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TIMBER/FISH/WILDLIFE ECOREGION BIOASSESSMENT PILOT PROJECT

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WASHINGTON STATE
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TIMBER FISH
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TIMBER/FISH/WILDLIFE
ECOREGION BIOASSESSMENT PILOT PROJECT

by
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July 1992

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ABSTRACT

Biological assessment of benthic macroinvertebrate communities was completed at forested stream reference sites in three ecoregions of Washington State: Puget Lowlands, Columbia Basin, and Cascades. Characteristic chemical and biological patterns were explored through reference sites within each ecoregion. Physical characteristics of the reference sites within an ecoregion were reflective of mid-order stream types and conformed, as closely as possible, to the predefined site selection criteria.

Habitat and biological conditions in each ecoregion were determined by using a modified version of the Environmental Protection Agency's Rapid Bioassessment Protocols (RBP). Habitat condition determined through the qualitative RBP scoring system indicated specific seasons that habitat availability to benthic macroinvertebrate communities was reduced due to changing wetted stream bottom surface areas. Each region had characteristic natural disturbances that determined timing of habitat instability.

Benthic macroinvertebrate communities and surface water conditions were examined for uniqueness by ecoregions and change by calendar seasons. The benthic macroinvertebrate information was initially examined by detrended correspondence analysis (DCA), and best distinctions among ecoregions occurred during the fall, spring, and summer seasons. Two-way indicator species analysis (TWINSPAN) produced lists of genera that were considered unique to each ecoregion. The functional attributes of these "unique assemblages" were used to relate water quality and physical habitat influences that were thought to shape community patterns. Seasonal taxonomic lists were also constructed for each ecoregion that included macroinvertebrates assumed to appear in streams similar to those used in this project.

Seven RBP biometrics were used to define ecoregion macroinvertebrate conditions. Each of the biometrics was examined individually during each calendar season. Three of the metrics commonly used by benthologists were problematic. The "shredders/total abundance of sample organisms" ratio had consistently low values in each ecoregion during the fall and winter. The "EPT/Chironomidae abundance" ratio was not useful for Cascades ecoregion reference streams because of highly variable results. The "scrapers/collector-filterer abundance" ratio was least useful during winter 1991 in this ecoregion, also.

Surface water information was examined through use of principal components analysis to define parameter relationships among the three ecoregions. Many of the parameters measured in this project revealed close associations between the Columbia Basin and Puget Lowland reference sites. The Cascade streams maintained distinct surface water conditions from the other two regions, probably due to increased streamflows and higher gradients. Biological, chemical, and physical instream information surveyed in this project contrasted the mountain ecoregion streams with the valley/plains ecoregion streams.

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INTRODUCTION

Biological Assessment

The past decade has been a prolific period for the introduction of environmental evaluation techniques. These methods are intended to give regulatory agencies a better understanding of the continued impact human society places on natural resources. The United States Environmental Protection Agency (EPA) has produced monitoring program guidance documents for the evaluation of water resources that are both understandable and have widespread distribution (Plafkin *et al.*, 1989). As a result, state agencies responsible for water resource surveys use this guidance to efficiently initiate integrated monitoring programs including chemical, physical, and biological components of aquatic systems. Development of environmental assessment methodology usually serves as a major obstacle for state regulatory agencies in implementing efficient monitoring programs.

Biological assessment, or bioassessment, can be applied at one or more levels within an ecosystem. For instance, monitoring for environmental effects may take place at the microorganism level, where algae and protozoa may be of primary interest (Cairns *et al.*, 1972; Cairns and Pratt, 1986; Cairns *et al.*, 1986). More commonly, bioassessment focuses on benthic macroinvertebrates, which are comprised mostly of aquatic insects. Current protocol in analysis of benthic communities examines both the structural and functional attributes (Klemm *et al.*, 1990). The structural features of a benthic community are abundance-based and so deal with the relative abundance of organisms present at a particular site. Functional attributes of a community are defined by the "feeding" mechanisms exhibited by the various taxa (Cummins, 1973; Cummins, 1974; Cummins and Klug, 1979). The same community analysis strategies are also applied to fish assemblages (Karr *et al.*, 1986; Miller *et al.*, 1988). These biological analyses help integrate monitoring information and aid state and federal agencies in designing their programs.

Integration of Monitoring Strategies

Physical and chemical water quality parameters are commonly used as surrogate criteria for beneficial uses of fresh and marine waters. Beneficial uses include water supply, recreation, and support of aquatic life. However, physical and chemical analyses should be integrated with direct biological assessment of stream communities for more complete resource evaluation. The integration of biological information with other analyses enhances water resource evaluation by: 1) validating water quality conditions indicated by physical and chemical analyses and criteria; 2) determining expected biological conditions in an aquatic environment; and 3) detecting the presence of intermittent toxic discharges or other limiting factors that may not be identified by periodic water quality monitoring. Incorporation of biological assessment into surface water evaluations further supports the water resource decision-making process by better estimating attainment of designated uses (Ohio EPA, 1990).

Existing State Programs

A number of states have developed and implemented integrated water quality and biological assessment programs. An impetus in developing an integrated monitoring strategy has resulted from the EPA's expectation that all states implement both narrative and numeric biocriteria within the next decade.

The Ohio EPA has pioneered a methodology for establishing effective biocriteria. Biosurveys have been conducted at more than 3,000 sites in Ohio since the late 1970's (Ohio EPA, 1990). These surveys include chemical and physical water quality measurements, fish and benthic macroinvertebrate collections, and physical habitat assessment. Ohio EPA has also implemented numerical biological criteria for both fish and macroinvertebrate assemblages for each of its five ecoregions.

The Maine Department of Environmental Protection has instituted a biological assessment program to support the aquatic life standards outlined in their Water Quality Classification Law (Courtemanch *et al.*, 1989; Davies, 1987). The Maine sampling strategy has focused on benthic macroinvertebrate communities upstream and downstream of significant dischargers. Impacted stream reaches were sampled in order to define the most degraded biological conditions. The integrated biological information was then used to implement and evaluate Maine's water quality management policy.

The North Carolina Division of Environmental Management has used a standardized qualitative benthic macroinvertebrate sampling approach for wadeable streams (Lenat, 1983). They have used a variety of biological metrics to determine the condition of water resources. Narrative biocriteria were developed for three ecoregions using total taxa richness and EPT taxa richness (EPT=Ephemeroptera, Plecoptera, Trichoptera). Good correlation between these biometrics and the Water Quality Index (WQI) on individual streams demonstrated that biological assessment was a useful indicator of changes in surface water conditions.

The Arkansas Department of Pollution Control and Ecology has used a modified version of the EPA Rapid Bioassessment Protocols for the past few years (Kathman and Brinkhurst, 1991; Shackleford, 1988). Their primary emphasis has been placed on streams possessing high resource value and reaches with the potential for water quality problems. Much information has been gathered from permitted point source dischargers where an upstream/downstream sampling strategy was implemented. Narrative biological criteria have been proposed for the six ecoregions of Arkansas.

Review of Federal Agency Guidance

The concept of biological assessment has also been embraced by federal agencies, which acknowledge its sensitivity in evaluating nonpoint source impacts on water resources. The United States Forest Service Intermountain Region developed a macroinvertebrate Biotic Condition Index (BCI) as a component of their General Aquatic Wildlife System. The BCI correlates taxon presence with a limited number of chemical and physical parameters (Winget and Mangum, 1979).

The United States Fish and Wildlife Service (USFWS) produced a guide for resource managers to evaluate water quality impacts through indicator aquatic organisms (Krueger *et al.*, 1988). The concept of an indicator organism encounters logical problems when applied within an ecological framework. The document does not discuss methodology for collection of macroinvertebrates, but does examine biometrics associated with analysis of each biological group (bacteria, algae, protozoans, macroinvertebrates, fish).

A Water Quality Indicators Guide has been compiled by the United States Department of Agriculture for use by Soil Conservation Service field personnel, particularly district conservationists (Terrell and Perfetti, 1989). The guidance relies on qualitative observations that are more effectively applied with increased evaluator experience. The qualitative evaluation is integrated with an existing water quality monitoring program. Biological groups used for this evaluation scheme include benthic macroinvertebrates, fish, algae, and aquatic plants.

The United States Environmental Protection Agency has developed a plan to monitor the status and trends of ecological conditions through the Environmental Monitoring and Assessment Program (EMAP) (Hunsaker and Carpenter, 1990). This federal program is aimed at confirming the maintenance and improvement of the nation's ecological resources. A similar plan implemented by the United States Geological Survey is the National Water Quality Assessment Program (NAWQA). The objectives for NAWQA projects are to provide consistent descriptions of the nation's water resources, define long-term water quality trends, and to determine major factors that affect water quality conditions and trends (Hirsch *et al.*, 1988).

Regional Stream Biological Assessment Approach

A number of monitoring methods have been developed to help identify attainable biological conditions in streams. Prior approaches have included sampling strategies confined by watershed boundaries or upstream/downstream and before/after study designs. Intensive investigations of biological impact are well suited for a site-specific monitoring approach, but information gained by this work is generally not applicable to other areas. A regional approach to biological assessment allows one to more broadly define community reference conditions. Regional biological assessment has applicability to: identification of natural ecological trends; provision of a reference condition for comparison to impacted sites; detection of obscured nonpoint source pollution impact; and development of reasonable chemical and biological standards (Omernik and Griffith, 1991).

Regional monitoring for the purpose of managing environmental resources is potentially an effective approach. Developing regional expectations for physical, chemical, and biological attributes is both time- and cost-efficient for the resources expended. The effectiveness of such a program relies on the ability to locate reference sites that are representative of the water resource being evaluated (Hughes *et al.*, 1986). A collection of reference sites within a region defines a range of physical, chemical, and biological characteristics to which streams suspected of being disturbed may be compared (Hughes and Larsen, 1988). The reference condition is not reflective of the ecological potential of all streams within a region. Unique conditions may occur on a site-specific basis, such as natural springs that sustain stream discharge, barriers to migration, and proximity to large waterbodies.

Regional management of water resources for the protection of beneficial uses has been approached by defining the inherent natural variability of environmental parameters. Biological assessment in Ohio streams has included analysis of fish assemblages as well as the benthic macroinvertebrate assemblages (Karr, 1981; Larsen *et al.*, 1986; Whittier *et al.*, 1987). Data sets that were partitioned using regional geographic characterization, defined macroinvertebrate assemblage patterns. Assemblage descriptors such as number of taxa or species diversity showed unique distribution measures on a regional basis. The same regional patterns existed for surface water quality parameters (Larsen *et al.*, 1988). Analytical methods such as multivariate analysis and biotic index scores have been applied in identifying distinct regional conditions. Ordination of fish, benthic macroinvertebrate, and periphyton assemblages have been used to define spatial patterns in Oregon stream ecosystems (Hughes *et al.*, 1987; Whittier *et al.*, 1988). Other examples of regional biological, chemical, and physical survey approaches include those from Arkansas, Minnesota, Wisconsin, and Nebraska (Rohm *et al.*, 1987; Heiskary, 1989; Lyons, 1989; Bazata, 1991).

Regions that may be used in defining water resource conditions should exhibit continuities in a number of physical, chemical, and biological attributes. Ideally, intra-regional variation should be less than inter-regional variation to permit effective delineation of spatial management units. Gallant *et al.* (1989) describe how regional delineation is used in determining physical, chemical, and biological similarities. The most effective regional strategy employed to date has been the ecological region or "ecoregion" delineation (Omernik, 1987). Omernik's ecoregions are defined by mappable quantitative characteristics including: land surface form, soil type, land use, and potential natural vegetation. These four characters have been used to define a national ecoregional map at a scale of 1:7,500,000 as well as a northwest regional map at a scale of 1:2,500,000 (Omernik and Gallant, 1986).

Objectives of the Ecoregion Bioassessment Pilot Project

An ecoregion bioassessment project was initiated in Washington to evaluate the usefulness of a monitoring protocol to detect water resource impacts due to forest practices. The Timber/Fish/Wildlife Program (T/F/W) funded Phase I of the project, which concentrated on defining a reference condition for three ecoregions in the state: Puget Lowlands, Cascades, and Columbia Basin. The planned second phase of this project will address streams that experience a gradient of forest practice impacts. Specific objectives for this pilot project included: 1) provision of complete data sets for surface water quality, benthic macroinvertebrates, and habitat in each ecoregion; 2) definition of reference conditions for water quality, macroinvertebrates, and habitat on a seasonal basis; and 3) description of a sampling and data analysis protocol for defining ecoregion reference conditions.

MATERIALS AND METHODS

Site Selection Criteria

Reference site selection in each ecoregion was based on historical physical habitat information and professional judgement of regional biologists. Existing physical habitat information was obtained from ongoing stream surveys of the United States Forest Service (USFS, 1990); United States Geological Survey (USGS, 1991), and the Timber/Fish/Wildlife Ambient Monitoring Program (T/F/W-AMP) (Cupp, 1989; Ralph, 1990; Ralph *et al.*, 1991). Regional biologists representing the United States Forest Service, Washington State Department of Wildlife, and Washington State Department of Fisheries were surveyed for suggestions of reference stream locations within their respective management jurisdictions.

Candidate and Final Site Selection

A list of "candidate" reference sites was compiled using existing quantified habitat information in addition to informed suggestions of the regional biologists surveyed. The criteria used for identifying potential candidate sites were:

1. availability of current or historical habitat information to expedite the screening process;

2. the drainage was mostly contained within a single ecoregion;

3. reference site condition was as completely undisturbed by typical regional land use activities;

4. potential site locations were situated on mid-order streams where forest practice activities elicit some of the greatest impacts (an exception to this rule were Puget Lowland streams); and

5. year-round accessibility.

Final reference site selection in each of the ecoregions focused on more detailed aspects of candidate streams, including elevation, gradient, substrate size, discharge, and broad spatial site locations within an ecoregion. Our ultimate goal was to select habitat conditions that were most representative of each ecoregion. Reference site locations in this project are displayed in Figure 1. A total of six stream reaches were identified in each of three ecoregions. The six sites were used as replicates to define baseline ecological reference conditions. On-site surveys were completed for final identification of reference stations before monitoring began.

