LANDSCAPE PATTERNS, NUTRIENT DISCHARGES AND BIOTA OF THE RHODE RIVER ESTUARY AND ITS WATERSHED: CONTRIBUTION OF THE SMITHSONIAN ENVIRONMENTAL RESEARCH CENTER TO THE PILOT INTEGRATED ECOSYSTEM ASSESSMENT

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EXECUTIVE SUMMARY

This report is submitted by the Smithsonian Environmental Research Center as its contribution to the collaborative effort led by the NOAA Chesapeake Bay Office to conduct a Mid-Atlantic Pilot Integrated Ecosystem Assessment. NOAA funding, subcontracted through Versar, supported analysis or report preparation of data presented, and in the case of the *Pragmites* study, collection of primary data. Authors of the various chapters are listed and can be contacted for additional details. We summarize the studies below.

An understanding of land use and watershed characteristics is important to management of nutrient discharges, and ultimately to restoration of water quality in Chesapeake Bay and its subestuaries. In Chapter 1, Dr. Donald Weller describes an analysis of land use changes from 1957 – 2000 within the Muddy Creek drainage of the Rhode River watershed. During that time period, forest cover, as well as 'yard and road' increased, but cropland and old field cover decreased. He also applied a geographic analyses developed in a previous study to describe variation among the subsystems *within* the Rhode River subestuary. Watershed and subestuary metrics for 11 subestuaries of the Rhode River were developed. These metrics can be used as independent variables in future IEA analyses that seek explain differences in estuarine responses among the subsystems of the Rhode River.

Knowledge of the sources and dynamics of nitrogen (N), phosphorus (P), and organic carbon (C) is essential for integrated assessment of eutrophic estuarine ecosystems. Since the 1970s, SERC has monitored concentrations of these materials throughout the Rhode River estuary and their inputs to the estuary from its watershed and from atmospheric deposition. In Chapter 2, Dr. Thomas Jordan highlights monitoring data since the 1990s. Publications summarize earlier data. Atmospheric deposition is a potentially important source N to the Rhode River. Deposition of nitrate, ammonium, and organic N in wetfall varied in step seasonally and interannually with the amount and distribution of precipitation. Concentrations of these N forms were higher in smaller volume precipitation events. However, more deposition occurred during time periods with more precipitation. There were no clear long-term trends in N deposition since 1992 despite earlier trends. Variations in watershed discharges of N, P, organic C, and total suspended solids (TSS) were mainly driven by variations in discharges of water. However, discharges of N and dissolved organic C discharges correlated with water discharge more closely than did discharges of P or suspended solids. There were no long-term changes in N concentrations in watershed discharge since 1980 despite declines in agricultural land, which is the main source of N in watershed discharge. Concentrations of P and TSS in watershed discharges showed long-term increases. Concentrations of N, P, chlorophyll, and TSS in the estuary showed distinct seasonal and spatial patterns. Nitrate concentration in the estuary was highest in winter and lowest in summer. Nitrate concentration reached a minimum in the mid-upper estuary and increased upstream toward the sources of local watershed inputs as well as downstream toward the adjacent Chesapeake Bay, which receives nitrate from the Susquehanna River watershed. Patterns of dissolved phosphate concentrations were opposite those of nitrate. Phosphate was highest in summer and lowest in winter. Phosphate was highest in the mid-upper estuary and decreased upstream or downstream. This suggests that dissolved phosphate is produced in the mid-upper estuary. Chlorophyll and TSS were highest in summer and increased upstream. Concentrations also showed interannual variation with nitrate being higher and phosphate being lower in years with more watershed discharge. Phosphate concentrations showed a long-term increase since 1986, while TSS and TN showed increases after 2002.

Chapter 3 describes work by Dr. Melissa McCormick and colleagues examining the spread of *Phragmites australis* within the Rhode River watershed. The rapid spread of an introduced genotype of *P. australis* has caused substantial concern along the east coast of the U.S. Introduced *Phragmites* can form dense, monospecific stands that have decreased floral and faunal diversity. The Rhode River subestuary provides an ideal opportunity to investigate the ways in which *Phragmites* is spreading and to contrast spread along developed versus forested shoreline. McCormick and colleagues mapped all patches of *Phragmites* within the Rhode River subestuary in fall 2007 and used microsatellite analysis to determine whether spread within and among patches was by rhizome or by seed. They then compared the distribution of *Phragmites* in 2007 with that in 1971-2 on developed and forested sides of the Rhode River. They found that, while more *Phragmites* was present on the forested side of the subestuary in both 1971 and 2007, the percentage increase from 1971 to 2007 was greater on the developed than on the forested side of the subestuary.

McCormick and colleagues found that nearly all patches were genetically unique and contained multiple genotypes, indicating that establishment of new patches was almost entirely by seed, rather than rhizome as many researchers have proposed, and that spread of individual patches also includes recruitment from seed. The decrease of genetic variation with increasing distance between samples indicates that most pollen and seed dispersal are local. This work demonstrates that seed is very important for the spread of non-native *Phragmites* and suggests that an accumulation of genetic variation within patches is necessary for the production of substantial numbers of viable seeds and subsequent rapid spread. The importance of seeds for *Phragmites* spread has substantial implications for management. For example, other ongoing *Phragmites* research in their laboratory confirms the Rhode River findings in other subestuaries of Chesapeake Bay and demonstrates the importance of understanding factors (e.g., natural and man-made disturbances) that influence the establishment of seedlings in brackish wetlands.

Chapter 4 summarizes sampling for gelatinous zooplankton in the Rhode River conducted by students and technicians in Dr. Denise Breitburg's laboratory that is being published in a volume of Smithsonian Contributions to Marine Science. The lobate ctenophore *Mnemiopsis leidyi* is an important predator of zooplankton and icthyoplankton both within and outside of its native range, and is a dominant consumer within the Chesapeake Bay food web. Breitburg's laboratory sampled the Rhode River, a subestuary of Chesapeake Bay, during 2004 and 2005 to quantify abundances of *M. leidy*, its scyphomedusan predators and its mesozooplankton prey, and conducted ctenophore egg production experiments in 2004. Despite low mesozooplankton densities, ctenophores produced up to 9,380 eggs ind^{-1} d^{-1}. Temporal patterns, as well as peak abundances, of copepods, ctenophores and sea nettles (*Chrysaora quinquecirrha*; the major predator of *M. leidyi*) varied considerably between years. This interannual variation may have been caused by direct and indirect effects of physical factors – especially low salinities during 2004 - on all components of the food web. In 2004, zooplankton abundances peaked in June, *M. leidyi* abundances steadily increased throughout the summer, and *C. quinquecirrha* was rare. In contrast, during 2005, *C. quinquecirrha* density increased during mid-summer. As this medusa increased in abundance, *M. leidyi* numbers declined and copepod abundances increased. Shallow systems with salinities near the minimum threshold for *C. quinquecirrha* ephyra production may exhibit more extreme interannual variability than deeper, higher salinity systems and may serve as models to provide insight into factors controlling gelatinous zooplankton dynamics.

In Chapter 5, Dr. Anson Hines and colleagues describe research on female blue crab migration. Spatial and temporal patterns of migration by mature female blue crabs in Chesapeake Bay were determined over a 9-year period (1999-2007) with a fishery-dependent tag-recapture program that paid rewards to individual fishers for providing recapture data. The study tagged 8,400 mature female blue crabs and released them in representative subestuaries along the Maryland and Virginia portions of the Bay. Recapture data for 1,526 crabs (18.2% average recovery rate) provided clear information about the timing, routes and depths of female migration to lower Bay spawning grounds. Most tagged crabs (947 females or 62.1%) were caught before they began to migrate, indicating that the impact of the fishery is greatest prior to female migration. Crabs moving more than 4.2 miles (7 km) from release sites comprised 37.9% of the recaptures. Of these migrating crabs, 19.3% were caught prior to September, while the majority of migrating crabs (56.1%) were caught during September to November, mostly along the mainstem of the Bay. Another 24.6% of migrating females were recaptured after November, mostly after they had already arrived in the lower Bay spawning area. Approximately half of the females migrated before mid-October, with approximately 36% caught before October 10 and 55% caught prior to October 23. Most migrating females were recaptured within one year of release, and only two crabs were recaptured more than 3 years after release, at estimated age 4.5-5 years. Most migrating females were recaptured along the shallow edges of the deeper tributaries (Potomac and York Rivers) and mainstem of the Bay. Most females (87%) migrating during September through November were caught in water shallower than approximately 30 ft (10 m). Recaptures for migrating females peaked at depths from 18 to 24 ft (6-8 m; 32%), and only 5% of females were caught deeper than 36 ft (12 m). However, these depth data may reflect fishery-dependent sampling, and fishery independent sampling will be necessary to test the route of migration for an unbiased population Improved mechanistic understanding of migration, particularly spatial variation in the onset of migration and factors limiting the depth of migration, would help advise management of this intense fishery to ensure that quotas and stock preservation can be achieved effectively.

Finally, Hines and colleagues report on long term sampling of living resources in the Rhode River in Chapter 6. SERC's on-going, long-term (25-30 years) monitoring program describes the population dynamics and community structure of fish and invertebrates throughout the Rhode River, a representative subestuary of Chesapeake Bay. This research tracks seasonal, annual and decadal variation in species composition and abundance of all fish and macro-invertebrates of the system. The SERC monitoring program includes four main components designed to sample the full range of habitats in the Rhode River subestuary:

- Fish and Crustaceans of a Tributary Creek – sampled weekly by a permanent fish weir.
- Epibenthic Fish and Crabs – sampled monthly by otter trawls at 4 stations.
- Infaunal Benthic Invertebrate Community – sampled 4-6 times per year by cores at 5 stations.
- Nearshore Fish Assemblage – sampled annually in summer by replicate seines at 13 stations.

Long-term measures of species composition and population dynamics provide important indicators of ecosystem function, the status of biotic resources, and possible anthropogenic impacts on community structure. As an example of their long-term monitoring, Hines and colleagues present analysis of the 28 year (1980-2007) data set for the nearshore fish assemblage provides in Rhode River, Maryland, a mesohaline subestuary of the upper Chesapeake Bay. A total of 53 fish species were caught and enumerated during yearly seine sampling (June –

September) at 13 stations distributed throughout the Rhode River. Multivariate statistical analysis indicated a shift in assemblage structure during three significant groups of years from the 1980s to 1990s and the 2000s. This shift was generally characterized by declines in spot *Leiostomus xanthurus* and menhaden *Brevoortia tyrannus* and marked increases in white perch *Morone americana* and striped bass *Morone saxatilis*. Multivariate statistical analysis also indicated three spatially distinct assemblages (i.e., headwater, salt creek, and mainstem stations) throughout the study period. These assemblages were characterized by increased numbers of freshwater and marsh associated fish at the headwater and creek sites and greater numbers of pelagic and transient species at mainstem sites. The assemblages at each group of stations exhibited different patterns of temporal change, resulting in a significant spatio-temporal interaction in variation of species composition. Species richness of rare species (species occurring with <10% prevalence in seines) was highly correlated with annual mean salinity, with species richness of freshwater and anadromous species being negatively correlated, and that of estuarine and marine species being positively correlated with salinity. These data illustrate the importance of long-term sampling programs, since it is often necessary to sample at large time scales (e.g., decadal) to adequately assess change in community structure and ecosystem dynamics.

I. LAND USE TRENDS IN THE RHODE RIVER WATERSHED

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We examined land use changes in the 2298 ha Muddy Creek drainage of the Rhode River watershed from 1957-2000. The 3332 ha Rhode River watershed and its estuary are in Anne Arundel County, Maryland near the cities of Washington DC, Baltimore, and Annapolis. Before European settlement, the area was covered by forests of the tulip poplar and basket oak associations (Brush et al. 1980). Since European settlement in the mid-17th century, the area has been primarily agricultural. The land was initially cleared for large tobacco plantations, which were gradually replaced from 1770-1840 with smaller, self-sufficient farms raising diverse food crops (Higman, unpublished manuscript). Modern chemical agriculture began in the 1940s. Currently, the landscape is a complex patchwork of forests of various ages, wetlands, agricultural land (cropland, pastures, and fallow fields), and rural to suburban housing.

We analyzed land use data from 1957, 1972, 1993, and 2000. Earlier data came from a report summarizing land use in 1957 and 1972 (Higman 1973). Beginning in 1990, we maintained land use records with a geographic information system (GIS). We digitized topographic contour lines, elevation benchmarks, roads, buildings, land-water boundaries, streams, and watershed divides from 1:2400 topographic maps (Anne Arundel County 1984). We digitized the boundaries of land use patches from 1:2400 prints of black and white aerial photographs taken in winter 1984 (Air Photographics, Inc., Morgantown, WV). We updated the boundaries in and 1993 using on-the-ground observations and low elevation color slides taken in June 1993 by the Anne Arundel County office of the USDA-ASCS (now the Farm Service Agency). Boundaries were updated in 2000 using a high resolution digital orthophotograph (VarGIS, LLC, Herndon, VA).

We used 13 land use categories in 1993 and 2000. We tentatively classified each land use feature on an aerial photograph into one of the 13 categories. The photo-based classifications were then verified and corrected with observations of all areas accessible on the ground and with land use data reported to the USDA-ASCS. We repeated the photo and ground-based classification in 1991, 1993, and 2000 to document changes in land use. Higman (1973) used 18 land use categories in 1957 and 1972, so we aggregated the data from different years to a common set of nine land use categories before analyzing land use changes (Fig. 1). Land use has not been remapped since 2000 because of lack of funding and the need to shift to less labor-intensive mapping methods based on remotely-sensed data.

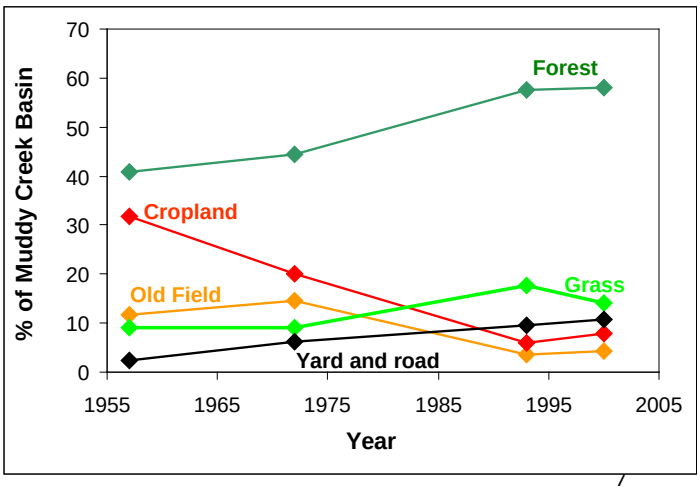

Figure 1. Land use trends in the Rhode River basin.

Cropland was the land use category showing the greatest change from 1957 to 1993, declining from 32% of the Muddy Creek basin to 6%, and then rising to 8% in 2000. Old fields fell from 12% to 4% in 2000. Forest increased from 41% to 58% while yards and roads rose from 2% to 11%. Grassed areas increased from 9% to 18% in 1993, then fell to 4% in 2000. The loss of cropland and pasture indicate a major decline of agriculture on the watershed. Land formerly used for food production is now occupied by forests, horse farms, non-commercial "farmettes," and housing.

Geographic Characteristics of Subestuaries of the Rhode River

Landscape analysis has been increasingly applied to identify watershed effects on estuarine responses (Comeleo et al. 1996; Dauer et al. 2000; Paul et al. 2002; Hale et al. 2004; King et al. 2005; Bilkovic et al. 2006; Rodriguez et al. 2007). Watershed land cover characteristics can be strong indicators of degraded estuarine health (Hale et al. 2004; Brooks et al. 2006). Watershed development has been associated with lower estuarine species diversity, altered food webs, and altered benthic community composition (Dauer et al. 2000; Lerberg et al. S2000; Breitburg 2002); and local watershed land cover provided significant indicators of marsh bird diversity (DeLuca et al. 2004), blue crab and bivalve abundances (King et al. 2005), and toxicants in fish (King et al. 2004).

Geographic characteristics of estuaries themselves may also be good indicators of estuarine condition. In a recent study, we combined watershed metrics with geographic descriptors of subestuaries to explain spatial variation in AV abundance among Chesapeake Bay subestuaries (Li et al. 2007). SAV abundance was strongly linked to subestuary and watershed characteristics. A regression tree model attributed 60% of the variance in SAV abundance among subestuaries to subestuary shoreline complexity (represented by fractal dimension), mean tidal range, the dominant land cover of the local watershed, watershed to subestuary area ratio, and mean wave height. SAV abundance declined with the dominant watershed land cover category in the order: forested, mixed-land > agricultural > developed.

In the present effort, We identified eight smaller systems that are tributary arms to the large Rhode River subestuary (Fig. 2, top). We also analyzed two aggregate systems that combined two or three of the smaller systems (Fig. 2, middle). Finally, we analyzed the entire Rhode River subestuary and its watershed (Fig. 2, bottom). As before (Li et al 2007), boundaries for the study subestuaries were taken from a 1:24,000 digital shoreline map of Chesapeake Bay (Federal Geographic Data Committee (FGDC) 2001; http://www.ngs.noaa.gov/newsys_ims/shoreline). For each subsystem, we added a line across the mouth to the digital shoreline to form a closed polygon representing the water surface. The local watershed boundaries were delineated from digital elevation maps and stream maps. We used the Spatial Analyst Tools of the ArcInfo. 9.1 (ESRI, Inc.) geographic information system (GIS) to analyze 1:24,000 DEM data (Caruso 1987; http://edc.usgs.gov/geodata/) and vector stream maps derived from the same 7.5 minute quadrangles (US Geological Survey (USGS) 1999; http://nhd.usgs.gov). We manually corrected the automated watershed delineation results (Baker et al. 2006). Areas and perimeters of the subestuaries and their watersheds are presented in Table 1.

Figure 1. Subsystems of the Rhode River watershed and subestuary. Top. Eight non-overlapping subsystems and their watersheds. Middle: Two intermediate-sized subsystems, each combining two or three subsystems from the top map. Bottom: The entire Rhode River system. The identifying numbers are used in the data tables.

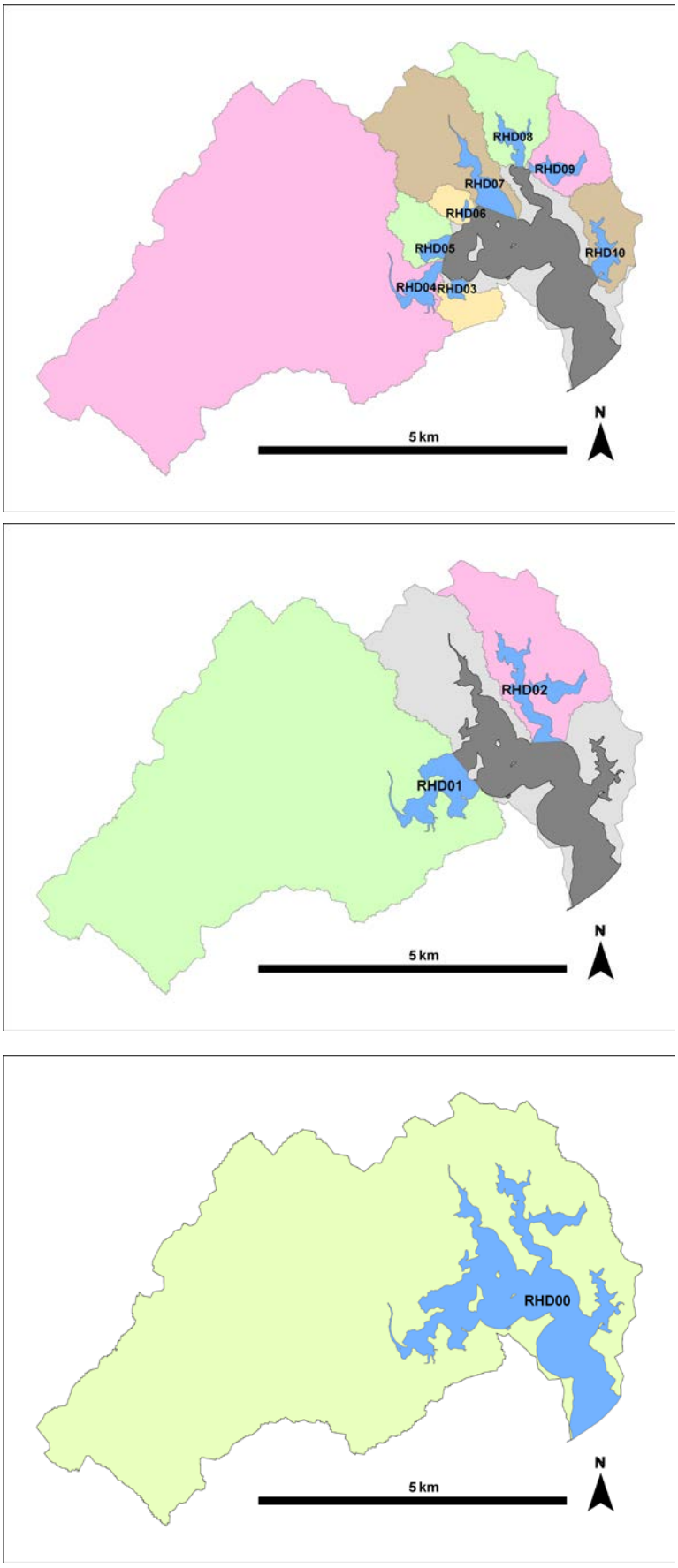

Table 1. Areas and Perimeters of Subsystems of the Rhode River.

	RHD00	RHD01	RHD02	RHD03	RHD04	RHD05	RHD06	RHD07	RHD08	RHD09	RHD10
Name	Rhode River	Muddy Creek	Bear Creek	Boathouse Creek	Upper Muddy Creek	Fox Creek	Sheephead Cove	Sellman Creek	Upper Bear Creek	Whitemarsh Creek	Cadle Creek
Estuary Surface Area (ha)	500.4	76.1	63.9	7.9	28.0	13.3	4.0	39.3	18.9	19.4	22.1
Shoreline Length (km)	39.0	9.4	10.1	1.3	5.7	1.7	1.2	5.2	3.8	3.1	4.7
Watershed Area (ha)	3247.6	2377.5	348.1	56.3	2238.8	70.6	29.7	289.9	201.6	112.2	104.6
Watershed Perimeter*	36.8	27.8	11.1	3.6	29.1	3.9	2.3	9.7	8.1	6.0	5.7

*Excluding shoreline.

Watershed land cover information was calculated from the second generation of the National Land Cover Dataset (NLCD 2001; 30 m resolution; Homer et al. 2004), which was derived from Landsat 7 satellite remote sensing imagery. In each watershed, we calculated the percentages of seven land cover categories created by aggregating the larger number of categories reported in the NLCD 2001 (Table 1).

Table 2. Land cover categories aggregated from NLCD categories.

Lumped Category	NLCD 2001 Categories
Developed	developed-open space, developed-low intensity, developed-medium intensity, developed-high intensity
Cropland	cultivated crops
Grassland	pasture/hay, grassland/herbaceous, shrub/scrub
Forest	deciduous forest, evergreen forest, mixed forest
Wetlands	emergent herbaceous wetlands, woody wetlands
Barren	barren
Water	water

As in earlier work (King et al. 2005; Brooks et al. 2006, Li et al. 2007), we classified each watershed into one of six land cover categories based on the dominant land cover type: (1) forested (≥60% forest + forested wetland), (2) developed (Dev., ≥50% developed land), (3) agricultural (Agr., ≥40% cropland), (4) mixed-developed (Mixed-Dev., 15-50% developed), (5) mixed- agricultural (Mixed-Agr., 20%-40% cropland), and (6) mixed-undisturbed. The last category includes all watersheds that can not be classified into any of the other five categories and are dominated by mixtures of forest, grassland, and wetland. Results of the watershed land cover analysis are in Table 3.

Table 3. Land cover characteristics of watersheds draining to Rhode River subestuaries, shown as the percentages of watershed areas (Table 1) in each of seven aggregated land cover categories (Table 2).

	RHD00	RHD01	RHD02	RHD03	RHD04	RHD05	RHD06	RHD07	RHD08	RHD09	RHD10
Forest	52.7	57.4	31.1	28.5	58.2	62.1	18.4	59.7	39.1	18.8	28.2
Developed	8.4	2.4	37.1	0.0	2.6	0.0	0.6	3.8	28.0	55.1	52.7
Grass	20.8	23.9	9.3	13.8	24.4	16.5	19.0	15.0	11.5	4.6	8.7
Cropland	6.9	6.9	4.0	13.7	6.9	2.2	42.0	8.5	5.3	0.5	1.9
Wetland	9.8	8.5	15.7	41.6	7.2	16.9	13.8	12.6	14.6	16.4	6.2
Bare	0.6	0.3	1.5	0.0	0.3	0.5	4.6	0.2	1.1	0.9	0.9
Water	0.8	0.6	1.3	2.4	0.4	1.8	1.5	0.3	0.5	3.6	1.4
Dominant Category	Forest	Forest	Mixed Developed	Forest	Forest	Forest	Agricultural	Forest	Mixed-Developed	Developed	Developed

We derived descriptive metrics for the size and shape of each subestuary and its watershed through GIS analysis of watershed boundaries, subestuary shorelines, and bathymetric data (Cohen 1994; http://www.ngdc.noaa.gov/mgg/bathymetry/maps). The metrics included: subestuary area, subestuary volume, subestuary mouth width, local watershed area, and other measures of shape or structure (Table 4).

Table 4. Geographic metrics for the Rhode River.

	RHD00	RHD01	RHD02	RHD03	RHD04	RHD05	RHD06	RHD07	RHD08	RHD09	RHD10
Mouth Width (km)	1.20	0.76	0.50	0.18	0.20	0.41	0.20	0.66	0.12	0.07	0.23
Shoreline Length (km)	37.81	8.60	9.64	1.13	5.47	1.30	1.01	4.58	3.65	3.02	4.45
Perimeter (Shoreline+Mouth, km)	39.01	9.36	10.14	1.31	5.67	1.71	1.21	5.24	3.77	3.09	4.68
Volume (km^3)	0.08	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Maximum Depth (m)	4.10	2.00	2.90	1.20	1.70	1.80	2.00	2.70	2.30	2.60	2.50
Mean Depth (m)	1.80	0.86	1.26	0.58	0.39	1.23	0.71	1.48	0.97	1.17	1.18
Median Depth (m)	1.80	0.80	1.30	0.60	0.40	1.40	0.70	1.60	1.00	1.40	1.30
1000*Perimeter(m)/Area(m^2)	7.80	12.30	15.87	16.50	20.24	12.85	30.45	13.32	19.94	15.91	21.15
1000*Perimeter(m)/Volume(m^3)	0.48	1.70	1.48	3.65	6.53	1.20	5.87	1.03	2.64	1.53	2.20
Elongation Ratio[a]	1262	492	451	159	299	206	112	354	245	249	265
Shoreline length/Diameter_Cir	29.95	17.47	21.36	7.11	18.31	6.33	9.00	12.93	14.89	12.14	16.76
Shoreline Fractal Dimension[b]	1.37	1.35	1.38	1.27	1.38	1.26	1.34	1.33	1.36	1.32	1.37
Watershed Area/Estuary Area	7.57	4.57	37.20	25.36	10.35	8.44	564.16	2.66	1.57	3.63	2.55
Watershed Area/Estuary Volume (km^2/km^3)	462	631	3474	5615	3340	786	108681	205	208	351	265

[a]The diameter of a circle of the same area as the estuary.

