

Chesapeake Bay Water Quality Monitoring Program

Long-term Benthic Monitoring and Assessment Component Level 1 Comprehensive Report

July 1984 – December 2021
(Volume 1)

Prepared for

**Maryland Department of Natural Resources
Resource Assessment Service
Tidewater Ecosystem Assessments
Annapolis, Maryland**

Prepared by

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July 2022

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**CHESAPEAKE BAY WATER QUALITY
MONITORING PROGRAM**

**LONG-TERM BENTHIC MONITORING
AND ASSESSMENT COMPONENT
LEVEL I COMPREHENSIVE REPORT**

JULY 1984 - DECEMBER 2021 (VOLUME 1)

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FOREWORD

This document, Chesapeake Bay Water Quality Monitoring Program: Long-Term Benthic Monitoring and Assessment Component, Level I Comprehensive Report (July 1984-December 2021), was prepared by Versar, Inc., at the request of Mr. Tom Parham of the Maryland Department of Natural Resources under Contract # K00R1600026 between Versar, Inc., and Maryland DNR. The report assesses the status of Chesapeake Bay benthic communities in 2021 and evaluates their responses to changes in water quality.

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ACKNOWLEDGEMENTS

We are grateful to the State of Maryland's Environmental Trust Fund which partially funded this work. The benthic studies discussed in this report were conducted from the University of Maryland's (R/V *Rachel Carson*) and Maryland DNR (R/V *Kerhin*) research vessels and we appreciate the efforts of their captains and crew. We thank Nancy Mountford and Tim Morris of Cove Corporation who identified benthos in many of the historical samples and provided current taxonomic and autoecological information. We also thank those at Versar whose efforts helped produce this report: the field crew who collected samples, including Field Coordinator Patrick Donovan, David Wong, Marc Molé, and Charles Tonkin; the laboratory staff who processed the samples and provided taxonomic identifications, Suzanne Arcuri, Istvan Turcsanyi, and Michael Winnell; and Allison Brindley for GIS and report preparation support. Mike Lane at Old Dominion University managed and analyzed the data.

We appreciate the efforts of Dr. Daniel M. Dauer, Mike Lane, and Anthony (Bud) Rodi of Old Dominion University who coordinate the activities of the Virginia Benthic Monitoring Program. Lastly, we thank Todd Beser who helped coordinate logistics for the sampling of the Aberdeen Proving Grounds.

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EXECUTIVE SUMMARY

Benthic macroinvertebrates have been an important component of the State of Maryland's Chesapeake Bay water quality monitoring program since the program's inception in 1984. Benthos integrate temporally variable environmental conditions and the effects of multiple types of environmental stress. They are sensitive indicators of environmental status. Information on the condition of the benthic community provides a direct measure of the effectiveness of management actions. The Long-Term Benthic Monitoring and Assessment Program contributes information to the Chesapeake Bay Health and Restoration Reports, and to the water quality characterization and list of impaired waters under the Clean Water Act. This report is one in a series of Level-One Annual Reports that summarize data up to the current sampling year. In this report, benthic community condition and trends in the Chesapeake Bay are assessed for 2021, with the following cautionary note:

"Due to an inadvertent misapplication of the random-site selection process, the Maryland 2021 data results cannot be assessed with confidence. Individual site data (species abundance and biomass; benthic index metrics and scores) are correct but summaries and interpretations of these data such as areal estimates of degradation and trends should be viewed with caution. Virginia data are unaffected."

Benthic community condition in Chesapeake Bay improved considerably in 2021, after two years of high degradation exceeding 60% of the bay's tidal area. Decreases in the percentage of degradation (decline in the number of acres failing the benthic community restoration goals) were observed in the Maryland and Virginia mainstems, Maryland Western Tributaries and the Patuxent River (with the caution noted above). The inferred improvement was greatest in the mainstem of the Chesapeake Bay, while the tributaries showed both increases and decreases in benthic degradation. These changes were consistent with those observed in years of moderate precipitation and river flow. Whereas large amounts of precipitation fell over the Chesapeake Bay watershed in the spring of 2019 resulting in massive flows, and pulses in river flow occurred in the spring of 2020, river flow in 2021 was below average early in the year. Excess nutrient runoff after heavy rains changes the balance of biological and chemical processes and the alteration of these processes often lead to hypoxia and loss of benthic biomass and productivity. Benthic condition varies annually depending on a variety of factors, among which nutrient loading, variability in spring river flow, physical forcing, and the timing of hypoxia play contributing and interacting roles.

The highlights for 2021 can be summarized as follows (but see cautionary note above; the confidence of tidal area estimates for 2021 is unknown):

- (1) The tidal area with degraded benthos in Chesapeake Bay decreased from 61% in 2020 to 48% in 2021.

- There was no statistically significant trend in percent area degraded over the 1996-2021 time period.
- (2) In Maryland degradation also decreased in 2021. By area, 68% of the Maryland Bay's tidal waters failed to meet the benthic community restoration goals in 2021.
- There was no statistically significant trend in percent area degraded over the 1995-2021 time period.
 - Degradation decreased in the Maryland mainstem, Maryland Western Tributaries, and the Patuxent River, and increased in the Upper Bay mainstem, Maryland Eastern Tributaries, and the Potomac River.
 - The Potomac River, Patuxent River, and Maryland mainstem were in poorest condition, with 68-72% of their tidal areas failing the restoration goals. The Upper Bay Mainstem was in best condition.
- (3) Benthic community condition (B-IBI scores averaged over the last 3 years of monitoring) remained within the same condition category at most of the monitoring fixed sites, improved at 2 sites from failing the goals to meeting the goals, and declined at 5 sites.
- Currently, 6 sites meet the benthic community restoration goals and 21 sites fail the goals.
- (4) Statistically significant B-IBI trends were detected at 17 of the 27 fixed sites.
- 5 sites had improving trends (significantly increasing B-IBI score): Upper Bay mainstem (Station 026), Elk River (Station 029), mesohaline Choptank River (Station 064), Bear Creek (Station 201), and Back River (Station 203).
 - 12 sites had declining trends (significantly decreasing B-IBI score): Mid Bay Mainstem at Calvert Cliffs (Station 001), Baltimore Harbor (Station 022), tidal freshwater Potomac River (Station 036), mesohaline Potomac River at Morgantown (Stations 043 and 047), deep mesohaline Potomac River at St. Clements Island (Station 052), Nanticoke River (Station 062), oligohaline Choptank River (Station 066), Patuxent River at Broomes Island (Station 071), Patuxent River at Holland Cliff (Station 077), Curtis Bay (Station 202), and Severn River (Station 204).
 - Changes in 2021 from 2020 results were limited to the appearance of a declining B-IBI trend in Curtis Bay -Patapsco River estuary.

Fixed-site and probability-based sampling strata in 2021 continued to show improvements in benthic condition from excess abundance (eutrophic condition). The percentage of sites in Maryland tidal waters scoring 1 for excess abundance (above

restorative thresholds) showed a statistically significant declining trend through 2021. This trend is important because it may signal favorable conditions in recent years associated with restoration efforts to reduce nutrient pollution.

Despite improvements in recent years, benthic condition remains largely degraded in Chesapeake Bay. Biomass-dominant species have declined over the years and low rates of benthic secondary production are observed in areas impacted by hypoxia. This background suggests that the recovery of the benthic communities, on which many fisheries and avian species depend, may be tied to factors in which not only management plays a role, but increasingly important aspects of climate change interact with species populations to provide patterns of benthic community change that mask the restoration efforts. The results of the benthic monitoring program, however, suggest that benthic communities are resilient to stress and respond quickly to improvements in water quality.

The use of probability-based sampling (but see cautionary note above for 2021) and fixed point monitoring allows us to provide an overall picture of benthic condition in Chesapeake Bay that helps track the success of efforts to clean up the bay. This picture would not have emerged if only water quality were monitored, and points to the value of long-term biological monitoring in the face of natural variability and variability from climate change.

Continuing monitoring on the appearance of non-indigenous species showed a spreading population of *Hermundura americana* (Polychaeta: Pilargidae). This species is now density-dominant in the low mesohaline portion of the Potomac River. *H. americana* is a warm-water polychaete worm in subtidal mud and sand bottoms of the Gulf of Mexico and Central America. It was first reported in Chesapeake Bay in the Southern Branch of the Elizabeth River in a single benthic sample in 2009. From the Elizabeth River this species spread into the James River in 2012 and is now found throughout the tidal James River and its tributaries. In 2018 *H. americana* was found in the Maryland portion of the Chesapeake Bay at five locations, three in the Potomac River near Morgantown and two in the Wicomico and Nanticoke rivers. *H. americana* has already colonized a wide range of salinity, depth, and sediment type in the James River, Rappahannock River, and Potomac River. The potential ecological community effects of this species as it expands throughout the Chesapeake Bay are unknown.

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1.0 INTRODUCTION

1.1 BACKGROUND

Monitoring is a necessary part of environmental management because it provides the means for assessing the effectiveness of previous management actions and the information necessary to focus future actions (NRC 1990). Towards these ends, the State of Maryland has maintained a water quality monitoring program for Chesapeake Bay since 1984. The goals of the program are to:

- quantify the types and extent of water quality problems (i.e., characterize the “state-of-the-bay”);
- determine the response of key water quality measures to pollution abatement and resource management actions;
- identify processes and mechanisms controlling the bay’s water quality;
- define linkages between water quality and living resources;
- contribute information to the Chesapeake Bay Health and Restoration Reports; and
- contribute information to the Water Quality Characterization Report (305b report) and the List of Impaired Waters (303d list).

The program includes elements to measure water quality, phytoplankton, and benthic macroinvertebrates (i.e., those invertebrates retained on a 0.5-mm mesh sieve). The monitoring program includes assessments of biota because the condition of biological indicators integrates temporally variable environmental conditions and the effects of multiple types of environmental stress. In addition, most environmental regulations and contaminant control measures are designed to protect biological resources; therefore, information about the condition of biological resources provides a direct measure of the effectiveness of management actions.

The Maryland program uses benthic macroinvertebrates as biological indicators because they are reliable and sensitive indicators of habitat quality in aquatic environments. Most benthic organisms have limited mobility and cannot avoid changes in environmental conditions (Gray 1979). Benthos live in bottom sediments, where exposure to contaminants and oxygen stress is most frequent. Benthic assemblages include diverse taxa representing a variety of sizes, modes of reproduction, feeding guilds, life history characteristics, and physiological tolerances to environmental conditions; therefore, they respond to and integrate natural and anthropogenic changes

in environmental conditions in a variety of ways (Pearson and Rosenberg 1978; Warwick 1986; Dauer 1993; Wilson and Jeffrey 1994).

Benthic organisms are also important secondary producers, providing key linkages between primary producers and higher trophic levels (Virnstein 1977; Holland et al. 1980, 1989; Baird and Ulanowicz 1989; Diaz and Schaffner 1990). Benthic invertebrates are among the most important components of estuarine ecosystems and may represent the largest standing stock of organic carbon in estuaries (Frithsen 1989). Many benthic organisms, such as clams, are economically important. Others, such as polychaete annelids and small crustaceans, contribute significantly to the diets of economically important bottom feeding juvenile and adult fishes, such as spot and croaker (Homer and Boynton 1978; Homer et al. 1980).

The Chesapeake Bay Program's decision to adopt benthic community restoration goals (Ranasinghe et al. 1994 updated by Weisberg et al. 1997) enhanced use of benthic macroinvertebrates as a monitoring tool. Based largely on data collected as part of Maryland's monitoring effort, these goals describe the characteristics of benthic assemblages expected at sites exposed to little environmental stress. The restoration goals provide a quantitative benchmark against which to measure the health of sampled assemblages and ultimately the Chesapeake Bay. Submerged aquatic vegetation (Dennison et al. 1993) and benthic macroinvertebrates are the only biological communities for which such quantitative goals have been established in Chesapeake Bay.

A variety of anthropogenic stresses affect benthic macroinvertebrate communities in Chesapeake Bay. These include toxic contaminants, organic enrichment, and low dissolved oxygen. While toxic contaminants are generally restricted to urban and industrial areas typically associated with ports, low dissolved oxygen (hypoxia) is the more widespread problem, encompassing an area of about 600 million m² mainly along the deep mainstem of the bay and at the mouth of the major Chesapeake Bay tributaries (Flemer et al. 1983). Organic enrichment, associated with excess phytoplankton growth and decay, is also a major problem in some regions of the Bay.

A variety of factors contribute to the development and spatial variation of hypoxia in Chesapeake Bay. Freshwater inflow, salinity, temperature, wind stress, and tidal circulation are primary factors in the development of hypoxia (Holland et al. 1987; Tuttle et al. 1987; Boicourt 1992). The development of vertical salinity gradients during the spring freshwater run off leads to water column density stratification. The establishment of a pycnocline, in association with periods of calm and warm weather, restricts water exchange between the surface and the bottom layers of the estuary, where oxygen consumption is large. This process is especially manifested along the Maryland mid-bay and Potomac River deep troughs. Formation or disruption of the pycnocline is probably the most important process determining intensity and extent of hypoxia (Seliger et al. 1985; Boicourt 1992), albeit not the only one. Biological processes contribute significantly to deep water oxygen depletion in Chesapeake Bay (Officer et al. 1984). Benthic metabolic rates increase during spring and early summer, leading to an increase of the

rate of oxygen consumption in bottom waters. This depends in part on the amount of organic carbon available for the benthos, which is derived to a large extent from seasonal phytoplankton blooms (Officer et al. 1984). Anthropogenic nutrient inputs to the Chesapeake Bay further stimulate phytoplankton growth, which results in increased deposition of organic matter to sediments and a concomitant increase in chemical and biological oxygen demand (Malone 1987). Winter to spring accumulation of phytoplankton biomass has been linked to depletion of bottom water oxygen in the Chesapeake Bay (Malone et al. 1988; Boynton and Kemp 2000).

The effects of hypoxia on benthic organisms vary as a function of the severity, spatial extent, and duration of low dissolved oxygen events. Oxygen concentrations down to about 2 mg L⁻¹ do not appear to significantly affect benthic organisms, although incipient community effects have been measured at 3 mg L⁻¹ (Diaz and Rosenberg 1995; Ritter and Montagna 1999). Hypoxia brings about structural and organizational changes in the community, and may lead to hypoxia resistant communities. With an increase in the frequency of hypoxic events, benthic populations become dominated by fewer and short-lived species, and their overall productivity is decreased (Diaz and Rosenberg 1995). Major reductions in species numbers and abundance in Chesapeake Bay have been attributed to hypoxia (Dauer et al. 1992, Llansó 1992). These reductions become larger both spatially and temporally as the severity and duration of hypoxic events increase. As hypoxia becomes persistent, mass mortality of benthic organisms often occurs with almost complete elimination of the macrofauna.

Hypoxia has also major impacts on the survival and behavior of a variety of benthic organisms and their predators (Diaz and Rosenberg 1995). Many infaunal species respond to low oxygen by migrating toward the sediment surface, thus potentially increasing their availability to demersal predators. On the other hand, reduction or elimination of the benthos following severe hypoxic and anoxic (absence of oxygen) events results in a reduction of food for demersal fish species and crabs. Therefore, the structural changes and species replacements that occur in communities affected by hypoxia may alter the food supply of important ecological and economical fish species in Chesapeake Bay. Given that dissolved oxygen stress and nutrient run-off are critical factors in the health of the biological resources of the Chesapeake Bay region, monitoring that evaluates benthic condition and tracks changes over time helps Chesapeake Bay managers assess the effectiveness of nutrient reduction efforts and the status of the biological resources of one of the largest and most productive estuaries in the nation.

1.2 OBJECTIVES OF THIS REPORT

This report is part of a series of Level I Comprehensive reports produced annually by the Long-Term Benthic Monitoring and Assessment Component (LTB) of the Maryland Chesapeake Bay Water Quality Monitoring Program. Level I reports summarize data from the latest sampling year and provide a limited examination of how conditions in the latest

year differ from conditions in previous years of the study, as well as how data from this year contribute to describing trends in the Bay's condition.

The report reflects the maturity of the current program focus and design. Approaches introduced when the new program design was implemented in 1995 continue to be extended, developed, and better defined. The level of detail in which changes are examined at the fixed stations sampled for trend analysis continues to increase. For example, we report on how species contribute to changes in condition and discuss trends in relation to changes in water quality. The Chesapeake Bay Benthic Index of Biotic Integrity (B-IBI) is applied to each sampling site, from tidal freshwater to polyhaline zones, and thus provides a uniform measure of ecological condition across the estuarine gradient. In describing baywide benthic community condition, estimates of degraded condition are presented for all subregions of the Bay, and community measures that contribute to restoration goal failure are used to diagnose the causes of failure.

The continued presentation of estimates of Bay area meeting the Chesapeake Bay Program benthic community restoration goals, rather than Maryland estimates only, reflects improved coordination and unification of objectives among the Maryland and Virginia benthic monitoring programs. The sampling design and methods in both states are compatible and complementary.

In addition to the improvements in technical content, we have enhanced electronic production and transmittal of data. Data and program information are available to the research community and the general public through the Chesapeake Bay Benthic Monitoring Program Home Page at <https://baybenthos.versar.com>. The 2021 data, as well as the data from previous years, can be downloaded from this website. The Benthic Monitoring Program Home Page represents the culmination of collaborative efforts between Versar, Maryland DNR, and the Chesapeake Information Management System (CIMS). The activities that Versar undertakes as a partner of CIMS were recorded in a Memorandum of Agreement signed October 28, 1999.

1.3 ORGANIZATION OF REPORT

This report has two volumes. Volume 1 is organized into five major sections and three appendices. Section 1 is this introduction. Section 2 presents the field, laboratory, and data analysis methods used to collect, process, and evaluate the LTB samples. Section 3 presents the results of analyses conducted for 2021, and consists of two assessments: an assessment of trends in benthic community condition at the fixed sites sampled annually by LTB in the Maryland Chesapeake Bay, and an assessment of the area of the Bay that meets the Chesapeake Bay Benthic Community Restoration Goals. Section 4 discusses the results and evaluates status and trends relative to changes in water quality. Section 5 is the literature cited in the report. Appendix A amplifies information presented in Table 3-2 and Table 3-3 by providing rates of change for the 1985-2021 fixed site trend analysis. Appendices B and C present the B-IBI values for the

2021 fixed and random sampling components, respectively. Appendix D presents newly collected data on benthic chlorophyll-a and phaeophytin. Finally, Volume 2 consists of the benthic, sedimentary, and hydrographic data appendices.

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2.0 METHODS

2.1 SAMPLING DESIGN

The LTB sampling program contains two primary elements: a fixed site monitoring effort directed at identifying trends in benthic condition and a probability-based sampling effort intended to estimate the area of the Maryland Chesapeake Bay with benthic communities meeting the Chesapeake Bay Program's benthic community restoration goals (Ranasinghe et al. 1994, updated by Weisberg et al. 1997; Alden et al. 2002). The sampling design for each of these elements is described below.

2.1.1 Fixed Site Sampling

The fixed site element of the program involves sampling at 27 sites, 23 of which have been sampled since the program's inception in 1984, 2 since 1989, and 2 since 1995 (Figure 2-1). Sites are defined by geography (within 1 km from a fixed location), and by specific depth and substrate criteria (Table 2-1).

The 2021 fixed site sampling continues trend measurements, which began with the program's initiation in 1984. In the first five years of the program, from July 1984 to June 1989, 70 fixed stations were sampled 8 to 10 times per year. On each visit, three benthic samples were collected at each site and processed. Locations of the 70 fixed sites are shown in Figure 2-2.

In the second five years of the program, from July 1989 to June 1994, fixed site sampling was continued at 29 sites and a stratified random sampling element was added. Samples were collected at random from approximately 25 km² small areas surrounding these sites (Figure 2-3) to assess the representativeness of the fixed locations. Sites 06, 47, 62, and 77, which are part of the current design, were not sampled during this five-year period. Stratum boundaries were delineated on the basis of environmental factors that are important in controlling benthic community distributions: salinity regime, sediment type, and bottom depth (Holland et al. 1989). In addition, four new areas were established in regions of the Bay targeted for management actions to abate pollution: the Patuxent River, Choptank River, and two areas in Baltimore Harbor. Each area was sampled four to six times each year.

From July 1994 through 2008, three replicate samples were collected in spring and summer at most of the current suite of 27 sites (Stations 203 and 204 were added in 1995, Table 2-1, Figure 2-1). This sampling regime was selected as being most cost effective after analysis of the first 10 years of data jointly with the Virginia Benthic Monitoring Program (Alden et al. 1997). Starting in 2009, spring sampling was eliminated due to budgetary constraints.

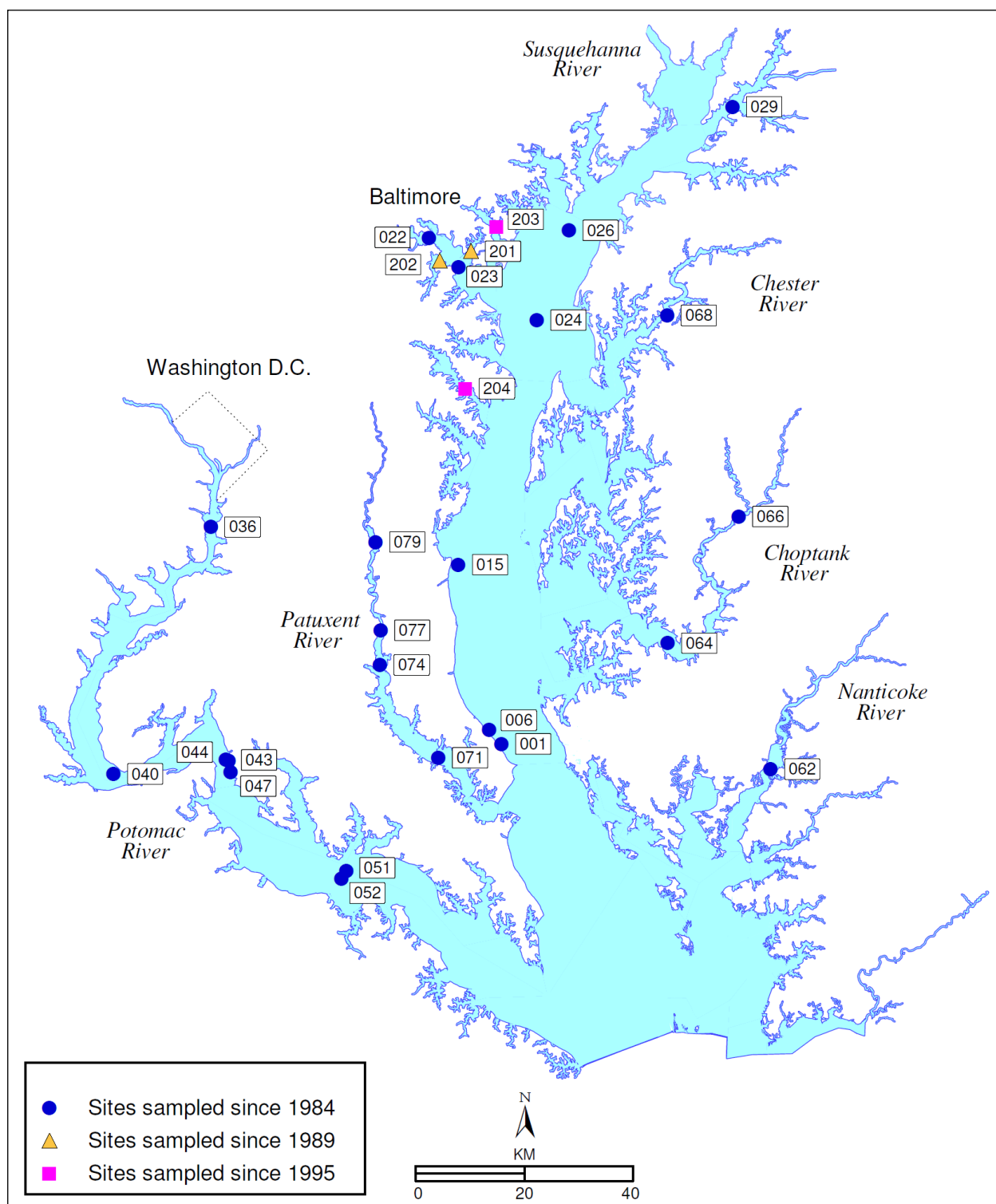


Figure 2-1. Fixed sites sampled in 2021

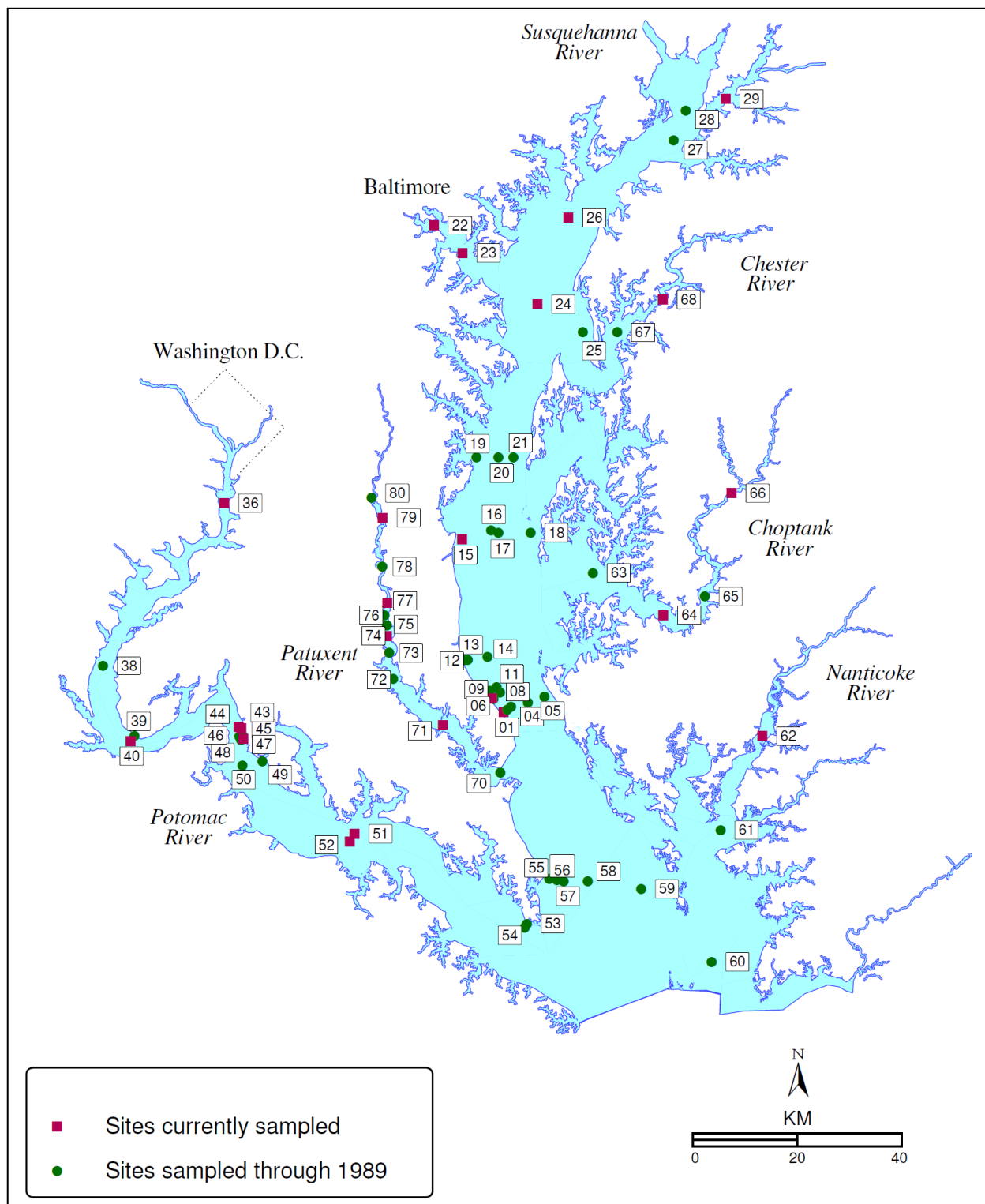


Figure 2-2. Fixed sites sampled from 1984 to 1989; some of these sites are part of the current design

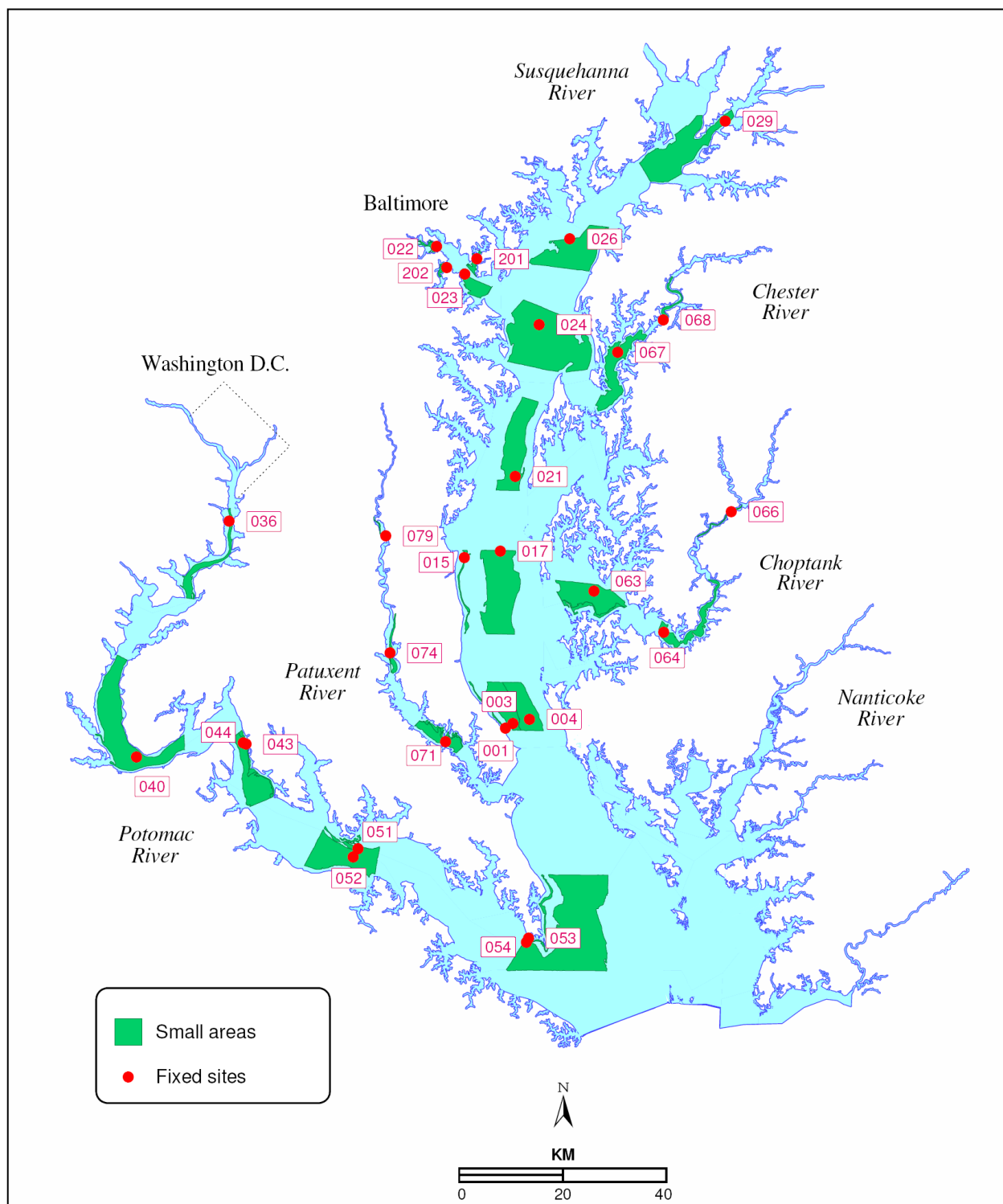


Figure 2-3. Small areas and fixed sites sampled from 1989 to 1994

Table 2-1. Location, habitat type (Table 5, Weisberg et al. 1997), sampling gear, and habitat criteria for fixed sites. ^(a) Sta. 047 temporally relocated to 38.37654, -76.98519 in 2020 and 2021 due to construction in the Potomac River Route 301 Bridge. ^(b) Sta. 022 permanently relocated across the channel during the 2010 field season because of construction at the old site. ^(c) Sta. 202 sampled in 2021 with a box corer.									
Stratum	Sub-Estuary	Habitat	Station	Latitude (WGS84)	Longitude (WGS84)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Potomac River	Potomac River	Tidal Freshwater	036	38.769788	-77.037534	WildCo Box Corer	<=5	>=40	1.0
		Oligohaline	040	38.357466	-77.230537	WildCo Box Corer	6.5-10	>=80	1.0
		Low Mesohaline	043	38.384479	-76.988329	Modified Box Corer	<=5	<=30	1.0
		Low Mesohaline	047 ^(a)	38.363825	-76.983737	Modified Box Corer	<=5	<=30	0.5
		Low Mesohaline	044	38.385633	-76.995698	WildCo Box Corer	11-17	>=75	1.0
		High Mesohaline Sand	051	38.205355	-76.738622	Modified Box Corer	<=5	<=20	1.0
		High Mesohaline Mud	052	38.192304	-76.747689	WildCo Box Corer	9-13	>=60	1.0
Patuxent River	Patuxent River	Tidal Freshwater	079	38.750457	-76.689023	WildCo Box Corer	<=6	>=50	1.0
		Low Mesohaline	077	38.604461	-76.675020	WildCo Box Corer	<=5	>=50	1.0
		Low Mesohaline	074	38.548962	-76.676186	WildCo Box Corer	<=5	>=50	0.5
		High Mesohaline Mud	071	38.395132	-76.548847	WildCo Box Corer	12-18	>=70	1.0

Table 2-1. (Continued)									
Stratum	Sub-Estuary	Habitat	Station	Latitude (WGS84)	Longitude (WGS84)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Upper Western Tributaries	Patapsco River	Low Mesohaline	023	39.208283	-76.523354	WildCo Box Corer	4-7	>=50	1.0
	Middle Branch	Low Mesohaline	022 ^(b)	39.258082	-76.59512	WildCo Box Corer	2-6	>=40	1.0
	Bear Creek	Low Mesohaline	201	39.234167	-76.497501	WildCo Box Corer	2-4.5	>=70	1.0
	Curtis Bay	Low Mesohaline	202	39.217839	-76.564171	WildCo Box Corer	5-8	>=60	1.0
	Back River	Oligohaline	203	39.275005	-76.444508	Young-Grab	1.5-2.5	>=80	1.0
	Severn River	High Mesohaline Mud	204 ^(c)	39.006954	-76.504955	Young-Grab	5-7.5	>=50	1.0
Eastern Tributaries	Chester River	Low Mesohaline	068	39.132509	-76.078780	WildCo Box Corer	4-8	>=70	1.0
	Choptank River	Oligohaline	066	38.801455	-75.921827	WildCo Box Corer	<=5	>=60	1.0
		High Mesohaline Mud	064	38.590459	-76.069331	WildCo Box Corer	7-11	>=70	1.0
	Nanticoke River	Low Mesohaline	062	38.383960	-75.849990	Petite Ponar Grab	5-8	>=75	1.0

Table 2-1. (Continued)									
Stratum	Sub-Estuary	Habitat	Station	Latitude (WGS84)	Longitude (WGS84)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Upper Bay	Elk River	Oligohaline	029	39.479505	-75.944836	WildCo Box Corer	3-7	>=40	1.0
	Mainstem	Low Mesohaline	026	39.271450	-76.290013	WildCo Box Corer	2-5	>=70	1.0
		High Mesohaline Mud	024	39.122004	-76.355673	WildCo Box Corer	5-8	>=80	1.0
Mid Bay	Mainstem	High Mesohaline Sand	015	38.715126	-76.513679	Modified Box Corer	<=5	<=10	1.0
		High Mesohaline Sand	001	38.419001	-76.418385	Modified Box Corer	<=5	<=20	1.0
		High Mesohaline Sand	006	38.442000	-76.444261	Modified Box Corer	<=5	<=20	0.5

2.1.2 Probability-based Sampling

The second sampling element, which was instituted in 1994, was probability-based summer sampling designed to estimate the area of the Maryland Chesapeake Bay and its tributaries that meet the Chesapeake Bay benthic community restoration goals (Ranasinghe et al. 1994, updated by Weisberg et al. 1997; Alden et al. 2002). Different probability sample allocation strategies were used in 1994 than in later years. In 1994, the design was intended to estimate impaired area for the Maryland Bay and one sub-region, while in later years the design targeted five additional sub-regions as well.

The 1994 sample allocation scheme was designed to produce estimates for the Maryland Bay and the Potomac River. The Maryland Bay was divided into three strata with samples allocated unequally among them (Table 2-2); sampling intensity in the Potomac was increased to permit estimation of degraded area with adequate confidence, while mainstem and other tributary and embayment samples were allocated in proportion to their area.

Table 2-2. Allocation of probability-based baywide samples, 1994			
Stratum	Area		Number of Samples
	km ²	%	
Maryland Mainstem (including Tangier and Pocomoke Sounds)	3,611	55.5	27
Potomac River	1,850	28.4	28
Other tributaries and embayments	1,050	16.1	11

In subsequent years, the stratification scheme was designed to produce an annual estimate for the Maryland Bay and six subdivisions. Samples were allocated equally among strata (Figure 2-4, Table 2-3). According to this allocation, a fresh new set of sampling sites were selected each year. Figure 2-5 shows the locations of the probability-based Maryland sampling sites for 2021. Regions of the Maryland mainstem deeper than 12 m were not included in sampling strata because these areas are subjected to summer anoxia and have consistently been found to be azoic.

A similar stratification scheme has been used by the Commonwealth of Virginia since 1996, permitting annual estimates for the extent of area meeting the benthic community restoration goals for the entire Chesapeake Bay (Table 2-3, Figure 2-6). These samples were collected and processed, and the data analyzed by the Virginia Chesapeake Bay Benthic Monitoring Program.

Note: The random sites were not selected using the same process for 2021. See cautionary note in page 3-36 of this report.

