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Review

Disentangling Effects of Natural Factors and Human Disturbances on Aquatic Systems—Needs and Approaches

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Abstract: Disentangling the effects of natural factors and human disturbances on freshwater systems is essential for understanding the distributions and composition of biological communities and their relationship with physicochemical and biological factors. As the spatial extent of ecological investigations increases from local to global scales, efforts to account for the increasing influence of natural factors become more important. This article synthesizes the current knowledge and commonly used approaches for disentangling these effects on aquatic systems. New understanding has been facilitated by the availability of large-scale geospatial landscape databases that facilitate regional analyses and classifications in conjunction with novel approaches to identify reference conditions and statistical partitioning analyses. This synthesis begins with a summary of how natural factors and human disturbances interactively affect aquatic systems. It then provides an overview of why it is essential to separate the effects of natural factors and human disturbances and a description of examples of landscape databases that make the separation of natural and human factors feasible. It last synthesizes currently-used common approaches for separating the effects of natural factors from human disturbances. Our synthesis assembles representative approaches to disentangling human disturbances in one place to provide new insights that stimulate integrated uses of multiple approaches and the development of new approaches so that management actions can be taken to protect and restore aquatic ecosystem health.

Keywords: aquatic system; human disturbance; natural factor; bioassessment; landscape; disentangling

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1. Introduction

Identifying key natural and anthropogenic landscape factors and understanding and measuring how they influence freshwater systems is a central theme of research and management of rivers and lakes [1,2]. It has been well accepted that the characteristics of a river segment are determined by river system longitudinal settings from headwaters to river mouths and lateral settings of water boundaries from their riparian floodplains to catchment divides (Figure 1) [1,3]. Similarly, it has also been well documented that characteristics of lake systems are strongly influenced by lake system spatial settings, including lake tributary influence, the upstream-downstream influence of chain lakes, lake catchment influence through surface runoff and groundwater inputs (in addition to the history of lake formation, Figure 1) [4]. Catchment landscape characteristics such as climate, elevation, vegetative cover, land use, soil permeability, landscape slope, topography, and overall surficial geology control the deliveries of water, nutrients, minerals, and sediments into the systems [1,5]. Such landscape influences determine regimes of flow, sediment inputs, nutrient levels, and water temperatures at local scales [1,6]. Therefore, effective management and a thorough understanding of freshwater systems rely on in-depth knowledge and effective analytical approaches to quantify the structural and functional components of waterbody-landscape systems.

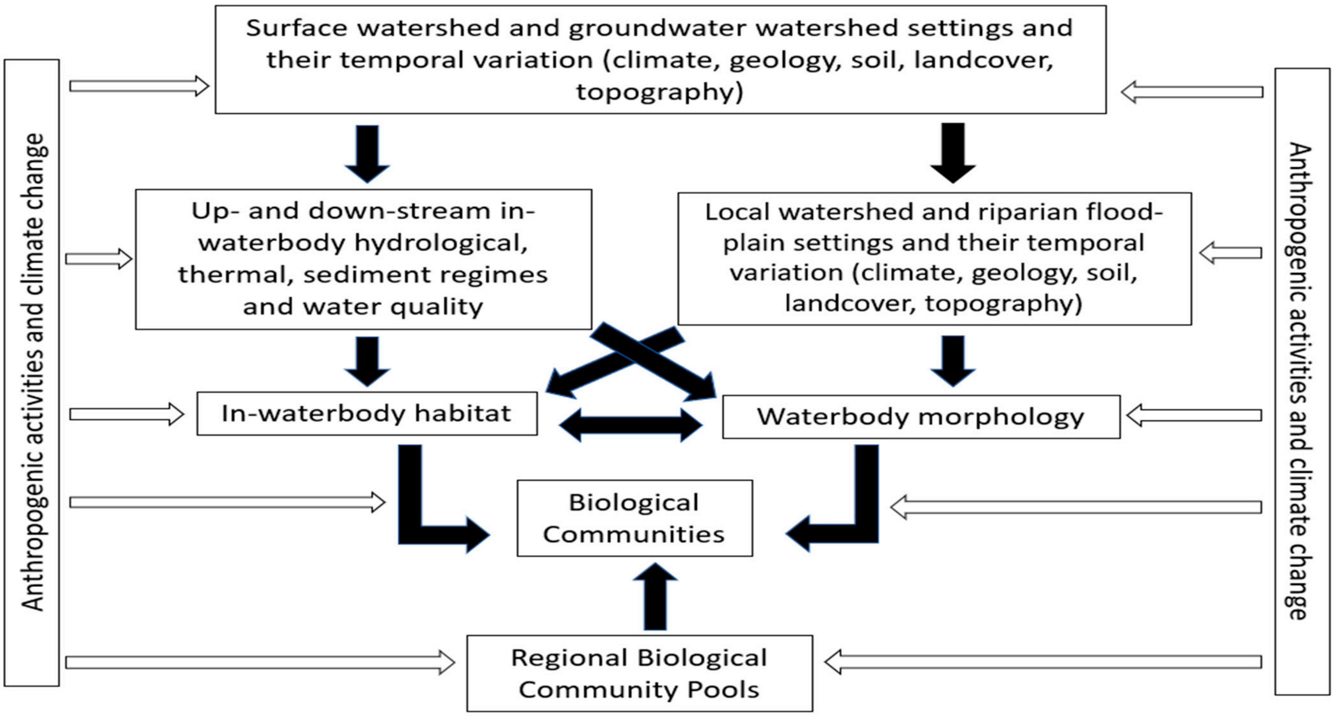

Figure 1. Conceptual linkages of landscape factors at multiple spatial scales and their influence on freshwater systems.

