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Multi-scaled drivers of ecosystem state: quantifying the importance of the regional spatial scale

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Abstract. The regional spatial scale is a vital linkage for the informed extrapolation of results from local to continental scales to address broad-scale environmental problems. Among-region variation in ecosystem state is commonly accounted for by using a regionalization framework, such as an ecoregion classification. Rarely have alternative regionalization frameworks been tested for variables measuring ecosystem state, nor have the underlying relationships with the variables that are used to define them been assessed. In this study, we asked two questions: (1) How much among-region variation is there for ecosystems and does it differ by regionalization framework? (2) What are the likely causes of this among-region variation? We present a case study using a large data set of lake water chemistry, uni- and multi-scaled hydrogeomorphic and anthropogenic driver variables that likely influence lake chemistry at the subcontinental scale, and seven existing regionalization frameworks. We used multilevel models to quantify and explain within- and among-region variation in lake water chemistry. Our models account for local driver variables of ecosystem variation within regions, differences in regional mean ecosystem state (i.e., random intercepts in multilevel models), and differences in relationships between local drivers and ecosystem state by region (i.e., random slopes in multilevel models). Using one of the best performing regionalization frameworks (Ecological Drainage Units), we found that for lake phosphorus and alkalinity: (1) a majority of all the variation in water chemistry among the studied lakes occurred among regions, (2) very few regional-scale landscape driver variables were required to explain among-region variation in lake water chemistry, (3) a much higher proportion of the total variation among lakes was explained at the regional scale than at the local scale, and (4) some relationships between local-scale driver variables and lake water chemistry varied by region. Our results demonstrate the importance of considering the regional spatial scale for broad-scale research and ecosystem management and conservation. Our quantitative approach can be easily applied to other response variables, ecosystem types, geographic areas, and spatial extents to inform ecosystem responses to global environmental stressors.

Key words: anthropogenic disturbance; ecoregions; hydrogeomorphic; lakes; landscape limnology; spatial scale.

Introduction

Ecological research and management have traditionally focused on understanding spatial variation among populations, communities, or ecosystems using driver variables quantified at relatively fine scales. Although such knowledge is important, the hydrogeomorphic and anthropogenic features that influence ecosystem variation occur at multiple scales (Levin 1992, Turner et al. 2001). In addition, there is increasingly a need to extrapolate finely scaled knowledge to continental spatial extents to address broad-scale environmental problems (Miller et al. 2004, Peters et al. 2006). To do this extrapolation, researchers and managers need to understand variation in both drivers and response variables across the full spectrum of spatial extents, from the local to the continental scale. Between these two scales is the intermediate (or "regional") scale that has been less well studied compared to other spatial extents, but may represent a critically important linkage between local and continental scales. For example, by quantifying variation within and among regions, both in ecosystem responses and hydrogeomorphic and anthropogenic driver variables, we can begin to account for interactions between local- and regional-scale variables that have so far limited our ability to extrapolate and understand ecosystem variation.

A commonly used approach to capture regional-scale spatial variation in driver and/or response variables is to place ecosystems within a regionalization framework (e.g., Omernik 1987, Bailey et al. 1994). A regionalization framework is created by dividing a continent into contiguous, often hierarchical, discrete spatial units of

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TABLE 1. Examples of regionalization frameworks currently being used for national–continental science, management, and conservation.

Organization	Name of framework	Reference
National Ecological Observatory Network	Eco-climatic Domains	Keller et al. (2008)
The Nature Conservancy	four-tiered hierarchical freshwater classification framework	Higgins et al. (2005)
World Wildlife Fund	Freshwater Ecoregions of the World	Abell et al. (2008)
Landscape Conservation Cooperatives	National Geographic Areas†	
U.S. Environmental Protection Agency	EPA Regions‡	

† http://www.fws.gov/landscape-conservation/lcc.html
‡ http://www.epa.gov/wed/pages/ecoregions.htm

similar landscape features that are referred to interchangeably as "ecological regions," "ecoregions," or "regions." Regionalization frameworks have proven useful in part because they provide a means by which to quantify and study spatial variation at broader spatial scales than the fine scale (McMahon et al. 2004, Cheruvelil et al. 2008).

An important assumption in the use of regionalizations is that a significant amount of variation among individual ecosystems is captured at the regional scale. Accordingly, using a regionalization framework to group similar ecosystems is often a first step when designing continental-scale research initiatives for management or conservation applications. However, choosing which regionalization framework to use is not obvious, partly because there are an infinite number of ways to carve up a continent, and each framework is created by using different combinations (and weighting functions) of variables representing climate, geomorphology, hydrology, and land use/cover (McMahon et al. 2001, Loveland and Merchant 2004, McMahon et al. 2004, Hargrove and Hoffman 2005, Thompson et al. 2005). As a result, some recent national- and continental-scale efforts have developed new regionalization frameworks, whereas others have either aggregated regions from an existing framework or integrated across multiple existing frameworks to create one that meets their needs (Table 1). However, only a few studies from a range of ecosystem types have compared whether and how these different regionalization frameworks capture variability in the many biotic and chemical variables that people are trying to understand, manage, and conserve (Van Sickle and Hughes 2000, Jenerette et al. 2002, McMahon et al. 2004, McDonald et al. 2005, Wickham et al. 2005, Cheruvelil et al. 2008, Snelder et al. 2009).

In addition to whether and which regionalization frameworks are useful for capturing variation among ecosystems, ecologists remain unsure about *why* particular regionalization frameworks do so. In fact, there is a lack of a mechanistic understanding of the specific hydrogeomorphic and anthropogenic features that are related to particular ecosystem response variables, both in the specific processes underlying the patterns and the scales at which the processes operate (Magnusson 2004, McMahon et al. 2004, Turner 2005). It is difficult to identify which landscape features, operating at which spatial scales, are important for capturing ecosystem variation. This difficulty is in part because many landscape features can be quantified at multiple spatial scales as opposed to uni-scaled variables that can only be quantified at one scale (Levin 1992, Turner et al. 2001), and because landscape features often co-vary within and among scales (and hence regions; King et al. 2005). Therefore, addressing the difficult task of teasing apart individual effects of regional-scale and local-scale landscape features on ecosystem states requires ecosystem-specific data collected consistently within and across many regions, and a statistical approach that accounts for the hierarchical structure of the corresponding multi-scaled landscape features.

In this study, we asked two questions related to understanding ecosystem variation at the subcontinental scale: (1) How much among-region variation is there for ecosystems and does it differ by regionalization framework? And, (2) what are the likely causes of this among-region variation? To answer our questions, we quantified within- and among-region variation in ecosystem states (Fig. 1A, B), and then built multilevel models that included both local- and regional-scale driver variables to account for (a) local drivers of ecosystem variation within regions, (b) differences in regional mean ecosystem state (i.e., random intercepts in a multilevel model; Fig. 1C), and (c) differences in relationships between local drivers and ecosystem state by region (i.e., random slopes in a multilevel model; Fig. 1D). We built the multilevel models using a large data set of lake water chemistry, uni- and multi-scaled hydrogeomorphic and anthropogenic variables that likely influence lake chemistry at the subcontinental scale, and seven existing regionalization frameworks.

For Question 1, we quantified among-region variation in two lake chemistry variables from 2319 north-temperate lakes in six northern U.S. states using seven

TABLE 1. Extended.

