

Macroinvertebrate Assemblages in Agriculture- and Effluent-dominated Waterways of the Lower Sacramento River Watershed

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Executive Summary

A large area of the Central Valley is characterized by irrigation-subsidized agriculture and water development projects. The natural flow regimes and physical aquatic habitats of most Central Valley waterways are significantly altered and/or modified due to current and historical practices (including mining, colonization/urbanization, and agriculture). Thousands of miles of canals have been constructed, and most natural channels have been altered and/or modified to move stored water from foothill and mountain reservoirs to water users throughout the valley, and state. Currently, the vast infrastructure of Central Valley waterways remain heavily managed to support current societal needs including irrigation of agricultural lands, municipal and industrial demands, and water quality/supply needs.

The Central Valley Regional Water Quality Control Board (CVRWQCB), is responsible for protecting beneficial uses of surface waters, and has relied on toxicity testing for monitoring and assessment of aquatic contaminants and aquatic life toxicity to determine compliance with water quality objectives in their Basin Plan. In 2000 the CVRWQCB funded an exploratory project with the University of California, Davis Aquatic Toxicology Laboratory that applied benthic macroinvertebrate (BMI) bioassessment to agriculture- (ADWs) and effluent-dominated waterways (EDWs) of the lower Sacramento River Watershed. BMIs constitute an important link in freshwater aquatic ecosystem structure (food web). BMI community integrity and health vary in response to a variety of stressors that affect physical habitat and water quality. Bioassessments provide indications of aquatic system ‘biotic integrity’ as well as physical habitat condition. BMIs are considered effective indicators of aquatic system ecological health.

The goal of this study was to explore the utility of the BMI bioassessment approach in assessing condition of two types of regionally important waterways, Central Valley ADWs and EDWs. Very little is known regarding biological condition in ADWs and EDWs. Objectives of this study included: 1) examine the physical habitat and water quality parameters that potentially determine BMI community integrity; 2) probe the nature and variability of BMI communities in ADWs and EDWs; 3) search for strong associations between biological communities and environmental parameters using a variety of statistical approaches; and 4) establish a baseline of BMI community composition and habitat conditions from which future assessments may be compared.

ADWs occur in the valley floor, and can be natural, modified natural, and/or constructed waterways. Aquatic life community integrity can be impacted in ADWs by physical habitat destruction or modification, hydrology regimes (e.g., modified and intermittent flow), sediment, elevated nutrients, contaminants (including organic chemicals, such as pesticides and other agricultural chemicals and inorganic chemicals) and organic wastes. EDWs are characterized as waterways that, due to low or intermittent flow, may consist of a majority of flow originating from discharged effluents. EDWs are common in the Central Valley foothills, where natural stream flows typically diminish during dry periods of summer and fall. Physical habitat destruction or modification, effluent contaminants, and contaminants in urban runoff likely impact biological communities in EDWs.

The two-year bioassessment investigation began fall 2000 and continued through spring 2002. Fall and spring BMI samples were collected from ADWs (Butte Creek, Gilsizer Slough, Jack

Slough, Main Drainage Canal, Wadsworth Canal) and EDWs (Auburn Ravine, Dry Creek, Pleasant Grove Creek) at a range of sites within each watershed. Most of the ADW sites were low gradient (slope <0.2) occurring within the valley floor. Most EDW sites occurred in the Sierra Nevada foothills northeast of the City of Sacramento and were mostly high gradient (containing riffles). Low and high gradient versions of the California Stream Bioassessment Protocol (CSBP) were used to guide collection of macroinvertebrates. Simultaneously, physical habitat and land use data were collected. Traditional water quality data were gathered monthly at the same sites during the two-year period of macroinvertebrate collection.

Bioassessments provided an indication of BMI community integrity in ADWs and EDWs of lower Sacramento River watershed. In general, the most severely impacted sites were located adjacent to the highest intensities of agricultural and urban land uses. At three of four municipal treatment facilities, BMI community integrity below the discharge was not significantly different compared to upstream community integrity. Seasonal differences (within site variability) were detected in BMI community integrity in ADWs and EDWs. These differences may be related to natural temporal variation, to seasonal influences of anthropogenic factors, or both.

The largest differences in BMI taxa composition in this investigation were between high and low gradient sites. A range of BMI community integrity was observed within each waterway low and high gradient site groups. Downstream sites on ADWs tended to manifest more robust BMI communities than upstream sites surrounded by agricultural land use. EDWs were characterized by more robust BMI communities at sites farther away from urban areas compared to sites within

urban areas. Environmental parameters likely to be determinants of BMI community integrity included substrate, several physical habitat parameters, and some water quality variables.

Although reports that describe reference metrics/conditions (determined at sites un-impacted or ‘least disturbed’ by human activities) for BMI community integrity have not been published for a majority of Central Valley waterways, comparisons among sites of similar gradient allowed assessment of relative range and ranking of community integrity. ADWs manifested a range of biological conditions suggesting that they could support more robust BMI community integrity if physical habitat and water quality were not degraded. Likewise, EDWs were characterized by a range of BMI community integrity conditions. Detrimental effects on BMI community integrity consequent to urban land used were evident in foothill streams.

Habitat variables including decreased riparian zone, increased channel alteration, and increased sedimentation, and loss of high quality benthic habitat were discovered as probable determinants of BMI community integrity. Of the environmental parameters measured, water quality parameters appeared to exert less effect on BMI community integrity than physical habitat factors. We hypothesize that effects of water quality variables were difficult to detect with the bioassessment procedure because physical habitat conditions were so poor at most sites, especially in ADWs. Bioassessment provided an indication of biotic integrity in ADWs and EDWs, but bioassessment alone is not capable of definitively identifying the stressors causes impacts. Compromised BMI community integrity and poor aquatic habitat conditions were identified in many ADWs and EDWs of the lower Sacramento River watershed.

Results of this study clearly reveal that BMI communities integrate ‘cumulative’ effects of various land uses and associated stressors, including physical habitat and water quality conditions. Partitioning the effects of various stressors on BMI communities and identification of those stressors will require carefully designed studies. Assessing habitat conditions with BMI bioassessment protocols is likely to be straightforward, while assessing water quality impairments may pose more of a challenge in waterways with poor physical habitat conditions. Contaminants and identification of aquatic life toxicity in surface waters can be measured directly by chemical analyses and toxicity testing. An understanding of strengths and limitations of methods will be important in designing monitoring projects. Bioassessment information can be an important component of comprehensive evaluations of aquatic ecosystem condition when used in concert with water and sediment chemistry and toxicity data (e.g., triad approach).

This report provides basic background and baseline information, which will be valuable the design of future bioassessment projects. Section 5 (see page 120) of this report offers 12 recommendations intended to refine, enhance, and focus future bioassessments and data analyses. Also included are bioassessments issues to be addressed. This project was funded through the Surface Water Ambient Monitoring Program (SWAMP) from the lower Sacramento River Basin of Region 5 SWAMP fund allocation.

1. Introduction

In the Central Valley of California the Sacramento River and San Joaquin River converge to form the Sacramento-San Joaquin Delta before flowing into San Francisco Bay (Fig. 1). The Sacramento River serves as a catchment for waters draining the entire northern portion of the Central Valley and drains approximately 70,000 km². Omernik (1987) designated the Sacramento River and San Joaquin River contiguous basins as the Central Valley ecoregion. This ecoregion is characterized by irrigation-subsidized agriculture, and water development activities have significantly modified stream flow regimes. All large rivers and most small streams are dammed for flood control and runoff storage. Stored water is transported through natural channels or constructed canals for irrigation of agricultural lands, municipal and industrial needs, and to fulfill environmental requirements. Annual precipitation in the Sacramento River basin averages 36 to 63 cm. This rainfall occurs primarily in the November through February period. The predominant landscape feature of the Sacramento River basin is agriculture (Domagalski et al., 1998; Groneberg et al., 1998). Urban land use and historical mining activities are also important influences in the basin. All of these activities and modifications in the Central Valley have resulted in widespread alteration of riparian zones, waterway geomorphology, flow, and water quality raising concerns about the health of the region's aquatic ecosystems.

Agriculture-dominated waterways (ADWs) receive greater than fifty percent of flow from irrigation runoff. Irrigation occurs primarily during the dry season (March through September). ADWs can be natural streams, constructed waterways, or a combination of both. There are over 9,173 km of natural and constructed ADWs in the Sacramento River watershed; natural ADWs constitute approximately 10 percent of the total. A wide range of physical and biological conditions exist in both natural and constructed agricultural drains. Waterways in which, due to low or intermittent flow, a majority of the flow is constituted by discharged effluent are designated as effluent-dominated waterways (EDWs). These waterways are common near areas of extensive urban development, and are a focus of concern because of possible water quality effects of the combination of effluent and urban runoff that they receive.

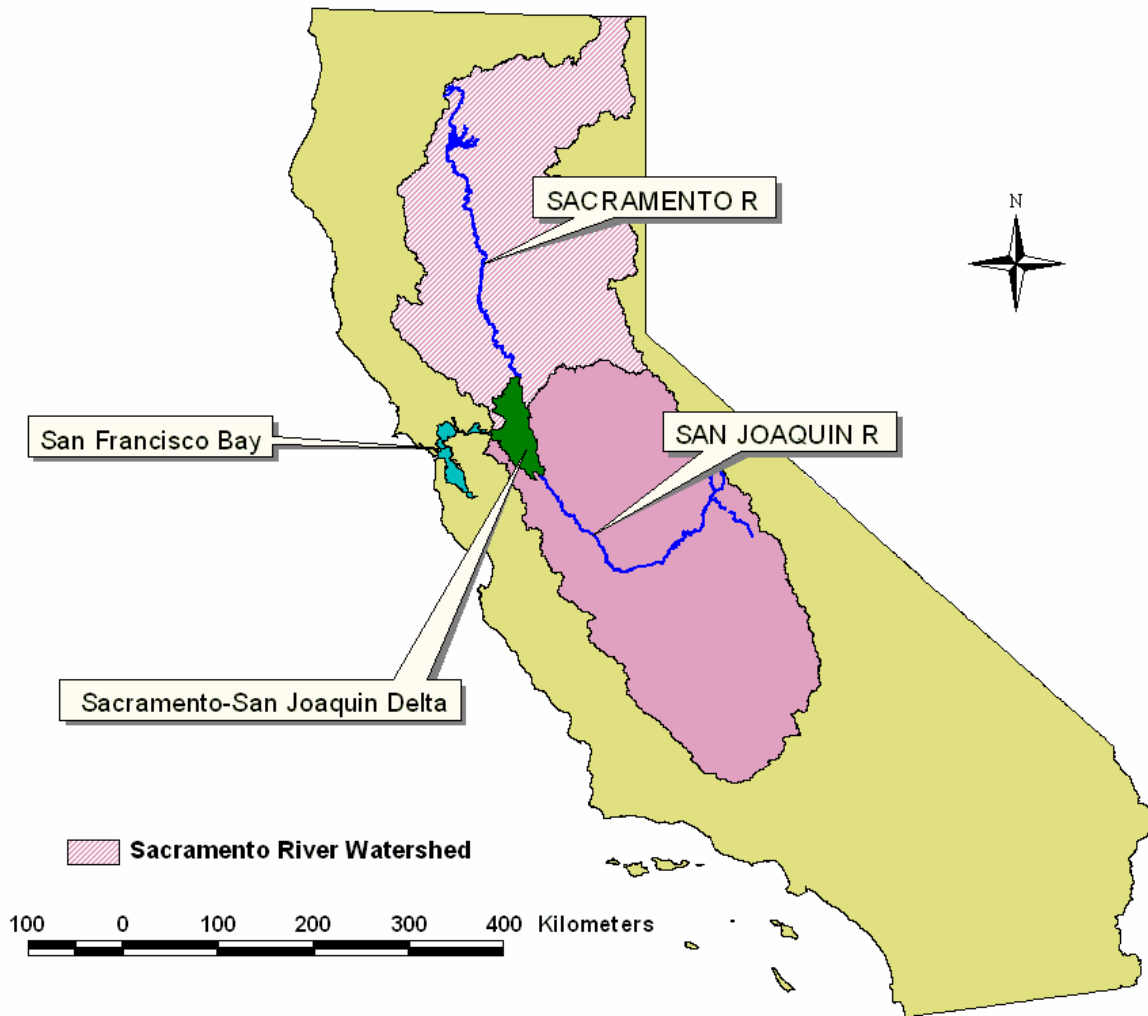


Fig. 1. California's Central Valley, Sacramento and San Joaquin River watersheds, and Delta and San Francisco Bay.

Due to the seasonality of rain and snowmelt, most wadeable (< 1.5 meters depth) streams and creeks in the Sacramento River watershed are intermittent unless supplemented by irrigation water, urban runoff, and/or discharge of treated effluent. Many streams, creeks, and rivers within the Sacramento River watershed are dominated either by water that will be used for irrigation or by irrigation runoff (ISWP, 1991). The agriculture-dominated segments of most waterways usually occur in the lower valley floor (< 165 m elevation).

The largest urban area in the Sacramento River basin is the City of Sacramento with a population of over a million. Placer County, north of Sacramento, has experienced population growth ranking among the highest in Northern California for several years. Most population growth in Placer County has occurred in the valley floor region of the county, expanding the cities of Roseville and Lincoln. The rapid population growth resulted in a net loss of irrigated agricultural land in the Sacramento River basin (CDWR, 1998).

Population growth over the next fifty years is expected to add another 1.7 million people in western Placer County. Numerous municipal wastewater treatment plants are being constructed to handle the large regional population growth. Many of these wastewater treatment facilities, both planned and existing, will discharge to low flow or intermittent Placer County EDWs. The low-flow intermittent nature of EDWs raises concern of possible aquatic community disturbance because there is little or no stream dilution of wastewater discharges.

Water column chemistry and aquatic species toxicity studies in ADWs of the Central Valley ecoregion documented impaired water quality conditions (e.g., Domagalski, 1996; de Vlaming et al., 2000; Holmes and de Vlaming, 2003). Pesticides (including herbicides, insecticides, fungicides) totaling millions of kilograms are applied annually in Sacramento River basin (CDPR, 2003). Much of the toxicity to aquatic species in ADWs has been linked to insecticides (e.g., de Vlaming et al., 2000). Ambient water toxicity testing and chemistry measurements conducted by National Pollutant Discharge

Elimination System (NPDES) dischargers have also indicated periodic water quality degradation in EDWs.

Bioassessments are a component of assessing the health and integrity of aquatic communities in several areas of the United States. These procedures have served as important tools for evaluating impacts from anthropogenic disturbances such as non-point source pollution and alterations of stream channels, riparian areas, and entire stream catchments (Fore et al., 1996; U.S. EPA, 2002). Benthic macroinvertebrate (BMI) communities are a critical component of stream ecosystems. Although there is considerable concern regarding health and integrity of biotic communities, few BMI community studies have been conducted on waterways in California's Central Valley.

Leland and Fend (1998) conducted artificial-substrate macroinvertebrate bioassessments in the lower San Joaquin River and associated tributaries applying a multivariate analysis approach, but did not sample in the Sacramento River Basin. Brown and May (2000) conducted biological assessments (1993-97) on the lower Sacramento and San Joaquin River drainages as a component of the U.S. Geological Surveys (USGS) National Water Quality Assessment Program. The focus of the study was on snag macroinvertebrate assemblages, with sampling according to Meador et al. (1993). Hall and Killen (2001) applied California Department of Fish and Game (Harrington, 1999) BMI procedures to assess benthic communities and habitat in a representative urban and agricultural waterway in the Central Valley. Applying the U.S. EPA Environmental Monitoring and Assessment Program (EMAP) procedure (Lazorchak et al., 1998), relationships between environmental gradients and macroinvertebrate assemblages in lotic habitats of the California Central Valley were examined by Griffith et al. (2003).

A primary goal of this study was to assess BMI community structure and physical stream habitat conditions in several ADWs and EDWs of the lower Sacramento River watershed. These regionally prominent aquatic ecosystems, including BMI communities, are poorly understood. A second objective was to establish a baseline of BMI community composition and habitat conditions from which future assessments may be compared.

Another intent was to identify environmental factors that potentially impact BMI assemblages, although limited resources precluded evaluation of all potential stressors on aquatic ecosystem biota. As stated, these types of waterways are characterized by highly modified, or unnatural, conditions. Another aim of this study was to examine the nature of variability among aquatic communities, physical habitat and water quality parameters in ADWs and EDWs. At the time of this investigation no reference metrics/conditions for Central Valley waterways were published for gauging relative BMI community integrity/health. Therefore, while it was impossible to determine the extent of biological impairment at the sites examined, we sought to measure the health of the BMI communities at these sites relative to one another.

2. Methods

2.1 Site selection rationale and locations

Sampling sites were in agriculture- and effluent-dominated tributaries to the lower Sacramento River (Figs. 2 and 3). Five ADWs (Butte Creek, Jack Slough, Gilsizer Slough, Main Drainage Canal, and Wadsworth Canal) and three EDWs (Auburn Ravine, Dry Creek, and Pleasant Grove Creek) were selected for sampling. Multiple sites were selected on each waterway for more complete characterization and to allow examination of possible impacts related to various land use practices. The basic rationale for waterway selection for ADWs was based upon historical and current toxicity and chemistry data for each waterway – although it was not our intent to evaluate the relationships between bioassessment and other monitoring data from other time periods and other special studies. Preference was given to ADWs that had such existing information available. Sites were generally selected in ADWs to reflect a gradient of agricultural land use and allow for possible examination and partitioning of different cropping patterns and intensity of those patterns within each watershed. EDWs were selected either because there were wastewater treatment plants in operation on such waterways or there are planned treatment plants. EDWs that had watershed groups working in those waterways were preferred as this allowed for coordination of monitoring and access to private property. In general, sampling sites in EDWs were

selected to reflect a gradient of urban land use within each watershed, and to bracket wastewater treatment plant effluent discharges. Access to sampling sites from road crossings was also a factor in site selection for all sites.

Land uses surrounding waterways included in this investigation are summarized in Table 1. Land use was calculated based on GIS layers and delimited by watershed boundary. Many agriculture-dominated watersheds were unnatural with boundaries identified from irrigation district historical records. Most ADWs consist of 70 percent or more agricultural land use. Urban land use in the EDWs ranged between 9 to 47 percent. Dry Creek and Pleasant Grove Creek EDWs consist of 30 percent or greater of agricultural land use in the downstream-most portions of each basin. Because natural vegetation predominates Butte Creek and Auburn Ravine, these sites were projected to manifest higher biological and habitat conditions. Native vegetation is a classification consisting of grasses, brush, timber, and forests (CDWR, 1993). Sampling site locations within each watershed are summarized in Table 2. Habitat assessments were conducted simultaneously with BMI collections. Most sites were sampled during spring and fall (2000/01; 2001/02) over the two-year study period.

2.2 Habitat assessments and water quality measurements

Habitat structure is one of five parameters (habitat structure, flow regime, water quality, energy source, and biotic interactions) that human activities can alter that, in turn, degrade water resources and impact BMI community health and integrity (Karr and Chu, 1999). For a more comprehensive understanding of spatial variations in BMI community structure/integrity and potential causes of biotic disturbances, habitat assessments were conducted simultaneously with BMI collections.

Physical habitat assessments were conducted at each site. These included two components: (1) the CSBP Worksheet that focuses on water quality and habitat parameters at the individual riffle/transect level and (2) the US EPA nationally standardized Habitat Assessment Field Data Sheet (Barbour et al., 1999) that targets

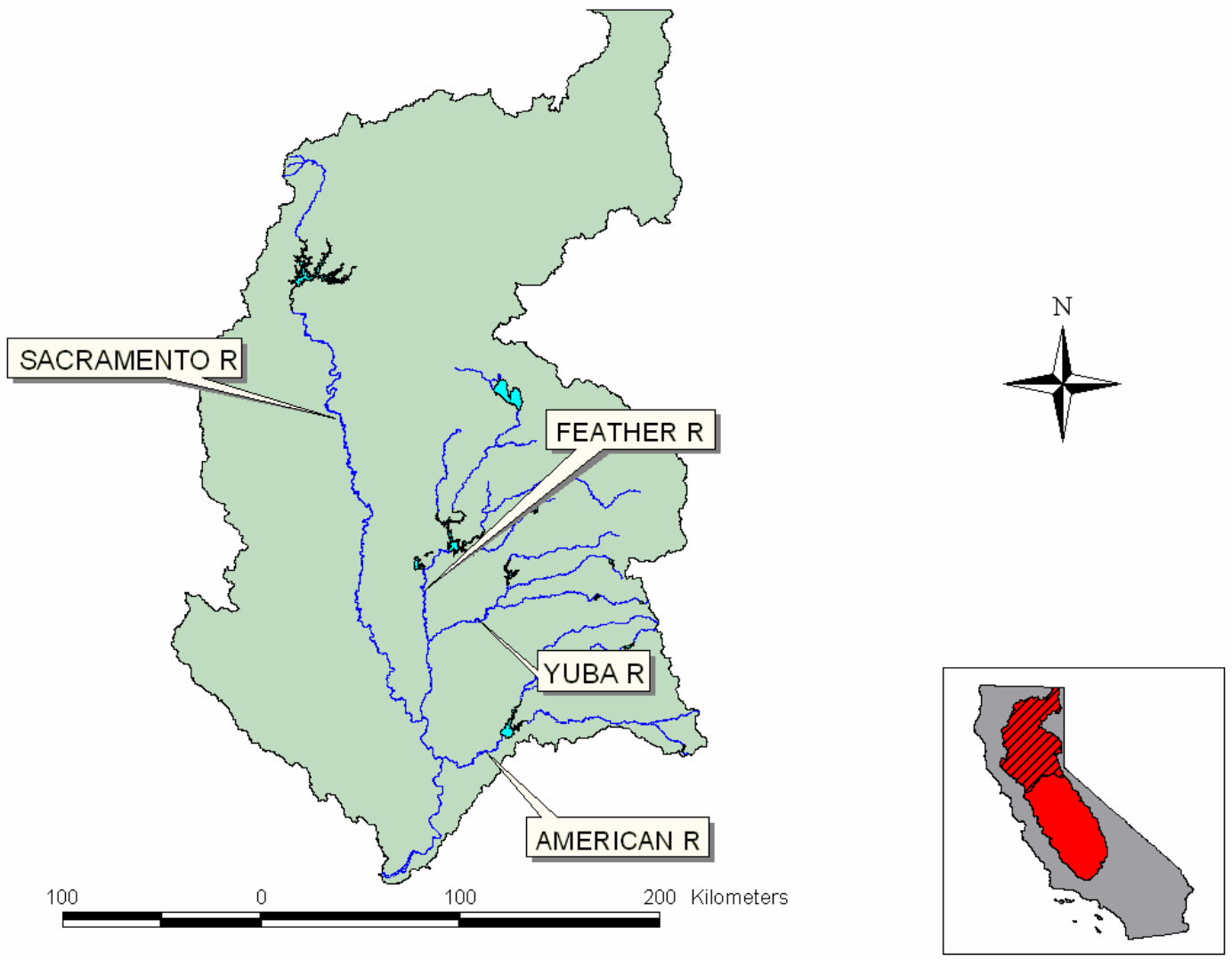


Fig 2. Sacramento River watershed.

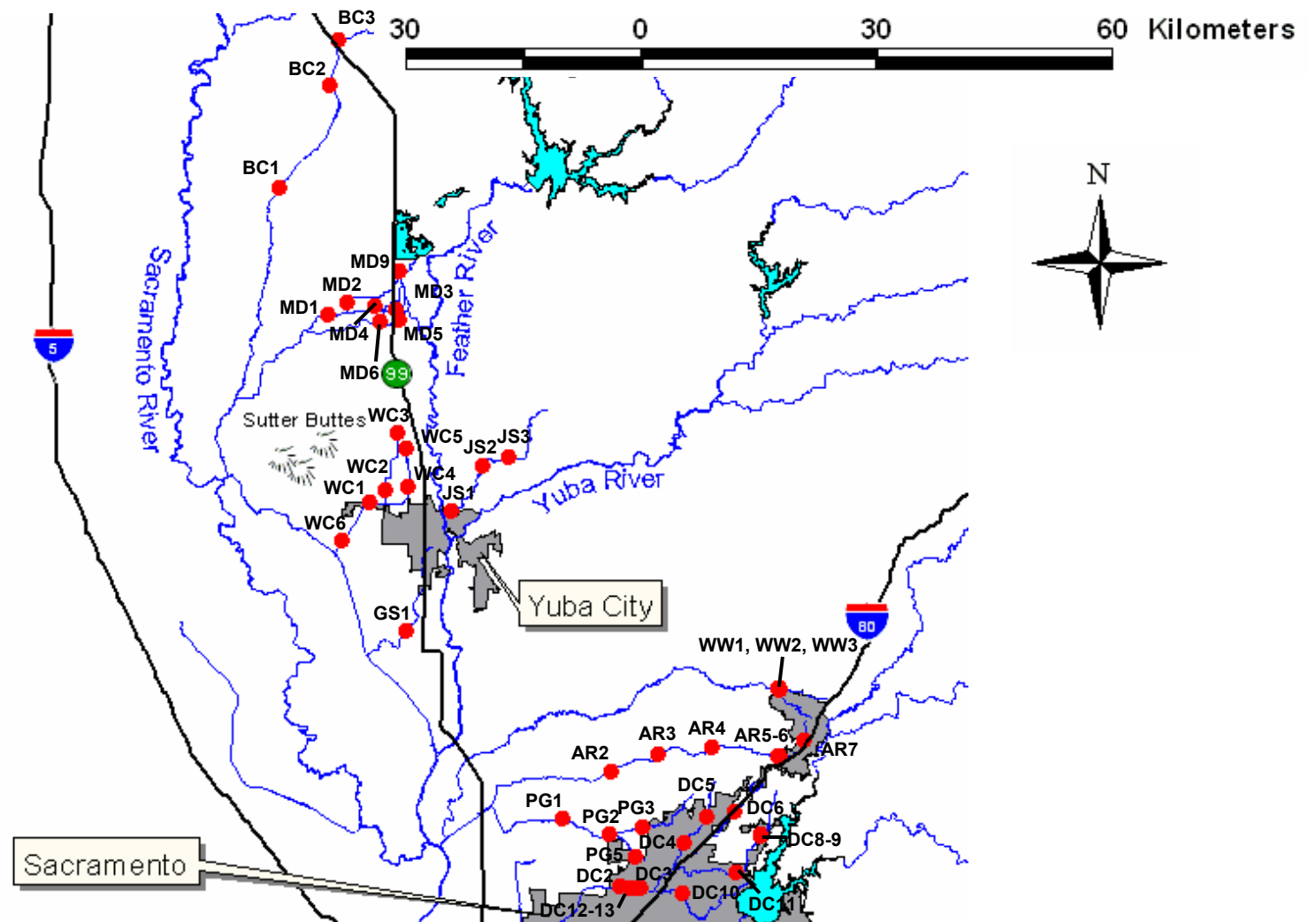


Fig. 3. Sampling sites included in the study of the lower Sacramento River watershed. The main waterbodies sampled included Butte Creek (BC), Main Drainage Canal (MD), Wadsworth Canal (WC), Jack Slough (JS), Gilsizer Slough (GS), Auburn Ravine (AR), Dry Creek (DC), and Pleasant Grove Creek (PG), as well as sites bracketing the SMD1 waste water treatment facility (WW).

Table 1
 Land use surrounding low gradient and high gradient waterways in the lower Sacramento River Watershed

	Low Gradient					High Gradient		
	Pleasant Grove Ck.	Jack Sl.	Wadsworth Canal	Main Canal	Gilsizer Sl.	Butte Ck.	Auburn Ravine	Dry Ck.
Agriculture	34%	72%	69%	78%	69%	21%	29%	9%
Urban	14%	3%	4%	4%	28%	6%	9%	47%
Native Vegetation	52%	25%	27%	18%	3%	73%	62%	44%
Total Acres	44,870	40,441	74,178	32,588	31,096	134,583	51,170	74,069

Table 2
 Sampling site locations and seasons sampled during fall 2000 – spring 2002

Waterbody	Sites	Location	Latitude	Longitude	Sampling Season		
					Fall	Spring	
Auburn Ravine	AR2	Moore Rd.	38.8700	121.3566	00, 01	01,02	
	AR3	Hwy 65	38.8885	121.2850	00, 01	01,02	
	AR4	Fowler Rd.	38.9011	121.2125	00, 01	01,02	
	AR5	Downstream Auburn WWTF	38.8897	121.1123	00, 01	01,02	
	AR6	Upstream Auburn WWTF	38.8891	121.1097	00, 01	01,02	
	AR7	Palm Avenue - Most Upstream	38.9064	121.0751	00, 01	01,02	
	Dry Creek	DC2	Dry Creek - Cook Riolo Rd.	38.7368	121.3383	00, 01	01,02
DC3		Dry Creek - Atkinson Rd.	38.7343	121.3087	00, 01	01,02	
DC4		Antelope Creek - Sunset Blvd.	38.7876	121.2489	00, 01	01,02	
DC5		Antelope Creek - Taylor Park	38.8183	121.2164	00, 01	01,02	
DC6		Secret Ravine - Loomis Park	38.8245	121.1755	00, 01	01,02	
DC8		Miners Ravine - d/s SMD3 WWTF	38.7968	121.1358		01,02	
DC9		Miners Ravine - u/s SMD3 WWTF	38.7982	121.1352		01,02	
DC10		Linda Creek - Champion Oaks Blvd.	38.7300	121.2493	00, 01	01,02	
DC11		Miners Ravine - Auburn Folsom Blvd.	38.7545	121.1702	00, 01	01,02	
DC12		Dry Creek - d/s Roseville WWTF	38.7343	121.3246	00, 01		
DC13		Dry Creek - u/s Roseville WWTF	38.7339	121.3187	00, 01		
Coon Creek		WW1	Coon Creek - u/s SMD1 WWTF	38.9663	121.1096	00, 01	01,02
		WW2	Coon Creek - d/s SMD1 WWTF	38.9657	121.1130		01,02
	WW3	Rock Creek - u/s SMD1 WWTF	38.9643	121.1101		01,02	
Pleasant Grove Ck.	PG1	Pleasant Grove Creek - Pettigrew Rd.	38.8124	121.4245	00, 01	01,02	
	PG2	Pleasant Grove Creek - Fiddymont Rd.	38.7959	121.3555	00, 01	01,02	
	PG3	Pleasant Grove Creek - Industrial Blvd.	38.8055	121.3087	00, 01	01,02	
	PG5	South Branch PGC - Pleasant Gr. Blvd.	38.7711	121.3159	00, 01	01,02	
Butte Creek	BC1	Butte Creek - Aguas Frias Rd.	39.5301	121.8584	00, 01	01,02	
	BC2	Butte Creek - Durham/Dayton HWY	39.6471	121.7870	00, 01	01,02	
	BC3	Butte Creek - HWY 99	39.6994	121.7771	00, 01	01,02	
Gilsizer Slough	GS1	Gilsizer Slough - O'Banion Rd.	39.0260	121.6592	00, 01	01,02	
Jack Slough	JS1	Jack Slough - Doc Adams Rd.	39.1623	121.5959	00, 01	01,02	
	JS2	Jack Slough - Woodruff Rd.	39.2149	121.5513	00, 01	01,02	
	JS3	Jack Slough - Loma Rica Rd.	39.2253	121.5116	00, 01	01,02	
Main Drainage Canal	MD1	Main Canal - Farris Rd	39.3747	121.7828	00, 01	01,02	
	MD2	Main Canal - Farris Rd. North Lateral	39.3996	121.7562	00, 01	01,02	
	MD3	Main Canal - South Ave. Lateral E	39.3924	121.6840	00, 01	01,02	
	MD4	Main Canal - West Biggs/Gridley HWY Lateral H	39.3952	121.7160	00, 01	01,02	
	MD5	Main Canal - Ord Ranch Rd. Lateral E-7	39.3804	121.6787	00, 01	01,02	
	MD6	Main Canal - West Biggs/Gridley HWY Lateral E-7	39.3779	121.7062	00, 01	01,02	

continued

Table 2 continued

Waterbody	Sites	Location	Latitude	Longitude	Sampling Season	
					Fall	Spring
Main Drainage Canal	MD9	Main Drain - Phil Fran Dr.	39.4359	121.6789		01,02
Wadsworth Canal	WC1	Wadsworth Canal - Butte House Rd.	39.1710	121.7165	00, 01	01,02
	WC2	Wadsworth Canal - Nuestro Rd. RD777 Lateral	39.1854	121.6950	00, 01	01,02
	WC3	Wadsworth Canal - Paseo Ave. RD777 Lateral	39.2498	121.6789	00, 01	01,02
	WC4	Wadsworth Canal - Eager/Larkin Rd. Lateral	39.1897	121.6620	00, 01	01,02
	WC5	Live Oak Slough - Clark Rd.	39.2331	121.6653	00, 01	01,02
	WC6	Wadsworth Canal - Franklin Rd.	39.1273	121.7566	00, 01	01,02

habitat conditions along the entire reach. Each of these physical habitat assessments has a low and high gradient version. Riffle/transect data collected included depth, velocity, and substrate composition. At each transect, these measurements were recorded as the mean of three measurements. Substrate composition was recorded as an observational estimate of percentages of mud (<0.2 cm), sand (<0.2 cm), gravel (0.2 to 5.0 cm), cobble (5.0 to 25.0 cm), boulder (>25.0 cm), and bedrock/hardpan (solid rock or clay forming a continuous surface). Substrate consolidation was determined to be either 'loose', 'moderate', or 'tight'.

Site habitat data included estimates of ten physical habitat parameters (epifaunal substrate, sediment deposition, channel sinuosity, riparian vegetative zone width, pool substrate, available cover, channel flow status, bank stability, pool variability, channel alteration, and vegetative protection). Each habitat parameter consists of 'poor', 'marginal', 'sub-optimal', and 'optimal' scoring categories. Each habitat parameter is scored using semi-qualitative criteria (Barbour et al., 1999). For analyses involving the combined high and low gradient datasets, the high gradient embeddedness, velocity/depth regime, and frequency of riffles scores were considered to be equivalent to the low gradient pool substrate, pool variability, and channel sinuosity measures.

Water quality parameters were recorded monthly at each site and at the times of BMI collection, except the following sites, where these measurements were taken only at the times of BMI collection: MD9, WC6, DC8, DC9, DC12, DC13, WW1, WW2, WW3. At the times of BMI collection, water quality measurements were recorded prior to collection of BMIs at the second riffle/transect (CDFG, 2003). Measurements included pH, specific conductance (SpC), dissolved oxygen (DO), and temperature. Turbidity, ammonia, hardness, and alkalinity measurements were performed on monthly water samples from each site, except AR5, AR6, and the sites listed above, where these measurements were not taken. Orthophosphate and nitrate-nitrogen nutrient analyses were conducted during fall 2001 using a HACH DR890 Colorimeter at all sites except those listed above.

Canopy cover was estimated with a hand held densiometer. At high gradient (slope > 0.2) sites, gradient was measured using a stadia rod and a clinometer. GPS coordinates were recorded at the second riffle/transect of all sites.

2.3 BMI sampling

BMI bioassessments were performed according to the California Stream Bioassessment Procedure (CSBP; Harrington, 1999). The CSBP is a California adaptation of the US EPA Rapid Bioassessment Protocol (Barbour et al., 1999) and focuses on richest stream habitat (generally riffles). Many of the streams, especially the ADWs, sampled were low gradient with no riffle habitat; substrate was primarily mud and fine grains. The non-riffle, low gradient (slope < 0.2) sites were sampled with a modified version of the CSBP (CDFG, 2003) for low gradient waterways.

Sampling riffle habitat consisted of identifying five riffles in a stream reach, then randomly selecting three of the five to sample. If only three riffles could be identified within the reach (<600 meters), those riffles were sampled. The distance between the first and last riffle determined reach length, and generally was less than 100 meters. Beginning at the most downstream area, sampling occurred across a transect in the top third of the riffle. Transects were chosen at random from all possible meter marks available in the upper third of the riffle. A 500 um mesh D-frame kick net was placed immediately downstream of the transect, and a 0.3 X 0.6 meter of substrate upstream of the net was disturbed. Disturbing the stream bottom included kicking, turning over and scrubbing of all large debris (cobble, wood chunks, gravel, leaves). A total of three, 0.3 X 0.6 meter areas, were sampled across each transect, composited into a sample container, and preserved with 95 percent ethanol. This process was repeated at the remaining two upstream riffles.

Sampling low gradient, fine substrate-dominated streams using a modified low gradient CSBP sampling adaptation required identification of 100 meter standardized reach lengths at each site. Three randomly-selected, meter mark transects were chosen for sampling within the 100 meter reach. Three, 0.3 X 0.6 meter, areas were sampled with

kick net across each transect as described above. The three collection areas usually were bank margins and the thalweg (deepest point of stream cross section). The three, 0.3 X 0.6 meter transect samples were composited. Gradient (percent slope) was determined as the change in elevation between upstream and downstream ends of a sampling reach.

2.4 Sub-sampling and taxonomy

In the laboratory, three hundred organisms were sub-sampled and removed from each transect composited sample for taxonomic identification, metric analyses, and abundance estimations. Sub-sampling consisted of: (1) transferring each sample to a 500 um sieve, gently rinsing to flush out fine particles, (2) removing large debris such as gravel, fresh leaves, and sticks after thoroughly inspecting for entangled BMIs, (3) submerging the sieve containing BMI's in a 2.5 liter container of water to homogenize the sample, (4) draining the sieve, and (5) inverting the sieve over a white tray with numbered grid lines. Samples were spread evenly over 5X5 cm grids so as to accommodate the entire sample volume. Grids to be examined by dissecting microscope were selected at random. BMIs were removed from grids and transferred to a vial containing 70% ethanol (EtOH) until a 300 count was achieved. The last grid examined to achieve the three hundred count was completely processed, with additional BMIs placed into an 'extras' vial. BMIs from the 'extra' vial are necessary for an accurate estimate of sample BMI abundance. Sample abundance was estimated as the total number of BMIs removed from a sample, divided by number of grids processed, multiplied by total number of grids covered by the sample.

