NYSG Completion Report Instructions & Required Format

Please include the following information for your project. The text of this report should be at least 5-8 pages and be composed for an audience of your peers. Other formats, or reports with incomplete sections, will not be accepted. The expectation is that information or material will be provided under each section.

REPORT WRITTEN BY: Gregory P. Dietl **DATE:** July 13, 2026

A. PROJECT NUMBER AND TITLE: # R/CMB-49-NYCT
"Using Geohistorical Baselines to Assess Responses of Benthic Macroinvertebrate Communities to the Nitrogen TMDL Management Intervention in Long Island Sound"

B. PROJECT PERSONNEL:

Table 1: Senior Personnel (list the PI, Co-PI(s), and AI(s), should match what was in the proposal (90-2 form). Changes in senior project personnel require prior notification and approval from NYSG.

Name	Role (PI, CO-PI, or AI)	Institution	Note
Gregory P. Dietl	PI	Paleontological Research Institution	

Table 2: Postdocs (list post-doctoral researchers who worked on the project and were supported by the grant).

Name	Institution	Note

Table 3: Research Support Specialists (list any professional staff who worked on the project and were paid by the grant).

Name	Institution	Note

Table 4: Sea Grant Scholar Fellows and Research Assistants (list all Scholar Fellows and graduate and undergraduate Research Assistants who were paid from the project's budget during this reporting period, i.e., on payroll).

Student Name	New or Continuing?*	Degree Program	Type (choose from list)	Graduation Date	Anticipated or Confirmed?+

*New students are working on the project for the first time during the reporting period. It would include all students in the first year and any students who begin in the second year or later. Students still working on the project in the second year are continuing.

+Anticipated or confirmed graduation date. For Scholar Fellows on the project, submit their thesis or dissertation after publication.

Student Types List (enter three-letter code in the Type column):
SGS – Sea Grant Scholar Fellow
GRA – Graduate Research Assistant
RA – Undergraduate Research Assistant or Technician

Table 5: Student Volunteers (list all graduate and undergraduate students who worked on the project and were not paid from the project's budget during this reporting period).

Student Name	New or Continuing?*	Degree Program	Graduation Date	Anticipated or Confirmed?+
Matthew J. Pruden	Continuing	PhD	8/31/2026	Anticipated

Table 6: Public Volunteers (list any volunteers from the public (e.g., citizens, high school students), if any, who worked on the project during this reporting period).

Volunteer Name	Estimated Hours
John C. Handley	134 hours

Student notes or details: Write details about the contribution to the project and other relevant details for each student listed in Table 4 as necessary.

Non-supported students: Provide details about each student's contribution to the project and any other relevant information as needed from Table 5. Students who receive payment from other sources for work done on this project are considered student volunteers on this project.

Matthew J. Pruden served as the Laboratory Technician and Taxonomist for the project. He was responsible for processing benthic grab samples, including washing, sieving, sorting, and picking mollusk shells from sediment residues. He identified specimens to the lowest practical taxonomic level using the project's reference collections and taxonomic literature, maintained and expanded the physical reference collection as new taxa were encountered, and selected suitable shell specimens for radiocarbon dating based on taxonomic identity, preservation, and analytical requirements. In addition, he assisted with data management and contributed to data analysis, visualization, and interpretation and final report drafting.

Volunteers: Write details about the contribution to the project from any volunteers listed in Table 6. Indicate their activity and estimate the amount of time (hours) they contributed.

John C. Handley served as the project's Data Manager and Statistician. He was responsible for managing the project database, including data entry and organization, overseeing data quality assessment procedures to identify transcription errors, inconsistencies, and missing information, ensuring data were formatted according to project reporting standards, verifying laboratory data prior to reporting, and evaluating data usability to ensure datasets met project quality objectives before analysis. He also led the statistical analyses used to evaluate project objectives and contributed to the interpretation of study results and final report drafting.

C. PROJECT RESULTS:

Complete the following sub-sections to discuss your results as they relate to the project's objectives:

C1. Meeting the Objectives:

> **Objective 1:** Summarize the results of meeting this objective (e.g., details of experiments, fieldwork, summary of results, graphs, tables, photos, etc.).
>
> **Next Objective:** Follow the same format for all objectives.

Objective: To use the remains of dead mollusks preserved in benthic grab samples to establish pre-Total Maximum Daily Load (TMDL) baselines of biological condition for benthic macroinvertebrate communities in Long Island Sound (LIS).

C1.1 Background and Justification

Summer hypoxia—< 3.0 mg/L dissolved oxygen—has been a persistent issue in Long Island Sound (LIS), with analysis of sediment core records suggesting that summer hypoxic events began as early as the mid-19th century (Varekamp et al., 2010). The increase in hypoxia has been linked to excess nitrogen, and resulting dissolved oxygen problems, from point-source pollution, such as municipal and industrial wastewater (NYSDEC and CT DEEP, 2000; Whitney and Vlahos, 2021). To address the water quality problems in LIS, the Connecticut Department of Environmental Protection (CT DEEP) and New York State Department of Environmental Conservation (NYS DEC) adopted a Total Maximum Daily Load (TMDL) strategy aimed to improve water quality and increase dissolved oxygen levels via the reduction of point-source nitrogen pollution by 58.5%, and nonpoint-source levels by 10%, by the year 2014 (NYS DEC and CT DEEP, 2000). Reducing the extent and duration of hypoxic conditions in LIS was expected to provide ecological and environmental benefits, including overall improvement in benthic habitat conditions (NYS DEC and CT DEEP, 2000). Ultimately, the TMDL proved successful as the nitrogen reduction goals were reached by 2017, and have since continued to decline (LISS, 2017).

While the TMDL targets were exceeded, a crucial knowledge gap remains: it is unknown whether the marine biota have responded as expected because long-term monitoring data needed to assess management success are lacking (LISS, 2020). The primary objective of this pilot study is to test whether the remains of shelled mollusks can establish pre-TMDL baseline conditions of biological condition—measured using the AZTI Marine Biotic Index (AMBI; Borja et al., 2000)—to assess benthic macroinvertebrate community response to improved water quality in LIS. We focus on mollusks because they are readily identifiable, a major functional component within the shallow marine benthos, differentially sensitive to environmental stressors (Zenetos, 1996; Marshal et al., 2018), and are reliable proxies of the whole benthic macroinvertebrate community (Dietl et al., 2016; Pruden et al., 2021). Mollusks also have high preservational potential, which allows for their remains to accumulate within the seafloor sediment. The molluscan geohistorical record thus has the potential to provide long-term, location-specific ecological information to establish baseline conditions (Dietl et al., 2016; Smith et al., 2020).

The baseline samples we analyze here provide a unique perspective on the composition of the pre-TMDL benthic macroinvertebrate community that is not available from any other source. We hypothesize that biological condition in LIS has changed since implementation of the TMDL in 2001, with the direction of change tracking site-specific trends in water quality.

C1.1.1 Mollusks as Reliable Proxies for the Whole-Community

To confirm the fidelity of a mollusk-only AMBI to that of the whole benthic macroinvertebrate community in LIS, we used the methods outlined in Pruden et al. (2021; see also Dietl et al. 2016) and compiled benthic survey data from the US Environmental Protection Agency's Environmental

Monitoring & Assessment Program (EMAP; 1990–1993; USEPA, 1999), National Coastal Assessment (NCA; 2000–2006; USEPA, 2005, 2008, 2012) and National Coastal Conditions Assessment surveys (NCCA; 2010, 2015, 2020; USEPA, 2016, 2021, in preparation). Sample sites were restricted to those with at least three molluscan species, following the guidelines set by Borja and Muxika (2005), and those experiencing polyhaline conditions due to the known effect of salinity on AMBI scores, resulting in 275 sample sites (Gillett et al., 2015). AMBI is calculated based on the weighted proportional abundance of taxa assigned to ecological groups (EGs) indicating increasing tolerance to anthropogenic stress (EG1 = stress sensitive; EG2 = stress indifferent; EG3 = stress tolerant; EG4 = second-order opportunistic; EG5 = first-order opportunistic; Figure 1; Borja et al., 2000). Taxa were assigned an EG using the U.S. hybrid designation scheme (Gillett et al., 2015), supplemented by the international AZTI EG designation scheme (http://ambi.azti.es; Species List v.Oct2024; Borja et al., 2012) and revised assignments for LIS taxa whose existing EG designations were reevaluated by Pruden et al. (2026). AMBI scores were calculated in R using the square-root transformed abundance data for both the mollusk-only and whole-community (Eq. 1; Borja et al., 2000; Sigovini et al., 2013; R Core Team, 2026).

$$AMBI = [(0 * \%EG1) + (1.5 * \%EG2) + (3 * \%EG3) + (4.5 * \%EG4) + (5 * \%EG5)]/100 \text{ (Eq 1.)}$$

The resulting AMBI scores were then evenly split into two groups (137 and 138 sample sites in the training and test datasets respectively). A Bayesian linear regression was applied to the first group to assess the relationship between the mollusk-only and whole-community AMBI scores. Subsequently, the calculated linear regression was applied to the mollusk-only scores in the second group of sample sites to calculate the corrected mollusk-only AMBI scores. The biological condition of the corrected mollusk-only and whole-community AMBI scores from the second group of sample sites was then designated as either Undisturbed, Slightly Disturbed, Moderately Disturbed, or Heavily Disturbed (Borja et al., 2000; Teixeira et al., 2012; Gillett et al., 2015). Because transforming the abundance data shortens the AMBI scale, thereby reducing the difference between biological condition classifications, the boundaries were adjusted based on the linear relationship between the raw, untransformed, and square-root transformed whole-community AMBI scores (Undisturbed: AMBI < 1.43, Slightly Disturbed: 1.43 ≤ AMBI < 2.77, Moderately Disturbed: 2.77 ≤ AMBI < 3.42, Disturbed: 3.42 ≤ AMBI; Figures 1, 2A; Warwick et al., 2010; Pruden et al., 2021).

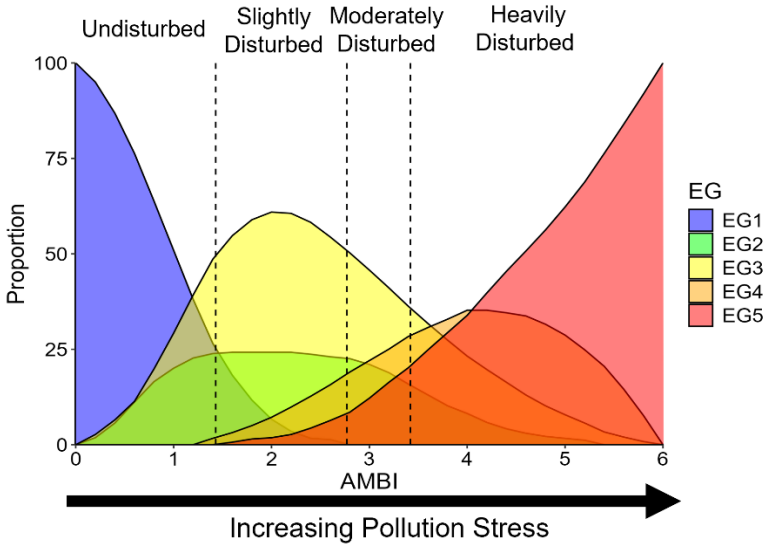


Figure1. Proportional abundance of the five ecological groups (EGs) used to calculate AMBI scores along a pollution gradient, ranging from sensitive (EG1) to highly opportunistic species (EG5). The vertical dotted lines represent the biological condition classification boundaries adjusted for square-root transformation.

Precision scores, which measure the accuracy of corrected mollusk-only biological condition designations in relation to the whole-community (e.g., the percentage of sites classified as Undisturbed according to the corrected mollusk-only scores that were also classified as Undisturbed by the whole-community), were calculated to determine the fidelity of the corrected mollusk-only designations (Goutte and Gaussier, 2005). Given that the boundary between Slightly Disturbed and Moderately Disturbed indicates whether a site is impaired, and therefore may require remediation effort, we focused on the fidelity of the corrected mollusk-only AMBI in discerning sample sites on either side of the impairment boundary.

The mollusk-only AMBI scores from the first group of sample sites were positively correlated with the whole-community (R^2 = 0.43 ± 0.01; Figure 2B). After applying the linear regression to the mollusk-only sample sites from the second group, the corrected mollusk-only scores indicated a high degree of fidelity, correctly classifying 86% and 72% of sample sites as unimpaired and impaired, respectively (Table 1; Figure 2C). These results confirm that mollusks are reliable proxies for the whole benthic macroinvertebrate community within LIS and highlight their potential to provide site-specific geohistorical baselines to assess changes in biological condition since the TMDL management intervention.

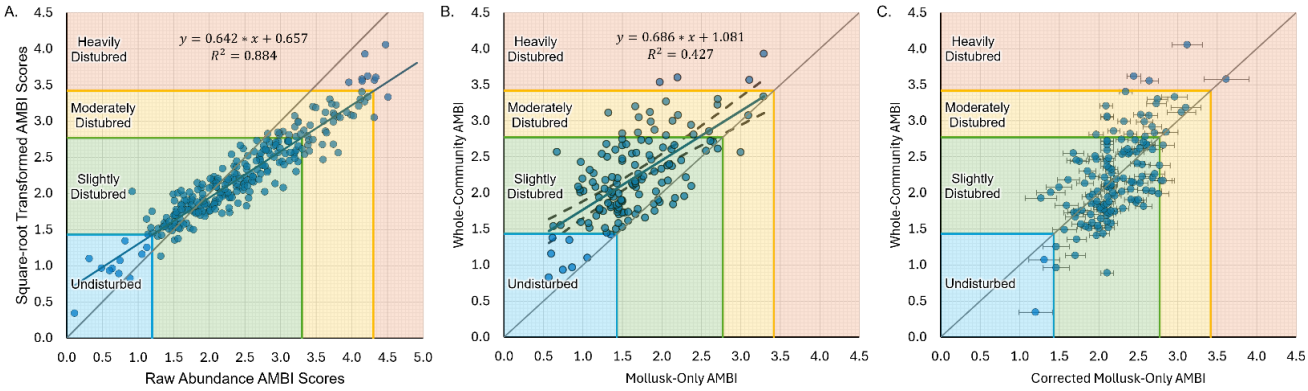


Figure 2. A. Linear regression between the raw, untransformed and square-root transformed whole-community AMBI scores. B. Linear regression between the whole-community and mollusk-only AMBI scores from the first group of sample sites. The blue line represents the linear regression, the dashed lines represent two standard deviations from the mean linear regression relationship, and the solid gray line represents a one-to-one relationship. C. Comparison between the corrected mollusk-only AMBI scores and the whole-community AMBI scores from the second group of sample sites. The error bars represent two standard deviations. The colored regions indicate the designated biological conditions: blue = Undisturbed, green = Slightly Disturbed, orange = Moderately Disturbed, red = Heavily Disturbed.

Table 1. Fidelity metric scores for the corrected mollusk-only AMBI.