Formation of framework	Other frameworks included in development (citation)
multivariate geographical clustering of ecoclimatic variables	none
freshwater classification using abiotic factors within a zoogeographic context	Aquatic Ecological Units (Maxwell et al. 1995), Freshwater Ecoregions of the World (Abell et al. 2000), Eight-digit Hydrologic Units (Seaber et al. 1987)
freshwater classification using regional fish species distributions	varies by region of the world, e.g., Aquatic Ecological Units for the USA (Maxwell et al. 1995)
aggregation of existing regions	Bird Conservation Regions (Bart 2006), Freshwater Ecoregions of the World (Abell et al. 2008), Level II Ecoregions (Omernik 1987)
aggregation of existing regions	Level III Ecoregions (Omernik 1987)

existing regionalization frameworks. The two water chemistry response variables are of interest for lake ecology, management, and conservation; are indicators of two of the major stressors on freshwater ecosystems (acidification and eutrophication); and have spatial patterns related to different landscape features that operate at different spatial scales. Alkalinity is closely related to geology, which varies regionally, and is an indicator of a lake's ability to resist acidification. Total phosphorus (hereafter, phosphorus) is influenced by land use/cover and other anthropogenic activities within the local catchment, and is an indicator of cultural eutrophication. For Question 2, we used a single regionalization framework that captured high amounts of among-region lake water chemistry variation (from Question 1) to build models to identify the underlying hydrogeomorphic and anthropogenic landscape features that are related to among-region variation in lake chemistry. To do so, we also accounted for local-scale (within-region) variation in lake chemistry by including commonly measured local driver variables in our models.

Lakes and their water chemistry provide a model system for answering our questions because a wealth of knowledge exists for lakes, and as spatially discrete gathering points of water, lakes present an ideal

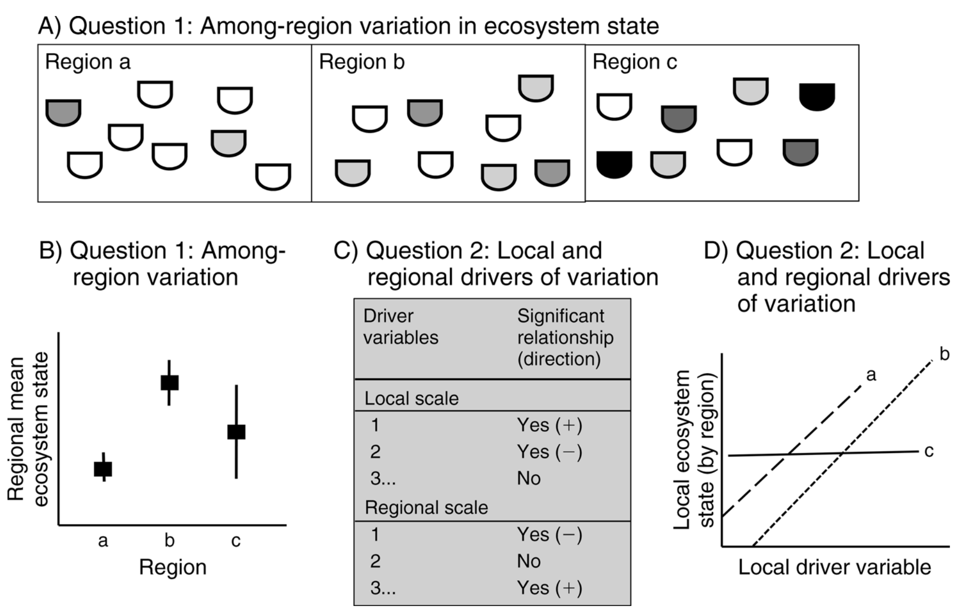

FIG. 1. Schematic representation of within- and among-region variation in ecosystem states and the local- and regional-scale drivers of that variation. (A) Each region (a–c) represents a discrete spatial unit, and each shape within a region represents an individual ecosystem. Different ecosystem shading represents the variation present in the ecosystem state of interest. (B) For Question 1, we quantified the percentage of among-region variation in ecosystem state (as shown in panel A). In this example, ecosystem state varies among regions (differently shaded shapes in region a, b, and c result in different regional mean ecosystem state), but within-region variation also exists (differently shaded shapes within each region). (C, D) For Question 2, we quantified driver–response relationships using both local and regional driver variables (1, 2, 3, . . .) to account for (C) local driver of ecosystem variation within regions and differences in regional mean ecosystem state (i.e., random intercepts in a multilevel model) and (D) differences in relationships between local drivers and ecosystem state by region (i.e., random slopes in addition to random intercepts in a multilevel model). See *Methods* for details on the multilevel modeling.

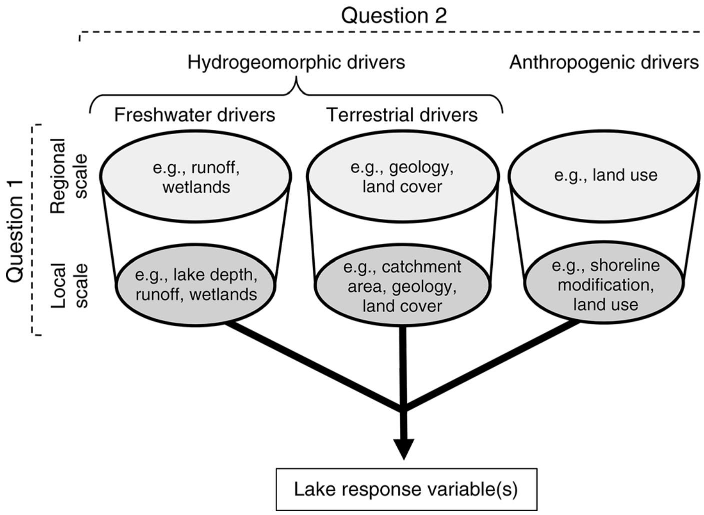

FIG. 2. The conceptual framework of landscape limnology and its relationship with our study questions. The vertical columns show the three broad categories of landscape drivers that can influence variation among lake ecosystems. The two rows represent spatial scale and the hierarchical nature of drivers of ecosystem variation, where "regional scale" refers to among-region drivers of lake ecosystem variation and "local scale" refers to within-region drivers of lake ecosystem variation. General examples of each type of driver at each spatial scale are provided (see Table 2 for driver variables used in this study). Note that some driver variable are multi-scale (i.e., are quantified at multiple scales), whereas others are uni-scale variables (i.e., can only be quantified at the local scale).

opportunity to study the spatial structure of ecosystem variation, the processes underlying that structure, and its implications. The conceptual framework for studies such as ours has been formalized as landscape limnology, defined as the spatially explicit study of lakes, streams, and wetlands as they interact with freshwater, terrestrial, and human landscapes (Fig. 2; Soranno et al. 2010). A great number of individual studies of lakes have been conducted at a single spatial scale; however, by looking across the body of accumulated knowledge, it is clear that lakes are influenced by multiple freshwater, terrestrial, and human drivers at multiple scales. Although it is well established that lakes can integrate the effects of hydrologic, land use, and climatic changes in their watersheds and airsheds at a range of spatial and temporal scales (Williamson et al. 2009), the relative importance of drivers, scales, and underlying processes is incompletely known, and represents a challenge of fundamental importance to the extrapolation of knowledge to regional- and continental-scale ecology research and conservation. Our study addresses this knowledge gap by including and building upon the rich local-scale understanding of lakes, covering a broad geographic range, including uni- and multi-scaled driver variables related to lake states to better identify relevant spatial scales, and adopting an analytical approach that explicitly recognizes and studies the spatial hierarchy in which lakes exist.

METHODS

Study lakes and lake response variables

We assembled data for 2319 north-temperate lakes in four lake-rich U.S. states (Maine, New Hampshire, Michigan, Wisconsin) and two reservoir-rich states (Ohio and Iowa) (Fig. 3, Table 2; data set *available online* on the ESA Data Registry).[5] We included lakes and reservoirs (hereafter lakes) with surface area $\geq$0.01 km^2 and maximum depth $\geq$2 m. Lake chemistry data came from databases maintained by state agencies responsible for monitoring lakes under the Federal Clean Water Act with the exception of Iowa; for Iowa, data came from lake monitoring conducted at Iowa State University (Ames, Iowa, USA). Sampling programs that provided us with data used standard methods and quality assurance/quality control protocols. We used only data that were collected from the epilimnion during the summer stratified period (July to September) to avoid variability due to seasonal and depth effects. In addition, we used only data from a single sampling date from each lake because most lakes had only single observations (Webster et al. 2008) and we wanted to avoid any bias introduced from multiple observations in a few lakes. For lakes with more than one sample date, we chose the most recent observation that had measurements for both phosphorus and alkalinity. Most lakes were sampled between 1990 and 2004 (75%), with the earliest samples collected during 1975. Phosphorus was measured as total phosphorus colorimetrically after persulfate digestion. Alkalinity was measured as $CaCO_3$ using titration to an electrometrically determined endpoint. Large ranges of both total phosphorus and alkalinity existed across the study area (1–765 μg/L and 0–302 mg/L, respectively; Table 2).

