

SPATIAL PATTERNS IN BENTHIC BIODIVERSITY OF CHESAPEAKE BAY, USA (1984–1999): ASSOCIATION WITH WATER QUALITY AND SEDIMENT TOXICITY

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Abstract—Non–point-source pollution is an increasing source of stress to aquatic, estuarine, and marine ecosystems. Such pollution may be of unknown etiology, distributed over extensive spatial scales, and comprised of multiple stressors. Current stressor-based paradigms for ecological risk assessment (ERA) may be insufficient to characterize risk from multiple stressors at regional spatial scales, necessitating the use of effects-based approaches. Historical data (1984–1999) for benthic macroinvertebrate biodiversity in Chesapeake Bay, USA, were incorporated into a geographic information system (GIS) and spatial analysis tools were used to model zones within the bay predicted to be of low or high anthropogenic impact. Data for benthic water quality and sediment toxicant concentrations from each of these zones were subsequently analyzed and compared to identify associations between benthic biodiversity and potential stressors. A number of stressors were significantly associated with high-impact zones, including increased nitrogen and phosphorus concentrations, low dissolved oxygen, heavy metals, pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls. The spatial autocorrelation among multiple stressors. Considering the effects of individual stressors rather than net effects of multiple stressors may result in underestimation of risk. The GISs are a useful tool for integrating multiple data sets in support of comprehensive regional ERA.

Keywords—Ecological risk assessment Geographic information systems Water quality Toxicity

INTRODUCTION

During the past decade, the use of risk-based decisionmaking in environmental management has increased significantly, as evidenced by the proliferation of the ecological risk assessment (ERA) paradigm as the standard method for characterizing ecological impacts associated with anthropogenic activities [1]. This paradigm largely centers on identifying potential ecological stressors, characterizing their toxicity through laboratory methods, and subsequently inferring effect concentrations and ecological consequences. As such, ERA currently focuses on characterizing the stressor while effects are estimated through various forms of data manipulation. This approach to risk assessment is valid when information regarding the potential ecological consequences of contaminant exposure is required before their release, such as in the development of novel chemical compounds or the siting of an industrial facility that will generate potentially toxic effluent. However, future challenges to environmental quality will likely be quite different from those that have historically been of concern. As water quality criteria become more rigorous and pollution-prevention controls more effective, acute pointsource pollution will have a decreasing influence on environmental quality. The future challenge to risk assessors and managers will be the cumulative effects of chronic exposure to multiple stressors of unknown etiology distributed over variable temporal and spatial scales [2]. To meet this challenge, development of effects-based approaches to ecological risk assessment will be necessary [3], whereby observed changes in ecosystem structure and function are used to monitor ecological stress and identify stressors.

A potential barrier to effects-based risk assessment is the

availability of sufficient data to characterize ecological effects. Natural populations are frequently associated with significant temporal and spatial variability [4–7], necessitating robust data sets to differentiate natural variability from anthropogenic impacts. If such data are to be used in hazard identification, then extensive data collection regarding the distribution and magnitude of a broad range of potential stressors must be performed as well. Although such data dependence frequently may be perceived as a prohibitive obstacle to effects-based ERA, such data sets are readily available for many regions of the United States. For example, state environmental agencies conduct routine biological monitoring, water quality assessment and toxicity screening in pursuance of the requirements of the Clean Water Act. Temporally or spatially extensive environmental monitoring projects also have been sponsored by federal agencies, such as the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program and the U.S. Geological Survey's National Water Quality Assessment Program [8,9]. However, historically few concerted attempts have been made to coordinate monitoring programs, even within the same region, and significant variation may exist with respect to spatial resolution and monitoring frequency among data sets [9]. Thus, a more difficult challenge is developing methods for integrating multiple large data sets into a construct amenable to ERA so that such valuable data resources can be used effectively in environmental management [9].

Benthos

Geographic information systems (GISs) are robust tools for managing data associated with natural landscapes, and their use in the analysis of environmental monitoring data may enhance the ERA process. The automated functions of commercial GISs allow rapid quantification of distance, area, and gradient, and more complex operations can be executed to

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construct single or multivariate spatial models [10,11]. The GISs also enable one to link spatially and temporally heterogeneous data sets for the purpose of quantitative statistical analyses. The GISs have been used increasingly in watershed analysis to understand interactions between land use and surface water hydrodynamics and quality [12–17]. However, little attempt has been made to use GISs for assessing stressorresponse phenomena within the water column [18]. With more emphasis placed on non–point-source stressors, the spatial scales over which ERAs are conducted will likely expand, making the utilization of tools such as the GISs more critical.

The objective of the current study was to perform a regional effects-based hazard identification of potential stressors to benthic communities of the Chesapeake Bay, USA. Historical data on the biodiversity of benthic macroinvertebrates in Chesapeake Bay was incorporated into a GIS and used to model zones of high and low ecological impact over the time period of 1984 through 1999. The GIS functions were then used to link these regions to data sets for benthic water quality and sediment toxicant concentrations over a similar time period. Data for 17 water quality parameters and 68 toxicants were compared between high- and low-impact zones to identify potential stressors or stressor interactions associated with observed ecological effects.

MATERIALS AND METHODS

Software

Data management and selection were conducted using Microsoft Excel® 97 (Redmond, WA, USA), Statview 4.0 (SAS Institute, Cary, NC, USA), and the database features of ArcView[®] GIS 3.2 (Environmental Systems Research Institute, Redlands, CA, USA). Mapping of all geographic and environmental monitoring data was performed using ArcView. Point-pattern analyses were performed using CrimeStat[®] 1.1 (Ned Levine and Associates, Annandale, VA, USA). Effect modeling and surface interpolation of benthic biodiversity were performed using the Spatial Analyst 2.0 extension of ArcView. Statistical operations were performed using Stat-View.

