2013

# Linking Land Use, In-Stream Stressors, and Biological Condition to Infer Causes of Regional Ecological Impairment in Streams

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Freshwater Science, 2013, 32(3):801–820

DOI: 10.1899/12-186.1
Published online: 18 June 2013

# Linking land use, in-stream stressors, and biological condition to infer causes of regional ecological impairment in streams

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**Abstract.** We used field-derived data from streams in Nevada, USA, to quantify relationships between stream biological condition, in-stream stressors, and potential sources of stress (land use). We used 2 freshwater macroinvertebrate-based indices to measure biological condition: a multimetric index (MMI) and an observed to expected (O/E) index of taxonomic completeness. We considered 4 categories of potential stressors: dissolved metals, total dissolved solids, nutrients, and flow alteration. For physicochemical factors that varied predictably across natural environmental gradients, we quantified potential stress as the site-specific difference between observed (O) and expected (E) levels of each factor ($O–E_{stress}$). We then used 2 sets of Random Forest models to quantify relationships between: 1) biological condition and potential stressors, and 2) stressor values and land uses. The 2 indices of biological condition were differentially responsive to stressors, indicating that no single measure of biological condition could fully characterize assemblage response to stress. Total dissolved solids (as measured by electrical conductivity [EC]) and metal contamination were the stressors most strongly associated with biological degradation. The most likely sources of these stressors were agriculture, urban development, and mining. Our findings highlight the need to develop EC criteria for streams. Measures of biological condition and stress that account for natural variability should reduce errors of inference and increase confidence in causal analyses. This approach will require development of robust models capable of predicting physical and chemical reference conditions. Causal analyses for individual sites require appropriate hypotheses about which stressors and what levels of stress can cause biological degradation. Our study demonstrates the usefulness of field data collected from multiple sites within a region for developing these hypotheses.

**Key words:** causal analysis, electrical conductivity, ecological assessment, flow modification, metals, models, nutrients, pollutants, Random Forests, stream ecosystems, stressors, temperature.

Many types of stressors can alter freshwater communities, and identifying the specific stresses causing biological alteration at individual sites can be challenging for at least 4 reasons. First, conducting in situ, stressor-removal experiments in the field generally is impractical, especially at the thousands of sites that have been biologically degraded. Second, quantifying stress in the field is not straightforward given that many types of stress represent alterations in natural physicochemical conditions at a site rather than the addition of a novel contaminant. Third, multiple potential stressors or alterations often co-occur, and each must be evaluated as a potential contributor to biological degradation. Last, managers often may lack realistic hypotheses about what types and levels of stress are likely to lead to biological degradation.

Several frameworks have been advanced recently to help managers identify causes of ecological degradation (e.g., Suter et al. 2010, Allan et al. 2011, Norris et al. 2012). Cause and effect can rarely be established from single studies (Norris et al. 2012), so a weight-of-evidence approach generally is needed to identify the most likely causes of impairment (Suter et al. 2010). Strong inferences regarding the causes of ecological degradation require, at a minimum, observed exposure of biota to a stressor, identification of a plausible causal mechanism (i.e., a causal chain starting with exposure and ending in a biological response), and a consistent and strong association between the hy-

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pothesized cause and effect (Norris et al. 2012). Plausible causal mechanisms have been identified for many stressors, but accurate quantification of associations between hypothesized causes and effects can be difficult to achieve. Simple (i.e., small-scale or laboratory-based) bioassays may not scale up to real systems (Kimball and Levin 1985, Crane et al. 2007), and inferences derived from field observations often are confounded by the presence of natural environmental gradients or complicated by the combined effects of co-occurring stressors (Allan et al. 2011). Models that quantify relationships between biological condition, stressors, and land uses among multiple sites could help quantify how biological communities respond to different stressors and identify the likely contribution of different land uses and waterway alterations as sources of stress. However, accurately modeling complex relationships between stressors and biological condition in multistressor environments can be problematic (Allan 2004, Townsend et al. 2008). Individual stressors may interact, and biota may respond in complex, nonlinear ways to stress (Townsend et al. 2008). Therefore, understanding the real-world effects of stressors will require analyses that are robust in detecting interactions among predictors and nonlinear responses.

The natural physicochemical properties of stream water are an important determinant of invertebrate assemblage composition (Allan and Castillo 2007). When human activities cause these properties to exceed their natural range of variation, they can be considered stressors (Townsend et al. 2008) that may alter and degrade ecological communities. Many types of stressors can adversely affect aquatic macroinvertebrate assemblages (e.g., Poff et al. 1997, Clements et al. 2000, Van Sickle and Paulsen 2008, Hentges and Stewart 2010, Nicola et al. 2010). However, the relative importance of different stressors and their sources often are not well understood. Anthropogenic activities that can alter physicochemical properties of stream water include urban development (e.g., Paul and Meyer 2001, Walsh et al. 2005), agriculture (e.g., Collins and Jenkins 1996, Johnson et al. 1997, Matthaei et al. 2010), impoundment and other flow modifications (e.g., Poff et al. 1997), and mineral extraction (e.g., Smolders et al. 2003, Clements 2004). The in-channel physicochemical alterations that result from these land and water uses are all potential causes of biological degradation.

Accurate association of degradation of biological condition with stressors in survey data requires separation of the effects of anthropogenic degradation from natural variation in both the biota and physicochemical properties of streams. Biota vary along natural and anthropogenic gradients, and failure to account for natural biotic variation will confound interpretations of the effects of stressors and increase both type I and type II errors of inference regarding whether streams are biologically impaired (Cao et al. 2007, Hawkins et al. 2010a). Biological indices adjusted for natural biotic variability among sites help isolate the effects of human-caused stress and enable investigators to attribute variation in index scores to anthropogenic factors with more confidence. Several types of stress represent alterations in the specific physicochemical conditions that naturally occur at sites. These naturally occurring conditions can vary markedly among locations and must be accounted for to associate environmental alteration accurately with both biotic responses and the sources of stress (human land uses). However, until recently, few attempts have been made to account for natural variability in the spatial distributions of physicochemical factors that, if modified, can act as stressors (Hawkins et al. 2010b). Alteration of naturally occurring levels of electrical conductivity (EC), nutrients, temperature, and flow can present significant stress, but these factors also vary markedly along natural climatic and geologic gradients. Similar to the reference-condition approach used in bioassessments, use of models that estimate expected natural physicochemical properties of individual streams could improve characterization of stressors occurring at different locations.

Our main objectives were to: 1) identify regionally important causes of biological impairment of streams in a highly heterogeneous region that has been exposed to a variety of potential stressors, and 2) develop realistic hypotheses about the levels of stress that can lead to biological degradation. First, we quantified relationships between biological condition and individual and multiple stressors. We then assessed the evidence for interactive effects of co-occurring stressors on biota. Last, we identified likely general sources of different types of stressors. Models that predict expected site-specific stream EC (Olson and Hawkins 2012) and total N and P concentrations at base flow (Olson and Hawkins 2013) in our study area allowed us to interpret stress associated with these factors in the context of natural variation.

## Methods

The state of Nevada (NV) is a highly heterogeneous region that varies greatly in both natural environmental characteristics and human-caused alteration. Environments in this region range from desert to montane. The Basin and Range terrain produces

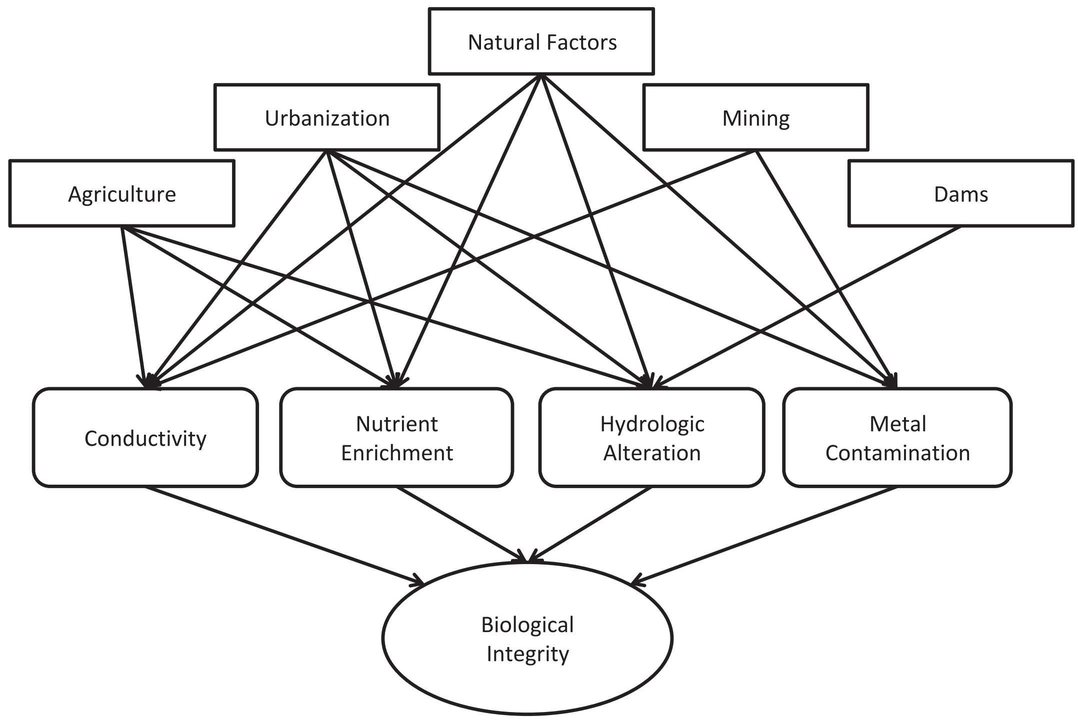



FIG. 1. Conceptual model for our study identifying possible linkages between land uses, in-stream stressors, and biological condition.

extreme climatic variability, and cool, wet climates are restricted to isolated high-elevation habitats. These high-elevation habitats are sometimes called sky islands, and despite their small size and isolation, they support most of the aquatic habitats and much of the total biodiversity of the region (Chambers et al. 2008). This region also has experienced a wide range of human-caused watershed and channel alterations, and individual streams range from heavily degraded to nearly pristine. Human-caused alterations in the region include agriculture, urbanization, hydrologic modification, and mineral extraction. We considered 4 types of potential stressors to stream biota of regional concern: total dissolved solids as measured by EC, nutrient enrichment, trace-metal contamination, and flow alteration. Initially, we had included a measure of stream temperature alteration as a 5$^{th}$ stressor. We have models that can predict reference-condition temperatures (Hill et al. 2013), but the spot temperature measurements available to us were not comparable to the predictions of seasonal and annual means that our models produce. Therefore, we excluded temperature alteration from further analyses. The wide ranges of environmental and anthropogenic characteristics in this region made a good test case for evaluating our ability to assess regional variation in stressor levels and to identify likely causes of biological degradation in the context of background physicochemical and biological stream characteristics.

