

A Multimetric Biological Index for Evaluating Ecological Condition in Long Island Sound

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Executive Summary

The Long Island Sound (LIS) Macroinvertebrate Multimetric Index (MMI) was developed to assess the biological condition of embayment sites using benthic macroinvertebrate communities. This effort, led by Tetra Tech in collaboration with the Connecticut Department of Energy and Environmental Protection (CT DEEP), builds on foundational work from Phase I of the project and aligns with the goals of the Long Island Sound Study's 2025 Comprehensive Conservation and Management Plan (CCMP).

The MMI integrates biological, chemical, and physical data collected from over 200 embayment sites between 2010 and 2021. Using macroinvertebrate trait data, sediment and water quality parameters, and land use information, a disturbance gradient was developed to classify sites from minimally to highly disturbed. This gradient informed the selection and scoring of biological metrics that reflect both structural and functional aspects of benthic communities.

From over 500 candidate metrics, 19 were shortlisted and rigorously evaluated. The final index model (6_10396) includes six metrics that capture key ecological responses to stress, including the presence of tolerant and sensitive taxa, bioturbation potential, and pollution tolerance. The index demonstrated strong performance, with a discrimination efficiency of 85.7% in the calibration dataset and 85% in the validation dataset, indicating its reliability in distinguishing between healthy and degraded sites.

To support implementation and stakeholder engagement, we developed two complementary tools: an R Markdown-based calculation [tool](#) for generating MMI scores and a dual-format ArcGIS StoryMap, one [technical](#) and one [non-technical](#), that visually communicates the project's context, methodology, and key findings. Together, these resources enhance the accessibility and utility of the MMI for regulatory assessments, long-term monitoring, and public outreach.

The LIS MMI provides a scientifically robust, ecologically meaningful, and regionally tailored approach to evaluating estuarine health. It will serve as a foundational tool for protecting and restoring the Long Island Sound, offering a model for future index development in other coastal systems. However, due to the limited availability of minimally disturbed sites, the disturbance gradient and reference conditions were defined using a combination of regional benchmarks and quantile-based thresholds, which may warrant refinement as additional data become available.

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1 INTRODUCTION

1.1 BACKGROUND

The Long Island Sound Study's Comprehensive Conservation and Management Plan (CCMP, LISS 2015) promotes effective management of Long Island Sound (LIS), with emphasis on protecting and improving water quality, habitat, and biological diversity. In 2020, the LISS supported Phase I of a two-phase project, conducted by Tetra Tech under the auspices of the Connecticut Department of Energy and Environmental Protection (CT DEEP), to develop a scientifically robust biological index for assessing the ecological condition of LIS embayments in both Connecticut and New York.

Phase I focused on a comprehensive review and synthesis of existing estuarine and coastal benthic macroinvertebrate indices used across the United States (Tetra Tech, 2020). The goal was to identify best practices and methodological frameworks that could inform the development of a regionally tailored index for LIS embayments. This effort included a review of over 30 published and unpublished studies on multi-metric indices (MMIs), tolerance indices (e.g., AMBI, M-AMBI), and predictive models (e.g., RIVPACS). It also evaluated sampling designs, reference site designation strategies, metric selection and scoring methods, and index validation approaches. Special attention was given to how natural variability, such as salinity, sediment type, and depth, was accounted for in index development, and how these factors could be addressed in LIS.

Key outcomes of Phase I included a detailed framework for identifying reference and degraded sites using independent stressor data such as sediment chemistry, dissolved oxygen, and toxicity. It provided guidance on metric screening and scoring, including the use of discrimination efficiency (DE), z-scores, and quantile regression, and emphasized the advantages of a multi-metric index approach over tolerance-only models due to its greater sensitivity to a range of stressors. The report also proposed a stepwise workflow for index development, including metric calculation, site classification, index calibration, and validation, and identified potential data sources and gaps. It recommended the use of standardized sampling protocols and consistent taxonomic resolution and highlighted the importance of a robust disturbance gradient to support index development.

These findings laid the groundwork for Phase II, which is the focus of this report. Phase II builds directly on the Phase I recommendations by applying them to a comprehensive dataset of over 200 embayment sites sampled between 2010 and 2021. The result is the development, calibration, and validation of a LIS-specific macroinvertebrate Multimetric Index (MMI) that is scientifically defensible, ecologically meaningful, and aligned with the goals of the LISS CCMP and Clean Water Act (CWA) biological assessment requirements. The structure and methodology of this report directly reflect the framework and recommendations outlined in the Phase I white paper, ensuring continuity and scientific rigor across both phases of the project.

1.2 LONG ISLAND SOUND INDEX DEVELOPMENT

A practical and well-founded indicator of conditions representing biological integrity is a quantitative description of the biota from minimally disturbed sites, defined as reference

conditions. Differences in the quantification of biota between the defined reference condition and other locations indicate degradation. The biological condition of minimally disturbed reference sites, quantified and characterized by individual biological metrics and the Multimetric Index (MMI), serves as a standard for assessing other sites. For site assessment based on such comparisons to be meaningful, it is crucial that reference sites are properly identified, biological data are of known and acceptable quality, natural variability is well described and accounted for, and the metric selection process is structured to be responsive to prevalent stressors. Adhering to these principles ensures that the resulting MMI is optimized for accurate comparisons and valid, defensible assessments, strengthening environmental management decision-making.

The objective of this project is to create an embayment MMI utilizing data collected as part of the National Coastal Condition Assessment (NCCA) and a related NCCA intensification study funded by the EPA LISS NEP to provide an improved understanding of the variability in embayment characteristics.

The MMI development process includes several data preparation and analytical steps:

1. Collect, compile, and subject data to Quality Control (QC) analysis
2. Identify reference and stressed conditions
3. Characterize and account, as needed, for natural variability (site classification)
4. Calculate metrics
5. Identify discriminating metrics
6. Score metrics
7. Combine scores in candidate indices
8. Assess candidate index performance
9. Select optimal index
10. Apply the index for biological assessment

These steps are generally linear but often iterative as analyses proceed and additional questions arise. The MMI development process focuses on optimizing the sensitivity, accuracy, and precision of individual metrics and the final index. This will allow CT DEEP and NY DEC to confidently apply the indices for biological assessments if they choose to do so. The purpose of this report is to summarize the analysis results of the MMI creation for Long Island Sound embayments and to demonstrate the applicability of the index to continued implementation of water quality standards.

2 DATA DESCRIPTION

The National Coastal Condition Assessment (NCCA; formerly known as the National Coastal Assessment, NCA) is a comprehensive monitoring program conducted by the U.S. Environmental Protection Agency (EPA) to evaluate the ecological health and recreational potential of the nation's

coastal and Great Lakes waters¹. NCCA monitoring surveys are conducted every 5 years, using standardized sampling procedures and rigorous quality assurance protocols to identify the percentage of coastal waters in good, fair, and poor condition and assess the impact of key stressors such as nutrients and contaminated sediments at national and regional scales. The assessment provides statistically valid estimates of water quality, sediment quality, benthic community condition, and fish tissue contaminants.

While the NCCA offers a broad overview of coastal waters, this project focuses specifically on developing a regionally tailored embayment biological index. Unlike the NCCA's Northeast coastal work, this work integrates LIS-specific data, local stressor gradient, and macroinvertebrate trait information to create a multimetric index (MMI) that is more sensitive to local ecological conditions. This allows for more precise assessment and management decisions at the embayment scale.

For this project, macroinvertebrate data collected as part of the NCCA from 2010 through 2020/2021 and from the intensification in 2020/2021, were sourced from the official EPA website², which hosts comprehensive information on the National Coastal Condition Assessment (NCCA) surveys. Coastal assessment data downloaded from this source include water chemistry, water quality depth profiles, sediment chemistry, toxicity, and macroinvertebrate abundance and diversity, for the NCCA years 2010 - 2020.

2.1 DATA TREATMENTS

All macroinvertebrate count files from the NCCA and NCA datasets were cleaned and standardized using R Studio (Version 4.3.0, Already Tomorrow) to ensure consistency across datasets from different NCCA years. Information on location (e.g., station identifier), unique sample identifier, visit number, date, and sample type information (e.g., grab area, grab depth, sieve size) were extracted and incorporated into a metadata file. A "Source" field was added to track the origin of each observation, indicating the year and specific survey from which the data were collected.

All benthic macroinvertebrate counts from the NCCA 2010 dataset were combined into a single dataset to streamline analysis and ensure comprehensive data coverage. A subset of the total available macroinvertebrate samples could not be used to evaluate ecological health because insufficient ancillary data were associated with the macroinvertebrate data.

2.1.1 Station Selection and Data Compilation

In collaboration with CT DEEP, the area of interest for MMI development was initially determined to extend from Buzzards Bay in the east to Raritan Bay in the west (Figure 1), with the possibility of narrowing this area if needed. The northernmost point was in Narragansett Bay, and the

¹ [What is the National Coastal Condition Assessment? | US EPA](#)

² [National Coastal Condition Assessment | US EPA](#)

southernmost was in Raritan Bay. This area included 313 stations with macroinvertebrate data, and was chosen to maximize the number of samples available for MMI development,

To be consistent with the objective of developing this index, only embayment sites were retained for further analyses (Figure 1). Tetra Tech selected a candidate list of stations, which were then approved by CT DEEP staff to finalize the list of embayment sites. This selection process involved identifying all stations intersecting the LIS embayment layer and manually selecting other stations with macroinvertebrate data that met the embayment definition developed by Jamie Vaudrey³.

After broadening the geographic scope for MMI development, we ensured that all included stations were comparable to the Connecticut LIS embayment sites by verifying their depth (Maximum Depth = 12.5 meters) and distance to the shoreline (Max Distance = 2,345 meters). Stations that did not meet these criteria were excluded, leaving 211 stations presented in Figure 1 for further consideration. Note that NY sites include LIS, South Shore, NY Harbor, and Hudson River sites.

³ “An embayment is defined as a recess in a coastline or an indentation off a shoreline which forms a bay. In Long Island Sound, the names of embayments often include the words Harbor (27%), River (23%), Cove (19%), Bay (10%), Creek (10%), and Pond (7%); with a few including the names Brook, Gut, Inlet, or Lake. These embayments are where people interact with Long Island Sound. They are the sites of our marinas and host our beaches and parks. People use embayments for kayaking, swimming, fishing, crabbing, and other recreational activities. These areas serve as nurseries and foraging grounds for many commercially and recreationally important species of animals.” – Vaudrey Lab, University of Connecticut

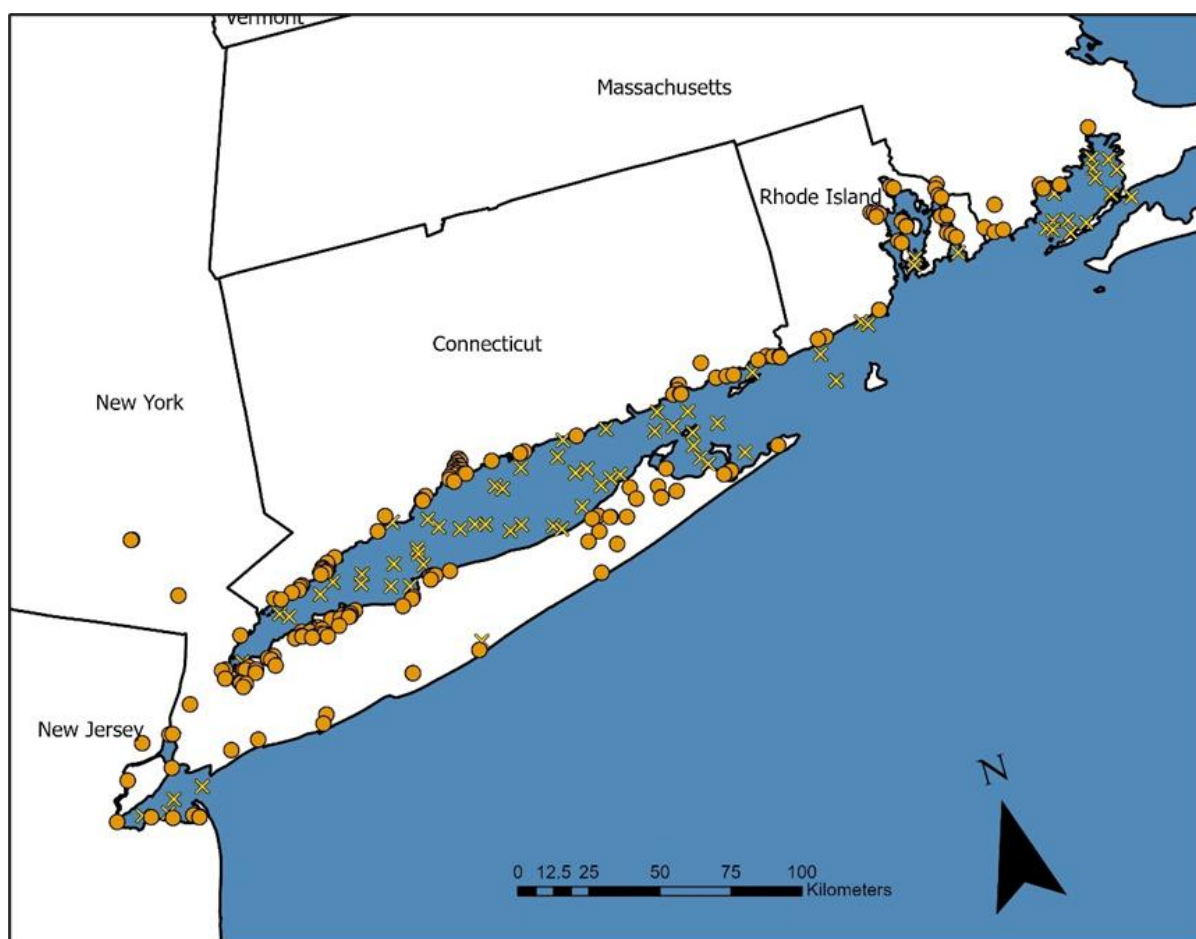


Figure 1. Stations used in the MMI development are shown as orange circles ($n=211$), where the X symbol denotes sites that had macroinvertebrate data available but were removed from further consideration. A total of 313 stations are shown.

Ultimately, the area of interest for this study was defined to encompass embayment sites within the broader Long Island Sound region, extending from Buzzards Bay in Massachusetts to Raritan Bay in New Jersey. This geographic scope was selected to maximize the number of embayment samples available for index development while maintaining ecological relevance to LIS. Sites were included based on their location within or adjacent to LIS embayments, as identified through GIS analysis and manual review. To ensure comparability, only sites with a maximum depth of 12.5 meters and within 2,345 meters of the shoreline were retained. This approach excluded deeper open-water sites and ensured that the selected stations reflected the shallow, nearshore conditions characteristic of LIS embayments. The final area of interest includes embayment sites from Connecticut, New York, Rhode Island, Massachusetts, and New Jersey, with LIS-specific sites forming the core of the index calibration and validation dataset.

The approach to station selection was informed by Phase I recommendations, which emphasized the importance of consistent sampling protocols, comparable gear types, and spatial representativeness across embayments. The use of depth and shoreline distance thresholds aligns with Phase I guidance on defining embayment-specific conditions.

2.1.2 Macroinvertebrate Data

Data compilation focused first on the availability of macroinvertebrate samples, essential for the index described in this report. A list of stations and samples was created to link biological data with sediment and water chemistry, as well as vertical profile data. This section describes the data compilation process to assign traits and taxonomic information to macroinvertebrates.

2.1.2.1 Macroinvertebrate Traits

Trait Compilation

During the initial phase of macroinvertebrate data compilation, all distinct taxa were extracted, and their taxonomic classifications were updated from the NCCA data files. Taxonomic levels were standardized where applicable, resulting in 420 different genera, 216 families, 72 orders, 30 classes, and 15 phyla. From this, a table of unique taxa ($n = 911$) was compiled from the initial 313 stations, retaining the original NCCA names. Trait information, such as body size, feeding habits, and mode of movement, was added for each taxon to enable the calculation of trait-based metrics. Except for AMBI traits, no comprehensive trait information had previously been compiled for macroinvertebrates in the New England/East Coast US region. Therefore, Tetra Tech sourced and compiled macroinvertebrate traits from various databases and studies, including:

- The Gulf of Mexico Traits from the 2011 Tetra Tech Report: Development and application of a multi-metric benthic index to assess estuarine and near-coastal ecological health in the Gulf of Mexico Alliance and the Mississippi DEQ from 2011.
- The New Zealand Trait Database for Marine Benthic Invertebrates: A comprehensive assessment of microbenthic traits from New Zealand, providing trait information for over 700 taxa.
- Gillett et al. (2015) AMBI: Examines the effect of ecological group classification schemes on the performance of the AZTI Marine Biotic Index in U.S. coastal waters.
- MacDonald et al. (2010) traits: Taxonomic and feeding guild classification for marine benthic macroinvertebrates in the Strait of Georgia, British Columbia.
- Queiros et al. (20130) traits: A bioturbation classification of European marine infaunal invertebrates, providing a functional classification for 1033 species to standardize the calculation of bioturbation potential.
- Polytraits: A database on biological traits of polychaetes (bristle worms) covering 47 traits and categories related to morphology, behavior, and reproduction. Maintained by the Hellenic Centre for Marine Research.
- Gulf of Mexico (GOM) trait information was used for taxa in common with the Long Island Sound (LIS), noting that the GOM database provided only functional feeding group information.

Additional resources, such as the San Francisco Bay Benthic Macroinvertebrate Atlas and Degen et al. (2019), were utilized as supplementary information.

Once traits were joined to the NCCA taxa, the following steps were applied to all traits except AMBI:

- Traits were standardized to common, broader groups to match between datasets. For instance, all functional feeding groups were grouped into one of six categories: deposit feeders, sub-surface deposit feeders, predators, filter/suspension feeders, scavengers/opportunists, and grazers/scrapers. The GOM, NZ, MacDonald, and Queiros datasets were used for this step, with other resources supplementing the missing data.
- Groups that were too broad (more than three different functional feeding groups) were classified as generalists in the main traits table. These groups included combinations such as deposit feeder; filter/suspension; predator; scavenger/opportunist.
- The New Zealand traits database included a greater level of detail than was available from most other sources; therefore, traits were sometimes consolidated for consistency. For example, the New Zealand database included four sub-categories under "bioturbation" (biodiffusor, bioirrigator, no bioturbation, surface modifier), which were combined into a single "Bioturbation" category. If a taxon had a value greater than 0 in more than one bioturbation column, all such sub-categories were included in the newly created column and separated by a semicolon.

This approach ensured that the trait data were standardized, facilitating taxonomic trait aggregation outlined in the next section.

Taxonomic Trait Aggregation

Tetra Tech aggregated traits at both the genus and family levels to compile and complete traits of the LIS taxa. While the approach was largely similar for both taxonomic classifications, logical differences were applied where necessary.

For genus-level trait standardization, Tetra Tech started with 911 taxa and their biological classification information. Taxa lacking genus-level information (either due to missing data or being identified only to a higher taxonomic level, such as family) were excluded. Genera that appeared only once in the taxa list were also removed from further analysis. This process was repeated for each trait separately (Functional Feeding Group (FFG), motility, bioturbation, and life habit). Distinct observations were recorded across three columns: genus, the trait under consideration, and the initial count of that genus. The number of unique Genus-Trait combinations was calculated, and genera missing trait values were identified. Genera with only one consistent trait value were noted (allowing for missing values), indicating that the trait was consistent within that genus and could be applied to all instances of that genus.

For example, the genus *Exogone* appeared in various forms (e.g., *Exogone* sp., *Exogone lourie*, *Exogone dispar*) and was classified into two different FFGs: predator and grazer/scrapper, with some instances having unknown FFG categories. Due to this inconsistency, *Exogone* was unsuitable for completing the FFG trait at the genus level. In contrast, the genus *Nephtys* appeared eight times and was classified as a predator in six out of the eight occurrences, allowing us to reasonably assume that all *Nephtys* species are predators. Family-level trait compilation followed the same procedure, with the key difference being the use of family-level rather than genus-level data. Family-level traits required a family to occur at least four times to be considered, and traits consistent across these occurrences were assigned to the taxa.

The traits were integrated with the unique genera in the dataset, producing the final set of traits to be used in metrics calculation. This process involved assigning available traits to each taxon, with

genus-level traits preferred over family-level traits. This integration significantly improved the proportion of available traits per category. Additional traits were filled from various sources for taxa frequently observed, especially those occurring in more than 10 of the 331 samples collected from 313 stations (including replicates in some stations).

2.1.3 Water and Sediment Quality Data

Water Chemistry

Water chemistry data (for 2010, 2015, and 2020) matching the samples and stations at which macroinvertebrates were collected were also compiled from the NCCA website. All data were consolidated into a single data frame, with a column added to each observation to track its origin. Values that were reported as not applicable/available (NA) were converted to half of the method detection limit, where available. All comments were concatenated into a single column. The processed dataset includes results for total phosphorus, total nitrogen, ammonia, nitrate and nitrite, dissolved inorganic nitrogen, soluble reactive phosphorus, pH, conductivity, chlorophyll a, nitrite, and nitrate. This step yielded water chemistry data for 220 samples across 211 stations, with a varying degree of completeness across all mentioned variables per sample.

Sediment Chemistry

Sediment chemistry data were compiled for the mentioned stations. One-half of the method detection limit was used where the result value was either not available (NA) or 0. Each observation includes a corresponding source file name in the Source column of the data frame. Chemical naming inconsistencies were addressed by standardizing these names in a new column for the chemicals of interest. Sediment chemistry data were available for 211 samples across 219 stations.

Sediment Toxicity

NCCA's sediment toxicity summary files were used to obtain the mean survival and control-corrected survival proportions for the samples of interest. This dataset yielded 199 samples across 207 stations, making sediment toxicity the dataset with the fewest available samples. All NA control-corrected survival values were removed, assuming the remaining data had undergone the QC process before publication, as indicated by the comments in the NCCA dataset.

Vertical Profile Data

The same steps taken with the above datasets were applied to the vertical profile data. This dataset allowed Tetra Tech to extract the station depth, and with that, bottom (maximum depth) salinity and dissolved oxygen for each sample. Vertical profile data was available for 211 samples across 219 stations.