Habitat Structure Survey

Reference stream reaches were 100 meters in length. Reference site location considered physical habitat characteristics that typified streams within each ecoregion. The reference stream reaches

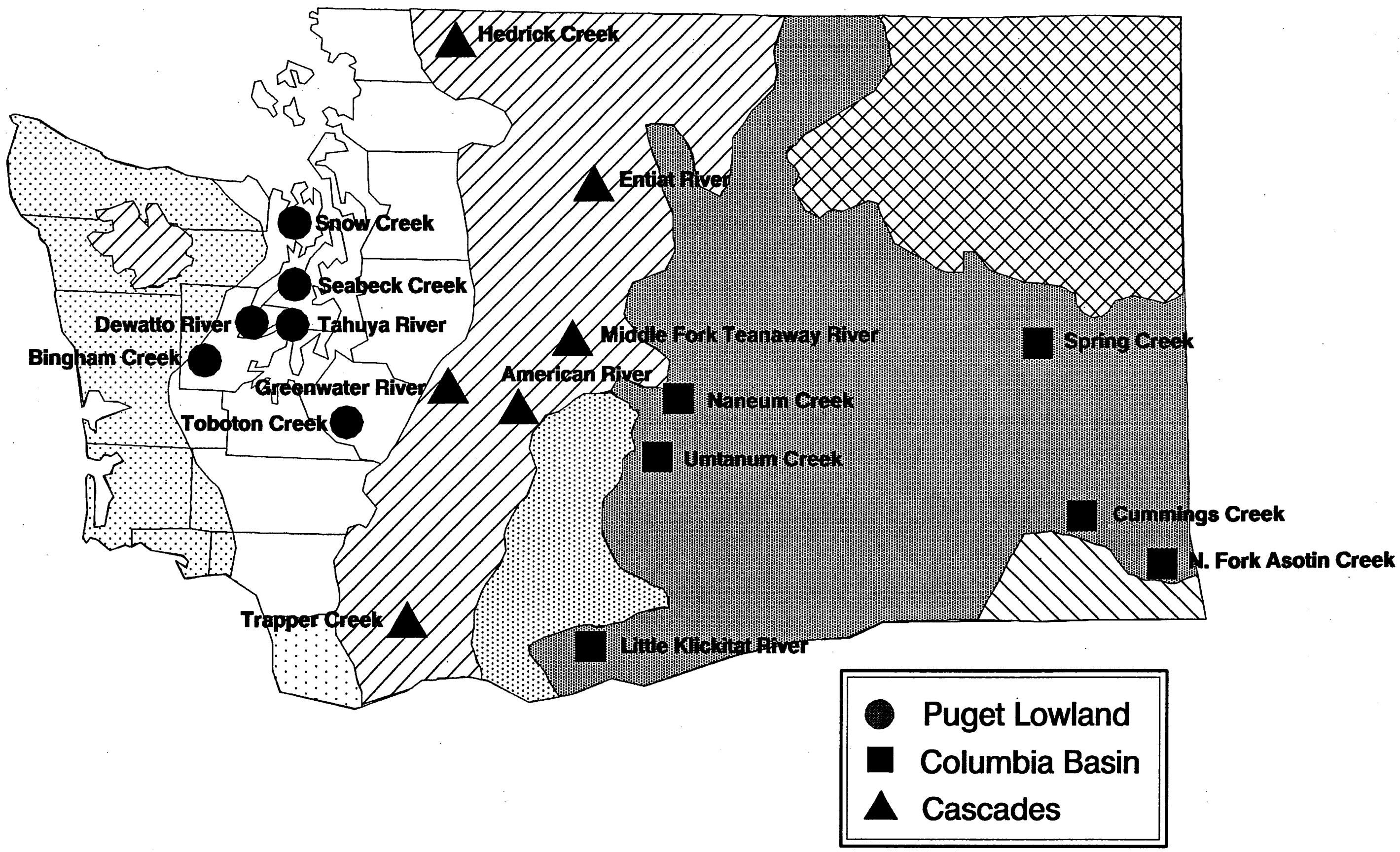

Figure 1. Location and identification of sites surveyed in the Ecoregion Bioassessment Pilot Project.

within each ecoregion were typified by a heterogeneous set of habitat characters. These physical habitat characters were reflective of natural stream conditions expected in the ecoregion.

The qualitative habitat evaluation used in this project was that described in the Rapid Bioassessment Protocols (Plafkin *et al.*, 1989). The habitat survey was comprised of three major components: primary parameters (substrate and instream cover), secondary parameters (channel morphology), and tertiary parameters (riparian and bank structure). Nine habitat parameters were scored on a numerical scale based on poor, fair, good, and excellent categories. A qualitative habitat assessment is limited to detecting substantial alterations from expected conditions.

The habitat survey form used by the evaluator was duplicated from the Rapid Bioassessment Protocols Document (Plafkin *et al.*, 1989) and is provided in Appendix A. Two evaluators participated in habitat assessment at each stream reach. Habitat assessment was completed from November 1990 to August 1991. Future use of qualitative habitat assessment will be guided by a scoring form reflective of Pacific Northwest stream conditions (Hayslip and Montgomery, 1992).

Habitat Analysis

Habitat information for this pilot project was summarized using notched box plots. The purpose for examining habitat score distributions was to provide a measure of habitat score expectations for each ecoregion. Notched box plots were used to display habitat score information on an ecoregion-by-season basis (SYSTAT, 1990). Habitat scores were then partitioned into primary, secondary, and tertiary components for further analysis of habitat-limiting regional features.

Benthic Macroinvertebrate Monitoring

Benthic macroinvertebrates were collected during four consecutive seasons from fall 1990 to summer 1991. Sampling was completed at the midpoint of each season (*i.e.* fall=November 1990, winter=February 1991, spring=May 1991, summer=August 1991). Seasonal reference sampling for invertebrates was essential in accounting for life cycle stage progression, identifying the influence of natural seasonal disturbance frequencies, and for direct comparison to other project samples collected during the same season. Months included within each season were as follows: fall (October-December), winter (January-March), spring (April-June), and summer (July-September).

<u>Field Sampling Equipment</u>

Macroinvertebrate sampling methodology was adopted from the U.S. EPA's Rapid Bioassessment Protocols (Plafkin *et al.*, 1989). A 1 square meter kick net was used. The kick net was constructed of nylon screen mesh with 500 micron openings. Two one-inch wooden dowels were attached at opposite sides of the net with plastic tie-downs strung through grommets spaced at eight inch intervals along each side. A weighted cord was placed along the bottom edge of the kick net to prevent organisms from passing under the net. An important aspect regarding net mesh size of the sampling apparatus is that it is a major determinant of collection abundances (Storey *et al.*, 1991; Minshall, pers. comm., 1992).

Site Sampling Methodology

Duplicate invertebrate samples were collected from each reference stream reach. Two transects were randomly located within each 100-meter reference reach. Two random numbers were generated with a hand-held calculator (Hewlett-Packard HP-32S). Each transect within the reach was then sampled by compositing material collected within the square meter kick net from the closest riffle and closest run either upstream or downstream of the transect location. A "riffle" was identified by broken surface water and a "run" was identified by unbroken continuously moving surface water. Thus the total area sampled at each transect from a stream reference site was 2 square meters. Composite samples were first collected from downstream portions of a reach, working in an upstream direction. Streams that are not dominated by riffles will present greater difficulty when locating the sampler under this project's guidelines. It is suggested for future studies that the investigator examine stream characteristics of a region and consider a multihabitat sampling approach. Duplicate samples were collected in order to eliminate investigator bias through stream sampler placement, and also to maximize the likelihood of collecting the greatest variety of taxa.

Sub-Sampling Methodology

Each 2 square meter benthos sample was emptied into a 24cm x 36cm sub-sampling tray gridded with 6cm x 6cm squares. The benthic material was then evenly spread over the bottom and benthic macroinvertebrates were sub-sampled by randomly selecting grid squares. All invertebrates were removed from one square at a time until at least one hundred organisms were collected. A minimum of two squares in the sub-sampling tray were picked using a lighted hand-held magnifying glass (magnification=5X). Organisms were placed into 250 mL Nalgene® jars with screw top lids. Field preservative was 10% formalin diluted from a stock solution of 37% formaldehyde. When field conditions were unsuitable for sub-sampling (*i.e.*, heavy rain, snow, high winds), kick net samples were placed in double Ziploc® freezer bags. Formalin preservative was added to the inner freezer bag containing the sample and a label with site, collection date, transect number, and preservative was placed in the dry space between the first and second freezer bag. These benthic collections were sub-sampled at a later date in the laboratory using the same procedure. The formalin preservative was replaced with 70% ethanol for subsequent laboratory sorting and identification. Attention was given to the Chironomidae (midges) and Elmidae (riffle beetles) when picking insects in the laboratory. Taxa representing these families tend to be easier to find in live samples.

Laboratory Equipment and Sample Processing

Sorting and identification of the benthic macroinvertebrate samples were completed in the laboratory with a Unitron® Dissecting Stereoscope (magnification range: 7X-45X). Taxa were identified to genus and sometimes species, where reasonably possible. An exception to generic taxonomic identification were the Chironomidae, Simuliidae, Lumbriculidae, Naididae, families of Coleoptera, Planariidae, and Hydracarina. The primary taxonomic keys used were Merritt and Cummins (1984), Pennak (1978), and Wiggins (1977). Additional taxonomic keys that were found useful in this project are listed in Appendix B. A comprehensive literature review for aquatic macroinvertebrate taxonomic keys can be found in Clark (1991).

Benthic Macroinvertebrate Data Analysis

Ordination: Detrended Correspondence Analysis and TWINSPAN

The benthic macroinvertebrate data set was analyzed using exploratory statistical techniques. Detrended Correspondence Analysis (DCA) and TWINSPAN (Two Way Indicator Species Analysis) were used for data sets comprised of counts of individuals (Hill, 1979a; Hill, 1979b; James and McCulloch, 1990). DCA and TWINSPAN analyses (Hill, 1979) are components of the Cornell Ecology Programs (CEP) (Mohler, 1987). A $\log_{10}(x+1)$ transformation was used because of the difference in magnitude between some taxa abundances (Zar, 1984). Otherwise, the ordination analyses used with the macroinvertebrate datasets would have weighted the more abundant taxa in favor of the rarer taxa (Gauch, 1982).

Ecoregion differentiation by season was examined from DCA results. The purpose was to determine uniqueness of community assemblages within the three ecoregions examined and to identify optimal biological sampling seasons for each ecoregion. TWINSPAN was used to determine site associations within each season and to identify distinct taxa associations. These taxa associations were further examined for relationships to other ecosystem components such as habitat and surface water characteristics. Consistent associations between taxa and environmental variables helped define "indicator assemblages".

Rapid Bioassessment Protocol Analysis

Rapid Bioassessment Protocol (RBP) metrics were calculated based on macroinvertebrate datasets identified to both the familial and generic taxonomic levels (Plafkin *et al.*, 1989). The purpose for comparison of metric information derived from family level and generic level identification was to evaluate the most time-efficient and cost-effective approach in applying the RBP's. A list of the biological metrics evaluated in this project is provided in Appendix C.

The distribution of values for each metric was described by notched box plots produced with the SYGRAPH® software statistical package (SYSTAT, 1990). The purpose for the "notched" boxplot was to detect significantly different median metric conditions at the 95% confidence level within particular sampling seasons.

Surface Water Monitoring

Physical and chemical surface water parameters were also characterized monthly in each ecoregion between November 1990 and August 1991. Water samples were collected at the downstream boundary of the 100 meter reference reach prior to collecting the macroinvertebrate samples. Table 1 describes the surface water parameters measured and methods of analysis. Water samples collected each day were shipped within 24 hours to Ecology's Manchester Environmental Laboratory.

Ecoregional Surface Water Patterns

Physical and chemical variables from surface water analysis were analyzed using Principal Components Analysis (PCA). PCA uses multiple variable data sets in constructing a multiple axis cloud of data points. The number of axes corresponds to the number of variables. The first component is a line through the cloud of points that represents the longest distance. PCA 1 now represents variance among the water quality variables and defines variable groups that may be associated with regional conditions. All variable observations are located somewhere along this line and explain contribution of each variable to total variance. The parameters used in this ordination analysis were not measured on the same scale (unit and magnitude differences) and thus were analyzed by using the correlation matrix (James and McCulloch, 1990). Interpretation of surface water parameter associations through ordination are made on the assumption that natural linear or near-linear relationships exist among some variables (Ludwig and Reynolds, 1988). Principal components analysis is useful when the objectives are in data reduction and interpretation (Johnson and Wichern, 1988).

Quality Control/Quality Assurance Procedures

Habitat Assessment

Qualitative habitat scoring was replicated by two evaluators at each reference station on a seasonal basis. Individual differences in the cumulative habitat scores were presumed to result from evaluator unfamiliarity with regional physical characteristics, evaluator experience, and individual habitat metrics that are not amenable to qualitative evaluation. Scores were compared between investigators and justifications for scoring decisions were discussed in order to make the scoring exercise consistent between evaluators.

Benthic Macroinvertebrate Assessment

Duplicate macroinvertebrate samples were collected from similar combinations of habitat types (riffle and run) at each reference station. The location of multiple reference stations within each ecoregion satisfied statistical requirements for sample independence, which was necessary to address the multivariate normal assumption associated with ordination analysis (Johnson and Wichern, 1988). Lack of independent sampling with adequate reference station replication may result in weak inferences of an ecoregion effect (Hurlbert, 1984).

Table 1. Parameters, analysis methods, and detection limits of water quality data evaluated for the Ecoregion Bioassessment Pilot Project.

Parameter	**Method**	**Detection Limits**
Temperature	Mercury-Filled Thermometer	± 0.1° Centigrade *
pH	Beckman pH Instrument	± 0.2 pH units *
Conductivity	YSI Conductivity Meter, Null Indicator	± 2.5 μmhos/cm at 25°C *
Dissolved Oxygen	YSI Membrane Electrode, Model 57	± 0.2 mg/L *
Discharge	Swoffer Flow Meter	± 20 percent of total *
Turbidity	Nephelometric	1 NTU
Alkalinity	Titrimetric	1 mg/L as $CaCO_3$
Hardness	EDTA Titrimetric	1 mg/L as Mg+Ca
Total Organic Carbon	Dohrman TOC Analyzer	0.1 mg/L
Ammonia-Nitrogen	Automated Phenate Method	0.01 mg/L
Nitrate+Nitrite-Nitrogen	Colorimetric, Automated, Cadmium Reduction	0.01 mg/L
Total Phosphorus	Colorimetric, Automated, Ascorbic Acid	0.01 mg/L
Ortho-Phosphate	Colorimetric, Automated, Ascorbic Acid	0.01 mg/L
Total Persulfate Nitrogen	Digestion Technique, EPA Method 353.2	0.02 - 0.2 mg/L

* Field parameter, value reflects instrument error rather than detection limit.

Analytical methods outlined by EPA (1983) and APHA (1989).

Precision of replicate macroinvertebrate sampling was determined at each reference reach by calculating the coefficients of variation (equivalent to the % relative standard deviation) for taxa richness in fall 1990 and spring 1991 samples. Individual reference reach coefficients of variation were partitioned by ecoregion and the root mean square of these were calculated. Distribution of the individual coefficients of variation within an ecoregion indicate the necessity for: 1) increased replication of macroinvertebrate samples at a site, or 2) reduction of sampling effort to fewer samples per site. The root mean square of the ecoregion coefficients of variation describes the expectation of ecoregional replicability between stream sites of similar physical condition (*i.e.* reference sites).

Surface Water Quality Assessment

Replication of surface water samples was achieved through independent sampling of different streams within the same ecoregion. Duplicate samples were collected from one station in each of two ecoregions every month in order to achieve ten percent replication overall. Stations were randomly chosen for duplicate sampling within the two ecoregions; also, the two ecoregions were never the same on consecutive months.

Field instruments were used to take *in situ* measurements for temperature, pH, dissolved oxygen, and conductivity. Calibration of the pH meter (Orion, Model 250A) was carried out at each site before water samples were collected. The dissolved oxygen probe (YSI, Model 57) was calibrated daily and at each station before use. Dissolved oxygen readings were taken from the sample container following collection. The conductivity meter (Beckman Solu Bridge, Model RB5) was calibrated at a frequency of once per month. Sample blanks of deionized water were also analyzed periodically with reference station sample sets in order to detect the presence of cross-contamination.

RESULTS

Physical Description of Reference Sites

Reference site descriptions were based on the "final site selection" criteria. A compilation of elevation information for each sample reach is provided in Table 2. Sample reaches in the Puget Lowlands ranged from 120-650 feet in elevation. Cascade reach elevations ranged from 1,000-2,950 feet. Columbia Basin reference sites were located within the elevation range of 1,600-2,600 feet.

Upstream drainage area was also calculated for each reference site in all three ecoregions (Table 2). Hughes and Omernik (1983) discussed alternatives for characterizing stream size and concluded that watershed area and mean annual discharge per unit area relayed a more accurate representation of stream size. The ratio of mean annual discharge per watershed area provides a standard by which hydrologic watershed characteristics may be compared. Upstream

Table 2. Physical characteristics of the basin area upstream of the reference sites.

