

Multivariate Analysis of Fish and Environmental Factors in the Grande Ronde Basin of Northeastern Oregon

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ABSTRACT

Management and restoration activities in the upper Grande Ronde Basin of Northeastern Oregon that focus on reducing the maximum annual stream temperature will be the most effective in creating stream conditions that support salmonid dominated fish assemblages. This paper outlines the analysis of 5 years of water quality; habitat and fish survey results from the 10 stream reaches in the upper Grande Ronde basin. The Environmental Protection Agency (EPA) through the Oregon Department of Environmental Quality has funded a long-term monitoring project that utilizes a paired watershed approach to assessing the biotic, habitat, and chemical conditions before, during, and after channel restoration activities. Multivariate and traditional statistical techniques were used to separate test streams from reference streams and identify indicator variables associated with fish assemblages. Seven-day moving average seasonal maximum temperature was shown to be a significant indicator variable for the fish assemblages in the upper Grande Ronde Basin.

Introduction

To assist the states in identifying management strategies that help improve streams limited by temperature and channel/riparian condition the Environmental Protection Agency (EPA) awarded the state funding under their National Monitoring Program. Since 1993 the EPA has funded Oregon Department of Environmental Quality (DEQ) to assess streams in the upper Grande Ronde watershed. The study is designed as a long-term monitoring project to assess the improvement in salmonid and aquatic communities in McCoy Creek, a tributary of the Grande Ronde River, by restoring habitat and lowering stream temperatures. McCoy Creek is located in the upper Grande Ronde basin of Northeastern Oregon (Figure 1). The upper Grande Ronde basin is in the Blue mountain ecoregion (Omernik, 1997) and has over 1000 miles of stream with a watershed area of 695 square miles. The elevation of the basin ranges from 2300 to 7800 feet. Land use is dominated by forest and grazing.

This project uses a paired watershed design to evaluate the benefits of channel restoration on McCoy Creek. Sites include high quality reference streams and different levels of impaired streams. Reference streams have stable, complex in-channel and riparian habitat, and are in watersheds where little or no grazing activity is present. The impaired streams are of two types: grazing-restored and active grazing. Grazing-restored sites are stream reaches where historical grazing occurred in the watershed and the active channel but prior to the study was fenced to exclude cattle from the active channel along the site

The success of the Grande Ronde study objectives relate to whether or not differences exist in the fish assemblages between the reference sites and the test sites and whether or not the environmental variables explaining the differences can be used to assess improvement in stream quality. Specifically, how effective is channel restoration in reducing stream temperature and improving habitat conditions for salmonids and other aquatic life, and how can these conditions be assessed? Correspondence and classification analysis is used to test both questions.

If differences in the fish assemblages between reference sites and impaired sites exist, then which environmental variables are largely responsible for the observed differences? Correlation and canonical correspondence analysis is used to test these questions.

Once variables are so identified, can the indicator variables be used to track stream improvement over time? Principle component analysis is used to test this question.

Methods

To monitor the effects of salmonid populations and habitat quality due to stream restoration activities on McCoy Creek data on temperature, fish assemblage, macroinvertebrates, physical habitat and water chemistry were collected. Study site conditions ranged from stable, high quality stream habitat to impaired, low quality stream habitat. A paired watershed approach was used in which a reference condition stream (Limber Jim Creek), restoration stream (McCoy Creek) and an untreated control (Dark Canyon) would be monitored over time. Additional sites were monitored to document basin-wide conditions (Table 1).

Site	Condition	Restoration	Area (ha)	Elevation (feet)	Comments
Limber Jim - Upper	Reference	No	2050	4650	Forested
Limber Jim – Lower	Reference	Passive	3670	4300	Grazing stopped in 1980
Lookout Creek	Reference	No	980	4700	Forested
Dark Canyon Creek – Upper	Intermediate	No	3480	3550	Seasonal grazing
Dark Canyon Creek – Lower [@]	Intermediate	No	4900	3350	Seasonal grazing
Meadow Creek – Starkey	Intermediate	No	12400	3770	Seasonal grazing
McCoy Creek – Restored Reach *	Intermediate	Yes	13840	3418	Stream put back into old channel
McCoy Creek – Middle	Impaired	No	12140	3550	
Meadow Creek – Lower	Impaired	Yes	26800	3380	Fencing along riparian
McCoy Creek – Lower #1	Impaired	Yes	14650	3378	Reach #1 – Fencing along riparian
McCoy Creek – Lower #2 [§]	Impaired	Yes	14650	3378	Reach #2 - Fencing along riparian

[@] 1994-1997 data, * 1998 data only, [§] 1995-1997 data only.