Most BMIs were identified to genus level. However, midges (Chironomidae) were identified to tribe, worms (oligochaetes) to family, and clams (bivalves) as well as crayfish (Decapoda) to superfamily. Our criterion for this study: at least 285, but no more than 315 BMIs were to be recovered from each sample.

2.5 Laboratory and field performance evaluation

To assure that data generated were of high quality and credible, performance evaluation (quality assurance) measures were included in this study. Both internal (University of California, Davis Aquatic Toxicology Laboratory; UCD ATL) and external performance

evaluations on taxonomic identification were a component of this study. Internal evaluation consisted of re-identification by a second taxonomist of BMIs randomly selected 10 percent of all samples. External performance evaluation was performed by the CDFG Bioassessment Laboratory, Rancho Cordova, CA on 20 percent of all year one (fall 2000, spring 2001) samples, and by Bioassessment Services Inc. (Folsom, CA) on 10 percent of all study year two (fall and spring) samples. A total of 56 samples were evaluated. There were no major discrepancies between UCD ATL identifications and those of CDFG (misidentifications in only 4 percent of 1,018 taxa vials) or Bioassessment Services, Inc. (misidentification in only 1.7 percent of 177 taxa vials). These performance evaluations lend credibility to the taxonomic identification presented herein.

Prior to actual sampling, field crews engaged in trial runs to assure consistency of sampling efforts and habitat scoring. During actual sampling events habitat scoring was completed individually by two field crew members at 20 percent of the sites. These individual scorings were compared and, if necessary, adjusted to achieve agreement. There was 95 to 100 percent agreement in individual scoring of habitat parameters at all sites.

For sub-sampling, only UCD ATL internal performance evaluation was conducted. Ten percent of all samples were subjected to re-evaluation. Our sub-sampling criterion for this investigation was that no more than ten percent of the BMIs in a sample could be overlooked. No remnant samples exceeded the ten percent criterion; in a majority of samples less than five percent of BMIs were overlooked.

2.6 Statistical analyses

Multivariate and multimetric analyses were applied to investigate spatial and temporal variability in BMI communities. Relationships between community structure, a range of environmental variables describing habitat and water quality, and a number of widely used metrics indicative of BMI community integrity also were examined. Metrics of BMI community integrity used in this study are described in Table 3.

In many cases, we found it informative to analyze data from high gradient waterways separately from data from low gradient waterways (low gradient = slope < 0.2, high gradient = slope \geq 0.2). This decision was supported by a cluster analysis which clearly separated the waterways by gradient (see Fig. 7). Note that the most downstream sites in two of the high gradient waterways were actually low gradient (AR2 and BC1), but these sites were analyzed in the high gradient dataset because of their placement on the cluster dendrogram.

Where data conformed to assumptions of normality and homogeneity of variance, parametric statistics were performed, otherwise nonparametric equivalents were used. The significance of multiple simultaneous tests was evaluated after sequential Bonferroni correction, which adjusts the tests to be less likely to indicate a significant difference where one does not exist. The composition and range of BMI communities were probed by cluster analysis, indicator species analysis, and nonmetric multidimensional scaling (NMS) ordination. Analysis of variance (ANOVA) was applied to distinguish the environmental variables and BMI metrics associated with the site groups (based on taxa similarities) identified by cluster analysis. Principal Component Analyses (PCAs) and multivariate linear models were applied to identify environmental variables accounting for most of the variability in BMI metrics at both high and low gradient sites. In a complimentary analysis, we conducted a Multiple Analysis of Variance (MANOVA) and developed a linear model to examine the statistical significance of relationships between BMI metrics and environmental variables. Transect data points were treated as replicates of their reach in analyses examining within reach versus between reach variability. For analyses which did not consider within reach, within event variability, a single data point for each site was calculated by taking the average of the measurements taken at the three transects.

Table 3
Description of the metrics of BMI community integrity used in this study

Metric	Description
Taxonomic Richness	<ul style="list-style-type: none"> • Number of taxa present
Shannon Diversity Index	<ul style="list-style-type: none"> • Measure of taxonomic diversity considering the number of taxa present and the relative abundances of all taxa
EPT Taxa	<ul style="list-style-type: none"> • Number of taxa present of the insect orders Ephemeroptera, Plecoptera, and Trichoptera
EPT Index	<ul style="list-style-type: none"> • Percentage of BMIs belonging to the EPT orders
Sensitive EPT Index	<ul style="list-style-type: none"> • Percentage of BMIs which belong to the EPT orders and with pollution tolerance (Hilsenhoff) scores not exceeding four
ETO Taxa	<ul style="list-style-type: none"> • Number of taxa present of the insect orders Ephemeroptera, Plecoptera, and Odonata
ETO Index	<ul style="list-style-type: none"> • Percentage of BMIs belonging to the ETO orders
Ephemeroptera Taxa	<ul style="list-style-type: none"> • Number of mayfly taxa present
Plecoptera Taxa	<ul style="list-style-type: none"> • Number of stonefly taxa present
Trichoptera Taxa	<ul style="list-style-type: none"> • Number of caddisfly taxa present
Odonata Taxa	<ul style="list-style-type: none"> • Number of damselfly and dragonfly taxa present
Tolerance Value	<ul style="list-style-type: none"> • Average pollution tolerance (Hilsenhoff Biotic Index) of the BMIs present (Range: 1 – 10)
% Intolerant	<ul style="list-style-type: none"> • Percentage of BMIs with pollution tolerance (Hilsenhoff) scores not exceeding four
% Tolerant	<ul style="list-style-type: none"> • Percentage of BMIs with pollution tolerance scores exceeding five
% Dominant Taxon	<ul style="list-style-type: none"> • Percentage of BMIs belonging to the most abundant taxon
% Hydropsychidae	<ul style="list-style-type: none"> • Percentage of BMIs belonging to the somewhat pollution tolerant Hydropsychidae taxa (Trichoptera)
% Baetidae	<ul style="list-style-type: none"> • Percentage of BMIs belonging to the somewhat pollution tolerant Baetidae taxa (Ephemeroptera)
% Chironomidae	<ul style="list-style-type: none"> • Percentage of BMIs belonging to the pollution tolerant Chironomidae taxa (Diptera)
% Tanytarsini / % Chironomini	<ul style="list-style-type: none"> • The ratio of the proportional abundances of the chironomid tribes Tanytarsini (somewhat tolerant of low DO) and Chironomini (highly tolerant of low DO)
% Insects	<ul style="list-style-type: none"> • Percentage of BMIs belonging to insect taxa
% Oligochaeta	<ul style="list-style-type: none"> • Percentage of BMIs belonging to highly pollution tolerant worm taxa
% Collectors	<ul style="list-style-type: none"> • Percentage of BMIs in the collector feeding group
% Filterers	<ul style="list-style-type: none"> • Percentage of BMIs in the filterer feeding group
% Filterers + Collectors	<ul style="list-style-type: none"> • Percentage of BMIs in the collector or filterer feeding groups
% Grazers	<ul style="list-style-type: none"> • Percentage of BMIs in the grazer feeding group
% Predators	<ul style="list-style-type: none"> • Percentage of BMIs in the predator feeding group
% Shredders	<ul style="list-style-type: none"> • Percentage of BMIs in the shredder feeding group
% Semivoltine	<ul style="list-style-type: none"> • Percentage of BMIs taking longer than one year to reach reproductive maturity
% Multivoltine	<ul style="list-style-type: none"> • Percentage of BMIs having more than one complete generation every year

2.6.1 Cluster analysis and ordination

Taxa composition was probed using hierarchical cluster analysis, indicator species analysis, and ordination by NMS to reveal the strongest patterns in BMI community structure across sites. Hierarchical cluster analysis lumped sites into groups based on similarity in BMI communities. Indicator species analysis (Dufrière and Legendre, 1997) revealed the taxa characteristic of each cluster of sites. NMS ordination created axes that summarize BMI assemblages based on the proportions of taxa at the sites. Correlations of environmental variables and BMI metrics indicative of community integrity with these axes indicated the strength and direction of associations. NMS was selected in preference to canonical correspondence analysis (CCA) because in CCA the pattern of biological samples is constrained by the environmental variables included in the analysis. With NMS, measured environmental variables do not bias the ordination of the biological data. This gives a more accurate picture of the overall community structure in the dataset.

Because there may be seasonal variation in diversity and abundance of BMI taxa, separate analyses were performed for fall and spring sampling events. The fall data subset included samples collected during 2000 and 2001, while the spring data subset included samples taken in 2001 and 2002. For each season, the proportional abundance of each taxon was averaged at each site across years. Only sites sampled during both sampling years were included in this analysis.

Proportional abundance ($\# \text{ taxon individuals} / \text{total } \# \text{ individuals collected}$) of taxa was used in all statistical analyses, as opposed to estimated absolute abundance, because the CSBP sampling and sample processing method is not designed to determine actual abundances at a site. The proportional abundance data were arcsine-square root transformed to moderate the influence of common and rare taxa. Taxa occurring only at one site (rare taxa) were excluded from statistical analyses to improve resolution of commonalities among sites.

Cluster analysis and ordination rely on calculation of a distance measure to quantify taxa composition similarities among sites. Sorenson distance, which has been shown to be a

more accurate representation of community structure than Euclidean distance, was used as a measure of overall site similarity (McCune and Grace, 2002). Cluster analyses and ordinations were performed using PC-ORD 4.0 (McCune and Mefford, 1999). Cluster analyses were performed using flexible beta linkage ($\beta = -0.25$) because the model is compatible with the Sorenson distance measure. Also, with $\beta = -0.25$, results are consistent with Ward's method (McCune and Grace, 2002). Ward's method accurately represents similarities in community structure and cluster discreteness.

A hierarchical cluster analysis based on similarity of site taxa composition was performed. This analysis connected the sites through a tree-like dendrogram, with more closely connected branches indicating sites with similar taxa proportionalities. At each branching of the dendrogram, indicator species analysis was applied to identify taxa associated with site clusters and to evaluate efficacy of the cluster analysis for differentiating sites representing discrete communities. Indicator values were computed for all taxa based on their relative abundance and frequency of occurrence in each cluster. The significance of indicator values was determined by Monte Carlo tests, randomizing sampling units 1000 times among the clusters.

Dendrograms based on site taxa composition were employed to identify suites of environmental variables and metrics associated with the various BMI assemblage clusters. For every split in a dendrogram, a MANOVA was conducted to evaluate statistical significance between the two branches in regards to environmental variables and BMI metrics. The MANOVA took into account all comparisons made at a single bifurcation when determining the significance of associations, but alpha levels were not Bonferroni corrected to take into account the multiple bifurcations in the dendrogram examined by this analysis. Therefore, those associations presented with $P < 0.05$ should be considered as fairly strong associations between metrics or environmental variables and clusters of sites, but these associations should not be considered to be statistically significant in the strictest sense. We use these results only for descriptive purposes, and not for hypothesis testing.

NMS ordination was used to graphically display site-to-site variation in BMI assemblages. NMS ordination transformed measurements of taxa proportional abundances at each site into coordinates in two or three dimensions that summarize taxa composition of each site relative to all other sites. Sites with similar taxa composition are given similar coordinates and appear in close proximity in the ordinations. NMS is appropriate for analysis of communities by taxa proportional abundance because it summarizes associations among abundances of a large number of taxa, many that occur only in a subset of sites (McCune and Grace, 2002). As with the method selected for cluster analysis, NMS is distance-preserving, maintaining the rank-order of similarity values among sites in ordination space. NMS is an iterative optimization method that improves the fit of the ordination to the original set of similarity measurements through a series of small steps until a stable, well-fitting, solution is obtained (Clarke, 1993).

NMS was performed with random starting coordinates and a step length of 0.20. Forty starting configurations were used, and for each starting configuration, solutions were computed with dimensionalities ranging from two to six. The lowest stress solution for each dimensionality (in which the distances in the ordination space most resemble distances in the original distance matrix) was compared to the lowest stress solution for the other dimensionalities by visual inspection of a scree plot (McCune and Grace, 2002). The solution accepted was the highest dimensionality solution with a final stress more than 5 units lower than the next lower dimension, provided that the solution had a stress lower than 95 percent of 50 solutions at that dimensionality with randomized data.

Ordinations were performed separately for fall and spring datasets. Each dataset was then separated into high and low gradient subsets by the first split of the cluster analysis dendrogram, and separate ordinations were performed for high and low gradient sites. These general and gradient-specific ordinations were utilized to examine associations among taxa composition and environmental variables, complementing the analysis of site clusters and environmental variables.

2.6.2 Relationship of BMI metrics to environmental variables

A Multiple Analysis of Variance (MANOVA) was administered to discriminate the environmental factors correlated with BMI metric variations. MANOVA is a multivariate linear model that appraises multiple response variables simultaneously. BMI metrics were considered response variables, whereas the environmental variables were potential predictor variables. Environmental variables included physical habitat, substrate, water quality, hydrographic, and land use variables, as well as season and project year. The MANOVA identified environmental variables that correlated with any of the BMI metrics. A backwards elimination method (exit if $P > 0.05$) was exercised to select the final subset of environmental variables included in the model. MANOVAs were performed on low and high gradient site data separately to probe for fundamental differences between these two site groups in relation to environmental variables.

2.6.3 Principle components analyses

Principal Components Analyses (PCAs) were performed on BMI metrics and on environmental variables for all sites and samplings, and for high and low gradient subsets of the data. Principle components analysis allows the examination of many variables at once by constructing a number of axes (principle components) that focus the variation in the full set of variables into a few major components. Data points (e.g. sites) represented in close proximity on the principle component axes have similar values in the original variables. The strengths and directions of associations between the original variables and the principle components are indicated by loadings of each variable onto each principle component. Loading scores range from -1 to 1, with extremely positive or negative loadings indicating a stronger influence of a variable on a principle component. The first principle component is the axis that summarizes the greatest part of the variation in the dataset, and each axis thereafter summarizes a smaller portion of variation. After performing the PCAs of the environmental parameters, pairwise correlations (Pearson product-moment correlations) between the BMI metrics and the first three PC axes were used to evaluate environmental variables important to BMI community integrity.

PCA differs from NMS ordination in that it requires that all variables conform to normal distributions, and output includes loadings of each original variable onto each Principle Component axis. While taxa abundance data do not conform to the assumption of normality, many other types of ecological data may, including water quality, habitat, and land use data, as well as metrics indicative of BMI community integrity. The normality of all variables included in the principle components analyses was tested using Shapiro-Wilks tests. Variables which deviated significantly from normality were $\log(x + 1)$ transformed and tested for normality again. Those variables that deviated grossly from normality after log transformation ($W < 0.70$ in a Shapiro-Wilks test) were excluded from principle components analyses.

2.6.4 Relationship between BMI metrics PC1 and environmental variables

A multivariate linear model was performed to identify the environmental variables that best predicted site scores on principle component 1 (PC1) derived from the PCA of BMI metrics. BMI metrics loading onto PC1 account for the majority of BMI metric variability. The backwards elimination method (exit if $P > 0.05$) was employed to select the final subset of environmental variables incorporated into the model. This method highlighted the subset of environmental variables that were most highly correlated with the combination of BMI metrics represented by PC1. Multivariate linear models on PC1 were performed on low and high gradient sites separately to discriminate fundamental differences between the two groups with regard to environmental variables.

The MANOVA, PCA and multivariate linear analyses were conducted according to SAS (SAS Institute Inc., Cary, North Carolina). The statistical approaches applied in these analyses were substantiated by Mitch Watnik at the UCD Statistics Laboratory. Two environmental variables, percent urban land use and percent native vegetation, were excluded from the analysis of high gradient sites. The three land use variables, Agriculture, Urban and Native are interrelated (they sum to 100%) and are only applicable at the watershed level. Since only three watersheds were represented in the high gradient stream data, including all three land use variables would have been redundant, impacting the performance of the model. A single land use variable, 'land

use', which is based on percent agricultural land, was included in the high gradient stream analyses.

2.6.5 BMI community integrity rankings (biotic index [BI] construction)

In watersheds where a large scale sampling effort (200 + sites) is devoted to characterizing the BMI fauna, the least impacted ('reference') sites can be identified, and the fauna metrics can be used to formulate an index of biological integrity (IBI). Such indices have been utilized to classify sites as 'unimpacted', 'marginal', or 'impacted' (Barbour et al., 1999; EPA, 2002). In the Central Valley, 'reference' BMI metrics have not been described and the sampling effort in this study was not large enough to construct an IBI. However, a BMI community integrity relative ranking system was constructed. Unlike an IBI, these Biotic Indices (BIs) are not applicable to sites outside of the current dataset. Nonetheless, this ranking system was useful for quantifying the range of BMI community integrity at sites included in this investigation.

To construct this ranking system, we developed a set of metrics indicative of BMI community integrity in the investigated waterways. Statements regarding degree of community integrity are relative to data collected in this project, but based upon diversity of taxa intolerant to anthropogenic stressors (related to inherent potential being realized). Initially, a group of metrics commonly incorporated into benthic macroinvertebrate IBIs was evaluated; these were supplemented with metrics likely to be informative in low gradient systems with various degrees of degradation. Supplemental metrics included ETO (Ephemeroptera, Trichoptera, and Odonata) taxa, an ETO index, percent insects, the ratio of percent Tanytarsini / percent Chironomini, and percent multivoltine taxa (taxa with short life cycles and multiple cohorts per year) (See Table 3). It is acknowledged that the voltinism data is very general at best. Very little voltinism data on California taxa has been published. Variability at the genus and species level is unknown and can be high. This would be especially true for taxa identified to a higher category, such as the Chironomidae, which were identified to tribe. Voltinism metrics were used based on the principles that less disturbed sites should contain a mixture of longer lived univoltine taxa, possibly some semivoltine (or longer lived) taxa and some multivoltine taxa.

Whereas frequently disturbed sites should mostly consist of multivoltine taxa, which can quickly recolonize disrupted areas after any disturbance has passed.

The BIs were constructed through a multistep process of metric exclusion. The metrics remaining at the termination of the exclusionary process were merged into a measure of BMI community integrity, a BI. Separate BIs were constructed for high gradient and low gradient sites. For each gradient-specific subset of sites three ranking systems (BIs) were constructed: one with combined fall and spring data, one with only fall data, and one with only spring data. This approach allowed evaluation of seasonal variation in (1) relative community integrity and of (2) reliable metrics most indicative of biotic integrity.

Shapiro-Wilks tests were used to examine the normality of distributions of all metrics. Metrics with distributions deviating significantly from normality were $\log(x + 1)$ transformed. Those deviating grossly from normality after log transformation (Shapiro-Wilks test, $W < 0.70$) were excluded from the BI.

Signal/noise ratios are good indicators of the sensitivity of metrics to BMI community differences between sites. Metrics were screened to ensure that the signal/noise ratio for each metric (between-site variation/within-site variation) exceeded 3:1. Screening was accomplished by one-way ANOVAs with site as the independent variable for each metric. F-ratios resulting from ANOVAs represented between site/within site metric variation and were considered the signal/noise ratios. Metrics with signal/noise ratios of less than 3 were excluded.

Final selection of metrics included in the site ranking index (BI) depended on metric redundancy and correlations with specific environmental variables. Screening for redundancy involved pair-wise correlations among all remaining metrics. When two metrics were correlated with a Pearson correlation coefficient of > 0.70 , only one of the metrics was incorporated into the final BI. Correlations of metrics determined to be reflective of BMI community integrity with environmental variables likely to modulate these metrics were computed. The environmental variables were: lowest DO

measurement recorded over a three month period, highest specific conductivity value observed over a three month period, epifaunal substrate observation taken at the time of sample collection, percent fine substrate estimated at the time of sample collection, and percent gravel estimated at the time of sample collection. When two metrics were redundant, the metric with the higher signal/noise ratio was included. In cases of equivalent signal/noise ratios, the metric with stronger correlations with the environmental variables was selected.

Scores on the metrics chosen for a BI were combined into the overall BI score as follows. For each metric in each transect, a metric rank score was calculated by subtracting the mean metric score from the metric score of that transect, and dividing the result by the standard error of that metric. This standardized the metrics with one another by giving them all the same mean (zero) and the same variability, which caused the metrics to be weighted equally when combined into the BI. The BI score of each transect was calculated by adding together the metric rank scores of all metrics thought to indicate high BMI community integrity, and subtracting the metric rank scores of all metrics thought to indicate low BMI community integrity. The BI score of a site for a given season (fall or spring) was calculated as the mean of the BI scores of the transects examined in any year during that season.

3. Results

Assessments of BMI communities and habitat conditions in ADWs and EDWs in the lower Sacramento River watershed revealed a wide range of habitat conditions and BMI assemblages. The most severe biological impacts observed in EDWs occurred around urban areas while the most compromised BMI community integrity in ADWs was observed at upstream sites. In this report biological impacts and degraded/compromised community integrity are used interchangeably; however neither are absolute measurements but rather relative to the BMI data gathered for this project. In both ADWs and EDWs, BMI metrics indicative of community integrity were correlated with

gradient, substrate, other habitat factors, land use, and water quality. Common and scientific names of taxa identified in this study are summarized in Appendix A.

3.1 Habitat conditions

Habitat conditions at low gradient ADW sites were poor to marginal (Fig. 4). Little to no riparian vegetative zones, lack of channel sinuosity, decreased bank stability, and a high level of physical channel alteration characterized ADWs. Habitat conditions and scores at Pleasant Grove Creek, a low gradient waterway with little agricultural influence, were higher than at low gradient ADW sites. Habitat conditions at high gradient sites were sub-optimal to optimal (Fig. 5). Riparian zones of three to six meters, moderate channel sinuosity, increased pool variability, and increased stream cover characterized these systems. Substrates at low gradient sites were heavily dominated by mud (Fig. 6A). Substrates at high gradient sites were more evenly comprised of gravel, sand, and cobble (Fig. 6B). Substrate composition at sites was consistent over seasons and years (Table 4).

3.2 Water quality

Water quality parameters are potential stressors on BMI communities. Mean values and within site ranges of all water quality parameters for low gradient and high gradient sites are presented in Table 5. The within site range is the difference between the minimum and maximum values of a water quality parameter measured at a given site. Mean within site range for a parameter represents the average within site variability of that parameter at sites of a given gradient. All water quality variables except temperature show wider ranges at low gradient (primarily agriculturally-dominated) sites than at high gradient sites. Minimum DOs were much lower at low gradient sites than at high gradient sites. It was not uncommon to see DO concentrations in the 2 to 3 mg/L range at ADW sites during late fall. Extremely high concentrations of ammonia were found occasionally at low gradient sites, but never at high gradient sites.

Water quality was compared between sites upstream and downstream of major municipal wastewater treatment facilities (WWTF) discharge points. The facilities monitored

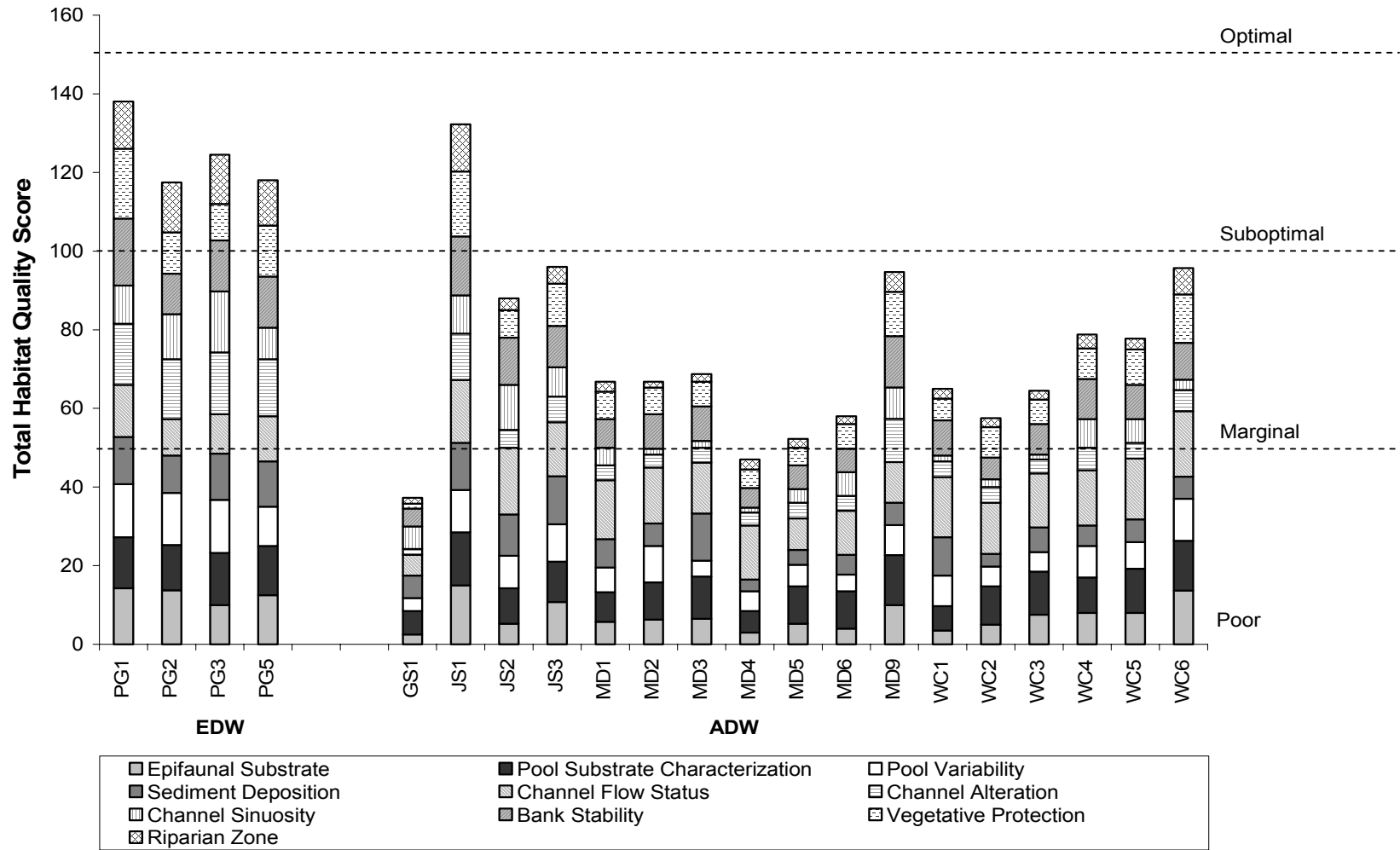


Fig. 4. Physical habitat scores of low gradient sites in the lower Sacramento River watershed. EDW: effluent-dominated waterways; ADW: agriculture-dominated waterways. Scores are an average of four samples taken over a two-year period: Two fall and two spring samples. See Table 2 for site codes and locations.

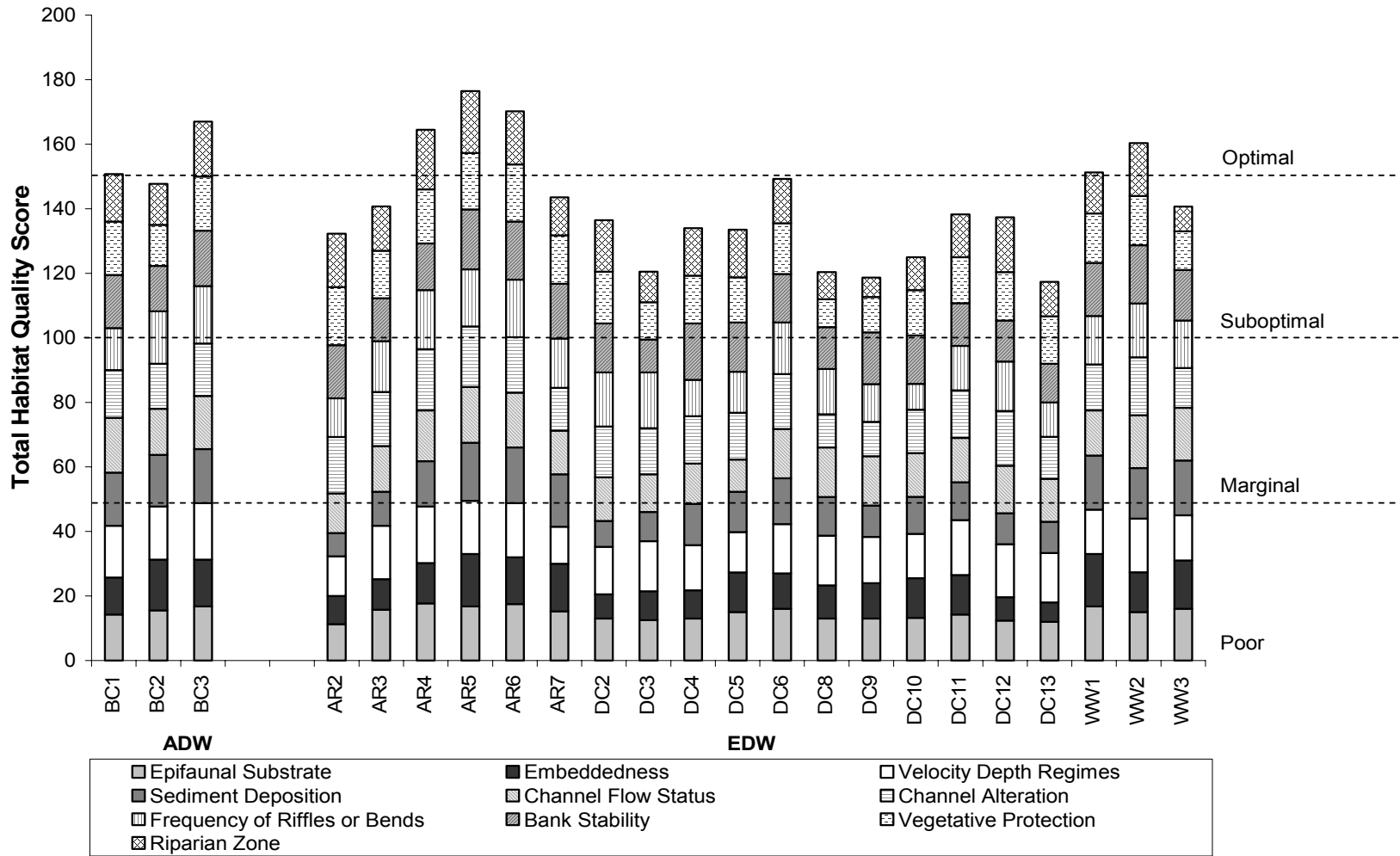
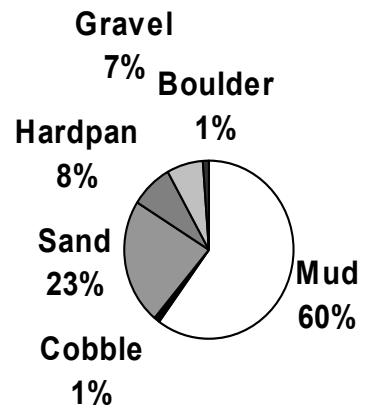


Fig. 5. Physical habitat scores of high gradient sites in the lower Sacramento River watershed. EDW: effluent-dominated waterways; ADW: agriculture-dominated waterways. Scores are an average of four samples taken over a two-year period: Two fall and two spring samples. See Table 2 for site codes and locations.

A **Low Gradient**



B **High Gradient**

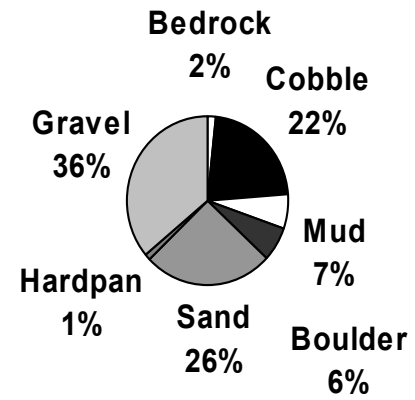


Fig. 6. Substrate composition of (A) low-gradient and (B) high-gradient sites.

Table 4

Means and standard deviations of percent fine substrates (mud and sand) and total physical habitat score by site

	Site Code	% Fine Substrates		Habitat Scores	
		Mean	SD	Mean	SD
Low Gradient Sites	PG1	83.3	13.6	138.0	16.8
	PG2	70.8	21.3	117.5	21.0
	PG3	88.8	15.4	124.5	7.3
	PG5	90.4	13.9	118.0	16.8
	GS1	97.5	5.0	37.3	8.7
	JS1	53.3	16.6	132.3	9.0
	JS2	58.9	10.7	88.0	9.1
	JS3	65.8	20.7	96.0	10.0
	MD1	60.8	13.7	66.8	14.7
	MD2	81.9	2.3	66.8	8.7
	MD3	64.8	14.8	68.8	11.9
	MD4	98.3	2.4	47.0	9.4
	MD5	96.3	4.4	52.3	7.9
	MD6	95.4	6.3	58.0	6.7
	MD9	87.2	16.7	94.7	17.7
	WC1	41.1	17.2	65.0	5.7
	WC2	99.5	1.0	57.5	7.0
	WC3	92.9	8.4	64.5	6.6
	WC4	75.0	24.2	78.8	11.5
	WC5	94.6	10.8	77.8	20.5
WC6	100.0	0	95.7	6.8	
High Gradient Sites	BC1	33.8	29.3	147.0	11.5
	BC2	19.0	8.9	147.8	10.4
	BC3	15.0	6.2	167.0	5.5
	AR2	56.3	30.8	132.3	19.1
	AR3	31.3	9.8	140.8	11.1
	AR4	16.6	6.7	164.5	12.4
	AR5	7.0	5.9	176.5	8.1
	AR6	12.9	4.6	170.3	9.1
	AR7	17.9	7.5	143.5	16.1
	DC2	52.1	10.3	136.3	7.8
	DC3	35.6	12.7	120.5	7.7
	DC4	52.3	12.0	134.0	11.3
	DC5	50.8	5.7	133.5	18.0
	DC6	15.8	11.4	149.3	5.1
	DC8	18.3	7.3	120.3	10.7
	DC9	27.2	9.6	118.7	3.1
	DC10	18.0	6.9	125.0	16.8
	DC11	31.7	9.5	138.3	7.7
	DC12	49.6	17.9	132.7	5.8
	DC13	46.1	9.2	117.3	5.8
WW1	16.7	4.4	151.3	10.7	
WW2	21.1	3.5	160.3	3.2	
WW3	15.6	4.2	140.7	3.5	

Table 5

Ranges, mean values (+/- standard deviation), and mean within-site ranges of water-quality variables at low gradient and high gradient sites in the lower Sacramento River watershed

	Low Gradient				High Gradient			
	Range	Mean	SD	Mean Within-Site Range	Range	Mean	SD	Mean Within-Site Range
Alkalinity (mg/L of CaCO ₃)	20 – 332	126	72	162	20 – 175	63	23	59
Ammonia (mg/L)	0.0 – 30.0	0.6	3.0	2.2	0.0 – 0.5	0.02	0.07	0.15
DO (mg/L)	0.7 – 19.0	7.8	2.8	9.3	5.3 – 14.0	9.5	1.7	5.4
Hardness (mg/L of CaCO ₃)	16 – 480	135	89	249	12 – 408	68	41	107
pH (pH units)	5.1 – 9.9	7.6	0.7	2.5	5.8 – 9.1	7.6	0.6	2.1
SpC (µS/cm)	48 – 991	295	185	408	47 – 465	165	79	155
Temperature (°C)	4.6 – 29.0	15.8	5.9	17.9	4.8 – 29.2	15.6	6.0	18.7
Turbidity (NTU)	0 – 97	15	18	42	0 – 32	5	6	18

included the City of Roseville WWTF, City of Auburn WWTF, Sewer Maintenance District (SMD) 1 WWTF Placer County, and SMD 3 WWTF Placer County. SpC measurements were significantly higher at downstream sites (paired t-test, $t_{12} = -4.07$, $P = 0.0016$), while temperature was not significantly different between upstream and downstream sites (paired t-test, $t_{12} = 1.24$, NS).

Nutrient data were collected during the fall of 2001. Reactive phosphate concentrations were above acceptable analytical test range (5.0 mg/L) at Dry Creek EDW sites below the City of Roseville WWTF and at one ADW site in a small lateral to Wadsworth Canal (WC3). Nitrate-nitrogen also was elevated at sites in Dry Creek below the City of Roseville WWTF (DC2). However, most concentrations of reactive phosphate and nitrate-nitrogen were between 0.3 to 0.6 mg/L in both types of waterways. All water quality and nutrient data are summarized in Appendix B.

Aseasonal relatively rapid changes in flow or depth can be stressful to BMI communities. Several water quantity parameters varied considerably over the course of this study, including flow velocity and wetted channel width. All sites were visited monthly throughout the duration of the study. During these visits one site (WC4) was found to be dry on one occasion (Dec 2001).

In summary, low gradient sites tended to have wider fluctuations in water quality parameters than high gradient sites. At low gradient sites, SpC, hardness, alkalinity, and turbidity were generally elevated compared to high gradient sites, and low gradient sites were subject to extremely low levels of DO and high concentrations of ammonia. Upstream/downstream comparisons showed that SpC tended to be increased at sites downstream of WWTFs.