Regionalization frameworks

We compared the magnitude of among-region variation for seven published regionalization frameworks, each of which is spatially contiguous and geographically dependent; these include four terrestrial frameworks and three aquatic frameworks (Table 3 and citations therein). Our goal was not a comprehensive test of all existing regionalization frameworks; rather, we chose seven frameworks that represented good contrasts in features that we expected to be important sources of regional lake variation: region size, landscape and

[5] http://data.esa.org/esa/style/skins/esa/index.jsp

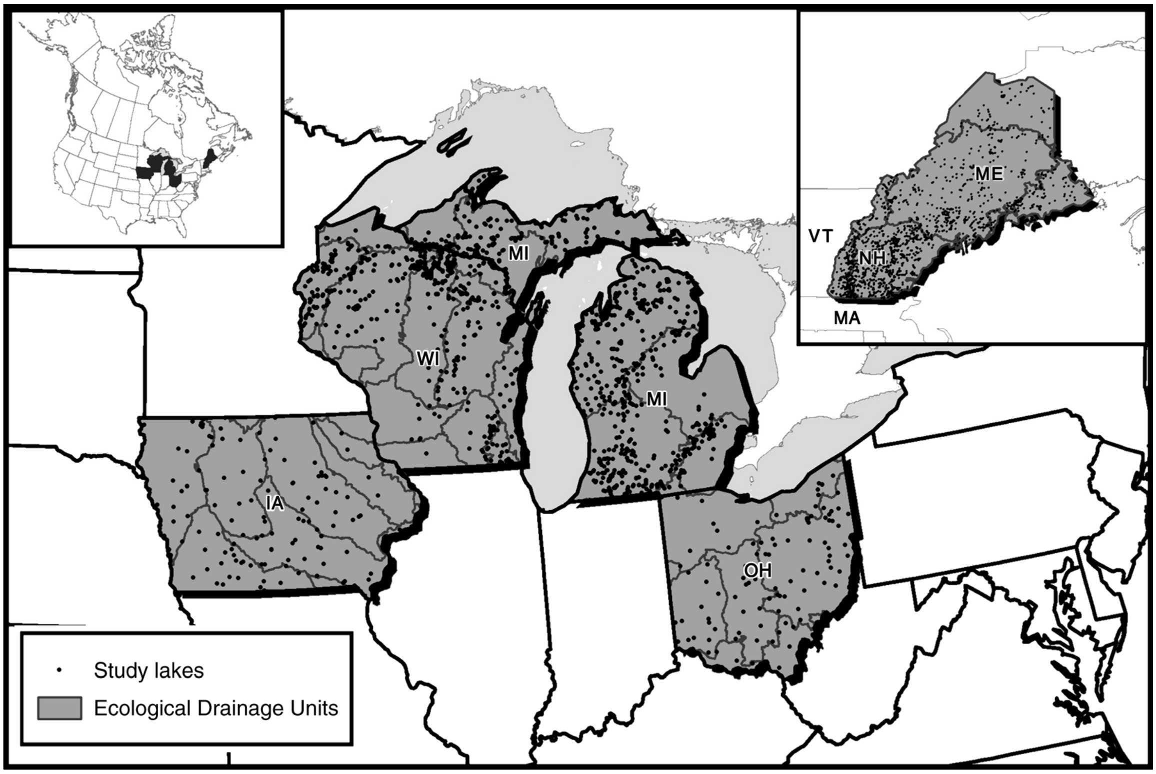

FIG. 3. Map of the United States, showing the six-state region (from west to east: Iowa [IA], Wisconsin [WI], Michigan [MI], Ohio [OH], New Hampshire [NH], and Maine [ME]), Ecological Drainage Units, and the study lakes. State lines are shown with black lines, and the Ecological Drainage Unit boundaries are shown with gray lines.

climate variables used to classify the regions, the types of ecosystems for which they were created, and the goals of the framework (Table 3). Each lake was spatially assigned to a region within each of the seven frameworks using geographic information system (GIS) coverages, and ArcGIS, Version 10 (ESRI 2010), and the sources listed in Table 3.

Local- and regional-scale driver variables

Uni-scaled local driver variables included lake and catchment morphometric data either provided by the state and university monitoring programs described in the previous section (total lake catchment area, lake maximum depth) or quantified from individual state GIS coverages at 1:24 000 resolution (lake area). The catchment areas provided by state sources were calculated using a variety of methods, and were not available as GIS coverages, so could not be used to calculate catchment-scale statistics.

Multi-scaled local and regional driver variables included hydrogeomorphic and anthropogenic landscape variables that potentially influence lake phosphorus and alkalinity. We used GIS databases available at the national scale to quantify metrics of these landscape variables using the sources listed in Table 2. For hydrogeomorphic variables, we quantified metrics associated with land use/cover, surficial geology, and hydrology (precipitation, runoff, baseflow). For anthropogenic landscape features, we quantified metrics associated with land use/cover and roads. We quantified these landscape metrics at two spatial scales: the 500-m buffer around each lake and the region (using Ecological Drainage Units; Higgins et al. 2005).

Due to data limitations, we made two assumptions. First, we used land use/cover data from the 1992 National Land Cover Dataset (*available online*)[6] because land use/cover data were not available for each year within the time range that our lakes were sampled (30 years) and 1992 was closest to the median sample year for the lakes in our data set. Second, we used the 500-m buffer around each lake as an indicator of local-scale landscape effects on lake water chemistry. We used the 500-m buffer rather than the catchment because no national database exists for lake catchments, there were no delineated state catchment GIS layers, and it was cost prohibitive to obtain catchment delineations for 2319 lakes. We tested the assumption that the 500-m buffer can be used as an indicator of land use/cover in a lake's catchment using two subsets of our database for which we had lake catchments delineated (461 lakes in

[6] http://landcover.usgs.gov/natllandcover.php

TABLE 2. Descriptive statistics for lake phosphorus and alkalinity and uni-scaled lake and catchment driver variables included in conditional multilevel models.

Variable (%, unless noted)	*N*	Mean	SD	Minimum–maximum
Lake response variable				
Total phosphorus (μg/L)	2319	22.3	40.2	1.0–765.0
Alkalinity (mg/L $CaCO_3$)	2037	46.1	57.9	0–302.0
Local driver variables				
Surface area (km^2)	2319	281.2	1508	1.3–53 367
Maximum depth (m)	2319	11.7	8.9	2.0–96.3
Catchment area (km^2)	2142	137.7	997.5	0.02–31 080
Water†	2320	3.8	4.4	0–39.6
Forest†	2320	66.6	25.1	0.6–100
Woody and herbaceous wetland†	2320	9.2	10.7	0–80.3
Runoff (cm)‡	2313	18.3	7.0	2–40
Baseflow§	2320	56.3	12.2	14–90
Precipitation (cm)¶	2320	39.7	6.8	25.7–72.3
Surf. geol., coarse grained#	2307	32.8	41.2	0–100
Surf. geol., fine grained#	2307	1.9	10.5	0–100
Till#	2307	51.4	42.4	0–100
Surf. geol., patchy Quaternary#	2307	11.9	28.0	0–100
Urban†	2320	4.0	9.1	0–93.5
Small grain/fallow ag and urb/rec grass†	2320	0.5	2.0	0–33.5
Pasture ag†	2320	5.6	10.1	0–79.6
Row crop ag†	2320	8.7	12.2	0–89.8
Road density (m/ha)‖	2320	33.3	19.5	0–119.2
Regional driver variables (EDU)				
Water†	35	2.5	2.1	0.5–10.6
Forest†	35	39.1	27.8	2.3–86.2
Woody and herbaceous wetland†	35	7.0	8.6	0.1–42.8
Runoff (cm)‡	35	13.1	6.3	3.7–27.7
Baseflow§	35	49.1	12.8	25.1–78.3
Precipitation (cm)¶	35	31.8	8.3	10.3–44.3
Surf. geol., coarse grained#	35	21.7	16.7	4.0–61.7
Surf. geol., fine grained#	35	4.4	7.5	0–30.9
Till#	35	56.7	23.4	8.9–91.4
Surf. geol., patchy Quaternary#	35	6.7	9.3	0–41.2
Urban†	35	3.5	4.3	0.3–19.9
Small grain/fallow ag and urb/rec grass†	35	0.6	0.6	0–2.6
Pasture ag†	35	14.3	10.5	0.3–38.6
Row crop ag†	35	30.8	25.0	0.6–79.6
Road density (feet/acre)‖	35	16.0	6.3	6.0–38.3

Notes: Variables were ln- or arcsine square-root transformed. Abbreviations are: ag, agriculture; urb, urban; rec, recreational; and EDU, Ecological Drainage Units. Surf. geol. (surficial geology) refers to coarse- and fine-grained stratified surficial deposits, and patchy Quaternary to patchy Quaternary sediments. Italicized variables were quantified at only one scale.

