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The Influence of Physical Habitat, Pyrethroids and Metals on Benthic Community Condition in an Urban and Residential Stream in California

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Running Head – Influence of Physical Habitat

ABSTRACT

This study was designed to characterize benthic communities (macroinvertebrates) and physical habitat in both an urban (Kirker Creek) and residential (Pleasant Grove Creek) stream in California in late spring of 2006 and 2007. Concurrent water quality evaluations, physical sediment parameters, pyrethroids, and bulk metals {including simultaneously extracted metals (SEM) and acid volatile sulfides (AVS) ratios} were also measured during both years of this study. The relationship of various benthic metrics to physical habitat metrics, pyrethroids, and metals was evaluated for each stream using stepwise multiple linear regressions with both years combined for each stream, as well as both years and both streams combined, to increase the statistical power for determining significant relationships. Physical habitat was determined to be poor in each stream during both years of sampling. Over 100 benthic taxa were reported annually for both streams based on 2006 and 2007 sampling. Dominant benthic taxa in both streams were generally moderate to highly tolerant organisms such as Chironomids, Oligochaetes and Physa (snails). Results of stepwise multiple linear regressions of 2006 and 2007 data combined by stream, for both Kirker Creek and Pleasant Grove Creek, showed: (1) both habitat metrics and metals have stronger statistical relationships with benthic metrics than pyrethroids in Kirker Creek and (2) habitat metrics (primarily velocity depth regimes) dominated in their effects on benthic metrics in Pleasant Grove Creek. In the statistical models that included all environmental metrics for 2006 and 2007 for both streams combined, the habitat metrics (primarily velocity depth regimes) tended to dominate the significant relationships with benthic metrics. In combination with habitat metrics, a few metals (i.e., arsenic and nickel) were also observed to display significant but moderately small relationships to benthic metrics. A significant result from this stepwise regression analysis combining data for two years across both streams is that when habitat metrics and to a lesser degree metals are considered in the statistical models pyrethroids do not display any significant relationships to the benthic metrics. In summary, it is apparent from this analysis that the health of benthic communities in both streams is primarily affected by habitat metrics.

Key Words: bioassessments, pyrethroids, metals, physical habitat, urban and residential streams

INTRODUCTION

Impacts from urbanization can degrade aquatic ecosystems by altering one or more of the following principal groups of attributes: water or sediment quality; habitat structure; flow regime; energy source (food); and biotic interactions (Karr and Chu 1999). Rhoades (1995) has reported that urbanization specifically leads to fundamental changes in the hydrologic, hydraulic, erosional, and depositional characteristics of fluvial systems causing increased channel instability. In the western United States, urbanization was reported to produce lower Index of Biotic Integrity (IBI) scores than activities such as logging and larger cities were reported to have lower IBI scores than smaller cities (Kleindl 1995; Fore *et al*. 1996; and Karr 1998). Expanded population growth in many urban and residential areas in states such as California is therefore a potential stressor to aquatic ecosystems that merits an investigation of multiple stressors that can exist.

Bioassessment, a quantitative survey of physical habitat and biological communities of a water body, is a well established approach for determining the ecological condition of stream and river systems (Yoder and Rankin 1995; Karr and Chu 1999; Barbour *et al.* 1996; Wright *et al.* 2000; Bailey *et al*. 2004). Assessments of benthic invertebrate assemblages and physical habitat (bioassessments) have been conducted in wadeable streams in California's Central Valley for a number of years (Bacey 2005; Brown and May 2004; Hall and Killen 2001; Hall and Killen 2002; Hall and Killen 2003; Hall and Killen 2004; Hall and Killen 2005a; Hall and Killen 2005b; Jim Harrington, California Department of Fish and Game, personal communication; Tetra Tech 2003). To date, most of the bioassessments conducted in California have been conducted in rural areas with minimal data available for urban streams (Hall and Killen 2001; Bacey and Spurlock 2007; Peter Ode, California Department of Fish and Game, personal communication).

Bioassessments provide a useful approach for integrating effects from physical, chemical, and biological stressors on aquatic organisms. The underpinnings of bioassessments are that the structure and function of an aquatic biological community can provide critical information about the quality of the surface water and sediment. Bioassessments are extremely valuable for determining the status of aquatic biological communities across large spatial scales and land use types (agricultural and urban). Information on the status of resident biological communities is particularly useful for determining impaired water bodies, developing Total Maximum Daily Loads (TMDLs), and measuring success of voluntary or regulatory actions. Bioassessments serve monitoring needs through three primary functions: (1) screening or initial assessment of conditions; (2) characterization of impairment and diagnosis; and (3) trend monitoring to evaluate improvements from mitigation practices or further degradation. In addition, bioassessments also provide a direct means of measuring compliance with the goal of biotic integrity stipulated under the Clean Water Act because assemblages of aquatic organisms (i.e., macroinvertebrates) are comprised of taxa that are differentially responsive to different environmental stressors.

In recent years, pyrethroid pesticides - replacements for the organophosphate pesticides that are used for structural pest control, landscape maintenance and residential home and garden use - have been identified at toxic concentrations in both an urban (Kirker Creek) and residential (Pleasant Grove Creek) stream in California (Weston *et al*. 2005a; Weston *et al*. 2005b; Amweg *et al*. 2006). The toxicity assessment of pyrethroids in these two streams was based on sediment toxicity test results with a single species, the amphipod *Hyalella azteca*. Uncertainty exists when using only one species as a benthic barometer for suggesting impairment of ecosystem health. Bioassessments that include assessing the entire benthic assemblage in concert with physical habitat assessments, as described above, are a preferred approach for determining the ecological status of these streams. In addition, the assumption that pyrethroids are the only stressor in urban waterbodies is also questionable as other investigators have reported that chemical stressors such as metals (Crunkilton *et al*. 1997; Pettigrove and Hoffman 2003a) and polycyclic aromatic hydrocarbons (PAHs) (Pettigrove and Hoffman 2003b) may also be present at concentrations that are potentially toxic to aquatic life.

The primary goal of this study was to characterize benthic communities (bioassessments) and physical habitat in Kirker Creek and Pleasant Grove Creek in California in the spring of 2006 and 2007. Basic water quality parameters, eight specific pyrethroids, Total Organic Carbon (TOC), grain size, and bulk metals {including simultaneously extracted metals (SEM) and acid volatile sulfides (AVS)} were also evaluated in sediment at each stream site in concert with the bioassessments for both years. The relationship between various benthic community metrics (species richness, abundance, etc.) and physical habitat metrics, pyrethroids, and metals were evaluated for combined years by stream as well as both years and both streams. Benthic community data was interpreted in the context of biological expectations for these urban/residential streams.

MATERIALS AND METHODS

Site Selection

A total of 14 sites covering approximately 6 miles were sampled in Kirker Creek in the late spring of 2006 and 2007 (Figure 1). Sample site coordinates are listed in Hall *et al*. 2008. Kirker Creek was previously sampled for pyrethroids in 2004 (Amweg *et al*. 2006). The Kirker Creek watershed encompasses residential, commercial and industrial areas of Pittsburg, California.

A total of 21 sites were sampled in Pleasant Grove Creek and its tributaries (South Branch and Kaseberg Creek) in late spring of 2006 and 2007 (Figure 2; see Hall *et al*. 2008 for site coordinates). Pleasant Grove Creek, located in Roseville, California, is characterized by numerous contiguous subdivisions of single family homes which are less than 10 years old. There is no industry in the area and sparse commercial development and agriculture. The distance from the upstream to downstream site was approximately 12 miles in the mainstem of Pleasant Grove Creek. The distance from the upstream to downstream site in South Branch was approximately 5 miles while the distance from the upstream to downstream site in Kaseberg Creek was approximately 6 miles. The 21 sites sampled in Pleasant Grove Creek were the same sites sampled by Weston *et al*. 2005a during their pyrethroid study in 2004.

Physical Habitat Assessments

Physical habitat was evaluated at each site during both years by the same experienced field biologist concurrently with benthic collections, water quality evaluations, sediment parameters, pyrethroids, and metals. The physical habitat evaluation methods followed protocols described in Harrington and Born (2000). The physical habitat metrics used for this study are based on nationally standardized protocols described in Barbour et al. (1999). A total of 10 continuous metrics scored on a 0-20 scale were evaluated (Harrington and Born 2000). Other non-continuous metrics including percent canopy, percent gradient, and substrate composition that were also measured are described in Harrington and Born (2000).

Benthic Macroinvertebrate Sampling

Benthic macroinvertebrates were collected in the late spring of 2006 and 2007 from three replicate samples at all sample sites in the two streams. The sampling procedures were conducted in accordance with methods described in Harrington and Born (2000). Within each of these sample reaches, a riffle was located (if possible) for the collection of benthic macroinvertebrates. Only Kirker Creek site 10 and Pleasant Grove Creek sites 2 and 5 were sampled using the riffle method (see non-riffle method described below). A tape measure was placed along the riffle and potential sampling transects were located at each meter interval of the tape. Using a random numbers table, three transects were randomly selected for sampling from among those available within the riffle. Benthic samples were taken using a standard D-net with 0.5 mm mesh starting with the most downstream portion of the riffle. A 1x2 foot section of the riffle immediately upstream of the net was disturbed to a depth of 4-6 inches to dislodge and collect the benthic macroinvertebrates. Large rocks and woody debris were scrubbed and leaves were examined to dislodge organisms clinging to these substrates. Within each of the randomly chosen transects, three replicate samples were collected to reflect the structure and complexity of the habitat within the transect. If habitat complexity was lacking, samples were taken near the side margins and thalweg of the transect and the procedures described above were followed. All samples were preserved with 95% ethanol.