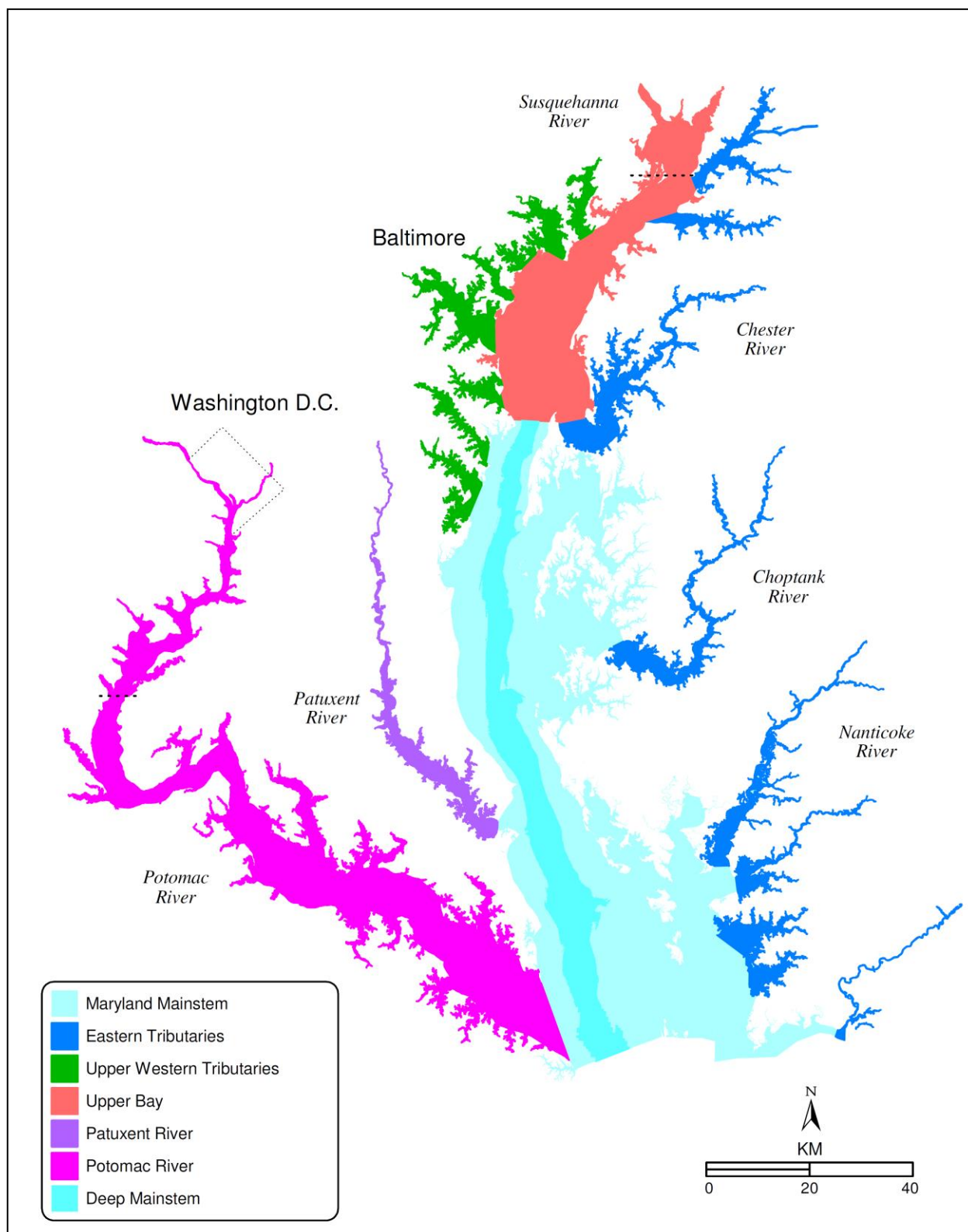


Figure 2-4. Maryland baywide sampling strata in and after 1995

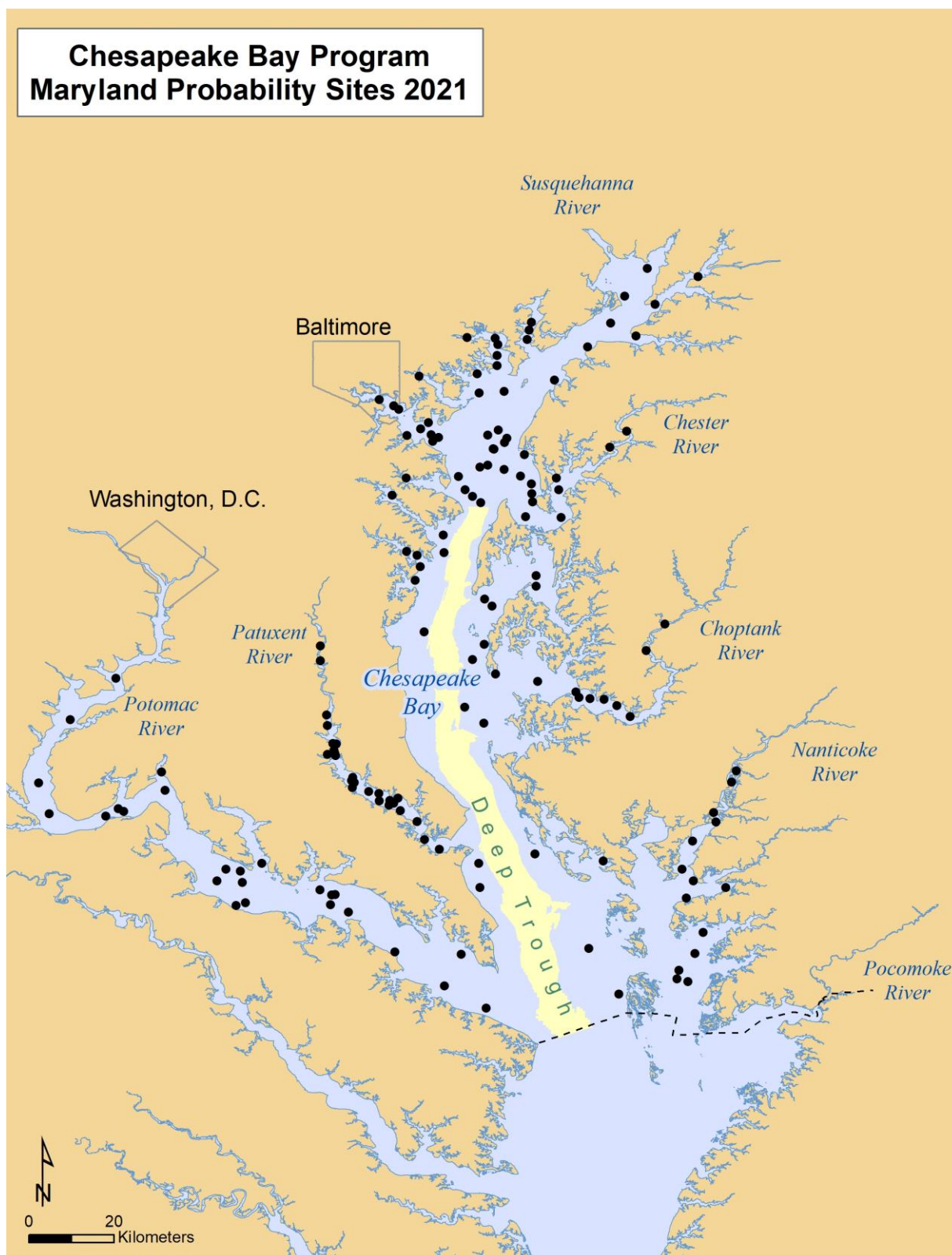


Figure 2-5. Maryland probability-based sampling sites for 2021 (see cautionary note in page 3-36)

Table 2-3. Allocation of probability-based baywide samples, in and after 1995. Maryland areas exclude 676 km ² of mainstem habitat deeper than 12 m. Virginia strata were sampled by the Virginia Chesapeake Bay Benthic Monitoring Program commencing in 1996.					
State	Stratum	Area			Number of Samples
		km ²	State %	Bay %	
Maryland	Deep Mainstem	676	10.8	5.8	0
	Mid Bay Mainstem	2,552	40.9	22.0	25
	Eastern Tributaries	534	8.6	4.6	25
	Western Tributaries	292	4.7	2.5	25
	Upper Bay Mainstem	785	12.6	6.8	25
	Patuxent River	128	2.0	1.1	25
	Potomac River*	1,276	20.4	11.0	25
	TOTAL	6,243	100.0	53.8	150
Virginia	Mainstem	4,120	76.8	35.5	25
	Rappahannock River	372	6.9	3.2	25
	York River	187	3.5	1.6	25
	James River	684	12.8	5.9	25
	TOTAL	5,363	100.0	46.2	100
*Excludes Virginia tidal creeks and district of Columbia waters					

2.2 SAMPLE COLLECTION

2.2.1 Station Location

From July 1984 to June 1996, stations were located using Loran-C. After June 1996 stations were located using a differential Global Positioning System. The WGS84 coordinate system (undistinguishable in practice from NAD83) is currently used.

2.2.2 Water Column Measurements

Water column vertical profiles of temperature, conductivity, salinity, dissolved oxygen concentration (DO), and pH were measured at each site. Oxidation reduction potential (ORP) was measured prior to 1996. For fixed sites, profiles consisted of water quality measurements at 1 m intervals from surface to bottom at sites 7 m deep or less, and at 3 m intervals, with additional measurements at 1.5 m intervals in the vicinity of the pycnocline, at sites deeper than 7 m. Surface and bottom measurements were made at all other sampling sites. In 2016, a modification to the fixed-site water quality profiles was introduced, whereby measurements were taken at 1 m intervals at sites 10 m deep or less, and at 2 m intervals, with additional measurements in the vicinity of the pycnocline,

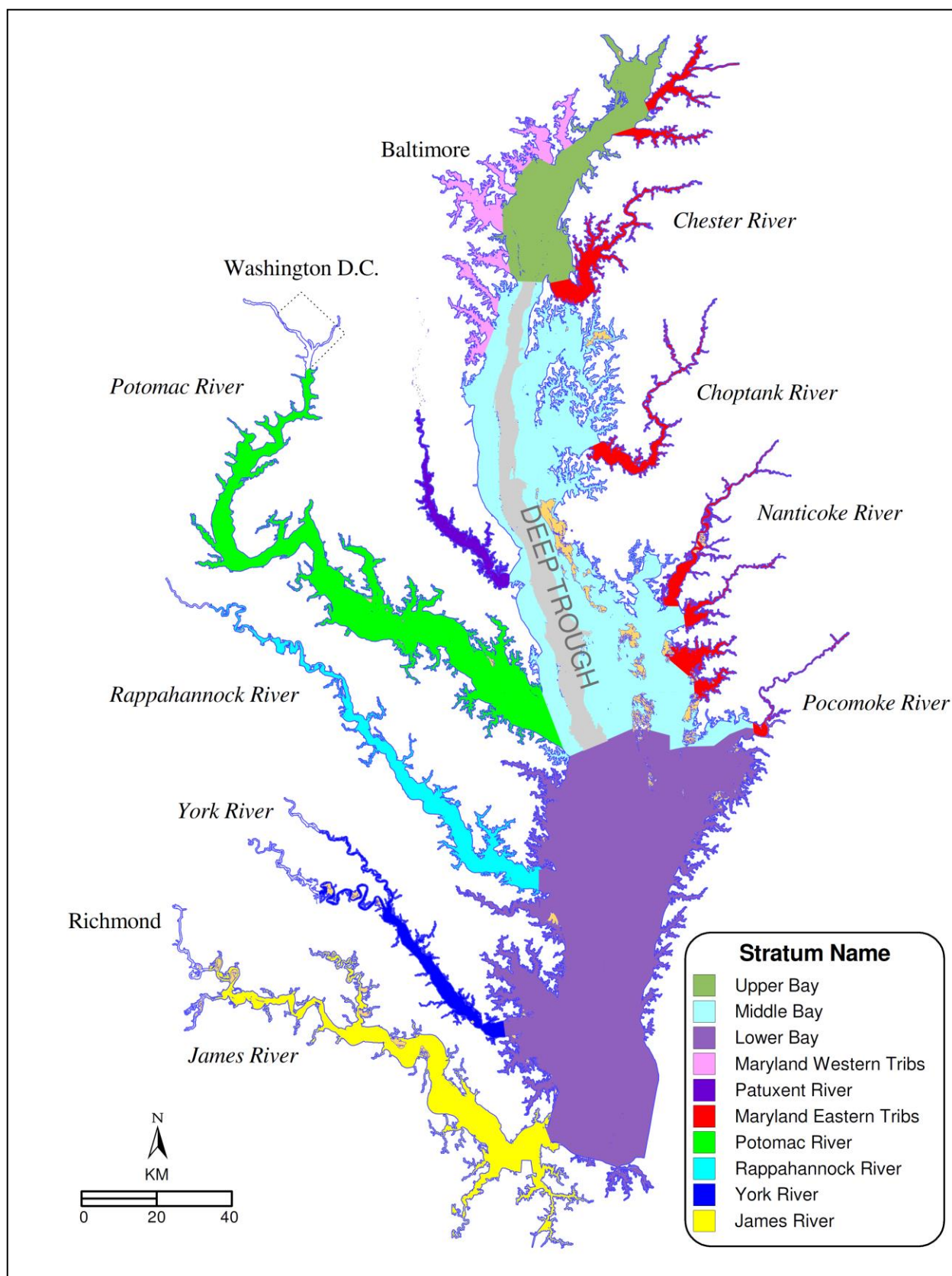


Figure 2-6. Chesapeake Bay stratification scheme

Table 2-4. Methods used to measure water quality parameters.		
Parameter	Period	Method
Temperature	July 1984 to November 1984	Thermistor attached to Beckman Model RS5-3 salinometer
	December 1984 to December 1995	Thermistor attached to Hydrolab Surveyor II
	January 1996 to present	Thermistor attached to Hydrolab DataSonde 4a, YSI 6600, or YSI EXO2 sonde
Salinity and Conductivity	July to November 1984	Beckman Model RS5-3 salinometer toroidal conductivity cell with thermistor temperature compensation
	December 1984 to December 1995	Hydrolab Surveyor II nickel six-pin electrode-salt water cell block combination with automatic temperature compensation
	January 1996 to present	Hydrolab DataSonde 4a four graphite electrode cell (open-cell design), YSI 6600, or YSI EXO2 four nickel electrode cell, with automatic temperature compensation
Dissolved Oxygen	July to November 1984	YSI Model 57 or Model 58 Oxygen Meter with automatic temperature and manual salinity compensation
	December 1984 to December 1995	Hydrolab Surveyor II membrane design probe with automatic temperature and salinity compensation
	January 1996 to present	Hydrolab DataSonde 4a membrane-design or optical DO sensor, YSI 6600 Rapid Pulse, or YSI EXO2 optical sensor, with automatic temperature and salinity compensation
pH	July to November 1984	Orion analog pH meter with Ross glass combination electrode manually compensated for temperature
	December 1984 to December 1995	Hydrolab Surveyor II glass pH electrode and Lazaran reference electrode automatically compensated for temperature
	January 1996 to present	Hydrolab DataSonde 4a, YSI 6600, or YSI EXO2 combined glass pH and reference sensor, automatically compensated for temperature
Oxidation Reduction Potential	December 1984 to December 1995	Hydrolab Surveyor II platinum banded glass ORP electrode

at sites deeper than 10 m. Table 2-4 lists the measurement methods used.

2.2.3 Benthic Samples

Samples were collected using four kinds of gear depending on the program element and habitat type. For the fixed site element (Table 2-1), a hand-operated box corer ("modified box corer"), which samples a 250 cm² area to a depth of 25 cm, was used in the nearshore shallow sandy habitats of the mainstem bay and tributaries. A Wildco box corer, which samples an area of 220 cm² to a depth of 23 cm, was used in shallow muddy or deep-water (> 5 m) habitats in the mainstem bay and tributaries. A Petite Ponar Grab, which samples 250 cm² to a depth of 7 cm, was used at the fixed site in the Nanticoke River to be consistent with previous sampling in the 1980s. At the two fixed sites first sampled in 1995 and at all probability-based sampling sites, a Young Grab, which samples an area of 440 cm² to a depth of 10 cm, was used.

Sample volume and penetration depth were measured for all samples; Wildco and hand-operated box cores penetrating less than 15 cm, and Young and Petite Ponar grabs penetrating less than 7 cm into the sediment were rejected and the site was re-sampled.

In the field, samples were sieved through a 0.5-mm screen using an elutriative process. Organisms and detritus retained on the screen were transferred into labeled jars and preserved in a 10% formaldehyde solution stained with Rose Bengal (a vital stain that aids in separating organisms from sediments and detritus).

One surface-sediment sub-sample of approximately 120 ml is collected for grain-size, carbon, and nitrogen analysis from an additional grab sample at each site. This sub-sample is maintained in the dark on wet ice while on board, and frozen until processed in the laboratory. In addition, starting in summer 2021 three surface sediment replicate samples (2.5 cm diameter x 1 cm sediment cores, 4.91 cm³) were collected from a separate grab sample at each fixed site for benthic chlorophyll-a analysis. These samples were stored frozen in the dark until processed.

2.3 LABORATORY PROCESSING

Organisms were sorted from detritus under dissecting microscopes, identified to the lowest practical taxonomic level (most often species), and counted. Oligochaetes and chironomids were mounted on slides and examined under a compound microscope for genus and species identification.

Ash-free dry weight biomass was determined by three comparable techniques during the sampling period. For samples collected from July 1984 to June 1985, biomass was directly measured using an analytical balance for major organism groups (e.g., polychaetes, molluscs, and crustaceans). Ash-free dry weight biomass was determined by

drying the organisms to a constant weight at 60 °C and ashing in a muffle furnace at 500 °C for four hours. For samples collected between July 1985 and August 1993, a regression relationship between ash-free dry weight biomass and size of morphometric characters was defined for 22 species (Ranasinghe et al. 1993). The biomass of the 22 selected species was estimated from these regression relationships. These taxa (Table 2-5) were selected because they accounted for more than 85% of the abundance (Holland et al. 1988). After August 1993, ash-free dry weight biomass was measured directly for each species by drying the organisms to a constant weight at 60 °C and ashing in a muffle furnace at 500 °C for four hours and re-weighing (ash weight). The difference between the dry weight and the ash weight is the ash-free dry weight. Bivalves were crushed to open the shells and expose the animal to drying and ashing (shells included).

Table 2-5. Taxa for which biomass was estimated in samples collected between 1985 and 1993	
Polychaeta	Mollusca
<i>Eteone heteropoda</i>	<i>Acteocina canaliculata</i>
<i>Glycinde solitaria</i>	<i>Corbicula fluminea</i>
<i>Heteromastus filiformis</i>	<i>Gemma gemma</i>
<i>Marenzelleria viridis</i>	<i>Haminoea solitaria</i>
<i>Neanthes succinea</i>	<i>Macoma balthica</i>
<i>Paraprionospio pinnata</i>	<i>Macoma mitchelli</i>
<i>Streblospio benedicti</i>	<i>Mulinia lateralis</i>
	<i>Mya arenaria</i>
	<i>Rangia cuneata</i>
	<i>Tagelus plebeius</i>
Crustacea	
<i>Cyathura polita</i>	
<i>Gammarus</i> spp.	
<i>Leptocheirus plumulosus</i>	
Nemertina	
<i>Carinoma tremaphoros</i>	
<i>Micrura leidy</i>	

Silt-clay composition was determined by wet-sieving through a 63-µm stainless steel sieve followed by pipetting of the silt and clay fraction using procedures described in the Versar, Inc., Laboratory Standard Operating Procedures (Versar 1999), and Folk (1974). Carbon and nitrogen content of dried sediments was determined using an elemental analyzer. Sediment carbon content was measured with a Perkin-Elmer Model 240B analyzer from 1984 to 1988, and an Exeter Analytical Inc., Model CE-440 analyzer in and after 1995. The results from both instruments are comparable. Samples were combusted at high temperature (975 °C) and the carbon dioxide and nitrogen produced were measured by thermal conductivity detection. Prior to combustion, each sample was

homogenized and oven-dried. No acid was applied. Remaining sediment was archived for quality assurance purposes (Scott et al. 1988). Chlorophyll-*a* concentrations were determined by fluorometry following procedures in EPA's Method 445.0.

2.4 DATA ANALYSIS

Analyses for the fixed site and probability-based elements of LTB were both performed in the context of the Chesapeake Bay Program's benthic community restoration goals and the Benthic Index of Biotic Integrity (B-IBI) by which goal attainment is measured. The B-IBI, the Chesapeake Bay benthic community restoration goals, and statistical analysis methods for the two LTB elements are described below.

2.4.1 The B-IBI and the Chesapeake Bay Benthic Community Restoration Goals

The B-IBI is a multiple-attribute index developed to identify the degree to which a benthic assemblage meets the Chesapeake Bay Program's benthic community restoration goals (Ranasinghe et al. 1994, updated by Weisberg et al. 1997; Alden et al. 2002). The B-IBI provides a means for comparing relative condition of benthic invertebrate assemblages across habitat types. It also provides a validated mechanism for integrating several benthic community attributes indicative of habitat "health" into a single number that measures overall benthic community condition.

The B-IBI is scaled from 1 to 5, and sites with values of 3 or more are considered to meet the restoration goals. The index is calculated by scoring each of several attributes as either 5, 3, or 1 depending on whether the value of the attribute at a site approximates, deviates slightly from, or deviates strongly from values found at the best reference sites in similar habitats, and then averaging these scores across attributes. The criteria for assigning these scores are numeric and depend on habitat. Data from seasons for which the B-IBI has not been developed were not used for B-IBI based assessment.

Benthic community condition was classified into four levels based on the B-IBI. Values less than or equal to 2.0 were classified as severely degraded; values from 2.0 to 2.6 were classified as degraded; values greater than 2.6 but less than 3.0 were classified as marginal; and values of 3.0 or more were classified as meeting the goals. Values in the marginal category do not meet the restoration goals, but they differ from the goals within the range of measurement error typically recorded between replicate samples.

2.4.2 Fixed Site Trend Analysis

Trends in condition at the fixed sites were identified using the nonparametric technique of van Belle and Hughes (1984). This procedure is based on the Mann-Kendall statistic and consists of a sign test comparing each value with all values measured in

subsequent periods. The ratio of the Mann-Kendall statistic to its variance provides a normal deviate that is tested for significance. Alpha was set to 0.1 for these tests because of the low power for trend detection for biological data. An estimate of the magnitude of each significant trend was obtained using Sen's (1968) procedure which is closely related to the Mann-Kendall test. Sen's procedure identifies the median slope among all slopes between each value and all values measured in subsequent periods.

2.4.3 Probability-based Estimation

The Maryland Bay was divided into three strata (Bay Mainstem, Potomac River, other tributaries and embayments) in 1994 (Table 2-2). It was divided into six strata in and after 1995 (Figure 2-4, Table 2-3). The Virginia Bay was divided into four strata, beginning in 1996 (Figure 2-6, Table 2-3).

To estimate the amount of area in the entire Bay that failed to meet the Chesapeake Bay benthic community restoration goals (P), we defined for every site i in stratum h a variable y_{hi} that had a value of 1 if the benthic community met the goals, and 0 otherwise. For each stratum, the estimated proportion of area meeting the goals, p_h , and its variance were calculated as the mean of the y_{hi} 's and its variance, as follows:

$$p_h = \bar{y}_h = \sum_{i=1}^{n_h} \frac{y_{hi}}{n_h} \quad (1)$$

and

$$\text{var}(p_h) = s_h^2 = \sum_{i=1}^{n_h} \frac{(y_{hi} - \bar{y}_h)^2}{n_h - 1} \quad (2)$$

Estimates for strata were combined to achieve a statewide estimate as:

$$\hat{P}_{ps} = \bar{y}_{ps} = \sum_{h=1}^6 W_h \bar{y}_h \quad (3)$$

where the weighting factor $W_h = A_h/A$; A_h is the total area of the h th stratum, and A is the combined area of all strata. The variance of (3) was estimated as:

$$\text{var}(\hat{P}_{ps}) = \text{var}(\bar{y}_{ps}) = \sum_{h=1}^6 W_h^2 s_h^2 / n_h \quad (4)$$

The standard error for individual strata is estimated as the square root of (2), and for the combined strata, as the square root of (4).

2.4.4 B-IBI Salinity Habitat Class Correction in 2018

Because of high precipitation in the Chesapeake Bay region, salinities were very low in summer 2018. Areas in the upper Chesapeake Bay that are in the low mesohaline range, had tidal freshwater bottom salinities at the time of sampling. The species composition of the 2018 probability-based sites was compared with the species composition of nearby sites sampled in 2017. The species composition was similar in both years. However, because of habitat salinity class differences, the B-IBI was quite different when calculated on the lower salinity classes of 2018; it tended to over-estimate benthic community condition. Therefore, a salinity habitat class correction was necessary for making the B-IBI more comparable to previous years. Box plots of bottom salinity were constructed for all sites, 1995-2017. Six years for which the salinity was clearly too high or too low (1995, 1996, 1999, 2002, 2004, and 2011) were removed. Using GIS, the bottom salinity values of the remaining years were mapped and the 2018 sites were superimposed on the map. The salinity class of the 2018 sites was then re-assigned to reflect the predominant salinity class of the average year. Some of the 2018 sites did not need re-assignment because their salinity, although low (e.g., 6) was still within the salinity class of the average year (e.g., 5-12). Affected sites included sites in each of the sampling strata in Maryland and Virginia (Table 2-6). Habitat class corrections were also made in 2011 because of very low salinity in Maryland after Hurricane Irene and Tropical Storm Lee (see the Methods sections of 2012-2018 Level-I reports).

Table 2-6. Salinity class correction for 2018.

Stratum	Site	Original	Corrected
Maryland Mid Bay Mainstem	MMS-25507	Low Mesohaline	High Mesohaline
	MMS-25509	Low Mesohaline	High Mesohaline
	MMS-25510	Low Mesohaline	High Mesohaline
	MMS-25512	Low Mesohaline	High Mesohaline
	MMS-25514	Low Mesohaline	High Mesohaline
	MMS-25515	Low Mesohaline	High Mesohaline
	MMS-25517	Oligohaline	Low Mesohaline
	MMS-25520	Low Mesohaline	High Mesohaline
	MMS-25523	Low Mesohaline	High Mesohaline
Maryland Eastern Tributaries	MET-25413	Oligohaline	Low Mesohaline
	MET-25415	Oligohaline	Low Mesohaline
	MET-25416	Oligohaline	Low Mesohaline
	MET-25422	Tidal Fresh	Oligohaline
	MET-25423	Tidal Fresh	Oligohaline
	MET-25425	Tidal Fresh	Oligohaline
Maryland Western Tributaries	MWT-25303	Oligohaline	Low Mesohaline
	MWT-25304	Oligohaline	Low Mesohaline
	MWT-25305	Oligohaline	Low Mesohaline

Table 2-6. (Continued)			
Stratum	Site	Original	Corrected
Maryland Western Tributaries (continued)	MWT-25306	Oligohaline	Low Mesohaline
	MWT-25307	Oligohaline	Low Mesohaline
	MWT-25308	Oligohaline	Low Mesohaline
	MWT-25309	Oligohaline	Low Mesohaline
	MWT-25310	Oligohaline	Low Mesohaline
	MWT-25311	Oligohaline	Low Mesohaline
	MWT-25312	Oligohaline	Low Mesohaline
	MWT-25313	Oligohaline	Low Mesohaline
	MWT-25317	Oligohaline	Low Mesohaline
	MWT-25318	Tidal Fresh	Oligohaline
	MWT-25319	Tidal Fresh	Oligohaline
	MWT-25320	Tidal Fresh	Low Mesohaline
	MWT-25321	Tidal Fresh	Oligohaline
	MWT-25322	Tidal Fresh	Oligohaline
	MWT-25324	Tidal Fresh	Oligohaline
	MWT-25325	Tidal Fresh	Oligohaline
	MWT-25326	Oligohaline	Low Mesohaline
Maryland Upper Bay Mainstem	UPB-25604	Oligohaline	Low Mesohaline
	UPB-25605	Oligohaline	Low Mesohaline
	UPB-25607	Oligohaline	Low Mesohaline
	UPB-25608	Oligohaline	Low Mesohaline
	UPB-25609	Oligohaline	Low Mesohaline
	UPB-25610	Oligohaline	Low Mesohaline
	UPB-25611	Oligohaline	Low Mesohaline
	UPB-25612	Oligohaline	Low Mesohaline
	UPB-25613	Oligohaline	Low Mesohaline
	UPB-25614	Oligohaline	Low Mesohaline
	UPB-25615	Oligohaline	Low Mesohaline
	UPB-25616	Oligohaline	Low Mesohaline
	UPB-25617	Tidal Fresh	Low Mesohaline
	UPB-25621	Tidal Fresh	Oligohaline
	UPB-25622	Tidal Fresh	Oligohaline
	UPB-25623	Tidal Fresh	Oligohaline

Table 2-6. (Continued)

Stratum	Site	Original	Corrected
Patuxent River	PXR-25201	Low Mesohaline	High Mesohaline
	PXR-25202	Low Mesohaline	High Mesohaline
	PXR-25203	Low Mesohaline	High Mesohaline
	PXR-25204	Low Mesohaline	High Mesohaline
	PXR-25205	Low Mesohaline	High Mesohaline
	PXR-25206	Low Mesohaline	High Mesohaline
	PXR-25207	Low Mesohaline	High Mesohaline
	PXR-25208	Low Mesohaline	High Mesohaline
	PXR-25209	Low Mesohaline	High Mesohaline
	PXR-25210	Low Mesohaline	High Mesohaline
	PXR-25221	Oligohaline	Low Mesohaline
	PXR-25222	Oligohaline	Low Mesohaline
	PXR-25223	Oligohaline	Low Mesohaline
Potomac River	PMR-25104	Low Mesohaline	High Mesohaline
	PMR-25105	Low Mesohaline	High Mesohaline
	PMR-25106	Low Mesohaline	High Mesohaline
	PMR-25108	Low Mesohaline	High Mesohaline
	PMR-25109	Oligohaline	Low Mesohaline
	PMR-25110	Low Mesohaline	High Mesohaline
	PMR-25112	Oligohaline	Low Mesohaline
	PMR-25114	Oligohaline	Low Mesohaline
	PMR-25115	Oligohaline	Low Mesohaline
	PMR-25116	Oligohaline	Low Mesohaline
	PMR-25117	Tidal Fresh	Low Mesohaline
	PMR-25118	Tidal Fresh	Low Mesohaline
	PMR-25119	Oligohaline	Low Mesohaline
	PMR-25120	Tidal Fresh	Oligohaline
	PMR-25121	Oligohaline	Low Mesohaline
	PMR-25122	Tidal Fresh	Oligohaline
Virginia Mainstem	VBY-25M04	High Mesohaline	Polyhaline
	VBY-25M09	High Mesohaline	Polyhaline
	VBY-25M11	High Mesohaline	Polyhaline
	VBY-25M22	Low Mesohaline	High Mesohaline
Rappahannock River	RAP-25R01	Low Mesohaline	High Mesohaline
	RAP-25R02	Low Mesohaline	High Mesohaline

Table 2-6. (Continued)			
Stratum	Site	Original	Corrected
Rappahannock River (continued)	RAP-25R04	Low Mesohaline	High Mesohaline
	RAP-25R06	Low Mesohaline	High Mesohaline
	RAP-25R07	Low Mesohaline	High Mesohaline
	RAP-25R09	Low Mesohaline	High Mesohaline
	RAP-25R11	Low Mesohaline	High Mesohaline
	RAP-25R14	Low Mesohaline	High Mesohaline
	RAP-25R15	Low Mesohaline	High Mesohaline
	RAP-25R18	Tidal Fresh	Oligohaline
	RAP-25R19	Tidal Fresh	Oligohaline
	RAP-25R20	Tidal Fresh	Oligohaline
	RAP-25R26	Tidal Fresh	Low Mesohaline
York River	YRK-25Y01	High Mesohaline	Polyhaline
	YRK-25Y03	High Mesohaline	Polyhaline
	YRK-25Y05	High Mesohaline	Polyhaline
	YRK-25Y23	Tidal Fresh	Oligohaline

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3.0 RESULTS

3.1 TRENDS IN FIXED SITE BENTHIC CONDITION

Trend analysis is conducted on 27 fixed sites located throughout the Bay and its tributaries to assess whether benthic community condition is changing. Through 2008 the sites were sampled yearly in the spring and summer. Since 2009, sites are sampled in the summer only. Trend analysis is performed on the summer data only in order to apply the B-IBI (Weisberg et al. 1997; Alden et al. 2002). B-IBI calculations and trend analysis methods are described in Section 2.4.

The B-IBI is the primary measure used in trend analysis because it integrates several benthic community attributes into a measure of overall condition. It provides context for interpretation of observed trends because status has been calibrated to reference conditions. Significant trends that result in a change of status (sites that previously met the Chesapeake Bay benthic community restoration goals which now fail, or vice versa) are of greater management interest than trends which do not result in a change. As a first step in identifying causes of changes in condition, trends on individual attributes are identified and examined.

Table 3-1 presents trends in benthic community condition from 1985 to the present. Although the Maryland benthic monitoring component began sampling in 1984, data collected in the first year of our program were excluded from analysis to facilitate comparison of results with other components of the monitoring program. Several components of the Maryland program as well as the Virginia Benthic Monitoring Program did not start sampling until 1985. Thirty seven-year (1985-2021) trends are presented for 23 of the 27 trend sites, 33-year trends are presented for two sites in Baltimore Harbor (Stations 201 and 202) first sampled in 1989, and 27-year trends are presented for two western shore tributaries (Back River Station 203, and Severn River Station 204) first sampled in 1995. Trend site locations are shown in Figure 2-1.

Statistically significant B-IBI trends (10% significance level) were detected at 17 of the 27 sites (Table 3-1). If a 5% significance level is chosen, the number of statistically significant B-IBI trends is 13. The 10% level is kept for consistency with previous reports. One trend was new with the addition of the 2021 data. Trends in benthic community condition declined at 12 sites (significantly decreasing B-IBI score) and improved at 5 sites (significantly increasing B-IBI score). Except for the new trend (declining), the direction of the trends did not change over the direction reported for 2020.

Sites with improving condition (Table 3-1) were located in the upper Bay mainstem (Station 026), Elk River (Station 029), mesohaline Choptank River (Station 064), Bear Creek (Station 201), and Back River (Station 203). Sites with declining condition (Table 3-1) were located in the Mid Bay Mainstem at Calvert Cliffs (Station 001), Baltimore Harbor (Station 022), tidal freshwater Potomac River (Station 036), mesohaline Potomac River at

Morgantown (Stations 043 and 047), deep mesohaline Potomac River at St. Clements Island (Station 052), Nanticoke River (Station 062), oligohaline Choptank River (Station 066), Patuxent River at Broomes Island (Station 071), Patuxent River at Holland Cliff (Station 077), Curtis Bay (Station 202), and Severn River (Station 204).

The only change in 2021 from 2020 results was the appearance of a declining B-IBI trend in Curtis Bay (Station 202). Using the last three years of data (2019-2021), the average B-IBI score remained within the same condition category at most sites, improved at 2 sites from failing the goals to meeting the goals (Potomac River Station 040 and Patuxent River Station 074), and declined at 5 sites from meeting the goals to failing the goals (Potomac River Stations 044 and 047, and Patuxent River Station 079), from marginal to degraded condition (Back River Station 203), and from degraded to severely degraded condition (Mid Bay Mainstem Station 015) (Table 3-1 shaded areas). Currently, 6 sites meet the benthic community restoration goals and 21 sites fail the goals.

Trends in community attributes that are components of the B-IBI are presented in Table 3-2 (mesohaline stations), Table 3-3 (oligohaline and tidal freshwater stations), and Appendix A. Sites with decreasing B-IBI trends had decreasing trends (below restorative thresholds) in abundance, biomass, or both, and usually in several other components of the B-IBI such as Shannon diversity and abundance or biomass of pollution sensitive species (Tables 3-2 and 3-3). The tidal fresh Potomac River (Station 036), Nanticoke River (Station 062), and oligohaline Choptank River (Station 066) had increasing trends in abundance but these trends indicated degrading conditions due to excess abundance relative to thresholds. The mesohaline Potomac River at Morgantown (Stations 043 and 047) had decreasing trends in abundance that indicated improving conditions from excess abundance.

Several sites without B-IBI trends also exhibited statistically significant, degrading trends in abundance, biomass, Shannon diversity, or (not shown in Table 3-2) numbers of species.

Figures 3-1 through 3-27 show patterns in abundance, biomass, number of species, and B-IBI at the fixed sites. A general pattern of declining trends has remained unchanged in the last few years of the monitoring record. Using the Mann-Kendall test, 12 sites had significant declining trends in abundance, 10 sites had significant declining trends in biomass, and 14 sites had significant declining trends in numbers of species. These sites tended to be in the mesohaline region of the estuary. Patterns in this region of the estuary also revealed overall lower abundance during the 1998-2021 period than during the 1984-1997 period.

When the data are examined in relation to the metric thresholds, some of the decreases in abundance over time were from values above the upper threshold for abundance (indicating benthic community degradation) to values within the good range for abundance. These changes reflected improvements in benthic condition. Declining

trends in abundance below the upper threshold were statistically significant in the shallow mesohaline region of the Potomac River (Stations 043 and 047), and in the Elk River (Station 029). Conversely, four sites had significant increasing trends in abundance in the direction of excess abundance (degrading). These sites were located in the tidal freshwater (Station 036) and oligohaline (Station 040) region of the Potomac River, in the Nanticoke River (Station 062), and in the oligohaline portion of the Choptank River (Station 066).

In 2021 eleven fixed sites showed increases in the B-IBI score and few sites showed decreases (Figures 3-1 to 3-27). Increased B-IBI scores were most pronounced in the mid-Bay mainstem (Stations 001 and 024), the Patapsco River (Stations 022 and 023), the Severn River (Station 204) and the lower mesohaline portion of the Choptank River, suggesting better water quality in 2021 than in 2020 in these regions of the Bay. The upper oligohaline portion of the Choptank River, however, showed a strong decline in the B-IBI.

The tidal freshwater Potomac River (Station 036) showed an increasing trend in abundance above the upper threshold (excess abundance), a decreasing trend in biomass, and a declining B-IBI (Figure 3-9). Benthic community at this site is numerically dominated by tubificid oligochaete worms, which account for most of the biomass (Figure 3-28). The benthic community was previously dominated by the bivalve *Corbicula fluminea*, but the abundance of this bivalve decreased from 4,500 individuals per m² in 1984 to zero in 2021 (Figure 3-28). During the period 2017-2019 *Corbicula* was found in low densities after years of absence in the samples. The sharp decline over time of *Corbicula* in the Potomac River may be related to patchiness, the normal post-invasion population decline of introduced species, or a reduction in the algal biomass on which the clams feed, through improving water quality conditions in the river. With this decline, *Corbicula fluminea* is no longer a biomass-dominant component of the benthic community in the tidal freshwater Potomac River.

Table 3-1. Summer trends in benthic community condition, 1985-2021. Trends were identified using the van Belle and Hughes (1984) procedure. Current mean B-IBI and condition are based on 2019-2021 values. Initial mean B-IBI and condition are based on 1985-1987 values, except where noted. NS: not significant; (a): 1989-1991 initial condition; (b): 1995-1997 initial condition. Shaded areas highlight changes in condition or trend direction over those reported for 2020 (light gray = better; deep gray = worse).

Station	Trend Significance	Median Slope (B-IBI units/yr)	Current Condition (2019-2021)	Initial Condition (1985-1987 unless otherwise noted)
Potomac River				
036	p < 0.001	-0.03	2.78 (Marginal)	3.14 (Meets Goal)
040	NS	0.00	3.07 (Meets Goal)	2.80 (Marginal)
043	p < 0.001	-0.03	2.51 (Degraded)	3.76 (Meets Goal)
044	NS	0.00	2.47 (Degraded)	2.80 (Marginal)
047	p < 0.01	-0.00	2.91 (Marginal)	3.89 (Meets Goal)
051	NS	0.00	2.52 (Degraded)	2.43 (Degraded)
052	p < 0.05	-0.00	1.11 (Severely Degraded)	1.37 (Severely Degraded)
Patuxent River				
071	p < 0.001	-0.03	1.26 (Severely Degraded)	2.52 (Degraded)
074	NS	0.00	3.31 (Meets Goal)	3.78 (Meets Goal)
077	p < 0.01	-0.02	2.47 (Degraded)	3.76 (Meets Goal)
079	NS	0.00	2.83 (Marginal)	2.75 (Marginal)
Choptank River				
064	p < 0.05	+0.01	3.15 (Meets Goal)	2.78 (Marginal)
066	p < 0.001	-0.02	2.22 (Degraded)	2.60 (Degraded)
Maryland Mainstem				
001	p < 0.10	-0.00	2.30 (Degraded)	2.93 (Marginal)
006	NS	0.00	2.11 (Degraded)	2.56 (Degraded)
015	NS	0.00	1.93 (Severely Degraded)	2.22 (Degraded)
024	NS	0.00	3.04 (Meets Goal)	3.04 (Meets Goal)
026	p < 0.05	+0.00	3.58 (Meets Goal)	3.16 (Meets Goal)
Maryland Western Shore Tributaries				
022	p < 0.001	-0.02	1.89 (Severely Degraded)	2.08 (Degraded)
023	NS	0.00	2.07 (Degraded)	2.49 (Degraded)
201	p < 0.10	+0.00	1.27 (Severely Degraded)	1.10 (Severely Degraded) (a)
202	p < 0.10	-0.00	1.13 (Severely Degraded)	1.40 (Severely Degraded) (a)
203	p < 0.001	+0.04	2.63 (Degraded)	2.08 (Degraded) (b)
204	p < 0.10	-0.02	2.48 (Degraded)	3.67 (Meets Goal) (b)
Maryland Eastern Shore Tributaries				
029	p < 0.05	+0.01	2.88 (Marginal)	2.38 (Degraded)
062	p < 0.001	-0.05	1.49 (Severely Degraded)	3.42 (Meets Goal)
068	NS	0.00	3.58 (Meets Goal)	3.51 (Meets Goal)

Table 3-2. Summer trends in benthic community attributes at mesohaline stations 1985-2021. Monotonic trends were identified using the van Belle and Hughes (1984) procedure. ↑: Increasing trend; ↓: Decreasing trend. *: $p < 0.1$; **: $p < 0.05$; ***: $p < 0.01$; shaded trend cells indicate increasing degradation; unshaded trend cells indicate unchanging or improving conditions; (a): trends based on 1989-2021 data; (b): trends based on 1995-2021 data; (c): attribute trend based on 1990-2021 data; (d): attributes are used in B-IBI calculations when species specific biomass is unavailable; NA: attribute is not part of the reported B-IBI. Blanks indicate no trend (not significant). See Appendix A for further detail.

