University of Nevada, Reno

Using Benthic Indicator Species and Community Gradients to Optimize Restoration in the Arid, Endorheic Walker River Watershed, Western USA

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

by

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Abstract

The challenge of restoring watersheds in arid regions often requires the development of novel scientific tools to guide management. The Walker Basin Program was created to reverse ecological decline in an arid, endorheic watershed through scientifically guided restoration. As part of this program, three years of benthic macroinvertebrate samples were collected seasonally at ten sites that represent the diversity of river environments from the high mountain headwaters to a desert terminal lake. Samples were analyzed to quantify baseline conditions in reference and degraded reaches of river and identify opportunities and constraints for aquatic community restoration. Naturally harsh environments in the lower river characterized by high temperatures and low base flow combined with a weak understanding of reference conditions to limit the utility of commonly used indices for quantifying biotic integrity. A flexible approach was employed using a combination of indicator species analysis, cluster analysis, canonical correspondence analysis, and community tolerance indices to evaluate the variation of benthic macroinvertebrate community composition across a set of environmental gradients. Results demonstrate that benthic communities in the watershed are primarily influenced by a longitudinal gradient related to elevation. A strong secondary community gradient caused by anthropogenic nutrient loading may constrain restoration effectiveness in some parts of the watershed. Restoration activities should improve water quality conditions and initially target areas of the watershed less affected by nutrient loading. Results also demonstrate that benthic communities shift longitudinally. These shifts should be monitored to inform adaptive management of restoration actions.
Acknowledgements

This study was funded by a grant under Public Laws 109-103, Section 208 (a) through the U.S. Bureau of Reclamation (Cooperative Agreement 06FC204044). BMIs were identified and enumerated by C. Rosamond with Desert Research Institute, Reno, Nevada. A. Schwaneflugel and K. Mehler assisted with field work, and BMI processing was conducted by J. Dillon, J. Dillon. The manuscript was greatly improved by constructive comments from an anonymous reviewer.
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Introduction

Arid watersheds of western North America provide unique challenges for the study, management, and restoration of aquatic ecosystems. Aridity restricts humans and economies to small areas with adequate surface or ground water often originating in wetter mountain areas. Aridity limits the extent of forest communities to high elevations and restricts aquatic communities to a small number of widely scattered water bodies. The water demands of even small human economies can have dramatic impacts on terrestrial and aquatic ecosystems. The hydrographic Great Basin is an expanse of endorheic basins that encompass approximately 20 percent of the United States between the Sierra Nevada on the west, the Wasatch Range on the east, and south of the Snake River Plain to the Colorado River drainage (Grayson 1993). It is the most arid region in the United States and includes more than 150 north-south oriented mountain ranges that receive most precipitation during winter storms. Water flows from mountains into intervening valleys where it accumulates in terminal lakes or percolates into the soil as ground water. Mountains support coniferous forests. Most valleys are covered by sagebrush. Forest and meadow areas in valleys are limited to riparian areas along streams, rivers, and spring brooks.

Though a moderate number of studies have examined limnology (e.g. Galat et al. 1981, Cooper and Koch 1984, Benson et al. 1991, Buetel et al. 2001), aquatic invertebrate diversity (e.g. Hershler 1998, Hershler and Sada 2002, Polhemus and Polhemus 2002), and fisheries (e.g. Hubbs and Miller 1948, Smith 1978, Dickerson and Vinyard 1999) in
the western Great Basin, few studies have considered river benthic macroinvertebrate (BMI) communities and there is little information available to assess how its aquatic life varies over the course of western Great Basin streams and rivers. These data gaps contribute to the challenge of planning and implementing watershed analysis, restoration, and monitoring in the western Great Basin.

Watershed analysis and monitoring are essential components of the watershed restoration process. Watershed analysis serves the primary purpose of identifying opportunities and constraints prior to a restoration project so that the project can be planned for optimum impact and cost-efficiency (Kershner 1997). Monitoring measures restoration effectiveness over time by comparing project outcomes with project goals and provides feedback for adaptive management (Downs and Kondolf 2002). Both of these components of the restoration process are often overlooked by restoration practitioners for the sake of cost savings, expediency, or perceived lack of importance (Palmer et al. 2007, Bernhardt et al. 2005, Bash and Ryan 2002). As a result, restoration projects are often planned and evaluated based on subjective, often aesthetic, measures of ecological improvement (Palmer et al. 2007, Kondolf et al. 2007). In cases where watershed assessments are conducted and monitoring is implemented, deficiencies of baseline data or scientific understanding can prove challenging to overcome, especially in watersheds that have not been well studied (Follstad Shah et al. 2007, Kondolf and Micheli 1995). The initial watershed analysis, the establishment of a baseline data set, and the implementation of monitoring are all made more complex by the dynamic nature of
aquatic communities. The cumulative effects of annual hydrograph structure aquatic communities both spatially and temporally (Lytle and Poff 2004, Strange et al. 1999).

Temperature has a strong effect on the structure of benthic communities and generally functions as a longitudinal gradient of increased thermal load in mountainous watersheds of western North America (Boyle and Strand 2003, Hawkins et al. 1997). Increased loads of nutrients and other pollutants can lead to sharp changes in BMI community structure often associated with land use (Cuffney et al. 2000). Abiotic factors such as flow, temperature, and water chemistry can be seen as a complex set of filters that determine the BMI community at a given location (Poff 1997). Changes to these abiotic ‘filters’ over space and time may lead to an expansion, contraction, or shift in communities.

The river continuum concept (Vannote et al. 1980) models watersheds as a longitudinal gradient of communities, with a different composition of functional feeding groups in the upper, middle, and lower reaches of a watershed. Anthropogenic changes to flow, temperature, or water chemistry may lead to a longitudinal shift in the community gradient (Voelz and Ward 1990). Understanding and predicting community shifts at the watershed level can be extremely valuable for optimizing restoration. Monitoring these shifts over time can lead to valuable insights that increase the effectiveness and cost efficiency of restoration actions (Kershner 1997) and adaptive management programs.

