Identifying Stressor Risk to Biological Health in Streams and Small Rivers of Western Washington

April 2014
Revised July 2014

King County

Department of Natural Resources and Parks
Water and Land Resources Division
Science and Technical Support Section
King Street Center, KSC-NR-0600
201 South Jackson Street, Suite 600
Seattle, WA 98104
206-477-4800 TTY Relay: 711
www.kingcounty.gov/EnvironmentalScience

Alternate Formats Available

Identifying Stressor Risk to Biological Health in Streams and Small Rivers of Western Washington

Submitted by:

Elene Dorfmeier
King County Department of Natural Resources and Parks
Water and Land Resources Division

King County
Department of
Natural Resources and Parks
Water and Land Resources Division

Acknowledgements

The research presented here was funded by the U.S. Environmental Protection Agency through grant number PC-J28401-0 to King County, Department of Natural Resources and Parks. This document has been subjected to peer-review and approved for publication; approval does not signify that the contents reflect the views or policy of King County. We wish to acknowledge Leska Fore (Statistical Design) for study design and analysis support. We thank Washington State Department of Ecology's Status and Trends Monitoring for Watershed Health and Salmon Recovery program for its publicly offered data made available through the Environmental Information Management database. We would like to acknowledge John Van Sickle for coding help and statistical advice and Glenn Merritt for assistance with database inquiries and habitat assessment questions. We thank everyone who participated in editorial review, including: Deb Lester, Jo Wilhelm, Jim Simmonds, Curtis DeGasperi, Kate Macneale, and Scott Stolnack of King County, and Chad Larson and Glenn Merritt of Washington State Department of Ecology.

Citation

King County. 2014. Identifying stressor risk to biological health in streams and small rivers of western Washington. Prepared by Elene Dorfmeier, King County Department of Natural Resources, Water and Land Resources Division. Seattle, Washington.

A note about this revised edition

This report was originally published April 2014. This July 2014 revised edition provides: (a) Bonferroni-corrected confidence intervals for attributable risk scores, (b) updated results and graphs, and (c) limited text edits to improve clarity and to update language as related to changes noted. No major findings or conclusions have been altered from the original April 2014 edition.

Table of Contents

Figures

Tables

Appendices

EXECUTIVE SUMMARY

The Puget Lowland benthic index of biotic integrity (B-IBI) is an index composed of 10 metrics that assess benthic macroinvertebrate community health. These metrics reflect specific and predictable responses of organisms to changes in landscape condition that stress biological systems. The B-IBI was developed as an integrative measure of the biological health, or a bioindicator, of wadeable streams in the Pacific Northwest. In 2010 King County was awarded a grant from the United States Environmental Protection Agency (EPA) to begin working towards a more standardized approach for benthic macroinvertebrate monitoring and data analysis in the Puget Sound region, in addition to improving collaboration between organizations conducting this type of monitoring. As one component of the larger project, this report documents the statistical analysis conducted to help identify which environmental stressors (water quality, nutrients, metals, physical habitat) consistently result in decreased macroinvertebrate community health in western Washington. The analysis presented here was conducted to enhance the use of macroinvertebrate data as a tool for focusing potential future restoration strategies.

For this project, water quality, sediment chemistry, and physical habitat data (146 sites) from the Washington State Department of Ecology's Status and Trends Monitoring for Watershed Health and Salmon Recovery program were used to determine the relative importance and strength of relationship between benthic macroinvertebrate metrics and environmental stressors in western Washington streams and small rivers. B-IBI was used to determine biological condition at each site. The statistical approach used assesses (1) the regional extent of each stressor in poor condition, (2) the relative risk, or strength of statistical association between each stressor and B-IBI scores, and (3) the overall impact (extent and biological impact), or attributable risk, for each stressor. Each of the 10 component metrics used to calculate the B-IBI were also assessed.

The results of this study indicate stream substrate composition, specifically sedimentation (percent fines, small gravel and cobble, sand-fines) and embeddedness, or the degree to which fine sediments surround coarse substrates on the surface of a streambed, presents the greatest attributable risk to B-IBI scores and 5 of the 10 individual B-IBI component metrics. Biological indices are also sensitive to a number of surface water quality parameters (dissolved oxygen, total phosphorous, turbidity, and pH). The sediment chemistry parameters evaluated by this effort had little effect on B-IBI scores in the regions examined.

An essential step in watershed management is the identification of key natural and anthropogenic stressors influencing important biological indicators of watershed health, such as B-IBI. Relative risk analysis provides quantifiable associations between stressors of concern and biological response, making this a useful tool to identify potential risks to aquatic biota, complimenting monitoring programs, and supporting watershed management decisions. These results will help assist King County and other jurisdictions in focusing key watershed restoration efforts associated with environmental stressors impacting B-IBI scores within the Puget Sound region. Specifically, results suggest that targeting restoration of physical habitat, specifically rebuilding riparian buffers and remediating excessive sources of sedimentation, could improve regional watershed health and water quality.

1.0. INTRODUCTION

Protection and management of ecological resources are key priorities for regulatory agencies. An important step in determining management objectives is to scientifically evaluate and interpret complex functional relationships between stressors and biota within an ecological system. Ecological risk assessment offers a way to quantify relationships between candidate stressors and biotic response to help identify, characterize, and prioritize important ecological risk factors that contribute to environmental degradation. Many types of environmental stress - chemical parameters, flow, nutrients, habitat structure – must be evaluated for both regional relevancy and relative importance to biota. By quantifying exposure-response models using bioindicators, resulting relationships can assist in predictive assessments and facilitate development of watershed restoration strategies.

Macroinvertebrates are ubiquitous in freshwater ecosystems and exhibit a wide range of sensitivities to chemical and physical changes such as water and sediment quality, nutrient input, sedimentation, and habitat structure. Macroinvertebrate communities are key indicators of watershed health because they integrate the cumulative impacts of biophysical changes in the watershed. Indices based on macroinvertebrate taxa assemblages provide a tool to identify and analyze environmental risks and can be used to help determine linkages between observed ecological effects and environmental stress. The Puget Lowland benthic index of biotic integrity (B-IBI) consists of 10 metrics used to measure the biological health of wadeable streams in the Pacific Northwest (Kleindl 1995; Fore et al. 1996; Karr 1998). Individual, component B-IBI metrics respond predictably to changes in environmental conditions associated with human disturbance (Fore et al. 2001; Morley and Karr 2002; Booth et al. 2004; DeGasperi et al. 2009) making B-IBI ideal for use as an ecological response indicator to identify regionally important risk factors affecting watershed health.

This report identifies and ranks major aquatic and habitat stressors for stream ecosystems in western Washington using a relative risk analysis approach. Relative risk offers an effective way to identify the major chemical and physical factors driving biotic changes in the aquatic environment, while providing a clear measure of stressor severity and relative extent (Van Sickle et al. 2006; Van Sickle and Paulsen 2008; Van Sickle 2013).

Relative risk is used extensively in the field of epidemiology to evaluate the relative importance, or risks, of various factors (diet, health, environment, genetics, etc.) on human health and disease occurrence at the population level. The statistical methods used here were adapted to assess which individual disturbance measures of site condition are associated most closely with poor biological condition (Van Sickle et al. 2006; Van Sickle and Paulsen 2008; Van Sickle 2013). Using relative risk with regional watershed data, we can estimate both the relative importance of an individual aquatic stressor and estimate that stressor's effect on the overall population of streams and small rivers.

Three ecological risk measurements – relative extent, relative risk, and attributable risk– allow us to test the effects of selected stressors on stream biological condition throughout western Washington. The first measure, relative extent, describes the proportion of poor stressor condition within a study region. The second measure, relative risk is an estimate of stressor effect on biological assemblages, in this case B-IBI. The relative risk ratio represents the likelihood that a poor B-IBI score is associated with poor stressor condition. And, the third measure, population attributable risk, combines relative risk and extent to describe the regional contribution of the stressor to poor biological condition. Any increase in stressor extent or relative risk will increase its attributable risk. Because of this combination, attributable risk can estimate the proportionate reduction of poor biological response that could be achieved by eliminating a stressor from a system (Van Sickle and Paulsen 2008).

To illustrate the utility of this type of analysis for watershed management and prioritization of restoration efforts, aquatic and physical habitat stressor risk was characterized for western Washington small rivers and streams using B-IBI. Biological, chemical and physical habitat data from 146 sites in western Washington sampled as part of the Washington State (WA) Department of Ecology (Ecology) Status and Trends Monitoring for Watershed Health and Salmon Recovery (WHSR) were utilized for analysis. Stream sites used in this monitoring program were randomly selected to provide an unbiased estimate of stream condition (Merritt and Hartman 2012); thus, results of this analysis are representative and applicable at the regional scale. These data were selected to characterize relative risk of key aquatic and habitat stressors on B-IBI scores and component B-IBI metrics. Risk analysis was used to estimate: (1) stressor extent, or the proportion of a study population of streams and small rivers within western Washington in poor biological condition; (2) the severity of individual stressor effect, or relative risk, in the region; and (3) regional-level impact, or attributable risk, of stressors on B-IBI scores and component B-IBI metrics.

2.0. METHODS

2.1 Site and data selection

Ecology's WHSR program includes a collection of standardized, probabilistic-based monitoring data aimed at assessing stream health and river corridor habitat to detect overall trends within WA watersheds. A variety of data are collected by this monitoring program, including benthic macroinvertebrate, water quality, sediment chemistry, and habitat assessment. These data are available through Ecology's Environmental Information Management database, which provides downloadable physical, chemical, and biological data from WA streams and rivers. Data collected in 2009 and 2010 from 146 small streams and river locations in three western WA basins (Puget Sound Basin, n = 47; Coastal n = 49; and Lower Columbia, n = 50) were used for analysis (Fig. 1). Habitat assessment, water quality, and sediment chemistry were collected using standardized protocols (Merritt 2009; Merritt et al. 2010; Merritt and Hartman 2012).

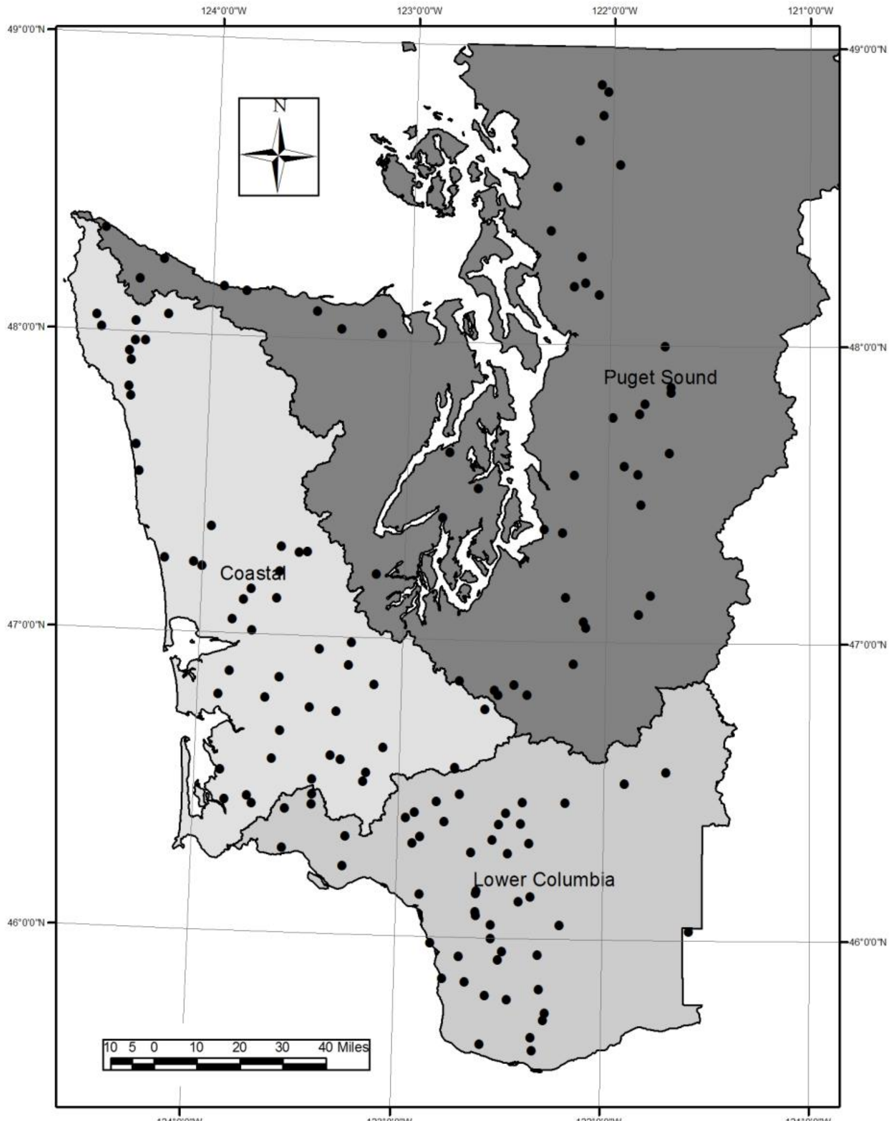

Figure 1. Map of Ecology's Status and Trends Monitoring for Watershed Health and Salmon Recovery sites used for analysis within Puget Sound, Coastal, and Lower Columbia basins. Black dots represent individual sampling sites.