† Data from the 1992 National Land Cover Dataset (http://landcover.usgs.gov/natllandcover.php).

‡ Average annual runoff in the United States, 1951–1980 (http://water.usgs.gov/GIS/metadata/usgswrd/XML/runoff.xml#stdorder).

§ Baseflow index grid for the conterminous United States (http://water.usgs.gov/GIS/metadata/usgswrd/XML/bfi48grd.xml).

¶ United States average monthly or annual precipitation, 1971–2000 (http://www.prism.oregonstate.edu/docs/meta/ppt_30s_meta.htm).

Character of Quaternary Sediments in the Glaciated United States East of the Rocky Mountains (Soller and Packard 1998; http://pubs.usgs.gov/ds/656/)

‖ Street map USA 2000 available in ArgGIS Desktop Release 10 (ESRI 2010).

Michigan and 128 lakes in Wisconsin). We found land use/cover percentages to be highly correlated between the 500-m buffer and the lake catchment scales (in Michigan and Wisconsin, respectively: percent agriculture cover, $r = 0.79$ and 0.94; percent forest cover, $r = 0.86$ and 0.94; and percent wetland cover, $r = 0.64$ and 0.87 with $P < 0.001$; P. A. Soranno and K. E. Webster, *unpublished data*). Therefore, the 500-m buffer provides a close approximation of land use/cover in lake catchments. To aid interpretation of the results and examine spatial patterns across regions, we mapped the regional values of a subset of the multi-scaled regional landscape drivers as well as the region-specific lake averages for phosphorus and alkalinity.

Statistical analysis

We performed all analyses in SAS, Version 9.2 (SAS Institute 2008). When assumptions of normality and homoscedasticity were not met, we ln-transformed the response, hydrogeomorphic, and anthropogenic disturbance variables, except for the land use/cover, surficial geology, and base flow proportions, which we arc sine square-root transformed.

To quantify among-region variation in lake water chemistry, we used unconditional multilevel models (also called mixed-effects models, hierarchical linear models, or one-way ANOVAs with random effects; Raudenbush and Bryk 2002). We modeled each response variable (phosphorus, alkalinity) with each of the seven regionalization frameworks separately, resulting in 14 individual models. Each model did not include predictor variables; rather, each included one regionalization framework as a grouping variable and partitioned the total among-lake variance into within-region (σ^2) and among-region (τ_{00}) variance components (see Cheruvelil et al. 2008 for additional modeling details). For each model, we then calculated the intraclass correlation coefficient (ICC, $\hat{\rho}$) as $\hat{\tau}_{00}/(\hat{\tau}_{00} + \hat{\sigma}^2)$. This statistic is the percentage of the total variation among lakes that is "among-region variation" (i.e., regional; as opposed to within-region or local variation). For Question 1, we compared the ICCs for lake total phosphorus and alkalinity among regionalization frameworks. The regionalization framework with the highest among-region variation was considered best at partitioning among-region variation in lake phosphorus or alkalinity.

To further study the among-region variation captured by the ICCs, we selected one of the regionalization frameworks that captured high among-region variation in both lake water chemistry variables (Ecological Drainage Units; Higgins et al. 2005). Using the Ecological Drainage Units unconditional model, we calculated the best linear unbiased predictors (BLUPs) for each region, defined as the regional deviation from the grand mean lake response (i.e., regional variation around the intercept; Robinson 1991). For each response variable, each region's BLUP depicts whether and how that region differs from the other regions and from the grand mean, making BLUPs a useful diagnostic and visualization tool.

For Question 2, we used Ecological Drainage Units to quantify which landscape variables explained the observed among-region variation in lake water chemistry. To do so, we built conditional multilevel models for phosphorus and alkalinity separately, and allowed for random intercepts and slopes among regions. We included local and regional driver variables in the models (i.e., uni-scaled lake and catchment morphometry variables and multi-scaled hydrogeomorphic and anthropogenic landscape driver variables). Including both scales in the model allowed us to quantify which lake and landscape features were related to lake variation in phosphorus and alkalinity and the scales at which they operate. We included only driver variables in candidate and final models that had pairwise Pearson correlation coefficients of less than 0.70. Throughout the model building process, after including each driver variable, we determined whether the more complex model was significantly better than the simpler model using a Likelihood Ratio Test ($\alpha < 0.05$). We group mean centered ($X_{ij} - X_{.j}$) local driver variables and grand mean centered ($X_{ij} - X_{..}$) regional driver variables to aid in interpretation (Enders and Tofighi 2007). We performed the multilevel modeling using the SAS MIXED procedure (SAS Institute 2008). Finally, we calculated the percentage of variation explained among- and within-regions and the total combined percentage of variation explained by the final conditional model.

Below is an example of the general form that our multilevel models took (Raudenbush and Bryk 2002), using just two driver variables, one local and one regional:

$$\begin{aligned} Y_{ij} = \gamma_{00} &+ \gamma_{10}(\text{lake area}_{ij} - \text{lake area}_j) \\ &+ u_{1j}(\text{lake area}_{ij} - \text{lake area}_j) \\ &+ \gamma_{01}(\text{regional ag}_j - \text{regional ag..}) + r_{ij} + u_{0j} \end{aligned}$$

where Y_{ij} is the alkalinity or phosphorus for lake$_i$ in region$_j$; γ_{00} is the intercept representing the grand mean alkalinity or phosphorus for all regions, controlling for lake area and regional agriculture; γ_{10} is the fixed slope representing the relationship between lake area and alkalinity or phosphorus; u_{1j} is the random effect of region$_j$ on the relationship (slope) between lake area and Y_{ij}, where $u_{1j} \sim N(0, \tau_{11})$ and τ_{11} represents the among-region variability in slopes (random slope); γ_{01} is the effect of regional agriculture on region-average alkalinity or phosphorus (the random intercepts); r_{ij} is the lake error for lake$_i$ in region$_j$, where $r_{ij} \sim N(0, \sigma^2)$ and σ^2 represents the within-region variability in alkalinity or phosphorus; and u_{0j} is the error for region$_j$, where $u_{0j} \sim N(0, \tau_{00})$, and τ_{00} represents the residual among-region variance in alkalinity or phosphorus after controlling for regional agriculture.

In this example, note that if the parameter estimate u_{1j} is not significantly different from zero, then that term is removed from the model and that parameter becomes a fixed estimate, and we have the simpler model as follows:

$$\begin{aligned} Y_{ij} = \gamma_{00} &+ \gamma_{10}(\text{lake area}_{ij} - \text{lake area}_j) \\ &+ \gamma_{01}(\text{regional ag}_j - \text{regional ag}) + r_{ij} + u_{0j}. \end{aligned}$$

The proportion of among-region variation explained by these final conditional models was calculated as $\Delta\tau_{00} = (\tau_{00\text{-U}} - \tau_{00\text{-C}})/\tau_{00\text{-C}}$, where U is unconditional and C is conditional, and the total combined proportion variation was calculated as $[\Delta\tau_{00} \times \rho] + [\Delta\sigma \times (1 - \rho)]$.