The abovementioned natural characteristics of waterbodies and their local and landscape settings have been increasingly modified by human activities since human civilization. Due to the enhanced advancement of modern technology, increased human population, and increased demand for materials and space per capita, the influences of human activities on the natural physicochemical and biological characteristics of waterbodies have been intensified at both local and landscape scales. Human-induced landscape modifications (e.g., urban development, agricultural practices, dams, and industrial and municipal waste generation) often bring excess amounts of nutrients, sediments, and toxicants into freshwater ecosystems; modify freshwater systems' physicochemical and morphological characteristics; alter thermal, sediment, and hydrologic regimes; and fragment waterway networks [1,7,8]. As a result, these landscape changes modify the characteristics of waterbodies established by the natural waterbody-landscape linkages and consequently degrade freshwater habitat and biological communities [1,9,10].

Although considerable progress has been made in linking landscapes and associated physicochemical and biological characteristics of freshwater systems, the development of conceptual frameworks and tools for quantifying such linkages at large spatial scales (e.g., state/provincial, regional, and national) is relatively recent and limited to a few countries [1,6,11]. Separating the influences of natural factors from human activities on waterbodies at landscape and local spatial scales is challenging. Research on this topic is still largely limited to small spatial scales and individual natural and human factors in the literature. Additionally, temporal variation in the characteristics of freshwater systems and modification of such characteristics by climate change complicates studying and understanding the influence of landscape factors at different spatial scales. Identifying and measuring key natural landscape and human factors and separating their influences on freshwater systems largely relies on the development of conceptual frameworks, greater availability of large spatial scale (regional or national) databases, development of geographic information systems, and advancement of database management and analytical technologies [1,3,6].

The overall goals of this article are to provide an overview of the need for disentangling natural influences and anthropogenic impacts on freshwater systems, to describe some spatial frameworks that have made such disentangling efforts feasible and effective, and to present examples to describe some of the approaches in disentangling effects of natural and human impacts at both local and landscape scales. This synthesis leads to several articles in this special issue to demonstrate specific approaches to disentangling the effects of natural and human impacts on freshwater systems. Although studies have published work individually elsewhere, this special issue assembles studies on the same topic in one place to aid in promoting research in the development, evaluation, and applications of conceptual frameworks and analytical approaches applicable for disentangling the effects of the natural environment and human disturbances on freshwater ecosystems.

2. The Need for Disentangling Natural Variation and Human Disturbance for Ecological Understanding, Biomonitoring, and Bioassessment

Ecological studies of individual organisms or biological communities require an understanding of relationships between organisms or communities and their natural environmental conditions. Such conditions are determined by different spatial scale landscapes and in-waterbody factors (e.g., river channel and floodplain form and lake morphology) defined by the history of earth and climate events at different temporal scales. For example, cold-water fishes only occur in cold climates and high-elevation regions or in other areas where streams and rivers are largely fed by groundwater, which often occurs in landscapes with coarse surficial geology [12]. Cold-water fishes also occur in deep lakes where deep-water layers can maintain suitable temperatures and dissolved oxygen for cold-water species, even if surface water has unsuitable temperatures [13]. Freshwater ecological studies require knowledge of what and how natural physicochemical and biological characteristics have determined the composition of biological communities, species presence or abundance, and the causes of a species abundance decline, a species extinction or invasion, or changes in community biodiversity. Such knowledge needs to be gained under natural conditions without human influences. However, human footprints and their influences on natural systems occur across the earth. Hence, disentangling natural and human effects is a critical step in ecological studies. We need to know how human activities have modified the natural waterbody-biota relationship so that the correct linkage can be established between organisms or biological communities and their natural environment for the development of ecological theory and modeling, reporting the health status of biological populations, assessing habitat loss or gain for specific populations, and protecting and restoring biodiversity and native communities.

Biomonitoring of freshwater system changes is an effective way of providing information essential to ecosystem protection, restoration, and regulation [14]. Many government organizations in the U.S. (e.g., U.S. Environmental Protection Agency, Ohio Environmental Protection Agency, and Minnesota Pollution Control Agency) and in European countries (e.g., European Commission, Joint Nature Conservation Committee in the United Kingdom) have obligated biomonitoring into their programs. Biomonitoring samples represent the composition, abundances, and health condition of species or communities and their associated environments (e.g., physicochemical habitat, waterbody morphology, surrounding land use and cover, and presence of other human disturbances) at selected locations and times. Adequate spatial and temporal representation of waterbodies' physicochemical and biological conditions and their associated human activities in the monitoring of waterbodies and their catchments are key components of biomonitoring.

