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MARINE AND ESTUARINE FINFISH ECOLOGICAL AND HABITAT INVESTIGATIONS

Maryland Department of Natural Resources

Fishing and Boating Services

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This report was completed during September, 2017. It consists of summaries of activities for Jobs 1–4 under this grant cycle. All pages are numbered sequentially; there are no separate page numbering systems for each Job. Job 1 activities are reported in separate numbered sections. For example, Job 1, section 1 would cover development reference points (Job 1) for stream spawning habitat of anadromous fish (Section 1). Tables in a Job are numbered as section number – table number (1-1, 1-2, etc). Figures are numbered in the same fashion. Throughout the report, multiple references to past annual report analyses are referred to. The complete PDF versions of many past annual reports can be found under the Publications and Report link on the Fisheries Habitat and Ecosystem (FHEP) website page on the Maryland DNR website. The website address is http://dnr.maryland.gov/fisheries/Pages/FHEP/pubs.aspx . Table 1 provides the page number for each job and section.

Table 1. Job and section number, topic covered, and page number.

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SURVEY TITLE: MARINE AND ESTUARINE FINFISH ECOLOGICAL AND HABITAT INVESTIGATIONS
PROJECT 1: FINFISH HABITAT AND MANAGEMENT

Job 1: Development of habitat-based reference points for recreationally important Chesapeake Bay fishes of special concern: development targets and thresholds

Executive Summary

Job 1 investigates two general alternative hypotheses relating recreationally important species to development and-or agriculture. The first hypothesis is that there is a level of a particular land-use that does not significantly alter habitat suitability and the second is that there is a threshold level of land-use that significantly reduces habitat suitability (production from this habitat diminishes). The null hypothesis would be an absence of differences. In general, we expect habitat deterioration to manifest itself as reduced survival of sensitive live stages (usually eggs or larvae) or limitations on use of habitat for spawning or growth (eggs-adults). In either case, we would expect that stress from habitat would be reflected by dynamics of critical life stages (abundance, survival, growth, condition, etc.).

Spatial Analyses - We used property tax map based counts of structures in a watershed (C) per hectare (C/ha), as our indicator of development. We developed an equation to convert annual estimates of C/ha to estimates of impervious surface (IS) calculated by Towson University from 1999-2000 satellite imagery. Estimates of C/ha that were equivalent to 5% IS (target level of development for fisheries; a rural watershed), 10% IS (development threshold for a suburban watershed), and 15% IS (highly developed suburban watershed) were 0.27, 0.83, and 1.59 C/ha, respectively (Uphoff et al. 2012). Percent of watershed in agriculture, forest, and wetlands were estimated from Maryland Department of Planning spatial data.

Correlation analysis suggested negative, associations of C/ha with agriculture, forest, and wetlands. Examination of scatter plots for these comparisons suggested a negative hyperbolic curve (power function) would provide a stronger description for the comparison of percent agriculture with C/ha. Remaining land use combinations were not significantly correlated with one another.

Section 1, Stream Ichthyoplankton - Proportion of samples with Herring eggs and-or larvae (P_{herr}; Blueback Herring, Alewife, and Hickory Shad) provided a reasonably precise estimate of habitat occupation based on encounter rate. Regression analyses indicated significant and logical relationships among P_{herr}, C/ha, and conductivity consistent with the hypothesis that urbanization was detrimental to stream spawning. Estimates of P_{herr} were consistently high in the three watersheds dominated by agriculture. Importance of forest cover could not be assessed with confidence since it was possible that forest cover estimates included residential tree cover. Conductivity was positively related with C/ha in our analysis and with urbanization in other studies. Herring spawning became more variable in streams as watersheds developed. The surveys from watersheds with C/ha of 0.46 (~7% IS) or less had high P_{herr}.

Ranges of P_{herr} in study streams may have indicated variability in suitable habitat rather than abundance of spawners. In developed watersheds, a combination of urban and natural stream processes may create varying amounts of ephemeral spawning habitat annually and dampen spawning migrations through increased conductivity. Observed variation in P_{herr} would indicate wide annual and regional fluctuations in population size. However, stock assessments of Alewife and Blueback Herring indicate they are in decline or are at depressed, stable levels rather than fluctuating.

Section 2, Yellow Perch Larval Presence-Absence Sampling - Annual L_p, the proportion of tows with Yellow Perch larvae during a standard time period and where larvae would be expected, provides a cost-effective measure of the product of egg production and survival through the early postlarval stage. General patterns of land use and L_p emerged from the expanded analyses conducted for this report: L_p was negatively related to development, positively associated with forest and agriculture, and not associated with wetlands. At least five factors can be identified that potentially contribute to variations in L_p: salinity, summer hypoxia, maternal influence, winter temperature, and watershed development. These factors may not be independent and there is considerable potential for interactions among them.

Amount of organic matter present and subsequent feeding success of first-feeding Yellow Perch larvae were negatively influenced by development in Chesapeake Bay subestuaries. Wetlands appeared to be an important source of organic matter for Yellow Perch larvae in subestuaries we studied. Years of high spring discharge favor anadromous fish recruitment in Chesapeake Bay and may represent episodes of hydrologic transport of accumulated organic matter from riparian marshes and forests of watersheds that fuel zooplankton production and feeding success.

RNA/DNA ratios for 6-9 mm (first feeding) larvae did not indicate consistent differences in larval condition between two watersheds below the target level of development and two at or slightly above the threshold and the latter two watersheds appeared to be holding their own on L_p and initial feeding success of larvae. These watersheds may be providing some indication of large scale factors that mitigate impacts of development on L_p and organic matter, zooplankton, and larval feeding linkages when development is beyond, but still near, the threshold. Mattawoman Creek was considered a “developed” treatment, but most of its watershed was classified by MD DOP as forested. Patuxent River, classified by MD DOP as urban, had more organic matter than other developed watersheds that were sampled in the feeding study. Much of the development in both watersheds occurs upstream along the fluvial region above the larval estuarine nursery and water moves through a more rural region with floodplain swamps before reaching the tidal-fresh larval nursery. The regions adjacent to the larval nursery are zoned for rural land use. Both developed watersheds had 1% or greater of their watershed area in wetlands. While wetland coverage in these two developed watersheds was less than encountered in the rural “treatments”, all four systems had fringing wetlands along the larval nursery region. Patuxent River had two drinking water reservoirs (Tridelphia and Rocky Gorge) upstream of the larval nursery. The worst performing watersheds in our studies (Piscataway Creek and South, Severn, and Magothy rivers) had higher levels of development, were classified by MD DOP as urban, and had 0-0.5% of their watershed in wetlands.

Significant annual differences in initial size of first-feeding larvae were exhibited by amount of DNA per sample during 2014-2016, indicating that maternal influence on size of first-feeding larvae was not constant among years.

Section 3, Estuarine Community Sampling in Summer - Dissolved Oxygen Dynamics - Correlation analyses of DO with temperature and C/ha in subestuaries sampled since 2003 indicated that DO responded to temperature and C/ha differently depending on salinity classification. Mean bottom DO in summer surveys declined with development in mesohaline tributaries, reaching average levels below 3.0 mg/L when development was beyond its threshold, but did not in oligohaline or tidal-fresh. Mean surface DO in summer surveys did not fall below the threshold for oligohaline and tidal-fresh subestuaries, but one mesohaline subestuary fell below target. The extent of bottom channel habitat that can be occupied does not appear to diminish with development in tidal-fresh and oligohaline subestuaries due to low DO.

Inspection of the scatter plot of percent of watershed in agriculture versus median bottom DO in mesohaline subestuaries indicated an ascending limb of median DO when agricultural coverage went from 6.0 to 40.9% that was comprised entirely of western shore subestuaries. Median DO measurements beyond this level of agricultural coverage (42.6-71.6% agriculture) were from eastern shore subestuaries and the DO trend appeared to be stable or declining. Agricultural coverage and C/ha were strongly and inversely correlated, so the positive trend of DO with agriculture when agricultural coverage was low was likely to reflect development's negative impact. A dome-shaped quadratic model of median bottom DO and agricultural coverage that did not account for regional differences fit the data well. Modest declines in bottom DO would occur with increases in agriculture in subestuaries with 45%-71% of their watershed covered in agriculture. Predicted median bottom DO at the highest level of agriculture observed would equal 4.2 mg/L, which is between the DO target and threshold.

Mattawoman Creek - The level of development in Mattawoman Creek's watershed more than doubled between 1989 (0.43 C/ha) and 2011 (0.91 C/ha; Figure 3-9). This watershed reached the threshold for suburban development (C/ha = 0.83) in 2006. Currently, Mattawoman Creek's level of watershed development is 0.93 structures per hectare.

Mattawoman Creek's finfish abundance appeared to exhibit boom and bust dynamics after 2001. "Busts" were concurrent with spikes (2002) or plateaus (2007-2009) of total ammonia nitrogen (TAN; ammonia toxicity is a complex function of pH, temperature, and DO). Collapses of the magnitude exhibited during 2002 and 2008-2009 were not detected previously and the collapse of abundance in 2008-2009 was local to Mattawoman Creek and not widespread in the Potomac River. Recovery of fish abundance since 2011 has coincided with moderate values of TAN. Shifts in ecosystem status observed in Mattawoman Creek may represent shifts to different unstable or stable states (shifting baselines or regime shifts, respectively) of ecological systems rather than steady declines.

Approaching and breaching the development threshold in Mattawoman Creek's watershed has been concurrent with changes in stream hydrology and water quality, increased sediment and nutrient loading from stream erosion and construction, decreased chlorophyll a, and DO. Water clarity has increased, as has TAN and SAV while finfish abundance has become more variable and less diverse (particularly planktivores) in the subestuary. When evaluated in the context of Chesapeake Bay Program's habitat goals, Mattawoman Creek superficially resembles a restored system, i.e., increased clarity, reduced chlorophyll a, and increased SAV. The fish community has become highly variable and less diverse under these conditions.

Choptank River Subestuaries - We have explored DO trends in mesohaline Broad Creek, Harris Creek, and Tred Avon River since 2007. These watersheds are similar in agricultural and forest cover, but these adjacent watersheds have undergone development at different levels. Broad and Harris creeks have just passed the target level of development, while Tred Avon River is approaching the development threshold. Seven percent of bottom DO measurements during 2006-2016 in Tred Avon River were below the DO threshold; 31% were below DO target. Harris Creek had 2.5% of bottom DO measurements below the target during 2012-2016 and 0% below the threshold. In Broad Creek, 17% of all DO values were below the target and 1% were below the threshold.

Gunpowder and Middle Rivers - Oligohaline Gunpowder and Middle Rivers were routinely sampled during 2009-2016. In the fall of 2015 and 2016, fish kills occurred in Middle River (2015) and in Gunpowder River (2016). The Maryland Department of the Environment reported that both fish kills were caused by high amounts of toxic algae, *Karlodinium veneficum*, whose toxin causes gill damage to fish when in high concentrations. In 2015, MD DNR biologists discovered and confirmed zebra mussel presence in the Middle River. We summarized water quality and fish data to examine if these parameters changed in response to these two events.

During 2009-2015, Secchi measurements in Gunpowder River ranged from 0.3m to 0.5m; lowest median Secchi depths were during 2012-2013 and highest during 2014-2016. Median Secchi depth in Middle River ranged from 0.5m to 0.6m during 2009-2013, and then increased to 0.8m to 1.2m. . In 2011-2012, SAV coverage was below or at the median in both systems. In 2016, coverage reached the highest point in the Middle River time-series, but hovered near the median in Gunpowder River. Bottom DO (mg/L) did not appear to fluctuate dramatically from year to year in Gunpowder and Middle rivers. Measurements of pH were typically between 7 and 8. During 2009-2016, median salinity ranged from 0.34 and 3.19 ‰ in Gunpowder River and 1.2 to 5.49 ‰ in Middle River. Salinity was high in 2016 and could have had negative or lethal effects on Zebra Mussels.

We separated all subestuaries sampled from 1989-2016 by salinity class, then ranked all annual trawl geometric means of catches of all species (GM) to find where Gunpowder and Middle Rivers fell against the other oligohaline subestuaries. Gunpowder River had two GMs within the top third of ranks; five GMs in the bottom third; and one GM in the middle third. Middle River had one GM within the top third; two in the bottom third); and five in the middle. The two years with confirmed zebra mussel presence in Middle River, 2015-2016, ranked in the middle third. Declines abundance and species composition in response to fish kills in Middle River during 2015 were not detected.

STATE: MARYLAND

SURVEY TITLE: MARINE AND ESTUARINE FINFISH ECOLOGICAL AND HABITAT INVESTIGATIONS

PROJECT 1: HABITAT AND ECOLOGICAL ASSESSMENT FOR RECREATIONALLY IMPORTANT FINFISH

JOB 1: Development of habitat-based reference points for recreationally important Chesapeake Bay fishes of special concern

COMMON BACKGROUND for Sections 1-3.

"*It is the whole drainage basin, not just the body of water, that must be considered as the minimum ecosystem unit when it comes to man's interests.*" (Odum 1971).

Fishing has been the focus of assessments of human-induced perturbations of fish populations (Boreman 2000) and biological reference points (BRPs) have been developed to guide how many fish can be safely harvested from a stock (Sissenwine and Shepherd 1987). Managers also take action to avoid negative impacts from habitat loss and pollution that might drive a fish population to extinction (Boreman 2000) and typically control fishing to compensate for these other factors. A habitat-based corollary to the BRP approach would be to determine to what extent habitat can be degraded before adverse conditions cause habitat suitability to decline significantly or cease.

Forests and wetlands in the Chesapeake Bay watershed have been converted to agriculture and residential areas to accommodate increased human populations since colonial times (Brush 2009). These watershed alterations have affected major ecological processes and have been most visibly manifested in Chesapeake Bay eutrophication, hypoxia, and anoxia (Hagy et al. 2004; Kemp et al. 2005; Fisher et al. 2006; Brush 2009). Human population growth since the 1950s added a suburban landscape layer to the Chesapeake Bay watershed (Brush 2009) that has been identified as a threat to the Bay (Chesapeake Bay Program or CBP 1999). Land in agriculture has been relatively stable, but fertilizer and pesticide use became much more intensive (use had increased) in order to support population growth (Fisher et al. 2006; Brush 2009). Management of farming practices has become more intense in recent decades in response to eutrophication (Kemp et al. 2005; Fisher et al. 2006; Brush 2009). Through previous research under F-63, we have identified many negative consequences of watershed development on Bay habitat of sportfish and have used this information to influence planning and zoning (Interagency Mattawoman Ecosystem Management Task Force 2012) and fisheries management (Uphoff et al. 2011). We have less understanding of the consequences of agriculture on sportfish habitat and have redirected some effort towards understanding impacts of agricultural land use on sportfish habitat.

Job 1 investigates two general alternative hypotheses relating recreationally important species to development and-or agriculture. The first hypothesis is that there is a level of a particular land-use that does not significantly alter habitat suitability and the second is that there is a threshold level of land-use that significantly reduces habitat suitability (production from this habitat diminishes). The null hypothesis would be an absence of differences. In general, we expect habitat deterioration to manifest itself as reduced survival of sensitive live stages (usually eggs or larvae) or limitations on use of habitat for spawning or growth (eggs-adults). In either case, we would expect that stress from habitat would be reflected by dynamics of critical life stages (abundance, survival, growth, condition, etc.).

Development associated with increased population growth converts land use typical of rural areas (farms, wetlands, and forests) to residential and industrial uses (Wheeler et al. 2005; National Research Council or NRC 2009; Brush 2009) that have ecological, economic, and societal consequences (Szaro et al. 1999). Ecological stress from development of the Bay watershed conflicts with demand for fish production and recreational fishing opportunities from its estuary (Uphoff et al. 2011; Uphoff et al 2015). Extended exposure to biological and environmental stressors affect fish condition and survival (Rice 2002; Barton et al. 2002; Benejam et al. 2008; Benejam et al. 2010; Branco et al. 2016).

Impervious surface is used as an indicator of development because of compelling scientific evidence of its effect in freshwater systems (Wheeler et al. 2005; NRC 2009) and because it is a critical input variable in many water quality and quantity models (Arnold and Gibbons 1996; Cappiella and Brown 2001). Impervious surface itself increases runoff volume and intensity in streams, leading to increased physical instability, erosion, sedimentation, thermal pollution, contaminant loads, and nutrients (Beach 2002; Wheeler et al. 2005; NRC 2009). Urbanization may introduce additional industrial wastes, contaminants, stormwater runoff and road salt (Brown 2000; NRC 2009; Benejam et al. 2010; McBryan et al. 2013; Branco et al. 2016) that act as ecological stressors and are indexed by impervious surface. The NRC (2009) estimated that urban stormwater is the primary source of impairment in 13% of assessed rivers, 18% of lakes, and 32% of estuaries in the U.S., while urban land cover only accounts for 3% of the U.S. land mass.

Impact of development on estuarine systems has not been well documented, but measurable adverse changes in physical and chemical characteristics and living resources have occurred at IS of 10-30% (Mallin et al. 2000; Holland et al. 2004; Uphoff et al. 2011). Habitat reference points based on IS have been developed (ISRPs) for Chesapeake Bay estuarine watersheds (Uphoff et al. 2011). They provide a quantitative basis for managing fisheries in increasingly urbanizing Chesapeake Bay watersheds and enhance communication of limits of fisheries resources to withstand development-related habitat changes to fishers, land-use planners, watershed-based advocacy groups, developers, and elected officials (Uphoff et al. 2011; Interagency Mattawoman Ecosystem Management Task Force 2012). These guidelines have held for Herring stream spawning, Yellow Perch larval habitat (they are incorporated into the current draft of Maryland's tidal Yellow Perch management plan), and summer habitat in tidal-fresh subestuaries (Uphoff et al. 2015). Preserving watersheds at or below 5% IS would be a viable fisheries management strategy. Increasingly stringent fishery regulation might compensate for habitat stress as IS increases from 5 to 10%. Above a 10% IS threshold, habitat stress mounts in brackish subestuaries and successful management by harvest adjustments alone becomes unlikely (Uphoff et al. 2011; Interagency Mattawoman Ecosystem Management Task Force 2012; Uphoff et al. 2015). We have estimated that impervious surface in Maryland's portion of the Chesapeake Bay watershed has increased from 3.3% in 1950 to 9.3% in 2011 and project it will exceed 10% by 2020. We expect adverse habitat conditions for important forage and gamefish to worsen with future growth. Managing this growth with an eye towards conserving fish habitat is important to the future of sportfishing in Maryland.

We now consider tax map derived development indices as the best source for standardized, readily updated, and accessible watershed development indicators in Maryland and have development targets and thresholds based on it that are the same as ISRPs (Uphoff et al. 2015; Topolski 2015). Counts of structures per hectare (C/ha) had strong relationships with IS in years when all were estimated (1999-2000; Uphoff et al. 2015). Tax map data can be used as the basis for estimating target and threshold levels of development in Maryland and these estimates can be converted to IS. Estimates of C/ha that were equivalent to 5% IS (target level of development for fisheries; a rural watershed), 10% IS (development threshold for a suburban watershed), and 15% IS (highly developed suburban watershed) were estimated as 0.27, 0.83, and 1.59 C/ha, respectively. Tax map data provide a development time-series that goes back to 1950, making retrospective analyses possible (Uphoff et al. 2015).

The area of major spawning tributaries used by Striped Bass, White Perch, Yellow Perch, Alewife, Blueback Herring, Hickory Shad, and American Shad are typically on the receiving end of large amounts of agricultural drainage because of their location at the junction of large fluvial systems and brackish estuaries. Trends in juvenile indices of these species are similar, indicating similar influences on year-class success (Uphoff 2008).

Agricultural pesticides and fertilizers were thought to be potential sources of toxic metals implicated in some episodic mortality of Striped Bass larvae in Bay spawning tributaries in the early 1980s (Uphoff 1989; 1992; Richards and Rago 1999; Uphoff 2008). A correlation analysis of Choptank River watershed agricultural best management practices (BMPs) and estimates of postlarval survival during 1980-1990 indicated that as many as four BMPs were positively associated with survival (Uphoff 2008). Two measures that accounted for the greatest acreage, conservation tillage and cover crops, were strongly associated with increased postlarval survival ($r = 0.88$ and $r = 0.80$, respectively). These correlations cannot explain whether toxicity was lowered by BMPs, but it is possible that reduced contaminant runoff was a positive byproduct of agricultural BMPs aimed at reducing nutrients (Uphoff 2008).

Agriculturally derived nutrients have been identified as the primary driver of hypoxia and anoxia in the mainstem Chesapeake Bay (or Bay; Hagy et al. 2004; Kemp et al. 2005; Fisher et al. 2006; Brush 2009). Hypoxia is also associated with transition from rural to suburban landscapes in brackish Chesapeake Bay subestuaries (Uphoff et al. 2011).

Hypoxia's greatest impact on gamefish habitat occurs during summer when its extent is greatest, but hypoxic conditions are present at lesser levels during spring and fall (Hagy et al. 2004; Costantini et al. 2008). Episodic hypoxia may elevate catch rates in various types of fishing gears by concentrating fish at the edges of normoxic waters, masking associations of landings and hypoxia (Kraus et al. 2015).

Habitat loss due to hypoxia in coastal waters is often associated with fish avoiding DO that reduces growth and requires greater energy expenditures, as well as lethal conditions (Breitburg 2002; Eby and Crowder 2002; Bell and Eggleston 2005). There is evidence of cascading effects of low DO on demersal fish production in marine coastal systems through loss of invertebrate populations on the seafloor (Breitburg et al. 2002; Baird et al. 2004). A long-term decline in an important Chesapeake Bay pelagic forage fish, Bay Anchovy, may be linked to declining abundance of the common calanoid copepod *Acartia tonsa* in Maryland's portion of Chesapeake Bay that, in turn, may be linked to rising long-term water temperatures and eutrophication that drive hypoxia (Kimmel et al. 2012). Crowding in nearshore habitat, if accompanied by decreased growth due to competition, could lead to later losses through size-based processes such as predation and starvation (Breitburg 2002; Eby and Crowder 2002; Bell and Eggleston 2005). Exposure to low DO appears to impede immune suppression in fish and blue crabs, leading to outbreaks of lesions, infections, and disease (Haeseker et al. 1996; Engel and Thayer 1998; Breitburg 2002; Evans et al. 2003). Exposure of adult Carp to hypoxia depressed reproductive processes such as gametogenesis, gonad maturation, gonad size, gamete quality, egg fertilization and hatching, and larval survival through endocrine disruption even though they were allowed to spawn under normoxic conditions (Wu et al. 2003). Endocrine disruption due to hypoxia that could reduce population spawning potential has been detected in laboratory and field studies of Atlantic Croaker in the Gulf of Mexico (Thomas and Rahman 2011) and Chesapeake Bay (Tuckey and Fabrizio 2016).

Impacts of hypoxia may not be entirely negative. Costantini et al. (2008) examined the impact of hypoxia on Striped Bass 2 years-old or older in Chesapeake Bay during 1996 and 2000 through bioenergetics modeling and concluded that a temperature-oxygen squeeze had not limited growth potential of Striped Bass in the past. In years when summer water temperatures exceed 28°C, hypoxia could reduce the quality and quantity of habitat through a temperature-oxygen squeeze. In cooler summers, hypoxia may benefit Striped Bass by concentrating prey and increasing encounter rates with prey in oxygenated waters (Costantini et al. 2008).

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General Spatial and Analytical Methods used in Job 1, Sections 1-3

Spatial Methods - We used property tax map based counts of structures in a watershed, standardized to hectares (C/ha), as our indicator of development (Uphoff et al. 2012; Topolski 2015). This indicator has been provided to us by M. Topolski (MD DNR). Tax maps are graphic representations of individual property boundaries and existing structures that help State tax assessors locate properties (Maryland Department of Planning or MD DOP 2013). All tax data were organized by county. Since watersheds straddle political boundaries, one statewide tax map was created for each year of available tax data, and then subdivided into watersheds. Maryland's tax maps are updated and maintained electronically as part of MDP's Geographic Information System's (GIS) database. Files were managed and geoprocessed in ArcGIS 9.3.1 from Environmental Systems Research Institute (ESRI 2009). All feature datasets, feature classes, and shapefiles were spatially referenced using the NAD_1983_StatePlane_Maryland_FIPS_1900 projection to ensure accurate feature overlays and data extraction. ArcGIS geoprocessing models were developed using Model Builder to automate assembly of statewide tax maps, query tax map data, and assemble summary data. MdProperty View tax data are annually updated by each Maryland jurisdiction to monitor the type of parcel development for tax assessment purposes. To create watershed land tax maps, each year's statewide tax map was clipped using the MD 8-digit watershed boundary file; estuarine waters were excluded. These watershed tax maps were queried for all parcels having a structure built from 1700 to the tax data year. A large portion of parcels did not have any record of year built for structures, but consistent undercounts should not have presented a problem since we were interested in the trend and not absolute magnitude (Uphoff et al. 2012). Mattawoman Creek C/ha declined between 2011 and 2012 and then returned to a higher level in 2013. We replaced the 2012 estimate of C/ha for Mattawoman Creek with the average of 2011 and 2013.

Uphoff et al. (2012) developed an equation to convert annual estimates of C/ha to estimates of impervious surface (IS) calculated by Towson University from 1999-2000 satellite imagery. Estimates of C/ha that were equivalent to 5% IS (target level of development for fisheries; a rural watershed), 10% IS (development threshold for a suburban watershed), and 15% IS (highly developed suburban watershed) were 0.27, 0.83, and 1.59 C/ha, respectively (Uphoff et al. 2012).

Percent of watershed in agriculture, forest, and wetlands were estimated from Maryland Department of Planning spatial data. The MD DOP forest cover estimates have a minimum mapping unit of 10 acres that mixes forest cover in residential areas (trees over lawns) with true forest cover, clouding interpretation of forest influence (R. Feldt, MD DNR Forest Service, personal communication). An urban category was available as well, but was not featured in many subsequent analyses since we have adopted C/ha as our preferred index of development. Land use and land cover (LULC) shapefiles for the years 2002 and 2010 were downloaded from http://planning.maryland.gov/OurProducts/downloadFiles.shtml. Maryland Department of Planning LULC shapefiles for the year 1994 were obtained from the Maryland Department of Natural Resources. The shapefiles are vector polygon projected in NAD_1983_StatePlane_Maryland_FIPS_1900. General categories of LULC queried were urban land uses, agriculture, forest, and wetlands. Metadata for the LULC categories is available for download from the Maryland Department of Planning. Shapefiles are provided for each Maryland jurisdiction and as an aggregated statewide file.

The statewide LULC shapefiles were clipped using boundary shapefiles for each watershed of interest. Once clipped, polygon geometry was recalculated. Polygons designated as water were omitted when calculating watershed area; that is only land was considered when calculating the ratio of LULC for each category. For each LULC category, polygons were queried and the total land area in hectares was calculated. The ratio of LULC was its total hectares divided by the total watershed hectares to the nearest tenth of a hectare.

Statistical Analyses – A combination of correlation analysis, plotting of data, and curve-fitting was used to explore trends among land use types (land that was developed or in agriculture, forest, or wetland) and among fish habitat responses. Fish habitat responses were the proportion of stream samples with Herring eggs and-or larvae (P_{herr}; Section 1), proportion of subestuary samples with Yellow Perch larvae (L_p; Section 2), or subestuary bottom dissolved oxygen in summer (Section 3).

Correlations among watershed estimates of C/ha and percent of watershed estimated in urban, agriculture, forest, and wetland based on Maryland's Department of Planning spatial data (Maryland Department of Planning 2013) were used to describe associations among land cover types. Urban land consisted of high and low density residential, commercial, and institutional acreages (Maryland Department of Natural Resources or MD DNR 1999) and was not a direct measure of IS. These analyses explored (1) whether C/ha estimates were correlated with another indicator of development, percent urban and (2) general associations among major landscape features in our study watersheds. Scatter plots were inspected to examine whether nonlinear associations were possible. Land use was assigned from Maryland Department of Planning estimates for 1973, 1994, 1997, 2002, or 2010 that fell closest to a sampling year. We were particularly interested in knowing whether these land uses might be closely correlated enough (r greater than 0.8; Ricker 1975) that only one should be considered in analyses of land use and L_p and P_{herr}. We further examined relationships using descriptive models as a standard of comparison (Pielou 1981). Once the initial associations and scatter plots were examined, linear or nonlinear regression analyses (power, logistic, or Weibull functions) were used to determine the general shape of trends among land use types. This same strategy was pursued for analyses of land use and L_p or P_{herr}. Default level of significance was $P \leq 0.05$, but we did not rigidly adhere to level of significance in judging ecological or biological significance (See Johnson 1999; Anderson et al. 2000; Nakagawa 2004; Nuzzo 2014). Residuals were inspected for trends, non-normality, and need for additional terms. A general description of equations used follows, while more specific applications will be described in later sections.

Linear regressions described continuous change in variable Y as X changed:

$$Y = (m \cdot X) + b;$$

where m is the slope and b is the Y-intercept (Freund and Littel 2006). Multiple regression models accommodated an additional variable (Z):

$$Y = (m \cdot X) + (n \cdot Z) + b;$$

where n is the slope for variable Z and other parameters are as described previously (Freund and Littel 2006). We did not consider multiple regression models with more than two variables. Potential dome- or U-shaped relationships were examined with quadratic models (Freund and Littell 2006):

$$Y = (m \cdot X) + (n \cdot X^2) + b.$$

The linear regression function in Excel or Proc REG in SAS (Freund and Littel 2006) was used for single variable linear regressions. Multiple linear and quadratic regressions were analyzed with Proc REG in SAS (Freund and Littell 2006).

Examination of scatter plots suggested that some relationships could be nonlinear, with the Y-axis variable increasing at a decreasing rate with the X-axis variable and we fit power, exponential, logistic growth, or Weibull functions to these data using Proc NLIN in SAS (Gauss-Newton algorithm). The power function described a relationship with a perceptible, but declining increase or decrease in Y with X by the equation:

$$Y = a \bullet (X)^b;$$

where a is a scaling coefficient and b is a shape parameter. The exponential function describes similar relationships as the power function:

$$Y = a \cdot (\exp (b \cdot X));$$

where a is the value of Y when X = 0 and b is the rate of growth or decline. The symmetric logistic growth function described growth to an asymptote through the equation:

$$Y = b / ((1 + ((b – c) / c) \bullet (\exp (-a \bullet X)));$$

where a is the growth rate of Y with X, b is maximum Y, and c is Y at X = 0 (Prager et al. 1989). The Weibull function is a sigmoid curve that provides a depiction of asymmetric ecological relationships (Pielou 1981). A Weibull curve described the increase in Y as an asymmetric, ascending, asymptotic function of X:

$$Y = K\{1 - \exp [-(Y / S)^b]\};$$

where K was the asymptotic value of Y as X approached infinity; S was a scale factor equal to the value of Y where $Y = 0.63 \bullet K$; and b was a shape factor (Pielou 1981; Prager et al. 1989).

Confidence intervals (typically 95% CIs) of the model parameters for each indicator species were estimated to examine whether parameters were different from 0 (Freund and Littel 2006). If parameter estimates were not different from 0, the model was rejected.

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Job 1, Section 1: Stream Ichthyoplankton Sampling

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Introduction

Urbanization associated with increased population growth became a factor in the decline of diadromous fishes in the late 20th century (Limburg and Waldman 2009). Increased impervious surfaces have altered hydrology and increased diadromous fish habitat loss (Limburg and Waldman 2009). Anadromous fish egg densities (Alewife and White Perch) in the Hudson River exhibited a strong negative threshold response to urbanization (Limburg and Schmidt 1990). We were interested in understanding how reference points for development (impervious surface reference points or ISRPs, or C/ha reference points) developed for Chesapeake Bay subestuaries (Uphoff et al. 2011) were related to anadromous fish spawning in streams in Maryland's portion of Chesapeake Bay.

Surveys to identify spawning habitat of White Perch, Yellow Perch and "Herring" (Blueback Herring, Alewife, American Shad, and Hickory Shad) were conducted in Maryland during 1970-1986. These data were used to develop statewide maps depicting anadromous fish spawning habitat (O'Dell et al. 1970; 1975; 1980; Mowrer and McGinty 2002). Many of these watersheds have undergone considerable development and recreating these surveys provided an opportunity to explore whether spawning habitat declined in response to urbanization. Surveys based on the sites and methods of O'Dell et al. (1975; 1980) were used to sample Mattawoman Creek (2008-2016), Piscataway Creek (2008-2009 and 2012-2014), Bush River (2005-2008 and 2014), Deer Creek (2012-2015), Tuckahoe Creek (2016), and Choptank River (2016; Figure 1-1).

Mattawoman and Piscataway Creeks are adjacent Coastal Plain watersheds along an urban gradient emanating from Washington, DC (Table 1-1; Figure 1-1). Piscataway Creek's watershed is both smaller than Mattawoman Creek's and closer to Washington, DC. Bush River is located in the urban gradient originating from Baltimore, Maryland, and is located in both the Coastal Plain and Piedmont physiographic provinces. Deer Creek is entirely located in the Piedmont north of Baltimore, near the Pennsylvania border (Clearwater et al. 2000). Bush River and Deer Creek are adjacent to each other. An agricultural watershed, Choptank River, was surveyed in 2016. This watershed is entirely within the Coastal Plain. Ichthyoplankton surveys were conducted in the upper reaches of the Choptank River and Tuckahoe Creek, a tributary of the Choptank River. Both systems are predominantly agricultural, and the Choptank River is a major tributary of the Chesapeake Bay (Table 1-1; Figure 1-1).

We developed two indicators of anadromous fish spawning in a watershed based on presence-absence of eggs and larvae: occurrence at a site (a spatial indicator) and proportion of samples with eggs and larvae (a spatial and temporal indicator). Occurrence of eggs or larvae of an anadromous fish group (White Perch, Yellow Perch, or Herring) at a site recreated the indicator developed by O'Dell et al. (1975; 1980). This spatial indicator was compared to the extent of development in the watershed (counts of structures per hectare or C/ha) between the 1970s and the present (Topolski 2015). An indicator of habitat occupation in space and time from collections in the 2000s was estimated as proportion of samples with eggs and-or larvae of anadromous fish groups. Proportion of samples with an anadromous fish group was compared to level of development (C/ha) and conductivity, an indicator of water quality strongly associated with development (Wang and Yin 1997; Paul and Meyer 2001; Wenner et al. 2003; Morgan et al. 2007; Carlisle et al. 2010; Morgan et al. 2012).

Methods

Stream sites sampled for anadromous fish eggs and larvae during 2005-2016 were typically at road crossings that O'Dell et al. (1975; 1980) determined were anadromous fish spawning sites during the 1970s. O'Dell et al. (1975; 1980) summarized spawning activity as the presence of any species group (White Perch, Yellow Perch, or Herring) egg, larva, or adult at a site. O'Dell et al. (1975; 1980) sampled eggs and larvae with stream drift ichthyoplankton nets and adults were sampled by wire traps.

All collections during 2005-2016, with the exception of Deer Creek during 2012-2015, and Choptank River and Tuckahoe Creek during 2016, were made by citizen volunteers who were trained and monitored by program biologists. During March to May, 2008-2015, ichthyoplankton samples were collected in Mattawoman Creek from three tributary sites (MUT3-MUT5) and four mainstem sites (MC1-MC4; Figure 1-2; Table 1-2). Tributary sites MUT4 and MUTX were selected based on volunteer interest and added in 2010 and 2014, respectively; MUTX was discontinued in 2015 due to restricted access and limited indication of spawning. All mainstem sites were sampled in 2016, while the only tributary site sampled was MUT3; beaver dams blocked spawning access to MUT4 and MUT5. Piscataway Creek stations were sampled during 2008-2009 and 2012-2014 (Figure 1-3; Uphoff et al. 2010). Bush River stations were sampled during 2005-2008 and 2014 (Figure 1-4; McGinty et al. 2009; Uphoff et al. 2015). Deer Creek sites SU01-SU04 were added to sampling in 2012 and sampling continued in 2013-2015 with the addition of site SU05 (Figure 1-5). Choptank River (CH100-CH111; Figure 1-6) and Tuckahoe Creek (TUC101-TUC110; Figure 1-7) sites were added to sampling in 2016. Table 1-2 summarizes sites, dates, and sample sizes in Mattawoman, Piscataway, Deer, and Tuckahoe Creeks, and Bush and Choptank Rivers during 2005-2016.

Ichthyoplankton samples were collected in all systems and years using stream drift nets constructed of 360-micron mesh. Nets were attached to a square frame with a 300 • 460 mm opening. The stream drift net configuration and techniques were the same as those used by O'Dell et al. (1975). The frame was connected to a handle so that the net could be held stationary in the stream. A threaded collar on the end of the net connected a mason jar to the net. Nets were placed in the stream for five minutes with the opening facing upstream. Collections in Choptank River and Tuckahoe Creek during 2016 were made using stream drift nets at wadeable sites or using a conical plankton net towed from a boat (see Section 2) at sites too deep to wade. This mimics collections made by O'Dell et al. (1980) within the Choptank River drainage, specifically Tuckahoe Creek. For both types of collection, nets were retrieved and rinsed in the stream by repeatedly dipping the lower part of the net and splashing water through the outside of the net to avoid sample contamination. The jar was removed from the net and an identification label describing site, date, time, and collectors was placed both in the jar and on top of the lid before it was sealed. Samples were fixed immediately after collection by DNR staff, or were placed in a cooler with ice for transport and preserved with 10% buffered formalin after a volunteer team was finished sampling for the day. Water temperature (°C), conductivity (µS/cm), and dissolved oxygen (DO, mg/L) were recorded at each site using either a hand-held YSI Model 85 meter or a YSI Pro2030 meter. Meters were calibrated for DO each day prior to use. All data were recorded on standard field data forms and double-verified at the site during volunteer collections. Approximately 2-ml of rose bengal dye was added to each sample in order to stain the organisms pink to aid sorting.

Ichthyoplankton samples were sorted in the laboratory by project personnel. All samples were rinsed with water to remove formalin and placed into a white sorting pan. Samples were sorted systematically (from one end of the pan to another) under a 10x bench magnifier. All eggs and-or larvae were removed and were retained in a small vial with a label (site, date, and time) and stored with 20% ethanol for later identification under a microscope. Each sample was sorted systematically a second time for quality assurance (QA). Any additional eggs and-or larvae found were removed and placed in a vial with a label (site, date, time, and QA) and stored with 20% ethanol for identification under a microscope. All eggs and larvae found during sorting (both in original and QA vials) were identified as either Herring (Blueback Herring, Alewife, and Hickory Shad), Yellow Perch, White Perch, unknown (eggs and-or larvae that were too damaged to identify) or other (indicating another fish species) and the presence or absence of each of the above species was recorded. The three Herring species' eggs and larvae are very similar (Lippson and Moran 1974) and identification to species can be problematic. American Shad eggs and larvae would be larger at the same stages of development than those identified as Herring (Lippson and Moran 1974) and none have been detected in our surveys. Quality assurance vials only contained additional eggs and-or larvae of target species already present in the original vials. No new target species were detected during the assessment of the QA vials.

Methods used to estimate development (C/ha) and land use indicators (percent of watershed in agriculture, forest, wetlands, and urban land use) are explained in **General Spatial and Analytical Methods used in Job 1, Sections 1-3.** Development targets and limits and general statistical methods (analytical strategy and equations) are described in this section as well. Specific spatial and analytical methods for this section of the report are described below.

Mattawoman Creek's watershed equaled 24,441 ha and estimated C/ha increased from 0.87 to 0.93 during 2008-2016; Piscataway Creek's watershed equaled 17,642 ha and estimated C/ha increased from 1.41 to 1.50 during 2008-2016; Bush River's watershed equaled 36,038 ha and estimated C/ha increased from 1.37 to 1.51 during 2005-2016; and Deer Creek, a spawning stream with low development, has a watershed equal to 37,697 ha and estimated C/ha was 0.24 during 2012-2016 (Table 1-1). The upper portion of the Choptank River (watershed area = 38,216 ha and developmental level = 0.18 C/ha) and a tributary of the Choptank River, Tuckahoe Creek (watershed area = 39,388 ha and developmental level = 0.07), were added in 2016 as spawning streams with high agricultural influence and low watershed development (Table 1-1; Figure 1-1). Deer Creek, and Choptank River and Tuckahoe Creek, collections were made by DNR biologists from the Fishery Management Planning and Fish Passage Program at no charge to this grant.

Conductivity measurements collected for each date and stream site (mainstem and tributaries) during 2008-2016 from Mattawoman Creek were plotted and mainstem measurements were summarized for each year. Mainstem sites would be influenced by development in Waldorf, while the monitored tributaries would not. Unnamed tributaries were excluded from calculation of summary statistics to capture conditions in the largest portion of habitat. Comparisons were made with conductivity minimum and maximum reported for Mattawoman Creek during 1991 by Hall et al. (1992). Conductivity data were similarly summarized for Piscataway Creek mainstem stations during 2008-2009 and 2012-2014. A subset of Bush River stations that were sampled each year during 2005-2008 and 2014 (i.e., stations in common) were summarized; stations within largely undeveloped Aberdeen Proving Grounds were excluded because they were not sampled every year. Conductivity was measured with each sample in Deer Creek in 2012-2015, and in the Choptank River and Tuckahoe Creek in 2016.

A water quality database maintained by DNR's Tidewater Ecosystem Assessment (TEA) Division (S. Garrison, MD DNR, personal communication) provided conductivity measurements for Mattawoman Creek during 1970-1989. These historical measurements were compared with those collected in 2008-2016 to examine changes in conductivity over time. Monitoring was irregular for many of the historical stations. Table 1-3 summarizes site location, month sampled, total measurements at a site, and what years were sampled. Historical stations and those sampled in 2008-2016 were assigned river kilometers (RKM) using a GIS ruler tool that measured a transect approximating the center of the creek from the mouth of the subestuary to each station location. Stations were categorized as tidal or non-tidal. Conductivity measurements from eight non-tidal sites sampled during 1970-1989 were summarized as monthly medians. These sites bounded Mattawoman Creek from its junction with the estuary to the city of Waldorf (Route 301 crossing), the major urban influence on the watershed. Historical monthly median conductivities at each mainstem Mattawoman Creek non-tidal site were plotted with 2008-2016 spawning season median conductivities.

Presence of White Perch, Yellow Perch, and Herring eggs and-or larvae at each station in 2016 was compared to past surveys to determine which sites still supported spawning. We used the criterion of detection of eggs and-or larvae at a site (O'Dell et al. 1975; 1980) as evidence of spawning. Raw data from early 1970s collections were not available to formulate other metrics.

Four Mattawoman Creek mainstem stations sampled in 1971 by O'Dell et al. (1975) were sampled by Hall et al. (1992) during 1989-1991 for water quality and ichthyoplankton. Count data were available for 1991 (C/ha = 0.46) in a tabular summary at the sample level and these data were converted to presence-absence. Hall et al. (1992) collected ichthyoplankton with 0.5 m diameter plankton nets (3:1 length to opening ratio and 363μ mesh set for 2-5 minutes, depending on flow) suspended in the stream channel between two posts instead of stream drift nets. Changes in spawning site occupation among the current study (2008-2016), 1971 (O'Dell et al. 1975) and 1991 (Hall et al. 1992) were compared to C/ha in Mattawoman Creek. Historical and recent C/ha were compared to site occupation for Piscataway Creek 1971 (O'Dell et al. 1975), 2008-2009 and 2012-2014; Bush River 1973 (O'Dell et al. 1975), 2005-2008 (McGinty et al. 2009; Uphoff et al. 2010) and 2014; Deer Creek 1972 (O'Dell et al. 1975) and 2012-2015; and Tuckahoe Creek 1976-77 (O'Dell et al. 1980) and 2016.

The proportion of samples where Herring eggs and-or larvae were present (P_{herr}) was estimated for Mattawoman Creek mainstem stations (MC1-MC4; Figure 1-2) during 1991 and 2008-2016. Sampling of ichthyoplankton in Piscataway Creek (2008-2009 and 2012-2014), Bush River (2005-2008 and 2014), Deer Creek (2012-2015), Choptank River (2016) and Tuckahoe Creek (2016) also provided sufficient sample sizes to estimate P_{herr}. Herring was the only species group represented with adequate sample sizes for annual estimates with reasonable precision. Mainstem stations (PC1-PC3) and Tinkers Creek (PTC1) were used in Piscataway Creek (Figure 1-3). Only sites in streams that were sampled in all years (sites in common) in the Bush River drainage were analyzed (Figure 1-4; see Uphoff et al. 2014 for sites sampled in other years). Deer Creek stations SU01, SU04, and SU05 correspond to O'Dell et al. (1975) sites 1, 2, and 3 respectively (Figure 1-5). Two additional sites, SU02 and SU03 were sampled and analyzed in this system as well. The mainstem of the Choptank has not been sampled previously so 12 stations (CH100-CH111; Figure 1-6) were added in that system for analysis. Tuckahoe Creek stations TUC101, TUC102, TUC103, and TUC108 correspond to O'Dell et al. (1980)

sites 4, 5, 6, and 8 respectively (Figure 1-7). Eight additional sites were sampled in this system and analyzed as well.

For the rivers and stations described above, the proportion of samples with Herring eggs and-or larvae present was estimated as:

$$^{(1)}\ P_{herr} = N_{present} / N_{total};$$

where $N_{present}$ equaled the number of samples with Herring eggs and-or larvae present and N_{total} equaled the total number of samples taken. The SD of each P_{herr} was estimated as:

$$^{(2)}\ \mathrm{SD} = [(P_{herr} \bullet (1- P_{herr})) / \mathrm{N}_{\mathrm{total}}]^{0.5}\ \text{(Ott 1977).}$$

The 90% confidence intervals were constructed as:

$$^{(3)}\ P_{herr} \pm (1.44 \bullet \mathrm{SD}).$$

White Perch and Yellow Perch have been present in samples at the downstream-most one or two stations in Mattawoman Creek during 1989-1991 (Hall et al. 1992) and 2008-2016. We pooled three year intervals (1989-1991, 2008-2010, 2011-2013, and 2014-2016) to estimate the proportion of samples with White or Yellow Perch eggs and larvae in order to gain enough precision to separate these estimates from zero. Formulae for estimating proportions were the same as for estimating P_{herr} and its SD and 90% CI's (see above). White Perch spawning occurred at MC1 and MC2. Yellow Perch spawning was only detected at Station MC1.

Regression analyses examined relationships of development (C/ha) with standardized conductivity measurements (median conductivity adjusted for Coastal Plain or Piedmont background level; see below), C/ha and Herring spawning intensity (P_{herr}), standardized conductivity with P_{herr}, and estimates of watershed percentage that was agriculture or forest with P_{herr}. Data were from Mattawoman, Piscataway, Deer and Tuckahoe Creeks, and Bush and Choptank Rivers. Twenty-six estimates of C/ha, percent agriculture or forest, and P_{herr} were available (1991 estimates for Mattawoman Creek could be included), while 25 estimates were available for standardized conductivity (Mattawoman Creek conductivity data were not available for 1991). Examination of scatter plots suggested that a linear relationship was the obvious choice for C/ha and P_{herr}, that either linear or curvilinear relationships might be applicable to C/ha with standardized conductivity and standardized conductivity with P_{herr}, and that quadratic relationships best described the relationships of percentage of a watershed that was either agriculture or forest and P_{herr}. Power functions were used to fit curvilinear models. Linear regressions were analyzed in Excel, while the non-linear regression analysis used Proc NLIN in SAS (Freund and Littell 2006). A linear or nonlinear model was considered the best description if it was significant at $\alpha < 0.05$ (both were two parameter models), it explained more variability than the other (r^2 for linear or approximate r^2 for nonlinear), and examination of residuals did not suggest a problem. We expected negative relationships of P_{herr} with C/ha and standardized conductivity, while standardized conductivity and C/ha were expected to be positively related.

Conductivity was summarized as the median for the same stations that were used to estimate P_{herr} and was standardized by dividing by an estimate of the background expected from a stream absent anthropogenic influence (Morgan et al. 2012; see below). Piedmont and Coastal Plain streams in Maryland have different background levels of conductivity (Morgan et al. 2012). Morgan et al. (2012) provided two sets of methods of estimating spring base flow background conductivity for two different sets of Maryland ecoregions, for a total set of four potential background estimates. We chose the option featuring Maryland Biological Stream Survey (MBSS) Coastal Plain and Piedmont regions and the 25th percentile background level for conductivity. These regions had larger sample sizes than the other options and background conductivity in the Coastal Plain fell much closer to the observed range estimated for

Mattawoman Creek in 1991 (61-114 µS/cm) when development was relatively low (Hall et al. 1992). Background conductivity used to standardize median conductivities was 109 µS/cm in Coastal Plain streams and 150 µS/cm in Piedmont streams.

Results

Development level of Piscataway, Mattawoman, and Deer Creeks, Bush River, and the Choptank River drainage (which includes Tuckahoe Creek) watersheds started at approximately 0.05 C/ha in 1950 (Figure 1-8). Surveys conducted by O'Dell et al. (1975, 1980) in the 1970s, sampled largely rural watersheds (C/ha < 0.27) except for Piscataway Creek (C/ha = 0.46). By 1991, C/ha in Mattawoman Creek was similar to that of Piscataway in 1970. By the mid-2000s, Bush River and Piscataway Creek were at higher suburban levels of development (~1.30 C/ha) than Mattawoman Creek (~0.80 C/ha). Deer Creek (zoned for agriculture and preservation) and the Choptank River drainage (predominantly agricultural) remained rural through 2016 (0.24 and 0.13 C/ha, respectively; Figure 1-8).

Conductivity measurements in mainstem Mattawoman Creek during 2008-2016 never fell within the range observed during 1991 (Figure 1-9). Conductivity in Mattawoman Creek tributaries sampled during 2008-2016 often fell within the range observed during 1991.

In 2016, conductivity measurements in mainstem Mattawoman Creek were elevated in March and April (> 150 µS/cm) and declined slightly in May, but never fell to the 1991 maximum (114 µS/cm; Figure 1-9). Conductivity measurements in tributary MUT3 in 2016 never went above the 1991 maximum, and had values similar to those observed in the tributaries during 2010-2013 (Figure 1-9). Conductivities in Mattawoman Creek's mainstem stations in 2009 were highly elevated in early March following application of road salt in response to a significant snowfall that occurred just prior to the start of the survey (Uphoff et al. 2010). Measurements during 2009 steadily declined for nearly a month before leveling off slightly above the 1989-1991 maximum. Temperatures were higher and snowfall lower in 2016 than the previous two years, with a conductivity pattern similar to 2010-2013 (Figure 1-9). During 2014 and 2015, temperatures were colder and snowfall was higher; conductivities were elevated and similar to 2009. In general, highest conductivity measurements were at the most upstream mainstem site (MC4) and declined downstream to the site on the tidal border. This, along with low conductivities typically seen at the unnamed tributaries, indicated that development at and above MC4 associated with Waldorf affected water quality (Figure 1-9).

Conductivity levels in Piscataway Creek and Bush River were elevated compared to Mattawoman Creek (Table 1-4). With the exception of Piscataway Creek in 2012 (median = 195 µS/cm), median conductivity estimates during spawning surveys were always greater than 200 µS/cm in Piscataway Creek and Bush River during the 2000s. Median conductivity in Mattawoman Creek was greater than 200 µS/cm during 2009, but was less than 155 µS/cm during the next four years. With increased snowfalls in 2014 and 2015, median conductivity rose to 166 and 173 µS/cm, respectively (Table 1-4). While 2016 did not have a maximum conductivity as high as those in the previous two years, elevated values were recorded continuously until May (Figure 1-9), resulting in the highest median conductivity since 2009 (189 µS/cm; Table 1-4).

During 1970-1989, 73% of monthly median conductivity estimates in Mattawoman Creek were at or below the background level for Coastal Plain streams; C/ha in the watershed increased from 0.25 to 0.41. Higher monthly median conductivities in the non-tidal stream were more frequent nearest the confluence with Mattawoman Creek's estuary and in the vicinity of

Waldorf (RKM 35; Figure 1-10). Conductivity medians were highly variable at the upstream station nearest Waldorf during 1970-1989. During 2008-2016 (C/ha = 0.87-0.93), median spawning survey conductivities at mainstem stations MC2 to MC4, above the confluence of Mattawoman Creek's stream and estuary (MC1), were elevated beyond nearly all 1979-1989 monthly medians and increased with upstream distance toward Waldorf. Most measurements at MC1 fell within the upper half of the range observed during 1970-1989 (Figure 1-10). None of the non-tidal conductivity medians estimated at any mainstem site during 2008-2016 were at or below the Coastal Plain stream background criterion.

Herring spawning was detected at all mainstem stations in Mattawoman Creek (MC1-MC4) during 1971 and 1991 (Table 1-5). Herring spawning in fluvial Mattawoman Creek was detected at two mainstem sites during 2008-2009 and all four mainstem stations during 2010-2016. Herring spawning was not detected at tributary site MUT3 during 2008-2009, but was consistently present afterwards. Spawning was intermittently detected at MUT4 and MUT5 in sampling during the 2000s. During 1971 and 1989-1991, White Perch spawning occurred annually at MC1 and intermittently at MC2. Stream spawning of White Perch in Mattawoman Creek was not detected during 2009, 2011, and 2012, but spawning was detected at MC1 during 2008, 2010 and 2013-2016, and at MC2 during 2013-2014 and 2016. Spawning was detected at MC3 during 1971 and 2016. Station MC1 was the only stream station in Mattawoman Creek where Yellow Perch spawning has been detected in surveys conducted since 1971. Yellow Perch spawning occurred at station MC1 every year except 2009 and 2012 (Table 1-5).

Herring spawning was detected at all mainstem sites in Piscataway Creek in 2012-2014. Stream spawning of anadromous fish had nearly ceased in Piscataway Creek between 1971 and 2008-2009 (Table 1-6). Herring spawning was not detected at any site in the Piscataway Creek drainage during 2008 and was only detected on one date and location (one Herring larvae on April 28 at PC2) in 2009. Stream spawning of White Perch was detected at PC1 and PC2 in 1971, was not detected during 2008-2009 and 2012-2013, but was detected at PC1 in 2014 (Table 1-6).

Changes in stream site spawning of Herring, White Perch, and Yellow Perch in the Bush River stations during 1973, 2005-2008, and 2014 were not obvious (Table 1-7). Herring eggs and larvae were present at three to five stations (not necessarily the same ones) in any given year sampled. Occurrences of White and Yellow Perch eggs and larvae were far less frequently detected during 2005-2008 than 1973 and 2014 (Table 1-7).

O'Dell et al. (1975) reported that Herring, White Perch, and Yellow Perch spawned in Deer Creek during 1972 (Table 1-8). Three sites were sampled during 1972 in Deer Creek and one of these sites was located upstream of an impassable dam near Darlington (a fish passage was installed there in 1999). During 1972, Herring spawning was detected at both sites below the dam (SU01 and SU03), while White and Yellow Perch spawning were detected at the mouth (SU01). During 2012-2015, Herring spawning was detected at all sites sampled in each year. White Perch spawning was not detected in Deer Creek in 2012 but was detected at three sites each in 2013 and 2014, and two sites in 2015. Yellow Perch spawning detection has been intermittent; evidence of spawning was absent in 2013 and 2015, while spawning was detected at two and three sites in 2012 and 2015 respectively (Table 1-8).

While the Choptank River itself had not been sampled previously (Table 1-9), O'Dell et al. (1980) reported Herring, White Perch, and Yellow Perch spawned in its drainage (Tuckahoe Creek) during 1976-1977 (Table 1-10). Twelve sites were sampled during 1976-77 after installation of a fish ladder at the dam for the lake at Tuckahoe State Park. Sampling sites were established above and below the dam to determine the effectiveness of the fish ladder in passing anadromous and estuarine species (O’Dell et al. 1980). During 1976-77, White Perch, Yellow Perch, and Herring were collected downstream of the dam/fishway, while White Perch were documented on the upstream side. O’Dell et al. (1980) noted that this species might have been trapped behind the dam when it was built and that its presence did not necessarily indicate successful migration through the fish ladder since no other species were documented on the upstream side. Sites in common between 2016 and the O’Dell et al. (1980) study include TUC101-TUC103 and TUC108. Herring spawning was detected at all sites sampled in 2016 with the exception of the most upstream site, TUC110 (furthest above the dam). A new fish ladder was installed in 1993 to replace the one referenced in O’Dell et al. (1980) and it passes Herring (J. Thompson, MD DNR, personal communication). White Perch spawning was detected in all but the two most upstream sites, both of which were located above the dam. In 2016 Yellow Perch spawning was detected at all sites below the dam and at none of those above.

The 90% confidence intervals of P_{herr} (Figure 1-11) provided sufficient precision for us to categorize four levels of stream spawning: very low levels at or indistinguishable from zero based on confidence interval overlap (level 0); a low level of spawning that could be distinguished from zero (level 1); a mid-level of spawning that could usually be separated from the low levels (level 2); and a high level (3) of spawning likely to be higher than the mid-level. Stream spawning of Herring in Mattawoman Creek was categorized at levels 1 (2008-2009), 2 (2010 and 2012), and level 3 (1991, 2011, and 2013-2016). Spawning in Piscataway Creek was at level 0 during 2008-2009, at level 2 during 2012, and at level 1 during 2013-2014. Bush River Herring spawning was characterized by levels 0 (2006), 1 (2005 and 2007-2008), and 2 (2014). Deer Creek (2012-2015), Tuckahoe Creek (2016), and Choptank River (2016) are the least developed watersheds and were characterized by the highest level of Herring spawning (level 3) in all years sampled (Figure 1-11).

The 90% CI’s of proportions of samples with White Perch eggs and larvae at Mattawoman Creek’s stations MC1 and MC2, pooled in 2-to-3-year intervals, indicated less stream spawning occurred during 2008-2010 than during 1989-1991 (Figure 1-12). Status of White Perch spawning in Mattawoman Creek during 2011-2016 was not clear since 90% CI’s of the proportion of samples with White Perch eggs and larvae during 2011-2016 overlapped both 1989-1991 and 2008-2010. The 90% CI’s for stream spawning of Yellow Perch (at MC1 only) overlapped for 1989-1991, 2008-2010, and 2011-2013 indicating significant change in stream spawning had not been detected up to that point. Stream spawning of Yellow Perch in 2014-2016 does appear to have increased significantly, however, with little overlap of its 90% CI with those in 1989-1991 and 2008-2013 (Figure 1-12). Anecdotally, fishermen targeting Yellow Perch just downstream of Mattawoman’s MC1 site indicated that 2016 had the highest number of adults seen and caught in recent (10+ year) memory (C. Hoover, MD DNR, personal communication).

Correlation analysis was used to examine associations among three land cover parameters in Table 1-1: C/ha, agricultural land cover, and forest cover. Unlike similar analyses in Job 1, Sections 2 and 3, wetlands were not a land use feature common in the portion of watershed where fluvial spawning streams were located. The percent of a watershed in agriculture was strongly and negatively correlated with C/ha ($r = -0.68$, $P = 0.0013$) and forest cover and agriculture were also strongly and negatively correlated ($r = -0.66$, $P = 0.0021$). Forest cover was poorly correlated with C/ha ($r = -0.05$, $P = 0.852$); MD DOP forest cover estimates mixes forest cover in residential areas (trees over lawns) with true forest cover, clouding interpretation of forest influence. Subsequent analyses with P_{herr} beyond comparisons with C/ha were likely to be confounded by the close negative correlations (close to the r = 0.8 criterion described in **General Spatial and Analytical Methods used in Job 1, Sections 1-3)** so statistical analyses with land uses other than C/ha were not pursued. Our preference for using C/ha in analyses was two-fold: we have already done considerable work using C/ha, and C/ha provides a continuous time-series rather than episodic. We did note, however, when these other land uses were predominant for particular P_{herr} outcomes.

Standardized conductivity increased with development, while P_{herr} declined with both development and standardized conductivity. Regression analyses indicated significant and logical relationships among P_{herr}, C/ha, and standardized median conductivity (Table 1-11). The relationship of C/ha with standardized median conductivity was linear, significant, and positive ($r^2 = 0.41$, P = 0.0006, N = 25; Figure 1-13). Estimates of P_{herr} were linearly, significantly, and negatively related to C/ha ($r^2 = 0.58$, P = <.0001, N = 26). Negative linear and curvilinear (power function) regressions similarly described the relationship of P_{herr} and standardized median conductivity ($r^2 = 0.29$, $P < 0.0055$; or approximate $r^2 = 0.26$, $P < 0.0001$, respectively), with linear regression explaining only slightly more variability (N = 25; Figure 1-14). Low estimates of P_{herr} were much more frequent beyond the C/ha threshold (0.83 C/ha) or when standardized conductivity was 1.5-times or more than the baseline level (Figure 1-14). Estimates of P_{herr} were consistently above 0.6 in the three watersheds dominated by agriculture (Deer Creek, Tuckahoe Creek, and Choptank River; Figure 1-14). The only watershed in this analysis dominated by forest cover was Mattawoman Creek and only one estimate (1991 at 62.6% forest cover and C/ha = 0.46) represented development below the C/ha threshold. This estimate of P_{herr} was above 0.6 and was consistent with watersheds dominated by agriculture. Remaining estimates for Mattawoman Creek were represented by 53.9% forest cover with C/ha increasing from 0.87 in 2008 to 0.93 in 2014. Estimates of P_{herr} exhibited a much greater range, 0.08-0.66, at these higher levels of development and lower forest cover (Figure 1-14).

Discussion

Proportion of samples with Herring eggs and-or larvae (P_{herr}) provided a reasonably precise estimate of habitat occupation based on encounter rate. Regression analyses indicated significant and logical relationships among P_{herr}, C/ha, and conductivity consistent with the hypothesis that urbanization was detrimental to stream spawning. Estimates of P_{herr} were consistently high in the three watersheds dominated by agriculture. Importance of forest cover could not be assessed with confidence since it was possible that forest cover estimates included residential tree cover. Conductivity was positively related with C/ha in our analysis and with urbanization in other studies (Wang and Yin 1997; Paul and Meyer 2001; Wenner et al. 2003; Morgan et al. 2007; Carlisle et al. 2010; Morgan et al. 2012).

Herring spawning became more variable in streams as watersheds developed. The surveys from watersheds with C/ha of 0.46 or less had high P_{herr}. Estimates of P_{herr} from Mattawoman Creek during 2008-2016 (C/ha was 0.87-0.93) varied from barely different from zero to high. Eggs and larvae were nearly absent from fluvial Piscataway Creek during 2008-2009, but P_{herr} rebounded to 0.45 in 2012 and then dropped again to 0.2 in 2013-2014 (C/ha was 1.41-1.50). The rebound in Herring spawning in Piscataway Creek during 2012 was concurrent with the lowest mean and median conductivities encountered there in the four years sampled. Variability of Herring spawning in Bush River during 2005-2008 and 2014 involved “colonization” of new sites as well as absence from sites of historical spawning (Uphoff et al.

2014). Limburg and Schmidt (1990) found a highly nonlinear relationship of densities of anadromous fish (mostly Alewife) eggs and larvae to urbanization in Hudson River tributaries, reflecting a strong, negative threshold at low levels of development.

Ranges of P_{herr} in study streams may have indicated variability in suitable habitat rather than abundance of spawners. In developed watersheds, a combination of urban and natural stream processes may create varying amounts of ephemeral spawning habitat annually and dampen spawning migrations through increased conductivity. Observed variation in P_{herr} would indicate wide annual and regional fluctuations in population size. However, stock assessments of Alewife and Blueback Herring along the Atlantic coast, including those in Maryland, indicate they are in decline or are at depressed, stable levels (ASMFC 2009a; 2009b; Limburg and Waldman 2009; Lipkey and Jarzynski 2015) rather than fluctuating.

Processes such as flooding, riverbank erosion, and landslides vary by geographic province (Cleaves 2003) and influence physical characteristics of streams. Unconsolidated layers of sand, silt, and clay underlie the Coastal Plain and broad plains of low relief and wetlands characterize the natural terrain (Cleaves 2003). Coastal Plain streams have slow flows and sand or gravel bottoms (Boward et al. 1999). The Piedmont is underlain by metamorphic rocks and characterized by narrow valleys and steep slopes, with regions of higher land between streams in the same drainage. Most Piedmont streams are of moderate slope with rock or bedrock bottoms (Boward et al. 1999). The Piedmont is an area of higher gradient change and more diverse and larger substrates than the Coastal Plain (Harris and Hightower 2011) that may offer greater variety of Herring spawning habitats.

Urbanization and physiographic province both affect discharge and sediment supply of streams (Paul and Meyer 2001; Cleaves 2003) that, in turn, could affect location, substrate composition, and extent and success of spawning. Alewife spawn in sluggish flows, while Blueback Herring spawn in sluggish to swift flows (Pardue 1983). American Shad select spawning habitat based on macrohabitat features (Harris and Hightower 2011) and spawn in moderate to swift flows (Hightower and Sparks 2003). Spawning substrates for Herring include gravel, sand, and detritus (Pardue 1983); these can be impacted by development. Strong impacts of urbanization on lithophilic spawners are well documented and range from loss of suitable substrate, increased embeddedness, lack of bed stability, and siltation of interstitial spaces (Kemp 2014). Broadcasting species, such as Herring, could be severely affected since they neither clean substrate during spawning nor provide protection to eggs and larvae in nests (Kemp 2014). Detritus loads in subestuaries are strongly associated with development (see Section 2) and urbanization affects the quality and quantity of organic matter in streams (Paul and Meyer 2001) that feed into subestuaries. Organic matter may be positively impacted by nutrients and negatively impacted by fine sediment from agriculture (Piggot et al. 2015).

Elevated conductivity, related primarily to chloride from road salt (but including most inorganic acids and bases; APHA 1979), has emerged as an indicator of watershed development (Wenner et al. 2003; Kaushal et al. 2005; Morgan et al. 2007; Morgan et al. 2012). Use of salt as a deicer may lead to both “shock loads” of salt that may be acutely toxic to freshwater biota and elevated baselines (increased average concentrations) of chloride that have been associated with decreased fish and benthic diversity (Kaushal et al. 2005; Wheeler et al. 2005; Morgan et al. 2007; 2012). Commonly used anti-clumping agents for road salt (ferro- and ferricyanide) that are not thought to be directly toxic are of concern because they can break down into toxic cyanide under exposure to ultraviolet light. Although the degree of breakdown into cyanide in nature is unclear (Pablo et al. 1996; Transportation Research Board 2007), these compounds have been implicated in fish kills (Burdick and Lipschuetz 1950; Pablo et al. 1996; Transportation Research Board 2007). Heavy metals and phosphorous may also be associated with road salt (Transportation Research Board 2007).

At least two hypotheses can be formed to relate decreased anadromous fish spawning to conductivity and road salt use. First, eggs and larvae may die in response to sudden changes in salinity and potentially toxic amounts of associated contaminants and additives. Second, changing stream chemistry may cause disorientation of spawning adults and disrupted upstream migration. Levels of salinity associated with our conductivity measurements are very low (maximum 0.2 ppt) and anadromous fish spawn successfully in brackish water (Klauda et al. 1991; Piavis et al. 1991; Setzler-Hamilton 1991). A rapid increase might result in osmotic stress and lower survival since salinity represents osmotic cost for fish eggs and larvae (Research Council of Norway 2009).

Elevated stream conductivity may prevent anadromous fish from recognizing and ascending streams. Alewife and Blueback Herring are thought to home to natal rivers to spawn (ASMFC 2009a; ASMFC 2009b), while Yellow and White Perch populations are generally tributary-specific (Setzler-Hamilton 1991; Yellow Perch Workgroup 2002). Physiological details of spawning migration are not well described for our target species, but homing migrations in anadromous American Shad and Salmon have been connected with chemical composition, smell, and pH of spawning streams (Royce-Malmgren and Watson 1987; Dittman and Quinn 1996; Carruth et al. 2002; Leggett 2004). Conductivity is related to total dissolved solids in water (Cole 1975) which reflects chemical composition.

An unavoidable assumption of regression analyses of P_{herr}, C/ha, and summarized conductivity was that watersheds at different levels of development were a substitute for time-series. Extended time-series of watershed-specific P_{herr} were not available. Mixing physiographic provinces in this analysis had the potential to increase scatter of points, but standardizing median conductivity to background conductivity moderated the province effect in analyses with that variable. Differential changes in physical stream habitat and flow with urbanization due to differences in geographic provinces could also have influenced fits of regressions. Estimates of C/ha may have indexed these physical changes as well as water chemistry changes, while standardized conductivity would only have represented changes in water chemistry. Estimates of C/ha explained more variation in P_{herr} (58%) than standardized conductivity (29%). Liess et al. (2016) developed a stress addition model for meta-analysis of toxicants and additional stressors of aquatic vertebrates and invertebrates and found that the presence of multiple environmental stressors could amplify the effects of toxicants 100-fold. This general concept may offer an explanation for the difference in fit of P_{herr} with C/ha and median conductivity, with conductivity accounting for water quality and C/ha accounting for multiple stressors.

Application of presence-absence data in management needs to consider whether absence reflects a disappearance from suitable habitat or whether habitat sampled is not really habitat for the species in question (MacKenzie 2005). Our site occupation comparisons were based on the assumption that spawning sites detected in the 1970s were indicative of the extent of habitat. O'Dell et al. (1975; 1980) summarized spawning activity as the presence of any species group's egg, larva, or adult (latter from wire fish trap sampling) for all samples at a site and we used this criterion (spawning detected at a site or not) for a set of comparisons. Raw data for the 1970s were not available to formulate other metrics. This site-specific presence-absence approach did not detect permanent site occupation changes or an absence of change since only a small number of sites could be sampled (limited by road crossings) and the positive statistical effect of repeated visits (Strayer 1999) was lost by summarizing all samples into a single record of occurrence in a sampling season. A single year's record was available for each of the watersheds in the 1970s and we were left assuming this distribution applied over multiple years of low development.

Proportion of positive samples (P_{herr}) incorporated spatial and temporal presence-absence and provided an economical and precise alternative estimate of habitat occupation based on encounter rate. Encounter rate is readily related to the probability of detecting a population (Strayer 1999). Proportions of positive or zero catch indices were found to be robust indicators of abundance of Yellowtail Snapper *Ocyurus chrysurus* (Bannerot and Austin 1983), age-0 White Sturgeon *Acipenser transmontanus* (Counihan et al. 1999; Ward et al. 2017), Pacific Sardine *Sardinops sagax* eggs (Mangel and Smith 1990), Chesapeake Bay Striped Bass eggs (Uphoff 1997), and Longfin Inshore Squid *Loligo pealeii* fishery performance (Lange 1991).

Unfortunately, estimating reasonably precise proportions of stream samples with White or Yellow Perch eggs annually would not be logistically feasible without major changes in sampling priorities. Estimates for Yellow or White Perch stream spawning would require more frequent sampling to obtain precision similar to that attained by P_{herr} since spawning occurred at fewer sites. Given staff and volunteer time limitations, this would not be possible within our current scope of operations. In Mattawoman Creek, it was possible to pool data across years to increase precision of estimates of proportions of samples with White Perch eggs and larvae (sites MC1 and MC2) or Yellow Perch larvae (MC1) for 1989-1991 collections to compare with 2008-2016 collections at the same combinations of sites. These estimates did not indicate a loss in stream spawning in these downstream sites.

Volunteer-based sampling of stream spawning during 2005-2016 used only stream drift nets, while O'Dell et al. (1975; 1980) and Hall et al. (1992) determined spawning activity with ichthyoplankton nets and wire traps for adults. Tabular summaries of egg, larval, and adult catches in Hall et al. (1992) allowed for a comparison of how site use in Mattawoman Creek might have varied in 1991 with and without adult wire trap sampling. Sites estimated when eggs and-or larvae were present in one or more samples were identical to those when adults present in wire traps were included with the ichthyoplankton data (Hall et al. 1992). Similar results were obtained from the Bush River during 2006 at sites where ichthyoplankton drift nets and wire traps were used; adults were captured by traps at one site and eggs and-or larvae at nine sites with ichthyoplankton nets (Uphoff et al. 2007). Wire traps set in the Bush River during 2007 did not indicate different results than ichthyoplankton sampling for Herring and Yellow Perch, but White Perch adults were observed in two trap samples and not in plankton drift nets (Uphoff et al. 2008). These comparisons of trap and ichthyoplankton sampling indicated it was unlikely that an absence of adult wire trap sampling would impact interpretation of spawning sites when multiple years of data were available.

The different method used to collect ichthyoplankton in Mattawoman Creek during 1991 could bias that estimate of P_{herr}, although presence-absence data tend to be robust to errors and biases in sampling (Green 1979; Uphoff 1997). Removal of 1991 data lowered the fit between C/ha and P_{herr} (from $r^2 = 0.58$, $P = <.0001$ to $r^2 = 0.56$, $P = <.0001$), but did not alter the negative relationship (95% CI's of slopes and intercepts of both models overlapped).

Absence of detectable stream spawning does not necessarily indicate an absence of spawning in the estuarine portion of these systems. Estuarine Yellow Perch presence-absence surveys in Mattawoman and Piscataway Creeks, and Bush River did not indicate that lack of detectable stream spawning corresponded to their elimination from these subestuaries. Yellow

Perch larvae were present in upper reaches of both subestuaries, (see Section 2). Yellow Perch do not appear to be dependent on non-tidal stream spawning, but their use may confer benefit to the population through expanded spawning habitat diversity. Stream spawning is very important to Yellow Perch anglers since it provides access for shore fisherman and most recreational harvest probably occurs during spawning season (Yellow Perch Workgroup 2002).

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Table 1-1. Summary of subestuaries and their watershed size, Department of Planning (DOP) land use designation and estimates of land use types, and level of development (C/ha) during years sampled. DOP Year = the year DOP estimated land use that best matches sample year.

River	Sample Year	DOP Year	C / ha	% Ag	% Forest	Watershed Size (ha)	Primary Land Use
Bush (w/o APG)	2005	2002	1.37	25.4	35	36,038	Urban
Bush (w/o APG)	2006	2002	1.41	25.4	35		
Bush (w/o APG)	2007	2010	1.43	18	29.9		
Bush (w/o APG)	2008	2010	1.45	18	29.9		
Bush (w/o APG)	2014	2010	1.51	18	29.9		
Choptank	2016	2010	0.18	55	27.8	38,216	Agriculture
Deer	2012	2010	0.24	44.6	28.4	37,697	Agriculture
Deer	2013	2010	0.24	44.6	28.4		
Deer	2014	2010	0.24	44.6	28.4		
Deer	2015	2010	0.24	44.6	28.4		
Mattawoman	1991	1994	0.46	13.8	62.6	24,441	Forest
Mattawoman	2008	2010	0.87	9.3	53.9		
Mattawoman	2009	2010	0.88	9.3	53.9		
Mattawoman	2010	2010	0.90	9.3	53.9		
Mattawoman	2011	2010	0.91	9.3	53.9		
Mattawoman	2012	2010	0.90	9.3	53.9		
Mattawoman	2013	2010	0.91	9.3	53.9		
Mattawoman	2014	2010	0.93	9.3	53.9		
Mattawoman	2015	2010	0.93	9.3	53.9		
Mattawoman	2016	2010	0.93	9.3	53.9		
Piscataway	2008	2010	1.41	10	40.4	17,642	Urban
Piscataway	2009	2010	1.43	10	40.4		
Piscataway	2012	2010	1.47	10	40.4		
Piscataway	2013	2010	1.49	10	40.4		
Piscataway	2014	2010	1.50	10	40.4		
Tuckahoe	2016	2010	0.07	66.6	25.4	39,388	Agriculture

Table 1-2. Summary of subestuary watersheds sampled, years sampled, number of sites sampled, first and last dates of sampling, and stream ichthyoplankton sample sizes (N).

Subestuary	Year	Number of Sites	1st Sampling Date	Last Sampling Date	Number of Dates	N
Bush	2005	13	18-Mar	15-May	16	99
Bush	2006	13	18-Mar	15-May	20	114
Bush	2007	14	21-Mar	13-May	17	83
Bush	2008	12	22-Mar	26-Apr	17	77
Bush	2014	6	22-Mar	1-Jun	10	60
Choptank	2016	12	17-Mar	18-May	10	101
Deer	2012	4	20-Mar	7-May	11	44
Deer	2013	5	19-Mar	23-May	19	87
Deer	2014	5	2-Apr	28-May	12	60
Deer	2015	5	23-Mar	26-May	15	75
Mattawoman	2008	9	8-Mar	9-May	10	90
Mattawoman	2009	9	8-Mar	11-May	10	70
Mattawoman	2010	7	7-Mar	15-May	11	75
Mattawoman	2011	7	5-Mar	15-May	14	73
Mattawoman	2012	7	4-Mar	13-May	11	75
Mattawoman	2013	7	10-Mar	25-May	12	80
Mattawoman	2014	8	9-Mar	25-May	12	87
Mattawoman	2015	7	15-Mar	24-May	11	60
Mattawoman	2016	5	13-Mar	22-May	11	55
Piscataway	2008	5	17-Mar	4-May	8	39
Piscataway	2009	6	9-Mar	14-May	11	60
Piscataway	2012	5	5-Mar	16-May	11	55
Piscataway	2013	5	11-Mar	28-May	11	55
Piscataway	2014	5	10-Mar	1-Jun	9	45
Tuckahoe	2016	10	16-Mar	16-May	12	97

Table 1-3. Summary of historical conductivity sampling in non-tidal Mattawoman Creek. RKM = site location in river kilometers from the mouth; Months = months when samples were drawn; Sum = sum of samples for all years.

RKM	Months	Sum	Years Sampled
12.4	1 to 12	218	1971, 1974-1989
18.1	4 to 9	8	1974
27	4 to 9	9	1970, 1974
30	8 and 9	2	1970
34.9	4 to 9	9	1970, 1974
38.8	8 and 9	2	1970

Table 1-4. Summary statistics of conductivity (µS/cm) for mainstem stations in Mattawoman, Piscataway, Deer, and Tuckahoe Creeks, and Bush and Choptank Rivers during 2005-2016. Unnamed tributaries were excluded from analysis. Tinkers Creek was included with mainstem stations in Piscataway Creek.

	Year											
Conductivity	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
						Bush						
Mean	269	206	263	237						276.7		
Standard Error	25	5	16	6						15		
Median	230	208	219	234						253.4		
Kurtosis	38	2	22	7						3.16		
Skewness	6	-1	4	0						1.56		
Range	1861	321	1083	425						606		
Minimum	79	0	105	10						107		
Maximum	1940	321	1187	435						713		
Count	81	106	79	77						60		
						Choptank						
Mean												130.7
Standard Error												1.4
Median												133.2
Kurtosis												2.41
Skewness												-1.07
Range												89
Minimum												74
Maximum												163
Count												101
						Deer						
Mean								174.9	175.6	170.3	191.8	
Standard Error								1.02	1.5	1.4	0.9	
Median								176.8	177.7	171.7	193.5	
Kurtosis								17.22	13.88	9.21	7.43	
Skewness								-3.78	-2.25	-2.42	-1.97	
Range								39.3	122	66	51	
Minimum								140.2	93	116	156	
Maximum								179.5	215	183	207	
Count								44	87	60	75	
						Mattawoman						
Mean				120.1	244.5	153.7	147.5	128.9	126.1	179.4	181.8	180.3
Standard Error				3.8	19.2	38	2.8	1.9	2.4	9.1	6.5	4.1
Median				124.6	211	152.3	147.3	130.9	126.5	165.8	172.5	188.8
Kurtosis				2.1	1.41	1.3	8.29	-0.26	5.01	0.33	1.49	-0.80
Skewness				-1.41	1.37	0.03	1.72	-0.67	-1.70	1.00	1.33	-0.68
Range				102	495	111	117	49	96	261	185	93
Minimum				47	115	99	109	102	63	88	130	121

Maximum	148	610	210	225	151	158	350	315	214
Count	39	40	43	44	44	48	48	44	44

Table 1-4 cont.

	Year											
Conductivity	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Piscataway												
Mean				218.4	305.4			211.4	245	249.4		
Standard Error				7.4	19.4			5.9	6.9	11.1		
Median				210.4	260.6			195.1	238.4	230		
Kurtosis				-0.38	1.85			0.11	-0.29	2.56		
Skewness				0.75	1.32			0.92	0.73	1.50		
Range				138	641			163	173	274		
Minimum				163	97			145	181	174		
Maximum				301	737			308	354	449		
Count				29	50			44	44	36		
Tuckahoe												
Mean												152.2
Standard Error												2.4
Median												159.6
Kurtosis												-0.29
Skewness												-0.68
Range												103
Minimum												85
Maximum												188
Count												97

Table 1-5. Site-specific presence-absence of Herring (Blueback Herring, Hickory Shad, and Alewife), White Perch, and Yellow Perch stream spawning in Mattawoman Creek during 1971, 1989-1991, and 2008-2016. 0 = site sampled, but spawning not detected; 1 = site sampled, spawning detected; and blank indicates no sample. Station locations are identified on Figure 1-2.

	Year												
Station	1971	1989	1990	1991	2008	2009	2010	2011	2012	2013	2014	2015	2016
							Herring						
MC1	1	1	1	1	1	1	1	1	1	1	1	1	1
MC2	1	1	1	1	0	0	1	1	1	1	1	1	1
MC3	1			1	1	1	1	1	1	1	1	1	1
MC4	1			1	0	0	1	1	1	1	1	1	1
MUT3	1				0	0	0	1	1	1	1	1	1
MUT4							0	0	1	0	0	0	
MUT5	1				1	0	0	0	0	0	1	0	
							White Perch						
MC1	1	1	1	1	1	0	1	0	0	1	1	1	1
MC2	0	0	1	0	0	0	0	0	0	1	1	0	1
MC3	1			0	0	0	0	0	0	0	0	0	1
							Yellow Perch						
MC1	1	1	1	1	1	0	1	1	0	1	1	1	1

Table 1-6. Site-specific presence-absence of Herring (Blueback Herring, Hickory and American Shad, and Alewife) and White Perch spawning in Piscataway Creek during 1971, 2008-2009, and 2012-2014. 0 = site sampled, but spawning not detected; 1 = site sampled, spawning detected; and blank indicates no sample. Station locations are identified on Figure 1-3.

	Year					
Station	1971	2008	2009	2012	2013	2014
			Herring			
PC1	1	0	0	1	1	1
PC2	1	0	1	1	1	1
PC3	1	0	0	1	1	1
PTC1	1	0	0	1	1	0
PUT4	1		0	0	0	0
			White Perch			
PC1	1	0	0	0	0	1
PC2	1	0	0	0	0	0

Table 1-7. Site-specific presence-absence of Herring (Blueback Herring, Hickory Shad, and Alewife), White Perch, and Yellow Perch spawning in Bush River streams during 1973, 2005-2008, and 2014. 0 = site sampled, but spawning not detected; 1 = site sampled, spawning detected; and blank indicates no sample. Station locations are identified on Figure 1-4.

	Year					
Station	1973	2005	2006	2007	2008	2014
			Herring			
BBR1	0	1	1	1	1	1
BCR1	1	0	0	1	0	1
BHH1	0	0	1	1	1	1
BJR1	0	1	1	1	0	1
BOP1	1	1	1	1	1	1
BWR1	1	0	0	1	0	1
			White Perch			
BBR1	1	0	0	0	0	1
BCR1	1	0	0	0	0	1
BHH1	0	0	0	0	0	0
BJR1	0	0	0	0	0	0
BOP1	1	0	0	1	0	1
BWR1	1	0	0	0	0	0
			Yellow Perch			
BBR1	1	0	0	0	0	0
BCR1	0	0	0	0	0	1
BHH1	0	0	0	0	0	1
BJR1	1	0	0	0	0	1
BOP1	0	0	0	0	0	0
BWR1	1	0	1	0	0	0

Table 1-8. Site-specific presence-absence of Herring (Blueback Herring, Hickory Shad, and Alewife), White Perch, and Yellow Perch stream spawning in Deer Creek during 1972 and 2012-2015. 0 = site sampled, but spawning not detected; 1 = site sampled, spawning detected; and blank indicates no sample. Station locations are identified on Figure 1-5.

	Year				
Station	1972	2012	2013	2014	2015
			Herring		
SU01	1	1	1	1	1
SU02		1	1	1	1
SU03		1	1	1	1
SU04	1	1	1	1	1
SU05	0		1	1	1
			White Perch		
SU01	1	0	1	1	1
SU02		0	1	0	1
SU03		0	0	1	0
SU04	0	0	1	1	0
SU05	0		0	0	0
			Yellow Perch		
SU01	1	1	0	1	0
SU02		1	0	1	0
SU03		0	0	1	0
SU04	0	0	0	0	0
SU05	0		0	0	0

Table 1-9. Site-specific presence-absence of Herring (Blueback Herring, Hickory Shad, and Alewife), White Perch, and Yellow Perch stream spawning in Choptank River during 2016. 0 = site sampled, but spawning not detected; 1 = site sampled, spawning detected; and blank indicates no sample. Station locations are identified on Figure 1-6.

	Year		
Station	2016		
	Herring	White Perch	Yellow Perch
CH100	1	1	1
CH101	1	1	1
CH102	1	1	1
CH103	1	1	1
CH104	1	1	1
CH105	1	1	1
CH106	1	1	1
CH107	1	1	0
CH108	1	1	0
CH109	1	1	1
CH110	1	0	0
CH111	0	0	0

Table 1-10. Site-specific presence-absence of Herring (Blueback Herring, Hickory Shad, and Alewife), White Perch, and Yellow Perch stream spawning in Tuckahoe Creek during 1976-77 and 2016. 0 = site sampled, but spawning not detected; 1 = site sampled, spawning detected; and blank indicates no sample. Station locations are identified on Figure 1-7.

	Year	
Station	1976-77	2016
	Herring	
TUC101	1	1
TUC102	1	1
TUC103	1	1
TUC104		1
TUC105		1
TUC106		1
TUC107		1
TUC108	0	1
TUC109		1
TUC110		0
	White Perch	
TUC101	1	1
TUC102	1	1
TUC103	1	1
TUC104		1
TUC105		1
TUC106		1
TUC107		1
TUC108	1	1
TUC109		0
TUC110		0
	Yellow Perch	
TUC101	1	1
TUC102	1	1
TUC103	1	1
TUC104		1
TUC105		1
TUC106		1
TUC107		1
TUC108	0	0
TUC109		0
TUC110		0

Table 1-11. Summary of best regression models for standardized conductivity (annual median/province background) versus development level (C/ha), proportion of samples with Herring eggs or larvae (P_{herr}) versus C/ha, and P_{herr} versus standardized conductivity.

Linear Model	Standardized conductivity = Structure density (C/ha)					
ANOVA	df	SS	MS	F	P	
Regression	1	1.22592	1.22592	16.02	0.0006	
Residual	23	1.75976	0.07651			
Total	24	2.98567				
$r^2 = 0.4106$						
	Estimate	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	1.10691	0.12193	9.08	<.0001	0.85467	1.35915
C / ha	0.45612	0.11395	4.00	0.0006	0.22040	0.69184

Linear Model	Proportion of samples with Herring eggs or larvae (P_{herr}) = Structure density (C/ha)					
ANOVA	df	SS	MS	F	P	
Regression	1	1.16925	1.16925	32.74	<.0001	
Residual	24	0.85720	0.03572			
Total	25	2.02645				
$r^2 = 0.5770$						
	Estimate	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.84233	0.08041	10.48	<.0001	0.67637	1.00829
C / ha	-0.43685	0.07635	-5.72	<.0001	-0.59443	-0.27927

Linear Model	Proportion of samples with Herring eggs or larvae (P_{herr}) = Standardized conductivity					
ANOVA	df	SS	MS	F	P	
Regression	1	0.56843	0.56843	9.41	0.0055	
Residual	23	1.38989	0.06043			
Total	24	1.95831				
$r^2 = 0.2903$						
	Estimate	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	1.09656	0.22480	4.88	<.0001	0.63153	1.56159
Standardized Conductivity	-0.43633	0.14227	-3.07	0.0055	-0.73063	-0.14203

Figure 1-1. Watersheds sampled for stream spawning anadromous fish eggs and larvae during 2005-2016. Coastal Plain and Piedmont Regions are indicated.