To identify important stressors and their sources, we developed 2 sets of predictive models: 1 set to link measures of biological condition to in-stream stress and 1 set to link spatial variation in stressor levels to gradients of land uses. Together these models link: 1) watershed-scale land uses to 2) reach-scale estimates of likely exposure of organisms to stress to 3) reach-scale estimates of biological condition (Fig. 1). We then interpreted quantitative associations between stressors and biological degradation within the context of existing conceptual models of biological response to different types of stressors to infer the most likely causes of biological degradation within the region.

*Biological indices, stressor data, and natural environmental data*

We used 2 bioassessment indices specifically developed to characterize biological condition of benthic invertebrate assemblages in NV streams: a multimetric index (MMI) and an observed to expected index of taxonomic completeness ($O/E_{taxa}$) (Vander Laan 2012). The MMI includes 7 metrics and quantifies biological condition in terms of overall

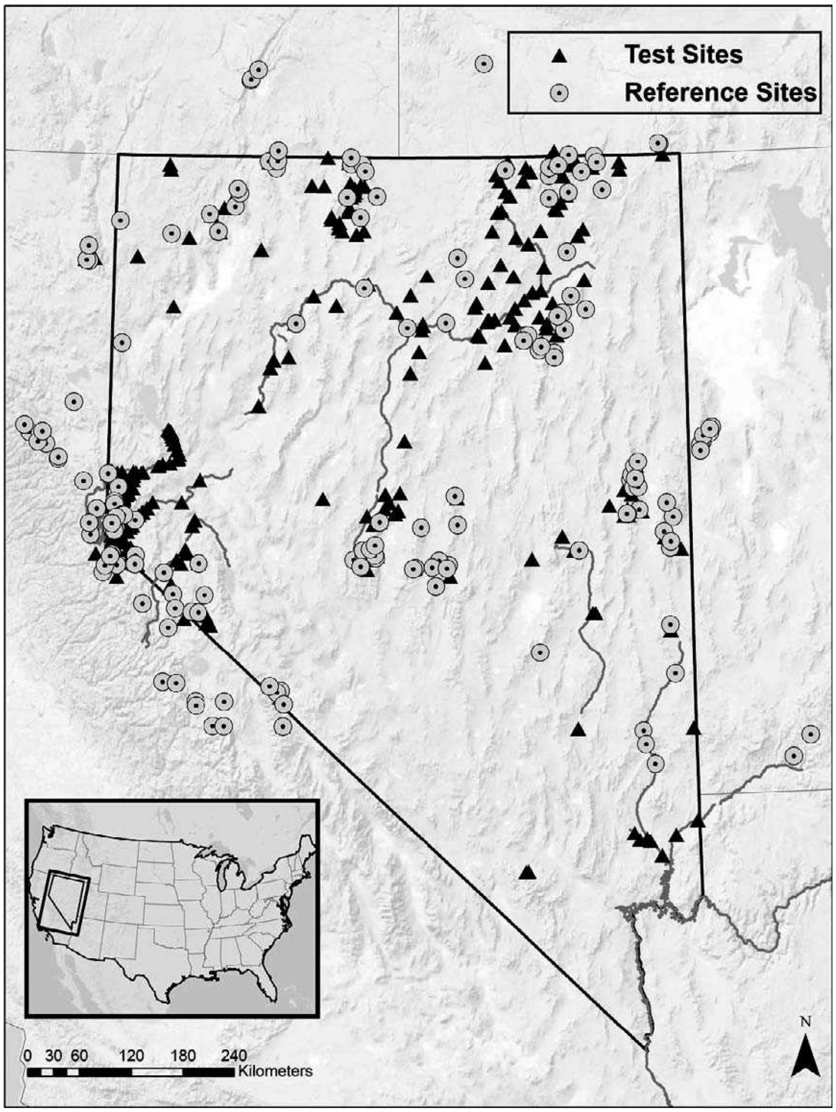



FIG. 2. Distribution of benthic invertebrate sampling locations for reference and test sites used in biological indices and stressor analyses. Some sites in close proximity to Nevada were included for index development and evaluations.

assemblage structure. The metrics included in the MMI are: insect richness, Ephemeroptera relative abundance, Shannon diversity, collector-filterer relative abundance, Plecoptera relative abundance, noninsect richness, and clinger richness. These metrics were selected based on their statistical and biological independence and their ability to discriminate between reference and degraded sites. $O/E_{taxa}$ measures biological condition in terms of the loss of expected taxa at individual sites. Both indices were developed from samples collected at 165 reference sites in NV and surrounding areas (Fig. 2). Samples were collected by personnel at the NV Department of Environmental Protection (NDEP), the US Environmental Protection Agency (EPA), and Utah State University. Both indices were based on a reference-condition approach in which models were used to predict site-specific biological expectations (taxonomic composition or assemblage metrics) based on naturally occurring environmental characteristics (Hawkins et al. 2000, Cao et al. 2007). By setting site-specific biological expectations, this approach accounts for variation in individual metric values or taxonomic composition associated with natural environmental gradients. We followed procedures similar to those of Herlihy et al. (2008) to select reference sites when developing the biological indices. Reference sites passed a screen for land uses and other potential human impacts and represent the least-disturbed conditions (sensu Stoddard et al. 2006) for streams in the study area. Specific criteria were given by Vander Laan (2012). Reference-site scores for both indices are centered on 1, and scores significantly $<1$ indicate anthropogenic degradation. In addition to the 165 reference samples used in index development, 401 invertebrate samples from nonreference sites (hereafter, test sites) were available from the NDEP database. We assessed biological condition for all test sites by calculating $O/E_{taxa}$ and MMI scores from the invertebrate sample data.

We assumed that an ecologically meaningful measure of the chronic stress at each site could be estimated as either the mean values of water-chemistry variables calculated over multiple years of data or the long-term hydrologic alteration associated with upstream reservoirs. We calculated mean values of potential dissolved stressors (EC, metal concentrations, and nutrient concentrations) from water-sample data provided by the NDEP. These samples generally were collected during low-flow conditions. We derived mean EC, dissolved metal concentrations, and nutrient concentrations from samples collected between the years 2000–2010 (average number of samples/site = 8). EC was measured as μS/cm, and nutrients were measured as concentrations (μg/L) of total N (TN) and total P (TP). Metal concentrations were measured as dissolved metals (μg/L). We used dissolved metals because this measure is generally considered to be most representative of the biologically available portion of metals in aquatic systems (Reiley 2007). We had data available for 10 metals: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), silver (Ag), and zinc (Zn). We also obtained water-chemistry data from the NDEP for 68 of the reference samples used for index development, and we included these samples in the models relating land uses, stressors, and biological condition to ensure that the full ranges of land uses and stressor gradients were represented. We used data from the National Inventory of Dams (USACE 2009) to estimate the total reservoir volume and the volume of the largest reservoir within each watershed and within 3 km upstream from each site (both standardized by dividing by watershed area).

We used a geographic information system (GIS) to characterize land use and the natural environmental characteristics of each watershed (Appendix 1). We obtained land-cover information from the National Land Cover Database (NLCD) produced by the Multi-Resolution Land Characteristics Consortium (Homer et al. 2007) to calculate the % area in each watershed that was classified as having agricultural or urban land use and the % area of the watershed within 3 km upstream from the sample site that was classified as having agricultural or urban land use. We used the whole-watershed measure of land use to characterize the potential cumulative effects of land uses on physicochemical conditions and the 3-km measure to characterize more proximate watershed conditions that may be important determinants of local stream physicochemical conditions. We obtained mining data from the US Geological Survey (USGS) mineral-resources data system (http://mrdata.usgs.gov) and characterized mining activity as the density of known mine sites in each watershed. Dams may influence physicochemical factors other than flow, so we also used the measures of reservoir volumes described above as potential predictors of the levels of other stressors. We extracted 30-y average air temperature and precipitation information from grids produced by the PRISM climate group (Daly et al. 2008). We used geologic (Reed and Bush 2001) and soil (Wolock 1997) data to define geology and soil properties of each watershed. We characterized watershed topography as watershed size; mean, minimum, and maximum watershed elevation; and watershed slope calculated as the change in elevation within a watershed (maximum elevation minus minimum elevation) divided by the maximum flow length. We also roughly characterized 2 aspects of the natural hydrologic regime: 1) mean baseflow index as provided by Wolock (2003), and 2) a measure of hydrologic stability calculated as the minimum mean monthly discharge divided by the maximum mean monthly discharge interpolated from the 12 closest USGS gauging stations within a 100-km radius.

*Modeling approach*

We developed Random Forest (RF) models to identify and quantify relationships between land uses, stressors, and biological condition. RF models combine predictions from numerous regression or classification trees based on bootstrap resamples of the data to produce robust and accurate predictions (Cutler et al. 2007). RF models can be used for classification and regression and have significant advantages over other statistical methods, including the ability to model complex interactions among predictors and resistance to model over-fitting (Cutler et al. 2007). Individual relationships between predictors and the response variable are assessed with variable importance measures and partial dependence plots. Variable importance in an RF model is measured as the % increase in mean squared error (MSE) of the model when that variable is randomly permuted. Partial dependence plots characterize the effect of an individual predictor on the response after accounting for the effects of all other predictors (Hastie et al. 2001). RF model performance is best evaluated based on out-of-bag (OOB) MSE and % variance explained. OOB refers to the samples that are left out of each of the bootstrap samples in each tree in an RF model. By predicting to these OOB samples, an unbiased estimate of error, similar to cross-validation, can be obtained (Cutler et al. 2007). Because RF can model complex interactions between predictors, it is well suited for assessing individual and cumulative effects of stressors on stream invertebrate assemblages.

*Accounting for natural variability in stressor levels*

Of the stressors we examined, EC, nutrients, and hydrology exhibited marked natural variation among streams. Therefore, we used existing models to estimate expected site-specific reference conditions for EC (Olson and Hawkins 2012) and TN and TP (Olson and Hawkins 2013). Similar to the biological indices, these models are based on a reference-condition approach in which relationships between environmental characteristics and water chemistry at reference sites are used to predict site-specific expectations for reference-condition water-chemistry values. For these factors, we subtracted expected (E) values from observed (O) values and used O–$E_{stress}$ values as estimates of physicochemical alteration in the biota–stressor and stressor–landuse models. We lacked a practical way of assessing natural variation in metal concentrations among sites, so we assumed that observed metal concentrations represented anthropogenic disturbance. We also lacked direct estimates of flow modification. Therefore, we used total upstream reservoir volume and the volume of the largest upstream reservoir as a proxy for hydrologic alteration. We conducted a principal components analysis (PCA) on all stressor variables followed by varimax rotation of the principal component axes to identify patterns of spatial covariation among stressors.

