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## PERSPECTIVES

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### Vulnerability of biological metrics and multimetric indices to effects of climate change

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**Abstract.** Aquatic ecosystems and their fauna are vulnerable to a variety of climate-related changes. Benthic macroinvertebrates are used frequently by water-quality agencies to monitor the status of aquatic resources. We used several regionally distributed state bioassessment data sets to analyze how climate change might influence metrics used to define ecological condition of streams. Many widely used, taxonomically based metrics were composed of both cold- and warm-water-preference taxa, and differing responses of these temperature-preference groups to climate-induced changes in stream temperatures could undermine assessment of stream condition. Climate responsiveness of these trait groups varied among states and ecoregions, but the groups generally were sensitive to changing temperature conditions. Temperature sensitivity of taxa and their sensitivity to organic pollution were moderately but significantly correlated. Therefore, metrics selected for condition assessments because taxa are sensitive to disturbance or to conventional pollutants also were sensitive to changes in temperature. We explored the feasibility of modifying metrics by partitioning components based on temperature sensitivity to reduce the likelihood that responses to climate change would confound responses to impairment from other causes and to facilitate tracking of climate-change-related taxon losses and replacements.

**Key words:** climate change, biological indicators, biological metrics, multimetric indices, vulnerability, biomonitoring, macroinvertebrates.

Water-quality agencies measure responses of biological indicators to assess the status and health of ecosystems and to establish biological criteria for defining acceptable condition of communities in rivers and streams regulated under the 1972 US Clean

Water Act (CWA; section 303[c][2][B]) and 304[a][8]). Stream benthic invertebrates are used frequently for biomonitoring in the US (USEPA 2002). Climate change has the potential to alter benthic invertebrate communities, and therefore, their use as the basis for assessments of stream condition and CWA-related management decisions. Thus, climate-related shifts in benthic community structure are relevant to bioassessment efforts (Dolédéc et al. 1996, Daufresne et al.

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2003, 2007, Mouthon and Daufresne 2006, Bêche and Resh 2007, Burgmer et al. 2007, Durance and Ormerod 2007, Collier 2008, Chessman 2009). However, the vulnerabilities of bioassessment/biomonitoring to climate-related shifts in community structure have not been evaluated.

Assessment of stream status requires distillation of data on macroinvertebrates, fish, or other stream assemblages into a format that reflects biological responses to environmental conditions. Multimetric indices (MMIs) and predictive modeling are 2 approaches frequently used to distill biomonitoring data. Both are grounded in the assumption that environmental conditions, both natural (e.g., climate, physiography, geology, soil type) and anthropogenic (e.g., land use, pollutant discharges), drive the structure and functioning of biological communities (e.g., Poff and Ward 1990, Allan 1995), so that expectations for reference-community composition and responses of disturbed communities can be compared as indicators of degradation (e.g., Barbour et al. 1999, Hawkins et al. 2010). Any metrics or indices of community condition must be readily compared between reference and test locations (Hering et al. 2006a, b). We focused on evaluating shifts in some commonly used metrics and in reference-community composition and assessed their potential effects on site-condition classifications.

MMIs generally are structured as composites of biological metrics selected to capture ecologically important community structural or functional characteristics and have been applied to fish and benthic macroinvertebrate communities (Karr 1991, Barbour et al. 1995, DeShon 1995, Yoder and Rankin 1998, Sandin and Johnson 2000, Böhmer et al. 2004, Norris and Barbour 2009). Component metrics are selected based on their responsiveness to the environmental effects most often evaluated (Barbour et al. 1999, Hering et al. 2006b, Johnson et al. 2006). Sites are assessed by comparing the MMI score for the test site to values at comparable reference sites. Predictive models use regional reference conditions to develop relationships between environmental predictor variables and macroinvertebrate taxon occurrence from which predictions for an expected (E) community are based. A commonly applied model for macroinvertebrate communities is the River InVertebrate Prediction And Classification System (RIVPACS) (Wright 2000). An important assumption is that the predictor variables are minimally affected by human disturbance and are relatively invariant over ecologically relevant time (Wright et al. 1984, Hawkins et al. 2000, Wright 2000, Tetra Tech 2008). The E community is then compared to various observed (O) communities

at nonreference locations. A basis for comparison is that any differences between O and E communities reflect biological responses to the range of environmental pollutants or alterations that are intended to be evaluated. For both approaches, the underlying assumption of site comparisons is that degradation in metrics or scores reflects responses of the aquatic community to stressors.

Climate change is a stressor that is likely to affect MMI scores. Thus, MMIs must be evaluated to determine: 1) their responsiveness to climate change, 2) whether responses to climate change can be differentiated from responses to conventional stressors, and 3) whether they will continue to be useful tools for attributing likely causes of degradation.

The International Panel on Climate Change (IPCC; IPCC 2001) defined vulnerability as the extent of susceptibility of a system to sustaining damage from climate change, including variability in climate (see also Hurd et al. 1999). Vulnerability is affected by degree of exposure and by sensitivity. Vulnerability of biological indices and metrics can be judged on the basis of existing evidence of biological responses to climate change (exposure), the range of metric responses to climate-related changes in temperature (sensitivity), and the effect of observed changes in metrics on site-condition classifications. We examined bioassessment data sets from 3 US states (Maine, North Carolina, Utah) to assess the vulnerability of biological metrics and indices to climate change. Bioassessment of wadeable streams is based on MMIs in Maine and North Carolina and on predictive modeling in Utah. These states are representative of major ecoregions of the US, and the data sets encompass large-scale variations in current and future climatic conditions, geography, topography, geology, and hydrology. Thus, our results provide a regional view of climate-change implications for commonly used MMIs and predictive models.

## Methods

### *State biomonitoring data sets*

We used biomonitoring data sets from Maine, North Carolina, and Utah for our analyses because they are relatively long-term data sets of high quality. Macroinvertebrate collection methods and assessment techniques differ among these states.

*Utah.*—The protocol used by Utah Division of Water Quality (DWQ) calls for quantitative samples collected from riffle habitats with the US Environmental Protection Agency (EPA) Environmental Monitoring and Assessment Program (EMAP) kick method (UTDWQ 2006). Samples are collected during

an autumn index period (typically September/October), and a RIVPACS-type model is used as a basis for site-condition classification. The model has 15 predictor variables, and 7 are related to climate (e.g., temperature, precipitation, freeze dates).

*Maine.*—The protocol used by Maine Department of Environmental Protection (DEP) calls for use of artificial substrates (rock bags or baskets) to collect quantitative samples during late-summer, low-flow periods (July 1–September 30). Site condition is rated with a set of 4 linear discriminant models that incorporate 30 input metrics or indices, and sites are assigned to 1 of 4 classes (A, B, C, and NA, where A is best condition and NA is nonattainment). The same criteria are applied to all sites (Davies and Tsomides 2002).

*North Carolina.*—The collection method used by North Carolina Department of Environment and Natural Resources (NC DENR) depends on the location and type of habitat. We limited our analyses to samples collected between June and September with the NC DENR full-scale collection method, which calls for 2 kick samples, 3 sweep samples, 1 leaf-pack sample, 2 rock- or log-wash samples collected in a fine-mesh sieve, 1 sand sample, and visual collections (NCDENR 2006). Macroinvertebrate abundance is rated as rare, common, or abundant. Site condition is rated based on Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa richness and the Hilsenhoff Biotic Index (HBI; Hilsenhoff 1987) modified for application in North Carolina (Lenat 1993). Typically, taxa are assigned pollution-tolerance values ranging from 1 (most sensitive) to 10 (most tolerant). Sites in North Carolina are assigned to 1 of 5 condition classes: excellent (5), good (4), good/fair (3), fair (2), or poor (1). Different scoring criteria are applied in each major ecoregion (Blue Ridge Mountain, Piedmont, Mid-Atlantic Coastal Plain).