The basis for the decision to combine data for sampling sites located west of the Cascade crest (Puget Sound Basin, Coastal, and Lower Columbia basins) was two-fold: additional sites were needed to meet the minimum statistical requirements of relative risk, and sites west of the Cascades are relatively similar in vegetation and forest cover, climate and physiogeography compared to sites east of the Cascades (Scott et al. 1989).

Relative risk analysis is a contingency based statistical procedure and requires a minimum of five observations within each contingency table cell. After partitioning biological and environmental data into condition classes, Puget Sound Basin sites (n = 47) did not meet the minimum statistical requirements of relative risk analysis and more sites were needed for the study design. Additional sites within the Coastal and Lower Columbia regions were added to develop a more robust analysis.

To evaluate possible differences in variable distributions, the distribution of each habitat, water quality, and sediment chemistry variable was examined and compared across basins before conducting risk analyses. Data were examined for completeness. If 25% or more total study sites were missing data for a specific environmental variable, it was not included in the analysis.

B-IBI data for each site were obtained from the Puget Sound Stream Benthos (PSSB) database (pugetsoundstreambenthos.org) by using the following search parameters: all rivers / all streams; Ecology-status and trends project; combined replicate handling; B-IBI score type 0-100; Fore, Wisseman (2012) taxa attributes; taxonomic resolution as defined by metadata; and organisms subsampled to a maximum of 500 per visit.

2.2 Risk analysis

All statistical analyses were performed using the *spsurvey* package developed by EPA in R statistical software version 3.0.1 (R Development Core Team 2013). Individual relative risk analyses for the B-IBI and its 10 component metrics were performed. Relative extent, relative risk, and population attributable risk were calculated for the biological response indicator and each parameter of interest. Definitions of relative risk calculations are summarized in Table 1.

Three result values are summarized for each environmental variable in this report:

Relative extent: Relative extent (%) is the proportion of sites (biological response or environmental stressor) classified in poor condition for a given variable.

Relative Risk: Relative risk is a ratio of two probabilities: (1) poor biological condition, given poor stressor condition, and (2) poor biological condition, given good stressor condition. Relative risk values greater than 1 demonstrate an increased risk of poor biological condition (based on B-IBI, or its component metrics). Relative risk values greater than one represent the amount of risk to B-IBI scores likely to occur when poor environmental conditions exist. For example, if excess sedimentation was found to have a relative risk of 1.8, poor B-IBI scores are 1.8 times more likely to occur in areas with excess sediment, than in areas without excess sedimentation.

Attributable Risk: Attributable risk (%) combines relative risk and relative extent into one number that can be used to rank stressors for a watershed population (Paulsen et al. 2008). Any increase in either extent or risk is reflected in the attributable risk. An attributable risk value of 0 (no effect) occurs when either the stressor extent is 0 or no association with the biological indicator response exists (relative risk no effect value of one). Because attributable risk is reflective of both extent and risk to biota, attributable risk can determine the regional-level impact of each variable within this study.

Attributable risk assumes both stressor causal effects and the reversibility of stressor condition within an ecological system. Since relative extent describes the proportion of streams in poor condition and relative risk estimates the impact on biological condition, attributable risk (the combination of both stressor extent and risk) can estimate the proportional decrease of poor biological condition extent that would result if the stressor was eliminated (Van Sickle and Paulsen 2008). For example, if excess sedimentation produced an attributable risk score of 40% and the original extent of sites determined to be in poor biological condition (poor B-IBI) was 30%, the reduction in poor B-IBI scores within the study region after the elimination of excess sedimentation would reduce the percentage of poor sites by 40%, a reduction from the original 30% to 18%. Of course, the elimination of any given variable may not be realistic, or even possible, but here, we used the attributable risk value to estimate the biological impact of a given variable in western Washington. The larger the attributable risk value, the greater the impact a given stressor has on regional biological response (B-IBI or component metrics).

Interpretation of relative risk and attributable risk significance are as follows: relative risk values are significant when confidence bounds do not cross the "no effect" value of 1; attributable risk values are significant when confidence bounds do not cross 0. When several tests of significance are performed using multiple, non-independent inferences, the probability of type I error increases. To control for this, Bonferroni-adjusted CI were used to determine significance of variables tested (Van Sickle 2003). Both relative risk and attributable risk values are reported with Bonferroni-adjusted confidence intervals (CI) with a familywise alpha level of 0.05. Familywise CI were adjusted as described in Van Sickle 2003 by using the $100(1-\alpha/2K)$ percentile of the standard normal distribution rather than the $100(1-\alpha)$ percentile. The familywise confidence level is used to ensure that confidence levels will be at least $100(1-\alpha)$ percentile. This is a highly conservative procedure that will favor accepting the null hypothesis.

To calculate the number of comparisons, variables were grouped into categories of substrate, riparian cover/woody debris, water quality, and sediment chemistry. Family comparisons (K) were calculated for each grouping of variables using the following equation:

$$K = I(I - 1)/2$$

Where K is the total family comparisons and I = number of variables within a family category

Table 1. Definitions of relative risk calculations (Van Sickle et al. 2006; Van Sickle and Paulsen 2008).

Relative Extent (%) describes the proportion of streams/rivers in poor condition within the study region.
Relative Risk estimates a stressor's association with biota in terms of the likelihood that poor stressor conditions and poor biological conditions co-occur in streams.
Population Attributable Risk (%) combines both the extent and risk of a given stressor to biota into one risk value. Additionally, it estimates the reduction in poor biological condition (poor B-IBI scores) that would be achieved if the stressor was "eliminated" from the watershed.

2.3 Considerations

Relative risk analysis can accommodate both weighted and unweighted statistical designs. In a probabilistic sampling design such as in the WHSR program, stream size (i.e., Strahler order) is used for unequal (probability) weighting. Sample site selection was based on a randomized sampling approach which targeted 50 sites per basin according to stream size (i.e., 10 sites each from the following Strahler orders: 1, 2, 3, >4) (Merritt and Hartman 2012). Within a randomized sample design, every possible sample location has an equal probability of being selected for sampling. Due to the inherent bias in site selection, each site within a probabilistic sampling framework is expressed as a statistical weight in order to make an unbiased assessments of biological or stressor condition across a large geographical area. An unweighted relative risk analysis biases results to be more reflective of larger water bodies, due to the increased probability of sampling large water bodies (higher stream orders) , rather than smaller ones relative to their occurrence. Final weighting information for Ecology's probabilistic sampling design was not yet available for the 2009 or 2010 survey data; therefore, an equally-weighted sample design was used. The results presented here assume that all sites are equally representative of streams in all three basins. To investigate this assumption, preliminary relative risk analysis using only data from the Puget Sound Status and Trends Region (n=47) were conducted using two approaches - weighted and un-weighted designs, and results were compared. Small differences in attributable risk values were seen, but the overall significance of stressors and the relative order of stressor importance was unchanged (data not shown). For reference, stream order frequency for this study is summarized in Fig. 2.

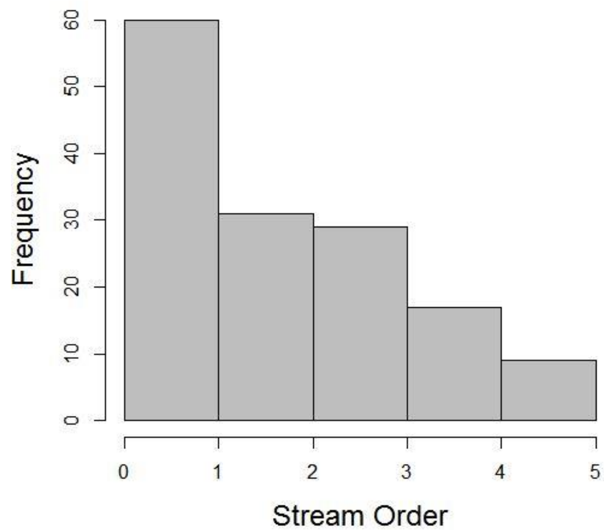

Figure 2. **Stream order distribution of 146 western Washington sites included in this assessment.**

Relative risk analysis assumes variable independence. Multicollinearity of water quality and habitat variables is inherent within these data. Risk estimates were not adjusted for each stressor and possible confounding by other covarying stressors may be included within these results. We chose to present these data without grouping variables to illustrate the relative importance and individual contributions of each variable to B-IBI indices within our study sites. Because of this, individual parameters should be interpreted with care since interactions among variables may be underrepresented and individual associations to B-IBI response may be overrepresented (Van Sickle 2013).

2.4 Threshold determination

An important step in this analysis involves setting thresholds that classify the condition of both the biological response, B-IBI, and each stressor variable of physical habitat, water quality, and sediment chemistry for subsequent analysis. All dataset values were split into one of two conditional categories: "poor" or "not poor," consistent with Van Sickle and Paulsen 2008. Ideally, thresholds for condition classes would be based on the distribution of stressor variables obtained from a range of reference sites representing least-disturbed sites within each basin (Stoddard et al. 2006a; 2006b). Ecology is currently developing a formal site guidance to identify randomly selected reference sites. Some reference site data within these basins are available, but site datasets were not complete by the time of analysis. In the absence of a reliable estimator of minimally disturbed conditions within all three basins, a mixed approach was used to determine threshold values. This approach included federal and state recommended criteria for water quality and sediment chemistry variables and an ambient distribution-based approach for physical habitat metrics.

Detailed information about B-IBI metric definitions, calculations, and scoring is available on the PSSB website (pugetsoundstreambenthos.org/About-B-IBI.aspx). Best professional judgment and ambient data distributions were used to determine B-IBI thresholds. The condition classes of overall B-IBI scores were divided using the 30th percentile value, meaning any value greater than the 30th percentile (a score of 51 or higher; B-IBI score range of 0-100), was designated as "not poor" while any site with a score of 50 or lower was classified as "poor" (Fig. 3; Table 2). A more stringent threshold was used to help identify major stressors to B-IBI component metrics. For each metric, the 25th percentile value was used to designate condition classes, meaning any value less than or equal to the 25th percentile was used to identify "poor" metric scores, while values over the 25th percentile were classified "not poor" (Table 2).

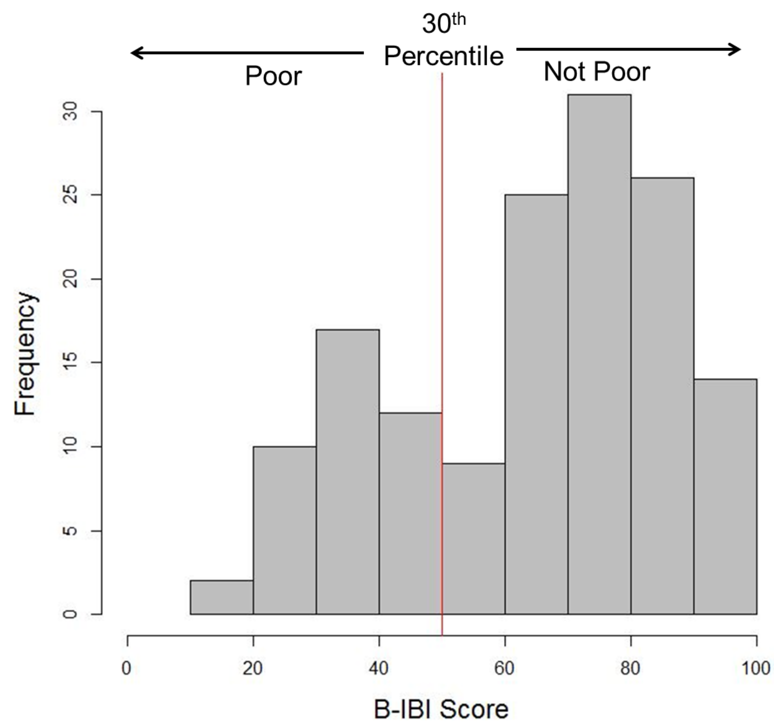

Figure 3. B-IBI score distribution of the 146 study sites. Red line denotes B-IBI threshold value (50), separating the condition classes of "poor" and "not poor", used in analysis.

Of the 58 habitat assessment variables evaluated by Ecology in 2009-2010, 26 were used for analysis (7 substrate and 19 riparian cover/woody debris). The remaining 32 variables were not used because condition class assignments could not be readily determined or similar to other variables. Definitions for each habitat variable are summarized in Appendix A. Ideally, reference site data would assist in determining threshold cutoff values for physical habitat variables. Since these data were not available at the time of analysis, ambient data distributions for each physical habitat variable were examined and the 25th or 75th percentile values (depending on the direction of poor condition of the parameter) were used to determine condition classes (Table 3; 4).