Station	B-IBI	Abundance	Biomass	Shannon Diversity	Indicative Abundance	Sensitive Abundance	Indicative Biomass (c)	Sensitive Biomass (c)	Abundance Carnivore/Omnivores
Potomac River									
043	↓ ***	↓ ***	↓ ***	↓ **	↑ ***	↓ *** (d)	NA	↓ ***	NA
044					↓ ***	(d)	NA		NA
047	↓ ***	↓ ***	↓ ***	↓ **	↑ ***	↓ *** (d)	NA	↓ ***	NA
051		↓ ***	↓ ***		↓ ***		NA	↓ ***	↑ ***
052	↓ **	↓ ***	↓ ***	↓ ***	(d)	↓ * (d)			↓ **
Patuxent River									
071	↓ ***	↓ ***	↓ ***	↓ ***	(d)	↓ *** (d)	↑ *		
074			↓ ***		↑ *	↓ *** (d)	NA	↓ ***	NA
077	↓ ***		↓ ***		↑ ***	(d)	NA	↑ *	NA
Choptank River									
064	↑ **	↓ *	↑ ***		↓ *(d)	↑ *** (d)			
Maryland Mainstem									
001	↓ *	↓ ***			↓ *		NA	NA	↓ ***
006						↓ **	NA	NA	↓ ***
015				↓ **	↓ **		NA	NA	
024			↑ ***	↓ ***	↓ *** (d)	↑ *** (d)		↑ ***	
026	↑ **					(d)	NA	↓ **	NA
Maryland Western Shore Tributaries									
022	↓ ***	↓ ***		↓ ***	↑ ***	(d)	NA	↓ *	NA
023		↓ ***		↓ ***		↑ *** (d)	NA		NA
201(a)	↑ *					(d)	NA		NA
202(a)	↓ *	↓ ***		↓ **		(d)	NA		NA
204(b)	↓ *	↓ ***			↑ ** (d)	(d)			
Maryland Eastern Shore Tributaries									
062	↓ ***	↑ ***	↓ ***	↓ ***	↑ ***	↓ *** (d)	NA	↓ ***	NA
068			↑ ***	↓ **		(d)	NA		NA

Table 3-3. Summer trends in benthic community attributes at oligohaline and tidal freshwater stations 1985-2021. Monotonic trends were identified using the van Belle and Hughes (1984) procedure. ↑: Increasing trend; ↓: Decreasing trend. *: p<0.1; **: p<0.05; ***: p<0.01; shaded trend cells indicate increasing degradation; unshaded trend cells indicate unchanging or improving conditions; (a): trends based on 1995-2021 data; NA: attribute not calculated. Blanks indicate no trend (not significant). See Appendix A for further detail.

Station	B-IBI	Abundance	Tolerance Score	Freshwater Indicative Abundance	Oligohaline Indicative Abundance	Oligohaline Sensitive Abundance	Tanypodinae to Chironomidae Ratio	Abundance Deep Deposit Feeders	Abundance Carnivore/Omnivores
Potomac River									
036	↓ ***	↑ ***	↑ ***	↑ **	NA	NA	NA	↑ ***	NA
040		↑ **		NA			↓ **	NA	
Patuxent River									
079			↓ *		NA	NA	NA		NA
Choptank River									
066	↓ ***	↑ ***	↑ ***	NA	↑ ***		↑ **	NA	
Maryland Western Shore Tributaries									
203(a)	↑ ***			NA				NA	↑ ***
Maryland Eastern Shore Tributaries									
029	↑ **	↓ **		NA				NA	↑ ***

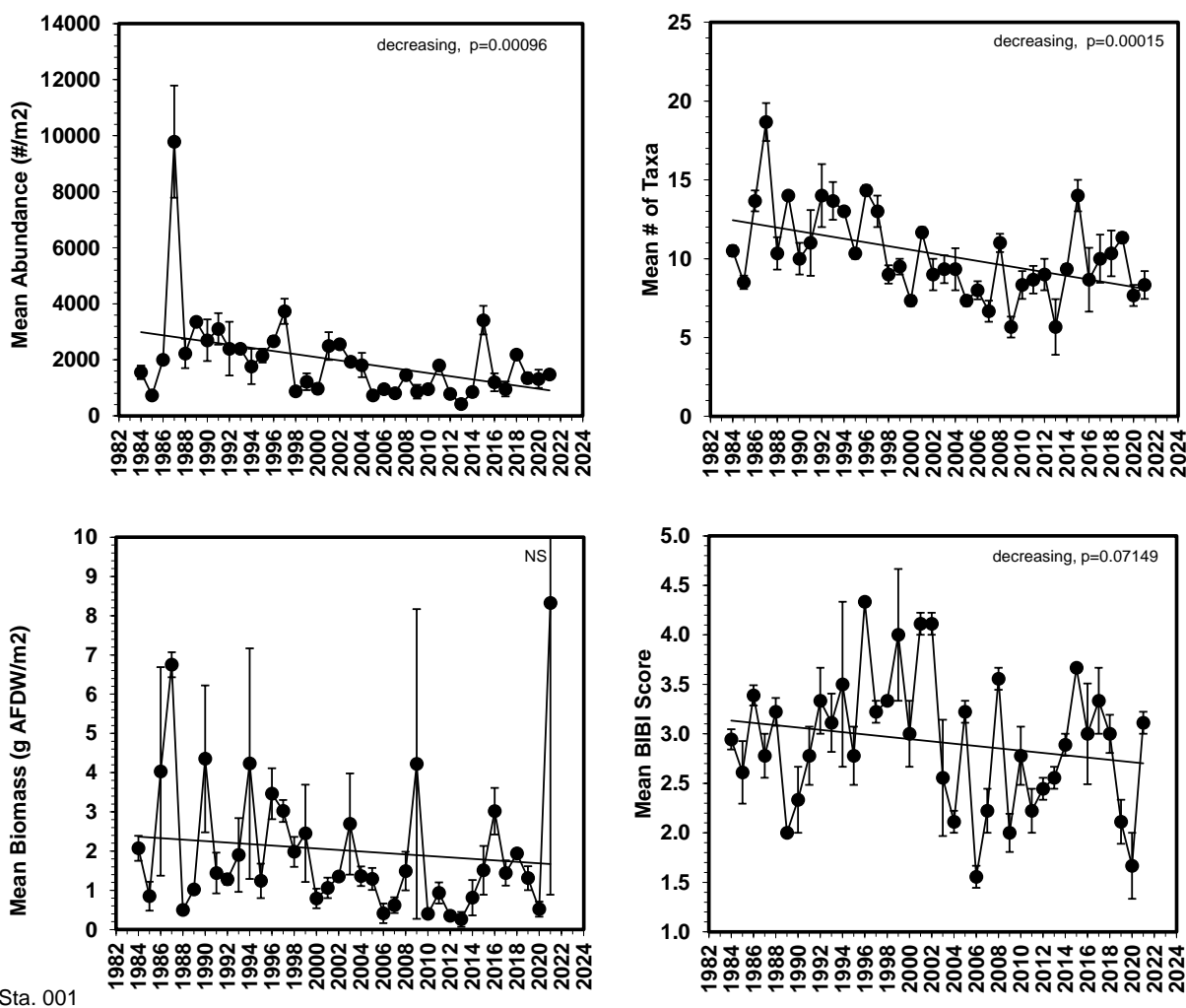


Figure 3-1. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 001 = Chesapeake Bay mainstem (≤ 5 m) at Calvert Cliffs.

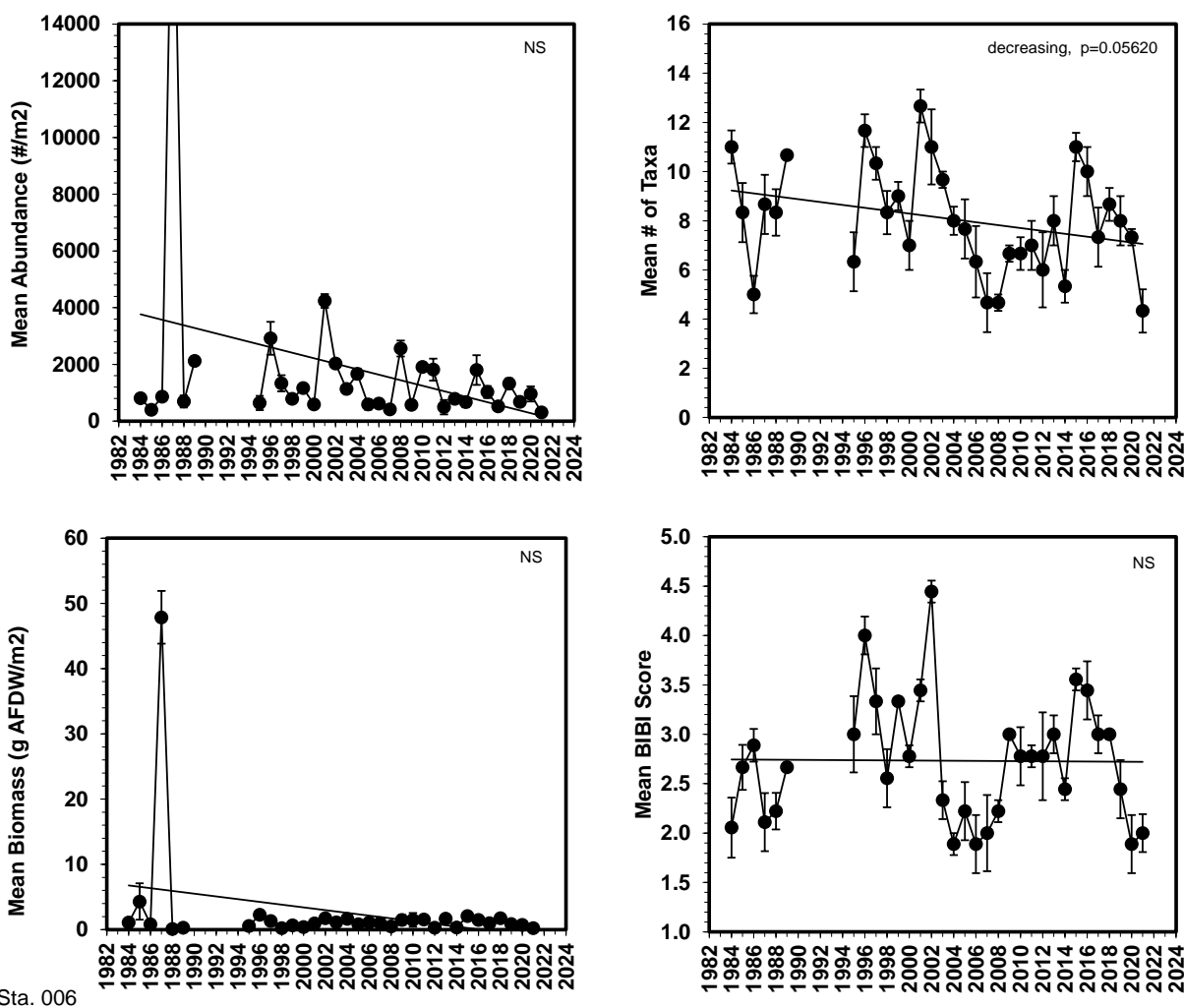


Figure 3-2. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 006 = Chesapeake mainstem (\leq 5 m) at Calvert Cliffs. Data gaps indicate periods where sampling was suspended because of program design changes

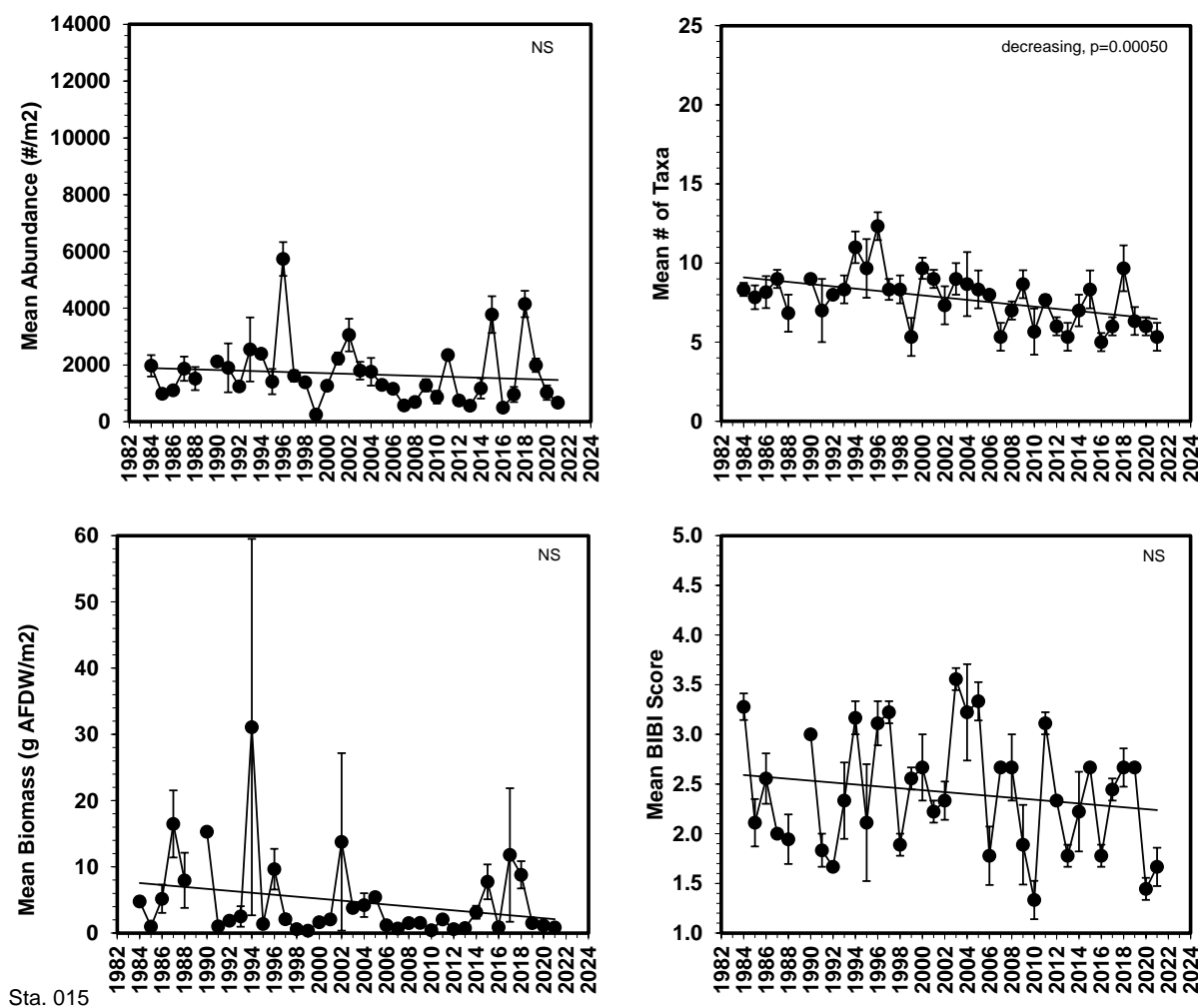


Figure 3-3. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 015 = Chesapeake mainstem (\leq 5 m), North Beach. Data gaps indicate periods where sampling was suspended because of program design changes

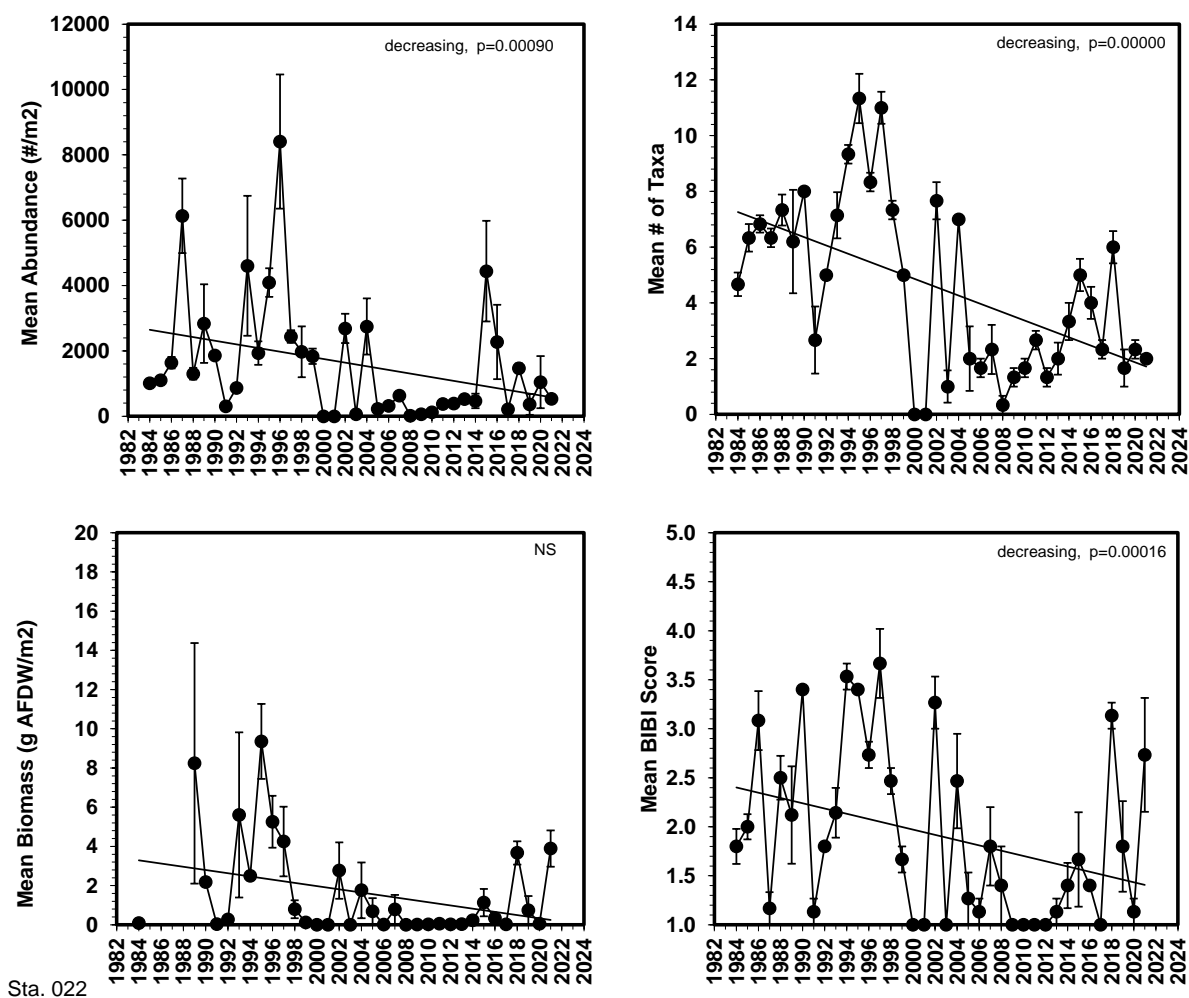


Figure 3-4. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 022 = Patapsco River estuary (2-6 m), Middle Branch. Data gaps indicate periods where sampling was suspended because of program design changes

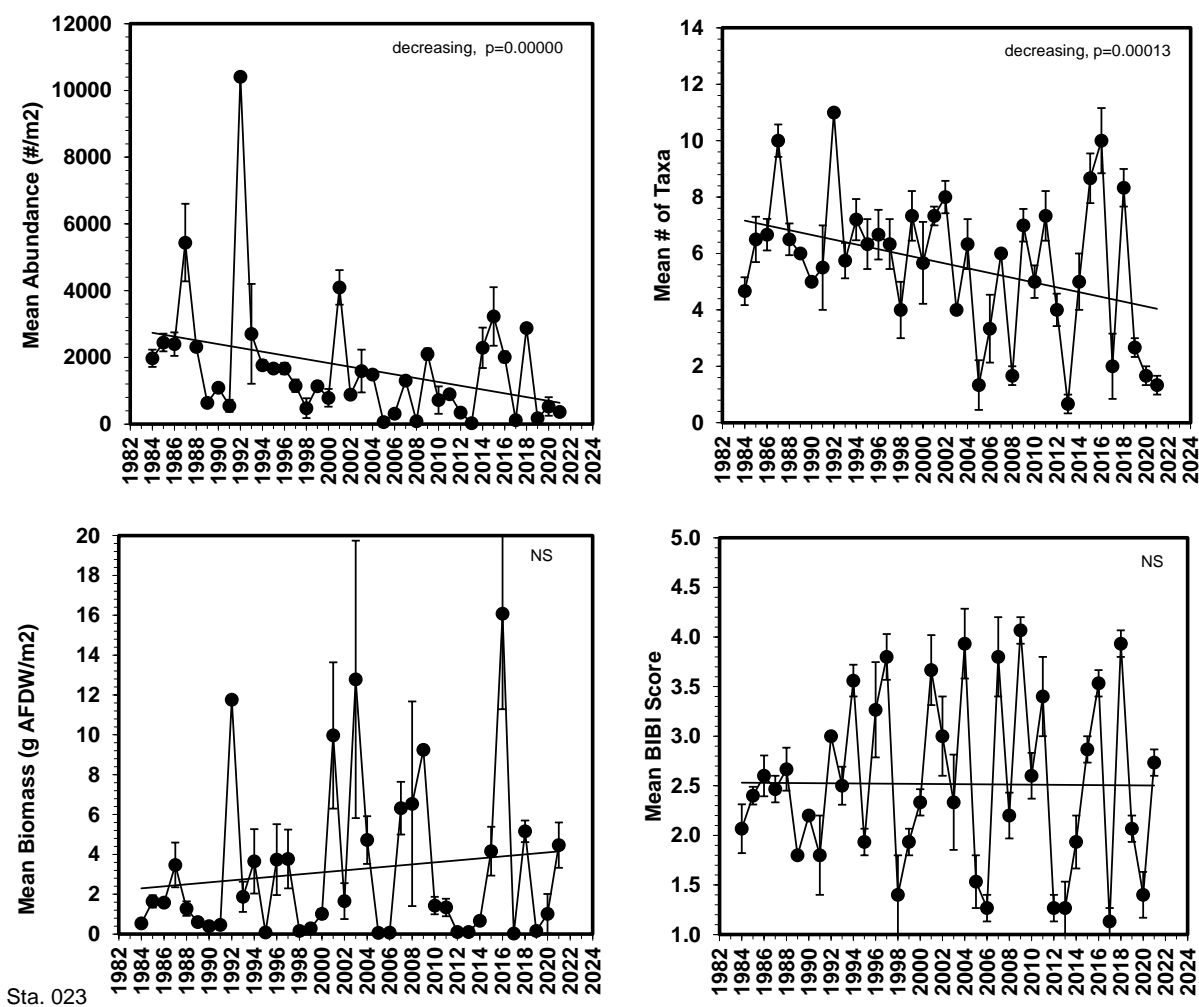


Figure 3-5. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 023 = Patapsco River estuary (4-7 m), lower mainstem

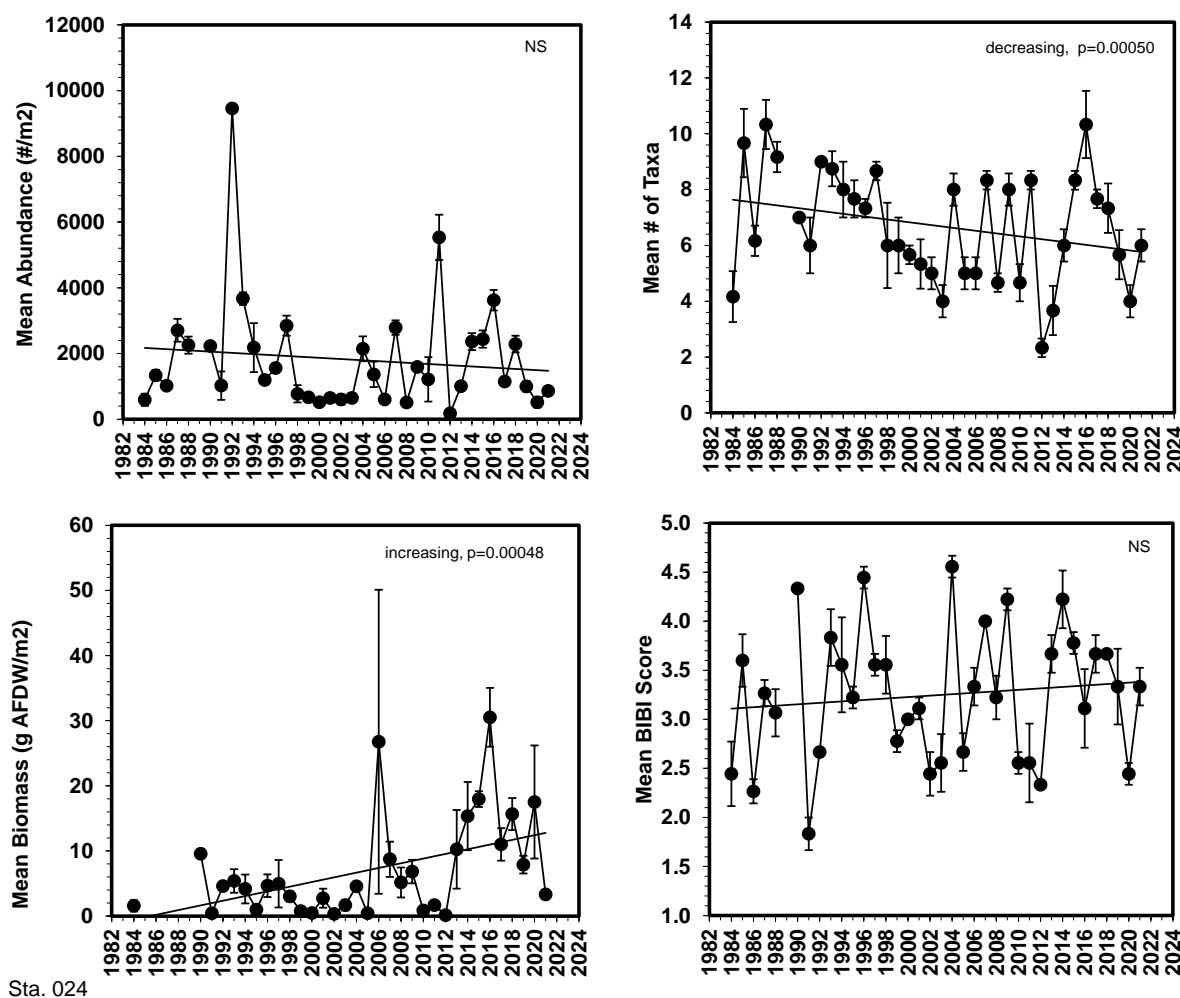


Figure 3-6. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 024 = Chesapeake Bay mainstem (5-8 m), near the mouth of the Patapsco River. Data gaps indicate periods where sampling was suspended because of program design changes

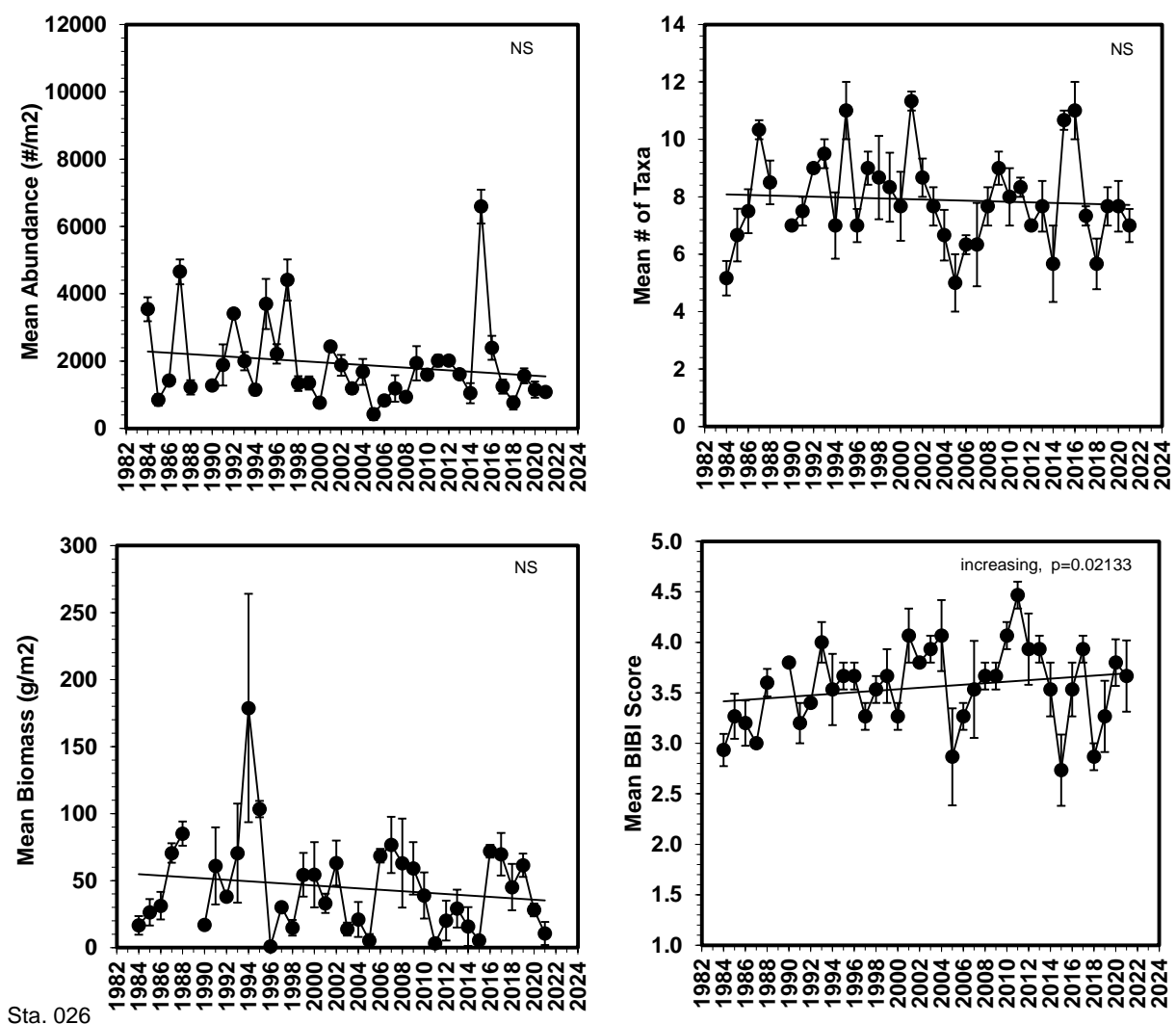


Figure 3-7. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 026 = Chesapeake Bay mainstem (2-5 m), Pooles Island. Data gaps indicate periods where sampling was suspended because of program design changes

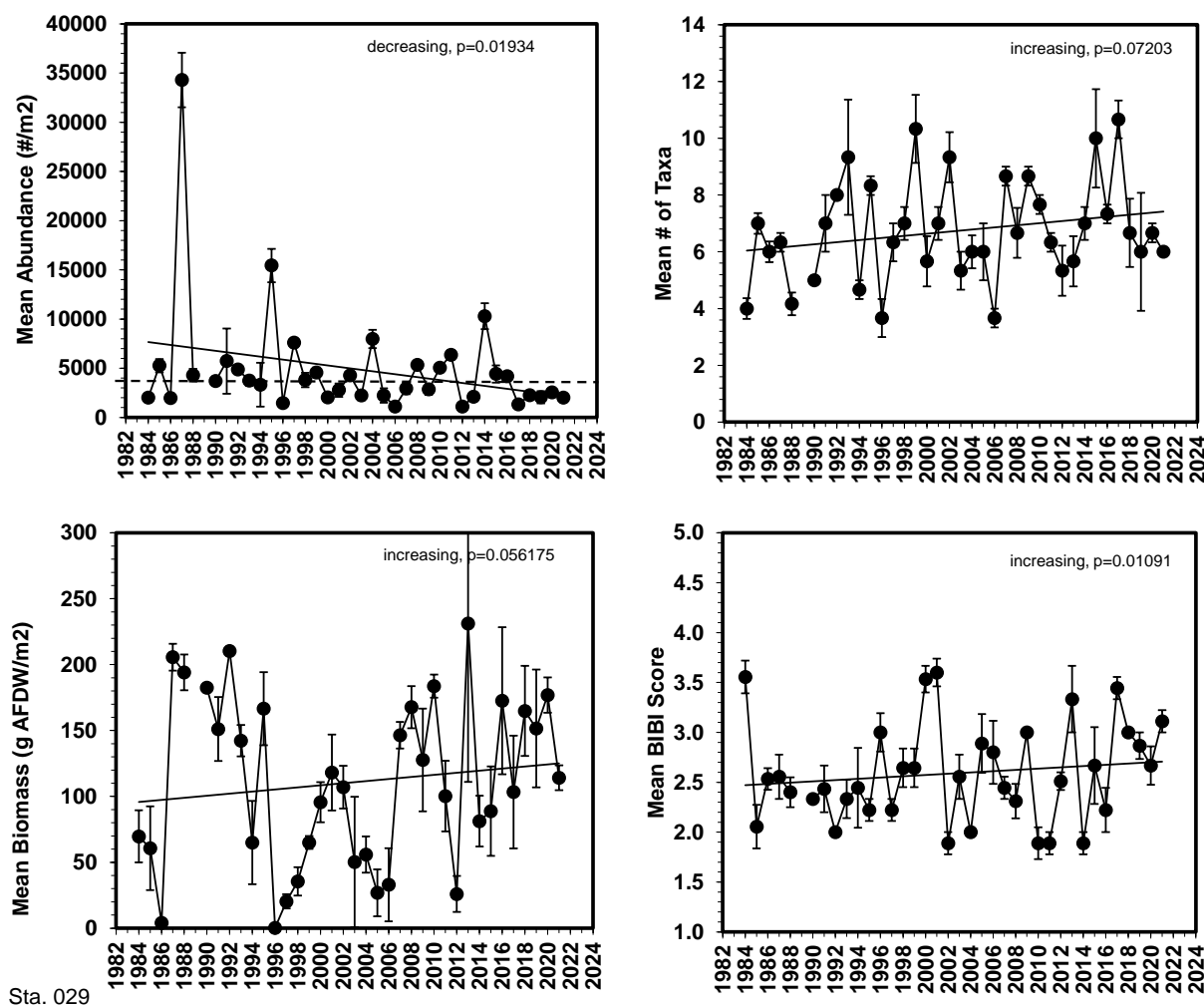


Figure 3-8. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 029 = Elk River. The dashed line in the abundance plot is the upper B-IBI threshold (scored as 1) for abundance. Data gaps indicate periods where sampling was suspended because of program design changes

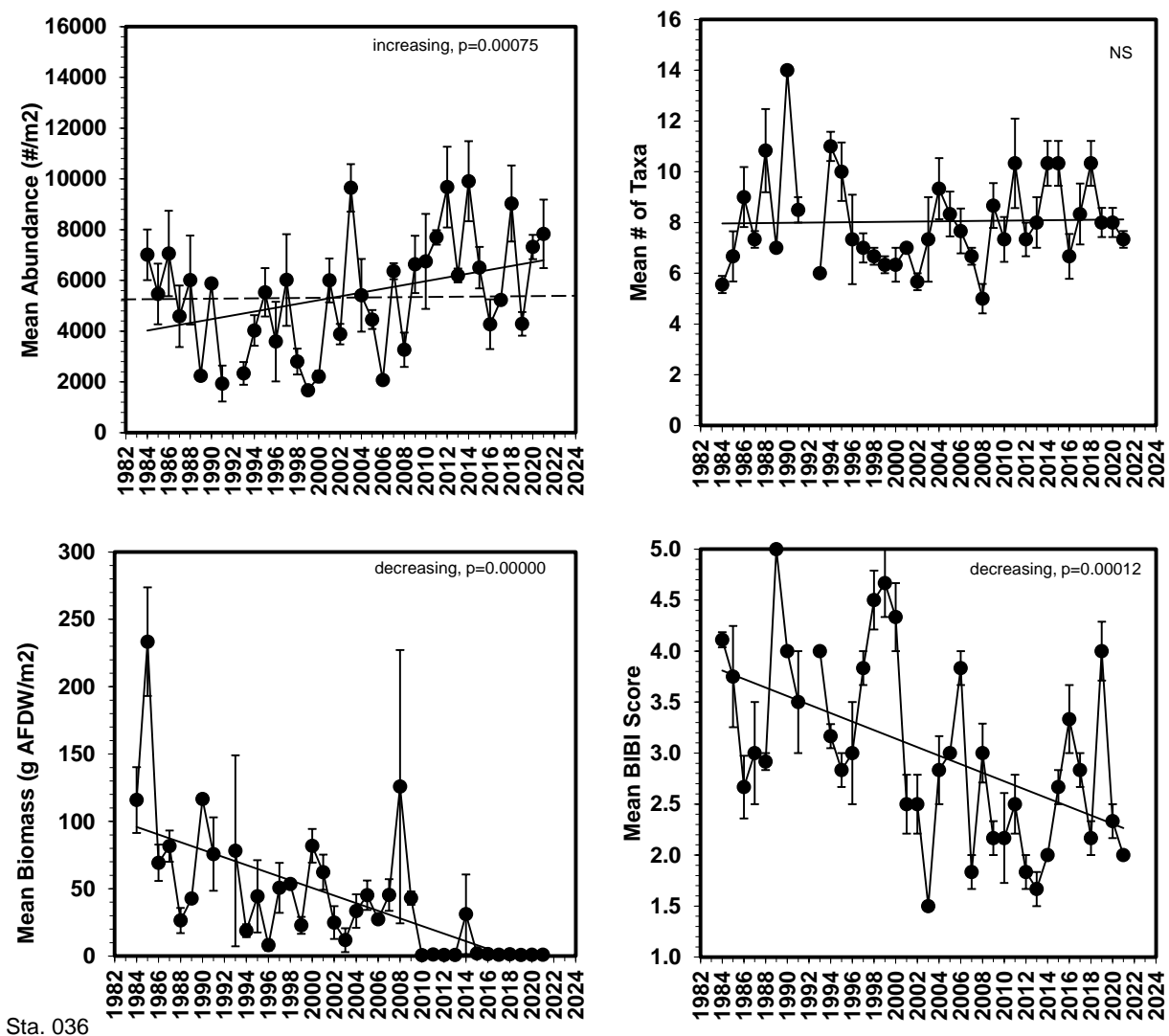


Figure 3-9. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 036 = Tidal freshwater Potomac River (≤ 5 m) at Rosier Bluff. The dashed line in the abundance plot is the upper B-IBI threshold (scored as 1) for abundance. Data gaps indicate periods where sampling was suspended because of program design changes

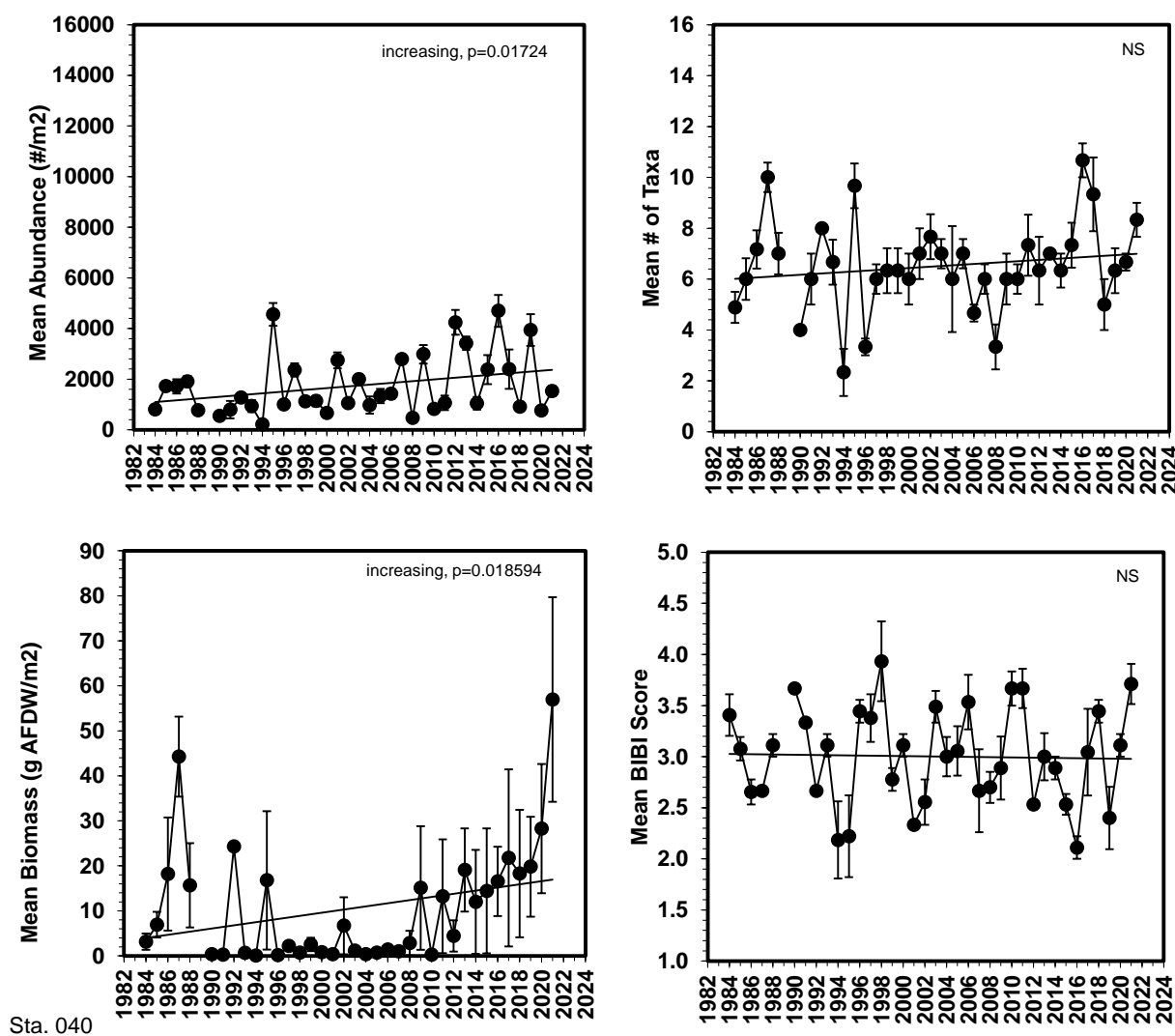


Figure 3-10. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 040 = Oligohaline Potomac River (6-10 m) at Maryland Point. Data gaps indicate periods where sampling was suspended because of program design changes