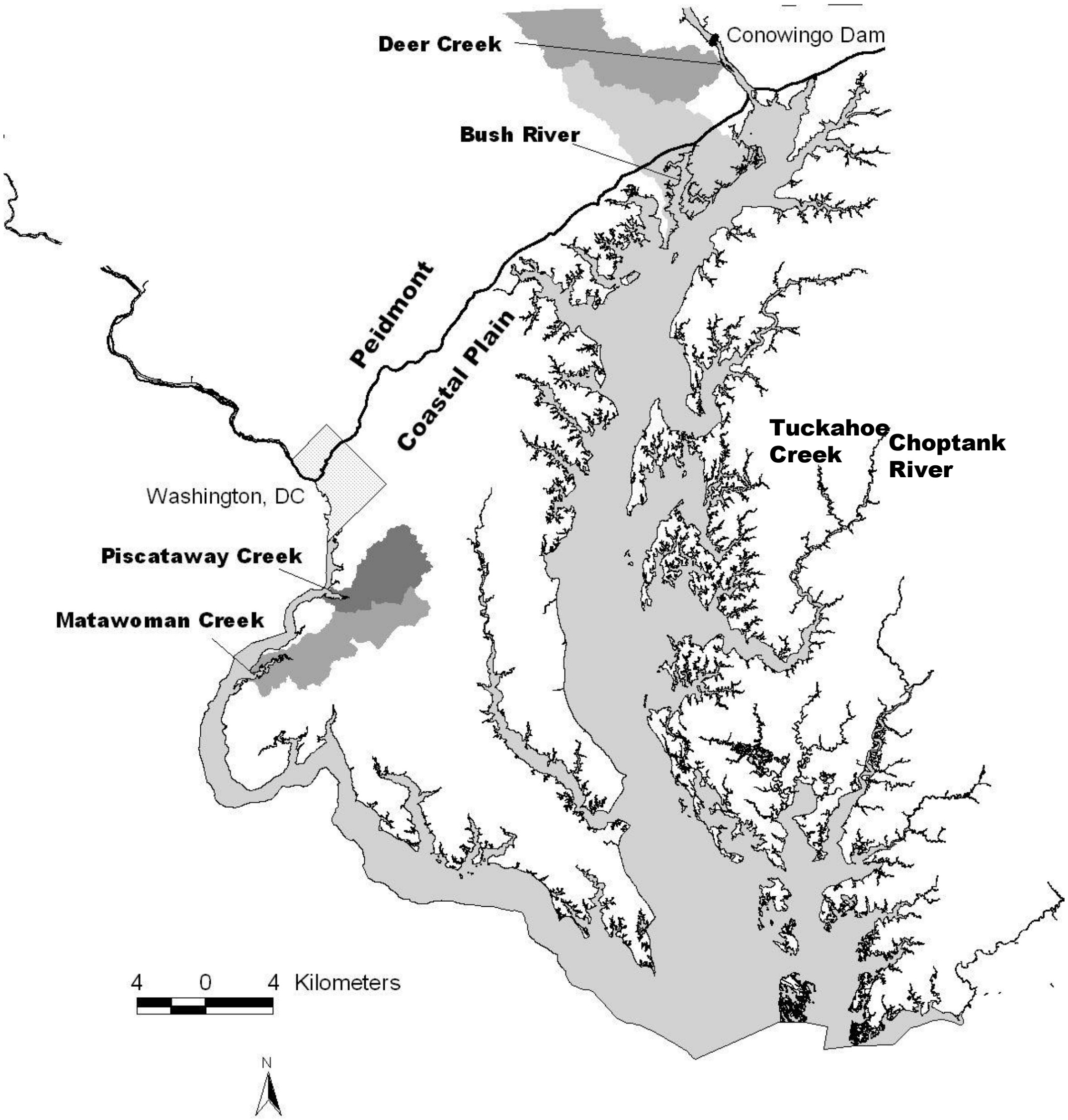


Figure 1-2. Mattawoman Creek's 1971 and 2008-2016 sampling stations. Bar approximates lower limit of development associated with the town of Waldorf.

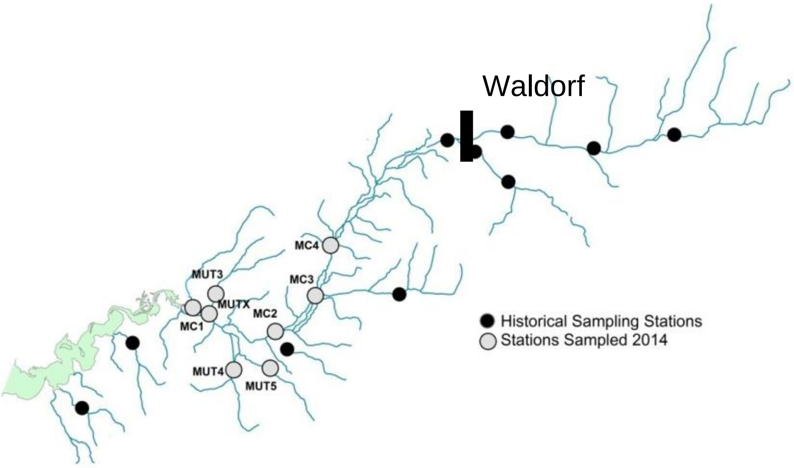


Figure 1-3. Piscataway Creek's 1971, 2008-2009, and 2012-2014 sampling stations.

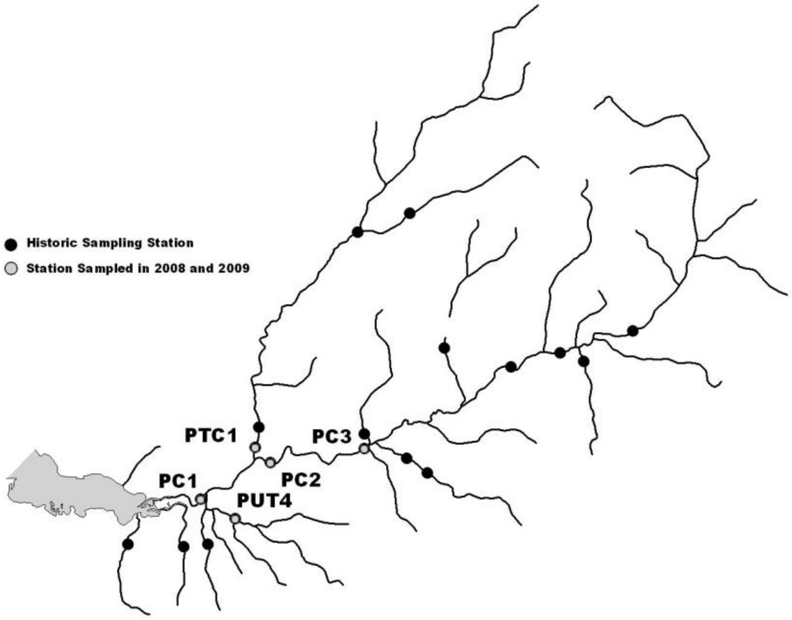


Figure 1-4. Bush River's 1973, 2005-2008, and 2014 sampling stations. Stations in Aberdeen Proving Grounds (APG) have been separated from other Bush River stations. Line delineates APG streams that were excluded.

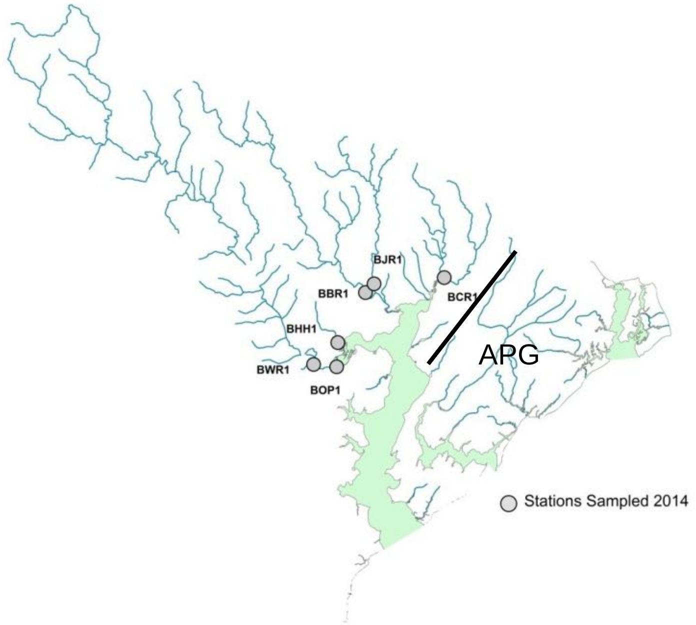


Figure 1-5. Deer Creek's 1972 and 2012-2015 sampling stations.

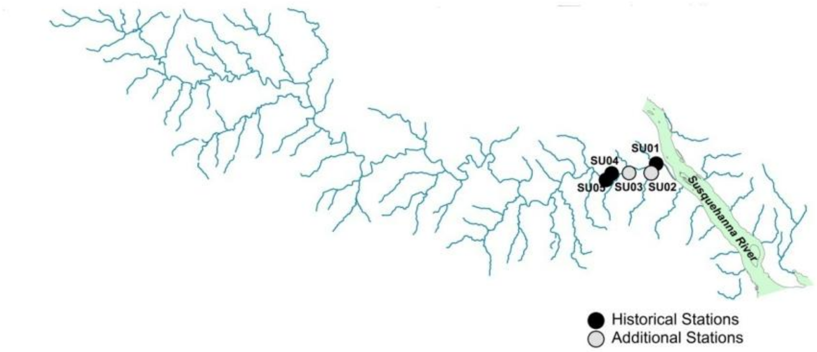


Figure 1-6. Choptank River’s 2016 sampling stations and their location in the watershed.

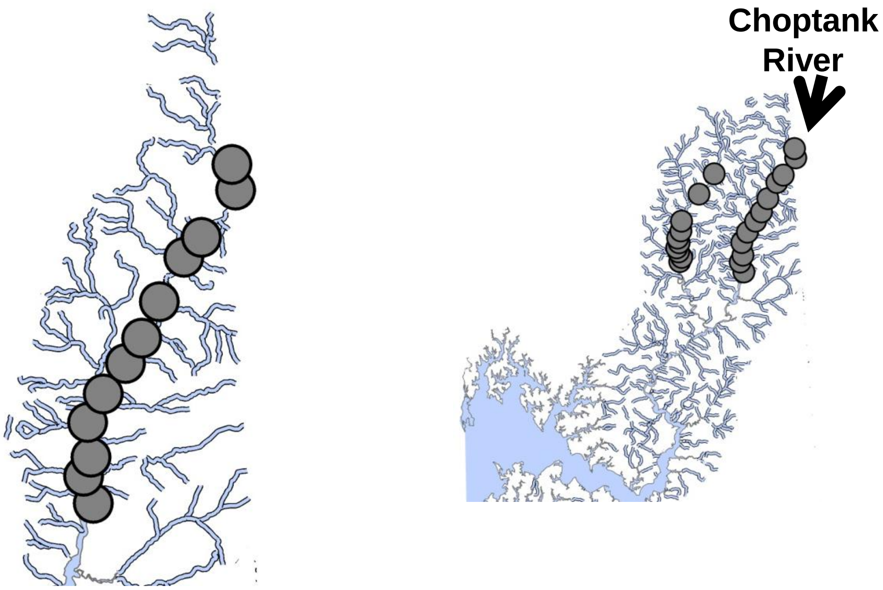


Figure 1-7. Tuckahoe Creek’s 2016 sampling stations and their location within the Choptank River watershed.

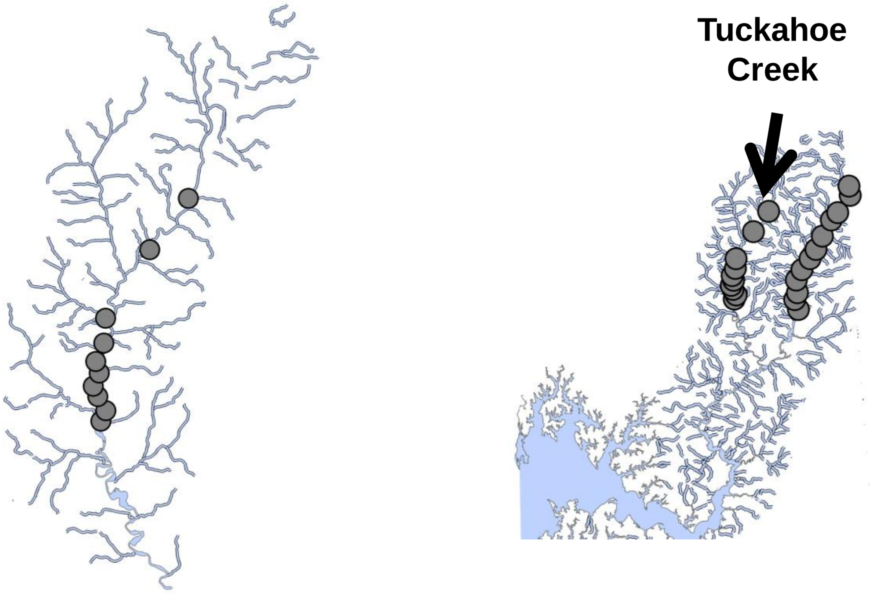


Figure 1-8. Trends in counts of structures per hectare (C/ha) during 1950-2016 in Mattawoman, Piscataway, and Deer Creeks, the Bush River, and the Choptank River drainage watersheds. Updated estimates of C/ha were not available for 2015 or 2016. Large symbols indicate years when stream ichthyoplankton was sampled.

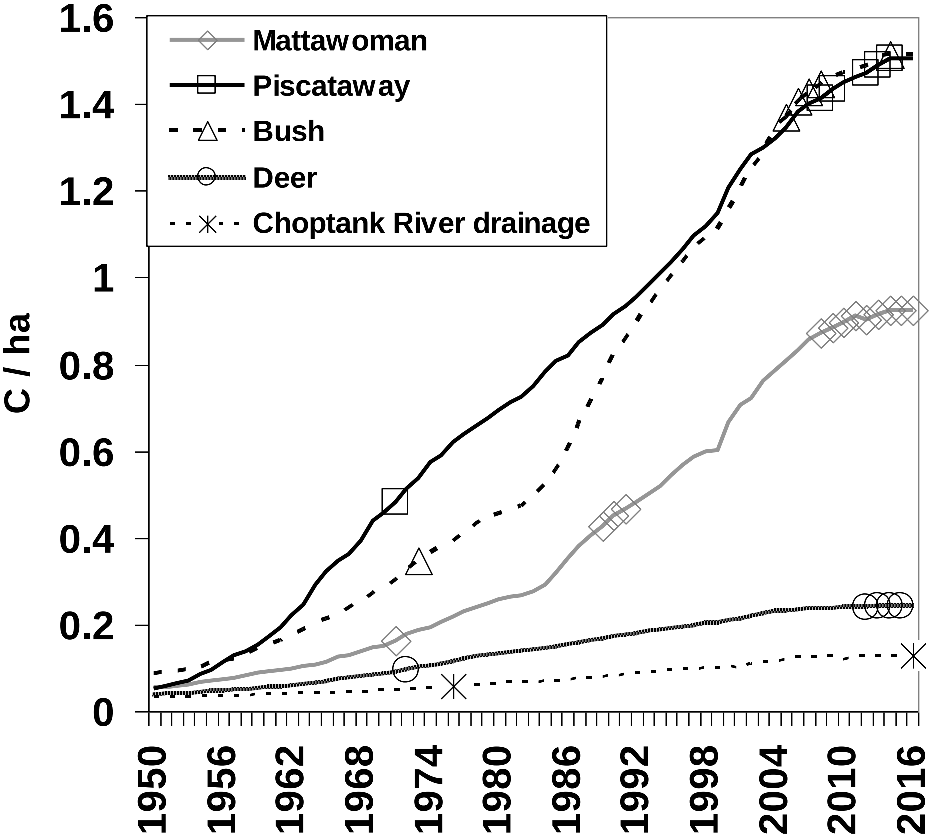


Figure 1-9. Stream conductivity measurements (µS/cm), by station and date, in Mattawoman Creek during (A) 2009, (B) 2010, (C) 2011, (D) 2012, (E) 2013, (F) 2014, (G) 2015, and (H) 2016. Lines indicate conductivity range measured at mainstem sites (MC1 – MC4) during 1991 by Hall et al. (1992).

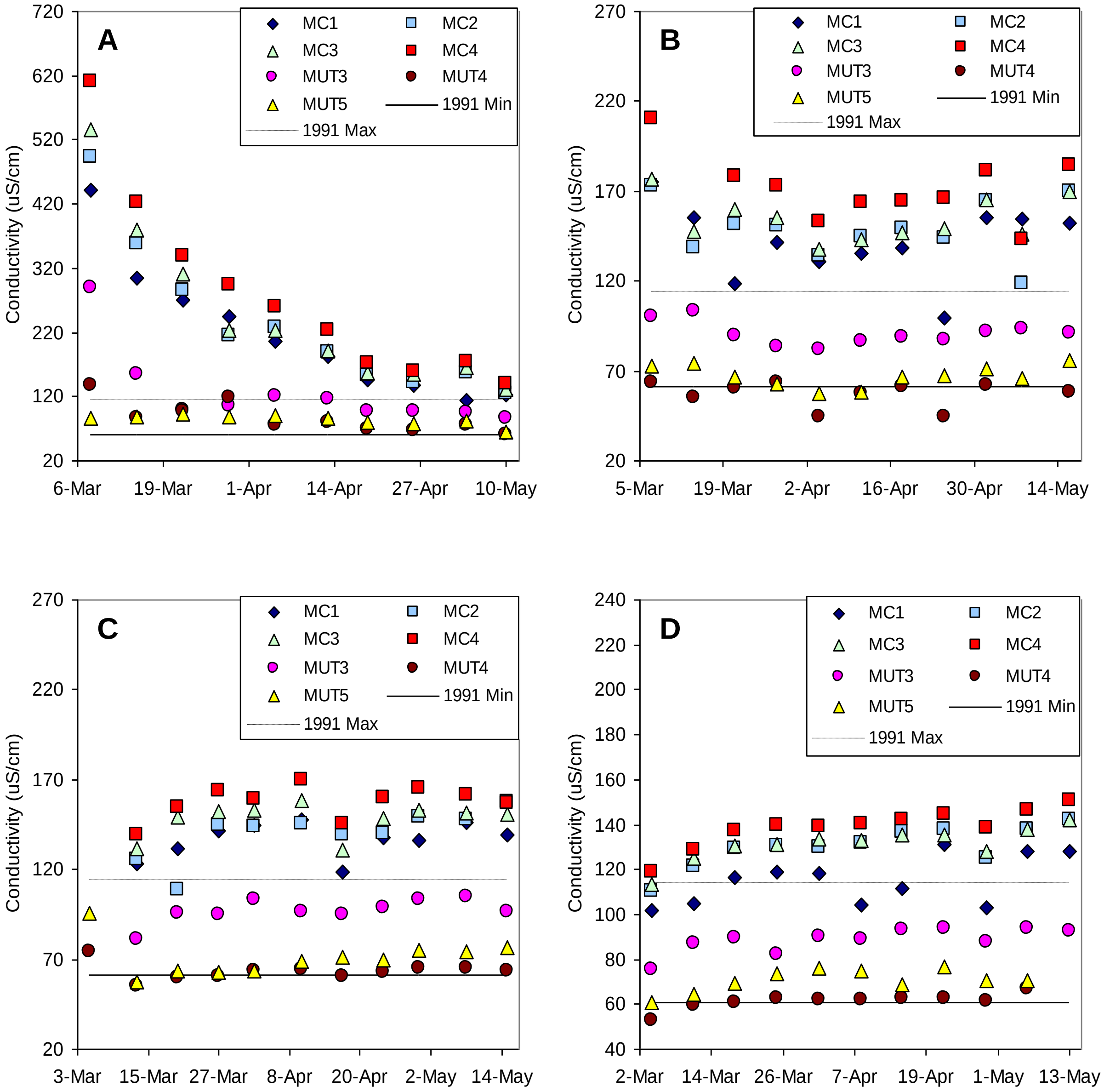


Figure 1-9 cont.

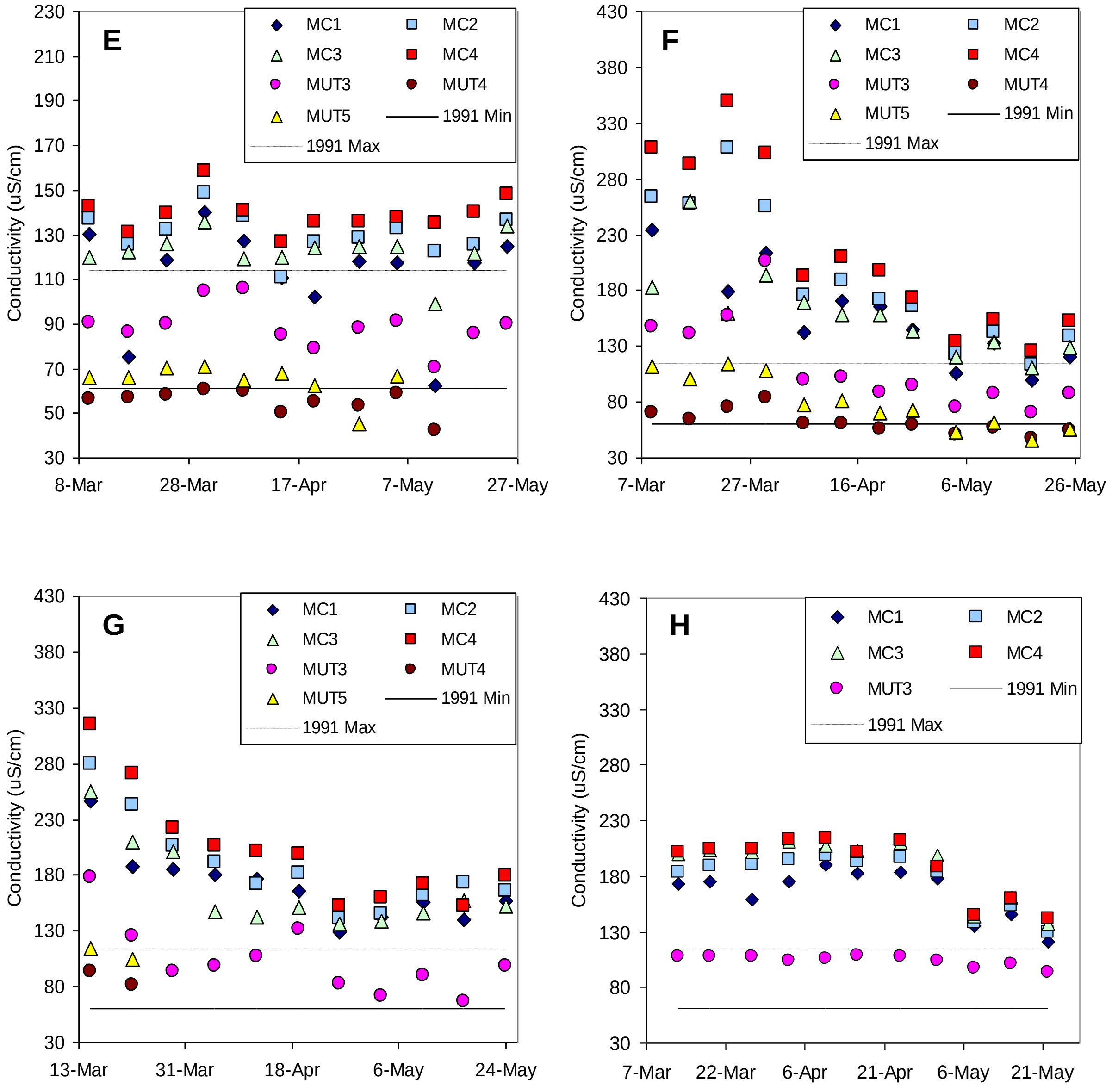


Figure 1-10. Historical (1970-1989) median conductivity measurements and current (2008-2016) anadromous spawning survey median conductivity in non-tidal Mattawoman Creek (between the junction with the subestuary and Waldorf) plotted against distance from the mouth. The two stations furthest upstream are nearest Waldorf. Median conductivity was measured during March-May, 2008-2016, and varying time periods (see Table 1-2) during 1970-1989.

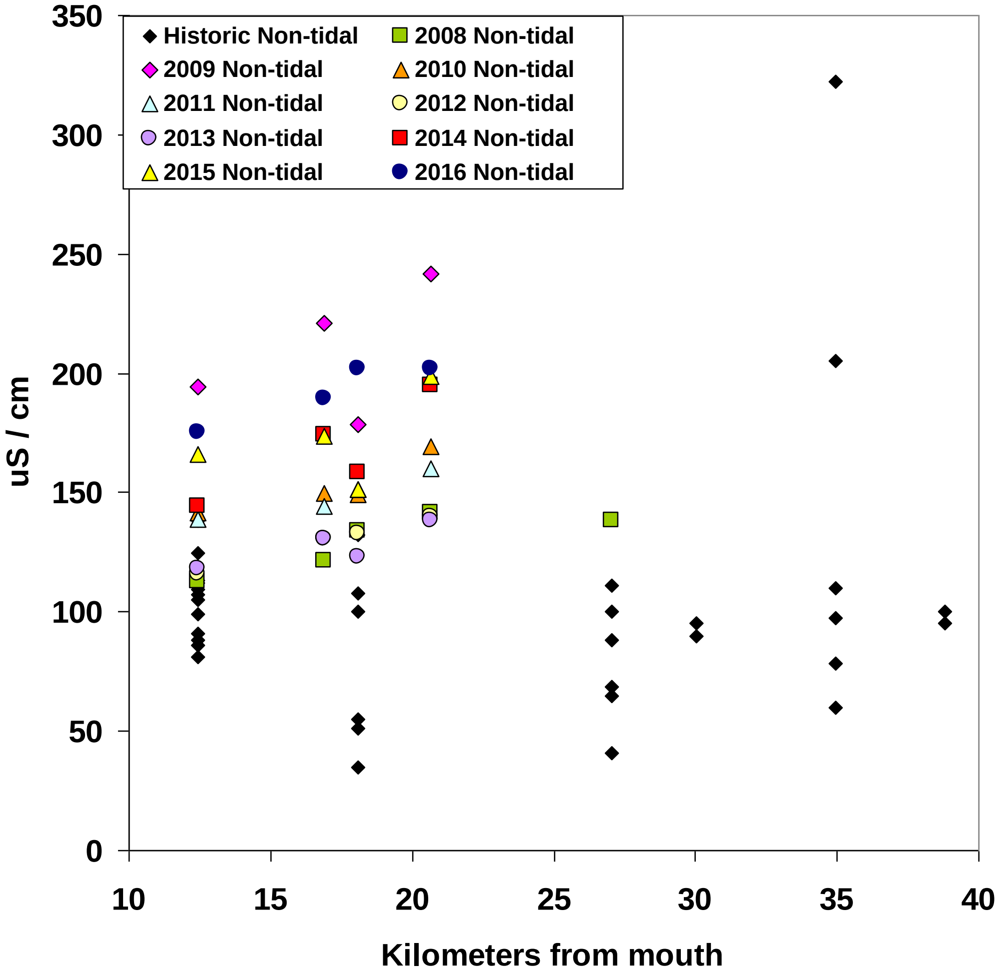


Figure 1-11. Proportion of samples (P_{herr}) with Herring and its 90% confidence interval for stream ichthyoplankton surveys in Mattawoman, Piscataway, Deer, and Tuckahoe Creeks, and Bush and Choptank Rivers.

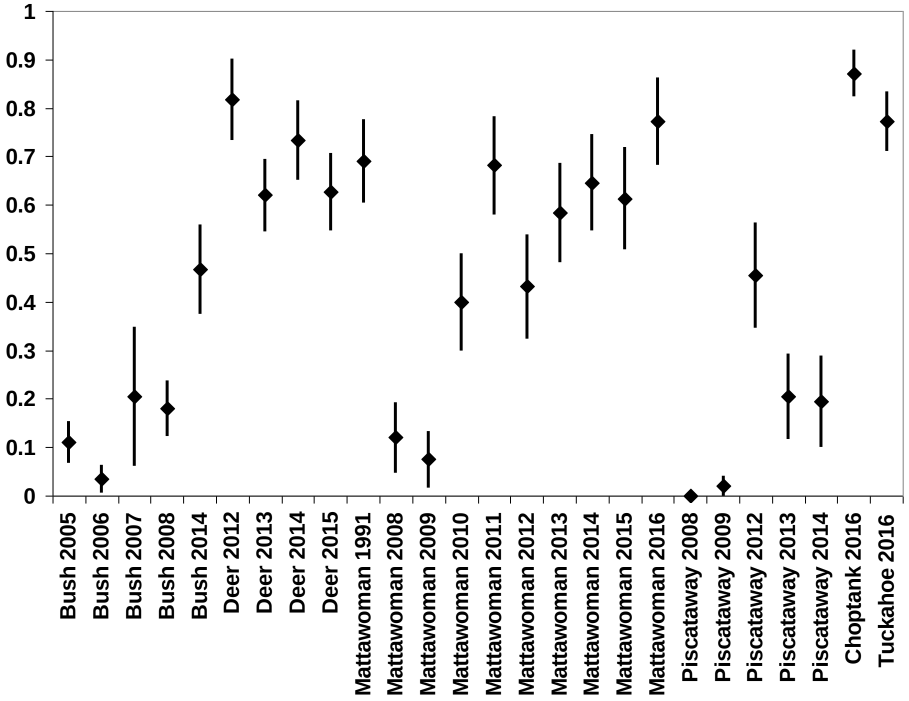


Figure 1-12. Mattawoman data pooled across years to form estimates of proportions of samples with White Perch (WP) eggs and-or larvae (sites MC1 and MC2) or Yellow Perch (YP) eggs and-or larvae (MC1) for 1989-1991 collections compared to 2008-2010, 2011-2013, and 2014-2016 collections at the same combination of sites.

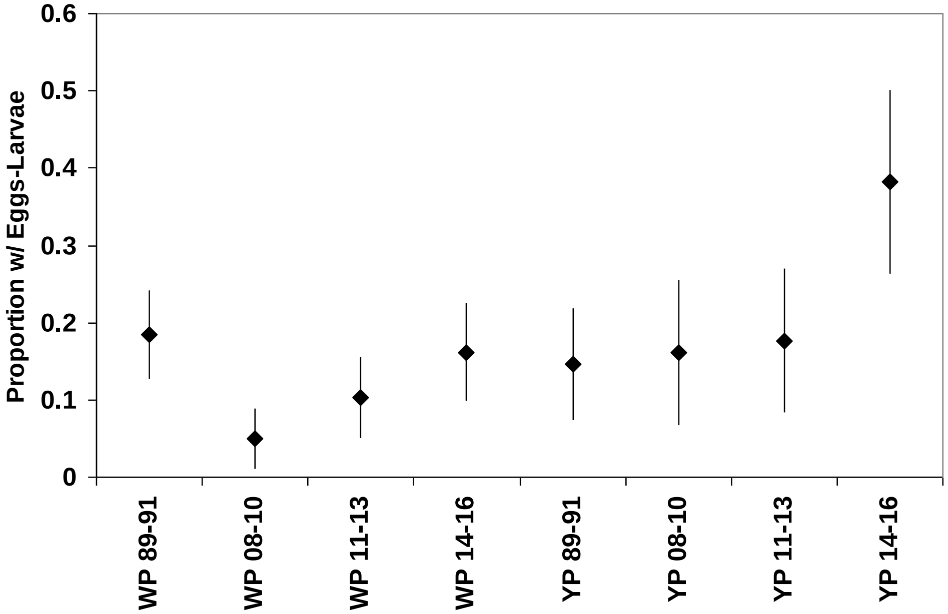


Figure 1-13. Standardized median conductivity during spring spawning surveys and level of development (C/ha). Median conductivity was standardized to background estimates for Coastal Plain and Piedmont regions based on estimates in Morgan et al. (2012).

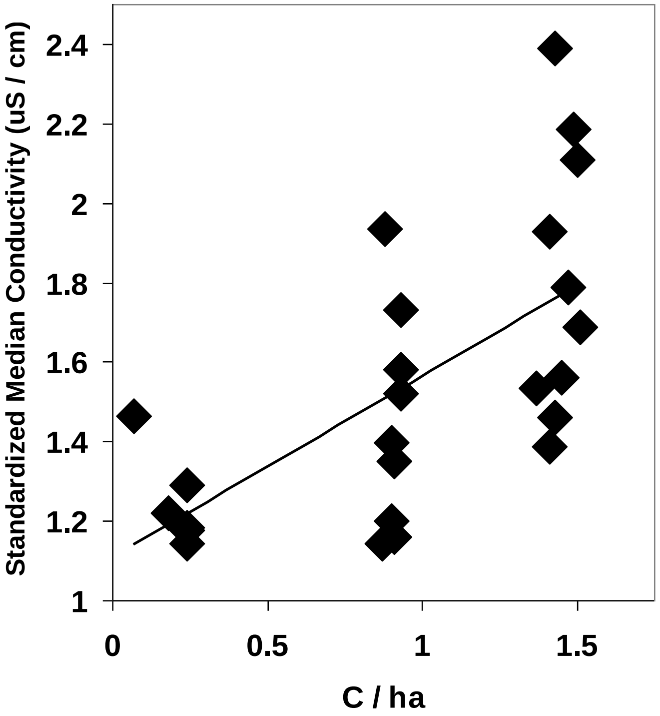
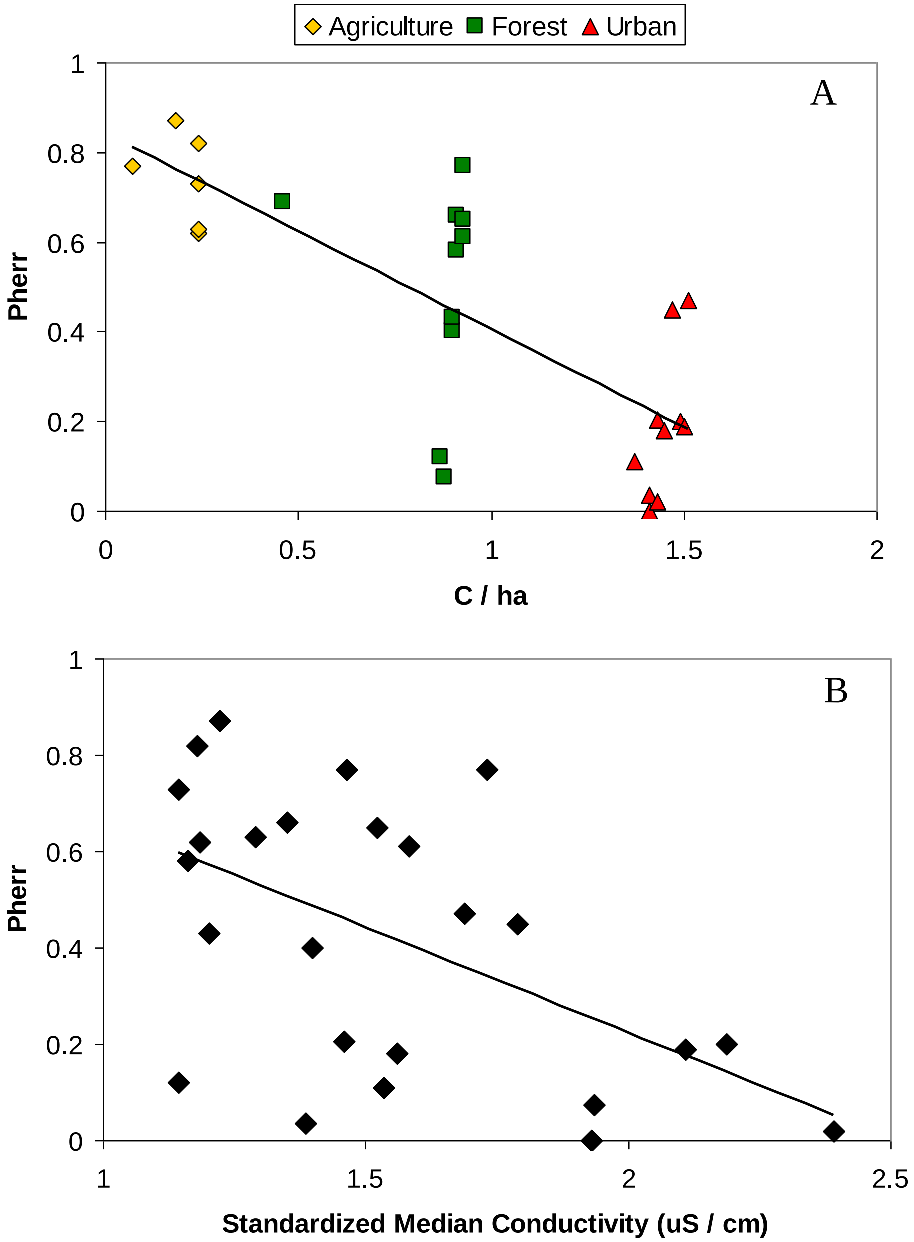


Figure 1-14. (A) Proportion of stream samples with Herring eggs and-or larvae (P_{herr}) and level of development (C/ha) with Department of Planning land use designations. (B) P_{herr} and standardized median spawning survey conductivity (uS/cm). Median conductivity was standardized to background estimates for Coastal Plain and Piedmont regions based on estimates in Morgan et al. (2012).



Section 2: Estuarine Yellow Perch Larval Presence-Absence Sampling

Carrie Hoover, Alexis Park, Jim Uphoff, Margaret McGinty, and Erik Yetter

Introduction

Annual L_p, the proportion of tows with Yellow Perch larvae during a standard time period and where larvae would be expected, provides a cost-effective measure of the product of egg production and survival through the early postlarval stage. Presence-absence sampling for Yellow Perch larvae in 2016 was conducted in the upper tidal reaches of the Choptank, Nanticoke, Magothy, and Patuxent rivers, and in Mattawoman Creek. Sampling started the last week of March in the Choptank and Patuxent rivers, and the first week of April in the Nanticoke River and Mattawoman Creek. Sampling continued through the end of April (Figure 2-1). Sampling in the Magothy River started the fourth week of March and continued through the second week in April. In 2016 we used regression and correlation analyses to examine associations and relationships among land use types (development, agriculture, forest, and wetlands), L_p, and organic matter availability.

We also examined a hypothesis that watershed development and wetland coverage impacted related organic matter (OM) dynamics, altering zooplankton production important for Yellow Perch larval feeding success and survival (the OM hypothesis). Urbanization was expected to negatively impact Yellow Perch larval feeding success because it affects quality and quantity of OM in streams (Paul and Meyer 2001), and was negatively associated with extent of wetlands in Chesapeake Bay subestuary watersheds (Uphoff et al. 2011). Riparian zones and floodplains that are sources of OM become disconnected from stream channels by stormwater management in suburban and urban watersheds (Craig et al. 2008; Kaushal et al. 2008; Elmore and Kaushal 2008; Brush 2009; NRC 2009), altering quantity and transport of OM (Paul and Meyer 2001; McClain et al. 2003; Stanley et al. 2012).

We used the empirical-statistical approach recommended by Austin and Ingham (1978) and Crecco and Savoy (1984) for resolving the effects of environment on fish recruitment. This approach offers a working hypothesis that is tested for validity with empirical data and a thorough statistical analysis. Shortage of appropriate food has been frequently hypothesized to cause high mortality of fish larvae (Martin et al. 1985; Miller et al. 1988; Heath 1992). Years of high spring discharge favor anadromous fish recruitment in Chesapeake Bay (Hoffman et al. 2007; Martino and Houde 2010) and may represent episodes of hydrologic transport of accumulated OM from watersheds that fuel zooplankton production and feeding success (McClain et al. 2003). Under natural conditions, riparian marshes and forests would provide OM subsidies in high discharge years, while phytoplankton would be a greater source of OM in years of lesser flow (Hoffman et al. 2007).

During 2012-2016, Yellow Perch were collected for analysis of the ratio of ribonucleic acid (RNA) concentration to deoxyribonucleic acid (DNA) concentration in body tissue (RNA/DNA ratio) to further explore the OM hypothesis. Samples were gathered from Choptank River and Patuxent River during 2016, two subestuaries of Chesapeake Bay with watersheds exhibiting rural (C/ha = 0.13) and suburban (C/ha = 1.22) levels of development, respectively. Tardif et al. (2005) used RNA/DNA ratios of Yellow Perch larvae and juveniles to determine differences in productivity of managed and natural wetlands of Lake St. Pierre, Canada.

We expected RNA/DNA ratios to decline with increased development and our hope was to develop RNA/DNA as an easy to apply metric for detecting effects of development on larval

Yellow Perch habitat. The quantity of DNA within a cell is constant within a species while the quantity of RNA varies with protein synthesis (Tardif et al. 2005). Since growth is a function of protein synthesis, RNA/DNA ratios provide a sensitive indicator of recent growth at any given time (Buckley 1984). This ratio is a useful indicator of nutritional status and somatic growth in larval fish (Buckley 1984) that provides a method for examining connections of feeding success and larval condition (Buckley 1984; Martin et al. 1985; Wright and Martin 1985; Clemmesen 1994; Blom et al. 1997) without requiring extensive sampling and sample processing needed to measure mortality directly.

We wanted to see if RNA/DNA could be linked to the OM hypothesis and compared variables linked to feeding success with RNA/DNA metrics. We expected feeding success of early Yellow Perch postlarvae examined for the OM hypothesis would be easily linked to RNA/DNA dynamics. We could also explore possible maternal effects by comparing the average amount of DNA present in larvae that had just begun feeding. Heyer et al. (2001) found that maternal effects were expressed in offspring of Yellow Perch in Lake Michigan as DNA amount.

Methods

Conical plankton nets were towed from boats in upper portions of subestuaries to collect Yellow Perch larvae. Nets were 0.5-m in diameter, 1.0-m long, and constructed of 0.5 mm mesh. Nets were towed with the current for two minutes at a speed that maintained the net near the surface (approximately 2.8 km per hour). Temperature, dissolved oxygen, conductivity, and salinity were measured at each site on each sample date.

Ten sites were sampled twice weekly in the Choptank, Patuxent, and Nanticoke rivers, and weekly in Mattawoman Creek and Magothy River (Figure 2-1). Boundaries of areas sampled were determined from Yellow Perch larval presence in estuarine surveys conducted during the 1970s and 1980s (O'Dell 1987). Larval sampling usually occurs during late March through mid-to-late April, depending on larval presence and catchability.

Each sample was emptied into a glass jar and checked for larvae. Yellow Perch larvae can be readily identified in the field since they are larger and more developed than Striped Bass and White Perch larvae with which they could be confused (Lippson and Moran 1974). Contents of the jar were allowed to settle and then the amount of settled OM was assigned a rank: 0 = a defined layer was absent; 1 = defined layer on bottom; 2 = more than defined layer and up to ¼ full; 3 = more than ¼ to ½ and; 4 = more than ½ full. If a jar contained enough OM to obscure seeing larvae, it was emptied into a pan with a dark background and observed through a 5X magnifying lens. Organic matter was moved with a probe or forceps to free larvae for observation. If OM loads, wave action, or collector uncertainty prevented positive identification, samples were preserved and taken back to the lab for sorting.

Choptank and Patuxent Rivers were sampled by program personnel in 2016, while Nanticoke River was voluntarily sampled by another Maryland Fishing and Boating Services project during its normal operations without charge to this grant. Mattawoman Creek and Magothy River were sampled by citizen scientist volunteers from the Mattawoman Watershed Society and the Magothy River Association, respectively, trained by our program biologists.

The proportion of tows with Yellow Perch larvae (L_p) for each subestuary was determined annually for dates spanning the first catch through the last date that larvae were consistently present as:

$$^{(1)}\ L_p = N_{present} / N_{total};$$

where $N_{present}$ equaled the number of samples with Yellow Perch larvae present and N_{total} equaled the total number of samples. The SD of L_p was estimated as:

$$^{(2)}\ \mathrm{SD} = [(L_p \cdot (1 - L_p)) / N_{total}]^{0.5}\ \text{(Ott 1977).}$$

The 95% confidence intervals were constructed as:

$$^{(3)}\ L_p \pm 1.96 \cdot \mathrm{SD};\ \text{(Ott 1977).}$$

In general, sampling to determine L_p began during the last days of March or first days of April and ended after larvae were absent (or nearly so) for two consecutive sampling rounds. In years where larvae disappeared quickly, sampling rounds into the third week of April were included in analysis even if larvae were not collected. Inclusion of these zeros reflected expectation (based on previous years) that larvae would be available to the sampling gear had they been there. This sampling schedule has been maintained for tributaries sampled by program personnel since 2006. Sampling by other Fisheries Service projects and volunteers sometimes did not adhere as strictly to this schedule.

Historical collections in the Choptank and Nanticoke Rivers targeted Striped Bass eggs and larvae (Uphoff 1997), but Yellow Perch larvae were also common (Uphoff 1991). Uphoff et al. (2005) reviewed presence-absence of Yellow Perch larvae in past Choptank and Nanticoke River collections and found that starting dates during the first week of April or early in the second week were typical and end dates occurred during the last week of April through the first week of May. Larval presence-absence was calculated from data sheets (reflecting lab sorting) for surveys through 1990. During 1998-2004, L_p in the Choptank River was determined directly in the field and recorded on data sheets (P. Piavis, MD DNR, personal communication). All tows were made for two minutes. Standard 0.5 m diameter nets were used in the Nanticoke River during 1965-1971 (1.0 • 0.5 mm mesh) and after 1998 in the Choptank River (0.5 mm mesh). Trawls with 0.5 m nets (0.5 mm mesh) mounted in the cod-end were used in the Choptank River during 1980-1990 (Uphoff 1997; Uphoff et al. 2005). Survey designs for the Choptank and Nanticoke Rivers were described in Uphoff (1997).

Methods used to estimate development (C/ha) and land use indicators (percent of watershed in agriculture, forest, wetlands, and urban land use) are explained in **General Spatial and Analytical Methods used in Job 1, Sections 1-3.** Development targets and limits and general statistical methods (analytical strategy and equations) are described there as well. Specific spatial and analytical methods for Section 2 are described below.

Estimates of C/ha and MD DOP land cover (agriculture, forest, and wetland) percentages were used as measures of watershed land use for analyses (Table 2-1). Whole watershed estimates were used with the following exceptions: Nanticoke, Choptank, and Patuxent River watersheds were truncated at the lower boundaries of their striped bass spawning areas (these coincide well with Yellow Perch nurseries; J. Uphoff, personal observation), and estimates for Choptank and Nanticoke River watersheds stopped at the Delaware border (latter due to lack of comparable land use data). Estimates of C/ha were available from 1950 through 2014 (M. Topolski, MD DNR, personal communication). Estimates of C/ha for 2014 were used to represent 2015 and 2016 for all systems.

Uphoff et al. (2012) developed L_p thresholds for brackish and tidal-fresh systems. Three brackish subestuaries with C/ha > 1.59 (10 estimates from Severn, South, and Magothy Rivers) exhibited chronically depressed L_p and their maximum L_p (0.40) was chosen as a threshold indicating serious deterioration of brackish subestuary larval nursery habitat. Similarly, tidal-fresh Piscataway Creek's four estimates of L_p (2008-2011) consistently ranked low when compared to other tidal-fresh subestuaries sampled (13th to 17th out of 17 estimates). The maximum for Piscataway Creek's four estimates, $L_p = 0.65$, was chosen as a threshold indicating serious deterioration of tidal-fresh larval habitat. Estimates of L_p would need to be consistently at or below this level to be considered "abnormal" as opposed to occasional depressions (Uphoff et al. 2012).

In this report, we have initiated analyses to explore the potential influence of the three major land uses on larval Yellow Perch dynamics: agriculture, forest, and wetland, as well as C/ha. Percents of watershed area in these land uses were based on MD DOP estimates. We examined scatter plots and used correlation or regression analyses to examine associations among land use types.

Two regression approaches were used to examine possible linear relationships between C/ha and L_p. First, separate linear regressions of C/ha against L_p were estimated for brackish and tidal-fresh subestuaries. If 95% CIs of slopes overlapped and 95% CIs of the intercepts did not overlap, we used the multiple regression of C/ha and salinity class against L_p. This latter approach assumed slopes were equal for two subestuary salinity categories, but intercepts were different (Freund and Littell 2006). Salinity was modeled as an indicator variable in the multiple regression with 0 indicating tidal-fresh subestuaries and 1 indicating brackish subestuary conditions. High salinity has been implicated in contributing to low L_p in Severn River (Uphoff et al. 2005). The association of mean salinity and IS can be significant and strong (Uphoff et al. 2010), and salinity is important to formation of stressful DO conditions in summer in mesohaline tributaries (see Section 3). Ricker (1975) warned against using well correlated variables in multiple regressions, so categorizing salinity for multiple or separate regressions of C/ha against L_p minimized confounding salinity with level of development. These same analyses were repeated using percent agriculture and percent forest land cover estimates in place of C/ha in regressions with L_p.

We used Akaike Information Criteria adjusted for small sample size, AICc, to evaluate the models that describe hypotheses that related changes in L_p to either C/ha, percent agriculture, or percent forest, for each salinity category (separate slopes) or to C/ha (percent agriculture or percent forest) and salinity category (common slopes, separate intercepts; Burnham and Anderson 2001):

$$^{(4)}\ AIC_c = -2(\text{log-likelihood}) + 2K + [(2K \cdot (K+1)) / (n-K-1)];$$

where n is sample size and K is the number of model parameters. Model parameters for the least squares regressions consisted of their mean square error estimates (variance), intercepts, slopes, and salinity category in the case of the multiple regression. We rescaled AIC_c values to Δ_i, (AIC_{ci} – minimum AIC_c), where i is an individual model, for the tidal-fresh or brackish regression compared to the multiple regression. The Δ_i values provided a quick "strength of evidence" comparison and ranking of models and hypotheses. Values of $\Delta_i \leq 2$ have substantial support, while those > 10 have essentially no support (Burnham and Anderson 2001).

An additional view of the relationship of L_p and C/ha was developed by considering dominant land use classification (land use type that predominated in the watershed) when interpreting plots of salinity classification (brackish or tidal-fresh), C/ha, and L_p. Dominant land use (agriculture, forest, or urban) was determined from Maryland Department of Planning estimates for 1973, 1994, 1997, 2002, or 2010 that fell closest to a sampling year (MD DOP 2013). Urban land consisted of high and low density residential, commercial, and institutional acreages (MD DNR 1999).

Composite samples of larvae were collected for feeding analyses from sites in Choptank, Nanticoke, and Patuxent Rivers during several sample trips in 2016. Subsamples of postlarvae

12 mm TL or less were examined for gut contents from each day's samples of each subestuary, although only larvae 6-9 mm were used for analyses. Larvae were measured to the nearest 0.5 millimeter. These 6-9 mm larvae represented early postlarvae that absorbed their yolk and began active feeding (first-feeding larvae; Hardy 1978). Gut fullness was judged visually and assigned a rank: 0 = empty; 1 = up to ¼ full; 2 = up to ½ full; 3 = up to ¾ full; and 4 = full. Major food items were classified as copepods, cladocerans, or other, and the presence (coded 1) or absence (coded 0) of each group was noted.

The mean of feeding success rank was calculated annually for each subestuary sampled in 2010-2016, as was mean total length (TL in mm) of larvae. The proportion of guts without food (P0) was estimated for each subestuary as was the proportion of larvae with copepods (P_{cope}), cladocerans (P_{clad}), or other (P_{other}) food items. The latter three proportions were not additive.

We used OM0 (proportion of samples without organic material, i.e., rank = 0) as our indicator of detritus availability. Proportions of samples without OM were only estimated during 2011-2016, so fewer observations were available for analyses than would be for feeding metrics. The distribution of OM ranks assigned to samples were highly skewed towards zero, and few ranks greater than one were reported. We regressed OM0 against C/ha, and associations of OM0 with mean feeding rank, P0, P_{cope}, P_{clad}, and P_{other} were tested with regression analysis as well. An additional set of regression analyses examined relationships among mean feeding success rank and P_{cope}, P_{clad}, and P_{other}. Correlation analysis was used to explore whether feeding success (P0 and mean feeding rank) was associated with L_p. Moderate or strong positive correlations would indicate that successful feeding of first-feeding larvae contributed to variation in L_p. An absence of positive associations would not necessarily mean that feeding was not important to overall year-class success however. Feeding success of larvae may influence survival of larvae size classes beyond those considered in the feeding study (6-9 mm) and this process would not be captured by L_p.

We were specifically interested in the relationships of the amount of organic matter to development and larval feeding success. Examination of the plot of OM0 and C/ha suggested that the relationship could be nonlinear, with OM0 increasing at a decreasing rate with C/ha. We fit a power and logistic growth function to these data. We used linear and quadratic regressions to explore relationships of feeding success (mean of feeding ranks) with OM0 (Freund and Littel 2006). Linear regression described a hypothesis about steady change, while the dome-shaped quadratic relationship would indicate an optimum region of OM values for feeding success.

We were interested in links among OM0, percent wetlands in a watershed, and C/ha. Examination of the plot of percent wetlands and C/ha suggested that the relationship was nonlinear, with percentage of wetlands decreasing at a decreasing rate with C/ha, and appeared to be a mirror image of the plot of OM0 and C/ha. Examination of the plot of OM0 and percent wetlands suggested a linear relationship, with proportion of samples without organic material decreasing as percent wetlands per watershed increased. We fit power, logistic growth, or a linear function to these data sets, respectively.

During 2016, we collected Yellow Perch larvae for RNA/DNA analysis from two tributaries of the Chesapeake Bay with watersheds exhibiting different levels of development: the rural, agricultural Choptank River watershed (C/ha = 0.13) and the suburban Patuxent River watershed (C/ha = 1.22). Based on previous years' collections, we anticipated that sampling from these two rivers on three occasions would provide 30 larvae per system, per date, for a minimum of 180 samples. Yellow Perch larvae were also collected from Choptank and Patuxent

Rivers in 2015 (Uphoff et al. 2016). In 2013 and 2014, larvae were collected from Nanjemoy Creek (C/ha = 0.09) and Mattawoman Creek (C/ha = 0.91; Uphoff et al. 2014; 2015). Samples for RNA/DNA analysis were collected when larvae were gathered for analysis of gut contents. In the field, Yellow Perch larvae were composited from several stations (where possible) that bracketed where larvae are abundant. Once a candidate jar had been checked for larvae and OM, the sample was poured through a 500 µ screen and larvae were transferred to a large tube with special preservative (RNAlater®). The vial was labeled with the subestuary name and sample date. Larvae from other sites from one subestuary were composited into the vial on the same date.

In the lab, larvae for each date were processed for both gut contents and RNA/DNA ratios. Yellow Perch larvae 12 mm TL or less were examined for gut contents from each sample, although only larvae 6-9 mm in size (sizes in common among all years and subestuaries) were used for analyses. These larvae had absorbed their yolk and began active feeding. Generally, 6 mm larvae were the smallest that contained food. Larvae were removed from the composite sample and placed in a Petri dish of water, examined for gut contents and then the guts were removed. The RNA/DNA ratio estimate did not contain food items. If a larva had not fed, the guts were teased away to be safe. Each processed larva was placed in a small individual vial of RNAlater® preservative. The vial was coded on the outside as follows: letter designating which river, number designating which sample date, and number designating which individual larva was placed in the vial.

In 2013-2015 RNA/DNA ratios were estimated by science staff at the Cooperative Oxford Laboratory and partners from the University of Maryland Eastern Shore, while 2016 estimations were made by project staff. Protocols for estimating RNA/DNA generally followed Kaplan et al. (2001). Larvae were stored at 4°C in RNAlater® for up to three weeks until processing. Whole body samples, minus gut contents, were digested with 1% sodium dodecylsulfate, proteinase K digestion buffer (66ug/ml), and 0.1M NaCl at 55°C for several hours until completely digested. Samples were centrifuged at 11,000 rpm for 10 minutes, and the supernatant containing the nucleic acids was removed and stored at -80°C until ready for processing.

A 400 µL portion of the supernatant was removed for digestion of DNA prior to analysis of RNA. Removal of DNA was accomplished by treating this portion of supernatant with DNase digestion buffer (0.2M Tris-HCl pH=7.5, 0.1M MgCl and 0.02M CaCl, and 10 U RNase-free DNase I). Samples incubated at 37°C for 45 minutes in a dry bath. Samples were centrifuged for five minutes at 8,000 rpm. The supernatant was removed and stored at -80°C until ready for processing.

Samples were analyzed for DNA and RNA using Quant-it™ PicoGreen® and Quant-it™ RiboGreen® (Molecular Probes, Oregon), respectively, according to the manufacturer's protocol. Samples were plated in triplicate on solid black 96-well microplates and fluorescence was measured at 480 nm excitation and 520 nm.

During sample processing in 2014 it was discovered that a dilution had been missed in the instructions used to estimate ratios for 2013 and that year. Samples had been retained for both years and it was hoped these could be used to develop adjustments for the missed dilution. In 2014, to quantify nucleic acids, sample fluorescent readings were compared to DNA and RNA standard curves. These curves were developed by creating eight separate solutions of tissue digestion buffer and nucleic acid standard solutions. Lamda phage DNA and E. coli ribosomal 16S and 23S RNA (Molecular Probes, Oregon) were used as DNA and RNA standards, respectively. Serial dilutions of the 16 standard solutions (eight solutions per nucleic acid) were plated on 96-well microplates followed by the addition of PicoGreen® for DNA and RiboGreen® for RNA. Fluorescence was read at 480 nm excitation and 520 nm. The $\log_e$-transformed fluorescent measures from each standard solution (F) were plotted against their respective nucleic acid concentration (C). A quadratic regression was used to determine the coefficients (Table 2-2) for each curve. The regression model used was

$$^{(5)}\ \mathrm{Log}_e\,F = (m \cdot C) + (n \cdot C^2) + b;$$

where F and C are as defined previously, m and n are coefficients and b is the intercept. These coefficients were used to determine sample concentrations of DNA and RNA after back-transformation. This same process was supposed to be followed, and adjustments made, for 2013 samples as well. The freezer they were being stored in failed, however, and samples were lost. An attempt to make standards using the original erroneous methodology was undertaken, and correction curves were developed and used to create new "outputs" for samples that contained fluorescence in 2013. This data set was judged to be unreliable, with a huge range of values that do not resemble any other year of this work, or anything found so far in the literature, and was excluded from analyses.

Ratios of RNA/DNA were expected to increase as larval fish grew (Rooker et al. 1997). We used linear regressions to explore the relationship of RNA/DNA ratios and larval lengths by system in 2014, 2015 and 2016. We compared 95% confidence intervals of slopes or intercepts to look for differences between rural and developed systems within and among years. RNA/DNA ratios for each subestuary were also plotted against larval TL and reference lines indicating starving (RNA/DNA < 2; Blom et al. 1997) and fed larvae (RNA/DNA > 3; Buckley 1984; Wright and Martin 1985) based on values from larvae of several marine species and Striped Bass were added to the plots.

The proportions of larvae with RNA/DNA ratios less than 2 (proportion starved or *Ps*) were estimated for each subestuary as

$$^{(6)}\ Ps = N_{<2} / N_{total};$$

where $N_{<2}$ equaled the number of samples with RNA/DNA ratios less than 2 and N_{total} equaled the total number of RNA/DNA samples. Proportions of larvae with RNA/DNA ratios greater than 3 (proportion fed or *Pf*) were estimated as in equation 6, but *Pf* was estimated with the number of larvae with RNA/DNA ratios greater than 3 ($N_{>3}$) in the numerator of equation 6.

Lengths of Yellow Perch larvae were assigned to 0.5 mm increments, but a more precise estimate of relative size could be derived from the amount of DNA present (Heyer et al. 2001). We compared 95% CI's of the amount of DNA present (ng DNA / mg supernatant in solution or ng DNA / sample) for each subestuary and year sampled to explore whether differences in larval size were present. The quantity of DNA within cells is relatively constant but larger larvae have more cells, so the amount of DNA should be related to size of larvae. Size of Yellow Perch larvae was positively associated with female size, so amount of DNA reflected maternal influence (Heyer et al. 2001).

Correlation analysis was used to explore the link between RNA/DNA and a subset of variables that were supportive of the OM hypothesis. Amount of DNA was included in the correlation analyses to examine possible maternal influence. Dynamics of RNA/DNA were represented by the intercepts and slopes of the RNA/DNA versus length regressions. The intercept indicated condition of larvae at 6 mm when they initiated feeding. The slope would indicate growth between 6 mm and 9 mm. These correlation analyses had small sample sizes (N = 6; 3 years and 2 levels of development each year). We did not consider level of significance an overriding factor for interpreting results. A fairly high degree of correlation (r greater than 0.7 or less than -0.7) was used to screen for meaningful associations. We estimated the mean ratio of RNA/DNA at the 6.0 and 6.5 mm increments (combined) for each year and development category and regressed them against their corresponding intercept estimates as a check on the accuracy of the intercept as a measure of initial condition. A corresponding estimate of growth to compare to the slope was not available.

Results

During 2016, sampling on Choptank River lasted from March 28 to May 18, while sampling on Patuxent River lasted from March 30 to May 19. Samples through April 11 and April 14 were used to estimate L_p in Choptank and Patuxent rivers, respectively. Sampling began on April 4 in the Nanticoke River and ended on April 29; dates between April 4 and 13 were used for estimating L_p. Mattawoman Creek was sampled between April 4 and April 25, and Magothy River was sampled between March 23 and April 13; all dates were used to estimate L_p in both systems.

Based on 95% CIs, estimates of L_p during 2016 were sufficiently precise to separate them from thresholds (Figure 2-2). Estimates of L_p for Choptank and Patuxent rivers (L_p = 0.90 and 0.82, respectively) were similar to the single estimate for tidal-fresh subestuary Mattawoman Creek in 2016 (L_p = 0.93) based on 95% CI overlap. The estimate of L_p for the Nanticoke River (0.67) overlapped a greater portion of confidence intervals of Patuxent River and Mattawoman Creek L_p than Choptank River L_p. The estimate of L_p for Magothy River, 0.10, was much lower than the other systems based on 95% CIs, and was below the brackish L_p threshold (Figure 2-2).

Comparisons of L_p during 2016 with historical estimates for brackish subestuaries is plotted in Figure 2-3 and for tidal-fresh values in Figure 2-4. The range of C/ha values available for analysis with L_p was 0.05-2.78 for brackish subestuaries and 0.45-3.33 for tidal-fresh (Table 2-1). Estimates of L_p in 2016 were among the top historical values for Choptank and Nanticoke rivers and Mattawoman Creek; historical values were not available for Patuxent River. Estimated L_p in Magothy River, 0.10, was the same as estimated in 2009 (Table 2-1; Figure 2-3).

Separate linear regressions of C/ha and L_p by salinity category were significant at $P \leq 0.0005$; Table 2-3; Figure 2-5). These analyses indicated that C/ha was negatively related to L_p and L_p was, on average, higher in tidal-fresh subestuaries than in brackish subestuaries. Estimates of C/ha accounted for 28% of variation of L_p in brackish subestuaries and 34% in tidal-fresh subestuaries. Based on 95% CI overlap, intercepts were significantly different between tidal-fresh (mean = 0.95, SE = 0.09) and brackish (mean = 0.60, SE = 0.04) subestuaries. Mean slope for C/ha estimated for tidal-fresh subestuaries (mean = -0.29, SE = 0.07) were steeper, but 95% CI's overlapped CI's estimated for the slope of brackish subestuaries (mean = -0.17, SE = 0.04; Table 2-3). Both regressions indicated that L_p would be extinguished between 3.0 and 3.5 C/ha (Figure 2-5).

Overall, the multiple regression approach offered a similar fit ($r^2 = 0.33$; Table 2-3) to separate regressions for each salinity type. Intercepts of tidal-fresh and brackish subestuaries equaled 0.95 and 0.60, respectively; the common slope was -0.19. Predicted L_p over the observed ranges of C/ha would decline from 0.59 to 0.14 in brackish subestuaries and from 0.82 to 0 in tidal-fresh subestuaries (Figure 2-5).

Estimates of L_p were positively, yet weakly, related to agriculture ($r^2 = 0.10$, P = 0.0163) and forest ($r^2 = 0.10$, P = 0.0213) in brackish tributaries (Table 2-3; Figure 2-5). Regressions of L_p and agriculture and forest in tidal-fresh subestuaries were very similar to that found in brackish ones, but sample sizes were lower so their level of significance was slightly above 0.05

(Table 2-3). Correlation analysis did not suggest an association of wetlands with L_p in subestuaries of either salinity type so additional analyses were not conducted.

Akaike's Information Criteria values equaled 9.4 for the regression of C/ha and L_p for brackish subestuaries, 9.9 for tidal-fresh estuaries, and 11.4 for the multiple regression that included salinity category. Calculations of Δi for brackish or tidal-fresh versus multiple regressions were approximately 2.04 and 1.56 (respectively), indicating that either hypothesis (different intercepts for tidal-fresh and brackish subestuaries with different or common slopes describing the decline of L_p with C/ha) were plausible (Table 2-4). These same calculations were performed from the regressions of percent agriculture or percent forest and L_p and results were almost identical to AIC values of C/ha and L_p (Table 2-4).

Although we have analyzed these data in terms of tidal-fresh and brackish subestuaries, inspection of Table 2-1 indicated an alternative interpretation based on primary land use estimated by MD DOP. Predominant land use at lowest levels of development may be influencing the intercept estimates. Rural watersheds (at or below C / ha target) were absent for tidal-fresh subestuaries analyzed and the lowest levels of development in tidal-fresh subestuary watersheds were dominated by forest (Figure 2-6). Dominant land cover estimated by MD DOP for watersheds of tidal-fresh subestuaries was equally split between forest (C/ha = 0.45-0.93; 18 observations) and urban (C/ha > 1.17; 14 observations). Nearly all rural land in brackish subestuary watersheds was in agriculture (C/ha < 0.22; 36 observations), while forest land cover (C/ha ~ 0.09) was represented by six observations from Nanjemoy Creek. The range of L_p was similar in brackish subestuaries with forest and agricultural cover, but the distribution seemed shifted towards higher L_p in the limited sample from Nanjemoy Creek. Urban land cover predominated in 13 observations of brackish subestuaries (C/ha > 1.22; Table 2-1; Figure 2-6). Tidal-fresh subestuary intercepts may have represented the intercept for forest cover and brackish subestuary intercepts may have represented agricultural influence. If this is the case, then forest cover provides for higher L_p than agriculture. Increasing suburban land cover leads to a significant decline in L_p regardless of rural land cover type.

Correlation analysis suggested negative, linear associations of C/ha with agriculture (r = -0.79, P < 0.0001), forest (r = -0.40, P = 0.0041, and wetlands (r = -0.34, P = 0.0156; Table 2-5; Figure 2-7). Examination of scatter plots for these comparisons (Figure 2-7) suggested a negative hyperbolic curve (power function) would provide a stronger description for the comparison of percent agriculture with C/ha. Remaining land use combinations were not significantly correlated with one another (Table 2-5).

We examined 156 larval guts of 6-9 mm TL Yellow Perch larvae during 2010, 337 in 2011, 442 in 2012, 618 in 2013, 1495 in 2014, 1417 in 2015, and 508 in 2016 (Table 2-6). A smaller sample size was available for correlations with OM0 (N = 28) than other variables (N = 34) because observations of OM did not start until 2011.

Larval Yellow Perch guts contained food in all years and subestuaries except Piscataway Creek during 2011 (Table 2-6). Copepods were not found in larval stomachs in Elk River in 2012 and Northeast River in 2013, but were the most prevalent food item in 18 out of 33 system-year combinations (Piscataway Creek in 2011 is excluded since guts there did not contain any food), and were found in 8-100% of stomachs sampled. In 2016, copepods were present 46-99% of the time. Cladocerans were not found in larval stomachs in five of the 33 system-year combinations, and were the most prevalent food item 15% of the time. In 2016 cladocerans were present in 45-52% of guts sampled. "Other" food items were present in a higher fraction of samples in all system-year combinations than cladocerans, and were the most prevalent category

30% of the time. This category was predominant in larval gut samples from all five subestuaries during 2012 (69-100%; Table 2-6), but it should be noted that most gut contents in that year were already too digested to be identifiable and could not be categorized any other way. Gut content identification was more straightforward in other years, and except for 2014 when large amounts of digested material again could not be identified, “other” food items were present in 0-67% of samples. In 2016 “other” food items were present in 0-13% of stomachs sampled.

During 2010-2016, percentage of guts without food (P0) ranged from 0 to 52% in all subestuary and year combinations except Piscataway Creek during 2011 (100%). Mean fullness rank ranged between 0.50 and 3.23 in all subestuary and year combinations except Piscataway Creek during 2011(it was 0; Table 2-6).

Estimates of C/ha and OM0 were significantly related. A non-linear power function fit the data (approximate $r^2 = 0.46$, $P < 0.0001$; N = 30), depicting OM0 increasing towards 1.0 at a decreasing rate as C/ha approached 1.50 (Figure 2-8). The relationship was described by the equation:

$$^{(7)}\ \mathrm{OM0} = 0.87 \cdot ((\mathrm{C/ha})^{0.14}).$$

Approximate standard errors were 0.04 and 0.05 for parameters a and b, respectively. A logistic growth function fit these data similarly, but one term was not significantly different from zero, so the model was rejected.

Linear and curvilinear regressions described how the amount and type of food present in larval Yellow Perch guts was related to OM (Table 2-7). Quadratic relationships were indicated for some comparisons, suggesting there might be too much OM (acting as a prey refuge), too little (not enough to support zooplankton), and an optimum amount. Estimates of mean fullness rank exhibited a dome-shaped quadratic relationship with OM0 ($r^2 = 0.40$, P = 0.002; Table 2-7; Figure 2-9). P_{cope} also had a significant dome-shaped relationship with OM0 ($r^2 = 0.34$, P = 0.006). The relationship of P_{other} to OM0 was linear and increasing ($r^2 = 0.19$, P = 0.02), although this relationship could be biased since there were years when stomach contents were too digested to identify and this was only way they could be categorized. Relationships of OM with P_{clad} or P0 were not detected (Table 2-7).

The amount of food present in larval guts was significantly related to presence of copepods (Table 2-7). Both mean fullness rank and P0 were linearly related with P_{cope} (positive slope, $r^2 = 0.69$, $P = <0.0001$ and negative slope, $r^2 = 0.41$, $P = <0.0001$, respectively). Estimates of P_{clad} were modestly and negatively related to P0 ($r^2 = 0.16$, P = 0.02; Table 2-7). Copepods represent a much larger food item than cladocerans typically encountered.

Mean fullness rank and P0 were not associated with L_p (r = 0.18, P = 0.31 and r = 0.07, P = 0.70, respectively), indicating initial feeding success was unlikely to influence L_p. Examination of scatter plots of these data (not shown) did not suggest that nonlinear associations were likely either.

Percent wetlands (determined from the most recent MD DOP estimates in 2010) and development were negatively related. An inverse power function fit the relationship of C/ha and percent wetland well (approximate $r^2 = 0.51$, P = 0.01, N = 9; Figure 2-10). This relationship suggested that wetlands could be the main source of organic material in our study areas. We do not know whether lower wetland percentages were normal for more developed watersheds or if wetlands were drained and filled during development prior to wetland conservation regulations.

Yellow Perch larvae were collected for RNA/DNA analysis from Patuxent River (N = 167: C/ha = 1.22) and Choptank River (N = 210; C/ha = 0.13) from March 28 to April 12, 2016 (see Table 2-8 for this and other years’ collection summaries). Collections had larvae less than 6 mm, and exceeding 12 mm in length, but analysis was restricted to sizes in common for all years (6-9 mm) and typical of that of first-feeding larvae (Figure 2-11). Choptank River's watershed was below the threshold development level, while Patuxent River has passed the suburban threshold. Estimates of OM0 were 0.56 and 0.45 in Patuxent and Choptank Rivers, respectively.

During 2016, all 6-9 mm TL Yellow Perch larvae examined from Patuxent River had RNA/DNA ratios above the starvation threshold (2; Figure 2-11) and 100% had a ratio greater than 3, indicating well fed larvae. Two percent of Choptank River had RNA/DNA ratios below 2 and 78% had ratios greater than 3 (Figure 2-11). During 2015, all 6-9 mm TL Yellow Perch larvae examined from Patuxent River had RNA/DNA ratios above the starvation threshold (Figure 2-11) and 97% had a ratio greater than 3, indicating first-feeding larvae were in good condition. During 2014, 15% of Choptank River larvae had RNA/DNA ratios below 2 and 67% had ratios greater than 3. In 2014, few larvae in Mattawoman and Nanjemoy creeks had RNA/DNA ratios greater than 3 (*Pf* = 0.08, N = 217 and *Pf* =0.05, N = 255, respectively). The majority of larvae collected were in starved condition (*Ps* = 0.57 and 0.66, respectively; Figure 2-11). In 2016, RNA/DNA increased with length in both Patuxent and Choptank Rivers (r^2 = 0.16, P = <0.0001 and r^2 = 0.32, P = <0.0001, respectively).

Differences in relationships of RNA/DNA ratios of 6-9 mm larvae with length were detected (95% CI's of slopes and intercepts were different). Confidence intervals of slopes overlapped during 2014 and 2015, and indicated growth was low or negative, and RNA/DNA did not change or declined (Figures 2-12 and 2-11, respectively). The 95% CI's of the slopes of RNA/DNA change with length were substantially higher during 2016 with the rural Choptank River exhibiting faster growth than the more developed Patuxent River (Figure 2-12). The 95% CI's of the intercepts indicated variation in initial RNA/DNA ratios (Figure 2-12). Intercepts (RNA/DNA at initial postlarval size) were not different between developed and rural watersheds during 2014 (Mattawoman Creek and Nanjemoy Creek, respectively), but were greater during 2015 and 2016 in the developed watershed (Patuxent River) than the rural watershed (Choptank River). The relationship of the intercept and mean RNA/DNA of larvae in the 6.0 and 6.5 mm categories was significant (r^2 = 0.94, P < 0.002) and indicated a 1 to 1 relationship; the 95% CI of the slope (mean = 1.02, SE = 0.13) overlapped one and the intercept was not different from zero (mean = 0.14, SE = 0.58). Since the intercepts and the mean ratios at the 6.0 and 6.5 mm increments were related on a 1 to 1 basis, only the intercept was considered for subsequent correlation analyses. Intercepts and slopes of the relationships were not correlated ($r \approx 0$), indicating absence of an interaction of condition at initiation of feeding at 6 mm and subsequent growth to 9 mm.

Substantial annual differences in 95% CI's of amount of DNA per ml sample (initial postlarval size and maternal influence indicator) were detected, with over a 5-fold difference between the highest and lowest means (Table 2-9, Figure 2-13). Confidence intervals clearly differentiated each year, with 2014 having the highest mean; 2015 fell in the middle; and 2016 was lowest. Differences in mean ng DNA / mg sample between rural and developed were not indicated for 2014 and 2015. During 2016, larvae from the developed Patuxent River were smaller than those from rural Choptank River (Table 2-9, Figure 2-13).

In the RNA/DNA data set, only C/ha provided contrast (0.09-1.22) similar to that found for the same variables in the whole feeding data set (Table 2-9). Mean amounts of DNA and slopes of the change in RNA/DNA with length were poorly associated with C/ha (r = -0.32 and, -0.21 and P = 0.53 and 0.68, respectively). The correlation of C/ha and intercept (r = 0.68 and P = 0.13) was stronger, but interpretation of a mechanism was not clear. Feeding success (P0 and mean fullness) and variables important to feeding success (P_{cope}, OM0, and percent wetland) represented a very limited range when compared to the whole feeding data set (Table 2-9) and these correlation analyses were removed from consideration.

Discussion

General patterns of land use and L_p emerged from the expanded analyses conducted for this report: L_p was negatively related to development, positively associated with forest and agriculture, and not associated with wetlands. Development, organic matter, and feeding metrics of 6-9 mm Yellow Perch larvae were negatively, and often nonlinearly, linked. Wetlands appeared to be an important source of organic matter for subestuaries. Correlation analyses did not suggest that processes covered in the feeding analysis of 6-9 mm larvae would influence L_p; L_p is not a measure of year-class success and the processes influencing feeding success could impact poorly sampled older larvae. RNA/DNA ratios for 6-9 mm larvae did not detect consistent differences in larval condition between watersheds below the target level of development and those at or slightly above the threshold (latter watersheds may have had significant mitigating features). Significant annual differences in initial size of first-feeding larvae were exhibited by amount of DNA per sample during 2014-2016, indicating that maternal influence on size of first-feeding larvae was not constant among years.

Rural features (agriculture, forest, and wetlands) were negatively correlated with development in the watersheds monitored for L_p. A broad range of L_p (near 0 to 1.0) was present up to 1.3 C/ha. Beyond 1.3 C/ha, estimates of L_p values were less than 0.65. A full range of L_p values occurred in subestuaries with agricultural watersheds (C/ha was $\leq$ 0.22). A forest cover classification in a watershed was associated with higher L_p (median L_p = 0.79) than agriculture (median L_p = 0.54) or development (median L_p = 0.32), but these differences may have also reflected dynamics unique to brackish or tidal-fresh subestuaries since all agricultural watersheds had brackish subestuaries and nearly all forested watersheds had tidal-fresh subestuaries.

At least five factors can be identified that potentially contribute to variations in L_p: salinity, summer hypoxia, maternal influence, winter temperature, and watershed development. These factors may not be independent and there is considerable potential for interactions among them.

Salinity may restrict L_p in brackish subestuaries by limiting the amount of available low salinity habitat over that of tidal-fresh subestuaries. Uphoff (1991) found that 90% of larvae collected in Choptank River (based on counts) during 1980-1985 were from 1‰ or less. Approximately 85% of Yellow Perch larvae collected by Dovel (1971) from Magothy and Patuxent rivers, and Head-of-Bay, during1963-1967 were collected at salinity 1‰ or less.

Severn River offers the most extensive evidence of salinity changes in a subestuary that were concurrent with development from 0.35 to 2.29 C/ha. During 2001-2003, salinity within Severn River's estuarine Yellow Perch larval nursery ranged between 0.5 and 13‰ and 93% of measurements were above the salinity requirement for eggs and larvae of 2‰ (Uphoff et al. 2005). Muncy (1962) and O'Dell's (1987) descriptions of upper Severn River salinity suggested that the nursery was less brackish in the 1950s through the 1970s than at present, although a single cruise by Sanderson (1950) measured a rise in salinity with downstream distance similar to what Uphoff et al. (2005) observed. Most Yellow Perch spawning in Severn River during 1958 occurred in waters of 2.5‰ or less (Muncy 1962). Mortality of Yellow Perch eggs and prolarvae in experiments generally increased with salinity and was complete by 12‰ (Sanderson 1950; Victoria et al. 1992). Uphoff et al. (2005) estimated that nearly 50% of the historic area of estuarine nursery for Yellow Perch was subject to salinities high enough to cause high mortality. Salinity in the estuarine nursery of Severn River varied without an annual pattern even though conditions went from extremely dry (2001-2002) to extremely wet (2003; Uphoff et al. 2005).

As development increases, rainfall flows faster across the ground and more of it reaches fluvial streams rather than recharging groundwater (Cappiella and Brown 2001; Beach 2002). In natural settings, very little rainfall is converted to runoff and about half is infiltrated into underlying soils and the water table (Cappiella and Brown 2001). These pulses of runoff in developed watersheds alter stream flow patterns and could be at the root of the suggested change in salinity at the head of the Severn River estuary where the larval nursery is located (Uphoff et al. 2005).

In our studies, suburban mesohaline subestuaries commonly exhibit summer hypoxia in bottom channel waters, but it is less common in agricultural watersheds (see Section 3). Stratification due to salinity is an important factor in development of hypoxia in mesohaline subestuaries, while hypoxia is rarely encountered in tidal-fresh and oligohaline subestuaries (see Section 3). Depressed egg and larval viability due to endocrine disruption may follow inadequate DO the previous summer (Wu et al. 2003; Uphoff et al. 2005; Thomas and Rahman 2011; Tuckey and Fabrizio 2016). Ovaries of Yellow Perch are repopulated with new germs cells during late spring and summer after resorptive processes are complete (Dabrowski et al. 1996, Ciereszko et al. 1997).

Hypoxia in coastal waters reduces fish growth and condition due to increased energy expenditures to avoid low DO and compete for reduced food resources (Zimmerman and Nance 2001; Breitburg 2002; Stanley and Wilson 2004). Reproduction of mature female fish is higher when food is abundant and condition is good (Marshall et al. 1999; Lambert and Dutil 2000; Rose and O'Driscoll 2002; Tocher 2003), but stress may decrease egg quality (Bogevik et al. 2012). A female Yellow Perch's energetic investment provides nutrition for development and survival of its larvae until first feeding (Heyer et al. 2001) and differences in Yellow Perch larval length, yolk volume, and weight were attributed to maternal effects in Lake Michigan (Heyer et al. 2001).

Widespread low L_p occurs sporadically in Chesapeake Bay subestuaries that appears to be linked to high winter temperatures (Uphoff et al. 2013). During 1965-2012, estimates of L_p less than 0.5 did not occur at when average March air temperatures were 4.7°C or less (N = 3), while average March air temperatures of 9.8°C or more were usually associated with L_p estimates of 0.5 or less (7 of 8 estimates). Estimates of L_p between this temperature range exhibited high variation (0.2 – 1.0, N = 27; Uphoff et al. 2013). In Yellow Perch, a period of low temperature is required for reproductive success (Heidinger and Kayes 1986; Ciereszko et al. 1997). Recruitment of Yellow Perch continuously failed in Lake Erie during 1973-2010 following short warm winters (Farmer et al. 2015). Subsequent lab and field studies indicated reduced egg size, energy and lipid content, and hatching success followed short winters even though there was no reduction in fecundity. Whether this reduced reproductive success was due to metabolic or maternal endocrine pathways could not be determined (Farmer et al. 2015).

Yellow Perch egg viability declined in highly developed suburban watersheds of Chesapeake Bay (C/ha above threshold level; Uphoff et al. 2005; Blazer et al. 2013). Abnormalities in ovaries and testes of adult Yellow Perch during spawning season were found most frequently in subestuaries with suburban watersheds and these abnormalities were consistent with contaminant effects (Blazer et al. 2013). Blazer et al. (2013) offered an explanation for low egg viability observed by Uphoff et al. (2005) in Severn River during 2001-

2003 and persistently low L_p detected in three western shore subestuaries with highly developed suburban watersheds (C/ha > 1.59; Severn, South, and Magothy Rivers). Endocrine disrupting chemicals were more likely to cause observed egg hatching failure in well-developed tributaries than hypoxia and increased salinity (Blazer et al. 2013). It is unlikely that low L_p has always existed in well-developed Magothy, Severn, and South rivers since all supported well known recreational fisheries into the 1970s (the C/ha thresholds were met during the late 1960s-1970s). Severn River supported a state hatchery through the first half of the twentieth century and hatching rates of eggs in the hatchery were high up to 1955, when records ended (Muncy 1962). News accounts described concerns about fishery declines in these rivers during the 1980s and recreational fisheries were closed in 1989 (commercial fisheries had been banned many years earlier; Uphoff et al. 2005). A hatchery program attempted to raise Severn River Yellow Perch larvae and juveniles for mark-recapture experiments, but egg viability declined drastically by the early 2000s and Choptank River brood fish had to be substituted (Uphoff et al. 2005). Estimates of L_p from Severn River were persistently low during the 2000s. Yellow Perch egg per recruit (EPR) analyses incorporating Severn River egg hatch ratios or relative declines in L_p with C/ha indicated that recovery of Yellow Perch EPR in Severn River (and other developed tributaries) by managing the fishery alone would not be possible (Uphoff et al. 2014). Angler reports indicated that viable recreational fisheries for Yellow Perch returned to Severn River and similarly impacted western shore subestuaries (Magothy and South rivers) in the mid-to-late 1990s.

These reconstituted fisheries were likely supported by juvenile Yellow Perch that migrated from the upper Bay nursery rather than internal production (Uphoff et al. 2005). A sudden upward shift in both Yellow Perch juvenile indices and mesozooplankton relative abundance occurred in the early 1990s in the Head-of-Bay region which coincided with a downward shift in annual chlorophyll a averages at two Head-of-Bay monitoring stations (Uphoff et al. 2013). This shift in Head-of-Bay productivity was followed by reports of increased angling success in Severn, South, and Magothy rivers. Trends in L_p between the Magothy, Severn, and South rivers indicate a loss of productivity. All estimates of L_p have been below the threshold in the three western shore subestuaries with well-developed watersheds during 2001-2016 (11 of 11 estimates), while estimates from Head-of-Bay subestuaries have typically been above the threshold (4 of 7 Bush River estimates, 2 of 3 Elk River estimates, and 5 of 5 Northeast River estimates). Trends in volunteer angler catch per trip in Magothy River matched upper Bay estimates of stock abundance during 2008-2014 (P. Piavis, MD DNR, personal communication). Recreational fisheries in these three subestuaries were reopened to harvest in 2009 to allow for some recreational benefit of fish that migrated in and provided a natural “put-and-take” fishery. The term “regime shift” has been used to suggest these types of changes in productivity are causally connected and linked to other changes in an ecosystem (Steele 1996; Vert-pre et al. 2013).

Amount of organic matter present and subsequent feeding success of first-feeding Yellow Perch larvae were negatively influenced by development. Estimates of C/ha and OM0 were significantly related and a non-linear power function depicted OM0 increasing towards 1.0 at a decreasing rate with C/ha. Linear and curvilinear regressions described how feeding of early larvae declined with OM0 and C/ha. Correlation analyses did not suggest that processes covered in the feeding analysis of 6-9 mm larvae were associated with L_p. The proportion of tows with larvae is not a measure of year-class success and its lack of an association with L_p does not indicate feeding success is unimportant to Yellow Perch year-class success. At least three pieces of additional information would be needed to fully evaluate how L_p and feeding success are linked to Yellow Perch year-class success: zooplankton abundance, larval survival, and measures of year-class success in at least some of the subestuaries we have sampled. All require more intensive field and lab work than we can accommodate.

Years of high spring discharge favor anadromous fish recruitment in Chesapeake Bay (Hoffman et al. 2007; Martino and Houde 2010) and may represent episodes of hydrologic transport of accumulated OM from watersheds (McClain et al. 2003) that fuel zooplankton production and feeding success. Under natural conditions in York River, Virginia, riparian marshes and forests would provide OM subsidies in high discharge years (Hoffman et al. 2007), while phytoplankton would be the primary source of OM in years of lesser flow. Stable isotope signatures of York River American Shad larvae and zooplankton indicated that terrestrial OM largely supported one of its most successful year-classes. Lesser year-classes of American Shad on the York River were associated with low flows, OM based on phytoplankton, and lesser zooplankton production (Hoffman et al. 2007). The York River watershed, with large riparian marshes and forest, was largely intact relative to other Chesapeake Bay tributaries (Hoffman et al. 2007). Multiple regression models provided evidence that widespread climate factors (March precipitation as a proxy for OM transport and March air temperature) influenced year-class success of Head-of-Bay Yellow Perch (Uphoff et al. 2013).

Zooplankton supply (cladocerans and copepods) for first-feeding Yellow Perch larvae has been identified as an influence on survival in Lake Michigan (Dettmers et al. 2003; Redman et al. 2011; Weber et al. 2011) and Canadian boreal lakes (Leclerc et al. 2011), and survival of European Perch *Perca fluviatis* in the Baltic Sea (Ljunggren et al. 2003). In a two-year study in Lake Saint Pierre, Canada, Tardif et al. (2005) attributed larval Yellow Perch RNA/DNA response to wetland types, cumulative degree days, and feeding conditions. The importance of adequate zooplankton supply and factors influencing zooplankton dynamics have been established for survival of Chesapeake Bay Striped Bass, White Perch, and American Shad larvae (North and Houde 2001; 2003; Hoffman et al. 2007; Martino and Houde 2010). Yellow Perch larvae share habitat in Chesapeake Bay subestuaries with these species, but little has been published on larval Yellow Perch dynamics and feeding ecology in Chesapeake Bay (Uphoff 1991). Trends among baywide juvenile indices of Striped Bass and White Perch, and Head-of-Bay Yellow Perch are similar (this regional index is considered reliable for Yellow Perch; Yellow Perch Workgroup 2002; Uphoff 2008; Piavis and Webb 2016; Durell and Weedon 2016), so it is not unreasonable to expect that factors that impact White Perch and Striped Bass year-class success in Chesapeake Bay (such as feeding success on zooplankton) would influence Yellow Perch as well.

Urbanization reduces quantity and quality of OM in streams (Paul and Meyer 2001; Gücker et al. 2011; Stanley et al. 2012). Riparian zones and floodplains that are sources of OM become disconnected from stream channels by stormwater management in suburban and urban watersheds (Craig et al. 2008; Kaushal et al. 2008; Brush 2009; NRC 2009). Small headwater streams in the Gunpowder River and Patapsco River watersheds (tributaries of Chesapeake Bay) were sometimes buried in culverts and pipes, or were paved over (Elmore and Kaushal 2008). Decay of leaves occurred much faster in urban streams, apparently due to greater fragmentation from higher stormflow rather than biological activity (Paul and Meyer 2001). Altered flowpaths associated with urbanization affect timing and delivery of OM to streams (McClain et al. 2003). Organic matter was transported further and retained less in urban streams (Paul and Meyer 2001). Uphoff et al. (2011) and our current analysis found that the percentage of Maryland's

Chesapeake Bay subestuary watersheds in wetlands declined as C/ha increased, so this source of OM diminishes with development.

Management for organic carbon is nearly non-existent despite its role as a great modifier of the influence and consequence of other chemicals and processes in aquatic systems (Stanley et al. 2012). It is unmentioned in the Chesapeake Bay region as reductions in nutrients (N and P) and sediment are pursued for ecological restoration (http://www.epa.gov/reg3wapd/pdf/pdf_chesbay/BayTMDLFactSheet8_6.pdf). However, most watershed management and restoration practices have the potential to increase OM delivery and processing, although it is unclear how ecologically meaningful these changes may be. Stanley et al. (2012) recommended beginning with riparian protection or re-establishment and expand outward as opportunities permit. Wetland management represents an expansion of effort beyond the riparian zone (Stanley et al. 2012).

Agriculture also has the potential to alter OM dynamics within a watershed and has been associated with increased, decreased, and undetectable changes in OM that may reflect diversity of farming practices (Stanley et al. 2012). In our study, agricultural watersheds (all eastern shore) had most of the lower OM0 scores (indicating more detritus), while OM0 levels were higher and distributed similarly among watersheds that were predominately in development (all western shore) or forest (eastern and western shore).

In our feeding analyses, we assumed that mainstem Potomac or Susquehanna River water was not a major influence on subestuary water quantity, water quality, and zooplankton supply. Sampling for Yellow Perch larvae occurred in the upper portions of subestuaries and this should have minimized the influence of mainstem waters in most cases, although some intrusion would have been possible at the most downstream sites in the smallest tidal-fresh subestuaries closest to major rivers (i.e., Northeast River for the Susquehanna River and possibly Piscataway Creek for the Potomac). Northeast River, with development between the target and threshold levels, very low coverage in wetlands, and modest coverage in forest and agriculture, has exhibited a full range in L_p, OM0 and feeding metrics. Mean fullness rank and P_{cope} estimated for the Northeast River were strongly correlated with OM0 ($r = -0.95$, $P = 0.05$ and $r = -0.96$, $P = 0.04$, respectively). This subestuary is adjacent to the mouth of the Susquehanna River and may receive a supplement of OM during years of high discharge. We explored this possibility by correlating mean Susquehanna discharge at Conowingo Dam or Elk River USG gauging stations in March (Northeast River did not have a gauge and Elk River was adjacent) (https://waterdata.usgs.gov/md/nwis/monthly/?search_site_no=01578310&agency_cd=USGS&referred_module=sw&format=sites_selection_links) with estimates of OM0 from the Northeast River during 2011-2014. This correlation was very strong ($r = -0.95$, $P = 0.05$), suggesting that the Susquehanna River could be an important source of OM for Northeast River. However, Elk River flow was also well correlated with Elk River OM0 ($r = 0.91$, $P = 0.09$), so a local contribution of OM0 cannot be ruled out.