#### Sites used for analyses

From each state database, we selected reference sites with the longest-term ( $\geq 9$  y) biological data for analysis of long-term trends and temperature-year patterns. Our data set included 2 sites in the Wasatch and Uinta Mountain ecoregion in Utah (UT-1 and UT-4) and 2 sites in the Colorado Plateau ecoregion in Utah (UT-2 and UT-3), 3 sites in the Laurentian Plains and Hills ecoregion in Maine (ME-1, ME-2, and ME-3), and 1 site in the Blue Ridge Mountain ecoregion in North Carolina (NC-1) (Table 1). We used 3 additional reference sites in North Carolina (NC-2 to 4, Table 1) with slightly shorter data records (7 y) to assess the potential effects of climate responses on

TABLE 1. Characteristics of long-term reference sites in Maine, Utah, and North Carolina. Percent urban and agricultural land use was calculated for a 1-km-wide buffer around each site (Homer et al. 2004). Years of data was based on the subset of years for which samples were collected in the same season with similar methods at a site. W. B. = West Branch, Mnts = mountains.

State	Site code	Water body	Latitude (°N)	Longitude (°W)	Level III ecoregion	Stream order	Elevation (m)	Drainage area (km <sup>2</sup> )	Years of data	Land use	
										% urban	% agriculture
Maine	ME-1	Sheepscot	44.22319	69.59334	Laurentian Plains and Hills	4	31.6	362.8	22	16.4	23.0
	ME-2	W. B. Sheepscot	44.36791	69.53129	Laurentian Plains and Hills	3	70.1	38.1	12	9.1	18.5
	ME-3	Duck Brook	44.39340	68.23461	Laurentian Plains and Hills	1	54.6	12.8	9	15.9	0.0
Utah	UT-1	Weber	40.75294	111.37358	Wasatch and Uinta Mnts	5	1846.6	740.7	17	4.5	21.1
	UT-2	Virgin	37.28483	112.94808	Colorado Plateau	4	1369.2	756.3	14	3.4	0.5
	UT-3	Duchesne	40.46139	110.83000	Colorado Plateau	4	2123.5	489.5	12	4.8	10.3
	UT-4	Beaver	38.28000	112.56711	Wasatch and Uinta Mnts	4	1904.8	236.2	9	3.9	0.0
North Carolina	NC-1	New River	36.55220	81.18330	Blue Ridge Mnts	5	713.6	835.0	11	25	13.4
	NC-2	Cataloochee	35.66722	83.07277	Blue Ridge Mnts	4	756.9	49.1	7	16.5	0
	NC-3	Nantahala	35.38638	79.8322	Piedmont	4	149.3	86.4	7	12.4	2.6
	NC-4	Barnes	35.43861	80.00055	Piedmont	3	106.7	23.3	7	1.6	3.6



site-condition classifications. These sites were designated by the respective state agencies as reference (least-disturbed, best-available) sites. We focused on reference sites to minimize possible influence of other anthropogenic stressors. However, the distribution of land uses within a 1-km buffer zone around each site suggested that anthropogenic influences, indicated by % urban and % agricultural land use, sometimes exceeded what might be ideal for a reference characterization (Table 1). Land use was ~16% urban at 2 sites in Maine and ~23% agricultural at 1 of these sites. Land use was ~3 to 5% urban at the 4 Utah sites, but was 21% agricultural at 1 site. Land use was 12 to 25% urban at the North Carolina reference sites, with ~13% agricultural at one of these, but only 0 to 3% agricultural at the other 2 North Carolina sites.

We also used data from sites in Maine and North Carolina as case studies with which to explore the potential effects of climate change on commonly used bioassessment metrics and assessment outcomes. We used 3 additional reference sites in North Carolina (1 in the Blue Ridge Mountain ecoregion, and 2 in the Piedmont ecoregion) to analyze effects of potential range shifts of taxa in response to climate change. In Maine, we used all bioassessment stations to describe the average and range of each metric among the 4 site-condition classes.

#### *Data management*

We screened and corrected data sets to reflect changes during the period of record in collection methods, sample processing/subsampling methods, taxonomists, and taxonomic protocols. We excluded ambiguous taxa from analyses by developing (as needed) operational taxonomic units (OTUs) (Cuffney et al. 2007). Genus-level OTUs generally were most appropriate, but some exceptions occurred (e.g., a family-level OTU was needed for Chironomidae in Utah to account for inconsistencies among taxonomic laboratories).

We used weighted averaging or maximum-likelihood inferences to assign invertebrates to temperature-preference categories in each biomonitoring database (see Stamp et al. 2010 for details). We ranked organisms based on percentiles of the distribution of temperature optima for all invertebrate taxa in each state data set. We categorized taxa with optima values <40<sup>th</sup> percentile as cold-water-preference taxa and taxa with optima values >60<sup>th</sup> percentile as warm-water-preference taxa. We modified these assignments as necessary after considering temperature-preference classifications in traits databases (Poff et al. 2006b, Vieira et al. 2006), weighted-averaging results

based on data from other states in the same region, taxon distributions among warmer and colder streams in the states analyzed (USEPA 2010), literature reviews, and best professional judgment from the regional advisory groups.

#### *Temperature and year trend analyses*

Annual point measurements of temperature made in conjunction with biological sample collections are inadequate to characterize annual average temperature regime, categorize hottest and coldest years, or analyze long-term temperature trends. We used Parameter-elevation Regressions on Independent Slopes Model (PRISM) annual average maximum and minimum air-temperature data (PRISM Climate Group, Oregon State University, Corvallis, Oregon; <http://www.prismclimate.org>) to supplement the limited water-temperature data available in the state data sets. The PRISM model uses a digital elevation model and point measurements of climate data to generate estimates of annual, monthly, and event-based climatic variables. We used geographical information system (GIS) software (ArcGIS 9.2; ESRI, Redlands, California) to obtain minimum and maximum annual site-specific air-temperature values from 1975 to 2006 (USEPA 2010). We used mean (average of maximum and minimum) annual air temperatures to analyze long-term temperature trends and to categorize years in terms of relative temperatures. Air and stream temperatures are correlated, but the magnitude and seasonal patterns of changes in stream water temperatures are likely to vary regionally because of factors such as the influence of water sources, watershed characteristics, and season (Daufresne et al. 2003, Caissie 2006). We assumed that mean air temperature was an acceptable surrogate for mean water temperature for comparison of relative temperature among years and grouped years as coldest, normal, or hottest based on PRISM annual average air temperature values for years during which the biological samples were collected (Stamp et al. 2010). Coldest years had mean annual air temperatures <25<sup>th</sup> percentile of the overall data set, normal years had temperatures between the 25<sup>th</sup> and 75<sup>th</sup> percentiles, and hottest years had temperatures >75<sup>th</sup> percentile values.

#### *Responses of commonly used metrics*

The HBI and EPT metrics (e.g., relative abundance or richness of EPT taxa, relative abundance or richness of taxa within the EPT) are used commonly in bioassessment indices. For example, in Maine, 8 of the input metrics used in the discriminant models are

related to EPT taxa and 1 is the HBI. In North Carolina, only EPT richness and the HBI are used in an MMI to classify site condition. Utah recently adopted use of a RIVPACS predictive model to assess site condition, but most other southwestern states currently use MMIs. Several southwestern states, including Idaho, New Mexico, Colorado, Nevada, Wyoming, Montana, and Arizona, incorporate richness or relative abundance of EPT taxa, Ephemeroptera taxa, Plecoptera taxa, or Trichoptera taxa in their MMIs. The HBI also is used in several southwestern states.

We used 1-way analysis of variance (ANOVA) to compare various EPT metrics and the HBI among hottest-, normal-, and coldest-year groups. We used Pearson product-moment correlations to test relationships among biological metrics (e.g., various EPT richness and abundance metrics, HBI values) and mean annual temperature or year. We examined correlations between HBI pollution-tolerance rankings and taxon temperature-preference optima (see Stamp et al. 2010 for details) to investigate potential vulnerability of the HBI metric to climate-change effects. We used Statistica software (version 8.0; StatSoft, Tulsa, Oklahoma) for all analyses.

