where the substrate was relatively stable. Diel drift was highest in areas where substrate consisted of gravel and cobble and was considerably lower in areas dominated by sand substrate. Chironomids, simuliids, baetid mayflies and oligochaetes comprised over 93 percent of drifting organisms.

For element 2 above, contribution of terrestrial organisms to drift as a food resource for steelhead was considerably higher (numerical abundance and biovolume) in canopied river reaches when compared to river reaches with no or little canopy cover.

For element 3 above, Fields reported the BMI assemblages of Pine Creek to be the most diverse and attributed the high diversity to the "unperturbed" condition of the site where samples were collected. Fields also found that while there was ample BMI drift downstream of San Clemente Reservoir, species diversity was low and almost all the food available as drift to steelhead consisted of black fly larvae.

For element 4 above, Fields found that trout inhabiting both San Clemente and Los Padres Reservoirs fed on invertebrates from three sources, in order of decreasing relative importance: riverine, lucustrine and terrestrial. By far, the terrestrial component was the least important food source to trout. Of the lucustrine food source, benthic invertebrates were more important than planktonic invertebrates.

2.0 METHODS

2.1 Monitoring Sites

To optimize time and budget constraints, originally only four sites were established by District staff. In fall of 2000, four monitoring sites on the Carmel River were chosen to conduct the CRBP. An additional site at the Sleepy Hollow Steelhead Rearing Facility's (SHSRF) rearing channel (SHRC) was sampled three times during the monitoring period. In 2004 a site was added upstream of Los Padres Reservoir (CRLP) and a site (CRSW) approximately one river mile upstream of site CRRR was added as an alternative to site CRRR during conditions of inadequate flow for sampling. A summary of all BMI sites monitored by the District is provided in **Table 1** where "B" indicates that benthic samples were collected and "H" indicates that a site scale habitat assessment was performed using the parameters shown in **Appendix A**. Site CRDD was sampled using a point-source design as part of a separate project, which precludes a site scale habitat assessment.

The sites are shown in **Figure 1**, along with the approximate locations of three of the District's streamflow gaging stations. Flow data for those stations, Below Los Padres (BLP), Sleepy Hollow Weir (SHW) and Don Juan Bridge (DJB) are provided in **Appendix I** along with continuous water temperature data monitored at three sites, upstream of Los Padres Reservoir, and downstream of Los Padres and San Clemente reservoirs. The four original invertebrate sampling sites were selected because they corresponded to established juvenile steelhead population survey sites and they were representative of most reaches of the Carmel River. Reaches farther downstream have lower gradients, a higher percentage of sand and fines, and frequently dry up during the dry season in response to pumping and low flows. The CRRW site was added in 2002 to determine if detrimental

effects were occurring as a result of the operation of the District's SHSRF, and to better detect effects of sedimentation from Tularcitos Creek. This site may also provide information on the effects of sedimentation and turbidity associated with the annual lowering of the water surface elevation of San Clemente Reservoir, which began in June 2003, in response to an order from the California Department of Water Resources, Division of Safety of Dams.

Site locations are summarized below:

- Los Padres – CRLP: upstream of Los Padres Reservoir;
- Cachagua - CRCA: between Los Padres Dam and Cachagua Creek;
- Sleepy Hollow - CRSH: about one mile downstream from San Clemente Dam, immediately above the SHSRF intake pumps;
- Sleepy Hollow Rearing Channel - SHRC: artificial off-channel steelhead rearing facility (sampled three times);
- Russell Wells - CRRW: added in 2002, between Sleepy Hollow and Stonepine;
- Stonepine - CRSP: just below confluence with Tularcitos Creek;
- DeDampierre - CRDD: sampled once in Spring 2001, prior to a restoration project that installed large-woody debris in channel;
- Scarlett Well – CRSW: alternate site sampled twice when the CRRR site was dry; and
- Red Rock - CRRR: Mid-Valley, below the Narrows; channel dries up here some years.

