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Seasonal Toxicity Observed with Amphipods (*Eohaustorius estuarius*) at Paleta Creek, San Diego Bay, USA

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Abstract: To assess potential impacts on receiving systems, associated with storm water contaminants, laboratory 10-d amphipod (*Eohaustorius estuarius*) survival toxicity tests were performed using intact sediment cores collected from Paleta Creek (San Diego Bay, CA, USA) on 5 occasions between 2015 and 2017. The approach included deposition-associated sediment particles collected from sediment traps placed at each of 4 locations during the 2015 to 2016 wet seasons. The bioassays demonstrated wet season toxicity, especially closest to the creek mouth, and greater mortality associated with particles deposited in the wet season compared with dry season samples. Grain size analysis of sediment trap material indicated coarser sediment at the mouth of the creek and finer sediment in the outer depositional areas. Contaminant concentrations of metals (Cd, Cu, Hg, Ni, Pb, and Zn) and organic compounds (polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], and pesticides) were quantified to assess possible causes of toxicity. Contaminant concentrations were determined in the top 5 cm of sediment and porewater (using passive samplers). Whereas metals, PAHs, and PCBs were rarely detected at sufficient concentrations to elicit a response, pyrethroid pesticides were highly correlated with amphipod toxicity. Summing individual pyrethroid constituents using a toxic unit approach suggested that toxicity to *E. estuarius* could be associated with pyrethroids. This unique test design allowed delineation of spatial and temporal differences in toxicity, suggesting that storm water discharge from Paleta Creek may be the source of seasonal toxicity. *Environ Toxicol Chem* 2020;39:229–239. © 2019 SETAC

Keywords: Sediment toxicity; Stormwater; Recontamination; Pyrethroids; Seasonal; Amphipods

INTRODUCTION

Sediment contamination and associated remediation measures are a major challenge for water and sediment program managers and regulators, resulting in significant financial liability (Strategic Environmental Research and Development Program/Environmental Security Technology Certification Program 2016). The potential for recontamination of sediment sites undergoing remediation is also a demonstrated concern, especially when sources of the contamination have not been effectively mitigated (e.g., runoff from storm events; Reible et al. 2018). It is critical not only to understand sources for the recontamination, but also to understand which contaminants are responsible, to better manage mitigation of sediment recontamination. Storm water is considered to be a likely source of this recontamination, although it can be difficult to characterize and identify sources (Brown et al. 1985; Strategic Environmental Research and Development Program/Environmental Security Technology Certification Program 2016). Storm water is comprised of multiple contaminants of concern, including heavy metals, pesticides, and hydrocarbons, present in both water and particulate fractions (Burton and Pitt 2001). Contaminants from storm water events can load contaminants into marine sediment systems (Strategic Environmental Research and Development Program/Environmental Security Technology Certification Program 2016).

The present study is part of a larger effort that characterized recontamination potential of urban sources to the Paleta Creek (San Diego, CA, USA) watershed adjacent to Naval Base San Diego in San Diego Bay (Reible et al. 2018). Paleta Creek is a natural urban/industrial creek with generally higher flows

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associated with winter storm events (measured during this project on the order of approximately 0.6 m^3/s during 2016, and not measured the other years), compared with extended periods with no surface flow during dry weather conditions. The 2161-acre watershed is primarily comprised of residential areas, with some commercial and military uses (Reible et al. 2018). Paleta Creek has been designated a toxic hotspot by the San Diego Regional Water Quality Control Board due to contamination of the sediment and benthic community impacts (SWRCB 1999; Southern California Coastal Water Research Project and Space and Naval Warfare Systems Center 2005). A toxicity identification evaluation concluded that toxicity to amphipods was due to an organic toxicant, but the authors were unable to identify the specific group (Greenstein et al. 2011). However, for any future clean-up action to be successful, continuing sources of contamination must be identified and mitigated to prevent recontamination.

At this site, copper (Cu) and polycyclic aromatic hydrocarbons (PAHs) have been traditionally assumed to be the cause of the observed toxicity (SWRCB 1999; Southern California Coastal Water Research Project and Space and Naval Warfare Systems Center 2005). However, several studies in southern California have attributed this seasonal toxicity of sediments to pyrethroids, using the standard 10-d amphipod acute toxicity test (US Environmental Protection Agency 1994, rather than either Cu or PAHs (e.g., Holmes et al. 2008; Anderson et al. 2010; Lao et al. 2012; Greenstein et al. 2014, 2019). Pyrethroids are commonly used insecticides for residential uses because organophosphates were phased out of household use in the United States (Amweg et al. 2006). Residential runoff in southern California is a major source of pyrethroid contamination in urban creeks, often more important than dry season irrigation runoff (Weston et al. 2009). Due to their chemical characteristics, specifically their hydrophobicity (log octanol/water partition coefficient [K_{OW}] > 5.9) and tendency to associate with sediment particles, sediments in receiving environments may act as a sink for pyrethroids in highly urbanized systems (Gan et al. 2005; Weston et al. 2009; Weston and Lydy 2010). Pyrethroids can cause toxicity in nontarget benthic organisms in the receiving environment (Anderson et al. 2008, 2010; Holmes et al. 2008; Hintzen et al. 2009; Lao et al. 2010, 2012; Van Geest et al. 2014). Thus it is possible that the input of these urban streams has resulted in the seasonal pyrethroid contamination of these receiving environments.

A modified 10-d amphipod acute toxicity test, the toxicity of intact core samples collected in wet and dry seasons over a 2-yr period (2015–2017), was performed with the aim of potentially further identifying the cause(s) of toxicity and the source(s) of contamination.

MATERIALS AND METHODS

Site description

The mouth of Paleta Creek is located on the eastern shoreline in the central portion of San Diego Bay, flowing directly into Naval Base San Diego. The mouth emerges into the bay in a relatively constricted channel area, which then expands into a broader area (Figure 1). The constricted channel area, where sites P11 and P17 were located, will be referred to as the Inner Creek area, whereas the area where the receiving water opens up is referred to as the Outer Creek area and is where site P08 is located. A nearby reference sediment from site P01 was collected to decouple effects from Navy and urban runoff by comparing contaminant concentrations of inner (P17 and P11) and outer creek (P08) sites with the reference sediment.