Due to the physical nature of these urban/residential streams, it was often difficult to locate a substantial number of riffles to sample. In numerous cases, there was only a single section of riffle available within a selected reach to sample and in most instances there were no riffles present. In cases where riffles were lacking, alternative sampling methods (reach wide sampling method) for non-riffle areas were used as outlined in Harrington and Born (2000). All sites except KC 10 and PGC 2 and 5 were sampled using the non-riffle method. This involved sampling the best available 1x2 foot sections of habitat throughout the reach using the same procedures described above. Nine 1x2 foot sections were randomly selected for sampling.

Groups of three 1x2 foot sections were composited for each replicate for a total of three replicates per site.

All benthic samples were identified to the species level if possible. Species level identifications will be particularly useful when Indices of Biotic Integrity (IBIs) are developed for wadeable streams in California. For taxa such as oligochaetes and chironomids, family and genus level, respectively, were often the lowest level of identification possible.

The benthic macroinvertebrate subsampling (resulting in a maximum of 300 individuals) and identifications were conducted by California's Department of Fish and Game (CDFG) in Rancho Cordova, California. The benthic macroinvertebrate samples were subsampled and sorted by personnel at the CDFG Laboratory located at Chico State University. Level 3 identifications (species level identifications) followed protocols outlined in Harrington and Born (2000). Slide preparations and mounting for species such as midges and oligochaetes followed protocols from the United States Geological Survey National Quality Control Laboratory described in Moulton *et al*. 2000.

Taxonomic information was used to develop benthic metrics. Benthic metrics for wadeable streams in the Central Valley were developed by California Department of Fish and Game as described by Harrington and Born (2000). The process of metric selection is driven by the goal of representing different categories of ecological information (i.e., richness, composition, tolerances and trophic measures). The various metrics were selected to maximize the effectiveness of detecting degradation in concert with communicating meaningful ecological information.

Water Quality and Sediment Measurements

The following water quality parameters were measured at each stream site in both years using procedures described in Kazyak (1997): temperature, pH, salinity, specific conductivity, dissolved oxygen, and turbidity (Hall *et al.* 2008).

Grain size (Plumb, 1981) and TOC (U. S. EPA, 2004) were measured on sediment samples collected at each site. Depositional areas - areas most likely to contain hydrophobic pesticides such as pyrethroids - were specifically sampled at each site and three to five sediment samples from depositional areas were composited for the final sample. A stainless steel spoon (similar to a scoop) was used to collect the top 2-3 cm of sediment from each site. Approximately one liter of sediment was collected from each site for grain size and TOC (as well as pyrethroids and metals). All sampling equipment was cleaned between sites using nitric acid, ethanol and distilled water. Sediment samples were stored in a cooler on ice in the field and later transferred to a refrigerator before shipment to the Applied Marine Sciences Laboratory in League City, Texas for grain size and TOC analysis.

Pyrethroid Analysis

Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-cyhalothrin and Permethrin residues were extracted from sediment by shaking with methanol/water mixture and hexane for one hour. The sample was centrifuged and an aliquot of the upper hexane layer evaporated to dryness and re-dissolved in a small volume of hexane. The hexane sample was then subjected to a silica solid phase extraction (SPE) procedure prior to residue determination by gas chromatography with mass selective detection using negative ion chemical ionisation (GC-MS/NICI). The limit of quantitation of the method was 0.1 ng/g wet weight for Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-cyhalothrin and 1.0 ng/g wet weight for Permethrin (see Robinson, 2005 for details).

Bulk Metals and SEM/AVS Analysis

The following bulk metals with existing Threshold Effects Levels (TELs), conservative protective benchmarks, as described by Buchman (1999) were measured on composited sediment samples for each site as previously described using EPA method 6020m: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn). The method detection limit (MDL) for these 7 metals was 0.025 ug/g dry weight. Mercury (Hg) was also measured on all sediment samples using EPA method 245.7m. The MDL for mercury was 0.01 ug/g dry weight.

Simultaneously extracted metals (SEM) analysis was conducted on Ni, Cu, Zn, Cd, Pb, and Hg using EPA method 200.8m. The MDLs (umol/dry g) for these SEMs were as follows: Ni (0.0033), Cu (0.0062), Zn (0.0015), Cd (0.0018), Pb (0.0002) and Hg (0.00005). Acid volatile sulfides (AVS) were evaluated on sediment samples from each site using procedures described by Plumb (1981). SEM/AVS ratios were then developed for each site to provide insight on the bioavailability of these metals in sediment. The principle of SEM/AVS is based on the observation that there are some components in sediment that bind certain metals such that they are no longer available and therefore not toxic to benthic organisms (DiToro et al. 1990 1992). Sulfides in sediments have the ability to bind with divalent metals such as cadmium, copper, lead, mercury, nickel and zinc and may render these metals unavailable to the extent sulfides are available. Sediments from the study sites were therefore analyzed for the amount of SEM and for the amount of freely available divalent metals as simultaneously extractable metals (SEM). Assuming that sulfides would bind with metals on a 1:1 molar basis, dividing SEM by the amount of AVS would suggest that these metals are available when the ratio is greater than 1.

Statistical Analysis

The Wilcoxon Rank-Sum Test was used to compare habitat and benthic metrics between the two streams for each year. Data for the key 14 benthic metrics were averaged across the three transects that were sampled for each site in Kirker Creek and Pleasant Grove Creek. These data were merged with data sets of pyrethroid concentrations, habitat metrics, and metals (bulk metal concentration in sediment to TEL ratio) for each site. The sediment concentration data for each pyrethroid were converted to toxicity units by standardizing them to 1% TOC and dividing by *Hyalella* LC50 values that were also standardized to 1% TOC (Erin Amweg, University of California at Berkeley, personal communication). A variable called "total toxicity units" was created by summing all of the toxicity units for pyrethroids at any given site.

A series of statistical models were conducted on the data to determine potential relationships between the benthic metrics and pyrethroids (in toxicity units), habitat metrics, and metals (bulk metal to TEL ratio). The overall hypthesis behind the models was that the benthic metrics are largely influenced by habitat metrics, with the alternate hypothesis being that benthic communities are significantly influenced by potential toxicants such as pyrethroids (as would be suggested by the single species toxicity tests, Weston et al. 2005a,b) and metals.

The statistical analyses were conducted on the data from the 2006 and 2007 collections combined by stream; and the data from both 2006 and 2007 collections combined across the two streams in order to examine the overall statistically meaningful relationships. Combining data from both streams and both years is appropriate because the habitat and geographic strata is similar for the two streams. Prior to the statistical analyses of the combined data set, all variables were assessed for homogeneity of variance between the two creeks. Any variable found to display heterogeneity of variance was appropriately transformed (e.g., logarithmic transformation). The variables were also assessed for creek-specific and year-specific effects using strepwise regression models. For any variables displaying these effects, the residuals of the regression models were used in place of the original variable, so that these large scale spatial and temporal effects were removed from the variables. In order to remove scale effects, all variables were standardized by unit deviate standardization prior to the multivariate statistical analyses. Due to the number of combinations of variables that were to be analyzed, it was decided *a priori* to use α = 0.01 rather than the more common 0.05 level as the statistical criterion for significance in all multivariate analyses.

A pair of complementary multivariate models was also developed to confirm the results of the stepwise regression analyses: Model 1 was designed to take the effects of the habitat metrics on the benthic metrics into account before the effects of potential toxicants (pyrethroids or metals) were assessed; and Model 2 was designed to take the effects of toxicants on the benthic metrics into account before the effects of the habitat metrics were assessed. A principal components analysis (Proc Factor, principal components method with a “varimax” rotation) was conducted on all environmental data (SAS Institute Inc. 2003). In the Model 1 confirmations, the principal components from the PCA that were most highly “loaded” by the toxicants (i.e. those PCs identified by salient factor loadings of pyrethroids and/or metals) were forced into regression models to remove their potential effects and the residuals were re-analyzed by the stepwise regression series to determine the effects of the habitat metrics. In Model 2 confirmations, the effects of the principal components that were most highly loaded by the habitat metrics were removed in a similar manner prior to re-assessing the effects of the toxicants by stepwise regression analyses. In each case, if the significant relationships between the benthic and the environmental metrics were observed to persist from the results of the original stepwise regression series to the results from Models 1 or 2, they were felt to be stronger (i.e. less confounded by the other environmental variables). These confirmation analyses were conducted on the data sets from the two creeks for both years and the combined data set.

RESULTS AND DISCUSSION

Physical Habitat

2006 Kirker creek

Based on 10 instream and riparian physical habitat metrics presented in Hall *et al*. 2008, total physical habitat scores in Kirker Creek ranged from 35 to 113 in 2006 (maximum possible total score is 200). Lower total physical habitat scores generally occurred at the downstream sites. Most habitat metrics were highly variable among Kirker Creek sites. Channel alteration and frequency of bends/riffles metric scores were generally lower at the downstream sites.

Other descriptive physical habitat metrics that were not scored on a 0-20 scale are also presented in Hall *et al*. 2008. These metrics are not scored on a 0-20 scale because some are bimodal (too much or too little canopy can be advantageous) and others are just descriptive. Mean flow ranged from 0.01 to 0.19 m/s for sites where flow measurements could be made. Percent canopy ranged from 0 to 99%. Gradient was consistent at all sites (1%) except upstream site KC14 (3%). The % fines ranged from 10 to 100%.

2007 Kirker creek

Based on 10 instream and riparian physical habitat metrics in Hall *et al*. 2008, total physical habitat scores in Kirker Creek ranged from 38 to 89 in 2007. Lower total physical habitat scores generally occurred at the downstream sites. Most habitat metrics were highly variable among Kirker Creek sites. Channel alteration and frequency of bends/riffles metric scores were generally lower at the downstream sites.

