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Organic contaminants in western pond turtles in remote habitat in California

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HIGHLIGHTS

- Organic pollutants are widespread in a California turtle with conservation status.
- Pesticides were prominent in Sequoia National Park downwind of heavy agriculture.
- PCBs and PAHs are associated with watersheds having historic mines and mills.
- HUP and PCB concentrations indicate potential bioaccumulation is occurring.

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ABSTRACT

Remote aquatic ecosystems are exposed to an assortment of semivolatile organic compounds (SOCs) originating from current and historic uses, of local and global origin. Here, a representative suite of 57 current- and historic-use pesticides, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons were surveyed in the plasma of the western pond turtle (*Emys marmorata*) and their potential prey items and habitat. California study sites included Sequoia National Park, Whiskeytown National Recreation Area, and Six Rivers National Forest. Each was downstream of undeveloped watersheds and varied in distance from agricultural and urban pollution sources. SOCs were detected frequently in all sites with more found in turtle plasma and aquatic macroinvertebrates in the two sites closest to agricultural and urban sources. Summed PCBs were highest in Whiskeytown National Recreation Area turtle plasma (mean; 1.56 ng/g ww) compared to plasma from Sequoia National Park (0.16 ng/g ww; $p = 0.002$) and Six Rivers National Forest (0.07 ng/g ww; $p = 0.001$). While no current-use pesticides were detected in turtle plasma at any site, both current- and historic-use pesticides were found prominently in sediment and macroinvertebrates at the Sequoia National Park site, which is immediately downwind of Central Valley agriculture. SOC classes associated with urban and industrial pollution were found more often and at higher concentrations at Whiskeytown National Recreation Area. These findings demonstrate a range of SOC exposure in a turtle species with current and proposed conservation status and shed additional light on the fate of environmental contaminants in remote watersheds.

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1. Introduction

Environmental contaminants present unique challenges for species conservation. Unlike habitat loss or other stressors that are often highly visible with discrete borders, environmental contaminants like pesticides and industrial pollution can be widely distributed to natural areas via overspray, drift, post-application volatilization, and windblown erosion (Majewski and Capel, 1995). Thus, even organisms in seemingly pristine areas, such as national parks, are exposed to harmful pollutants of local and global origin (Landers et al., 2008). One noteworthy example involves the San Joaquin Valley and adjacent Sierra Nevada foothills of California, USA. In 2011 and 2012, over 73 million kg of pesticides were

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applied to agricultural lands in the four counties upwind of Sequoia and Kings Canyon National Parks, accounting for nearly half of the state's total agricultural pesticide use for those years (CDPR, 2012, 2013). Despite a record drought and demand for limited water resources, California agriculture has still continued to grow to over 80,000 farms and ranches recording $44.7 billion in revenue (CDFA, 2015). This continued agricultural growth can be partly attributed to pesticides, which are used prominently in the state for pest management. The problems associated with heavy pesticide use are well documented, and in some California National Parks, pesticides are found ubiquitously across diverse biological and physical endpoints (LeNoir et al., 1999; Landers et al., 2008; Flanagan Pritz et al., 2014; Sparling et al., 2015).

At sublethal concentrations, many semivolatile organic compounds (SOCs), such as chlorinated historic-use pesticides (HUPs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), cause deleterious impacts to organisms after exposure, resulting in effects that range from immunosuppression, genotoxicity, loss of secondary sex characteristics, decreased reproductive and endocrine function, and agonistic or antagonistic actions on hormone receptors (de Solla, 2010). Additionally, current-use pesticides (CUPs) such as organophosphates and carbamates, are prominently applied to California agriculture and can inhibit cholinesterase (ChE) enzymes in non-target wildlife, leading to acute or delayed neurological disorders (Sparling and Fellers, 2007). Possibly because of such effects, pesticides transported downwind to the Sierra Nevada foothills have been linked to declines and extirpations of amphibians in otherwise protected natural areas (Davidson et al., 2002). One species of state conservation concern, the Foothill Yellow-legged Frog (*Rana boylii*), has not been seen since the 1970s in the southern Sierra Nevada, where upwind pesticide use and agriculture has been prevalent and linked to their declines (Davidson et al., 2002; Sparling and Fellers, 2007) Although the relationship between amphibian declines and pesticide use has received much attention, other species may offer more promise for studying the accumulation and effects of environmental contaminants in wildlife.

Turtles can be key indicators of exposure to environmental contaminants; they readily bioaccumulate contaminants due to their long lifespans and generalist diets, integrating a lifetime of exposure from multiple sources, interactions with sediments, and ingestion of macroinvertebrates or aquatic plants (Bergeron et al., 2007; Rowe, 2008). Turtle populations can also persist in areas after other species have disappeared, partly because of their long lives and low generational turnover (Rowe, 2008). Thus, while it may be too late to study the accumulation of pesticides and their physiological effects in frogs that have been extirpated from the Sierra Nevada foothills, the western pond turtle (*Emys marmorata*), native to this region, still persists and may prove to be an appropriate biosentinel. In fact, a recent study of *E. marmorata* from the southern Sierra Nevada foothills found they had significantly reduced ChE activity compared to turtles from northern sites farther from agricultural areas (Meyer et al., 2013), offering support for the idea that heavy pesticide use in the San Joaquin Valley may be negatively affecting downwind ecosystems.