[b]A measure of shoreline complexity (calculation in Ferrarini et al. 2005).

Finally, we intersected the subestuary boundaries with the VIMS digital data on shoreline situations (VIMS Comprehensive Coastal Inventory, www.vims.edu/ccrm.) to quantify shoreline land use, shoreline physical condition (bank height, bank cover, bank stability, and the presence of natural buffers at the bank toe; Table 5), and shoreline modification (piers, riprap, breakwaters boat ramps; Tables 6 and 7).

The watershed and subestuary metrics (Tables 1-7) have been developed to explore spatial patterns in estuarine responses. The metrics can be used as independent variables in future IEA analyses that seek explain differences in estuarine responses among the subsystems (Fig. 1) of the Rhode River.

Table 5. Rhode River shoreline conditions mapped by the VIMS Comprehensive Coastal Survey. Conditions are given as percentages of the shoreline lengths in the first row.

	RHD00	RHD01	RHD02	RHD03	RHD04	RHD05	RHD06	RHD07	RHD08	RHD09	RHD10
Shoreline Length (km)[a]	36.80	9.38	10.48	1.25	5.25	1.43	1.27	3.81	4.18	3.14	4.18
Shoreline Land Cover (%)											
Forest	49.3	73.1	35.3	100.0	27.8	100.0	47.3	89.2	51.5	11.1	6.3
Residential	33.6	0.9	55.3	0.0	0.0	0.0	29.4	1.8	46.7	82.4	73.6
Commercial	4.9	0.4	7.0	0.0	0.0	0.0	0.0	6.5	0.0	3.7	9.7
Scrub-shrub	2.2	0.0	0.8	0.0	0.0	0.0	23.3	2.5	0.0	0.0	1.8
Grass	3.3	0.0	1.6	0.0	0.0	0.0	0.0	0.0	1.9	2.7	7.3
Paved	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
No Data	6.5	25.6	0.0	0.0	72.2	0.0	0.0	0.0	0.0	0.0	0.0
Bank Vegetation Cover (%)											
Complete Cover	86.3	74.4	88.5	100.0	27.8	100.0	100.0	73.7	93.6	98.0	100.0
Partial Cover	6.7	0.0	11.5	0.0	0.0	0.0	0.0	22.6	6.4	2.0	0.0
Bare	0.4	0.0	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0
No Data	6.5	25.6	0.0	0.0	72.2	0.0	0.0	0.0	0.0	0.0	0.0
Marsh (%)											
No Marsh	60.9	27.8	72.3	47.6	14.9	18.1	91.9	52.1	50.8	83.0	87.1
Marsh (not eroded)	27.0	46.6	23.8	52.4	12.9	81.9	8.1	24.8	48.4	10.4	6.6
Marsh (eroded)	5.5	0.0	3.9	0.0	0.0	0.0	0.0	23.1	0.8	6.6	6.3
No Data	6.5	25.6	0.0	0.0	72.2	0.0	0.0	0.0	0.0	0.0	0.0
Phragmites (%)											
No	74.9	47.6	78.7	47.6	14.9	100.0	68.9	89.2	71.9	81.2	99.5
Yes	18.5	26.8	21.3	52.4	12.9	0.0	31.1	10.8	28.1	18.8	0.0
No Data	6.6	25.6	0.0	0.0	72.2	0.0	0.0	0.0	0.0	0.0	0.5
Bank Height Classes (%)											
0-5 m	87.7	73.5	93.8	100.0	27.8	100.0	100.0	97.9	94.3	94.4	93.2
5-10 m	4.6	0.0	5.2	0.0	0.0	0.0	0.0	2.1	4.3	4.4	5.6
10-30 m	1.2	0.9	1.0	0.0	0.0	0.0	0.0	0.0	1.4	1.2	1.1
No Data	6.5	25.6	0.0	0.0	72.2	0.0	0.0	0.0	0.0	0.0	0.0
Beach (%)											
No Beach	87.0	74.4	95.1	100.0	27.8	100.0	100.0	96.3	97.5	98.5	98.0
Beach (not eroded)	3.8	0.0	0.7	0.0	0.0	0.0	0.0	3.7	0.7	1.5	1.4
Beach (eroded)	2.7	0.0	4.2	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.6
No Data	6.5	25.6	0.0	0.0	72.2	0.0	0.0	0.0	0.0	0.0	0.0
Erosion (%)											
Low Erosion	66.0	47.0	75.4	52.4	12.9	81.9	66.6	43.2	81.6	80.2	93.9
High Erosion	27.4	27.4	24.6	47.6	14.9	18.1	33.4	56.8	18.4	19.8	6.1
No Data	6.5	25.6	0.0	0.0	72.2	0.0	0.0	0.0	0.0	0.0	0.0

Table 6. Shoreline modifications in the Rhode River as percentages of total shoreline length.

	RHD00	RHD01	RHD02	RHD03	RHD04	RHD05	RHD06	RHD07	RHD08	RHD09	RHD10
Riprap	18.27	0.00	9.02	0.00	0.00	0.00	4.85	5.05	10.23	7.40	31.43
Marina, <50 Slips	3.88	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.90	11.56
Marina, >50 Slips	2.28	0.00	4.65	0.00	0.00	0.00	0.00	0.00	0.00	3.74	0.00
Bulkhead	3.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.67
Dilapidated Bulkhead	0.22	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Groin Field	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Jetty	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Miscellaneous	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.15
Total Modified	28.99	0.00	14.24	0.00	0.00	0.00	4.85	5.05	10.23	12.04	56.81

Table 7. Shoreline structures in the Rhode River expressed as structures per km of shoreline.

	RHD00	RHD01	RHD02	RHD03	RHD04	RHD05	RHD06	RHD07	RHD08	RHD09	RHD10
Dock	6.20	0.00	3.24	0.00	0.00	0.00	0.79	0.00	3.59	2.55	5.02
Dilapadated Dock	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boat House	0.49	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.24	0.64	0.00
Private Boat Ramp	0.35	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.24
Outfall	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24
Total Structures	7.17	0.00	3.72	0.00	0.00	0.00	0.79	0.00	3.83	3.51	5.50

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II. Atmospheric Deposition, Watershed Discharges, and Long-Term Variability of Nutrients, Suspended Solids, and Chlorophyll in the Rhode River

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Knowledge of the sources and dynamics of nitrogen (N), phosphorus (P), and organic carbon (C) is essential for integrated assessment of eutrophic estuarine ecosystems. Since the 1970s, SERC has monitored concentrations of these materials throughout the Rhode River estuary and their inputs to the estuary from its watershed and from atmospheric deposition. Here we highlight monitoring data since the 1990s and cite our publications summarizing earlier data.

Atmospheric deposition:

SERC samples bulk precipitation with a funnel and wetfall with an Aerochem Metric sampler, which opens only during wetfall events. These samplers are mounted on a tower above the surrounding tree canopy. The samples are collected after events of more than 0.5 cm precipitation. Smaller volume events are allowed to accumulate in the sampler until at least 0.5 cm has fallen. The samples are analyzed for pH, major ions, and forms of N, P, and organic C (see Jordan et al., 1995, for methods).

Atmospheric N inputs are the most important to ecosystem function in the Rhode River. Direct inputs of N from atmospheric deposition can exceed N inputs from the watershed during periods of low watershed discharge (Correll and Ford 1982; Jordan et al. 1991a). Moreover, atmospheric deposition is an important N load to the watershed that can be passed through in watershed discharges to the estuary (e.g., Castro et al. 2003). In contrast, atmospheric inputs of P and organic C are trivial compared to other sources in the Rhode River estuary (Jordan et al. 1991a).

Volume-weighted mean concentrations of dissolved nitrate plus nitrite N (NO_3^-), dissolved ammonium N (NH_4^+), and total particulate plus dissolved organic N (TON) in wet deposition span similar ranges and show interannual and seasonal variability (Fig. 1). For this analysis we define winter as January-March, spring as April-June, summer as July-September, and fall as October-December. The seasonal mean concentrations of the different N forms are correlated partly because concentrations of all materials decrease as the volume of the precipitation event increases (Jordan et al. 1995). This is due to wash out of the atmosphere and dilution as precipitation events continue. The variability of rainfall patterns can effect the flow-weighted mean concentrations even at seasonal and annual time scales. Thus, concentrations are higher for seasons or years dominated by smaller-volume precipitation events. After controlling for the effect of varying rain volume, Jordan et al. (1995) found that concentrations of all N forms peak in the spring. High TON concentrations are often linked with pollen deposition (Jordan et al. 1995). TON from pollen would not represent a net N input to the land surface from which it originated but it would be an external N input to the estuary. NO_3^- and NH_4^+ concentrations may also be influenced by the origin of the precipitation-bearing air mass (Jordan et al. 1995). Precipitation from areas with high fossil fuel combustion would

carry more NO_3^-, while precipitation from areas with high animal waste production would carry more NH_4^+. Concentrations in bulk deposition are higher than in wet deposition but bulk deposition samples are much more subject to contamination with insects or bird droppings (Jordan et al. 1995). There were no clear long-term trends in concentrations of any N form after the 1990s (Fig. 1), despite the increasing trends of NO_3^- in the 1970s and of NH_4^+ in the 1980s (Jordan et al. 1995). The fluxes of N forms in wetfall vary mainly with fluctuations in the volume of wetfall, which ranged from 8 cm in the fall of 2001 to 59 cm in the summer of 2003 (Fig. 2), but typically does not show a regular seasonal pattern.

Watershed discharges:

SERC monitors watershed discharges into Muddy Creek, the largest tributary of the Rhode River estuary. Discharges have been monitored since 1974 with a network of automated samplers that measure water flow from several subwatersheds (Fig. 3) and take samples in volumes proportional to the flow (e.g. Correll et al. 1992). The flow-weighted composite samples are collected after fixed sampling periods (mostly weekly) and are analyzed for NO_3^-, and the total of particulate and dissolved N (TN), P (TP), organic C (TOC), phosphate (TPO_4^{3-}), and ammonium (TNH_4^+) (see Jordan et al., 1991a, for methods). For this analysis we combined the measured discharges from the largest subwatersheds to estimate the discharge rate from the entire Muddy Creek watershed.

Watershed discharges of all materials vary greatly among years and seasons (Figs. 4 and 5). As with atmospheric deposition, the variations in fluxes of materials are mainly driven by differences in water flow. However, unlike precipitation volumes, water flow from the watershed shows a strong seasonal pattern with high flows in winter and early spring and low flows in summer and fall due to seasonal changes in evapotranspiration and groundwater supply. Watershed discharges often cease during late summer and early fall during dry years. Thus, watershed discharges often differ by orders of magnitude among different seasons within a year (Figs. 4 and 5). During periods of low watershed discharge, direct atmospheric deposition may be a more important source of N to the estuary than is watershed discharge. Most or all of the interannual differences in watershed discharges can be attributed to differences in annual water discharge.

The discharges of all materials strongly correlate with water flow, but the correlations are stronger for forms of N and organic C than for total suspended solids (TSS) and forms of P (Fig. 6). The concentrations of TSS and forms of P are more variable than those of N and organic C. The factors controlling the concentrations of TSS and the predominantly particulate forms of P are not well understood (Boomer et al. 2008). High concentrations of particulate materials often occur when water flow rates are high (e.g., Boomer et al. 2008), because higher current velocities lead to higher turbulence and a greater capacity to maintain particles in suspension.

We compared concentrations in watershed discharges averaged at annual time scales rather than seasonal time scales because seasons with low discharges are under sampled and have little influence on the annual discharge. The long-term declines in agriculture and increases in residential development that have occurred in the watershed would be expected to cause changes in concentrations of N and P forms. Croplands are important sources of N and P discharges in the Rhode River watershed (e.g. Correll et al. 1992). However, there were no clear long-term trends in the concentrations of N forms

(Fig. 7). The lack of trends could reflect the slow release of groundwater enriched in N from past agriculture, or it might reflect offsetting effects of increasing residential development. TP, TPO_4^{3-}, and TSS concentrations showed long-term increases over time (Fig. 8). This may be partly due to several years with high water flow occurring near then end of the time series and to the tendency of particulate materials to increase in concentration with increasing water flow.

Concentrations in the estuary:

SERC has monitored water quality in the Rhode River estuary since the early 1970s, but most intensively since 1980. For sampling purposes the estuary is divided into segments along its axis (Fig. 3). Spatially integrated samples were taken by pumping surface water while cruising the length of each segment. Profiles of salinity and temperature are measured *in situ* at the boundaries and centers of the segments. Sampling took place from March-November at irregular intervals in the 1970s, weekly 1980-1986, and every other week after 1986. Spatially integrated samples are analyzed for NO_3^-, NH_4^+, particulate ammonium (PNH_4^+), PO_4^{3-}, particulate phosphate (PPO_4^{3-}), dissolved and total Kjeldhal N, dissolved and total organic C (DOC, TOC), TSS, and chlorophyll *a* (chla). Particulate organic P, N, and C (POP, PON, POC) were calculated by subtracting dissolved from total concentrations (see Jordan et al. 1991a for methods).

Concentrations of many materials show distinctive seasonal and spatial patterns (e.g. Jordan et al. 1991a). For example, NO_3^- concentrations in the estuary reflect inputs from watershed discharges, with highest concentrations in winter and spring and with concentration increasing closer to the watershed inputs (Fig. 9). Thus, NO_3^- concentration increases moving up-estuary from segment 5 toward segments 7 and 8 where the local watershed inputs enter. However, NO_3^- concentration also increases moving down-estuary from segment 5 toward segment 3, indicating a source of NO_3^- input from adjacent upper Chesapeake Bay, which in turn receives NO_3^- from the Susquehanna River. Apparently, biotic uptake of NO_3^- in the upper Rhode River draws NO_3^- from both the local and distant watersheds. Detailed time series analyses have resolved the separate influences of the Susquhanna and local watersheds on nutrients and chla in the Rhode River (Jordan et al. 1991b).

In contrast to NO_3^-, PO_4^{3-} concentration peaks in summer in the upper estuary in segment 6 (Fig. 9) indicating a source of PO_4^{3-} release in the upper estuary with dilution of the PO_4^{3-} up-estuary by local watershed inputs and down-estuary by mixing with adjacent Chesapeake Bay water. The opposing spatial and seasonal patterns of PO_4^{3-} and NO_3^- result in changes in the relative availability of N and P to phytoplankton and spatial and seasonal changes in nutrient limitation (Jordan et al. 1991a).

Increase in chla and TSS up-estuary (Fig. 9) may reflect effects of shoaling. Greater phytoplankton may be supported by more light availability in shallower waters, while higher TSS concentration may reflect the resuspension of bottom sediments and the increase in bottom area relative to water volume. Both chla and TSS peak in summer (Fig. 9) perhaps partly due low flushing rates from low watershed discharges during summer but also due to the seasonality of phytoplankton growth and bioturbation.

There is also much interannual variation in concentrations and some apparent long-term trends. Some interannual variation may be related to changes in watershed discharges. For example, NO_3^- concentrations are lower during years with lower

discharge rates (Fig. 10). Note the especially low NO_3^- concentrations during 2001 and 2002 (Fig. 10) when watershed discharges were also low (Fig. 4). In contrast, PO_4^{3-} concentration tends to be higher years with lower watershed discharge (Figs. 11 and 4) because PO_4^{3-} is produced within the estuary and flushed out by watershed discharges. Flushing could be quantified using a mixing model based on salinity as in previous analyses (Jordan et al. 1991a).

A few materials showed long-term trends in concentration. For example, the summer peaks in PO_4^{3-} concentration seem to be lower after 1998 than before (Fig. 11). This could partly reflect the recent occurrence of several summers with high watershed discharges (Fig. 11) that would flush out PO_4^{3-}. However, PO_4^{3-} concentration did not reach comparable pre-1998 concentrations in the summers of 2001 and 2002 (Fig. 11) when watershed discharges were very low (Fig. 4). This trend in PO_4^{3-} concentration is in puzzling contrast to the increasing trend in TPO_4^{3-} concentration in watershed discharges (Fig. 8). Increasing inputs of TPO_4^{3-} from the watershed would be expected to increase PO_4^{3-} concentration in the estuary because dissolved PO_4^{3-} in the estuary is supposedly released from particulate PO_4^{3-}, which is the largest component of TPO_4^{3-} (Jordan et al. 1991a). There seems to be decadal scale fluctuations in estuarine TN, which decreased during the 1990s and increased after 1999 (Fig. 10). Also, TSS seemed to increase steadily from 2002-2008, in step with the TN increase. A previously observed decrease in DON from 1982 to 1986 (Jordan et al. 1991b) did not continue later on (Fig. 10). Instead, DON increased back to pre-1982 levels and remained there.

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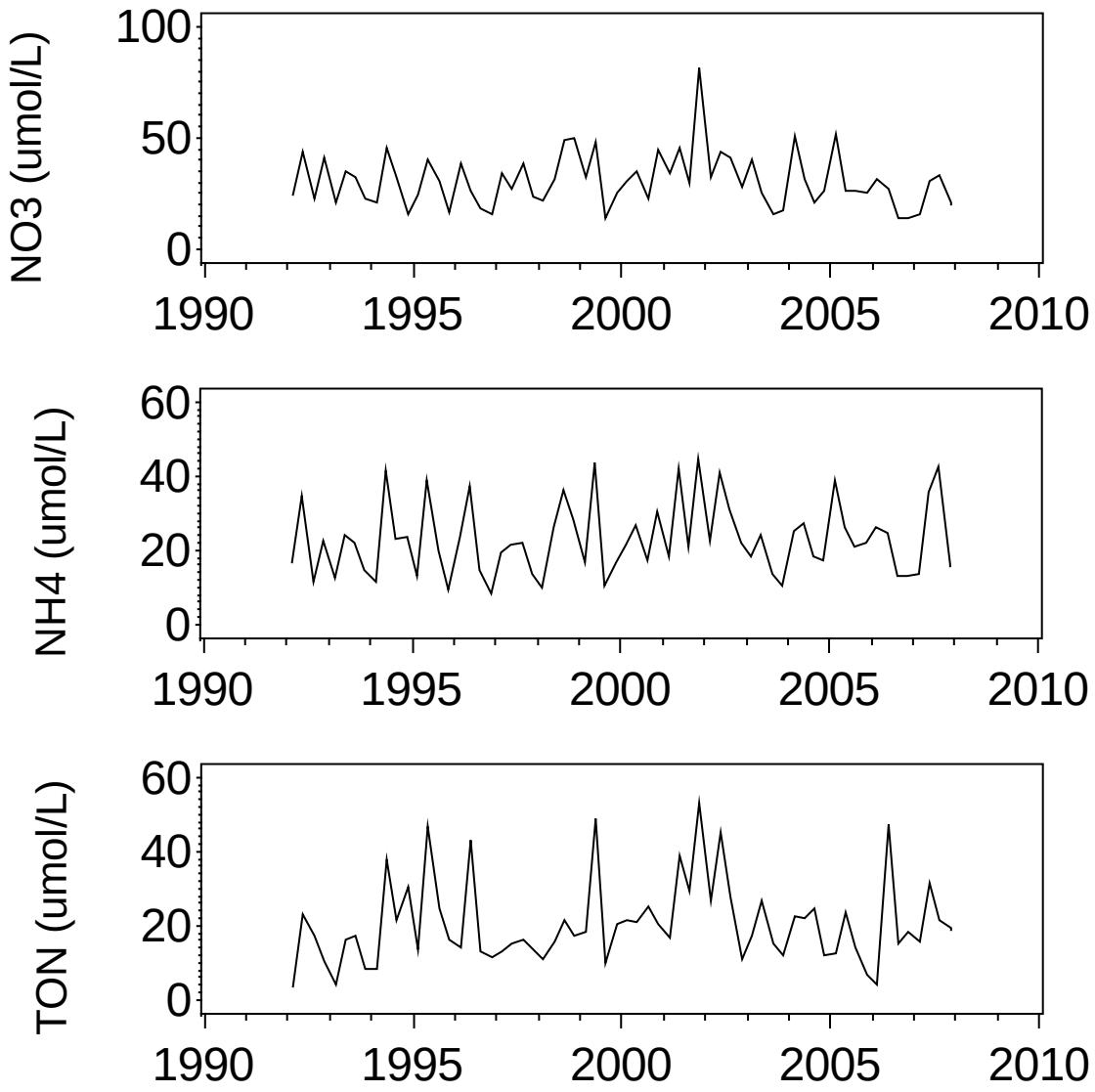

Fig. 1. Volume weighted seasonal mean concentrations of NO_3^-, NH_4^+, and TON (μmol N/L) in wetfall versus time.

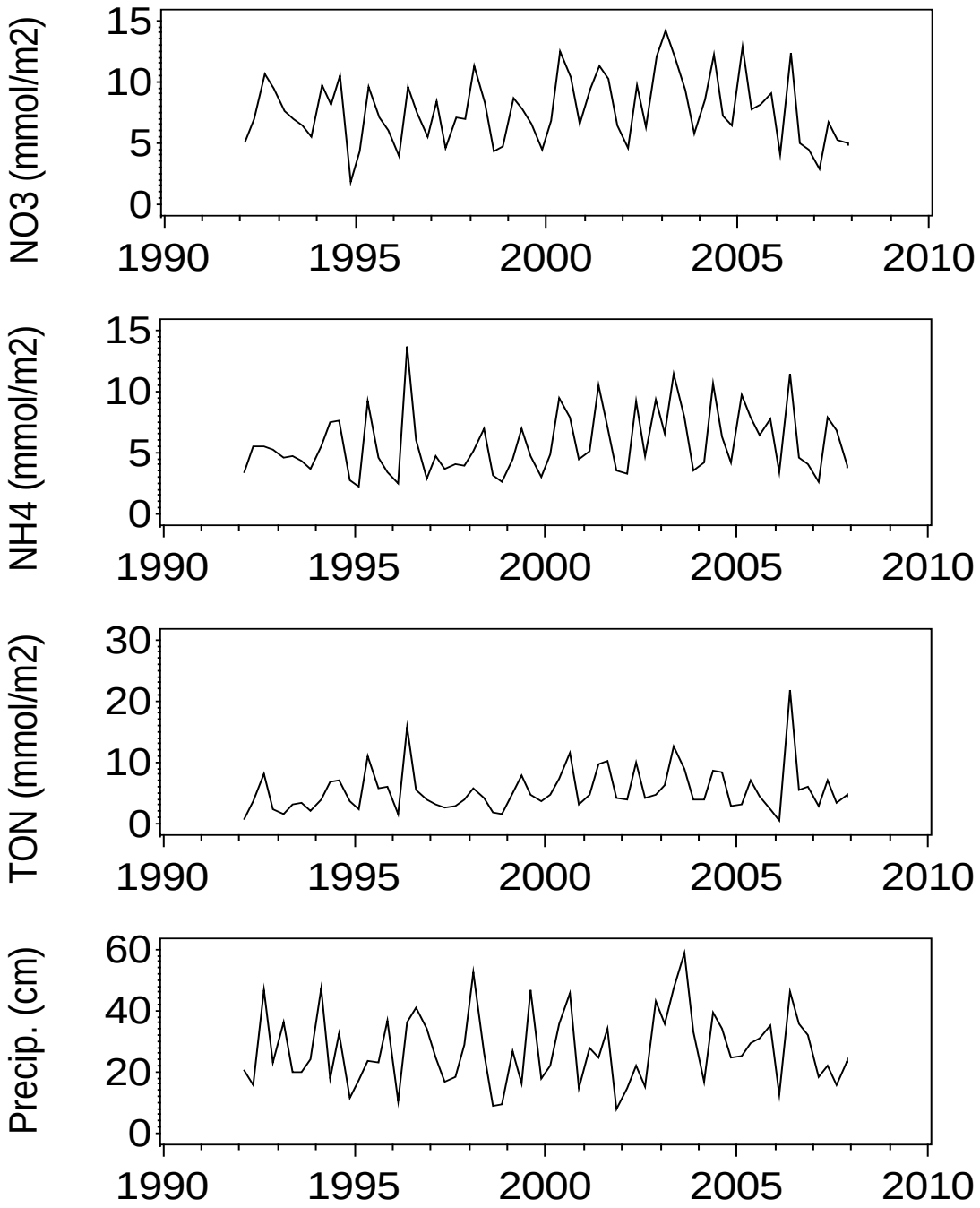

Fig. 2. Fluxes of NO_3^-, NH_4^+, and TON (mmol N/m^2) in wetfall and precipitation volume (cm) versus time.

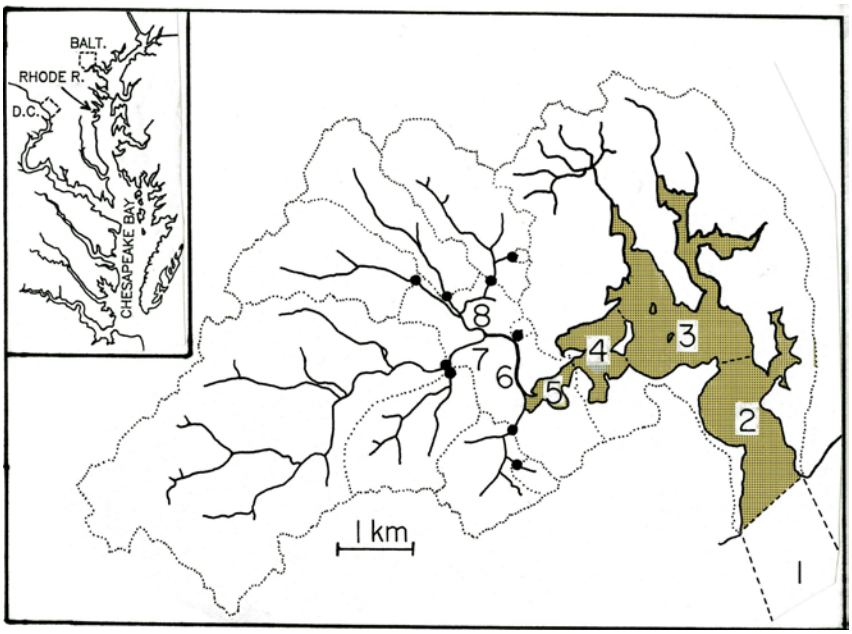

Fig. 3. The Rhode River estuary and watershed. Monitored subwatersheds are outlined with dotted lines and large dots show locations of watershed discharge monitors. Numbered segments extend from Chesapeake Bay (1) to the forks of Muddy Creek (7, 8), the largest tributary. Sampling of the estuary focused on segments 3-8. Water quality in segments 1 and 2 was generally similar to that in segment 3.