Comparisons of RNA/DNA ratios of first-feeding larvae for the six development and year combinations during 2014-2016 provided little direct insight on the impact of watershed development, OM levels and feeding success. In a way, this is good news since two urbanizing watersheds appear to be holding their own on two facets of Yellow Perch productivity (L_p and success of first-feeding larvae). These watersheds may be providing some indication of large scale factors that mitigate impacts of development on L_p and OM, zooplankton, and larval feeding linkages when development is beyond, but still near, the threshold. Mattawoman Creek was considered as a "developed" treatment, but most of its watershed was classified by MD DOP as forested (although estimates underwent a 14% decline between 2002 and 2010). Patuxent River, classified by MD DOP as urban, had OM0 much lower than other developed watersheds that were sampled in the feeding study. Much of the development in both watersheds occurs upstream along the fluvial region above the larval estuarine nursery and water moves through a more rural region with floodplain swamps before reaching the tidal-fresh larval nursery. The regions adjacent to the larval nursery are zoned for rural land use. Both developed watersheds had 1% or greater of their watershed area in wetlands. While wetland coverage in these two developed watersheds were less than encountered in the rural “treatments”, all four systems had fringing wetlands along the larval nursery region. Patuxent River had two drinking water reservoirs (Tridelphia and Rocky Gorge) upstream of the larval nursery. The worst performing watersheds in our studies (Piscataway Creek and South, Severn, and Magothy rivers) had higher levels of development, were classified by MD DOP as urban, and had 0-0.5% of their watershed in wetlands.

Larvae examined for RNA/DNA exhibited little contrast in feeding metrics derived from gut contents, and OM0 happened to all fall around the optimum for feeding described by the quadratic equation of mean fullness rank and OM0. Intercepts and slopes of RNA/DNA with length only indicated differences between rural (Choptank River) and developed (Patuxent River) watersheds in 2016, but they exhibited opposing trends. The intercept was higher in the Patuxent River, while the increase in RNA/DNA was greater in the Choptank River.

RNA/DNA varied substantially among years. RNA/DNA ratios for 6-9 mm Yellow Perch larvae from Patuxent and Choptank Rivers during 2015 and 2016 indicated that most were in the well-fed category, while ratios in Mattawoman and Nanjemoy Creeks in 2014 indicated that most were in the starved category. A significant decline in RNA/DNA with length was observed in 2014 and change was not indicated in 2015. In 2016, RNA/DNA increased with length in both Patuxent and Choptank Rivers, but a difference between subestuaries was not observed.

Changes in RNA/DNA ratios with length exhibited by 6-9 mm Yellow Perch were expected to reflect external nutrition, but there was little difference in mean fullness rank, P0, and P_{cope} in the three years and two treatments sampled for RNA/DNA. However, we observed a full spectrum of RNA/DNA change (increase, no change, and decrease) in spite of similar feeding metrics. Ratios of RNA/DNA of better fed larvae in our study were expected to increase with body size (Clemmensen 1994; Rooker et al. 1997). The RNA/DNA ratio reflects the condition of larvae during the few days prior to sampling (Kimura et al. 2000), and the response time of RNA/DNA ratios of larval fishes characterizes the feeding environment within a week of sampling (Tardif et al. 2005). It may be that the lengths we analyzed (6-9 mm), because they were common among years and watershed types, did not provide a full picture of the effect of feeding success that might have emerged by examining larger larvae. We believe that efficiency of our plankton nets declined rapidly with length (although large larvae could be caught in some years) and a more efficient net would be needed to capture larger larvae (see Uphoff 1991).

Prey availability, in terms of quality and quantity, is expected to lead to higher growth and better nutritional condition of fish larvae (Buckley 1984; Canino 1994, Diaz et al. 2011). Chicharo et al. (2003) did not find significant correlations between RNA/DNA ratios and length, while Diaz et al. (2011) found a negative correlation between RNA/DNA and length. RNA/DNA ratios of Atlantic Herring larvae fed shortly after hatching were in the same range as those found for starved larvae and were thought to result from problems in changing from internal to external nutrition (Clemmenson 1994). There was no difference in RNA/DNA ratios for starved and fed Atlantic Herring larvae up to an age of 10 days, but after 10 days, deprivation of food lead to a significant decrease in RNA/DNA ratios in comparison to fed Atlantic Herring larvae (Clemmensen 1994). Poor nutritional condition of larvae not only contributes to increased mortality rates through starvation, but can lead to an increase in predation mortality through diminished escape responses or increased duration of larval stage (Caldarone et al. 2003).

Laboratory studies of RNA/DNA ratios of fed and starved larval Yellow Perch have not been conducted and we have relied on general guidelines from other species (Blom et al. 1997). Tardif et al. (2005) determined that RNA/DNA ratios of Yellow Perch in Lake Saint Pierre, Canada, averaged below 2, but did not provide indication of nutritional state of these larvae. Surveys of larval Striped Bass RNA/DNA in 1981 in the Potomac River estuary exhibited a declining pattern, but ratios stabilized above starvation values (Martin et al. 1985). Blom et al. (1997) detected a decline in RNA/DNA ratios of Atlantic herring *Clupea harengus*; but few herring larvae were observed with ratios indicating starvation.

Significant annual differences in initial size of first-feeding larvae were exhibited by amount of DNA per sample during 2014-2016, indicating that maternal contribution to larval condition was not constant among years. These disparities could reflect general year-to-year differences in condition of stocks in the various rivers or demographic shifts due to year-class differences. In Lake Michigan, female Yellow Perch gonadosomatic index, age, size, and egg production were traits that explained the DNA content of offspring (Heyer et al. 2001), i.e., larger, older, more fecund females would have larger larvae. A significant part of variation in early life history traits of fish can be attributed to maternal influences on individual larvae, including important traits such as length and weight (Heyer et al 2001; Bang et al. 2006). While these important traits are linked to maternal effects, traits that provide a survival advantage likely vary from year to year relative to biotic and abiotic conditions present in the environment (Heyer et al. 2001). Larger larvae may have an advantage over smaller larvae in that they can swim faster, avoid predation better, search greater distances for food, and capture larger sizes and higher numbers of prey. This does not take into consideration, however, initial starvation if there is little spatial and temporal overlap with prey items. Heyer et al. (2001) indicated that a trade-off exists between larval size and yolk sac volume. Under circumstances where food is limited after hatching it would be expected that smaller larvae with larger yolk sacs would have a survival advantage over larger larvae with less body reserves. While larger larvae would again be expected to have a survival advantage as feeding and predator avoidance become important, it is suggested that bigger might not necessarily be better in all cases (Heyer et al. 2001).

Annual L_p (proportion of tows with Yellow Perch larvae during a standard time period and where larvae would be expected) provided an economically collected measure of the product of egg production and egg through early postlarval survival. We used L_p as an index to detect “normal” and “abnormal” larval dynamics. We considered L_p estimates from subestuaries that were persistently lower than those measured in other subestuaries indicative of abnormally low survival. Remaining levels were considered normal. Assuming catchability does not change greatly from year to year, egg production and egg through early postlarval survival would need to be high to produce strong L_p, but only one factor needed to be low to result in lower L_p.

High estimates of L_p that were equal to or approaching 1.0 have been routinely encountered in the past, and it is likely that counts would be needed to measure relative abundance if greater resolution was desired. Mangel and Smith (1990) indicated that presence-absence sampling of eggs would be more useful for indicating the status of depleted stocks and count-based indices would be more accurate for recovered stocks. Larval indices based on counts have been used as a measure of year-class strength of fishes generally (Sammons and Bettoli 1998) and specifically for Yellow Perch (Anderson et al. 1998). Tighter budgets necessitate development of low cost indicators of larval survival and relative abundance in order to pursue ecosystem-based fisheries management. Characterizations of larval survival and relative abundance normally are derived from counts requiring labor-intensive sorting and processing. Estimates of L_p were largely derived in the field and only gut contents and RNA/DNA required laboratory analysis. These latter two analyses represented separate studies rather than a requirement for estimating L_p.

We have relied on correlation and regression analyses to judge the effects of watershed development on Yellow Perch larval dynamics. Ideally, manipulative experiments and formal adaptive management should be employed (Hilborn 2016). In large-scale aquatic ecosystems these opportunities are limited and are not a possibility for us. Correlations are often not causal, but may be all the evidence available. Correlative evidence is strongest when (1) correlation is high, (2) it is found consistently across multiple situations, (3) there are not competing explanations, and (4) the correlation is consistent with mechanistic explanations that can be supported by experimental evidence (Hilborn 2016).

Interpretation of the influence of salinity class or major land cover on L_p needs to consider that our survey design was limited to existing patterns of development. All estimates of L_p at or below target levels of development (forested and agricultural watersheds) or at the threshold or beyond high levels of development (except for one sample) were from brackish subestuaries; estimates of L_p for development between these levels were from tidal-fresh subestuaries with forested watersheds. Larval dynamics below the target level of development primarily reflected eastern shore agricultural watersheds. Two types of land use would be needed to balance analyses: (1) agricultural, tidal-fresh watersheds with below target development and (2) forested, brackish watersheds with development between the target and threshold. We do not believe that these combinations exist where Yellow Perch spawning occurs in Maryland's portion of Chesapeake Bay. The MD DOP forest cover estimates have a minimum mapping unit of 10 acres that mixes forest cover in residential areas (trees over lawns) with true forest cover, clouding interpretation of forest influence (R. Feldt, MD DNR Forest Service, personal communication).

Development was an important influence on Yellow Perch egg and larval dynamics and negative changes generally conformed to impervious surface reference points developed from distributions of dissolved oxygen, and juvenile and adult target fish in mesohaline subestuaries (Uphoff et al. 2011). Hilborn and Stokes (2010) advocated setting reference points related to harvest for fisheries (stressor) based on historical stock performance (outcome) because they were based on experience, easily understood, and not based on modeling. We believe applying IS or C/ha watershed development reference points (stressor) based on L_p (outcome) conforms to the approach advocated by Hilborn and Stokes (2010).

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Table 2-1. Estimates of proportions of ichthyoplankton net tows with Yellow Perch larvae (L_p) during 1965-2016 and data used for correlation and regression with counts of structures per hectare (C/ha), percent agriculture, percent forest, and percent wetland. Salinity class 0 = tidal-fresh (≤ 2.0‰) and 1 = brackish (> 2.0‰). Land use percentages and overall primary land use were determined from Maryland Department of Planning estimates for 1973, 1994, 1997, 2002, or 2010 that were closest to a sampling year.

River	Sample Year	DOP Year	C/ha	% Ag	% Forest	% Wetland	Primary Land Use	Salinity	Lp
Bush (w/ APG)	2006	2002	1.17	21	36.3	5.5	Urban	0	0.79
Bush (w/ APG)	2007	2010	1.19	14.9	32.1	5.5	Urban	0	0.92
Bush (w/ APG)	2008	2010	1.2	14.9	32.1	5.5	Urban	0	0.55
Bush (w/ APG)	2009	2010	1.21	14.9	32.1	5.5	Urban	0	0.86
Bush (w/ APG)	2011	2010	1.23	14.9	32.1	5.5	Urban	0	0.96
Bush (w/ APG)	2012	2010	1.24	14.9	32.1	5.5	Urban	0	0.28
Bush (w/ APG)	2013	2010	1.25	14.9	32.1	5.5	Urban	0	0.15
Choptank	1986	1994	0.07	58.5	32.4	1.3	Agriculture	1	0.53
Choptank	1987	1994	0.08	58.5	32.4	1.3	Agriculture	1	0.73
Choptank	1988	1994	0.08	58.5	32.4	1.3	Agriculture	1	0.8
Choptank	1989	1994	0.08	58.5	32.4	1.3	Agriculture	1	0.71
Choptank	1990	1994	0.08	58.5	32.4	1.3	Agriculture	1	0.66
Choptank	1998	1997	0.1	57.9	31.3	1.2	Agriculture	1	0.6
Choptank	1999	1997	0.1	57.9	31.3	1.2	Agriculture	1	0.76
Choptank	2000	2002	0.1	58.2	30.8	1.1	Agriculture	1	0.25
Choptank	2001	2002	0.1	58.2	30.8	1.1	Agriculture	1	0.21
Choptank	2002	2002	0.11	58.2	30.8	1.1	Agriculture	1	0.38
Choptank	2003	2002	0.11	58.2	30.8	1.1	Agriculture	1	0.52
Choptank	2004	2002	0.12	58.2	30.8	1.1	Agriculture	1	0.41
Choptank	2013	2010	0.13	55	27.8	1.4	Agriculture	1	0.47
Choptank	2014	2010	0.13	55	27.8	1.4	Agriculture	1	0.68
Choptank	2015	2010	0.13	55	27.8	1.4	Agriculture	1	0.82
Choptank	2016	2010	0.13	55	27.8	1.4	Agriculture	1	0.9
Corsica	2006	2002	0.21	64.3	27.4	0.4	Agriculture	1	0.47
Corsica	2007	2010	0.22	60.4	25.5	0.1	Agriculture	1	0.83
Elk	2010	2010	0.59	28	38.7	1.1	Forest	0	0.75
Elk	2011	2010	0.59	28	38.7	1.1	Forest	0	0.79
Elk	2012	2010	0.59	28	38.7	1.1	Forest	0	0.55

Table 2-1 cont.

River	Sample Year	DOP Year	C/ha	% Ag	% Forest	% Wetland	Primary Land Use	Salinity	Lp
Langford	2007	2010	0.07	20.4	70.2	1.5	Agriculture	1	0.83
Magothy	2009	2010	2.73	1.2	21	0	Urban	1	0.1
Magothy	2016	2010	2.78	1.2	21	0	Urban	1	0.1
Mattawoman	1990	1994	0.45	13.8	62.6	0.9	Forest	0	0.81
Mattawoman	2008	2010	0.87	9.3	53.9	2.8	Forest	0	0.66
Mattawoman	2009	2010	0.88	9.3	53.9	2.8	Forest	0	0.92
Mattawoman	2010	2010	0.9	9.3	53.9	2.8	Forest	0	0.82
Mattawoman	2011	2010	0.91	9.3	53.9	2.8	Forest	0	0.99
Mattawoman	2012	2010	0.9	9.3	53.9	2.8	Forest	0	0.2
Mattawoman	2013	2010	0.91	9.3	53.9	2.8	Forest	0	0.47
Mattawoman	2014	2010	0.93	9.3	53.9	2.8	Forest	0	0.78
Mattawoman	2015	2010	0.93	9.3	53.9	2.8	Forest	0	1
Mattawoman	2016	2010	0.93	9.3	53.9	2.8	Forest	0	0.82
Middle	2012	2010	3.33	3.4	23.3	2.1	Urban	0	0
Nanjemoy	2009	2010	0.09	12.4	68.7	4.1	Forest	1	0.83
Nanjemoy	2010	2010	0.09	12.4	68.7	4.1	Forest	1	0.96
Nanjemoy	2011	2010	0.09	12.4	68.7	4.1	Forest	1	0.99
Nanjemoy	2012	2010	0.09	12.4	68.7	4.1	Forest	1	0.03
Nanjemoy	2013	2010	0.09	12.4	68.7	4.1	Forest	1	0.46
Nanjemoy	2014	2010	0.09	12.4	68.7	4.1	Forest	1	0.82
Nanticoke	1965	1973	0.05	46.6	43.4	8.1	Agriculture	1	0.5
Nanticoke	1967	1973	0.05	46.6	43.4	8.1	Agriculture	1	0.43
Nanticoke	1968	1973	0.05	46.6	43.4	8.1	Agriculture	1	1
Nanticoke	1970	1973	0.06	46.6	43.4	8.1	Agriculture	1	0.81
Nanticoke	1971	1973	0.06	46.6	43.4	8.1	Agriculture	1	0.33
Nanticoke	2004	2002	0.11	46.3	40.7	7.4	Agriculture	1	0.49
Nanticoke	2005	2002	0.11	46.3	40.7	7.4	Agriculture	1	0.67
Nanticoke	2006	2002	0.11	46.3	40.7	7.4	Agriculture	1	0.35
Nanticoke	2007	2010	0.11	45	39.4	7.4	Agriculture	1	0.55
Nanticoke	2008	2010	0.11	45	39.4	7.4	Agriculture	1	0.19
Nanticoke	2009	2010	0.11	45	39.4	7.4	Agriculture	1	0.41
Nanticoke	2011	2010	0.11	45	39.4	7.4	Agriculture	1	0.55

Table 2-1 cont.

River	Sample Year	DOP Year	C/ha	% Ag	% Forest	% Wetland	Primary Land Use	Salinity	Lp
Nanticoke	2012	2010	0.11	45	39.4	7.4	Agriculture	1	0.04
Nanticoke	2013	2010	0.11	45	39.4	7.4	Agriculture	1	0.43
Nanticoke	2014	2010	0.11	45	39.4	7.4	Agriculture	1	0.35
Nanticoke	2015	2010	0.11	45	39.4	7.4	Agriculture	1	0.64
Nanticoke	2016	2010	0.11	45	39.4	7.4	Agriculture	1	0.67
Northeast	2010	2010	0.46	31.1	38.6	0.1	Forest	0	0.68
Northeast	2011	2010	0.46	31.1	38.6	0.1	Forest	0	1
Northeast	2012	2010	0.47	31.1	38.6	0.1	Forest	0	0.77
Northeast	2013	2010	0.47	31.1	38.6	0.1	Forest	0	0.72
Northeast	2014	2010	0.48	31.1	38.6	0.1	Forest	0	0.77
Patuxent	2015	2010	1.22	20.5	35.1	1	Urban	1	0.72
Patuxent	2016	2010	1.22	20.5	35.1	1	Urban	1	0.82
Piscataway	2008	2010	1.41	10	40.4	0.2	Urban	0	0.47
Piscataway	2009	2010	1.43	10	40.4	0.2	Urban	0	0.39
Piscataway	2010	2010	1.45	10	40.4	0.2	Urban	0	0.54
Piscataway	2011	2010	1.46	10	40.4	0.2	Urban	0	0.65
Piscataway	2012	2010	1.47	10	40.4	0.2	Urban	0	0.16
Piscataway	2013	2010	1.49	10	40.4	0.2	Urban	0	0.5
Severn	2002	2002	2.02	8.6	35.2	0.2	Urban	1	0.16
Severn	2004	2002	2.09	8.6	35.2	0.2	Urban	1	0.35
Severn	2005	2002	2.15	8.6	35.2	0.2	Urban	1	0.4
Severn	2006	2002	2.18	8.6	35.2	0.2	Urban	1	0.27
Severn	2007	2010	2.21	5	28	0.2	Urban	1	0.3
Severn	2008	2010	2.24	5	28	0.2	Urban	1	0.08
Severn	2009	2010	2.25	5	28	0.2	Urban	1	0.15
Severn	2010	2010	2.26	5	28	0.2	Urban	1	0.03
South	2008	2010	1.61	10.2	39.2	0.5	Urban	1	0.14

Table 2-2. Regression coefficients for DNA and RNA standard curves used for quantification of sample concentrations of nucleic acids in 2014.

Model	Intercept	Slope ([DNA or RNA])	Slope ([DNA or RNA]^2	p-value	R^2
DNA	2.111	4.15E-03	-2.71E-06	<0.0001	0.90
RNA	5.802	6.85E-03	-4.16E-06	<0.0001	0.95

Table 2-3. Summary of results of regressions of proportions of tows with Yellow Perch larvae (L_p) and (A) counts of structures per hectare (C/ha), (B) percent agriculture, and (C) percent forest. Separate regressions by salinity (tidal-fresh ≤ 2.0 ‰ and brackish > 2.0 ‰) and a multiple regression using salinity as a class variable (tidal-fresh = 0 and brackish = 1) are presented.

ANOVA			(A) Brackish			
Source	df	SS	MS	F	P	
Model	1	1.14514	1.14514	20.9	<.0001	
Error	53	2.90407	0.05479			
Total	54	4.04921				
r^2	0.2828					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.59776	0.03773	15.84	<.0001	0.52208	0.67343
C / ha	-0.16635	0.03639	-4.57	<.0001	-0.23933	0.09336

ANOVA			(A) Tidal-Fresh			
Source	df	SS	MS	F	P	
Model	1	0.77363	0.77363	15.51	0.0005	
Error	30	1.49597	0.04987			
Total	31	2.2696				
r^2	0.3409					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.94825	0.08595	11.03	<.0001	0.7727	1.12379
C / ha	-0.28944	0.07348	-3.94	0.0005	-0.43951	-0.13937

ANOVA			(A) Multiple Regression			
Source	df	SS	MS	F	P	
Model	2	2.22519	1.11259	20.7	<.0001	
Error	84	4.51443	0.05374			
Total	86	6.73962				
r^2	0.3302					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.84368	0.05316	15.87	<.0001	0.73797	0.94939
C / ha	-0.1888	0.03258	-5.79	<.0001	-0.2536	-0.12401
Salinity	-0.23317	0.05378	-4.34	<.0001	-0.34011	-0.12622

Table 2-3 cont.

ANOVA	(B) Brackish					
Source	df	SS	MS	F	P	
Model	1	0.4211	0.4211	6.15	0.0163	
Error	53	3.62811	0.06845			
Total	54	4.04921				
r^2	0.104					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.35032	0.07105	4.93	<.0001	0.20782	0.49282
% Ag	0.00416	0.00168	2.48	0.0163	0.0007952	0.00752

ANOVA	(B) Tidal-Fresh					
Source	df	SS	MS	F	P	
Model	1	0.21286	0.21286	3.1	0.0883	
Error	30	2.05674	0.06856			
Total	31	2.2696				
r^2	0.0938					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.49679	0.09725	5.11	<.0001	0.29818	0.69541
% Ag	0.00944	0.00536	1.76	0.0883	-0.0015	0.02038

ANOVA	(B) Multiple Regression					
Source	df	SS	MS	F	P	
Model	2	0.99404	0.49702	7.27	0.0012	
Error	84	5.74559	0.0684			
Total	86	6.73962				
r^2	0.1475					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.57362	0.05281	10.86	<.0001	0.4686	0.67864
% Ag	0.00463	0.0016	2.89	0.0048	0.00145	0.00781
Salinity	-0.24065	0.06701	-3.59	0.0006	-0.37391	-0.10739

Table 2-3 cont.

ANOVA			(C) Brackish			
Source	df	SS	MS	F	P	
Model	1	0.38879	0.38879	5.63	0.0213	
Error	53	3.66042	0.06906			
Total	54	4.04921				
r^2	0.096					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.25063	0.11222	2.23	0.0298	0.02553	0.47572
% Forest	0.00655	0.00276	2.37	0.0213	0.00101	0.01208

ANOVA			(C) Tidal-Fresh			
Source	df	SS	MS	F	P	
Model	1	0.22878	0.22878	3.36	0.0766	
Error	30	2.04082	0.06803			
Total	31	2.2696				
r^2	0.1008					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.26392	0.21418	1.23	0.2274	-0.1735	0.70134
% Forest	0.00908	0.00495	1.83	0.0766	-0.00103	0.0192

ANOVA			(C) Multiple Regression			
Source	df	SS	MS	F	P	
Model	2	1.02471	0.51235	7.53	0.001	
Error	84	5.71491	0.06803			
Total	86	6.73962				
r^2	0.152					
	Coefficients	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.346	0.11121	3.11	0.0025	0.12486	0.56715
% Forest	0.00714	0.0024	2.98	0.0038	0.00237	0.0119
Salinity	-0.1183	0.05864	-2.02	0.0468	-0.23492	-0.00169

Table 2-4. Summary of Akaike's Information Criteria from regressions of proportions of tows with Yellow perch larvae (Lp) and (A) counts of structures per hectare (C/ha), (B) percent agriculture, and (C) percent forest for each salinity category and a multiple regression using salinity as a class variable.

Model (A)	MSE	n	K	-2loge(MSE)	2K	2K(K+1)	(n-K-1)	AICc	Delta brackish	Delta fresh
Categorical	0.05374	87	4	2.92360	8	40	82	11.4	2.04	1.56
Fresh	0.04987	32	3	2.99834	6	24	28	9.9		
Brackish	0.05479	55	3	2.90425	6	24	51	9.4		

Model (B)	MSE	n	K	-g2loge(MSE)	2K	2K(K+1)	(n-K-1)	AICc	Delta brackish	Delta fresh
Categorical	0.0684	87	4	2.682382454	8	40	82	11.2	2.02	1.63
Fresh	0.06856	32	3	2.680046005	6	24	28	9.5		
Brackish	0.06845	55	3	2.681651727	6	24	51	9.2		

Model (C)	MSE	n	K	-2loge(MSE)	2K	2K(K+1)	(n-K-1)	AICc	Delta brackish	Delta fresh
Categorical	0.06803	87	4	2.687806495	8	40	82	11.2	2.03	1.63
Fresh	0.06803	32	3	2.687806495	6	24	28	9.5		
Brackish	0.06906	55	3	2.672779587	6	24	51	9.1		

Table 2-5. Correlations among major watershed land uses in watersheds sampled for L_p (see Table 2-1 for values).

Parameter	Statistic	C / ha	% Agriculture	% Forest
% Agriculture	r	-0.7897		
	P	<.0001		
	N	51		
% Forest	r	-0.3953	-0.1790	
	P	0.0041	0.2089	
	N	51	51	
% Wetland	r	-0.3370	0.1569	0.1867
	P	0.0156	0.2717	0.1897
	N	51	51	51

Table 2-6. Summary of estimates used in regression analyses of Yellow Perch larval feeding success. C/ha = counts of structures per hectare. Mean full = mean of fullness ranks assigned to larval guts. OM0 = proportion of samples without organic matter (detritus). P0 = proportion of guts without food. Pclad = proportion of guts with cladocerans. Pcope = proportion of guts with copepods. Pother = proportion of guts with "other" food items. Mean TL = mean TL of larvae in mm. N = number of Yellow Perch larvae examined. Lp = estimates of proportions of ichthyoplankton net tows with Yellow Perch larvae. 2016 analyses are restricted to sizes-in-common of 6-9 mm for all years.

River	Year	C / ha	Mean full	OM0	P0	Pclad	Pcope	Pother	Mean TL	N	Lp
Elk	2010	0.59	2.43		0.00	0.00	1.00	0.14	8.7	7	0.75
Mattawoman	2010	0.90	1.42		0.15	0.15	0.67	0.09	8.4	33	0.82
Nanjemoy	2010	0.09	2.89		0.00	0.08	1.00	0.19	8.6	36	0.96
Northeast	2010	0.46	1.89		0.28	0.30	0.57	0.32	7.5	47	0.68
Piscataway	2010	1.45	2.24		0.03	0.00	0.52	0.67	8.4	33	0.54
Elk	2011	0.59	2.98	0.76	0.07	0.00	0.93	0.00	8.1	60	0.79
Mattawoman	2011	0.91	0.59	0.78	0.48	0.00	0.41	0.11	8.2	54	0.99
Nanjemoy	2011	0.09	2.00	0.56	0.07	0.05	0.77	0.13	7.5	84	0.99
Nanticoke	2011	0.11	3.23	0.55	0.08	0.70	0.93	0.13	7.9	40	0.55
Northeast	2011	0.46	2.43	0.58	0.10	0.00	0.89	0.08	8.0	72	1.00
Piscataway	2011	1.46	0.00	1.00	1.00	0.00	0.00	0.00	8.2	27	0.65
Bush	2012	1.24	2.33		0.00	0.52	0.52	1.00	8.2	33	0.28
Elk	2012	0.59	0.76	0.77	0.25	0.02	0.00	0.69	7.6	190	0.55
Mattawoman	2012	0.90	1.69	1.00	0.00	0.38	0.85	1.00	8.3	13	0.20
Northeast	2012	0.47	1.17	0.99	0.02	0.05	0.08	0.98	7.5	198	0.77
Piscataway	2012	1.47	1.63	0.98	0.00	0.50	0.75	1.00	8.5	8	0.16
Choptank	2013	0.13	1.00	0.33	0.22	0.34	0.35	0.32	7.5	302	0.47
Mattawoman	2013	0.91	1.66	0.79	0.00	0.81	0.66	0.05	7.2	80	0.47
Nanjemoy	2013	0.09	1.60	0.65	0.00	0.60	0.44	0.23	7.3	62	0.46
Nanticoke	2013	0.11	0.97	0.13	0.37	0.35	0.23	0.24	7.8	116	0.43
Northeast	2013	0.47	0.50	1.00	0.52	0.13	0.00	0.41	7.9	46	0.72
Piscataway	2013	1.49	2.33	0.74	0.00	0.33	0.75	0.17	7.6	12	0.50
Choptank	2014	0.13	1.56	0.60	0.006	0.87	0.54	0.88	7.8	539	0.68
Mattawoman	2014	0.93	1.88	0.72	0.00	0.95	0.87	1.00	7.1	241	0.78
Nanjemoy	2014	0.09	2.43	0.53	0.00	0.34	0.73	0.42	7.7	292	0.82
Nanticoke	2014	0.11	1.36	0.11	0.00	0.61	0.43	0.04	8.1	28	0.35
Northeast	2014	0.48	1.40	0.86	0.05	0.65	0.53	0.69	7.9	395	0.77
Choptank	2015	0.13	2.89	0.54	0.00	0.34	0.93	0.29	7.2	227	0.82
Mattawoman	2015	0.93	1.58	0.74	0.06	0.11	0.73	0.22	7.5	738	1.00
Nanticoke	2015	0.11	1.51	0.25	0.09	0.34	0.76	0.27	7.8	237	0.64
Patuxent	2015	1.22	2.33	0.56	0.00	0.87	0.90	0.36	7.4	215	0.72
Choptank	2016	0.13	2.72	0.45	0.008	0.45	0.75	0.12	8.0	247	0.90
Nanticoke	2016	0.11	1.79	0.19	0.08	0.50	0.46	0.13	9.3	24	0.67
Patuxent	2016	1.22	2.35	0.56	0.008	0.52	0.99	0.00	8.5	237	0.82

Table 2-7. Summary of regression results for Yellow Perch larval feeding success. Mean full = average feeding rank of larvae. OM0 = proportion of samples without organic matter (detritus). P0 = proportion of guts without food. Pclad = proportion of guts with cladocerans. Pcope = proportion of guts with copepods. Pother = proportion of guts with “other” food items. Independent/dependent = how variables were assigned in regression analyses, shape = shape of plotted data with ND indicating none detected, r^2 = coefficient of determination, and P = level of significance. Gray shading indicates significance of interest at $P \leq 0.05$.

Independent	Dependent	Relationship	Shape	r^2	P	Intercept	SE	Parameter a	SE	Parameter b	SE
OM0	P0	Quadratic	U	0.20	0.07	0.3078	0.1774	-1.1217	0.6355	1.1277	0.5297
OM0	Pclad	Quadratic	Dome	0.08	0.36	0.3891	0.2513	0.4466	0.9002	-0.6118	0.7503
OM0	Pcope	Quadratic	Dome	0.34	0.006	0.0897	0.2234	2.4862	0.8004	-2.2881	0.6671
OM0	Pother	Linear	Increase	0.19	0.02	-0.0029	0.1573	0.5667	0.2302	---	---
OM0	Mean full	Quadratic	Dome	0.40	0.002	0.5096	0.5397	6.4350	1.9333	-6.1175	1.6113
Pclad	P0	Linear	Decline	0.16	0.02	0.2161	0.0524	-0.2872	0.1158	---	---
Pcope	P0	Linear	Decline	0.41	<.0001	0.3912	0.0649	-0.4469	0.0951	---	---
Pother	P0	Linear	Decline	0.06	0.16	0.1711	0.0521	-0.1516	0.1053	---	---
P0	Mean full	Linear	Decline	0.48	<.0001	2.1034	0.1100	-2.5309	0.4658	---	---
Pclad	Mean full	Linear	ND	0.03	0.35	1.6597	0.2061	0.4309	0.4557	---	---
Pcope	Mean full	Linear	Increase	0.69	<.0001	0.4992	0.1705	2.1284	0.2498	---	---
Pother	Mean full	Linear	ND	0.02	0.46	1.9166	0.1947	-0.2931	0.3937	---	---

Table 2-8. Summary of feeding success, larval length, sample size, and RNA/DNA characteristics, by subestuary and sample date (A = 2014, B = 2015, C = 2016). Data only for dates with feeding information and with RNA/DNA analysis are summarized. Mean fullness = mean feeding rank. Mean TL is in mm. N = the sample size of larvae processed for gut contents and with RNA/DNA ratios available for the date. Mean RNA/DNA is the average for the date. SE RNA/DNA is the standard error for the date. *Pf* RNA/DNA ≥ 3 is the number of ratios above the fed criterion. *Ps* RNA/DNA ≤ 2 is the number of ratios below the starvation criterion.

Subestuary (A)	Variable	9-Apr	14-Apr	17-Apr	22-Apr	24-Apr
Mattawoman	Mean Fullness	1.7	2	2.3		2.2
	Mean TL	6.3	7.5	9		10.8
	N	102	122	6		6
	Mean RNA/DNA	2.37	1.69	1.22		1.06
	SE RNA/DNA	0.06	0.06	0.07		0.08
	Pf (RNA/DNA ≥ 3)	13	6	0		0
	Ps (RNA/DNA ≤ 2)	35	90	6		6
Nanjemoy	Mean Fullness	1.8	2.8	2.8	2.9	3
	Mean TL	6.8	8.1	8.5	11	11.1
	N	89	102	78	67	16
	Mean RNA/DNA	2.27	1.54	1.52	1.27	1.09
	SE RNA/DNA	0.07	0.05	0.07	0.08	0.04
	Pf (RNA/DNA ≥ 3)	10	1	2	2	0
	Ps (RNA/DNA ≤ 2)	31	87	62	62	1

Subestuary (B)	Variable	13-Apr	15-Apr	22-Apr	24-Apr	29-Apr	
Choptank	Mean Fullness	2.7	3.0	3.2	2.7	3.0	
	Mean TL	6.9	7.4	9.6	11.1	11.8	
	N	50	60	13	7	6	
	Mean RNA/DNA	3.89	4.00	4.29	4.73	5.36	
	SE RNA/DNA	0.29	0.18	0.35	0.31	0.72	
	Pf (RNA/DNA ≥ 3)	0.50	0.82	0.77	1	1	
	Ps (RNA/DNA ≤ 2)	0.38	0.10	0.08	0	0	
		14-Apr	16-Apr	21-Apr	23-Apr	28-Apr	30-Apr
Patuxent	Mean Fullness	2.2	2.4	2.1	2.2	2.6	3.3
	Mean TL	7.2	7.4	9.8	10.8	12.3	13.3
	N	50	49	45	10	9	4
	Mean RNA/DNA	4.68	5.10	4.07	5.61	5.35	5.75
	SE RNA/DNA	0.15	0.11	0.29	0.18	0.29	0.43
	Pf (RNA/DNA ≥ 3)	1	1	0.60	1	1	1
	Ps (RNA/DNA ≤ 2)	0	0	0.18	0	0	0

Table 2-8 cont.

Subestuary (C)	Variable	28-Mar	31-Mar	4-Apr	7-Apr	
Choptank	Mean Fullness	2.6	3.1	3.0	2.2	
	Mean TL	6.9	7.5	8.1	8.7	
	N	60	60	60	30	
	Mean RNA/DNA	3.37	3.74	8.72	8.47	
	SE RNA/DNA	0.13	0.18	0.32	0.48	
	Pf (RNA/DNA ≥ 3)	0.57	0.68	1	1	
	Ps (RNA/DNA ≤ 2)	0.05	0.02	0	0	
		30-Mar	1-Apr	6-Apr	8-Apr	12-Apr
Patuxent	Mean Fullness	2.2	2.6	2.8	1.5	2.7
	Mean TL	7.2	6.8	8.3	8.7	8.9
	N	42	30	59	30	6
	Mean RNA/DNA	7.54	8.90	9.12	10.41	9.27
	SE RNA/DNA	0.18	0.31	0.18	0.39	0.95
	Pf (RNA/DNA ≥ 3)	1	1	1	1	1
	Ps (RNA/DNA ≤ 2)	0	0	0	0	0

Table 2-9. Summary of variable values for land use, feeding, and RNA/DNA analyses and a comparison with the range encountered during monitoring of feeding during 2010-2016 (see Table 2.6). C/ha = counts of structures per hectare in the watershed. % Wetland is the percent of watershed in wetlands. OM0 = proportion of samples without organic matter (detritus). Mean full = mean of fullness ranks assigned to larval guts. P0 = proportion of guts without food. Pcope = proportion of guts with copepods. Lp is the proportion of tows with Yellow Perch larvae. DNA is the mean amount of DNA (ng / ml) measured in larvae in 6.0 and 6.5 mm length categories. RNA/DNA is the mean RNA/DNA ratio in 6.0 and 6.5 mm length categories. Slope is the rate of change with length of the RNA/DNA ratio estimated by regression and intercept is the initial ratio at 6 mm estimated by the same regression.

	2014		2015		2016		2010-2016 All feeding studies	
Land Class	Rural Nanjemoy Creek	Developed Mattawoman Creek	Rural Choptank River	Developed Patuxent River	Rural Choptank River	Developed Patuxent River	Min	Max
C/ha	0.09	0.93	0.13	1.22	0.13	1.22	0.09	1.49
% Wetland	4.1	2.8	1.4	1	1.4	1	0.1	7.4
OM0	0.53	0.72	0.54	0.56	0.45	0.56	0.11	1
Mean full	2.43	1.88	2.89	2.33	2.72	2.35	0	3.23
P0	0	0	0	0	0.01	0.01	0	1
Pcope	0.73	0.87	0.93	0.9	0.75	0.99	0	1
Lp	0.82	0.78	0.82	0.72	0.9	0.82	0.16	1
DNA	6183	5736	3885	3382	2240	1160		
RNA/DNA	2.2	2.4	4.0	4.9	3.3	8.1		
Slope	-0.39967	-0.40391	-0.00652	-0.17545	2.15235	0.78574		
Intercept	2.46577	2.40369	3.95018	5.11454	2.17883	7.45221		

Figure 2-1. Areas sampled Yellow Perch larval presence-absence studies, 2006-2016. Areas sampled in 2016 are highlighted in green. Nanticoke River watershed delineation was unavailable for Delaware and Northeast and was unavailable for Pennsylvania.

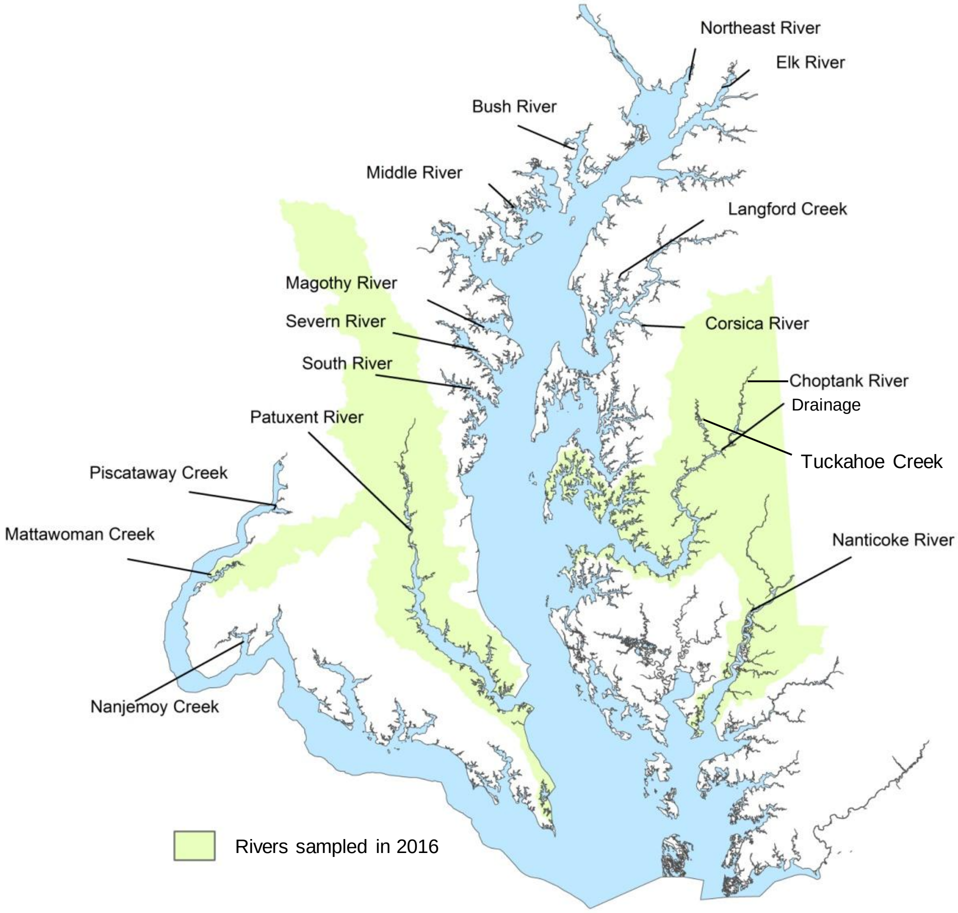


Figure 2-2. Proportion of tows with larval Yellow Perch (*L*p) and its 95% confidence interval in systems studied during 2016. Mean *L*p of brackish tributaries indicated by diamond and tidal-fresh mean indicated by dash.

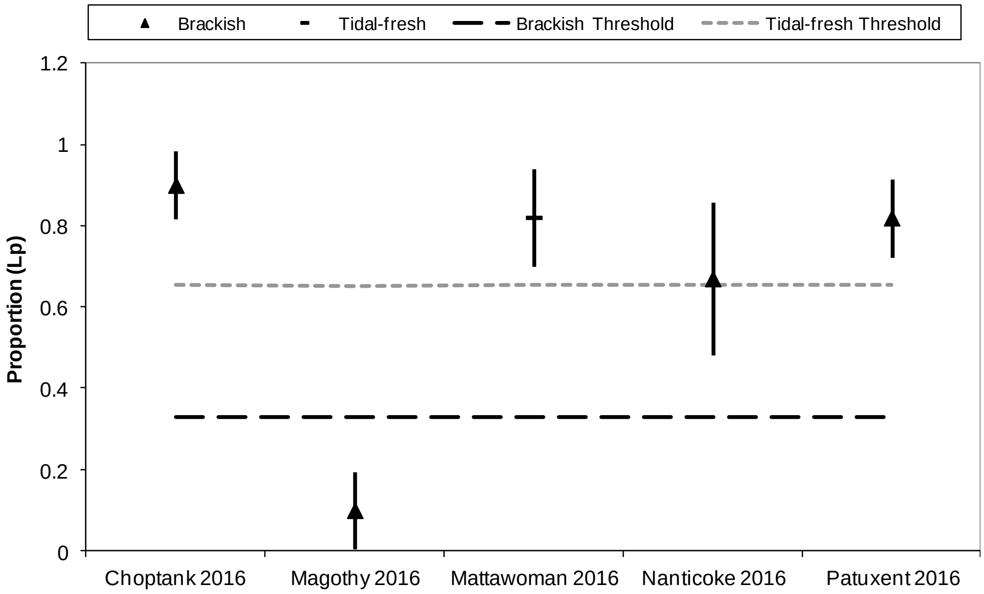


Figure 2-3. Proportion of tows with Yellow Perch larvae (*Lp*) for brackish subestuaries, during 1965-2016. Dotted line provides reference for persistent poor *Lp* exhibited in developed brackish subestuaries. Dominant Department of Planning land use is indicated by symbol color (gold = agriculture, green = forest, and red = urban).

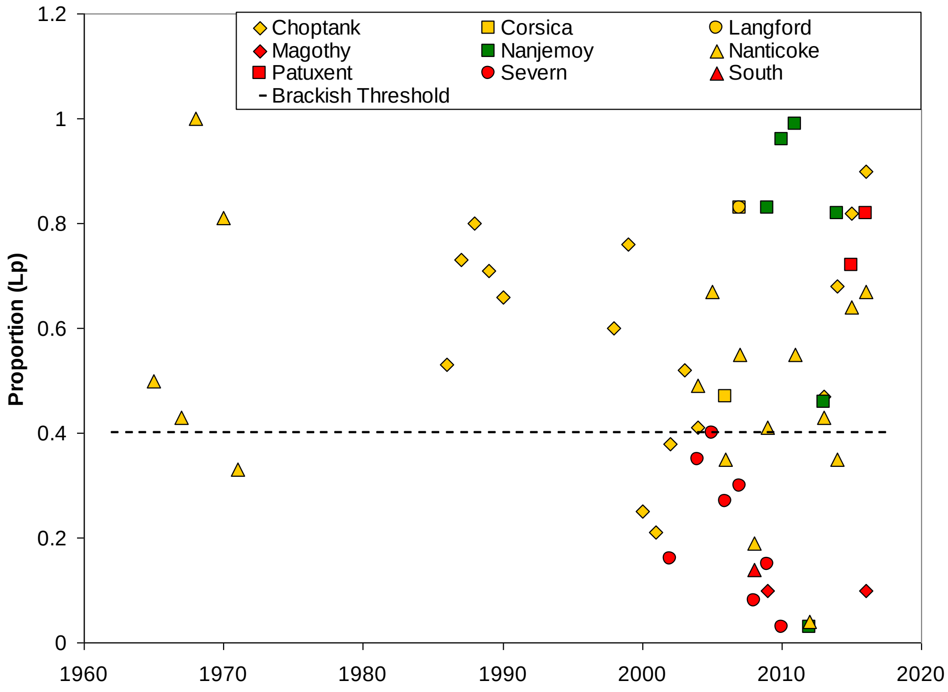


Figure 2-4. Proportion of tows with Yellow Perch larvae (*Lp*) for tidal-fresh subestuaries, during 1990-2016. Dotted line provides reference for consistent poor *Lp* exhibited in a more developed tidal-fresh subestuary (Piscataway Creek). Dominant Department of Planning land use is indicated by symbol color (green = forest and red = urban).

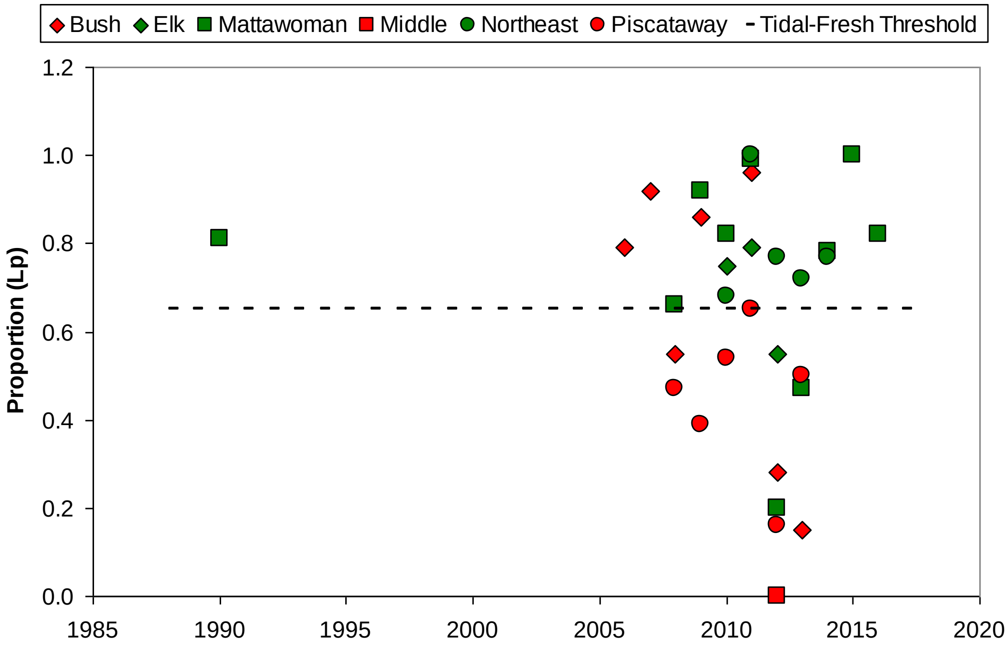


Figure 2-5. Relationship of proportion of plankton tows with Yellow Perch larvae and development (structures per hectare or C/ha) indicated by multiple regression of fresh and brackish subestuaries combined (prediction = MR) and separate linear regressions for both (prediction = LR).

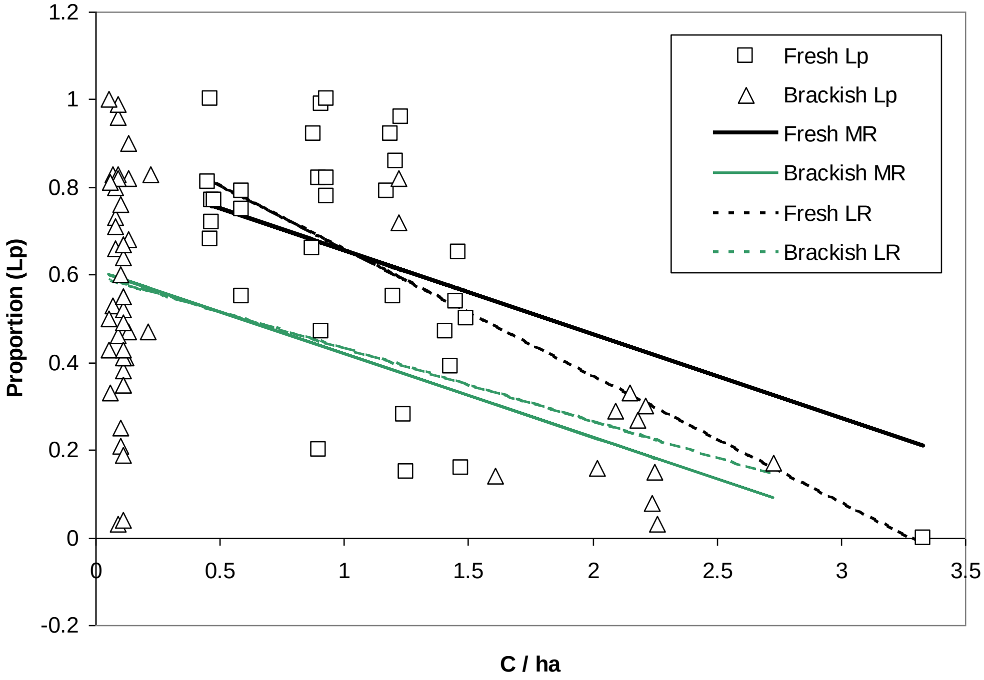


Figure 2-6. Proportion of plankton tows with Yellow Perch larvae plotted against development (C/ha) with Department of Planning land use designations and salinity class indicated by symbols. Diamonds and a “1” behind land use in the key indicate brackish subestuaries, while squares and a “0” indicate tidal-fresh.

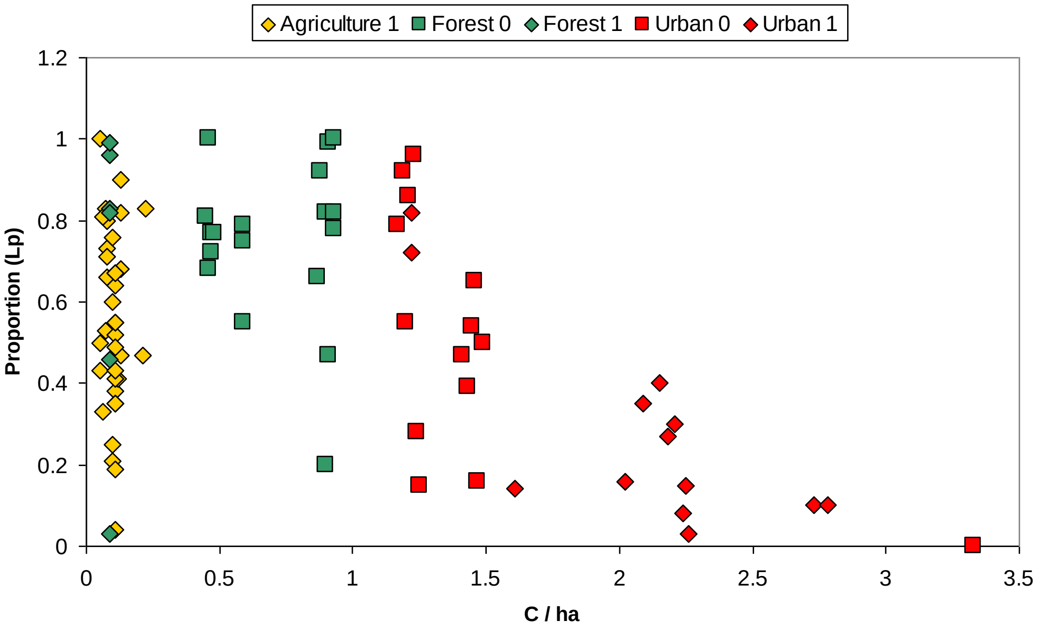


Figure 2-7. Bivariate plots of major land uses in watersheds sampled for *Lp* (A = agriculture, B = forest, and C = wetlands) versus development (structures per hectare or C/ha).

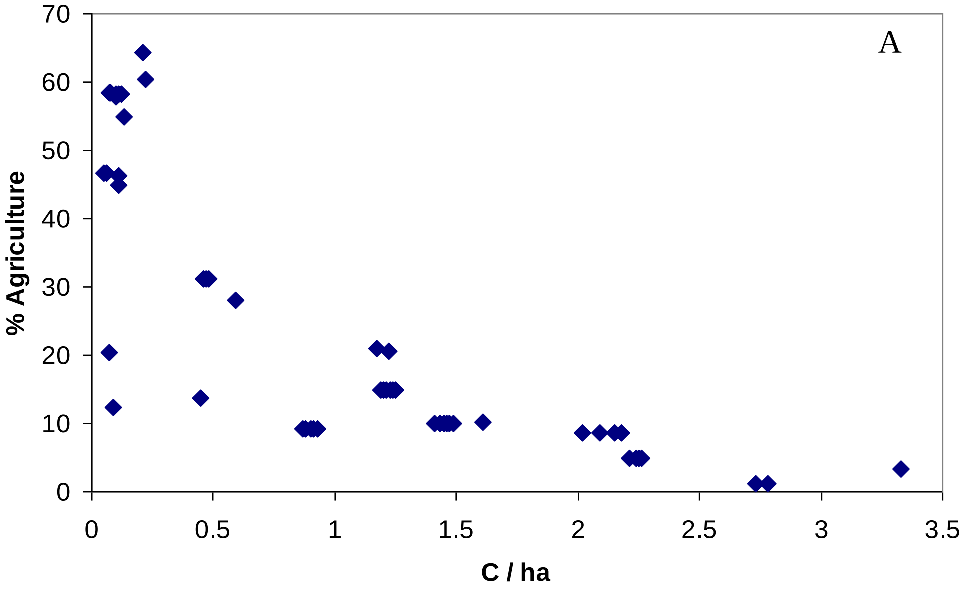
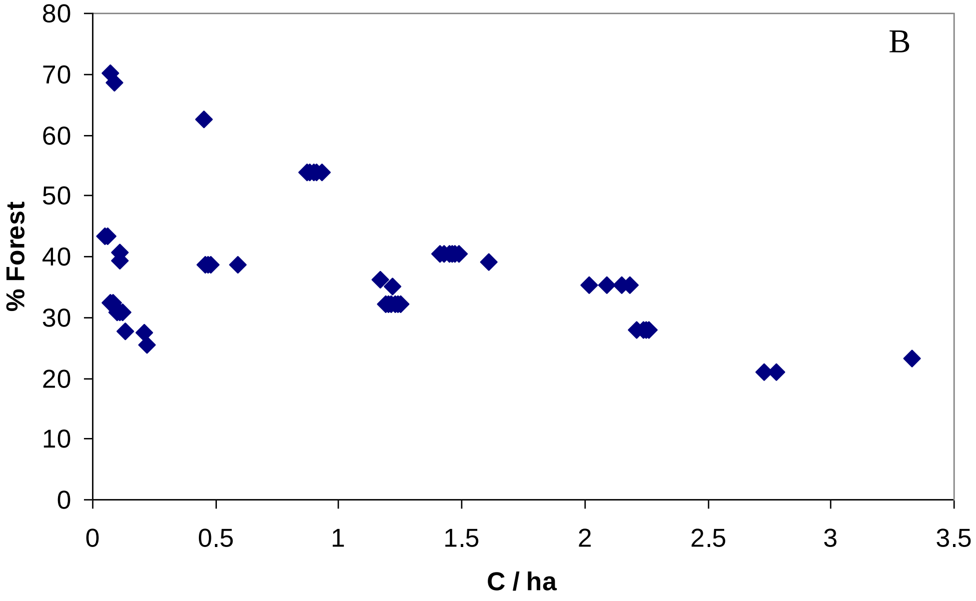


Figure 2-7 cont.



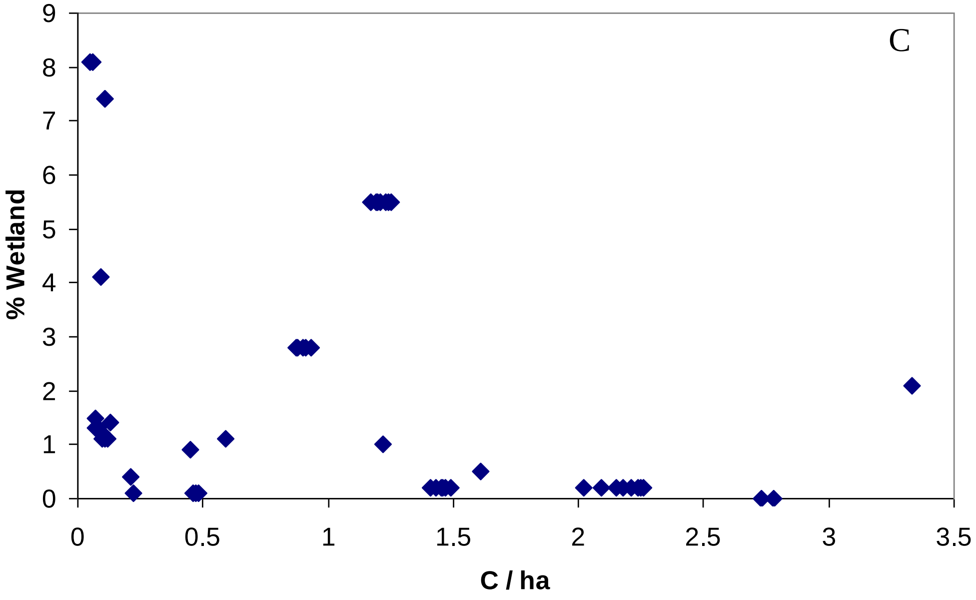


Figure 2-8. Relationship of proportion of plankton tows without detritus (OM0) and development (structures per hectare or C/ha). Dominant Department of Planning land use is indicated by symbol color (gold = agriculture, green = forest, and red = urban).

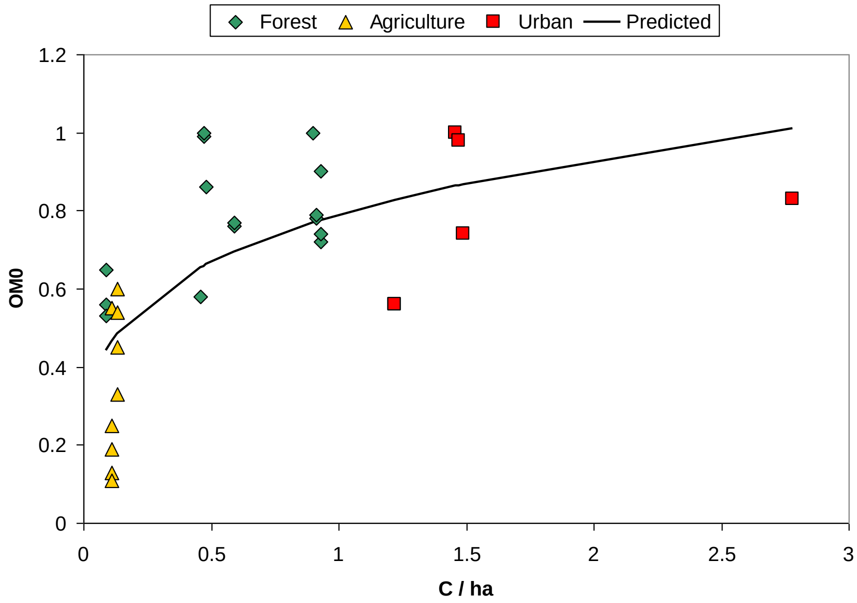


Figure 2-9. Suggested relationship of mean fullness rank of larval Yellow Perch and proportion of plankton tows without detritus (OM0) during 2011-2016. Dominant Department of Planning land use is indicated by symbol color (gold = agriculture, green = forest, and red = urban).

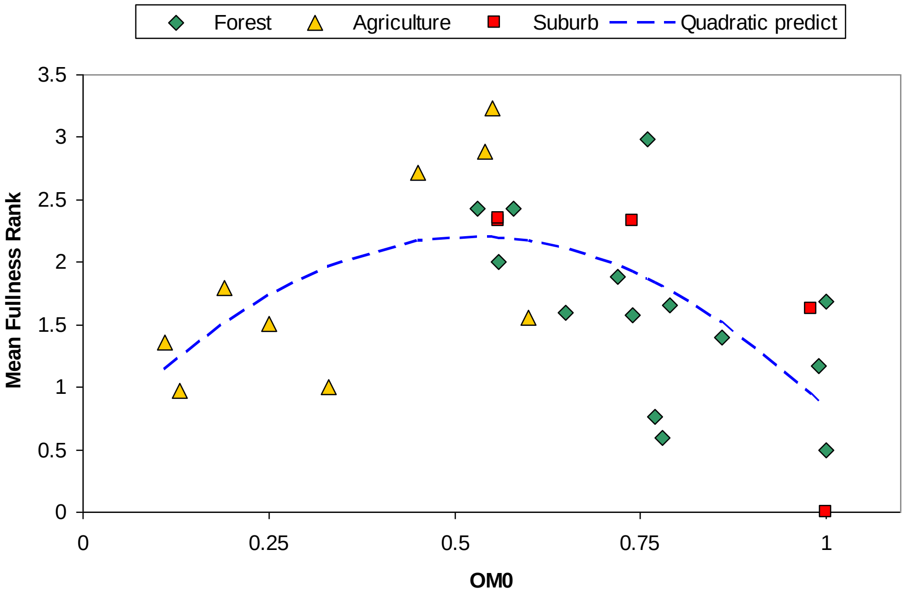


Figure 2-10. (A) Relationship of percent wetlands per watershed obtained from 2010 Department of Planning estimations and level of development (C/ha). (B) Proportion of samples without organic material (OM0) and percent wetlands per watershed.

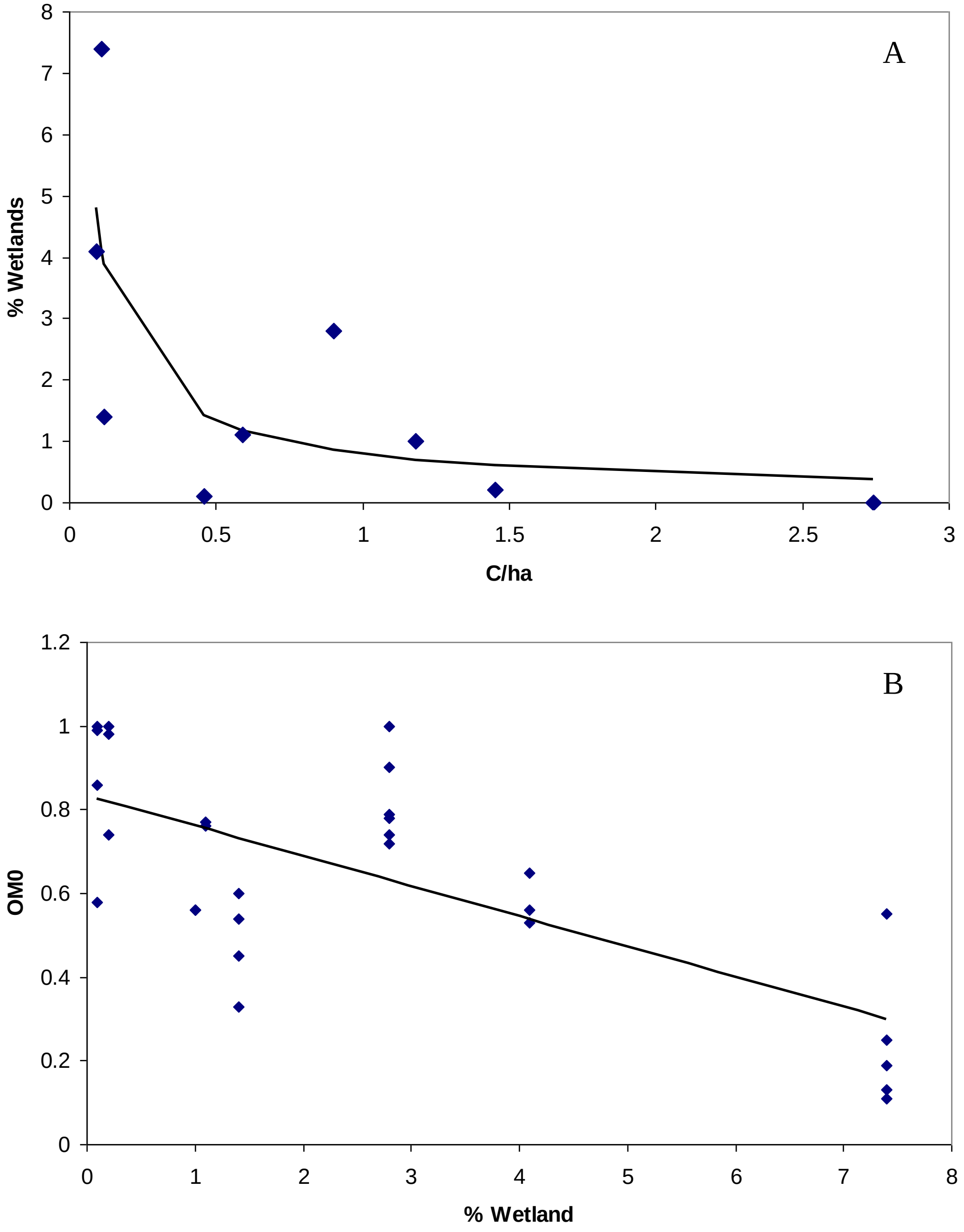


Figure 2-11. RNA/DNA ratios for Yellow Perch larvae by total length. (A) Larvae collected from Mattawoman and Nanjemoy Creeks during 2014. (B) Larvae collected from Patuxent and Choptank Rivers during 2015. (C) Larvae collected from Patuxent and Choptank Rivers during 2016. Subestuaries are indicated by symbols. Reference lines are provided for ratios indicative of starved and fed conditions.

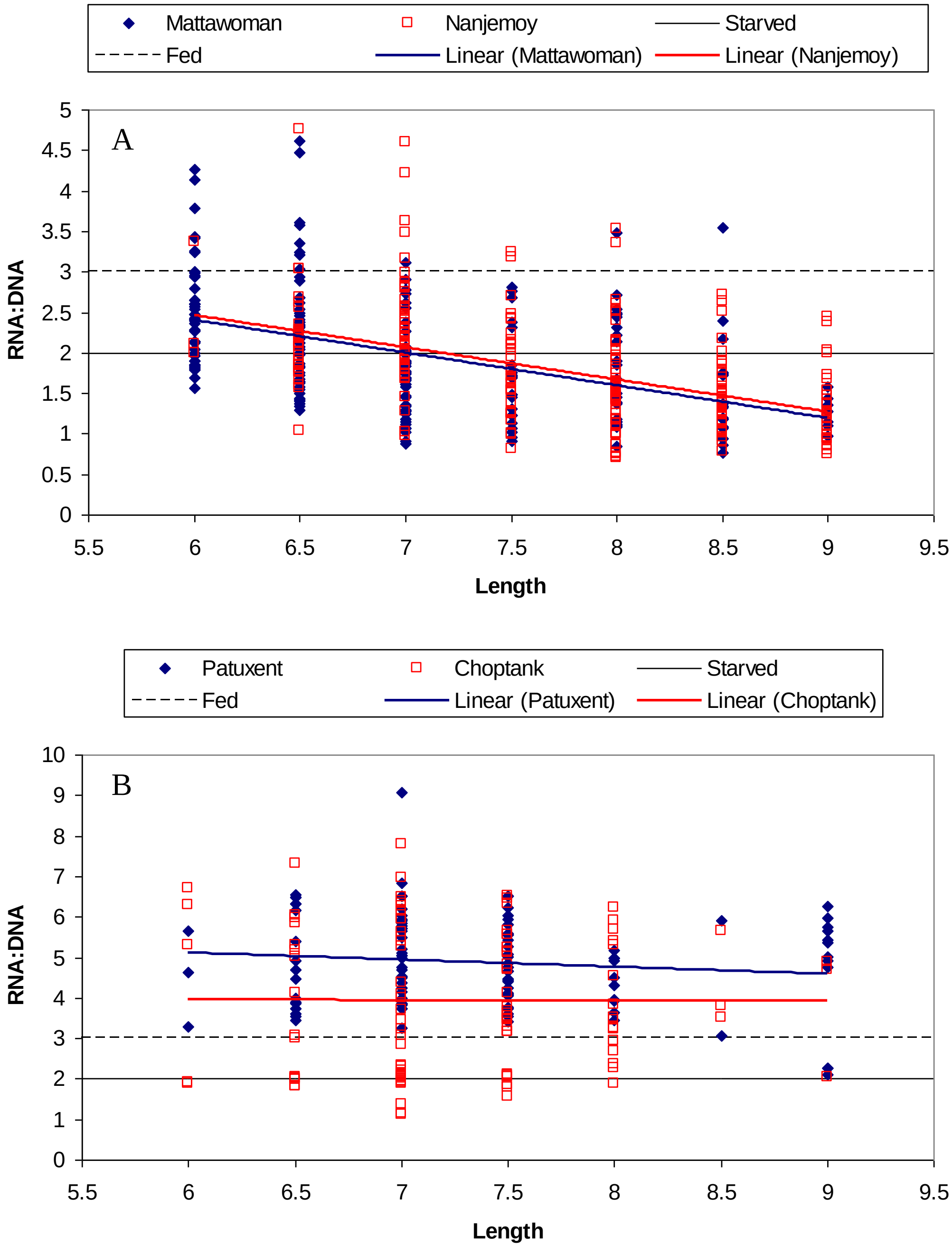


Figure 2-12 cont.

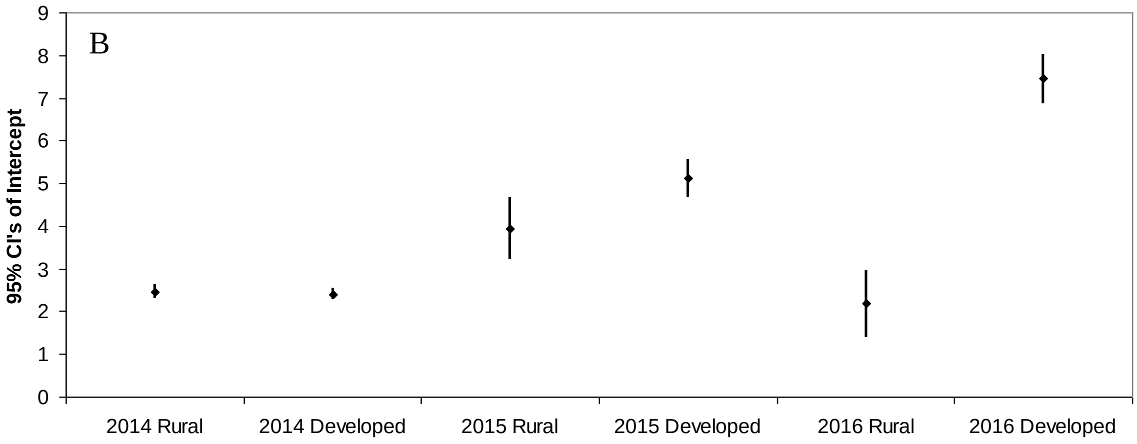


Figure 2-13. The 95% confidence intervals for annual of amount of DNA per ml sample.

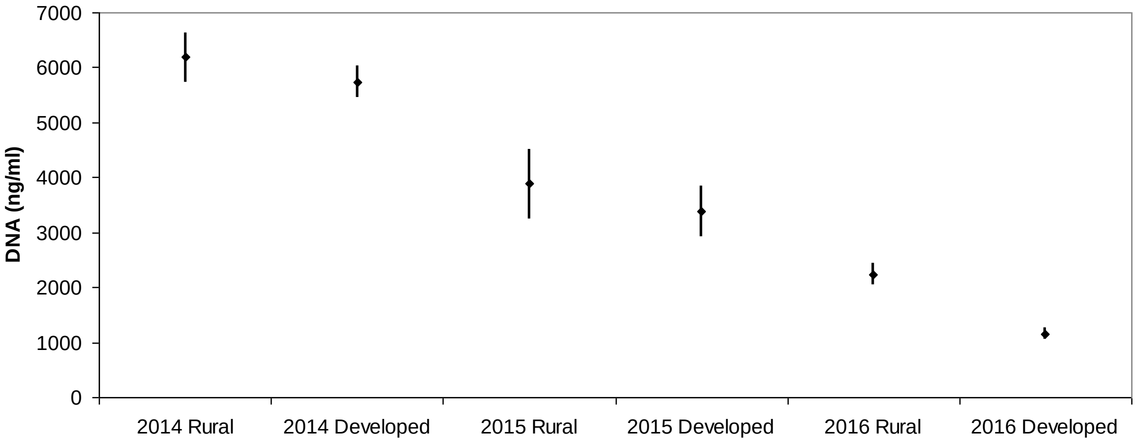


Figure 2-11 cont.

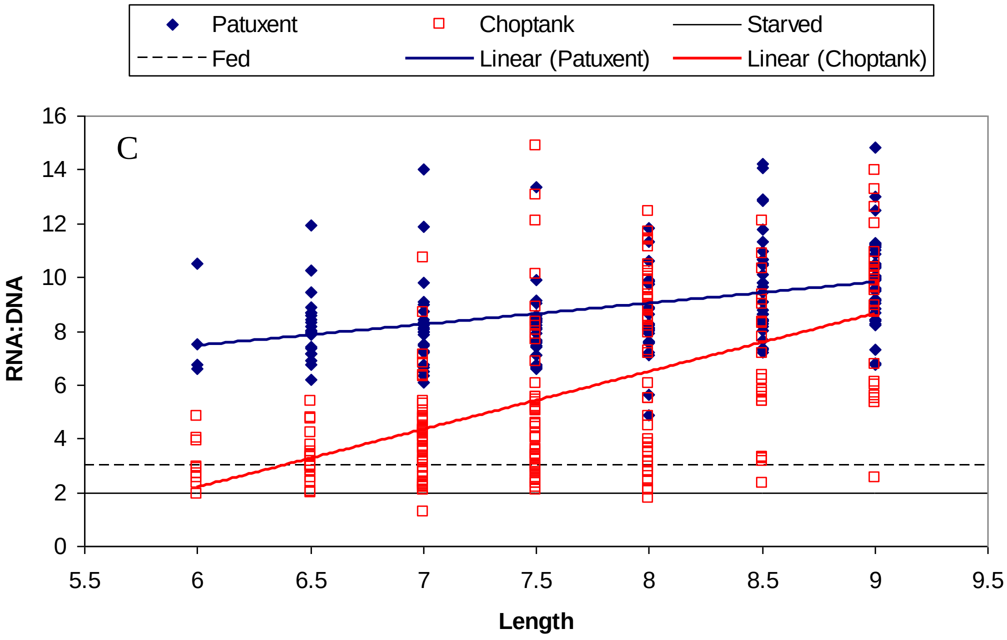
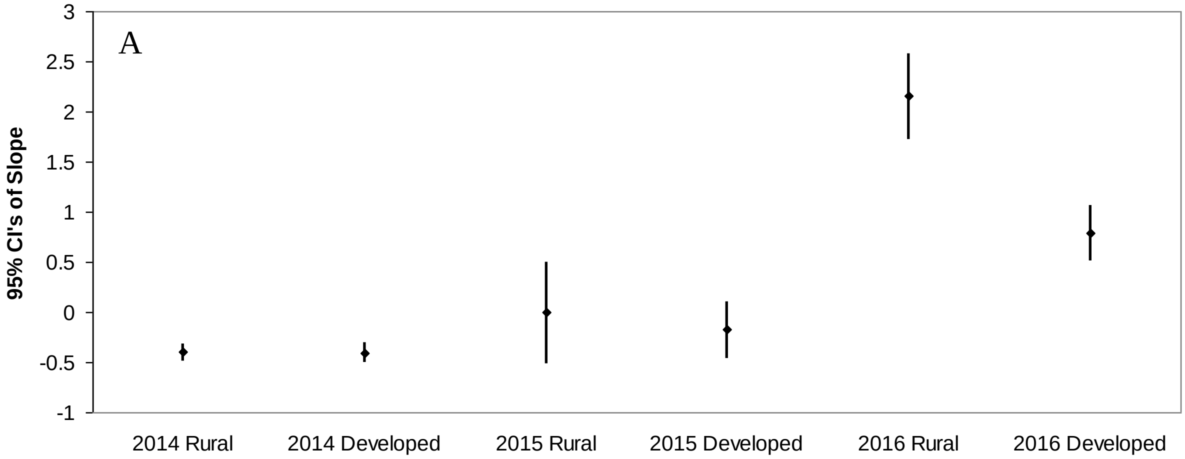


Figure 2-12. Relationships of 95% confidence intervals for (A) slopes and (B) intercepts of annual RNA/DNA ratios of 6-9 mm larvae with length.



Section 3 - Estuarine Fish Community Sampling

Alexis Park, Carrie Hoover, Margaret McGinty, Jim Uphoff, Patrick Donovan, Michael Macon, and Alexis Walls

Introduction

Human population growth in the Chesapeake Bay (or Bay) watershed since the 1950s added a suburban landscape layer to the Chesapeake Bay watershed (Brush 2009) that has been identified as a threat to the Bay (Chesapeake Bay Program or CBP 1999). Development converts land use typical of rural areas (farms, wetlands, and forests) to residential and industrial uses (Wheeler et al. 2005; National Research Council or NRC 2009; Brush 2009; Meals et al. 2010; Sharpley et al. 2013; Zhang et al. 2016). These are the basic trade-off in land use facing Maryland as its population grows (Maryland Department of Planning 2015) and they have ecological, economic, and societal consequences (Szaro et al. 1999).

Water quality and aquatic habitat is altered by agricultural activity and urbanization within watersheds. Both land-uses include pesticide and fertilizer application. Agriculturally derived nutrients have been identified as the primary driver of hypoxia and anoxia in the mainstem Chesapeake Bay (or Bay; Hagy et al. 2004; Kemp et al. 2005; Fisher et al. 2006; Brush 2009; Zhang et al. 2016). Land in agriculture has been relatively stable, but farming itself has become much more intensive (fertilizer and pesticide use has increased) in order to support population growth (Fisher et al. 2006; Brush 2009).

Urbanization may introduce additional industrial wastes, contaminants, stormwater runoff and road salt (Brown 2000; NRC 2009; Benejam et al. 2010; McBryan et al. 2013; Branco et al. 2016) that act as ecological stressors. Extended exposure to biological and environmental stressors affect fish condition and survival (Rice 2002; Barton et al. 2002; Benejam et al. 2008; Benejam et al. 2010; Branco et al. 2016). Reviews by Wheeler et al. (2005), the National Research Council (NRC 2009) and Hughes et al. (2014a; 2014b) documented deterioration of non-tidal stream habitat with urbanization.

Development of the Bay watershed brings with it ecologically stressful factors that conflict with demand for fish production and recreational fishing opportunities from its estuary (Uphoff et al. 2011a; Uphoff et al 2015). Uphoff et al. (2011a) estimated target and limit impervious surface reference points (ISRPs) for productive juvenile and adult fish habitat in brackish (mesohaline) Chesapeake Bay subestuaries based on dissolved oxygen (DO) criteria, and associations and relationships of watershed impervious surface (IS), summer DO, and presence-absence of recreationally important finfish in bottom waters. Watersheds of brackish subestuaries at a target of 5.5% IS (expressed as IS equivalent to that estimated by the methodology used by Towson University for1999-2000) or less (rural watershed) maintained mean bottom DO above 3.0 mg / L (threshold DO), but mean bottom DO was only occasionally at or above 5.0 mg / L (target DO). Mean bottom DO seldom exceeded 3.0 mg / L above 10% IS (suburban threshold; Uphoff et al. 2011a). Although bottom DO concentrations were influenced by development (indicated by IS) in brackish subestuaries, Uphoff et al. (2011b; 2012; 2013; 2014; 2015; 2016) have found adequate concentrations of DO in bottom channel habitat of tidal-fresh and oligohaline subestuaries with watersheds at suburban and urban levels of development. They suggested these bottom channel waters were not succumbing to low oxygen because stratification due to salinity was weak or not present, allowing for more mixing.

In 2016, we continued to evaluate summer nursery and adult habitat for recreationally important finfish in tidal-fresh (0-0.5 ‰), oligohaline (0.5-5.0 ‰) and meshohaline (5.0-18.0 ‰; Oertli, 1964) subestuaries of Chesapeake Bay. In this report, we evaluated the influence of watershed development on target species presence-absence and abundance, total abundance of finfish, and finfish species richness. We analyzed the associations of land use (i.e., agriculture, forest, urban, and wetlands) and C/ha (structures per hectare) on the annual median bottom DO (mg/L) among subestuaries sampled during 2003-2016 using correlation analysis (Pearson correlation coefficients). We summarized work to date on Mattawoman Creek in this report as part of Maryland DNRs' effort to assist Charles County with its comprehensive growth plan in order to conserve natural resources of its watershed, including its recreational fisheries (Interagency Mattawoman Ecosystem Management Task Force 2012). We evaluated development's effect on three Choptank River subestuaries: Tred Avon River, Harris Creek and Broad Creek. We added analyses of conditions in Gunpowder and Middle Rivers following discovery of Zebra Mussels in 2015 and major fish kills in 2015 and 2016.

Methods

Each subestuary sampled in the past or present was classified into a salinity category based on the Venice System for Classification of Marine Waters (Oertli1964). Salinity influences distribution and abundance of fish (Hopkins and Cech 2003; Cyrus and Blaber, 1992; Allen 1982) and DO (Kemp et al. 2005). Uphoff et al. (2012) calculated an arithmetic mean of all bottom salinity measurements over all years available to determine salinity class of each subestuary. Tidal-fresh ranged from 0-0.5 ‰; oligohaline, 0.5-5.0 ‰; and meshohaline, 5.0-18.0 ‰ (Oertli 1964). We grouped data by these classifications when examining effects of development.

We sampled eight subestuaries in Chesapeake Bay during 2016: Broad Creek, Harris Creek, and Tred Avon River, mesohaline subestuaries of the Choptank River; Mattawoman Creek and Nanjemoy Creek, fresh-tidal and oligohaline subestuaries (respectively) of the Lower Potomac River; and Northeast River (fresh-tidal), Middle River (oligohaline), and Gunpowder River (oligohaline) subestuaries located in the upper Chesapeake Bay (Table 3-1; Figure 3-1). This is the fifth year of sampling of Broad Creek and Harris Creek. These watersheds, downstream of Tred Avon River (sampled since 2006) on the Choptank River, represented rural to near suburban development within a single major watershed. Harris Creek underwent an extensive Eastern Oyster restoration effort in 2011(MD DNR 2014). Mattawoman Creek has been sampled since 1989, Nanjemoy Creek since 2008, Northeast River since 2007, Middle River since 2009, and Gunpowder River since 2009.

We obtained compatible data from Bush River monitoring by citizen volunteers and staff from the Anita C. Leight Estuary Center (Table 3-1; Figure 3-1). The Bush River has been sampled since 2006; the Estuary Center and its citizen volunteers, trained in 2011 by the Fisheries Service staff, have taken over sampling. We included their data in this report.

We used property tax map based counts of structures in a watershed (C), standardized to hectares (C/ha), as our indicator of development (Table 3-1; Uphoff et al. 2012; Topolski 2015). Estimates of C/ha and MD DOP land use percentages were used for analyses of data from mesohaline subestuaries sampled during 2003-2016 (Table 3-2). Methods used to estimate development (C/ha) and land use indicators (percent of watershed in agriculture, forest, wetlands, and urban land use) are explained in **General Spatial and Analytical Methods used in Job 1, Sections 1-3.** Development targets and limits and general statistical methods (analytical strategy and equations) are described in this section as well. Specific spatial and analytical methods for this section of the report are described below.

Tidal water surface area of each subestuary was estimated using the planimeter function on MDMerlin satellite photographs and maps (www.mdmerlin.net; Table 3-1). Shorelines were traced five-times for each system, and an average area was calculated. The lower limit of each water body was determined by drawing a straight line between the lowest downriver points on opposite shores (the mouth of each system) and the upper limits were to include all waters influenced by tides.

Surveys focused on eleven target species of finfish that fell within four broad life history groups: anadromous (American Shad, Alewife, Blueback Herring, Striped Bass), estuarine residents (semi-anadromous White Perch, Yellow Perch), marine migrants (Atlantic Menhaden and Spot), and tidal-fresh forage (Spottail Shiner, Silvery Minnow, Gizzard Shad). With the exception of White Perch, adults of the target species were rare and juveniles were common. Use of target species is widespread in studies of pollution and environmental conditions (Rice 2003). These species are widespread and support important recreational fisheries in Chesapeake Bay (directly or as forage); they are well represented in commonly applied seine and-or trawl techniques (Bonzek et al. 2007); and the Chesapeake Bay serves as an important nursery for them (Lippson 1973; Funderburk et al. 1991; Deegan et al. 1997). Gear specifications and techniques were selected to be compatible with past and present MD DNR Fishing and Boating Service surveys (Carmichael et al. 1992; Bonzek et al. 2007; Durell 2007).

Ideally, four evenly spaced haul seine and bottom trawl sample sites were located in the upper two-thirds of each subestuary. Nanjemoy Creek was covered sufficiently by three sites. However, during 2011 and 2012, NOAA sampled the Nanjemoy Creek fish community with compatible gear as part of a coastal ecosystem assessment and added a site upstream of our three sites; the data collected during those years were added into our analyses. Broad and Harris creeks lacked shoreline for a fourth comparable seine site, both systems have four trawl sites and three seine sites. Sites were not located near a subestuary’s mouth to reduce influence of mainstem waters on fish habitat. We used GPS to record latitude and longitude at the middle of the trawl site, while latitude and longitude at seining sites were taken at the seine starting point on the beach.

Sites were sampled once every two weeks during July-September, totaling six annual visits per a system. The number of total samples collected from each system varied due to number of sites, SAV and weather/tidal influences, and equipment issues. All sites on one river were sampled on the same day, usually during morning through mid-afternoon. Sites were numbered from upstream (site 1) to downstream (site 4). The crew leader flipped a coin each day to determine whether to start upstream or downstream. This coin-flip somewhat randomized potential effects of location and time of day on catches and DO. However, sites located in the middle would not be as influenced by the random start location as much as sites on the extremes because of the bus-route nature of the sampling design. If certain sites needed to be sampled on a given tide then the crew leader deviated from the sample route to accommodate this need. Trawl sites were generally in the channel, adjacent to seine sites. At some sites, seine hauls could not be made because of permanent obstructions, SAV beds, or lack of beaches.

Water quality parameters were recorded at all sites. Temperature (ºC), DO (mg / L), conductivity (mS / cm), salinity (‰), and pH were recorded at the surface, middle, and bottom of the water column at the trawl sites and at the surface of the seine site. Mid-depth measurements were omitted at sites with less than 1.0 m difference between surface and bottom. Secchi depth was measured to the nearest 0.1 m at each trawl site. Weather, tide state (flood, ebb, high or low slack), date, and start time were recorded for all sites.

A 4.9 m headrope semi-balloon otter trawl was used to sample fish in mid-channel bottom habitat. The trawl was constructed of treated nylon mesh netting measuring 38 mm stretch-mesh in the body and 33 mm stretch-mesh in the codend, with an untreated 12 mm stretch-mesh knotless mesh liner. The headrope was equipped with floats and the footrope was equipped with a 3.2 mm chain. The net used 0.61 m long by 0.30 m high trawl doors attached to a 6.1 m bridle leading to a 24.4 m towrope. Trawls were towed in the same direction as the tide. The trawl was set up tide to pass the site halfway through the tow, allowing the same general area to be sampled regardless of tide direction. A single tow was made for six minutes at 3.2 km / hr (2.0 miles / hr) per site on each visit. The contents of the trawl were emptied into a tub for processing.

During 2009-2016, a 3.1 m box trawl made of 12.7 mm stretch-mesh nylon towed for five minutes was used on the same day sampling was conducted with a 4.9 m trawl in Mattawoman Creek to create a catch-effort time-series directly comparable to monitoring conducted during 1989-2002 (Carmichael et al. 1992). The net size at the start of a sampling day in Mattawoman Creek alternated between visits.

A 30.5 m × 1.2 m bagless beach seine, constructed of untreated knotted 6.4 mm stretch mesh nylon, was used to sample inshore habitat. The float-line was rigged with 38.1 mm by 66 mm floats spaced at 0.61 m intervals and the lead-line rigged with 57gm lead weights spaced evenly at 0.55 m intervals. One end of the seine was held on shore, while the other was stretched perpendicular from shore as far as depth permitted and then pulled with the tide in a quarter-arc. The open end of the net was moved towards shore once the net was stretched to its maximum. When both ends of the net were on shore, the net was retrieved by hand in a diminishing arc until the net was entirely pursed. The section of the net containing the fish was then placed in a washtub for processing. The distance the net was stretched from shore, maximum depth of the seine haul, primary and secondary bottom type, and percent of seine area containing aquatic vegetation were recorded.