Other descriptive physical habitat metrics evaluated in 2007 that were not scored on a 0-20 scale are also presented in Hall *et al*. 2008. Mean flow ranged from 0.01 to 0.04 m/s for sites where flow measurements could be made. Percent canopy ranged from 0 to 97%. Gradient was consistent at all sites (1%) except upstream site KC14 (3%). The % fines ranged from 20 to 100%.

2006 Pleasant Grove creek

Pleasant Grove Creek total habitat scores ranged from 40 to 127 in 2006. Most habitat metrics were variable among the creek sites (Hall *et al*. 2008). Spatial trends in physical habitat metrics were lacking in Pleasant Grove Creek.

Descriptive physical habitat metrics presented in Hall *et al*. 2008 showed the following: (1) mean flow ranged from 0 to 0.50 m/s; (2) % canopy ranged from 0 to 94%; (3) % gradient ranged from 1 to 2 %; and (4) % fines ranged from 10 to 100%.

2007 Pleasant Grove creek

Pleasant Grove Creek total habitat scores ranged from 55 to 140 in 2007. Most habitat metrics were variable among the creek sites (Hall *et al*. 2008). Spatial trends in physical habitat metrics were not reported in Pleasant Grove Creek.

Descriptive physical habitat metrics presented in Hall *et al*. 2008 showed the following for all sites: (1) mean flow ranged from 0.01 to 0.44 m/s for sites where flow measurements could be made; (2) % canopy ranged from 0 to 91%; (3) % gradient ranged from 1 to 2 %; and (4) % fines ranged from 0 to 100%.

2006 and 2007 comparison of habitat metrics for both creeks

A comparison of mean habitat metric scores and total score for each creek showed that there were no statistical differences among either habitat metrics scores or total score for these two creeks in 2006 (Table 1). However, a comparison of mean habitat metric scores and total score for each creek showed that there were statistical differences between the two creeks for four metrics as well as the total score based on 2007 sampling (Table 2). Epifaunal substrate, channel alteration, riparian buffer, channel flow and total habitat scores were greater in Pleasant Grove Creek than Kirker Creek. This result is in contrast to the 2006 data where there were no significant differences among either habitat metrics or total score for these two creeks. These results suggest that physical habitat conditions have generally declined in Kirker Creek from 2006 to 2007. The total habitat score for Kirker Creek in 2007 (66) was lower that the total score (101) reported for another California urban creek (Arcade Creek) sampled in 2001 (Hall and Killen 2001). The total mean physical habitat score for Kirker Creek in 2007 is also lower than the 6 year mean for Orestimba Creek (112) and the five year mean for Del Puerto Creek (104) (Hall and Killen 2006). Both of these streams are located in the California's San Joaquin River watershed and are dominated by agricultural activity.

Benthic Macroinvertebrates

2006 Kirker creek

Approximately 12,000 individual macroinvertebrates were picked and identified from 110 taxa collected from 14 Kirker Creek sites in 2006. The five most abundant taxa **–** *Cyprididae* (seed shrimp), *Physa sp* (snails). *Micropsectra sp*. (Chironomids), *Tubificidae* (Oligochaetes) and *Simulium sp* (Black flies), - comprised ~ 67% of the total individuals collected (Table 3). These five taxa are generally considered tolerant to moderately tolerant of environmental stressors (Harrington and Born, 2000; Stribling *et al*., 1998). Pollution sensitive species such as Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), i.e. EPT taxa, were rare at all sites.

Total taxa richness ranged from 16 at downstream site KC1 to 41 at upstream site KC13 (Hall *et al*. 2008). The % dominant taxa – a metric that increases with disturbance – was reported to be higher at downstream site KC2 and lower at upstream site KC13. Tolerance value – a metric that increases with disturbance – was found to be greater at downstream site KC4 and lower at upstream site KC11. Percent tolerant taxa were reported to be greater at downstream site KC1 and lower at upstream site KC11. Percent collectors/gatherers – a feeding guild that is dominant is stressed environments - were reported to both lower and higher at downstream sites KC2 and KC4, respectively. Percent collectors/filterers – a feeding guild that is dominant in stressed environments – was reported to be lower at downstream site KC2 and higher at upstream site KC11. The total abundance metric was reported to be higher at KC5 and lower at KC8.

2007 Kirker creek

Approximately 9,500 individual macroinvertebrates were picked and identified from 114 taxa collected from 14 Kirker Creek sites in 2007. The five most abundant taxa – *Cyprididae* (seed shrimp), *Physa sp* (snails), *Tubificidae* (Oligochaetes) – unidentified immature, Tubificidae with hair chaete, and *Chironomus sp*. - comprised ~ 61% of the total individuals collected (Table 3). These taxa are generally considered tolerant to moderately tolerant of environmental stressors (Harrington and Born, 2000; Stribling *et al*., 1998). As reported in 2006, pollution sensitive species such as Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), i.e. EPT taxa, were rare at all sites.

Total taxa richness ranged from 10 at KC8 to 43 at KC13 (Hall *et al*. 2008). The % dominant taxa – a metric that increases with disturbance – was reported to be higher at upstream site KC11 and lower at downstream site KC2. Tolerance value – a metric that increases with disturbance – was found to be greater at downstream sites KC3, KC4, and KC5. Percent tolerant taxa were reported to be greater at downstream site KC3 and lower at upstream site KC11. Percent collectors/gatherers – a feeding guild that is dominant in stressed environments – were lower at KC11 when compared with other sites. Percent collectors/filterers – a feeding guild that is dominant in stressed environments – was reported to be higher at KC2 when compared with other sites. The total abundance metric was reported to be higher at upstream site KC14 when compared with other sites.

2006 Pleasant Grove creek

Approximately 18,000 individual macroinvertebrates were picked and identified from 142 taxa collected from 21 sites in Pleasant Grove Creek in 2006. The following taxa comprised ~ 56% of the total number of individuals collected: *Micropsectra* (Chironomids), *Tubificidae* unidentified immatures (Oligochaetes), *Paratanytarsus sp*. (Chironomids), *Physa sp*. (snails) and *Nais communis/variabilis* (Oligochaetes) (Table 3). All of these taxa are generally tolerant of environmental degradation (Harrington and Born 2000; Stribling *et al*. 1998).

Total taxa richness ranged from 17 at PGC12 to 52 at PGC4 (Hall *et al*. 2008). Percent dominant taxa ranged from 19% at PGC4 to 70% at PGC9. EPT taxa, which are generally considered sensitive to environmental stressors, were found at low numbers in all sites with highest number found at PGC2 (8), PGC6 (7) and PGC4 (6). The tolerance value metric was reported to be higher at PGC11 and lower at PGC5. The % tolerant taxa were reported to range from 24% at PGC3 to 62% at PGC8. Percent collectors/gatherers ranged from 23% at PGC5 to 96% at PGC8 while percent collectors/filterers ranged from 0 at PGC8 to 61% at PGC5. Total abundance ranged from 3,725 at PGC22 to 30,928 at PGC3.

2007 Pleasant Grove creek

Approximately 18,000 individual macroinvertebrates were picked and identified from 145 taxa collected from 21 sites in Pleasant Grove Creek in 2007. The following taxa comprised ~ 37% of the total number of individuals collected: *Tubificidae* unidentified immatures (Oligochaetes), *Physa sp*. (snails), *Hyalella sp.* (amphipod), *Dugestia tigrina* (Planariidae), and *Dero digitata* (Naididae) (Table 3). All of these taxa are generally tolerant of environmental degradation (Harrington and Born 2000; Stribling *et al*. 1998). However, it is also noteworthy that the 3rd most dominant species (*Hyalella sp*.) is extremely sensitive to pyrethroids as reported by Giddings (2006).

Total taxa richness ranged from 19 at PGC16 to 51 at PGC3 (Hall *et al*. 2008). Percent dominant taxa ranged from 13.5% at PGC10 to 53.2% at PGC7. EPT taxa, which are generally considered sensitive to environmental stressors, were found at low numbers in all sites with highest number found at PGC2 (8), PGC4 (7) and PGC5 (7). The lowest tolerance value metric was reported at PGC5 (5.1) and the highest value was reported at PGC16 (9.1). The % tolerant taxa were reported to range from 24% at PGC5 to 67% at PGC17. Percent collectors/gatherers ranged from 19% at PGC5 to 83% at PGC6 while percent collectors/filterers ranged from 0% at PGC11 to 32% at PGC2. Total abundance ranged from 892 at PGC8 to 22,254 at PGC17.

2006 and 2007 Comparison of benthic metrics for both creeks

A comparison among benthic metrics in Kirker Creek and Pleasant Grove Creek in Table 4 for 2006 showed that number of Trichoptera taxa, sensitive EPT index, and % predators were statistically (p-value $\leq$ 0.05) higher in Pleasant Grove Creek than Kirker Creek. If a significance level of p $\leq$ 0.10 was used as a cutoff, the following comparisons could be made: (1) EPT Index (%) and % Hydropsychidae are higher in Pleasant Grove Creek than Kirker Creek; (2) the tolerance value is higher in Kirker Creek than Pleasant Grove Creek; and (3) taxonomic richness is higher in Pleasant Grove Creek than Kirker Creek.

A comparison among benthic metrics in Kirker Creek and Pleasant Grove Creek in Table 5 for 2007 showed that sensitive EPT index, EPT index (%), Shannon Diversity, percent collectors-filterers, and taxonomic richness were statistically (p-value ≤ 0.05) higher in Pleasant Grove Creek than Kirker Creek. In contrast, the following metrics were higher in Kirker Creek than Pleasant Grove Creek: percent collectors-gatherers; percent dominant taxa; percent shredders; and tolerance value. The 2006 and 2007 benthic data suggests that higher quality benthic communities exist in Pleasant Grove Creek than Kirker Creek.