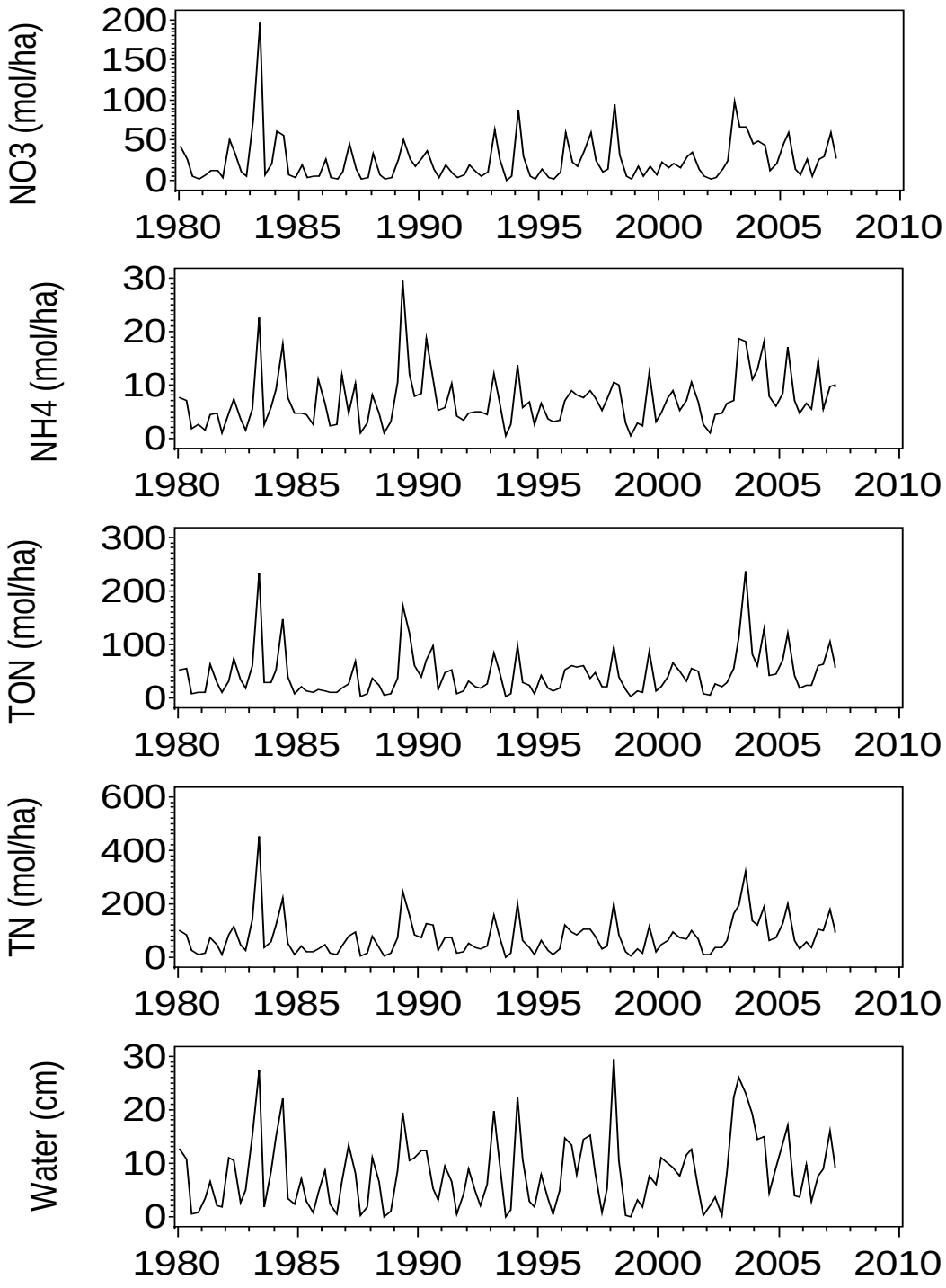

Fig. 4. Seasonal total watershed discharges of N forms (mol N/ha per season) and water (cm per season) versus time. Note that 1 cm of water discharge is equivalent to 100 m^3/ha.

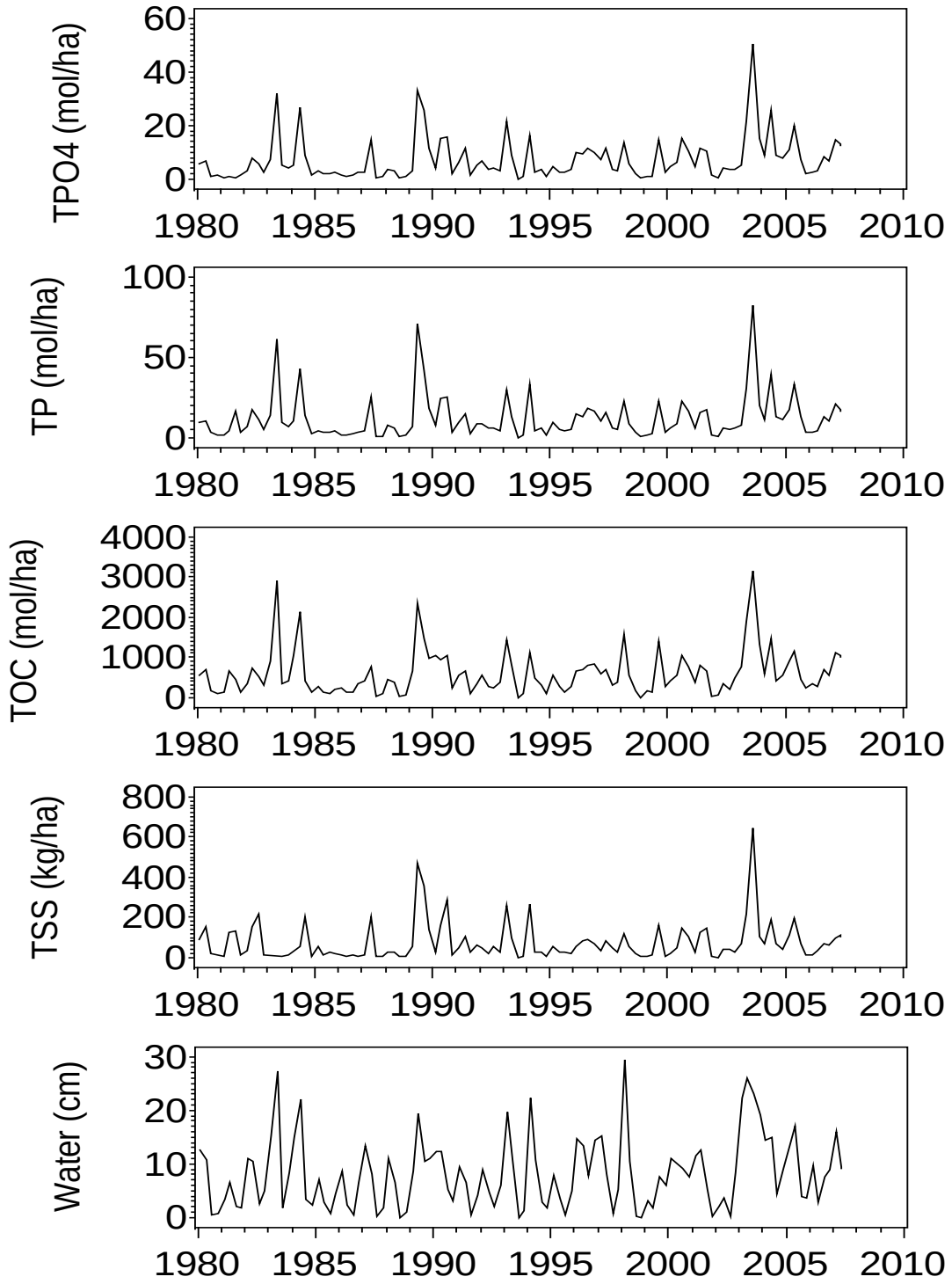

Fig. 5. Seasonal total watershed discharges of P forms, TOC (mol P or C/ha per season), TSS (kg/ha per season) and water (cm per season) versus time. Note that 1 cm of water discharge is equivalent to 100 m^3/ha.

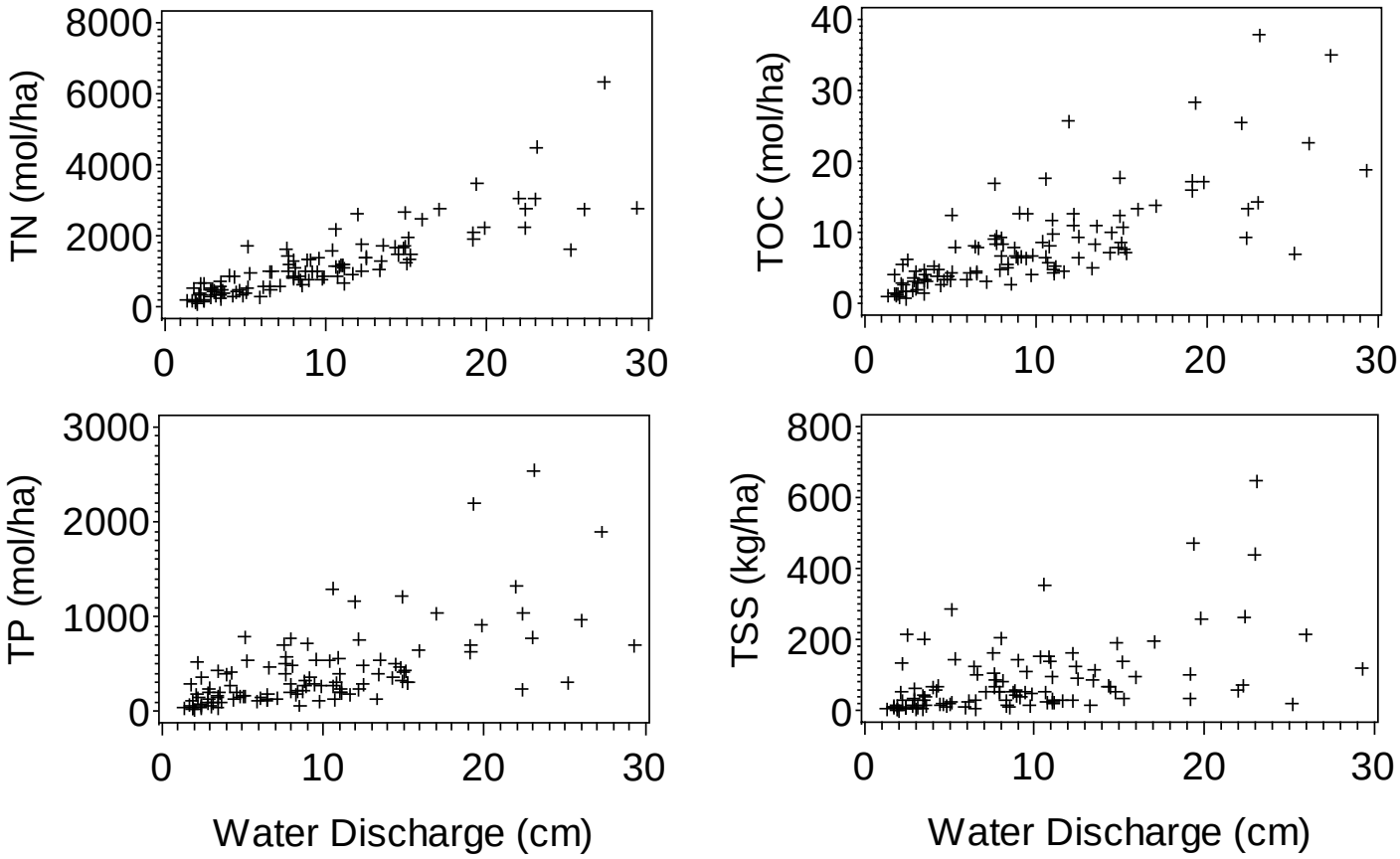

Fig. 6. Seasonal total watershed discharges of TN, TP, TOC (mol N, P or C/ha per season), and TSS (kg/ha per season) versus water discharge (cm).

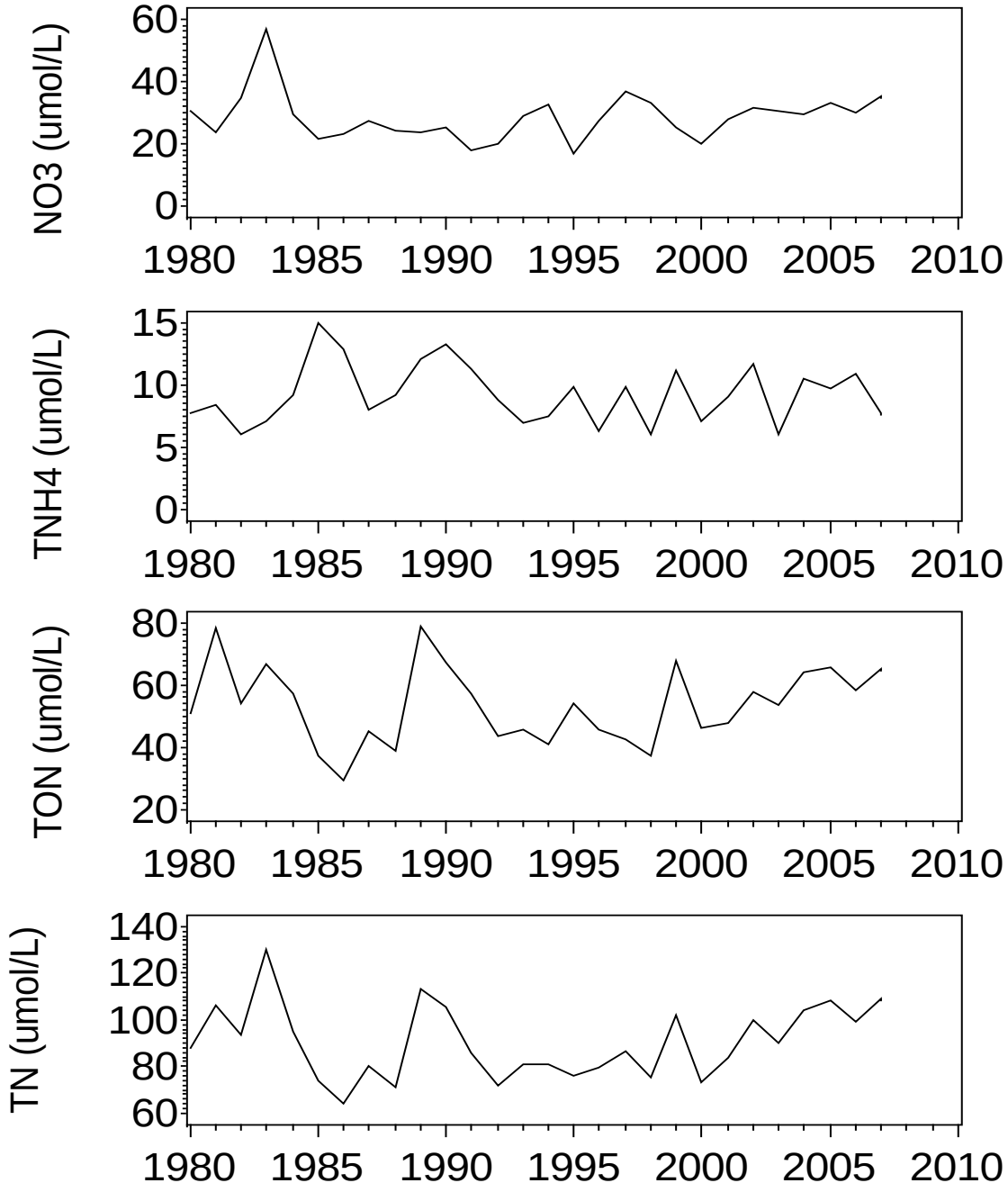

Fig. 7. Annual flow weighted mean concentrations of N forms (μmol N/L) in watershed discharges versus time.

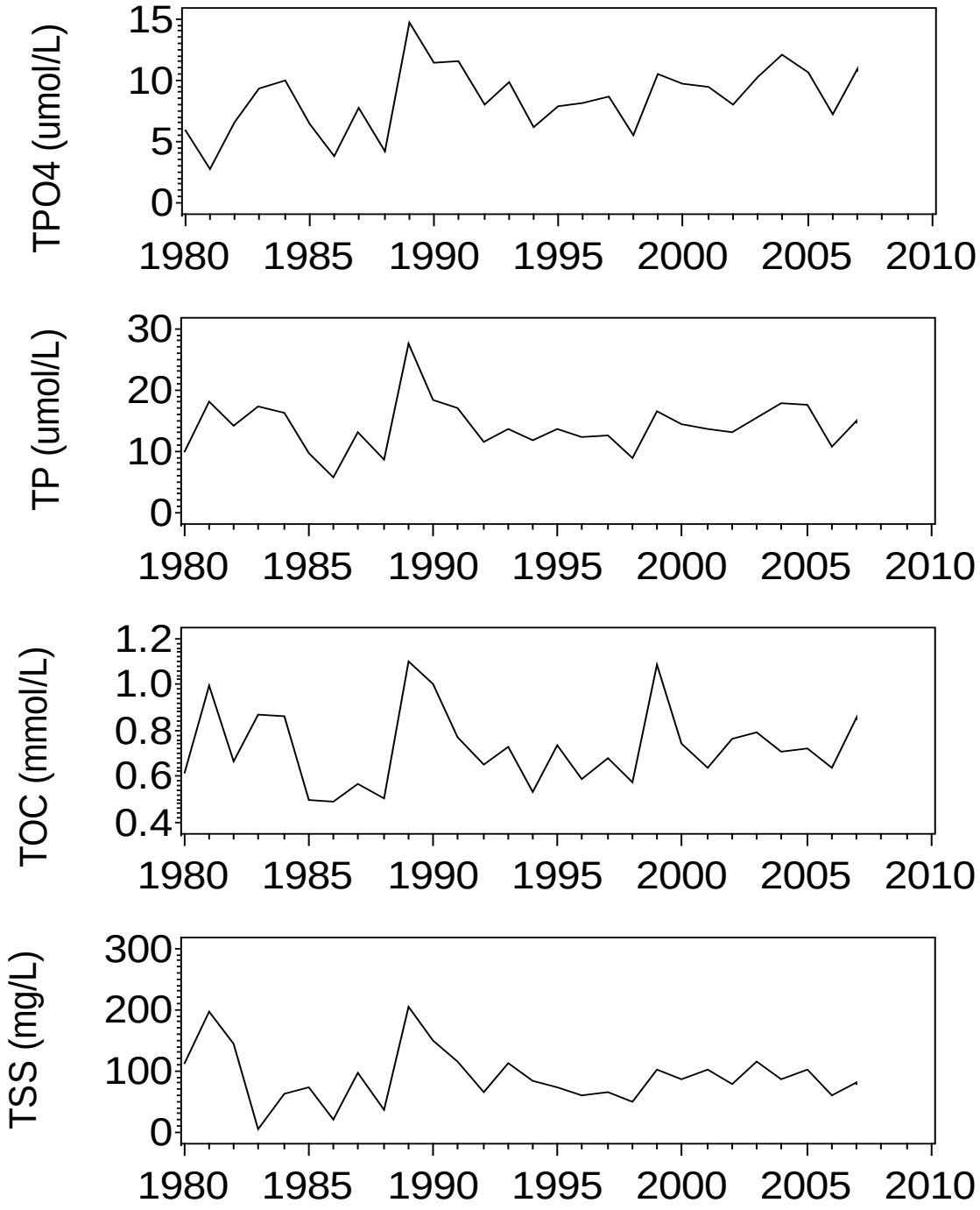

Fig. 8. Annual flow weighted mean concentrations of P forms, TOC (μmol P or C/L) and TSS (mg/L) in watershed discharges versus time.

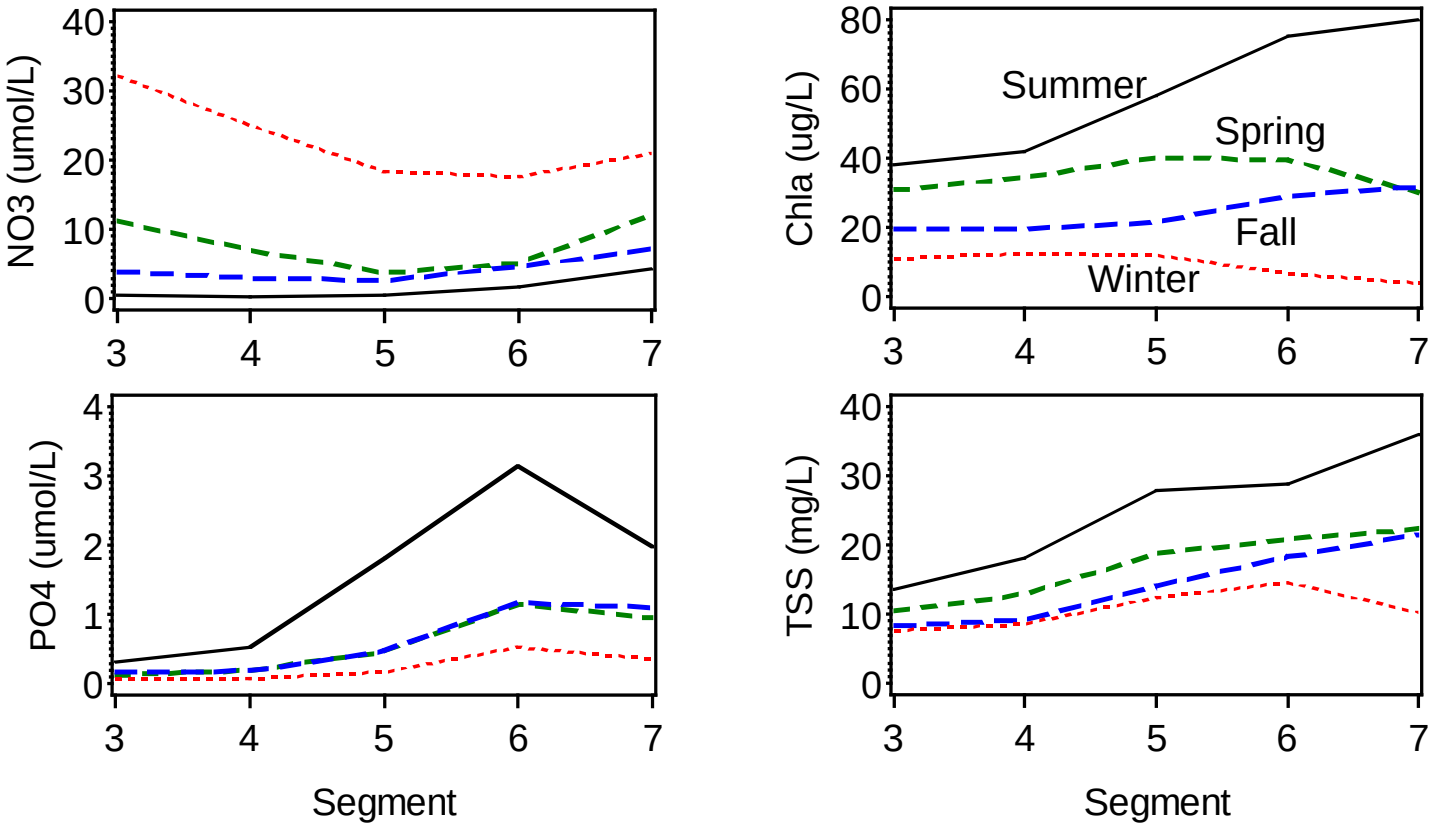

Fig. 9. Seasonal mean concentrations of NO_3^-, PO_4^{3-} (μmol N or P/L), chla (μg/L) and TSS (mg/L) averaged over 1986-2008 and plotted versus segment. The different line types representing each season follow the convention as labeled on the chla plot. Values plotted as segment 7 are the combined means of both forks of Muddy Creek, segments 7 and 8 (Fig. 3).

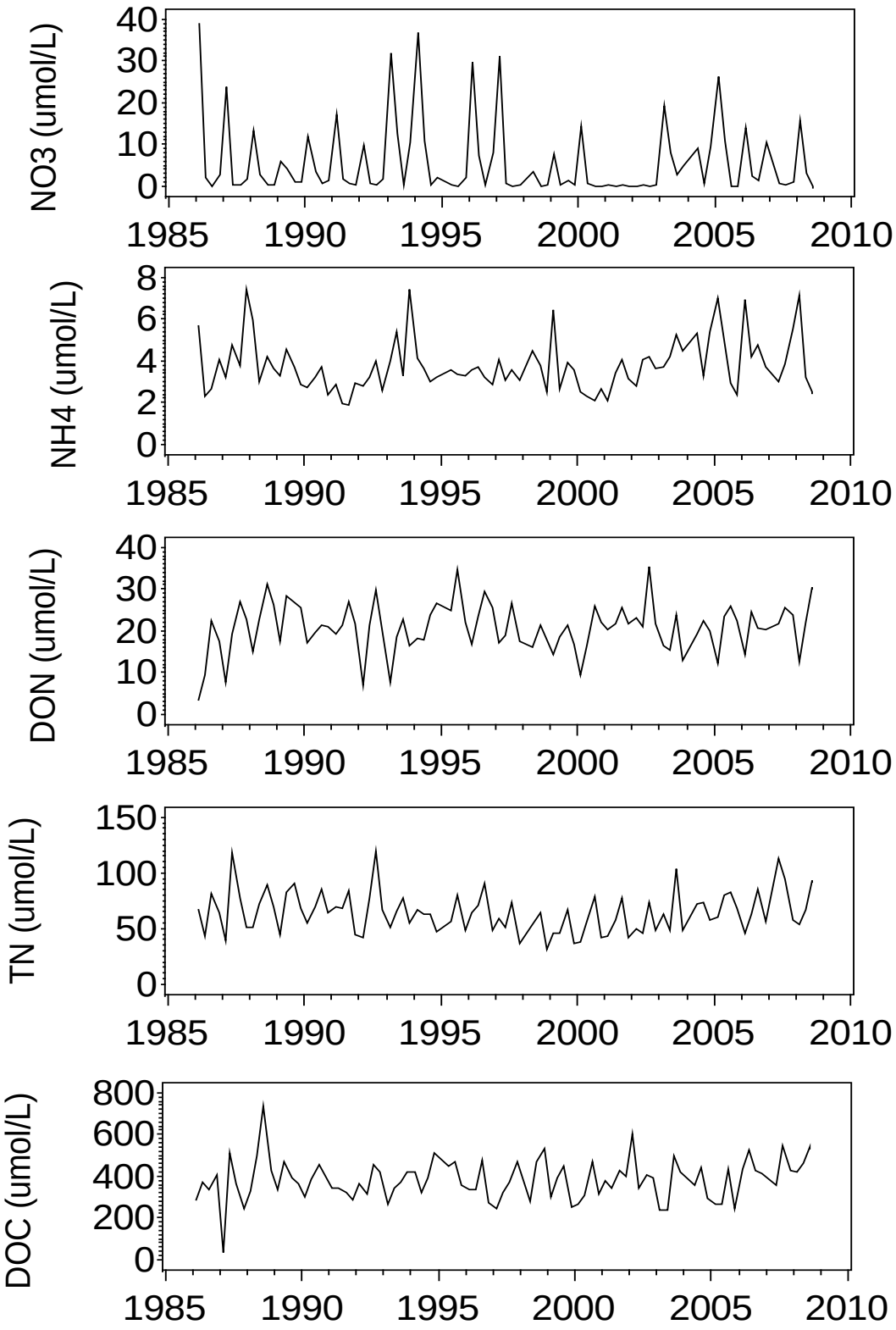

Fig. 10. Seasonal mean concentrations of N forms and DOC (μmol N or C/L) in segment 5 of the Rhode River versus time.