Sample collection

Two types of sediment samples were collected: intact sediment cores and sediment trap material. Figure S1 in the

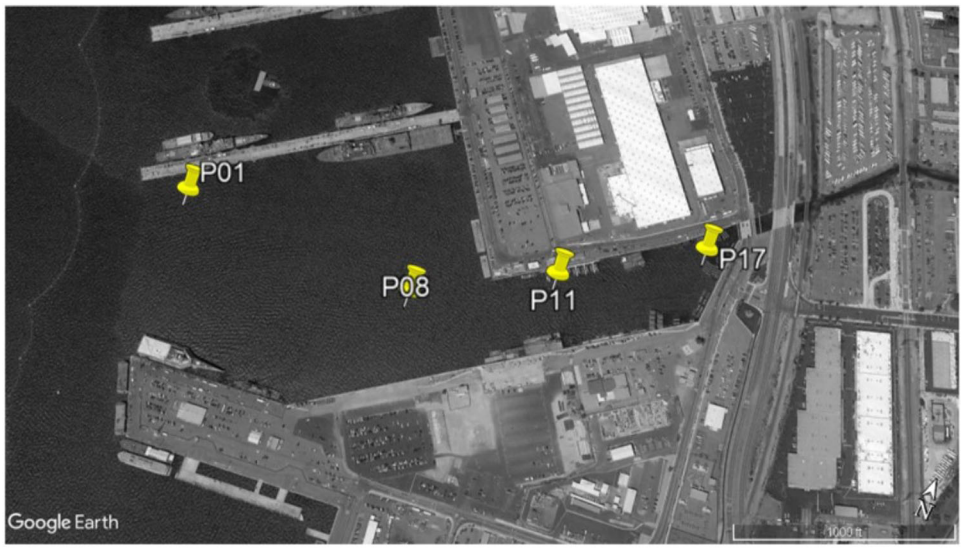

FIGURE 1: Map of the Paleta Creek receiving environment (adjacent to Naval Base San Diego, USA) with study sites indicated. P17 (32.67376, –117.11601) is located near the creek mouth and P11 (32.67265, –117.11800), P08 (32.67165, –117.12000), and P01 (32.67170, –117.12395) are increasingly further away from the creek mouth. Map from Google Earth.

Supplemental Data indicates the timing of each sediment collection (a total of 5 events for intact cores, and 1 event for sediment trap material) in relation to rainfall recorded in San Diego during the study period of June 2015 to March 2017. There were a total of 2 dry and 3 wet weather intact sediment core sampling events. The specific methods for collecting each type of sediment sample are described in the following sections, *Intact sediment core collection* and *Sediment trap material collection*).

Intact sediment core collection

Of the 5 sampling events for intact cores, 2 were dry weather (July 2015 and September 2016) and 3 were wet weather (October 2015, February 2016, and March 2017). All events involved core collection with scuba diver assistance. Following GPS verification of each station location, divers collected cores by pushing cellulose acetate butyrate core liners (7-cm diameter × 28-cm length) approximately 10 cm into the sediment. The divers then capped the ends of the cores. On the boat, overlying water was removed, and samples were placed vertically in coolers. Cores were transported to the Naval Information Warfare Center Pacific for storage at 4 °C until initiation of toxicity and passive sampler exposures.

Sediment trap material collection

Sediment traps consisted of schedule 40 polyvinyl chloride pipe with a trap height of 76.2 cm and a trap diameter of 12.7 cm (5:1 aspect ratio) as described by Blake et al. (2007). A 5-mm stainless steel mesh cover was included on the top end to reduce colonization by macrofauna. The traps were filled with hypersaline brine to retain captured sediment particles, and deployed on the sediment surface for 5 mo, using scuba divers to assist with placement and ensure vertical positioning. Traps were deployed between October 2015 and March 2016 (the 2015/2016 wet season). Two traps were deployed at each of 4 sites: P01 (a reference station), P08, P11, and P17 (Figure 1). The homogenized material from one trap was sent to Texas Tech University (Lubbock, TX, USA) for the same chemical analyses as the intact cores samples, including grain size and other physical parameters, and the homogenized material from the second trap was used for the amphipod toxicity test, described in the following section, *Amphipod toxicity test*. One of the 2 sediment traps from P01 was not recovered successfully, so that material was not assessed for toxicity.

Amphipod toxicity test

For all core samples collected, a 10-d acute amphipod toxicity test was conducted with *Eohaustorius estuarius* using standard methods (US Environmental Protection Agency 1994), with one modification. Instead of homogenizing and sieving sediment samples, cores were collected and tested intact to preserve vertical stratification (Rosen et al. 2017; Kirtay et al. 2018; Fetters et al. 2019), thus more realistically evaluating effects of freshly deposited sediment particles and better replicating in situ conditions. Four toxicity experiments were conducted including cores from: 1) July 2015, 2) October 2015 and February 2016, 3) September 2016, and 4) March 2017.

The day prior to test initiation, all cores were set up in an environmental chamber at 15 °C by adding approximately 500 mL of uncontaminated 0.45-μm filtered seawater and trickle-flow aeration. There were 4 to 6 replicate cores/station, depending on the sampling event. After an overnight equilibration period, 20 amphipods were added to each core. During the test period, water quality (dissolved oxygen, pH, salinity, and temperature) was measured daily, and all parameters were within acceptable ranges (US Environmental Protection Agency 1994). In addition, ammonia was quantified in the overlying water prior to addition of amphipods and prior to test.

During the testing for wet season 2015/2016 cores, 2 additional treatments were added. The first treatment consisted of the October 2015 core with sediment trap material placed on the top of the sediment to mimic particle deposition between the start (October 2015) and end (February 2016) of the wet season. A proportional amount of sediment trap material was added to the top of the core, based on how much material was collected in the recovered sediment trap. This resulted in the height of added sediment on top of October 2015 cores being 2, 1.2, and 3 cm to P08, P11, and P17 cores, respectively. A second additional treatment consisted of the sediment trap material alone. This resulted in a total of 4 treatments, including the intact cores from October 2015 and intact cores from February 2016.