This study was conducted as part of the Walker Basin Science Program for the purpose of informing and guiding future hydrologic and geomorphic restoration actions in the Walker River basin.
Methods

Study Area

The Walker River originates above 3,000 m elevation on the east side of the Sierra Nevada, California (Figure 1) and flows to its terminus at Walker Lake. Walker Lake is one of three terminal lakes on the west side of the Great Basin with a native population of the threatened Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*). Agricultural diversions over the past century have decreased discharge and altered the natural hydrograph. Decreased discharge has elevated river water temperature in this arid environment and caused the lake level to decline approximately 43 m. This has resulted in an increase in total dissolved solids from 2,500 mg/L to 15,000 mg/L, thereby changing Walker Lake limnology and ultimately causing a precipitous decline in all fish populations (Cooper and Koch 1984, Beutel et al. 2001, Dickerson and Vinyard 1999). Furthermore, the Walker River and many of its tributaries are designated as ‘impaired’ under the clean water act in both Nevada and California due to nutrients, temperature, metals, and other factors (EPA 2012).
Figure 1: Map of the Walker basin showing sampling sites (site abbreviations are as defined in Table 1).

The natural hydrograph of the Walker River is primarily influenced by high elevation snowmelt, and highest flows normally occur in the late spring and early summer. Two forks, the West Walker (WW) and the East Walker (EW), flow out of the Sierra Nevada and through valleys and canyons of varying gradients before joining in Mason Valley where water is diverted for agricultural uses. The lower portions of each fork are regulated by a reservoir. Bridgeport reservoir, on the East Walker, is wide and shallow and surrounded by actively grazed pasture. The West Walker is free-flowing upstream
from Topaz Lake, an off channel reservoir used for water storage and flow regulation. Stream gradients and substrate sizes are highest upstream from Mason Valley. After reaching Mason Valley the two forks join and the Walker River main stem (W) flows in a wide arc through sandy alluvium and highly altered agricultural landscape before reaching its terminus at Walker Lake.

**Approach**

In watersheds that have not been well studied, such as the Walker River and other watersheds of the western Great Basin, a greater understanding of aquatic communities and ecological gradients is needed effectively assess biotic integrity and river health (Kennedy et al. 2000). Community composition of BMIs is often used as a measure of anthropogenic impact in watersheds (Karr 1999), and likewise an important measure of restoration priorities and successes (Poff et al. 2010, Gore et al. 2001). Baseline data sets can be used to generate biotic indices to measure present and future conditions (Karr 1981). These methods require the comparison of some component of the community (the index) at the site of interest to the same community component at undisturbed or unimpaired ‘reference’ sites (Reynoldson et al. 1997). This comparison involves two potentially inaccurate assumptions: 1) the reference site is undisturbed or the relative degree of disturbance can be accurately assessed so as to function as a precise benchmark by which to measure disturbance at other sites and 2) the component of the community that makes up the index measurably responds to the to the specific impairment or disturbance being addressed. Data from reference sites is considered ‘baseline’ data to which other data can be compared.
In many ecosystems of the world there are no truly undisturbed sites, so the reference sites selected are therefore limited to the least disturbed of available sites based on surveys of attributes related to disturbance (Stoddard et al. 2006). Tetra Tech (2007) found that, though the upper (montane) reaches of western Great Basin watersheds contained many sites that were mostly pristine, the lower reaches of these watersheds contained no sites that were free of anthropogenic disturbance or ecological impairment. The synergistic effects of extensive agricultural development and water delivery infrastructure have not missed any aquatic habitats in this arid region. For example, even sites that lie within the relatively undisturbed confines of wildlife refuges or other public lands are inherently affected by hydrologic alteration and pollution from upstream.

Tetra Tech, Inc. (2007) developed a common set of BMI indices to be used for the Walker River and the nearby Truckee and Carson Rivers. Tetra Tech concluded that existing biotic indices based largely on Hilsinhoff tolerance values (Hisenhoff 1988) and EPT (Ephemeroptera, Plecoptera, Trichoptera) diversity cannot accurately describe river health for lower watersheds as they can for upper watersheds. The difficulty of establishing tolerance values that accurately describe reference conditions is a common problem in arid, mountainous, and poorly studied regions of western North America (Blinn and Ruiter 2006). Lower reaches of these systems are naturally harsh environments, and their physical, chemical, and hydrologic characteristics have been altered for many decades by human activity. This makes it difficult to quantify reference conditions and to assess the efficacy of restoration. An ideal biotic index to assess restoration should accurately measure changes in anthropogenic factors structuring BMI
communities throughout the watershed and be sensitive enough to identify the effects of hydrograph, temperature, and pollution. Existing biotic indices do not accurately gauge all of these factors in the Walker River watershed.

To more accurately inform restoration design and assessment, we analyzed Walker River BMI communities, the river environment, and their seasonal and inter-annual shifts over three years using indicator species analysis (IndVal), cluster analysis, canonical correspondence analysis (CCA), and community tolerance indices. Using multiple analyses is a way to overcome the limitations of singular use of ordination or biotic indices. BMI community datasets on a watershed scale are often characterized by high dimensionality due to the large number of taxa in the data set (Miserendino 2001). Graphical representation through ordination in two or three dimensions may be insufficient to accurately represent community structure (McCune and Grace 2002). IndVal serves to describe communities and how they differ from each other (Dufrêne and Legendre 1997). Cluster analysis provides a hierarchical grouping framework on which to overlay the IndVal analysis. CCA determines which environmental variables have the greatest influence on these community differences (ter Braak and Verdonschot 1995). We compared results from multiple analytical methods to determine if observed community gradients and shifts are natural or anthropogenic. The result is a flexible approach useful for describing a minimally studied arid watershed and analyzing opportunities and constraints for restoration planning.

Sample Methods

Benthic Macroinvertebrates
Ten riffle sites were sampled in 2007, 2008, and 2010 ranging from headwater streams to the lowest reaches above Walker Lake (Figure 1, Table 1). These sites were located at or near sites used for discharge and water quality monitoring (DRI 2010). A single transect was surveyed at each site up to three times each year (spring, summer, autumn). ‘Spring’ sampling was conducted prior to the onset of the snowmelt pulse. ‘Summer’ sampling was conducted after the snowmelt driven flows had dropped to wadeable levels but before reaching base flow. Autumn sampling was conducted at base flow levels prior to the onset of winter weather events. Headwater sites and the lowest two sites were not sampled in all seasons or in all years (see Table 1). Sampling of headwater sites was expanded later in the study to illustrate reference conditions at the upstream end of the watershed and was not conducted in the spring due to deep snowpack. Sampling of the lowest two sites was precluded in some seasons and years due to limitations on access to tribal property.
Table 1: Site codes for Walker River sample locations, site elevations, and seasons and years that BMIs and river environments were sampled.