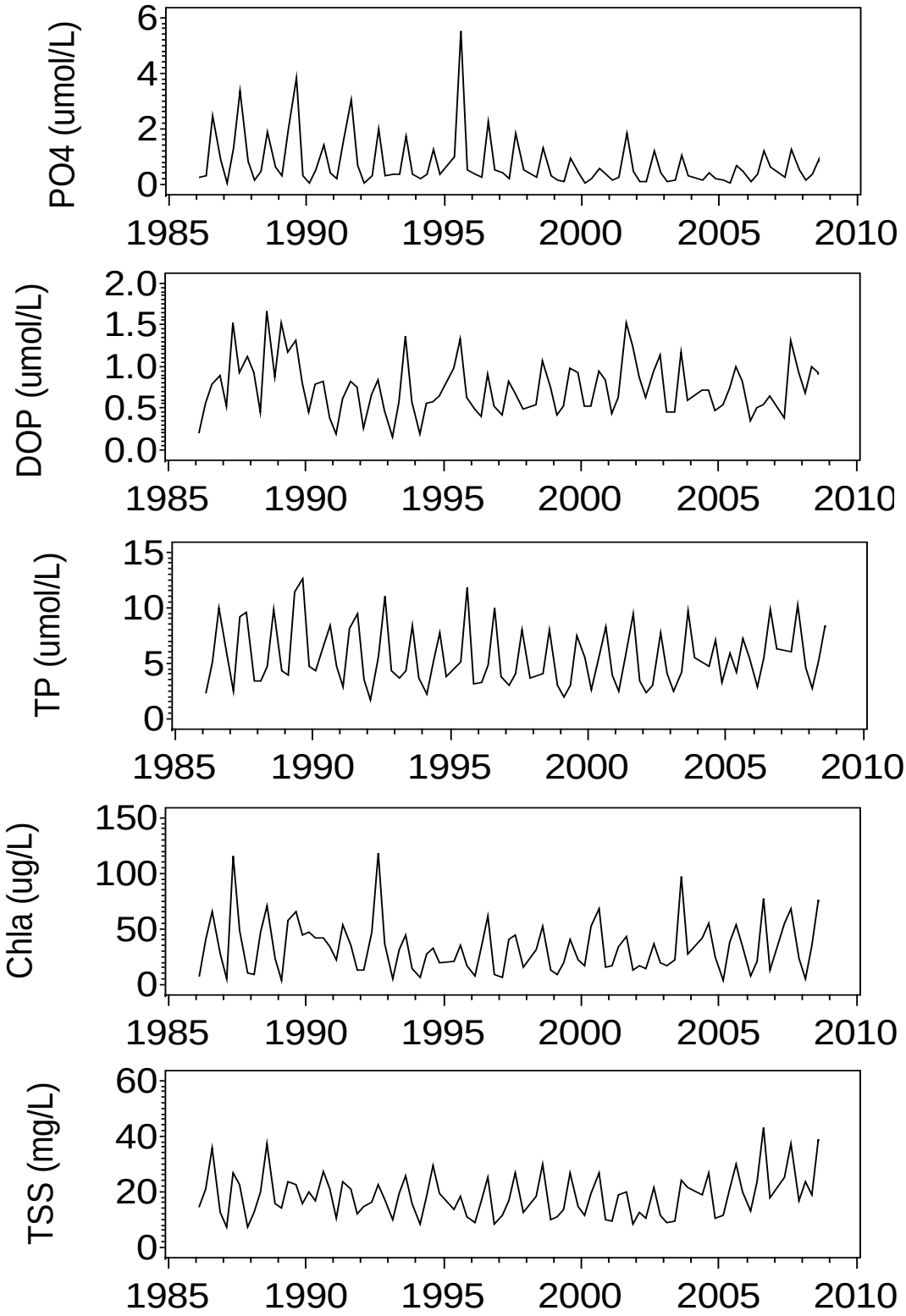

Fig. 11. Seasonal mean concentrations of P forms (μmol P/L), Chla (μg/L), and TSS (mg/L) in segment 5 of the Rhode River versus time.

III. TIMING, EXTENT, AND MECHANISMS OF *PHRAGMITES AUSTRALIS* SPREAD IN THE RHODE RIVER SUBESTUARY

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Introduction

Phragmites australis (Cav.) Trin. Ex Steud. is a circumboreal, perennial grass that is rapidly expanding into freshwater and brackish wetlands across North America (Marks *et al.* 1994). Although *Phragmites* is native to this region, a non-native haplotype of *Phragmites*, likely introduced from Europe in the late 1700's or early 1800's, was recently identified (Saltonstall 2002) and is thought to be responsible for the aggressive and widespread invasion of this plant (Saltonstall 2003a, b, Lelong *et al.* 2007). Spread of non-native haplotypes of *Phragmites australis* has dramatically changed a wide range of estuarine and freshwater wetland communities throughout the United States, especially along the Atlantic coast and Chesapeake Bay regions (Saltonstall 2003b).

In contrast to native types of *Phragmites*, which can form relatively loose patches and are only one component of multispecies stands of vegetation, the non-native genotypes often form dense mono-specific stands in fresh and brackish wetlands. The non-native, invasive form of *Phragmites* is of concern to citizens and scientists alike because it can alter floral and faunal diversity and wetland nutrient dynamics as well as affect property values (Keller 2000, Meyerson *et al.* 2000, Lathrop *et al.* 2003, Windham *et al.* 2003, Minchinton *et al.* 2006). Although non-native types of *Phragmites* have been spreading throughout the eastern United States since at least the 1800s, their rate of spread has increased dramatically in recent decades (Saltonstall 2002).

Concern caused by this recent rapid expansion has prompted investigations into how *Phragmites* is spreading and to what extent its spread has increased in recent decades. This information is similarly important for conservation and management of *Phragmites* in Europe, where many patches have been declining (Clevering and Lissner 1999). In the Chesapeake Bay, King *et al.* (2007) found that *Phragmites* was more abundant in wetlands in subestuaries that had largely developed watersheds than in wetlands in subestuaries with largely forested watersheds. Furthermore, the degree of development on the watershed was reflected by nutrient loading in the water, such that estuarine wetlands associated with developed watersheds had higher nutrient concentrations in the water and *Phragmites* had higher foliar nitrogen levels compared to wetlands in forested watersheds. Similarly, development and eutrophication both were positively related to the abundance of *Phragmites* in coastal wetlands of Rhode Island (Bertness et al. 2002, Minchinton and Bertness 2003, Silliman and Bertness 2004). However, to understand the mechanisms by which increased development and nutrient loads may increase *Phragmites* spread, we first need to understand how *Phragmites* is spreading.

In many plant species there is only one mode of spread, by seed. However, most aquatic and many invasive species are at least facultatively clonal (Barrett *et al.* 1993), raising questions about the reproductive mode responsible for their spread. *Phragmites* can reproduce both

clonally and sexually. Plants form dense mats of rhizomes that are largely responsible for excluding other species from *Phragmites* patches (Minchinton *et al*. 2006). Each rhizome has multiple nodes from which new plants can sprout. Rhizome pieces that break off can be carried in the water to lodge in wrack piles to sprout and start new patches (Minchinton 2002). *Phragmites* also produce windborne seeds that can travel long distances in the air and can float for extended distances in the water (Minchinton 2002).

Despite the substantial interest in understanding the ecology and management of non-native *Phragmites*, we still lack an understanding of what factors contribute to its spread. The extent to which seeds and rhizomes are responsible for patch growth and establishing new patches of *Phragmites* is unclear. Studies in Europe have found a mixture of seed and rhizome establishment. Haslam (1972) found that population dynamics were largely driven by rhizome growth and sexual reproduction (i.e., seed recruitment) was rare. In contrast, Alvarez *et al*. (2005) found that population dynamics of *Phragmites* in the Mediterranean were driven by a combination of slow rhizome growth and episodic seed recruitment. Koppitz (1999) found that there was substantial genetic diversity among patches worldwide, suggesting substantial sexual reproduction, at least among patches. Multiple studies report both monoclonal and polyclonal patches (e.g., Clevering and Lissner 1999, Koppitz 1999, Alvarez *et al.* 2005). Guo *et al.* (2003) found multiple clonal types within every population they investigated.

Spread of invasive species in their non-native habitats could be achieved differently than in their native habitats. Studies of *Phragmites* in North America have also provided mixed results. Pellegrin and Hauber (1999) found that patches of (most likely native) *Phragmites* along the U.S. Gulf Coast were produced almost exclusively vegetatively, as evidenced by fixed heterozygosity and very limited genetic variation. Studying non-native *Phragmites* in Massachusetts, Keller (2000) found substantial genetic variation among patches but attributed this to accumulation of somatic mutations followed by asexual propagation, rather than recruitment from seed. Similarly, Hudon and Gagnon (2005) attributed most spread of *Phragmites* in the Saint Lawrence River, Québec, to be primarily a result of vegetative spread, although they acknowledged that some long-distance seed spread was also likely. Gervais *et al.* (1993) suggested that generally poor seed set and slow development of seedlings made establishment of patches from seed unlikely.

Differences in the mode of spread in different habitats within *Phragmites*' invasive range may also explain differences in their invasive abilities. As described above, in a study of *Phragmites* cover in the Chesapeake Bay, King *et al*. (2007) found that *Phragmites* cover within a subestuary correlated with watershed development; the more highly developed the land within the watershed of a subestuary, the greater the abundance of *Phragmites* and the higher the levels of leaf tissue nitrogen of the plants in that subestuary. Kettenring and Whigham (in prep) and Kettenring *et al*. (in prep) found that some *Phragmites* patches in subestuaries with developed watersheds produced more viable seeds than did patches in forested watersheds.

In this study, we used comparisons between *Phragmites* patches on the forested vs. developed sides of the Rhode River subestuary to determine if there were differences in the spread of the species between the two very different parts of the subestuary. We also compared patches on the two sites of the estuary to determine if there were differences in the genetic characteristics of patches. We mapped all existing patches of *Phragmites* in the subestuary, compared patch locations and extent to those previously mapped by McCormick and Somes (1982), and examined the distribution of genetic variation within and among patches to identify the mode by which *Phragmites* is spreading in the Rhode River. We calculated the area of

Phragmites cover on developed and forested sides of the Rhode River and compared the genetic variation in patches on either side. We used microsatellite analysis to distinguish sexual from clonal spread within and among patches and tested the extent to which mode of spread differed with degree of development within the watershed. We then compared these results to findings in other subestuaries.

Methods

Study area: This study was conducted in brackish tidal wetlands of the Rhode River, a subestuary of the Chesapeake Bay, near Edgewater, Maryland, USA (38°53'N, 76°32'W). The Rhode River watershed contains a mixture of forested, agricultural, and developed land. The Rhode River is somewhat unique because virtually half of the watershed is developed and the other half is dominated by forests.

Defining and mapping patches: From September to November 2007 we traveled all branches of the Rhode River by boat and noted the locations of all patches of *Phragmites*. We defined a *Phragmites* "patch" as being a robust stand of plants isolated from other stands by a distance of at least 5 meters. Alternatively, the stands had to be separated by a distance of at least 10 meters if there were sparse *Phragmites* stems between robust stands.

We used a global-positioning system (Garmin GPS 12CX; Olathe, Kansas) to determine the geographic location of each patch by, in most cases, walking its perimeter. Occasionally, if access to a patch was limited, we estimated the size and/or location in the field by using select GPS waypoints and delineating the stand on a 1998 natural color 1"=200' scale aerial photograph (MrSid, VarGIS, Herndon, VA, USA). Using the GPS data and field sketches, we digitized all *Phragmites* patches using the geographic information system, ArcGIS 9.2 (ESRI; Redlands, California).We used a similar digitization of McCormick and Somes' (1982) vegetation maps obtained from Maryland Department of the Environment to relate the positions of patches in 1971-2 to current patches. We then used ArcGIS to calculate the total area of patches in 1971-2 and 2007 overall and on the developed and forested sides of the river.

Genetic variation assessment: We used microsatellite analysis to determine the amount of genetic diversity within and among patches of *Phragmites* in the Rhode River following the methods of Saltonstall (2003). To ensure adequate extraction of lysate, we collected the freshest leaf of sufficient size available, which was usually from a non-reproductive (vegetative) plant. Leaves were kept in plastic storage bags in a refrigerator at 4°C until DNA was extracted. DNA was extracted from approximately 20 mg of fresh tissue using a BioSprint 96 (QIAGEN, Inc.; Valencia, California) adhering to the supplied protocol.

Multilocus gene phenotypes of individual *Phragmites* plants were assessed using microsatellite markers. Gene phenotypes (i.e., each allele is classified as present or absent) were used because determination of the number of copies of each allele is unreliable in polyploid species (Becher *et al.* 2000, Saltonstall 2003). Eight different primer pairs (developed by Saltonstall 2003a) were used to target different regions of the DNA (Table 1). Annealing temperatures for the primer pairs were determined during trials prior to analysis of samples for maximum yield of amplification product. PCR amplification was performed using a PTC-200 DNA Engine thermalcycler (MJ Research, Inc.; Waltham, Massachusetts) programmed using the following conditions: an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 30 sec, 50-58°C (depending on primer; See Table 1) for 30 sec, and 72°C for 10 sec, with a final polymerization step at 72°C for 2 min. PCR was run as 12.5µl volume reactions with concentrations as follows: 1.25 µl template DNA (diluted 1:5-1:100 depending on fluorophor

and primer pair, see Table 1), 3.2 µl distilled water, 0.75 µl of each primer, 0.3 µl 25 mM $MgCl_2$, 6.25 µl RedMix Plus (Gene Choice, Inc.; Frederick, MD, USA).

Amplified samples were subjected to analysis on an ABI 3100 Automated Capillary DNA Sequencer (Applied Biosystems, Inc.; Foster City, California) using a custom ROX size standard to determine fragment sizes. After amplification, PCR product amplified with different fluorophores and with different expected fragment sizes were combined prior to fragment size analysis as follows: primers 4+9+16, primers 12+13+22, and primers 14+21. Fragment sizes were determined using GeneMapper v4.0 (Applied Biosystems, Inc.; Foster City, California).

Fragments for all samples were aligned using a TRFLP peak sorting function for Excel (Rees *et al.* 2004, http://www.wsc.monash.edu.au/~cwalsh/treeflap.xls) and shadow peaks were removed manually.

The probability of a repeated gene phenotype arising by chance sexually rather than asexually was calculated as $P = (\Pi p_i q_i) 2^h$, where p_i and q_i are the frequencies of the two alleles at the *i*th locus and *h* is the number of heterozygous loci in the genotype (Parks and Werth 1993, Pollux *et al*. 2006) modified for gene phenotypes (i.e., X1/X2 in a tetraploid could be 1, 2, or 3 copies of allele X1 and 3, 2, or 1 copies of allele X2). We used a moderately conservative cutoff of $P<0.001$ to identify repeated gene phenotypes arising through asexual reproduction rather than through seed because inbreeding can greatly increase the probability of repeated gene phenotypes arising through sexual reproduction.

Data analysis: DNA samples with identical multilocus genotypes were assumed to result from asexual reproduction. We compared all multilocus gene phenotypes found within and among patches to identify all repeated types, indicative of expansion and establishment of new patches by rhizome fragments, respectively. Because *Phragmites* is polyploid (all samples in Rhode River appeared to be tetraploid) many calculations of genetic similarity were not appropriate so we calculated genetic similarity as a function of distance using Moran's I statistic (SPAGeDi v1.2g, Hardy and Vekemans 1999, 2002) at distances of 10, 50, 100, 200, 500, 1000, 5000m.

All identical gene phenotypes among patches were identified. The genetic similarity of gene phenotypes were compared within patches and to other patches in the Rhode River using F- and R-statistics. F-statistics were calculated both with and without repeated samples removed (Halkett *et al.* 2005) using the method of Weir and Cockerham (1984), which is appropriate for polyploid samples. R-statistics (Slatkin 1995) were also calculated for comparison. F- and R-statistics differ in the model they assume for mutation accumulation. F-statistics assume an infinite allele model and R-statistics assume a stepwise mutation model. In particular, the two statistics differ in how they are affected by the mutation rate. Substantial mutation rates, as might be expected for microsatellite loci, depress F_{ST} values. In contrast, R_{ST} is unaffected by mutation rates but suffers from much higher variation. The two statistics represent opposing extremes of mutation models, neither of which is likely to strictly match the mutation of microsatellite loci, rather each is more appropriate in some conditions, so both are reported here as suggested by Balloux and Lugon-Moulin (2002).

Results

Defining and mapping patches: We identified 212 *Phragmites* patches in the Rhode River subestuary in 2007, 49 on the developed and 159 on the forested side of the subestuary. This represents a dramatic increase from the 5 patches identified in 1971. *Phragmites* coverage expanded rapidly from 7,294 m^2 in 1971-2 to 183,369 m^2 in 2007 (Fig. 1). Much of this growth

occurred on the forested side of the river, largely due to a greater area of available wetland habitat. In 2007, *Phragmites* covered 135,131 m^2 on the forested side and 48,218 m^2 on the developed side of the Rhode River. More *Phragmites* was also present on the forested side of the river in 1971 (5947 m^2 on the forested side versus 1346 m^2 on the developed side of the river) so *Phragmites* increased to 36 times its 1971 coverage on the developed side of the river, while increasing coverage 15 times on the forested side of the river.

Spread within patches and establishment of new patches: Overall, 55 of 57 patches from which we obtained multiple DNA samples contained multiple gene phenotypes. In 33 patches all samples had unique gene phenotypes. This did not differ with degree of local development.

Six pairs of patches had identical gene phenotypes. Calculation of the probability of repeated gene phenotypes arising by chance revealed that five of the six pairs of identical gene phenotypes could have reasonably have arisen by seed ($P>0.01$). In five of these repeated pairs the high probability of arising by chance resulted from missing data at 3-5 of the 8 loci and the presence of common alleles at the loci for which data were present in both samples being compared. The single remaining case of repeated gene phenotypes had very low probability of arising by chance ($P=0.00002$) with respect to the overall frequency of alleles in the subestuary and so was considered to be a case where a patch could have been established by rhizome. However, note that these patches could have been established by growth of a single rhizome mat rather than requiring transport of rhizome fragments (Fig. 1).

Genetic similarity with distance: Across the Rhode River subestuary plants that were physically closer together were genetically more similar than those farther apart (Figure 2). Moran's I decreased quickly with distance separating samples, however, Moran's I was significantly different from 0 (no relation) even out to 1000m. This was true regardless of whether repeated samples within patches (likely repeated sampling of the same genet) were included in the analysis, so the data presented do not include within-patch repetitions.

F-statistics jackknifed across all eight loci indicated that substantial genetic variation was distributed among patches ($F_{ST} = 0.225 \pm 0.025$ SE), while individual samples showed lower heterozygosity ($F_{IS} = 0.161 \pm 0.077$), indicative of inbreeding, and samples within patches were significantly inbred (local pollen exchange) relative to the subestuary as a whole ($F_{IT} = 0.351 \pm 0.072$). Removing repeated gene phenotypes produced a slight decrease in F_{ST}, a corresponding increase in F_{IS} and little change in F_{IT}. For comparison, R-statistics were also calculated without repeated gene phenotypes and jackknifed across all eight loci. R_{IS} and R_{IT} values were similar to F_{IS} and F_{IT} ($R_{IS} = 0.222 \pm 0.182$ SE, $R_{IT} = 0.319 \pm 0.145$ SE) but R_{ST} was lower than F_{ST} ($R_{ST} = 0.129 \pm 0.026$ SE).

Discussion

Spread of *Phragmites* in the Rhode River subestuary has been substantial since McCormick and Somes (1984) mapped tidal wetland vegetation in 1971-2. Although *Phragmites* has been present in the Chesapeake Bay system since the 1800's, few patches were present as late as the early 1970's and they were relatively small. This suggests that some recent change of conditions in the watershed, changes in wetland conditions within the subestuary or changes in *Phragmites* populations has resulted in the dramatic recent increases in *Phragmites* coverage.

Increases of *Phragmites* in other systems have been tied to nutrient conditions (King *et al*. 2007), climatic conditions favoring seed recruitment, especially dry-downs (Alvarez *et al.* 2005), alteration of hydrologic conditions (Hudon and Gagnon 2005), shoreline disturbance (Bertness *et al*. 2003, Minchinton and Bertness 2004), and placement of construction fill (Keller 2000,

Lelong *et al.* 2007). King *et al.* (2007) found increased *Phragmites* coverage in brackish wetlands that occurred in subestuaries with developed as opposed to forested watersheds and related this to nutrient levels in water, which were also reflected in the nitrogen content of *Phragmites* leaves. Baron *et al.* (in prep.) found that nitrogen and phosphorus levels did not differ in *Phragmites* leaves between the developed and forested sides of the Rhode River, suggesting that nutrients in a subestuary are well-mixed, causing developed and forested sides of the Rhode River to respond as a single system, rather than differing as a result of local landuse.

Despite suggestions from multiple authors (e.g., Pellegrin and Hauber 1999, Keller *et al.* 2000, Saltonstall 2002) that spread of invasive *Phragmites* in the U.S. occurs primarily through vegetative reproduction, we found that *Phragmites* in the Rhode River has spread primarily through seed. Nearly all discrete patches were genetically unique, indicating they were most likely established by seed. Furthermore, even spread of individual patches seems to include substantial recruitment from seed, as we found that 96% of patches were composed of multiple genotypes.

The one case where two patches displayed a repeated gene phenotype could also have arisen from growth of a single genet or from self-fertilized or from inbred seed, since inbreeding would dramatically increase the probability of repeated genotypes arising through sexual reproduction. If we consider the possibility that the repeated gene phenotype could have arisen from self-fertilized seed, we need to recalculate the probability of one putative parent producing a seed that has a gene phenotype identical to itself. In the one case where we found a gene phenotype that was repeated among patches, 3 of the 7 loci with shared gene phenotypes (one sample had missing data at one locus) were homozygous, so self-fertilized seed would necessarily match the parental type. The other four loci each had two alleles that, depending on the number of gene copies in the gene phenotype of the parent and offspring (something we do not know) could have as high as a 0.94 probability of arising in a self-fertilized seed. So, if self-fertilization occurs, the probability of a seed-established population displaying a gene phenotype identical to the parent patch (with 3 homozygous and 4 heterozygous loci) could be as high as 0.80.

Furthermore, because *Phragmites* is thought to be an allopolyploid, gene copies originating from different hybrid parents may not segregate independently. The probability of a parent heterozygous for 2 alleles producing a self-fertilized seed having a gene phenotype identical its own could be as high as 1.0. Although self-fertilization is thought to be uncommon, Casagrande and Lambert (2007) have found that it can occur in *Phragmites*. Even if self-fertilization does not occur, our genetic analysis demonstrates that there is substantial inbreeding among close relatives so, while the probabilities will not be as high as for selfing, they will be substantially higher than those calculated based on random mating. Because the production of seed with gene phenotypes identical to a parent may be quite high, we must interpret the evidence for asexual spread of *Phragmites* (i.e., patches with identical gene phenotypes) with caution.

Regardless of whether any cases of spread via rhizomes were detected, it is clear that the production of viable seeds is a major factor in the spread of invasive *Phragmites*. The relationship between genetic similarity and distance between samples can be used to understand the scale of gene flow. The high genetic similarity (Moran's I values, Fig. 2) at 10 and 50 m and significant F_{IT} values demonstrate that most pollen and/or seed flow has been very local, primarily within a patch. This has important implications for understanding factors involving spread and seed production.

Other researchers have found that *Phragmites* is at least partially self-incompatible both within its native and invasive ranges (Ishii and Kadono 2002, Lambert and Casagrande 2007) and Ishii and Kadono (2002) suggested that outcrossed pollen was important for seed production. Our results also suggest that availability of outcrossed pollen is important for *Phragmites* spread. Furthermore, in a companion study Baron *et al.* (in prep.) found that the percent of viable seeds produced by *Phragmites* was positively correlated with the number of genotypes in a patch. Combined, these data suggest that the increased rate of *Phragmites* spread in recent decades, despite many prior years of presence in the subestuary, may be explained by the accumulation of genetic variation and accompanying increased seed set. In this scenario, low seed set when only self pollen is available would be followed by a dramatic increase in seed set when, over time, a few genetically distinct seedlings were able to establish in an existing patch or when adjacent genetically distinct patches grew to the point where they merged, making outcross pollen available for seed set 10-50 m away.

All 2007 patches in locations where *Phragmites* was located in 1971-2 were composed entirely of unique gene phenotypes (i.e., all four samples were distinct), suggesting that greater genetic variation might have accumulated over time. However many patches where *Phragmites* was absent in 1971 also contained this level of variation. In order to definitively say that genetic variation increased over time we would need to dramatically increase the number of samples per patch and to have a better determination of patch age at multiple time points. We attempted this with aerial photographs prior to and since 1971 but were unable to consistently discern patch outlines or to distinguish *Phragmites* from *Spartina cynosuroides*.

It is possible, as suggested by Keller (2000) that genetic variation within and among patches could have arisen as accumulated somatic mutations rather than from seed recruitment. Frequent mutation is common at microsatellite loci and it is just this high level of variation that makes them so useful for population genetic studies (Balloux and Lugon Moulin 2002). However the distribution of genetic variation seen in this study seems unlikely to have been generated by mutation accumulation for several reasons. 1. If establishment of new patches was primarily vegetative, a substantial number of cases of repeated gene phenotypes would be expected even in the face of high levels of somatic mutation. 2. Somatic mutation would be expected to produce a high proportion of heterozygotes relative to homozygotes (Balloux *et al.* 2003), whereas observed heterozygosity in Rhode River *Phragmites* was 0.59. 3. High mutation rates deflate F_{ST} values (Balloux and Lugon-Moulin 2002) relative to R_{ST} values, which are independent of mutation rate, yet F_{ST} values for Rhode River samples ($F_{ST} = 0.225$) were relatively high and were higher, rather than lower, than R_{ST} ($R_{ST} = 0.129$).