After 10 d, amphipods were sieved from cores using a 0.5-mm stainless steel sieve, and the surviving amphipods were enumerated. All controls met acceptability criteria, with 90% or greater survival (US Environmental Protection Agency 1994).

Chemical and physical analyses

Intact cores collected for chemistry were sent to Texas Tech University for metals (As, Cd, Pb, Zn, Cu, Ni, and Hg), PAHs, polychlorinated biphenyls (PCBs), and pesticide analyses. Pyrethroids were analyzed by Weck Laboratories (Hacienda Heights, CA, USA) for all events, except for March 2017, which was analyzed by Texas Tech University. In general, only the top 5 cm of sediment of the intact core was analyzed. Specifics (including quality assurance/quality control) regarding chemical analyses are available in Reible et al. (2018). All organic contaminants were reported as organic carbon normalized values; metal concentrations were not normalized.

These intact cores were similarly analyzed for physical parameters, including total organic carbon, black carbon, and percentage of moisture. In addition, for cores collected in September 2016 and March 2017, particle size fractionation was performed using a combination of wet sieving and pipette methods to distinguish between coarse sand (>63 μm in diameter), fine sand (20–63 μm in diameter), silt (2–20 μm in diameter), and clay (<2 μm in diameter) following standard protocols as defined in Reible et al. (2018).

Diffusive gradients in thin films (DGT) samplers were deployed in the same containers as amphipods, except for July 2015, when they were exposed in a surrogate chamber (i.e., no amphipods). Single DGT samplers were deployed for 2 d in sediment cores to quantify porewater concentrations of trace metals (Harper et al. 1998; Davison and Zhang 2016).

In addition, triplicate solid-phase microextraction (SPME) fibers were deployed for 28 d to quantify porewater concentrations of PAHs, chlordane, and PCBs (Arthur and Pawliszyn 1990; Mayer et al. 2000) in additional intact cores. Methods to analyze passive samplers from this study are described by Reible et al. (2018).

Bulk sediment chemical and physical (e.g., grain size and total organic carbon) parameters for intact cores were analyzed in the top 5 cm to best estimate the sediment fraction that may have been exposed to the shallow burrowing *E. estuarius* (US Environmental Protection Agency 1994). All parameters (grain size, total organic carbon, black carbon, and percentage of moisture) were measured using standard methods (Reible et al. 2018).

Statistical analyses

Analyses were performed using SYSTAT Ver 12 (SYSTAT Software) and Microsoft Excel 2016. Two series of one-way analyses of variance (ANOVAs) were conducted, one to evaluate the effect of station for each sample date, and the other to assess the effect of sample date at each station. To control for type 1 error, Bonferroni corrections were performed resulting in $\alpha = 0.01$ and 0.0125, respectively. Post hoc Tukey's honestly significant difference tests were performed when one-way ANOVAs revealed significant effects. These data met all relevant assumptions and were not transformed.

Sediment bulk concentrations of trace metals and PCBs were compared with conservative risk thresholds (effect range low [ERL] and effect range median [ERM]) by screening quick reference tables (Buchman 2008), because the relevant literature median lethal concentration (LC50) values were not always available for these contaminants for 10-d amphipod sediment exposures. Sediment concentrations below the ERL are unlikely to elicit toxic effects, and values above the ERM are likely to elicit toxic effects in sensitive species (Long et al. 1995, 1998; Buchman 2008). The ERL and ERM values are based on field observations of a wide range of species responses and do not necessarily indicate a causal relationship (i.e., Zn may exceed the ERM, but is not necessarily the cause of observed toxicity; Long et al. 1995, 1998). Some of these species are more or less sensitive than *E. estuarius*, so these thresholds may not indicate toxicity, although they have been shown to be reasonably reliable with amphipod acute toxicity bioassays (Long et al. 1998). The ERL and ERM values are presented for PAHs as well, but not for pyrethroids (because they have not been developed for these pesticides) or for total chlordane or 4,4′-dichlorodiphenyldichloroethylene (4′,4-DDE; because these contaminants were not detected in sediments).

In addition, toxicity unit analyses were performed for pyrethroids and PAHs, summing them to understand bulk sediment concentrations in terms of toxicity (Holmes et al. 2008; Greenstein et al. 2019). Similar to these studies, relevant pyrethroid literature LC50 values from a similar freshwater amphipod *Hyalella azteca* (when not available for *E. estuarius*) were used to calculate toxicity units by dividing the concentrations of each constituent by literature LC50 values (Table 1). Because these toxicity units were relatively high, the percentage contribution of each pyrethroid analyte to the sum toxicity unit value was calculated. For PAHs, porewater concentrations were calculated using K_{OW} values and bulk sediment concentrations (Swartz et al. 1995). Based on those values, and LC50 values calculated from an amphipod model outlined by Swartz et al. (1995), sum toxicity units were calculated for bulk sediment PAHs. The same analysis was performed again using porewater values obtained from SPME measurements. There were insufficient LC50 literature values to perform toxicity unit analyses for metals or PCBs. Finally, Pearson correlation analyses were performed to determine correlation of the contaminant concentrations with amphipod survival.

TABLE 1: Median lethal concentration (LC50) values of pyrethroids from the literature used in the sum toxic unit calculations

	Literature LC50 (µg/g organic carbon)	
Pyrethroid	*Hyallela azteca*	*Eohaustorius estuarius*
Bifenthrin	—	1.05[a]
Cyfluthrin	—	0.33[b]
Cypermethrin	—	1.41[a]
Deltramethrin/tralomethrin	0.79[d]	—
Fenvalerate/esfenvalerate	0.89[c]	—
L-cyhalothrin	0.45[c]	—
Permethrin		11.16[a]

[a]Anderson et al. 2008.
[b]Greenstein et al. 2014.
[c]Li et al. 2017.
[d]Amweg et al. 2006.