Data sources

Digital geographic data for surface features were obtained from several sources. Land and water features were obtained as ArcView shapefiles from the U.S. Environmental Protection Agency's Better Assessment Science Integrating Point and Non-point Sources (BASINS) geographic information system (available over the Internet at http://www.epa.gov/OST/ BASINS). These features were based upon digitized images of U.S. Geological Survey base maps (1:250,000 scale). These features were used to construct a polygon theme representing the primary basin of the Chesapeake Bay and the lower regions of major tributaries comprising an area of 11,170 km² (Fig. 1). All subsequent analyses were restricted to this study area. State boundaries were also obtained as ArcView shapefiles from the U.S. Environmental Protection Agency's BASINS system, based upon U.S. Geological Survey digital line graphs (1:2,000,000 scale). Data for the general location of major cities in close proximity to the Chesapeake Basin were obtained as ArcView shapefiles from Environmental Systems Research Institute.

Shannon's biodiversity index for the benthic macroinvertebrate community was used as an effect indicator. Shannon's



Fig. 1. Distribution of benthic macroinvertebrate biodiversity monitoring stations (n = 1,054) and calibration points for effect modeling (n = 40) within Chesapeake Bay, USA (1984–1999).

biodiversity index describes the equitability with which individuals in a community are allocated among species and was calculated with Equation 1 [19]

$$SBI = \sum p_i \ln p_i \tag{1}$$

where SBI is Shannon's biodiversity index and p_i is the percentage of all individuals in the *i*th species. Shannon's biodiversity index is a useful metric in regional biological assessments where spatial heterogeneity in the distribution of specific species may be significant [20]. Biodiversity data for summer months (May-September) between 1984 and 1999, inclusive, were obtained from the Chesapeake Bay Program's (CBP's) data clearinghouse (available over the Internet at http://www.chesapeakebay.net). Data were originally derived from benthic community samples collected from 1,054 fixed and random monitoring stations located throughout the basin, resulting in a total of 2,596 individual observations (Fig. 1). The locations of monitoring stations in decimal degrees were obtained by loran-C (accurate to \pm 500 m) with the geographic coordinate system North American Datum 1927 between 1984 and 1996, after which locations were based upon global positioning system receivers using the North American Datum 1983. Samples were obtained by the use of a box-coring device that collected from a benthic surface area of 0.04 m². Counts of individuals were subsequently normalized to the square meter. Three replicate samples were taken per monitoring event. Samples were then transferred to a 0.5-mm sieve bucket to recover benthic organisms, after which samples were fixed with a 10% formalin solution. Benthic specimens were returned to the laboratory and sorted using a dissecting micro-



Fig. 2. Distribution of water quality monitoring stations (n = 112) and sediment toxicant monitoring stations (n = 353) within Chesapeake Bay, USA (1984–1999).

scope. All specimens were then identified to the lowest practical taxonomic level. Identification was aided by stereoscopic zoom dissecting microscopes, fiber-optic illuminators, magnifying lamps, and a phase-contrast compound microscope. Five percent of all samples were reworked independently for quality control of taxonomic identification, enumeration, and biomass estimation. All values for Shannon's biodiversity index available for the study area over the time period specified were utilized.

Sixteen water quality parameters were analyzed including physical and chemical conditions such as temperature, dissolved oxygen, pH, and total suspended solids as well as nutrient concentrations. Water quality data for summer months between 1984 and 1999 also were obtained from the CBP. Data were originally collected by monthly sampling from the basin floor at 112 fixed monitoring stations located throughout the basin (Fig. 2). Geographic locations in decimal degrees for monitoring stations were obtained in a similar fashion as described above. At each station, a hydrographic profile was made (every 1-2 m) and water samples for chemical analysis were collected from the surface and the bottom layers via a pumping system. Quality assurance and control was maintained within laboratories through replication of field samples and replication of laboratory analysis. Among-laboratory calibration and validation also were performed routinely to ensure comparability of results among cooperating institutions within the region. Specific methods used in the analysis of individual water quality parameters have been previously reported by the CBP [21]. The total number of observations for each parameter ranged from approximately 200 to 2,000 depending on how frequently data for individual variables were collected. All data available for the 16 parameters included in the current study over the specified time period were utilized.

Sediment concentrations of 68 toxicants were analyzed, including total concentrations for 17 metals, 14 pesticides, 19 polycyclic aromatic hydrocarbons (PAHs), and 17 polychlorinated biphenyl (PCB) congeners. Sediment toxicant data for summer months between 1984 and 1998 were obtained from the CBP. Data originally were collected by yearly sediment sampling at 353 fixed monitoring stations located around the perimeter of the basin and in most of its major tributaries (Fig. 2). Geographic locations in decimal degrees for monitoring stations were obtained in a similar fashion as described above. Because one of the goals of the current study was to compare sediment toxicant concentrations among regions of the bay associated with low or high anthropogenic impacts (based upon biodiversity modeling: see below), at least 10 samples must have been collected from each predicted impact zone (i.e., high and low) to be included in the current study. The total number of observations for each toxicant varied, ranging from approximately 50 to 150 (see the Appendix for a complete list of toxicants, sample size, and detection limits). Because no formal toxicant monitoring program currently exists for the watershed as a whole, data on sediment concentrations of toxicants available from the CBP are synthesized from approximately 50 independent assessment reports, a complete list of which was published previously by the CBP [22]. All sediment toxicant data that were available and met the above criteria were utilized.

Point-pattern analysis

The distribution of monitoring stations for benthic biodiversity, water quality, and sediment toxicant data were analyzed to assess sampling density and clustering among monitoring stations. The mean distance among monitoring stations was calculated by averaging the distance of each individual site to its first order (i.e., nearest) neighboring site. The basinwide site density for each data set was calculated as the ratio of the total number of monitoring stations to the total area of the basin. In addition, spatial clustering of monitoring stations was analyzed by calculating the *K*-statistic (a measure of the nonrandomness with which points are distributed in space) with Equation 2 [23,24]

$$K(d_s) = (A/N^2) \left(\sum_i \sum_j I(d_{ij}) \right)$$
(2)