*Quantifying relationships between biological condition and stressors*

We built RF regression models with index scores as responses and stressors as predictors (hereafter biota–stressor models) to quantify individual relationships between biological condition and individual stressors. We developed 2 biota–stressor models: one with O/E scores as the response and the other with MMI scores as the response. We developed these models iteratively by first including all stressors as potential predictors. We then conducted a stepwise removal of the least important predictors until performance was maximized (as measured by % variance explained). We assessed the performance of these models by comparing the % variance in index scores explained by the models to the maximum possible variance explained given the variability in standardized biological index scores. We calculated the maximum possible % variance explained as

$$100 \times \frac{S:N}{(S:N)+1}$$

where *S:N* is the signal-to-noise ratio calculated by dividing the variance of biological index scores observed at all sites by the variance of index scores observed at only the reference sites used to calibrate the biological indices (J. Van Sickle, Department of Fisheries and Wildlife, Oregon State University, personal communication). The variance among reference-site scores is associated with sampling variance and model error and represents the noise that will limit the amount of variation in index scores that can be associated with predictors. We also modeled associations between individual component metrics and stressors. We used univariate and bivariate partial-dependence plots to assess the associations between biological index values and different stressors, check for interactions among stressors, and visually identify thresholds associated with biological degradation. We also used *t*-tests to assess whether biological index values differed between sites with a dam within 3 km upstream from the sample site and those without dams this close. Many factors affect the length of stream that a dam will influence. Our 3-km threshold was arbitrary, but should encompass the sections of streams most likely to be affected by upstream dams, i.e., tailwaters.

*Relating stressors to land uses*

We developed several RF models to relate stressor levels to land uses within watersheds. We included both land use and naturally occurring environmental characteristics (including model-derived estimates of EC and nutrients expected under natural conditions) as predictors of altered physicochemical conditions for 3 reasons: 1) the effect of land uses on in-stream physicochemical conditions may depend on the natural environmental setting, 2) we were interested in assessing the effects of land use on stressor levels in context of natural background conditions, and 3) land use and naturally occurring environmental characteristics may be correlated. By including natural environmental characteristics or estimates of naturally occurring physicochemical conditions as predictors in the RF models, the partial dependence plots should more accurately describe how stress varied across gradients of different land uses.

We used the same modeling approach described earlier to develop models iteratively through stepwise removal of the weakest predictors. For nutrients and EC, potential predictors included model-estimated reference-condition values of these factors and land-use variables. For metals, potential predictors included all measures of land uses and all measures of natural environmental characteristics (Appendix 1). We retained landuse predictors with interpretable relationships with the response variables even if they were relatively weak predictors so that we could assess the relative strength of associations between different land uses and stressors. We used the % variance in each stressor explained by the models, variable importance measures, and partial dependence plots to assess the performance of models and interpret relationships. We could not model relationships between actual flow modification and the source of flow modification (dams and land uses) because we did not have direct measures of hydrologic alteration. If stressors were not continuously related to gradients of specific land use, we used *t*-tests to determine if stressor levels were related to general watershed alteration, i.e., if mean stressor levels differ between reference and test sites.

TABLE 1. Minima, maxima, and coefficients of variation (CV) of potential stressors among sites. Both observed minus expected (O–E) and raw observed values of electrical conductivity (EC), total P (TP), and total N (TN) are listed for context. Negative values of O–E are caused by model error at low levels of expected concentrations of total dissolved solids (EC), TP, and TN.

| Stressor | Minimum | Maximum | CV |
|---|---|---|---|
| $O–E_{EC}$ (μS/cm) | −241 | 2743 | 2.51 |
| EC (μS/cm) | 22 | 3256 | 1.35 |
| $O–E_{TP}$ (μg/L) | −32.6 | 2571 | 2.95 |
| TP | 4.16 | 2600 | 1.97 |
| $O–E_{TN}$ (μg/L) | −231 | 3891 | 2.05 |
| TN | 0 | 4200 | 1.05 |
| Watershed maximum reservoir volume ($m^3/km^2$) | 0.0 | 363,250 | 6.34 |
| Maximum reservoir volume within 3 km ($m^3/km^2$) | 0.0 | 12,854 | 20.5 |
| Watershed sum reservoir volume ($m^3/km^2$) | 0.0 | 539,608 | 6.25 |
| Sum reservoir volume within 3 km ($m^3/km^2$) | 0.0 | 12,854 | 19.9 |
| As (μg/L) | 1.9 | 250.7 | 2.10 |
| Cd (μg/L) | 1.0 | 2.0 | 0.14 |
| Cr (μg/L) | 1.5 | 4.0 | 0.10 |
| Cu (μg/L) | 1.7 | 5.7 | 0.17 |
| Pb (μg/L) | 1.0 | 2.3 | 0.26 |
| Hg (μg/L) | 0.1 | 0.5 | 0.34 |
| Ni (μg/L) | 2.8 | 16.0 | 0.22 |
| Se (μg/L) | 1.5 | 7.6 | 0.34 |
| Ag (μg/L) | 1.3 | 2.0 | 0.09 |
| Zn (μg/L) | 8.1 | 157.0 | 0.66 |

## Results

### *Stressor levels*

Stressor levels varied considerably among sites (Table 1), but they were relatively low at most sites. Reservoir volume was the most variable potential stressor followed by $O–E_{TP}$, As, and $O–E_{EC}$. PCA showed that stressors tended to covary along 7 axes of variation (Table 2).

### *Relationships between biological condition and stressors*

Four stressors were important predictors of $O/E_{taxa}$ scores (Table 3). $O/E_{taxa}$ scores were negatively associated with $O–E_{EC}$, Cu, As, and Zn concentrations (Fig. 3A–D). These stressors accounted for 20% of the variance in $O/E_{taxa}$ scores out of a maximum possible 64% variance. $O/E_{taxa}$ scores declined as $O–E_{EC}$ increased from near 0 to ~400 μS/cm, but showed little further response to $O–E_{EC}$ values >600 μS/cm (Fig. 3A). $O/E_{taxa}$ declined sharply and uniformly across the full range of observed As values (~0–80 μg/L) (Fig. 3B). $O/E_{taxa}$ was insensitive to Cu concentrations between 0 and 10 μg/L, but dropped precipitously at concentrations >10 μg/L (Fig. 3C). $O/E_{taxa}$ was weakly associated with variation in Zn concentrations (Fig. 3D). We did not observe evidence for interactive effects of stressors on $O/E_{taxa}$.

Four stressors were important predictors of MMI scores: As, $O–E_{EC}$, $O–E_{TN}$, and $O–E_{TP}$ (Table 3). These 4 stressors accounted for 13% of the variance in MMI scores out of a maximum possible 80%. As observed for the $O/E_{taxa}$ index, MMI scores declined sharply and continuously with increasing concentrations of As (Fig. 3E). However, the MMI was much less responsive to variation in $O–E_{EC}$ than was $O/E_{taxa}$ (Fig. 3F), and it was not responsive to variation in either Zn or Cu. In contrast to the $O/E_{taxa}$ index, the MMI did vary with excess nutrient concentrations (Fig. 3G, H). MMI values declined ~0.1 units (10% of the mean reference-site value) across a 0 to 1000 μg/L range of excess TN, but increased slightly as $O–E_{TP}$ increased from 0 to 20 μg/L. We did not observe obvious evidence for interactive effects of stressors on MMI values. $O–E_{EC}$, $O–E_{TN}$, and $O–E_{TP}$ accounted for 2 to 17% of the variance in the component metrics of

TABLE 2. Varimax rotated principal components loadings for all stressors. Bolded values highlight strongly correlated stressors. Abbreviations are as in Table 1.

| Stressor | Axis 1 | Axis 2 | Axis 3 | Axis 4 | Axis 5 | Axis 6 | Axis 7 |
|---|---|---|---|---|---|---|---|
| Cu | **0.94** | 0.09 | −0.01 | 0.00 | 0.05 | 0.22 | 0.03 |
| Pb | **0.94** | 0.07 | −0.01 | −0.01 | 0.14 | 0.10 | 0.02 |
| Hg | **0.90** | 0.11 | −0.01 | 0.00 | 0.36 | −0.04 | 0.00 |
| Cd | **0.87** | 0.16 | −0.01 | −0.01 | 0.13 | 0.00 | −0.04 |
| Zn | **0.86** | 0.02 | −0.02 | −0.05 | −0.10 | 0.12 | 0.05 |
| $O–E_{TP}$ | 0.02 | **0.94** | 0.01 | −0.01 | 0.08 | 0.07 | 0.02 |
| $O–E_{EC}$ | 0.24 | **0.80** | −0.02 | 0.00 | 0.01 | 0.14 | 0.14 |
| $O–E_{TN}$ | 0.11 | **0.67** | 0.01 | −0.02 | −0.19 | 0.43 | 0.11 |
| Maximum reservoir volume within 3 km | −0.02 | 0.00 | **1.00** | 0.01 | 0.01 | 0.00 | 0.00 |
| Sum reservoir volume within 3 km | −0.02 | 0.00 | **1.00** | 0.01 | 0.01 | 0.00 | 0.00 |
| Watershed maximum reservoir volume | −0.03 | −0.01 | 0.01 | **1.00** | 0.03 | 0.00 | −0.01 |
| Watershed sum reservoir volume | −0.03 | −0.01 | 0.01 | **1.00** | 0.03 | −0.01 | −0.01 |
| Ag | 0.10 | 0.02 | 0.01 | 0.04 | **0.91** | −0.05 | 0.03 |
| Cr | 0.20 | −0.06 | 0.00 | 0.01 | **0.83** | 0.05 | 0.05 |
| Se | 0.21 | 0.20 | 0.00 | 0.00 | −0.10 | **0.87** | 0.06 |
| Ni | 0.08 | 0.34 | 0.00 | −0.01 | 0.43 | **0.61** | −0.13 |
| As | 0.04 | 0.17 | −0.01 | −0.02 | 0.06 | 0.01 | **0.97** |

the MMI (Table 3). The most responsive (17% of variance) metrics were clinger richness and Ephemeroptera relative abundance.