A combination of published guidelines and standards were used to set thresholds for water and sediment quality parameters (MacDonald et al. 2000; Smith et al. 1996; Washington Administrative Code [WAC] 173-201A-240; WAC 173-204). Water quality and sediment chemistry samples were collected as outlined in Merritt et al. 2009; 2010. Water quality data for temperature, dissolved oxygen (DO), pH, and conductivity represent mean values of two *in situ* measurements at each site. Sediment samples were collected from three locations within the stream that contain sediment particles less than 2 mm in diameter, have water depth <30 cm, and within a continuously wetted area (Merritt et al. 2010). Whenever possible, the most stringent available value was used for each sediment chemistry and water quality parameter (Table 5; 6). Water quality criteria for total nitrogen (TN) and total phosphorus (TP) have not been adopted by Washington State; the TN and TP thresholds used were established by EPA based on the 25th percentile of nutrient concentrations within the Puget Sound Basin and not specifically associated with an adverse condition (EPA 2000). Currently, only instantaneous temperature measurements are available for these study sites, therefore temperature was not used in the analysis. Continuous temperature measurements or a 7-day moving average of the daily maximum temperature would provide more accurate data to use for future analyses.

Data for 34 sediment chemistry variables were available for most sites; however; 23 variables were not used for analyses for one of the following reasons: (1) no threshold was available to set a condition class; (2) all resulting analyte values for a given variable were below the threshold; therefore, no sites were ranked in poor stressor condition (2-methylnaphthalene, acenaphthene, acenaphthylene, anthracene, benzo(g,h,i)perylene, dibenzo(a,h)anthracene, dibenzofuran, indeno(1,2,3-cd)pyrene, lead, naphthalene); or (3) >25% of sites did not contain results for the variable.

Table 2. Thresholds for B-IBI scores and component metrics. Poor condition classification based on 30th (overall B-IBI) and 25th percentile values (component metrics). Overall B-IBI range 0 – 100; Component B-IBI metric score range 0 – 10. Threshold value indicates direction of poor condition class.

Parameter Name	Threshold Value > or < indicate direction of poor condition class
Overall B-IBI	≤50
Taxa Richness	≤5.6
Ephemeroptera Richness	≤4.3
Plecoptera Richness	≤2.9
Trichoptera Richness	≤3.8
Clinger Richness	≤2.9
Long-Lived Richness	≤2.5
Intolerant Richness	≤2.9
Percent Dominant	≤4.6
Predator Percent	≤2.85
Tolerant Percent	≤9.1

Table 3. Substrate variables and threshold values. Poor condition classification based on the 25th percentile values of variable. Threshold value indicates direction of poor condition class. See Appendix A for definitions.

Parameter Name	Threshold Value > or < indicate direction of poor condition class
% Cobble	≤1.91
% Fines	≥22.15
% Gravel Coarse	≤13.90
% Gravel Fine	≥15.19
% SandFines	≥41.67
% Wood	≤0.40
Mean % Embed	≥60.17

Table 4. Riparian cover/woody debris variables and threshold values. Poor condition classification based on the 25th percentile values of variable. Threshold value indicates direction of poor condition class. See Appendix A for definitions.

Parameter Name	Threshold Value > or < indicate direction of poor condition class
FishCv Algae	≥18.20
FishCv Big	≤81.80
FishCv Brush	≤54.50
FishCv LWD	≤18.20
FishCv Natural	<90
FishCv NoAqVeg	<100
FishCv OvHgVeg	≤63.60
FishCv TreesRoots	≤9.1
FishCv Undercut	≤10.24
Mean % ShadeBnk	≤85.26
Mean % FishCv Algae	≥5.00
Mean % FishCv Big	≤11.40
Mean % FishCv Brush	≤4.10
Mean % FishCv LWD	≤1.40
Mean % FishCv Natural	≤25.82
Mean % FishCv NoAqVeg	≤24.60
Mean % FishCv OvHgVeg	≤4.50
Mean % FishCv TreesRoots	≤0.50
Mean % FishCv Undercut	≤0.55

Table 5. Sediment chemistry variables and threshold values. Threshold value indicates direction of poor condition class.

Parameter	Threshold Value > or < indicate direction of poor condition class	Source
2-Methylnaphthalene	≥470 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Acenaphthene	≥1060 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Acenaphthylene	≥470 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Anthracene	≥600 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Arsenic	≥5.9 mg/kg	Smith et al. 1996
Benz[a]anthracene	≥31.7 ug/kg	Smith et al. 1996
Benzo(a)pyrene	≥31.9 ug/kg	Smith et al. 1996
Benzo(ghi)perylene	≥4020 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Chrysene	≥57.1 ug/kg	Smith et al. 1996
Copper	≥35.7 mg/kg	Smith et al. 1996
Dibenzo(a,h)anthracene	≥33 ug/kg	MacDonald et al. 2000
Dibenzofuran	≥400 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Fluoranthene	≥111.3 ug/kg	Smith et al. 1996
Fluorene	≥77.4 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Indeno(1,2,3-cd)pyrene	≥4120 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Lead	≥35 mg/kg	Smith et al. 1996
Naphthalene	≥100 ug/kg	Chapter 173-204 WAC WA Sediment Management Standards
Phenanthrene	≥41.9 ug/kg	Smith et al. 1996
Pyrene	≥53 ug/kg	Smith et al. 1996
Zinc	≥123.1 mg/kg	Smith et al. 1996

Table 6. Water quality variables and threshold values. Threshold value indicates direction of poor condition class.

Parameter	Threshold Value > or < indicate direction of poor condition class	Source
Dissolved Oxygen	≤9.5 mg/L	WA Water Quality Standard for Aquatic Life Surface Freshwater Criteria (WAC-173-201A-200)
pH	≤6.5 / ≥8.6 pH units	WA Water Quality Standard for Aquatic Life Surface Freshwater Criteria (WAC-173-201A-200)
Total Nitrogen†	≥0.34 mg/L‡	EPA 2000, EPA reference conditions Level 3, Ecoregion 2 (Puget Lowlands)
Total Phosphorus	≥0.0195 mg/L‡	EPA 2000, EPA reference conditions Level 3, Ecoregion 2 (Puget Lowlands)
Turbidity	≥1.95 NTU‡	EPA 2000, EPA reference conditions Level 3, Ecoregion 2 (Puget Lowlands)

† Total nitrogen calculation is based on the sum of TKN + NO_2 + NO_3

‡ Median 25^{th} percentile value derived by EPA from all available data for the region.

3.0. RESULTS

Overall, differences were relatively small compared to the possible range of metric and B-IBI scores, which supported the decision to include data for sites from all three basins in the analysis. The three WHSR regions showed similar distributions of most habitat, water, and sediment quality variables. Of the 146 stream and small river sites used in this study, 29% were assigned "poor" biological condition based on an overall B-IBI score of <50 (0-100 scale). Mean B-IBI score across all sites was 64.

Compared with sites in the Puget Sound and Coastal basins, the Lower Columbia basin had the highest mean B-IBI score (69) and component metric scores (individual metrics 0-10 scale) with the exception of percent predator (mean 4.5) and percent dominant (mean 6.9) (Fig. 4). Sites in the Puget Sound basin had the lowest overall mean B-IBI score (60) and component B-IBI metric scores with the exception of percent predator (mean 6.0) and percent tolerant (mean 5.9). Coastal sites tended to have mid-range scores for both the overall B-IBI (mean B-IBI score 64) and the component metrics.

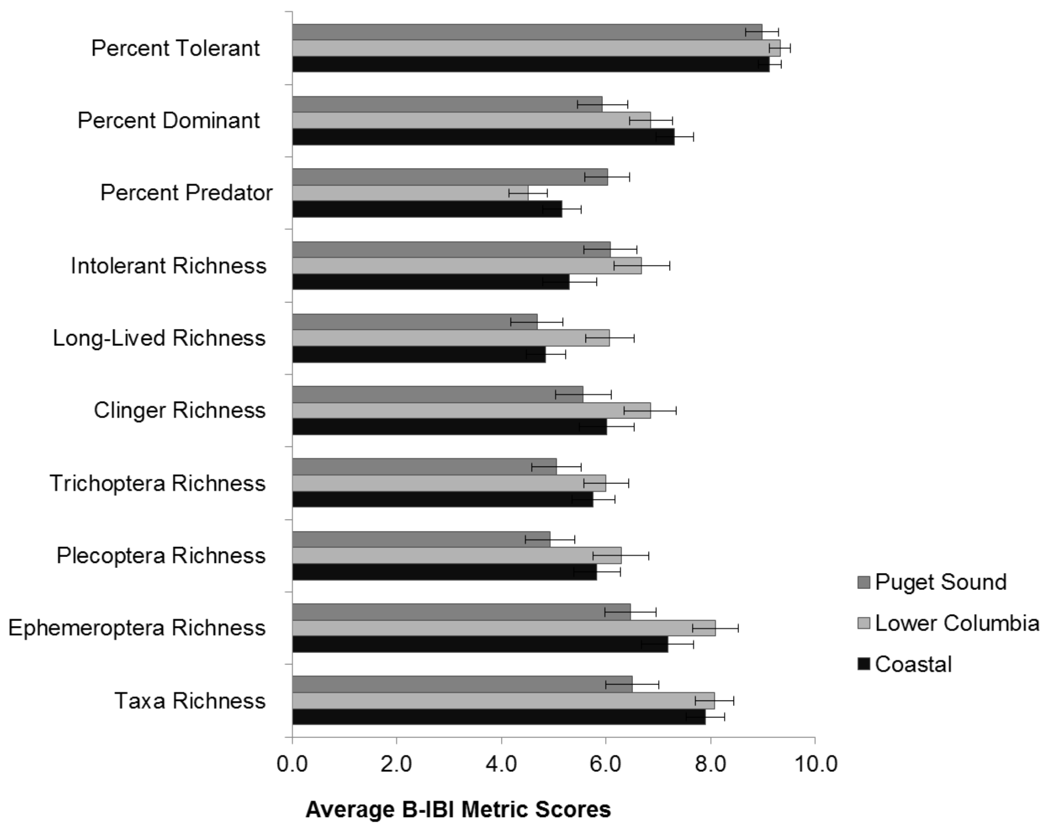

Figure 4. Mean B-IBI component metric scores by Watershed Health and Salmon Recovery region (Puget Sound Basin n = 47; Coastal n = 49; and Lower Columbia n = 50). Metric scores are calculated on a scale of 0 – 10. For each metric, a score of 0 represents the lowest possible metric score, indicating poor biological condition, while 10 indicates the highest score, and best biological condition. Error bars = Standard Error.

3.1 Physical habitat

Population extent, relative risk, and attributable risk were calculated for each physical habitat variable (Figs. 5- 10). The habitat variables in poor condition with the greatest relative extent within the study population included sites with low amounts of large woody debris (32%), low mean fish cover by brush (31%), and sites with low percent woody substrate (31%) (Fig. 5; 6).

The highest relative risk for poor B-IBI scores included conditions reflective of finer substrate (Fig. 7): high mean percent embeddedness (4.5 (95%CI 2.3, 8.8)), excess percent sand-fines (4.1 (95%CI 2.1, 7.9)), substrates with poor coarse gravel scores (sites with low percent coarse gravel) (4.1 (95%CI 2.1, 7.9)), and poor cobble scores (sites with low percent cobble) (3.7 (95%CI 2.0, 7.0)), .Variables of riparian cover/woody debris were mostly non-significant, with the exception of mean percent fish cover by algae (2.4 (95% CI 1.0, 5.5)) (Fig. 8).

Attributable risk values also indicated that variables associated with finer substrates pose risk to B-IBI and its metrics (Fig. 9). The highest attributable risks included: mean percent embeddedness (sites with high percent embeddedness) (47% (95%CI 20, 65)), sand-fines (sites with high percent sand-fines) (44% (95%CI 28, 57)), cobble (sites with low percent cobble) (41% (95%CI 14, 59)), and sites with low percent coarse gravel (37% (95%CI 10, 56)).

No riparian/woody debris variables resulted in significant attributable risk scores, but the highest scores included mean percent fish cover by tree roots (18% (95%CI -13, 44)) and fish cover by undercut banks (16% (95%CI -14, 38)) (Fig. 10).