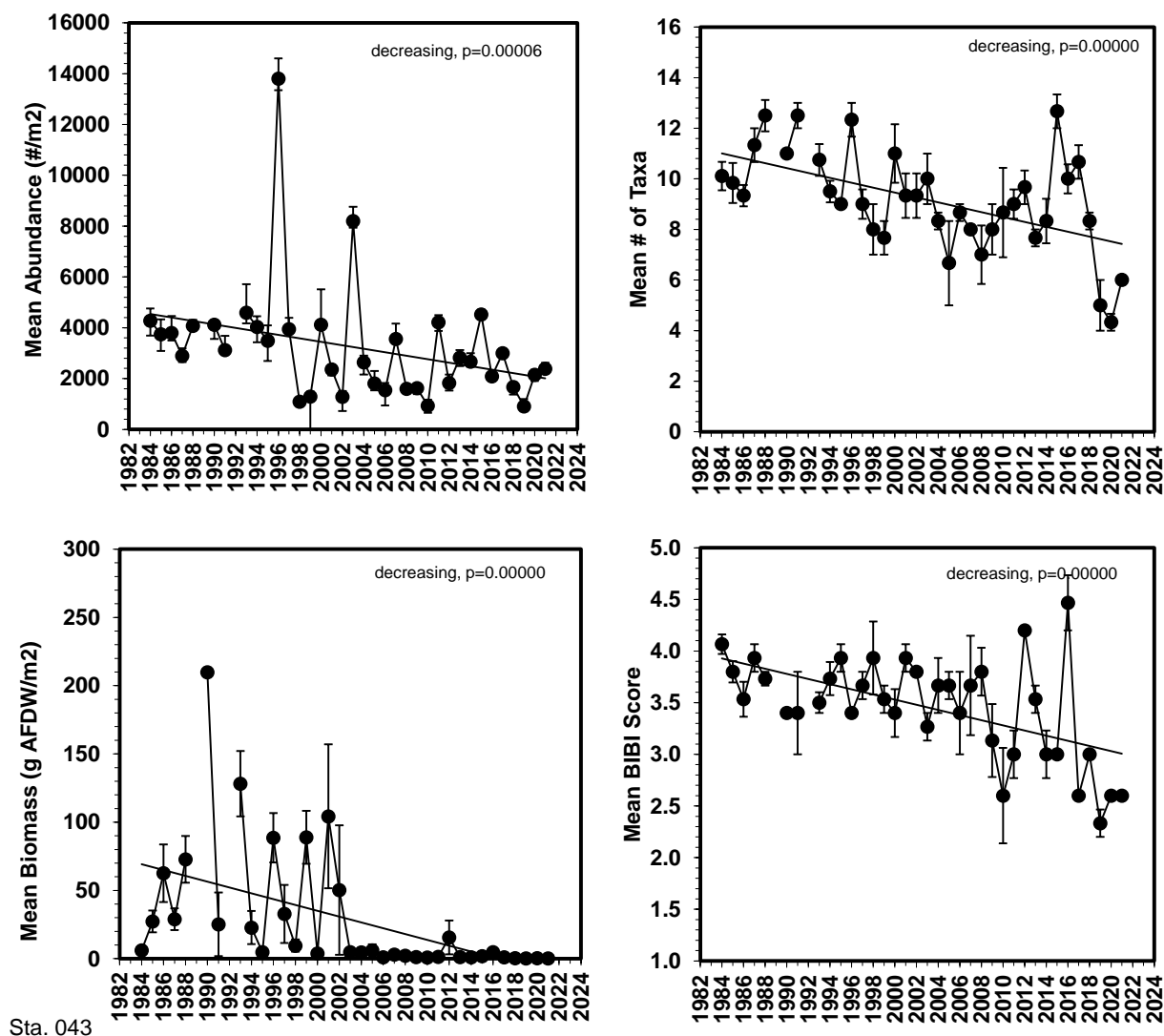
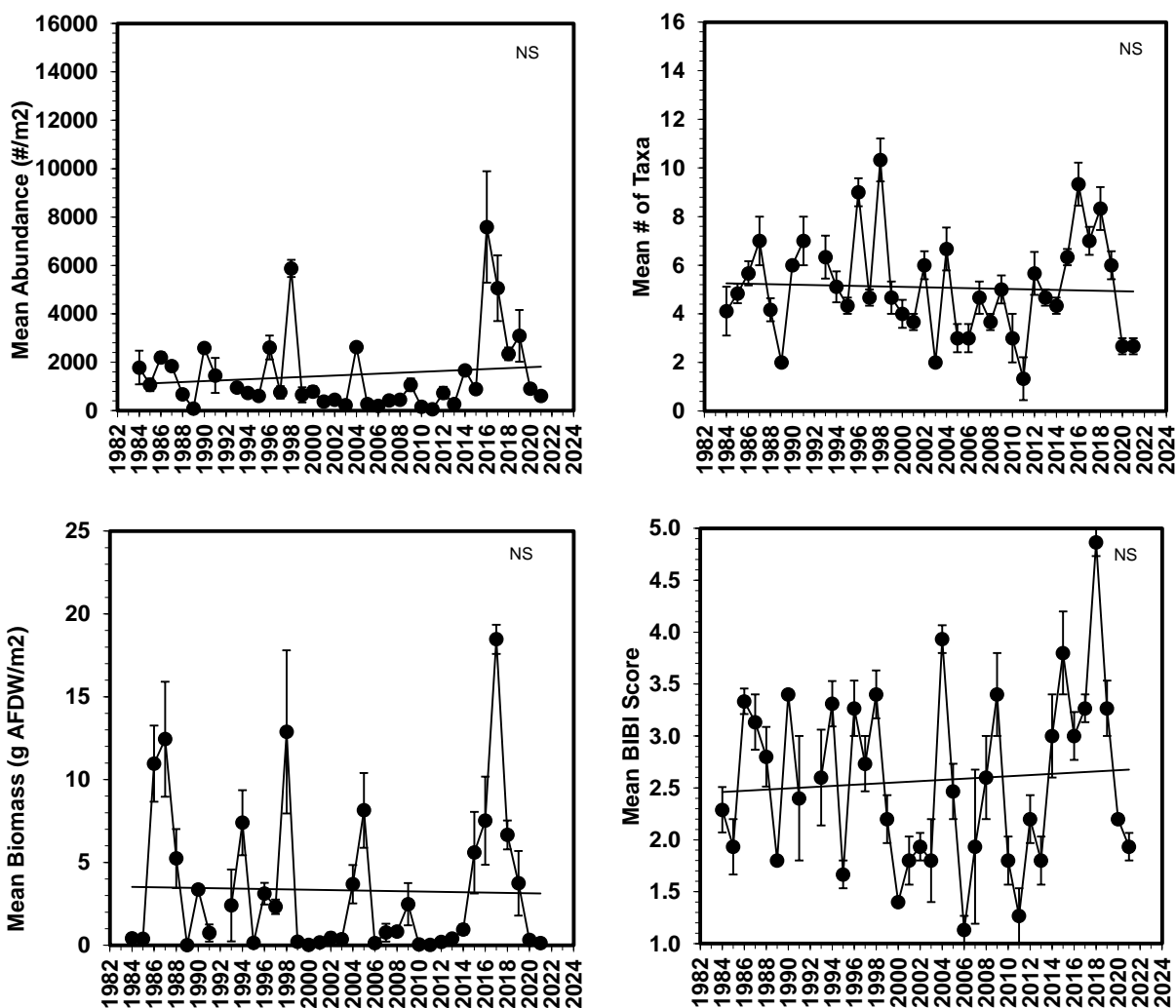


Figure 3-11. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 043 = Shallow mesohaline Potomac River (\leq 5 m) at Morgantown. Data gaps indicate periods where sampling was suspended because of program design changes



Sta. 044

Figure 3-12. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 044 = Deep mesohaline Potomac River (11-17 m) at Morgantown. Data gaps indicate periods where sampling was suspended because of program design changes

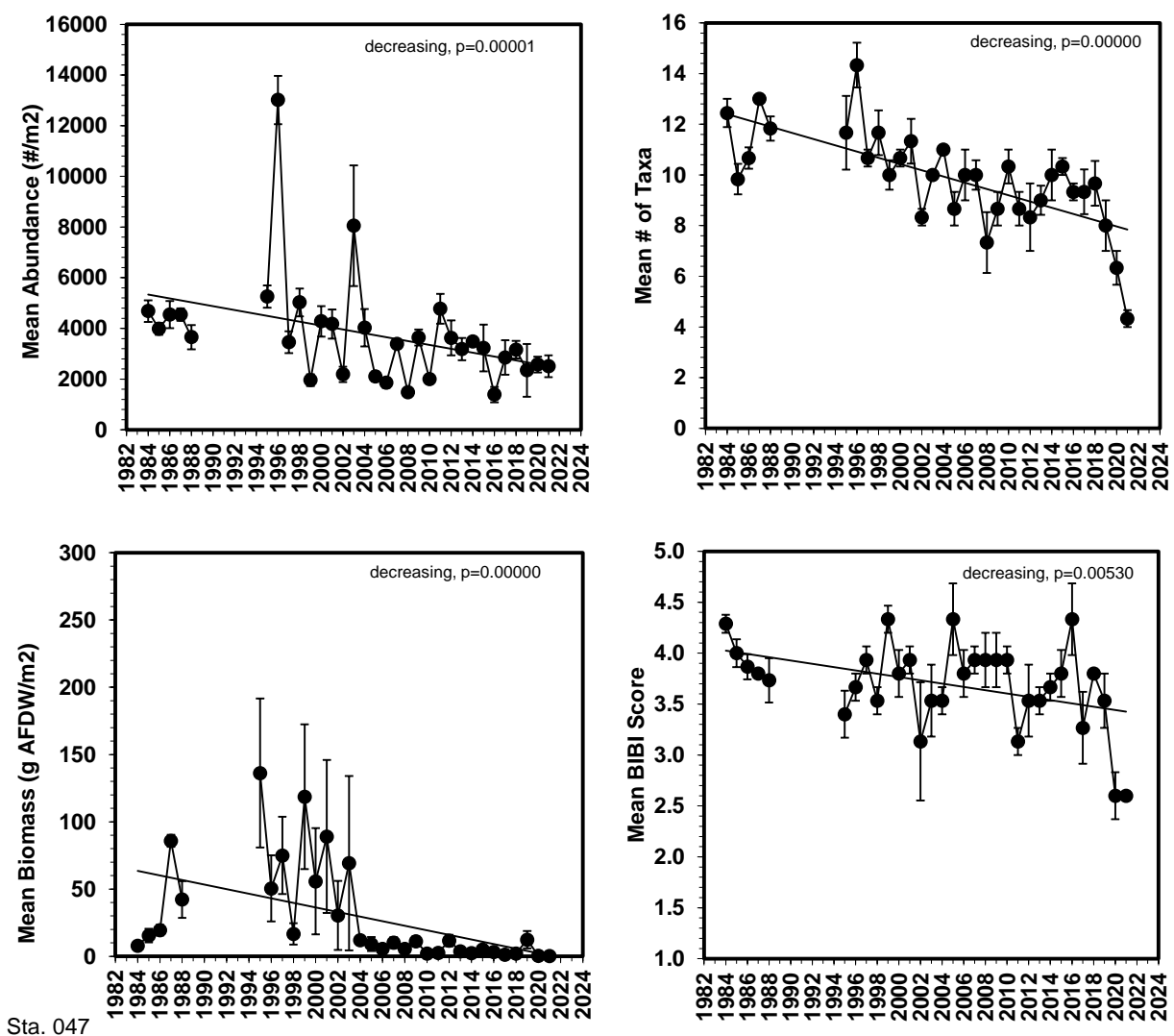


Figure 3-13. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 047 = Shallow mesohaline Potomac River (\leq 5 m) at Morgantown. Data gaps indicate periods where sampling was suspended because of program design changes

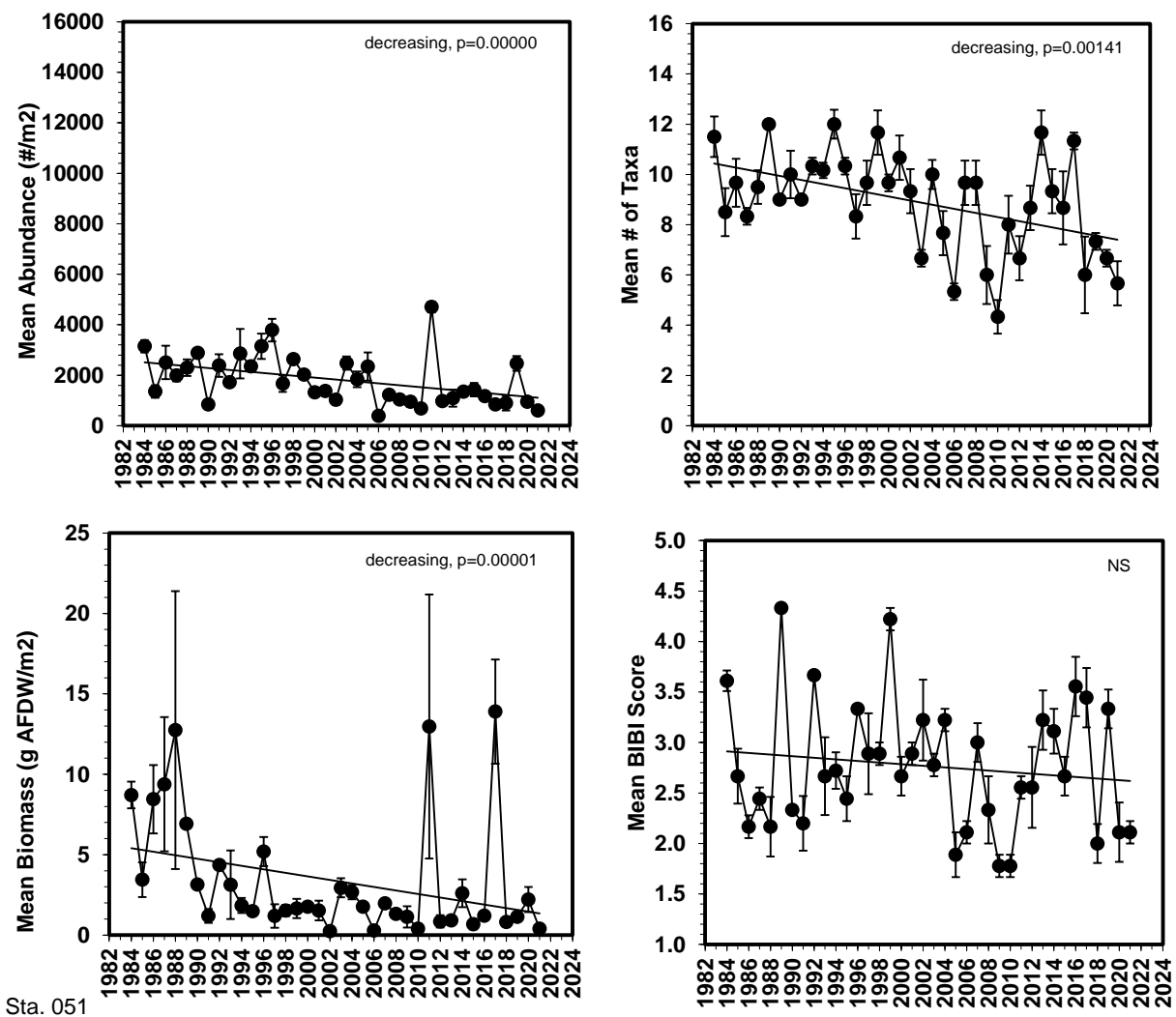


Figure 3-14. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 051 = Shallow mesohaline Potomac River (≤ 5 m), St. Clements Island

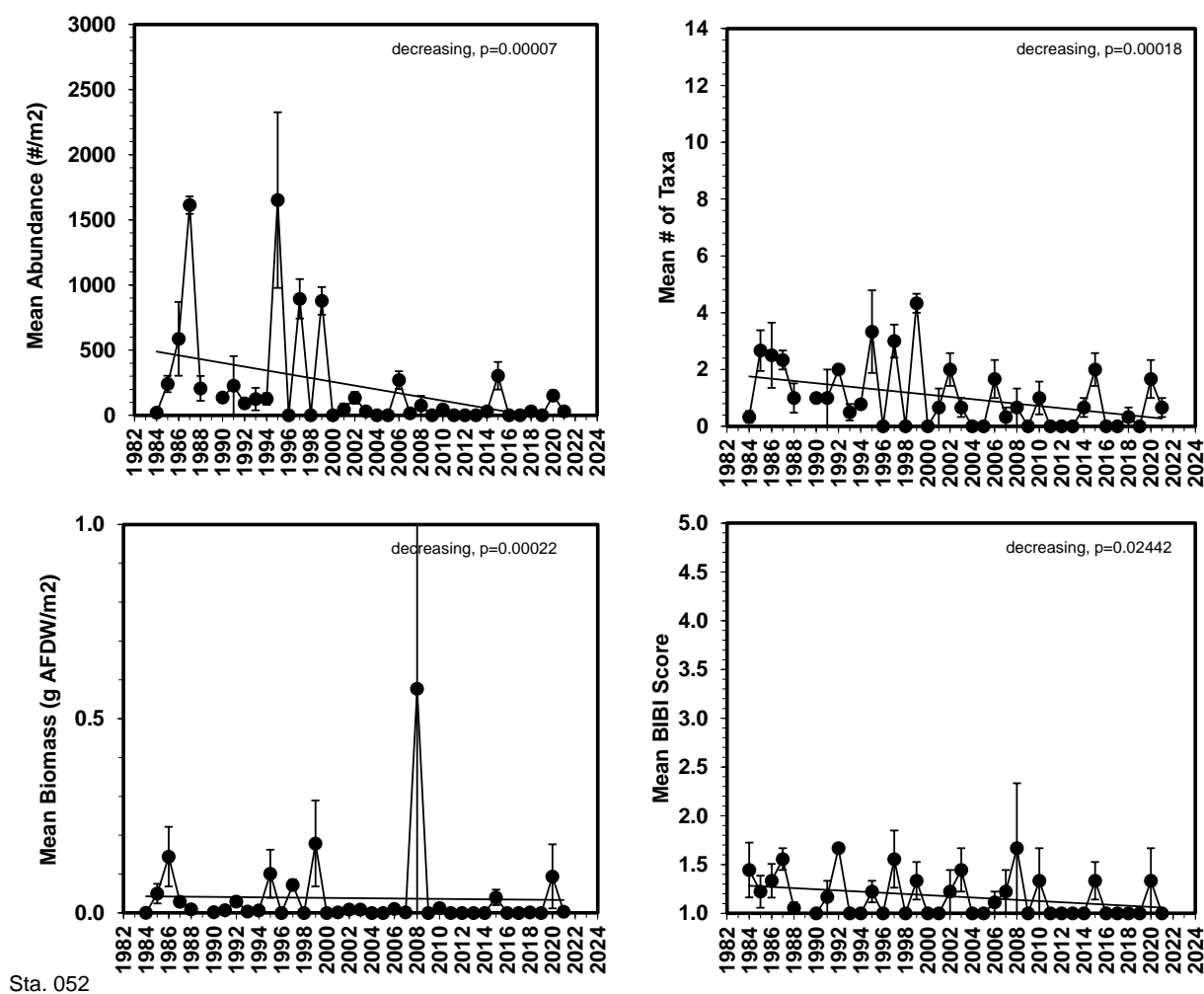


Figure 3-15. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 052 = Deep mesohaline Potomac River (9-13 m), St. Clements Island. Data gaps indicate periods where sampling was suspended because of program design changes

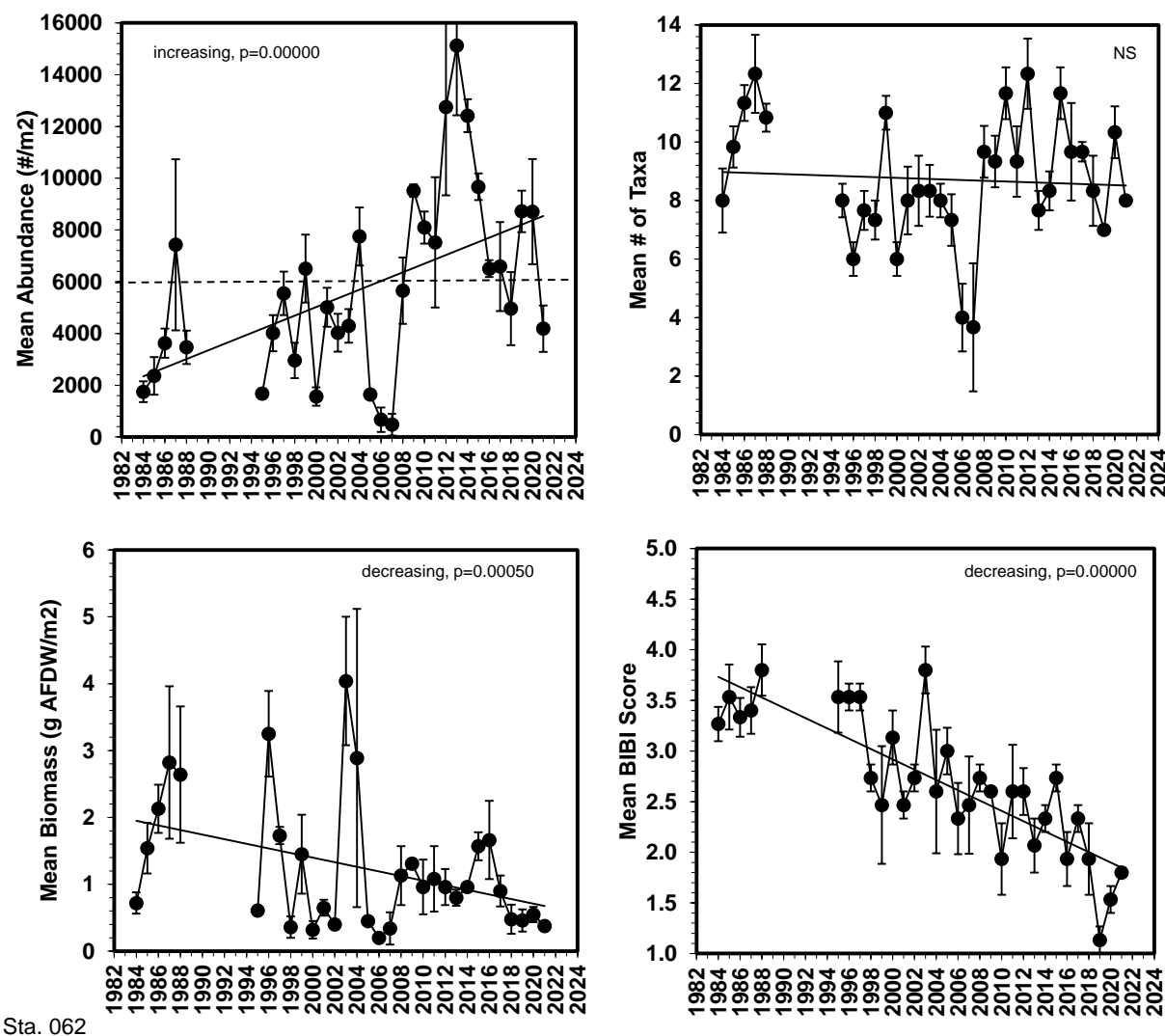


Figure 3-16. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 062 = Nanticoke River. The dashed line in the abundance plot is the upper B-IBI threshold (scored as 1) for abundance. Data gaps indicate periods where sampling was suspended because of program design changes

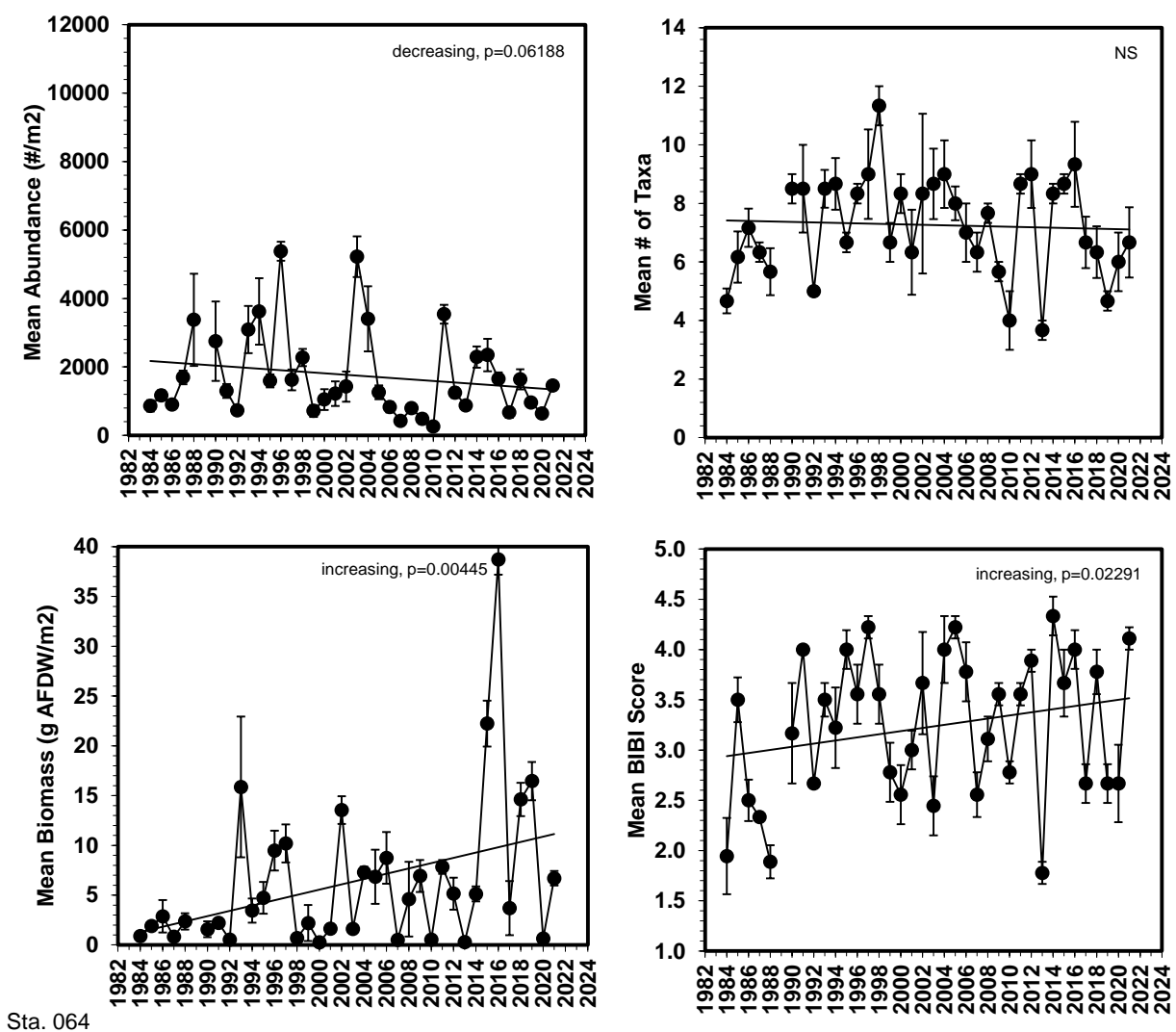


Figure 3-17. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 064 = Mesohaline Choptank River. Data gaps indicate periods where sampling was suspended because of program design changes

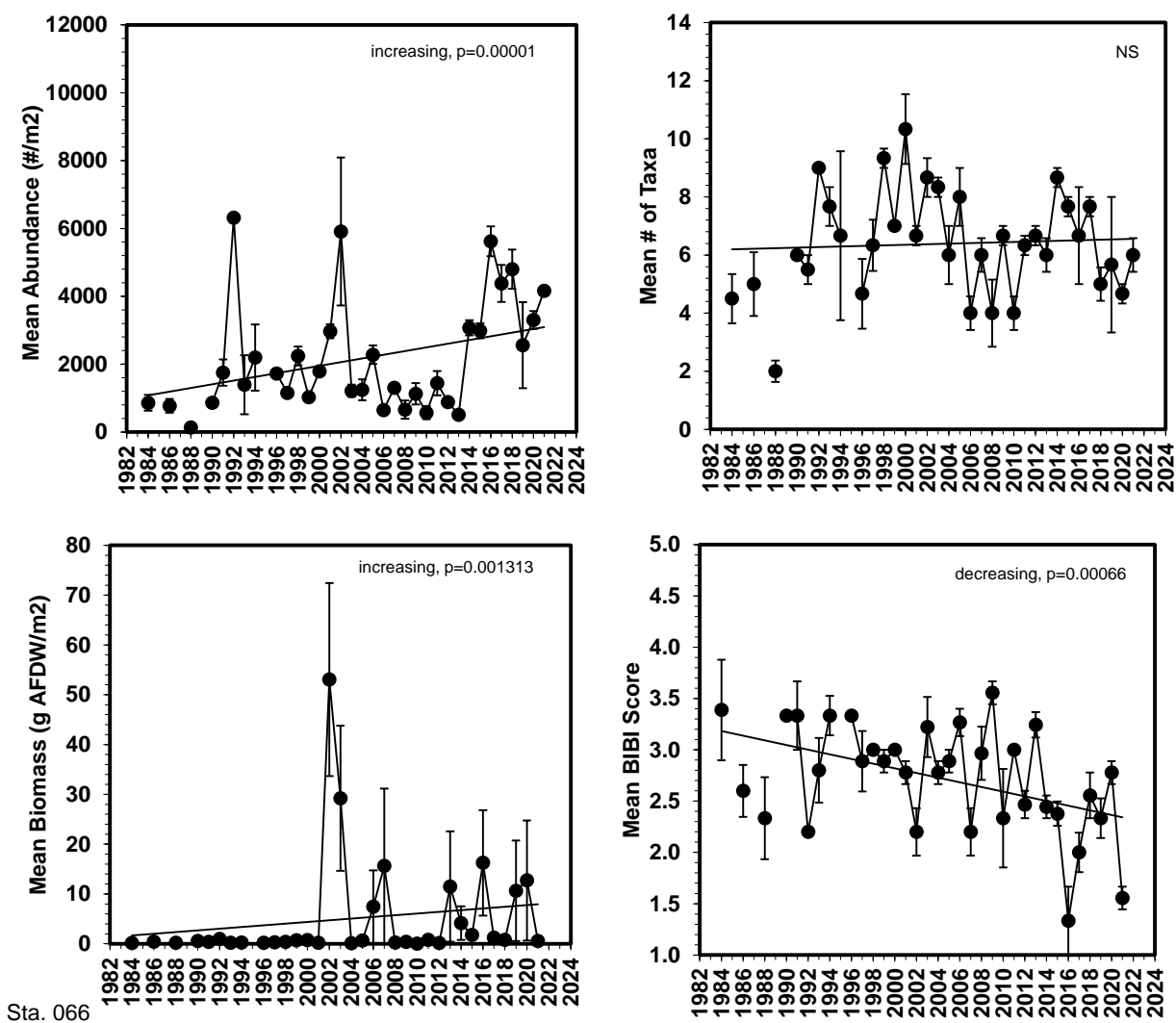


Figure 3-18. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 066 = Oligohaline Choptank River. Data gaps indicate periods where sampling was suspended because of program design changes

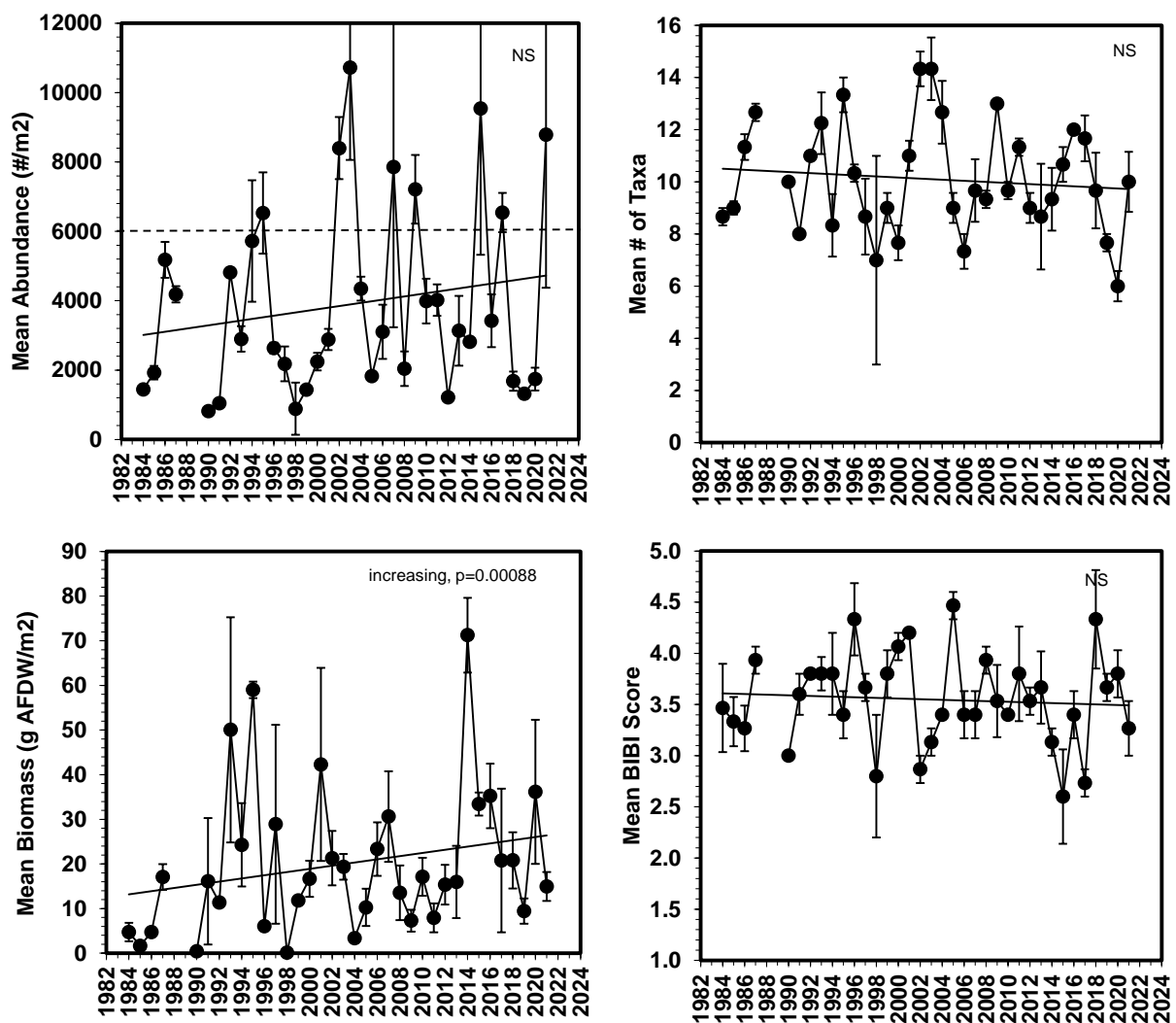


Figure 3-19. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 068 = Chester River. The dashed line in the abundance plot is the upper B-IBI threshold (scored as 1) for abundance. Data gaps indicate periods where sampling was suspended because of program design changes

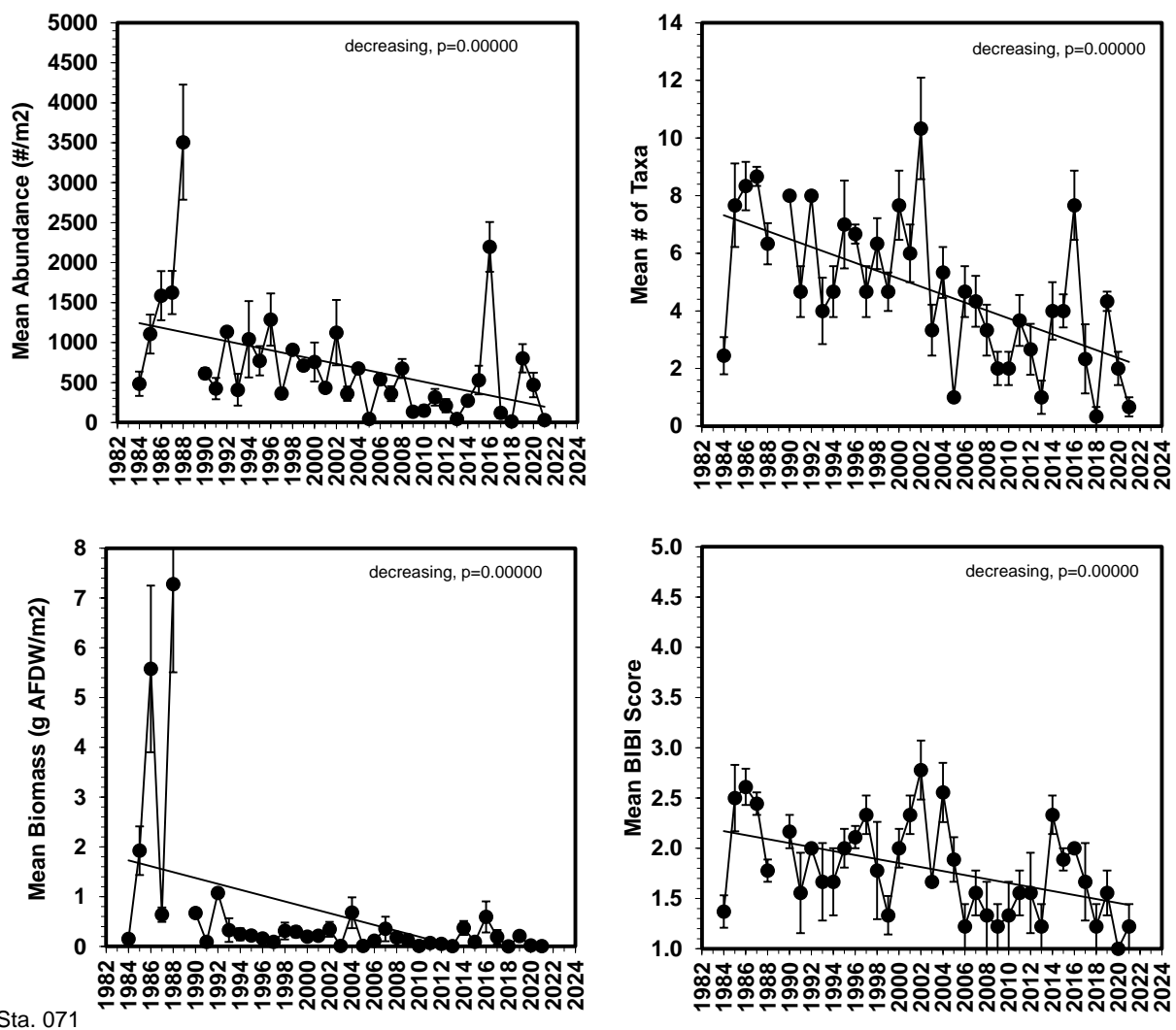


Figure 3-20. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 071 = Mesohaline Patuxent River (12-18 m), Broomes Island. Data gaps indicate periods where sampling was suspended because of program design changes