#### *MMI vulnerabilities*

*Maine.*—Vulnerabilities of linear discriminant models to long-term temperature changes were difficult to evaluate because discriminant models test multiple variables simultaneously. Therefore, extrapolating the effect of climate-change on an individual input variable to assessments of site condition is problematic. Moreover, no firm thresholds or values of individual metrics can be identified at which an assessment of condition will change. We used ANOVA to identify component metrics that were particularly influential in differentiating between site-condition classes (see USEPA 2010 for detailed results) in conjunction with tests of climate-related sensitivities of these metrics (see *Responses of commonly used metrics* above) to infer vulnerabilities of the models to climate change.

*North Carolina.*—Observed biological responses to climate change include shifts in geographical ranges of sensitive taxa. These shifts often involve movements to higher latitudes or elevations. One consequence of such movements is that communities at higher latitudes or altitudes tend to become more similar to communities at lower latitudes or elevations (Bonada et al. 2007a). We used the North Carolina MMI to assess potential consequences of this type of climate-change effect on site-condition

classifications. In one scenario, we removed all cold-water-preference taxa from the annual data set for sites in the Blue Ridge Mountain ecoregion (on average, cold-water-preference taxa are more abundant in Blue Ridge Mountain sites than in Piedmont or Mid-Atlantic Coastal Plain sites; Table 2) and recalculated the HBI, EPT richness, and site-condition scores. In another scenario, we applied Blue Ridge Mountain scoring criteria to data from 2 Piedmont sites and evaluated the degree to which site-condition scores changed.

#### *Modified metrics using temperature-preference traits*

We modified 2 common invertebrate metrics to assess their ability to account for climate-related trends in cold- or warm-water-preference taxa separately from other stressors. We examined the ratio of cold- or warm-water-preference taxa to total invertebrate taxon richness (cold-to-total, warm-to-total) as an addition to the commonly used total invertebrate community richness metric. We also examined the ratio of cold- or warm-water-preference EPT taxon to total EPT taxa (cold-to-total EPT, warm-to-total EPT). We applied these modified metrics to the reference-site data sets from Utah, Maine, and North Carolina. We used 1-way ANOVA to compare these modified metrics among hottest-, normal-, and coldest-year groups.

## Results

### *Temperature and year trend analyses*

At sites UT-1 and UT-2, richness of total, EPT, Ephemeroptera, and Plecoptera taxa was significantly lower in the hottest- than in the coldest-year group (Table 3; USEPA 2010). The linear relationship between EPT richness and temperature can be used to infer a loss rate of  $\sim 3$  EPT taxa for every  $1.0^{\circ}\text{C}$  increase in air temperature in the Wasatch and Uinta Mountain ecoregion (Fig. 1A). The median number of EPT taxa at site UT-1 was  $\sim 13$  to 14 taxa. Based on a projected temperature increase of  $2^{\circ}\text{C}$  over the next 40 y (i.e., by 2050; National Center for Atmospheric Research website: <http://rcpm.ucar.edu>), an average of 6 taxa could be lost ( $>40\%$  of total EPT richness). The inferred loss rate ( $\sim 1.5$  EPT taxa/ $1.0^{\circ}\text{C}$ ) was lower at site UT-2, which is at a lower elevation than site UT-1 (Fig. 1B). At site ME-1, total richness and EPT richness did not differ among hottest-, coldest-, or normal-year groups. This site is in the Laurentian Hills and Plains, with a relatively low elevation and has few cold-water-preference taxa. At the shorter-duration reference station in the Maine Northeast

TABLE 2. Differences in elevation, Parameter-elevation Regressions on Independent Slopes Model (PRISM) mean annual air temperature, and mean ( $\pm 1$  SD) richness and relative abundance of cold- and warm-water-preference taxa among selected level III ecoregions in each state.

State	Ecoregion	Elevation (m)	Air temperature (°C)	Richness				Relative abundance	
				Cold-water- preference	Warm-water- preference	Cold-water- preference	Warm-water- preference	Cold-water- preference	Warm-water- preference
Maine	Laurentian Plains and Hills	65.2	6.5	1.1 $\pm$ 1.4	4.7 $\pm$ 3.3	2.8 $\pm$ 6.6	22.4 $\pm$ 22.0		
	Northeastern Highlands	690.3	42.5	1.7 $\pm$ 2.0	3.2 $\pm$ 2.7	7.1 $\pm$ 11.8	15.1 $\pm$ 17.5		
	Colorado Plateau	1729.4	9.1	3.8 $\pm$ 2.8	1.2 $\pm$ 1.2	9.8 $\pm$ 11.5	6.1 $\pm$ 11.6		
Utah	Wasatch and Uinta Mtns	2131.1	5.4	5.5 $\pm$ 4.0	1.0 $\pm$ 1.3	13.1 $\pm$ 15.4	3.8 $\pm$ 11.0		
	Piedmont	183.5	15.0	1.5 $\pm$ 2.0	5.2 $\pm$ 3.1	1.8 $\pm$ 2.7	6.7 $\pm$ 4.7		
	Mid-Atlantic Coastal Plain	173	15.4	0.1 $\pm$ 0.2	4.7 $\pm$ 5.1	0.1 $\pm$ 0.4	12.3 $\pm$ 6.4		
North Carolina	Blue Ridge Mountains	714.5	12.1	8.0 $\pm$ 4.5	2.8 $\pm$ 2.4	11.4 $\pm$ 7.9	3.1 $\pm$ 3.7		

TABLE 3. Results of analyses of variance testing for differences in standard and modified metrics among hottest-, coldest-, and normal-temperature years. Results are presented as values for hottest-year groups relative to values in coldest- or normal-year groups. + = significantly higher ( $p < 0.05$ ) and - = significantly lower ( $p < 0.05$ ) in hottest- than in coldest- or normal-year groups. NS = no significant difference among groups, \* indicates no analysis because no warm-water-preference taxa occurred at that site. EPT = Ephemeroptera, Plecoptera, Trichoptera, cold-to-total = ratio of cold-water-preference taxa to total taxa richness, warm-to-total = ratio of warm-water-preference taxa to total taxa richness.

Site	Cold-water-preference taxa			Warm-water-preference taxa			Standard metrics			Modified richness metric		
	Richness	Relative abundance	Richness	Richness	Relative abundance	Total taxa	Total EPT taxa	Cold-to-total	Warm-to-total	Cold-to-total	Warm-to-total	Warm-to-total
ME-1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
ME-2	NS	-	+	NS	NS	NS	+	NS	NS	NS	-	-
ME-3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
UT-1	-	NS	NS	NS	NS	-	-	-	NS	NS	NS	NS
UT-2	-	-	+	NS	NS	-	-	-	+	-	+	+
UT-3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
UT-4	NS	NS	*	*	*	NS	NS	NS	*	NS	*	*
NC-1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

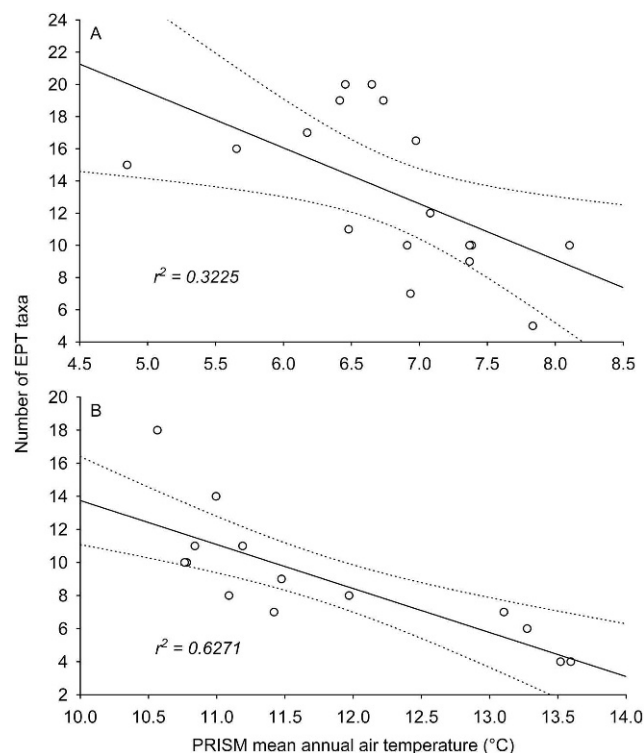


FIG. 1. Correlations of Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa richness (EPT taxa) with mean annual Parameter-elevation Regressions on Independent Slopes Model (PRISM) air temperature at Wasatch and Uinta Mountain long-term reference site UT-1 ( $r = 0.5679$ ,  $r^2 = 0.3225$ ,  $p = 0.0174$ ) (A) and Colorado Plateau long-term reference site UT-2 ( $r = 0.7919$ ,  $r^2 = 0.6271$ ,  $p = 0.0007$ ) (B). Dashed curves indicate 95% confidence intervals.