Site Identification:		Basin Descriptors: Elevations (ft. above mean sea level)		Upstream Drainage Basin	Reference Reach Locations: Latitude			Longitude			Legal Description			
Ecoregion	Station	Basin Maximum	Sample Reach	Area (sq.mi.)	Deg	Min	Sec	Deg	Min	Sec	Township	Range	Sec.	Sub-sec.
2	Bingham	2600	650	4.6	47	16	36	123	20	36	T21N	R5W	29	S2
2	Snow	4250	300	11.4	47	56	25	122	53	13	T28N	R2W	11	NE4
2	Seabeck	540	120	2.2	47	37	15	122	50	17	T25N	R1W	31	NE4
2	Dewatto	400	180	5.44	47	31	20	122	57	38	T23N	R2W	5	N2
2	Tahuya	1600	400	8.03	47	31	3	122	52	44	T23N	R2W	1	NW4
2	Toboton	800	460	2.2	46	50	17	122	29	9	T16N	R2E	25	SE4
4	Hedrick	4900	1000	1.98	48	53	41	121	58	9	T39N	R6E	1	
4	Greenwater	4900	2300	52.1	47	7	26	121	31	57	T19N	R10E	21	NE4
4	American	6500	2950	79.1	46	58	38	121	10	4	T17N	R13E	12	
4	Entiat	6500	1950	158	47	54	12	120	28	22	T28N	R19E	29	N2
4	Trapper	3900	1800	6.9	45	53	44	122	0	55	T5N	R6E	23	SE4
4	MFTeanaway	5900	2600	26	47	17	43	120	57	34	T21N	R15E	21	
10	Naneum	5900	2600	66.8	47	8	21	120	28	19	T19N	R19E	16	W2
10	Umtanum	3900	1600	52	46	36	19	120	29	19	T16N	R19E	19	SE4
10	LKlickitat	4600	1800	78	45	51	5	120	47	1	T5N	R16E	10	NE4
10	Cummings	4900	2300	19	46	34	55	117	39	14	T10N	R41E	22	
10	NFAsotin	4900	2400	42	46	14	32	117	19	12	T9N	R44E	23	
10	Spring	2800	1600	18	47	45	22	117	53	16	T26N	R39E	16	NE4

2 = Puget Lowland Ecoregion

4 = Cascades Ecoregion

10 = Columbia Basin Ecoregion

watershed area and the discharge regime of a reference site are variables that can be used to relate similar streams within an ecoregion. Table 3 summarizes the water yield per unit area for each reference site. Water yields were higher in the Puget Lowland and Cascade streams. Streams with larger watershed areas generally yielded smaller quantities of water to surface flow probably due to the variety of associated hydrologic processes. Surveys of mid-order streams in this project were chosen based on a hypothesis that greatest macroinvertebrate taxonomic richness exists in these reaches (Vannote *et al.*, 1980; Minshall *et al.*, 1985).

Substrate size in reference reaches of the Puget Lowland were predominantly cobble, gravel, and sand. The Cascade substrates were cobble, pebble, and boulder, with intermittent gravel dispersion at some sites. Columbia Basin substrates were primarily cobble and gravel. The aforementioned substrate categories are based on the Wentworth Substrate Particle Size Classification (Cummins, 1962). Detailed descriptions of substrate size at reference sites are contained in Appendix D.

Stream gradient was measured previously by surveyors participating in the T/F/W-AMP at sites in the vicinity of each reference reach. Continuity in stream gradient was maintained among the replicate sites within each ecoregion. Discharge rates measured at each reference site are presented in Appendix F, and a summary plot of results is shown in Appendix J15. Discharge in the Cascades ecoregion was considerably higher than in the Puget Lowland and Columbia Basin ecoregions.

Seasonal Habitat Scores

Seasonal habitat scores were summarized using notched box plots. The box plots provided distributional information for the qualitative habitat condition within each ecoregion and examined changes that occurred seasonally (Figure 2). The notched boxplot diagrams exhibit some folding; meaning that the 95% confidence interval about the median lies beyond either the 25th or 75th interquartile interval. The highest habitat score possible using the Rapid Bioassessment Protocol survey form was 135 points. Seasonal partitioning of habitat scores within the Puget Lowland ecoregion showed very similar median values (Figure 3). The Cascades ecoregion had larger seasonal differences in total habitat scores (Figure 3). Significant median differences existed between fall 1990 and winter 1991 habitat conditions ($p=0.05$). The Columbia Basin possessed the greatest habitat score differences between successive seasons (Figure 3).

As mentioned earlier, the habitat assessment method used in the U.S. EPA Rapid Bioassessment Protocols is based on categories defined by: 1) primary parameters (substrate and instream cover); 2) secondary parameters (channel morphology); and 3) tertiary parameters (riparian and bank structures). The potential cause of the differences in habitat scores between seasons was explored by examining these habitat score components.

Table 3. Water yield per unit basin area estimated from watershed area above the reference site location.

Reference Stream	Mean Annual Discharge (cfs)	Basin Area (mi^2)	Water Yield/ Basin Area (cf/mi^2)
Puget Lowland			
Bingham Creek	21.23	4.6	4.62
Snow Creek	18.86	11.4	1.65
Seabeck Creek	8.36	2.2	3.8
Dewatto River	15.49	5.44	2.85
Tahuya River	32.02	8.03	3.99
Toboton Creek	6.11	2.2	2.78
Cascades			
Hedrick Creek	13.0	1.98	6.57
Greenwater River	175.39	52.09	3.37
American River	247.11	79.05	3.13
Entiat River	188.2	158.4	1.19
Trapper Creek	41.74	6.9	6.05
Middle Fork Teanaway River	62.71	26.0	2.41
Columbia Basin			
Naneum Creek	43.47	66.8	0.65
Umtanum Creek	1.56	52.0	0.03*
Little Klickitat River	54.84	78.0	0.70
Cummings Creek	7.2	19.03	0.38
North Fork Asotin Creek	41.83	42.0	0.99
Spring Creek	0.91	18.03	0.05*

* Note: Umtanum Creek and Spring Creek have sustained flows through contribution of groundwater input.

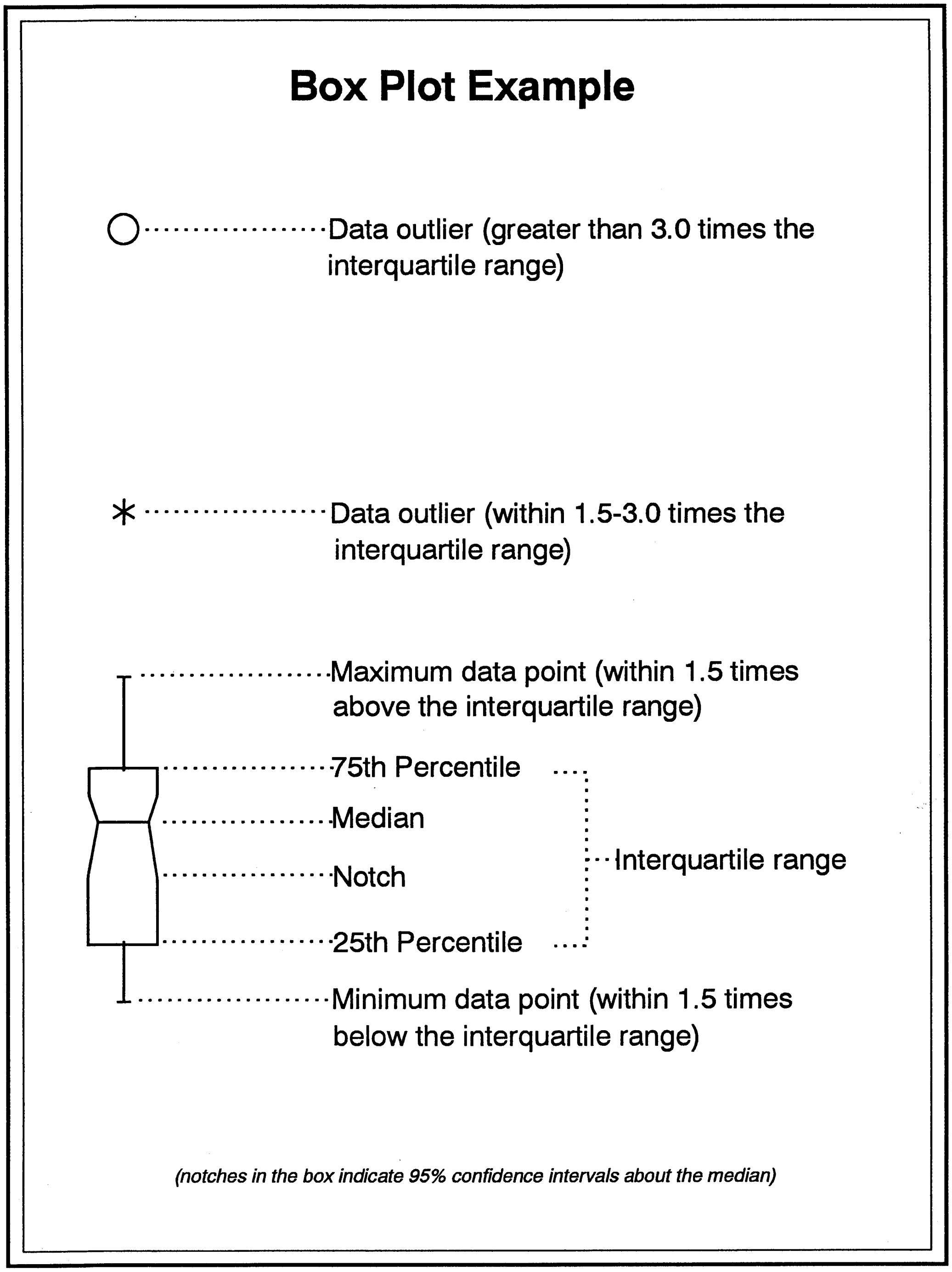

Figure 2. Interpretation of the notched boxplot characteristics

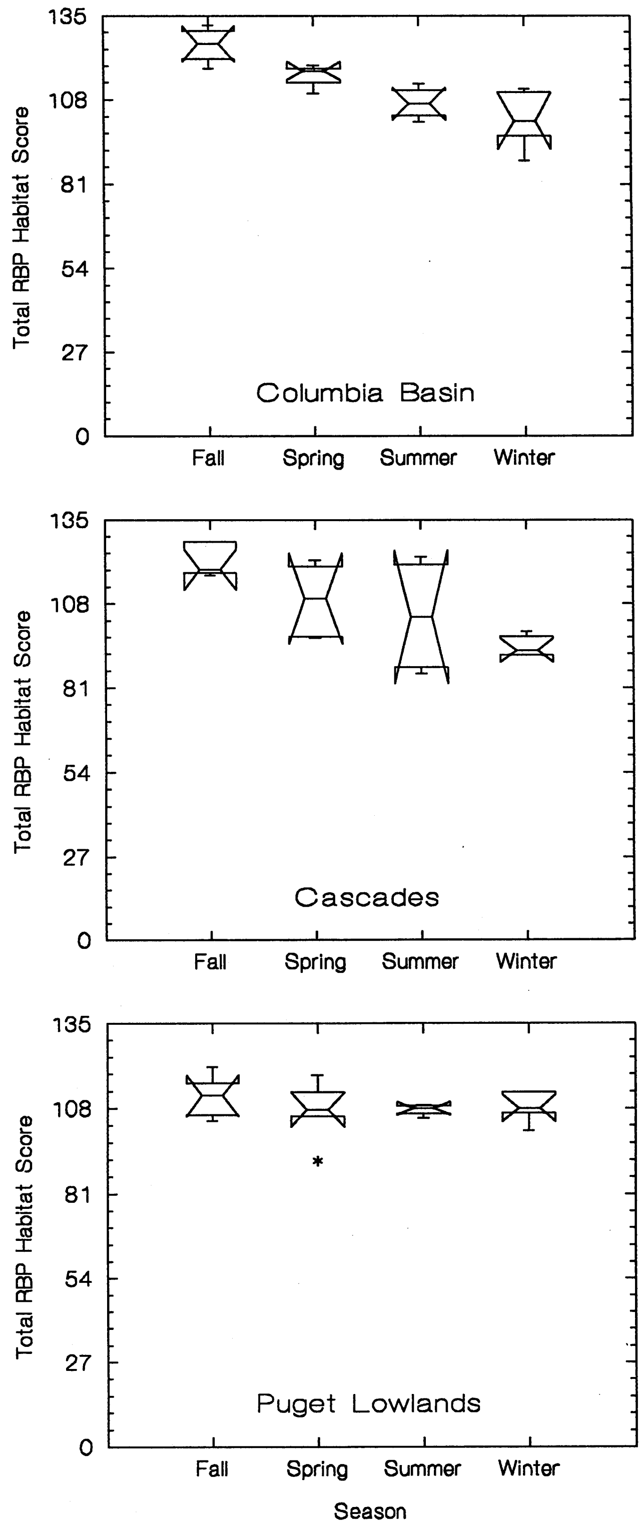

Figure 3. Total RBP habitat scores for each season in three ecoregions (Puget Lowland, Cascade, Columbia Basin) (n=6 observations per season).

Partitioned median habitat scores were highly consistent for Puget Lowland reference sites among all four seasons. Primary, secondary, and tertiary habitat parameters revealed no digressive trends (Figure 4). Partitioned habitat score distributions for the Cascades and Columbia Basin ecoregions showed the same general parameters trend as for the habitat score totals except for tertiary parameters (Figures 5 and 6). The seasonal habitat changes that were identified by this evaluation constitute physical constraints imposed on the macroinvertebrate community.

Benthic Macroinvertebrate Data Analysis

Detrended Correspondence Analysis (DCA)

Detrended correspondence analysis was performed on seasonal macroinvertebrate abundance data sets (Figures 7-10). The most distinct separation of ecoregion reference sites occurred for fall 1990, spring 1991, and summer 1991 benthic macroinvertebrate assemblages. The Cascades ecoregion invertebrate assemblages during the fall season were completely distinct from the other two ecoregions (Figure 7). Further statistical examination was limited to fall and spring assemblages. The summer benthic macroinvertebrate assemblage was not further analyzed because climatic conditions may have favored emergence for some populations and the remaining taxa collected during summer 1991 were similar to those collected in spring of 1991. A Columbia Basin stream outlier occurred in each of the fall 1990, spring 1991, and summer 1991 detrended correspondence analysis (Figures 7, 9, and 10). Naneum Creek (fall 1990), Umtanum Creek (spring 1991), and Little Klickitat River (summer 1991) were not closely clustered with other replicate Columbia Basin streams.

Two-Way Indicator Species Analysis (TWINSPAN)

TWINSPAN was used to produce benthic macroinvertebrate taxa lists that discriminated between each ecoregion during fall 1990 and spring 1991. The two taxonomic lists represent benthic macroinvertebrates that define an "indicator assemblage." A summer 1991 indicator assemblage list was not produced because TWINSPAN results did not reveal strong clusters of taxa that were consistently associated with single ecoregions. Lack of distinct taxa assemblages in each ecoregion during summer 1991 could have been a result of insect emergence timing and, therefore, a transition period for macroinvertebrate population patterns. TWINSPAN analyses were based on the percentage composition of taxa at each reference station. Only taxa that had 5% or greater representation in a reference site community were included and considered dominant in streams within an ecoregion. Frequency of taxa appearance was identified by percent representation of total sample abundance.