Water Quality and Sediment Parameters

2006 Kirker creek

All water quality parameters, with the exception of pH (7.4 - 8.4) and salinity (0 – 1 ppt), were variable in Kirker Creek in 2006 (Hall *et al*. 2008). Ranges of various water quality parameters were as follows: temperature (15.6 - 23.2 C), specific conductivity (252- 3970 uS), dissolved oxygen (4.7 - 10.88 mg/L), and turbidity (4.4 - 47.8 ntu).

In sediment, the percent TOC in Kirker Creek ranged from 0.70 to 2.8% with a mean value of 1.2% (Hall *et al*. 2008). Percent sand ranged from 12.3% to 69.3% with a mean value of 46%. Mean values for % gravel, % silt and % clay were 1.4, 25.4 and 27.2%, respectively.

2007 Kirker creek

All water quality parameters, with the exception of pH (7.3 - 8.3), were variable in Kirker Creek in 2007 (Hall *et al*. 2008). Ranges of various water quality parameters were as follows: temperature (15 - 24 C), specific conductivity (427-5080 uS), dissolved oxygen (1.3-15.7 mg/L), salinity (0.2 to 3 ppt) and turbidity (4.5-143 ntu).

Percent TOC in Kirker Creek sediment ranged from 0.93 to 4.7% with a mean value of 1.95% (Hall *et al*. 2008). Percent sand ranged from 7.1% to 58.6% with a mean value of 35.6%. Mean values for % gravel, % silt and % clay were 1.7%, 29.8% and 32.9%, respectively.

2006 Pleasant Grove creek

With the exception of salinity (0 – 0.4 ppt), all water quality parameters in Pleasant Grove Creek were highly variable in 2006 (Hall *et al.* 2008). Ranges of water quality conditions across the 21 sites were as follows: temperature (18.2 - 34.6 C), specific conductivity (105 – 903 uS), pH (6.4 – 9.1), dissolved oxygen (2.9 - 13 mg/L), and turbidity (2.6 – 27.8 ntu).

Percent TOC in Pleasant Grove Creek sediment ranged from 0.3 to 8.4 % with a mean value of 2.2% (Hall *et al*. 2008). The percent sand across sites ranged from 5.5 to 86.8% with a mean value of 60.4%. Mean percent values for % gravel, % silt and % clay were as follows 1.5, 23.5, and 15 %, respectively.

2007 Pleasant Grove creek

With the exception of salinity (0.1 – 0.2 ppt), all water quality parameters in Pleasant Grove Creek were highly variable in 2007 (Hall *et al*. 2008). Ranges of water quality conditions across the 21 sites were as follows: temperature (14 – 30 C), specific conductivity (117 – 407 uS), pH (6.7-9.6), dissolved oxygen (1.1 to 11.9 mg/L), and turbidity (1.9 to 67.7 ntu).

Percent TOC in Pleasant Grove Creek sediment ranged from 0.7 to 8.5 % with a mean value of 2.7% (Hall *et al.* 2008). The percent sand across sites ranged from 2.4 to 77.4% with a mean value of 51.6%. Mean percent values for % gravel, % silt and % clay were as follows 1.6%, 27.7%, 19.1%, respectively.

Pyrethroids

2006 Kirker creek

Ranges of pyrethroid concentrations (ng/g dry weight) presented in detail in Hall *et al.* 2008 and normalized to 1% TOC in Kirker Creek for 2006 were as follows: Bifenthrin (0.04 – 8.6); Fenpropathrin (< detection limit); Lambda-cyhalothrin (0.01 – 0.332); Permethrin (0.09 – 4.2); Cyfluthrin (0.06 – 2.3); Cypermethrin (0.04 – 1.7); Esfenvalerate (0.003 – 0.194) and Deltamethrin (0.01 – 2.8). Highest concentrations of pyrethroids (1% TOC normalized) in descending order from sediment samples in Kirker Creek were reported for Bifenthrin, Permethrin, Deltamethrin, Cyfluthrin, Cypermethrin, Lambda-cyhalothrin, Esfenvalerate and Fenpropathrin.

2007 Kirker creek

Ranges of pyrethroid concentrations (ng/g dry weight) presented in detail in Hall *et al*. 2008 and normalized to 1% TOC in Kirker Creek for 2007 were as follows: Bifenthrin (0.1 – 15.9); Fenpropathrin (< detection limit); Lambda-cyhalothrin (0.01- 0.267); Permethrin (0.24 – 7.56); Cyfluthrin (0.024 – 11.9); Cypermethrin (0.03 – 2.6); Esfenvalerate (0.009 – 0.252) and Deltamethrin (0.03 – 3.7). Highest concentrations of pyrethroids (based on 1% TOC normalized maximum values) in descending order from sediment samples in Kirker Creek were reported for Bifenthrin, Cyfluthrin, Permethrin, Deltamethrin, Cypermethrin, Lambda-cyhalothrin, Esfenvalerate and Fenpropathrin.

2006 Pleasant Grove creek

Ranges of pyrethroid concentrations (ng/g dry weight) presented in detail in Hall *et al*. 2008 and normalized to 1% TOC in Pleasant Grove Creek in 2006 were as follows: Bifenthrin (0.226 – 52.3); Fenpropathrin (0.002 – 0.062 with only detected values used); Lambda-cyhalothrin (0.012 – 3.3); Permethrin (0.122 – 106.6); Cyfluthrin (0.060 – 11.2); Cypermethrin (0.019 – 5.2); Esfenvalerate (0.009 – 1.5) and Deltamethrin (0.012 – 8.9). Highest concentrations of pyrethroids (1% TOC normalized) in descending order were as follows: Bifenthrin, Permethrin, Cyfluthrin, Cypermethrin, Deltamethrin, Lambda-cyhalothrin, Esfenvalerate, and Fenpropathrin. Station mean concentrations of all 8 pyrethroids were generally higher in Pleasant Grove Creek than Kirker Creek in 2006.

2007 Pleasant Grove creek

Ranges of pyrethroid concentrations (ng/g dry weight) presented in detail in Hall *et al.* 2008 and normalized to 1% TOC in Pleasant Grove Creek for 2007 were as follows: Bifenthrin (0.165 – 75.1); Fenpropathrin (0.003 – 0.098 with only detected values used); Lambda-cyhalothrin (0.020 – 3.6); Permethrin (0.29 – 53.6); Cyfluthrin (0.056 – 47.1); Cypermethrin (0.045 – 14.6); Esfenvalerate (0.020 – 1.6) and Deltamethrin (0.044 – 15.4). Highest concentrations of pyrethroids (based on 1% TOC normalized maximum concentrations) in descending order were as follows: Bifenthrin, Permethrin, Cyfluthrin, Deltamethrin, Cypermethrin, Lambda-cyhalothrin, Esfenvalerate, and Fenpropathrin. As reported for 2006, station mean concentrations of the various pyrethroids were generally higher in Pleasant Grove Creek than Kirker Creek in 2007.

2006 Toxic unit (TU) calculations of pyrethroids for both creeks

Toxic units (TU) calculations were determined in 2006 for each pyrethroid in both creeks by dividing the 1% TOC normalized concentration by the *Hyalella* LC50 concentration (a species highly sensitive to pyrethroids as reported by Giddings 2006) that was also 1% TOC normalized (Table 6). TU concentrations exceeding 1.0 were considered potentially toxic. Using the TU approach for individual pyrethroids at Kirker Creek showed that only Bifenthrin concentrations at the two downstream sites were potentially toxic. Total TUs at the five downstream Kirker Creek sites were also potentially toxic based on summing the TUs for all pyrethroids. A ranking of total TUs for all Kirker Creek sites also showed that pyrethroid toxicity was greater at the five downstream sites.

Bifenthrin TUs in Pleasant Grove Creek also showed potential toxicity at 6 sites (Table 6). Toxic Units calculations exceeding 1.0 were also reported for Cyfluthrin (1 site), Cypermethrin (3 sites) and Deltamethrin (1 sites). Total TUs for all pyrethriods combined exceeded 1.0 at nine Pleasant Grove Creek sites. Total TUs at a backwater site (PGC16), a site that is not located in the mainstem, were particularly high (15.3). High total TUs were reported at three sites in Pleasant Grove Creek (PGC 16, PGC 17 and PGC 10) that were located in the fairly close proximity to each other (Figure 2).

A ranking of total TUs across both streams showed that the top 6 sites (highest TUs) were all reported in Pleasant Grove Creek (Table 6). These data demonstrate that potential pyrethroid toxicity is greater in Pleasant Grove Creek compared to Kirker Creek in 2006.

2007 Toxic unit (TU) calculations of pyrethroids for both creeks

Using the TU approach described above for Kirker Creek showed that Bifenthrin concentrations at the three sites were potentially toxic based on 2007 data (Table 7). Total pyrethroid TUs at eight Kirker Creek sites suggested potential toxicity in this creek.

Bifenthrin TUs in Pleasant Grove Creek also showed potential toxicity at four sites (Table 7). Toxic Units calculations exceeding 1.0 were also reported for Cyfluthrin (1 site), Cypermethrin (4 sites) and Deltamethrin (1 site). Total TUs for all pyrethriods combined exceeded 1.0 at 10 Pleasant Grove Creek sites. Total TUs at site PGC17 (24.3) and PGC8 (17.6) were particularly high.

A ranking of total TUs across both streams showed that 5 of the top 6 sites (highest TUs) were all reported in Pleasant Grove Creek (Table 7). These data suggest that potential pyrethroid toxicity is greater in Pleasant Grove Creek compared to Kirker Creek in 2007. A similar result was reported in 2006. These pyrethroid data would suggest that benthic community conditions should be more depressed in Pleasant Grove Creek when compared to Kirker Creek if pyrethroids are a major stressor. However, as reported previously in this paper the converse is true, i.e. benthic community condition is higher in Pleasant Grove Creek. These data would therefore suggest that pyrethroids are not a major stressor as discussed and confirmed by statistical analysis presented later in this paper.