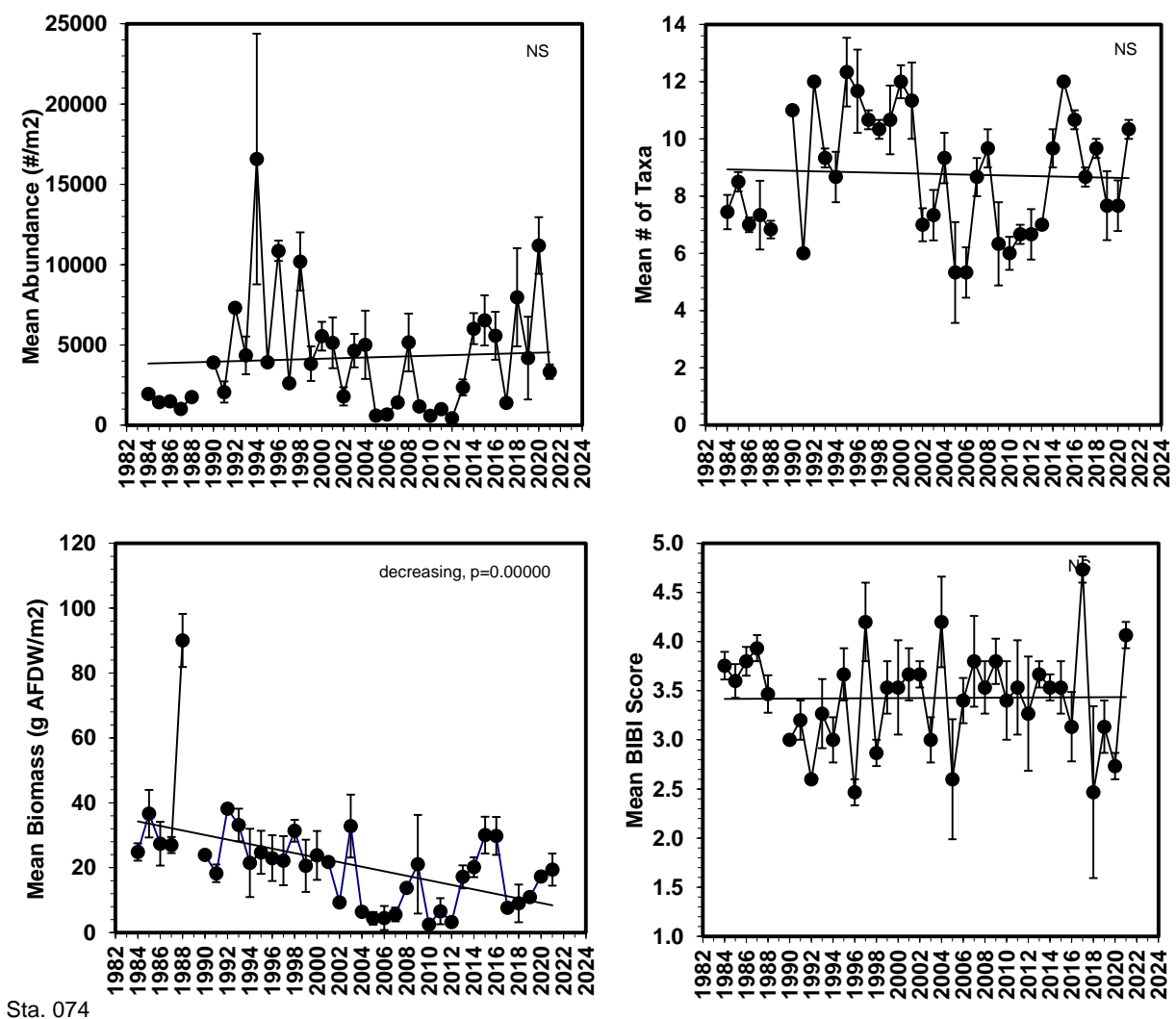
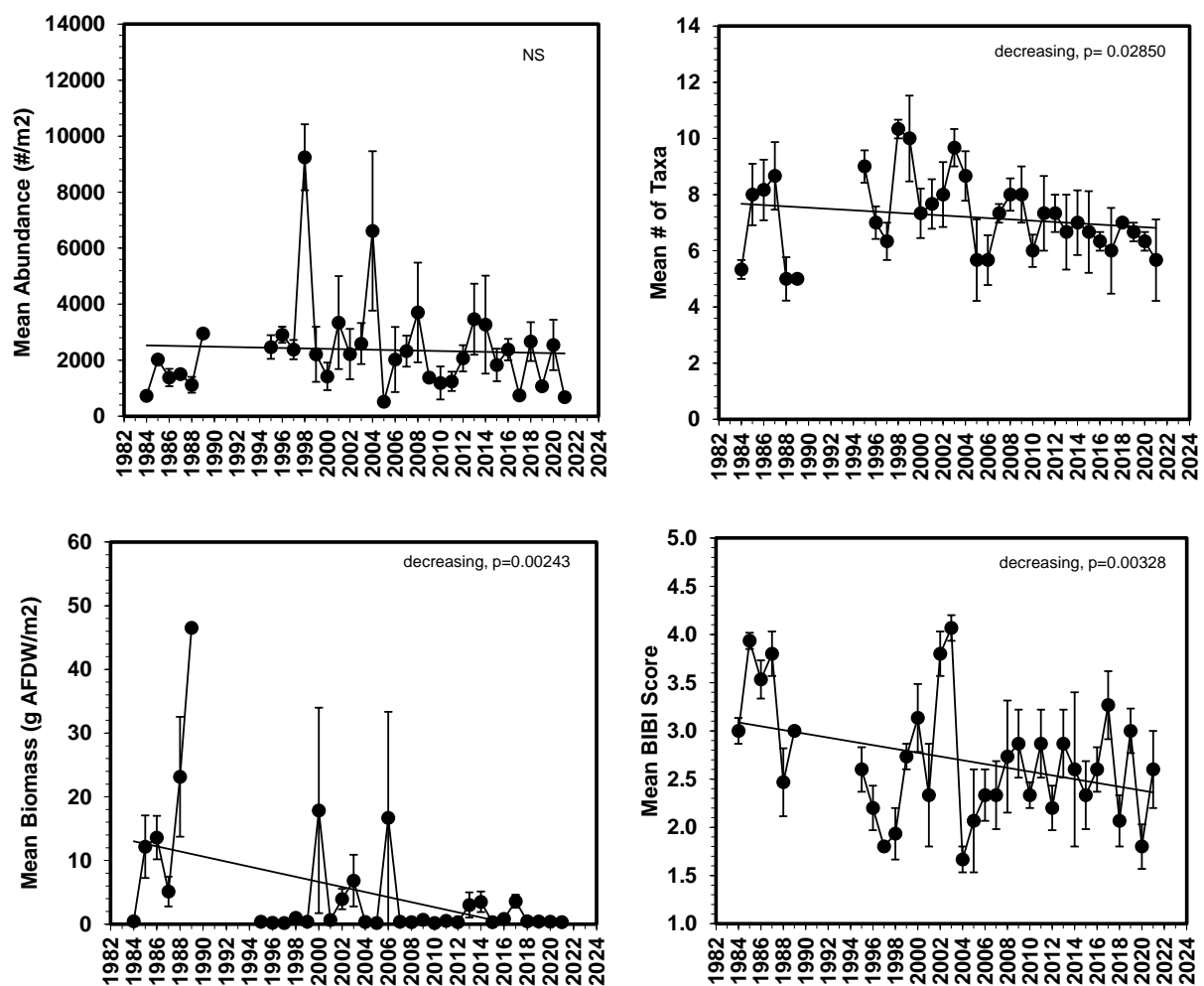


Figure 3-21. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 074 = Mesohaline Patuxent River (\leq 5 m), Chalk Point. Data gaps indicate periods where sampling was suspended because of program design changes



Sta. 077

Figure 3-22. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 077 = Mesohaline Patuxent River (\leq 5 m), Holland Cliff. Data gaps indicate periods where sampling was suspended because of program design changes

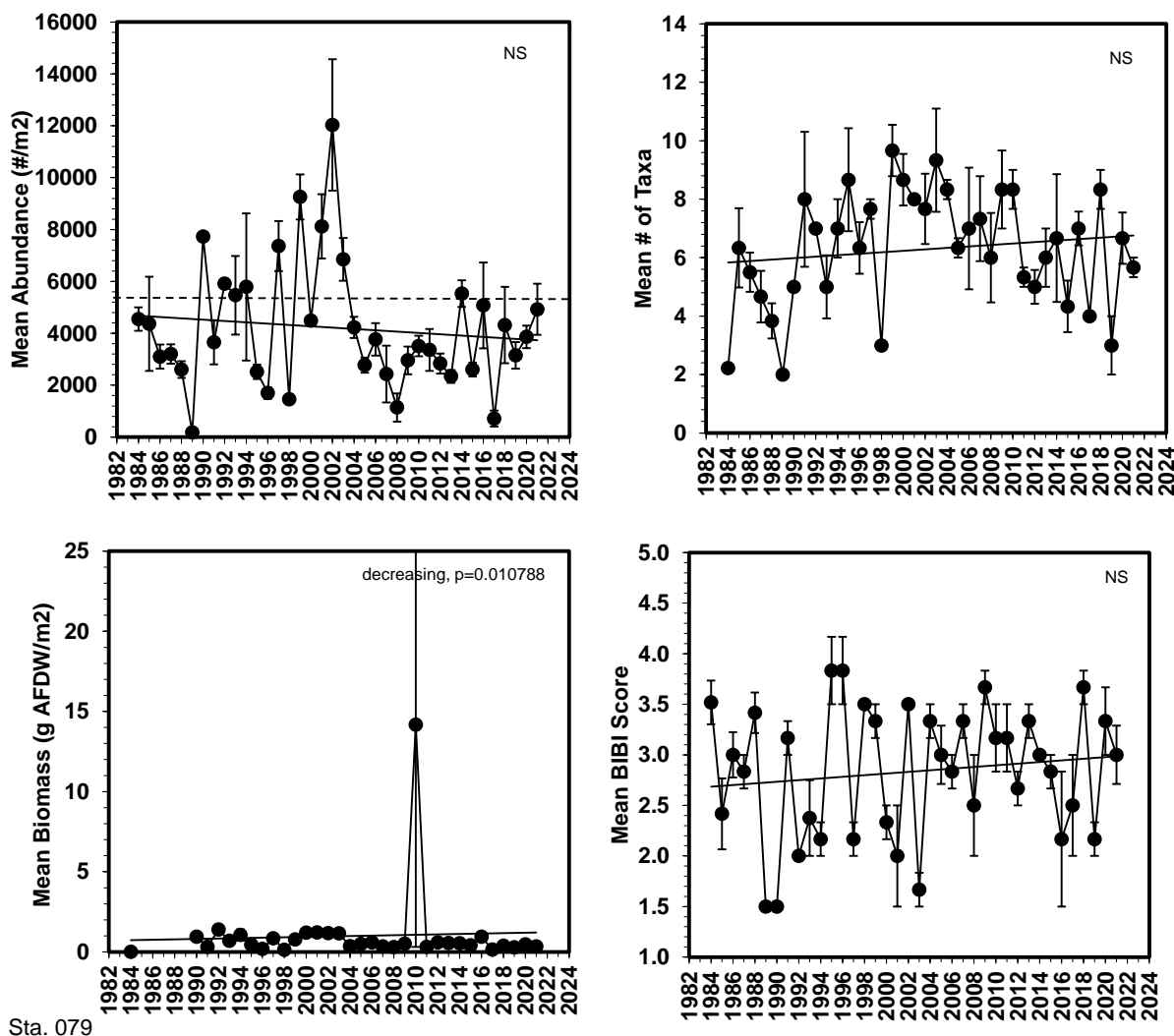


Figure 3-23. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 079 = Tidal freshwater Patuxent River (≤ 6 m), Lyons Creek. The dashed line in the abundance plot is the upper B-IBI threshold (scored as 1) for abundance. Data gaps indicate periods where sampling was suspended because of program design changes

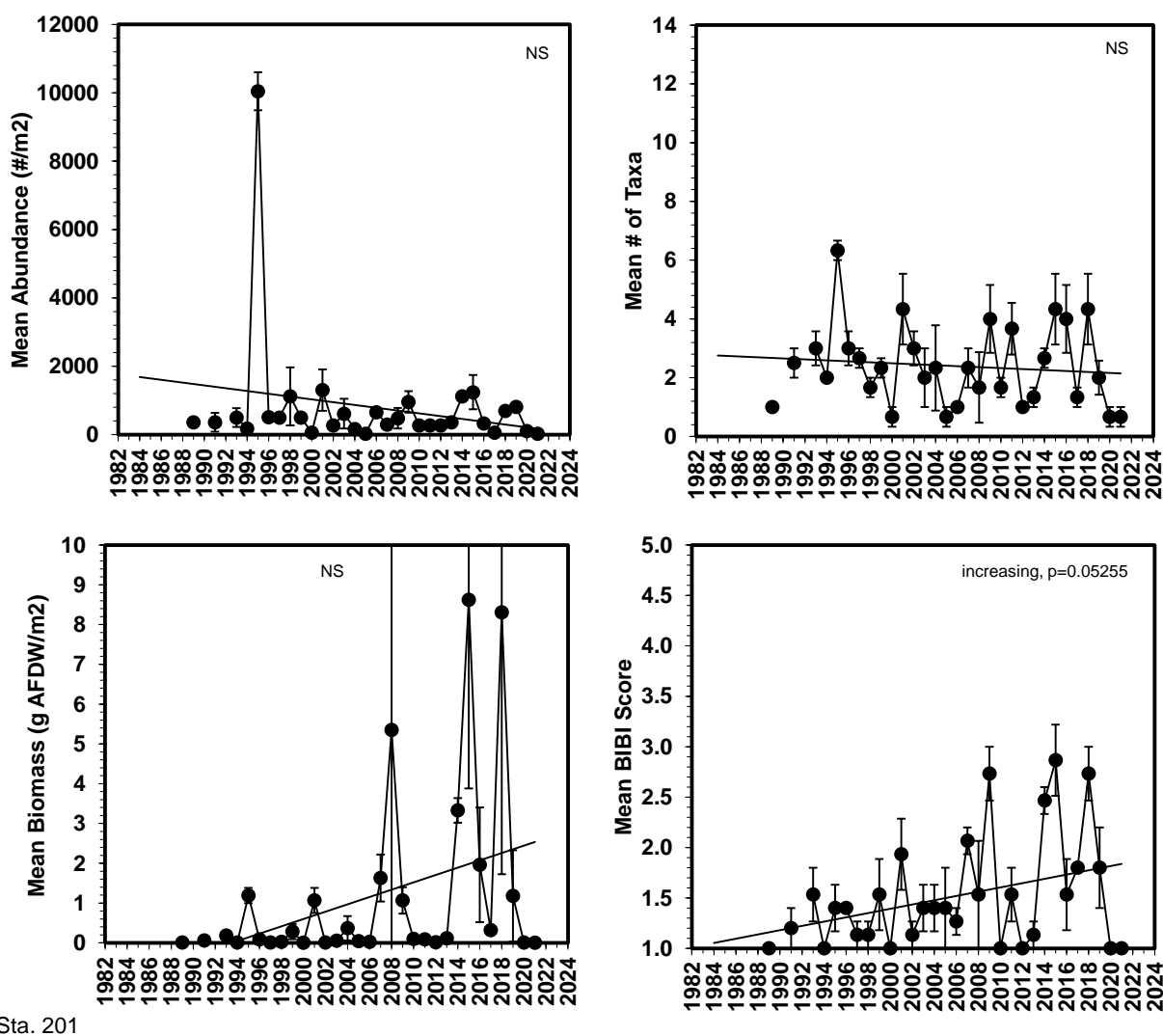


Figure 3-24. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 201 = Patapsco River estuary, Bear Creek. Data gaps indicate periods where sampling was suspended because of program design changes

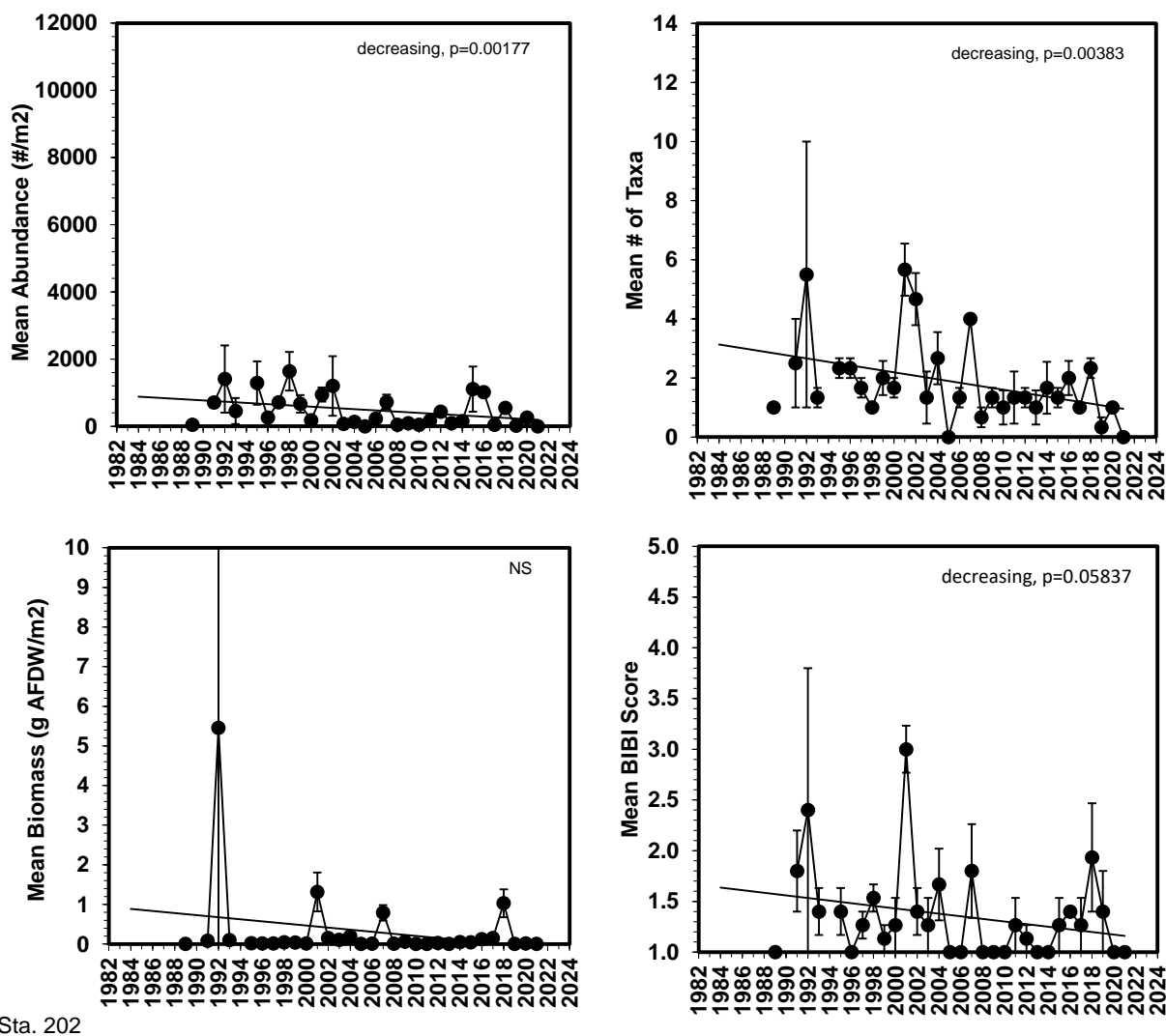
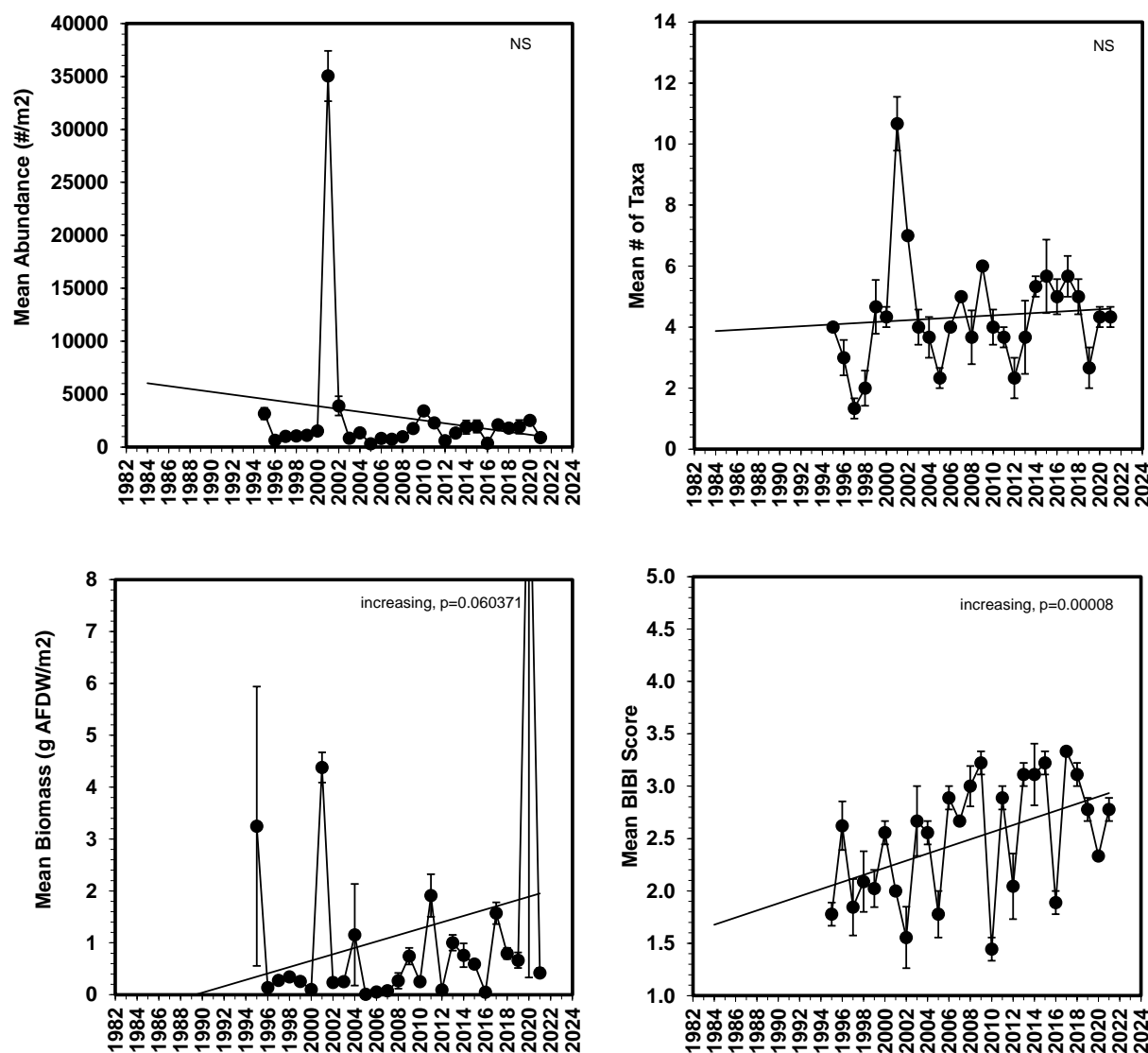


Figure 3-25. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 202 = Patapsco River estuary, Curtis Creek. Data gaps indicate periods where sampling was suspended because of program design changes



Sta. 203

Figure 3-26. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 203 = Back River. Note change in scale in abundance compared to Stations 201, 202, and 204. Mean biomass in 2020 was 11.67 g AFDW/m².

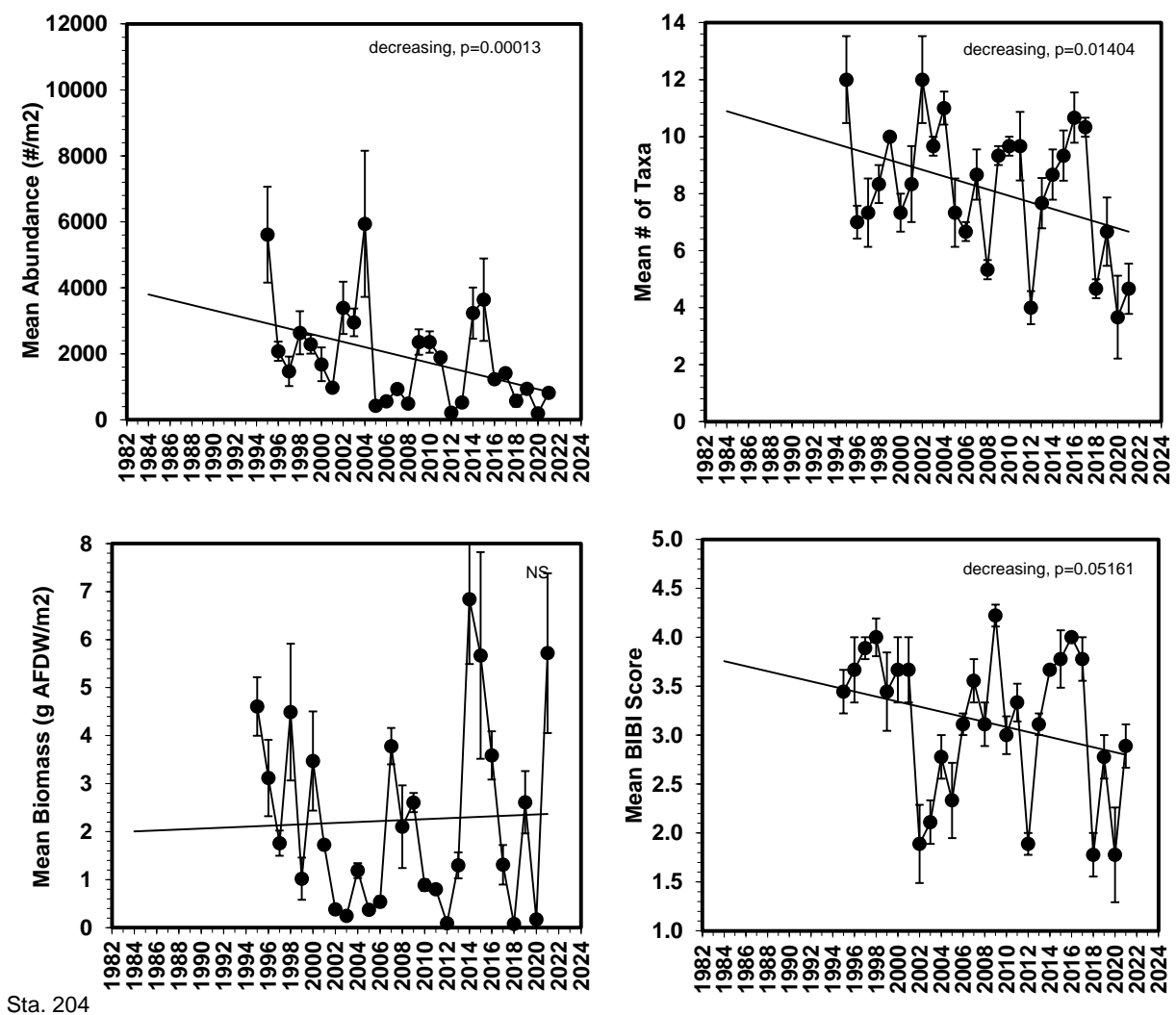


Figure 3-27. Trends in abundance, biomass, number of species, and B-IBI (mean \pm 1 SE) at fixed sites. Station 204 = Severn River

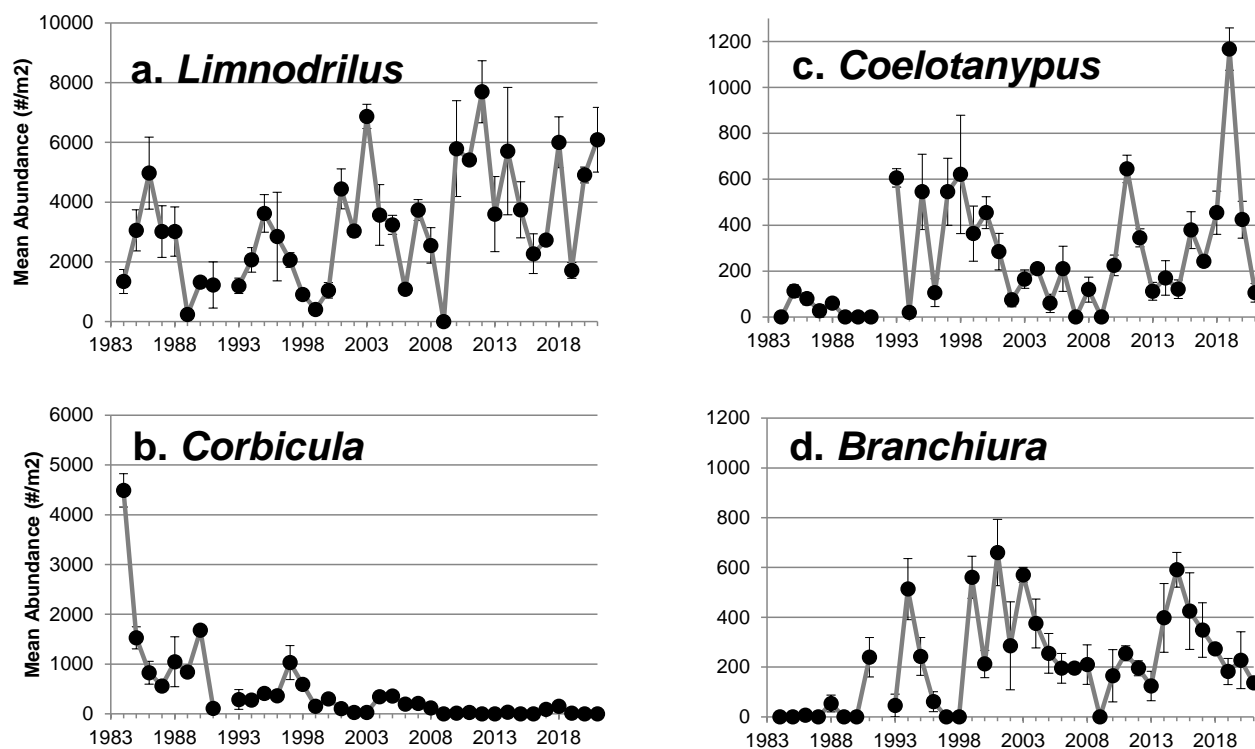


Figure 3-28. Trends in abundance (mean \pm 1 SE) of four numerically dominant species in the tidal freshwater Potomac River at Station 036, 1984-2021. (a) *Limnodrilus hoffmeisteri*, a tubificid oligochaete worm; (b) *Corbicula fluminea*, a bivalve; (c) *Coelotanypus* spp., a midge larva; and (d) *Branchiura sowerbyi*, another tubificid oligochaete worm.

3.2 BAYWIDE BOTTOM COMMUNITY CONDITION

The fixed site monitoring provides useful information about trends in the benthic community condition at 27 locations in the Maryland Chesapeake Bay but it does not provide an integrated assessment of the Bay's overall condition. The fixed sites were selected for trend monitoring because they are located in areas subject to management action and, therefore, are likely to undergo change. Because these sites were selected subjectively, there is no objective way of weighting them to obtain an unbiased estimate of Maryland baywide status.

An alternative approach for quantifying status of the bay, which was first adopted in the 1994 sampling program, is to use probability-based sampling to estimate the bottom area populated by benthos meeting the Chesapeake Bay benthic community restoration goals. Where the fixed site approach quantifies change at selected locations, the probability sampling approach quantifies the spatial extent of problems. While both approaches are valuable, developing and assessing the effectiveness of a Chesapeake Bay management strategy requires understanding the extent and distribution of problems throughout the Bay, instead of only assessing site-specific problems. Our probability-based sampling element is intended to provide that information, as well as a more widespread baseline data set for assessing the effects of unanticipated future contamination (e.g., oil or hazardous waste spills). Probability-based sampling information is used annually in the Bay Report Card and for Chesapeake Bay aquatic life use support decisions under the Clean Water Act (Llansó et al. 2005, 2009a).

Probability-based sampling was employed prior to 1994 by LTB, but the sampled area included only 16% of the Maryland Chesapeake Bay (Ranasinghe et al. 1994) which was insufficient to characterize the entire Bay. Probability-based sampling was also used in the Maryland Bay by the U.S. EPA Environmental Monitoring and Assessment Program (EMAP) and by the U.S. EPA National Coastal Condition Assessment, but at a sampling density too low to develop precise condition estimates for the Maryland Bay. The 2021 sampling continues with efforts initiated in 1994 to develop area-based bottom condition statements for the Maryland Bay.

Estimates of tidal bottom area meeting the benthic community restoration goals are included for the entire Chesapeake Bay. The estimates were enabled by including a probability-based sampling element in the Virginia Benthic Monitoring Program starting in 1996. The Virginia sampling is compatible and complementary to the Maryland effort and is part of a joint effort by the two programs to assess the extent of "healthy" tidal bottom baywide.

This section presents the results of the 2021 Maryland and Virginia probability-based sampling and provides twenty-eight years (1994-2021) of benthic community monitoring in tidal waters of the Maryland Chesapeake Bay. The analytical methods for estimating the areal extent of bay bottom meeting the restoration goals were presented

in Section 2.0. The physical data associated with the benthic samples (bottom water salinity, temperature, dissolved oxygen, and sediment silt-clay and organic carbon content) can be found in the Appendices Section of this report (Volume 2). Only summer data (July 15-September 30) are used for the probability-based assessments.

The 2021 results are presented here to help managers better understand the general level of impact on benthic communities last year. However, the following cautionary note should be kept in mind throughout this section:

“Due to an inadvertent misapplication of the random-site selection process, the Maryland 2021 data results cannot be assessed with confidence. Individual site data (species abundance and biomass; benthic index metrics and scores) are correct but summaries and interpretations of these data such as areal estimates of degradation and trends should be viewed with caution. Virginia data are unaffected.”

Of the 150 Maryland samples collected with the probability-based design in 2021, 53 met and 97 failed the Chesapeake Bay benthic community restoration goals (Figure 3-29). Of the 250 probability samples collected in the entire Chesapeake Bay in 2021, 101 met and 149 failed the restoration goals. The Virginia sampling results are presented in Figure 3-30. In terms of number of sites meeting the goals in Chesapeake Bay (Maryland plus Virginia), fewer sites met the goals in 2021 (40%) than in 2020 (42%).

The area with degraded benthos in the Maryland Bay decreased in 2021 relative to 2020 (Maryland Tidal Waters, Figure 3-31 left panel), and the magnitude of the severely degraded condition also decreased (Maryland Tidal Waters, Figure 3-31 right panel). This change, however, was within the margin of error of the estimate (but see cautionary note above). Results from the individual sites were weighted based on the area of the stratum represented by the site in the stratified sampling design to estimate the tidal Maryland area failing the restoration goals. In 2021, 68% ($\pm 4.7\%$ SE) of the Maryland Bay was inferred to fail the restoration goals (Figure 3-31). Expressed as area, $4,227 \pm 296$ km² of the Maryland tidal waters in Chesapeake Bay remained to be restored in 2021 (Table 3-4). There was no statistically significant trend in percent area degraded over the 1995-2021 time period (ANOVA: $F = 1.50$, $p = 0.2319$).

The Potomac River and the Maryland mainstem were among the Maryland strata in poorest condition, with 76% and 68% area degraded in 2021, respectively (Figures 3-32 and 3-34). The estimate for the Maryland mainstem includes the mid-bay deep trough, which is perennially hypoxic and accounts for 21% of the area of the stratum. The Patuxent River also had a large percentage of degradation in 2021 (72% area degraded). In 2021 degradation decreased in the Maryland mainstem, Maryland Western Tributaries, and Patuxent River, and increased in the Upper Bay mainstem, Maryland Eastern Tributaries, and the Potomac River (Figure 3-32), but these changes should be viewed with caution because of the misapplication of the random-site selection process in 2021. The Upper Bay mainstem was in best condition (Figure 3-34); however, the large increase

in degradation observed in 2021 in this region of the bay was due to an excessive concentration of sampling sites in deep water near the mouth of the Chester River and the Baltimore Harbor navigation channel. The 2021 estimate for the Upper Bay mainstem should not be considered representative of the stratum.

Over the 1995-2021 time period, more than half of the mid-bay mainstem (1,697-2,718 km²) and the tidal Potomac River (714-1,173 km²) (Table 3-4) failed the restoration goals each year, and a large portion of that area, ranging from 52% to 85% in the mainstem and 46% to 93% in the Potomac River, was severely degraded. In 2021, 63% of the Potomac River bottom failing the restoration goals was severely degraded. In the Patuxent River, both the percent degraded and percent severely degraded condition increased over the 1995-2021 time series (ANOVA: Percent degraded, $F = 11.13$, $p = 0.0027$; percent severely degraded, $F = 8.34$, $p = 0.0079$).

For the Chesapeake Bay, degradation in 2021 decreased relative to 2020 (Chesapeake Bay, Figure 3-31 left panel), and the magnitude of the severely degraded condition also decreased (Chesapeake Bay, Figure 3-31 right panel). Weighting results from the 250 probability sites in Maryland and Virginia, 48% ($\pm 3.5\%$) or 5,553 \pm 410 km² of the tidal Chesapeake Bay was estimated to fail the restoration goals in 2021, and 66% of that area (3,642 km²) was severely degraded (Table 3-4).

In Virginia, degradation remained high in all strata except in the Virginia mainstem (Figure 3-33). Benthic community condition in the Virginia mainstem showed a large recovery compared to the previous two years.

Stream flow into Chesapeake Bay was overall lower in the spring of 2021 than in the spring of 2020 (Figure 3-35). In summer, stream flow was low in June, higher in July, and very high with the passing of Hurricane Ida in September, with a peak of 219,000 cfs on September 2, 2021. The average flow for the water year (October 1, 2020-September 30, 2021) was higher than normal.

Hypoxic volume in 2021 was low in June and July but above average in August and September (Figure 3-36). Increased precipitation in mid July and again in early September in combination with warm water temperatures and diminished wind speed during the summer probably contributed to the higher hypoxic volume observed in August and September. The average water-column temperature at the time of the benthic sampling was 2.2 degrees warmer in 2021 (26.8 °C) than in 2020 (24.6 °C).

The average abundance, biomass, and number of species in Maryland tidal waters were lower in 2021 than in 2020. The average B-IBI score was about the same (Figure 3-37). Over time, statistically significant declining trends in mean abundance, mean number of species, and mean B-IBI remained in Maryland tidal waters with the addition of the 2021 data, with the caution noted above (Figure 3-37). Baywide, abundance and biomass were also lower in 2021, and the mean number of species declined significantly

over the 1996-2021 time series (Figure 3-38). In Maryland, the percentage of sites scoring 1 for excess abundance continued to decline strongly (Figure 3-37, ANOVA: $F = 15.10$, $p = 0.0007$), indicating improvements in benthic community condition from excess abundance (eutrophic condition).

In addition to percent area degraded, results can be summarized by the type of stress experienced by the benthic communities. Low abundance, low biomass, and the level of widespread failure in most metrics necessary to classify a site as severely degraded is usually expected on exposure to catastrophic events such as prolonged dissolved oxygen stress. Conversely, excess abundance and excess biomass are phenomena usually associated with eutrophic conditions and organic enrichment of the sediment in the absence of low dissolved oxygen stress. For the period 1996-2021, four strata (Potomac River, Patuxent River, Mid Bay Mainstem, and Maryland Western Tributaries) had a large percentage ($\geq 70\%$) of sites failing the goals because of insufficient abundance or biomass of organisms relative to reference conditions (Table 3-5). These regions were the most dissolved-oxygen stressed. These strata also had a high percentage ($>50\%$) of failing sites classified as severely degraded (Table 3-5). These results contrast with those of the James, York, and Rappahannock rivers, which had fewer depauperate sites but excess abundance, excess biomass, or both in $>20\%$ of the failing sites (Table 3-6).

Table 3-4. Estimated tidal area (km²) failing to meet the Chesapeake Bay benthic community restoration goals. In this table, the area of the mainstem deep trough is included in the estimates for the severely degraded condition. The Potomac River area sampled in 1994 differs (See Table 2-2). See cautionary note in page 3-36.

Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Chesapeake Bay	1996	3,080	1,388	1,056	5,524	47.6
	1997	2,941	2,093	856	5,890	50.7
	1998	3,771	1,689	1,271	6,731	58.0
	1999	3,164	1,660	1,020	5,844	50.3
	2000	2,704	1,538	1,474	5,715	49.2
	2001	3,123	1,187	1,749	6,060	52.2
	2002	3,424	1,584	1,170	6,178	53.2
	2003	3,351	2,537	964	6,852	59.0
	2004	2,902	1,940	650	5,492	47.3
	2005	4,664	1,550	614	6,829	58.8
	2006	4,336	1,779	756	6,871	59.2
	2007	4,120	1,529	1,064	6,713	57.8
	2008	3,459	1,570	1,759	6,788	58.5
	2009	3,164	898	1,032	5,094	43.9
	2010	3,199	1,492	1,485	6,177	53.2
	2011	3,686	1,534	1,132	6,353	54.7
	2012	3,125	2,039	1,173	6,337	54.6
	2013	3,650	1,760	800	6,210	53.5
	2014	2,601	1,660	505	4,767	41.1
	2015	2,595	1,485	349	4,428	38.2
	2016	3,071	1,031	1,169	5,271	45.4
	2017	3,073	1,116	563	4,752	40.9
	2018	2,769	1,377	689	4,835	41.7
	2019	3,750	1,642	1,503	6,895	59.4
	2020	4,463	1,610	1,059	7,131	61.4
	2021	3,642	1,157	755	5,554	47.8

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Maryland Tidal Waters	1994	2,684	1,152	497	4,332	66.5
	1995	2,872	605	182	3,659	58.6
	1996	2,614	700	155	3,469	55.6
	1997	2,349	719	462	3,529	56.5
	1998	2,663	1,016	623	4,302	68.9
	1999	2,423	1,137	374	3,935	63.0
	2000	2,455	1,137	236	3,828	61.3
	2001	2,313	582	644	3,538	56.7
	2002	2,444	713	928	4,086	65.4
	2003	2,571	1,288	228	4,086	65.4
	2004	2,037	985	226	3,248	52.0
	2005	2,771	1,014	295	4,080	65.3
	2006	3,077	1,013	504	4,595	73.6
	2007	3,088	851	513	4,452	71.3
	2008	2,727	767	854	4,348	69.6
	2009	2,484	580	540	3,605	57.7
	2010	2,656	1,171	355	4,182	67.0
	2011	2,320	1,027	703	4,050	64.9
	2012	2,620	1,161	785	4,565	73.1
	2013	2,549	1,269	184	4,001	64.1
	2014	2,110	1,402	241	3,753	60.1
	2015	1,997	1,071	254	3,322	53.2
	2016	2,813	650	685	4,148	66.4
	2017	2,223	832	278	3,333	53.4
	2018	2,416	1,163	215	3,794	60.8
	2019	2,860	1,052	328	4,240	67.9
	2020	3,255	845	425	4,525	72.5
	2021	2,940	978	309	4,227	67.7

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Virginia Tidal Waters	1996	466	688	901	2,055	38.3
	1997	592	1,375	394	2,361	44.0
	1998	1,107	673	648	2,429	45.3
	1999	741	523	646	1,909	35.6
	2000	249	401	1,238	1,888	35.2
	2001	810	606	1,106	2,522	47.0
	2002	980	871	242	2,092	39.0
	2003	780	1,249	736	2,766	51.6
	2004	866	955	424	2,245	41.9
	2005	1,893	536	319	2,748	51.2
	2006	1,259	765	252	2,276	42.4
	2007	1,031	678	552	2,261	42.2
	2008	732	803	905	2,440	45.5
	2009	680	318	491	1,489	27.8
	2010	543	321	1,130	1,994	37.2
	2011	1,366	508	429	2,303	42.9
	2012	505	878	389	1,772	33.0
	2013	1,101	491	616	2,208	41.2
	2014	490	259	264	1,013	18.9
	2015	598	413	95	1,106	20.6
	2016	258	380	484	1,123	20.9
	2017	850	284	286	1,419	26.5
	2018	353	214	474	1,041	19.4
	2019	889	591	1,175	2,655	49.5
	2020	1,208	765	634	2,606	48.6
	2021	702	179	446	1,327	24.7

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Maryland Eastern Tributaries	1995	107	128	0	235	44.0
	1996	21	150	21	192	36.0
	1997	43	86	0	128	24.0
	1998	21	64	64	150	28.0
	1999	43	150	86	278	52.0
	2000	64	150	21	235	44.0
	2001	128	64	86	278	52.0
	2002	64	107	64	235	44.0
	2003	128	214	0	342	64.0
	2004	86	107	21	214	40.0
	2005	86	64	86	235	44.0
	2006	86	128	43	257	48.0
	2007	150	86	128	363	68.0
	2008	86	86	64	235	44.0
	2009	192	64	64	321	60.0
	2010	150	171	43	363	68.0
	2011	86	86	86	257	48.0
	2012	128	128	0	257	48.0
	2013	64	150	43	257	48.0
	2014	86	64	21	171	32.0
	2015	64	86	21	171	32.0
	2016	86	150	107	342	64.0
	2017	64	192	21	278	52.0
	2018	43	128	21	192	36.0
	2019	107	43	107	257	48.0
	2020	128	107	64	299	56.0
	2021	86	192	64	342	64.0

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Maryland Mid-Bay Mainstem	1995	1,799	204	102	2,106	65.2
	1996	1,595	306	102	2,004	62.1
	1997	1,493	306	306	2,106	65.2
	1998	1,799	204	408	2,412	74.7
	1999	1,391	715	102	2,208	68.4
	2000	1,493	510	204	2,208	68.4
	2001	1,289	102	408	1,799	55.7
	2002	1,595	204	613	2,412	74.7
	2003	1,289	613	204	2,106	65.2
	2004	983	510	204	1,697	52.6
	2005	1,595	613	204	2,412	74.7
	2006	1,697	613	306	2,616	81.0
	2007	1,799	510	306	2,616	81.0
	2008	1,799	306	613	2,718	84.2
	2009	1,595	204	408	2,208	68.4
	2010	1,697	510	204	2,412	74.7
	2011	1,391	408	510	2,310	71.5
	2012	1,595	408	510	2,514	77.9
	2013	1,697	613	102	2,412	74.7
	2014	1,085	919	102	2,106	65.2
	2015	1,187	408	102	1,697	52.6
	2016	1,493	102	510	2,106	65.2
	2017	1,493	204	102	1,799	55.7
	2018	1,391	715	102	2,208	68.4
	2019	1,493	715	204	2,412	74.7
	2020	2,208	408	204	2,820	87.4
	2021	1,799	204	204	2,208	68.4

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Maryland Upper Bay Mainstem	1995	345	63	0	408	52.0
	1996	126	126	31	283	36.0
	1997	126	94	31	251	32.0
	1998	157	188	31	377	48.0
	1999	188	63	63	314	40.0
	2000	94	126	0	220	28.0
	2001	157	31	31	220	28.0
	2002	94	126	31	251	32.0
	2003	188	157	0	345	44.0
	2004	220	31	0	251	32.0
	2005	31	0	0	31	4.0
	2006	188	31	31	251	32.0
	2007	188	31	0	220	28.0
	2008	126	188	94	408	52.0
	2009	31	31	63	126	16.0
	2010	157	31	31	220	28.0
	2011	94	126	0	220	28.0
	2012	126	157	31	314	40.0
	2013	94	157	0	251	32.0
	2014	94	63	94	251	32.0
	2015	94	63	63	220	28.0
	2016	157	188	0	345	44.0
	2017	63	94	126	283	36.0
	2018	94	63	63	220	28.0
	2019	126	63	0	188	24.0
	2020	94	94	94	283	36.0
	2021	283	157	0	440	56.0

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Maryland Upper Western Tributaries	1995	58	47	23	129	44.0
	1996	117	47	0	164	56.0
	1997	105	23	12	140	48.0
	1998	94	23	12	129	44.0
	1999	117	47	12	175	60.0
	2000	140	70	0	211	72.0
	2001	70	12	47	129	44.0
	2002	94	47	47	187	64.0
	2003	47	105	23	175	60.0
	2004	70	117	0	187	64.0
	2005	140	47	0	187	64.0
	2006	187	47	12	246	84.0
	2007	94	35	12	140	48.0
	2008	94	23	12	129	44.0
	2009	94	35	0	129	44.0
	2010	152	70	0	222	76.0
	2011	35	70	0	105	36.0
	2012	199	23	23	246	84.0
	2013	70	23	23	117	40.0
	2014	70	70	23	164	56.0
	2015	105	35	12	152	52.0
	2016	164	47	12	222	76.0
	2017	47	35	23	105	36.0
	2018	82	58	23	164	56.0
	2019	94	94	12	199	68.0
	2020	105	82	12	199	68.0
	2021	94	47	35	175	59.9

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Patuxent River	1995	51	10	5	67	52.0
	1996	41	20	0	61	48.0
	1997	20	5	10	36	28.0
	1998	31	26	5	61	48.0
	1999	20	10	10	41	32.0
	2000	51	26	10	87	68.0
	2001	56	15	20	92	72.0
	2002	36	26	20	82	64.0
	2003	51	46	0	97	76.0
	2004	15	67	0	82	64.0
	2005	51	36	5	92	72.0
	2006	51	41	10	102	80.0
	2007	41	36	15	92	72.0
	2008	61	10	20	92	72.0
	2009	61	41	5	108	84.0
	2010	41	31	26	97	76.0
	2011	51	31	5	87	68.0
	2012	61	36	15	113	88.0
	2013	61	20	15	97	76.0
	2014	61	31	0	92	72.0
	2015	36	20	5	61	48.0
	2016	46	10	5	61	48.0
	2017	46	51	5	102	80.0
	2018	41	46	5	92	72.0
	2019	72	36	5	133	88.0
	2020	56	51	0	108	84.0
	2021	67	20	5	92	71.9

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Potomac River	1994	793	330	0	1,123	60.7
	1995	510	153	51	714	56.0
	1996	714	51	0	765	60.0
	1997	561	204	102	867	68.0
	1998	561	510	102	1,173	92.0
	1999	663	153	102	918	72.0
	2000	612	255	0	867	68.0
	2001	612	357	51	1,020	80.0
	2002	561	204	153	918	72.0
	2003	867	153	0	1,020	80.0
	2004	663	153	0	816	64.0
	2005	867	255	0	1,122	88.0
	2006	867	153	102	1,122	88.0
	2007	816	153	51	1,020	80.0
	2008	561	153	51	765	60.0
	2009	510	204	0	714	56.0
	2010	459	357	51	867	68.0
	2011	663	306	102	1,071	84.0
	2012	510	408	204	1,122	88.0
	2013	561	306	0	867	68.0
	2014	714	255	0	969	76.0
	2015	510	459	51	1,020	80.0
	2016	867	153	51	1,071	84.0
	2017	510	255	0	765	60.0
	2018	765	153	0	918	72.0
	2019	969	102	0	1,071	84.0
	2020	663	102	51	816	64.0
	2021	612	357	0	969	75.9

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Rappahannock River	1996	119	60	0	179	48.0
	1997	149	74	15	238	64.0
	1998	60	134	45	238	64.0
	1999	89	89	74	253	68.0
	2000	149	104	15	268	72.0
	2001	30	60	60	149	40.0
	2002	134	45	0	179	48.0
	2003	89	104	0	194	52.0
	2004	60	89	30	179	48.0
	2005	253	60	30	343	92.0
	2006	223	15	45	283	76.0
	2007	209	104	15	328	88.0
	2008	179	60	45	283	76.0
	2009	119	104	45	268	72.0
	2010	209	45	45	298	80.0
	2011	134	119	30	283	76.0
	2012	179	60	30	268	72.0
	2013	194	30	60	283	76.0
	2014	89	104	30	223	60.0
	2015	60	89	30	179	48.0
	2016	119	89	15	223	60.0
	2017	134	60	119	313	84.0
	2018	89	74	74	238	64.0
	2019	149	89	60	298	80.0
	2020	45	134	15	194	52.0
	2021	164	74	60	298	80.1

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
York River	1996	45	52	22	120	64.0
	1997	60	37	22	120	64.0
	1998	60	45	0	105	56.0
	1999	75	22	22	120	64.0
	2000	45	22	15	82	44.0
	2001	67	52	30	150	80.0
	2002	22	30	22	75	40.0
	2003	60	75	22	157	84.0
	2004	37	15	37	90	48.0
	2005	75	37	15	127	68.0
	2006	75	37	15	127	68.0
	2007	82	52	15	150	80.0
	2008	60	30	37	127	68.0
	2009	67	22	7	97	52.0
	2010	60	30	15	105	56.0
	2011	52	60	15	127	68.0
	2012	52	22	30	105	56.0
	2013	112	22	7	142	76.0
	2014	45	45	15	105	56.0
	2015	45	22	37	105	56.0
	2016	30	45	30	105	56.0
	2017	30	60	30	120	64.0
	2018	45	30	15	90	48.0
	2019	0	7	45	52	28.0
	2020	37	0	15	52	28.0
	2021	45	22	30	97	52.0

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
James River	1996	137	82	55	273	40.0
	1997	219	109	27	355	52.0
	1998	164	164	109	437	64.0
	1999	82	246	55	383	56.0
	2000	55	109	55	219	32.0
	2001	219	164	27	410	60.0
	2002	164	137	55	355	52.0
	2003	137	246	55	437	64.0
	2004	109	191	27	328	48.0
	2005	82	109	109	301	44.0
	2006	137	219	27	383	56.0
	2007	246	191	27	465	68.0
	2008	164	219	164	547	80.0
	2009	164	191	109	465	68.0
	2010	109	82	82	273	40.0
	2011	355	164	55	574	84.0
	2012	109	137	164	410	60.0
	2013	301	109	55	465	68.0
	2014	191	109	55	355	52.0
	2015	164	137	27	328	48.0
	2016	109	246	109	465	68.0
	2017	191	164	137	492	72.0
	2018	219	109	55	383	56.0
	2019	246	164	82	492	72.0
	2020	137	137	109	383	56.0
	2021	328	82	27	437	63.9

Table 3-4. (Continued)						
Region	Year	Severely Degraded	Degraded	Marginal	Total Failing	% Failing
Virginia Mainstem	1996	165	494	824	1,483	36.0
	1997	165	1,154	330	1,648	40.0
	1998	824	330	494	1,648	40.0
	1999	494	165	494	1,154	28.0
	2000	0	165	1,154	1,318	32.0
	2001	494	330	989	1,813	44.0
	2002	659	659	165	1,483	36.0
	2003	494	824	659	1,977	48.0
	2004	659	659	330	1,648	40.0
	2005	1,483	330	165	1,977	48.0
	2006	824	494	165	1,483	36.0
	2007	494	330	494	1,318	32.0
	2008	330	494	659	1,483	36.0
	2009	330	0	330	659	16.0
	2010	165	165	989	1,318	32.0
	2011	824	165	330	1,318	32.0
	2012	165	659	165	989	24.0
	2013	494	330	494	1,318	32.0
	2014	165	0	165	330	8.0
	2015	330	165	0	494	12.0
	2016	0	0	330	330	8.0
	2017	494	0	0	494	12.0
	2018	0	0	330	330	8.0
	2019	494	330	989	1,813	44.0
	2020	989	494	494	1,977	48.0
	2021	165	0	330	495	12.0

Table 3-4. Sites severely degraded (B-IBI \leq 2) and failing the restoration goals (scored at 1) for insufficient abundance, insufficient biomass, or both as a percentage of sites failing the goals (B-IBI \leq 3), 1996 to 2021. Strata are listed in decreasing percent order of sites with insufficient abundance/biomass.

Stratum	Sites Severely Degraded		Sites Failing the Goals Due to Insufficient Abundance, Biomass, or Both	
	Number of Sites	As Percentage of Sites Failing the Goals	Number of Sites	As Percentage of Sites Failing the Goals
Potomac River	338	70.0	412	85.3
Patuxent River	240	54.8	362	82.6
Mid Bay Mainstem	219	54.6	312	77.8
Western Tributaries	229	60.9	262	69.7
Upper Bay Mainstem	108	50.0	144	66.7
Virginia Mainstem	71	36.2	118	60.2
Rappahannock River	230	52.9	260	59.8
Eastern Tributaries	107	34.4	173	55.6
York River	185	48.6	131	34.4
James River	166	43.6	97	25.5

Table 3-5. Sites failing the restoration goals (scored at 1) for excess abundance, excess biomass, or both as a percentage of sites failing the goals (B-IBI \leq 3), 1996 to 2021. Strata are listed in decreasing percent order of sites with excess abundance/biomass.

Stratum	Number of Sites	As Percentage of Sites Failing the Goals
James River	135	35.4
York River	95	24.9
Rappahannock River	97	22.3
Eastern Tributaries	60	19.3
Upper Bay Mainstem	38	17.6
Western Tributaries	55	14.6
Mid Bay Mainstem	48	12.0
Patuxent River	36	8.2
Virginia Mainstem	16	8.2
Potomac River	38	7.9

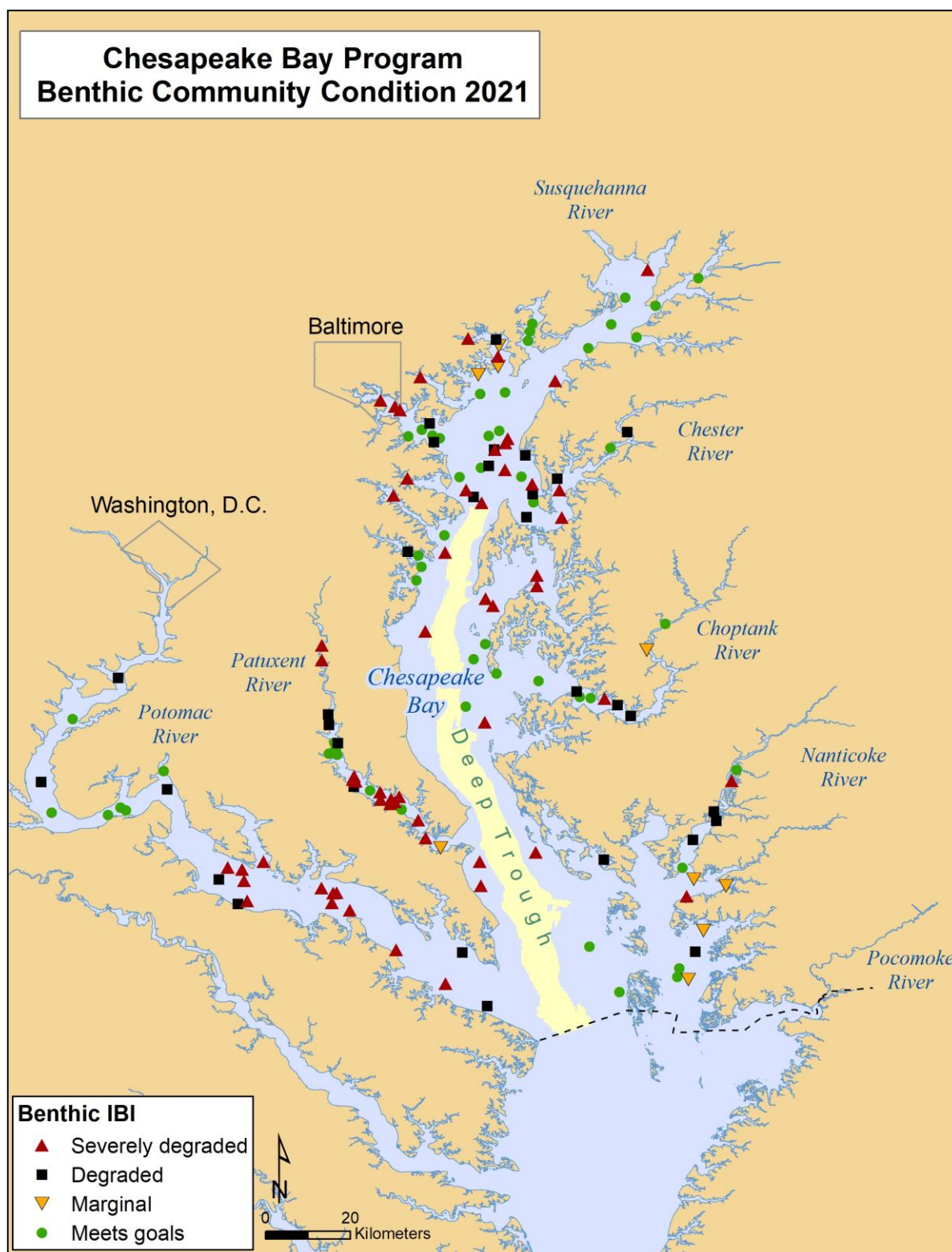


Figure 3-29. Results of probability-based benthic sampling of the Maryland Chesapeake Bay and its tidal tributaries in 2021. Each sample was evaluated in context of the Chesapeake Bay benthic community restoration goals

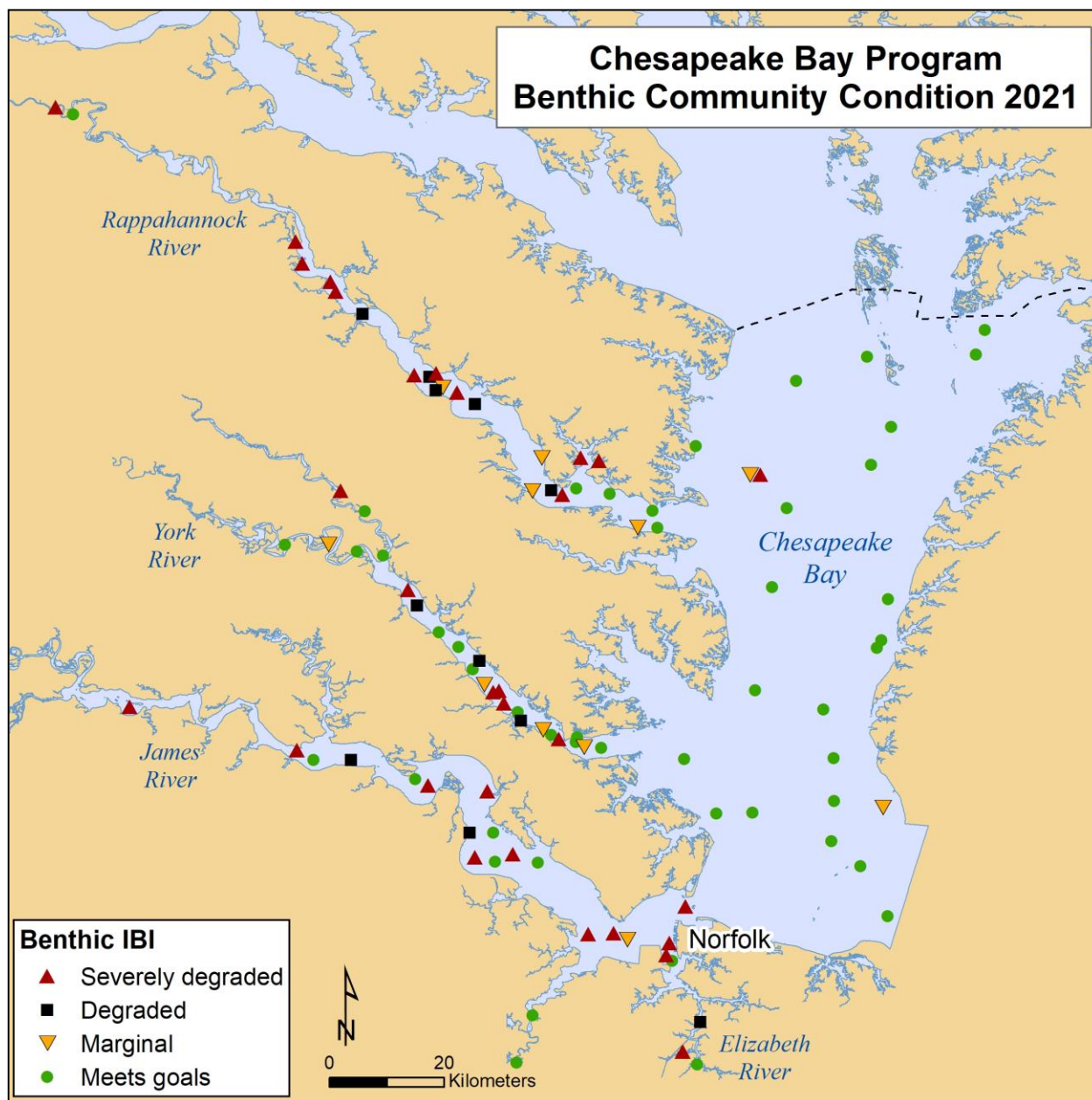


Figure 3-30. Results of probability-based benthic sampling of the Virginia Chesapeake Bay and its tidal tributaries in 2021. Each sample was evaluated in context of the Chesapeake Bay benthic community restoration goals

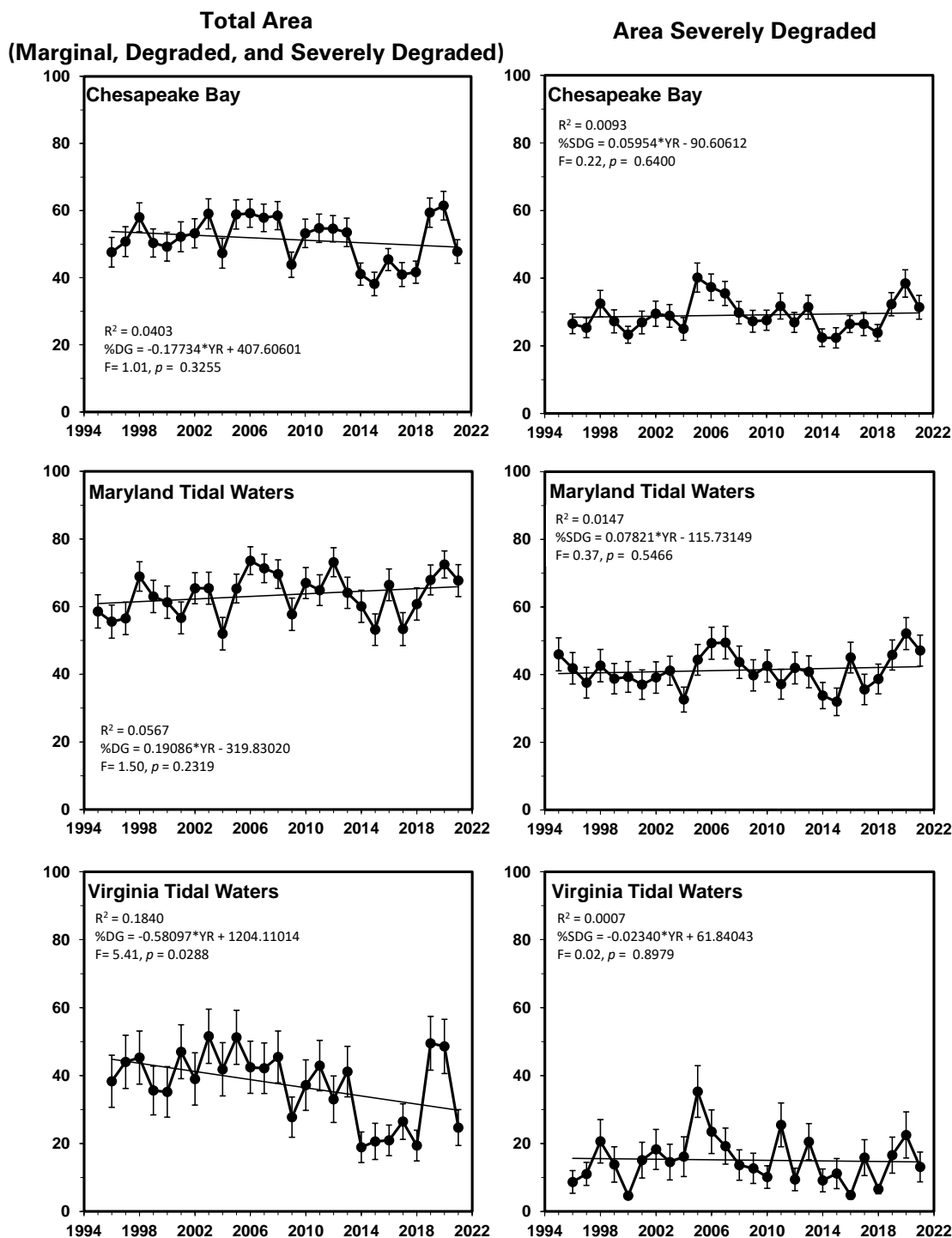


Figure 3-31. Proportion of the Chesapeake Bay, Maryland tidal waters, and Virginia tidal waters failing the Chesapeake Bay benthic community restoration goals, 1996 to 2021 (1995-2021 for Maryland). Panels on left show percent total area degraded ($B-IBI < 3.0$); panels on right show percent area severely degraded ($B-IBI \leq 2.0$). Error bars indicate ± 1 standard error. The mainstem deep trough is included in the severely degraded condition estimates. See cautionary note in page 3-36

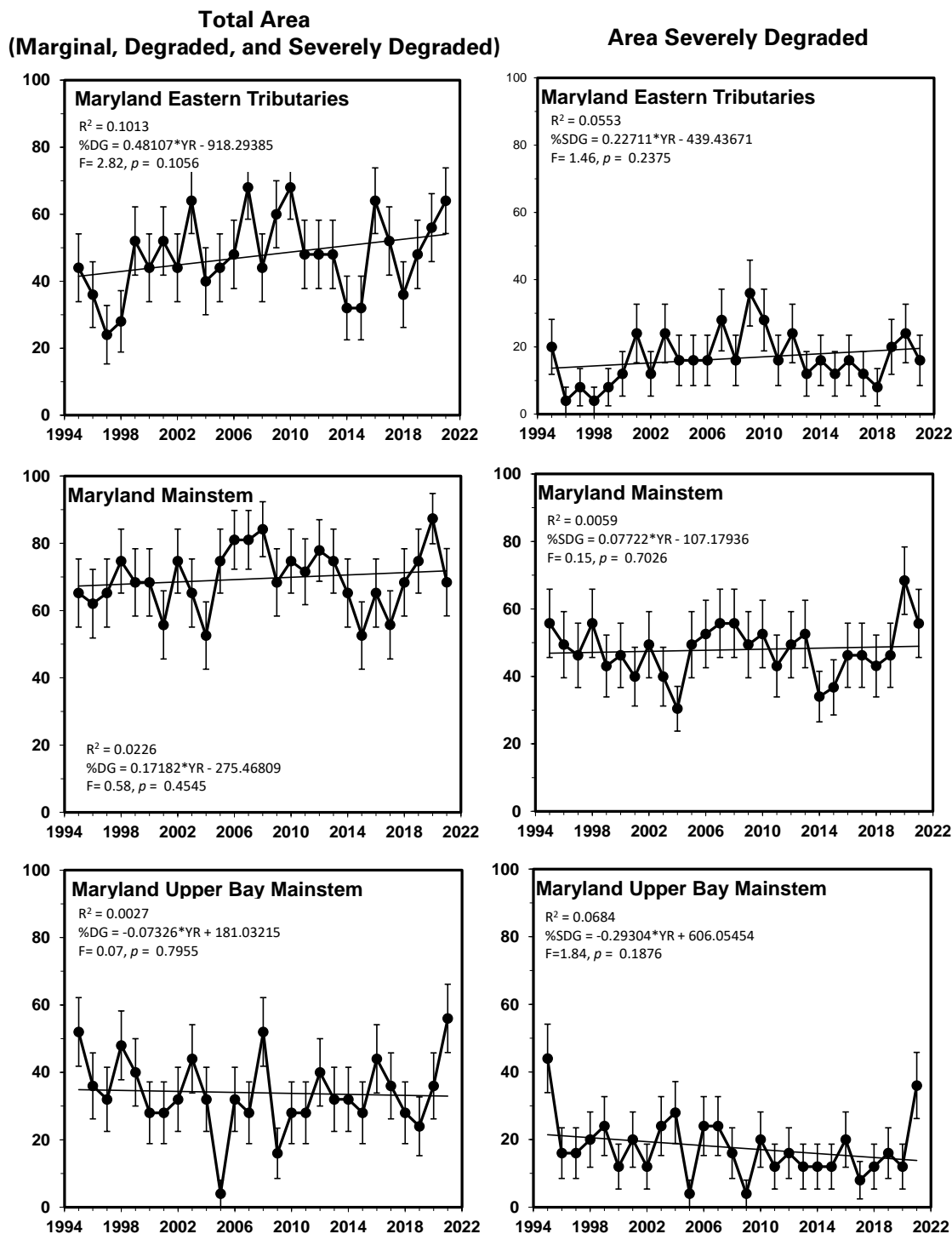


Figure 3-32. Proportion of the Maryland sampling strata failing the Chesapeake Bay benthic community restoration goals, 1995 to 2021. Panels on left show percent total area degraded ($B-IBI < 3.0$); panels on right show percent area severely degraded ($B-IBI \leq 2.0$). Error bars indicate ± 1 standard error. The deep trough is included in the Maryland mainstem stratum estimates. See cautionary note in page 3-36

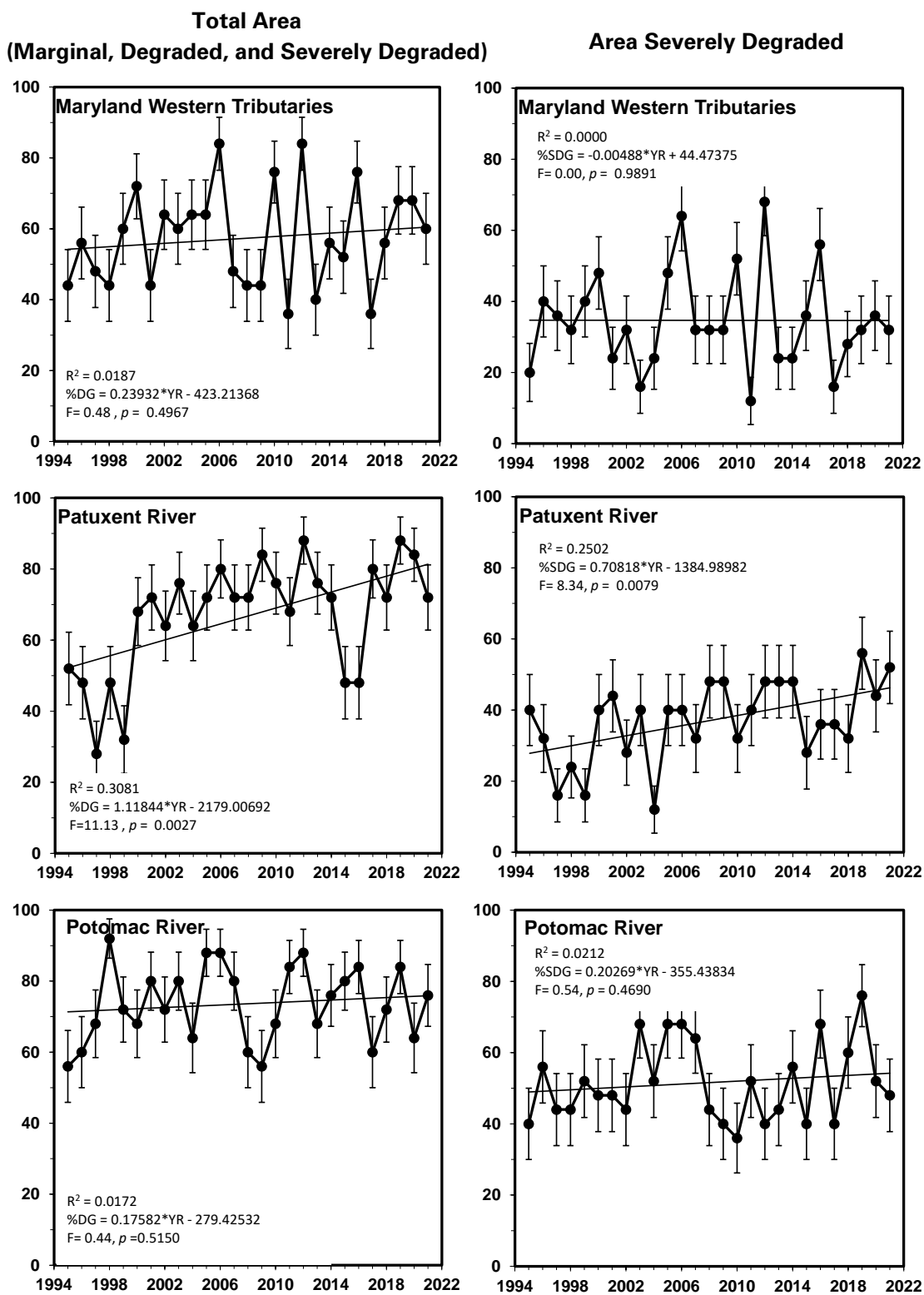


Figure 3-32. (Continued)

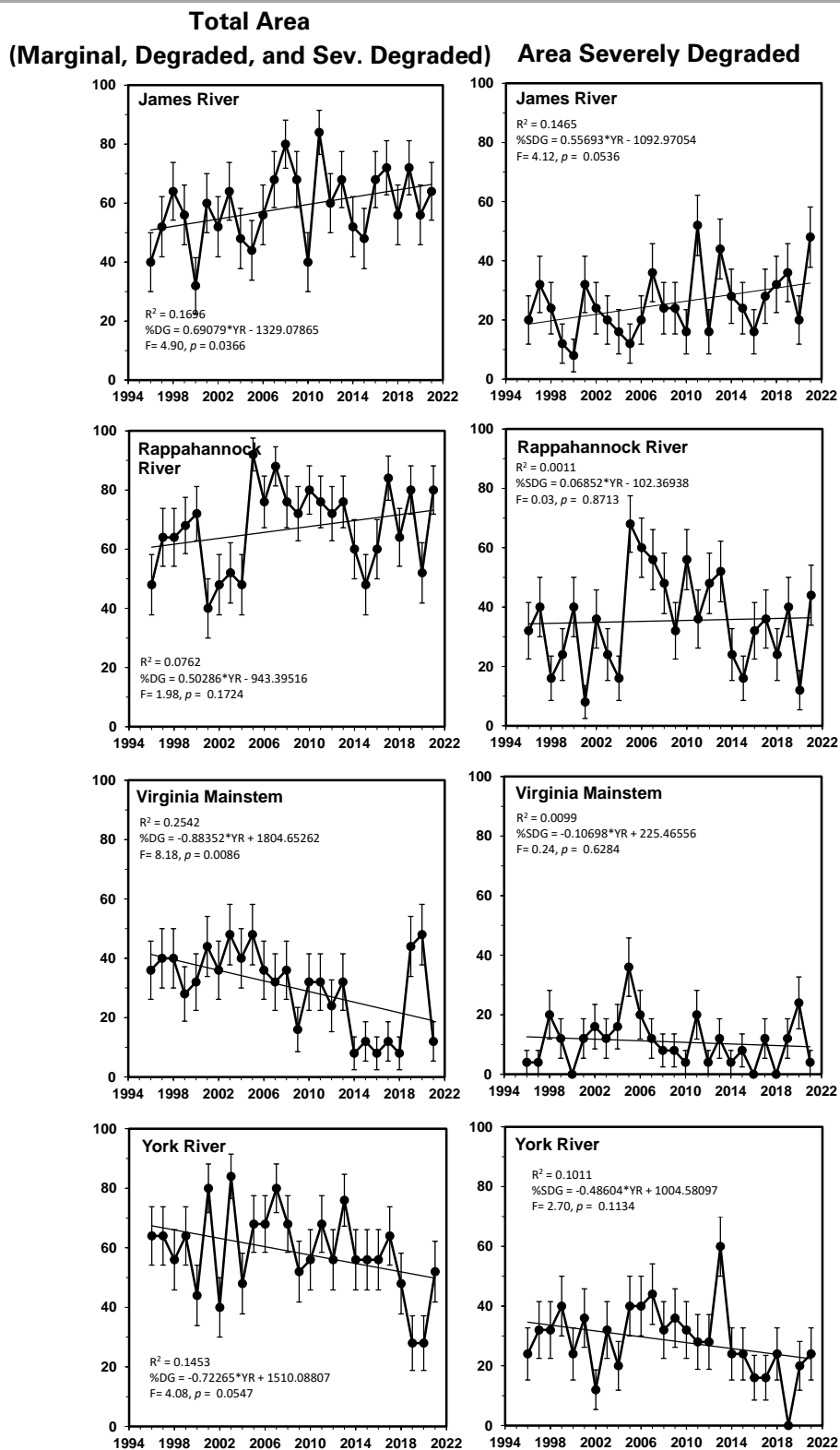


Figure 3-33. Proportion of the Virginia sampling strata failing the Chesapeake Bay benthic community restoration goals, 1996 to 2021. Panels on left show percent total area degraded (B-IBI<3.0); panels on right show percent area severely degraded (B-IBI≤2.0). Error bars indicate ± 1 standard error

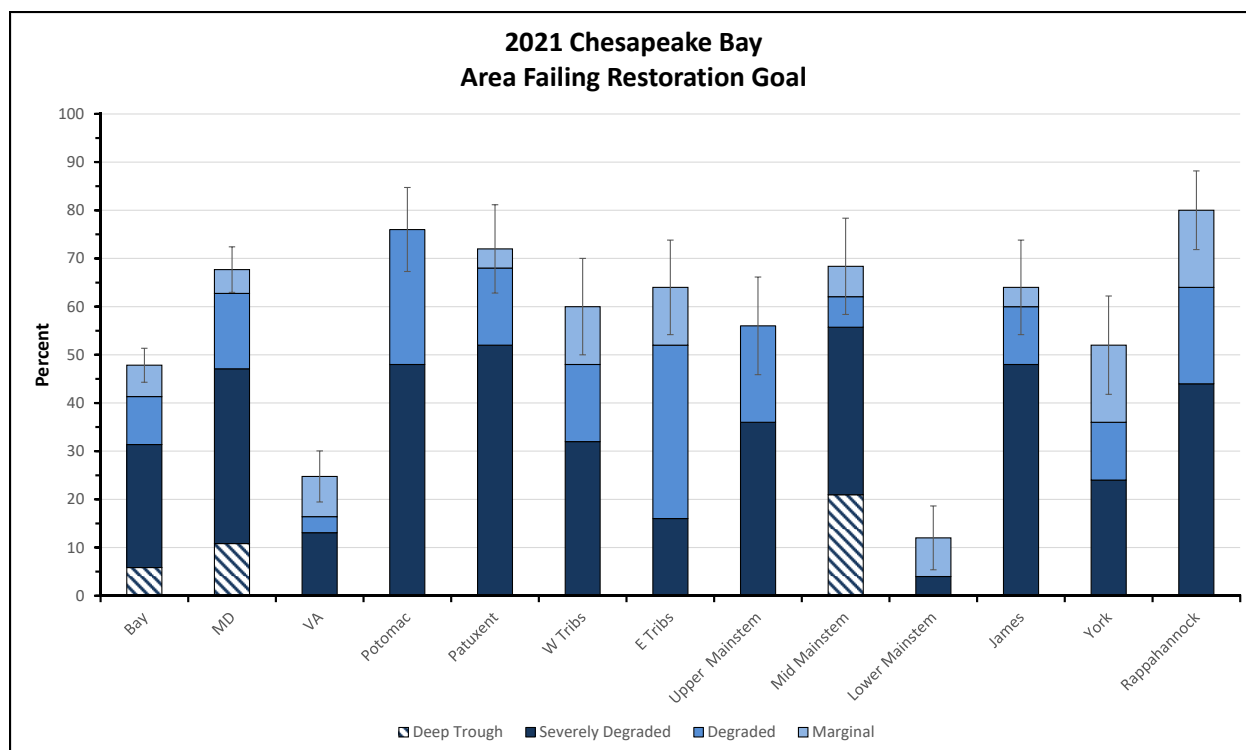


Figure 3-34. Proportion of the Chesapeake Bay, Maryland, Virginia, and the 10 sampling strata failing the Chesapeake Bay benthic community restorations goals in 2021. The deep trough is considered severely degraded. Error bars indicate ± 1 standard error. See cautionary note in page 3-36