3 REFERENCE AND STRESSED SITE IDENTIFICATION

The foundation of biological assessment lies in comparing conditions observed at a test site to those found at high-quality reference sites. These reference conditions represent the best available ecological status, undisturbed, or with very little disturbance by human activities, and serve as benchmarks for evaluating other sites. Developing a disturbance gradient is crucial when creating an MMI because it quantifies the extent of human impact on a site, providing a clear measure of

deviation from reference, or unstressed conditions. This index helps in identifying and categorizing stressors such as pollution and habitat alteration, which can significantly affect biological communities.

The criteria used to define reference and stressed sites were adapted from the Phase I review, which highlighted the use of dissolved oxygen, sediment chemistry, and toxicity thresholds as standard practice in estuarine index development. The decision to use a combination of these parameters, along with quantile-based thresholds, reflects the multi-criteria approach recommended in Phase I.

By incorporating a disturbance gradient, we can more accurately assess the health of aquatic ecosystems. It allows for the differentiation between natural variability and human-induced changes, ensuring that the MMI reflects true ecological conditions rather than anomalies. This approach enhances the reliability and validity of the MMI. This section outlines the process of development of the LIS MMI.

3.1 PARAMETERS

All candidate parameters mentioned in this section were considered for inclusion in the disturbance gradient.

3.1.1 Landscape Development Intensity and Imperviousness

The calculation of a landscape development intensity (LDI) closely followed the approach outlined in a 2011 study in the Virgin Islands (Oliver et al., 2011), where an LDI, first developed by Brown and Vivas (2005), was used to assign each watershed an index reflecting its effects on near-shore communities. Watersheds were delineated using geospatial tools and various datasets, and an LDI coefficient was calculated based on land-use categories and their cumulative, nonrenewable energy input. Sites were assigned to watersheds based on proximity, considering the effects of currents on downstream sites.

A similar approach was used in Puerto Rico's Canal Luis Pera Natural Reserve (Hernandez et al., 2017), where sediment-laden and nutrient-loaded runoff significantly impacted coastal coral reefs. Studies found that sites frequently disturbed by such runoff were dominated by macroalgae and had lower coral species richness. In 2018, another study in the Virgin Islands used LDI (Oliver et al., 2018), sedimentation threat, and water quality impairments to characterize anthropogenic activity. An integrated stressor index (ISI) was assigned to each watershed, considering factors like dissolved oxygen, bacteria, and nutrient concentrations.

The common theme among these studies is the emphasis on the importance of a station's proximity to watersheds. For example, Carlson et al. (2019) provide a comprehensive analysis of the impacts of these stressors, highlighting several key points. Forest and vegetative land cover play a critical role in absorbing rainfall, thereby reducing freshwater inputs to marine habitats. They also stabilize soils, minimize erosion and sediment flux, absorb and fix soil nutrients, mineralize heavy metals, and provide essential habitat for fish. Repeatedly, research has shown a positive correlation between native forest cover and coral condition metrics.

While many studies address macroinvertebrates in coastal embayments, few investigate the process of categorizing each site by disturbance level. The Massachusetts Department of

Environmental Protection (Mass DEP, 2014) outlines methods for such assessments, classifying habitats as healthy, moderately impaired, significantly impaired, or severely degraded. Challenges in estuarine studies, such as the "estuarine quality paradox" (Elliot and Quintino, 2007), complicate the detection of human-induced stress due to natural variability in estuarine communities.

Additionally, in a detailed review written by Borja et al. (2011), significant challenges in estuarine studies, some of which may also apply to embayments, are discussed. The review mentions the "estuarine quality paradox" (developed by Dauvin, 2007 and Elliott & Quintino 2007), which suggests that the natural variability in estuarine communities — including naturally stressed areas — can be confused with anthropogenically-stressed regions. Additionally, since estuaries are naturally organic-rich environments, their biota can resemble those in human-impacted areas, potentially leading to flawed indices for environmental improvement. The review also examines various groups of organisms and how stress manifests among them. For example, nutrient enrichment can result in seagrass loss and low dissolved oxygen levels due to increased phytoplankton primary production (Chlorophyll a).

The review also references Remane's diagram, illustrating the presence of "true estuarine organisms" flanked by freshwater and marine species along the salinity gradient. The review also discusses the strengths and limitations of using macroinvertebrates in such studies. Macroinvertebrates are relatively sedentary, with long lifespans, and encompass diverse species with varying stress tolerances. They play a crucial role in nutrient and material cycling between sediments and the water column. However, they provide a static snapshot of ecological conditions and are not explicitly linked to changes in ecological function, which may limit their specificity in relation to different stressors.

3.1.1.1 Watersheds

The Watershed Boundary Dataset (WBD), as briefly explained by the EnviroAtlas fact sheet ([Link](#)), includes over 90,000 local sub-watersheds that capture tributary systems smaller than subbasin and large river basin watersheds (HUC 8 and HUC 4, respectively). It is important to note the distinction between watersheds and hydrologic unit codes (HUCs): the area contributing to the downstream outlet point within a single HUC may extend beyond its boundaries in an upstream direction, encompassing several other subbasin HUCs.

Given the absence of HUC 14 watersheds in the area of interest (Figure 1), we utilized HUC 12 watersheds to delineate immediate, adjacent watershed zones around each embayment where monitoring stations are located. Each designated watershed was given a unique identifier. Watersheds were merged to better represent their effect on the embayments (Figure 2A). They were also clipped using state boundaries, so no HUC 12 watershed extended into the embayment or ocean (Figure 2B).

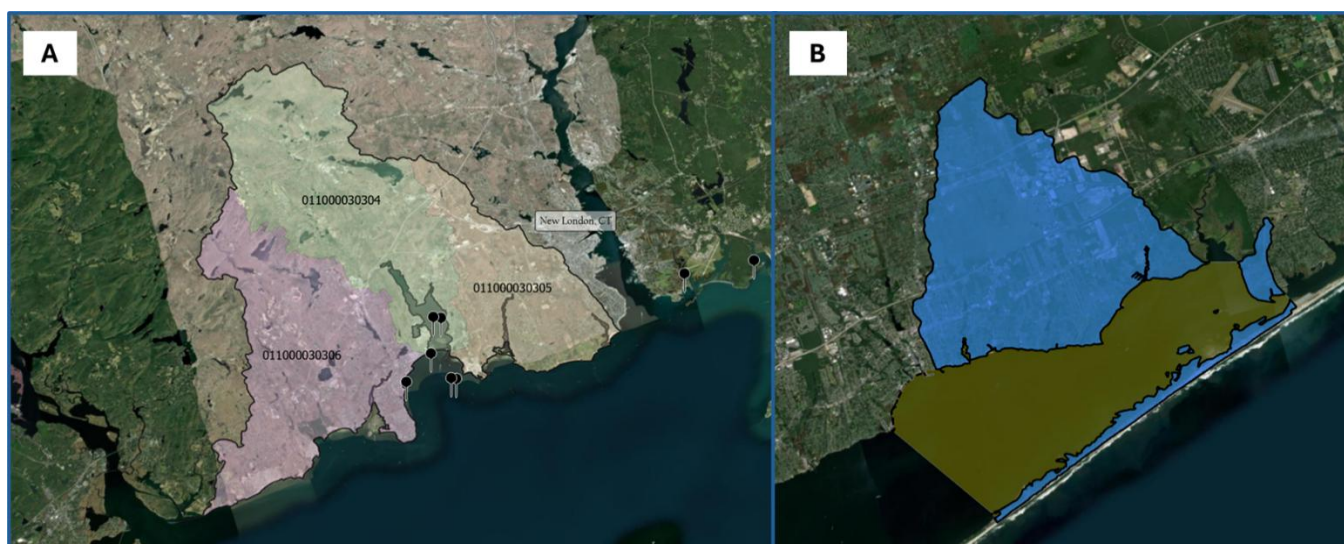


Figure 2. Satellite imagery depicting (A) how three HUC12 watersheds with their IDs shown were combined to create a single, embayment-specific watershed (Unique ID = 7) just west of New London, CT, and (B) the extent of the original HUC 12 watershed (blue and yellow area combined), and the clipped area of the watershed (blue) that will be used for land use summarization. Note that the yellow area is fully overlaying the water.

3.1.1.2 Calculation

Land Use and Land Cover

Given that samples from the selected stations (Figure 1) were collected over 11 years (2010-2021), it was essential to assess whether significant changes in land cover had occurred. To address this, we utilized the National Land Cover Database (NLCD) Land Cover Change Index to evaluate temporal changes in land cover. The proportion of each watershed that experienced change was calculated as a percentage, with a focus on urban and agricultural land covers. This analysis informed Tetra Tech of the necessity to incorporate NLCD data for each year. Several watersheds exhibited substantial land cover changes, prompting the decision to provide a summary of land cover layers for the years 2011, 2016, and 2021 to account for these changes.

Land use and land cover (LULC) data for the years 2011, 2016, and 2021 were obtained from the Multi-Resolution Land Characteristics Consortium website⁴. These data were imported into ArcGIS and analyzed using the “Tabulate Area” tool from the Spatial Analyst toolbox for each respective year. The raster cell counts per area were converted into percentages representing the land cover for each specific category.

It is important to note that the percentages for imperviousness and NLCD land use categories were derived from two separate ArcGIS layers, both with a 30-meter resolution. When adding the percentage of impervious land to other land cover categories, the total percentage per unit area

⁴ [Data | Multi-Resolution Land Characteristics \(MRLC\) Consortium](#)

may exceed 100%. This is because imperviousness is already incorporated in the developed low-, medium-, and high-intensity land use categories, as well as the developed open space category.

Landscape Development Intensity

Categories from the National Land Cover Database (NLCD) were aligned with the land cover categories presented in Oliver et al. (2011) (Table 1), ensuring that each category had a corresponding LDI coefficient. The LDI was calculated using the following formula:

$$LDI_{total} = \sum \%LUi \times LDI_i$$

where LDI_{total} is the LDI score for the watershed, $\%LUi$ is the percent of the total area of influence of a given land use type, and LDI_i is the landscape development intensity coefficient.

Table 1. A translation table of NLCD's LULC categories obtained for the LIS watersheds to those categories used by Oliver et al., 2011 to enable the calculation of LDI.

<i>NLCD Category</i>	<i>Oliver 2011 Category</i>	<i>Oliver 2011 LDI</i>
<i>PCTIMPV</i>	Impervious surface	8.28
<i>OPEN_WATER</i>	Open water	1
<i>DEVELOPED_OPEN_SPACE</i>	Developed, open space	1.85
<i>DEVELOPED_LOW_INTENSITY</i>	Impervious surface	8.28
<i>DEVELOPED_MEDIUM_INTENSITY</i>	Impervious surface	8.28
<i>DEVELOPED_HIGH_INTENSITY</i>	Impervious surface	8.28
<i>BARREN_LAND</i>	Bare land	1.85
<i>DECIDUOUS_FOREST</i>	Deciduous forest	1
<i>EVERGREEN_FOREST</i>	Evergreen forest	1
<i>MIXED_FOREST</i>	Mixed forest	1
<i>SHRUB_SCRUB</i>	Scrub/shrub	2.06
<i>HERBACEOUS</i>	Grassland/Herbaceous	2.06
<i>HAY_PASTURE</i>	Pasture/Hay	3.03
<i>CULTIVATED_CROPS</i>	Cultivated crops	4.42
<i>WOODY_WETLANDS</i>	Forested wetland	1
<i>EMERGENT_HERBACEOUS_WETLANDS</i>	Emergent wetland	1

Accounting for Percent Imperviousness

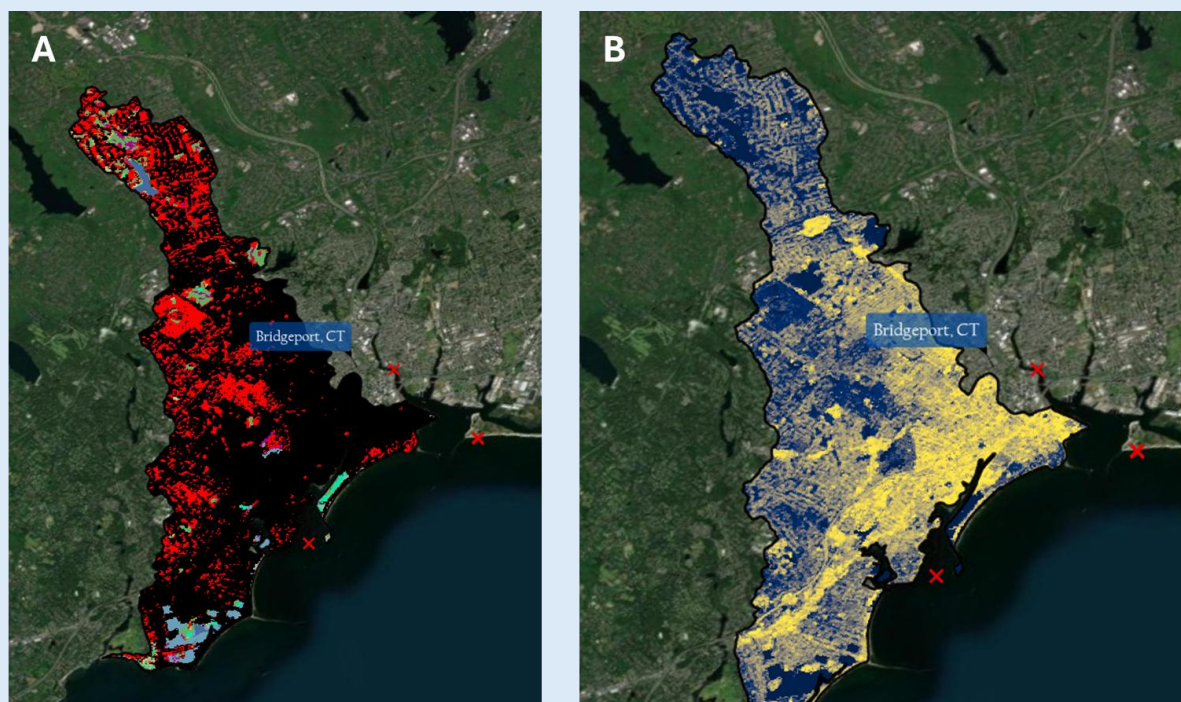
The percent imperviousness within a watershed often overlaps with areas classified as low-, medium-, and high-intensity developed land, leading to potential double counting when these categories are combined with impervious surfaces. To address this, it was decided to exclude

developed land categorized as low-, medium-, and high-intensity since these categories were not included in Table 2 of Oliver et al. (2011). This exclusion occasionally resulted in the total land use/land cover (LULC) percentage being less than 100%.

To rectify this, the Tetra Tech team decided that the remaining percentage of watershed LULC should be assigned to the "Developed, open space" category, as all impervious surfaces were already accounted for in the other developed land cover groups. For instance, in watershed 11 (each watershed was assigned a unique number ID) in 2011, the total LULC sums to approximately 55% after excluding low-, medium-, and high-intensity developed land, and after accounting for impervious surfaces. The remaining 45% was allocated to the "Developed, open space" category (see Info Box 1).

Info Box 1

When examining the land use cover of Watershed 11 in the Bridgeport, CT area, it becomes clear that there is significant overlap between low-, medium-, and high-intensity developed land (Map A, black area) and the percent imperviousness (Map B, bright yellow). This overlap suggests that it is logical to consider one while accounting for the other, as they complement each other for the most part. Ultimately, every pixel (30 m x 30m) that is not covered by the imperviousness or other land use type was counted as "Developed, open space" land use category.



The NLCD defines developed, open space land cover category as: "Areas with a mixture of some constructed materials but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20% of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes." This definition supported our decision to use

this category over others. While "Grassland/herbaceous" was considered as an alternative, it was not chosen due to its higher LDI coefficient, which could have led to an overestimation of the LDI. Ultimately, the suggested category was added to the previously calculated land cover categories and its values were combined with the existing open space category values.

3.1.1.3 Results

The LDI is expected to most accurately represent the levels of imperviousness and development in a watershed, given that these land use categories have the highest LDI coefficients, per Brown and Vivas (2005). This was the case in the 79 watersheds associated with the embayment stations (Figure 3), where the highest correlation between LDI and a land use variable was with percent imperviousness (mean of 2011, 2016, and 2021 NLCD imperviousness).

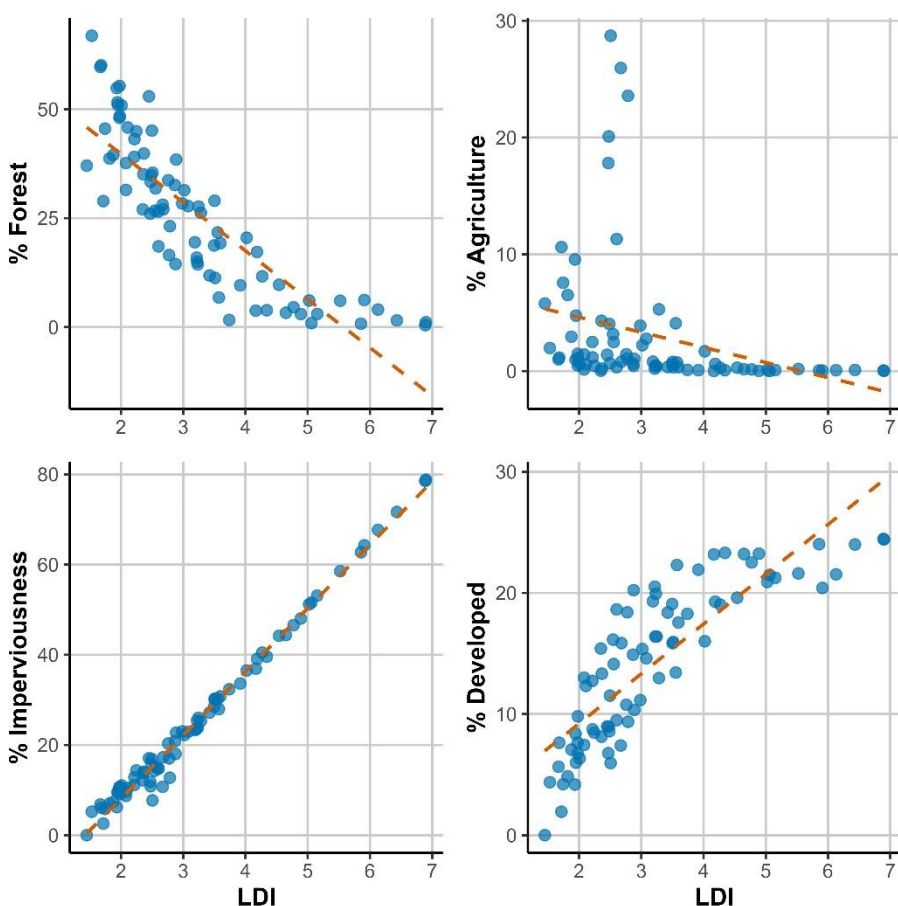


Figure 3. Scatter plots of LDI with % forest, agriculture, imperviousness, and development across 79 watersheds attributed to the selected sites.

A closer spatial investigation of watershed LDI across the region of interest reveals somewhat of a clustered pattern throughout the area of interest (AOI). In Connecticut, stressed watersheds appear to be around Bridgeport, Stamford, and New Haven, and become scarcer in the central and eastern portion of the state (Figure 4). A large portion of Narragansett Bay is surrounded by more developed watersheds, while this is not the case in the Buzzards Bay watersheds, with only the

New Bedford area having higher LDI. As expected, all NYC-adjacent watersheds have a high LDI as well, with several smaller upstream watersheds being an exception to this pattern.

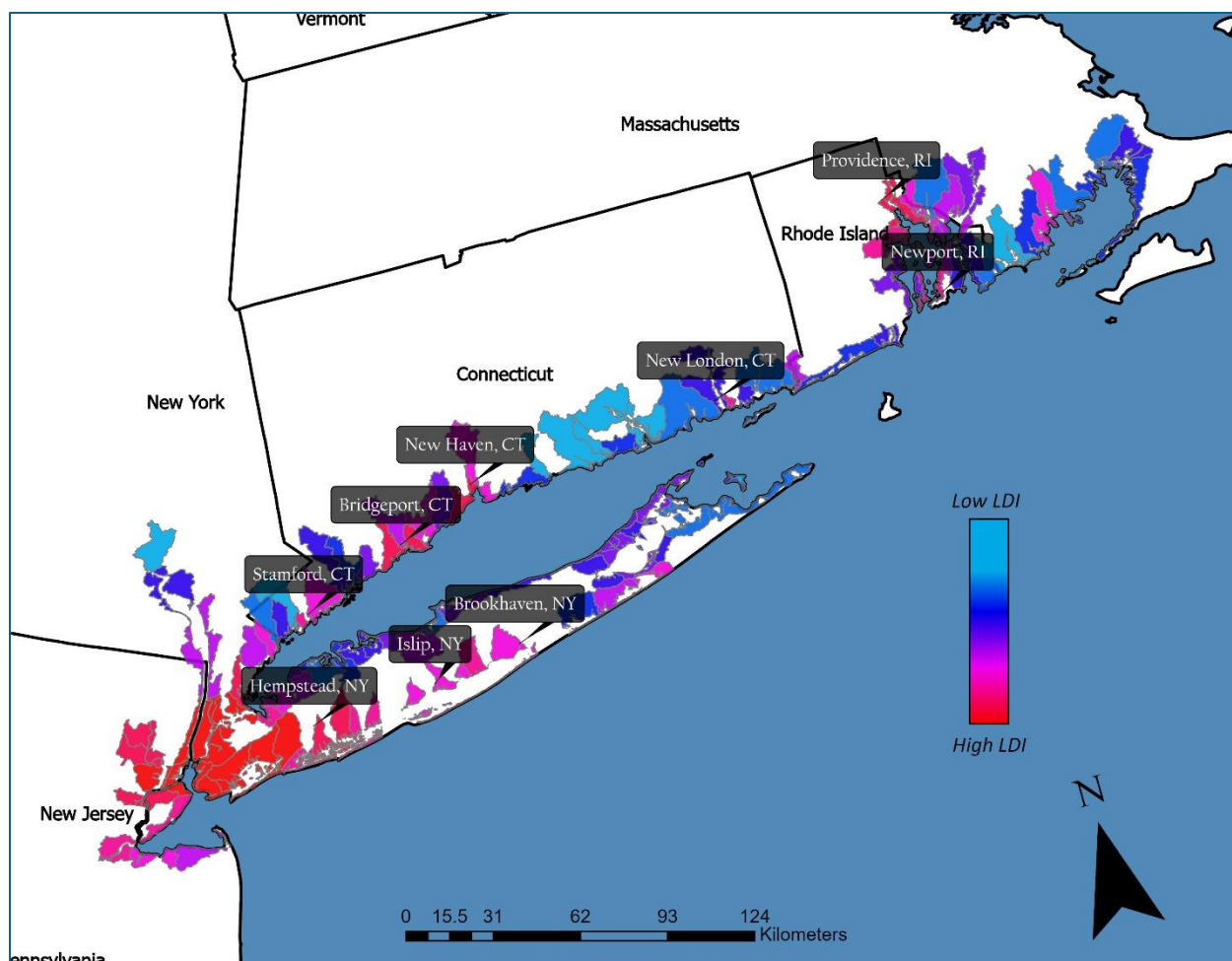


Figure 4. A map of the area of interest shows more stressed, higher LDI watersheds in red and orange, and less stressed, lower LDI watersheds in blue and light blue.