Highlands (ME-2), EPT taxa richness was significantly positively correlated with temperature; however, the trend with year was not significant (USEPA 2010). The remaining bioassessment data records did not show significant trends in EPT taxa over time or with temperature (USEPA 2010).

The correlations between temperature-preference optima and HBI tolerance values were statistically significant but weak (Maine:  $r = 0.29$ ,  $p = 0.0013$ ; North Carolina:  $r = 0.53$ ,  $p = 0.000$ ; Utah:  $r = 0.2851$ ,  $p = 0.0034$ ). Except for the chironomids *Larsia* and *Natarsia*, most cold-water-preference taxa in Maine had low ( $\leq 3$ ) HBI tolerance values. However, warm-water-preference taxa in Maine had a mix of HBI tolerance values (9 had values  $\geq 7$ , 10 had values  $\leq 3$ ). In North Carolina, most (22 of 30) of the cold-water-preference taxa had low tolerance values ( $< 3$ ). Only one cold-water-preference taxon (the chironomid *Diamesa*) had a tolerance value  $> 7$ . In contrast, 12 of the warm-water-preference taxa had tolerance values  $> 7$ , and only one warm-water-preference taxa, *Chimarra*, had a tolerance value  $< 3$ .

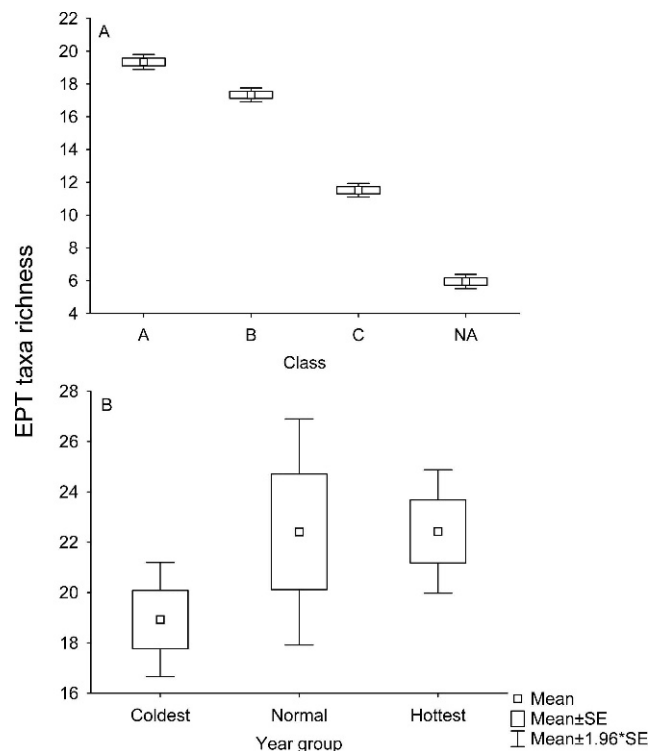


FIG. 2. Box plots of the Maine Ephemeroptera, Plecoptera, Trichoptera (EPT) generic taxon richness metric averaged among site-condition classes (A is best, NA is nonattainment) (A) and among coldest-, normal-, and hottest-year groups at site ME-1 (B).

Based on this information alone, a loss of cold-water-preference taxa and an increase in warm-water-preference taxa probably would result in higher HBI scores, which would contribute to lower site-condition classifications. For example, in North Carolina, an increase in the HBI score of 0.1 would reduce the classification of an excellent site from 5 to 4. At lower-quality sites (score  $\leq 4$ ), an increase in the HBI score of 0.6 would reduce the classification 1 full level.

#### Responses of commonly used metrics and MMI vulnerabilities

**Maine.**—Many of the discriminant model input metrics were related to EPT taxa and were influential in defining site-condition classifications. On average, higher values for the EPT richness metric occurred at A-quality sites than at sites in other condition classes (Fig. 2A). We explored the mix of cold- and warm-water-preference taxa within these EPT and related metric groups to understand potential vulnerability of these metrics to climate change. In Maine, 28 of 39 cold-water-preference taxa were EPT taxa, whereas 18



of 40 warm-water-preference taxa were EPT taxa (Appendix). Richness of EPT taxa was higher during warm years (Fig. 2B), in part because a large number of EPT taxa were warm-water-preference taxa. The difference between A-quality and B-quality sites in the mean number of EPT taxa (2–3 taxa) was well within the range of difference between coldest and hottest years in the mean number of EPT taxa (Fig. 2A, B). Thus, increases in the number of warm-water-preference EPT taxa as temperature increases with climate change could result in an apparent improvement in site-condition classifications.

Two of the model input metrics used in Maine were related specifically to Ephemeroptera (abundance and relative abundance). More warm-water-preference Ephemeroptera taxa (9) than cold-water-preference Ephemeroptera (3) occurred in Maine. Mean values of the Ephemeroptera abundance metric were highest at B-quality sites and lower at both A- and C-quality sites (Fig. 3A). Thus, the site-condition classification of a C-quality site might improve, whereas that of an A-quality site might degrade if the abundance of warm-water-preference Ephemeroptera increased consequent to climate change. However, the relative abundance (% composition) of Ephemeroptera was greatest at A-quality sites and decreased with decreasing site condition (Fig. 3B). Thus, the influence of increases in warm-water-preference Ephemeroptera caused by climate change on this metric will depend on the net responses of other warm- and cold-water-preference taxa.

Cold-water-preference taxa like Plecoptera also were expected to be sensitive to climate change. Three Plecoptera metrics (Plecoptera abundance, Perlidae abundance, and relative Plecoptera richness) were used as inputs to the Maine discriminant model. Highest Plecoptera abundances or richness occurred at A-quality sites, and site-condition classifications decreased as values of Plecoptera metrics decreased (USEPA 2010). Many more Plecoptera taxa were cold- than warm-water-preference taxa in Maine (Appendix), but Plecoptera metrics were not correlated with temperature and did not differ among hottest-, normal-, or coldest-year groups (USEPA 2010). Thus, changes are not expected in Plecoptera metrics in response to climate change.

Two model input metrics related to Trichoptera, *Hydropsyche* abundance and *Cheumatopsyche* abundance, were not correlated with temperature and did not differ among hottest-, normal-, or coldest-year groups (USEPA 2010). Neither taxon was a cold- or warm-water-preference taxon in Maine and neither is viewed as particularly sensitive to temperature. Thus, these taxa are likely to be resilient to climate change.

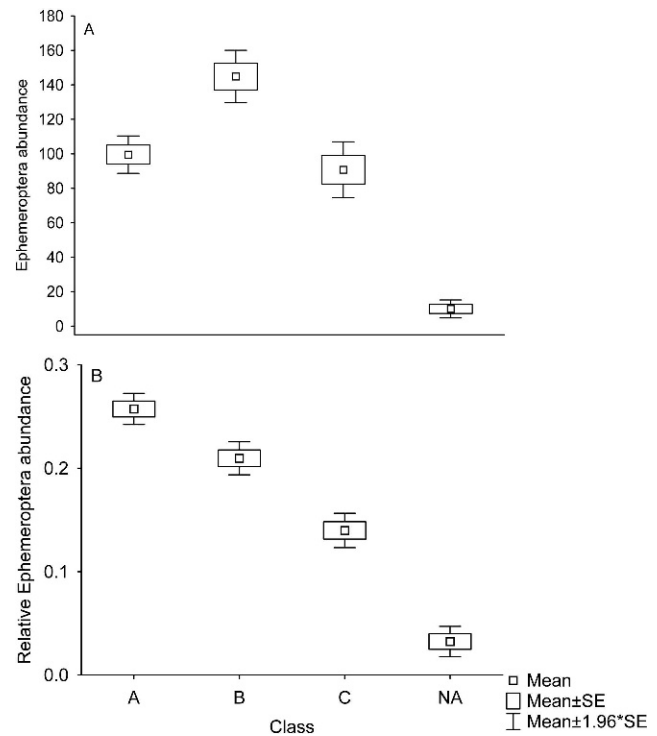


FIG. 3. Box plots of Ephemeroptera abundance (A) and relative abundance (B) averaged among site-condition classes (A is best, NA is nonattainment) in Maine.