Except for temperature and fish, all parameters were collected three times annually in April, July and September. Temperature was recorded continuously from June through September. Fish were sampled once each year during mid summer. A summary of sampling methods for specific parameters is listed below.

Temperature - From June to September continuous (every hour) data-loggers recorded temperature at the upper and lower boundaries of each sample reach as well. Each data logger was audited with a NIST verified thermometer at the logger placement, mid-season, and prior to pick-up. Seven-day moving average seasonal maximum (TEMP7DM) statistics were calculated for each site. Seven-day moving average is used because that statistic is the value used as the temperature criterion in the state water quality rules.

Fish - Starting in 1994 fish community surveys were conducted each August by snorkeling each reach and identifying and counting all species observed. Species were identified to the lowest taxonomic level possible. Family level identification was easily achieved, however hider/clinger species such as sculpins (cottidae) were difficult to observe and are under represented in the data set.

For this analysis the August fish data were compared with the water chemistry and physical habitat of the July sampling. The period between these two sampling events was typically less than two weeks.

Physical habitat - Physical habitat measurements are made at six transects on a reach. Transects are laid out so a sequence of three riffles and three pools make up a reach, with the midpoint of each riffle or pool making up the transect. Substrate type, embeddedness, densiometer (mid-channel shade), widths (bankful and wet), depth, and velocity are measured at each transect. Reach averages were calculated, as well as width to depth ratio values.

Large woody debris, bank erosion and undercut bank presence or absence at each transect is also recorded. Proportion of the reach with these variables present was calculated (1.0 = present at all transects, 0.0 absent at all transects). Finally, a qualitative habitat assessment was performed using the EPA Rapid Bioassessment Protocol (Plafkin et al, 1989).

Water chemistry – Chemical analysis is done on grab samples. Temperature, dissolved oxygen, pH, and specific conductance were measured in the field. Nutrients, alkalinity, organic carbon, chemical and biological oxygen demand, and turbidity were field sampled, transported to the laboratory and analyzed. All analysis followed EPA and DEQ standard operating procedures (DEQ, 1998).

Data analysis/screening - Fish species data were converted to relative abundance values for correspondence analysis (CA), canonical correspondence analysis (CCA) and Two Way Indicator SPecies ANalysis (TWINSPAN) analysis. Because so few taxa (n=7) were involved, no species were deleted from the analysis. The same relative abundance

values used for CA were used for TWINSpan. Environmental data were log transformed (except for pH) prior to CANOCO analysis. Transformation was performed on raw values plus one ($n + 1$) to prevent the creation of undefined values due to having zeros in the data set. Outliers were examined and deleted if necessary.

Statistical analysis – Several multivariate and traditional statistical techniques were used to analyze the Grande Ronde data set. A brief description of each follows:

Fish community – Fish data were analyzed with correspondence analysis and TWINSpan to distinguish reference sites from impaired sites. Correspondence Analysis (CA) is a type of ordination that calculates site and species “scores” based on reciprocal averaging. Reciprocal averaging is weighted averages based on species abundances or weighted on known species environmental optima or tolerances. It assumes a unimodal species response curve. Two Way INDicator Species ANalysis (TWINSpan) is a divisive classification method derived from correspondence analysis (Hill, 1993). TWINSpan relies on the idea that each group of sites can be distinguished by a “indicator” species.

Fish and environmental variables - Pearson product moment correlation was used to isolate environmental variables strongly associated with the fish ordination axis scores. Forward selection CCA was used to select a combination of environmental variables that explained most of the variation observed in the fish species matrix. For variables that were significantly correlated with the fish ordination axes, a series of constrained CCA permutations was performed to determine which variables best explained the fish assemblage variation. The variables were put through the automatic forward selection with a Monte Carlo permutations tests (permutations = 199). Monte Carlo permutation tests are used to judge the statistical significance of selected variables (ter Braak, 1998). This process chooses variables that explain significant ($p \leq 0.05$) and independent directions of total variation in the distribution of the fish taxa, in a manner that is analogous to the selection process found in step-wise multiple regression (Christie and Smol, 1993).

