

Multitaxon distribution models reveal severe alteration in the regional biodiversity of freshwater invertebrates

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Abstract: Knowledge of current and historical conditions is needed to guide conservation and restoration policies, but such information is lacking for most taxa. This problem is especially severe for small, inconspicuous taxa, such as the thousands of invertebrate species that inhabit stream and other freshwater ecosystems. We describe a novel application of River Invertebrate Prediction and Classification System (RIVPACS)-type multitaxon distribution models that, when applied to the biological survey data routinely collected in support of water-quality monitoring programs, can quantify the regional biodiversity status of hundreds of taxa. We used models developed for 2 stream data sets (North Carolina and the Mid-Atlantic Highlands, USA) to illustrate the potential of this approach. The models were calibrated with data collected from sites considered to be in reference condition and predict how probabilities of capture for each taxon in a data set vary across natural environmental gradients. When applied to survey data from multiple sites, predicted probabilities of capture can be summed across sites to estimate the taxon-specific frequencies of collection (F_e) expected under reference conditions. Comparison of observed frequencies (F_o) with F_e provides a quantitative measure of how individual taxon frequencies of collection have shifted relative to estimated reference conditions. In these 2 data sets, F_e was statistically different from F_o for >70% of taxa, implying wholesale changes have occurred in the stream invertebrate biodiversity of both regions. F_o was < F_e for most taxa, including those predicted to have been the historically most common taxa, indicating significant biodiversity loss. This type of multitaxon, regional-scale assessment of biodiversity status complements the site-specific, community-level assessments typically used by water resource managers to assess the biological integrity of individual water bodies. Together, the 2 approaches can provide a more robust, multi-scale understanding of the effects landscape and waterway alteration have had on the diversity of freshwater biota.

Key words: biodiversity status, multi-species distribution models, freshwater invertebrates, environmental alteration

Human activities have displaced and extirpated thousands of species (Master et al. 2000, Dirzo and Raven 2003, Thomas et al. 2004, Sauer et al. 2005, Butchart et al. 2010, Wood et al. 2013, Newbold et al. 2015). In freshwater ecosystems, little systematically compiled and summarized quantitative information has been published regarding the status of most species of freshwater invertebrates, a condition typical of invertebrates across different types of habitats (Bossart and Carlton 2002, Revenga and Kura 2003, Wilcove and Master 2005, Gerlach et al. 2014), but see Warren et al. (2001) and Thomas et al. (2004) for important terrestrial exceptions. Those estimates that have been made for freshwater invertebrates are based largely

on best professional judgment or semiquantitative compilations of survey data for a few conspicuous taxa groups (e.g., mussels, crayfish, and dragonflies; Master 1990, Allan and Flecker 1993, Stein and Flack 1997, Master et al. 1998, Ricciardi and Rasmussen 1999, Master et al. 2000, Dudgeon et al. 2006, Clausnitzer et al. 2009, Richman et al. 2015). Furthermore, we usually have limited context for interpreting contemporary survey data because we typically lack estimates of how prevalent most species were within a region in the past (DeWalt et al. 2005, 2009, Magurran et al. 2010, Willis et al. 2005).

Ideal assessments of regional changes in biodiversity status would be based on spatially balanced surveys that

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are repeated over time. However, such spatially and temporally extensive data are extremely rare for most taxa and regions and are especially lacking for invertebrate taxa in most types of ecosystems (Gerlach et al. 2014, Wilcove and Master 2005). The availability of data for freshwater biota has greatly improved as agencies responsible for monitoring aquatic life conditions have increasingly implemented large-scale survey programs in response to aquatic monitoring recommendations or directives, e.g., the US Clean Water Act (33 U.S.C. § 1251) and the European Union Water Framework Directive (WFD 2000). The survey data collected in support of these programs consist of counts of the taxa observed in samples, and these data are typically aggregated into assemblage-level indices of overall biological integrity or condition (Gerritsen 1995, National Research Council 2000, Hawkins 2006) before being published. Site-specific assessments of biological condition are produced by comparing the index score calculated for a specific site with scores observed at appropriate reference sites (Stoddard et al. 2006, Hawkins et al. 2010). These site-specific scores can be aggregated to quantify regional-scale conditions, e.g., percentage of water bodies in different condition categories as presented in the US Environmental Protection Agency's (USEPA) National Aquatic Resource Assessments reports (www.epa.gov/national-aquatic-resource-surveys). These assessments are useful in that they quantify important aspects of biological water quality, but the summary indices used provide no direct information on the status of either individual species or overall biodiversity at either local (site) or regional scales. Direct and accurate measures of species status are needed to inform policies designed to protect and restore biodiversity. Moreover, we need to understand which species are increasing and decreasing in prevalence over time in response to environmental alteration given that shifts in the specific mix of taxa present could potentially influence ecosystem processes (e.g., Cardinale et al. 2012), and hence ecosystem services, at both local and regional scales.

In this paper, we show how River Invertebrate Prediction and Classification System (RIVPACS)-type, multitaxon distribution models can be used to simultaneously estimate the historical prevalence of hundreds of stream invertebrate taxa in environmentally heterogeneous landscapes. RIVPACS-type models typically are used to compute site-specific, assemblage-level indices of local taxonomic completeness (Moss et al. 1987, Hawkins 2006), but here we describe a novel approach for interpreting the raw outputs from the models to assess changes in regional biodiversity. More specifically, we compared observed frequencies of collection with those predicted by the model to occur under reference conditions to estimate how regional frequencies of collection of individual taxa and overall biodiversity patterns have changed in response to landscape and waterway alteration. This type of analysis could greatly enhance

the information content provided by the USEPA's National Aquatic Resource Assessments and similar surveys at negligible additional cost. We discuss current limitations and uncertainties regarding the use of these types of models for predicting expected regional frequencies of collection and identify ways that predictions could be improved.