The goal of the present study was to examine baseline SOC concentrations by evaluating a representation of CUPs, HUPs, PAHs, and PCBs in *E. marmorata* at seemingly pristine locations that differed in their proximity to urban and agricultural land uses. *Emys marmorata* in the southern Sierra Nevada are considered to be in decline (Jennings and Hayes, 1994), and they have recently been petitioned for listing under the US Endangered Species Act (Dreher, 2015). Historical over-harvesting, habitat loss, and invasive species have been implicated as the primary factors threatening *E. marmorata* (Bury et al., 2008). However, negative biological effects from environmental contaminants have only recently received attention as a contributing factor, and the present study sheds further light on this possible cause of decline in a sensitive status species (Meyer et al., 2013, 2014). With these goals in mind, the presence of prominent environmental contaminants was examined at three California locations in three distinct sample types: 1) blood plasma of free ranging *E. marmorata*; 2) a composite sample of potential *E. marmorata* macroinvertebrate prey; and 3) stream sediments collected from *E. marmorata* habitat. The highest concentrations of CUPs and HUPs were expected at locations downwind of agriculture, and PCBs and PAHs were expected to be elevated at a location with legacy mines and mills.

2. Methodology

2.1. Sampling

2.1.1. Animal care and use

Animal handling and care approval was obtained through the University of California, Davis Institutional Animal Care and Use Committee (#16505). Research was also completed under approval from a California Department of Fish and Game scientific collecting permit (SCP 11633) and National Park Service (NPS) permits (CLCK-2011-SCI-0011, SEKI-2012-SCI-0456).

2.1.2. Study sites

Turtles, macroinvertebrates, and sediments were sampled at three lotic sites encompassing a 700 km latitudinal gradient across California (Fig. 1). The southernmost sampling site was the North Fork Kaweah River (NFKR), within Sequoia National Park in the southern Sierra Nevada foothills. The NFKR site (UTM 11S E 330211 N 4044134) lies to the east of, and downwind from, extensively cultivated agricultural areas of the San Joaquin Valley. This area is known to be influenced by atmospherically deposited pollutants originating from nearby San Joaquin Valley agricultural lands and surrounding urban areas (LeNoir et al., 1999; Landers et al., 2008). The topography of this region brings winds that transport pollution from the San Joaquin Valley east into the mountains during each day, and this diel wind pattern is more pronounced at foothill sites like NFKR than in the higher elevations of the Sierra Nevada (Ewell et al., 1989; Davidson et al., 2002). One northern California site was located at Clear Creek (CLCK) in Whiskeytown National Recreation Area (UTM 10T E 531265 N 4501103). This site is oriented facing south at the northernmost extent of the Sacramento Valley, and exhibits northerly and southerly predominant wind directions (Davidson et al., 2002). The winds blowing from the south have the potential to transport regional agricultural-related contaminants, but prior studies have indicated that environmental contaminants in this area are less pronounced than in the southern Sierra Nevada (Davidson et al., 2012; Smalling et al., 2013). Clear Creek is the most developed of the study locations, given its parallel proximity to California Highway 299 and its placement downstream of historic mines and mills responsible for elevated non-essential metals (National Park Service, 2014; Hothem et al., 2015). The third site was the South Fork Trinity River (SFTR), located in Six Rivers National Forest (UTM 10T E 448425 N 4525528). This site lies even farther west of the Sacramento Valley in the Klamath Mountains and is not immediately downwind of any industrial or agriculture areas. There is little expected influence from atmospheric deposition of contaminants from local sources given that northerly winds predominate over a landscape lacking traditional agricultural land-use (Davidson et al., 2002).