Table 1. Carmel River monitoring locations including year of sampling for benthic macroinvertebrates (B) and habitat assessment (H). Fall season unless indicated otherwise.

Site Name	Monitoring Sites							Other Sites	
	Los Padres	Cachagua	Sleepy Hollow	Russell Wells	Stonepine	Scarlett Well	Red Rock	DeDampierre	Sleepy Hollow Rearing Channel
Site Code	CRLP	CRCA	CRSH	CRRW	CRSP	CRSW	CRRR	CRDD	SHRC
River Mile	26.0	23.5	17.6	16.2	15.7	8.9	7.7	13.9	17.5
Site Elev. (ft)	1,100	820	380	360	280	200	110	250	400
2000		BH	BH		BH		BH		BH
2001 spring		BH	BH		BH		BH	B	
2001		BH	BH		BH		BH		
2002 spring		BH	BH		BH		BH		
2002		BH	BH	BH	BH		BH		
2003 spring		BH	BH	BH	BH		BH		
2003		BH	BH	BH	BH		BH		
2004	BH	BH	BH		BH		BH		BH
2005	BH	BH	BH		BH		BH		
2006	BH	BH	BH		BH		BH		
2007	BH	BH	BH		BH	BH			
2008	BH	BH	BH		BH	BH			BH
2009	BH	BH	BH		BH		BH		