METHODS

Study area

We conducted analyses on data obtained from the USEPA's Environmental Monitoring and Assessment Program (EMAP) (Herlihy et al. 2000) and North Carolina Department of Environment and Natural Resources (NCDENR) (NCDENR 2003) (Fig. 1). The USEPA data were collected between 1993 and 1995 from 547 randomly selected sites in the Mid-Atlantic Highlands (MAH) area of the northeastern USA, which encompasses several states. Seventy-two MAH sites were identified as being in least-disturbed (*sensu* Stoddard et al. 2006) reference condition after screening sites based on their water chemistry, channel physical habitat, and surrounding land use (Herlihy et al. 2000). The MAH samples contained 432 taxa in 78 families, most of which were identified to genus. The North Carolina (NC) data consisted of collections made at 209 reference-quality sites and 943 non-reference sites between 1983 and 1998. The NC reference sites were selected based on best-professional judgment of NCDENR staff. Both reference and nonreference sites were selected from all river basins within the state, which ensured broad coverage across the state. The distribution of sites across all river basins and the large number (>1000) of sites should enable approximate estimation of regional conditions in NC streams. The NC samples contained 910 invertebrate taxa, 78% of which were identified to species or near species level, occurring in 100 families and 249 genera. The similarity in mean individual site assessment scores for nonreference sites in NC and MAH (genus-based RIVPACS observed/expected [O/E] index of local taxonomic completeness = 0.65 for NC and 0.64 for MAH) implies that the distribution and magnitude of environmental alteration is similar in the 2 regions (Hawkins 2006).

RIVPACS-type multitaxon distribution models

Models that simultaneously predict the probabilities of observing all taxa of interest in a sample are especially useful in biodiversity surveys (Olden et al. 2006), in which the status of hundreds or thousands of species must be assessed. RIVPACS-type models are multitaxon distribution models that use information on local environmental features to predict the probabilities of observing the hundreds of taxa found in a region in standard samples collected from individual water bodies. These types of models have been used successfully for almost 30 y to assess the degree to which observed assemblage composition in samples

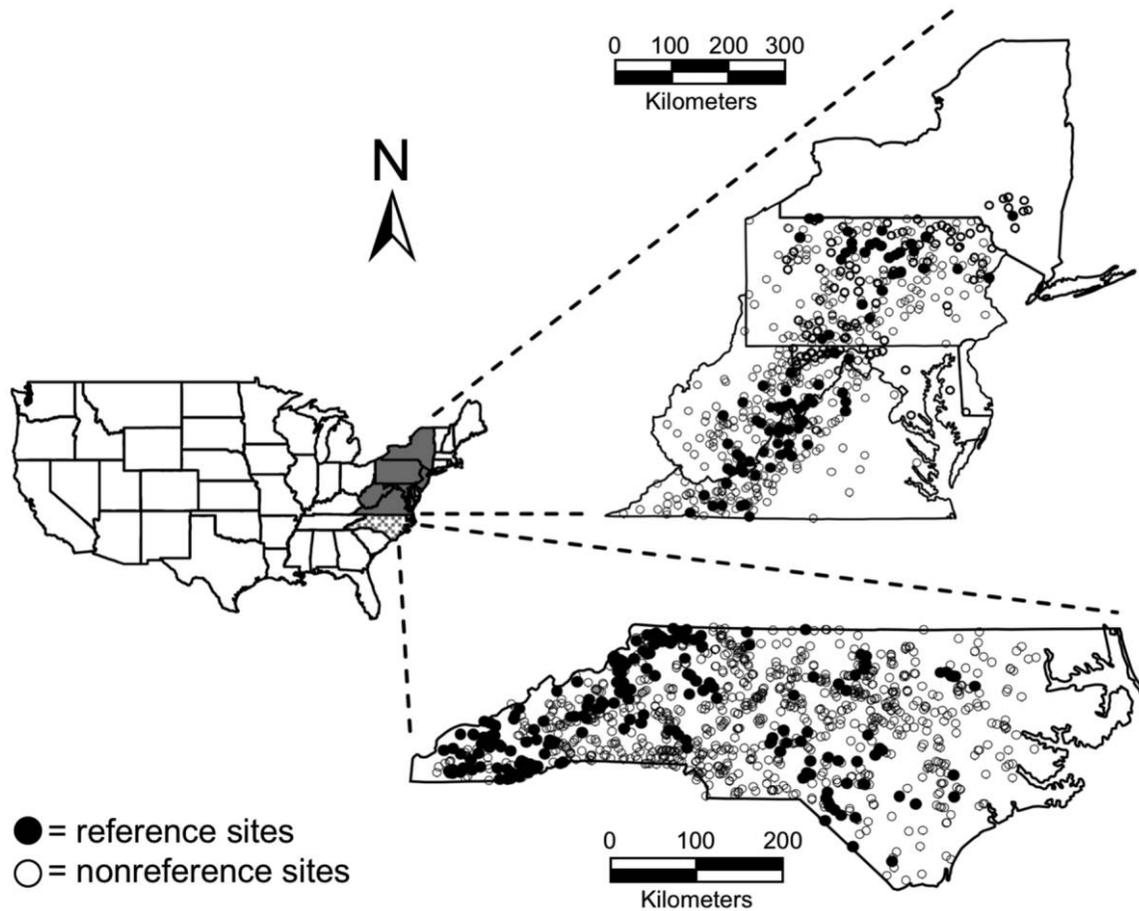


Figure 1. Locations of stream sampling sites in North Carolina and the Mid-Atlantic Highlands.