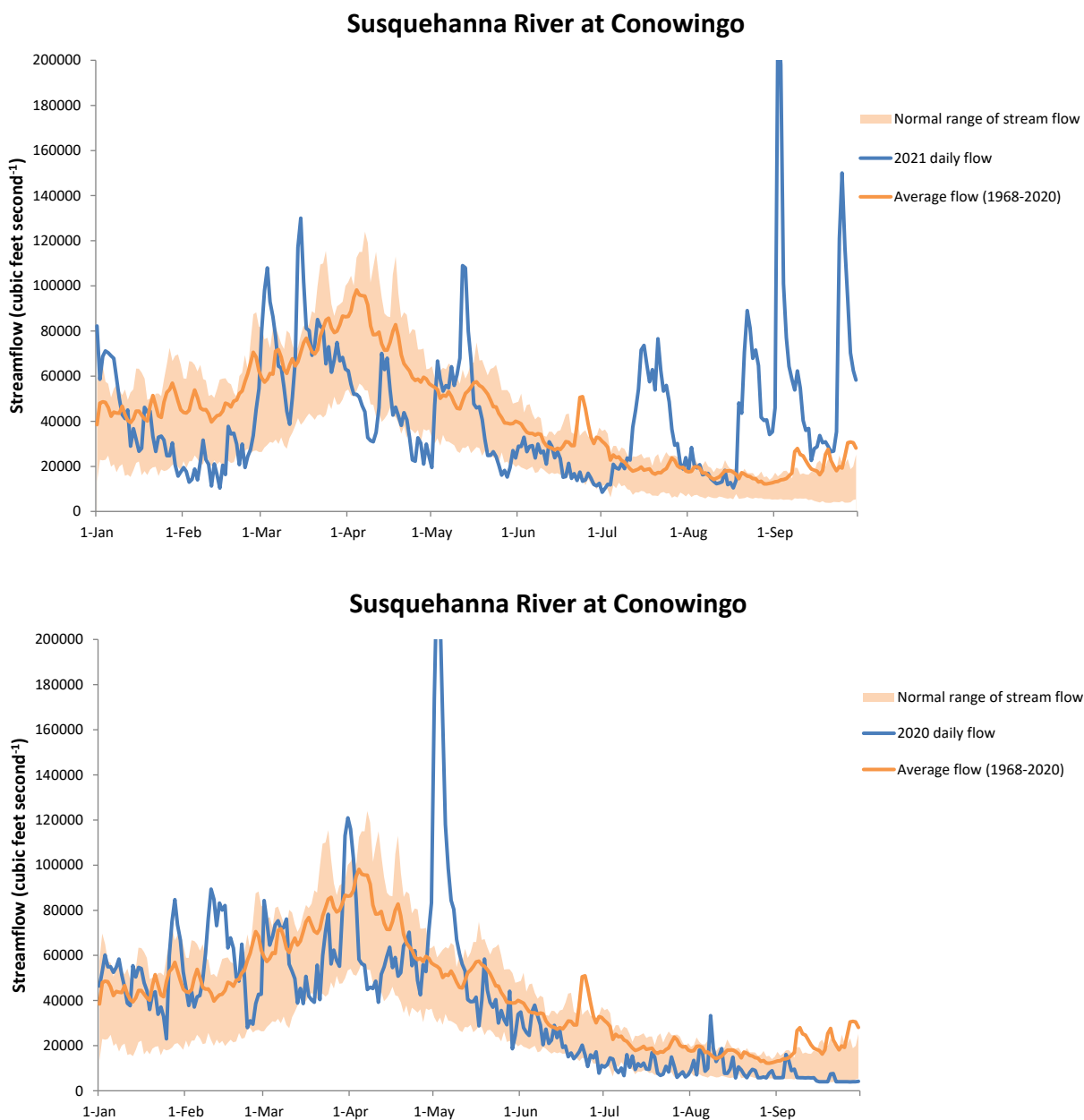


Figure 3-35. Daily flow entering Chesapeake Bay from the Susquehanna River at Conowingo in 2021 (top panel) and 2020 (bottom panel) compared to the long-term average, January through September. Normal range of stream flow: 25%-75%. Data source: United States Geological Survey

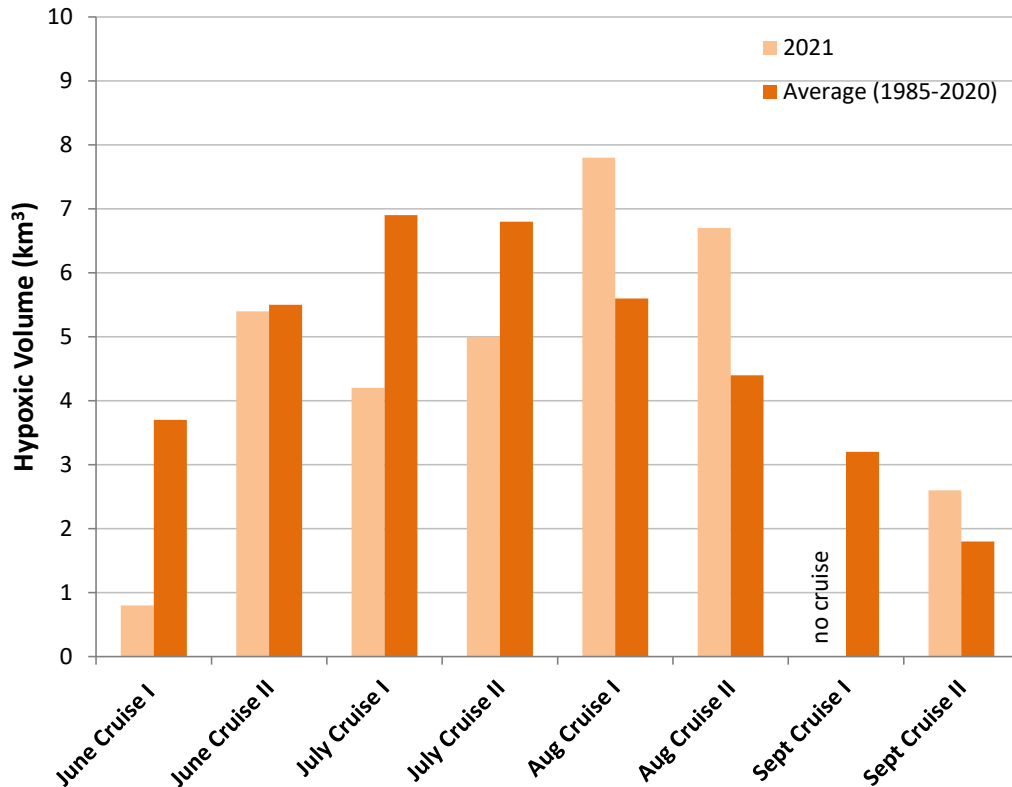


Figure 3-36. Hypoxic volume in Chesapeake Bay in 2021 compared to the long-term average. Data provided by Mark Trice, Maryland Department of Natural Resources (DNR). Cruises conducted by Maryland DNR and Virginia DEQ

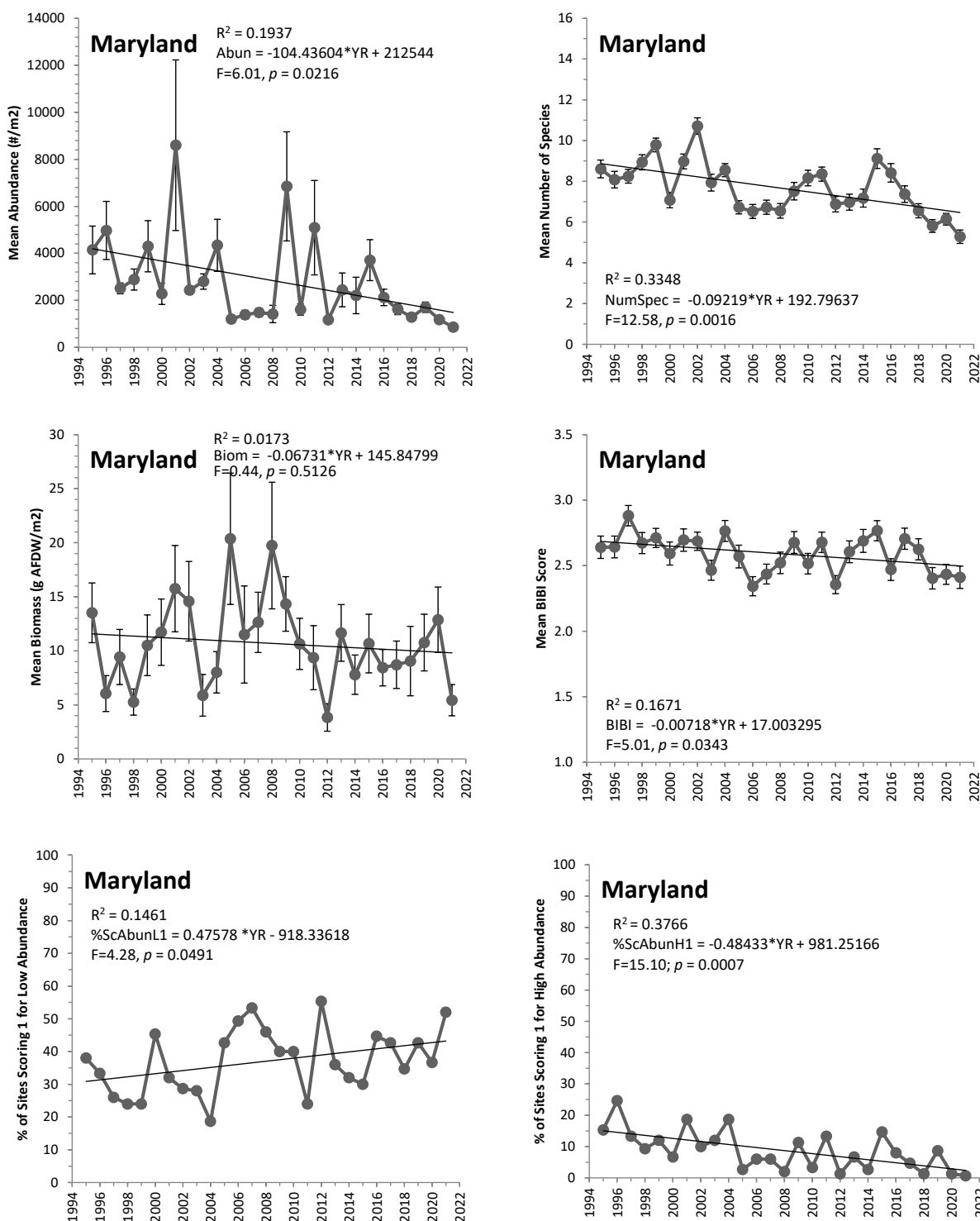


Figure 3-37. Trends in abundance, biomass, number of species, B-IBI (mean \pm 1 SE), and percent sites scoring "1" for low abundance and "1" for high abundance in Maryland tidal waters, 1995-2021 (N = 150 sites per year)

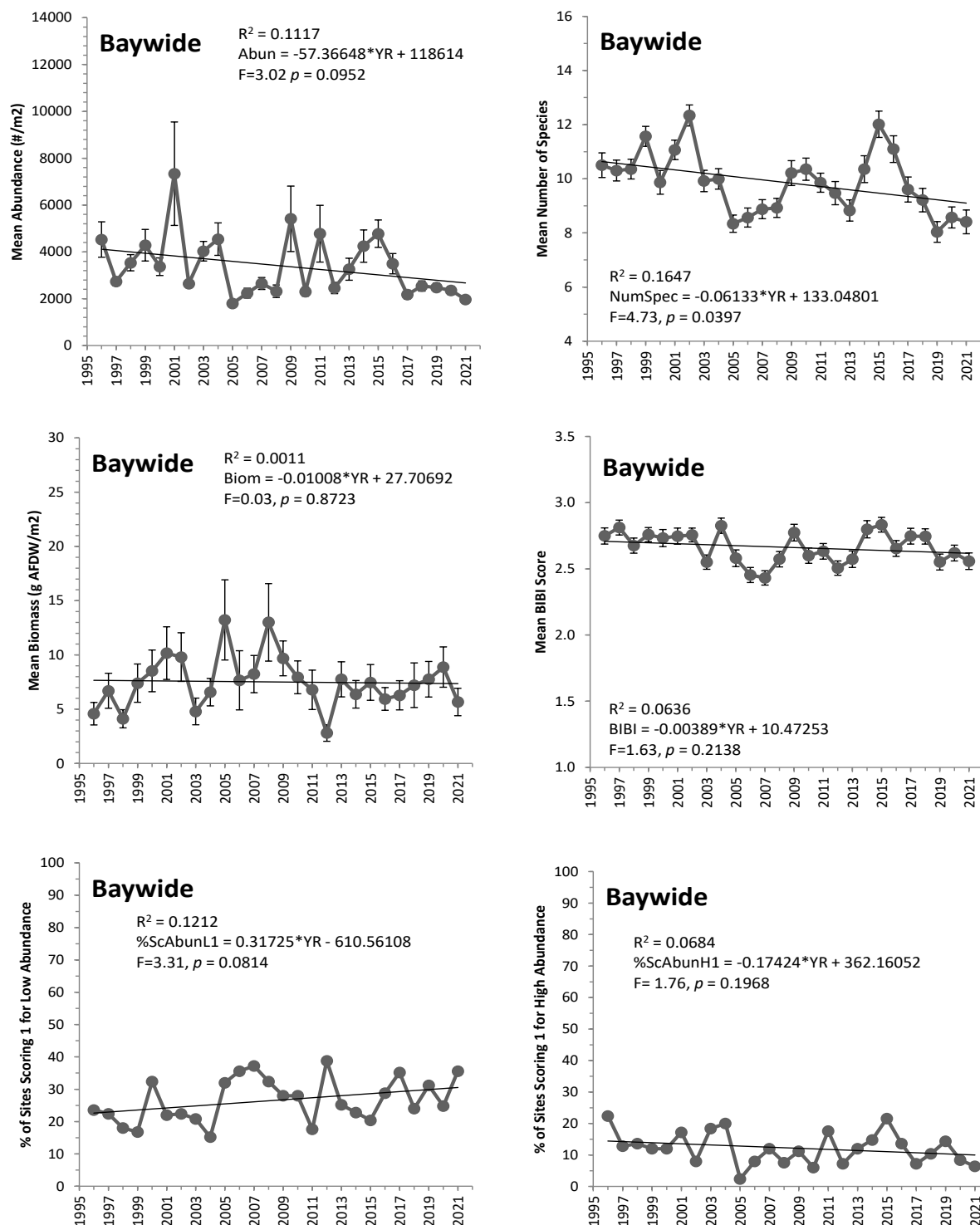


Figure 3-38. Trends in abundance, biomass, number of species, B-IBI (mean \pm 1 SE), and percent sites scoring "1" for low abundance and "1" for high abundance in Chesapeake Bay, 1996-2021 (N = 250 sites per year)

3.3 BASIN-LEVEL BOTTOM COMMUNITY CONDITION

Probability-based sampling can be used to produce areal estimates of degradation for regions of interest. The 2021 random sites were post-stratified into 15 reporting regions used by the Chesapeake Bay Program to assess the health of the Bay's ecosystem (Figure 3-39). The Bay Program conducts an annual integrated assessment for the Bay and its tidal tributaries using indicators of water quality conditions (chlorophyll-*a*, dissolved oxygen, water clarity, total nitrogen, total phosphorus), living resources (plankton and benthos), and habitat (bay grasses) combined into a Bay Health Index (BHI, Williams et al. 2009). The BHI is a spatially explicit management tool that was developed to evaluate the status of water quality, habitat quality, and biotic condition in Chesapeake Bay. This information is linked to nutrient and sediment pollution sources and is intended to assist in setting restoration goals at the level of tributary basins.

Probability-based estimates for each region followed the methods described in Section 2.4.3 for single Benthic Monitoring Program strata (formulae 1 and 2), except for regions that overlapped strata (Maryland Upper Eastern Shore, Choptank River, Maryland Lower Eastern Shore, and Mid Bay regions). Regions that overlapped benthic program strata were partitioned into the portions corresponding to each stratum, and the estimates for each portion or sub-region were weighted by area and combined into region-wide estimates, as described in Section 2.4.3 (formulae 3 and 4). For example, the Choptank River reporting region consisted of two sub-regions: the Choptank River proper (Bay Program segments CHOTF, CHOOH, and CHOMH2) and the open waters of the Choptank and Little Choptank Rivers (Bay Program segments CHOMH1 and LCHMH). While the former sub-region is part of the Maryland Eastern Tributaries stratum, the latter is part of the Maryland Mid Bay Mainstem stratum. Thus, degradation estimates were produced for each of the Choptank River sub-regions, weighted by the proportion of area represented by each sub-region, and combined.

At the BHI reporting region level, degradation in 2021 was largest in the Chesapeake Mid Bay Mainstem (Table 3-7) (but see cautionary note on page 3-36). However, the percent area degraded in the mainstem decreased by 28% in 2021 relative to 2020. The Potomac River and the Maryland Lower Eastern Shore tributaries had the next largest area degraded. This area increased by 5% in the Potomac River and decreased by 48% in the Lower Eastern Shore tributaries relative to 2020. Large decreases in degradation were also recorded in the Choptank River (45% decrease), Patapsco/Back rivers (30% decrease), and Lower Bay (28% decrease). Large increases in degradation were recorded in the Rappahannock River (28% increase), York River (24% increase), and Upper Bay mainstem (19% increase). Changes in the Maryland regions should be viewed with caution because of the potential effects of a problem with the 2021 random site selection process (see page 3-36 of this report). Nonetheless, the uncertainty associated with regional estimates is usually large because of small sample size or poor data coverage in some of the regions. Thus, at the BHI reporting region level, large changes in benthic condition are likely to be observed from year to year.

Table 3-6. Estimated tidal area failing to meet the Chesapeake Bay benthic community restoration goals in 2021 by Bay Health Index (BHI) Reporting Region and Tributary Basin. See Figure 3-39 for reporting regions. *Northeast River (part of the Maryland Upper Eastern Shore) is not included in the estimates because of insufficient data. See cautionary note in page 3-36.

Region/Basin	Percent Failing	Km² Failing	SE	N
Rappahannock River	80	298	8.2	25
Maryland Upper Eastern Shore*	78	359	8.6	13
Potomac River	75	952	6.3	25
Patuxent River	72	92	9.2	25
Maryland Upper Western Shore	67	59	16.7	9
James River	65	416	10.9	20
Elizabeth River	60	28	24.5	5
Patapsco/Back Rivers	60	66	16.3	10
Mid Bay	58	1,372	7.9	12
Upper Bay	56	442	10.1	25
York River	52	97	10.2	25
Maryland Lower Western Shore	50	50	22.4	6
Maryland Lower Eastern Shore	41	614	9.9	20
Lower Bay	15	466	8.2	20
Choptank River	14	61	4.2	10

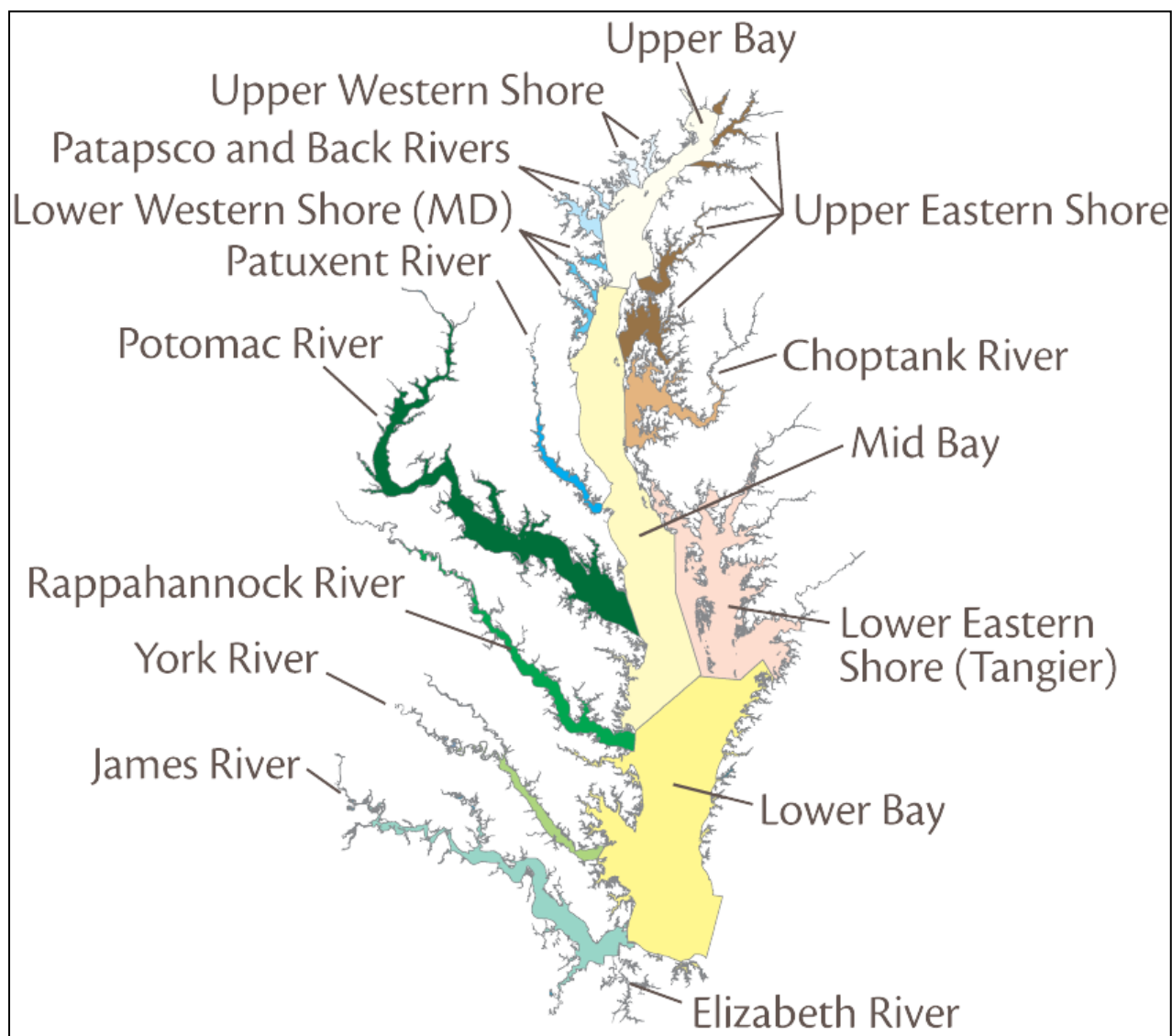


Figure 3-39. Bay Health Index Reporting Regions and Tributary Basins. Source: *EcoCheck*, University of Maryland Center for Environmental Science

3.4 RELATIONSHIP OF BENTHIC CONDITION MEASURES WITH FLOW

Water quality is usually influenced by years of high and low precipitation and hence river flow. Because dry and wet years can mask most pollution trends, changes in water quality resulting from management actions for which freshwater flow is factored out are of greatest interest to environmental managers. The present study was conducted in 2018 but it is included in this report as a reference to the other analytical sections. In this study 23 years of probability-based, benthic community data were analyzed to evaluate the correspondence between measures of benthic community condition and river flow as categorical predictor variable. This study is a re-run of an original study conducted in 2010 (Llanso et al. 2011). The objective is to assess whether the original results hold with additional data through 2017.

General linear models (GLM) were used to evaluate the correspondence between measures of benthic condition and river flow. Analysis of Variance (ANOVA) was used in the GLM with river flow as categorical predictor variable. Flow was represented by spring (February-June), summer (July-September), and annual (January-September 30) averages of daily fall-line gage measurements from the Susquehanna River at Conowingo, and alternatively from the Patuxent, Potomac, Rappahannock, York, and James rivers. The original analysis did not include February in the spring average. Analysis regions are the random sites in each sampling strata segregated into the tidal fresh, oligohaline, and mesohaline portions of the strata using the Bay Program segmentation to define the boundaries of each salinity portion. Spring, summer, and annual mean flows above the 75th percentile of the normal range of mean flows for the baseline period were categorized as high; otherwise, flows were categorized as normal or low. The baseline period was the longest period of record available in the USGS National Water Information System (<http://waterdata.usgs.gov/nwis>). River flow in years of heavy precipitation lasting only a few days but contributing to near-record precipitation levels exhibit high standard deviations. To capture this variability, spring flow was categorized as of high or low standard deviation and used as an independent variable in the GLM analysis. The period of record for the random-site B-IBI used in this analysis is 23 years: 1995-2017 (1996-2017 for Virginia).

Table 3-8 presents the results for statistically significant variables. Few B-IBI metrics differed significantly between years of high and low or normal spring, summer, and annual mean flow. However, metrics in the mainstem differed significantly between years of high and low standard deviation (s.d.) of spring flow. Pulses in Susquehanna River flow were significantly associated with higher benthic community degradation in the Chesapeake Bay mainstem. B-IBI scores, number of species, and Shannon diversity were lower in years of high s.d. in spring river flow than in years of low s.d. in spring river flow. Abundance of suspension feeders in the Upper Bay and the James River (bivalve habitat) was also lower in years of high s.d. in spring river flow. This last result is in agreement with observed declines in bivalve abundance at the Upper Bay mainstem fixed Station 026. The results of the present study confirm a relationship between pulses in river flow and benthic community condition in the Chesapeake Bay mainstem.

Table 3-8. General linear model results of B-IBI metrics for river flow scenarios, with river flow as categorical predictor variable. River flow was the average of spring (February-June), summer (July-September), or annual (January-September) daily fall-line gage measurements from the Susquehanna River at Conowingo (mainstem strata), Patuxent River, Potomac River, York River, and James River. S.D. of Spring Flow was the standard deviation of spring (February-June) daily fall-line gage measurements from same tributaries as above. Statistically significant results ($p \leq 0.05$) are included in the table. High = High flow or S.D., Low = Low flow or S.D. See methods for flow classification. Abundance and biomass metrics are log-transformed. Strata: UPB = Upper Bay, MMS = Maryland mainstem, VBY = Virginia mainstem, PXR = Patuxent River, PMR = Potomac River, YRK = York River, JAM = James River. TF = Tidal freshwater, OH = oligohaline, MH = mesohaline, PH = polyhaline.

Factor	Stratum	Metric	Overall ANOVA										Fit Statistics	Mean Metric for Levels of Factor			
			Model					Error			Corrected Total			High		Low	
			DF	SS	MS	F Value	Prob. F	DF	SS	MS	DF	SS	R ²	N	Mean	N	Mean
Spring Flow	PMR MH	B-IBI	1	0.312	0.312	4.932	0.037	21	1.328	0.063	22	1.640	0.190	6	1.55	17	1.82
	VBY MH	B-IBI	1	1.678	1.678	9.353	0.006	20	3.588	0.179	21	5.266	0.319	2	2.19	20	3.15
	VBY MH	N of Species	1	109.189	109.189	6.282	0.021	20	347.650	17.383	21	456.839	0.239	2	9.13	20	16.87
	VBY MH	Shannon Diversity	1	2.535	2.535	13.127	0.002	20	3.862	0.193	21	6.397	0.396	2	1.79	20	2.97
Summer Flow	UPB OH	Biomass/m2	1	0.299	0.299	9.703	0.005	21	0.648	0.031	22	0.947	0.316	7	1.38	16	1.63
	PMR MH	B-IBI	1	0.397	0.397	6.699	0.017	21	1.244	0.059	22	1.640	0.242	6	1.53	17	1.83
	YRK MH	N of Species	1	11.343	11.343	7.288	0.014	20	31.129	1.556	21	42.471	0.267	6	10.86	16	12.47
Annual Flow	UPB OH	Biomass/m2	1	0.407	0.407	15.818	0.001	21	0.540	0.026	22	0.947	0.43	4	1.27	19	1.62
	PMR MH	B-IBI	1	0.312	0.312	4.932	0.037	21	1.328	0.063	22	1.640	0.19	6	1.55	17	1.82
	PMR TF	Biomass/m2	1	2.887	2.887	7.070	0.016	19	7.760	0.408	20	10.647	0.27	4	0.74	17	1.68
	VBY MH	B-IBI	1	1.115	1.115	5.371	0.031	20	4.152	0.208	21	5.266	0.21	4	2.58	18	3.17
S.D. of Spring Flow	UPB MH	Abun Susp Feeders	1	0.048	0.048	4.611	0.044	21	0.220	0.010	22	0.269	0.180	7	0.132	16	0.232
	MMS MH	B-IBI	1	0.248	0.248	6.124	0.022	21	0.850	0.040	22	1.098	0.226	7	2.411	16	2.637
	MMS MH	N of Species	1	26.966	26.966	9.062	0.007	21	62.487	2.976	22	89.453	0.301	7	8.794	16	11.148
	MMS MH	Shannon Diversity	1	0.316	0.316	5.789	0.025	21	1.148	0.055	22	1.464	0.216	7	2.012	16	2.267
	VBY MH	B-IBI	1	1.170	1.170	5.714	0.027	20	4.096	0.205	21	5.266	0.222	7	2.723	15	3.218
	VBY MH	N of Species	1	149.296	149.296	9.709	0.005	20	307.543	15.377	21	456.839	0.327	7	12.357	15	17.950
	VBY MH	Shannon Diversity	1	2.349	2.349	11.604	0.003	20	4.048	0.202	21	6.397	0.367	7	2.386	15	3.087
	VBY PH	B-IBI	1	0.384	0.384	8.525	0.008	20	0.900	0.045	21	1.284	0.299	7	3.111	15	3.395
	VBY PH	N of Species	1	106.140	106.140	21.608	0.000	20	98.240	4.912	21	204.380	0.519	7	18.845	15	23.561
	VBY PH	Shannon Diversity	1	0.329	0.329	5.999	0.024	20	1.096	0.055	21	1.424	0.231	7	2.999	15	3.261
	PXR MH	Shannon Diversity	1	0.607	0.607	5.996	0.023	21	2.127	0.101	22	2.735	0.222	9	1.760	14	2.093
	JAM MH	Abun Susp Feeders	1	0.009	0.009	8.861	0.007	20	0.021	0.001	21	0.030	0.307	5	0.114	17	0.163

3.5 BENTHIC CHLOROPHYLL-A AND PHAEOPHYTIN

The monitoring of microphytobenthos was initiated in 2021 with samples collected in summer to determine surface chlorophyll-a concentrations as an index of algal biomass. Biomass of microphytobenthos varies with irradiance, temperature, and nutrient availability (Jacobs et al. 2021). Microphytobenthos help regulate the flux of nutrients and oxygen at the sediment-water interface (Sundback et al. 2000) and may be useful in tracking eutrophication (Kemp et al. 2005). Benthic chlorophyll-a concentrations at the fixed sites (mg/m^2) were determined from three surface sediment replicate samples (2.5 cm diameter x 1 cm sediment cores, 4.91 cm^3).

Chlorophyll-a is corrected for the presence of its degradation product phaeophytin, and the results are presented separately for chlorophyll-a and phaeophytin in Figure 3-40. Individual replicate sample concentrations are presented in Appendix D. Mean chlorophyll-a concentrations per site ranged between 5 and $85 \text{ mg}/\text{m}^2$ at 25 sites, and concentrations $>100 \text{ mg}/\text{m}^2$ were measured at sites 006 and 051 (Figure 3-40). The latter two sites, Mid Bay mainstem at Calvert Cliffs and the lower shallow Potomac River, may indicate bloom conditions. These are also sandy, shallow sites with low silt-clay concentrations. Mean phaeophytin concentrations varied between $33 \text{ mg}/\text{m}^2$ in the mainstem at Calvert Cliffs (Site 001) and $300 \text{ mg}/\text{m}^2$ in the lower Patuxent River (Site 071).

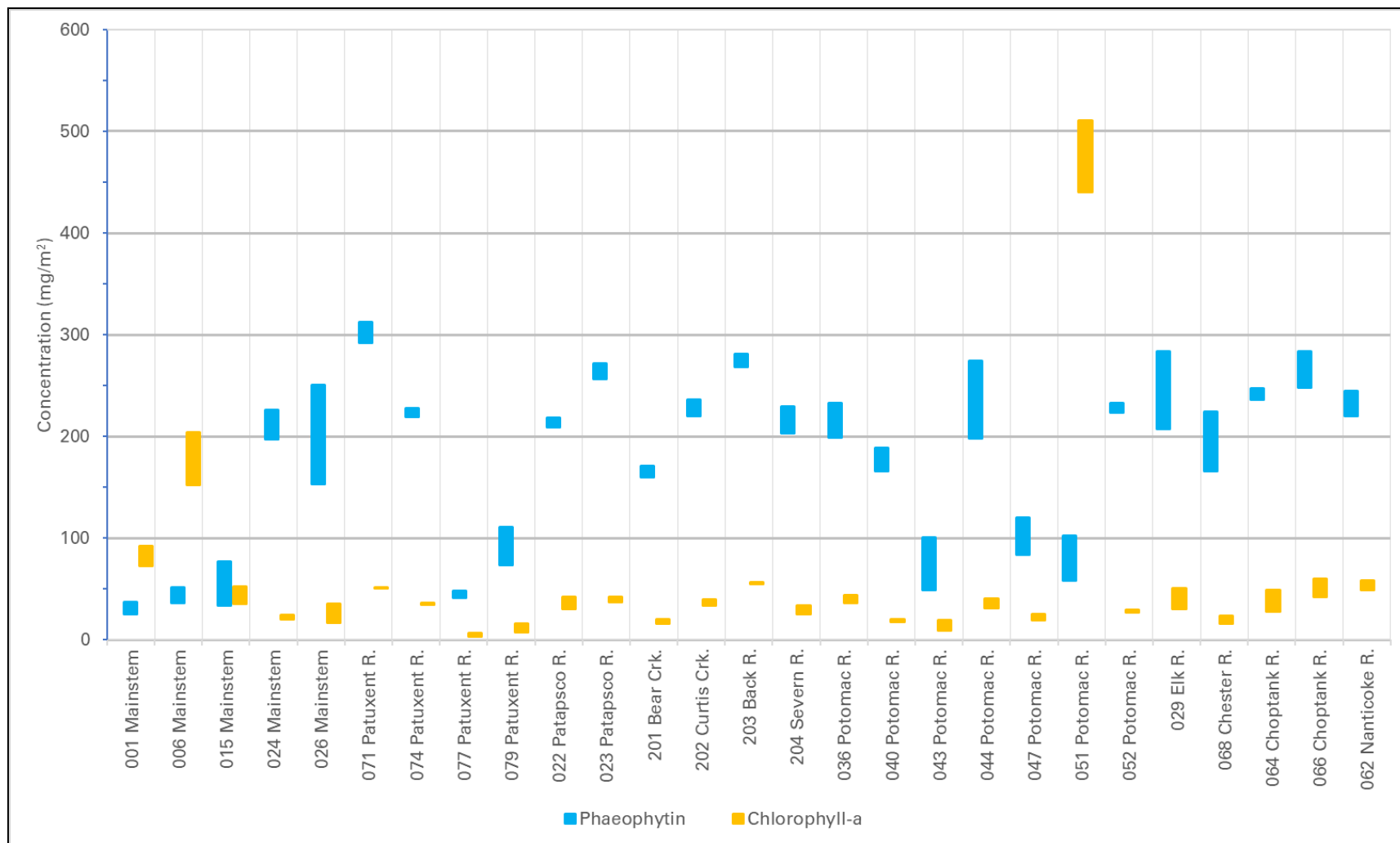


Figure 3-40. Benthic chlorophyll-*a* and phaeophytin concentration ranges (n = 3) at fixed sites, 2021

4.0 DISCUSSION

The highlights for 2021 can be summarized as follows (but see cautionary note on page 3-36 of this report):

(1) The tidal area with degraded benthos in Chesapeake Bay decreased from 61% in 2020 to 48% in 2021. There was no statistically significant trend in percent area degraded over the 1996-2021 time period.

(2) In Maryland degradation also decreased in 2021, but the change was within the margin of error of the estimate (however, see cautionary note). By area, 68% of the Maryland Bay's tidal waters failed to meet the benthic community restoration goals in 2021. In 2020, 73% of the Maryland Bay's tidal waters failed the goals. There was no statistically significant trend in percent area degraded over the 1995-2021 time period.

(3) In 2021 degradation decreased in the Maryland mainstem, Maryland Western Tributaries and Patuxent River, and increased in the Upper Bay mainstem, Maryland Eastern Tributaries, and the Potomac River (with the caution noted on page 3-36). The Potomac River, Patuxent River, and Maryland mainstem were in poorest condition in 2021, with 68-72% of their tidal areas failing the restoration goals. The Upper Bay Mainstem was in best condition.

(4) Benthic community condition (B-IBI scores averaged over the last 3 years of monitoring) remained within the same condition category at most of the fixed sites, improved at 2 sites from failing the goals to meeting the goals, and declined at 5 sites. Currently, 6 sites meet the benthic community restoration goals and 21 sites fail the goals.

(5) Statistically significant B-IBI trends were detected at 17 of the 27 fixed sites, with 12 sites exhibiting declines in benthic condition and 5 sites exhibiting improvements. Changes in B-IBI trends between 2020 and 2021 were limited to the appearance of a declining B-IBI trend in Curtis Bay (Patapsco River estuary).

Benthic community condition in Chesapeake Bay improved considerably in 2021 after two years of high degradation exceeding 60% of the bay's tidal area. The Virginia mainstem contributed largely to this improvement, although the Maryland mainstem also improved. Although the improvement is likely to be real, its magnitude cannot be determined with confidence because of an inconsistent application of the random-site selection process in 2021 that affected Maryland results. The improvement appears to have been greatest in the mainstem of the Chesapeake Bay, while the tributaries showed both increases and decreases in benthic degradation. Virginia tributaries, not affected by the random-site selection misapplication, showed declines in benthic condition (increasing degradation) in 2021, with the Rappahannock River exhibiting the largest decline.

An improvement was also seen at the fixed monitoring sites. The B-IBI score for a majority of the sites in 2021 increased from the previous year or remained unchanged. Only three sites exhibited declines in the B-IBI, two in the Potomac River (tidal freshwater and low mesohaline portions) and one in the Choptank River (oligohaline portion). The tidal fresh Potomac River and the oligohaline Choptank River also had increasing trends in abundance above the upper threshold for excess abundance. Typically, in years of high precipitation and stream runoff many of the fixed sites exhibit low B-IBI scores, and this was not the case in 2021. This result contrasts with the lower overall abundance, biomass, and number of species at the probability-based sites. Although the average B-IBI score did not change at these sites, the lower abundance, biomass, and taxa metric values may have been a result of the inconsistent application of the random-site selection process in 2021, so these latter results cannot be held with confidence.

Improvements in benthic condition in 2021 are consistent with those observed in years of moderate precipitation and river flow. Whereas large amounts of precipitation fell over the Chesapeake Bay watershed in the spring of 2019 resulting in massive flows, and pulses in river flow occurred in the spring of 2020, river flow in 2021 was below average early in the year. Excess nutrient runoff after heavy rains changes the balance of biological and chemical processes and the alteration of these processes often lead to hypoxia and loss of benthic biomass and productivity. In 2021 hypoxic water volume was below average in early summer, increasing only above average in August and September. High water temperatures and diminished wind speeds are likely to have contributed to the higher hypoxic volume in August (MDNR 2021). Temperature and winds are significant factors modulating hypoxia in Chesapeake Bay (Scully 2010, Lee et al. 2013, Zhou et al. 2014).

Nutrient inputs typically fuel benthic and phytoplankton growth in the spring as waters warm up, leading to oxygen consumption and development of summer-time hypoxia (Tuttle et al. 1987, Kemp et al. 2005). In years with pulses in spring river flow effects on benthic communities are manifested through low B-IBI scores as revealed by general linear model analysis of flow and benthic data (Llansó et al. 2011, this report). The intensity and periodicity of the spring river flow appear to be factors affecting benthic condition. Pulses in spring river flow were moderate in 2021, and this is likely to have been a contributing factor to the better overall benthic condition observed in Maryland tidal waters in 2021.