where $K(d_s)$ is the K-statistic for a distance d_s . A is the total area of interest (here $11,170 \text{ km}^2$), N is the total number of points (i.e., monitoring stations), and $I(d_{ii})$ is the number of points (j) found within the distance d_s summed over all points (i). Thus, a circle of radius d_s is placed over each point *i*, and the number of other points *ij* within that circle is counted. In other words, the statistic compares the number of neighboring points within a given radius to the number expected on the basis of complete spatial randomness. The $K(d_s)$ can be calculated over a range of d_s (here 0.5–60 km) to determine the extent of spatial clustering (nonrandomness) in the distribution of points at different spatial scales. The relationship between $K(d_s)$ and d_s can be presented graphically for interpretation. However, because the relationship is often nonlinear, $K(d_s)$ is transformed to a square root function $L(d_s)$ with Equation 3 [25]

$$L(d_s) = [(K(d_s)/\pi)]^{1/2} - d_s$$
(3)

where $L(d_s)$ is the transformed *K*-statistic, $K(d_s)$ is the *K*-statistic for distance d_s (from Eqn. 2), π is pi, and d_s is the distance. The function $L(d_s)$ was plotted against a range of d_s values for the monitoring stations associated with each data set (biodiversity, water quality, or sediment toxicant concentrations) to assess the extent of spatial clustering among monitoring stations that might influence the study results. Values greater than zero are indicative of clustering, whereas values less than zero are indicative of dispersion.

Effect modeling

To determine values for biodiversity throughout the study area, values for biodiversity had to be estimated for locations where biological monitoring was not performed. This was accomplished by interpolating values for benthic biodiversity at locations where samples were not collected based upon observed values where samples were collected. A variety of statistical models can be used to perform such interpolations. The simplest is simply the unweighted average of observed values within a specified proximity to unsampled locations [26]. However, this assumes that all observed values are equally representative of the true value at the unsampled location, an assumption that is often not valid for environmental monitoring data [26]. Instead, a weighted average can be used where the weights are based upon a distance-decay function that gives greater weight to observed values that are closer to the unsampled location than those values that are farther away. A simple form of such weighted models is an inverse distanceweighted (IDW) model, where the value of the weights decreases with some power function of distance from the interpolated area [26]. Therefore, all observed values within the same distance from the interpolated area are assigned the same weight, with no consideration for spatial correlation among observed values. Most GIS and geostatistical software will perform IDW modeling or calculations can be performed by hand if data sets are sufficiently small. Alternatively, kriging models are also distance-weighted, but the weights can be adjusted to account for spatial correlation among observed values, such as directional trends in the modeled variable or spatial clustering of observations [26-28].

In the current study, an IDW model with the weights determined by the inverse square law (i.e., value of the weights decrease with the square of distance) was utilized to estimate values for biodiversity throughout the study area. Early experiments with various model parameters indicated that higherorder IDW models (e.g., distance³ or distance⁴) yielded nearly identical results as a second-order model. Before interpolation, values for Shannon's biodiversity index were transformed to a cumulative probability distribution. Data were then projected using an Albers equal-area–conic projection to account for curvature of the earth. Surface interpolation was based upon a grid system comprised of cells of equal area. Biodiversity values were assigned to each grid cell with Equation 4 [29]

$$X_0 = \sum w_i X_i \tag{4}$$

where X_0 is the interpolated value for a grid cell, X_i is the transformed value for Shannon's biodiversity index, and w_i is $D(X_0, X_i)^{-2/W}$, where $D(X_0, X_i)$ is the distance from X_0 to X_i and W is a normalization factor that enables $\sum w_i = 1$. This method of interpolation helps minimize bias caused by spatial heterogeneity in the distribution of biodiversity monitoring stations around each grid cell. Although IDW models tend to cause

spatial smoothing of the modeled variable, given that the model was used to identify general regions of the basin consistent with low or high biodiversity rather than the value of biodiversity at any single location, this smoothing was seen as advantageous.

Both the size (i.e., area) of the grid cells as well as the neighborhood size (i.e., number of observations used to interpolate the value of a grid cell) were important considerations. Spatial resolution decreases with increasing cell size, as data are aggregated over a larger area. Therefore, small grid cells increase the likelihood that the interpolated value for that cell will be representative of local phenomenon. Similarly, the accuracy of the interpolated value for a grid cell is enhanced by maximizing the number of biodiversity observations (i.e., neighborhood size) used in the interpolation. However, as neighborhood size increases, the interpolation includes observed values at increasing distances from the grid cell. Therefore, large neighborhood sizes decrease the likelihood that the interpolated value for that cell will be representative of local phenomenon. Because of these constraints, the interpolated value for a grid cell may vary significantly depending upon how the IDW model is parameterized. Therefore, a sensitivity analysis was conducted to optimize grid cell and neighborhood size. Forty calibration points (independent of biotic sampling points) distributed throughout the basin were selected (Fig. 1). The interpolated value for Shannon's biodiversity index at each of these points was calculated assuming a range of cell sizes (0.01-625 km²) and neighborhood sizes (1-20). No statistically significant differences were observed in mean biodiversity among the 40 grid cells associated with the calibration points over the range of cell and neighborhood sizes tested. However, cell-specific interpolated values for biodiversity varied with cell and neighborhood size. Therefore, optimal cell and neighborhood sizes were determined by quantifying the variation associated with each interpolated cell value with stepwise decreases in cell size or increases in neighborhood size and selecting neighborhood and cell sizes that minimized this variation. At cell sizes of 0.0625 km², further reductions in cell size caused no further significant decreases in within-cell variation. No significant variation was defined as a less than 1% change in mean values among the 40 calibration points and a less than 5% change in value at 95% of individual calibration points. At neighborhood sizes of 10, further increases in neighborhood size caused no further significant decrease in within-cell variation. Thus, effect modeling of benthic biodiversity was performed using grid cell and neighborhood sizes of 0.0625 km² and 10, respectively.

Confidence in the model was also assessed by validating both the appropriateness of the IDW method of interpolation and Shannon's biodiversity index as an indicator of ecological stress. Validation of the IDW model was performed by using an alternative interpolation method, the results of which could be compared to those of the IDW model. By using the same parameters for neighborhood size and cell size as above, a benthic biodiversity model was constructed with universal kriging (see above). Validation of Shannon's biodiversity index was achieved by using an alternative indicator of benthic ecological integrity in the IDW model. The predicted spatial patterns for Shannon's biodiversity index were compared to predicted patterns of benthic total macroinvertebrate biomass, species richness, and the CBP's benthic index of biotic integrity with similar GIS-based modeling tools. The benthic index of biotic integrity is a qualitative indictor incorporating multiple biotic indicators normalized for salinity and substrate [20], and, thus, is designed to yield a more robust, comprehensive measure of ecological integrity than the use of a single indicator. The results of both of these modeling exercises were subsequently compared to the results generated for the IDW model utilizing Shannon's biodiversity index.