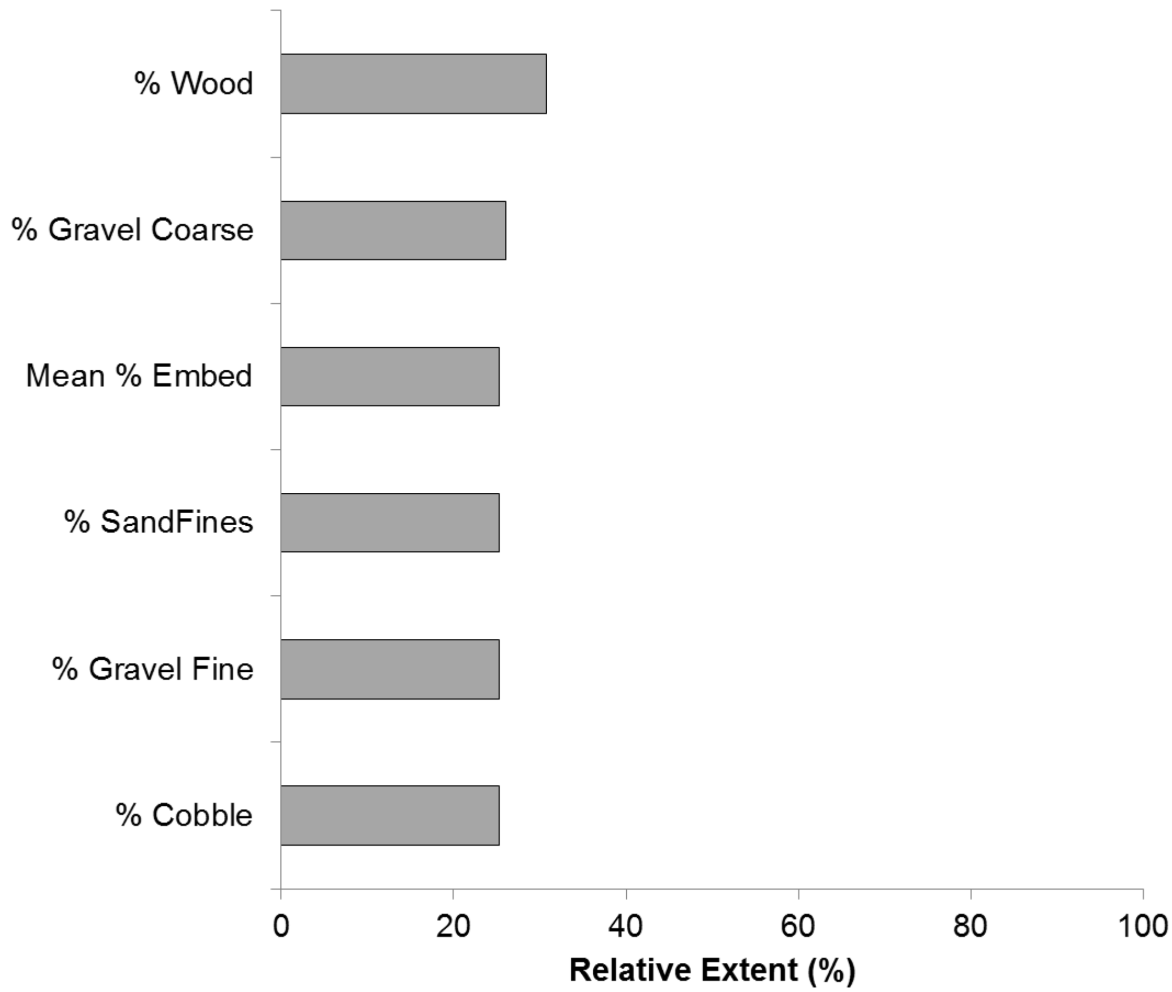

Figure 5. Relative extent of substrate variables in poor condition for 146 sites in western Washington. See Appendix A for habitat definitions.

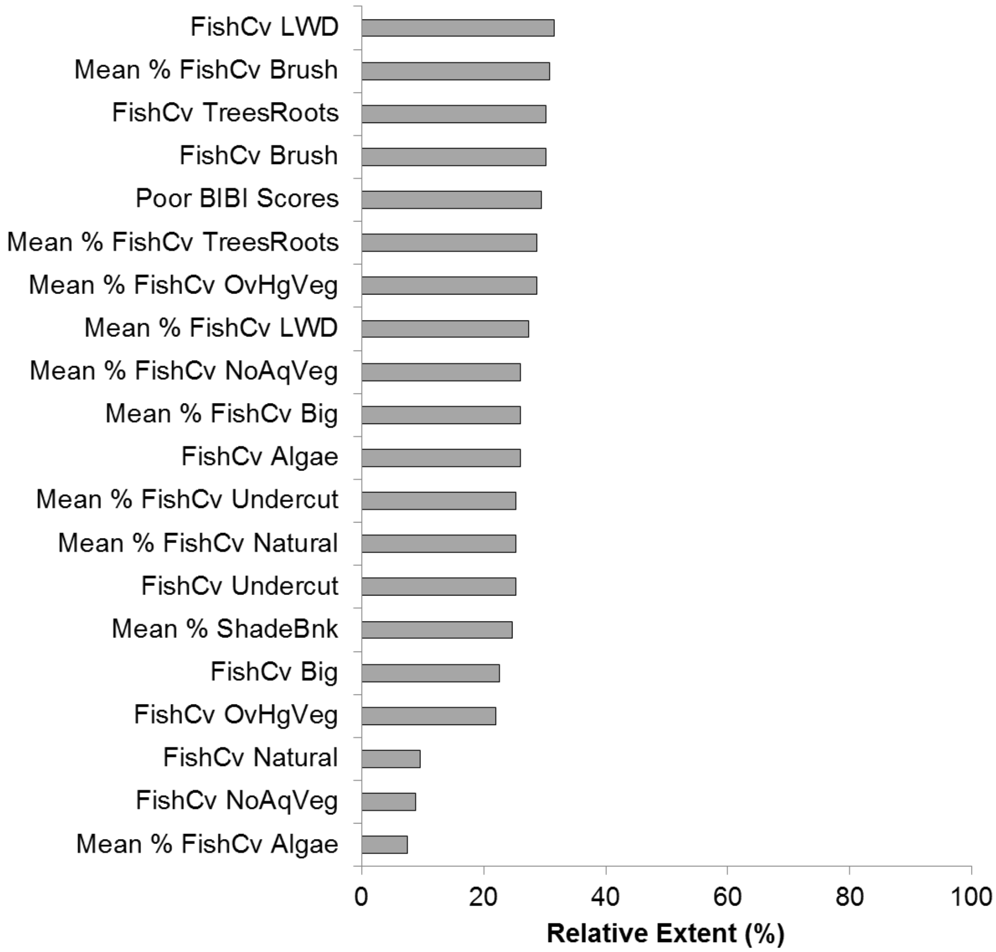

Figure 6. Relative extent of riparian cover/woody debris variables in poor condition for 146 sites in western Washington. See Appendix A for habitat definitions.

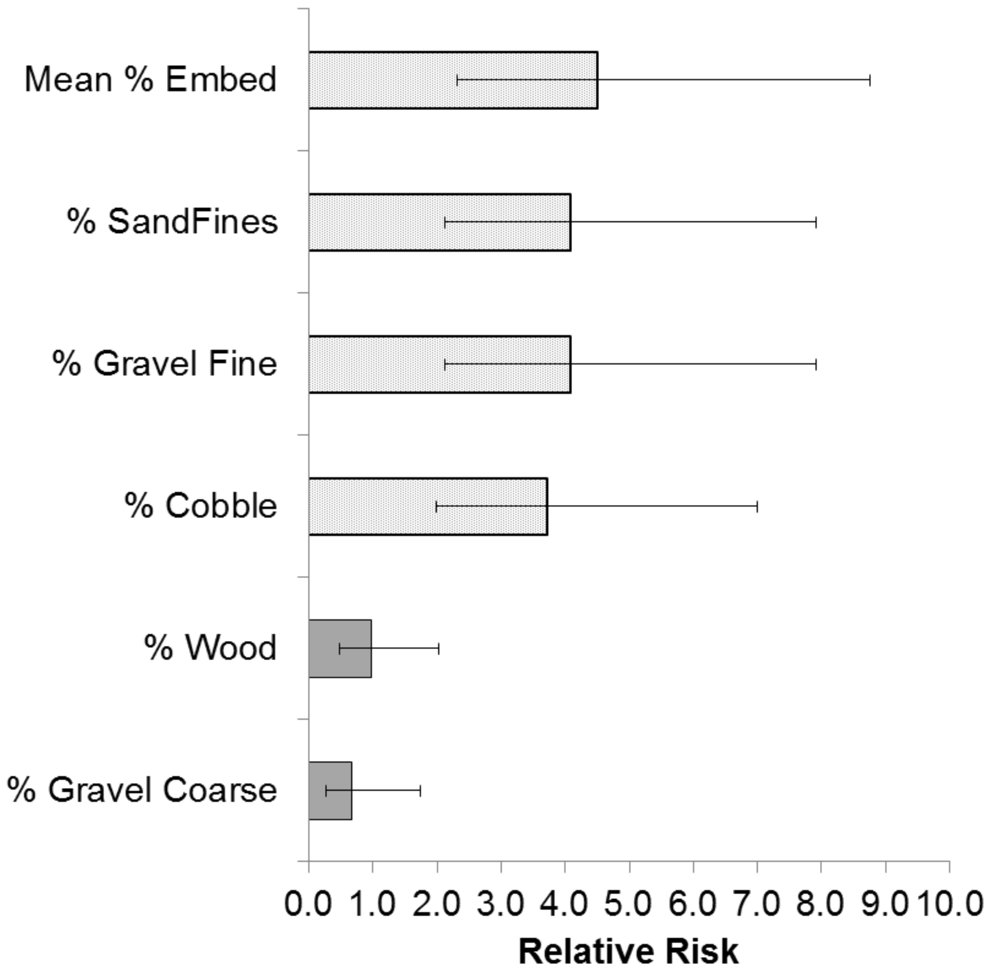

Figure 7. Relative risk of substrate variables to overall B-IBI scores. Error bars ± 95% CI. Light-colored bars denote significant relative risk values (95% CI does not cross 1). See Appendix A for habitat definitions.

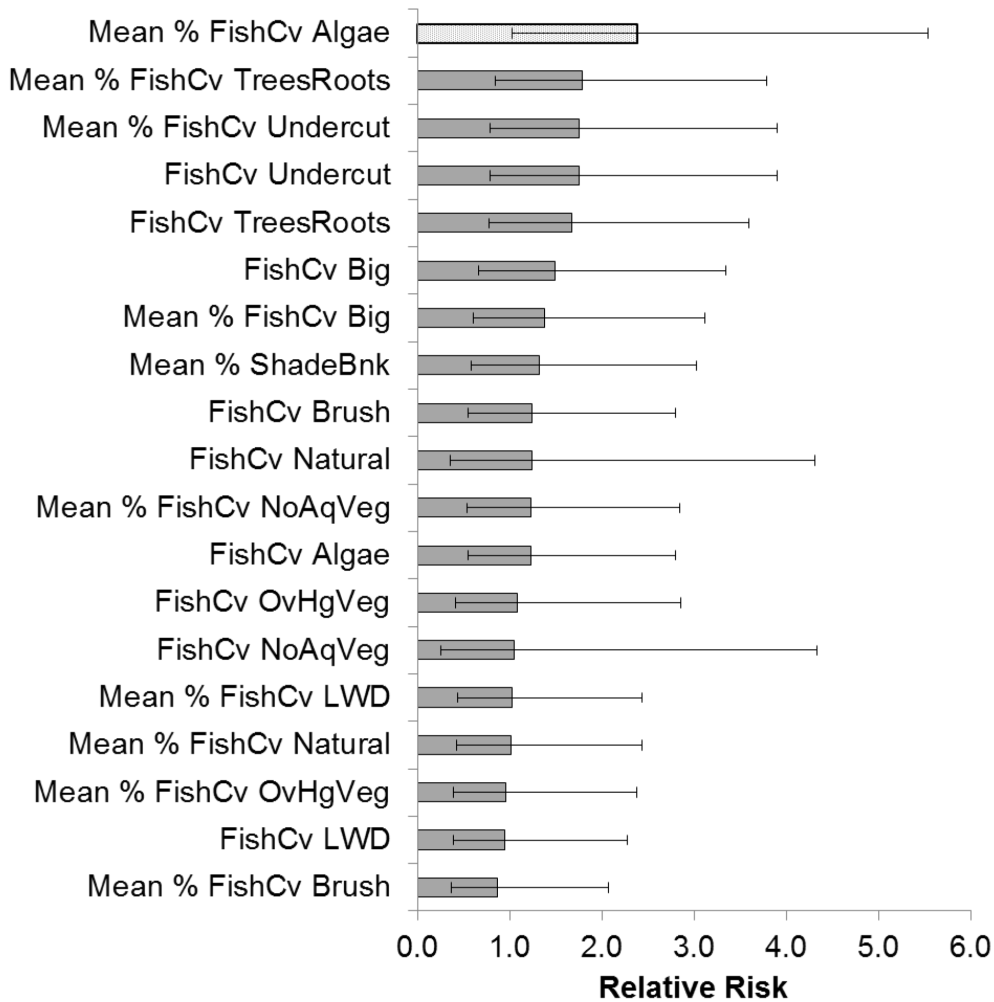

Figure 8. Relative risk of riparian cover/woody debris variables to overall B-IBI scores. Error bars ± 95% CI. Light-colored bars denote significant relative risk values (95% CI does not cross 1). See Appendix A for habitat definitions.