RESULTS

Amphipod survival

There was no significant effect of sampling date at the reference site ($p = 0.086$), but there was a significant effect at 3 other sites, P08 ($p < 0.001$), P11 ($p < 0.001$), and P17 ($p < 0.001$; Figure 2). The observed toxicity (determined by a one-tailed *t* test between the negative control of sediment from amphipod collection site and sample) is noted in Figure 2. To understand the effect of site (reference/P01, P08, P11, P17) on survival, a series of 5 one-way ANOVAs for each sampling date was conducted. There was no significant effect of site for either dry season event, July 2015 ($p = 0.503$) and September 2016 ($p = 0.0444$), although the September 2016 event did show reduced survival at P17 (Figure 3). There were significant effects for the 3 wet weather sampling events, October 2015 ($p < 0.001$), February 2016 ($p < 0.001$), and March 2017 ($p < 0.001$; Figure 3).

Due to 100% mortality of amphipods exposed to the P11 and P17 sediment trap material, and 100% mortality observed in the October 2015 sediment + sediment trap material

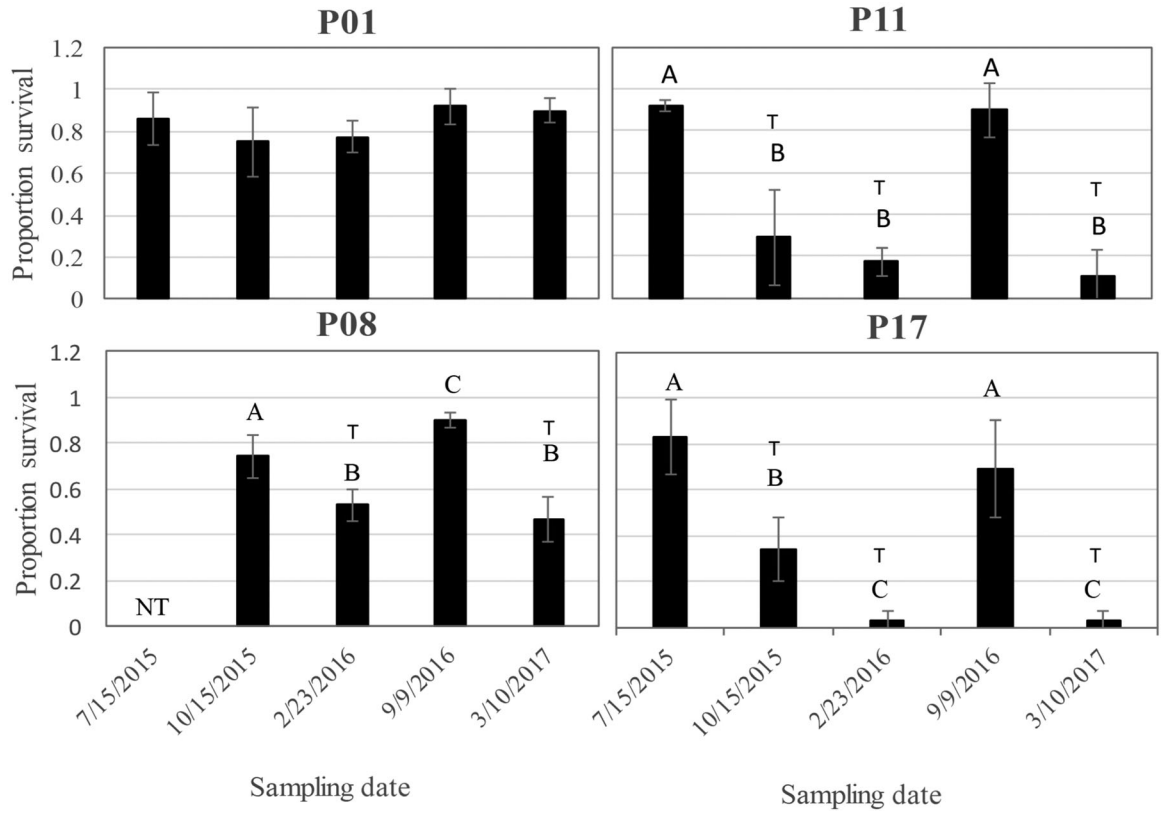

FIGURE 2: *Eohaustorius estuarius* survival at each of 4 sites grouped by sampling location. The letters represent the results of post hoc Tukey tests, indicating differences between sampling dates. Plots with no letters indicate nonsignificant effects of site on the survival of amphipods. "NT" indicates samples that were not tested. A "T" above the bar indicates a significant toxic effect.

treatments, statistical analyses were not performed to compare these treatments with the October 2015 sediment cores (without sediment trap material) or February 2016 cores. However, it is clear that the addition of sediment trap material to P08, P11, and P17 cores collected in October 2015 resulted in reduced amphipod survival compared with unamended October 2015 cores. Furthermore, this addition of trap material resulted in similar, or smaller, survival rates in February 2016 cores (Figure 4).

Physiochemistry of sediment cores

Given the strong seasonal toxicity observed with *E. estuarius*, a variety of toxicants present in the system were measured to determine which ones occurred at 1) concentrations high enough to potentially cause significant toxicity, and 2) concentrations in bulk sediments and/or porewater that correlated with observed toxicity. All measured contaminant concentrations are presented in the Supplemental Data for measured metals, PCBs, PAHs, and pesticides for both bulk sediment concentrations and as results from DGT and SPME measurements (used as proxies for porewater concentrations).

To address the first requirement (that toxicants occurred at concentrations high enough to cause potentially significant toxicity to *E. estuarius*), the ERL and ERM concentrations were used to determine which contaminants were present at concentrations that might result in toxicity if LC50 values could not be located. Values below the ERL are unlikely to elicit toxic effects, whereas values above the ERM may elicit toxic effects (Long et al. 1998).

Organic contaminants measured in the collected sediments were generally below ERL concentrations and well below ERM concentrations, including total PCBs (13% exceeded ERLs, and none exceeded ERMs), total PAHs (11% exceeded ERLs, and none exceeded ERMs), chlordane (not detected in any samples), and 4′,4-DDE (not detected in any samples; Supplemental Data, Tables S1–S3). In addition, SPME-derived porewater PCBs, PAHs, chlordane, and 4′,4-DDE concentrations were well below LC50 values for this species, where data were available (Swartz et al. 1995; Anderson et al. 2010; Phillips et al. 2011; Supplemental Data, Tables S3–S5). Pyrethroids were not targeted for analysis in SPMEs, and thus no porewater data are available for pyrethroids.