	Precision
Unimpaired	0.86 ± 0.01
Impaired	0.72 ± 0.17

C1.1.2 Molluscan Death Assemblages as Records of Past Conditions

During the sorting process of benthic samples (e.g., collected by a Young-modified Van Veen grab), the remains of dead individuals are separated and retained in unsorted sample residues. These residues are also referred to as death assemblages and are the product of the accumulation of recently dead individuals (shells) from local standing populations (or living assemblages; Kidwell,

2013). As new shells are accumulated, sediment reworking (e.g., bioturbation) is constantly mixing new and older shells together within the top layer of the sediment, a process called time-averaging. Therefore, as biological condition changes within a living assemblage, the retention of past biological information via sediment reworking results in the taxonomic composition of the death assemblage lagging behind that of the living assemblage (Kidwell, 2007). In other words, the time-averaged nature of the death assemblage retains a "memory" of the former abundance of species.

C1.1.3 Project Importance and LISP CCMP Topic Areas

This project supports the LIS Comprehensive Conservation and Management Plan (CCMP) Implementation Action WW-8: "Conduct studies and research to better understand the ecosystem's response to nitrogen reductions to support an evaluation of the 2000 Dissolved Oxygen TMDL" (LISS, 2020). The Long Island Sound Study (now the Long Island Sound Partnership; LISP) identified several ecological benefits from reduced nitrogen loading from the TMDL, including the reduction of maximum area of LIS that is unhealthy for aquatic life by 75%, the reduction of larval mortality by 67%, and the improvement of ecological conditions in LIS's embayments (NYS DEC and CT DEEP, 2000). Knowledge of pre-TMDL baselines of biological condition and directions of change fill an identified gap in our understanding of whether the TMDL intervention was successful or if more progress on the reduction of nitrogen is needed to meet water quality standards in LIS that stimulate the desired ecological benefits. Also, our project supports the CT DEEP priority to develop biotic indices to assess biological condition in LIS under their 2015-2024 Ambient Water Quality Monitoring Program Strategy and Clean Water Act mandate (CT DEEP, 2015), such as the Benthic Macroinvertebrate Sampling Methodology Embayment project (Tetra Tech, 2020).

C1.2 Research Design and Methods

C1.2.1 Sample Source and Selection

To test our hypothesis that benthic macroinvertebrate communities responded to the TMDL management intervention, we analyzed ten 0.04m^2 Young-modified Van Veen grab samples collected in 2020 as part of the National Coastal Conditions Assessment (NCCA) (USEPA, 2020a, in preparation) (Figure 3). Samples were selected from the Western Open Sound, the Narrows, and several New York embayments based on their proximity to long-term water quality monitoring stations (Figure 3). To minimize potential differences in sediment accumulation rates and sediment mixing, and thus variation in time-averaging, we restricted analyses to samples from fine grained sediments (i.e., sandy-silt to silty-clay).

NCCA samples were processed by the USEPA and its contractors, who sieved each sample through a 500-µm mesh, sorted living benthic macroinvertebrates, identified them to the lowest possible taxonomic level, counted individuals, and preserved them in 1-L jars of 95% ethanol (USEPA, 2020a). The shelled remains of dead mollusks were retained in the unsorted residue and preserved separately in 1-L jars of ethanol (USEPA, 2020a). Each grab sample is associated with environmental data (USEPA, 2020a).

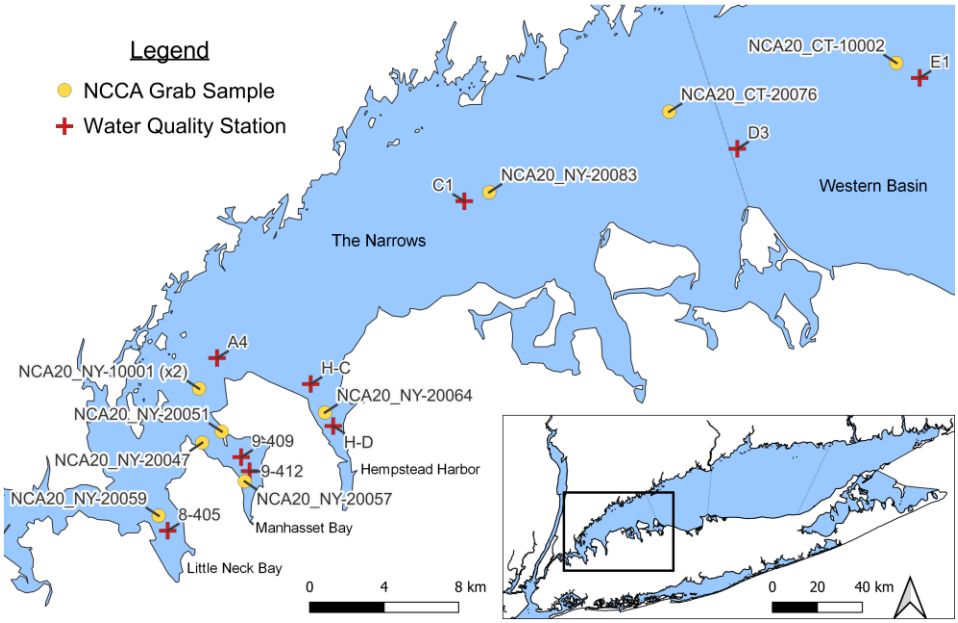


Figure 3. Map of the NCCA grab samples (yellow circles) and water quality monitoring station locations (red plus sign). Note: NCA20_NY-10001 visit 1 and NCA20_NY-10001 visit 2 share the same location. Coastline shapefile downloaded from USGS (201403).

Salinity, water depth, and substrate type were classified according to the Coastal and Marine Ecological Classification Standard (USFGDC, 2012). Sediment quality was evaluated using the Sediment Contamination Index (SCI), which integrates the mean effects range median quotient (mERM-Q) and maximum probability of toxicity (Pmax), while water quality was evaluated using the Eutrophication Index (EI), which integrates bottom-water dissolved oxygen (DO), chlorophyll-*a* (CHLA), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), and water transmissivity (TRANS) following the National Coastal Condition Assessment protocol (USEPA, 2020b). Thresholds used in this study to classify the SCI and EI for each sampling site as Good, Fair, or Poor are summarized in Table 2.

The distance between each benthic sampling site and its associated water-quality monitoring station was calculated in QGIS and is reported in the site summary tables (see Results and Discussion section) to document the proximity of each sample to the station used for reconstructing hypoxia trends.

Table 2: Thresholds used to classify the Eutrophication Index (EI) and Sediment Contamination Index (SCI) metrics into Good, Fair, and Poor condition classes following the National Coastal Condition Assessment (USEPA, 2020b).

EI Metrics	**Good**	**Fair**	**Poor**
DO (mg/L)	> 5	≤ 5 and > 2	≤ 2
CHLA (µg/L)	≤ 5	> 5 and ≤ 20	> 20
DIN (mg/L)	≤ 0.1	> 0.1 and ≤ 0.5	> 0.5
SRP (mg/L)	≤ 0.01	> 0.01 and ≤ 0.05	> 0.05
TRANS (%)	> 20	≤ 20 and > 10	≤ 10
EI	No metrics are rated 'Poor' and a maximum of one metric is rated 'Fair'	One metric is rated 'Poor'; or two or more metrics are rated 'Fair'	Two or more metrics are rated 'Poor'
SCI Metrics	**Good**	**Fair**	**Poor**
mERM-Q	< 0.1	≥ 0.1 and ≤ 0.5	> 0.5
Pmax	≤ 0.5	> 0.5 and < 0.75	≥ 0.75
SCI	Both mERM-Q and Pmax are rated as 'Good'	Either mERM-Q or Pmax are rated as 'Fair'	Either mERM-Q or Pmax are rated as 'Poor'

C1.2.2 Modeling the Probability of Summer Hypoxic Events

To assess expected changes in biological condition based on long-term trends in water quality, we used dissolved oxygen (DO) data collected through the CT DEEP LIS Ambient Water Quality Monitoring program and the Interstate Environmental Commission WLIS monitoring program (CT DEEP, 2015; IEC, 2022). We defined a hypoxic event as a measured DO concentration < 3 mg/L at the maximum depth recorded on a sampling day during July and August.

Because reductions in hypoxia are expected to improve benthic biological condition, we quantified long-term trends in the probability of hypoxic events at each monitoring station to generate site-specific expectations for ecological change. Temporal trends in hypoxia events were estimated using a binomial model, where the number of "trials" was the total number of observations over July and August in a given year and a "success" was defined as a hypoxic event. For a given year t,

$$Y_t \sim Binomial(n_t; p_t) \text{ (Eq. 2)}$$

where n_t is the total number of observations in a given year, Y_t is the number of hypoxic events, and p_t is the probability of a hypoxic event. The log-odds of hypoxia were modeled as:

$$logit(p_t) = \beta_0 + \beta_1(t - t_0) \text{ (Eq. 3)}$$

where t_0 is the earliest year in the dataset. The corresponding probability of a hypoxic event was obtained using the inverse-logit transformation:

$$p_t = 1/1 + e^{-(\beta_0 + \beta_1(t - t_0))} \text{ (Eq. 4)}$$

A negative slope ($\beta_1 < 0$) indicates that the probability of a hypoxic event decreases over time, whereas a positive slope ($\beta_1 > 0$) indicates an increasing probability of hypoxia. Slopes centered near zero indicate little evidence for directional change over time. Because the sample sizes are relatively small, we adopted a Bayesian approach to explicitly quantify uncertainty rather than relying solely on null hypothesis significance testing with a strict p-value cut-off (e.g., 0.05). Bayesian inference yields a posterior distribution for β_1, from which the probability that the slope is negative, positive, or near zero can be estimated. These posterior probabilities provide a more informative measure of evidence for changing hypoxia conditions than a binary significance test.

C1.2.3 Sample Processing

The NCCA grab sample residues were processed at the Paleontological Research Institution (PRI), where all bivalve and gastropod remains were sorted, counted, and identified to the lowest possible taxonomic level. Because articulated bivalve specimens were rare, indicating that shell disturbance was sufficiently high for most valves to represent different individuals, each disarticulated valve was counted as a unique individual (Gilinsky and Bennington, 1994).

C1.2.4 Geochronology

To confirm whether the dead mollusk samples represent pre-TMDL baseline conditions, we estimated the ages of shells within each grab sample residue. Twenty shells of the dominant mollusk species, *Mulinia lateralis,* were randomly selected and radiocarbon dated (^{14}C) at the ACE Isotope Laboratory, Northern Arizona University, using a Mini Carbon Dating System (MICADAS; Synal et al., 2007; Gottschalk et al., 2018). Because sample NY-20051 contained fewer than 20 *Mulinia* shells, radiocarbon dating included seven *Crepidula fornicata* and eight *Mulinia* specimens to obtain a sufficient number of age determinations.

To estimate sample age and time averaging, we used the Bayesian radiocarbon calibration approach developed by Heaton (2022). Radiocarbon calibration uses a calibration curve to map radiocarbon measurements to calendar dates; however, uncertainty in both radiocarbon measurement and the calibration curve results in a probability distribution of possible calendar dates for each shell. Traditional calibration approaches treat each shell in a sample independently and then average resulting distributions to estimate an age distribution of the sample. However, this approach often produces multimodal distributions and does not explicitly account for shared structure among dated shells within a sample. In contrast, Heaton's Bayesian method assumes that all dated shells within a sample are drawn from a common probability distribution. The full set of ^{14}C measurements is jointly modeled with the calibration curve to estimate a calendar age distribution represented as a random mixture of normal distributions. This approach is well suited to time-averaged death assemblages, which are expected to approximate unimodal age distributions due to sediment mixing processes (Tomasovych et al., 2014). The resulting calendar age distributions are usually unimodal as well, simplifying interpretation of sample age and time averaging. We implemented this approach using modified R-code from Heaton (2022; Github repository cited therein).

C1.2.5 Calculating Biological Condition

Biological condition was estimated for both the live and dead mollusk components of each grab sample residue using the AZTI Marine Biotic Index (AMBI; Borja et al., 2000). Analysis included all identifiable individuals within each sample. As with the mollusk-only AMBI validation for LIS (see Section C1.1.1 Mollusks as Reliable Proxies for the Whole-Community) taxa were assigned an EG using the U.S. hybrid designation scheme (Gillett et al., 2015), supplemented by the international AZTI EG designation scheme (http://ambi.azti.es; Species List v.Oct2024; Borja et al., 2012) and revised assignments for LIS taxa whose existing EG designations were reevaluated by Pruden et al. (2026). AMBI scores were calculated in R using the square-root transformed abundance data for both the mollusk-only and whole-community (Eq. 1; Borja et al., 2000; Sigovini et al., 2013; R Core Team, 2026). The resulting AMBI scores ranged from zero to six (Figure 1), with higher values indicating more degraded biological conditions. For example, a high proportion of EG1 (stress sensitive) taxa in a grab sample would indicate an undisturbed habitat ($0.0 \leq AMBI < 1.43$), while a dominance of EG 4 and EG 5 opportunistic taxa would suggest a heavily disturbed habitat ($3.42 \leq AMBI$).

To assess temporal change in biological condition, we calculated the difference in AMBI between the living and death assemblages within each grab sample (living assemblage AMBI − death assemblage AMBI). Because higher AMBI scores indicate more degraded biological conditions, negative differences indicate that the contemporary living mollusk assemblage has improved biological condition relative to the time-averaged dead mollusk assemblage, whereas positive differences indicate deterioration.

C1.2.6 Copula

Our goal is to evaluate confidence in differences in biological condition by estimating the joint probability that 1) hypoxic conditions have improved over time and 2) death assemblages predate implementation of the TMDL. Both components are uncertain and are estimated independently. Hypoxia trends are subject to model and estimation uncertainty and are represented by Bayesian posterior distributions of slopes. Ages of mollusk samples are likewise uncertain owing to sampling and radiocarbon dating uncertainty. The challenge is therefore to combine these two measures while preserving their inherent uncertainty.

For example, a given sample may be associated with a nearby water quality monitoring station exhibiting an improving hypoxia trend and have an observed AMBI-difference value indicative of

relatively good biological condition; however, that same sample may still have a 60% probability of being younger than 2001 (the year of TDML implementation). Copulas provide a formal approach to combining uncertainty (Bhatti and Do, 2019; Tootoonchi et al. 2022).

Therefore, we adopted a simple copula-based approach to combine the posterior distributions of hypoxia trend slopes and sample ages. That is, if F(x) is the cumulative probability distribution of the slope posterior and G(y) is the cumulative probability distribution of sample age, then the joint distribution is H(x,y) = F(x)G(y). While this represents the simplest case, it provides a transparent means of propagating and visualizing joint uncertainty. “Iso-probability” contours of H(x,y) illustrate trade-offs between uncertainty in hypoxia trend direction and uncertainty in sample age.

C1.3 Results and Discussion

C1.3.1 Overarching Results

Sample ages

Radiocarbon-based age estimates indicate that molluscan death assemblages are predominantly time-averaged prior to TMDL implementation. Across sites, median calibrated ages cluster well before 2001 CE, with a mean median age of approximately 1985 CE (Figure 4; NCA20_CT-10002 contained too few shells to derive a reliable age). Most samples show broad, unimodal age distributions spanning multiple decades, consistent with within-sample time averaging. Although some samples include minor contributions of post-2001 specimens, probability density is strongly weighted toward pre-TMDL deposition in nearly all cases. This pattern confirms that death assemblages primarily integrate historical benthic conditions from the pre-TMDL era, providing an appropriate baseline for evaluating ecological change associated with TMDL implementation. Limited inter-site variation in central tendency suggests broadly similar temporal integration across LIS, despite differences in local depositional environments.