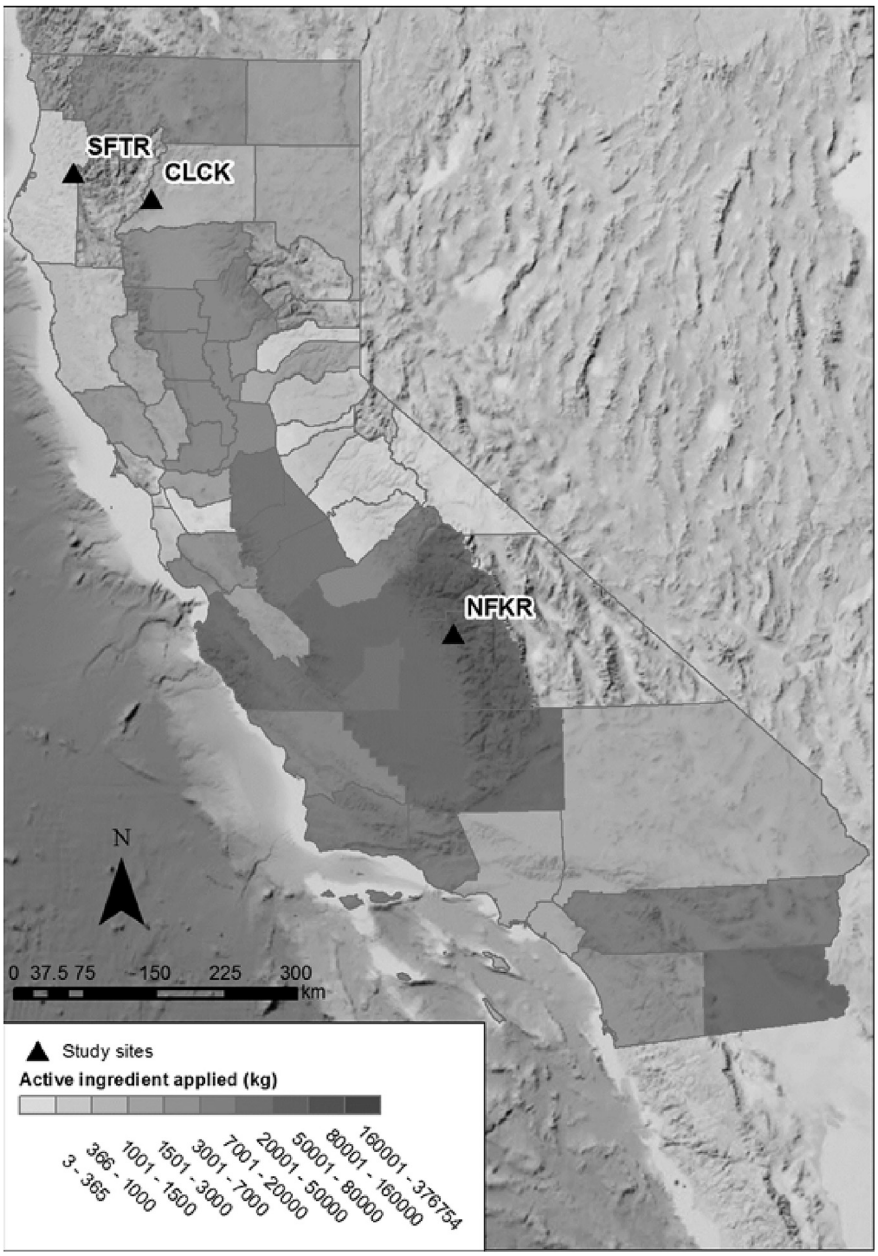

Fig. 1. Map of study sites North Fork Kaweah River (NFKR), Clear Creek (CLCK), and South Fork Trinity River (SFTR) in relation to total pesticide applications by California counties. Pesticides included active ingredients measured in the present study and are applied using aerial and ground methods.

2.1.3. Field sampling

We collected all samples at each study location from July–August 2012. Turtles were captured by hand following methods previously described (Meyer et al., 2013). A total of 80 turtles were captured in relatively comparable abundances and sex ratios across sites (male:female:juvenile ratio: NFKR = 14:11:2, CLCK = 9:11:5, SFTR = 11:12:5). Each animal was weighed to the nearest tenth of a gram using a digital Ohaus® scale, and carapace length (mm) was measured using calipers. No more than 1% of an adult or large juvenile turtles body mass in blood (typically < 5 cc) was collected from the subcarapacial venous sinus (Meyer et al., 2013). Whole blood samples were centrifuged at 1170 g for 15 min to separate plasma from red blood cells. To obtain sufficient plasma volume for later chemical analysis (up to 5 g), we combined individual samples based upon similar body mass, sex, and capture location. Blood was collected from 63 turtles and consolidated down to 10 samples from each site (male:female:juvenile sample ratio: NFKR = 7:3:0, CLCK = 4:5:1, SFTR = 4:5:1), which were made up of one to five (mean = 2.1) individuals per sample.

Three (NFKR, CLCK) to four (SFTR) benthic macroinvertebrate samples weighing 5–10 g each were collected along evenly distributed sections of each study area. Species collected represented potential *E. marmorata* prey items. Samples were collected by hand or using a D-net while overturning rocks in riffle areas and pools. There was slight variation in macroinvertebrate assemblages across the study areas, though we attempted to obtain samples with similar composition when possible. Across all sites, one sample included aquatic insect larvae of the orders Ephemeroptera, Plecoptera, and Trichoptera. At least one sample at each site was composed of mollusks: *Anodonta* spp. at NFKR and a composite of

freshwater snails (unknown Gastropoda) at CLCK. At SFTR we collected two mollusk samples: a composite of freshwater limpets (*Ferrissia* spp.) and a composite of the western pearlshell mussel (*Margaritifera falcata*). At CLCK and SFTR, where Megaloptera were absent, crayfish (*Orconectes virilis*) were collected. Finally, at NFKR, where crayfish were absent, one sample of Megalopteran larvae was collected.

At each study site, three composite sediment samples were collected along the stream reach where turtles were captured. Each composite sediment sample represented three 50 g samples drawn from the upper 3 cm of the streambed spanning the width of the stream.

All plasma, macroinvertebrate, and sediment samples were stored on ice or refrigerated immediately after sample collection. All samples were then sent overnight on dry ice to Oregon State University (Corvallis, OR) where they were stored at −80 °C until chemical analysis.

2.2. Pollutant analysis

Standard solutions for pesticides and PAHs were purchased from AccuStandard (New Haven, CT), Restek (Bellefonte, PA) and Chem Service (West Chester, PA); isotopically-labeled standards used as surrogates and internal standards were purchased from CDN Isotopes (PointClaire, Quebec) and Restek (Bellefonte, PA), and are provided in the supporting information (Table S1). Fifty seven SOCs were assessed in turtle plasma samples, and 56 were assessed for in macroinvertebrate and sediment samples.

2.2.1. Plasma extraction

The procedure for extraction of turtle plasma was slightly modified from a previous method to accommodate larger extraction masses (Keller et al., 2009). Briefly, 5 g of plasma was spiked with isotopically-labeled surrogate standards and allowed to equilibrate for 2 h at 4 °C. After equilibration, 5 mL hexane-rinsed Millipore water and 5 mL formic acid were added. The mixture was sonicated for 20 min at 25 °C. Plasma samples were then extracted using 3 mL OASIS HLB (Waters, Milford, MA) solid phase extraction columns with 20 mL dichloromethane. Plasma extracts were concentrated to a volume of 200 μL using a stream of N_2 gas (Turbovap II, Caliper Life Sciences, MA), and isotopically-labeled internal standards were added prior to gas chromatographic/mass spectrometry (GC/MS) analysis.