Taken together, the results of this study provide strong support for spread of invasive *Phragmites* by seed. Seed set appears to be responsible for both establishment of new patches and also some amount of patch spread and the establishment of genetically diverse patches. While spread of individual patches is, without question, largely through rhizome growth and spread by rhizome fragments has been documented (e.g., Bart and Hartman 2003) increased production of viable seeds coupled with local pollen dispersal, may be in large part responsible for the increased spread of *Phragmites* in the Rhode River. The spread of *Phragmites* by seed does not appear to be limited to the Rhode River subestuaries. We have found similar dependence on seed for both patch establishment and expansion for more limited studies in nine other Chesapeake Bay subestuaries with land-use that ranged from largely forested to highly developed (McCormick *et al.* in prep).

The substantially greater role of seed in supporting *Phragmites* invasion suggests a new management emphasis. Three recommendations emerge from these data. 1. It is important to remove new *Phragmites* stands before they have had time to become genetically diverse and commence production of viable seeds. 2. Removal of large, established stands will require repeated treatments to remove both resprouts from rhizomes and seedlings emerging from a seedbank. 3. Preventing establishment of new patches will require limiting viable seed production in nearby patches and also minimizing conditions for seed establishment (e.g., by minimizing availability of exposed soil during disturbances and drydowns and windows of low salinity).

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Table 1. Microsatellite primers and PCR conditions used in the current study. Primer names reference Saltonstall (2003a).

Primer pair	annealing temperature (°C)	fluorophor	DNA dilution
PaGT4	50	FAM	1:100
PaGT9	50	HEX	1:50
PaGT12	56	FAM	1:100
PaGT13	50	HEX	1:50
PaGT14	58	FAM	1:100
PaGT16	56	NED	1:10
PaGT21	58	HEX	1:50
PaGT22	50	NED	1:10

Figure 1: Map of *Phragmites australis* patches in the Rhode River in 1971-2 (pink, from McCormick and Somes 1982) and 2007 (yellow). The one pair of identical gene phenotypes is identified by a pair of red arrows.

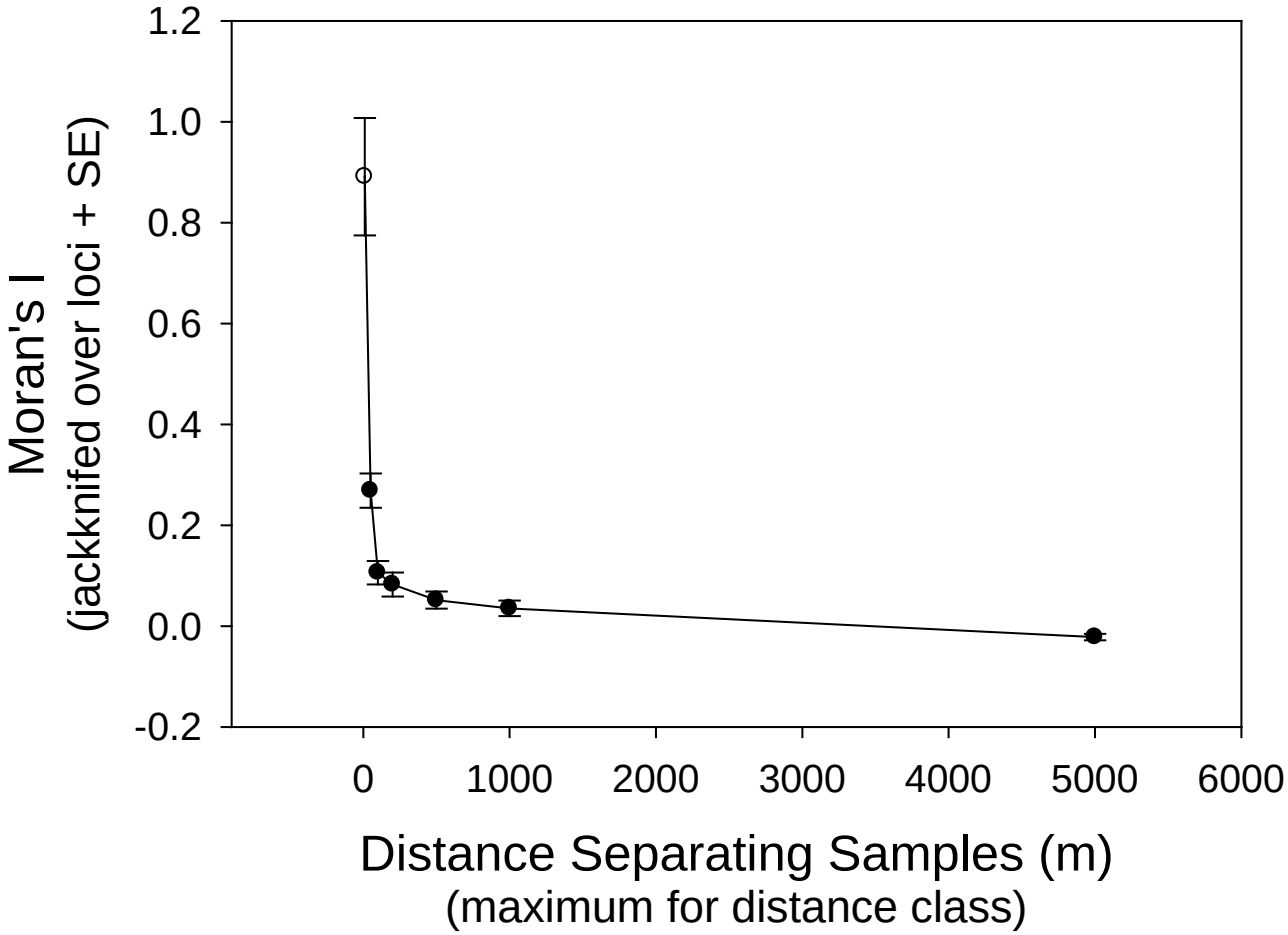

Figure 2: Genetic similarity (Moran's I) as a function of distance separating plant samples from Rhode River *Phragmites* patches.

IV. INTERANNUAL VARIATION IN GELATINOUS ZOOPLANKTON AND THEIR PREY IN THE RHODE RIVER, MARYLAND

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Introduction

The lobate ctenophore, *Mnemiopsis leidyi*, is native to Atlantic and Caribbean estuaries and coastal waters from Massachusetts to southern Argentina, and has been introduced to several Eurasian systems including the Black, Caspian, Baltic and North Seas (Purcell et al., 2001; Kube et al., 2007). *M. leidyi* can tolerate a wide range of temperatures, salinities and dissolved oxygen (DO) concentrations. It occurs in waters with salinities ranging <5 to over 36 (Purcell et al., 2001; Purcell and Decker, 2005), and can survive exposure to DO concentrations of 0.5 mg L^{-1} for at least 4d (Decker et al., 2004). Optimal temperatures for *M. leidyi* reproduction are approximately 18-20°C (Costello et al., 2006).

In late spring and early summer, *M.* leidyi can be abundant in Chesapeake Bay and its tributaries, where it is a dominant consumer, potentially capable of clearing much of the daily standing stock of zooplankton and ichthyoplankton (Cowan et al., 1992; Cowan and Houde, 1993; Purcell et al., 1994; Purcell and Decker, 2005). In mesohaline portions of the Chesapeake Bay system, the major predator of *M. leidyi*, the scyphomedusa *Chrysaora quinquecirrha*, usually becomes abundant in early July and persists through the end of summer (e.g., Cargo and King, 1990). As *C. quinquecirrha* population densities increase, *M. leidyi* abundances typically decline and zooplankton populations rebound (Purcell and Cowan, 1995). However, in years when *C. quinquecirrha* populations are low, *M. leidyi* may exert much greater and prolonged control within the food web. *Chrysaora quinquecirrha* polyps are generally found in salinities 7-20 psu and strobilate when temperatures exceed 17°C (Cargo and Schultz, 1967; Cargo and King, 1990). Medusae are most abundant at salinities of 10-16 psu and temperatures of 26-30^{o}C (Decker et al., 2007). Thus, interannual variation in salinity and temperature can strongly affect the timing and spatial distribution of *C. quinquecirrha* and its control of *M. leidyi.*

The Rhode River is a small, shallow subestuary on the western shore of Chesapeake Bay (Figure 1) characterized by summer salinities that vary interannually in both absolute maxima and timing of these maxima. Like other tributaries in the Chesapeake Bay system, it supports a gelatinous zooplankton food web throughout late spring and summer months. The most abundant gelatinous species are the zooplanktivorous *M. leidyi* and its scyphomedusan predator and competitor *C. quinquecirrha*. Average spring-summer salinity in the Rhode River is near the lower limit required for strobilation by *C. quinquecirrha*. In addition, interannual variation in water temperature has the potential to cause variation in the timing of initial and peak occurrences of these gelatinous species and their prey. As a result, the Rhode River can have two distinct gelatinous food webs: one in which the top predator (*C. quinquecirrha*) exerts control

over the intermediate consumer (*M. leidyi*) and one in which the intermediate consumer is not controlled by predation.

The objectives of this study were to examine temporal and spatial patterns in abundances of *M. leidyi* and *C. quinquecirrha* within and near the Rhode River, and to examine how those patterns varied in relation to water temperature, salinity, and the abundance of mesozooplankton prey. We also examined temporal and spatial variation in egg production by *M. leidyi*.. This study was conducted during the summers of 2004 and 2005, years with very different temporal patterns of *M. leidyi* and *C. quinquecirrha* densities.

Methods

We sampled seven sites: six within the Rhode River and one just beyond the mouth of the river in the mainstem Chesapeake Bay. Sites were chosen based on prior research conducted in the Rhode River and designed to cover its entire length. At each site, weather conditions were noted and temperature, DO, and salinity were recorded at the surface and subsequent 1 m depth intervals with a YSI 600QS meter. Additional temperature, DO and salinity data were available from the monitoring station located at the dock of the Smithsonian Environmental Research Center (SERC) in the Rhode River, which was equipped with a YSI 6600 meter (C. Gallegos, SERC, unpublished).

Gelatinous zooplankton samples were collected in duplicate 3 minute stepped oblique tows using a 0.5 m diameter 202 μm mesh hoop plankton net towed at approximately 2 knots and equipped with a General Oceanics flowmeter (Model 2030). Excess water was strained from the sample, total volume of gelatinous zooplankton was measured, and all individuals were identified to species and enumerated. *C. quinquecirrha* bell diameters and oral-to-aboral lengths of up to 15 *M. leidyi* were recorded. Remaining specimens of *M. leidyi* were classified as > or $\leq$3.0 cm.

Mesozooplankton samples were collected using 0.3 m diameter 202 μm mesh paired hoop-nets. Samples were rinsed through a 2 mm sieve to remove gelatinous zooplankton, preserved with 10% buffered formalin, and mesozooplankton species were subsequently identified and enumerated.

Whole water column chlorophyll data were collected by another research group (C. Gallegos, SERC) at the four central Rhode River sites (1A, 2A, 3A, 4B; Figure 1) on different days during each sampling week. Chlorophyll *a* (chl *a*) was measured with a Spectronics Genesis 5 spectrophotometer and converted into $\mu g\ l^{-1}$.

Mnemiopsis leidyi egg production assays were conducted in 2004 using established methodology (Kremer, 1976; Grove and Breitburg, 2005). Undamaged individuals covering the size range from each site (3-8 cm) were randomly assigned to jars containing 3 L of filtered Rhode River water and left overnight at ambient water temperatures. At approximately 0900 the following morning, adult ctenophores were removed and lengths and volumes recorded. Water from each jar was strained through a 35 μm sieve, preserved with 10% acid Lugol's solution (Sullivan and Gifford, unpublished; Grove and Breitburg, 2005) and eggs were enumerated. Egg production was normalized by ctenophore volume to facilitate comparisons among individuals.

Data were analyzed using analysis of variance (Proc GLM: SAS v 9.1) on rank-transformed data. Student-Newman-Keuls tests were used for *a posteriori* comparisons. Regression models were used to examine the effects of ctenophore volume, site, date and interactions between these factors on egg production. Non-significant interaction terms with $p \geq 0.25$ were dropped from statistical models.

Results

Physical Parameters

Temperature, salinity and DO all varied among sites and between years (Table 1, Figure 2; 2-way ANOVA). Surface water temperature varied among sites ($F = 38.21$, $p < 0.01$), and was cooler adjacent to, and near the mouth of, the Rhode River and at the deeper sites. Surface salinity also varied significantly among sites ($F = 3.55$, $p < 0.01$), and was generally highest at the Bay site (Site 73) and at sites near the mouth of the Rhode River. Minimum DO concentration varied among sites ($F = 7.33$, $p < 0.01$) and was significantly lower at the Bay site than elsewhere.

Measurements at the SERC dock indicated that surface water temperatures reached 25°C more than 3 weeks earlier in 2004 than in 2005, but exceeded 30°C only during 2005. Salinity remained below 8 except for a brief period in 2004 but exceeded 8 for most of the summer in 2005. Daytime low DO concentrations (< 2 mg l^{-1}) were occasionally recorded in the bottom waters during cruises; all low daytime DO measurements in 2004 and all but one in 2005 were recorded at the Bay site. The continuous YSI 6600 monitor at the SERC dock indicated that low DO concentrations occurred near the surface within the Rhode River in the early morning hours of both years (C. Gallegos, SERC, pers. comm.). Analysis of our weekly sampling data indicated that temperature ($F = 5.38$, $p = 0.02$), salinity ($F = 135.18$, $p < 0.01$), and DO concentrations ($F = 6.39$, $p = 0.01$) were all significantly higher in 2005 than in 2004.

2004 Biota

Chlorophyll *a* concentrations peaked in early June, declined, and then rose continually during the period sampled from mid-June through early September 2004 (Figure 2). Mesozooplankton samples in both years were dominated (> 95% of individuals) by the calanoid copepod *Acartia tonsa*. During 2004, mesozooplankton densities varied significantly among dates ($F = 6.28$, $p < 0.01$). Peak densities of 4-7 ind l^{-1} occurred on 21 June and 7 July and then declined to approximately 1.0 ind l^{-1} for the rest of the season (Figure 2).

Mnemiopsis leidyi volumes also varied significantly among dates (one-way ANOVA on ranks, $F = 6.08$, $p < 0.01$). Numerical densities and volumes were lowest in mid-June ($\leq 0.62 \pm 0.25$ ind m^{-3} and $\leq 2.3 \pm 0.77$ ml m^{-3}, respectively), and then gradually increased to a maximum of 51 (± 30.2) ind m^{-3} and 58 (± 33.5) ml m^{-3} on 19 August (Figure 2), the date that coincided with highest densities of 'recruits' (individuals ≤ 1 cm in length). Regression analyses indicated a significant relationship between the prior week's zooplankton density and both *M. leidyi* volume ($r^2 = 0.13$, $p < 0.01$) and the density of recruits ($r^2 = 0.21$, $p < 0.01$). However, the previous week's chl *a* concentration explained a greater percentage of the variation in both of these measures of *M. leidyi* abundance for the sites at which chl *a* data were available (1A, 2A, 3A, 4B) (volume: $r^2 = 0.33$, $p < 0.01$; density of new recruits: $r^2 = 0.25$, $p < 0.01$). *Chrysaora quinquecirrha* abundances were low during 2004. A few medusae were seen in the field during August and early September, but were never caught with either the 0.5 m diameter hoop nets or the larger 1 m^2 neuston net, which was deployed in an attempt to more accurately sample the low density *C. quinquecirrha* population.

2005 Biota

Temporal patterns and peak abundances of most biota in 2005 differed from those in 2004 (Figure 2). Mid-June chl *a* concentrations in 2005 were similar to those in the corresponding time period in 2004, and as in 2004 generally increased during the remainder of the season. However, sampling did not detect an early June chl *a* peak in 2005 and maximum chl *a* concentrations in late-summer 2005 reached only about two-thirds the concentrations reached in 2004 (Figure 2). Mesozooplankton densities varied among dates (one-way ANOVA on ranks, $F = 4.87$, $p < 0.01$). The 21 July peak density of 2.3 ind. l^{-1} was both later and lower than peak densities in 2004. Early June through early July mesozooplankton densities remained below 1 ind l^{-1}, and were similar to mid-July – early September densities in 2004.

The timing of the increase in mesozooplankton densities in 2005 corresponded to a decrease in *M. leidyi* densities and the appearance of *C. quinquecirrha*. *M. leidyi* densities varied significantly among dates (one-way ANOVA on ranks, $F = 13.98$, $p < 0.01$). Peak *M. leidyi* densities were higher and occurred earlier in 2005 than in 2004. Volumes peaked on 16 June (279 ± 205 ml m^{-3}), declined substantially by the 21 July sample date, and then remained low throughout the rest of the season (Figure 2). Medusae of *Chrysaora quinquecirrha* were first caught in our sample nets on 18 July, 2005 and numbers continually increased over the season reaching a maximum on the last sample date, 7 September (Figure 2). *Mnemiopsis leidyi* densities declined as *C. quinquecirrha* abundances increased. Regression analysis was run on *C. quinquecirrha* density, and the prior week's zooplankton density and chl *a* concentrations. Partial r-squared values indicated that *C. quinquecirrha* number explained 41% of the variation in the number of *M. leidyi* recruits while prior week's zooplankton explained only 17% and 13% of the variation in number of recruits and *M. leidyi* volume respectively.

***Mnemiopsis leidyi* Egg Production**

Egg production assays were performed on three dates in July 2004. *Mnemiopsis leidyi* produced between zero and 668 eggs ml^{-1} of ctenophore. There was a significant positive correlation between *M. leidyi* volume and the number of eggs produced both on each date and for the three dates, combined (Figure 3). Egg production on each date in 2004 differed significantly from all others. Egg production was highest on 7 July, 355 ± 28.2 eggs ml^{-1} (n=36); lower on 1 July, 274 ± 25.7 eggs ml^{-1} (n=33); and lowest on 22 July, 50 ± 8.71 eggs ml^{-1} (n=35) (Table 2).

Mesozooplankton prey density during the week leading up to the reproduction assays was estimated by averaging mesozooplankton densities measured in samples collected on the day of the egg production assay and from the week prior in order to include food immediately available as well as prey quantity potentially affecting prior growth and reproduction. Two week average zooplankton densities were 4.13 ± 1.03 (n=6), 2.72 ± 0.56 (n=7), and 1.03 ± 0.34 (n=7) ind l^{-1} for 7 July, 1 July and 22 July, respectively (Table 2). These zooplankton densities corresponded directly with the ranked egg production rates on these dates. ANOVA indicated that the total number of eggs produced per individual increased significantly with ctenophore volume ($F = 201.24$, $p < 0.01$) and average zooplankton density ($F = 34.87$, $p < 0.01$), and varied among sites ($F = 5.70$, $p < 0.01$), dates ($F = 13.82$, $p < 0.01$), and the interaction between sites and dates ($F = 3.39$, $p < 0.01$); the model r^2 was 0.80 ($p < 0.01$).

Discussion

Temporal patterns of mesozooplankton, *M. leidyi*, and *C. quinquecirrha* in the Rhode River differed strongly between 2004 and 2005. In 2004 mesozooplankton abundances peaked in early summer and then declined as ctenophores gradually increased throughout the season. *C. quinquecirrha* medusae were rare and their appearance did not result in a decline in ctenophore density or biomass. In contrast, in 2005, late spring through early summer mesozooplankton densities were low and ctenophore density and biomass were high. As *C. quinquecirrha* abundances increased in late summer, *M. leidyi* decreased and mesozooplankton densities increased. Peak densities of *M. leidyi* measured during this study in the Rhode River (approximately 200 ind. m^{-3} and nearly 300 ml m^{-3}) are higher than those reported in the Pamlico River, NC (just over 60 ml m^{-3}; Miller, 1974) or the mid-Chesapeake Bay (Purcell et al. 2001), but similar to abundances reported for Narragansett Bay, RI (Deason, 1982; Sullivan et al., 2001). Peak Rhode River densities measured in this study were lower, however, than those reported for systems such as the Black and Caspian Seas to which *M. leidyi* has been introduced (Kideys and Romanova, 2001; Bilio and Niermann, 2004).

Interannual variation in salinity likely contributed to observed interannual differences in gelatinous zooplankton densities and food web interactions, but the effect of interannual variation in water temperatures is less clear. Low salinities in 2004 likely resulted in the low densities of *C. quinquecirrha* in that year. *Chrysaora quinquecirrha* polyps are generally not found in salinities less than 7 psu, and become more abundant as salinities increase between 7 and 10 (Cargo and King, 1990). During 2004, surface salinity did not reach 5 until mid June, or 7 until July, and never reached 10. In contrast, surface salinity reached 7 by mid-June and 10 by early August in 2005. We suggest that salinities below 5 in May and early June also delayed or reduced early season *M. leidyi* reproduction in Rhode River in 2004 (Purcell et al., 2001). We were unable to find published studies that report *M. leidyi* reproductive rates at salinities below 5. However, if this hypothesis is correct, there is a very narrow margin between salinities that prevent recruitment of *C. quinquecirrha* and allow *M. leidyi* populations to grow unchecked by predation, and salinities that hinder *M. leidyi* populations by limiting reproduction. The combined effects of salinity on these two gelatinous species in Rhode River in 2004 appears to have resulted in a persistent *M. leidyi* population that did not become abundant until mid-to-late July, but then remained abundant at least through early September.

Although surface waters warmed earlier in the season during 2004 than during 2005, the effect of this warming on gelatinous zooplankton seasonal abundances is not clear, and may have been overwhelmed by other factors. Spring temperatures were 5°C higher in 2004 than in 2005. But by early May of both years, temperatures exceeded the 9-13°C minimum temperature required for *M. leidyi* reproduction (P. Kremer, University of Connecticut, unpublished), and by mid-May of both years, temperatures exceeded the 17°C threshold required for strobilation by *C. quinquecirrha* (Cargo and King, 1990; Purcell and Decker, 2005). In addition, there are no data to suggest that temperatures that occurred during the warmer 2004 spring should have reduced growth or reproduction of either gelatinous species. By late July 2005, surface water temperatures exceeded 30°C, the temperature at which *M. leidyi* suffers mortality in laboratory experiments (D. Breitburg, unpublished). However, *M. leidyi* could have avoided high mid-day surface temperatures by moving lower in the water column, and the appearance of predatory *C. quinquecirrha* is a more parsimonious explanation as the major cause of the seasonal ctenophore decline during 2005 given the high percentage of *M. leidyi* with damage indicative of encounters with medusae (Purcell and Cowan, 1995; Kreps et al. 1997).

With a mean depth of 2 m, the shallow bathymetry of the Rhode River may limit the potential for co-existence of *M. leidyi* and *C. quinquecirrha*. In the Rhode River, densities of *M. leidyi* averaged <2 ml m^{-3} in August and September 2005 when *C. quinquecirrha* densities reached an average of 2-6 ml m^{-3}. In contrast, Keister et al. (2000) found 26.6 ml *M. leidyi* m^{-3} in the Patuxent River, MD when *C. quinquecirrha* density averaged 11.8 ml m^{-3}. The deeper water column of the Patuxent, which includes a bottom layer with variable and sometimes severely hypoxic DO concentrations (Breitburg et al., 2003) may provide greater opportunity for spatial separation of *M. leidyi* and *C. quinquecirrha* and increase survival of *M. leidyi* at moderate *C. quinquecirrha* densities.

Prey availability could limit *M. leidyi* abundance and production, but our data do not suggest that low mesozooplankton densities were likely to have caused the large interannual variation in ctenophore abundances. Mesozooplankton densities were higher in 2004 than in 2005, and the temporal pattern of mesozooplankton and ctenophore abundances was more suggestive of ctenophore control of mesozooplankton than the reverse. An inverse relationship between copepod densities and ctenophore abundance has been noted previously in both Chesapeake Bay (Feigenbaum and Kelly 1984; Purcell and Cowan, 1995) and Narragansett Bay (Sullivan et al., 2001. In both years high densities of *M. leidyi* recruits were found in the Rhode River during periods of lowest mesozooplankton densities. We did not sample microzooplankton, however, and cannot rule out their potential influence on ctenophore abundance.

The maximum egg production we measured in the Rhode River (9,000 eggs ind^{-1} *M. leidyi* day^{-1}) was lower than the maximum reported value of 14,000 eggs ind^{-1} day^{-1} (Kremer, 1976; Reeve et al., 1989), but well within the range of values reported elsewhere. *Mnemiopsis leidyi* egg production in the Rhode River was similar to that of field-collected ctenophores from elsewhere in Chesapeake Bay (Purcell et al., 2001), including the Patuxent River (D. Breitburg and R. Burrell, unpublished). *Mnemiopsis leidyi* from the Patuxent produced a maximum of 610 eggs ml^{-1} of ctenophore at mesozooplankton abundance of 1 ind l^{-1}, which is very close to the rate found in this study of 668 eggs ml^{-1} at 2.2 mesozooplankton ind l^{-1}. Variation among dates in the relationship between zooplankton density and egg production suggests an interesting pattern of trade-offs in energy allocation to somatic growth versus reproduction, or nutritional constraints.

Predicted changes in sea surface temperatures and rainfall throughout the world may lead to changes in the geographic ranges of many aquatic organisms. The Rhode River provides an interesting model that may aid predictions of climate-change related shifts in ranges and predator-prey dynamics because it is often near the threshold of salinity tolerances and the dynamics of the system can fluctuate markedly from year to year. These characteristics of the Rhode River allowed us to examine the gelatinous zooplankton food web within the river during two distinct years: one with, and one without, strong influence by a top predator. Differences in species abundances and food web interactions observed here may help to predict dynamics in other systems as environmental conditions, and the range of *C. quinquecirrha*, change. Although generally considered a nuisance species by swimmers and fishermen, *C. quinquecirrha* may benefit fisheries and habitat by controlling densities of *M. leidyi*, which is an important predator of oyster larvae – a prey not utilized by *C. quinquecirrha* (Purcell et al., 1991; Breitburg and Fulford, 2006).