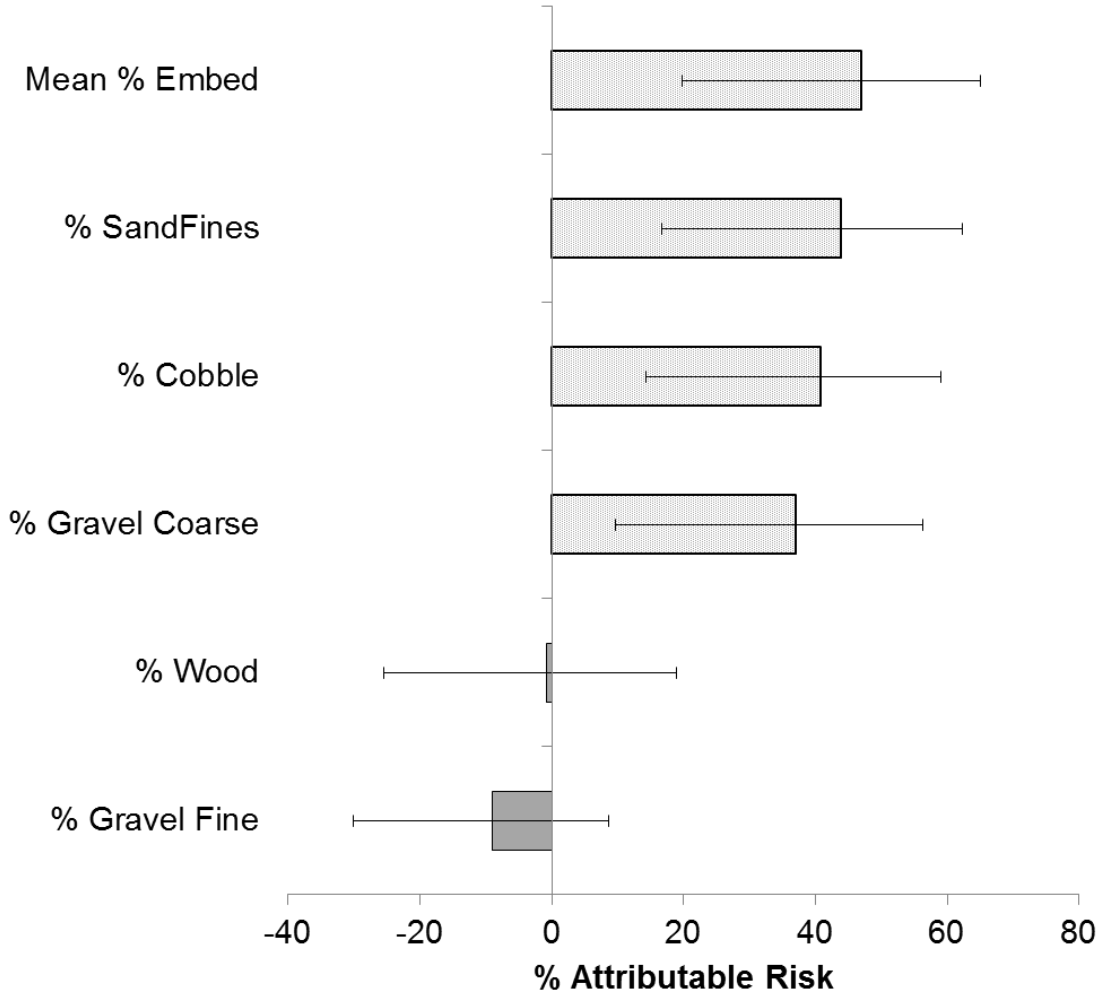

Figure 9. Population attributable risk of substrate variables in poor condition to overall B-IBI scores. Error bars ± 95% CI. Light-colored bars denote significant attributable risk values (95% CI do not cross 0). See Appendix A for habitat definitions.

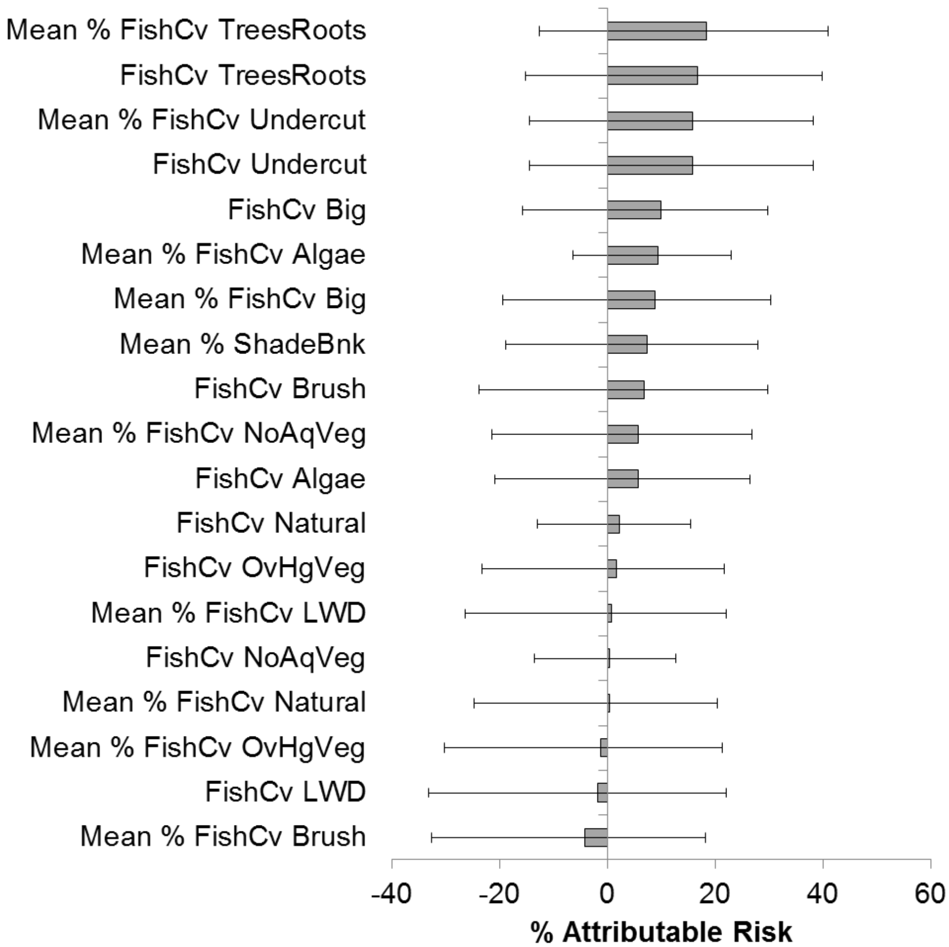

Figure 10. **Population attributable risk of riparian cover/woody variables in poor condition to overall B-IBI scores. Error bars ± 95% CI. See Appendix A for habitat definitions.**

3.2 Water quality

Concentrations of TP, turbidity, and DO exceeded their respective thresholds or criteria by over 30% (Fig. 11a). Water quality stressors that pose significant relative risk to overall B-IBI scores included TP (2.6 (95%CI 1.4, 4.9)), pH (2.6 (95%CI 1.4, 4.7)), DO (2.5 (95%CI 1.4, 4.6)), and turbidity (2.5 (95%CI 1.3, 4.7)) (Fig. 11b).

Highest attributable risk values were associated with TP (37% (95%CI 7, 57)), DO (35% (95%CI 6, 55)), turbidity (32% (95%CI 4, 52)), and pH (17% (95%CI 1, 31)) (Fig. 11c). pH, while having the lowest proportion of streams and rivers in poor condition (13%), had significant attributable risk for overall B-IBI. TN did not result in significant risk (relative or attributable risk) to overall B-IBI scores.

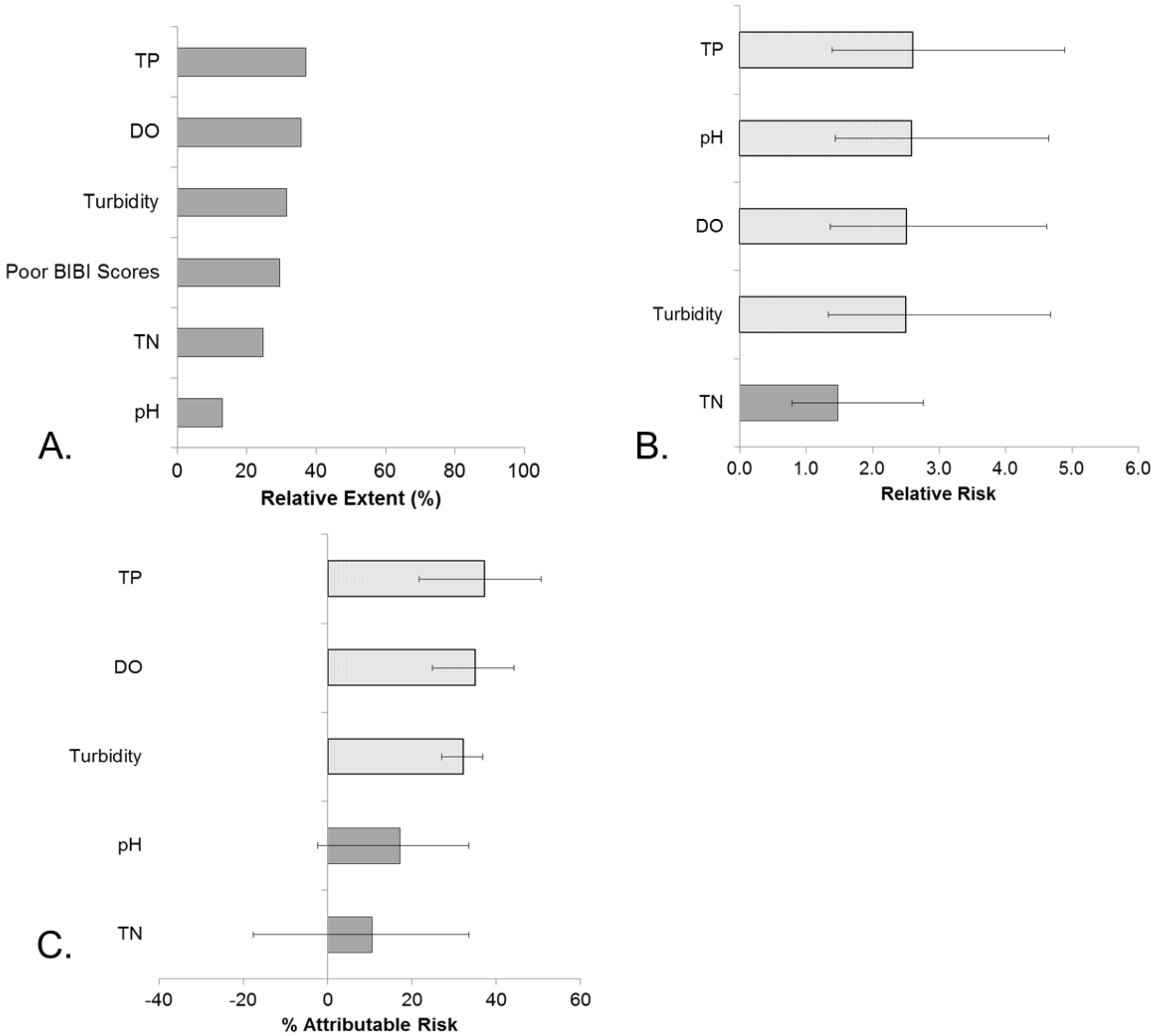

Figure 11. **Relative extent (A), relative risk (B), and population attributable risk (C) of water quality parameters in poor condition to overall B-IBI scores. TN = total nitrogen; TP = total phosphorous; DO = dissolved oxygen. Light-colored bars denote significant results. Error bars ±95% CI.**

3.3 Sediment chemistry

A number of sediment chemistry parameters exceeded their respective standards or thresholds resulting in numerous sites being classified in poor condition (Fig. 12a). Sediment chemistry data revealed that study sites had the highest regional exceedance of copper (40%), followed by arsenic (21%), benzo(a)pyrene (16%), and benz[a]anthracene (16%). Lead was not examined due to the extremely low number of sites in poor condition for this metal.

Sediment chemistry variables examined did not result in significant attributable risk to overall B-IBI scores (Fig 12b; 12c). Of the sediment chemistry variables, fluoranthene resulted in the highest relative risk (2.3 (95%CI 0.7, 8.3)). The highest attributable risk to B-IBI was chrysene (4% (95%CI -5, 12)).

The regional distribution of copper was relatively high (extent = 40%), yet resulted in an extremely low attributable risk value[1] (-27 (95%CI -60, -4)). Copper exhibited a weakly positive correlation to overall B-IBI scores (Pearson's R = 0.37, p <0.0001), indicating that higher concentrations of copper are associated with higher B-IBI. Out of 146 study sites, only 7% contained both poor copper conditions and poor B-IBI scores, which is quite low considering the high percentage of sites in poor copper condition (40%). Due to the unexpected relationship between B-IBI and sediment concentrations of copper, adjusting thresholds of the analyte or using dissolved copper values in the water column rather than metal deposition of copper within sediment may clarify copper impact to watershed biota.

[1] Negative attributable risk values suggest that the relationship between a given stressor and the biological indicator, B-IBI, is positive.