2.2.2. Macroinvertebrate extraction

Whole invertebrate tissue and shells were used except for the western pearlshell mussel (*Margaritifera falcata*) in which only the soft tissue was used. The samples were homogenized and extracted following an existing protocol (Ackerman et al., 2008). In brief, the samples were frozen with liquid nitrogen and blended using a high-powered industrial blender into a homogenized fine powder. Approximately 20 g of the frozen homogenate was mixed with sodium sulfate and packed into 100 mL stainless steel cells, spiked with isotopically-labeled surrogate standards, and extracted with dichloromethane at 100 °C and 1500 psi using pressurized liquid extraction (PLE). Purification of the extract was performed using silica solid phase extraction, as well as gel permeation chromatography (GPC). Isotopically-labeled internal standards were spiked prior to GC/MS analysis.

2.2.3. Sediment extraction

For sediment samples, excess water was decanted from the top of the sediment sample jar and the remaining sediment thoroughly stirred prior to extraction. Approximately 20 g of sediment was mixed with sodium sulfate until the mixture was free-flowing. The sediment was extracted with 75:25 hexane:acetone using previously described PLE methods (Primbs et al., 2008; Genualdi et al., 2009). The extracts were further purified with silica solid phase extraction, and spiked with isotopically-labeled internal standards prior to GC/MS analysis.

2.2.4. Chemical analysis

Gas chromatographic/mass spectrometry analyses were performed using an Agilent 6890 GC system coupled to a 5973 mass spectrometer. A DB-5MS (30 m × 0.25 mm I.D. × 0.25 μm) was used for all analytes. Electron impact (EI) (for pesticides and PAHs) and electron chemical negative ionization (ECNI) (for pesticides and PCBs) modes were used with selective ion monitoring for quantitation. Helium was used as the carrier gas at 0.9 mL min^{-1}, and the interface and ion source temperatures were maintained at 300 and 250 °C, respectively.

Turtle plasma samples utilized a programmed temperature vaporizer (PTV) inlet to increase sensitivity in solvent vent mode. The parameters of the PTV injection methods were: injection volume 4 μL, initial injection temperature 20 °C (hold 0.1 min), to 300 °C at 600 °C min^{-1}, and hold for 5 min. The purge valve was opened after 7.10 min (purge flow 300 mL min^{-1}). The oven temperature program was 60 °C (hold 0.10 min) to 250 °C at 4 °C min^{-1}, to 320 °C at 15 °C min^{-1}, and hold for 7 min. Macroinvertebrate and soil samples were analyzed following previously described methods (Usenko et al., 2007). The oven temperature program for GC/EI-MS was 60 °C (hold 1 min), to 300 °C at 6 °C min^{-1} (hold 3 min), to 320 °C at 20 °C min^{-1} (hold 9 min). The oven temperature program for GC/ECNI-MS was 120 °C (hold 1 min), to 275 °C at 4 °C min^{-1} (no hold), to 320 °C at 6 °C min^{-1} (hold 4.75 min). The inlet temperature for both methods was 300 °C.

2.2.5. Quality control and assurance

Laboratory blanks consisting of hexane and Millipore water (for turtle plasma) and of sodium sulfate (for sediment and macroinvertebrates) were prepared at a frequency of 30%. Calibration verification standards were analyzed at 20% frequency to ensure accurate quantification of sample concentrations. Estimated detection limits (EDLs) following EPA method 8280A were calculated for turtle plasma, macroinvertebrates, and sediment using a representative sample from each matrix. The table of EDLs in Supporting Information (Table S2) does not include an EDL for total PCBs. EDLs were calculated for each individual PCB. If an EDLs was needed for total PCBs, it would be the sum of the EDLs for the individual PCBs. Turtle plasma methodology was validated for pesticides (Hexachlorobenzene, cis-Chlordane, cis-Nonachlor, trans-Nonachlor, and Mirex) and PCBs (74, 101, 118, 138, 153, 183, and 187) using replicate human serum samples of the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1958, and measured concentrations were generally within 30% of the certified NIST value. Surrogate standard recoveries (used for analyte quantification) for pesticides and PAHs were between 30 and 130%.

2.3. Statistical analysis

All statistical analyses were performed in R, version 3.2.0 (R Core Team, 2015). To investigate contaminant prominence in each sample, a generalized linear model (Poisson family and log link) was used to model detection counts of non-censored data. For all sample media, detection counts in each replicate were the response variables. For turtle plasma, turtle mass, sex, and site were explanatory variables. For macroinvertebrates and sediments, site was the only explanatory variable. Homogeneity of model residuals was tested using a Shapiro-Wilk test ($p > 0.05$). All model residuals

passed this normality test and thus were not transformed.

Using the measured concentrations EDLs (Table S2), the Kaplan-Meier method (*cenfit* in the NADA package) was applied to estimate mean, median, standard error, and standard deviation for each compound by site (Lee, 2015). This methodology was beneficial for our dataset due to the prominence of censored data (Helsel, 2005). As a result, non-detects were always included in statistical analysis, and the only values excluded were samples that had laboratory blank concentrations exceeding 33% of the measured value (resulting in missing data). Due to non-normally distributed data and heterogeneity of errors, the non-parametric (Kruskal-Wallis) *cendiff* function was used to test for site differences for each compound and contaminant class for turtle plasma, macroinvertebrate, and sediment results. Multiple comparison post-hoc tests (also *cendiff*) were run on compounds where the Kruskal-Wallis test resulted in $p \leq 0.05$; significant differences among sites were evaluated using Bonferroni adjusted P-values ($p \leq 0.017$). The most complete data were of PCB congeners in macroinvertebrates and turtle plasma. To assess total burdens of this class, PCB concentrations were summed ($\sum$ PCBs) for each sample, and site differences were tested with an ANOVA (followed by Wilcoxon post-hoc test) and Kruskal-Wallis test for turtle plasma and macroinvertebrate data, respectively. For only this test, non-detects of specific congeners were set at zero.