Because of the high LDI-imperviousness correlation, it was determined that both the LDI and imperviousness would be further investigated for inclusion in the disturbance gradient.

3.1.2 Sediment Contamination

The sediment contamination analysis was conducted through a systematic approach aimed at evaluating the presence and impact of various contaminants in sediment samples. This approach closely followed the methods outlined in the 2015 NCCA Technical Report (US EPA, 2020). In brief, sediment samples were collected from designated sites and analyzed using standardized laboratory methods. The collected sediment was analyzed in the lab for physical characteristics of the sediment, including total organic carbon, percent solids, and grain size. To assess potentially toxic levels of chemicals/compounds in the sediments, sediment quality guidelines (SQGs) were applied to calculate mean effects-range median quotients (mERM-Q). Additionally, a logistic regression model (LRM) was utilized to predict the probability of toxicity based on contaminant

concentrations, generating a maximum probability of toxicity (Pmax). All contaminants were tested for method detection limit (MDL) and LRM T25 (threshold concentration at which there is a 25% probability of observing a toxic effect on benthic organisms) exceedance. The contaminants used for calculations of the mERM-Q and LRM are shown in Table 2.

Table 2. Contaminants with estuarine sediment quality guidelines (SQG) included in calculating the mERM-Q and LRM.

<i>Contaminant Name</i>	<i>Included in mERM-Q</i>	<i>Included in LRM</i>
<i>Antimony</i>		X
<i>Arsenic</i>	X	X
<i>Cadmium</i>	X	X
<i>Chromium</i>	X	X
<i>Copper</i>	X	X
<i>Lead</i>	X	X
<i>Mercury</i>	X	X
<i>Nickel</i>		X
<i>Silver</i>	X	X
<i>Zinc</i>	X	X
<i>Acenaphthene</i>	X	X
<i>Acenaphthylene</i>	X	X
<i>Anthracene</i>	X	X
<i>Benz(a)anthracene</i>	X	X
<i>Benzo(b)fluoranthene</i>		X
<i>Benzo(k)fluoranthene</i>		X
<i>Benzo(a)pyrene</i>	X	X
<i>Biphenyl</i>		X
<i>Chrysene</i>	X	X
<i>Dibenz(a,h)anthracene</i>	X	X
<i>2,6-dimethylnaphthalene</i>		X
<i>Fluoranthene</i>	X	X
<i>Fluorene</i>	X	X
<i>Indeno(1,2,3-c,d)pyrene</i>		X
<i>1-methylnaphthalene</i>		X
<i>2-methylnaphthalene</i>	X	X
<i>1-methylphenanthrene</i>		X
<i>Naphthalene</i>	X	X
<i>Perylene</i>		X
<i>Phenanthrene</i>	X	X
<i>Pyrene</i>	X	X
<i>TotalPCBcongeners*</i>	X	X
<i>4,4'-DDD</i>		X

Contaminant Name	Included in mERM-Q	Included in LRM
4,4'-DDE		x
4,4'-DDT		x
TotalDDT**	x	
Dieldrin		x

*Total PCBs included the following congeners: 8, 18, 28, 44, 52, 66, 77, 101, 105, 110, 118, 126, 128, 138, 153, 170, 180, 187, 195, 206, 209

**Total DDT represents the sum of 4,4'-DDT; 2,4'-DDT; 4,4'-DDE; 2,4'-DDE; 4,4'-DDD; 2,4'-DDD

Using the existing benchmarks for the NCCA Sediment Contamination Index (SCI), out of 219 samples sampled within the greater Long Island Sound area (Figure 1) 140 of them were deemed as good, 17 as fair, 5 as poor, and 57 remained uncategorized (i.e., did not fall into any of the three groups outlined in Table 6.4 of the 2015 NCCA Technical Report). This suggested that these thresholds needed to be adjusted for the Long Island Sound disturbance gradient since there were too few sites in the poor category. Figure 5 shows the distribution of samples across the mERM-Q and P maximum gradients, illustrating the distribution of sediment contamination levels across various states. The visual representation allows for a clear comparison of contamination levels between states.

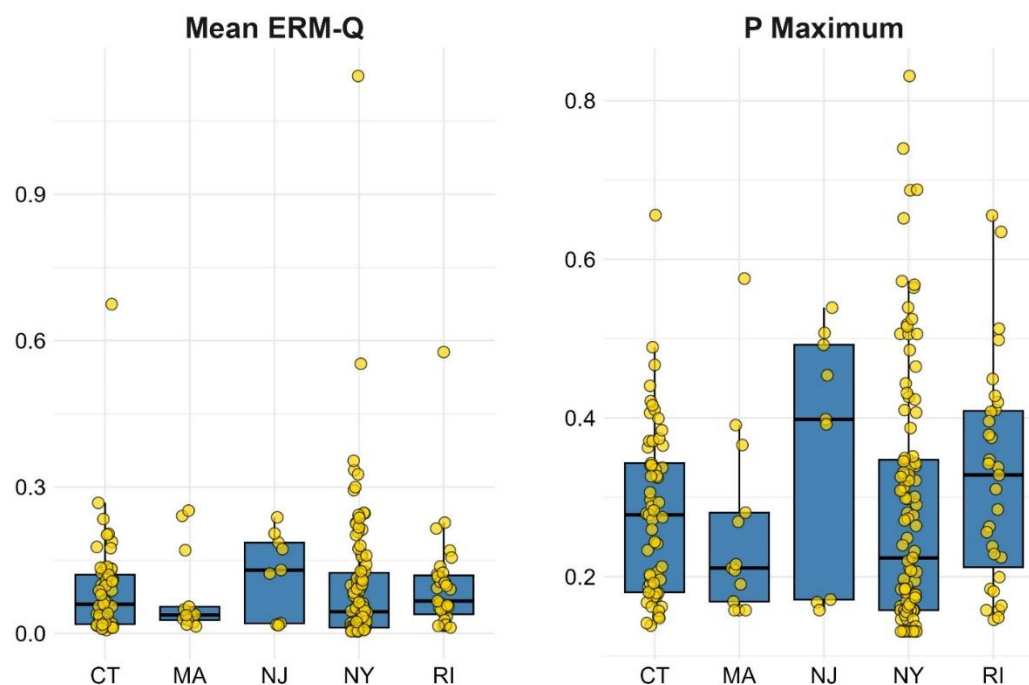


Figure 5. Boxplots show the distribution of mERM-Q and P maximum across all samples in the area of interest. An outlier from sample 2014950 is not shown (mERM-q = 10.7).

From the analysis, distinct patterns in sediment contamination can be observed. For instance, certain states exhibited lower contamination levels than others (e.g. Massachusetts). The presence of outliers in some boxplots further highlights the variability in contamination levels, which could be attributed to specific anthropogenic activities (e.g. New York City) or natural factors affecting

sediment quality (e.g. Cadmium, Lead, and Mercury). Interestingly, neither of the two parameters is significantly different across states (One-way ANOVA; $p > 0.05$).

3.1.3 Sediment Toxicity

Laboratories subcontracted by NCCA conducted sediment toxicity tests to assess the survival rate of laboratory amphipods after 10 days of exposure to sediment samples. They ran control tests simultaneously, using reference sediments. The same batch of organisms was utilized for both the sample and control tests, with water sourced from the same locations. Estuarine samples had 5 replicate chambers with 20 organisms each, with a minimum of 90% survival of control organisms needed to meet test acceptability criteria (NCCA Technical Report, 2015).

These tests provided a second line of evidence to assess whether sediments can support healthy benthos. Control-corrected survival was used to assess the sediment samples and was calculated by dividing the sample mean percent survival by the control mean percent survival. Some replicates were removed from the analysis due to QA/QC concerns, and, in such instances, those samples were not used to calculate the control-adjusted survival for the sample (Figure 6).

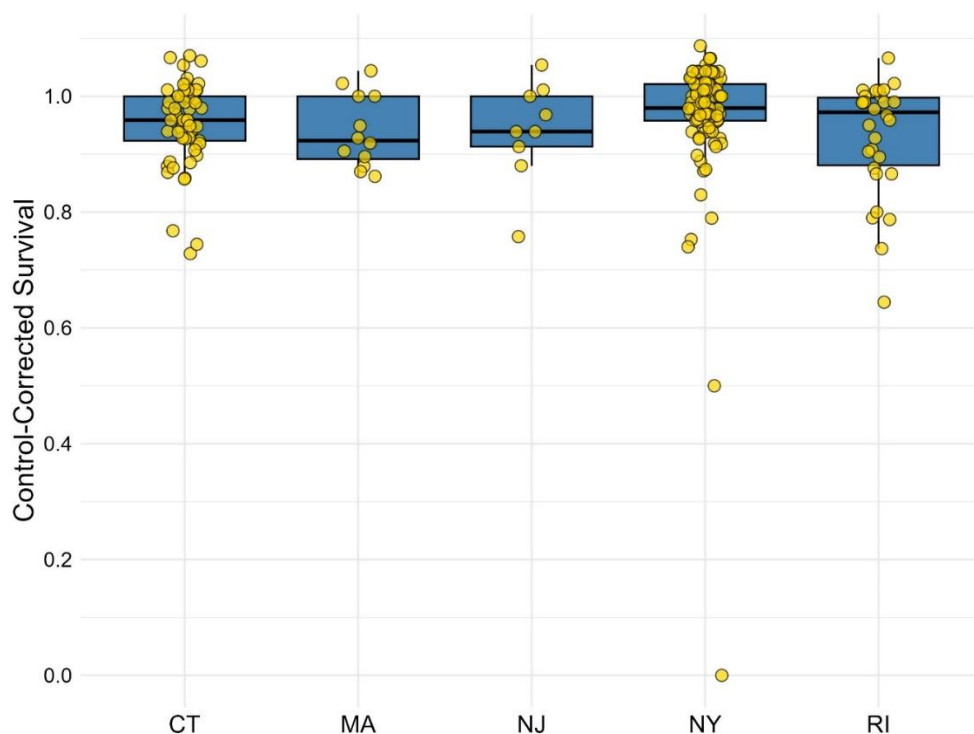


Figure 6. Boxplots illustrating the control-corrected survival rate (proportion) across the 5 states.

The analysis of control-corrected survival rates revealed no statistically significant differences in survival outcomes between states (One-way ANOVA; $p > 0.05$). The presence of outliers in both groups indicates some variability in survival responses.

3.1.4 Water Quality

The NCCA has employed a standardized methodology for calculating the Estuarine Eutrophication Index, which incorporates key factors such as surface nutrients, chlorophyll-a levels, bottom water

dissolved oxygen (DO), and water clarity. After compiling these variables, Tetra Tech wanted to calculate a light attenuation factor (K_d), which is the measure of how quickly light diminishes in intensity as it travels through water (i.e., it indicates the rate at which light is absorbed or scattered in the water column). However, when these calculated K_d values were compared to those from the 2015 dataset, significant discrepancies were observed, leading to their classification as inaccurate. Despite numerous attempts to reconcile these differences and produce comparable values, these efforts were unsuccessful. Nevertheless, data for all other variables were successfully compiled and are presented in Figure 7, providing valuable insights into the overall conditions of the estuarine environment.

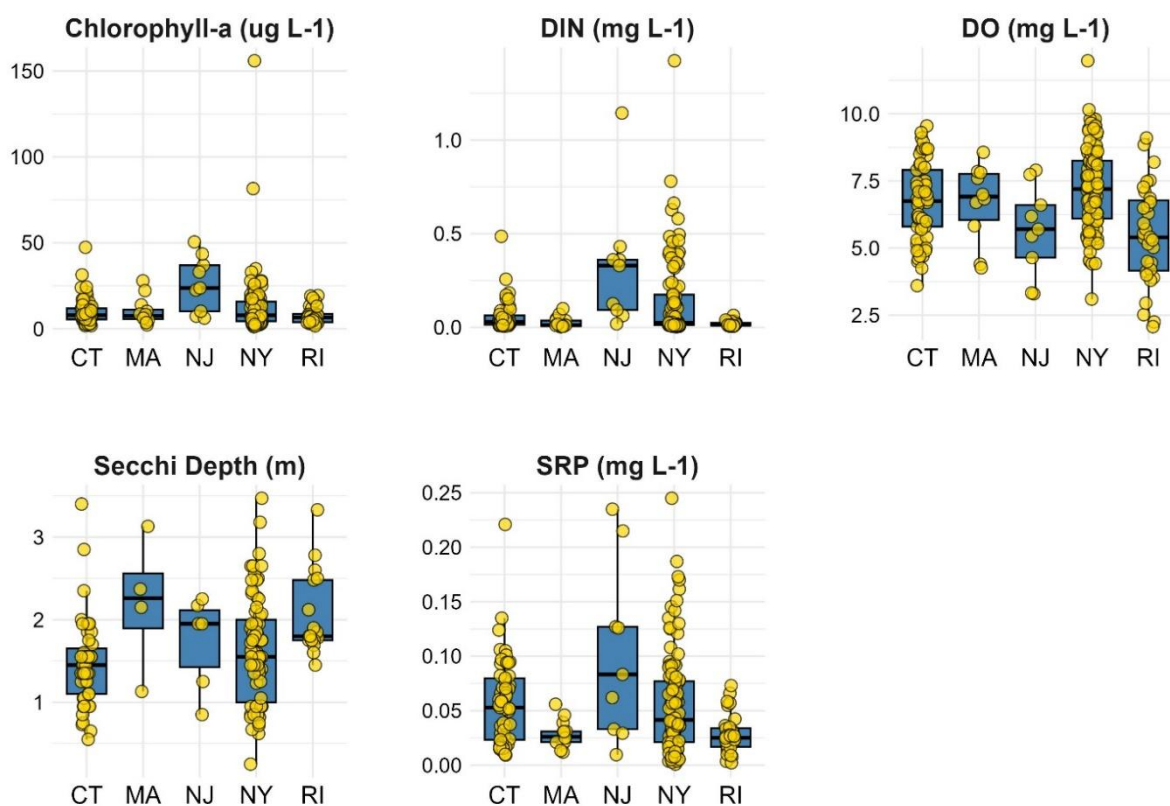


Figure 7. Boxplots of five variables from selected samples used in the calculation of the NCCA's eutrophication index. Note that while the NCCA utilized light transmittance at 1m depth to represent water clarity, Tetra Tech was unable to align the calculated light transmittance with the available NCCA values. Therefore, these values were dropped, and Secchi depth was used instead. These boxplots and variables were compiled and created by Tetra Tech. DIN (Dissolved Inorganic Nitrogen), DO (Dissolved Oxygen), and SRP (Soluble Reactive Phosphorus).

Figure 7 provides a detailed visualization of various water quality parameters across states in the region spanning from Raritan Bay to Buzzard Bay, highlighting notable differences. Connecticut's water quality data shows significant differences compared to other states in several parameters: Dissolved Oxygen with Rhode Island ($p < 0.01$), Chlorophyll-a with New Jersey ($p < 0.01$), Dissolved Inorganic Nitrogen with New Jersey ($p < 0.01$) and New York ($p < 0.05$), Soluble Reactive Phosphorus with New Jersey ($p < 0.05$) and Rhode Island ($p < 0.05$), and Secchi Depth

with Rhode Island ($p < 0.05$). Additionally, the observed differences may be exaggerated due to the lower number of samples in Rhode Island and New Jersey, as well as the area bias for New Jersey and Massachusetts, since only Raritan Bay and Buzzards Bay sites were considered, respectively.

New Jersey stands out with a notably higher median Chlorophyll-a concentration compared to Connecticut ($p < 0.01$) and also shows significantly higher levels than New York and Rhode Island ($p < 0.05$). Dissolved Inorganic Nitrogen (DIN) varies significantly, with New Jersey exhibiting higher concentrations than Connecticut ($p < 0.01$) and Massachusetts ($p < 0.01$). Conversely, Rhode Island has significantly lower DIN concentrations compared to New Jersey and New York ($p < 0.01$). For Soluble Reactive Phosphorus (SRP), New Jersey again shows higher levels than Connecticut ($p < 0.05$) and Massachusetts ($p < 0.001$), while Rhode Island has lower SRP concentrations compared to New Jersey ($p < 0.001$) and New York ($p < 0.05$). Secchi Depth, a measure of water clarity, is significantly higher in Rhode Island compared to Connecticut ($p < 0.01$).

These findings underscore the variability in water quality across the region and highlight the importance of considering regional data to enhance the robustness of environmental assessments. By incorporating data from neighboring states, we can better identify overarching patterns and influences that affect water quality, leading to a more effective index that addresses both local and broader regional factors.

3.2 STRESSOR SELECTION

All variables commonly utilized by the NCCA to evaluate the health of a specific site were consolidated into a single data frame, allowing for a comprehensive exploration of their interrelationships. The primary objective of this analysis is to identify and eliminate any redundancy among the disturbance variables, particularly as we develop a disturbance gradient. By understanding these relationships, we can enhance the accuracy and effectiveness of the index.

In our investigation, we focused on two critical aspects of the selected variables: their correlations (Figure 8) and their ability to cluster sites within the multivariate space defined by Principal Component Analysis (PCA; Figure 9). This approach enabled us to assess how these variables collectively influence site disturbance classification when all effects are considered. By examining both the correlations and the PCA groupings, we aimed to gain deeper insights into the underlying patterns of disturbance and site health, ultimately leading to a better-informed MMI.

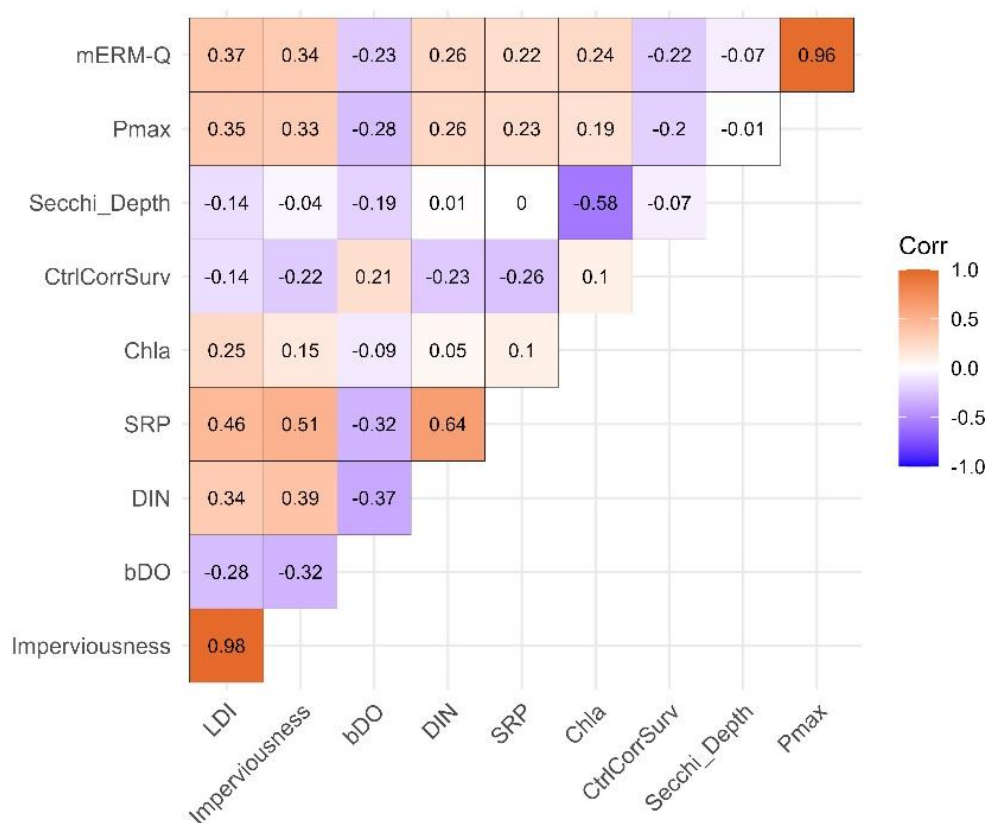


Figure 8. A correlation plot between all variables considered for the disturbance gradient that NCCA commonly employs for such decisions. bDO = bottom dissolved oxygen.

The correlation plot reveals several important relationships among the variables considered. A strong positive correlation between imperviousness and LDI indicates that increased urbanization is the main driver of the LDI, introducing redundancy in using both variables for the disturbance gradient. Additionally, a strong positive relationship between DIN and SRP shows that higher levels of one nutrient often coincide with another; however, chlorophyll displays weak correlations with both of these nutrients, suggesting that other limiting factors may affect productivity in these sites. Notably, Secchi depth, which serves as an indicator of water clarity and can reflect the presence of phytoplankton and suspended particles, shows no relationship with DIN and SRP, in addition to the expected strong negative correlation with chlorophyll. This further supports the statement about other potential factors limiting productivity. While not strong, bottom dissolved oxygen has a negative correlation with these nutrients, implying that elevated nutrient levels could contribute to near-bottom hypoxia. Similarly, a moderate positive relationship between sediment contamination metrics, mERM-Q and Pmax, suggests potential negative effects of urbanization on sediment quality.

To explore these relationships further, we analyzed the distribution of sites within a multivariate space by employing a Principal Component Analysis (PCA; Figure 9). This statistical technique simplifies complex datasets by reducing their dimensionality while retaining the most important information. It transforms the original variables into a new set of uncorrelated variables called

principal components, which are ordered by the amount of variance they explain in the data. PCA allows researchers to identify patterns, visualize relationships, and highlight the most significant factors influencing the dataset. It is particularly useful in exploratory data analysis, enabling clearer insights into the structure of the data and facilitating further analysis or modeling. PCA helps summarize large datasets and uncover underlying trends that may not be immediately apparent.