Bulk Metals and SEM/AVS

2006 Kirker creek

For all Kirker Creek sites in 2006 at least one bulk metal concentration as presented in Hall *et al.* 2008 exceeded a sediment Threshold Effect Level (TEL) for freshwater (Buchman 1999). TELs are conservative highly protective biological benchmarks. Sediment TELs for nickel were exceeded at all sites and arsenic TELs were exceeded at all sites except one. The number of TEL exceedences for various metals by site were chromium (3 sites), copper (2 sites), mercury (1 site), and zinc (3 sites). There were two sites where TELs for four metals were exceeded and four sites where TELs for three metals were exceeded.

The SEM/AVS data suggests that for at least nine of the fourteen sites ratios are greater than one; therefore, metals are bioavailable and potentially toxic (Hall *et al.* 2008). Sites where metals toxicity may occur are KC 2, KC 3, KC 7, KC 8, KC 9, KC 10, KC 11, KC 12, and KC 13.

2007 Kirker creek

For 11 of 14 Kirker Creek sites at least one bulk metal concentration presented in Hall *et al.* 2008 exceeded a sediment Threshold Effect Level (TEL) for freshwater in 2007 (Buchman

1999). Sediment TELs for arsenic were exceeded at 11 sites and nickel TELs were exceeded at 9 sites. The number of TEL exceedences for various metals by site were cadmium (7 sites), copper (3 sites), mercury (1 site), and zinc (3 sites). There was one site (KC4) where TELs for six metals were exceeded and two sites (KC 3 and KC 5) where TELs for five metals were exceeded.

The SEM/AVS data suggests that for at least two of the fourteen sites ratios are greater than one; therefore, metals are bioavailable and potentially toxic (Hall *et al.* 2008). Sites where metals toxicity may occur are KC 5 and KC 11.

2006 Pleasant Grove creek

Eight sites in Pleasant Grove Creek in 2006 had at least one bulk metal TEL exceedence as presented in Hall *et al.* 2008. The frequency of TEL exceedences was as follows: zinc (7 sites), copper (6 sites), nickel (4 sites), chromium (3 sites) and cadmium (1 site). The number of metals exceeding TELs by site were 5 metals for PGC 17, 4 metals for PGC8 and PGC 16, 3 metals for PGC 15, 2 metals for PGC 11, and 1 metal for PGC 12, PGC 18 and PGC 21.

The SEM/AVS data suggests that for at least 13 of the 21 sites ratios are greater than one; therefore, metals are bioavailable and potentially toxic if reported above TELs (Hall *et al*. 2008). Sites where metals toxicity may occur are PGC 8, PGC 11, PGC 12, PGC 15, PGC 17, PGC 18, and PGC 21.

2007 Pleasant Grove creek

Five sites in Pleasant Grove Creek in 2007 had at least one bulk metal TEL exceedence as presented in Hall *et al*. 2008. The frequency of TEL exceedences was as follow: zinc (4 sites), copper (4 sites), cadium (3 sites), nickel (3 sites), and arsenic (2 sites). The number of metals exceeding TELs by site were 5 metals for PGC 17, 4 metals for PGC 15 and PGC 22, 2 metals for PGC 18, and 1 metal for PGC 4.

The SEM/AVS data suggests that for at least 12 of the 21 sites ratios are greater than one; therefore, metals are bioavailable and potentially toxic if reported above TELs (Hall *et al*. 2008). Sites where metals toxicity may occur are PGC 4, PGC 15, PGC 17, and PGC 22.

Relationship of Benthic Metrics to all Stressors

2006 and 2007 Data combined by creek

Data from both 2006 and 2007 were combined for each creek to increase the statistical power of the analysis for determining the relative importance of the various stressors on benthic community metrics. Extensive univariate analysis designed to evaluate the influence of each stressor (pyrethroids, metals and habitat metrics) on benthic metrics has been presented in detail in Hall *et al*. 2008. The section below is a summary of the results of the stepwise multiple regression models that includes the influence (relationships) of all three stressors concurrently on benthic community condition expressed as benthic metrics.

For Kirker Creek, the stepwise regression models that included pyrethroids, metals to TELs and habitat metrics displayed results (Table 8a) that were similar to some of those observed in the univariate regressions as reported in Hall *et al*. 2008. Taxonomic Richness was directly related to Frequency of riffles/bends (R^2=0.26) and inversely related to Vegetative protection (R^2=0.14). In addition, % Tolerant Taxa was inversely related to Frequency of riffles/bends (R^2=0.46). The persistence of this habitat metric in the multiple regression models tends to re-enforce that it is directly related to benthic community health and inversely related to the dominance of pollution tolerant taxa. Ephemeroptera taxa and % Predators were both directly related to Chromium (R^2=0.25 and 0.29, respectively), although minimal significance should be attributed to either of these relationships, because both of the benthic metrics had only about ¼ of the data represented by non-zero values. The Tolerance Value metric was directly related to Cypermethrin TUs (R^2=0.37), although, as was previously pointed out in Hall *et al*. 2008, this was only one of 6 highly correlated pyrethroids (including Total TUs) that could have been selected by the stepwise procedure, if their R^2 values had been only slightly higher (e.g., the R^2 values for Total TUs and Bifenthrin were both 0.34, while the R^2 for Cypermethrin was 0.37; see Hall *et al*. 2008). In addition, examination of the data suggests that only a few samples from sites KC3 to KC5 appeared to be responsible for the significant regression relationship (i.e., they displayed both above average Tolerance Values and above average Cypermethrin TU values for one of the two years). Morever, the Cypermethrin concentrations did not exceed 70% of a toxicity unit in any of the samples. Conversely, some of the other highly correlated pyrethroids did display TUs that exceeded 1 in a number of samples. Thus, not too much ecological significance should be attributed to this specific relationship.

There were a few other relationships displayed by stepwise regression analyses that included all of the potential independent variables from Kirker Creek (Table 8a): % Collector/Filterers were inversely related to % fines (R^2=0.35), % Shredders were inversely related to Sediment deposition and Nickel to TEL ratios (R^2=0.17 for both), and Abundance was inversely related to Lead to TEL and % Canopy cover (R^2=0.17 for both). While only accounting for approximately a third of the variance in the benthic metrics (R^2~0.34), the relationships tended to make ecological sense.

The stepwise regression models with all independent variables from the Pleasant Grove Creek data sets from 2006 and 2007 indicated that the habitat metrics dominated in their effects on benthic metrics (Table 8b). Velocity depth regimes was the principal metric that displayed significant effects on the benthic metrics. Taxonomic Richness, Ephemeroptera Taxa, EPT Taxa, EPT Index, and % Collector/Filterers all displayed direct relationships to this metric (R^2 values ranging from approximately 0.3 to 0.5, indicating that this habitat metric "explained" from 1/3 to ½ of the variance in these benthic metrics). In contrast, the Tolerance Value metric, and % Tolerant Taxa displayed inverse relationships to this metric (R^2 values of 0.34 and 0.53, also in the same range). The direction of effects of Velocity depth regimes on these benthic metrics was logical from an ecological perspective. The benthic metrics that were indicators of benthic community health (e.g., Taxonomic Richness, Ephemeroptera Taxa, EPT Taxa, EPT Index, and % Collector/Filterers) were directly related to Velocity depth regimes, while those metrics that were associated with stress tolerant communities (e.g. Tolerance Value, % Tolerant Taxa) were inversely related to this habitat metric. Other metrics displayed weaker relationships with benthic metrics in combination with Velocity depth regimes. The Tolerance Value metric was inversely related to Sediment deposition (R^2=0.13) and directly related to % Gravel (R^2=0.10). Percent Tolerant Taxa was directly related to Mercury to TEL ratios (R^2=0.16).

2006 and 2007 Combined across both creeks

In the stepwise regression models that included all environmental metrics for 2006 and 2007 for both creeks combined, the habitat metrics (particularly velocity depth regimes) tended to dominate the significant relationships with benthic metrics (Table 9). Taxonomic Richness, Ephemeroptera Taxa, EPT Taxa, EPT Index, Shannon Diversity and % Collector/Filterers all displayed direct relationships with Velocity depth regimes (R^2 values ranging from 0.16 to 0.33, with most at or above 0.30). Percent Dominant Taxon, a metric that tends to increase in stressed environments, displayed a small inverse relationship to this metric (R^2=0.05). The % Dominant Taxon variable also displayed a small direct relationship with sediment deposition, while

Shannon Diversity displayed a small inverse relationship to this habitat metric (R^2=0.08 and 0.09, respectively). The Tolerance Value and % Tolerant Taxa metrics were inversely related to Total Score (R^2=0.33 and 0.45, respectively). The other significant relationships between benthic metrics and habitat metrics were rather weak (i.e., characterized by low R^2 values). EPT Index (%) was inversely related to Frequency of riffles/bends (R^2=0.06). Percent Collectors/Gatherers was inversely related to Epifaunal substrate/available cover (R^2=0.10) and % Grazers was inversely related to Channel alterations (R^2=0.08).

In combination with habitat metrics, a few metals (e.g. arsenic, nickel and cadmium) were also observed to display significant but moderately small relationships to the benthic metrics. The % Dominant Taxon metric was directly related to Nickel to TEL ratios (R^2=0.10), while Shannon Diversity was inversely related to Nickel to TEL ratios (R^2=0.05). The EPT Taxa and EPT Index metrics both displayed small inverse relationships to Arsenic to TEL ratios (R^2=0.10 and 0.08, respectively). The Tolerant Taxa metric displayed a small direct relationship to Cadmium to TEL (R^2=0.10). While the direction of the relationships between benthic metrics and nickel, arsenic and cadmium all make ecological sense (i.e., the greater the relative metal concentrations, the greater the indications of stress in the benthic communities), the strength of these relationships are rather weak (R^2 values $<=0.10$). Thus, causality of impacts associated with these specific metals should not be inferred.