We did observe evidence that hydrologic alteration negatively affected biological condition even though reservoir volume was not an important predictor of index scores in the RF models. $O/E_{taxa}$ scores were lower for samples from sites with a dam within 3 km upstream (mean = 0.56) than for sites without dams within 3 km upstream (mean = 0.91) ($p < 0.005$). However, MMI scores from sites near dams did not differ significantly from scores from sites that were not in close proximity to dams.

TABLE 3. Percent variance (% var) accounted for by each of the biota–stressor Random Forest models. Predictors are listed in order (left to right) of their importance in the model. Abbreviations are as in Table 1.

| Index | % var | Predictors |
|---|---|---|
| $O/E_{taxa}$ | 20 | $O–E_{EC}$, Cu, As, Zn |
| MMI | 13 | As, $O–E_{EC}$, $O–E_{TN}$, $O–E_{TP}$ |
| Insect richness | 2 | As, $O–E_{TP}$, $O–E_{EC}$, $O–E_{TN}$ |
| Ephemeroptera relative abundance | 17 | $O–E_{EC}$, $O–E_{TP}$, As, $O–E_{TN}$ |
| Shannon diversity | 8 | As, $O–E_{EC}$, $O–E_{TP}$, $O–E_{TN}$ |
| Collector/filterer relative abundance | 11 | As, $O–E_{EC}$, $O–E_{TN}$, $O–E_{TP}$ |
| Plecopteran relative abundance | 6 | As, $O–E_{TP}$, $O–E_{TN}$, $O–E_{EC}$ |
| Noninsect richness | 10 | $O–E_{EC}$, As, $O–E_{TN}$, $O–E_{TP}$ |
| Clinger richness | 17 | $O–E_{EC}$, As, $O–E_{TP}$, $O–E_{TN}$ |

Biological index scores also were related to overall human impacts. Mean scores for both indices were ~10% lower at test sites that failed a screen for land use and other potential human impacts ($O/E_{taxa}$ = 0.89, MMI = 0.90) than at reference sites (1.0 for both indices).

*Relationships between stress and land uses*

Variation in biologically important stressor values among sites was associated (32–96% of variance) with a combination of land use and natural features (Table 4, Appendix 2). Altered EC was the most predictable stressor and was positively related to agriculture, mine density, and urbanization. With the distinct exception of As, land use accounted for most of the variation in in-stream stressor levels (Table 4). As levels were significantly ($p < 0.005$) higher at test sites (mean = 7.8 μg/L) than reference sites (mean = 5.0 μg/L), but As concentrations were not related to any measure of land use used in RF models. In contrast to As, in-channel dissolved concentrations of other metals, such as Cu and Zn, were associated with urban development and mining within watersheds. Altered EC and nutrient levels were most strongly, and positively, associated with agriculture and urban development. For all stressors, % area of the entire watershed classified as urban or agriculture was a better predictor of stressor levels than the % area of the watershed within 3 km upstream from the sample site.

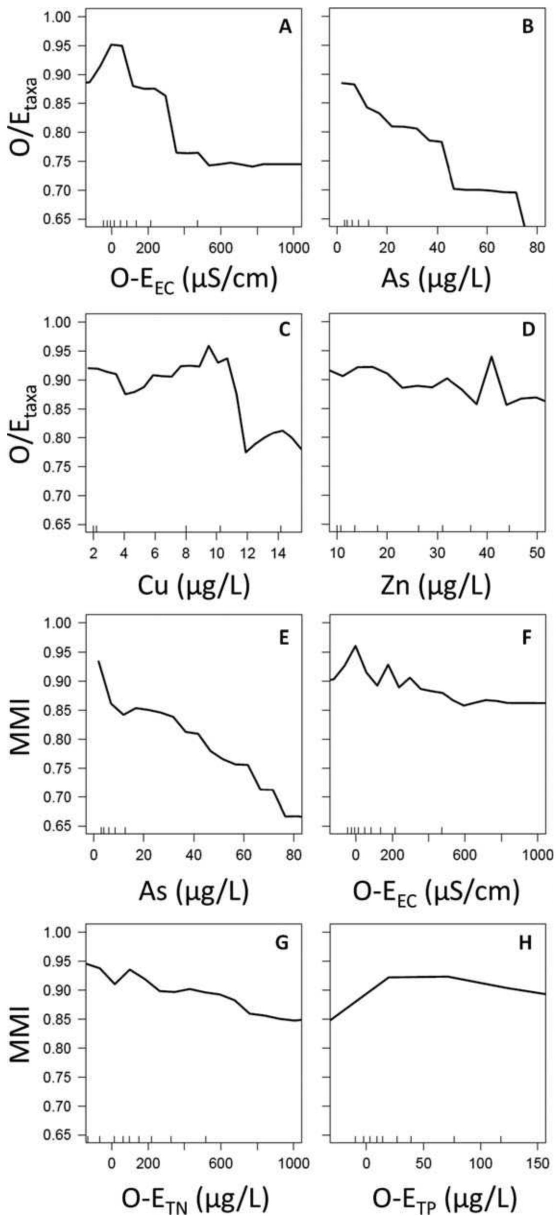



FIG. 3. Partial dependence plots of biological indices on stressors from the Random Forest biota–stressor models that describe how biological index scores for observed/expected taxa at a site (O/E$_{taxa}$) (A–D) and the multimetric index (MMI) (E–H) vary along the stressor gradients. A.—O–E$_{EC}$. B.—As. C.—Cu. D.—Zn. E.—As. F.—O–E$_{EC}$. G.—O–E$_{TN}$. H.—O–E$_{TP}$. EC = electrical conductivity, TN = total N, TP = total P.

Some stressors also varied with natural watershed features (Table 4). As levels were negatively related to precipitation and elevation and positively related to air temperature. Cu and Zn concentrations decreased with either precipitation or elevation, but not as strongly as observed for As. Little of the variation in O–E values for EC, TN, and TP was associated with

TABLE 4. Summary of Random Forest (RF) models predicting biologically important stressors. % var = % variation in each stressor accounted for by the model. Values on the left of the slash indicate model performance with both natural and landuse predictors. Values on the right of the slash indicate model performance without natural predictors. Predictors are listed from left to right in order of importance in the model. Signs in parentheses indicate the general direction of the stressor in response to a predictor. Abbreviations are as in Table 1.

| Stressor | % var | Predictors |
|---|---|---|
| O–E$_{EC}$ | 96/93 | Predicted EC (+), % watershed agriculture (+), mine density (+), % watershed urban (+) |
| O–E$_{TN}$ | 52/46 | % watershed urban (+), % watershed agriculture (+), mine density (+), predicted total N (+) |
| O–E$_{TP}$ | 43/41 | % watershed agriculture (+), % watershed urban (+), predicted total P (+) |
| As | 32/0 | watershed mean annual maximum precipitation (−), site elevation (−), watershed mean annual minimum temperature (+) |
| Cu | 80/69 | % watershed urban (+), watershed mean annual maximum wet days (−), site elevation (−), watershed mean hydrologic stability (−), mine density (+) |
| Zn | 62/56 | % watershed urban (+), mine density (+), site elevation (−) |

predicted reference-condition concentrations, results indicating that the magnitude of stress (O–E values) for these factors generally was not related to the natural background concentrations of these factors at a site.

## Discussion

Restoring the biological integrity of degraded streams requires that we identify the stressors causing degradation and the sources of those stressors. Our use of a modeling approach to quantify relationships between biological index scores, potential stressors, and measures of watershed alteration enabled us to address 2 major challenges for quantifying relationships between biota, stressors, and land uses: 1) separating anthropogenic effects on physicochemical conditions and biota from natural variation, and 2) assessing relative, cumulative, and interactive effects of co-occurring stressors on biotic condition. Our interpretations of the modeling results were guided by the degree to which observed relationships were consistent with established causal mechanisms (e.g., Suter et al. 2010, Allan et al. 2011, Norris et al. 2012). Our approach is similar in concept to that used by others who have tried to relate land uses, stressors, and biota (e.g., Dauer et al. 2000, Volstad et al. 2003, Yuan and Norton 2004, Novotny et al. 2005), but our use of models to account for natural variation in both stressors and biota, coupled with a nonparametric modeling technique improved our ability to interpret these relationships.

*Biological index responsiveness to stressors*

Our use of 2 established types of biological indices revealed that different biological indices may be differentially responsive to the same stressors. These differences in responsiveness have implications for interpreting the biological effects of stressors on aquatic ecosystems. Differences in index responsiveness to stressors could lead to different assessments of biological condition and potentially could cause managers to ignore stressors that are actually causing biological harm. To interpret biological responses to stressors adequately, understanding what the biological indices we use actually measure and how they are likely to change along stressor gradients is critical.

The higher responsiveness of the $O/E_{taxa}$ index than the MMI to stressors may have occurred for at least 2 reasons. First, $O/E_{taxa}$ and an MMI are based on different biological properties of the same assemblage that may differ in their responsiveness to stress for ecological or statistical reasons. For example, the responsiveness of the MMI may have been dampened by aggregation of information from individual taxa into composite metrics that describe community-level attributes (trophic structure, diversity, etc.). If sensitivity to specific stressors varies among taxa that contribute information to a metric, the overall responsiveness of a metric will be some average function of responses of those specific taxa that contribute to a metric. Furthermore, an MMI as a whole comprises individual metrics that may differ in their response to any given stress. $O/E_{taxa}$ is not prone to these averaging effects because reductions in $O/E_{taxa}$ occur when individual taxa expected at a site are lost, theoretically because of stress and in order of their sensitivities to local stressors. Second, differences in how indices are calibrated may affect their responsiveness (Hawkins et al. 2010a). For example, $O/E_{taxa}$ indices are calibrated with only reference data, whereas MMIs are calibrated with both reference sites and predefined degraded sites. Calibrating an index with data from disturbed sites generally should lead to high responsiveness, but MMIs may show dampened response to high levels of stress or to novel stressors if the degraded sites used in calibration do not adequately characterize the complete mix or levels of stressors within a region (Hawkins et al. 2010a). However, the differential sensitivities of the 2 types of indices cannot necessarily be generalized to other studies or types of stressors. MMIs measure a somewhat different aspect of biological condition than $O/E_{taxa}$ indices, and may respond more strongly than $O/E_{taxa}$ to types of stressors that were not present in our study region. Furthermore, the differential responsiveness of component metrics implies that some individual metrics may be more useful in assessing biological response to different stressors than aggregate indices. In general, more comparisons of index and metric responsiveness to different stressors are needed.