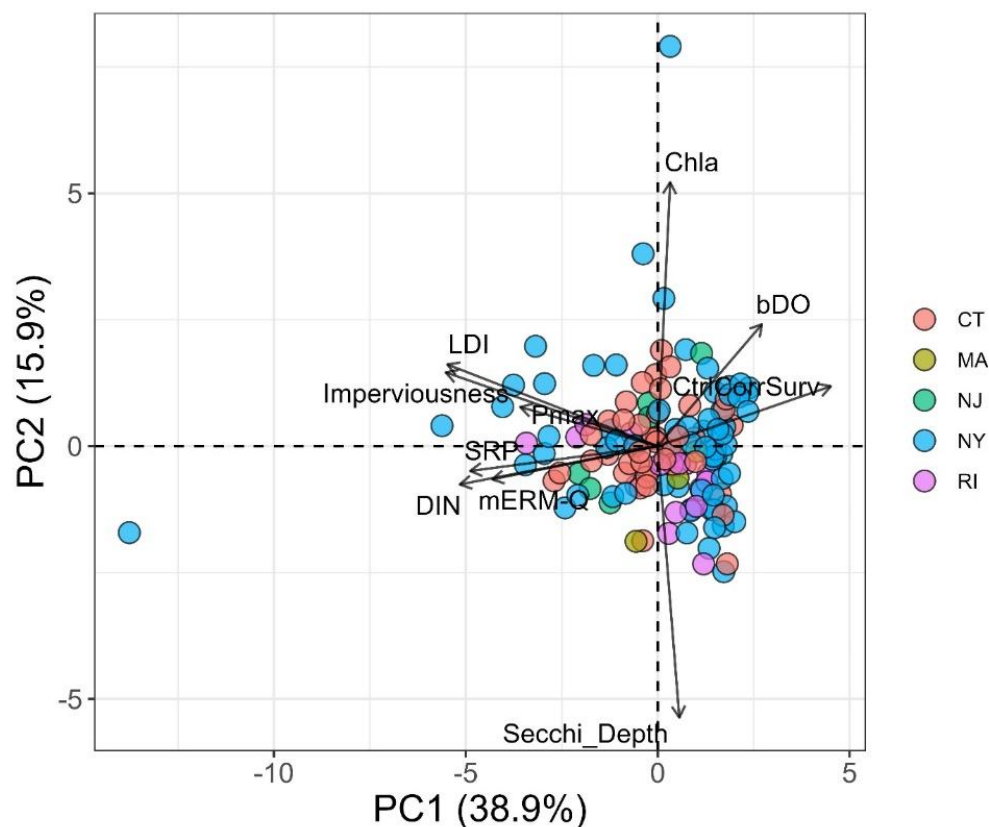


Figure 9. PCA biplot illustrating data from 130 stations and 133 samples (CT = 42; MA = 3; NJ = 6; NY = 66; RI = 16) showing candidate disturbance gradient variables that account for 54.8% of the variability in site distribution within the multivariate space. The x-axis outlier is a New York station NCA20_NY-10018 and sample 2014950.

In the PCA biplot, imperviousness, soluble reactive phosphorus (SRP), and dissolved inorganic nitrogen (DIN) are grouped, indicating a relationship among these variables. This clustering suggests that urbanization potentially increases nutrient runoff into water bodies. Chlorophyll-a is positioned orthogonally from this nutrient group, reflecting a weak response to SRP and DIN, implying that factors beyond nutrient availability, such as light conditions or grazing pressure, may limit chlorophyll-a concentrations. Additionally, bottom dissolved oxygen is located on the opposite side of the biplot, suggesting a potential for hypoxic conditions and a negative correlation with the nutrients. The low angle of control-corrected survival when compared to mERM-Q and nutrients suggests the expected, which is that higher sediment contamination, and nutrients, may lead to lowered survival rates.

The biplot also points to the fact that not all of the variables presented are necessary to capture the gradient of stress across all these sites. For instance, it is rather redundant to use both chlorophyll-

a and Secchi depth, since they are negatively correlated ($r = -0.58$; $p < 0.01$). Secondly, there is no utility in using both DIN and SRP, since these two are strongly and positively correlated to one another ($r = 0.64$; $p < 0.01$). Despite mERM-Q plotting closely together with the nutrients, the correlation between these two, while significant ($p < 0.01$), is rather weak ($r < 0.26$). Similarly, while LDI and imperviousness are well-correlated ($r = 0.98$; $p < 0.01$), and are significantly correlated with Pmax ($p < 0.01$), their correlation with Pmax is on the weaker part of the gradient ($r < 0.35$). Lastly, while the relationship between Pmax and mERM-Q is not apparent in the PCA biplot, their correlation of 0.96 makes using both unnecessary and introduces redundancy into the disturbance gradient.

3.2.1 Selection Rationale

Variables were selected based on several criteria that made developing a region-specific disturbance gradient possible and feasible. Those variables that met the following criteria were given priority for inclusion into the disturbance gradient.

Sufficient Data: Variables had to have sufficient data available across the 219 samples, and, if a dilemma existed between two variables, the one with the higher number of available data was selected. For example, given the high correlation between chlorophyll-a and Secchi depth, one of those variables had to be excluded. Secchi depth readings were missing for 74 samples, with most of them coming from the 2010 NCCA year, while chlorophyll-a was available for all but three samples. Therefore, Secchi depth was dropped from further consideration.

Simplicity and Communication: Variables that were simpler to calculate were prioritized over those requiring a multi-step process. For instance, while the LDI and Pmax necessitate extensive data compilation and analysis for each site, they are highly correlated with imperviousness and mERM-Q, respectively. Therefore, it is unnecessary to include the time-consuming calculations when the same information can be obtained through more straightforward and easily calculable variables. Additionally, mERM-Q is easier to communicate to the public.

Known Stressors: Those variables that are known to be stressors to the system have been prioritized over those that are not. For example, SRP and DIN are extremely highly correlated, but DIN appears to be a limiting nutrient in Long Island Sound (e.g. Anderson and Taylor, 2001; Varekamp et al., 2018; Vlahos et al., 2020). DIN therefore took precedence over SRP.

Using this rationale, six variables were ultimately selected to represent the gradient of stress across the available sites: bottom dissolved oxygen, sediment contamination, sediment toxicity, dissolved inorganic nitrogen, watershed imperviousness, and chlorophyll a.

3.3 DISTURBANCE GRADIENT

3.3.1 Purpose

Bioassessment compares the conditions of waterbodies to those of relatively natural environments, known as reference sites. These reference sites can vary from truly natural to as natural as possible given widespread disturbances. In some datasets, reference sites represent minimally disturbed conditions, which might be truly natural. However, in datasets where even the best sites are

considerably disturbed, reference sites represent the least disturbed or best observed conditions, which may not reflect the truly natural state.

When developing new biological indices, the first step is to identify reference and stressed sites. Reference sites are crucial for index calibration and validation, site classification, and setting biocriteria thresholds. Biotic indices are calibrated and validated along a disturbance gradient, making it essential to capture the full range from best to worst conditions. Reference sites help establish metric expectations with minimal disturbance. Conversely, stressed sites, identified using criteria at the opposite end of the disturbance scale, allow for evaluating metric responses along the stressor gradient. The direction and strength of these responses help select and score candidate metrics for inclusion in a biotic index.

Reference sites also aid in site classification. The natural variability in biological characteristics, influenced by environmental factors like salinity, proximity to land, or depth is best understood when not confounded by human disturbance. During site classification, the distribution and abundance of biota or metric values in minimally or least disturbed streams are used to identify distinct biological groups and their responses to natural gradients.

3.3.2 Approach

To establish a disturbance gradient for embayments, it's essential to define criteria for identifying the least and most disturbed sites. These criteria should be clearly documented and be independent of biological factors (USEPA 2013).

Initially, we attempted to apply the Northeast thresholds outlined in the 2015 NCCA Report. However, these thresholds resulted in a large number of undisturbed sites and very few stressed sites. For instance, using the NCCA thresholds for DIN of 0.1 and 0.5 mg L⁻¹, we categorized 133 samples as “good,” 34 as “other,” and only four as “poor.” Similarly, applying the benchmarks for the sediment contamination index from the 2015 NCCA report, we classified 140 samples as “good” and only five as “poor.” This evidence indicates that the thresholds need reassessment to better reflect the stressor gradient of the samples.

To address this, we employed quantiles to estimate the extent of stress for certain parameters and used predefined thresholds for others (e.g., NCCA’s sediment toxicity, LIS Report Card⁵ chlorophyll a and DO thresholds). This quantile-based approach ensures that thresholds reflect the actual distribution of stressor values across the LIS region, capturing ecologically meaningful variation. Thresholds were iteratively refined in collaboration with CT DEEP to ensure they align with regional management goals and accurately distinguish between minimally and highly disturbed sites. Six variables were selected: imperviousness, dissolved inorganic nitrogen, mean ERM-Q, chlorophyll a, bottom DO, and control-corrected survival. A sample could only be deemed a reference if it passed the control-corrected survival threshold of 0.9, in addition to the established criteria outlined below.

⁵ [Long Island Sound Report Card - Save the Sound](#)

Exploring the distribution of data using the quantile approach, thresholds were iteratively selected for each parameter to produce an acceptable number of stressed and reference samples on which MMI performance statistics can be calculated, without compromising the true condition of undisturbed and disturbed sites. Ultimately, parameter thresholds were selected (Table 3) once an acceptable number of reference and stressed samples were produced, with revisions and agreement from the CT DEEP partners.

Table 3. A table of parameter thresholds used to categorize parameters as good, poor, or moderate. Note that the values highlighted in red match those used by the LIS Report Card.

<i>Parameter</i>	<i>Good</i>	<i>Poor</i>
<i>Imperviousness (%)</i>	≤ 6	≥ 17
<i>mERM-Q</i>	≤ 0.06	≥ 0.25
<i>Chlorophyll a ($\mu\text{g L}^{-1}$)</i>	≤ 5.6	≥ 8.2
<i>Bottom DO (mg L^{-1})</i>	≥ 5.49	≤ 4.29
<i>DIN (mg L^{-1})</i>	≤ 0.02	≥ 0.1
<i>Sed Toxicity</i>	≥ 0.9	≤ 0.8

The thresholds from Table 3 were utilized to classify each value as either good or poor for each parameter. Subsequently, we developed a set of criteria to position each site along a stress gradient, ranging from very poor to very good samples. These criteria, known as "Combination Rules," are the standards a sample must meet to be classified accordingly. When creating combination rules, it is crucial to be stringent enough to minimize overlap between reference and stressed sites, yet not so strict that the number of representative samples in each group becomes too low to produce reliable results. The rules we established for the LIS region are detailed in Table 4 and further elaborated in the following section.

Table 4. A set of combination rules used to establish the degree of disturbance for each sample.

<i>Degree of Disturbance</i>	<i>Criteria</i>
<i>Very Good</i>	# Good ≥ 3 & Survival = Good & No Poor Parameters
<i>Good</i>	# Good ≥ 2 & No Poor Parameters
<i>Moderate</i>	All other combinations of Good and Poor indications
<i>Poor</i>	# Poor ≥ 2
<i>Very Poor</i>	# Poor ≥ 3

With the information in Table 4, one can deduce that very good and good sites are likely to be those with very little to no stress, since at least two out of six parameters must meet the "good" condition, and no parameters are allowed to show poor conditions. Moderate sites have a mix of conditions, not meeting the criteria for "good" or "very good," but also not falling into the "poor"

category. Poor sites show significant stress, with at least two out of six parameters meeting the "poor" condition. Very poor sites exhibit high levels of stress across multiple parameters, with at least half of the six parameters meeting the "poor" condition. By using these combination rules, one can categorize sites based on the overall health and stress levels indicated by the six selected parameters. The results of the calculated disturbance gradient based on these combination rules are shown in Table 5 and Figure 10.

Table 5. The number of samples in each disturbance category using the developed CT LIS disturbance gradient. Note that these 180 samples with complete disturbance data are taken across 174 stations.

<i>Disturbance Category</i>	<i>CT</i>	<i>MA</i>	<i>NJ</i>	<i>NY</i>	<i>RI</i>	<i>Totals</i>
<i>Very Good</i>	9	0	0	19	6	34
<i>Good</i>	4	0	0	0	1	5
<i>Moderate</i>	16	3	0	36	10	65
<i>Poor</i>	24	1	3	16	3	47
<i>Very Poor</i>	5	1	3	18	2	29
<i>Totals</i>	58	5	6	89	22	180

The disturbance gradient for LIS highlights some possible differences in environmental conditions across the states, keeping in mind that many stations from both New Jersey and Massachusetts were not in the defined area of interest and were therefore disregarded. Connecticut and New York have the highest number of samples, with a wide range of site conditions from very good to very poor. In contrast, Massachusetts and New Jersey have fewer samples, with New Jersey showing only poor and very poor conditions. This may be due to the fact that most of the New Jersey sites selected are located in Raritan Bay, a known area of poor water quality. Rhode Island presents a well-balanced distribution of site conditions. Overall, the number of minimally disturbed sites is sufficient, and may be expanded by consolidating sites from the "Good" category. Similarly, many sites are available in the "Poor" category if we deem that expanding the number of "Very Poor" sites necessary. Only stations NCA20_RI-10001 and NCA20_RI-10002 show differing disturbance categories based on the two samples collected at each station in 2020. Station NCA20_RI-10001 has samples classified as moderate and poor, while station NCA20_RI-10002 has samples classified as very poor and moderate.

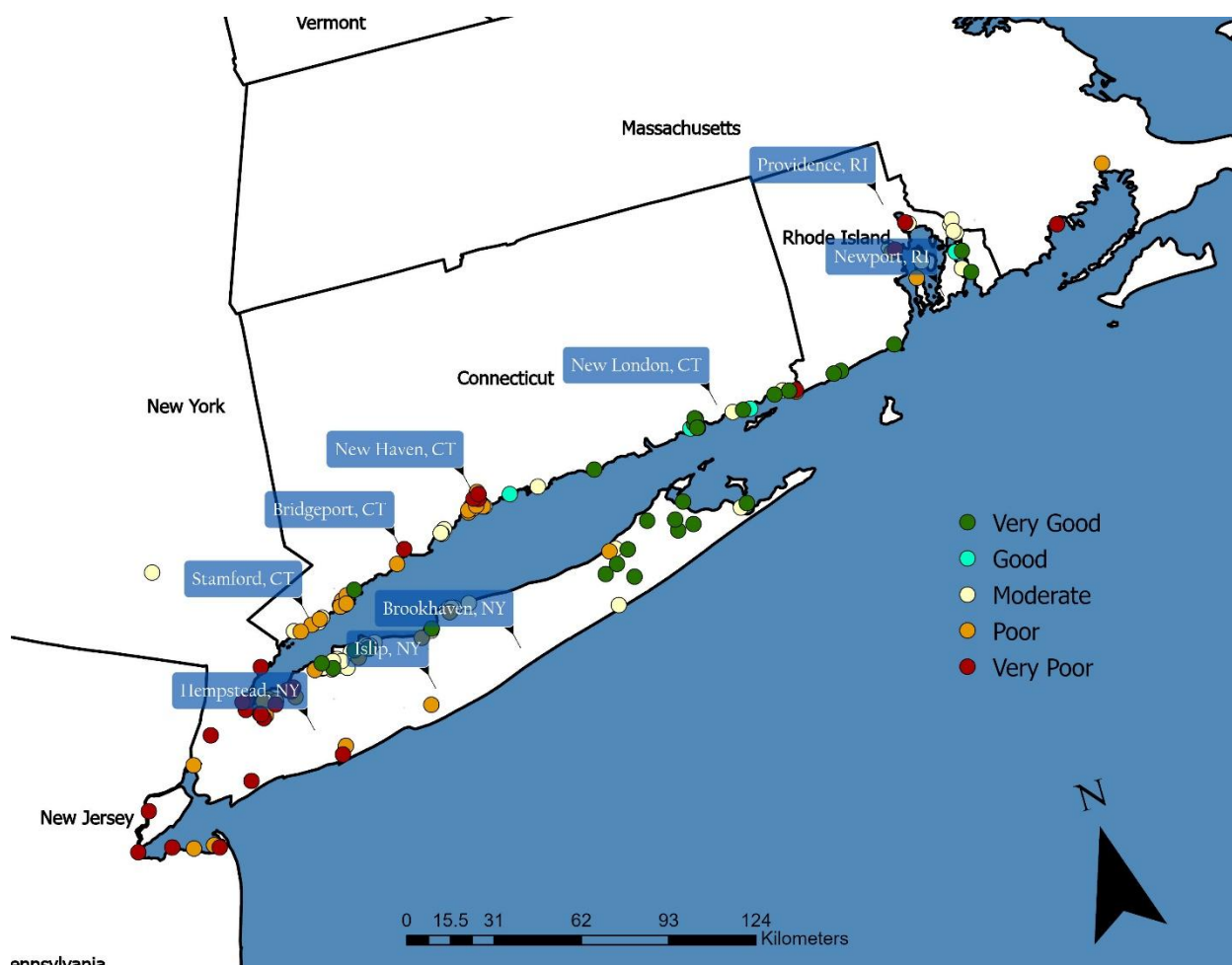


Figure 10. A map of the defined area of interest showing the degree of stress in the disturbance categories for the 180 sites (for which the disturbance variables were available). The number of each disturbance group for each state is shown in Table 5.

After selecting the sites in the “Good” category, Tetra Tech conducted a site classification analysis to examine the natural variability among biological communities within the area of interest. The analysis revealed no distinct or continuous site classes in the selected area for the MMI application (Appendix A).

The construction of the disturbance gradient follows the framework outlined in Phase I, which recommended integrating multiple independent stressor variables and using ordination techniques (e.g., PCA) to validate the gradient’s ecological relevance.

4 METRIC CALCULATION AND SELECTION

Macroinvertebrate metrics were calculated in R Studio (version 4.4.2; Pile of Leaves) to associate sample taxa lists with traits. These metrics included taxa richness by group, composition of individuals by group, richness and composition of feeding or trophic groups, richness and composition of habit types (methods of attachment or locomotion), and richness and composition

of pollution tolerance groups (The AZTI Marine Biotic Index; AMBI). Community structure metrics were calculated for all genera, families, orders, and classes that occurred in more than 50 samples. In total, 526 metrics were calculated (Appendix B), and the best candidate metrics were selected by closely following the steps described by Riato et al. (2022) and the references therein. The number of individuals and AMBI ratio metrics were immediately dropped from further consideration as they were inconsistent, resulting in 401 possible candidate metrics. A stepwise screening procedure was then employed to: (1) remove metrics with strongly skewed distributions or very narrow ranges, specifically those where the 75th percentile is 0, the 75th percentile is equal to the minimum value, or the 25th percentile is equal to the maximum value; (2) remove metrics with a low signal-to-noise ratio (S/N), calculated as the ratio of a given metric's variance among sites (S) to the variance within a site (N), with the noise calculated as the variance across two different visits in the same year; and (3) remove metrics that do not sufficiently discriminate between good (very good and good) and poor (very poor and poor) sites, using a t-score to compare mean values, with metrics having a t-score $> |3|$ being further considered. A higher t-score indicated that a given metric is sensitive to stress and significantly different between the two site groups.

Metric performance was primarily evaluated using the Discrimination Efficiency (DE) statistic, following the methodology outlined by Flotemersch et al. (2006) and Maxted et al. (2000). DE was calculated using the `metric.stats` and `metric.stats2` functions from the 'BioMonTools' R package, which quantify a metric's ability to distinguish between reference and stressed sites based on quartile thresholds. It represents the percentage of metric values in stressed sites that fall beyond the "worst" quartile of those in reference sites. The "worst" quartile of reference values varies depending on whether a metric decreases or increases with environmental stress. For metrics that decrease with increasing stress (called *decreasers*; Figure 11), DE is the percentage of stressed values below the 25th percentile of reference values. Conversely, for metrics that increase with stress (*increasers*), DE is the percentage of stressed values above the 75th percentile of reference values. DE can be illustrated using box plots of reference and stressed values. A higher DE indicates a more accurate association of metric values with site conditions.

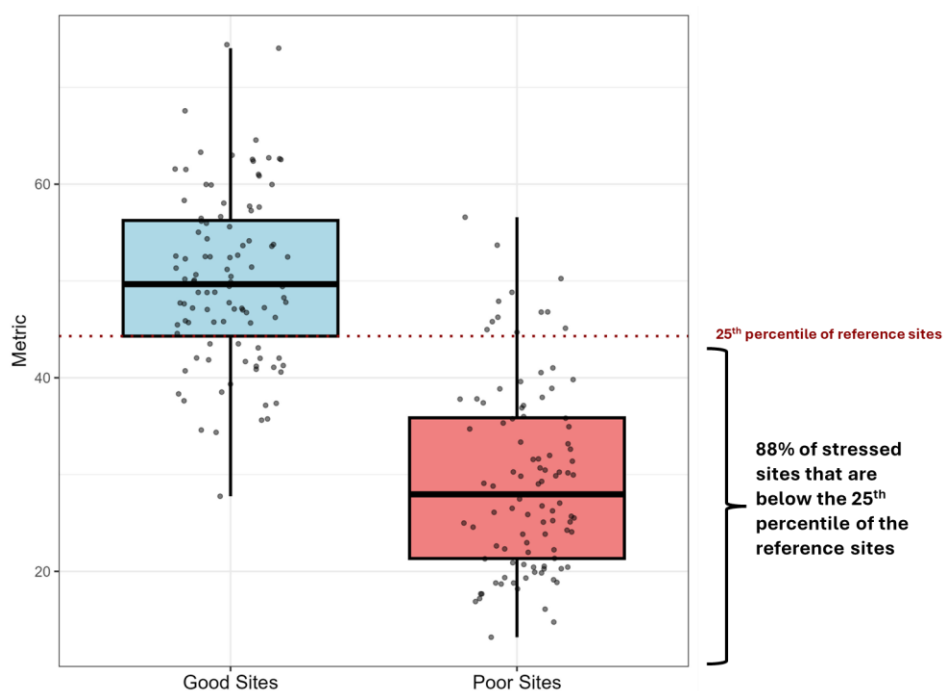


Figure 11. An example discrimination efficiency (DE) plot. The fictional metric here is a 'decreaser' metric so the DE is the percentage of stressed samples (Strs) that have values lower than the 25th percentile of good sites. In this case, the discrimination efficiency is 88.

