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Original Articles

The first statewide stream macroinvertebrate bioassessment in Washington State with a relative risk and attributable risk analysis for multiple stressors

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ABSTRACT

We report results from the first statewide assessment of biological health in perennial streams in Washington State. Using a probabilistic sampling survey design, we were able to make unbiased estimates of biological condition of macroinvertebrate communities throughout the state based on 346 sites sampled from 2009 to 2012. Results from randomly sampled sites were classified as either good, fair, poor in comparison with 75 regional reference sites that were sampled concurrently. We determined that approximately 34 percent of stream kilometers assessed were in poor biological condition as measured with a multi-metric index, the Benthic Index of Biotic Integrity. Additionally, we evaluated a variety of chemical and physical habitat stressors known to negatively influence macroinvertebrate communities and determined that poor substrate conditions were the most prevalent and important stressors impacting stream macroinvertebrates, with relative bed stability and percent sand/fines being the most prevalent. A relative risk/attributable risk analysis suggests that improving physical habitat conditions in streams, most notably a reduction in percent sand/fines, will have the greatest impact for improving biological condition for macroinvertebrate communities. It is estimated that approximately 60% of stream kilometers now classified as in poor biological condition in Washington could be improved by reducing the amount of percent sand/fines in the substrate. These results are consistent with those obtained from EPA's national stream surveys and suggest that poor habitat conditions are the most prevalent stressors impacting stream macroinvertebrates in Washington State.

1. Introduction

Aquatic resources are under an increasing threat of biodiversity loss due to human modifications to the landscape and climate (Vörösmarty et al., 2010; Kuemmerlen et al., 2015; Pyne and Poff, 2017). Many human activities have measureable deleterious impacts on aquatic resources, with streams particularly prone to the influences of human development and agricultural practices (Allan, 2004). Streams impacted by agriculture and/or urbanization are subject to modifications affecting the natural condition, including, but not limited to, altered flow regimes (Rosburg et al., 2017; Marshalonis and Larson, 2018), loss of riparian habitat (Osborne et al., 1993), and elevated delivery of fine sediments, nutrients and toxic substances (Paul and Meyer, 2001). These factors alone or in combination can alter aquatic community structure and function (Woodward et al., 2010; Pyne and Poff, 2017), beginning with replacement of sensitive taxa by more tolerant ones, followed by significant diversity loss (Dudgeon et al., 2006; Vörösmarty et al., 2010).

Evaluating biodiversity patterns in freshwater streams and rivers across broad geographic scales is necessary for elucidating questions about biodiversity loss. Biological monitoring programs with environmental data encompassing large spatial extents benefit from wide environmental gradients from which to make meaningful associations between stressors and biodiversity loss. Furthermore, comparison of results obtained from multiple monitoring programs encompassing various spatial scales (e.g. U.S. Environmental Protection Agency's National River and Streams Assessment (NRSA), https://www.epa.gov/national-aquatic-resource-surveys/nrsa) has great potential to inform efforts aimed at addressing biological impairment in streams and rivers. Freshwater macroinvertebrate communities are relatively good indicators of water quality and stream biological health and often the focus of monitoring efforts by agencies charged with evaluating water and habitat quality. In many applied situations, linking alterations in biological communities with environmental stressors is vital,

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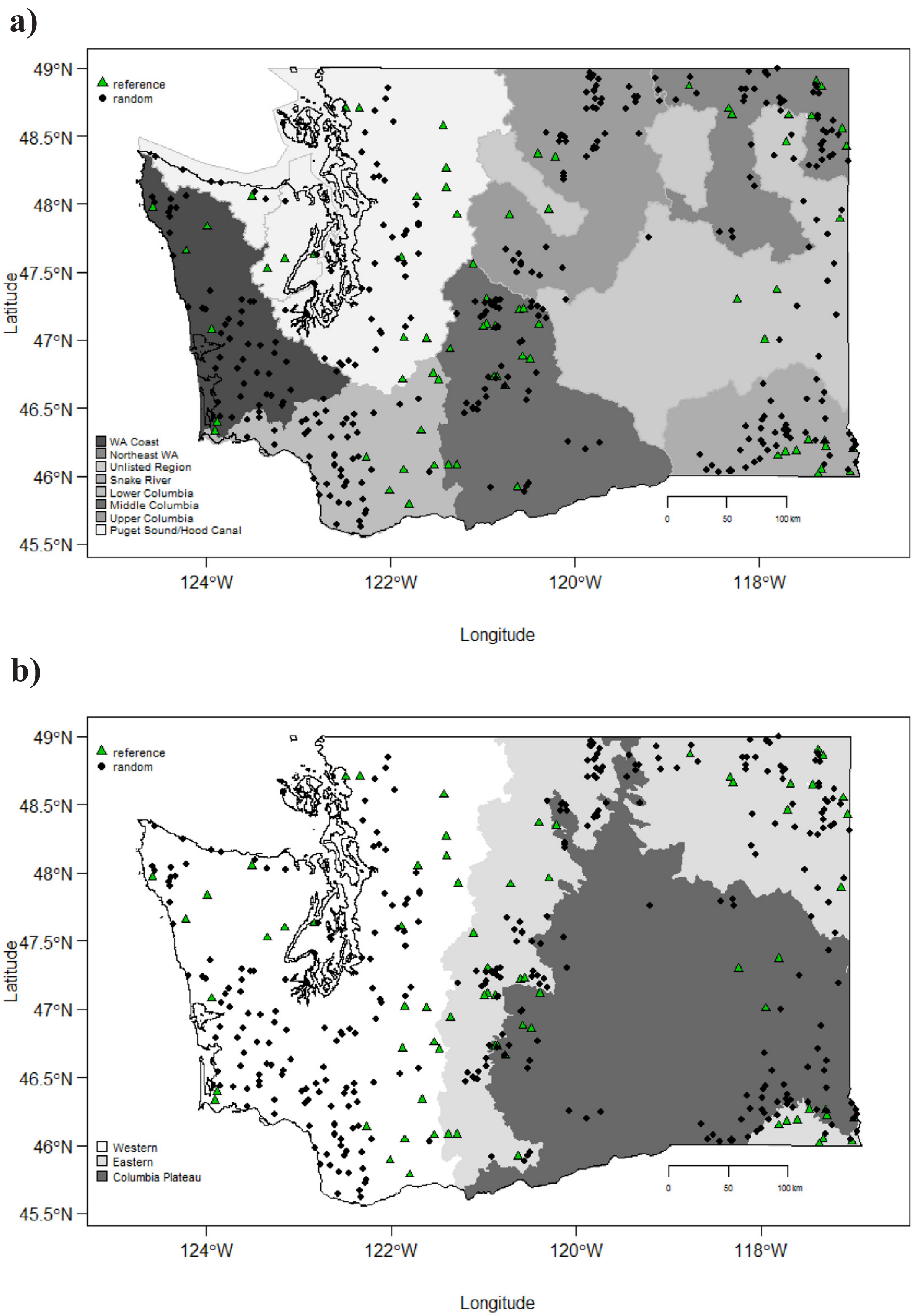

Fig. 1. (a) Random and reference sites sampled in eight Status and Trends Regions and (b) three assessment regions.

potentially giving stakeholders the necessary information for the implementation of more effective restoration efforts and management practices aimed at minimizing degradation. However, practical demonstrations of the successful implementation of effective management strategies and subsequent recovery of biological communities are relatively uncommon, with limited examples documented in the literature. Therefore, there is great need for scientists to provide better practical information about the causes and consequences of biodiversity losses in streams and rivers.