Results

Among-region variation in lake water chemistry and regionalization frameworks

Among-region variation accounted for equal to or greater than half of the total variation in lake phosphorus and alkalinity (50–75%) across all regionalization frameworks except for the coarsest framework (EPA regions, no significant among-region variation; Table 4). Therefore, a significant and large proportion

TABLE 3. Descriptions of the regionalization frameworks tested.

Regionalization framework	Reference	N	Mean area (km^2)
Terrestrial ecoregions			
EPA Regions†		4	163 616
Omernik Level III	Omernik (1987)	17	38 462
Bailey Sections	Bailey et al. (1994)	23	28 365
Major Land Resource Areas	USDA (2006)	29	22 277
Aquatic ecoregions			
Freshwater Ecoregions	Abell et al. (2000, 2008)	6	109 009
Six-digit Hydrologic Units	Seaber et al. (1987)	47	13 868
Ecological Drainage Units	Higgins et al. (2005)	35	18 544

Notes: The frameworks are grouped according to the type of ecosystems they were created for. Mean area for each regionalization framework is across all regions in our study area.

† Omernik Level III Ecoregions were agglomerated into nine ecological regions in the conterminous United States by the U.S. EPA for uses such as the Wadeable Stream Assessment (EPA 841-B-06-002; www.epa.gov/owow/streamsurvey) and the National Lake Assessment (EPA 841-R-09-001; http://water.epa.gov/type/lakes/lakessurvey_index.cfm).

of variation among lakes for both response variables was at the regional spatial scale. The percentage of among-region variation was relatively similar for phosphorus and alkalinity (50–60% and 58–75% among-region variation, respectively; Table 4). The regionalization frameworks that captured the highest, and very similar amounts of, among-region variation were the same for the two response variables: Bailey's ecoregions, Six-digit Hydrologic Units, and Ecological Drainage Units (Table 4). These results indicate that these three regionalization frameworks may be particularly useful for grouping together lakes with similar phosphorus and alkalinity at spatial extents similar to ours.

Drivers of among-region variation in lake water chemistry

We chose Ecological Drainage Units to quantify which multi-scaled drivers explained variation in lake water chemistry. Although Bailey's Sections and Six-digit Hydrologic Units captured similarly high amounts of regional variation in lake phosphorus and alkalinity (Table 4), we used Ecological Drainage Units because they were created by aggregating Eight-digit Hydrologic Units (major river watersheds; Seaber et al. 1987) according to natural hydrologic, physiographic, and climatic features (Higgins et al. 2005). Therefore, they represent a hybrid approach to delineating ecoregions that combines both the freshwater and terrestrial landscapes. We hypothesized that delineating ecoregions in this way would be useful for understanding what drives variation in lake chemistry at the subcontinental scale.

Phosphorus and alkalinity both exhibited southwest to northeast gradients of generally decreasing value across our spatial extent (Fig. 4). The BLUPs from the unconditional mixed models show that the majority of the 35 Ecological Drainage Unit regions had a significant deviation from the grand mean for both response variables (Fig. 4). To better understand which landscape drivers are related to this variation in water chemistry among lakes and regions, we next built conditional multilevel models that included variables that were uni- and multi-scaled, local and regional, and hydrogeomorphic and anthropogenic. At the local scale, we found that nine commonly measured drivers explained 31.0% and 25.4% of the within-region variation in lake phosphorus and alkalinity, respectively (Table 5). These local-scale variables included a combination of hydrogeomorphic and anthropogenic, uni- and multi-scale lake and landscape features. For phosphorus, these nine local-scale variables were fixed in the model because we found that the relationship (i.e., slope) between each driver variable and lake phosphorus did not vary by region. However, for alkalinity, we found that the relationship between local precipitation and lake alkalinity varied among regions (i.e., random slope; Tables 5 and 6).

At the regional scale, and consistent with the BLUPs (Fig. 4), we found that mean phosphorus and alkalinity values varied among regions (i.e., random intercepts). For phosphorus and alkalinity, respectively, only three and one regional landscape driver variable(s) were included in the final models that explained 80.7% and 74.0% of the among-region variation. For phosphorus, the three regional drivers were one of each of the

TABLE 3. Extended.

Landscape and climate variables used to classify the regions	Goals that each framework was projected to meet
land use, landform, natural vegetation, soils	design monitoring programs, assess ecosystem state, and report the results of regional and national monitoring efforts
land use, landform, natural vegetation, soils	assess regional patterns and trends in the extent and quality of environmental resources and relations with natural and human-related factors
climate, landform, vegetation, soils	provide information for the development of resources and conservation of the environment
soils, geology, climate, water resources, land use	for agricultural planning; forms a basis for: decisions regarding regional–national land resource issues; identifying research and monitoring needs; extrapolating research results across political boundaries; organizing and operating land resource, management, and conservation programs
freshwater fish biogeography	assess and conserve freshwater diversity.
surface hydrologic features; drainage areas of a series of major river drainage basins	reporting of water resource state
aggregated Eight-digit Hydrologic Units (Seaber et al. 1987) by hydrology, physiography, and climate	capture representative freshwater biodiversity for regional conservation planning

freshwater, terrestrial, and anthropogenic driver types: proportion baseflow (negative association), fine-grained surficial geology (negative association), and row crop agriculture (positive association). Both proportion baseflow and fine-grained surficial geology exhibited a northeast to southwest gradient of generally decreasing value across our spatial extent, whereas row crop agriculture exhibited a northeast to southwest gradient of generally increasing proportion across our spatial extent (Fig. 5). However, these three landscape features did not follow the exact same (or converse) spatial pattern (Fig. 5). For alkalinity, the proportion of forest land cover within the region, which exhibits a northeast to southwest gradient of generally decreasing value across our spatial extent, was negatively associated with region-average alkalinity. Combining within- and among-region variation explained, we found that these local and regional landscape drivers accounted for a total of 60.0% and 54% of the variation in lake phosphorus and alkalinity, respectively (Table 6), the majority of which was at the regional spatial scale.

DISCUSSION

Regionalization frameworks

Using 2319 lakes in six northeastern U.S. states and seven regionalization frameworks, we found a significant and large amount of among-region variation in two lake response variables. For both phosphorus, an indicator of cultural eutrophication, and alkalinity, a measure of a lake's ability to resist acidification, we found that $\geq$50% of the variation among lakes was captured at the regional spatial scale using all but one of the regionalization frameworks tested. Therefore, using a regionalization framework can be an important first step in capturing and developing a better understanding of intermediate-scale variation in ecosystem state. The choice of *which* regionalization framework to use for broad-scale scientific, management, and conservation applications may differ depending on the response variable. For our two contrasting lake chemistry variables, it was possible to choose a single regionalization framework that captured among-region variation in both variables quite well. However, a quantitative evaluation of candidate regionalization frameworks should be conducted for all response variables of interest before using a framework.

TABLE 4. Results of unconditional multilevel models that quantify among-region variation, sorted by region type (terrestrial, aquatic; see Table 2).

Category and regionalization framework	Variation among regions (%)	
	Phosphorus	Alkalinity
Terrestrial		
EPA Regions	n/s	n/s
Omernik Level III	54.0	58.3
Bailey sections	**58.8**	**73.3**
Major Land Resource Areas	57.0	64.6
Aquatic		
Freshwater Ecoregions	49.6	57.5
Six-digit Hydrologic Units	**60.3**	**74.6**
Ecological Drainage Units	**58.5**	**73.2**

Notes: Response variables were ln-transformed. Percentage of variation among regions refers to the percentage of total variation that was "regional" using the interclass correlation coefficient (ICC; see *Methods* for details). Boldface type indicates frameworks with the highest percentage of among-region variation; n/s indicates insignificant among-region variation with $P > 0.05$; $n = 2319$ and 2037 lakes for phosphorus and alkalinity, respectively. See Table 2 for units of response variables.