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Table 1. Mean environmental conditions measured at each site sampled for 2004 and 2005. Chlorophyll *a* concentrations are whole water integrated values (C. Gallegos, SERC); minimum DO values are based on near-bottom measurements; temperature and salinity are from the surface waters (<1m depth). Sites are ordered from the mainstem Chesapeake Bay to the closest site to Muddy Creek. Because of sea state conditions, Site 73 was not sampled as frequently during mid-late summer 2004 as other sites. As a result, annual averages below are not necessarily representative of physical conditions at Site 73 relative to those at other sites measured on the same dates. - = no data

Year:	**2004**				**2005**			
Site:	Chlorophyll *a* (µg l^{-1})	Minimum DO (mg l^{-1})	Temperature (^{o}C)	Salinity	Chlorophyll *a* (µg l^{-1})	Minimum DO (mg l^{-1})	Temperature (^{o}C)	Salinity (psu)
73	-	2.31	25.40	7.76	-	3.46	26.26	9.79
1A	24.55	5.20	25.95	8.20	21.37	6.61	26.64	9.26
2A	24.76	5.77	26.66	8.04	37.02	6.88	27.58	9.44
CC	-	4.70	29.14	7.35	-	5.14	28.91	9.10
3A	32.43	4.73	27.54	7.94	28.20	5.21	28.00	8.87
SC	-	4.23	28.09	7.78	-	5.31	27.97	9.38
4B	44.34	5.22	28.41	7.29	32.29	5.19	28.62	9.08

Table 2 Mean Mnemiopsis leidyi egg production (eggs ml^{-1} ctenophore ± SE) and two-week mean mesozooplankton density (no. l^{-1} ± SE) for each of the reproduction assay dates

Assay Date	*M. leidyi* Egg Production	Mesozooplankton Density
1 July	274 (± 25.7)	2.72 (± 0.56)
7 July	355 (± 28.2)	4.13 (± 1.03)
22 July	50 (± 8.7)	1.03 (± 0.34)

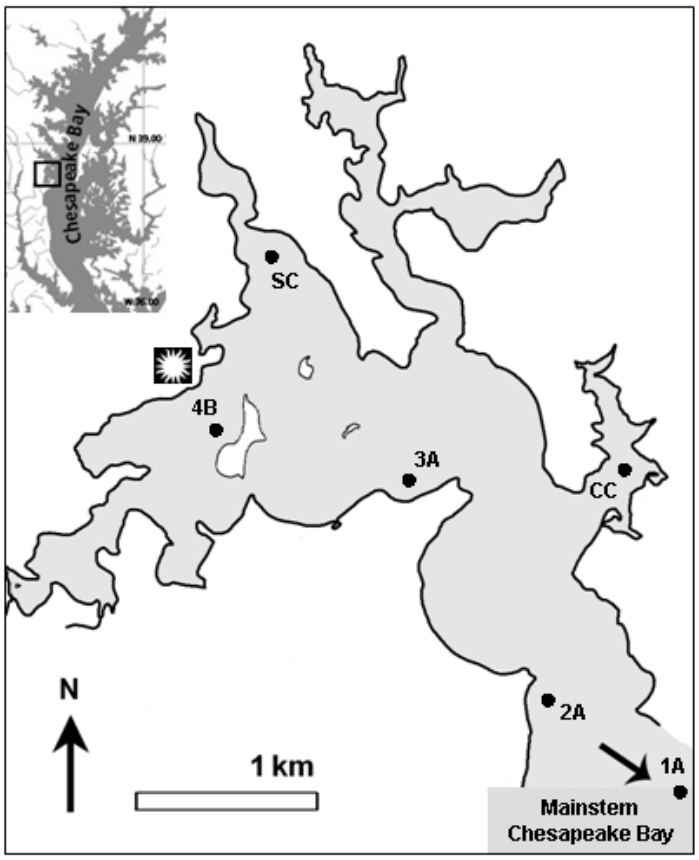

Fig. 1 The Rhode River and its location in the Chesapeake Bay. Dots indicate location of sampling sites; Smithsonian Institution logo denotes location of SERC dock

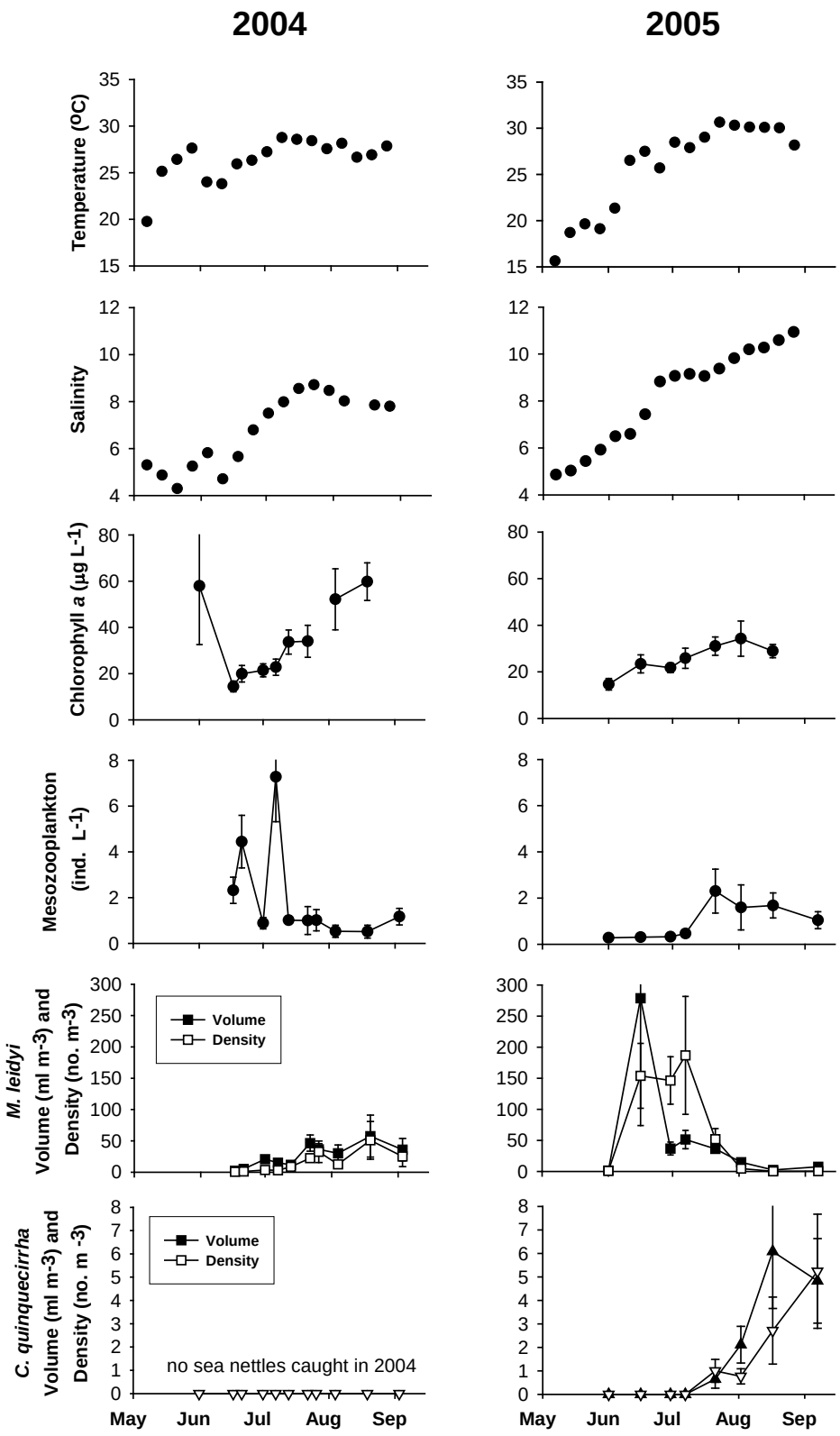

Fig. 2 Weekly mean temperature (°C) and salinity at the SERC dock (C. Gallegos, unpublished), and river-wide mean (± SE) chlorophyll *a* concentration (µg L^{-1}), mesozooplankton abundance (no. l^{-1}), and *Mnemiopsis leidyi* and *Chrysaora quinquecirrha* abundance (volume: ml m^{-3} and density: no. m^{-3}).

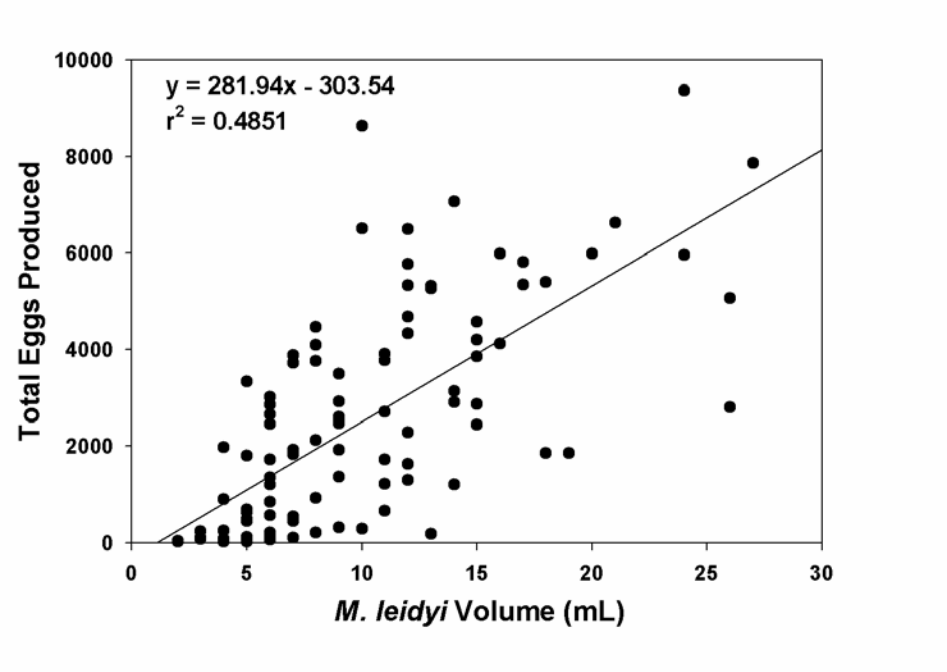

Fig. 3 Total number of eggs produced and volume (ml) of each *Mnemiopsis leidyi* in all three reproduction assays

V. FEMALE BLUE CRAB MIGRATION IN CHESAPEAKE BAY

From 20 May 2008 Report

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Introduction

Blue crabs move among various ecosystems and salinity zones of estuaries during the progression of their complex life history (see review by Hines 2007). Female blue crabs mate once in their lifetime when they molt to maturity in juvenile nursery areas of tributaries dispersed throughout Chesapeake Bay (Jivoff et al. 2007, Hines 2007). Following the maturation molt and mating, inseminated females harden their exoskeleton and typically forage in the low salinity subestuaries for a period of time before they migrate to high salinity areas of the lower mainstem of the Bay for brood production (sometimes termed "spawning") (Turner et al. 2003).

Migration by mature females typically occurs in two phases (Tankersley et al. 1998, Carr et al. 2004, 2005). Phase I of migration involves movement from mating locations to the lower estuary before brood production. In large estuaries like Chesapeake and Delaware Bays, phase I migration may occur over distances of 150 miles (250 km) or more, whereas in smaller estuaries and for females mating in the tributaries adjacent to the spawning regions of large systems, crabs may migrate fewer than 10 miles (16 km) during phase I migration. Phase II occurs during brood incubation just before egg hatching and involves movement to the mouth of, or off-shore from, the estuary. In phase II of migration, ovigerous females may exhibit a tidal rhythm of swimming at the surface on nocturnal ebbing tides, to move near to, or out of, the mouth of estuaries, where they hatch their eggs (Tankersley et al. 1998, Forward et al. 2003a, b, Forward and Cohen 2004, Carr et al. 2004, 2005, Ziegler et al. in review). For very large estuaries, phase II of the female spawning migration out of the mouth of the bay is not well documented or understood. For Chesapeake Bay, trawl surveys out onto the continental shelf have not captured females off-shore, but some egg-bearing females do move outside the mouth of Chesapeake Bay along the nearshore zone (<50 ft) southward toward North Carolina (R. Lipcius, Virginia Institute of Marine Science, personal communication).

The purpose of this paper is to provide information about migratory movement of mature female blue crabs in Chesapeake Bay. Information on female migration was obtained over a 9-year period with a fishery-dependent tag-recapture program that paid rewards to individual fishers for providing recapture data. The tagging program was designed to determine spatial and temporal variation in movement of female crabs from several representative nursery areas in Maryland and Virginia subestuaries along the mainstem of the Bay to ultimately arrive at lower Bay spawning grounds. This information is particularly relevant to recently proposed blue crab management regulations in Maryland which target protection and restoration of the blue crab spawning stock during the fall migration as a critical aspect of blue crab fishery management in Maryland.

Methods

Mature female blue crabs were tagged in a series of batches and released into several subestuaries of Chesapeake Bay during 9 years, 1999-2007. Within each year, releases generally occurred at monthly or bi-monthly intervals during the summer and autumn months (Table 1). Mature female blue crabs were obtained in the vicinity of each release area by a variety of methods. Freshly caught crabs were either purchased from local fishers using trotlines and crab pots or caught by researchers using trawl, crab pot, or trotline. Tagging proceeded immediately upon collection, and we tagged only lively post-molt crabs in good condition with no indication of disease and missing no more than one chela or 5th periopod (swimming leg). From 1999-2002, crab tags consisted of a double-faced 1 by 3 inch (25 by 75 mm) aluminum tag (Forestry Supplies, Inc.) with a waterproof label affixed to the outward surface that was coated with epoxy resin. From 2003 to 2007 tags consisted of 1 by 2 inch (25 by 50 mm) pink laminated vinyl disks

Figure 1. Example of plastic crab tags used during 2003 to 2007.

(Floy Tag; Fig. 1). Each tag was inscribed with a unique identification number, contact information for the Smithsonian Environmental Research Center (SERC), and "REWARD". The tags were attached to the dorsal surface of the carapace with malleable aluminum wire (1999-2002) or annealed stainless steel wire (2003-2007). The following data were recorded for each crab: unique tag number; carapace width (CW to the nearest millimeter); and autotomy (limb loss). Crabs were released immediately after tagging and the date and GPS coordinates (longitude and latitude) were recorded for each release event. Tagged crabs were released at four sites in Maryland (Rhode River vicinity including Rhode River, South River and adjacent mainstem Bay, Back River and Potomac River) and a single site in Virginia (York River).

To encourage recreational and commercial fishers to provide data upon recapture of tagged crabs, we disseminated detailed information about our tagging program at numerous public meetings (Annual Watermen's Expo in Ocean City, MD, Annual research update for local watermen at SERC,civic groups, NOAA Chesapeake Bay fishery workshops) and through publications targeting commercial and recreational fishers (Maryland Watermen's Association newsletter, newspapers, magazines). An internet web site on the SERC homepage (http:\\www.serc.si.edu) explained the study and how to report recaptures. An automated telephone message system allowed callers to report recaptures 24-hours per day, 7 days per week. All persons reporting tagged crabs were asked to provide the following data: tag number,

capture date, capture location, capture depth, and capture gear. From 1999-2002 captors received a $2 reward for each tag reported; rewards were increased to $5 per tag during 2003-2007. In addition, beginning in 2005 a small subset (5%) of clearly marked high value tags ($100 in 2005 and $50 in 2006 and 2007) were released to estimate tag reporting rate. During all years, all tag returns were entered into several $100-200 lotteries. Rewards and additional information about the study were mailed promptly to callers after receiving the capture data.

Distance that recaptured females moved from their release site was determined by latitude and longitude coordinates using geographic information systems (GIS; ArcView and ArcGIS). Females that moved more than 4.2 miles (7 km), which is approximately twice the length of the Rhode River subestuary (the main release area), were categorized as "migrating". Migrating females were also categorized by date of recapture into three groups: "before September"; "during September through November" (the main migration season); and "after November". Migrating females were also partitioned into recaptures before and after October 10 or before and after October 23, which correspond with dates identified by the Maryland Department of Natural Resources for seasonal closure of the mature female fishery in Maryland under two proposed regulatory changes. Frequencies of depths of recapture sites were tabulated for migrating females.

Results

Recapture Rates. During 9 years of study from 1999-2007, a total of 8,400 mature female blue crabs were tagged (Table 1), with numbers tagged ranging annually from 231 females in 2005 to 2,391 females in 2007. A total of 1,526 females were recaptured, with recapture rates averaging 18.2% and ranging from 3.7% in 2002 to 48.3% in 2006. Most females (6,393 or 76.1%) were released in the vicinity of the Rhode River; 1,507 females were released in the York River; 271 females in the Potomac River; and 169 females in the Back River of the upper Chesapeake Bay. Of the 1,526 recaptured female crabs that were reported, most (947 females or 62.1%) were caught before they began to migrate.

Table 1. Release and recapture information for individually tagged mature female blue crabs in Chesapeake Bay, 1999-2007.

Year	No. of Mature Females Tagged	No. Re-covered	Return Rate (%)	Release Months	Release Area	Capture Methods	Non-migrating[a]	Moving > 4.2 km from release site			Uncertain[b]
								Before Sept	During Sept - Nov	After Nov	
1999	388	49	12.6	Aug - Oct	Rhode River vicinity	Purchased	25	0	14	10	0
2000	367	58	15.8	June, Sept, Oct	Rhode River vicinity	Purchased	24	4	26	0	4
2001	361	29	8.0	June, July, Sept, Oct	Rhode River vicinity	Purchased	12	5	8	3	1
2002	324	12	3.7	July, Oct	Rhode River vicinity	Purchased	2	0	8	1	1
2003	990	115	11.6	June - Oct	Rhode River vicinity	Purchased	60	34	11	2	8
2003	783	188	24.0	June, Oct	York River	Purchased	135	15	15	15	8
2004	944	114	12.1	June - Oct	Rhode River vicinity	Purchased	47	33	27	5	2
2004	784	32	4.1	June, Oct	York River	Purchased	16	11	3	2	0
2005	139	41	29.5	June - Aug	Rhode River vicinity	Trawl	29	1	2	7	2
2005	92	41	44.6	Sept - Nov	Rhode River vicinity	Crab Pot	30		2	9	0
2006	877	424	48.3	July - Sept	Rhode River vicinity	Trotline	313	4	72	35	0
2007	1642	325	19.8	Sept	Rhode River vicinity	Trotline	204		82	39	0
2007	169	20	11.8	Sept	Back River	Purchased	8		12	0	0
2007	271	64	23.6	Sept	Potomac River	Purchased	34		23	7	0
2007	269	14	5.2	Sept	Rhode River vicinity	Purchased	8		5	1	0
								Migrating[c]			
Total	**8400**	**1526**	**18.2**				**947**	**107**	**310**	**136**	**26**
					Percent of Recaptures		**62.1**	**7.0**	**20.3**	**8.9**	**1.7**
					Percent of Migrating Females			**19.3**	**56.1**	**24.6**	

a Non-migrating indicates crabs caught less than 4.2 miles (7km) from release site
b Uncertain indicates crabs where migration status (i.e., migrating or non-migrating) and/or recapture date was unknown
c Migrating indicates crabs that moved greater than or equal to 4.2 miles from release site

Timing of Migration. Females that were caught more than 4.2 miles (7 km) away from their release site comprised 36.2% (553 females) of the recaptures (Table 1). Of these, 19.3% were caught prior to September but had moved long distances from their release sites. The majority of migrating crabs (56.1%) were caught during September to November, mostly along the mainstem of the Bay. Another 24.6% of migrating females were recaptured after November; most of these females (92%) had already arrived in the lower Bay spawning zone. A small percentage (5.5%) of migrating females did not have complete recaputure data reported, and were not include in further analyses. The timing of female migration was nearly evenly split in mid-October (Fig. 2). Of 310 fall-migrating females, approximately 36% were caught prior to, and 64% were caught after October 10. Similarly, approximately 55% were caught prior to, and 45% were caught after October 23.

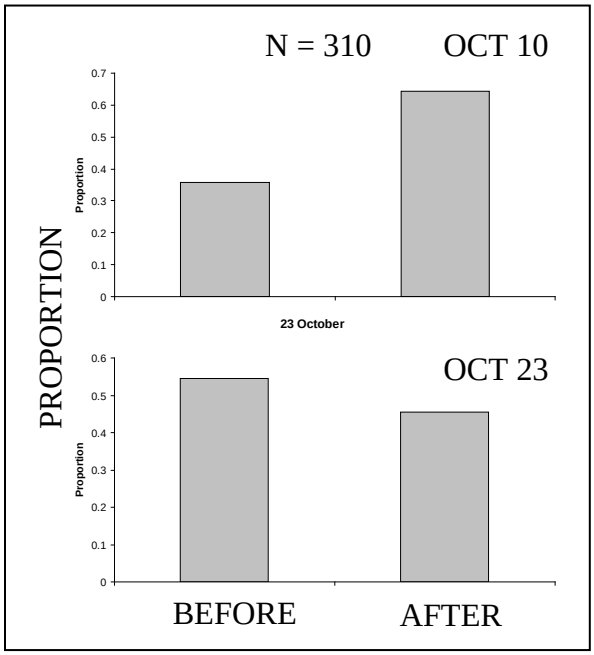

Figure 2. Proportion of tagged female blue crabs migrating during September-November that captured before and after October 10 (top) and October 23 (bottom) in Chesapeake Bay.

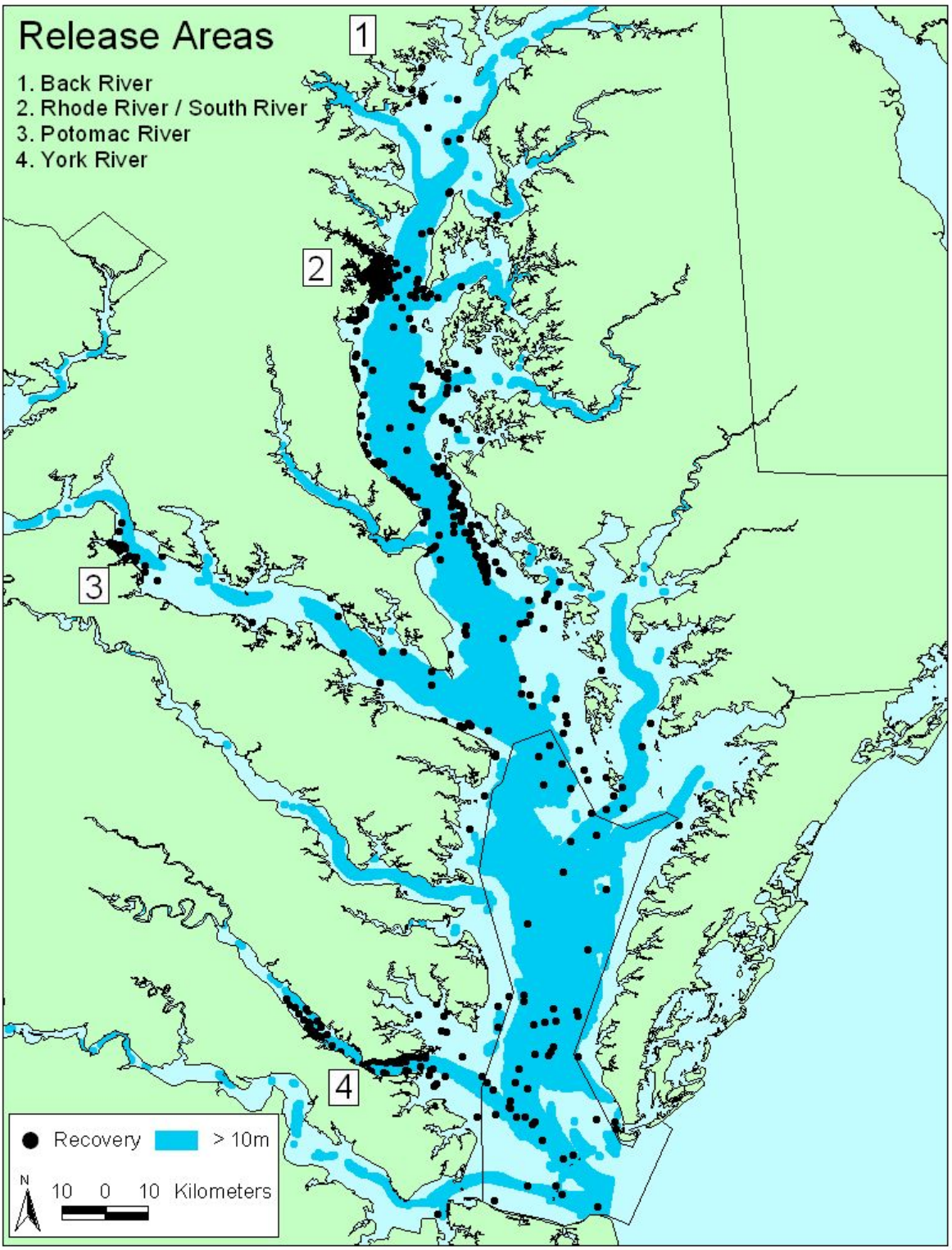

Figure 3. Map of Chesapeake Bay showing locations of release sites (numbers) and recaptured tagged crabs (dots). 1 = Middle River; 2 = Rhode, South Rivers; 3 = Potomac River; 4 = York River. Polygon outline shows location of spawning stock sanctuary for blue crabs in Virginia waters. Darker shading contrast indicates depths greater than 30 ft (approx. 10 m).

Maximum Age of Female Recaptures. Most migrating females were recaptured within one year of release. Only two crabs were recaptured more than 3 years after release. One lower bay female was recaptured 1,300 days (3.5 years) after release. She was tagged in the upper York River in October 2004 and caught by crab pot in the James River in May 2008. An upper bay female was recaptured 3.3 years after release. She was tagged in August 2004 in the Rhode River and subsequently captured in the winter dredge fishery in January 2008 in the York River Channel. Therefore, because females molt to maturity at approximately 1-1.5 years old (Hines 2007), both of these crabs were 4.5-5 years old.

Migration Route. Recapture locations indicated that migration routes of mature females extended along both sides of the release tributaries and on both sides of the mainstem of Chesapeake Bay even though all of the release locations were only on the western shore of the Bay (Fig. 3). Clearly, in addition to moving down the western shore, migrating females readily moved across the bay to move down the eastern shore. Most migrating females were recaptured along the shallow edges of the deeper tributaries (Potomac and York Rivers) and mainstem of the Bay.

Migration Depth. Most females (87%) migrating during September through November were caught in water shallower than approximately 30 ft (10 m; Fig. 3). Frequencies of recaptures for migrating females peaked at depths from 18 to 24 ft (6-8 m; 32%), and only 5% of females were caught deeper than 36 ft (12 m; Fig 4). For females recaptured before October 23, 91% were caught shallower than 24 ft (6 m), with most (84%) caught in a broad depth zone from 6 to 24 ft (2-6 m); and only 9% were caught deeper than 24 ft (6 m; Fig. 5). For females recaptured after October 23, in addition to a clear peak of capture frequencies (31%) at 18-24 ft (6-8 m) depths, more females (34%) were caught at 24-36 ft depths; but 93% were caught shallower than 36 ft (12 m) and only 7% were caught deeper (Fig. 5).