Upon the completion of the IDW interpolation, the areas corresponding with the upper and lower 20th percentiles of the cumulative probability distribution for benthic biodiversity were selected and digitized as polygon features. The area of each polygon was subsequently calculated for each tail of the distribution (i.e., upper or lower 20th percentile) and designated as low-impact and high-impact zones, assuming that low values for benthic biodiversity were indicative of ecological stress. The upper and lower 20th percentiles were selected because the modeled biodiversity values for these areas are greater than one standard deviation from the basinwide observed mean for benthic biodiversity and can be assumed to be ecologically atypical because of their local environmental conditions. Water quality and sediment toxicant monitoring stations intersecting with either low-impact or high-impact zones were subsequently selected and organized into separate subsets for statistical comparison.

Stressor identification

Statistical comparisons of water quality parameters and sediment toxicant concentrations between the high- and low-impact zones were used to identify stressors that may account for observed patterns of biodiversity. The assumption was made that if the values for an individual water quality parameter of a toxicant were comparable between the high- and lowimpact zones, that variable likely could be excluded as a source of stress to the benthic community within the high-impact zones. Comparisons were made among mean values with a two-tailed *t* test ($\alpha = 0.05$) to account for both variance and sample size, because sample sizes differed among different variables and effect zones. For sediment toxicant data, the percentage of samples above the detection limit was estimated when sufficient data were available.

Methodological uncertainties and limitations

The above methods produce several uncertainties and limitations that should be noted. The dominant limitation is the use of secondary data rather than the collection of original data by the investigator. Historical monitoring data were not collected specifically for the purposes for which they were utilized in the current study, and verifying the quality of the data and consistency in analysis methods is problematic. For example, whether statistical outliers for monitoring parameters are accurate representations of environmental phenomenon or errors in analysis or reporting cannot be ascertained. Also, the current study was retrospective, and thus, may not necessarily accurately reflect future trends. Shannon's biodiversity index has been criticized because of its assumption that all species are represented in the sample from a population of effectively infinite size, and the value of the index does not vary with sample size [19]. The strengths of alternative biodiversity measures, such as Brillouin's index, have been described for nonrandom field sampling, such as that performed by the CBP [19]. In addition, other biotic indicators such as species richness (i.e., the number of species in a community) and species evenness (i.e., the equitability of abundances among species in a community) have been suggested for such applications



Fig. 3. Square-root-transformed *K*-statistics for the distribution of biodiversity, water quality, and sediment toxicant monitoring stations used in the current study at increasing spatial scales. Values greater than zero indicate that the spatial distribution of monitoring stations is more clustered than would be expected given complete spatial randomness. Values less than zero indicate that the spatial distribution of stations is more dispersed than would be expected given complete spatial randomness.

[19]. Regardless of the methods used to parameterize and construct the biodiversity model, uncertainty is an unavoidable consequence. Thus, any interpretation of the model must be made with reported variation and uncertainty in mind. Although field data were used as a potential indicator of adverse effects, these effects cannot necessarily be attributed to water quality or toxicant parameters considered in the current study. Other environmental factors such as temperature or salinity, may affect biodiversity [30–35]. Toxicant concentrations were not adjusted to reflect modifying factors such as hardness or dissolved organic carbon that might affect bioavailability or toxicity, and ecotoxicologic data were not analyzed to estimate community effect concentrations for stressors that could be compared to environmental concentrations.

RESULTS

Point-pattern analysis

Monitoring stations for benthic biodiversity had a spatial density of 0.09 monitoring stations/km² with a mean distance between monitoring stations of 1.38 km. Monitoring stations for water quality had a spatial density of 0.01 monitoring stations/km² with a mean distance between monitoring stations of 7.5 km. Monitoring stations for sediment toxicants had a spatial density of 0.032 monitoring stations/km² and a mean distance between monitoring stations of 2.27 km. The calibration sites used for sensitivity analysis for the interpolation of biodiversity had a density of 0.004 sites/km² and a mean distance between sites of 17.86 km.

Analysis of the *K*-statistics indicated that monitoring stations for biodiversity, water quality, and sediment toxicants all exhibited high spatial clustering over small spatial scales (<10-20 km; Fig. 3). This indicates a spatial bias in monitoring stations that is a result of oversampling in certain regions of the basin, particularly the lower Potomac, York, and James rivers and the greater Baltimore and Annapolis, Maryland, USA, areas (Figs. 1 and 2). This pattern is likely the result of



Fig. 4. Results of effect modeling based upon observed benthic biodiversity within the study area (1984–1999). Low- and high-impact zones were interpolated from observed values for Shannon's biodiversity index at each biodiversity sampling location using a secondorder inverse distance-weighted model.

intensive sampling in regions of the bay historically considered to be adversely impacted or located near areas of dense human development. Monitoring stations for water quality and sediment toxicants were generally fewer in number, less dense, and, thus, of lower spatial resolution than biodiversity monitoring stations. However, at spatial scales above 10 to 20 km, monitoring stations for all three data sets were significantly more dispersed than would be expected from a random distribution, indicating that monitoring stations were distributed uniformly with respect to one another (Fig. 3). The *K*-statistics for the calibration sites used for sensitivity analysis were also highly dispersed (data not shown), because these sites were selected by the investigator to be uniformly distributed.