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>Site Code</th>
<th>Elevation (M)</th>
<th>Seasons Sampled</th>
<th>Years Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker A</td>
<td>WA</td>
<td>1251</td>
<td>Spring (not 2008), summer, autumn</td>
<td>2007,2008</td>
</tr>
<tr>
<td>Walker C</td>
<td>WC</td>
<td>1320</td>
<td>Spring, summer, autumn</td>
<td>2007,2008,2010</td>
</tr>
<tr>
<td>East Walker A</td>
<td>EWA</td>
<td>1394</td>
<td>Spring, summer, autumn</td>
<td>2007,2008,2010</td>
</tr>
<tr>
<td>East Walker C</td>
<td>EWC</td>
<td>2183</td>
<td>Summer (not 2008), autumn</td>
<td>2008, 2010</td>
</tr>
<tr>
<td>West Walker A</td>
<td>WWA</td>
<td>1417</td>
<td>Spring, summer, autumn</td>
<td>2007,2008,2010</td>
</tr>
<tr>
<td>West Walker C</td>
<td>WWC</td>
<td>2175</td>
<td>Summer, autumn</td>
<td>2010</td>
</tr>
</tbody>
</table>

Benthic macroinvertebrates were collected in six, one square foot kick-net samples (spaced evenly across the wetted width) along each transect and combined into a single composite sample. Composite samples were sub-sampled by plankton splitter and all organisms were picked from each sub-sample before beginning a new sub-sample. This method minimized body-size related bias in sampled picking. BMIs were identified to the lowest taxonomic level possible - which was to genus for most groups. Approximately 300 randomly selected organisms were identified from each sample (Vinson and Hawkins 1996). Organisms that could not be identified to the same taxonomic level as
other organisms within a taxonomic group were considered ‘non-distinct’ and not used in the analysis. Non-distinct organisms were generally early instars or damaged specimens. Counts of all species were standardized to 300 for each sample.

Rare taxa were defined as all taxa that did not total more than 50 organisms in the entire data set, or more than 5 percent (>15 organisms) of any one sample after the counts were standardized to 300. Rare taxa were not used in the CCA, cluster, and indicator species analyses because rare taxa known to have a disproportionate effect on community gradient analyses (Legendre and Gallagher 2001) and can therefore obscure important community trends in gradient analyses conducted at large spatial scales (Cao et al. 2001, Marchant 2002). A single community matrix was utilized for the CCA, cluster, and indicator species analyses. This species matrix was LOG(n+1) transformed to stabilize the variance. Rare taxa were included in the tolerance analysis because this utilized whole-community indices calculated using a separate community matrix from the other analyses.

River Environment

Physical and chemical characteristics of the river environment were quantified during each sample. Physical characteristics were determined along transects (were BMIs were also collected) that spanned the wetted width and included 25 depth and mean water column velocity measurements at even intervals across each transect using a Marsh-McBirney Model 2000 flow meter with a top set rod. Substrate size, embeddedness, and the depth of submerged vegetation and detritus were also measured at 100 points along each transect at the four corners surrounding a 30.5 cm² (1 ft²) area that was centered on
each depth/velocity point (Table 2). Substrate size was estimated visually using the
markings on the topset rod. Embeddedness was estimated visually as the percentage of
each measured substrate piece embedded in fine (< 2mm) sediment. The thickness of the
layers of submerged vegetation and detritus covering the substrate at each point were
measured using the top set rod. Water temperature was measured at 15 minute intervals
from March through September using Hobo® Water Temp Pro Loggers. Some loggers
were lost due to vandalism or high flows. In these cases temperature was estimated by
regression of nearby weather station data (WRCC, http:\www.wrcc.dri.edu) with water
temperature at upstream sites or ambient air temperature depending on which relationship
provided the greatest $R^2$. All temperature regressions used had $R^2$ values greater than 0.9.
Water samples were collected during benthic sampling and habitat surveys.
Concentrations of total phosphorus, total nitrogen, NO₂ + NO₃, and total suspended
sediments were determined by the Desert Research Institute Analytical Chemistry
Laboratory.
Table 2: Habitat variables used in the CCA. All % values scaled for analysis.

<table>
<thead>
<tr>
<th>Habitat Variable</th>
<th>Code</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>SEASON</td>
<td>1,2,3</td>
</tr>
<tr>
<td>Elevation</td>
<td>ELEV</td>
<td>M</td>
</tr>
<tr>
<td>Wetted Width</td>
<td>WW</td>
<td>M</td>
</tr>
<tr>
<td>Mean Water Depth</td>
<td>MnWD</td>
<td>CM</td>
</tr>
<tr>
<td>Mean Substrate Size</td>
<td>MnSUB</td>
<td>MM</td>
</tr>
<tr>
<td>Fines</td>
<td>F</td>
<td>%</td>
</tr>
<tr>
<td>Sand</td>
<td>SA</td>
<td>%</td>
</tr>
<tr>
<td>Gravel</td>
<td>GR</td>
<td>%</td>
</tr>
<tr>
<td>Cobble</td>
<td>CO</td>
<td>%</td>
</tr>
<tr>
<td>Boulder</td>
<td>BO</td>
<td>%</td>
</tr>
<tr>
<td>Aquatic Vegetation</td>
<td>VEG</td>
<td>%</td>
</tr>
<tr>
<td>Detritus</td>
<td>DETR</td>
<td>%</td>
</tr>
<tr>
<td>Mean Embeddedness</td>
<td>MnEmb</td>
<td>%</td>
</tr>
<tr>
<td>Mean Water Velocity</td>
<td>MnWV</td>
<td>M/S</td>
</tr>
<tr>
<td>Maximum Water Temperature 60 days</td>
<td>MaxT60</td>
<td>°C</td>
</tr>
<tr>
<td>Minimum Water Temperature 60 days</td>
<td>MinT60</td>
<td>°C</td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td>TP</td>
<td>Mg/L</td>
</tr>
<tr>
<td>Nitrate + Nitrite</td>
<td>NO3+NO2</td>
<td>Mg/L</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>TN</td>
<td>Mg/L</td>
</tr>
<tr>
<td>Total Suspended Solids</td>
<td>TSS</td>
<td>Mg/L</td>
</tr>
</tbody>
</table>

Data Analysis

Indicator Species Analysis and Cluster Analysis

Dufrêne and Legendre (1997) define indicator species as taxa that indicate the presence of a discrete community based on two proportions: 1) the number of occurrences of the species in samples within a sample group (Nindividuals_{ij}) over the total of all occurrences
of that species in the data set (Nindividuals_i) and 2) the number of samples within the
group that contain the species (Nsamples_ij) out of the total number of samples in the
group (Nsamples_j) where:

\[ A_{ij} = \frac{\text{Nindividuals}_{ij}}{\text{Nindividuals}_{i}} \]

\[ B_{ij} = \frac{\text{Nsamples}_{ij}}{\text{Nsamples}_{j}} \]

\[ \text{IndVal}_{ij} = A_{ij} \times B_{ij} \times 100 \]

The resulting IndVal index ranges from 0 to 100 and reflects both group fidelity and
regularity of presence, but not numerical abundance. The presence or absence of taxon
primarily influences the results, though the total number sampled of a taxon is relevant to
the analysis. In this study, each ‘group’ was a group of samples representing a
community type. The samples that comprised each group were usually related spatially or
temporally.