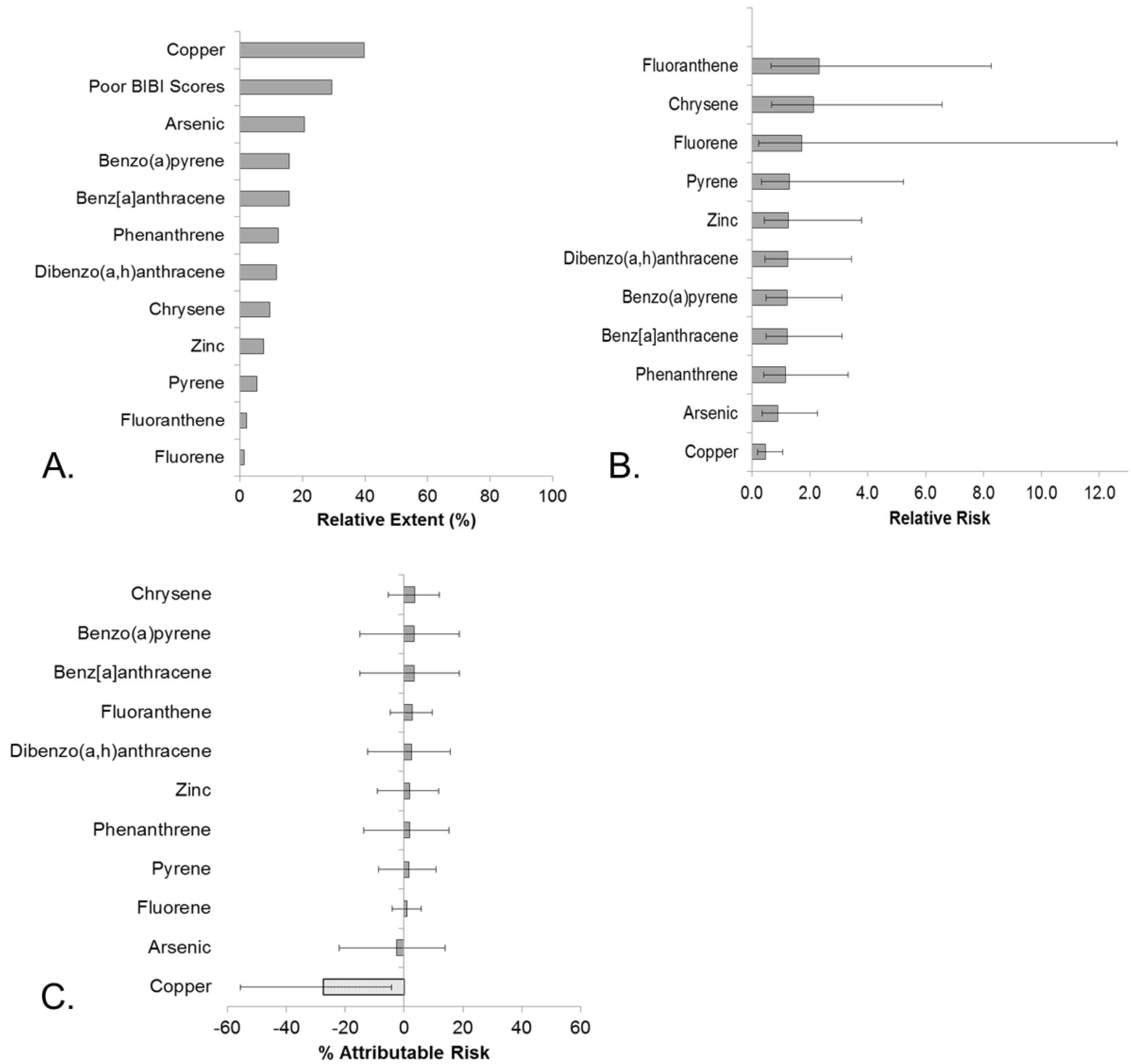

Figure 12. Relative extent (A), relative risk (B), and population attributable risk (C) of sediment chemistry results in poor condition to overall B-IBI scores. Light-colored bars denote significant results. Error bars ± 95% CI.

3.4 Attributable risk and B-IBI component metric summary

Attributable risk values for overall B-IBI and each component metric of B-IBI are presented and summarized in Table 7. Although attributable risk values differ in impact between overall B-IBI scores and its individual metrics overall, habitat variables, specifically poor condition for substrate: embeddedness, sand-fines, cobble, and gravel, produced the highest attributable risk values (mean of all substrate variables and metrics: 19%, ±0.07 SE) (Table 7). Two substrate variables: high percent fine gravel and low percent wood did not show association with poor B-IBI, or any component metrics. Variables of riparian cover/woody debris did not result in significant attributable risk values for B-IBI or individual metrics. The highest scores of riparian cover were those of percent mean fish cover by tree roots (mean 14%, ±0.2 SE) and undercut banks (mean 10%, ±0.3 SE).

All water quality variables examined, except TN, resulted in significant attributable risk to either overall B-IBI or its component metrics (Table 7). The highest attributable risk to biota included water quality variables of TP, DO, and turbidity (TP: mean 27%, ±0.4 SE; DO: mean 26%, ±0.3 SE; turbidity: mean 24%, ±0.3 SE)). pH (≤ 6.5 or ≥ 8.6) was found to have significant risk to overall B-IBI and 3 metrics, although risk values were low compared to other water quality parameters of turbidity, DO, and TP (mean 10%, ±0.2SE). TN resulted in the lowest attributable risk values (mean 6%, ± 0.3 SE) and did not show significant influence on B-IBI, or its component metrics.

Sediment chemistry was not associated with poor overall B-IBI condition within the study population.

Table 7. Heat map of attributable risk (%) results of each environmental variable to B-IBI scores and/or component metrics. Attributable risk combines both stressor prevalence and its associated relative risk into one value of regional-level impact. Attributable risk to overall B-IBI is shown in purple and attributable risk to component metrics in blue. Darker colors indicate higher attributable risk. Variables exhibiting significant risk to B-IBI scores and/or component metrics are indicated in bold. See Appendix A for habitat definitions.

		OVERALL B-IBI	Taxa Richness	Ephemeroptera Richness	Plecoptera Richness	Trichoptera Richness	Clinger Richness	Long-Lived Richness	Intolerant Richness	Percent Dominant	Percent Predator	Percent Tolerant
Substrate	**% Cobble**	**41**	24	**43**	**35**	**37**	**38**	21	**48**	6	17	0
	% Gravel Coarse	**37**	20	**45**	21	**28**	**44**	17	**39**	5	12	2
	% Gravel Fine	-9	-5	1	-15	-11	-1	-13	3	-5	-16	3
	% SandFines	**44**	24	**52**	**35**	**40**	**44**	18	**51**	6	17	0
	% Wood	-1	2	-10	23	14	-6	11	5	2	18	-16
	Mean % Embed	**47**	**31**	**49**	**31**	**40**	**51**	21	**48**	9	13	0
Riparian / Fish Cover / Woody Debris	**FishCv Algae**	6	1	3	9	8	-2	2	8	1	12	9
	FishCv Big	10	6	5	16	20	9	9	9	-5	9	3
	FishCv Brush	7	7	-9	7	9	-1	12	-11	7	19	-4
	FishCv LWD	-2	-7	-8	8	1	-3	0	4	-10	9	-13
	FishCv Natural	2	7	0	5	6	3	7	-3	1	4	0
	FishCv NoAqVeg	0	5	-2	6	7	1	8	-5	2	5	1
	FishCv OvHgVeg	2	3	-10	5	2	-9	19	-14	7	17	-9
	FishCv TreesRoots	17	11	8	13	18	16	15	9	15	26	-7
	FishCv Undercut	16	20	8	10	15	9	27	-10	17	13	-11
	Mean % FishCv Algae	9	1	10	7	10	5	4	7	6	9	3
	Mean % FishCv Big	9	5	0	9	17	4	8	2	1	1	-12
	Mean % FishCv Brush	-4	2	-10	-4	-8	-9	-5	-18	-2	14	-12
	Mean % FishCv LWD	1	3	-5	7	-3	-1	6	3	-8	11	-17
	Mean % FishCv Natural	0	6	-12	3	-3	-5	12	-16	2	13	-11
	Mean % FishCv NoAqVeg	6	12	-3	9	5	1	14	-11	1	16	-8
	Mean % FishCv OvHgVeg	-1	9	-10	9	-2	-10	17	-12	5	28	-9
	Mean % FishCv TreesRoots	18	13	10	15	19	18	17	11	13	28	-5
	Mean % FishCv Undercut	16	20	8	10	15	9	27	-10	17	13	-11
	Mean % ShadeBnk	7	-4	5	26	21	3	10	13	3	17	0
Surface Water	**Dissolved Oxygen**	**35**	20	**41**	**46**	21	24	21	**40**	20	16	3
	Total Phosphorus	**37**	23	**43**	22	19	**42**	6	**53**	10	14	29
	Total Nitrogen	10	14	18	1	10	16	-9	16	-15	-4	4
	Turbidity	**32**	29	**41**	22	**25**	**39**	7	**37**	9	5	16
	pH	**17**	7	**21**	12	9	**19**	3	**16**	7	4	-9
Sediment	**Benz[a]anthracene**	3	-3	12	3	4	10	-11	8	-9	-12	-4
	Benzo(a)pyrene	3	-3	12	3	4	10	-11	8	-9	-12	-4
	Chrysene	4	2	4	4	3	4	1	4	-1	-4	-1
	Dibenzo(a,h)anthracene	3	-10	8	-3	1	3	-13	5	-4	-7	-5
	Fluoranthene	3	3	5	5	4	5	3	5	1	-2	3
	Fluorene	1	-1	1	1	1	1	-1	1	-1	-1	-1
	Phenanthrene	2	-2	8	5	3	5	4	4	-2	1	0
	Pyrene	2	0	7	4	3	5	-1	4	-3	-6	5
	Zinc	2	1	2	-6	-1	2	-1	-1	-2	-8	-5
	Arsenic	-2	-5	-2	-5	1	-1	-6	-3	-5	-9	-4
	Copper	-27	-17	-26	-23	-27	-25	-24	-28	-30	-17	-12

4.0. DISCUSSION

The utilization of relative risk analysis with western Washington stream and small river data has facilitated the identification and prioritization of major physical and chemical stressors that impact the regional index of watershed health, B-IBI. This analysis is important to resource management in several ways: it evaluates risk factors independently, stressor risks are ranked by strength of association to biota response, and interpretation of the resulting analysis are simple and interpretable measures of regional stressor severity. The results from these data indicate that physical habitat parameters were found to be major drivers of B-IBI scores, while measures of water quality were important, but less influential. Sediment chemistry had the smallest impact to aquatic biota.

Of all the physical and chemical variables examined in this study, substrate variables in poor condition significantly contributed to B-IBI decline. Reducing habitat complexity from diverse heterogeneous substrates to a homogenous sand/silt or fine particle substrate can have deleterious effects on benthic macroinvertebrates. Increased sedimentation deposited in rivers and streams can contribute to substrate embeddedness and reduce habitat complexity in stream and river channels. Excessive sedimentation has been associated with reduced feeding activity (Arruda et al. 1983), invertebrate movement (Rosenberg and Wiens 1978), and abundance and fecundity (Kirk 1992), in addition to changes in overall community structure (Tebo 1955; Gammon 1970; Gray and Ward 1982; Kreutzweiser et al. 2005). Although concentrations of both suspended solids and nutrients are controlled by a variety of natural factors, anthropogenic disturbances from urbanization, deforestation, and agriculture have the greatest impact on sediment runoff and accumulation in streams and rivers (Waters 1995; Wood and Armitage 1997; Zweig and Rabeni 2001; Parkhill and Gulliver 2002; Allan 2004). The importance of physical substrate to macroinvertebrate assemblages was reflected in attributable risk scores across B-IBI and its individual component metrics. Physical habitat variables indicative of more homogeneous substrates, or low habitat complexity (embeddedness, low percent cobble and coarse gravels, high percent of fines) resulted in the highest attributable risk values for overall B-IBI scores and many component B-IBI metrics in this study.

Based on attributable risk results, surface water quality parameters, although not as influential as habitat substrate, were also significantly associated with poor B-IBI scores (Table 7). Of the surface water parameters evaluated here, elevated phosphorous concentrations had the strongest relationship to poor B-IBI scores (overall B-IBI and 6 metrics). Because phosphorous is a limiting factor in most freshwater aquatic environments, an excess of this nutrient, can result in excessive periphyton growth (a mixture of green algae, diatoms and cyanobacteria), which can subsequently cause fluctuations in DO and pH and may negatively impact biota (Jones et al. 1998; Biggs 2000; Dodds et al. 2002). Natural phosphorous sources include leafy debris, natural weathering of rock, soils, and organic material; however, elevated TP levels in watersheds associated with eutrophication are primarily linked with agriculture and urban development (Nixon 1995; Carpenter et al. 1998). Concentrated levels of phosphorous are commonly used in fertilizers for agricultural practices. Non-point source runoff or wastewater discharge (including leaking sewer conveyance and onsite septic systems), particularly from agriculture and impervious substrates, can increase primary productivity and degrade stream condition, a consequence of eutrophication (i.e., nutrient enrichment).

