Cascade – Siskiyou National Monument spring aquatic invertebrates and their relation to environmental and management factors

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Executive summary

The purpose of this study is to document the biodiversity of aquatic invertebrates in the Cascade–Siskiyou National Monument (CSNM) springs, and relate distribution patterns to environmental and management factors. Of particular interest is the influence of past and current management activities on aquatic macroinvertebrate distribution in the springs of the monument.

The springs of the CSNM are highly diverse, with 92 different genera being identified in the total number of 10,427 individuals collected. This diversity is similar to invertebrate diversity of other spring systems – even when sampling efforts of the other systems are much more extensive, or when the spring system covers a much larger geographic area.

External factors used to explore interactions of spring invertebrates and environmental and management factors are many, and showed some general patterns – mainly high utilization (by native and non-native ungulates) excludes certain invertebrate species considered intolerant of disturbance. However, high diversity and species indicative of clean water (mainly Ephemeroptera, Plecoptera and Trichoptera) are compatible with low to moderate amounts of utilization. Although these patterns are clear, distinct and supported by multiple analytical methods, there is a wide amount of variance associated with the data in general. Continued sampling, incorporation of quantitative sampling, and collection of more direct factors relating to macroinvertebrate distribution will help clarify the relationship of invertebrate biodiversity to management factors.

Introduction

The proclamation for the Cascade-Siskiyou National Monument (CSNM) requires studies to determine potential impacts by livestock on objects of biological interest including plant communities, wildlife populations, aquatic organisms, and ecological processes. The ecological and biological importance of the area has long been recognized (Detling 1961, Prevost et al. 1990, Carroll et al. 1998), and the stream invertebrates in several stream systems located within the monument have been well described (e.g. ABA 1991, 1992, 1993, 1995), but the invertebrates of the numerous spring-seeps have remained uncatalogued.

Concerns over livestock impacts (see Hosten et al. (2007) for review of livestock usage in the CSNM) to spring-seep habitats are derived from observations that livestock grazing is detrimental to many components of stream ecosystems (Minshall et al. 1989). Chief among these is the impact livestock have on riparian zones; causing loss of streamside vegetation, resulting in increased solar radiation (and increased water temperature) and sedimentation, reducing habitat quality and with the potential to smother aquatic invertebrates (Kauffman et al. 1983a, 1983b, Gamougoun et al. 1984, Marlow et al. 1987, Waters 1995). Additional evidence has shown that 182 organisms currently listed as threatened or endangered by the USFWS can be related to livestock impacts (Czech et al. 2000).

Despite the aforementioned impacts, livestock grazing has also been shown to have little effect on invertebrates. Steinman et al. (2003) found that

cattle stocking rates were unrelated to invertebrate assemblages, and that
vegetation data was much more important as factors in determining
invertebrates. However, this study was restricted to sub-tropical Florida, but
Steinman et al. point out the scarcity of literature discussion livestock impacts in
wetlands, as compared to the large amount of literature on lotic streams. The
small, low outflow spring-seeps of the CSNM may exhibit more habitat
characteristics of wetlands than lotic streams, so that livestock effects on spring-
seep invertebrates may respond differently that lotic invertebrates.

Depending on context, invertebrate assemblages have been found to be
compatible with low-levels of grazing (Braccia and Voshell 2006). The
continuation of ranching and its suppression of urban development may favor
conservation objectives (Jensen 2001).

This study addresses the overall levels of biodiversity of aquatic
invertebrates in the CSNM, their relation to vegetation, environmental and
management factors, and general patterns of biodiversity so that future, sound
management decisions can be implemented.

Methods

A subsets of seeps and springs were selected from survey results conducted in
the summer prior to aquatic macro-invertebrate sampling. A survey team
completed the ocular estimates of vegetation, chemical, and physical parameters
described by Table 2. Seeps and springs were rejected in they had been
influenced by the creation of stockponds or were adjacent to roads. Most lower
elevation springs had been disturbed, leaving mostly higher elevation seeps and

springs within a conifer matrix available for sampling. No sampled springs had fish populations.

Aquatic invertebrate collection

Qualitative invertebrate collections were performed at each sampling location. The objective of the sampling was to collect as many different kinds of invertebrates living at a site as possible. We collected 500 individuals or collected for 2 hours, whichever was accomplished first.   Samples were collected with a rectangular kicknet with a 500 micron mesh net, and by hand picking invertebrates from vegetation and substrate. All connected habitats were sampled (standing water at spring site, outflow, small water pockets surrounding spring) and all samples were composited to form a single sample for each site. Collected invertebrates were stored in a 70% ethanol. Sample labels were written in pencil on waterproof paper.

All of the samples were processed in the entirety, i.e., all the organisms were removed and identified. We attempt to identify each organism to the lowest possible taxonomic level, except for Chironomidae (identified to subfamily) and Hydrobiidae (retained at family level). All samples were retained in our collection and will be made available to other researchers upon request.

Invertebrate data summarization

A number of metrics or ecological summaries were provided for each sampling station (Table 1). These metrics were calculated following (Hilsenhoff 1987, 1988; Magurran 1988; Ludwig and Reynolds 1988; and Plafkin et al. 1989), and are described below. These summarizations were used to explore the relation of observed assemblage presence/absence patterns to the biotic and environmental variables in Non-metric Multidimensional Scaling (NMS) ordinations. See analytical methods for more explanation.

Taxa richness - Richness is a component and estimate of community structure and spring health based on the number of distinct taxa. Taxa richness normally decreases with decreasing water quality. In some situations organic enrichment can cause an increase in the number of pollution tolerant taxa. Taxa richness was calculated for operational taxonomic units (OTUs) and the number of unique genera or families. The values for operational taxonomic units may be overestimates of the true taxa richness at a site if individuals were the same taxon as those identified to lower taxonomic levels or they may be underestimates of the true taxa richness if multiple taxa were present within a larger taxonomic grouping but were not identified. All individuals within all samples were generally identified similarly, so that comparisons in operational taxonomic richness among samples within this dataset are appropriate, but comparisons to other data sets may not. Comparisons to other datasets should be made at the genus or family level.

Table 1. Variables used to explore biological patterns in macroinvertebrate distributions. Range refers to minimum and maximum values encountered in survey sites. Variable name is code for NMS biplot figures (see Figures 7).

Variable Class	Variable description	Average (range)	Variable name
Diversity summary	OTU richness	23.58 (7-48)	RICH
Diversity summary	Number of distinct families	15.35 (5-29)	FAMILIES
Diversity summary	Number of distinct genera	23.30 (7-47)	GENT
Tolerance assessment index	Number of Ephemeroptera, Plecoptera, and Trichoptera taxa	8.53 (0-23)	EPTT
Tolerance assessment index	Number of intolerant taxa	5.58 (0-13)	HBIINT
Tolerance assessment index	% of total taxa categorized as intolerant	0.23 (0-0.52)	HBIIPER
Tolerance assessment index	Number of tolerant taxa	0.30 (0-2)	HBITOL
Natural history characteristic	Number of clinger taxa	3.35 (0-12)	CLINGER
Natural history characteristic	Number of long-lived taxa	4.30 (0-10)	LLTAXA
Specific taxa richness	Number of Elmidae taxa	0.30 (0-6)	ELMIDS
Functional feeding groups	Number of shredder taxa	2.89 (0-8)	SHT
Functional feeding groups	Number of scraper taxa	1.07 (0-5)	SCT
Functional feeding groups	Number of collector-filter taxa	0.87 (0-2)	CFT
Functional feeding groups	Number of collector-gather taxa	6.51 (3-15)	CGT
Functional feeding groups	Number of predatory taxa	8 (0-15)	PRT
Specific taxa richness	Number of unidentified taxa	3.32 (1-10)	UNT
Specific taxa richness	Number of Ephemeroptera taxa	1.71 (0-9)	EPHET
Specific taxa richness	Number of Plecoptera taxa	3.26 (0-9)	PLECT
Specific taxa richness	Number of Trichoptera taxa	3.55 (0-10)	TRICT
Specific taxa richness	Number of Coleoptera taxa	4.28 (0-9)	COLET
Specific taxa richness	Number of Megaloptera taxa	0.14 (0-1)	MEGAT
Specific taxa richness	Number of Diptera taxa	8.10 (3-16)	DIPTT
Specific taxa richness	Number of Chironomidae taxa	2.58 (0-4)	CHIRT
Specific taxa richness	Number of Crustacea taxa	0.19 (0-2)	CRUST
Specific taxa richness	Number of Oligochaete taxa	0.37 (0-1)	OLIGT
Specific taxa richness	Number of Mollusca taxa	0.87 (0-3)	MOLLT
Specific taxa richness	Number of miscellaneous other taxa	0.62 (0-5)	OTHERT

EPT - A summary of the taxa richness among the insect Orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). These orders are commonly considered sensitive to pollution.

Number of families - All families are separated and counted. The number of families normally decreases with decreasing water quality.