3.3 BMI community composition

BMI communities in low gradient sites were comprised primarily of tolerant taxa (high Hilsenhoff tolerance values). Tubificidae and Naididae, both oligochaetes (segmented worms), were the dominant taxa while Chironomidae (Diptera) were third most dominant

in low gradient sites. High gradient sites also were dominated by moderately tolerant taxa (Table 6). The moderately tolerant taxa in the high gradient sites were mostly baetid mayflies, chironomid midges and hydroptychid caddisflies.

Table 6
Most common taxa in low gradient and high gradient waterways

Abund. Rank	Taxa	
	Low Gradient	High Gradient
1	Tubificidae	<i>Baetis</i>
2	Naididae	Orthoclaadiinae
3	Tanytarsini	Tanytarsini
4	Chironomini	<i>Hydropsyche</i>
5	Cyprididae	<i>Simulium</i>
6	Orthoclaadiinae	Naididae
7	<i>Crangonyx</i>	<i>Tricorythodes</i>
8	Nematoda	Chironomini
9	Corbiculacea	Corbiculacea
10	Tanypodinae	Planariidae

A total of 168 and 161 distinct taxa were identified in the EDWs and ADWs of the lower Sacramento River watershed, respectively. A third (56 of 161) of the total distinct taxa in ADWs were in Butte Creek, the only high gradient ADW examined. Chironomids were common, occurring at every site. At high gradient sites, chironomids comprised from 2 to 68% of invertebrates collected. At low gradient sites, chironomids comprised from 2 to 71% of invertebrates. Oligochaetes (segmented worms, 1 to 88% of taxa) and amphipods (primarily *Crangonyx* and *Hyalella*, 0 to 82%) were common at low gradient sites. Fewer oligochaetes and amphipods (oligochaetes: 1 to 38%; amphipods: 0 to 32%) were collected at high gradient sites. Stoneflies sensitive to environmental degradation were rare, found only at some sites in Auburn Ravine and upper sites on Butte Creek. Sensitive Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa were absent in ADWs except at a high gradient upstream headwater site (BC3) less influenced by agricultural irrigation return flow.

3.4 Cluster analyses

Cluster analyses were applied to divide sites into groups with similar BMI communities. These analyses served to render very complex data into a more comprehensible form and to facilitate the exploration of community integrity and identification of environmental variables that potentially determine community structure and integrity. Sites were clustered by taxonomic similarity rather than similarity in BMI metrics scores because we sought to characterize the range of BMI communities at selected sites; clusters based on BMI metrics could result in dissimilar communities appearing identical if they scored similarly on BMI metrics.

Analysis of both fall and spring data revealed a fundamental split in taxonomic composition between sites in high gradient streams (Auburn Ravine, Dry Creek, and Butte Creek) and sites in low gradient streams (Pleasant Grove, Jack Slough, Wadsworth Canal, Main Drain, and Gilsizer Slough) (Figs. 7 and 8, respectively). The most downstream site in Auburn Ravine (AR2) was relatively low gradient, yet this site was taxonomically similar to the high gradient sites in that waterway.

Analysis of data collected in fall revealed site clusters that followed watersheds. The three high gradient site clusters were related to Auburn Ravine (HG Native 1), Dry Creek (HG Urban), and Butte Creek (HG Native 2) waterways, while the three low gradient site clusters were identifiable as Pleasant Grove / Gilsizer Slough (LG Ag/Urban), Jack Slough (LG Ag 1), and Wadsworth Canal / Main Drain (LG Ag 2). Site clusters of the spring analysis did not follow watershed divisions.

3.5 Associations of site taxa clusters with indicator taxa, environmental variables, and BMI metrics

Cluster dendrograms corresponding to fall and spring analyses depict significant indicator taxa for each site cluster group along with environmental variables and metrics significantly associated with each bifurcation in the dendrogram (MANOVA, $P < 0.05$) (Figs. 9 and 10, respectively). Indicator taxa are listed from most to least abundant. Taxa identified as indicators for a particular cluster occurred in high abundances and at a large

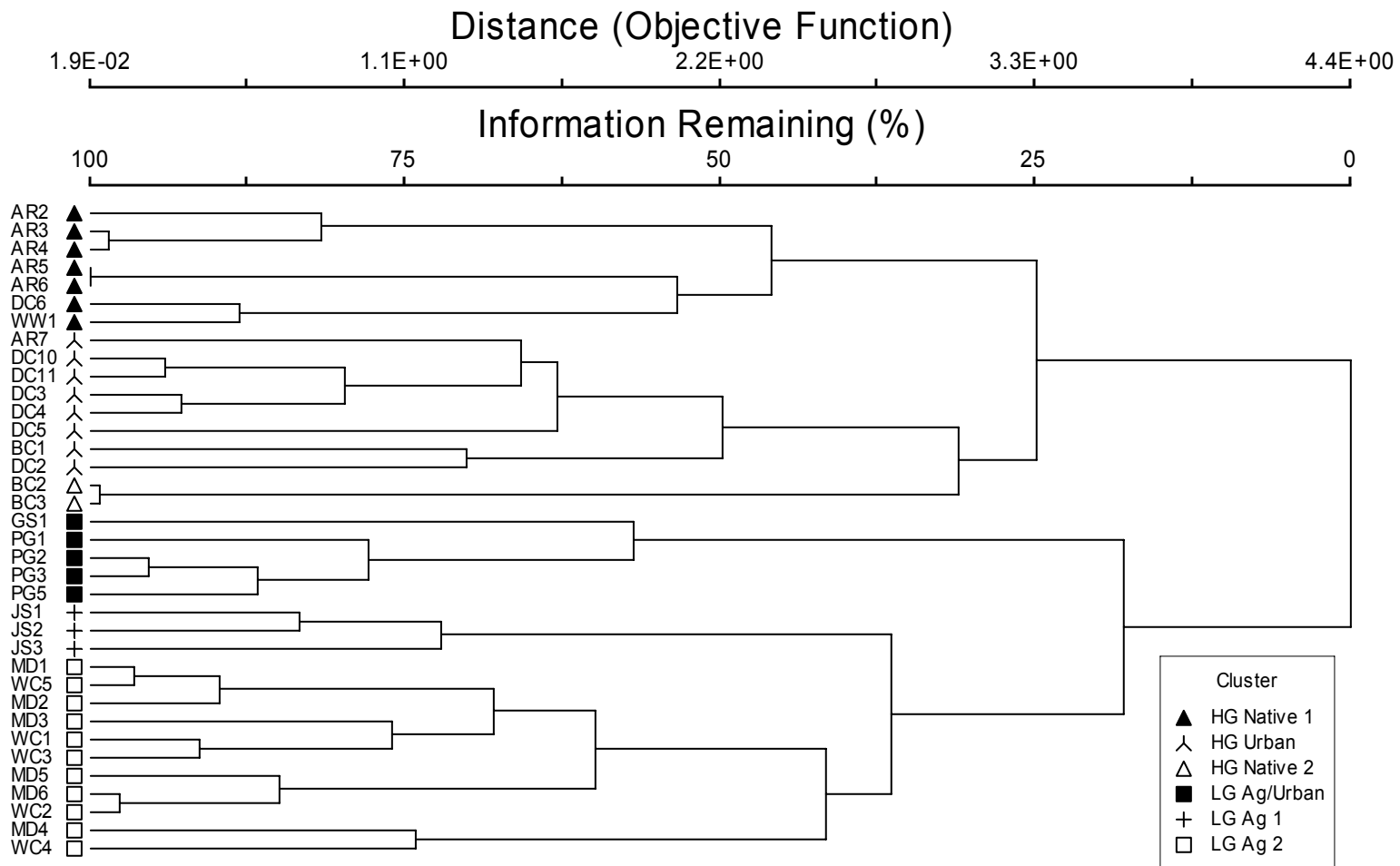


Fig. 7. Cluster analysis dendrogram of fall samples based on taxonomic similarity.

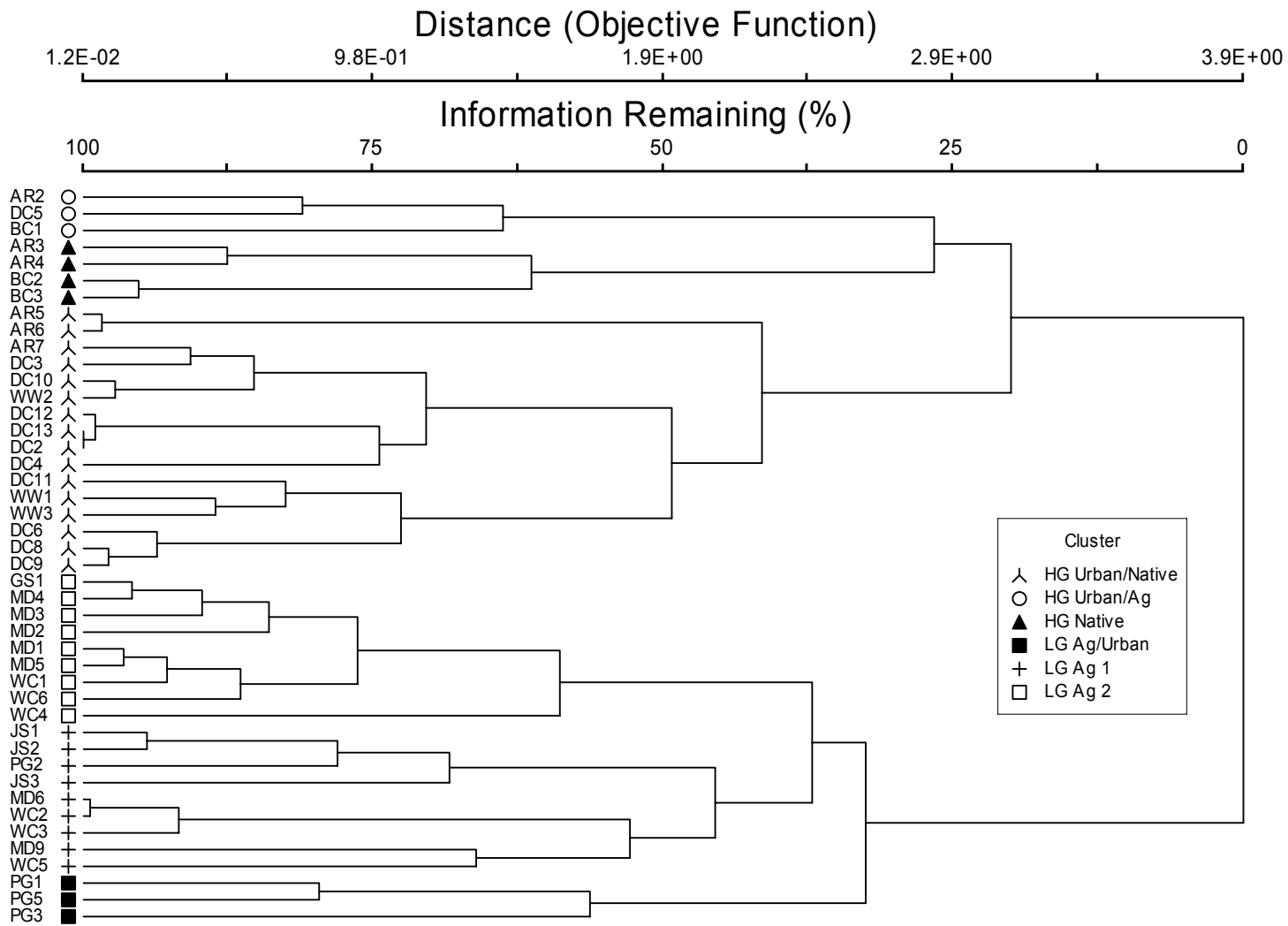


Fig. 8. Cluster analysis dendrogram of spring samples based on taxonomic similarity.

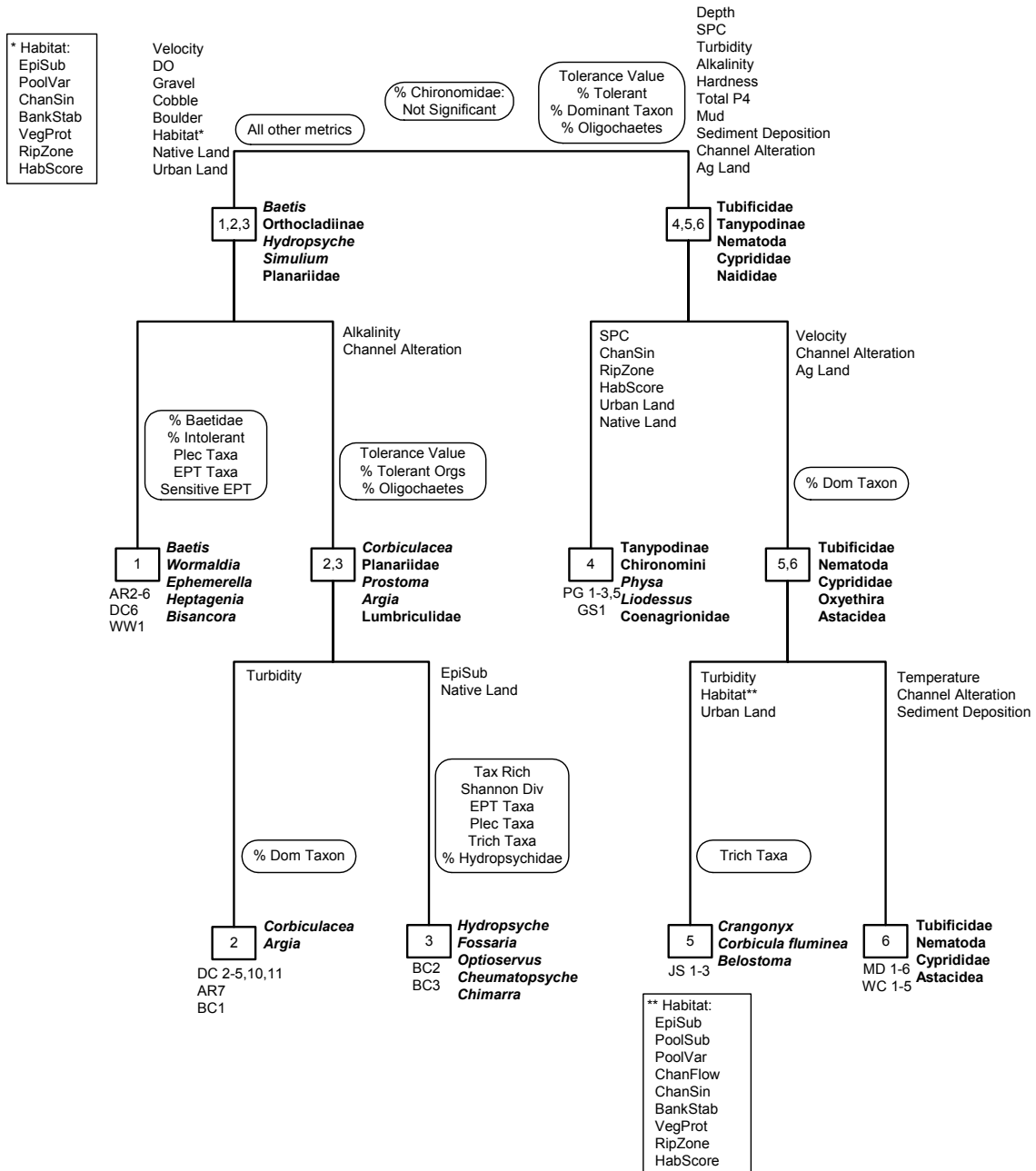


Fig. 9. Site cluster dendrogram based on taxonomic similarity of fall samples. Depicted are the five most common indicator taxa of each cluster, as well as environmental variables and BMI metrics significantly different between branches of the dendrogram (MANOVA, $P < 0.05$). The initial split in the dendrogram is between low- and high-gradient sites. Site clusters are identified as: 1: high gradient native 1; 2: high gradient urban; 3: high gradient native 2; 4: low gradient agricultural/urban; 5: low gradient agricultural 1; 6: low gradient agricultural 2.

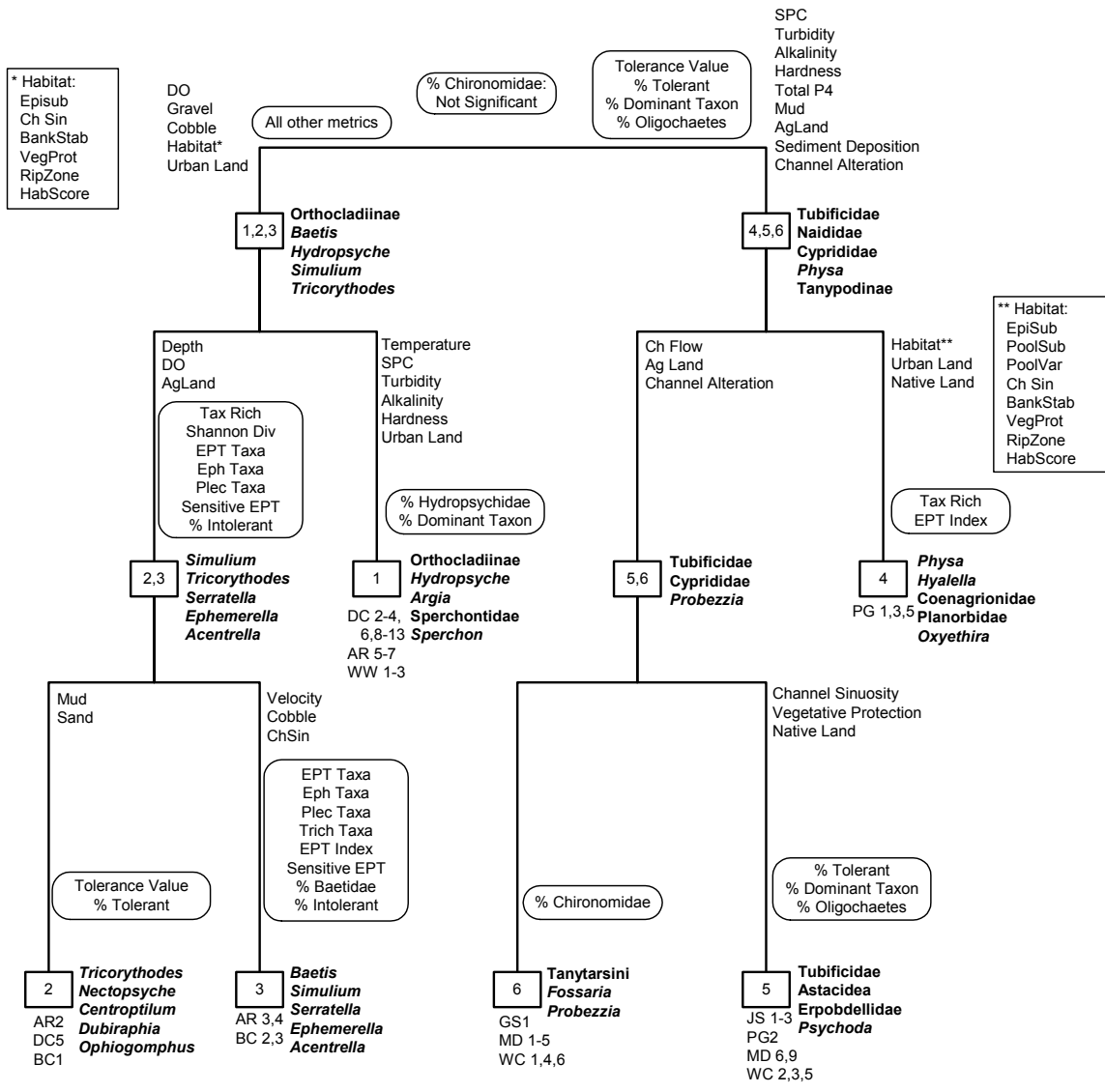


Fig. 10. Site cluster dendrogram based on taxonomic similarity of spring samples. Depicted are the five most common indicator taxa of each cluster, as well as environmental variables and BMI metrics significantly different between branches of the dendrogram (MANOVA, $p < 0.05$). The initial split in the dendrogram is between low- and high-gradient sites. Site clusters are identified as follows: 1: high gradient urban; 2: high gradient urban/agricultural; 3: high gradient native; 4: low gradient agricultural/urban; 5: low gradient agricultural 1; 6: low gradient agricultural 2.

number of sites in that cluster, but were relatively rare at sites outside of the cluster. The spring analysis is outlined in Fig. 10, and patterns seen were broadly similar to those seen in the fall.

Overall, BMI communities were more robust at high gradient sites than at low gradient sites. Metrics indicative of BMI community integrity (e.g. Taxa Richness, EPT Taxa, and Percent Intolerant Organisms) and environmental variables suggestive of relatively unimpacted conditions were significantly associated with high gradient site clusters. Indicator taxa of high gradient sites were primarily insects, with a dominant fraction of EPT taxa (Fig. 9). In contrast, indicator taxa of low gradient site clusters were primarily oligochaetes and chironomids, which tend to be associated with impacted waterways. Environmental variables associated with low gradient sites indicated possible impacts of sedimentation, nutrient loading, heightened conductivity, and channel alteration. Metrics associated with these sites (e.g. Tolerance Value and Percent Dominant Taxon) indicated more tolerant BMI communities.

3.5.1 Taxonomic and environmental differences among high gradient sites

Within the high gradient site dataset, robust associations exist between BMI metrics, environmental variables, and individual high gradient site clusters. This suggests that high gradient site clusters differ in degree of BMI community integrity. In contrast, associations of BMI metrics and measured environmental variables with low gradient site clusters were less distinct. Moreover, the low gradient site cluster was less divergent in terms of BMI community integrity.

Indicator species of the HG Native 1 cluster (constituted predominantly of Auburn Ravine sites) were entirely EPT taxa, including one trichopteran (*Wormaldia*), three ephemeropterans (*Baetis*, *Ephemerella*, and *Heptagenia*), and one plecopteran (*Bisancora*). EPT diversity metrics were significantly higher at sites in the HG Native 1 cluster than at other high gradient sites. These sites were characterized by significantly less alkaline waters and less drastically altered channels than other high gradient sites. The HG Urban cluster was composed primarily of Dry Creek sites and was characterized

by an abundance of clams (Corbiculacea) and damselflies (*Argia*). The HG Native 2 cluster, comprised of two upstream Butte Creek sites, was characterized by three trichopterans (*Hydropsyche*, *Cheumatopsyche*, and *Chimarra*), a snail (*Fossaria*) and a beetle (*Optioservus*). Water at the HG Urban cluster sites was significantly more turbid than at the HG Native 2 sites. HG Native 2 sites also were characterized by higher quality epifaunal substrates and a higher proportion of land devoted to native vegetation. The fraction of the BMI community comprised by the most dominant taxon was higher in the HG Urban cluster than in HG Native 2, while metric values were higher at HG Native 2 sites, indicating a diverse BMI fauna and a predominance of EPT Taxa.

These results show that the communities in high gradient waters ranged from being dominated completely by EPT Taxa (HG Native 1) to urban sites where EPT has been replaced by clams and damselflies. Differences in invertebrate communities among high gradient sites were correlated with differences in alkalinity, turbidity, and channel alteration.

3.5.2 Taxonomic and environmental differences among low gradient sites

The LG Ag/Urban cluster was composed of Pleasant Grove sites and the site on Gilsizer Slough. Sites in this cluster were dominated by Chironomidae (Tanypodinae and Chironomini), snails (*Physa*), beetles (*Liodessus*), and damselflies (Coenagrionidae). Waters at the LG Ag/Urban cluster sites were more stagnant (lentic) than at other low gradient sites and had higher SpC. The Pleasant Grove sites were characterized by more sinuous channels and less impacted instream habitats than other low gradient sites. The LG Ag 1 cluster, consisting of the sites on Jack Slough, was characterized by numerous amphipods (*Crangonyx*), clams (*Corbicula fluminea*), and water bugs (*Belostoma*). LG Ag 1 sites were associated with a higher diversity of Trichoptera, more turbid waters, less impacted habitat conditions, and more surrounding urbanized land than LG Ag 2 sites. LG Ag 2 sites (Main Drain and Wadsworth Canal) were dominated by oligochaetes (Tubificidae), nematodes, ostracods (Cyprididae) and decapod crustaceans (Astacidae).

These results show that communities in low gradient waters range from those with many chironomids and other insects to those where insects are rare, and oligochaetes and crustaceans dominate. Differences in invertebrate communities among low gradient sites were correlated with differences in flow velocity, SpC, instream habitat, and turbidity.

3.6 Nonmetric multidimensional scaling (NMS) ordination

Relationships between BMI site taxa composition, environmental variables, and BMI metrics were examined with NMS ordination. Proximity of sites relative to one another on NMS ordination plots indicates similarity in taxonomic composition. The positions of sites relative to NMS axes can change between different ordinations of the same dataset. In context of ordinations based on taxa lists, the axes on NMS plots represent major components of variation in taxonomic composition. NMS plots are useful for exploring correlations between environmental variables, BMI metrics, and between site taxonomic variation represented by NMS axes. These associations are illustrated by overlays of rays emanating from the center of an NMS plot. The direction of a ray indicates the direction of the line of best fit for the most positive correlation between a variable and the NMS axes, and the length of the ray represents the strength of that correlation. As seen in the cluster analyses, the spring NMS ordinations revealed patterns broadly similar to those shown by ordinations of fall data. Therefore, analysis focused on the fall NMS ordinations.

The ordination plot of the fall dataset, including both low- and high- gradient sites, demonstrated two biologically distinct site groups differentiated by stream gradient (Fig. 11). Pleasant Grove Creek was shown as taxonomically similar to other, more intensively agricultural low gradient waterbodies. The ordination clustered high gradient waterways together, including the agriculture-influenced Butte Creek, as well as the urban Auburn Ravine and Dry Creek. The high gradient Butte Creek and low gradient Pleasant Grove waterbodies may have ordinated and clustered with other sites of the same gradient but differing land use because BMI communities are strongly influenced by substrate and instream habitat, which are closely related to the gradient of the stream. Within high and low gradient clusters, sites in the same waterway tended to occur near

one another. In the low gradient clusters, this was particularly true of the non-agricultural Pleasant Grove waterway. The patterns seen in the fall ordination are in accord with the fall cluster analysis.

NMS ordinations revealed patterns that did not emerge in cluster analysis. Of all high gradient sites, those in the urban-dominated Dry Creek ordinated in proximity to the low gradient site clusters. Thus, Dry Creek was the high gradient waterway most taxonomically similar to low gradient ADWs. This similarity to ADWs may occur because the effects of urban land use are broadly similar to the effects of agricultural land use, including loss of instream habitat through sedimentation and channelization, loss of riparian vegetation, and changes in water quality due to runoff. High gradient site clusters generally separated from each other along the same axis that separated high from low gradient waterways. This indicates that aspects of BMI community differentiating high gradient site clusters from one another are the same as those differentiating high and low gradient waterways. In contrast, low gradient site clusters aligned along an axis perpendicular to the axis separating low from high gradient site clusters. Aspects of the BMI community that distinguish low gradient sites from one another are therefore likely to be distinct from those that distinguish low from high gradient sites.

Similar to the fall ordination, the spring ordination of the full dataset (Fig. 12) revealed a wide separation of low and high gradient site samples. Also, Dry Creek samples were again seen to be the high gradient sites most similar to low gradient sites, and low gradient site clusters differed from each other along an axis perpendicular to the axis separating low from high gradient sites.

In agreement with associations of environmental variables, BMI metrics and site clusters, correlations with ordination axes revealed that agricultural land use, mud substrate, and metrics indicative of impacted BMI communities are associated with low gradient sites, while more favorable habitat conditions, coarse substrates, and metrics indicative of less impacted conditions are associated with high gradient sites.

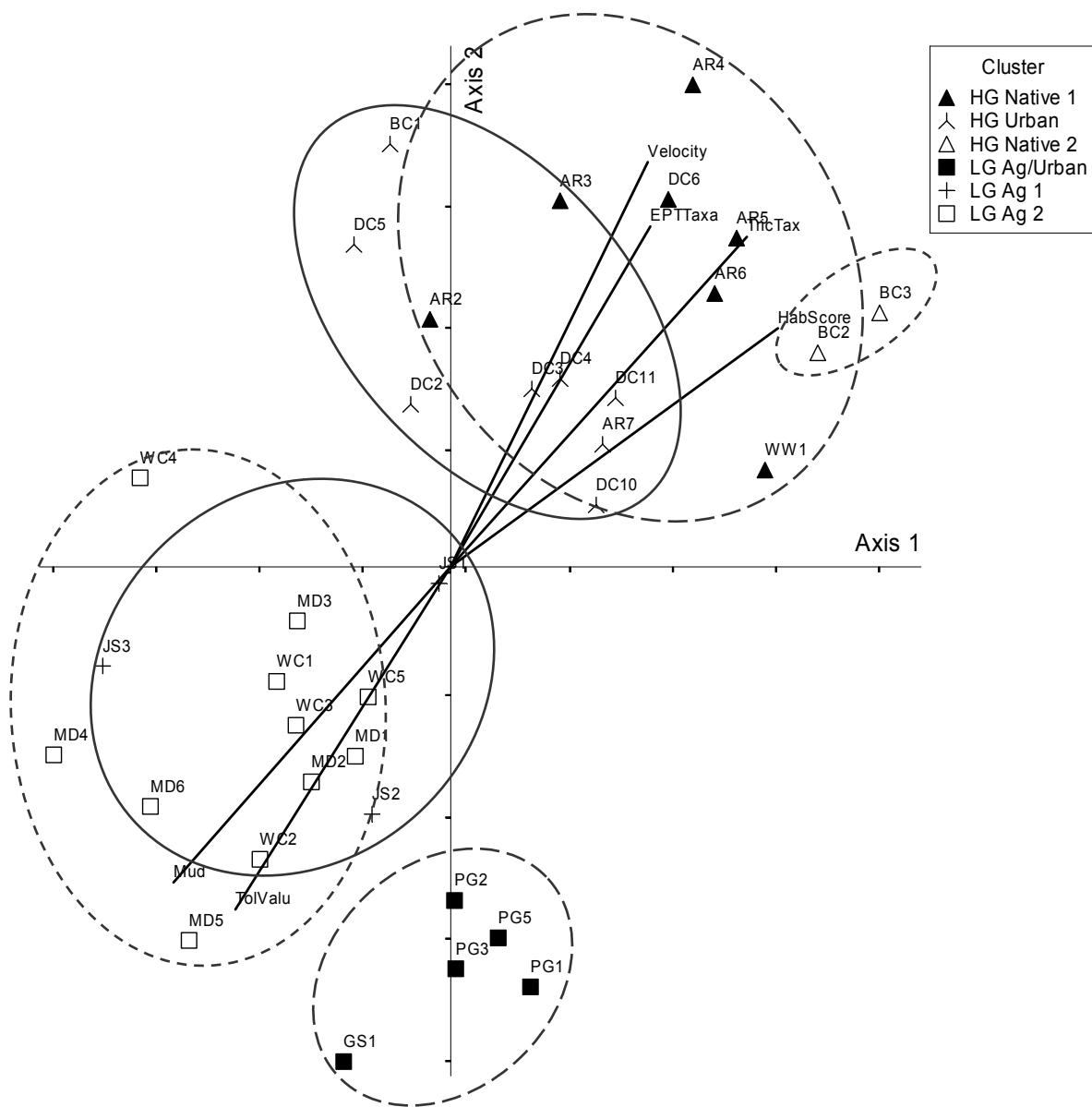


Fig. 11. Non-metric multidimensional scaling (NMS) ordination based on taxonomic similarity of fall samples. Each ordinated site was sampled both in fall 2000 and 2001. Overlay shows environmental variables and BMI metrics correlated with the NMS axes at $R^2 > 0.80$. The value the environmental variable or BMI metric increases in the direction of the ray; the length of the ray reflects the strength of the correlation. Rays parallel to an axis are highly correlated with that axis. Rays perpendicular to an axis reveal little association with that axis. Axis 1 accounts for 32.8% of the variance in the dataset, while axis 2 explains 53.7% of the variance in the dataset.

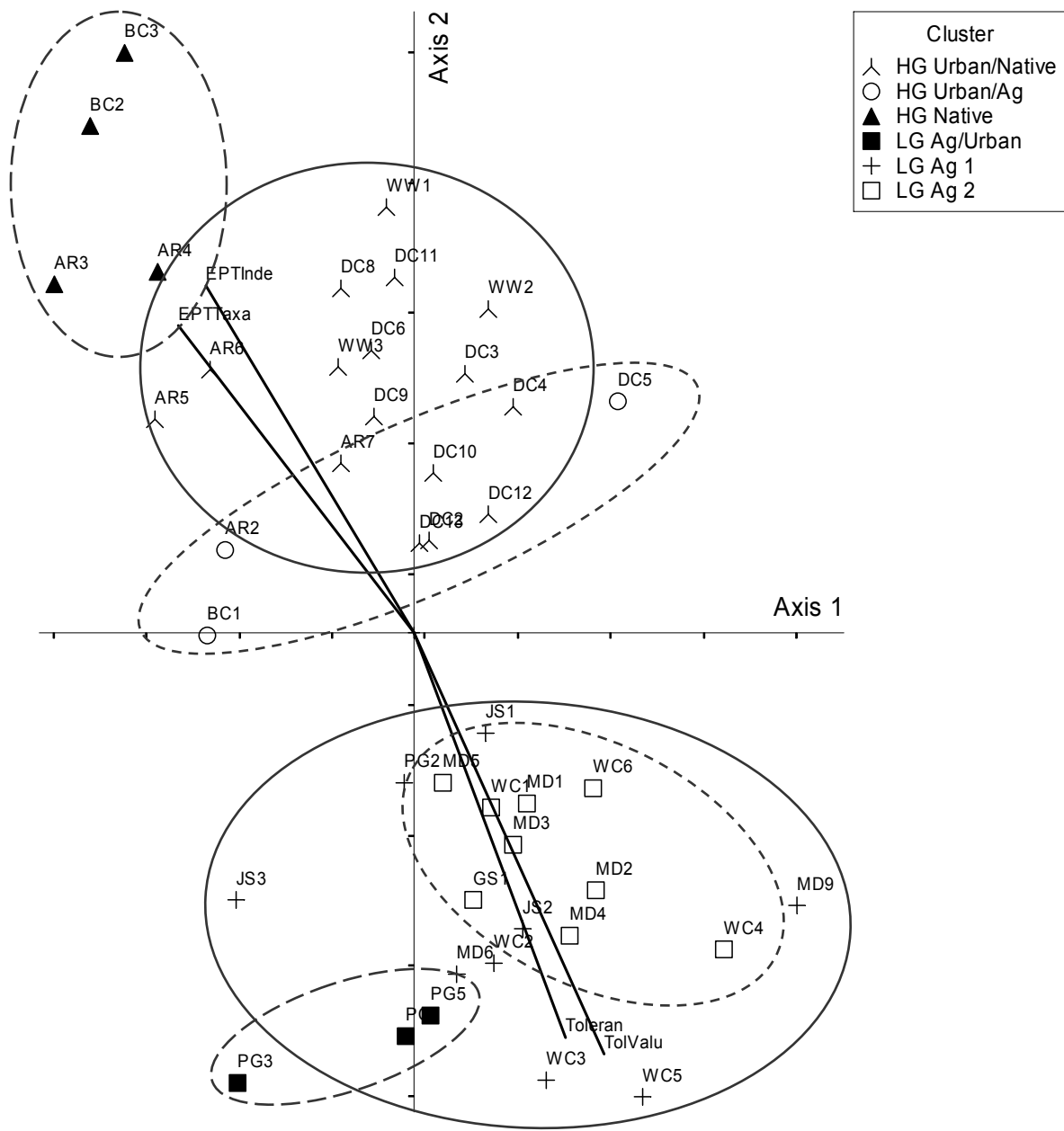


Fig. 12. Non-metric multidimensional scaling (NMS) ordination based on taxonomic similarity of spring samples. Each ordinated site was sampled in spring 2001 and 2002. Overlay shows environmental variables and metrics of BMI community integrity correlated with the NMS axes at $R^2 > 0.80$. Axis 1 accounts for 25.0% of the variance in the dataset, while axis 2 explains 63.0% of the variance in the dataset.

3.6.1 Ordinations of low gradient site taxa data

Three-dimensional plots were derived from ordinations of fall and spring low gradient site taxa composition data. Axes 1 and 3 of fall data ordination (Fig. 13a) separated the same three low gradient site clusters produced by cluster analysis. However, habitat factors, substrate, land use and BMI metrics were most strongly correlated with axis 2 (Fig. 13b). There was therefore significant divergence in BMI community integrity among low gradient sites, however these differences were not site-cluster specific. Fig. 13 shows that axes 1 and 3 separated clusters most effectively, while BMI metrics were most strongly correlated with axis 2. It is noteworthy that the ADW site GS1 was stagnant and clustered with the low flow Pleasant Grove sites, yet separated from these sites along axis 2, the axis most correlated with BMI metrics indicative of community integrity. Such a position indicates that GS1 was more severely impacted than other low-flow sites.

In spring ordination, site cluster correlations with environmental variables and metrics revealed that the major gradient of BMI community integrity was related to both NMS axes 2 and 3, while axes 1 and 3 separated the clusters most effectively (Fig. 14). This indicates that taxa site clusters were partially reflective of BMI community integrity, while part of the variation in BMI community integrity was not reflected in the clusters. The spring ordination illustrated that, though cluster analysis assigned PG2 to a cluster separate from the remainder of the Pleasant Grove sites, PG2 was actually taxonomically similar to the other sites in the Pleasant Grove waterway.