collected from streams and lakes matches that expected under reference conditions (Moss et al. 1987, Simpson and Norris 2000, Johnson 2003, Knapp et al. 2005, Hawkins 2006). RIVPACS-type models typically are calibrated by first statistically grouping samples collected from reference-quality sites based on similarity in their taxonomic composition and then developing a statistical model to predict the probabilities that a site belongs to each of the groups from a set of physiochemical variables little affected by human activities (e.g., elevation, stream size, channel slope, climate, geology). Reference sites are selected to represent the range of naturally occurring environmental conditions found in the region of interest. Taxon-specific probabilities of collection are then estimated for each site by weighting the frequencies of collection of each taxon within each calibration-site group by the predicted probabilities of membership of the new site in each group (Moss et al. 1987):

$$p_i = \sum_{j=1}^m p_j c_{j,i}, \quad (\text{Eq.1})$$

where p_i is the probability of capture of each taxon i in a sample taken at a site, p_j is the probability of a site belong-

ing to group j of m total groups, and $c_{j,i}$ is the proportion of sites in group j that contain taxon i . Details regarding the specific models we used here were described elsewhere (Van Sickle et al. 2005, Hawkins 2006). The specific predictor variables used by the 2 models were: NC = latitude, longitude, elevation, stream wetted width, stream depth, % stream bed as boulder, % stream bed as rubble, and day of year the sample was collected; MAH = elevation, day of year the sample was collected, catchment area, concentration of carbonate water chemistry, and dummy variables (0 or 1) for North Central Appalachian and Central Appalachian Ridge and Valley ecoregions.

We extended the application of RIVPACS-type models by using their primary output (predicted probabilities of observing each taxon in a sample collected at a site), to estimate the expected frequencies (F_e) of observing taxa across samples collected from a set of sites within a region. We and others have previously used this approach to examine taxon-specific responses to habitat alteration in streams and lakes (Armitage et al. 1987, Knapp et al. 2005, De Zwart et al. 2006, Carlisle et al. 2008, Carlisle and Hawkins 2008, Hawkins et al. 2015). F_e is derived by aggregat-

ing site-specific estimates of p_i as $F_e = S_i/N$, where S_i is the sum of p_i for a given taxon over all sampled sites, and N is the number of sites sampled. Assuming perfect detection, the difference between observed (F_o) and expected (F_e) frequencies, D_f , measures the degree to which a species' actual frequency of collection in samples collected within a region agrees with that expected under reference conditions. Thus, any significant departures of D_f from 0 should be associated with changes in environmental conditions (e.g., land use, habitat alteration, or pollution) that have influenced the viability of species. The assumption of perfect detection is unrealistic in most survey data, and inferences that do not incorporate estimates of detection probabilities must be interpreted with caution (MacKenzie et al. 2003, Kéry and Schmidt 2008). In general, data from small samples will underestimate the true occurrence of freshwater invertebrate species in a reach, but the replicate samples needed to account for imperfect detection when modeling p are seldom available from routine survey data. Therefore, we present estimates of p , F_o , and F_e as first approximations of how environmental alteration has caused shifts in the relative commonness and rareness of stream invertebrate taxa in these regions.

Modeling generally is needed to allow meaningful comparisons of F_o and F_e . Valid comparisons of F_o and F_e can be calculated directly if both are derived from randomly collected samples. In this case, F_e would be calculated from reference sites, and F_o would be calculated from all sites. In such a situation, reference and nonreference sites would occur in the same relative frequency across all naturally occurring environmental gradients within a region of interest. However, the latter assumption seldom holds because more reference sites occur in areas that have not undergone extensive human development (e.g., high elevation, steep slopes) than elsewhere, and the most-altered regions tend to be flat and low in elevation. In such cases, inferences regarding D_f must be based on estimates of F_e derived from species distribution models.

Testing the null hypothesis that $F_o = F_e$

We tested the null hypothesis that $F_o = F_e$ by computing the expected range of F_o values, given the assumption that probabilities of collection of each taxon at each site were as predicted by the model. We simulated a set of presence/absence data consistent with the probabilities of collection (p) provided by the model for each taxon in each data set that either occurred in or was predicted to occur in ≥ 5 samples (547 taxa for NC and 251 for MAH). That is, for each taxon and each site, we drew a random number between 0 and 1, and if this random number was $< p$, we designated that taxon as being present at the site. We then computed a value of S_o (i.e., the sum of p_i for a given taxon over all sampled sites) based on these simulated data.

We repeated the simulation $5000\times$ and accumulated an empirical distribution of S_o values for the taxon. The 2.5th and 97.5th percentiles of S_o/N then provided the 95% confidence limits for F_o in the case in which $F_o = F_e$. Values of F_o that were outside of these confidence limits failed the null hypothesis test. To check whether the modeling approach accurately predicted F_e , we conducted similar tests on an independent set of samples collected at the 202 North Carolina reference sites (i.e., repeat samples collected in different years). If models were accurate, only $\sim 5\%$ of the taxa should have been incorrectly identified as having statistically different F_o and F_e values.