Time series analysis of the fixed sites has revealed a shift in summer hypoxia from midsummer to early summer (Llansó et al. 2011). This shift appeared in 1998 and coincided with decreases in abundance and species numbers at many of the fixed sites in the Maryland tributaries and the mainstem. Likewise, Murphy et al. (2011) observed increasing hypoxia in June over time. The implications of such a shift is the potential for cumulative impacts on the benthic community through suppression of recruitment processes and an inability of the community to recover from previous years hypoxic

events. Management actions that help mitigate factors that lead to early hypoxia, such as runoff and excess nutrient delivery in years of high spring flow, thus become critical in Bay restoration efforts.

Fixed-site and probability-based sampling strata in 2021 continued to show improvements in benthic community condition from excess abundance (eutrophic condition). The percentage of sites in Maryland tidal waters scoring 1 for excess abundance declined significantly through 2021. This trend is important because it may signal favorable conditions in recent years associated with restoration efforts to reduce nutrient pollution. We will continue to track eutrophic conditions and examine B-IBI changes in the Bay that might be directly attributed to declining nutrient inputs.

Diverging patterns in benthic condition also suggest water quality influences that differ between the upper (land-base, agricultural) and the lower (open water, bay-influenced) reaches of the estuary. For example, the upper Choptank River (Station 066) exhibited a degrading trend in the B-IBI through 2021. This trend reflected increases in abundance of pollution-indicative organisms above restorative thresholds at this site. In the lower Choptank River, however, an improving trend in the B-IBI reflected increases in the biomass of the bivalve *Macoma balthica*, suggesting the establishment and development of a mature community.

Despite the improvements observed in recent years, benthic community condition in Chesapeake Bay remains largely degraded. Biomass-dominant species have declined over the years (Llansó et al. 2013, Seitz et al. 2009) and low rates of benthic secondary production are observed in areas impacted by hypoxia (Sturdivant et al. 2014), most dramatically in the Patuxent and Potomac rivers (Dauer et al. 2011, Llansó et al. 2012). This background suggests that the recovery of the benthic communities, on which many fisheries and avian species depend, may be tied to factors in which not only management plays a role but increasingly important aspects of climate change (sensu Lee et al. 2013) interact with species populations to provide patterns of benthic community change that mask the restoration efforts. Inter-annual variability in benthic condition, however, suggests that benthic communities are resilient and respond quickly to improvements in water quality.

The results presented in this report were enabled by the combination of probability-based sampling and fixed point monitoring. Probability-based sampling allows determination of levels of benthic community degradation at multiple spatial scales, from strata and Bay Health Index reporting regions (this report) to tidal creeks (Dauer and Llansó 2003) and Chesapeake Bay Program segments (Llansó et al. 2003). Probability-based data are also useful for reporting overall condition and identification of impaired waters (305b report) under the Clean Water Act (Llansó et al. 2005, 2009a). These assessments are dependent on fully validated thresholds for assessing benthic community condition at sampling sites. The thresholds were established and validated by Ranasinghe et al. (1994) and updated by Weisberg et al. (1997). The thresholds and

the B-IBI and its component metrics allow for a validated, unambiguous approach to characterizing conditions in the Chesapeake Bay. The B-IBI has been shown by Alden et al. (2002) to be sensitive, stable, robust, and statistically sound. Its performance was verified by Llansó et al. (2009b) using data independent of those used in the initial index development effort. This last study revealed good classification performance of the B-IBI, balanced Type I and Type II errors, and the influence of a variety of metrics in the final B-IBI score, characteristics that made assessments in Chesapeake Bay more reliable with the B-IBI than with any of the alternative benthic indicators.

The use of probability-based sampling and fixed point monitoring allows us to provide an overall picture of benthic condition in Chesapeake Bay that helps track the success of efforts to clean up the bay. This picture would not have emerged if only water quality were monitored, and points to the value of long-term biological monitoring in the face of natural variability and variability from climate change.

Finally, continuing monitoring on the appearance of non-indigenous species showed a spreading population of *Hermundura americana* (Polychaeta: Pilargidae). This species is now density-dominant in the low mesohaline portion of the Potomac River. *H. americana* is a warm-water polychaete worm in subtidal mud and sand bottoms of the Gulf of Mexico and Central America. It was first reported in Chesapeake Bay in the Southern Branch of the Elizabeth River in a single benthic sample in 2009. From the Elizabeth River this species spread into the James River in 2012 and is now found throughout the tidal James River and its tributaries. In 2018 *H. americana* was found in the Maryland portion of the Chesapeake Bay at five locations, three in the Potomac River near Morgantown and two in the Wicomico and Nanticoke rivers. *H. americana* has already colonized a wide range of salinity, depth, and sediment type in the James River, Rappahannock River, and Potomac River. The potential ecological community effects of this species as it expands throughout the Chesapeake Bay are unknown.

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APPENDIX A**FIXED SITE COMMUNITY ATTRIBUTE
1985-2021 TREND ANALYSIS RESULTS**

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Appendix Table A-1. Summer trends in benthic community attributes at mesohaline stations 1985-2021. Shown is the median slope of the trend. Monotonic trends were identified using the van Belle and Hughes (1984) procedure. (a) trends based on 1989-2021 data; (b) trends based on 1995-2021 data; (c) attribute trend based on 1990-2021 data; (d): attributes are used in B-IBI calculations when species specific biomass is unavailable; (e): attribute and trend are not part of the reported B-IBI. Probability values shown in Table A-3.

Station	B-IBI	Abundance	Biomass	Shannon Diversity	Indicative Abundance	Sensitive Abundance	Indicative Biomass (c)	Sensitive Biomass (c)	Abundance Carnivore/ Omnivores
Potomac River									
43	-0.0250	-49.4678	-0.5622	-0.0077	0.1896	-0.7359 (d)	0.0277 (e)	-1.8852	0.0924 (e)
44	0.0000	-6.3637	-0.0148	-0.0055	-0.3831	0.0000 (d)	0.0000 (e)	0.0000	0.4257 (e)
47	0.0000	-53.3333	-0.7757	-0.0080	0.1530	-0.7703 (d)	0.0364 (e)	-1.7004	0.0834 (e)
51	0.0000	-32.8992	-0.0636	-0.0013	-0.5293	0.0419	0.0000 (e)	-0.6144 (e)	0.3968
52	0.0000	0.0000	0.0000	0.0000	0.0000 (d)	0.0000 (d)	0.0000	0.0000	0.0000
Patuxent River									
71	-0.0256	-29.0000	-0.0155	-0.0360	0.2032 (d)	-0.0000 (d)	0.5148	0.0000	0.0000
74	0.0000	25.8000	-0.6337	-0.0025	0.1031	-0.5048 (d)	0.0018 (e)	-0.2664	-0.1709 (e)
77	-0.0211	-8.7311	-0.0282	-0.0009	0.6415	-0.1055 (d)	-0.2311 (e)	0.8592	-0.0718 (e)
Choptank River									
64	0.0133	-14.0909	0.1265	0.0073	-0.2547 (d)	0.7599 (d)	-0.0020	0.0409	0.1097
Maryland Mainstem									
01	0.0000	-26.1538	-0.0123	-0.0011	-0.1302	-0.1786	0.0000 (e)	-0.1061 (e)	-0.4781
06	0.0000	-3.4534	0.0028	-0.0040	-0.0471	-0.3730	0.0275 (e)	-0.3271 (e)	-0.4772
15	0.0000	-9.0909	-0.0171	-0.0085	-0.2864	-0.0113	0.0857 (e)	-0.3133 (e)	0.0106
24	0.0000	-9.0000	0.1980	-0.0195	-0.3400 (d)	0.5063 (d)	-0.0011	0.6518	0.1582
26	0.0000	-0.9625	-0.3746	0.0058	0.0402	-0.1707 (d)	0.0000 (e)	-0.0166	0.1190 (e)
Maryland Western Shore Tributaries									
22	-0.0192	-28.1433	-0.0023	-0.0418	0.6326	0.0000 (d)	0.0096 (e)	0.0000	-0.2579 (e)
23	0.0000	-48.1579	-0.0056	-0.0190	0.0000	0.4464 (d)	0.0000 (e)	0.0000	0.0000 (e)
201(a)	0.0000	-5.2607	0.0011	0.0000	0.0000	0.0000 (d)	0.0000 (e)	0.0000	0.0000 (e)
202(a)	0.0000	-9.7732	0.0000	0.0000	0.0000	0.0000 (d)	0.0000 (e)	0.0000	0.0000 (e)
204(b)	-0.0196	-61.9636	-0.0222	-0.0054	0.4358 (d)	0.0000 (d)	0.0100	-0.0978	-0.1832
Maryland Eastern Shore Tributaries									
62	-0.0522	156.1818	-0.0255	-0.0211	0.2841	-0.3311 (d)	0.1527 (e)	-1.8152	-0.2056 (e)
68	0.0000	7.0960	0.3480	-0.0117	0.1135	0.1780 (d)	0.0005 (e)	0.0204	-0.0794 (e)

Appendix Table A-2. Summer trends in benthic community attributes at oligohaline and tidal freshwater stations 1985-2021. Shown is the median slope of the trend. Monotonic trends were identified using the van Belle and Hughes (1984) procedure. (a): trends based on 1989-2021 data; NA: attribute not calculated. Probability values shown in Table A-4.

Station	B-IBI	Abundance	Tolerance Score	Freshwater Indicative Abundance	Oligohaline Indicative Abundance	Oligohaline Sensitive Abundance	Tanypodinae to Chironomidae Ratio	Abundance Deep Deposit Feeders	Abundance Carnivore/Omnivores
Potomac River									
36	-0.0263	76.8645	0.0134	0.4719	NA	NA	NA	0.3810	NA
40	0.0000	22.0737	-0.0020	NA	0.1803	0.0000	0.0000	NA	-0.0131
Patuxent River									
79	0.0000	-3.6482	-0.0076	-0.0425	NA	NA	NA	0.0272	NA
Choptank River									
66	-0.0190	67.2964	0.0620	NA	0.7011	0.0000	0.0000	NA	0.0460
Maryland Western Shore Tributaries									
203(a)	0.0361	9.0218	0.0000	NA	0.0000	0.0000	0.0000	NA	1.1388
Maryland Eastern Shore Tributaries									
29	0.0101	-43.1955	0.0031	NA	-0.3563	0.0789	0.0000	NA	0.2704

Appendix Table A-3. Summer trends in benthic community attributes at mesohaline stations 1985-2021. Shown is the probability for each trend. See Table A-1 for attribute information.

Station	B-IBI	Abundance	Biomass	Shannon Diversity	Indicative Abundance	Sensitive Abundance	Indicative Biomass (c)	Sensitive Biomass (c)	Abundance Carnivore/Omnivores
Potomac River									
43	0.00000	0.00006	0.00000	0.03627	0.00011	0.00002	0.00001	0.00000	0.21793
44	0.71208	0.36872	0.12048	0.24385	0.00151	0.89505	0.34147	0.81579	0.00005
47	0.00530	0.00001	0.00000	0.03296	0.00157	0.00002	0.00000	0.00000	0.36354
51	0.34450	0.00000	0.00001	0.70467	0.00001	0.47584	0.80340	0.00017	0.00326
52	0.02442	0.00007	0.00022	0.00039	0.11033	0.06575	0.82951	0.72653	0.04789
Patuxent River									
71	0.00000	0.00000	0.00000	0.00000	0.24142	0.00198	0.05152	0.25788	0.24138
74	0.78610	0.15771	0.00000	0.54135	0.06307	0.00100	0.15544	0.00000	0.02135
77	0.00328	0.34228	0.00243	0.83064	0.00101	0.43284	0.08497	0.07184	0.59383
Choptank River									
64	0.02291	0.06188	0.00445	0.11786	0.08805	0.00006	0.64843	0.77418	0.33472
Maryland Mainstem									
01	0.07149	0.00096	0.11796	0.77999	0.06249	0.11737	0.58554	0.33051	0.00221
06	0.58965	0.58184	0.57809	0.40501	0.18588	0.01507	0.15104	0.00357	0.00976
15	0.54668	0.19669	0.17816	0.01162	0.02828	0.66650	0.11609	0.06321	0.86611
24	0.38137	0.26908	0.00048	0.00001	0.00001	0.00024	0.13092	0.00303	0.28091
26	0.02133	0.84046	0.18996	0.19943	0.24184	0.41927	0.71586	0.03640	0.18902
Maryland Western Shore Tributaries									
22	0.00016	0.00090	0.15327	0.00000	0.00773	0.76461	0.30872	0.07465	0.00004
23	0.48598	0.00000	0.60302	0.00180	0.86124	0.00001	0.98669	0.78580	0.89149
201(a)	0.05255	0.18686	0.14285	0.41241	0.29111	0.25409	0.44137	0.50887	0.19771
202(a)	0.05837	0.00177	0.81216	0.03497	0.14997	0.83979	0.14847	0.58377	0.28935
204(b)	0.05161	0.00013	0.29982	0.35415	0.01260	0.74642	0.17007	0.61177	0.30745
Maryland Eastern Shore Tributaries									
62	0.00000	0.00000	0.00050	0.00005	0.00000	0.00000	0.00000	0.00017	0.00000
68	0.33604	0.69809	0.00088	0.01058	0.14948	0.24895	0.69821	0.48190	0.26361

Appendix Table A-4. Summer trends in benthic community attributes at oligohaline and tidal freshwater stations 1985-2021. Shown is the probability for each trend. See Table A-2 for attribute information. NA: attribute not calculated.

Station	B-IBI	Abundance	Tolerance Score	Freshwater Indicative Abundance	Oligohaline Indicative Abundance	Oligohaline Sensitive Abundance	Tanypodinae to Chironomidae Ratio	Abundance Deep Deposit Feeders	Abundance Carnivore/Omnivores
Potomac River									
36	0.00012	0.00075	0.00013	0.00265	NA	NA	NA	0.00078	NA
40	0.71319	0.01724	0.13490	NA	0.36512	0.82376	0.02229	NA	0.87758
Patuxent River									
79	0.61989	0.84061	0.05719	0.82511	NA	NA	NA	0.69115	NA
Choptank River									
66	0.00066	0.00001	0.00000	NA	0.00105	0.29250	0.03450	NA	0.62522
Maryland Western Shore Tributaries									
203(a)	0.00008	0.61273	0.99339	NA	0.46734	0.63655	0.27879	NA	0.00012
Maryland Eastern Shore Tributaries									
29	0.01091	0.01934	0.75063	NA	0.13178	0.12793	0.82169	NA	0.00000

APPENDIX B**FIXED SITE B-IBI VALUES, SUMMER 2021**

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Appendix Table B-1. Fixed site B-IBI values, Summer 2021.					
Station	Sampling Date	Latitude (WGS84 Decimal Degrees)	Longitude (WGS84 Decimal Degrees)	Mean B-IBI	Status
001	9/27/2021	38.41876902	-76.41851822	3.11	Meets Goal
006	9/27/2021	38.44201229	-76.44450212	2.00	Severely Degraded
015	9/27/2021	38.71518446	-76.51412037	1.67	Severely Degraded
022	8/23/2021	39.25805946	-76.59502392	2.73	Marginal
023	8/30/2021	39.20830314	-76.52337122	2.73	Marginal
024	8/18/2021	39.12201667	-76.35568333	3.33	Meets Goal
026	8/26/2021	39.27148333	-76.29001666	3.67	Meets Goal
029	9/9/2021	39.47947539	-75.94482854	3.11	Meets Goal
036	9/13/2021	38.76975535	-77.03753661	2.00	Severely Degraded
040	9/13/2021	38.357487	-77.23052582	3.71	Meets Goal
043	9/30/2021	38.38459952	-76.9880185	2.60	Degraded
044	9/7/2021	38.38549186	-76.99555417	1.93	Severely Degraded
047	9/30/2021	38.37655465	-76.9852166	2.60	Degraded
051	9/30/2021	38.20557263	-76.73917665	2.11	Degraded
052	8/17/2021	38.19235	-76.7477	1.00	Severely Degraded
062	9/27/2021	38.38393374	-75.85012394	1.80	Severely Degraded
064	9/15/2021	38.59057389	-76.06926033	4.11	Meets Goal
066	9/10/2021	38.80152	-75.92177	1.56	Severely Degraded
068	9/21/2021	39.13253853	-76.07877848	3.27	Meets Goal
071	8/24/2021	38.39512769	-76.54885791	1.22	Severely Degraded
074	8/27/2021	38.54902	-76.67625	4.07	Meets Goal
077	8/27/2021	38.60450923	-76.67500689	2.60	Degraded
079	8/27/2021	38.75048443	-76.68921346	3.00	Meets Goal
201	8/30/2021	39.23424463	-76.49753996	1.00	Severely Degraded
202	8/30/2021	39.21784862	-76.56413963	1.00	Severely Degraded
203	8/12/2021	39.27499694	-76.44441101	2.78	Marginal
204	8/31/2021	39.0069684	-76.50506665	2.89	Marginal

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APPENDIX C**RANDOM SITE B-IBI VALUES, SUMMER 2021**

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Appendix Table C-1. Random site B-IBI values, Summer 2021.					
Station	Sampling Date	Latitude (WGS84 Decimal Degrees)	Longitude (WGS84 Decimal Degrees)	B-IBI	Status
MET-28402	9/28/2021	38.12349	-75.88720	2.67	Marginal
MET-28404	9/28/2021	38.25726	-75.93216	3.00	Meets Goal
MET-28406	9/28/2021	38.31665	-75.90933	2.20	Degraded
MET-28407	9/27/2021	38.35707	-75.86061	2.60	Degraded
MET-28408	9/27/2021	38.37676	-75.86480	2.20	Degraded
MET-28409	9/27/2021	38.44134	-75.82745	1.50	Severely Degraded
MET-28410	9/27/2021	38.46457	-75.81722	3.00	Meets Goal
MET-28411	9/15/2021	38.60257	-76.06971	2.20	Degraded
MET-28412	9/15/2021	38.61524	-76.09710	1.40	Severely Degraded
MET-28413	9/15/2021	38.61722	-76.12602	4.20	Meets Goal
MET-28414	9/15/2021	38.61999	-76.14922	3.80	Meets Goal
MET-28415	9/15/2021	38.63140	-76.15647	2.60	Degraded
MET-28416	9/10/2021	38.71838	-76.00736	2.67	Marginal
MET-28417	9/10/2021	38.77498	-75.96781	4.50	Meets Goal
MET-28418	9/21/2021	38.99953	-76.18712	1.00	Severely Degraded
MET-28419	8/26/2021	39.00108	-76.26258	2.20	Degraded
MET-28420	9/21/2021	39.05787	-76.19197	1.40	Severely Degraded
MET-28422	9/21/2021	39.18160	-76.04882	2.20	Degraded
MET-28423	8/26/2021	39.38263	-76.02950	3.33	Meets Goal
MET-28424	9/9/2021	39.44952	-75.98874	3.00	Meets Goal
MET-28425	9/9/2021	39.50766	-75.89794	5.00	Meets Goal
MET-28426	9/15/2021	38.57918	-76.04152	2.20	Degraded
MET-28427	9/27/2021	38.21853	-75.83955	2.67	Marginal
MET-28428	9/21/2021	39.08287	-76.19760	2.20	Degraded
MET-28429	9/21/2021	39.14816	-76.08402	3.00	Meets Goal
MMS-28501	8/17/2021	37.99378	-76.06553	3.67	Meets Goal
MMS-28502	9/28/2021	38.01972	-75.91990	2.67	Marginal
MMS-28503	9/28/2021	38.02561	-75.94285	4.33	Meets Goal
MMS-28504	9/28/2021	38.04406	-75.93847	3.00	Meets Goal
MMS-28505	9/28/2021	38.07878	-75.90446	2.33	Degraded
MMS-28506	8/17/2021	38.09023	-76.12890	3.33	Meets Goal
MMS-28507	8/17/2021	38.21847	-76.35845	1.00	Severely Degraded
MMS-28508	9/28/2021	38.23206	-75.90788	2.67	Marginal
MMS-28509	8/17/2021	38.26982	-76.36102	1.67	Severely Degraded
MMS-28510	9/20/2021	38.27421	-76.09863	2.33	Degraded
MMS-28512	9/20/2021	38.28968	-76.24254	2.00	Severely Degraded
MMS-28513	8/18/2021	38.56543	-76.35053	1.00	Severely Degraded
MMS-28514	8/18/2021	38.59898	-76.39060	3.00	Meets Goal

Appendix Table C-1. (Continued)					
Station	Sampling Date	Latitude (WGS84 Decimal Degrees)	Longitude (WGS84 Decimal Degrees)	B-IBI	Status
MMS-28515	8/18/2021	38.65387	-76.23713	3.33	Meets Goal
MMS-28516	8/18/2021	38.66882	-76.32557	3.00	Meets Goal
MMS-28517	8/18/2021	38.69943	-76.37460	3.00	Meets Goal
MMS-28518	8/18/2021	38.73168	-76.35008	3.00	Meets Goal
MMS-28520	8/18/2021	38.75817	-76.47662	1.00	Severely Degraded
MMS-28521	8/18/2021	38.82738	-76.34928	1.00	Severely Degraded
MMS-28522	9/29/2021	38.85442	-76.24039	1.00	Severely Degraded
MMS-28523	9/29/2021	38.87656	-76.24032	1.00	Severely Degraded
MMS-28524	8/18/2021	38.92573	-76.43457	2.00	Severely Degraded
MMS-28525	8/18/2021	38.96240	-76.43625	3.00	Meets Goal
MMS-28526	8/18/2021	38.81283	-76.33308	1.67	Severely Degraded
MMS-28527	9/27/2021	38.19611	-75.92287	2.00	Severely Degraded
MWT-28301	8/31/2021	38.86689	-76.49597	3.40	Meets Goal
MWT-28302	8/31/2021	38.89609	-76.48483	3.80	Meets Goal
MWT-28303	8/31/2021	38.91935	-76.49157	3.80	Meets Goal
MWT-28304	8/31/2021	38.92825	-76.51340	2.20	Degraded
MWT-28306	8/31/2021	39.04691	-76.54390	1.00	Severely Degraded
MWT-28307	8/31/2021	39.08305	-76.51480	1.80	Severely Degraded
MWT-28308	8/10/2021	39.16043	-76.45888	2.60	Degraded
MWT-28309	8/10/2021	39.16856	-76.44579	3.00	Meets Goal
MWT-28310	8/10/2021	39.17280	-76.51302	3.00	Meets Goal
MWT-28311	8/10/2021	39.17451	-76.46171	3.00	Meets Goal
MWT-28312	8/10/2021	39.18677	-76.48402	3.40	Meets Goal
MWT-28314	8/10/2021	39.19955	-76.46731	2.60	Degraded
MWT-28315	8/30/2021	39.22829	-76.52992	1.00	Severely Degraded
MWT-28316	8/30/2021	39.23547	-76.54073	1.00	Severely Degraded
MWT-28317	8/12/2021	39.29768	-76.48752	1.00	Severely Degraded
MWT-28318	9/14/2021	39.30252	-76.36438	2.67	Marginal
MWT-28319	9/15/2021	39.34130	-76.32239	2.00	Severely Degraded
MWT-28321	9/15/2021	39.36431	-76.32076	2.67	Marginal
MWT-28322	9/17/2021	39.37552	-76.25887	3.67	Meets Goal
MWT-28323	9/15/2021	39.37816	-76.32634	2.33	Degraded
MWT-28324	9/14/2021	39.37965	-76.38576	2.00	Severely Degraded
MWT-28325	9/17/2021	39.41129	-76.24966	3.00	Meets Goal
MWT-28326	8/30/2021	39.24810	-76.57114	1.00	Severely Degraded
MWT-28327	9/15/2021	39.32034	-76.32271	2.67	Marginal
MWT-28328	9/17/2021	39.39494	-76.25532	3.00	Meets Goal

Appendix Table C-1. (Continued)					
Station	Sampling Date	Latitude (WGS84 Decimal Degrees)	Longitude (WGS84 Decimal Degrees)	B-IBI	Status
PMR-28101	8/17/2021	37.96358	-76.34557	2.33	Degraded
PMR-28102	8/17/2021	38.01115	-76.43403	2.00	Severely Degraded
PMR-28103	8/17/2021	38.07715	-76.39867	2.33	Degraded
PMR-28106	8/17/2021	38.16613	-76.63687	1.67	Severely Degraded
PMR-28107	9/7/2021	38.18060	-76.87384	2.20	Degraded
PMR-28108	8/17/2021	38.18233	-76.67453	1.00	Severely Degraded
PMR-28109	9/7/2021	38.18671	-76.85417	1.00	Severely Degraded
PMR-28110	8/17/2021	38.20330	-76.66428	1.00	Severely Degraded
PMR-28112	8/17/2021	38.21313	-76.69670	1.00	Severely Degraded
PMR-28113	9/7/2021	38.22889	-76.86044	1.00	Severely Degraded
PMR-28114	9/7/2021	38.23239	-76.91382	2.20	Degraded
PMR-28115	9/7/2021	38.25326	-76.86482	1.00	Severely Degraded
PMR-28116	9/7/2021	38.25748	-76.89505	1.00	Severely Degraded
PMR-28117	9/7/2021	38.26949	-76.81954	1.00	Severely Degraded
PMR-28119	9/13/2021	38.36954	-77.14901	4.00	Meets Goal
PMR-28120	9/13/2021	38.37404	-77.26815	5.00	Meets Goal
PMR-28121	9/13/2021	38.37928	-77.11122	3.00	Meets Goal
PMR-28122	9/13/2021	38.38499	-77.12300	3.80	Meets Goal
PMR-28124	9/7/2021	38.42329	-77.02404	2.33	Degraded
PMR-28125	9/7/2021	38.46244	-77.03112	3.00	Meets Goal
PMR-28126	9/13/2021	38.57248	-77.22425	3.50	Meets Goal
PMR-28127	8/17/2021	38.08220	-76.53878	2.00	Severely Degraded
PMR-28128	8/17/2021	38.20262	-76.67175	1.00	Severely Degraded
PMR-28129	9/30/2021	38.65988	-77.12722	2.50	Degraded
PMR-28130	9/28/2021	38.43926	-77.29096	2.50	Degraded
PXR-28201	8/18/2021	38.29892	-76.44485	2.67	Marginal
PXR-28202	8/24/2021	38.31957	-76.47566	1.67	Severely Degraded
PXR-28204	8/24/2021	38.35765	-76.49126	1.00	Severely Degraded
PXR-28205	8/24/2021	38.38082	-76.52728	4.00	Meets Goal
PXR-28206	8/24/2021	38.39238	-76.55051	1.00	Severely Degraded
PXR-28207	8/24/2021	38.39671	-76.54129	2.00	Severely Degraded
PXR-28208	8/25/2021	38.40160	-76.57101	1.00	Severely Degraded
PXR-28209	8/24/2021	38.40195	-76.54905	2.00	Severely Degraded
PXR-28210	8/24/2021	38.40728	-76.53221	2.00	Severely Degraded
PXR-28211	8/25/2021	38.41686	-76.57188	1.67	Severely Degraded
PXR-28212	8/25/2021	38.42149	-76.59333	3.00	Meets Goal
PXR-28213	8/25/2021	38.42942	-76.62845	2.60	Degraded
PXR-28214	8/25/2021	38.44018	-76.62437	1.33	Severely Degraded
PXR-28215	8/25/2021	38.44439	-76.63029	1.00	Severely Degraded

Appendix Table C-1. (Continued)					
Station	Sampling Date	Latitude (WGS84 Decimal Degrees)	Longitude (WGS84 Decimal Degrees)	B-IBI	Status
PXR-28217	8/25/2021	38.49930	-76.68118	4.20	Meets Goal
PXR-28218	8/25/2021	38.50184	-76.66522	3.80	Meets Goal
PXR-28219	8/25/2021	38.50794	-76.66665	3.40	Meets Goal
PXR-28221	8/25/2021	38.52182	-76.66209	2.60	Degraded
PXR-28222	8/25/2021	38.52233	-76.66976	3.80	Meets Goal
PXR-28223	8/25/2021	38.58297	-76.68236	2.33	Degraded
PXR-28224	8/27/2021	38.69742	-76.69606	1.00	Severely Degraded
PXR-28225	8/27/2021	38.72862	-76.69576	2.00	Severely Degraded
PXR-28226	8/25/2021	38.56055	-76.68134	2.20	Degraded
PXR-28227	8/25/2021	38.49658	-76.66389	3.80	Meets Goal
UPB-28601	8/18/2021	39.03077	-76.35710	1.00	Severely Degraded
UPB-28602	8/26/2021	39.03270	-76.24727	3.00	Meets Goal
UPB-28603	8/18/2021	39.04382	-76.37447	2.33	Degraded
UPB-28604	8/26/2021	39.05003	-76.24918	2.60	Degraded
UPB-28605	8/18/2021	39.05852	-76.39032	1.00	Severely Degraded
UPB-28606	8/26/2021	39.07045	-76.24987	1.40	Severely Degraded
UPB-28607	8/18/2021	39.08637	-76.40390	4.20	Meets Goal
UPB-28608	8/26/2021	39.08707	-76.27343	3.40	Meets Goal
UPB-28609	8/18/2021	39.10118	-76.30758	1.00	Severely Degraded
UPB-28610	8/18/2021	39.10965	-76.34267	2.60	Degraded
UPB-28611	8/26/2021	39.14415	-76.32923	1.00	Severely Degraded
UPB-28612	8/26/2021	39.14498	-76.33060	2.33	Degraded
UPB-28613	8/26/2021	39.15807	-76.30723	1.67	Severely Degraded
UPB-28614	8/26/2021	39.16652	-76.30212	1.00	Severely Degraded
UPB-28615	8/26/2021	39.17355	-76.34228	3.80	Meets Goal
UPB-28616	8/26/2021	39.18432	-76.32038	3.80	Meets Goal
UPB-28617	9/14/2021	39.26208	-76.36022	3.40	Meets Goal
UPB-28618	8/26/2021	39.26602	-76.30780	3.00	Meets Goal
UPB-28619	8/26/2021	39.28957	-76.20115	1.40	Severely Degraded
UPB-28620	8/26/2021	39.35957	-76.13165	5.00	Meets Goal
UPB-28621	8/26/2021	39.41010	-76.08245	4.50	Meets Goal
UPB-28622	9/9/2021	39.46683	-76.05345	3.00	Meets Goal
UPB-28625	9/9/2021	39.52509	-76.00522	2.00	Severely Degraded
UPB-28626	8/18/2021	39.10607	-76.35918	3.80	Meets Goal
UPB-28627	8/26/2021	39.13267	-76.26480	2.60	Degraded

APPENDIX D**CHLOROPHYLL-A AND PHAEOPHYTIN
LABORATORY RESULTS
SUMMER 2021**

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Appendix Table D-1. Benthic Chlorophyll-*a* and Phaeophytin laboratory analysis results.
 Three replicate samples were collected and analyzed for each parameter.

Station	Sampling Date	Parameter	Result (mg/m ²)	Station	Sampling Date	Parameter	Result (mg/m ²)
001	9/27/21	Active	73.09	023	8/30/21	Active	39.86
001	9/27/21	Active	89.27	023	8/30/21	Active	37.47
001	9/27/21	Active	92.49	023	8/30/21	Active	41.84
001	9/27/21	Phaeo	25.71	023	8/30/21	Phaeo	258.75
001	9/27/21	Phaeo	36.87	023	8/30/21	Phaeo	256.39
001	9/27/21	Phaeo	35.02	023	8/30/21	Phaeo	271.43
001	9/27/21	Total	87.47	023	8/30/21	Total	182.37
001	9/27/21	Total	109.87	023	8/30/21	Total	178.68
001	9/27/21	Total	112.06	023	8/30/21	Total	191.33
006	9/27/21	Active	152.56	024	8/17/21	Active	24.61
006	9/27/21	Active	184.31	024	8/17/21	Active	23.86
006	9/27/21	Active	204.16	024	8/17/21	Active	20.19
006	9/27/21	Phaeo	36.45	024	8/17/21	Phaeo	225.61
006	9/27/21	Phaeo	36.35	024	8/17/21	Phaeo	196.9
006	9/27/21	Phaeo	51.52	024	8/17/21	Phaeo	202.43
006	9/27/21	Total	173.05	024	8/17/21	Total	148.89
006	9/27/21	Total	204.82	024	8/17/21	Total	132.32
006	9/27/21	Total	233.1	024	8/17/21	Total	131.71
015	9/27/21	Active	52.09	026	8/26/21	Active	17.09
015	9/27/21	Active	45.71	026	8/26/21	Active	26.53
015	9/27/21	Active	35.17	026	8/26/21	Active	35.14
015	9/27/21	Phaeo	77.36	026	8/26/21	Phaeo	161.13
015	9/27/21	Phaeo	35.44	026	8/26/21	Phaeo	250.37
015	9/27/21	Phaeo	33.71	026	8/26/21	Phaeo	153.06
015	9/27/21	Total	95.02	026	8/26/21	Total	105.85
015	9/27/21	Total	65.42	026	8/26/21	Total	164.45
015	9/27/21	Total	53.91	026	8/26/21	Total	119.41
022	8/23/21	Active	30.09	029	9/9/21	Active	50.52
022	8/23/21	Active	39	029	9/9/21	Active	51.01
022	8/23/21	Active	42.04	029	9/9/21	Active	30.1
022	8/23/21	Phaeo	209.37	029	9/9/21	Phaeo	283.8
022	8/23/21	Phaeo	212.39	029	9/9/21	Phaeo	264.84
022	8/23/21	Phaeo	218.73	029	9/9/21	Phaeo	207
022	8/23/21	Total	145.41	029	9/9/21	Total	207.74
022	8/23/21	Total	155.96	029	9/9/21	Total	197.74
022	8/23/21	Total	162.49	029	9/9/21	Total	144.77

Appendix Table D-1. (Continued)							
Station	Sampling Date	Parameter	Result (mg/m ²)	Station	Sampling Date	Parameter	Result (mg/m ²)
036	9/13/21	Active	36.04	047	9/30/21	Active	25.07
036	9/13/21	Active	43.95	047	9/30/21	Active	19.45
036	9/13/21	Active	42.44	047	9/30/21	Active	19.36
036	9/13/21	Phaeo	199.28	047	9/30/21	Phaeo	120.2
036	9/13/21	Phaeo	212.09	047	9/30/21	Phaeo	119.09
036	9/13/21	Phaeo	232.64	047	9/30/21	Phaeo	83.63
036	9/13/21	Total	146.44	047	9/30/21	Total	91.66
036	9/13/21	Total	161.45	047	9/30/21	Total	85.42
036	9/13/21	Total	171.32	047	9/30/21	Total	65.7
040	9/13/21	Active	20.51	051	9/30/21	Active	499.27
040	9/13/21	Active	19.15	051	9/30/21	Active	510.8
040	9/13/21	Active	17.54	051	9/30/21	Active	440.44
040	9/13/21	Phaeo	188.97	051	9/30/21	Phaeo	58.93
040	9/13/21	Phaeo	179.85	051	9/30/21	Phaeo	58.31
040	9/13/21	Phaeo	166.11	051	9/30/21	Phaeo	102.69
040	9/13/21	Total	125.17	051	9/30/21	Total	532.91
040	9/13/21	Total	118.75	051	9/30/21	Total	544.12
040	9/13/21	Total	109.54	051	9/30/21	Total	498.19
043	9/30/21	Active	19.37	052	8/18/21	Active	29.41
043	9/30/21	Active	9.42	052	8/18/21	Active	26.81
043	9/30/21	Active	14.18	052	8/18/21	Active	28.53
043	9/30/21	Phaeo	100.38	052	8/18/21	Phaeo	223.3
043	9/30/21	Phaeo	48.89	052	8/18/21	Phaeo	225.82
043	9/30/21	Phaeo	88.28	052	8/18/21	Phaeo	233.22
043	9/30/21	Total	74.98	052	8/18/21	Total	152.42
043	9/30/21	Total	36.51	052	8/18/21	Total	151.21
043	9/30/21	Total	63.08	052	8/18/21	Total	157
044	9/7/21	Active	40.65	062	9/27/21	Active	58.52
044	9/7/21	Active	30.83	062	9/27/21	Active	48.75
044	9/7/21	Active	34.03	062	9/27/21	Active	57.31
044	9/7/21	Phaeo	274.72	062	9/27/21	Phaeo	232.17
044	9/7/21	Phaeo	264.23	062	9/27/21	Phaeo	220.04
044	9/7/21	Phaeo	198.46	062	9/27/21	Phaeo	244.85
044	9/7/21	Total	192.82	062	9/27/21	Total	187.17
044	9/7/21	Total	177.18	062	9/27/21	Total	170.67
044	9/7/21	Total	143.97	062	9/27/21	Total	192.98

Appendix Table D-1. (Continued)							
Station	Sampling Date	Parameter	Result (mg/m ²)	Station	Sampling Date	Parameter	Result (mg/m ²)
064	9/15/21	Active	27.64	074	8/27/21	Active	34.89
064	9/15/21	Active	43.41	074	8/27/21	Active	36.44
064	9/15/21	Active	49.19	074	8/27/21	Active	35.99
064	9/15/21	Phaeo	247.35	074	8/27/21	Phaeo	219.48
064	9/15/21	Phaeo	238.07	074	8/27/21	Phaeo	225.53
064	9/15/21	Phaeo	236.25	074	8/27/21	Phaeo	227.46
064	9/15/21	Total	164.63	074	8/27/21	Total	155.77
064	9/15/21	Total	175.3	074	8/27/21	Total	160.65
064	9/15/21	Total	180.08	074	8/27/21	Total	161.26
066	9/10/21	Active	51.36	077	8/27/21	Active	5.71
066	9/10/21	Active	59.68	077	8/27/21	Active	6.31
066	9/10/21	Active	42.35	077	8/27/21	Active	3.72
066	9/10/21	Phaeo	247.84	077	8/27/21	Phaeo	41.31
066	9/10/21	Phaeo	283.51	077	8/27/21	Phaeo	42.96
066	9/10/21	Phaeo	260.93	077	8/27/21	Phaeo	47.76
066	9/10/21	Total	188.68	077	8/27/21	Total	28.46
066	9/10/21	Total	216.76	077	8/27/21	Total	29.97
066	9/10/21	Total	186.89	077	8/27/21	Total	30.03
068	9/21/21	Active	23.93	079	8/27/21	Active	16.33
068	9/21/21	Active	18.99	079	8/27/21	Active	8.83
068	9/21/21	Active	16.32	079	8/27/21	Active	7.55
068	9/21/21	Phaeo	224.51	079	8/27/21	Phaeo	111.12
068	9/21/21	Phaeo	217.32	079	8/27/21	Phaeo	81.1
068	9/21/21	Phaeo	165.57	079	8/27/21	Phaeo	73.57
068	9/21/21	Total	148.27	079	8/27/21	Total	77.53
068	9/21/21	Total	139.34	079	8/27/21	Total	53.5
068	9/21/21	Total	108.02	079	8/27/21	Total	48.08
071	8/24/21	Active	51.81	201	8/30/21	Active	16.6
071	8/24/21	Active	51.08	201	8/30/21	Active	19.91
071	8/24/21	Active	50.69	201	8/30/21	Active	15.85
071	8/24/21	Phaeo	296.4	201	8/30/21	Phaeo	160.25
071	8/24/21	Phaeo	312.43	201	8/30/21	Phaeo	171.21
071	8/24/21	Phaeo	292.07	201	8/30/21	Phaeo	166.54
071	8/24/21	Total	215.04	201	8/30/21	Total	104.88
071	8/24/21	Total	223.15	201	8/30/21	Total	114.22
071	8/24/21	Total	211.54	201	8/30/21	Total	107.6

Appendix Table D-1. (Continued)			
Station	Sampling Date	Parameter	Result (mg/m ²)
202	8/30/21	Active	39.54
202	8/30/21	Active	33.55
202	8/30/21	Active	34.62
202	8/30/21	Phaeo	219.87
202	8/30/21	Phaeo	236.42
202	8/30/21	Phaeo	229.55
202	8/30/21	Total	160.63
202	8/30/21	Total	163.77
202	8/30/21	Total	161.05
203	8/12/21	Active	54.86
203	8/12/21	Active	55.64
203	8/12/21	Active	56.4
203	8/12/21	Phaeo	281.06
203	8/12/21	Phaeo	267.98
203	8/12/21	Phaeo	270.16
203	8/12/21	Total	209.63
203	8/12/21	Total	203.21
203	8/12/21	Total	205.16
204	8/31/21	Active	27.69
204	8/31/21	Active	24.93
204	8/31/21	Active	34.18
204	8/31/21	Phaeo	229.67
204	8/31/21	Phaeo	211.49
204	8/31/21	Phaeo	203.36
204	8/31/21	Total	154.89
204	8/31/21	Total	142.06
204	8/31/21	Total	146.84