The low gradient ordinations demonstrated that metrics strongly correlated with site-to-site community variability at low gradient sites were measures of diversity (Shannon Diversity and Taxonomic Richness) and Percent EPT Taxa. Percent Chironomidae also varied strongly between sites, but Chironomid variability was unrelated to variability in other metrics, and was not associated with measured environmental variables.

Environmental variables that correlated strongly with taxonomic differences among sites were primarily measures of substrate and physical habitat, including percent mud substrate, extent of sedimentation, pool variability, and width of the riparian zone.

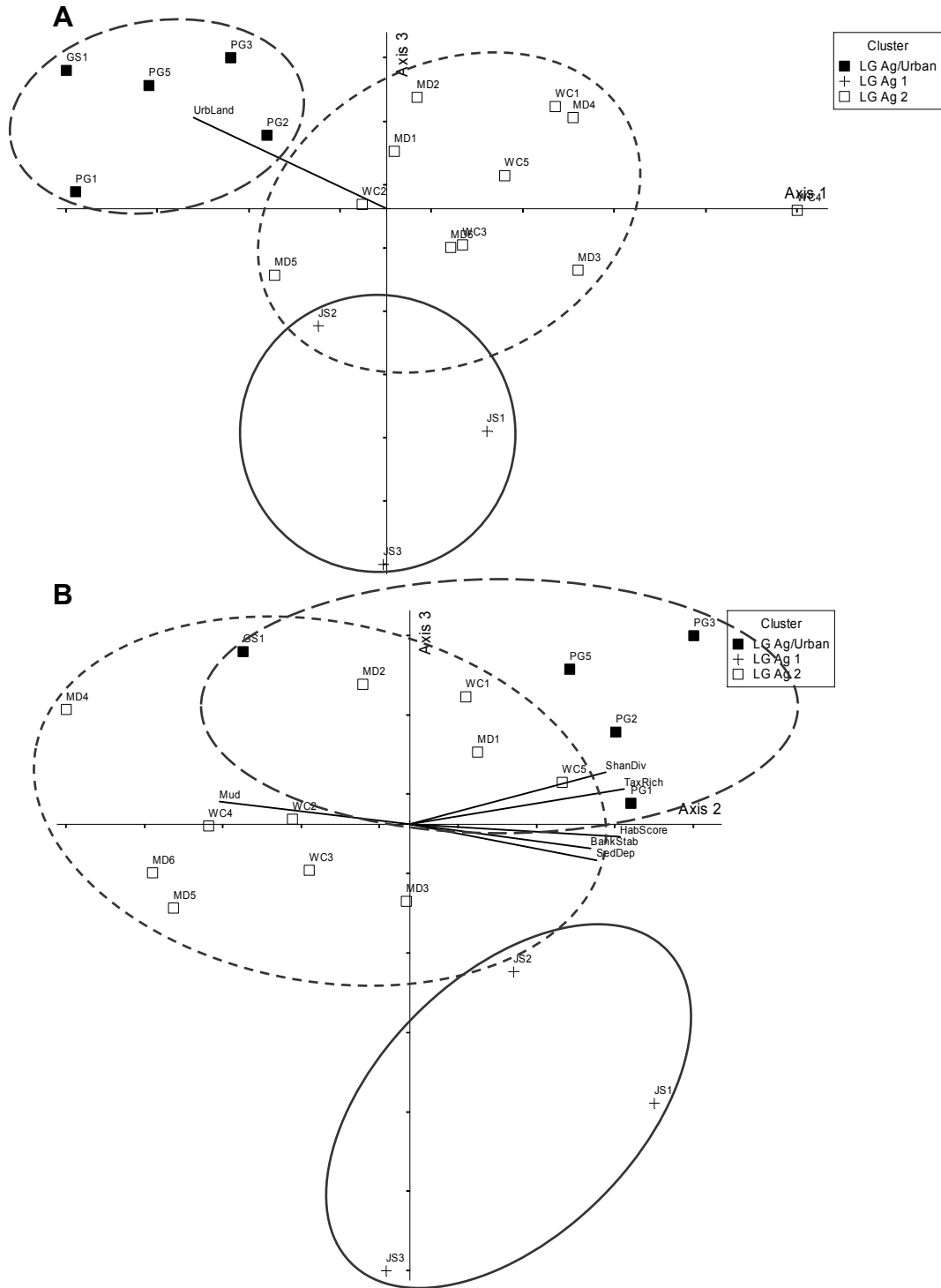


Fig. 13. NMS ordination based on taxonomic similarity of fall samples collected at low gradient sites. Samples were collected at each site in fall 2000 and 2001. Overlay shows environmental variables and metrics of BMI community integrity correlated with the NMS axes at $R^2 > 0.50$. Two views of a 3-dimensional plot are shown. A—Axes 1 and 3 plot. B—Axes 2 and 3 plot. Axis 1 accounts for 47.4% of the variance in the dataset, axis 2 explains 19.0% of the variance in the dataset, and axis 3 explicates 24.2% of the variance in the dataset.

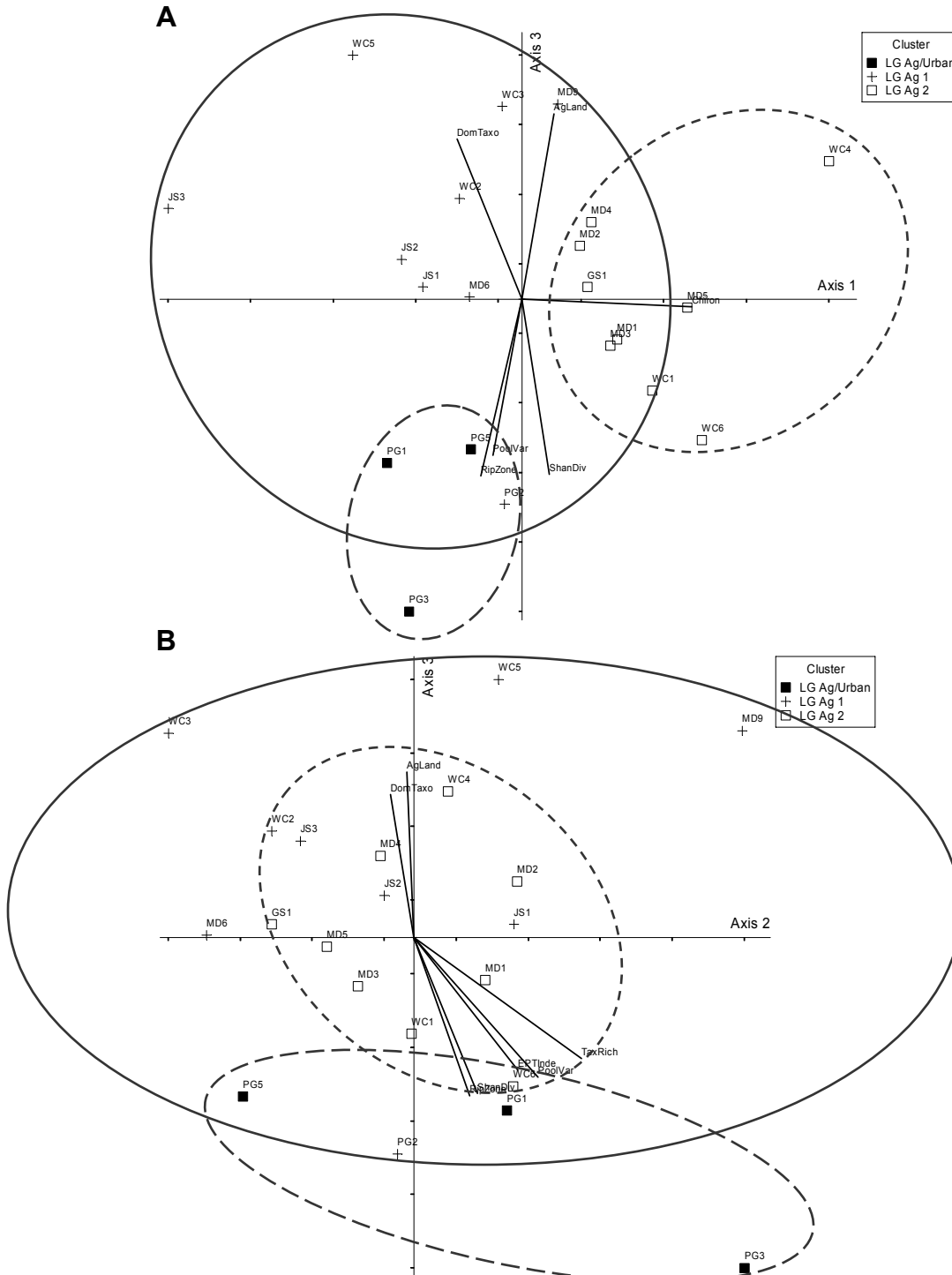


Fig. 14. NMS ordination based on taxonomic similarity of spring samples collected at low gradient sites. Samples were collected at each site in spring 2001 and 2002. Overlay shows environmental variables and metrics of BMI community integrity correlated with the NMS axes at $R^2 > 0.40$. Two views of a 3-dimensional plot are shown. A—Axes 1 and 3 plot. B—Axes 2 and 3 plot. Axis 1 explains 21.4% of the variance in the dataset, axis 2 accounts for 37.7% of the variance in the dataset, and axis 3 explicates 24.0% of the variance in the dataset.

3.6.2 Ordinations of high gradient site taxa data

As in the low gradient dataset, seasonal ordinations of high gradient site data yielded three dimensional plots (Figs. 15 and 16). Ordination of the fall high gradient taxa data exhibited best separation of clusters along axes 2 and 3 (Fig. 15). Both of these axes were strongly correlated with habitat variables and BMI metrics. Axis 1 includes a gradient of substrate between boulder- and sand-dominated sites, but BMI metrics were not correlated with this axis, suggesting that these types of substrates may not necessarily have an impact on community integrity. Ordination of spring high gradient site data also resulted in best separation of site taxa clusters along axes 2 and 3 (Fig. 16). Land use variables and metrics correlated with axis 2 indicating greater BMI community integrity at less urbanized sites. Axis 3 represents a strong substrate gradient, from mud-dominated to cobble-dominated substrates. BMI metrics signified that community integrity was associated with cobble-dominated substrates. Measures of species diversity (taxonomic richness and Shannon diversity) correlated with this axis. Indicators of BMI community integrity varied independently of one another and correlated with many divergent environmental parameters.

Overall, the metrics most strongly correlated with taxonomic variability at high gradient sites were those that measure components of EPT taxa, while pool variability and substrate types were the environmental variables most associated with high gradient site taxonomic variability.

3.7 Relationship of BMI metrics to environmental variables

A MANOVA was performed on the entire dataset, as well as on low and high gradient datasets individually, to identify environmental variables that explain variability in BMI metrics (Table 7).

Waterway gradient, land use, substrate, and many other habitat variables were significant factors in explaining variation of metrics in the full dataset. Velocity also was a significant factor in accounting for metric variations in the entire dataset, because the low gradient agricultural sites were more impacted than the high gradient sites and had lower

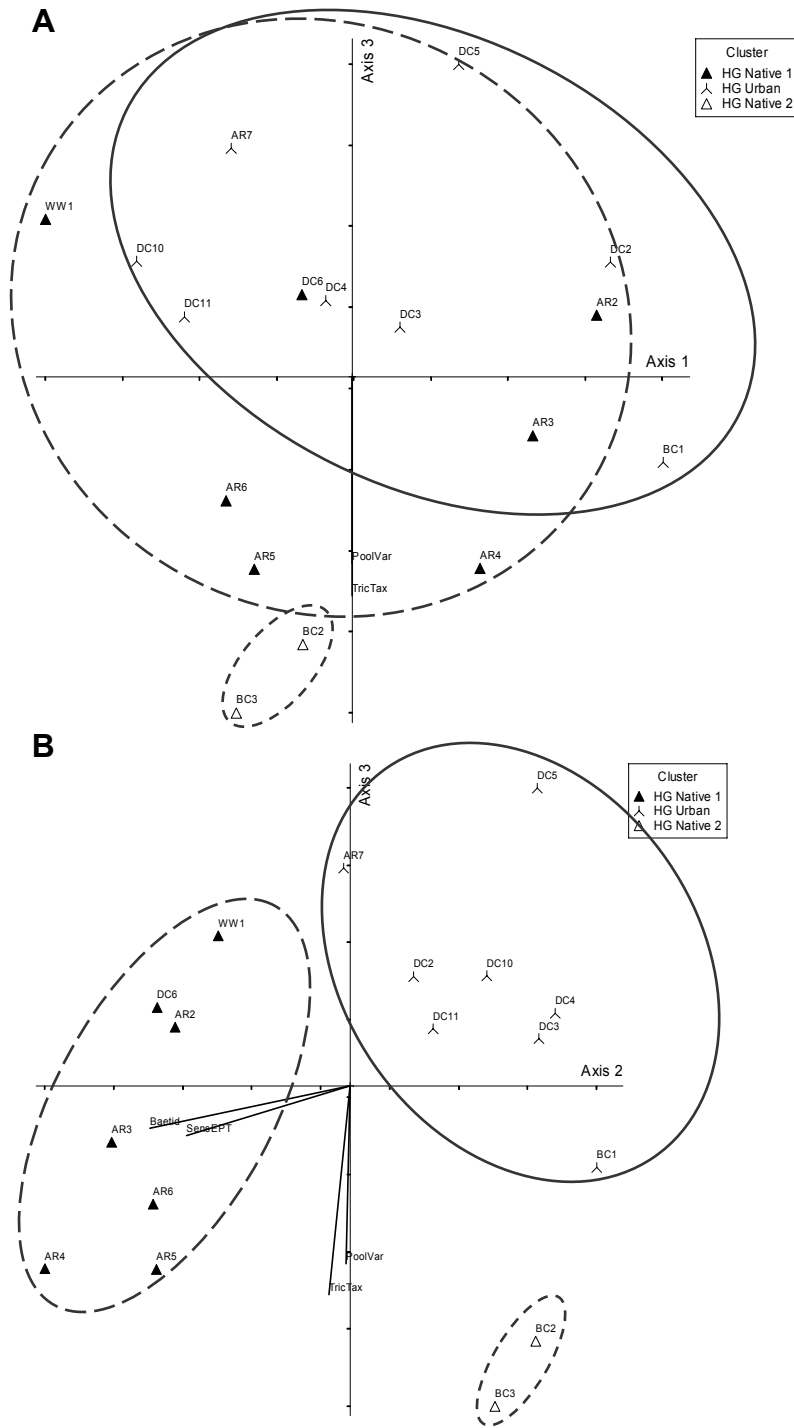


Fig. 15. NMS ordination based on taxonomic similarity of fall samples collected at high gradient sites. Samples were collected at each site in fall 2000 and 2001. Overlay shows environmental variables and metrics of BMI community integrity correlated with the NMS axes at $R^2 > 0.60$. Two views of a 3-dimensional plot are shown. A—Axes 1 and 3 plot. B—Axes 2 and 3 plot. Axis 1 accounts for 16.7% of the variance in the dataset, axis 2 explains 34.3% of the variance in the dataset, and axis 3 explains 34.2% of the variance in the dataset.

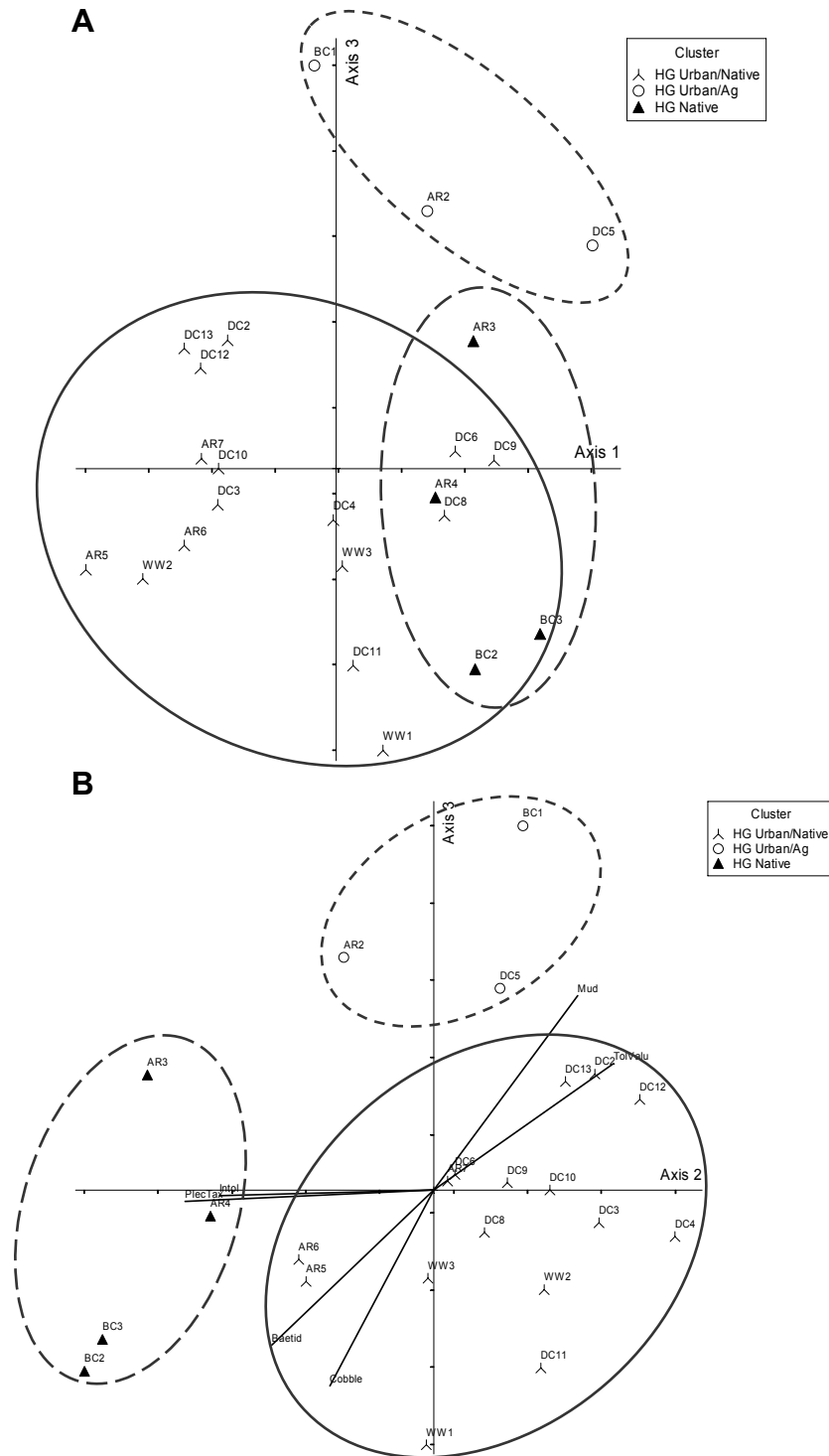


Fig. 16. NMS ordination based on taxonomic similarity of spring samples collected at high gradient sites. Samples were collected at each site in spring 2001 and 2002. Overlay shows environmental variables and metrics of BMI community integrity correlated with the NMS axes at $R^2 > 0.62$. Two views of a 3-dimensional plot are shown. A—Axes 1 and 3 plot. B—Axes 2 and 3 plot. Axis 1 accounts for 21.4% of the variance in the dataset, axis 2 explains 38.8% of the variance in the dataset, and axis 3 elucidates 32.8% of the variance in the dataset.

Table 7

Environmental variables included in the final linear models of MANOVA analyses using all BMI metrics as response variables. P-values for all effects of the models are in parentheses. Three multiple regression models were constructed: one for the entire dataset, one for the low gradient sites, and one for the high gradient sites.

All Sites	High Gradient Sites	Low Gradient Sites
Gradient (< 0.0001)	--	--
Season (< 0.0001)	Season (< 0.0001)	Season (< 0.0001)
Project Year (< 0.0001)	Project Year (< 0.0001)	Project Year (< 0.0001)
% Ag Land Use (< 0.0001)	% Ag Land Use (< 0.0001)	% Ag Land Use (< 0.0001)
% Urban Land Use (< 0.0001)	--	% Urban Land Use (< 0.0001)
Cobble (0.0011)		Cobble (0.0310)
		Gravel (< 0.0001)
		Bedrock (< 0.0001)
		Hardpan (< 0.0001)
	Boulder (0.0277)	Boulder (0.0018)
	Mud (< 0.0001)	Mud (< 0.0001)
	Sand (0.0008)	Sand (< 0.0001)
Velocity (0.001)		
Depth (< 0.0001)	Depth (< 0.0001)	
Epifaunal Substrate (0.0003)	Epifaunal Substrate (0.0004)	Epifaunal Substrate (0.0009)
Pool Variability (0.0008)	Pool Variability (< 0.0001)	
Pool Substrate (0.0001)	Pool Substrate (0.0002)	Pool Substrate (< 0.0001)
Sediment Deposition (< 0.0001)	Sediment Deposition (0.0168)	Sediment Deposition (0.0003)
Channel Flow (< 0.0001)	Channel Flow (0.0001)	Channel Flow (0.0009)
Channel Sinuosity (0.0041)	Channel Sinuosity (0.0001)	Channel Sinuosity (< 0.0001)
Bank Stability (0.0135)	Bank Stability (0.0002)	
Vegetative Protection (< 0.0001)		Vegetative Protection (< 0.0001)
Riparian Zone (0.0119)	Riparian Zone (< 0.0001)	

velocity flows. Velocity was not a significant factor in either the high or the low gradient site data subsets. While aspects of flow and water volume such as velocity and depth are likely to influence BMI community composition, point measurements at sites only at the time of BMI sample collection may not effectively represent such parameters. Therefore, caution should be used when analyzing and interpreting data involving these parameters. Metrics showed significant seasonal differences in all three MANOVA analyses ($P < 0.0001$). This analysis, in combination with others performed on this dataset provide evidence that BMI communities in high and low gradient waterways are significantly different between fall and spring seasons, and that metrics indicative of BMI community integrity change between seasons. Therefore, sites must be sampled during the same index period for valid comparison of metrics scores.

3.8 Metrics principle components analyses (PCA)

Principle Components Analyses (PCA) based on BMI metrics were performed on the entire, low gradient, and high gradient data sets individually. Fig. 17 depicts the sites ordinated on the first three principle components of the metrics PCA of the entire dataset. Tables 8 and 9 present the loadings of metrics onto of the first three principle components of the three analyses. The first principle component accounted for 40 to 57% of the variability in the datasets.

The first principle component (PC1) for each of the three analyses represents a contrast between metrics indicating sensitive taxa and biological diversity (positive loadings; EPT Taxa, Trichoptera Taxa, Ephemeroptera Taxa, Shannon Diversity, Taxa Richness) and those representing tolerant taxa (negative loadings; Percent Multivoltine, Percent Oligochaeta, Percent Tolerant Organisms). Metrics with the most extremely positive and negative loadings were the most influential on the principle components. The magnitude and sign (+/-) of the metrics were relatively consistent among all analyses. Only percent Filterers and Percent Chironomidae changed sign among analyses.

The high gradient analysis shows a strong negative PC1 loading for Percent Chironomidae, making that metric one of the indicators of biologically impacted

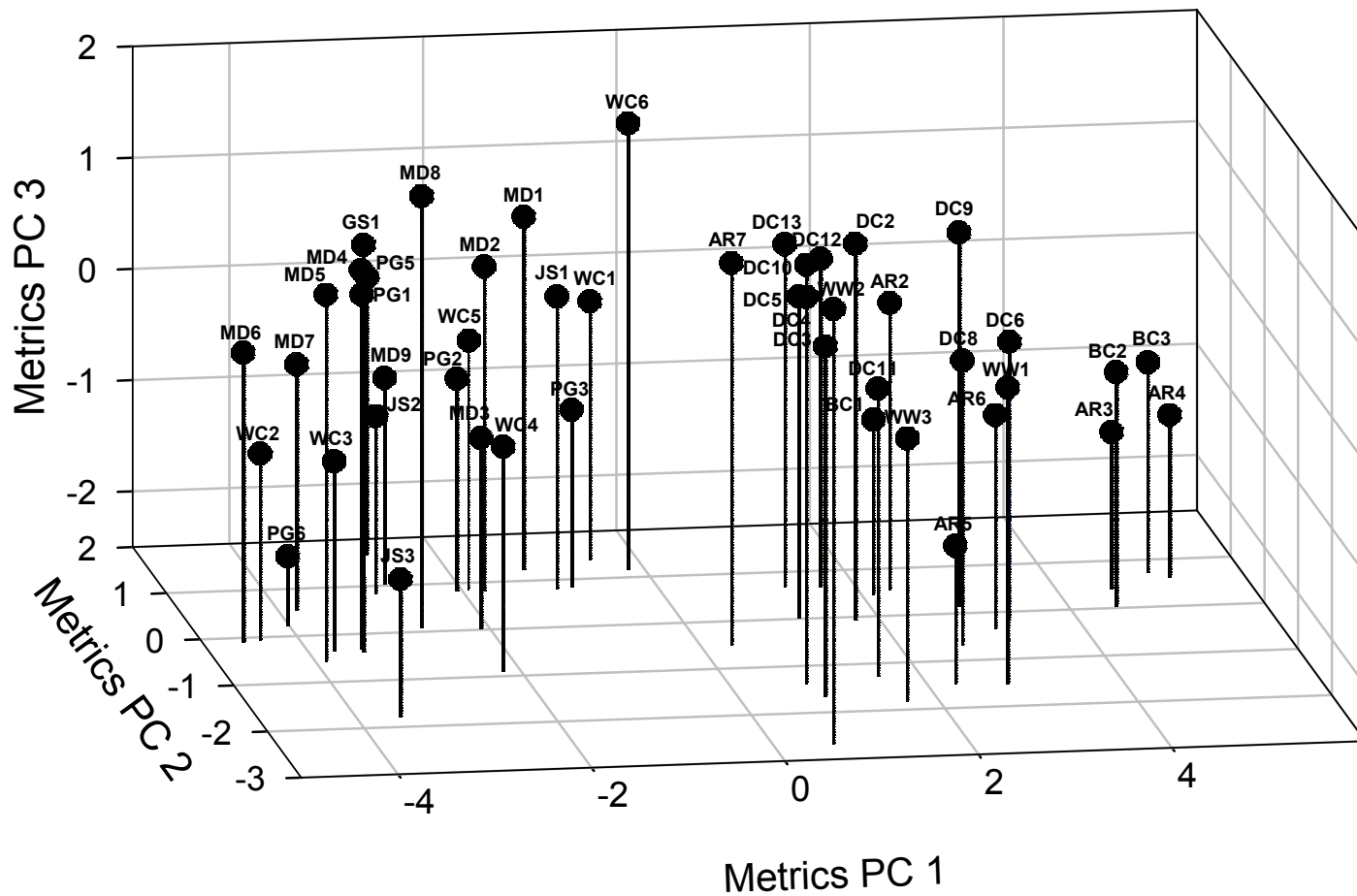


Fig. 17. Three-dimensional ordination of BMI metrics data by Principle Components Analysis. Proximity of sites indicates similarity of metric scores. Axis PC1 summarizes 57.2% of the variance in the dataset, while axis PC2 summarizes 11.0% and axis PC3 summarizes 8.4%.

Table 8
 Loadings of BMI metrics onto the first three principle components of full data set PCA analyses (light shading indicates $r > 0.5$, dark shading indicates $r < -0.5$)

	PC 1	PC2	PC3
Eigenvalue	9.1441	1.7804	1.3376
Percent Variance	57.2	11.0	8.4
	Loadings		
Metric	PC 1	PC2	PC3
EPT Taxa	0.9410	0.0602	-0.0738
EPT Index	0.9107	-0.1416	-0.1530
Tric Taxa	0.8992	0.0254	-0.0010
Eph Taxa	0.8519	0.0944	-0.0763
% Baetidae	0.8261	-0.2686	-0.1956
Taxa Richness	0.8013	0.3954	-0.0074
% Intolerant	0.7543	0.3258	-0.2373
Sensitive EPT	0.7325	0.3301	-0.2598
Shannon Diversity	0.7224	0.5617	0.2201
% Hydropsychidae	0.7077	-0.3783	0.1377
% Filterers	0.4196	-0.1538	0.5482
% Chironomidae	-0.0276	-0.0445	0.7798
% Dominant Taxon	-0.5874	-0.5233	-0.3964
% Oligochaeta	-0.7675	0.4201	-0.0513
% Tolerant	-0.7882	0.4668	-0.0233
Tolerance Value	-0.8426	0.3620	-0.0664

Table 9

Loadings of BMI metrics onto the first three principle components of the low- and the high-gradient PCA analyses. Dashes indicate metrics not included in the low gradient analysis because distribution varied grossly from normality, even after log transformation (Shapiro-Wilks test, $W < 0.70$). (light shading indicates $r > 0.5$, dark shading indicates $r < -0.5$)

	Low Gradient			High Gradient		
	PC 1	PC2	PC3	PC 1	PC2	PC3
Eigenvalue	5.4213	2.2413	1.7715	6.5830	2.6873	1.8288
Percent Variance	45.2	18.7	14.8	41.1	16.8	11.4
Metric	Loadings			Loadings		
	PC 1	PC2	PC3	PC 1	PC2	PC3
EPT Taxa	0.8748	0.3470	-0.1901	0.8798	0.2773	0.1498
EPT Index	0.7900	0.3348	-0.3175	0.7392	-0.4815	-0.1078
Tric Taxa	0.7807	0.1891	-0.1016	0.7427	0.1783	-0.0497
Eph Taxa	0.7969	0.3510	-0.2068	0.6639	0.3596	0.1663
% Baetidae	-	-	-	0.5599	-0.5451	0.1523
Taxa Richness	0.7763	0.4054	0.2063	0.7490	0.4916	-0.1183
% Intolerant	-	-	-	0.7759	0.1233	0.4166
Sensitive EPT	-	-	-	0.7895	0.0987	0.4260
Shannon Diversity	0.6998	0.1535	0.6173	0.6928	0.5786	-0.2811
% Hydropsychidae	-	-	-	0.2511	-0.2427	-0.8085
% Filterers	0.4083	-0.3086	0.3449	0.0012	0.1249	-0.6422
% Chironomidae	0.2018	-0.7033	0.4346	-0.5271	0.2249	0.2942
% Dominant Taxon	-0.5796	0.1070	-0.7285	-0.5117	-0.5217	0.3427
% Oligochaeta	-0.6643	0.4916	0.2110	-0.4775	0.5160	0.1513
% Tolerant	-0.5872	0.6712	0.3526	-0.5747	0.6100	-0.0063
Tolerance Value	-0.6046	0.6070	0.3877	-0.7276	0.5323	-0.0218

conditions. In contrast, the low gradient analysis shows a strong loading for Percent Chironomidae on PC2 but not PC1, indicating that Percent Chironomidae varies independently of many other metrics. In all three analyses, EPT Taxa is a consistently very high loading metric on PC1, indicating that it is a consistently strong indicator of less impacted communities.

3.8.1 Associations between metrics PC 1 and environmental variables

Similar to applying MANOVA to investigate the relationship between metrics and environmental variables, ANOVAs were utilized to examine environmental variables that potentially determine or influence the first principle component (PC1) of the metrics PCA (metrics PC1 summarizes the largest portion of the variability in BMI metrics in the dataset). Three ANOVAs were performed relating the environmental variables (the predictor variables) to metrics PC1 (the response variable): one on the entire dataset and one on each gradient-specific subset. The environmental variables significantly related to the first principle component in each of the three analyses are summarized in Table 10. Results were similar, although not identical, to the outcome of the MANOVA applied to all metrics. Site gradient ($P < 0.0001$) was the most significant factor explaining variation of metrics across sites in the full dataset. The inclusion of the gradient effect in the model made it unnecessary to include substrate or land use factors. Among habitat variables, riparian zone width ($P = 0.0051$) and vegetative protection ($P = 0.0157$) significantly increased the fit of the model. In accordance with the MANOVA, examination of the first principle component identified season as the only environmental variable ($P < 0.0001$) statistically significant in all three analyses. Analysis of both low and high gradient site data identified land use, substrate, and hydrological variables as significantly associated with metrics PC1. Project year was significantly associated with PC 1 only at the high gradient sites ($P < 0.0001$).

3.9 Environmental parameters principle components analysis

Principle components analyses were performed to determine the major environmental gradients in the entire dataset and the high- and low gradient subsets. Sites ordinated on the first three principle components of the environmental parameters PCA performed on

Table 10

Environmental variables significantly associated with BMI metrics Principle Component 1 in final multiple regression models. P-values for all effects of the models are in parentheses. Three multiple regression models were constructed: one for the entire dataset, one for the low gradient sites, and one for the high gradient sites

All Sites	Low Gradient	High Gradient
Gradient (> 0.0001)		
Season (>0.0001)	Season (> 0.0001)	Season (> 0.0001)
Project Year (> 0.0001)		Project Year (> 0.0001)
	% Urban Land Use (> 0.0001)	
	% Native Land Use (> 0.0001)	
	% Ag Land Use (> 0.0001)	Land Use (> 0.0001)
	Sand (0.0021)	
	Hardpan (> 0.0001)	
	Boulder (0.0015)	
	Gravel (> 0.0001)	Gravel (0.0045)
	Bedrock (0.0055)	
Vegetative Protection (0.0051)		
Riparian Zone (0.0157)	Channel Flow (0.0011)	Channel Flow (> 0.0001) Channel Alteration (0.0171) Pool Variability (> 0.0001)
	Sediment Deposition (0.0272)	

the entire dataset are exhibited in Figs. 18 and 19. The first principle components of these analyses accounted for 20 to 40% of the variance in the environmental variables dataset. Details of percent variance explained by and loadings of variables onto the first three principle component axes of each analysis are summarized in Tables 11 and 12. BMI metrics correlated strongly with PC1 in all of the PCAs of environmental variables, but did not correlate strongly with the other principle component axes. The correlations of BMI metrics with the first principle components are summarized in Table 13.

In analysis of the entire dataset, PC1 included a gradient between agricultural and non-agricultural environments. Loadings of habitat factors and gravel substrates were positive and loadings of SpC, alkalinity, hardness, and mud substrates were negative. Several metrics indicative of water quality, as well as both high and low gradient biotic indices, were positively correlated with this axis, while tolerance metrics were negatively correlated with this axis. PC1 therefore shows associations of water quality, substrate type, and physical habitat with BMI metrics. SpC, ammonia, and nutrients loaded heavily onto the second principle component axis, and this axis was positively correlated with percent Hydropsychidae and percent Chironomidae. PC2 therefore indicates an association between these taxa and higher readings of SpC, ammonia, and nutrients.

Similar to the analysis of the entire dataset, the environmental parameters PC1 of the low gradient site dataset (Fig. 19A) indicated strong positive loadings for sand and gravel substrates, native vegetation, and measures of intact instream and riparian habitat contrasted with strong negative loadings for SpC, alkalinity, hardness, P4, and mud substrates. Since many BMI metrics were highly correlated with the PC1 axis, the environmental parameters with strong loadings on this axis are likely to include those important to BMI community integrity. Interestingly, the Percent Chironomidae metric exhibited a strong negative loading onto the low gradient environmental variables PC1, although PCA of BMI metrics demonstrated that this metric was not strongly correlated with many other metrics at low gradient sites. This analysis indicated that water quality,

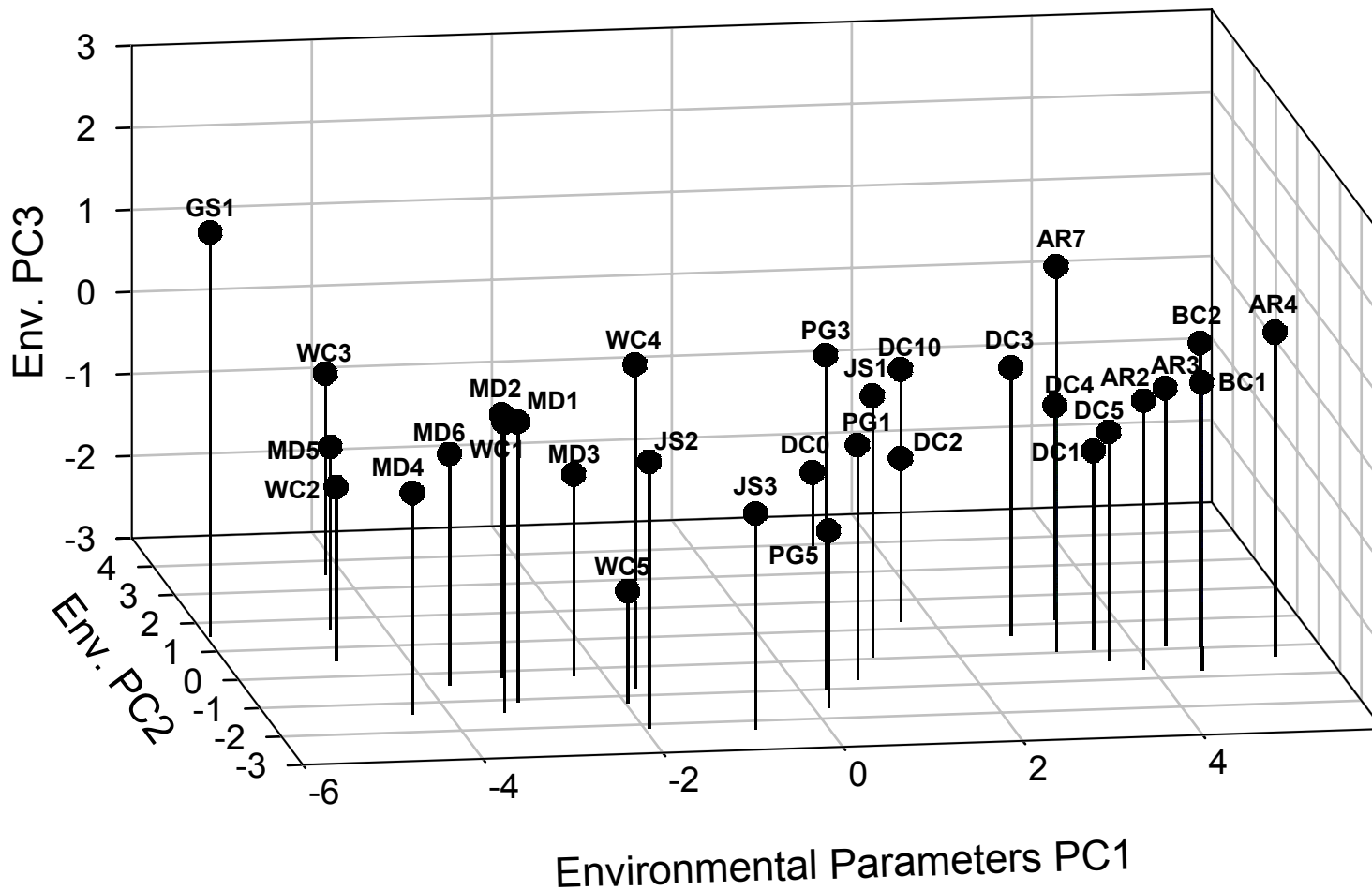


Fig. 18. Three-dimensional ordination by Principle Components Analysis of environmental data, including water quality, substrate, nutrients, physical habitat, and land use. Proximity of sites indicates similarity in environmental variables. Axis PC1 summarizes 37.3% of the variance in the dataset, while axis PC2 summarizes 9.8% and axis PC3 summarizes 6.3%.