*Accounting for natural variability in both biota and stressors*

Many bioassessments and causal analyses are potentially confounded by spatial covariation of naturally occurring features and human alteration of the environment. For example, more human-associated alteration has occurred in lowland than upland settings. Our use of modeled bioassessment indices (Vander Laan 2012) that measure biological condition as the deviation from expected reference condition (Hawkins et al. 2010a) allowed us to account for the effects of naturally occurring environmental variability on biota and to attribute changes in biological condition scores to anthropogenic stressors with more confidence. We were able to use a similar approach to understand when physicochemical conditions probably exceeded levels expected at individual sites, which allowed us to describe both biota–stress relationships and stress–land use relationships more accurately.

Quantification of stressors can be an especially difficult problem when analyzing field data. For novel stressors, direct measures of the concentrations observed at each site should be a meaningful estimate of exposure because natural background concentrations must be 0. We assumed that observed metal concentrations were associated with anthropogenic disturbance. However, this assumption may not have been completely robust because relatively high levels of metals in streams can result from natural geologic sources (Schmidt et al. 2012). We were not able to account for potential natural sources of trace metals in our study area. However, the levels of metal concentrations that we observed at reference sites were below those considered to be toxic (USEPA 1996), which suggests that natural background metal concentrations may be generally low in the study region. Many other forms of stress represent human-caused changes in physicochemical conditions that can naturally vary among locations, e.g., water chemistry, temperature, sediment, nutrients, and flow modification. In these cases, potential stress is best measured as deviation from natural conditions. We were able to estimate deviation from expected reference condition for EC (Olson and Hawkins 2012) and nutrients (Olson and Hawkins 2013), so we were able to strengthen inferences regarding the effect of alterations in these factors on invertebrate assemblages and the landuse activities that are associated with their alteration. However, we did not have direct estimates of hydrologic regimes and, therefore, had to use a proxy (reservoir volume) to estimate flow alterations. Better methods of estimating flow alterations would greatly improve our ability to analyze the effects of altered hydrology on stream biota. Omitting natural predictors from the land use–stressor models had only a small effect on stressor predictability (with the exception of As), suggesting that watershed alteration accounts for most spatial variation in stressors in our study region. However, stressors should be interpreted in the context of natural environmental gradients, and including natural predictors increased our confidence in interpreting relationships between land uses and stressors.

### *Stressor-specific relationships*

*Metals.*—Zn, Cu, and As were associated with degraded biological condition in our data set, a result consistent with results of several other studies in which negative associations between the condition of invertebrate assemblages and metal contamination were reported in field (e.g., Clements et al. 2000, Cain et al. 2004, Pollard and Yuan 2006) or laboratory settings (e.g., Richardson and Kiffney 2000, Clements et al. 2002, Clements 2004). Modes of biological uptake and toxicity are less broadly understood, but several studies have identified possible pathways (e.g., Xie et al. 2009, 2010). In general, metal toxicity can increase mortality or reduce the fitness of aquatic invertebrates (e.g., Thorp et al. 1979, Wicklum and Davies 1996) and increase population loss by increasing emigration through invertebrate drift (Clements 2004). This information coupled with the negative relationships we and others have observed between measures of biological condition and metal concentrations supports the inference that metal contamination is a stressor of concern in NV streams. Zn and Cu also tended to co-occur spatially with other metals like Cd, Pb, and Hg (Table 2), results suggesting that the relationships we observed between biota and Zn and Cu may represent the combined effects of this suite of heavy metals. Zn and Cu were both strongly associated with human land uses, such as urbanization and mining, a result implying that these land uses are sources of contamination by these metals and the ultimate cause of biological degradation. These source-channel relationships are consistent with previous observations (e.g., Paulson 1997, Beasley and Kneale 2002, Macklin et al. 2006, Wong et al. 2006, Xiao and Ji 2007).

The US EPA recommends a chronic water-quality criterion of 150 $\mu g/L$ of dissolved As to protect aquatic life (USEPA 1996). Our results suggest that dissolved As affects stream invertebrates at concentrations well below this value and that this recommendation should be reevaluated. However, our results also imply that setting a single, ecologically meaningful As criterion may be difficult if high As concentrations occur naturally in some streams (e.g., Wilkie and Hering 1998). Given that we found no relationships between As concentrations and variation in specific types of land use, the biological associations with As that we observed may not be associated with anthropogenic activities. However, the moderate difference in mean As concentrations between reference and test sites does imply that human activity is generally associated with increased concentrations of As in streams. Therefore, we probably did not characterize the specific human activities associated with increased delivery of As to these streams (e.g., atmospheric deposition, As-based pesticide use). Determining how much of the As concentrations in these streams result from natural conditions and human activities will require a detailed and more-refined analysis of potential sources of As than we were able to conduct in our study. Even though the sources of As in many streams are uncertain, the associations between biological condition and As levels that we observed should be useful in setting criteria for those streams known to have naturally low levels of As. Improved ways to account for background metal concentrations in bioassessments and causal analyses (e.g., Schmidt et al. 2012) will help our understanding of both the sources of metal contamination and its effects on stream biota.

Climatic and geographic factors also may be important determinants of a stream's exposure and susceptibility to metal contamination. The fact that As, Cu, and Zn concentrations were negatively associated with site elevation and cooler, wetter climates implies that the natural environmental setting can influence dissolved metal concentrations. Higher-elevation sites are generally more remote and have smaller watersheds, which generally should result in a lower probability of metal contamination from urban development or mineral extraction. However, watershed area was not a good predictor of metal concentrations, suggesting that the patterns in metal concentrations we observed along elevation and climate gradients are not associated simply with the size of the watershed and the probability that reaches receive metal contaminants from up-watershed sources. We suspect this pattern may be the result of the evaporative concentration of dissolved metals at lower elevation sites that, in this region, generally experience hotter and drier climates than high elevation sites.

*Hydrologic alteration.*—Hydrologic alteration is considered one of the most serious threats to stream ecosystems (Bunn and Arthington 2002). The low $O/E_{taxa}$ scores that we observed at sites in close proximity (3 km) of dams is consistent with this generalization, but the lack of relationships between $O/E_{taxa}$ and the MMI with upstream reservoir volume implies that overall hydrologic alteration may not be as important a stressor as chemical alteration in NV streams. However, only 8 samples in our study were taken from sites within 3 km of a dam, and small sample size may explain why reservoir volume was not an important predictor in RF models. In addition, use of reservoir volume as a proxy for hydrological alteration does not fully characterize flow alterations. Quantitative characterizations of natural hydrologic regimes have been developed (e.g., Carlisle et al. 2010, Chinnayakanahalli et al. 2011), but we did not have comparable observations of flow regimes to quantify site-specific hydrologic modification. Estimates of the deviation of observed hydrologic conditions from expected reference conditions would strengthen causal arguments for biological responses to flow modification.

*Nutrients.*—Nutrient enrichment is an important stressor in many stream ecosystems, but we did not observe strong changes in invertebrate assemblages in response to elevated nutrients. Indices based on algae (e.g., Smith et al. 2007) may be better indicators of changes in ecological condition resulting from nutrient enrichment than indices based on invertebrates because nutrient enrichment in streams is more likely to have direct effects on algal assemblages than on invertebrate assemblages. In fact, in naturally nutrient-poor streams, modest increases in nutrients may increase macroinvertebrate abundance and richness and potentially could compensate for the adverse effects of other stressors (e.g., Hawkins et al. 2000).

*Total dissolved solids.*—In our study area, increases in EC above expected natural levels ($O–E_{EC} > 0$) translate to absolute conductivities that are $>\sim$300 μS/cm, which is well below levels associated with acute toxicity for most freshwater invertebrates (Blasius and Merritt 2002, Kefford et al. 2003, 2005, Benbow and Merritt 2004). Therefore, we suspect that the biological alterations we observed with elevated EC probably have resulted from shifts in chemical niche space associated with taxon-specific differences in osmoregulatory ability. Increased EC improves conditions for taxa that are intolerant of very low-ionic-strength water, whereas taxa that are adapted to low EC may be outcompeted and excluded from systems with elevated EC (Olson 2012). This 300 μS/cm threshold is the same as that observed in Central Appalachian streams (USEPA 2011), and may indicate a major natural threshold between freshwater invertebrate assemblage types. Our results showing strong associations between excess EC and land use are consistent with the observations of others who also report increases in stream EC associated with agriculture (e.g., Johnson et al. 1997, Pan et al. 2004), urbanization (e.g., Wang and Yin 1997, Hatt et al. 2004), and mining (e.g., Pond et al. 2008, Palmer et al. 2010).

Additional research on how changes in EC affect freshwater communities is especially needed. We and others (e.g., USEPA 2011, Pond et al. 2008) have linked changes in EC to changes in invertebrate assemblages, but the causal mechanisms by which EC in excess of natural conditions affects the fitness and survival of specific invertebrate taxa are not well understood. Some investigators have suggested that EC constituents, such as $Cl^-$, rather than total EC, may be responsible for associated biological degradation (Soucek et al. 2011), but others have argued that it is the mixture of all ions that leads to biological degradation (Cormier et al. 2013). Further work that establishes the chemical, physiological, and ecological bases for invertebrate assemblage alterations in response to changes in the individual and combined constituents of EC would greatly strengthen our understanding of this potentially critical stressor and benefit development of EC criteria that are protective of freshwater ecosystems.

### *Implications for causal analysis of stream degradation*

We used a modeled reference-condition approach to quantify several types of stress that represent human alterations of naturally occurring physicochemical conditions. To assess levels of these stressors accurately, we needed to separate anthropogenic alterations of physicochemical conditions from natural variation. We were able to separate these components by using models that predict expected natural physicochemical reference conditions (e.g., Hawkins et al. 2010b, Olson and Hawkins 2012, 2013). However, easily applied models do not yet exist for many important stressors like sedimentation or acidification, and widely applicable EC and nutrient models have been developed only for streams in the western USA. In contrast, for some stressors, the ability to assess alteration of physicochemical characteristics will be limited by inadequate characterizations of observed conditions, not by our inability to estimate conditions expected under reference conditions. For example, models to estimate expected reference-stream temperature (Hill et al. 2013) and hydrologic characteristics (Carlisle et al. 2010) have been developed, but for our study, insufficient data existed to produce comparable characterizations of observed conditions for temperature and hydrologic alteration. Temperature and hydrology are both potentially important determinants of biological patterns (Jacobsen et al. 1997, Vinson 2001, Bunn and Arthington 2002, Olden and Naiman 2010) and the ability to assess alterations in these factors quantitatively will be crucial for future causal analyses.