One of the most significant observations with the overall results from the combined data sets is that, when habitat metrics and, to a lesser extent, metals are considered in the statistical models, pyrethroids do not display any significant relationships to the benthic metrics. This observation tends to confirm the previous speculation that the rather weak relationships observed for pyrethroids when they were considered alone (see Hall et al. 2008) may have been simple "markers" of anthropogenic disturbance of certain sites at which benthic communities may have been stressed by overall human activity and habitat alteration, rather than by toxic effects of the pyrethroids. When the habitat metrics were considered directly, these relationships between benthic metrics and pyrethroids disappeared, so inferences of causality associated with these pesticides do not appear warranted.

In summary, it is apparent that the health of the benthic communities in Kirker Creek and Pleasant Grove Creek is primarily affected by habitat metrics. Velocity depth regimes and Total Habitat Score metrics appear to be directly related to healthier benthic communities, while, to a lesser extent, sediment deposition and certain metals (e.g. nickel, arsenic and cadmium) appear to be associated with more stressed or stress tolerant communities. When these factors are taken into account, pyrethroids do not appear to play a significant role in the spatiotemporal patterns of the benthic community conditions in these two urban/residental creeks.

CONCLUSIONS

Urban and residential water bodies are subjected to numerous environmental stressors - such as altered water and sediment quality, physical habitat conditions, energy sources and biotic interactions - that can interact jointly to potentally impact aquatic life. This study was designed address the influence of multiple stressors (physical habitat, pyrethroids and metals) on resident benthic communities in both an urban and residential stream in California in order to tease out the magnitude of risk posed by each stressor when subjected concurrently. The chemical stressors, particularly pyrethroids and to a lesser degree metals, were found to have no significant statistical relationships with benthic metrics in either the urban or residential stream when data sets were combined by year and stream to increase the statistical power for discrimination. The most important stressor impacting resident benthic communities for both streams was altered physical habitat. This result is not surprising as altered physical habitat is considered one of the major stressors of aquatic ecosystems throughout the United States resulting in extinctions, local expirations and population reductions of aquatic fauna (Karr *et al*. 1986; Rankin 1995). Studies designed to only assess chemical stressors from toxicity testing in wadeable streams, without evaluating physical habitat, risk reporting a significant chemical stressor impact when one does not exist (false positive) as previously discussed by Rankin (1995). Physical habitat evaluations are not intended to replace other tools used for assessing impairment such as biological assessments, toxicity testing or chemical monitoring but rather add another line of evidence for assessing the conditions of lotic wadeable aquatic systems and determining possible causes of impairment. A key concept of the Clean Water Act is the protection of biological integrity of streams and rivers of the United States. Preservation of natural physical habitat of these ecosystems is critical for maintaining diverse and functional aquatic communities.

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Table 1. Mean scores for each physical habitat metric and the total for each creek with the p-values for comparing the means among creeks based on the Wilcoxon rank-sum test for 2006 sampling.

Habitat Metric	Pleasant Grove Mean	Pleasant Grove N	Kirker Mean	Kirker N	Wilcoxon p-value
VEL DPTH	5.57	21	7.07	14	0.3707
EPI SUB	7.57	21	6.79	14	0.5412
BENRIFF	5.86	21	6.71	14	0.8872
CHAN ALT	14.00	21	10.71	14	0.2008
BANKVEG	13.62	21	12.29	14	0.3917
RIPBUFF	11.19	21	9.36	14	0.2498
SED DEP	4.95	21	6.21	14	0.3520
EMBEDD	2.10	21	2.43	14	0.7746
BANKSTAB	11.33	21	11.79	14	0.6689
CH FLOW	12.76	21	11.43	14	0.1517
Total	88.95	21	84.79	14	0.9597

* $p < 0.05$

Table 2. Mean scores for each physical habitat metric and the total for each creek with the p-values for comparing the means among creeks based on the Wilcoxon rank-sum test for 2007 sampling.

Habitat Metric	Pleasant Grove Mean	Pleasant Grove N	Kirker Mean	Kirker N	Wilcoxon p-value
VEL DPTH	5.05	21	1.93	14	0.1501
EPI SUB	9.14	21	4.79	14	0.0018*
BENRIFF	5.62	21	2.79	14	0.0567
CHAN ALT	15.05	21	11.00	14	0.0235*
BANKVEG	14.71	21	12.50	14	0.1165
RIPBUFF	10.81	21	7.36	14	0.0027*
SED DEP	5.62	21	3.50	14	0.2106
EMBEDD	4.00	21	1.43	14	0.2948
BANKSTAB	15.00	21	14.50	14	0.7917
CH FLOW	13.24	21	6.07	14	0.0002*
Total	98.24	21	65.86	14	0.0002*

* $p < 0.05$

Table 3. Total and taxon abundance for benthic macroinvertebrates in Kirker Creek (KC) and Pleasant Grove Creek (PGC) in 2006 and 2007. Only the top five dominant taxa are listed for each year and stream. A detailed presentation of all taxa are available in Hall *et al*. 2008

Year and Stream	Lowest Taxa	Higher Taxa	Total N	Total %	Cumulative %
2006 – KC	*Cyprididae*	Cyprididae	2352	19.03	19.03
	Physa sp.	Physidae	2236	18.09	37.12
	Micropsectra sp.	Chironomidae	1964	15.89	53.01
	Tubificidae unid.imm.	Tubificidae	1057	8.55	61.57
	Simulium sp.	Simuliidae	680	5.50	67.07
2007 – KC	*Cyprididae*	Cyprididae	1466	15.56	15.56
	Physa sp.	Physidae	1436	15.24	30.80
	Tubificidae Unident. immature	Tubificidae	1219	12.94	43.73
	Tubificidae with hair chaetae	Tubificidae	904	9.59	53.33
	Chironomus sp.	Chironomidae	718	7.62	60.95
2006 - PGC	*Micropsectra sp.*	Chironomidae	3788	20.66	20.66
	Tubificidae unid.imm.	Tubificidae	2315	12.63	33.29
	Paratanytarsus sp.	Chironomidae	1645	8.97	42.26
	Physa sp.	Physidae	1325	7.23	49.49
	Nais communis/ variabilis	Naididae	1139	6.21	55.70
2007 - PGC	Unidentified immature	Tubificidae	1863	10.35	10.35
	Physa sp.	Physidae	1413	7.85	18.21
	Hyalella sp.	Hyalellidae	1390	7.72	25.93
	Dugesia tigrina	Planariidae	1110	6.17	32.10
	Dero digitata	Naididae	825	4.58	36.68

Table 4. Benthic metric means for Kirker Creek and Pleasant Grove Creek with statistical comparisons by Wilcoxon Test for 2006.

Benthic Metric	Kirker Creek Mean	Pleasant Grove Creek Mean	Wilcoxon p-value
Number Trichoptera Taxa	0.07	1.19	0.0055*
Number Ephemeroptera Taxa	0.36	0.90	0.5937
Sensitive EPT Index (%)	0.00	0.48	0.0251*
EPT Index (%)	0.29	4.14	0.0886
Percent Hydropsychidae	0.00	2.90	0.0647
Percent Chironomidae	35.36	41.95	0.4439
Percent Collectors Gatherers	63.14	58.10	0.4245
Percent Intolerant Taxa (0-2)	0.00	0.33	0.1642
Percent Tolerant Taxa (8-10)	44.00	41.14	0.3961
Shannon Diversity	1.88	2.17	0.1552
Percent Dominant Taxon	43.79	36.24	0.1865
Percent Predators	6.07	12.86	0.0268*
EPT Taxa	0.43	2.10	0.1159
Percent Baetidae	0.29	0.33	0.7028
Percent Scrapers	18.21	9.67	1.0000
Percent Shredders	0.00	0.00	1.0000
Percent Collector-Filterers	7.93	14.52	0.2204
Tolerance Value	7.59	7.19	0.0651
Taxonomic Richness	27.86	33.43	0.0829
Abundance	882.79	873.05	0.1867

* $p < 0.05$

Table 5. Benthic metric means for Kirker Creek and Pleasant Grove Creek with statistical comparisons by Wilcoxon Test for 2007.

Benthic Metric	Kirker Creek Mean	Pleasant Grove Creek Mean	Wilcoxon p-value
Number Trichoptera Taxa	0.43	1.14	0.1064
Number Ephemeroptera Taxa	0.14	1.14	0.0956
Sensitive EPT Index (%)	0.00	1.95	0.0158*
EPT Index (%)	0.29	9.05	0.0263*
Percent Hydropsychidae	0.00	3.14	0.0646
Percent Chironomidae	19.64	26.38	0.2333
Percent Collectors Gatherers	75.57	57.38	0.0170*
Percent Intolerant Taxa (0-2)	1.57	0.71	0.3418
Percent Tolerant Taxa (8-10)	51.86	44.24	0.0827
Shannon Diversity	1.85	2.36	0.0164*
Percent Dominant Taxon	42.66	29.70	0.0253*
Percent Predators	6.93	12.86	0.0546
EPT Taxa	0.57	2.29	0.1477
Percent Baetidae	0.29	0.95	0.2365
Percent Scrapers	14.00	12.10	0.4040
Percent Shredders	0.86	0.05	0.0228*
Percent Collector-Filterers	1.79	11.57	0.0056*
Tolerance Value	8.08	7.10	0.0085*
Taxonomic Richness	23.71	32.71	0.0215*
Abundance	673.07	856.86	0.0778

* p-value < 0.05

Table 6. Toxic units (TU) calculations for pyrethroids (1% TOC normalized) by site for Kirker Creek (KC) and Pleasant Grove Creek (PGC) sites in 2006. The sum of TUs by site and ranking by stream and all sites is also included. Toxic units > 1.0 are in bold type.

