.2016.03.229

Eagles-Smith CA, Silbergeld EK, Basu N, Bustamante P, Diaz-Barriga F, Hopkins WA, Kidd KA, Nyland JF (2018) Modulators of mercury risk to wildlife and humans in the context of rapid global change. Ambio 47:170–197. https://doi.org/10.1007/s13280-017-1011-x

Eagles-Smith CA, Willacker JJ, Nelson SJ, Flanagan Pritz CM, Krabbenhoft DP, Chen CY, Ackerman JT, Grant EHC, Pilliod DS (2020) A national-scale assessment of mercury bioaccumulation in United States national parks using dragonfly larvae as biosentinels through a citizen-science framework. Environ Sci Technol 54:8779–8790. https://doi.org/10.1021/acs.est.0c01255

Gerson JR, Walters DM, Eagles-Smith CA, Bernhardt ES, Brandt JE (2020) Do two wrongs make a right? Persistent uncertainties

regarding environmental selenium–mercury interactions. Environ Sci Technol 54:9228–9234. https://doi.org/10.1021/acs.est.0c01894
Harris HH, Pickering IJ, George GN (2003) The chemical form of mercury in fish. Science 301:1203. https://doi.org/10.1126/science.1085941
Hawkins CP (1985) Food habits of species of ephemerellid mayflies (Ephemeroptera: Insecta) in streams of Oregon. American Midland Naturalist 113:343–352. https://doi.org/10.2307/2425580
Hawkins CP (1990) Relationships between habitat dynamics, food availability, and growth patterns of Ephemerellid mayflies from western North America. In: Campbell IC (ed) Mayflies and stoneflies: life histories and biology. Series Entomologica, vol 44. Springer, pp 35–42. https://doi.org/10.1007/978-94-009-2397-3_5.
Hopkin SP, Hardisty GN, Martin MH (1986) The woodlouse *Porcellio scaber* as a 'biological indicator' of zinc, cadmium, lead and copper pollution. Environ Pollut 11:271–290. https://doi.org/10.1016/0143-148X(86)90045-5
Jackson AK, Eagles-Smith CA, Robinson WD (2021) Differential reliance on aquatic prey subsidies influences mercury exposure in riparian arachnids and songbirds. Ecol Evol 11:7003–7017. https://doi.org/10.1002/ece3.7549
Jardine TD, Kidd KA, Rasmussen JB (2012) Aquatic and terrestrial organic matter in the diet of stream consumers: implications for mercury bioaccumulation. Ecol Appl 22:843–855. https://doi.org/10.1890/11-0874.1
Jardine TD, Kidd KA, O'Driscoll N (2013) Food web analysis reveals effects of pH on mercury bioaccumulation at multiple trophic levels in streams. Aquat Toxicol 132:46–52. https://doi.org/10.1016/j.aquatox.2013.01.013
Konietschke F, Friedrich S, Brunner E, Pauly M (2021) rankFD: rank-based tests for general factorial designs. R package version 0.1.0. https://CRAN.R-project.org/package=rankFD.
Kraus JM, Walters DM, Mills MA (2020) Contaminants and ecological subsidies: the land-water interface. Springer Nature, Switzerland, 10.1007/978-3-030-49480-3
Lemly AD (2002) A procedure for setting environmentally safe total maximum daily loads (TMDLs) for selenium. Ecotoxicol Environ Saf 52:123–127. https://doi.org/10.1006/eesa.2002.2176
Lenth R (2021) emmeans: estimated marginal means, aka least-squares means. R package version 1.7.0. https://CRAN.R-project.org/package=emmeans.
Lescord GL, Johnston TA, Branfireun BA, Gunn JM (2018) Percentage of methylmercury in the muscle tissue of freshwater fish varies with body size and age and among species. Environ Toxicol Chem 37:2682–2691. https://doi.org/10.1002/etc.4233
Merritt RW, Cummins KW (1996) An introduction to the aquatic insects of North America, 3rd edn. Kendall Hunt, Dubuque IA
Moyle PB (2002) Inland fishes of California: revised and expanded. University of California Press, Berkeley CA
Nakano S, Murakami M (2001) Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proc Natl Acad Sci 98:166–170. https://doi.org/10.1073/pnas.98.1.166
Neubert K, Brunner E (2007) A studentized permutation test for the non-parametric Behrens–Fisher problem. Comput Stat Data Anal 51:5192–5204. https://doi.org/10.1016/j.csda.2006.05.024
Ortiz C, Weiss-Penzias PS, Fork S, Flegal AR (2015) Total and monomethyl mercury in terrestrial arthropods from the central California coast. Bull Environ Contamin Toxicol 94:425–430. https://doi.org/10.1007/s00128-014-1448-6