Spatial covariation and potential interactive and nonlinear effects of stressors on biota can make it difficult to attribute biological degradation to specific stressors. These issues also may make simple correlation- or linear-regression-based approaches (e.g., de Zwart et al. 2006, Kapo et al. 2008, Atkinson et al. 2009, Brown et al. 2012) less successful in characterizing the true effects of stressors on biota. Assessing interactive effects of stressors with linear models requires large sample sizes and a priori hypotheses about potentially interactive stressors (e.g., de Zwart et al. 2006). However, most causal analyses are done with relatively small sample sizes (e.g., $<100$) and many possible stressors, resulting in little statistical power to assess interactive effects of stressors with linear models. A multivariate modeling approach capable of accounting for the interactive and nonlinear effects of spatially covarying stressors, such as RF, should increase statistical power and improve causal analyses of stream degradation.

The overall goal of causal analysis is to determine the causes of degradation at individual sites. However, this goal cannot be accomplished without having realistic hypotheses about what stressors and stressor levels probably are causing biological degradation. Our study showed that analyses of field data from multiple sites within a region can provide important insights regarding the likely effects of stressors on real biological communities and can provide the realistic hypotheses necessary to guide site-specific causal analyses. Laboratory experiments are often thought to produce the most reliable inferences regarding the effect of a stressor on biota because they control for extraneous variables and isolate the effects of individual stressors. However, they may lack the realism needed to identify the levels of stress that cause degradation in real ecosystems. In particular, laboratory experiments often cannot test the responsiveness of either the most sensitive taxa or the most sensitive life stages because these organisms are difficult to maintain in the laboratory (e.g., Buchwalter et al. 2007). Instead, these experiments often rely on standard test organisms, which are less sensitive to stress and, therefore, overestimate safe stressor levels. For example, metal concentrations that elicit toxicity in the laboratory can be much higher than those associated with degraded aquatic communities in the field (Buchwalter et al. 2007). Similarly, the levels of excess EC that we found to be associated with biological alteration are much lower than those found to be toxic from experiments (Blasius and Merritt 2002, Kefford et al. 2003, 2005, Benbow and Merritt 2004). More realistic laboratory experiments that incorporate sensitive species and life stages could result in better estimates of safe levels of stress. The use of field data ensures realism and is probably a better starting point for identifying the levels of stress relevant to the measures of biological condition that resource managers use to assess aquatic life. Realistic experiments, conducted under field conditions, could then be used to test these field-derived hypotheses and refine criteria.

To restore and protect stream ecosystems, managers must be able to assess biological condition and to identify likely causes of degradation. Causal analyses must overcome significant challenges, such as separating natural variation in physicochemical and biological properties from human-caused changes and accurately quantifying relationships between biota and potential stressors in complex, multistressor environments. We recommend use of a modeled reference-condition approach to separate natural from anthropogenic effects by predicting biological and physicochemical characteristics that would be expected in the absence of human disturbance. This approach will require development of models to predict reference-condition levels of several important stressors and monitoring programs that adequately measure observed physiochemical conditions. In addition, for some stressors, such as EC, further research is needed to establish or confirm the causal mechanisms associated with biological harm, or for stressors, such as As, to identify realistic levels of exposure that cause biological degradation. Last, more studies that seek to quantify relationships between stressors and stream biota and identify likely causes of degradation at regional spatial scales would improve our understanding of important stressors and thresholds of degradation. These causal analyses will require comprehensive monitoring programs that simultaneously quantify biological assemblages and stressor levels.

## Acknowledgements

We thank Robin Jones, Iva Sokolovska, 3 anonymous referees, and Associate Editor Will Clements for suggestions that improved the manuscript. Our research was supported by the Nevada Division of Environmental Protection.

## Literature Cited

Allan, J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35:257–284.

Allan, J. D., and M. M. Castillo. 2007. Stream ecology: structure and function of running waters. 2nd edition. Springer, Dordrecht, The Netherlands.

Allan, J. D., L. L. Yuan, P. Black, T. Stockton, P. E. Davies, R. H. Magierowski, and S. M. Read. 2011. Investigating the relationships between environmental stressors and the stream condition using Bayesian belief networks. Freshwater Biology 57:58–73.

Atkinson, S. F., D. R. Johnson, B. J. Venables, J. L. Slye, J. R. Kennedy, S. D. Dyer, B. B. Price, M. Ciarlo, K. Stanton, H. Sanderson, and A. Nielsen. 2009. Use of watershed factors to predict consumer surfactant risk, water quality, and habitat quality in the upper Trinity River, Texas. Science of the Total Environment 407:4028–4037.

Beasley, G., and P. Kneale. 2002. Reviewing the impact of metals and PAHs on macroinvertebrates in urban watercourses. Progress in Physical Geography 26: 236–270.

Benbow, M. E., and R. W. Merritt. 2004. Road-salt toxicity of select Michigan wetland macroinvertebrates under different testing conditions. Wetlands 24:68–76.

Blasius, B. J., and R. W. Merritt. 2002. Field and laboratory investigations on the effects of road salt (NaCl) on stream macroinvertebrate communities. Environmental Pollution 120:219–231.

Brown, L. R., J. T. May, A. C. Rehn, P. R. Ode, I. R. Waite, and J. G. Kennen. 2012. Predicting biological condition in southern California streams. Landscape and Urban Planning 108:17–27.

Buchwalter, D. B., D. J. Cain, W. H. Clements, and S. N. Luoma. 2007. Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. Environmental Science and Technology 41:4821–4828. Correction in: Environmental Science and Technology 42:3117.

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management 30: 492–507.

Cain, D. J., S. N. Luoma, and W. G. Wallace. 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. Environmental Toxicology and Chemistry 23:1463–1473.

Cao, Y., C. P. Hawkins, J. Olson, and M. A. Kosterman. 2007. Modeling natural environmental gradients improves the accuracy and precision of diatom-based indicators. Journal of the North American Benthological Society 26: 566–585.

Carlisle, D. M., J. Falcone, D. M. Wolock, M. R. Meador, and R. H. Norris. 2010. Predicting the natural flow regime: models for assessing hydrological alteration in streams. River Research and Applications 26:118–136.

Chambers, J. C., N. Devoe, and A. Evenden (editors). 2008. Collaborative management and research in the Great Basin: examining the issues and developing a framework for action. General Technical Report RMRS-GTR-204. Rocky Mountain Research Station, US Department of Agriculture Forest Service, Fort Collins, Colorado.

Chinnayakanahalli, K. J., C. P. Hawkins, D. G. Tarboton, and R. A. Hill. 2011. Natural flow regime, temperature and the composition and richness of invertebrate assemblages in streams of the western United States. Freshwater Biology 56:1248–1265.

Chinnayakanahalli, K., C. Kroeber, R. A. Hill, D. G. Tarboton, and C. P. Hawkins. 2006. The multi-watershed delineation tool: GIS software in the support of regional watershed analyses. Utah State University, Logan, Utah.

Clements, W. H. 2004. Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. Ecological Applications 14:954–967.

Clements, W. H., D. M. Carlisle, L. A. Courtney, and E. A. Harrahy. 2002. Integrating observational and experimental approaches to demonstrate causation in stream biomonitoring studies. Environmental Toxicology and Chemistry 21:1138–1146.

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

Collins, R., and A. Jenkins. 1996. The impact of agricultural land use on stream chemistry in the Middle Hills of the Himalayas, Nepal. Journal of Hydrology 185:71–86.

Cormier, S. M., G. W. Suter, L. Zheng, and G. J. Pond. 2013. Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions. Environmental Toxicology and Chemistry 32:277–287.

Crane, M., G. A. Burton, J. M. Culp, M. S. Greenberg, K. R. Munkittrick, R. Ribeiro, M. H. Salazar, and S. D. St-Jean. 2007. Review of aquatic in situ approaches for stressor and effect diagnosis. Integrated Environmental Assessment and Management 3:234–245.

Cutler, D. R., T. C. Edwards, K. H. Beard, A. Cutler, and K. T. Hess. 2007. Random forests for classification in ecology. Ecology 88:2783–2792.

Daly, C., M. Halbleib, J. I. Smith, W. P. Gibson, M. K. Doggett, G. H. Taylor, J. Curtis, and P. P. Pasteris. 2008. Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous

United States. International Journal of Climatology 28: 2031–2064.

DAUER, D. M., J. A. RANASINGHE, AND S. B. WEISBERG. 2000. Relationships between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. Estuaries 23:80–96.

DE ZWART, D., S. D. DYER, L. POSTHUMA, AND C. P. HAWKINS. 2006. Predictive models attribute effects on fish assemblages to toxicity and habitat alteration. Ecological Applications 16:1295–1310.

HASTIE, T. J., R. J. TIBSHIRANI, AND J. H. FRIEDMAN. 2001. The elements of statistical learning: data mining, inference, and prediction. Springer Series in Statistics. Springer, New York.

HATT, B. E., T. D. FLETCHER, C. J. WALSH, AND S. L. TAYLOR. 2004. The influence of urban density and drainage infrastructure on the concentrations and loads of pollutants in small streams. Environmental Management 34:112–124.

HAWKINS, C. P., Y. CAO, AND B. ROPER. 2010a. Method of predicting reference condition biota affects the performance and interpretation of ecological indices. Freshwater Biology 55:1066–1085.

HAWKINS, C. P., R. H. NORRIS, J. N. HOGUE, AND J. W. FEMINELLA. 2000. Development and evaluation of predictive models for measuring the biological integrity of streams. Ecological Applications 10:1456–1477.

HAWKINS, C. P., J. R. OLSON, AND R. A. HILL. 2010b. The reference condition: predicting benchmarks for ecological and water-quality assessments. Journal of the North American Benthological Society 29:312–343.

HENTGES, V. A., AND T. W. STEWART. 2010. Macroinvertebrate assemblages in Iowa prairie pothole wetlands and relation to environmental features. Wetlands 30: 501–511.

HERLIHY, A. T., S. G. PAULSEN, J. VAN SICKLE, J. L. STODDARD, C. P. HAWKINS, AND L. L. YUAN. 2008. Striving for consistency in a national assessment: the challenges of applying a reference-condition approach at a continental scale. Journal of the North American Benthological Society 27:860–877.

HILL, R. A., C. P. HAWKINS, AND D. M. CARLISLE. 2013. Predicting thermal reference conditions in USA streams. Freshwater Science 32:39–55.

HOMER, C., J. DEWITZ, J. FRY, M. COAN, N. HOSSAIN, C. LARSON, N. HEROLD, A. MCKERROW, J. N. VANDRIEL, AND J. WICKHAM. 2007. Completion of the 2001 National Land Cover Database for the conterminous United States. Photogrammetric Engineering and Remote Sensing 73: 337–341.