For metals (As, Cd, Pb, Zn, Cu, Ni, and Hg), many bulk sediment concentrations were above the ERL (all samples had at least one ERL exceedance) and in some cases, above the ERM (41% had at least one metal above the ERM). Copper and Zn were notably elevated, suggesting possible metal-associated toxicity (Supplemental Data, Table S6). Although there is a published Cu 10-d sediment LC50 (Anderson et al. 2008), toxicity unit calculations showed a maximum value of 0.63, suggesting that Cu had not made a large contribution to the toxicity of these samples to amphipods, especially considering that this maximum value occurred during a dry

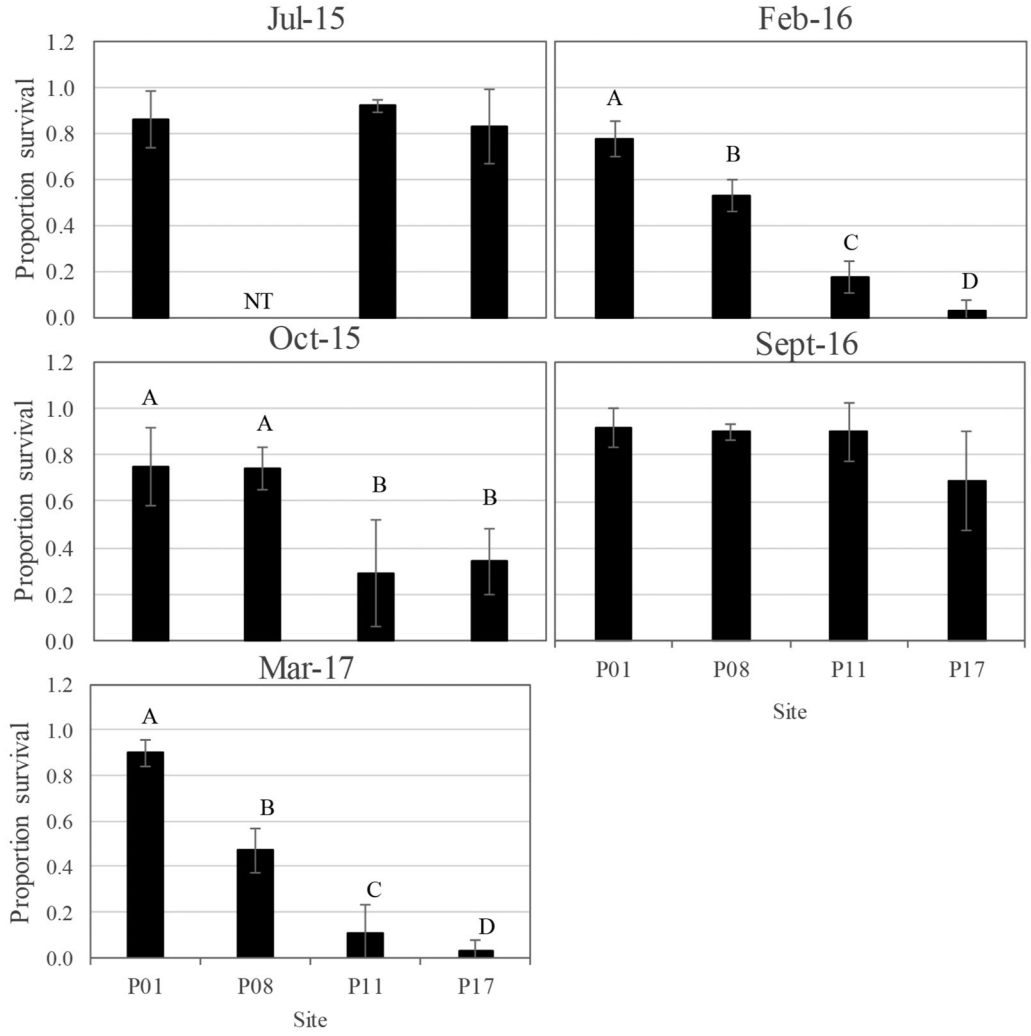

FIGURE 3: *Eohaustorius estuarius* survival at each of 4 sites grouped by sampling date. The letters represent the results of post hoc Tukey tests, indicating differences between sampling dates. Plots with no letters indicate nonsignificant effects of site on the survival of amphipods. "NT" indicates samples that were not tested.

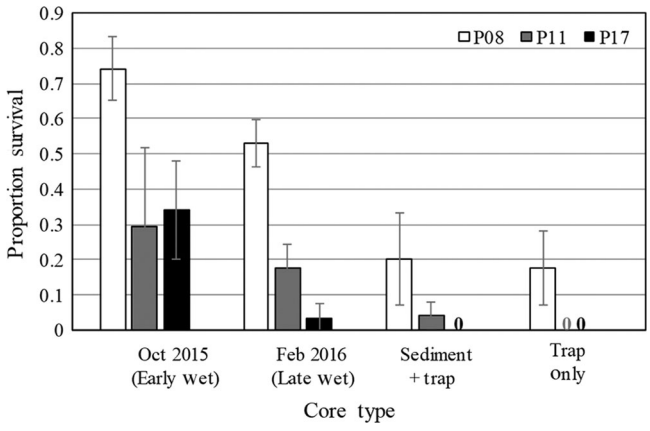

FIGURE 4: *Eohaustorius estuarius* survival for sediment trap material treatments. The first 2 groups of columns are data presented in Figures 2 and 3, shown for comparison with sediment trap treatments. Sediment + Trap indicates sediment + sediment trap material treatment. Trap Only indicates the results from exposure to just the sediment trap material.

weather sampling event (when amphipod survival was >90%). Measured LC50 values for another estuarine amphipod, *Rhepoxynius abronius*, were much lower (generally by at least a factor of 100) than reported LC50 values for Cd and Hg, which were 9810 and 13.1 mg/kg, respectively (Phillips et al. 2011). However, for Zn, with a reported LC50 of 276 mg/kg for *R. abronius* (Phillips et al. 2011), almost every core taken in this study met, or exceeded, this value (Supplemental Data, Table S6). However, this was the case for cores in which no toxicity was observed, so this value is likely too low to be relevant to *E. estuarius*. In our review, no other measured metal concentrations could be associated with reliable or relevant LC50 values from the literature. The DGT-derived metal porewater values were low (highest values for Pb, Cu, and Zn were 20.9, 1.44, and 5.18 μg/L, respectively). These were generally a factor of 10^3 lower than reported 4-d water LC50 values (reviewed in Phillips et al. 2011; Supplemental Data, Table S7).