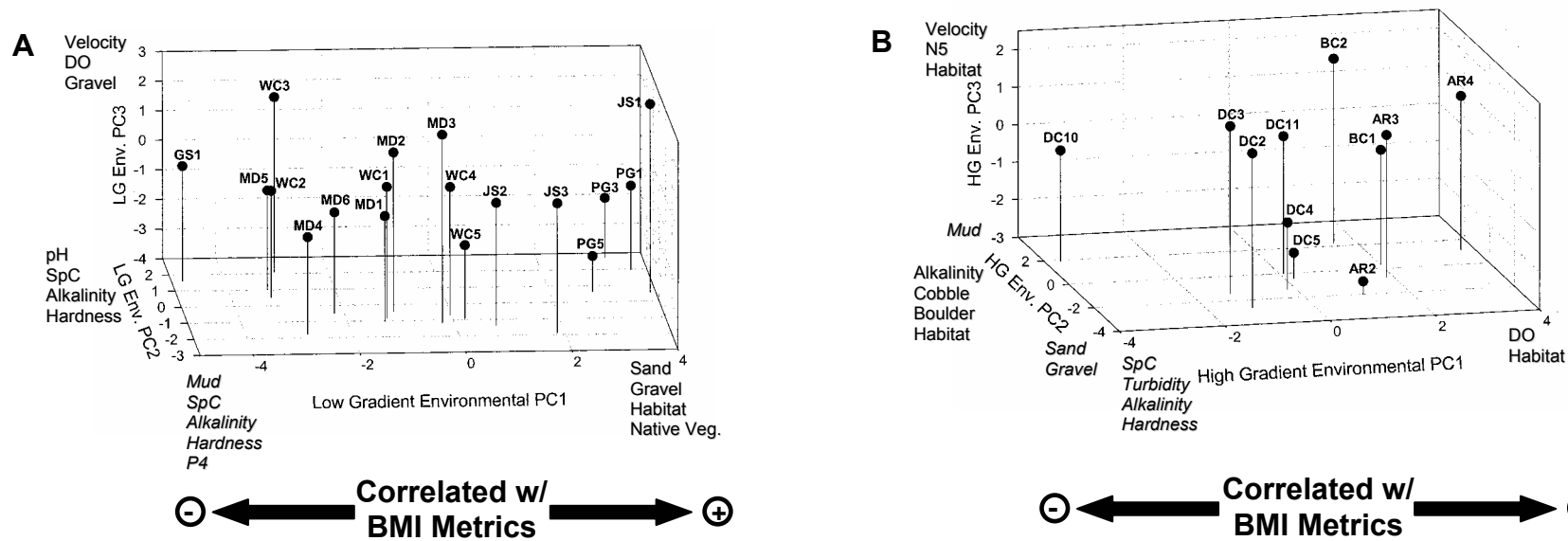


Fig. 19. (A) Low gradient and (B) high gradient principle components analyses of environmental variables. Environmental parameters with absolute value loadings > 0.5 are shown next to each principle component axis. Italics indicate negatively correlated environmental parameters. In each analysis, correlations with BMI community metrics indicated better community integrity at sites with more positive values on PC1.

Table 11
 Loadings of environmental variables onto the first three principle components of the full dataset PCA analysis (light shading indicates $r > 0.5$, dark shading indicates $r < -0.5$)

	PC 1	PC2	PC3
Eigenvalue	12.6895	3.3271	2.3609
Percent Variance	37.3	9.8	6.9
	Loadings		
Environmental Parameter	PC 1	PC2	PC3
Velocity	0.6308	0.1385	-0.2573
Depth	-0.2235	-0.4380	0.4305
DO	0.4474	0.1286	-0.4969
pH	-0.1935	0.1691	-0.7022
Temperature	-0.0601	0.1637	0.3496
SpC	-0.7286	0.4062	0.0167
Turbidity	-0.5210	-0.1126	0.1002
Alkalinity	-0.7926	0.1745	-0.1741
Hardness	-0.7157	0.1934	-0.2321
Ammonia	-0.2792	0.4338	0.0953
P2	-0.3797	0.7377	0.3238
Nitrate	-0.1549	0.6261	0.2945
P4	-0.6049	0.4322	0.2660
N5	-0.0751	0.7453	0.1003
Mud	-0.8488	-0.2405	0.0864
Sand	0.6258	0.0999	0.3209
Gravel	0.8006	0.2676	-0.1003
Cobble	0.6085	0.0811	-0.4813
Boulder	0.2768	0.0292	-0.4852
Bedrock	0.2610	-0.0034	-0.1054
Hardpan	-0.2822	-0.5267	0.1121
Epifaunal Substrate	0.8615	0.0744	0.1684
Pool Substrate	0.4043	-0.0755	-0.0447
Pool Variability	0.8531	0.0175	0.1128
Sediment Deposition	0.6809	-0.1881	0.0551
Channel Flow	0.2796	-0.2680	0.2695
Channel Alteration	0.8680	0.0809	0.0787
Channel Sinuosity	0.7531	0.0078	-0.0269
Bank Stability	0.8074	-0.0314	0.1704
Vegetative Protection	0.7962	-0.0184	0.2307
Riparian Zone Width	0.8864	0.0826	0.1046
Ag Land	-0.7726	-0.3829	-0.0030
Urban Land	0.4980	0.4741	-0.0614
Native Veg. Land	0.8250	-0.0525	0.1930

Table 12

Loadings of environmental variables onto the first three principle components of the low and high gradient PCA analyses (light shading indicates $r > 0.5$, dark shading indicates $r < -0.5$)

	Low Gradient			High Gradient		
	PC 1	PC2	PC3	PC 1	PC2	PC3
Eigenvalue	9.5145	2.7360	2.4677	5.1888	4.8839	2.8591
Percent Variance	36.6	10.5	9.5	20.8	19.5	11.4
Environmental Parameter	Loadings			Loadings		
	PC 1	PC2	PC3	PC 1	PC2	PC3
Velocity	0.2197	-0.2090	0.7857	0.1514	-0.1396	0.6611
Depth	0.2567	-0.1265	-0.1236	0.1875	-0.4543	0.4861
DO	0.1694	0.0860	0.6163	0.5675	0.1140	-0.1800
pH	-0.3250	0.5441	0.3477	-0.0274	0.3159	-0.0564
Temperature	-0.1659	-0.2338	-0.2805	0.1950	-0.1079	-0.2251
SpC	-0.6191	0.6831	-0.1112	-0.8059	-0.0625	0.1710
Turbidity	-0.0644	0.1866	0.1099	-0.7581	-0.3543	0.0305
Alkalinity	-0.6616	0.6210	0.0242	-0.7596	0.5087	0.0097
Hardness	-0.5967	0.6856	-0.0410	-0.8555	0.2688	0.0114
Ammonia	-	-	-	-	-	-
P2	-0.4418	0.3574	0.3814	-	-	-
Nitrate	-	-	-	-	-	-
P4	-0.5339	0.0590	0.4806	-	-	-
N5	-0.3524	0.2954	0.0867	-0.2707	-0.2853	0.5086
Mud	-0.7494	0.1141	-0.3559	-0.0231	-0.1078	-0.6035
Sand	0.5722	0.2361	-0.0849	-0.0919	-0.8633	-0.2316
Gravel	0.5461	0.0205	0.6634	0.2019	-0.5038	0.3556
Cobble	-	-	-	0.1159	0.8255	0.3013
Boulder	-	-	-	-0.3363	0.7510	-0.1008
Bedrock	-	-	-	-	-	-
Hardpan	-	-	-	-	-	-
Epifaunal Substrate	0.8388	0.1759	0.0280	0.5171	0.5698	0.1325
Pool Substrate	0.6019	0.3725	0.0822	0.1333	0.8248	0.0196
Pool Variability	0.8189	0.0695	-0.0841	0.4999	0.0684	0.5097
Sediment Deposition	0.7797	0.0611	0.1657	0.3434	0.7574	-0.0028
Channel Flow	0.3071	-0.1783	0.2623	0.2182	0.1410	0.5161
Channel Alteration	0.8002	0.4163	-0.2008	0.7515	-0.2477	-0.1174
Channel Sinuosity	0.6989	0.2363	-0.2080	0.4899	0.0604	0.2549
Bank Stability	0.8424	0.1530	0.0085	0.1268	0.1799	-0.5473
Vegetative Protection	0.8109	0.2341	0.0262	0.4323	-0.1115	-0.4327
Riparian Zone Width	0.8315	0.3216	-0.1524	0.5284	-0.1504	-0.2358
Ag Land	-	-	-	-	-	-
Urban Land	-	-	-	-	-	-
Native Veg. Land	0.7995	0.2295	-0.2293	-	-	-

Table 13

Pair-wise correlations of BMI metrics with PC1 of environmental parameter PCAs of the entire dataset, low-, and high-gradient site data subsets (light shading indicates $r > 0.5$, dark shading indicates $r < -0.5$)

Metric	Correlation with PC1 of Environmental Parameter PCAs		
	All Sites	Low Gradient	High Gradient
ETO Index	0.7659	0.5444	0.4092
ETO Taxa	0.7549	0.4977	0.6719
EPT Index	0.7454	0.3174	0.4352
EPT Taxa	0.7429	0.4808	0.6767
Ephemeroptera Taxa	0.7017	0.2551	0.6947
Trichoptera Taxa	0.7006	0.288	0.4092
Taxa Richness	0.6923	0.5733	0.6514
% Baetidae	0.6595	0.1113	0.3015
% Hydropsychidae	0.6542	0.2519	-0.1179
Shannon Diversity	0.5679	0.4457	0.5462
% Insects	0.5636	-0.0753	0.1605
% Intolerant	0.5078	0.1487	0.5974
Sensitive EPT	0.4579	0.1405	0.6169
% Shredders	0.3618	0.2032	0.1198
% Filterers	0.2994	-0.0987	-0.1588
% Grazers	0.1757	0.3861	0.3244
Tanytars / Chironomini	0.0209	0.0322	-0.395
% Predators	0.0141	0.2341	0.1621
% Filterers + Collectors	-0.0591	-0.2526	-0.1786
% Chironomidae	-0.171	-0.4519	-0.4206
% Collectors	-0.2369	-0.0994	0.0023
% Dominant Taxon	-0.3946	-0.2682	-0.3251
% Multivoltine	-0.4503	-0.4722	-0.0724
% Oligochaeta	-0.6461	-0.2617	-0.254
% Tolerant	-0.6667	-0.0706	-0.3921
Tolerance Value	-0.7338	-0.2063	-0.4083

sediment type, and physical habitat were all correlated with BMI metrics at low gradient sites.

The environmental variables PCA of the high gradient site data subset differed from the PCAs of the entire dataset and the low gradient site data subset. Rather than one overwhelmingly important PC axis, the first two PCs of the high gradient site PCA accounted for approximately equal portions of variance in the dataset. There were strong positive loadings of some physical habitat parameters and DO and strong negative loadings of SpC, alkalinity, hardness, and turbidity onto PC1. Strong positive loadings of some physical habitat parameters as well as boulder and bedrock substrates onto PC2 were also evident, contrasted with strong negative loadings of sand and gravel substrates. Despite this difference in the high gradient site PCA, BMI metrics similar to those in the other two analyses were correlated with PC1 in this analysis. This indicates stronger associations of BMI metrics with water quality and physical habitat than with substrate at high gradient sites.

All three environmental variable PCAs detected a strong pattern wherein high physical habitat scores and coarser substrates were contrasted with a correlated set of water quality parameters (SpC, hardness, alkalinity, and turbidity) and finer substrates. In all cases, high physical habitat scores and coarse substrates were correlated with BMI metrics, indicating a less impacted BMI fauna. To the contrary, the set of water quality parameters and finer substrates were correlated with BMI metrics indicating impacted conditions.

3.10 Indices of BMI community integrity

Community integrity rankings were developed separately for high and low gradient site data subsets (Table 14). After eliminating metrics with low signal/noise ratios and redundant metrics, similar sets of metrics remained; these were incorporated into community integrity rankings (a biotic index, BI) for the gradient-specific subsets. For the high gradient ranking system, more metrics were included that reflect specific

Table 14

BMI metrics included in the Biotic Indices created to discriminate more impacted sites from less impacted sites. Metrics marked with an asterisk (*) were considered indicators of impacted fauna, and scores on these metrics were weighted negatively in calculation of the Biotic Index.

Low Gradient	High Gradient
Taxonomic Richness	Shannon Diversity
EPT Taxa	EPT Taxa
ETO Index	EPT Index
	Plecoptera Taxa
	% Hydropsychidae
% Insects	% Insects
% Intolerant	
Tanytarsini / Chironomini	
% Multivoltine *	% Multivoltine *
Tolerance Value *	Tolerance Value *
% Dominant Taxon *	
% Collectors *	% Grazers
	% Oligochaeta *
	% Chironomidae *

Table 15

Signal to noise (S/N) ratios of BMI metrics chosen for the low gradient and high gradient Biotic Indices, and Pearson product-moment correlations between these metrics and five environmental variables possibly indicating anthropogenic stress

Low Gradient						
Metric	S/N Ratio	Minimum DO	SpC	Epifaunal Substrate	Fines	Gravel
Tax Rich	9.45	-0.1388	-0.2561	0.3994	-0.3025	0.2724
EPT Taxa	9.31	0.1074	-0.2173	0.0627	-0.4432	0.2202
% Insects	5.33	-0.1565	0.2518	0.0287	0.0487	0.0260
ETO Index	5.63	-0.2149	-0.2037	0.3708	-0.1649	0.2563
T/C	4.78	0.2204	-0.3590	-0.0690	-0.2156	0.1124
%Intolerant	4.42	0.0637	-0.2184	-0.0143	-0.1645	-0.0247
Tol Value	3.85	-0.0865	0.2156	-0.1664	0.2589	-0.1651
%DomTaxon	3.83	0.1319	0.0427	-0.2730	0.0895	-0.2164
% Collectors	9.32	-0.0072	0.1975	-0.0688	-0.0704	0.0604
% Multivolt	7.14	-0.0017	0.3633	-0.3179	0.0502	-0.1376
High Gradient						
Metric	S/N Ratio	Minimum DO	SpC	Epifaunal Substrate	Fines	Gravel
EPT Taxa	23.32	0.5239	-0.3970	0.3139	-0.2433	-0.0220
Plec Taxa	9.35	0.4151	-0.3899	0.3514	-0.1885	-0.0606
EPT Index	6.80	0.2024	-0.2748	0.2525	-0.2313	0.0603
Sens EPT	7.98	0.4352	-0.3116	0.2946	-0.0354	0.0057
Shan Div	3.25	0.3792	-0.4002	0.3089	-0.1917	-0.0165
Hydro	3.08	-0.2464	0.0366	0.0505	-0.3055	-0.0701
% Insects	8.18	0.2003	-0.1219	0.2863	-0.2773	-0.1062
% Grazers	7.19	0.4163	-0.2935	0.3244	-0.3529	-0.1087
% Multivolt	3.41	0.0157	0.0985	-0.0667	0.1414	0.0905
% Oligos	3.22	-0.1271	0.3035	-0.3316	0.1899	0.1798
% Chiros	5.97	-0.2117	0.4676	-0.2784	0.2177	-0.0866
Tol Value	9.48	-0.1739	0.3352	-0.4708	0.4416	0.0404

portions of intolerant EPT orders, including the number of Plecoptera taxa and percent Hydropsychidae. The metrics chosen for low gradient ranking system included the ratio of Tanytarsini to Chironomini midges (expected to increase with higher dissolved oxygen concentrations). The concept is that this ratio is a useful indicator of relative BMI community integrity in areas impacted by sedimentation, where midges thrive. Both ranking systems included measures of BMI diversity, EPT diversity and taxa tolerance. Correlations of candidate metrics with five environmental variables possibly indicative of anthropogenic stress were used to choose which of two metrics to include in a BI, when two metrics were highly correlated. These correlations are shown in Table 15.

BI scores were calculated separately for each transect, providing three scores per site for each sampling event. The BI scores are summarized by site in Tables 16 and 17 and are mapped by waterway in Figs. 20 to 28. In both low and high gradient site datasets, fall BI scores were significantly higher than spring scores (low gradient: matched pairs t-test, $t_{20} = -4.06$, $P = 0.0006$; high gradient: matched pairs t-test, $t_{24} = -3.17$, $P = 0.0041$).

While fall BI scores were higher than spring scores in both low and high gradient datasets, a comparison of the ability of BI scores to distinguish site-to-site differences based on F-score magnitude from between-site ANOVAs suggested that resolution of between-site differences was higher in the spring at low gradient sites, resolution of between-site differences was higher in the fall at high gradient sites (Low Gradient fall: 1-way ANOVA, $F_{21,101} = 5.9417$; Low Gradient spring: 1-way ANOVA, $F_{22,109} = 9.8593$; High Gradient fall: 1-way ANOVA, $F_{26,102} = 16.4742$; High Gradient spring: 1-way ANOVA, $F_{24,119} = 7.6293$). At some sites the BI was relatively high or low independent of season, while other sites fluctuated widely in relative BMI community integrity between seasons (Tables 16 and 17). BI scores at high gradient sites were lower in urban areas. At ADW low gradient sites BI scores tended to be lower in upstream compared to downstream sites.

Among low gradient waterways, BI scores were highest in Pleasant Grove Creek (Fig. 28). Pleasant Grove Creek is an urban low gradient waterway in western Placer County exposed to minimal agricultural influence. Of the low gradient ADWs, BI score was

highest in Wadsworth Canal (Fig. 26). Among the high gradient sites, BI score was highest in Auburn Ravine (Fig. 20), while Dry Creek and its tributaries scored lowest (Fig. 22). Dry Creek has a higher percentage of urban land use than other high gradient waterways, and is effluent-dominated during low-flow periods.

Pair-wise correlations between BI scores and physical habitat scores showed that both low gradient and high gradient BI scores were correlated with the total habitat score (Pearson product-moment correlations, low gradient: $r^2 = 0.0871$, $P = 0.0061$; high gradient: $r^2 = 0.1806$, $P < 0.0001$; see figs. 29 and 30). Among low gradient sites, the physical habitat components most strongly correlated with BI score were riparian zone width, pool variability, and epifaunal substrate (Pearson product-moment correlations, pool variability: $r^2 = 0.0939$, $P = 0.0043$; epifaunal substrate: $r^2 = 0.0895$, $P = 0.0054$; riparian zone width: $r^2 = 0.0466$, $P = 0.0478$). Among high gradient sites, the physical habitat components most strongly correlated with BI score were epifaunal substrate, pool variability, and sediment deposition (Pearson product-moment correlations, epifaunal substrate: $r^2 = 0.1806$, $P < 0.0001$; pool variability: $r^2 = 0.1377$, $P = 0.0003$; sediment deposition: $r^2 = 0.1158$, $P = 0.0009$).

3.10.1 Seasonal site comparisons

To evaluate possible variation and reliability of metrics between seasons, a separate biotic index was constructed for spring and fall in each gradient-specific dataset (Table 18). A notable difference between the general and season-specific BIs for low gradient sites was the inclusion of a greater number of EPT taxa component measures in the season-specific BIs. Percent grazers was a metric included in every high gradient site BI, while the ratio of Tanytarsini/Chironomini was a metric included in all low gradient site BIs. Percent multivoltine organisms and percent insects metrics were included in all BIs except at high gradient sites in spring.

BI site scores were higher in fall than spring at most high and low gradient sites. In the high gradient dataset, the taxonomic basis of this pattern is clear. That is, EPT taxa occurred at higher taxonomic richness and abundances in fall. Taxa following this

Table 16
Mean biotic index scores of fall and spring samples at low gradient sites,
including 95% confidence intervals

Site	Fall		Spring	
	Mean BI	95% CI	Mean BI	95% CI
GS1	49	5.4	29	3.2
JS1	56	7.5	51	6.7
JS2	50	14.2	28	6.6
JS3	31	7.4	27	2.2
MD1	66	8.3	60	12.1
MD2	64	12.6	52	7.1
MD9	50	4.4	36	9.0
MD3	58	21.8	36	9.2
MD4	46	8.1	37	4.8
MD5	22	2.6	42	3.8
MD6	31	5.2	24	6.6
PG1	57	13.3	55	16.6
PG2	50	25.0	43	7.3
PG3	64	10.5	57	9.4
PG5	50	9.2	47	5.5
WC6	91	5.6	63	8.4
WC1	64	20.2	58	10.6
WC2	28	6.8	20	4.8
WC3	40	10.0	15	8.7
WC4	62	16.3	44	7.3
WC5	79	8.5	27	15.0

Table 17
 Mean biotic index scores of fall and spring samples at high gradient sites, including 95% confidence intervals

Site	Fall		Spring	
	Mean BI	95% CI	Mean BI	95% CI
AR2	49	8.2	29	11.4
AR3	70	7.3	63	16.6
AR4	85	11.0	59	15.1
AR5	65	2.6	34	8.9
AR6	66	3.2	34	10.0
AR7	31	8.8	26	12.7
BC1	50	5.4	32	11.2
BC2	64	5.8	68	9.2
BC3	68	7.6	74	10.0
DC02	40	8.7	26	14.2
DC03	35	4.1	33	16.4
DC04	32	5.1	44	4.3
DC05	34	5.5	27	3.1
DC06	52	3.7	40	8.4
DC08	43	1.4	46	8.0
DC09	36	2.5	44	6.3
DC10	35	4.0	30	11.9
DC11	38	11.5	45	1.8
DC12	44	4.1	33	11.1
DC13	40	6.0	23	5.8
WW1	43	4.1	56	3.6
WW2	40	5.2	31	8.5
WW3	45	1.0	43	7.2

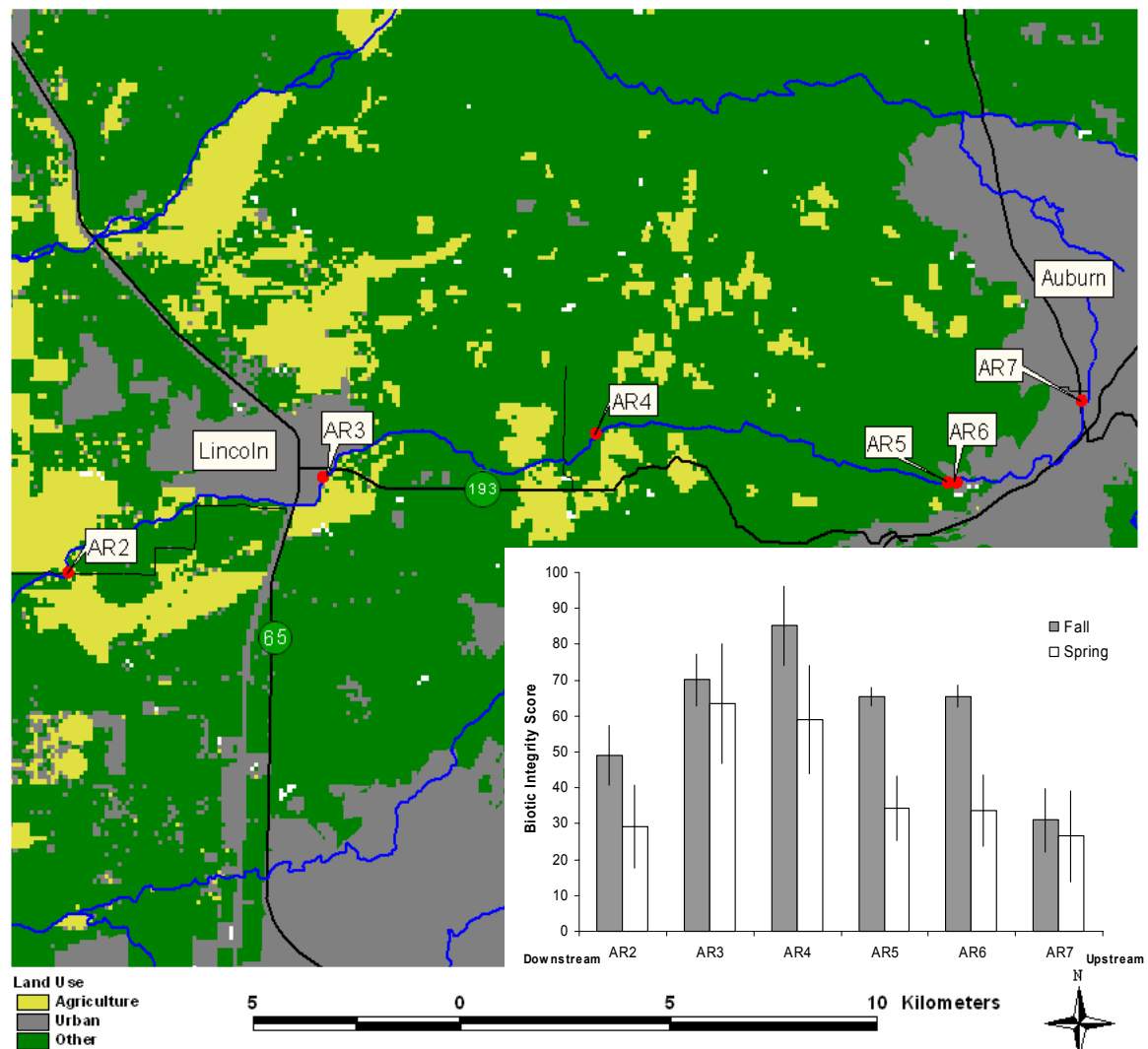


Fig. 20. Biotic index scores in Auburn Ravine waterway. Each bar represents the mean BI score and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

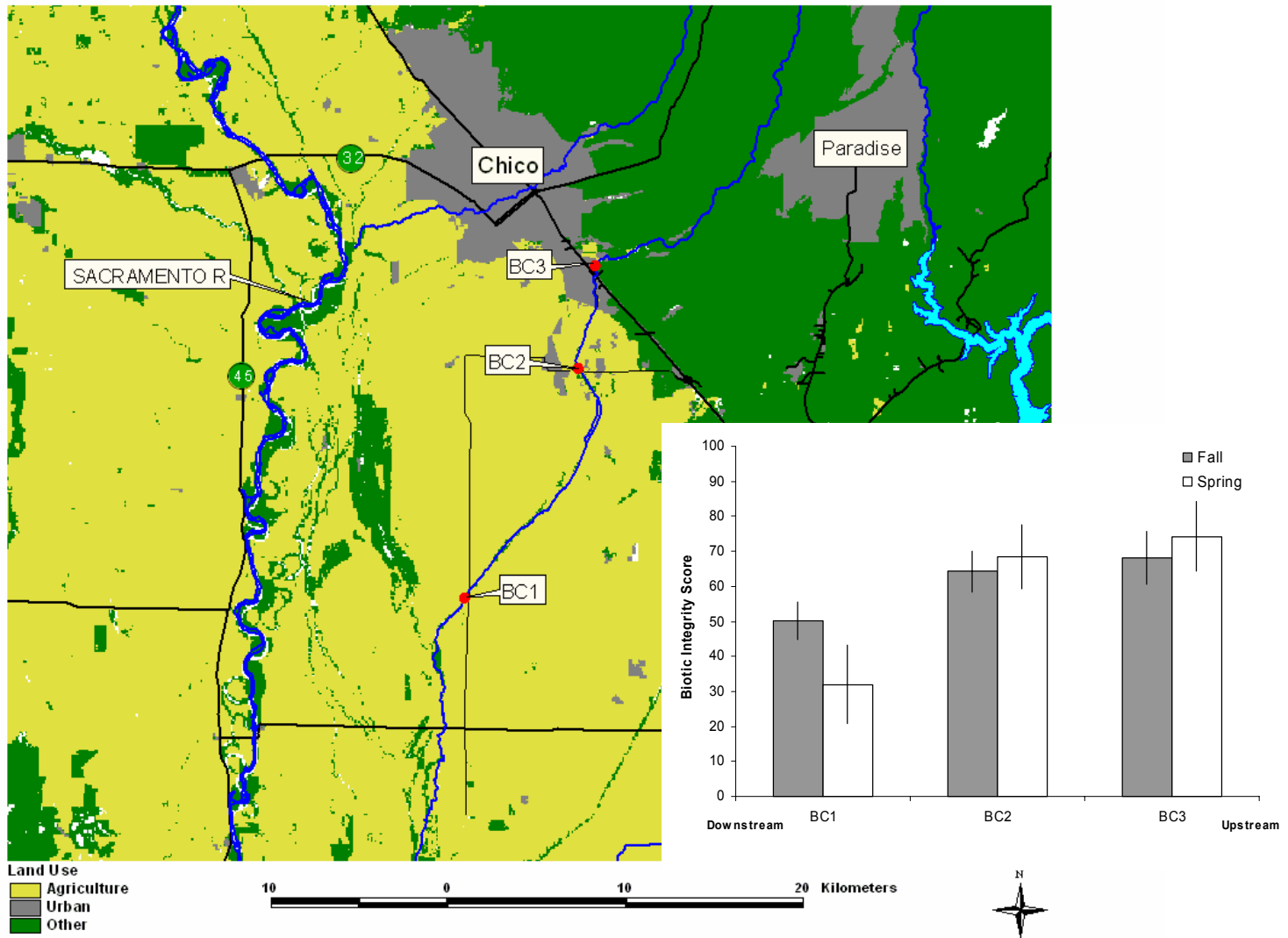


Fig 21. Biotic index scores in the Butte Creek waterway. Each bar represents the mean and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

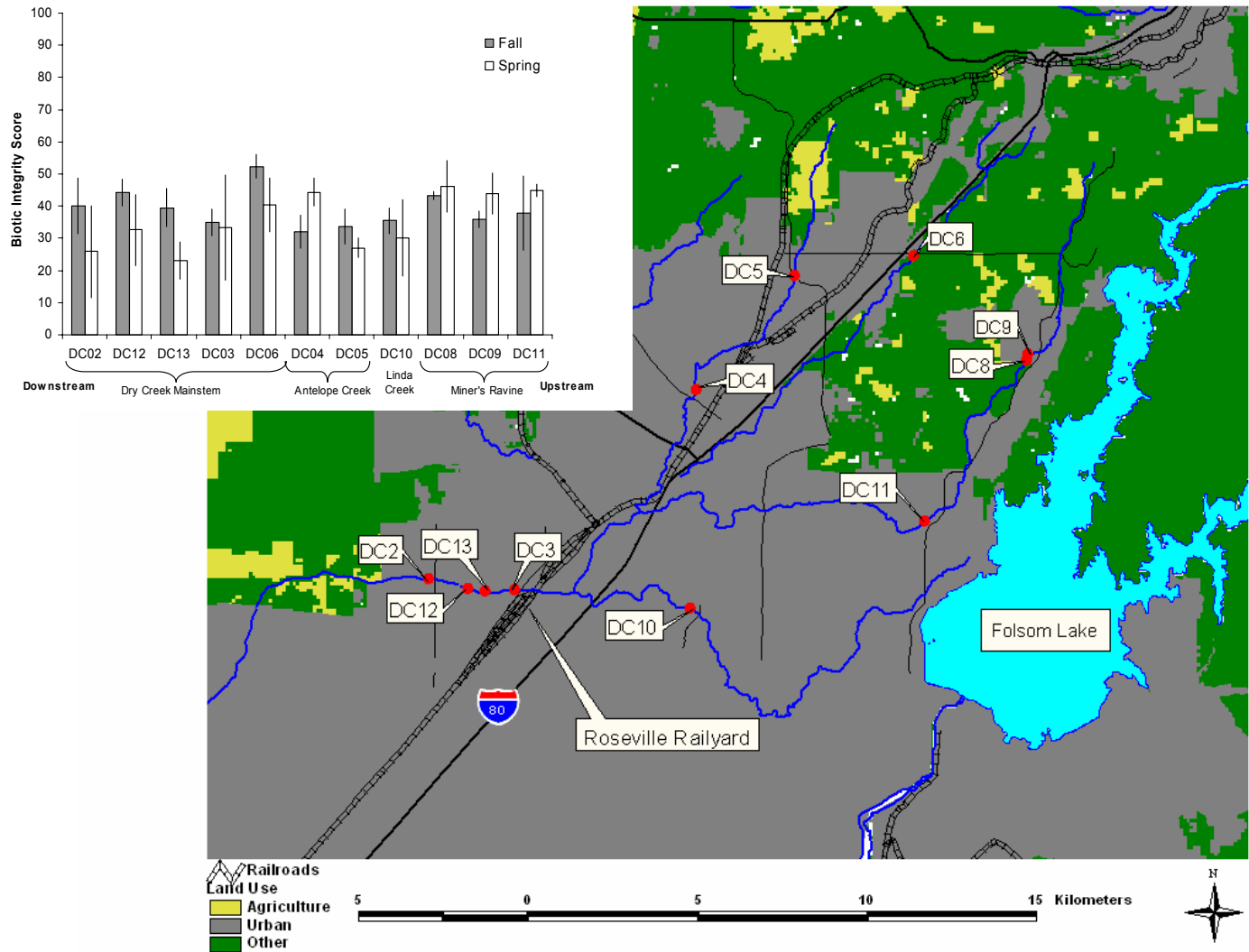


Fig. 22. Biotic index scores in Dry Creek waterway. Each bar represents the mean BI score and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

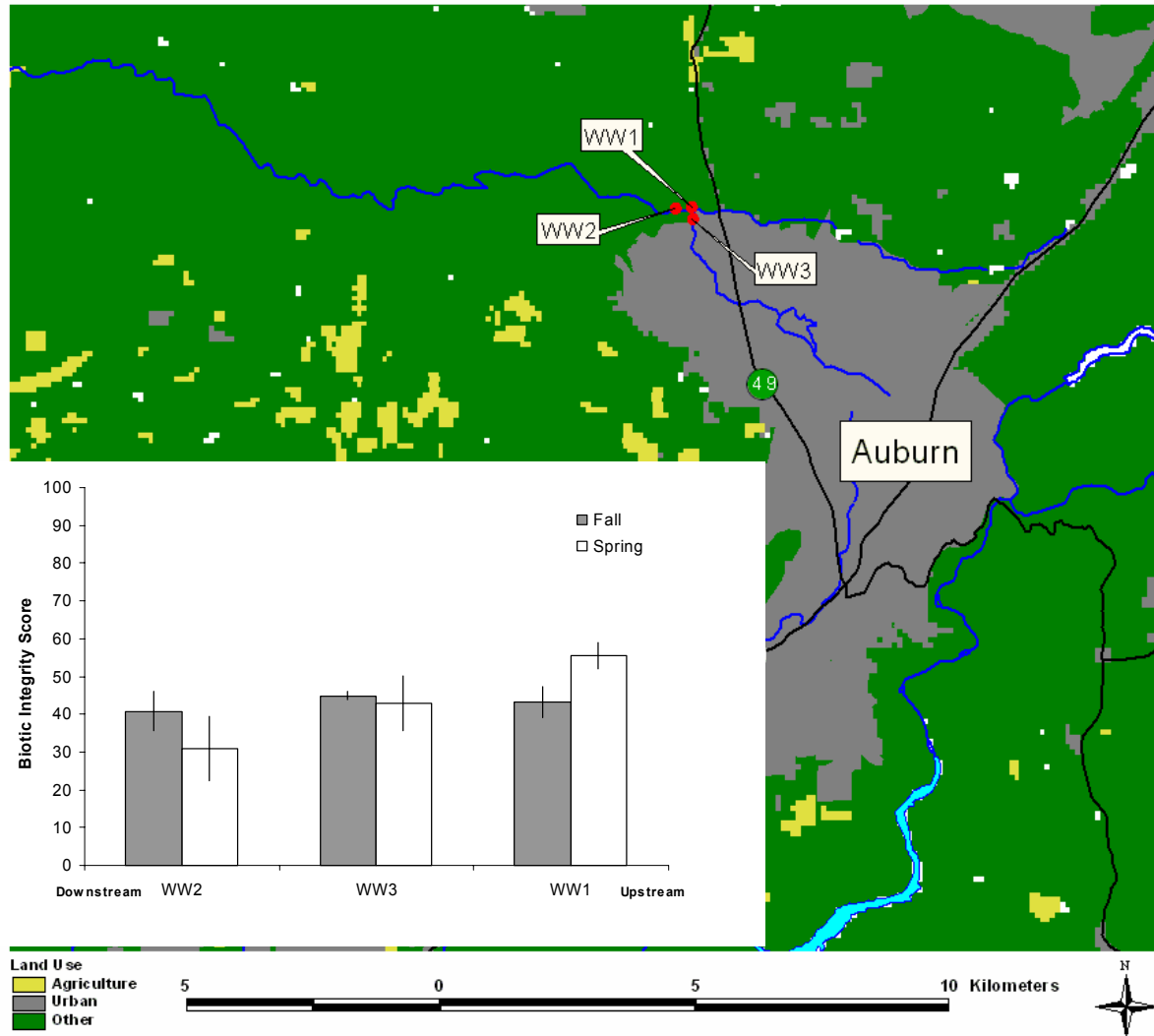


Fig. 23. Biotic index scores in Coon Creek waterway. Each bar represents the mean BI score and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