JACOBSEN, D., R. SCHULTZ, AND A. ENCALADA. 1997. Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude. Freshwater Biology 38:247–261.

JOHNSON, L. B., C. RICHARDS, G. E. HOST, AND J. W. ARTHUR. 1997. Landscape influences on water chemistry in Midwestern stream ecosystems. Freshwater Biology 37:193–208.

KAPO, K. E., G. A. BURTON, D. DE ZWART, L. POSTHUMA, AND S. D. DYER. 2008. Quantitative lines of evidence for screening-level diagnostic assessment of regional fish community impacts: a comparison of spatial database evaluation methods. Environmental Science and Technology 42:9412–9418.

KEFFORD, B. J., C. G. PALMER, AND D. NUGEGODA. 2005. Relative salinity tolerance of freshwater macroinvertebrates from the south-east Eastern Cape, South Africa compared with the Barwon Catchment, Victoria, Australia. Marine and Freshwater Research 56:163–171.

KEFFORD, B. J., P. J. PAPAS, AND D. NUGEGODA. 2003. Relative salinity tolerance of macroinvertebrates from the Barwon River, Victoria, Australia. Marine and Freshwater Research 54:755–765.

KIMBALL, K. D., AND S. A. LEVIN. 1985. Limitations of laboratory bioassays: the need for ecosystem level testing. BioScience 35:165–171.

MACKLIN, M. G., P. A. BREWER, K. A. HUDSON-EDWARDS, G. BIRD, T. J. COULTHARD, I. A. DENNIS, P. J. LECHLER, J. R. MILLER, AND J. N. TURNER. 2006. A geomorphological approach to the management of rivers contaminated by metal mining. Geomorphology 79:423–447.

MATTHAEI, C. D., J. J. PIGGOTT, AND C. R. TOWNSEND. 2010. Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. Journal of Applied Ecology 47: 639–649.

NICOLA, G. G., A. ALMODÓVAR, AND B. ELVIRA. 2010. Effects of environmental factors and predation on benthic communities in headwater streams. Aquatic Sciences 72: 419–429.

NORRIS, R. H., J. A. WEBB, S. J. NICHOLS, M. J. STEWARDSON, AND E. T. HARRISON. 2012. Analyzing cause and effect in environmental assessments: using weighted evidence from the literature. Freshwater Science 31:5–21.

NOVOTNY, V., A. BARTOSOVÁ, N. O'REILLY, AND T. EHLINGER. 2005. Unlocking the relationship of biotic waters to anthropogenic integrity of impaired stresses. Water Research 39:184–198.

OLDEN, J. D., AND R. J. NAIMAN. 2010. Incorporating thermal regimes into environmental flows assessments: modifying dam operations to restore freshwater ecosystem integrity. Freshwater Biology 55:86–107.

OLSON, J. R. 2012. The influence of geology and other environmental factors on stream water chemistry and benthic invertebrate assemblages. PhD Dissertation, Utah State University, Logan, Utah.

OLSON, J. R., AND C. P. HAWKINS. 2012. Predicting natural base-flow stream water chemistry in the western United States. Water Resources Research 48:W02504.

OLSON, J. R., AND C. P. HAWKINS. 2013. Developing site-specific nutrient criteria from empirical models. Freshwater Science 32:000–000.

PALMER, M. A., E. S. BERNHARDT, W. H. SCHLESINGER, K. N. ESHLEMAN, E. FOUFOULA-GEORGIOU, M. S. HENDRYX, A. D. LEMLY, G. E. LIKENS, O. L. LOUCKS, M. E. POWER, P. S. WHITE, AND P. R. WILCOCK. 2010. Mountaintop mining consequences. Science 327:148–149.

PAN, Y. D., A. HERLIHY, P. KAUFMANN, J. WIGINGTON, J. VAN SICKLE, AND T. MOSER. 2004. Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: a multi-spatial scale assessment. Hydrobiologia 515:59–73.

PAUL, M. J., AND J. L. MEYER. 2001. Streams in the urban landscape. Annual Review of Ecology and Systematics 32:333–365.

PAULSON, A. J. 1997. The transport and fate of Fe, Mn, Cu, Zn, Cd, Ph and $SO_4$ in a groundwater plume and in downstream surface waters in the Coeur d'Alene Mining District, Idaho, USA. Applied Geochemistry 12:447–464.

POFF, N. L., J. D. ALLAN, M. B. BAIN, J. R. KARR, K. L. PRESTEGAARD, B. D. RICHTER, R. E. SPARKS, AND J. C. STROMBERG. 1997. The natural flow regime. BioScience 47: 769–784.

POLLARD, A. I., AND L. YUAN. 2006. Community response patterns: evaluating benthic invertebrate composition in metal-polluted streams. Ecological Applications 16: 645–655.

POND, G. J., M. E. PASSMORE, F. A. BORSUK, L. REYNOLDS, AND C. J. ROSE. 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. Journal of the North American Benthological Society 27:717–737.

REED, J. C., AND C. A. BUSH. 2001. Generalized geologic map of the conterminous United States. US Geological Survey, Denver, Colorado.

REILEY, M. C. 2007. Science, policy, and trends of metals risk assessment at EPA: how understanding metals bioavailability has changed metals risk assessment at US EPA. Aquatic Toxicology 84:292–298.

RICHARDSON, J. S., AND P. M. KIFFNEY. 2000. Responses of a macroinvertebrate community from a pristine, southern British Columbia, Canada, stream to metals in experimental mesocosms. Environmental Toxicology and Chemistry 19:736–743.

SCHMIDT, T. S., W. H. CLEMENTS, R. B. WANTY, P. L. VERPLANCK, S. E. CHURCH, C. A. SAN JUAN, D. L. FEY, B. W. ROCKWELL, E. H. DEWITT, AND T. L. KLEIN. 2012. Geologic processes influence the effects of mining on aquatic ecosystems. Ecological Applications 22:870–879.

SMITH, A. J., R. W. BODE, AND G. S. KLEPPEL. 2007. A nutrient biotic index (NBI) for use with benthic macroinvertebrate communities. Ecological Indicators 7:371–386.

SMOLDERS, A. J. P., R. A. C. LOCK, G. VAN DER VELDE, R. I. M. HOYOS, AND J. G. M. ROELOFS. 2003. Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. Archives of Environmental Contamination and Toxicology 44: 314–323.

SOUCEK, D. J., T. K. LINTON, C. D. TARR, A. DICKINSON, N. WICKRAMANAYAKE, C. G. DELOS, AND L. A. CRUZ. 2011. Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. Environmental Toxicology and Chemistry 30:930–938.

STODDARD, J. L., D. P. LARSEN, C. P. HAWKINS, R. K. JOHNSON, AND R. H. NORRIS. 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16:1267–1276.

SUTER, G. W., S. B. NORTON, AND S. M. CORMIER. 2010. The science and philosophy of a method for assessing environmental causes. Human and Ecological Risk Assessment 16:19–34.

THORP, J. H., J. P. GIESY, AND S. A. WINERITER. 1979. Effects of chronic cadmium exposure on crayfish survival, growth, and tolerance to elevated temperatures. Archives of Environmental Contamination and Toxicology 8:449–456.

TOWNSEND, C. R., S. S. UHLMANN, AND C. D. MATTHAEI. 2008. Individual and combined responses of stream ecosystems to multiple stressors. Journal of Applied Ecology 45:1810–1819.

USACE (US ARMY CORPS OF ENGINEERS). 2009. National inventory of dams US Army Corps of Engineers, Washington, DC. (Available from: http://geo.usace.army.mil/pgis/f?p=397:1:0:)

USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 1996. 1995 Updates: water quality criteria documents for the protection of aquatic life in ambient water. EPA-820-B-96-001. Office of Water, US Environmental Protection Agency, Washington, DC.

USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 2011. A field-based aquatic life benchmark for conductivity in central Appalachian streams. EPA/600/R-10/023. Office of Research and Development, National Center for Environmental Assessment, US Environmental Protection Agency, Washington, DC.

VANDER LAAN, J. J. 2012. Environmental assessment of streams: linking land use, instream stressors, and biological indices to infer likely causes of ecological impairment. MS Thesis, Utah State University, Logan, Utah.

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.

VINSON, M. R. 2001. Long-term dynamics of an invertebrate assemblage downstream from a large dam. Ecological Applications 11:711–730.

VOLSTAD, J. H., N. E. ROTH, G. MERCURIO, M. T. SOUTHERLAND, AND D. E. STREBEL. 2003. Using environmental stressor information to predict the ecological status of Maryland non-tidal streams as measured by biological indicators. Environmental Monitoring and Assessment 84:219–242.

WALSH, C. J., A. H. ROY, J. W. FEMINELLA, P. D. COTTINGHAM, P. M. GROFFMAN, AND R. P. MORGAN. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society 24:706–723.

WANG, X. H., AND Z. Y. YIN. 1997. Using GIS to assess the relationship between land use and water quality at a watershed level. Environment International 23:103–114.

WICKLUM, D., AND R. W. DAVIES. 1996. The effects of chronic cadmium stress on energy acquisition and allocation in a freshwater benthic invertebrate predator. Aquatic Toxicology 35:237–252.

WILKIE, J. A., AND J. G. HERING. 1998. Rapid oxidation of geothermal arsenic (III) in streamwaters of the eastern Sierra Nevada. Environmental Science and Technology 32:657–662.

WOLOCK, D. M. 1997. STATSGO soil characteristics for the conterminous United States. US Geological Survey Open-File Report 97-656. US Geological Survey, Lawrence, Kansas.

WOLOCK, D. M. 2003. Base-flow index grid for the conterminous United States. US Geological Survey Open-File Report 03-263. US Geological Survey, Reston, Virginia.

WONG, C. S. C., X. D. LI, AND I. THORNTON. 2006. Urban environmental geochemistry of trace metals. Environmental Pollution 142:1–16.

XIAO, H., AND W. JI. 2007. Relating landscape characteristics to non-point source pollution in mine waste-located watersheds using geospatial techniques. Journal of Environmental Management 82:111–119.

XIE, L., J. L. FLIPPIN, N. DEIGHTON, D. H. FUNK, D. A. DICKEY, AND D. B. BUCHWALTER. 2009. Mercury (II) bioaccumulation and antioxidant physiology in four aquatic insects. Environmental Science and Technology 43:934–940.

XIE, L., D. H. FUNK, AND D. B. BUCHWALTER. 2010. Trophic transfer of Cd from natural periphyton biofilms to the grazing mayfly Centroptilum triangulifer in a life cycle test. Environmental Pollution 158:272–277.