Biotic index - Biotic indices use the indicator taxa concept. Taxa are assigned water quality tolerance values based on their specific tolerances to pollution. Scores are typically weighted by taxa relative abundance, but this only applies to quantitative samples. In the United States the most commonly used biotic index is the Hilsenhoff Biotic Index (Hilsenhoff 1987, Hilsenhoff 1988). The Hilsenhoff Biotic Index (HBI) summarizes the overall pollution tolerances of the taxa collected. This index has been used to detect nutrient enrichment, high sediment loads, low dissolved oxygen, and thermal impacts. It is best at detecting organic and nutrient pollution that reduces dissolved oxygen levels. Families were assigned an index value from 0 to 10, with taxa normally found only in high quality unpolluted water ranked at 0, and taxa found only in severely polluted waters ranked at 10. Family level values were taken from Hilsenhoff (1987, 1988) and a family level HBI was calculated for each sampling location for which there were a sufficient number of individuals and taxa collected to perform the calculations. Sampling locations with HBI values of 0-2 are considered clean, 2-4 slightly enriched, 4-7 enriched, and 7-10 polluted. Individual taxon HBI values can be used to determine the number of pollution intolerant and tolerant taxa occurring at a site. In this report, taxa with HBI values of 0-2 were

considered intolerant clean water taxa and taxa with HBI values of 9-10 were considered pollution tolerant taxa. The number of tolerant and intolerant taxa were calculated for each sampling location. The number of intolerant taxa divided by the total number of taxa found was then used to calculate %intolerant taxa, a index used to assess overall biodiversity patterns.

Functional feeding groups – A common classification scheme for aquatic macroinvertebrates is to categorize them by feeding acquisition mechanisms. Categories are based on food particle size and food location, e.g., suspended in the water column, deposited in sediments, leaf litter, or live prey. This classification system reflects the major source of the resource, either within the stream itself or from riparian or upland areas and the primary location, either erosional or depositional habitats. The number of taxa of the following feeding groups were calculated for each sample based on designations in Merritt and Cummins (1996).

Shredders - Shredders use both living vascular hydrophytes and decomposing vascular plant tissue - coarse particulate organic matter (CPOM). Shredders are sensitive to changes in riparian vegetation. Shredders can be good indicators of toxicants that adhere to organic matter.

Scrapers - Scrapers feed on periphyton - attached algae and associated material. Scraper populations increase with increasing abundance of diatoms and can decrease as filamentous algae, mosses, and vascular plants increase. Scrapers decrease in relative abundance in response to sedimentation and organic pollution.

Collector-filterers - Collector-filterers feed on suspended fine particulate organic matter (FPOM). Collector-gatherers are sensitive to toxicants in the water column and deposited in sediments.

Collector-gatherers - Collector-gatherers feed on deposited fine particulate organic matter. Collector-gatherers are sensitive to deposited toxicants.

Predators - Predators feed on living animal tissue.

Environmental data

A number of metrics describing land use patterns, management actions and other physio-chemical parameters were collected (Table 2). The variables are broadly grouped into topographic (slope and heatload), water quality (conductivity, temperature, and pH), spring characteristics (total area of the riparian area, percent bare soil, and percent surface water), and management. Topographic variables were derived from existing digital elevation data, and heatload calculated using algorithms from McCune & Keon (2002). Water quality data were collected on site – conductivity was determined using an Oakton TDS Testr 40 hand held meter and pH was determined using an Oakton pH Testr. Spring characteristics were also noted in the field. Some management variables were calculated in GIS (distance from roads, years since last grazed, average utilization, and maximum utilization). Bare soil and vegetated area of the riparian zone influenced by ungulates (hoof prints) were estimated as a percent of the riparian area on a ocular basis during the summer of the year prior to aquatic macr0-invertebrate sampling.

Table 2. Variables used to explore environmental/management influences on macroinvertebrate distributions. C = Categorical variable, Q = Quantitative variable. Range refers to minimum and maximum values encountered in survey sites. Variable name is code for NMS biplot figures (Figure 8).

Variable class	Variable description	Type of Data	Measurement Units and Range	Average	Variable name
Ownership	Public, permitted sampling on private	C	Yes or No	NA	cole
Management	Years since last grazed	Q	Years, 1-6	2.5	lastgrzd
Management	Vegetation cover change	C	Yes or No	NA	logging
Management	% riparian vegetation marked by ungulate hoofprints	Q	% (0 -85)	10.6	plstkinf
Management	Protected	C	Yes or No	NA	protecte
Management	Distance from road	Q	meters, (100 - 400)	130.6	rddist
Management	Average utilization over 12 years	Q	Scale (1 = least, 5 = most)	2.5	util_avg
Management	Maximum utilization over 12 years	Q	Scale (1 = least, 5 = most)	3.34	util_max
Spring characteristic	Outflow (presence/absence)	C	Yes or No	NA	outflo
Spring characteristic	% bare soil	Q	%, (0 - 90)	16.6	pbarsoil
Spring characteristic	Spring area	Q	Square feet, (0 - 2500)	383.5	sprarea
Spring characteristic	Surface water	Q	%, (0 - 20)	1.05	surfwat
Spring characteristic	Water temperature	Q	degrees C (7 - 30)	9.84	temperat
Topgraphic	Slope	Q	%, (3 - 49)	23.75	sloped
Topographic	Heatload	Q	unitless, (4654 - 9209)	7300	hloade2
Water quality	Conductivity	Q	µS/cm (72 - 850)	192	conducti
Water quality	pH	Q	unitless, (6 - 9)	7.16	ph

Analyses

      Regional biodiversity patterns were examined using taxa accumulation curves. Taxa accumulation curves (Hayek and Buzas 1996) show the number of new taxa accrued as a function of increased sampling. A curve that rises steeply indicates that many more taxa are being found with each new sampling effort (by repeated sampling or new sites) – i.e. a highly diverse area. A curve that rises slowly would indicate that few additional species are identified with each new sampling effort – i.e a depauperate area. When sampling efforts have adequately described the species of an area, the curve generally levels out and obtains an asympotote. At this point, increased sampling will generally add a small number of fauna, regardless of overall diversity levels. We estimated the total number of taxa present, but not collected using the Chao 2 richness estimator (Chao 1987). The Chao 2 richness estimator is a correction based speices estimator that incorporates measure or rarity (singletons and doubletons, species that only occurred in one or two samples, respectively) to calculate total species richness.

      Testing of factors most related to invertebrate species presence/absence was done using Mantel tests. The Mantel test calculates a cross-product variable between similarity matrices. Values are then randomized and rerandomized within each matrix to generate a distribution of cross-products. The observed cross-product is then mapped against the distribution to determine significance. An important option in the Mantel test is how the distances in the similarity matrices are calculated. Here we used the Sorenson (Bray-Curtis)

dissimility measure for any biological data and Euclidean distance for environmental data considered most suitable for our data (Faith et al. 1997). Another important output of the Mantel test is the Mantel statistic, interpreted in the same manner as the Pearson correlation coefficient (i.e. a high value indicates a high degree of concordance or relatedness, and a low value indicates that the matrices are not related).

We tested what secondary factors were the best explanatory variables using the Primer routine, BIOENV (PRIMER-E Ltd., Plymouth, UK). This routine matches subsets of secondary variables (in this case environmental variables) to find the subset that has the higher correlation coefficient. BIOENV does not, however, provide information on how these variables influence observed biodiversity patterns. Hence, once we determined which factors were important, we followed up with appropriate graphing techniques to elucidate their role, e.g. bar graphs of utilization scales against overall invertebrate diversity.

Patterns of invertebrate distributions were also examined using Non-metric Multi-Dimensional Scaling (NMS) ordinations. Ordinations offer insight into the relation of invertebrate site assemblages. In our ordinations, we used Sorenson/Bray-Curtis distance measures to determine site differences in invertebrate assemblages. NMS ordination routines then provides a graphical interpretation in 2 or 3 dimensions, providing a "map" of invertebrate assemblages. In the map, sites that map close to each other are very similar, and sites far apart are dissimilar. We used two programs for NMS ordinations,

Primer (version 5.2.8, PRIMER-E Ltd., Plymouth, UK) and PC-ORD (version 4.41, McCune and Mefford. 1999).

We then used NMS ordination to look at the factor that geographic affinity (e.g. Soda Mountain, Pilot Rock areas) has in invertebrate distribution, and also used the biplot function in PCord , which correlate secondary variables to the invertebrate assemblages to further determine the influence of secondary variables in determining invertebrate assemblages. The ordinations were varimax rotated so that the strongest correlation patterns was shown.

Habitat Models

The relation of management and environmental factors on invertebrate assemblages was also explored using Nonparametric Multiplicative Regression (NPMR, McCune and Mefford 2004). However, instead of running NPMR on every single genus, we limited our analyses to several indexes and summaries of biological diversity to give a more robust, integrated view of overall assemblage response. Summaries examined were: 1) Number of Ephemeroptera, Plecoptera, and Trichoptera taxa, 2) % of total taxa categorized as intolerant; and 3) OTU richness. Predictor variables included environmental factors, vegetative descriptors, and management activities prevalent across the CSNM, and are the same set as used in BIOENV models (Table 2). Nonparametric Multiplicative Regression as implemented in Hyperniche (McCune 2006) was used to derive best-fit models describing the pattern of the above defined response variables relative to the predictor variables. The Local Mean form of the NPMR regression

was used to enable the incorporation of binary or quantitative data The modeling process includes an initial screening for variables of interest followed by an exhaustive modeling approach. As the number of predictor variables increases, a stepwise search is initiated. All predictor variables are assessed in one-variable models to determine the best one-variable model. Additional variables are added stepwise, assessing improvement at each step. This approach evaluates all possible combinations of predictors and tolerances.

In addition to identifying important variables, the modeling process provides several measures for assessing importance of individual variables and overall model quality. When a response variable is declared as quantitative, model quality is evaluated in terms of the size of the cross-validated residual sum of squares in relation to the total sum of squares. The HYPERNICHE manual calls this the "cross r^2" (xr^2) because the calculation incorporates a cross validation procedure. The xr^2 value is a measure of variability captured by the best fit model.