Taxa that are frequently present in an ecoregion during a particular season can be used as a "fingerprint" to describe the structural and functional characteristics of regional macroinvertebrate conditions. Thus the seasonal lists of macroinvertebrate occurrence frequencies reported in Appendix G provide some indication of biological expectation for other streams within the same ecoregion. A tabulation of represented functional attributes describes the expected macroinvertebrate conditions in an ecoregion during each season. Taxa included

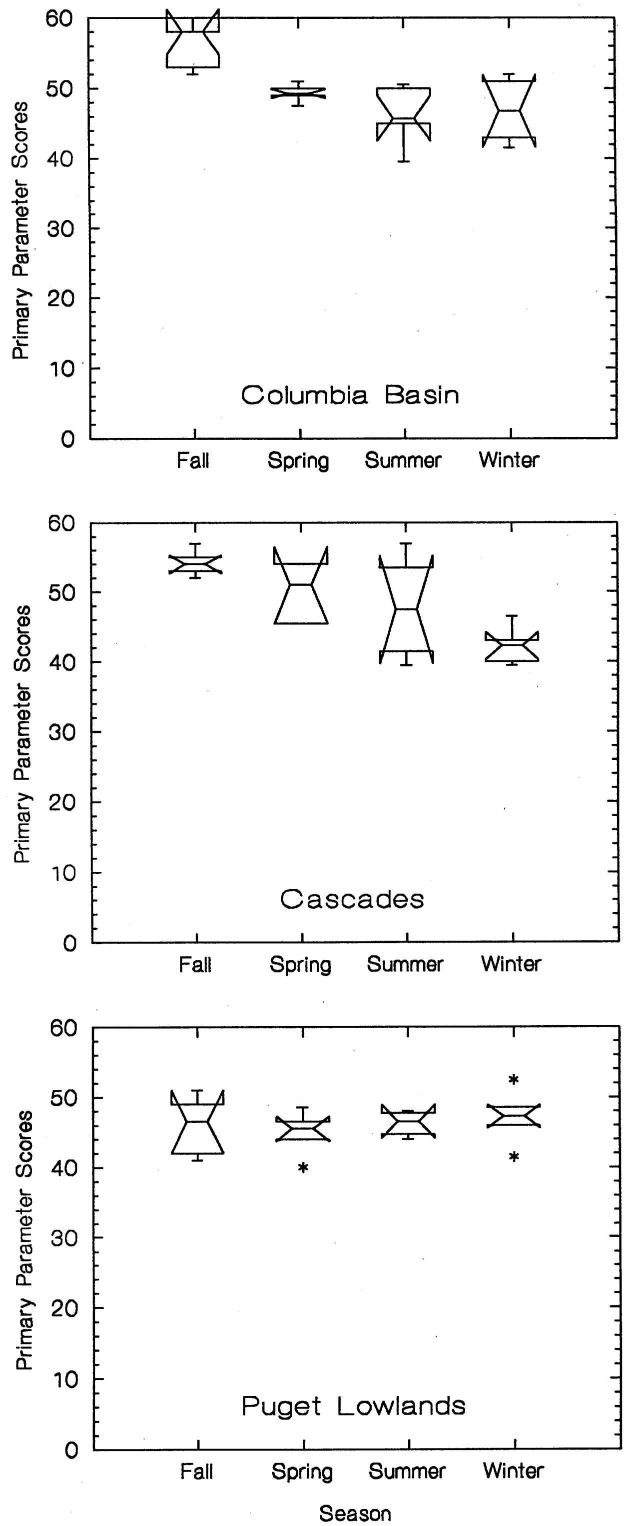

Figure 4. Primary RBP habitat parameter scores for each season in three ecoregions (Puget Lowland, Cascade, Columbia Basin) (n=6 observations per season).

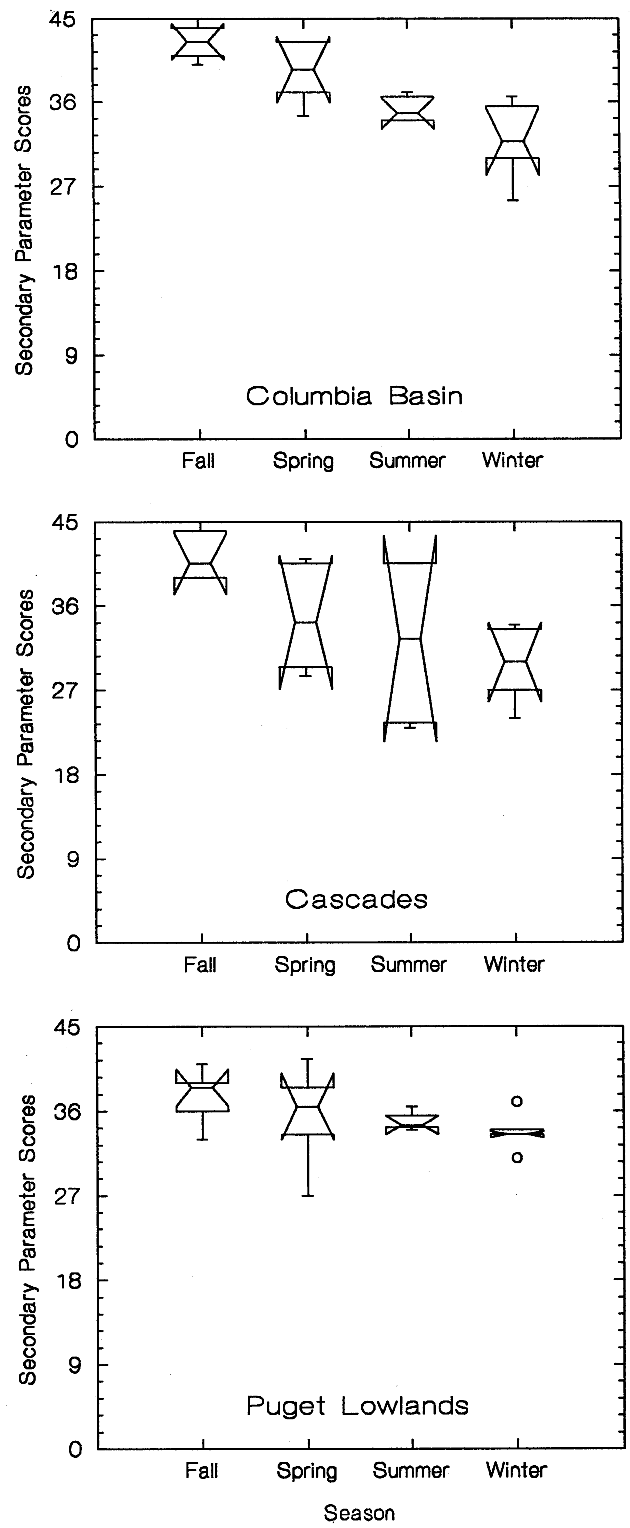

Figure 5. Secondary RBP habitat parameter scores for each season in three ecoregions (Puget Lowland, Cascade, Columbia Basin) (n=6 observations per season).

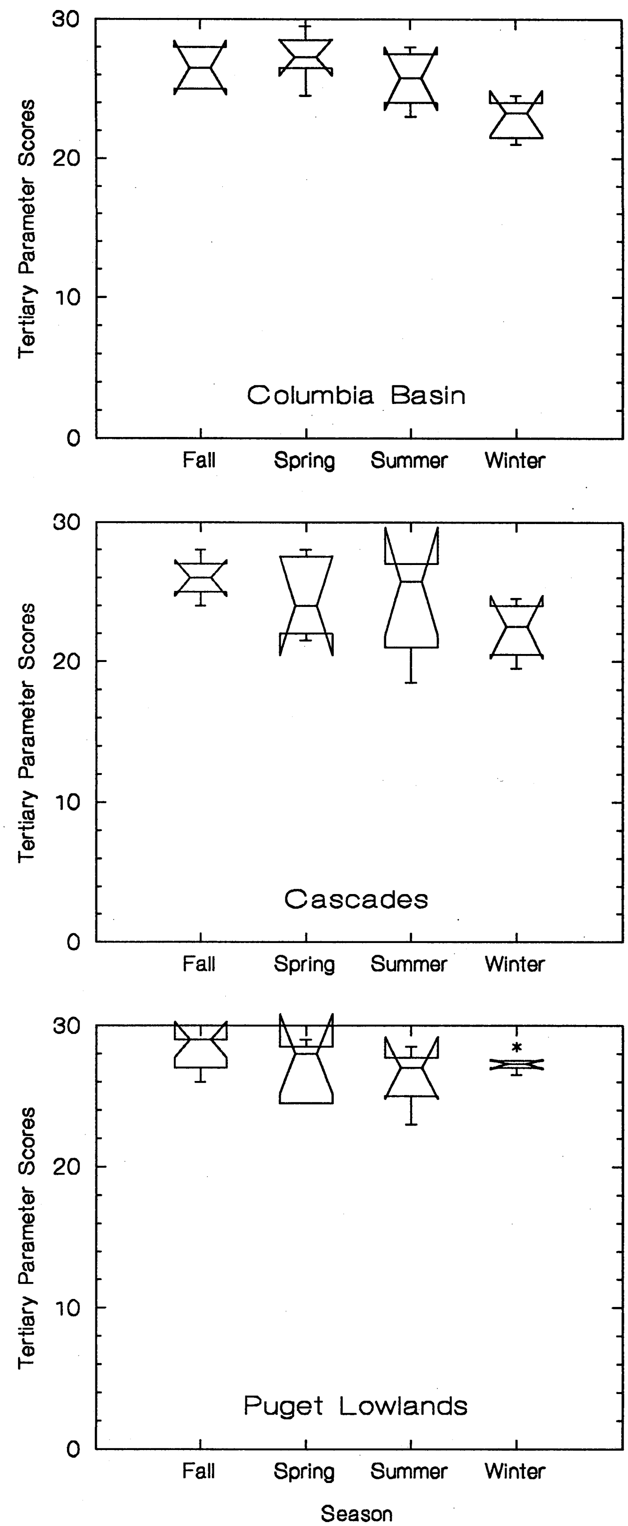

Figure 6. Tertiary RBP habitat parameter scores for each season in three ecoregions (Puget Lowland, Cascade, Columbia Basin) (n=6 observations per season).

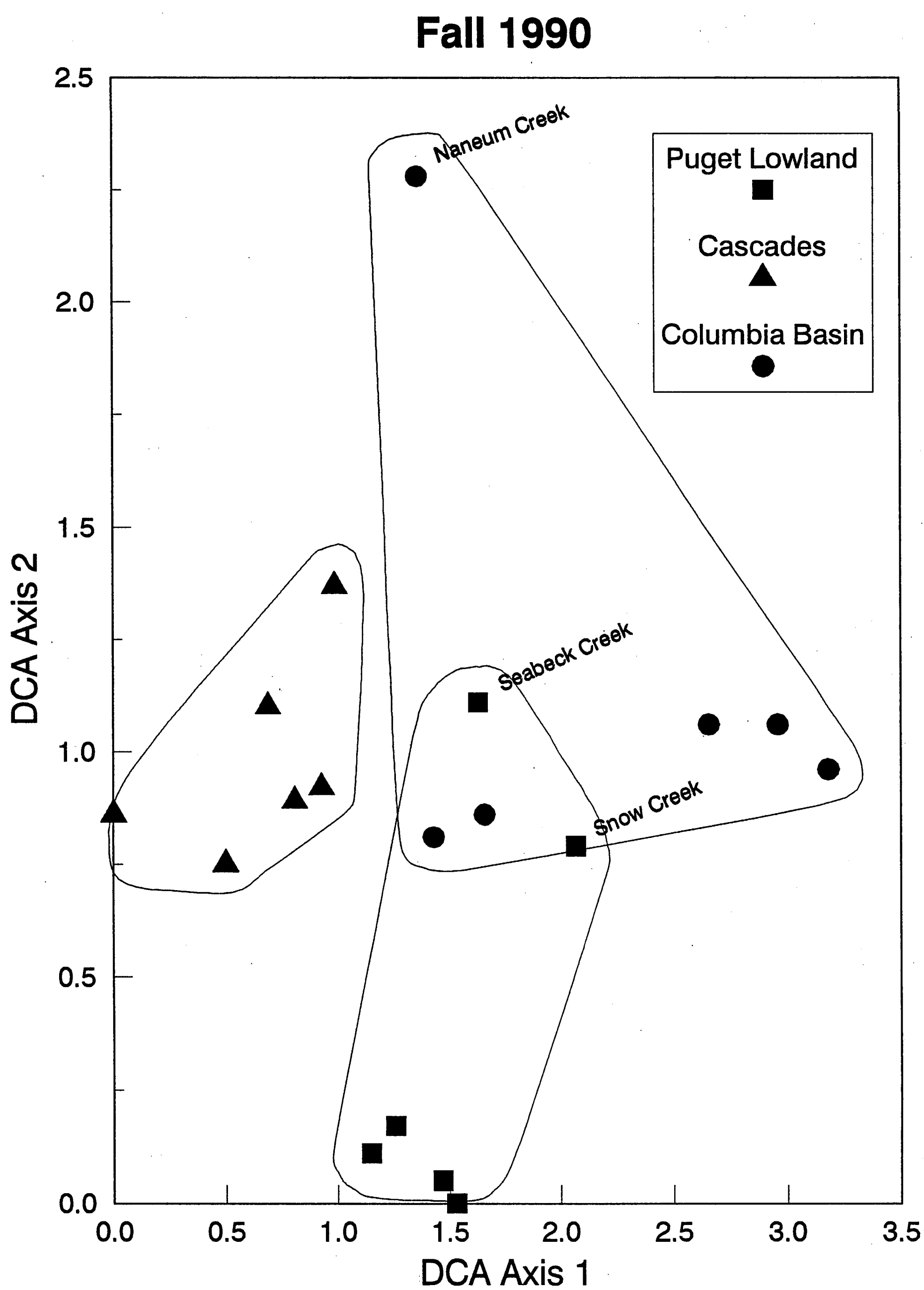

Figure 7. Detrended Correspondence Analysis of benthic macroinvertebrate communities during fall 1990.

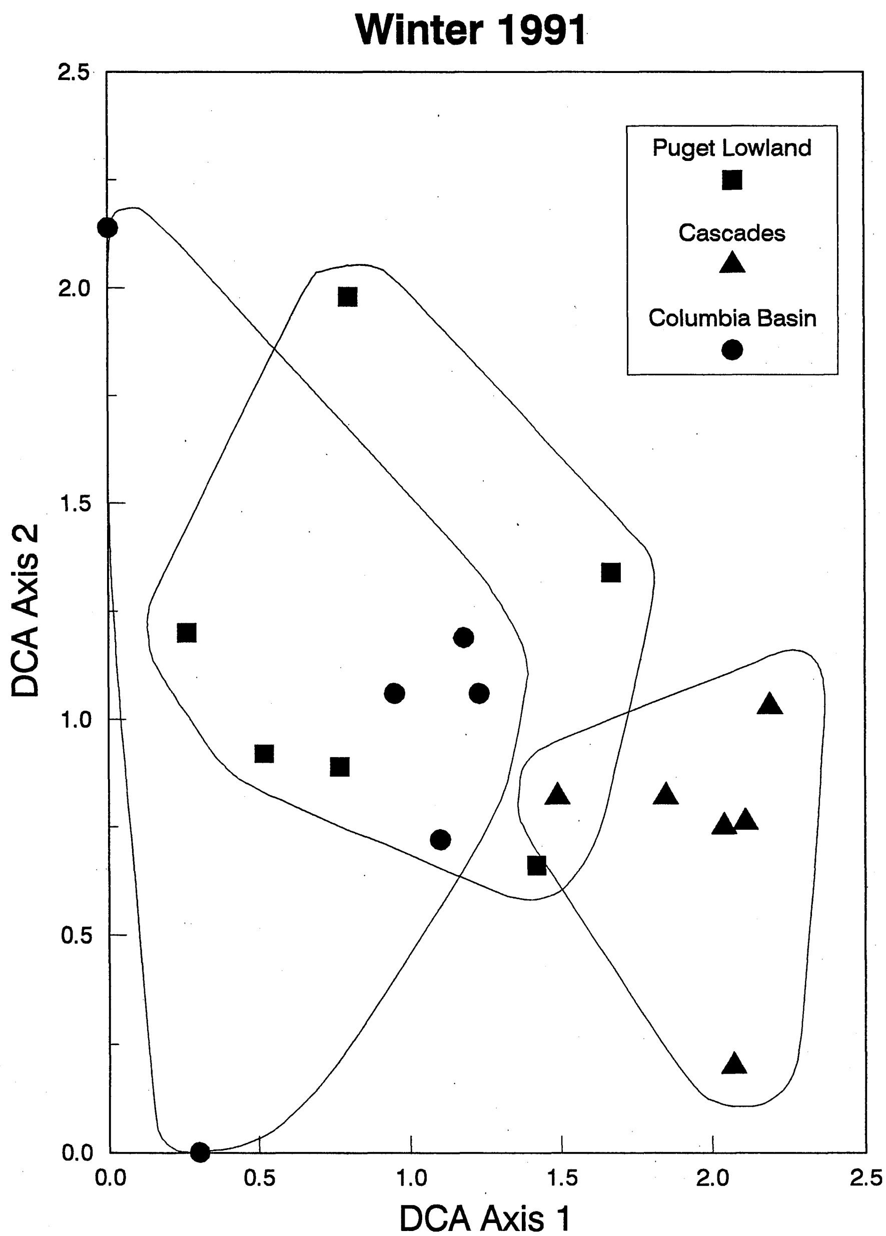

Figure 8. Detrended Correspondence Analysis of benthic macroinvertebrate communities during winter 1991.