In the United States, several tools have been developed for evaluating the impacts of stressors on biological communities at local (U.S. EPA, 2000, 2007; Yuan and Norton, 2004; http://cfpub.epa.gov/caddis) and broader regional and national scales (Van Sickle and Paulsen, 2008). These types of exercises can be valuable for focusing efforts and limited resources on the practices that will be most effective at improving conditions at a local or regional scale (see Marshalonis

Table 1
Condition class thresholds for B-IBI and other potential stressors for three assessment regions in Washington State. Poor and good condition classes are defined in each cell, by the 1st and 2nd inequalities, respectively, with values between these two thresholds designated as fair condition. Unless denoted with superscript, all thresholds were determined using regional 'minimally impacted' reference sites as described in Section 2.4. LRBS = log relative bed stability, DgmLog10 = average substrate size, LWDSiteVolume = volume of large woody debris standardized to 100 m of stream reach.

Variable	Western WA	Eastern WA	Columbia Plateau
B-IBI (0–100)	49.98, 73.73	53.44, 63.0	36.37, 48.7
Water			
Conductivity (µS/cm)	162.25, 143.8	349.39, 117.65	309.24, 264.3
Dissolved Oxygen (mg/L)	9.77, 10.25	9.19, 9.7	7.44, 8.81
pH (Low)	6.5, 7.0	6.5, 7.0	6.5, 7.0
pH (High)	8.5, 7.5	8.5, 7.5	8.5, 7.5
Water Temperature (°C)	15.06, 13.65	14.2, 12.0	20.14, 17.23
Turbidity (NTU)	4.475, 1.73	9.96, 2.7	20.47, 15.95
Chloride (mg/L)	12.45, 5.61	1.86, 0.74	13.72, 6.07
Total Nitrogen (µg/L)[a]	229, 131	229, 131	462, 246
Total Phosphorus (µg/L)[a]	36, 14	36, 14	70, 36
Total Suspended Solids (mg/L)	5.47, 2	22.65, 6.0	44.4, 11.33
Sediment[b]			
Arsenic (mg/kg)	33, 9.8	33, 9.8	33, 9.8
Copper (mg/kg)	149, 32	149, 32	149, 32
Lead (mg/kg)	128, 36	128, 36	128, 36
Zinc (mg/kg)	459, 120	459, 120	459, 120
Total PAHs	22800, 1610	22800, 1610	22800, 1610
Habitat[c]			
DgmLog10	0.80, 1.37	−0.08, 0.69	0.03, 0.98
LRBS	0.26, −0.21	−0.28, −0.66	−0.51, −0.44
Reach Slope %	0.5, 2	0.5, 2	0.5, 2
Sinuosity	1.06, 1.11	1.03, 1.09	1.03, 1.09
Sand/Fines %[d]	25.5, 15.5	25.5, 15.5	25.5, 15.5
Embeddedness %	46.93, 35.10	70.27, 54.38	63.99, 45.63
LWDSiteVolume (m^3 per 100 m)	7.80, 25.11	2.15, 6.39	0.19, 1.64
Canopy Cover (proportion)	0.87, 0.95	0.93, 0.98	0.46, 0.82

[a] Thresholds set using values from Wadable Streams Assessment (Van Sickle and Paulsen, 2008).
[b] Sediment Quality Standard/Screening Level 1 values from Michelsen (2011) (Arsenic = 14 mg·kg^{-1}, Copper = 400 mg·kg^{-1}, Lead = 360 mg·kg^{-1}, Zinc = 3200 mg·kg^{-1} Total PAHs = 17,000 µg·kg^{-1}).
[c] Detailed descriptions of habitat metrics in Janisch (2013).
[d] Thresholds set using EMAP West survey (Bryce et al., 2010).

and Larson, 2018 for a recent example). When available data encompass broad spatial scales, relative risk and the associated attributable risk analyses have been used to link poor biological conditions in streams to environmental stressors. These types of analyses have great potential for informing restoration efforts (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). Additionally, comparison of results from regional assessments with those obtained from broader national surveys (e.g. NRSA) will aid efforts to better understand the response of stream biological communities to human induced stressors across multiple scales.

In Washington State, one tool for assessing the biological health of streams is the Benthic Index of Biological Integrity (B-IBI), a macroinvertebrate multi-metric index (Karr, 1998, Morley and Karr, 2001). The B-IBI is composed of 10 individual diversity metrics quantifying different components of the macroinvertebrate community (more information at: https://www.pugetsoundstreambenthos.org/About-BIBI.aspx). However, in Washington, there has not yet been a statewide assessment of the biological health of perennial streams, or evaluations to determine the most frequent set of stressors impacting these streams. This has hampered efforts to develop standard protocols for addressing stream rehabilitation. Once biological impairment has been determined for a site, evaluating the most likely set of stressors contributing to poor biological condition will give decision makers the ability to focus on those stressors having the greatest potential for improving conditions (Yuan and Norton, 2004).

Here, we report the first statewide evaluation of stream macroinvertebrate communities in Washington State using a probabilistic sampling design, implemented by the Environmental Assessment Program at the Washington State Department of Ecology (ECY). The design allows for unbiased estimates of biological and habitat conditions. Our objectives were threefold: 1) determine the proportion of stream kilometers within Washington that are in 'good', 'fair' and 'poor' biological condition using macroinvertebrate communities as a proxy for biological health, 2) evaluate the proportion of stream kilometers in 'poor' condition for a variety of stressors known to impact stream macroinvertebrate communities, and 3) conduct a relative risk/attributable risk analysis for establishing the most probable set of stressors impacting the biological health of macroinvertebrates in perennial streams. By establishing the status of biological health in streams across the state at various spatial scales and employing techniques that link stressors and impairment, we will inform discussions focused on addressing the maintenance and rehabilitation of biological diversity of stream communities.