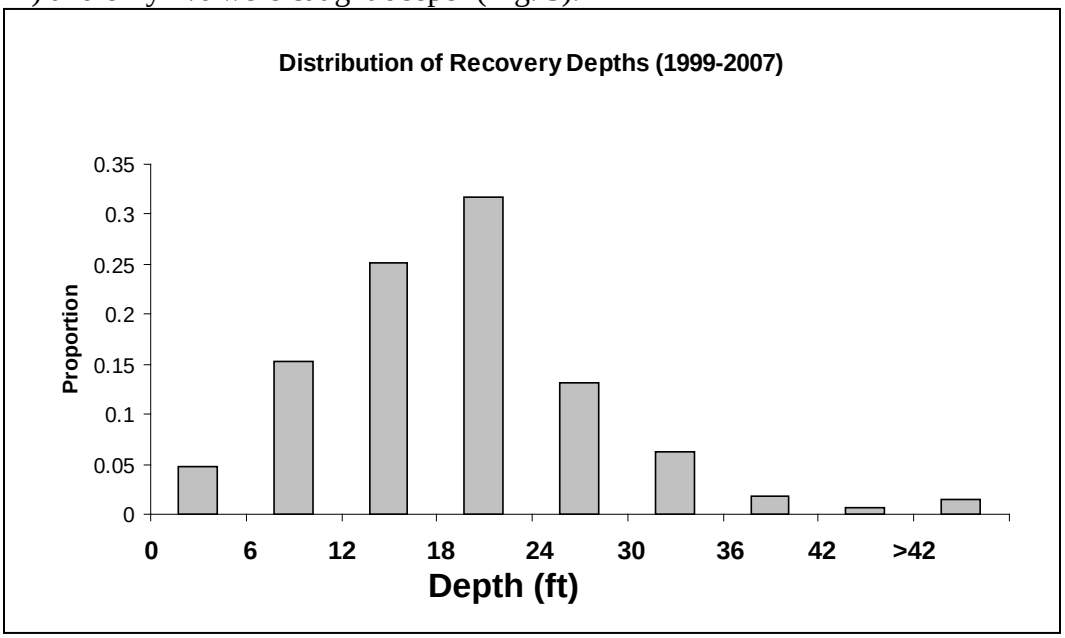

Figure 4. Depth distribution of migrating female blue crabs recaptured during September to November in Chesapeake Bay.

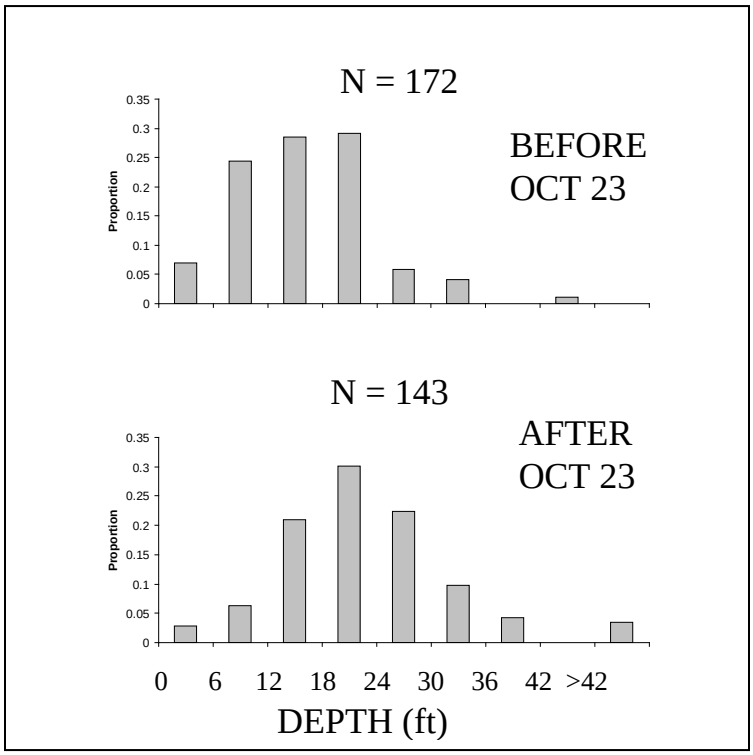

Figure 5. Depth distribution of migrating female blue crabs recaptured before and after October 23 in Chesapeake Bay.

Discussion

This 9-year tagging study suggests that the impact of the fishery on mature female blue crabs is greatest prior to migration, as 62.1% of recaptured crabs were caught within 4.2 miles (7 km) of their release site and before migration. In addition, most migrating females (75.4%) were caught while moving down the Bay, especially during the fall months (September-November, 56.1%). Of the 24.6% of females caught after November, most had already arrived successfully in the spawning zone of the Bay, as approximately 92% of these were caught by winter dredge fishing and spring crab pots prior to the start of the brood production (spawning) season during the following summer.

However, although our recapture rate was relatively high (averaging 18.2%) for this type of fishery-dependent mark-recapture studies and was comparable to similar tagging studies of blue crabs in Chesapeake Bay (e.g., Lambert et al. 2006a, b) and elsewhere (summarized by Hines 2007), we cannot be sure of the fate of the 81.8% of tagged crabs that were not reported to us. Among the factors that comprise the fate of unreported tagged crabs are: natural mortality (predation and senescence) before or after spawning; handling mortality from the capture and tagging process; tag loss; and unreported fishery capture. Our unpublished preliminary data suggest that handling mortality (especially by the fishery) can be appreciable, but highly variable, although we took care to minimize this factor. We estimated tag loss rates directly by holding tagged mature females in captivity; these experiments determined that mechanical tag loss for mature females, which have ceased molting, is negligible. By using high-reward tagging (Pollock et al. 2002), we estimated that reporting rates were greater than 80% during 2006 and 2007 within the Rhode River subestuary, where we have worked to develop cooperative

relationships with the local fishers. On the other hand, reporting rates over large areas of the Bay may be substantially less in a fishery that is suspicious of research. Nevertheless, tagging studies conducted in the lower Bay also indicate very high fishing mortality on mature females when not protected by the Virginia spawning sanctuary (Lambert et al. 2006a, b, Hewitt et al. 2007).

This tagging study indicates that the first phase (phase I) of migration by mature female blue crabs in Chesapeake Bay occurs during September through November, which is consistent with our earlier studies (Turner et al. 2003, Aguilar et al. 2005). Approximately half of the females migrated before mid-October, with approximately 36% caught before October 10 and 55% caught prior to October 23. Both over-the-back tags and ultrasonic telemetry show that after mating during July through September, mature females continue to forage and move within subestuaries in a typical small-scale pattern of alternating meander and short directional movement (Turner et al. 2003; Aguilar et al. 2005). Although this period of foraging before migration allows a female to recover from her molt to maturity, the migration appears to occur in seasonal synchrony rather than being triggered by completing a non-synchronized period of physiological recovery after molting to acquire sufficient energy reserves (Aguilar et al. 2005). However, we do not have good estimates of the annual or spatial variation in timing of the onset of migration, nor the cues that may trigger it.

In Chesapeake Bay, our fishery-dependent data indicate that mature females migrate along the shallow edges of the deep tributaries and the mainstem of the bay. Frequencies of migrating crabs peaked at depths of 18-24 ft (6-8 m), and nearly 90% were caught at depths less than 30 ft, while very few (5%) crabs were caught deeper than 36 ft (12 m). However, these depths may reflect sampling bias of the fishery and fishery-independent sampling is needed to test the migration route(s) of an unbiased population. Phase I migration for Chesapeake females appears to consist of relatively rapid (1-2 month transit time down the Bay) seaward movement (walking or swimming) on or near the bottom, with only a small percentage of crabs occasionally swimming near the surface (Wolcott et al. 2004, Johnson and Hines unpublished data).

In Chesapeake Bay, females cease migrating for the winter and settle into bottom sediments of the mainstem as water temperatures drop below about 9° to 10°C, with some females remaining in the mesohaline zone and others arriving in the polyhaline zone for winter. As water temperature rises in spring and females become active, those that over-wintered in the mesohaline zone complete phase I seaward migration. Although fishers in Chesapeake Bay report a “wave” of mature females moving up the estuary in spring, this reflects increases in female activity and feeding with increasing temperatures progressing northward, increasing their vulnerability to fishing rather than reflecting actual movement of females up-estuary.

Phase II of female migration in Chesapeake Bay is not well understood. Although females preparing to hatch their eggs exhibit selective tidal stream transport of swimming rhythms on nocturnal ebb tides in small inlets of North Carolina and Florida estuaries, this behavior is not documented in the large spawning area of Chesapeake Bay. After their eggs hatch, some females in North Carolina estuaries reverse their tidal-stream transport on flooding tides to move back into the lower estuary, where they may produce subsequent broods (Tankersley et al. 1998, Carr et al. 2004, 2005; R.A. Tankersley, Florida Institute of Technology, pers. comm.). Other females may remain outside the estuary (D. Ritschoff, Duke University Marine Laboratory, pers. comm.), which may account for some of them moving to neighboring estuaries. However, mature females do not move back to lower salinity zones of estuaries (Fischler 1965; Hines et al. 1987, 1990).

Prager (1996) assumed that females had a mean residence time of 4 to 21 d in the spawning area of Chesapeake Bay, but there are no empirical measures of this, and other studies indicate that mature females remain in the lower Bay spawning sanctuary throughout the summer (Lambert et al. 2006a, b). For Chesapeake Bay during the summer, peak abundances of egg-bearing females tend to move from northern to southern portions of the lower bay spawning area (R. Lipcius, Virginia Institute of Marine Science, personal communication). Although there is little evidence of females migrating out of the mouth of the bay onto the continental shelf, some egg-bearing females move out of the bay mouth southward along the nearshore zone (Lipcius, Virginia Institute of Marine Science, personal communication). In Delaware Bay, which has a large, progressively widening mouth, spawning females occur over a broad area of the mouth and may move out onto the shelf as well (C. Epifanio, University of Delaware College of Marine studies, pers. comm.).

Several factors indicate that the patterns of migration presented here are robust for Chesapeake Bay: i.) the relatively large sample size of our cumulative multi-year study (8,400 females tagged and released, with 1526 recaptured); ii.) temporal consistency of recaptures over the seasons of a 9 year period; and iii.) spatial consistency of recaptures among a wide range of subestuaries. However, much remains unknown. Spatial variation in migration is poorly defined, because 76% of our releases occurred in the vicinity of one subestuary (Rhode River) and no releases were conducted in Eastern Shore subestuaries. Cues triggering migratory behavior and variation in the onset of migration are not known, and we do not know how or if these may relate to stressful conditions that could impact females during migration, such as direct and indirect effects of low dissolved oxygen levels. Moreover, we lack good quantitative information to determine if upper Bay females initiate migration sooner than middle and lower Bay females; or if they do by how much time. The factor(s) limiting the depth of migrating females is (are) not known, and fishery-independent sampling is needed to test the route(s) and depth(s) of migration reflected by our fishery-dependent data. We know that females feed during their migration (Wolcott et al. 2004), but we do not know if the summer mortality of benthic food resources by low oxygen levels restricts females to depths shallower than the pycnocline that coincides with the observed depth limit of migration. Improved mechanistic understanding of migration would help advise management of the intense fishery to ensure that quotas and stock preservation can be achieved effectively.

Acknowledgments

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VI. LIVING RESOURCES OF THE RHODE RIVER SUBESTUARY

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Introduction

SERC's on-going, long-term (25-30 years) monitoring program describes the population dynamics and community structure of fish and invertebrates throughout the Rhode River, a representative subestuary of Chesapeake Bay. This research tracks seasonal, annual and decadal variation in species composition and abundance of all fish and macro-invertebrates of the system. Temporal variation is related to ecosystem change (weather, land-use, water quality, fisheries impacts, predator dynamics). The long-term descriptive data bases, in combination with our experimental studies (e.g., see chapter on Female Blue Crab Migration in this report), provide an unusual overview of estuarine population dynamics and community structure of living resources. This research has been funded by SI Environmental Sciences Program, external funding for a broad range of research projects, and SERC's Fellowship/Professional Training Program. The structure of this monitoring program was described initially in Hines et al. (1987).

There are four main components to our long-term studies:

- Fish and Crustaceans of a Tributary Creek.

More than 65 species of fish and blue crabs use Muddy Creek (a small tidal tributary of the Rhode River) for seasonal reproduction, nursery habitat, molting refuge, and year-round residence. At weekly intervals for more than 25 years (since 1983), we have utilized a permanent fish weir to sample the abundance and species composition of all fish and crabs moving up and down the creek.

- Epibenthic Fish and Crabs.

Epibenthic fish and crabs (including blue crabs, Norfolk spot, croaker, various flat fish) comprise the dominant predators on benthic communities. We sample these fish, blue crabs, and other epibenthic species using triplicate otter trawls at 4 stations arrayed along the axis of the Rhode River. This data set extends nearly 30 years (since 1981). See Hines et al. (1990).

- Infaunal Benthic Invertebrate Community.

Infaunal macro-invertebrates are dominated by deposit feeders and small suspension feeders that process the particles at the sediment-water interface. These species undergo large seasonal fluctuations with spring and fall recruitment and high summer mortality from fish and crab predation. We have sampled community dynamics with cores at 4 stations along the axis of the Rhode River for nearly 30 years (since 1979). Diversity is low with about 20 common species and a total of about 55 species, but secondary production is high, forming a major link in benthic-pelagic coupling, as these invertebrates are the major food resource for epibenthic fish and crabs. See Hines and Comtois (1985).

- Nearshore Fish Assemblage.

Juvenile fish and crustaceans utilize the nearshore shallow fringe of Chesapeake Bay as a nursery habitat and refuge from large predatory fish and adult blue crabs. For nearly 30 years (since 1980) we have sampled the summertime abundance and species composition of nearshore fish communities using seines pulled at 13 stations arrayed throughout the major shoreline habitats of the Rhode River. The major long-term patterns of species composition and dynamics of the nearshore fish assemblage of the Rhode River is described below.

Long-term Species Composition and Dynamics of Nearshore Fish Assemblage

The nearshore zone, with its shallow depths and variable habitats, can play a vital role in the survival, nurturing and growth of its juvenile fish and crustaceans. Our research has shown that small fish, juvenile blue crabs and grass shrimp use the shallow depths 1) as a refuge from larger predators, 2) as nursery grounds for larval fish and small crustaceans and 3) as a resource for food. Our on-going long-term data data provides important background information for detailed experimental studies about the life history of species, habitat partitioning, reproduction and recruitment patterns and requirements, trophic interactions, and the effect of environmental changes on their behavior, population dynamics and species composition.

Sampling Methods for Nearshore Fishes

Study Area

The Rhode River is a small (550 ha) mesohaline subestuary in the upper Chesapeake Bay (Lat long). Located approximately 135 km from the Bay mouth, the mean tidal range is 30 cm, but weather conditions, particularly barometric pressure and wind, can often cause substantially greater fluctuations in water level (up to 2.5 m) (Jordan et al. 1991; Hines, personal observations). Like many portions of the Chesapeake Bay and the temperate eastern United States, water temperature of the Rhode River can vary fluctuate greatly among seasons from 0-33° C. Waters of the upper reaches of the Rhode River (i.e., up stream of the SERC dock; Figure 1) can be affected by local runoff (Jordan et al. 1991), but salinity in the lower Rhode River is primarily driven by that of the mainstem of the bay, which is markedly affected by flow from the Susquehanna River into the upper bay (Han 1974). These conditions can lead to substantial yearly and spatial variation in salinity. In dry years, salinity can vary from 0 psu in the headwaters to nearly 20 psu near the mouth of the Rhode River, whereas, in wetter years salinity near the river mouth can drop to < 10 psu.

Nearshore Fish Sampling

From 1980-2006, the nearshore fish assemblage in the Rhode River was sampled by a seining survey conducted yearly in summer months at 13 stations. Sampling stations were distributed throughout the Rhode River, with 2 stations in the headwaters (Muddy Creek), 4 stations in other subtributary creeks and 7 stations along the main axis of the subestuary (Fig. 1, Table 1). Stations were categorized by three dominant shoreline habitat types: *Spartina /Phragmites* marsh, woody debris (shoreline with fringing forest and fallen dead branches and trees in the intertidal zone), and sandy beach. Shoreline habitat types were distributed among the locations in the Rode River. However, stations located in headwaters and other subtributary creeks had shorelines that were predominantly lined by marshes. Each station was sampled yearly with exception of 1981, when only 4 stations were sampled. Prior to 1999, at least 3

replicate samples were conducted at each station. From 1999 to 2007, 2 replicate samples were conducted per station. The seine survey was conducted during the summer months from June to early September. Stations were not sampled in the same order among years to reduce possible temporal sampling bias within the summer season.

The nearshore fish assemblage was sampled with a 15 m-long, 2 m-high flat seine. Each seine sample was swept parallel to shore for 30 m at a distance of 12 m from the shoreline. Thus, approximately 360 m^2 of shoreline was sampled per seine haul. All captured individuals were identified to the species level, counted, and recorded with the following exceptions. Atlantic silverside *Menidia menidia* and inland silverside *Menidia beryllina* were not individually identified in the field and subsequently grouped as *Menidia spp*. Prior to 2002, anchovies were not individually identified in the field and were assumed to be bay anchovy *Anchoa mitchilli*. Since 2002, anchovies were identified to species, but since < 10 striped anchovies *Anchoa hepsetus* have been caught from 2002-2007, we pooled all catches of anchovy as *Anchoa spp*. Pipefish were not individually identified and all were assumed to be northern pipefish *Syngnathus fuscus*. After capture, all recorded individuals were returned to the water alive. However, any individual that could not be identified confidently in the field was taken to the lab for identification and preserved as a reference specimen. Length measurements were recorded for the first 20 individuals of every species captured per seine haul.

Statistical Analyses

Multivariate statistical analysis of the 28-year data set was performed using Primer v6 (Primer-E). Mean catches-per-unit-effort (CPUE) were generated by year and station for all 'species' (Table X). To reduce the impact of abundant fish species, data were log (x+1) transformed. Bray-Curtis similarity matrices were then created to quantify the relationships among samples. The Bray-Cutis coefficient is widely used in ecological studies because it satisfies many of the guidelines for handling species assemblage data, with some limited exceptions (e.g., heavily denuded samples; Clarke et al. 2006b).

One-way Analysis of Similarities (ANOSIM) tests were performed using the Bray-Curtis similarity matrices to test for significant differences in species composition among years (across all stations) and among all stations (across all years). ANOSIM is a nonparametric permutation procedure that can be used to test for significant differences in *a priori* defined groups (Clarke and Gorley 2006). It produces a value of a statistic, R, which can vary from -1 to +1, where zero represents the null hypothesis that there is no difference among sample groups, and negative values are rare but theoretically possible.

Non-metric Multi-Dimensional Scaling (hereafter referred to as MDS) plots were created to visually examine differences among years and stations. MDS is a non-parametric ordination technique which displays the rank order of the distances among samples from any resemblance matrix in low-dimensional space (Clarke and Gorley 2006). Although the MDS technique is computationally involved, interpretation is fairly straightforward: points that are closer in the plot are more similar to each other (i.e., larger Bray-Curtis coefficient) than points farther away. This procedure produces a goodness-of-fit value (called "stress") that represents how well the n-dimensional multivariate data are represented in low-dimensional space, i.e., plots in 2- or 3-dimensions. MDS plots in low dimensions are generally considered to represent the n-dimensional data set adequately when the stress value is <2.0.

Hierarchical cluster analysis (which generates group averages for multiple dimensional data) was also used to examine differences in species composition among years and stations. Cluster analysis is a complementary technique to MDS and is often used to check the accuracy of MDS plots, particularly when stress values are high (Clarke and Gorley 2006). The SIMPROF procedure was used in conjunction with cluster analysis to identify statistically significant evidence of clustering among sampling. The SIMPROF procedure is a permutation test of the null hypothesis that a set of samples with no *a priori* defined groupings do not differ from each other in multivariate structure (Clarke and Gorley 2006). When SIMPROF detected significant clustering of samples the Similarities Profile (SIMPER) procedure was used to identify which subset of species contributed most to the dissimilarity between groups. The Index of Multivariate Dispersion (IMD) was used to estimate the amount of variation among samples for each significant SIMPROF grouping.

Interactions among categorical variables, which are common in ecological processes, can be difficult to analyze using non-parametric statistics. Due to the inherent parametric nature of an interaction term, it is incompatible with standard non-parametric analysis (Clarke et al. 2006a). However, certain types of interaction (e.g., spatio-temporal interaction) can be examined in fully non-parametric multivariate analyses, such as second-stage MDS. Second-stage MDS compares data trajectories among samples (irrespective of species composition), in which a second set of MDS analyses is performed on multiple MDS plots. With this technique we compared how the species assemblage at each station responded through time. That is, we compared the temporal variation in the pattern of species composition among the 13 stations to determine how individual stations responded and how that relates to the first-stages MDS. Individual Bray-Curtis similarity matrices were created from the log transformed mean yearly CPUE data for all 13 stations. A MDS plot was generated from a correlation matrix based on r values among the stations. Stations that responded similarly through time will have higher correlation values, and thus be closer together in ordination space. One-way ANOSIM tests were performed to test for significant differences in species composition among SIMPROF station groupings.

Salinity categories of species

Each of the species sampled was assigned to one four affinity groups based on salinity occurrence and life history:

- estuarine resident group;
- marine group (primarily fishes that use the estuary as a nursery ground and/or migrate from ocean waters to the estuary);
- salt-tolerant freshwater group; and
- diadromous species, subdivided into 3 subgroups:
    - anadromous,
    - semi-anadromous, and
    - catadromous species.

Environmental Variables

Environmental variables (water temperature, salinity, oxygen concentration, etc.) were recorded throughout the study period. The methods and these data are not described in this report, but see Han (1974) and Jordan et al. (1991). Correlations of species richness with salinity was tested for certain affinity groups of nearshore fishes.

Results

Species composition

A total of 53 fish species (i.e., species or species groups) were sampled in the nearshore zone of the Rhode River during the 28-year period from 1980-2007 (Table 2). Estuarine-resident species were the most abundant, but many other species were sampled from the marine, salt-tolerant freshwater, and diadromous groups. However, the majority of species were quite rare, and the six most abundant species comprised over 92% of the total catch: mummichog *Fundulus heteroclitus*; menhaden *Brevoortia tyrannus*; silverside *Menidia spp*.; sheepshead minnow *Cyprinodon variegates*; striped killifish *Fundulus majalis*; and spot *Leiostomus xanthurus*. These six species were also extremely prevalent and occurred in 47-95% of all seine hauls (Table 2).

Temporal variation

Species composition of the nearshore fish assemblage exhibited significant temporal variation during this study period. The stress statistic of the 2-dimensional solution was moderate (<0.2), and thus considered to be an appropriate simplification of the multi-dimensional data set. Moreover, detailed comparison with the 3-dimensional solution (stress = 0.09; not shown) and Cluster Analysis showed no substantial difference in interpretation. Thus, the 2-dimensional analysis was considered a good representation and is presented here (Fig. 2). Nonparametric multivariate statistical analyses indicated marked shifts in species composition (Fig. 2), with significant differences among yearly species assemblages (one-way ANOSIM: Global R= 0.332; P=0.001). In addition, species similarity of the assemblage differed among three distinct periods of years (1) 1980-1990, excluding 1987 as an outlier (2) 1991-2002, excluding 1993 (3) 2003-2007 and 1993 (MDS and Cluster analysis, SIMPROF procedure; Fig. 2). Because these groups roughly corresponded with 3 decadal periods, 1980s, 1990, and 2000s, respectively, we refer to these temporal groups for convenience in the rest of this paper.

These temporal shifts in assemblage structure also corresponded with marked changes in abundances of many common species (Fig. 3). The 1980s assemblage was generally characterized by high numbers of *L. xanthurus* and *B. tyrannus*; however, in the 1990s, these two species experienced precipitous declines in abundance. By contrast, several species that had very low abundances in the 1980s showed dramatic increases in abundance in the assemblages of the 1990s: white perch *Morone americana*, striped bass *Morone saxatilis*, Atlantic croaker *Micropogonias undulatus*, *C. variegates*; and to a lesser extent pumpkinseed *Lepomis gibbosus*, *Menidia spp.*, *F. heteroclitus*, and rainwater killifish *Lucania parva*. The 2000s assemblages were characterized by declines in many of the more common species, particularly *B. tyrannus*, *C. variegatus*, *F. heteroclitus*, *L. parva*, *M. undulatus*, *F. majalis* and *Menidia spp.*, which were also (barring *Menidia spp*.) below abundance levels in the 1980s assemblage. A few species increased in abundance in the 2000s, principally *L. gibbosus*, banded killifish *Fundulus diaphanus* and bluegill *Lepomis macrochirus*, but also some rarer species, such as alewife *Alosa pseudoharengus*. The 1987 outlier year was characterized by high abundances of *C. variegatus* and *F. majalis* combined with low abundances of *L. xanthurus* and *B. tyrannus*, particularly compared with years in the 1980s assemblage. The 1980s assemblage was the most stable (IMD = 0.835), followed by the 1990s and 2000s assemblages (IMD = 0.992 and 1.54, respectively).

Spatial variation

The fish assemblage also exhibited significant spatial variation in species composition (Fig. 4). The stress statistic of the 2-dimensional solution was low (stress = 0.02) and was considered a good representation of the multi-dimensional data set. Non-parametric multivariate statistical analyses indicated marked shifts in assemblage structure (Fig. 4) (one-way ANOSIM test, Global R= 0.308; P=0.001; and MDS and cluster analysis), with 3 clear, significant spatial groups of stations in the Rhode River subestuary (SIMPROF): (1) all headwater stations; (2) all the subtributary creek stations with the addition of the mudflats station; and (3) the mainstem stations excluding the mudflats station. The Headwater stations assemblage was dominated by estuarine-resident marsh and freshwater associated fishes, such as *F. heteroclitus*, *C. variegatus*, *F, majalis*, *L. gibbosus*, *L. macrochirus*, *L. parva*, fourspine stickleback *Apeltes quadracus*, green sunfish *Lepomis cyanellus*, *F. diaphanus*, yellow perch *Perca flavescens*, chain pickerel *Esox niger*, golden shiner *Notemigonus crysoleucas*, and common carp *Cyprinus carpio*. A small number of highly abundant species were also fairly common, principally *Menidia spp*. and *B. tyrannus*. The Creek stations assemblage was also dominated by marsh associated fishes.

However, with the notable exception of *E. niger*, there was a decline in abundance in the overwhelming majority freshwater fishes. There was also a clear increase in the abundance of pelagic and transient species, such as *B. tyrannus*, *L. xanthurus*, *Anchoa spp.*, *M. americana*, *M. saxatilis*, and *M. undulatus*. The Mainstemstations assemblage was dominated by pelagic and transient species, but frequent catches of *F. majalis* and *F heteroclitus* also occurred. With the exception of *B. tyrannus*, *L. xanthurus*, and *M. americana* there was an increase in the abundances of pelagic and transient species and barring *F. Majalis* the abundance of marsh associated fishes generally decreased in comparison to the Creek assemblage. In general, there was a gradient of increasing abundances of freshwater- and marsh-associated fishes from the mainstem to the subtributary creeks to the headwaters. Similarly, there reverse gradient for pelagic and transient fishes. The Headwaters assemblage was the variable (IMD=1.793), followed by the Mainstem (IMD=1.008) and Creek assemblage (IMD=0.839). However, it should be noted that the Headwaters and Creek assemblage consisted of 2 and 4 samples, respectively.