Indicator species analysis and cluster analysis of all BMI communities sampled in 2007,
2008, and 2010 was conducted using PC-ORD (McCune and Grace 2002). A cluster
dendrogram of all samples was created using a Sorenson (Bray-Curtis) distance measure
and a flexible beta of -0.25. At each branching level of the dendrogram a new IndVal
index was calculated for each species at every sample grouping existing at that level. For
example, two groups existed at the first branching level and an IndVal index was
generated for every species relative to each of these two groups. In general, IndVal
indices for an individual taxon were low for most groupings but would peak for a
particular grouping associated with a new branch in the dendrogram. IndVal indices
were tested against the distribution of indices from 4999 permutations of randomized grouping. Taxa with IndVal indices greater than 50 and $\alpha$ less than 0.05 were selected as indicator species at a particular grouping level. The lowest grouping level at which to assign indicator species was determined subjectively based on the size and spatial structure of sample groupings. Groups needed to be spatially discrete so that they could be assigned an easily identifiable geographic description useful for interpretation of discrete BMI community types. Groups also needed to be large enough to represent a pattern or trend in community type: a group of less than three samples could not represent a broader pattern.

*Canonical Correspondence Analysis*

Canonical Correspondence Analysis examining relationships between 69 species and 20 environmental variables was carried out using the software *PC-ORD* (McCune and Grace 2002). Results were plotted to optimize samples using biplot scaling, three axes of variation were interpreted, and the significance of the first axis was tested using 999 Monte Carlo tests of randomized data. Sites WWC and EWC were not included in CCA to reduce total variation and focus the analysis of environmental eigenvectors on parts of the watershed of primary interest for restoration. The proportion of each substrate type, vegetation, detritus, and substrate embeddedness were categorized into 12 classes based on range means to avoid violation of covariate assumptions.

*Community Tolerance Indices*
Community Tolerance Indices were calculated using taxon-specific tolerance values from a regularly updated regional database (Ode 2003). Community tolerance was calculated by multiplying the number of individuals of each distinct taxon picked from the sample by that taxon’s tolerance score. The sum of all of these products was divided by the total number of organisms picked to generate the community tolerance value. Indices for every sample from a site in all seasons and years were averaged to determine the mean tolerance index for each site. Sites WWC and EWC were not included due to the low number of samples (N = 1 or 2) taken at these sites.

Results

A total of 25,250 individual organisms were identified from 342 taxa in 68 samples. Many of these taxa occurred sporadically and were too ‘rare’ to be included in the analyses. Sixty-nine taxa (Table 3) in 68 samples were used for the indicator species and cluster analyses. 69 taxa in 64 samples were used for the CCA and community tolerance analyses.
<table>
<thead>
<tr>
<th>Code</th>
<th>Taxon</th>
<th>Code</th>
<th>Taxon</th>
<th>Code</th>
<th>Taxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMEL</td>
<td>Ameletus</td>
<td>ARCT</td>
<td>Arctopsyche</td>
<td>CRTN</td>
<td>Cricotopus cf triannulatus</td>
</tr>
<tr>
<td>APO</td>
<td>Apobaetis</td>
<td>HYD</td>
<td>Hydropsyche</td>
<td>CRTF</td>
<td>Cricotopus trifascia</td>
</tr>
<tr>
<td>ACE</td>
<td>Acenterella</td>
<td>HDT</td>
<td>Hydroptila</td>
<td>EUKD</td>
<td>Eukiefferiella (devonica group)</td>
</tr>
<tr>
<td>BBI</td>
<td>Baetis bicaudatus</td>
<td>NEC</td>
<td>Nectopsyche</td>
<td>EUKG</td>
<td>Eukiefferiella (gracei group)</td>
</tr>
<tr>
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<td>Baetis tricaudatus</td>
<td>OEC</td>
<td>Oecetis</td>
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<td>Hydrobaenus</td>
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<td>CAL</td>
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<td>OPT</td>
<td>Optioservus quadrimeculatus</td>
<td>LOP</td>
<td>Lopescladius</td>
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<td>CAM</td>
<td>Camelobaetidius</td>
<td>BZPA</td>
<td>Bezzia/ Palpomyia</td>
<td>RCR</td>
<td>Rheocricotopus</td>
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<td>CEPR</td>
<td>Centroptilum/Procloeon</td>
<td>CUL</td>
<td>Culicoides</td>
<td>RSM</td>
<td>Rheosmittia</td>
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<tr>
<td>FCQ</td>
<td>Fallecon quilleri</td>
<td>PPFL</td>
<td>Polypedilum cf fallax/laetum</td>
<td>THI</td>
<td>Thienemanniella</td>
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<td>Labiobaetis</td>
<td>PPFV</td>
<td>Polypedilum cf flavum</td>
<td>TVT</td>
<td>Tvtenia (discoloripes group)</td>
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<td>Paracloeodes</td>
<td>ROB</td>
<td>Robackia claviger</td>
<td>PRK</td>
<td>Parochlus kieferi</td>
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<td>CLT</td>
<td>Cladotanytarsus</td>
<td>THMY</td>
<td>Thienemannimyia group</td>
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<td>PAT</td>
<td>Paratanytarsus</td>
<td>SIM</td>
<td>Simulium</td>
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<td>STIB</td>
<td>Serratella tibialis</td>
<td>TAN</td>
<td>Tanytarsus</td>
<td>LEB</td>
<td>Lebertia</td>
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<td>CINY</td>
<td>Cinygmula</td>
<td>RTA</td>
<td>Rheotanytarsus</td>
<td>SPE</td>
<td>Sperchon</td>
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<td>Stempelinella</td>
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<td>Tricorythodes</td>
<td>PAG</td>
<td>Pagastia</td>
<td>TRCL</td>
<td>Tricladida</td>
</tr>
<tr>
<td>PRLE</td>
<td>Paraleptophaelia</td>
<td>COR</td>
<td>Corynoneura</td>
<td>NEM</td>
<td>Nematoda</td>
</tr>
<tr>
<td>ISO</td>
<td>Isoperla</td>
<td>CRBI</td>
<td>Cricotopus (cf Bicinctus)</td>
<td>LUM</td>
<td>Lumbriculidales</td>
</tr>
<tr>
<td>ANAG</td>
<td>Anagapetus</td>
<td>CROR</td>
<td>Cricotopus/Orthocladius complex</td>
<td>NAI</td>
<td>Naididae</td>
</tr>
<tr>
<td>CUL</td>
<td>Culoptila</td>
<td>CRNO</td>
<td>Cricotopus nostocicola</td>
<td>TUB</td>
<td>Tubificidae</td>
</tr>
</tbody>
</table>
Indicator Species and Cluster Analyses