3. Results

3.1. SOC prominence

Detections of unique compounds and contaminant classes varied by site and media. The sediment medium detected the most diverse analyte list, followed by macroinvertebrates and turtle plasma. The NFKR site had the most diverse and abundant analyte detections for macroinvertebrates and turtle plasma, whereas CLCK had the most diverse and abundant analyte detections for the sediment medium. After statistically accounting for average turtle body mass per sample ($p > 0.05$) and sex ($p > 0.05$), significantly fewer compounds were detected at SFTR than at NFKR ($p = 0.008$) and at CLCK ($p = 0.001$; Fig. 2A). Both NFKR ($p = 0.006$) and CLCK ($p = 0.03$) had significantly more compounds detected in macroinvertebrates as well (Fig. 2B). In sediment, there were significantly more compounds detected at CLCK and NFKR ($p = 0.051$) than at and SFTR ($p = 0.004$; Fig. 2C).

3.2. Current-use pesticides

Current-use pesticides were the least commonly detected analytes across all sites and sample types (Tables S3–S5). However, NFKR had the most CUP detections at the highest concentrations in sediment and macroinvertebrates. Mean chlorpyrifos concentrations were highest at NFKR in sediments (mean ± standard error; 0.064 ± 0.004 ng/g dw) but were not statistically different from CLCK (0.048 ± 0.009 ng/g dw; $p = 0.27$; Table S5). Among macroinvertebrates, dacthal concentrations were highest in samples from NFKR (0.23 ± 0.007 ng/g ww) but were not statistically different from the other sites ($p = 0.245$, Table S4). Endosulfan sulfate was found at two locations in macroinvertebrates (NFKR and CLCK) and was found at higher concentrations in NFKR sediments than at CLCK and SFTR ($p = 0.034$), although these differences were not statistically significant. There were no CUPs detected in turtle plasma from any of the study sites (Table S3).

3.3. Historic-use pesticides

Historic-use pesticides were found less frequently across all sites in sediments compared to all other compound classes. Hexachlorobenzene (HCB) was the only compound found in sediments at more than one site (NFKR and CLCK), while methoxychlor (3.0 ± 0.00 ng/g dw) at SFTR and p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE; 0.273 ± 0.073 ng/g dw) at NFKR were found at the highest concentrations. Among macroinvertebrates, HUPs were found at all three sites. Hexachlorobenzene ($p = 0.723$) was found in all macroinvertebrate samples at all sites and was highest at NFKR (0.055 ± 0.015 ng/g ww), followed by SFTR (0.045 ± 0.015 ng/g ww) and CLCK (0.035 ± 0.015 ng/g ww). While there were no statistically significant differences among sites with macroinvertebrate samples, the most frequently detected compounds (% > reporting limit) and those with the highest concentrations were all found at NFKR, followed by CLCK and SFTR (Table S4). This trend remained true for turtle plasma as well, where the most frequently detected HUP was p,p′-DDE, which was found above the detection limit in 90% of NFKR (0.886 ± 0.474 ng/g ww) and 88% of CLCK samples (1.299 ± 0.321 ng/g ww; $p = 0.152$). Among HUPs, HCB and p,p′- DDE generally increased from sediment, to macroinvertebrates, and up to turtle plasma samples, suggesting that bioaccumulation is occurring (Tables S3–S5).

3.4. Polycyclic aromatic hydrocarbons

Detection of PAH analytes were especially common in sediment samples from CLCK, where 11 of 16 PAH compounds were detected (Table S5). Retene was found in all sediment samples at all sites and was highly elevated at CLCK, though concentrations did not differ significantly among study sites ($p = 0.06$; Table S5). PAH

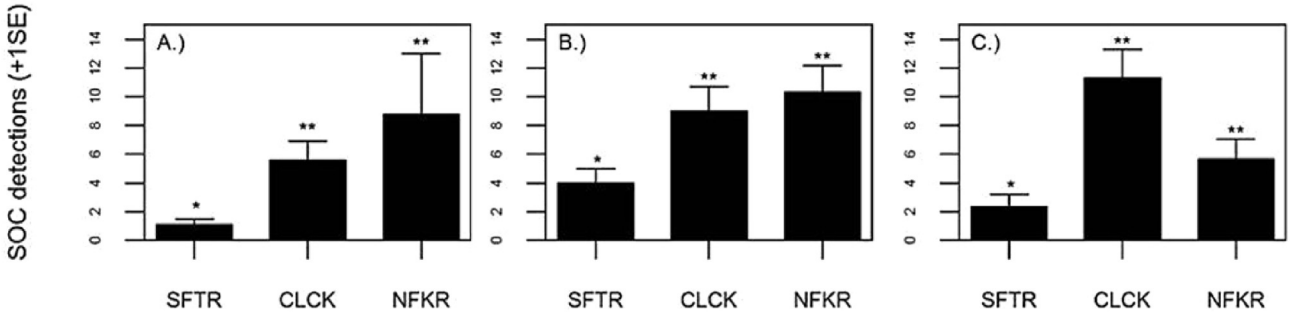

Fig. 2. Estimated detections of 57 possible semivolatile organic compounds (SOCs, y-axis) in A.) turtle plasma samples and 56 possible SOCs in B.) macroinvertebrate and C.) sediment samples at three locations in California, USA. Detections were modeled using a generalized linear model with a Poisson outcome distribution. Back-transformed least squared means (+1 standard error) are shown, and significant differences are represented by asterisks. SFTR = South Fork Trinity River, CLCK = Clear Creek, NFKR = North Fork Kaweah River.

compounds were detected less frequently in macroinvertebrates (Table S4). However, in turtle plasma, NFKR had the highest concentrations of five PAHs, including benzo(*a*)anthracene, fluoranthene, fluorene, acenaphthene, and phenanthrene (Table S3). The highest mean concentration of a PAH compound found in turtle plasma was pyrene, at SFTR (53.0 ± 0.00 ng/g ww), and SFTR had significantly higher benzo(*b*)fluoranthene concentrations than NFKR ($p = 0.002$).