Bioassessment uses biomonitoring-derived information to identify indicators, such as nutrients, toxicants, dissolved oxygen, presence or abundance of target species, community composition or abundance, or biological integrity indices to assess the health status of sampled waterbodies. Sometimes, the result of evaluations from sampled water-bodies can also be applied to unsampled waterbodies that have similar natural environmental settings and have readily available human activity measures to assess their health conditions because monitoring agencies never have enough resources and time to sample all waterbodies [3]. The assessment of a waterbody, river network, or ecosystem requires knowledge of the natural condition of a waterbody and which and how much human activities have altered a waterbody's natural condition at different spatial and temporal scales [1]. Without a thorough understanding of how the natural factors and human disturbances interactively determine a waterbody's condition will confound the outcomes of biomonitoring and bioassessment, leading to inadequate management practice and environmental regulation.

Bioassessment provides tools for regulatory policy-making and management operation. Freshwater resource management and regulatory agencies are responsible for protecting freshwater systems largely in their natural conditions, enhancing systems that are modified from their natural conditions, and restoring systems that are degraded by human activities. In order to do so, managers and policymakers need methods to identify which waterbodies are in natural, modified, or degraded conditions. Bioassessment, and consequent disentangling natural and human effects on freshwater ecosystems, can provide tools for separating the influences of natural factors from human disturbances on water quality and biological communities, hence identifying the degradation of human activities on the health of ecosystems and ecoservices they provide so that management actions can be taken.

3. The Need for Integrated Freshwater System Network Databases for Disentangling Natural Variation and Human Disturbances

Analyzing and interpreting linkages between freshwater systems and their associated landscape networks requires dissecting the continuous networks of rivers and lakes into meaningful spatial units ("waterbodies") for attributing data, linking landscape information, and carrying out analysis [3,15]. Traditionally, sampling sites where biota or physicochemical habitat data have been collected are used as such units, which are usually from less than 100 m to several hundred meters for stream or kilometers for river sampling. Such sampling sites are used as the lowest elevation points to delineate catchment boundaries and capture catchment information upstream of sampling sites [10]. This approach has been proven useful in linking in-waterbody measures and catchment information, but the delineation of catchment boundaries for each sampling location of a region or multiple regions is very time-consuming and costly. This approach does not provide catchments and their associated information for unsampled sites; hence, it does not allow extrapolating the results of sampled sites to unsampled areas [16]. Additionally, catchments created on a site-by-site basis usually do not produce readily comparable catchment boundaries across different projects or programs that usually have different sampling site locations for different sampling purposes.

Recent advances in geospatial frameworks in the U.S. and Europe have provided approaches in the development of datasets that provide the capability of linking waterbody spatial units with river catchments, lake subbasins, and political boundaries. For example, the spatial framework and datasets developed in the U.S. fill the gaps in linking basic spatial units of waterbodies with their catchments for the entire State of Michigan [17]) and for all rivers of the conterminous U.S. [3]. The most recent effort of high-resolution global hydrographic dataset that calculated upstream flow accumulation and flow direction to delineate a total of 1.6 million drainage basins, extracted a total of 726 million unique stream segments with their corresponding sub-catchments, and computed stream topographic variables is an attempt to provide such an approach at a global scale [18].

3.1. An Example of River System Network Database

The U.S. Hierarchical Spatial Framework and Database (USHFD) for the national river fish habitat condition assessment uses inter-confluence river reaches and their associated catchments as fundamental spatial river units and a series of ecological and political spatial descriptors as hierarchy structures for data attribution and analysis [14]. This geospatial dataset uses river networks and associated catchments of the National Hydrography Database Plus Version 1 (NHDPlusV1) to build the geospatial framework and database (this geospatial framework is replicated on the NHDPlusV2 described later). The USHFD is a 1:100,000-scale streamlined database that includes all streams, rivers, and impoundments captured at this resolution. Streamlines in the USHFD are divided into segments that are defined from the origin of a stream to the first downstream confluence, from this confluence to the next downstream confluence, and so on, then from a confluence to the upstream end of an impoundment or a lake, from the downstream end of an impoundment or a lake to a downstream confluence, and finally from a confluence to a pour point at the sea or a lake without an outlet [3]. These river segments or impoundments are the smallest spatial units in the USHFD.

Within the USHFD, the local catchment boundaries of a river segment (the land area where surface runoff flows directly into a river segment) are delineated based on the 30-m National Elevation Dataset (Figure 2). The network catchment of each river segment (the entire catchment area upstream of the downstream end of the segment) is defined by merging all upstream segment local catchments (Figure 2). This database includes all streams and rivers captured at the 1:100,000-scale resolution for the entire conterminous U.S.