RESULTS

Model performance

The models appeared to produce reasonably precise and generally unbiased estimates of F_e based on the 202 independent validation samples from the NC reference-quality streams (Figs 2, 3). For the 350 taxa that we assessed from these samples, the mean difference between F_o and F_e was 0.0053 (standard deviation [SD] = 0.032). However, F_e was significantly either under- or over-predicted for 66 taxa ($p < 0.05$), which was $\sim 4\times$ as many as would be expected based on random chance. The distribution of these errors was not symmetrical. Under-prediction occurred $\sim 2\times$ as often as over-prediction. However, in all cases, prediction of F_e rarely differed from observed F_o by > 0.1 (mean absolute difference = 0.024, SD = 0.022), so the absolute errors were not substantial. Independent validation data were not available for the MAH.

Region-wide changes in frequencies of collection

The number of significant differences between F_o and F_e , the direction of change, and the magnitude of change in common taxa suggests that stream invertebrate faunas in both NC and MAH have undergone marked changes in biodiversity patterns. Overall, F_o estimates for 416 of 547 (76%) assessed taxa were different from expected in NC (Fig. 4A), and F_o estimates for 177 out of 251 taxa (71%) were different from expected in the MAH (Fig. 4B). Of the 547 NC taxa, more (50%) occurred less frequently than expected than either occurred more frequently (26%) or were statistically indistinguishable (24%) from expectation. Of the 251 MAH taxa, about the same percentages of taxa occurred less (36%) as occurred more (35%) frequently than expected. If we consider only relatively common taxa (i.e., $F_e > 0.1$), 74% of 176 NC taxa and 67% of the 101 common MAH taxa occurred less frequently than expected. The most conspicuous differences in both regions occurred with taxa that were expected to be most common throughout each region (i.e., $F_e > 0.5$). Eighty-six percent of the 22 most common NC taxa and 95% of the

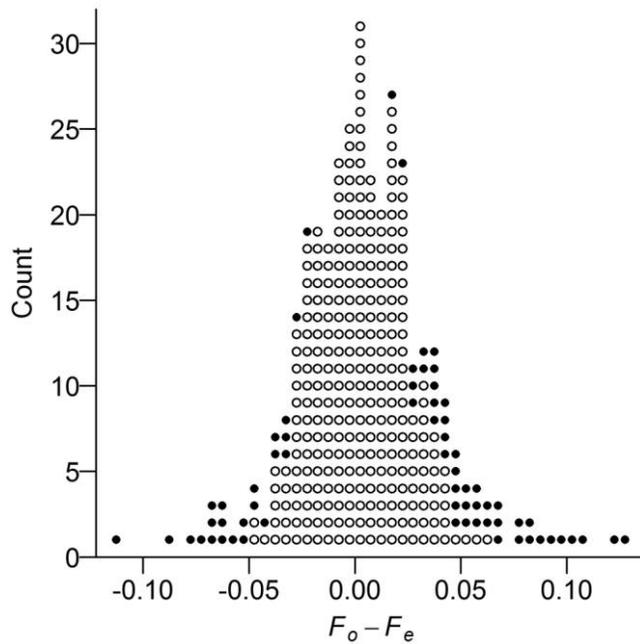


Figure 2. Frequency distribution of the difference between observed (F_o) and expected (modeled) (F_e) frequencies (i.e., D_f) of 350 North Carolina stream invertebrates in standard samples collected from the 202 reference-quality streams that were used to validate model performance. The mean (\pm SD) of $F_o - F_e$ was 0.0053 ± 0.032 . Filled symbols represent taxa for which F_e was either over- or under-predicted.

22 most common MAH taxa occurred less frequently than expected (Table 1). When data were aggregated by major taxonomic group, taxa in some groups deviated from expectation more strongly than did taxa in other groups (Table 2). For example, Plecoptera (stonefly), Trichoptera (caddis fly), and Ephemeroptera (mayfly) taxa generally were observed less frequently than expected in both regions, whereas Megaloptera (dobson and alder fly), Hemiptera (true bug), Odonata (dragonfly and damselfly), Coleoptera (beetle), Diptera (true fly), Crustacea (crayfish, amphipod, and isopod), Oligochaeta (worm), Hirudinea (leech), and Turbellaria (flatworm) taxa had mixed responses with taxa in some groups clearly observed more frequently than expected.

DISCUSSION

Baselines, models, and assessments

A critical challenge in estimating regional alterations in biodiversity status is the necessity of establishing baseline conditions from which consistent comparisons with existing conditions can be made. Historical information on distributions and abundance is lacking for the vast majority of taxa on Earth, so the taxa that probably occurred at currently altered locations before disturbance must be predicted from environment–occurrence relationships observed at ref-

erence sites. Empirical species distribution models are obvious tools for such tasks, but their utility will depend on how well they predict known distributions.

The confidence with which we can infer that region-wide changes in stream invertebrate biodiversity have occurred in association with regional environmental alteration depends on how well 4 critical assumptions are met: 1) data and models adequately assess the status of all taxa, 2) the reference sites used in building the models adequately characterize the historical condition of all other locations in the region of interest, 3) the reference sites adequately represent the historical biological condition of these sites, and 4) the nonreference sites represent an unbiased selection of the population of all streams in the region.