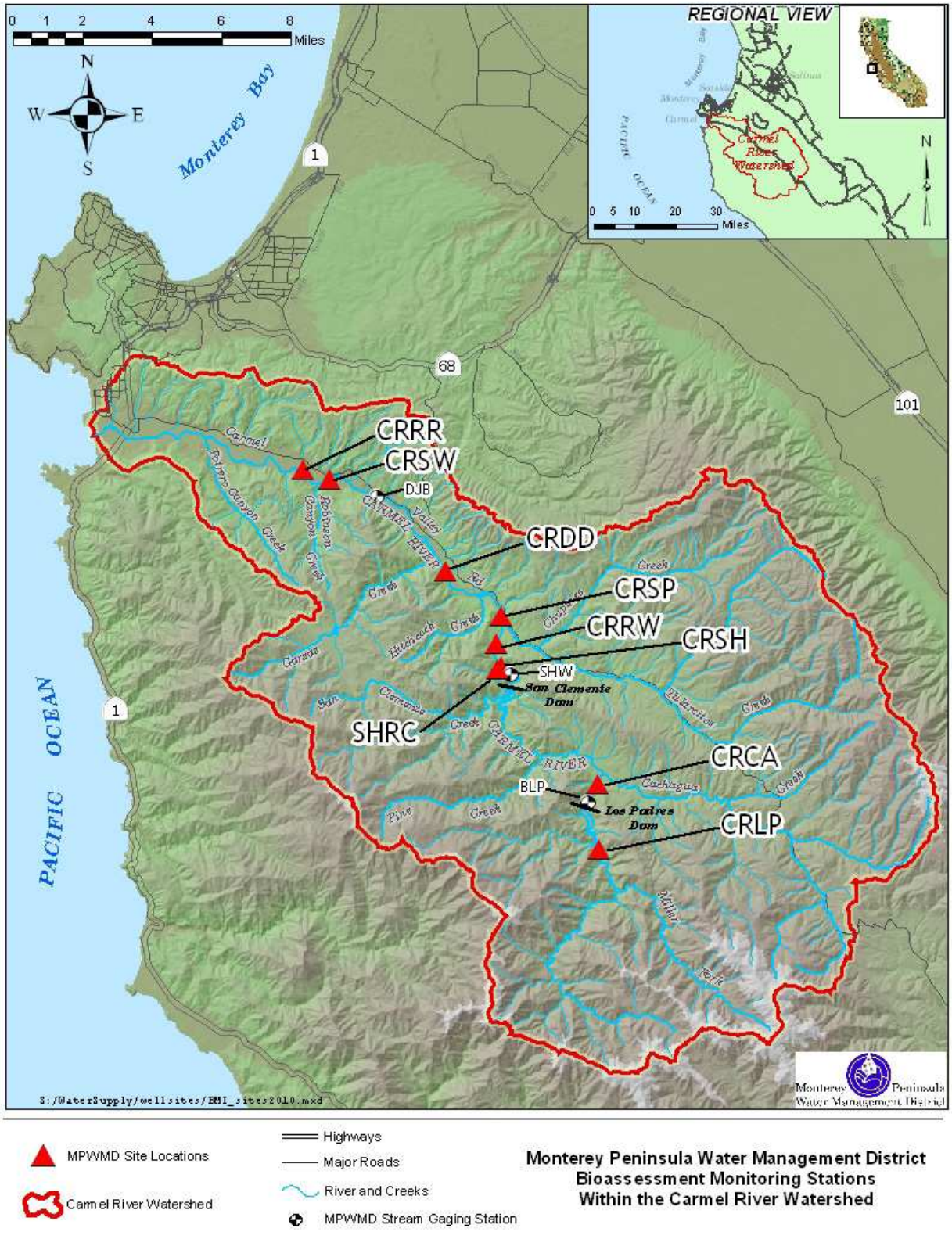

Figure 1. Benthic macroinvertebrate sampling stations in the Carmel River system.

2.2 Benthic Sampling

The non-point source portion of the CSBP was applied to this monitoring effort for documenting and describing BMI assemblages and physical habitat within the selected sites. The non-point sampling strategy is used to monitor general conditions along a stream segment or watershed where potential perturbations are diffuse and of variable magnitude. In contrast, the point source sampling strategy is used to assess changes in BMI assemblages upstream and downstream of a specific location where a potential perturbation, such as a storm drain, could affect water quality condition of the receiving stream. For both sampling strategies, a targeted riffle approach is used as specified in the CSBP.

The sampling strategy used for the CRBP is described as follows. Each sample reach consisted of riffle habitat units of varying number. Three riffles were randomly chosen for sampling when possible but for some sites with fewer than three riffles, samples were collected from different sections of the same riffle. Three subsamples were collected along a transect established perpendicular to the current, one near each bank, and a third near the thalweg. Samples collected from the three distinct riffles or riffle areas comprised the total samples for each site during each monitoring event.

Benthic samples were collected by rubbing cobble and boulder substrates and disturbing finer substrates for 90 seconds within a 2 square foot (sq. ft.) area upstream of a D-frame kicknet fitted with a 0.5 mm mesh net. The total area sampled per transect was 6 sq. ft. Each sample was transferred to a plastic jar, preserved with 95 percent ethanol and labeled. At each transect, where benthic samples were collected, several local habitat parameters were assessed including substrate composition, percent canopy, average stream velocity, average water depth and riffle gradient (**Appendix A**). A substrate index was developed where each composite benthic sample was collected from riffle habitat. The substrate index was calculated as a weighted mean midpoint substrate size as described by Quinn and Hickey (1990). The following categories were used to classify substrate: sand/fines (<2 mm) gravel (2-64 mm), cobble (64-256 mm), boulder (256-330 mm) and bedrock. Bedrock was assigned a nominal size of 400 mm (Quinn and Hickey 1990).

2.3 Habitat and Water Quality Assessment

At each site, physical characteristics of the riparian zone were documented using the CDFG's Aquatic Biological Laboratory's Physical/Habitat field Data Sheet (May 1999 revision), which in turn is based on the US EPA's Rapid Bioassessment Protocols for high gradient streams (Barbour et al. 1999). Criteria for scoring the habitat parameters are shown in **Appendix A**. In addition, sites were photographed and water quality measurements recorded. Dissolved oxygen, pH and temperature were measured using either a Hach test kit or YSI 85 multi-meter. Specific conductance was measured with a calibrated Cole-Parmer TDSTestr, model 20, and YSI 85 multi-meter, which were calibrated prior to the sampling trip and checked daily.