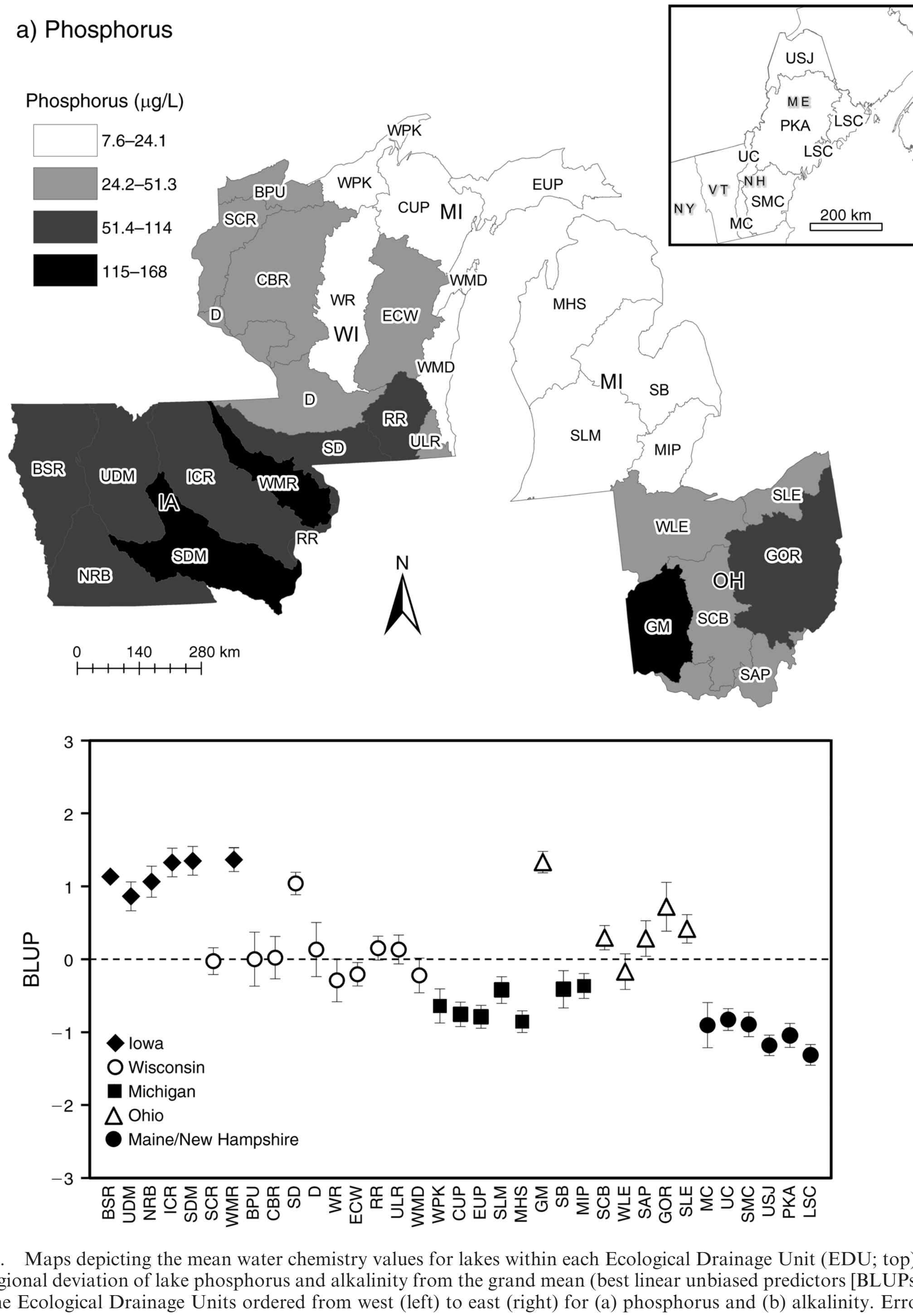

FIG. 4. Maps depicting the mean water chemistry values for lakes within each Ecological Drainage Unit (EDU; top) and plots of the regional deviation of lake phosphorus and alkalinity from the grand mean (best linear unbiased predictors [BLUPs]; bottom) across the Ecological Drainage Units ordered from west (left) to east (right) for (a) phosphorus and (b) alkalinity. Error bars are the standard error of the BLUP estimate. See Fig. 3 for state identifiers and Appendix A for EDU names.

We found that Bailey's ecoregions, Six-digit Hydrologic Units, and Ecological Drainage Units were consistently the "best" regionalization frameworks for capturing among-region variation in lake phosphorus and alkalinity. These latter two frameworks are both agglomerations of Eight-digit Hydrologic Units, which are approximations of major river watersheds (Seaber et al. 1987). It makes sense that both frameworks would work well to capture regional variation among lakes. It is heartening that Ecological Drainage Units were

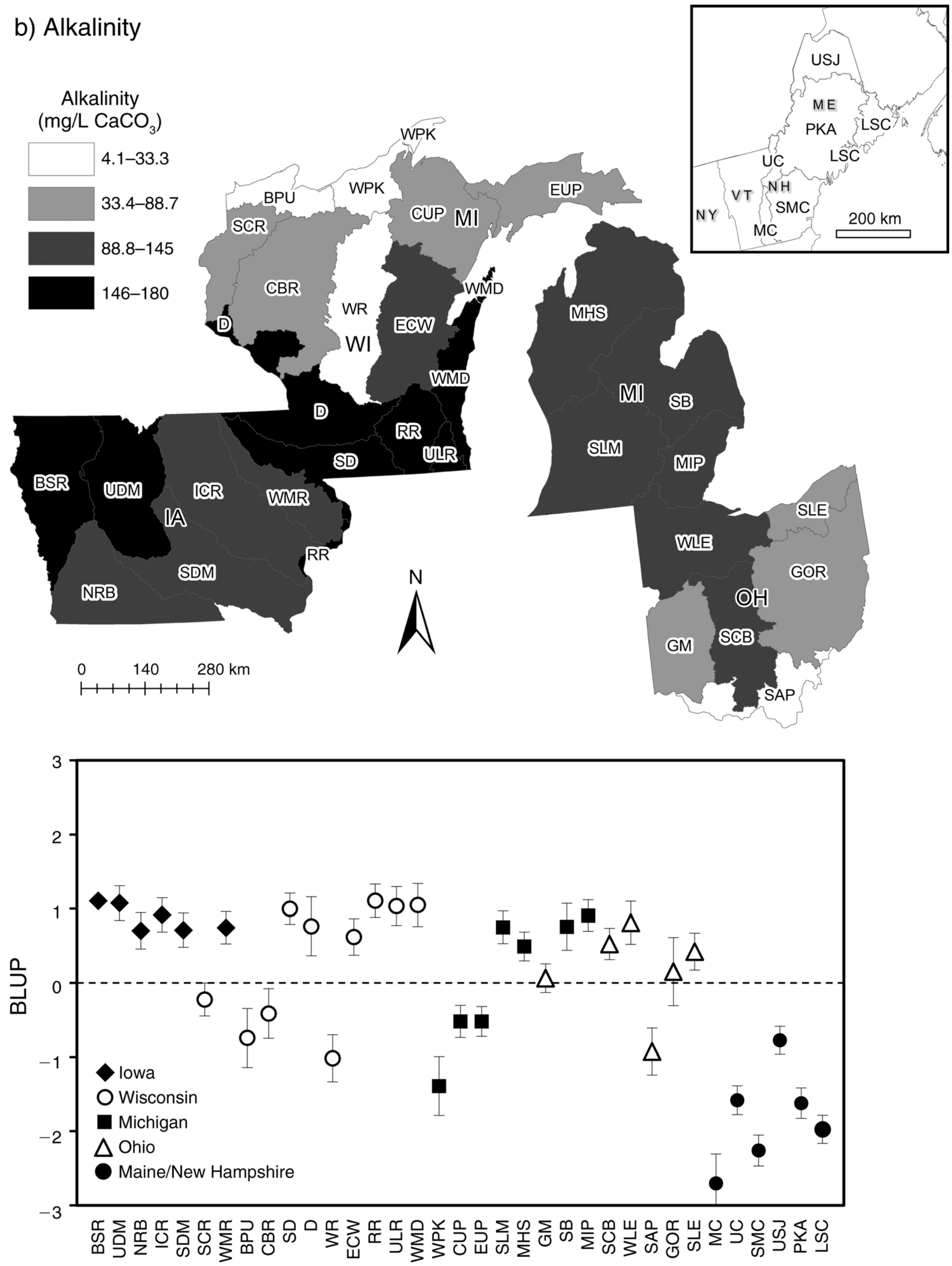

FIG. 4. Continued.

successful at partitioning variation in lake chemistry because they are currently being used for assessment, management, and conservation purposes for freshwaters (e.g., The National Fish Habitat Action Plan [*available online*][7] and The Nature Conservancy for identifying lake conservation priorities [Blann and Cornett 2008]).