With respect to the 1st assumption, our assessment of model performance showed that the empirical modeling approach we used estimated F_e across samples well for most taxa, although these models may slightly over- or under-predict F_e for some taxa. (We assumed that modeled probabilities were correct when we did the statistical tests for differences between F_o and F_e .) Therefore, model precision did not greatly influence our ability to detect differences between F_o and F_e . The bias in predicting F_e for some taxa probably resulted in a slight underestimate of the number of taxa that have declined in their frequencies of collection. However, this bias did not affect assessments of the direction of change in frequencies of collection for any common

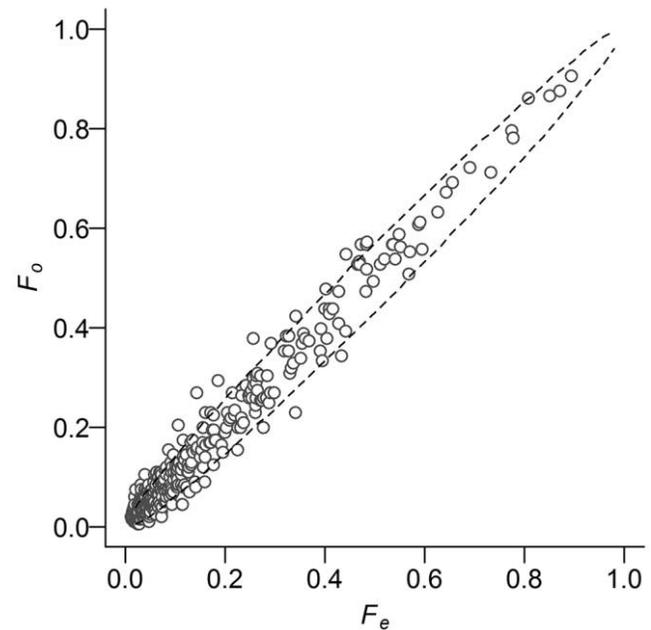


Figure 3. Relationships between observed (F_o) and expected (modeled) (F_e) frequencies for data from North Carolina reference-quality, model-validation samples. The ellipse represents the 95% confidence intervals associated with estimates of F_e .

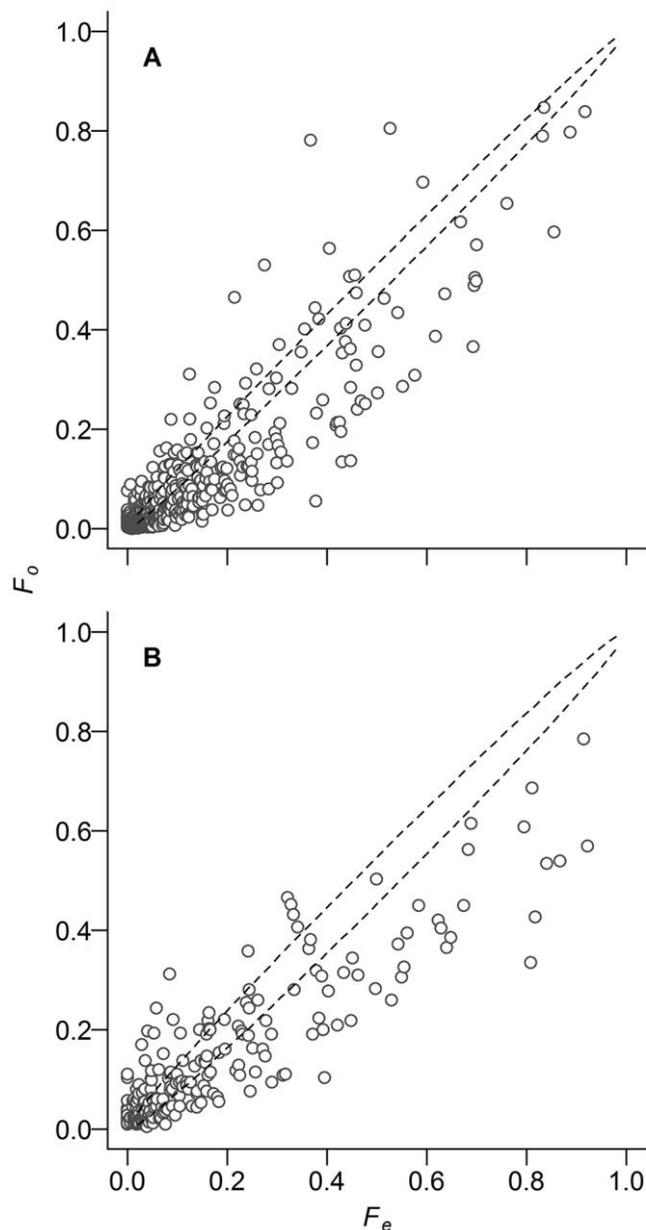


Figure 4. Relationships between observed (F_o) and expected (modeled) (F_e) frequencies for data from North Carolina (A) and Mid-Atlantic Highland (B) nonreference sites. The ellipses represent the 95% confidence intervals associated with estimates of F_e .

taxa. Other alternative modeling approaches (e.g., ensembles of individual species models or multispecies response models) may have produced better predictions for some taxa (cf., Olden et al. 2006, Baselga and Araujo 2010, Chapman and Purse 2011, Rose et al. 2016) and should be evaluated in the future. Multispecies response models (Rose et al. 2016) appear to be particularly promising, but the effort involved in building, managing, and applying ensembles of models for hundreds of individual species might greatly limit their practical application with respect to biodiver-

sity assessments (Chapman and Purse 2011). Moreover, aggregating the predictions of individual species distribution models can markedly overestimate the number of species present at a site (e.g., Labay et al. 2015), which implies they may overestimate the frequencies of collection of many species within a region.