2.4 Sample Processing and Data Analysis

Samples were processed according to a standard level of analysis as per the California Stream Bioassessment Procedure. At the laboratory, each sample was rinsed in a standard no. 35 sieve (0.5 mm) and transferred to a tray with twenty, 4 $in.^2$ (25 cm^2) grids for subsampling. Benthic material in

the subsampling tray was transferred from randomly selected grids (or half grids if BMI densities were high) to Petri dishes where the BMIs were removed systematically with the aid of a stereomicroscope and placed in vials containing 70 percent ethanol and 30 percent water. From 2000 to 2003, at least 300 BMIs were subsampled from a minimum of three grids. If there were more BMIs remaining in the last grid after 300 were archived, then the remaining BMIs were tallied and archived in a separate vial. This was done to assure a reasonably accurate estimate of BMI abundance based on the portion of benthos in the tray that was subsampled. These "extra" BMIs were not included in the taxonomic lists and metric calculations. From 2004 to 2009 the three samples collected at each site were composited at the laboratory and 500 (±5%) organisms were subsampled. This latter procedure change was consistent with the methods outlined in the 2003 version of the CSBP.

Starting in 2005, the subsampling procedure was supplemented to accommodate an estimate of BMI biovolume. Biovolume measurements were made by calculating the volume of liquid displaced by the subsampled BMIs from each sample prior to sorting by taxon. Subsampled BMIs were transferred to a 35% ethanol solution prior to volumetric displacement measurements. Surface liquid was removed from the BMIs using blotting paper after the BMIs were transferred to a 5.0 ml graduated cylinder. The blotting paper was rolled into a cylinder of suitable diameter to facilitate insertion into the graduated cylinder to the level of the BMIs. The graduated cylinder was then inverted to facilitate the wicking effect of the blotting paper. The endpoint of removing surface liquid from the BMIs occurred when the wicking action of the blotting paper ceased. A 35% ethanol solution was dispensed from a 10 ml capacity burette to the graduated cylinder to the 5.0 ml mark. The volume of organisms was determined by subtracting the volume of liquid/organism mixture contained in the graduated cylinder (5.0 mls) from the volume of liquid dispensed from the burette. For example, if 3.2 mls of ethanol solution were dispensed from the burette to fill the 5.0 ml graduated cylinder, then the volume of the BMIs was 1.8 mls. After biovolume measurements, the BMIs were preserved in an 80% ethanol, and 20% water solution. BMI volume of the sample was then estimated and reported as mls per m^2 of benthos sampled.

Subsampled BMIs were identified using taxonomic keys (Merritt and Cummins 1996; Stewart and Stark 1993; Thorp and Covich 2001 and Wiggins 1996) and unpublished references. A standard level of taxonomic effort was used as specified in the California Aquatic Macroinvertebrate Laboratory Network (CAMLnet, http://www.nps.gov/yose/naturescience/upload/Macroinvertebrates.2003.pdf) short list of taxonomic effort, January 2003 revision and the Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT, http://www.safit.org/). Exceptions were made for some early instar organisms and organisms in poor condition. Other exceptions included the identification of midges to subfamily/tribe. The subsampled BMIs identified from each sample were archived in labeled vials with a mixture of 70 percent ethanol and 30 percent water.

2.4.1 Macroinvertebrate Metrics

BMI taxa and the number of BMIs comprising each taxonomic group were entered into a Microsoft Access® database. Database queries generated taxonomic lists which were transferred to a spreadsheet program where a suite of biological metrics was calculated. Data sets from year 2000 to year 2003 consisted of three samples of 300 organisms each, resulting in a 900 organism subsample for each site. Since the current protocol yields a 500 organism subsample for each site, the 900

organism subsamples were reduced to 500 organisms for the purpose of equalizing processing effort. Two methods were used to standardize the data set consisting of 900 organism subsamples. First, for presentation of taxonomic lists, 900 organism subsamples were reduced to 500 organisms by proportion to avoid loss of taxa. The second method was applied to the original taxonomic list for metric calculations and consisted of converting 900 organism subsamples to 500 organisms using software that resampled the data without replacement. This latter resampling technique resulted in the probability of lost taxa but was necessary so that metrics associated with richness could be compared for all years using the same subsample size of 500 organisms. Richness metrics are influenced by subsample size and are part of the suite of metrics used in the application of indices of biotic integrity (Section 2.4.2). It is therefore necessary to apply an equal subsampling effort across all sample units when indices of biotic integrity are used.