Out of 400 macroinvertebrate metrics calculated, 24 of them passed the screening process. However, only 10 of these metrics were kept, with 16 of them removed for various reasons:

- Twelve metrics related to the AMBI score passed the initial screening process. These scores were either derived directly from the AZTI Marine Biological Index website or represented national-level interpretations. In cases where redundancy existed, such as measuring high AMBI scores (e.g., IV and V) using both percent individuals and percent taxa, the metric with stronger performance was retained. Ultimately, only three AMBI metrics were selected for inclusion.
- If a metric was redundant and outperformed by a similar metric, it was excluded from further consideration. For example, Margalef diversity performed worse than Gleason diversity, and the number of Polychaeta taxa performed better when the Spionidae family was excluded.

Due to the limited number of stations revisited (only eight), the results of the S/N screening step were considered with less stringency. Eleven metrics that failed the screening process were also considered for inclusion in the MMI because their performance and distribution were excellent. Of these eleven metrics, ten failed the S/N screening step, while only four failed the t-test. Ultimately, 19 metrics were considered for inclusion in the multimetric index (

Table 6).

Table 6. A list of metrics considered for inclusion in the final MMI ordered by decreasing discrimination efficiency (DE). Category and direction are shown for each metric. *pi* = percent of individuals; *pt* = percent of taxa; *nt* = number of taxa; *prop* = proportion of group in a sample

<i>Metric Code</i>	<i>Category</i>	<i>Direction</i>	<i>DE</i>
<i>pi Syllidae</i>	Structural	Decreaser	75.68
<i>pt Heteromastus & Mediomastus</i>	Structural	Increaser	74.32
<i>pt AMBI (IV and V)</i>	AMBI or MAMBI	Increaser	66.22
<i>nt bioturbators</i>	Functional	Decreaser	67.57
<i>pt Clitellata</i>	Structural	Increaser	66.22
<i>pi Heteromastus & Mediomastus</i>	Structural	Increaser	64.86
<i>pi Streblospio</i>	Structural	Increaser	63.51
<i>pt Hypereteone</i>	Structural	Increaser	62.16
<i>pi AMBI (I and II)</i>	AMBI or MAMBI	Decreaser	64.86
<i>nt AMBI (I and II)</i>	AMBI or MAMBI	Decreaser	59.46
<i>Gleason Diversity</i>	DRE	Decreaser	58.11
<i>pt Capitellidae</i>	Structural	Increaser	56.76
<i>Nt Polychaeta (Spionidae excluded)</i>	Structural	Decreaser	56.76
<i>nt Predators</i>	Functional	Decreaser	54.05
<i>nt Heteromastus & Mediomastus</i>	Structural	Increaser	51.35
<i>nt freeliving</i>	Functional	Decreaser	51.35
<i>pt AMBI (I and II)</i>	AMBI or MAMBI	Decreaser	48.65
<i>pt Idoteidae</i>	Structural	Increaser	47.3
<i>pi AMBI (IV and V)</i>	AMBI or MAMBI	Increaser	33.78

Metrics considered in index compilation from

Table 6 showed that they distinguish well between good and poor sites, that they are statistically sound and informative concerning their range across samples, that they are not redundant when used together (i.e., do not covary), and that they represent various components (categories) of biological health. Furthermore, of the 19 metrics, 9 are decreaseers, and 10 are increasers.

4.1 METRIC SCORING

To standardize the metrics to a 100-point scoring scale, we applied specific formulae as outlined by Barbour et al. (1999). The scoring scale was derived from the percentile statistics of metric values across reference and stressed samples from the calibration dataset. To mitigate the impact of extreme metric values on scoring, the 5th and the 95th percentiles were set as the minimum and maximum values, respectively (Blocksom, 2003). Below, we describe the scoring methods for metrics based on whether they are increasers or decreaseers. For a practical example of how to manually calculate metric scores, refer to Info Box 2.

Decreasers: Decreasers are metrics that decrease with increasing stress (e.g. number of intolerant taxa – taxa with low AMBI scores). To derive the score, the following equation was used, and, in this equation, the 95th percentile of all samples represents the upper limit of the scoring scale, and the 5th percentile represents the lower limit:

$$\text{Decreaser Metric Score} = 100 \times \frac{\text{Metric value} - 5\text{th percentile}}{95\text{th percentile} - 5\text{th percentile}}$$

Increasers: Increasers are metrics that increase with increasing stress (e.g., number of tolerant taxa – taxa with high AMBI scores), the following equation was applied. In this case, the 95th percentile of all samples represents the lower limit of the scoring scale, and the 5th percentile represents the upper limit. This inversion ensures that increaser metrics are scored on a 0-100 scale similar to decreaseers:

$$\text{Increaser Metric Score} = 100 \times \frac{95\text{th percentile} - \text{Metric Value}}{95\text{th percentile} - 5\text{th percentile}}$$

These scored metrics were then used to calculate the index and estimate index performance.

Info Box 2: Standardizing Metrics to a 100-Point Scale**Decreaser Metrics**

Percent of individuals from the Syllidae family is a decrease metric with high discrimination efficiency. This means that the percentage of individuals from this family decreases with increasing stress (higher values indicate healthier samples). To standardize this metric to a 0-100 scale without inverting its values, follow the example below:

1. Metric Value for a given sample is 17%
2. 5th percentile of the metric value distribution is 10%
3. 95th percentile of the metric value distribution is 70%

$$\text{Decreaser Metric Score} = 100 \times \frac{17 - 10}{70 - 10} = \frac{100 * 7}{60} = 11.66$$

4. The result of 11.66, which represents the standardized score for the original value of 17%.

Increaser Metrics

An increaser metric, such as percent of taxa from the Mediomastus genus, increases with stress (higher values indicate more polluted sites). Since the Multimetric Index (MMI) is calculated by summing selected metrics, higher scores represent healthier sites. Therefore, we must invert the values for increaser metrics so that high values represent better sites and low values represent poorer sites.

Example 1: High Original Metric Value

1. Metric Value for a given sample is 73%
2. 5th percentile of the metric value distribution is 12%
3. 95th percentile of the metric value distribution is 82%

$$\text{Increaser Metric Score} = 100 \times \frac{82 - 73}{82 - 12} = \frac{100 * 9}{70} = 12.85$$

4. The result is 12.85, which is now the metric score representative of the original value of 73% (notice the high to low inversion in the value).

Example 2: Low Original Metric Value

1. Metric Value for a given sample is 15%
2. 5th percentile of the metric value distribution is 12%
3. 95th percentile of the metric value distribution is 82%

$$\text{Increaser Metric Score} = 100 \times \frac{82 - 15}{82 - 12} = \frac{100 * 67}{70} = 95.7$$

4. The result is 95.7, which is now the metric score representative of the original value of 15% (notice the low to high inversion in the value).

To create exact metric scores, 39 samples in good condition and 56 samples in poor condition from the calibration dataset were used to derive the 95th and 5th percentile values for metric scoring formulae outlined above. Metrics were scored based on the final formulas shown in Table 7.

Table 7. Final scoring formulae for those metrics considered for inclusion in the final index.

<i>Metric Name</i>	<i>Formula</i>
<i>nt AMBI I&II</i>	$100 * (\text{metric} - 3) / (28 - 3)$
<i>nt bioturbators</i>	$100 * (\text{metric} - 7.4) / (33.3 - 7.4)$
<i>nt Predators</i>	$100 * (\text{metric} - 1.7) / (13.3 - 1.7)$
<i>nt HeteroMedio</i>	$100 * (2 - \text{metric}) / (2 - 0)$
<i>nt freelifving</i>	$100 * (\text{metric} - 3.7) / (18.3 - 3.7)$
<i>nt Polychaeta (Spionidae excluded)</i>	$100 * (\text{metric} - 3) / (21.3 - 3)$
<i>pi AMBI I&II</i>	$100 * (\text{metric} - 3.91) / (83.5 - 3.91)$
<i>pi AMBI IV&V</i>	$100 * (51.26 - \text{metric}) / (51.26 - 0.63)$
<i>pi HeteroMedio</i>	$100 * (49.11 - \text{metric}) / (49.11 - 0)$
<i>pi Streblospio</i>	$100 * (44.37 - \text{metric}) / (44.37 - 0)$
<i>pi Syllidae</i>	$100 * (\text{metric} - 0) / (14.55 - 0)$
<i>pt AMBI I&II</i>	$100 * (\text{metric} - 25.76) / (65.79 - 25.76)$
<i>pt AMBI IV&V</i>	$100 * (29.69 - \text{metric}) / (29.69 - 5.88)$
<i>pt Capitellidae</i>	$100 * (13.81 - \text{metric}) / (13.81 - 0)$
<i>pt Clitellata</i>	$100 * (7.1 - \text{metric}) / (7.1 - 0)$
<i>pt HeteroMedio</i>	$100 * (10.54 - \text{metric}) / (10.54 - 0)$
<i>pt Hypereteone</i>	$100 * (7.49 - \text{metric}) / (7.49 - 0)$
<i>pt Idoteidae</i>	$100 * (5.99 - \text{metric}) / (5.99 - 0)$
<i>Gleason Diversity</i>	$100 * (\text{metric} - 2.17) / (7.53 - 2.17)$

5 INDEX CALCULATION, SELECTION, AND PERFORMANCE

The index calculation process involves selecting the combination of the best-performing metrics across various categories, which represent different components of overall site and ecosystem health. Metrics are standardized to a 0-100 scale, where higher values indicate healthier stations. These standardized metrics are then summed and averaged to derive the final index score.

However, not all 19 candidate metrics from Table 6 can be included in the final index, and only 5-8 metrics were considered for inclusion. Using 20 candidate metrics to produce an index with between five to eight metrics yields over 164,000 different models (i.e., different combinations of

non-redundant metrics for the final index). To generate this large number of models, Tetra Tech has developed an automated all-subsets model that evaluates all possible metric combinations and generates relevant statistics and information to aid in the selection of the final index.

Each candidate index was assessed for performance using DE, the number of metric categories, the redundancy of component metrics, model sensitivity, and standard deviation. Models with two or more metric pairwise Spearman correlations greater than 0.75 were excluded from further consideration. Additionally, only models with a mean pairwise correlation among all metrics of <0.5 were retained. While several metrics should be included to represent biological integrity, redundant metrics can bias an index to reflect responses specific to certain environmental stressors or taxonomic responses. Therefore, indices containing conceptually redundant metrics were removed from consideration.

To ensure the robustness and accuracy of the selected MMI, validation samples from poor sites were selected ($n = 20$) across salinity gradients, maximum depth, latitude and longitude, and distance to shore (Figure 12). These poor validation samples are crucial for verifying the index's performance. By comparing these validation samples to the 25th percentile of the index derived from good samples, we can assess the index's ability to identify poor conditions accurately.

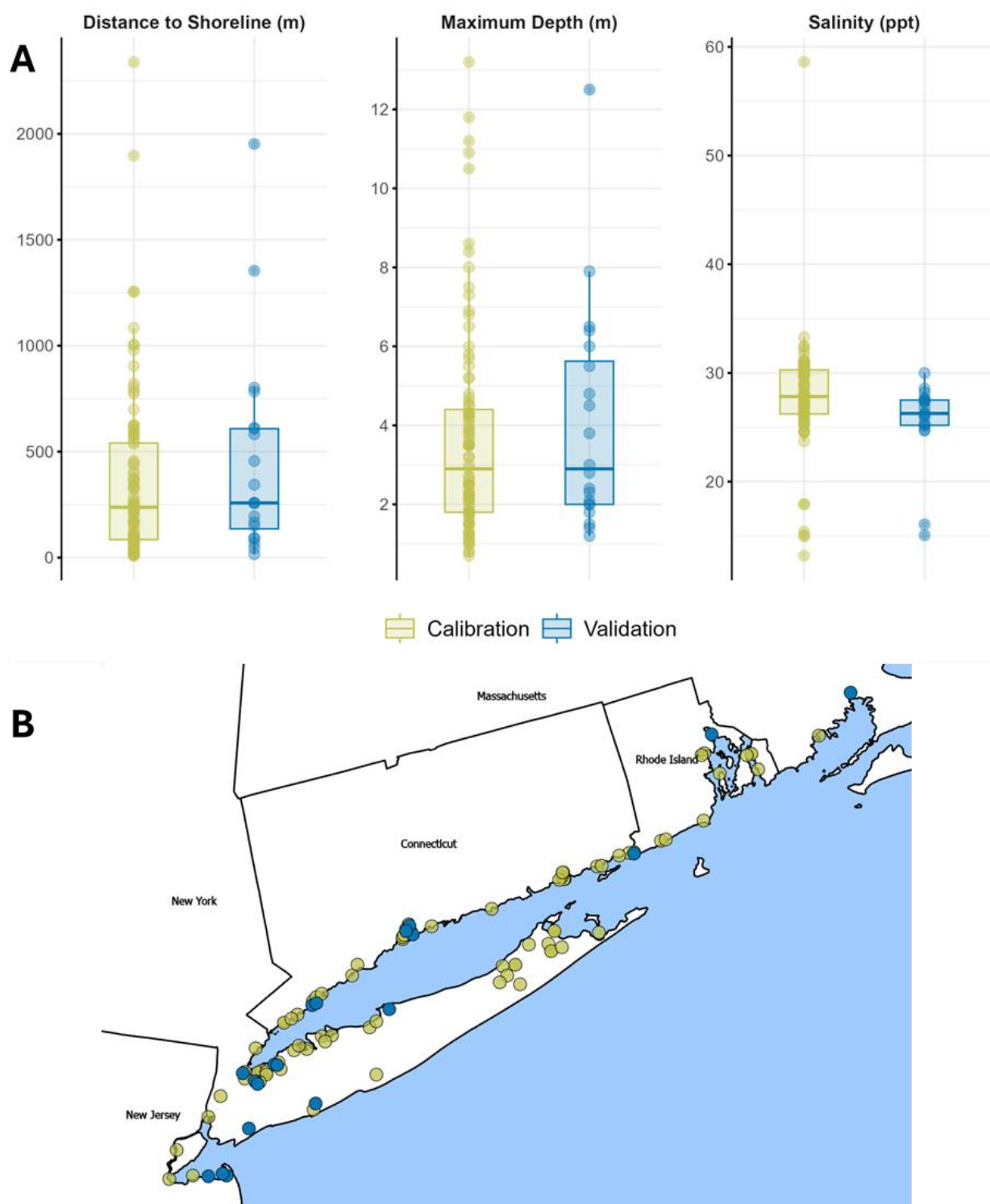


Figure 12. Boxplots (A) showing how well the validation samples ($n=20$) represent three major site characteristics across the area of interest when compared to the calibration data ($n=95$) and a map of validation samples (B). Only those samples with a poor disturbance gradient were used in the validation dataset.

Once all candidate models were generated, they were further scrutinized for their performance and the information they provided. Several performance metrics were calculated for each model:

- **Discrimination Efficiency (DE):** calculated for calibration and validation data. Represents a percentage of poor samples that are below the 25th percentile of good samples.
- **Model standard deviation (SD):** a statistic taken from Riato et al. (2022) that represents a standard deviation of reference samples in the calibration data, with lower SD indicating higher model precision.
- **Sensitivity:** This metric represents the percentage of most-disturbed sites evaluated as being in poor condition based on the MMI. A higher percentage indicates greater sensitivity of the MMI. The sensitivity is determined using an interval test (Kilgour et al., 1998), which checks if the MMI score for a site is significantly lower ($P < 0.05$) than the 5th percentile of MMI scores for least-disturbed sites. This test assumes a normal distribution of MMI scores among least-disturbed sites and uses a non-central F distribution to account for uncertainty in the 5th percentile estimate. This method is more conservative than simply comparing the MMI to a 5th percentile threshold, as it considers the uncertainty around the 5th percentile estimate.

With these statistics available, the selection process followed a set of sequential steps to arrive at the lower number of candidate models since 29,019 feasible models were generated, even after the QC process that took place before producing these models. These steps are the following:

- No models with $DE < 80$ were considered since a significant number of models performed better
- No models with more than two AMBI metrics were selected as there were only low- and high-AMBI score metrics, meaning that more than two would most definitely introduce redundancy into the model
- All models that had fewer than three metric categories were not considered
- All models with a standard deviation > 13 were not considered, as better precision was available in other models
- All models with sensitivity < 12 were not considered for inclusion in the index

Tetra Tech undertook a comprehensive evaluation process, initially reducing the pool of candidate models from 29,019 to 15. These 15 models were then subjected to rigorous scrutiny and in-depth analysis. The expert's review highlighted that all 15 candidate indices performed acceptably, with the top seven models demonstrating strong performance and a good variety of metrics. All 15 models included some form of *Heteromastus* & *Mediomastus* and *Idoteidae* metrics, 14 out of 15 included AMBI and a *Streblospio* metric. Following this thorough examination, six candidate models (Table 8) were selected and presented to the CT DEEP working group for further review. The working group reviewed these models, ultimately selecting the final model.

Table 8. This table presents the performance statistics and includes metrics for six different candidate MMI models (i.e., candidate indices).

<i>Model_ID</i>	5_3337	6_10396	5_997	6_10180	6_10399
<i>DE (calibration)</i>	85.7	85.7	83.9	82.1	82.1
<i>DE (validation)</i>	85.0	85.0	80.0	100.0	90.0
<i>Reference 25th</i>	58.2	64.3	67.6	69.1	63.1
<i>SD</i>	12.6	11.6	12.2	12.4	12.4
<i>Sensitivity</i>	12.5	12.5	12.5	12.5	12.5
<i>pt Heteromastus & Mediomastus</i>			X		
<i>pt Clitellata</i>	X	X		X	X
<i>pi Streblospio</i>		X	X	X	X
<i>pt Hypereteone</i>				X	
<i>nt Heteromastus & Mediomastus</i>	X	X		X	X
<i>pt Idoteidae</i>	X	X	X		X
<i>pi AMBI (IV & V)</i>				X	
<i>nt Bioturbators</i>	X	X	X	X	
<i>pi AMBI (I & II)</i>	X	X	X		X
<i>Gleason Diversity</i>					X

Model 6_10396 was selected as the best index, given its DE of 85.7% (Figure 13). No other models had a better standard deviation. Furthermore, the selected model had four structural metrics, one AMBI metric, and one functional/behavioral metric. As evident in the figure mentioned, the index distinguishes well across the gradient of stress, from the best (least disturbed) sites deemed as “Good”, across those sites with moderate stress (“Moderate”), and sites with the highest degree of stress (“Poor”). The validation dataset also showed a good DE of 85%.

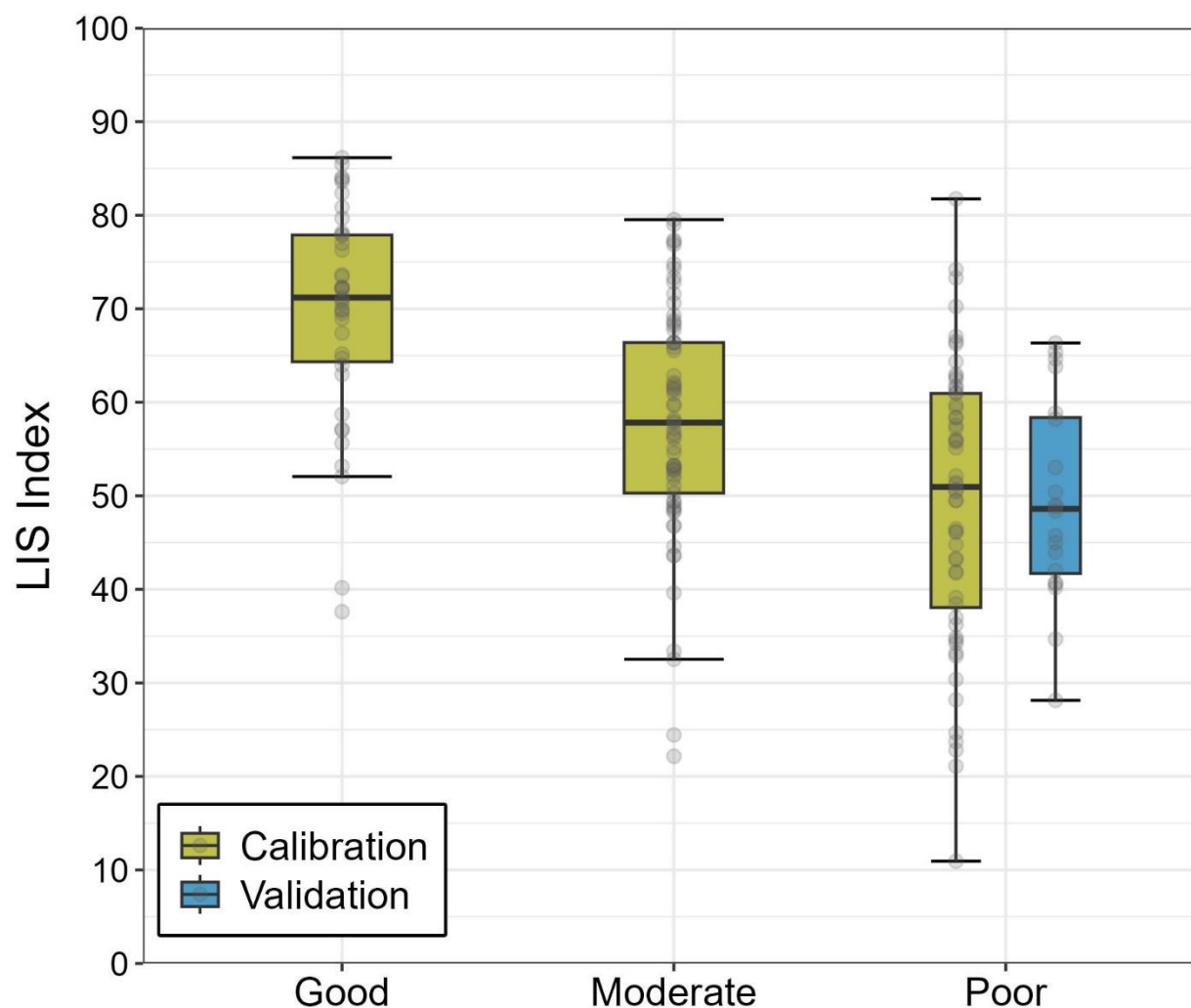


Figure 13. Distribution of index scores for index 6_10396 in reference and stressed sites in the Long Island Sound, showing calibration and validation datasets. No reference samples were selected for the validation dataset.