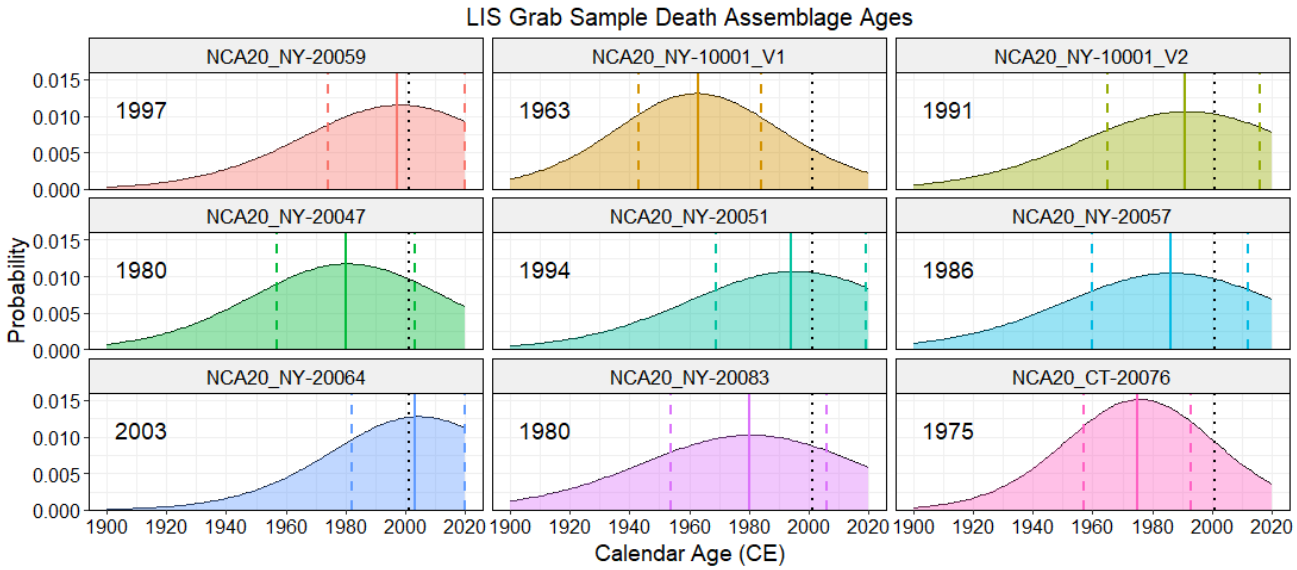


Figure 4. Calibrated calendar age distributions of the molluscan death assemblages. Dotted black like = 2001; Solid color lines = median age; Dashed colored lines = Interquartile range.

AMBI differences

Although AMBI scores generally increased from east to west, indicating more disturbed biological conditions toward the western end of LIS (Figure 5), differences between the living assemblages (LA) and death assemblages (DA) displayed a more nuanced pattern. Increases in AMBI from the DA to LA, indicating declining biological condition, occurred at several western sites (e.g., NY-10001_1, NY-

10001_2, and NY-20051_1), whereas decreases in AMBI, consistent with improved biological condition, were observed at NY-20064_1, NY-20083_1, and CT-20076_1. Changes at NY-20057_1, NY-20059_1, NY-20047_1, and CT-10002_1 were consistent with little change in biological condition. Despite documented improvements in water quality following implementation of the TMDL, the westernmost portion of the Sound continues to exhibit limited recovery. This apparent disconnect may reflect persistent sediment degradation, as western sites generally had poorer Sediment Contamination Index (SCI) ratings than sites farther east (Figure 5; see also section C1.3.2 Site-specific Interpretations).

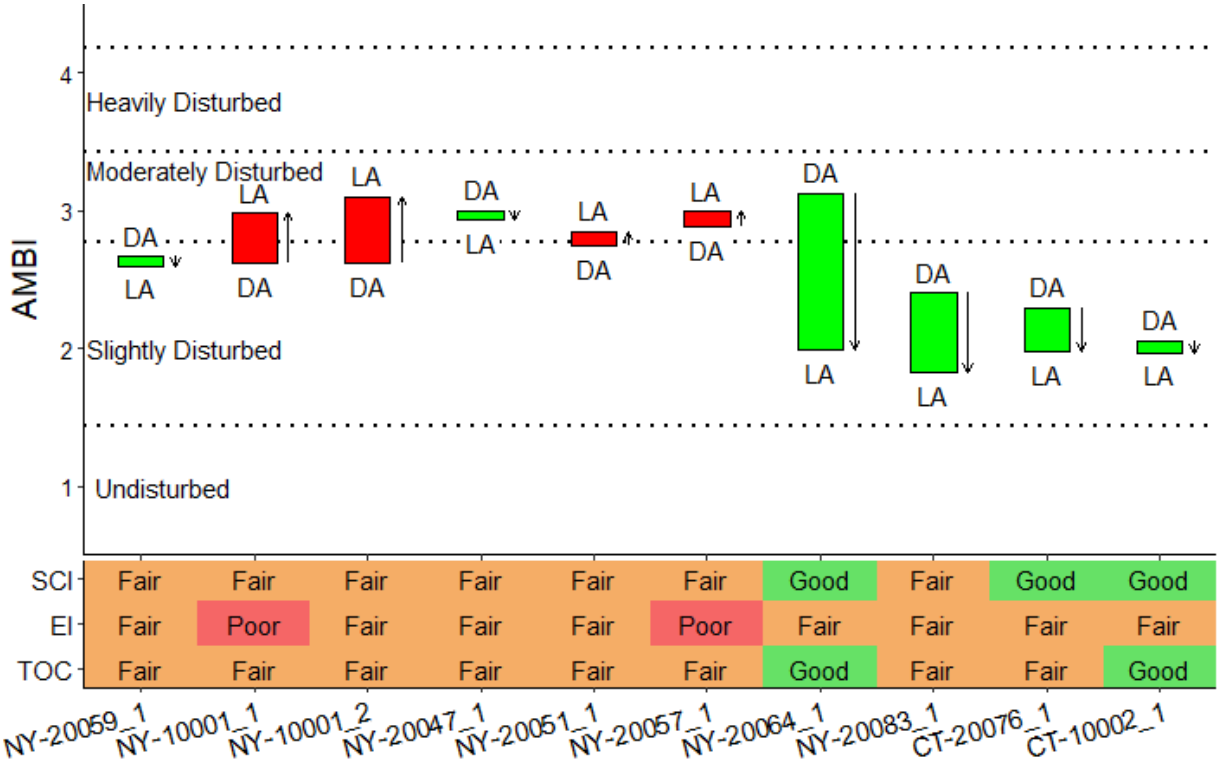


Figure 5. Top: Candlestick plot indicating the change in AMBI values from the DA to the LA. Bright red = decline in benthic conditions (increase in AMBI score); bright green = improved benthic conditions (decrease in AMBI score). Bottom: Environmental quality indicators calculated from the LA and rated Good (green), Fair (orange), or Poor (red). SCI = Sediment Contamination Index; EI = Eutrophication Index; TOC = sediment concentration of total organic carbon.

C1.3.2 Site-specific Interpretations

To better understand the local factors underlying the spatial patterns in biological condition shown in Figure 5, we evaluated each grab sample individually. For each sample, the observed change in biological condition (LA–DA AMBI) was compared with the expected response based on reconstructed trends in summer hypoxia (Table 2) and interpreted in the context of sediment- and water-quality conditions at the site.

NCA20_NY-20059: No change in biological condition

At site NY-20059 (Little Neck Bay; Figure 3), AMBI values remained effectively stable between the death and living assemblages (LA-DA = −0.07; Table 3; Figure 5), indicating little net change in biological condition despite strong evidence for improved water quality conditions over time, including a substantial decline in the probability of summer hypoxia (27% pre-TMDL to 2% in 2020; Table 3; Figure 6) and a strongly negative hypoxia trend (copula analysis: Pr[slope < 0] = 0.99; Figure 7). The median age of the death assemblage (1997 CE; joint probability = 0.53) suggests that it largely

represents conditions immediately preceding TMDL implementation, making the lack of biological response particularly notable (Table 3).

One possible explanation for the lack of response is the persistence of degraded sediment conditions and associated environmental stressors. Little Neck Bay exhibits relatively poor sediment quality (SCI = Fair; Table 3) and the highest contaminant burden among sampled stations. Consistent with these indicators, the living assemblage contained relatively high abundances of EG 4 second-order opportunists (*Mulinia lateralis*), and EG 2 stress-indifferent taxa, particularly *Nucula proxima*, suggesting continued disturbance-mediated community structure despite improved water-quality conditions.

Table 3. Summary of site characteristics, environmental indicators, and benthic condition for NY-20059.

NCA20_NY-20059 Visit 1								
Sample No.: 7989.17-13			**Location:** Little Neck Bay, NY					
Date Collected: 09/21/2020			**Water Quality Station:** 8-405					
Sediment: Sandy Mud (87.9% SICL)			**Salinity:** Mesohaline (15.0 psu)					
Depth: Shallow Infralittoral (2.5 m)			**Acidity:** Slightly basic (7.7 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
1.07 km	27%		2%		-0.122 ± 0.058		Improved	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
1997	47		2.6		2.67		-0.07	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.33	0.56	Fair	6.83	3.79	0.46	0.15	42.91	Fair

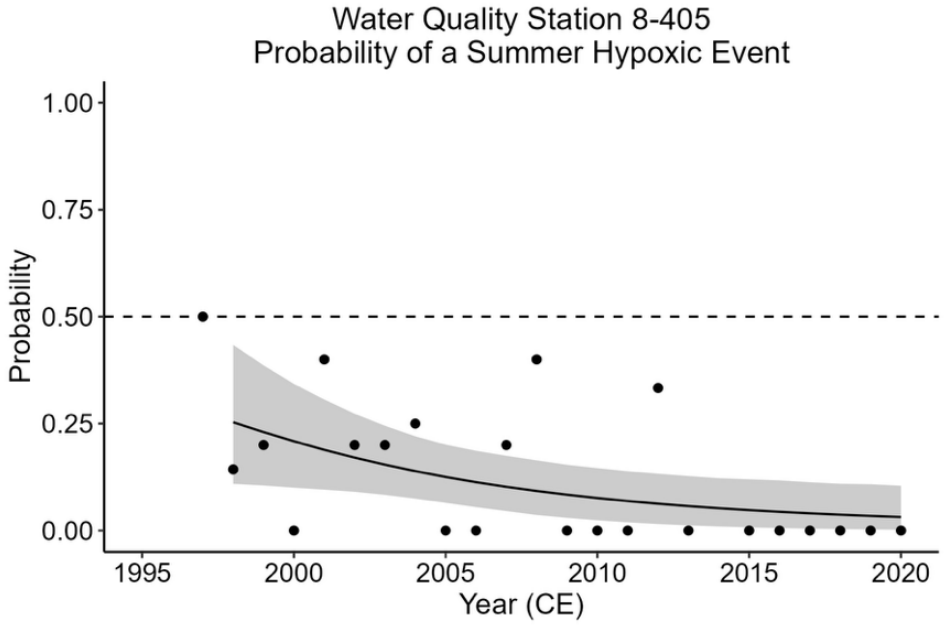


Figure 6. Hypoxic event probability trend line for Water Quality Station 8-405.

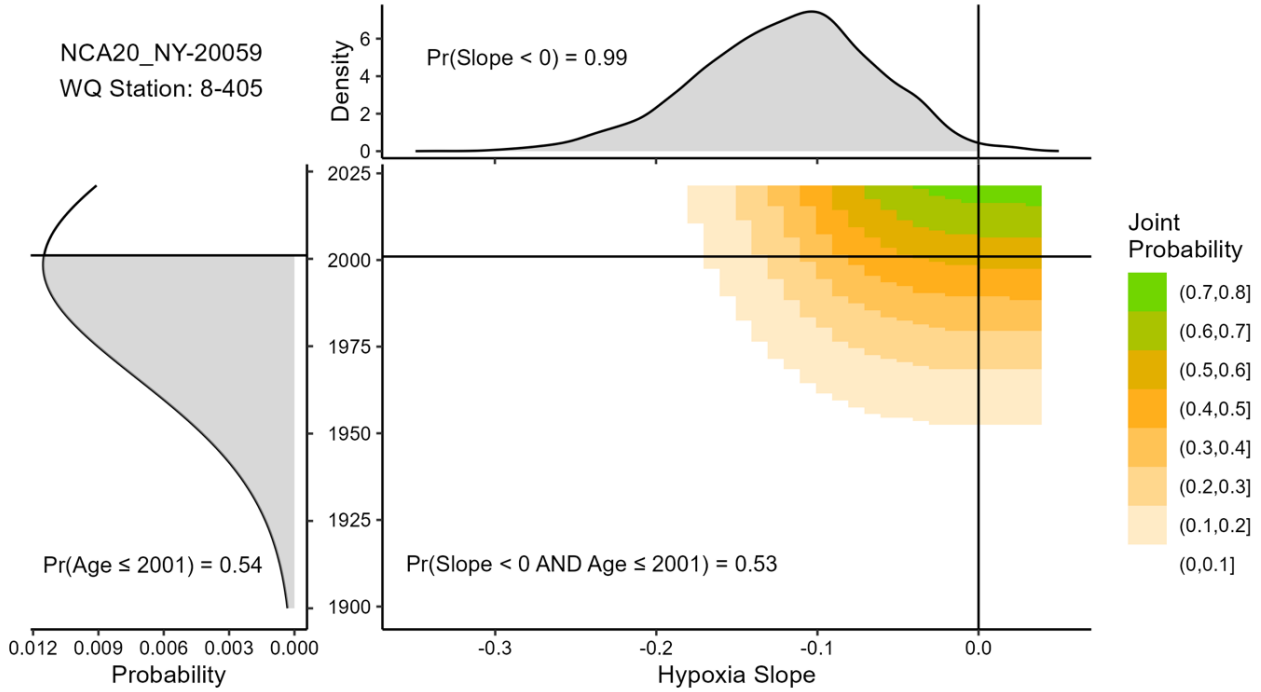


Figure 7. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-20059 (Little Neck Bay). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_NY-10001 Visit 1: Degraded biological condition

At site NY-10001 (Visit 1; The Narrows; Figure 3), the probability of a summer hypoxic event declined from 79% before the TMDL to 53% in 2020, indicating some improvement in water quality conditions. The copula analysis similarly supported a declining hypoxia trend (Pr[slope < 0] = 0.96; Table 4; Figure 8) and indicated a high probability that the death assemblage represents pre-TMDL conditions (median age = 1963 CE; Pr[Age ≤ 2001] = 0.87; joint probability = 0.83; Figure 9), providing a well-constrained baseline for comparison with the living assemblage. Despite these improvements, biological condition deteriorated from slightly disturbed in the death assemblage (AMBI = 2.62) to moderately disturbed in the living assemblage (AMBI = 2.98; LA-DA = 0.36; Table 4; Figure 5). The living assemblage was dominated by EG 4 second-order opportunistic taxa, particularly *M. lateralis*, and EG 2 stress-indifferent taxa, including *N. proxima* and *Yoldia* spp., consistent with continued environmental stress. At the time of sampling, water quality was rated as poor (EI = Poor), and sediment quality was only Fair (Table 4), reflecting persistent legacy sediment contamination. Together, these results suggest that reductions in hypoxia have not been sufficient to overcome continuing water-quality impairment and legacy sediment contamination, both of which likely continue to limit biological recovery.