2. Materials and methods

2.1. Sample frame

Using a spatially balanced probabilistic sampling design (Generalized Random Tessellation Stratified (GRTS); Stevens and Olsen, 2004), 346 randomly selected sites (278 wadeable streams and 68 larger non-wadeable rivers, *see* Section 2.3) were sampled once in eight Status and Trends Regions and an Unlisted Region in Washington State from 2009 to 2012 (Fig. 1a; more information at: https://ecology.wa.gov/Research-Data/Monitoring-assessment/River-stream-monitoring/Habitat-monitoring/Watershed-health). Status and Trends regional boundaries represent state watersheds for coordinating monitoring efforts and developing recovery plans for threatened or endangered salmonid fishes (more information at: https://rco.wa.gov/salmon_recovery/regions/regional_orgs.shtml). Sample sites were chosen from a 1:24,000 scale statewide master sample frame, with the goal of allocating sampled sites equally among regions and five stream order classes, i.e., 0-order, 1st-order, 2nd-order, 3rd-order, and 4th-order+. In order to supplement rather than duplicate ongoing monitoring efforts on Federal and tribal lands in Washington (e.g. USFS Pacfish/Infish Biological Opinion (Roper et al., 2010) and USFS Aquatic and Riparian Effectiveness Monitoring Program (Gallo et al., 2005)), we chose to exclude sites in these parts of Washington and focus more on non-Federal, as well as publicly and privately owned lands. We also excluded tidal streams, streams in constructed channels, and great rivers (i.e. the Columbia River and lower Snake River) since samples from these types of sites would likely have contained very different macroinvertebrate communities. One advantage of employing a GRTS survey design is the ability to interpret data at multiple spatial scales, either at the scale the samples were collected, i.e. Status and Trends Regions in our situation, or at larger scales, e.g. statewide.

Simultaneous to the randomly sampled sites, 75 targeted 'minimally impacted' reference sites were also sampled across the state (Fig. 1). These targeted sites were chosen based on best professional judgement after previous visits and after evaluation for meeting conditions of low human influence (e.g., minimal road density, impervious cover, etc.; Wilmoth et al., 2015) and were sampled for the purpose of establishing expectations under minimal human influence and for setting regional thresholds (*see* Section 2.4) for the variables used in the Relative Risk/Attributable Risk (RR/AR) analyses (Table 1).

2.2. Biological and chemical data

Composite samples of macroinvertebrate communities along each stream reach were collected from 0.74 square meters of surface area across eight randomly sampled pool/riffle transects at each stream reach using a D-frame kick net with a 500 μm net. All samples were preserved in ethanol and sent to Rhithron Associates, Inc. (Missoula, Montana) for sorting, identification and counting. In the lab, a subsample of 500 organisms was counted and identified for each sample, typically to genus or species. In addition to collecting samples of macroinvertebrates, at the beginning of each sampling visit, water samples were also collected. These samples for total phosphorus, total nitrogen, turbidity, total suspended solids, and chloride concentration were analyzed at Washington Department of Ecology/EPA's Manchester Environmental Laboratory (https://ecology.wa.gov/About-us/Get-to-know-us/Our-Programs/Environmental-Assessment/Manchester-Environmental-Laboratory). Sediment samples were also collected at the beginning of each sampling visit, from a composite of three random locations in the stream reach. Samples for arsenic, copper, lead, zinc and poly-aromatic hydrocarbons (PAHs) were analyzed at the Manchester Environmental Laboratory. Furthermore, at the beginning and end of each sampling visit, water temperature, dissolved oxygen, conductivity, and pH were measured with a Hach® portable meter that had been calibrated for each parameter.

2.3. Physical habitat metrics

Each sample stream reach extended 20× average bankfull width (not less than 150 m and not more than 2 km). Eleven equidistant transects across the stream channel were evaluated for a variety of factors, including substrate size, channel dimensions, in-stream cover, and riparian cover.

For measures of substrate at waded streams, eleven observations of substrate size and embeddedness were performed across the stream channel at each of the eleven transects. Substrate size was also assessed at ten midpoint transects. In all, substrate size for waded streams was evaluated at 231 points among these 21 equidistant transects. Substrate at larger rivers was assessed at eleven transects, among a variable number of observation points per transect. More detailed descriptions of all physical habitat measures collected can be found at: https://ecology.wa.gov/Research-Data/Monitoring-assessment/River-stream-monitoring/Habitat-monitoring/Habitat-monitoring-methods.

During field sampling events, where sites were sampled once, additional habitat measurements were conducted while traversing up through the thalweg of the reach. These included measures of thalweg water depth (100 equidistant points) bearing (20 observations), reach slope, and a large woody debris tally. Habitat metrics, calculated from the field data, were downloaded from the Watershed Health Monitoring database (see https://fortress.wa.gov/ecy/eimreporting/WHM/WHMSearch.aspx). Calculation methods for physical habitat metrics are described in Janisch (2013, as revised in 2018).

2.4. Classification of stressor and response variables

All variables used in the Relative Risk/Attributable Risk (RR/AR) analyses were placed into one of three condition classes: good, fair, or poor at each of the random sites based on either regional thresholds established using reference sites, or from available literature sources (see Table 1). From each of three assessment regions (Fig. 1b, *see* Section 2.5), regional thresholds were established using the 5th and 25th percentile of the reference distribution for stressors for which values decrease with impairment, and the 95th and 75th percentiles for stressors for which values increase with impairment. Consistent with Van Sickle and Paulsen (2008), good, fair, and poor classifications represented ranges of response variables representing either: not different from, somewhat different from, and markedly different from the range of values from minimally impacted reference sites. Additionally, for each biological sample, several additional metrics were calculated, including a Fine Sediment Biotic Index (FSBI, where high and low values indicate greater or lower abundance of taxa sensitive to fine sediment deposition, respectively; Relyea et al., 2012), EPT taxa richness (sum of taxa from the Orders: Ephemeroptera, Plecoptera, and Trichoptera, generally considered sensitive taxa, with high values indicating greater numbers of sensitive taxa), taxa richness of intolerant taxa and relative abundance of tolerant taxa (stress intolerant taxa and relative abundance of tolerant taxa, respectively; information at: https://www.pugetsoundstreambenthos.org/About-BIBI.aspx#Tolerant).

Notably, for the presented analyses, we added a value of 5.5% to our measures of percent sand/fines. We were interested in applying the recommendations for percent sand/fines in Bryce et al. (2010) for macroinvertebrates, yet our monitoring assessed substrate across the bankfull channel, whereas the United States Environmental Protection Agency methods (Bryce et al., 2010) assess substrate across the wetted channel. Therefore we regressed our wetted data against our bankfull data for percent sand/fines, which resulted in a coefficient of 0.98 and a y-intercept of negative 5.5% (wet estimate = 0.98 × bankfull estimate – 5.5%), so we added 5.5% to our measure of percent sand/fines.