Overlaying the sample and species dendrograms (Figure 2) illustrates the species-sample relationships that group similar communities. Collectively, these groups, the indicator species that represent them, and the habitat conditions they are associated with can be viewed as BMI community types in large areas of the watershed that may shift seasonally or inter-annually. These areas of the watershed were given descriptive names that are identified on the watershed map (Figure 1).
Figure 2: Two-way dendrogram of Walker River taxa (horizontal axis) and samples (vertical axis) created using a Sorenson (Bray-Curtis) distance measure and a flexible beta of -0.25. The darkness of a square represents the relative abundance of each taxon in a sample. Taxon codes are shown in Table 2. Samples were assigned a code consisting of the site in capital letters followed by two lower case letters indicating the season (sp=spring, su=summer, au=autumn) followed by two numbers indicating the year (07=2007, 08=2008, 10=2010).
Eleven indicator species assemblages were identified by IndVal and cluster analyses, including high level and sub-groupings (Figure 3, Table 4). Table 4 describes the groups identified by the bold numbers in Figure 3. Each group (Figure 3) was characterized by a set of indicator species (Table 4) and each indicator species was additionally described by its tolerance value, functional feeding group, and behavioral category to characterize its ecological niche (Table 4).

Groups that separated higher on the sample distance dendrogram (Figure 3) were more distinct from each other than those that split lower. The highest level split in the dendrogram (Figure 3) occurred between a large group titled ‘Upper and Middle Watershed’ (Figure 3, Group 1) and a smaller sample group titled ‘Lower Watershed’ (Figure 3, Group 4). Group 1 was associated with taxa that were regularly distributed through most samples and therefore ubiquitous in the watershed, such as the mayfly *Baetis tricaudatus* (BTR) (Table 4). The split between Group 1 (Upper and Middle Watershed) and Group 4 (Lower Watershed) was followed by a sequence of lower level splits associated with more geographically specific groupings; such as a split between EWB (Group 5) and WWB, and headwater sites WWC and EWC (Group 6). Samples from the lower East Walker, lower West Walker, and upper main stem formed a major group with fewer indicator species than upstream groups (Group 3 and sub-group 7).

Most dendrogram splits occurred between two groups that were each associated with indicator species. However, some lower level groups did not include indicator species that met the criteria for both IndVal index and statistical significance. The lowest level group identified with corresponding indicators was titled ‘lowest site – WA’ (Figure 3,
Group 11). Cluster analysis measures a gradient of contiguous communities across the watershed and need not be analyzed at one particular grouping level. Patterns that distinguish a higher level grouping from its component sub-groupings can be indicative of community gradients. For example, Group 5 (WWB and montane headwaters) contained a sub-group consisting of WWB springtime and montane headwaters group (Group 9) and a WWB summer and autumn group (Group 10). This pattern indicates that the WWB communities in the spring resemble the summertime communities found at higher elevation sites but diverge toward a more unique community during the summer and fall.
Figure 3: Bray-Curtis dendogram of Walker River BMI benthic samples. Numbers in bold designate indicator species assemblages described in Table 4.
Table 4. Indicator species assemblages sorted by group based on the Bray-Curtis dendrogram (Figure 3). Peak IndVal indices for taxa in each group are marked with a ‘*’. Tolerance values, functional feeding groups, and behavior type are from Ode (2003).