Sensitivity analysis provides a measure of the relative importance of individual quantitative predictors in NPMR models. The sensitivity measure used here refers to the mean absolute difference resulting from nudging the predictors, expressed as a proportion of the range of the response variable. The greater the sensitivity, the more influence that variable has in the model. With this sensitivity measure, a value of 1.0 implies a change in response variable equal to that of change in a predictor. A sensitivity of 0.5 implies that the change of response variable magnitude is half that of the predictor variable. A sensitivity of 0.0

implies that nudging the value of a predictor has no detectable effect on the response variable.

NPMR models can be applied in the same way that traditional regression models are used (McCune 2006). A major difference is that estimates from the model require reference to the original data. Three-dimensional plots of select predictor and response variables provide a visual assessment of how the relationship of predictor variables to response variables. The modeling approach as utilized by HYPERNICHE works well with variables defined in GIS as ASCII grids, allowing the formulation of probability estimate maps for response variables.

Results and Discussion

Invertebrate surveys

Preliminary surveys in 2005 identified 120 potential springs from USGS topographic maps, BLM inventories and World Wildlife Fund surveys. Early ground visits to these springs indicated that not all would be suitable for sampling Springs that were generally not converted to stock-ponds were generally the higher elevation, perennial springs and sampling efforts focused on 56 of these springs (Figure 1). By limiting the sampling to this particular class of springs, we increased our knowledge of the biodiversity of these sites with a high number of replicates, but the results and conclusions of this report should be limited to this class of springs. The excluded springs, however, do not represent "natural" springs and are hence unlikely to be a high source of biodiversity or management

action. Nevertheless, we urge that the results presented here should not be applied to low elevations spring systems.

Overall, we collected 10,427 individual invertebrates of 179 different operational taxa units (OTUs which include specimens only identified to family level). For the CSNM springs, we observed a total of 92 genera after collecting 56 samples, but the CHAO 2 genera estimator suggest that this is an understimate of the genus richness (Figure 2). The CHAO 2 suggest that there were potentially 118 different genera, indicating that we missed 26 genera in our sampling. The 78% of the total genera located in these springs should allow an accurate analysis of the regional biodiversity relative to other systems.
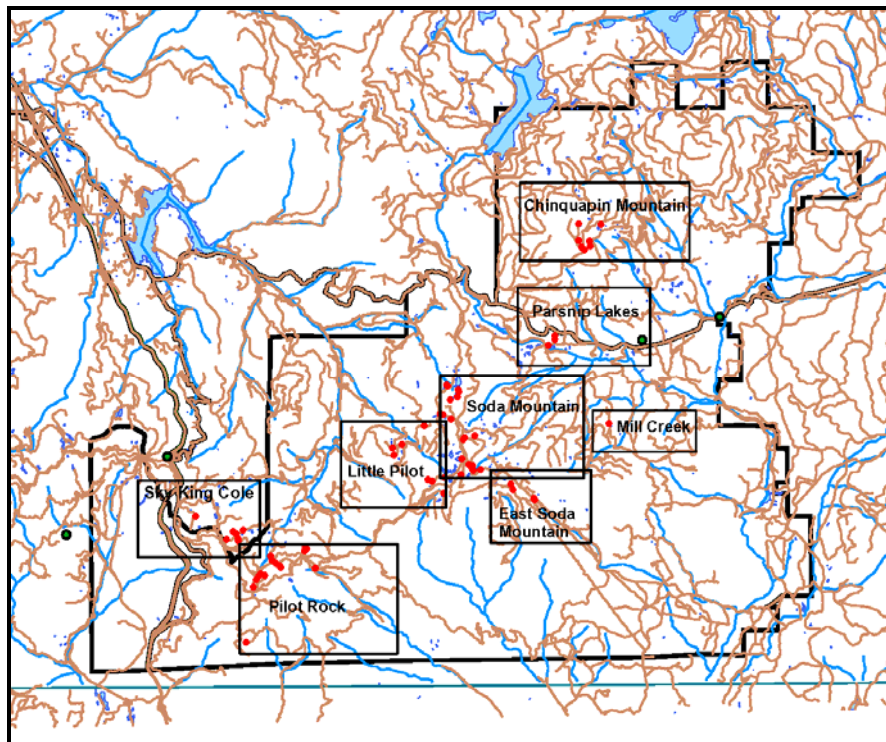
Figure 1. Map of the Cascade-Siskiyou National Monument showing sampling locations (red dots) and geographic affinities (e.g. Soda Mountain).
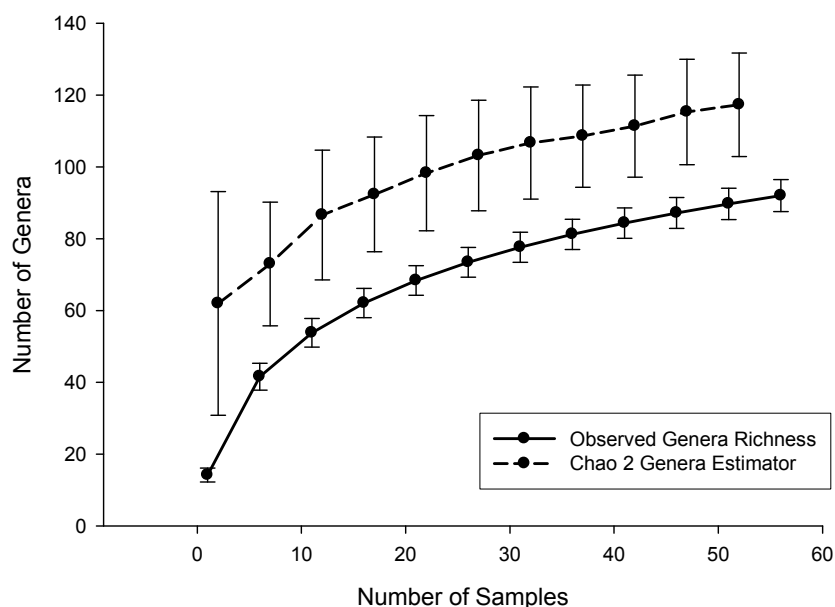
Figure 2. Species-area curves (in this case genus) and genera estimator for springs in the Cascade-Siskiyou National Monument.

Comparisons to other western US spring systems indicates that the springs of CSNM have a high amount of biodiversity (Figure 3). For example, compared to the springs of the Cache Valley, Utah, a similar number of total genera (92 versus 100) were identified in approximately half the sampling effort (56 versus 117). Additionally, at this sampling effort, the CSNM compares favorably to the springs of the Bonneville Basin. However, the total number of genera in the Bonneville basin is considerably higher due to a large sampling effort (over 250 samples)! It is likely that continued sampling of CSNM springs

Comparison of Genera Richness to other spring systems
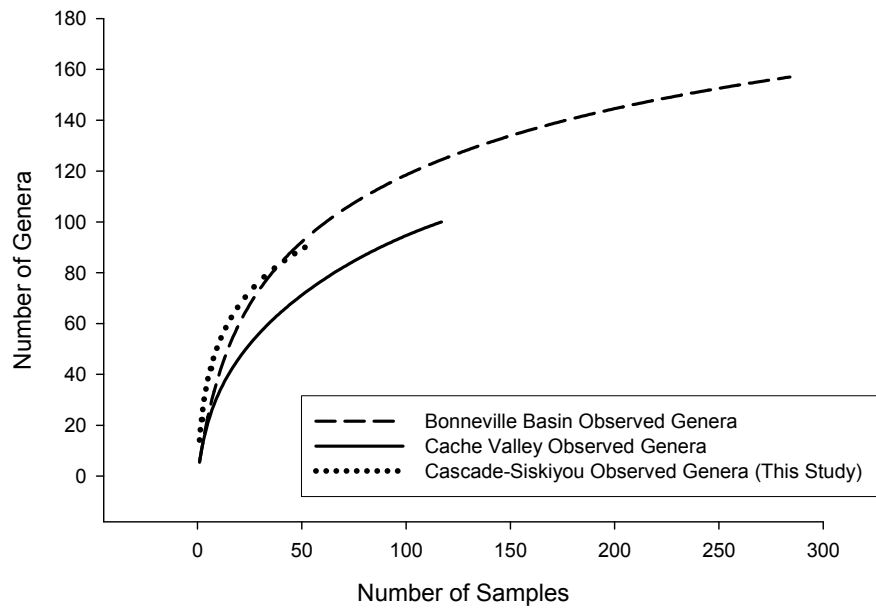
Figure 3. Comparisons of CSNM genus richness to other western spring systems.

would increase our overall knowledge of aquatic macroinvertebrate biodiversity by adding additional genera.