Table 4. Summary of site characteristics, environmental indicators, and biological condition for NY-10001 Visit 1.

NCA20_NY-10001 Visit 1								
Sample No.: 7989.8-42			**Location:** Narrows					
Date Collected: 07/29/2020			**Water Quality Station:** A4					
Sediment: Mud (94.9% SICL)			**Salinity:** Upper Polyhaline (27.1 psu)					
Depth: Deep Infralittoral (15 m)			**Acidity:** Slightly basic (7.3 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
1.91 km	79%		53%		-0.047 ± 0.027		Improved	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
1963	41		2.98		2.62		0.36	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.33	0.38	Fair	1.46	25	0.02	0.07	52.28	Poor

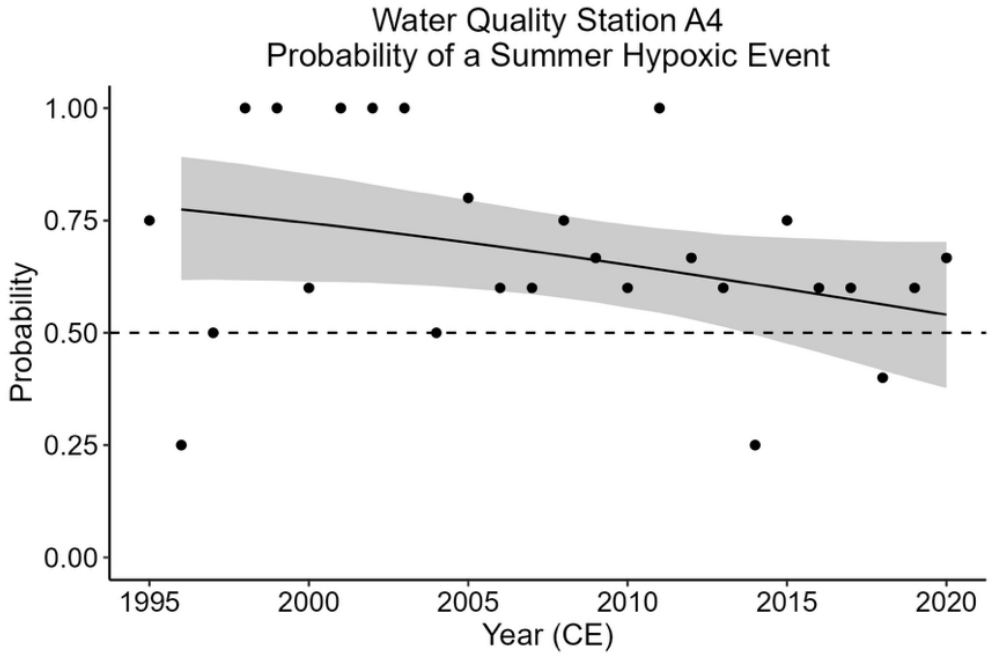


Figure 8. Hypoxic event probability trend line for Water Quality Station A4.

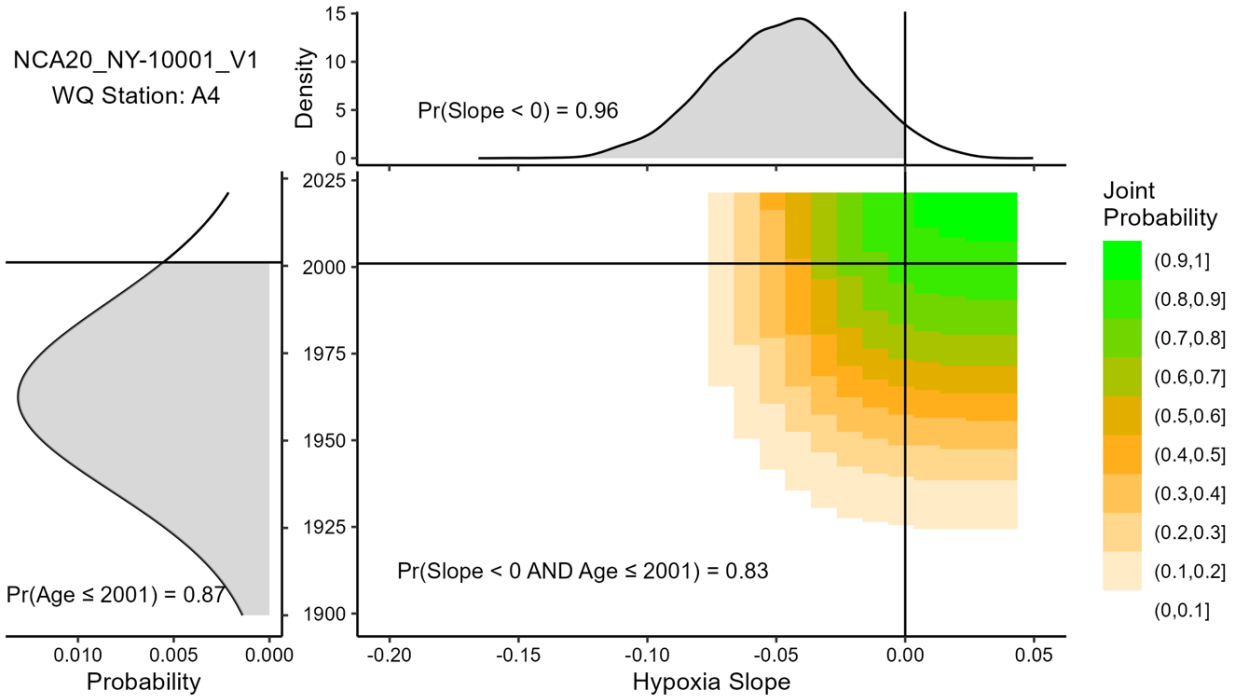


Figure 9. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-10001 (Visit 1; The Narrows). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_NY-10001 Visit 2: Degraded biological condition

Because both of the Narrows sampling sites (NY-10001 Visit 1 and 2; Figure 3) were located close (<2 km; Table 5) to the same water-quality monitoring station (A4; Figure 3), they shared an identical reconstructed hypoxia trend, indicating a decline in the probability of summer hypoxic events from 79% before implementation of the TMDL to 53% in 2020 (Table 5; Figure 8). The death assemblage at site NY-10001 Visit 2 again provided support for representing pre-TMDL conditions (median age = 1991 CE; joint probability = 0.58; Figure 10). Despite slight improvement in hypoxia conditions, biological condition deteriorated from slightly disturbed in the death assemblage (AMBI = 2.62) to moderately disturbed in the living assemblage (AMBI = 3.10; LA-DA = 0.48; Table 5; Figure 5), representing an even greater decline than observed in the Visit 1 sample. Although water quality at the time of sampling for Visit 2 was somewhat better than at Visit 1 (EI = Fair versus Poor; Tables 4, 5), the living assemblage was again dominated by EG 4 second-order opportunistic taxa (*M. lateralis*), and EG 2 stress-indifferent taxa, including *N. proxima* and *Yoldia* spp. Sediment quality also was rated as Fair (Table 5), suggesting that persistent sediment contamination, together with ongoing water-quality impairment, may continue to limit biological recovery.

Table 5: Summary of site characteristics, environmental indicators, and benthic condition for NY-10001 Visit 2.

NCA20_NY-10001 Visit 2								
Sample No.: 7989.16-34			**Location:** Narrows					
Date Collected: 09/01/2020			**Water Quality Station:** A4					
Sediment: Mud (95.1% SICL)			**Salinity:** Upper Polyhaline (27.6 psu)					
Depth: Deep Infralittoral (17.7 m)			**Acidity:** Slightly basic (7.8 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
1.91 km	79%		53%		-0.047 ± 0.027		Improved	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
1991	51		3.1		2.62		0.48	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.16	0.41	Fair	3.42	12.4	0.14	0.09	49.1	Fair

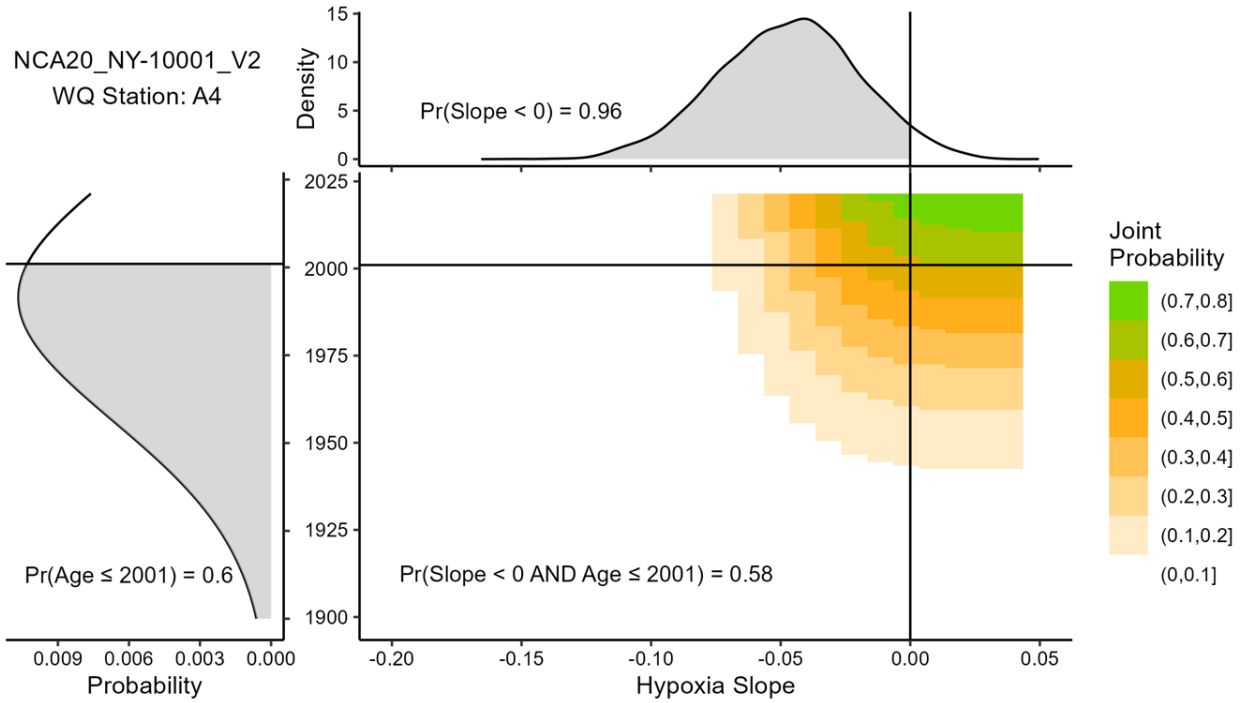


Figure 10. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-10001 (Visit 2; The Narrows). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_NY-20047: No change in biological condition

At site NY-20047 (Manhasset Bay; Figure 3), the probability of a summer hypoxic event declined substantially from 31% before the TMDL to 6% in 2020 (Table 6; Figure 11), and the copula analysis indicated strong support for improving hypoxia conditions (Pr[slope < 0] = 0.98; Figure 12). The death assemblage also provided strong support for representing pre-TMDL conditions (median age = 1980 CE; joint probability = 0.71; Table 6; Figure 12). Although AMBI decreased slightly from 2.99 in the death assemblage to 2.93 in the living assemblage (LA-DA = −0.06), both assemblages remained classified as moderately disturbed (Figure 5), providing little evidence for meaningful improvement in biological condition.

Consistent with this limited response, the living assemblage was dominated by the EG 4 second-order opportunist *M. lateralis* and contained abundant EG 2 stress-indifferent taxa, including *Japonactaeon punctostriatus*. Although sediment contamination was lower than at the westernmost LIS sites, sediment quality was only rated as Fair Table 6), suggesting that persistent legacy sediment contamination may continue to limit biological recovery despite substantial improvements in hypoxia conditions.

Table 6: Summary of site characteristics, environmental indicators, and biological condition for NY-20047.

NCA20_NY-20047 Visit 1									
Sample No.: 7989.16-20			**Location:** Manhasset Bay, NY						
Date Collected: 09/17/2020			**Water Quality Station:** 9-409						
Sediment: Sandy Mud (87.0% SICL)			**Salinity:** Upper Polyhaline (26.2 psu)						
Depth: Shallow Infralittoral (5.5 m)			**Acidity:** Slightly basic (7.6 pH)						
Probability of a Summer Hypoxic Event									
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**		
2.22 km	31%		6%		-0.085 ± 0.046		Improved		
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**						
Median	**IQR**		**LA**		**DA**		**LA-DA**		
1980	46		2.93		2.99		-0.06		
Sediment Contamination			**Water Quality**						
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**	
0.2	0.44	Fair	5.37	7.04	0.37	0.14	52.06	Fair	

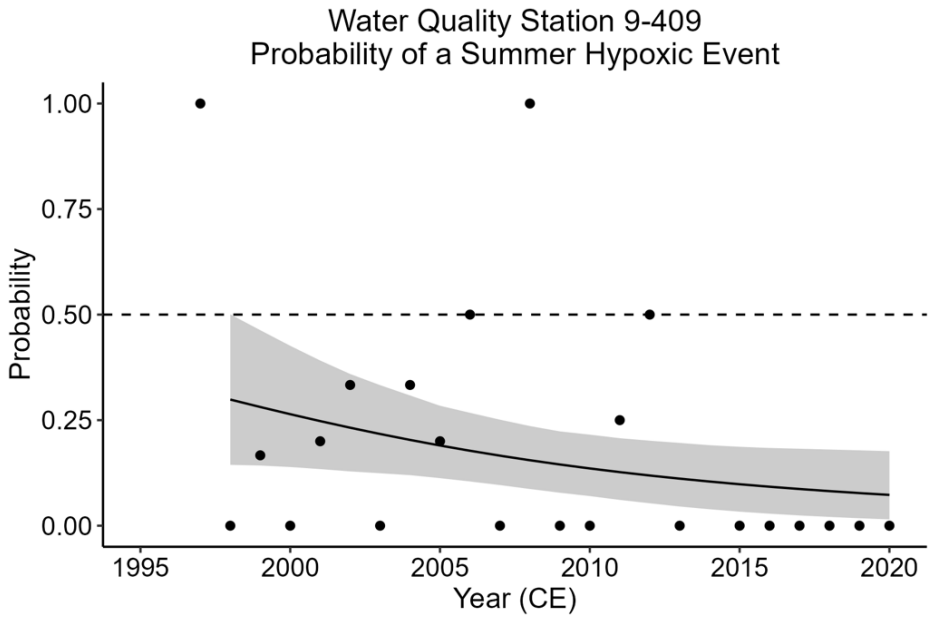


Figure 11. Hypoxic event probability trend line for Water Quality Station 9-409.