To explore multivariate patterns among ecological metrics, PCA was conducted using scaled data from a curated set of environmental indicators across 217 samples (note that the index was calculated for all 219 samples with available biological data). Before analysis, two outlier samples were excluded due to their disproportionate influence on the first principal component. Several variables contained missing values, which precluded direct use of the `'prcomp'` function. To address this, we employed the `'missMDA'` package to perform a robust imputation using a regularized iterative PCA approach. The optimal number of dimensions for imputation was estimated via cross-validation, and missing values were subsequently imputed using `'imputePCA'`. This method preserves the underlying structure of the data while minimizing bias introduced by imputation. The resulting PCA was visualized using individual samples colored by their LIS Index (6_10396), allowing for interpretation of ecological gradients in relation to this index.

The PCA conducted on a suite of environmental metrics reveals that the first two principal components (PC1 and PC2) account for 36.2% and 22.4% of the total variance, respectively. PC1 captures a dominant gradient of anthropogenic stress, with strong positive loadings from nutrient enrichment (DIN), sediment contamination (Mean ERM-Q), and phytoplankton biomass (Chlorophyll a), while exhibiting negative associations with indicators of ecological integrity such as Secchi depth and bottom dissolved oxygen (botDO). PC2 reflects a secondary axis of variation, potentially linked to site-specific biological responses or natural variability, with notable contributions from Secchi depth and chlorophyll concentration.

The biplot clearly demonstrates that sites are distributed along these axes in a manner consistent with environmental conditions. When colored by the selected LIS Index (6_10396), a strong pattern emerges: sites with low MMI scores (indicating degraded conditions) cluster in regions of the PCA space associated with elevated stressor levels, while high MMI scores align with areas characterized by clearer water, higher oxygen levels, and lower contaminant loads. This spatial coherence underscores the MMI's effectiveness as a multimetric index that integrates diverse stressor signals into a single, ecologically meaningful gradient. The alignment of MMI scores with the principal stressor axes in the PCA space provides compelling evidence that the index is both sensitive and robust in capturing the cumulative impacts of environmental degradation across Long Island Sound.

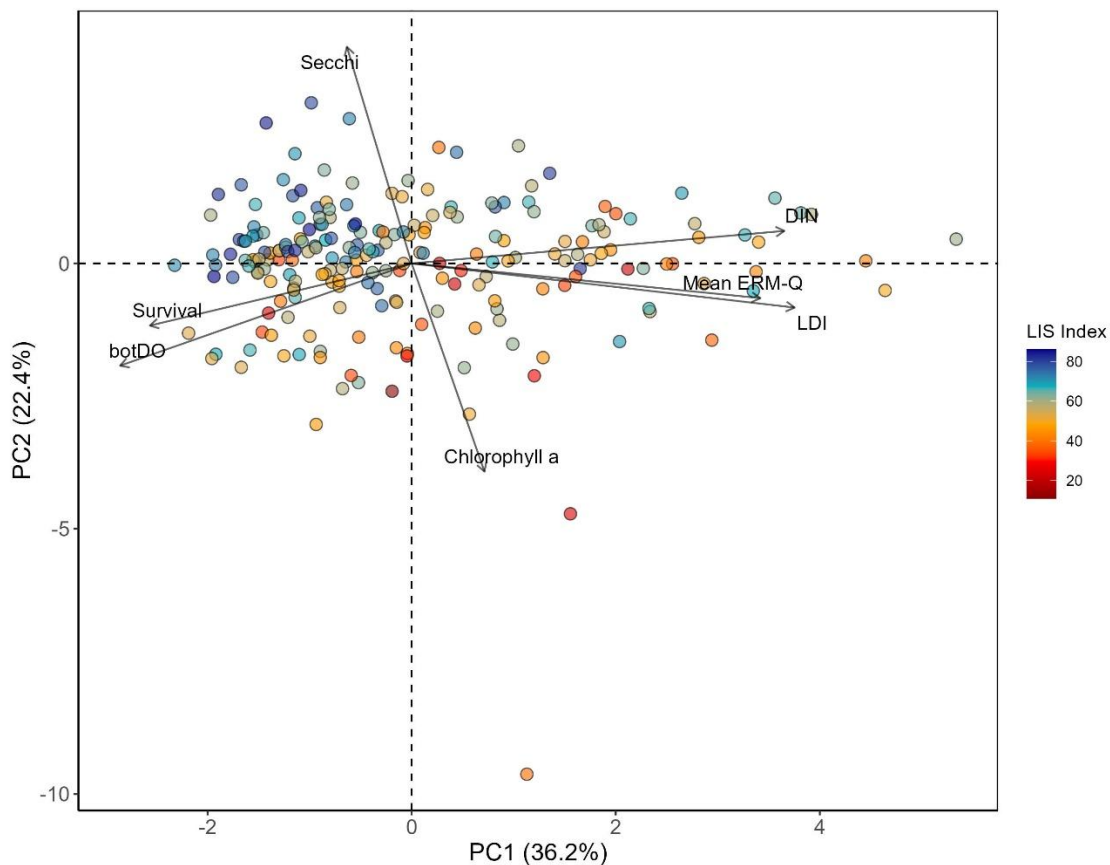


Figure 14. MMI response to known stressors in Long Island Sound.

This spatial coherence observed in the PCA biplot is further reinforced by the geographic distribution of MMI scores across the Long Island Sound region (Figure 15). The map of macroinvertebrate multimetric scores reveals a clear spatial gradient that mirrors the stressor patterns identified in the PCA. Embayments adjacent to highly urbanized areas, such as those near New York City and New Haven, consistently exhibit lower MMI scores, aligning with regions of elevated nutrient enrichment, sediment contamination, and reduced water clarity and oxygen levels. In contrast, higher-scoring sites are predominantly located in less developed areas, particularly in eastern Connecticut and parts of Rhode Island and Massachusetts, where environmental conditions are more favorable. This alignment between multivariate environmental gradients and spatial MMI patterns underscores the index's sensitivity to cumulative anthropogenic stress and its utility in identifying both degraded and relatively undisturbed sites.

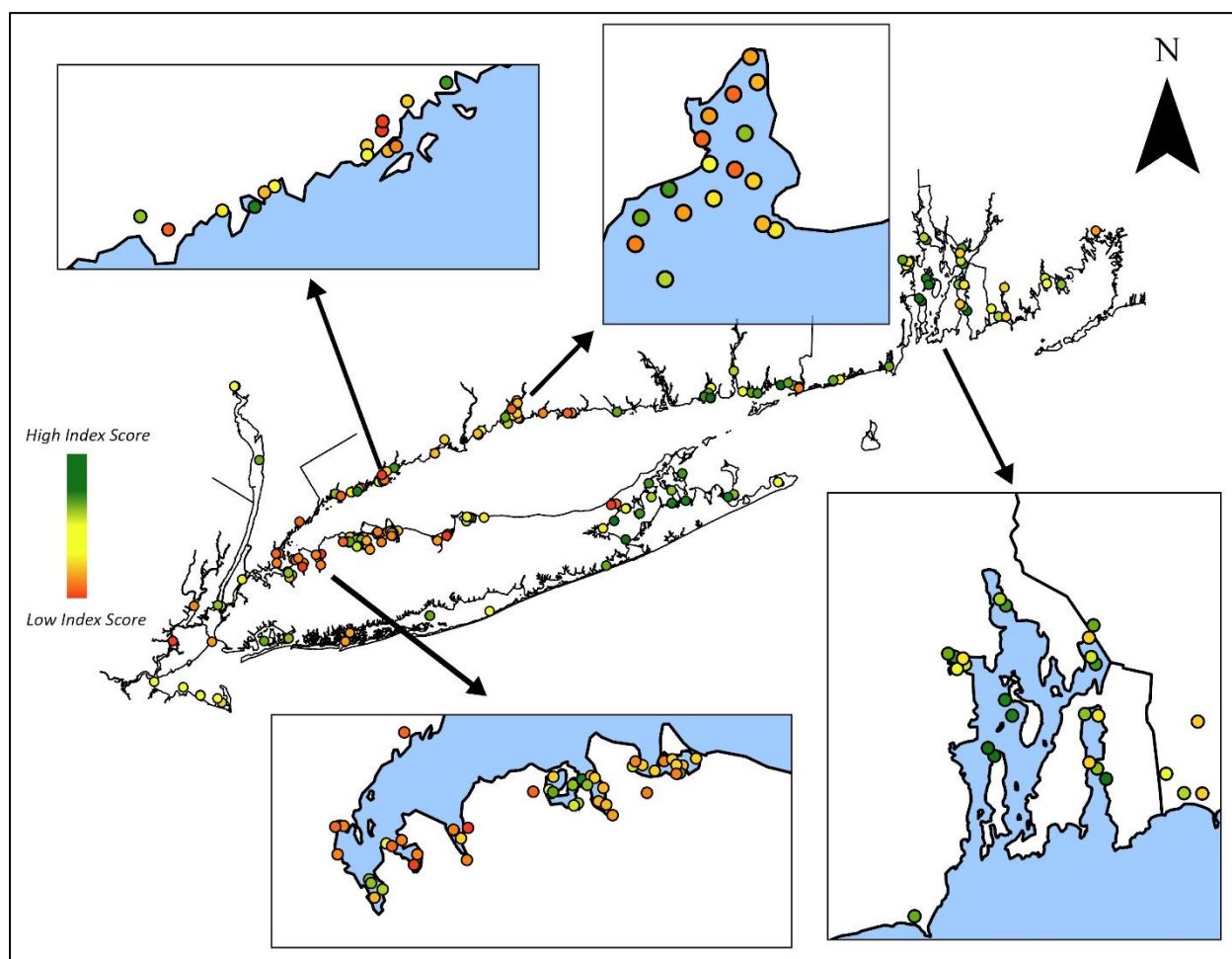


Figure 15. Spatial distribution of macroinvertebrate multimetric index (MMI) scores across Long Island Sound and surrounding embayments. Colored points represent site-specific MMI scores, with green indicating high ecological condition and red indicating degraded condition.

6 DISCUSSION

Tetra Tech developed an MMI model that Connecticut and New York can apply within the Long Island Sound to assess the biological integrity of sampled locations based on benthic invertebrate community condition relative to the average condition observed at reference locations. The selected MMI model (6_10396) performs exceptionally well statistically, with a discrimination efficiency (DE) of 85.7% in the calibration dataset and 85% in the validation dataset. These values indicate a high capacity to distinguish between reference and stressed sites and exceed the performance of many comparable indices developed for other estuarine systems.

Despite the strong performance of the selected MMI, several limitations should be acknowledged. Most notably, due to the limited number of minimally disturbed sites in the dataset, no reference sites were available for inclusion in the validation dataset. As a result, validation was conducted using only stressed sites, which limits the ability to fully assess the index's performance across the entire condition gradient. This constraint may affect the generalizability of the index, particularly in distinguishing between high-quality and moderately impacted sites. Additionally, while the disturbance gradient was carefully constructed using multiple independent stressor variables, the thresholds used to define “good” and “poor” conditions were based on quantile distributions rather than regionally validated ecological benchmarks. This introduces some uncertainty in the classification of sites, especially in transitional or moderately disturbed conditions. Furthermore, the metric based on the number of *Heteromastus* and *Mediomastus* taxa functions as a presence-absence indicator, given that only three species (*Mediomastus ambiseta*, *Mediomastus californiensis*, and *Heteromastus filiformis*) are typically encountered. Nonetheless, this metric was retained due to its strong discriminatory power and consistent performance across the calibration dataset. Finally, while the index was calibrated using a diverse set of metrics, the relatively small sample size and geographic concentration of reference-quality sites may limit the robustness of the index in underrepresented embayments or salinity regimes. These limitations highlight the importance of continued data collection and refinement of the index as additional monitoring data become available.

For context, Hale and Heltshe (2008) evaluated four candidate MMI models for the nearshore Gulf of Maine (Acadian Province Benthic Indices), which achieved classification efficiencies ranging from 72% to 85%. Similarly, the Chesapeake Bay Benthic Index of Biotic Integrity (B-IBI) developed by Weisberg et al. (1997) reported classification efficiencies of 84% on average, with performance varying by salinity zone - 82% in low mesohaline mud habitats (5–12 ppt) and up to 96% in high mesohaline mud habitats (>12–18 ppt). The LIS MMI's performance falls at the upper end of this range, demonstrating its robustness across a moderately broad range of estuarine and near-coastal salinities and sediment types.

The ecological composition of the selected MMI is also noteworthy. It includes six metrics that collectively represent a balanced spectrum of community structure and function, as well as sensitivity to environmental stress. Four of the six metrics are “increasers,” meaning they have higher values under stressed conditions, while two are “decreasers,” which decline in response to stress. This balance ensures that the index captures both the loss of sensitive taxa and the proliferation of tolerant forms, key indicators of ecological degradation. The ecological interpretation of the six metrics is as follows:

Clitellata: This metric represents the percent of taxa in the class Clitellata, primarily estuarine oligochaetes such as Tubificoides. These taxa are known for their tolerance to organic enrichment and low oxygen conditions due to their hemoglobin content (Martin et al., 2008). Their increased presence at stressed sites makes this a reliable increaser metric.

Streblospio: This metric represents the percentage of individuals from a sample in the genus *Streblospio*, a group of estuarine and near-coastal polychaetes in the family Spionidae. These worms construct tubes in muddy sediments and use their buccal palps to feed on phytoplankton and detritus from both the sediment surface and bottom waters. *Streblospio* species are broadly tolerant of a wide range of salinities, temperatures, and organic pollution (Levin 1986), and are highly resilient under low dissolved oxygen conditions (Llansó 1991), making them well-suited as indicators of environmental stress. Conde et al. (2013) identified *S. shrubsolii* as one of the most abundant species in the upper Tagus Estuary (Portugal), where it persisted under extreme conditions including salinity fluctuations and fluid mud deposition, and was consistently associated with low-diversity, high-stress environments. While *S. shrubsolii* is the dominant species in European estuaries, the only *Streblospio* species observed in Long Island Sound is *S. benedicti*. According to da Fonseca-Genevois and Cazaux (1987), these two species are morphologically distinct but ecologically similar, both exhibiting opportunistic life histories and thriving in organically enriched, disturbed habitats. These traits support the use of *S. benedicti* as an “increaser” metric indicative of degraded benthic conditions.

Heteromastus and Mediomastus: This metric counts the number of taxa from two genera of Capitellid polychaetes. These burrowing deposit feeders are opportunistic and thrive in organically enriched sediments, making them strong indicators of disturbance. They are also believed to be tolerant of heavy metal pollution, organic input, and hypoxia (Ryu et al., 2011; Hiscock et al., 2005).

Idoteidae: This metric captures the percentage of taxa in the family Idoteidae, a group of aquatic isopods. These crustaceans are common in LIS and are generally tolerant of environmental stress, contributing to the structural diversity of the index.

AMBI I & II: This decreaser metric represents the percentage of individuals classified in the two most sensitive AMBI pollution tolerance categories. These include a wide range of polychaetes, crustaceans, and mollusks that decline in abundance as stress increases, making this a critical component for detecting ecological degradation.

Bioturbators: This functional metric counts the number of taxa that engage in sediment mixing and oxygenation. Bioturbators are sensitive to disturbance due to their reliance on stable sediment conditions. Their decline is indicative of impaired benthic function and reduced ecosystem resilience.

Together, these metrics ensure that the LIS MMI is both ecologically grounded and statistically robust. The inclusion of both structural (taxonomic composition) and functional (bioturbation) indicators enhances the index’s diagnostic power and ecological relevance. Furthermore, the PCA analysis confirms that the MMI aligns well with known environmental stress gradients, reinforcing its utility as a tool for regulatory assessment, long-term monitoring, and adaptive management in Long Island Sound and the adjacent regions.

7 CONCLUSIONS

The development of the Long Island Sound Macroinvertebrate Multimetric Index (LIS MMI) marks an important step forward in the region's capacity to assess and manage estuarine health. This effort successfully translated a complex array of biological, chemical, and physical data into a practical, scientifically defensible tool for environmental assessment. The MMI not only reflects the current state of embayment conditions but also provides a scalable framework for future monitoring and adaptive management. By integrating diverse metrics and grounding them in a clearly defined disturbance gradient, the index offers a nuanced understanding of ecological condition that is both regionally specific and broadly applicable. Its development process, rooted in transparency and rigorous statistical validation, ensures that the MMI is not only technically sound but also aligned with regulatory and conservation goals. It is important to note that the disturbance gradient and reference site definitions were constrained by data availability and may not fully capture the range of natural variability across all embayments. Future updates to the index should revisit these thresholds as more monitoring data become available.

To ensure the MMI is accessible and actionable, two digital tools have been developed: an R Markdown [tool](#) for calculating index scores and an ArcGIS StoryMap ([technical](#) and [non-technical](#) versions) for communicating the project's context and findings (Appendix C). These tools will support regulatory implementation, stakeholder engagement, and ongoing monitoring efforts across the region. Looking ahead, the MMI developed here will serve as a foundational element in efforts to protect and restore the Long Island Sound. It also sets a precedent for future index development, including potential applications to fish and plankton communities, and offers a replicable model for other estuarine systems seeking to enhance their biological assessment capabilities.

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9 APPENDIX A: SITE CLASSIFICATION

Site classification involves comparing conditions at test sites to reference conditions, which vary across different natural settings. This process ensures that test sites are evaluated against similar site classes, allowing for accurate bioassessment by recognizing natural variability and evaluating sites according to their natural potential. By examining the structure and composition of samples in the least disturbed reference sites, distinct classes of sites with similar biological expectations can be identified. This reduces variability and allows for a more precise assessment of anthropogenic disturbances.

In the site classification analysis, natural variability among biological community types is accounted for by exploring sample characteristics in reference sites. Biological characteristics associated with natural environmental settings are best recognized when not confounded by human disturbance. This approach allows for the calibration of MMIs to specific site types and their unique responses to disturbances. Environmental variables, such as water temperature, salinity, substrate type, and water depth (Figure A), are used to determine the basis for biological structure, helping to define site classes.

This classification simplifies the development of biological indices, enabling a focused analysis of biological responses within each site type. For the LIS, this method ensures that the biological potential of each site is accurately assessed, facilitating effective conservation and management efforts.

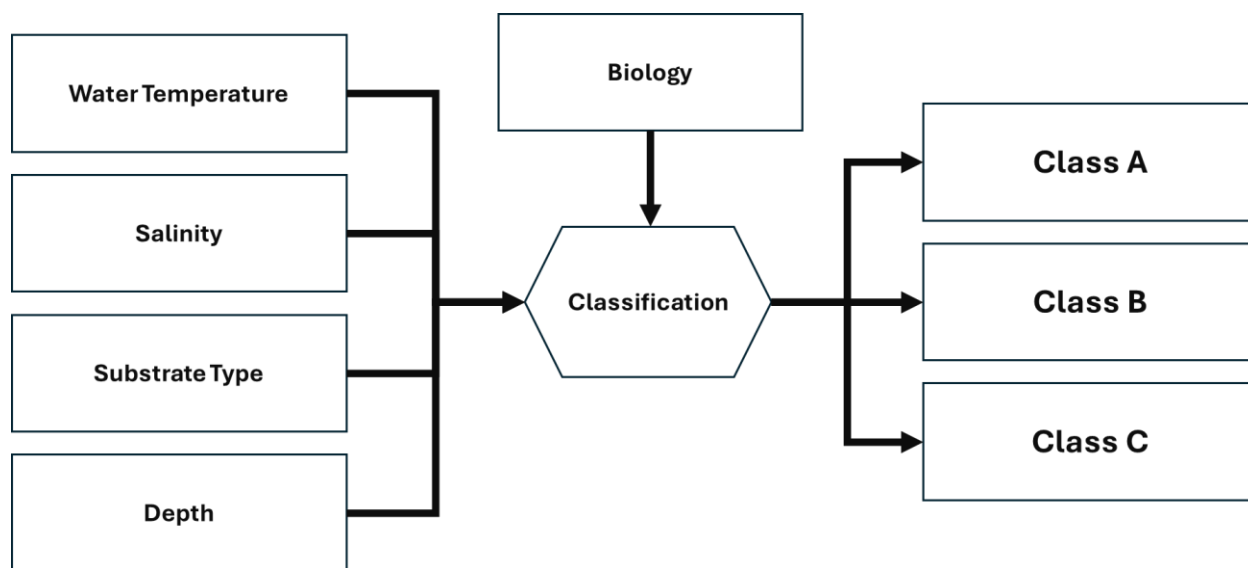


Figure A1. Potential inputs and results in a discrete classification process for coastal embayments. Inputs must include natural environmental variables. Resulting site classes are discrete groups where a site can be placed in only one of them using one, or a combination of natural variables.

Tetra Tech typically attempts to do two types of site classification to identify any potential natural variables that may be driving the differences in biological communities in the absence of, or with minimal stress. One approach is to create distinct classes of sites that have similar biological

expectations within each class. By examining the structure and composition of samples in least disturbed reference sites, a reasonable number of biological types can be recognized so that variability is reduced relative to the variability in all sites. Assigning sites into classes that reflect the biological types is accomplished by identifying the environmental conditions that determine the biological variation. The second approach is to utilize random forest models to adjust metrics based on natural variability, however, in this case, the classification is continuous and site-specific, with each metric compared to the model-predicted value to evaluate whether each metric is achieving the reference levels or is underperforming. For example, a site may not be classified into the deep or shallow category in combination with the salinity group, but rather its expectations are continuously adjusted based on multiple predictor variables (e.g. station depth and salinity).

Tetra Tech employed both of these approaches and found no evidence of site classes in the selected area of interest spanning from Raritan Bay to Buzzards Bay. Our findings are shown in the following two sections.