3.5. Polychlorinated biphenyls

PCB analytes were detected in all three sample media but most ubiquitously in the biological media (Table S3–S5). Congeners PCB 153, PCB 183, and PCB 187 all increased in concentration from sediment to macroinvertebrate and up to turtle plasma, indicating a pattern of bioaccumulation similar to HUPs. Among sediments, PCB analytes were only found above the EDLs in samples from CLCK (Table S5). For macroinvertebrates, five analytes were found in samples from all three of the study sites, although concentrations did not differ significantly ($p > 0.05$) where comparisons were made between sites for each congener (Table S4). However, across sites, the highest mean macroinvertebrate concentrations of all PCB congeners were found at NFKR. The highest mean $\sum_6$ PCBs were also found in NFKR macroinvertebrates but no significant site differences ($p = 0.427$; Fig 3B). Five different PCB analytes were detected in samples of turtle plasma, with more detections in samples from CLCK than the other two sites (Table S3). PCB 153 was highest at CLCK (0.929 ± 0.203 ng/g ww; $p < 0.001$) but did not differ between NFKR and SFTR (Table S3). CLCK turtle plasma also had the highest concentrations of PCB 118, 138, 183, and 187. Further, CLCK had significantly higher concentrations of $\sum_7$ PCBs compared to NFKR ($p = 0.002$) and SFTR ($p = 0.001$; Fig. 3A).

4. Discussion

This is the first study to evaluate the occurrence of common organic pollutants in juvenile and adult *E. marmorata*, their potential prey, and their habitat. While novel for this species, similar pollutants have received attention in a variety of tissues of other free-ranging freshwater turtles and sea turtle species (de Solla, 2010). Residues of HUPs and PCBs were previously found in *E. marmorata* eggs at one location in Oregon (Henny et al., 2003), which, in combination with the present study, suggests that turtle environment, diet, and maternal transfer are typical pathways for contaminant exposure for this species in natural areas.

Across all sites, CUPs and HUPs were found more frequently at concentrations above EDLs at NFKR. While the most removed, protected study site, this trend is consistent with NFKR's geographic location if regional sources are considered, given atmospheric transport is the primary contaminant source within the watershed. In turtle plasma, chlorinated HUPs, including o,p′-DDE, p,p′-DDE, p,p′-DDD, and HCB were detected, confirming continued persistence in the environment as a result of widespread agricultural use historically. Each HUP except p,p′-DDE were found at higher concentrations at NFKR than in plasma of two species of freshwater turtles in the Tennessee River Gorge (Table 1). Concentrations of p,p′-DDE at Clear Creek were higher than those in Tennessee turtles, but much lower than common snapping turtles in the Canadian Great Lakes region (Table 1). Macroinvertebrate samples contained more pesticide compounds than turtle plasma, but when a particular HUP was found in both sample types, concentrations were always higher in plasma. In general, HUPs are lipophilic and bioaccumulate in the food web, which would explain a positive association between contaminant concentration and trophic levels (de Solla, 2010). However, the absence of several pesticides paired in both biological media suggest turtles are consuming different prey than what was collected or that other tissues would have been better at detecting low concentration HUPs based on lipid content or temporal feeding habits (Keller et al., 2004).

Much like HUPs, PAHs have a greater likelihood to bioaccumulate based on their stability and high lipophilic properties, and in reptiles, known routes of exposure include skin contact, diet, transfer through eggshells from contaminated soil, and maternal transfer (de Solla, 2010). PAHs, which are derived from petroleum and its combustion, can arise from the use of fossil fuels but can also be released naturally from forest fires (Usenko et al., 2010). PAHs were most frequently found at highest concentrations in sediments from CLCK, which might be explained by the site's location along a state highway, where fossil fuels from automobiles are likely deposited in streams as runoff from impervious surfaces. Macroinvertebrates were poor indicators of PAH pollution, which was unexpected given that dietary consumption was the hypothesized route of PAH exposure for turtles. Furthermore, PAH compounds found in invertebrates typically co-occur with those in sediments (Gewurtz et al., 2000; Arias et al., 2009). This was not the case in the present study, where PAHs were found prominently in CLCK sediments, but not turtles, and NFKR turtles, but not sediments. Mean concentrations of the seven PAH compounds that were detected in NFKR turtles were higher than those found in plasma of three of four recent sea turtle studies (Table 1). At sediment and tissue concentrations measured more than 3 orders of magnitude higher than the present study, summed PAHs were implicated in increased deformities in common snapping turtles (*Chelydra serpentina*) and