The assumption of perfect detection is clearly problematic because we know it is false for RIVPACS and nearly all other species distribution models. No single sample of a few hundred individuals will contain all of the taxa occurring in an entire stream reach. Therefore, our results cannot be extrapolated directly to occupancy at the scale of a stream reach, the general spatial unit of inference in most stream surveys. More robust estimates of differences in regional occupancy will be possible if either techniques for more completely characterizing biodiversity are developed (e.g., environmental DNA-based metabarcoding; Taberlet et al. 2012, Yu et al. 2012, Zhan et al. 2013) or replicate sampling can be incorporated into routine multispecies surveys. Unless near-census-quality surveys can be conducted, replicate sampling will be needed to develop multispecies occupancy models that predict occupancy based on estimates of species-specific probabilities of detection (Zipkin et al. 2012, Mihaljevic et al. 2015). Until that time, the sample-scale estimates of F_o and F_e presented here represent significant improvements over the qualitative and spatially coarse summaries that have been used previously to estimate biodiversity loss and alteration.

The 2nd assumption that the reference sites used in building the models adequately characterized the historical condition of all other locations in the region of interest was addressed by not allowing the RIVPACS models to extrapolate beyond the range of predictor variable values used in the models (Clarke 2000, Clarke et al. 2003, Moss et al. 1987). This feature effectively restricts model application to only those locations in the region for which representation by reference sites is adequate. In general, reference-site representation is poor for streams that are likely to be most degraded (Stoddard et al. 2006, Ode et al. 2016), e.g., large rivers or streams in lowland areas with a long history of human occupation and modification. This constraint prevented assessment of many highly degraded sites. Thus, our assessments should underestimate changes that have occurred in these regions as a whole.

The 3rd assumption that the reference sites adequately represent the historical biological condition of these sites is almost certainly not true. However, the biological quality of the reference sites used is unlikely to have been any better than their true historical condition given that essentially all water bodies have experienced at least some minimal human-caused environmental alteration (e.g., atmospheric deposition of pollutants). Therefore, the use of these least-impaired reference sites (Stoddard et al. 2006) should have resulted in underestimates of the actual amount of bio-

logical alteration that has occurred at nonreference-quality water bodies in the region. In many respects, it is less critical that these assessments be based on pristine reference conditions than it is to establish fixed, quantitative baselines that ensure comparable assessments will be possible in the future (Pauly 1995, National Research Council 2000, Thomas et al. 2004, Gatti et al. 2015). Meaningful estimates of biodiversity trends must be anchored to a fixed baseline. Ideally, that baseline will be the historical, pristine condition, but in practice, a less-than-pristine baseline will still allow us to assess if biodiversity patterns are changing.

Our interpretations of the causes of biodiversity shifts also assume that the taxa currently present at reference sites are similar to the taxa that occurred in these sites historically. We know that stream invertebrate faunas can change over time in response to natural environmental shifts (e.g., Woodward et al. 2002, Collier 2008), but too few long-term data sets exist from reference-quality streams to fully understand patterns of faunal persistence and the natural and anthropogenic factors that affect persistence. Moreover, we have few historical records (i.e., >200 y ago) that we can compare with contemporary records. For some species, it might be possible to model historical biodiversity patterns from museum records (e.g., DeWalt et al. 2009, Labay et al. 2015), but adequate museum records are lacking for the vast majority of freshwater taxa.

The 4th assumption that the nonreference sites represent an unbiased selection of the population of all streams in the region is valid for the MAH but less certain for the NC data. By design, the sites sampled in the MAH were a true probabilistic sample of wadeable streams in the region (Herlihy et al. 2000). The NC sites were not sampled based on the same type of survey design used in the MAH. However, the NCDENR sampling did cover all major river basins in the state (NCDENR 2003). Given the breadth of coverage (Fig. 1) and density of sampling in NC, these samples may be generally representative of the state's streams and small rivers, but we cannot be certain of how well these sites represented the entire population of streams and rivers in NC. These problems in survey design should diminish as more data emerge from surveys that are based on robust, probabilistic sampling designs (e.g., USEPA 2013).

Regional status of freshwater invertebrate taxa

Few investigators have attempted to quantify and summarize the extent to which the regional biodiversity status of the many invertebrate taxa found in stream ecosystems have been affected by landscape and waterway modifications (Carlisle et al. 2008, Carlisle and Hawkins 2008, Hawkins et al. 2015), and our study is the first to show how regional changes in the frequencies of collection of these taxa vary across major taxonomic groups of stream invertebrates. Assessments for the MAH and NC regions

were generally consistent with one another in suggesting that marked changes have occurred in the status of many taxa (Tables 1, 2, Fig. 4A, B) and that some of these taxa may be at high risk of extinction across much of the regions we studied given that their observed frequencies across samples were 0 or ~0 at nonreference sites. In general, these quantitative assessments suggest that changes in the status of stream invertebrate taxa in these 2 regions have been both severe and pervasive.