Canonical Correspondence Analysis (CCA) is a widely used method for direct gradient analysis, best developed by ter Braak (1986). It is essentially a constrained correspondence analysis with selected environmental variables. The software program CANOCO (ter Braak, 1998) was used for CA and CCA.

Indicator variable analysis – Boxplots (median, 25th and 75th percentiles, and minimum-maximum) and analysis of variance (ANOVA) tests were done to evaluate the significance of each “indicator” variable. A principal components analysis (PCA) plotted ordination of these variables was done to test the utility of trending the improvement in fish and stream habitat condition. PCA is another ordination method that uses linear equations of variables to explain the variation in an environmental data set by finding the dominant gradient(s). PCA assumes a linear response model. The statistical package STATISTICA was used for correlation and PCA (Statsoft, 1993).

Results and Discussion

Correspondence analysis – CA showed that the reference sites ordinate together in a tight group to the right hand of the origin (Figure 2). The intermediate and impaired sites show more dispersion but tend to group in to the left of the origin. The sites to the right of the origin contain rainbow trout and sculpin taxa, whereas sites to the left contain lesser numbers of trout and sculpin as well as three or four additional taxa. This division of groups suggests that the first CA axis may represent a temperature-pollution gradient. The reference streams are comprised of only temperature-pollution sensitive species, while the intermediate and impaired sites are comprised of temperature-pollution tolerant species. This clear division confirms that differences in fish assemblages between reference and test sites exist. Table 2 summarizes the average fish abundances for each site across all years (See appendix 1 for complete fish results).

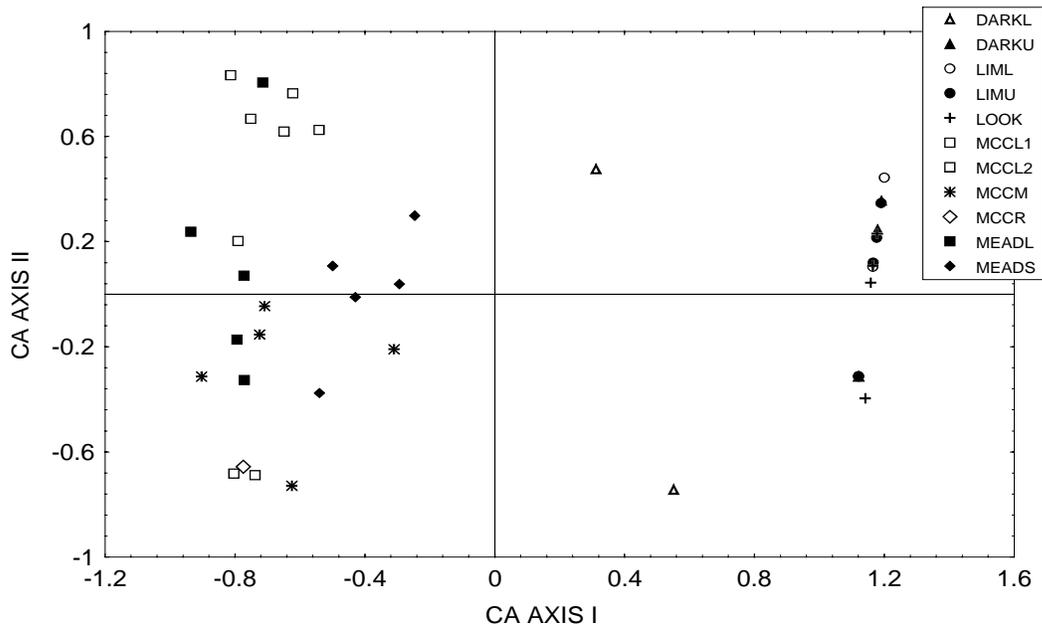


Figure 2. Correspondence Analysis biplot of Grande Ronde fish. DARKL; Dark Canyon Creek – lower site, DARKU; Dark Canyon Creek– Upper site, LIML; Limber Jim Creek– Lower site, LIMU; Limber Jim Creek– Upper site LOOK; Lookout Creek, MCCL1 & 2; McCoy Creek – Lower site 1 & 2, MCCM; McCoy Creek – Middle site, MCCR; McCoy Creek – Restored reach, MEADL; Meadow Creek – Lower site, MEADS; Meadow Creek at Starkey.