2.5. Statistical analyses

All statistical analyses were performed in R, version 3.3.3 (R Core Team 2017), with adjusted spatial weights of sites, all extent estimates and RR/AR analysis implemented using spsurvey (version 3.3, Kincaid and Olsen 2016). Briefly, initial spatial weights (the reciprocal of the inclusion probability) were adjusted to account for sites that were dropped either due to land owner denied permission or to sites determined to be non-target (Stevens and Olsen, 2003). Extent estimates were the proportion of total stream length in poor condition for a particular stressor (Van Sickle and Paulsen, 2008). Relative risk (RR) is a ratio, measuring the strength of association between a condition class of a biological response indicator (here 'poor' B-IBI scores) and a stressor, with values > 1.0 indicating an increased risk of poor biological condition whenever a stressor is encountered (Van Sickle and Paulsen, 2008). The associated attributable risk (AR) represents a combination of RR and stressor extent, with values indicating the proportional reduction in the extent of poor biological condition expected with the elimination of the particular stressor (Van Sickle and Paulsen, 2008). We evaluated biological condition of random sites at three spatial scales: 1) statewide, 2) each of the eight Status and Trends Regions (Fig. 1a; *see also* Section 2.1), and 3) three assessment regions, corresponding roughly to precipitation gradients in Washington, namely Western Washington (west of the Cascade crest, which receives the most precipitation), Eastern Washington (east of the Cascade crest exclusive of the Columbia Plateau EPA Level III Ecoregion, which receives far less precipitation than Western Washington), and the Columbia Plateau (Columbia Plateau EPA Level III Ecoregion, which receives the least amount of precipitation in Washington; Fig. 1b). RR/AR analyses and stressor extents were evaluated statewide. One-way ANOVA was used to examine differences between condition categories for several biological metrics (FSBI, EPT taxa richness, intolerant taxa richness, relative abundance of tolerant taxa), with differences between factor levels evaluated with Tukey pair-wise comparisons.

3. Results

3.1. Macroinvertebrate biological health

An estimated 26,361 stream kilometers were assessed in Washington State using a probabilistic sampling design. Estimates from these random samples of stream macroinvertebrate communities show that 8869 stream kilometers (33.6%) were in poor biological condition, with 11,256 (42.7%) and 6236 (23.7%) stream kilometers in good and

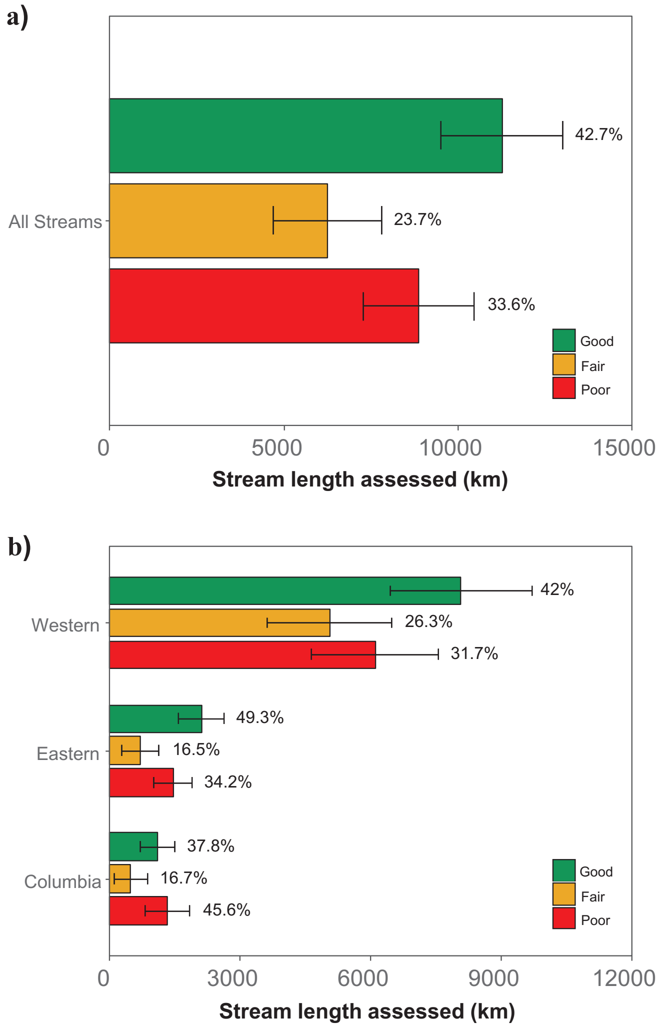

Fig. 2. (a) Number of stream kilometers assessed as either good, fair, or poor biological condition based on B-IBI scores for all sites in Washington and (b) for the same sites by each of the assessment regions. Percent of kilometers for each category are presented next to error bars. Error bars represent 95% confidence intervals.

fair condition, respectively (Fig. 2a). In the three assessment regions, the Columbia Plateau had the highest proportion of stream kilometers classified as poor biological condition (45.6%), with estimates for Eastern and Western Washington having similar values of 34.2% and 31.7%, respectively (Fig. 2b). Conversely, the highest proportion of stream kilometers in good biological condition were observed in Eastern and Western Washington, with 49.3% and 42%, respectively. An estimated 37.8% of stream kilometers in the Columbia Plateau are estimated to be in good biological condition. Across the Status and Trends Regions, poor biological condition was highest in the Unlisted region, with nearly 50% of assessed stream kilometers estimated as having poor biological condition. The Puget Sound, Snake River and Northeast regions have similar estimates of poor biological condition (Fig. 3). The highest proportion of stream kilometers assessed in good biological condition was observed in the Lower Columbia region.

3.2. Stressor extents

Of the stressors evaluated here, Log Relative Bed Stability (LRBS), percent sand/fines and total Nitrogen (TN) were the top three stressors in terms of the statewide extent categorized as being in poor condition (Fig. 4). Over 50% of stream kilometers assessed were in poor condition for substrate, with LRBS and % sand/fines at 78% and 54%, respectively. The extent of stream kilometers in poor condition for elevated total nitrogen levels was also at 49%. Conversely, very few sites had levels of sediment metals that were considered in poor condition, with only one site categorized as poor for lead and three sites categorized as poor for copper. Additionally, none of the sites assessed had levels of total PAHs considered to be in poor condition. In the different assessment regions, the variables with the highest proportion of streams in poor condition generally came from the Columbia Plateau, with high values for TN, total Phosphorus (TP), % embeddedness, large woody debris volume, slope, conductivity and sinuosity (Supplementary Fig. 1).

3.3. Relative risk/Attributable risk

A majority of the variables evaluated had relative risk ratios greater than one, with most of those having ratios between 2 and 4 (Fig. 5). The variable with the highest relative risk for the B-IBI was percent sand/fines. Taking into account the relative risk and extent, we obtained the attributable risk for the evaluated variables and four substrate variables had the largest attributable risk for B-IBI, with percent sand/fines and LRBS having very similar values. These values can be interpreted to mean that approximately 60% (95% CI: 38–75.6%) of streams classified as currently being in poor condition could be improved to either fair or good if percent sand/fines or LRBS were improved. After the four substrate variables, proportion of canopy cover (PPNCanopy) and nutrients (total N and P, respectively) had the highest attributable risk to B-IBI.