We also examined the genus-area curve of the CSNM to all samples we have collected in Jackson County, Oregon (Figure 4). Here, the genera of CSNM is low compared to all habitats. For instance, after 56 samples of all Jackson County sites, our observed genus richness was 128 genera, compared to the total of 92 for the CSNM study. However, considering that Jackson County sites include many different habitat types (e.g. other springs, lotic, lentic,

wetlands, etc) and multiple years, the CSNM sites should still be considered as having high biodiversity.
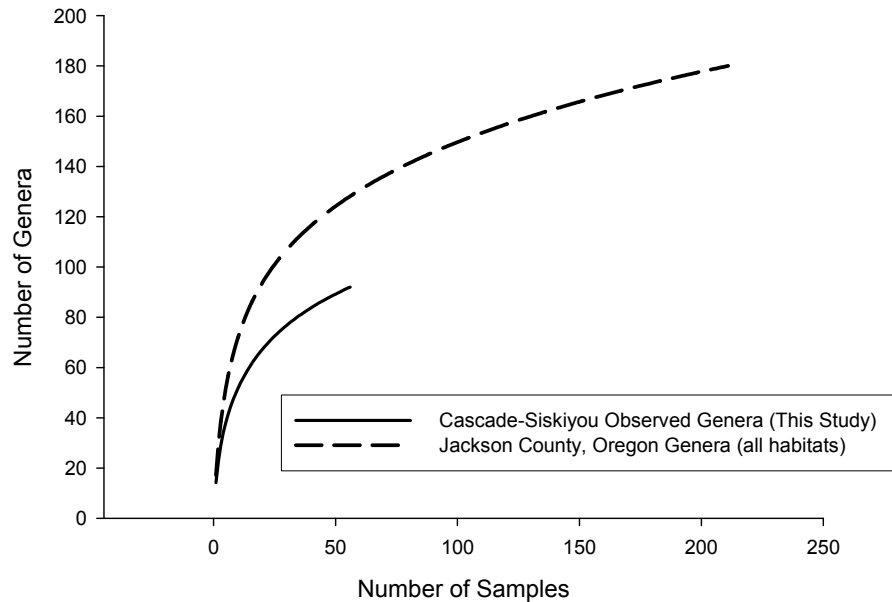
Figure 4. Comparison of Genus – area curves for CSNM springs and all Jackson County, Oregon invertebrate samples.

Exploration of invertebrate assemblages with NMS show apparent grouping based on habitat locality (Figure 5). The first ordination was based on relative abundances collected, but was standardized to species maximum. This standardization allows the ordination routine to view all species as equal (i.e. numerically dominant species do not overshadow other less dominant species). Because plotting of sites next to each other indicates similarity, there is obvious grouping and overlapping of sites, for example Parsnip Lake sites are similar to sites within the Soda Mountain and East Soda Mountain areas. Also, the Soda Mountain locations are all very similar, and form a separate group from those of Pilot Rock localities. However, the advantage of using this technique is that it

can identify sites that are very dissimilar to other sites within the same

geographic locality – in this case R100919A (upper right of Figure 5) is unlike

any of the other Chinaquapin Mountain sites. In this case, this site might have

unusual species, or might be either heavily impact (or pristine).

Macroinvertebrate relations to external factors

Macroinvertebrate presence/absence was most related to geographical

parameters using the Mantel Test (Table 4). Distribution was also related to

environmental factors, but was not related to vegetation characteristics. Because

of this, effect of vegetation on macroinvertebrate distribution was not investigated

further, although a Mantel test for relatedness between environmental factors
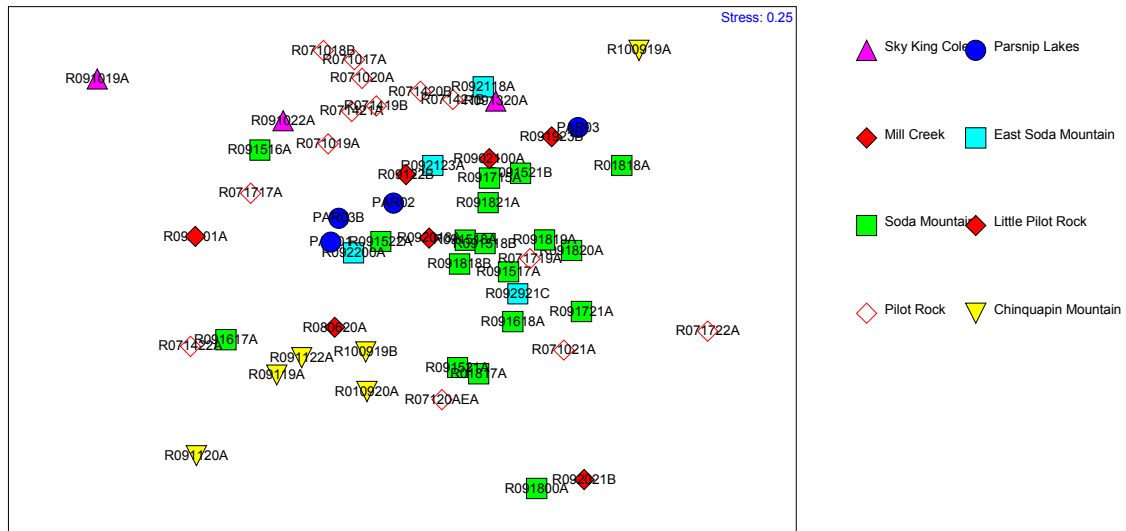
Figure 5. NMS ordination of collected invertebrates in CSNM area, using relative abundance of invertebrates collected. Points close to each other indicate similar invertebrate assemblages, whereas points distant from each other indicate dissimilar invertebrate assemblages.

Table 4. Mantel test results for relation between invertebrate presence/absence and secondary variables. In all tests, the primary matrix was invertebrate presence/absence based on Sorenson (Bray-Curtis) distance measures. Distance measure refers to the measure used for the secondary matrix. The r statistic is the standardized Mantel statistic, interpreted the same way as a Pearson Correlation (i.e. strength of relation). The *p*-value is determined through Monte Carlo randomization tests.

Secondary Matrix	Distance Measure	r statistic	*p*-value
Environmental	Euclidean	0.17495	0.0048
Geographical	Euclidean	0.20799	0.0004
Vegetation	Sorenson (Bray-Curtis)	0.07756	0.16

and vegetation was significant (r = 0.148, *p* = 0.0214) suggesting simultaneous effects of environmental factors on both invertebrates and vegetation.

The results from the mantel test can also be visualized through the Primer routine, 2STAGE, which shows the graphical representation of multiple matrices where the closer the plotted matrices, the more correlated the matrices. This analysis of the four matrices (invertebrate presence/absence, environmental, geographical and vegetation) shows that invertebrate presence and absence is most closely related to geography, but that environmental and vegetative factors are also important (Figure 6).

Non-Metric Multidimensional scaling ordinations combined with biplots show that Pilot Rock site localities are generally high in Ephemeroptera taxa, and other indicators of high biological diversity (Figure 7). Additionally, 3 sites in the Chinaquapin Mountains are high in Plecoptera diversity. Sites to the left of the
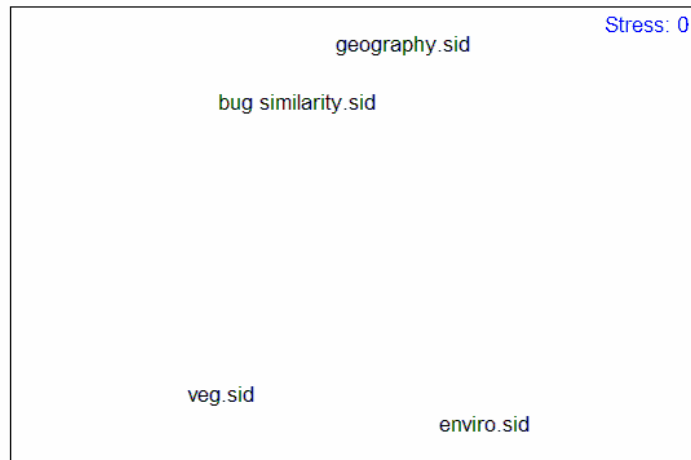
Figure 6. Second stage non-metric multidimensional scaling ordination of the four matrices. Bug similarity = Invertebrate matrix, Geography = UTM coordinates and elevation, Veg = Vegetation matrix, Enviro = Environmental and management matrix.

centroid, are inversely related to the biplots, meaning that they are lower in Ephemeroptera diversity and other diversity measures. Note that only biplots with a correlation coefficient greater than 0.2 are shown.