Many Diptera occurred on both cold- and warm-water-preference lists (Appendix). Seven of 39 cold-water-preference taxa were Diptera (Chironomidae), and 10 of 40 warm-water-preference taxa were Diptera (Appendix). In the discriminant model, high abundance or richness of Diptera taxa tended to cause a low site-condition classification, even though several Diptera were classified as cold-water-preference taxa (USEPA 2010). Cold- and warm-water-preference Diptera are expected to respond differently to climate change. Thus, the effects on Maine model outcomes are likely to be variable and unpredictable and might depend on whether cold-water-preference taxa are replaced by warm-water-preference taxa.

*North Carolina.*—The North Carolina MMI is composed of an EPT richness metric and the North Carolina HBI. Twenty of 32 cold-water-preference taxa (genus-level OTUs) in North Carolina are EPT taxa, whereas only 5 EPT taxa in North Carolina are warm-water-preference taxa (Appendix). Removal of cold-water-preference EPT taxa from the data set for 1 Blue Ridge Mountain reference site (NC-1) resulted in the loss of 1 to 4 EPT taxa and a reduction in the EPT richness score of up to 0.6 (Fig. 4A), where each unit score represents the difference between a site-condition classification (e.g., excellent to good, good to fair).

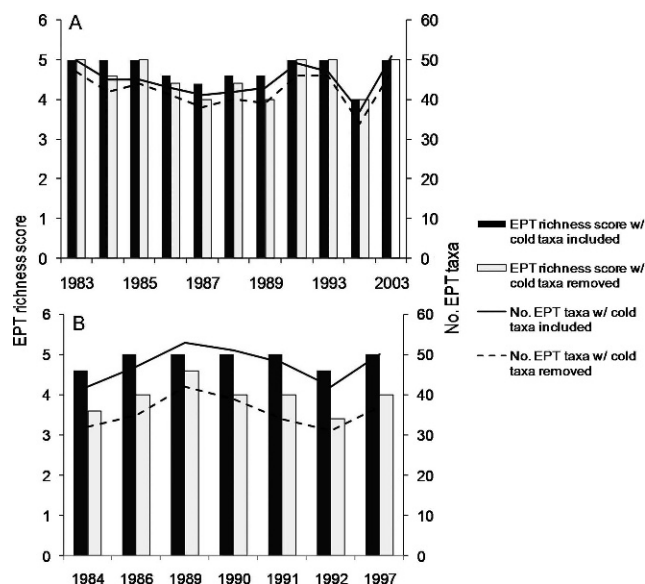


FIG. 4. Ephemeroptera, Plecoptera, Trichoptera (EPT) richness scores before and after all cold-water-preference taxa (cold taxa) were removed from the data sets for reference sites NC-1 (A) and NC-2 (B). w/ = with.

At a 2<sup>nd</sup> Blue Ridge Mountain reference site (NC-2), 10 to 14 EPT taxa were lost when cold-water-preference taxa were removed, and the EPT richness score decreased by 0.4 to 1.2 (Fig. 4B). A loss of 3 (Mid-Atlantic Coastal Plain ecoregion) or 4 (Blue Ridge Mountain or Piedmont sites) EPT species from high-quality sites would lower the EPT richness score from 5 (excellent) to 4 (good) (NCDENR 2006). A loss of 10 EPT taxa at Blue Ridge Mountain sites, 8 taxa at Piedmont sites, or 7 at Mid-Atlantic Coastal Plain sites would be needed to decrease EPT richness scores by 1 level at sites currently rated good or lower (NCDENR 2006).

Removal of cold-water-preference taxa from the data sets for these 2 North Carolina Blue Ridge Mountain reference sites resulted in increases in HBI values from 0.03 to 0.24 at NC-1, and from 0.58 to 0.86 at NC-2. These changes corresponded to decreases in HBI scores of up to 0.2 and 1, respectively (Fig. 5A, B). Cold-water-preference taxa were less abundant at NC-1 than at NC-2. In combination, the changes in EPT richness and HBI metric scores from removal of cold-water-preference taxa resulted in no net change in site-condition classification in some years, a change of up to 1 level (excellent to good) in 3 of 11 y at NC-1 and in 5 of 7 y at NC-2 (Fig. 6A, B).

Blue Ridge Mountain-ecoregion scoring criteria were applied to Piedmont-ecoregion reference sites (equivalent to replacing the Mountain ecoregion taxa with the Piedmont ecoregion taxa) as an approxima-

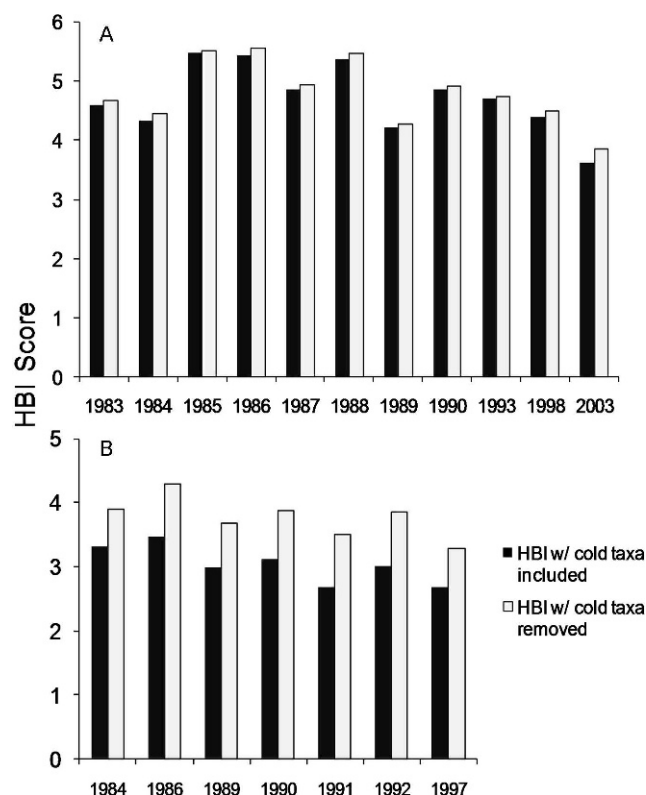


FIG. 5. Hilsenhoff Biotic Index (HBI) scores before and after all cold-water-preference taxa (cold taxa) were removed from the data sets for reference sites NC-1 (A) and NC-2 (B). w/ = with.

tion of the outcome of range shifts of sensitive taxa. In the most extreme case (i.e., complete community replacement), site-condition classification of 1 Blue Ridge Mountain site decreased 1 level (from 5 to 4; Fig. 7A, B).

#### Modified metrics using temperature-preference traits

At site ME-1, the cold-to-total and warm-to-total ratios did not differ significantly among coldest-, hottest-, and normal-temperature years for the reference site (Table 3). The cold-to-total ratio appeared to be slightly higher during the hottest years. However, the number of cold-water-preference taxa at this site was low, and the cold-to-total ratio was so low that any apparent trend is misleading. The number of cold-water-preference EPT taxa at this site was too low to permit calculation of the cold-to-total EPT ratio.