3.4. Various biological metrics

FSBI values were highest in streams classified as ‘good’, intermediate for streams classified as ‘fair’, and lowest in streams classified as ‘poor’ (one-way ANOVA, $F_{2,343} = 197.5$, $p \leq 0.0001$, Fig. 6a). Pair-wise comparisons revealed significant differences between all three classifications ($p < 0.05$). EPT taxa richness was highest in streams classified as ‘good’, intermediate for streams classified as ‘fair’, and lowest in streams classified as ‘poor’ (one-way ANOVA, $F_{2,343} = 405.0$, $p \leq 0.0001$, Fig. 6b). Pair-wise comparisons revealed significant differences between all three classifications ($p < 0.05$). Taxa richness of species categorized as ‘intolerant’ was highest in streams classified as ‘good’, intermediate for streams classified as ‘fair’, and lowest in streams classified as ‘poor’ (one-way ANOVA, $F_{2,343} = 58.3$, $p \leq 0.0001$, Fig. 6c). Pair-wise comparisons revealed significant differences between all three classifications ($p < 0.05$). The relative abundance of tolerant taxa was lowest in streams classified as ‘good’, intermediate for streams classified as ‘fair’, and highest in streams classified as ‘poor’ (one-way ANOVA, $F_{2,343} = 8.61$, $p = 0.0002$, Fig. 6d). Pair-wise comparisons revealed significant differences only between the ‘good’ and ‘poor’ classifications ($p < 0.05$).

4. Discussion

We report results from the most comprehensive statewide assessment to date of macroinvertebrate communities in Washington State using a probabilistic sample design. Because we employed a GRTS design, our results should represent unbiased estimates of perennial stream biological condition on non-federal and non-tribal lands across the state of Washington. Because we concurrently sampled targeted reference sites, we were able to interpret the results from our randomly sampled sites in context with expectations under minimal human impacts and perform a broad assessment of stressors with the potential to impact stream macroinvertebrates using RR/AR. We cannot overstate the value of having a dataset as comprehensive as what is reported here and collected at the scale that it was collected, for evaluating patterns of impairment and linking it to potential environmental stressors.

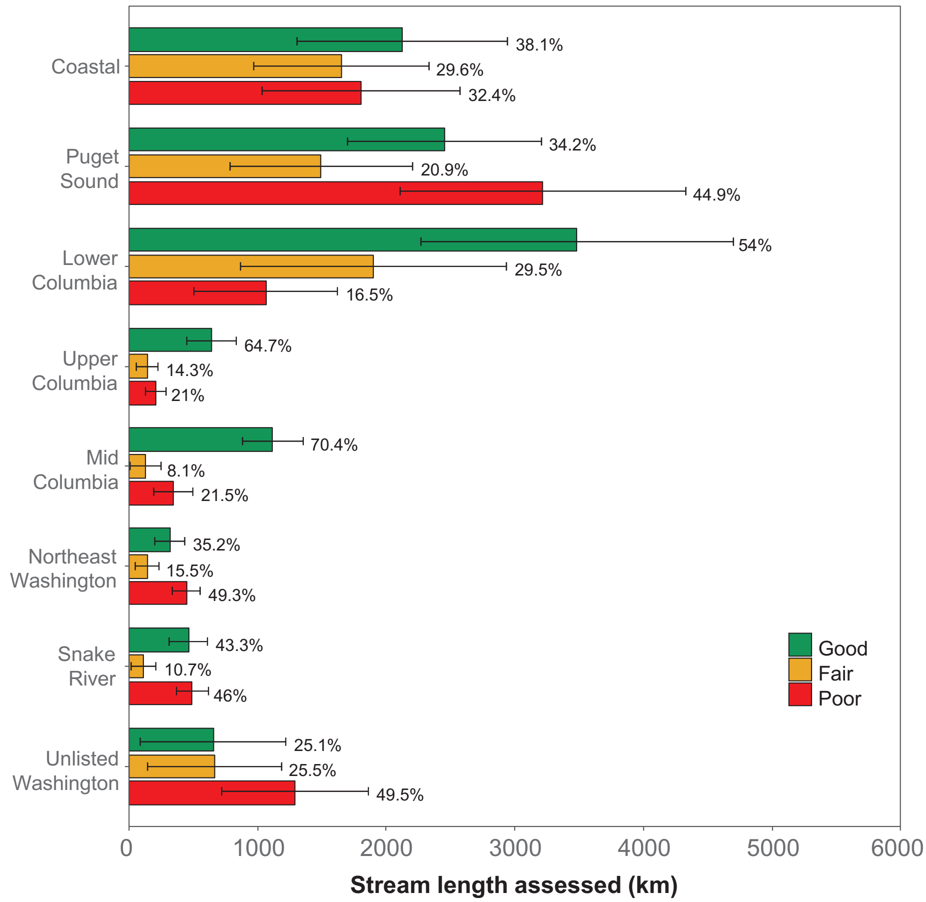

Fig. 3. Number of stream kilometers assessed as either good, fair, or poor biological condition based on B-IBI scores for eight Status and Trends Regions in Washington. Percent of kilometers for each category are presented next to error bars. Error bars represent 95% confidence intervals.

In the United States and elsewhere, the reality is that beyond Federal surveys, most stream surveys are local or regional in scale, i.e., matching the spatial scale of the jurisdiction or agency involved and often focused on specific management objectives, e.g., answering specific questions related to endangered species, etc. (Dobbie et al., 2008; Jackson and Fuereder, 2006; Buss et al., 2015). Often, due to budget and time constraints, many of these surveys collect only a limited number of physico-chemical measures in addition to the biological data. While informative for that particular stream or narrow region, these studies can be limited in their capacity for making broad, general conclusions about diversity patterns and/or impairment at larger spatial scales. Additionally, many stream surveys are targeted, i.e., sampling sites where there is known impairment or where remediation efforts are being implemented, which limits the scope of findings and biases the types of questions that can be addressed with larger probabilistic surveys (Stevens and Olsen, 2004). Likewise, because there are typically inconsistencies in methodologies, combining data from multiple smaller regional surveys can be problematic if not impractical for evaluating patterns at broader spatial scales and for making general observations (Carter and Resh, 2001). The ability to make broad, more generalizable conclusions benefits from data collected by probabilistic surveys conducted at larger spatial scales employing consistent methodologies (Stevens and Olsen, 2004; Dobbie et al., 2008).