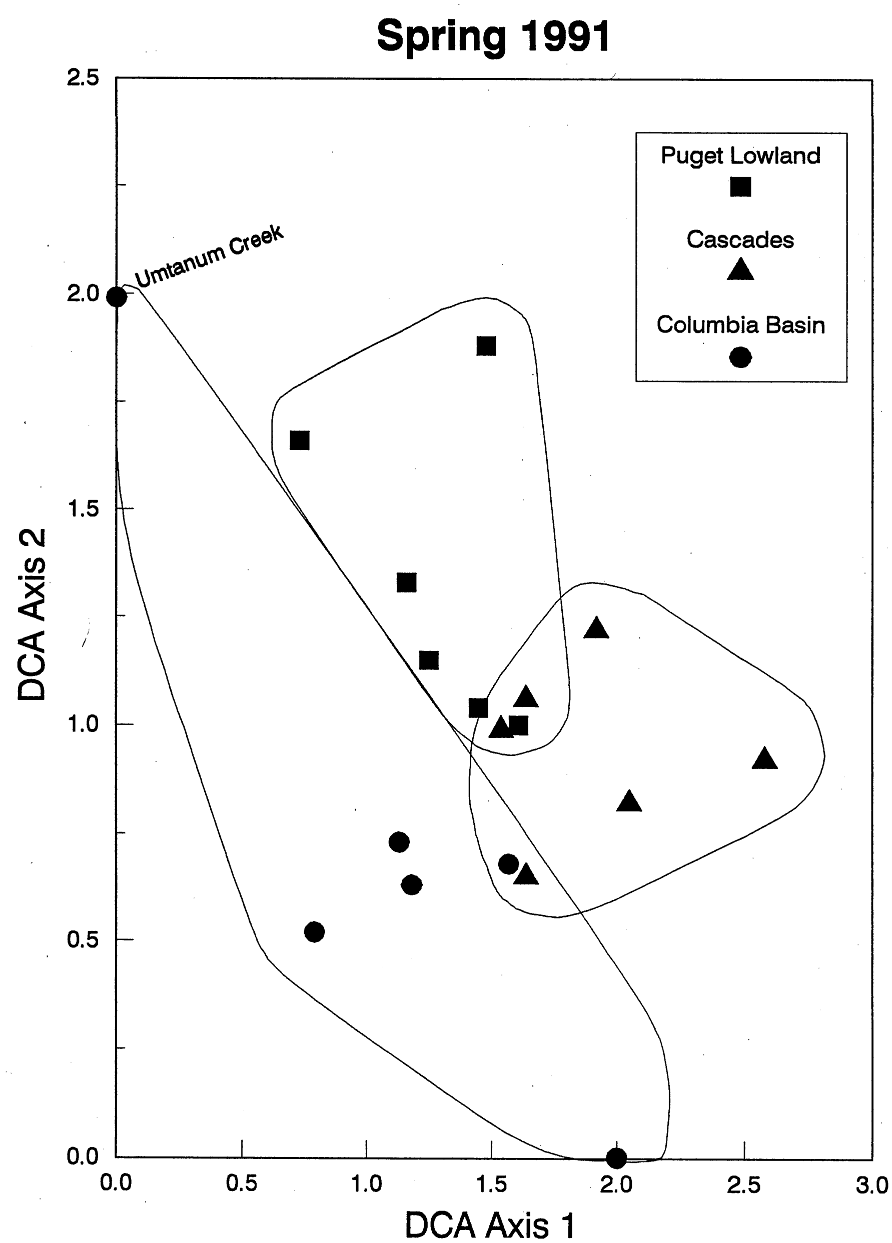

Figure 9. Detrended Correspondence Analysis of benthic macroinvertebrate communities during spring 1991.

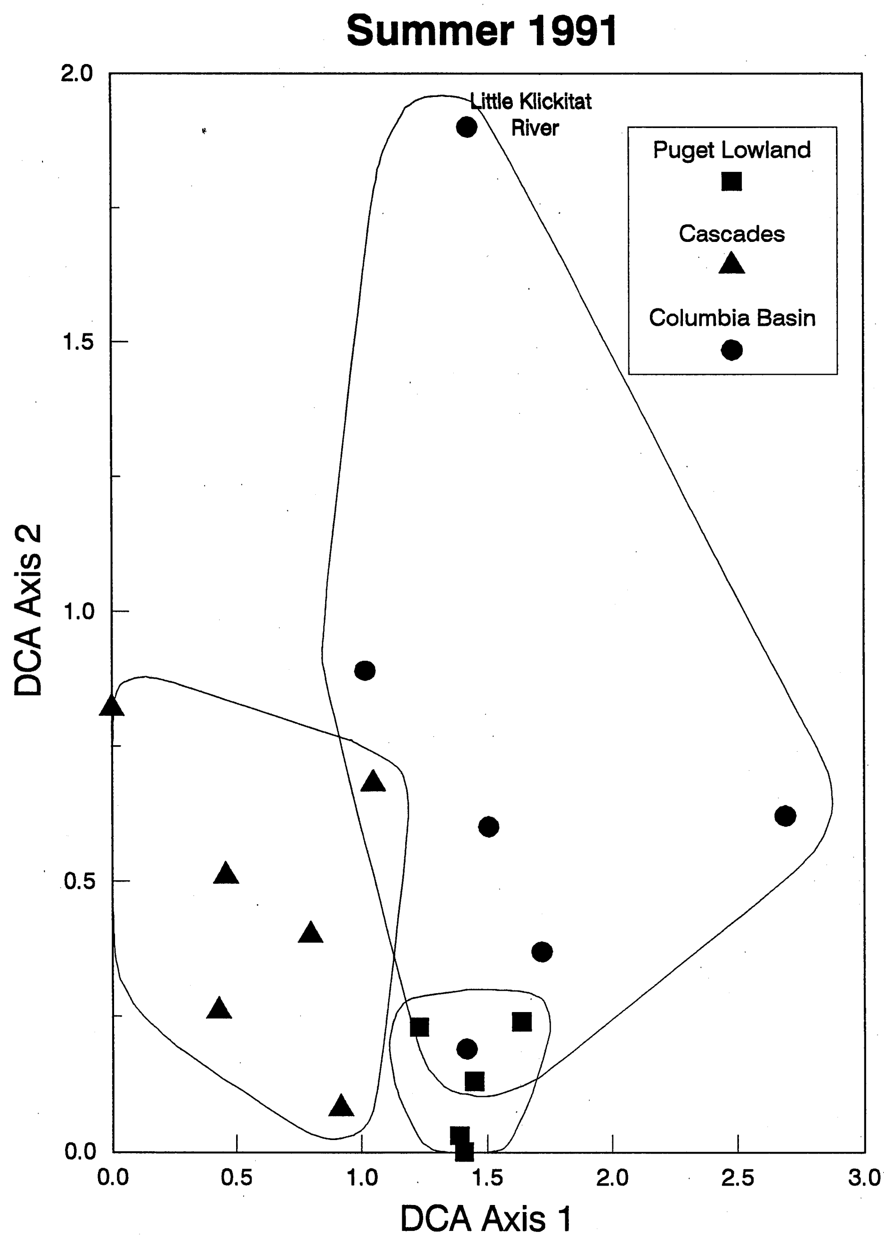

Figure 10. Detrended Correspondence Analysis of benthic macroinvertebrate communities during summer 1991.

in this description had 5% and greater representation of total relative abundance per site collection. These taxa were likely to be collected at a reference site and had a smaller likelihood of chance collection.

Ecoregion Indicator Assemblages

Taxa that were dominant and unique to the Puget Lowlands during fall 1990 are listed in Table 4. The assemblage includes a variety of Plecoptera (stoneflies), Trichoptera (caddisflies), and Diptera (midges, mosquitoes, blackflies). The functional description of Puget Lowlands macroinvertebrate indicator taxa during fall 1990 was that of a "shredder/collector-gatherer" community. The Columbia Basin indicator assemblage during fall 1990 included one mayfly, *Cinygmula #2*, and a host of other taxa, most of which were functionally classified as predators or scrapers. The Cascades ecoregion contained the highest representation of mayfly and stonefly taxa as unique indicators during fall 1990. Only a single dipteran taxon was characteristic of Cascades reference sites. Overall, the Cascades macroinvertebrate assemblage was functionally characterized as a "scraper/collector-gatherer" community.

The Puget Lowlands contained the smallest number of indicator taxa during the spring 1991 season (Table 5). As in fall 1990, mayfly indicator taxa were absent. This assemblage was functionally represented by all the primary and secondary macroinvertebrate consumers (shredders, scrapers, collector-filterers, collector-gatherers), but predators were most common. The Columbia Basin indicator assemblage in spring 1991 contained considerably more taxa than either the Puget Lowlands or Cascades. This ecoregion was characterized primarily by the "collector-gatherers" with good representation from other functional groups. The Cascades ecoregion, like the Puget Lowlands, produced an indicator assemblage dominated by predators.

A set of tables was prepared that describes the frequency of macroinvertebrate taxa occurrence both seasonally and spatially within an ecoregion (Appendix G). These tables identify "frequently present" and "occasionally present" taxa for each ecoregion by season. The utility of these taxonomic lists is to provide an indication of expected taxa in forested reference areas within each ecoregion. Appendix G also lists macroinvertebrates that appeared in all three ecoregions during the same season. These ubiquitous taxa represent tolerant or generalist benthic macroinvertebrates that may represent basic functional characterizations of all ecoregions surveyed in this project.

The functional classification of feeding strategies changed within each ecoregion as seasons progressed (Table 6). The most notable change in the Puget Lowlands reference condition occurred between fall 1990 and winter 1991 macroinvertebrate communities, when a community dominated by predators and collector-gatherers was joined by the other major functional groups (shredders, scrapers, and collector-filterers). Seasonal changes occurred in functional groups other than the predators and collector-gatherers which tended toward dominating the taxonomic composition of the Columbia Basin and Cascade ecoregions.

Biological Metrics: Rapid Bioassessment Protocols (RBP)

Single community measures such as diversity, total abundance, and species richness do not individually portray an accurate image of biological condition. However, combining a variety

Table 4. Unique taxa defined for each ecoregion: Puget Lowland, Columbia Basin, and Cascades (Fall 1990).

Puget Lowland

Plecoptera
- Capniidae
- *Doddsia*
- *Pteronarcella*
- *Nemoura*

Trichoptera
- *Hydatophylax*
- *Moselyana*
- *Micrasema*

Diptera
- Chironomidae (Pupa)
- *Glutops*
- Psychodidae (Pupa)
- *Ptychoptera*
- *Tipula*

Coleoptera
- *Cleptelmis*
- *Lara*

Amphipoda

Isopoda

Cascades

Ephemeroptera
- *Drunella doddsi*
- *Drunella spinifera*
- *Drunella coloradensis*
- *Eurylophella*

Plecoptera
- *Setvena*
- *Alloperla*
- *Doroneuria*
- *Haploperla*
- *Kathroperla*
- *Utaperla*
- *Podmosta*

Trichoptera
- *Ochrotrichia*
- *Ecclisomyia*
- *Glossosoma*
- *Neophylax*
- *Parapsyche*
- *Psychomyia*

Diptera
- *Pericoma*

Columbia Basin

Ephemeroptera
- *Cinygmula #2*

Plecoptera
- *Kogotus*
- *Hesperoperla*
- *Skwala*
- *Diura*

Trichoptera
- *Cheumatopsyche*
- *Helicopsyche*
- *Polycentropus*

Diptera
- *Dixa*
- *Tabanus*

Coleoptera
- *Stenelmis*
- *Psephenus*

Megaloptera
- *Sialis*

Acari
- Hydracarina

Oligochaeta
- Naididae
- *Rhynchelmis*

Odonata (Zygoptera)
- *Argia*

Gastropoda
- *Physa*

Table 5. Unique taxa defined for each ecoregion: Puget Lowland, Columbia Basin, and Cascades (Spring 1991).

Puget Lowland

- Plecoptera
  - *Kogotus*
- Trichoptera
  - *Ceratopsyche*
  - *Ecclisomyia*
- Diptera
  - *Chelifera*
  - *Pseudolimnophila*
- Odonata
  - Anisoptera
- Gastropoda
  - *Juga*

Cascades

- Ephemeroptera
  - *Drunella coloradensis*
  - *Attenella*
- Plecoptera
  - *Skwala*
- Trichoptera
  - *Lepidostoma*
  - Limnephilidae (Pupa)
  - *Pedomoecus*
  - *Parapsyche*
- Diptera
  - *Atherix*
  - *Bibiocephala*
  - *Molophilus*
  - *Oreogeton*
- Lepidoptera
  - Pyralidae
- Acari
  - Hydracarina
- Turbellaria
  - Planariidae
- Nematoda

Columbia Basin

- Ephemeroptera
  - *Cinygmula #2*
  - *Ironodes*
- Plecoptera
  - *Cultus*
  - *Skwala*
  - *Amphinemura*
  - Capniidae
  - *Podmosta*
- Trichoptera
  - *Amiocentrus*
  - *Cheumatopsyche*
  - *Moselyana*
  - *Arctopsyche*
  - *Neophylax*
- Diptera
  - *Clinocera*
  - *Pericoma*
  - Dixidae
- Coleoptera
  - *Lara*
  - *Psephenus*
  - *Optioservus* (Adult)
  - *Heterlimnius*
  - *Heterlimnius* (Adult)
- Megaloptera
  - *Sialis*
- Oligochaeta
  - Naididae

Table 6. Macroinvertebrate community characterization using the trophic descriptions for frequently occurring taxa in each ecoregion.

Trophic Function	Puget Lowland	Columbia Basin (no. taxa/ecoregion)	Cascades
Fall 1990			
Predators	8	8	7
Shredders	0	2	0
Scrapers	0	3	4
Collector-filterers	1	3	2
Collector-gatherers	4	7	4
Piercers	0	0	0
Winter 1991			
Predators	9	9	5
Shredders	2	4	3
Scrapers	4	1	3
Collector-filterers	3	4	2
Collector-gatherers	6	8	4
Piercers	0	0	0
Spring 1991			
Predators	4	6	7
Shredders	1	2	4
Scrapers	2	5	7
Collector-filterers	2	5	4
Collector-gatherers	3	10	8
Piercers	0	0	0
Summer 1991			
Predators	7	8	10
Shredders	2	8	1
Scrapers	2	6	3
Collector-filterers	1	2	3
Collector-gatherers	4	10	5
Piercers	0	0	06

of biological metrics or "biometrics" enables a more comprehensive evaluation of biological data sets because ineffectiveness of one biometric may be supplemented by more sensitive information in another. The biometrics used in this project are described in Appendix C. Each of the metrics describes an ecological aspect of the macroinvertebrate community collected from streams used in this survey. Seven of the eight original metrics listed in Plafkin *et al.* (1989) were used. The "Community Similarity Index" was not calculated because impaired site information was not available for comparison to the reference condition.