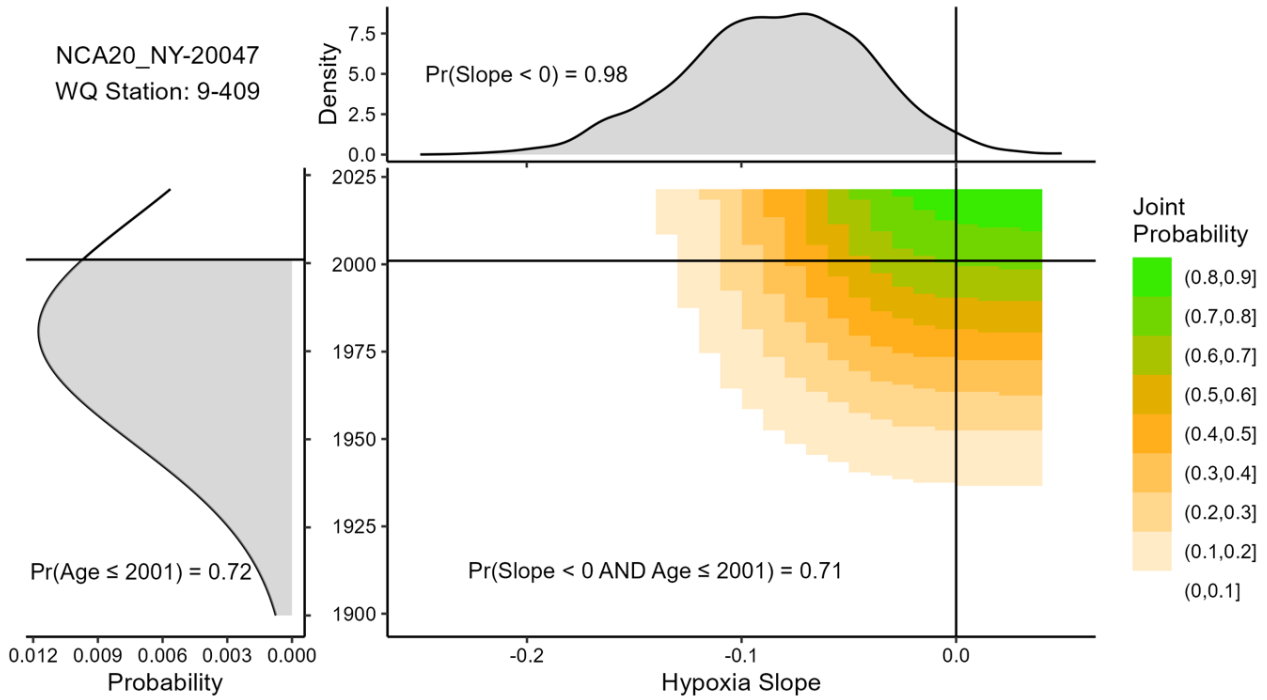


Figure 12. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-20047 (Manhasset Bay). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_NY-20051: *Degraded biological condition*

Site NY-20051 (Manhasset Bay; Figure 3) was associated with the same estimated hypoxia trend as NY-20047 because both sites were located close (~2 km) to water-quality station 9-409 (Figure 3; Table 6, 7). The probability of a summer hypoxic event declined substantially from 31% before implementation of the TMDL to 6% in 2020 (Table 7; Figure 11), and the copula analysis indicated strong support for a declining hypoxia trend (Pr[slope < 0] = 0.98; Table 7; Figure 13). The death assemblage provided support for representing pre-TMDL conditions (median age = 1994 CE; joint probability = 0.56; Table 7; Figure 13). Despite improved hypoxia conditions, biological condition showed little evidence of recovery. AMBI increased marginally from 2.74 (slightly disturbed) in the death assemblage to 2.85 (moderately disturbed) in the living assemblage (LA-DA = 0.11; Table 7; Figure 5). The living assemblage was dominated by EG 3 (stress-tolerant) taxa, particularly *Crepidula fornicata*, indicating continued ecological disturbance. Although water quality at the time of sampling was rated Fair (EI = Fair), sediment quality also was rated as Fair (Table 7), consistent with the continued influence of legacy contamination. Together, these results suggest that improvements in hypoxia conditions have not translated into measurable biological recovery.

Table 7: Summary of site characteristics, environmental indicators, and biological condition for NY-20051.

NCA20_NY-20051 Visit 1								
Sample No.: 7989.16-21			**Location:** Manhasset Bay, NY					
Date Collected: 09/17/2020			**Water Quality Station:** 9-409					
Sediment: Muddy Sand (49% SICL)			**Salinity:** Upper Polyhaline (26.2 psu)					
Depth: Shallow Infralittoral (3.0 m)			**Acidity:** Slightly basic (7.6 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
1.73 km	31%		6%		-0.085 ± 0.046		Improved	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
1994	50		2.85		2.74		0.11	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.13	0.34	Fair	6	7.58	0.35	0.14	40.12	Fair

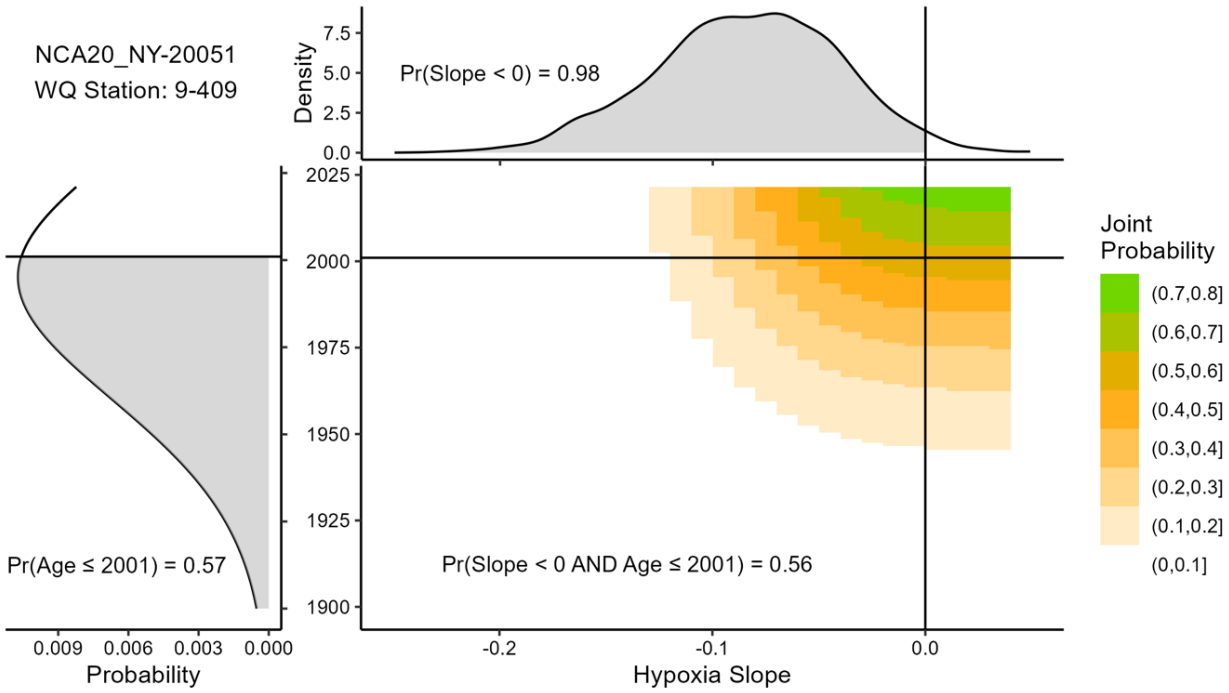


Figure 13. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-20051 (Manhasset Bay). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_NY-20057: *No change in biological condition*

Consistent with the other two Manhasset Bay sites (NY-20047 and NY-20051), the probability of a summer hypoxic event for site NY-20057 (Figure 3) declined from 32% before implementation of the TMDL to 15% in 2020 (Table 8; Figure 14), and the copula analysis supported a declining hypoxia trend (Pr[slope < 0] = 0.88; Figure 15). The death assemblage also provided support for representing pre-TMDL conditions (median age = 1986 CE; joint probability = 0.57; Table 8; Figure 15). Despite these improvements in hypoxia, biological condition showed little evidence of recovery. AMBI increased slightly from 2.88 in the death assemblage to 2.99 in the living assemblage (LA-DA = 0.11), although both assemblages remained classified as moderately disturbed. The living assemblage was dominated by EG 2 stress-indifferent taxa, particularly *J. punctostriatus* and *Acteocina canaliculata*. The site was rated as Poor for eutrophication and Fair for sediment quality (Table 8), suggesting that persistent eutrophication, together with legacy sediment contamination, may have constrained biological recovery.

Table 8: Summary of site characteristics, environmental indicators, and biological condition for NY-20057.

NCA20_NY-20057 Visit 1								
Sample No.: 7989.16-27			**Location:** Manhasset Bay, NY					
Date Collected: 09/18/2020			**Water Quality Station:** 9-412					
Sediment: Sandy Mud (58.7% SICL)			**Salinity:** Upper Polyhaline (25.8 psu)					
Depth: Shallow Infralittoral (3.0 m)			**Acidity:** Slightly basic (7.8 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
0.58 km	32%		15%		-0.043 ± 0.037		Improved	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
1986	52		2.99		2.88		0.11	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.22	0.49	Fair	7.26	27.9	0.15	0.1	20.77	Poor

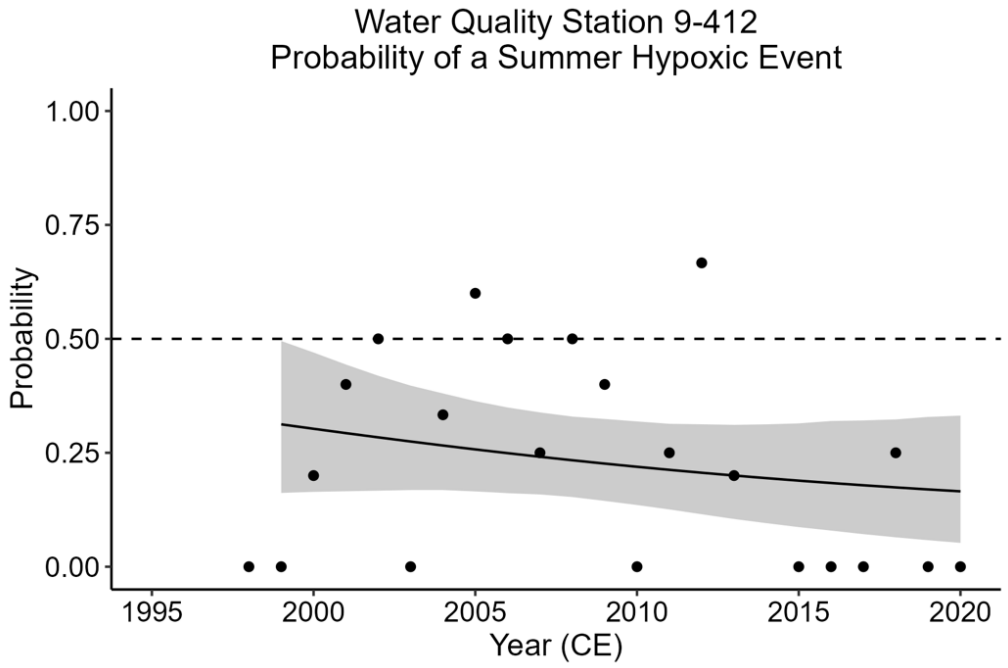


Figure 14. Hypoxic event probability trend line for Water Quality Station 9-412.

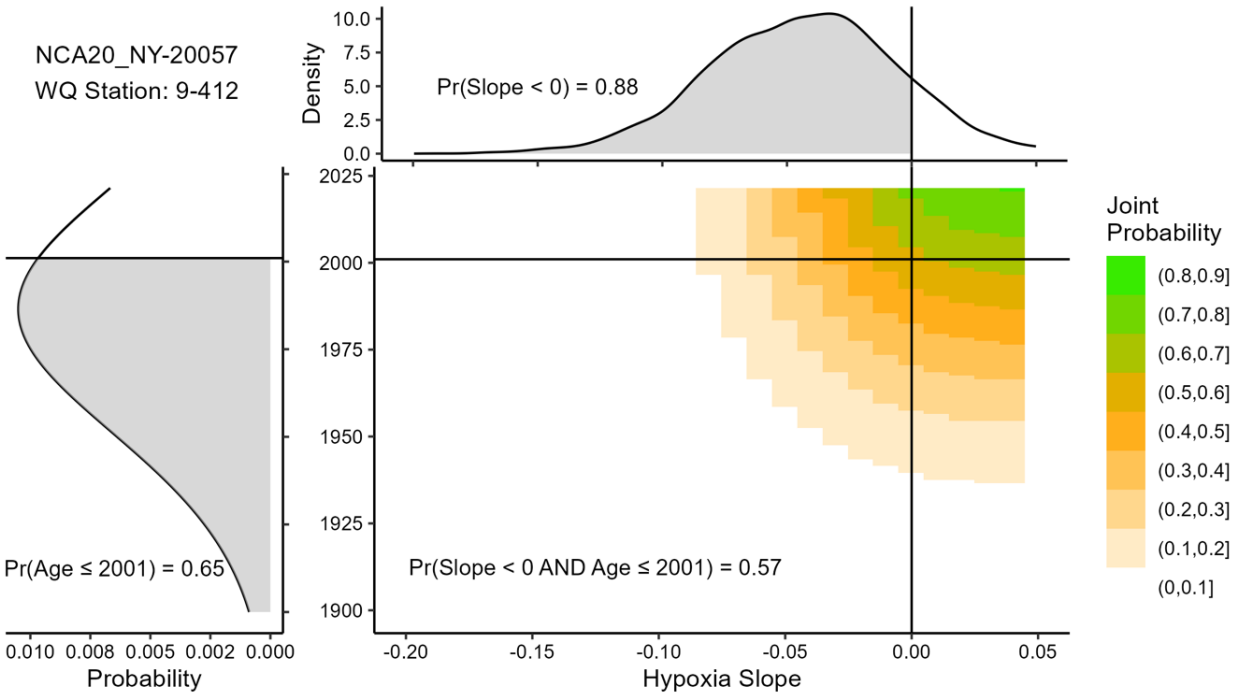


Figure 15. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-20057 (Manhasset Bay). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_NY-20064: Improved biological condition

The estimated hypoxia trend suggested that biological condition at site NY-20064 (Hempstead Harbor; Figure 3) should have remained relatively unchanged (or worsened), as the probability of a summer hypoxic event increased slightly from 41% before the TMDL to 55% in 2020 (Table 9; Figure 16), and the copula analysis supported little improvement in hypoxia conditions (Pr[slope > 0] = 0.87; Figure 17). Despite continued stress from summer hypoxia, AMBI decreased markedly from 3.12 in the death assemblage to 1.99 in the living assemblage (LA-DA = −1.14; Table 9; Figure 5), representing a shift from moderately disturbed to slightly disturbed conditions. However, the copula analysis provided only weak support that the death assemblage represents pre-TMDL conditions (median age = 2003 CE; joint probability = 0.40; Figure 17), suggesting caution when interpreting the magnitude of ecological change. The EG 2 stress-indifferent taxon *J. punctostriatus* dominated the living assemblage. Unlike other embayment sampling locations, this site exhibited the highest sediment quality (SCI = Good; Table 9). These observations suggest that local environmental conditions, particularly the absence of substantial sediment contamination, may have facilitated improved biological condition despite little evidence for a corresponding improvement in the reconstructed hypoxia record.