YUAN, L. L., AND S. B. NORTON. 2004. Assessing the relative severity of stressors at a watershed scale. Environmental Monitoring and Assessment 98:323–349.

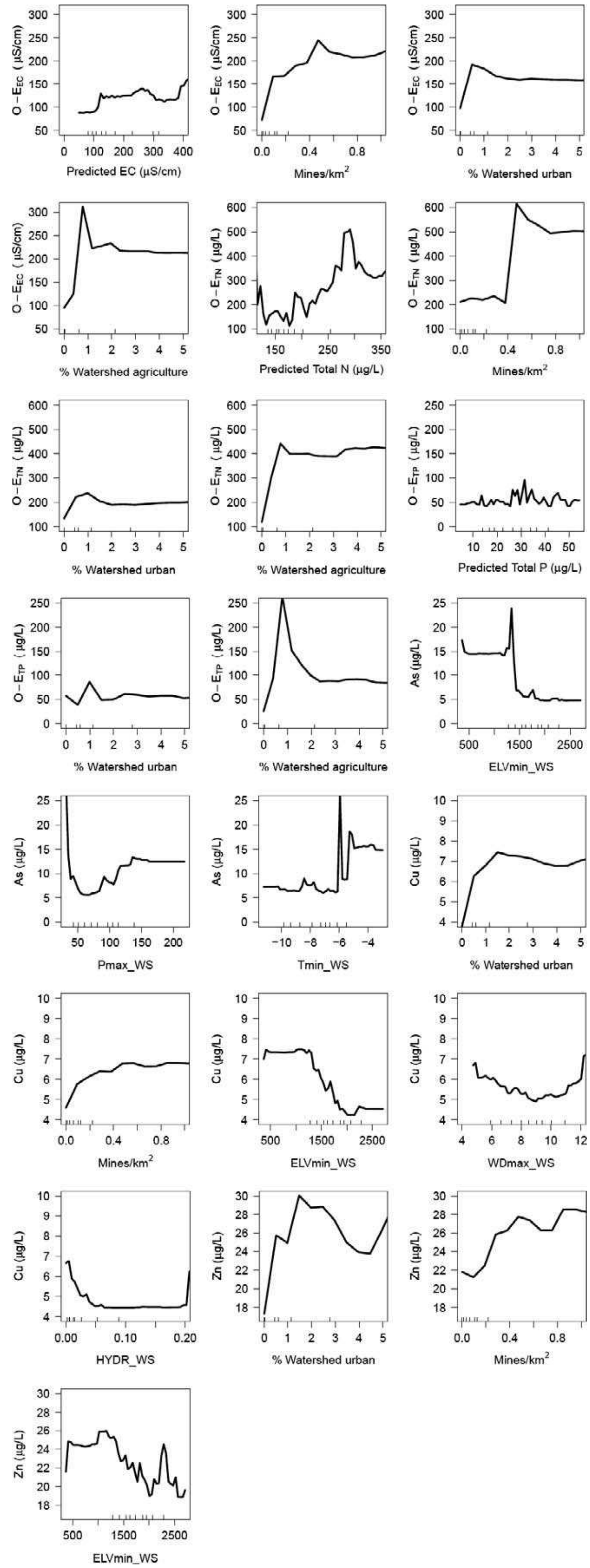
*Received: 27 November 2012*
*Accepted: 19 April 2013*

APPENDIX 1. Natural and anthropogenic variables used as potential predictors. USGS = US Geological Survey, GIS = geographic information system.

| Variable | Description |
|---|---|
| AG_WS | % area of watershed classified as agricultural (Homer et al. 2007) |
| BFI_WS | Mean of all baseflow-index pixel values within the watershed; estimates % stream flow composed of ground water relative to event flow; calculated from USGS-generated 1-km-resolution grid of base flows derived by interpolating calculated base flows at 19,000 USGS stream-flow gauging stations distributed across the conterminous USA (Wolock 2003) |
| CaO_Mean | Mean of all cells within the watershed, where cells represent % underlying bedrock composed of calcium oxide (CaO); percentages are the average % CaO for all lithologies within a cell, weighted by lithology prevalence; lithologies and their prevalences were derived from the USGS Preliminary Integrated Geologic Map of the USA |
| DOM_GEOL | Geology type with largest % cover within the watershed derived from a simplified version of Reed and Bush (2001): Generalized Geologic Map of the Conterminous United States |
| ELVcv_PT | Coefficient of variation of elevations within a radius of 5 digital elevation model cells (30 $\times$ 30-m resolution) of the sample site |
| ELVmax_WS | Maximum watershed elevation (m) |
| ELVmean_WS | Mean watershed elevation (m) |
| ELVmin_WS | Minimum watershed elevation (m) |
| HYDR_PT | GIS raster calculated as $(\mathrm{MIN}x_i)/(\mathrm{MAX}x_i)$, where $x_i$ = mean monthly discharge for month $i$ for the period of record and $x_i \geq 12$ mo of record; values were calculated for each of 9,941 USGS gauging stations in the western USA, and values for unmeasured locations were interpolated using inverse-distance-squared weighting of the 12 closest gauging stations within 100 km; each interpolated value represents a 4 $\times$ 4-km cell |
| HYDR_WS | Mean of all HYDR_PT values within the watershed |
| NEAR_AG | % watershed within 5 km of sample location classified as agricultural (Homer et al. 2007) |
| NEAR_URB | % watershed within 5 km of sample location classified as urban (Homer et al. 2007) |
| Max_ResVol | The volume ($km^3$) of the largest reservoir within the watershed (USACE 2009) standardized by dividing by watershed area |
| MgO_Mean | Mean of all cells within the watershed, where cells represent % underlying bedrock composed of magnesium oxide (MgO); percentages are the average % MgO for all lithologies within a cell, weighted by lithology prevalence; lithologies and their prevalences were derived from the USGS Preliminary Integrated Geologic Map of the United States |
| MINEperSQKM | Watershed mine density calculated as the number of mines divided by watershed area (USGS mineral resources data system; http://mrdata.usgs.gov) |
| Pmax_PT | Sample site value from the GIS raster calculated as $\sum(\mathrm{MAX}x_i)/30$ at the sampling point, where $x_i$ = the modeled total precipitation (mm) for month $i$ (1–12); values based on 30 y (1971–2000) of PRISM (http://www.prism.oregonstate.edu) climate estimates; each value represents a 900 $\times$ 900-m cell |
| Pmax_WS | Mean of all Pmax_PT values within the watershed |
| Pmin_PT | GIS raster calculated as $\sum \mathrm{MIN}x_i/30$ at the sampling point, where $x_i$ = the modeled total precipitation (mm) for month $i$ (1–12); values based on 30 y (1971–2000) of PRISM climate estimates; each value represents a 900 $\times$ 900-m cell |
| Pmin_WS | Mean of all Pmin_PT values within the watershed |
| PrdCond | Expected conductivity at sampling point (Olson and Hawkins 2012) |
| RHmean_PT | Sample-site value from the GIS raster calculated as $\sum(\mathrm{SUM}x_i/12)/30$ at the sampling point, where $x_i$ = the modeled mean relative humidity (%) for month $i$ (1–12); values based on 30 y (1961–1990) of PRISM climate estimates; each value represents a 2 $\times$ 2-km cell |
| RHmean_WS | Mean of all Rhmean_PT values within the watershed |
| S_Mean | Mean of all cells within the watershed, where cells represent the % underlying bedrock composed of S; percentages are the average % S for all lithologies within a cell, weighted by lithology prevalence; lithologies and their prevalences were derived from the USGS Preliminary Integrated Geologic Map of the United States |
| Slope_WS | Watershed slope measured as the (ELVmax_WS – ELVmin_WS)/maximum flow length; calculated from statistics produced by the multiwatershed delineation tool (Chinnayakanahalli et al. 2006) |
| Sum_ResVol | The total volume ($km^3$) of all reservoirs within the watershed (USACE 2009) standardized by dividing by watershed area |
| Tmax_PT | Sample-site value from the GIS raster calculated as $\sum \mathrm{MAX}x_i/30$ at the sampling point, where $x_i$ = the modeled monthly average maximum air temperature (°C) for month $i$ (1–12); values based on 30 y (1971–2000) of PRISM climate estimates; each value represents a 900 $\times$ 900-m cell; note that these values are modified from the PRISM annual maximum air temperature grid (http://www.prism.oregonstate.edu), which are calculated as $\sum(\mathrm{SUM}x_i/12)/30$, where $x_i$ = the modeled monthly average maximum air temperature (°C) for month $i$ (1–12) |

APPENDIX 1. Continued.

| Variable | Description |
| --- | --- |
| Tmax_WS | Mean of all Tmax_PT values within the watershed |
| Tmean_PT | Sample site value from the GIS raster calculated as $\sum(\mathrm{SUM}x_i/12)/30$ at the sampling point, where $x_i$ = the modeled mean air temperature (°C) for month $i$ (1–12); the modeled monthly mean air temperature ($x_i$) is the average of the minimum and maximum monthly air temperatures (http://www.prism.oregonstate.edu/faq.phtml). Values based on 30 y (1971–2000) of PRISM climate estimates; each value represents a 900 × 900-m cell. |
| Tmean_WS | Mean of all Tmean_PT values within the watershed |
| Tmin_PT | Sample site value from the GIS raster calculated as $\sum \mathrm{MIN}x_i/30$ at the sampling point, where $x_i$ = the modeled monthly average minimum air temperature (°C) for month $i$ (1–12); values based on 30 y (1971–2000) of PRISM climate estimates; each value represents a 900 × 900-m cell; note that these values are modified from the PRISM annual maximum air temperature grid (http://www.prism.oregonstate.edu), which are calculated as $\sum(\mathrm{SUM}x_i/12)/30$, where $x_i$ = the modeled monthly average minimum air temperature (°C) for month $i$ (1–12) |
| Tmin_WS | Mean of all Tmin_PT values within the watershed |
| UCS_Mean | Mean of all cells within the watershed, where cells represent the average of uniaxial compressive strength (UCS) of the underlying bedrock; cell values are the average UCS for all lithologies within that cell, weighted by lithology prevalence; lithologies and their prevalences were derived from the USGS Preliminary Integrated Geologic Map of the United States |
| URB_WS | % area of watershed classified as urban (Homer et al. 2007) |
| WSA | Watershed area in $km^2$ |

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APPENDIX 2. Partial dependence plots of all stressors on land uses and natural characteristics. EC = electrical conductivity. See Appendix 1 for other abbreviations.