Biplots with the NMS ordination using the environmental matrix shows very few strong correlations of invertebrates with environmental and management variables (Figure 8). Only temperature and conductivity showed correlation coefficients greater than 0.2, and they had no strong association with any particular geographic group. The fact that the only strong correlates were
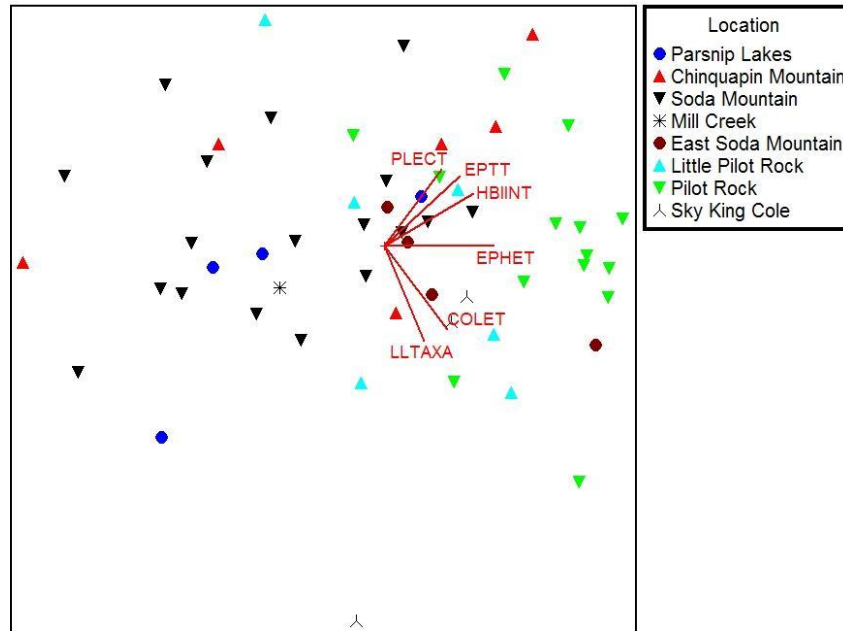
Figure 7. Non-metric Multidimensional scaling ordination with biplots of diversity measures. Only factors with a correlation greater than 0.2 are shown. Definitions of plotted factors are given in Table 1.
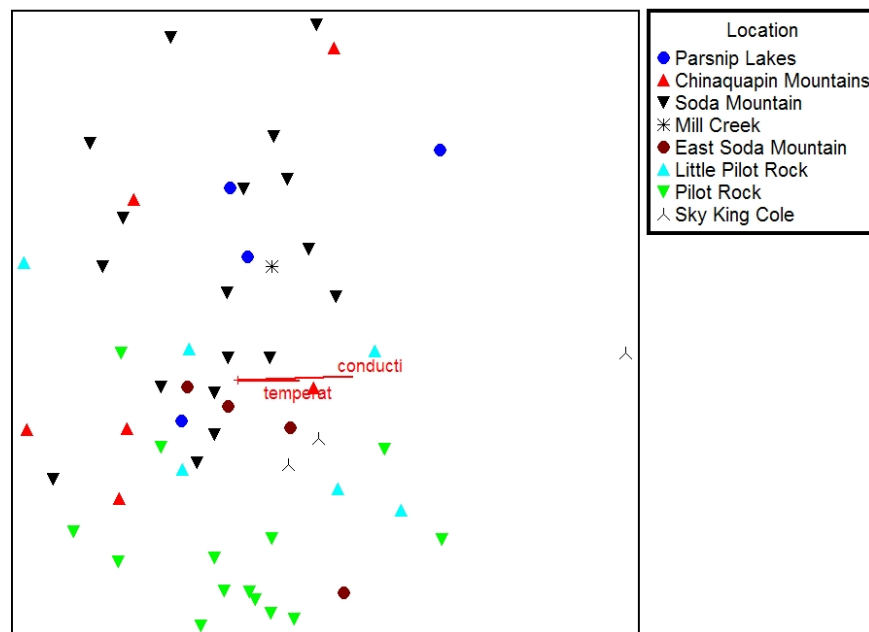

Figure 8. Non-metric Multidimensional scaling ordination with biplots of environmental and management measures. Only factors with a correlation greater than 0.2 are shown. Definitions for plotted factors are given in Table 2.

with water chemistry parameters suggests that the best determinants of invertebrate assemblages are the ones most directly associated with invertebrate habitat. Increased understanding of how management activities affect habitat directly should give insight into how management can indirectly affect invertebrate distributions.

Results of the BIOENV shows that when all springs are examined, environmental and management factors are poorly correlated with invertebrate distribution (r = 0.256, Table 5). However, when individual localities were examined (e.g. Parsnip Lakes), higher values of correlation was found. Certain factors were a common theme in a large percentage of the regional

Table 5. Best explanatory variables for all springs of the CSNM and individual geographic regions within the CSNM as determined with BIOENV analysis.

Locality	# of Springs	# of Variables	Correlation coefficient	Best explanatory variables
All springs	56	5	0.256	Outflow, Maximum utilization, Average utilization, pH, Temperature
Parsnip Lakes	4	3	0.943	Distance from road, Conductivity, % riparian vegetation influenced by livestock
Chinquapin Mountain	6	3	0.681	Slope, % riparian vegetation influenced by livestock, Conductivity
Soda Mountain	18	5	0.357	Outflow, Maximum utilization, Average utilization, Protected, Temperature
East Soda Mountain	4	1	0.765	Temperature or Conductivity (both are valid "best explanatory variables"
Little Pilot Rock	6	2	0.145	Property, Temperature
Pilot Rock	14	5	0.548	Spring area, Vegetation cover change, Protected, % riparian vegetation influenced by livestock, Temperature
Sky King Cole	3	1	1.000	Conductivity

analyses: temperature, outflow, % riparian vegetation influenced by livestock, conductivity, utilization (average and maximum), etc. Since BIOENV does not explicity address how these factors influence invertebrate distribution, we followed up these analyses with univariate examination to determine how some of these selected factors influence measures of invertebrate diversity (see below).

Hyperniche analyses of NPMR provided best fit models for the three response variables examined [the number of Ephemeroptera, Plecoptera, and Trichoptera taxa (EPTT); % of total taxa categorized as intolerant (HBIIPER); and OTU richness (RICH)] and indicated a relatively low amount of variability explained in the models, as measured by xR2 (Table 6). The model for OTU richness explained only 20% of the variability within the dataset and included topographic (slope), spring characteristics (area and whether the spring had outflow), water characteristics, and a measure of direct ungulate influence (% bare soil). The best fit model for EPTT captured 32% of the variability in the dataset and a similar set of variables representing topography (heatload, a combination of slope and aspect), spring characteristics (area and whether the spring had outflow), water quality (pH and temperature) and a direct measure of livestock influence expressed as a percentage of the remaining vegetation appearing impacted by ungulates. The model for the % of total taxa categorized as intolerant showed the highest xR2 (0.36) and was dominated by measures of management activities (logging, average utilization, and a direct measure of

livestock influence expressed as a percentage of the remaining vegetation appearing impacted by ungulates), and spring characteristics (area and whether the spring had outflow).

To assist in interpreting the models, Hyperniche outputs a series of three dimensional graphs to describe the effects of the model components on the response variable. The number of EPPT are lowest at low heatload (Figure 9), which was the strongest predictor variable included in the best fit model, but restricted to a subset of the dataset (sensitivity = 0.19, tolerance = 15, Table 6). Temperature shows a similar strong though restricted influence on EPPT (Table 6), though graphic output shows a mixed response (Figure 9). The influence of ungulates on vegetation is most marked at the lower range of recorded pH values (Figure 9) resulting in a decline in EPPT (sensitivity = 0.04, tolerance = 50).

The percent intolerant taxa changed little with logging activity, but declined with spring area (Figure 10). The percent intolerant species declined with increasing livestock influence on extant vegetation and for average utilizations ranging from 2 to 4. The percent intolerant species increased with average utilization at lower utilization levels (1-2, Figure 10) declined at higher average utilization levels. The percent intolerant taxa declined with increasing temperature, particularly at higher levels of ungulate influence on extant vegetation.

A

No. of
Ephem.,
Plec., and
Trich. taxa

Heatload

Temperature

B

No. of
Ephem.,
Plec., and
Trich. taxa

Spring
area

Heatload

C

No. of
Ephem.,
Plec., and
Trich. taxa
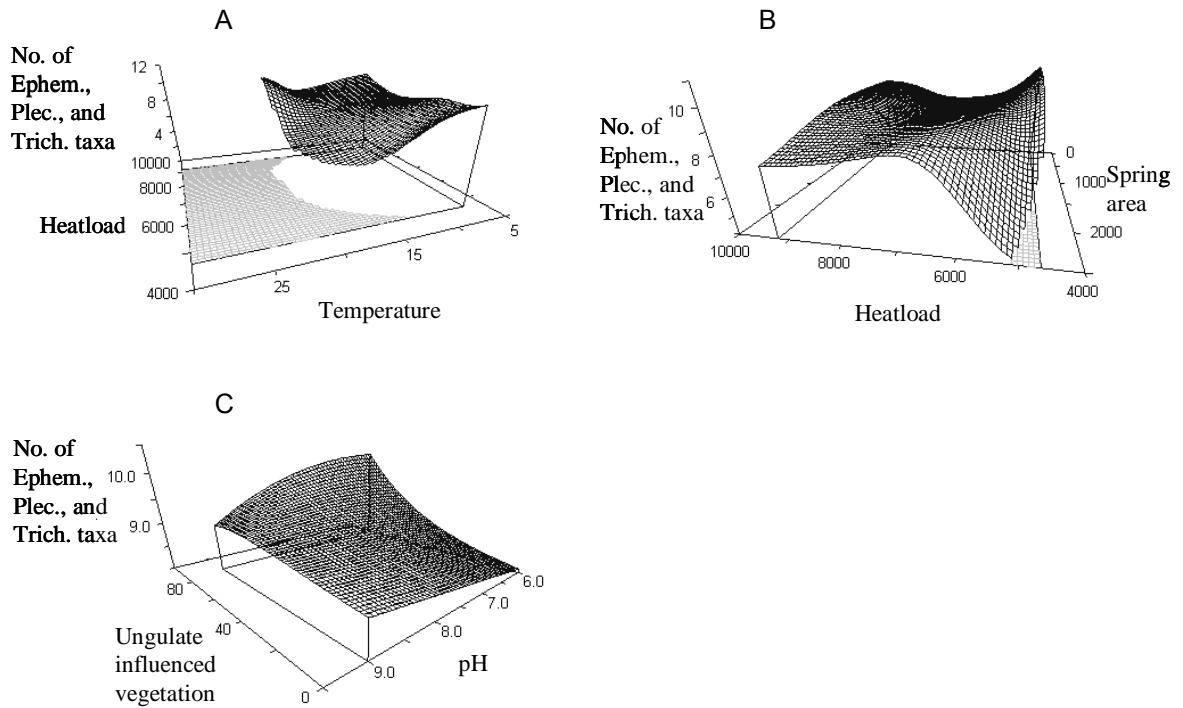
Ungulate
influenced
vegetation