The biological metrics were calculated for each reference station in an ecoregion during each season. The biometric values were then displayed as box plots to compare the three ecoregions each season (Appendix H). All biometrics generally performed similarly throughout the seasons. Two biometrics were of questionable value on an ecoregional basis. The ratios of "Shredder Abundance/Total Number of Sample Organisms" were very low and thus would likely be of little value in detecting substantial changes in the reference communities. The ratios of "Total EPT Taxa Abundance/Chironomidae Abundance" produced acceptable distribution ranges for the Puget Lowlands and Columbia Basin ecoregions, but this metric was not well suited for the Cascades ecoregion during fall, winter, and spring due to the high variability. Seasonal variation of "Scraper Abundance/Collector-filterer Abundance" in the Cascade streams produced a wide distribution of values during winter 1991, but improved in summer 1991. Problems with the ratio biometrics occur when either of the numerator or denominator do not reflect regional consistency within macroinvertebrate assemblage structural or functional attributes.

Some of the RBP III biometrics delineated ecoregional conditions quite clearly. Spring 1991 macroinvertebrate conditions were best described by the "Hilsenhoff Biotic Index," "EPT Index," and "Taxa Richness." The EPT Index was effective in separating ecoregion condition during fall 1990, while the Hilsenhoff Biotic Index was the only biometric that differentiated ecoregion conditions during winter 1991. The RBP II biometrics displayed strongest ecoregion delineation with "Family Richness" and "% Contribution of Dominant Family" during summer 1991. Family Richness was also a useful biometric in delineating biological conditions in spring 1991 benthic macroinvertebrate surveys.

Comparison of RBP III and RBP II Biological Metric Results

Family-level RBP II and generic-level RBP III biometric results were compared to determine the potential gain or loss of biological information associated with evaluating data at two different taxonomic levels. Three biometrics were compared: Taxa Richness, EPT Index, and Percent Contribution by the Dominant Taxon.

Differences between RBP III and RBP II metrics were reviewed by using the medians produced in box plots for each metric (Appendices H and I). The Cascades ecoregion generally contained the greatest differences for taxa richness and the EPT Index values when RBP III and RBP II were compared. The Puget Lowlands maintained the smallest score differences between the RBP III and II comparisons. Fall 1990 and summer 1991 macroinvertebrate RBP score differences were largest overall.

Quality Assurance Results

Distribution of the coefficients of variation within each ecoregion was generally below 20 percent. This meant that taxa richness estimates in replicate samples within a site varied by less than 20 percent. The trend toward lower coefficients of variation in taxa richness was consistent for fall 1990 and spring 1991 benthic macroinvertebrate samples (Figures 11 and 12, respectively). The root mean square of the coefficients of variation in each ecoregion was between 10 and 20 percent for the Cascades and Columbia Basin during both seasons. The ecoregional sampling precision estimate for the Puget Lowlands was higher for spring 1991 macroinvertebrate samples than the fall season. The same streams from each ecoregion had the highest coefficients of variation for fall 1990 and spring 1991. The outliers had a tendency to increase the regional replicate sampling precision estimate.

Principal Components Analysis (PCA) of Surface Water Parameters

Surface Water Parameter Associations

Principal components analysis was used for examining surface water quality and quantity data from two perspectives. First, parameter associations were defined by examining the spatial correlations displayed in Figure 13. Principal component 1 explained 43.6 percent of the data set variance and principal component 2 explained an additional 23.4 percent of the variance. A point of perspective was defined for this two-dimensional analysis of surface water parameters which shall be termed the "origin." The origin from which lines are drawn to each parameter indicates that nutrients and other chemical parameters are separated to the right on principal component 1. Left of the origin lies discharge, and to a lesser degree, dissolved oxygen and percent oxygen saturation. Relative position of the chemical/physical parameters to the origin indicates the nature of relationship between one or groups of parameters (direct or inverse relationship). Each of the surface water quality parameters were further examined by relating the parameter medians defined in notched box plots (Appendix J) to the ecoregion(s) that demonstrated significantly higher median estimates. Parameters to the right of the origin on principal component 1 had significantly higher medians in the Puget Lowland and Columbia Basin ecoregions. Median discharge was located to the left of the origin on principal component 1 and was significantly higher in the Cascades ecoregion streams.

An overall examination of surface water parameter separation revealed Puget Lowlands and Columbia Basin (valley/plains) ecoregion separation from the Cascades (mountains). Dissolved oxygen and percent oxygen saturation were similar among all three ecoregions and were spatially separated from the other two groups of parameters. Additionally, discharge seemed to be inversely related to the nutrients and most of the chemical parameters while water temperature was inversely related to dissolved oxygen concentrations. Explanation of the total variance for each principal component is defined by the chemical/physical parameter covariances. A list of these parameters and corresponding covariances (or eigenvectors) is displayed in Table 7. The eigenvectors are further grouped by similar loading values for each component and may, in part, discriminate logical regional surface water patterns.

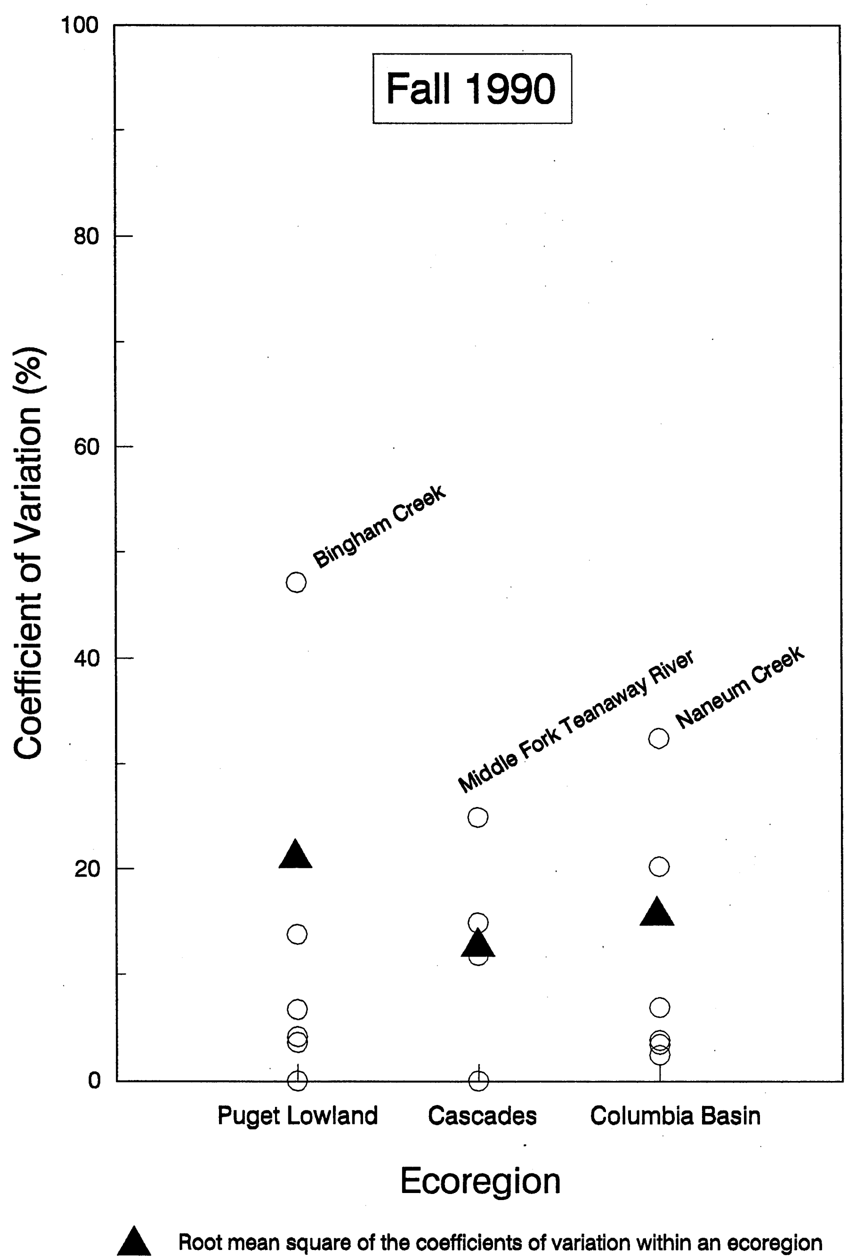

Figure 11. Distributions for coefficient of variation at each reference site within an ecoregion using total number of taxa from replicate macroinvertebrate samples (fall 1990).

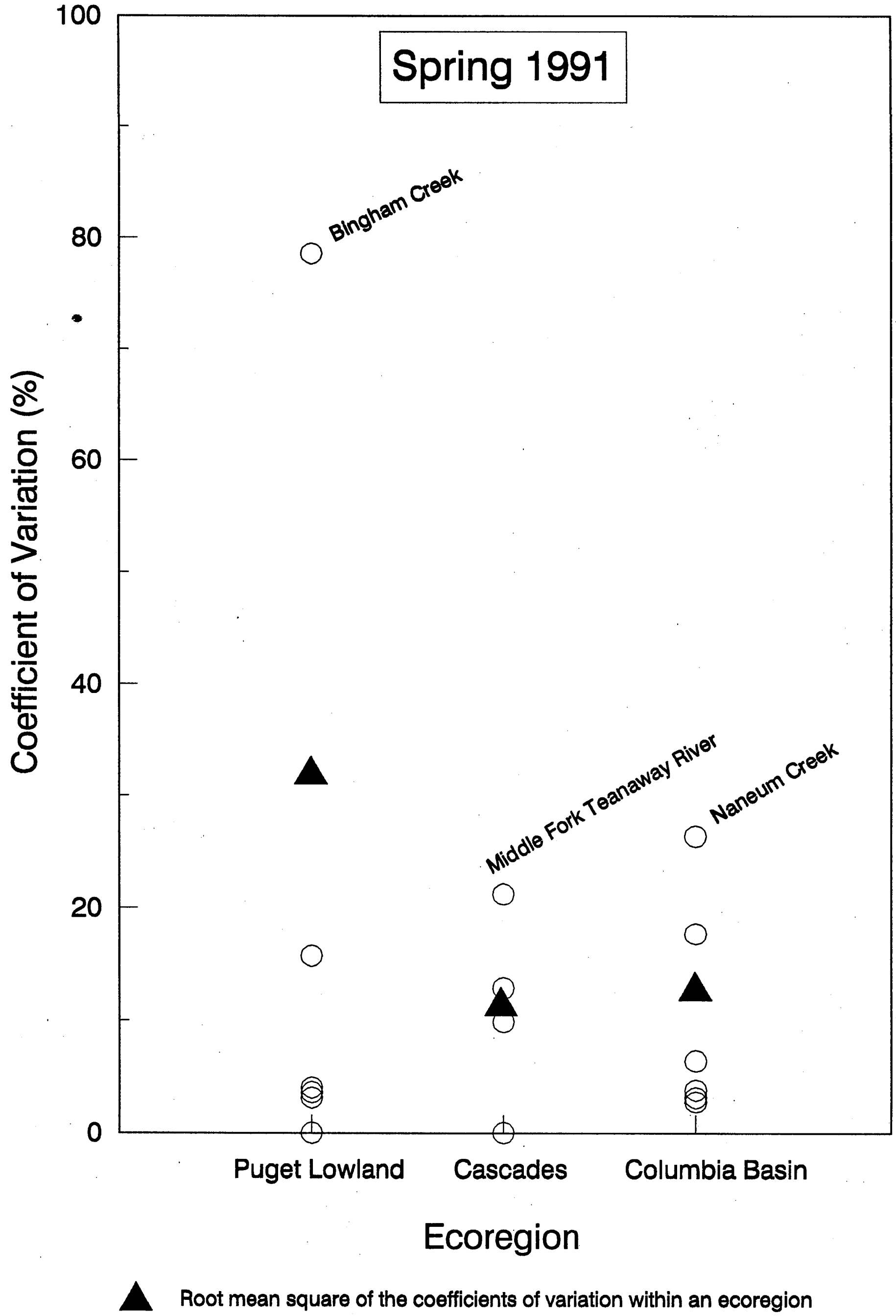

Figure 12. Distributions for coefficient of variation at each reference site within an ecoregion using total number of taxa from replicate macroinvertebrate samples (spring 1991).

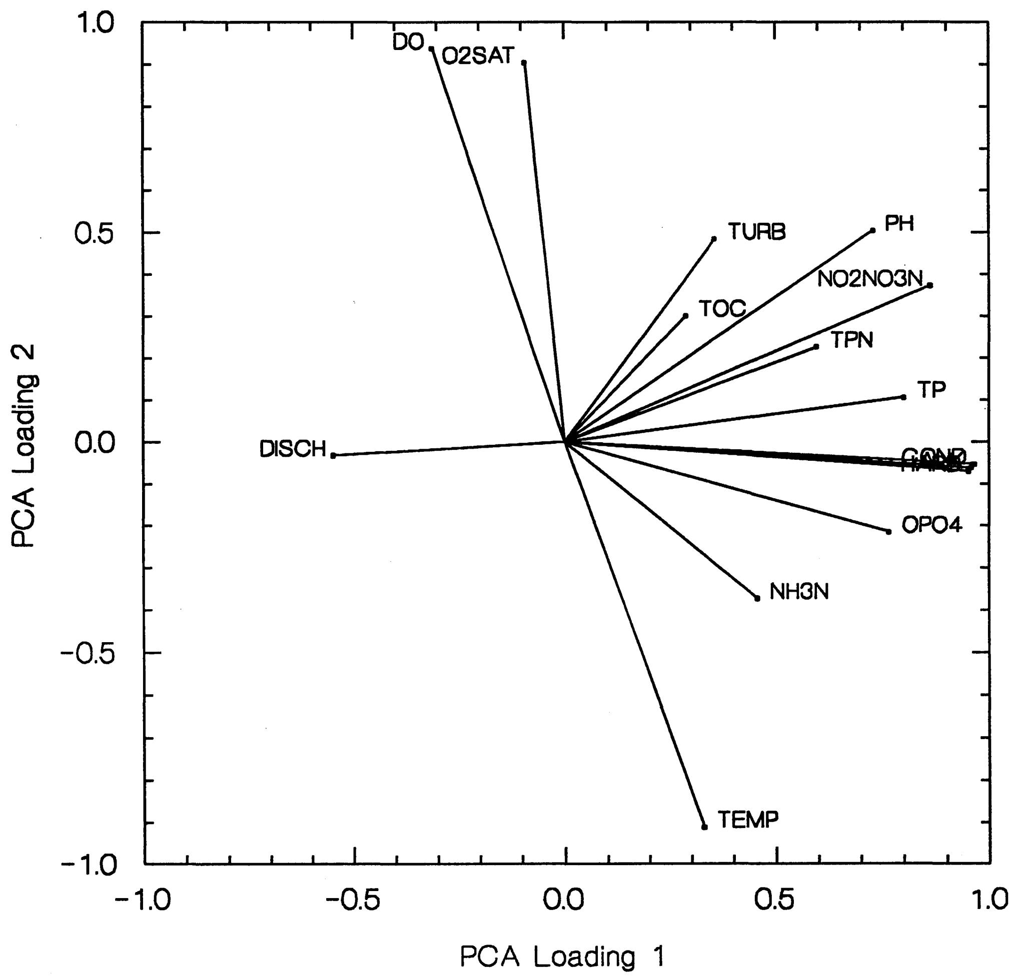

Figure 13. Principal components analysis parameter associations.

Table 7. Principal component analysis loadings for the surface water quality parameters measured at ecoregional reference sites.

Parameters	Loadings	
	Component 1	Component 2
Conductivity	0.965	-0.054
Alkalinity	0.957	-0.062
Hardness	0.952	-0.070
Nitrate+Nitrite-nitrogen	0.863	0.373
Total Phosphorus	0.801	0.105
Ortho-Phosphate	0.766	-0.215
pH	0.729	0.505
Total Persulfate Nitrogen	0.596	0.226
Ammonia-nitrogen	0.456	-0.372
Turbidity	0.357	0.485
Temperature	0.328	-0.912
Total Organic Carbon	0.287	0.301
Percent Oxygen Saturation	-0.091	0.904
Dissolved Oxygen	-0.311	0.937
Discharge	-0.548	-0.032

Note: The loadings are equivalent to the covariances which estimates each parameter's contribution to the explanation of the principal component variance.