Biological metrics provide numerical attributes of biotic assemblages and are described in **Appendix B**. Tolerance values and functional feeding group designations were obtained from the California Macroinvertebrate Laboratory network (CAMLnet) short list of taxonomic effort, January 2003 revision. The SAFIT, which replaced CAMLnet in 2006, is a network of professional taxonomists that conducts taxonomic workshops and establishes standard taxonomic effort guidelines. Where possible, all taxa identified for the CRBP were standardized to the SAFIT level 1 standard taxonomic effort. Biological metric values were tabulated by sample and summarized at the project scale and sample scale.

The various metrics can be categorized into five main types:

- Richness Measures (reflects one component of diversity);
- Composition Measures (reflects the distribution of individuals among the taxonomic groups);
- Tolerance/Intolerance Measures (reflects the relative sensitivity of the assemblage to disturbance);
- Functional Feeding Groups (shows the balance of feeding strategies in the aquatic assemblage);
- Abundance and biovolume (estimate of total number and volumetric displacement of organisms in a sample based on the area sampled)

2.4.2 Index of Biotic Integrity

To assess the biological integrity of the sites, the coastal southern California index of biotic integrity (IBI) (Ode et al. 2005) was applied to the 10-year data set. Development of the IBI included the screening and testing of 61 possible metrics from 275 sites exhibiting a wide range of condition, from reference sites to severely impaired sites. Seven metrics were selected and were scored and combined into a composite index. The objectives of a regional IBI are to incorporate metrics that measure distinct attributes of the BMI assemblage, and are responsive to stressor gradients while maintaining a high signal-to-noise ratio. The spatial extent of the coastal southern California IBI includes the Carmel River watershed (Ode et al. 2005).

The seven metrics used to develop the IBI are:

1. Coleoptera (beetle) taxa
2. EPT [Ephemeroptera (mayfly), Plecoptera (stonefly) and Trichoptera (caddisfly)] taxa

3. Predator taxa
4. Percent collector individuals
5. Percent intolerant individuals
6. Percent non-insect taxa
7. Percent tolerant taxa

The product of the IBI yields scores and narrative descriptions of biotic integrity as follows: 0 to 19 (very poor), 20 to 39 (poor), 40 to 59 (fair), 60 to 79 (good), and 80 to 100 (very good). The IBI values generated for the CRBP were used as a primary biological signal to assess site quality and to explore interactions with other variables relating to physical habitat and other factors such as seasonal differences.

2.4.3 Ordination

Nonmetric multidimensional scaling (NMS) ordination was used to evaluate relative similarity of samples based on BMI taxonomic composition. Unlike other ordination methods that require normal data distributions, NMS ordination is based on ranked distances, which make it suitable for ecological data that are often not normally distributed nor measured on continuous scales (McCune and Grace 2002). The output of NMS is a plot, which shows sample units oriented in relative space along one or more ordination axes where the distance between the samples increases with increasing taxonomic dissimilarity. In addition, quantitative environmental variables can be included as an overlay of lines (termed 'joint plot') radiating from the center of the graph, with each line indicating both the direction and strength of correlation with the graph axes. The graph axes represent the unit-less numeric 'scores' generated during the 12-step ordination procedure that orients the sample units along the graph axes based on relative taxonomic dissimilarity. The numeric 'scores' are used for correlation with quantitative environmental variables (section 2.4.4). In addition, the application of categorical variables can be used to identify ecologically meaningful site groupings. While NMS consists of many steps involving complex mathematical algorithms, the output is visually straightforward and is useful for screening multiple variables for relationships, identifying patterns in ecological data and summarizing results in graphical formats. For additional information on NMS applications and procedures see McCune and Grace (2002), Clarke (1993), and Mather (1976).