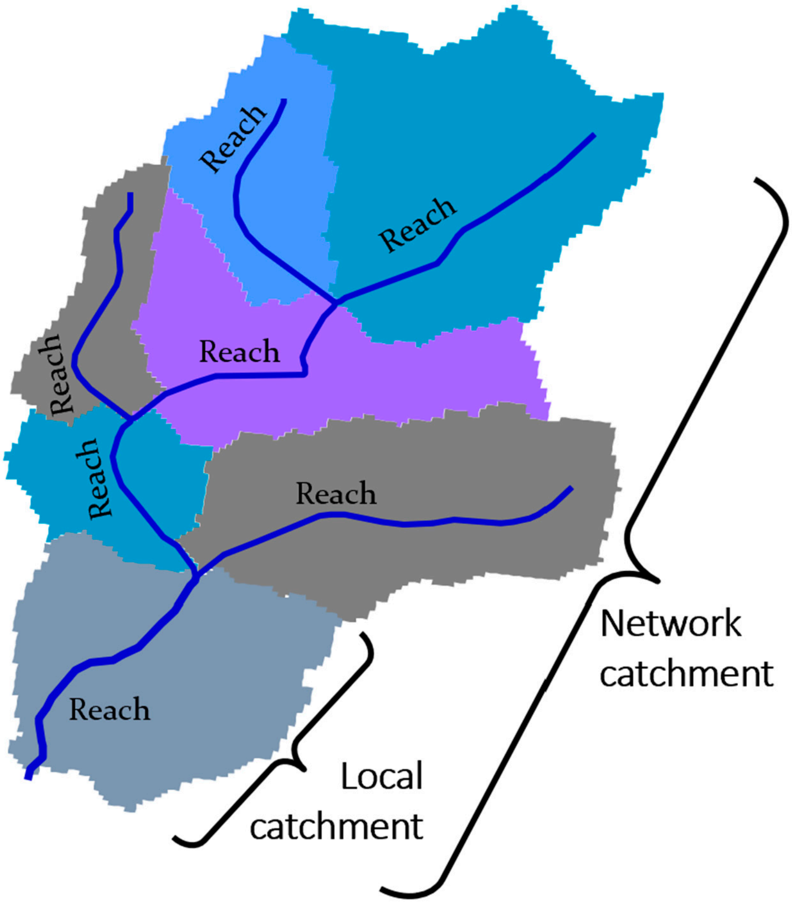

Figure 2. Stream reaches and the local and network catchments of the reaches.

For assessing natural characteristics and human disturbances, data on natural and human-disturbance variables are attributed to each spatial unit (i.e., river segment) of the USHFD database. The variables describing values of natural variation in climate, elevation, geology, soil, land cover, groundwater contribution, and river connectivity represent surrogates of river segment-scale natural variation in physicochemical and biological characteristics. Some of the natural variation descriptors, such as network catchment size, Strahler order, segment length, mean elevation, and gradient, are calculated or obtained from the NHDPlusV1 database. The other natural descriptors, such as mean annual air temperature and precipitation, soil permeability, types of surficial geology, and groundwater contribution, are calculated based on readily available databases using GIS. The human disturbance variables, such as land use, population density, area of roads, nutrient enrichment, agricultural pollutants, dams, and point source pollution in river channels, riparian and floodplain, and catchments, are also calculated from readily available databases using GIS. The natural and human activity descriptors in the local catchment are attributed to each river segment first, and then these descriptors for network catchment are aggregated by summarizing values of each variable of all upstream segments' local catchments [3,19,20].

3.2. An Example of Lake System Network Database

The catchment boundaries of lakes (or impoundments) are not consistently defined nationally in the current version of USHFD. However, lake catchment boundaries have been consistently delineated based on the 30-m National Elevation Dataset for all lakes that are 2 ha or larger for the State of Michigan in the U.S. [5,21]. Similar to the river catchment delineation described above, the local catchment of a lake is defined as land areas where surface runoff flows directly into the lake, and the tributary catchment of a lake is defined as the network catchments of all tributaries that flow into the lake (Figure 3). The local and tributary catchments of lakes are also generated based on the 1:100,000-scale NHDPlusV1 dataset. The descriptors of lake morphology and the other physicochemical, biological, and catchment natural and human activity measures described for river segments are also attributed to each lake.

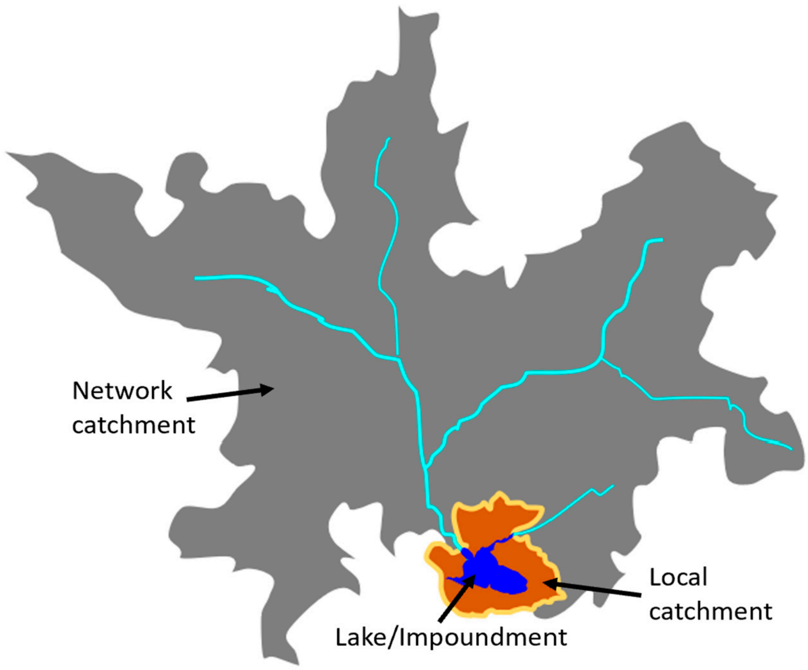

Figure 3. Lake or impoundment and its associated local and tributary catchments.