<table>
<thead>
<tr>
<th>Sample Group Taxon</th>
<th>IndVal Index</th>
<th>Tolerance Index</th>
<th>Functional Feeding Group</th>
<th>Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Upper and Middle Watershed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baetis tricaudatus</td>
<td>68.8*</td>
<td>5</td>
<td>COLLECTER-GRAZER</td>
<td>SWIMMER</td>
</tr>
<tr>
<td>Hydropsyche*</td>
<td>50.8*</td>
<td>4</td>
<td>COLLECTER-FILTERER</td>
<td>CLINGER</td>
</tr>
<tr>
<td>Cricotopus/Orthocladius</td>
<td>66.0*</td>
<td>7</td>
<td>COLLECTER-GRAZER</td>
<td>CLINGER</td>
</tr>
<tr>
<td>Thienemanniella</td>
<td>60.0*</td>
<td>6</td>
<td>COLLECTER-GRAZER</td>
<td>SPRAWLER</td>
</tr>
<tr>
<td>Sperchon</td>
<td>71.2*</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2. Upper Watershed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optioservus quadrimaculatus</td>
<td>84.5*</td>
<td>4</td>
<td>SCRAPER</td>
<td>CLINGER</td>
</tr>
<tr>
<td>Rheocricotopus</td>
<td>62.1</td>
<td>6</td>
<td>COLLECTER-GRAZER</td>
<td>SPRAWLER</td>
</tr>
<tr>
<td>Baetis tricaudatus</td>
<td>61.0</td>
<td>5</td>
<td>COLLECTER-GRAZER</td>
<td>SWIMMER</td>
</tr>
<tr>
<td><strong>3. Middle Watershed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheotanytarsus</td>
<td>54.8*</td>
<td>6</td>
<td>COLLECTER-FILTERER</td>
<td>CLINGER</td>
</tr>
<tr>
<td>Thienemanniella</td>
<td>56.9</td>
<td>6</td>
<td>COLLECTER-GRAZER</td>
<td>SPRAWLER</td>
</tr>
<tr>
<td><strong>4. Lower River</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracloeodes</td>
<td>56.1</td>
<td>4</td>
<td>COLLECTER-GR A ZER</td>
<td>SWIMMER</td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>51.1*</td>
<td>6</td>
<td>COLLECTER-FILTERER</td>
<td>CLINGER</td>
</tr>
<tr>
<td>Cricotopus (Bicinctus)</td>
<td>64.5*</td>
<td>8</td>
<td>COLLECTER-GRAZER</td>
<td>CLINGER</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>68.4*</td>
<td>8</td>
<td>COLLECTER-GRAZER</td>
<td>SPRAWLER</td>
</tr>
<tr>
<td><strong>5. East Walker B (all seasons)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheocricotopus</td>
<td>75.2*</td>
<td>6</td>
<td>COLLECTER-GRAZER</td>
<td>SPRAWLER</td>
</tr>
<tr>
<td>Tvtenia</td>
<td>52.7*</td>
<td>5</td>
<td>COLLECTER-GR A ZER</td>
<td>SPRAWLER</td>
</tr>
<tr>
<td>Optioservus quadrimaculatus</td>
<td>62.6</td>
<td>4</td>
<td>SCRAPER</td>
<td>CLINGER</td>
</tr>
<tr>
<td>Baetis tricaudatus</td>
<td>54.2</td>
<td>5</td>
<td>COLLECTER-GR A ZER</td>
<td>SWIMMER</td>
</tr>
<tr>
<td><strong>6. West Walker B and Montane Headwaters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ameletus</td>
<td>80.5*</td>
<td>0</td>
<td>COLLECTER-GR A ZER</td>
<td>SWIMMER</td>
</tr>
<tr>
<td>Species</td>
<td>Percent</td>
<td>Behavior</td>
<td>Family</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------</td>
<td>----------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td><em>Attenella delantala</em></td>
<td>57.1*</td>
<td>2</td>
<td>COLLECTER-GRAZER</td>
<td></td>
</tr>
<tr>
<td><em>Epeorus</em></td>
<td>60.7</td>
<td>0</td>
<td>SCRAPER</td>
<td></td>
</tr>
<tr>
<td><em>Rithrogena</em></td>
<td>71.4*</td>
<td>0</td>
<td>SCRAPER</td>
<td></td>
</tr>
<tr>
<td><em>Paraleptophlebia</em></td>
<td>71.4</td>
<td>4</td>
<td>COLLECTER-GRAZER</td>
<td></td>
</tr>
<tr>
<td><em>Pagastia</em></td>
<td>52.8*</td>
<td>1</td>
<td>COLLECTER-GRAZER</td>
<td></td>
</tr>
</tbody>
</table>

7. Confluence Region

- *Acentrella* 56.8 4 COLLECTER-GRAZER SWIMMER
- *Camelobaetidius* 50.7 4 COLLECTER-GRAZER SWIMMER

8. Valley Sites

- *Paracloeodes* 71.5* 4 COLLECTER-GRAZER SWIMMER

9. Headwaters and WWB Springtime

- *Epeorus* 74.4* 0 SCRAPER CLINGER

10. WWB Summer and Autumn

- *Sublettea* 67.0* 0 COLLECTER-GRAZER SWIMMER
- *Ameletus* 55.3 0 COLLECTER-GRAZER SWIMMER
- *Pagastia* 51.0 1 COLLECTER-GRAZER SPRAWLER

11. WA

- *Paratanytarsus* 57.6* 6 COLLECTER-GRAZER SPRAWLER

The dendrogram exhibited distance patterns attributable to multiple factors that are most influential in determining sample similarity. Samples from the same site tended to group together and samples from sites at similar elevations tended to group. Grouping by season was also apparent in the dendrogram. Grouping by site was most apparent in the upper watershed. In the middle and lower watershed, groupings at all levels were more influenced by season and year.

Groups were characterized by indicator species representing diverse ecological niches, but patterns were apparent when the tolerance, behavior, and functional feeding group of indicator species were compared across groups (Table 4). Longitudinal watershed-scale
gradients were apparent in tolerance, behavior, and functional feeding group. The ecological niches of indicator species were most similar within groups and between groups at similar elevation. However, there was considerable variation within groups and overlap between groups.

CCA Analysis

The CCA ordination plot (Figure 4) was not representative of the full range of community variation (ter Braak and Verdonschot 1995). However, CCA effectively determined the relative contribution of habitat variables in structuring community variation seen in the IndVal analysis (Figure 3). All axis were characterized by high species environment correlations greater than 0.9 (Table 5). Mean species-environment correlations based on 998 runs of a Monte Carlo test were greater than 0.7 for all three axes. Due to the properties of the CCA ordination method only the first axis was tested for statistical significance (p=0.001). Other axis were sufficiently close in both eigenvalue and species-environment correlation to verify their importance in structuring the observed community patterns.
Figure 4: CCA biplot of benthic samples with eigenvectors of habitat variables displayed along axes 1 and 2. Only eigenvectors with $R^2 > 0.300$ are shown. Abbreviations for environmental parameters are shown in Table 3.
Table 5: Species-environment correlations for the first three axis of the CCA ordination and the results of 998 runs of a Monte Carlo test using randomized data. Only the first axis could be tested for significance because randomization tests on subsequent axis could yield biased P-values.

<table>
<thead>
<tr>
<th>Axis</th>
<th>Real Data</th>
<th>Randomized Data Monte Carlo Test</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Spp.-Env. Correlation</td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>0.978</td>
<td>0.748</td>
</tr>
<tr>
<td>2</td>
<td>0.910</td>
<td>0.745</td>
</tr>
<tr>
<td>3</td>
<td>0.935</td>
<td>0.754</td>
</tr>
</tbody>
</table>