At site UT-1, the cold-to-total ratio was significantly lower for the hottest-year group than for the other year groups, but the warm-to-total ratio did not differ among year groups (Table 3). The cold-to-total EPT ratio was lower and the warm-to-total EPT ratio was

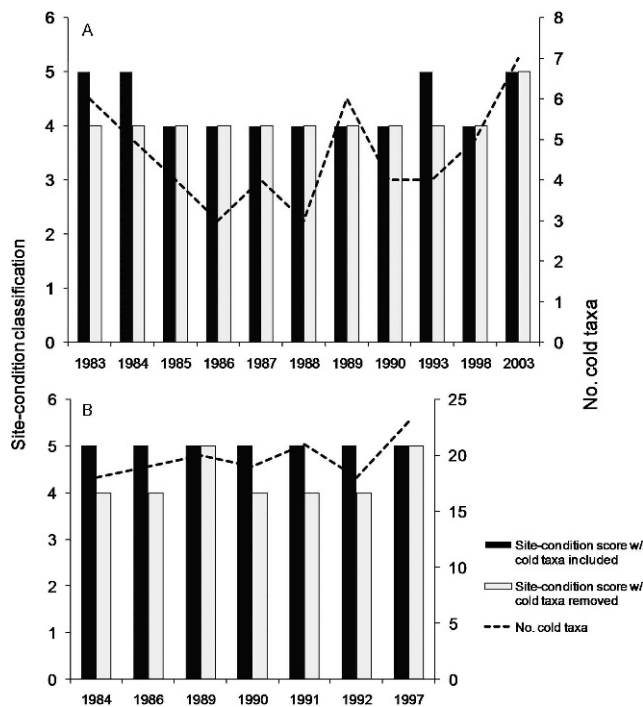


FIG. 6. Final site-condition classification scores before and after all cold-water-preference taxa (cold taxa) were removed from the data sets for reference sites NC-1 (A) and NC-2 (B). w/ = with, Mountain = Blue Ridge Mountain.

higher for the hottest-year group than for other year groups, but the number of cold- and warm-water-preference EPT was low, so these trends were not statistically significant. At UT-2, the cold-to-total and cold-to-total EPT ratios were significantly lower and warm-to-total and warm-to-total EPT was significantly higher for the hottest-year group than for the other year groups (Table 3).

At site NC-1, none of the modified metrics differed among year groups (Table 3).

To visualize how much of the variation in the traditional EPT richness metric could be explained by the temperature-preference modified metrics, we plotted all 3 metrics (EPT taxa richness, cold-to-total EPT, and warm-to-total EPT) by year for UT-1 and UT-2 (Fig. 8A, B). At site UT-1, EPT richness declined significantly over time (Fig. 8A). The warm-to-total EPT ratio did not change significantly over time. However, the cold-to-total EPT ratio also decreased significantly over time, and the regression slope was similar to the slope for total EPT richness. At site UT-2, total EPT richness and cold-to-total EPT declined significantly over time, but the slope of the regression for cold-to-total EPT was steeper (Fig. 8B). Moreover, the warm-to-total EPT ratio increased over time (Fig. 8B).

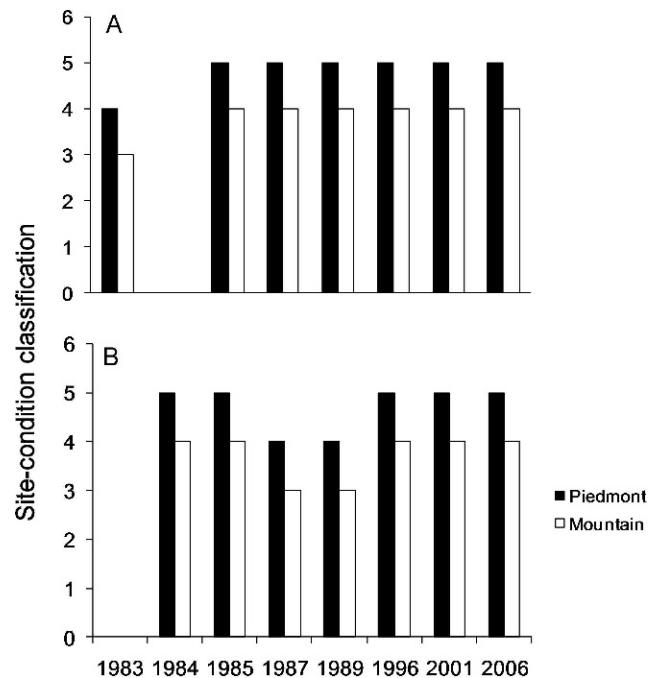


FIG. 7. Final site-condition classifications at 2 reference sites, NC0075 (A) and NC0248 (B), in the Piedmont ecoregion of North Carolina when Piedmont and Blue Ridge Mountain (Mountain) scoring criteria were applied to the site data sets.

## Discussion

### *Interactive effects of ecoregional characteristics and climate change on bioassessment metrics*

Evidence of invertebrate and other aquatic community responses to climate change is accumulating, but the magnitude of effects from climate change is often low compared to the magnitude of effects of other large-scale spatial (e.g., land use) and temporal (e.g., the North American Oscillation [NAO]) influences (Bradley and Ormerod 2001, Collier 2008, Chessman 2009, Sandin 2009). We found temperature-related responses of benthic indicators and metrics that are consistent with long-term climate-change effects and that could be used to establish future expectations for responses and to understand implications of these responses to bioassessment-based decisions. Responses were variable among sites and ecoregions, but they included decreases in richness or relative abundance of cold-water-preference taxa, and in some areas, increases in warm-water-preference taxa with increasing temperatures. These results are consistent with those of other studies that have reported significant increasing or decreasing trends in macroinvertebrates based on the thermophilic characteristics of the taxonomic group

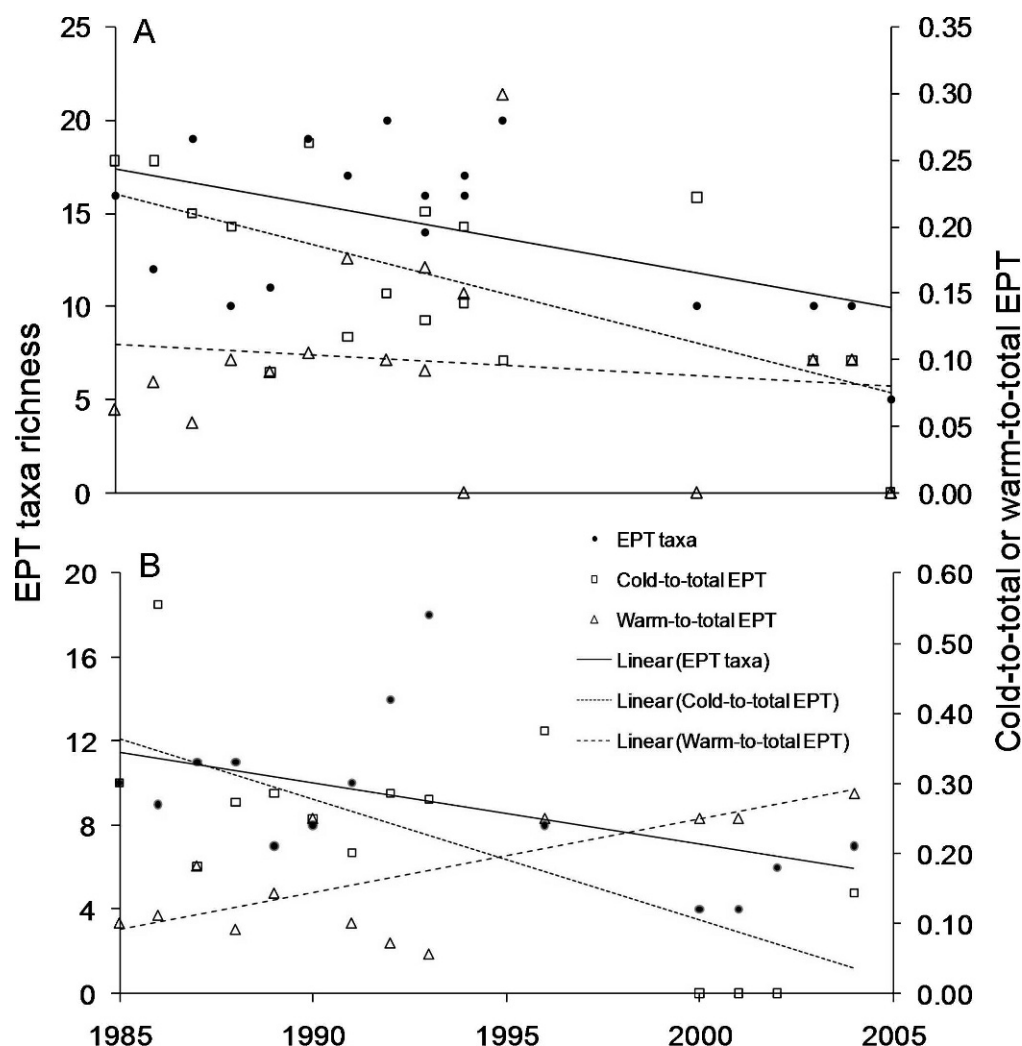


FIG. 8. Linear regressions for Ephemeroptera, Plecoptera, Trichoptera (EPT) taxon richness and the ratio of cold- or warm-water-preference EPT taxon richness to total EPT taxon richness vs year for reference sites UT-1 in the Uinta and Wasatch Mountains ecoregion (A) and UT-2 in the Colorado Plateau ecoregion (B) in Utah over the period 1985 to 2005.