9.1 Distinct Site Classification

Distinct site classification begins with a detailed description of the natural conditions and landscape variability in the study area. This involves using maps to display ecoregions, river basins, hydrological units, salinity, depth, and other variables. Ecoregions, which incorporate key characteristics like topography, soils, vegetation, and more, serve as a foundational element for classification due to their regional distinctiveness and relevance to aquatic organisms. Additionally, non-metric multidimensional scaling (NMS) is used to analyze taxa presence/absence, plotting samples with similar taxonomic compositions close together based on Bray-Curtis distance measures. This method helps in visualizing the similarities among samples in a multidimensional space.

When identifying taxa at the order level, issues can arise if the specimens are immature, damaged, or from obscure groups. To address this, higher-level identifications are either eliminated or reassigned to a more general level to minimize information loss. Rare and ubiquitous taxa are also screened out to avoid skewing the ordination results. The process involves examining taxa counts and sums to assign operational taxonomic units (OTUs).

The NMDS algorithm in PC-ORD uses randomization to find configurations with minimal stress, starting with results from a Detrended Correspondence Analysis (DCA). The final ordination stress should be below 20, decreasing with more axes.

Environmental variables are then related to the principal axes through graphic overlays and correlations. Samples with similar taxonomic compositions are plotted close together, and evident site groupings linked to environmental characteristics suggest potential classification variables. Suitable environmental variables for site classification are those that are available, replicable, and minimally influenced by human activity, including both categorical and continuous variables like ecoregions, elevation, and climate data. An example of a single NMDS biplot is shown in Figure A2-B, where variable correlations with axis 1 and axis 2 are shown in Figure A2-A, and Figure A2-C, respectively.

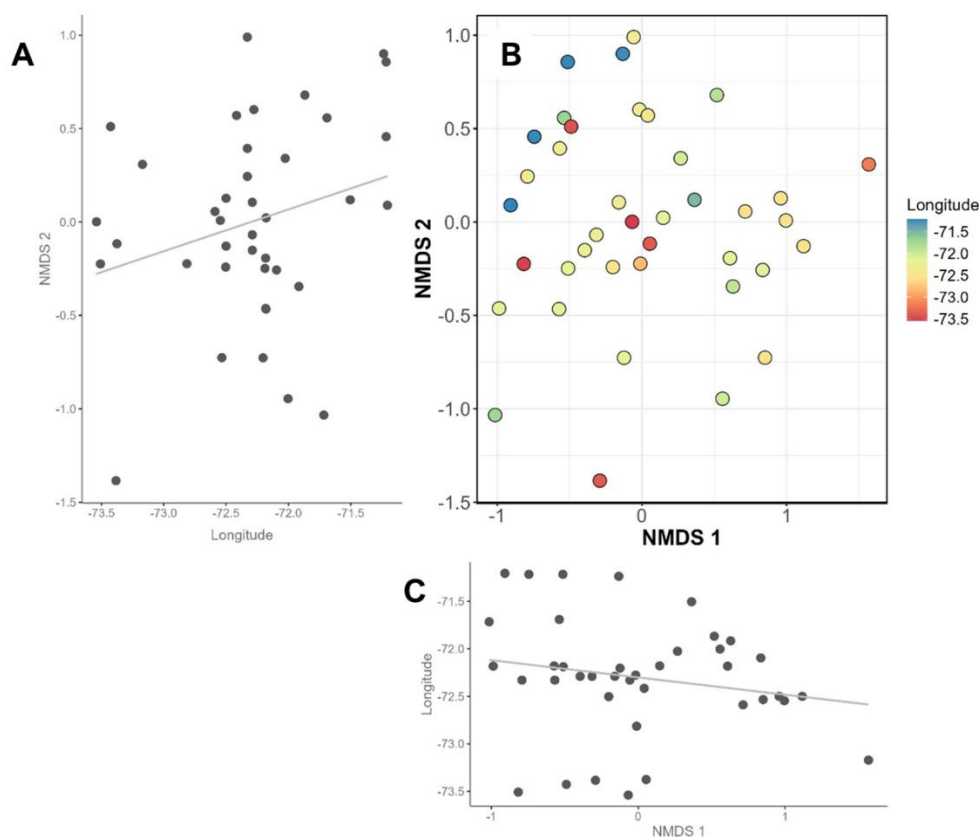


Figure A2. A figure showing the distribution of reference stations along NMDS 1 and NMDS 2 axes indicating their respective similarity in the macroinvertebrate community. No distinct site class exists concerning Longitude, which is also shown by the weak correlations among Longitude and NMDS1(C) and NMDS2 (A).

The presence of a distinct site class is evident when sites with similar macroinvertebrate communities exhibit comparable environmental variability. This is visually represented in Plot B of Figure A2, where sites of similar color indicate the variable in question. Additionally, such grouping would be reflected by a strong correlation in the correlation plots shown in Figures A2-A or A2-C. However, no such grouping is observed in Figure A2. Multiple figures identical to this one were produced to test any potential distinct site classes in the reference sites of the area in question, and no evidence was found that a distinct site class is necessary. The following variables were considered in the site classification analysis: day of the year, station depth, distance to shoreline, bottom salinity, bottom dissolved oxygen, percent silt and clay, percent sand, percent granules, and longitude.

9.2 Continuous Site Classification

A method inspired by Carlisle et al. (2022) was employed to predict site-specific metric expectations using a random forest model, leveraging multiple natural environmental variables from reference sites. This continuous classification approach compares observed metric values to model-predicted values, enhancing precision and reducing variability by accounting for natural gradients. For example, site expectations are dynamically adjusted based on variables such as site salinity, site depth, and distance to shoreline, rather than being categorized as deep or shallow.

In the random forest analysis, predictor variables account for natural gradient effects on metrics. The model uses decision trees to predict metric values based on site characteristics. Site-specific natural metrics from the NCCA dataset were also utilized for this step. Random forest models were developed for candidate metrics using the *randomForest* package in R. A random forest comprises 500 classification and regression tree models, each constructed with subsets of training data. Bootstrap samples are used to build each tree, and each split considers a random subset of predictors. The final model averages the metric values from all 500 trees to make predictions. Pseudo R^2 value is produced as a part of a random forest model to estimate the model performance, with higher values indicating greater predictor influence on a given metric. Each metric for which the random forest model explains 20% variation or more requires that particular metric to be adjusted. This approach also yielded no significant results, and no metric needs continuous adjustment. All metrics considered with their respective pseudo- R^2 are shown in Table A1.

Table A9. A table showing the results of the random forest metric adjustment analysis. Note that no metric variation explained by the random forest model exceeds 20%.

Metric Name	pseudo Rsq	Metric Name	pseudo Rsq
nt_AMBI_I	-15.2	nt_PolyNoSpion	-15.5
nt_AMBI_IandII	-28.6	nt_total	-22
nt_AMBI_II	-12.5	pi_SYLLIDAE	-5.3
nt_AMBIInat_I	-17.1	pt_AMBI_IandII	-55.6
nt_AMBIInat_IandII	-23.5	pt_AMBI_II	15.3
nt_AMBIInat_II	-21.6	pt_AMBI_IVandV	-34.1
nt_AMPHIPODA	-21.4	pt_AMBIInat_IandII	-47.9
nt_bioturb_gen	-18.1	pt_AMBIInat_II	7.3
nt_bioturb_surfmod	-17.9	pt_AMBIInat_IVandV	-29.5
nt_ffg_pred	-39.8	pt_EDOTIA	10.6
nt_ffg_scav	-41.2	pt_IDOTEIDAE	10.6
nt_livhabit_freeliving	-30.3	x_Gleason	-0.3
nt_POLYCHAETA	-19	x_Margalef	-2.4

Based on the two approaches employed to investigate potential site classifications in the Long Island Sound region, Tetra Tech has determined that there is no evidence to support the need for such site treatments. A single multimetric index is deemed sufficient to describe stress levels without interference from natural site variables.

10 APPENDIX B: LIST OF ALL METRICS

Table B1. A list of metrics screened for performance. Note: ni = number of individuals, nt = number of taxa, pi = percentage of individuals, pt = percentage of taxa; li = log number of individuals, prop = proportion of individuals; ri = ratio.

Note: The metrics listed in this appendix are provided for transparency and reproducibility. Only a subset of these metrics was selected for inclusion in the final LIS MMI based on screening and performance criteria.

Metric Name	Category	Formula
li_total	Diversity, Richness, or Evenness	log Number of Individuals
ni_total	Diversity, Richness, or Evenness	Number of Individuals
ni_ACTEOCINA	Structural	Number of Individuals
ni_ALITTA	Structural	Number of Individuals
ni_AMPELISCA	Structural	Number of Individuals
ni_CAPITELLA	Structural	Number of Individuals
ni_CLYMENELLA	Structural	Number of Individuals
ni_CREPIDULA	Structural	Number of Individuals
ni_EDOTIA	Structural	Number of Individuals
ni_EXOGONE	Structural	Number of Individuals
ni_GEMMA	Structural	Number of Individuals
ni_GLYCERA	Structural	Number of Individuals
ni_GLYCINDE	Structural	Number of Individuals
ni_GRANDIDIERELLA	Structural	Number of Individuals
ni_HETEROMASTUS	Structural	Number of Individuals
ni_HYPERETEONE	Structural	Number of Individuals
ni_ILYANASSA	Structural	Number of Individuals
ni_JAPONACTAEON	Structural	Number of Individuals
ni_KIRSTEUERIELLA	Structural	Number of Individuals
ni_LEITOSCOLOPLOS	Structural	Number of Individuals
ni_LEUCON	Structural	Number of Individuals
ni_MEDIOMASTUS	Structural	Number of Individuals
ni_MICRODEUTOPUS	Structural	Number of Individuals
ni_MONOCOROPHIUM	Structural	Number of Individuals
ni_NEPHTYS	Structural	Number of Individuals
ni_NUCULA	Structural	Number of Individuals
ni_OXYUROSTYLIS	Structural	Number of Individuals
ni_PECTINARIA	Structural	Number of Individuals
ni_POLYCIRRUS	Structural	Number of Individuals
ni_POLYDORA	Structural	Number of Individuals
ni_SCOLETOMA	Structural	Number of Individuals

Metric Name	Category	Formula
ni_SPIOCHAETOPTERUS	Structural	Number of Individuals
ni_STREBLOSPIO	Structural	Number of Individuals
ni_THARYX	Structural	Number of Individuals
ni_ACTEONIDAE	Structural	Number of Individuals
ni_AMPELISCIDAE	Structural	Number of Individuals
ni_AORIDAE	Structural	Number of Individuals
ni_CALYPTRAEIDAE	Structural	Number of Individuals
ni_CAPITELLIDAE	Structural	Number of Individuals
ni_CHAETOPTERIDAE	Structural	Number of Individuals
ni_CIRRATULIDAE	Structural	Number of Individuals
ni_COROPHIIDAE	Structural	Number of Individuals
ni_DIASTYLIDAE	Structural	Number of Individuals
ni_GLYCERIDAE	Structural	Number of Individuals
ni_GONIADIDAE	Structural	Number of Individuals
ni_IDOTEIDAE	Structural	Number of Individuals
ni_LEUCONIDAE	Structural	Number of Individuals
ni_LUMBRINERIDAE	Structural	Number of Individuals
ni_MACTRIDAE	Structural	Number of Individuals
ni_MALDANIDAE	Structural	Number of Individuals
ni_NASSARIIDAE	Structural	Number of Individuals
ni_NEPHTYIDAE	Structural	Number of Individuals
ni_NEREIDIDAE	Structural	Number of Individuals
ni_NUCULIDAE	Structural	Number of Individuals
ni_ORBINIIDAE	Structural	Number of Individuals
ni_PECTINARIIDAE	Structural	Number of Individuals
ni_PHYLLODOCIDAE	Structural	Number of Individuals
ni_POSEIDONEMERTIDAE	Structural	Number of Individuals
ni_PYRAMIDELLIDAE	Structural	Number of Individuals
ni_SPIONIDAE	Structural	Number of Individuals
ni_SYLLIDAE	Structural	Number of Individuals
ni_TELLINIDAE	Structural	Number of Individuals
ni_TEREBELLIDAE	Structural	Number of Individuals
ni_TORNATINIDAE	Structural	Number of Individuals
ni_VENERIDAE	Structural	Number of Individuals
ni_AMPHIPODA	Structural	Number of Individuals
ni_CARDIIDA	Structural	Number of Individuals
ni_CEPHALASPIDEA	Structural	Number of Individuals
ni_CUMACEA	Structural	Number of Individuals
ni_DECAPODA	Structural	Number of Individuals
ni_EUNICIDA	Structural	Number of Individuals

Metric Name	Category	Formula
ni_HETEROSTROPHA	Structural	Number of Individuals
ni_ISOPODA	Structural	Number of Individuals
ni_LITTORINIMORPHA	Structural	Number of Individuals
ni_MONOSTILIFERA	Structural	Number of Individuals
ni_NEOGASTROPODA	Structural	Number of Individuals
ni_NUCULIDA	Structural	Number of Individuals
ni_PHYLLODOCIDA	Structural	Number of Individuals
ni_PYRAMIDELLIDA	Structural	Number of Individuals
ni_SPIONIDA	Structural	Number of Individuals
ni_TEREBELLIDA	Structural	Number of Individuals
ni_VENERIDA	Structural	Number of Individuals
ni_BIVALVIA	Structural	Number of Individuals
ni_CLITELLATA	Structural	Number of Individuals
ni_GASTROPODA	Structural	Number of Individuals
ni_HOPLONEMERTEA	Structural	Number of Individuals
ni_MALACOSTRACA	Structural	Number of Individuals
ni_POLYCHAETA	Structural	Number of Individuals
ni_ffg_dep	Functional; FFG	Number of Individuals
ni_ffg_pred	Functional; FFG	Number of Individuals
ni_ffg_filt	Functional; FFG	Number of Individuals
ni_ffg_scav	Functional; FFG	Number of Individuals
ni_ffg_graz	Functional; FFG	Number of Individuals
ni_ffg_subsudep	Functional; FFG	Number of Individuals
ni_ffg_gen	Functional; FFG	Number of Individuals
ni_bioturb_surfmod	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_biodiff	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_convey	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_nobiot	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_upwardconv	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_downwardconv	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_bioirr	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_gen	Functional; bioturbation - specific	Number of Individuals
ni_bioturb_nobiot_gen	Functional; bioturbation - specific	Number of Individuals

Metric Name	Category	Formula
ni_livhabit_freeliving	Functional; living habit/movement	Number of Individuals
ni_livhabit_burrower	Functional; living habit/movement	Number of Individuals
ni_livhabit_tubedwelling	Functional; living habit/movement	Number of Individuals
ni_livhabit_freeliving_commensal	Functional; living habit/movement	Number of Individuals
ni_livhabit_attached	Functional; living habit/movement	Number of Individuals
ni_AMBI_I	AMBI or MAMBI	Number of Individuals
ni_AMBI_II	AMBI or MAMBI	Number of Individuals
ni_AMBI_III	AMBI or MAMBI	Number of Individuals
ni_AMBI_IV	AMBI or MAMBI	Number of Individuals
ni_AMBI_V	AMBI or MAMBI	Number of Individuals
ni_AMBI_IandII	AMBI or MAMBI	Number of Individuals
ni_AMBI_IVandV	AMBI or MAMBI	Number of Individuals
ni_AMBInat_I	AMBI or MAMBI	Number of Individuals
ni_AMBInat_II	AMBI or MAMBI	Number of Individuals
ni_AMBInat_III	AMBI or MAMBI	Number of Individuals
ni_AMBInat_IV	AMBI or MAMBI	Number of Individuals
ni_AMBInat_V	AMBI or MAMBI	Number of Individuals
ni_AMBInat_IandII	AMBI or MAMBI	Number of Individuals
ni_AMBInat_IVandV	AMBI or MAMBI	Number of Individuals
nt_total	Diversity, Richness, or Evenness	Number of Taxa
nt_PolyNoSpion	Structural	Number of Taxa
nt_ACTEOCINA	Structural	Number of Taxa
nt_ALITTA	Structural	Number of Taxa
nt_AMPELISCA	Structural	Number of Taxa
nt_CAPITELLA	Structural	Number of Taxa
nt_CLYMENELLA	Structural	Number of Taxa
nt_CREPIDULA	Structural	Number of Taxa
nt_EDOTIA	Structural	Number of Taxa
nt_EXOGONE	Structural	Number of Taxa
nt_GEMMA	Structural	Number of Taxa
nt_GLYCERA	Structural	Number of Taxa
nt_GLYCINDE	Structural	Number of Taxa
nt_GRANDIDIERELLA	Structural	Number of Taxa
nt_HETEROMASTUS	Structural	Number of Taxa
nt_HYPERETEONE	Structural	Number of Taxa
nt_ILYANASSA	Structural	Number of Taxa

Metric Name	Category	Formula
nt_JAPONACTAEON	Structural	Number of Taxa
nt_KIRSTEUERIELLA	Structural	Number of Taxa
nt_LEITOSCOLOPLOS	Structural	Number of Taxa
nt_LEUCON	Structural	Number of Taxa
nt_MEDIOMASTUS	Structural	Number of Taxa
nt_MICRODEUTOPUS	Structural	Number of Taxa
nt_MONOCOROPHIUM	Structural	Number of Taxa
nt_NEPHTYS	Structural	Number of Taxa
nt_NUCULA	Structural	Number of Taxa
nt_OXYUROSTYLIS	Structural	Number of Taxa
nt_PECTINARIA	Structural	Number of Taxa
nt_POLYCIRRUS	Structural	Number of Taxa
nt_POLYDORA	Structural	Number of Taxa
nt_SCOLETOMA	Structural	Number of Taxa
nt_SPIOCHAETOPTERUS	Structural	Number of Taxa
nt_STREBLOSPIO	Structural	Number of Taxa
nt_THARYX	Structural	Number of Taxa
nt_ACTEONIDAE	Structural	Number of Taxa
nt_AMPELISCIDAE	Structural	Number of Taxa
nt_AORIDAE	Structural	Number of Taxa
nt_CALYPTRAEDAE	Structural	Number of Taxa
nt_CAPITELLIDAE	Structural	Number of Taxa
nt_CHAETOPTERIDAE	Structural	Number of Taxa
nt_CIRRATULIDAE	Structural	Number of Taxa
nt_COROPHIIDAE	Structural	Number of Taxa
nt_DIASTYLIDAE	Structural	Number of Taxa
nt_GLYCERIDAE	Structural	Number of Taxa
nt_GONIADIDAE	Structural	Number of Taxa
nt_IDOTEIDAE	Structural	Number of Taxa
nt_LEUCONIDAE	Structural	Number of Taxa
nt_LUMBRINERIDAE	Structural	Number of Taxa
nt_MACTRIDAE	Structural	Number of Taxa
nt_MALDANIDAE	Structural	Number of Taxa
nt_NASSARIIDAE	Structural	Number of Taxa
nt_NEPHTYIDAE	Structural	Number of Taxa
nt_NEREIDIDAE	Structural	Number of Taxa
nt_NUCULIDAE	Structural	Number of Taxa
nt_ORBINIIDAE	Structural	Number of Taxa
nt_PECTINARIIDAE	Structural	Number of Taxa
nt_PHYLLODOCIDAE	Structural	Number of Taxa

Metric Name	Category	Formula
nt_POSEIDONEMERTIDAE	Structural	Number of Taxa
nt_PYRAMIDELLIDAE	Structural	Number of Taxa
nt_SPIONIDAE	Structural	Number of Taxa
nt_SYLLIDAE	Structural	Number of Taxa
nt_TELLINIDAE	Structural	Number of Taxa
nt_TEREBELLIDAE	Structural	Number of Taxa
nt_TORNATINIDAE	Structural	Number of Taxa
nt_VENERIDAE	Structural	Number of Taxa
nt_AMPHIPODA	Structural	Number of Taxa
nt_CARDIIDA	Structural	Number of Taxa
nt_CEPHALASPIDEA	Structural	Number of Taxa
nt_CUMACEA	Structural	Number of Taxa
nt_DECAPODA	Structural	Number of Taxa
nt_EUNICIDA	Structural	Number of Taxa
nt_HETEROSTROPHA	Structural	Number of Taxa
nt_ISOPODA	Structural	Number of Taxa
nt_LITTORINIMORPHA	Structural	Number of Taxa
nt_MONOSTILIFERA	Structural	Number of Taxa
nt_NEOGASTROPODA	Structural	Number of Taxa
nt_NUCULIDA	Structural	Number of Taxa
nt_PHYLLODOCIDA	Structural	Number of Taxa
nt_PYRAMIDELLIDA	Structural	Number of Taxa
nt_SPIONIDA	Structural	Number of Taxa
nt_TEREBELLIDA	Structural	Number of Taxa
nt_VENERIDA	Structural	Number of Taxa
nt_BIVALVIA	Structural	Number of Taxa
nt_CLITELLATA	Structural	Number of Taxa
nt_GASTROPODA	Structural	Number of Taxa
nt_HOPLONEMERTEA	Structural	Number of Taxa
nt_MALACOSTRACA	Structural	Number of Taxa
nt_POLYCHAETA	Structural	Number of Taxa
nt_ffg_dep	Functional; FFG	Number of Taxa
nt_ffg_pred	Functional; FFG	Number of Taxa
nt_ffg_filt	Functional; FFG	Number of Taxa
nt_ffg_scav	Functional; FFG	Number of Taxa
nt_ffg_graz	Functional; FFG	Number of Taxa
nt_ffg_subsupdep	Functional; FFG	Number of Taxa
nt_ffg_gen	Functional; FFG	Number of Taxa
nt_bioturb_surfmud	Functional; bioturbation - specific	Number of Taxa