Pedrini-Martha V, Sager M, Werner R, Dallinger R (2012) Patterns of urban mercury contamination detected by bioindication with terrestrial isopods. Arch Environ Contam Toxicol 63:209–219. https://doi.org/10.1007/s00244-012-9766-3
Peterson SA, Van Sickle J, Herlihy AT, Hughes RM (2007) Mercury concentration in fish from streams and rivers throughout the western United States. Environ Sci Technol 41:58–65. https://doi.org/10.1021/es061070u
Peterson SA, Ralston NVC, Whanger PD, Oldfield JE, Mosher WD (2009) Selenium and mercury interactions with emphasis on fish tissue. Environ Bioindic 4:318–334. https://doi.org/10.1080/15555270903358428
Pinheiro J, Bates D, DebRoy S, Sarkar S, et al (2021) nlme: linear and nonlinear mixed effects models. R package version 3.1-152, URL: https://CRAN.R-project.org/package=nlme.
Polis GA, Anderson WB, Holt RD (1997) Toward and integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu Rev Ecol Syst 28:289–316. https://doi.org/10.1146/annurev.ecolsys.28.1.289
R Core Team. (2021) R: A language and environment for statistical computing. Version 4.1.1. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
Reinitz G (1983) Relative effect of age, diet, and feeding rate on the body composition of young rainbow trout (*Salmo gairdneri*). Aquaculture 35:19–27. https://doi.org/10.1016/0044-8486(83)90067-4
Rimmer CC, Miller EK, McFarland KP, Taylor RJ, Faccio SD (2010) Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. Ecotoxicology 19:697–709. https://doi.org/10.1007/s10646-009-0443-x
Riva-Murray K, Chasar LC, Bradley PM, Burns DA, Brigham ME, Smith MJ, Abrahamsen TA (2011) Spatial patterns of mercury in macroinvertebrates and fishes from streams of two contrasting forested landscapes in the eastern United States. Ecotoxicology 20:1530–1542. https://doi.org/10.1007/s10646-011-0719-9
Riva-Murray K, Bradley PM, Brigham ME (2020) Methylmercury–total mercury ratios in predator and primary consumer insects from Adirondack streams (New York, USA). Ecotoxicology 29:1644–1658. https://doi.org/10.1007/s10646-020-02191-7
Rodenhouse NL, Lowe WH, Gebauer RLE, McFarland KP, Bank MS (2019) Mercury bioaccumulation in temperate forest food webs associated with headwater streams. Sci Tot Environ 665:1125–1134. https://doi.org/10.1016/j.scitotenv.2019.02.151
Rundio DE, Lindley ST (2008) Seasonal patterns of terrestrial and aquatic prey abundance and use by *Oncorhynchus mykiss* in a California coastal basin with a Mediterranean climate. Trans Am Fish Soc 137:467–480. https://doi.org/10.1577/T07-076.1
Rundio DE, Lindley ST (2019) Diet variability of steelhead/rainbow trout in a coastal basin in central California: relative importance of seasonal, spatial, and ontogenetic variation. Trans Am Fish Soc 148:88–105. https://doi.org/10.1002/tafs.10121
Rundio DE, Lindley ST (2021) Importance of non-native isopods and other terrestrial prey resources to steelhead/rainbow trout *Oncorhynchus mykiss* in coastal streams in Big Sur, California. Ecol Freshw Fish 30:419–432. https://doi.org/10.1111/eff.12594
Rundio DE, Williams TH, Pearse DE, Lindley ST (2012) Male-biased sex ratio of nonanadromous *Oncorhynchus mykiss* in a partially migratory population in California. Ecol Freshw Fish 21:293–299. https://doi.org/10.1111/j.1600-0633.2011.00547.x
Sandheinrich MB, Wiener JG (2011) Methylmercury in freshwater fish: recent advances in assessing toxicity of environmentally relevant exposures. In: Beyer WN, Meador JP (eds) Environmental contaminants in biota: interpreting tissue concentrations, 2nd edn. CRC/Taylor and Francis, Boca Raton, FL, p 169–190
Streets DG, Horowitz HM, Jacob DJ, Lu Z, Levin L, ter Schure AFH, Sunderland EM (2017) Total mercury released to the environment by human activities. Environ Sci Technol 51:5969–5977. https://doi.org/10.1021/acs.est.7b00451
Sullivan SMP, Rodewald AD (2012) In a state of flux: the energetic pathways that move contaminants from aquatic to terrestrial environments. Environ Toxicol Chem 31:1175–1183. https://doi.org/10.1002/etc.1842