PC-ORD® version 5 software (McCune and Mefford 2006) was used to perform NMS in "autopilot mode", utilizing the "slow and thorough" setting (500 iterations) and the Sorensen (Bray-Curtis) distance measure. Plots of stress versus iteration (scree plots) were evaluated to assure that improvement in fit was achieved with added dimensions and exceeded a cumulative coefficient of determination of 0.6.

2.4.4 Analyses

Data analyses were primarily exploratory, utilizing graphics and tables of pertinent summary information, with the objective of revealing patterns in biological data across sites and their relationships with environmental variables. Hypothesis testing was used in some cases to detect significant differences but these analyses should be considered with caution because a priori hypotheses were not developed as part of the CRBP and budget constraints limited sample sizes. Sample size limitations were partially overcome by combining samples from sites in close proximity:

samples from site CRRW were combined with samples from CRSP and samples from CRSW were combined with samples from CRRR.

Statistical analyses included the application of the non-parametric Wilcoxon paired sample test to evaluate significant seasonal effects on IBI, EPT taxa, and Predator taxa values for the time period between 2001 and 2003 when both spring and fall samples were collected. One-factor analysis of variance (ANOVA) was used to test for significant differences in log transformed abundance and biovolume data across sites. The non-parametric alternative to ANOVA, Kruskal-Wallis, was applied when assumptions of normal data distributions and homogeneity of variance were not met. Pearson correlations were used to test for significant increases or decreases in IBI values at the monitoring sites through the 10-year monitoring period.

NMS ordination was applied to the CRBP data set for examining potential effects of categorical and quantitative environmental variables on taxonomic composition. Categorical variables included seasonality of sampling (spring and fall), sample type (reference and non-reference), year of sampling, and water-year type. Quantitative variables included elevation, total habitat score, gradient, canopy, substrate index, substrate classes, water temperature and specific conductance. The IBI values were included as a quantitative biological variable. A threshold coefficient of determination of 0.20 was used to screen quantitative variables for the joint plot; coefficient of determination values less than 0.20 were excluded from the joint plot. Numbers of organisms comprising each taxon and quantitative environmental variables were log transformed prior to running ordination.

The RWQCB, in association with the CDFG, collected and processed benthic samples using the CSBP from sites on the Carmel River from 2001 to 2004, and again in 2007. The sites were located near the mouth of the Carmel River at the Highway 1 crossing and at river mile 14.5 at Esquiline Road. BMI data were obtained through the CCAMP. IBIs were calculated for the CCAMP sites after standardizing subsample size to 500 organisms when necessary.

Methods employed by Fields (1984) in the spring season of 1982 for characterizing BMI fauna of the Carmel River were evaluated for applicability to methods used for this current monitoring program. Factors considered for data set compatibility included sampling sites, sampling method and sample processing method including standard taxonomic level.

3.0 RESULTS

3.1 Benthic Macroinvertebrates

The ten-year CRBP yielded a total of 133 samples from which 46,378 BMIs were processed. After site compositing and standardization of subsample size, 66 composite samples were generated comprising 111 total taxa, 42 EPT taxa, 13 mayfly taxa, six stonefly taxa, 23 caddisfly taxa, and 14 beetle taxa (**Table 2**). Tolerance and Shannon Diversity for the pooled samples were 5.1 and 2.7, respectively. Median sample taxa richness was 21 (range 13 - 41), median EPT richness was 7 (range 4 – 22), median mayfly richness was 2 (range 1 – 9), median stonefly richness was 0 (range 0 – 6), median caddisfly richness was 5 (range 2 – 12), and median beetle richness was 1 (range 0 – 5).