3.3. More Recent Development of Freshwater System Network Databases

Other large spatial databases with hydrological network delineation and landscape data attribution capability similar to the above-described examples of river and lake databases have also been developed. At a national scale, for example, the NHDPlusV2 developed using the National Hydrography Dataset medium resolution data at 1:100,000 scale (https://nhdplus.com/NHDPlus/; accessed on 24 March 2023) and the NHDPlusHR developed using the National Hydrography Dataset High Resolution data at 1:24,000 scale (https://www.usgs.gov/national-hydrography/nhdplus-high-resolution; accessed on 24 March 2023) by the U.S. Environmental Protection Agency (USEPA) with the assistance of U.S. Geological Survey are the significant improvement of national hydrological river network databases [22]. These databases have identified river segments, defined local and network catchment boundaries, and attributed estimated flow volume and velocity, precipitation, temperature, and land cover for each river segment. These databases allow users to calculate and attribute additional natural (e.g., geology, soil, slopes of catchment and river segment, and more) and human impact factors (e.g., in-river network barriers, habitat modification, water deviation, and farming animal density, fertilizer, and herbicide application, impervious area, road density, and others). These databases enable and improve our capability to disentangle impacts of natural and human factors.

At a continental scale, the pan-European River and Catchment Database is a hierarchically structured and fully integrated database of rivers and catchments for hydrological analysis and is fully linked to the catchment at each level of the hierarchy [23,24]. Series data such as statistics on terrain and climate parameters, river stretch descriptor, and river gradient are attributed. This database covers the entire pan-European continent from the Atlantic to the Urals and from the Mediterranean to northern Scandinavia, which is complementary to the national and regional datasets that cover only limited areas with greater detail [23].

On a global scale, the high-resolution global hydrographic dataset includes 1.6 million drainage basins and 726 million unique stream segments with their corresponding sub-catchments. This dataset includes an upstream contributing area as small as 5 ha that allows the extraction of headwater stream channels in great detail [18]. This database contains stream segments and their associated catchments and sub-catchments, flow accumulation, and topographic and topological variables such as stream slope, reach length, and stream order. Although this database contains only natural descriptors, many human activity measurement databases are available [25,26] and could be incorporated into this database for the analysis of disentangling effects of natural and human activities.

4. Approaches for Disentangling Natural Variation and Human Disturbance

Due to the importance of disentangling the effects of natural factors from human disturbances, several approaches have been developed to separate natural variation in freshwater system physicochemical and biological characteristics from anthropogenic degradation. Because freshwater systems are largely separated by drainage divides, catchments are commonly used as ecological boundaries. Freshwater system management and environmental protection policy are often implemented by different jurisdictions, and hence, political boundaries are often incorporated into the analysis process. For studies over large spatial scales (e.g., a state, province, region, nation, continent) with broad ranges in natural variations, an integration of regionalization, classification, and statistical techniques have often been used. For studies of smaller spatial scales with relatively narrower ranges of natural variations (e.g., one specific type of stream or river, a catchment, or a subbasin), a single approach (e.g., statistical technique, reference condition) is often used.

4.1. Regionalization

Regionalization has been used for dividing a large region with a broad natural variation into smaller regions with similar natural variations. Several regionalization approaches have been developed in the North America. The most widely used regionalization is the USEPA's Level-III ecoregions that were developed based on land surface form, land use, natural vegetation, and soil types for the conterminous U.S. [27]. These Level-III ecoregions were further refined to Level-IV ecoregions more recently based on geology, landforms, soils, vegetation, climate, land use, wildlife, and hydrology and were expanded to include entire North America [28]. The ecoregions were developed as a spatial framework for research, assessment, and monitoring of freshwater ecosystems. The ecoregions represent areas of similarity in biotic, abiotic, terrestrial, and freshwater ecosystem components, with humans being considered part of the biota [28]. There are 12 Level-I, 25 Level-2, 105 Level-III, and 967 Level-IV nested ecoregions in the continental U.S. [28]. These two versions of ecoregions have been broadly used for biomonitoring design and bioassessment that requires separating the influences of natural variation from human activities on the health of freshwater systems [29,30].

In Europe, the Water Framework Directive endorsed the use of an ecoregional map adapted from Illies' [31] zoogeographical delineations to standardize surface water typology for ecological assessment and reporting for all European inland waters. This ecoregion map delineates Europe into 25 regions with coarsely defined boundaries based on a combination of distribution limits and prevailing endemism of freshwater organisms [32]. In practical uses, some European countries, such as Austria, have refined the boundaries more precisely using geological and ecological features similar to the delineation process used for the U.S. ecoregion delineation [27] for reference condition identification and reference-based bioassessments [33].

Several other regionalization efforts that have been used less frequently for separating the influences of natural variation from human activities on the health of freshwater systems are biological community or biodiversity-focused regionalization. For example, the freshwater zoogeographic regions of North America were developed by recognizing the variation in the form and function (species similarity) of biological communities [34,35]. These zoogeographic regions consist of hierarchical subzones, regions, and subregions. Zoogeographic regions also incorporate the effects of climate and physiography on the hydrologic processes that create patterns in freshwater systems [34]. Another example is the freshwater ecoregions of the world that developed maps of biogeographic units for freshwater biodiversity conservation [36]. These ecoregion maps were created using the best available information on freshwater biogeography by mainly analyzing information describing freshwater fish species distribution. Although these zoogeographic regionalization approaches have been designed for regional conservation planning and resource management, they have been adapted for freshwater system bioassessment [30].