Table 9: Summary of site characteristics, environmental indicators, and biological condition for NY-20064.

NCA20_NY-20064 Visit 1								
Sample No.: 7989.16-25			**Location:** Hempstead Harbor					
Date Collected: 09/16/2020			**Water Quality Station:** H-D/H-C					
Sediment: Muddy Sand (41.7% SICL)			**Salinity:** Upper Polyhaline (27.3 psu)					
Depth: Deep Infralittoral (7.4 m)			**Acidity:** Slightly basic (7.8 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
0.86 km /1.69 km	41%		55%		0.018 ± 0.015		No Change	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
2003	39		1.99		3.12		-1.14	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.04	0.22	Good	6.94	14.7	0.1	0.08	42.53	Fair

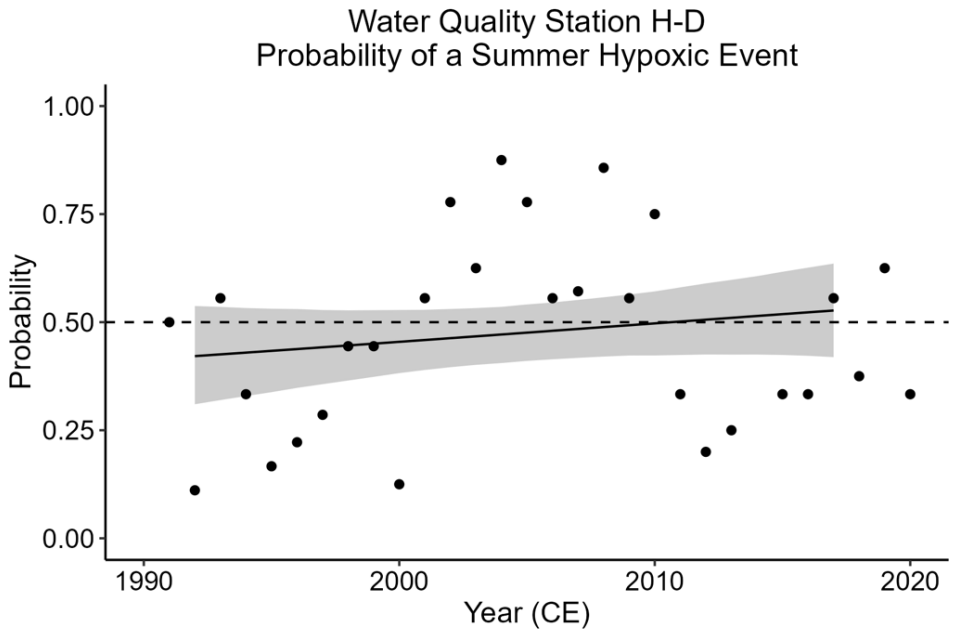


Figure 16. Hypoxic event probability trend line for Water Quality Station H-D.

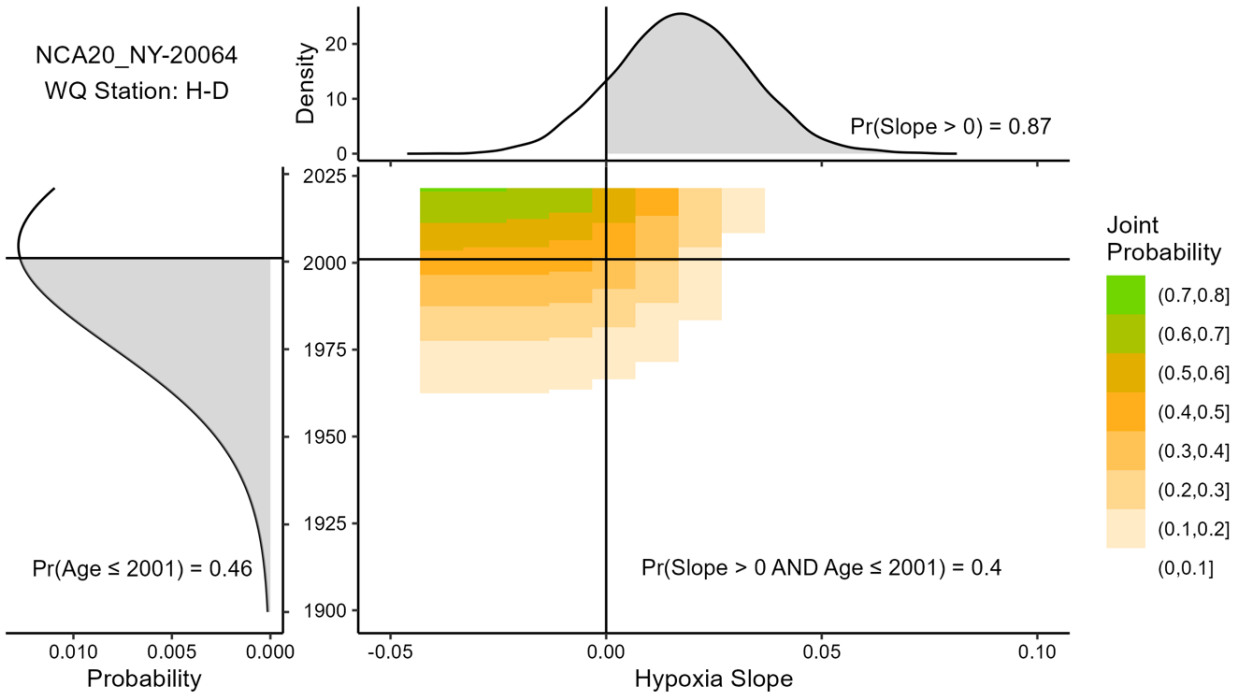


Figure 17. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-20064 (Hempstead Harbor). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_NY-20083: Improved biological condition

Unlike the westernmost Long Island Sound sites, the probability of a summer hypoxic event declined at site NY-20083 (Western Open Sound; Figure 3) from 45% before implementation of the TMDL to 25% in 2020 (Table 10; Figure 18), and the copula analysis supported a declining hypoxia trend (Pr[slope < 0] = 0.90; Figure 19). The death assemblage also provided strong support for representing pre-TMDL conditions (median age = 1980 CE; joint probability = 0.63; Figure 19). Consistent with improved hypoxia conditions, biological condition also improved, with AMBI decreasing from 2.40 in the death assemblage to 1.82 in the living assemblage (LA-DA = −0.85), representing a shift from moderately disturbed to slightly disturbed conditions. Unlike the westernmost LIS sites, where living assemblages were often dominated by EG 4 second-order opportunistic taxa, the living assemblage at this site was dominated by EG 2 stress-indifferent taxa, including *Yoldia* spp. and *N. proxima*, and EG 1 stress-sensitive taxa, particularly *Argripomma morrhuanum*, indicating a less disturbed benthic community. Although this site exhibited moderate sediment contamination (SCI=Fair; Table 10), contaminant concentrations were substantially lower than those observed at the western LIS sites. Together, these results suggest that improvements in hypoxia conditions, coupled with lower levels of sediment contamination, may have facilitated some recovery of the benthic community.

Table 10: Summary of site characteristics, environmental indicators, and biological condition for NY-20083.

NCA20_NY-20083 Visit 1								
Sample No.: 7989.8-43			**Location:** Western Open Sound					
Date Collected: 07/29/2020			**Water Quality Station:** C1					
Sediment: Mud (92.5% SICL)			**Salinity:** Upper Polyhaline (27.5 psu)					
Depth: Deep Infralittoral (18.0 m)			**Acidity:** Slightly basic (7.5 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
1.44 km	45%		25%		-0.034 ± 0.026		Improved	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
1980	52		1.82		2.40		-0.85	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.11	0.29	Fair	3.1	9.02	0.01	0.04	54.67	Fair

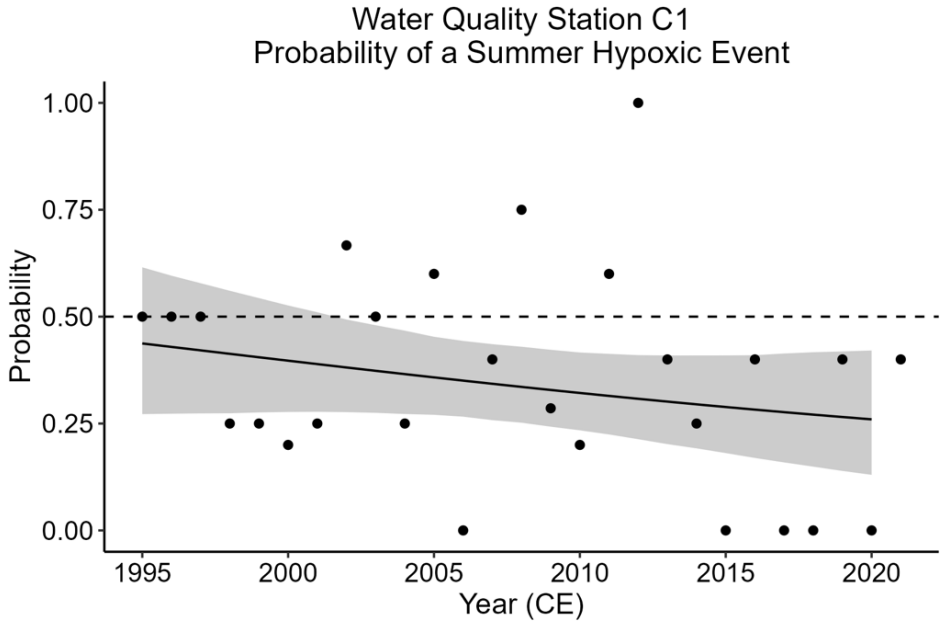


Figure 18. Hypoxic event probability trend line for Water Quality Station C1.

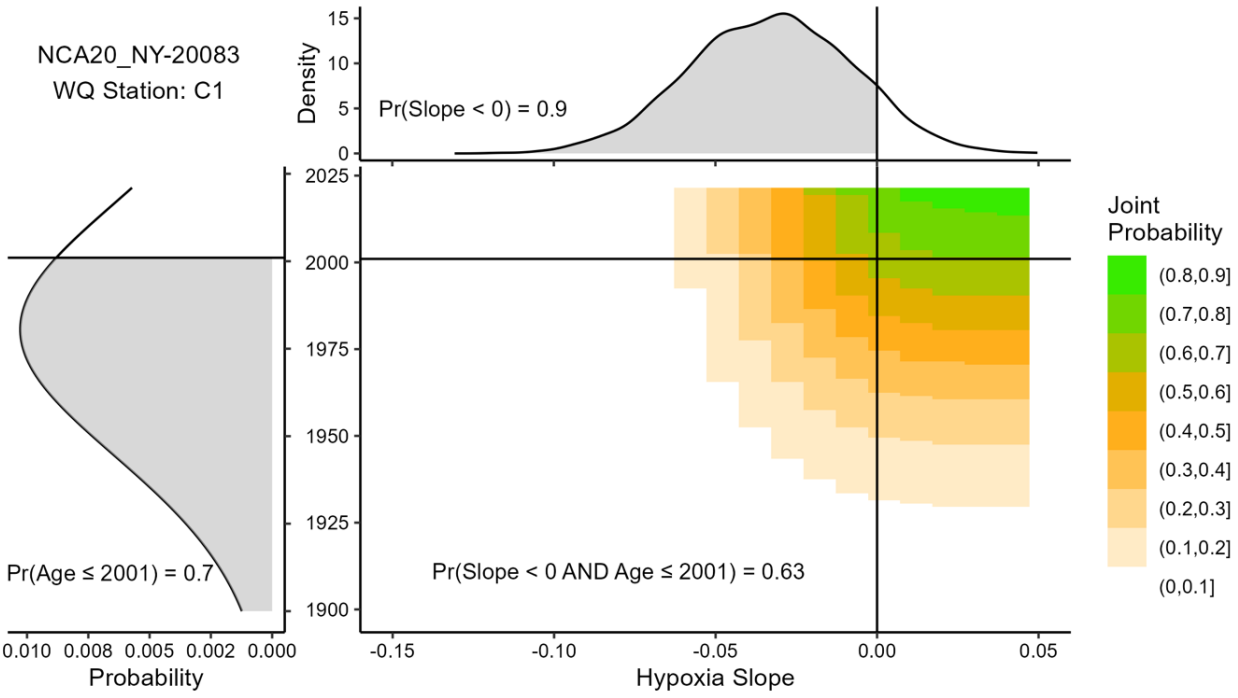


Figure 19. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for NY-20083 (Western Open Sound). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_CT-20076: Improved biological condition

At site CT-20076 (Western Open Sound; Figure 3), the probability of a summer hypoxic event declined from 22% before implementation of the TMDL to 9% in 2020 (Table 11; Figure 20), and the copula analysis supported a declining hypoxia trend (Pr[slope < 0] = 0.87; Figure 21). The death assemblage also provided strong support for representing pre-TMDL conditions (median age = 1975 CE; joint probability = 0.72; Figure 21). Consistent with the predicted response, biological condition improved, with AMBI decreasing from 2.29 in the death assemblage to 1.98 in the living assemblage (LA-DA = −0.32; Table 11; Figure 5), representing a shift from moderately disturbed to slightly disturbed conditions. Similar to NY-20083, this site was characterized by Good sediment quality (Table 11), suggesting that lower levels of sediment contamination may have facilitated recovery of the benthic community. Consistent with this interpretation, the living assemblage lacked strong dominance by opportunistic taxa and was instead dominated by EG 2 stress-indifferent taxa, including *Yoldia* spp. and *N. proxima*, and contained several EG 1 stress-sensitive taxa, including *A. morrhuanum* and *Sayella fusca*. Although water quality at the time of sampling was rated as Fair (Table 11), biological condition improved in the direction predicted by the reconstructed decline in summer hypoxia.

Table 11: Summary of site characteristics, environmental indicators, and biological condition for CT-20076.