The pattern observed in both regions of a general decline in the frequencies of collection of common taxa (Table 1) and an increase in frequencies for at least some rare taxa (Fig. 4A, B) is consistent with the pervasive environmental alteration (e.g., channel habitat structure; contaminant loads; and thermal, hydrologic, sediment, and nutrient regimes) that has occurred in both the study regions and in most other regions on Earth (Vörösmarty et al. 2010, Carlisle et al. 2013, USEPA 2013). Such alterations would most negatively affect taxa that are best adapted to predisturbance environments, while improving conditions for at least some taxa for which historical conditions were not optimal (Hawkins et al. 2015). Considering that up to 2× as many taxa decreased in frequencies of collection as increased (Table 1) and that in both regions individual samples contained ~65% of the taxa expected (Van Sickle et al. 2005, Hawkins 2006), regional environmental alterations have probably been so severe that these ecosystems have lost capacity to support both historically dominant taxa and many less common taxa that often make up much of the biodiversity present in individual water bodies. Even taxa that are generally thought to be tolerant of pollution (e.g., the filter-feeding caddis fly genus *Cheumatopsyche*) declined in prevalence (Table 1).

Our analyses also demonstrated that several groups of freshwater taxa (Table 2) may be even more vulnerable than the more conspicuous or charismatic taxa (e.g., mussels, crayfish, dragonflies/damselflies) whose status has been previously assessed by other methods (Richter et al. 1997, Stein and Flack 1997, Master et al. 1998, Ricciardi and Rasmussen 1999, Stein et al. 2000, Wilcove and Master 2005, Clausnitzer et al. 2009, Richman et al. 2015). Stoneflies, caddis flies, and mayflies appear especially vulnerable given that almost 9× as many of these taxa declined in prevalence as increased (Table 2).

Taxa in NC and the MAH appeared to differ somewhat in overall departure from expected frequencies, with more NC than MAH taxa appearing to have been negatively affected. However, differences among these data sets prevent direct comparisons. The finer taxonomic resolution (mostly species), the more precise model (Van Sickle et al. 2005, Hawkins 2006), and the more targeted survey used in the NC samples all could have resulted in stronger responses than observed in the MAH, for which a probabilistic survey and a coarser, genus-level taxonomic resolution were used (Hawkins 2006). We cannot evaluate the

Table 1. Comparison of observed (F_o) and expected (F_e) cross-sample frequencies of observing taxa in each region. Estimates are for the 22 taxa predicted to be most common ($F_e \geq 0.5$) before environmental alteration in each region. Taxa are ranked in decreasing order of F_e . All differences (D_f) between F_o and F_e but 2 were statistically significant (*), and all but 4 values of D_f were negative.

Taxon	Taxonomic group	F_o	F_e	D_f
North Carolina				
<i>Cheumatopsyche</i>	Trichoptera	0.839	0.917	-0.078*
<i>Stenonema modestum</i>	Ephemeroptera	0.797	0.887	-0.090*
<i>Acroneuria abnormis</i>	Plecoptera	0.597	0.855	-0.258*
<i>Conchapelopia</i> ^a	Diptera	0.847	0.835	0.012
<i>Boyeria vinosa</i>	Odonata	0.790	0.832	-0.042*
<i>Rheotanytarsus</i>	Diptera	0.654	0.761	-0.106*
<i>Macronychus glabratus</i>	Coleoptera	0.571	0.700	-0.129*
<i>Isonychia</i>	Ephemeroptera	0.498	0.699	-0.201*
<i>Nigronia serricornis</i>	Trichoptera	0.505	0.696	-0.191*
<i>Corydalus cornutus</i>	Megaloptera	0.489	0.695	-0.206*
<i>Polycentropus</i>	Trichoptera	0.366	0.693	-0.327*
<i>Calopteryx</i>	Odonata	0.617	0.667	-0.050*
<i>Palpomyia</i>	Diptera	0.472	0.636	-0.164*
<i>Antocha</i>	Diptera	0.387	0.617	-0.230*
<i>Tipula</i>	Diptera	0.697	0.592	0.105*
<i>Hexatoma</i> ^a	Diptera	0.309	0.576	-0.267*
<i>Symphitopsyche sparna</i>	Trichoptera	0.286	0.551	-0.265*
<i>Baetis intercalaris</i>	Ephemeroptera	0.435	0.542	-0.107*
<i>Rheocricotopus robacki</i>	Diptera	0.805	0.526	0.279*
<i>Ancyronyx variegatus</i>	Coleoptera	0.463	0.514	-0.051*
<i>Pseudocloeon</i> ^b	Ephemeroptera	0.356	0.503	-0.146*
<i>Psephenus herricki</i>	Coleoptera	0.273	0.501	-0.228*
Mid-Atlantic Highlands				
<i>Ephemerella</i>	Ephemeroptera	0.569	0.923	-0.354*
<i>Polypedilum</i>	Diptera	0.784	0.915	-0.130*
<i>Baetis</i>	Ephemeroptera	0.539	0.867	-0.328*
<i>Leptophlebiidae</i>	Ephemeroptera	0.534	0.841	-0.306*
<i>Stempellinella</i>	Diptera	0.427	0.817	-0.391*
<i>Leuctridae</i>	Trichoptera	0.686	0.811	-0.126*
<i>Epeorus</i>	Ephemeroptera	0.335	0.808	-0.473*
<i>Tanytarsus</i>	Diptera	0.608	0.795	-0.187*
<i>Parametriocnemus</i>	Diptera	0.615	0.689	-0.074*
<i>Amphinemura</i>	Plecoptera	0.562	0.683	-0.121*
<i>Microtendipes</i>	Diptera	0.450	0.584	-0.134*
<i>Hexatoma</i>	Diptera	0.385	0.648	-0.263*
<i>Isoperla</i>	Plecoptera	0.365	0.640	-0.275*
<i>Oulimnius</i>	Coleoptera	0.404	0.629	-0.225*
<i>Simulium</i>	Diptera	0.420	0.623	-0.203*
<i>Micropsectra</i>	Diptera	0.450	0.584	-0.134*
<i>Stenonema</i>	Ephemeroptera	0.394	0.561	-0.166*
<i>Rhyacophilidae</i>	Trichoptera	0.326	0.554	-0.229*
<i>Drunella</i>	Ephemeroptera	0.305	0.550	-0.244*
<i>Acentrella</i>	Ephemeroptera	0.372	0.543	-0.171*
<i>Acroneuria</i>	Plecoptera	0.259	0.529	-0.270*
<i>Cricotopus</i>	Diptera	0.502	0.499	0.003