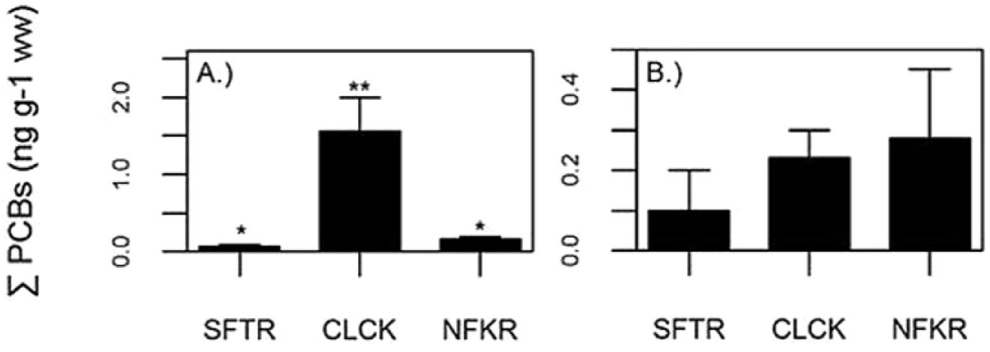

Fig. 3. Site differences of $\sum_7$ polychlorinated biphenyls (PCBs) in turtle plasma and $\sum_6$ PCBs in macroinvertebrates. Sediment results are excluded since there were zero detections at two sites (SFTR, NFKR). The number of asterisks represents significant differences, and means and standard errors are displayed. SFTR = South Fork Trinity River, CLCK = Clear Creek, NFKR = North Fork Kaweah River.

Table 1
Mean (standard deviation) plasma concentrations ng g^{-1} ww of the most frequently detected (a) historic-use pesticides and polychlorinated biphenyls, and (b) polycyclic aromatic hydrocarbons in western pond turtles in California. Recent studies reporting similar contaminants in turtle plasma are included for comparison.

(a) Species	Stage/sex[a]	Location	Historic-use pesticides		Polychlorinated biphenyls					Reference
			HCB	p,p′-DDE	PCB 118	PCB 138	PCB 153	PCB 183	PCB 187	
Western pond turtle[b]	J, AM&F	Sequoia NP, CA	0.079 (0.017)	0.886 (0.474)	0.02	0.066 (0.009)	0.108 (0.021)	<LOD	<LOD	This study
Western pond turtle[b]	J, AM&F	Whiskeytown NRA, CA	NA	1.299 (0.321)	0.227 (0.049)	0.64 (0.131)	0.929 (0.203)	0.117 (0.029)	0.132 (0.031)	This study
Western pond turtle[b]	J, AM&F	Six Rivers, NF, CA	0.37	<LOD	0.055 (0.025)	<LOD	0.093 (0.013)	<LOD	<LOD	This study
Common snapping turtle	AM	Canada Great Lakes	<0.1[c]	27 (6)	8.5 (0.957)	22.3 (0.957)	20.2 (0.85)	NA	NA	(Letcher et al., 2015)
Loggerhead sea turtle[d]	NA	Eastern Atlantic Ocean	0.03 (0.04)	0.16 (0.31)	0.05 (0.11)	1.00 (1.56)	0.38 (0.55)	NA	NA	(Bucchia et al., 2015)
Loggerhead sea turtle[d]	NA	Adriatic Sea	0.02 (0.04)	1.13 (0.95)	1.15 (1.40)	24.36 (27.23)	9.46 (11.63)	NA	NA	(Bucchia et al., 2015)
Green turtle[d]	J	Cape Verde	0.12 (0.20)	0.06 (0.12)	0.21 (0.51)	0.02 (0.04)	0.09 (0.36)	NA	NA	(Camacho et al., 2014)
Hawksbill turtle[d]	J	Cape Verde	0.11 (0.26)	0.05 (0.16)	0.005 (0.02)	0.04 (0.14)	0.09 (0.30)	NA	NA	(Camacho et al., 2014)
Loggerhead sea turtle	AF	Cape Verde	0.012 (0.01)	0.075 (0.07)	0.005 (0.01)	0.08 (0.09)	0.047 (0.06)	NA	NA	(Camacho et al., 2013)
Common musk turtle	AM	Tennessee	<LOD	1.28 (0.72)	0.377 (0.205)	1.27 (0.552)	NA	NA	NA	(Moss et al., 2009)
Common musk turtle	AF	Tennessee	<LOD	0.738 (0.08)	0.830 (0.218)	1.24 (0.606)	NA	NA	NA	(Moss et al., 2009)
Cumberland slider	AM	Tennessee	<LOD	0.538 (0.237)	1.11 (0.541)	0.578 (0.263)	NA	NA	NA	(Moss et al., 2009)
Cumberland slider	AF	Tennessee	<LOD	0.576 (0.328)	0.671 (0.470)	0.451 (0.372)	NA	NA	NA	(Moss et al., 2009)

(b) Species	Stage/sex[a]	Location	Polycyclic aromatic hydrocarbons						Reference
			Benzo-(*b*) fluoranthene	Fluoranthene	Fluorene	Phenanthrene	Pyrene	Retene	
Western pond turtle[b]	J, AM&F	Sequoia NP, CA	<LOD	1.667 (0.539)	1.567 (0.467)	3.31 (1.109)	8.733 (3.041)	1.3	This study
Western pond turtle[b]	J, AM&F	Whiskeytown NRA, CA	NA	NA	NA	NA	NA	0.883 (0.113)	This study
Western pond turtle[b]	J, AM&F	Six Rivers, NF, CA	2.55 (0.725)	NA	NA	NA	53.0	1.6	This study
Loggerhead sea turtle[d]	NA	Eastern Atlantic Ocean	NA	0.029 (0.06)	0.87 (1.1)	4.62 (3.52)	0.036 (0.07)	NA	(Bucchia et al., 2015)
Loggerhead sea turtle[d]	NA	Adriatic Sea	NA	0.15 (0.25)	2.25 (1.38)	11.35 (5.05)	0.16 (0.20)	NA	(Bucchia et al., 2015)
Green turtle[d]	J	Cape Verde	NA	0.42 (0.51)	0.70 (0.80)	2.81 (3.00)	0.67 (0.61)	NA	(Camacho et al., 2014)
Hawksbill turtle[d]	J	Cape Verde	NA	0.12 (0.23)	0.02 (0.042)	1.28 (1.78)	0.08 (0.22)	NA	(Camacho et al., 2014)
Loggerhead sea turtle[d]	AF	Cape Verde	NA	0.03 (0.04)	0.24 (0.16)	1.21 (0.74)	0.04 (0.04)	NA	(Camacho et al., 2013)
Loggerhead sea turtle[d]	J	Canary Islands	<LOD	0.03 (0.17)	0.13 (0.35)	4.19 (3.48)	0.04 (0.21)	NA	(Camacho et al., 2012)
Loggerhead sea turtle[d]	AF	Cape Verde	<LOD	0.09 (0.19)	0.27 (0.45)	3.94 (3.7)	0.13 (0.39)	NA	(Camacho et al., 2012)