NCA20_CT-20076 Visit 1								
Sample No.: 7989.8-26			**Location:** Western Open Sound					
Date Collected: 07/29/2020			**Water Quality Station:** D3					
Sediment: Sandy Mud (60.2% SICL)			**Salinity:** Upper Polyhaline (27.6 psu)					
Depth: Deep Infralittoral (25.0 m)			**Acidity:** Slightly basic (7.6 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
3.40 km	22%		9%		-0.034 ± 0.031		Improved	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
1975	36		1.98		2.29		-0.32	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.1	0.3	Good	3.15	7.76	0.02	0.03	57.3	Fair

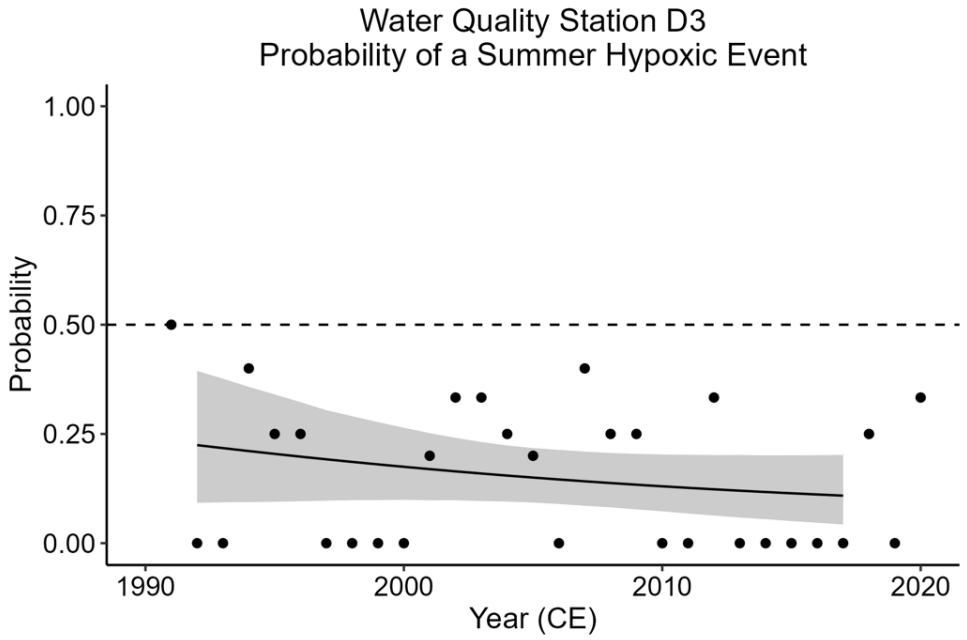


Figure 20. Hypoxic event probability trend line for Water Quality Station D3.

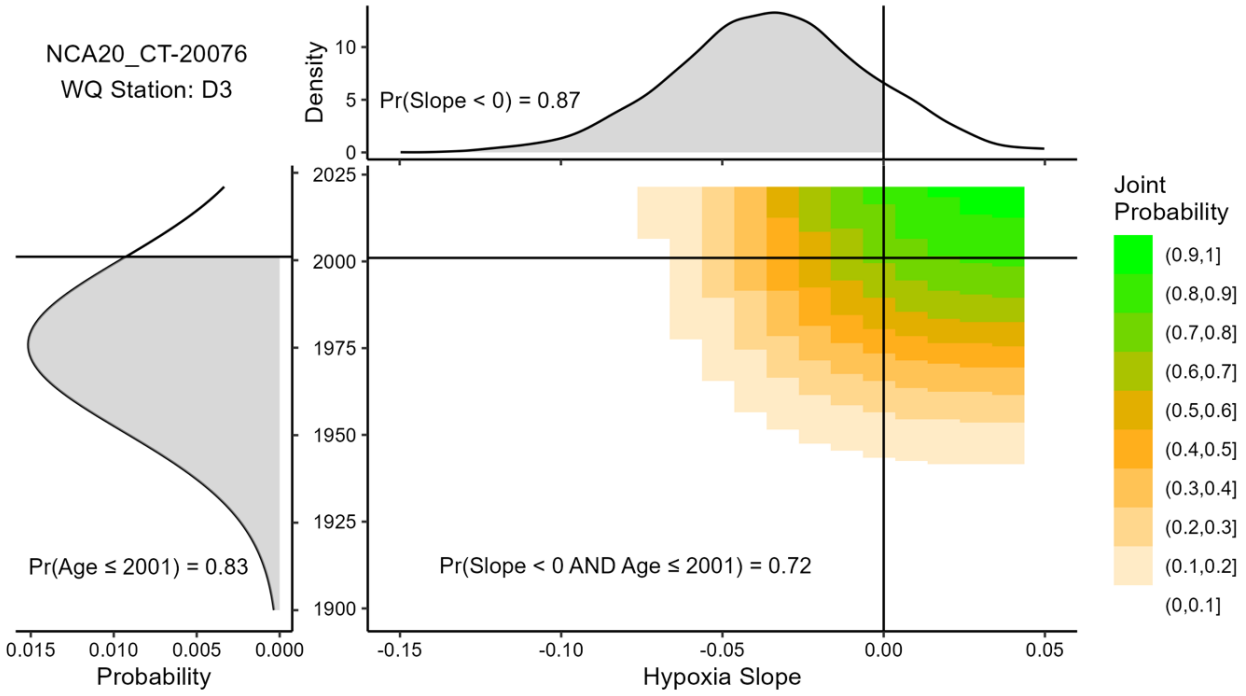


Figure 21. Posterior probability distributions of the temporal trend in summer hypoxia probability (top) and median death assemblage age (left), and their joint probability distribution (center) for CT-20076 (Western Open Sound). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical and horizontal reference lines denote a slope of zero and a death assemblage median age of 2001 CE, respectively. Warmer colors indicate lower joint probability and cooler colors indicate higher joint probability.

NCA20_CT-10002: No change in biological condition

Consistent with the other Western Open Sound sites, the reconstructed hypoxia trend for CT-10002 (Figure 3) indicated little change in hypoxia conditions, with the probability of a summer hypoxic event declining slightly from 12% before implementation of the TMDL to 7% in 2020 (Table 12; Figure 22, 23). Biological condition similarly changed little between the death and living assemblages, with AMBI decreasing slightly from 2.06 to 1.97 (LA-DA = −0.09; Table 12; Figure 5), with both assemblages remaining classified as slightly disturbed. The living assemblage was dominated by EG 1 stress-sensitive taxa, particularly *A. morrhuanum*, and EG 2 stress-indifferent taxa, including *Yoldia* spp. and *N. proxima*. Sediment quality was rated Good (Table 12). However, because no geochronologic age could be obtained for the death assemblage, it was not possible to determine whether it provides a pre-TMDL baseline. Consequently, comparisons between the death and living assemblages should be interpreted cautiously. Nevertheless, the similarity in biological condition and the presence of good sediment quality are broadly consistent with the pattern observed at the neighboring western Open Sound stations.

Table 12: Summary of site characteristics, environmental indicators, and biological condition for CT-10002.

NCA20_CT-10002 Visit 1								
Sample No.: 7989.8-25			**Location:** Western Open Sound					
Date Collected: 07/28/2020			**Water Quality Station:** 8-405					
Sediment: Mud (91.7% SICL)			**Salinity:** Upper Polyhaline (27.8 psu)					
Depth: Deep Infralittoral (25.0 m)			**Acidity:** Slightly basic (7.6 pH)					
Probability of a Summer Hypoxic Event								
Distance to WQ	**Pre-TMDL**		**2020**		**Slope**		**Hypothesis**	
1.50 km	12%		7%		-0.022 ± 0.09		No Change	
Death Assemblage Age (CE)			**AZTI Marine Biotic Index**					
Median	**IQR**		**LA**		**DA**		**LA-DA**	
—	—		1.97		2.06		-0.09	
Sediment Contamination			**Water Quality**					
mERM-Q	**Pmax**	**SCI**	**DO**	**CHLA**	**DIN**	**SRP**	**TRANS**	**EI**
0.04	0.22	Good	3.48	5.75	0.03	0.03	54.8	Fair

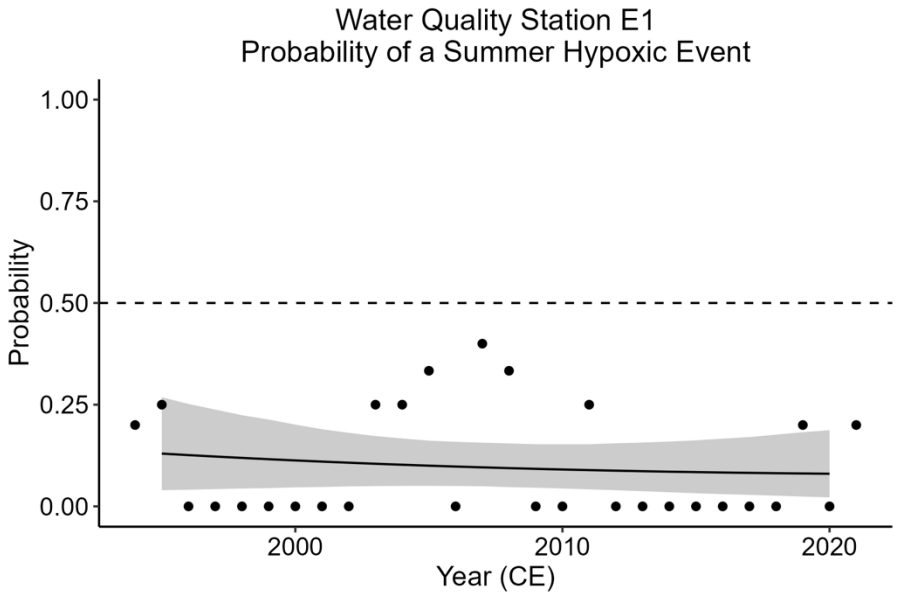


Figure 22. Hypoxic event probability trend line for Water Quality Station E1.

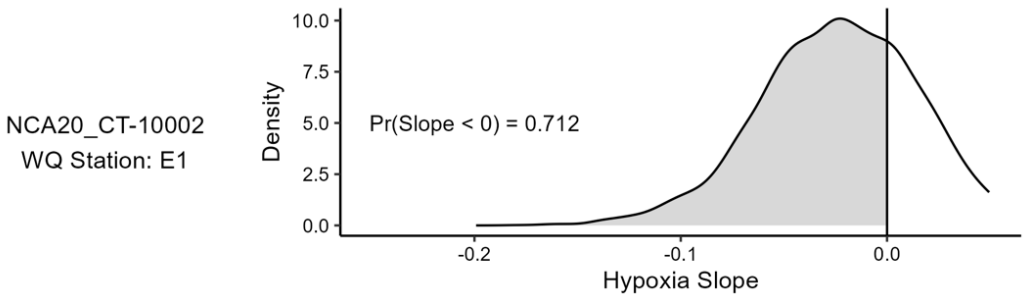


Figure 23. Posterior probability distributions of the temporal trend in summer hypoxia probability for NCA20_ CT-10002 (Western Open Sound). Negative slope values indicate declining probability of summer hypoxia through time (i.e., improving DO conditions). Vertical reference line denotes a slope of zero.

Sample-specific interpretation summary

Collectively, the site-specific analyses demonstrated that changes in biological condition did not consistently track reconstructed improvements in hypoxia. Although several sites exhibited the predicted biological response, many western embayment sites showed little evidence of recovery—or continued deterioration—despite substantial declines in the probability of summer hypoxia. These contrasting responses suggest that local environmental conditions strongly mediate the relationship between water-quality improvement and biological recovery.

A second recurring pattern was the association between sediment quality and biological response. Sites that failed to exhibit biological recovery were typically characterized by poor to fair sediment quality, greater legacy contaminant burdens, and living assemblages dominated by opportunistic or pollution-tolerant taxa. In contrast, sites exhibiting improved biological condition generally had lower contaminant concentrations, good sediment quality, and living assemblages dominated by disturbance-indifferent taxa.

One potential source of uncertainty in the interpretation of individual sites is that reconstructed hypoxia trends were derived from nearby long-term monitoring stations rather than from the benthic sampling locations themselves. Because local water-quality conditions can vary over short distances, especially in embayments, the monitoring records may not fully capture the environmental conditions experienced by the benthic communities. Although samples linked to the same monitoring station sometimes showed similar biological responses, this does not independently demonstrate that the reconstructed hypoxia trends were locally representative. Therefore, some of the unexplained variation in biological response may reflect spatial mismatch between monitoring stations and sampling sites, in addition to differences in sediment quality, legacy contamination, and other local benthic habitat conditions.

Finally, the site-specific analyses highlight the importance of establishing robust pre-TMDL baselines. Geochronologic uncertainty varied among sites, influencing confidence in the magnitude of inferred ecological change. Nevertheless, the overall spatial patterns remained consistent, indicating that benthic recovery following the TMDL has been heterogeneous across western LIS and is likely influenced by both improvements in water quality and the persistence of legacy sediment conditions.

C1.4 Next Steps

C1.4.1 Replicate Sampling

This pilot study relied on a single grab sample at each site. Future work should expand sampling to include replicate samples to better characterize within-site spatial variability and quantify uncertainty in AMBI estimates. Replicate sampling would improve confidence in estimates of biological condition in comparisons of mollusk living and death assemblages, allow statistical partitioning of spatial and temporal variation, and provide a more reliable basis for detecting biological responses to water quality improvements across Long Island Sound.

C1.4.2 Application of geoAMBI

This pilot study used AMBI, a widely used biotic index, to quantify biological condition in both living and death assemblages of mollusks and to assess differences between them in response to TMDL implementation in LIS. Future work could build on this approach by applying geoAMBI (Pruden et al., 2026), a newly developed geohistorical extension of AMBI designed to explicitly account for potential taphonomic biases in time-averaged death assemblages. By incorporating the effects of differential preservation and accumulation over time, geoAMBI provides a means of refining estimates of past biological condition while maintaining comparability with standard AMBI-based assessments. A useful next step would be to directly compare geoAMBI and AMBI results across LIS to evaluate the influence of taphonomic bias on estimates of biological condition and to assess whether geoAMBI improves agreement between living- and death-assemblage based inference. Applying geoAMBI across LIS would extend this pilot approach and improve confidence in retrospective biological condition reconstructions.

C1.4.3 Developing a Visualization Tool for End-users

This pilot study used copulas to quantify the joint uncertainty associated with temporal trends in water quality (indexed by the estimated rate of change in hypoxia probability) and the estimated ages of death assemblage samples. For this study, we evaluated these joint probabilities using fixed thresholds (i.e., a negative slope indicating improving water quality and a sample age predating TMDL implementation in 2001). However, resource managers might want to evaluate alternative scenarios. For example, they might be interested in the probability that water quality improved substantially (i.e., a slope well below zero) or that ecological responses occurred only after allowing several years following TMDL implementation (e.g., pre-2004 rather than pre-2001). Exploring how these thresholds influence joint probabilities would provide a more flexible approach for evaluating management outcomes and explicitly characterizing trade-offs among alternative definitions of success. Such functionality could be implemented through an interactive decision-support tool, for example using R Shiny (Chang et al., 2026), allowing users to explore these scenarios in real time.

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C2. Scientific Abstract: Describe your entire project's results as if intended for a scientific meeting or publication.