Axis 1 had a significant (p = 0.01) eigenvalue of 0.318 and was generally associated with geomorphic (i.e., cobble, sand, embeddedness, sand, fines) and elevation related (i.e., elevation, maximum temperature) variables. Elevation and substrate composition are closely related covariates along the longitudinal profile of most watersheds due to the effect of stream size and gradient on the patterns of erosion and deposition that structure benthic habitats. Higher elevation stream reaches are characterized by higher gradients and larger substrate. Lower elevation reaches are characterized by lower gradients with sand and high embeddedness. Many Walker River BMIs occupy the surface of riffle substrates, and are therefore highly affected by substrate size and the availability of interstitial space. Substrate size, composition, and embeddedness are all proximate predictors of habitat type and availability. Elevation has an overarching influence on habitat conditions in this basin, and it is correlated with multiple proximate predictors including substrate composition, peak water temperature, and may other physical habitat variables (e.g., current velocity, water depth, wetted width, etc.).
Axis 2 had an eigenvalue of 0.193 and was primarily associated with nutrient related variables (i.e., total nitrogen, total phosphorus, NO$_3$+NO$_2$). Gradients of nutrient concentration naturally occur in watersheds, but the gradients associated with axis were not consistent with patterns of nutrient accumulation that would be expected without anthropogenic pollution. First, the nutrient gradients associated with axis 2 had a stronger effect on community structure than seasonal effects. This is surprising, given the freezing winter temperatures and hot summer temperatures inherent to the western Great Basin. Second, nutrient gradients identified in the CCA analysis appeared to be working ‘across’ the watershed, rather than longitudinally as would be expected under natural conditions. Sites at a similar elevation, particularly sites EWB and WWB, had markedly differing communities at opposite ends of the nutrient gradients. These two lines of evidence indicate that anthropogenic pollution is primarily responsible for the gradients represented by axis 2. These gradients were easily observed and quantified in the CCA analysis (Figure 5) but not as easily distinguished in the cluster analysis (Figure 3) where they were primarily represented by peculiar clustering patterns that do not seem compatible with the predicted effects of elevation and season. Anthropogenic nutrient input may have been responsible for less predictable grouping patterns in middle and lower Walker River and is almost certainly responsible for the distinct split between the EWB group (Group 5) and the ‘WWB and Montane Headwaters’ group (Group 6).
Figure 5: Magnitude of total nitrogen (TN) variable overlaid on a CCA biplot. Relative size of the ‘site’ symbols in the biplot represents the relative magnitude of the associated TN value. Scatterplots below and to the left of the biplot represent regressions of TN values relative to axis 1 and axis 2 respectively. Only eigenvectors with $R^2 > .300$ are shown.

Axis 3 had an eigenvalue of 0.142 and was primarily associated with season and temperature. Samples largely grouped by site in the biplot. Though this axis of variation had a lesser effects on community structure than axes 1 and 2 it was comparably
predictive. Seasonal effects on BMI community structure may include biological effects due to community succession or timing of life histories. Seasonal effects are generally large in temperate rivers. Samples taken at the same site and year under similar flow conditions but in different seasons may not be directly comparable (Reece et al. 2001). Clustering patterns seemed to support CCA results indicating an important seasonal effect. Samples from the same site were often grouped by season rather than year. Maximum water temperature was associated with both elevation and season related axes, which complicated the interpretation of this variable. Water temperature was influenced by a combination of seasonal cycles and longitudinal thermal loading. CCA results indicate the relative contributions of each of these factors to maximum temperature values were similar.

Community Tolerance Indices

Mean community tolerance generally increased along a longitudinal gradient from higher elevations to lower elevations (Figure 6). This trend was correlated with a gradient of increasing anthropogenic disturbance in downstream valley reaches. Mean tolerance based on a Hilsenhoff scale was above 6.0 at all of the main stem river sites but was below 5.0 at WWB. Likewise, tolerance of indicator species alone tended to be higher at downstream sites than upstream sites, but the trend was less obvious compared to that of BMI communities (Table 4).
Figure 6: Boxplot of mean community tolerance indices (based on Ode 2003) for all samples plotted by site and elevation along the longitudinal profile of the watershed.

The tolerance gradient was associated with increasing anthropogenic disturbance downstream, but may also have been influenced by a covariate trend in species traits associated with higher tolerance even under natural conditions without anthropogenic influences. Tolerance values encompass tolerance of pollution, fine sediment, water temperature, and a variety of factors both natural and anthropogenic. The BMI species assemblage occupying the sandy desert reaches of Great Basin rivers may have a higher tolerance for the abundant fine sediment, decreased dissolved oxygen, high peak water
temperatures, and accumulated organic material associated with such habitats. The average tolerance value of the sub-set of species that can occupy lower river habitats may be naturally higher than the average tolerance value for other species in the watershed. In this way the hierarchical nature of community-environment relationships may complicate the direct comparison of tolerance indices longitudinally on a watershed scale (Poff 1997, Ciesielka and Bailey 2007). It is probable that the longitudinal watershed gradient observed for mean community tolerance is a natural phenomenon combined with anthropogenic watershed impacts. However, mean community tolerance was slightly higher at EWB than WWB. This trend does not follow the longitudinal gradient and is entirely due to anthropogenic causes.

The overall trends in tolerance indices reflected the same gradients as the CCA and the indicator species analysis. There was a dominant longitudinal gradient of BMI communities from upstream to downstream characterized by increasing tolerance and affinity for warmer maximum temperatures, lower gradients, and finer substrates. There was also a secondary gradient associated with higher nutrient loads in the East Walker and main stem river. This secondary gradient was evidenced by higher tolerance values for the BMI communities and indicator species in these areas of the watershed. Tolerance indices varied widely between seasons and years at a given site, underscoring the importance of seasonal cycles, inter-annual community shifts, and environmental variability.
Discussion

A dominant gradient of discrete community types was apparent along the longitudinal profile of the watershed from the ‘montane headwaters’ to WA. The dominant effect of geomorphic and elevation related factors such as substrate type, embeddedness, and maximum temperature is not surprising given the range of elevations and slopes encompassed by the watershed. The greatest community differences apparent in the IndVal and cluster analyses are between the highest and lowest sites in the watershed. Strong longitudinal community gradients are characteristic of streams in mountainous topography of western North America (Boyle and Strand 2003, Finn and Poff 2005). The relative contribution of different functional feeding groups within the indicator species assemblages varied from upstream to downstream as would be predicted by the river continuum concept (Table 4). Scrapers such as Rithrogena and Epeorus were indicative of the upper reaches of the watershed while collector-filters such as Tanytarsus and collector-grazers such as Paracloeodes were more indicative of the lower river. Though essential for understanding the watershed, this gradient of community types from the mountains to the desert is not entirely anthropogenic. This community gradient is the combined outcome of a mountainous watershed and increased agricultural development at lower elevations.