[a] Stage/sex abbreviations: A = adult; J = juvenile; M = male; F = female.
[b] Mean (standard error) reported.
[c] Estimated from graph.
[d] Mean (SD); ng mL^{-1}.

painted turtles (*Chrysemys picta*) in Philadelphia, Pennsylvania (Bell et al., 2006). Moreover, sediment PAH concentrations 1 to 2 orders of magnitude greater than reported in the present study were associated with genotoxicity in turtles (Matson et al., 2005). To our knowledge, this is the first study to measure plasma PAH concentrations in freshwater turtles. Three-, 4-, and 5-ring PAHs were detected most frequently across all study sites and sample types, and the primary sources attributed to the these PAH profiles are highway runoff, wood combustions, and diesel and petrol emissions (Ravindra et al., 2008).

PCBs are also lipophilic, persistent in the environment, and entirely man-made; they are readily found in aquatic ecosystems in sediments, and concentrations generally increase up the food chain (McFarland and Clarke, 1989). PCBs were historically used in a variety of industrial products such as electrical insulators and surfactants, but their use has largely been phased out globally over the past two decades (Porta and Zumeta, 2002). They are transported ubiquitously in the atmosphere but are often elevated in urban areas (Cotham and Bidleman, 1995). In turtles, PCBs have been linked to hatchling abnormalities, altered sex determination, and increased juvenile mortality (Bishop et al., 1991; Bergeron et al., 1994; Eisenreich et al., 2009; Matsumoto et al., 2014). In the present study, PCBs were found elevated in all sample types in CLCK compared to the other sites and followed the expected pathway of biomagnification. Although the upstream watershed at CLCK is almost entirely undeveloped, a historic sawmill site was located

nearby, and its effluent may have created a reservoir effect of legacy PCB pollution (Elliott et al., 2001). Of the 209 potential congeners, PCB 153 is one of the most prominent and environmentally threatening (McFarland and Clarke, 1989). This study detected the congener PCB 153 most frequently, and it was found to be proportionally higher among PCB congeners across all sites and sample types. PCBs 138 and 118 in turtle plasma and PCBs 183 and 187 in macroinvertebrates and sediment were the next most frequently detected congeners in the respective media. Each of these congeners has been identified as a highest concern pollutant based upon their potential for toxic effects (McFarland and Clarke, 1989). Concentrations of many individual congeners were higher in CLCK turtle plasma than in several sea turtle species, but lower than those found in recent freshwater turtle studies (Table 1). While CLCK had the most diverse and prominent PCB results across sample types, the NFKR macroinvertebrate samples had the highest $\sum_{6}$PCB concentrations of all sites. This suggests that potential *E. marmorata* feeding ecology was more accurately represented at CLCK than at NFKR.

4.1. Conclusions

As a biosentinel for recent and historic contaminant exposure, *E. marmorata* may provide insight on the ecological health of aquatic systems over broad regions (i.e., their range in California and/or the western US). The present study found that representative CUPs, HUPs, PAHs, and PCBs were found universally across most sample types and study areas, and the classes of contaminants followed expected trends of regional use (NFKR > CLCK > SFTR). Concentrations were below many diagnostic thresholds; however, it is imperative to consider the unknown toxicological effects of exposure to multiple environmental contaminants at relatively low concentrations. For example, in this study, six CUPs were detected of 9 CUPs analyzed, whereas 878 active ingredients were applied to agricultural lands during the study duration (CDPR, 2012, 2013). Although each unique pesticide applied to California agriculture has a range of toxic effects and potential to drift into natural ecosystems, it is likely that many additional unmeasured pesticides are being transported to these remote ecosystems. Because many CUPs are lipophobic, they are hard to detect in biota and thus cause subtle physiological effects that are difficult to monitor. It is likely for this reason that compounds such as chlorpyrifos were only detected in sediments in the present study, but the physiological impacts of these compounds have been in observed in *E. marmorata* at NFKR (Meyer et al., 2013).

In examining the role that environmental pollution may play in the conservation efforts of *E. marmorata*, consideration should be given to the risk associated with exposure to multiple pollutants, including effects from current and past agricultural practices, land use, and management (PAHs via roads and forest fires), as well as PCBs and mercury from the atmosphere, and current and legacy mill and mining operations (Meyer et al., 2014). Residues of CUPs in biological and physical media may evade detection, so biomarkers of exposure such as ChE activity may provide more insight into toxicological risk (Handy et al., 2003). The present study provides a baseline contaminant survey and highlights the ubiquitous nature of many pollutants found in *E. marmorata*, their prey, and their habitat. This work lays a foundation for more focused research that could expand upon dose-response relationships, trophic position and routes of exposures, biomarker responses, and chemical synergistic effects.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.03.128.

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