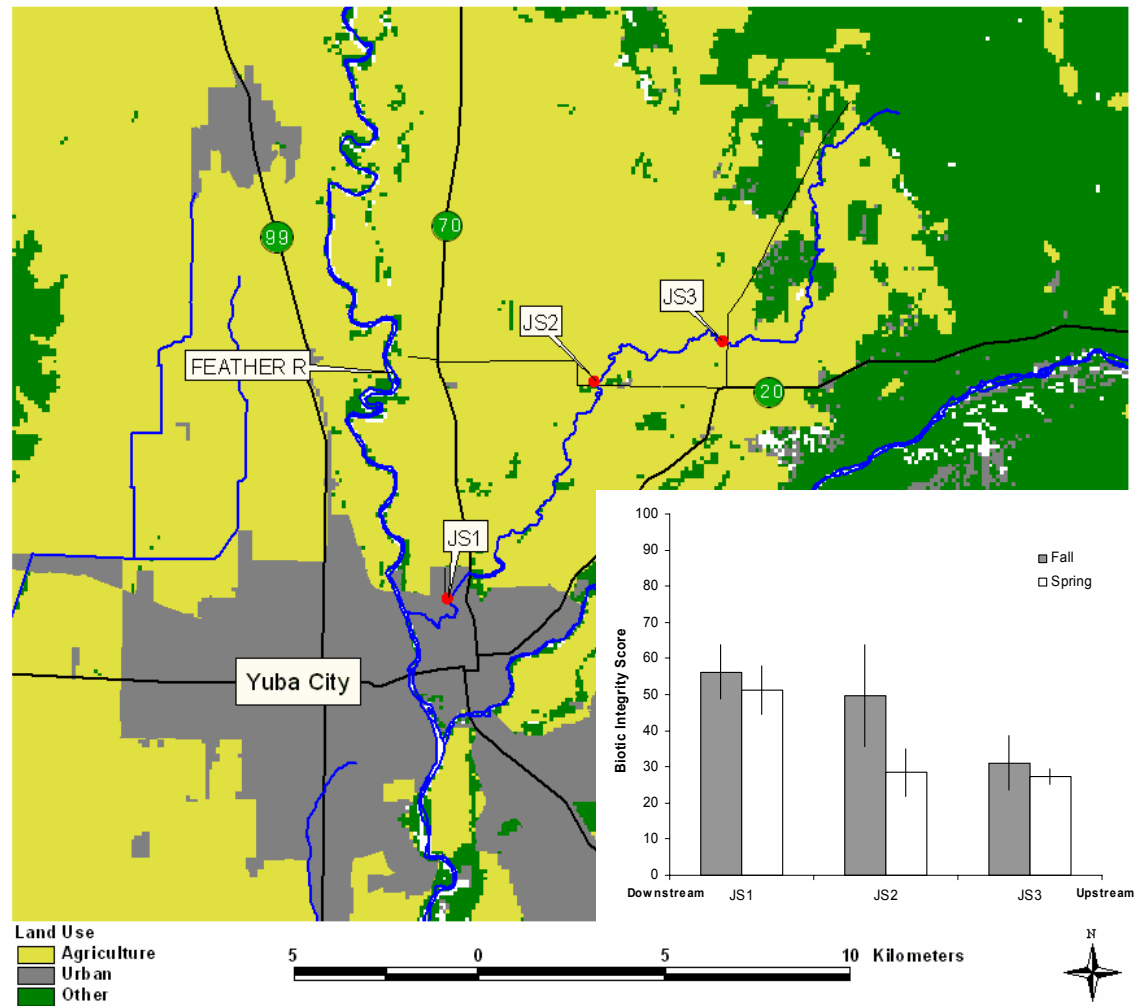


Fig. 24. Biotic index scores in the Jack Slough waterway. Each bar represents the mean and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

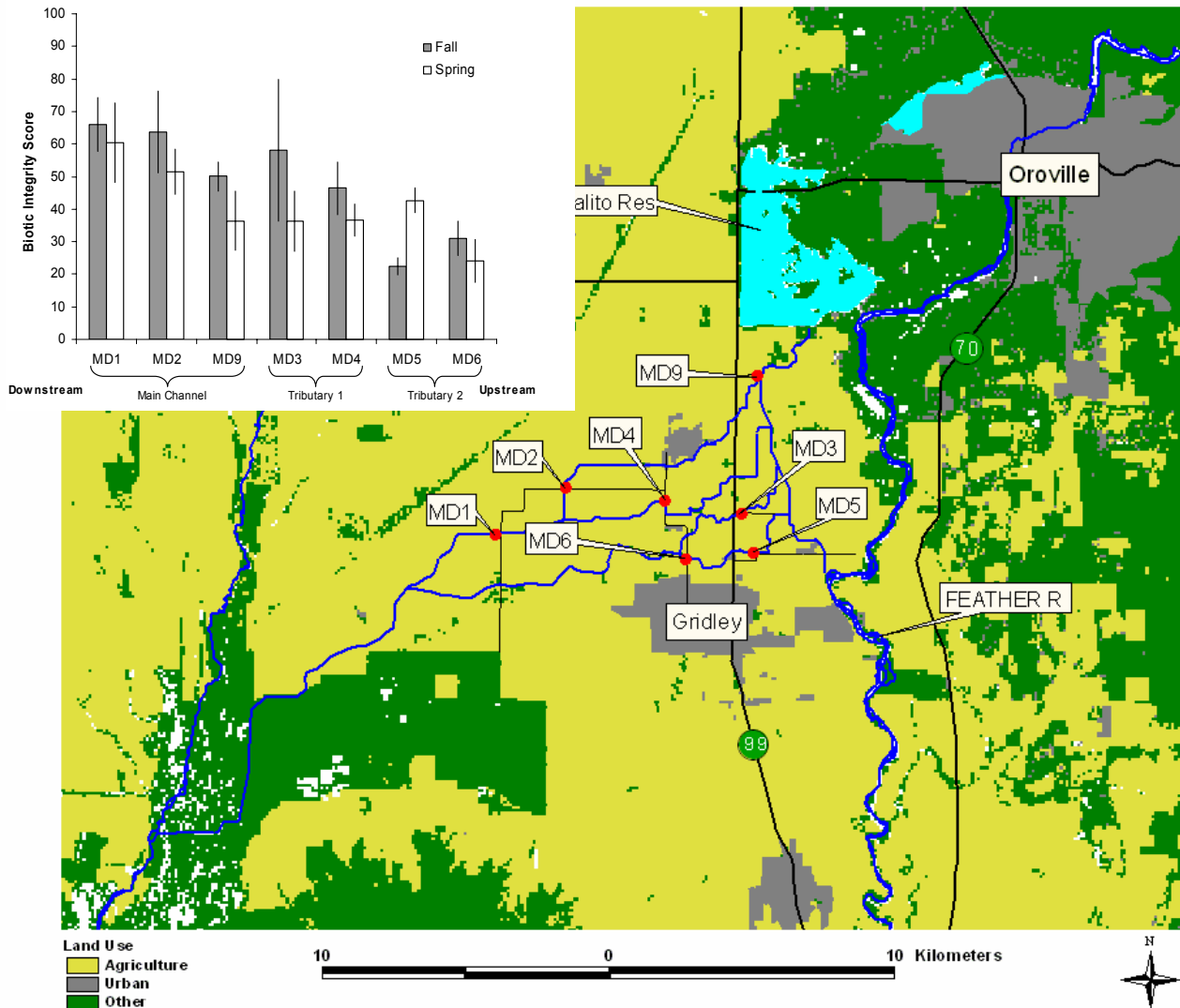


Fig. 25. Biotic index scores in Main Drainage Canal waterways. Each bar represents the mean and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

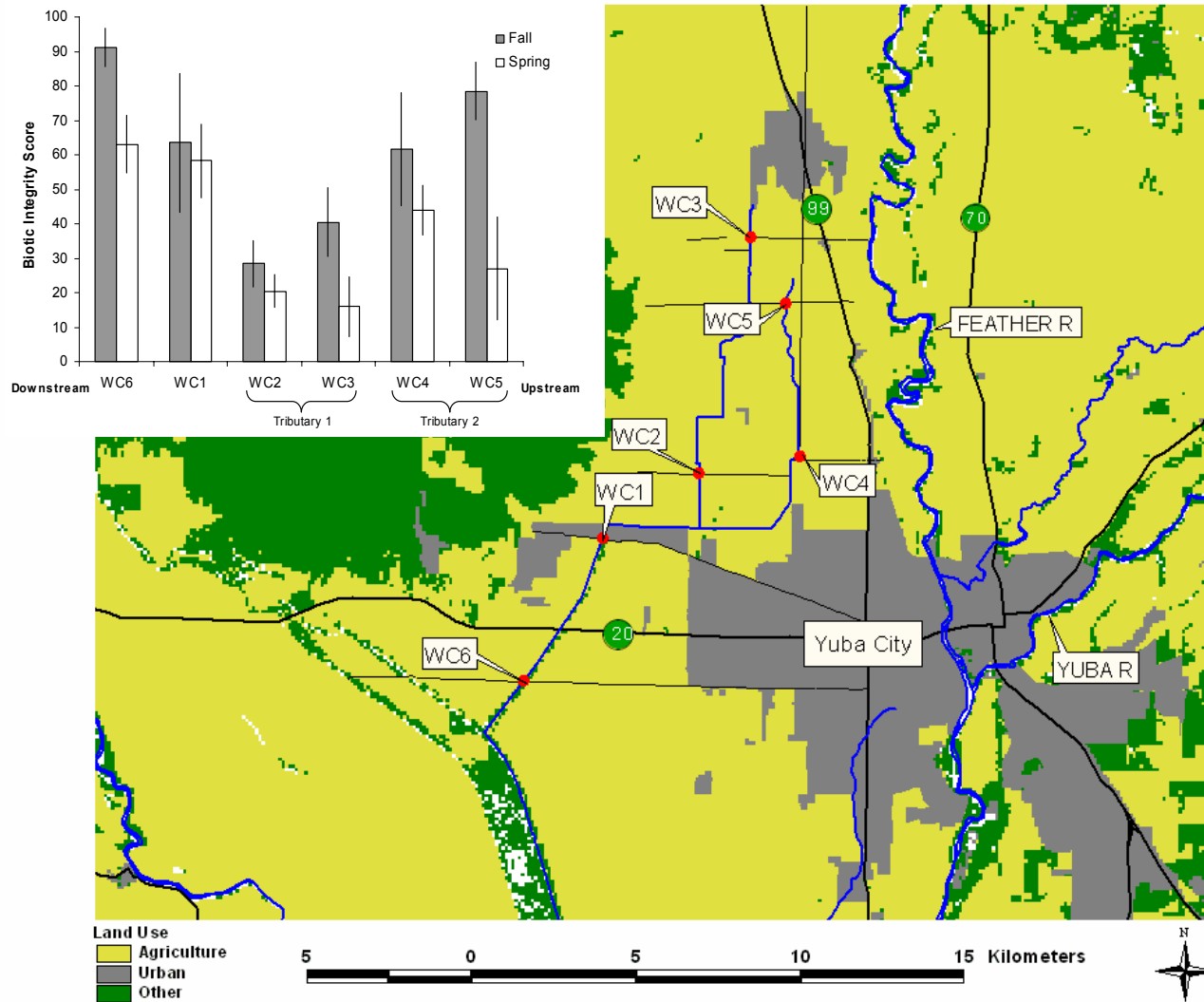


Fig. 26. Biotic index scores in Wadsworth Canal waterway. Each bar represents the mean and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

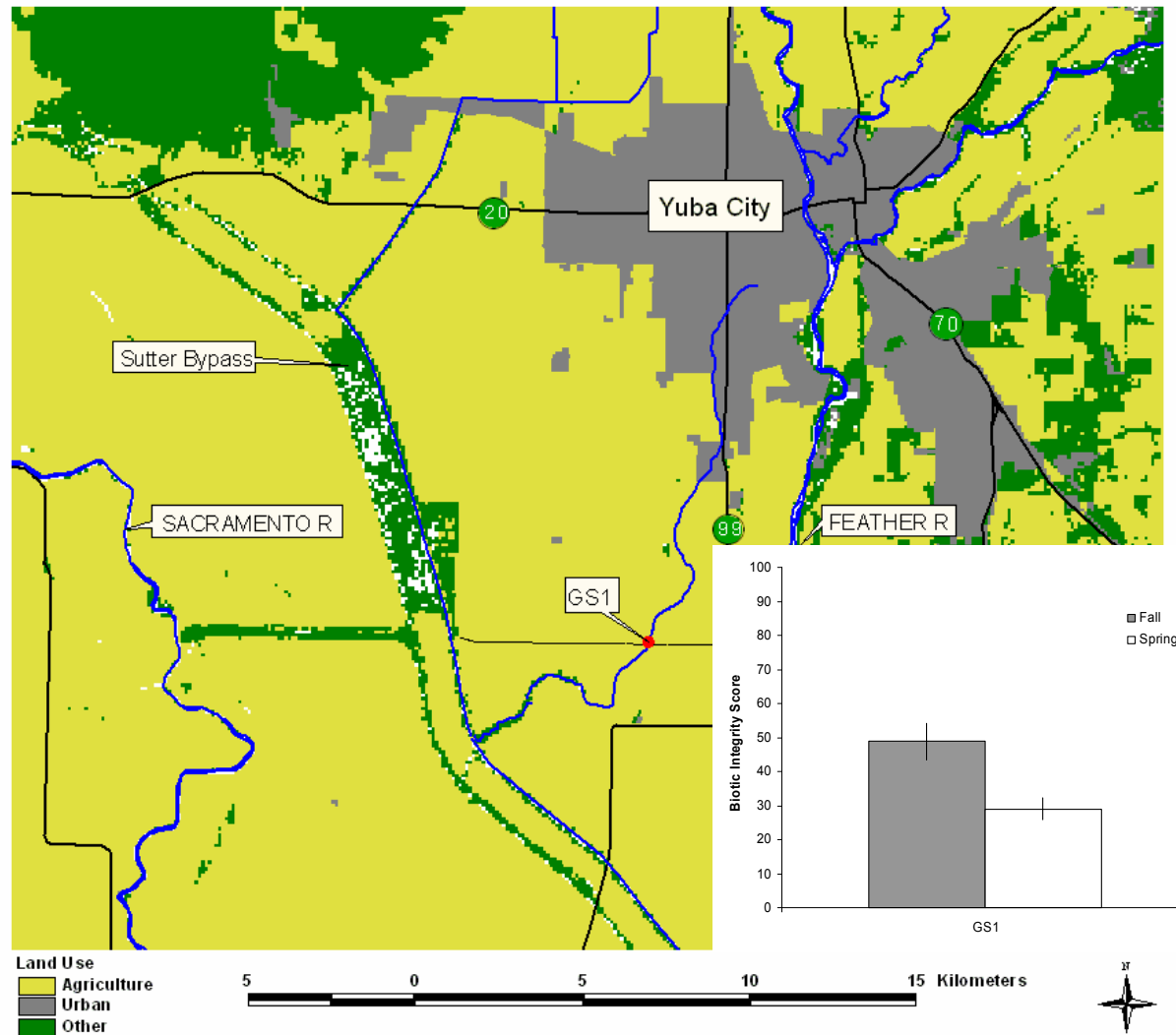


Fig. 27. Biotic index scores in the Gilsizer Slough waterway. Each bar represents the mean and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

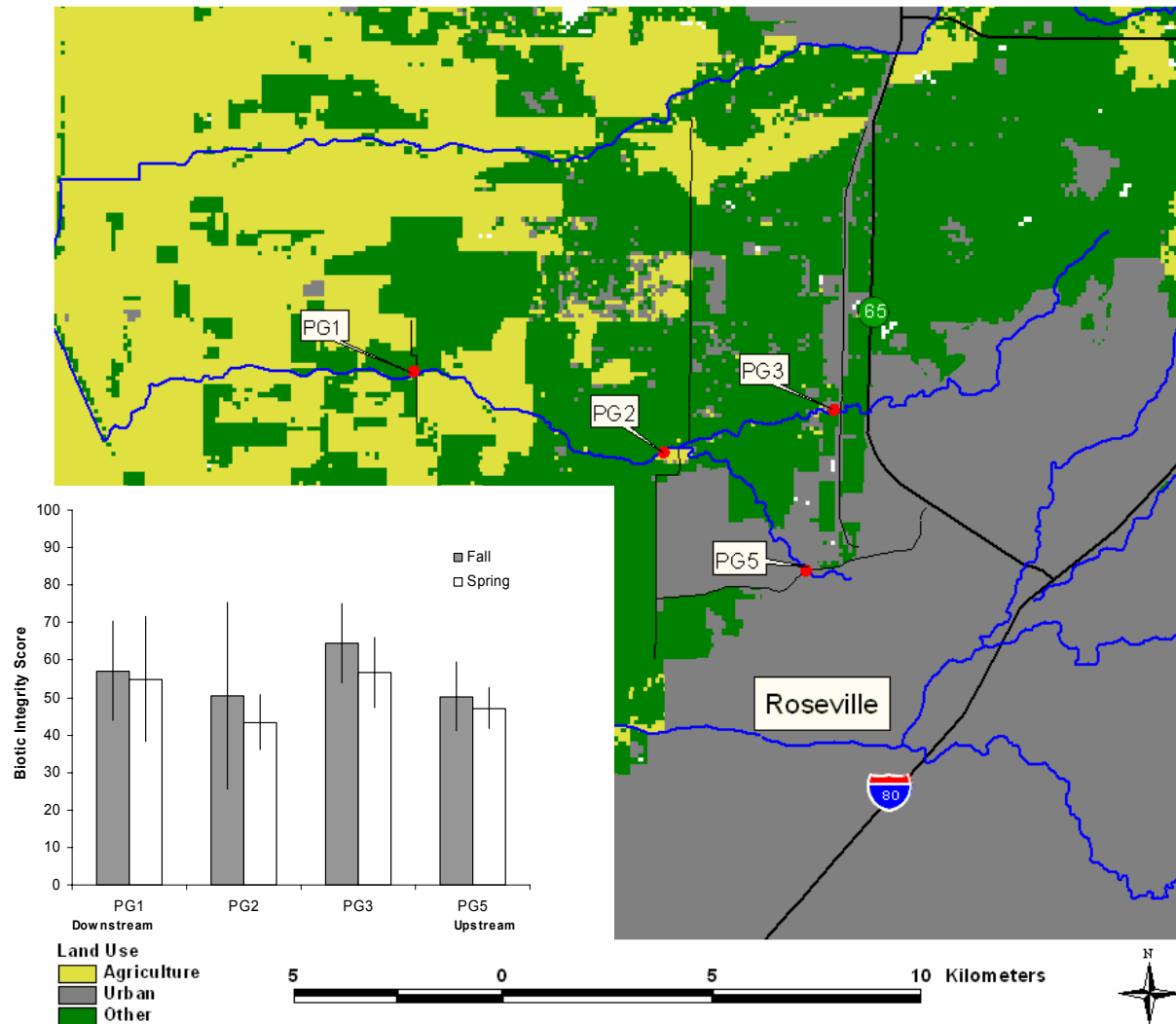


Fig. 28. Biotic index scores in the Pleasant Grove waterway. Each bar represents the mean and 95% confidence interval of the BI scores measured in a site during a given season (6 transects, 3 transects in each of 2 years).

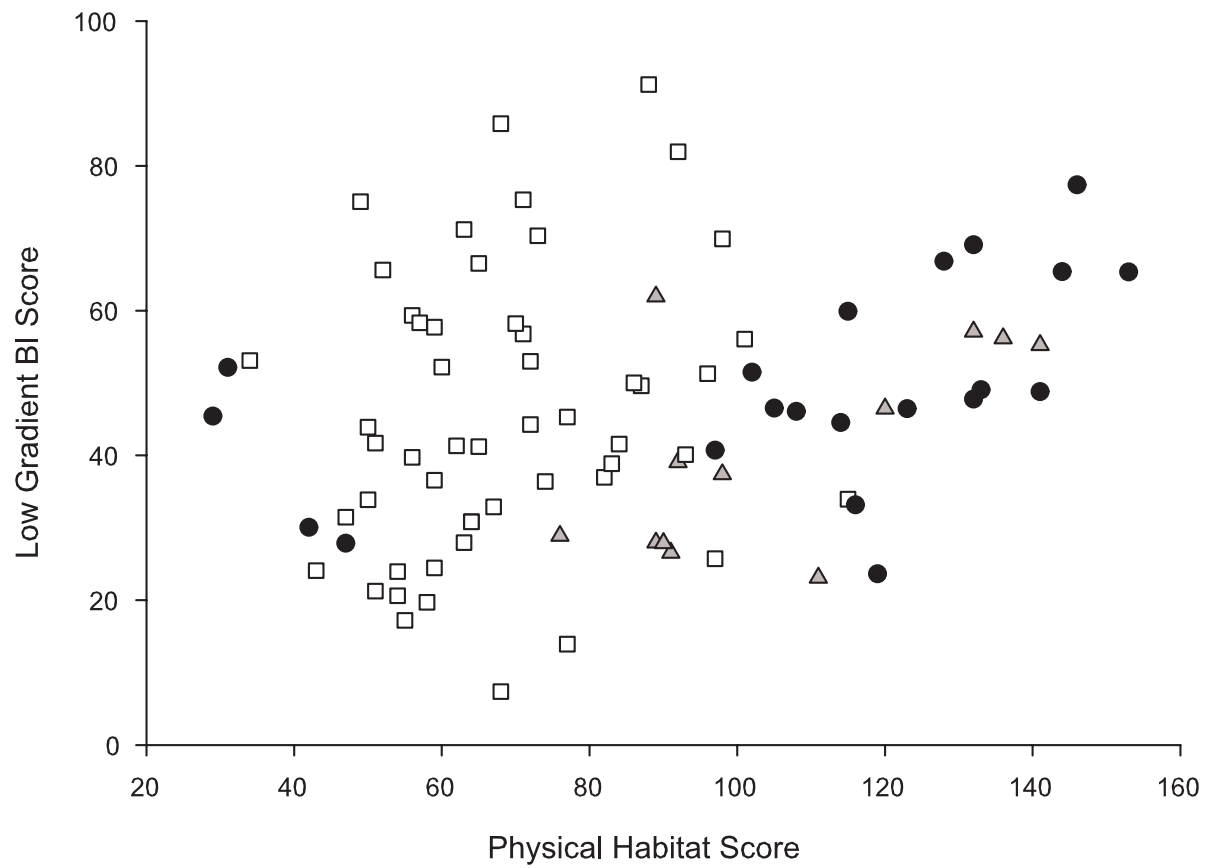


Fig. 29. Correlation of physical habitat score with low gradient BI score ($r^2 = 0.0871$). Points indicate fall cluster membership: ▲ = LG Ag 1, □ = LG Ag 2, ● = LG Ag/Urban.

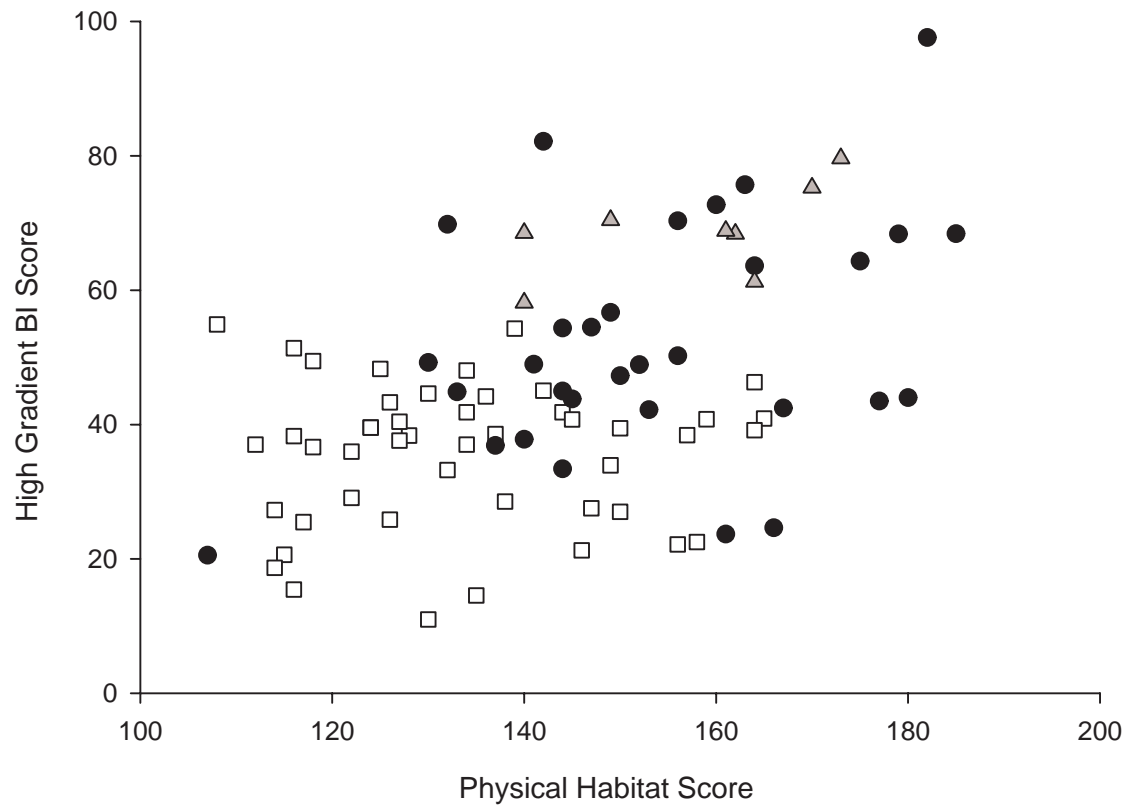


Fig. 30. Correlation of physical habitat score with high gradient BI score ($r^2 = 0.1806$). High sediment deposition score indicates little sediment deposition. Points indicate fall cluster membership: ● = HG Native 1, ▲ = HG Native 2, □ = HG Urban.

Table 18

BMI metrics included in season- and gradient-specific biotic indices created to discriminate more impacted sites from less impacted sites. Metrics marked with an asterisk (*) were considered indicators of impacted fauna, and scores on these metrics were weighted negatively in calculation of the biotic indices.

Low Gradient		High Gradient	
Fall	Spring	Fall	Spring
Taxonomic Richness	Shannon Diversity	EPT Taxa	Shannon Diversity
Ephemeroptera Taxa	EPT Index	EPT Index	EPT Taxa
Trichoptera Taxa	Trichoptera Taxa		EPT Index
ETO Index	ETO Taxa		
% Intolerant		% Intolerant	
% Baetidae		% Grazers	% Grazers
% Insects	% Insects	% Insects	
Tanytarsini/Chironomini	Tanytarsini/Chironomini		
% Multivoltine *	% Multivoltine *	% Multivoltine *	
% Dominant Taxon *	% Oligochaeta *	% Chironomidae *	
Tolerance Value *		Tolerance Value *	% Tolerant *
	% Collectors *	% Filterers *	% Filterers *

pattern included Baetidae, *Tricorythodes*, *Hydropsyche*, *Heptagenia*, *Wormaldia*, and Odonata. In general, the filter feeding niche was filled by *Hydropsyche* in fall, but by *Simulium* in spring. Since *Hydropsyche* is a trichopteran and *Simulium* is a dipteran, this shift reduces scores on metrics measuring the richness and abundance of EPT taxa. The pattern of low BI scores in spring at high gradient sites appeared to be related to higher abundances of Naidid oligochaetes and chironomid taxa. Both oligochaetes and chironomids factored negatively into the BI scores at high gradient sites.

Other common seasonal shifts that occurred in both low and high gradient sites were Tanytarsini in fall giving way to Orthoclaadiinae in spring (both chironomids), and Tubificidae in fall shifting to Naididae in spring (both oligochaetes). Since these shifts were between related taxa, they did not affect BI scores, except for the Tanytarsini/Chironomini ratio.

The signal/noise ratios of fall and spring BIs were compared to evaluate which BI gives more power to detect site to site differences in BMI community integrity. The low gradient spring BI had more power to detect differences between sites, while the high gradient fall BI had more power to detect differences between sites (One-way ANOVAs: low gradient fall: $F_{21,101} = 5.9417$, low gradient spring: $F_{22,109} = 9.8593$, high gradient fall: $F_{26,102} = 16.4742$, high gradient spring: $F_{24,119} = 7.6293$).

3.10.2 Summary by watershed/waterway

Auburn Ravine and Butte Creek, the two waterways with the least impacted land use profiles and instream habitats, scored highest on measures of BMI community integrity (BIs). Dry Creek is a more urbanized, more impacted high gradient waterway, and sites on Dry Creek received lower BI scores. In the comparison of sites surrounding Coon Creek SMD 1 WWTF, decreases in BI score were detected downstream of the WWTF. Differences in BI scores between low gradient waterbodies were small, but sites on Pleasant Grove Creek, the low gradient waterbody with the least amount of agricultural land use and the greatest amount of native vegetation, manifested higher BI scores than

most ADW sites. Some of the sites on low gradient ADWs scored lowest on the low gradient biotic index.

3.10.2.1 Auburn Ravine (Fig. 20)

Auburn Ravine is a natural waterway that originates in the Sierra Nevada foothills near the City of Auburn (396 meters elev.). The stream flows west until discharging into the East Side Canal, and finally the Sacramento River. The upstream reaches of Auburn Ravine are dominated by wastewater treatment plant effluent and urban runoff from the City of Auburn. The most downstream portion of Auburn Ravine, below the City of Lincoln (50 meters elev.), is dominated by agricultural activities during irrigation season. All BMI sampling sites were located upstream of major agricultural activities in the valley floor.

As a whole the Auburn Ravine waterway scored high in measures of BMI community integrity. Sites AR3 and AR4, both high gradient sites with favorable instream habitat and distant from urban areas, manifested the highest BIs. BIs at AR2, the most downstream Auburn Ravine site, were somewhat lower. AR2 is lower gradient than the other Auburn Ravine sites and consisted mostly of fine substrates, some gravel, and some woody debris. AR5 and AR6, immediately downstream of the city of Auburn and bracketing the Auburn WWTF, were comprised of similar taxa and characterized by equivalent BI scores. BMI integrity scores were not significantly different between the two sites (fall: Wilcoxon rank sum test, $N = 9$, $P = 0.1113$; spring: t-test, $t_{10} = 0.555$, $P = 0.5909$). Few gastropods and many Chironomids and *Wormaldia* (Trichoptera) were noted at AR6 (upstream of the WWTF), while at AR5, gastropods (Planorbidae) were numerous and Chironomids and *Simulium* were uncommon. The lowest BIs in Auburn Ravine were at AR7, in the center of the Auburn urban area. A large portion of water flowing through AR7 originates in storm drains of roads and parking lots. AR7 was relatively taxa rich, but not EPT rich. A large influx of water enters the stream at a Pacific Gas and Electric hydroelectric power station between AR7 and AR6. This water is likely to be unpolluted relative to the storm drain water from the city of Auburn, and may be the cause of the sizable increase in BI scores downstream of AR7.

3.10.2.2 Butte Creek (Fig. 21)

Butte Creek originates in the Sierra Nevada range east of the City of Chico (60 meters elev.). Butte Creek is a natural waterway that flows into Butte Slough, the heavily aligned Sutter Bypass, and Sacramento Slough before reaching the Sacramento River. For the majority of the irrigation season, April through September, lower Butte Creek and Butte Slough are dominated by supply and return flows for agricultural irrigation.

Butte Creek is the only snowmelt driven agriculture-dominated waterway, as opposed to irrigation supply and return water driven, sampled in this study. The upstream most BMI sampling site, BC3, is located adjacent to the City of Chico. At sampling site BC2, Butte Creek is considered agriculture-dominated due to volume of irrigation supply and return flows, and is surrounded by intensive agricultural land use. However, large (> 5 meters high) levees are located between the creek and surrounding agricultural land (rice and orchards). The most downstream BMI sampling site on Butte Creek (BC1) is located at the transition from Butte Creek into Butte Slough. This transition zone is characterized by a slower flowing, deeper, and warmer waterway. Further, the substrate changes from coarse boulder, cobble, and gravel to more fine material such as silt and clay in this transition zone.

BI scores of upstream sites BC2 and BC3 were high, while BIs of BC1 were lower. Habitat at BC2 and BC3 was very good, with large percentages of cobble substrates. These sites are upstream of most agriculture, though some orchards are nearby. While BIs at most other sites were high in fall, BC2 and BC3 scored somewhat higher in spring. These two sites receive a high volume of cold water from snowmelt in spring. While many other sites were characterized by fewer EPT taxa in spring, cold water Ephemeroptera taxa such as ephemereids and heptageniids increased at BC2 and BC3 in spring. BC1 is surrounded by agricultural land. BC1 followed the usual seasonal pattern of lower BIs in spring. The fall to spring taxonomic shift included a change from many Ephemeroptera (particularly *Tricorythodes*) to many Chironomidae.

3.10.2.3 Dry Creek (Fig. 22)

Dry Creek is an urban-dominated watershed that originates from a number of small creeks in the Sierra Nevada foothills east of Roseville (49 meters elev.). Dry Creek flows into Natomas East Main Canal and eventually the Sacramento River at Discovery Park. The Dry Creek Watershed covers approximately 74,000 acres, 8,700 acres of which are located within the City of Roseville. The primary streams in the Dry Creek Watershed include Dry Creek, Cirby Creek, Linda Creek, Strap Ravine, Miner's Ravine, Secret Ravine, Antelope Creek, and Clover Valley Creek. Portions of Dry Creek are effluent-dominated certain times of the year (primarily during low flow summer conditions). There are a number of wastewater treatment plants discharging within the Dry Creek Watershed. BMI community integrity was assessed above and below the Placer County Sewer District 3 (SMD3) wastewater treatment plant in Miner's Ravine and the City of Roseville wastewater treatment plant on the lower mainstem reach of Dry Creek. In addition, BMI sampling sites were selected in most major tributary streams of Dry Creek.

The Dry Creek watershed is almost completely urbanized with many point and non-point sources of possible contamination. BMI community integrity at all sites along Dry Creek with the possible exception of DC6, the most upstream site, appeared impacted relative to the most diverse BMI communities examined in this study. DC2 and DC12 were located downstream of a WWTF. DC13 was immediately upstream of the WWTF, while DC3 was located upstream of the WWTF and downstream of a railroad yard in the city of Roseville. Communities at sites DC12 and DC13 were similar, with dominant populations of Chironomidae and Naididae. Filter-feeding *Hydropsyche* and *Nectopsyche* were more common at DC12, as would be expected at a site enriched with organic matter. Higher populations of filter-feeding trichopteran may explain the somewhat higher fall BI score at DC12 relative to DC13. BI scores of the upstream sites were not significantly different than those of downstream sites (fall DC3 – DC2: t-test, $t_{10} = 0.236$, $P = 0.8185$; fall DC13 – DC12: t-test, $t_4 = 2.538$, $P = 0.0641$; spring DC3 – DC2: t-test, $t_{10} = -0.339$, $P = 0.7417$; spring DC13 – DC12: t-test, $t_{10} = -0.352$, $P = 0.7319$). The similar BI scores of these four sites suggest that the WWTF they surround had no discernable effect on BMI community integrity. DC4 and DC5 are located in

Antelope Creek, a natural creek with some cobble substrate and some depositional habitat. At DC4, BIs indicated relatively impacted biological conditions despite cobble substrate. The higher BI score in spring was related to a seasonal shift from clams and flatworms (fall) to *Hydropsyche* and *Baetis* (spring). Though water at DC5 was fairly clear and many crayfish were present, BIs at this site indicated an impacted community. DC6 was the least biologically impacted site on Dry Creek. Substrate consisted of cobble that was well-consolidated. BMI fauna was diverse. DC10, on Linda Creek, was located in central Roseville; BI scores were low and equivalent to sites on Dry Creek. Sites DC8, DC9, and DC11 were located in Miner's Ravine. At all these sites BI scores were higher in spring than in fall, divergent from the common pattern. DC8 and DC9 bracketed a WWTF, but BI scores were similar at both sites. DC8 had marginally higher BI scores in the fall, but BIs did not differ in spring (fall: t-test, $t_4 = 2.867$, $P = 0.0456$; spring: t-test, $t_{10} = -0.523$, $P = 0.6122$). The seasonal pattern at sites DC8 and DC11 relate to higher EPT abundances in the spring, particularly *Hydropsyche*. The higher spring BI at DC9 was related to greater taxonomic diversity.