Another nutrient associated with urbanization, nitrogen, did not show the same relationship to overall B-IBI scores as phosphorous, TN did not exhibit significant risk to macroinvertebrate taxa overall, although higher AR values were seen in sensitive taxa including, Ephemeroptera (18%), clinger (16%), and intolerant species (16%). The lack of relationship between excess TN and poor overall B-IBI scores may be related to the fact that, unlike phosphorus, nitrogen is typically not a liming nutrient in stream systems. Ecosystem response to the addition of excess nutrients, in this case the addition of phosphorous with existing nitrogen, may induce excessive periphyton proliferation where the presence of nitrogen alone may not elicit the same periphyton growth. Although nutrient input has been linked with periphyton production, multiple studies have indicated that other factors, such as light intensity (Triska et al. 1983), flow-related disturbances (Dodds et al. 2002) and their interactions may influence periphyton biomass in addition to nutrient input alone. These results may reflect the correlative, confounded, or interactive effects of the two nutrients and may point to further synergistic relationships with other physical and chemical variables, such as riparian cover, temperature, DO, and pH that should be examined further.

Other water quality parameters, DO, turbidity, and pH were also found to contribute to low B-IBI scores. DO, essential for the most basic of aquatic macroinvertebrate biological functions, was determined to have one of the highest mean attributable risk of all other water quality parameters affecting overall B-IBI scores and three metric components (mean attributable risk 26%). Elevated turbidity, indicative of increased sedimentation, was found to be an important risk to overall B-IBI scores and four component metrics and consistent with the results described above for the physical habitat portion of this evaluation. This analysis also indicated that more acidic (<6.0 pH) or alkaline (>8.5 pH) waters were highly associated with poor overall B-IBI scores and three component metrics containing chemically sensitive taxa: Ephemeroptera, clinger, and intolerant richness. Elevated or low pH levels may also contribute to reduced macroinvertebrate diversity and can possibly limit survivorship of the most intolerant taxa, including Ephemeroptera. Although variable effect on regional biota is examined here individually, synergistic effects of nutrient input (TN, TP) with alterations in conditions of DO, pH and turbidity are well known factors influencing eutrophication and this relationship should not be overlooked (Yang et al. 2008).

Sediment chemistry variables had very little impact on B-IBI scores compared to the other stressor groups evaluated in this study. None of the variables examined had significant contribution to poor overall B-IBI scores. Other analytes at these sites were low in concentration and could not be examined due to the minimal number of sites in poor stressor condition; therefore, impact from additional sediment chemistry variables to the B-IBI is unknown. But it is likely that the low extent of poor sediment chemistry conditions is most likely beneficial to regional macroinvertebrate communities.

Metal concentrations in stream sediments (arsenic, copper, zinc) did not yield significant risk to B-IBI. Copper exhibited an unexpected relationship to B-IBI: out of all study sites, only 7% contained both poor copper conditions and associated poor B-IBI scores, which was extremely low considering the high percentage of sites in poor copper condition (40%). This poor association was reflected in the resulting risk values (relative risk = 0.5; attributable risk = -27%). Further evaluation of the data showed that copper was weakly correlated with B-IBI scores (Pearson's R = 0.38), suggesting that sediment copper concentration was not only associated with B-IBI, but actually associated with higher B-IBI scores. This result was unexpected and, with further data exploration, the interpretation of the relationship of copper deposition and B-IBI is questionable. The risk results for copper levels in stream sediments may highlight the differences, and perhaps disconnect, between sediment chemistry results, usually sampled within fine sediments (Merritt et al. 2010), and the macroinvertebrate-based metrics, which are typically sampled in coarser substrates (Merritt 2009). Here, we do not conclude that high metal deposition, specifically copper, in sediments is low risk or beneficial to benthic macroinvertebrates; previous studies demonstrate the opposite (Clements et al. 2000; Hickey and Clements 1998; Ruse and Hermann 2000; Giddings et al. 2001). Rather, relative risk results may reflect the weak relationship of sediment-deposited metal analytes and B-IBI scores or, alternatively, possibly low regional deposition of metals, causing minimal impact to macroinvertebrate assemblages, although these hypotheses remain to be investigated.

Alternative thresholds for copper should also be considered. The most stringent thresholds were used in this study to analyze sediment-deposited metals (Table 4). For copper results, sites containing ≥ 35.7 mg/kg copper in sediment samples were considered "poor." This value was published by Smith et al. (1996) who used a metadata approach, matching biological and chemical data from numerous modelling, laboratory, and field studies performed on freshwater sediments, to determine sediment quality criteria. This threshold is extremely low compared to the 400 mg/kg value given by WA Ecology's Sediment Quality Standard (SQS), where values at or below the SQS criteria are expected to have no adverse effects on biological resources (WAC 173-204). If the WA state SQS was used to designate condition class of copper instead of the value published in Smith et al. 1996, none of the sites evaluated would be designated with a poor condition class. This finding highlights the importance of threshold determination. All thresholds used in this study were determined by a combination of published sources, when available, ambient data distributions, and best professional judgment. For future risk analyses, adjusting thresholds to either reference conditions, or state criteria, may provide a more accurate representation of the relationship between sediment-deposited metal risk and B-IBI or, at the very least, allow the comparison of B-IBI risk from sediment-deposited metals at different concentrations. Alternatively, analyzing dissolved metal concentrations in the water column instead of sediment metal deposition may elucidate the risk of metals and biota.

The results presented here summarize major stressors to western Washington watershed biota. While the results of this study are relatively straightforward in ranking stressor risk, the data must be interpreted carefully. A number of factors may influence result interpretation, including correlation of environmental variables, threshold determination, and the addition of more environmental variables not analyzed in this study. Additionally, the confidence intervals reported in this study were adjusted to avoid type I errors. This statistical adjustment is known to be quite conservative (Legendre & Legendre 2012), meaning some environmental variables reported as non-significant to B-IBI or its metrics using this approach may, in fact, have significant impact to benthic macroinvertebrate taxa using another, less conservative statistical method to control for type I errors. Trends of increased attributable risk values across B-IBI metrics, such as the results reported for riparian cover by tree roots or undercut banks, should be further examined for importance to macroinvertebrate taxa, even if they are non-significant.

Interpretation of relative risk results can be difficult due to existing multicollinearity between the various physical and chemical stressor variables. Past relative risk studies have worked around this issue by grouping highly correlated variables prior to analysis to ease interpretation and to facilitate resource management decisions (Van Sickle and Paulsen 2008; Van Sickle 2013). Here we chose to present these data without adjusting attributable risk for covarying stressors to illustrate the relative importance and individual contributions of each variable to B-IBI indices within our study region. Because we did not group correlated variables, individual parameters, such as TN, TP, or highly correlated physical habitat metrics, such as sedimentation and embeddedness, should be interpreted with care since complex, synergistic relationships of variables may be underrepresented and individual variable associations to biological response may be overrepresented (Van Sickle 2013).

Data distributions were used to determine thresholds of B-IBI and ambient distribution-based criteria used to set physical habitat thresholds. Other studies may choose to approach this analysis differently and adjusting the thresholds that determine condition class for each variable and biological response may influence relative risk results. Future analyses may choose to incorporate thresholds specific to regional conditions, or establish alternative thresholds using reference stream conditions to validate the results presented here. The approach to threshold determination used in this study was our best effort to use a combination of published criteria and data distributions to set thresholds as objectively as possible. These conditions do not limit the ability of relative risk to reveal the major environmental variables driving B-IBI response in western Washington. Major patterns of biological disturbance are evident, giving us a broad picture of the most influential environmental variables affecting B-IBI and provide direction for management or restoration efforts in the region. As more reference information becomes available from EPA and Ecology in western Washington, physical habitat variable thresholds can be adjusted to be representative of "least-disturbed" sites, rather than by ambient distributions alone.

Additionally, we recognize that stream flow is a major physical attribute of stream ecology because it exerts control over multiple structural attributes including habitat volume, current velocity, channel geomorphology, and bank stability (Poff and Ward 1989). There is great interest in expanding this analysis to include hydrological measurements to explore the relationship between stream dynamics and biology. Although flow data was available for each site, these measurements were taken from discrete flow meter readings (Merrit

2009). Continuous flow was preferred over discrete measurements and, therefore, these data were not used. Watershed urbanization can not only influence local-scale stream and channel morphology, substrate, and physiochemical properties but can also influence pollutant and sedimentation deposition, impacting freshwater biota. Changes in flow due to urban development or impervious surfaces can have a major impact on stream hydrology, including increased flashiness, shorter, more frequent peak flows, higher magnitude and increased total runoff volume (EPA 1997; McMahon et al. 2003), which can directly impact freshwater invertebrate taxa richness, recruitment, and community structure (Poff and Ward 1989; Clausen and Biggs 1997).

We recommendation that future study designs include variables we were unable to incorporate here, specifically stream flow characteristics, land use, basin size and basin-level characteristics, and riparian inventory at multiple geographical scales. Differences in basin-level characteristics such as drainage area, drainage density, shape, and relief within each basin may influence which environmental variables drive biological response. Effects of urbanized areas on watershed biology may be captured with additional metrics associated with land use changes, forest cover fragmentation, and impervious surfaces.

4.1 Conclusions and Suggestions

Risk analysis of the major physical and chemical environmental variables with B-IBI and its component metrics in western Washington revealed:

1) Several variables were significantly associated with biological response:
    - Variables associated with physical habitat, specifically fine substrate conditions, were the most influential to B-IBI scores and component metrics.
    - Water quality parameters of TP, DO, turbidity, and pH were strongly associated with poor B-IBI, but less influential than substrate.
    - The sediment chemistry parameters evaluated had the least influence on biological condition.
2) Relative risk analysis offers an unbiased way to examine biological importance and prioritize stressors.
3) Caveats of the analysis include multicollinearity of variables and simplification of chemical and physical dynamics of water systems. Multicollinearity may overemphasize associations with biota. Synergistic relationships between variables should be further explored or the most correlated variables consolidated.

Relative risk analysis was utilized to determine regionally significant stressors driving B-IBI scores and can assist in directing restoration strategies for western Washington sites. The study results suggest that targeting restoration of physical habitat, specifically rebuilding riparian buffers and remediating excessive sources of sedimentation, could improve regional watershed health and water quality. Prioritization of water quality monitoring and management of parameters, such as nutrient enrichment, DO, and pH, shown to significantly impact biota throughout these basins, can provide critical information needed to protect sites in excellent biological health and help identify potential sources of impairment. To help minimize the effects of eutrophication in highly urbanized areas, efforts to understand the mechanisms of nutrient input (excess phosphorous, nitrogen) linked with periphyton production within western Washington watersheds may improve water quality within the region. Additionally, rebuilding and maintaining vegetative areas to restore bank stabilization, reduce sedimentation and erosion, and limit excess nutrient/contaminants from entering the watershed may offer protection from nonpoint source pollution.

This analysis can be built upon for future study. As more survey data are published and habitat metrics updated, continued characterization of physical and chemical stressor impact to watershed health is necessary. Relative risk analysis can be adjusted to incorporate more information as it becomes available and future, iterative relative risk analyses may build on the results presented here. Ecology is currently incorporating additional habitat assessment measures into its monitoring program. These supplementary metrics will allow future assessments to examine variables that may be more representative of overall riparian conditions. Grouping highly correlated stressors, or reducing the numbers of correlated variables in analyses, may give a clearer representation of the relationships between major contributing environmental variables and B-IBI. The results presented here reflect the major associations between physical and chemical attributes and freshwater biota in the region; however, using a weighted statistical approach, when data are available, would allow us to present results as though every stream and river in the region had been sampled, regardless of stream size or length. Additionally, relative risk can be expanded to other biological indicators, such as fish biological indices, to better understand how stressors impacting macroinvertebrate communities differ from fish assemblages within the same geographic areas.

5.0. REFERENCES

Allan, J.D. 2004. Landscapes and riverscapes: The influences of land use on stream ecosystems. Annual Review of Ecological Systems 35:257- 284.

Arruda, J.A., G.R. Marzolf, R.T. Faulk. 1983. The role of suspended sediments in the nutrition of zooplankton in turbid reservoirs. Ecology 64:1225-1235.

Biggs, B.J.F. 2000. Eutrophication of streams and rivers: dissolved nutrient chlorophyll relationships for benthic algae. Journal of the North American Benthological Society 19:17–31.

Booth, D.B., J.R. Karr, S. Schauman, C.P. Konrad, S.A. Morley, M.G. Laron, S.J. Burges. 2004. Reviving urban streams: land use, hydrology, biology, and human behavior. Journal of the American Water Resources Association 40:1351-1364.

Carpenter, S., N. Caraco, D.L. Correll, R. Howarth, A. Sharply, V. Smith. 1998. Nonpoint source pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8: 559-68.

Clausen, B., B.J.F. Biggs. 1997. Relationship between benthic biota and hydrological indices in New Zealand streams. Freshwater Biology 38: 327-342.

Clements W.H., D.M. Carlisle, J.M. Lazorchak, P.C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications 10:626–638.