Excess nitrogen input has contributed to recurring summer hypoxia in Long Island Sound (LIS), with long-term impacts on the structure and condition of benthic macroinvertebrate communities over time. In 2001, the Connecticut Department of Energy and Environmental Protection and New York State Department of Environmental Conservation adopted a Total Maximum Daily Load (TMDL) management strategy to reduce nitrogen pollution and hypoxia in the Sound. Reduced hypoxia was predicted to result in ecological benefits, including overall improvement of benthic habitat conditions. Although TMDL targets were met by 2017, no consistent benthic monitoring data exist to assess management success. Time-averaged molluscan death assemblages (DAs) that readily preserve and accumulate within the sediment provide a unique opportunity to reconstruct past habitat condition. Here we use the remains of dead mollusk shells retained in 10 benthic grab samples collected from the Sound as part of the Environmental Protection Agency-led 2020 National Coastal Conditions Assessment (NCCA) to evaluate the response of benthic communities to the TMDL intervention. We hypothesized that habitat condition—measured using the AZTI Marine Biotic Index (AMBI)—improved over time as water quality (indexed as the frequency of summer hypoxic events at each sampling location) improved. Post-TMDL reductions in hypoxia improved benthic conditions in eastern LIS (indexed by lower AMBI scores), but some westernmost LIS sites showed no improvement, likely due to poor sediment quality conditions. This pilot study demonstrates that molluscan DAs collected with standard benthic sampling protocols can provide a cost-effective means of retrospectively acquiring data to assess restoration success when long-term biological monitoring data are unavailable or limited.

C3. Problems Encountered: Describe difficulties encountered during the project, e.g., problems with experimental protocols, and how they were resolved.

Project progress was temporarily delayed because the initial analyses relied on a preliminary version of the National Coastal Condition Assessment (NCCA) dataset. Subsequent revisions by the U.S. Environmental Protection Agency required postponing final analyses until the quality-assured dataset was released. During this period, sample metadata were verified through direct communication with EPA staff and independent review of the associated records. Following release of the finalized dataset in September 2024, analyses were updated and the project proceeded as planned.

A second issue arose during radiocarbon dating. Sample NCA20_CT-10002 contained too few suitable molluscan specimens for reliable dating. As a result, no radiocarbon age could be estimated for this sample. This limitation could not be resolved within the scope of the project and reflects constraints associated with variable shell abundance and preservation within individual grab samples.

Although the project originally proposed using the multivariate AZTI Marine Biotic Index (M-AMBI; Muxika et al., 2007), we instead used AMBI to assess biological condition. The original research plan recognized that time-averaging could complicate comparisons between molluscan living and death assemblages but anticipated that its effects on richness and diversity could be accounted for analytically. However, a reliable method could not be developed during the project. M-AMBI combines AMBI with measures of taxonomic richness and diversity, with richness and diversity weighted more heavily than AMBI in determining the final index score. Because diversity is itself partly influenced by richness, taxonomic richness has a disproportionate influence on M-AMBI scores. This presents a particular problem for death assemblages, in which time-averaging inherently increases richness and diversity (Kidwell, 2013). Consequently, M-AMBI could assign higher biological condition to death

assemblages largely because of time-averaging rather than differences in biological condition. The problem is further compounded by the need to define a reference condition based on high richness and diversity. Whereas these attributes generally indicate good biological condition in living assemblages, in death assemblages they may instead reflect greater time-averaging, making an appropriate reference condition difficult to define. AMBI is not immune to time-averaging because differences in shell preservational potential can bias the relative abundance of ecological groups in death assemblages; however, these effects can be reduced through abundance transformations. AMBI was therefore considered the more suitable biotic index for comparing living and death assemblages in this pilot study (see also section C1.4.2 Application of geoAMBI).

C4. New Research Directions: Identify any additional, new research directions pursued during the project and reasons for adding them to the original research plan.

During the project, a new methodological research direction emerged involving improved approaches for estimating the age and time-averaging of death assemblages from radiocarbon-dated shells. The original research plan followed the approach of Kowalewski et al. (2018), which estimates the age of a sample by averaging the posterior distributions of individually dated shells. However, because many of the shells in this study likely lived during the post-bomb period (after intense atmospheric testing of nuclear weapons in the mid-1950s through the mid-1960s), calibrated radiocarbon ages can exhibit bimodal posterior distributions (Kowalewski et al., 2018). As a result, time-averaging statistics, such as interquartile range (IQR), often span both modes of the posterior distribution, artificially inflating time-averaging estimates. During this project, we became aware of a recently developed Bayesian method (Heaton, 2022) that jointly models all radiocarbon determinations from a sample to estimate a common calendar-age distribution. Rather than averaging individual posteriors, this approach uses a prior on the calendar date posterior that allows for multimodal distributions only when strongly supported by the data, typically resulting in posterior age distributions that are unimodal. Because this approach offered a more reliable approach for estimating sample age and time averaging, we incorporated it into the present study in place of the originally proposed method.

C5. Outreach & Interactions: Describe activities and results of the project's Extension and Outreach Plan. What audiences were reached, and what products were produced? Describe any other interactions with industry, agency, or other stakeholder representatives.

This pilot project benefited from interactions with several key collaborators and agency partners. Katie O'Brien-Clayton, the lead for the Benthic Macroinvertebrate Sampling Methodology Embayment project, and Chris Bellucci, the Assistant Director of the CT DEEP Water Planning and Management Division, provided guidance on site selection, ensuring alignment between benthic sampling locations and long-term water quality monitoring stations. In addition, coordination with Hugh Sullivan, National Coastal Conditions Assessment (NCCA) Project Lead at the U.S. Environmental Protection Agency (EPA), facilitated access to the NCCA dataset and supported the transfer of grab samples, while also helping resolve preliminary NCCA dataset inconsistencies. These interactions were essential for ensuring appropriate data use and for integrating federal benthic monitoring resources into the study design.

C6. Presentations and Publications: Describe efforts to disseminate information about this research not previously reported (e.g., conferences, journal articles, presentations, books/book chapters, proceedings, invited papers or seminars, workshops, symposia, fact sheets, bulletins, brochures, student theses, unpublished reports, responses to inquiries, news releases, newspaper coverage, web articles, newsletters, television or radio appearances, governmental testimony, or interviews with the media). Either attach or send when available one copy of each publication/product (PDF preferred or web link). Please list as outlined below:

Publications: List publications here in standard citation format (please do not include publications submitted or in review, only list in-press and published publications). Include the abstract if possible.

Example: Last Name, First Initial. Second Initial., First Initial. Second Initial. Last Name, and First Initial. Second Initial. Lastname. Year. Article title. *Journal name*. Vol(No): pages or article number.

Presentations: List presentations here, include the name of the conference or meeting and indicate if oral or poster presentation, and if it was invited. Include abstract(s) if available. For posters, include a

PDF copy if possible.

Example: Last Name, First Initial. Second Initial., and First Initial. Second Initial. Lastname (underline presenter). Year. Title. Conference name and organizing group (e.g., professional society or agency if applicable). City, State, day, month. Presentation type (talk or poster, indicate if invited). Note any awards.

Dietl, G. P., Pruden, M. J., and J. C. Handley. 2024. Responses of molluscan communities to improved water quality in Long Island Sound. National Conference on Ecosystem Restoration. Albuquerque, New Mexico, 14-19, April. Talk (invited)

Dietl, G. P., Pruden, M. J., and J. C. Handley. 2025. Effect of improved water quality on molluscan communities in Long Island Sound. Geological Society of America. San Antonio, Texas, 19-22, October. Poster

Dietl, G.P., Pruden, M. J., and J. C. Handley. 2024. Effect of improved water quality on molluscan communities in Long Island Sound: preliminary results. 2024 Long Island Sound Research Conference. Port Jefferson, New York, 15, May. Poster

Dietl, G. P., Pruden, M. J., and J. C. Handley. 2026. Ecological responses to declining hypoxia in Long Island Sound. 92nd Annual Meeting American Malacological Society. Syracuse, New York, 15-19, June. Poster

Dietl, G. P., Pruden, M. J., and J. C. Handley. 2026. Effect if improved water quality on molluscan communities in Long Island Sound. 2026 Long Island Sound Research Conference. Mystic, Connecticut, 4-5, June. Poster

D. ACCOMPLISHMENTS:

Complete the following sub-sections:

D1. Impacts & Effects: Describe any significant impacts/effects that the project is expected to have or had on business or industry development, resource management, the behavior of user groups, and the advancement of scientific knowledge. Provide information on direct socioeconomic gains realized because of the project if available. For example, these might include new businesses created, businesses retained, new tools or information created, cost savings, new products or expanded markets, jobs created or retained, or social benefits resulting from new resource uses. Benefits must be documentable, and where possible, quantifiable. List anyone we could contact regarding the accomplishments of this project. Much of this information should be available because of the extension and outreach activities from the Extension and Outreach Plan.

This pilot project is expected to have significant impacts on the assessment of benthic biological recovery in LIS by providing a practical method for evaluating biological responses to nitrogen management where long-term baseline data are lacking. The 2025 Long Island Sound Comprehensive Conservation and Management Plan (LIS CCMP) (LISP, 2025; p. 52) identified no progress toward the 2015 LIS CCMP Priority Implementation Action WW-8 ("Conduct studies and research to better understand the ecosystem's response to nitrogen reductions to support an evaluation of the 2000 Dissolved Oxygen TMDL"), in part because appropriate assessment tools have been unavailable. This pilot study demonstrates that molluscan death assemblages derived from the same grab samples collected for routine benthic monitoring can be integrated with living community data to reconstruct pre-TMDL biological condition and evaluate ecological change over management-relevant timescales.

This scalable approach provides resource managers with a practical means of assessing restoration outcomes and tracking biological recovery without requiring additional field sampling. In doing so, it directly addresses recommendations in the 2025 LIS CCMP to develop methods for evaluating ecosystem targets where existing approaches are lacking and to establish indicators consistent with a SMART (Specific, Measurable, Achievable, Relevant, and Time-Bound) management framework.

D2. Patents: Describe patents awarded or pending, including number, date, and licenses granted.

D3. Leveraged Funding: List and describe any funding or grants (e.g. from NSF, etc.) you received

with the help of the work or results from this project. Provide project title, project dates, funding amount, and funding source.

Title	Start Date	End Date	Funded Amount	Funding Source

E. Stakeholder Summary: Provide a summary of your project as if you were selling or describing your project to a potential "stakeholder" (a non-peer audience). Focus on the major issues and their importance, your results, and what impact they have. Be sure to include "take-home messages" from your project.

Evaluating the success of the Long Island Sound (LIS) Total Maximum Daily Load (TMDL) strategy requires tools that can demonstrate whether investments are translating into real ecological recovery. In this project, we tested whether improvements in water quality have translated into measurable recovery of benthic (seafloor) biological communities.

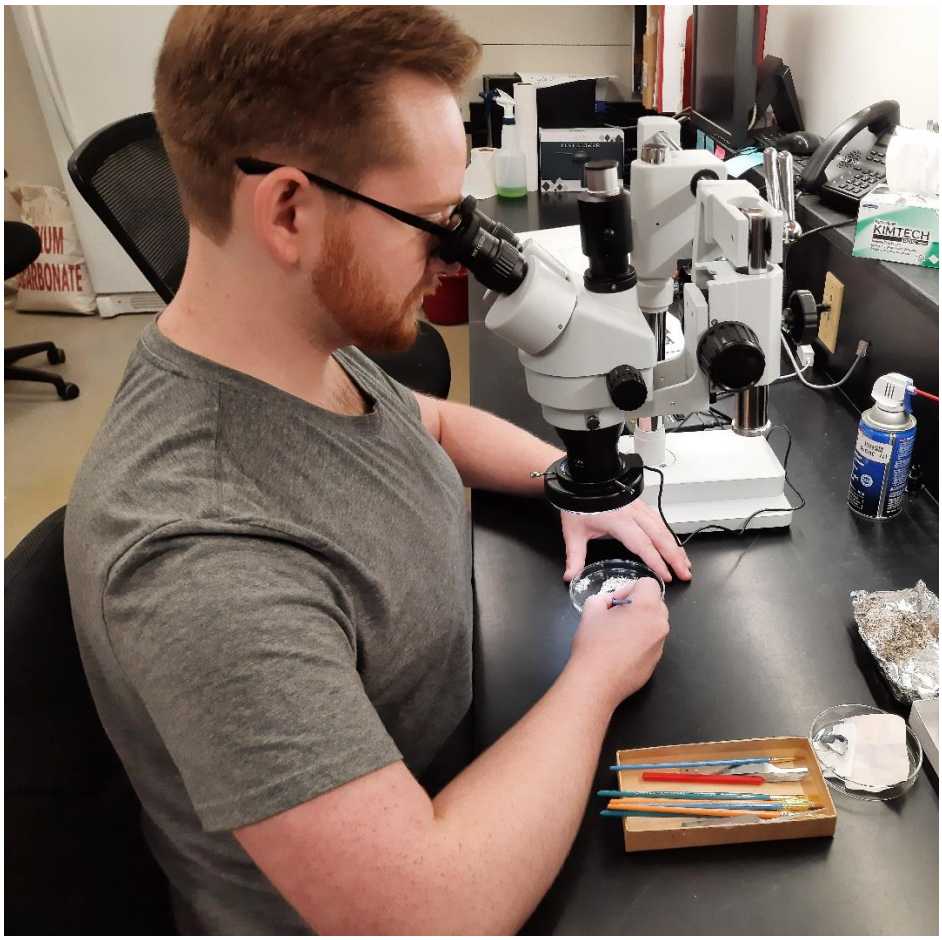
We show that dead remains of mollusks (shells) collected using standard benthic sampling protocols provide a cost-effective way to retrospectively evaluate biological responses to water quality management actions. These preserved shell assemblages serve as a natural archive (memory) of past habitat conditions, allowing ecological change to be assessed even in the absence of long-term monitoring data of living communities.

Overall, we find evidence that reductions in hypoxia following the TMDL have led to improved biological condition in eastern LIS. However, this improvement is not uniform across the Sound. Several sites in the western Narrows show little to no improvement, likely due to persistent legacy effects of historically degraded sediment quality that continue to influence benthic communities even after water quality has improved.

Importantly, this study demonstrates a proof of concept: routinely collected monitoring data from living benthic communities can be paired with dead mollusk shells from the same sampling events to assess restoration success. This approach can be scaled across LIS and extended to other estuaries, providing a transferable tool for evaluating the long-term outcomes of environmental management actions.

F. Pictorial: Provide any additional images/photos of personnel at work, in the field or laboratory, equipment being used, field sites, organism(s) of study or links to websites, etc. Please include proper photo credits and a caption with the date, location, names of people, and activity. By submitting photos for this report, you acknowledge that these images can be used by NYSG to document your project in future NYSG publications, websites, NYSG social media, and presentations or shared with the media. For any photos with people, please obtain each person's consent by informing them of how NYSG can use any photos submitted.

Please contact our Communications Director, Paul Focazio (paul.focazio@stonybrook.edu) for information about photo consent/release forms and other policies regarding photographs that may be publicly used.



Matthew Pruden (Graduate Student, Cornell University) sorting NCA20_NY-20047 Visit 1 sample residue at the Paleontological Research Institution, Ithaca, NY (09/27/2023). Image credit: Kiera Crowley.

K. OTHER:

Provide other relevant information that you would like to include. This section is optional.