^a Predictions of F_e were biased for only 2 taxa (*Conchapelopia*: under-prediction = 0.052, *Hexatoma*: over-prediction = 0.061). Adjusting for this bias would not have affected the statistical inference regarding a change in frequency of collection for either taxon.

^b Recent taxonomic revisions within the family Baetidae now place individuals originally identified as *Pseudocloeon* in the North Carolina database into either *Baetis* or *Acentrella*, which could not be separated in this analysis.

Table 2. Number of taxa in major taxonomic groups that were either predicted to occur in or were observed at ≥ 5 sites (No. taxa), the percentage of those taxa that were encountered less frequently ($p < 0.05$) than expected (%D = % decrease), and the percentage of those taxa that were encountered more frequently ($p < 0.05$) than expected (%I = % increase). Four taxa were lumped as Other: aquatic moths, hydras, aquatic mites, and nematodes. – indicates group was not included in surveys.

Group	North Carolina			Mid-Atlantic Highlands		
	No. taxa	%D	%I	No. taxa	%D	%I
Total	547	50	26	251	36	35
Plecoptera	46	74	0	22	68	5
Trichoptera	102	79	7	29	52	21
Ephemeroptera	91	67	10	23	61	13
Diptera	174	36	36	115	30	34
Coleoptera	28	46	36	14	29	57
Odonata	29	21	59	9	11	44
Megaloptera	7	29	57	3	67	0
Hemiptera	5	20	40	4	0	100
Crustacea	9	22	44	4	0	100
Oligochaeta	25	16	56	18	11	72
Hirudinea	9	11	56	–	–	–
Turbellaria	5	0	60	1	0	0
Gastropoda	10	30	40	3	67	33
Pelecypoda	3	0	67	3	0	100
Other	4	25	50	3	0	33

effect of the different survey designs, but differential sensitivities of species within the same genus would potentially obscure real changes in species-level biodiversity (Lenat and Resh 2001). More consistent and finely resolved taxonomy across data sets would improve our ability to compare the conservation status of taxa in different regions.

Outlook

The need to quantify and monitor the status of all of the Earth's biota is critical (Andelman and Willig 2003, Mooney et al. 2004, Nielsen et al. 2007, Lamb et al. 2009). Doing so for small inconspicuous taxa is particularly challenging because thousands of taxa exist, they have to be assessed by field sampling, and sorting and identifying individuals is time consuming and expensive. We also have lacked quantitative methods for estimating the historical or baseline condition for most taxa. The increasing use of multitaxon distribution models to assess the biological condition of individual water bodies means that regional assessments of biodiversity status are possible in other regions of the USA and elsewhere where appropriate data exist. The performance of these models is well documented, and many states and countries collect data that could be mined to conduct this type of regional assessment. Incorporating replicate sampling into these models to account

for imperfect detection (Zipkin et al. 2012, Mihaljevic et al. 2015) and improving detection techniques (Yoccoz 2012) will further improve their utility in biodiversity monitoring and assessment.

Region-wide assessments of biodiversity status are needed to inform us of the consequences of past and ongoing environmental alterations, to identify taxa at potentially high risk of extinction within a region, and to aid in identifying specific regions either at risk and in need of restoration or of unique biodiversity value and in need of protection. The USEPA now supports statistically defensible, national-scale assessments of the biological, physical, and chemical status of the USA's waters (<https://www.epa.gov/national-aquatic-resource-surveys>). The survey data on which these assessments are based probably represent the most comprehensive data set in existence for describing the biotic condition of freshwater ecosystems at regional-to-national scales. However, the regional and national summaries produced from these surveys represent scaled-up, site-specific, assemblage-wide condition scores (e.g., % stream length in good or poor condition; Paulsen et al. 2008), which are not informative of the status of either specific taxa or overall regional biodiversity. The type of analyses described here could complement ongoing assessments by explicitly documenting the regional- and national-scale status of hundreds, perhaps thousands, of different taxa. Furthermore, when coupled with knowledge regarding taxon- and stressor-specific tolerances

(Liess et al. 2008, Meador et al. 2008), analysis of those taxa that either decrease or increase in frequency of collection should help diagnose what specific stressors are of regional concern. Such analyses could help managers target those human activities that most strongly and pervasively degrade freshwater biodiversity when developing management plans designed to protect or restore aquatic life across entire regions.

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