(Daufresne et al. 2003, Durance and Ormerod 2007, Chessman 2009). Other widespread responses among commonly used, taxonomically based metrics were declining richness with temperature or over time of Ephemeroptera, Plecoptera, EPT, and total taxa. These responses can alter reference communities to a degree that would affect decisions about site condition. Site condition could be deemed degraded or even improved simply because of taxon-specific responses to climate change.

Temperature-preference groups generally were sensitive to changing temperature conditions, but responsiveness varied among ecoregions. Regional variations in projected climate-change effects indicate that not all ecoregions are equally vulnerable to climate change (USEPA 2007, NCAR 2008, Schoof et al. 2010). Many factors can influence susceptibility to

changing water temperature or hydrologic regime from climate change. These factors include elevation (Cereghino et al. 2003, Diaz et al. 2008, Chessman 2009), stream order (Minshall et al. 1985, Cereghino et al. 2003), degree of groundwater influence, or factors that affect water depth and flow rate, such as water withdrawals (Poff 1997, Poff et al. 2006a, Chessman 2009).

Many taxonomic metrics are based on mixtures of cold- and warm-water-preference taxa, and the degree of mixing is related, in part, to ecoregional characteristics, notably elevation. In all 3 states evaluated, a greater proportion of cold-water-preference taxa occurred in higher-elevation ecoregions and a greater proportion of warm-water-preference taxa occurred in low-elevation ecoregions (Table 2). Our results suggested that elevation is one factor that



drives the temperature-trait composition of regional benthic communities. In turn, the temperature-trait composition of a community affects the vulnerability of metrics and MMIs. State or tribal bioassessment managers should consider focusing efforts to evaluate and modify MMIs first in the most vulnerable, higher-elevation ecoregions.

#### *Responses of commonly used metrics and MMI vulnerabilities*

*Utah.*—Fairly predictable losses in EPT taxon richness (especially cold-water-preference taxa) with increasing temperatures have occurred at high- and intermediate-elevation sites in Utah. Projected EPT losses are as high as 40% by 2050. The potential effect of this loss on bioassessment capabilities is too high to ignore. Moreover, this projection of future losses was based on a linear estimate over time, but we have no reason to assume that the actual rate of taxon losses will be linear, especially given year-to-year and decadal-scale climatic variations. Thus, a linear estimate might be a poor predictor of when southwestern regional MMIs or predictive models might become incapable of differentiating reference from impaired sites.

*Maine.*—The Maine bioassessment protocol is based on a series of discriminant models that require inputs of ~30 bioindicators. Therefore, other components of the decision-making process regarding site condition are vulnerable to climate change. For example, Maine uses a group of Class-A indicator taxa as one metric for separating Class-A from Class-B condition ratings. Class-A indicator taxa are evenly divided between cold- and warm-water-preference taxa. As temperature increases, application of this metric could confound results of the models because some of the Class-A indicators could decrease with increasing temperatures, whereas others could increase (USEPA 2010). In contrast, North Carolina uses only the HBI and EPT richness to classify site condition. Both of these are vulnerable to effects of climate change on temperature-sensitive taxa, but their effects on the MMI are direct and easy to understand. Thus, size of the effect ultimately realized in MMI-based site-condition classifications will, in some part, be modified by the complexity of the MMI used.

*North Carolina.*—The HBI is vulnerable to expected increases in water temperature because changes in the temperature-trait composition of the community will be confounded by the relationship between temperature preferences and pollution tolerance. Decreases in cold-water-preference taxa with low HBI tolerance values or increases warm-water-preference taxa with

higher tolerance values will cause an increase in the HBI. Because higher HBI values impart a more-impaired site-condition classification, an increase in HBI driven by shifts in temperature-trait composition would result in a concomitant decrease in site-condition classification. This vulnerability will be stronger in regions like North Carolina where the correlation between pollution tolerance and temperature preferences of taxa was strong and consistent. In other regions, such as Maine, a more variable relationship, especially between warm-water-preference taxa and HBI tolerances, could lead to variability in HBI vulnerability caused by spatial differences in community composition of warm-water-preference taxa.

#### *Modified metrics using temperature-preference traits*

Cause cannot be determined from field observations or retrospective correlative analyses. Moreover, causal assessment, like risk assessment, is retrospective rather than prospective. However, causes must be considered to formulate corrective actions or to make management or regulatory decisions. Therefore, interpretation of bioassessment results often includes a process of inferring likely causes from environmental information on the area being evaluated (e.g., chemistry, land use, watershed conditions, discharges), species autecological information, and toxicological information (e.g., Beyers 1998, Suter et al. 2002). The USEPA has structured this approach into a stressor-identification process (USEPA 2000). In general, biological indicators, which are combined into MMIs, are used for their diagnostic value (Verdonschot and Moog 2006). Sensitivity to climate change and diagnostic capabilities of invertebrate indicators for this stressor have received little consideration because climate change was not considered a stressor of concern until recently. Thus, the effects of progressive changes in temperature and hydrological regimes on existing metrics and MMIs are untested.

We focused on the relative contribution of cold- and warm-water-preference taxa to particular component metrics with the intent of tracking climate-related taxon losses or replacements. Our preliminary evaluation indicates that a temperature-modified EPT richness metric shows promise as a way to achieve this goal. Separate tracking of cold-to-total EPT and warm-to-total EPT richness metrics successfully accounted for trends in total EPT richness over time regardless of whether changes in total EPT richness were caused by losses of cold-water-preference taxa (UT-1; Fig. 8A) or by losses of cold-water-preference taxa plus gains of warm-water-preference taxa (taxon replacements; UT-2, Fig. 8B).

Long-term increases in temperature have the potential to confuse the diagnosis of altered conditions as defined by many bioassessment metrics. For example, decreases in total richness or EPT richness, as observed at Utah reference sites, would be evaluated in relation to a conventional stressor, such as organic pollution. However, we found that decreases in cold-water-preference taxa and perhaps increases in warm-water-preference taxa caused by increasing temperatures changed total and EPT richness metrics. The magnitude of these changes was similar to the magnitude of changes caused by conventional stressors that would lead to classification of a site as impaired. The additional information provided by a temperature-modified metric could alter the way in which site condition and probable causes are interpreted. In this example, the additional information would support a shift from a presumption of pollution as the cause of the reduction in the EPT richness metric to consideration of a temperature-related effect. Without some attempt to modify traditional metrics to help characterize the contribution of climate change to changes in metrics, erroneous conclusions might be drawn, and conclusions of pollution effects or habitat degradation will be difficult to support.

We found a consistent moderate but significant relationship between temperature sensitivity and sensitivity to organic pollution, defined in the context of the HBI. A similar relationship was reported for the Ohio state biomonitoring data set (Rankin and Yoder 2009, USEPA 2010). Thus, metrics selected because their component taxa are generally sensitive or respond to conventional pollutants (Hilsenhoff 1987, Lenat and Penrose 1996), also will be sensitive to climate-related changes in temperature and flow conditions. Increasing organic pollution could alter the richness or relative abundances of cold- or warm-water-preference taxa. Partitioning the HBI metric according to the temperature-preference classification of component taxa could provide evidence to distinguish probable effects of climate change from effects of conventional pollution. This approach could be used in a weight-of-evidence context, supported by documentation of both temperature trends and pollution status at a site.