DeGasperi, C.L., H.B. Berge, K.R. Whiting, J.J. Burkey, J.L. Cassin, R.R. Fuerstenberg. 2009. Linking hydrologic alteration to biological impairment in urbanizing streams of the Puget Lowland, Washington, USA. Journal of the American Water Resources Association 45:512–533.

Dodds, W.K., V.H.Smith, K. Lohman 2002. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Canadian Journal of Fisheries and Aquatic Sciences, 59: 865-874.

Environmental Protection Agency 1997. Urbanization and streams—Studies of hydrologic impacts: EPA Report 841–R–97–009, Washington, D.C., 15 p.

Environmental Protection Agency 2000. Ambient water quality criteria recommendations information supporting the development of state and tribal nutrient criteria for rivers and streams in nutrient ecoregion II. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, DC EPA Document 2000- 822-B-00-015.

Fore, L.S., K. Paulsen, K. O'Laughlin. 2001. Assessing the performance of volunteers in monitoring streams. Freshwater Biology 46:109-123.

Fore, L.S., J.R. Karr, R.W. Wisseman. 1996. Assessing invertebrate responses to human activities: evaluating alternative approaches. Journal of the North American Benthological Society 15:212-231.

Gammon, J.R. 1970. The effect of inorganic sediment on stream biota. Water Pollution Control Research Series 18050 DWC 12/70, U. S. Environmental Protection Agency, Washington, D.C. 141 p.

Giddings, E.M., M.I. Hornberger, H.K. Hadley. 2001. Trace-metal concentrations in sediment and water and health of aquatic macroinvertebrate communities of streams near Park City, Summit County, Utah. U. S. Geological Survey. Water-Resources Investigations Report 01–4213. http://pubs.usgs.gov/wri/wri014213/pdf/wri014213.pdf

Gray, L.J. and J.V. Ward. 1982. Effects of sediment releases from a reservoir on stream macroinvertebrates. Hydrobiologia 96:177-184.

Hickey, C.W. and Clements, W.H. 1998. Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. Environmental Toxicology and Chemistry 17:2338–2346.

Karr, J.R. 1998. Rivers as sentinels: using the biology of rivers to guide landscape management. Pages 502-528. In Naiman, R.J. and R.E. Bilby (editors). River Ecology and Management: Lessons from the Pacific Coastal Ecosystem. Springer, New York, NY.

Kleindl, W.J. 1995. A benthic index of biotic integrity for Puget Sound lowland streams, Washington, USA. Page 64. College of Forest Resources. University of Washington.

Kreutzweiser, D.P., S.S. Capell, K.P. Good. 2005. Effects of fine sediment inputs from a logging road on stream insect communities: a large-scale experimental approach in a Canadian headwater stream. Aquatic Ecology 39:55-66.

Legendre, P and L. Legendre. 2012. *Numerical Ecology*. 3rd Edition. Elsevier, Oxford, UK. 905 p.

MacDonald, D.D., C.G. Ingersoll, T.A. Berger. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Archives of Environmental Contamination and Toxicology 39:20-31.

McMahon, G., J.D. Bales, J.F. Coles, E.M.P. Giddings, H. Zappia. 2003. Use of stage data to characterize hydrologic conditions in an urbanizing environment. Journal of the American Water Resources Association 39:1529–1546.

Merritt, G. 2009. Status and Trends Monitoring for Watershed Health and Salmon Recovery: Field Data Collection Protocol. Wadeable Streams. Draft, May 14, 2009. Environmental Assessment Program. Washington State Department of Ecology, Olympia, WA. www.ecy.wa.gov/programs/eap/stsmf/docs/01SnTWadeableManA-Vv3bhfl.pdf

Merritt, G., D. Monahan, C. Hartman. 2010. Status and Trends monitoring for watershed health and salmon recovery: field data collection protocol. Wide streams and rivers. Draft, January 27, 2010. Environmental Assessment Program. Washington State Department of Ecology, Olympia, WA. www.ecy.wa.gov/programs/eap/stsmf/docs/01-27-10DRAFT_WHSR_Rivers_Manual.pdf

Merritt, G. and C. Hartman. 2012. Status of Puget Sound Tributaries 2009. Biology, Chemistry, and Physical Habitat. Publication No. 12-03-029. Environmental Assessment Program. Washington State Department of Ecology, Olympia, WA. https://fortress.wa.gov/ecy/publications/publications/1203029.pdf

Morley, S.A. and J.R. Karr. 2002. Assessing and restoring the health of urban streams in the Puget Sound basin. Conservation Biology. 16:1498-1509.

Nixon, S.W. 1995. Coastal marine eutrophication: a definition social causes, and future concerns. Ophellia 41:199-219.

Parkhill, K.L. and J.S. Gulliver. 2002. Effect of inorganic sediment on whole- stream productivity. Hydrobiologia 472: 5- 17.

Paulsen, S.G., A. Mayio, D.V. Peck, J.L. Stoddard, E. Tarquinio, S.M. Holdsworth, et al. 2008. Condition of stream ecosystems in the US: an overview of the first national assessment. Journal of the North American Benthological Society 27:812-821.

Poff, N. L., J. V. Ward. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. Canadian Journal of Fish and Aquatic Science 46:1805-1817.

R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3- 900051-07-0, URL: http://www.r-project.org/.

Rosenberg, D.M. and A.P. Wiens. 1978. Effects of sedimentation on macrobenthic invertebrates in a northern Canadian river. Water Research 12:753-763.

Ruse, L.P. and S.J. Herrmann. 2000. Plecoptera and Trichoptera species distribution related to environmental characteristics of the metal-polluted Arkansas River, Colorado. Western North American Naturalist 60:57–65.

Scott, J.W., C.R. Vasquez, J.G. Newman, B.C. Sargent. 1989. Washington Centennial Atlas. Washington: Western Washington University Center for Pacific Northwest Studies.

Smith, S.L., D.D. MacDonald, K.A. Keenleyside, C.G. Ingersoll, J. Field. 1996. A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. Journal of Great Lakes Research 22:624-638.

Sprague, L.A., R.E. Zuellig, J.A. Dupree. 2006. Effects of urbanization on stream ecosystems in the South Platte River Basin, Colorado and Wyoming. In Chapter A of Effects of Urbanization on Stream Ecosystems in Six Metropolitan Areas of the United States. U.S. Geological Survey Scientific Investigations Report 2006–5101–A, 139 p.URL: http://pubs.water.usgs.gov/SIR2006–5101A/

Stoddard, J.L., A.T. Herlihy, B.H. Hill, R.M. Hughes, P.R. Kaufmann, D.J. Klemm et al. 2006a. Mid-Atlantic Integrated Assessment (MAIA)—State of the Flowing Waters Report. EPA/620/R-06/001, U.S. Environmental Protection Agency, Washington, DC.

Stoddard, J.L., D.P. Larsen, C.P. Hawkins, R.K. Johnson, R.H. Norris 2006b. Setting expectations for the ecological condition of streams: The concept of reference condition. Ecological Applications 16:1267–1276.

Tebo, L.B. Jr. 1955. Effects of siltation, resulting from improper logging, on the bottom fauna of a small trout stream in the southern Appalachians. Progressive Fish Culturist 17:64-70.

Triska, F.J., V.C. Kennedy, R.J. Avanzino, B.N. Reilly. 1983. Effect of simulated canopy cover regulation of nitrate uptake and primary production by natural periphyton assemblages. In Dynamics of lotic ecosystems. Edited by T.D. Fontaine III and S.M. Bartell. Ann Arbor Science Publishers, Ann Arbor, Michigan. pp. 129–160.

Van Sickle, J. 2003. Analyzing correlations between stream and watershed attributes. Journal of the American Water Resources Association 39:717-726.

Van Sickle, J., J.L. Stoddard, S.G. Paulsen, A.R. Olsen. 2006. Using relative risk to compare the effects of aquatic stressors at a regional scale. Environmental Management 38:1020–1030.

Van Sickle, J. and S.G. Paulsen. 2008. Assessing the attributable risks, relative risks, and regional extents of aquatic stressors. Journal of the North American Benthological Society 27:920–931.

Van Sickle, J. 2013. Estimating the risks of multiple, covarying stressors in the National Lakes Assessment. Freshwater Science 32:204-216.

Washington State Department of Ecology. 2012. Water Quality standards for surface waters of the state of Washington, Chapter 173-201A WAC. Publication 06-10-091. Washington State Department of Ecology, Olympia, WA. http://www.ecy.wa.gov/programs/wq/swqs/criteria.html

Washington State Department of Ecology. 2013. Sediment Management Standards, Chapter 173-204 WAC. Publication 13-09-055. Washington State Department of Ecology, Olympia, WA. https://fortress.wa.gov/ecy/publications/publications/1309055.pdf

Waters, T.F. 1995. Sediment in streams: sources, biological effects and control. Bethesda, Maryland, American Fisheries Society Monograph 7.

Wood, P.J. and P.D. Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environmental Management. 21:203- 217.

Yang, X., X. Wu, H. Hao, Z. He. 2008. Mechanisms and assessment of water eutrophication. Journal of Zhejiang University Science: Biomedicine and Biotechnology 9:197-209.

Zweig, L.D. and C.F. Rabeni. 2001. Biomonitoring for deposited sediment using benthic invertebrates: a test on 4 Missouri streams. Journal of the North American Benthological Society 20:643-657.

Appendix A

Status & Trends Monitoring for Watershed Health and Salmon Recovery physical habitat variable definitions.

Variable Abbreviation	Relative Risk Abbreviation	WA Ecology Definition (Merritt and Hartman 2012)
Substrate		
PCT Cobble	% Cobble	Percent of all substrate-stations observed in a site with a substrate size class of cobble (CB).
PCT Fines	% Fines	Percent of all substrate-stations observed in a site with a substrate size class of fines (FN).
PCT GravelC	% Gravel Coarse	Percent of all substrate-stations observed in a site with a substrate size class of coarse gravel (GC).
PCT GravelF	% Gravel Fine	Percent of all substrate-stations observed in a site with a substrate size class of fine gravel (GF).
PCT SandFines	% SandFines	Percent of all substrate-stations observed in a site with a substrate size class of sand (SA) or fines (FN).
PCT Wood	% Wood	Percent of all substrate-stations observed in a site with a substrate class of wood (WD).
X Embed	Mean % Embed	Mean percent embeddedness for a site. An average for all observed substrate-stations.
Riparian / Fish Cover / Woody Debris		
PFC Algae	FishCv Algae	Percent of transects observed at each site that contain any fish cover from filamentous algae.
PFC Big	FishCv Big	Percent of transects observed at each site that contain any fish cover from artificial structures, boulders, live trees or roots, large woody debris, or undercut banks.
PFC Brush	FishCv Brush	Percent of transects observed at each site that contain any fish cover from brush.
PFC LWD	FishCv LWD	Percent of transects observed at each site that contain any fish cover from large woody debris.
PFC Natural	FishCv Natural	Percent of transects observed at each site that contain any fish cover, excluding cover from artificial structures.

PFC NoAqVeg	FishCv NoAqVeg	Percent of transects observed at each site that contain any fish cover, excluding cover from bryophytes, macrophytes or filamentous algae.
PFC OvHgVeg	FishCv OvHgVeg	Percent of transects observed at each site that contain any fish cover from overhanging vegetation.
PFC TreesRoots	FishCv TreesRoots	Percent of transects observed at each site that contain any fish cover from large trees or roots.
PFC Undercut	FishCv Undercut	Percent of transects observed at each site that contain any fish cover from undercut banks.
X ShadeBnk	Mean % ShadeBnk	Mean percent shade at a site's bankfull margin.
XFC Algae	Mean % FishCv Algae	Mean percent fish cover (water surface area) provided by filamentous algae, averaged across all observed transects.
XFC Big	Mean % FishCv Big	Sum of mean percent fish cover provided by these five types: Artificial + Boulders + TreesRoots + LWD + Undercut.
XFC Brush	Mean % FishCv Brush	Mean percent fish cover (water surface area) provided by brush, averaged across all observed transects.
XFC LWD	Mean % FishCv LWD	Mean percent fish cover (water surface area) provided by large woody debris, averaged across all observed transects.
XFC Natural	Mean % FishCv Natural	Sum of mean percent fish cover provided by these nine types: Boulders + Brush + Bryophytes + Algae + LWD + TreesRoots + Macrophytes + OvHangVeg + Undercut.
XFC NoAqVeg	Mean % FishCv NoAqVeg	Sum of mean percent fish cover provided by these seven types: Artificial + Boulders + Brush + LWD + TreesRoots + OvHangVeg + Undercut (excludes bryophytes, macrophytes and filamentous algae).
XFC OvHgVeg	Mean % FishCv OvHgVeg	Mean percent fish cover (water surface area) provided by overhanging vegetation, averaged across all observed transects.
XFC TreesRoots	Mean % FishCv TreesRoots	Mean percent fish cover (water surface area) provided by live trees or roots, averaged across all observed transects.
XFC Undercut	Mean % FishCv Undercut	Mean percent fish cover (water surface area) provided by undercut banks, averaged across all observed transects.