Site	Taxa*						
	Rainbow Trout	Sculpin spp.	Redside Shiner	Dace spp.	Sucker spp.	Northern pikeminnow	Catfish fam.
Lookout Creek	52	2	0	0	0	0	0
Limber Jim Lower	48	3.4	0	0	0	0	0
Limber Jim Upper	17	1	0	0	0	0	0
Dark Canyon Upper	48	2.3	0	0	0	0	0
Dark Canyon Lower	37	0	0	4	.4	0	0
Meadow @ Starkey	70	5.6	273	296	113	43	0
McCoy Restored	16	0	1570	21	330	73	122
McCoy Middle	11	.4	469	55	65	17	0
McCoy Lower 1	5.8	.8	385	75	22	0	0
Meadow Lower	4.8	2	1109	216	58	35	0
McCoy Lower 2	3	0	397	192	118	0	67

* Rainbow trout = *Oncorhynchus mykiss*, Sculpin spp.= Cottidae, Redside shiner = *Richardsonius balteatus*, Dace spp. = Rhinichthys spp., Sucker spp. = Catostomidae, Northern pike minnow = *Ptychocheilus oregonensis*, Catfish fam. = Ictaluridae.

TWINSPAN – Two Way INDicator SPecies ANalysis was used to classify the Grande Ronde fish assemblages from each site into distinct groups (Hill et al, 1993). Site by species table summarizes the *TWINSPAN* output (Figure 3). Figure 3 shows two groups (split in table); a trout dominated group and a redbside shiner dominated group. These two groups were split into four (circled below the matrix).

The *TWINSPAN* analysis agrees with the CA ordination and helps to isolate some “indicator” species or groups of species. This data set is less robust then necessary to adequately describe definitive indicator groups, but the four groups were retained to discern whether they held any predictive information. Specifically, are dace, sculpins, and suckers describing an additional environmental gradient? A plot of the initial CA site ordination labeled with the fish groups was done to examine any patterns (Figure 4). The labeled biplot suggests that some “trout only” sites occur between the more distinct groups and dace and sucker groups show more separation with axis II. This may either be an artifact of the sampling (species misidentified or undersampled) or indicate a secondary environmental gradient.

Pearson correlation analysis – Fifty environmental variables were available to analyze for correlations. Many of the variables were significantly correlated with one another (Appendix 3). To reduce the number of variables and redundancy in the environmental dataset, a Pearson correlation analysis was performed on the CA axis I and II sites scores and the environmental variables (Table 3).

Thirty-two variables were significantly correlated with axis I (27 at $p=0.01$, 5 at $p=0.05$), while only three were significantly correlated ($p=0.05$) with Axis II. The number of variables correlated with axis I indicates that this axis is responsible for the most of variation in the observed in fish assemblage. The variables that correlate with axis I are dominated by temperature and/or eutrophic related parameters. Temperature, channel and riparian disturbance characteristics related to increases in temperature, and water quality parameters indicative of disturbance are all correlated to the fish axes scores. Seasonal temperature maximum (Seven Day moving average seasonal maximum – “TEMP7DM”) is the most strongly correlated variable with fish axis I score.

Table 3. Pearson correlation coefficients of environmental variables with fish CA Axis I and Axis II sites scores of July–August 1994-1998 Grande Ronde project. This list includes only those variables significant at $p=0.05$ (bolded) or $p=0.01$ (shaded) levels. $n = 48$ for all variables. Indicator variables used in the trend model are underlined.

Category	Variable	CA AXIS I	Category	Variable	CA AXIS II
Landscape	AREA	-0.87	Riparian/Bank	BANKERO	0.33
	ELEV	0.67	Habitat	%FINES	0.31
	SLOPE	0.73			
Seasonal Temp	<u>TEMP7DM</u>	-0.91	Seasonal Temp	TEMP7DdT	0.31
	TEMP7DdT	-0.67			
Channel	<u>XWIDTH</u>	-0.75			
	XBKF_W	-0.63			
	XDEPTH	-0.48			
	XVEL	0.47			
	CHANSHP	0.44			
	WIDDEPTH	0.32			
	POOLRIF	0.32			
Habitat	LWDB	0.69			
	INSTCOV	0.54			
	XEMBED	-0.39			
	EMBED	0.30			
Riparian/Bank	<u>XC DEN</u>	0.54			
	UNDBNK	0.44			
	BANKSTAB	0.30			
Water Quality	COND	-0.81			
	TEMP	-0.81			
	ALK	-0.78			
	OPO4	0.75			
	PH	-0.66			
	<u>NPRAT</u>	-0.61			
	UNNH3	-0.58			
	TPO4	0.56			
	DOSAT	-0.53			
	COD	-0.48			
	TKN	-0.43			
	TOC	-0.40			
	NH3	0.29			

CCA forward selection – Forward selected Canonical Correspondence Analysis (CCA) ordination was used to identify the best set of environmental variables explaining the variance in the fish data. The variables (n=35) showing a significant correlation with the CA axis were used in the CCA. Two variables were added to the list; WDRAT (width to depth ratio) and PCT_SAFN (Percent sand and fines substrate) in an attempt to create composite variables with more explanatory power.

Five variables were significant contributors to the model; (in descending order) AREA, pH, PCT_SAFN, BANKVEG, and WDRAT. When this combination of variables is entered into the CCA model the cumulative percentage variance of fish data for the first and second axis is 48.9% and 52.3%, respectively. The large amount of variance explained in the first axis agrees with the previous findings. However, the parameters explaining the variance are problematic. AREA is the watershed area for each site and in terms of restoration activities is not a stream characteristic that can be “restored”. Though not evaluated, it is likely that some feature (e.g. land use, % grazing) of the watershed would be a better predictor of impairment and more suited to changes in management practices. For these reasons AREA was removed from subsequent CCA models. BANKVEG (bank vegetation score) from the Rapid Bioassessment Protocol (RBP) habitat assessment was also removed. Previous screening of the data showed this RBP component to be the least repeatable of the data set.

To facilitate selecting a more ecologically relevant list of indicator variables, forward selection was performed on subsets of the water quality and physical habitat variables. Landscape variables (area, elevation, and aspect) and RBP parameter scores were removed from consideration. Out of this process two water quality and three physical habitat variables explained 53.7% cumulative percentage (axis I and axis II) of the fish data. This is similar to the variance explained from the original ordination which had selected from 35 parameters. The final five variables chosen as indicators for this data set are:

- TEMP7DM (seasonal seven day moving average maximum in °Celsius)
- NPRAT (total inorganic nitrogen(ammonia+nitrate/nitrite):orthophosphate ratio)
- PCT_SAFN (percent sand and fines substrate)
- XWIDTH (mean stream wet width in feet)
- XCDEN (mean canopy shade – densiometer)

Indicator variable analysis – Box and whisker plots were constructed to note the differences between the fish groups and the five indicator variables. Figures 5 show the median, 25th, 75th and maximum and minimum values for the five variables by fish group. As is seen with these box-plots; temperature, shade, width and N:P ratio separate the trout and shiner groups, while percent sand and fine and, to some extent, nitrogen:phosphorus ratio begin to separate the sub-groups of trout only from trout/sculpin, and shiner/dace from shiner/sucker.

Analysis of variance (ANOVA) shows significant differences between the trout groups and shiner groups for these five variables. Furthermore when watershed area is used as a covariable (effects of area are “removed”) these five variables are still significantly different. However, because the assumption that the observations are independent is not met (same sites sampled in differing years) those results are not reported here. An ANOVA test on each year of data could be performed if the differences still need to be tested.

Principle components analysis (PCA) ordination was performed on the five indicator variables selected using CCA forward selection (Figure 6). PCA assumes a linear response model so it is more appropriate for ordination of environmental variables. The five variables explained 70.2% of the variation in the first two axes. As with CA ordination the first axis dominates the explained variability (50.0%). Shade, temperature, N:P ratio, and width are the strongest axis I variables, which again supports the CA and box-plot analysis. These variables also are highly correlated, but demonstrate that variables related to stream temperature make up the axis I environmental gradient. Percent sand and fines is a stronger axis II variables. This points to in-stream habitat as important axis II variables.

The PCA results are consistent with the previous analysis and in maintaining the transition of reference to intermediate to impaired condition. In terms of ordination space the restored McCoy site is separated from the other sites. This site exists in what was the historic streambed. The stream was rerouted and reintroduced into its present course just prior to the 1998 sampling. It is much more of a willow-vegetated beaver pool meadow stream than any of the other sites and is actively revegetating. It is located on the “impairment” side of the ordination space largely because it has more sand and fine substrate and warm temperatures.

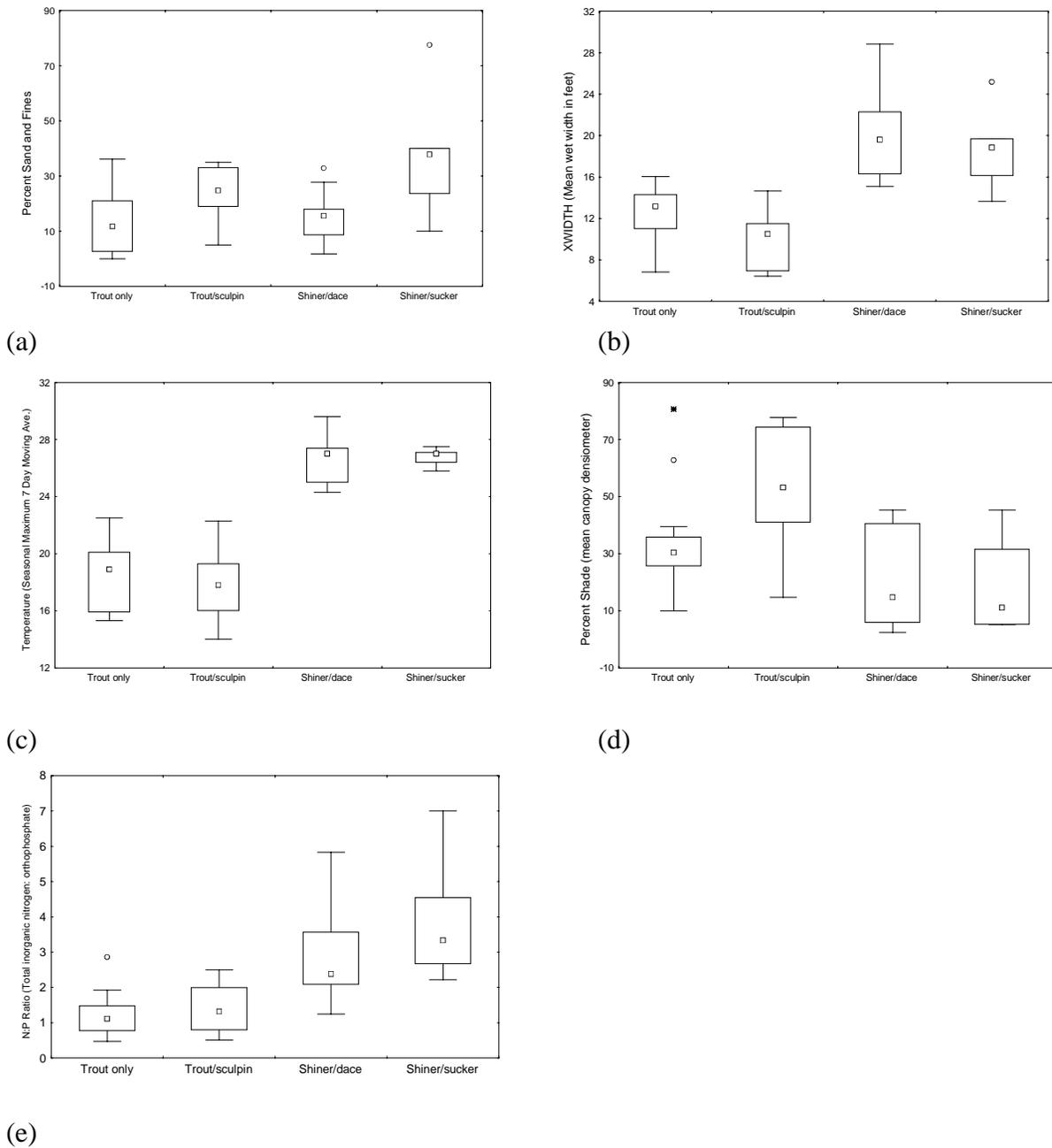


Figure 5. Box-plots of Grande Ronde 1994-1998 indicator variables by fish group. Plots show mean, 25th, 75th, and non-outlier maximum and minimum. (a) & (b) Percent sand and fines and mean width variables shows separation of 'trout only' from 'trout/sculpin' and 'shiner/dace' from 'shiner/sucker' fish groups. (c), (d), and (e) Temperature, percent shade, and N:P ratio shows separation of trout groups from shiner groups. Percent shade does not separate 'trout only' groups from the shiner groups. The 'trout only' group includes the lower Limber Jim site, an exposed reach just downstream of a forested reach.

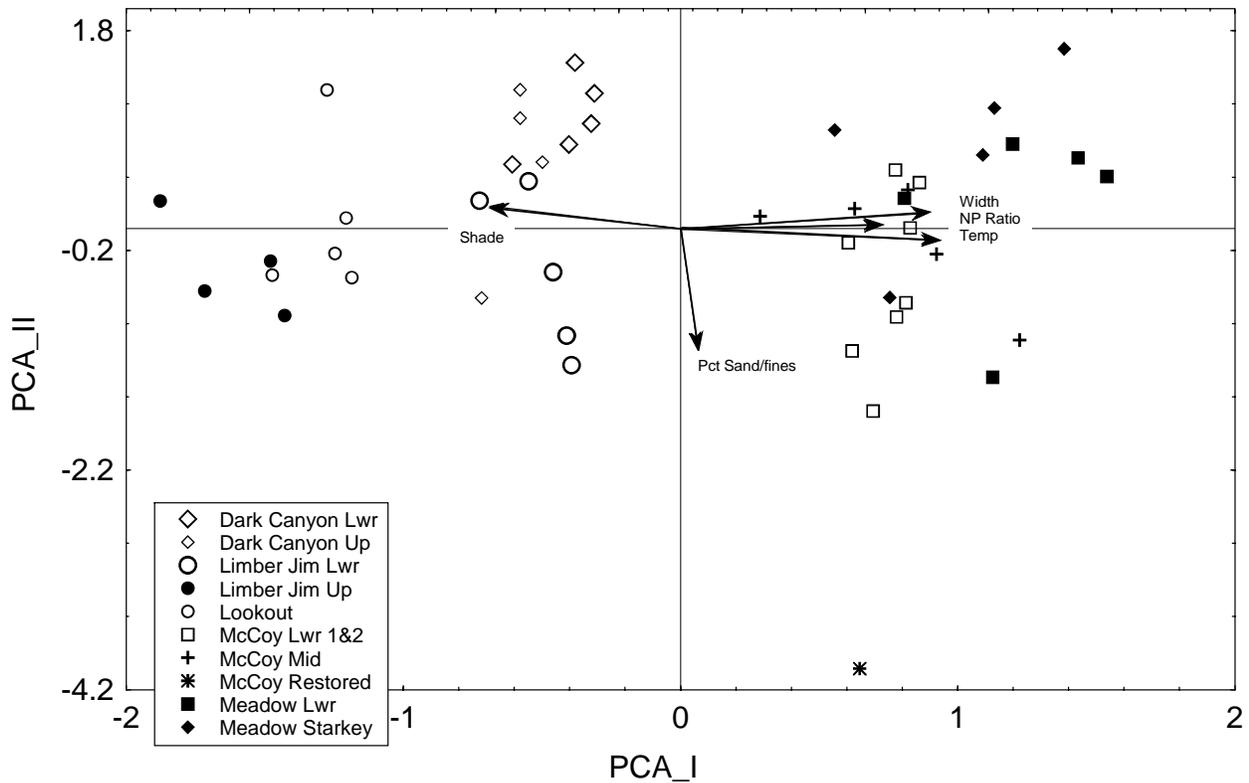


Figure 6. PCA ordination of five indicator environmental variables for the Grande Ronde Long Term Monitoring project. Lookout and Limber Jim Creek sites are reference site (circles); McCoy Lower 1 & 2 and Meadow Lower are impaired sites (squares) and intermediate sites are Dark Canyon, Meadow Starkey, McCoy restored and middle (diamonds, asterisk, and plus symbols, respectively).

Conclusions

Multivariate (ordination, classification) and traditional statistics (correlation, box-plots of descriptive statistics) support the conclusion that temperature is a critical limiting variable for the Grande Ronde Long-Term Monitoring project. Seasonal maximum temperature, and variables related to it, as a significant explanatory variable for the fish is not unexpected. To illustrate the relationship between fish and temperature for this data set, a plot of percent rainbow trout and redbreast sunfish versus the seasonal maximum temperature was made (Figure 7).