4.2. Ecological Classification

Classification is useful for understanding the freshwater system components' composition, structure, and process; hence, it is an effective tool for studying and managing rivers and lakes. A classification will minimize natural variation within a class and maximize natural variation among classes in physicochemical and biological measures. In a classification process, natural variation should be distinguished from human alterations at different spatial scales. In the environmental assessment of waterbodies for degradation rehabilitation, health status assessment, and regulation enforcement, scientists and managers often classify waterbodies into types. Waterbodies of each type have similar ecological expectations.

Longitudinal zonation and location-independent approaches are the most common approaches for freshwater system classifications [37]. Longitudinal zonation accounts for changes in freshwater systems' physicochemical and biotic conditions from headwaters to river mouths [38], while location-independent approaches split freshwater systems into discontinued groups based on their differences in physicochemical and biotic factors [39]. Spatial hierarchical classifications are also described as a type of freshwater system classification, which is similar to the aforementioned regionalization since they group freshwater systems into spatially contiguous regions at a variety of spatial scales based on the variation in natural variables. All three classification approaches are based on natural variations in physicochemical and biological characteristics; hence, the three classification approaches are often integrated at a variety of spatial scales [40]. Although these stream classifications and underlying data are intended to provide tools for freshwater system research and management, they can be valuable resources for separating the impact of natural and human activities on freshwater ecosystems.

Classification has been used worldwide for disentangling natural and human effects. At a state scale, the U.S. Clean Water Act (CWA) Section 305(b) requires states to assess the overall quality of waters in their jurisdiction, identify impaired waters, and take management actions to restore such waters. Under Section 303(d) of the CWA, states develop lists of all impaired waterbodies and prioritize these waters for the establishment of total maximum daily load allocations designed to restore degraded areas. When determining which waterbodies are impaired, states need to establish waterbodies' designated use because different types of water have different natural physicochemical and biological characteristics. For example, several U.S. Midwestern States (Michigan, Minnesota, and Wisconsin) have classified streams into different thermal types of cold-, cool-, and warm-water streams for establishing designated uses [41]. These states' monitoring and bioassessment programs also stratify streams based on a combination of stream size and thermal classes for the purposes of data representation and data extrapolation [39]. Such a classification scheme should be based only on stream natural thermal regimes. Because human impacts, such as urban land use, can turn a cold-water stream into a cool- or warm-water stream, discriminating human impacts from natural stream conditions is a critical step for accurate ecological classification.

At a national scale, for example, the USEPA's Environmental Monitoring and Assessment Program stratified streams based on ecoregion and stream order for its random site selection design [42] with the purpose of minimizing natural variation in physicochemical and biological measures so that human disturbances can be assessed. In combination with ecoregionalization and stream order classification with state jurisdiction boundaries, the USEPA's National Rivers and Streams Assessment program is able to evaluate the condition of rivers and streams across the nation and identify leading problems that impact rivers and streams [43]. This assessment reported 46% of the nation's rivers and streams are in poor biological condition, 25% in fair condition, and 28% in good condition based on benthic macroinvertebrate sampling [43].

At a continental scale, for example, the Europe's Water Framework Directive requires EU Member States to develop typologies (classes) for lakes and rivers based on natural environmental variables [44]. These typological classification variables need to be natural characteristics and not respond to human activities (e.g., altitude, size, basin geology), and represent the minimally disturbed abiotic conditions that are most important to determine the natural variation of the biological components and to support abiotic components (e.g., nutrients, transparency, oxygen, sediment, flow, waterbody morphology) [44,45]. The Europe's Water Framework Directive uses abiotic factors to typologically classify streams and rivers into types. For example, the EU Water Framework Directive "System A" typology is defined by ecoregions, catchment areas, catchment geology, and altitude [44,46]. Such a typology classification assumes biological communities in undisturbed sites within a typology are similar, and hence it stratifies natural spatial variability for freshwater system monitoring and assessment. The Europe's Water Framework Directive's typological classification of streams and rivers has been tested using diatoms, macrophytes, invertebrates, fish, and hydromorphological features and has proven useful [47]. Additionally, the Water Framework Directive also requires its Member States to assess and report on the ecological (health) status using biological measures (e.g., phytoplankton, macrophytes, phytobenthos, benthic invertebrate, and fish fauna) for each waterbody type defined by the abiotic factors [44].

4.3. Statistical Variance Partitioning and Probability Estimate Approaches

To understand which and how much each of the natural and human disturbance factors contribute to the determination of freshwater system health status, variances partitioning approaches have often been used. Although many approaches have been developed and used, multivariate analysis, structural equation modeling, and multiple regression analysis are commonly used for disseminating natural and human influences.

Canonical correspondence analysis (CCA) and redundancy analysis (RDA) are the most commonly used multivariate analyses for such a purpose [48]. The advantage of this approach is that it can partition out the variances of a set of response variables (e.g., fish assemblage measures) explained by a set of independent variables (e.g., environmental measures). It generates variance explained by each set of the measured independent variables and the common contribution of two or more sets of the independent variables jointly. The difference between these two analyses is that RDA assumes linear relations between multiple dependent variables and multiple independent variables, while CCA is more suitable for the relationship between multiple dependent and multiple independent variables that is unimodal [49,50]. Multivariate analyses have been broadly used to calculate the percentages of freshwater indicator variances explained by environmental factors at different spatial scales [9,51,52]. More details of this approach are provided by Cao and Wang [53].