PCA Ecoregion-by-Season Relationships

Principal components analysis was also used to generate a spatial plot of how ecoregion water quality varies by season (Figure 14). In this analysis, the Cascades ecoregion completely separated from the other two ecoregions. This pattern reflects the surface water quality parameter associations presented above. Seasonal water quality information partitioning distinguished the valley/plains ecoregions (Puget Lowlands and Columbia Basin) from the mountains (Cascades). Close association of the reference sites in valleys/plains ecoregions results from minimal differences in water quality measurements collected throughout the year.

Cluster Analysis Using the Ecoregion-by-Season Matrix

Closer confirmation of ecoregion-by-season relationships was demonstrated with cluster analysis using the average-linkage method and Euclidean distances (Figure 15). The dendrogram produced from the cluster analysis confirmed that seasonal water quality conditions were more characteristic of a particular ecoregion. Specific seasonal associations within each ecoregion were also defined by cluster analysis. For instance, fall and winter surface water parameters were more similar to each other than to other seasons or other ecoregions in both the Cascades and Puget Lowlands.

DISCUSSION

Seasonal Habitat Scores

Evaluations of Puget Lowland reference sites were completed before the fall 1990 flood events began. There typically were higher surface water discharge rates at Puget Lowland stations following the summer due to increased rainfall frequency. Fall 1990 habitat conditions were improved with increased flow by creating additional useable instream habitat (Figure 3). The hydrologic year in Cascade streams culminated in an extreme low discharge period during winter 1991 while precipitation was bound in the form of snowpack. The low winter 1991 habitat condition in the Cascades may also have been influenced by ice formation and general loss of useable instream habitat (Figure 3). The best Cascades ecoregion habitat score occurred during fall 1990 when sufficient water discharge and existing riparian and bank structure were major influences. The Columbia Basin ecoregion had similar seasonal patterns as those occurring within the Cascades. Riparian and bank structure habitat scores increased in the Columbia Basin during fall 1990 due to higher discharge rates that provided additional habitat availability (Figure 3).

Benthic Macroinvertebrate Patterns

Detrended Correspondence Analysis (DCA)

Detrended correspondence analysis identified fall 1990, spring 1991, and summer 1991 macroinvertebrate communities as more distinct within each ecoregion than were the winter 1991

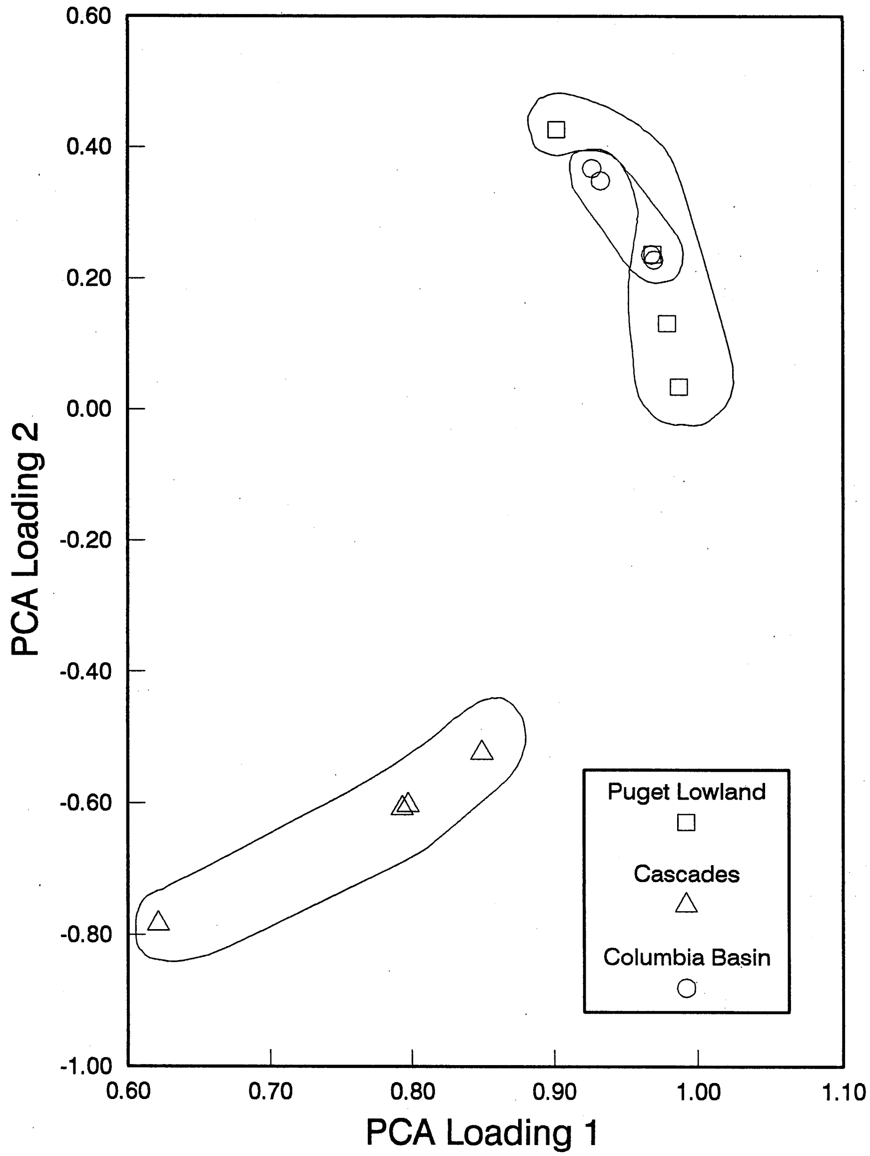

Figure 14. Principal components analysis of ecoregions by seasonal surface water quality information (fall, winter, spring, summer).

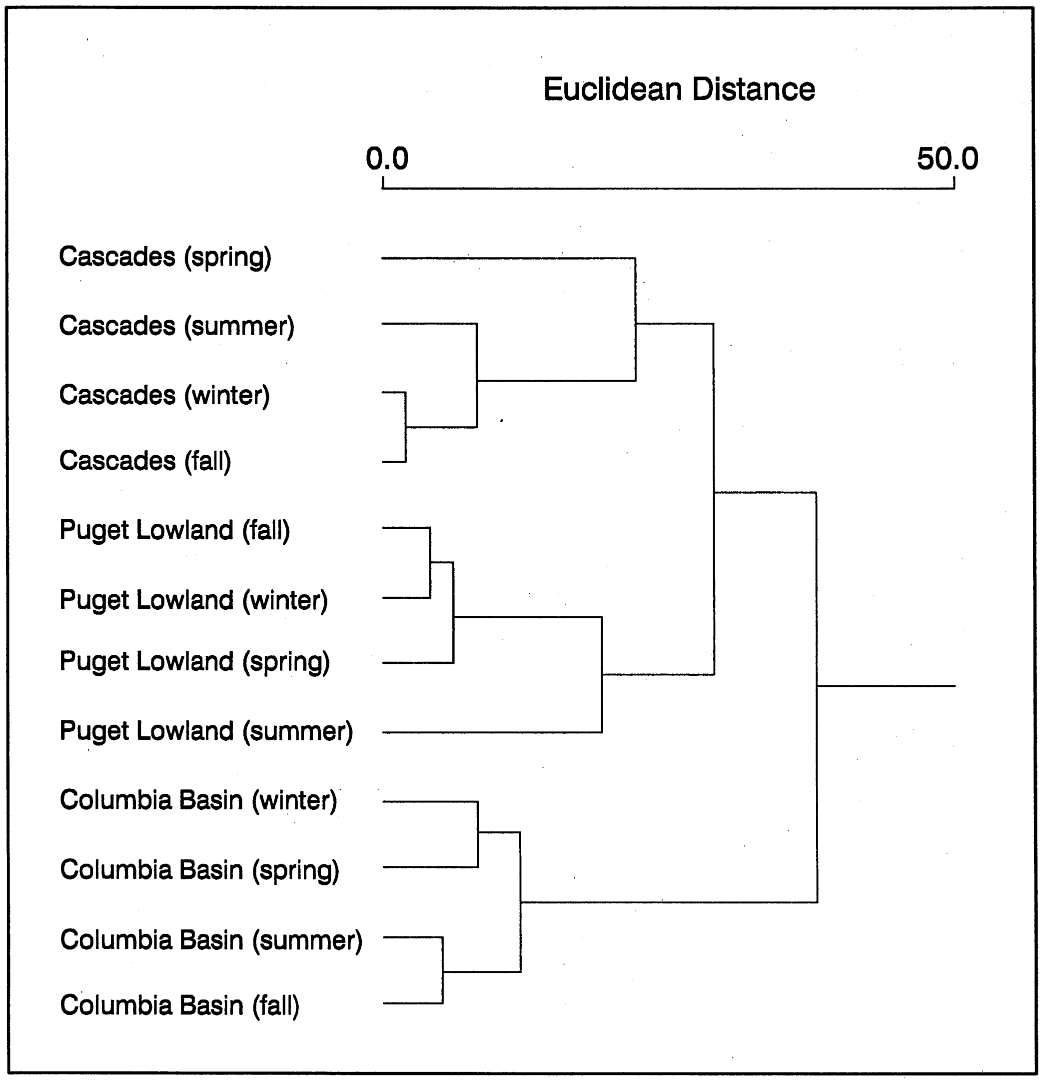

Figure 15. Cluster analysis (average-linkage) of seasonal ecoregion surface water quality parameters.

collections (Figures 7, 9, and 10). Hynes (1970) summarized the determinants of macroinvertebrate community structure and function that best explain regional distinctions as: current speed, temperature, substratum, and dissolved substances. Water temperature is known to be influenced by seasonality and altitude.

The distinction of the fall 1990 macroinvertebrate communities collected in each ecoregion may have been a result of good instream habitat conditions and the maturation of larval instars or winter emerging insects. Natural disturbance frequencies were also low in each ecoregion during fall 1990. Moderate surface water temperatures and favorable current velocities may have contributed to the distinction of each ecoregion's reference streams by allowing efficient macroinvertebrate use of instream resources.

The return of more stable physical habitat conditions in each ecoregion during spring 1991 marked another season where macroinvertebrate communities were most distinct. Numerous populations of macroinvertebrates appear from development of diapausing eggs during spring conditions. The development of diapausing eggs is strongly influenced by increasing surface water temperatures (Sweeney, 1984).

Winter 1991 reference conditions were marked by frequent natural disturbances in the Puget Lowlands (flooding and erosion), Columbia Basin (ice formation, peak flows), and Cascades (ice formation, snow load, torrential flows). Although some macroinvertebrates can withstand environmental extremes, life-cycle strategies such as egg diapause and hyporheic residence may occur during highly variable seasons (Vogl, 1980; Butler, 1984; Williams, 1984).

The summer season is typically a period of mass emergence of many species (Williams, 1984). Summer stream conditions within the Columbia Basin may become temperature limiting, which would promote life-cycle progression to emergence. The hyporheos also provides temporary refuge of cooler water temperatures for macroinvertebrate habitation (Butler, 1984; McElravy and Resh, 1991). Summer months may be appropriate for sampling when conditions are favorable. The regionally distinct summer macroinvertebrate assemblages determined by DCA demonstrate good biological characterization of ecoregions.

Two-Way Indicator Species Analysis: Indicator Assemblages

An important aspect of the distinct regional assemblages are their functional characteristics. The fall 1990 indicator macroinvertebrates collected from Puget Lowland reference sites were represented by shredders and collector-gatherers (Table 4). Energy input and available detrital material may have been primarily from allochthonous input; that is, organic material contributed from outside of the stream. Allochthonous input from riparian vegetation may also have encouraged the presence of certain feeding groups such as the shredders and collector-gatherers (Ward, 1984). The presence of many shredder and collector-gatherer taxa would infer that coarse particulate organic matter (CPOM) was abundant in Puget Lowland streams and also that microbial decay of freshly input material was efficient. Leaf processing by shredders produces fine particulate organic matter (FPOM) that is readily used by collector taxa. Microbial activity, including rates of processing, is known to be dependent on water temperature and the type of allochthonous material entering the stream (Cairns *et al.*, 1972; Cummins 1974; Lamberti and Moore, 1984; Merritt *et al.*, 1984; Chergui and Pattee, 1990; Quinn and Hickey, 1990). The Columbia Basin and Cascades macroinvertebrate assemblages were also represented by many gatherer taxa during fall 1990, but were additionally characterized by "scraper" taxa (Table 4). These two ecoregions maintained three conditions that would favor a scraper/collector-gatherer community: 1) cooling water temperatures, 2) stable current regime, and 3) adequate photoperiod. Favorable flows and instream detrital retention encourage algal proliferation and an accumulated food base, respectively (Minshall et al., 1983; McCormick and Stevenson, 1991; Richardson and Neill, 1991).

The Puget Lowland reference streams experienced functional feeding group diversification during spring 1991 (Table 5), probably because of an increased variety in food resources and stabilizing instream habitat conditions. The collector-gatherers and predators were present during all seasons in the three ecoregions (Table 6). Alterations in other functional feeding groups were examined to provide evidence of seasonal change. Spring 1991 macroinvertebrate communities in the Columbia Basin and Cascades ecoregions progressed to shredder/collector-gatherer communities. Richardson (1991) demonstrated that increases in shredder abundance and biomass resulted from increased availability of food. Forest leaf litter may have been transported via snowmelt and gusting wind events to reference streams in these two ecoregions (Merritt *et al.*, 1984). Shredders were most active in the Cascades during spring 1991 while Columbia Basin shredders reached peak abundance in the summer. Also, adequate microbial processing of instream leaf litter may be seasonally delayed in these ecoregions until spring months. Decomposition rates of instream leaf litter vary depending on leaf type and may not be substantially decreased by a low water temperature. Cascade streams contained a larger number of predators than Columbia Basin streams. High substrate heterogeneity in Cascade streams may provide a variety of habitable substrate surfaces for prey items which sustain the diverse number of predator taxa (Peckarsky, 1984). The Baetidae were a ubiquitous taxon in Cascade region streams during spring 1991 and characteristically exhibit rapid generation succession (Anderson and Wallace, 1984). Predator populations may also have been sustained by this large prey population comprised by the baetid mayflies during the spring.

The taxa assemblages listed in Appendix G have been delineated as either frequently present or occasionally present in an ecoregion during each season. These lists were compiled to indicate the potential taxa that would likely be distributed within each ecoregion. Physical/chemical tolerances as well as individual pollution tolerances may largely account for those benthic macroinvertebrate community patterns (Beck, 1977; Harris and Lawrence, 1978; Hubbard and Peters, 1978; Surdick and Gaufin, 1978; Klemm *et al.*, 1990).

Biological Metrics: Rapid Bioassessment Protocols (RBP)

The biometric "shredder abundance/total number of sample organisms" was found to be exceptionally low in all ecoregions over all seasons (Appendix H). Shredder abundance should not be confused with the greater shredder taxa richness found under fall 1990 conditions in Puget

Lowland streams. Difficulty in shredder collection was the primary reason for this metric's poor performance. Collection timing is important because shredders appear with litter drop and population increases occur following leaf litter conditioning (Cummins *et al.*, 1989). Loss of smaller shredder taxa during sample collection may be a function of the sampler net mesh size. Other surveys in Washington have similarly found shredder taxa and abundance to be represented in smaller quantities than other functional feeding groups (Munn *et al.*, 1990). A seasonally-focused, multihabitat sampling approach that includes detrital deposition zones may be needed to adequately characterize the shredder community.