pH

Figure 9. Three dimensional views of the relationship of select predictor variables to the Number of Ephemeroptera, Plecoptera, and Trichoptera taxa.
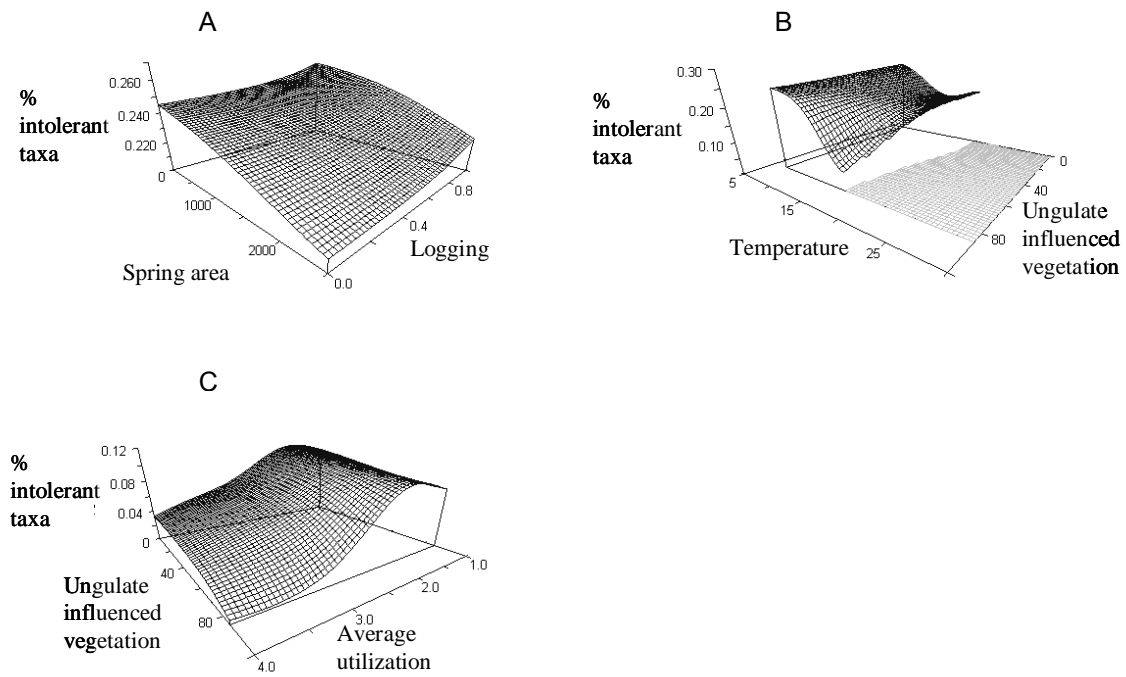
A

%
intolerant
taxa

Spring area

Logging

B

%
intolerant
taxa

Temperature

Ungulate
influenced
vegetation

C

%
intolerant
taxa

Ungulate
influenced
vegetation

Average
utilization

Figure 10. Three dimensional views of the relationship of select predictor variables to the percent of taxa classified as intolerant.

Surface water appears to contribute significantly to OTU richness (Figure 11). The percent bare soil has little influence on OTU richness for smaller seeps and springs. For larger springs, OTU richness declines sharply (sensitivity = 0.1, the second highest of all variables incorporated within the best fit model) with increases in bare soil. This influence is predominant for the larger springs examined (Tolerance = 25). Moderate sized springs appear to have the highest number of collected OTUs. Overall taxonomic unit richness is inversely related to slope. OTU richness is strongly influenced by electrical conductivity (sensitivity = 0.313) at a threshold of approximately 400 µS/cm. While sensitivity of bare soil (0.1) as a measure of influence on OTU richness is the second highest of all variables incorporated in the best fit model, graphic depiction (Figure 11) showed only a moderate positive influence.
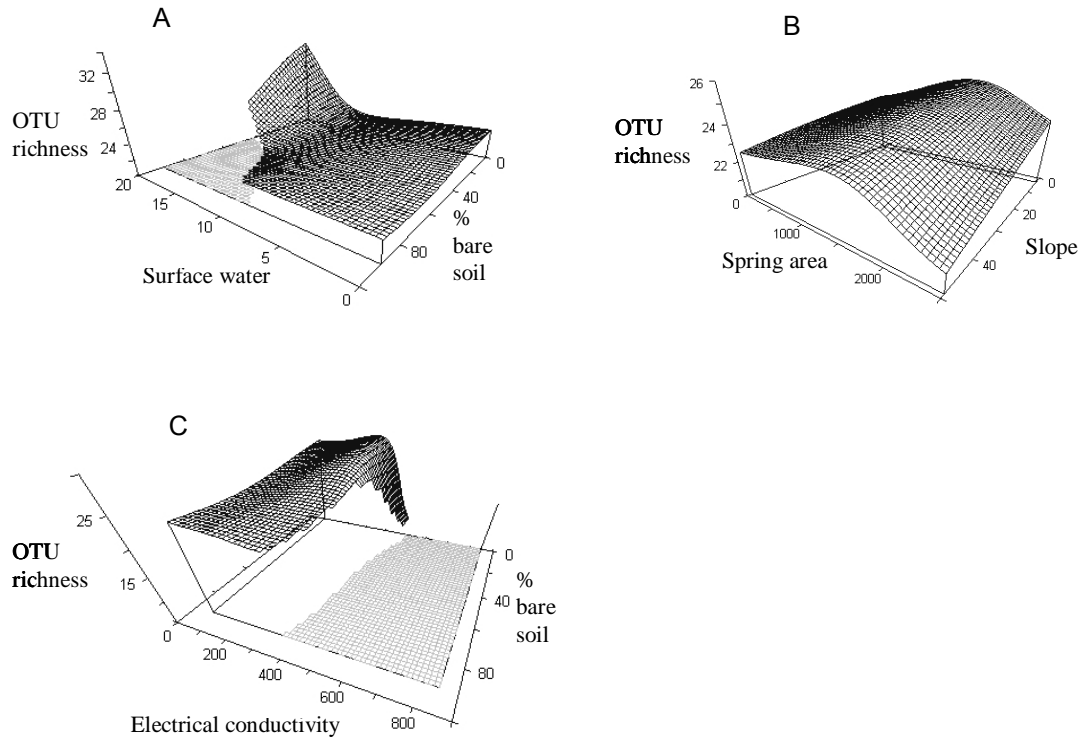
Figure 11. Three dimensional views of the relationship of select predictor variables to OTU richness.

Table 5. Tolerance and sensitivity of predictor variables included in models of the Number of Ephemeroptera, Plecoptera, and Trichoptera taxa, the percent of total taxa categorized as intolerant, and OTU Richness.

Ephemeroptera, Plecoptera and Trichoptera Taxa			% of taxa categorized as intolerant			OTU Richness		
$xR2 = 0.3263$			$xR2 = 0.3611$			$xR2 = 0.2072$		
Predictor	Tolerance (%)	Sensitivity	Predictor	Tolerance(%)	Sensitivity	Predictor	Tolerance (5)	Sensitivity
Heatload	15	0.1921	Temperature	10	0.5288	Conductivity	10	0.313
Temperature	15	0.1619	Average utilization		0.2991	% bare soil	25	0.1033
Spring area	35	0.0535	Spring area	40	0.0726	Spring area	30	0.078
% rip veg influenced by ungulates	50	0.0386	% rip veg influenced by ungulates	20	0.0562	Slope	45	0.0615
pH	50	0.0319	logging	60	0.017	Surface water		0.0503
Outflow	categorical		Outflow	40	categorical	Outflow	categorical	

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The relatively low xR2 values for best fit models examined could be explained by several different possibilities. First, the predictor variables used have little influence or predictive power on invertebrate distribution. Considering the high amount of variation common in invertebrate assemblages, any combination of variables will always be somewhat inadequate. Second, geography or other variables may be better predictors of invertebrate distribution. However, the goal of these analyses were not to maximize the predictive power of the models, but to understand the relationship of the management and environmental factors in the CSNM to the invertebrate distributions.

The incorporation of outflow in all three response variables examined highlights the direct influence of water flow on total OTU richness, percent intolerant species, and EPPT. It is likely that water flow (or the lack of) also influence temperature, electrical conductivity, and pH. The only other variable incorporated in all models (the area of the spring) also emphasizes the importance of spring characteristics in defining invertebrate assemblages.

As a response variable, the percent intolerant species showed the highest xR2 and the incorporation of greatest number of indicators of disturbance (logging and influence by ungulates). As such, taxa considered intolerant of degraded environments were higher at lower utilization levels, before declining at higher utilization levels.

The count of OTUs showed continuing declines with increased ungulate disturbance measured as bare soil for larger springs. The lower OTU richness for small springs implies that a fluctuating environment (springs drying out, widely

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varying temperature and other variables defining the aquatic environment)
prevent the attainment of higher species richness represented by larger springs.

Effects of important variables

Because BIOENV and Hyperniche do not implicitly address the overall effect of management or environmental factors on invertebrate assemblages, we examined the influence of identified important variables (using BIOENV and Hyperniche) in univariate graphs.