Effect modeling

The basinwide mean and standard error for Shannon's biodiversity index was 2.05 ± 0.02 . Based upon the modeling of benthic biodiversity within the basin, $1,815 \text{ km}^2$ were identified as low-impact zones (Fig. 4). These zones were predominantly located in the central and southern regions of the basin, although smaller dispersed areas of high biodiversity were located throughout the basin. The low-impact zones contained nine water quality monitoring stations and 24 sediment toxicant monitoring stations. In comparison, $1,412 \text{ km}^2$ of the basin were identified as high-impact zones (Fig. 4). These zones were predominantly located in the northern regions of the basin and the lower Potomac River, downstream of Baltimore and Washington, DC. However, smaller areas of low biodiversity were



Fig. 5. Results of uncertainty analysis for effect modeling conducted on the subset of grid cells associated with the 40 calibration points used in sensitivity analysis (see Materials and Methods; Fig. 1). Uncertainty in the interpolated value for each cell was expressed as the coefficient of variation among the 10 observed values for Shannon's biodiversity index used to interpolate each grid cell value.

located throughout the basin. The high-impact zones contained 20 water quality monitoring stations and 64 sediment toxicant monitoring stations. The means and standard errors for observed values for Shannon's biodiversity index in the low- and high-impact zones were 3.51 ± 0.03 and 0.93 ± 0.03 , respectively, confirming that the modeled areas identified as high- and low-impact zones represented the observed values for biodiversity within each.

An uncertainty analysis was subsequently conducted to assess confidence in the model. The uncertainty associated with the interpolated value for Shannon's biodiversity index was quantified for the subset of 40 grid cells associated with the 40 calibration points used in the sensitivity analysis (see above). For each of these 40 grid cells, the coefficient of variation (CV) was calculated for the 10 observed values for Shannon's biodiversity index used to interpolate the value for that grid cell. The CVs varied from a low of 11% to a high of 167% (mean = 42%). Three fourths of the 40 grid cells used in uncertainty analysis were associated with CVs of 50% or less (Fig. 5).

Results of model validation indicated that the IDW model agreed closely with results generated by universal kriging. A high percentage of the areas identified as low- and high-impact zones by the IDW model were also classified similarly by kriging (Table 1). In addition, 70 to 100% of the biodiversity, water quality, and sediment toxicant monitoring stations that were contained in the low- or high-impact zones with the IDW model were also contained in those zones when the kriging model was used (Table 1). The IDW results for low-impact areas generally agreed better with the kriging model than those from the high-impact areas (Table 1). The IDW model results generated from the use of Shannon's biodiversity index also agreed reasonably well with results generated from the use of other biological indicators. Seventy-eight percent and 62% of the areas identified as high-impact zones by the use of Shannon's biodiversity index were also similarly classified by the use of the benthic index of biotic integrity or total biomass,

Table 1. Validation of the inverse distance-weighted (IDW) model by comparison to the kriging model. The % common area indicates the percentage of the areas predicted to be of low or high anthropogenic impact using the IDW model that were similarly identified using the kriging model. The % biodiversity stations, % water quality stations, and % sediment toxicant stations represent the percentage of monitoring stations associated with low- or high-impact zones as predicted by the use of the IDW model that were similarly associated with low- or high-impact zones with the kriging model

	Low impact	High impact
 % Common area % Biodiversity stations % Water quality stations % Sediment toxicant stations 	96 99 100 96	73 81 70 93

respectively (Table 2). However, both the benthic index of biotic integrity and biomass were biased toward low values, resulting in a poor fit with the low-impact zones predicted by Shannon's biodiversity index. In contrast, species richness was biased toward high values, and, thus, 95% of the areas identified as low-impact zones by Shannon's biodiversity index were also identified as low-impact zones by species richness (Table 2). However, high-impact zones identified by Shannon's biodiversity index were a poor fit with those identified with species richness. Because of the inherent biases and conflicting results of the benthic index of biotic integrity, biomass, and species richness, Shannon's biodiversity index was the most useful biological indicator of the four. However, other indicators provided evidence in support of the delineation of highor low-impact areas based upon the use of Shannon's biodiversity index.

Stressor identification

Comparison of benthic water quality data identified several potential sources of stress for the benthic communities in the Chesapeake Bay. Most significant were the disparities in nutrient concentrations between the low- and high-impact zones (Table 3). Total nitrogen and phosphorus concentrations were higher in the high-impact zones by 87 and 55%, respectively. Similarly, dissolved organic nitrogen and phosphorus were higher by 34% and 57%, respectively, in the high-impact zones. Dissolved inorganic nitrogen was more than threefold higher and dissolved inorganic phosphorus was 79% higher in the high-impact zones. Total and dissolved organic carbon were also significantly higher in the high-impact zones, by 41 and 34%, respectively. The availability of nutrients in the high-impact zones was reflected in the photosynthetic biota, as indicated by a twofold increase in chlorophyll concentrations

compared to the low-impact zones. Dissolved oxygen in the high-impact zones was significantly lower by 34% than in the low-impact zones, averaging only 3.69 μ g/L during summer months. Although pH varied significantly between the two zones, the values were relatively similar (between 7.6 and 7.9), suggesting this disparity may not necessarily be ecologically relevant. In addition, salinity and conductivity were significantly higher in the low-impact zones, which is likely a function of the geographic distribution of the low-impact zones and their closer proximity on average to the Atlantic Ocean (Fig. 4).

Of the 17 metals considered in the current study, 14 were significantly increased in the high-impact area (Fig. 6). Cadmium concentrations were more than an order of magnitude higher in sediments from the high-impact zones (1,001.6 μ g/kg) compared to the low-impact zones (73.6 μ g/kg), and near order of magnitude disparities were observed for other heavy metals, including mercury and lead (Fig. 6). Other potentially toxic metals such as arsenic, copper, aluminum, zinc, and manganese were also significantly increased in the high-impact zones (Fig. 6).

Concentrations of all pesticides considered in the current study were increased in the high-impact zones with the exception of DDT (4,4') (Fig. 7). However, observed differences were only statistically significant for dichlorodiphenyldichloroethylene (DDE, 4,4') and dieldrin, both of which were approximately an order of magnitude higher in the high-impact zones (Fig. 8). Mirex was present at concentrations on the order of 100 ng/kg in the high-impact zones and was undetectable in the low-impact zones, but this disparity could not be addressed statistically (Fig. 7). The mean concentrations for individual pesticides in low- and high-impact zones were 0.07 µg/kg and 0.5 µg/kg, respectively, and this difference was highly significant (p = 0.001; Fig. 7). Total pesticide concentrations among monitoring stations in the high-impact zones were also significantly higher than those in the lowimpact zones (p = 0.03; Fig. 7).