Based on a random sample of surveyed streams from across Washington, we conclude that approximately one third of all stream kilometers assessed were in poor biological condition as determined with macroinvertebrate communities. Unsurprisingly, we also observed regional differences in the proportion of streams determined to be in poor biological condition, with nearly 46% of streams assessed in the Columbia Plateau classified as impaired relative to regionally targeted reference sites. At an even finer scale, i.e., Status and Trends Monitoring Regions, the proportion of stream kilometers in poor biological condition was highest in the Puget Sound region and far eastern portions of the state. For example, in eastern Washington, in each of the Northeast, Snake River and Unlisted Regions, we observed over 40 percent of stream kilometers assessed as being in poor biological condition. For context, the Puget Sound region is strongly influenced by urban influences, namely the Seattle-Tacoma metropolitan area (Hepinstall-Cymerman et al., 2013), while eastern Washington has lower population density, receives far less precipitation than western Washington (Bond and Vecchi, 2003), and is largely influenced by agricultural practices (Stöckle et al., 2010).

We sampled about fifty sites per Status and Trends Monitoring Region, to describe conditions at that scale. It is important to note that this sampling frequency might not describe some localized urban effects that are clumped and intense and it is likely that our data actually underestimated the proportion of streams in poor biological condition within the Puget Sound region because of this. From the random data, we observed that a majority of sites (313 or 90%) were located in watersheds with urbanization levels less than 3.56% (based on 2011 National Land Cover Database (Homer et al., 2015), summing low, moderate and high urban development). Conversely, only 9 (2.6%) sites were located in watersheds with high urban development (> 17.2%). Results from a more recent regional probabilistic study in the Puget Sound using the same methods as our survey, suggests the impacts of greater urbanization on stream macroinvertebrate communities are more intense, finding that approximately 82% of sites inside urban growth boundaries had B-IBI scores considered to reflect poor biological condition (DeGasperi et al., 2018).

We simultaneously measured a wide variety of physico-chemical

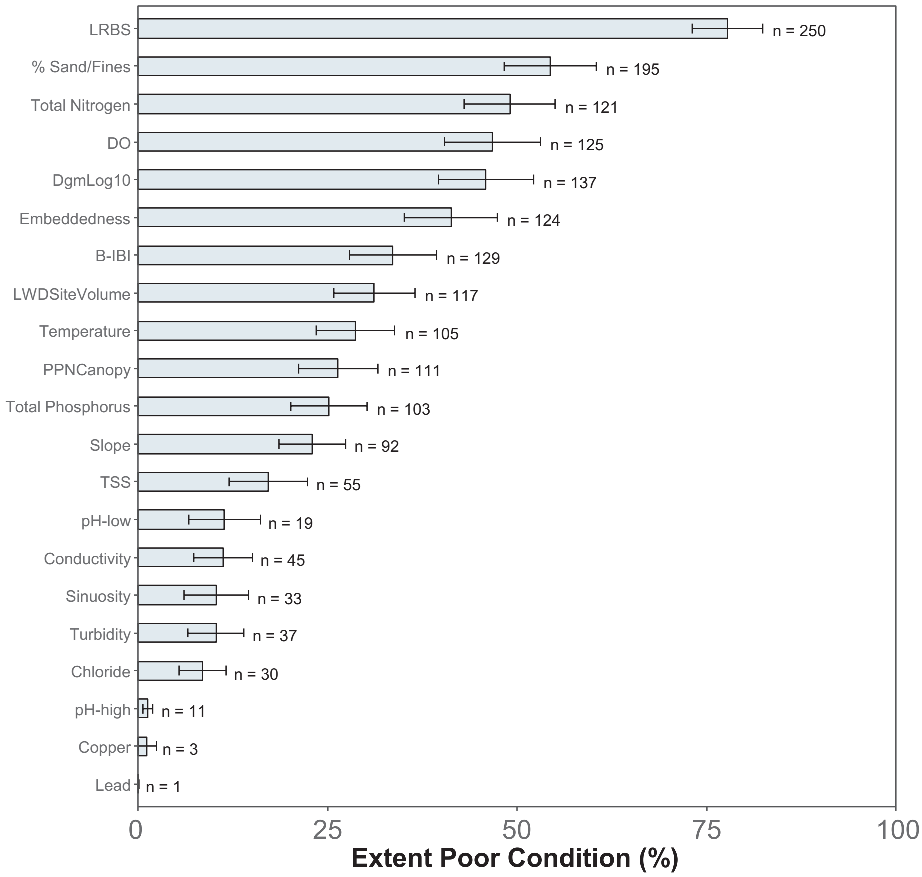

Fig. 4. Percent of statewide stream kilometers determined to be in poor condition for various chemical and physical habitat stressors. Condition determined relative to regional reference sites. Values next to error bars represent the number of sites determined to be in poor condition. Error bars represent 95% confidence intervals. Parameters from Table 1 with no samples categorized as being in poor condition were not included in figure.

and physical habitat parameters along with the biological data, which gave us the ability to evaluate the prevalence of possible impairment for multiple variables known to influence stream macroinvertebrate communities. We determined that many of the aquatic stressors with the greatest statewide prevalence were those tied to substrate condition. Based on statewide extent, four of the top six stressors evaluated were variables related to the condition of the substrate, with LRBS and percent sand/fines being the most prevalent. Additionally, the prevalence of poor condition across the state for water quality variables, including total nitrogen and low dissolved oxygen levels were also noteworthy. These findings are consistent with those from a national survey in the U.S., where the most prevalent stressors observed were excessive nutrients (i.e., total N and total P) and fine streambed sediments (Paulsen et al., 2008).

Having co-occurring biological and environmental data, as well as a fairly large sample size, allowed us to evaluate the potential influence of these variables on macroinvertebrate communities using conditional probabilities. Using RR analysis, we observed that poor B-IBI scores were four times more likely when observed with elevated percent sand/fines. A similar RR ratio for excessive streambed sediments and a macroinvertebrate MMI was also observed in the western U.S. (Paulsen et al., 2008), indicating consistent findings between our program and those from a national survey. Other notable variables in our study also associated with poor B-IBI scores based on RR analyses were lead concentrations in sediment, proportion of riparian zone with canopy cover, conductivity, turbidity, chloride and total phosphorus. While relative risk assessment has yet to be implemented widely in stream surveys beyond several national surveys in the U.S., recent findings from a probabilistic survey in Brazil employing RR determined that poor biological condition within the benthos was much greater when associated with the loss of riparian canopy cover (Jiménez-Valencia et al., 2014). Additionally, in our study, the frequency with which some variables were observed in poor condition was relatively low, e.g., only one site with elevated lead levels in sediment, indicating that when these stressors were observed in poor condition, the probability of poor biological condition increased, yet given how infrequently some of these variables occurred in poor condition within our dataset, the problems associated with these variables could be considered of immediate concern when encountered rather than a general problem. However, one benefit of the AR analyses is the incorporation of relative extent and RR, which helps to identify key regional stressors and estimate the potential benefits of stressor remediation.