#### *Potential effects of losses of cold-water-preference taxa on MMI-based assessments*

Estimated quantitative effects on site-condition classifications of changes in community composition caused by changes in temperature-trait composition varied. In some cases, changes in metric values were

not sufficient to affect site-condition classifications. In other cases, they changed by 1 level (e.g., from excellent to good, good to fair). For example, in North Carolina, simulated loss of all cold-water-preference EPT taxa because of increasing temperatures or community replacement (mimicking migration of a warmer-water Piedmont community into the Blue Ridge Mountain ecoregion) reduced site-condition classifications by 1 level. Full realization of either of these 2 scenarios is unlikely and certainly would not occur in the near term. However, they represent an upper bound on expected vulnerability of MMIs in the near future and illustrate the immediate importance of testing and adopting temperature-modified metrics into bioassessment analysis frameworks.

We evaluated preliminarily the ability of a modified metric to track temperature-related species replacements, but we have not fully explored its ability to quantify the proportion of changes caused by climate change from the proportion caused by other stressors. Such an analysis would require investigation of modified metrics at a wide variety of nonreference and reference sites, i.e., along a gradient of stressed conditions, to examine combined responses to other stressors and to climate change. Proportional changes in temperature-trait groups (using modified metrics) could be compared between nonreference (conventional stressors plus climate change) and reference (climate change only) sites to differentiate contributing causes.

The most valuable approach for incorporating modified metrics into an analytical approach might be to continue calculating the traditional metric (e.g., EPT richness, HBI), while adding new cold- and warm-water-preference metrics. In this way, proportional changes in cold- and warm-water-preference taxa could be used to assess how much of the difference in the total metric can be accounted for by changes in temperature-trait groups. This comparison could be made over time or among locations or groups of sites (e.g., reference and nonreference). This traits-based approach for detecting and tracking effects of climate change is promising (Poff et al. 2010, Stamp et al. 2010), given that few taxa (genera or species) in our study showed consistent climate-related trends across the multiple sites and states analyzed.

We tested only a temperature-modified EPT richness metric, but other climate-vulnerable and influential metrics (such as the HBI), metrics related to Ephemeroptera, Plecoptera, or Trichoptera taxa, and community diversity metrics also should be modified into new metrics that account for temperature preferences and tested within the bioassessment

framework. For example, a climate-tolerant metric could be used to help separate responses to climate change and conventional stressors. Odonata, Coleoptera, and Hemiptera (OCH) taxa have been used as a high-temperature/low-flow tolerant indicator because of their prevalence in summer conditions, higher temperatures, and lower flows (Bonada et al. 2007b). OCH taxa increased over time, with increasing temperatures, or with lower precipitation at some sites in a study related to ours (USEPA 2010), results that support potential use of OCH as a climate-tolerant metric.

### *Limitations*

Lack of information on temperature preferences for many taxa currently limits development of climate-sensitive or climate-tolerant metrics. We used biomonitoring data to develop temperature-preference and tolerance information for many taxa common to Maine, Utah, and North Carolina (Stamp et al. 2010). Our approach could be used more broadly to support development of temperature-modified metrics in other states.

Data limitations prevent differentiation among interannual, cyclical, and long-term, directional climate changes. However, the larger issue might be how to use biomonitoring data to distinguish effects of climate change from effects of other natural and anthropogenic stressors. Durance and Ormerod (2009) discounted climate as the cause of changes in stream benthic assemblages that were correlated with long-term (18 y) temperature increases at sites in southern England. They argued that some of the faunal changes included taxa with traits (e.g., preferences for high flows and high dissolved O<sub>2</sub>) that were contrary to expected responses to climate-driven increases in stream temperatures. However, the observed biological responses used to project biological-indicator and MMI vulnerabilities in our study were based on temperature traits that are mechanistically linked to expected increases in water temperature consequent to climate change. Our expectation of increasing temperature was corroborated by significant ( $p < 0.05$ ) long-term increasing trends in air or water temperatures in several of the ecoregions evaluated and in numerous streams in the US (Kaushal et al. 2010, USEPA 2010). Many observed metric and MMI responses were consistent with temperature increases.

We used data from reference sites to minimize effects from conventional stressors so that responses of benthic indicators to climatic variables could be evaluated independently. However, several of our study sites potentially were affected by human land

uses (agricultural and urban). Landuse changes in the watersheds of reference sites could have affected our long-term ability to separate climate change from other landscape-scale stressors. Intensity of development-related land use at some of our long-term reference sites has been relatively stable over the period of record (USEPA 2010). Thus, the influence of urban and agricultural land use at those sites probably has been consistent over the period of record. Nevertheless, the interactions of climate change and development pose a substantial concern for biomonitoring programs in terms of data interpretation and protection of sites in reference condition.

Few state biomonitoring programs have adopted landuse criteria for defining and selecting reference sites, and no widely accepted criteria exist. However, some southeastern states (e.g., Georgia, Alabama, and South Carolina) apply landuse criteria for selection of reference sites. These criteria are <15% urban plus <20% agricultural for high-gradient streams, and <15% urban plus <30% agricultural for low-gradient streams (Barbour and Gerritsen 2006). A broad spatial analysis of the relationships among population density, land uses, water-chemistry constituents, and benthic community characteristics in New England states led Snook et al. (2007) to associate best-available reference conditions with  $\leq 5\%$  urban and  $\leq 10\%$  agricultural land use, a result that supports a lower threshold for defining reference sites. Several of the reference sites used in our study (2 in Maine and 1 in North Carolina) had >15% urban land uses, and only a few reference sites available for our study, mostly in Utah, met the more stringent criteria. Additional study and objective documentation to support specific landuse criteria for definition of reference condition will be important for developing and adapting biomonitoring programs to incorporate climate-change detection.

The period of record of a data set might determine our ability to use it to assess effects of climate change. For example, in the Wasatch and Uinta Mountains and Colorado Plateau ecoregions in Utah, detection of significant trends appeared to be determined by the period of record of the data sets (trends detected at >14 y but not at  $\leq 12$  y). In the higher-elevation Northeast Highlands ecoregion in Maine, a period of record of 11 y was apparently too short to define significant trends despite a predominance of cold-water-preference taxa (USEPA 2010).

We rarely had >1 or 2 reference sites within an ecoregion with sufficient data to conduct long-term trends analyses, even within the relatively extensive biomonitoring data sets used in our study. For



example, 105 reference sites were in the North Carolina biomonitoring data set, but long-term data was available at only 3 of them. One site was in the Blue Ridge Mountain ecoregion (11 y; Table 1), and 2 others had only 5 to 9 y of data (USEPA 2010). Regional consistency of observed responses is difficult to determine when spatial coverage and temporal replication are limited. We will need to understand how many sites and how long a period of record are needed to detect effects of climate change before we can modify and adapt biomonitoring programs to account for climate change.

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APPENDIX. Number of cold- and warm-water-preference taxa in each order in each state bioassessment data set. CWP = cold-water preference, WWP = warm-water-preference.

Order	Maine		North Carolina		Utah	
	CWP	WWP	CWP	WWP	CWP	WWP
Arhynchobdellida		1		1		
Basommatophora		4		1		
Coleoptera	1	1	1	2	1	3
Decapoda		1		1		
Diptera	7	10	10	5	8	2
Dorylaimida					1	
Ephemeroptera	4	9	6	1	6	2
Haplotaxida		1				
Hemiptera				1		1
Hoplonemertea		1				
Hydroida		1				
Isopoda				1		1
Megaloptera	1			7		
Mesogastropoda		1				
Odonata	2	1	1			1
Plecoptera	14	3	8		10	1
Rhynchobdellida				2		
Trichoptera	10	6	6	4	7	5
Unionoida				1		