Tsui MTK, Blum JD, Kwon SY, Finlay JC, Balogh SJ, Nollet YH (2012) Sources and transfers of methylmercury in adjacent river and forest food webs. Environ Sci Technol 46:10957–10964. https://doi.org/10.1021/es3019836

Tsui MTK, Blum JD, Finlay JC, Balogh SJ, Nollet YH, Palen WJ, Power ME (2014) Variation in terrestrial and aquatic sources of methylmercury in stream predators as revealed by stable mercury isotopes. Environ Sci Technol 48:10128–10135. https://doi.org/10.1021/es500517s

Tsui M, Liu S, Brasso RL, Blum JD, Kwon SY, Ulus Y, Nollet YH, Balogh SJ, Eggert SL, Finlay JC (2019) Controls of methylmercury bioaccumulation in forest floor food webs. Environ Sci Technol 53:2434–2440. https://doi.org/10.1021/acs.est.8b06053

U.S. EPA (1998) Method 1630: methyl mercury in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry. https://www.epa.gov/sites/default/files/2015-08/documents/method_1630_1998.pdf

U.S. EPA (2000) Guidance for assessing chemical contaminant data for use in fish advisories, volume 1: fish sampling and analysis, third edition. https://www.epa.gov/sites/default/files/2018-11/documents/guidance-assess-chemical-contaminant-vol1-third-edition.pdf

U.S. EPA (2007) Method 7473: Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. https://www.epa.gov/sites/default/files/2015-12/documents/7473.pdf

U.S. EPA (2021) 2021 revision to: aquatic life ambient water quality criterion for selenium –freshwater 2016. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

U.S. Office of the Federal Register (2014) Endangered and threatened wildlife: final rule to revise the code of federal regulations for species under the jurisdiction of the National Marine Fisheries Service. Federal Register 79:71(14 April 2014):20802–20817.

Walters DM, Fritz KM, Otter RR (2008) The dark side of subsidies: adult stream insects export organic contaminants to riparian predators. Ecol Appl 18:1835–1841. https://doi.org/10.1890/08-0354.1

Ward DM, Nislow KH, Folt CL (2010) Bioaccumulation syndrome: identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. Ann NY Acad Sci 1195:62–83. https://doi.org/10.1111/j.1749-6632.2010.05456.x

Ward DM, Nislow KH, Folt CL (2012) Do low-mercury terrestrial resources subsidize low-mercury growth of stream fish? Differences between species along a productivity gradient. PLoS One 7:e49582. https://doi.org/10.1371/journal.pone.0049582

Weiss-Penzias PS, Ortiz C, Acosta RP, Heim W, Ryan JP, Fernandez D, Collett JL, Flegal AR (2012) Total and monomethyl mercury in fog water from the central California coast. Geophys Res Lett 39:L03804. https://doi.org/10.1029/2011GL050324

Weiss-Penzias PS, Bank MS, Clifford DL, Torregrosa A, Zheng B, Lin W, Wilmers CC (2019) Marine fog inputs appear to increase methylmercury bioaccumulation in a coastal terrestrial food web. Sci Rep 9:17611. https://doi.org/10.1038/s41598-019-54056-7

Wickham H (2016) ggplot2: elegant graphics for data analysis. Version 3.3.5. Springer-Verlag, New York