Our AR analyses determined that generally, statewide, the greatest potential for improving poor biological condition for stream macroinvertebrates lies in improving substrate conditions, riparian canopy cover and nutrients. AR revealed that the top four stressors with the largest attributable risk were all measures related to condition of the substrate and that approximately 60% of stream kilometers now classified as being in poor biological condition could be improved if conditions relating to elevated percent sand/fines were also improved. This regional scale estimate does not mean that biological condition at sites currently classified as poor would improve to the point where they would be considered to be in good condition, but that improvement would be such that they would no longer be classified as being in poor condition, i.e., either fair or good. This conclusion is based on the assumptions of causality and reversibility, which are the expectations that if a stressor is eliminated, the degree of ecosystem recovery will be commensurate (Van Sickle and Paulsen, 2008). While these assumptions may not be completely reasonable in a practical sense, our findings still implicate poor substrate conditions, i.e., elevated sediment deposition as the most likely stressor contributing to poor macroinvertebrate community health in our dataset.

The findings that substrate conditions were important contributors to stream macroinvertebrate health at a regional scale were not

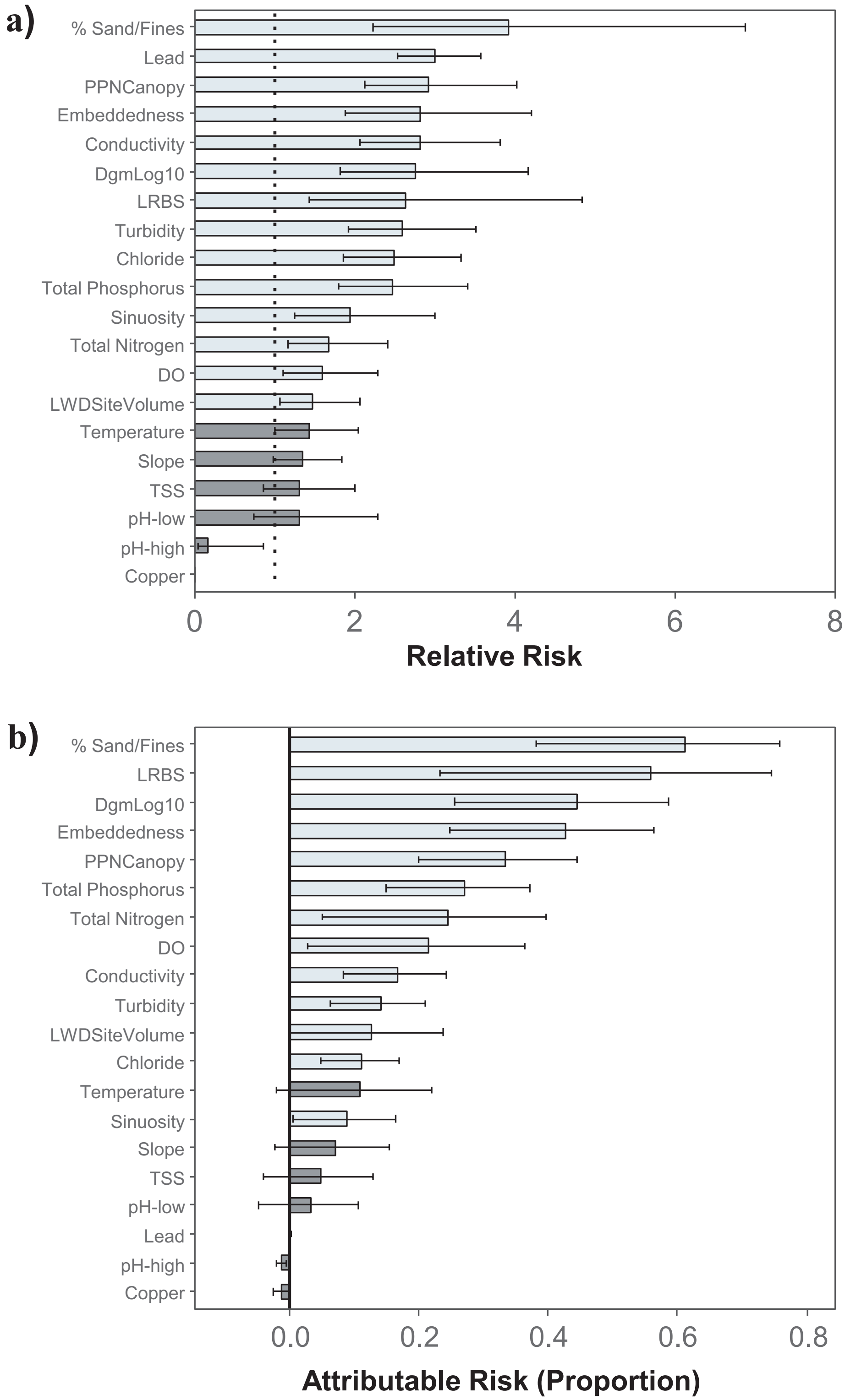

Fig. 5. (a) Relative risk analysis, where values greater than one indicate an increased risk of poor B-IBI scores associated with poor conditions of the evaluated environmental parameters and (b) attributable risk analysis, where values greater than zero indicate the proportion of stream kilometers assessed that could be improved to 'not poor' if the environmental parameter were improved. Light colors indicate variables with significant impacts and error bars represent 95% confidence intervals. Parameters from Table 1 with no samples categorized as being in poor condition were not included in Relative Risk or Attributable Risk analyses.

surprising, yet they are intuitive, as many sensitive stream invertebrate taxa (e.g., EPT taxa) require hard substrate with adequate interstitial spaces to thrive. Excessive inputs of fine sediments and sand to stream substrates can fill interstitial spaces, leading to a loss of functional habitat and shifts in community composition and/or biodiversity loss (Bryce et al., 2010; Burdon et al., 2013). In support of this reasoning, we observed that between biological condition classes, there was a distinct loss of taxa sensitive to fine sediment deposition as measured with the FSBI. We also observed a significant loss of sensitive taxa across biological condition classes as measured with EPT and intolerant taxa richness, respectively, while also observing a trend towards sensitive taxa being replaced by more tolerant ones. Given that multiple diversity measures responded predictably and consistently, we believe that this speaks to the generality and applicability of our results outside our region of study. Additionally, EPT taxa richness, a common variable evaluated in many stream studies (Kerans et al., 1992; Wagenhoff et al., 2012), was highly correlated with B-IBI scores (Pearson correlation coefficient = 0.95) in our dataset, which suggests that had we