[7] http://www.fws.gov/midwest/fisheries/nfhap.html

It is likely that the importance of using a regionalization framework in general and the choice of framework in particular, are highly sensitive to the spatial extent and location of the study area. We examined this issue by comparing our results to a study that was conducted on the same response variables as ours within a spatial extent that was nested within, and covering approximately one-sixth of, our spatial extent (Cheru-

TABLE 5. Conditional multilevel model results.

Variable	Phosphorus (ln(μg/L)) Coefficient	SE	Alkalinity (ln(mg/L $CaCO_3$)) Coefficient	SE
Local driver variables				
Surface area	...	...	−0.068	0.014
Max depth	−0.509	0.020	...	...
Catchment area	0.044	0.007	0.094	0.010
Forest	−0.312	0.102	...	...
Baseflow	−0.738	0.221	1.038	0.257
Surf. geol., coarse grained	−0.124	0.028	...	...
Surf. geol., fine grained	0.195	0.080	...	...
Surf. geol., patchy Quaternary	...	...	−0.194	0.037
Urban	0.354	0.108	0.758	0.106
Agriculture, pasture	0.453	0.145	0.424	0.156
Agriculture, row crop	0.328	0.112	0.571	0.113
Road density	...	...	0.044	0.0143
Random slope (precipitation)†	...	...	12.783	5.433
Random intercept‡	2.695	0.078	3.299	0.121
Regional driver variables (EDU)				
Forest	...	...	−39.985	4.141
Baseflow	−2.092	0.559	...	...
Surf. geol., fine grained	−19.580	4.340	...	...
Agriculture, row crop	18.417	3.021	...	...

Notes: All variables with coefficients were significant at $P < 0.02$ for phosphorus and $P < 0.006$ for alkalinity; ellipses indicate variables not included in that model. Units for raw driver variables are as in Table 2, but they were ln- or arcsine square-root transformed and then either group- or grand-mean centered (local and regional variables, respectively). Abbreviations are: EDU, Ecological Drainage Units; Surf. geol., surficial geology. Coarse and fine grained refer to coarse- and fine-grained stratified surficial deposits, and patchy Quaternary to patchy Quaternary sediments. Italicized variables were quantified at only one scale. $N = 2130$ and 1890 for phosphorus and alkalinity, respectively. See Table 2 for data sources.

† The relationship between local precipitation and lake alkalinity varies among regions.

‡ The mean lake phosphorus or alkalinity varies among regions.

velil et al. 2008). Our results both agree and disagree with results of this previous study. For example, Cheruvelil et al. (2008) found that among-region variation was predominantly <50% for the tested regionalization frameworks, as compared to our study that found among-region variation was ≥50%. Such a result makes sense because at smaller spatial extents, such as within a single U.S. state, there is likely to be less variation both among regional-scale driver variables and among ecosystem response variables (as seen in Figs. 4 and 5). Despite these differences between studies, Ecological Drainage Units were found to capture a significant amount of among-region variation in both phosphorus and alkalinity for the smaller spatial extent studied by Cheruvelil et al. (2008), suggesting this framework's usefulness at a range of spatial extents.

Because regionalization frameworks assume that ecosystems that are closer to one another are more similar than ecosystems that are further apart (Seelbach et al. 2006), spatial autocorrelation of the response variables is likely and, in fact, necessary for their usefulness (Legendre et al. 2002). Therefore, the statistically "best" regionalization framework(s) might be the one(s) that divides the landscape into many small regions to maximize the spatial autocorrelation structure of the response variables. For our study lakes and extent, the percentage of among-region variation was positively related to the number of regions in each regionalization framework for both response variables, but it did not explain all of the variation in these relationships ($r^2 = 0.64$ and 0.69, $P < 0.05$, for phosphorus and alkalinity, respectively). Therefore, there is more than simply spatial autocorrelation accounting for regionalization frameworks' success at grouping similar lakes, and the "best" regionalization frameworks are not necessarily those that divide the landscape into the smallest regions. This result is important because to be useful, a regionalization framework must strike a balance between having few regions with high within-region variation, and many regions with low within-region variation.

TABLE 6. Variation and variation explained by the conditional multilevel models in Table 5.

Variation (%)	Phosphorus (ln(μg/L))	Alkalinity (ln(mg/L $CaCO_3$))
a) Within-region variation	41.5	28.8
b) Within-region variation explained	31.0	25.4
c) Among-region variation	58.5	73.2
d) Among-region variation explained	80.7	74.0
Total variation explained	60.0	53.8

Note: Total percentage of variation explained = (a × b) + (c × d).