A sum toxicity unit method for PAHs, first demonstrated by Swartz et al. (1995), was used as another line of evidence to

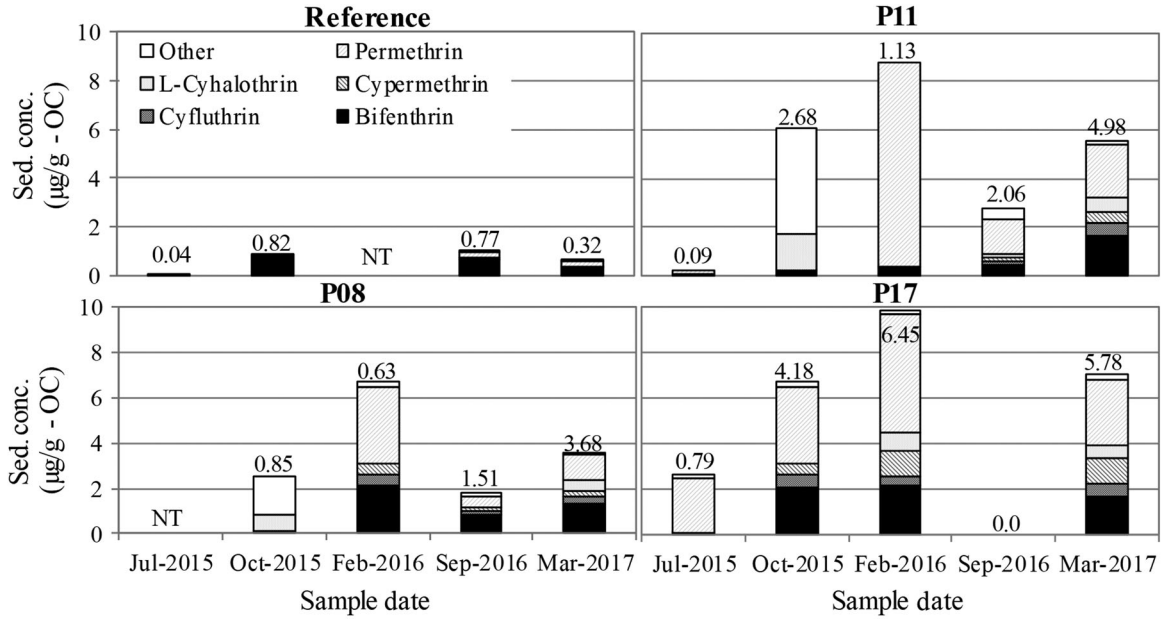

FIGURE 5: Bulk sediment concentrations for pyrethroids in sediment cores, normalized to organic carbon (OC). Pyrethroids detected infrequently were not included in the sum toxicity unit calculations due to insufficient toxicity data and are grouped as "Other." These include deltamethrin/tralomethrin, dichloran, fenvalerate/esfenvalerate, and pendimethalin. Values above the bars represent the calculated sum toxicity unit for the pyrethroids used for analyses. "NT" indicates samples that were not tested.

assess PAH toxicity, because it has been documented that these contaminants are possibly responsible for toxicity observed at this site (Southern California Coastal Water Research Project and Space and Naval Warfare Systems Center 2005; Greenstein et al. 2011). The sum toxicity unit analysis employed both modeled PAH porewater measurements (with a model developed by Swartz et al. [1995] using K_{OW} values and bulk sediment measurements) and porewater values measured using SPME (Supplemental Data, Table S4) and compared those values with porewater LC50 values calculated using an amphipod model from Swartz et al. (1995) that utilized data from 4 marine amphipods, including *E. estuarius* (Supplemental Data, Table S8). Sum toxicity unit values were low (<0.77) for modeled porewater measurements and exceedingly low (<0.039) for porewater concentrations measured using SPME (Supplemental Data, Table S9).

To assess whether pyrethroids might result in significant toxicity to *E. estuarius,* a sum toxicity unit approach was used that has been used previously for evaluating pyrethroid toxicity (Holmes et al. 2008; Lao et al. 2010; Greenstein et al. 2014, 2019). Organic carbon normalized LC50 values from sediment spiking studies were used to determine toxicity units. Pyrethroid data for *E. estuarius* are relatively limited, so when data for *E. estuarius* were not available, LC50 values derived for the freshwater amphipod *H. azteca* were used, because this species has a similar life history (epibenthic, deposit feeder). It is noteworthy, however, that *H. azteca* has been reported to be somewhat more sensitive than *E. estuarius* to pyrethroids (Lao et al. 2010). Regardless, several studies have incorporated *H. azteca* LC50 values when no *E. estuarius* data were available (e.g., Greenstein et al. 2014, 2019), so the results we present are comparable. The LC50 values used in our study are shown in Table 1. The summed toxicity units were as high as 6.35; a value of approximately 2.8 toxicity units corresponded to a 50% effect (Figure 5), and generally the values were higher at inner creek sites and during the wet seasons. The summed toxicity units remained relatively low at the reference site (<1 toxicity unit). The percentage of contributions of each individual pyrethroid toxicity unit to the sum toxicity unit was calculated (Supplemental Data, Table S10).

The percentage of fines in the sediment was determined in cores collected in September 2016 and March 2017, because some studies have indicated that high fines percentage (70–100%) may cause mortality to *E. estuarius,* although there is a lack of agreement regarding an actual fines threshold (Dewitt et al. 1989; Tay et al. 1998; Anderson et al. 2017). Anderson et al. (2017) found that survival was reduced when clay concentrations exceeded 50%, with smaller *E. estuarius* showing less sensitivity than larger ones. However, all the collected cores and sediment trap materials were characterized as having <70% fines (<63 µm in diameter), with the exception of P08 cores collected in March 2018, which had a fines content of 84.2% (Supplemental Data, Table S11). Similarly, clay percentages were all <50%, except for P08 collected in March 2018, which had a clay content of 50.8% (Supplemental Data, Table S11).