Structural equation modeling is another approach used more broadly in recent years for environmental variance partition [54]. It is a causal modeling approach for examining the strengths of correlations within a predefined relational model. It allows users to apply accumulated knowledge and theoretical concepts to build the model empirically. It is a statistical technique based upon a generalized linear equation system used to examine causal relationships that each independent variable has direct and indirect effects via a mediating variable on the dependent variable. From the strength of the direct and indirect correlations between dependent and independent variables, one can interpret how strongly natural factors or human disturbances impact the dependent variables (e.g., physicochemical or biological measures). The strength of structural equation modeling is that it estimates the multiple and interrelated dependence in a single analysis and allows the construction of the model empirically. The latter is also a weakness because if the model is not constructed correctly, it may result in weak or misleading relationships. Structural equation modeling has been used to partition out the percentages of freshwater indicator variances explained by environmental factors [55–57].

Multiple regression analysis has long been used as a statistical tool for predicting a dependent variable from multiple independent variables and has been used for interpreting how much independent variables individually and jointly contribute to the explanation of the variation of a dependent variable. For freshwater system analysis, multicollinearity often occurs, which makes the interpretation of regression results complex and can contribute to misinterpretation of results. In order to overcome this problem; commonality analysis has been recommended alongside regression analysis [58]. Commonality analysis is an approach to partition the explained variance in a multiple regression analysis into variances explained by each independent variable uniquely and jointly by two or more independent variables [59]. Unlike stepwise regression, commonality analysis is not affected by the order in which the predictors are entered into the model [58]. One of the weaknesses of commonality analysis is that it can become complex as the number of independent variables increases, but the use of computer software has enabled such an analysis of more independent variables efficiently. Similar to commonality analysis, hierarchical partitioning also allows the partition of the contribution of each predictor to the total explained variance in a regression model. Both independently and in conjunction with the other predictors, hierarchical partitioning allows the calculation of all possible candidate regression models. Hierarchical partitioning also has been recommended for estimating the relative importance of predictors in CCA and RDA [60].

Many other statistical approaches have also been used to separate the natural variation and human disturbances of freshwater systems. For example, the propensity score approach, commonality analysis, sums of Akaike information criterion weight, random forest, and boosted regression tree, and other approaches have been applied to separate the natural variation from human impacts. These approaches are relatively new, promising but less used, or commonly used but controversial, which have been discussed in detail by Cao and Wang [53].

4.4. Reference Approach

Reference conditions of freshwater systems have been broadly used for environmental bioassessment [14]. Reference conditions are often established in conjunction with regionalization or classification, or both (described above), which have been proven effective for bioassessment [61]. Reference condition has been used as the standard or benchmark for comparing the condition of a to-be-assessed waterbody for evaluating the direct or indirect effects of human disturbances.

Due to the variation in the purpose of use, geographic region, and assessment target, different reference conditions have been developed and used. Some examples are historical conditions, least disturbed conditions, best attainable conditions, and expected conditions. The historical condition describes the conditions of waterbodies at some point in their history before the start of human disturbances (e.g., pre-settlement, pre-industrial, or pre-intensive agriculture). The least disturbed condition describes the best available physicochemical and biological conditions at a given state of the landscape today. The best attainable condition describes the condition that can be obtained if the best possible management practices have been implemented. Specific reference conditions are often defined within a specific region or waterbody type (class). Because of the variation in natural conditions within a region or class, reference condition describes a range of values, instead of a single value, that measure waterbody health conditions using physicochemical and/or biological indicators.

Reference conditions can be identified by assessing the natural variability of indicator measures at waterbodies where human disturbances are minimal or absent. Reference conditions can also be identified by analyzing historical data before human disturbance occurs in regions where such data are available. In areas where neither historical data nor undisturbed waterbodies exist, reference conditions can also be identified using the best professional judgment by experienced freshwater professionals who have expertise in evaluating and managing freshwater systems' physicochemical and biological conditions. For areas where there are no historical data or all waterbodies have been degraded at some levels, the best professional judgment can also be used by borrowing reference conditions from their neighbor regions with similar natural conditions (e.g., climate, geology, soil, vegetation, topography).

4.5. Other Approaches

Disentangling effects of natural and human disturbance in bioassessments is based on the principle of comparing waterbodies' current condition to the natural condition in the absence of human disturbance or alterations (i.e., comparison to a pristine, unpolluted, or anthropogenically undisturbed state). However, as part of natural-human ecosystems, humans and their activities will never go away, nor will their impacts on freshwater systems. For example, freshwater systems in a region that has been heavily modified by human activities require an approach to identify the best remaining conditions. Several approaches have been developed to address this issue, such as the frequency of distribution ecological conditions [43], biological condition gradient [62], and landscape-based identification of human disturbance gradients [17,63].

The frequency of distribution ecological conditions of all sampled sites approach is to characterize the central tendency and dispersion in measured ecological attributes [43]. This approach delineates a frequency distribution that is either the maximum or minimum ecological condition for a specific percentage of sampled sites (e.g., 75th percentile of all sites representing 25% of sites condition in the region). This approach rates all sampled sites of a region (e.g., a state) that represent the entire range of natural and human disturbance conditions to discrete health groups based on the frequency of distribution.