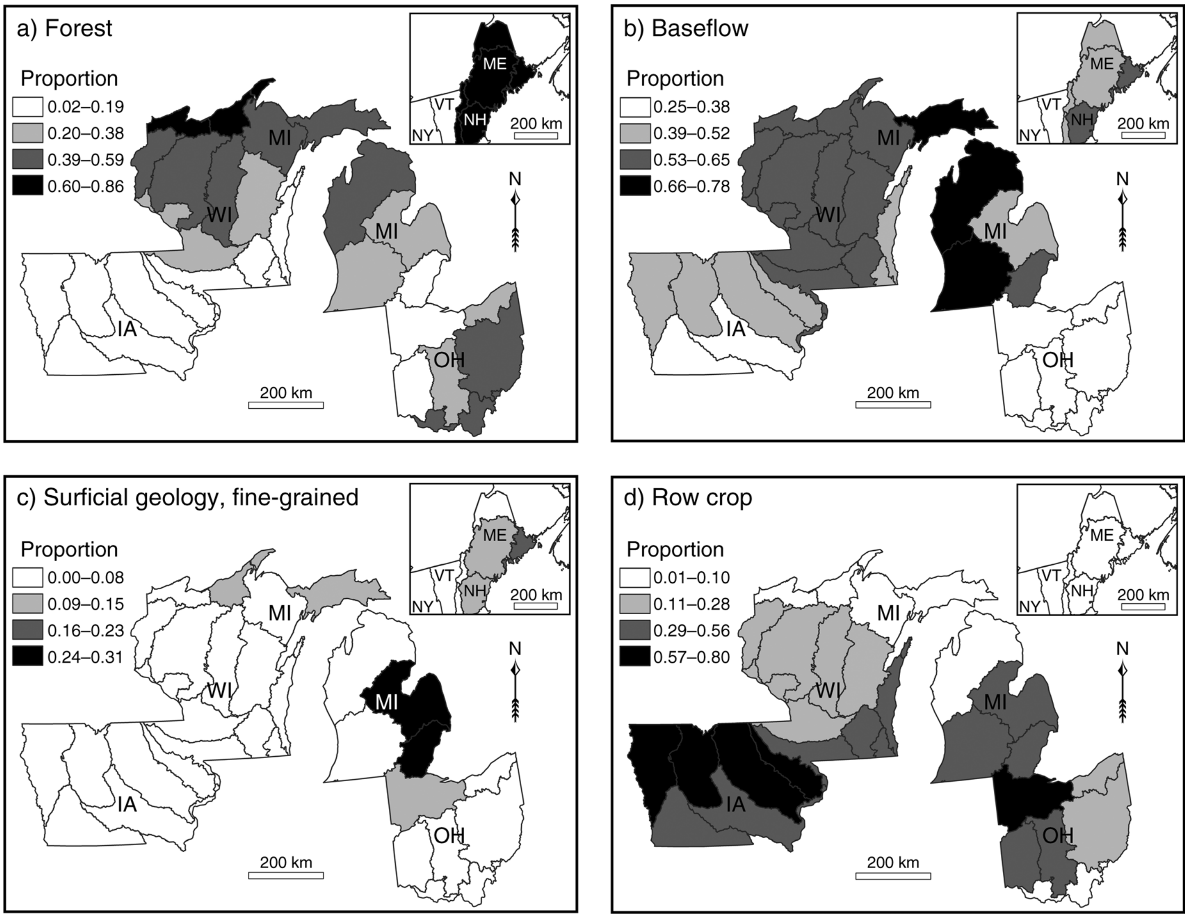

FIG. 5. Maps depicting the regional hydrogeomorphic and anthropogenic landscape features of each Ecological Drainage Unit (EDU) for (a) the proportion forest land cover, (b) the proportion of baseflow, (c) the proportion of fine-grained stratified surficial deposits, and (d) the proportion of row crop agriculture. Sources are as in Table 2; see Fig. 4 for EDU codes and Appendix A for EDU names.

The results of our conditional multilevel models can also be used to design new regionalization frameworks specifically for lakes. We found that regional-scale land use/cover, surficial geology, and hydrology, representing the three types of landscape features shown in Fig. 2, appear to be the key landscape features driving broad-scale patterns in lake phosphorus and alkalinity across our study's spatial extent. Therefore, these landscape variables could be used to create a new regionalization framework for lakes. It will also be necessary, however, to test whether such custom-made regionalization frameworks based on readily-available landscape features capture among-region variation in ecosystem states better than existing frameworks.

The regional spatial scale

We found that a majority of the total variation in lake water chemistry occurred at the regional scale, and that regional variation was explained by just a few regional-scale landscape driver variables. These results support the use of regionalization frameworks to account for such variation when studying or managing lakes at these spatial extents. If we ignored the regional spatial scale by not using a regionalization framework or by not including regional-scale landscape features in our models, we would wrongly assume that all of the variation in lake water chemistry is at the local scale and occurs within regions. This mistake has important statistical implications (Wagner et al. 2006), and would result in our failure to recognize that spatial variation among lakes occurs at multiple spatial scales. Others have argued for a multi-scaled perspective to understand global change biology, which our results support (Peters et al. 2011).

In addition to average ecosystem state varying across regions, we found that the relationship between local-scale driver variables and lake chemistry can vary regionally. For example, the relationship between local precipitation and lake alkalinity varied across EDUs (i.e., significant random slope estimate). Precipitation is well known as a strong driver of variability in lake chemistry, including alkalinity. However, lake chemistry responds differently to climate signals such as drought, not only within a region, but also across regions that differ in hydromorphology (Webster et al. 2000). Although our study only examines spatial patterns in the static relationship between alkalinity and each lake's average precipitation, we can speculate that the regional relationship may depend on the type, magnitude and diversity of both the hydrogeomorphic setting(s) and rainfall intensity, both of which vary greatly across our study extent. This is an intriguing result with no concrete explanation at this time, requiring further analysis of precipitation and alkalinity data through time and across regions to identify the causal mechanisms.

Our overall results show that both fine- and broad-scaled features are related to among-lake variation. Although our objectives did not explicitly include an exhaustive interpretation of each driver variable's relationship with lake chemistry, we found that the local-scale driver variables of both the phosphorus and alkalinity models made limnological sense. Both models included local driver variables found to be important in studies conducted within individual regions such as catchment area, forest cover, geology, and land use/cover (e.g., Hakanson 1996, Jones et al. 2004, Taranu and Gregory-Eaves 2008). However, our results also suggest that many of the landscape variables that are typically quantified only at the local scale (and modeled only as local-scaled effects) also have a regional component (i.e., are multi-scaled) with substantial explanatory power. Unfortunately, it is not yet clear at which spatial scale(s) the important ecological interactions or the underlying mechanisms play out.

This type of research is an important first step to identify hypotheses related to the mechanisms at these broad scales. For example, in our lake alkalinity model, forest land cover was not significant at the local scale (as we may have expected), but was significant at the regional scale. We hypothesize that regional forest land cover is related to two processes that influence lake alkalinity. First, regions with very high forest cover likely have surficial geology and soils that are not amenable to agriculture and that are not highly weatherable, thus resulting in poorly buffered, low-alkalinity lakes (Baker et al. 1991). Fig. 5a shows that the regions with the highest forest cover in our study area are areas with geology that is known to be poorly weathered (granite bedrock in the northeast and low-calcareous till in the western portion of the upper peninsula of Michigan). Thus, it could be that regional forest cover is a better indicator of geology related to water chemistry than was the surficial geological data used in our models. Second, regions with very low forest cover also have intensive agricultural activity (Fig. 5d), likely due to more calcareous surficial geologies and soils, resulting in high-alkalinity lakes (Johnson et al. 1997, Raymond and Cole 2003, Bremigan et al. 2008). Such complexities add to the challenges in defining regional- and continental-scale reference conditions or nutrient criteria in water bodies (Herlihy et al. 2008, Soranno et al. 2011) and highlight the need for considering multi-scaled driver variables and spatial heterogeneity within and across regions for future research and applications.

Further complicating our ability to identify mechanisms at broad spatial scales is the fact that multi-scaled local and regional landscape variables can be highly correlated with each other. For example, in our study extent, regional-scale forest cover was highly negatively correlated with regional-scale row crop and pasture agriculture land use and highly positively correlated with patchy surficial geology ($-0.7 < r > 0.7$). Although we did not include highly correlated variables in any single model, interpretation and future use of our final models must recognize these correlations and their implications for identifying underlying mechanisms. Our results demonstrate the need for additional multi-scaled research with multiple response and driver variables across broad geographic extents that carefully considers multicolinearity to distinguish the individual effects of hydrogeomorphic and anthropogenic features on ecosystem state (McNally 2002, King et al. 2005).

Conclusions

Our study demonstrated (1) that a large amount of the ecologically relevant variation existing among ecosystems at the regional spatial scale can be captured by regionalization frameworks, (2) the importance of, and the difficulty in, understanding the separate roles of local and regional landscape drivers of variation in ecosystem state, and (3) that both hydrogeomorphic and anthropogenic features explain among-region variation in ecosystem state across a wide geographic area. We emphasize the regional spatial scale because, for many ecosystems (including lakes), local-scale drivers have been historically better studied, and our results demonstrate the importance of considering the regional spatial scale for broad-scale ecology research and applications.

Regionalization frameworks are almost always the first step in continental-scale research or management and conservation efforts that rely on extrapolation of local knowledge to broad spatial scales. Therefore, it is critical to have a well-developed understanding of the importance of the regional spatial scale and the landscape variables that drive patterns and processes at this scale (Hargrove and Hoffman 2005). Our approach can serve as a model for testing frameworks for any terrestrial or aquatic ecosystem response variable or practical application. Multilevel models offer an excellent quantitative framework to do so because they incorporate the inherently hierarchical nature of regionalization frameworks as spatial classifications of individual ecosystems nested within subregions, which are nested within regions, which are nested within a continent. In our example, we partitioned the variation into within- and among-region components to compare regionalization frameworks for quantifying regional variation in two important lake response variables and then used conditional multilevel models to model driver–response relationships at both local and regional spatial scales (i.e., within regions, as well as across regions). This approach is becoming more common for lakes (e.g., Fergus et al. 2011, Wagner et al. 2011, Sadro et al. 2012) and streams (Qian et al. 2010). However, the need for similar approaches and research applies to all ecosystem types and practical applications, particularly those efforts conducted at broad spatial scales (Peters et al. 2008).

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Supplemental Material

Appendix

Names and codes of the Ecological Drainage Units depicted in Figs. 3–5 (*Ecological Archives* A023-083-A1).