There appeared to be no biologically significant effect of either land ownership or protected status on three measures of invertebrate assemblage health (Figure 12). On private lands where permission was granted for sampling, it should be noted that most measures of health were improved over non-private lands – private land had higher levels of OTU richness, and EPT taxa, although on average there were reduced numbers of intolerant taxa (indicating more pollution tolerant taxa). Results may also be confounded because springs examined on private land had not been heavily utilized for many years, but happened to be strongly influenced the year the sampling took place.

The effects of % vegetation affected by livestock and temperature on invertebrate appear distinct, but are highly influenced by outliers (Figure 13). For example, overall taxonomic diversity (OTU richness) appears to be higher in site with a high amount of livestock use (Figure 13A), but this pattern is driven by only
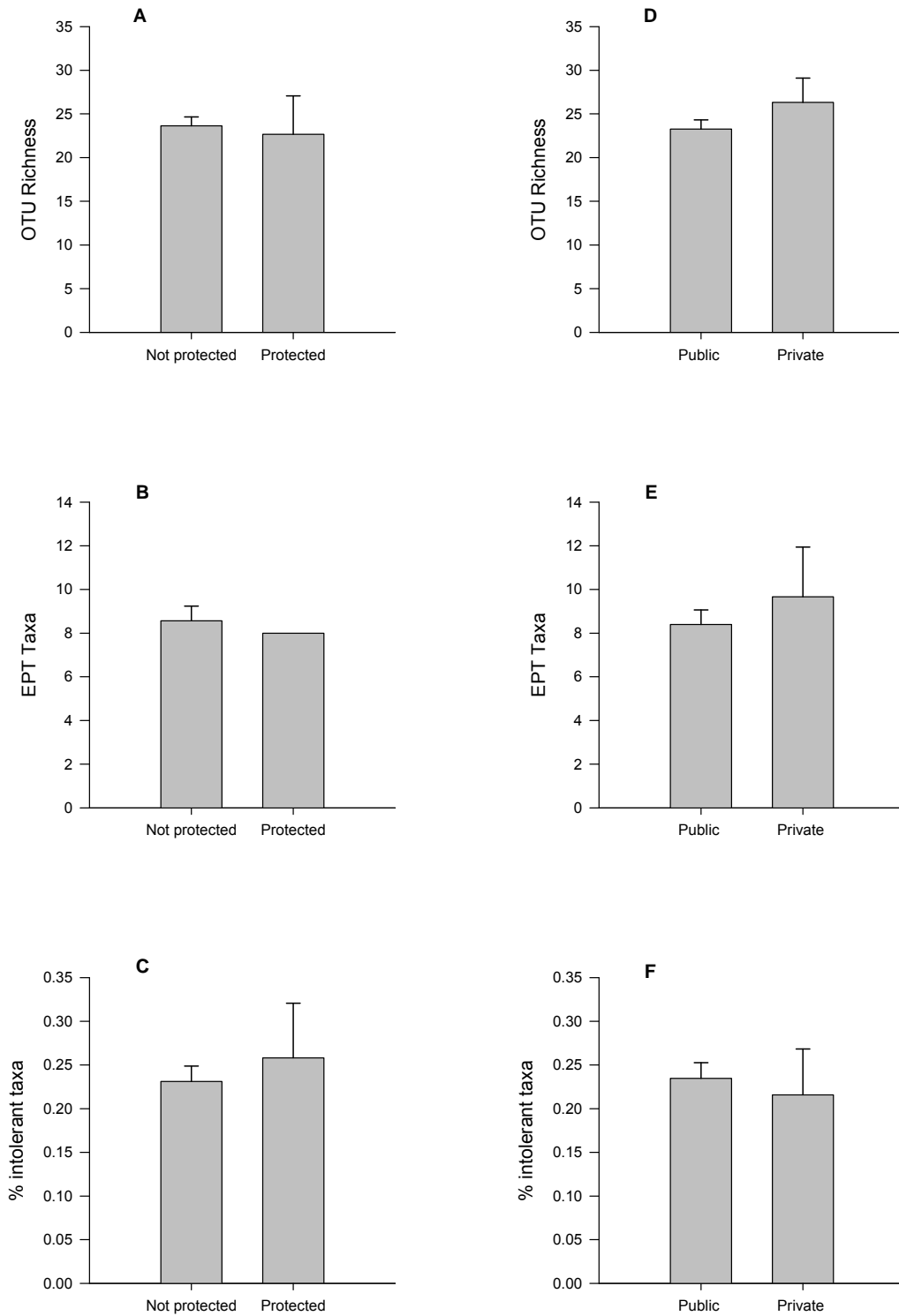
Figure 12. The relations of protection (A-C) and landownership (D-F) on 3 measures of invertebrate assemblage health.
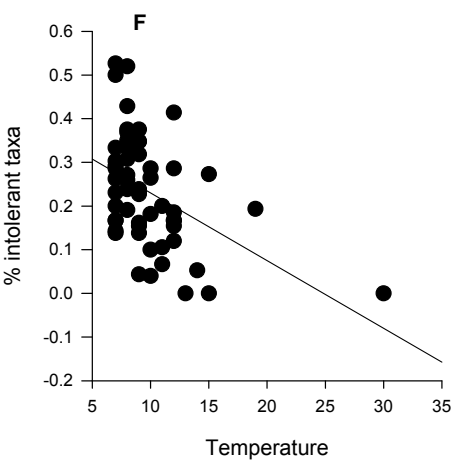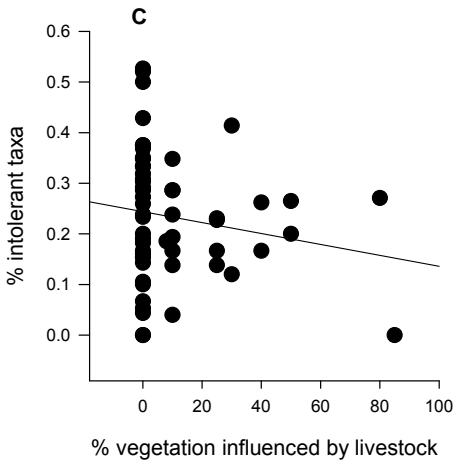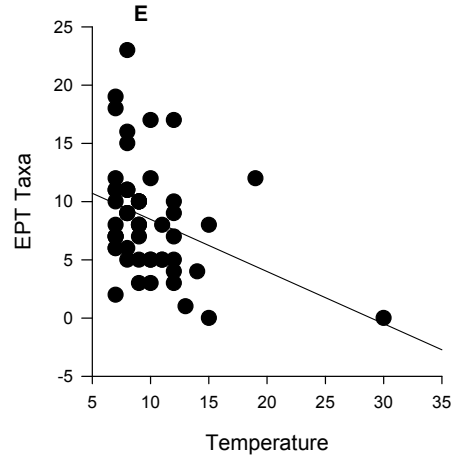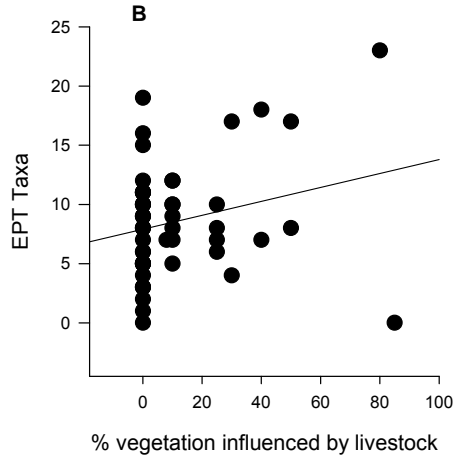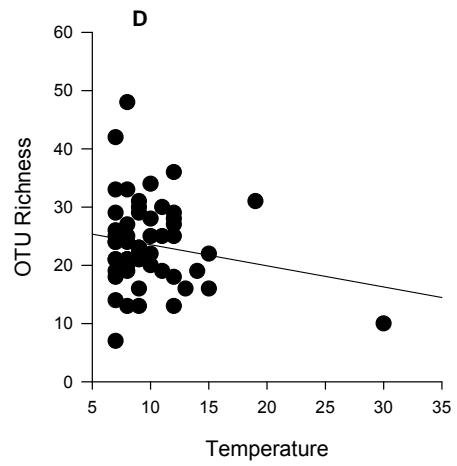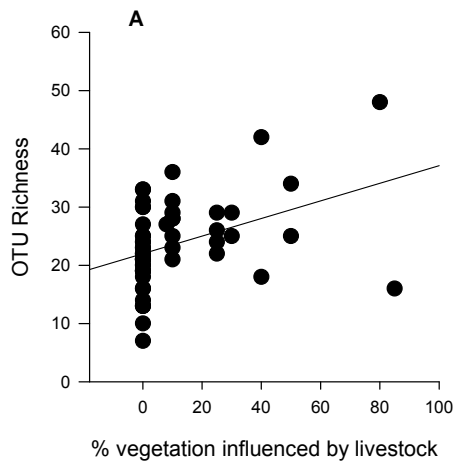
Figure 13. The relations of livestock (A-C) and temperature (D-F) on 3 measures of invertebrate assemblage health.

two sample sites at high use, one with high diversity and the other with low diversity, creating the appearance of relatively high diversity under high grazing conditions, which should be interpreted that under certain, unknown conditions it is possible for high grazing conditions to contain high invertebrate diversity, but other times there can be a large negative effect on invertebrate diversity. This pattern also holds true in relation to EPT taxa and % intolerant taxa – although some insight into this patterns is that the large diversity apparent in the one site is driven by a large number of pollution tolerant taxa (Figure 13C). The effects of temperature are also driven by low number of replicates at high temperature. This pattern of low diversity of invertebrates at high temperature, however, is consistent with other observed patterns, because high temperature causes lower concentrations of dissolved oxygen, prohibiting many species of invertebrates from inhabiting these locations (Sweeney 1984, Allan 1995).