All PAHs were significantly increased in the high-impact zones compared to the low-impact zones, many by an order of magnitude or more (Fig. 8). However, only increases of fluorene and perylene were statistically significant. The mean concentrations for individual PAHs in low- and high-impact zones were 50 μ g/kg and 110 μ g/kg, respectively, and this difference was highly significant (p < 0.0001; Fig. 8). Total PAH concentrations in the high-impact zones were approximately fourfold higher than the in low-impact zones (Fig. 8). However, this difference was not statistically significant.

All PCB congeners were elevated in the high-impact zones

Table 2. Validation of the use of Shannon's biodiversity index as an effect indicator. The % common area indicates the percentage of the areas predicted to be of low or high anthropogenic impact using Shannon's biodiversity index that were similarly identified by the other three indicators. The % biodiversity stations, % water quality stations, and % sediment toxicant stations represent the percentage of monitoring stations associated with low- or high-impact zones as predicted by the use of Shannon's biodiversity index that were similarly associated with low- or high-impact zones with each of the other three indicators^a

	Benthic IBI		ic IBI Biomass Species richness			
	Low impact	High impact	Low impact	High impact	Low impact	High impact
% Common area	9	78	0	69	99	2
% Biodiversity stations	40	64	2	62	95	11
% Water quality stations	11	60	0	55	100	5
% Sediment toxicant stations	4	73	0	48	100	3

^a IBI = index of biotic integrity.

Table 3. Comparison of mean benthic water quality parameters between low-impact and high-impact zones of Chesapeake Bay, USA, during summer months (May–September)^a

	Low-impact zones		High-impact zones			
Parameter	n	Mean ± SE	п	Mean \pm SE	High/low	
Dissolved oxygen (mg/L)	599	5.62 ± 0.08	1,321	3.69 ± 0.09	0.66*	
Water temperature (°C)	614	23.20 ± 0.15	1,335	22.70 ± 0.11	0.98	
pH	589	7.96 ± 0.01	1,312	7.63 ± 0.01	0.96*	
Salinity (g/L)	614	22.26 ± 0.18	1,335	14.26 ± 0.18	0.64*	
Conductivity (µmhos/cm)	614	$34,473.80 \pm 249.16$	1,335	$23,311.90 \pm 274.44$	0.68*	
Total suspended solids (mg/L)	601	20.85 ± 0.66	1,324	19.89 ± 0.67	0.95	
Turbidity (NTU)	66	16.12 ± 1.75	111	20.29 ± 2.52	1.26	
Chlorophyll (µg/L)	361	5.67 ± 0.31	986	12.30 ± 0.85	2.17*	
Total organic carbon (mg/L)	441	2.62 ± 0.07	943	4.06 ± 0.09	1.55*	
Dissolved organic carbon (mg/L)	380	2.32 ± 0.04	943	3.26 ± 0.06	1.41*	
Total nitrogen (mg/L)	405	0.50 ± 0.01	1,005	0.94 ± 0.02	1.87*	
Total dissolved nitrogen (mg/L)	164	0.36 ± 0.01	985	0.69 ± 0.02	1.94*	
Dissolved organic nitrogen (mg/L)	153	0.26 ± 0.01	972	0.35 ± 0.01	1.34*	
Dissolved inorganic nitrogen (mg/L)	488	0.10 ± 0.00	1,147	0.32 ± 0.01	3.29*	
Total phosphorus (mg/L)	494	2.62 ± 0.05	1,164	4.06 ± 0.03	1.55	
Total dissolved phosphorus (mg/L)	408	0.03 ± 0.03	1,101	0.04 ± 0.04	1.33*	
Dissolved organic phosphorus (mg/L)	404	0.01 ± 0.00	1,082	0.01 ± 0.00	1.57*	
Dissolved inorganic phosphorus (mg/L)	404	0.01 ± 0.00	1,082	0.03 ± 0.00	1.79*	
Sample depth (m)	614	12.93 ± 0.30	1,338	14.21 ± 0.26	1.10	

a n = number of observations for each parameter; SE = standard error of the mean; High/low = ratio of mean values in high-impact to low-impact zones; NTU = nephelometric turbidity unit.

* Significant difference ($\alpha = 0.05$) between means in low- and high-impact zones by two-sided t test.

(data not shown). However, when individual congeners were considered, no significant differences were observed between mean sediment concentrations in the low- and high-impact zones. The mean concentrations for individual PCBs in low- and high-impact zones were 0.110 μ g/kg and 1.05 μ g/kg, respectively, and this difference was highly significant (p = 0.004). Total PCB concentrations in the high-impact zones were approximately fivefold higher than in the low-impact zones. However, this difference was not statistically significant.

DISCUSSION

The analysis of the spatial distribution of benthic biodiversity in Chesapeake Bay indicates that a significant area of



Fig. 6. Comparison of sediment concentrations in the low- and highimpact zones for 17 metals considered in the current study. Error bars represent the standard error of the mean. Asterisks indicate that the mean concentration in the high-impact zones differed significantly (p < 0.05) from that in the low-impact zones by two-tailed *t* test.

the basin (\sim 13% of the study area) is predicted to have low biodiversity. Those regions of principle concern are the main channel of the upper Chesapeake Bay, downstream of Baltimore and Annapolis, and the lower Potomac River, downstream of Washington. The proximity of these high-impact zones to areas of dense human population, development, and industry suggests an anthropogenic influence on the spatial distribution of biodiversity within the basin. The high-impact regions identified in the current study are areas historically associated with high organic and inorganic toxicant concentrations [36–38]. However, some high-impact zones overlapped with salinity values between 5 and 8 g/L that have been associated with low estuarine species abundance [35]. Thus,



Fig. 7. Comparison of sediment concentrations in the low- and highimpact zones for 15 pesticides considered in the current study. Error bars represent the standard error of the mean. Asterisks indicate that the mean concentration in the high-impact zones differed significantly (p < 0.05) from that in the low-impact zones by two-tailed *t* test. DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene.