The second axis of variation was strongly associated with anthropogenic nutrient gradients including total nitrogen, nitrate plus nitrite, and total phosphorus. This gradient was most apparent in community differences between EWB and WWB. The most likely driver of this gradient was nonpoint source pollution from agriculture and grazing.
Bridgeport Reservoir receives runoff from a large area of highly productive pasture land. The nutrient loading that occurs around the reservoir influences trophic dynamics downstream (DRI 2010). Although, EWB is similar to WWB in both elevation and geomorphology, they are characterized by very different benthic communities. Nutrient input from pasture is complemented by agricultural runoff in the middle watershed around sites EWA, WWA, and WD. Site WC lies in Mason Valley Wildlife Management Area and is not immediately adjacent to agriculture. Sites WB and WA lie on Paiute tribal land where some agriculture occurs. The general pattern of proximity to agriculture and grazing explains much of the BMI community variation attributable to nutrient loading (Figure 5).

The significant effect of anthropogenic nutrient loading in structuring benthic communities has important implications for flow restoration. The strength of the nutrient pollution gradient in structuring benthic communities shows that this gradient has the potential to constrain the outcomes of restoration actions targeting fisheries. Hydrologic restoration in the East Walker may result in different ecological impacts than equal restoration actions in the West Walker. Additional hydrologic and water quality modeling would be needed to quantify and predict such differences with respect to specific restoration actions.

Increased attention to improving water quality in the East Walker and main stem river would result in broad and lasting impacts to aquatic communities at multiple trophic levels. Increased BMI diversity and decreased community tolerance would be likely outcomes of such an approach. Furthermore, because existing water quality conditions
influence BMI communities so strongly, they are also likely to influence fisheries that are sensitive to water quality. Anthropogenic nutrient and thermal pollution may present challenges for the restoration of native Lahontan cutthroat trout in the watershed. Variation in BMI communities and environmental conditions due to anthropogenic factors may lead to variation in native trout restoration success across the watershed.

The unique nature of site WC with respect to nutrient concentrations may provide a useful comparison for monitoring and adaptive management. WC lies within the Mason Valley Wildlife Refuge. Within the lower watershed WC is the site least modified by agriculture. WC is characterized by lower nutrient loads, especially Total Nitrogen, than WD, WB, or WA (Figure 5). The simplest explanation for this is that much of the anthropogenic nutrient load apparent at WD has been biologically assimilated before reaching WC. Nonpoint source agricultural pollution downstream of the Wildlife Refuge is then responsible for the higher nutrient concentrations observed at WA. The ‘insulating’ effect of the wildlife refuge should put WC in the unique position of being an effective reference site for the lower river with respect to nutrient pollution. However, community tolerance values do not support this distinction. Community tolerance values calculated for WC samples are similar to, or even higher than, values for other sites on the lower river (Figure 6). This curious paradox highlights the limitations of the biotic index approach used alone without benefit of supporting community analyses. Subtle community and water quality differences that were apparent in the CCA ordination did not translate into a similar pattern of tolerance values. More data will be needed from
lower river sites to identify what the ‘reference condition’ actually is and to gauge the response of lower river sites to restoration actions relative to each other.

The effect of thermal loading, and maximum water temperature, in structuring aquatic communities is of particular importance for restoration optimization (Null et al. 2009). The complicated relationship of maximum temperature with both season and elevation underscores the need for a working temperature model for the watershed to help predict the outcome of restoration actions with respect to temperature. While the effect of maximum temperature on benthic communities may be predominantly a function of elevation, the more subtle interactions between season, hydrology, and elevation cannot be discounted. Maximum water temperatures at EWB are generally higher than at WWB due to thermal loading caused by a broad, shallow upstream reservoir. The elevated maximum temperatures at EWB are clearly anthropogenic. At downstream sites the distinction between natural and anthropogenic thermal loading becomes blurred with the addition of tributary sources. Improved temperature models for the Walker River would yield valuable insights on how to mitigate the anthropogenic thermal loading for the benefit of aquatic communities.

BMI communities exhibit inter-annual variation, independent of site or season, most commonly attributed to changes in hydrograph (Monk et al. 2008, Carter and Fend 2001). Inter-annual variation in benthic communities is important to quantify for the purposes of long term watershed management and hydrologic restoration, but few studies exist with sufficient long-term data sets (Monk et al. 2008). Only three annual hydrographs were represented in our data set – an insufficient sample size to quantify or predict the effects
of annual hydrograph on the structure of benthic communities. A long term monitoring program would be needed to predict the impacts of incremental changes in the annual hydrograph of the Walker River and develop new biotic indices of restoration success. However, based on the current data set alone, some inferences can be made regarding community changes that would be evidence of restoration success. Upper watershed indicator taxa moving into middle watershed, and middle watershed indicator taxa moving into lower watershed, would imply a gradual shift in the longitudinal community continuum and possibly a new community stable state more consistent with restoration goals. In the cluster analysis site WD grouped with downstream sites in some seasons and years and upstream sites in other seasons and years (Figure 3). The grouping of autumn samples was particularly indicative of a major community shift because autumn samples represent an annual climax BMI community under minimum flow conditions. During the dry years of 2007 and 2008 the autumn samples from WD grouped with the lowest reaches of the river including WA and WB. In the wet year of 2010 autumn samples from WD grouped with upstream sites including EWA and WWA. A BMI community that shifts so widely and predictably may be indicative of an opportunity to permanently shift the upper main stem river toward a community resembling upstream sites through hydrologic and geomorphic restoration.

In the context of future Walker Basin Program studies, indicator species analysis can also be used to identify and inform the use of model organisms. For example, growth and productivity curves for specific model organisms can be interpreted and applied to the parts of the watershed where the model organism is most representative. *Beatis*
*Beatis tricaudatus* (Ephemeroptera) is often used as a model organism in lotic systems due to its abundance, multivoltine life history, and widespread occurrence (Irving et al. 2003, Robinson et al. 1992). The species can be used to develop detailed models linking changes in discharge, temperature, or water quality to changes in annual production that can be extrapolated to the larger BMI community. *Beatis tricaudatus* may not be representative of the entire Walker River watershed though, and the application of temperature-productivity regressions to reaches of the river where the species does not frequently occur would introduce error into predictive models. Using the IndVal analysis, we can demonstrate that *B. tricaudatus* is broadly representative of ‘upper and middle watershed’ (Figure 3, Group 1) and apply predictive models accordingly.

For restoration practitioners, the primary strength of the approach described here is its flexibility. From identifying watershed-scale gradients of pollution to guiding the use of model organisms, a wide variety of questions pertinent to restoration planning and adaptive management were addressed with only a three-year data set. There is a high probability that restoration objectives and priorities will continue to evolve over time with the Walker Basin Program as with other restoration programs. Flexible tools are likely to remain valuable and find new applications over time.
Literature Cited