All fish captured were identified to species and counted. Striped Bass and Yellow Perch were separated into juveniles and adults. White Perch were separated into three categories (i.e., juvenile, small adults, and harvestable size adults) based on size and life stage. The small adult White Perch category consisted of ages 1+ White Perch smaller than 200 mm.

2016 Sampling Summary - Three basic metrics of community composition were estimated for subestuaries sampled: geometric mean catch of all species, total number of species (species richness), and species comprising 90% of the catch. The geometric mean (GM) was estimated as the back-transformed mean of $\log_e$-transformed catches (Ricker 1975; Hubert and Fabrizio 2007). The GM is a more precise estimate of central tendency of fish catches than the arithmetic mean, but is on a different scale (Ricker 1975; Hubert and Fabrizio 2007). We noted which target species were within the group that comprised 90% of fish collected. We summarized these metrics by salinity type since some important ecological attributes (DO and high or low SAV densities) appeared to reflect salinity class (Uphoff et al. 2012).

We plotted species richness collected by seine and by 4.9 m trawl against C/ha by salinity class. A greater range of years (1989-2016) was available for seine samples than the 4.9 m trawl (2004-2016) due to a change from the 3.1 m trawl used during 1989-2002 (Carmichael et al. 1992). We set a minimum number of samples (15) for a subestuary in a year to include estimates of species richness based on analyses in Uphoff et al. (2014). This eliminated years where sampling in a subestuary ended early due to site losses, typically from SAV growth.

Dissolved Oxygen Dynamics - Dissolved oxygen concentrations were evaluated against a target of 5.0 mg / L and a threshold of 3.0 mg / L (Batiuk et al. 2009; Uphoff et al. 2011a). These criteria were originally derived from laboratory experiments, but were also associated with asymptotically high presence of target species in trawl samples from bottom channel habitat in mesohaline subestuaries (Uphoff et al. 2011a). Target DO was considered sufficient to support aquatic life needs in Chesapeake Bay (Batiuk et al. 2009) and has been used in a regulatory framework to determine if a water body is meeting its designated aquatic life uses. Presence of target species declined sharply when bottom DO fell below the 3.0 mg / L threshold (Uphoff et al. 2011a). We estimated the percentages of DO samples in each subestuary that did not meet the target or threshold for all samples (surface to bottom) and for bottom waters alone. The percentages of DO measurements that met or fell below the 5 mg / L target (V_{target}) or fell at or below the 3 mg / L threshold ($V_{threshold}$) were estimated as:

$$V_{target} = (N_{target} / N_{total})*100;$$

and

$$V_{threshold} = (N_{threshold} / N_{total})*100;$$

where N_{target} was the number of measurements meeting or falling below 5 mg / L, $N_{threshold}$ was the number of measurements falling at or below 3 mg / L, and N_{total} was total sample size.

Separate Pearson correlation analyses were conducted for surface or bottom temperature or C/ha with surface or bottom DO for all subestuaries sample since 2003. This analysis explored multiple hypotheses related to DO conditions. Structure per hectare estimates were considered proxies for nutrient loading and processing due to development in the subestuaries in this analysis (Uphoff et. al 2011a). Water temperature would influence system respiration and stratification (Kemp et al. 2005; Murphy et al. 2011; Harding et al. 2016). Conducting correlation analyses by salinity classification provided a means of isolating the increasing influence of salinity on stratification from temperature. Our primary interest was in associations of C/ha to DO in surface and bottom channel waters. Temperature and salinity were potential influences on DO because of their relationships with DO saturation and stratification (Kemp et al. 2005; Murphy et al. 2011; Harding et al. 2016). We correlated mean surface temperature with mean surface DO, mean bottom temperature with mean bottom DO, and C/ha with surface and bottom DO for each salinity class. We chose annual means of surface or bottom DO and water temperature in summer at all sites within a subestuary for analyses to match the geographic scale of C/ha estimates (whole watershed) and characterize chronic conditions.

Land Use Categories, C/ha, and Mesohaline Subestuary Bottom Dissolved Oxygen - We analyzed the associations of land use (i.e., agriculture, forest, urban, and wetlands) and

C/ha (structures per hectare) on the annual median bottom DO (mg/L) among mesohaline systems sampled during 2003-2016 using correlation analysis (Pearson correlation coefficients). We obtained land use estimates for our watersheds from the Maryland Department of Planning for 2002 and 2010 (MD DOP 2002 and 2010). The MD DOP provides agriculture, forest, urban, and wetlands estimates periodically rather than annually, but C/ha is estimated annually. Median summer bottom DO estimates made before 2010 were compared with 2002 DOP land use estimates and those made for 2010-2016 were matched with 2010 DOP estimates. Four categories of land use were present for all mesohaline tributaries, agriculture, forest, urban, and wetlands were estimated for only the land portion of the watershed (water area was not included).

Mattawoman Creek - We continued to track bottom DO, SAV area, finfish abundance and finfish species richness in 3.1 m and 4.9 m trawl samples from Mattawoman Creek and compared them to changes in C/ha. We used Virginia Institute of Marine Science (2017) estimates of SAV area. We obtained measurements of total ammonia nitrogen (TAN; NH_3 plus NH_4), chlorophyll a, and pH in Mattawoman Creek during the SAV growing season (April-October) from Chesapeake Bay Program (CBP; 2016; 2017) monitoring site MAT0016, located in the channel between our stations 3 and 4 (W. Romano, MD DNR, personal communication). The TAN estimates were available for 1986-2016, but we eliminated 1986-1990 from analysis because of methodology differences. During 1991-2009, TAN samples were collected twice a month, only the first TAN sample of each month was selected for analysis (except in 1991, the first sample during May was not available so the second sample was selected) to correspond equally with the number of samples in the following years 2010-2015 (N=7). In 2014, only 6 TAN samples were used in analysis because samples were not collected in July. In 2016, only 3 samples were used in analysis; no samples were taken after June. Measurements of growing season TAN were annually summarized as minimum, median, and maximum and compared to US EPA ambient water quality criteria for TAN (US EPA 2013) to capture the potential for acute and chronic toxicity.

Sampling with 3.1 m trawls was conducted in Mattawoman Creek during 1989-2002 and 2009-2016; 4.9m trawls have been continuously used since 2003. Geometric means of total fish abundance and their 95% CI's were estimated for the 3.1 m and 4.9 m trawls for samples from Mattawoman Creek. We compared trends of GMs of total fish abundance and YOY White Perch (juveniles) abundance in the years in common for the 3.1 m and 4.9 m trawls (2009-2016) in Mattawoman Creek using linear regression. We also compared trends during 1989-2016 of YOY White Perch (juveniles) in Mattawoman Creek to the Potomac River with linear regression to assess how similar they were. Low coherence (low r^2) could indicate local processes were more important to White Perch in Mattawoman Creek than processes in the mainstem Potomac River. High coherence could indicate similar processes between the two or that YOY from the Potomac River predominated in Mattawoman Creek.

Estimates of species richness in Mattawoman Creek (number of species encountered) were made for 3.1 m trawl samples during 1989-2002 and 2009-2016. Sampling during 1989-2002 was based on monthly sampling of five stations (Carmichael et al. 1992). Station 5, the furthest downstream station sampled during 1989-2002, was dropped because it was outside the range of stations 1-4 sampled during 2009-2016. Remaining stations were the same throughout the time-series, but were sampled monthly during 1989-2002 (annual N = 12) and bi-monthly during 2009-2016 (annual N = 24). In order to match the annual sample sizes of 1989-2002, we made two sets of estimates for each sample year during 2009-2016:

one for the first round of the month and one for the second. As a result, all comparisons of species richness in Mattawoman Creek were based on the same annual sample size.

Choptank River Subestuaries - The trajectories of C/ha since 1950 were plotted for each of the three Choptank River tributaries. Bottom DO measurements during 2006-2016 were plotted against C/ha for each Choptank River subestuary. The percentage of target and threshold violations (violations meant that target or threshold criteria were not met; they did not have a legal meaning) were estimated using all DO measurements combined (surface, middle, and bottom) and for bottom DO measurements alone. Annual mean bottom DO in Tred Avon River at each station during 2006-2016 summer sampling was estimated and plotted by year.

A modified Proportional Stock Density (PSD; Anderson 1980; Neumann and Allen 2007) was calculated for White perch for each of the three Choptank River subestuaries to compare relative proportions of the adult population that would be of interest to anglers. Proportional stock density is calculated using length-frequency data and provides population dynamics information (Neumann and Allen 2007). It is normally calculated by dividing the number of fish at or greater than minimum quality length by the number of fish at or greater than minimum stock length x 100. Stock length refers to the number of fish at the minimum length of fish that provides a recreational value. Quality length refers to the number of fish at the minimum length most anglers like to catch (Anderson 1980; Neumann and Allen 2007).

White Perch greater than or equal to 200 mm were considered to be of harvestable size and all captured were measured to the nearest millimeter. White Perch of this size or larger corresponded to the quality length category minimum (36-41% of the world record TL) proposed by Anderson (1980) for proportional stock density (PSD) indices; 200 mm TL is used as the length cut-off for White Perch in Chesapeake Bay PSD assessments (Piavis and Webb 2013). We substituted the total number of small adult plus harvestable length White Perch for stock lengths to estimate a modified PSD (MPSD) since we did not measure small adults. These data provided an opportunity to evaluate the influence of development on the availability of fish for anglers to harvest.

Annual proportions of seine or trawl samples in a subestuary with quality length or greater (≥ 200mm) White Perch and their 95% CI were calculated. The proportion of samples with quality length or greater White Perch was estimated as $MPSD = N_{Quality} / N_{total}$; where $N_{Quality}$ equaled the number of samples with quality length or greater White Perch present and N_{total} equaled the total number of small adults + harvestable White Perch. The standard deviation (SD) of each MPSD was estimated using the normal distribution approximation of the bionomial distribution (converting MPSD to a proportion):

$$SD = \{[(1 - (MPSD/100) \bullet (1- (MPSD / 100))) / N_{total}]^{0.5}\} \times 100 \text{ (Ott 1977).}$$

Middle and Gunpowder Rivers - We analyzed Gunpowder River and Middle River subestuaries in more detail in response to fish kills that occurred there in 2015 and 2016. We assembled our time-series of Secchi depth, SAV area, bottom DO (mg/L), pH, and salinity (ppt), and to look for changes in variables that might have preceded the fish kills. Geometric means (GM) of total fish abundance and their 95% CI's were estimated for 4.9 m trawl for samples from Gunpowder and Middle Rivers for 2009-2016. Compositions of all finfish species caught by seine for all time-series (2009-2016) were graphed for Gunpowder and

Middle Rivers. The top 90% of finfish species occurring in annual trawl catches was estimated for 2009-2106.

Errata - We discovered an error in Nanjemoy Creek data during 2011-2012 due to station identifications being switched; stations were correctly identified using the latitudes-longitudes recorded at the time of sampling and matched to the correct sites. Errors were also discovered in Mattawoman 2009-2011 trawl data, the 3.1 m box trawls and 4.9 m headrope semi-balloon otter trawls were not correctly identified in entered data; trawls were correctly identified from field data sheets.

Conductivity measurements in 2012-2013 were recorded incorrectly. The raw conductivity was recorded instead of the specific conductivity, which compensates for temperature. An equation was used to correct the error and convert the raw conductivity measurements that were recorded to specific conductivity (Fofonoff and Millard 1983):

$$\text{Specific Conductivity} = \text{Conductivity} / (1 + ((0.02 \cdot T) - 25));$$

for each ºC change in water temperature (T) there was a 2% change in conductivity.

Results and Discussion

2016 Sampling Summary - The Northeast River was the only subestuary that did not have any DO readings less than the target level (5.0 mg / L) during 2016 (Table 3-3). Sixteen percent of all DO measurements in 2016 from Nanjemoy Creek were below the target; Tred Avon River had 13%; Bush River, 10%; Middle River, 9%; Mattawoman Creek, 5%; Broad Creek, 4%; Harris Creek, 3%; and Gunpowder River, 2%. In 2016, only two subestuaries did not have any bottom DO estimates below the target level; Gunpowder and Northeast Rivers. The remaining seven subestuaries had target bottom DO violations: Nanjemoy Creek, 57%; Tred Avon, 38%; Bush River, 24%; Broad and Harris Creeks, 9%; Mattawoman Creek, 7%; and Middle River, 2%. Only one subestuary had measurements of bottom DO below the 3 mg / L threshold: Tred Avon River at 13%. Oligohaline Nanjemoy Creek, which was the least developed watershed sampled during 2016 and is mostly forested, had a higher frequency of target bottom DO violations than the most developed system Middle River, also oligohaline (Table 3-3).

Geometric mean catch per seine haul ranged from 64 to 398 fish among subestuaries sampled during 2016, with some indication that salinity class or development level exerted an influence (Table 3-4). Out of seven subestuaries sampled during 2016, oligohaline subestuaries were least productive, ranking sixth and seventh. Rankings of tidal-fresh (ranked = 3 and 4 out of 7) and mesohaline subestuaries (1, 2, and 5) were interspersed.

Between 26 and 29 species were encountered in seine samples from three mesohaline subestuaries (Tred Avon River, Broad and Harris creeks). The three oligohaline subestuaries that had sufficient sample sizes (Bush and Gundpowder rivers, and Nanjemoy Creek) had 22-27 species. Northeast River, the only tidal-fresh subestuary with sufficient seine samples, had 27 species (Table 3-4). During 2016, dense SAV prevented seining in Mattawoman Creek and Middle River. Seining in Broad Creek was very restricted because of high tides that limited beach availability and dense SAV in two seine sites (BROS02 and BROS04); BROS02 was moved down river in order to obtain a sample. Additional seine sites sampled in Middle River and Nanjemoy Creek for NOAA's Integrated Assessment were dropped in

2013 once NOAA ended their field collections. In Gunpowder River, one seine site (GURS02) has not been sampled since 2012 after it was roped off for swimming.

A plot of species richness in seine samples and C/ha did not suggest a relationship in tidal-fresh or oligohaline, or mesohaline subestuaries (Figure 3-2). Tidal-fresh subestuary watersheds were represented by a limited range of C/ha (0.48 - 0.93). Oligohaline subestuary watersheds were represented by the widest range of C/ha (0.09 - 3.34, rural to urban) of the three salinity classes (Figure 3-2).

A total of 46,307 fish representing 58 species were captured by beach seine in 2016 (Table 3-4). Ten species comprised 90% of the total fish caught in 2016, including (from greatest to least) Atlantic Menhaden, Atlantic Silverside, Gizzard Shad, Striped Killifish, Blueback Herring, Banded Killifish, White Perch (adult), Mummichog, White Perch (juveniles), Bay Anchovy, and Sheepshead Minnow. Atlantic Menhaden, Gizzard Shad, Blueback Herring, and White Perch (juvenile and adult) represented target species among the species comprising 90% of the total catch. A total of 6 target species were present among species comprising 90% of the seine catch throughout all subestuaries: Atlantic Menhaden were present in seine samples in six of the seven subestuaries; White Perch (juveniles and/or adults) in five; Gizzard Shad in three; Spottail Shiner and Blueback Herring in two; Alewife in one. Atlantic Menhaden were present in the top 90% of species for all systems except Northeast River. Target species comprised of all 90% of the catch in Bush River.

Geometric mean trawl catches during 2016 were between 40 and 296 (Table 3-5). Subestuaries had 17 – 24 samples. Nanjemoy Creek had the greatest GM (296) and Harris Creek had the least (40). Oligohaline subestuaries were the most productive, ranking 1-4 out of 9 in magnitude of their GM's. Rankings of tidal-fresh (ranks = 5 and 8) and mesohaline subestuaries (6, 7, and 9) were interspersed. Number of species captured by trawl in subestuaries sampled during 2016 (17 – 24) overlapped for all three salinity classifications (Table 3-5). A plot of species richness in trawl samples against C/ha (2003-2016) did not indicate a relationship of development and number of species for tidal-fresh or oligohaline subestuaries (Figure 3-3). Species richness declined in mesohaline subestuaries as C/ha advanced beyond the threshold (C/ha = 0.83; Figure 3-3). Species richness ranged from 12 to 23 when development was below the threshold. Four of 39 species richness estimates were at or slightly below 11 when development was above the threshold. The four estimates of species richness in mesohaline subestuaries fell steadily when C/ha was over the threshold (Severn River: 7 at 2.09 C / ha and 3 at 2.15 C/ha; South: 11 at 1.25 C / ha and 1.26 C / ha; Figure 3-3).

Sampling with a 4.9 m headrope bottom trawl was conducted in all nine subestuaries in 2016 (Table 3-5). Unlike seining, all trawl sites could be sampled (except in bad weather). A total of 47,190 fish and 51 fish species were captured. Four species comprised 90% of the total catch for 2016 (from most to least): Bay Anchovy, White Perch (juveniles and adults), Spottail Shiner, and Pumpkinseed. Bay Anchovy, White Perch (juveniles and adults) and Spottail Shiner were target species. Target species comprising 90% of the catch in one or more of the nine subestuaries sampled during 2016 were White Perch (juveniles and/or adults) and Bay Anchovy in seven subestuaries; Spottail Shiner in two; and Gizzard Shad in one.

Dissolved Oxygen Dynamics - Correlation analyses of DO with temperature and C/ha in subestuaries sampled since 2003 (Table 3-6) indicated that DO responded to temperature and C/ha differently depending on salinity classification (Table 3-7). Mean bottom DO in summer surveys declined below the threshold level in mesohaline tributaries, but did not in oligohaline or tidal-fresh (Figure 3-4). There were a few years where mean survey bottom DO fell below the target in oligohaline subestuaries, but remained above 4.0 mg / L; these below target conditions would not affect the use of this habitat (Uphoff et al. 2011a). Mean surface DO in summer surveys did not fall below the threshold for oligohaline and tidal-fresh subestuaries, but one mesohaline subestuary fell below target (Figure 3-5).

Negative associations of surface and bottom DO with corresponding mean water temperatures at depth were detected for oligohaline subestuaries by correlation analyses (surface: r = -0.35, *P*= 0.030, N= 38; bottom: r = -0.56, *P*= 0.0002, N= 38; Table 3-7), suggesting respiration was a major consideration oligohaline subestuaries. Oligohaline subestuaries that have been monitored have been shallower than most subestuaries of the other salinity categories. Associations of temperature and DO were not detected in mesohaline or tidal-fresh subestuaries. The strongest and only negative association between bottom DO and C/ha was found in mesohaline subestuaries (r = -0.58, *P* < 0.0001, N= 62); mesohaline subestuaries were where strongest stratification was expected. Positive associations of surface DO with development were suggested for oligohaline subestuaries (r = 0.36, *P* = 0.025, N= 38). A positive association between bottom DO and C/ha in fresh-tidal subestuaries was significant (r = 0.41, *P* = 0.0173, N= 33). Given that multiple comparisons were made, correlations that were significant at *P* < 0.02 might be considered spurious if one rigorously adheres to significance testing (Nakagawa 2004). However, oligohaline and tidal-fresh subestuaries were less likely to stratify because of low or absent salinity and the biological consequences of no or positive relationships would be similar (i.e., a negative impact on habitat would be absent). Sample sizes of mesohaline subestuaries (N = 62) were over twice as high as oligohaline (N = 38) or tidal-fresh subestuaries (N = 33), so ability to detect significant associations in mesohaline subestuaries was greater.

Levels of bottom DO were not negatively associated with development in tidal-fresh or oligohaline subestuaries, but were in mesohaline subestuaries (Table 3-7). Depletion of bottom DO in mesohaline subestuaries to hypoxic or anoxic levels represented a direct loss of habitat to be occupied. Uphoff et al. (2011a) determined that the odds of adult and juvenile White Perch, juvenile Striped Bass, Spot, and Blue Crabs being present in shore zone seine samples from mesohaline subestuaries were not influenced by development, but odds of target species being present in bottom channel trawl samples were negatively influenced by development.

The extent of bottom channel habitat that can be occupied does not appear to diminish with development in tidal-fresh and oligohaline subestuaries due to low DO. Sampling of DO in dense SAV beds in tidal-fresh Mattawoman Creek in 2011 indicated that shallow water habitat could be negatively impacted by low DO within the beds (Uphoff et al. 2012; 2013; 2014; 2015); it was not feasible for us to routinely monitor fish within the beds so the impact on target finfish could not be estimated. The summer fish community of tidal-fresh Mattawoman Creek underwent drastic changes in abundance and species richness as development threshold was approached that were unrelated to adequacy of DO in channel waters, indicating other stressors (see *Mattawoman Creek* section, below) were important (Uphoff et al. 2009; 2012; 2013; 2014; 2015; 2016). During November, 2015, the oligohaline Middle River subestuary (the most heavily developed watershed in our study) experienced an extensive fish kill attributable to harmful algal blooms (MDE 2016). In December 2016, Gunpowder River experienced a fish kill attributable to harmful algal blooms (MDE 2017).

Both Middle River and Gunpowder River have exhibited diverse and abundant fish communities over the course of our monitoring.

Land Use Categories, C/ha, and Mesohaline Subestuary Bottom Dissolved Oxygen - We correlated percent of watershed in MD DOP land use categories (agriculture, forest, urban, and wetlands), and C/ha (structures per hectare) to explore associations among land uses. Correlations of agriculture with C/ha and urban cover were negative and strong (r = -0.74, P <0.0001 and r = -0.73, P = 0.001, respectively); the correlation of urban land cover with C/ha was positive and strong (r = 0.86, P < 0.0001); and forest cover was moderately and negatively correlated with agriculture (r = -0.59, P = 0.012; Table 3-8). Remaining pairings of categories were not well correlated (Table 3-8).

After inspection of scatter plots, forest cover was further divided into regional categories, East (eastern shore of Chesapeake Bay: Broad Creek, Corsica River, Harris Creek, Langford Creek, Miles River, Tred Avon River, and Wye River) and West (western shore of Chesapeake Bay: Breton Bay, Magothy River, Rhode/West Rivers, Severn River, South River, St. Clements River, and Wicomico River) reflecting lower percentages of forest cover on the eastern Shore (Figure 3-6). A multiple linear regression of the change in forest cover with C/ha using region as a categorical variable indicated a strong region effect (lower forest cover intercept, reflecting greater area in agriculture on the eastern shore) and similar negative slopes for forest cover with C/ha (r^2 = 0.919, *P* < 0.0001, N = 49; Table 3-9).

Inspection of the scatter plot of percent of watershed in agriculture versus median bottom DO in mesohaline subestuaries indicated an ascending limb of median DO when agricultural coverage went from 6.0 to 40.9% that was comprised entirely of western shore subestuaries (Figure 3-7). Median DO measurements beyond this level of agricultural coverage (42.6-71.6% agriculture) were from eastern shore subestuaries and the DO trend appeared to be stable or declining (Figure 3-7). Development was predominant at low levels of agriculture (< 20%), agricultural coverage and C/ha were strongly and inversely correlated, so the positive trend of DO with agriculture when agricultural coverage was low was likely to reflect development's negative impact.

We split agricultural coverage and median DO data into western and eastern regions and used a linear regression for each region to describe regional changes in DO with agriculture (model equation was median DO = % agriculture). The relationship was positive for the western shore (slope = 0.132, SE = 0.022; r^2 = 0.689, P < 0.0001, N = 19; Table 3-10) and negative for the eastern shore (slope = -0.047, SE = 0.013; r^2 = 0.267, P = 0.001, N = 37; Table 3-10). Predictions of median DO for western shore subestuaries rose from 0.8 mg / L at 6.0% agricultural coverage to 5.5mg/L at 40.9% (Figure 3-7). Predictions of median DO for eastern shore subestuaries fell from 5.8 mg / L at 42.5% agricultural coverage to 4.4 mg/L at 71.6% (Figure 3-7).

A dome-shaped quadratic model of median bottom DO and agricultural coverage that did not account for regional differences fit the data well (r^2 = 0.61, P < 0.0001, N = 56; Table 3-11). Predictions of median DO for these subestuaries based on the full data set were very similar to those for separate regional linear regressions (Figure 3-8); indicating region was probably not a factor. Both approaches indicated that modest declines in bottom DO would occur with increases in agriculture in subestuaries with 45%-71% of their watershed covered in agriculture. Predicted median bottom DO at the highest level of agriculture observed would equal 4.2 mg/L, which is between the DO target and threshold. Uphoff et al. (2011a) developed a Weibull function to describe the relationship of presence-absence of five target species (data pooled) to bottom DO; the proportion of samples with indicator species was estimated to be 0.52 at 5 mg/L DO. At 4.4 mg/L DO, the predicted proportion was 0.51. At 3.0 mg/L DO or less, the proportion of samples with indicator species fell rapidly (Uphoff et al. 2011a); levels of DO this low are much more common in mesohaline subestuaries with developed watersheds (see Figure 3-4).

Mattawoman Creek - The level of development in Mattawoman Creek's watershed more than doubled between 1989 (0.43 C/ha) and 2011 (0.91 C/ha; Figure 3-9). This watershed reached the threshold for suburban development (C/ha = 0.83) in 2006. Currently, Mattawoman Creek's level of watershed development is 0.93 structures per hectare.

There appeared to be two periods of bottom DO in the Mattawoman Creek time-series (Figure 3-10). Mean bottom DO was near or above the median for the time-series (8.39 mg / L) during 1989-2000 (C/ha < 0.67) and then fell below the time-series median afterward (with exceptions of 2003, 2013, and 2014). Mean bottom DO during summer sampling has never fallen below the target of 5.0 mg / L and excursions below this level have been rare. Since 1989, only nineteen bottom DO values have fallen below the target of 5.0 mg / L; in 2006, 2007, and 2011, four bottom DO values were below target; in 2012 and 2016, three bottom DO values were below target; and in 2008, only one bottom DO value was below target. There has only been one recorded bottom DO value that has fallen below the threshold of 3.0 mg / L since 1989; in 2006, one value was below the threshold level in Mattawoman Creek. These shifts in mean bottom DO corresponded to a downward shift in Mattawoman Creek's subestuary median chlorophyll a levels (Figure 3-11). The relationship of mean bottom DO (DO) to median chlorophyll a (Chl) in Mattawoman Creek was described by the equation:

$$DO = 0.0636Chl + 7.0674,$$

($r^2 = 0.3318$, $P = 0.0013$, N = 28; Figure 3-12). A shift in SAV acreage from low (coverage < 10% of water area; 1989-1998) to high (coverage > 30%; 2002-2012, 2015, 2016) coincided with the shift in mean bottom DO (Figure 3-13; Uphoff et al. 2011b; 2012; 2013; 2014; 2015; 2016). The increasing SAV coverage and mean DO (1989-2016) were negatively related ($r^2 = 0.6788$, $P < 0.0001$, N = 28). SAV may have higher respiration than the phytoplankton it has replaced or provides more organic biomass that fuels respiration of decomposers, lowering DO.

The TAN measurements collected by Chesapeake Bay Program (2017) at MAT0016 ranged from 0.005 mg/L to 0.078 mg/L and had a median of 0.01 mg/L during 2016 (Figure 3-14). The relationship of median TAN (mg/L) readings from 1990-2016 and the percentage of SAV coverage in Mattawoman Creek was best described by an exponential function ($r^2 = 0.46$, $P = 0.0061$, N = 26; Figure 3-15). Measurements of pH at MAT0016 during April through October have fluctuated between 6.3 and 9.5 since 1989 (Figure 3-16). In 1998, pH peaked at 9.5. Years when pH values exceeded 9.0 were 1989–1999, 2003–2004, 2014, and 2016 and few of these years match years of high TAN, indicating pH was a poor indicator of TAN toxicity.

Geometric mean catches for all finfish in 3.1m and 4.9m trawls in Mattawoman Creek were calculated from 1989 to 2016 (Figure 3-17). The linear regression of GM catches of all fish combined to predict the GM for the 3.1 m trawl from the 4.9 m trawl during 2009-2016 indicated that their trends were closely and linearly related ($r^2 = 0.9768$, $P < 0.0001$, N = 8). The slope and its SE were 0.359 and 0.022, respectively. The intercept and its SE were -8.731 and 5.861 (not different from 0 based on its 95% CI). We predicted the missing portion of the 3.1 m trawl GM time-series from the slope alone. The span of GMs in the regression was similar to those that were predicted, so values did not have to be extrapolated beyond bounds of data. The full 3.1 m GM time-series (observations and predictions) suggested abundance of all species became much more variable after 2001. During 1989-2002, minimum, maximum, and median GM catches of 3.1m for all species were 12, 108, 50, respectively; during 2003-2016, minimum, maximum, and median GM catches of all species (predictions for missing years included) in 3.1 m trawls were 0, 200, and 45, respectively.

Geometric mean catches for YOY White Perch (juveniles) in 3.1m and 4.9m trawls in Mattawoman Creek were calculated for 1989-2016 (Figure 3-18). YOY White Perch (juveniles) were consistently present in both trawls throughout 1989–2016. The linear regression of GM catches of YOY White Perch (juveniles) to predict 3.1 m trawl GM catch from 4.9 m trawl GM catch during 2009-2016 indicated that their trends were closely and linearly related ($r^2 = 0.9484$, $P < 0.0001$, N = 28). The slope estimate and its SE were 0.327 and 0.031, respectively. The intercept estimate and its SE were -2.019 and 3.323, indicating it was not different from zero. The minimum, maximum, and median GM catches of 3.1m trawl for the time-series 1989-2002 for YOY White Perch (juveniles) were 2, 28, and 10, respectively; in 2003-2016, minimum, maximum, and median GM catches of 4.9m trawl were 1, 82, and 15, respectively.

The Mattawoman Creek YOY White Perch (juveniles) geometric means (GM) for 3.1m trawl catches were compared to YOY White Perch (juveniles) GMs for the Potomac River seine survey catches (Durrell and Weedon 2016) during 1989-2016 (Figure 3-19). The 3.1m trawl GMs for Mattawoman Creek included predicted GMs The linear regression of GMs of catches for YOY White Perch in Mattawoman Creek and Potomac River for 1989-2016 explained a small amount of variation and had a marginal level of significance ($r^2 = 0.109$, $P = 0.086$, N = 28). It did not provide strong evidence that processes that influenced Mattawoman Creek YOY White Perch abundance were similar to mainstem Potomac River. The slope estimate and its SE were 1.165 and 0.654, respectively. The intercept estimate and its SE were 7.645 and 5.538.

During 1989-2002, annual minimum, maximum, and median numbers of species collected in 3.1-m trawls at Mattawoman Creek were 8, 19, and 14 respectively (Figure 3-20); during 2009-2016, minimum, maximum, and median were 5, 20, and 11, respectively. Species comprising 90% of the 3.1m trawl catch (dominant species) changed between 1989-2002 and 2009-2016 at Mattawoman Creek (Figure 3-21a). White Perch (YOY) were usually a significant fraction of catch during both periods; adult White Perch were only a part of the top 90% in 1991, 1995, 1999, 2000, and 2016. Planktivorous Blueback Herring, Alewife, Gizzard Shad, and Bay Anchovy, dominant during 1989-2002, were replaced by Spottail Shiners. Percentage contributions of YOY White Perch and Spottail Shiners were similar during 2013–2015. In 2016, Bay Anchovy and adult White Perch re-appeared in the top 90% of species, along with Spottail Shiners and juvenile White Perch.

White Perch (YOY) and Spottail Shiners became the only target species in Mattawoman Creek to qualify as dominant in 4.9 m trawls after 2011 (Figure 3-21b). Since 2003, planktivores have been uncommon and adult White Perch have dropped out of the dominant species category. Bluegill and Pumpkinseed, have dropped out the top 90% of species since 2012 and 2008, respectively. Although sunfish are still present in 2013–2016 catches, they are not abundant. Blue Spotted Sunfish were rare, but present, in 2004-2005, 2009–2011, and 2015. Largemouth Bass were present in 75% of the sampling years (exceptions: 1989, 1993, 1995, and 1997–2000). Striped Bass were present in 93% of years sampled (exceptions: 1991 and 2001). Channel Catfish have been consistently present in samples since 2009. Invasive Blue Catfish were first observed during sampling in 2003 and have been consistently present since 2014. Yellow Perch, juveniles or adults, have been constantly been sampled since 2003.

Mattawoman Creek's finfish abundance appeared to exhibit boom and bust dynamics after 2001. "Busts" were concurrent with spikes (2002) or plateaus (2007-2009) of TAN (Figure 3-14). Collapses of the magnitude exhibited during 2002 and 2008-2009 were not detected previously (Figure 3-17). Uphoff et al. (2010) determined that the collapse of abundance in 2008-2009 was local to Mattawoman Creek and not widespread in the Potomac River. Recovery of fish abundance since 2011 has coincided with moderate values of median TAN and TAN medians in 2015 and 2016 were within the range of those estimated in the 1990s. Shifts in ecosystem status observed in Mattawoman Creek may represent shifts to different unstable or stable states (shifting baselines or regime shifts, respectively) of ecological systems rather than steady declines (Steele and Henderson 1984; Duarte et al. 2009; Cloern et al. 2016). The term "regime shift" has been used to suggest jumps between alternative equilibrium states are nonlinear, causally connected, and linked to other changes in an ecosystem (Steele 1996; Duarte et al. 2009). The regime shift concept implies that different regimes have inherent stability, so that significant forcing is required to flip the system into alternative states (Steele 1996). Eutrophication is one of these forcing mechanisms (Duarte et al. 2009), while urbanization creates a set of stream conditions (urban stream syndrome; Hughes et al. 2014a; 2014b; Mackintosh et al. 2016) that qualifies as a shift as well. Both of these processes (eutrophication and urban stream syndrome) are inter-related products of development in Mattawoman Creek's watershed. Sediment loads in Mattawoman Creek from construction and stream bank erosion were high (Gellis et al. 2009) and increased nutrient loading there was strongly associated with sediment level increases that occurred after 2003 (J. Uphoff, MDDNR, unpublished analysis of USGS data obtained by W. Romano, MDDNR). Approaching and breaching the development threshold in Mattawoman Creek's watershed has been concurrent with changes in stream hydrology and water quality, increased sediment and nutrient loading from stream erosion and construction, decreased chlorophyll a (a powerful indicator of ecosystem response to nutrients; Duarte et al. 2009) and DO. Water clarity has increased, as has TAN and SAV while finfish abundance has become more variable and less diverse (particularly planktivores) in the subestuary (Gellis et al. 2009; Uphoff et al 2009; 2010; 2011b; 2012; 2013; 2014; 2015; 2016). When evaluated in the context of Chesapeake Bay Program's habitat goals, Mattawoman Creek superficially resembles a restored system with reduced nutrient loads, i.e., increased clarity, reduced chlorophyll a, and increased SAV. Together, these factors were expected to increase habitat for fish (Chesapeake Bay Program 2014). However, Chanat et al. (2102) reported that nutrient and sediment loads in Mattawoman Creek were nearly twice those of the Choptank River, an agriculturally dominated watershed twice the size of Mattawoman Creek. Boyton et al. (2012) modeled nutrient inputs and outputs in Mattawoman Creek and found that nutrients were not exported out of the subestuary, suggesting that wetlands, emergent vegetation, and SAV in Mattawoman Creek were efficiently metabolizing and sequestering nutrients. The fish community has become highly variable and less diverse under these conditions. Duarte et al. (2009) analyzed responses of phytoplankton of four coastal ecosystems to eutrophication and oligotrophication and found diverse, idiosyncratic responses. An expectation that ecosystems would revert to an expected reference condition was unsupported (Duarte et al. 2009). During 2014, we further explored a hypothesis that water quality dynamics in Mattawoman Creek's extensive SAV beds (low DO, high pH, and high organic matter) may be creating episodes of ammonia toxicity for fish (Uphoff et al. 2014). A24-hour study in a single SAV bed suggested that fish could be caught in a habitat squeeze in SAV from high ammonia at the surface and low DO at the bottom (Uphoff et al. 2014).

Ammonia is considered one of the most important pollutants in the aquatic environment because it is both common and highly toxic (US EPA 2013). Ammonia toxicity in fish is heavily influenced by pH; temperature and salinity are considered minor influences (Randall and Tsui 2002). Low DO may lead to positive feedback of nutrient cycling and enhance NH_4 levels (Testa and Kemp 2012). The toxic substance profile for ammonia developed by the United Kingdom's Marine Special Areas of Conservation Project (2001) determined that toxicity of ammonia increased with low DO.

Breakdown of organic matter is a source of ammonia (US EPA 2013). Macrophyte beds have high primary productivity and are an important source of organic matter (Caraco and Cole 2002). The microorganisms of decay assimilate some of the organic material in the dead remains to build their cells (Cole 1975). Other organic material is converted to ammonia. This, in turn, is oxidized to nitrite and then to nitrate. Both aerobic and anaerobic bacteria function in ammonification, while only aerobic forms participate in nitrification (Cole 1975). Low DO in SAV beds can impact this biogeochemical cycle within the bed (Caraco and Cole 2002).

Some species of SAV create low DO conditions and introduced species, in particular, may induce hypoxia (Caraco and Cole 2002). Uphoff et al. (2011b) found low DO patches were not uncommon within an extensive SAV bed in Mattawoman Creek and DO conditions were generally worse within the SAV bed than in bottom channel waters. Introduced Hydrilla and Eurasian Milfoil are commonly encountered in Mattawoman Creek and often form dense beds. In general, SAV are two orders of magnitude less sensitive to ammonia than aquatic invertebrates and vertebrates (US EPA 2013).

Toxicity of ammonia to fish increases with pH (Randall and Tsui 2002; US EPA 2013) and conditions within SAV beds are in a range where enhanced toxicity could be expected. Growing season (April-October) median pH during 2004-2013, measured at the continuous monitor within the Sweden Point Marina SAV bed ranged between 7.7 and 8.2, while maximum pH varied from 8.9 to 9.6 (Uphoff et al. 2013).

If toxic ammonia caused episodic "disappearances" of Mattawoman Creek's estuarine fish community, it either did so at levels well below EPA's acute criteria for aquatic life (US EPA 2013) or at levels much greater than indicated by TAN monitoring at MAT0016. Under the temperature and pH conditions used by US EPA (2013) for chronic ammonia conditions (longer term reductions in survival, growth, or reproduction), the range of TAN maximum measurements at MAT0016 and the Sweden Point continuous monitor measurements of pH and temperature indicate a potential match for pH from 8.6 to 9.0 at water temperatures from 21 to 30 ºC during 2004-2011(Uphoff et al. 2013). Measurements of TAN from the Chesapeake Bay Program's monitoring site MAT0016, while adjacent to the continuous monitor at Sweden Point Marina, are channel measurements. These measurements may be diluted by mainstem Potomac River tidal inflow. Randall and Tsui (2002) criticized ammonia criteria for fish because they were based on starved, resting, non-stressed fish. These criteria did not protect swimming and stressed fish, nor did criteria reflect that feeding reduced the toxicity response. Fish may employ strategies, such as reduced ammonia excretion, that ameliorate ammonia toxicity (Randall and Tsui 2002). Anecdotally, we have observed multiple fish kills in Mattawoman Creek since the early 2000s. Some have followed tournament releases of Largemouth Bass at Sweden Point Marina; at least one was fairly widespread and involved multiple species.

Choptank River Subestuaries - In 2015 and 2016, we explored DO trends in mesohaline Broad Creek, Harris Creek, and Tred Avon River. These adjacent watersheds have undergone development at different levels, with two having just passed the target level of development and one approaching the development threshold. We have monitored the Tred Avon in anticipation of measuring DO and fish community changes in a mesohaline subestuary as its watershed develops over time and contrast it with less developed watersheds in the same region.

Percentages of land in agriculture (43-45%), forest (20-25%), and urban (30-34%) categories were similar (MD DOP 2010; Table 3-12; Figure 3-22). However, wetlands varied among the three systems, comprising 0.4% of Broad Creek's watershed, 5.6% of Harris Creek's, and 0.8% of Tred Avon's watershed (Table 3-12). Water comprised a larger fraction of the area considered by MD DOP (2010) in Broad and Harris Creeks (57% and 61%, respectively) than Tred Avon River (27%, respectively; i.e., water to watershed ratios were higher in the former).

Tax map estimates of C/ha indicated that the Tred Avon River watershed has been subject to more development than Harris Creek or Broad Creek watersheds and more than indicated by the MD DOP urban category (Figure 3-22). Time-series for all three watersheds started at a rural level of development (C/ha ranged from 0.1 to 0.2) in 1950 (Figure 3-23). Broad Creek has been subject to the least growth (C/ha = 0.29 in 2014), followed by Harris Creek (C/ha = 0.39 in 2014), and Tred Avon River (C/ha = 0.76 in 2014; Figure 3-23). Development accelerated noticeably in the Tred Avon watershed during 1999-2007 and then slowed. Tred Avon River's watershed has been approaching the suburban threshold, (C/ha > 0.87). The other two watersheds have passed the rural development target (C / ha = 0.27).

During 2016, bottom DO readings below the threshold (DO < 3.0 mg / L) were more frequent in the more developed Tred Avon River than the other two subestuaries (Figure 3-24). Seven percent of bottom DO measurements during 2006-2016 in Tred Avon River were below the DO threshold; 31% were below DO target. Harris Creek had 2.5% of bottom DO measurements below the target during 2012-2016 and 0% below the threshold (Figure 3-24). In Broad Creek, 17% of all DO values were below the target and 1% were below the threshold. Below target (≤ 5.0 mg / L) and threshold (≤ 3.0mg/L) bottom DO conditions were both most common the Tred Avon River, followed by Broad Creek, then Harris Creek (Table 3-13).

An ANOVA of Tred Avon River stations and bottom DO indicated significant differences were present (F = 32.83; DF = 3; $P < 0.0001$; N = 259). Tukey Studentized Range and Tukey Honestly Significant Difference (HSD) tests indicated that bottom DO at station 1 (station at Easton, Maryland) was significantly lower than downstream stations 2, 3, and 4 (critical value of studentized range = 3.65723). This result was consistent with other mesohaline tributaries with high impervious surface; DO declines as you move upstream (Uphoff et al. 2011a). The mean and SE for bottom DO at all stations in Tred Avon River were 5.36 mg / L and 0.09, respectively. Mean and SE for station 1 were 4.07 mg / L and 0.21; station 2 was 5.76 mg / L and 0.12; station 3 was 5.81 mg / L and 0.13; and station 4 was 5.84 mg / L and 0.12. Deterioration of DO at the uppermost station (station 1; Figure 3-25) during 2006-2015 indicated that watershed development around Easton was the source of poor water quality rather than water intruding from downstream. During 2016, DO at station 1 was above target value and the overall median for the time-series. Likewise, stations, 2 and 4 had mean bottom DO between 6 and 7 mg / L in 2016, while station 3 was just below the mean of the overall time series. ANOVAs of Broad Creek (F = 1.51; DF = 3; *P* = 0.2185; N = 94) and Harris Creek (F = 1.13; DF = 3; *P* = 0.3418; N = 94) stations and bottom DO concentrations did not indicate significant differences among stations. The overall means (SE) for bottom DO in Broad Creek and Harris Creek were 6.03 mg / L (0.12) and 6.26 mg / L (0.08), respectively.

Modified Proportional stock densities (MPSD) for White Perch (Table 3-14) during 2012-2016 were higher in less developed Harris (MPSD = 7%, SD = 0.60%) and Broad Creeks (MPSD = 13%, SD = 0.96%), than more developed Tred Avon River (MPSD = 2%, SD = 0.12% for 2006-2016). Size quality of White Perch directly aligned with the percentage of all DO measurements below the target level (5.0 mg / L). However, sample sizes observed from 2012-2016 indicate that White Perch were more abundant in Tred Avon River, so diminished size quality may reflect density-dependence. Tred Avon River is both the most developed watershed of the three Choptank River subestuaries and is closer to the Choptank River spawning area. Abundance of adult White Perch in trawl samples was negatively influenced by development and distance from their spawning area (Uphoff et al. 2011a).

Gunpowder and Middle Rivers - Oligohaline Gunpowder and Middle Rivers were routinely sampled during 2009-2016. In the fall of 2015 and 2016, fish kills occurred in Middle River (2015) and in Gunpowder River (2016; MDE 2016; 2017). The Maryland Department of the Environment reported that both fish kills were caused by high amounts of toxic algae, *Karlodinium veneficum*, whose toxin causes gill damage to fish when in high concentrations (MDE 2016; 2017). In 2015, MD DNR biologists discovered and confirmed zebra mussel presence in the Middle River. Nogaro and Steinman (2014) indicated that invasive mussels can change a system's ecosystem and promote toxic algae blooms. These events triggered a review of our data collected during 2009-2016.

In both Gunpowder and Middle Rivers we examined trends in Secchi depth to see if water clarity was increasing with colonization by Zebra Mussels (Figure 3-26). During 2009-2015, Secchi measurements in Gunpowder River ranged from 0.3m to 0.5m; lowest median Secchi depths were during 2012-2013 and highest during 2014-2016. Median Secchi depth in Middle River ranged from 0.5m to 0.6m during 2009-2013, and then increased to 0.8m to 1.2m.

Starting in 1989 and continuing through 2000, SAV coverage was low in both subestuaries (Figure 3-27), usually well below the median of the time-series (1989-2016 medians: Gunpowder River = 9% and Middle River = 12.8%; Figure 3-27). SAV coverage was typically well above the time-series median after 2000, except in 2002, 2003, 2011, and 2012 for Gunpowder and in 2006 for Middle. In 2011-2012, SAV coverage was below or at the median in both systems. In 2016, coverage reached the highest point in the Middle River time-series, but dropped to the median in Gunpowder River (Figure 3-27).

Bottom DO (mg/L) did not appear to fluctuate dramatically from year to year in Gunpowder and Middle rivers (Figure 3-28). Measurements of pH were typically between 7 and 8 (Figure 3-29), but Gunpowder River pH measurements appeared to have a wider range than Middle River pH measurements.

During 2009-2016, median salinity ranged from 0.34 and 3.19 ‰ in Gunpowder River and 1.2 to 5.49 ‰ in Middle River (Figure 3-30). Salinity was high in 2016, with measurements reaching 4.27 ‰ in Gunpowder River and 7.36 ‰ in Middle River (Figure 3-30). Increased salinity could have negative or lethal effects on Zebra Mussels depending on variability of salinity measurements and exposure time. USGS (2017) indicated Zebra Mussels in North America can tolerate salinity up to 4 ‰ and Strayer and Smith (1993), Spidle (1994), and Walton (1996) indicated repeated salinity levels greater than 5 ‰ were lethal. Zebra Mussels within the Chesapeake Bay subestuaries are usually found at 3 ‰ or less (M. Ashton, MD DNR *personal communication*).

Gunpowder and Middle Rivers geometric mean trawl catches of all finfish (GM) and their 95% CI's were plotted for 2009-2016 (Figure 3-31). Relative abundance was highest during 2009-2011. Since 2011, both rivers exhibited lower finfish catches. Geometric means were of similar magnitude in the two rivers (Figure 3-31).

We separated all subestuaries sampled from 1989-2016 by salinity class, then ranked all annual trawl geometric means of catches of all species (GM) to find where Gunpowder and Middle Rivers fell against the other oligohaline subestuaries (Table 3-15). Gunpowder River had two GMs within the top third of ranks; five GMs in the bottom third; and one GM in the middle third (Table 3-15). Middle River had one GM within the top third; two in the bottom third); and five in the middle. The two years with confirmed zebra mussel presence in Middle River, 2015-2016, ranked in the middle third (Table 3-15).

Finfish composition for seine catches was compiled together for all species for the time-series, 2009–2016, for both Gunpowder and Middle Rivers (Figure 3-32). White Perch (juveniles) and Gizzard Shad were the predominant species in seine catches in Middle River time-series. Atlantic Menhaden and Atlantic Silverside were the predominant species in seine catches in Gunpowder River time-series. Blueback Herring, Banded Killifish, Bay Anchovy, Gizzard Shad, Spottail Shiner, and Pumpkinseed were present in the top 90% of seine catches in both Gunpowder and Middle Rivers. White Perch, both adults and juveniles, were only observed in the top 90% in the Middle River. Atlantic Menhaden were only present in the top 90% in the Gunpowder River. Both Gunpowder and Middle Rivers had 8 species of finfish that made up 90% of seine catches, the remaining 10% comprised of 37 species in Gunpowder River and 25 species in Middle River (Figure 3-32).

Finfish in trawl catches that comprised the annual total catches for Gunpowder and Middle Rivers were graphed (Figure 3-33). Bay Anchovy and White Perch juveniles) were the most predominant species during 2009-2016. In the Gunpowder River, "other species" consists of 19 species in 2009; 22 species in 2010-2011; 19 species in 2012-2013; 22 species in 2014-2015; and 21 species in 2016. In the Middle River, "other species" consist of 14 species in 2009; 19 species in 2010-2011; 15 species in 2012-2013; 20 species in 2014; 18 species in 2015; and 14 species in 2016. Adult White Perch were present at low percentages in both Gunpowder and Middle Rivers in 2016 (Figure 3-33). Declines abundance and species composition in response to fish kills in Middle River during 2015 were not detected.

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Table 3-1. General location (area), percent impervious cover (IS), structures per hectare (C/ha), watershed area (Total Hectares), area of tidal water (Water Hectares), and salinity class for the subestuaries sampled in 2016.

Area	Subestuary	IS	C/ha	Total Hectares	Water Hectares	Salinity Class
Upper-Bay	Bush River	14.3	1.51	36,038	2,962	Oligohaline
Upper-Bay	Gunpowder River	9.0	0.73	113,760	4,108	Oligohaline
Upper-Bay	Northeast River	6.9	0.48	16,342	1,579	Tidal Fresh
Mid-Bay	Middle River	23.5	3.34	2,753	982	Oligohaline
Mid-Bay	Broad Creek	5.1	0.29	4,730	3,148	Mesohaline
Mid-Bay	Harris Creek	6.1	0.39	3696	2,919	Mesohaline
Mid-Bay	Tred Avon River	9.2	0.76	9,563	2,429	Mesohaline
Potomac	Mattawoman Creek	10.5	0.93	24,441	729	Tidal Fresh
Potomac	Nanjemoy Creek	2.5	0.09	18,893	1,131	Oligohaline

Table 3-2. Estimates of C/ha and land use percentage from Maryland Department of Planning (2002 and 2010) for subestuaries sampled 2003-2016.

River	Year	C/ha	Agriculture	Wetland	Forest	Urban
Breton Bay	2003	0.265021	26.30	0.50	61.50	11.30
Breton Bay	2004	0.281742	26.30	0.50	61.50	11.30
Breton Bay	2005	0.298533	26.30	0.50	61.50	11.30
Broad Creek	2012	0.293475	42.55	0.36	25.39	31.47
Broad Creek	2013	0.2958	42.55	0.36	25.39	31.47
Broad Creek	2014	0.296435	42.55	0.36	25.39	31.47
Broad Creek	2015	0.296435	42.55	0.36	25.39	31.47
Broad Creek	2016	0.296435	42.55	0.36	25.39	31.47
Corsica River	2003	0.171949	64.32	0.43	27.36	7.89
Corsica River	2004	0.184452	64.32	0.43	27.36	7.89
Corsica River	2005	0.193959	64.32	0.43	27.36	7.89
Corsica River	2006	0.211423	64.32	0.43	27.36	7.89
Corsica River	2007	0.224649	64.32	0.43	27.36	7.89
Corsica River	2008	0.23705	64.32	0.43	27.36	7.89
Corsica River	2011	0.250586	60.40	0.10	25.51	13.20
Corsica River	2012	0.254	60.40	0.10	25.51	13.20
Harris Creek	2012	0.387979	44.87	5.61	19.72	29.80
Harris Creek	2013	0.387708	44.87	5.61	19.72	29.80
Harris Creek	2014	0.387979	44.87	5.61	19.72	29.80
Harris Creek	2015	0.387979	44.87	5.61	19.72	29.80
Harris Creek	2016	0.387979	44.87	5.61	19.72	29.80
Langford Creek	2006	0.072884	71.63	1.48	23.04	3.85
Langford Creek	2007	0.073608	71.63	1.48	23.04	3.85
Langford Creek	2008	0.073504	71.63	1.48	23.04	3.85
Magothy River	2003	2.678242	6.00	0.00	32.80	61.10
Miles River	2003	0.23851	56.10	1.40	30.40	12.10
Miles River	2004	0.243382	56.10	1.40	30.40	12.10
Miles River	2005	0.244374	56.10	1.40	30.40	12.10
Rhode/West Rivers	2003	0.5484	36.40	1.00	44.90	17.70
Rhode/West Rivers	2004	0.5549	36.40	1.00	44.90	17.70
Rhode/West Rivers	2005	0.5611	36.40	1.00	44.90	17.70
Severn River	2003	2.058995	11.10	0.20	41.20	47.30
Severn River	2004	2.09118	11.10	0.20	41.20	47.30
Severn River	2005	2.148981	11.10	0.20	41.20	47.30
South River	2003	1.234149	19.90	0.40	50.50	29.00
South River	2004	1.2497	19.90	0.40	50.50	29.00
South River	2005	1.26471	19.90	0.40	50.50	29.00

Table 3-2. Continued.

St. Clements River	2003	0.192976	40.90	0.80	51.30	7.00
St. Clements River	2004	0.19621	40.90	0.80	51.30	7.00
St. Clements River	2005	0.198532	40.90	0.80	51.30	7.00
Tred Avon River	2006	0.691286	50.08	1.00	21.58	27.23
Tred Avon River	2007	0.713035	50.08	1.00	21.58	27.23
Tred Avon River	2008	0.724433	50.08	1.00	21.58	27.23
Tred Avon River	2009	0.736144	50.08	1.00	21.58	27.23
Tred Avon River	2010	0.74681	43.20	0.85	21.63	33.57
Tred Avon River	2011	0.750993	43.20	0.85	21.63	33.57
Tred Avon River	2012	0.75298	43.20	0.85	21.63	33.57
Tred Avon River	2013	0.754025	43.20	0.85	21.63	33.57
Tred Avon River	2014	0.757267	43.20	0.85	21.63	33.57
Tred Avon River	2015	0.757267	43.20	0.85	21.63	33.57
Tred Avon River	2016	0.757267	43.20	0.85	21.63	33.57
Wicomico River	2003	0.193906	34.71	4.58	48.52	12.03
Wicomico River	2011	0.212462	31.60	4.57	44.94	18.74
Wicomico River	2012	0.213493	31.60	4.57	44.94	18.74
Wye River	2007	0.095131	67.72	0.66	23.51	8.06
Wye River	2008	0.095424	67.72	0.66	23.51	8.06

Table 3-3. Percentages of all DO measurements (surface, mid-depth, and bottom) and bottom DO measurements that did not meet target (= 5.0 mg/L) and threshold (= 3.0 mg/L) conditions during July – September 2016, for each subestuary. The N refers to the total number of DO measurements for each system for all DO and bottom DO measurements. C/ha = structures per hectare.

			All DO		Bottom DO		
Subestuary	Salinity Class	C/ha	N	% < 5.0 mg/L	N	% < 5.0 mg/L	% < 3.0 mg/L
Broad Creek	Mesohaline	0.29	76	4	22	9	0
Harris Creek	Mesohaline	0.39	79	3	23	9	0
Tred Avon River	Mesohaline	0.76	96	13	24	38	13
Middle River	Oligohaline	3.34	59	9	22	2	0
Gunpowder River	Oligohaline	0.73	50	2	8	0	0
Nanjemoy Creek	Oligohaline	0.09	45	16	7	57	0
Bush River	Tidal Fresh	1.51	51	10	17	24	0
Mattawoman Creek	Tidal Fresh	0.93	96	5	42	7	0
Northeast River	Tidal Fresh	0.48	84	0	24	0	0

Table 3-4. Beach seine catch summary for 2016. C/ha = structures per hectare. GM = Geometric mean. *Italicized species are target species*.

River	Stations Sampled	Number of Samples	Number of Species	Comprising 90% of Catch	C / ha	Total Catch	GM CPUE
Broad Creek	3	14	26	Atlantic Silverside Banded Killifish Striped Killifish Sheepshead Minnow Mummichog *Atlantic Menhaden*	0.29	6904	398
Bush River	4	24	28	*Atlantic Menhaden* *Gizzard Shad* *White Perch (JUV)* *Spottail Shiner* *White Perch (Adult)*	1.51	7508	210
Gunpowder River	3	18	28	*Atlantic Menhaden* Pumpkinseed *Spottail Shiner* *Gizzard Shad* Banded Killifish *Blueback Herring* *White Perch (Adult)* Bluegill Atlantic Silverside	0.73	3093	133
Harris Creek	3	17	29	Atlantic Silverside Striped Killifish *Atlantic Menhaden* Mummichog Banded Killifish Rainwater Killifish	0.39	8087	385
Nanjemoy Creek	3	18	27	*Atlantic Menhaden* *White Perch (JUV)* Atlantic Silverside *White Perch (Adult)* Inland Silverside Bay Anchovy Pumpkinseed	0.09	2342	64
Northeast River	4	24	27	*Gizzard Shad* *Blueback Herring* *White Perch (JUV)* *White Perch (Adult)* Bay Anchovy *Alewife*	0.48	9118	240

Table 3-4. Continued.

Tred Avon River	4	24	29	*Atlantic Menhaden* Atlantic Silverside Mummichog *White Perch (Adult)* Striped Killifish	0.76	9255	203
Grand Total	24	139	58	*Atlantic Menhaden* Atlantic Silverside *Gizzard Shad* Striped Killifish *Blueback Herring* Banded Killifish *White Perch (Adult)* Mummichog *White Perch (JUV)* Bay Anchovy Sheepshead Minnow		46307	

Table 3-5. Bottom Trawl (4.9 m headrope) catch summary for 2016. C/ha = structures per hectare. GM = Geometric. *Italicized species are target species.*

River	Stations Sampled	Number of Samples	Number of Species	Comprising 90% of Catch	C / ha	Total Catch	GM CPUE
Broad Creek	4	23	22	Bay Anchovy *Weakfish*	0.29	4277	127
Bush River	3	17	21	*White Perch (Adult)* *Gizzard Shad* *White Perch (JUV)* Bay Anchovy Brown Bullhead	1.51	5726	249
Gunpowder River	4	24	22	Bay Anchovy *Spottail Shiner* *White Perch (Adult)* Pumpkinseed	0.73	6144	205
Harris Creek	4	23	23	Bay Anchovy *White Perch (Adult)*	0.39	2705	40
Mattawoman Creek	4	24	22	*White Perch (JUV)* *Spottail Shiner* *White Perch (Adult)*	0.93	5785	145
Middle River	4	23	15	Bay Anchovy Pumpkinseed *White Perch (Adult)*	3.34	7048	260
Nanjemoy Creek	3	18	17	*White Perch (JUV)* Bay Anchovy	0.09	7349	296
Northeast River	4	24	17	*White Perch (JUV)* *White Perch (Adult)* Bay Anchovy Brown Bullhead	0.48	2906	96
Tred Avon River	4	24	19	Bay Anchovy *Weakfish*	0.76	5250	103
Grand Total	34	200	51	Bay Anchovy *White Perch (JUV)* *White Perch (Adult)* *Spottail Shiner* Pumpkinseed		47190	

Table 3-6. Mean surface and bottom temperatures, mean surface and bottom dissolved oxygen (mg/L), and C/ha of subestuaries sampled during summer 2003-2016, by salinity class.

			Temperature		Dissolved Oxygen	
River	Year	C / ha	Surface	Bottom	Surface	Bottom
Mesohaline						
Blackwater River	2006	0.04	28.14	27.98	5.27	4.12
Breton Bay	2003	0.27	26.40	25.69	8.10	3.75
	2004	0.28	27.01	25.95	7.36	3.73
	2005	0.30	28.62	27.51	6.98	3.99
Broad Creek	2012	0.29	27.50	26.60	8.30	5.97
	2013	0.30	27.30	26.49	7.26	5.76
	2014	0.30	27.62	26.64	7.65	5.78
	2015	0.30	28.05	27.05	7.93	6.63
	2016	0.30	29.16	28.33	7.30	6.16
Corsica River	2003	0.17	25.90	26.13	6.50	4.67
	2004	0.18	27.18	26.88	5.57	4.57
	2005	0.19	28.54	28.14	6.48	3.08
	2006	0.21	27.39	26.84	7.55	4.05
	2007	0.22	25.94	25.82	6.24	4.22
	2008	0.24	26.20	25.22	7.32	4.21
	2011	0.25	27.00	27.01	5.30	3.28
	2012	0.25	27.79	27.47	4.71	3.40
Fishing Bay	2006	0.03	26.23	25.28	7.24	6.79
Harris Creek	2012	0.39	26.55	26.42	7.44	6.35
	2013	0.39	26.39	26.05	7.02	6.01
	2014	0.39	26.89	26.21	6.78	4.54
	2015	0.39	26.62	26.62	7.19	6.56
	2016	0.39	27.82	27.75	6.65	6.02
Langford Creek	2006	0.07	27.05	26.52	6.95	5.68
	2007	0.07	26.23	25.48	6.69	5.68
	2008	0.07	27.14	26.69	6.68	4.79
Magothy River	2003	2.68	25.70	25.31	7.30	2.04
Miles River	2003	0.24	25.50	25.60	6.50	4.09
	2004	0.24	25.75	25.64	6.08	5.47
	2005	0.24	28.03	27.44	5.96	3.31
Rhode River	2003	0.47	25.00	24.69	7.10	4.80
	2004	0.47	27.00	26.95	6.58	5.39
	2005	0.48	27.78	27.16	6.50	4.03
Severn River	2003	2.06	26.30	24.75	7.60	1.57
	2004	2.09	27.42	26.18	7.05	2.64
	2005	2.15	28.01	26.23	7.07	0.96
South River	2003	1.23	25.40	24.56	7.60	2.61
	2004	1.25	25.79	25.48	6.46	3.77
	2005	1.26	27.57	26.67	6.02	2.49

Table 3-6. Continued.

St. Clements River	2003	0.19	26.00	25.29	8.20	3.48
	2004	0.20	26.08	25.78	6.84	4.61
	2005	0.20	26.94	26.42	6.73	4.35
Transquaking River	2006	0.03	26.68	22.75	5.75	5.85
Tred Avon River	2006	0.69	27.12	26.72	6.18	5.34
	2007	0.71	26.85	26.59	6.49	5.39
	2008	0.72	26.28	25.61	6.90	4.83
	2009	0.74	26.15	26.03	7.37	6.31
	2010	0.75	27.47	26.93	7.08	5.26
	2011	0.75	28.48	28.18	6.82	5.11
	2012	0.75	27.27	27.16	7.02	5.47
	2013	0.75	26.79	26.39	7.15	5.00
	2014	0.75	26.66	26.51	6.12	5.90
	2015	0.75	28.00	27.60	6.92	5.54
	2016	0.76	28.89	28.44	7.27	5.15
West River	2003	0.64	24.90	24.31	7.40	4.84
	2004	0.65	26.83	26.59	7.37	5.58
	2005	0.66	27.96	27.15	6.72	3.99
Wicomico River	2003	0.19	25.40	23.83	7.00	5.85
	2011	0.21	27.08	26.89	5.57	4.30
	2012	0.21	27.57	27.38	6.59	5.44
Wye River	2007	0.10	26.75	26.45	7.08	5.70
	2008	0.10	26.98	26.22	5.70	5.11
Oligohaline						
Bohemia River	2006	0.11	26.79	26.02	7.01	6.41
Bush River	2006	1.41	25.48	24.28	7.96	7.47
	2007	1.43	27.02	26.42	7.68	6.54
	2008	1.45	26.59	24.20	9.00	5.43
	2009	1.46	25.88	24.34	9.41	8.54
	2010	1.47	27.72	23.80	7.79	7.04
	2011	1.48	26.98	26.94	6.47	5.50
	2012	1.49	26.79	26.17	6.63	5.20
	2013	1.51	25.11	24.73	9.98	6.73
	2014	1.51	26.79	25.92	7.21	5.12
	2015	1.51	26.82	25.92	7.24	5.25
	2016	1.51	27.98	27.48	7.97	6.34
Gunpowder River	2009	0.72	25.71	26.05	7.39	6.79
	2010	0.72	25.17	25.91	7.89	7.13
	2011	0.72	25.09	25.56	8.28	7.14
	2012	0.73	26.48	25.93	8.19	6.71
	2013	0.73	25.85	27.46	8.05	6.10
	2014	0.73	26.70	25.96	7.23	5.24
	2015	0.73	27.51	27.65	8.02	6.63
	2016	0.73	27.70	26.46	7.43	6.18

Table 3-6. Continued.

Middle River	2009	3.30	26.50	25.78	7.27	6.07
	2010	3.32	24.65	24.20	8.44	7.11
	2011	3.33	27.13	26.42	8.35	7.33
	2012	3.33	28.05	26.60	8.82	5.21
	2013	3.34	27.12	26.46	7.58	5.79
	2014	3.34	26.75	26.12	7.76	6.27
	2015	3.34	28.47	27.20	8.20	6.23
	2016	3.35	28.87	27.82	7.56	5.69
Nanjemoy River	2003	0.08	25.90	28.80	7.30	4.96
	2008	0.09	27.53	26.58	7.85	6.65
	2009	0.09	26.31	24.64	7.05	7.49
	2010	0.09	26.50	24.80	7.66	7.02
	2011	0.09	29.34	28.55	6.13	5.30
	2012	0.09	26.18	25.92	6.73	5.98
	2013	0.09	26.88	26.30	6.76	5.86
	2014	0.09	26.85	26.41	7.66	6.28
	2015	0.09	27.40	27.10	7.16	6.32
	2016	0.09	28.49	28.21	6.86	5.16
Tidal-fresh						
Mattawoman Creek	2003	0.76	26.00	25.75	9.00	8.81
	2004	0.79	27.33	27.14	8.34	7.95
	2005	0.81	28.77	28.09	7.74	7.27
	2006	0.83	27.05	26.44	7.10	6.50
	2007	0.86	26.89	26.85	6.70	6.48
	2008	0.87	26.40	24.52	7.97	6.33
	2009	0.88	26.20	26.64	7.92	7.86
	2010	0.90	26.21	26.10	6.95	6.62
	2011	0.91	27.08	27.46	6.33	6.51
	2012	0.90	26.70	26.82	7.40	7.00
	2013	0.91	26.35	25.94	9.22	8.40
	2014	0.91	26.90	26.34	7.56	6.26
	2015	0.91	27.91	26.84	8.66	7.74
	2016	0.93	28.47	28.03	6.96	6.54
Northeast River	2007	0.44	26.83	26.43	9.73	7.75
	2008	0.44	25.35	24.98	8.43	7.70
	2009	0.45	26.33	25.55	9.35	7.36
	2010	0.46	25.90	26.21	7.76	6.78
	2011	0.46	25.97	25.71	6.87	5.79
	2012	0.47	27.78	27.59	7.88	6.03
	2013	0.47	26.61	26.11	9.33	7.06
	2014	0.47	26.83	26.46	7.74	6.67
	2015	0.47	26.66	26.23	7.84	6.17
	2016	0.48	27.95	26.86	8.81	7.10

Table 3-6. Continued.

Piscataway Creek	2003	1.30	25.60	24.63	10.20	8.33
	2006	1.38	28.16	24.97	8.70	6.85
	2007	1.40	27.47	26.00	8.57	7.60
	2009	1.43	26.72	27.07	8.56	6.62
	2010	1.45	27.07	25.08	9.36	7.63
	2011	1.46	28.25	30.07	9.05	9.47
	2012	1.47	27.92	25.51	9.53	9.34
	2013	1.49	27.19	26.22	9.87	7.65
	2014	1.49	27.01	26.31	8.63	7.31

Table 3-7. Correlations (r), level of significance (*P*), and sample size (N) of comparisons of 2003-2016 mean annual surface or bottom DO (mg/L) with mean water temperatures (surface and bottom) or watershed development (C/ha = structures per hectare), by salinity class. Bold numbers indicate a significant relationship at $\alpha < 0.05$.

DO Depth	Statistics	Temperature	C / ha
Mesohaline			
Surface	r	-0.13858	0.19945
	P	0.2828	0.1201
	N	62	62
Bottom	r	0.08271	**-0.57376**
	P	0.5227	**<.0001**
	N	62	**62**
Oligohaline			
Surface	r	**-0.35155**	**0.36207**
	P	**0.0304**	**0.025**
	N	**38**	**38**
Bottom	r	**-0.56287**	-0.00681
	P	**0.0002**	0.9676
	N	**38**	38
Tidal Fresh			
Surface	r	-0.03087	0.31096
	P	0.8646	0.0782
	N	33	33
Bottom	r	0.05528	**0.41152**
	P	0.76	**0.0173**
	N	33	**33**

Table 3-8.Correlations (r) among land use categories and C/ha, level of significance (*P*), and sample size for mesohaline subestuaries sampled during 2003-2016. Land cover estimates were estimated by Maryland Department of Planning for 2002 and 2010. Bold numbers indicate a significant relationship at $\alpha < 0.05$.

			Land Use Categories			
	Statistics	**C/ha**	**Agriculture**	**Wetland**	**Forest**	**Urban**
C/ha	r	1				
	P					
	N					
Agriculture	r	**-0.7442**	1			
	P	**<0.0001**				
	N	**49**				
Wetland	r	-0.26497	0.0292	1		
	P	0.0658	0.9114			
	N	49	17			
Forest	r	0.13755	**-0.59347**	0.00743	1	
	P	0.3459	**0.012**	0.9774		
	N	49	**17**	17		
Urban	r	**0.85969**	**-0.72596**	-0.14936	-0.116	1
	P	**<0.0001**	**0.001**	0.5672	0.658	
	N	**49**	**17**	17	17	

Table 3-9. Statistics and parameter estimates for regional (western and eastern shore) multiple linear regression of change in forest cover with structure density (C / ha).

Linear Model	Region = Forest cover with structure density (C / ha)					
ANOVA	df	SS	MS	F	Significance F	
Regression	2	10.68579	5.3429	259.57	<.0001	
Residual	46	0.94686	0.02058			
Total	48	11.63265				
$r^2 = 0.9186$						
	Estimate	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-0.87308	0.05918	-14.75	<.0001	-0.99219	-0.75397
C/ha	0.23841	0.03514	6.78	<.0001	0.16767	0.30915
Forest	0.03341	0.00162	20.61	<.0001	0.03015	0.03668

Table 3-10. Statistics and parameter estimates for regional (western and eastern shore) linear regressions of median bottom DO versus percent agricultural coverage.

Linear Model	Western Shore: Median DO = Agriculture (%)					
ANOVA	df	SS	MS	F	Significance F	
Regression	1	40.35023	40.35023	37.63	<.0001	
Residual	17	18.22754	1.07221			
Total	18	58.57777				
r^2 = 0.6888						
	Estimate	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.04701	0.62427	0.08	0.9408	-1.27009	1.36412
Agriculture (%)	0.13253	0.0216	6.13	<.0001	0.08695	0.17812
Linear Model	Eastern Shore: Median DO = Agriculture (%)					
ANOVA	df	SS	MS	F	Significance F	
Regression	1	8.7531	8.7531	12.76	0.0011	
Residual	35	24.01556	0.68616			
Total	36	32.76866				
r^2 = 0.2671						
	Estimate	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	7.75011	0.71192	10.89	<.0001	6.30483	9.19539
Agriculture (%)	-0.04699	0.01316	-3.57	0.0011	-0.0737	-0.02028

Table 3-11. Statistics and parameter estimates for a quadratic regression of median bottom DO versus agricultural (%) coverage.

Linear Model	Median Bottom DO = Agriculture (%) Coverage					
ANOVA	df	SS	MS	F	Significance F	
Regression	2	76.54702	38.27351	40.87	<.0001	
Residual	53	49.633	0.93647			
Total	55	126.18				
r^2 = 0.6066						
	Estimate	SE	t Stat	P-value	Lower 95%	Upper 95%
Intercept	-1.26141	0.67554	-1.87	0.0674	-2.61638	0.09356
Agriculture (%)	0.27238	0.03323	8.2	<.0001	0.20574	0.33902
Agriculture (%)^2	-0.00274	0.000388	-7.06	<.0001	-0.00352	-0.00196

Table 3-12. Estimated percent of land cover in three Choptank River subestuary watersheds. Estimates are determined from Department of Planning 2010 data. The top four land use categories are for land only. Water is the percent of water + land surface area that water represents.

	Subestuary		
Land Use Category	**Broad Creek**	**Harris Creek**	**Tred Avon River**
Agriculture	42.55	44.87	43.2
Forest	25.39	19.72	21.63
Urban	31.47	29.8	33.57
Wetlands	0.36	5.61	0.85
Water	57.28	61.18	24.4

Table 3-13. Percentages of all DO measurements (surface, middle, and bottom) and bottom DO measurements that did not meet target (= 5.0mg/L) and threshold (= 3.0mg/L) conditions during July – September for all sampling years, for each Choptank River subestuary. The N refers to the total number of DO measurements for each system by year for all DO and bottom DO measurements.

				All DO		Bottom DO	
Subestuary	Year	C / ha	N	% < 5.0 mg/L	N	% < 5.0 mg/L	% < 3.0 mg/L
Broad Creek	2012	0.29	83	7	24	17	4
	2013	0.30	78	10	23	30	0
	2014	0.30	81	6	24	21	0
	2015	0.30	82	1	23	0	0
	2016	0.29	76	4	22	9	0
Harris Creek	2012	0.39	82	0	23	0	0
	2013	0.39	83	0	24	0	0
	2014	0.39	84	1	23	4	0
	2015	0.39	85	0	24	0	0
	2016	0.39	79	3	23	9	0
Tred Avon River	2006	0.69	91	19	24	38	0
	2007	0.71	93	11	23	26	4
	2008	0.72	89	24	21	48	14
	2009	0.74	95	6	24	13	0
	2010	0.75	89	20	24	38	13
	2011	0.75	82	22	21	48	10
	2012	0.75	94	10	24	29	0
	2013	0.75	103	15	26	31	15
	2014	0.75	96	11	24	21	0
	2015	0.75	96	8	24	21	13
	2016	0.76	96	13	24	38	13

Table 3-14. Modified proportional stock density (MPSD) of White Perch in Choptank River subestuaries.

Subestuary	Years	N Stock	Quality (200mm)	MPSD	SD
Broad Creek	2012-2016	1,738	118	7%	0.60%
Harris Creek	2012-2016	1,374	174	13%	0.96%
Tred Avon River	2006-2016	12,272	236	2%	0.12%

Table 3-15. Subestuaries sampled during 2003-2016, by salinity class and ranked by annual 4.9 m trawl geometric mean (GM) catches.

River	Year	GM	Rank
Mesohaline			
Corsica River	2010	845	1
Wicomico River	2003	616	2
Broad Creek	2014	384	3
Broad Creek	2012	274	4
Langford Creek	2007	271	5
Langford Creek	2006	256	6
Tred Avon River	2010	251	7
Corsica River	2004	250	8
Corsica River	2011	237	9
Tred Avon River	2014	181	10
Corsica River	2006	173	11
Wye River	2007	167	12
Harris Creek	2014	161	13
Corsica River	2012	160	14
Langford Creek	2008	156	15
Rhode River	2005	148	16
Tred Avon River	2008	139	17
Broad Creek	2013	137	18
Corsica River	2007	131	19
Broad Creek	2016	127	20
Tred Avon River	2012	126	21
Tred Avon River	2007	119	22
Harris Creek	2012	117	23
Corsica River	2005	109	24
Wye River	2008	106	25
Fishing Bay	2006	104	26
West River	2005	103	27
Wicomico River	2012	103	28
Tred Avon River	2016	103	29
Corsica River	2008	101	30
Transquaking River	2006	94	31
Broad Creek	2015	91	32
Harris Creek	2013	87	33
Tred Avon River	2011	82	34
Tred Avon River	2009	81	35
Miles River	2004	76	36
Tred Avon River	2015	74	37
Tred Avon River	2013	72	38
Miles River	2005	68	39
Wicomico River	2011	60	40
Tred Avon River	2006	55	41

Table 3-15. Continued.

St. Clements River	2005	51	42
Harris Creek	2016	40	43
Breton Bay	2005	33	44
South River	2005	33	45
Harris Creek	2015	30	46
Rhode River	2004	25	47
Blackwater River	2006	22	48
St. Clements River	2004	20	49
West River	2004	18	50
Breton Bay	2004	16	51
South River	2004	16	52
South River	2004	5	53
South River	2005	3	54
Oligohaline			
Bush River	2011	665	1
Nanjemoy Creek	2013	574	2
Bush River	2014	528	3
Middle River	2011	519	4
Bush River	2010	465	5
Nanjemoy Creek	2015	416	6
Nanjemoy Creek	2014	395	7
Gunpowder River	2011	393	8
Gunpowder River	2010	391	9
Nanjemoy Creek	2011	376	10
Bush River	2007	323	11
Bush River	2015	321	12
Bush River	2009	319	13
Middle River	2010	309	14
Nanjemoy Creek	2016	296	15
Nanjemoy Creek	2010	294	16
Middle River	2009	291	17
Gunpowder River	2009	286	18
Middle River	2015	286	19
Nanjemoy Creek	2009	280	20
Middle River	2016	260	21
Middle River	2014	251	22
Bush River	2016	249	23
Bush River	2012	248	24
Nanjemoy Creek	2012	231	25
Gunpowder River	2012	222	26
Gunpowder River	2014	218	27
Gunpowder River	2015	215	28
Bush River	2013	215	29
Bush River	2008	210	30
Nanjemoy Creek	2008	210	31

Table 3-15. Continued.

Gunpowder River	2016	205	32
Middle River	2013	180	33
Bush River	2006	152	34
Middle River	2012	146	35
Gunpowder River	2013	146	36
Bohemia River	2006	111	37
Tidal-Fresh			
Mattawoman Creek	2014	580	1
Northeast River	2010	391	2
Piscataway Creek	2011	320	3
Northeast River	2014	291	4
Northeast River	2011	290	5
Piscataway Creek	2010	289	6
Mattawoman Creek	2013	283	7
Piscataway Creek	2014	221	8
Mattawoman Creek	2015	217	9
Mattawoman Creek	2011	209	10
Mattawoman Creek	2004	202	11
Northeast River	2009	198	12
Northeast River	2012	191	13
Northeast River	2013	186	14
Mattawoman Creek	2005	185	15
Piscataway Creek	2013	184	16
Northeast River	2008	152	17
Northeast River	2015	150	18
Northeast River	2007	148	19
Mattawoman Creek	2016	145	20
Mattawoman Creek	2003	143	21
Piscataway Creek	2012	119	22
Piscataway Creek	2009	105	24
Northeast River	2016	96	26
Mattawoman Creek	2010	78	27
Mattawoman Creek	2006	73	28
Mattawoman Creek	2012	69	30
Mattawoman Creek	2007	55	33
Piscataway Creek	2006	28	42
Mattawoman Creek	2008	25	43
Mattawoman Creek	2009	10	45
Piscataway Creek	2007	8	46

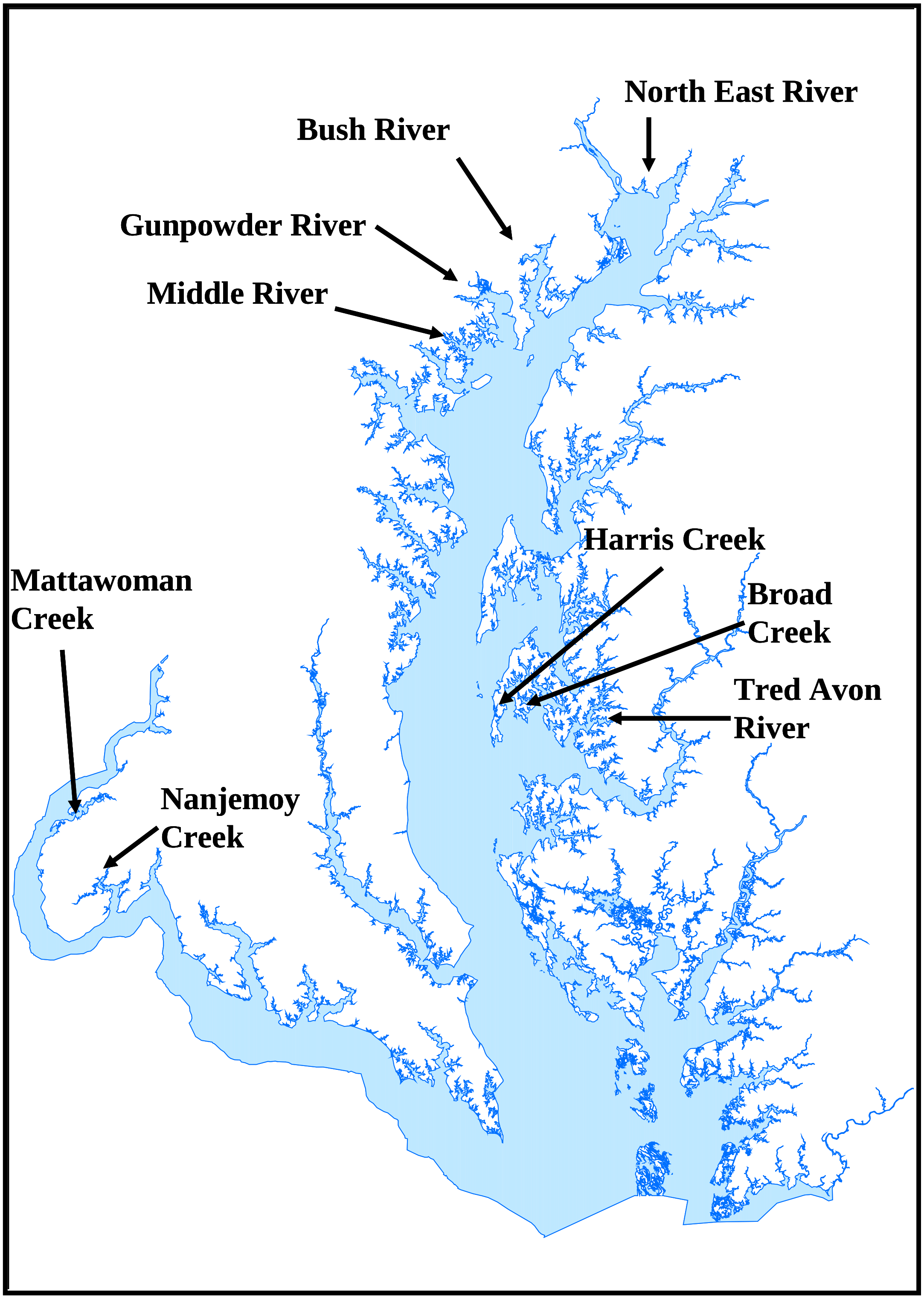

Figure 3-1. Subestuaries sampled in 2016.

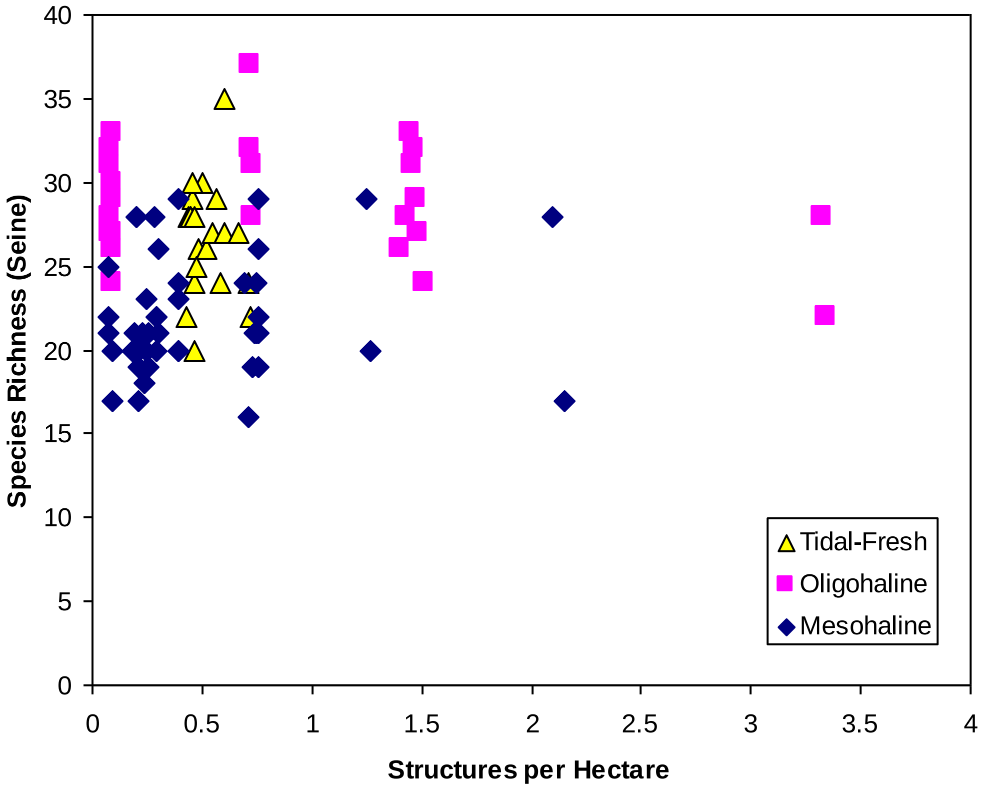

Figure 3-2. Number of finfish species collected by seining in tidal-fresh, oligohaline, and mesohaline subestuaries versus intensity of watershed development (C/ha = structures per hectare). Points were omitted if seine effort <15 hauls.

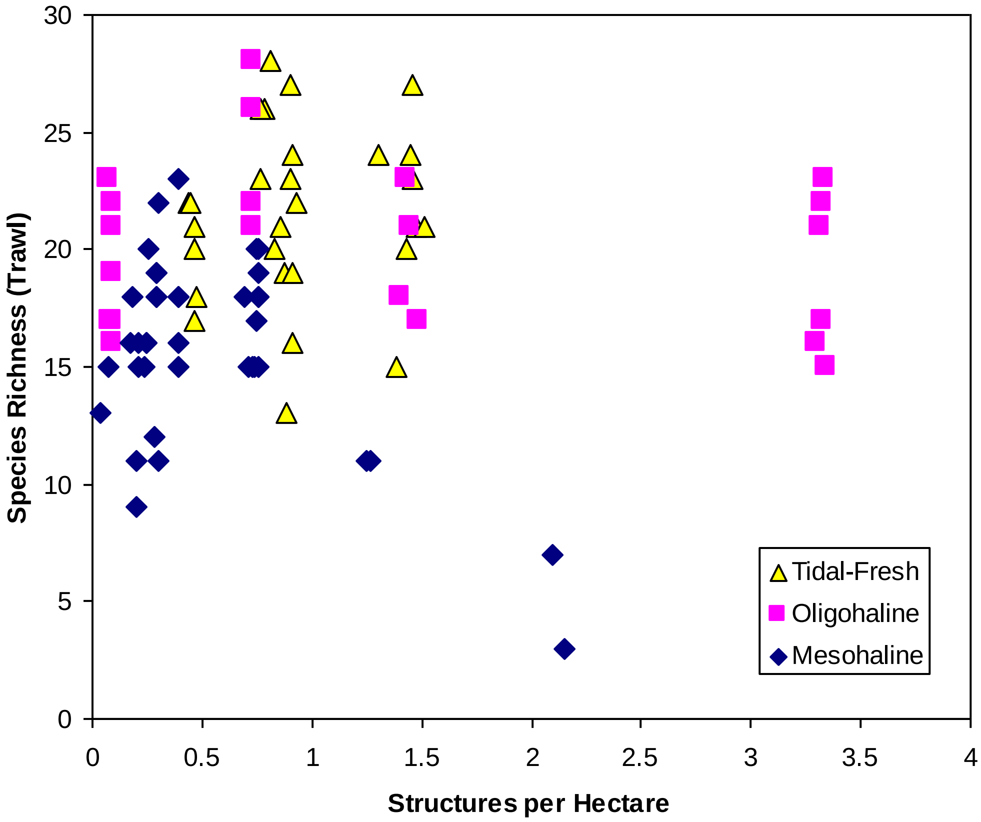

Figure 3-3. Number of finfish species collected by 4.9m trawl in tidal-fresh or oligohaline subestuaries versus intensity of development (C/ha = structures per hectare). Points were omitted if trawl effort < 15 hauls.

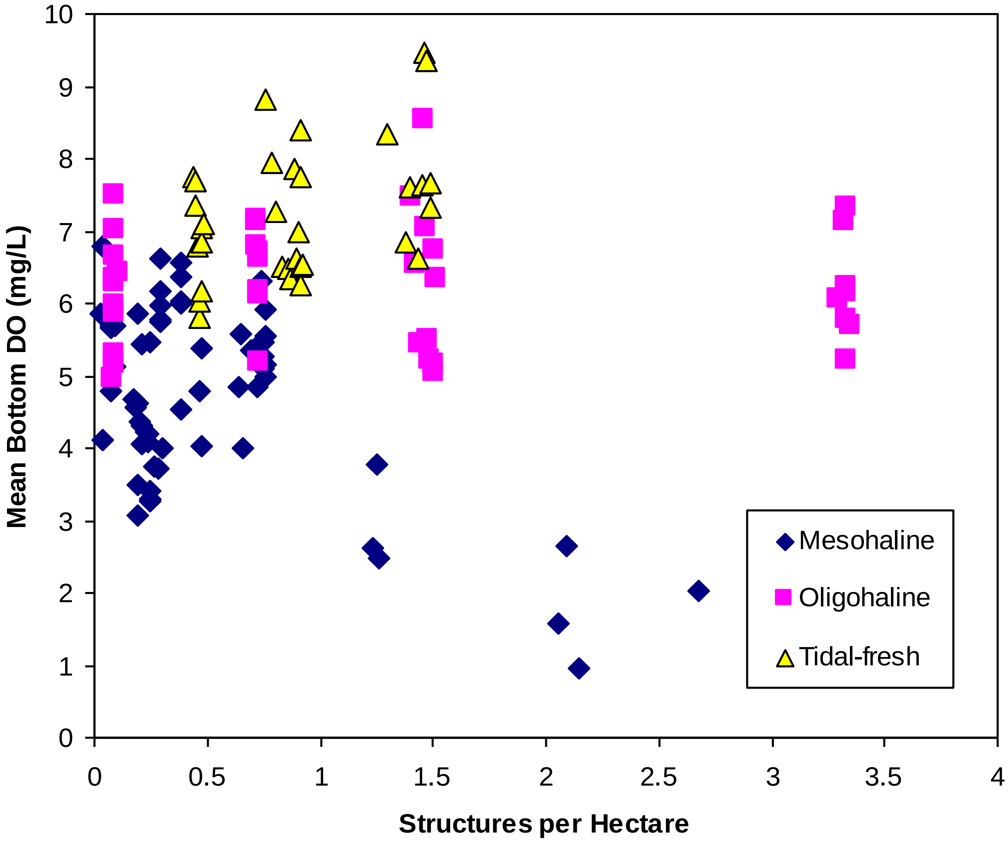

Figure 3-4. Mean subestuary bottom DO during summer sampling, 2003-2016, plotted against C/ha (structures per hectare).

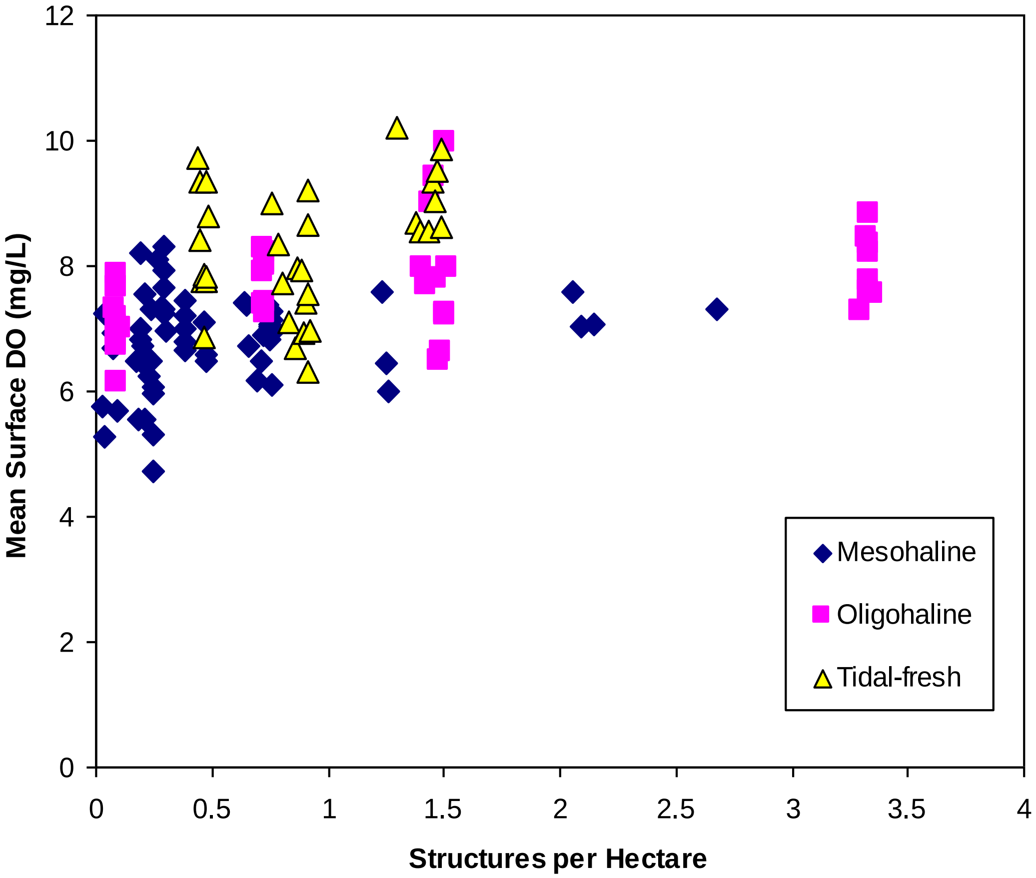

Figure 3-5. Mean subestuary surface DO during summer sampling, 2003-2016, plotted against C/ha (structures per hectare).

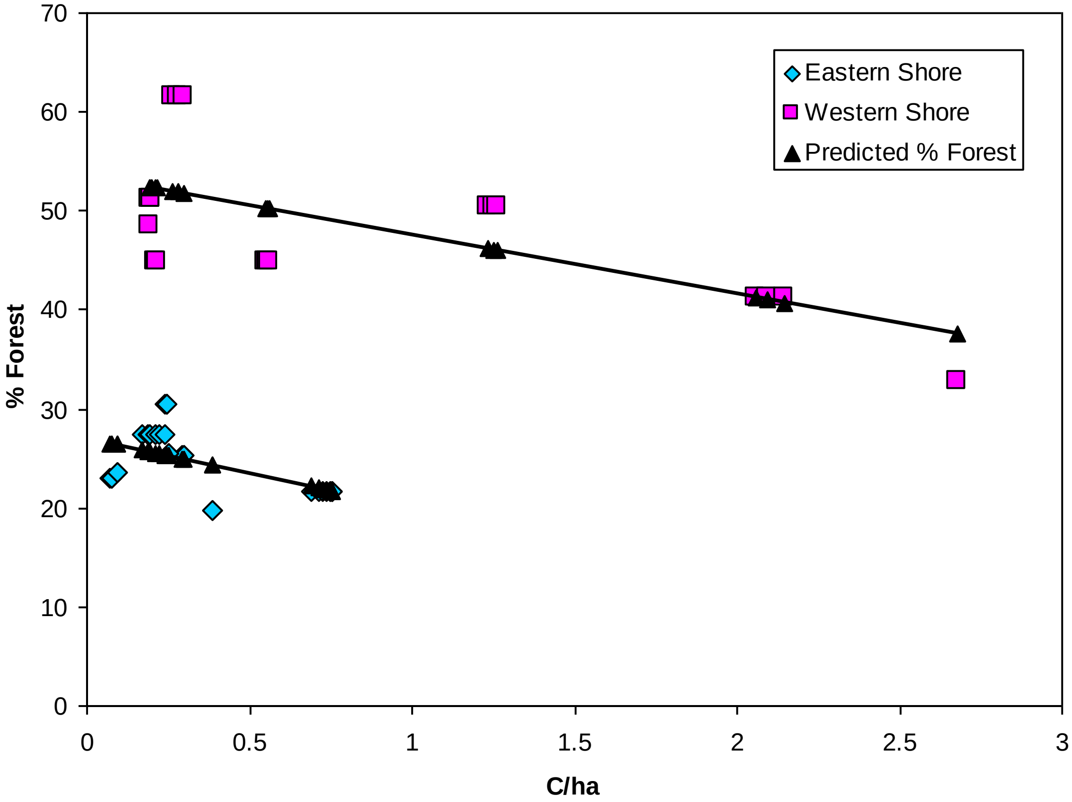

Figure 3-6. Trend in forest versus C/ha after accounting for regional differences for fourteen subestuaries sampled during 2003-2016.

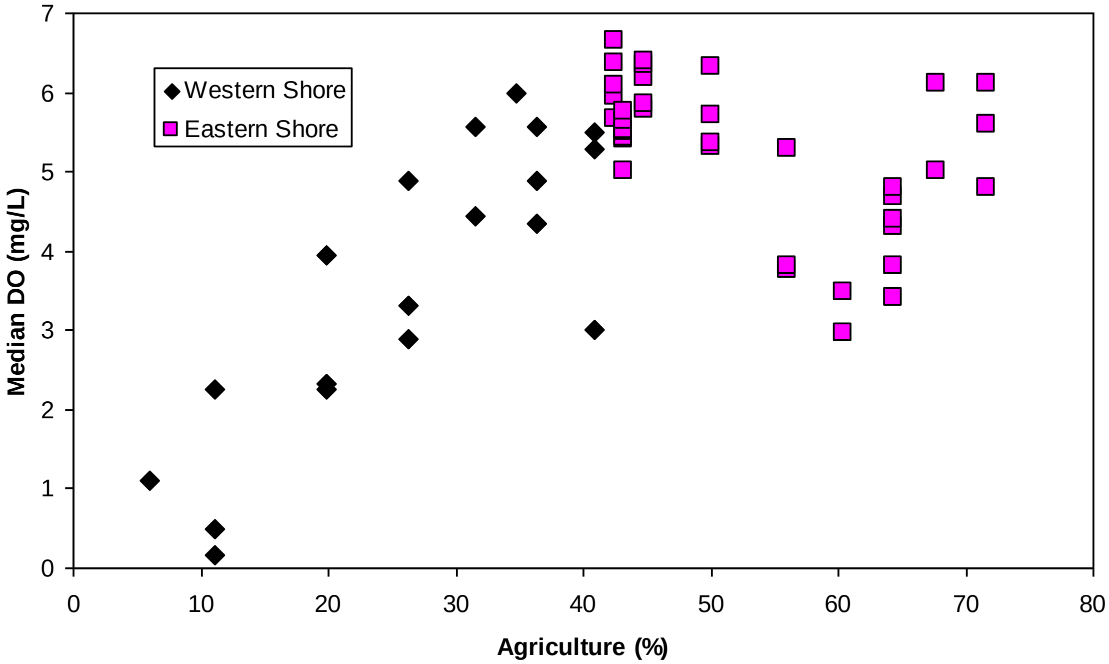

Figure 3-7. The percentage of agriculture land used by region (i.e., Western shore and Eastern shore) versus median dissolved oxygen in mesohaline subestuaries of the Chesapeake Bay.

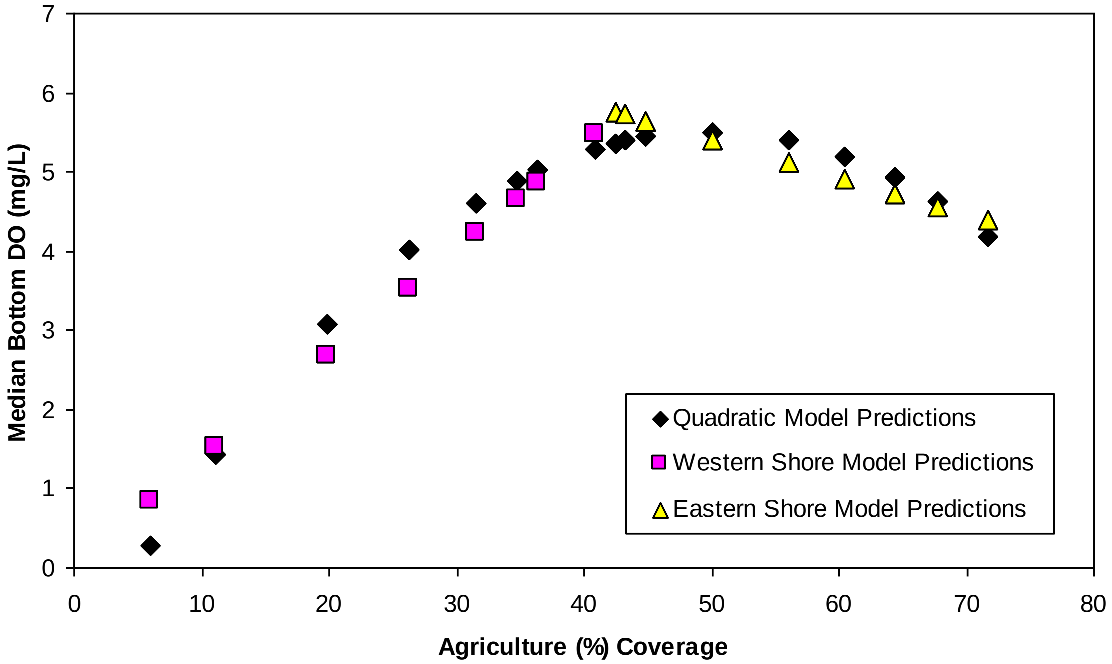

Figure 3-8. Predictions of median bottom DO and agricultural coverage (%) for the all data quadratic model, Western Shore linear, and Eastern Shore linear models in mesohaline subestuaries of the Chesapeake Bay.

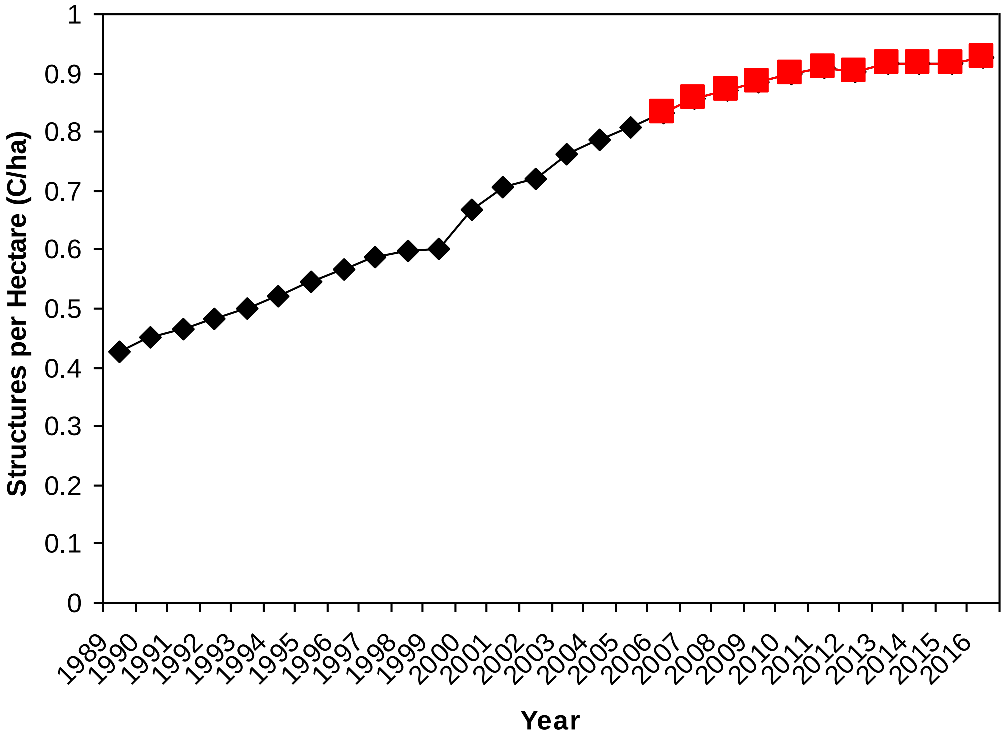

Figure 3-9. Trend in development (C/ha = structures per hectare) of Mattawoman Creek's watershed during 1989-2016. Red squares indicate C/ha values that are at or beyond the threshold for a suburban watershed.

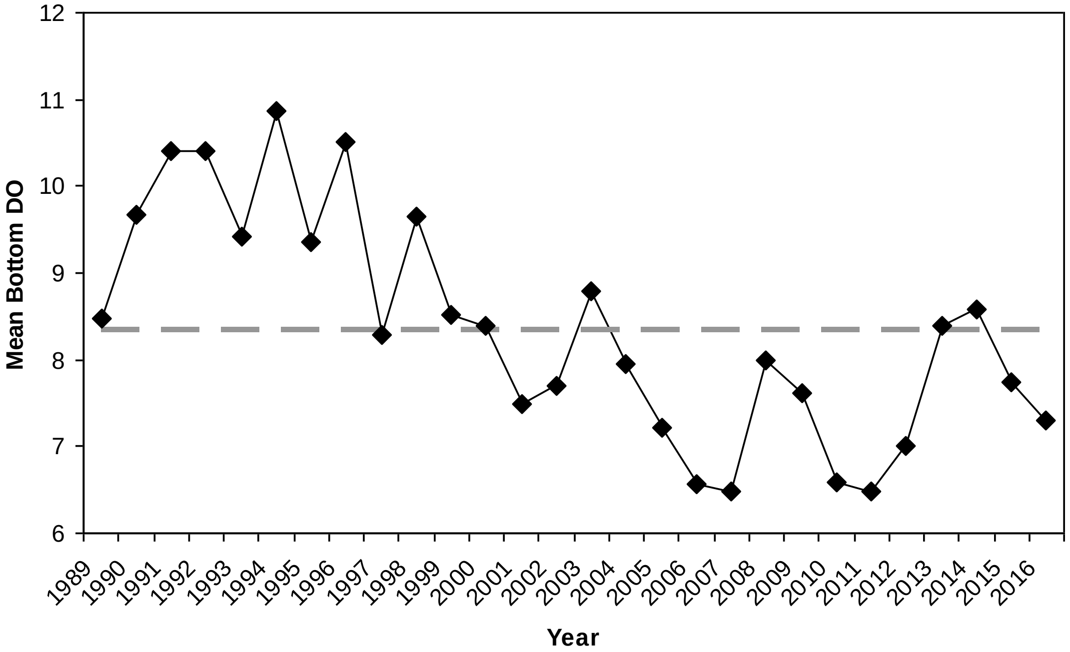

Figure 3-10. Mean bottom DO during July – September in Mattawoman Creek's subestuary, 1989-2016. Dashed line indicates median for the time-series of annual means.

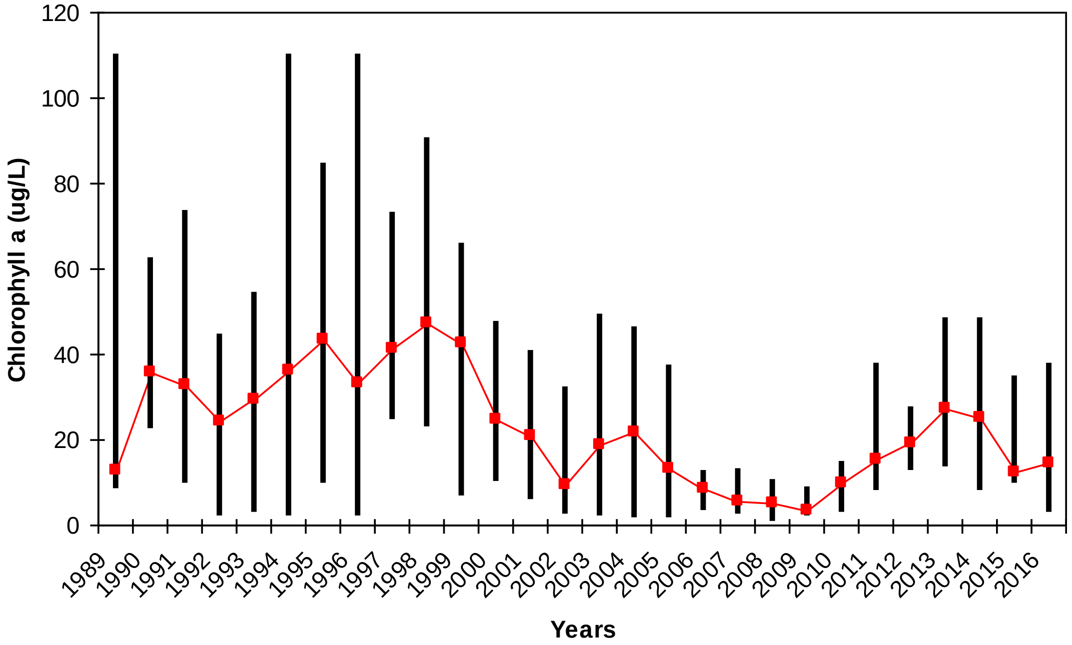

Figure 3-11. Range (solid black bars) of chlorophyll a (ug/L) and the median chlorophyll a (ug/L; red line with squares) at a Chesapeake Bay Program monitoring station in Mattawoman Creek (MAT0016) during SAV growing season (April – October).

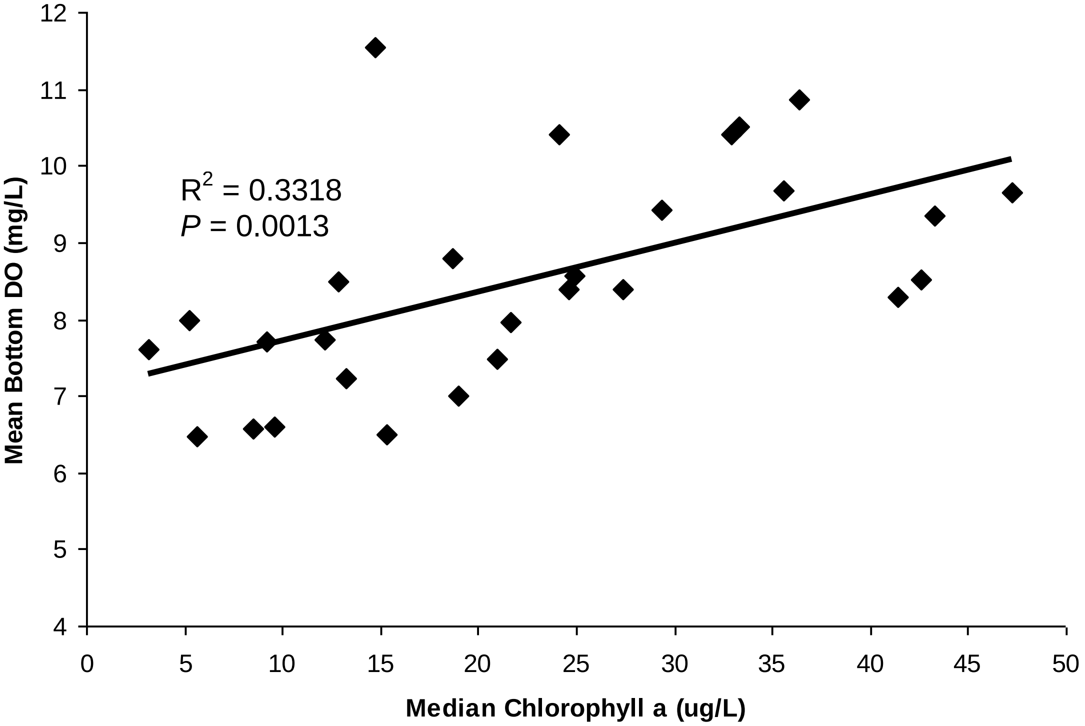

Figure 3-12. Mean bottom DO (mg/L) versus the median chlorophyll a (ug/L) at the Chesapeake Bay program monitoring station in Mattawoman Creek (MAT0016).

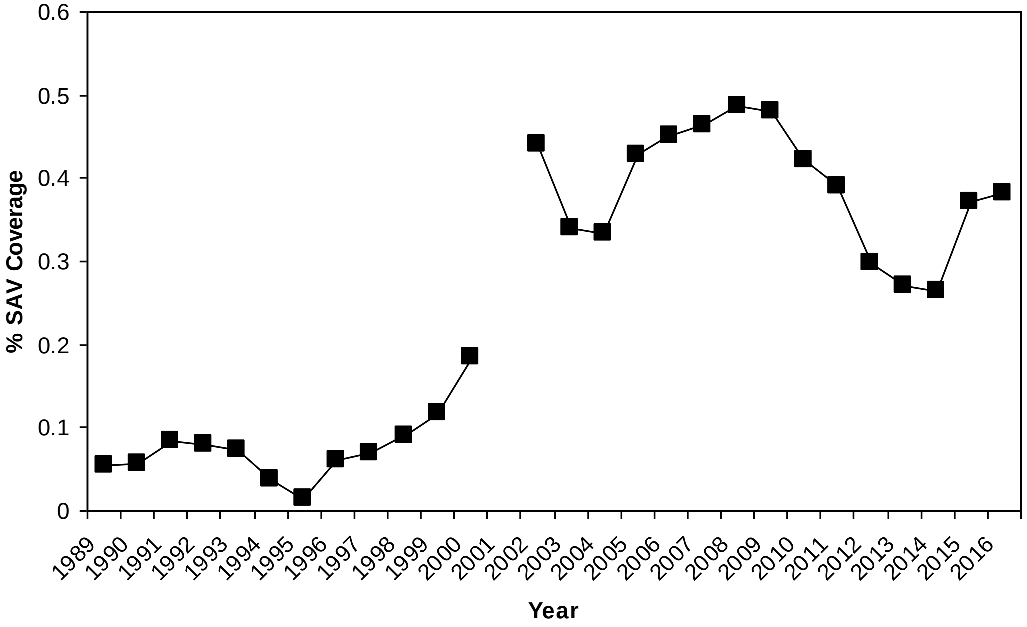

Figure 3-13. Percent of Mattawoman Creek's subestuary covered by SAV during 1989-2016 (2001 was only partially mapped). Estimates are from Virginia Institute of Marine Science (2017).

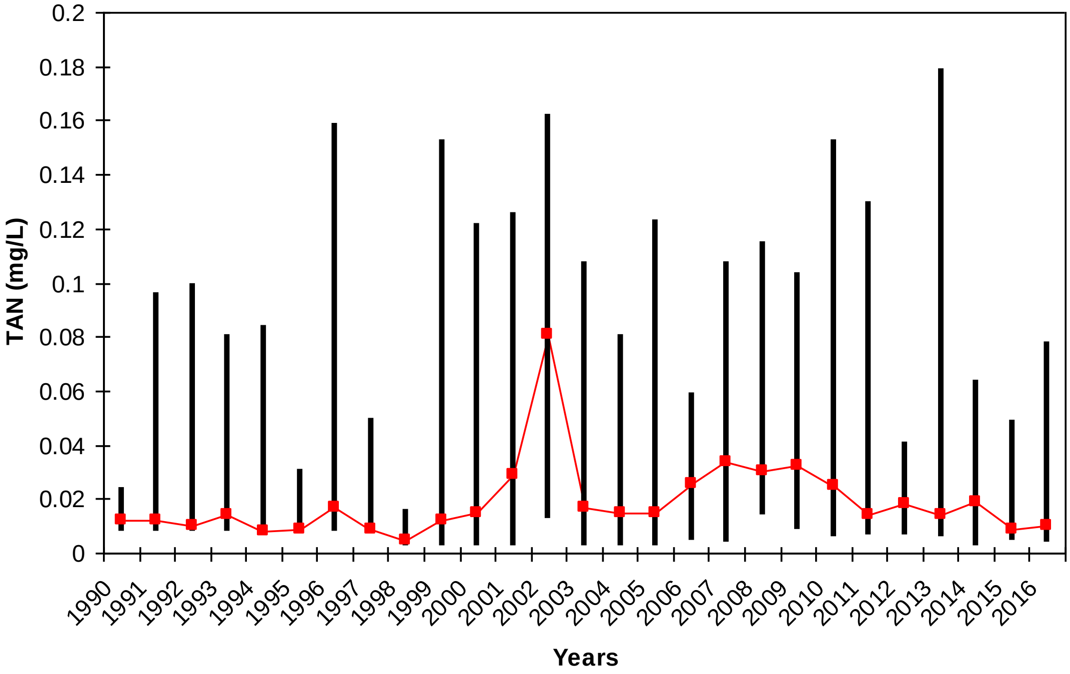

Figure 3-14.Annual range (solid black bars) of total ammonia nitrogen (TAN; mg/L) and median total ammonia nitrogen (TAN; mg/L; solid red line with squares) at the Chesapeake Bay program monitoring station in Mattawoman Creek (MAT0016) during SAV growing season (April – October).

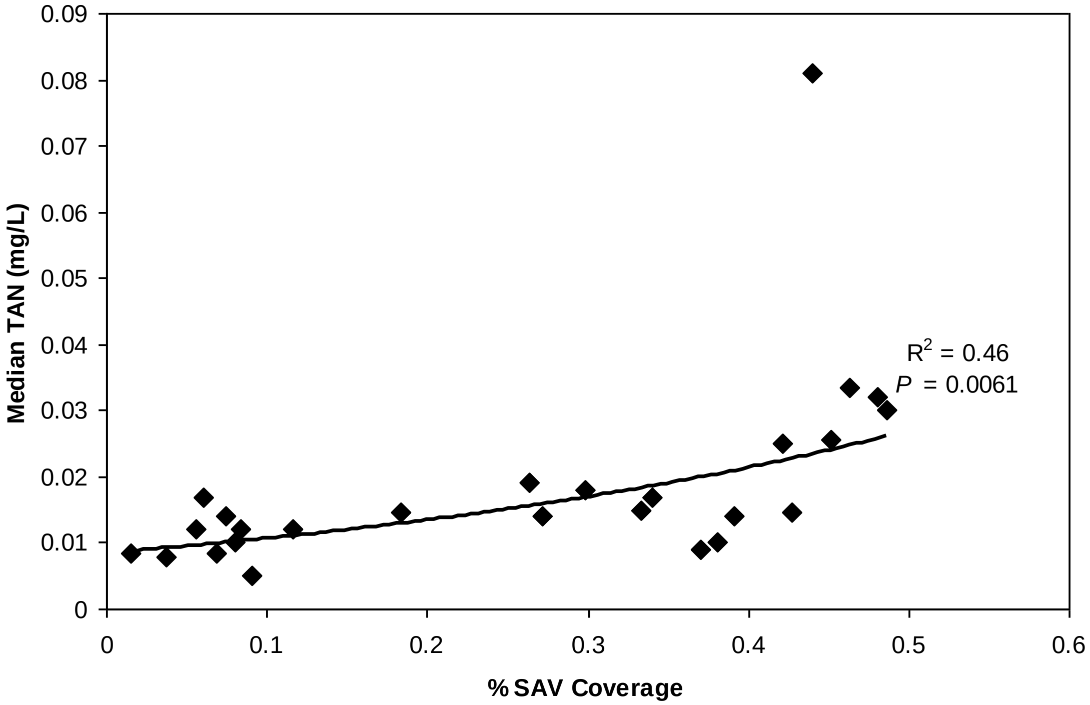

Figure 3-15. Relationship of median Total Ammonia Nitrogen (TAN, mg/L) versus the percentage of SAV coverage 1990-2016 at the Chesapeake Bay program monitoring station in Mattawoman Creek (MAT0016) during SAV growing season (April – October).

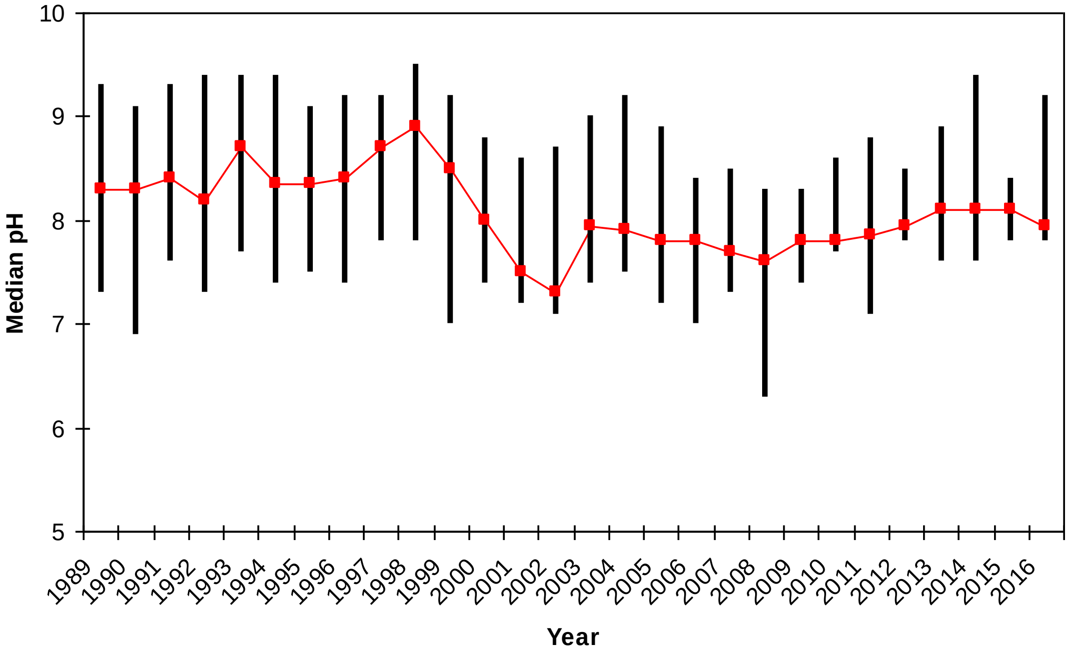

Figure 3-16. Median pH (red line with squares) and its range (vertical solid black bar) at a Chesapeake Bay program monitoring station in Mattawoman Creek (MAT0016) during SAV growing season (April – October), 1989-2016.

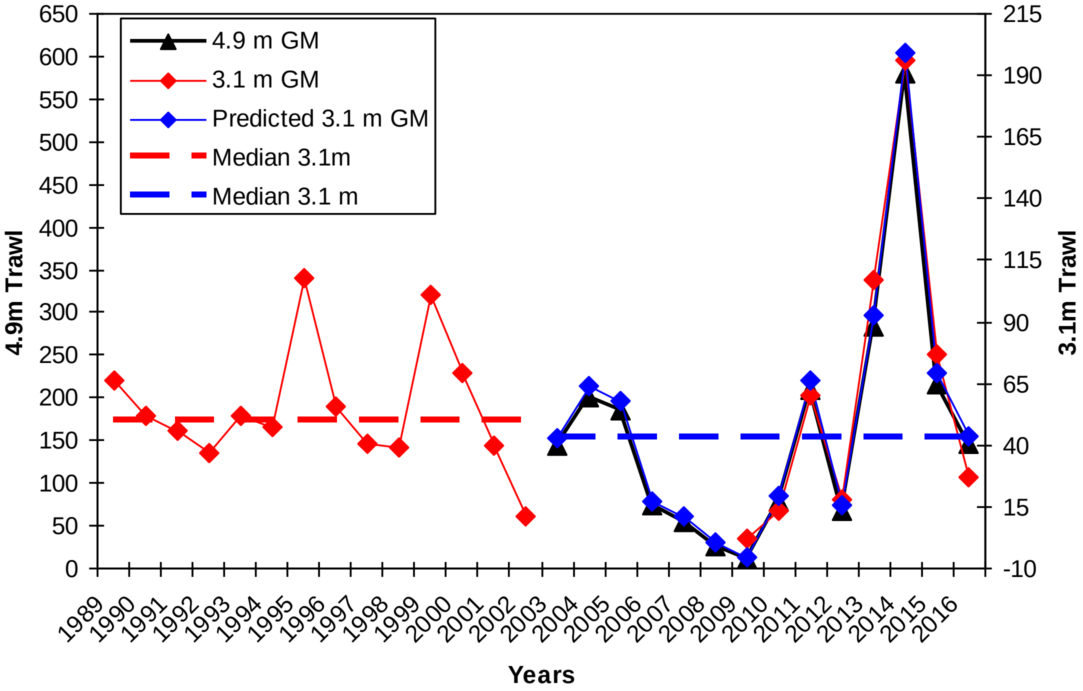

Figure 3-17. Geometric mean (GM) catches per trawl of all species of finfish in Mattawoman Creek during 1989-2016. Note dual axes for 3.1m and 4.9m trawls. Predicted 3.1m GM is based on a linear regression of 3.1m and 4.9m GMs during 2009-2016. Dotted horizontal lines indicate median GM of 3.1m trawl samples for 1989-2001 (red dotted line) and 2002-2016 (blue dotted line).

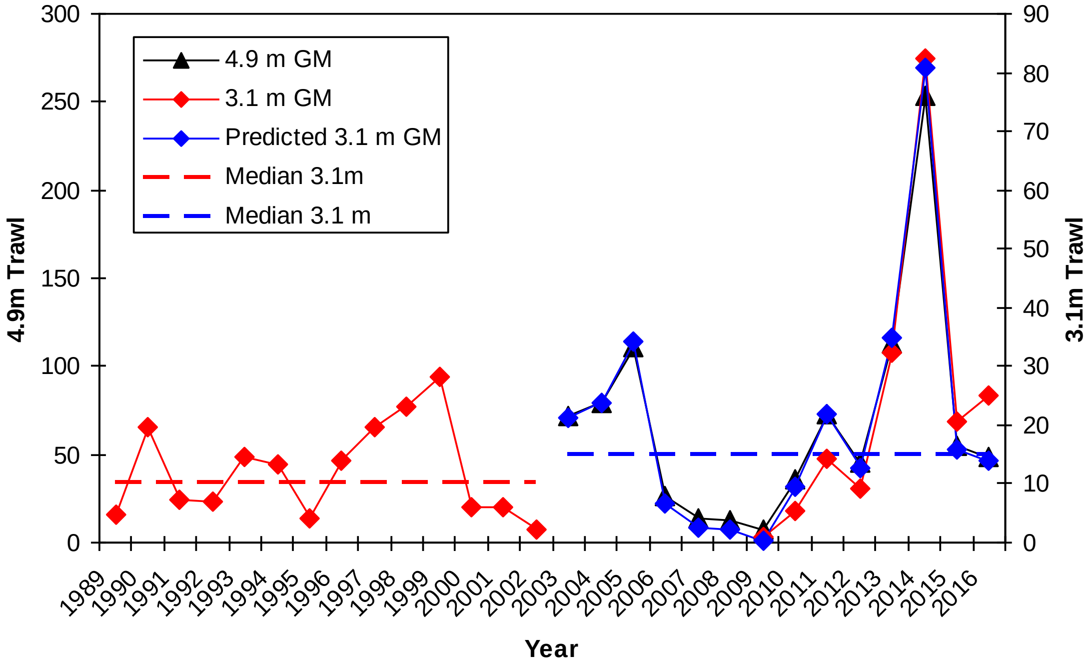

Figure 3-18. Geometric mean (GM) catches per trawl of YOY White Perch (juveniles) in Mattawoman Creek during 1989-2016. Note dual axes for 3.1m and 4.9m trawls. Predicted 3.1m GM is based on a linear regression of 3.1m and 4.9m GMs during 2009-2016. Dotted horizontal lines indicate median GM of 3.1m trawl samples for 1989-2001 (red dotted line) and 2002-2016 (blue dotted line).

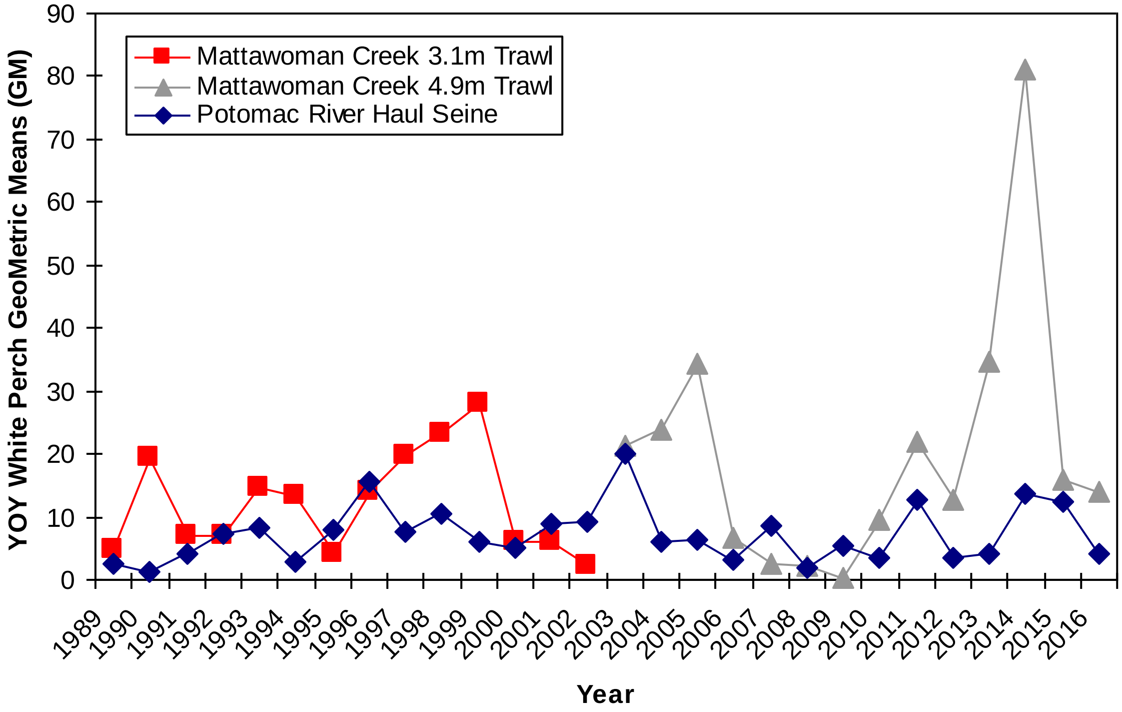

Figure 3-19. Geometric mean (GM) catches of YOY White Perch (juveniles) for 3.1 m trawl in Mattawoman Creek (1989-2002, red squares), 4.9 m trawl in Mattawoman Creek (2003-2016, grey triangles), and haul seine in Potomac River (1989-2016, blue diamonds).

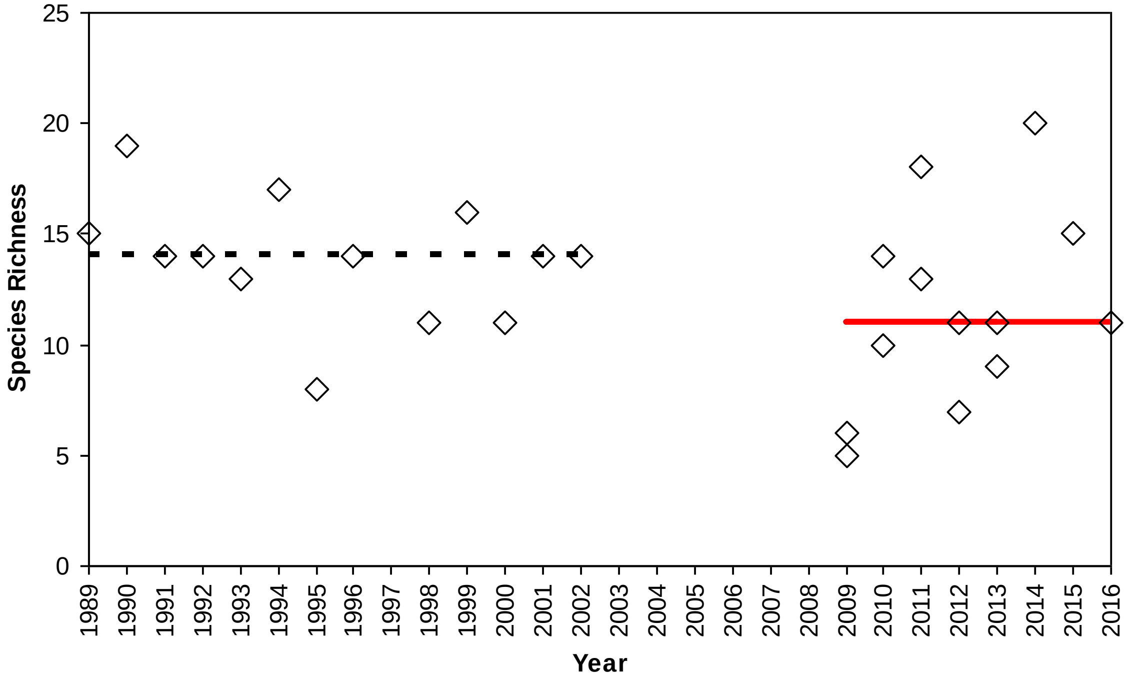

Figure 3-20. Mattawoman Creek species richness (number of species) in 3.1m trawl samples during summer sampling based on 12 samples each year. Bimonthly sampling during 2009-2016 allowed for two estimates of N = per year. Median number of species during 1989-2002 is indicated by the dashed black line; median number of species during 2009-2016 is indicated by the solid red line.

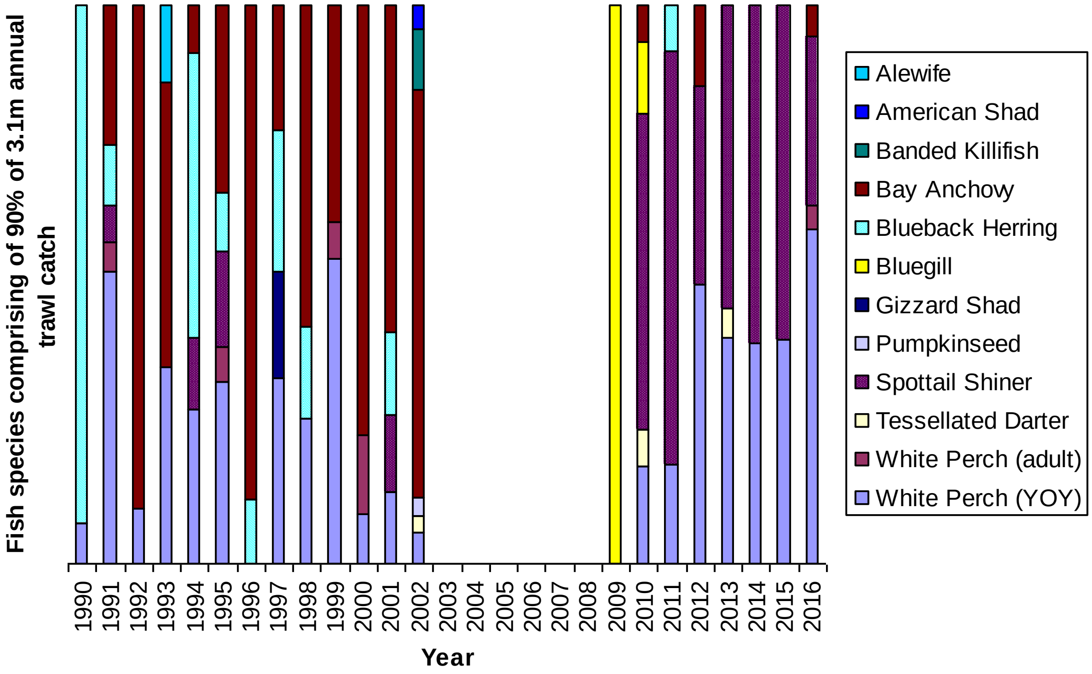

Figure 3-21a. Fish species comprising of 90% of 3.1m trawl catches by year in Mattawoman Creek.

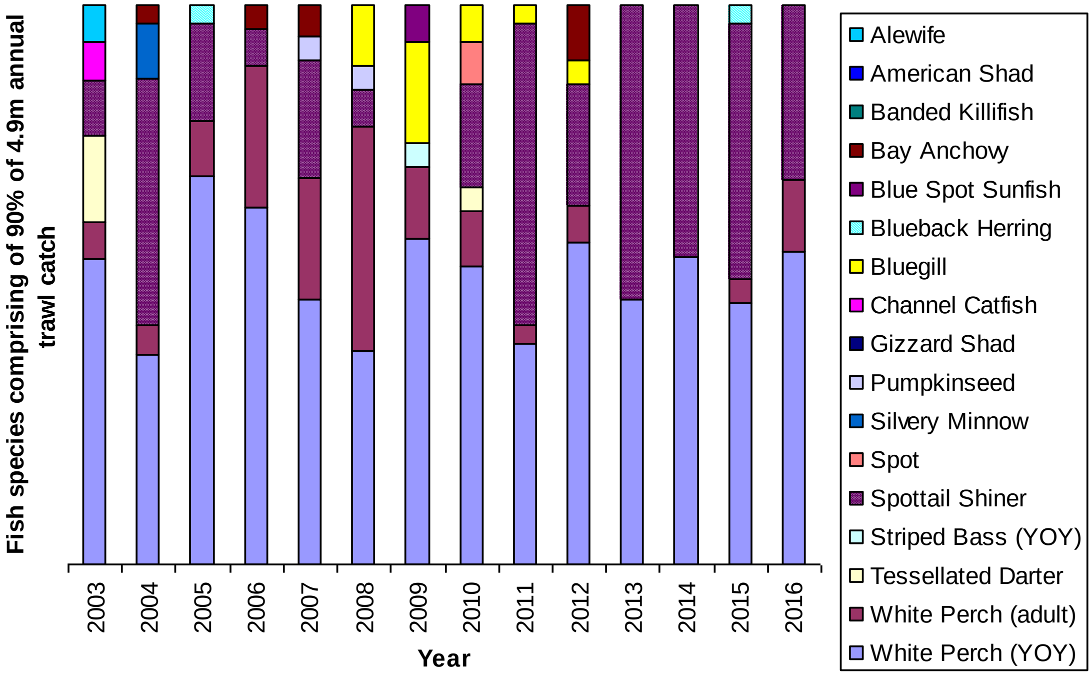

Figure 3-21b. Fish species comprising of 90% of 4.9m trawl catches by year in Mattawoman Creek.

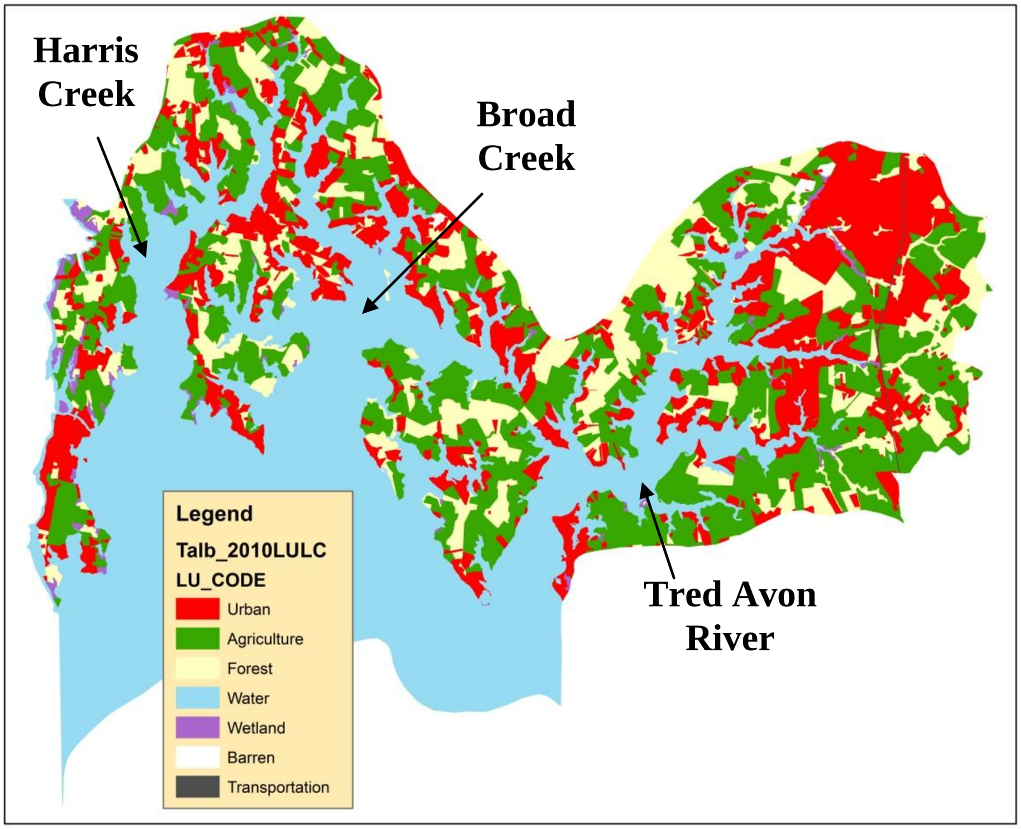

Figure 3-22. Map illustrating land use categories for the lower Choptank River subestuaries, Harris Creek, Broad Creek, and Tred Avon River.

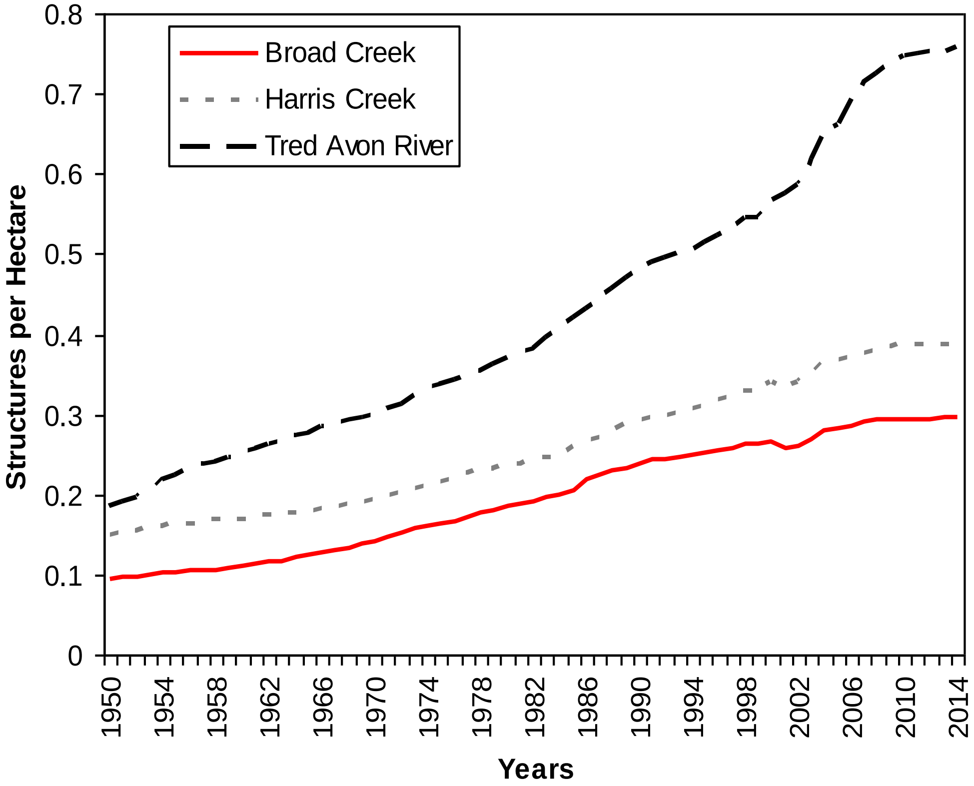

Figure 3-23. Trends in development (structures per hectare) in watersheds of three adjacent subestuaries in the Choptank River drainage, 1950-2014.

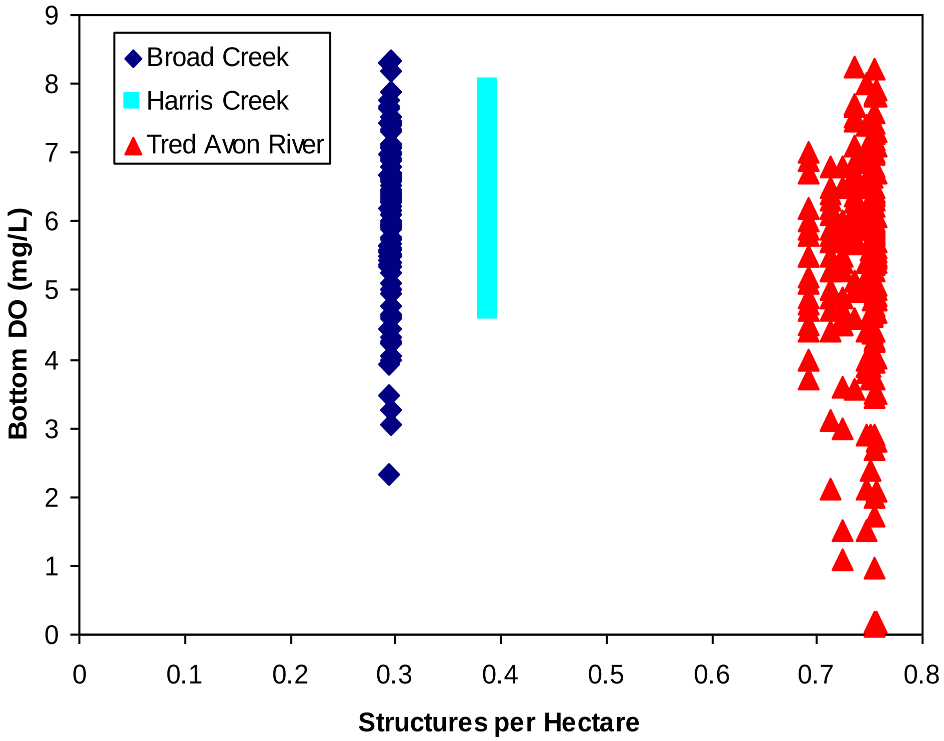

Figure 3-24. Bottom dissolved oxygen (mg/L) readings (2006-2016) versus intensity of development (C/ha = structures per hectare) in Harris Creek, Broad Creek, and Tred Avon River.

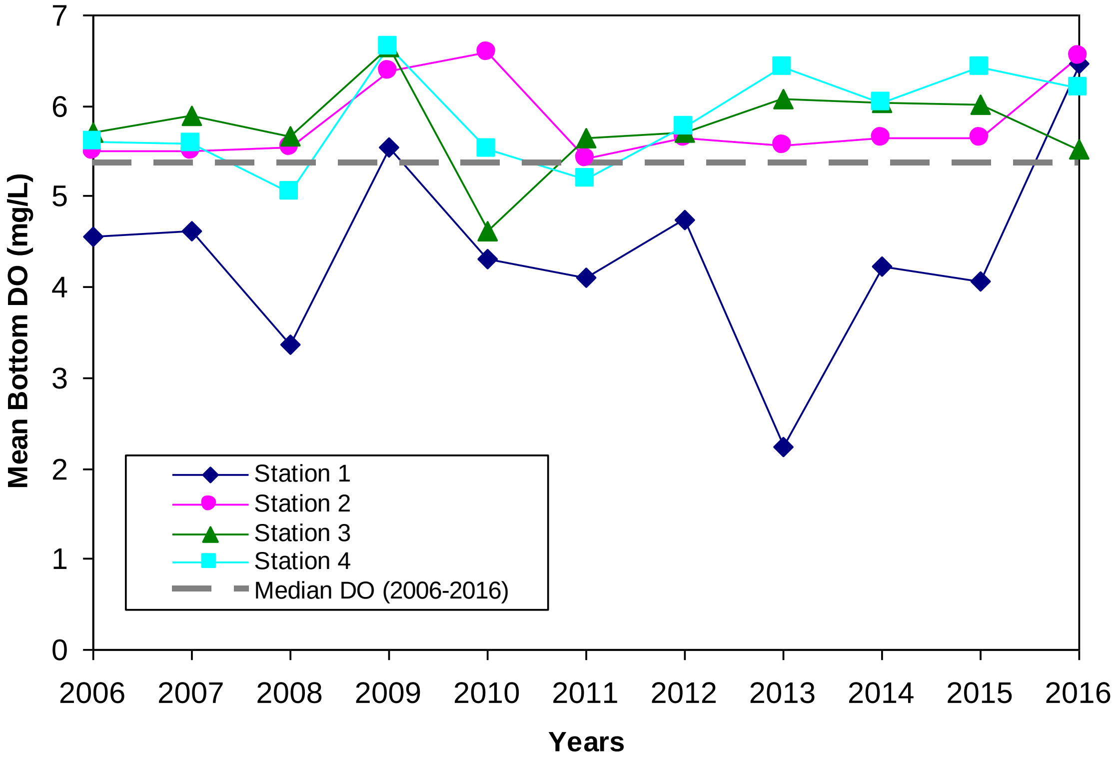

Figure 3-25. Mean bottom DO (mg/L) in Tred Avon River's subestuary by station, 2006-2016. Dotted line indicates the median for the time-series.

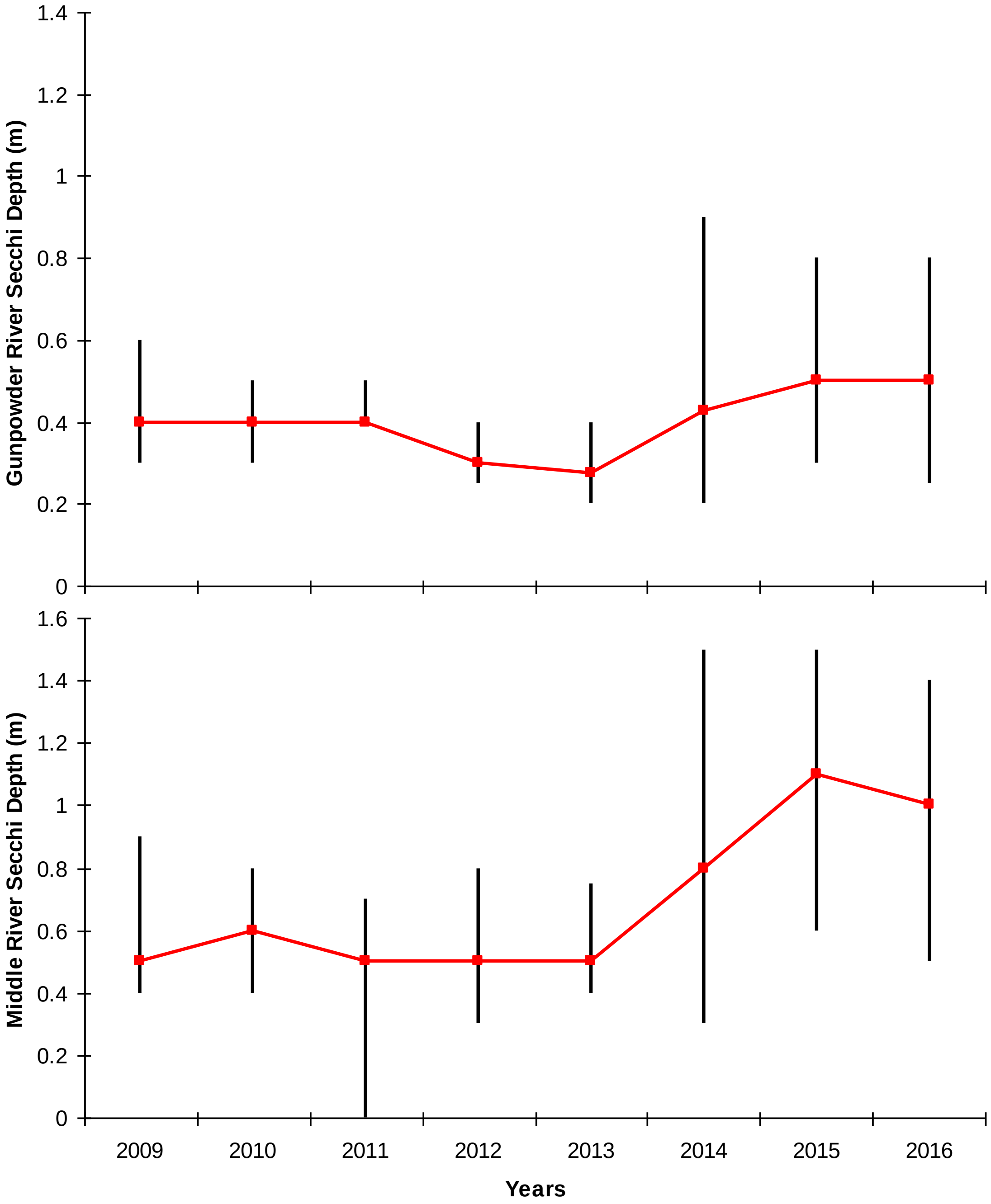

Figure 3-26. Median Secchi depth (m) by year for Gunpowder and Middle Rivers (2009-2016). Solid black bars indicate range of Secchi depth measurements.

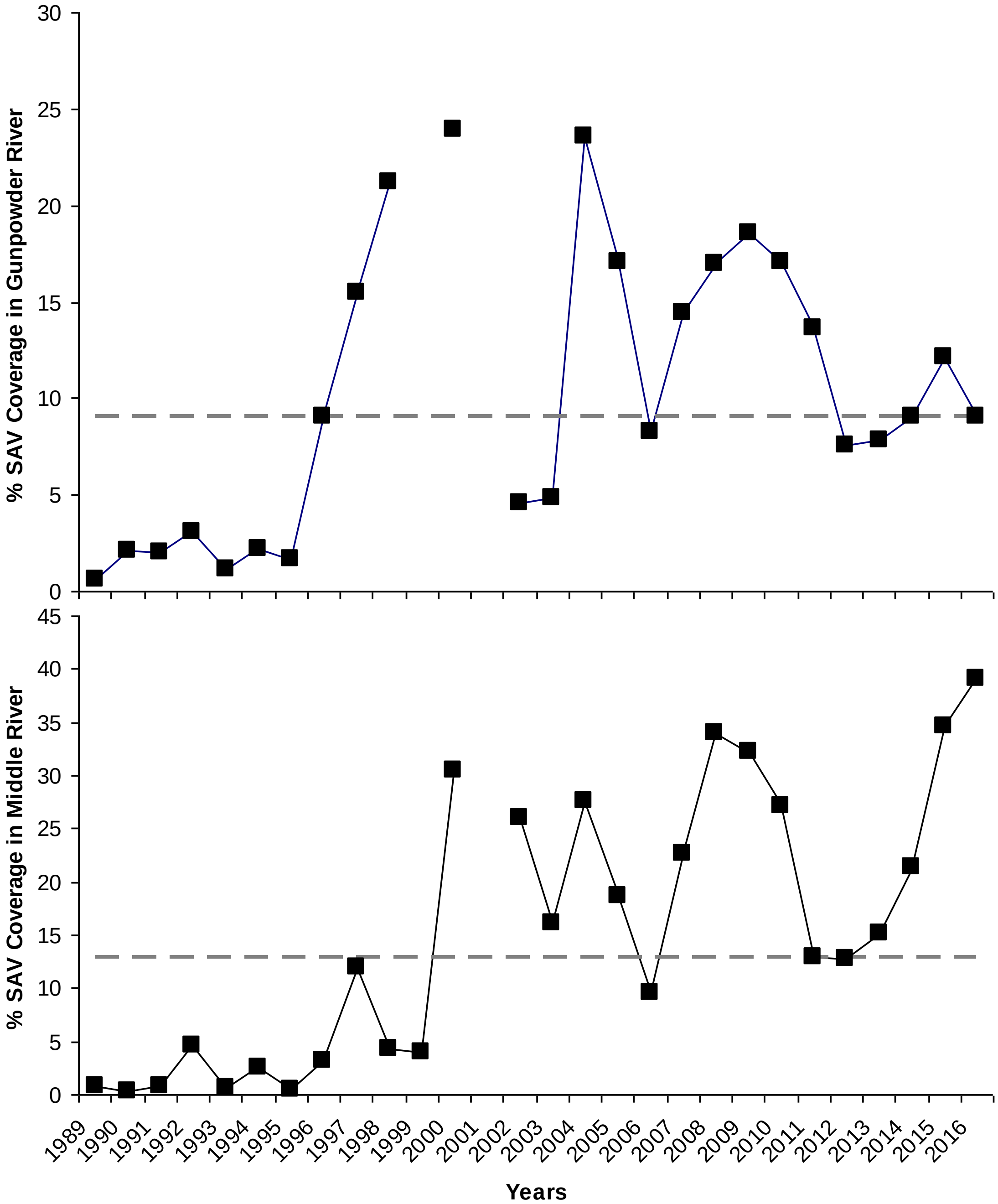

Figure 3-27. Percent of Gunpowder and Middle River's subestuary covered by SAV during 1989-2016 (excluded were 1999 Gunpowder River due to partial mapping; neither river was mapped in 2001). Median of time-series is indicated by the dashed line.

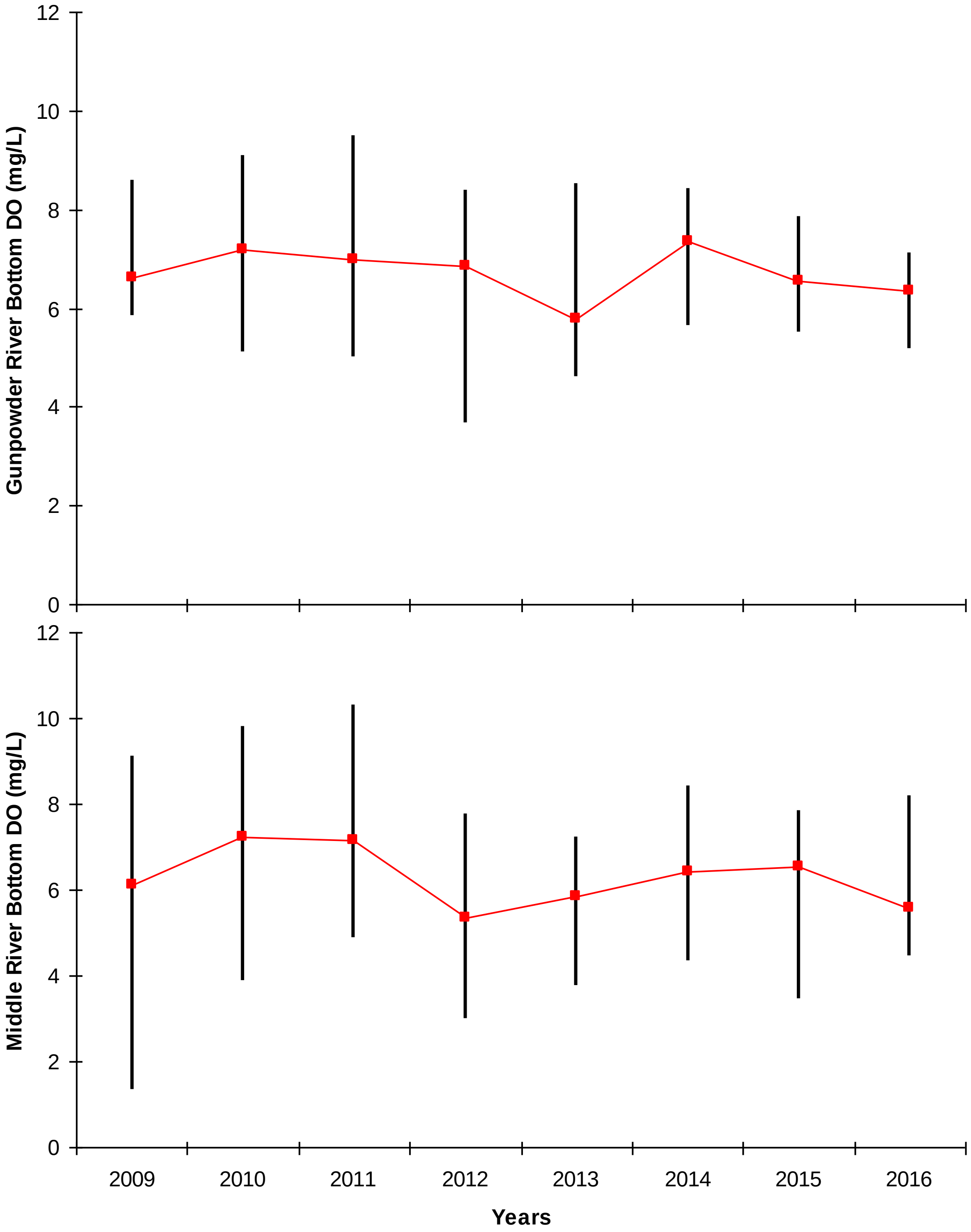

Figure 3-28. Median bottom DO (red squares and line; mg/L) by year for Gunpowder and Middle Rivers (2009-2016). Solid black bars indicate range of bottom DO measurements.

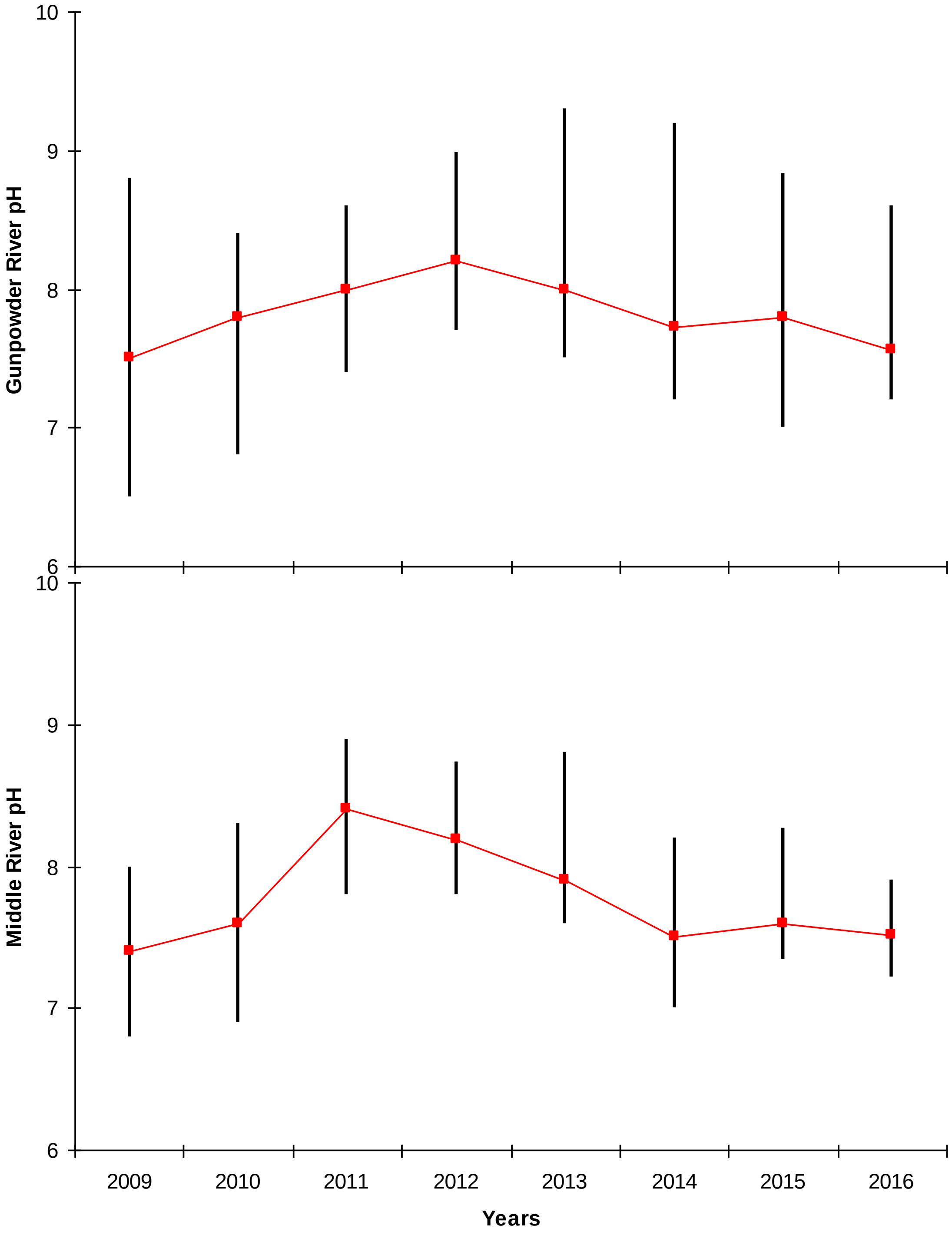

Figure 3-29. Gunpowder and Middle River median surface pH (red squares and line) during 2009-2016. Solid black bars indicate range of pH measurements.

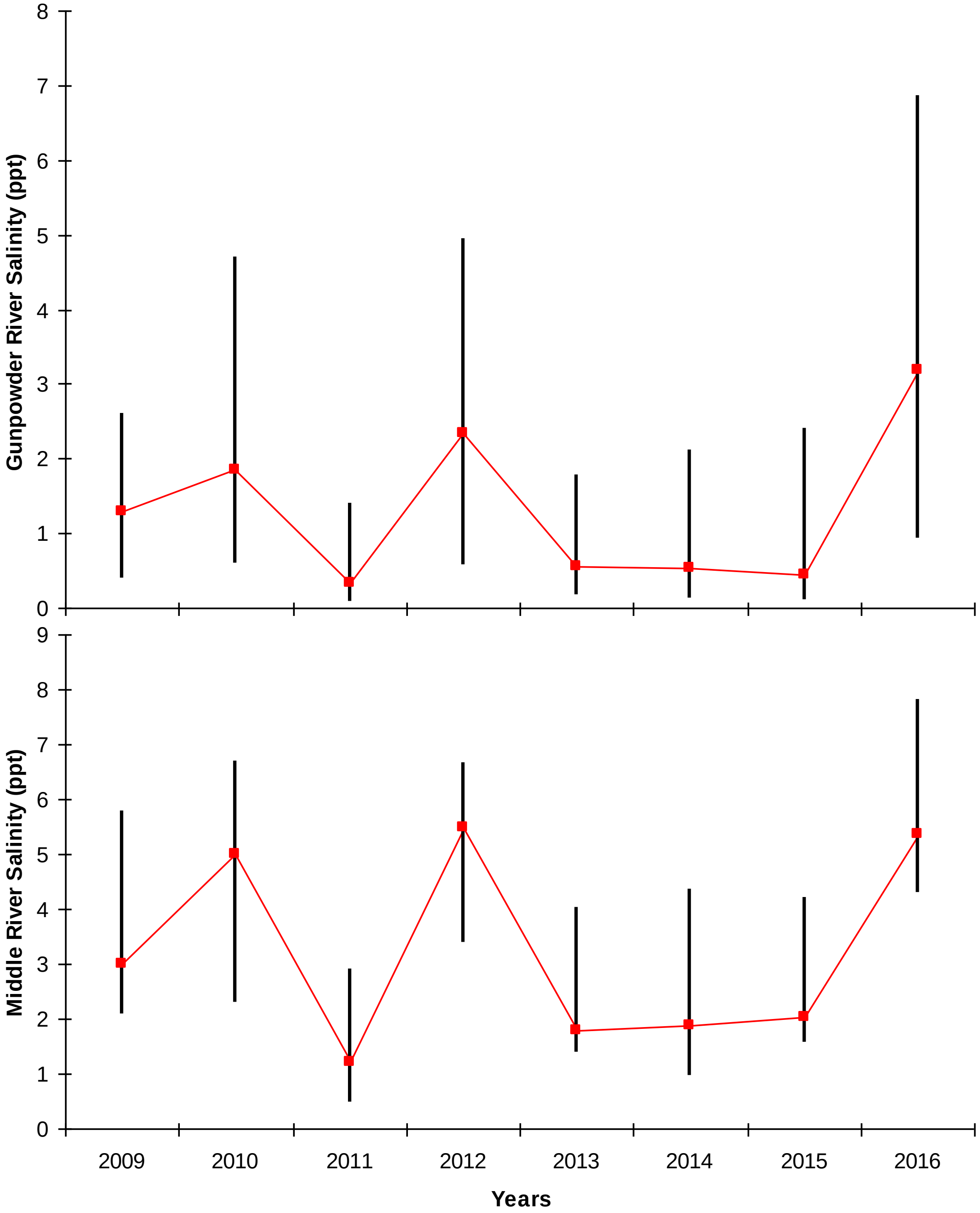

Figure 3-30. Median salinity (red squares and line; ppt = ‰) by year for Gunpowder and Middle Rivers (2009-2016). Solid black bars indicate range of salinity measurements.

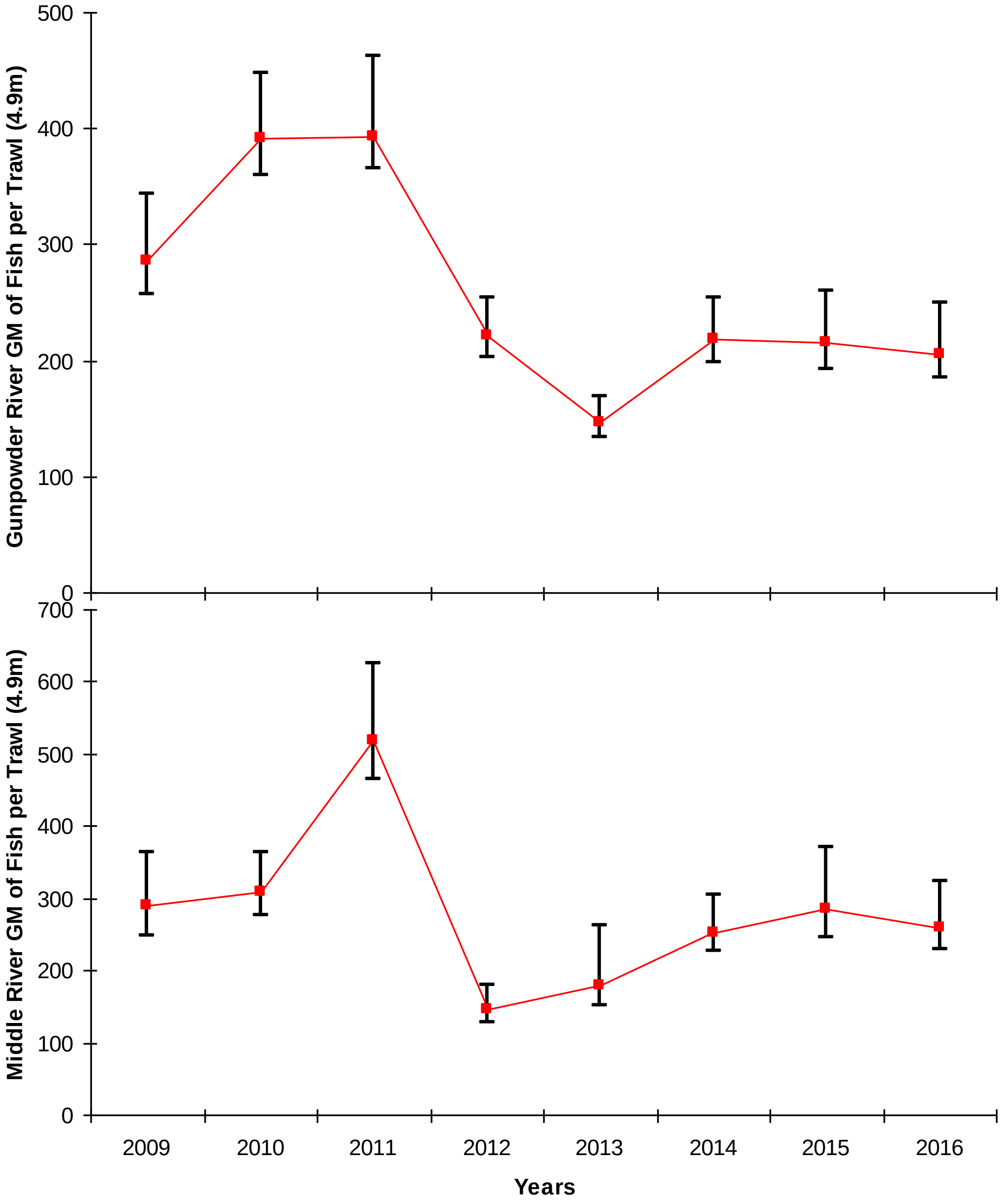

Figure 3-31. Annual geometric mean catches per 4.9m trawl of all species of finfish (GM; red squares and line) in Gunpowder and Middle Rivers during 2009-2016. Black bars indicate the 95% confidence intervals.

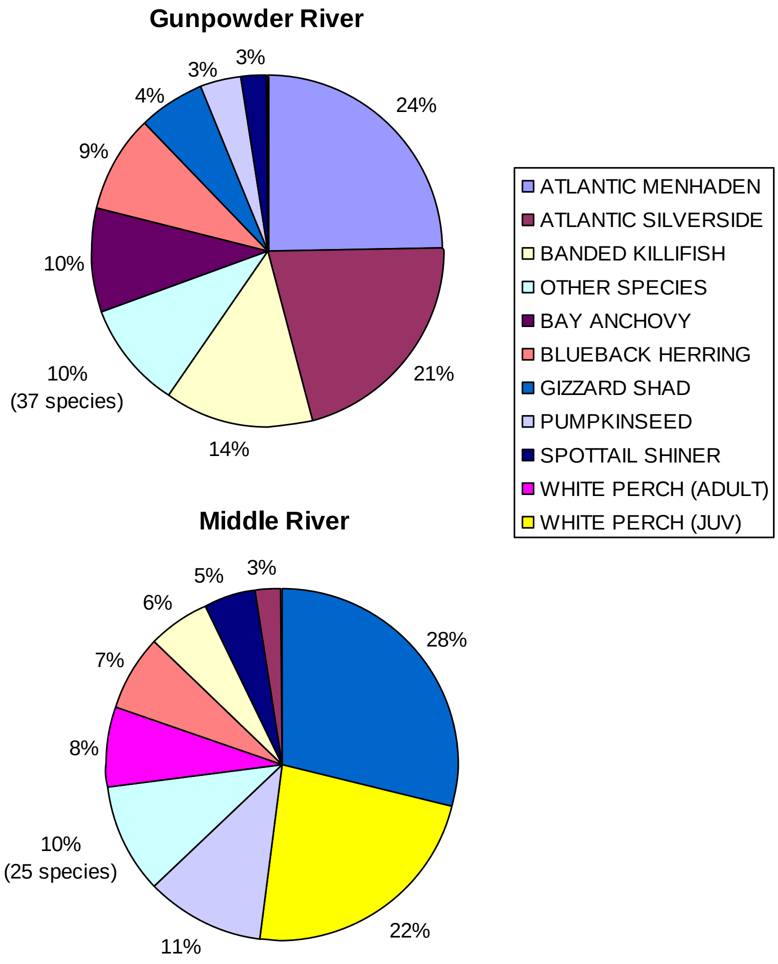

Figure 3-32. Species composition for seine catches of all species of finfish in Gunpowder and Middle Rivers during 2009-2016. Species that define the top 90% are identified and the remainder of species are grouped and labeled as “other species”.

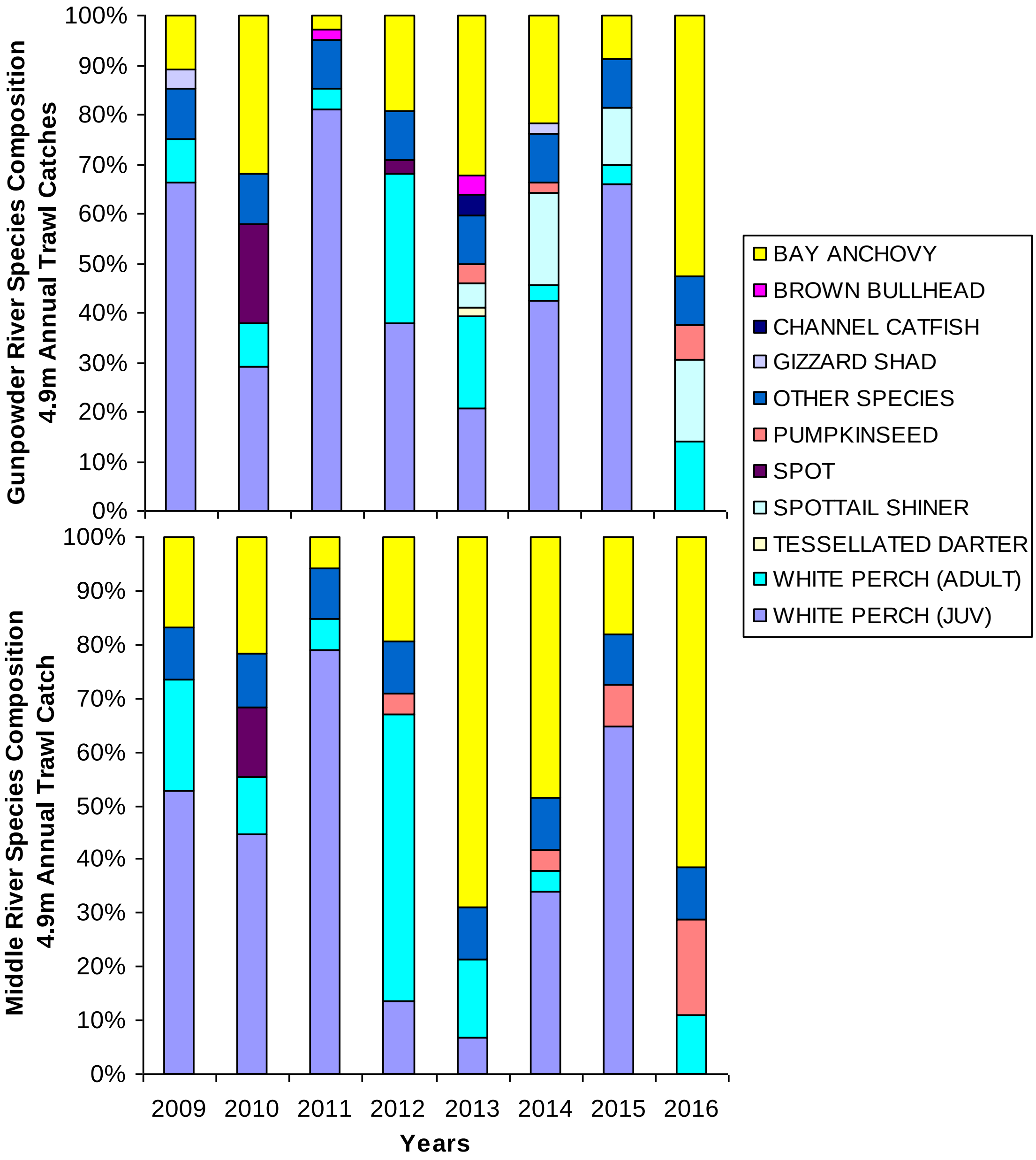

Figure 3-33. Finfish species composition, by year, for 4.9m trawl catches in Gunpowder and Middle Rivers for 2009-2016. Composition is defined as 100% of all species, the species that define the top 90% of the composition are identified and the remainder of species are grouped and labeled as “other species”.

JOB 2: Support multi-agency efforts to assess finfish habitat and ecosystem-based fisheries management.

Jim Uphoff, Margaret McGinty, Alexis Park, Carrie Hoover, Alexis Walls, Michael Macon, Patrick Donovan, and Charles Yetter

Introduction

The objective of Job 2 was to document participation of the Fisheries Habitat and Ecosystem Program (FHEP) in habitat, multispecies, and ecosystem-based management approaches important to recreationally important finfish in Maryland's Chesapeake Bay and Atlantic coast. Activities in this job used information generated by F-63 in communication and fisheries management or were consistent with the goals of F-63. Contributions to various research and management forums by Program staff through data collection and compilation, analysis, and expertise are vital if Maryland is to successfully develop an ecosystem approach to fisheries management.

Fisheries Habitat and Ecosystem Program Website - We continued to populate the website with new reports to keep it up to date with project developments. The web site was redesigned in April 2015 to help with navigation. Currently, we are working on compiling reports, maps, and presentations to add to the FHEP website.

Publications -

Kornis, M. S., and coauthors. 2017. Linking the abundance of estuarine fish and crustaceans in nearshore waters to shoreline hardening and land cover. Estuaries and Coasts 40:1464-1486.

Environmental Review Unit Bibliography Database - We maintain an Environmental Review Unit database, adding additional literature when it becomes available.

Review of County Comprehensive Growth Plans - We reviewed and commented on three comprehensive growth plans (Kent County and New Market and Ocean City municipalities) , providing recommendations consistent with maintaining viable fish habitat. These efforts included an assessment of local fisheries resources that represent recreational opportunities and the importance to consider fish habitat protection in planning. We also met with Queen Anne’s County planning staff to highlight the importance of fishing in the county and offer assistance to incorporate fish habitat needs in future planning activities.

MD DNR Interagency Effort on Mattawoman Creek - We continued to assist with Charles County with analyses of impervious surface by zoning category. Ultimately, the cumulative work on Mattawoman Creek under F-63 helped lead to adoption of new zoning designed to conserve remaining fish habitat. The following is an abridged version of a press release by the County.

The Charles County Board of Commissioners voted to approve the Mattawoman Creek Watershed Conservation District (WCD) and Zoning Map Amendment (ZMA), with changes. The Board’s approval creates new development standards for watershed conservation zoning and rezones approximately 36,000 acres of land in the northwestern part of Charles County.

The purpose of the WCD is to implement zoning rules in line with the Charles County Comprehensive Plan. The WCD, as well as the Comprehensive Plan, supports the Commissioners' goals of addressing school overcrowding, enhancing transportation, promoting economic development, improving public safety, and preserving environmental resources throughout Charles County.

The approved WCD zoning:

- Limits impervious surfaces in the WCD to 8 percent (based on direct measurement from aerial photographs).
- Restricts development in new residential subdivisions to one housing unit per 20 acres of land.
- Includes an intra-family transfer provision permitting two additional housing units on residential lots larger than 10 acres.
- Allows areas previously zoned for commercial and business park use to maintain their zoning.
- Maintains WCD zoning for Bryans Road and the area outside of the Bryans Road Village Center.

Cooperative Research - M. McGinty, A. Park, C. Hoover, A. Walls, M. Macon, P. Donovan, and C. Yetter supported field sampling efforts of various state and federal projects including: the DNR’s Coastal Bays Program, Resident Fish Species, Fish Passage Program, the Alosid Project, Resource Assessment Services, Artificial Reefs Initiative, Hatcheries Division Program, Inland Fisheries Division, Striped Bass Program, APAIS, and the Fish Health Program.

J. Uphoff, A. Park, C. Hoover, and A. Walls collaborated with the Fish Health Program at the Oxford Lab assessing Striped bass stomach contents collected from the upper, middle, and lower Chesapeake Bay.

A. Park and C. Hoover assisted the Coastal Bays Program with the highly migratory species tagging and data collection at the Mid-Atlantic and the White Marlin Fishing Tournaments held in Ocean City, Maryland.

J. Uphoff, C. Hoover, and A. Park worked with NOAA, UMES staff, and a UMES graduate student on initiating RNA/DNA lab protocols and field sampling. C. Hoover completed the RNA/DNA lab work.

J. Uphoff and M. McGinty continued working with NOAA and TNC on linking Choptank River land use practices to fish habitat in order to set priorities for restoration activities. Choptank River was chosen by NOAA as a habitat focus area (HFA), one of five nationwide.

M. McGinty, A. Park, C. Hoover, and C. Yetter met with staff from MBSS and MDE to learn the best approach to collecting chemistry samples to identify the source of elevated conductivity in streams.

J. Uphoff supplied comments for the draft Yellow Perch Management Plan update on the impact of habitat degradation due to development, food web issues, and climate change.

Presentations and Outreach - J. Uphoff presented to the Magothy River Association on the status of Yellow perch.

M. McGinty and A. Park presented to the Magothy River Association volunteers a presentation detailing field and data collection methods for larval Yellow perch training at AACC. Volunteers from the Magothy River Assoc. will be sampling for Yellow perch larval by conducting larval tows from late-March to late-April. Volunteers will also be conducting Yellow perch egg strand counts in March.

J. Uphoff and M. McGinty presented *Managing Chesapeake Bay's Land Use, Fish Habit and Fisheries: Developing and Applying Impervious Surface Reference Points*; *Indicator-based Assessment of Forage Status and Well-being of Striped Bass in Upper Chesapeake Bay*.at the Northeast Fish and Wildlife Association Conference.
J. Uphoff, M. McGinty, A. Park, and C. Hoover presented a poster at the Northeast Fish and Wildlife Association Conference titled *The Mutual Benefits of Working with Citizen Scientist.*

M. McGinty presented *Evaluating Effects of Conductivity Increases on Herring Spawning Habitat* .at the MWMC Workshop titles Road Salt Usage and Environmental Impacts.

A. Park presented on 2015 Bush River summer sampling results and illustrated what summer juvenile fish sampling involved for the Anita C. Leight Estuary Center staff and volunteers. The Bush River is one of FHEP's sampling areas and has been sampled since 2006 by staff and volunteers. The volunteer group samples the Bush River and provides data to FHEP staff.

J. Uphoff presented on development and fish habitat in the Tred Avon River to the Tred Avon Yacht Club. The presentation featured information on deterioration of dissolved oxygen at a station closest to Easton. Local government engineers were contacted beforehand and the Mid-Shore Riverkeeper afterwards.

M. McGinty attended a meeting with Anne Arundel County Staff and community representatives to discuss expanding the Habitat Protected Areas to nontidal water. Staff is working on providing maps as a first step to evaluating resources in the county.

M. McGinty worked with Harford County and Inland staff to target streams in Deer Creek for restoration. This was prompted by comments we made to the County's Comprehensive Growth Plan. The County is submitting a proposal to do restoration work within the Deer Creek watershed and is looking to DNR Fisheries for guidance in identifying the best candidates for restoration, and support in conducting the restoration.

J. Uphoff reviewed a MD Dept of Planning annual summary of Patuxent River oysters and fish in relation to water quality. Generally, links with water quality were not suggested by the data. However, something interesting popped out suggesting urbanization may be impacting herring there.

J. Uphoff reviewed a Choptank River digital ecological atlas for NOAA.

J. Uphoff, A. Park, C. Hoover, and A. Walls attended a presentation about Projecting and Protecting Quality of Life in the Baltimore Washington Region focusing on Smart Growth, biodiversity, climate change resiliency and ecosystem service valuation and their influence on quality of life in the BW region.

A. Park and C. Hoover attended a Chesapeake Bay River Herring Workshop at SERC to discuss past/future regional work and data findings.

J. Uphoff, A. Park, C. Hoover, and A. Walls attended the MWMC 22nd Annual Conference. J. Uphoff presented *Managing Chesapeake Bay's Land Use, Fish Habit and Fisheries: Developing and Applying Impervious Surface Reference Points* and co-presented *Has Striped Bass Movement Changed in the Potomac and Lower Bay?* at the MWMC 22nd Annual Conference.

J. Uphoff worked with T. Parham (Tidewater Ecosystem Assessment Division of MD DNR) on the presentation *Has Striped Bass Movement Changed in the Potomac And Lower Bay?* This presentation has proved to be of great interest and has introduced ecological considerations affecting fisheries management to general audiences. It has been presented at the DNR's Sportfish Advisory Commission, a CCA seminar on fisheries management, at Bass Pro Shops, and the Potomac River Fisheries Commission.

Program and Staff Development - A. Park attended the Bleiker Webinar titled *Getting Useful Input without Enflaming Anti-Government Attitudes*.

J. Uphoff, M. McGinty, A. Park, C. Hoover, and C. Yetter attended the MWMC Workshop titled Road Salt Usage and Environmental Impacts.

J. Uphoff attended a symposium sponsored by the Harry Hughes Center for Agro-Ecology on agriculture and water quality in the Choptank River.

A. Park and C. Hoover attended the fisheries index standardization class using R.

A. Park, C. Hoover, and A. Walls attended ArcGIS training at Towson University.

A. Walls attended the Freshwater Benthic Macroinvertebrate Identification Workshop to enhance taxonomic skills that can be used to identify the major taxonomic groups found in Maryland freshwater streams and rivers.

A. Park attended a MPEC workshop titled Dealing with Difficult People.

ASMFC - J. Uphoff attended meetings of the ASMFC's Biological and Ecological Reference Point Work Group to discuss ecological reference points for Atlantic menhaden. The ASMFC work group recognized that menhaden management requires additional precautions because of their importance as forage. A biomass dynamic model that includes Striped Bass and Spiny Dogfish predation developed by J. Uphoff is being considered for development of forage reference points. J. Uphoff wrote a working paper for the workgroup describing the multispecies model (menhaden, striped bass, and spiny dogfish) that was presented to the ASMFC subcommittee developing forage reference points for menhaden. Indicator approaches developed in Job 4 are of interest as well.

A. Park participated in ASMFC Shad and River Herring Technical Committee meeting to learn how states develop/renew/alter their Sustainable Fishery Management Plans (SFPs).

Chesapeake Bay Agreement - J. Uphoff developed indicators (Job 4) to address the forage fish outcomes of the new Bay Agreement, "By 2016, develop a strategy for assessing the forage fish base available as food for predatory species in the Chesapeake Bay."

M. McGinty outlined a strategy for Maryland's effort to support the Chesapeake Bay Agreement, Habitat Goal. This approach involves identifying key sentinel species that represent various habitats and ecoregions in the Bay, mapping them according to their natural limiting factors and applying know stressor information to develop spatial tools.

M. McGinty continues to serve as the Maryland lead on the team to support development of regional approaches to identify effective management strategies design to conserve and restore fish habitat. This requires monthly meetings and communications to develop work plans, review public comment, shape research proposals and track progress toward meeting the 2017 milestones outlined in the plan.

M. McGinty participated in several meetings to explore criteria to develop Healthy Watersheds designations in Maryland, to support the Healthy Watersheds Goal. The priority fish habitat map was referenced in the discussion to include fish habitat needs, but the watershed scale used in the approach was considered too large and intractable. Presently, Healthy Watersheds are designated at a small stream catchment scale. This is considered practical to assure protection through regulations. We address this issue in the Mapping section of this report, noting that stressors influence fish habitat at all scales, and if we want to protect large tracts of anadromous spawning habitat, we will have to conserve large tracts of rural lands to assure watersheds do not exceed the impervious target developed by our program.

M. McGinty reviewed and commented on a scope of work the CBP Fish Habitat Team developed for a contractor to gather information to develop a habitat criteria matrix.

M. McGinty met with consultants developing NOAA Environmental Sensitivity Inventory maps to review their work to date. Staff provided anadromous spawning habitat maps that delineate habitat based on prevalence of spawning use.

M. McGinty provided NOAA CBP staff with spawning habitat maps to support cross-GIT efforts to map habitats in the Bay.

M. McGinty reviewed and commented on a scope of work for a job to support the Fish Habitat Goal. This work will involve deriving criteria important for early life stages of key species, so we can map sensitive habitats in the Bay and its tributaries.

M. McGinty is serving on a steering committee to develop a workshop titled, “Factors Influencing Tidal and Non-Tidal Fish Habitat Function in the Chesapeake Bay Watershed: Application to Restoration and Management Decisions.

JOB 3: Developing Priority Fish Habitat Spatial Tools

Margaret McGinty, Alexis Park, Carrie Hoover, Jim Uphoff

Introduction

As the human population in the Chesapeake Bay watershed increases, we need to understand its effects on aquatic systems. Identifying and quantifying stressors associated with development that can be applied as stressor criteria in a visual spatial framework will promote more effective management of habitat and fisheries. In 2016, we developed maps of suitable habitat for juvenile target species and continued to monitor conductivity in spawning areas to determine if there was potential to develop conductivity criteria for anadromous spawning habitat mapping. We present results of this effort by topic in the following narrative.

Target Species Juvenile Distribution Maps

Introduction

Use of maps and mapping tools to guide decisions has become commonplace in resource management. Maps provide spatial depictions of distribution of species or habitats within a studied ecosystem and provide visual representations of how these distributions change over time in response to stressors. They are important and useful tools in conservation planning, because they delineate areas based on a chosen set of focal attributes that support conservation approaches (Bedward et al. 1992). Maryland Fishing and Boating Services has developed and distributed maps depicting species ranges, spawning habitat delineation, oyster grounds and fishing areas. These maps were created from historical data and updated with new information to fashion a modern view of conditions of mapped resources. They have been applied as a regulatory tool to designate fishing area closures and identify vulnerable habitats needing special protective consideration. More recently, maps were developed to communicate fisheries habitat condition and promote management approaches geared toward sustainable fisheries. Maps are useful tools and, when used properly, they can help managers focus action in areas where management goals are more likely to be met.

Our initial interest in developing maps was focused on updating historical anadromous fish spawning maps. Our work indicated that urbanized watersheds that had historically supported anadromous fish spawning habitat were had become much less productive (Uphoff et al. 2012, 2013, 2014) but were still being targeted to protect spawning habitat in permit reviews s. This served as an impetus to develop maps that more accurately reflected present conditions by accounting for stress from urbanization. The first several iterations of maps used historical salinity data along with historical stream spawning maps to categorize spawning habitats into preferred, acceptable, marginal and areas of no occurrence (Uphoff et al. 2014, 2015, 2016). We applied information about known stressors to score habitats in order to prioritize areas based on present condition. This prioritization approach allowed land managers to visualize areas in the state where conservation measures can be applied to sustain production of target species. In 2015 (Uphoff et al. 2016), we looked at other stressor information and provided a demonstration of how stressor information can be used to target areas for management action.

This year, our efforts have focused on developing juvenile habitat maps of target species. This report documents the work done to interpret these maps for the anadromous species (American Shad, Alewife, Blueback Herring and Striped Bass). We are in the process of reviewing literature and compiling information on habitat limits and stressors on the remaining target species. Due to time limitations, maps of these remaining species are included in this report, but documentation will be in the next report. Methodology is similar to that described for anadromous juvenile habitat.

Methods

We focused this report on mapping summer habitat occupation of anadromous target species juveniles in summer (Table 3-1). We used long-term (1959-2016) Striped Bass Juvenile Seine Survey data (Durell and Weedon 2017) to evaluate salinity preferences for each target species. Salinity influences distribution and abundance of fish (Hopkins and Cech, 2003; Cyrus and Blaber, 1992; Allen, 1982) and DO (Kemp et al. 2005; see Job 1, Section 3). The database contains catch and salinity records in tidal areas spanning head-of-tide regions to mouths of major Striped Bass spawning rivers and areas (Durell and Weedon 2017). We calculated presence of target species for each sampling event and developed frequency distributions of frequency of occurrence by salinity.

Although the data base contains records on all species collected, there were only a few species where juvenile (age 0) and older fish (ages 1+) were designated. The Striped Bass Juvenile Seine Survey recorded minimum and maximum total length of each species captured through 1990. Where juveniles and ages 1+ fish were not designated, we examined maximum length data to develop a range of maximum sizes and then compared the distribution of size to reported lengths found in the literature to determine if catches were predominately juvenile or ages 1+, or a mix of both. Salinity ranged from 0.0 to 20.0 parts per thousand (ppt) but data were skewed toward lower salinities (Uphoff et al. 2013).

To remain consistent with past mapping efforts (Uphoff et al. 2013; 2014), we rounded salinity to the nearest whole number and summed presence of each species and life stage by each whole salinity bin. We adjusted for unequal effort by dividing total presence by effort for each salinity concentration and then plotted frequency distributions for each species and life stage. We calculated proportion of samples with species (S*p*) present by life stage for each 1 ppt increment between 0 and 20 ppt. Because sampling effort varied with salinity, we divided this proportion by effort (n) to adjust for sampling effort (adjusted S*p*= S*p*/n*)*, summed the adjusted *Sp* estimates and recalculated the cumulative percentages by salinity from effort-adjusted estimates to classify habitat for each species.

We observed points where slopes changed by fitting lines to the frequency distributions. We considered these inflection points to represent cut offs and assigned habitat categories as follows: preferred habitat represents the salinities where the greatest percentage change of the cumulative distribution was observed (segment with the highest linear slope); acceptable, the second greatest; and marginal the smallest percentage change of the distribution (last segment up to 100%). No occurrence represents salinities where the species and/or life stage was not observed. We explored reported values in literature to assure these observations were consistent with previously reported values for each species.

We then mapped each species by category to provide a visualization of summer habitat potential. We developed maps of dissolved oxygen criteria and combined these with the summer habitat maps to evaluate the potential loss of habitat. The Chesapeake Bay Program has given considerable attention to effects of hypoxia on living resources (Batiuk et al. 2009; Uphoff et al. 2011). Jordan et al. (1992) published dissolved oxygen goals for various target species. Jordan et al. (1992) selected ten key finfish species along with several shellfish species that they considered representative of various Bay habitats and concluded if habitat needs were met for these species, habitat needs for all species would be satisfied. Of the ten focal finfish species Jordan et al. (1992) selected to develop oxygen criteria, our project focused on nine, including the anadromous American Shad, Alewife, Blueback Herring and Striped Bass; estuarine resident White Perch, Yellow Perch; and marine migrants, Atlantic Menhaden and Spot. We added three more species that reside predominately in tidal-fresh habitat and are important forage species: Gizzard Shad, Silvery Minnow and Spottial Shiner. Because Bay Anchovy is a keystone species in the Bay, providing valuable forage for gamefish (Job 4), we included maps of their distribution. Batuik et al. (2009) conducted a literature review to examine the impact of hypoxia on reproduction, growth and survival of target species. Concentrations of DO 5 mg/L or greater were considered desirable for many Chesapeake Bay living resources (Batiuk et al. 2009). Chesapeake Bay DO criteria for deep-water fish and shellfish call for maintaining a 30 day mean of 3 mg/L during June 1 – September 30 in bottom waters (Batiuk et al. 2009). Uphoff et al. (2011) used 5.0 mg/L as a target dissolved oxygen level and 3.0 mg/L as a limit to suitable finfish habitat. We applied these criteria to examine the potential impact of low oxygen on target species.

Water quality data used for mapping were obtained from the Chesapeake Bay Program. Maps were derived from averaged 1998-2003 bottom habitat parameters by season for each tidal monitoring station (Uphoff et al. 2014). These years were chosen because wet, dry and average rainfall years are represented in the data (USGS, 2017). Map layers for each season were developed by interpolating the averaged data from each station. Therefore, the maps we used were static representations of average conditions for tidal waters of the Bay. We acknowledge that conditions are not static but these maps do provide a general picture of key habitat areas for target species. They also allow us to estimate the percentage of area based on bottom habitat conditions that may be compromised by a particular stressor. The purpose of developing these maps was to assess present habitat quality for target species and to support development of management recommendations that promote sustainable fish habitat.

Results

American Shad (Alosa sapidissima):

American Shad was once the most valuable commercial species in Chesapeake Bay. Hildebrand and Schroeder (1927) reported they were the most valuable food fish with a value exceeding the "*four next most important species*" in the Chesapeake Bay. Maryland Department of Research and Education (1955) identified Shad as a cheap and abundant source of protein that had historically supported fisheries operated by George Washington on the Potomac. Declines in the fisheries were reported by Hildebrand and Schroeder (1927) and they reported that stocking efforts were not successful in reestablishing the Shad's former abundance. Historical reports (Hildebrand and

Schroeder 1927; MD Dept of Research and Education 1955) attribute the species' decline to harvest pressure, ruination of nursery areas, and pollution. More recently, Maryland Sea Grant (2011) identified migratory barriers, flow changes, water quality, physical habitat alteration, and land use as major factors influencing habitat quality for Alosine species (American Shad, Hickory Shad, Alewife and Blueback Herring). We focused on habitat losses to determine the potential impact of habitat change on juvenile fish. Klauda et al. (1991b) conducted an extensive literature review to identify potential habitat stressors contributing to population declines. Most of the studies reported stressors and losses related to spawning (impediments reducing available habitat) and survival of early life stages (reduced hatching rates from metal toxicity, malformed larvae in hypoxic waters and increased larval mortality in relation to elevated suspended solids). In relation to juvenile habitat, Klauda et al. (1991b), cited studies conducted in the Delaware River that implicated low oxygen as a factor impeding emigration of juveniles. In an effort to identify priority habitat and examine potential stressor impacts on juveniles American Shad habitat in the Bay, we mapped their preferred, acceptable, and marginal habitat, and then overlaid dissolved oxygen targets and thresholds.

American Shad is an anadromous species migrating from marine waters to the Chesapeake to spawn. Adults return to oceanic waters after spawning. Eggs, larvae and juveniles reside in the bay, using it as a nursery. Summer seine survey data document American Shad juvenile presence. Maximum size recorded over the entire record ranged from 40 to 132 mm with a mean maximum length of 81mm. Hildebrand and Schroeder (1927) reported American Shad juvenile lengths ranging from 30 to 94 mm, but reported observations of two juvenile fish taken in November measuring 149 mm. They postulated these were second year fish, because they were larger than their cohorts, but noted they could not verify that based on aging attempts. They did note that juveniles in other collections of fish "under favorable conditions" were able to reach lengths of 150-177 mm. Tuckey (2009) reported juvenile growth rates are density-dependent and can vary among rivers; therefore, it is possible that the larger fish we observed are juveniles in habitats favoring accelerated growth. Based on this and the common thought that juveniles emigrate to the ocean in fall and late winter, we assumed American Shad recorded in the seine survey were juveniles.

Presence of American Shad was observed throughout the sampling period (Figure 3-1). Proportion of positive seine hauls declined in the 1970's consistent with reports of dwindling stocks around the same time frame (ASMFC, 2009). This pattern of low presence persisted through the early 1990's when proportion of positive seine hauls returned to similar levels of the pre-1970 timeframe. Klauda et al. (1991b) cited Hurricane Agnes as a contributing factor to the swift and persistent decline in presence. Major changes in Maryland's landscape associated with suburban development and changes in agricultural practices (see **COMMON BACKGROUND for Job 1, Sections 1-3)** occurred at this time as well. This suggests that habitat change influenced stock success and both highlights the importance of habitat in evaluating stock dynamics and demands greater attention to understanding natural and anthropogenic limits to habitat.

This decline in the early 1970's prompted aggressive coastal management with Maryland issuing a fishing moratorium in 1980 and initiating stock recovery efforts including stocking (Stence 2017). Maryland experienced positive results in stocking while the coastal population continued to decline (Stence2017). Proportion of seine hauls with juvenile American Shad recovered to previous high levels beginning in the early 1990's, although they appeared to be less consistent than 1960-1971 (Figure 3-1). We compared proportion of seine hauls with American Shad present to annual baywide geometric mean abundance and found a fairly strong relationship between presence and abundance ($r^2 = 0.62$, $P = 0.0001$; Figure 3-2). Presence and abundance track fairly well. In regard to defining habitat criteria, presence is a preferred measure, because data are at times sparse. Presence-absence is ecologically meaningful, minimizes errors and biases in sampling, and reduces statistical concerns about lack of normality and high frequency of zero catches that were expected in stressed habitats (Uphoff et al. 2011).

Preferred salinity for juvenile American Shad in summer was 0-4 ppt, accounting for 82.5% of the occurrences (Figure 3-2). This salinity range corresponds to tidal-fresh and oligohaline regions of the Bay (see Job 1, Section 3). Spatially, this represents 11.6% of tidal habitat in Maryland's portion of Chesapeake Bay. Acceptable habitat (4 -7 ppt) represented 12.5% of occurrences. Marginal habitat occurred at 7-13ppt and accounted for the remaining 5% of occurrences (Figure 3-2). Juvenile American Shad were not present in salinity greater than 13 ppt in seine survey samples (Table 3-2). Because juvenile shad eventually migrate to oceanic waters, all tidal waters support juvenile habitat at some point, but dissolved oxygen should be most limited in summer.

Seine data are limited to July through September sampling, so late emigration to higher salinity would not be reflected in catch data. Klauda et al. (1991b) reported salinity for juvenile American Shad ranged from 0–30 ppt without preference. We found most of juveniles in low salinity habitat in summer, consistent with other studies reporting that juvenile Shad remain in fresher waters unit fall migration (Sea Grant 2011).

We overlaid mean dissolved oxygen at <5.0 and <3.0 mg/L to examine the potential impact low oxygen could have on American Shad juvenile habitat. We observed a small overlap of the <5.0mg/L distribution with preferred habitat in the Potomac River and one occurrence in the Upper Bay (Figure 3-3). There was no overlap between dissolved oxygen < 3.0 mg/L and preferred habitat. We looked at presence by month and salinity (Figure 3-4) to determine if shifts in presence toward areas with low oxygen could make American Shad vulnerable to oxygen stress and did not observe a major shift in presence by salinity during the summer sampling timeframe. Though low oxygen volume changes annually and monthly in the Bay (Bill Romano, MD DNR, personal communication) there is little evidence that low oxygen impacts preferred juvenile American Shad habitat to a major extent.

Though juvenile Shad eventually migrate to the sea in their first year of life (Table 3-1), Murdy et al. (1997) reported that juveniles inhabit fresh to brackish water feeding on copepods and insect larvae. O'Leary and Kynard (1986) found that juvenile shad seaward migrations began at 19 °C in the Connecticut River. Klauda et al. (1991b) reported peak emigration of shad occurred in October and November. Mean temperature of all stations sampled declined over the sampling period, but did not reach 19°C before the survey was completed in September (Figure 3-5). Klauda et al. (1991b) reported that juvenile American Shad prefer water temperatures ranging from 15.6 to 23.9 °C. We evaluated temperature data from the Striped Bass Juvenile Seine survey in preferred habitat areas to examine the range of temperature exposure in these areas and found that only 13% of temperature values were at or below the 23.9 °C reported preferred upper limit for temperature.

Alewife (Alosa psuedoharengus) and Blueback Herring (Alosa aestivalis)

Alewife and Blueback Herring, collectively known as River Herring are anadromous species that exhibit similar life histories to American Shad. They were a popular commercial species at the turn of the century that followed a similar precipitous decline in harvest as American Shad, reaching a critical low in the 1970's (Klauda et al. 1991). Proportion of positive tows for Alewife and Blueback juveniles followed a similar pattern to American Shad, showing declines in presence beginning in the 1970's and extending into the early 1990's (Figure 3-6). High values of these indices returned to levels seen during 1960-1970, but became more variable (exhibiting lower lows; Figure 6). Declines in production have been attributed to overfishing and degradation of spawning and nursery habitats (Klauda et al. 1991).

River Herring follow similar spawning migration patterns as American Shad, but ascend further upstream than American Shad into non-tidal freshwater streams. Like American Shad, River Herring migrate to fresh areas to spawn and adults quickly return to oceanic waters after spawning. Juveniles remain in brackish waters of the Bay during their first summer and begin seaward migration in the fall. Job 1, Section 1 documented negative associations of River Herring stream spawning habitat occupation with increased urbanization and conductivity.

We examined the reported maximum sizes of Alewife and Blueback Herring during July-September in the Striped Bass Seine Survey data. Alewife maximum length ranged from 46 mm to 290 mm in length, with a mean maximum length of 87.6 mm and 145mm marking the 99th percentile of the distribution. Historical records of juvenile Alewife length ranges suggested juveniles in the summer ranged from 45 to 85 mm length (Hildebrand and Schroeder, 1927). Present records identified a mean maximum length of 87.6 mm. This could suggest that growth rates increased or these large fish are age 1 fish that remained in the Bay from the previous year. Hildebrand and Schroeder (1927) noted a small fraction of fish remained in the Bay for a second year.

Blueback Herring ranged from 25-292 mm in length with a mean of 64.2 mm and 101 mm falling at the 99th percentile of the distribution. In 1928, Hildebrand and Schroeder reported summer juvenile lengths were between 30 and 70 mm. This suggests most fish captured were juveniles and is consistent with our observations in our summer seining efforts.

We found that the majority of Alewife (Figure 3-7) and Blueback Herring (Figure 3-8) remain in lower salinity waters (tidal-fresh and oligohaline; see Job 1, Section 3) over the summer. Preferred habitat for Alewife was 0-5 ppt salinity, accounting for 70% of occurrences. Preferred habitat for Blueback Herring was 0-4 ppt, representing 65% of occurrences. These preferred habitat areas coincide with optimal salinity of 0-5 ppt reported for Alewife and Blueback Herring. (Klauda et al. 1991). Only 12.8% of tidal water in Maryland was preferred habitat for juvenile Alewife (Figure 3-7) and 11.6% preferred habitat for juvenile Blueback Herring (Figure 3-8).

Several studies conducted on oxygen limits for juvenile Alewife and Blueback Herring suggest dissolved oxygen limits of 4.0 mg/L and 3.6 mg/L (respectively) is limiting (Klauda et al. 1991). We overlaid oxygen layers at the 5.0 and 3.0 mg/L criteria, but also at 4.0 mg/L for Alewife and 3.6 mg/L for Blueback Herring to examine the impact low oxygen may have on juvenile habitat.

A very small portion of juvenile Alewife preferred habitat was prone to effects from low oxygen: 0.2% at 5.0 mg/L, and 0% at 3.0 mg/L and 4.0 mg/L (Figure 3-9). However, total juvenile habitat (salinity of 0-16 ppt) showed various levels of impairment from low oxygen; 26.6% of total habitat had DO < 5.0 mg/L, 10.4% was associated with DO < 3.0 mg/L and 17.2% was impacted at DO < 4.0 mg/L (Figure 3-9). Mesohaline regions (> 5 ppt) are subject to low bottom oxygen conditions not seen in tidal-fresh and oligohaline regions (Job 1, Section 3) and the increased percentages of potentially impaired habitat reflect the addition of this salinity region to the overlays.

Blueback Herring habitat was mapped with 3.0 mg/L, 3.6 mg/L, and 5.0 mg/L dissolved oxygen overlays (Figure 3-10). Blueback Herring preferred habitat (0-4 ppt salinity) did not overlap with low oxygen at any of the three levels. In evaluating total habitat (0-15 ppt salinity) 21.7% of the area was affected by DO < 5.0 mg/L, 7.5% with DO < 3.0 mg/L and 7.8% with DO < 3.6 mg/L (Figure 3-10). Again, addition of mesohaline waters that comprise marginal habitat and are more conducive to low dissolved oxygen created the increase in affected area.

Potential juvenile habitat losses may be over-exaggerated in the overlays because much of the low oxygen area was in deeper waters of the main Bay and larger rivers and may not directly impact juvenile River Herring habitat. That said, we need to give more consideration to low oxygen effects on juvenile habitat as Loesch et al. (1982) documented depth segregation by species (Alewife juveniles preferred deeper water) and reported shifts in water column orientation associated with diel cycles. There is a potential for low oxygen in deeper waters to impose sublethal effects on fish or influence distribution or even seasonal patterns of emigration. The present water quality data set represents an interpolation of average summer bottom dissolved oxygen between 1998 and 2003, therefore it lacks the resolution needed to explore these interactions and dynamics. However, we will explore other water quality and biological datasets to determine if there is sufficient data to explore potential influences of low oxygen on distribution and water column orientation, along with potential influences on fall migration of river herring.

Klauda et al. (1991) reported suitable temperature for Alewife was 10-28 °C with 17-24 °C the preferred range. Blueback Herring suitable temperatures were 10-30 °C, with 20-28 °C the preferred range. When we evaluated temperature at sites with Alewife present, we found suitable temperatures were met 69.6% of the time and preferred temperatures 14.9% of the time. In Alewife preferred habitat (salinity 0-5 ppt), preferred temperatures were met 69.1% of the time and suitable temperatures 14.7%. Preferred temperatures for Blueback Herring were met 68.4% of the time, while suitable temperatures were met 95.3% of the time. In Blueback Herring preferred habitat, suitable temperatures were met 93.7% of the time and preferred temperature 66.2%. As with American Shad, increased temperatures could enhance growth and increase distribution (Klauda et al. 1991).

Striped Bass (Morone Saxatilis)

Striped Bass are the most important gamefish in Maryland's portion of the Bay today. Like the alosines, Striped Bass production declined in the early 1970's, prompting strong management measures. Figure 3-11 shows presence declined in the 1970's but began a recovery to previous levels in 1993. Numerous habitat stressors of concern have been identified as having potential to impair future production, including climate driven water temperature changes that can disrupt timing of natural cycles, flow changes that limit habitat, increased hypoxic volumes, contaminants and urbanization of landscapes (Maryland Sea Grant 2011b). Hall (1991) reported Striped Bass juveniles can tolerate a wide range of water quality. Secor et al. (2000) found that salinity was a factor in juvenile growth rates, associating salinity of 7 ppt at 28 °C with higher growth rates than fish occupying 0.5 and 15 ppt salinity.

Our examination of distribution with salinity indicated that 60% of juvenile Striped Bass were found in salinity from 0-9 ppt. (preferred category; a mix of tidal-fresh, oligohaline, and mesohaline), 30% occurred in areas with 10-16 ppt salinity (acceptable category; mesohaline) and the remaining 10% in areas with salinity greater than 16 ppt (marginal category; mesohaline). Figure 3-12 shows the distribution of habitat by salinity category.

We overlaid dissolved oxygen criteria at 5.0 and 3.0 mg/L to evaluate percentage of habitat potentially impacted by low oxygen. Figure 3-13 shows the distribution of habitat with low oxygen areas. In juvenile preferred habitat with DO < 5 mg/L, 16.7% is potentially compromised and 0.8% when DO < 3.0 mg/L. Of the total habitat for Striped Bass, 36.9% is potentially compromised at DO < 5.0 mg/L and 22.6% at DO < 3.0 mg/L.

In addition to the 3.0 and 5.0 mg/L oxygen criteria, we evaluated the effect of oxygen below 6.0 mg/L. Fay et al. (1983) reported the optimum range for all Striped Bass life stages was 6.0-12.0 mg/L DO. We evaluated the percentage of preferred habitat with DO < 6.0 mg/L and found that 39.6% of preferred habitat area would be affected and, for total habitat, 48.5% of the area would be impaired. As previously stated, these oxygen evaluations are based on bottom habitat conditions and most of the oxygen depleted waters are associated with deeper areas of bays and tributaries. And though there may not be direct exposure to low oxygen habitat, there could be secondary impacts caused by reduced volumes of suitable summer habitat in the Bay. Uphoff et al. (2011) observed that the odds of striped bass occupying the shore zone (seine sampling) in mesohaline Chesapeake Bay subestuaries did not change with increased impervious surface (DO in bottom channel waters was negatively related to impervious surface), whereas the odds that they would occupy bottom channel waters was strongly and negatively influenced by impervious surface. This loss of habitat could increase predator-prey encounters and impact trophic dynamics. We need to continue to evaluate additional water quality data to determine the exposure level of juvenile Striped Bass to low oxygen in shallow waters, along with sublethal impacts of these exposures.

We will continue to consider other habitat criterion and stressors and mine data to provide the best assessment of habitat condition possible for all target species. We intend to develop a compendium of collected information for each species to present a full picture of habitat stressors by life stage in key habitats to allow managers to develop strong policies to support sound management promoting sustainable fisheries.

Additional Species Mapping:

We evaluated salinity preferences for all target species. Salinity preferences for Estuarine, Marine Migrants and Tidal Fresh Forage species are presented in Table 3-3. Figures 3-14 to 3-21 show the distribution by habitat category for each species. We will continue to evaluate habitat stressors and map these in the future to develop habitat criteria that can be applied to manage these species.

Discussion

There may be a potential flaw, discovered late in report preparation, in the technique used to determine habitat preference based on salinity. Habitat classification by salinity was determined by cumulative distribution changes and this may lead to a bias for including low salinity areas as preferred habitat. We will be revisiting this technique for categorizing habitat and considering others. However, we believe that the strategy of categorizing habitat by salinity is sound, but the method may (or may not) change in future applications.

The Chesapeake Bay Program cites excess nutrients as the main factor in degradation of the Bay’s health (http://www.chesapeakebay.net/issues/issue/nutrients#inline). Numerous programs have evolved to implement nutrient reduction strategies to restore oxygen to bottom waters of the Bay (Butt and Brown 2010), with a focus on the main-Bay “dead zone”. The intent is to restore habitat for living resources, particularly key charismatic species that support the fishing industry. In our evaluation of preferred habitat for American Shad, Alewife, Blueback Herring, and Striped Bass juveniles, we found that low oxygen was a stressor in limited portions of their juvenile habitat.

Juvenile American Shad, Alewife, and Blueback Herring were primarily encountered in tidal-fresh and oligohaline regions of Chesapeake Bay. Portions of mesohaline region, as well as fresh-tidal and oligohaline, were within the preferred region for Striped Bass. Tidal-fresh and oligohaline subestuaries monitored for Job 1, Section 3 (summer monitoring) have rarely exhibited low dissolved oxygen levels in open waters. Mesohaline waters exhibited declines in mean bottom dissolved oxygen to levels below its target and threshold with increased development and increased stratification due to salinity is an important factor (Uphoff et al. 2011).

Although bottom channel waters of tidal-fresh and oligohaline subestuaries exhibit little hypoxia, dense SAV beds in Mattawoman Creek’s shallow waters (primarily introduced species) have exhibited low dissolved oxygen and levels of ammonia that are of concern. American Shad, Alewife, and Blueback Herring were commonly among dominant species prior to the advent of dense SAV and have rarely appeared afterward (see Job 1, Section 3). If these shallow regions were important habitat, then impenetrably high plant density or stressful habitat conditions within the beds may be precluding habitat occupation.

Temperatures have a potential to increase as Maryland’s climate becomes warmer (Boesch and Greer 2003), yet this may favor American Shad (and possibly other alosine species) according to McCormick et al. (1996) who suggested increased temperatures could allow juveniles to remain in nursery areas longer, increasing growth and potentially expanding the species habitat range. However, negative associations of surface and bottom DO with corresponding mean water temperatures at depth were detected for oligohaline subestuaries, suggesting system respiration could be a major consideration in oligohaline subestuaries (Job 1, Section3). Associations of temperature and DO were not detected in mesohaline or tidal-fresh subestuaries (Job 1, Section 3).

While dissolved oxygen may not limit alosine juveniles, increased hypoxia is an indicator of ecological change. We need to consider how other habitat and ecological features interact with land use practices that contribute to increased nutrient loads. In the coming year, we will compile information on multiple stressors to fish habitat for target species. We will also explore other data sets to attempt to evaluate effects of other factors on habitat, while also finalizing present maps to provide better information on preferred habitat of target species. Though these maps lack the resolution to explore the extensive influence of stressors, they can allow mangers to visualize key habitat for various life stages of managed fish species and thereby target management actions in areas where habitat conservation or recovery can maximize benefits to multiple species.

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Table 3-1. Classification of anadromous species with a summary of their life history and value in context of fishing. Life history summaries were derived based on descriptions found in Murdy et al. 1997.

Species	**Life Cycle Summary**	**Importance**
American Shad	Adults enter the Bay in the spring to spawn; eggs, larvae and juveniles occur in Chesapeake Bay from Spring through Fall	Forage Species; Commercial and Recreational Harvest (Moratorium imposed)
Alewife	Adults enter the Bay in the spring to spawn; eggs, larvae and juveniles occur in Chesapeake Bay from Spring through Fall	Forage Species; Commercial and Recreational Harvest (Moratorium imposed)
Blueback Herring	Adults enter the Bay in the spring to spawn; eggs, larvae and juveniles occur in Chesapeake Bay from Spring through Fall	Forage Species; Commercial and Recreational Harvest (Moratorium imposed)
Striped Bass	Immature males are widespread throughout the Bay year-round. Mature adults migrate from oceanic waters to tidal freshwaters to spawn. Larvae develop in tidal fresh areas. Juveniles disperse to higher salinity areas through the summer.	Iconic Commercial and Recreational Species

Table 3-2. Anadromous species with salinity (ppt) range categorized. Parenthetical values show the percentage of the distribution associated with each salinity range.

Species	**Life Stage**	**Preferred**	**Acceptable**	**Marginal**	**No Occurrence**
American Shad	Juvenile	0-4 (82.5)	4-7 (12.5)	7-13 (5.0)	>13
Alewife	Juvenile	0-5 (70)	5-10 (25)	10-16 (5)	>16
Blueback Herring	Juvenile	0-4 (65)	4-10 (30)	10-15 (5)	>15
Striped Bass	Juvenile	0-9 (60)	9-16 (30)	>16 (10)	

Table 3-3. Estuarine, Marine Migrant and Tidal Fresh Forage species with salinity (ppt) range categorized. Parenthetical values show the percentage of the distribution associated with each salinity range.

	Species	**Life Stage**	**Preferred ppt (%)**	**Acceptable ppt (%)**	**Marginal ppt (%)**	**No Occurrence**
Estuarine	White Perch	Juvenile	0-6 (60)	6-13 (35)	13-17 (5)	>17
	Yellow perch	Juvenile	0-4 (75)	4-6 (15)	6-11 (10)	>11
	Bay Anchovy	Juvenile/Adult	0-9 (65)	9-15 (25)	>15 (10)	
Marine Migrant	Atlantic Menhaden	Adult	0-9 (70)	9-12 (15)	>12 (15)	
	Spot	Adult	3-15 (65)	>15 (30)	1-3 (5)	
Tidal Freshwater Forage	Gizzard Shad	Juvenile/Adult	0-8 (75)	8-13 (20)	13-18 (5)	>18
	Silvery Minnow	Juvenile/Adult	0-2 (60)	2-6 (35)	6-14 (5)	>14
	Spottail Shiner	Juvenile/Adult	0-3 (75)	3-5 (15)	5-13 (10)	>13

Figure 3-1. Proportion of seine hauls with American Shad present (positive hauls) by year.

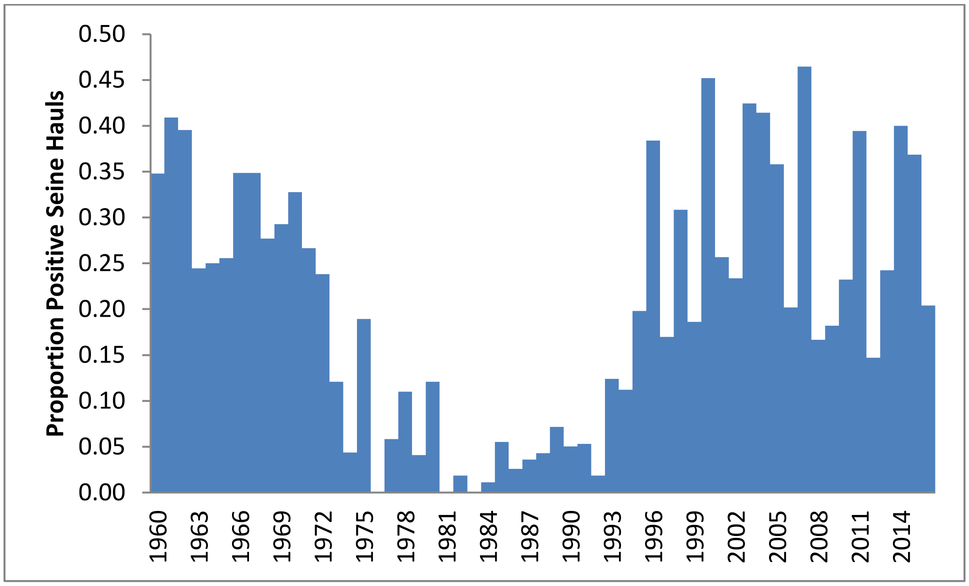


Figure 3-2. American Shad summer habitat based on frequency of presence by 1 ppt salinity increment.

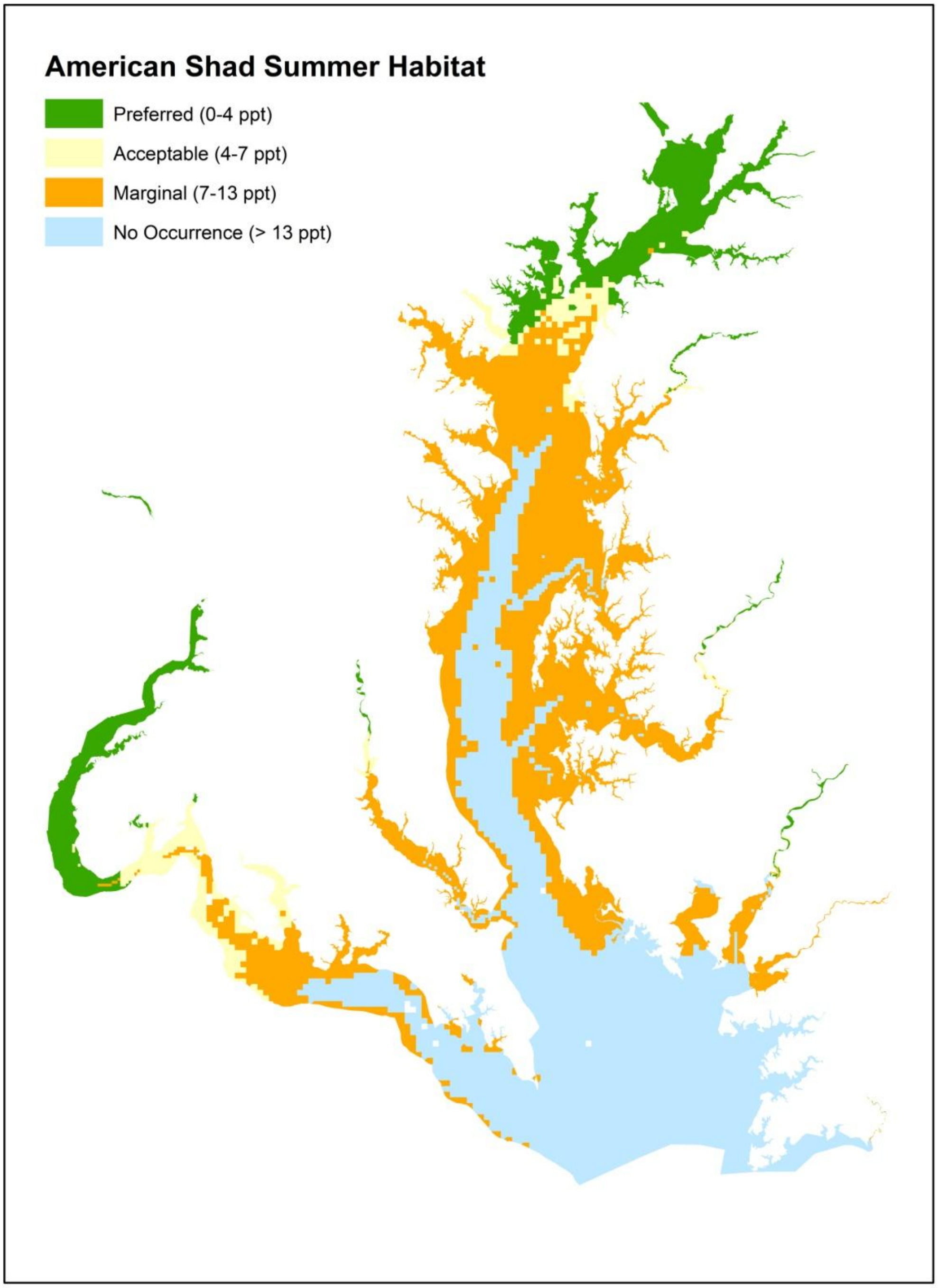


Figure 3-3. Preferred American Shad habitat with low oxygen areas overlaid.

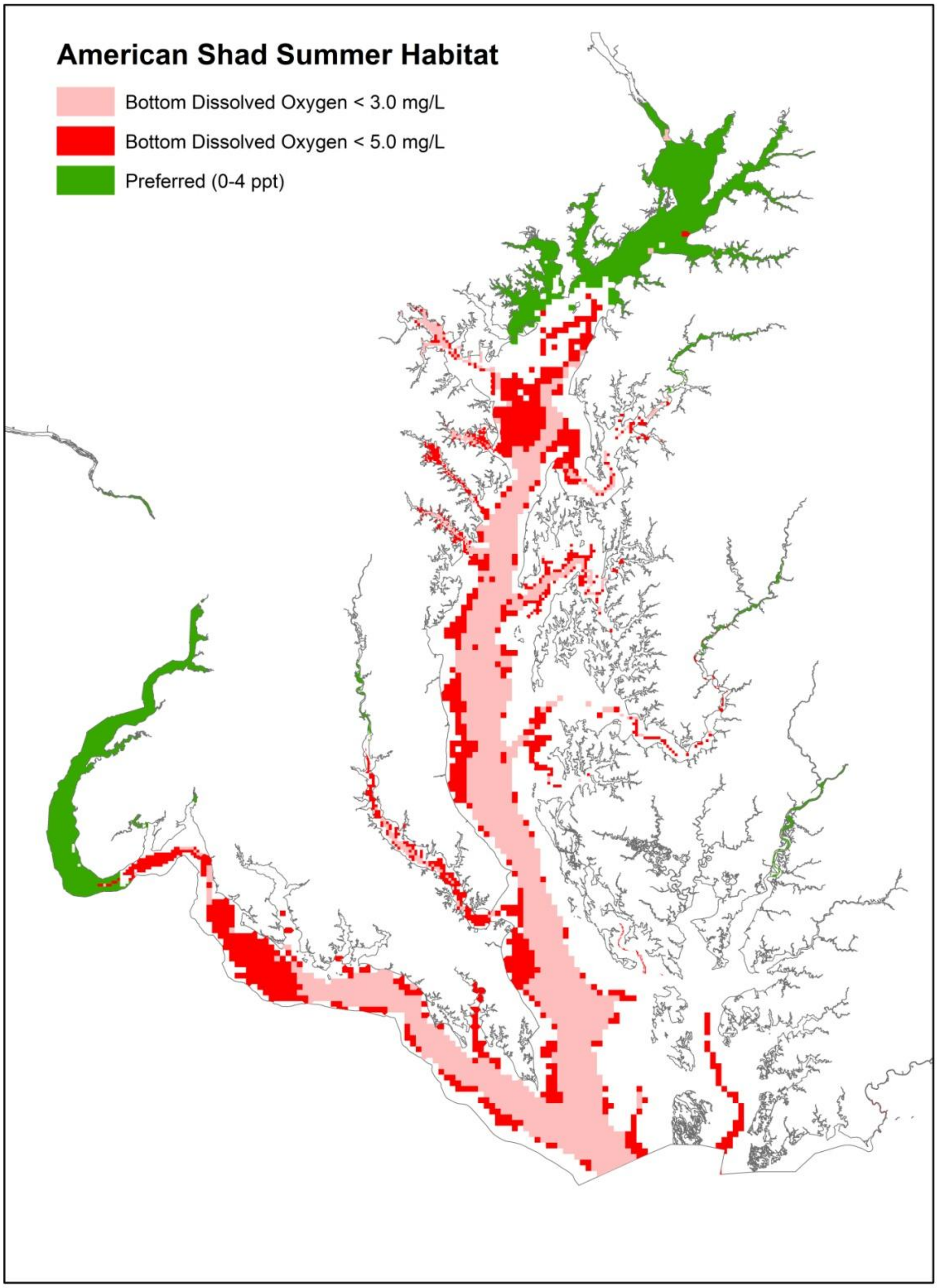


Figure 3-4. Proportion of positive seine hauls by month and salinity for juvenile American Shad.

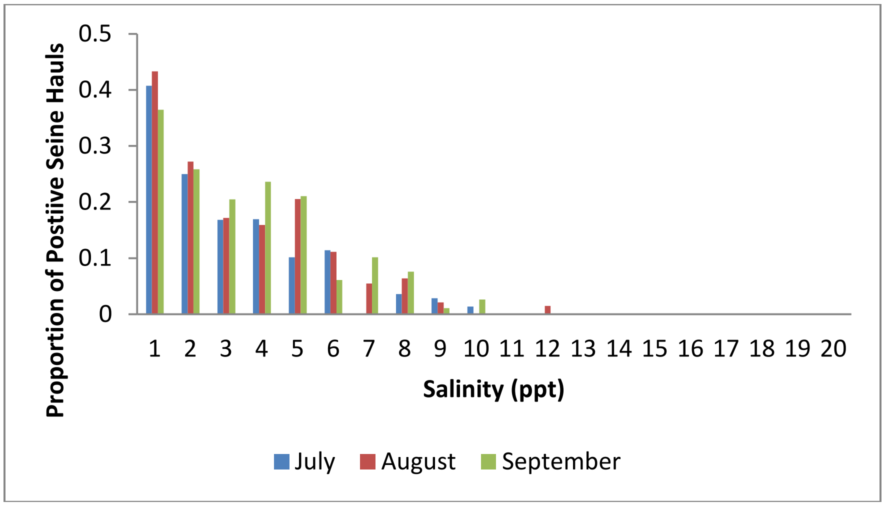


Figure 3-5. Mean water temperature by month from Striped Bass Juvenile Seine data, 1960-2016.

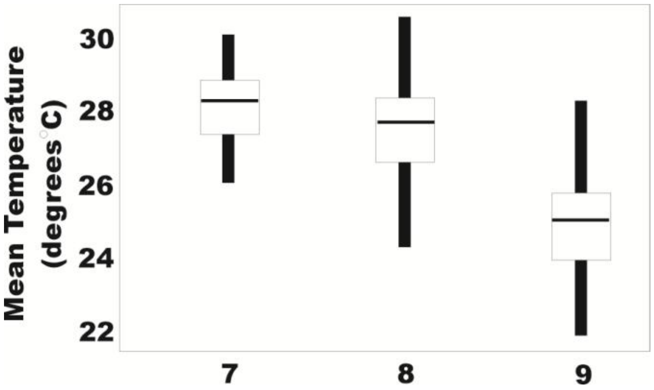


Figure 3-6. Proportion of positive seine hauls for Alewife and Blueback Herring by year.

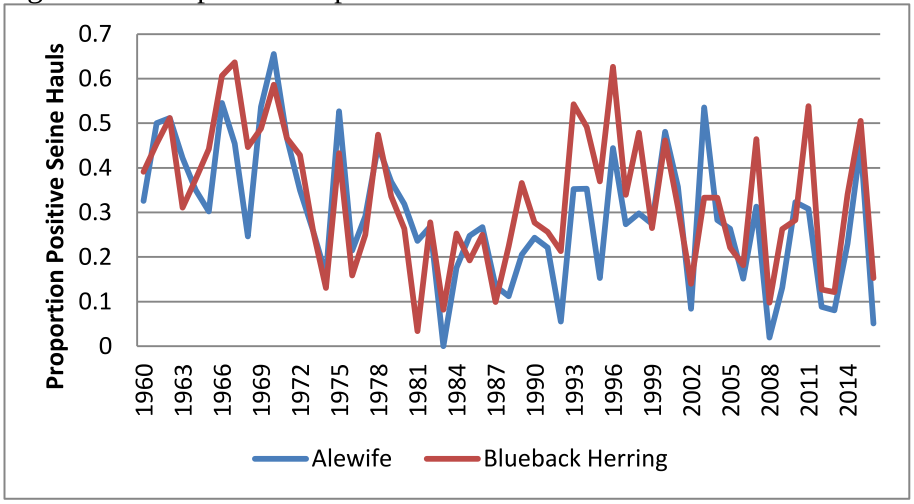


Figure 3-7. Alewife summer habitat based on frequency of occurrence by 1 ppt salinity increment.

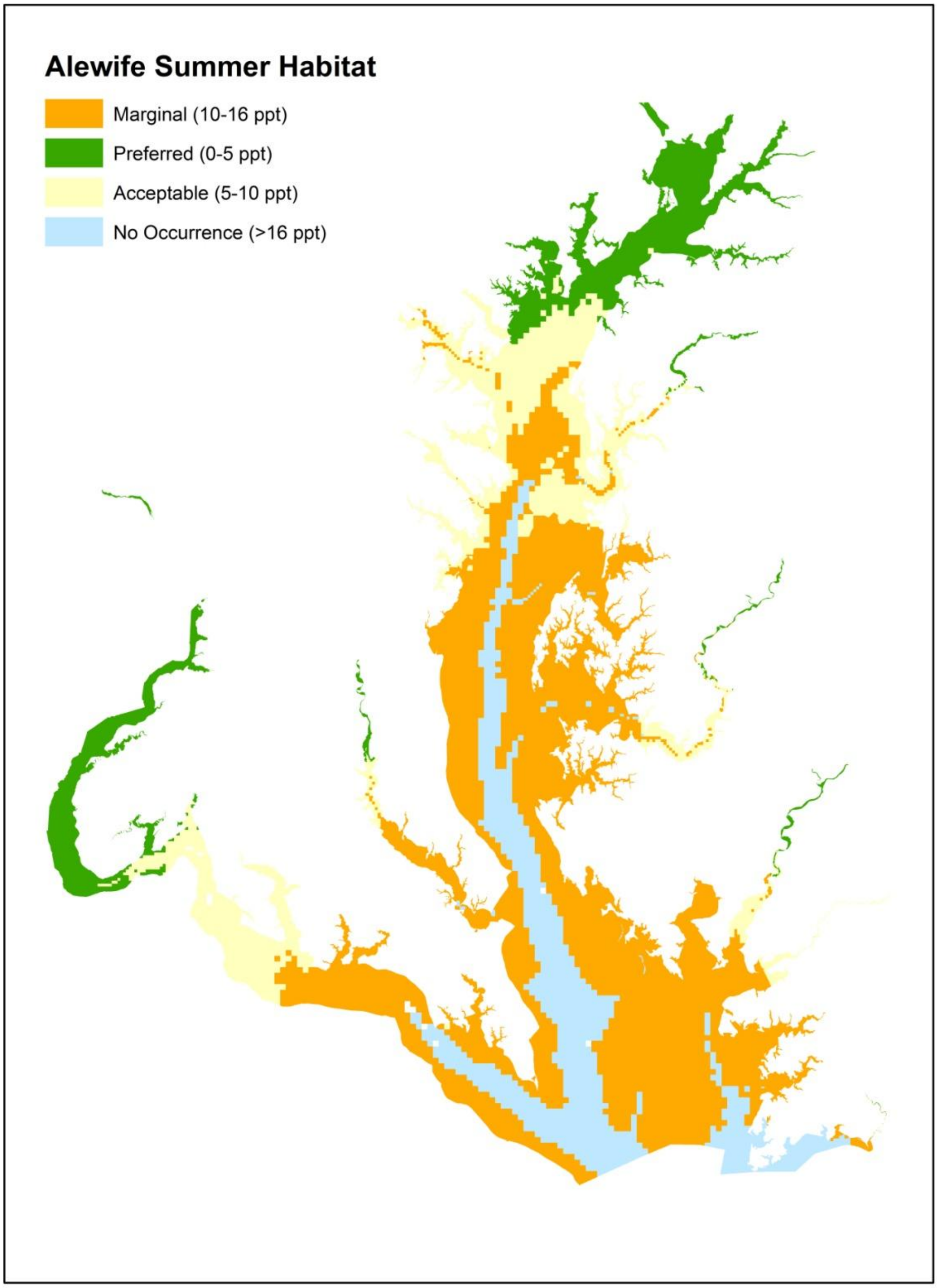


Figure 3-8. Blueback Herring summer habitat based on frequency of occurrence by 1 ppt salinity increment.

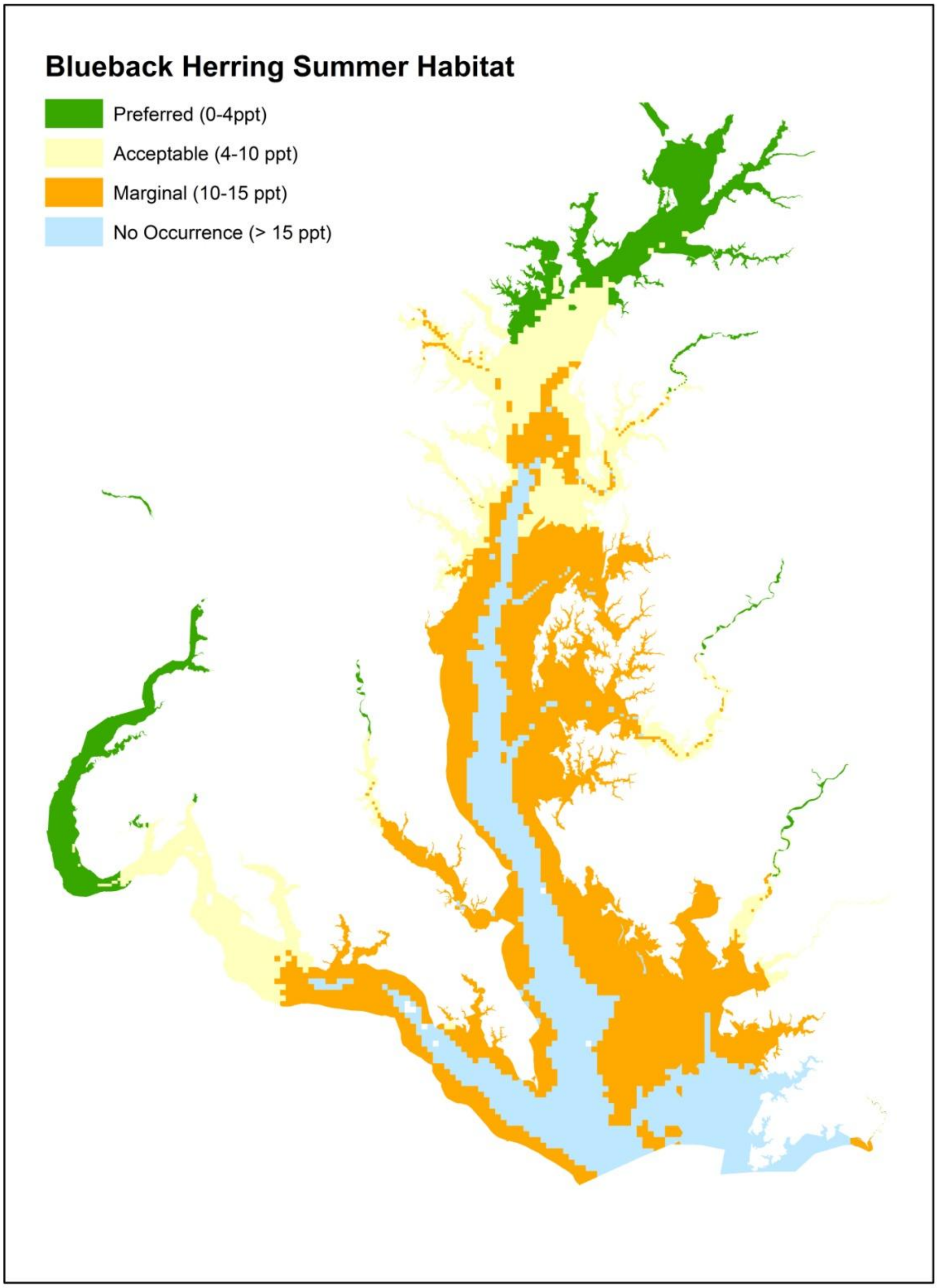


Figure 3-9. Summer juvenile Alewife habitat with various oxygen overlays of areas less than 5 mg/L (top left), less than 3.0 mg/l (top right) and less than 4.0 mg/L bottom.

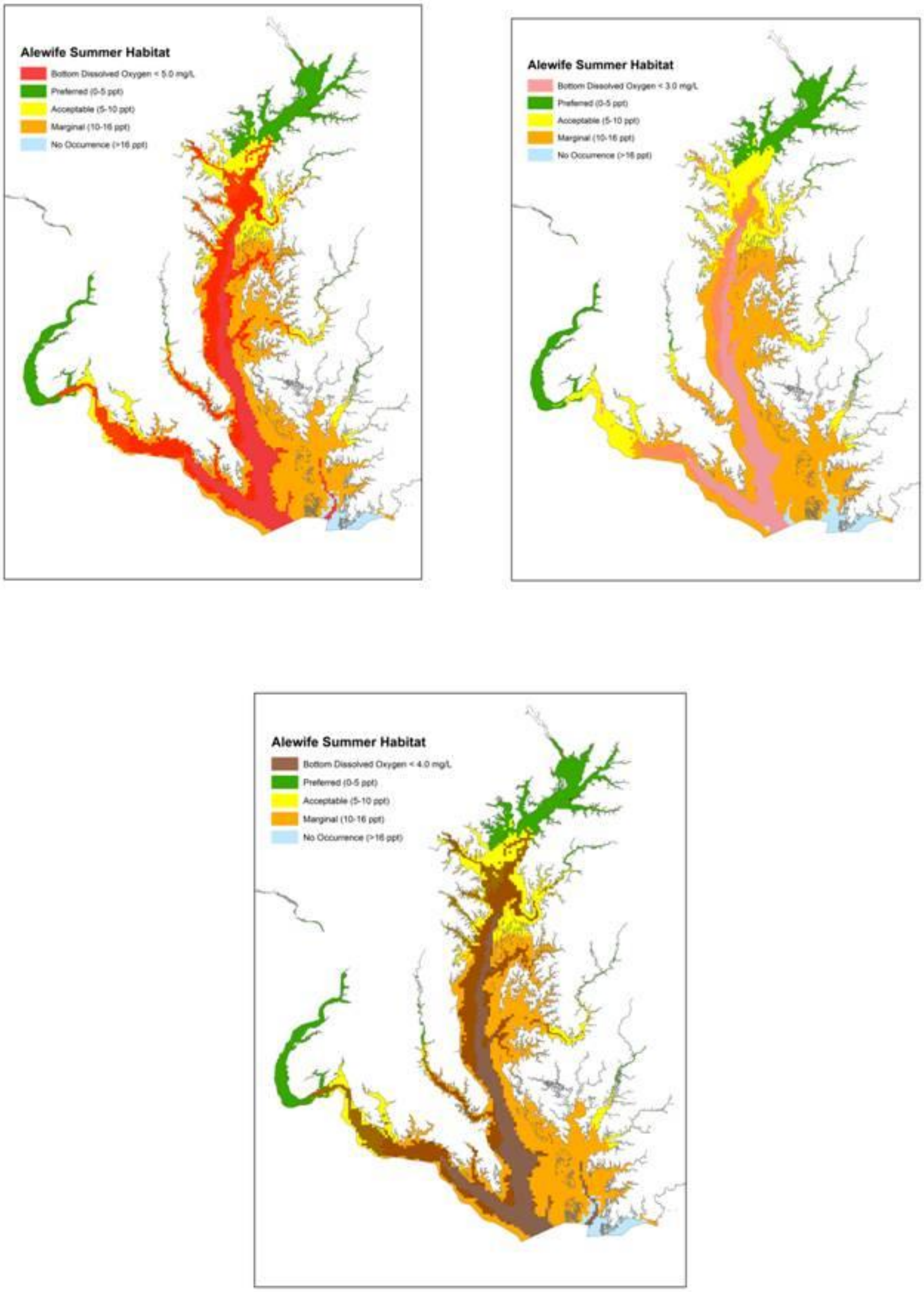


Figure 3-10. Summer juvenile Blueback Herring habitat with various oxygen overlays of areas less than 5 mg/L (top left), less than 3.0 mg/l (top right) and less than 4.0 mg/L bottom.

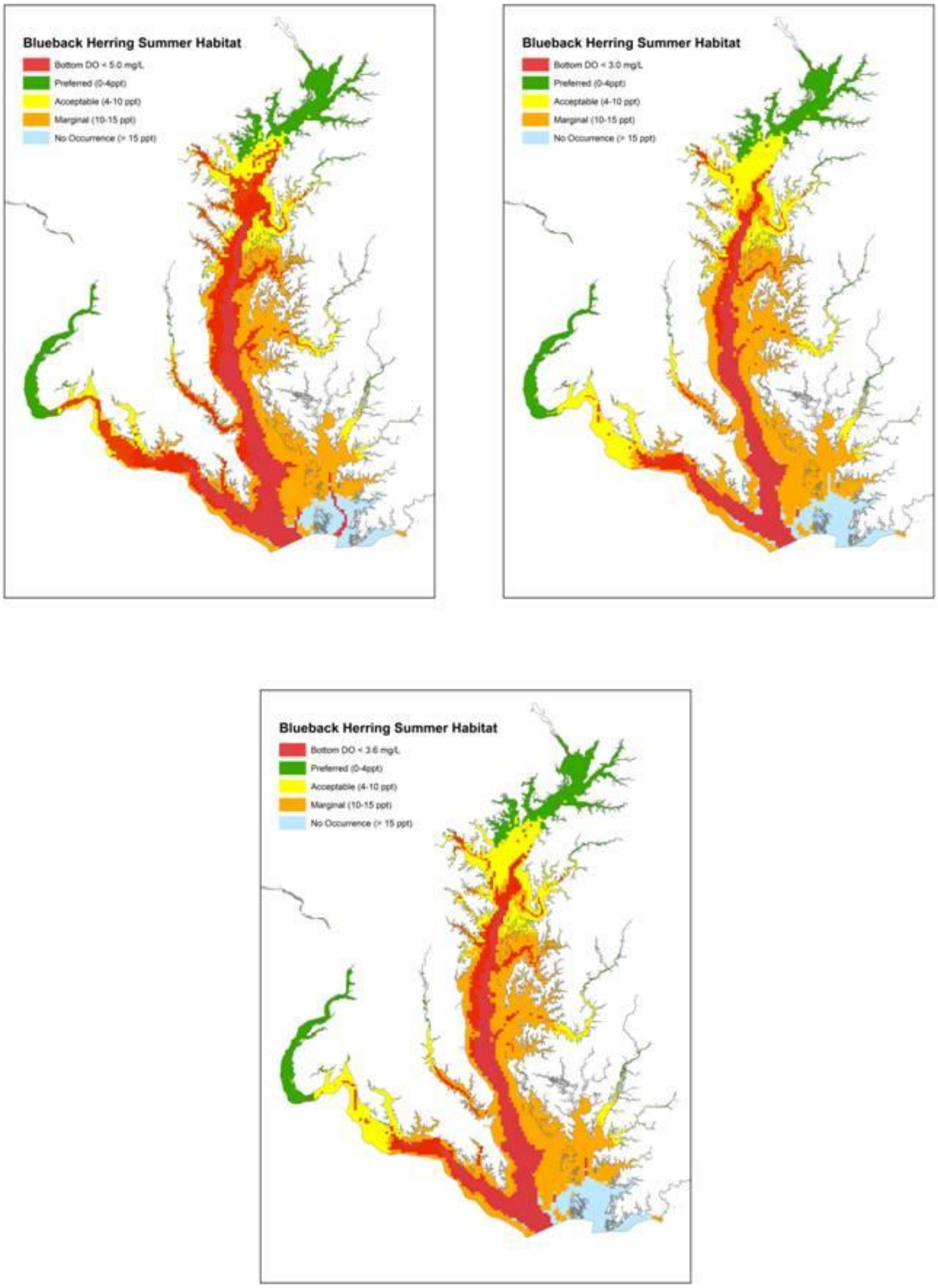


Figure 3-11. Proportion of positive hauls for Striped Bass juveniles by year.

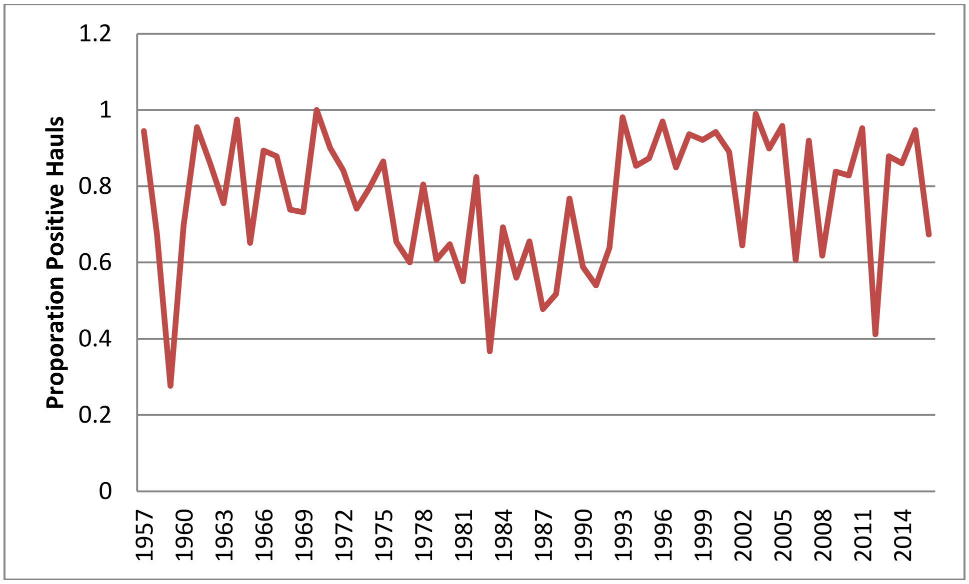


Figure 3-12. Striped Bass summer habitat based on frequency of occurrence by 1 ppt salinity increment.

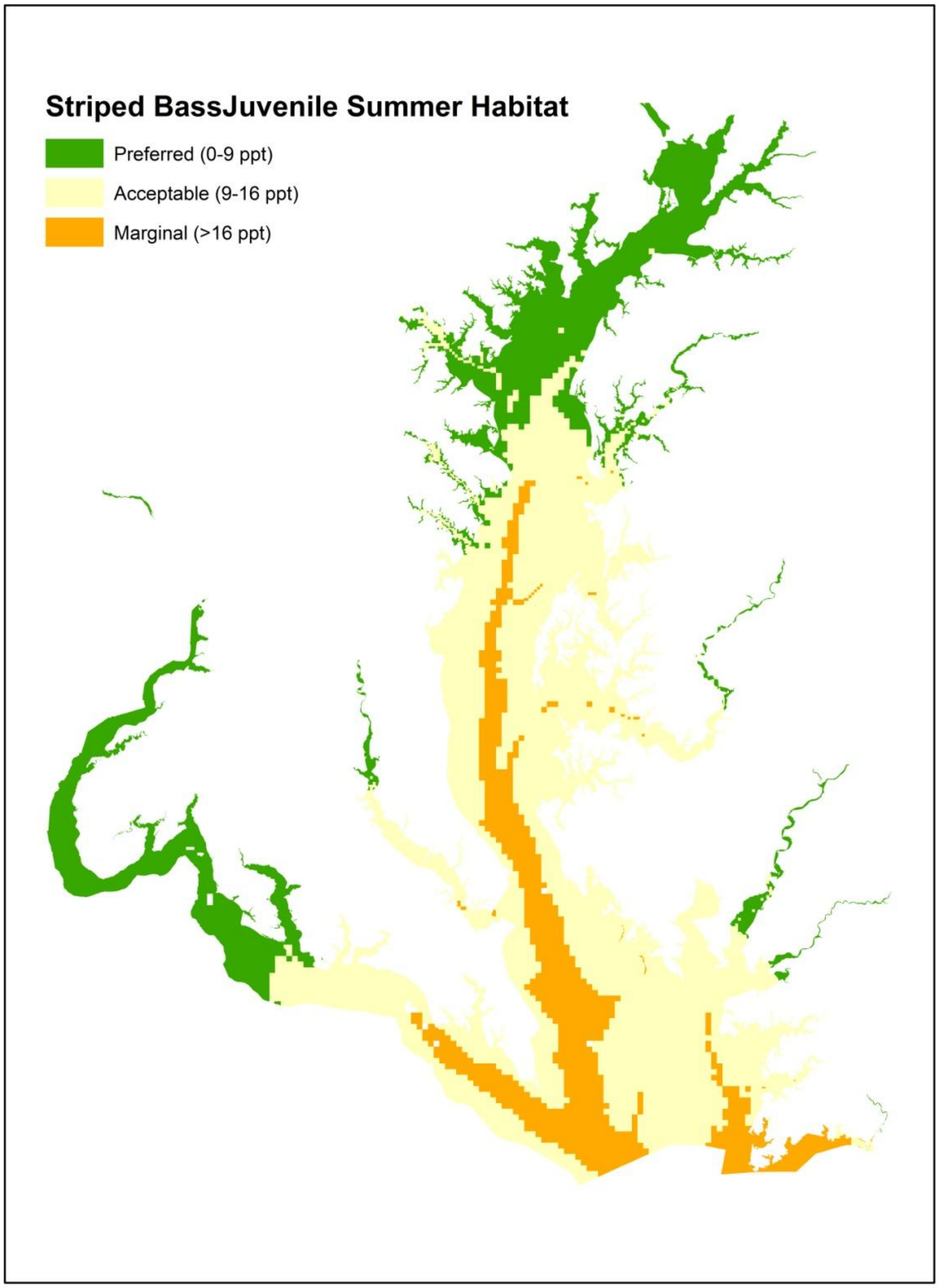


Figure 3-10. Striped Bass juvenile habitat with dissolved oxygen < 5.0 mg/L (left) and dissolved oxygen < 3.0 mg/L (right) with percentage of preferred and total habitat lost at each DO concentration.

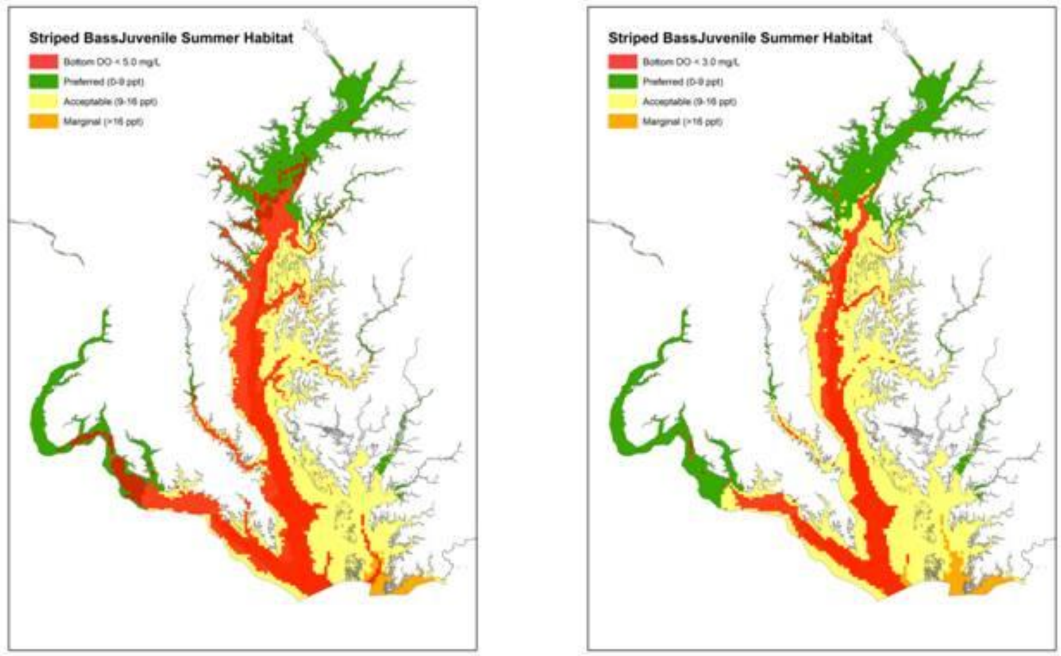


Figure 3-14. Bay Anchovy summer habitat based on frequency of occurrence by 1 ppt salinity increment.

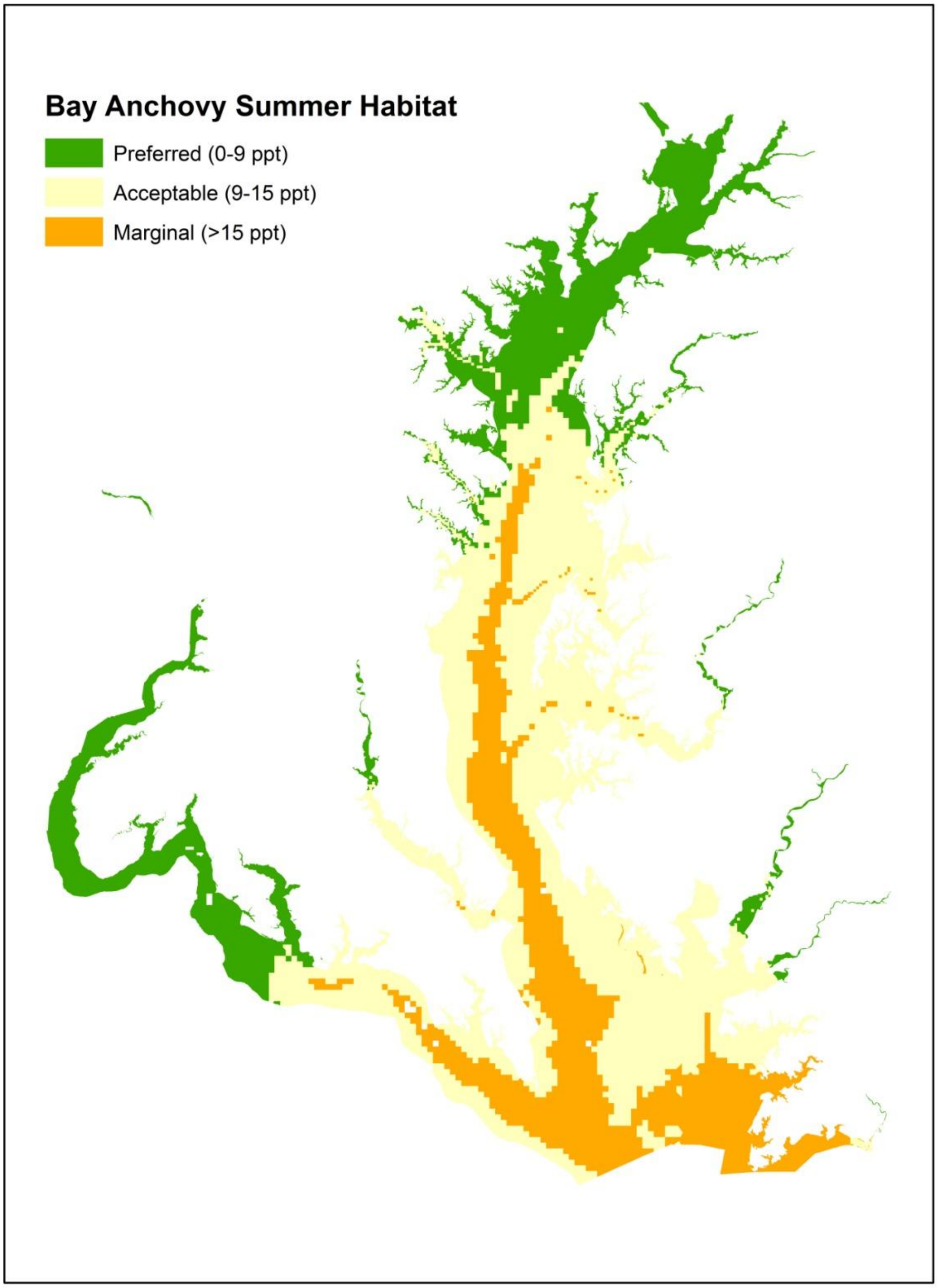


Figure 3-15. White Perch juvenile summer habitat based on frequency of occurrence by 1 ppt salinity.

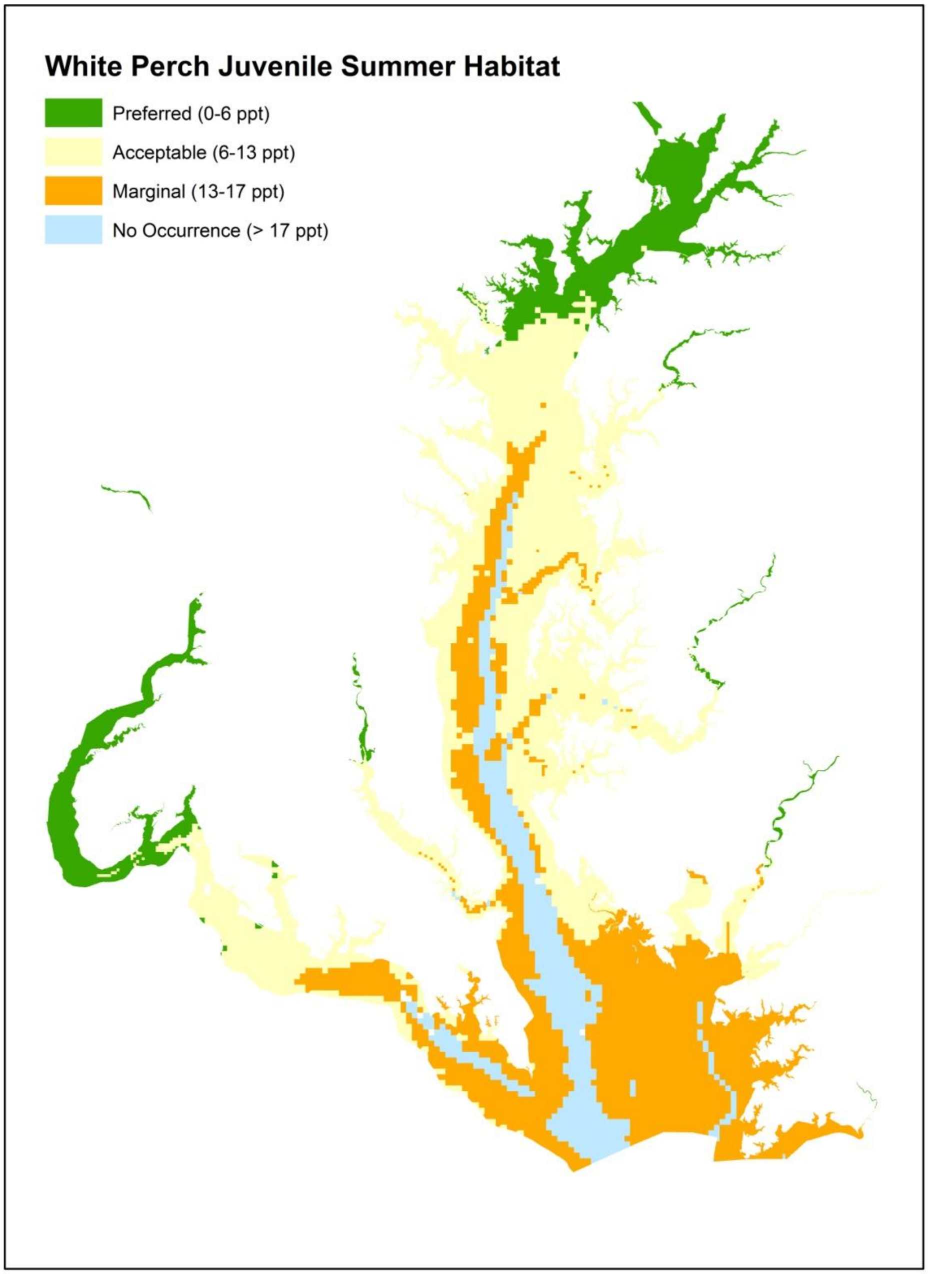


Figure 3-16. Yellow Perch juvenile summer habitat based on frequency of occurrence by 1 ppt salinity increment.

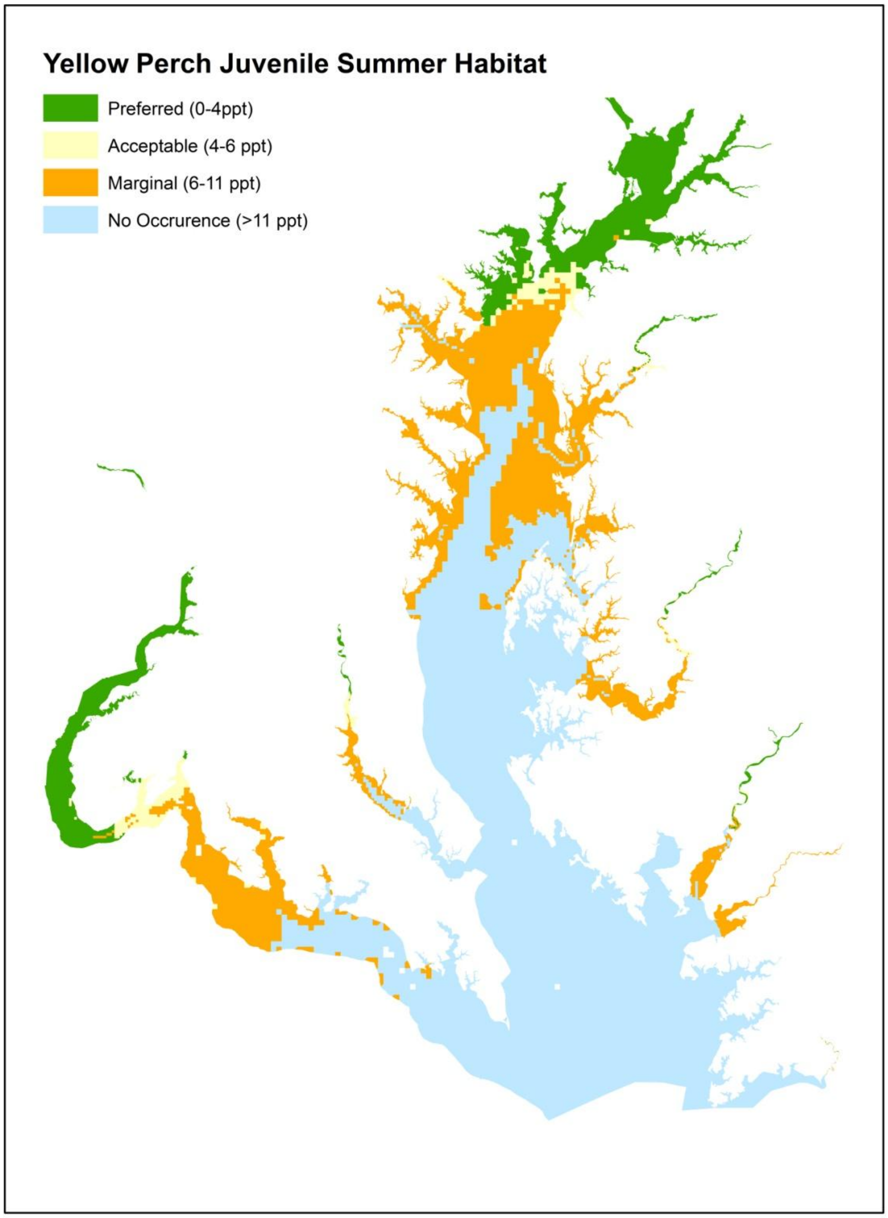


Figure 3-17. Atlantic Menhaden summer habitat based on frequency of occurrence by 1 ppt salinity.

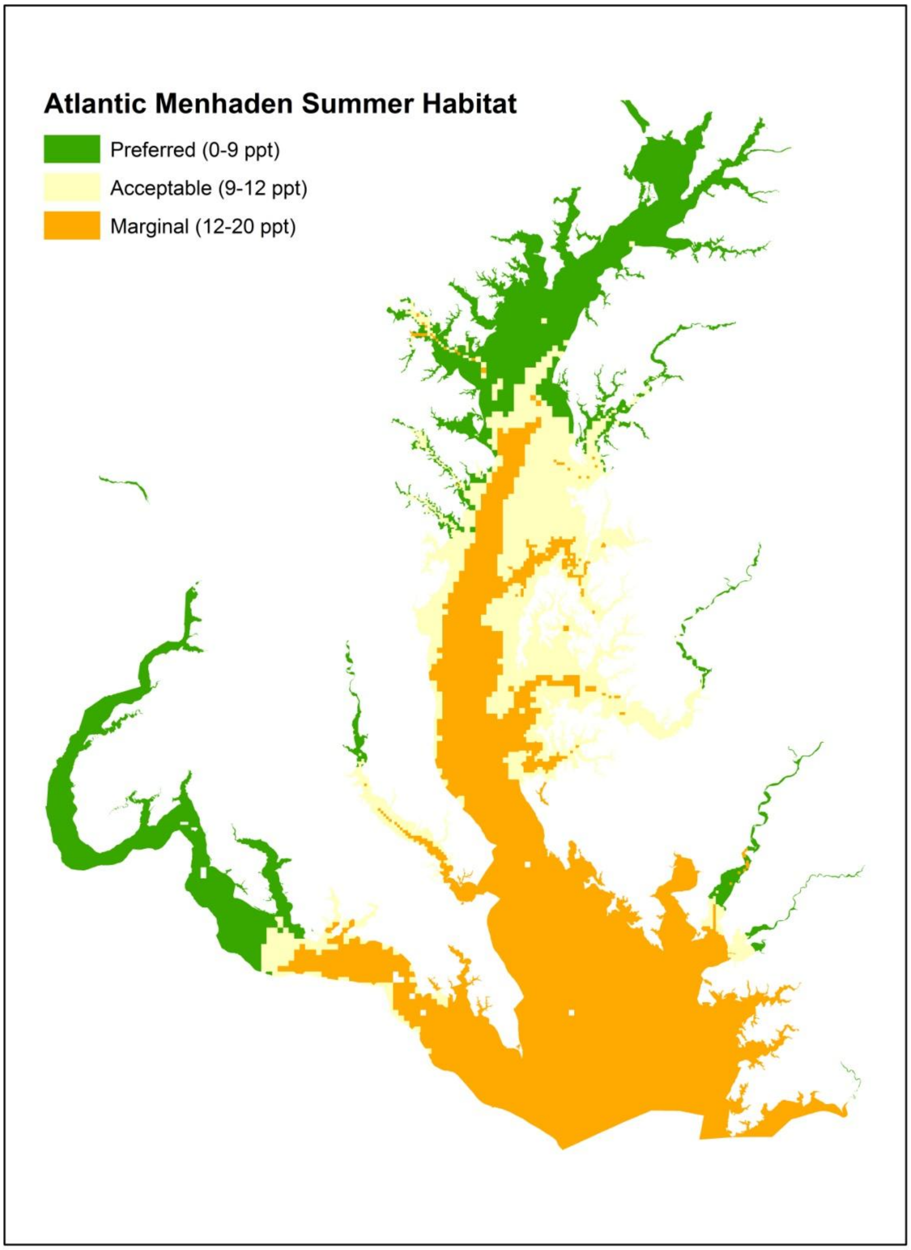


Figure 3-18. Spot summer habitat based on frequency of occurrence by 1ppt salinity.

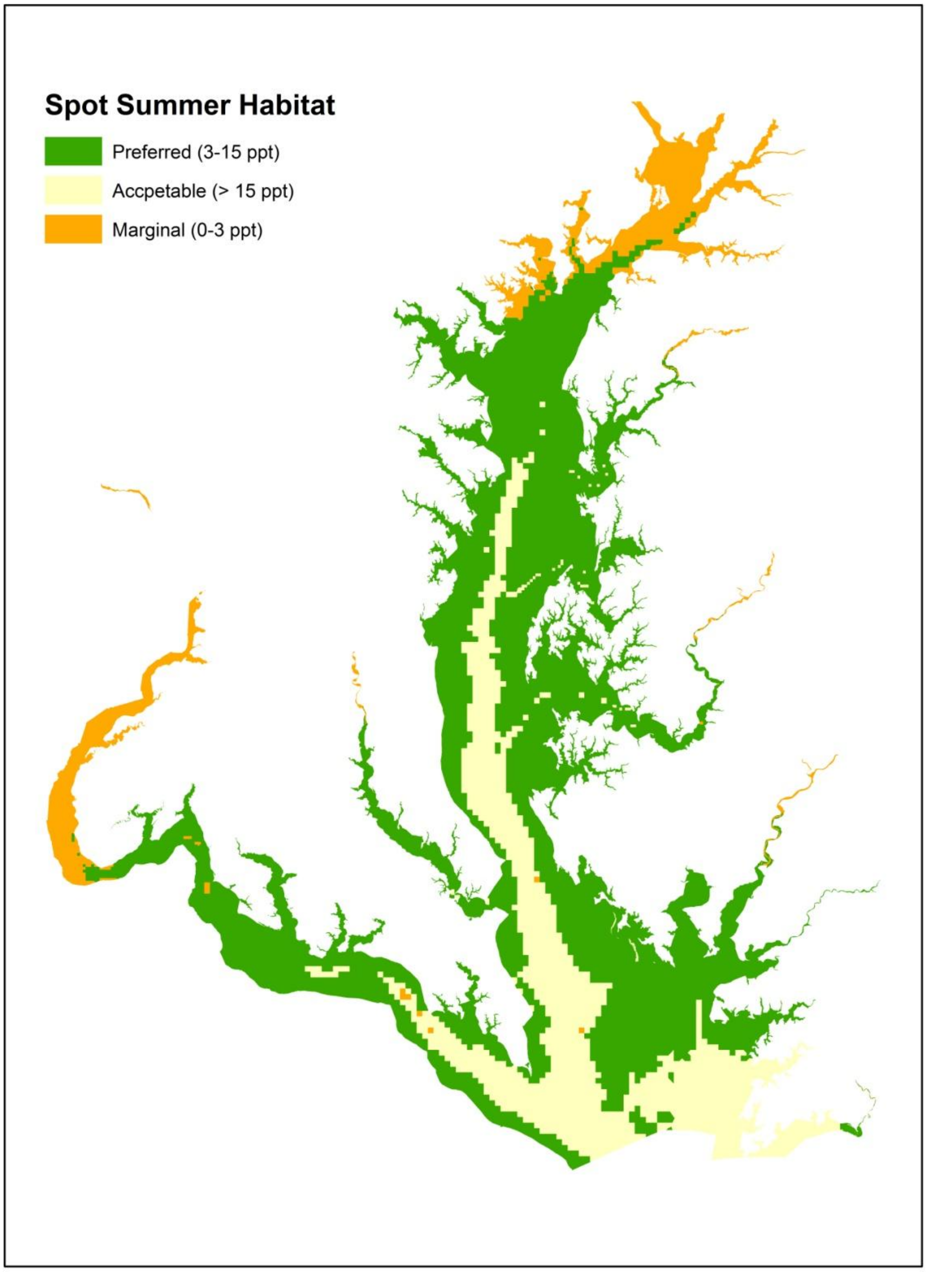


Figure 3-19. Gizzard Shad summer habitat based on frequency of occurrence by 1 ppt salinity.

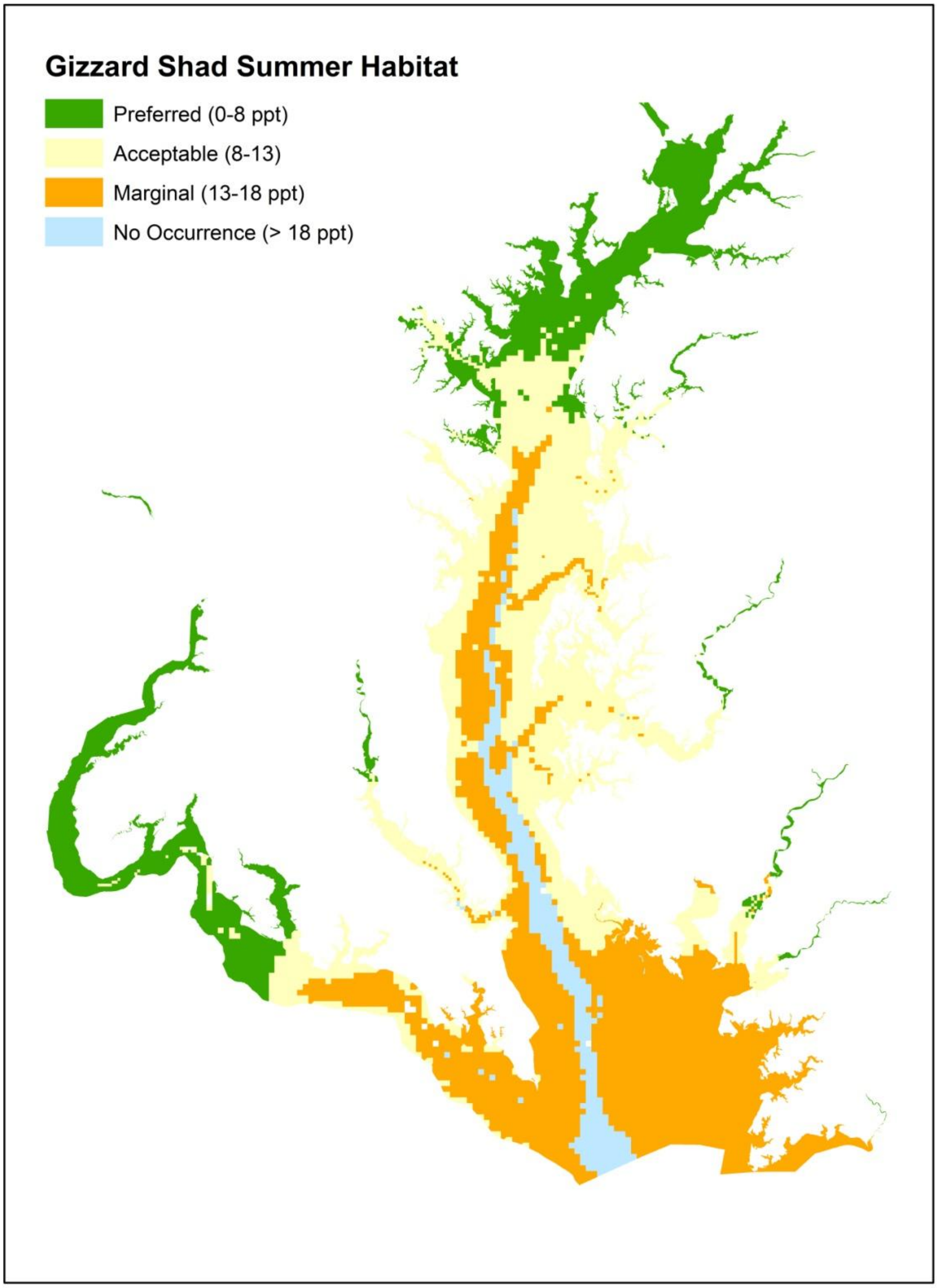


Figure 3-20. Silvery Minnow summer habitat based on frequency of occurrence by1 ppt salinity.

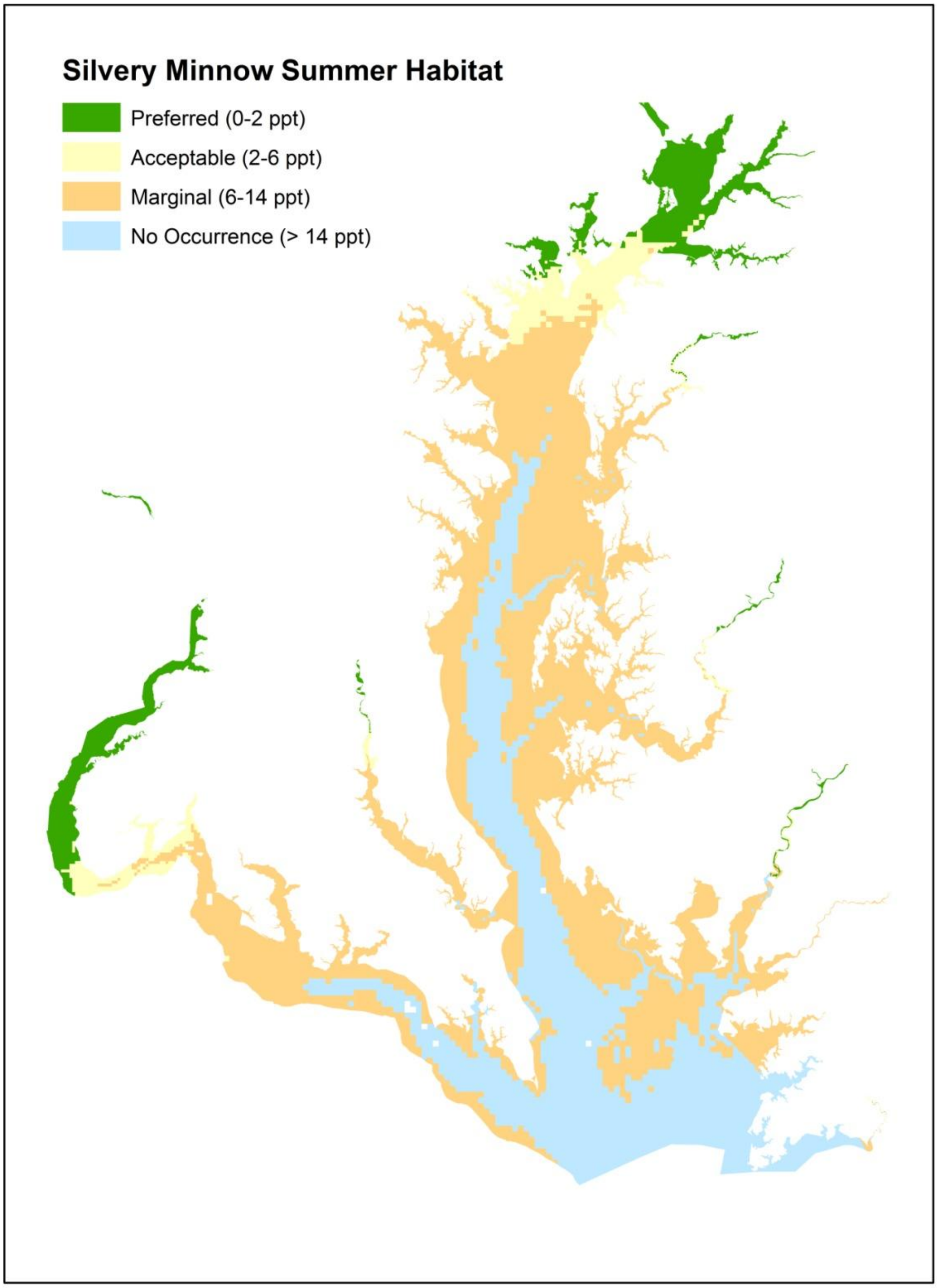
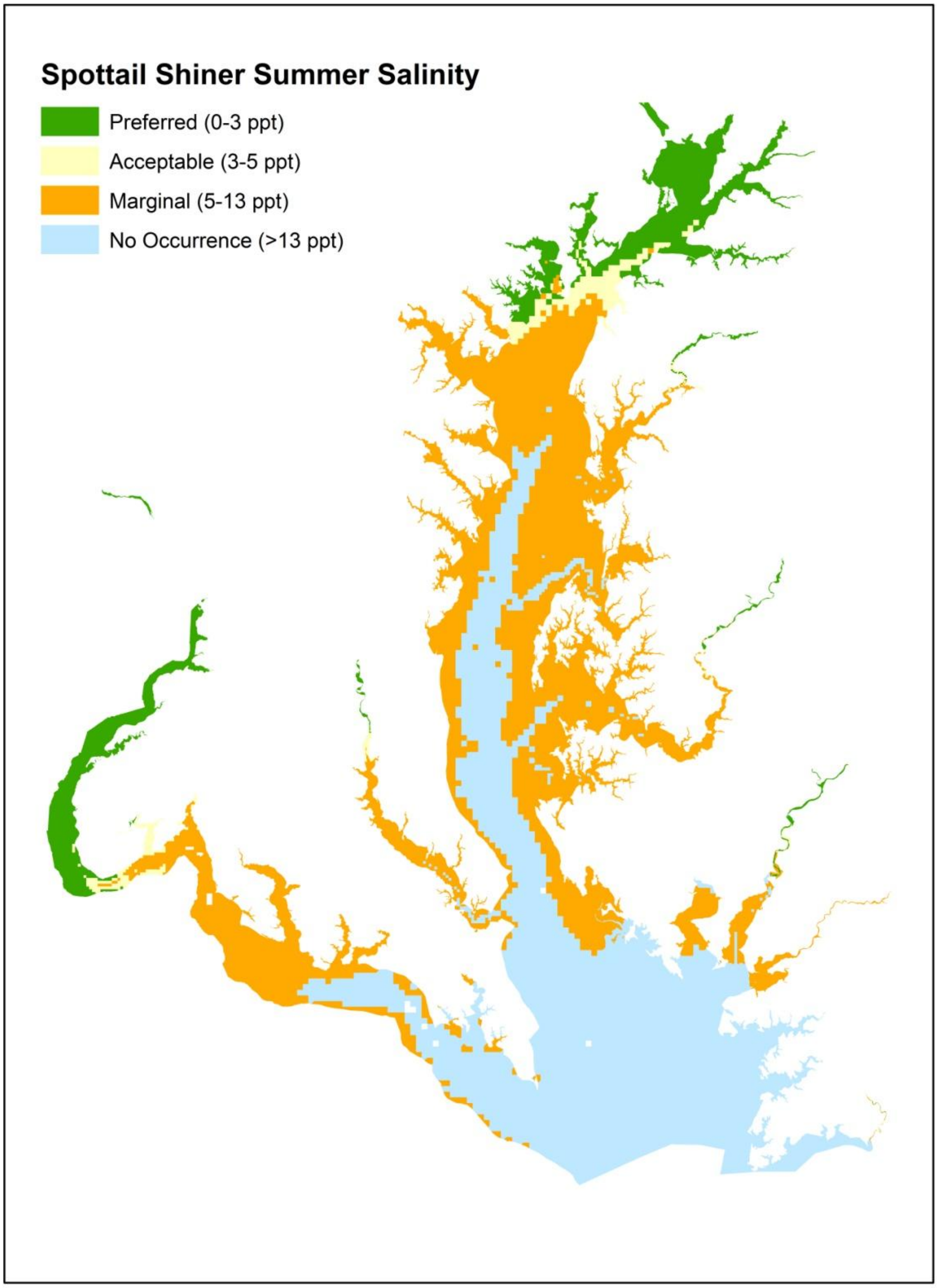


Figure 3-21. Spottail Shiner summer habitat based on frequency of occurrence by 1 ppt salinity.



Conductivity Criteria for Anadromous Fish Stream Spawning

Introduction

Increased salinization of freshwater is a growing global concern (Cañedo-Argüelles et al. 2013). Salinization of freshwater naturally occurs in response to weathering of soils, deposition of salt from sea spray in coastal areas and deposition in rainfall (Cañedo-Argüelles et al. 2013). Anthropogenic sources of salt from irrigation, mining and use of deicing agents to treat road surfaces can also contribute to a rise in salt concentrations in freshwater (Cañedo-Argüelles et al. 2013). Research has focused on defining impacts of increased salt loads to aquatic systems to identify the source and persistence of salts in surface and ground water (Kaushal et al. 2005; Kelley et al. 2008; Casey et al. 2013) and to understand impacts to biological systems (Blasius and Merritt 2002; Gillis 2011; Endreny et al. 2012). In the United States, salt usage in road deicing has increased significantly since first applications in the 1940's (The Cary Institute, www.caryinstitute.org). Morgan et al. (2007; 2012) evaluated chloride effects on stream biota in Maryland and found elevated chloride concentrations associated with increased road deicing operations. Uphoff et al. (2010; 2011; 2012) explored conductivity levels in anadromous fish spawning areas in Mattawoman Creek and documented an increase over historical (1970s) levels. They identified increased conductivity as an indicator of habitat change associated with declining anadromous fish spawning. In 2015, we began continuous monitoring streams in subwatersheds of Mattawoman Creek with mixed levels of land use and found higher conductivity associated with urban areas (Uphoff et al. 2016).

We were interested in the relationship of road density to conductivity, postulating that higher conductivity would be observed in subwatersheds with higher road densities. In 2016, we continued to evaluate the conductivity in five subwatersheds of Mattawoman Creek to explore the seasonal persistence of elevated conductivity and the source of increased conductivity in the watershed. In cooperation with citizen volunteers, we collected continuous and monthly conductivity data along with monthly (July through December) water samples collected to evaluate individual ion concentrations. Evaluation of dominant ions in a sample provided a means to identify the source of salts in the sample.

Methods

We deployed HOBO™ continuous monitors in 2015 at five stations on Mattawoman Creek. Four stations were in streams off of the mainstem, one station was on the mainstem of the creek, just above head of tide (Figure 3-23). Stations were chosen to examine input of road salt from different road densities in the subwatersheds and were located at the lowest accessible point on the stream to capture as much influence coming from the watershed as possible.

To calculate road density, road centerline shapefiles were obtained from Charles County and Prince George's County 2014 editions of MD Property View and then merged to create a single road centerline shapefile. In 2015 the road centerline shapefile was imported into Google Earth and edited to include roads that had been built since 2014. The modified road centerline shapefile was clipped to include only roads within the Mattawoman Creek watershed. All centerlines were assumed to represent two lanes and each lane was assumed to be 10 feet in width (Southworth and Ben-Joseph 1995; Stein and Neuman 2007; Institute of Transportation Engineers and Congress for the New Urbanism. 2010). A 10 foot buffer was applied to each side of the road centerlines, all buffer segments were dissolved to form one road polygon, the resulting road polygon shapefile was clipped to the Mattawoman Creek watershed, and polygon geometry was recalculated. Road density was calculated for each station by delineating the watershed where it intersected the station. Density estimates reflect total road area in the watershed catchment area above the station. Road density was calculated as follows:

$$RD_{DEN} = T\_RD_{area} / T\_CT_{area};$$

Where RD_{DEN} is road density; T_RD_{area} is total hectares of roads in the catchment and T_CT_{area} is the total hectares of land in the catchment.

We examined conductivity data from continuous monitoring at the five fixed stations for the period covering July 1, 2015 through December 31, 2016. These dates were chosen to assure equal sample size at all stations and to extend through the period when water samples were collected. Data were recorded hourly with a total number of 13,200 conductivity measurements recorded for each station over the sampling period.

We evaluated basic statistics: mean, median, maximum, and minimum. We plotted median catchment conductivity against catchment road density to view the trend.

We estimated the percent of conductivity measurements that exceeded the background benchmark (109 mS/cm) for Coastal Plain streams proposed by Morgan et al. (2012; see Job 1, Section 1) during the entire period of record and the March through May spawning period for Herring. This conductivity benchmark was interpreted to represent expected conditions in coastal plain streams experiencing minimal anthropogenic disturbance. Uphoff et al. (2016) examined conductivity limits to spawning and showed that the likelihood of observing spawning declined once conductivity exceeded 174 mS/cm. We evaluated percentage of time this spawning season threshold was violated for the entire year, the spawning season and monthly during the spawning season (March-May). We reviewed whether Herring eggs and larvae had been detected in or near the catchments monitored for conductivity in past and present spawning surveys.

Citizen scientists sampled conductivity at additional sites along the mainstem of Mattawoman Creek. They measured conductivity at nine stations along the longitudinal gradient of the creek on a monthly basis to examine potential sources or regions of the watershed that could be contributing to increased conductivity (Figure 3-23). They compared averaged baseflow conductivity to conductivity during a high discharge event. They determined baseflow verses storm flow by examining stream gage data from a nearby USGS gage located near station MC3.

Water samples were taken monthly from June through December, 2016. At each site, one 1-L polyethylene bottle was rinsed three times with sample water and then filled under the surface to minimize headspace in the bottle. A clean 60-cc syringe was triple rinsed and filled with sample water, then closed with a Luer-Lok valve. All samples were placed on ice, cooled to 4° C and then delivered to University of Maryland Appalachian Environmental Laboratory within 48 hours of collection. The laboratory filtered samples using a 0.45 µm membrane filter, aliquoted, preserved and analyzed samples within holding times. Each sample was analyzed for conductivity, anions including chloride, bromide, nitrate and sulfate; cations including sodium, potassium, magnesium, calcium, total alkalinity; and closed pH. The analytical methods and holding times for sample processing are outlined in Table 3-4 provided by the laboratory.

Total specific conductance and proportion contribution from each ion was calculated using the method of superposition (Granato and Smith 1999). This method is based on the law of independent migration of ions where molar conductivity is the sum of contributions from individual ions. We calculated ionic contribution of each ion by applying the equation:

$$cc = \sum_{i=1}^{n}(c_i * ec_i),$$

where cc = specific conductance calculated by superposition, in microsiemens per centimeter at 25° C; n is number of major ions in solution; c_i is the concentration of the ith major ionic constituent in milliequivalents per liter; and ec_i is the equivalent ionic conductance at infinite dilution for the *i*th ionic constituent, in microsiemens-liters pers centimeter-milliequivalent at 25°C (Granato and Smith 1999). We then calculated the proportion of conductance that each ion contributed to total specific conductance by dividing individual ionic conductivity by total conductivity.

Results

Table 3-5 presents summary statistics by station. MUT4 had the lowest median and mean conductivity followed by MUT3, MC1, MPB1 and MTB1 (Table 3-5).

Median conductivity increased steadily from 70 uS/cm to 308 uS/cm across four stations as road density rose from 0.007 to 0.024 and then fell to 181 uS/cm at the highest road density (0.044; Figure 3-24). Road density at MTB1(0.024) was 21% higher than at MC1 (road density = 0.019), yet the median conductivity at MTB1 was almost twice as high as MC1. Median conductivity at MTB1 was nearly twice that of MPB1 (road density = 0.044). When we examined satellite imagery of the three drainages (Figures 3-25-3-27), we saw that MPB1 has a significant network of housing developments (Figure 3-25), MTB1 has some large commercial features that may be point sources of salt (Figure 3-26); one of the commercial areas was a large warehouse store. In contrast, MC1 includes the other two drainages, and at the scale that captures the full drainage of MC1, localized landscape features are lost and the drainage area appears to be largely forested (Figure 3-27). It is possible that commercial shopping areas take a more liberal salting approach than county road crews and it would be worth investigating to determine if the load from these commercial areas is higher and could be better managed. It would also help to evaluate the density of stormwater ponds to determine if drainages with higher density of stormwater catchments are associated with higher conductivity.

All stations sampled exceeded the background 109 mS/cm conductivity benchmark proposed by Morgan et al. (2012) at least some of the time (Table 3-5). Station MUT4 (road density =0.007) met the benchmark conductivity 98.1% of the time and 99.5% of the time during the Herring spawning season. MUT3 (road density = 0.010) had the second highest occurrences of meeting the benchmark with 46.0% of observations meeting year round and 80.9% of observations during the spring spawning seasons. Station MC1 (road density = 0.019), the one station on the lower mainstem of the creek only met the benchmark 0.6% of the time year round, and 1.4% of the time during the spawning season. The two tributaries with high road density (MPB1 with road density = 0.44 and MTB1 with road density = 0.024) met the benchmark 0.5% and 0.0%

of the time respectively, while both sites exceeded the benchmark during the entire spawning season.

Site MUT4 (road density = 0.007) met the 174 mS/cm threshold 99.8% of the time year round and 100% of the time during the spawning season. MUT3 (road density =0.010) also met the threshold 100% of the time during spawning season with 97.2% of observations meeting the threshold year round. MC1 (0.019) met the threshold 52.8% of the time year round and 47.2% of the time during the spawning period. MPB1 (road density = 0.044) met the threshold 37.9% of the time year round and did not meet the threshold during the spawning season. MTB1 (road density = 0.024) met the spawning season threshold 0.2% of the time year round and 0% of the time during the spawning period. MUT3 and MUT4 met the threshold 100% of the time during spawning season for anadromous fish (March-May), while MC1 met the threshold most consistently in May, with lowest number of observations meeting the threshold in April (7.5%) and 37.5% of observations meeting the threshold in March (Table 3-6).

We obtained discharge data from the USGS gage near station MC3 (Figure 3-23) and compared it to monthly average discharge during 1949-2016 and found that March and April discharge was lower than average while May was above average (Figure 3-28). It is possible that conductivity was higher than the spawning threshold because flows were lower than average and salts were concentrated. It is also possible that at low flow conditions, groundwater with elevated conductivity could be influencing conductivity (Long 2017).

Site MC1 has consistently supported Herring spawning since1971 based on presence of eggs or larvae (O’Dell eta al. 1975; Uphoff, et al. 2016). Herring eggs and larvae were present at MUT3 in 1971 and again in 2012. MUT4 was not sampled in 1971, but spawning was indicated in 2012 (Uphoff et al. 2016). MPB1was sampled for egg and larval presence in 1971, but samples did not indicate presence of herring spawning (O’Dell et al. 1975). MTB1 was not sampled during the historical survey, but samples were taken in the mainstem just below and above the confluence of Timothy Branch where MTB1 was located; Herring spawning was not observed at these stations (O’Dell et al. 1975).

Citizen scientists (Long 2017) evaluated conductivity collected at nine sites along the longitudinal axis of Mattawoman Creek from May 2015 through 2016. Baseflow conditions had higher conductivity than a higher flow event (Figure 3-29), suggesting high flows dilute concentrations of ions and lower the specific conductance. Higher conductivity at baseflow is a driven by groundwater infiltrated by salt. Mean conductivity slightly increased with upstream distance until station MC8 (upstream of Waldorf and adjacent to state managed land, Cedarville State Forest; Long 2017). Uphoff et al. (2011) observed increased conductivity in Mattawoman Creek as measurements progress from the head of tide towards Waldorf, MD.

Sodium and chloride were the dominant ions in all samples collected except for MUT4 (Figure 3-30), where calcium and bicarbonate were prevalent. Sodium and chloride exhibited similar responses to road density as conductivity (increasing rapidly as road density increased to 0.02 with little change afterward; Figure 3-31).

Discussion

Our initial focus was to develop conductivity criteria for anadromous fish stream spawning habitat, but we cannot conclude that conductivity directly limits spawning in the absence of other factors that contribute to the cumulative impact of development. However, conductivity indicated degradation of anadromous fish stream spawning habitat and is a useful indicator of potential for stream spawning when data on eggs, larvae, and spawning adults is absent.

Conductivity varied among sites and over seasons in Mattawoman Creek, but increased near the urban center of the watershed (Uphoff et al. 2009). Herring spawning is present at the lowest stream monitoring site on Mattawoman Creek (MC1), in spite of frequent violations of proposed spawning conductivity threshold (Uphoff et al. 2016; see Job 1, Section 1) and benchmark conductivity established by Morgan et al. (2012). Evaluation of long term discharge data showed that flows in 2016 were below average in March and April and above average in May. Depressed flows could be a factor in elevated conductivity as higher conductivity groundwater could contribute salts raising conductivity during low flow conditions. Kaushal et al. (2005) identified ground water as a source of salts to freshwater streams. If this is the case, higher flows could dilute these effects in spring and magnify them during low flow years. While we have not associated increased conductivity as a direct stress to spawning habitat occupation, these fluctuations in conductivity are an indicator of habitat change that can be exacerbated by annual weather conditions and should be evaluated to determine if changing weather patterns related to climate change can decrease spawning habitat suitability.

Herring were not frequently present at MUT3 and MUT4 even though these stations met conductivity criteria more frequently during the Herring spawning season. These tributaries were small and may have been less suitable habitat for Herring spawning. These results suggest that conductivity provides an indication of spawning habitat change (salinization) for Herring spawning, but its impact on spawning may be confounded by other factors that limit spawning habitat use.

Chloride from road salt does not readily biodegrade. It persists and accumulates in groundwater and receiving waters; associated ecological changes can be pervasive and irreparable. Daley et al. (2009) documented increased chloride concentrations in groundwater. Roy and Bickerton (2012) concluded that groundwater contaminants, including chloride from road salts, needed to be evaluated to determine the risk they pose to urban streams. Cooper et al. (2014) suggested that road salt can limit effectiveness of stream restoration by impairing biological processes.

Other habitat impacts that affect fisheries may result from increased conductivity, particularly if the source of salts is related to road deicing. High chloride concentrations can alter aquatic food webs and affect diversity and productivity of aquatic organisms (Betts et al. 2014). Stranko et al. (2013) found that benthic macroinvertebrate community integrity declined with increased conductivity. Losses in macroinvertebrates can alter stream food webs and production, affecting food subsidies of downstream receiving waters (Stranko et al. 2013).

We will continue to monitor conductivity, evaluate its effect on fish habitat quality, and explore the potential to use conductivity as a stressor variable to identify management priorities. Conductivity data can be used as a screening tool for anadromous fish and spawning stream restoration where spawning data is absent or dated, because there is a robust statewide conductivity data set. Where road salt loads are localized in an otherwise suitable spawning stream, we may be able to communicate with other state and local government agencies to see if de-icing can be better managed. Conductivity data can pinpoint urban streams where investments are not likely to improve anadromous fish stream spawning, particularly when stormwater management approaches cannot reduce salt loads (Casey et al. 2013). While this is not a publicly favorable message, it does allow us to communicate realistic expectations, while promoting an understanding of how anthropogenic influences impact ecological services, particularly fish production. This information can be used effectively in an ecosystem based management framework to equip managers and the public to make informed decisions.

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Table 3-4. Analytical parameters, methods, and holding times for water samples collected during 2016.

PARAMETER(S)	METHOD/REFERENCE	MAXIMUM HOLDING TIME
Specific Conductance	Conductivity Meter and Cell/APHA (2005) 2510	7 days at 4°C
Cations (Na, K, Mg, Ca)	Perkin Elmer AAnalyst 800 Atomic Absorption Spectrometer/ APHA (2005) 3111	6 months preserved with nitric acid
Anions (Cl, Br, NO_3-N, SO4)	Dionex DX-500 Ion Chromatograph/APHA (2005) 4110 (B)	14 days at 4°C
Total Alkalinity	Fixed endpoint potentiometric titration/APHA (2005) 2320 (B)	14 days at 4°C
Closed pH	pH meter w/glass electrode/ EPA (1987) Method 19	7 days at 4°C

Table 3-5. Mean, median, minimum, and maximum conductivity from continuous monitoring of the five fixed stations, during July 1, 2015, through December 31, 2016, with 0 values eliminated (n=13,200 for stations MC1 and MTB1, n= 12637 for MPB1, n= 11968 for MUT3 and n=12062 for MUT4)). % < 109 uS/cm and % < 109 uS/cm March-May = percent of observations meeting the benchmark for coastal plain streams for the entire record and for the Herring spawning season (March-May), respectively. % < 174 uS/cm and % < 174 uS/cm March-May = percent of observations meeting spawning habitat threshold during the entire record and during the spawning period for Herring (March-May), respectively. * Represents the lowest value recorded above 0.0 mS/cm. The actual lowest value recorded was 0.0 mS/cm during periods when the site was dewatered.

Station	Road Density	Mean (uS/cm)	Median (uS/cm)	Minimum (uS/cm)	Maximum (uS/cm)	% < 109 uS/cm	% < 109 uS/cm March-May	% < 174 uS/cm	% < 174 uS/cm March-May
MC1	0.019	195.7	171.7	83.6	1063.3	0.64	1.40	52.83	47.15
MUT3	0.010	115.6	110.7	27.6*	798.0	46.00	80.90	97.20	100.00
MUT4	0.007	63.0	69.7	16.6*	361.9	98.10	99.50	99.80	100.00
MPB1	0.044	235.0	181.2	75.5*	1254.5	0.50	0.00	37.90	0.00
MTB1	0.024	317.0	308.4	142.8	2519.0	0.00	0.00	0.20	0.00

Table 3-6. Percentage of observations below the 174 uS/cm proposed spawning threshold (Uphoff et al. 2016); n=744 for March, n=720 for April and n=744 for May.

Station	Road Density	March	April	May
MC1	0.019	37.5	7.5	95.16
MUT3	0.010	100	100	100
MUT4	0.007	100	100	100
MPB1	0.044	0	0	0
MTB1	0.024	0	0	0.13

Figure 3-23. Sampling stations in Mattawoman Creek with road density estimated by catchment delineated above each station.

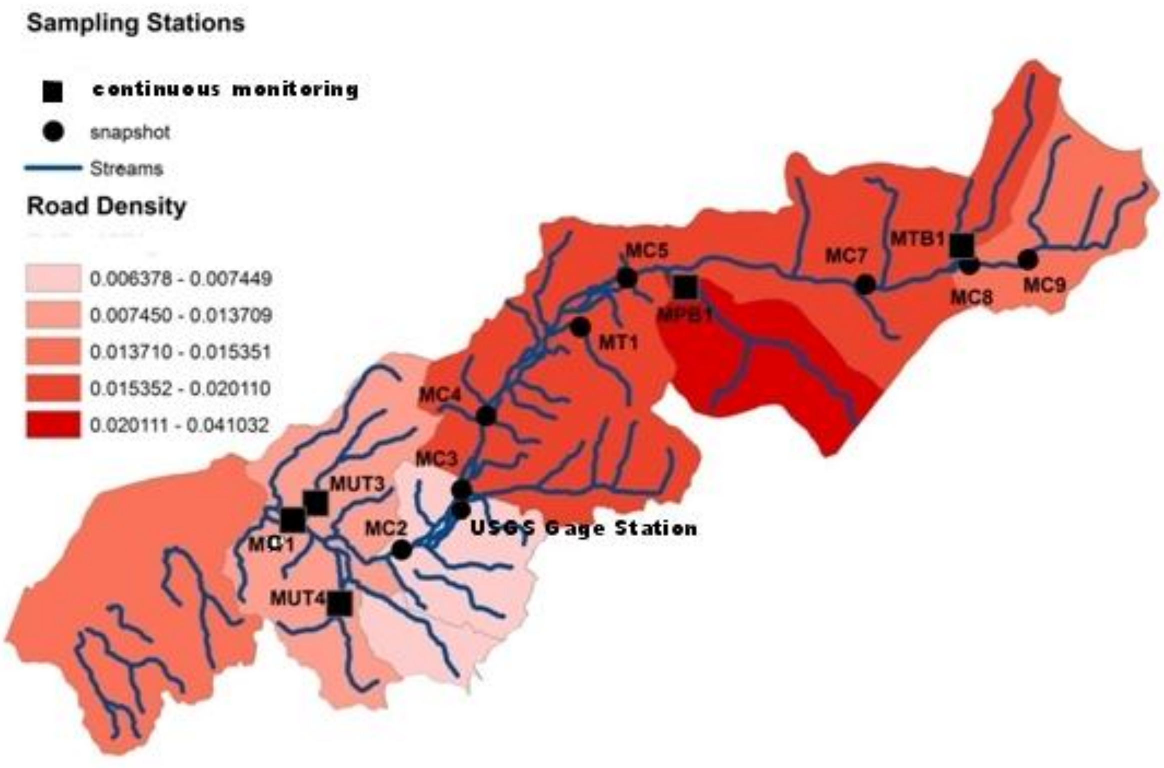


Figure 3-24 Median conductivity (ms/cm) as a function of road density.

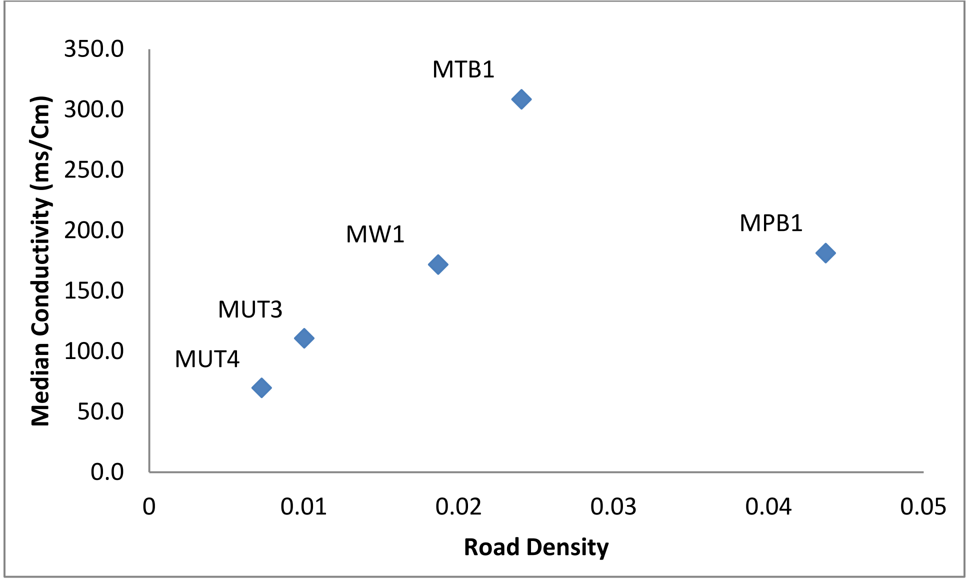


Figure 3-25. Google Earth image of Piney Branch watershed draining to station MPB1. (Source “Piney Branch Watershed” Google Earth 2017).

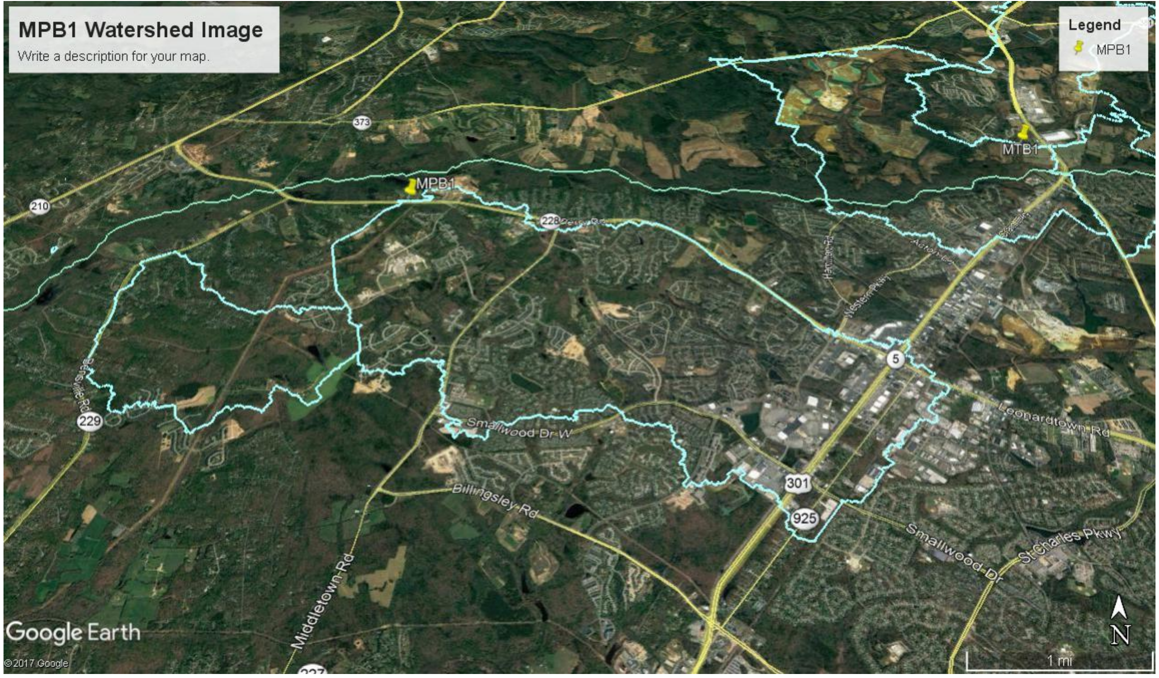


Figure 3-26. Google Earth image of Timothy Branch watershed draining to station MTB1. (Source “Timothy Branch Watershed” Google Earth 2017).

Figure 3-27. Google Earth image of Mattawoman Creek watershed draining to station MC1. (Source “Mattawoman Creek Watershed” Google Earth 2017).

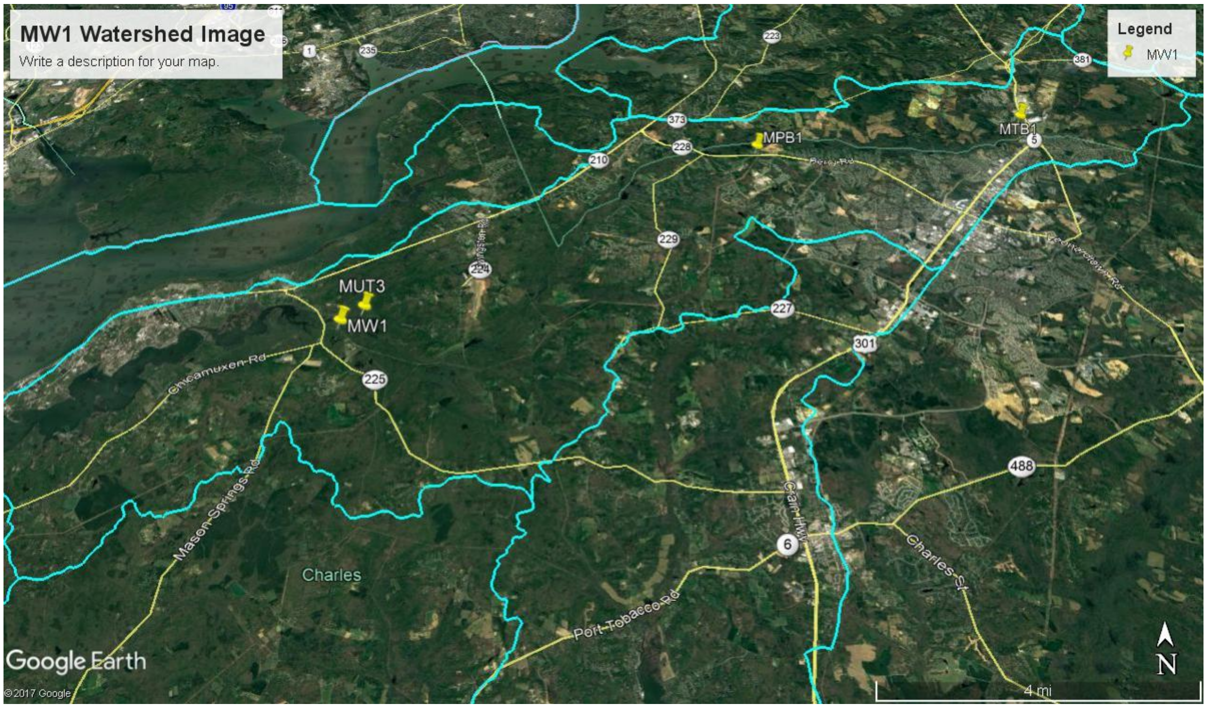


Figure 3-28. Daily discharge (blue line) at the Mattawoman Creeks’ USGS gauge during anadromous fish spawning season, 2016) with monthly average discharge (red line) estimated from long term data records (1949-2017).

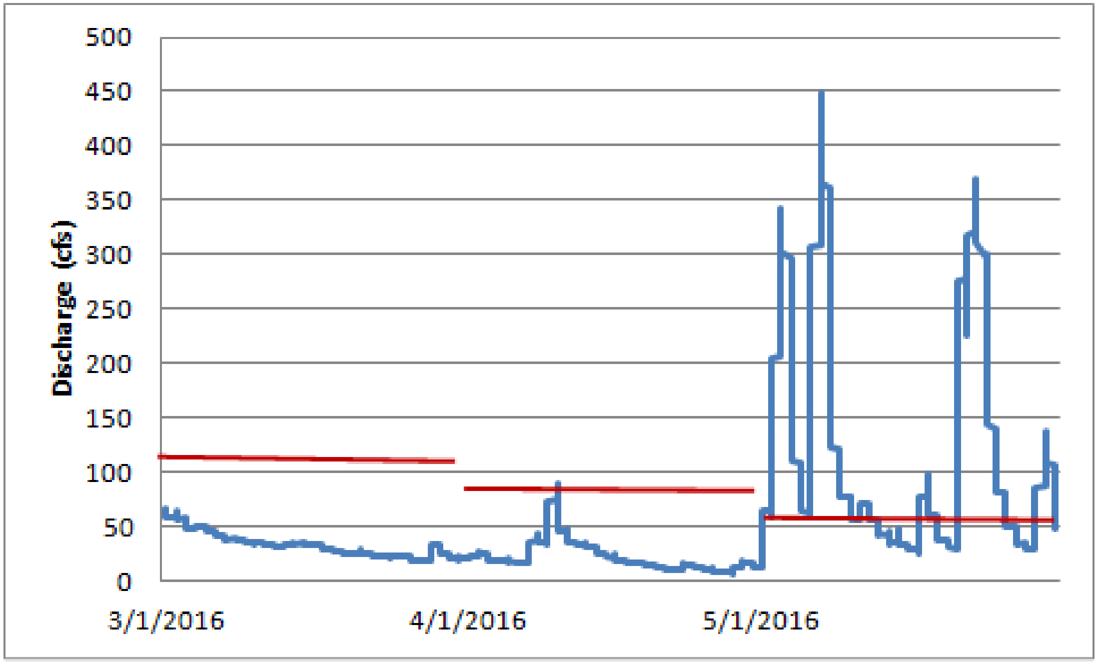


Figure 3-29. Comparing annual mean baseflow conductivity (Baseflow; bars indicate one standard deviation) to conductivity during a high discharge event. The selective baseflow represents conductivity measured at instances where actual baseflow conditions were observed based on gage data. Mean annual conductance was calculated over an entire year of sampling (2015-2016) with values that were associated with dewatering events removed. Selective baseflow conductivity was plotted to verify that means were correctly estimating baseflow conditions. (Adapted from Long 2017).

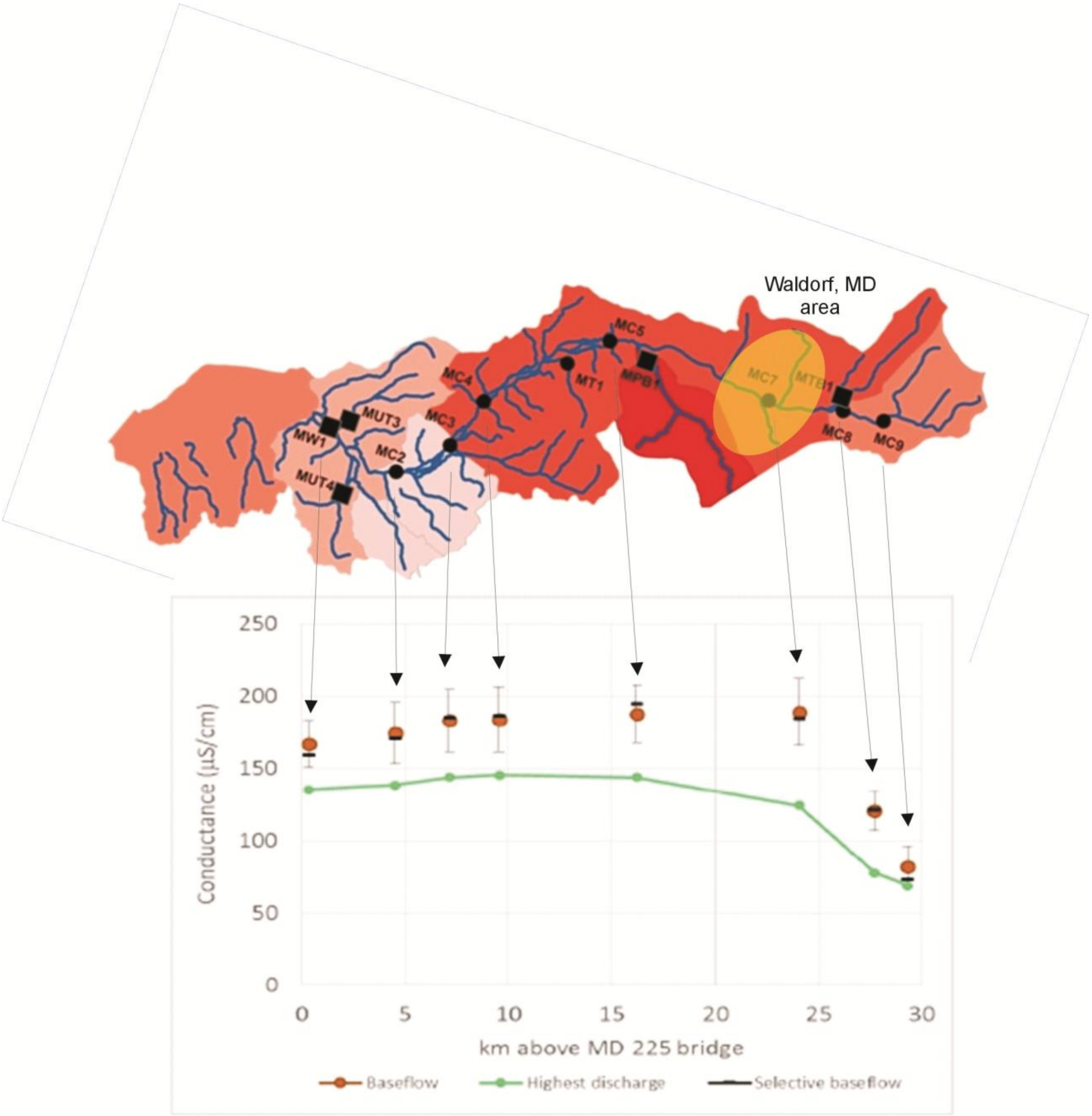


Figure 3-30. Percent contribution of each ion to total conductivity (uS/cm) by station.

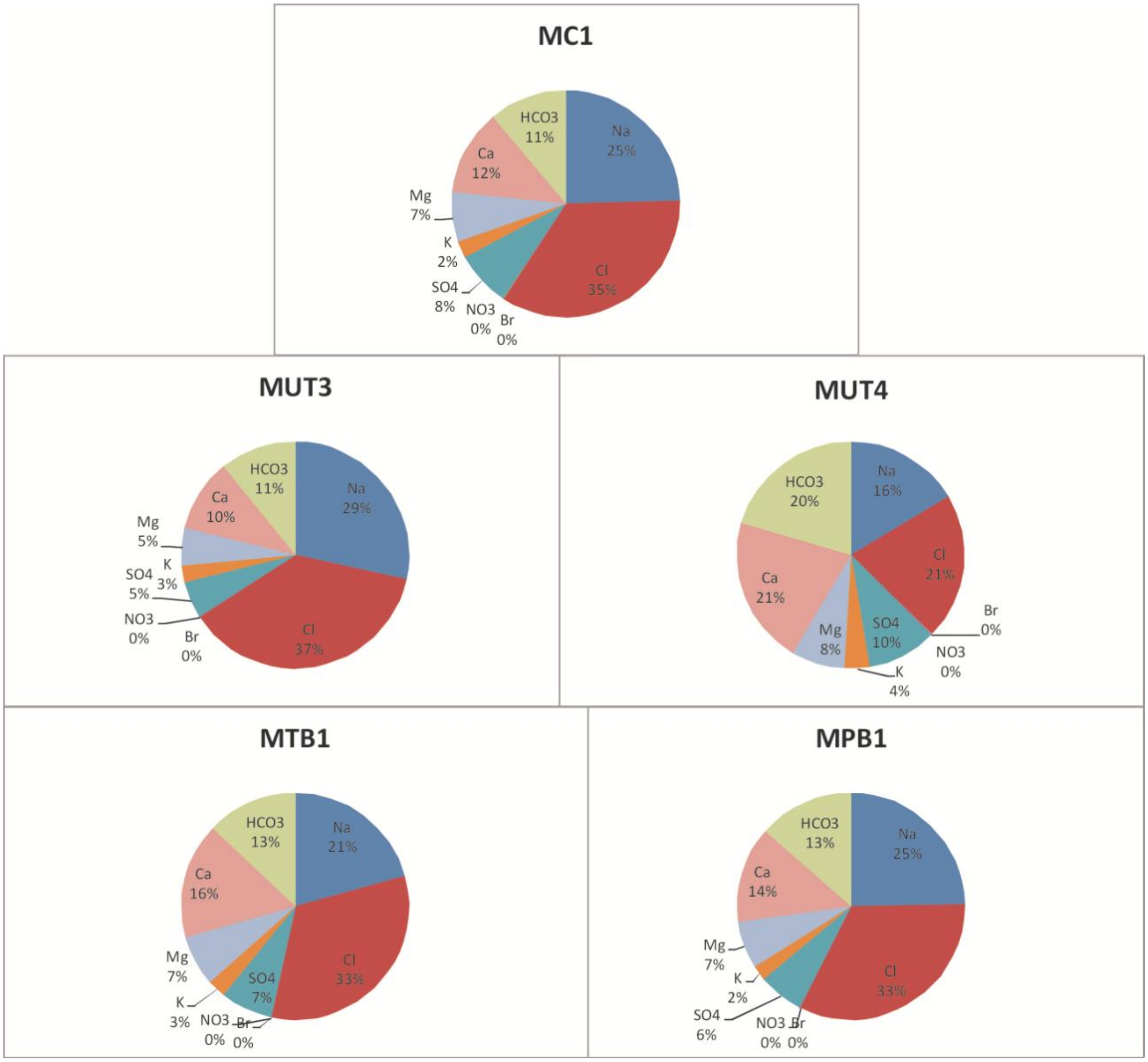
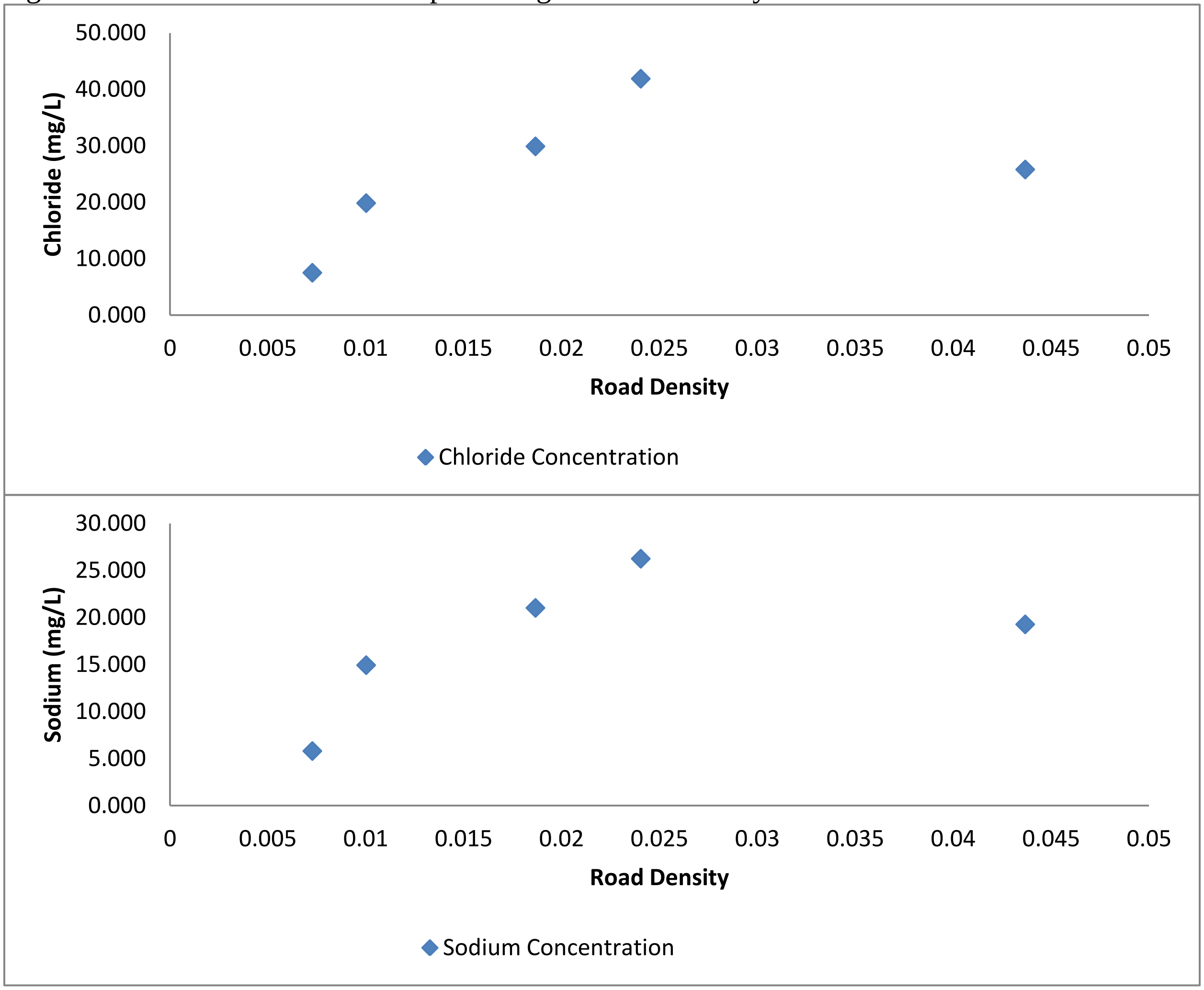


Figure 3-31. Chloride and Sodium plotted against road density.



Job 4: Development of ecosystem-based reference points for recreationally important Chesapeake Bay fishes of special concern: Striped Bass nutrition and forage availability benchmarks

Jim Uphoff, Jim Price (Chesapeake Bay Ecological Foundation), Alexis Park, Carrie Hoover, Alexis Walls

Executive Summary

Maryland's fisheries managers and stakeholders want to know whether there is enough forage to support Striped Bass in Maryland's portion of Chesapeake Bay (upper Bay). Formal assessments of abundance and biomass of Striped Bass and most forage species in upper Bay are lacking due to cost and difficulty in mathematically separating migration effects from mortality. Past efforts to launch ecosystem based fisheries management in Chesapeake Bay have been comprehensive and complex, but have not resulted in integration into management. An index-based (Index of Forage or IF) approach could integrate forage into Maryland's resident Striped Bass management at low complexity and cost. The IF represents a framework for condensing complex ecological information so that it can be communicated simply to decision makers and stakeholders.

Monitoring of Striped Bass health (1998-2016), relative abundance (1983-2016), natural mortality (1987-2016), and forage relative abundance in surveys (1959-2016) and fall diets of Striped Bass (1998-2000 and 2006-2016) provided indicators to assess forage status and Striped Bass well-being in Maryland's portion of Chesapeake Bay. A Striped Bass recreational catch per trip index provided an index of relative abundance (forage demand). Forage-to-Striped Bass ratios (focal species = Atlantic Menhaden, Bay Anchovy, Spot, and Blue Crab) and proportion of Striped Bass in fall with empty guts provided trends in supply relative to demand based on relative abundance and diet sampling, respectively. Proportion of resident Striped Bass without visible body fat and an index of natural mortality based survival were indicators of Striped Bass well-being. Analyses were split into two size classes, sublegal (<457 mm TL) and legal (≥ 457 mm TL). The sublegal class was most sensitive to forage and indicators were mostly based on the smaller size class. Statistical analyses provided evidence that forage and Striped Bass abundance and well-being were inter-related. The proportion of Striped Bass without body fat, anchored our approach, providing a measure of condition and potential for starvation that was well-related to feeding of Striped Bass in the laboratory.

Targets and thresholds were then developed for each of these indicators to assign them scores. A score of 1 indicated threshold conditions; a score of 5 indicated target conditions; and scores of 2-4 indicated grades between. Time-periods where body fat indicators (1998-2016) were at target or threshold conditions provided a time-frame for evaluating other indicators. Annual scores for each variable were averaged for a combined annual IF score.

The IF indicated poor foraging conditions during 1998-2004 and improvement since 2004. Best IF scores occurred during 2008-2010 (~4 and above) and all components reached their targets in 2010. The IF has fallen to between 2 and 3 (near or avoids threshold) in 2015-2016. High variability in component scores was evident as IF improved after 2004 that may have reflected sampling issues, nonlinear relationships among variables, lagged responses, potential insensitivity of some indices, behavioral changes that could increase feeding efficiency, influence of episodes of good foraging conditions outside of those monitored in fall, and combinations of the above.

Introduction

The Chesapeake Bay stock of Striped Bass *Morone saxatilis* supports major commercial and recreational fisheries within Chesapeake Bay and along the Atlantic coast of the United States (Richards and Rago 1999; Maryland Sea Grant 2009). Recovery of Atlantic coast Striped Bass was declared in 1995 after the stock grew seven-fold between1982 and 1994 (Richards and Rago 1999; ASMFC 2016). Concern emerged about the impact of high Striped Bass population size on its prey-base shortly after recovery (Hartman and Margraf 2003; Hartman 2003; Uphoff 2003; Overton et al. 2015). Striped Bass appear capable of limiting prey populations along the Atlantic coast and in its estuaries (Hartman 2003; Uphoff 2003; Savoy and Crecco 2004; Heimbuch 2008; Davis et al. 2012; Overton et al. 2015).

A large contingent of Chesapeake Bay Striped Bass that do not participate in the Atlantic coast migration (mostly males along with some young, immature females; Setzler et al. 1980; Kohlenstein 1981; Dorazio et al. 1994; Secor and Piccoli 2007) constitute a year-round population of predators that provides the upper Bay’s major recreational fishery and an important commercial fishery (Maryland Sea Grant 2009). Reports of Striped Bass in poor condition and with ulcerative lesions increased in Chesapeake Bay shortly after recovery was declared (Overton et al. 2003; Uphoff 2003; Gauthier et al. 2008; Overton et al. 2015). These reports spurred concerns about the effect of low forage fish abundance on Striped Bass health (Uphoff 2003; Overton et al. 2015). Linkage between these phenomena and poor feeding success on Atlantic Menhaden and other prey in upper Bay was considered plausible (Uphoff 2003; Overton et al. 2015). Mycobacteriosis, a chronic wasting disease, became an epizootic in Chesapeake Bay (Maryland and Virginia) in the late 1990s and was concurrent with lesions and poor condition (Overton et al. 2003; Jiang et al. 2007; Gauthier et al. 2008; Jacobs et al. 2009b). Challenge studies with Striped Bass linked nutrition with progression and severity of the disease, and survival (Jacobs et al. 2009a). Tagging models indicated that annual instantaneous natural mortality rate (M) of Striped Bass in Chesapeake Bay increased substantially during the mid-1990s while fishing mortality remained low (Jiang et al. 2007; ASMFC 2013).

In addition to top-down pressure on forage, a long-term decline in major pelagic prey, Bay Anchovy *Anchoa mitchilli* and Atlantic Menhaden *Brevoortia tyrannus* in Maryland’s portion of Chesapeake Bay (hereafter, upper Bay) since 1993. The decline in Bay Anchovy may be linked to declining abundance of the common calanoid copepod *Acartia tonsa* that, in turn, was linked to rising long-term water temperatures and eutrophication (Kimmel et al. 2012). Age-0 Atlantic Menhaden abundance *Brevoortia tyrannus* in upper Bay underwent a decline that was concurrent with that of Bay Anchovy. Houde et al. (2016) found primary production,Chl a, and variables associated with freshwater flow, e.g. Secchi disk depth and zooplankton assemblages, were correlated with age-0 Menhaden abundance in the upper Bay.

Maintaining a stable predator-prey base is a challenge for managing Striped Bass in lakes (Axon and Whitehurst 1985; Sutton et al. 2012; Wilson et al. 2012). In lakes, Striped Bass exhibit high fidelity for clupeids and reduced growth and poor condition are a common problem when supply decreases (Axon and Whitehurst 1985; Matthews et al. 1988; Cyterski and Ney 2005; Raborn et al. 2007).

Uphoff et al. (2016) described a fall in upper Bay Striped bass survival that was consistent with a compensatory response to high Striped Bass abundance, low forage, and poor condition. Management of Chesapeake Bay Striped Bass fisheries attempts to balance a trade-off of yield with escapement of females to the coastal migration by controlling fishing mortality, and compensatory mortality would undercut both objectives. Increased M over time may have serious implications for Striped Bass management since Chesapeake Bay is the main contributor to Atlantic coast fisheries (Richards and Rago 1999; ASMFC 2013). The degree that M compensates with fishing mortality (F) may reduce effectiveness of management measures since total mortality (Z) may not be reduced by harvest restrictions when M increases as F decreases (Hilborn and Walters 1992; Hansen et al. 2011; Johnson et al. 2014). Single species stock assessments typically assume that M is constant and additive with F to keep calculations tractable (Hilborn and Walters 1992). Animal populations may exhibit additive mortality at low abundance and compensatory mortality at high abundance or compensatory mortality that changes continuously with density (Hansen et al. 2011).

Maryland's fisheries managers and stakeholders want to know whether there is enough forage to support Striped Bass in upper Bay. Formal assessments of abundance and biomass of Striped Bass and most forage species in upper Bay are lacking due to cost and difficulty in mathematically separating migration from mortality.

The Atlantic States Marine Fisheries Commission (ASMFC) is moving to develop reference points for Atlantic Menhaden's forage role along the Atlantic coast and Striped Bass is a predator of concern (SEDAR 2015). Prevalence of mycobacteriosis and natural mortality appear to be less outside Chesapeake Bay (Matsche et al. 2010; ASMFC 2013), leaving open the possibility that a forage issue in the upper Bay would be diluted in a coastal approach. In 2014, a forage fish outcome was included in the Chesapeake Bay Agreement (Chesapeake Bay Program): "By 2016, develop a strategy for assessing the forage fish base available as food for predatory species in the Chesapeake Bay." Resident Striped Bass offered an immediate opportunity to develop an indicator-based assessment approach based on existing monitoring.

Indicators based on monitoring, such as forage indices, prey-predator ratios, Striped Bass condition indices, and prey abundance in diet samples have been suggested as a basis for assessment (Maryland Sea Grant 2009; SEDAR 2015) and formed the foundation of our approach. Indicators are widely used for environmental reporting, research, and management support (Rice 2003; Jennings 2005; Fogarty 2014).

Uphoff et al. (2014) devised five annual forage indicators for resident Striped Bass in Maryland's portion of Chesapeake Bay. A Striped Bass recreational catch per trip index provided an index of relative abundance (demand). A forage-to-Striped Bass ratio (focal species combined) and grams of forage consumed per gram of Striped Bass (replaced by proportion of Striped Bass in fall with empty guts; Uphoff et al. 2016) provided trends in supply relative to demand based on relative abundance and diet sampling, respectively. Proportion of resident Striped Bass without visible body fat and an index of natural mortality based survival were indicators of Striped Bass well-being. Statistical analyses provided evidence that forage and Striped Bass abundance and well-being were inter-related (Uphoff et al. 2013; 2014; 2015). Targets and thresholds were then developed for each of these indicators to assign them scores. A score of 1 indicated threshold conditions; a score of 5 indicated target conditions; and scores of 2-4 indicate grades between (Uphoff et al. 2014). This report provides a complete set of indicators through 2016. Some indicators were revised and all were summarized into a single score to serve as a quick reference for managers and the public.

A nutritional indicator, proportion of Striped Bass without body fat, anchored our approach, providing a measure of condition and potential for starvation that was well-related to feeding of Striped Bass in the laboratory (Jacobs et al. 2013). Lipids are the source of metabolic energy for growth, reproduction, and swimming for fish and relate strongly to foraging success, subsequent fish health, and survival of individual fish and fish populations (Tocher 2003; Jacobs et al. 2013).

While upper Bay Striped Bass feed on a wide range of prey, Atlantic Menhaden, Bay Anchovy, Spot *Leiostomus xanthurus*, and Blue Crab *Callinectes sapidus* have consistently accounted for most annual diet biomass in Chesapeake Bay studies (Hartman and Brandt 1995b; Griffin and Margraf 2003; Walter et al. 2003; Overton et al. 2009; Overton et al. 2015). We selected these species as focal prey for forage indices. Indices of focal prey availability were estimated from fishery-independent surveys and fall diets of Striped Bass. Trends in prey index-to-Striped Bass index ratios were examined for each focal prey since forage indices alone would not consider the possibility of predator interference or the vulnerability exchange process of foraging arena theory (Ginzburg and Akçakaya 1992; Yodzis 1994; Ulltang 1996; Uphoff 2003; Walters and Martell 2004; Walters et al. 2016).

The ratio of age-3 relative abundance of male Striped Bass in spring gill net surveys (Versak 2015) to their year-class-specific juvenile indices (Durell and Weedon 2017) were used as indicators of change in survival due to natural mortality (SR) prior to recruitment to the fishery (Uphoff et al. 2015; 2016). Confining the gill net relative abundance indices to 3 year-old males makes it likely that trends in SR will reflect resident Striped Bass survival before harvest (i.e., natural mortality). Age-3 male Striped Bass in the spring gill net survey were nearly always well below legal-size in years when harvest was allowed (Versak 2015), but they could be subject to catch-and-release mortality. We expected SR to vary without trend if M remained constant. Unfortunately, it became apparent that SR estimates used in Uphoff et al. (2015) were biased because age-3 gill net indices were not reflecting expected trends in abundance of age-3 fish indicated by the stock assessment, juvenile indices, and other indicators. Uphoff et al. (2016) developed adjusted gill net indices that reflected expected stock changes and used these as the numerator in the SR estimates. We have revisited the approach in Uphoff et al. (2016) in this report.

Statistical analyses can provide insight into important processes related to predation (Whipple et al. 2000). We used correlation and regression analyses to examine whether indicators of upper Bay Striped Bass abundance, forage abundance, consumption, and relative survival estimates were linked to the body fat condition indicator.

The IF approach was based on a suite of statistically linked indicators. Status would be judged by whether target or threshold reference points were met for each indicator. Time-periods where body fat indicators (1998-2016) were available provided a time-frame for developing targets and thresholds for other indicators. Targets and limits based on historical performance are desirable because they are based on experience and easily understood (Hilborn and Stokes 2010).

Methods

Definitions of abbreviations can be found in Table 4-1.

Nutritional status (condition) for upper Bay Striped Bass was estimated as the proportion of fish without visible body fat during October-November (P0; Jacobs et al. 2013). Body fat data were collected by the Fish and Wildlife Health Program (FWHP) as part of comprehensive Striped Bass health monitoring in upper Bay initiated after an outbreak of lesions began in the late 1990s. Fish were collected by hook-and-line from varying locations during fall, 1998-2016, between Baltimore, Maryland (northern boundary), and the Maryland-Virginia state line (southern boundary; Figure 4- 1).

Estimates of P0 were made for two size classes of Striped Bass separately and combined: Striped Bass less than 457 mm total length (or TL; hereafter, sublegal sized Striped Bass or fish) and fish 457 mm TL or larger (hereafter, legal sized Striped Bass or fish). The sublegal and legal designations maintain nomenclature used in previous reports, but does not reflect current length limits (the length limit was raised to 508 mm TL in 2015). Standard deviations and confidence intervals (95% CI) of P0 were estimated using the normal distribution approximation of the binomial distribution (Ott 1977).

As Striped Bass experience starvation, lipids are replaced by water, conserving weight loss and hampering the interpretation of weight at length condition indices (Jacobs et al. 2013). Jacobs et al. (2013) presented a target for body moisture (25% or less of fish with starved status) as a surrogate for lipid content estimated from proximate composition of well-fed Striped Bass. This target was derived from fall 1990 field collections by Karahadian et al. (1995) - the only field samples available from favorable feeding conditions (high forage to Striped Bass ratios; Uphoff et al. 2016). A target for visible body fat was not presented in Jacobs et al. (2013) because the index was not applied in the 1990 collection. However, mean tissue lipid of Striped Bass without visible body fat was reported to be identical to that estimated from percent moisture in the remainder of the data set, meaning that P0 related strongly to the proportion exceeding the moisture criteria (Jacobs et al. 2013). A level of P0 of 0.30 or less was used to judge whether Striped Bass had fed successfully during October-November. Variation of tissue lipids estimated from body fat indices was greater than for moisture and the P0 target accounted for this additional variation plus a buffer for misjudging status (J. Jacobs, NOAA, personal communication). Jacobs et al. (2013) stressed that comparisons of Striped Bass body fat to a nutritional target or threshold in Chesapeake Bay should be based on October-November data since they were developed from samples during that time span. Uphoff et al. (2014) estimated the P0 threshold as 0.68 (average of the lower 95% CI of high Pf0 estimates during 1998-2004).

Uphoff et al. (2016) examined bivariate plots of proportion with empty guts (PE) or grams of forage consumed per gram of Striped Bass (C) with P0 and found potential for previous feeding history and condition to influence P0 change in fall. We examined the relationships of P0 in fall of year t with P0 in the fall of t-1. We also estimated P0 for late spring-summer samples (May-September) taken by the FWHP during 1999-2012 and tried linear and nonlinear asymptotic functions (logistic and Weibull functions) to describe relationships of P0 in summer of year t with P0 in fall of year t and the relationship of P0 in fall of year t with P0 in summer of year t+1. These analyses would provide insight on lagged responses of condition.

We used geometric mean catches from fixed station seine and trawl surveys as indicators of relative abundance of most major prey species in upper Bay. A shoreline seine survey targeting age-0 Striped Bass during 1959-2016 provided indices for Atlantic Menhaden, Bay Anchovy, and Spot *Leiostomus xanthurus* (Goodyear 1985; Richards and Rago 1999; Kimmel et al. 2012; Durell and Weedon 2017; Houde et al. 2016). Additional indices for Spot and Bay Anchovy were estimated from a Blue Crab trawl survey conducted during 1989-2016 (Uphoff 1998; Rickabaugh and Messer 2015; MD DNR 2017a; estimates for 2016 were provided by H. Rickabaugh, MD DNR, personal communication). These surveys sampled major and minor tributaries, sounds adjacent to the mainstem upper Bay, but not the mainstem itself (Figure 4- 1). Sampling occurred during summer through early fall. Density of juvenile Blue Crabs *Callinectes sapidus* in a stratified random winter dredge survey (1989-2016) that sampled Chesapeake Bay-wide (Maryland and Virginia) was our indicator of Blue Crab relative abundance (Sharov et al. 2003; Jensen et al. 2005; MD DNR 2017b). Spot and Blue Crabs were classified as benthic forage, while Atlantic Menhaden and Bay Anchovy were pelagic (Hartman and Brandt 1995b; Overton et al. 2009). Correlation analyses explored associations of forage indices and P0. Each forage index was divided by its mean for years in common among all surveys (1989-2016) to place them on the same scale.

Indicators of feeding success and diet composition during October-November were developed using data from a citizen-science based Striped Bass diet monitoring program conducted by Chesapeake Bay Ecological Foundation (CBEF) during 2006-2015. During 2014-2016, Striped Bass collected as fish health samples by the Fish and Wildlife Health Program (FWHP) were processed by Fish Habitat and Ecosystem Program personnel for diet information. Methods for CBEF and FWHP collections have been described in Uphoff et al. (2014; 2015; 2016) and will be briefly repeated here.

Striped Bass diet collections by CBEF and FWHP were made in a portion of upper Bay bounded by the William Preston Lane Bay Bridge to the north, the mouth of Patuxent River to the south, and into the lower Choptank River (Figure 4- 1). Striped Bass were collected for diet samples by hook and line fishing.

Conditions of the collectors permit issued to CBEF allowed for samples of up to 15 Striped Bass less than 457 mm total length (or TL; hereafter, sublegal sized Striped Bass or fish) and 15 fish 457 mm TL or larger (hereafter, legal sized Striped Bass or fish) per trip during 2006-2014. Most active trips by CBEF occurred in Choptank River, but some occurred in the mainstem Chesapeake Bay. These trips were our source of sublegal sized fish, but legal sized fish were caught as well. Striped Bass kept as samples during active trips were placed in a cooler and either processed immediately or held on ice for processing the next day. Legal sized Striped Bass collections were supplemented by charter boat hook and line catches sampled at a fish cleaning business by CBEF. These fish were predominately from the mainstem Chesapeake Bay. These fish were iced immediately and cleaned at the station upon return to port. Fish, minus fillets, were held on ice over one to several days by the proprietor of the fish cleaning service and processed at the check station.

Diet collections by FWHP during 2014 were not constrained by collectors permit conditions like CBEF collections. Sampling by FWHP was designed to fill size class categories corresponding to age-classes in an age-length key to assess Striped Bass health. Some trips occurred where fish in filled out length classes were discarded (typically sublegal sized fish). Samples were usually obtained by fishing on a charter boat using the techniques considered most effective by the captain (bait or artificial lures). During fall, 2015, Striped Bass were obtained on nine trips made in Choptank River and mainstem middle and lower Chesapeake Bay (between Eastern Bay and mouth of Patuxent River). Feeding data for fall 2016 were available, but the data set did not contain location and date information, but the same general region described for 2015 was sampled. Location data will be available once FWHP completes their analysis and feeding data will be merged with it.

Total length of each Striped Bass was recorded and whole fish were weighed on a calibrated scale for CBEF and FWHP samples. Striped Bass length-weight regressions based on that year's October-November samples were used to estimate missing weights from filleted fish in CBEF collections. Diet items of each fish were identified to the lowest taxonomic group. Contents were classified as whole or partially intact. In CBEF collections, total length of intact fish and shrimp, carapace width of crabs, and shell length of intact bivalves were measured. Non-linear allometry equations for converting diet item length to weight (Hartman and Brandt 1995a) were used. In a few cases, equations for a similar species were substituted when an equation was not available. These equations were used to reconstruct diets for Overton et al. (2009) and Griffin and Margraf (2003), and were originally developed and used by Hartman and Brandt (1995a). Soft, easily digested small items such as amphipods or polychaetes that could not be weighed were recorded as present. Empirical relationships developed by Stobberup et al. (2009) were used to estimate relative weight from frequency of occurrence of their general taxonomic category. These soft items were not common in our fall collections, but were more common during other seasons (J. Uphoff, personal observation).

Striped Bass diets were analyzed separately for sublegal and legal sized fish. These categories accounted for ontogenic changes in Striped Bass diet, but also reflected unbalanced sample availability to CBEF (sublegal fish could only be collected by fishing for them directly, while legal sized fish were supplemented by cleaning station samples). The lower limit of fish analyzed in the sublegal category, 286 mm, was the minimum length in common among years during 2006-2013. An upper limit of 864 mm avoided inclusion of large, migratory Striped Bass that reentered upper Bay in late fall.

We confined analysis of food items to those considered recently consumed in an attempt to keep odds of detection as even as possible. Items with "flesh", including whole or partial fish and invertebrates, and intact crab carapaces were considered recently consumed. Hard, indigestible parts such as gizzards, mollusk shells, and backbones were excluded. Partially intact items with flesh were identified to lowest taxonomic group and assigned the mean weight estimated for intact items in the same group. Bait was excluded.

Percentage of food represented by an item (excluding bait) during 2006-2016 was estimated for each Striped Bass size class in numbers and weight based on fish with stomach contents (Pope et al. 2001). Two feeding metrics were calculated for each size class for each year. Relative availability of prey biomass (C) was estimated by dividing the sum of diet item weights by the sum of weight of all Striped Bass sampled (including those with empty stomachs; Pope et al. 2001). Estimates of C were subdivided by contribution of each major prey to overall diet mass (species-specific C). Proportion of Striped Bass with empty stomachs (PE) was also estimated as an indicator of total prey availability (Chipps and Garvey 2007). Standard deviations and confidence intervals (95% CI) of PE were estimated using the normal distribution approximation of the binomial distribution (Ott 1977).

An fishery-independent index of relative abundance of upper Bay resident Striped Bass was not available; therefore, we developed a catch-per-private boat trip index (released and harvested fish) for 1981-2016 from the National Marine Fisheries Service's (NMFS) Marine Recreational Information Program (MRIP; NMFS Fisheries Statistics Division 2017) database. Similar recreational catch per trip indices have been used as abundance indicators in Atlantic coast stock assessments of major pelagic finfish predators: Striped Bass, Bluefish *Pomatomus saltatrix*, and Weakfish *Cynoscion regalis* (ASMFC 2009; NEFSC 2012; ASMFC 2013).

This index was estimated as a catch-effort ratio for private and rental boat anglers in Maryland in the MRIP inland fishing area (inshore saltwater and brackish water bodies such as bays, estuaries, sounds, etc, excluding inland freshwater areas; NMFS Fisheries Statistics Division 2017). This Striped Bass recreational fishing index (RI) equaled September-October recreational private and rental boat catch divided by estimates of trips for the private and rental boat sector. Recreational survey estimates are made in two month waves and September-October constituted the fifth wave (NMFS Fisheries Statistics Division 2017). The September-October wave was chosen because portions or the whole wave were continuously open for fishing for legal sized Striped Bass following the 1985-1990 moratorium, making it less impacted by regulatory measures than other waves that opened later. Recreational fishing by boat occurs over the entire portion of the upper Bay and this index would be as close to a global survey of non-migratory upper Bay Striped Bass as could be obtained. Migratory fish were unlikely to have been present during this wave. The RI was related to juvenile indices 2-5 years earlier (determined by multiple regression) and to Atlantic coast abundance estimates (Uphoff et al. 2014). Confidence intervals for RI were estimated using a spreadsheet developed by Desmond Kahn and Mary Chrisman based on Goodman's (1960) formula for the variance of two random variables. This method is explained in Appendix 1.

The relationship of upper Bay resident Striped Bass relative abundance (RI) to condition (P0) was examined using linear regression. Examination of the plot of P0 and RI suggested that an asymptotic relationship might be possible, so a reciprocal transformation (1 / RI; Sokal and Rohlf 1969) was used to linearize data. Therefore, two models were used: (1) P0 = RI and (2) P0 = 1 / RI.

We used forage indices divided by RI (forage index-to-Striped Bass index ratios) as indicators of forage supply relative to Striped Bass demand (relative attack success). Ratios were standardized by dividing each year's estimate by the mean of ratios during 1989-2016, a time-period in common among all data. The ratios covered 1983-2016. Correlation analysis of the ratios for each of the major species and P0 provided an indication of how strongly associated each ratio was with condition. Standardized ratios were, in turn, averaged to generate a single trend in major forage-to-Striped Bass ratios (or major forage ratios). Targets and limits for major forage ratios were drawn from periods of three or more years where forage ratios coincided with target or limit Pf0, respectively. A target for major forage ratios was estimated as the lowest average of the standardized ratio that coincided with Pf0 meeting its target. The limit was estimated as the highest major forage ratio during the Pf0 threshold period.

We estimated relative survival for age-3 Striped Bass in upper Bay as relative abundance at age-3 divided by age-0 relative abundance three years prior (juvenile index in y-3). Versak (2015) estimated age-specific indices of Striped Bass relative abundance from spawning season gill net surveys on the Potomac River and Head-of-Bay spawning areas (~39% and 47%, respectively, of Maryland's total spawning area; Hollis 1967) and we used the age-3 index for the numerator. An update through 2016 was provided (B. Versak, MD DNR, personal communication). To combine regional estimates, we first standardized each area's time series of gill net catch per unit effort to its time-series mean (1985-2016). These standardized estimates were then averaged for each year (Potomac River was not sampled in 1994) and this average of standardized CPUE was divided by its respective JI three years prior to estimate SR.

Gill net indices used in the numerator of SR in Uphoff et al. (2015) were suggesting either no change in abundance since 1985 or a decrease; Uphoff et al. (2016) considered both implausible when viewed against stock assessment estimates, juvenile indices, and harvest trends. Uphoff et al. (2016) determined that gill net survey catchability (q; estimated by dividing the index by the stock assessment abundance estimate) of 3 year-old male Striped Bass changed as an inverse nonlinear function of population size. A multiple regression approach was used to create an adjusted gill net index. The spring gill net time-series was split into two categories, one indicating catchability at low abundance (1985-1995; high catchability category = 0) and the other at high abundance (1996-2014; low catchability category = 1), and used in the multiple regression model:

$$\text{(4) Gill net index} = \text{catchability category} \cdot \text{juvenile index};$$

where juvenile index was the geometric mean juvenile indices from three years prior. Both model terms were significant. An adjusted gill net index time-series was estimated by setting category equal to one for the entire time-series and the regression equation predicted a gill net time-series for 1985-1995. This adjusted gill net index strongly mimicked the trend in year-class success indicated by juvenile indices three years prior. However, we felt it would be better to rely on actual indices for as much of the time-series as possible. For this report, we created a "hybrid" time-series that used adjusted indices for 1985-1995 and the original estimates from Versak et al. (2015) plus the estimates through 2016 (B. Versak, MD DNR, personal communication) afterwards.

Relative survival (SR) in year t was estimated as the hybrid gill net index for age-3 in year t (HI_t) divided by its respective juvenile index three years earlier (JI_{t-3});

$$SR_t = HI_t / JI_{t-3}.$$

We considered two approaches for developing target and threshold SR values: one used means of SR for time periods of high or low stable SR (target or limit, respectively) or one used medians. We adopted the approach using medians to estimate SR targets and limits. Means would be more susceptible to influence of anomalous values resulting from changes in catchability from either survey than the median.

Trends in SR were compared to P0 using regression analysis. Relative survival was estimated in spring, while P0 was estimated in fall, so we used SR estimates in the following year in these analyses.

Tag-based estimates of survival for legal-size (457-711 mm) Striped Bass from Chesapeake Bay in ASMFC stock assessment (ASMFC 2013) were compared to SR. Tag-based estimates of M were determined for two time periods in the ASMFC (2013) stock assessment (early period = 1987-1996 and late period = 1997-2011) and we converted the estimates of M in ASMFC (2013) to survival (S) using the equation $S = e^{-M}$ (Ricker 1975). The relative differences survival (early period estimate / later period estimate) were compared for the two approaches / size classes.

There was some variation in which size classes were used for indicators. All size classes of Striped Bass were used to estimate P0 since Uphoff et al. (2016) did not detect meaningful differences in trend among size-specific estimates. While size classes could not be specified for RI, Uphoff et al. (2014) found that Maryland Striped Bass juvenile indices for ages 2-5 (corresponding to both size classes) predicted trends in the RI. Forage to Striped Bass ratios would reflect availability to both size classes since RI was used in the denominator. Sublegal classes of Striped Bass had a more varied diet than legal sized fish (their fall diet was dominated by Atlantic Menhaden) and PE of the sublegal size class was used as an indicator of forage availability. Estimates of SR reflected survival of sublegal sized fish.

During 1998-2016, each indicator was assigned an annual score from 1 to 5 (bad to good); a score of 1 indicated bad or threshold conditions; a score of 5 indicated good or target conditions; and scores of 2-4 indicated grades of status in between. Scores between 2 and 4 were assigned by breaking the interval between a target and limit into 3 equal increments: a score of 3 represented the mid-increment (avoids the threshold); 2 represented the increment between the threshold and midpoint (approaching the threshold); and 4 indicated the increment between the midpoint and threshold (approaching the target).

Annual scores for each variable were averaged for a combined annual IF score. An average was necessary since five years were unavailable for the PE time-series. Two graphical depictions of uncertainty were developed for the IF. One presented the mean trend as a line and the scores for the individual components as points. This approach presented full variation of the component scores. The other used a “leave one out” approach where annual means were estimated by leaving one component out (i.e., a mean without P0, a mean without PE, etc.). Each set of means was compared to the overall mean and depicted variation in the means.

Scatter plots were examined for the need for data transformations and to identify candidate models. Residuals of regressions were inspected for outliers, trends, and non-normality. If a large outlier was identified, the data from that year was removed and the analysis was rerun. Levels of significance of correlations were not adjusted for multiple comparisons as there is no formal consensus as to when these adjustment procedures should be applied (Nakagawa 2004). A general description of equations used follows, while more specific applications will be described in later sections.

Linear regressions described continuous change in variable Y as X changed:

$$Y = (m \cdot X) + b;$$

where m is the slope and b is the Y-intercept (Freund and Littel 2006).

Potential dome-shaped relationships were examined with quadratic models (Freund and Littell 2006):

$$Y = (m \cdot X) + (n \cdot X^2) + b.$$

The linear regression function in Excel or Proc REG in SAS (Freund and Littel 2006) was used for single variable linear regressions. Multiple linear and quadratic regressions were analyzed with Proc REG in SAS (Freund and Littell 2006).

Examination of scatter plots suggested that some relationships could be nonlinear, with the Y-axis variable increasing at a decreasing rate with the X-axis variable and we fit power, logistic growth, or Weibull functions to these data using Proc NLIN in SAS (Gauss-Newton algorithm). The power function described a relationship with a perceptible, but declining increase in Y with X by the equation:

$$Y = a \bullet (X)^b;$$

where a is a scaling coefficient and b is a shape parameter. The symmetric logistic growth function described growth to an asymptote through the equation:

$$Y = b / ((1 + ((b – c) / c) \bullet (\exp (-a \bullet X)));$$

where a is the growth rate of Y with X, b is maximum Y, and c is Y at X = 0 (Prager et al. 1989).

The Weibull function is a sigmoid curve that provides a depiction of asymmetric ecological relationships (Pielou 1981). A Weibull curve described the increase in Y as an asymmetric, ascending, asymptotic function of X:

$$Y = K\{1 - \exp [-(Y / S)^b]\};$$

where K was the asymptotic value of Y as X approached infinity; S was a scale factor equal to the value of Y where Y = 0.63 • K; and b was a shape factor (Pielou 1981; Prager et al. 1989).

Confidence intervals (typically 95% CIs) of the model parameters for each indicator species were estimated to examine whether parameters were different from 0 (Freund and Littel 2006). If parameter estimates were not different from 0, the model was rejected.

Results

Striped Bass in the upper Bay during fall 1998-2016 were usually in poor condition (P0 $\geq$ threshold; threshold = 0.68) during 1998-2004 and at or near the target level of condition (P0 $\leq$ target; target = 0.30) during 2008-2010 and 2014-2015 (Table 4-2; Figure 4- 2). The 95% confidence intervals of P0 allowed for separation of years meeting the target condition from remaining estimates (Figure 4- 2). A IF score of 1 was assigned to P0 at or more than 0.68; a score of 2 was assigned for P0 between 0.68 and 0.55; a score of 3 was assigned to P0 between 0.55 and 0.43; a score of 4 was assigned to P0 between 0.43 and 0.30; and a score of 5 was assigned when P0 was 0.30 or less.

A combined P0 index for all sizes of Striped Bass was adopted in Uphoff et al. (2016) based on 1998-2014 data; however, in 2016 a pronounced difference in condition was evident between sublegal (sublegal P0 = 0.83) and legal sized fish (P0 = 0.25; Figure 4- 3).

Body fat data representing summer were collected during May-September, 1999-2012 by the FWHP (Table 4-3). Data were not collected during all months in all years. Sample sizes were less than 50 in 1999, 2001, and 2003, but estimates of summer P0 were sufficiently precise that their 95% CI’s did not overlap zero (Table 4-3).

Estimates of P0 in fall of year t were influenced by indices up to a year earlier, but not two years earlier (Table 4-4). The equations describing the influence of the P0 index in the fall of year t with indices in the previous summer and fall (year t-1) were linear and had similar slopes and intercepts; they explained a moderate amount of variation (r^2 = 0.34 and 0.43, respectively). A Weibull function best described the relationship of P0 in summer of year t with P0 in year t and explained 75% of the variation (Table 4-4; Figure 4- 4). The asymptote of this relationship (0.77) was greater than the fall P0 threshold (0.68). Predicted fall P0 improved over summer P0 when summer P0 was 0.30 or less and afterwards became progressively poorer than summer P0 until the asymptote was reached (Figure 4- 4).

Bay Anchovy seine indices (1959-2016) following the early to mid-1990s were typically at or below the bottom quartile of indices during 1959-1993. Highest Bay Anchovy trawl indices (1989-2016) occurred in 1989-1992 and 2001-2002, while lowest indices occurred during 2006-2011 (Figure 4- 5). There was little agreement between the two sets of Bay Anchovy indices; however, there were few data points representing years of higher abundance and contrast may have been an issue (comparisons are of mostly low abundance points). Atlantic Menhaden seine indices (1959-2016) were high during 1971-1994 and much lower during 1959-1970 and 1995-2016 (Figure 4- 5).

Benthic forage indices were low after the 1990s, but years of higher relative abundance were interspersed during the 2000s (Figure 4- 6). Seine (1959-2016) and trawl (1989-2016) indices for Spot were similar in trend and indicated high abundance during 1971-1994 and low abundance during 1959-1970 and 1995-2013. Blue Crab densities (1989-2016) were highest during 1989-1996, 2009, and 2011 (Figure 4- 6).

In general, relative abundance of Striped Bass (RI) during 1981-2016 was lowest prior to 1994 (mean RI < 0.7 fish per trip; Figure 4- 7). Estimates of RI then rose very rapidly to a high level and remained there during 1995-2006 (mean = 2.6). Estimates of RI fell to about half the 1995-2006 mean during 2008-2013 (mean = 1.2) and then rose to 2.5 in 2014 and 2.7 in 2015-2016. Confidence interval overlap indicated that RI was much lower during 1981-1993 than afterward and that there was some chance that RI during 2008-2013 was lower than other years during 1994-2016 (Figure 4- 7).

An inverse transformation was used to linearize curvature in the relationship of P0 with RI during 1998-2016. A linear regression of P0 against 1/RI accounted for a modest amount of variation ($r^2 = 0.27$, $P < 0.02$; Figure 4- 8). We identified 2015 as a possible outlier and reran the regression; fit was improved considerably by omitting 2015 (r^2 = 0.48, $P < 0.0015$; Figure 4- 11). This latter equation was used to describe the dynamics of condition and abundance:

$$P0 = (-0.43 \cdot (1/RI)) + 0.81;$$

standard errors of the slope and intercept were 0.07 and 0.11, respectively. Predicted back-transformed P0 rapidly increased from 0.25 to 0.5 as RI increased from 0.8 to 1.2 and then increased slowly towards an asymptote for P0 of approximately 0.68 when RI was 3.3 or more. Target P0 would be reached when predicted RI was less than 0.9 (met during 1983-1993, 2008 and 2010; Figure 4- 11).

Target P0 was met during 2008-2010 and average RI was approximately 1.1 or less (IF score = 5). Threshold P0 was met when average RI was approximately 2.7 or more (IF score =1). An IF score of 2 was assigned for RI between 2.7 and 2.17; a score of 3 was assigned for RI between 2.17 and 1.63; and a score of 4 was assigned for RI between 1.63 and 1.1.

Species-specific standardized forage-to-Striped Bass ratios exhibited similar patterns during 1983-2016 (Figure 4- 9). Ratios were high during 1983-1994. A nadir in the ratios appeared during 1995-2004, followed by occasional "spikes" of Spot and Blue Crab ratios and a slight rise in Atlantic Menhaden and Bay Anchovy ratios through 2014. Forage to Striped Bass ratios were lower during 2015-2016, particularly for Spot (Figure 4- 9).

During 1998-2016, P0 exhibited the strongest negative correlation with the ratio of Atlantic Menhaden to Striped Bass (r = -0.52, P = 0.02), followed by the Blue Crab ratio (r = -0.39, P = 0.11), the ratio based on the Spot trawl index (r = -0.37, P = 0.11), the Spot ratio based on the seine index (r = -0.32, P = 0.18), and the Bay Anchovy ratio using the seine index (-0.21, P = 0.40). The ratio of Bay Anchovy to Striped Bass based on the trawl index was positively correlated with P0 (r = 0.45, P = 0.06).

We considered calculating a mean of the standardized forage ratios based on all available surveys but we identified flaws associated with this approach. High weight would be assigned to Spot and Bay Anchovy by including two ratios while remaining major forage only had a single ratio. Spot indices were well correlated with one another (r = 0.81, P < 0.0001) and one could provide similar information as the other. Bay Anchovy ratios were poorly correlated (r = 0.17, P = 0.38) and one would contain different trends than the other. We chose to calculate the mean of the standardized ratios of major forage from seine data alone (FR) for finfish plus the Blue Crab survey; this combined forage ratio covered 1989-2016 (Figure 4- 10). The general trends described for the species-specific forage ratios were evident in the FR (Figure 4- 10).

Linear regression was used to describe the relationship of P0 to the mean major forage ratio (FR). The relationship was described by the equation:

$$P0 = (-0.260 \cdot FR) + 0.639 \ (r^2 = 0.18, P = 0.06);$$

standard errors of the slope and intercept were 0.131 and 0.069, respectively. Inspection of residuals indicated that 2015 was a potential outlier and the regression was run again without 2015. Removal of 2015 improved the fit considerably ($r^2 = 0.34$, P = 0.011). The relationship was described by the equation:

$$(-0.314 \cdot FR) + 0.687;$$

standard errors of the slope and intercept were 0.109 and 0.058, respectively.

Target P0 was met during 2008 and 2010 when FR was more than 0.82 (IF score = 5). Threshold P0 was met when FR was 0.25 or less (IF score =1). An IF score of 2 was assigned for FR between 0.25 and 0.44; a score of 3 was assigned for FR between 0.44 and 0.63; and a score of 4 was assigned for FR between 0.63 and 0.82.

Samples from 1,310 sublegal and 2,388 legal sized Striped Bass were analyzed for diet composition during October-November, 2006-2016 (Table 4-5). Numbers examined each year ranged from 47 to 221 sublegal fish and 49 to 327 legal fish. Fewer dates were sampled within similar time spans after the FWHP became the platform for sampling in 2014 since number collected per trip was not confined by the terms of the CBEF collector's permit (Table 4-5).

In combination and by number, Atlantic Menhaden, Bay Anchovy, Spot, and Blue Crab (major forage items) accounted for 96.3% of diet items encountered in sublegal Striped Bass collected from upper Bay during fall, 2006-2016. Bay Anchovy accounted for the highest percentage by number (59.6%, annual range = 19.1-87.9%); Atlantic Menhaden, 13.8% (annual range = 0.8-48.8%); Spot 7.5% (annual range = 0-70.7%); Blue Crab, 15.4% (annual range = 0.8-34.6%); and other items accounted for 3.7% (annual range = 0-7.6%; Figure 4- 11). The vast majority of major prey were YOY (Uphoff et al. 2016).

Major prey accounted for 92.5% of diet items, by number, encountered in legal sized Striped Bass diets during fall 2006-2016. Atlantic Menhaden accounted for 44.0% (annual range =12.4-76.4%); Bay Anchovy, 16.0% (annual range = 3.7-32.5%); Spot, 8.7% (annual range = 0-52.4%); Blue Crab, 23.3% (annual range = 2.6-59.4%); and other items, 7.5% (annual range = 0-36.2%; Figure 4- 12). The vast majority of major prey were young-of-year fish and Blue Crabs (Uphoff et al. 2016).

By weight, sublegal Striped Bass diets in fall 2006-2016 (combined) were dominated by Atlantic Menhaden (70.1%), followed by Spot (11.9%), Bay Anchovy (9.9%), Blue Crab (2.9%) and other items (5.2%). Estimates of relative availability of prey biomass (C, total grams of prey consumed per gram of Striped Bass) for sublegal Striped Bass varied as much as 8.17-times during 2006-2016 (Figure 4- 13). During years of lowest C (2007 and 2011) varying items contributed to the diet of sublegal fish. Atlantic Menhaden and, secondarily, Bay Anchovy accounted for most diet weight during 2016, the third lowest year for C. During remaining years of higher C, either Spot (2010) or Atlantic Menhaden (remaining years) dominated diet mass (Figure 4- 13).

By weight, Atlantic Menhaden predominated in legal fish sampled (83.6% of diet weight during 2006-2016; all years combined); Bay Anchovy accounted for 1.4%; Spot, 4.6%; Blue Crab, 4.5%; and other items, 5.9%. Estimates of C for legal sized Striped Bass varied as much as 3.4-times among years sampled (Figure 4- 14). Atlantic Menhaden dominated diet weight of legal sized fish during October-November (Figure 4- 14).

Estimates of proportion of empty stomachs (PE) of sublegal sized Striped Bass during fall, 2006-2016, ranged between 0.10 and 0.57 (Figure 4- 15). Lowest estimates of PE for sublegal fish (2009-2011 and 2014) could be separated from remaining estimates (except 2008) based on 95% confidence interval overlap. Estimates of PE steadily fell for sublegal sized fish during 2006-2011 and have varied greatly between the target and threshold PE since then (Figure 4- 15).

A linear regression was used to test whether estimates of PE for sublegal fish were related to P0. Model fit was poor based on all estimates for 1998-2000 and 2006-2016 ($r^2 = 0.15$, $P = 0.17$; Figure 4- 16). Examination of residuals indicated that 2015 was a possible outlier and the regression was rerun without 2015. Omitting 2015 improved fit considerably ($r^2 = 0.39$, $P = 0.02$; Figure 4- 16) and the relationship for sublegal Striped Bass was described by the equation:

$$P0 = (0.80 \cdot PE) + 0.24;$$

standard errors of the slope and intercept equaled 0.30 and 0.12, respectively.

The estimate of PE during 1998-2000 (PE = 0.54) developed for sublegal Striped Bass from Overton et al. (2009; Uphoff et al. 2016) was adopted as a threshold (IF score = 1) for sublegal fish; annual estimates of P0 for sublegal Striped Bass were at the threshold during 1998-2000. Maximum PE estimate during 2008-2010 (PE ranged from 0.19 to 0.31) when P0 was at its target was selected as the PE target ($PE \leq 0.31$ is assigned an IF score of 5). An IF score of 2 was assigned to estimates greater than 0.310

and less than or equal to 0.387; a score of 3 was assigned to estimates greater than 0.387 and less than or equal to 0.463; and 4 was assigned to estimates greater than 0.463 and less than 0.540.

Estimates of proportion of empty stomachs (PE) of legal sized Striped Bass during fall, 2006-2013, ranged between 0.40 and 0.63 (Figure 4- 17). Estimates of PE of legal sized fish fell to 0.10-0.29 during 2014-2016. Lowest estimates of PE for legal fish (2013-2016) could be separated from remaining higher estimates based on 95% confidence interval overlap (Figure 4- 8). Overton et al. (2009) provided an estimate of the percent of Striped Bass in their large size class (501-700 mm, TL) with food (42%) during 1998-2000 and we used this estimate to derive a threshold PE for legal sized fish (0.58). The 95% CI's during 2006-2008 and 2011-2012 overlapped this threshold.

A linear regression was used to test whether estimates of PE for legal fish were related to P0. Model fit was reasonable based on all estimates for 1998-2000 and 2006-2016 ($r^2 = 0.57$, $P = 0.0018$; Figure 4- 18). The relationship for legal sized Striped Bass was described by the equation:

$$P0 = (1.34 \cdot PE) - 0.15;$$

standard errors of the slope and intercept were 0.34 and 0.15, respectively.

The multiple regression of the age 3 standardized gill net index of male relative abundance on the spawning grounds (GI_3) with catchability category (category) and juvenile index (JI_{t-3}) three years prior was significant ($r^2 = 0.51$, $P < 0.0001$; 1985-2016). The equation describing the relationship was

$$GI_3 = 1.10 + (0.11 \cdot JI_{t-3}) - (1.00 \cdot \text{category});$$

standard errors of the intercept, JI_{t-3} coefficient and category coefficient equaled 0.15, 0.02 and 0.21, respectively. A linear regression of GI_3 against JI_{t-3} (without catchability categories) explained 12% of variation ($P = 0.047$) and exhibited serial patterning of residuals (generally positive during 1985-1996 and negative afterwards). Serial patterning was not evident with the residuals of the multiple regression.

The hybrid age 3 gill net index of male relative abundance (HI_3) on the spawning grounds indicated a dearth of high indices during 1985-1995, while the standardized index without adjustment in catchability (GI_3) indicated a full range of abundance during this early period with some of the highest GI_3 indices of the time-series occurring in 1985-1987 (Table 4-6). These low HI_3 year-classes were followed by the appearance of intermittent large year-classes at age 3 (1996, 1998, 1999, 2004, 2006, 2010, and 2014; Table 4-6). Estimates of q for the $HI_3$when abundance estimated by the updated SCAM was under $20.0 \cdot 10^6$ fish (25 of 31 observations) were about half of the average at higher abundance (Table 4-6). The HI_3 indicated sharper changes in relative abundance of age 3 Striped Bass from year-to-year than the updated SCAM (ASMFC 2016; Figure 4- 19). Peaks generally aligned, but years of low abundance in the SCAM tended to be higher than would have been indicated by the hybrid gill net index. Trends in the HI_3 were linearly related to trends in estimated abundance of three year-old Striped Bass (N_3) from the updated SCAM (1982-2015; ($r^2 = 0.67$, $P < 0.0001$; ASMFC 2016). The linear relationship was described by the equation:

$$N_3 = (8{,}708{,}241 \cdot HI_3) + 8{,}576{,}232;$$

standard errors for the slope and intercept were 1,126,991 and 970,200, respectively.

Relative survival (SR; HI_3 / JI_{t-3}) was consistently high during 1986-1996, shifted to consistently low during 1999-2004, and varied afterwards (Figure 4- 20). The 36%

percent reduction in median relative survival to age 3 between1986-1996 (median SR = 0.18) and 1997-2016 (median SR = 0.11) was of similar magnitude as changes in tag-based estimates of survival of legal-sized fish during the same period (from 77% annual survival to 44%, a 43% reduction).

The target for SR was derived as the median estimate for the period 1986-1996 (target SR = 0.18; IF score = 5) and the threshold was the median during 1999-2004 (threshold SR = 0.09; IF score = 1). A score of 2 was assigned to SR between 0.09 and 0.12; a score of 3 was assigned when SR was between 0.12 and 0.15; and SR between 0.15 and 0.18 was given a score of 4. After 1998, target SR was reached in 2010, 2011, and 2014. After 2004, threshold conditions were met in 2007 and 2008 (Figure 4- 20).

Linear regression was used to compare SR in year t with P0 in year t-1. Estimates of SR_t were weakly related to $P0_{t-1}$ ($r^2 = 0.15$, $P = 0.11$, 1998-2016; Figure 4-21). The equation for the regression was

$$SR_t = (-0.18 \cdot P0_{t-1}) + 0.24;$$

standard errors of the slope and intercept were 0.10 and 0.06, respectively.

Two points (2004 and 2010) were potential outliers and each was removed to examine their impact on the relationship. Removal of 2004 improved fit considerably ($r^2 = 0.29$, $P = 0.026$; Figure 4- 21), while the removal of 2010 worsened the fit ($r^2 = 0.06$, $P = 0.33$; Figure 4-). The equation for the regression with 2004 removed was

$$SR_t = (-0.23 \cdot P0_{t-1}) + 0.26;$$

standard errors of the slope and intercept were 0.06 and 0.09, respectively. With both points removed, fit was similar to when 2004 was removed ($r^2 = 0.30$, $P = 0.027$), but the slope (-0.13, SE = 0.05) and intercept (0.19, SE = 0.03) were considerably reduced (Figure 4- 21).

The IF varied from 1.2 to 5 during 1998-2016 (Figure 22). During 1998-2004, the IF was low, between 1 and 2. The IF increased to 3 in 2005, fell back to around 2 in 2006-2007, and then increased to near 4 to 5 during 2008-2010. After 2010, it varied from above 2 to just below 4. IF was between 2 and 3 during 2015-2016. Spread of annual component scores was generally narrower (no more than 2 units) during 1998-2004 when the IF was consistently low. Spread was typically wider as scores improved after 2004 (Figure 4- 22).

Estimates of mean IF with each component removed indicated little variation from the overall mean IF (Figure 4- 23). The maximum deviation from the overall mean in any given year ranged between 0.45 and 0.8 (Figure 4- 23). This approach suggested that IF means could be separated into high, medium, and low categories.

Discussion

The IF has fallen to between 2 and 3 (near or avoids threshold, respectively) in 2015-2016, reflecting the 2011 dominant year-class's demand for forage. The IF indicated threshold to near threshold foraging conditions during 1998-2004 and improvement since 2004. Best IF scores occurred during 2008-2010 (~4 and above) and all scores reached their targets in 2010. High variability in component scores was evident as IF improved after 2004. This variability may have reflected sampling issues, nonlinear, asymptotic relationships among variables, lagged responses, potential insensitivity of some indices, behavioral changes that could increase feeding efficiency, episodes of good foraging conditions outside of those monitored in fall, and combinations of the above. Many of these issues were discussed in Uphoff et al. (2016) and the reader is referred to that report. Some issues that were not discussed in Uphoff et al. (2016) will be covered in this discussion.

A rapid rise in Striped Bass abundance in upper Bay during the mid-1990s followed by a dozen more years at high abundance coincided with declines in indices of Atlantic Menhaden, Bay Anchovy, Spot, and Blue Crab (i.e., major pelagic and benthic prey) to low levels. Striped Bass were often in poor condition during fall 1998-2004 and vulnerable to starvation. Improvements in condition afterward coincided with lower abundance after 2007, spikes or slight increases in some major forage indices, and higher availability of prey in fall diets. Survival of sublegal and legal sized Striped Bass in upper Bay shifted downwards in the mid-1990s shortly after upper Bay major forage-to-Striped Bass ratios, an indicator of attack success, reached a nadir. Poor survival of age 3 Striped Bass persisted through 2004 and occasional years of above target survival appeared afterwards.

Ecosystem based fisheries management has been criticized for poor tractability, high cost, and difficulty in integrating ecosystem considerations into tactical fisheries management (Fogarty 2014). It has been the principal investigator's unfortunate experience that complex and comprehensive ecosystem based approaches to fisheries management for the entire Chesapeake Bay i.e., Chesapeake Bay Ecopath with Ecosim and MD Sea Grant's Ecosystem Based Fisheries Management for Chesapeake Bay (Christensen et al. 2009; MD Sea Grant 2009) have not gained much of a foothold in Chesapeake Bay's fisheries management. The index-based IF approach represents a less complex, low cost attempt to integrate forage into Maryland's fisheries management. Given the high cost of implementing new programs to obtain unbiased estimates of forage and forage demand with known statistical properties, we have combined effort with and information from existing sampling programs and indices (convenience sampling and proxies for population level estimates, respectively; Falcy et al. 2016). This trade-off is very common in fisheries and wildlife management (Falcy et al. 2016).

The IF represents a framework for condensing complex ecological information so that it can be communicated simply to decision makers and stakeholders. The science of decision making has shown that too much information can lead to objectively poorer choices (Begley 2011). The brain's working memory can hold roughly seven items and any more causes the brain to struggle with retention. Decision science has shown that proliferation of choices can create paralysis when the stakes are high and the information complex (Begley 2011). For this report, the IF condensed five elements into a combined score (sixth element) that, hopefully, can alert busy fisheries managers and stakeholders about the status of forage and whether this issue merits further attention and action.

The IF is similar to traffic light style representations for applying the precautionary approach to fisheries management (Caddy 1998; Halliday et al. 2001). Traffic light representations can be adapted to ecosystem based fisheries management (Fogarty 2014). The strength of the traffic light method is its ability to take into account a broad spectrum of information, qualitative as well as quantitative, which might be relevant to an issue (Halliday et al. 2001). It has three elements – a reference point system for categorization of indicators, an integration algorithm, and a decision rule structure based on the integrated score (Halliday et al. 2001). In the case of the IF, it contains the first two elements, but not the last. Decision rules would need input and acceptance from managers and stakeholders.

Some form of integration of indicator values is required in the traffic light method to support decision making (Halliday et al. 2001). Integration has two aspects, scaling the indicators to make them comparable (ranking them from 1-5 in the IF) and applying an operation to summarize the results from many indicators (averaging the elements of the IF; Halliday et al. 2001). Although it is intrinsic to integration that some information is lost, the loss is not necessarily of practical importance (Halliday et al. 2001). The original indicators are still available for decision rules that might require more information than is contained in the characteristics. Simplicity and communicability are issues of over-riding importance (Halliday et al. 2001). Caddy (1998) presented the simplest case for single-species management where indicators were scaled by converting their values to traffic lights, and decisions were made based on the proportion of the indicators that were red. While the IF is numeric, it could easily be converted to a traffic light using the strict (three distinct colors) or fuzzy (blended colors) methods. A prototype of the IF used a traffic light color scheme (Uphoff et al. 2014).

Outliers were identified for comparisons of PE, RI, and forage ratios with P0 (2015 in all three cases) and SR with P0 (2004 and 2010). Fit was considerably improved by removing these outliers (r^2 was increased by a factor of 1.8 to 3.5), except when 2010 was removed from the regression of SR with P0 (fit deteriorated by a factor of 2.5). Even with outliers removed, the relationships were not particularly strong ($r^2 \leq 0.53$) and indicated the elements were not redundant.

Outliers and divergences occurred once or twice in 19 years, indicating a roughly 5-10% chance of a non-conforming value in a given index. If managers decide to use the IF for decision making, they should consider multiple years of IF scores to make a judgment rather than a single year to avoid false positives or negatives.

While some could argue that we went “fishing for good statistics”, removal of 2015 from analyses may represent removal of the impact of a combination of an episode of high alternative forage in spring, 2015, and a lagged response to successful feeding the previous fall (PE at target condition). During late May to early July, 2015, Striped Bass of both size classes fed heavily on small clams, apparently by feeding around the surge of Cownose Rays that swarm the sandy shallows of Chesapeake Bay during late spring and early summer (J. Uphoff, MD DNR, personal observation). Striped Bass gained body fat in early summer by feeding heavily on these alternative prey items.

Our concentration on fall diets did not directly consider some prey items in the “other” category that could be important in other seasons. White Perch (*Morone americana*) and invertebrates other than Blue Crab are important diet items during winter and spring, respectively (Walter et al. 2003; Hartman and Brandt 1995b; Overton 2009). These species did not usually make a large contribution to diet mass during fall, but White Perch from the 2011 dominant year-classes made a large contribution (15.6%) to legal sized Striped Bass diet biomass in fall, 2012.

The two years that may have been outliers for SR and P0, 2004 and 2010 (lower and higher SR than expected, respectively) may best be explained as observation error or change in catchabilities of the spring gill net and juvenile surveys. The SR index has an added complication in that it is a measure of survival over about 2.5 years, while other IF indices are annual or have potential lags less than 2.5 years. They would not be relevant to this whole SR period since fish less than about 2-years old are poorly represented in diet samples.

Uphoff et al. (2015) had considered estimating P0 for the two size classes used in diet analyses, but found that little information would be lost by combining the estimates based on 2006-2013 data. Differences in values between size classes were minor, trends were closely followed one another up through 2015. Estimates of P0 in 2016 for the two size classes diverged greatly, with legal sized fish meeting the target and sublegal sized fish meeting the threshold. However, substituting sublegal P0 for P0 of all sizes in analyses seemed to have little effect on overall results (J. Uphoff, MD DNR, unpublished analysis). Should this divergence persist, P0 will need to be estimated for both size classes.

We chose PE as an indicator of feeding success over C because confidence intervals could be easily calculated for it and estimates from Overton et al. (2009) were available to estimate threshold conditions during 1998-2000. The CBEF and FWHP based feeding estimates time series (2006-2016) did not cover any portion of the period when P0 was low. In addition, this indicator could be derived from diet information from the 1930s (Hollis 1952) and the 1950s (Griffin and Margraf 2003). However, PE is insensitive to size of items. This may lead to positive bias in terms of mass consumed if small items predominate and negative bias if large items predominate.

Forage indices and forage to Striped Bass ratios were placed on the same scale by dividing them by arithmetic means over a common time period (ratio of means). Conn (2009) noted in several scenarios that the arithmetic mean of scaled indices performed as well as the single index estimated by a hierarchal Bayesian technique. Falcy et al. (2016) found that ratios of means provided a reasonable method for combining indices into a composite index to be calibrated with population estimates of Chinook Salmon *Oncorhynchus tshawytscha*, but there was no one optimal method among the four techniques applied.

The spreadsheet provided by Mary Chrisman and Desmond Kahn for estimating the confidence intervals for the RI was very much appreciated. However, applying the spreadsheet to forage ratios appears to be complicated by the use of geometric means for the forage indices (95% confidence intervals can be viewed in Durell and Weedon 2017). We intend on using @Risk (Monte Carlo simulation software for Excel spreadsheets; http://www.palisade.com/risk/) to develop confidence intervals for these ratios and, perhaps, revisit the confidence intervals for RI if time permits.

Due to Mr. Price’s health concerns, CBEF collections ended during late summer 2015. Collections by CBEF for 2014 and 2015 have been entered and need to be edited. Once edited, they will allow for a comparison with fall consumption estimates based on FWHP sampling. Mr. Price passed away in December 2016, and we are greatly indebted to him for the vast amount of data he collected and shared, and his insights on Striped Bass feeding ecology in Upper Chesapeake Bay.

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Table 4-1. Abbreviations and definitions of indicators and important parameters.

Abbreviation	Definition
C	Grams of prey consumed per gram of Striped Bass, an indicator of feeding success and prey availability.
F	Instantaneous annual fishing mortality rate.
FI	Forage index. Mean score for five indicators of forage status (FR, PE, P0, RI, and SR)
FR	Mean major forage ratio (mean of standardized seine based major finfish to Striped Bass ration and the standardized Blue Crab ratio)
GI	Unmodified gill net index of relative abundance of age 3 male Striped Bass.
HI	Hybrid gill net index of relative abundance of age 3 male Striped Bass that has been adjusted for catchability change with population size.
JI	Juvenile index of relative abundance of a species.
M	Instantaneous annual natural mortality rate.
PE	Proportion of Striped Bass with empty stomachs, an indicator of feeding success and prey availability.
P0	Proportion of Striped Bass without visible body fat, an indicator of nutritional status (condition).
q	Catchability (efficiency of a gear).
RI	Catch (number harvested and released) of Striped Bass per private and rental boat trip, a measure of relative abundance.
SR	Relative survival of sublegal sized resident Striped Bass to age-3.

Table 4-2. Proportion of Striped Bass without body fat (P0) during fall estimated from Fish and Wildlife Health Program sampling. N = sample size and SD is the standard deviation of P0.

Year	P0	N	SD
1998	0.749	338	0.024
1999	0.779	344	0.022
2000	0.773	290	0.025
2001	0.745	224	0.029
2002	0.605	316	0.028
2003	0.700	237	0.030
2004	0.746	414	0.021
2005	0.596	524	0.021
2006	0.600	863	0.017
2007	0.500	662	0.019
2008	0.137	629	0.014
2009	0.312	1107	0.014
2010	0.270	693	0.017
2011	0.531	1202	0.014
2012	0.658	333	0.026
2013	0.576	441	0.024
2014	0.312	398	0.023
2015	0.124	347	0.018
2016	0.476	429	0.024

Table 4-3. Proportion of Striped Bass without body fat (P0) during summer estimated from the Fish and Wildlife Health Program sampling. Months = the months that samples were taken, N = sample size, and SD is the standard deviation of P0.

Year	Months	P0 summer	N	SD
1999	5,6,8	0.49	35	0.08
2000	5,6,7,8	0.98	50	0.02
2001	8,9	0.8	20	0.09
2002	5,6,7,8,9	0.54	80	0.06
2003	7,9	0.68	19	0.11
2004	5,6,7,8,9	0.67	104	0.05
2005	5,7,9	0.38	134	0.04
2006	7,8,9	0.5	134	0.04
2007	6,7,8,9	0.44	180	0.04
2008	6,7,8	0.13	101	0.03
2009	6,7,8,9	0.34	370	0.02
2010	8,9	0.39	133	0.04
2011	6,7,9	0.44	638	0.02
2012	7,8,9	0.41	477	0.02

Table 4-4. Summary of regression analyses of the influence of previous body fat indices (P0) on condition in fall of year t. Function indicates the model that provided the best fit to the data.

Predictor	Predicted	Parameters					
Season, year	Season, year	Function	r^2	P	m (SE)	b (SE)	K (SE)
Summer, t	Fall, t	Weibull	0.75	<0.0001			0.76, (0.08)
Fall, t-1	Summer t	Linear	0.34	0.038	0.62 (0.26)	0.17 (0.16)	
Fall, t-1	Fall, t	Linear	0.43	0.003	0.63 (0.18)	0.18 (0.11)	
Fall, t-2	Fall, t	Linear	0.12	0.17	0.36 (0.26)	0.30 (0.15)	

Table 4-5. Number of dates sampled and number of sublegal (<457 mm, TL) and legal sized Striped Bass collected in each size category, by year.

Year	N dates	Sublegal N	Legal N
2006	19	118	49
2007	20	76	203
2008	15	29	207
2009	17	99	240
2010	22	112	317
2011	19	74	327
2012	11	47	300
2013	14	191	228
2014	7	221	84
2015	8	174	173
2016	12	169	260

Table 4-6. Summary of data used to estimate the hybrid gill net index for age 3 Striped Bass (HI_3). Std index = age 3 gill net index standardized to its time period mean. Age 3 N = estimated abundance of age 3 fish on the Atlantic coast. q = catchability. JI_{t-3} = the Maryland seine index of age 0 Striped Bass three years earlier. q category = the catchability category used in the multiple regression. HI_3 = the hybrid gill net index for age 3 Striped Bass. HI_3 q = catchability of the hybrid index.

Year	Std index	Age 3 N	q	JI_{t-3}	q category	HI_3	HI_3 q
1985	2.36	7,138,020	3.30E-07	3.6	0	0.50	7.06E-08
1986	2.04	6,587,420	3.10E-07	0.6	0	0.17	2.56E-08
1987	1.87	6,451,940	2.90E-07	1.6	0	0.29	4.42E-08
1988	0.52	5,198,420	9.96E-08	0.9	0	0.20	3.89E-08
1989	1.23	6,921,510	1.77E-07	1.3	0	0.25	3.62E-08
1990	1.33	9,092,900	1.46E-07	1.5	0	0.26	2.91E-08
1991	1.30	10,110,700	1.28E-07	0.7	0	0.18	1.79E-08
1992	1.45	13,519,100	1.07E-07	4.9	0	0.65	4.82E-08
1993	0.98	11,102,900	8.79E-08	1.0	0	0.22	1.94E-08
1994	0.42	11,334,100	3.71E-08	1.5	0	0.27	2.40E-08
1995	0.92	14,668,000	6.24E-08	2.3	0	0.36	2.484E-08
1996	2.47	29,162,700	8.47E-08	14.0	1	2.47	8.467E-08
1997	0.34	18,527,900	1.83E-08	6.4	1	0.34	1.83E-08
1998	1.10	20,214,300	5.46E-08	4.4	1	1.10	5.46E-08
1999	1.41	24,988,100	5.64E-08	17.6	1	1.41	5.64E-08
2000	0.40	16,002,500	2.47E-08	3.9	1	0.40	2.47E-08
2001	0.28	16,319,600	1.73E-08	5.5	1	0.28	1.73E-08
2002	0.33	12,906,400	2.59E-08	5.3	1	0.33	2.59E-08
2003	0.78	19,143,500	4.09E-08	7.4	1	0.78	4.09E-08
2004	1.37	22,289,000	6.13E-08	12.6	1	1.37	6.13E-08
2005	0.66	12,330,400	5.378E-08	2.2	1	0.66	5.37E-08
2006	1.75	26,957,500	6.497E-08	10.8	1	1.75	6.497E-08
2007	0.16	15,358,100	1.037E-08	4.9	1	0.16	1.037E-08
2008	0.58	14,227,000	4.06E-08	6.9	1	0.58	4.06E-08
2009	0.30	10,562,300	2.8465E-08	1.8	1	0.30	2.846E-08
2010	1.05	13,721,800	7.64E-08	5.1	1	1.05	7.646E-08
2011	0.56	9,800,980	5.70E-08	1.3	1	0.56	5.70E-08
2012	0.37	11,993,800	3.11E-08	3.9	1	0.37	3.11E-08
2013	0.38	15,324,600	2.49E-08	2.5	1	0.38	2.49E-08
2014	1.96	20,125,700	9.72E-08	9.6	1	1.96	9.72E-08
2015	0.06	5,070,610	1.17E-08	0.5	1	0.06	1.17E-08
2016	0.54			3.4	1	0.54	

Figure 4-1. Upper Bay (Maryland's portion of Chesapeake Bay) with locations of forage index sites (black dots = seine site and grey squares = trawl site), and regions sampled for Striped Bass body fat and diet data.

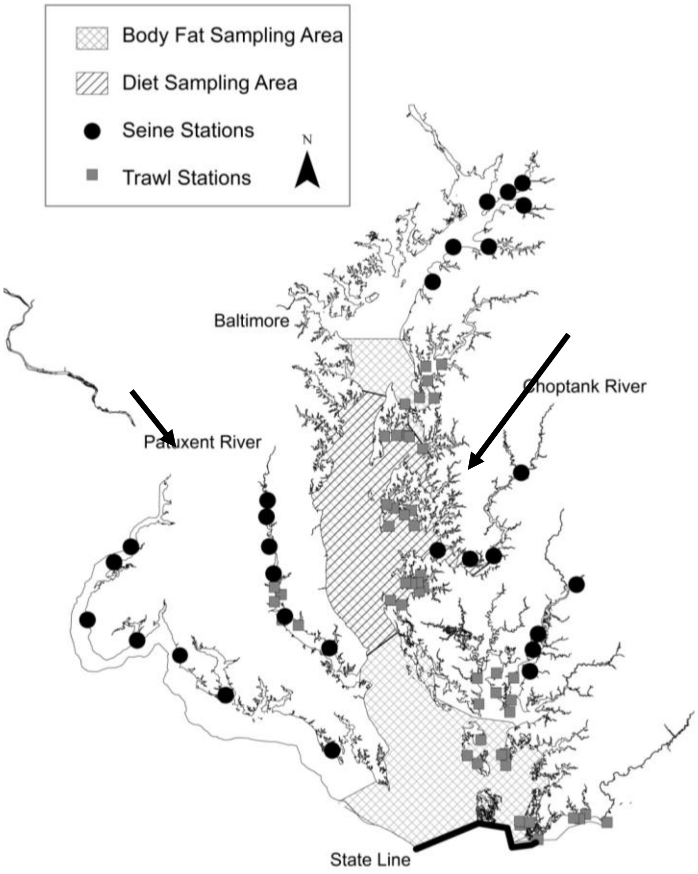


Figure 4-2. Proportion of Striped Bass (280 - 800 mm) without body fat during October-November (MD DNR Fish and Wildlife Health Program monitoring), with body fat targets and limits.

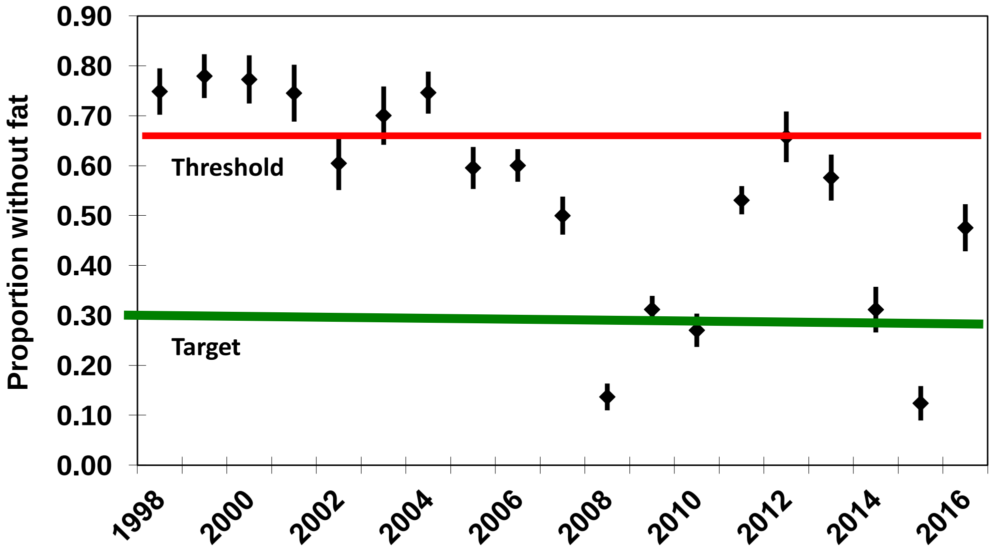


Figure 4-3. Trends in fall body fat indices for sublegal (280-456 mm) and legal striped bass

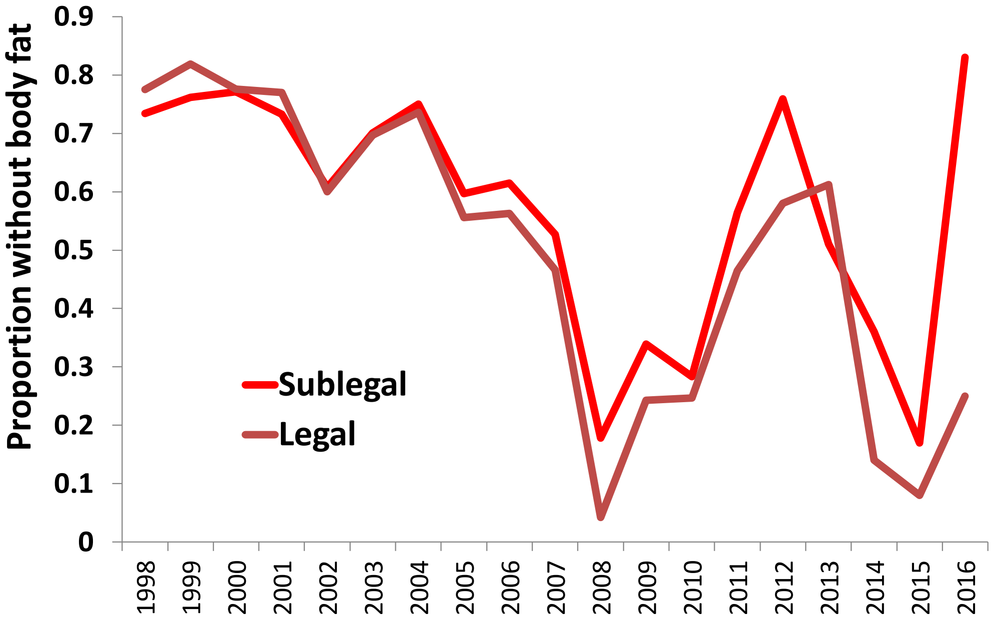


Figure 4-4. Proportion of striped bass without body fat in summer versus fall, 1999-2012 (FWHP sampling). Summer sampling was discontinued after 2012.

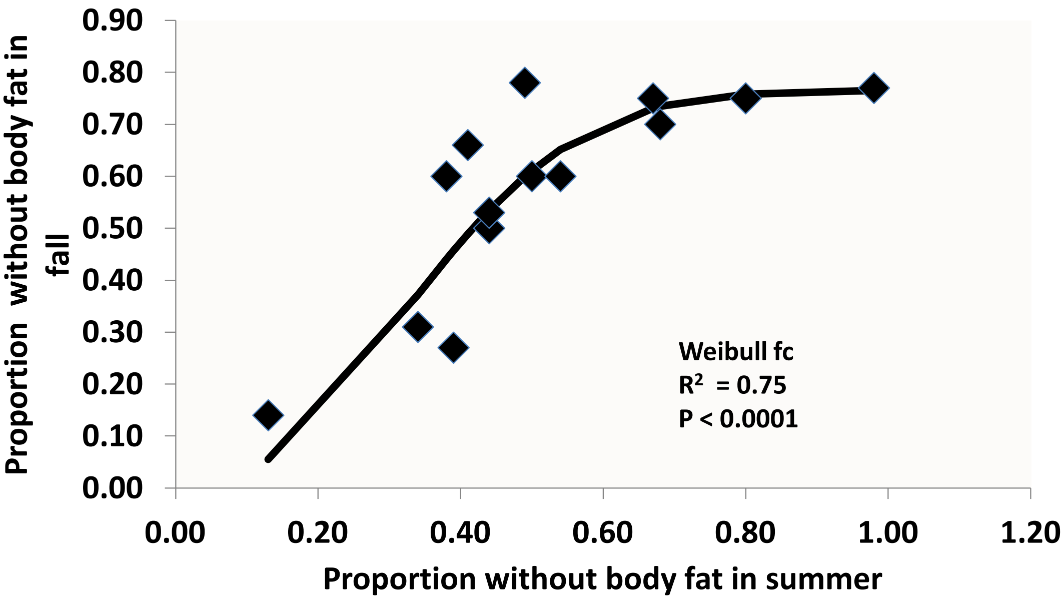


Figure 4-5. Trends in major pelagic prey of Striped Bass in upper Bay surveys, 1959-2014. Indices were standardized to their 1989-2016 means (time-series in common). Menhaden = Atlantic Menhaden and Anchovy = Bay Anchovy.

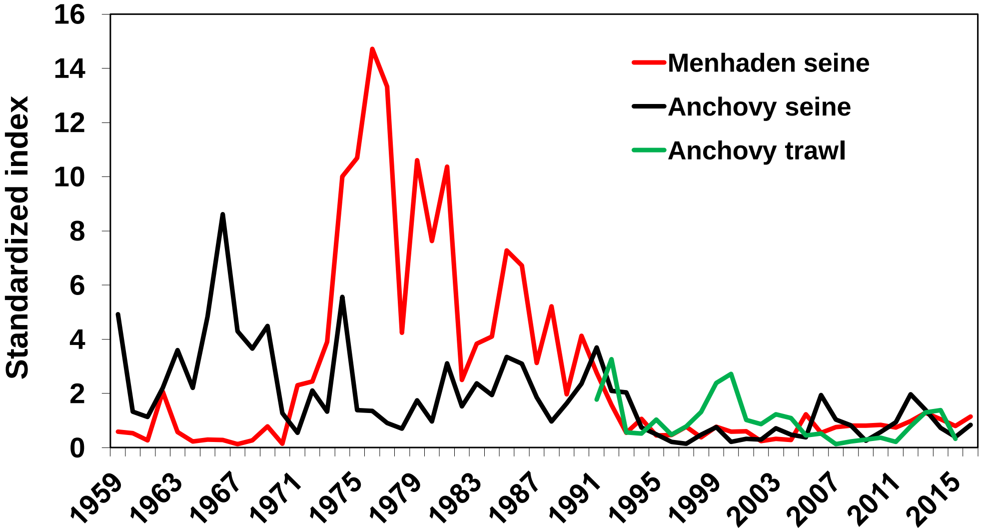


Figure 4-6. Trends in major benthic prey of Striped Bass in upper Bay surveys. Indices were standardized to their 1989-2016 means (time-series in common).

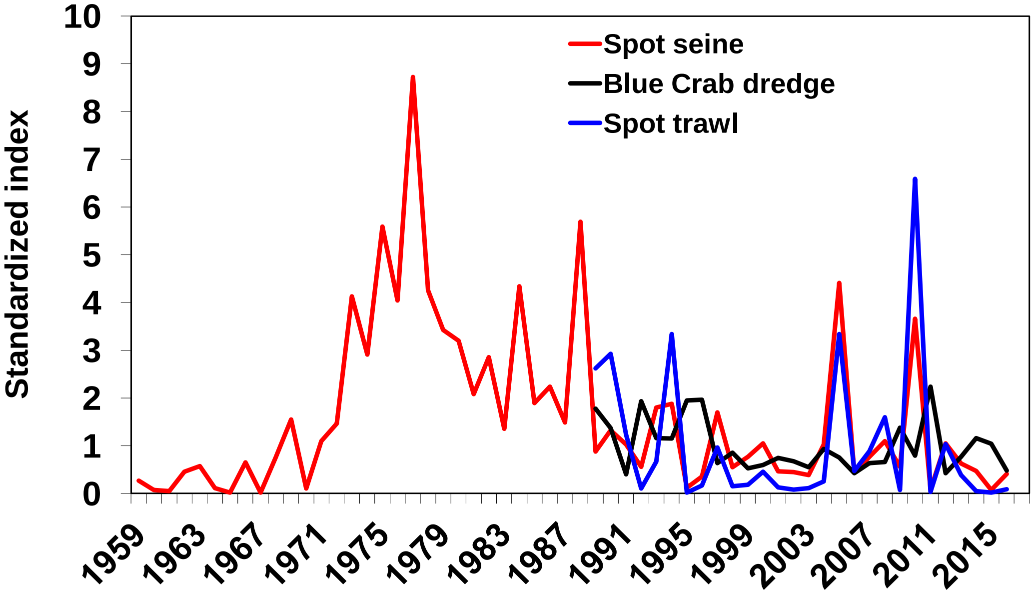


Figure 4-7. Upper Bay Striped Bass index (RI; MD MRIP recreational catch per private boat trip; mean = black line) and its 95% CI (grey lines). Catch = number harvest and released.

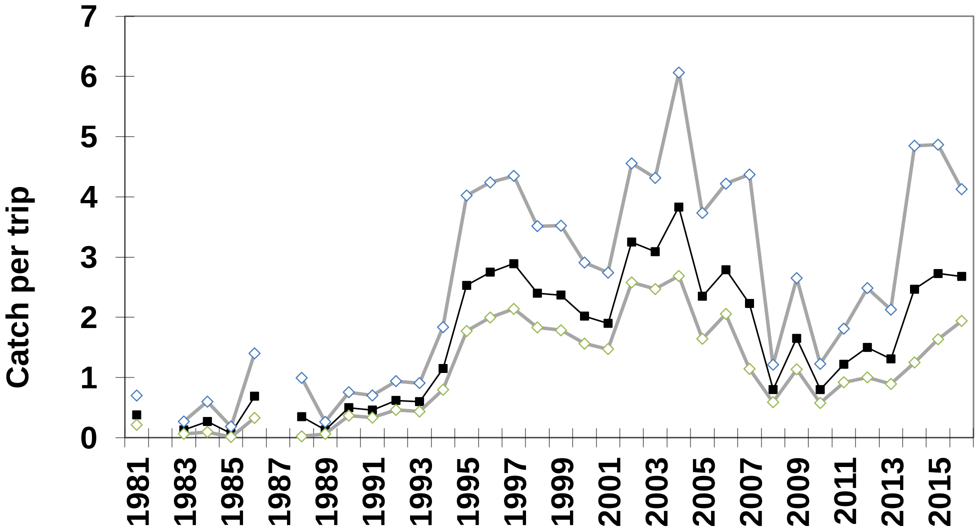


Figure 4-8. The relationship of condition (P0) to relative abundance of upper Bay resident Striped Bass (RI). Regression statistics and predicted line are for the regression with one point (2015) removed.

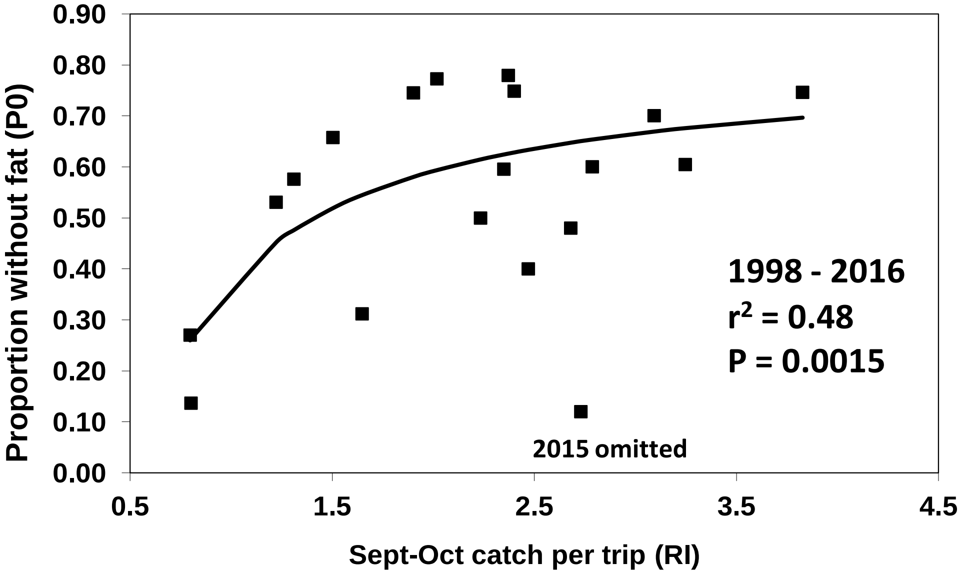


Figure 4-9. Trends of standardized ratios major upper Bay forage species indices to Striped Bass relative abundance (RI). Forage ratios have been standardized to place them on the same scale. S indicates a seine survey index; T indicates a trawl survey index; and D indicates a dredge index.

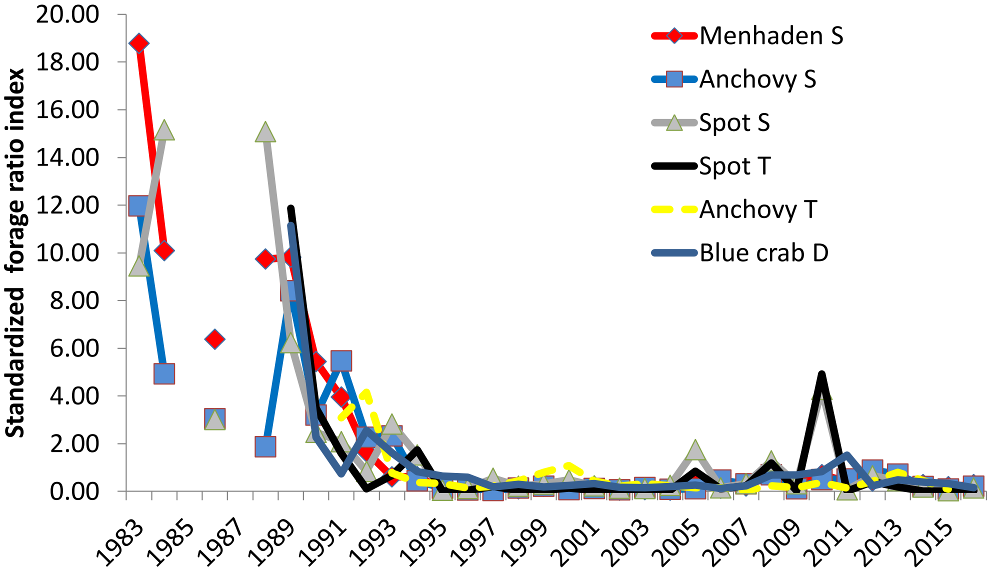


Figure 4-10. Standardized ratios of major forage and Striped Bass compared to the mean of seine based ratios used to summarize relative forage conditions.

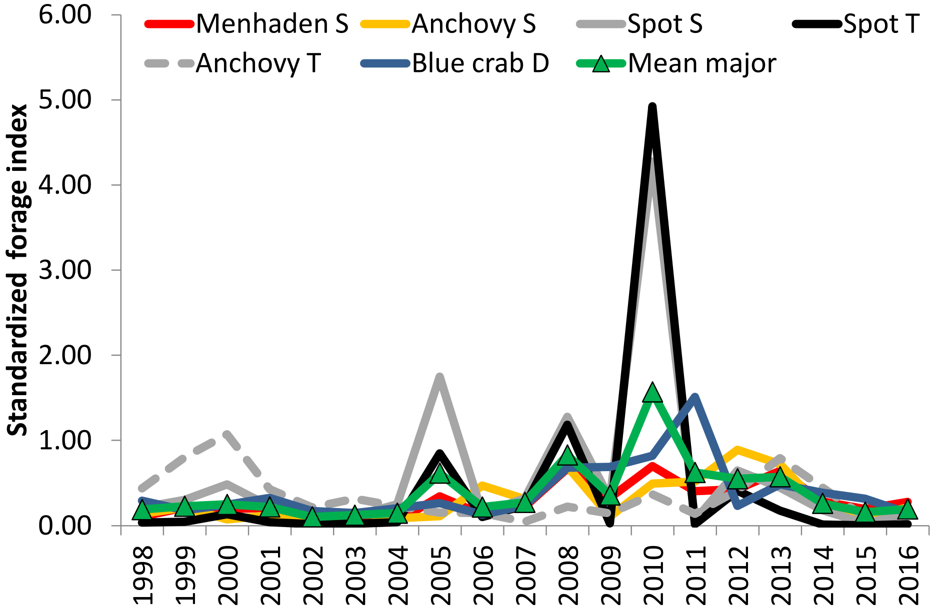


Figure 4-11 . Percent of sublegal (< 457 mm TL) Striped Bass diet, by number, in fall, represented by major forage groups

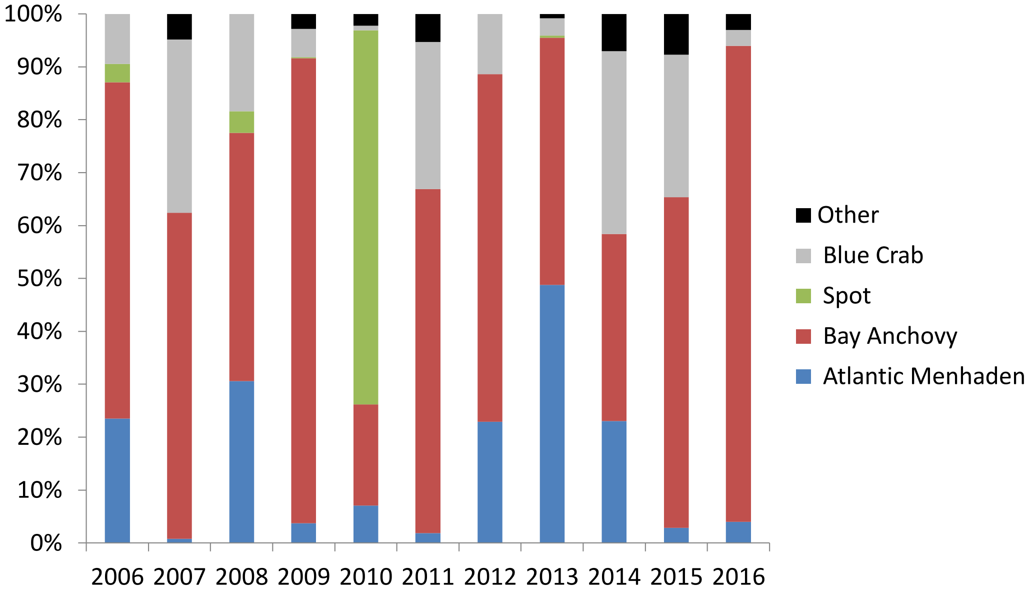


Figure 4-12 . Percent of legal (≥ 457 mm TL) Striped Bass diet, by number, in fall, represented by major forage groups

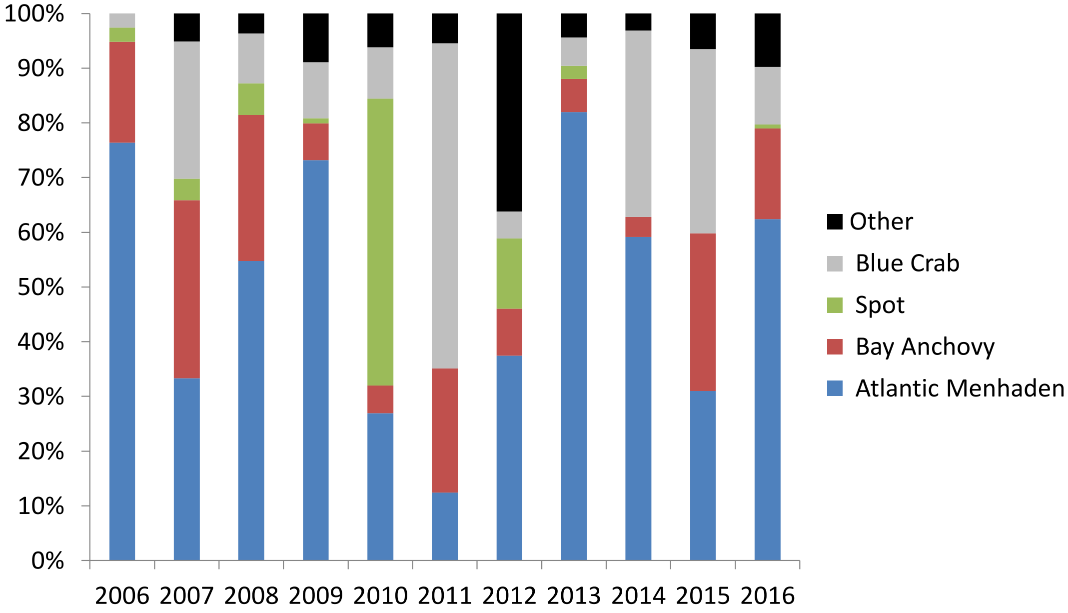


Figure 4-13. Gram prey consumed per gram of sublegal (< 457 mm or 18 in, TL) Striped Bass in fall hook-and-line samples. Age-0 forage dominate the diet. Arrow indicates color representing Atlantic Menhaden which disappeared on the figure legend.

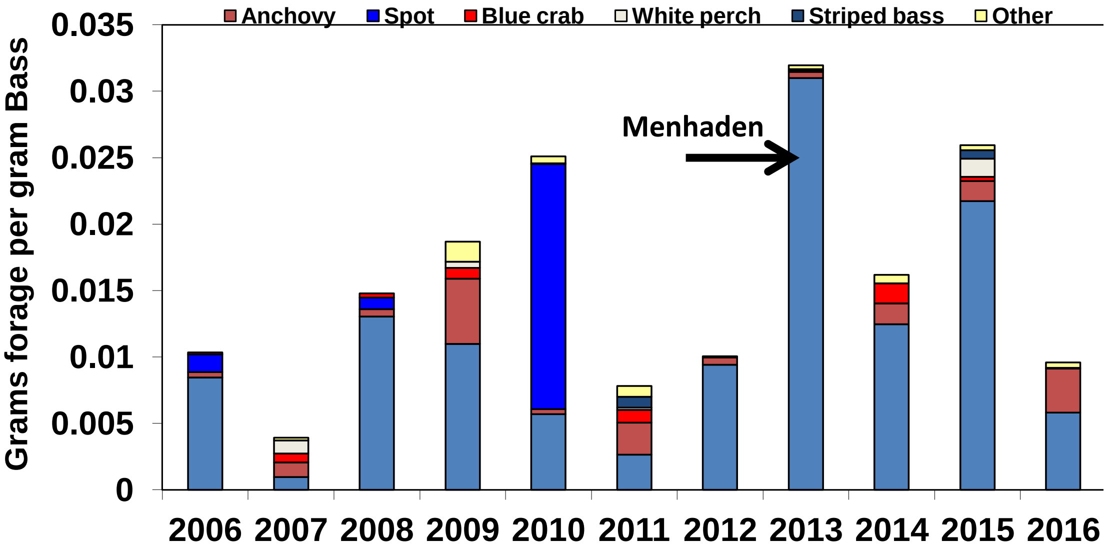


Figure 4-14. Grams of prey consumed per gram of legal (≥ 457 mm or 18 in TL) Striped Bass during October-November. Fall consumption dominated by YOY forage. Arrow indicates color representing Atlantic Menhaden which disappeared on the figure legend.

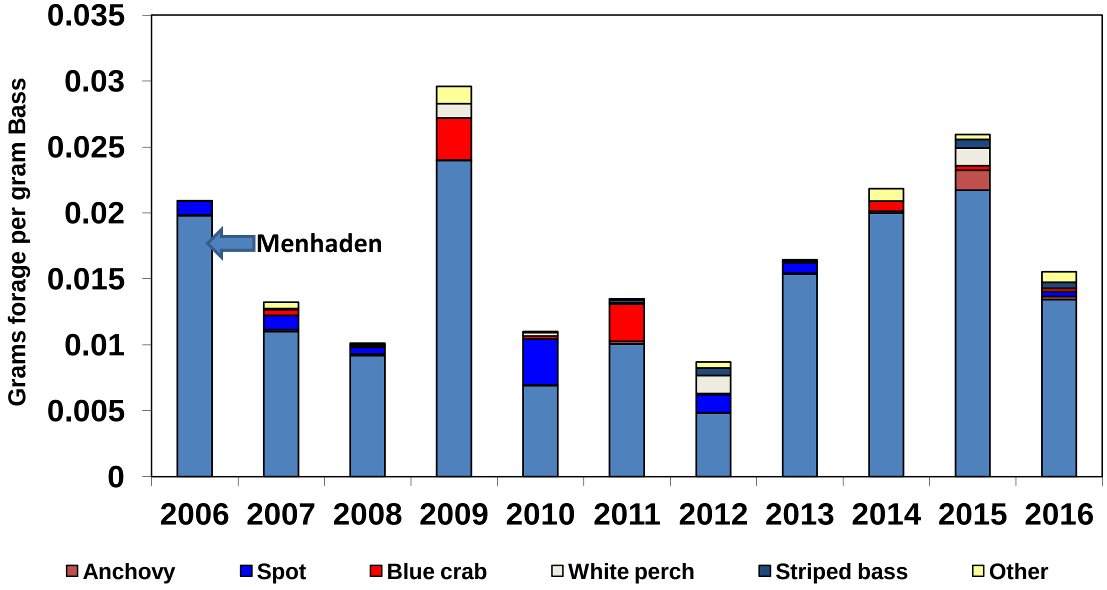


Figure 4-15. Proportion of sublegal Striped Bass guts without food (PE) in fall. Red diamond represents threshold PE and green diamond indicates the PE target.

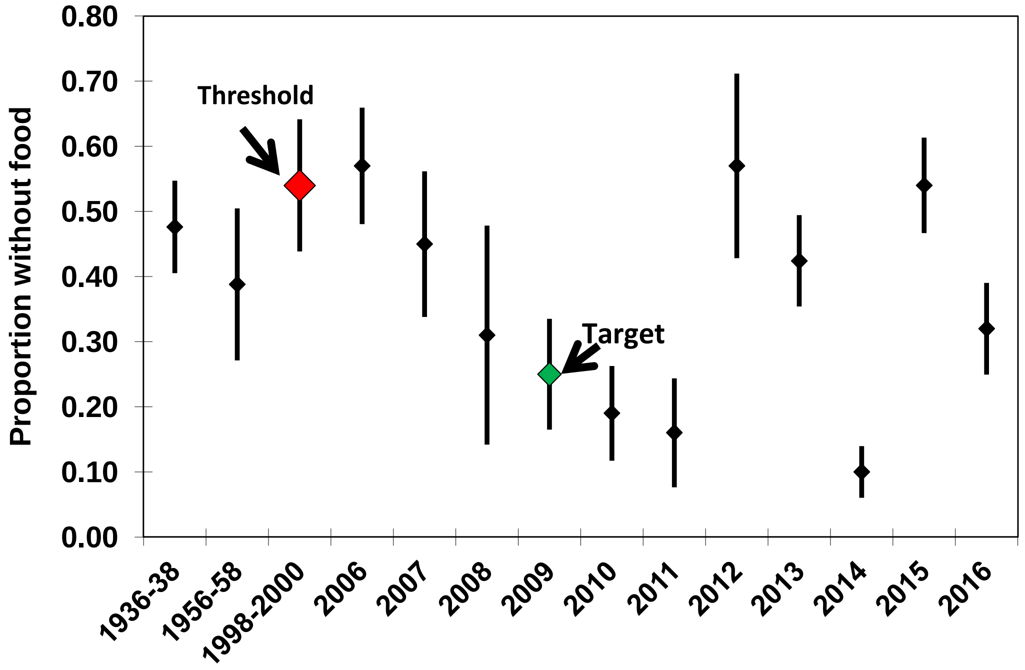


Figure 4-16. Relationship of the proportion (P) of all Striped Bass without fat in fall and proportion sublegal Striped Bass with empty stomachs in fall. An outlier (2015) was removed.

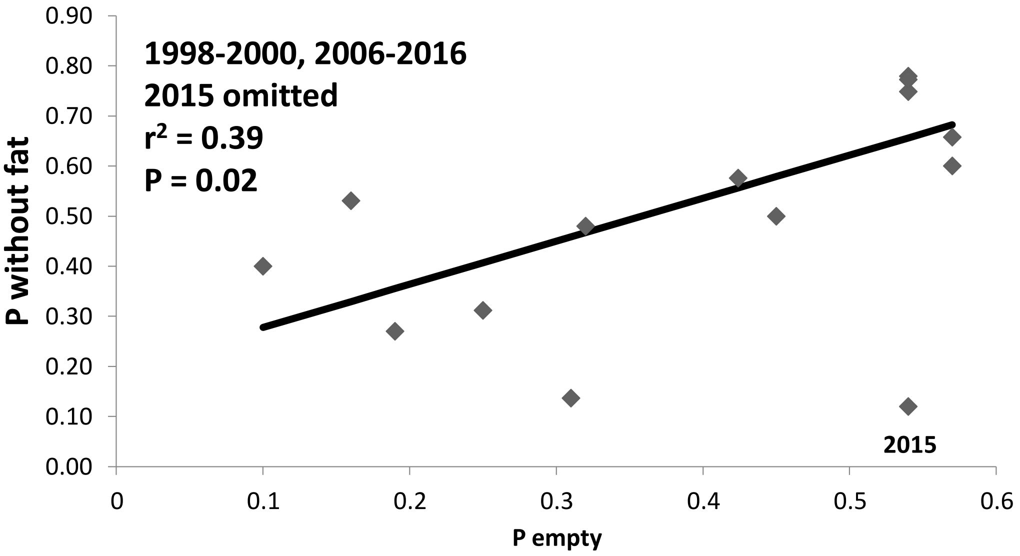


Figure 4-17. Proportion of legal sized Striped Bass (> 456 mm or 18 in, TL) guts without food in fall.

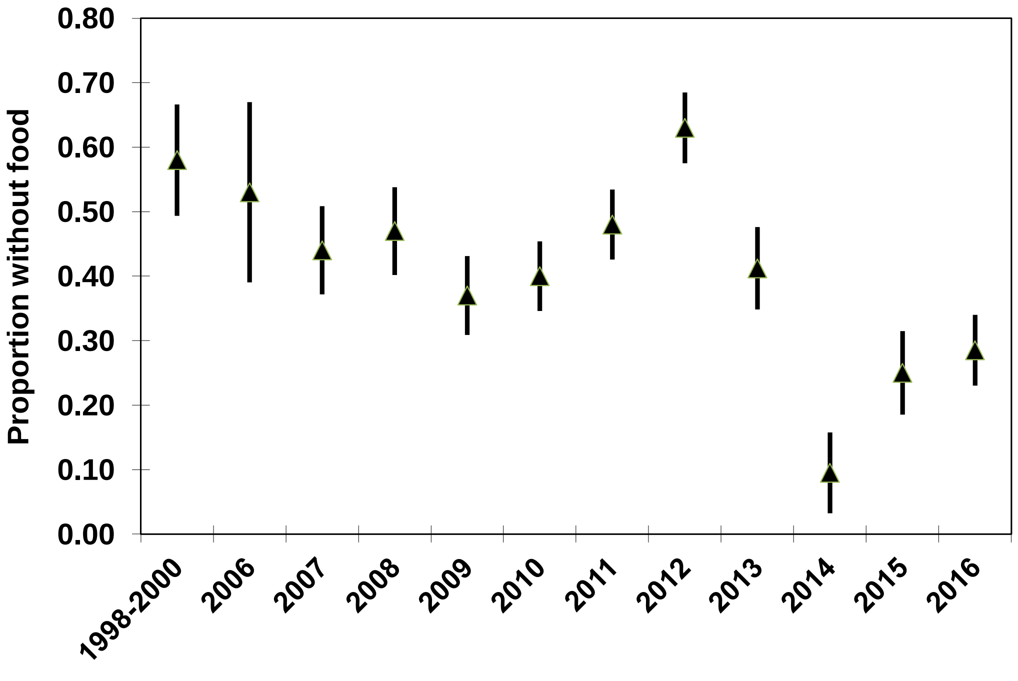


Figure 4-18. Relationship of proportion of legal sized Striped Bass (> 457 mm or 18 in, TL) without fat and proportion of empty stomachs.

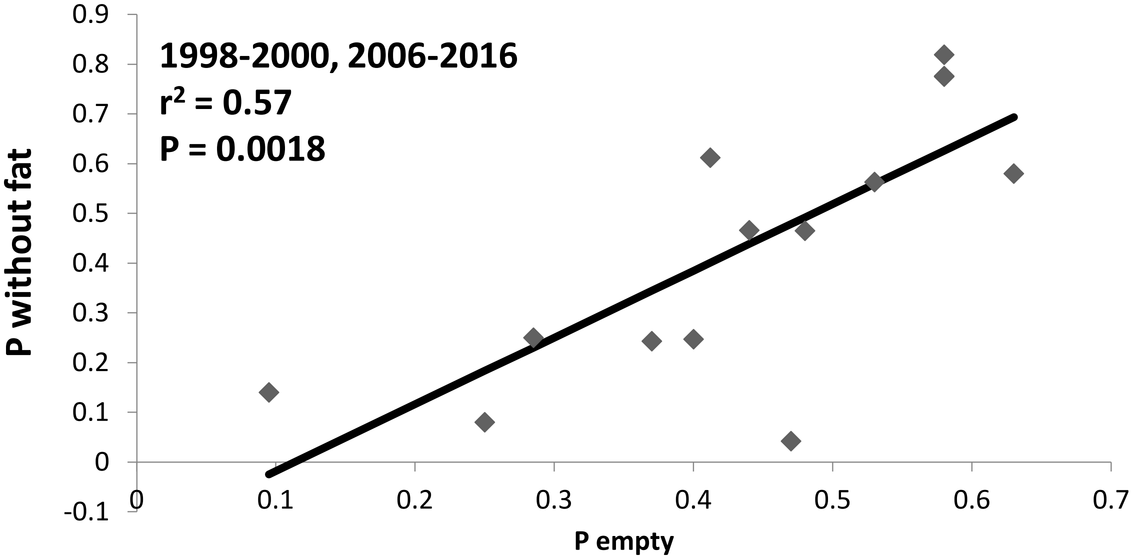


Figure 4-19. Time-series of age 3 male Striped Bass relative abundance on two major Maryland spawning areas (Hybrid index) and abundance (N) of age 3 Striped Bass along the Atlantic Coast estimated by the ASMFC (2016) statistical catch-at-age model.

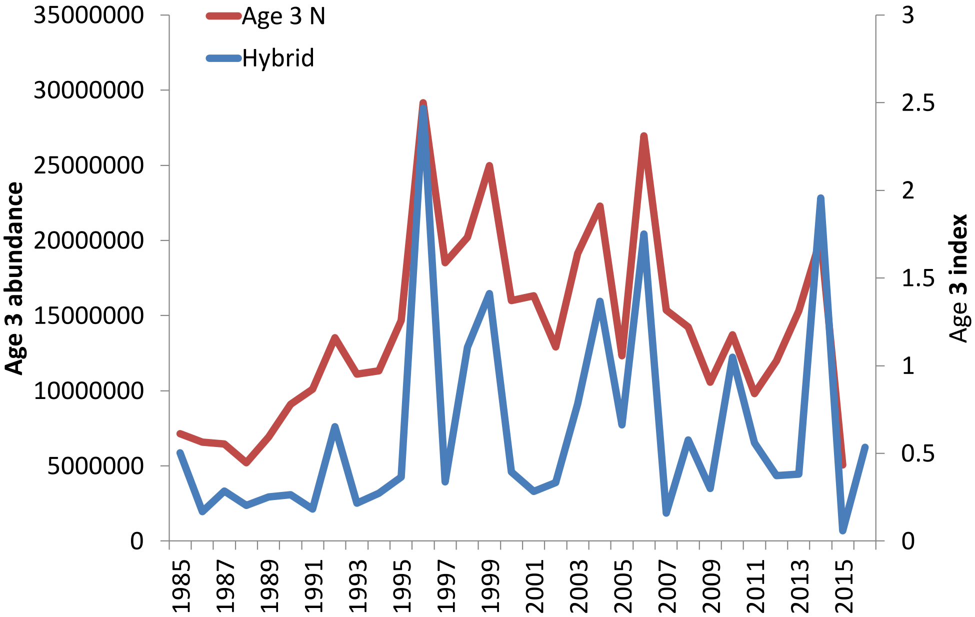


Figure 4-20. Relative survival (SR) of male Striped Bass to age-3 (hybrid gill net index / JI in yr-3) with targets and thresholds. Scale is arbitrary.

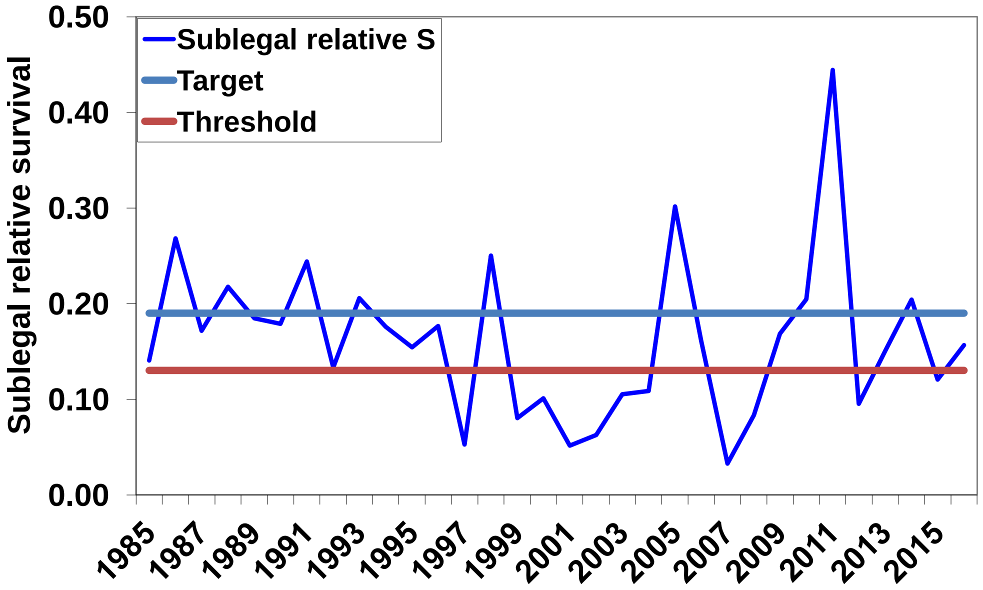


Figure 4-21. Predicted relationships of proportion of Striped Bass without body fat (P0) and relative survival to age 3 (SR). Red line = all data relationship; black line = 2004 removed; and blue line = 2004 and 2010 removed.

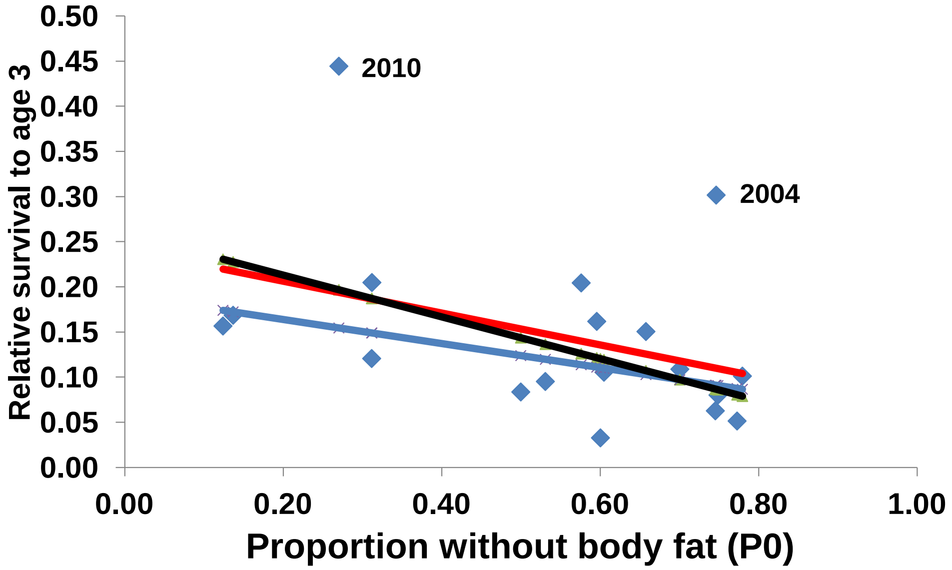


Figure 4- 22. Index of Forage (Mean IF) and its component scores. IF averages scores given to five indicators of forage status in upper Bay. A score of 5 indicates target conditions were met; 1 indicates threshold conditions; 4 indicates target was approached; 3 indicates threshold conditions were avoided; and 2 indicates threshold conditions were approached. RI = index of relative abundance of resident Striped Bass; FR = ratio of averaged major forage indices to RI; P0 = proportion of Striped Bass without body fat in fall; SR is relative survival of male Striped Bass to age 3; and PE = proportion of Striped Bass with empty guts in fall.

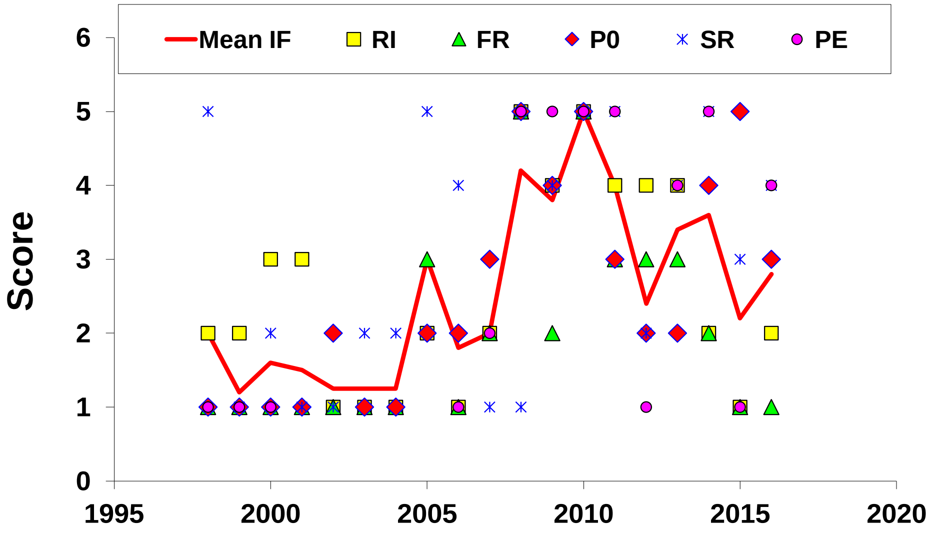
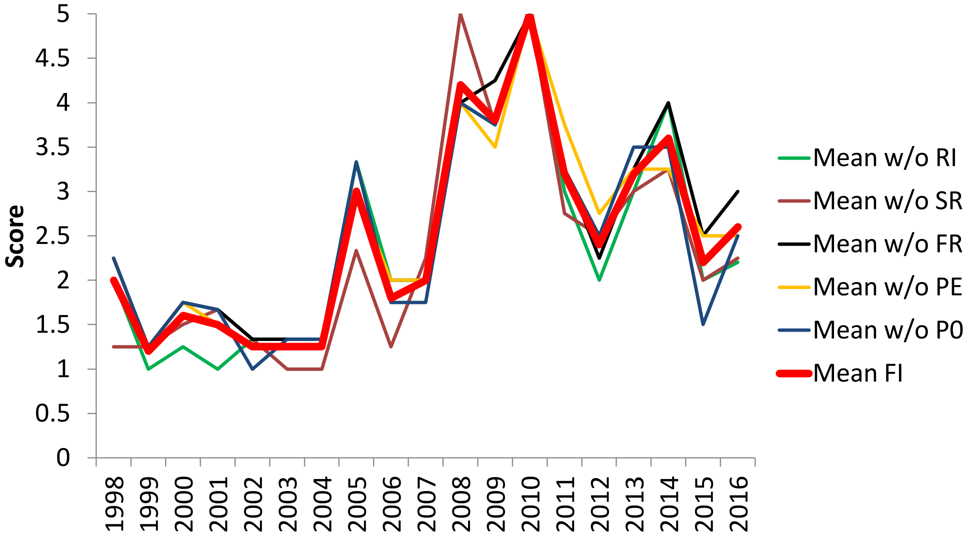


Figure 4-23. Forage index with all components averaged (Mean FI) and with each component removed. See Figure 22 for explanation of scores and abbreviations.



Appendix 1: Variance Estimation for a Ratio of Means (or Totals) from Mary Christman, University of Florida, May, 2016 personal communication to Desmond Kahn (Fisheries Investigations Inc.; Delaware Fish and Game, retired)

$$\hat{R} = \frac{\bar{Y}}{\bar{X}}$$

The $\widehat{\ }$ simply refers to the fact that this is an estimate of the true ratio and $\bar{Y}$ and $\bar{X}$ are sample means.

I am not sure what data you have so I am giving you two approaches to estimating a variance for your ratio.

Method 1. Design-Based Estimation (no assumptions about statistical properties except that randomness arises from the fact that the data are a random sample from a population of measurements).

Suppose you have a sample of n pairs $\{X_i,\ Y_i\}$ of data, e.g. Y_i is catch on the i^{th} trip and X is hrs fished on the i^{th} trip. Then, the sample CPUE (catch/hrs fished) is as above and an approximate estimator of its variance is given by

$$\widehat{var}\left(\hat{R}\right) = \frac{s_r^2}{n\bar{X}^2}$$

where

$$s_r^2 = \frac{1}{(n-1)} \sum_{i=1}^{n} \left(Y_i - \hat{R}\, X_i \right)^2.$$

The standard error of $\hat{R}$ is simply $SE\left(\hat{R}\right) = \sqrt{\widehat{var}\left(\hat{R}\right)}$.

Now, if you want the total catch for the fleet and you know the total effort over that fleet ($E = \sum_{i=1}^{N} X_i$ where N is the total number of trips in the fleet from which n was sampled) then the total catch is estimated to be

$$\hat{T} = E \times \hat{R}$$

where E is assumed to be known without error (i.e. has no variance). The estimated variance of $\hat{T}$ is simply $\widehat{var}\left(\hat{T}\right) = E^2 \times \widehat{var}\left(\hat{R}\right)$ and its standard error is $SE\left(\hat{T}\right) = E \times \sqrt{\widehat{var}\left(\hat{R}\right)}$.

Method 2. Model-Based Estimation (assumes asymptotic normality of the estimator for which the Delta Method is used).

Suppose the only information you have available are the sample means and their standard errors. If you have what most documents in fisheries refer to as a CV instead of a SE you can calculate the SE as SE = CV*Mean assuming the "CV" is actually the relative standard error. Then, there are two steps to be taken to get an estimate of the variance of $\hat{R}$.

Before starting, convert the standard errors (SE) into variances (var = SE^2).

Step 1: use the Delta Method to get an estimate of the variance of $\frac{1}{\bar{X}}$. The result is

$$\widehat{var}\left(\frac{1}{\bar{X}}\right) = \frac{1}{[\bar{X}^2]^2}\, \widehat{var}(\bar{X})$$

Step 2: use the result from Goodman (1960) for the variance of a product of two random variables.

If the two quantities used in the ratio are independent then the estimate of the variance of the ratio is given in equation 5 of Goodman (reworked here for this case):

$$\widehat{var}\left(\hat{R}\right) = \frac{\bar{Y}^2}{[\bar{X}^2]^2}\widehat{var}(\bar{X}) + \frac{1}{\bar{X}^2}\widehat{var}(\bar{Y}) - \frac{1}{[\bar{X}^2]^2}\widehat{var}(\bar{X})\widehat{var}(\bar{Y}).$$

I do not know if your quantities are in fact independent but I am assuming so here. It that is not the case, it is unlikely that you have an estimate of their covariance (or correlation) and so could only use this anyway. If you do actually have an estimate, then follow the methods described in Section 3 of Goodman.