The biological condition gradient descriptive model approach [62] is based on a quantitative understanding of the relationship between human disturbances and ecological measures. This disturbance-response relationship is then used for identifying sampled sites that are minimal, moderate, and severely impacted by human activities. This disturbance-response model represents the best waterbody conditions found at a region at one end of a biological-condition gradient and the worst condition at the other end of the gradient. The model divides this gradient of biological condition into six tiers ranging from tier 1 in a natural condition to tier 6, severely altered biological structure and function communities. Since the model does not incorporate natural variation factors, it assumes that the influences of the natural variation of target waterbodies on biological measures are minimal. Hence, a disturbance-response model needs to be developed in conjunction with regionalization and/or classification. This model has been used in the context of implementing the U.S. Clean Water Act by U.S. states to designate freshwater life uses and the development of biocriteria [64–66].

The landscape-based identification of human disturbance gradients [17,63] is based on the process of assessing disturbance gradients using readily available, geo-referenced stream and human disturbance databases for large regions (e.g., the State of Michigan, Mississippi River basin, the conterminous U.S.). The process involves three steps, the first of which is attributing readily available human disturbance data (e.g., urban and agricultural land uses, imperviousness, population density, road density, farming animal density, manure farming application, fertilizer and herbicides applications, and others) to the local and network catchments or buffers and then to each stream reach as described in Section 3.1. The second step requires identifying the weight of each disturbance factor based on the relationship between multiple fish measures (e.g., species occurrence, biotic integrity, % intolerant individuals) and human disturbance factors from local and network for sampled stream reaches using multiple regression or other variance partitioning statistical techniques. This step can also involve combining (using multivariate analysis) or removing (using correlation analysis) highly correlated disturbance factors. The last step involves calculating the accumulative impacts of the selected or combined key disturbance factors on each stream reach. This process involves calculating the product of the weight and amount of each disturbance factor for local and network separately, calculating the ratio (weight) of disturbance contribution of local and network using disturbance-biota relation variance partition, and summarizing all the local-network disturbance weighted products for all stream reaches. Because the influences of disturbances from local and network are similar for smaller streams and the influence of the network is greater than that of the local catchment for large rivers [63], all disturbance calculation steps are carried out separately for different sizes of streams or rivers (e.g., headwater streams (catchment area [A] $\leq$ 10 km^2), creeks (10 < [A] $\leq$ 100 km^2), small rivers (100 < [A] $\leq$ 1000 km^2), medium rivers (1000 < [A] $\leq$ 10,000 km^2), large rivers (10,000 < [A] $\leq$ 25,000 km^2), and great rivers ([A] > 25,000 km^2) [63]. The advantage of this approach is to use fish or other biota from limited sampling sites to assess disturbance for all sampled and unsampled stream reaches in a region. Because the overall disturbance index was a summation of multiple disturbance factors, this approach can also determine which disturbance factors are primarily responsible for any specific stream reach that is in poor health. The weakness of this approach is it requires the availability of key disturbance factor data with reasonable accuracy. In reality, some of the disturbance factors, such as farming animal density, manure application, and fertilizer and herbicide applications in local and network catchments, are from a very coarse level, which reduces the accuracy of the assessment.

5. Summary

It is critically important to disentangle the effects of natural factors and human disturbances for the understanding of biological community distribution and composition and their relationship with physicochemical and other biological factors. This synthesis presents the need to disentangle the effects of natural and human disturbance factors, the requirement of large-scale geospatial landscape databases for meeting the disentangling need, and the approaches used to separate the effects of natural factors from human disturbances.

Several large-scale geospatial databases for separating natural variation from human degradation at a regional, national, and global scale have been developed. This development provides the framework and technical tools how to develop similar databases elsewhere. These large geospatial databases make it possible to conduct regionalization and classification that are essential for separating the effects of natural factors and human disturbances. The multivariate analysis and structural equation modeling approaches allow the use of multiple dependent variables since the health of freshwater can rarely be measured by a single indicator. The multiple regression analyses use a single dependent variable, such as an integrated health measure index (e.g., the biological integrity index). These statistical approaches are for identifying the contribution of human disturbance to the total variance of the health indicators of freshwater systems. Reference condition is the standard or benchmark for comparing the condition of a to-be-assessed waterbody for evaluating the effects of human disturbances, and reference identification and designation are critical for bioassessment and for establishing the target of restoration. The frequency distribution ecological condition and biological condition gradient provide methods to rate all sampled sites in a region without the designation of references. The landscape-based identification of human disturbance gradients allows for the assessment health status of all the sampled and unsampled sites in a region.

Our synthesis provides an overview of why it is essential to disentangle the effects of natural and human impacts on freshwater systems and a description of some geospatial frameworks that make such disentangling efforts feasible at both local and landscape scales. Our synthesis assembles examples of approaches to disentangling human disturbances of freshwater systems in one place in the hope of stimulating research in the development and evaluation of conceptual frameworks and statistical methods and their applications to disentangling effects of the natural environment and human disturbances on freshwater ecosystems so that management actions can be taken to protect and restore high conservation and economically valued biological populations, reduce or prevent species decline and distinction, and maintain and enhance freshwater ecosystem health.

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