Fig. 8. Comparison of sediment concentrations in the low- and highimpact zones for 19 polycyclic aromatic hydrocarbons (PAHs) considered in the current study. Error bars represent the standard error of the mean. Asterisks indicate that the mean concentration in the high-impact zones differed significantly (p < 0.05) from that in the low-impact zones by two-tailed *t* test.

one cannot completely eliminate natural variation in water chemistry as a confounder in effects assessment.

Comparison of water quality parameters between the lowand high-impact zones identified potential direct and indirect sources of stress to the basin's benthos. Both nitrogen and phosphorus concentrations were elevated in the high-impact zones by approximately two- to threefold, indicating increased delivery of nutrients to the high-impact zones. Likely sources of such nutrients are urban and agricultural runoff associated with the various land usages within the watershed, as well as atmospheric deposition [38,39]. Although some discussion of the potential toxicity of nitrogenous compounds such as nitrates has occurred [40], the concentrations reported in the current study likely are not sufficient to cause direct toxicity. However, they are a potential indicator of runoff and environmental degradation and may be indirectly responsible for other stressors. For example, chlorophyll concentrations were also elevated in the high-impact zones, probably as a result of greater nutrient availability. Toxic algal blooms during summer months resulting from eutrophication are a well-documented source of stress to aquatic organisms [41-43]. Hypoxic or anoxic conditions also have been associated with eutrophication [44-47], and the oxygen demands in the benthic environment may be sufficient to cause periodic or sustained oxygen depletion. Dissolved oxygen concentrations similar to those in the high-impact zones (3.69 mg/L) have been demonstrated to cause stress to aquatic organisms [48-50].

The differences in sediment toxicant concentrations between the high-impact and low-impact zones were generally much greater than those observed for any water quality parameter. These differences were most readily apparent with metal concentrations. Cadmium concentrations in the highimpact zones were more than an order of magnitude greater than those in the low-impact zones. Concentrations of other heavy metals such as mercury and lead were also several-fold higher in the high-impact zones. The consistent elevation of all metals in the high-impact zones raises questions regarding whether such observations could be the result of geologic factors resulting in an inherently greater relative background metal concentration in the high-impact zones. However, previous work identified areas of elevated metal concentrations similar in extent to the high-impact zones defined in the current study, even after accounting for geologic phenomena [38]. In some cases, elevated metal concentrations were linked to pointsources such as steel mills or sewage treatment plants [36,37].

The concentrations of organic toxicants, including pesticides, PAHs, and PCBs were also consistently elevated in the high-impact zones. Because of the small sample sizes associated with these compounds as well as high or unreported detection limits, a significant degree of uncertainty must be associated with these data. However, the fact that the majority of organic compounds were elevated in the high-impact zones, often by an order of magnitude, indicates that delivery of these toxicants into these zones is greater than delivery into other zones. As anthropogenic compounds, background levels largely can be excluded as a confounder. Thus, the assumption can be made that benthic organisms within the high-impact zones are exposed to a complex mixture of organic and inorganic toxicants. However, determination of the ecological significance of such exposures is difficult. Average concentrations in the high-impact zones for individual pesticides, PAHs, and PCBs were on the order of 0.5 μ g/kg, 50 μ g/kg, and 1 μ g/ kg, respectively. If one considers additivity among those compounds included in the current study, their sum concentrations are on the order of 10 µg/kg for pesticides, 4,000 µg/kg for PAHs, and 20 µg/kg for PCBs. Persistent hydrophobic compounds as well as metals also may bioconcentrate or bioaccumulate, leading to more severe exposures for certain trophic levels than indicated by sediment concentrations alone [38,51,52].

The use of ecological effects data to drive the ERA process offers several advantages over the more common stressorbased approach to ERA. For example, Hall et al. [53] conducted an ERA of copper and cadmium in surface water of the Chesapeake Bay. Although several sites of potential risk were identified, the authors could not validate their risk estimates with observations of effects from the field. If indeed adverse effects had been observed in situ they could not have been attributed to cadmium and copper, for other potential stressors were not assessed and excluded. As a result, both the predicted effects as well as the predicted stressors are associated with significant uncertainty, identifying limitations of such an approach to ERA. An effects-based approach to ERA alleviates these problems in two ways. First, the estimates of adverse ecological effects are less ambiguous because they are based upon direct measurements and are the result of the net direct and indirect effects of all relevant stressors and stressor interactions. Thus, the a priori identification of the stressor(s) is not necessary for quantifying the effects [3]. Second, the ERA becomes more amenable to the consideration of multiple stressors, because potential stressors are not excluded by the investigators from the outset. Effects-based ERA also may be utilized to monitor ecosystem responses to long-term cumulative impacts or impact-mitigation management strategies [3]. However, effects-based ERA is not a replacement for traditional ERA methods. Although the identification of a suite of stressors that may affect the biota is advantageous for developing a comprehensive conceptual model of ecological stress, the ability to quantify and estimate the cumulative effects of multiple stressors remains an important challenge. For example, although the disparities in toxicant concentrations between the low- and high-impact zones were greater than the observed disparities in water quality parameters such as dissolved oxygen, one cannot conclude that toxicants pose a

greater risk to the biota. Therefore, use of an effects-based approach to screen or identify candidate stressors that can be investigated further through laboratory toxicity testing or body-burden analysis from field populations to prioritize their risk may be a rigorous approach to regional ERA.

CONCLUSIONS

The development of regional environmental assessment methods is a critical task in light of future challenges to both terrestrial and aquatic ecosystems. However, ERA at the regional level has significant data requirements necessitating the integration of data from all available sources. The availability of GIS technology and its increasing application in environmental management and decision-making makes GIS technology a useful tool for overcoming some of the current challenges to conducting regional ERA. Based upon available data, the current study identifies multiple potential stressors to the benthic biota of the Chesapeake Bay, including dissolved oxygen, cadmium, mercury, lead, copper, and the cumulative effects of pesticides, PAHs, and PCBs. Additive or synergistic interactions have been observed for combinations of these stressors as well [54-56]. A number of other toxicants likely are present, suggesting that continued sampling is necessary. The spatial autocorrelation among multiple stressors at regional scales suggests that a priori assumptions regarding the identity of stressors or the consideration of stressors individually may result in incomplete cataloging of hazards. As a result, effects-based approaches to retrospective ERA may assist in preventing the exclusion of potential effects, their causes, or both.