The correlation analysis showed twenty-six variables that were significantly correlated with temperature, but the canonical analysis selected temperature as the best explanatory variable of the fish data after watershed area. In the future, the addition of landscape level variables (watershed area, vegetation cover, soils, etc.) may help to explain more of the variability; however, temperature appears to be an essential limiting variable. The number of variables that correlate with temperature support the ecological relationship between this fundamental parameter and stream processes like channel modification, riparian condition, shade, in-stream large woody debris, and eutrophication.

These results suggest that management and restoration activities that focus on reducing the stream temperature will be the most effective in creating stream conditions that will support salmonid production.

Future monitoring may require that additional environmental data be collected. In particular, landscape level information about land use and watershed condition may be useful in more completely discriminating the variance in the fish assemblages. Additional data on channel condition (bankfull width and height, residual pool depth, etc.) may prove useful in explaining fish variance and tracking channel improvements.

Tracking stream improvement with PCA ordination is not clearly demonstrated with this data set. The intention is to observe impaired sites in ordination space move over time toward intermediate and/or reference site space. The Restored McCoy reach is the site most likely to show this improvement with this method. However, at this stage in the project, only one year of data at the restored reach was available for analysis. It appears that it is too early to see the results of the restoration activities in terms of the variables selected in this analysis. The rate of improvement in streams varies widely. However, this technique will allow certain variables to be identified and, either individually or as a group, demonstrate tangible improvements in stream quality that relate directly to the fish community.

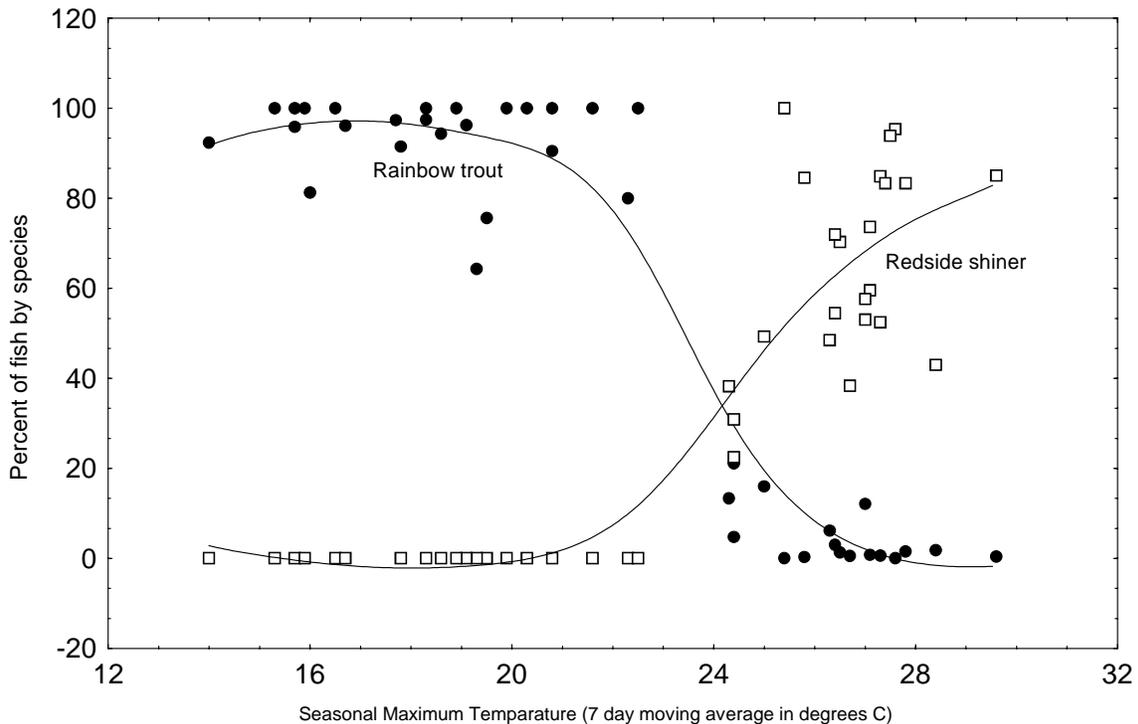


Figure 7. Relationship between percent of Rainbow trout (*Oncorhynchus mykiss*) and Redside shiner (*Richardsonius balteatus*) versus the seasonal maximum temperature. A distance weighted least squares line was used as a best fit model.

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