Utilization patterns were also shown to be important factors in BIOENV analyses, and in general the invertebrate health indices were higher (indicating more diversity) at lower levels of average utilization (Figure 14). Likewise, these measures were generally lower at higher levels of utilization, with the notable exception that springs with the highest average utilization (Figure 14D) generally had the highest overall OTU diversity. This pattern is perhaps best explained by the increased diversity being comprised of more tolerant species, because the highest average utilization sites also had the lowest proportion of intolerant species (Figure 14F). Another important pattern is that the invertebrate indices (OTU Richness; Ephemeroptera, Plecoptera and Trichoptera Taxa) were often higher in sites that experienced a rating of "2" in maximum utilization (Figure 14A-C).
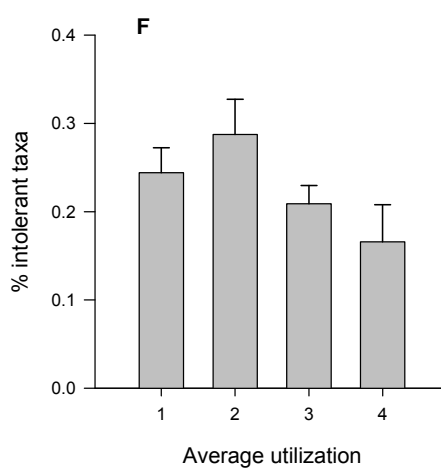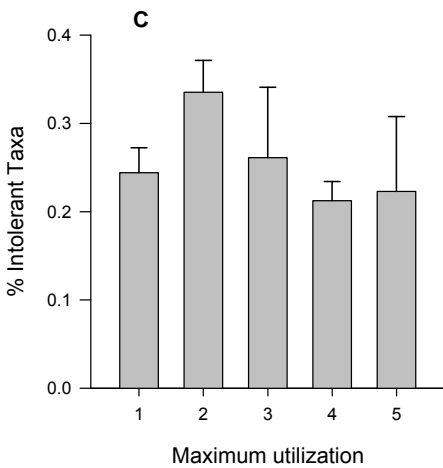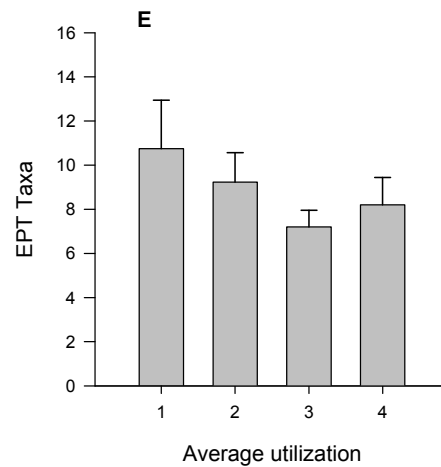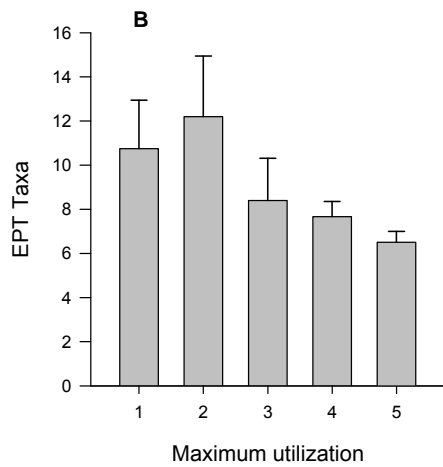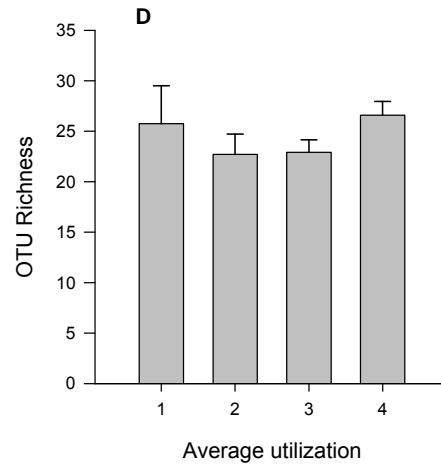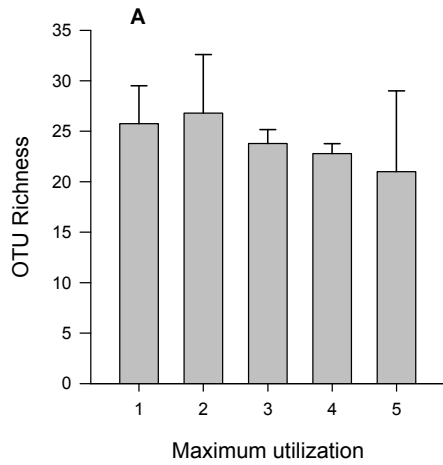
Figure 14. The relations of maximum utilization (A-C) and average utilization (D-F) on 3 measures of invertebrate assemblage health.

This is likely caused by low-level impacts disturbing the habitats, allowing colonization and prevention of climax assemblages, ala Connell's intermediate disturbance hypothesis (Connell 1978).

Conclusions

The consideration and integration of this report into management plans should include a discussion on the limitations of the data. First, the macroinvertebrates in the springs were collected qualitatively – a cost efficient method for developing species lists and gaining regional knowledge of biodiversity. However, this limits the data to a basic binary form: presence/absence. Although these data are important, they are limited for studying interrelationships of invertebrates to external factors, in that there is no flexibility allowed for sites that may have low populations living in sub-optimal conditions versus high populations living in ideal conditions. It also prohibits the calculation of useful diversity measures, such as Shannon Diversity. However, this indicates that patterns detected using presence/absence are strong, robust patterns.

Secondly, most of the external factors available and used in this report are *indirect* factors that would affect macroinvertebrate distributions. For example, one such factor is %bare soil, a measure of the amount of grazing. While a potential relationship between how increased soil and reduced vegetative cover can indirectly affect aquatic invertebrates, there are also measures of direct impacts that could be measured instead – e.g. amount of siltation in the spring, and the amount of daily fluctuation in water temperatures (plus other factors other than livestock could also be responsible for bare

soil). Hence, the collection of more direct variables may be helpful in determining causal linkage between invertebrates and environmental factors.

Thirdly, this survey offers only a snapshot of these systems. Continued seasonal and annual surveys of these springs would enhance our knowledge of the biodiversity and the relationship of the biota to external factors.

Despite these qualifications, it is clear that the springs of the CSNM are highly diverse ecosystems. Additionally, management actions appear to influence the diversity of the springs in the CSNM (see Table 7 for summary of analyses). Perhaps the best example of this is the effect of utilization patterns on invertebrate diversity – which is highest at lower levels of average utilization. Two important points are that these diversity measures are not maximized at the lowest level of utilization, but are often maximized at levels of "2", indicating that high levels of diversity may be compatible with moderate levels of use. Also, the differences between the levels of utilization on invertebrate diversity measures may not be statistically significant, but that the average difference between a maximum utilization usage of "2" and usage of "5" on EPT taxa (Mayflies, stoneflies and caddisflies) is approximately 5 species – which is definitely biologically significant.

Overall diversity (measured in OTU taxa richness) is often high in springs that show signs of degraded habitat (i.e. high % of bare soil, high temperatures, high max and average utilitization, etc.), but much of this increase is comprised of species tolerant to disturbance – which are generally Coleoptera, Diptera (Blackflies, Horseflies, and Mosquitos), Oligochaetes. While these organisms are certainly part of the overall

biodiversity and important components of ecosystems, increases in these tolerant organisms should not be mistaken as being identifiers of "healthy ecosystems".

The importance of geographic area in describing patterns of aquatic macro-invertebrates implies springs from all areas need to be conserved. No single area should be subjected to excessive livestock influence so as to prevent a reduction in beta and gamma macro-invertebrate diversity across the monument

Table 7. Summary of different methods of analyzing the invertebrate distributions and secondary factors used in current study.

Method of analyses	Analyses advantage	Conclusion
NMS Ordination	Well developed methodology	Geographic location of spring important in determing assemblage
NMS Ordination with biplot	Well developed methodology	**Against biodiversity measures:** Pilot Rock springs are among the most diverse, healthiest springs in the study area
		Against environmental measures: no strong correlations of environmental variables with invertebrates, except Sky King Cole spring high in both temperature and conductivity
Species-area curves	Allows comparison to other stream/spring systems	Springs of CSNM are as diverse as other spring systems, including systems covering much larger geographic areas
Mantel Test	Tests for broad associations with other factors	Invertebrate associations highest for geography and environmental factors
BIOENV	Tests for "best explanatory' variables - searches for most important secondary variables, within a geographical area	Variety of factors related to invertebrate distributions, various depending on geographic location: overall patterns of utilization, pH, temperature , outflow important
Hyperniche multiplicative modeling	Relates secondary factors to non-parametric, multiplicative best fit models	Low overall varibility described by models for invertebrate indices, but %influenced by livestock, temperature, spring area important variables, among others.

Acknowledgements

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