3.10.2.4 Coon Creek SMD 1 WWTF (Fig. 23)

Coon Creek originates in the Sierra Nevada foothills north of the City of Auburn (396 meters elev.). Coon Creek is a natural, ephemeral, creek that flows west to the East Side Canal, and finally the Sacramento River. Upstream reaches of Coon Creek are dominated by wastewater treatment plant effluent and urban runoff. The BMI sampling sites in the upper Coon Creek watershed were selected to assess BMI community integrity upstream and immediately downstream of the Placer County Sewer Maintenance District 1 (SMD1) Wastewater Treatment Facility (WWTF). Site WW1 is located in the upper Coon Creek watershed upstream of the Placer County SMD 1. WW2 is downstream of the WWTF and WW3 is situated on a tributary that flows into the main waterway between the WWTF and WW2. A reverse seasonal pattern was observed at WW2 due to high populations of *Hydropsyche* and *Wormaldia* (Trichoptera) in spring. Overall, the downstream site WW2 showed lower BMI community integrity than the upstream sites. In fall, WW1 and WW2 did not have significantly different BI scores (t-test, $t_4 = 1.852$, P

= 0.1376), but WW2 tended to score lower. In spring, BIs were much lower at WW2 than at WW1 (t-test, $t_{10} = 7.999$, $P < 0.0001$). BI scores at WW2 were lower than at WW3 in both seasons (fall: t-test, $t_4 = -6.061$, $P = 0.0037$; spring: t-test, $t_{10} = 0.0006$, $P = 0.0006$).

3.10.2.5 Jack Slough (Fig. 24)

Jack Slough is a small agriculture-dominated watershed north of Marysville (19 meters elev.) that is tributary to the Feather River. The slough is bounded between the Feather River on the north and west, and the Yuba River on the south. The slough is a modified natural waterway that has been extensively realigned and reconstructed along its lower portion for flood control and transport of agricultural supply and return water. The upper portion of Jack Slough, upstream of Trainer Hills, flows in its original channels and contains irrigation supply water from the Yuba River. We did not sample this portion of the slough.

During irrigation season (March – August) the lower portion of Jack Slough is composed of supply water mixed with return water from surrounding rice fields, and likely would not flow without agricultural inputs. During the winter months, Jack Slough contains stormwater runoff from surrounding agricultural lands and contains drainage from local waterfowl wetland areas. BMI sampling sites on Jack Slough were selected to reflect a gradient of agricultural land use. Sites JS2 and JS3 are immediately adjacent to rice fields. JS1, the most downstream sampling site, is located in a semi-natural, less managed, riparian area that is less than 2.5 kilometers upstream of the confluence into the Feather River. A BI score gradient was observed in Jack Slough, from relatively intact downstream BMI communities to relatively impacted upstream communities. This gradient was associated with declining abundance of coarse substrates, lower water quantities in the channel, and increasing abundance of submerged macrophytes. While submerged macrophytes created more potential BMI habitat, the abundance of macrophytes probably created anoxic conditions that were partially responsible for lower BIs. Jack Slough BMI fauna was dominated by *Crangonyx* amphipods, oligochaetes, and chironomids.

3.10.2.6 Main Drainage Canal (Fig. 25)

The Main Drainage Canal (Main Drain) consists of a network of modified natural (Hamlin Slough) drainage channels, historically natural drainage channels, and partially constructed laterals that have been extensively aligned and modified for conveying irrigation supply and return water for surrounding agricultural land practices. Irrigation water is supplied to the Main Drain from the Sutter Butte Canal that contains Feather River water from the Thermalito Afterbay. Flow in Main Drain goes into Cherokee Canal and then enters Butte Sink, Butte Slough, and eventually Sacramento Slough and Sacramento River. Peach and prune orchards are prominent in the upper watershed, while rice dominates in the lower Main Drain watershed. The primary channel of the Main Canal (Hamlin Slough) flows through the small town of Gridley (28 meters elevation) at sampling site MD7.

Stream bank channels in the Main Canal are unstable due to a lack of riparian cover related to agricultural practices including close proximity of crops, and heavy physical stream channel maintenance conducted to maintain adequate irrigation and return water flows. All sampling sites within the Main Drain have substrates dominated by mud. Tolerant taxa are ubiquitous in the Main Drain, especially chironomids and oligochaetes. Similar to other low gradient agriculture-dominated waterways, the Main Drain manifested the trend of having higher BI scores at the more downstream sites. This pattern was most likely related to the increase in substrate and physical habitat quality at the downstream sites (MD 1 and MD 2) compared to the upstream sites (MD3 to MD8).

MD1 was the most downstream site on the Main Drain system and is subject to the cumulative effects of seasonal irrigation and return water augmentation, stormwater pulses, and local land management practices throughout the watershed. MD1 and MD2 are both depositional habitats, although MD 1 is subject to extremely variable flow conditions. BI scores at MD1 and MD2 were the highest in the Main Drain. These sites also consisted of the best habitat and substrates in this waterway, including some gravel and abundant submerged macrophytes. BI scores at sites MD3 and MD4, both located on

one lateral of the Main Drain, were intermediate. Sites MD5 and MD6, both located on another lateral of the Main Drain, were characterized by extremely impacted BMI communities. Sites MD3, MD4, MD5, and MD6 were adjacent to agricultural land use. While site MD3 consisted of some gravel and rip-rap substrates mixed with mud and site MD5 appeared to be more eutrophic than other Main Drain sites, no major differences in habitat or substrate were noted. This may indicate that differences in water quality were responsible for much of the variation in BI scores among upstream sites in the Main Drainage Canal.

MD9 was located at the nexus of the Main Drain and the Sutter Butte supply water canal, and upstream of most agricultural return water inputs. BIs at MD9 were not particularly high compared to other sites on the Main Drain. Regardless of intermediate BI scores, taxonomic diversity at MD9 was greater than at all other agriculture-dominated, low gradient sites. During this study, MD9 was not entirely removed from agricultural practices effects. On 18 January 2001, a cropduster applying esfenvalerate (a pyrethroid insecticide) to orchards oversprayed resulting in direct application on the Main Drain at MD9. At or near this site, esfenvalerate concentrations exceeded 2070 ng/L in the surface microlayer and subsurface esfenvalerate concentrations reached 23 ng/L (Kuivila, pers. comm.). Large numbers of dead aquatic insects were observed drifting in the insecticide plume at MD8, including many Ephemeroptera, Plecoptera, Trichoptera, Odonata, and Diptera (located < 1 km downstream from MD9).

To examine temporal and spatial trends in the benthic community after the overspray, BMI samples were collected at MD9 and at the downstream sites MD8, MD7, and MD2 on 6 February (19 days post event), 27 March (48 days post event), and 10 May (104 days post event). While no clear pattern of recovery was observed, clear differences were observed among sites. These data show clear site-to-site changes in BMI community integrity, with MD9 and MD2, the most upstream and downstream sites, characterized by higher BI scores than MD7 and MD8 (Fig. 31). BI scores at MD2 were significantly higher in February than at later dates, while BIs were significantly lower in May than at

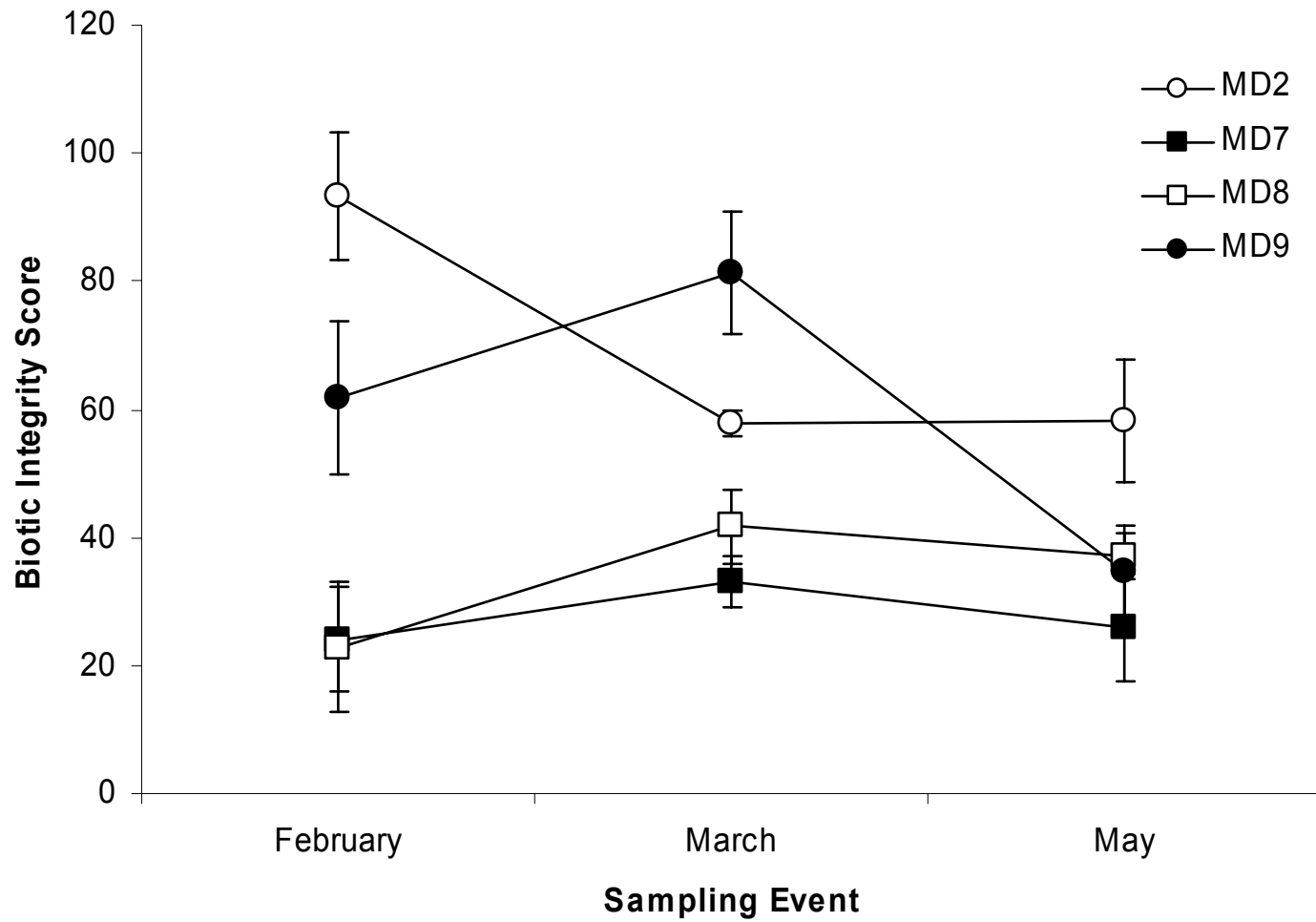


Fig. 31. Mean biotic integrity scores, bracketed by 95% confidence intervals (N = 3), at sites downstream of 18 January 2001 Main Drain over-spray event.

earlier dates. BI scores did not change significantly over time at either MD7 or MD8 (all comparisons: 2-way ANOVA with Tukey's multiple comparisons, $P < 0.05$).

Substrate and habitat were very poor at MD7 and MD8. This could account for the extremely impacted BMI communities (consistently low BI scores). These sites did not show "recovery" from the overspray. Substrate and habitat were relatively good at MD2, and this site manifested overall higher BI scores and more temporal fluctuation. There was no apparent recovery from the overspray event.

3.10.2.7 Wadsworth Canal (Fig. 26)

Wadsworth Canal is an agriculture dominated waterway that originates from water diverted from the Feather River, a natural creek, irrigation return water from extensive agricultural land east of the Sutter Buttes, and rainfall events. Wadsworth Canal consists of a number of small laterals that, historically, may have served as natural flow routes for rainfall runoff. Wadsworth Canal flows into the Sutter Bypass and Sacramento Slough before reaching the Sacramento River. Live Oak Slough is a natural tributary of Wadsworth Canal, which is a historical flood channel of the Feather River. There are currently no natural flows in Live Oak Slough. Live Oak Slough carries urban runoff and agricultural return water.

Habitat scores and substrates at Wadsworth Canal sites were comparable to those in the Main Drain, yet BMIs at some Wadsworth Canal sites were more diverse and less tolerant than at Main Drain sites. The spatial pattern of BI scores in Wadsworth Canal was as in the Main Drain and Jack Slough, with downstream sites manifesting higher scores. BI scores were highest at the two most downstream sites, WC6 and WC1. Sites WC2 and WC3, a tributary to Wadsworth Canal, were both extremely biologically impacted. These sites contained little water and their substrates were entirely composed of mud. In contrast, sites WC4 and WC5, on another Wadsworth Canal tributary, appeared only marginally more impacted than downstream sites. WC4 contained some cobble and gravel substrates and some emergent vegetation. WC5 was characterized by entirely sand substrates and masses of instream vegetation. WC5 was subject to some of

the lowest DO levels measured, possibly due to the large quantities of floating vegetation. It is striking that despite very anoxic conditions, WC5 harbored a more intact BMI community than sites WC2 and WC3.

3.10.2.8 Gilsizer Slough (Fig. 27)

Gilsizer Slough originates in Yuba City (18 meters elev.) and flows west in its original channel into the Sutter Bypass. Gilsizer Slough is a natural waterway that has an intensely managed riparian zone and channel. The slough is dominated by agricultural return flows during the irrigation season (March – October). Urban runoff from Yuba City at its headwaters and stormwater runoff during rain events are other sources of flow to Gilsizer Slough. The sampling site (GS1) on Gilsizer Slough was located in the lower agricultural dominated reach, and is adjacent to orchards. Flow is typically none to low in the slough, and aquatic habitat most resembles lentic waterbodies except during the winter rainy season.

Habitat scores at the Gilsizer Slough (GS1) site were the lowest of any in this study. Flow was very low. Chironomids and oligochaetes dominated.

3.10.2.9 Pleasant Grove (Fig. 28)

Pleasant Grove Creek originates near Loomis (121 meters elev.) and flows west before discharging into the Cross Canal, and finally the Sacramento River. Pleasant Grove Creek is a natural, ephemeral, creek that is dominated by urban runoff from the surrounding expanding development in Placer County. Agricultural return and supply water dominates Pleasant Grove Creek during the irrigation season (March – October) in the lower 4.5 miles from Pettigrew Rd. to the west. Only sampling site PG1 (Pleasant Grove Creek at Pettigrew Rd.) and PG2 (Pleasant Grove Creek at Fiddymment Rd.), the most downstream sites, are influenced by agricultural activities.

The Pleasant Grove waterway is low gradient, but surrounded by more native vegetation than the other low gradient waterways, and is subject to urban inputs including technology industry dischargers. The upstream-downstream gradient of increasing BI

scores seen in the more agriculture-dominated waterways was not observed on Pleasant Grove Creek. Among the Pleasant Grove sites, BI scores were highest at PG1 and PG3. PG1 is surrounded by native vegetation and characterized by the highest habitat scores of all low gradient sites. PG3 is located at a large pond with clear water and abundant macrophytes and algae. BI scores at PG2 and PG5 were slightly lower than at PG1 and PG3. Site PG2 is a relatively deep pond with low flow. Chironomids and worms (both tolerant of low DO) were dominant in both seasons at these sites. Site PG5 was near an urban area, receiving much urban runoff. This site was eutrophic and turbidity was high; chironomids, snails, and oligochaetes dominated. Despite the lack of intensive agriculture in the Pleasant Grove drainage, the BI scores at Pleasant Grove sites were lower than those at the least impacted agricultural drain sites (WC6 and MD1).

4. Discussion

This study was designed to describe BMI communities and aquatic habitats in agriculture- and urban (effluent)-dominated waterways in the lower Sacramento River watershed. Within these waterways sampling sites were selected to reflect a gradient of land use. Effluent dominated waterways included sampling sites above and below WWTFs. Analyses revealed that while the overriding difference in BMI communities occurred between high and low gradient waterways, much variation in BMI communities is present among sites of each gradient type. A major contribution of this study was to provide baseline information on BMI community structure and habitat information in high gradient and low gradient waterways of the Central Valley. In both high and low gradient waterways, cumulative effects of practices associated with agricultural and urban land uses were linked to differences in the BMI community. Agriculture and urbanization both affect waterway physical habitat and water quality that, in turn, impact biological communities. Following is a discussion and interpretation of our results and results of other relevant investigations including land use, physical habitat, water quality, identification of stressors, bioassessment data variability, seasonal variation, and level of taxonomic identification.

4.1 Waterway gradient

A large number of insect taxa, including many sensitive species of the EPT taxa, depend on fast-flowing, cold water for survival and health. Such sensitive taxa occur in high gradient streams, but conditions in low gradient, low elevation streams typically exclude the more sensitive EPT taxa. Our analyses identified indicator species representative of both high and low gradient waterways. Such information will be useful for future investigations in the lower Sacramento River watershed. Low gradient communities consisted primarily of chironomid midges, damselflies, beetles, and non-insects such as crustaceans, clams, and oligochaetes. High gradient communities included many mayflies and caddisflies, stoneflies of the genus *Bisancora*, Corbiculacea clams, *Argia* damselflies, and the pollution-sensitive beetle *Optioservus*.

When evaluating BMI communities or extent of perturbation, BMI data from sites with different gradients should not be compared. When constructing Indices of Biological Integrity (IBIs), care should be taken to select reference sites with equivalent gradient. Either separate IBIs should be created for low and high gradient streams in a region, or a multivariate model (e.g. RIVPACS, Clarke et al., 2003) should be constructed that accounts for gradient-specific differences in BMI communities. This conclusion is an extension of recommendations that ecoregion and watershed be considered in designing bioassessment studies and in interpreting data (Bournand et al., 1996; Bowman and Bailey, 1997; Omernik and Bailey, 1997; Brown and May, 2000).

BMI taxa at sites within individual waterways/watersheds tended to be similar. We are not suggesting that BMI data be independently interpreted for each waterway. Rather, an awareness of possible waterway-wide effects of stressors on BMIs may suggest that solutions to BMI community integrity issues require consideration of all activities in the watershed.

4.2 Land use

Wherever land is utilized intensively for agricultural or urban development, such activities affect the ecology of both terrestrial and aquatic systems. In wadeable streams such as those sampled in this study and in many other bioassessments conducted using similar methods, BMI metrics indicate impacted communities in areas of agricultural and urban development, where habitat, substrate, and water quality are degraded. Low-gradient streams subject to more intensive agriculture were characterized by lower diversity of insects, and higher numbers of chironomids and oligochaetes. High-gradient stream reaches subject to more intensive urban development consisted of fewer EPT taxa (especially pollution-sensitive EPT taxa) and greater numbers of chironomids. BI scores indicated that sites subject to intensive agriculture or urban development manifested lower BMI community integrity.

Other investigators have examined the association of agricultural and urban land use with BMI community structure and metrics. Brown and May (2000) discovered that agricultural and urban land uses were strongly associated (negative correlation) with macroinvertebrate community structure and metrics in the lower San Joaquin River watershed. Three streams in the Piedmont ecoregion of North Carolina were studied to evaluate the effect of land use on water quality and aquatic biota (Lenat and Crawford, 1994).

Lenat and Crawford (1994) examined three streams: one forest-dominated, one urban-dominated, and one dominated by agriculture. Only one site on each stream was sampled, but sites were sampled in January, April, June, and November. The three streams differed in regards to BMI community structure. The stream surrounded primarily by forests was characterized by high BMI richness, especially intolerant EPT groups (dominant taxa—mayflies), many unique species, and many intolerant species. Similar to our findings, the agriculture-dominated stream was characterized, as in the current study, by low EPT taxa richness, many tolerant taxa, and dominant populations of chironomid midges. Because land use in this watershed was 48% row crops and 31% forested, it is questionable that this stream effectively represented an agriculture-

dominated watershed. The urban stream site was the most highly stressed, characterized by low taxa richness, many tolerant species, low abundance values, and few unique species. Most species of mayflies, stoneflies, and caddisflies, numerous in forested areas, disappeared in agricultural and urban waterways. They were replaced by Chironomidae in agricultural areas and oligochaetes in urban areas. These authors attributed differences in BMI community structure in the three streams to water quality factors. However, neither instream habitat conditions nor riparian habitat were assessed in this study. Absence of such data confounds inferences regarding effects of water quality on BMI community structure.

Dance and Hynes (1980) studied invertebrate community composition in two stream reaches in Ontario, Canada where agricultural land use adjacent to the reaches varied considerably. These investigators concluded that intensive agricultural land use had profound effects on BMI community integrity. BMI community integrity was more impacted in the stream reach with more intense agriculture than in the reach with less intense agricultural activities. Further, BMI communities were less diverse in both agriculture-influenced streams than in unmodified streams of similar size and substrate.

A battery of *in situ* toxicity tests were used by Crane et al. (1995) to assess the effects of agricultural land runoff on water quality in a stream in the United Kingdom. The primary goal was to determine whether the *in situ* tests provided information consistent with BMI bioassessment data. Results of *in situ* toxicity tests with an amphipod and a midge (larval dipteran) were consistent with bioassessment data. Both confirmed impacts of agricultural runoff.

Roy et al. (2003a) investigated the effects of urban land use on BMI community integrity in the state of Georgia, and found that sites surrounded by more than 20 percent urban land use scored no higher than a “fair” rating on an IBI.

While the application of bioassessments to assess the effects of agricultural land use on aquatic ecosystem biological communities has been somewhat limited, other BMI

investigations document that farming activities degrade stream/river water quality and habitat, significantly impacting BMI communities (Kendrick, 1976; Corkum, 1990; Quinn and Hickey, 1990; Schofield et al., 1990; Delong and Brusven, 1998; Kay et al., 2001).

Both agricultural and urban land use can cause sedimentation, pollution by runoff, and loss of riparian vegetation, all of which can lead to impaired BMI community integrity. As other authors have concluded (e.g. Roy et al., 2003a), this relationship between land cover and reach-scale variables makes it difficult to separate the effects of various stressors. The effects of land use correlated variables will be discussed in terms of stressors to low and high gradient communities in following sections.

4.3 BMI community integrity

We were able to develop BMI community integrity relative ranking systems (Biotic Indices – BIs) were developed for low and high gradient streams. Both ranking systems showed that sites more drastically affected by anthropogenic stressors such as sedimentation, runoff pollution, and loss of riparian vegetation were characterized by more biologically compromised conditions than sites exposed to less intense anthropogenic stress. Accurate estimates of BMI community integrity at sites depends on the existence of reference metrics determined from reference (or ‘least disturbed/impacted’) sites sampled multiple times through time to account for spatial and temporal variation (Barbour et al., 1996-1999; Gibson et al., 1996; Bailey et al., 2004). In this report statements regarding BMI community integrity or BI scores at sites are not ‘absolute’, but rather relative to data collected in this project.

4.3.1 Low gradient waterways

In the current study, low gradient sites ranged from those with fauna dominated by damselflies, chironomid midges, and snails (Pleasant Grove) to sites dominated by oligochaetes and crustaceans (Main Drain and Wadsworth Canal). While sites on agricultural drains were generally more impacted than sites on the less agricultural Pleasant Grove waterway, some sites along agricultural drains, such as WC6 and MD1,

were characterized by higher BI scores than any site on Pleasant Grove. Downstream sites in agriculture-dominated waterways tended to have higher BI scores than upstream sites.

A study on the lower San Joaquin River watershed (Brown and May, 2000) yielded results similar to our observations on low gradient sites. This analysis revealed two types of low gradient sites in agriculture-influenced waterways, one dominated by baetid mayflies and gastropods, the other dominated by amphipods and oligochaetes.

The Central Valley is a vast region of mostly low gradient waterways dominated by intensively farmed land. Agricultural land use on such a scale is associated with chronic and extreme sedimentation, reduced or absent riparian vegetation, and water quality contamination by sediment, metals, nutrients, organic and inorganic pollutants, organic wastes that affect oxygen availability and agricultural chemicals, including pesticides. Compared to historical standards, the lack of or poor riparian zone vegetation, particularly trees, has dramatically reduced the amounts of allochthonous coarse particulate organic matter (CPOM- such as dead leaves and woody debris) entering aquatic ecosystems.

Many studies documented that presence of allochthonous CPOM to be very important to maintaining healthy BMI communities. CPOM is important both as a food source for many BMI taxa, and as a source of habitat. Richardson (1991) confirmed the importance of CPOM for maintaining high densities of invertebrates, and suggested that seasonal food limitations may lead to seasonal changes in BMI biota. By excluding terrestrial litter inputs and removing small woody debris, Wallace et al. (1999) reduced the biomass, abundance, and total secondary production of BMIs in areas of mixed substrate. The BMI communities of reaches dominated by bedrock substrates were not affected by the removal and exclusion of CPOM.

In low gradient agricultural areas of the Central Valley, CPOM has been replaced as an energy source by fine particulate organic matter (FPOM) originating from fertilizers and animal waste products. The increase in sedimentation and change in energy source

resulted in BMI communities dominated by FPOM utilizing mud-dwelling organisms. Riparian vegetation, instream habitat, water quality, and the dominant pathways of energy flow through these ecosystems are therefore intimately intertwined, and the combination of these factors determines the integrity of the aquatic ecosystem, including the BMI community.

4.3.2 High gradient waterways

High gradient sites generally scored higher on measures of community integrity than did low gradient sites. However, a wide range of BMI integrity was noted at high gradient sites. The major gradient in community integrity appeared to be associated with land use. Sites in more intensively developed urban areas showed lower community integrity than those surrounded by more native vegetation. Sites with more intact BMI communities consisted of higher proportions of sensitive EPT taxa, while more impacted sites contained fewer EPT and more chironomids.

This study reflects trends similar to those observed by other examinations of BMI communities in urban areas, confirming that urban development can have deleterious effects on BMI communities. One case is that of the Buffalo River in the metropolitan area of Buffalo, New York, where pollution caused virtual extinction of the entire BMI community by the 1960's. Forty years of remediation have improved the community to the point where oligochaetes and chironomids (pollution-tolerant taxa) are now plentiful, and many other insect taxa are beginning to re-establish populations (Diggins and Snyder, 2003).

Rogers et al. (2002) measured both metals contamination and habitat degradation in urban areas. They found that physical and chemical stressors arising from urban development act additively to increase impairment of BMI communities. Moreover, they found that physical impairment often masks effects of metals contamination.

4.4 Physical habitat

Physical habitat, including riparian vegetation, is a major determinant of aquatic biological community potential (e.g., Karr, 1991; Richards et al., 1997; Barbour et al., 1999). While chemical pollution continues to be an issue in many freshwater ecosystems, habitat degradation is considered responsible for more biological impairment than that caused by chemicals (Rankin, 1995). According to Cooper (1993), protection and remediation of habitat are the most effective means of conserving and restoring aquatic ecosystem biological diversity. Irrespective of this knowledge, little regulatory attention has focused on protection of aquatic and riparian habitat (Hughes et al., 1990; Karr, 1991-1993; Rankin, 1995), the focus being primarily on contaminants.

4.4.1 Low gradient waterways

Even among highly degraded low gradient waterways of the Central Valley, a range of habitat quality is evident. Coarse substrates and higher physical habitat scores were associated with higher BI scores at low gradient sites. Sites with plentiful submerged or emergent macrophytes (e.g. MD1) or relatively ample gravel substrates (e.g. WC4) contained more diverse BMI fauna than mud-dominated sites with few plants (e.g. WC2). Correlations of BI scores with physical habitat measures indicated that, along with pool variability and epifaunal substrate, riparian zone width significantly associated with BMI community integrity. Riparian zone width affects the amount of allochthonous plant matter that washes into a stream. Perhaps more importantly, the riparian zone can improve water quality by filtering many of the contaminants present in runoff from the land surrounding a waterway, including excess nutrients, sediment, metals, and pesticides (Muscutt et al., 1993; Lin et al., 2002). This finding suggests that increased protection of riparian areas, including the widening of undeveloped riparian zones, would help to improve the integrity of aquatic ecosystems in the Central Valley.

Downstream ADW sites, with greater flow and larger channels, manifested higher BI scores. For example, a notable increase of BI scores was observed in three Jack Slough sites from upstream to downstream. This trend also was observed in the Main Drain Canal and Wadsworth Canal. Further investigation is necessary to elucidate whether

factors other than stream flow and size are responsible for the higher downstream BI scores.

The importance of coarse substrates, instream habitat, and instream vegetation suggests that sedimentation, prevalent in ADWs, is a major obstacle to BMI community integrity. Relatively robust BMI communities can exist without coarse substrates in the presence of appropriate vegetation (e.g. MD1); a fruitful avenue to restoring BMI community integrity in low gradient waterways may include ensuring the presence of healthy plant communities. Prior to implementing such a restoration strategy, investigations into factors that preclude adequate vegetation in these waterways as well as into inhospitable DO levels resulting from overgrowth of instream vegetation (e.g., nutrients, low flow, high temperatures, etc.) would be needed.

There can be little doubt that hydrology factors (no flow, little flow, fluctuating flows) and physical alteration (plus maintenance activities) of waterways in the Central Valley have considerable impacts on biological communities. This study was not designed to assess the effects of these factors on BMI community integrity.

4.4.2 High gradient waterways

In urban watersheds chemical contamination commonly accompanies physical alteration of stream and riparian habitat. Correlations of high gradient BI scores with physical habitat signified that good instream conditions (including woody debris, root mats, leaf litter, and coarse substrates) and lack of sedimentation were the most important components to healthy BMI communities. This implies that sediment in urban runoff has the potential to significantly decrease BMI community integrity. Controlling erosion and inputs of sediment from construction and other urban activities would protect BMI community integrity. Apart from controlling sedimentation, we recommend preservation of existing instream and riparian habitat and restoration of instream and riparian habitat at sites with impacted BMI communities. Other investigations (e.g., Jones and Clark, 1987; Diamond et al., 2002; Rogers et al., 2002) documented that urbanization has severe impacts on aquatic biological communities.

4.4.3 Relevant studies

Similar to our results, other investigators have identified physical habitat factors as significant determinants of BMI community structure and metrics in California's Central Valley. Brown and May (2000) investigated associations between macroinvertebrate assemblages on large woody debris and environmental variables in the lower San Joaquin and Sacramento River drainages. Analogous to our results, their analyses indicated that dominant substrate type, stream gradient, specific conductance, water temperature, percentage of basin in agricultural land use, and percentage of basin in combined agricultural and urban land use were the likely determinants of macroinvertebrate assemblages. The importance of substrate to BMI communities was documented by observations that the communities of two natural channels came to resemble the communities of agricultural drains after a flooding event resulted in massive deposition of sediment. The change in community composition involved a loss of many EPT taxa and an increase in abundance of chironomids and oligochaetes.

Griffith et al. (2003) examined relationships between environmental gradients and macroinvertebrate assemblages in the Central Valley portions of the Sacramento and San Joaquin River watersheds. According to these authors the probable primary environmental determinants of BMI assemblages in the Central Valley are instream habitat, including substrate type: (1) By metrics analysis—channel morphology and substrate, and (2) By taxa abundance analyses—specific conductivity, channel morphology, and substrate. Channel management activities and landscape scale alterations of catchments by agriculture were identified by these authors as the major activities responsible for the environmental factors determining BMI assemblages. In this study, comparable to our results, more homogenous instream habitat and substrate was associated with lower taxa richness and higher mean tolerance value. Although site cluster analyses were performed, there was no information provided regarding location of the sites in the clusters. No inferences were made regarding BMI community integrity at sites in the study. One of their goals was to identify metrics and indicator taxa indicative of specific stressors. This goal was not achieved. Griffith et al. recommended a

multivariate approach that assesses sites with the BMI assemblage as an indicator unit instead of specific indicator taxa. According to these authors BMI metrics determined in their study could be incorporated into an ecoregion-specific index of biotic integrity that could be applied to diagnose associated environmental variables. However, this application was not incorporated into their article. Neither was there a ranking of sites relative to an index of biotic integrity. Neither seasonal nor year-to-year variations in BMI assemblages or metrics were assessed in this study.

The primary goal of a study conducted by Hall and Killen (2001) was to characterize physical habitat and BMI communities in Orestimba Creek (an agriculture-dominated stream that discharges into the San Joaquin River) and Arcade Creek (an urban creek in Sacramento that discharges into Steelhead Creek) a tributary to the Sacramento River. A second objective of this study was to assess potential impacts of organophosphorus (OP) insecticides, particularly chlorpyrifos, on BMI communities in these two streams. The CSBP procedure was applied to ten sites on each creek. The Hall and Killen report provides a qualitative characterization of some physical habitat factors in Orestimba and Arcade Creeks in late spring. BMI communities in both creeks were relatively impoverished and dominated by oligochaetes and chironomids. Bank stability, bank vegetation, and sinuosity were reported to be the physical habitat variables most likely influencing BMI community metrics.

Results of the current study are consistent with findings of Griffith et al. (2003) as well as Hall and Killen (2001). Stream size, the extent of channel alteration, extent of channel sinuosity, vegetation, and bank stability, along with other aspects of physical habitat, are important determinants of BMI community integrity.

Roy et al. (2003b) examined the effects of sedimentation on BMI communities, reporting that sedimentation reduced taxa richness. The effects of sedimentation were more drastic in riffle habitats than in bank habitats. Roy et al. suggested that bank and snag habitats act as refugia for BMIs during periods of sedimentation, and that taxa that facultatively inhabit bank habitats are more likely to survive sedimentation than taxa obligately

inhabiting riffles. Maintaining or restoring taxa richness and optimum health to high gradient waterway BMI communities will require minimizing sources of sedimentation (such as erosion from urban development, agriculture, and silviculture) to maintain populations of insect taxa obligately inhabiting riffles. The findings of Roy et al. (2003b) strengthen the argument that CPOM is important to BMI communities as a source of habitat. After a waterway has experienced a large sedimentation event that covers coarse substrates, allochthonous CPOM is likely to be the only source of new habitat for taxa that inhabit coarse substrates. CPOM is, therefore, crucial to maintaining taxa richness during periods of sedimentation.

4.5 Water Quality

According to Barbour et al. (1996), 'a clear distinction between impacts due to a combination of large-scale habitat alteration and water-quality degradation is often not possible.' Design of the current study was not such that water quality and physical habitat effects on BMI community integrity could be definitively distinguished. Further, few bioassessment studies, including the current one, have thorough/complete water quality data. The range of variables included is usually incomplete and detection limits for many variables inadequate. Frequency of measurement is typically sub-optimal. While physical habitat factors remain relatively constant temporally, many water quality variables vary considerably through time. Habitat features and water quality parameters often covary, confounding attempts to discriminate the factors most responsible for BMI community perturbations. Each water quality variable is measured independently, but BMI communities almost certainly respond to additive, synergistic, and/or cumulative effects. Statistical analyses cannot detect all water quality variable interactions. For example, water quality variables a, b, c, and d may be at concentrations that do not correlate with (and individually, are below published effect levels) with BMI metrics, but a+b+c+d evoke an additive, synergistic, or cumulative effect on BMI community integrity. This study confirmed depauperate physical habitat conditions in many Central Valley waterways, especially low gradient waterways. These conditions constrain the ability of bioassessments to discern water quality impacts on BMI communities. Given these limitations, we do not recommend standard BMI bioassessment as a stand-alone in

attempts to evaluate water quality (including the presence of contaminants), especially at sites with poor to moderate physical habitat conditions.

4.5.1 Low gradient waterways

Some associations between water quality variables and BMI metrics were discovered in this investigation. Correlations implicated SpC, hardness, alkalinity, and phosphorus as specific water quality components likely influence BMI community integrity. DO and pH also varied between sites in the low gradient dataset, but neither of these parameters was associated with differences in BMI community integrity. Downstream sites tended to have higher BI scores than upstream sites. Water quantity improvements between upstream and downstream sites may be responsible for this downstream increase in the integrity of BMI communities, possibly because larger volumes of water downstream dilute contaminants that were more concentrated in some upstream reaches. It should be emphasized that this study did not include streams containing source water, upstream from any agricultural influence. The majority of the upstream sites in the low gradient dataset were small tributaries containing return water, likely to be most intensively exposed to stressors from agricultural activities. Some indications of tributary-specific water quality effects on BMI communities were noted. For example, at Main Drain sites MD5 and MD6, situated on the same tributary, habitat quality was equivalent to that occurring at site MD4, yet BI scores at these two sites were lower than at MD4. Potentially degraded water quality in the MD5 /MD6 tributary may have been responsible for the lower BI scores.

To more effectively use bioassessments to evaluate low gradient waterway biotic conditions, procedures and study designs should be refined so that water quality and physical habitat variable effects on BMI community integrity can be more effectively distinguished. Probabilistic-based sampling designs are inappropriate when a primary objective is to assess potential water quality effects on macroinvertebrate communities because such an approach is unlikely to include gradients of water quality parameters of interest. Approaches that may be useful for discriminating water quality effects include use of artificial substrates, trend monitoring (frequent, consistent, and long-term

monitoring of water quality parameters) and event-based sampling to track changes in BMI community integrity at specific sites, point source studies bracketing areas suspected to be the origin of water quality concerns, and studies designed to minimize habitat variation while investigating water quality parameter gradients.

The basic concept of artificial substrate studies is to standardize substrate so that water quality parameters are the primary variables. While artificial substrate procedures have several limitations, they can be very useful components for assessing potential water quality issues if objectives remain simple and if inferences from data gathered do not exceed procedure capabilities. Taxonomic diversity and proportional abundance are measures that are usually within the capacity of artificial substrate procedures.