Sample ID	% TOC	Bifen TU	Fen TU	Lam-cy TU	Perm TU	Cyflu TU	Cyper TU	Esfen TU	Delt TU	Sum TU	Rank Stream	Rank All
KC 1	1.05	0.606	n/a	0.027	0.022	0.156	0.107	0.003	0.210	**1.13**	5	14
KC 2	1.45	0.728	n/a	0.034	0.014	0.098	0.131	0.003	0.327	**1.34**	3	11
KC 3	1.07	0.696	n/a	0.023	0.010	0.048	0.224	0.005	0.292	**1.30**	4	13
KC 4	2.77	**1.560**	n/a	0.064	0.038	0.208	0.460	0.011	0.357	**2.70**	1	7
KC 5	1.54	**1.645**	n/a	0.074	0.038	0.170	0.308	0.013	0.344	**2.59**	2	8
KC 6	1.12	0.325	n/a	0.011	0.005	0.020	0.103	0.003	0.084	0.55	6	17
KC 7	0.92	0.183	n/a	0.016	0.003	0.028	0.031	0.002	0.059	0.32	8	23
KC 8	1.90	0.182	n/a	0.005	0.002	0.018	0.040	0.002	0.031	0.28	9	24
KC 9	0.91	0.163	n/a	0.005	0.002	0.018	0.085	0.002	0.051	0.33	7	22
KC 10	0.71	0.137	n/a	0.002	0.001	0.063	0.019	0.001	0.036	0.26	11	26
KC 11	0.91	0.137	n/a	0.002	0.005	0.009	0.010	0.001	0.038	0.20	12	27
KC 12	1.07	0.201	n/a	0.003	0.005	0.013	0.017	0.001	0.033	0.27	10	25
KC 13	0.80	0.007	n/a	0.003	0.006	0.006	0.016	0.000	0.002	0.04	14	35
KC 14	0.78	0.012	n/a	0.014	0.006	0.006	0.017	0.004	0.003	0.06	13	34
PGC 1	0.62	0.105	n/a	0.003	0.001	0.008	0.005	0.005	0.010	0.14	17	29
PGC 2	0.37	0.044	n/a	0.003	0.013	0.006	0.036	0.009	0.017	0.13	20	32
PGC 3	0.83	0.131	n/a	0.003	0.006	0.006	0.010	0.001	0.008	0.16	16	28
PGC 4	1.16	0.120	n/a	0.008	0.002	0.014	0.210	0.001	0.012	0.37	14	20
PGC 5	0.26	0.210	n/a	0.030	0.018	0.071	0.109	0.004	0.044	0.49	13	19
PGC 6	0.39	0.059	n/a	0.007	0.012	0.009	0.024	0.002	0.016	0.13	18	30
PGC 7	0.73	0.077	n/a	0.014	0.001	0.006	0.020	0.001	0.009	0.13	19	31
PGC 8	3.80	**7.785**	n/a	0.125	0.092	0.484	**1.373**	0.029	0.551	**10.44**	2	2
PGC 9	1.02	0.837	n/a	0.056	0.013	0.126	0.265	0.003	0.032	**1.33**	9	12
PGC 10	0.93	**1.124**	n/a	0.095	0.026	0.751	**1.307**	0.008	0.138	**3.45**	4	4
PGC 11	2.03	**2.143**	n/a	0.160	0.043	0.445	0.423	0.017	0.133	**3.36**	5	5
PGC 12	2.47	0.190	n/a	0.028	0.006	0.040	0.064	0.004	0.031	0.36	15	21
PGC 14	1.10	**1.839**	n/a	0.141	0.037	0.232	0.336	0.019	0.150	**2.75**	6	6
PGC 15	6.26	0.760	n/a	0.090	0.026	0.229	0.287	0.010	0.085	**1.49**	7	9
PGC 16	3.66	**10.062**	n/a	0.732	0.987	**1.035**	**1.257**	0.096	**1.122**	**15.29**	1	1
PGC 17	8.36	**1.641**	n/a	0.217	0.052	0.487	0.894	0.013	0.338	**3.64**	3	3
PGC 18	2.27	0.685	n/a	0.024	0.008	0.102	0.502	0.004	0.035	**1.36**	8	10
PGC 19	1.04	0.501	n/a	0.043	0.003	0.038	0.099	0.003	0.126	0.81	10	15
PGC 20	0.81	0.043	n/a	0.005	0.001	0.010	0.014	0.001	0.002	0.08	21	33
PGC 21	1.77	0.419	n/a	0.028	0.014	0.100	0.077	0.004	0.060	0.70	11	16
PGC 22	5.97	0.245	n/a	0.040	0.009	0.105	0.080	0.003	0.031	0.51	12	18

Table 7. Toxic units (TU) calculations for pyrethroids (1% TOC normalized) by site for Kirker Creek (KC) and Pleasant Grove Creek (PGC) sites in 2007. The sum of TUs by site and ranking by stream and all sites is also included. Toxic units > 1.0 are in bold type.

Sample ID	% TOC	Bifen TU	Fen TU	Lam-cy TU	Perm TU	Cyflu TU	Cyper TU	Esfen TU	Delt TU	Sum TU	Rank Stream	Rank All
KC 1	1.55	0.447	n/a	0.025	0.032	0.229	0.145	0.003	0.182	**1.063**	8	18
KC 2	1.26	0.218	n/a	0.003	0.004	0.024	0.073	0.001	0.015	0.338	10	27
KC 3	3.11	0.705	n/a	0.020	0.014	0.137	0.402	0.004	0.110	**1.392**	3	12
KC 4	3.39	**1.157**	n/a	0.031	0.030	0.247	0.209	0.009	0.212	**1.895**	2	7
KC 5	4.72	**3.072**	n/a	0.059	0.070	1.103	0.691	0.016	0.472	**5.483**	1	3
KC 6	1.12	**1.147**	n/a	0.031	0.013	0.043	0.081	0.005	0.019	**1.339**	4	13
KC 7	2.13	0.126	n/a	0.021	0.018	0.016	0.026	0.001	0.006	0.214	12	30
KC 8	1.21	0.648	n/a	0.249	0.009	0.090	0.058	0.005	0.098	**1.157**	6	16
KC 9	1.29	0.391	n/a	0.008	0.007	0.037	0.197	0.002	0.040	0.682	9	21
KC 10	1.37	0.790	n/a	0.010	0.011	0.071	0.165	0.004	0.024	**1.075**	7	17
KC 11	0.93	0.215	n/a	0.005	0.005	0.016	0.018	0.002	0.024	0.285	11	28
KC 12	2.67	0.965	n/a	0.005	0.010	0.036	0.185	0.004	0.012	**1.217**	5	15
KC 13	2.12	0.019	n/a	0.002	0.002	0.002	0.008	0.0006	0.003	0.037	14	35
KC 14	1.50	0.021	n/a	0.007	0.003	0.005	0.009	0.0006	0.004	0.050	13	34
PGC 1	1.12	0.147	n/a	0.006	0.004	0.017	0.012	0.003	0.017	0.206	19	31
PGC 2	1.64	0.215	n/a	0.014	0.006	0.038	0.070	0.005	0.016	0.364	17	26
PGC 3	0.66	0.452	n/a	0.009	0.007	0.045	0.098	0.003	0.022	0.636	14	23
PGC 4	1.49	0.453	n/a	0.024	0.004	0.058	0.174	0.005	0.049	0.767	12	20
PGC 5	1.42	0.141	n/a	0.008	0.003	0.014	0.060	0.004	0.010	0.240	18	29
PGC 6	0.89	0.032	n/a	0.004	0.005	0.005	0.017	0.001	0.007	0.071	21	33
PGC 7	1.75	0.078	n/a	0.017	0.002	0.005	0.042	0.002	0.006	0.152	20	32
PGC 8	1.14	**14.456**	n/a	0.099	0.156	0.902	**1.586**	0.026	0.343	**17.568**	2	2
PGC 9	0.79	0.801	n/a	0.014	0.008	0.110	0.285	0.003	0.031	**1.252**	10	14
PGC 10	1.41	0.929	n/a	0.046	0.017	0.426	**1.851**	0.005	0.109	**3.383**	4	5
PGC 11	3.21	0.965	n/a	0.077	0.012	0.183	0.415	0.005	0.070	**1.727**	6	8
PGC 12	3.73	**1.093**	n/a	0.042	0.014	0.127	0.159	0.006	0.068	**1.509**	8	10
PGC 14	3.18	**2.848**	n/a	0.544	0.172	0.229	**0.698**	0.024	0.337	**4.852**	3	4
PGC 15	3.60	0.443	n/a	0.022	0.008	0.047	0.117	0.004	0.036	0.677	13	22
PGC 16	7.09	0.654	n/a	0.019	0.012	0.032	0.033	0.001	0.020	0.771	11	19
PGC 17	1.66	**12.743**	n/a	0.796	0.496	**4.362**	**3.852**	0.104	**1.944**	**24.297**	1	1
PGC 18	8.53	0.336	n/a	0.014	0.005	0.087	0.987	0.004	0.020	**1.453**	9	11
PGC 19	3.64	0.224	n/a	0.015	0.004	0.051	0.111	0.002	0.025	0.432	16	25
PGC 20	1.10	0.318	n/a	0.014	0.006	0.094	0.110	0.003	0.053	0.598	15	24
PGC 21	1.74	0.998	n/a	0.069	0.020	0.543	0.487	0.011	0.148	**2.276**	5	6
PGC 22	3.31	0.715	n/a	0.058	0.038	0.252	0.324	0.006	0.149	**1.542**	7	9

Table 8. Results of stepwise multiple linear regression models of benthic metrics versus toxicity units for pyrethroids, habitat metrics, and metals to TEL ratios for: a) Kirker Creek; b) Pleasant Grove Creek; both in 2006 and 2007. Only variables that were significant at α=0.01 were included in the models. The direction of the relationship for each significant variable is indicated (+ = direct; - = inverse), as is the contributed R^2 values.

a) Models for benthic metrics versus toxicity units for pyrethroids, habitat metrics, and metals to TEL ratios for Kirker Creek.