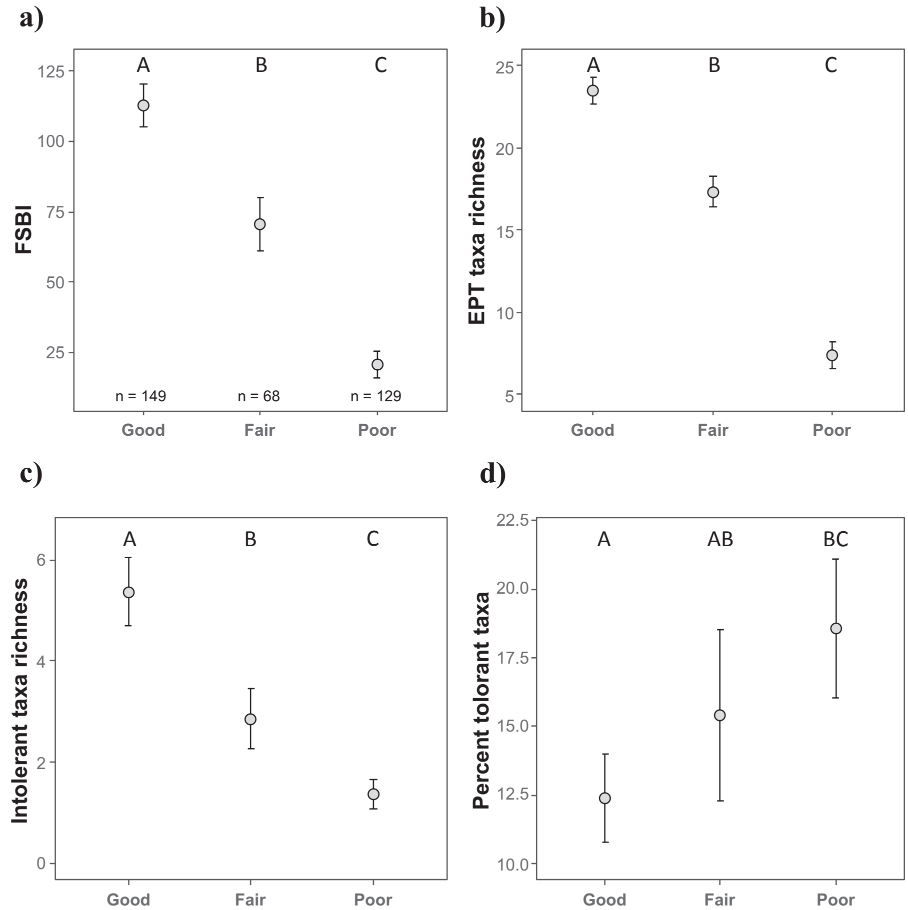

Fig. 6. (a) Average FSBI scores (b) EPT taxa richness, (c) intolerant taxa richness, and (d) percent of tolerant taxa for sites classified as 'good', 'fair', 'poor' based on B-IBI scores. Error bars represent 95% confidence intervals. Letters denote statistically significant differences between groups.

performed RR/AR on EPT richness, the major conclusions would likely have been quite similar to those we observed using the B-IBI.

Many of the findings reported here are consistent with those from the U.S. EPA's national stream surveys (e.g., Wadeable Streams Assessment and NRSA), which have found that elevated nutrients, loss of riparian vegetative cover and elevated fine sediment are common stressors leading to poor biological condition in the western mountains and xeric west of the U.S., which includes Washington (Paulsen et al., 2008; U.S. EPA, 2016). ECY employs very similar methodology, procedures and analyses to the U.S. EPA's national survey, which likely contributed to consistent findings between the two surveys. However, several of our findings are also consistent with those obtained from other regional stream surveys, despite differences in approaches, protocols and biological end points. Notably, elevated fine sediment accumulation has been shown to contribute to impairment of macroinvertebrate communities in many places, including Australia (Harrison et al., 2007; Harrison et al., 2008), Spain (Buendia et al., 2013), China (Zhao et al., 2011) and elsewhere. Additionally, elevated nutrient concentrations have been observed to be important stressors in European streams (Johnson and Hering, 2009) and loss of riparian vegetation has been shown to contribute to impairment of stream macroinvertebrate communities in regions such as South America (Jiménez-Valencia et al., 2014), Canada (Rios and Bailey, 2006), and Europe (Johnson and Hering, 2009). Therefore, our findings that elevated sediment deposition, loss of riparian cover and elevated nutrients build on those from a variety of other stream monitoring programs and manipulative studies (Wagenhoff et al., 2011; Wagenhoff et al., 2012) that have shown that macroinvertebrate communities respond consistently and reliably to a fairly predictable set of stressors resulting from human influences.

Loss of riparian cover can contribute to elevated fine sediment deposition and nutrient inputs to streams, as well as increased flashy flows (Poff et al., 1997; Coles et al., 2012) leading to greater scouring and bank erosion (Gellis and Gorman Sanisaca, 2018), all of which can negatively influence the composition and diversity of stream macroinvertebrate communities (Naiman et al., 1993; Rios and Bailey, 2006). Therefore, efforts aimed at preserving riparian buffers and maintaining/restoring stream flows which more closely mimic natural patterns would facilitate attempts to preserve stream biodiversity (Naiman et al., 1993). Conversely, in highly impacted areas, efforts which help restore and protect riparian vegetation and the natural flow regime should reduce inputs of fine sediment, nutrients and various toxics into the stream channel, contributing to their restoration (Naiman et al., 1993). Notably, fine sediment deposition can increase in low gradient streams, yet poor B-IBI scores were not significantly associated with low slope in our dataset, suggesting that low stream gradient by itself was not a major contributing factor to poor biological condition of macroinvertebrate communities. Additionally, elevated nutrient inputs and increased light resulting from loss of riparian cover can also lead to increased probability of nuisance algal growth in streams, which may reduce habitat complexity (i.e., fill interstitial spaces) and negatively impact aquatic biota. Nutrients, particularly phosphorus, have been observed to be increasing in the U.S., contributing to a significant loss of oligotrophic streams (Stoddard et al., 2016) and highlighting the need to increase efforts to monitor and evaluate nutrient inputs to streams.

5. Conclusions

Whether measured as the percent sand/fines in the substrate, relative bed stability, or average substrate size, data from the first statewide stream biological survey of perennial streams in Washington State suggests that the most prevalent stressors negatively impacting macroinvertebrate communities are poor substrate conditions. These results were corroborated with observations of reductions in taxa sensitive to fine sediment deposition and a losses of EPT taxa in sites with poor biological condition. All of this information has the potential for informing those entities charged with managing streams, as it suggests that generally, the most successful approaches for maintaining or improving biodiversity and biological condition of macroinvertebrate communities will be through effective management of those factors influencing substrate conditions, namely reducing the amount of fine sediment entering stream channels.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.02.032.

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