In the absence of reference sites, frequent, consistent, and long-term monitoring of water quality parameters of interest (trend monitoring), coupled with event-based macroinvertebrate sampling (e.g., rain event or ‘first flush’ sampling), could be informative because each site would serve as its own control when examining temporal variations in BMI community integrity. Such sampling requires identification of sites appropriate to the larger population of sites that the bioassessment results are intended to represent, and also necessitate recognition of events that potentially affect BMI communities or a large investment in routine sampling of a large number of sites. Continued trend monitoring can be especially useful in detecting effects of water quality parameters because, at a given site, physical habitat features are less likely to change over time whereas temporal variation of water quality can be considerable. Thus, frequent sampling of macroinvertebrates and water quality parameters allows for correlation analysis between BMI metrics and water quality parameters.

Point source study designs bracket suspected sources of water quality problems to investigate possible impacts resulting from those sources. With such designs care must be taken to select sites with similar habitat, including substrate, flow, vegetation in the channel, and riparian zone characteristics. It is also possible to design studies that assess BMI community integrity between populations of sites differing in water quality

parameters, but in such cases it is essential to control or account for, not only differences in BMI communities resulting from habitat differences, but also differences related to watershed-level factors and processes.

Lack of historical data prior to intensive agricultural land use and extensive use of pesticides, as well as other agricultural chemicals, precludes an understanding of BMI communities that did and should exist in ADWs. A study by Heckman (1981) cataloged the taxa of an orchard's irrigation system. This cataloging was performed because, just prior to the use of DDT, another scientist thoroughly cataloged taxa in the same irrigation system. The only major difference between the two cataloging events is decades of modern pesticide use. Results show that many taxa were extirpated from the system; however, several taxa apparently adapted and thrived in the presence of pesticides. Various resistance mechanisms and natural immunity weigh heavily in explaining the presence of the remaining taxa. The predominant taxa consisted of midges, oligochaetes, and flatworms. In support of Heckman's findings, a similar taxa list was garnered by Lugthart and Wallace (1992) after repeatedly treating a stream with methoxychlor to purposefully remove BMIs. Further investigations may reveal that BMI communities in ADWs are unaffected by low-magnitude agricultural chemical water quality degradation and/or that communities (dominated by resistant taxa with short life cycles) recover quickly to high concentration spikes of these chemicals. On the other hand, BMI communities in particular ADWs may have been degraded to a baseline condition such that little or no response to further water quality degradation can be detected. If this is the case, BMI bioassessments will be of little value to regulatory agencies in these types of waterways, with the possible exception of restoration projects. Rogers et al. (2002) suggested that effects of contaminants on macroinvertebrate communities can be masked if stream channelization, loss of riparian vegetation, or other physical stressors exert comparable or larger influences.

During the course of this study an insecticide overspray of the Main Drain was observed. Our field observations suggest that such events are relatively common. Immediately after the event large numbers of dead macroinvertebrates were collected. Preliminary

evidence concerning changes in BMI community integrity following the event indicates no notable ‘recovery’ in BMI communities downstream of the overspray over the following months. Worms and chironomid midges that reproduce and mature quickly dominated the downstream communities. These taxa could have re-established before our first samples were collected, weeks after the overspray. Agricultural chemical use in this area has been extensive for at least 40 years, so these BMI populations are likely to be highly tolerant. Note, however, that no downstream samples were collected prior to the overspray event, so the actual extent of effects is unknown.

4.5.2 High gradient waterways

At high gradient streams, robust BMI communities were correlated with high minimum DO measurements and low SpC. Alkalinity, N5, and flow velocity varied between sites, but were not associated with differences in BMI community integrity. The only WWTF found to have a potential effect on BMI community integrity was the Coon Creek SMD1 WWTF. SpC was significantly higher at sites downstream of WWTFs than at upstream sites. Thus, SpC may be an important determinant of BMI community integrity at high gradient sites. Because sites in the most urbanized waterway (Dry Creek) manifested the lowest BI scores, it is possible that contaminants in urban runoff (not measured in this study) affected BMI community integrity. Future studies of BMI community integrity in urbanized areas should be designed to distinguish effects of SpC and contaminants.

4.5.3 Relevant studies

Leland and Fend (1998) investigated the relationship of some water quality factors with invertebrate fauna in the lower San Joaquin River. They used artificial substrate and BMI approaches, applying canonical correspondence (CCA), metrics, and indicator species analyses. A basin-wide pattern in community response (metrics) to salinity (total dissolved solids-TDS) was detected with the standardized stable artificial substrate. TDS accounted for a large part of variance in artificial substrate assemblages over all seasons, flow conditions, and irrigation regimes. Biota communities on stable (artificial) and unstable substrate were highly dissimilar. Compared to the artificial substrate findings, there was a weaker statistical relationship between BMI community metrics and water

quality variables. That is, artificial substrate data were superior to BMI surveys in distinguishing water quality variables that influence biotic community structure/integrity. According to Brown and May (2000), specific conductance and temperature were water quality variables most important to BMI community (collected from snags) differences in low gradient waterways in the lower San Joaquin River watershed. Because the habitat/substrate sampled was rather consistent (snags), ability to distinguish potentially important water quality variables was enhanced. Griffith et al (2003) suggested that specific conductivity was an important determinant of BMI community composition, but not necessarily associated with BMI metrics, in the Sacramento and San Joaquin River watersheds. Gradients of specific conductivity are more pronounced in the San Joaquin River watershed than in the Sacramento River watershed. Thus, studies that include the San Joaquin River watershed are more likely to identify specific conductivity as an influence on BMI communities.

In ADWs, as well in urban streams, pesticides, especially insecticides, are potential BMI community stressors. While pesticides may have contributed to impoverished BMI community integrity in ADWs, our study was not designed to distinguish pesticide impacts on BMIs. However, there is evidence that insecticides have significant impacts on BMI communities. Runoff-related insecticide (ethyl-parathion and fenvalerate) input and resulting effects on BMIs were investigated by Schulz and Liess (1999). Results revealed that BMI communities were substantially impacted by irrigation runoff-related insecticide input from agriculture fields under conventional practices. Impacts were observed for the entire stream section investigated (up to 1.5 Km from the runoff location). Comparison of runoff events with and without insecticide contamination substantiated the importance of insecticide impacts on BMI. That is, hydraulic (discharge), turbidity, and nutrient components of the runoff were not correlated with effects on BMIs.

Liess and Schulz (1999) also documented that insecticides (ethyl-parathion and fenvalerate) in storm runoff from agricultural lands had significant negative impacts on stream BMI communities. The effects of insecticides in runoff were independent of

hydraulic stress, suspended particulates, and nutrients. Recovery of BMI communities required 6 to 11 months. A noteworthy finding in this study was that BMI community assessments revealed more severe impacts than predicted by laboratory toxicity test results. A significant aspect of these two studies was that sampling was event-based. Determination of an association of insecticides in agricultural runoff with effects on BMI has perhaps been a methodological issue. Event-based sampling (e.g., sampling associated with peak irrigation after insecticide application) is more likely to define the effects of insecticides on BMI communities than is random or probabilistic sampling.

Runoff from rice fields was shown to impact BMIs in a river in Japan (Tada and Shiraishi, 1994). The author postulated that rice pesticides were the cause of impacts, but there was no confirmation of this hypothesis. Nonetheless, some constituents in the runoff were responsible for the impacts. Leonard et al. (1999, 2001) investigated invertebrate species at eight sites on the Namoi River (southeastern Australia) in relation to endosulfan runoff from cotton fields. Study results linked population dynamics of the six dominant species to endosulfan contamination.

Increased BMI drift rate in streams following insecticide contamination has been confirmed in several studies: (Cuffney et al., 1984; Scherer and McNicol, 1986; Dossall and Lehmkuhl, 1989; Sibley et al., 1991). *Gammarus pulex* drift during runoff contaminated with insecticides was significantly increased compared to runoff without insecticide contamination (Liess et al., 1993). Several studies have documented that BMI drift is a significant determinant of BMI community dynamics (Dermott and Spence, 1984; Liess et al., 1993; Taylor et al., 1994).

A weight of evidence approach was applied to assess the effects of pollutants entering the Salinas River (California) from a tributary draining an agricultural watershed (Anderson et al., 2003a, b; Hunt et al., 2003; Phillips et al., 2004). Data were collected at stations upstream and downstream of the agricultural input. Analyses included water column chemical analyses, water column toxicity testing with *C. dubia* plus toxicity identification evaluations (TIE), sediment toxicity testing with *Hyaella azteca* (a resident species), in

situ toxicity tests, and benthic macroinvertebrate bioassessments. Both chemical analysis and toxicity bioassays indicated that concentrations of chlorpyrifos downstream of the input exceeded the lethality threshold of *C. dubia* while upstream chlorpyrifos concentrations were low. Sediment samples downstream of the creek also were toxic to *H. azteca*, whereas sediment upstream of the agricultural input was not toxic. Chlorpyrifos concentrations in the sediment collected downstream of the input exceeded the lethality threshold of this species. TIEs identified chlorpyrifos as the cause of toxicity. Benthic macroinvertebrate data revealed that downstream stations were impacted relative to upstream stations, containing lower percentages of Ephemeroptera (mayflies) and chironomid midges. All lines of evidence linked chlorpyrifos in the irrigation runoff dominated stream to impacts on Salinas River biota.

Lugthart and Wallace (1992) experimentally treated a stream in the Appalachian Mountains with the insecticide methoxychlor. Three streams were sampled in the first year of the study, none of which were treated with insecticide. In the second year of the study, two streams were re-sampled, one of which was treated with methoxychlor. The insecticide-treated stream showed a decrease in secondary production, and a shift in dominant taxa to collector-gatherers, predominantly worms and midges.

Several other studies provide evidence of agriculture-derived insecticide impacts on stream BMI communities (Liess et al., 1993; Liess and Schulz, 1996; Schulz and Liess, 1997; Schulz, 2004). Cuffney et al. (1984) documented that a pyrethroid insecticide contamination of an aquatic ecosystem not only altered BMI community structure, but also ecosystem processes.

In their investigation of the San Joaquin River watershed Leland and Fend (1998- summarized above) proposed that invertebrate community structures were unrelated to 'pesticide distributions'. Further, the authors suggested that BMI communities are not likely susceptible to seasonal changes in concentrations of anthropogenic constituents. However, only some pesticide constituents were measured (on only two occasions during the three-year study). Sediment pesticide concentrations were not analyzed. A second

objective of the Hall and Killen (2001) study (outlined above) on Orestimba and Arcade Creeks was to assess potential impacts of organophosphorus (OP) insecticides, particularly chlorpyrifos, on BMI communities in these two streams. Hall and Killen concluded that habitat factors likely explained the differences in BMI communities, and suggested that contaminants played a minor role. However, the Hall and Killen study did not include reference streams nor did they report instream insecticide concentrations or sampling site relationship to insecticide use. Associations of water quality factors, including contaminants with BMI metrics were not evaluated so contaminant effects cannot be ruled out.

Hayworth et al. (2004) applied BMI bioassessments to reservoirs, lakes, and agricultural canals in an attempt to assess potential community impacts of aquatic herbicide applications. The authors concluded that their initial results indicated impacts of copper, fluridone, and triclopyr. However, the inferences made about pesticide and metals contamination relied heavily on the equivalency of 'reference' sites to treatment sites. Data presented did not establish this equivalency. Another major component of data interpretation was based on abundance estimates. Abundance is not always a reliable indicator of BMI community integrity (Carlisle and Clements, 1999). Inferences also relied heavily on BMI tolerance values. Tolerance values relate mostly to organic pollution and DO. How these values reflect aquatic herbicide impacts is unknown. Before attributing the cause of the impact on BMI communities to anthropogenic factors, in this case aquatic herbicides, it is crucial to understand, and partition out, natural temporal and spatial variability. In this data set there were clear indications of temporal variation. BMI variability among replicates or between stations was not characterized effectively or discussed. This compromises comparison between sites and at sites through time. Singling out aquatic pesticides as the causative factor for differences in BMI metrics or abundance is very risky given that many habitat and water quality variables were not determined.

Urban development affects stream water quality by input of nutrients and contaminants from point-source discharges, as well as from runoff. Hannaford and Resh (1995)

reported that an urban stream reach exposed to organic pollution from runoff and a large spill of raw sewage suffered reduced BMI community integrity. Roy et al. (2003a) reported that high SpC (associated with more intense urban land use) was correlated with lower BMI community integrity. In a waterway near Boston, Massachusetts, Rogers et al. (2002) used physical, chemical, and biological indices to discern relative impacts of physical and chemical stressors. Their objective was to determine whether simple indices of chemical, physical, and biological conditions could be used to estimate the influence of chemical and physical degradation on macroinvertebrate communities. Macroinvertebrate condition was significantly dependent on contaminants in vegetative habitats, but not on contaminants in the stream bottom. Their observations demonstrate that physical habitat features can mask contaminant impacts.

WWTF discharges have been shown to impact BMI community integrity (de Vlaming and Norberg-King, 1999—a review). In a study characterizing the gradient of BMI communities in a stream receiving discharge from a secondary-treatment WWTF, Birge et al. (1989) observed that abundance, diversity, and trophic ecology of the BMI community were all affected by the WWTF effluent. The community recovered 37 to 50 Km downstream of the discharge site. Kosmala et al. (1999) examined BMI communities upstream and downstream of a WWTF discharge. This study identified a reduction in BMI community integrity caused by WWTF effluent. Contaminants identified in this effluent included ammonia, nutrients, metals, and polyaromatic hydrocarbons.

4.6 Identification of stressors

Data presented herein suggested that several habitat and water quality factors influence BMI community structure and integrity. However, all these relationships were determined by correlation. No cause-and-effect was established. According to the National Research Council (2001) bioassessments do not provide precise enough determination of causes and sources of impacts to satisfy water quality management needs. Further, several researchers (e.g., Barbour et al., 1996; Clements and Kiffney, 1996; Holdway, 1996; McCarty and Munkittrick, 1996; Wolfe, 1996; Power, 1997; Bart

and Hartman, 2000; Adams, 2003) have addressed the inability of bioassessment to establish a direct cause-and-effect relationship between stressors and biological communities. Reasons cited by these authors include the complex nature of ecosystems, the many biotic factors that influence or modify biological systems, the orders of magnitude involved in extrapolations over temporal and spatial scales, compensatory mechanisms that operate in natural populations, the many modes and pathways by which stressors disrupt and destabilize aquatic ecosystems, high variability of environmental factors, synergistic and cumulative interactions of factors that influence biological systems, and high unaccounted for variability in bioassessment data. Nonetheless, bioassessment is an important tool to be incorporated into a weight-of-evidence approach that includes several procedures (e.g., chemical analysis and toxicity testing).

Some authors caution against reliance on bioassessment approaches based on pattern detection in biotic communities for predicting biological integrity. Bunn and Davies (2000) contend that most of these pattern-detection approaches assume high temporal persistence (see Seasonal variation section, below) of biotic communities in the absence of anthropogenic disturbances. According to these authors, ‘Changes in patterns (abundance, richness, species composition) do not always equate to changes in ecological integrity. Marked changes can and do occur as a result of natural biological processes and may falsely lead to conclusions that impacts have occurred when they have not (i.e., Type I statistical error). Biomonitoring approaches reliant solely on pattern detection may be unable to detect changes in ecological integrity (i.e., Type II statistical error.). Direct measures of ecosystem processes are often neglected in river health assessment programs. However, they are sensitive to factors that are known to directly influence river health and it is possible to develop simple, yet powerful, predictive models. Strategies for remediation are more obvious as the causal processes are better known. The ultimate success of biomonitoring approaches depends on how well we understand the biophysical processes that influence the structure and dynamics of stream and river systems, and they way they function.’

4.7 Tolerance values

Tolerance values indicate pollution-tolerance of a taxon. For the most part, tolerance values represent resistance to organic waste, low DO, and nutrients. Generally, tolerance values do not apply to all classes of chemical pollutants, especially pesticides. However, these values provide a useful metric of the average degree of pollution-tolerance of taxa if not applied inappropriately. For this study, we used the region-specific tolerance values set forth in guidelines provided by the California Aquatic Bioassessment Laboratory Network (CAMLnet). The importance of refining tolerance values is recognized.

4.8 Bioassessment data variability

When comparing BMI assemblages or metrics between or among sites it is invaluable to have multiple samples/replicates taken at each site. This allows determination of within-site variability in BMI parameters and, subsequently, statistical comparison between or among sites. While statistical differences do not necessarily reflect biological/ecological differences, such statistical comparisons enhance reliability and credibility of inferences and conclusions.

In this study each of three transects sampled was treated as a replicate. This provided an estimate of within-site variability, and allowed the use of statistics such as ANOVA for examining differences between sites. More ideal replication could be obtained by collecting three replicate samples per site. This approach would minimize variability between replicates, giving greater statistical power to detect between-site differences. Although collection and analysis of replicate samples increases costs, collection of replicates is necessary when project objectives call for statistical comparisons between individual sites (e.g. upstream/downstream comparisons).

For BMI bioassessment results to be useful in making decisions regarding water quality, it is important that variability (precision), representativeness, and repeatability of results be known. Reliability of results is critical. In rapid bioassessment protocols (RBP) there is the implicit assumption that one sample (pooled transect samples) constitutes a precise and representative of BMI community and habitat. However, analysis of RBP replicates

documented high variability (e.g., Barbour et al., 1992; Resh, 1994; Hannaford and Resh, 1995). If within site/reach variability is high, comparisons of site data may be compromised. Field sampling and laboratory sub-sampling appear to be the sources of greatest variability in BMI bioassessment results. Within-reach replication would enhance understanding of variability. Precision and repeatability of results should be reported in bioassessment studies related to regulatory issues. Data variability also affects the sensitivity of a procedure (ability to discern differences between sites and at a site through time). The lower the sensitivity of monitoring procedure, the less its utility, especially in the regulatory arena. Unfortunately, sensitivity of bioassessment procedures is almost never reported or discussed.

In their investigation of the lower San Joaquin River watershed, Brown and May (2000) addressed sampling variability. Variability of macroinvertebrate data among replicate reaches was low. That samples were collected from relatively consistent habitat/substrate (snags) is almost certainly responsible for low variability. In an investigation of the lower San Joaquin River watershed Leland and Fend (1998) found that within-site macroinvertebrate data variability was low with the artificial substrate procedure, but high among BMI replicates.

4.9 Seasonal variation

Multiple statistical procedures applied to BMI data from the lower Sacramento River watershed revealed that season of sampling impacts estimated biotic integrity at sites. Biotic Index scores were systematically lower in spring than in fall at a majority of sites. For the most part, statistical differences were the result of higher abundances and EPT taxa diversity in fall. Whether this seasonal variation was natural temporal variation, anthropogenic-stressor caused, or a combination of both is unknown. This is consequent to no sites being free from human influences. The spring/fall shift was not found in upper Butte Creek (BC2 and BC3) or in Miner's Ravine (DC 8, 9, and 11). In upper Butte Creek, cold spring snowmelt created favorable conditions for coldwater EPT taxa in spring. Although Miner's Ravine did not receive cold water in spring, BMI communities there contained more EPT taxa spring than in fall.

These findings have implications for future bioassessment projects. To gain an accurate perspective of BMI community integrity, our concept is that sites should be sampled as often as possible during a year. However, given that there are typically economic constraints on projects, we advise that, if site comparisons are a component of the study, all sampling be confined to a specific season and that efforts be made to conduct sampling in a narrow timeframe. Constructing a multimetric measure of biotic integrity that is insensitive to seasonal fluctuations may be possible, but this could prove difficult to apply to the Sacramento River watershed, because EPT taxa occurring in fall are replaced by chironomids and oligochaetes in spring.

Selection of index period for sampling sites may alter sensitivity of bioassessments. Our evaluation of BI signal/noise ratios indicates that differences in biotic integrity among sites in the lower Sacramento River watershed may be more effectively detected in spring at low gradient sites, but in the fall at high-gradient sites. Building season-specific BIs for specific index periods could attain improved signal/noise ratios for a season. Whatever season is chosen as an index period, it is important to note that bioassessment data from the lower Sacramento River watershed are comparable only if collected during the same index period.

Lenat and Crawford (1994) observed seasonal variability in BMI communities sampled in January, April, June, and November. Particularly notable is that BMI taxa richness and abundance varied seasonally in all three streams. However, the seasonal variation differed in the three streams dominated by different land use. Whether the seasonal variation observed in this investigation related to natural temporal variation, seasonal changes of anthropogenic stressors, or a combination of both was not addressed.

Year-to-year variation of macroinvertebrate metrics was noted by Brown and May (2000) in a study on the lower San Joaquin River watershed. These shifts were attributed to variation in environmental parameters. While environmental variables may very well have been responsible for differences in metrics, it is impossible to distinguish natural

temporal variation from anthropogenic-caused variation. In bioassessment studies that do not include reference sites, such as the Lenat and Crawford as well as the Brown and May investigations, more attention must be focused on seasonal and year-to-year variation of macroinvertebrate metrics. That is, caution must be applied to inferences regarding biological integrity and cause(s) of observed metrics scores. In the absence of an understanding of natural temporal and spatial variation such inferences are neither credible nor reliable. According to Townsend and Riley (1999), ‘A full understanding of changes to river ecosystem structure and functioning along the continuum from relatively pristine to profoundly perturbed requires knowledge of physical, chemical and ecological properties at many spatial and temporal scales. Practical considerations limit most evaluations of river health to a small suite of indices though it is important that researchers continue to evaluate the spatial, temporal, and biological limitations of these indices.’

Several authors (Kearns et al., 1992; Osenberg et al., 1994; Karr and Chu, 1999; Dorward-King et al., 2001; Luoma et al., 2001) proposed that the primary goal of a monitoring and assessment program is to distinguish anthropogenic-caused impacts from natural temporal variation. Without reference sites that are minimally influenced by anthropogenic stressors, obtaining this type of essential information in California’s Central Valley will be difficult. Defining natural temporal and spatial variation in BMI communities of the Central Valley, especially in low gradient waterways, will be complicated because essentially all waterways and sites are influenced by anthropogenic activities. If natural temporal and spatial variation cannot be defined, separating anthropogenic impacts from natural variation will be a challenge.

4.10 Taxonomic Effort

Brown and May (2000) proposed that macroinvertebrate identification to family level is sufficient for evaluating effects of environmental variables on community assemblages in the Central Valley of California. These authors did not evaluate the taxonomic level needed to distinguish levels of BMI community impairment. In marine systems, family-level taxonomy is sufficient for evaluating community integrity (Bowman and Bailey,

1997). However, the applicability of this finding to freshwater bioassessments is uncertain because diversity in marine systems occurs on a different taxonomic scale compared to freshwater systems. In most cases, family level taxonomy is likely to be sufficient for a rapid assessment of the general level of community integrity. However, more fine-grained substrate distinctions of community integrity or evaluations of community integrity in areas of high diversity or moderate impairment will require more precise taxonomy. For example, in some low gradient Central Valley datasets, it may be that the more “pristine” sites differ from the more impacted sites in overall percentage of insects, whereas in datasets involving mountain streams, the number of genera of pollution-sensitive EPT taxa families may distinguish poorer sites from more ecologically intact sites. Another consideration in selecting level of taxonomic effort should be compatibility with other datasets. For example, those taken in the same region potentially could be merged. A more precise level of taxonomic effort can always be translated into a less precise level of effort, but a less precise level of effort can never be made more precise without costly re-examination of collected specimens.

5. Recommendations

- BMI bioassessments provide very useful information regarding waterway biotic integrity and should continue to be included with other assessment procedures in a weight-of-evidence approach to evaluate aquatic ecosystem health.
- BMI community integrity assessments of data collected from low gradient waterways should be analyzed separately from data gathered from high gradient waterways.
- Reference sites or ‘best attainable’ sites must be identified for low and high gradient waterways in both the lower Sacramento and San Joaquin River waterways. Indices of Biological Integrity (IBIs) should be developed using such sites.
- So that anthropogenic effects on BMI communities can be more effectively distinguished from natural variation, studies are needed to define natural temporal variation of BMI metrics, IBIs, and BIs. Without reference or best attainable sites this essential task will be difficult to impossible.

- For accurate assessment of BMI community integrity at sites we recommend repeated samplings at each site over time. If this is not feasible, an index period, ideally with a relatively short duration (e.g., September –October) is advised to avoid probable confounding effects of seasonal variation of BMI metrics, IBIs, and BIs.
- Field sample replication should become a standard component of bioassessment procedures. This will provide data that can be subjected to statistical analyses so that comparisons between or among sites, as well as temporal comparisons at a site, do not rely on best professional judgment or subjective criteria. Such measures will enhance the credibility and reliability of bioassessment data.
- Because bioassessment data tend to manifest considerable variability, precision and sensitivity (magnitude of response necessary to be statistically detected) should be determined and reported for bioassessment studies, especially if the data may be used for regulatory actions. Such measures will enhance the credibility and reliability of bioassessment data.
- If bioassessments are to be applied to evaluation of low gradient waterway biotic condition, procedures and study designs should be refined so that water quality and physical variable effects on BMI community integrity can be more effectively distinguished.
- BMI bioassessments should be used as a component of monitoring projects intended to assess potential water quality impacts. This recommendation applies particularly to low gradient waterways with marginal to poor habitat conditions. We recommend a weight of evidence approach that may include toxicity testing (sediment and water column) with associated toxicity identification evaluations (TIE), chemical analyses, or other appropriate procedures. This is consistent with the National Research Council (2001) conclusion that bioassessment data should be used in conjunction with physical, chemical, and toxicological data in assessing aquatic ecosystem condition. This approach is particularly essential if a study objective is to specifically identify the cause(s)/stressors responsible for community perturbations.

- Bioassessment approaches that may be useful for discriminating water quality effects include use of artificial substrates, upstream/downstream studies bracketing areas suspected to be the origin of water quality concerns, and studies designed to minimize physical habitat variables while investigating water quality parameter gradients.
- If biological community integrity in low gradient waterways of the Central Valley is to be improved, we recommend that the initial focus be on reducing sedimentation and on protection of existing riparian vegetation plus restoration of riparian zones. These activities would affect several other parameters that determine BMI community health.
- We recommend that the following questions be addressed:
 - (1) How different do IBIs or BIs have to be before we are confident that there are meaningful biological/ecological differences at sites/waterways? What is the IBI or IB threshold that indicates that a site is truly impaired?
 - (2) What is impaired, what is not? What are justifications for impairment thresholds? In rapid bioassessment protocols impairment thresholds are used as surrogates for the traditional statistical tests to detect site differences. Therefore, defining/selecting and calibration of thresholds are crucial. In a multimetric approach each metric will have a unique series of thresholds that identify various levels of perturbation. Consequently, variation in the bioassessment as a whole will be the additive variability of the component metrics.

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Appendix A

Table 1A
Common names for the taxa identified in this study

Common Name	Order	Family	Genus/Final ID
Mayflies	Ephemeroptera	Ameletidae	Ameletus
	Ephemeroptera	Baetidae	Acentrella
	Ephemeroptera	Baetidae	Baetis
	Ephemeroptera	Baetidae	Callibaetis
	Ephemeroptera	Baetidae	Camelobaetidius
	Ephemeroptera	Baetidae	Centroptilum
	Ephemeroptera	Baetidae	Dipheter hageni
	Ephemeroptera	Baetidae	Fallceon quilleri
	Ephemeroptera	Baetidae	Paracloeodes minutus
	Ephemeroptera	Baetidae	Procloeon
	Ephemeroptera	Caenidae	Caenis
	Ephemeroptera	Ephemerellidae	Drunella
	Ephemeroptera	Ephemerellidae	Ephemerella
	Ephemeroptera	Ephemerellidae	Eurylophella lodi
	Ephemeroptera	Ephemerellidae	Serratella
	Ephemeroptera	Ephemerellidae	Timpanoga hecuba
	Burrowing Mayflies	Ephemeroptera	Ephemeridae
Flat Headed Mayflies	Ephemeroptera	Heptageniidae	Epeorus
	Ephemeroptera	Heptageniidae	Heptagenia
	Ephemeroptera	Heptageniidae	Leucrocuta
	Ephemeroptera	Heptageniidae	Rhithrogena
	Ephemeroptera	Isonychiidae	Isonychia velma
	Ephemeroptera	Leptohyphidae	Asioplax
	Ephemeroptera	Leptohyphidae	Tricorythodes
	Ephemeroptera	Leptophlebiidae	Paraleptophlebia
Stoneflies	Plecoptera	Capniidae	Capniidae
	Plecoptera	Chloroperlidae	Bisancora
	Plecoptera	Nemouridae	Zapada
	Plecoptera	Perlidae	Calineuria californica
	Plecoptera	Perlidae	Hesperoperla
	Plecoptera	Perlodidae	Cultus
	Plecoptera	Perlodidae	Isoperla
	Plecoptera	Perlodidae	Osobenus yakimae
	Plecoptera	Perlodidae	Skwala parallela
Giant Stoneflies	Plecoptera	Pteronarcyidae	Pteronarcys
Caddisflies	Trichoptera	Brachycentridae	Amiocentrus aspilus
Humpless Case Makers	Trichoptera	Brachycentridae	Brachycentrus
	Trichoptera	Brachycentridae	Micrasema
Saddle Case Makers	Trichoptera	Glossosomatidae	Glossosoma
	Trichoptera	Glossosomatidae	Protoptila
Snail Case Makers	Trichoptera	Helicopsychidae	Helicopsyche borealis
Net Spinning Caddisflies	Trichoptera	Hydropsychidae	Cheumatopsyche
	Trichoptera	Hydropsychidae	Hydropsyche
Micro-Caddisflies	Trichoptera	Hydroptilidae	Agraylea

	Trichoptera	Hydroptilidae	Hydroptila
	Trichoptera	Hydroptilidae	Leucotrichia pictipes
	Trichoptera	Hydroptilidae	Ochrotrichia
	Trichoptera	Hydroptilidae	Oxyethira
	Trichoptera	Lepidostomatidae	Lepidostoma
	Trichoptera	Leptoceridae	Mystacides
	Trichoptera	Leptoceridae	Nectopsyche
	Trichoptera	Leptoceridae	Oecetis
	Trichoptera	Leptoceridae	Trienodes
	Trichoptera	Philopotamidae	Chimarra
	Trichoptera	Philopotamidae	Wormaldia
	Trichoptera	Polycentropodidae	Polycentropus
	Trichoptera	Psychomyiidae	Tinodes
Free Living Caddisflies	Trichoptera	Rhyacophilidae	Rhyacophila
	Trichoptera	Sericostomatidae	Gumaga
	Coleoptera	Dryopidae	Postelichus
Predaceous Diving Beetles	Coleoptera	Dytiscidae	Copelatus
	Coleoptera	Dytiscidae	Coptotomus longulus
	Coleoptera	Dytiscidae	Hydaticus aruspex
	Coleoptera	Dytiscidae	Laccophilus
	Coleoptera	Dytiscidae	Liodessus obscurellus (adult)
	Coleoptera	Dytiscidae	Rhantus
	Coleoptera	Dytiscidae	Thermonectus
Riffle Beetles	Coleoptera	Elmidae	Ampumixis dispar
	Coleoptera	Elmidae	Cleptelmis addenda
	Coleoptera	Elmidae	Dubiraphia
	Coleoptera	Elmidae	Heterlimnius
	Coleoptera	Elmidae	Microcyллоepus
	Coleoptera	Elmidae	Narpus
	Coleoptera	Elmidae	Optioservus
	Coleoptera	Elmidae	Ordobrevia nubifera
	Coleoptera	Elmidae	Zaitzevia
Whirlygig Beetles	Coleoptera	Gyrinidae	Gyrinus
Water Scavenger Beetles	Coleoptera	Hydrophilidae	Cymbiodyta
	Coleoptera	Hydrophilidae	Enochrus
	Coleoptera	Hydrophilidae	Hydrobius (adult)
	Coleoptera	Hydrophilidae	Tropisternus
Water Pennies	Coleoptera	Psephenidae	Eubrianax edwardsii
	Coleoptera	Psephenidae	Psephenus falli
Toe Winged Beetles	Coleoptera	Ptilodactylidae	Stenocolus scutellaris
	Coleoptera	Staphylinidae	Staphylinidae (adult)
Net Winged Midges	Diptera	Blephariceridae	Agathon
	Diptera	Blephariceridae	Blepharicera
	Diptera	Blephariceridae	Blephariceridae (pupa)
Biting Midges	Diptera	Ceratopogonidae	Atrichopogon
	Diptera	Ceratopogonidae	Bezzia/ Palpomyia
	Diptera	Ceratopogonidae	Ceratopogon
	Diptera	Ceratopogonidae	Culicoides
	Diptera	Ceratopogonidae	Dasyhelea
	Diptera	Ceratopogonidae	Mallochohelea

	Diptera	Ceratopogonidae	Monohelea
	Diptera	Ceratopogonidae	Probezzia
	Diptera	Ceratopogonidae	Sphaeromias
Non-Biting Midges	Diptera	Chironomidae	Chironomini
	Diptera	Chironomidae	Diamesinae
	Diptera	Chironomidae	Orthoclaadiinae
	Diptera	Chironomidae	Tanypodinae
	Diptera	Chironomidae	Tanytarsini
Mosquitoes	Diptera	Culicidae	Aedes
	Diptera	Culicidae	Anopheles
	Diptera	Culicidae	Culex
Mountain Midges	Diptera	Deuterophlebiidae	Deuterophlebia
Meniscus Midges	Diptera	Dixidae	Dixa
	Diptera	Dixidae	Dixella
Thick Headed Flies	Diptera	Dolichopodidae	Dolichopodidae
Dance Flies	Diptera	Empididae	Chelifera
	Diptera	Empididae	Clinocera
	Diptera	Empididae	Hemerodromia
	Diptera	Empididae	Metachela
	Diptera	Empididae	Trichoclinocera
	Diptera	Empididae	Wiedemannia
Shore Flies	Diptera	Ephydriidae	Scatella
	Diptera	Muscidae	Muscidae
Humpbacked Flies	Diptera	Phoridae	Phoridae
Moth/Sandflies	Diptera	Psychodidae	Maruina
	Diptera	Psychodidae	Pericoma
	Diptera	Psychodidae	Psychoda
Black Flies	Diptera	Simuliidae	Simulium
Soldier Flies	Diptera	Stratiomyidae	Caloparyphus
	Diptera	Stratiomyidae	Nemotelus
	Diptera	Stratiomyidae	Odontomyia
Deer Flies	Diptera	Tabanidae	Tabanidae
	Diptera	Tanyderidae	Tanyderidae
Crane Flies	Diptera	Tipulidae	Antocha
	Diptera	Tipulidae	Erioptera
	Diptera	Tipulidae	Limonia
	Diptera	Tipulidae	Ormosia
	Diptera	Tipulidae	Tipula
	Diptera		Brachycera
Giant Water Bugs	Hemiptera	Belostomatidae	Belostoma
	Hemiptera	Naucoridae	Ambrysus
Moths	Lepidoptera	Pyrilidae	Parapoynx
	Lepidoptera	Pyrilidae	Petrophila
Dragonflies-Darners	Odonata	Aeshnidae	Anax
Damselflies-Ruby Spots	Odonata	Calopterygidae	Hetaerina americana
Damselflies-Dancers	Odonata	Coenagrionidae	Argia
	Odonata	Coenagrionidae	Coenagrionidae
	Odonata	Coenagrionidae	Zoniagrion exclamationis
Dragonflies-Clubtails	Odonata	Gomphidae	Erpetogomphus
	Odonata	Gomphidae	Gomphus kurilis

	Odonata	Gomphidae	Octogomphus specularis
	Odonata	Gomphidae	Ophiogomphus
	Odonata	Gomphidae	Progomphus borealis
	Odonata	Gomphidae	Stylurus
Dragonflies-Skimmers	Odonata	Libellulidae	Brechmorhoga mendax
	Odonata	Libellulidae	Erythemis collocata
	Odonata	Libellulidae	Libellula
	Odonata	Libellulidae	Pachydiplax longipennis
	Odonata	Libellulidae	Pantala flavescens
	Odonata	Libellulidae	Plathemis
	Odonata	Libellulidae	Tramea
Water Mites	Acari	Hygrobatidae	Hygrobatidae
	Acari	Lebertiidae	Lebertiidae
	Acari	Sperchontidae	Sperchon
	Acari	Torrenticolidae	Torrenticolidae
Scuds	Amphipoda	Crangonyctidae	Crangonyx
	Amphipoda	Hyaellidae	Hyaella
Crayfish	Decapoda		Astacidea
Seed Shrimp	Ostracoda	Cyprididae	Cyprididae
Clams	Pelecypoda		Corbiculacea
Fresh Water Limpets	Gastropoda	Ancylidae	Ferrissia
Snails	Gastropoda	Lymnaeidae	Fossaria
	Gastropoda	Lymnaeidae	Radix
	Gastropoda	Physidae	Physa/ Physella
	Gastropoda	Planorbidae	Gyraulus
	Gastropoda	Planorbidae	Helisoma
	Gastropoda	Planorbidae	Planorbidae
Leeches	Hirudinea	Erpobdellidae	Erpobdellidae
	Hirudinea	Erpobdellidae	Mooreobdella
	Hirudinea	Glossiphoniidae	Helobdella
	Hirudinea	Glossiphoniidae	Placobdella/Oligobdella
Round Worms			Nematoda
Flatworms	Tricladida	Planariidae	Planariidae
Proboscis Worms	Nemertea	Tertastemmatidae	Prostoma
	Hydroida	Hydridae	Hydra
Segmented Worms	Tubificida	Lumbriculidae	Lumbriculidae
	Tubificida	Naididae	Naididae
	Tubificida	Tubificidae	Tubificidae
	Tubificida	Enchytraeidae	Enchytraeidae