Benthic Metrics	Prob.	R^2	Significant Variables (R^2)
Taxonomic Richness	0.002	0.40	+Frequency of riffles/bends* (0.26), -Vegetative protection (0.14)
% Dominant Taxon	NS		
Ephemeroptera Taxa	0.006	0.25	+Chromium to TEL(0.25)
EPT Taxa	NS		
EPT Index (%)	NS		
Shannon Diversity	NS		
Tolerance Value	<0.001	0.37	+Cypermethrin** (0.37)
% Tolerant Taxa (8-10)	<0.001	0.46	-Frequency of riffles/bends* (0.46)
% Collectors/Filterers	<0.001	0.35	-% fines* (0.35)
% Collectors/Gatherers	NS		
% Grazers	NS		
% Predators	0.003	0.29	+Chromium to TEL* (0.29)
% Shredders	0.006	0.34	-Sediment deposition* (0.17) -Nickel to TEL (0.17)
Abundance (#/sample)	0.008	0.34	-Lead to TEL (0.17), -% Canopy cover (0.17)

* Variables that remained significant after the principal components associated with pyrethriods and metals were forced into the stepwise regressions prior to the testing of the habitat variables (see text for details).
** Variables that remained significant after the principal components associated with habitat metrics were forced into the stepwise regressions prior to the testing of the toxicant variables (see text for details).

Table 8. - continued.

b) Models for benthic metrics versus toxicity units for pyrethroids, habitat metrics, and metals to TEL ratios for Pleasant Grove Creek.

Benthic Metrics	Prob.	R^2	Significant Variables (R^2)
Taxonomic Richness	<0.001	0.31	+Velocity depth regimes* (0.31)
% Dominant Taxon	NS		
Ephemeroptera Taxa	<0.001	0.44	+Velocity depth regimes* (0.44)
EPT Taxa	<0.001	0.52	+Velocity depth regimes* (0.52)
EPT Index (%)	<0.001	0.48	+Velocity depth regimes* (0.48)
Shannon Diversity	NS		
Tolerance Value	<0.001	0.57	-Velocity depth regimes* (0.34), -Sediment deposition (0.13), +%Gravel (0.10)
% Tolerant Taxa (8-10)	<0.001	0.69	-Velocity depth regimes* (0.53), + Mercury to TEL** (0.16)
% Collectors/Filterers	<0.001	0.39	+Velocity depth regimes* (0.39)
% Collectors/Gatherers	NS		
% Grazers	NS		
% Predators	NS		
% Shredders	NS		
Abundance (#/sample)	NS		

* Variables that remained significant after the principal components associated with pyrethroids and metals were forced into the stepwise regressions prior to the testing of the habitat variables (see text for details).
** Variables that remained significant after the principal components associated with the habitat metrics were forced into the stepwise regressions prior to the testing of the toxicant variables (see text for details).

Table 9. Results of stepwise multiple linear regression models of benthic metrics versus toxicity units for pyrethroids, habitat metrics, and metals to TEL ratios in Kirker Creek and Pleasant Grove Creek for 2006 and 2007. If significant creek-specific or year-specific effects were detected for benthic metrics, these effects (not shown) were corrected for prior to analyses of effects associated with pyrethroids, habitat indices or metals. Only variables that were significant at α=0.01 were included in the models. The direction of the relationship for each significant variable is indicated (+ = direct; - = inverse), as is the contributed R^2 value.

Benthic Metrics	Prob.	R^2	Significant Variables (R^2)
Taxonomic Richness	<0.001	0.33	+ Velocity depth regimes* (0.33)
% Dominant Taxon	0.004	0.23	+Nickel to TEL (0.10), + Sediment deposition* (0.08), - Velocity depth regimes* (0.05)
Ephemeroptera Taxa	<0.001	0.28	+ Velocity depth regimes* (0.28)
EPT Taxa	<0.001	0.40	+ Velocity depth regimes* (0.30), -Arsenic** (0.10)
EPT Index (%)	<0.001	0.44	+Velocity depth regimes* (0.30), -Arsenic** (0.08), -Frequency of riffles/bends (0.06)
Shannon Diversity	<0.001	0.30	+Velocity depth regimes* (0.16), -Sediment deposition (0.09), -Nickel to TEL (0.05)
Tolerance Value	<0.001	0.33	-Total Score* (0.33)
% Tolerant Taxa (8-10)	<0.001	0.61	-Total Score* (0.45), +Cadmium to TEL* (0.10)
% Collectors/Filterers	<0.001	0.32	+Velocity depth regimes* (0.32)
% Collectors/Gatherers	0.007	0.10	-Epifaunal substrate/available cover (0.10)
% Grazers	0.006	0.08	-Channel alteration (0.08)
% Predators	NS		
% Shredders	NS		
Abundance (#/sample)	NS		

* Variables that remained significant after the principal components associated with pyrethroids and metals were forced into the stepwise regressions prior to the testing of the habitat variables (see text for details).

** Variables that remained significant after the principal components associated with the habitat metrics were forced into the stepwise regressions prior to the testing of the toxicant variables (see text for details).

Figure Captions

Figure 1. Kirker Creek (KC) sample sites.

Figure 2. Pleasant Grove Creek (PGC) sample sites.

Figure 1. Kirker Creek (KC) sample sites.

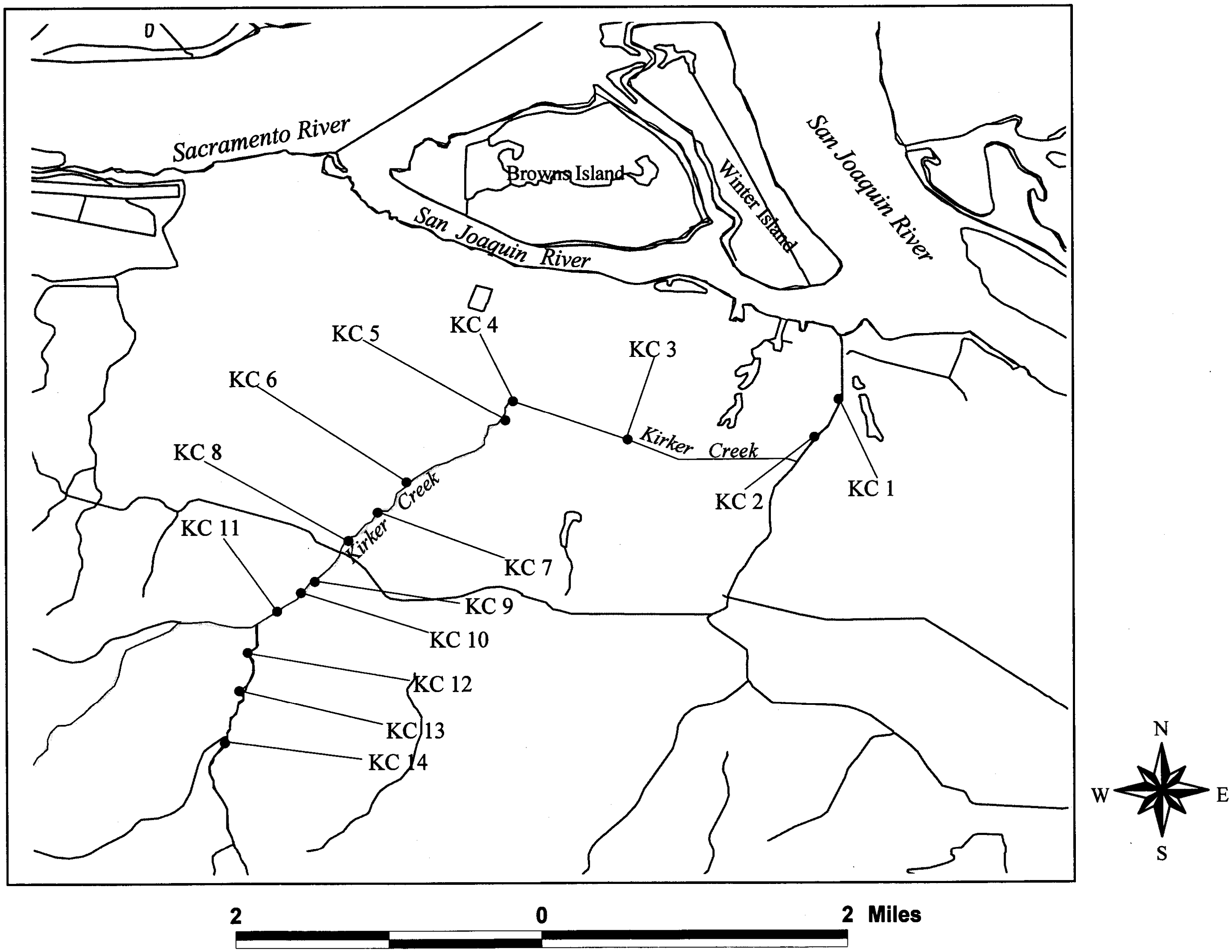

Figure 2. Pleasant Grove Creek (PGC) sample sites.