The "total EPT taxa abundance/Chironomidae abundance" metric was not effective in the Cascades ecoregion. Variability for the metric was generally high during all seasons except summer 1991, when a reduction of EPT taxa abundance from adult emergence minimized variability. This metric should only be used in the Cascade ecoregion streams when depositional areas are included in the sampling approach. Chironomid taxa are not typically abundant in higher gradient, non-depositional stream areas (Hynes, 1970).

The "scraper abundance/collector-filterer abundance" metric indicated a wide distribution of values from Cascade reference streams during winter 1991. Improvement in metric performance in summer 1991 may have been due to increased abundance of the collector-filterer community. Increased particulate transport following late-spring/early-summer runoff in Cascade streams can provide the food base to sustain collector-filterer abundance (Minshall *et al.*, 1983). The scraper/collector-filterer abundance and EPT/Chironomidae abundance metrics were not able to discern mountain streams from the valleys/plains streams in the three ecoregions. Barbour *et al.* (1992) demonstrated this same difficulty with these two metrics.

Rapid Bioassessment Protocols: Comparison of RBP II and RBP III

Taxa richness and EPT index medians in the Cascades ecoregion had the largest RBP II and RBP III comparison differences. This occurred because Cascade streams have smaller taxonomic variety at the family level, but numerous genera within each family. Information loss in moving from a generic to familial level ultimately lowers sensitivity of these two metrics in impact assessment.

"Percent Contribution of Dominant Taxa" metric values were larger for RBP II than RBP III. The reason for this is that with RBP II, multiple genera were represented under one Family while RBP III retained information from a single Genus.

Biological screening level activities for detecting heavily impacted stream conditions can be addressed through use of RBP II. Resolution between expected biological conditions and impaired biological condition is probably detectable when using Family levels of taxonomic identification. Detection of subtle anthropogenic stream disturbances must use the RBP III methodology where generic taxonomic identification would provide adequate resolution of biological impairment.

Sampling Quality Assurance

Low site-specific coefficients of variation for taxa richness in both fall 1990 and spring 1991 indicated that single samples from a site may be adequate to characterize conditions. In terms of efficiency, single samples collected at each site would allow additional sites to be monitored for benthic macroinvertebrates. If the purpose for conducting bioassessments is to define general regional conditions, then single samples at each site following the compositing methodology outlined in this project would be appropriate. Near-field intensive surveys require replicate sampling, regardless, in order to evaluate ecological conditions with a much higher level of sampling precision. Intensive surveys may be performed in response to a compliance regulatory action and are, therefore, scrutinized more carefully.

One stream in each ecoregion is identified in Figures 11 and 12 as an extreme outlier beyond the root mean square of the coefficients of variation. The same streams were outliers in both the fall 1990 and spring 1991 samples (Puget Lowlands-Bingham Creek; Cascades-Middle Fork Teanaway River; Columbia Basin-Naneum Creek). Precision in replicate sampling was not satisfactory at these sites for the following possible reasons: 1) small number of taxa present in the stream, 2) a high frequency of natural stream disturbance, 3) an existing impact that was not immediately evident, or 4) a combination of the prior conditions. Sampling precision may have future application in further reference site selection procedures. Highly variable precision estimates within a candidate site may indicate a potential problem that warrants further investigation.

Surface Water Patterns

Surface Water Parameter Associations

The Columbia Basin surface waters were characterized by higher concentrations in hardness, alkalinity, ortho-phosphate, total phosphorus and high conductivities. Columbia Basin soils, primarily loess, may release substantial portions of adsorbed phosphorus nutrients in this ecoregion's surface waters (Omernik and Gallant, 1986). The origin of phosphorus associated with the soil would have been derived from organic decay, primarily grasses or grazing activity in previous decades. Loess is comprised of clay and various calcareous components which may have been historically deposited by overlying water or transported by wind in arid regions (Loomis, 1948; Tweney and Hughes, 1965). A naturally occurring hard pan layer of secondary carbonates may contribute to the high alkalinity and hardness concentrations in Columbia Basin surface waters. Deep percolation water that generally supplies a high percentage of base flow in surface waters during low flow seasons may transport ionic constituents derived from the hard pan soil layer to surface waters (Keller, pers. comm., 1992). Low stream discharges were observed during the summer season where deep percolation groundwater formed a larger percentage of the base flow, as well, contributed in greater percentage of flow to the alkalinity and hardness concentrations.

Puget Lowlands streams generally maintained higher nitrate+nitrite-nitrogen concentrations than streams in the other two ecoregions, perhaps due to input and processing of substantial quantities of leaf litter. There were many higher nitrate+nitrite-nitrogen concentration outliers in Columbia Basin stream observations (Appendix J4). These outliers were recorded from Spring Creek (Appendix F). Some eastern Columbia Basin streams, particularly those associated with palouse soils, carry much of the current and historic nonpoint source impacts due to agricultural practices. These high nitrate+nitrite-nitrogen concentrations perhaps reflect these land use impacts and may be an indelible effect on eastern Columbia Basin streams. The most distinct stream characteristic in the Cascades was discharge; a likely indicator of the increased precipitation, snowpack, and highly variable watershed sizes in this ecoregion.

Prevailing water quality conditions within each of the ecoregions can be related to observed biological conditions. The valley/plains regions contain streams that typically act as catchments from piedmont and mountainous areas. Accumulation of nutrients in lowland streams may be derived from higher elevation sources as well from regional land use impacts. Higher nutrient concentrations provide conditions under which periphyton communities flourish (Hynes, 1970). The potential for increased algal community development in Columbia Basin streams may, in part, explain the presence of season specific indicator taxa that belong to the scraper functional feeding group.

Prevalence of total organic carbon (TOC) in surface waters may be used as an estimate for the presence of consumable detrital material. An indirect relationship between presence of macroinvertebrate collectors and TOC concentrations may be defined if increase in macroinvertebrate collector presence is directly proportionate to TOC increases. TOC is a measure of organic particulates larger than 450 micromillimeters which corresponds to the subclasses of particles UPOM (ultrafine particulate organic matter) and smaller quantities of FPOM (fine particulate organic matter) (Cummins, 1980; APHA, 1989). Macroinvertebrate collectors use both FPOM and UPOM where there is a tendency toward increased concentrations in downstream reaches. The benthic macroinvertebrate collector community also increases proportionately with increases in the small organic particle size classes. Either external organic allochthonous (from outside the stream) input or macroinvertebrate shredder processing will contribute to production of this particle size class (Vannote *et al.*, 1980; Merritt *et al.*, 1984; Wallace *et al.*, 1991). TOC in Cascade reference streams was probably less prevalent because increased flow generally moves organic particulates further downstream before it is processed to this particle size class and a useable form by the collectors functional group (Newbold *et al.*, 1981; Minshall *et al.*, 1983).

PCA Ecoregion-by-Season Relationships

The spatial PCA plot of ecoregions by season in Figure 14 reveals the separation of reference site conditions, into valleys and plains versus mountains which confirms the water quality parameter associations described above. A cluster analysis of these same ecoregion-by-season variables revealed complete separation of all seasonal water quality information by ecoregion (Figure 15). Within the clusters, both fall 1990 and winter 1991 surface water parameters for the Cascades and Puget Lowlands reference streams were most similar. Fall and winter conditions in the Puget Lowlands streams were frequently disturbed by flooding, while Cascades streams experienced much more stable flow conditions. A chart was created to better define the physical/chemical relationships among the three ecoregions on a seasonal basis (Figure 16). These relationships were important determinants of biological community composition in each ecoregion's streams.

CONCLUSIONS AND RECOMMENDATIONS

Habitat Information

1. The qualitative habitat information collected for this project was suitable in detecting seasonal differences. However, this same information may not maintain an adequate degree of sensitivity for detecting subtle instream impacts.

2. Quantitative habitat evaluation should occur on an occasional basis at each reference station for purposes of calibrating the qualitative assessment methodology.

Benthic Macroinvertebrate Information

1. The sampling and analysis methods used in this project were effective in producing biological data that were supported by water quality and habitat information. Sampler type, net mesh size, and sampling intensity are major determinants of the sampling efficiency in a benthic macroinvertebrate survey.

2. The ecoregion approach to defining reference sites produced a representative taxonomic list.

3. The most distinct seasons for benthic macroinvertebrate sampling were fall, spring, and summer. Early fall season sampling in the Puget Lowlands streams is recommended due to increasing flood frequencies when the wet season begins. Early spring sampling in the Cascades should be conducted prior to snowmelt (mid-May was suitable for sampling east side Cascade streams, while later March or April was suitable for west side Cascade streams). Timing of spring snowmelt will vary, therefore, sampling during this season should be determined by predicted climatological patterns for that year.

4. The Rapid Bioassessment Protocol biometrics that were inconsistent in performance were: 1) shredder abundance/total number of sample organisms, 2) total EPT taxa abundance/Chironomidae abundance, and 3) scraper abundance/collector-filterer abundance. The shredder/total sample abundance metric may be improved by either using a sampler type with a small net mesh size (250 microns) or by adopting a multihabitat sampling approach.

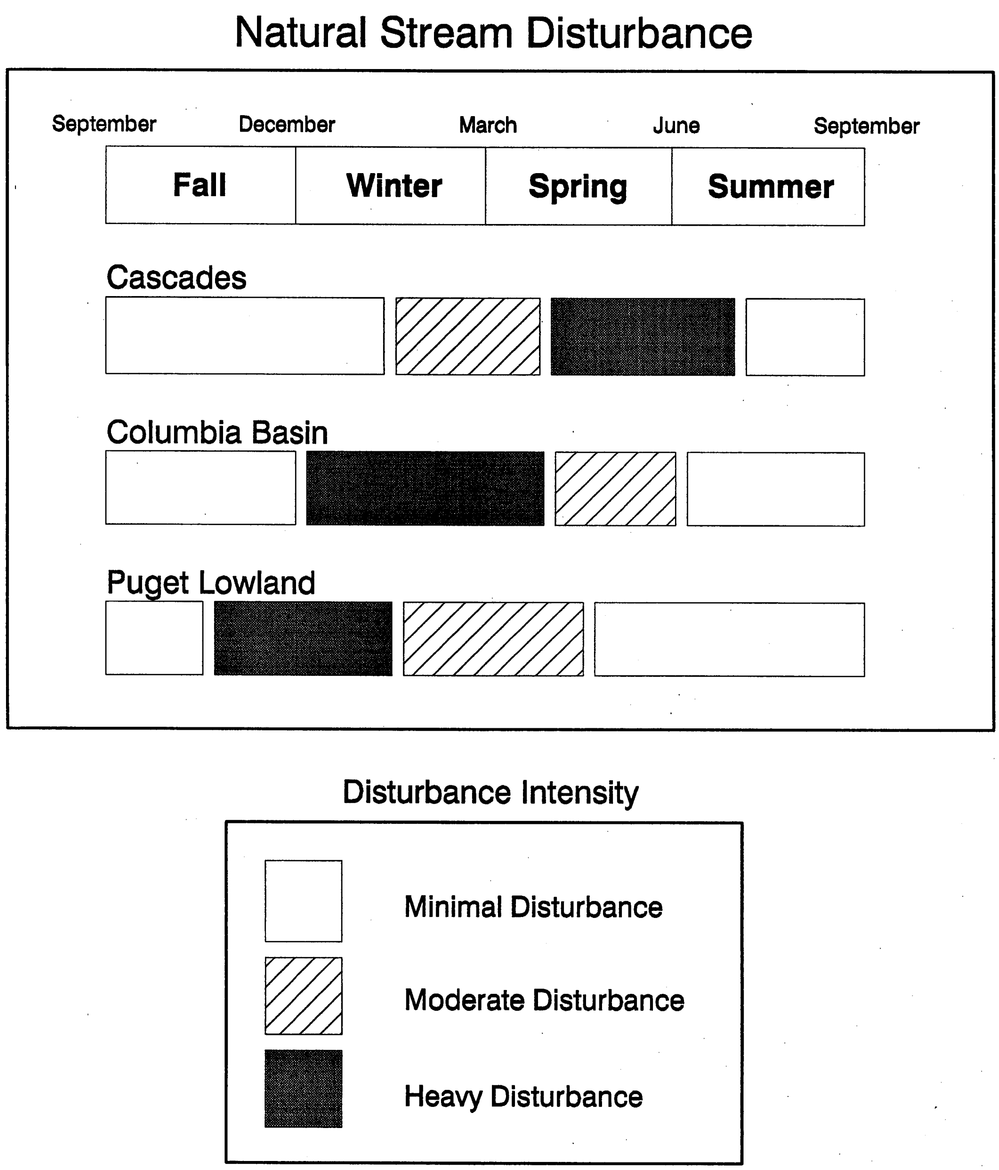

Figure 16. Natural stream disturbance intensity and seasonal timing in three ecoregions of Washington: Cascades, Columbia Basin, and Puget Lowlands.

5. RBP III biometrics that distinguished ecoregions were: Hilsenhoff Biotic Index, EPT Index, and Taxa Richness. All three of these biometrics distinguished ecoregion conditions during spring 1991, whereas only single biometrics differentiated the regional biological conditions in other seasons. Further modification and development of biometrics is required in order to determine ecoregion differences on a seasonal basis.

6. Site-specific and ecoregion-wide precision estimates for sample replicates indicate that single composite macroinvertebrate samples could be collected at a site. Coefficients of variation for taxa richness were generally less than 20 percent at each reference reach and for each ecoregion. Regional biological sampling could be expanded through reduced site-specific sampling and by sampling additional reference reaches.

General Synopsis

1. The modified Rapid Bioassessment Protocol sampling methodology for benthic macroinvertebrates was effective in discerning ecoregion community differences. The methods for collection and analysis of macroinvertebrates are described in this document. Further modification of sampling methodology and development of additional biometrics may be necessary when impacted stream conditions are surveyed.

2. Similarity of reference stations between ecoregions in this project seemed to be related to two categories: mountains or valley/plains. In choosing reference stations for extrapolation to other streams within the ecoregion, attention should be given to maintaining reference site selection in mountain, piedmont, or valley bottoms.

3. Cooperative monitoring among government agencies, private interests, and academic research institutions should be maintained. A standard database should be developed to promote sharing of biological assessment data.

Future Effort

1. The next logical phase of this project is an expansion of sampling to include a gradient of impacted sites for comparison to reference sites. This information is a necessary prerequisite to development of biocriteria.

2. Additional ecoregions should be monitored for biological, chemical, and physical characterization. Seasonal partitioning of biological monitoring into fall, spring, and summer periods is deemed most appropriate based on observations from the current project. Summer sampling should be conducted before substantial emergence activity appears. Drought years will accelerate insect life cycle progression that leads to early emergence.

3. An integrated freshwater ecosystem monitoring approach should be further refined to a systematic methodology. Simultaneous monitoring of physical, chemical, and biological attributes of a stream should be used to indicate relative "health" which would then guide future pollution abatement procedures and evaluation monitoring.

4. The number of chemical parameters monitored could be reduced by measuring one of a set of highly intercorrelated variables (*i.e.*, alkalinity, hardness, conductivity, pH). Other useful diagnostic indicators of surface water quality are ortho-phosphate, ammonia-nitrogen, total organic carbon, temperature, and discharge.

5. Reference site selection in this project was constrained by having continuous annual accessibility which, in some cases, resulted in choices of mid-elevation reaches that had experienced historical impact and minor current activity. Future biological assessment activities should expand the number of reference sites by locating in roadless areas. Access to the more remote sites would be necessary during the fall and spring seasons when macroinvertebrate assemblages are considered most distinct between the ecoregions surveyed in this project. Stream reaches chosen for the Columbia Basin and Puget Lowland survey sites may presently be the least impacted. Cascade stream sites may be improved by locating in seasonally accessible roadless areas. Reference streams in ecoregions not surveyed in this project should be sited in the roadless areas initially.

6. Stream conditions in remote areas should be compared to the stream conditions surveyed in this project in order to evaluate possible information loss due to the accessibility of a stream.

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