Metric Name	Category	Formula
nt_bioturb_biodiff	Functional; bioturbation - specific	Number of Taxa
nt_bioturb_convey	Functional; bioturbation - specific	Number of Taxa
nt_bioturb_nobiot	Functional; bioturbation - specific	Number of Taxa
nt_bioturb_upwardconv	Functional; bioturbation - specific	Number of Taxa
nt_bioturb_downwardconv	Functional; bioturbation - specific	Number of Taxa
nt_bioturb_bioirr	Functional; bioturbation - specific	Number of Taxa
nt_bioturb_gen	Functional; bioturbation - specific	Number of Taxa
nt_bioturb_nobiot_gen	Functional; bioturbation - specific	Number of Taxa
nt_livhabit_freeliving	Functional; living habit/movement	Number of Taxa
nt_livhabit_burrower	Functional; living habit/movement	Number of Taxa
nt_livhabit_tubedwelling	Functional; living habit/movement	Number of Taxa
nt_livhabit_freeliving_commensal	Functional; living habit/movement	Number of Taxa
nt_livhabit_attached	Functional; living habit/movement	Number of Taxa
nt_AMBI_I	AMBI or MAMBI	Number of Taxa
nt_AMBI_II	AMBI or MAMBI	Number of Taxa
nt_AMBI_III	AMBI or MAMBI	Number of Taxa
nt_AMBI_IV	AMBI or MAMBI	Number of Taxa
nt_AMBI_V	AMBI or MAMBI	Number of Taxa
nt_AMBI_IandII	AMBI or MAMBI	Number of Taxa
nt_AMBI_IVandV	AMBI or MAMBI	Number of Taxa
nt_AMBInat_I	AMBI or MAMBI	Number of Taxa
nt_AMBInat_II	AMBI or MAMBI	Number of Taxa
nt_AMBInat_III	AMBI or MAMBI	Number of Taxa
nt_AMBInat_IV	AMBI or MAMBI	Number of Taxa
nt_AMBInat_V	AMBI or MAMBI	Number of Taxa
nt_AMBInat_IandII	AMBI or MAMBI	Number of Taxa
nt_AMBInat_IVandV	AMBI or MAMBI	Number of Taxa
pi_Spion2Poly	Structural	Percent of Individuals
pi_ACTEOCINA	Structural	Percent of Individuals
pi_ALITTA	Structural	Percent of Individuals
pi_AMPELISCA	Structural	Percent of Individuals

Metric Name	Category	Formula
pi_CAPITELLA	Structural	Percent of Individuals
pi_CLYMENELLA	Structural	Percent of Individuals
pi_CREPIDULA	Structural	Percent of Individuals
pi_EDOTIA	Structural	Percent of Individuals
pi_EXOGONE	Structural	Percent of Individuals
pi_GEMMA	Structural	Percent of Individuals
pi_GLYCERA	Structural	Percent of Individuals
pi_GLYCINDE	Structural	Percent of Individuals
pi_GRANDIDIERELLA	Structural	Percent of Individuals
pi_HETEROMASTUS	Structural	Percent of Individuals
pi_HYPERETEONE	Structural	Percent of Individuals
pi_ILYANASSA	Structural	Percent of Individuals
pi_JAPONACTAEON	Structural	Percent of Individuals
pi_KIRSTEUERIELLA	Structural	Percent of Individuals
pi_LEITOSCOLOPLOS	Structural	Percent of Individuals
pi_LEUCON	Structural	Percent of Individuals
pi_MEDIOMASTUS	Structural	Percent of Individuals
pi_MICRODEUTOPUS	Structural	Percent of Individuals
pi_MONOCOROPHIUM	Structural	Percent of Individuals
pi_NEPHTYS	Structural	Percent of Individuals
pi_NUCULA	Structural	Percent of Individuals
pi_OXYUROSTYLIS	Structural	Percent of Individuals
pi_PECTINARIA	Structural	Percent of Individuals
pi_POLYCIRRUS	Structural	Percent of Individuals
pi_POLYDORA	Structural	Percent of Individuals
pi_SCOLETOMA	Structural	Percent of Individuals
pi_SPIOCHAETOPTERUS	Structural	Percent of Individuals
pi_STREBLOSPIO	Structural	Percent of Individuals
pi_THARYX	Structural	Percent of Individuals
pi_ACTEONIDAE	Structural	Percent of Individuals
pi_AMPELISCIDAE	Structural	Percent of Individuals
pi_AORIDAE	Structural	Percent of Individuals
pi_CALYPTRAEIDAE	Structural	Percent of Individuals
pi_CAPITELLIDAE	Structural	Percent of Individuals
pi_CHAETOPTERIDAE	Structural	Percent of Individuals
pi_CIRRATULIDAE	Structural	Percent of Individuals
pi_COROPHIIDAE	Structural	Percent of Individuals
pi_DIASTYLIDAE	Structural	Percent of Individuals
pi_GLYCERIDAE	Structural	Percent of Individuals
pi_GONIADIDAE	Structural	Percent of Individuals

Metric Name	Category	Formula
pi_IDOTEIDAE	Structural	Percent of Individuals
pi_LEUCONIDAE	Structural	Percent of Individuals
pi_LUMBRINERIDAE	Structural	Percent of Individuals
pi_MACTRIDAE	Structural	Percent of Individuals
pi_MALDANIDAE	Structural	Percent of Individuals
pi_NASSARIIDAE	Structural	Percent of Individuals
pi_NEPHTYIDAE	Structural	Percent of Individuals
pi_NEREIDIDAE	Structural	Percent of Individuals
pi_NUCULIDAE	Structural	Percent of Individuals
pi_ORBINIIDAE	Structural	Percent of Individuals
pi_PECTINARIIDAE	Structural	Percent of Individuals
pi_PHYLLODOCIDAE	Structural	Percent of Individuals
pi_POSEIDONEMERTIDAE	Structural	Percent of Individuals
pi_PYRAMIDELLIDAE	Structural	Percent of Individuals
pi_SPIONIDAE	Structural	Percent of Individuals
pi_SYLLIDAE	Structural	Percent of Individuals
pi_TELLINIDAE	Structural	Percent of Individuals
pi_TEREBELLIDAE	Structural	Percent of Individuals
pi_TORNATINIDAE	Structural	Percent of Individuals
pi_VENERIDAE	Structural	Percent of Individuals
pi_AMPHIPODA	Structural	Percent of Individuals
pi_CARDIIDA	Structural	Percent of Individuals
pi_CEPHALASPIDEA	Structural	Percent of Individuals
pi_CUMACEA	Structural	Percent of Individuals
pi_DECAPODA	Structural	Percent of Individuals
pi_EUNICIDA	Structural	Percent of Individuals
pi_HETEROSTROPHA	Structural	Percent of Individuals
pi_ISOPODA	Structural	Percent of Individuals
pi_LITTORINIMORPHA	Structural	Percent of Individuals
pi_MONOSTILIFERA	Structural	Percent of Individuals
pi_NEOGASTROPODA	Structural	Percent of Individuals
pi_NUCULIDA	Structural	Percent of Individuals
pi_PHYLLODOCIDA	Structural	Percent of Individuals
pi_PYRAMIDELLIDA	Structural	Percent of Individuals
pi_SPIONIDA	Structural	Percent of Individuals
pi_TEREBELLIDA	Structural	Percent of Individuals
pi_VENERIDA	Structural	Percent of Individuals
pi_BIVALVIA	Structural	Percent of Individuals
pi_CLITELLATA	Structural	Percent of Individuals
pi_GASTROPODA	Structural	Percent of Individuals

Metric Name	Category	Formula
pi_HOPLONEMERTEA	Structural	Percent of Individuals
pi_MALACOSTRACA	Structural	Percent of Individuals
pi_POLYCHAETA	Structural	Percent of Individuals
pi_dom01	Diversity, Richness, or Evenness	Percent of Individuals
pi_dom02	Diversity, Richness, or Evenness	Percent of Individuals
pi_dom03	Diversity, Richness, or Evenness	Percent of Individuals
pi_dom04	Diversity, Richness, or Evenness	Percent of Individuals
pi_dom05	Diversity, Richness, or Evenness	Percent of Individuals
pi_ffg_dep	Functional; FFG	Percent of Individuals
pi_ffg_pred	Functional; FFG	Percent of Individuals
pi_ffg_filt	Functional; FFG	Percent of Individuals
pi_ffg_scav	Functional; FFG	Percent of Individuals
pi_ffg_graz	Functional; FFG	Percent of Individuals
pi_ffg_subsudep	Functional; FFG	Percent of Individuals
pi_ffg_gen	Functional; FFG	Percent of Individuals
pi_bioturb_surfmod	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_biodiff	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_convey	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_nobiot	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_upwardconv	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_downwardconv	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_bioirr	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_gen	Functional; bioturbation - specific	Percent of Individuals
pi_bioturb_nobiot_gen	Functional; bioturbation - specific	Percent of Individuals
pi_livhabit_freeliving	Functional; living habit/movement	Percent of Individuals
pi_livhabit_burrower	Functional; living habit/movement	Percent of Individuals
pi_livhabit_tubedwelling	Functional; living habit/movement	Percent of Individuals

Metric Name	Category	Formula
pi_livhabit_freeliving_commensal	Functional; living habit/movement	Percent of Individuals
pi_livhabit_attached	Functional; living habit/movement	Percent of Individuals
pi_AMBI_I	AMBI or MAMBI	Percent of Individuals
pi_AMBI_II	AMBI or MAMBI	Percent of Individuals
pi_AMBI_III	AMBI or MAMBI	Percent of Individuals
pi_AMBI_IV	AMBI or MAMBI	Percent of Individuals
pi_AMBI_V	AMBI or MAMBI	Percent of Individuals
pi_AMBI_IandII	AMBI or MAMBI	Percent of Individuals
pi_AMBI_IVandV	AMBI or MAMBI	Percent of Individuals
pi_AMBInat_I	AMBI or MAMBI	Percent of Individuals
pi_AMBInat_II	AMBI or MAMBI	Percent of Individuals
pi_AMBInat_III	AMBI or MAMBI	Percent of Individuals
pi_AMBInat_IV	AMBI or MAMBI	Percent of Individuals
pi_AMBInat_V	AMBI or MAMBI	Percent of Individuals
pi_AMBInat_IandII	AMBI or MAMBI	Percent of Individuals
pi_AMBInat_IVandV	AMBI or MAMBI	Percent of Individuals
pt_ACTEOCINA	Structural	Percent of Taxa
pt_ALITTA	Structural	Percent of Taxa
pt_AMPELISCA	Structural	Percent of Taxa
pt_CAPITELLA	Structural	Percent of Taxa
pt_CLYMENELLA	Structural	Percent of Taxa
pt_CREPIDULA	Structural	Percent of Taxa
pt_EDOTIA	Structural	Percent of Taxa
pt_EXOGONE	Structural	Percent of Taxa
pt_GEMMA	Structural	Percent of Taxa
pt_GLYCERA	Structural	Percent of Taxa
pt_GLYCINDE	Structural	Percent of Taxa
pt_GRANDIDIERELLA	Structural	Percent of Taxa
pt_HETEROMASTUS	Structural	Percent of Taxa
pt_HYPERETEONE	Structural	Percent of Taxa
pt_ILYANASSA	Structural	Percent of Taxa
pt_JAPONACTAEON	Structural	Percent of Taxa
pt_KIRSTEUERIELLA	Structural	Percent of Taxa
pt_LEITOSCOLOPLOS	Structural	Percent of Taxa
pt_LEUCON	Structural	Percent of Taxa
pt_MEDIOMASTUS	Structural	Percent of Taxa
pt_MICRODEUTOPUS	Structural	Percent of Taxa
pt_MONOCOROPHIUM	Structural	Percent of Taxa

Metric Name	Category	Formula
pt_NEPHTYS	Structural	Percent of Taxa
pt_NUCULA	Structural	Percent of Taxa
pt_OXYUROSTYLIS	Structural	Percent of Taxa
pt_PECTINARIA	Structural	Percent of Taxa
pt_POLYCIRRUS	Structural	Percent of Taxa
pt_POLYDORA	Structural	Percent of Taxa
pt_SCOLETOMA	Structural	Percent of Taxa
pt_SPIOCHAETOPTERUS	Structural	Percent of Taxa
pt_STREBLOSPIO	Structural	Percent of Taxa
pt_THARYX	Structural	Percent of Taxa
pt_ACTEONIDAE	Structural	Percent of Taxa
pt_AMPELISCIDAE	Structural	Percent of Taxa
pt_AORIDAE	Structural	Percent of Taxa
pt_CALYPTRAEIDAE	Structural	Percent of Taxa
pt_CAPITELLIDAE	Structural	Percent of Taxa
pt_CHAETOPTERIDAE	Structural	Percent of Taxa
pt_CIRRATULIDAE	Structural	Percent of Taxa
pt_COROPHIIDAE	Structural	Percent of Taxa
pt_DIASTYLIDAE	Structural	Percent of Taxa
pt_GLYCERIDAE	Structural	Percent of Taxa
pt_GONIADIDAE	Structural	Percent of Taxa
pt_IDOTEIDAE	Structural	Percent of Taxa
pt_LEUCONIDAE	Structural	Percent of Taxa
pt_LUMBRINERIDAE	Structural	Percent of Taxa
pt_MACTRIDAE	Structural	Percent of Taxa
pt_MALDANIDAE	Structural	Percent of Taxa
pt_NASSARIIDAE	Structural	Percent of Taxa
pt_NEPHTYIDAE	Structural	Percent of Taxa
pt_NEREIDIDAE	Structural	Percent of Taxa
pt_NUCULIDAE	Structural	Percent of Taxa
pt_ORBINIIDAE	Structural	Percent of Taxa
pt_PECTINARIIDAE	Structural	Percent of Taxa
pt_PHYLLODOCIDAE	Structural	Percent of Taxa
pt_POSEIDONEMERTIDAE	Structural	Percent of Taxa
pt_PYRAMIDELLIDAE	Structural	Percent of Taxa
pt_SPIONIDAE	Structural	Percent of Taxa
pt_SYLLIDAE	Structural	Percent of Taxa
pt_TELLINIDAE	Structural	Percent of Taxa
pt_TEREBELLIDAE	Structural	Percent of Taxa
pt_TORNATINIDAE	Structural	Percent of Taxa

Metric Name	Category	Formula
pt_VENERIDAE	Structural	Percent of Taxa
pt_AMPHIPODA	Structural	Percent of Taxa
pt_CARDIIDA	Structural	Percent of Taxa
pt_CEPHALASPIDEA	Structural	Percent of Taxa
pt_CUMACEA	Structural	Percent of Taxa
pt_DECAPODA	Structural	Percent of Taxa
pt_EUNICIDA	Structural	Percent of Taxa
pt_HETEROSTROPHA	Structural	Percent of Taxa
pt_ISOPODA	Structural	Percent of Taxa
pt_LITTORINIMORPHA	Structural	Percent of Taxa
pt_MONOSTILIFERA	Structural	Percent of Taxa
pt_NEOGASTROPODA	Structural	Percent of Taxa
pt_NUCULIDA	Structural	Percent of Taxa
pt_PHYLLODOCIDA	Structural	Percent of Taxa
pt_PYRAMIDELLIDA	Structural	Percent of Taxa
pt_SPIONIDA	Structural	Percent of Taxa
pt_TEREBELLIDA	Structural	Percent of Taxa
pt_VENERIDA	Structural	Percent of Taxa
pt_BIVALVIA	Structural	Percent of Taxa
pt_CLITELLATA	Structural	Percent of Taxa
pt_GASTROPODA	Structural	Percent of Taxa
pt_HOPLONEMERTEA	Structural	Percent of Taxa
pt_MALACOSTRACA	Structural	Percent of Taxa
pt_POLYCHAETA	Structural	Percent of Taxa
pt_ffg_dep	Functional; FFG	Percent of Taxa
pt_ffg_pred	Functional; FFG	Percent of Taxa
pt_ffg_filt	Functional; FFG	Percent of Taxa
pt_ffg_scav	Functional; FFG	Percent of Taxa
pt_ffg_graz	Functional; FFG	Percent of Taxa
pt_ffg_subsudep	Functional; FFG	Percent of Taxa
pt_ffg_gen	Functional; FFG	Percent of Taxa
pt_bioturb_surfmod	Functional; bioturbation - specific	Percent of Taxa
pt_bioturb_biodiff	Functional; bioturbation - specific	Percent of Taxa
pt_bioturb_convey	Functional; bioturbation - specific	Percent of Taxa
pt_bioturb_nobiot	Functional; bioturbation - specific	Percent of Taxa
pt_bioturb_upwardconv	Functional; bioturbation - specific	Percent of Taxa

Metric Name	Category	Formula
pt_bioturb_downwardconv	Functional; bioturbation - specific	Percent of Taxa
pt_bioturb_bioirr	Functional; bioturbation - specific	Percent of Taxa
pt_bioturb_gen	Functional; bioturbation - specific	Percent of Taxa
pt_bioturb_nobiot_gen	Functional; bioturbation - specific	Percent of Taxa
pt_livhabit_freeliving	Functional; living habit/movement	Percent of Taxa
pt_livhabit_burrower	Functional; living habit/movement	Percent of Taxa
pt_livhabit_tubedwelling	Functional; living habit/movement	Percent of Taxa
pt_livhabit_freeliving_commensal	Functional; living habit/movement	Percent of Taxa
pt_livhabit_attached	Functional; living habit/movement	Percent of Taxa
pt_AMBI_I	AMBI or MAMBI	Percent of Taxa
pt_AMBI_II	AMBI or MAMBI	Percent of Taxa
pt_AMBI_III	AMBI or MAMBI	Percent of Taxa
pt_AMBI_IV	AMBI or MAMBI	Percent of Taxa
pt_AMBI_V	AMBI or MAMBI	Percent of Taxa
pt_AMBI_IandII	AMBI or MAMBI	Percent of Taxa
pt_AMBI_IVandV	AMBI or MAMBI	Percent of Taxa
pt_AMBInat_I	AMBI or MAMBI	Percent of Taxa
pt_AMBInat_II	AMBI or MAMBI	Percent of Taxa
pt_AMBInat_III	AMBI or MAMBI	Percent of Taxa
pt_AMBInat_IV	AMBI or MAMBI	Percent of Taxa
pt_AMBInat_V	AMBI or MAMBI	Percent of Taxa
pt_AMBInat_IandII	AMBI or MAMBI	Percent of Taxa
pt_AMBInat_IVandV	AMBI or MAMBI	Percent of Taxa
prop_AMBI_I	AMBI or MAMBI	Proportion of Individuals
prop_AMBI_II	AMBI or MAMBI	Proportion of Individuals
prop_AMBI_III	AMBI or MAMBI	Proportion of Individuals
prop_AMBI_IV	AMBI or MAMBI	Proportion of Individuals
prop_AMBI_V	AMBI or MAMBI	Proportion of Individuals
prop_AMBInat_I	AMBI or MAMBI	Proportion of Individuals
prop_AMBInat_II	AMBI or MAMBI	Proportion of Individuals
prop_AMBInat_III	AMBI or MAMBI	Proportion of Individuals
prop_AMBInat_IV	AMBI or MAMBI	Proportion of Individuals
prop_AMBInat_V	AMBI or MAMBI	Proportion of Individuals

Metric Name	Category	Formula
x_Shan_e	Diversity, Richness, or Evenness	$-\text{SUM}((N_TAXA / ni_total) * \text{LOG}((N_TAXA / ni_total)))$
x_Shan_2	Diversity, Richness, or Evenness	$x_Shan_e / \text{LOG}(2)$
x_Shan_10	Diversity, Richness, or Evenness	$x_Shan_e / \text{LOG}(10)$
x_Simpson	Diversity, Richness, or Evenness	$1 - \text{SUM}((N_TAXA / ni_total)^2)$
x_Gleason	Diversity, Richness, or Evenness	$(nt_total) / \text{LOG}(ni_total)$
x_Margalef	Diversity, Richness, or Evenness	$(nt_total - 1) / \text{LOG}(ni_total)$
x_PielouEvenness	Diversity, Richness, or Evenness	$x_Shan_e / \text{LOG}(nt_total)$
AvgTaxDistinct	Diversity, Richness, or Evenness	Average taxonomic distinctness (indepdent of the number of species)
TotTaxDistinct	Diversity, Richness, or Evenness	Total taxonomic distinctness (influenced by the number of species)
x_Chao1	Diversity, Richness, or Evenness	Chao richness
ri_AMBI_IandII_to_IVandV	AMBI or MAMBI	Ratio of individuals
rt_AMBI_IandII_to_IVandV	AMBI or MAMBI	Ratio of individuals
AMBI	AMBI or MAMBI	$(0 * pi_AMBI_I + 1.5 * pi_AMBI_II + 3 * pi_AMBI_III + 4.5 * pi_AMBI_IV + 6 * pi_AMBI_V) / 100$
AMBI_nat	AMBI or MAMBI	$(0 * pi_AMBI_{nat}_I + 1.5 * pi_AMBI_{nat}_II + 3 * pi_AMBI_{nat}_III + 4.5 * pi_AMBI_{nat}_IV + 6 * pi_AMBI_{nat}_V) / 100$
AMBI_Score_east_pckg	AMBI or MAMBI	Sample AMBI score from https://www.sccwrp.org/about/research-areas/additional-research-areas/sediment-quality/sediment-quality-assessment-tools/

Metric Name	Category	Formula
MAMBI_Score_east_pckg	AMBI or MAMBI	Sample MAMBI score from https://www.sccwrp.org/about/research-areas/additional-research-areas/sediment-quality/sediment-quality-assessment-tools/
AMBI_Score_nat_pckg	AMBI or MAMBI	Sample AMBI score from https://www.sccwrp.org/about/research-areas/additional-research-areas/sediment-quality/sediment-quality-assessment-tools/
MAMBI_Score_nat_pckg	AMBI or MAMBI	Sample MAMBI score from https://www.sccwrp.org/about/research-areas/additional-research-areas/sediment-quality/sediment-quality-assessment-tools/
nt_HeteroMedio	Structural	Number of Taxa
pt_HeteroMedio	Structural	Percent of Taxa
ni_HeteroMedio	Structural	Number of Individuals
pi_HeteroMedio	Structural	Percent of Individuals

11 APPENDIX C: LINKED RESOURCES AND TOOLS

To support information, outreach, and continued development, the following tools and documents are available:

GitHub Repository (<https://github.com/ismarbiberovic/LIS-Macroinvertebrate-MMi.git>):

- R Markdown tool for MMI Calculation: Calculate MMI scores for new sites using your macroinvertebrate data.
- Phase I Report: Provides the scientific foundation and recommendations that guided index development.
- Phase II Report: Includes detailed methods, results, and appendices.

StoryMaps:

- Technical StoryMap: Explore the scientific foundation of the index through detailed methods, data analysis, and performance metrics.

Link: <https://storymaps.arcgis.com/stories/b544679aecfc486d968472293c424746>

- Non-Technical StoryMap: Discover the project's goals, methods, and results through interactive maps, engaging visuals, and clear, plain-language explanations designed for a general audience.

Link: <https://storymaps.arcgis.com/stories/56a0779009b04e03a7fc5e4c4f0199cb>