Pearson correlations were used to assess the correlation between contaminant concentrations in either sediment or porewater (metals, total PCBs, chlordane) or sum toxicity unit (total PAHs and pyrethroids) and untransformed amphipod survival data (Supplemental Data, Table S12). The following contaminants demonstrated significantly negative correlations (i.e., chemistry parameter measurements increased as amphipod survival decreased) in the sediment phase: Zn ($r = -0.53$, $p < 0.05$), total

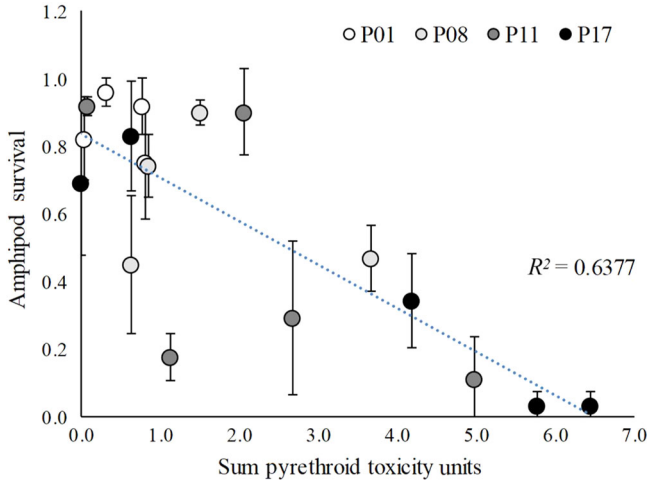

FIGURE 6: Correlation between the sum pyrethroid toxicity units and the proportion of amphipod survival.

PCBs ($r = -0.51$, $p < 0.05$), and pyrethroids ($r = -0.80$, $p < 0.001$). The following contaminants demonstrated significantly negative correlations in the porewater phase: SPME-measured total PAH ($r = -0.51$, $p < 0.05$), and total chlordane ($r = -0.73$, $p < 0.001$). Pyrethroids are the only contaminant to both occur at concentrations high enough to cause the observed toxicity and be highly correlated with amphipod survival (Figure 6).

Physiochemical parameters of sediment traps

Similar to the cores, sediment trap material did not exceed the ERM for any of the measured organic contaminants (total PCBs, total PAHs, and pesticides excluding pyrethroids), although some ERL concentrations were exceeded infrequently (Supplemental Data, Table S13–S15). In addition, the sediment trap material did exceed the ERL (for at least one metal in all samples), and in some cases the ERM (either Cu or Zn in all samples), for the measured trace metals (As, Cd, Pb, Zn, Cu, Ni, and Hg; Supplemental Data, Table S16). The bulk concentrations of pyrethroids, and the resulting sum toxicity unit values, are presented in Figure 7, showing an increase in sum toxicity unit values at sites closer to the mouth of Paleta Creek, although these values were not as high as those reported for the intact cores. The relative contribution of individual pyrethroid analytes to the sum toxicity unit value are summarized in the Supplemental Data, Table S17. Grain-size analysis of the sediment trap material (Supplemental Data, Table S11) indicated that the percentage of fines were 93.1, 90.3, 89.8, and 53.7% for stations P01, P08, P11, and P17, respectively. Although these values may suggest the potential to cause *E. estuarius* mortality, these data were not included in the correlation analysis because the sediment trap material frequently resulted in total mortality of the amphipods (i.e., no variation), making these data inappropriate for analysis.

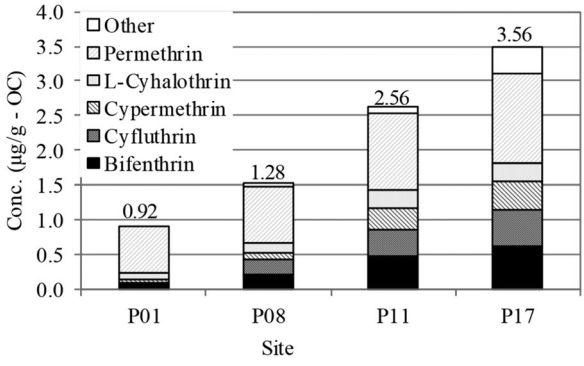

FIGURE 7: Bulk sediment concentrations for pyrethroids in sediment trap material, collected over the 2015/2016 wet weather season (October 2015–February 2016), separated by station. Pyrethroids detected infrequently were not included in sum toxicity unit calculations due to insufficient toxicity data and are grouped as "Other." These include deltamethrin/tralomethrin, dichloran, fenvalerate/esfenvalerate, and pendimethalin. Values above the bars represent the calculated sum toxicity unit for pyrethroids used for analyses. OC = organic carbon.

DISCUSSION

In the present study, a clear seasonal pattern in acute toxicity to the estuarine amphipod *E. estuarius* was observed over a 2-yr period at the mouth of Paleta Creek. In cores collected after significant antecedent dry periods (July 2015 and August 2016), high survival was generally observed at all stations (Figure 2). However, in cores collected after rain events (October 2015, February 2016, and March 2017) there was significant mortality at P08, P11, and P17, but not at P01 (Figure 2). In addition, reduced amphipod survival was greater in late wet season samples (February 2016 and March 2017) than earlier in the wet season (October 2015). This may have resulted from differences in rainfall and runoff quantity (Figure 2). Furthermore, cores collected during the wet season showed a clear spatial trend of amphipod survival, with reduced survival closer to the creek mouth, whereas this trend was not apparent in the dry season (Figure 3). In combination, these results suggest that contaminant toxicity is related to storm water discharge and that Paleta Creek may be the source. Finally, it appears that this effect is ephemeral, because it did not occur during dry season monitoring events.

Pyrethroid pesticides are the most likely cause for seasonal toxicity. They were the only organic contaminant class likely to cause toxicity to *E. estuarius* (demonstrated by sum toxicity unit values), and there was a strong correlation of sum toxicity unit values and amphipod survival (Figures 6 and 7). The sum toxicity unit analysis with pyrethroids demonstrated a similar pattern, resulting in a strong correlation between amphipod mortality and summed pyrethroid toxicity units (Figure 5). The coefficient of determination (r^2) calculated from this relationship was 0.638, which suggests that a majority of the variation in amphipod survival can be described by the sum toxicity unit values, although it is possible that other factors are also involved. Furthermore, available data regarding degradation of pyrethroids (30–90 d, with the exception of bifenthrin at 629 d), agree with observed ephemeral toxicity (Li et al. 2017). It is apparent from the pyrethroid analyses that whereas bifenthrin was consistently the largest contributor to toxicity in all core sediments (20–100%), during the second

season (2016/2017) cyfluthrin, and to a lesser degree other pyrethroids, became larger contributors to the overall sum toxicity unit values (Supplemental Data, Table S10). Although PCB sediment concentrations and chlordane porewater concentrations correlated significantly with amphipod toxicity, both were at relatively low concentrations, and were unlikely responsible for the toxic response (reviewed by Phillips et al. 2011).

Although some metals were elevated (i.e., Zn, Hg, and Cu, notably), previous research has suggested that *E. estuarius* is relatively insensitive to Cu (McPherson and Chapman 2000; Anderson et al. 2008) and likely other metals, although appropriate LC50 values of *E. estuarius* are not available for either Zn or Hg. Further DGT measurements were relatively low (in the low ppb range), and were a factor of 10^3 lower than reported water LC50 values for this species for 4-d exposures. Note that direct comparisons between DGT and porewater LC50 values are not possible, because these measurements are operationally different (some dissolved organic carbon–bound metal ions present in dissolved metal measurements are excluded by DGT; Davison and Zhang 2016). However, DGT measurements have been shown to be relevant to the bioavailable fraction (e.g., Degryse and Smolders 2016). Thus these low values suggest that most of the metals were likely not bioavailable in these sediments.

The only metal that was correlated significantly with amphipod mortality was Zn, possibly because Zn concentrations were higher during the wet season: increased storm water runoff generally increases Zn concentrations in sediment, and thus is not indicative of a causal relationship with amphipod survival. Given the apparently low bioavailability of Zn (from DGT measurements) and the much stronger correlation with pyrethroids (present at concentrations likely to cause toxicity), it is unlikely that Zn was driving this seasonal toxicity. Furthermore, although many of the sediment core measurements were above the reported sediment LC50 values for *R. abronius*, these samples included those without observable toxicity, suggesting that this LC50 value is too low to be applicable to *E. estuarius*.

The sediment + trap material treatment simulated particle deposition during the wet season and provided another line of evidence of the contribution of Paleta Creek contaminants as a potential source of seasonal toxicity (Figure 4). Trap material increased the toxicity of all stations (P08, P11, and P17) relative to unmanipulated intact cores, suggesting that particles contributed to the increase in mortality (Figure 4). Fines were unlikely responsible for mortality, given that they were not at levels as high as literature values that report only modest reductions in survival (Anderson et al. 2017). Furthermore, fines content generally increased in sediment traps further from the creek mouth, whereas toxicity was highest nearest the mouth. Thus, amphipod mortality and high fines/clay were not well correlated, suggesting that fine/clay percentages were not driving the observed toxicity in these treatments. The data suggest that storm water–associated particles from the creek may be a source of pyrethroids because pyrethroid sum toxicity units from the sediment traps decreased as they moved further from the creek mouth, matching the toxicity pattern (Figure 7). Note that the sediment trap material + core treatments were representative of a worst case scenario. It is possible that amphipods were unable to burrow past the sediment trap material (all of these specific laboratory treatments consisted of at least 1 cm of trap material), resulting in trap material bias. Finally, the percentage of contribution of each individual pyrethroid to the sum toxicity unit value in the sediment material suggests that Paleta Creek discharge is primarily loading cyfluthrin (44–56%) and *L*-cyhalothrin (16–23%), and, to a lesser degree, bifenthrin (11–18%; Supplemental Data, Table S17).

Our results are similar to others in Southern California, including San Diego Bay, suggesting that pyrethroids may be the cause of seasonal toxicity (Holmes et al. 2008; Anderson et al. 2010; Greenstein et al. 2014, 2019). Pyrethroids may be especially relevant in highly urbanized watersheds, such as Paleta Creek, because they have replaced organophosphates as a common household insecticide (Amweg et al. 2006; Weston and Lydy 2010; Tang et al. 2018) and are becoming increasingly relevant in agricultural settings as well. They have been detected worldwide in a variety of environmental media, such as surface water and sediment, making them critical to consider at potentially impacted sites (Tang et al. 2018). Following the correlation analysis, 63.8% of the variation in amphipod survival was described by pyrethroid sum toxicity units; this finding suggests that although pyrethroids are a significant driver of toxicity, there are other contaminants or parameters that may explain the observed toxicity, including some of those documented in the present study, such as Zn.

Understanding not only the responsible contaminants, but also the source of contamination, is critical to efforts to mitigate sediment contamination. Utilizing multiple lines of evidence, the results of the present study strongly suggest that Paleta Creek is an active source of pyrethroid contamination during the wet seasons. Without considering the source, and appropriately mitigating it, remediation efforts may fail due to recontamination of the site (Strategic Environmental Research and Development Program/Environmental Security Technology Certification Program 2016). For example, in the Paleta Creek receiving environment, if pyrethroid contamination from the creek is not considered, future amendments to reduce sediment toxicity may fail, because pyrethroids may continue to be a contaminant source at the site. Our study demonstrates that a better understanding of potential linkages between the source (storm water in the present study) and sediment contamination can be developed through delineation of spatial and temporal trends combined with novel storm particle treatment techniques. The novel combination of methods that we employed could increase the likelihood of appropriate determination of the contaminants of concern (including others yet to be determined) responsible for causing the observed toxicity, and its source, ultimately leading to effective use of available resources to assist remediation and regulatory compliance.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4619.

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