Methods similar to those used in the current study could be extended readily to address additional research questions related to regional ERA. For example, although the current study considered spatial patterns of environmental data, temporal patterns were not assessed. Temporal variation or trends in toxicant concentrations or effects are an important consideration, particularly in the development or assessment of management goals or strategies. Application of more advanced spatial modeling tools may lead to the development of probabilistic models of ecological risk derived from spatial as well as temporal patterns of multiple stressors. Additional work also must be performed regarding the data requirements for GISbased analyses methods, such as the optimal distribution and density of monitoring stations, the types of data that should be collected, and the frequency of data collection. Establishing links between the distribution of stressors and terrestrial sources, both point and nonpoint, would yield valuable information for watershed management. Finally, the rapid application of tools such as GIS technology to ERA as they become available will assist in the timely development of more robust assessment methods.

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APPENDIX

List of toxicants considered in the current study. Detection limits reported by the Chesapeake Bay Program for each toxicant are listed as a range (minimum–maximum). Sample sizes and the estimated percentage of samples above detection limits (% > DL) are also listed for each toxicant and impact zone (low or high)^a

Toxicant Name		Low-impact zones		High-impact zones	
	(mg/kg)	No. samples	% > DL	No. samples	% > DL
Metals					
Aluminum	0-1,500,000	25	100	57	100
Antimony	0-10,000	22	50	44	81
Arsenic	0–9,000	32	80	84	84
Beryllium	1,000-5,000	21	NA	37	NA
Cadmium	0-5,000	43	12	112	68
Chromium	0-6,000	33	85	88	96
Copper	0-5,000	43	96	112	91
Iron	0-500,000	26	100	66	100
Lead	0-6,000	43	91	112	95
Manganese	0-5,000	23	100	61	100
Mercury	0-1,000	34	27	88	60
Nickel	0-5,000	43	87	112	89
Selenium	0-8,000	31	20	70	40
Silver	6.5-5,000	25	13	51	27
Thallium	0-10,000	21	NA	37	8
Tin	2-1,000	14	80	53	75
Zinc	0-5,000	43	100	112	98
Mean		31	69	76	76
Pesticides					
Aldrin	0.004-150	22	NA	58	4
Chlordane	0.001-1.16	12	NA	42	100
DDD (4,4')	0.003-130	23	11	59	18
DDD(O,P)	0.49-1.36	12	NA	39	NA
DDE (4,4')	0.003-140	25	18	61	22

APPENDIX

Continued

Toxicant Name	Detection limit (mg/kg)	Low-impa	Low-impact zones		High-impact zones	
		No. samples	% > DL	No. samples	% > DL	
DDE (O, P)	0.68-1.11	12	NA	39	NA	
DDT (4,4')	0.002-120	23	10	59	16	
DDT(O,P)	0.18-1.22	12	NA	40	6	
Dieldrin	0.009 - 150	23	NA	60	8	
Endrin	0.008-230	20	NA	40	NA	
Heptachlor	0.003-120	23	NA	60	4	
Heptachlor epoxide	0.002-140	22	NA	53	2	
Lindan	0.004 - 16.1	12	0	49	NA	
Mirex	0.025 - 1.2	12	0	43	3	
Trans-nonachlor	0.031-0.87	12	NA	40	7	
Mean		18	8	49	17	
Polycyclic aromatic hydrocarbons						
Acenaphthene	0.002-225	25	50	59	43	
Acenaphthylene	0.05-212	16	10	51	20	
Anthracene	0.0002-265	28	62	63	58	
Benzo[B+K]fluroanthene	9 6-145	12	50	36	75	
Benzo[<i>a</i>]pyrene	0.0002-502	28	76	64	59	
Benzo[<i>b</i>]fluroanthene	0.19-459	11	NA	35	42	
Benzo[<i>e</i>]pyrene	0.05-153	14	71	43	63	
Benzo[<i>ah</i>]pervlene	0.0001-545	16	56	52	54	
Benzo[k]fluroanthene	0.306-459	10	NA	31	25	
Benzo[a]anthracene	0.0002-587	27	80	63	74	
Chrysene	0.0002-387	28	84	64	74	
Dibenzo[<i>a h</i>]anthracene	0.0002-475	18	42	58	30	
Fluroanthene	0.0003-350	10	42	50	71	
Fluorene	0.003-330	28	62	63	55	
Indeno[1 2 3 cd]pyrene	0.002-227	26	74	63	55	
Naphthalana	0.0001-545	20	18	55	56	
Parylana	0.05 189	17	10	13	50	
Dhananthrana	0.001 200	14	45	45	50	
Puropo	0.001-290	17	45	55	39 74	
Mean	0.005-550	20	70 59	54	74 55	
Polychlorinated binhenyls		21	57	57	55	
	0.00.2.0	10	NT A	20	7	
PCD 0	0.09-2.9	12	INA	39	7	
PCD 10	0.074-5.45	12	INA	27	7	
PCD 44	0.1 2.0	12	INA	57	22	
PCB 52	0.1-2.0	12	INA NA	40	33	
PCB 00	0.08-1.01	12	INA NA	57	20	
PCB 101	0.026-0.72	12	INA NA	40	43	
PCD 119	0.0/-0.7/	12	INA NA	40	19	
PCD 118	0.01-0.49	12	INA NA	40	20	
PCB 128	0.037-0.95	12	INA NA	41	20	
PCD 153	0.02 0.77	12	INA 20	41	43	
ГСБ 133 DCD 170	0.08 - 0.77	12	29 NI 4	41	30	
PCB 1/0	0.1-8.12	12	INA NA	40	14	
PCB 180	0.01-0.75	12	INA NA	5/	24	
PCB 18/	0.01-0.76	12	INA NA	40	40	
PCB 193	0.043-0.72	12	INA	5/	16	
PCB 200	0.068-0.81	12	NA	37	20	
РСВ 209	0.068-0.65	12	10	31	29	
Mean		12	20	39	24	

 a NA = not available (sufficient data were not available to make the calculation because of underreporting of detection limits); DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene.