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Report Organization

This report consists of summaries of activities for Jobs 1 – 3 under this grant. All pages are numbered sequentially; there are no separate page numbering systems for each Job. Job 1 activities are reported in separate numbered sections (Sections 1-3). Tables in Job 1 are numbered as section number – table number (1-1, 2-1, 2-2, etc). Figures are numbered in the same fashion. Jobs 2 and 3 are less complex and do not require sections.

Throughout the report, multiple references to past annual report analyses are referred to and are interrelated to current data throughout this report. 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/index.aspx.

Table of Abbreviations

°C	Celsius, temperature
α	Level of significance
μ (micron)	micrometer or one millionth of a meter
μg/L	Micrograms per liter
μmho/cm or μS/cm	Conductivity measurement as micromhos per centimeter or micro-Siemens per centimeter.
A	Area
A/ha	Structure area per hectare
ASMFC	Atlantic States Marine Fisheries Commission
BI	Blue Infrastructure
BRP	Biological reference point
C	Structures in a watershed
C / ha	Structure counts per hectare
CAD	Computer Aided Design
CBP	Chesapeake Bay Program
cfs	Cubic feet per second, measurement of flow volume
CI	Confidence Interval
COL	Cooperative Oxford Laboratory, NOAA
CPE	Catch per effort
CV	Flow coefficient of variation
DO	Dissolved oxygen
EBFM	Ecosystem-Based Fisheries Management
ER	Environmental Review Program in MD DNR
ESRI	Environmental Systems Research Institute
FERC	Federally Energy Regulatory Commission
FIBI	Fish Index of Biological Integrity (see reference Morgan et al. 2007)
GIS	Geographic Information System
gm	Gram
ha	Hectares
hr	Hour
P_i	Proportion of samples with target species *i*
IA	Impervious surface area estimated in the watershed
in	Inches
IS	Impervious surface
ISRPs	Impervious surface reference points
km	Kilometer
km^2	Square kilometers
L_P	Proportion of Tows with yellow perch larvae during a standard time period and where larvae would be expected
M	Median flow

m	Meter
Max	Maximum
MD DNR	Maryland Department of Natural Resources
MDE	Maryland Department of Environment
MDP	Maryland Department of Planning
mg/L	Milligrams per liter
Min	Minimum
mm	Millimeter
MT	Metric ton
$N_{present}$	Number of samples with herring eggs and-or larvae present
N_{total}	Total sample size
N	Sample size
NAD	North American Datum
NAJFM	North American Journal of Fisheries Management
N_i	Number of samples containing target species
NOAA	National Oceanic and Atmospheric Administration
NRC	National Research Council
OM	Organic matter
OM0	Proportion of samples without organic matter
P	Level of significance
P_{herr}	Proportion of samples where herring eggs and-or larvae were present
Pclad	Proportion of guts with cladocerans
Pcope	Proportion of guts with copepods
Pothr	Proportion of guts with “other” food items
P_0	Proportion of guts without food
P_i	Proportion of samples with a target species
pH	Concentration of hydrogen ions; the negative base-10 logarithm of hydrogen ion concentration.
ppt or ‰	Parts per thousand, salinity measurement unit
QA	Quality assurance
r	Correlation coefficient, statistical measurement
RKM	River kilometer
SAS	Statistical Analysis System
SAV	Submerged aquatic vegetation
SD	Standard deviation
SE	Standard error
TA	Estimate of total area of the watershed
TEA	Tidal Ecosystem Assessment Division in MD DNR
TL	Total length
USACOE	United States Army Corps of Engineers
USFWS	United States Fish and Wildlife Service
USGS	United States of Geological Services
V_{target}	Percentage of DO measurements that met or fell below the 5 mg/L target

$\mathbf{V}_{\mathbf{threshold}}$	Percentage of DO measurements that fell at or below the 3 mg/L threshold

Definitions

Alosines — American shad, hickory shad, blueback herring, and alewife are Alosines, which belong to the herring family, Clupeidae.

Anadromous Fish (Spawning) — Fish, such as shad, herring, white perch, and yellow perch, ascend rivers from the Chesapeake Bay or ocean for spawning.

Brackish Water — Water that has more salinity than freshwater. The salinity of brackish water is between 0.5 – 30 ppt.

Coastal Plain — An area underlain by a wedge of unconsolidated sediments including gravel, sand, silt and clay and is located in the eastern part of Maryland, which includes the Chesapeake Bay's eastern and western shores, up to the fall line roughly represented by U.S. Route 1.

Development — Refers to land used for buildings and roads.

Estuary — A body of water in between freshwater and the ocean; an estuary can be subject to both river and ocean influences, such as freshwater, tides, waves, sediment, and saline water.

Finfish — Referring to two or more species of fish and excludes shellfish.

Floodplain — Refers to land that is adjacent to a stream or river that experiences flooding during periods of high flow.

Fluvial — Of or pertaining to rivers.

Fresh-Tidal Sub estuary — An area containing mainly freshwater with salinity less than 0.5 ppt, but tidal pulses may bring higher salinity.

Hypoxia — Occurrence of low oxygen conditions.

Icthyoplankton — Refers to the eggs and larvae of fish.

Impervious surface (IS) — Hard surfaces that are not penetrated by water such as pavement, rooftops, and compacted soils.

Mesohaline	A region within an estuary with a salinity range between 5 and 18 ppt.
Non-Tidal Waters (Stream)	Areas that are not influenced by tides.
Oligohaline Subestuary	A brackish region of an estuary with a salinity range between 0.5 and 5 ppt.
Piedmont	A plateau region located in the eastern United States and is made up of low, rolling hills that contain clay-like and moderately fertile soils.
Planktivores	Animals that feed primarily on plankton (organisms that float within the water column).
Richness	The number of different species represented in a collection of individuals.
Riparian zone	The area between land and a river and/or stream, also known as a river bank.
Rural	Referring to areas undeveloped such as farmland, forests, wetlands and areas with low densities of buildings.
Stock Assessments	Assessments of fish populations (stocks); studies of population dynamics (abundance, growth, recruitment, mortality, and fishing morality).
Stock Level	Refers to the number or population weight (biomass) of fish within a population.
Subestuary	A smaller system within a larger estuary such as a branching creek or tributary within the estuary.
Suburb	An area that has mostly residential development located outside of city or town boundaries.
Threshold	A breaking point of an ecosystem and when pressures become extreme can produce abrupt ecological changes.
Tidal Waters	Waters influenced by tides.

Urban	A developed area characterized by high population, building, and road densities; may be considered a city or town.
Urbanization	Process of conversion of rural land to developed land.
Watershed	Defines a region where all of the water on and under the land drains into the same body of water.
Wetlands	An area of ground that is saturated with water either permanently or seasonally; they have unique vegetation and soil conditions and can either be saltwater, freshwater, or brackish depending on location.
Zooplankton	Animals that drift within the water column; these animals are typically very small, but may be large (jellyfish and comb jellies).

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

Jim Uphoff, Margaret McGinty, Alexis Maple, Carrie Hoover and Paul Parzynski

Executive Summary

Stream Ichthyoplankton - Historical collection methods (stream drift nets) were used to sample of anadromous fish eggs and larvae in Mattawoman Creek (2008-2012), Piscataway Creek (2008-2009 and 2012), Bush River (2005-2008) and Deer Creek (2012; Figure 1-1). All of these watersheds started at approximately 0.05 C / ha in 1950. In 2009, Bush River (without largely undeveloped Aberdeen Proving Grounds or APG) and Piscataway Creek were at substantially higher levels of development (≈1.40 C / ha, respectively) than Mattawoman Creek (0.88 C / ha). Deer Creek was added in 2012 as a spawning stream with low watershed development (0.24 C /ha). We compared detection of eggs or larvae of white perch, yellow perch, and herring eggs and-or larvae at stream sites in the 2000s to the 1970s (and 1989-1991 in Mattawoman Creek) and to C / ha. We also used another indicator of relative abundance, proportion of samples with eggs and-or larvae of anadromous fish groups, from collections in the 2000s and compared it to C / ha and summarized conductivity data. Conductivity (considered a water quality indicator of development) was standardized to the background level expected in Coastal Plain and Piedmont provinces.

Proportion of samples with herring eggs or larvae (P_{herr}) provided an estimate of relative abundance based on encounter rate that was sufficiently precise for analyses with C / ha and conductivity. Correlation analyses indicated significant and logical

associations among P_{herr}, C / ha and conductivity consistent with the hypothesis that urbanization was detrimental to stream spawning. Conductivity was positively associated with C / ha in our analysis and P_{herr} was negatively associated with C / ha and conductivity. Increased conductivity from urbanization is likely to reflect road salt use. At least two hypotheses can be formed to relate decreased herring spawning to 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 and disrupted upstream migration. Urbanization and physiographic province affect discharge and sediment supply of streams that could affect location, substrate composition, extent and success of herring spawning as well.

Herring spawning became more variable in streams as watersheds developed. The two surveys from watersheds with C / ha of 0.46 or less both had high P_{herr}. Estimates of P_{herr} from Mattawoman Creek 2008-2012 varied from barely different from zero to moderate to high (C / ha was 0.85-0.90). Eggs and larvae were nearly absent from fluvial Piscataway Creek during 2008-2009, but P_{herr} rebounded to 0.45 in 2012 (C / ha was 1.39-1.45). Variability of herring spawning in Bush River during 2005-2008 involved detection of spawning at new sites as well as absence from sites where spawning was detected in the past. Variability in P_{herr} at higher levels of development could signify creation and deterioration of ephemeral spawning habitat resulting from a combination of urban and natural stream processes. Magnitude of P_{herr} may indicate how much habitat is available from year to year rather than abundance of spawners.

Summarizing spawning activity as the presence of any species group's egg or larvae at a site encompassed more species groups (white perch, yellow perch and herring) than proportion of samples. However, it represented a presence-absence design with limited ability to detect population changes or conclude 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 was lost by summarizing all samples into a single record of occurrence in a sampling season. Loss of yellow perch stream spawning sites coincided with increased development. We demonstrated changes in stream spawning site occupation of white perch and herring between the 1970s and 2000s, but were unable to conclude that development had an impact with this estimator.

Estuarine Yellow Perch Larval Sampling - We examined hypotheses that development negatively influenced two processes important for yellow perch year-class formation: egg-larval survival and larval feeding success. Emphasis was on the latter hypothesis. Urbanization was expected to negatively impact yellow perch larval feeding success because it affects the quality and quantity of organic matter (OM) in streams and was negatively associated with extent of wetlands (an estuarine source of OM) in many subestuary watersheds. We hypothesized that development negatively influenced watershed OM supply and transport, altering zooplankton production important for yellow perch larval feeding success and survival (the OM hypothesis). We tested the OM hypothesis with analyses of 2010-2012 larval feeding. We also used statistical analyses of historical relative abundance of larvae (1965-2012) or juveniles (1968-2012) and environmental data that would reflect processes important for OM dynamics.

Presence-absence sampling for yellow perch larvae during 2012 was conducted in the upper tidal reaches of the Nanticoke, Northeast, Elk, Middle, Patuxent, and Bush rivers and Mattawoman, Nanjemoy, and Piscataway creeks during late March through April. We also ranked the relative level of organic matter (OM) in each sample. Annual L_p (proportion of tows with yellow perch larvae during a standard time period and where larvae would be expected) was used as an index of relative abundance of early postlarvae.

During 2010-2012, we collected composite samples of early feeding larvae from several sites on several subestuaries during several sample trips and examined them to rank how full their guts were. Major food items were classified as copepods, cladocerans, or other and their presence or absence was noted. Approximately 1,500 larvae were examined during 2010-2012.

Long-term environmental data from rural watersheds were analyzed to explore if OM transport would positively influence L_p and yellow perch juvenile indices (YPJ) under low levels of development. Detection of a positive influence of precipitation would support the OM hypothesis. Precipitation is frequently used in statistical analyses as an indicator related to the basic processes of OM transfer. Both L_p and YPJ data sets had observations back to the 1960s. Regression analyses of YPJ were confined to the Head-of-Bay since this was the only reliable regional time-series available. Estimates of L_p were drawn from several rural watersheds. Air temperature and Susquehanna River flow (Head-of-Bay only) were added as candidates. Zooplankton data from Chesapeake Bay monitoring was available for Head-of-Bay comparisons.

During 2012, we collected yellow perch larvae for analysis of the ratio of ribonucleic acid (RNA) concentration to deoxyribonucleic acid (DNA) concentration in body tissue when larvae were gathered for food analysis. This ratio is a useful indicator of nutritional status (starved or fed) and growth in larval fish that provided a method for examining connections of feeding success and larval condition. We expected RNA/DNA ratios to be lower in more developed watersheds.

In this report, we provided evidence that (1) development negatively influenced L_p, OM supply, and first feeding success; (2) March temperature conditions influenced L_p; and (3) low L_p in well developed watersheds was consistent with contaminant-related biological changes implicated in low egg hatching success. Our results suggest a general sequence of stressors that impacted yellow perch larvae as development increased. Feeding success declined as development proceeded past the target level of development, implying initial stress related to disruption of OM dynamics. Persistent reductions of egg and prolarval survival followed in highly developed subestuaries and endocrine disrupting contaminants are suspected as the major stressor.

Estimates of L_p declined perceptibly once development exceeded the threshold (0.83 C / ha or 10% IS). Extensive forest cover in a watershed generally resulted in higher L_p (median $L_p = 0.80$) than agriculture or development. Estimates of L_p from agricultural watersheds below the target level of development (median $L_p = 0.52$) were variable, but generally higher than suburban watersheds (median $L_p = 0.30$). Interpretation of the influence of primary 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 (0.27 C / ha or 5% IS; forested and agricultural watersheds) or at and beyond high levels of development (1.59 C / ha or 15% IS; urban watersheds) were from brackish subestuaries; estimates of L_p for development between these levels were from fresh-tidal subestuaries with forested watersheds.

Statistically significant results of all analyses were consistent with the OM hypothesis. Our index of early larvae relative abundance (L_p) was negatively related to development level (C / ha). Development level was positively correlated with an absence of organic matter in sample jars (OM0 or proportion of samples without OM) and OM0 was negatively correlated with how successful larvae were feeding on zooplankton. The forward selection procedure that parameterized multiple regression models of historical time-series of L_p and YPJ in rural watersheds from a set of potential environmental factors selected March precipitation, a variable representing mobilization of OM. The multiple regression models used to describe relationships of L_p or YPJ versus precipitation (and other significant environmental factors) in rural watersheds explained modest amounts of variation.

Estimates of L_p in the most rural subestuaries sampled during 2012 appeared anomalously low (except Elk and Northeast rivers) compared to other years, particularly in the most rural and southerly subestuaries (Nanjemoy and Nanticoke rivers). Average air temperatures in March 2012 were higher than other years (1965-2012), while precipitation was low but not the lowest. Average March air temperatures were 1.2 and 1.8 °C higher in weather stations for Nanjemoy Creek and Nanticoke River, respectively, than for the Head-of-Bay region. Yellow perch require a period of low temperature for reproductive success and warm temperatures may have precluded that from occurring in all but the most northerly subestuaries studied in 2012.

Our RNA/DNA sampling during 2012 found that most yellow perch larvae collected from subestuaries over a broad geographic expanse and throughout the season were in the starved category. We did not interpret RNA/DNA ratios as rejecting or supporting the OM hypothesis since there was very little variation in OM among systems in 2012, very low sample sizes in southern MD, and an indication that *ad hoc* collections in the Head-of-Bay region may have induced bias due to date of collection. We intended to collect larvae for RNA/DNA analysis from a regional urban gradient represented by Piscataway, Mattawoman and Nanjemoy creeks' watersheds. This design, based on several previous years' collections, had to be abandoned because L_p was so low in these systems due to poor egg and prolarval survival that *ad hoc* collections were added from Northeast River and Bush River.

Estuarine Fish Community Sampling – We sampled summer fish assemblages using seines and bottom trawls in twelve subestuaries. Mattawoman Creek, Piscataway Creek and Northeast River were classified as tidal-fresh; Gunpowder River, Middle River, Bush River and Nanjemoy Creek were oligohaline, and Tred Avon, Broad Creek, Harris Creek, Middle River and Wicomico River were mesohaline (Figure 3-1). Harris Creek and Piscataway Creek were the only subestuaries that did not have violations in oxygen criteria (0 observations of violations of $V_{threshold}$). Bush River, Corsica River, Middle River and Wicomico River had bottom oxygen concentrations below V_{target} more than 10% of the time. When we examined the relationship of temperature and C / ha on dissolved oxygen conditions, we did not find significant associations between temperature, dissolved oxygen and C / ha in oligohaline subestuaries, but found a positive association between both surface temperature and surface dissolved oxygen and C / ha.

Surface dissolved oxygen was positively correlated with temperature, and bottom oxygen was negatively correlated with C / ha in mesohaline subestuaries. This suggested that stratification in mesohaline waters is important for persistent hypoxic condition in urbanized subestuaries.

Effects of urbanization on white perch adults were documented by exploring the relationship between C / ha and P_i in bottom trawls. We found a negative relationship (r^2 =0.55, P , 0/0001) between white perch adult P_i and C / ha. Examination of residual output suggested white perch respond to the development threshold in a boom or bust fashion in tidal fresh subestuaries.

Median bottom oxygen has declined in Mattawoman since 1989; however, median concentrations have not fallen below $V_{target.}$ Over this same time-series, housing development continued to increase. We regressed C / ha against year for two different time periods (1989-2000 and 2001-2010) and found that the slope of the regression after 2000 was significantly higher in the latter time frame, indicating more rapid development of the landscape. We observed a marked increase in SAV occurring around 2000, attended by a decrease in chlorophyll *a* concentrations and accelerated decline in relative abundance of fish in trawl samples after 2000. Fish community comparisons among these two timeframes, using spearman rank correlation, did not prove to be statistically different. However, several changes in the fish community may have ecological significance. After 2000, total fish abundance in bottom off shore habitat declined, along with presence of three important pelagic plankton feeders (bay anchovy, gizzard shad and blueback herring). During this same timeframe, white perch, spottail shiner, bluegill and pumpkinseed have remained the same or increased in presence. These latter species feeding strategies are not dependant on pelagic food sources. These species shift suggest a major trophic shift potentially related to increased coverage of SAV driven by development. When evaluated by the Chesapeake Bay Program's habitat goals, Mattawoman Creek meets water quality goals associated with a restored system, however its tidal fish community diversity and function as an anadromous fish spawning and nursery habitat have declined.

Job 1 Introduction

Fisheries management uses biological reference points to determine how many fish can be safely harvested from a stock (Sissenwine and Shepherd 1987). The primary objective of Project 1 was to devise reference points for development as a similar tool for fish habitat management. Creating reference points that indicate safe and unsafe watershed stress from development involves determining functional relationships between an indicator of watershed development and habitat quality (water quality, physical structure, etc) or a species response (habitat occupation, abundance, distribution, mortality, recruitment success, growth, fish condition, etc). Quantitative, habitat-based reference points are envisioned as a basis for strategies for managing fisheries in increasingly urbanizing coastal watersheds and for communicating the limits of fisheries resources to withstand development-related habitat changes to stakeholders and agencies involved in land-use planning.

Maryland Fisheries Service has chosen counts of structures per watershed hectare from Maryland Department of Planning property tax map data as our indicator of development. Tax map indicators are standardized, annually updated, readily accessible and based on observed quantities (structure counts or structure area), and are strongly related to impervious surface estimated from satellite images (Uphoff et al. 2012).

Fisheries managers in Maryland do not have authority to manage land-use, so they need to consider managing fish differently at different levels of development if productivity diminishes. The target level of development for fisheries is indicated by about 0.27 structures per hectare (C / ha) or less (~ 5% impervious surface or IS; Uphoff et al. 2012). This target level of development in Maryland is characterized by forests, working farms, and wetlands that support productive fish habitat and fisheries. Land-use at this level does not undermine effectiveness of harvest controls for sustaining fish populations. Conserving watersheds at this level of development would be ideal. Once above this level of development, increasing consideration has to be given to habitat conservation, watershed revitalization (small scale ecological re-engineering), and watershed reconstruction (large scale ecological re-engineering). Revitalization and reconstruction could consist of measures such as road salt management, stemming leaks in sewage pipes, improving septic systems, stormwater retrofits, stream rehabilitation, replenishment of riparian buffers, creation of wetlands, planting upland forests, and "daylighting" of buried streams. Lowering harvest levels may be able to offset habitat degradation, but places the burden of development on anglers.

The threshold of development of 0.83 C / ha (10% IS) represents a suburban landscape where serious aquatic habitat degradation becomes apparent (Uphoff et al. 2012). At this point, conservation of remaining natural lands and habitat revitalization and reconstruction will be the primary tools for fishery sustainability. Harvest restrictions may be ineffective in stemming fishery declines. By 1.59 C / ha (15% C / ha), serious habitat problems make fish habitat revitalization very difficult. Managers must deal with substantially less productive fisheries.

Job 1 activities in 2012 included spring stream anadromous fish icthyoplankton collections, spring yellow perch larval presence-absence sampling, and summer sampling of estuarine fish communities and habitat. These activities are reported as separate sections in Job 1. These efforts were collectively aimed at defining the impact of development on target fish species populations and habitats and judging how development reference points proposed by Uphoff et al. (2011a) for brackish subestuaries (based on dissolved oxygen and habitat occupation by juveniles and adults of our target species) apply to Tax Map data, and other life stages and habitats.

Job 1 Section 1 - Stream Ichthyoplankton Sampling

Introduction

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 provides an opportunity to explore whether spawning habitat declined in response to urbanization. Surveys based on the sites and methods of O'Dell (1975) were used to sample Mattawoman Creek (2008-2012), Piscataway Creek (2008-2009 and 2012), Bush River (2005-2008) and Deer Creek (2012; Figure 1-1).

Mattawoman and Piscataway Creeks are adjacent Coastal Plain watersheds along an urban gradient emanating from Washington, DC (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 (Figure 1-1; Clearwater et al. 2000).

We developed two sets of indicators of anadromous fish spawning. Occurrence of anadromous fish group's (white perch, yellow perch, and herring) eggs and-or larvae was considered evidence of spawning at sites, recreating the indicator developed by O'Dell et al.(1975; 1980). This indicator was compared to the extent of development in the relevant watershed (counts of structures per hectare or C / ha) between the 1970s and the present. We also developed an indicator of relative abundance, proportion of samples with eggs and-or larvae of anadromous fish groups, from collections in the 2000s and compared it to C / ha and summarized conductivity data. Conductivity was monitored during these volunteer surveys and these data were used to examine whether urbanization had affected stream water quality. Increases in conductivity have been strongly associated with urbanization (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 the anadromous fish eggs and larvae during 2005-2012 were typically at road crossings that O'Dell et al. (1975) determined were anadromous fish spawning sites during the 1970s. O'Dell et al. (1975) summarized spawning activity as the presence of any species group egg, larva, or adult at a site. Eggs and larvae were sampled from stream drift ichthyoplankton nets and adults were sampled by wire traps.

All collections during 2005-2012, with the exception of Deer Creek during 2012, were made by citizen volunteers trained and monitored by program biologists. During March-May, 2008-2012, ichthyoplankton samples were collected in Mattawoman Creek from three tributary sites (MUT3-MUT5) and four mainstem sites (MC1-MC4; Figure 1-2; Table 1-1). Tributary site (MUT4) was selected based on volunteer interest and added in 2010. Piscataway Creek stations were sampled during 2008-2009 and 2012 (Figure 1-3; Uphoff et al. 2010). Bush River stations were sampled during 2005-2008 (Figure 1-4; McGinty et al. 2009). Deer Creek was added to sampling in 2012 (Figure 1-5). Table 1-

1 summarizes sites, dates, and sample sizes in Mattawoman, Piscataway and Deer Creeks, and Bush River during 2005-2012.

Ichthyoplankton samples were collected at each site 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. 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 in the jar. The jar was sealed and placed in a cooler with ice for transport when collections were made by volunteers. Preservative was not added by volunteers at a site because of safety and liability concerns. Formalin was added on site by DNR personnel. Water temperature (°C), conductivity (µS / cm), and dissolved oxygen (DO, mg / L) were recorded at each site using a hand-held YSI Model 85 meter. Meters were calibrated for DO each day prior to use. All data were recorded on standard field data forms and verified at the site by a volunteer.

After a team finished sampling for the day, the samples were preserved with 10% buffered formalin. Approximately 2-ml of rose bengal dye was added in order to stain the organisms red 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 fixed with formaldehyde 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 small labeled (site, date, time, and QA) vial and fixed with formaldehyde 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, hickory shad, and American shad), yellow perch, white perch, unknown (eggs and-or larvae were too damaged to identify) or other (indicating another fish species) and a total count (combining both original and QA vials) for each site was recorded, as well as the presence and absence of each of the above species. The four herring species' eggs and larvae are very similar (Lippson and Moran 1974) and identification to species can be problematic.

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). This indicator has been provided to us by Marek Topolski of the Fishery Management Planning and Fish Passage Program. Tax maps are graphic representations of individual property boundaries and existing structures that help State tax assessors locate properties (Maryland Department of Planning or MDP 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. Each year's statewide tax map was clipped using the MD 8-digit watershed boundary file, modified to exclude estuarine waters, to create watershed land tax maps. 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 present a problem since we are interested in the trend and not absolute magnitude (Uphoff et al. 2012).

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 estimated as 0.27, 0.83, and 1.59 C / ha, respectively (Uphoff et al. 2012).

Estimates of C / ha were available from 1950 through 2010 (M. Topolski, MDDNR, personal communication). Estimates of C / ha for 2010 were used to represent 2011 and 2012.

Mattawoman Creek's watershed equaled 25,168 ha and estimated C / ha was 0.85-0.90 during 2008-2012; Piscataway Creek's watersheds equaled 17,999 ha and estimated C / ha was 1.37-1.45 during 2008-2012; and Bush River's watershed equaled 44,167 ha and estimated C / ha was 1.37-1.43 during 2005-2008; M. Topolski, MD DNR, personal communication). Deer Creek (Figure 1-1), a tributary of the Susquehanna River, was added in 2012 as a spawning stream with low watershed development (watershed area = 37,701 ha and development level = 0.24 C /ha; M. Topolski, MD DNR, personal communication). It was sampled 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 2008-2012 from Mattawoman Creek were plotted and mainstem measurements were summarized for each year. 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. A subset of Bush River stations that were sampled each year during 2005-2008 (i.e., stations in common) were summarized and stations within largely undeveloped Aberdeen Proving Grounds were excluded (these were not sampled every year). Conductivity measurements were also collected for each date and stream site in Deer Creek in 2012.

A water quality database maintained by DNR's Tidewater Ecosystem Assessment (TEA) Division (S. Garrison, MD DNR TEA, personal communication) provided conductivity measurements for Mattawoman Creek during1970-1989. These historical measurements were compared with those collected in 2008-2012 to examine changes in conductivity over time. Monitoring was irregular for many of the historical stations.

Table 1-2 summarizes site location, month sampled, total measurements at a site, and what years were sampled. Historical stations and those sampled in 2008-2012 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- 2012 spawning season median conductivities.

Presence of white perch, yellow perch, and herring eggs and-or larvae at each station in 2012 was compared to past surveys to determine which sites still supported spawning. We used the criterion of detection of eggs or larvae at a site (O'Dell et al. 1975) 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 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 (2008-2012), 1971 (Odell 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 and 2008-2009) and Bush River (1973 and 2005-2008 (McGinty et al. 2009; Uphoff et al. 2010).

The proportion of samples where herring eggs and-or larvae were present (P_{herr}; was estimated for Mattawoman Creek mainstem stations (MC1-MC4) during 1991 and 2008-2012. Volunteer sampling of ichthyoplankton in Piscataway Creek (2008-2009 and 2012), Bush River (2005-2008; McGinty et al. 2009), and Deer Creek (2012) also provided sufficient sample sizes to estimate P_{herr} for those locations and years. 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. Streams that were sampled in all years in Bush River were analyzed.

For the rivers and stations described above, the proportion of samples with herring eggs and-or larvae present was estimated as $P_{herr} = N_{present} / N_{total}$; where $N_{present}$ equaled the number of samples with herring eggs or larvae present and N_{total} equaled the total number of samples taken. The SD of each P_{herr} was estimated as

$$SD = [(P_{herr} \bullet (1- P_{herr})) / N_{total}]^{0.5} \quad \text{(Ott 1977).}$$

The 90% confidence intervals were constructed as $P_{herr} \pm (1.44 \bullet SD)$.

Correlation analysis was used to examine associations among development (C / ha), summarized conductivity measurements (median conductivity adjusted for Coastal Plain or Piedmont background level; see below), and herring spawning intensity (P_{herr}) in Bush River and Mattawoman, Piscataway, and Deer Creeks. Fourteen estimates of C / ha and P_{herr} were available (1991 estimates for Mattawoman Creek could be included), while thirteen estimates were available for conductivity (Mattawoman Creek data were not available for 1991). 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).

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 with the other option 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 adjust median conductivities was 109 μS / cm in Coastal Plain streams and 150 μS / cm in Piedmont streams.

Correlations were considered significant at $P \leq 0.05$. We expected negative correlations of P_{herr} with C / ha and standardized conductivity, while standardized conductivity and C /ha were expected to be positively correlated.

Results

Development level of the watersheds of Piscataway, Mattawoman, and Deer creeks and Bush River started at approximately 0.05 C / ha in 1950, (Figure 1-6).

Surveys conducted by O'Dell in the 1970s sampled largely rural watersheds (C / ha $\leq$ 0.27) except for Piscataway creek (C / Ha = 0.47). By 1991, C / ha in Mattawoman Creek was similar to that of Piscataway in 1971. 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, remained rural through 2012 (0.24 C / ha; Figure 1-6).

In 2012, conductivity in mainstem Mattawoman Creek was steady after mid-March and was slightly higher than the 1991 maximum (114 μS / cm; Figure 1-7). Five of 11 measurements at MC1 and one measurement each at MC2 and MC3 (March 1) fell below the 1991 maximum. Conductivity in the tributaries MUT 3-5 all fell within or below the range reported by Hall et al. (1992) for the mainstem. This general pattern has held for years that conductivity was monitored. 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. There was a general pattern among years of higher conductivity at the most upstream mainstem site (MC4) followed by declining conductivity downstream to the site on the tidal border. This pattern and low conductivities at the unnamed tributaries indicated that development at and above MC4 was affecting water quality (Figure 1-7).

Conductivity levels in Piscataway Creek and Bush River were elevated when compared to Mattawoman Creek (Table 1-3). 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 in excess of 200 µS / cm during 2009 and was less than 155 µS / cm during the remaining four years.

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 1-8). Conductivity medians were highly variable at the upstream station nearest Waldorf during 1970-1989. During 2008-2012 (C / ha = 0.85-0.90), 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 1-8). None of the site conductivity medians estimated at any site during 2008-2012 were at or below the Coastal Plain stream background criterion.

Anadromous fish spawning site occupation in fluvial Mattawoman Creek improved during 2008-2012 but was less consistent than during 1971 and 1989-1991 (historical spawning period; Table 1-4). Herring spawning was detected during 2008-2012 at historical mainstem stations. Herring spawning was absent at stations MC2, MC4, and MUT3 during 2008-2009. Site occupation has increased since 2009 and all four mainstem stations had herring eggs and-or larvae during 2010-2011. Herring spawning was detected at MUT 3 in 2011, and MUT3 and MUT 4 in 2012. Herring spawning was detected at all mainstem stations in 1971 and 1991. Stream spawning of white perch in Mattawoman Creek was not detected during 2009, 2011, and 2012, but spawning was detected at MC1 during 2008 and 2010. During 1971 and 1989-1991, white perch spawning occurred annually at MC1 and intermittently at MC2; these two stations were represented every year. Prior to 2008-2012, MC3 was sampled in 1971 and 1991 and white perch were only present during 1971. Yellow perch spawning occurred at station MC1 every year except 2009 and 2012. Station MC1 was the only stream station in Mattawoman Creek where yellow perch spawning has been detected in surveys conducted since 1971 (Table 1-4).

Herring spawning was detected at all mainstem sites in Piscataway Creek in 2012. Stream spawning of anadromous fish had nearly ceased in Piscataway Creek between 1971 and 2008-2009 (Table 1-5). 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 but has not been detected during 2008-2009 and 2012 (Table 1-5).

There was no obvious decline in herring spawning in the Bush River stations between 1973 and 2005-2008, but occurrences of white and yellow perch became far less frequent (Table 1-6). During 1973, herring spawning was detected at 7 of 12 Bush River stream sites sampled; however, during 2005-2008 herring spawning was detected in as few as 5 of 12 sites or as many as 8 of 8 sites sampled in the Bush River. White perch spawning in the Bush River was detected at 8 of 12 sites sampled during 1973 and at one site in one year during 2005-2008. The pattern of stream spawning site occupation of yellow perch in Bush River was similar to that of white perch spawning. Yellow perch spawned at 5 of 12 sites during 1973. Yellow perch spawning was not detected during 3 of 4 surveys during 2005-2008, but was detected at 4 of 12 sites during 2006 (Table 1-6).

Deer Creek was added to the study in 2012 and herring spawning was detected at all sites sampled (Table 1-7). White perch spawning was not detected in Deer Creek, while yellow perch spawning was detected at the two stations closest to the mouth (Table 1-7). Only three sites were sampled during 1972 in Deer Creek, with one of these sites located upstream of an impassable dam near Darlington. Herring spawning was detected at both sites below the dam, while yellow perch and white perch were only detected at the first site, located closest to the mouth (Table 1-7).

The 90% confidence intervals of P_{herr} (Figure 1-9) 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 in Mattawoman Creek was categorized at levels 1 (2008-2009), 2 (2010 and 2012), and level 3 (1991, and 2011). Spawning in Piscataway Creek was at level 0 during 2008-2009 and level 2 during 2012. Bush River spawning was characterized by levels 0 (2006) and 1 (2005 and 2007-2008). Deer Creek, with the least developed watershed, was characterized by the highest level of spawning (level 3) during 2012 (Figure 1-9).

Correlation analyses indicated significant and logical associations among $P_{herr,}$ C / ha, and standardized median conductivity. The correlation of C / ha with standardized median conductivity was significant and positive (r = 0.55, P = 0.05, N = 13; Figure 1-10). Estimates of P_{herr} were significantly and negatively correlated with C / ha (r = -0.76, P = 0.002, N = 14) and standardized median conductivity (r = -0.56, P = 0.05, N = 13; Figure 1-11).

Discussion

Proportion of samples with herring eggs or larvae (P_{herr}) provided an estimate of relative abundance based on encounter rate that was sufficiently precise for analyses with C / ha and conductivity (considered a water quality indicator of development). Correlation analyses indicated significant and logical associations among P_{herr}, C / ha and conductivity consistent with the hypothesis that urbanization was detrimental to stream spawning. Conductivity was positively associated 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). 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 with a strong negative threshold at low levels of development.

An unavoidable assumption of the correlation analysis of P_{herr}, C / ha, and summarized conductivity was that watersheds at different levels of development were used as a substitute for time-series. Extended time-series of watershed specific data were not available. Mixing physiographic provinces in this analysis had the potential to increase scatter of points, although standardizing median conductivity to background conductivity should have moderated the province effect in analyses with that variable.

Differential changes in physical stream habitat and flow due to differences in geographic provinces could also have affected correlations.

Elevated conductivity, related primarily to chloride from road salt, has emerged as an indicator of watershed development (Wenner et al. 2003; Kaushal 2005; Morgan et al. 2007; Morgan et al. 2012). Most inorganic acids, bases, and salts are relatively good conductors, while organic compounds that do not dissociate in aqueous solution conduct current poorly (APHA 1979). 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 2005; Wheeler et al. 2005; Morgan et al. 2007; 2012). Commonly used anti-clumping agents (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 and disrupted upstream migration.

Levels of salinity associated with our conductivity measurements would be very low and anadromous fish spawn successfully in brackish water (Klauda et al. 1991; Piavis et al. 1991; Setzler-Hamilton 1991), although 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 could prevent anadromous fish from recognizing and ascending streams. Alewife and 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 migration in anadromous American shad and salmon has 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).

Physical characteristics of streams are influenced by geographic province. Processes such as flooding, riverbank erosion, and landslides vary by geographic province (Cleaves 2003). Unconsolidated sediments (layers of sand, silt, and clay) underlie the Coastal Plain and broad plains of low relief and wetlands characterize the terrain (Cleaves 2003). Coastal Plain streams have low 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) and 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, extent and success of spawning. Alewife spawn in sluggish water 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). Detritus loads in subestuaries are strongly associated with development (see Section 1-3) and urbanization affects the quality and quantity of organic matter in streams (Paul and Meyer 2001) that feed into subestuaries.

Herring spawning became more variable in streams as watersheds developed. The two surveys from watersheds with C / ha of 0.46 or less both had high P_{herr}. Estimates of P_{herr} from Mattawoman Creek 2008-2012 varied from barely different from zero to moderate to high (C / ha was 0.85-0.90). Eggs and larvae were nearly absent from fluvial Piscataway Creek during 2008-2009, but P_{herr} rebounded to 0.45 in 2012 (C / ha was 1.39-1.45). Variability of herring spawning in Bush River during 2005-2008 involved “colonization” of new sites as well as absence from sites of historical spawning.

Variability in P_{herr} at higher levels of development could signify creation and deterioration of ephemeral spawning habitat resulting from a combination of urban and natural stream processes. Magnitude of P_{herr} may indicate how much habitat is available from year to year more-so than abundance of spawners and egg and larval survival.

Stock assessments have identified that many populations of river herring (alewife and blueback herring) along the Atlantic coast, including those in Maryland, are in decline or are at depressed stable levels (ASMFC 2009; 2009b; Limburg and Waldman 2009; Maryland Fisheries Service 2012). However, variation in P_{herr} would indicate wide annual and regional fluctuations in population size.

Application of presence-absence data in management needs to consider whether absence reflects a disappearance from 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) summarized spawning activity as the presence of any species group's egg, larva, or adult (latter from wire trap sampling) 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 approach represented a presence-absence design with limited ability to detect population changes or conclude 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. Site occupation in Mattawoman Creek changed little, if at all, between 1971 and 1989-1991 when development was below threshold level; this represents the only data set available for this comparison.

Loss of yellow perch stream spawning sites coincided with increased development. When watersheds development was above the threshold (C / ha $\geq$ 0.83), yellow perch stream spawning was not detected in some years in Mattawoman Creek (C / ha = 0.85-0.90) and most years in Bush River. Sites occupation was steady when C / ha was 0.47 or less. We can demonstrate changes in stream spawning site occupation of white perch and herring between the 1970s and 2000s, but are unable to conclude that development had an impact. White perch stream spawning has largely ceased in our study streams between the 1970s and the 2000s. However, it disappeared in every watershed regardless of development level, except in Aberdeen Proving Grounds where white perch occupation was observed at three of the four historical sites sampled. Herring spawning has not occurred at some sites where it was documented in the 1970s, occurred at sites where it had not been detected previously, or continued at sites where it had been detected.

Proportion of positive samples (P_{herr} for example) provided an economical and precise alternative estimate of relative abundance based on encounter rate rather than counts. 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.

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), 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 will 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 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 appears possible to pool data across years to form 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 2008-2012 collections at the same combinations of sites.

Volunteer-based sampling of stream spawning during 2005-2012 used only stream drift nets, while O'Dell et al. (1975) 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 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/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 icthyoplankton 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). Removal of 1991 data lowered the correlation between C / ha and P_{herr} (from r = -0.75, P = 0.002 to r = -0.68, P = 0.01), but did not alter the conclusion that there was a negative association.

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).

Table 1-1. Summary of subestuaries, years sampled, number of sites, 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
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
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
Deer	2012	4	20-Mar	7-May	11	44

Table 1-2. Summary of historical conductivity sampling in non-tidal Mattawoman Creek. RKM = site location in river km from mouth; months = months when samples were drawn; Sum = sum of samples for all years.

RKM	12.4	18.1	27	30	34.9	38.8
Months	1 to 12	4 to 9	4 to 9	8,9	4 to 9	8,9
Sum	218	8	9	2	9	2
	Years Sampled					
1970			70	70	70	70
1971	71					
1974	74	74	74		74	
1975	75					
1976	76					
1977	77					
1978	78					
1979	79					
1980	80					
1981	81					
1982	82					
1983	83					
1984	84					
1985	85					
1986	86					
1987	87					
1988	88					
1989	89					

Table 1-3. Summary statistics of conductivity (μS / cm) for mainstem stations in Piscataway, Mattawoman and Deer Creeks, and Bush River during 2005-2012. 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
				Mattawoman				
Mean				120.1	244.5	153.7	116.3	128.9
Standard Error				3.8	19.2	38	4.6	1.9
Median				124.6	211	152.3	131	130.9
Kurtosis				2.1	1.41	1.3	-0.92	-0.26
Skewness				-1.41	1.37	0.03	-0.03	-0.67
Range				102	495	111	170	49
Minimum				47	115	99	55	102
Maximum				148.2	610	210	225	151
Count				39	40	43	69	44
				Bush				
Mean	269	206	263	237				
Standard Error	25	5	16	6				
Median	230	208	219	234				
Kurtosis	38	2	22	7				
Skewness	6	-1	4	0				
Range	1861	321	1083	425				
Minimum	79	0	105	10				
Maximum	1940	321	1187	435				
Count	81	106	79	77				
				Piscataway				
Mean				218.4	305.4			211.4
Standard Error				7.4	19.4			5.9
Median				210.4	260.6			195.1
Kurtosis				-0.38	1.85			0.11
Skewness				0.75	1.32			0.92
Range				138	641			163
Minimum				163	97			145
Maximum				301	737			308
Count				29	50			44

Table 1-3 continued.

Conductivity	2005	2006	2007	2008	2009	2010	2011	2012
	Year							
	Deer							
Mean								174.9
Standard Error								1.02
Median								176.8
Kurtosis								17.22
Skewness								-3.78
Range								39.3
Minimum								140.2
Maximum								179.5
Count								44

Table 1-4. Presence-absence of herring (blueback herring, hickory and American shad, and alewife), white perch, and yellow perch stream spawning in Mattawoman Creek during 1971, 1989-1991, and 2008-2012. 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.

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

Table 1-5. Presence-absence of herring (blueback herring, hickory and American shad, and alewife) and white perch stream spawning in Piscataway Creek during 1971, 2008-2009, and 2012. 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
	Herring			
PC1	1	0	0	1
PC2	1	0	1	1
PC3	1	0	0	1
PTC1	1	0	0	1
PUT4	1		0	0
	White Perch			
PC1	1	0	0	0
PC2	1	0	0	0

Table 1-6. Presence-absence of herring (blueback herring, hickory and American shad, and alewife), white perch, and yellow perch stream spawning in Bush River during 1973 and 2005-2008. 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
	Herring				
BBR1	0	1	1	1	1
BBR2	0	0	0		
BCR1	1	0	0	1	0
BGR1	0	1	1	1	
BGR2	1	0	0		
BGRT					0
BHH1	0	0	1	1	1
BHHT					0
BJR1	0	1	1	1	0
BOP1	1	1	1	1	1
BSR1	1	0	0		
BWR1	1	0	0	1	0
BWR2	1	0	0		
BWRT					1
BUN1	1	1	1	1	

Table 1-6 continued.

Station	Year 1973	2005	2006	2007	2008
			White Perch		
BBR1	1	0	0	0	0
BBR2	0	0	0		
BCR1	1	0	0	0	0
BGR1	1	0	0	0	
BGR2	1	0	0		
BGRT					0
BHH1	0	0	0	0	0
BHHT					0
BJR1	0	0	0	0	0
BOP1	1	0	0	1	0
BSR1	0	0	0		
BWR1	1	0	0	0	0
BWR2	1	0	0		
BWRT					0
BUN1	1	0	0	0	
			Yellow Perch		
BBR1	1		0		0
BBR2	1		1		
BCR1	0		0		0
BGR1	1		1		
BGR2	0	0	1	0	
BGRT					0
BHH1	0	0	0		0
BHHT					0
BJR1	1	0	0	0	0
BOP1	0	0	0	0	0
BSR1	0	0	0	0	
BWR1	1	0	1	0	0
BWR2	0	0	0		
BWRT					0
BUN1	0	0	0	0	

.

Table 1-7. Presence-absence of herring (blueback herring, hickory and American shad, and alewife) and white perch stream spawning in Deer Creek during 1972 and 2012. 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
	Herring	
SU01	1	1
SU02		1
SU03		1
SU04	1	1
	White Perch	
SU01	1	0
SU02		0
SU03		0
SU04	0	0
	Yellow Perch	
SU01	1	1
SU02		1
SU03		0
SU04	0	0

Figure 1-1. Watersheds sampled for stream spawning anadromous fish eggs and larvae in 2005-2012. Coastal Plain and Piedmont Regions are indicated.

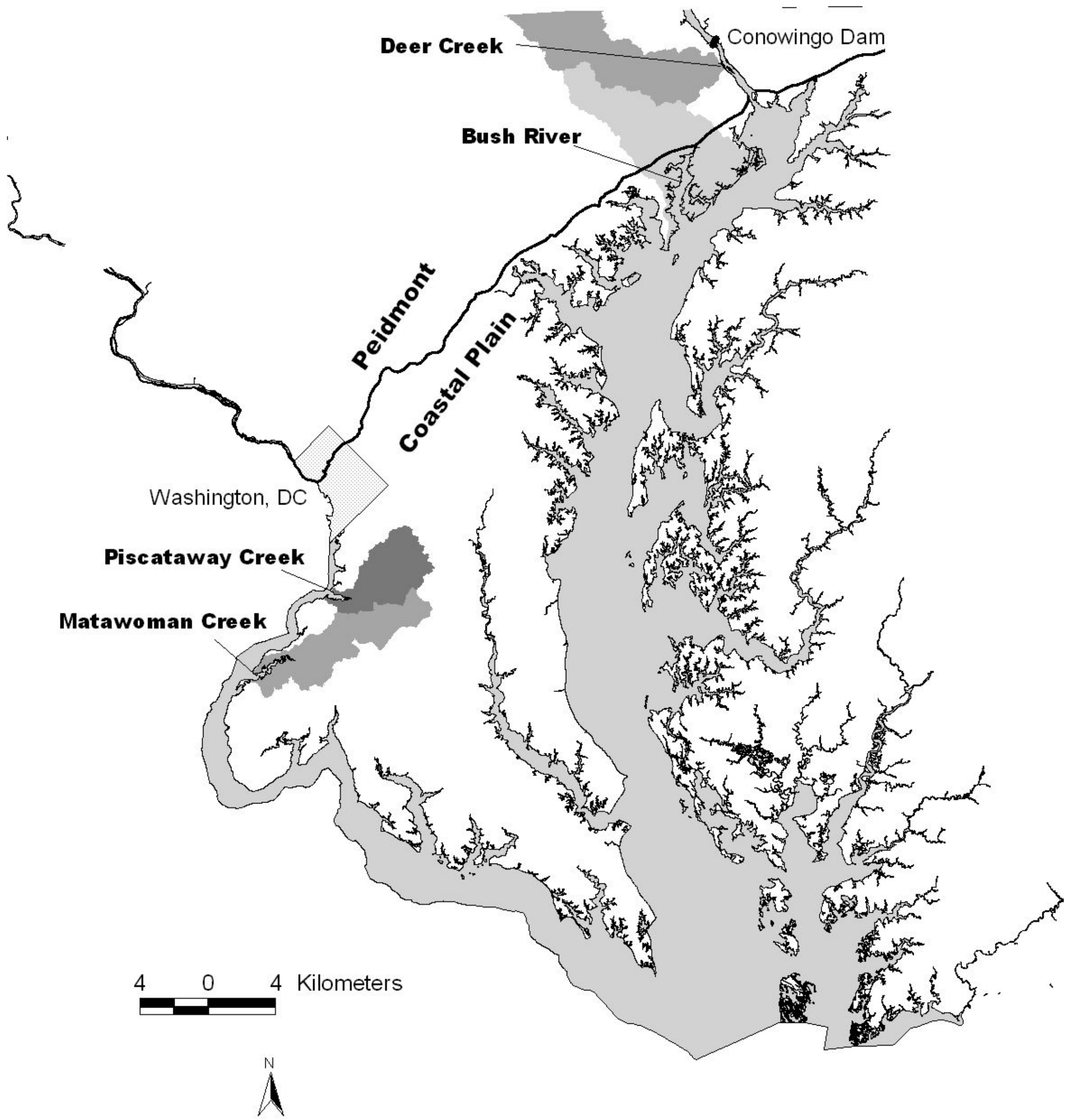


Figure 1-2. Mattawoman Creek's 1971 and 2008-2012 sampling stations.

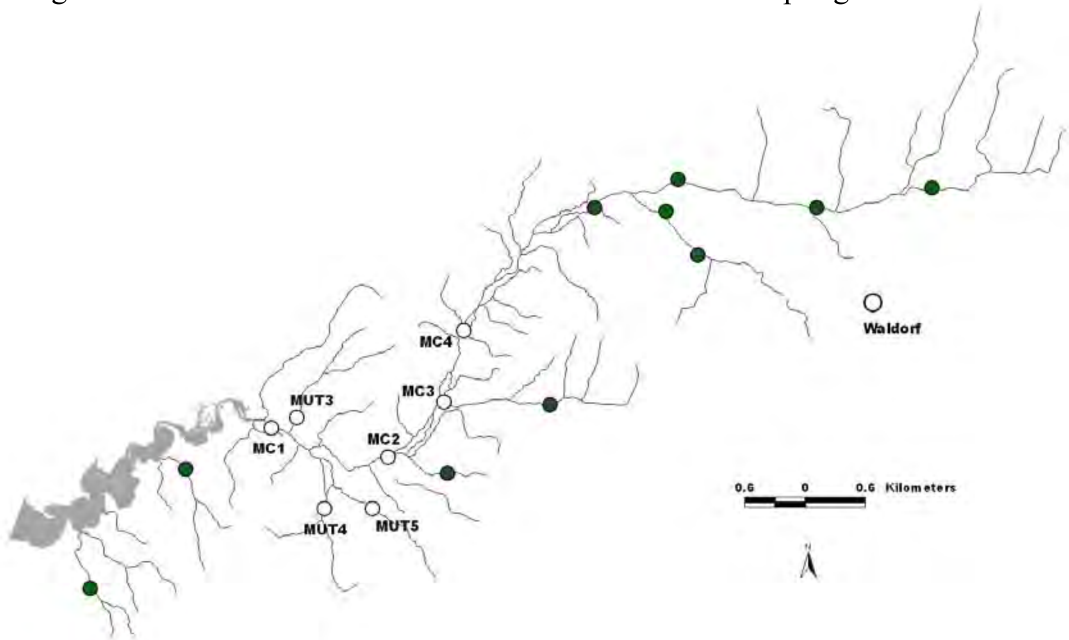


Figure 1-3. Piscataway Creek's 1971, 2008-2009, and 2012 sampling stations.

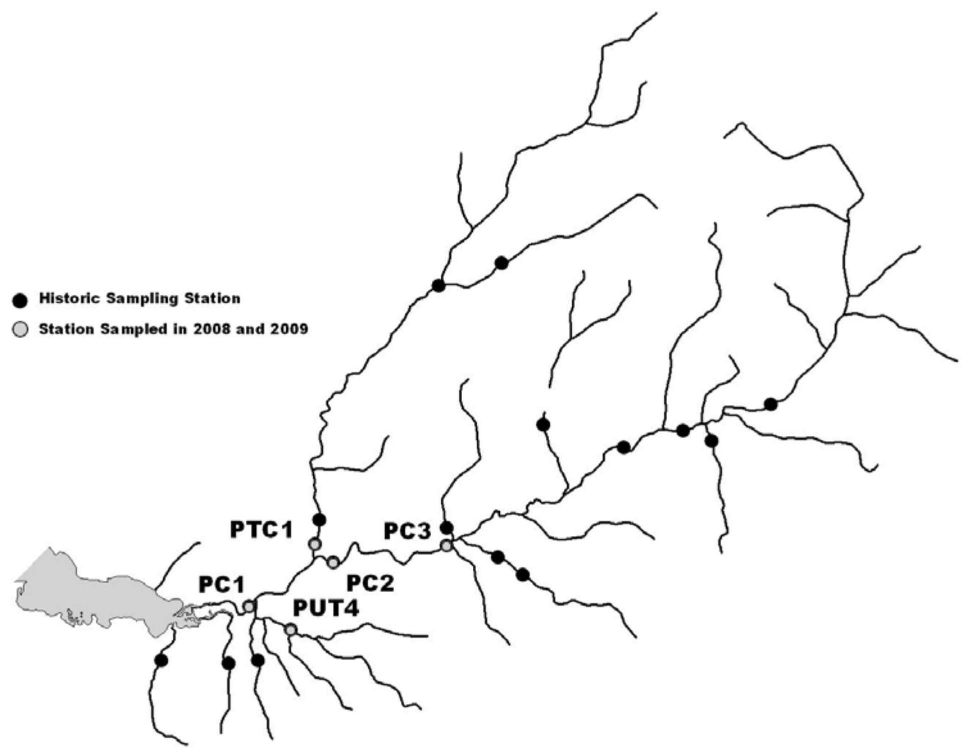


Figure 1-4. Bush River's 1973 and 2005-2008 sampling stations. Stations in Aberdeen Proving Grounds have been separated from other Bush River stations.

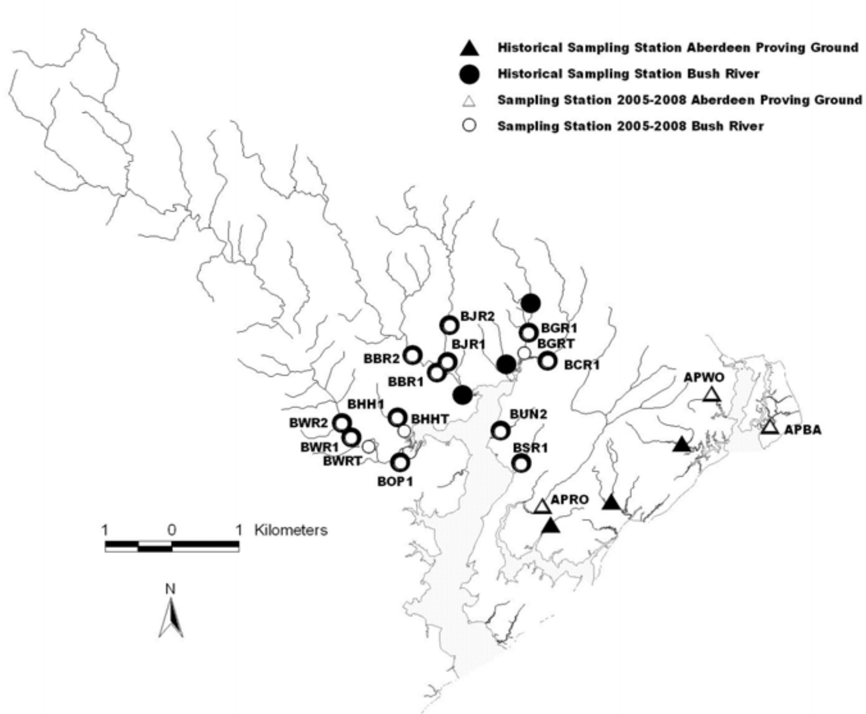


Figure 1-5. Deer Creek's 1972 and 2012 sampling stations.

Figure 1-6. Trends in counts of structures per hectare (C / ha) during 1950-2010 in Piscataway Creek, Mattawoman Creek, Deer Creek, and Bush River watersheds. Updates estimates of C / ha were not available for 2011 or 2012. Large symbols indicate years when stream ichthyoplankton was sampled.

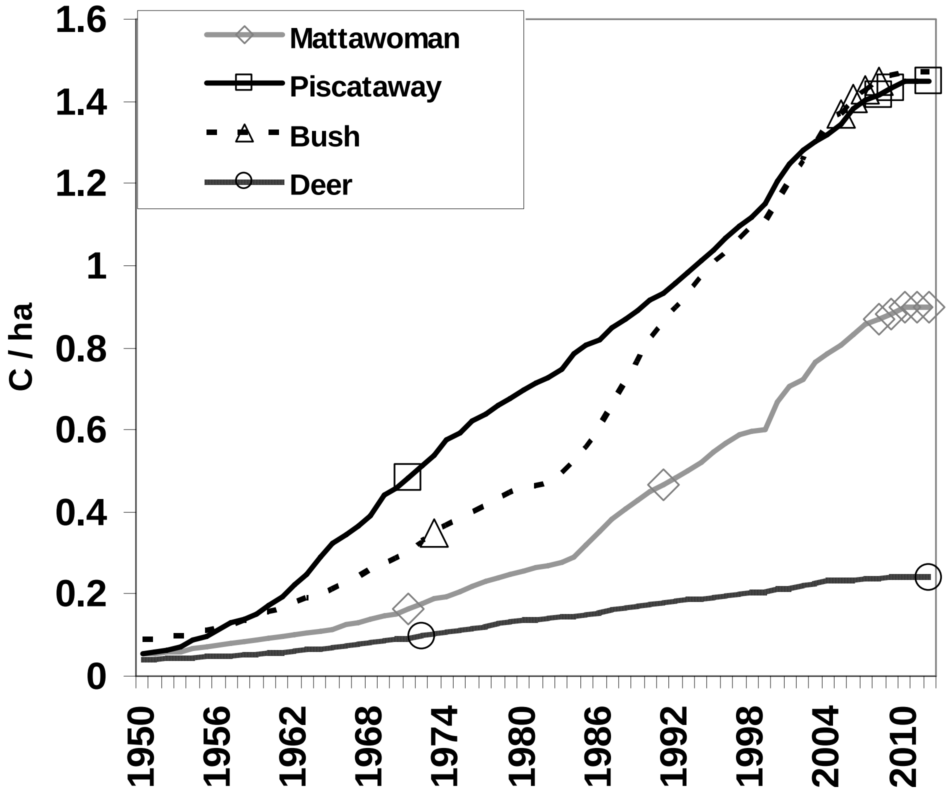


Figure 1-7 Stream conductivity measurements (µS / cm), by station and date, in Mattawoman Creek during (A) 2009, (B) 2010, (C) 2011, and (D) 2012. Lines indicate conductivity range measured at mainstem sites (MC1 – MC4) during 1991 by Hall et al. (1992)

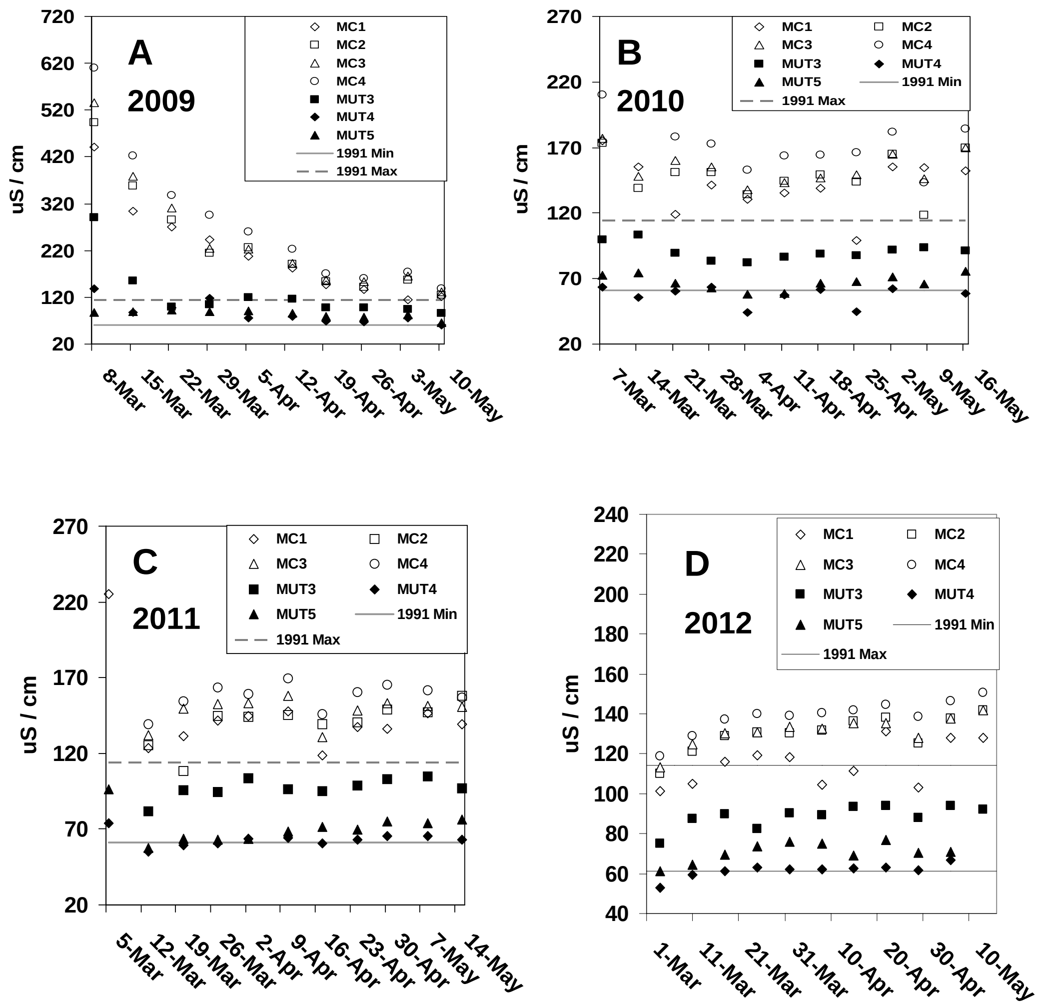


Figure 1-8. Historical (1970-1989) median conductivity measurements and current (2008-2012) anadromous spawning survey median conductivity in non-tidal Mattawoman Creek (between the junction with the subestuary and Waldorf) plotted against distance from the mouth.. The two stations furthest upstream are nearest Waldorf. Median conductivity was measured during March-May, 2008-2012, and varying time periods (see Table 2-2) during 1970-1989.

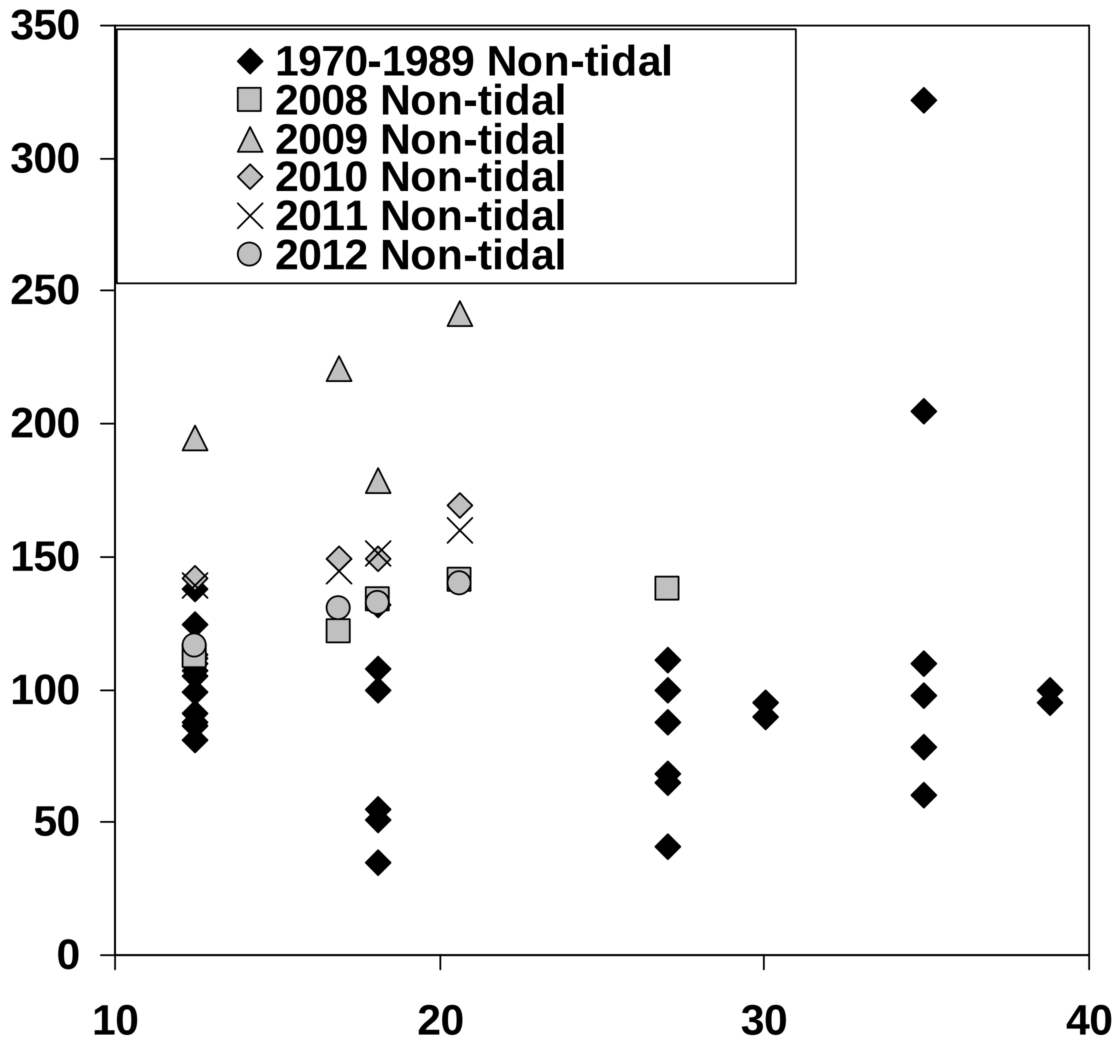


Figure 1-9. Proportion of samples (P_{herr}) with herring and its 90% confidence interval for stream ichthyoplankton surveys in Mattawoman, Piscataway and Deer Creeks, and Bush River.

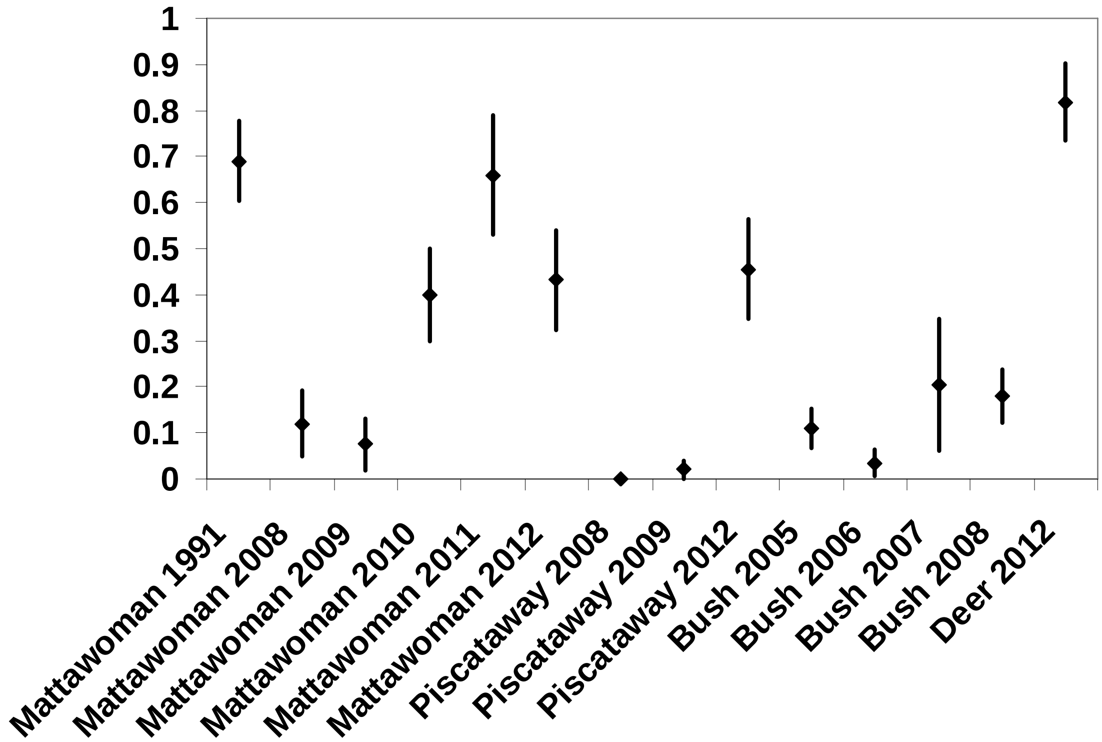


Figure 1-10. Standardized median conductivity during spring spawning surveys and level of development (C / ha). Median conductivity was standardized to background estimates for Coastal Plain and Piedmont regions based on estimates in Morgan et a. (2012)

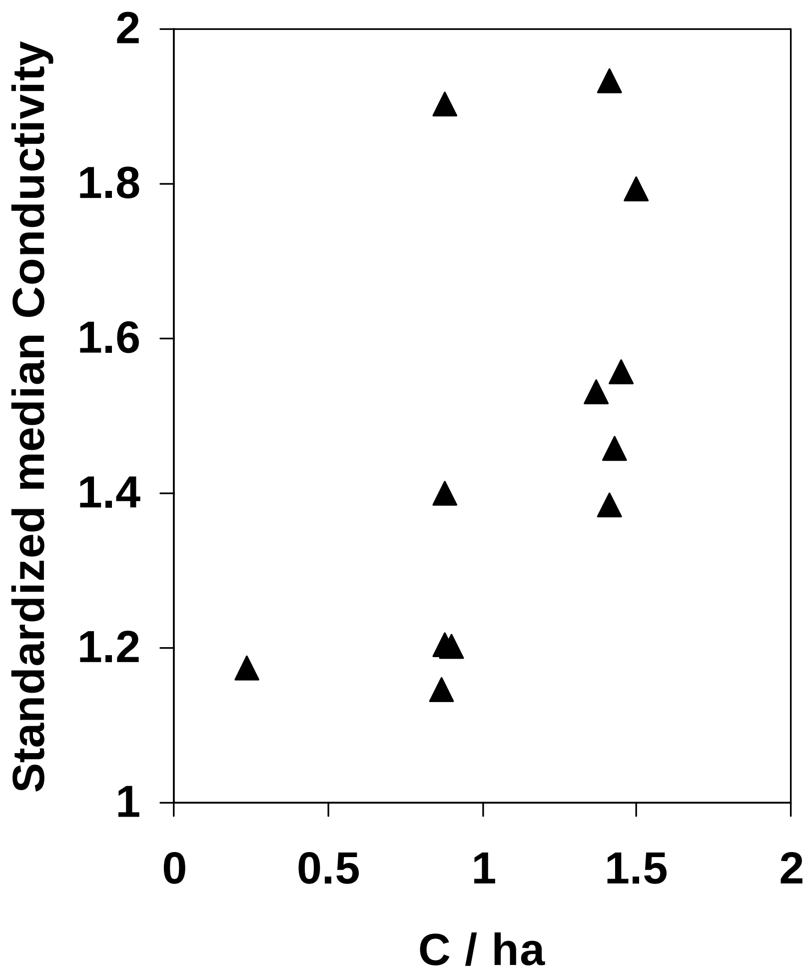
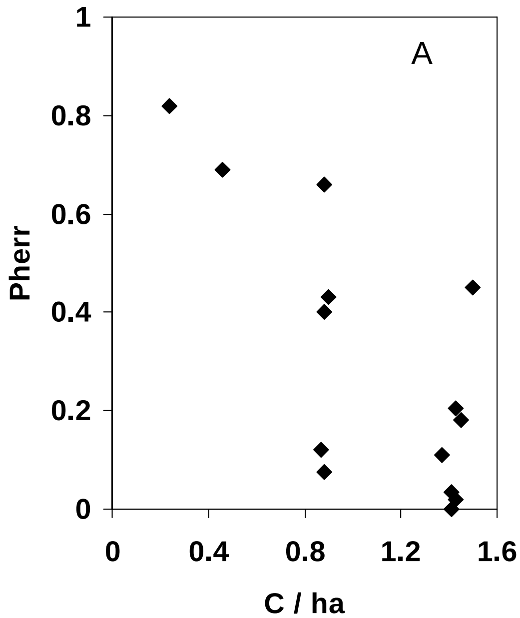
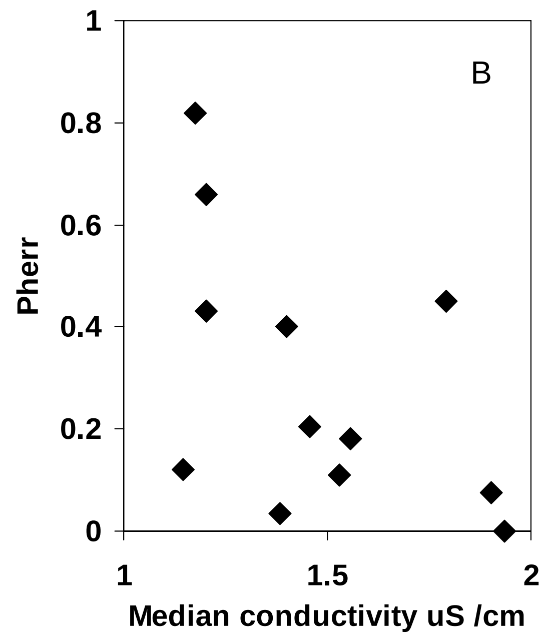


Figure 1-11. (A) Proportion of stream samples with herring eggs or larvae (P_{herr}) and level of development (C / ha). (B) Pherr and standardized median spawning survey conductivity. Median conductivity was standardized to background estimates for Coastal Plain and Piedmont regions based on estimates in Morgan et a. (2012).





Job 1 Section 2 - Estuarine Yellow Perch Presence-Absence Sampling

Introduction

Presence-absence sampling for yellow perch larvae was conducted in the upper tidal reaches of the Nanticoke, Northeast, Elk, Middle, Patuxent, and Bush rivers and Mattawoman, Nanjemoy, and Piscataway creeks during late March through April, 2012 (Figure 2-1). 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. In 2012, we continued examining relationships of L_p with estimates of development and other land uses.

In addition, we evaluated the role of development on yellow perch larval feeding success using 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.

We examined a hypothesis that development negatively influenced watershed organic matter (OM) dynamics, altering zooplankton production important for yellow perch larval feeding success and survival (the OM hypothesis). 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 (Hoffman et al.2007), while phytoplankton would be the primary source of OM in years of lesser flow. Shortage of appropriate food has been frequently hypothesized to cause high mortality of fish larvae (Martin et al. 1985; Miller et al. 1988; Heath1992).

Urbanization was expected to negatively impact yellow perch larval feeding success because it affects the quality and quantity of OM in streams (Paul and Meyer 2001) and was negatively associated with extent of wetlands in many subestuary watersheds encompassed in this study (Uphoff et al. 2011a). 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).

Correlation analyses examined associations of C / ha and 2010-2012 feeding success, L_p, larval TL, feeding success, diet composition, and relative detritus levels collected during spring surveys. Larval fish size was included because it can be critical to larval feeding and starvation (Miller et al. 1988). Uphoff et al (2012) included factors in addition to C / ha in analyses of 2010-2011 feeding success: relative amounts of OM, larval TL, mean water temperature, and mean conductivity in analyses of feeding success. Organic matter and larval length were found to be significant influences on feeding success, but water temperature and mean conductivity were not. Analyses of 2010-2012 feeding data in this report concentrated on variables that were significant in Uphoff et al. (2012).

Long-term environmental data from rural watersheds was analyzed to explore if OM transport would positively influence L_p and yellow perch juvenile indices (YPJ) under "natural" conditions. Detection of a positive influence of precipitation or flow would support the OM hypothesis. Both L_p and YPJ data sets had observations back to the 1960s. Analyses of YPJ were confined to the Head-of-Bay since this was the only reliable regional time-series available (Yellow Perch Workgroup 2002; Piavis and Webb 2011). Estimates of L_p were drawn from several rural watersheds (C / ha < 0.27) and discharge information was not available for many of them. Regional average precipitation was substituted for flow in this analysis. Precipitation is frequently used in statistical analyses related to the basic processes of OM transfer (Stanley et al. 2012). Air temperature was analyzed as well to maintain consistency with Martino and Houde's (2010) analyses for striped bass. We felt that observed coherency between juvenile indices of striped bass and yellow perch justified including analyses of regional air temperature, but its inclusion was not related to the OM hypothesis. We assumed that averages for March would provide a reasonable indicator of important environmental conditions for yellow perch since spawning occurs during mid-February-March (Piavis 1991). Estimates of L_p typically encompass the end of March through the second to third weeks of April, and significant reductions in larval presence become apparent by the first or second week of April. In addition to precipitation, average March flow of the Susquehanna River at Conowingo Dam was considered in YPJ analyses since it delivers large amounts of freshwater to the Head-of-Bay region. We considered precipitation to indicate conditions in our subestuaries' watersheds. Mesozooplankton trends were available for 1984-2001 from Chesapeake Bay Program monitoring and these data were included in YPJ analyses. Linear regression evaluated environmental factors.

In addition to estimating L_p and feeding success, we collected yellow perch larvae in 2012 for analysis of the ratio of ribonucleic acid (RNA) concentration to deoxyribonucleic acid (DNA) concentration in body tissue. The quantity of DNA within a cell is constant within a species while the quantity of RNA varies with protein synthesis. Since growth is a function of protein synthesis, RNA/DNA ratios provide a sensitive indicator of growth potential at any given time. 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 L_p as an indicator of survival, feeding success, and larval condition without requiring extensive sampling and sample processing needed to measure mortality directly.

Samples were gathered from subestuaries exhibiting a gradient of development from rural to suburban (C / ha ranged from 0.09 to 1.43) during 2012. We expected RNA/DNA ratios to decline with increased development. 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.

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, conductivity, and salinity were measured at each site on each sample date.

Ten sites were sampled in Nanjemoy and Mattawoman creeks, Middle River, Elk River, Northeast River, and Nanticoke River (Figure 2-1). Seven sites were sampled in Piscataway Creek. Five to ten stations were sampled on the Patuxent River (Figure 2-1). Elk, Patuxent, and Middle rivers were sampled once a week and all other subestuaries were sampled twice per week. Larval sampling occurred during late March through mid-to-late April. Boundaries of areas sampled were determined from yellow perch larval presence in estuarine surveys conducted during the 1970s and 1980s (O'Dell 1987). Sampling in Middle River was exploratory and covered the upper two-thirds of this subestuary. Middle River was not sampled by O'Dell et al. (1975). Therefore, documentation of spawning or larval occurrence there did not exist, but yellow perch juveniles and adults have been found regularly in this highly urbanized subestuary (C / ha = 3.32 in 2010). Three visits at ten sites were made to detect spawning in Middle River. Sites in all subestuaries (except the Nanticoke River) were sampled with little spacing between tows because their larval nurseries were small. Three upstream sites in Piscataway Creek could not be sampled at very low tides.

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 *Morone* larvae that could be confused with them (Lippson and Moran 1974).

Contents of the jar were allowed to settle and then the amount of settled OM was assigned a rank:

0 = clear to not enough to define a layer;

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 brought back to the lab for sorting.

Nanjemoy, Piscataway, and Mattawoman creeks were sampled by program personnel. Nanticoke, Elk, and Northeast rivers were voluntarily sampled by other Maryland Fisheries Service projects without charge to this grant. Patuxent and Bush rivers were sampled by staff from the Chesapeake Bay National Estuarine Research Reserve Program and volunteers trained by our program biologists.

Composite samples of larvae were collected for feeding analyses from several sites in Piscataway, Mattawoman, and Nanjemoy creeks, and Elk, Bush, and Northeast rivers during several sample trips. Subsamples of postlarvae 12 mm TL or less were examined for gut contents from each day's samples of each subestuary. These larvae represented first-feeding and early postlarvae - larvae that absorbed their yolk and began active feeding (Hardy 1978). Larvae were measured to the nearest millimeter. 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 proportion of tows with yellow perch larvae (L_p) 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

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

The 95% confidence intervals were constructed as

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

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. 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.

Uphoff et al. (2012) developed L_p thresholds for brackish and fresh-tidal systems. Three brackish subestuaries with C / ha $\geq$ 1.59 (10 estimates from Severn, South, and Magothy rivers) exhibited chronically depressed L_p and their maximum L_p (0.33) was chosen as a threshold indicating serious deterioration of brackish subestuary larval nursery habitat. Similarly, fresh-tidal Piscataway Creek's four estimates of L_p (2008-2011) consistently ranked low when compared to other fresh-tidal subestuaries sampled (13^{th} to 17^{th} 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 fresh-tidal 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).

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 1986-1990 (Uphoff et al. 2005). Survey designs for the Choptank and Nanticoke rivers were described in Uphoff (1997).

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). This indicator has been provided to us by Marek Topolski of the Fishery Management Planning and Fish Passage Program. Tax maps are graphic representations of individual property boundaries and existing structures that help State tax assessors locate properties (Maryland Department of Planning or MDP 2010). 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. Each year's statewide tax map was clipped using the MD 8-digit watershed boundary file, modified to exclude estuarine waters, to create watershed land tax maps. 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 present a problem since we are interested in the trend and not absolute magnitude (Uphoff et al. 2012).

Estimates of C / ha were used as a measure of watershed development intensity for analysis with L_p. Generally, whole watershed estimates were used with the following exceptions: Nanticoke and Choptank river watersheds were truncated at the lower boundaries of their striped bass spawning areas and at the Delaware border (latter due to lack of comparable data). Elk River was confined to subwatersheds designating the Elk River proper above the C and D Canal. Estimates of C / ha were available from 1950 through 2010 (M. Topolski, MDDNR, personal communication). Estimates of C / ha for 2010 were used to represent 2011 and 2012.

Estimates of C / ha for the IS target and limit were estimated from the power function that converts C / ha to IS based on Towson University satellite data interpretation (Uphoff et al. 2012). The target proposed in Uphoff et al. (2011a), 5.5% IS, was reduced to 5% to meet IS guideline being developed by Maryland's Department of Natural Resources (MDDNR 2012). The IS threshold of 10% in Uphoff et al. (2011a) remained unchanged. An estimate equivalent to 15% IS was also made to designate suburban watersheds that were developed well beyond the threshold. Estimates of C / ha that were equivalent to 5% IS, 10% IS, and 15% IS were estimated as 0.27, 0.83, and 1.59 C / ha, respectively by Uphoff et al. (2012).

Two regression approaches were used to examine the relationship between C / ha and L_p. First, separate linear regressions of C / ha against L_p were estimated for brackish and fresh-tidal 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 fresh-tidal conditions and 1 indicating brackish conditions. High salinity has been implicated in contributing to low L_p in Severn River (Uphoff et al. 2005) and the association of mean salinity and IS can be significant and strong (Uphoff et al. 2010). 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. Level of significance was set at $\alpha \leq 0.05$. Residuals were inspected for trends, non-normality, and need for additional terms.

We used Akaike information criteria adjusted for small sample size, AIC_c, to evaluate the models that describing hypotheses that related changes in L_p to C / ha for each salinity category (separate slopes) or to C / ha 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 $\Delta_{i,}$ ($AIC_{c\ i}$ – minimum AIC_c), where i is an individual model, for the fresh-tidal 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 when interpreting salinity classification (brackish or fresh-tidal), C / ha and *Lp* regressions. Primary land use (forest, agriculture, or urban) was determined from Maryland Department of Planning estimates for 1973, 1994, 1997, 2002, or 2010 that fell closest to a sampling year. These latter categories were not used in regression analyses, but were considered in the interpretation of results. Urban land consisted of high and low density residential, commercial, and institutional acreages (MD DNR 1999).

The mean of feeding success rank was calculated annually for each subestuary sampled in 2010-2012, 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_{othr}) food items. The latter three proportions were not additive.

Associations of C / ha with mean feeding rank, P0, mean TL, P_{cope}, P_{clad}, and P_{othr} (2010-2012 estimates) were tested with correlation analysis. Correlations of L_p with P0 and mean feeding rank were used to evaluate whether larval relative abundance was associated with feeding success. An additional set of correlation analyses examined associations among mean feeding success rank, mean TL, P_{cope}, P_{clad}, and P_{othr}.

We used OM0 (proportion of samples without OM, i.e., rank = 0) as our indicator of detritus availability and correlated OM0 against C / ha and feeding parameters that were significantly associated with C / ha. Proportions of samples without OM were estimated during 2011-2012, so fewer observations were available for analysis. The distribution of OM ranks assigned to samples in 2011-2012 was highly skewed towards zero and few ranks greater than 1 were reported.

Regression analyses of the influences of March air temperature (°C) and precipitation (cm) on L_p were confined to the following rural or near rural watersheds: Nanticoke, Choptank, Corsica, Elk, and Northeast rivers, and Langford and Nanjemoy creeks. Four regional sets of air temperature and precipitation records observations were available for the years and regions from Historical Climate Summaries for Maryland (http://www.sercc.com/climateinfo/historical/historical_md.html). Climate records from Salisbury were used for Nanticoke and Choptank rivers; records from Chestertown were used for the Corsica River and Langford Creek; Mechanicsville observations were used for Nanjemoy Creek; and Aberdeen observation were used for Elk and Northeast rivers (Figure 2-1).

Regressions used $\log_e$-transformed air temperature and precipitation. Log_e-transformation of climate variables was justified given the potential multiplicative influences of these environmental variables on fish production (Crecco and Savoy 1984). Estimates of L_p were not transformed because quantitative data were already truncated by the binary system (Green 1979). A salinity indicator variable was included (0 = fresh and 1 = brackish); however, the fresh-tidal subestuaries (Northeast and Elk rivers) were confined to the Head-of-Bay region and regional rather than salinity influence was possible. Hereafter, this indicator variable is referred to as a salinity/regional indicator.

The general form of the full multiple regression was

$$(5)\ L_p = a \cdot \log_e T + b \cdot \log_e P + c \cdot S + C;$$

where T was air temperature: P was precipitation; S was salinity category; C was the intercept, and a, b and c were coefficients. If a coefficient was not significant, then the regression was simplified by removing the term. Forward selection was used to select variables for the multiple regression (Freund and Littell 2006).

Head-of-Bay yellow perch juvenile indices (YPJ) were obtained from Durell and Weedon (2012) and analyzed by the same general process as specified in equation 5, with YPJ substituted for L_p. A categorical variable for salinity was not included since this region was fresh-tidal. Estimates of YPJ and climate variables were $\log_e$-transformed (Crecco and Savoy 1984); $\log_e$-transformed YPJ is denoted as YPT. Estimates of average Susquehanna River discharge (m^3) at Conowingo Dam during March were obtained from the US Geological Survey (http://waterdata.usgs.gov/nwis/monthly/?search_site_no=01578310&agency_cd=USGS&referred_module=sw&format=sites_selection_links) and considered in addition to air temperature and precipitation. Average March discharge was $\log_e$-transformed. An additional hypothesis regarding influence of Susquehanna River discharge on Head-of-Bay yellow perch was included since it is the largest source of freshwater in Chesapeake Bay (Kemp et al. 2005) and this large quantity of water is discharged into the Head-of-Bay. This discharge has been found to be an important influence on larval striped bass and white perch in the Head-of-Bay (North and Houde 2003; Martino and Houde 2010). The full model was described by the equation:

$$(5)\ YPT = a \cdot \log_e T + b \cdot \log_e P + c \cdot \log_e R + C;$$

where YPT, T, P, C, a, b, and c are as defined previously and R is average March Susquehanna River flow. These data were available for 1968-2012.

Estimates of mesozooplankton per liter available as striped bass food in the Head-of-Bay region for 1985-2001 (Versar 2002) were compared with the YPJ. These estimates were from Chesapeake Bay Program site CB1.1 (mouth of Susquehanna River) from 1985-1996 and CB2.1 (off Turkey Point) 1993-2001 (Versar 2002).

Level of significance was set at $\alpha \leq 0.05$ for analyses and retention of variables by the forward selection procedure. Mutltiple regressions used $P \leq 0.20$ for initial variable entry (Freund and Littell 2006). Residuals were inspected for trends, non-normality and need for additional terms.

During 2012, we collected yellow perch larvae for analysis of the ratio of ribonucleic acid (RNA) concentration to deoxyribonucleic acid (DNA) concentration in body tissue. 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).

Our intent was to collect larvae from a regional urban gradient represented by the watersheds of Piscataway Creek (C / ha = 1.45), Mattawoman Creek (C / ha = 0.90), and Nanjemoy Creek (C / ha = 0.09). This design, based on several previous years' collections, anticipated that sampling from these three rivers on three occasions would provide 30 larvae per date for a total of 180. Unfortunately, L_p was so low in these systems that this design had to be scrapped and *ad hoc* collections were added from Northeast River (C / ha = 0.46) and Bush River (C / ha = 1.22).

Samples for RNA/DNA analysis were collected when larvae were gathered for food analysis. In the field, yellow perch larvae were composited from several stations (where possible) that bracketed where larvae are abundant. Once a candidate jar has been checked for larvae and OM, the sample was poured 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, up to 30 larvae for each date were processed for both gut contents and RNA/DNA ratio. Yellow perch larvae 11 mm TL or less were examined for gut contents from each sample. These larvae represented first-feeding and early postlarvae, larvae that absorbed their yolk and began active feeding.. Generally, 7 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 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 creek, number designating which sample date, and number designating which individual larva) was placed in the vial.

RNA/DNA ratios were estimated by J. Brush at the Cooperative Oxford Laboratory. Protocols for estimating RNA/DNA generally followed Kaplan et al. (2001). Larvae were stored at 4°C in RNAlater® (Sigma-Aldrich, St. Louis, MO) for a few weeks until ready for 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^{o}C for several hours until completely digested. Samples were centrifuged at 11,000rpm for 10 minutes, and the supernatant containing the nucleic acids was removed and stored at -80^{o}C until ready for processing.

DNA was removed from a subsample of each sample's supernatant using 10X DNase digestion buffer (0.2M Tris-HCl pH=7.5, 0.1M MgCl and 0.02M CaCl) and Rnase-free Dnase I. Samples incubated at 37^{o}C for 45 minutes in a dry bath. Samples were centrifuged for 5 minutes at 8,000 rpm. The supernatant was removed and stored at -80 °C until ready for processing.

Samples were fluorometrically analyzed for DNA and RNA quantification using a 96-well microplate, 45% TE Buffer, 50% Quant-it™ PicoGreen® for DNA and 50% Quant-it™ RiboGreen® for RNA (Molecular Probes, Oregon), and Synergy 2 microplate reader. Samples were analyzed in triplicate using a black microplate (Corning).

Fluorescence was measured at 480nm excitation and 520 nm emission for both DNA and RNA quantification.

The RNA/DNA ratios for each subestuary were plotted against larval TL or date. Reference lines indicating starving (RNA/DNA < 2; Blom et al) 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. A tabular summary of C / ha, median RNA/DNA ratio, mean fullness rand, N, N < 2, and N > 3 was constructed. The proportions of larvae with RNA/DNA ratios less than 2 (proportion starved or *Ps*) and their 90% confidence intervals were estimated for each subestuary as

$$(7)\ Ps = N_{<2} / N_{total};$$

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. The SD of *Ps* was estimated as

$$(8)\ SD = [(Ps \bullet (1- Ps)) / N_{total}]^{0.5}\ \ \text{(Ott 1977).}$$

The 90% confidence intervals were constructed as

$$(9)\ Ps \pm (1.44 \bullet SD;\ \text{Ott 1977).}$$

Proportions of larvae with RNA/DNA ratios greater than 3 (proportion fed or *Pf*) were estimated as in equations 6-8, but *Pf* was estimated with the number of larvae with RNA/DNA ratios greater than 3 ($N_{>3}$) in the numerator of equation 6 and *Pf* was substituted for *Ps* in the remaining equations.

For each subestuary and sample date, RNA/DNA ratio means and the number of samples in the *Pf* and *Ps* categories were summarized along with mean fullness rank, mean TL, and total samples size. Mean fullness rank, mean TL, and total sample size for dates when samples were collected for gut analysis but not for RNA/DNA ratios were added to display feeding history.

The estimates of *Ps* and *Pf* were plotted and differences among watersheds (dates combined) were judged from 90% confidence interval overlap (Johnson 1999). Confidence interval comparisons were limited to larvae with a common TL range among all tributaries. Distribution of gut fullness ranks, P_{clad}, P_{cope}, and P_{othr} were compared for larvae (all sizes) in the *Pf* and *Ps* categories.

Results

During 2012, sampling began on March 27 in Piscataway, Mattawoman and Nanjemoy creeks, and they were sampled through April 19; samples through April 19 were used to estimate L_p. Sampling began on March 22 in the Northeast and Elk rivers, respectively and ended on April 26; samples through April 11 were used to estimate L_p. Nanticoke River was sampled between April 2 and 30 and samples taken during April 2-16 were used to estimate L_p. Bush River was first sampled on April 3 and last sampled on April 19; dates between April 3 and 16 were used for estimating L_p. Middle River samples were taken on March 28, April 2, and April 9. Patuxent River was sampled on four visits between March 20 and April 10. Sampling in the Patuxent River was inconsistent (5-10 sites per date) and was not used to estimate L_p. However, it is worth noting that only prolarvae were encountered and they were detected at four of five sites on March 20 and two of six on March 27. Yellow perch larvae were not encountered at 10 sites on either April 3 or April 10.

Estimates of L_p during 2012 were sufficiently precise for detecting significant differences among subestuaries based on 95% CIs (Figure 2-2). Estimates of L_p for brackish subestuaries (Nanjemoy Creek and Nanticoke River) with rural watersheds were extremely low (0.03 and 0.04, respectively), not significantly different from zero, and below estimates for fresh-tidal subestuaries (Mattawoman and Piscataway creeks, and Elk, Northeast, and Bush rivers; Figure 2-2). These two extremely low estimates from Nanticoke River and Nanjemoy Creek indicated very poor egg and prolarval survival since postlarvae should have been the predominant life stage encountered during sampling for L_p (Figure 2-3; Uphoff 1991). These two estimates were the lowest on record for rural subestuaries. Change between 2012 and other years was especially marked for Nanjemoy Creek, the least developed system sampled (C / ha = 0.09), where L_p had ranged between 0.83 and 0.99 in the previous three years (Figure 2-3). Estimates of L_p from fresh-tidal subestuaries ranged from 0.18 to 0.77 and indicated at least some survival of postlarvae (Figure 2-4). The only fresh-tidal subestuary where L_p exceeded the fresh-tidal L_p threshold (0.65) was Northeast River. Yellow perch larvae were not captured in Middle River during the three sample cruises and we concluded that successful spawning did not occur there despite adult and juvenile yellow perch presence during summer sampling (Figure 2-4).

The range of C / ha values available for analysis with L_p was more shifted towards the y-axis in brackish subestuaries (C / ha range = 0.05-2.73) than fresh-tidal (0.45-3.32; Table 2-1). None of the fresh-tidal estuaries analyzed were at or below the target condition (Table 2-1).

Separate linear regressions of C / ha and L_p by salinity category were significant at $P \leq 0.0017$; Table 2-2). These analyses indicated that C / ha was negatively related to L_p and L_p was, on average, higher in fresh-tidal subestuaries than in brackish subestuaries. Estimates of C / ha accounted for 31% of variation of L_p in brackish subestuaries and 37% in fresh-tidal subestuaries. Based on 95% CI overlap, intercepts were significantly different between fresh-tidal (mean = 0.94, SE = 0.10) and brackish (mean = 0.57, SE = 0.04) subestuaries. Mean slope for C / ha estimated for fresh-tidal subestuaries (mean = -0.28, SE = 0.08) were steeper, but 95% CI's overlapped CI's estimated for the slope of brackish subestuaries (mean = -0.17, SE = 0.04; Table 2-2). Both regressions indicated that L_p would be extinguished between 3.0 and 3.5 C / ha (Figure 2-5).

A leverage plot for the fresh-tidal analysis indicated that the single point representing Middle River (L_p = 0 at C / ha = 3.32) was highly influential. A regression of C / ha and L_p for fresh-tidal subestuaries without Middle River was significant at P = 0.06 (r^2 = 0.16) and mean estimates of the intercept (mean = 0.94, SE = 0.14) and slope (mean = -0.27, SE = 0.13) were very close to those estimated with Middle River included, but were much less precise. With Middle River removed, there was a slight overlap of 95% confidence intervals of the intercepts developed for brackish (upper limit = 0.65) and fresh-tidal subestuaries (lower limit = 0.66).

Overall, the multiple regression approach offered a similar fit (R^2 = 0.37; Table 2-2) than separate regressions for each type of subestuary. Intercepts of fresh-tidal and brackish subestuaries equaled 0.85 and 0.59, respectively; the common slope was -0.20. Predicted L_p over the observed ranges of C / ha would decline from 0.56 to 0.10 in brackish subestuaries and from 0.81 to 0.01 in fresh-tidal subestuaries (Figure 2-5). The common slope appeared to over-predict L_p for Middle River (urbanized tidal-fresh subestuary, C / ha = 3.32; Figure 2-5).

Akaike's Information Criteria values equaled 9.5 for the regression of C / ha and L_p for brackish subestuaries, 10.2 for fresh-tidal estuaries, and 11.6 for the multiple regression that included salinity category. Calculations of Δ_i for brackish or fresh-tidal versus multiple regressions were approximately 2.1 and 1.4 (respectively), indicating that either hypothesis (different intercepts for fresh-tidal and brackish subestuaries with different or common slopes describing the decline of L_p with C / ha) were plausible.

Although we have analyzed these data in terms of fresh-tidal and brackish subestuaries, inspection of Table 2-1 indicated an alternative interpretation based on primary land. Predominant rural land use may be influencing the intercept estimates since all rural land in fresh-tidal subestuaries analyzed was dominated by forest, while nearly all rural land in brackish tributaries was dominated by agriculture. Dominant land cover estimated by MD DOP for watersheds of fresh-tidal subestuaries was equally split between forest (C / ha $\leq$ 0.90) and urban (C / ha $\geq$ 1.17; 12 observations each), while brackish subestury watershed rural lands were dominated by agriculture (C / ha $\leq$ 0.22; 28 observations), while forest land cover (C / ha ~ 0.09) was represented by four observations. Urban land cover predominated in nine watersheds of brackish subestuaries (C / ha $\geq$ 1.61; Table 2-1). Fresh-tidal 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

A total of 332 larval guts were examined during 2010, 532 were examined in 2011, and 466 were examined in 2012. Samples were drawn primarily from fresh-tidal subestuaries (13 of 16 subestuary and year combinations). Larvae were too scarce in Nanticoke River and Nanjemoy Creek in 2012 to include in this analysis. The estimate of OM0 for Bush River in 2012 was omitted; observations by program biologists indicated that filamentous algae comprised most of the OM (C. Hoover, MD DNR, personal observation), while the purpose of estimating OM0 was to investigate the role of detritus from the watershed on larval feeding success. A smaller sample size was available for correlations with OM0 (N = 10) than other variables (N = 16) because observations of OM did not start until 2011.

Minimum and maximum average TL of larvae from subestuaries within a year fell from 8.4-11.1 mm in 2010, to 8.3-9.3 mm in 2011, and 7.7-8.8 mm in 2012 (Table 2-3). Larval yellow perch guts contained food in all years and subestuaries except Piscataway Creek during 2011. Copepods were the most prevalent food item during 2010 and 2011, and were found in 51-100% of guts sampled (excluding Piscataway Creek). Copepods were not as prevalent in 2012 and only Piscataway and Mattawoman creeks had P_{cope} estimates within the range observed in 2010-2011. During 2012, copepods were not observed in larval guts in Elk River and were very scarce in Northeast River larvae (8% of guts). Cladocerans were found in 71% of guts in the Nanticoke River and 0-22% of guts in the remaining year and subestuary combinations during 2010-2011. Cladocerans were found in 44-55% of guts in Mattawoman and Piscataway creeks, and Bush River during 2012 but were scarce in Northeast and Elk rivers. The “other” food item category represented a high fraction of guts in Piscataway Creek (53%) in 2010 and 1-30% of guts in remaining subestuaries during 2010-2011. This category was predominant in larval gut samples from all five subestuaries during 2012 (70-100%; Table 2-3).

During 2010-2012, percentage of guts without food ranged from 0 to 24% in all subestuary and year combinations except Mattawoman and Piscataway creeks during 2011 (42% and 100%, respectively). Mean fullness rank ranged between 0.8 and 3.3 in all subestuary and year combinations except Piscataway Creek during 2011 (where it was 0; Table 2-3). In comparison with 2010 and 2011, feeding success was low in 2012 (with the exception of Northeast River (Table 2-3).

Estimates of C / ha, OM0, and mean fullness rank were significantly and logically correlated with one another, supporting the OM hypothesis (Table 2-4). As development increased, the presence of OM declined as did the relative fullness of larval guts. Estimates of C / ha were positively correlated with OM0 (r = 0.75, P = 0.01) and negatively correlated with mean fullness (r = -0.51, P = 0.04), while OM0 was negatively correlated with mean fullness (r = -0.64, P = 0.05; Table 2-4).

Estimates of OM0 were not significantly associated with P0 (r = 0.25, P = 0.49) even though P0 and mean fullness rank were significantly associated (r = -0.71, P = 0.002; Table 2-4). Generally, P0 estimates were skewed towards low values (14 of 16 were 0.25 or less with a maximum of 1.00 possible), whereas mean fullness rank was more broadly distributed and approximated a normal distribution (median of 2.0 with a range of 0.0 to 3.3). Within this data set, and similar to previous analyses, C / ha was negatively associated with L_p (r = -0.56, P = 0.02; Table 2-4).

The type of food present in larval yellow perch guts was not significantly associated with development or OM, but the amount of food present in larval guts was correlated with the presence of copepods (Table 2-4). Both mean fullness rank and P0 were significantly and positively associated with P_{cope} (r = 0.86, P = <0.0001 and r = -0.57, P = 0.02, respectively). Mean TL was positively correlated with P_{cope} (r = 0.55, P = 0.03), indicating larger larvae had copepods present in their diets more often. Estimates of L_p were significantly and negatively correlated with P_{clad} (r = -0.72, P = 0.002) and P_{othr} (r = -0.75, P = 0.001; Table 2-4).

All candidate variables ($\log_e$-transformed March air temperature in °C or $\log_e T$; $\log_e$-transformed March precipitation in inches or $\log_e P$; and salinity/region indicator or S; Table 2-5) were retained in the multiple regression with L_p by the forward selection process (R^2 = 0.30, P = 0.01; Table 2-6). This relationship was described by the equation:

$$L_p = 4.92 - (0.31 \cdot \log_e T) + (0.11 \cdot \log_e P) - (0.22 \cdot S).$$

The $\log_e P$ and S terms were significant at P < 0.04, while $\log_e T$ was significant at P = 0.06 (Table 2-6). Retention of the precipitation term supported the OM hypothesis. A plot of residuals versus observed L_p indicated an overall increasing trend (Figure 2-6). Residuals at L_p below 0.33 were all negative (N = 6), while residuals when L_p was above 0.80 were all positive (N = 8). Positive and negative residuals were present for observed L_p between 0.33 and 0.80 (N = 22; Figure 2-6) and this may have indicated a range where the multiple regression was relatively unbiased.

Regression analyses of YPT ($\log_e$-transformed YPJ) with $\log_e$ T, $\log_e$ P, and $\log_e$ R (log-transformed March air temperature, precipitation, and Susquehanna River flow, respectively: untransformed variables are listed in Table 2-7) required the addition of a time category variable (Y) that split the time-series into 1967-1992 (time category 0) and 1993-2012 (time category 1) to remove serial patterning of residuals. The period of 1967-1992 was characterized by YPJ means of 0.37 or less (mean YPJ = 0.08), while ten YPJ means were greater than 0.37 after 1992 (mean = 0.54). Rose et al. (1986) proposed using categorized variables and ordinary least squares regression as a time-series analysis method for long-term ecological data.

Forward selection retained variables Y and $\log_e$ P for the multiple regression with YPT ($R^2 = 0.38$, $P < 0.0001$; Table 2-8). This relationship was described by the equation:

$$YPT = (0.09 \cdot \log_e P) + (0.28 \cdot Y) - 0.07.$$

Standard errors of the coefficients for $\log_e$P, Y, and the intercept were 0.045, 0.064, and 0.043, respectively (Table 2-8). Selection of $\log_e$ P as a term supported the OM hypotheses. This multiple regression captured the upward shift in YPJ after 1992, but did not capture the highs and lows well (Figure 2-7). Seven of the eight most recent years were lower than predicted (Figure 2-7) and it is possible that YPT shifted into a persistently lower state than exhibited during 1993-2004.

Mesozooplankton availability was persistently low during 1985-1992 and typically higher during 1993-2001 (Figure 2-8). The upward shift in the YPJ after 1992 corresponded to a similar general upward shift in mesozooplankton per liter in the Head-of-Bay region, although year-to-year variation was not particularly well matched (Figure 2-8).

Larvae were scarce for RNA/DNA analysis in Mattawoman (N = 12: C / ha = 0.90), Piscataway (N = 8: C / ha = 1.45), and Nanjemoy creeks (N = 1; C / ha = 0.09) on

March 27 and 29, 2012. Larvae were collected from Bush River on April 5 (N = 40; C /ha = 1.22) and Northeast River on April 6 (N = 27; C / ha = 0.46). Nanjemoy Creek, with only a single sample, was dropped from analysis and the four remaining subestuaries were compared (Table 2-9). None of these remaining subestuaries had watersheds below the development target. Northeast River's watershed was below the threshold development level and remaining watersheds were all above the development threshold. All four subestuaries had very low levels of OM (OM0 = 0.98 to 1.0).

Ratios of RNA/DNA declined with TL (Figure 2-9). They were highest for 7-9 mm TL postlarvae with values greater than 3 (fed larvae) and less than 2 (starved larvae) in all subestuaries evaluated. Ratios greater than 3 were not present for 10 or 11 mm TL larvae and RNA/DNA ratios of 11 mm TL larvae were all less than 2. Ten and 11 mm TL larvae were collected from Mattawoman and Piscataway creeks, and Bush River. Northeast River collections did not have larvae greater than 9 mm (Figure 2-9); lack of larger and presumably older larvae from Northeast River was likely a function of later timing of spawning and the *ad hoc*, opportunistic nature of sampling rather than mortality.

Comparisons of feeding success for larvae falling within *Ps* and *Pf* categories (subestuaries, sizes, and sample dates pooled) are tentative because of low samples within the *Pf* category and because this category was largely comprised of Northeast River larvae (10 of 16 total, with an additional 4 from Bush River and 1 larva each from Mattawoman and Piscataway creeks). Larvae without food in their guts were not detected in either RNA/DNA category, while a feeding rank of 2 was most common for both RNA/DNA categories. Proportions of guts containing cladocerans were similar for

Pf (P_{clad} = 0.25, SD = 0.11) and *Ps* (P_{cope} = 0.35, SD = 0.07) categories as were the proportions of guts containing copepods (P_{cope} = 0.62 and SD = 0.12 for *Pf* larvae, and P_{cope} = 0.64 and SD = 0.07 for *Ps* larvae). All larvae contained items categorized as "other".

Construction of 90% CI's of *Ps* and *Pf* was confined to 7-9 mm TL larvae, the size in common in all four systems. Confidence intervals of *Ps* overlapped for all subestuary combinations except Mattawoman Creek and Bush River, but these subestuaries were within the 90% CI's of the other subestuaries (Figure 2-10). The 90% CI's of *Ps* were interpreted as not indicating differences among subestuaries since there was not a clear separation (Figure 2-10). The 90% CI's of *Pf* did exhibit a clear separation that indicated *Pf* in Northeast River (C / ha = 0.41) was higher than the remaining subestuaries (C / ha $\geq$ 0.88; Figure 2-11). Estimates of *Pf* in southern Maryland subestuaries (Piscataway and Mattawoman creeks) were not significantly different from zero, but those for the two Head-of-Bay subestuaries were (Figure 2-11). Examination of mean fullness rank in Northeast River by date indicated that RNA/DNA samples drawn on April 6, 2012, came from the only sample date between March 22 and April 11 with a mean fullness rank (2.1) greater than 1.1 (Table 2-8).

Discussion

In this report, we provide evidence that (1) development negatively influenced L_p, OM supply, and first feeding success; (2) March temperature conditions influenced L_p; and (3) low L_p in well developed watersheds was consistent with contaminant-related biological changes implicated in low egg hatching success (see below).

Estimates of L_p declined perceptibly once development exceeded the threshold (0.83 C / ha or 10% IS). Extensive forest cover in a watershed generally resulted in higher L_p (median $L_p = 0.80$) than agriculture or development. Estimates of L_p from agricultural watersheds below the target level of development (median $L_p = 0.52$) were variable, but generally higher than suburban watersheds (median $L_p = 0.30$). Interpretation of the influence of primary 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 (0.27 C / ha or 5% IS; forested and agricultural watersheds) or at and beyond high levels of development (1.59 C / ha or 15% IS; urban watersheds) were from brackish subestuaries; estimates of L_p for development between these levels were from fresh-tidal 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) primarily agricultural, fresh-tidal 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.

Salinity may restrict L_p in brackish subestuaries by limiting the amount of available low salinity habitat over that in a fresh-tidal subestuaries. Uphoff (1991) found that 90% of larvae collected in Choptank River during 1980-1985 were from 1 ‰ or less. Mortality of yellow perch eggs and prolarvae in experiments generally increased with salinity and was complete by 12‰ (Sanderson 1950; Victoria et al. 1992). The range of suitable salinities for prolarvae was lower than that for eggs (Victoria et al. 1992).

Development was an important influence on yellow perch egg and larval dynamics and negative changes generally conformed to ISRP guidelines in Uphoff et al. (2011a). Hilborn and Stokes (2010) advocated setting reference points related to harvest for fisheries (stressor) based on historical stock performance (outcome) because they are based on experience, easily understood, and not based on modeling. We believe applying IS or C / ha watershed development reference points (stressor) based on yellow reproductive success (outcome) conforms to the approach advocated by Hilborn and Stokes (2010).

We used analyses of current and historical surveys to examine the OM hypothesis. Statistically significant results of all analyses were consistent with the OM hypothesis. Our index of early larvae relative abundance (L_p) was negatively related to development level (C / ha). Development level was positively correlated with OM0 and OM0 was negatively correlated with how successful larvae were feeding on zooplankton. The forward selection procedure that parameterized multiple regression models of L_p and YPJ in rural watersheds from a set of potential environmental factors selected March precipitation, a variable representing mobilization of OM. Precipitation often appears as a predicator related to hydrological connection in statistical models describing OM processes (Stanley et al. 2012).

The multiple regression models used to describe relationships of L_p or YPJ versus precipitation (and other significant environmental factors) in rural watersheds explained modest amounts of variation and precipitation accounted for, at best, a modest amount of that explained variation. These results do not support using these models to predict L_p or YPJ. However, they provided evidence that widespread climate factors (March precipitation as a proxy for OM transport and air temperature) influenced survival of yellow perch egg and larvae in Chesapeake Bay subestuaries and supported the OM hypothesis. Large amounts of unexplained variation may indicate that precipitation was not an exact proxy for local flow or OM conditions, other factors not included might have been an influence (spawning stock size, zooplankton levels and composition, rural land use issues, etc.), nonlinear ecological processes may not be represented well with $\log_e$ – transformation of variables, or timing of events was not well captured by March averages or totals.

Estimates of L_p in the most rural subestuaries sampled during 2012 appeared anomalous compared to other years. In particular, L_p during 2012 was lower in the most rural and southerly subestuaries (Nanjemoy and Nanticoke rivers) than L_p in more developed subestuaries located to the north. Remaining estimates of L_p were quite low in subestuaries, except in Elk and Northeast rivers. Average air temperatures in March 2012 were higher than any other years (1965-2012) used in the multiple regression of L_p, air temperature, and precipitation, while precipitation was low but not the lowest (Table 2-5). Average March air temperatures were 1.2 and 1.8 °C higher in weather stations for Nanjemoy Creek and Nanticoke River, respectively, than for the Head-of-Bay region (Table 2-5). It appears that temperatures at either extreme may be a primary influence on L_p. Estimates of L_p of 0.5 or less did not occur at average March air temperatures 4.7 °C or less (N = 3) and average March air temperataures 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 exhibited a large range of variation (0.2 – 1.0) in between this temperature range (N = 27). In yellow perch, a period of low temperature is required for reproductive success (Heidinger and

Kayes 1986; Ciereszko et a. 1997) and warm temperatures may have precluded that from occurring in all but the most northerly subestuaries studied in 2012.

Air temperatures in the L_p multiple regression data set increased over time. A linear regression of year against mean March air temperature indicated an average increase of 0.5 °C per year since 1965 ($r^2 = 0.14$, $P = 0.02$), from a prediction of 5.9 °C in 1965 to 8.3 °C in 2012. This estimate may be negatively biased since an uneven mix of weather observation stations was used. March temperature data (N = 38), particularly early and middle points in the time-series, were dominated by Nanticoke River (N = 13) and Choptank River (N = 12), so only the most southerly station, Salisbury, MD, was represented. After 2006, the mix of subestuaries studied required the addition of three stations (Mechanicsville, Chestertown, and Aberdeen); the latter two are well to the north of Salisbury and have lower average March temperatures. Projections of average annual air temperature increase in the Chesapeake Bay region due to global climate change indicated that a rise of 1.0-1.5 °C is possible by 2030 and 2.7-5.3 °C is possible by 2095 (Boesch and Greer 2008). These projections, combined with the observation of more frequent poor L_p in rural subestuaries with March air temperatures greater than 9.8 °C, indicated poor survival of yellow perch eggs and larvae may be more common in a warmer future.

A sudden upward shift in both YPJ and mesozooplankton relative abundance occurred in the early 1990s in the Head-of-Bay region. The term “regime shift” has been used to suggest these types of changes are causally connected and linked to other changes in an ecosystem (Steele 1996; Vert-pre et al. 2013). Previous analysis of annual chlorophyll a averages at two Head-of-Bay monitoring stations (CB1.1 and CB2.1;

provided by W. Romano, MD DNR, Tidewater Ecosystem Assessment; Table 2-10) indicated that a downward shift occurred between 1978 and the early 1990s; yellow perch stock biomass had declined during the same period (J. Uphoff, MD DNR, unpublished analysis). Shortly after the downward shift in chlorophyll a leveled off, YPJ rose. Annual mean chlorophyll a was always greater than 8 mg/L during 1978-1989 at both stations (N = 16, monitoring not conducted during 1980-1983); YPJ was, on average, lower during this period and stronger year-classes were less frequently detected. During 1990-2008, only 27% of annual means of chlorophyll a at CB1.1 and CB2.1 were greater than 8 mg/L (N = 44; Table 2-10) and there were only three years where both stations had chlorophyll a greater than 8 mg / L. Mesozooplankton indices were higher, and strong YPJ was more frequent during this period of lower chlorophyll a. Annual mean chlorophyll a has been greater than 8 mg/L after 2009 (4 of 6 estimates), but it is too early to know if this represents a shift. Zooplankton monitoring was discontinued in 2002 so zooplankton data to match against chlorophyll a or YPJ in subsequent years does not exist. If the regime shift detected for Head-of-Bay yellow perch YPJ was induced by a chlorophyll a changes, then autochthonous production may play a large role there. An exploration of actions that may have reduced chlorophyll a or other factors (such as toxics) that may have influenced chlorophyll a, zooplankton, and YPJ in the Head-of-Bay region has not been conducted. Regime shifts in productivity are common for marine stocks, more-so than responses due to shifts in spawning stock size that represent a single equilibrium (Vert-pre et al. 2013). Stocks exhibiting rapid productivity shifts require different harvest strategies than those based on an assumption of a single equilibrium (Vert-pre et al. 2013).

Versar (2002) noted that linear relationships between striped bass or white perch juvenile indices and mesozooplankton indices in several Bay subestuaries were not consistently detected. Searching for linear patterns may not have provided an adequate model for testing a regime shift hypothesis (Samhouri et al. 2010). Sudden shifts in population status can be regarded as nonlinear jumps between alternative equilibrium states of ecological systems (Steele and Henderson 1984) rather than linear transitions. Sampling of chlorophyll a and zooplankton occurred monthly, while their dynamics and those of larval fish occurred on a scale of days. This frequency of monitoring may have been enough to detect a general change in chlorophyll a and zooplankton in the Head-of-Bay region (from low to high), but not measure the magnitude and timing important to yellow perch. The use of a categorical variable in the multiple regression with YPT may have provided a more suitable representation of non-linear ecological change (Rose et al. 1986) than linear regressions that attempted to match a juvenile index endpoint with monthly "snapshots" of highly variable zooplankton abundance.

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 (Hoffman et al.2007), while phytoplankton would be the primary source of OM in years of lesser flow. Stable isotope signatures of York River, Virginia, 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 largely 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).

Zooplankton supply (cladocerans and copedpods) 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). 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).

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 and Patapsco rivers 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 the 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. (2011a) found that the percentage of Maryland's Chesapeake Bay subestuary watersheds in wetlands declined hyperbolically as IS increased, so this source of OM diminished with development.

Management for OM (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 has the potential to alter OM dynamics within a watershed (Stanley et al. 2012) and the effect of this major land use on fish habitat warrants further study. Agriculture has been associated with increased, decreased, and undetectable changes in OM that may reflect the diversity of farming practices (Stanley et al. 2012). As indicated earlier, extensive forest cover in a watershed may be linked to higher L_p than agriculture. However, Uphoff et al (2011a) noted that agricultural watersheds had more area in wetlands than urban watersheds and this could buffer loss of OM from decreased forest cover. Streams in agricultural watersheds were unlikely to become disconnected since urban stormwater controls would not be in use (Uphoff et al. 2011a).

In addition to feeding success, yellow perch egg viability declined greatly in highly developed suburban watersheds of Chesapeake Bay (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) explained the biology behind 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). Endrocrine disrupting chemicals were more likely to cause observed egg hatching failure in well developed tributaries than hypoxia and increased salinity (Blazer et al. 2013); these factors were identified as potential contributors to poor egg hatching success in Severn River (Uphoff et al. 2005). Low L_p occurs sporadically in subestuaries with rural watersheds and appears linked to high temperatures.

It is unlikely that low L_p had always existed in these suburban subestuaries since all supported productive and lightly regulated recreational fisheries into the 1970s (the C / ha threshold was met in Severn River during 1972). Severn River supported a state hatchery through the first half of the twentieth century and hatching rates of eggs in the hatchery were high into 1955, when records ended (Muncy 1962). Egg hatching success of Severn River yellow perch had declined drastically by the early 2000s when estimates of L_p were persistently low (Uphoff et al. 2005).

We used a general indicator of development (C / ha) in our analyses because negative effects of development involved multiple stressors difficult to isolate. Effects of multiple stressors are usually worse than the worst single stressor alone (Breitburg et al. 1998; Folt et al. 1999).

Our results suggest a general sequence of stressors impacted yellow perch larvae as development increased. Feeding success declined as development proceeded past the target level of development and was followed by reduced egg hatching in highly developed subestuaries, implying initial stress related to disruption of OM dynamics followed by endocrine disrupting contaminants

The response time of RNA/DNA ratios of larval fishes characterizes the feeding environment within a week of sampling (Tardif et al. 2005). 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. Hopefully, RNA/DNA response to development will be detectable in 2013 if conditions allow for successful application of our original sampling design.

We did not interpret RNA/DNA ratios as rejecting or supporting the OM hypothesis since there was very little variation in OM among systems in 2012, very low sample sizes in some systems, and an indication that *ad hoc* collections in the Head-of-Bay region may have induced bias due to date of collection.

Our RNA/DNA sampling during 2012 found that most yellow perch larvae collected from subestuaries over a broad geographic expanse and throughout the season were in the starved category. Some smaller TL larvae were in the fed category, but nearly all larger larvae (10-11 mm TL) had ratios indicating starved condition. The

RNA/DNA ratios of fed larvae were expected to increase with body size (Clemmensen 1994). Surveys of larval striped bass RNA/DNA in 1981 in the Potomac River estuary exhibited a similar declining pattern that we detected for yellow perch larvae, but striped bass 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. 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.

Low RNA/DNA ratios exhibited by some yellow perch at 7-9 mm may have reflected problems as they changed to external nutrition. 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 the 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. After 10 days, deprivation of food lead to a significant decrease in RNA/DNA ratios in comparison to fed Atlantic herring larvae (Clemmensen 1994). Low RNA/DNA ratios of larger and presumably older yellow perch larvae sampled from our subestuaries may have been more indicative of poor feeding conditions, although it was possible that bias may have resulted from starving, weaker, poorly growing larvae being more vulnerable to our plankton nets than fed larvae.

The proportion of RNA/DNA ratios indicative of fed larvae ($\geq$ 3) was greater in the least developed watershed sample (C / ha = 0.41, *Pf* = 0.30) than three subestuaries with above threshold development (*Pf* = 0.09 combined). However, *Pf* in the least developed watershed (Northeast River) may have reflected growth of a larval cohort rather than a summary of all cohorts produced there. Differences in *Ps* were not evident among the four watersheds (*Ps* = 0.62, all combined). Analysis and interpretation beyond comparison of 90% CI's of *Pf* and *Ps* were not conducted because of uneven and low sample sizes in some systems, and the *ad hoc* sampling needed after unexpected failure of eggs and prolarvae to survive in southern Maryland subestuaries.

In our 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, although some intrusion would have been possible at the most downstream sites in the smallest systems closest to the major rivers (i.e., Piscataway Creek for the Potomac River and Northeast River for the Susquehanna River). The forward selection procedure that selected variables for the multiple regression of YPJ and environmental factors did not retain March Susquehanna River flow in the final model, but did retain precipitation. We interpreted precipitation as a local watershed signal for subestuaries. Strong correlations of C / ha, L_p, and OM0 also indicated that local conditions prevailed.

Estimates of mean conductivity in subestuaries sampled during 2010-2011 (Uphoff et al. 2012) offered further evidence that local conditions were captured. Increases in stream conductivity have been strongly associated with urbanization (Wang and Yin 1997; Paul and Meyer 2001; Wenner et al. 2003; Morgan et al. 2007; Carlisle et al. 2010; Morgan et al. 2012) and have been noted in anadromous fish spawning streams in Maryland's portion of Chesapeake Bay (see Section 2-1). During 2010-2012, mean daily conductivities (219-249 μS / cm) in fresh-tidal Piscataway Creek's subestuary were elevated over those of fresh-tidal Mattawoman Creek's subestuary (range = 139-188 μS / cm) in spite of Piscataway Creek's upstream location on the Potomac River. In 2010, mean conductivities at two Chesapeake Bay Program monitoring stations corresponding to the mouths of Piscataway and Mattawoman creeks averaged 211-212 μS / cm (once-monthly measurements at six depths during March and April; W. Romano, MD DNR, personal communication). Elevated conductivity in Piscataway Creek indicated that urbanization impacted estuarine water quality as well as stream water quality.

High estimates of L_p that were equal to or approaching 1.0 have been routinely encountered 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 generally (Sammons and Bettoli 1998) and specifically for yellow perch (Anderson et al. 1998). Tighter budgets necessitate development of less costly 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.

Table 2-1. Estimates of proportions of ichtyoplankton net tows with yellow perch larvae (L_p) during 1965-2012 and data used for regression with counts of structures per hectare (C / ha). Salinity class 0 = tidal-fresh ($\leq$ 2.0 ‰) and 1 = brackish (> 2.0 ‰). Year is the year a subestuary was sampled. Primary landuse was determined from Maryland Department of Planning estimates for 1973, 1994, 1997, 2002, or 2010 that were closest to a sampling year. These latter categories were not used in regression analyses.

River	Year	C / Ha	Primary land use	Salinity	Lp
Bush	2006	1.17	Urban	0	0.79
Bush	2007	1.19	Urban	0	0.92
Bush	2008	1.20	Urban	0	0.49
Bush	2009	1.21	Urban	0	0.86
Bush	2011	1.22	Urban	0	0.96
Bush	2012	1.22	Urban	0	0.28
Choptank	1986	0.09	Agriculture	1	0.53
Choptank	1987	0.09	Agriculture	1	0.73
Choptank	1988	0.10	Agriculture	1	0.80
Choptank	1989	0.10	Agriculture	1	0.71
Choptank	1990	0.10	Agriculture	1	0.66
Choptank	1998	0.13	Agriculture	1	0.60
Choptank	1999	0.13	Agriculture	1	0.76
Choptank	2000	0.13	Agriculture	1	0.25
Choptank	2001	0.13	Agriculture	1	0.21
Choptank	2002	0.14	Agriculture	1	0.38
Choptank	2003	0.14	Agriculture	1	0.52
Choptank	2004	0.15	Agriculture	1	0.41
Corsica	2006	0.21	Agriculture	1	0.47
Corsica	2007	0.22	Agriculture	1	0.83
Elk	2010	0.59	Forest	0	0.75
Elk	2011	0.59	Forest	0	0.79
Elk	2012	0.59	Forest	0	0.53
Langford	2007	0.07	Agriculture	1	0.83
Magothy	2009	2.73	Urban	1	0.17
Mattawoman	1990	0.45	Forest	0	0.81
Mattawoman	2008	0.87	Forest	0	0.66
Mattawoman	2009	0.88	Forest	0	0.92
Mattawoman	2010	0.90	Forest	0	0.82
Mattawoman	2011	0.90	Forest	0	0.99
Mattawoman	2012	0.90	Forest	0	0.20
Middle	2012	3.32	Urban	0	0.00

Table 2-1 continued.

Nanjemoy	2009	0.09	Forest	1	0.83
Nanjemoy	2010	0.09	Forest	1	0.96
Nanjemoy	2011	0.09	Forest	1	0.99
Nanjemoy	2012	0.09	Forest	1	0.03
Nanticoke	1965	0.05	Agriculture	1	0.50
Nanticoke	1967	0.05	Agriculture	1	0.43
Nanticoke	1968	0.05	Agriculture	1	1.00
Nanticoke	1970	0.06	Agriculture	1	0.81
Nanticoke	1971	0.06	Agriculture	1	0.33
Nanticoke	2004	0.11	Agriculture	1	0.49
Nanticoke	2005	0.11	Agriculture	1	0.67
Nanticoke	2006	0.11	Agriculture	1	0.35
Nanticoke	2007	0.11	Agriculture	1	0.55
Nanticoke	2008	0.11	Agriculture	1	0.19
Nanticoke	2009	0.11	Agriculture	1	0.41
Nanticoke	2011	0.11	Agriculture	1	0.52
Nanticoke	2012	0.11	Agriculture	1	0.04
Northeast	2010	0.46	Forest	0	0.68
Northeast	2011	0.46	Forest	0	1.00
Northeast	2012	0.46	Forest	0	0.76
Piscataway	2008	1.41	Urban	0	0.47
Piscataway	2009	1.43	Urban	0	0.39
Piscataway	2010	1.45	Urban	0	0.54
Piscataway	2011	1.45	Urban	0	0.65
Piscataway	2012	1.45	Urban	0	0.18
Severn	2002	2.02	Urban	1	0.16
Severn	2004	2.09	Urban	1	0.29
Severn	2005	2.15	Urban	1	0.33
Severn	2006	2.18	Urban	1	0.27
Severn	2007	2.21	Urban	1	0.30
Severn	2008	2.24	Urban	1	0.08
Severn	2009	2.25	Urban	1	0.15
Severn	2010	2.26	Urban	1	0.03
South	2008	1.61	Urban	1	0.14

Table 2-2. Summary of results of regressions of proportions of tows with yellow perch larvae (L_p) and counts of structures per hectare (C / ha). 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		Brackish				
Source	*df*	*SS*	*MS*	*F*	*P*	
Model	1	1.007339	1.007339	18.27849	0.000114994	
Error	40	2.204425	0.055111			
Total	41	3.211763				
$r^2 = 0.31$						
	Coefficients	*SE*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.573645697	0.043682	13.13226	4.33E-16	0.485360744	0.661930649
Count/Ha	-0.174125679	0.040728	-4.27533	0.000115	-0.256439967	-0.091811392

ANOVA		Fresh-tidal				
Source	*df*	*SS*	*MS*	*F*	*P*	
Model	1	0.648134	0.648134	12.72007	0.001725107	
Error	22	1.120981	0.050954			
Total	23	1.769115				
$r^2 = 0.37$						
	Coefficients	*SE*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.94527613	0.096374	9.808433	1.71E-09	0.745409076	1.145143183
Count/Ha	-0.281081391	0.078811	-3.56652	0.001725	-0.444525606	-0.117637175

ANOVA		Multiple regression				
Source	*df*	*SS*	*MS*	*F*	*P*	
Model	2	2.043209	1.021604	18.92602	3.6573E-07	
Error	63	3.400666	0.053979			
Total	65	5.443875				
$R^2 = 0.37$						
	Coefficients	*SE*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0.853150087	0.061255	13.92789	6.71E-21	0.730742159	0.975558015
Count/Ha	-0.195305255	0.036097	-5.41061	1.04E-06	-0.267438847	-0.123171663
Salinity	-0.266809228	0.06187	-4.31245	5.79E-05	-0.390445725	-0.14317273

Table 2-3. Summary of estimates used in correlation analysis of yellow perch larval feeding success. C / ha = counts of structures per acre. Mean full = mean of fullness ranks assigned to larval guts. OM0 = proportion of samples with organic matter (detritus). P0 = proportion of guts without food. Pclad = proportion of guts with cladocerans. Pcope = proportion of guts with copedpods. Pothr = proportion of guts with "other" food items. Mean TL = mean TL of larvae in mm. Mean fullness = average feeding rank of larvae. *N* = number of yellow perch larvae examined.

River	Year	C / ha	Mean_full	OM0	P0	Pclad	Pcope	POther	Mean TL	N
Elk	2010	0.56	2.75		0.05	0.02	0.95	0.13	11.1	110
Mattawoman	2010	0.88	2.00		0.09	0.15	0.78	0.09	9.2	55
Nanjemoy	2010	0.09	2.88		0.00	0.10	1.00	0.15	9.1	48
Northeast	2010	0.41	2.34		0.19	0.22	0.72	0.30	8.4	64
Piscataway	2010	1.43	1.85		0.13	0.00	0.55	0.53	9.4	55
Elk	2011	0.56	2.81	0.76	0.08	0.00	0.96	0.01	8.9	90
Mattawoman	2011	0.88	0.90	0.78	0.42	0.02	0.51	0.07	9.3	110
Nanjemoy	2011	0.09	2.18	0.56	0.07	0.03	0.83	0.20	9.0	150
Nanticoke	2011	0.14	3.27	0.55	0.08	0.71	0.92	0.16	8.6	51
Northeast	2011	0.41	2.44	0.58	0.08	0.00	0.91	0.09	8.3	90
Piscataway	2011	1.43	0.00	1.00	1.00	0.00	0.00	0.00	8.4	32
Bush	2012	1.22	2.47		0.00	0.55	0.52	1.00	8.6	40
Elk	2012	0.56	0.77	0.77	0.24	0.02	0.00	0.70	7.7	198
Mattawoman	2012	0.88	1.81	1.00	0.00	0.44	0.88	1.00	8.8	16
Northeast	2012	0.41	1.17	1.00	0.01	0.04	0.08	0.99	7.5	203
Piscataway	2012	1.43	1.67	0.98	0.00	0.55	0.66	1.00	8.7	9

Table 2-4. Correlation matrix for yellow perch larval feeding success. C / ha = counts of structures per acre. Mean fullness = average feeding rank of larvae. OM0 = proportion of samples with organic matter. P0 = proportion of guts without food. P Clad = proportion of guts with cladocerans. P Cope = proportion of guts with copepods. P othr = proportion of guts with "other" food items. Mean TL = mean TL of larvae in mm. L_p = proportion of plankton tows with larvae. Statistic r = Pearson correlation coefficient, P = level of significance, and N = number of observations. Gray shading indicates correlation of interest at P ≤ 0.05.

Parameter	Statistic	C / ha	Mean fullness	OM0	P0	P_{clad}	P_{cope}	P_{othr}	Mean TL
Mean fullness	r	-0.51							
	P	0.04							
	N	16							
OM0	r	0.75	-0.64						
	P	0.01	0.05						
	N	10	10						
P0	r	0.38	-0.71	0.25					
	P	0.15	0.002	0.49					
	N	16	16	10					
P_{clad}	r	0.12	0.35	0.03	-0.34				
	P	0.65	0.18	0.93	0.20				
	N	16	16	10	16				
P_{cope}	r	-0.40	0.86	-0.52	-0.57	0.24			
	P	0.13	<.0001	0.13	0.02	0.37			
	N	16	16	10	16	16	16		
P_{othr}	r	0.34	-0.19	0.61	-0.39	0.46	-0.34		
	P	0.20	0.48	0.06	0.13	0.07	0.20		
	N	16	16	10	16	16	16		
Mean TL	r	0.07	0.37	-0.25	-0.11	-0.11	0.55	-0.39	
	P	0.80	0.15	0.48	0.67	0.68	0.03	0.13	
	N	16	16	10	16	16	16	16	
L_p	r	-0.56	0.08	-0.55	0.14	-0.72	0.17	-0.75	0.15
	P	0.02	0.77	0.10	0.61	0.002	0.53	0.001	0.57
	N	16	16	10	16	16	16	16	16

Table 2-5. Estimates of mean March air temperature (°C), salinity classification of subestuary (0 = tidal-fresh ≤ 2.0 ‰ and 1= brackish > 2.0 ‰), total March precipitation (cm) and proportion of tows with yellow perch larvae (L_p).

Year	Subestuary	Air temperature	Salinity type	Precipitation	Lp
1965	Nanticoke	4.7	0	1.25	0.5
1967	Nanticoke	5.9	0	0.72	0.43
1968	Nanticoke	8.8	0	1.91	1
1970	Nanticoke	4.6	0	1.61	0.81
1971	Nanticoke	5.6	0	1.31	0.33
2004	Nanticoke	10.4	0	0.00	0.49
2005	Nanticoke	4.1	0	1.59	0.67
2006	Nanticoke	6.1	0	0.06	0.35
2007	Nanticoke	7.0	0	1.38	0.55
2008	Nanticoke	7.8	0	0.95	0.19
2009	Nanticoke	6.1	0	0.56	0.41
2011	Nanticoke	9.9	0	1.39	0.52
2012	Nanticoke	12.2	0	0.44	0.04
1986	Choptank	7.9	0	0.26	0.53
1987	Choptank	7.1	0	1.58	0.73
1988	Choptank	7.5	0	1.42	0.8
1989	Choptank	7.5	0	2.80	0.71
1990	Choptank	9.1	0	1.34	0.66
1998	Choptank	8.4	0	1.88	0.6
1999	Choptank	6.3	0	1.90	0.76
2000	Choptank	9.8	0	2.45	0.25
2001	Choptank	5.8	0	2.50	0.21
2002	Choptank	7.8	0	2.25	0.38
2003	Choptank	7.6	0	1.92	0.52
2004	Choptank	10.4	0	0.00	0.41
2006	Corsica	6.4	0	0.14	0.47
2007	Corsica	7.0	0	1.51	0.83
2007	Langford	7.0	0	1.51	0.83
2009	Nanjemoy	5.7	0	1.26	0.83
2010	Nanjemoy	8.4	0	1.93	0.96
2011	Nanjemoy	6.8	0	2.39	0.99
2012	Nanjemoy	11.6	0	0.93	0.03
2010	Elk	8.5	1	2.21	0.75
2011	Elk	6.3	1	1.40	0.79

Table 2-5 continued.

2012	Elk	10.4	1	0.68	0.53
2010	Northeast	8.5	1	2.21	0.68
2011	Northeast	6.3	1	1.40	1
2012	Northeast	10.4	1	0.68	0.76

Table 2-6. Final multiple regression model of proportions of tows with yellow perch larvae (L_p) against $\log_e$-transformed mean regional March air temperature ($\log_e$-T, °C), $\log_e$-transformed mean regional March precipitation ($\log_e$-P, cm), and salinity category (0 = tidal-fresh ≤ 2.0 ‰ and 1= brackish > 2.0 ‰).

ANOVA

Source	df	SS	MS	F	P
Model	3	0.6521	0.21737	4.02769	0.01542
Error	32	1.72699	0.05397		
Total	35	2.37909			
R^2 =	0.27				

Parameter

Variable	Coefficient	SE	t-value	Pr > t
Intercept	1.38	0.35	3.93	0.0004
$\log_e$-T	-0.31	0.16	-1.93	0.0626
$\log_e$-P	0.11	0.05	2.21	0.0343
Salinity	-0.22	0.11	-2.07	0.0466

Table 2-7. Annual Head-of-Bay precipitation (cm), air temperature (°C), and Susquehanna River flow at Conowingo Dam (m^3) during March and the Head-of-Bay yellow perch juvenile index, 1968-2012.

Year	Precipitation	Air Temperature	Flow	Juvenile index
1968	2.23	8.2	1,701	0
1969	0.41	5.2	850	0
1970	1.26	4.0	1,618	0
1971	0.70	5.2	3,117	0
1972	1.17	4.9	3,321	0
1973	1.50	8.6	1,976	0
1974	1.31	7.3	1,843	0.18
1975	1.53	5.7	2,475	0.11
1976	0.40	8.7	1,961	0.03
1977	1.72	9.8	3,849	0.37
1978	1.95	5.1	3,438	0.24
1979	0.77	8.5	4,284	0.17
1980	2.29	5.3	2,111	0
1981	0.55	5.9	1,064	0.11
1982	0.77	6.2	2,584	0.07
1983	2.88	8.0	1,520	0.03
1984	1.55	4.6	1,675	0.29
1985	0.94	8.3	1,686	0.03
1986	0.38	8.4	3,051	0.07
1987	0.59	7.8	1,729	0.05
1988	0.81	7.5	1,467	0.05
1989	1.69	6.8	1,266	0.1
1990	0.72	8.7	1,169	0.22
1991	1.52	9.0	2,229	0.03
1992	1.67	6.2	1,885	0
1993	2.62	5.0	2,496	2.21
1994	2.50	6.2	4,434	0.42
1995	0.60	8.8	1,423	0.46
1996	1.67	5.9	2,138	1.44
1997	2.08	8.0	2,215	0.12
1998	2.45	7.5	2,950	0.64
1999	2.00	6.7	1,557	0.25
2000	2.61	10.4	2,456	0.9
2001	1.90	5.1	1,418	0.71
2002	1.31	6.7	1,222	0

Table 2-7 continued.

2003	1.74	6.5	3,132	1.2
2004	0.89	7.8	2,699	0.69
2005	1.81	4.8	2,037	0.29
2006	0.06	8.1	943	0.09
2007	1.76	7.9	3,072	0.18
2008	0.92	6.0	3,552	0.05
2009	0.77	5.1	1,438	0.18
2010	2.21	8.5	2,390	0.06
2011	1.40	6.3	4,680	0.64
2012	0.68	10.4	1,322	0.19

Table 2-8. Final Head-of-Bay multiple regression model of $\log_e$-transformed juvenile index (YPT) against $\log_e$-transformed mean regional March precipitation ($\log_e$-P, cm), and year category (Y; 0 1968-1992 and 1= 1993-2012).

ANOVA

Source	df	SS	MS	F	P
Model	2	1.18	0.59	13.07	<0.0001
Error	42	1.89	0.05		
Total	44	3.07			
R^2 =	0.38				

Parameter

Variable	Coefficient	SE	t-value	Pr > t
Intercept	0.072	0.043	1.696	0.097
$\log_e$-P	0.090	0.045	2.005	0.051
Y	0.282	0.064	4.400	<0.0001

Table 2-9. Summary of feeding success, larval length, sample size and RNA/DNA characteristics, by subestuary and sample date. Data for dates with feeding information only and with RNA/DNA analysis are summarized. Mean fullness = mean feeding rank. Mean TL is in mm. N = total sample size of larvae processed for gut contents. Mean RNA/DNA is the average for the date. SE RNA/DAN is the standard error for the date. N RNA/DNA > 3 is the number of ratios above the fed criierion. N RNA/DNA < 2 is the number of ratios below the starvation criterion.

Subestuary	Variable	22-Mar	27-Mar	29-Mar	30-Mar	3-Apr	5-Apr	6-Apr	11-Apr
Northeast	Mean fullness	1.0	1.1		1.0	1.0		2.1	1.0
	Mean TL	7.6	7.2		7.3	8.8		7.4	8.5
	N	7	78		61	26		27	4
	Mean RNA/DNA							2.18	
	SE RNA/DNA							0.252	
	N RNA/DNA > 3							8	
	N RNA/DNA < 2							17	
Bush	Mean fullness						2.5		
	Mean TL						8.8		
	N						40		
	Mean RNA/DNA						1.989		
	SE RNA/DNA						0.228		
	RNA/DNA > 3						4		
	RNA/DNA < 2						22		
Mattawoman	Mean fullness		1.4	2.0					
	Mean TL		8.5	9.2					
	N		10	5					
	Mean RNA/DNA		1.36	1.31					
	SE RNA/DNA		0.23	1.17					
	RNA/DNA > 3		0	1					
	RNA/DNA < 2		10	3					
Piscataway	Mean fullness		1.4	2.0					
	Mean TL		8.6	8.8					
	N		5	4					
	Mean RNA/DNA		1.99	2.50					
	SE RNA/DNA		1.04	0.29					
	RNA/DNA > 3		1	0					
	RNA/DNA < 2		4	0					

Table 2-10. Trends in annual mean chlorophyll a at two Head-of-Bay water quality monitoring stations (CB1.1 Mean = the mouth of the Susquehanna River and CB2.1 Mean =t Turkey Point) and the Head-of-Bay yellow perch juvenile index. Chlorophyll a data were provided by W. Romano (MD DNR, Tidewater Ecosystem Assessment)

Year	CB1.1 Mean	CB2.1 Mean	YPJ
1978	15.0	17.1	0.24
1979	15.0	18.0	0.17
1984	9.0	12.3	0.29
1985	8.9	9.0	0.03
1986	8.0	12.1	0.07
1987	10.2	11.3	0.05
1988	9.7	10.5	0.05
1989	10.8	11.3	0.10
1990	6.8	7.9	0.22
1991	8.8	7.2	0.03
1992	5.0	3.6	0.00
1993	5.2	3.6	2.21
1994	5.7	4.3	0.42
1995	10.0	6.1	0.46
1996	6.5	13.2	1.44
1997	9.9	8.4	0.12
1998	7.6	7.8	0.64
1999	7.9	5.2	0.25
2000	5.3	3.7	0.90
2001	7.1	6.0	0.71
2002	7.0	4.5	0.00
2003	8.2	10.4	1.20
2004	6.3	4.9	0.69
2005	6.2	4.5	0.29
2006	3.9	7.0	0.09
2007	4.4	8.3	0.18
2008	6.6	5.3	0.05
2009	6.3	11.0	0.18
2010	9.0	13.4	0.06
2011	7.4	10.8	0.64

Figure 2-1. Sampling areas for the 2011 yellow perch larval presence absence study. Nanticoke River watershed delineation was unavailable for Delaware and Northeast and upper Elk River were unavailable for Pennsylvania.

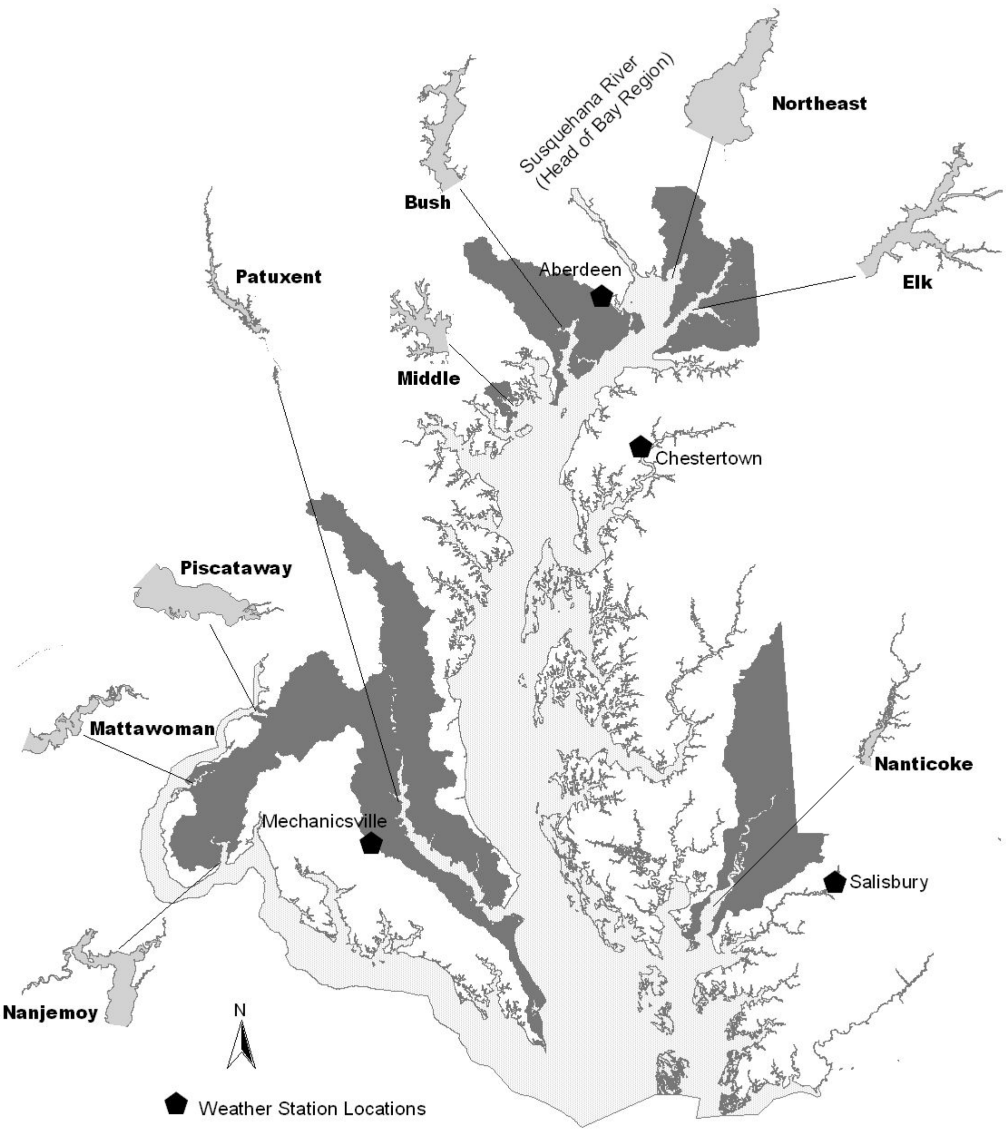


Figure 2-2. Proportion of tows with larval yellow perch (L_p) and its 95% confidence interval in systems studied during 2012. Mean *L*p of brackish tributaries indicated by diamond and fresh-tidal mean indicated by dash.

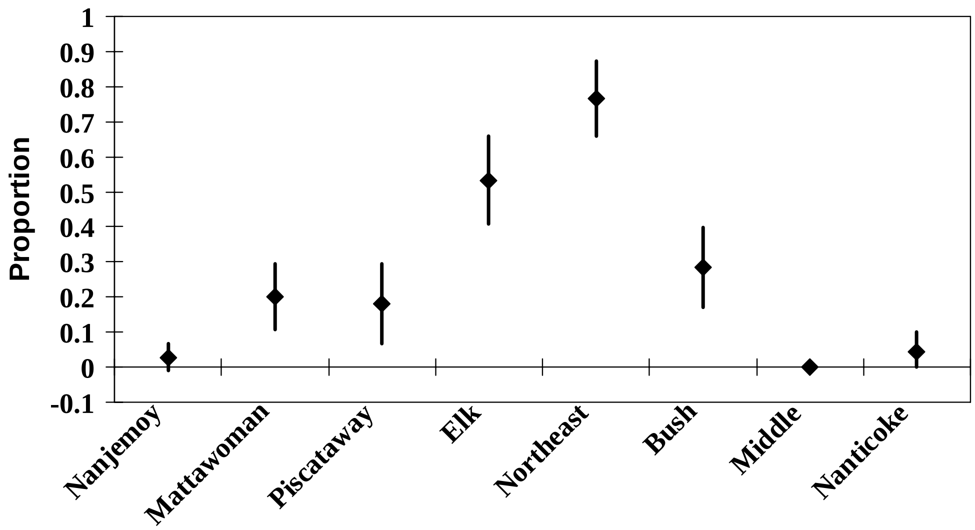


Figure 2-3. Proportion of tows with yellow perch larvae (L_p) for brackish subestuaries, during 1965-2012. Dotted line provides reference for persistent poor *Lp* exhibited in developed brackish subestuaries.

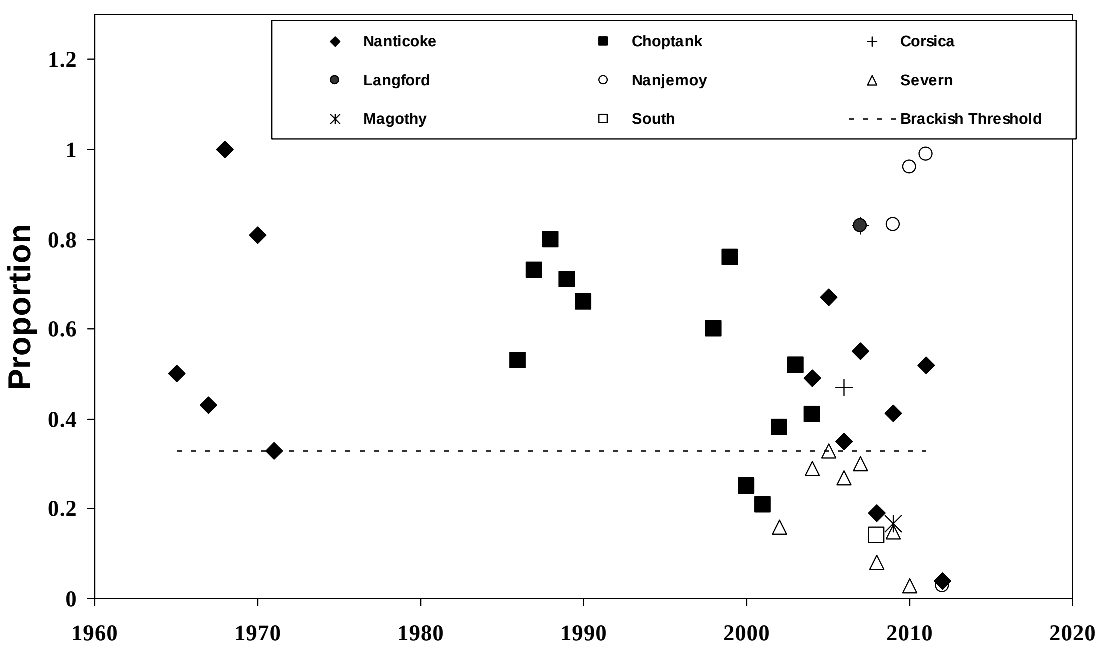


Figure 2-4. Proportion of tows with yellow perch larvae (*Lp*) for fresh-tidal subestuaries, during 1990-2011. Dotted line provides reference for consistent poor *Lp* exhibited in a more developed fresh-tidal subestuary (Piscataway Creek).

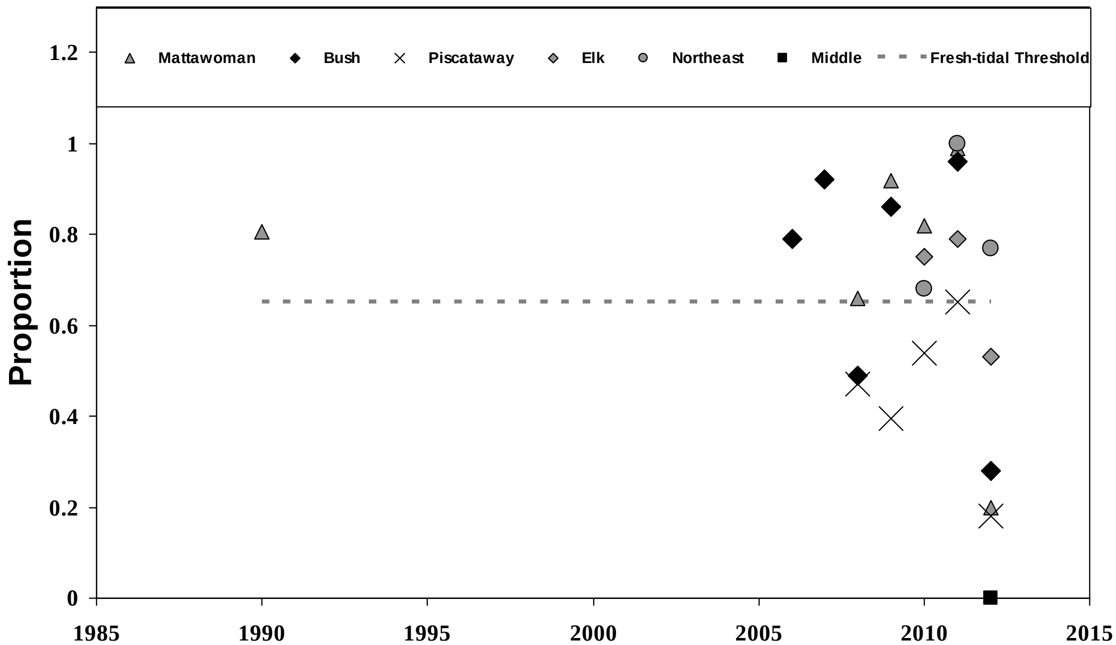


Figure 2-5 . Relationship of proportion of plankton tows with yellow perch larvae and development (structures per hectare or C / ha) indicated by multiple regression of fresh and brackish subestuaries combined (prediction = MR) and separate linear regressions for both (prediction = LR) .

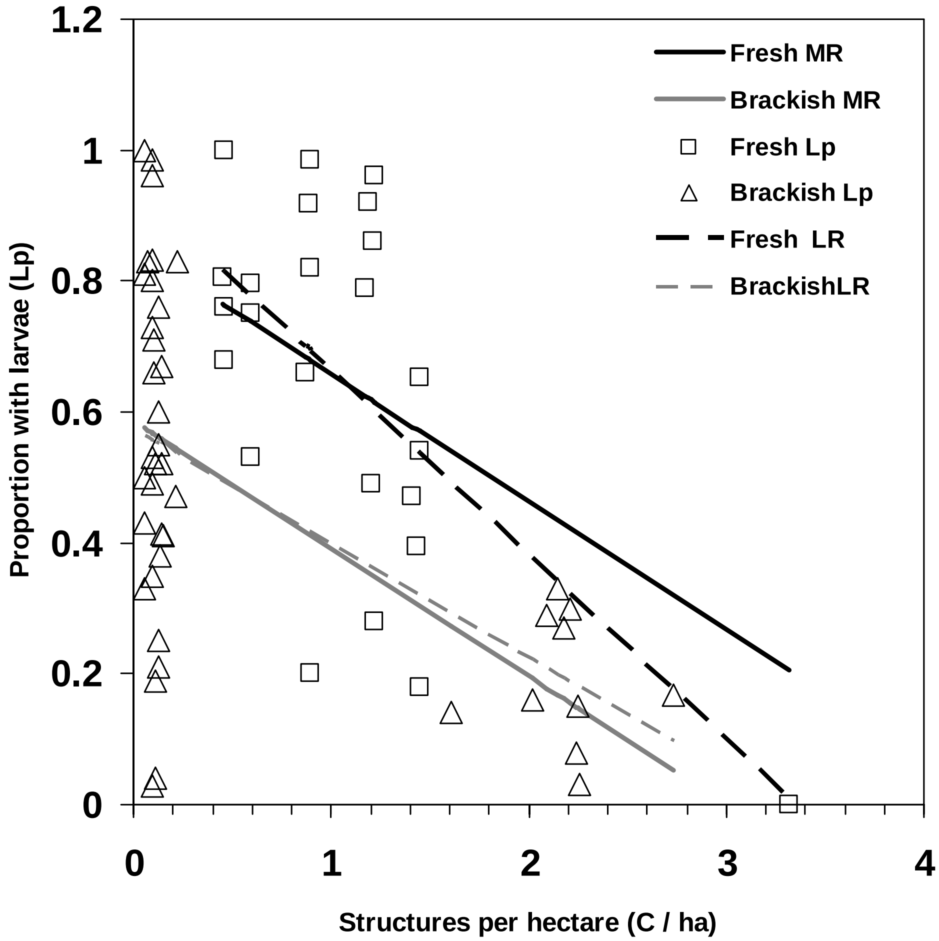


Figure 2-6. Residuals of the multiple regression of proportion of plankton tows against yellow perch larvae with March air temperature and precipitation, and salinity classification in rural subestuaries.

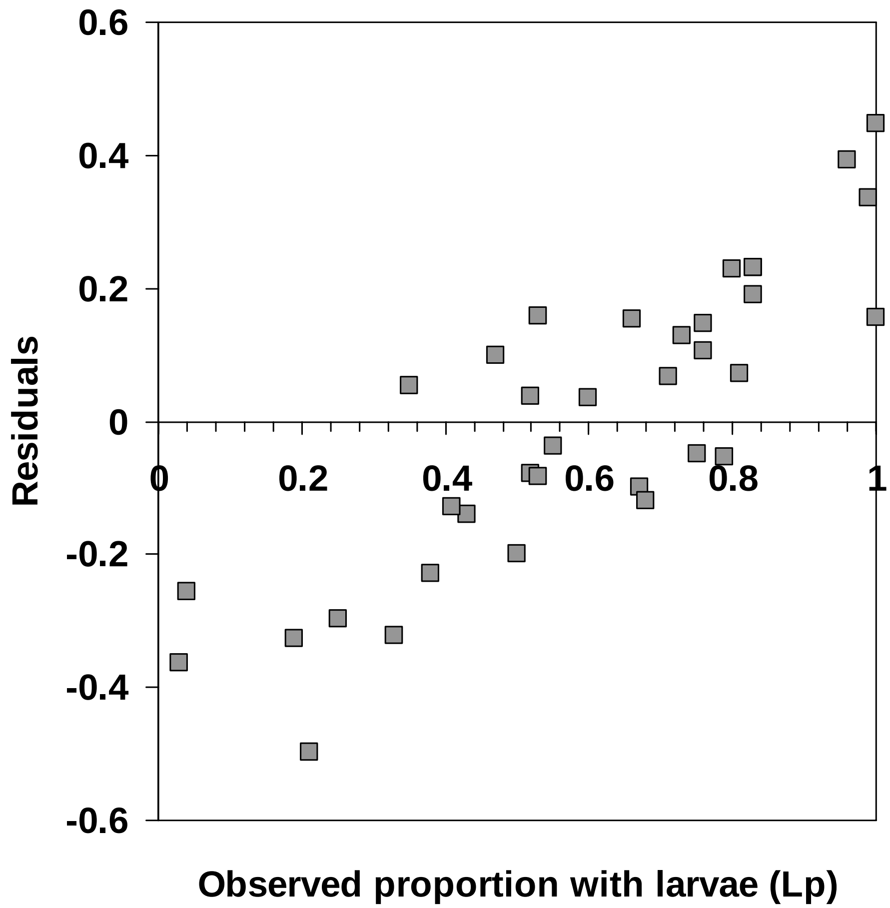


Figure 2-7. Observed and predicted $\log_e$-transformed yellow perch juvenile indices for Head-of-Bay during 1968-1012 from a multiple regression with $\log_e$-transformed March precipitation and a categorical variable indicating a regime shift.

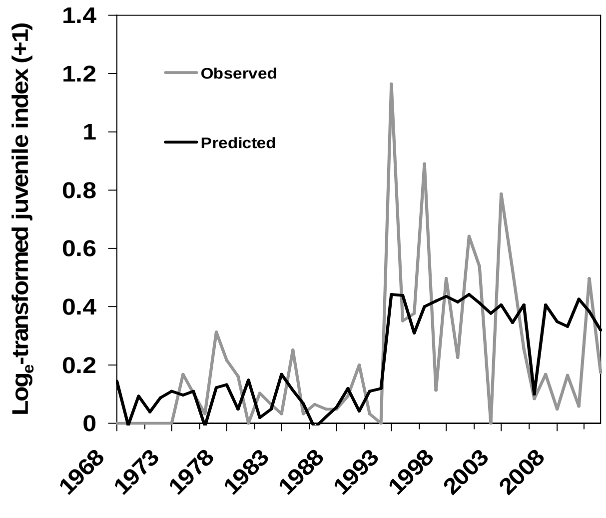


Figure 2-8. Mesozooplankton (Versar 2002) and yellow perch juvenile indices for Head-of-Bay during 1985-2001.

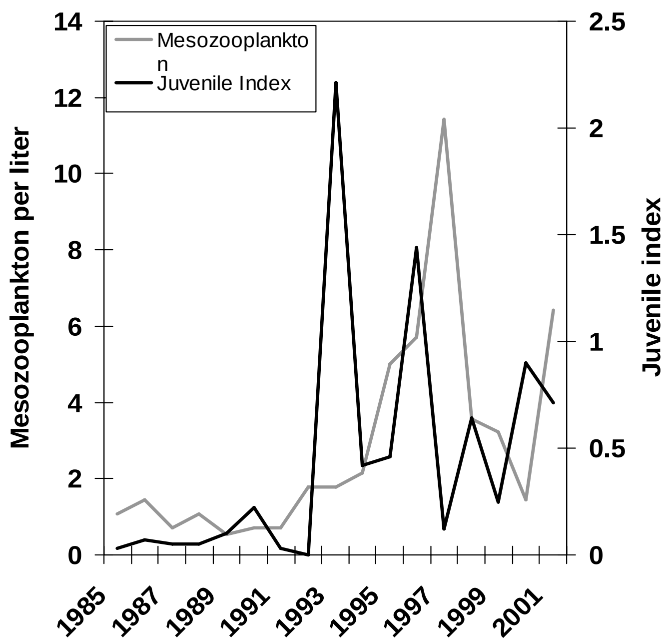


Figure 2-9. RNA / DNA ratios for yellow perch larvae by total length. Larvae were collected during 2012. Subestuaries are indicated by symbols. Reference lines are provided for ratios indicative of starved and fed conditions.

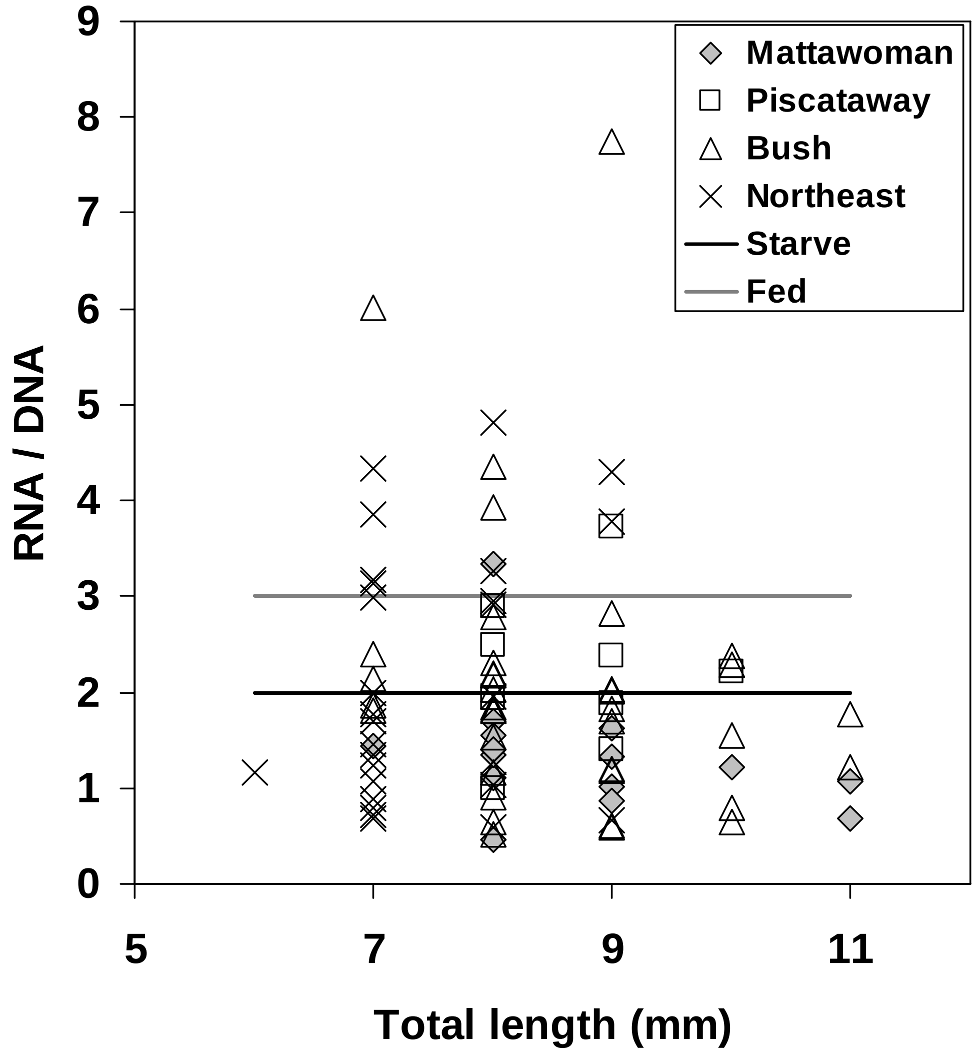


Figure 2-10. Proportion of sampled larvae with RNA / DNA ratios less than or equal to the starvation criterion (RNA / DNA ≤ 2) by subestuary watershed development level (structures per hectare).

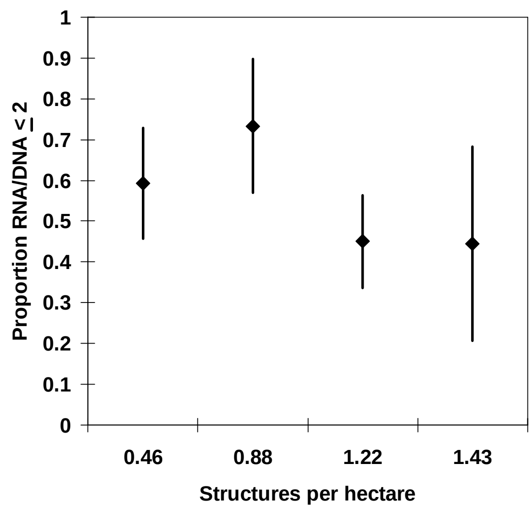
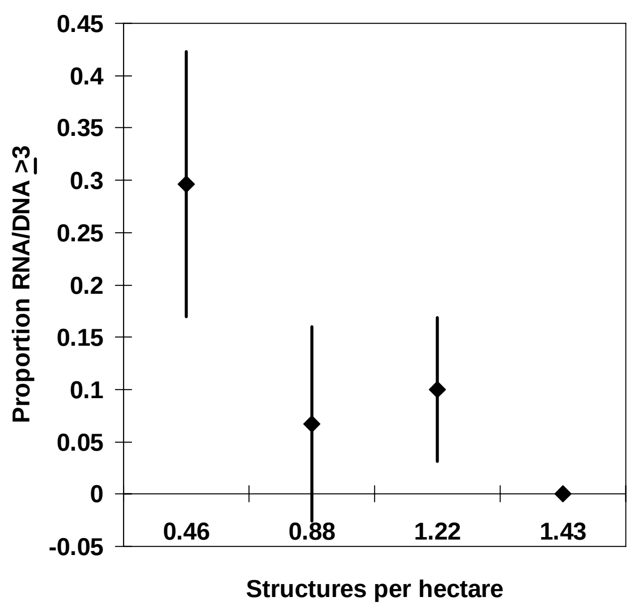


Figure 2-11. Proportion of sampled larvae with RNA / DNA ratios greater than or equal to the fed criterion (RNA / DNA ≥ 3) by subestuary watershed development level (structures per hectare).



Job 1 Section 3 - Estuarine Fish Community Sampling

Introduction

Reviews by Wheeler et al. (2005) and the National Research Council (NRC 2009) documented deterioration of non-tidal freshwater aquatic habitat as IS occupied more than 10% of watershed area. Uphoff et al. (2011a) estimated target and limit ISRPs for productive fish habitat in brackish (mesohaline) Chesapeake Bay subestuaries based on Chesapeake Bay DO criteria, and associations and relationships of watershed IS, summer DO, and presence-absence of recreationally important finfish in bottom waters. Watersheds at a target of 5.5% IS 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 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 respond to IS in brackish subestuaries, Uphoff et al. (2011b; 2012) have found adequate concentrations of DO in bottom channel habitat of fresh-tidal and oligohaline subestuaries with watersheds at suburban levels of development. They suggested bottom channel waters were not succumbing to low oxygen because stratification due to salinity was weak, allowing them to become well mixed. However, low DO was more frequent in shallow waters in dense SAV than in bottom channel waters of Mattawoman Creek during 2011 (Uphoff et al. 2012).

Water quality and aquatic habitat within watersheds is altered by agricultural activity and urbanization; both include use of pesticides and fertilizers, while the latter may have additional industrial wastes, contaminants, stormwater runoff and road salt (Brown 2000; NRC 2009; Benejam et al. 2010) 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).

In 2012, we continued to evaluate nursery and adult habitat for recreationally important finfish in fresh-tidal, oligohaline, and mesohaline subestuaries of Chesapeake Bay. We have emphasized Mattawoman Creek in this report as part of Maryland DNRs' efforts to influence Charles County into modifying its comprehensive growth plan to conserve natural resources of its watershed (See section 3; MDDNR 2012).

Methods

Data from twelve subestuaries of the Chesapeake Bay were evaluated in 2012 (Figure 3-1). Our program sampled eight subestuaries, including three tributaries to the Potomac (Piscataway and Mattawoman creeks, and Wicomico River), three subestuaries to the Choptank River (Broad and Harris creeks, and Tred Avon River), and two subestuaries of mainstem Chesapeake Bay (Middle and Gunpowder Rivers). Broad Creek and Harris Creek were added in 2012. These watersheds, downstream of Tred Avon River, represented a gradient of development from 0.293 (Broad Creek) to 0.747 (Tred Avon) within a single watershed (Table 3-1).

Four additional tributaries were sampled for us: Corsica and Northeast Rivers sampled by Alosine Project Staff; Nanjemoy Creek, sampled by NOAA's Integrated Assessment Project staff, and Bush River, sampled by Chesapeake Bay National Estuarine Research Reserve staff and volunteers.

Housing density (C / ha) and impervious surface (IS) were estimated for each watershed (Table 3-1). 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). This indicator has been provided to us by Marek Topolski of the Fishery Management Planning and Fish Passage Program.

Tax maps are graphic representations of individual property boundaries and existing structures that help State tax assessors locate properties (Maryland Department of Planning or MDP 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 ArcGIS Model Builder to automate assembly of statewide tax maps, query tax map data, and assemble summary data. Each year's statewide tax map was clipped using the Maryland 8-digit watershed boundary file to create watershed land tax maps. Watershed area estimates excluded estuarine waters,. 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).

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. The relationship of C / ha and IS was well described by the equation

$$IS = 10.98 \bullet (C / ha)^{0.63}, (r^2 = 0.96; P < 0.0001).$$

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 (Uphoff et al. 2012).

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 arbitrarily 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.

Ideally, four evenly spaced haul seine and bottom trawl sample sites were located in the upper two-thirds of each subestuary. Nanjemoy and Piscataway were covered sufficiently by three sites. Sites were not located near a subestuary's mouth to reduce influence of mainstem waters on fish habitat.

Sites were sampled once every two weeks during July-September. All sites on one river were sampled on the same day. 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. We used GPS to record the latitude and longitude at the middle of the trawl site, while seine latitude and longitude were taken at the exact seining location.

Target species included striped bass, yellow perch, white perch, alewife, blueback herring, American shad, spot, Atlantic croaker, and Atlantic menhaden. With the exception of white perch, adults of the target species were rare and juveniles were common. Gear specifications and techniques were selected to be compatible with other Fisheries Service surveys.

Water quality parameters were recorded at all sites. Temperature (ºC), dissolved oxygen or DO (mg/L), conductivity (µS / cm), salinity (‰), and pH were recorded for 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.

Dissolved oxygen concentrations were evaluated by watershed against a target of 5.0 mg/L and a threshold of 3.0 mg/L (Uphoff et al. 2011a). This target DO is 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. This criterion was associated with asymptotically high presence of target species in bottom channel habitat in brackish subestuaries (Uphoff et al. 2011a). Presence of target species declined sharply when bottom DO fell below the 3.0 mg / L threshold (Uphoff et al. 2011a). In each subestuary, we estimated the percentages of DO samples 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 $[(N_{target} / N_{total})•100]$ or $[(N_{threshold} / N_{total}) •100]$, respectively; 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.

Each subestuary was classified into a salinity category, based on the Venice System for Classification of Marine Waters (Oertli, 1964). Salinity influences distribution and abundance of fish (Hopkins and Cech, 2003; Cyrus and Blaber, 1992; Allen, 1982) and DO (Kemp et al. 2005). We calculated mean bottom salinity to determine salinity classification for each subestuary. We pooled all annual sets of bottom salinity data by watershed to calculate mean bottom salinity and used mean bottom salinity to classify each subestuary as tidal-fresh, oligohaline, or mesohaline. We grouped analyses by these classifications when examining effects of development.

Tidal-fresh ranged from 0 to 0.5 ‰; oligohaline, 0.5 -5.0 ‰; and meshohaline, 5.0 -18.0 ‰ (Oertli, 1964).

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 2009-2012, 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 initial choice of net for each day in Mattawoman Creek was decided by a coin flip.

An untreated 30.5 m • 1.2 m bagless knotted 6.4 mm stretch mesh beach seine, the standard gear for Bay inshore fish surveys (Carmichael et al. 1992; Durell 2007), 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 57 gm lead weights spaced evenly at 0.55 m intervals. One end of the seine was held on shore, while the other was stretched perpendicular to 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 (juvenile, small adults and harvestable size) based on size and life stage. The small adult white perch category consisted of ages-1+ white perch smaller than 200 mm. 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. Small and harvestable white perch were combined when catches were summarized as adults. Catch data were summarized and catch statistics were reported for both gears combined and each gear separately.

Target species catch data were treated as presence-absence to estimate relative abundance of each indicator species as P_i, the proportion of trawl or seine samples with a target species. Proportions of samples with a target species (P_i) and their SD's were calculated as $P_i = N_{present} / N_{total}$, where $N_{present}$ equaled the number of samples with a target species present and N_{total} equaled the total number of samples taken. The SD of each P_i was estimated as

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

The 95% confidence intervals were constructed as $P_{herr} \pm (1.96 \bullet SD)$.

We used linear regression to examine the relationship of C / ha and P_i in tidal-fresh subestuaries. We used annual estimates of P_i from trawl sampling during 2003-2012. Analyses were considered significant at $\alpha \leq 0.05$. Residuals were examined for normality and outliers.

Presence-absence was ecologically meaningful, minimized errors and biases in sampling, and reduced statistical concerns about lack of normality and high frequency of zero catches that were expected given the hypothesis that increased development leads to reduced habitat suitability (Green 1979; Bannerot and Austin 1983; Mangel and Smith 1990; Uphoff et al. 2011a).

Relative abundance of all finfish combined was summarized as an arithmetic mean catch per unit effort (CPUE) separately for trawl and seine for each subestuary sampled during 2012.

We compared long-term changes in fish abundance and community composition in Mattawoman Creek to changes in C / ha and SAV (Virginia Institute or Marince Science 2012) Estimates of SAV coverage were not available for Mattawoman Creek in 2001 because of air space restrictions precluded aerial surveys.

Annual estimates of central tendency of total fish abundance were calculated as geometric means (based on loge-transformed catches) of all fish species in 3.1 m headrope trawl samples (Ricker 1975; Green 1979). Geometric means and their 95% CI's were plotted against the progression of C / ha starting in 1989.

To examine changes in community compositions, we established 1989-2000 as a pre-disturbance period and 2001-2012 as a post-disturbance period. Uphoff et al. (2010) described a threshold effect of C / ha on total abundance of all species and species richness in Mattawoman Creek. Previous to 2002, abundance and richness were not responsive to development, but a sudden decrease in both occurred afterwards (Uphoff et al. 2010). Analysis of community composition had to consider changes in trawls used for monitoring. We had data from 1989-2002 and 2009-2012 using 3.1 m headrope otter trawls (Carmichael et al. 1992) and data from 2003-2012 using a 4.9 m headrope otter trawl. We calculated the proportion of positive trawls for each of the most common species during 1989-2000 and plotted them in descending order. For post-disturbance comparisons, we calculated gear-specific proportions of positive trawls in the same order as for the pre-disturbance period. Spearman's rank correlation analysis was used to determine if the pre-disturbance and post-disturbance community ranks were similar or different. Spearman rank correlation is a commonly used nonparametric test for ordinal data that measures statistical resemblance between two species assemblages (Kwak and Peterson 2007). Spearman's rank correlation coefficients range from 1 (identical assemblages) to -1 (different assemblages; Kwak and Peterson 2007).

Results and Discussion

Mattawoman Creek, Piscataway Creek, and Northeast River were classified as tidal-fresh (Table 3-2). Gunpowder River, Middle River, Bush River, and Nanjemoy Creek were considered oligohaline (Table 3-2). Broad Creek, Harris Creek, Middle River, Tred Avon River, and Wicomcio River were mesohaline subestuaries. Salinity data for Broad and Harris Creeks were only available for 2012, but we assumed that their classification would not be different than Tred Avon River since all three sets of summarized salinities in 2012 were similar (Table 3-2).

All rivers except Harris and Piscataway Creeks had non-zero estimates of V_{target} and $V_{threshold}$ in surface and bottom waters during 2012 (Table 3-3). Corsica River had the highest V_{target} followed by the Bush River, Middle River, and Wicomico River. These mesohaline or oligohaline systems had V_{target} values greater than 10%, whereas remaining rivers all fell below 10% (Table 3-3). When we evaluated V_{target} in bottom channel waters, Corsica River had the highest estimate, followed by Bush River, Middle River, Tred Avon River, Wicomico River, Northeast River, and Broad Creek; all other systems had V_{target} estimates below 10% (Table 3-3). Out of twelve systems, only three systems, Corsica River, Nanjemoy Creek, and Broad Creek, had non-zero estimates of $V_{threshold}$, during 2012 (Table 3-3).

Correlation analyses of 2003-2012 data suggested that the sign and significance ($P \leq 0.05$) of associations of mean surface or bottom DO with C / ha were influenced by salinity classification in a manner consistent with potential for stratification. In mesohaline subestuaries, where strongest stratification was expected, associations of surface DO with surface water temperature and bottom DO with C / ha were negative and significant ($r = -0.47$ and -0.45, respectively), while remaining comparisons (bottom temperature with bottom DO and C / ha with surface DO) were not (Table 3-4). Associations of mean bottom DO were only significant and negative in mesohaline subestuaries, indicating stratification was important for development of poor DO conditions as development increased. None of the variables were significantly correlated in oligohaline subestuaries. In tidal-fresh subestuaries, neither surface nor bottom DO was significantly correlated with temperature, but both were significantly and positively correlated with C / ha (r = 0.44 and 0.49, respectively). Sample sizes of mesohaline subestuaries (N = 48-49) were over twice as high as oligohaline or tidal-fresh subestuaries (N = 23 for both), so ability to detect significant associations in mesohaline subestuaries was greater Table 3-4).

A total of 28,864 fish representing 50 species were captured in the seine (Table 3-5). During 2012, dense submerged aquatic vegetation (SAV) prevented seining in Mattawoman and Piscataway creeks. Seining in Middle River was sporadic because of high tides that limited beach availability and dense SAV in seine sites. Nine species groups comprised 90% of the catch: white perch adults, white perch YOY, gizzard shad, Atlantic silverside, spottail shiner, bay anchovy, striped killifish, alewife, mummichog, and inland silverside. Three target species groups were within the groups comprising 90% of the catch: white perch adults and YOY, and alewife (Table 3-5). White perch (adults or YOY) was the only target species present in the species comprising 90% of the seine catch in every subestuary seined. Gunpowder River, with 31 species, ranked highest in species richness of all subestuaries seined during 2012 and Bush River ranked second with 28 species (Table 3-5).

Bottom trawling was conducted in all system during 2012 and a total of 63,747 fish and 48 fish species were captured (Table 3-6). Four species groups consistently comprised 90% of the total catch for the trawl: bay anchovy, white perch (adults and YOY), spot, and spottail shiner. Atlantic croaker was among the top species in Nanjemoy Creek. Broad and Harris Creeks were the only systems where white perch were not present in the species comprising of 90% of trawl catch. Piscataway Creek had the highest number of species in trawl samples, 23; Bush and Gunpowder rivers' species richness were also high, 22 and 21 species (respectively; Table 3-6).

Regression analyses indicated that bottom trawl P_i of juvenile white perch in tidal-fresh subestuaries was not linearly related to C / ha ($r^2 = 0.11$, $P = 0.09$, $N = 26$; Figure 3-2). A linear decline of P_i may be a poor choice of a model for describing a decline of tidal-fresh subestuary bottom channel habitat use by juvenile white perch. The plot of juvenile white perch P_i against C / ha indicated that once the threshold (C / ha = 0.83) had been breached, annual variation in P_i increased substantially. All 12 estimates of juvenile white perch P_i at development levels less than the threshold were clustered between 0.91 and 1.00. Beyond the threshold, the range expanded to 0.30 – 1.00; 6 of 14 estimates were between 0.91 and 1.0 (Figure 3-2).

The relationship of C / ha and P_i in bottom trawls was significant for adult white perch ($r^2 = 0.55$, $P < 0.0001$, $N = 26$; Figure 3-2). The equation describing the relationship of C / ha and P_i for adult white perch was

$$P_i = (-0.47 \bullet C / ha) + 1.20;$$

where P_i = the proportion of trawl samples with adult white perch. Standard errors of the slope and intercept were 0.08 and 0.09, respectively. Residuals of this regression plotted against C / ha (Figure 3-2c) suggest that points at lower and higher development (C / ha ≈ 0.45 and 1.40, respectively) were well described by the regression, but the points surrounding the threshold (C / ha = 0.83) were mostly clustered above zero. This suggests that stressors in tidal-fresh subestuaries affect adult white perch P_i in the region of the threshold in a "boom or bust" fashion.

We continued to evaluate long-term changes in habitat quality in Mattawoman Creek. Median DO in Mattawoman Creek bottom channel habitat has declined since 1989; however, it has never fallen below the target DO of 5.0 mg/L (Figure 3-3). Non-zero estimates of V_{target} for bottom channel habitat first appeared in 1997 and alternated with V_{target}, equaling zero through 2005 (Figure 3-4). Non-zero V_{target} was estimated every year after 2005. Non-zero $V_{threshold}$ was estimated during 1997, 2003, 2006, and 2008-2010 (Figure 3-4).

Over the last twenty-five years, Mattwoman has experienced increased development (Figure 3-5). When we regressed C / ha against year during 1989 to 2000 and 2001 to 2010, the slope was significantly higher during 2001-2010 (1989-2000 slope = 0.0185, SE = 0.0006 and 2001-2010 slope = 0.0232 and SE = 0.0011; Table 3-7); development accelerated after 2000. Housing density increased from 0.60 C / ha in 1999 to 0.67 C / ha in 2000. This sudden increase in housing density corresponded with a sudden increase in SAV coverage after 1999 that was complete by 2002 (from 50-100 ha of SAV to 250-350 ha; Figure 3-6). A survey of DO within a large bed at Sweden Point Marina during 2011 indicated that stressful conditions were more likely in Mattawoman Creeek's SAV bed than in bottom channel waters (Uphoff et al. 2012). High growth of SAV in Mattawoman Creek appeared to represent a manifestation of DO stress from development unique to tidal-fresh subestuaries. Uphoff et al. (2012) established a development threshold of 0.83 C / ha, beyond which changes in habitat are associated with declining fisheries resources. However, if this increase in SAV is an indication of an ecosystem change associated with development, that threshold may be lower for summer habitat in fresh-tidal subestuaries.

A marked change in relative abundance of all species of fish occurred once development accelerated after 2000. Geometric mean abundance fell precipitously within two years to a level well below the minimum observed during 1989-1999 (7.1 fish per trawl in 2002 versus the 1989-1999 minimum of 29.3 fish per trawl; Figure 3-7). Only one year (2011) during 2009-2012 had a geometric mean within the range typically observed during 1989-1999; the remaining years were well below the 1989-1999 range (Figure 3-7). Rapid changes in total abundance began when C / ha was approximately 0.70 C / ha. Wide 95% CI's during 1989-2000 may have reflected monthly sampling.

Comparisons pre- and post-disturbance community order based on samples from the 3.1 m headrope trawl (Figures 3-8 and 3-9) were weakly dependent and not statistically significant (Spearman's rank correlation = 0.47; P = 0.08). Results were similar with comparisons of pre-disturbance 3.1 m headrope trawl samples with the post-disturbance 4.9 m headrope trawl samples (Spearman's rank correlation = 0.36, P = 0.19). We also compared community order of 3.1 m headrope and 4.9 m headrope trawl samples during the post-disturbance period and found a strong and significant dependence between these variables (Spearman's rank correlation = 0.80; P = 0.0003), indicating that the community structure was similar between gears.

Even though community composition changes were not statistically significant, several changes of the fish community may be ecologically important. First, total abundance of all fish in bottom channel habitat in the post-disturbance period was much lower. Three pelagic plankton feeders (bay anchovy, blueback herring and gizzard shad) have drastically declined. White perch, spottail shiner, bluegill, and pumpkinseed, species that have remained or climbed in rank, feed on a variety of food items not associated with pelagic habitat: snails, benthic crustaceans, insects, worms, zooplankton, fish eggs, and small fish (www.fishbase.org). These fish species shifts suggest a major trophic shift potentially related to predominance of SAV.

Decreased fish abundance, loss of pelagic species, decline in species richness, increased representation of spottail shiner and sunfish corresponded with increase in SAV coverage as development has proceeded in Mattawoman Creek's watershed (Uphoff et al. 2010; 2011; 2012). Bottom dissolved oxygen has declined, though it is rarely below the 5.0 mg/L criteria with declining chlorophyll a and increasing water clarity (Uphoff et al. 2010; 2011; 2012). In Mattawoman Creek, below threshold and target levels of DO are more likely in shallow habitat with SAV than bottom channel waters.

Kraus and Jones (2011) conducted a study in Gunston Cove, (Northwest of Mattawoman Creek on the Virginia shore of the Potomac River) to determine how SAV influences fish abundance and richness in shoreline habitats. They compared species composition and abundance in SAV and non-vegetated beaches using dropnet and seine sampling, respectively. They reported that bay anchovy, Atlantic menhaden, gizzard shad, alewife, blueback, American shad, golden shiner and inland silversides were only present in shoreline habitats with low or no densities of SAV. Kraus and Jones (2011) also reported high abundance of bluegill and pumpkinseed in SAV. In comparing shoreline and SAV, they found both species occupying both habitats, but abundance was orders of magnitude higher in SAV. Their study results are consistent with increased sunfish presence we have observed in our trawl samples. We were unable to examine changes in shoreline occurrence of these species in Mattawoman Creek because thick SAV precludes seining. Gear specifically designed for fish sampling in SAV is labor intensive and logistically unsuitable for our frequent sampling of multiple subestuaries.

When we evaluated Mattawoman's habitat in the context of Chesapeake Bay Program's habitat goals, Mattawoman superficially resembles a restored system with reduced nutrient loads, i.e., increased clarity, reduced chlorophyll a, and increased SAV. Together, these factors are expected to increase habitat for fish (Chesapeake Bay Program 2013). However, Chanat et al, (2102) reported that nutrient and sediment loads were nearly twice those of the Choptank River, an agriculturally dominated watershed twice the size of Mattawoman Creek. Boyton et al (2012) modeled of nutrient inputs and outputs in Mattawoman Creek and have found that nutrients were not exported out of the subestuary. This result suggested that wetlands, emergent vegetation, and SAV in Mattawoman Creek were efficiently metabolizing and sequestering nutrients. Unfortunately, the response of the fish community may not be wholly positive. Mattawoman Creek's estuary provides a reservoir-like freshwater fishery for largemouth bass, sunfish, crappie, and catfish; the introduced northern snakehead and blue catfish have risen in popularity with anglers (MDDNR 2012). However, its tidal fish community diversity and function as an anadromous fish spawning area and nursery both have declined (MDDNR 2012). These observations in Mattawoman Creek may represent the script for how tidal-fresh systems respond to watershed development. This response may hinder the goal of the Chesapeake Bay Program to improve management and recovery of Atlantic menhaden, and alosines (pelagic filter feeders) by removing urbanized subestuaries from available habitiat. Freshwater habitats may have high value to contingents of white perch and trophic and habitat changes could diminish benefit of behavioral flexibility that may represent an adaptation for persistence (Kerr and Secor 2012)

Table 3-1. Percent impervious cover (IS), structures per hectare (C / ha), total non-water hectares, and area of tidal water for the watersheds sampled in 2012. Impervious surface was estimated from C / ha using a nonlinear power function developed in Uphoff et al. (2012). Water hectares are for the subestuary and do not include fluvial streams.

Area	Watershed	IS	C / ha	Total Hectares	Water Hectares
Mid-Bay	Broad Creek	3.7	0.18	4,730	3,148
Mid-Bay	Corsica River	4.5	0.244	9,677	537
Mid-Bay	Harris Creek	6.0	0.387	3696	2,919
Mid-Bay	Middle River	23.4	3.32	2,753	982
Mid-Bay	Tred Avon River	9.1	0.747	9,563	2,429
Potomac	Mattawoman Creek	10.3	0.898	24,441	729
Potomac	Nanjemoy Creek	2.4	0.092	18,893	1,131
Potomac	Piscataway Creek	13.9	1.449	17,642	361
Potomac	Wicomico River	5.6	0.34	58,389	4,012
Upper-Bay	Bush River	14.0	1.471	44,167	2,962
Upper-Bay	Gunpowder River	8.7	0.69	113,760	4,108
Upper-Bay	Northeast River	6.7	0.459	16,342	1,579

Table 3-2. Mean, minimum, and maximum bottom salinity (‰) for 2012, and 2003-2012 summer sampling and salinity classifications based on 2003-2012 mean bottom salinity. Broad and Harris Creeks were not sampled before 2012. *NA* = not applicable.

Sampling Location	Mean 2012	Mean 2003-2012	Minimum 2012	Minimum 2003-2012	Maximum 2012	Maximum 2003-2012	Class 2003-2012
Broad Creek	12.37	*NA*	11.25	*NA*	13.44	*NA*	Mesohaline
Bush River	1.56	1.06	0.49	0.10	4.60	4.60	Oligohaline
Corsica River	9.56	7.78	8.10	0.30	10.70	11.96	Mesohaline
Gunpowder River	3.16	1.98	0.67	0.13	5.11	5.11	Oligohaline
Harris Creek	12.53	*NA*	11.34	*NA*	13.97	*NA*	Mesohaline
Mattawoman Creek	0.39	0.37	0.19	0.00	0.57	1.70	Tidal-Fresh
Middle River	5.43	3.94	3.41	0.50	6.89	6.89	Oligohaline
Nanjemoy Creek	4.80	4.07	2.04	0.31	8.60	9.16	Oligohaline
Northeast River	0.12	0.23	0.10	0.06	0.20	3.39	Tidal-Fresh
Piscataway Creek	0.17	0.16	0.16	0.10	0.17	0.21	Tidal-Fresh
Tred Avon River	11.53	10.57	9.90	6.32	12.51	14.17	Mesohaline
Wicomico River	11.73	9.27	10.30	0.00	13.64	14.50	Mesohaline

Table 3-3. Percentages of all DO measurements and bottom DO measurements that did not meet target (≤ 5.0 mg / L) and threshold (≤ 3.0 mg / L) conditions during July-September, 2012, for each subestuary. C / ha = structures per hectare.

Subestuary	Salinity class	C / ha	All depths DO % ≤ 5.0 mg / L	Bottom DO % ≤ 5.0 mg / L	Bottom DO % ≤ 3.0 mg / L
Broad Creek	Mesohaline	0.293	6.0	12.5	4.2
Corsica River	Mesohaline	0.244	69.5	80.0	60.0
Harris Creek	Mesohaline	0.387	0.0	0.0	0.0
Middle River	Mesohaline	3.32	13.0	41.7	0.0
Tred Avon River	Mesohaline	0.747	9.6	29.2	0.0
Wicomico River	Mesohaline	0.211	12.2	26.7	0.0
Gunpowder River	Oligohaline	0.723	1.7	6.7	0.0
Nanjemoy Creek	Oligohaline	0.092	5.0	10.0	5.0
Bush River	Tidal-fresh	1.471	27.3	75.0	0.0
Mattawoman Creek	Tidal-fresh	0.898	4.3	4.8	0.0
Northeast River	Tidal-fresh	0.459	7.3	20.8	0.0
Piscataway Creek	Tidal-fresh	1.449	0.0	0.0	0.0

Table 3-4. Correlations of mean annual surface and bottom DO with matching water temperatures at depth (surface and bottom) or watershed development (C / ha = structures per hectare), by salinity class.

DO Depth	Statistics	Temperature Depth	C / ha
		Mesohaline	
Surface	r	-0.47	-0.13
	P	0.001	0.386
	N	49	48
Bottom	r	0.15	-0.45
	P	0.289	0.001
	N	49	48
		Oligohaline	
Surface	r	-0.20	0.32
	P	0.354	0.134
	N	23	23
Bottom	r	-0.17	-0.12
	P	0.449	0.575
	N	23	23
		Tidal-fresh	
Surface	r	0.08	0.44
	P	0.709	0.037
	N	23	23
Bottom	r	-0.10	0.49
	P	0.654	0.018
	N	23	23

Table 3-5. Seine catch summary, 2012. Fish per seine is the arithmetic mean of all fish species. C / ha = structures per hectare.

Subestuary	Stations Sampled	N	Species	Species comprising 90% of catch	C / ha	Total catch	Fish per seine
Broad Creek	3	18	22	Atlantic silveside	0.293	1879	104.4
				White perch			
				Striped killifish			
				Mummichog			
				Atlantic menhaden			
Bush River	4	20	28	Gizzard shad	1.471	3748	187.4
				Alewife			
				White perch			
				Spottail shiner			
				YOY White perch			
				Pumpkinseed			
Corsica River	3	18	21	White perch	0.244	1482	82.3
				Spottail shiner			
				Bay anchovy			
				Atlantic silverside			
				Mummichog			
				Atlantic menhaden			
				Inland silverside			
				Striped killifish			
Gunpowder River	4	24	31	Gizzard shad	0.723	3599	150
				YOY White perch			
				White perch			
				Spottail shiner			
				Atlantic menhanden			
				Bay anchovy			
				Pumpkinseed			
Harris Creek	3	18	24	Atlantic silverside	0.387	2514	139.7
				Striped killifish			
				Bay anchovy			
				White perch			
				Mummichog			
Middle River	3	18	25	YOY White perch	3.32	1577	87.6
				White perch			
				Pumpkinseed			
				Spottail shiner			
				Banded killifish			
				Gizzard shad			
				Bay anchovy			

				Inland silverside			
Nanjemoy Creek	4	28	24	YOY White perch	0.092	2146	76.6
				White perch			
				Inland silverside			
				Spottail shiner			
				Atlantic silverside			
				Atlantic croaker			
				Spot			
				Bay anchovy			
				Mummichog			
North East River	4	24	20	Gizzard shad	0.459	5574	232.3
				YOY White perch			
				White perch			
Piscataway Creek	1	1	11	Bluegill	1.449	53	53
				YOY White perch			
				Tesselated darter			
				Pumpkinseed			
				Banded killifish			
				Gizzard shad			
				Spottail shiner			
				Black crappie			
Tred Avon River	4	24	26	White perch	0.747	3723	155.1
				Atlantic silverside			
				Mummichog			
				Striped killifish			
				Atlantic menhaden			
Wicomico River	4	24	20	Atlantic silverside	0.211	2569	107
				White perch			
				Bay anchovy			
				Gizzard shad			

Table 3-6. Trawl catch summary, 2012. Fish per trawl is the arithmetic mean of all fish species. C / ha = structures per hectare.

Subestuary	Stations Sampled	Number of Samples	Species	Species Comprising 90% of Catch	C / ha	Total Catch	Fish per Trawl
Broad Creek	4	24	18	Bay anchovy	0.293	7543	314.3
Bush River	3	12	22	White perch	1.471	4933	411.1
				YOY White perch			
				Gizzard shad			
				Brown bullhead			
Corsica River	4	24	14	White perch	0.244	6551	273
				Bay anchovy			
Gunpowder River	4	24	21	YOY White perch	0.723	5805	241.9
				White perch			
				Bay anchovy			
				Spot			
Harris Creek	4	24	18	Bay anchovy	0.387	3840	160
				Green goby			
				Spot			
				Hogchoker			
Mattawoman Creek	4	24	17	YOY White perch	0.898	4454	185.6
				Spottail shiner			
				Bay anchovy			
				White perch			
				Bluegill			
Middle River	4	24	17	White perch	3.32	4108	171.2
				Bay anchovy			
				YOY White perch			
				Pumpkinseed			
Nanjemoy Creek	4	28	16	YOY White perch	0.092	8161	291.5
				Bay anchovy			
				White perch			
				Atlantic croaker			
North East River	4	24	17	YOY White perch	0.459	7566	315.3
				White perch			
				Brown bullhead			
Piscataway Creek	3	18	23	YOY White perch	1.449	3000	166.7

				Bay anchovy Spottail shiner Tessalated darter			
Tred Avon River	4	24	18	Pumpkinseed Bay anchovy White perch Hogchoker Spot	0.747	4067	169.5
Wicomico River	4	24	16	Bay anchovy White perch Spot	0.211	3719	155

Table 3.7. Regression statistics for years against structures per hectare (C / ha) in Mattawoman Creek's watershed during 1989-1999 and 2000-2010.

Pre disturbance (1989-1999)

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0375734	0.0375734	893.16541	2.57E-10
Residual	9	0.0003786	4.207E-05		
Total	10	0.037952			

r2 = 0.99

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-36.330522	1.2331153	-29.462387	2.918E-10	-39.120022	-33.541021	-39.120022	-33.541021
Year since 1989	0.0184818	0.0006184	29.885873	2.57E-10	0.0170828	0.0198807	0.0170828	0.0198807

Post disturbance (2000-2010)

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0590113	0.0590113	421.47283	7.209E-09
Residual	9	0.0012601	0.00014		
Total	10	0.0602714			

r2 = 0.98

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-45.639839	2.2620459	-20.176354	8.403E-09	-50.756943	-40.522736	-50.756943	-40.522736
Years since 2000	0.0231617	0.0011282	20.529804	7.209E-09	0.0206096	0.0257139	0.0206096	0.0257139

Figure 3-1. Tributaries sampled by seining and trawling during summer, 2012. Watershed area has been indicated by grey shading. Watershed of Tred Avon was not delineated.

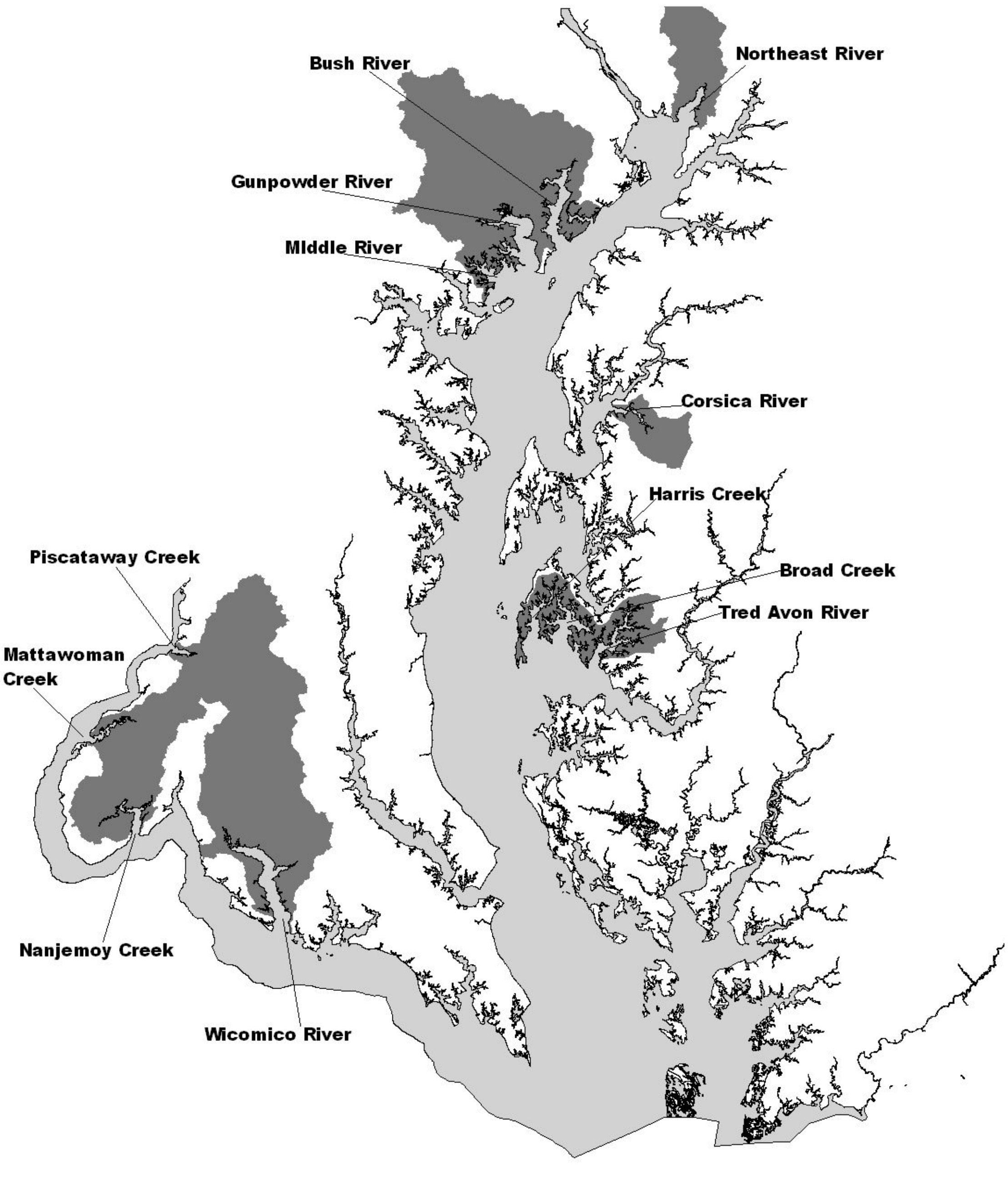


Figure 3-2. Bivariate plots of development (C/ha) and proportions of trawl samples taken in tidal fresh subestuaries with White perch juveniles (A) and adults (B). Absence of line for adults indicates linear regression was not significant at P ≤ 0.05. Predicted line for adults indicates a significant regression. Residuals of the linear regression with C/ha for White perch adults © plotted against C/ha.

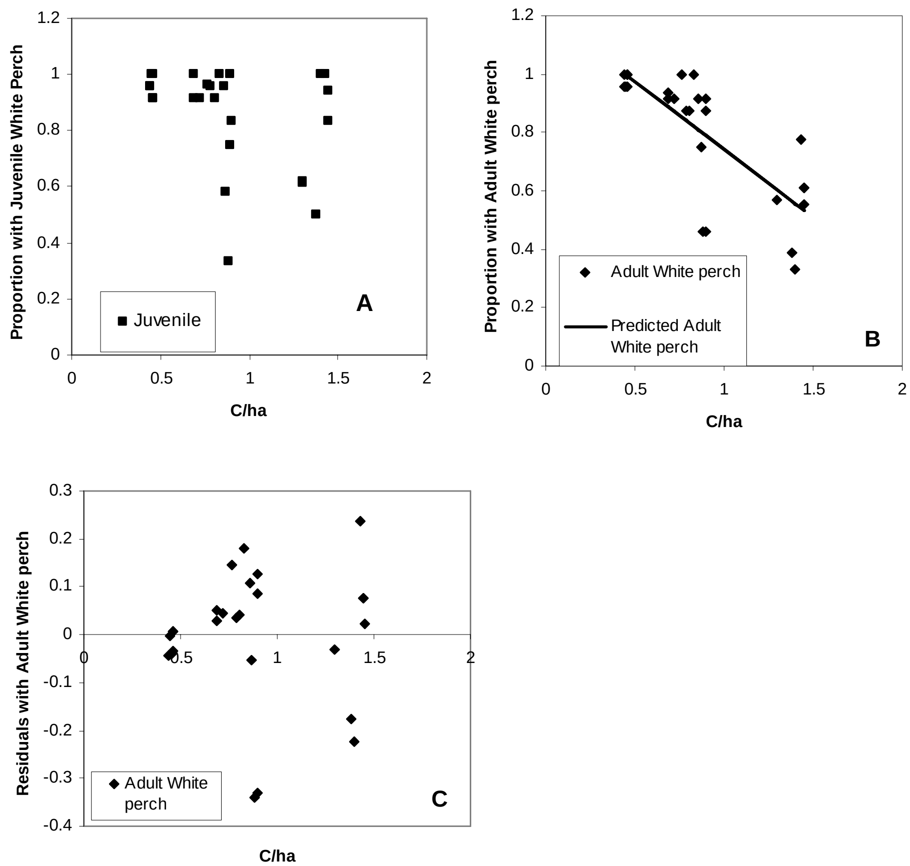


Figure 3-3. Box plot of bottom DO in Mattawoman Creek during 1989-2012. In this plot, the mean DO for each year is represented by cross marker and located on the secondary y-axis. The DO range, median, and quartiles for each year are located on the primary y-axis. The mean DO is added to indicate the difference between median and mean, both the mean and median DO never fall below the threshold DO (<3 mg/L).

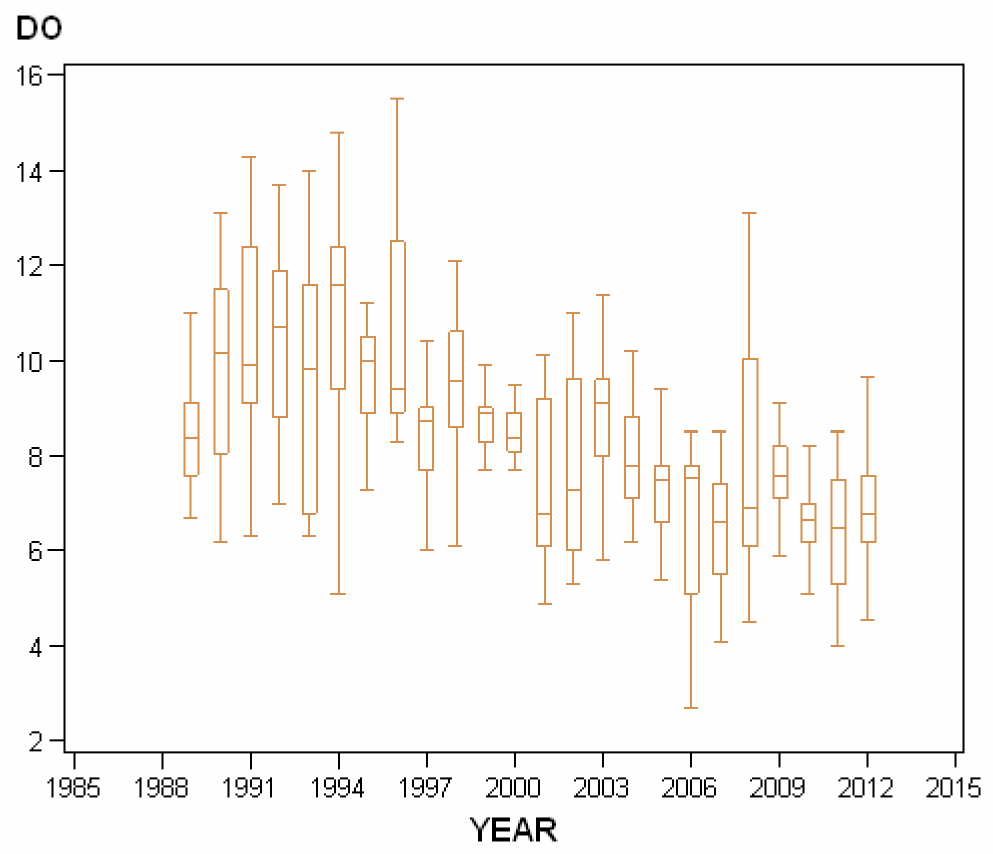


Figure 3-4. Proportion of measurements below target and limit DO in Mattawoman Creek bottom channel habitat during summer, 1989-2012.

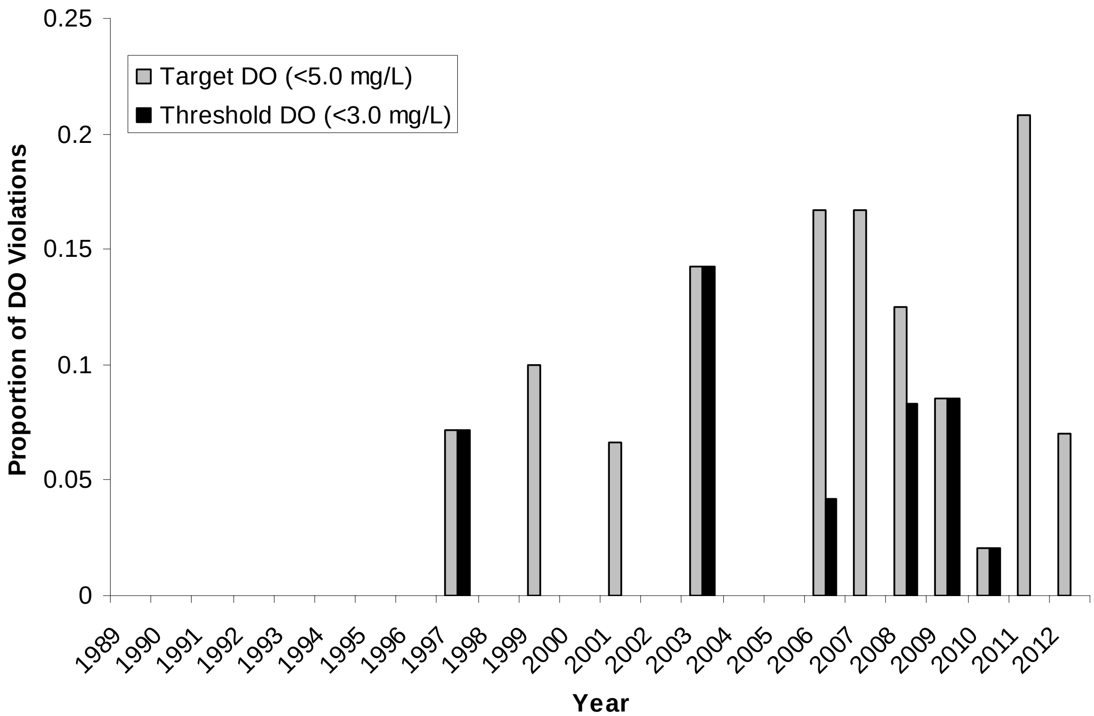


Figure 3-5. Housing density change by year in the Mattawoman watershed.

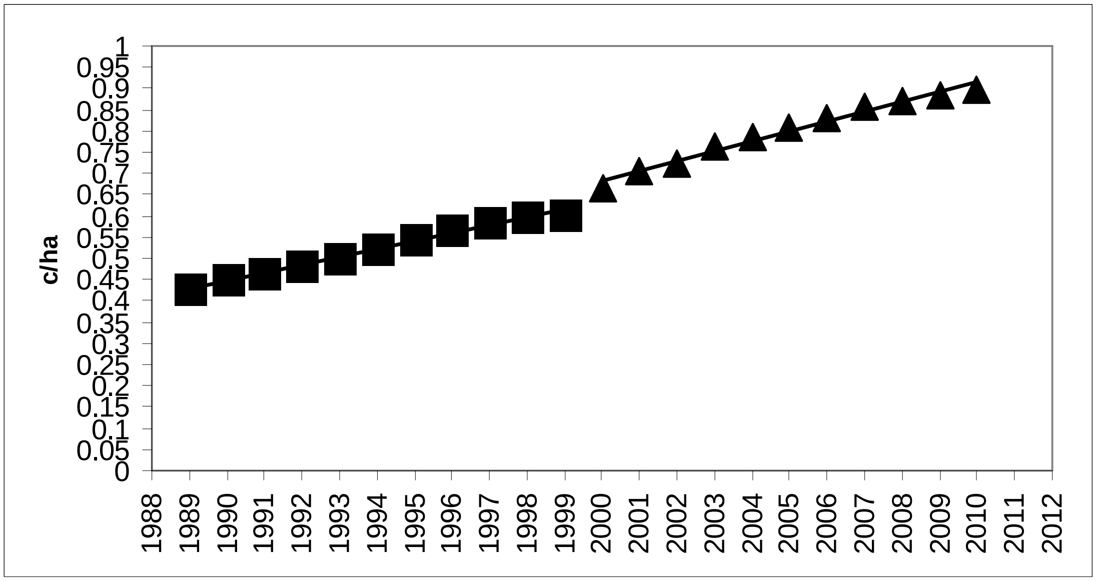


Figure 3-6. SAV coverage from 1989-2010 in Mattawoman Creek.

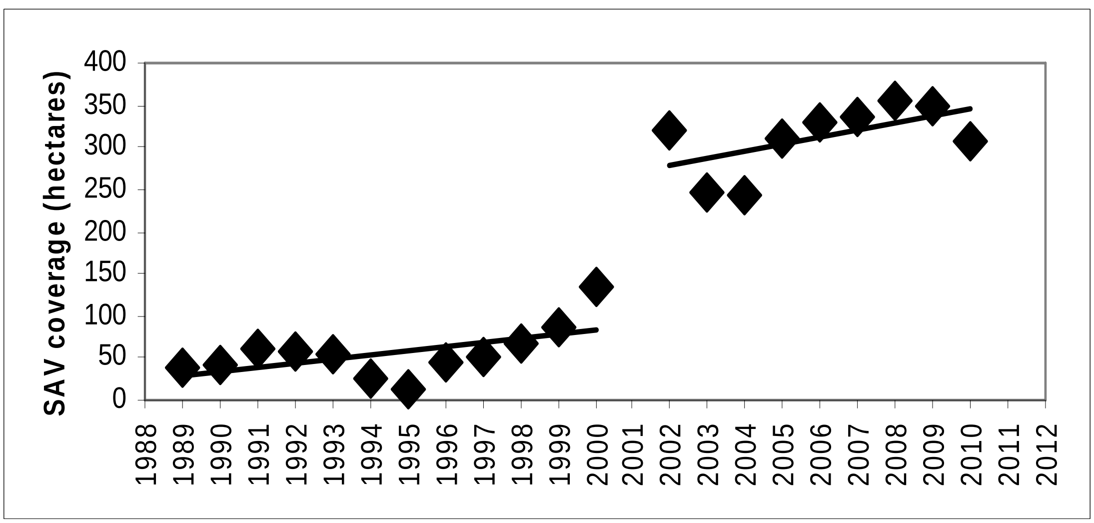


Figure 3-7. Geometric mean catch per trawl in Mattawoman Creek and it's 95% confidence interval for 3.1 m headrope trawl plotted against structures per hectare (C / ha). Time series begins in 1989 and time-series gap begins in 2002. Sampling with this trawl began again in 2009; 2012 is the last year of the time-series. Estimates of C / ha end in 2010 and estimates for 2011-2012 use the 2009-2010 C / ha increment to advance the time-series to prevent 2011 and 2012 points from laying over top of 2010 .

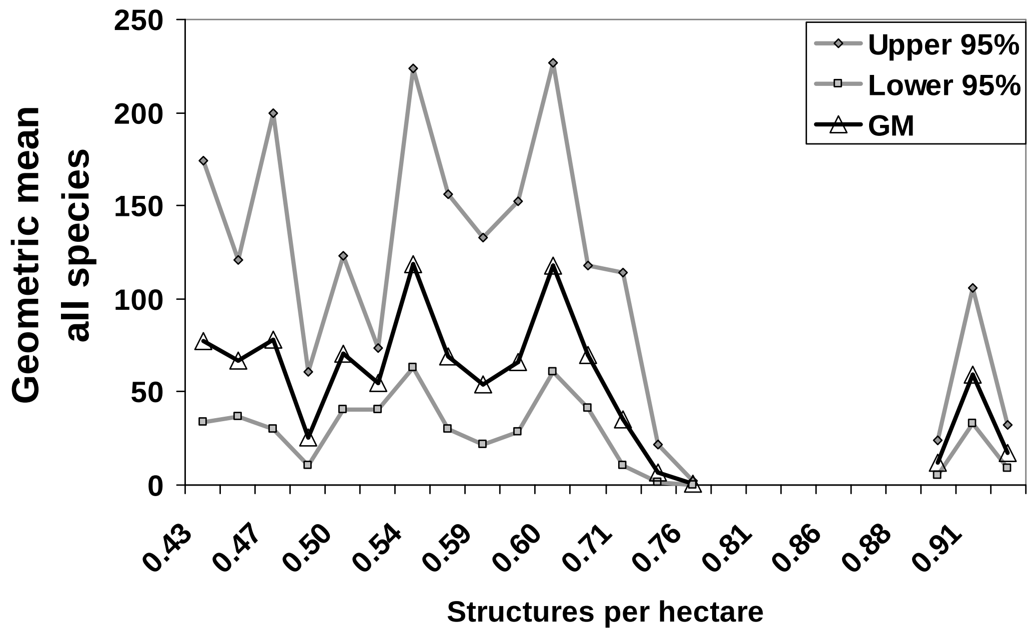


Figure 3-8 Proportion of positive catch of most common species in Mattawoman Creek (a)10’ trawl and (b) 10’ trawl, 2001-2002, 2009-2012. Species: 1, white perch juvenile, 2 white perch adult, 3 bay anchovy, 4 blueback herring, 5 spottail shiner, 6 gizzard shad, 7 American eel, 8 tesselated darter, 9 striped bass juvenile, 10 alewife, 11 brown bullhead, 12 bluegill, 13 pumpkinseed, 14 bluegill,15 silvery minnow.

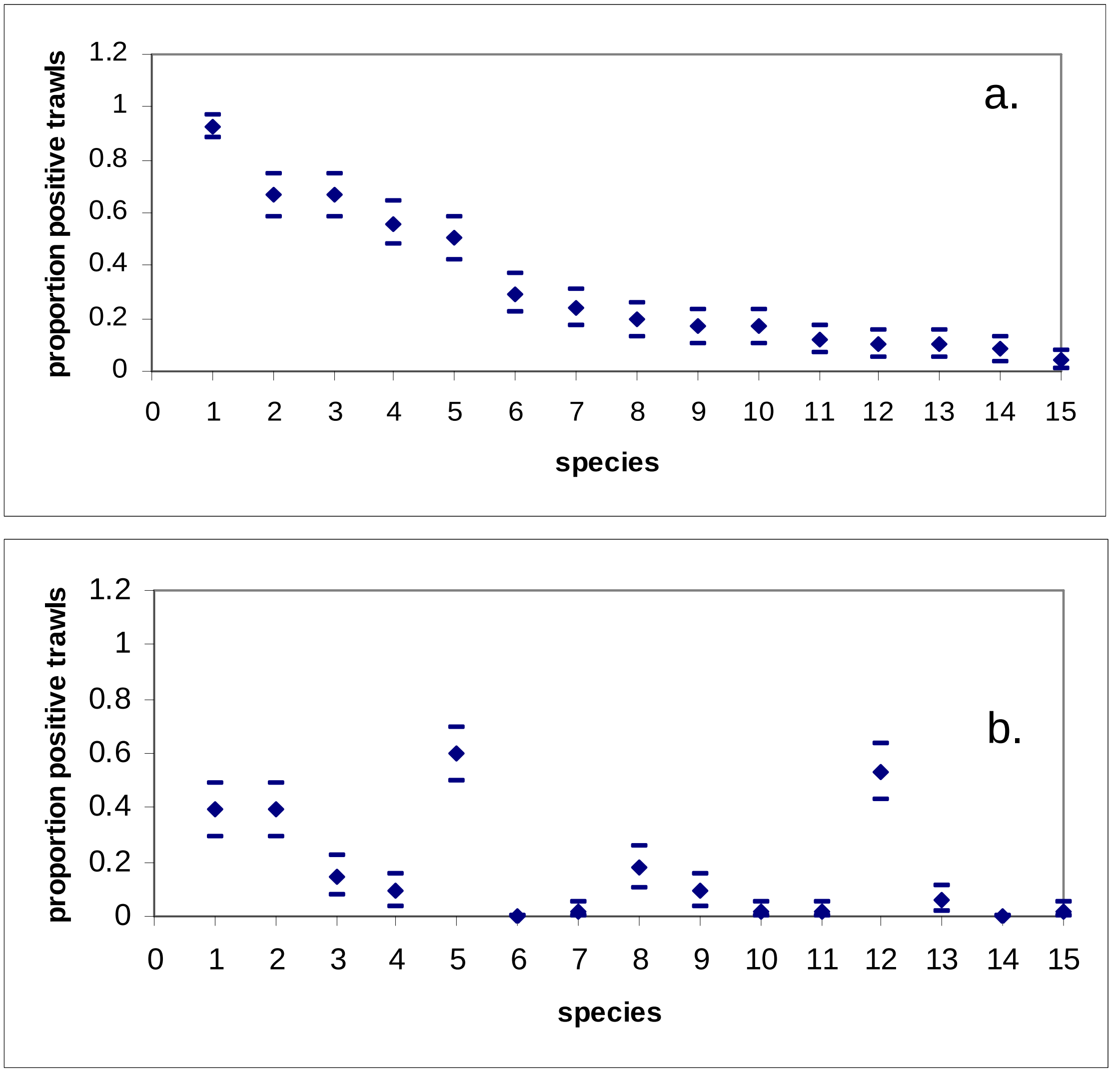
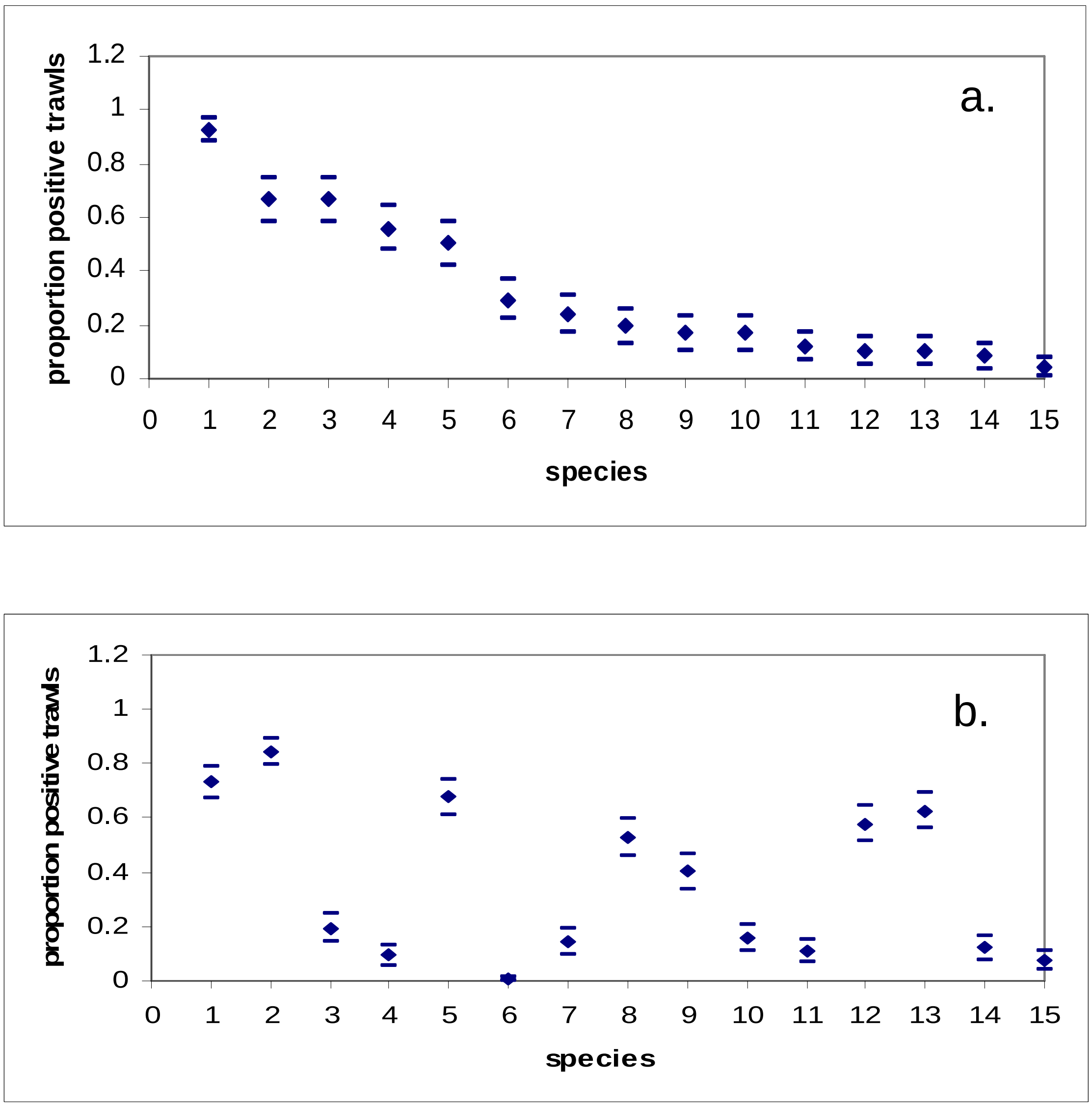


Figure 3-9 Proportion of positive catch of most common species in Mattawoman Creek 10' trawl, (a) 1989-2000 and (b) 16' trawl 2003-2012. Species: 1, white perch juvenile, 2 white perch adult, 3 bay anchovy, 4 blueback herring, 5 spottail shiner, 6 gizzard shad, 7 American eel, 8 tesselated darter, 9 striped bass juvenile, 10 alewife, 11 brown bullhead, 12 bluegill, 13 pumpkinseed, 14 bluegill, 15 silvery minnow.



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Job 2: Environmental Review Support for Estuarine and Marine Habitat

Bob Sadzinski

Introduction

Environmental review and planning represents the "frontline" of habitat management. The direct link between land-use, ecological condition of downstream receiving water and environmental review provides the opportunity to mitigate the impacts of land-based projects on aquatic resources through the permitting process.

The Task Force for Fishery Management recognized that Maryland Department of Natural Resources' (DNR's) Environmental Review (ER) Program was critically understaffed (Task Force on Fisheries Management 2008). An Integrated ER Team was created by assigning personnel from various units throughout DNR to address this critical staffing shortfall. Fisheries Service has provided one reviewer and an advisor who provides additional expertise to project review topics as well as guidance in setting environmental review policy for the Department. The activities of these positions are funded through this federal aid grant: ER activities were entirely funded under Job 2, while advisory and support activities were also covered under Jobs 1 and 3.

The Environmental Review unit has been charged by the Secretary of Natural Resources with both conducting routine reviews and taking a lead role in proactively using habitat criteria in project review activities. Routine reviews may be streamlined by developing habitat criteria for triage, such as impervious surface reference points and greater application of GIS technology.

The purpose of environmental review is to work proactively with partners (other DNR agencies, Maryland's Department of Environment and Department of Planning, local governments, and federal agencies) to protect key habitats and ecosystem functions and limit environmental impacts while making better natural resource data available to agencies at the state, county and local levels. Environmental review must identify the natural resources potentially impacted, assess the extent of the impacts on resources, review for regulatory requirements, and as applicable, identify and attempt conflict resolutions. The review agency is responsible for providing comments based on potential impacts of the project on the resources of concern to that agency and recommends avoiding, minimizing or mitigating project impacts as appropriate.

Major Activities in 2012

In 2010, DNR had assigned two staff members as the primary environmental reviewer and planner (Bob Sadzinski) and the other as the liaison for the Fisheries Service (Jim Uphoff), but in 2011, Bob Sadzinski became both the reviewer and the Fisheries Service liaison.

For the environmental reviewer and planner, duties included estuarine and marine environmental reviews for Charles, St. Mary's and Calvert counties for and all statewide landfill, reef and aquaculture applications. Table 1 presents an overview of the number of projects by permit type. In summary, 194 applications were reviewed, many of which required significant DNR coordination. The Fisheries Service liaison served as the "clearinghouse" for environmental review applications that require input from Fisheries Service programs.

In addition, the environmental reviewer/planner served as an advisor for programs including Smart Growth, Green Infrastructure, Blue Infrastructure, BayStat/StateStat, and Plan Maryland. We cooperated and coordinated the various landscape-based DNR habitat initiatives and utilized information developed by these programs. These programs were responsible for providing multi-disciplinary information to key partners;

- Codifying regulatory standards for water quality, especially for the key quantitative parameters that define limits of acceptable habitat quality for important species
- Identifying and prioritizing high quality aquatic habitats for protection, and
- Developing key stream management strategies and comprehensible living shorelines, climate change and comprehensive plan policies.

One of the most significant project developments was the streamlining of the oyster aquaculture review. This process enables the applicant to work cooperatively with DNR oyster personnel prior to the application submittal process to select potential oyster aquaculture sites that meet criteria including absence of submerged aquatic vegetation and minimum boating and recreational fishing activities and has resulted in decreased applicant waiting period and improved public relations. In addition, several of the applications were in important recreational fishing areas and we strongly supported maintaining or improving fishing access through minimizing sedimentation and surface runoff from these sites.

Potential future projects include developing a framework to enhance sound coastal and marine resource conservation, management and restoration by:

- Completing detailed spatial assessments of coastal habitat, critical natural resources, and associated human uses
- Identification and prioritization of areas containing concentrations of sensitive aquatic habitats and resources and
- Continue to restructure the current GIS system to include additional pertinent data layers including aquatic bottom types and navigational channels.

Table 1. Overview of the projects by application type and year.

Application Type	Number of Projects Reviewed		
	2010	**2011[1]**	**2012**
Aquaculture	24	14[2]	7
Reef	1	4	2
Living Shoreline	NA	64	36
County - Specific	141	250	296
Surface Mine	10	16	4
Landfill	18	14	6
Total	**194**	**362**	**351**

[1] Two additional counties were assigned to the reviewer in 2011.
[2] The environmental review unit ceased reviewing aquaculture permits in April 2011 because of the streamlined process with MDE, request occasionally come from the Corp of Engineers.

Job 3: Support multi-agency efforts to assess and delineate interjurisdictional finfish habitat and ecosystems

Jim Uphoff, Margaret McGinty, Alexis Maple, Carrie Hoover, Bruce Pyle, Jim Mowrer, Paul Parzynski

Introduction

The objective of Job 3 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. 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.

Maryland Fisheries Service – Fisheries Habitat and Ecosystem Progarm Website

We continued to populate the website with new reports and information to keep it up to date with project developments.

Environmental Review Unit Bibliography Database

We continued to compile a database, adding recent literature and additional topics including effectiveness of Best Management Practices. We also purchased Endnote Software to house the Bibliography and are in the process of entering the bibliography into Endnote.

DNR Interagency Effort on Mattawoman Creek

FHEP continued to support efforts to promote conservation of Mattawoman Creek. Staff attended meetings of citizen groups and local government officials to communicate the ecological value of Mattawoman Creek and recommend planning strategies conducive to conservation.

Spatial Planning

Applying Impervious Thresholds in a Fisheries Management Context

Fisheries service has developed and applied impervious surface targets and limits to assist fisheries managers in understanding the impacts of watershed development on fisheries habitat (Uphoff et al. 2009). These targets and limits are meant to alert managers to the need to compensate for fisheries losses related to habitat limitation in developed areas. This information can assist in developing assessments that include a term for habitat related losses. This information can also clarify understanding of management options in various habitats. For example, in highly urbanized watersheds, where habitat is limited and reproduction is impaired, managers may decide to open a previously closed fishery to anglers, so they can enjoy a fishery while it exists. While this option is not preferred, it does allow public access to a resource in urban areas. This may become common practice in the future, as development of the Bay watershed increases.

To promote understanding of these potential fisheries impacts, we have developed priority habitat maps and applied management options to watersheds. We applied three management options, conservation, rehabilitation and reengineering to help landscape managers and planners understand needs from a fisheries point of view. Conservation is the preferred management strategy. Watersheds targeted for conservation still support adequate spawning and impervious cover is less than the target impervious cover of 5% in the watershed. Areas targeted for rehabilitation support spawning but impervious cover falls between the target (5%) and the limit of impervious cover which is 10%. We recommend managers focus rehabilitation efforts to restore natural hydrology and limit pollutant loads to downstream receiving waters, while promoting conservation of existing rural lands in the watershed. Watershed exceeding the limit of 10% impervious cover were not associated with high quality fish habitat and we recommend that land managers focus on reengineering aquatic habitat to meet social needs and potentially reduce pollutant loads to downstream habitats.

We applied these thresholds to tidal watersheds in Maryland (Uphoff et al, 2012). These maps have been integrated into a statewide Green Print tool that is being used to guide land management efforts at the state level.

These maps were used as based maps to prioritize watersheds for management focus. Many watersheds were identifies as high priority habitat areas. However, there was general consensus from local, state and regional partners, that we need to prioritize the watersheds to direct our focus on key watersheds to promote conservation.

We developed a prioritization approach using available spatial data. This approach applied, data representing other key fisheries resources, ecological value of watersheds, vulnerability to development and opportunity.

We were able to overlay a series of fisheries data layers to assess additional fisheries values. These layers represented key trout resources in the target watersheds, oyster resources, high value waters supporting freshwater resources, and access (Figure 1).

We used data from the Green Print tool to assess ecological value of landscapes and nearshore aquatic habitats. These data include metrics related to high value landscapes (occurrence of rare, threatened or endangered species, forest conservation lands) and metrics related to high value aquatic lands (streams with high biodiversity, wetlands, Submerged Aquatic Vegetation, and natural shorelines) (Figure 2).

We used the MD Department of Planning (MDP, 2011) Rural Conservation Zoning map to asses vulnerability by county. We examined zoning in areas designated for rural conservation and found, zoning for these lands differs by county ranging from 1:5 to 1:20 houses per acre. We identified counties with the lowest zoning (1:5) designation as highly vulnerable to habitat degradation. We labeled watersheds within these counties as most vulnerable to impacts of development. We also used growth projections to identify counties with the greatest growth pressure (Figure 3).

Each county in Maryland is required by law to review and or update their comprehensive growth plan every six years to assure local planning is consistent with state laws. We used the review schedule to assess opportunity to work with counties and municipalities. We chose counties that were scheduled for review after 2014, reasoning this would give us time to communicate fisheries habitat priorities, before plans were completed and approved. We reason the earlier we invest in the process, the more successful we will be in communicating and assisting counties to plan growth in a way that protects resources. (Figure 4).

We then used these layers at a regional meeting of the Chesapeake Bay Program's Sustainable Fisheries Goal Implementation Team to identify watersheds in Maryland that are important to conserving fish habitat.. The maps were useful in honing in on several areas. Mapped guidance criteria, along with expert knowledge of areas, helped us identify the Northeast River as a target watershed. The Northeast still supports quality fish habitat along with key recreational fisheries like yellow perch and largemouth bass. The comprehensive plan update and review is due in 2016, so this gives us ample time to fashion a strategy to communicate fisheries habitat concerns to county planning staff and citizens.

We also worked within DNR to incorporate our maps into a DNR tool, Green Print, which is intended to identify high quality lands and watersheds for conservation. We worked with a cross agency team to present this tool to county staff and planners, to describe the data available and communicate the value of using this tool to identify areas for conservation in local planning efforts.

In the coming year, we will incorporate additional fish habitat layers to assess key species juvenile and adult habitat preferences. We will also work in house to develop communication messages and strategies, so we can effectively educate government and public constituents on the benefits of conservation for fisheries. We will capitalize on our message "Land conservation is fish conservation", by coordinating with other resource managers to present a holistic message underscoring the value of watershed conservation for conserving key aquatic and terrestrial habitats and resources.

Figure 1. Additional fisheries resources used to examine added value for fisheries.

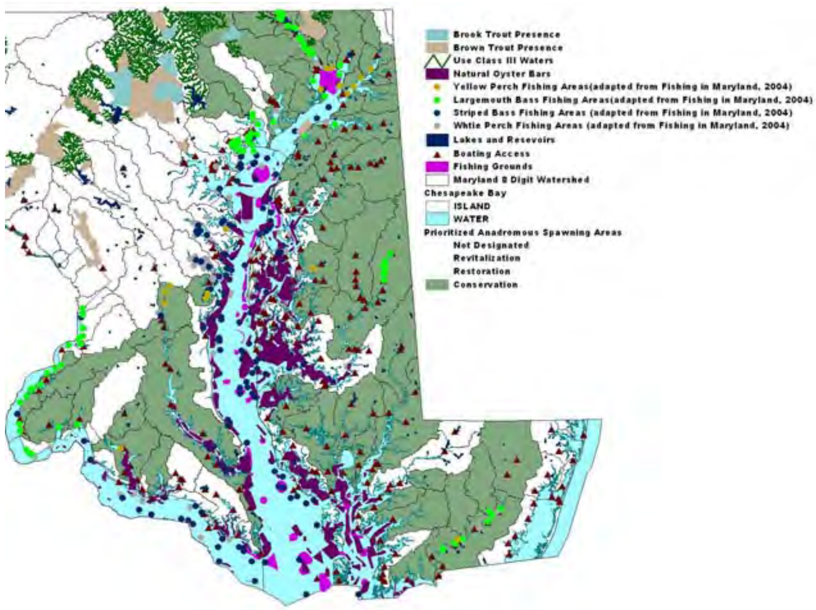


Figure 2: Ecologically important terrestrial and aquatic areas in Maryland.

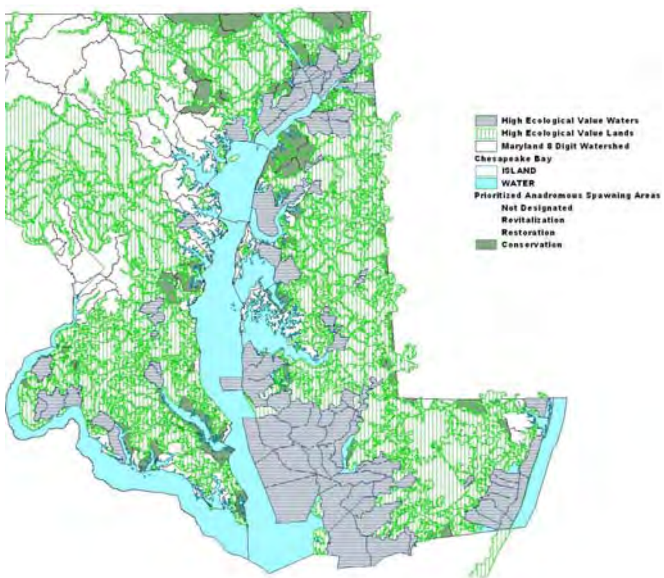


Figure 3. Maryland counties most vulnerable to growth.

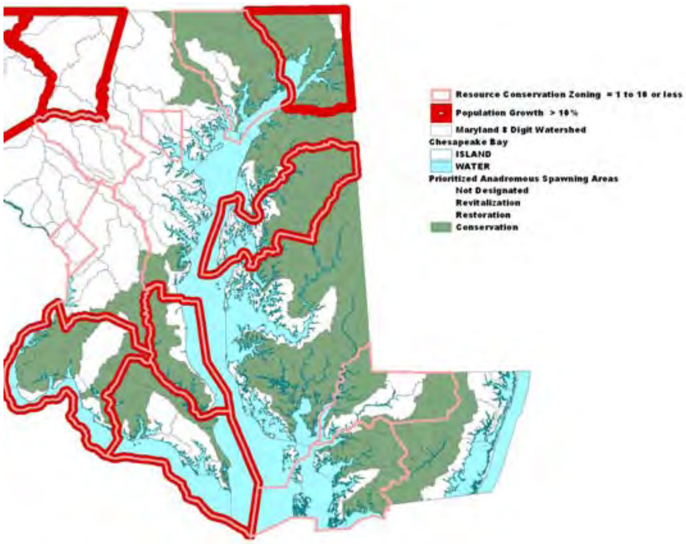
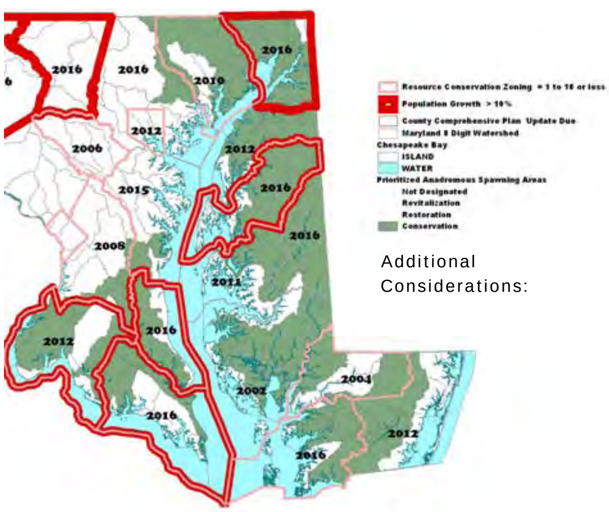


Figure 4. County Comprehensive Plan review schedule, to determine political opportunity.



Database Development

Scientific Collection Permits (SCP) are issued by the State to groups (agencies, organizations, individuals) who wish to legally try to collect finfish, shellfish, other target species, or data in the State of Maryland waters. They in return submit a report on their findings providing the location, date, species collected, number count, and gear used, or any other parameters collected.

Through the auspices of the State of Maryland, the permit coordinator authorizes the validity of the requests based on standard parameters consisting of location, time of year, gear used, type of species targeted and number collected, and use of such data. Other restrictions may be applied based on newly updated regulations.

These findings from the collectors are then scrutinized to identify which data would be appropriate to include in a database. Data from 2003 to present has been archived and the invaluable information is now being prioritized and recorded.

After ascertaining whether the data is from tidal or nontidal waters the tidal waters finfish data is being entered on an Excel worksheet. It consists of permit number, location (coordinates if available), scientific (genus/species) as well as common name of fish, number of fish, collecting agency and any pertinent comments.

The SCP data from year 2003 to present will be used in conjunction with MBSS's nontidal data for future map plotting and verifications.

Corsica River Restoration Support

We continued to support the Corsica River Restoration effort in 2012. The Corsica River watershed was selected as a targeted watershed by MDDNR in 2005 to demonstrate the effectiveness of restoration practices. Land use in the watershed is predominately agriculture and much of the restoration focus has centered on reducing nutrient loads, by applying agricultural best management practices. Extensive monitoring is being conducted to track changes in both habitat and biota. Fisheries Service has monitored the tidal fish community since 2003. Presently, there is no indication that the Corsica River is exhibiting improvements or declines in habitat quality based on water quality and fish assemblages. Habitat and fish surveys indicated that Corsica River functions as spawning, nursery, and adult habitat for important Bay species.

A conservative approach to development in the Corsica River's watershed is recommended. This watershed is currently near the target level of development recommended by Maryland's Fisheries Service for maintaining fisheries in Chesapeake Bay subestuaries.

Cooperative Research

We continued to collaborate with NOAA's Integrated Aseesment project and the NOAA funded project, 'Assessment of Stressors at the Land-Water Interface'.

We continued to collaborate with U.S. Fish and Wildlife Service and U.S. Geological Survey to support their efforts in evaluating impacts of contaminants and land use on yellow perch reproduction in Chesapeake Bay.

We supported field sampling efforts of various state and federal projects including: U.S. Fish and Wildlife Service American shad Restoration work, Maryland 's Coastal Bays Program, Maryland's Alosid Project and Maryland's Fall Oyster Survey.

Presentations and Outreach

FHEP staff organized and led sampling and fish identification training at the 15th Annual Bush River Wade in. FHEP staff also presented sampling results and led volunteer trainng for the Anita C. Leight Estuary Center staff and volunteers. The Bush

River is one of our sampling areas. This volunteer group samples the Bush River and provides data to the project.

Staff participated in various outreach events to demonstrate seining techniques and familiarize students and the public with common fish species of the Chesapeake Bay.

Staff organized and participated in a session held at the Alliance for the Chesapeake Bay's Annual Watershed Forum. The session presented the science supporting the linkages between development and fish habitat and how we are applying the science to management.

Staff Development

Staff participated in a day long training workshop sponsored by Maryland Biological Stream Survey Staff. The training focused on taxonomical identification of freshwater species. This training was helpful in preparing new staff for sampling tidal-fresh estuaries.

FHEP staff participated in a day long training introductory course in using ArcGIS. ArcGIS is increasingly becoming an invaluable tool for in both landscape and fisheries management.

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