Spatio-temporal variation

The nearshore fish assemblage exhibited significant spatio-temporal interaction in that the species composition of the spatial groups of stations differed in their patterns of temporal variation (Fig. 5). The 2-stage MDS plot indicated a clear grouping of samples (Fig. 5A), with significant differences among the station groupings (ANOSIM test of the SIMPROF groupings, Global R= 0.454, P=005). Each of the three spatial groups of stations (Headwater, Creek, and Mainstem) had distinct assemblages of species that responded differently through time (1st Stage MDS plots; Figs. 5B, C, D). The Headwater assemblage showed little pattern of temporal variation, except in the most recent years when the abundances of several freshwater species increased (Fig. 5B). The Creek stations (Fig. 5C) differed between the 1980s and 1990s-2000s when high abundances of B. tyrannus and L. xanthurus were replaced by high abundances of *M. americana*, and *M. saxatilis*. The Creek stations also possessed considerable numbers of several freshwater associated fishes that increased in abundances from the 1990's to the 2000s. The Mainstem assemblage (Fig. 5D) showed separation between the 1980s and 1990s assemblages, but little separation for the 2000s assemblage. Similar to the Creek stations, the Mainstem stations generally possessed (when present) high abundances of variable species such as *B. tyrannus*, *L. xanthurus*, *M. americana*, and *M. saxatilis*. However, they generally contained low

abundances of more freshwater associated species, which tended to increase in abundance over the last several years of this study.

Species Diversity Response to Salinity

The abundant species in the assemblage tolerated large fluctuations in salinity and did not exhibit clear patterns of response to annual variations in salinity. However, diversity of rare species (those that comprised less than 10% in prevalence; Table 2) showed clear correlations with annual variation in salinity for two combinations of affinity groups (Fig. 6). Species richness of freshwater species and anadromous species combined (Fig. 6A) decreased significantly with salinity; whereas species richness and for marine and estuarine species combined increased significantly (Fig 6B).

Conclusions

Large oscillations in forcing functions of weather patterns appeared to drive fluctuations in some of the common species of the fish assemblage, causing shifts in dominance between "fall shelf spawners" (e.g., spot) and "spring tributary spawners" (e.g., white perch and striped bass) (Wood 2000). These fluctuations exhibited general decadal patterns of variation among dry years of the 1980s, wetter years of the 1990s, and more variable periods of rainfall in the 2000s. However, many of the abundant estuarine species resident in the subestuary (e.g., mummichog) varied without clear temporal pattern, as they are adapted to tolerate wide fluctuations in environmental conditions. An important part of the change in species composition of the nearshore fish community was explained by the rare species in the community in response to annual variation in salinity. Species richness of freshwater species and anadromous species combined decreased significantly with salinity; whereas species richness and for marine and estuarine species combined increased significantly. The species composition of the nearshore assemblage showed significant spatial variation from the low salinity areas of the creek to the more open and higher salinity shorelines of the mainstem of the Rhode River subestuary.

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Table 1. Summary of habitat categories and station designation codes for the 13 seine stations sampled for nearshore fish composition in the Rhode River, 1980-2007. DFM indicates the linear distance from the mouth of the Rhode River in km. See Fig. 1 for map of station locations.

Name	Code	*a priori* Areas	Habitat	DFM (km)	Simprof groups
Dutchman's Point	DP	Mainstem	Beach	0.5	Mainstem
Cheston Point	CP	Mainstem	Woody Debris	0.75	Mainstem
Canning House Bay	CHB	Mainstem	Beach	1.43	Mainstem
Locust Point	LP	Mainstem	Woody Debris	2	Mainstem
Camp Letts	CL	Mainstem	Beach	2.76	Mainstem
Murry's Wharf	MW	Mainstem	Marsh	3.31	Mainstem
Dock	DK	Mainstem	Woody Debris	3.57	Mainstem
Bear Neck Creek	BNC	Creek	Marsh	4.24	Creek
Sellman Creek	SC	Creek	Woody Debris	4.24	Creek
Whitemarsh Creek	WC	Creek	Marsh	4.52	Creek
Mudflats	MF	Headwaters	Marsh	4.55	Creek
Railroad	RR	Headwaters	Marsh	5.38	Headwaters
Forks	FK	Headwaters	Marsh	5.89	Headwaters

Table 2. Summary of the catch composition of the nearshore fish community of the Rhode River sampled by summer seining surveys at 13 stations during the 28-year period, 1980-2007. Columns indicate mean values of percent abundance per seine, cumulative species composition, and prevalence of species occurrences in seines for the entire data set. Affinity guild indicates the salinity range of the species.

Species	abundance %	cumm %	prevalence %	affinity guild
mummichog *Fundulus heteroclitus*	34.6	34.6	91.8	Estuarine
menhaden *Brevoortia tyrannus*	21.9	56.5	47.1	Estuarine-marine
silverside *Menidia spp.*[a]	17.0	73.5	94.9	Estuarine
sheepshead minnow *Cyprinodon variegatus*	8.8	82.3	51.9	Estuarine
striped killifish *Fundulus majalis*	6.0	88.3	75.7	Estuarine
spot *Leiostomus xanthurus*	4.3	92.6	60.9	Estuarine-marine
white perch *Morone americana*	1.5	94.1	37.0	Semi-anadromous
striped bass *Morone saxatilis*	1.2	95.4	35.7	Anadromous
anchovy *Anchoa spp.*[b]	0.8	96.2	32.6	Estuarine
pumpkinseed *Lepomis gibbosus*	0.8	97.0	21.4	Freshwater
Atlantic croaker *Micropogonias undulatus*	0.6	97.6	9.5	Estuarine-marine
rainwater killifish *Lucania parva*	0.4	98.0	27.2	Estuarine
fourspine stickleback *Apeltes quadracus*	0.4	98.3	6.2	Estuarine
hogchoker *Trinectes maculatus*	0.3	98.6	27.2	Estuarine
bluegill L*epomis macrochirus**	0.2	98.9	9.0	Freshwater
threespine stickleback *Gasterosteus aculeatus*	0.2	99.1	5.0	Semi-anadromous
banded killifish *Fundulus diaphanus*	0.1	99.2	14.5	Estuarine
northern pipefish *Syngnathus fuscus*	0.1	99.3	18.6	Estuarine
chain pickerel *Esox niger*	0.1	99.5	9.8	Freshwater
skilletfish *Gobiesox strumosus*	0.1	99.5	8.9	Estuarine
Atlantic needlefish *Strongylura marina*	0.1	99.6	14.2	Estuarine
naked goby *Gobiosoma bosci*	0.1	99.6	15.4	Estuarine
American eel *Anguilla rostrata*	0.1	99.7	10.2	Catadromous
yellow perch *Perca flavescens*	<0.1	99.7	5.5	Freshwater
green sunfish *Lepomis cyanellus*	<0.1	99.8	3.4	Freshwater
spotted seatrout *Cynoscion nebulosus*	<0.1	99.8	3.1	Estuarine-marine
eastern mosquitofish *Gambusia holbrooki*	<0.1	99.9	1.0	Freshwater
bluefish *Pomatomus saltatrix*	<0.1	99.9	8.0	Estuarine-marine
common carp *Cyprinus carpio*[*]	<0.1	99.9	4.4	Freshwater
green goby *Microgobius thalassinus*	<0.1	99.9	4.3	Estuarine
golden shiner *Notemigonus crysoleucas*	<0.1	99.9	3.6	Freshwater
banded sunfish *Enneacanthus obesus*	<0.1	99.9	0.6	Freshwater
blueback herring *Alosa aestivalis*	<0.1	>99.9	0.9	Anadromous
brown bullhead *Ameiurus nebulosus*	<0.1	>99.9	2.3	Freshwater
striped blenny *Chasmodes bosquianus*	<0.1	>99.9	3.0	Estuarine
summer flounder *Paralichthys dentatus*	<0.1	>99.9	2.2	Estuarine-marine
largemouth bass *Micropterus salmoides*[*]	<0.1	>99.9	2.2	Freshwater
alewife *Alosa pseudoharengus*	<0.1	>99.9	1.5	Anadromous
black drum *Pogonias cromis*	<0.1	>99.9	1.2	Estuarine-marine
hickory shad *Alosa mediocris*	<0.1	>99.9	0.1	Anadromous
bluespotted sunfish *Enneacanthus gloriosus*	<0.1	>99.9	0.2	Freshwater

winter flounder *Pseudopleuronectes americanus*	<0.1	>99.9	1.0	Estuarine-marine
oyster toadfish *Opsanus tau*	<0.1	>99.9	0.9	Estuarine
gizzard shad *Dorosoma cepedianum*	<0.1	>99.9	0.5	semi-anadromous
cownose ray *Rhinoptera bonasus*	<0.1	>99.9	0.2	Estuarine-marine
inshore lizardfish *Synodus foetens*	<0.1	>99.9	0.2	Estuarine-marine
Spanish mackerel *Scomberomorus maculatus*	<0.1	>99.9	0.1	Estuarine-marine
spottail shiner *Notropis hudsonius*	<0.1	>99.9	0.2	Freshwater
black crappie *Pomoxis nigromaculatus*[*]	<0.1	>99.9	0.1	Freshwater
northern puffer *Sphoeroides maculatus*	<0.1	>99.9	0.1	Estuarine-marine
silver perch *Bairdiella chrysoura*	<0.1	>99.9	0.1	Estuarine

[a] includes Atlantic silverside *Menidia menidia* and inland silverside *Menidia beryllina*

[b] includes Bay anchovy *Anchoa mitchilli* and striped anchovy *Anchoa hepsetus*

* indicates invasive species, based on Fuller et al. (1999)

Figure 1. Map of the Rhode River indicating the locations of the 13 seine stations sampled from 1980-2007. See Table 1 for station names and habitat categories.

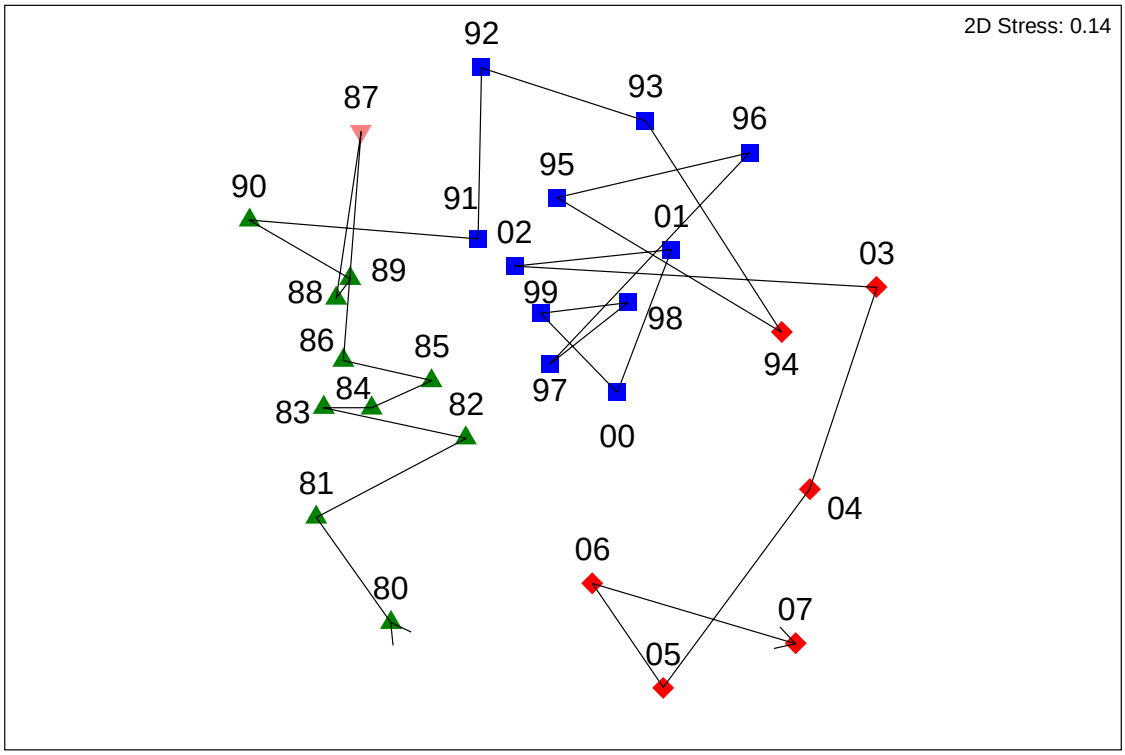

Figure 2. Annual variation in similarity of fish species composition across 13 nearshore seining stations of the Rhode River subestuary, Maryland. The figure shows a two-dimensional MDS plot of the yearly mean abundances of nearshore fish species during summer sampling for the 28-year period from 1980-2007. The line represents the yearly trajectory of species similarity. Symbols denote the statically significant clusters of similar species composition identified in the SIMPROF procedure.

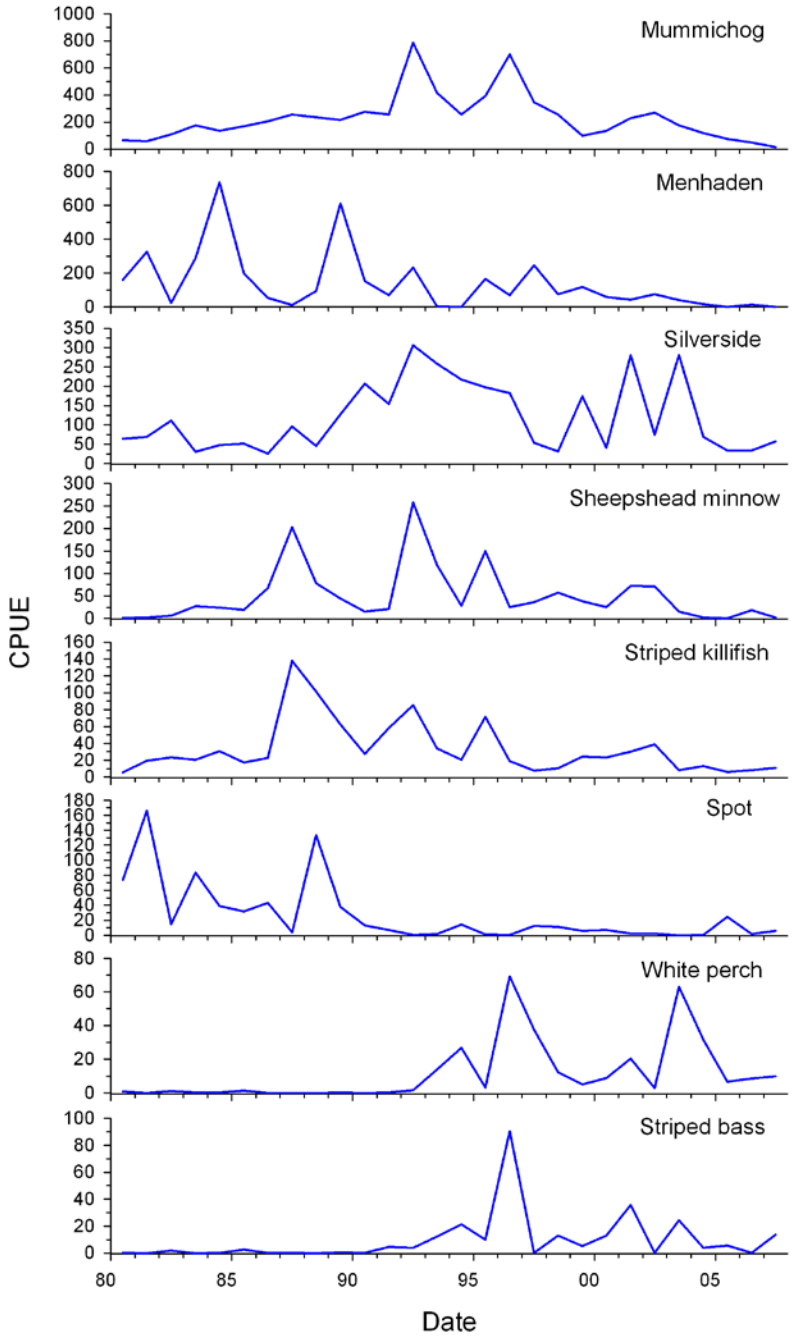

Figure 3. Long-term variation in abundance (mean CPUE) across 13 stations for the eight most abundant fish species caught by the nearshore seine survey in the Rhode River for the 28-year period, 1980-2007.

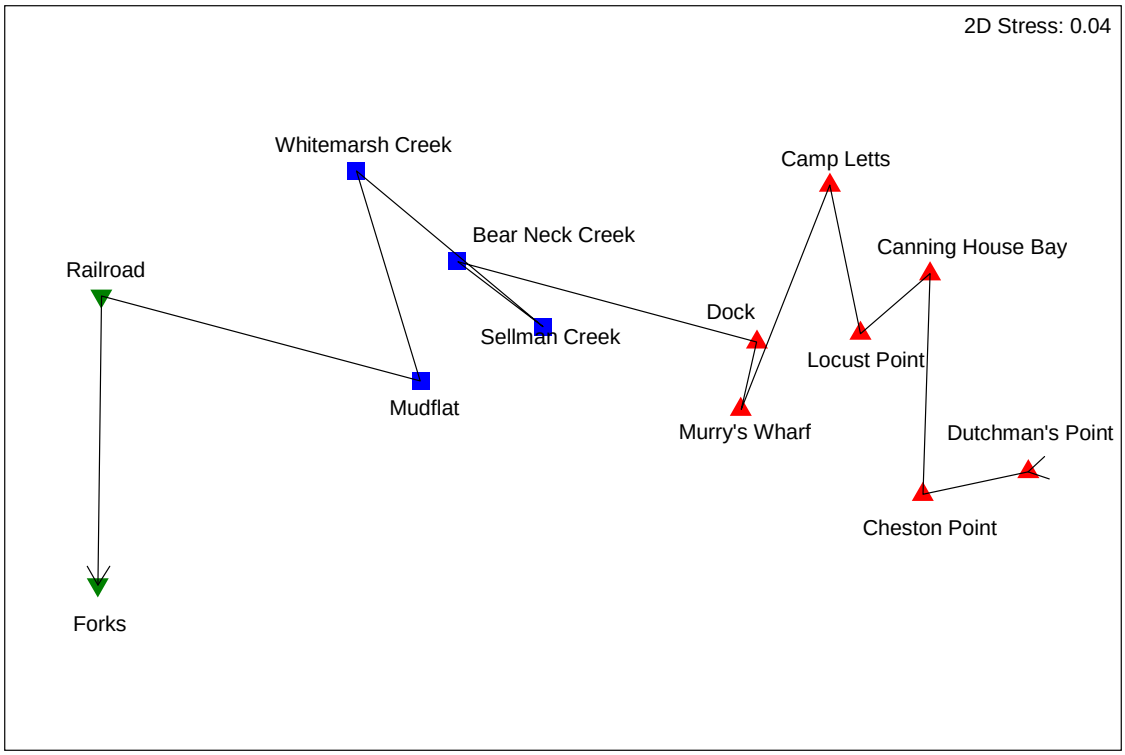

Figure 4. Spatial variation in similarity of fish species composition among 13 nearshore seining stations of the Rhode River subestuary, Maryland (Table 1, Fig. 1). The figure shows a two-dimensional MDS plot of the mean abundances by station of nearhsore fish species of summer sampling in the Rhode River over the 28-year period of 1980-2007. The line represents the linear trajectory of distance from the mouth of the Rhode River (see Table 1, Fig. 1). Symbols denote the statistically significant clusters of species similarity identified in the SIMPROF procedure.

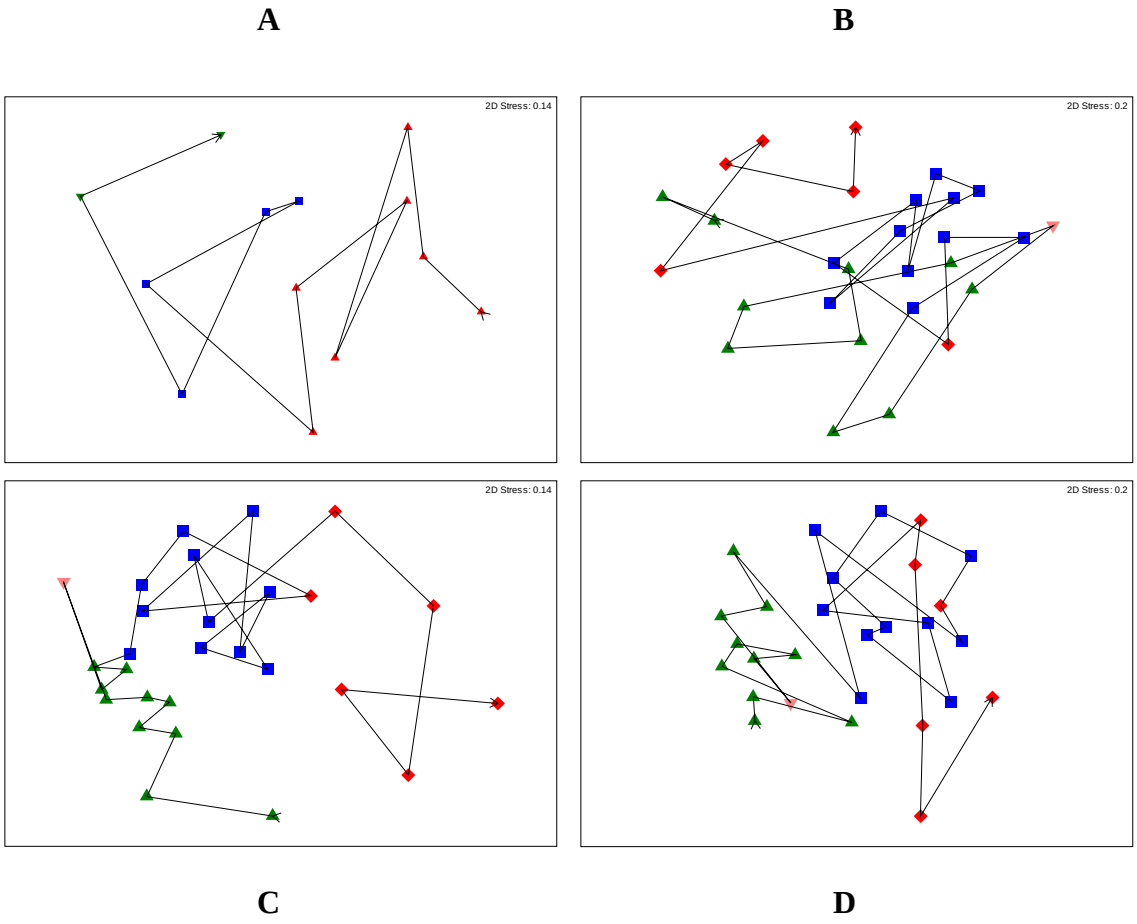

Figure 5. Spatio-temporal interaction of species composition of nearshore fish assemblage of the Rhode River subestuary. Second- and first-stage MDS plots examining spatio-temporal interaction. (A) is the 2nd-stage MDS plots comparing the temporal trajectories of the 13 seine stations in the Rhode River. The symbols represent the spatial SIMPROF groupings: red triangles indicate the Mainstem stations; blue squares indicate the Creek stations; and the green upside-down triangles indicate the Headwaters stations. The line represents the linear trajectory from the mouth of the Rhode River to the headwaters. (B-D) show the 1st-Stage MDS plots of temporal change for the Headwater, Creek, and Mainstem assemblages, respectively. The symbols represent the temporal SIMPROF groupings: green triangles indicate the 1980s assemblage, the pink upside-down triangles indicates the 1987 outliner, blue squares indicate the 1990s assemblage, and red diamonds indicate the 2000s assemblage (see Fig. 2 and text).

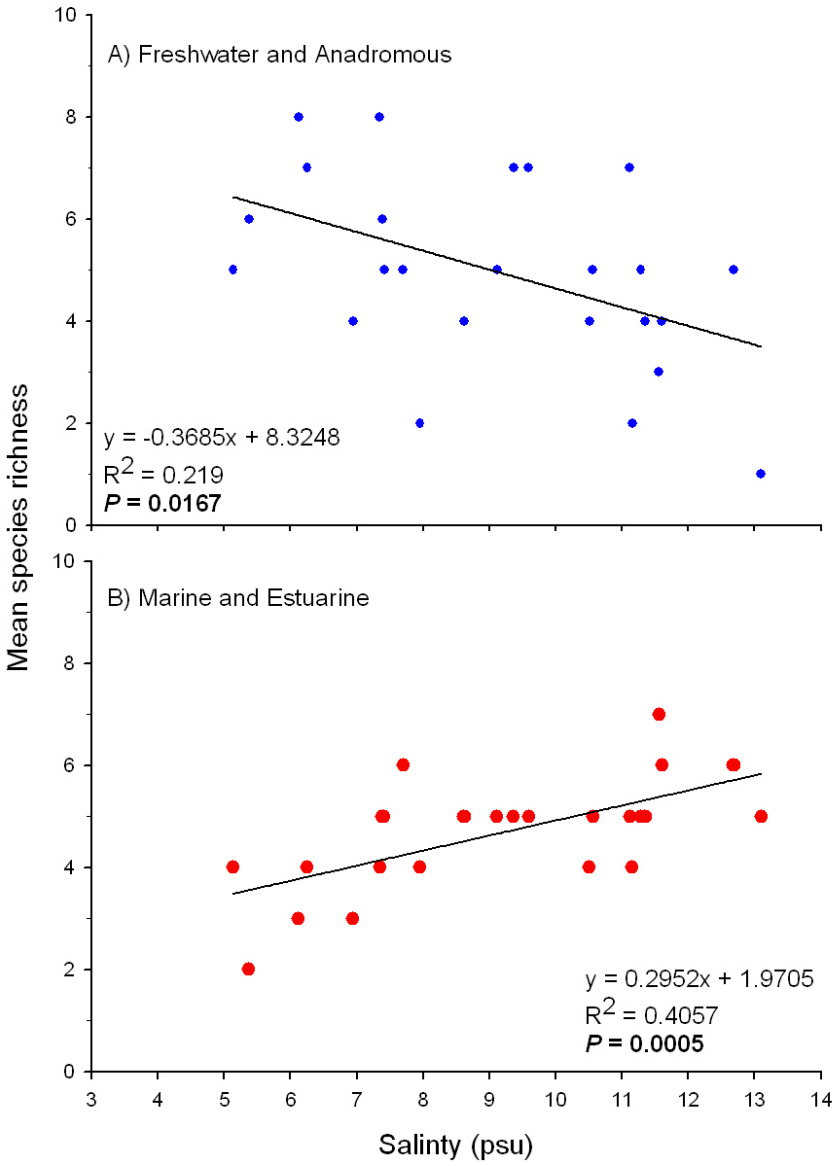

Figure 6. Correlations of species richness of rare species with salinity in nearshore fish communities across 13 stations of the Rhode River subestuary. Yearly mean species richness of rare species as a function of annual mean salinity is plotted for two affinity groups of species (Table 2): (A) freshwater species and anadromous species; and (B) marine and estuarine species. Rare species are fishes that occurred with a prevalence of less than 10% of all seine hauls (Table 2), which represented approximately 2% of total abundance.