American Shad Restoration in Three Maryland Rivers

F-57-R Segment 22 Progress Report
Reporting period January 1, 2021, to December 31, 2021

Charles P. Stence[*]
Matthew W. Baldwin
Ashlee N. Horne
William T. Stoker

Maryland Department of Natural Resources
Fishing and Boating Services
301 Marine Academy Drive
Stevensville, MD 21666

[*]*Corresponding author: chuck.stence@maryland.gov*

Reporting Timeline

This progress report will cover calendar year 2021 sub-projects one, two, three, and overall restoration progress. Elements of the project initiated in 2022 will be briefly reported. A comprehensive progress report for 2022 will be submitted in 2023.

OUTLINE

Need

American Shad *Alosa sapidissima* was once the most important commercial and recreational fish species in the Chesapeake Bay. In response to severe population declines from 1900 to the 1970s, Maryland closed its fishery in 1980. Various factors that contributed to the decline include over-fishing, stream blockages and poor water quality (Hildebrand and Schroeder 1928). Severely depressed or extirpated native adult stocks do not presently utilize most Chesapeake Bay tributaries, including the Choptank River (Klauda et al. 1991) and Patapsco River (USFWS 2013). The Choptank River historically supported commercially fished spawning stocks (Mansueti and Kolb 1953). Improvements in water quality, sustained fishing moratorium, and removal of many stream blockages has reopened potential shad spawning habitat in the Chesapeake Bay. Since shad populations indicate evidence of density dependent spawning behavior, self-sustaining shad populations are not likely to return to tributaries without hatchery stocking. Development of spawning, culture, marking, and stocking techniques could restore spawning populations of American Shad to these target tributaries.

Objective

The overall objective for this proposed scope of work is to restore self-sustaining American Shad populations to the Choptank River and Patapsco River. Prior to project inception, the depressed native stocks in the Choptank River did not exhibit any evidence of spawning activity, according to exploratory sampling efforts conducted by the Maryland Department of Natural Resources in the early 1990s. This tributary supported spawning runs and active commercial and recreational fisheries in the past (Mansueti and Kolb 1953). Sampling conducted by the United States Fish and Wildlife Service (USFWS) Maryland Fish and Wildlife Conservation Office (MDFWCO) on the Patapsco River in 2013 indicated that only a small remnant population of American Shad remained in the river (USFWS 2013).

Expected Results and Benefits

Hatchery stocked larvae and early juveniles are intended to provide adult spawners that will produce self-sustaining populations in the target tributary. These fish have tremendous value for stock assessment purposes at the larval, juvenile, and adult life stages since all stocked shad receive a unique otolith mark to identify either the larval or juvenile life stage stocking. Strip spawning culture techniques allow for the production of large numbers of larval and juvenile shad for stocking and assessment purposes.

Upper Bay and Potomac River shad populations currently support active catch and release recreational fishing. Restoring shad stocks to tributaries that historically supported runs will increase fishing opportunities for anglers. Recreational fishing that targets American Shad now occurs in the Patuxent River (previously stocked) and Choptank River (currently stocked). Angling groups have expressed interest in future angling opportunities in the Patapsco River. An indirect benefit of restoring shad populations to self-sustainable levels is the increased prey availability provided by both adult and juvenile shad for larger, more economically important recreational species such as Striped Bass *Morone saxatilis,* Bluefish *Pomatomus saltatrix,* and Weakfish *Cynoscion regalis***.**

Approach

Maryland Department of Natural Resources began a pilot project in 1993 to assess the response of American Shad adult broodstock during collection, handling, and captive holding. In 1994, experimental spawning was conducted using timed-release hormone implants. The success of these trials encouraged development of a long-term spawning, culture, stocking, and assessment program. In 1995, a non-funded, full-scale hatchery production effort was conducted with positive results. The project continued over the next three years through various short-term funding sources. In 1998, it was determined that a long-term funding source would be required, since it would take years of additional stocking and assessment to successfully support restoration. United States Fish & Wildlife Service (USFWS) Wildlife and Sport Fish Restoration (WSFR) funds are now used to conduct this long-term effort.

The project consists of three sub-projects:

1. *Produce, mark, and stock cultured American Shad in the Choptank and Patapsco rivers.*
2. *A. Assess the contribution of hatchery-produced fish to the resident/pre-migratory stock in the Choptank and Patapsco rivers.*
   B. Monitor the abundance and mortality of larval and juvenile shad using marked hatchery-produced fish.
3. *Analyze the contribution of hatchery origin American Shad to the adult spawning*

population and monitor the recovery of naturally produced stocks.

Location

Restoration efforts will occur in the Choptank River (Figure 1.1). The Choptank River watershed is rural-impacted by agricultural activities and low urban development. Choptank River efforts include the tributary Tuckahoe Creek.

Restoration efforts will also occur in the Patapsco River (Figure 1.2). The Patapsco River watershed is agriculturally impacted in headwaters and heavily urban-impacted in the lower reaches but has been the subject of numerous mitigation efforts due to its designation as a targeted watershed (e.g., sewage treatment upgrades, dam removal).

2021 Sub-Project 1

Produce, mark, and stock cultured American Shad in the Choptank and Patapsco rivers.

Objectives

The Maryland Department of Natural Resources produced, marked, and stocked American Shad larvae and early juveniles into the Choptank and Patapsco rivers (Figure 1.1; Figure 1.2). American Shad production needs were met by strip spawning broodstock from the Potomac River.

Materials and Methods

Broodstock Collection

The goal of broodstock collection is to catch ripe fish on spawning grounds and manually strip eggs and milt from American Shad. The Potomac River was chosen as the source population due to its healthy American Shad spawning population. The channel adjacent to Fort Belvoir concentrates a substantial number of American Shad and was used as the primary collection location (Figure 1.3). Broodstock collection was carried out aboard a 7.0 m flat-bottom, center console skiff equipped with an outboard motor.

Weather and temperature conditions in late March and early April greatly influence the timing of American Shad spawning on the Potomac River. It is essential to begin sampling in early April to ensure that collections occur during peak shad spawning. Sampling begins when water temperatures reach approximately 15°C. In early April, the majority of captured American Shad females are gravid, but not yet ripe for egg collection, and are identified as green. In early May, most captured females are ripe and suitable for egg collection. After a female has released her eggs, it is identified as spent. As the spawning season progresses, the composition gradually shifts from predominantly green females, to mostly spent females. Once the shift in ratio occurs, and contribution to hatchery production is low, broodstock collections cease.

Two different gill net configurations were used for brood collections. One net was a 5.5 m deep by 91.4 m long floating monofilament gill net, with 12.7 cm stretch mesh panels. The second net was the same length and depth (91.4 m by 5.5 m), but of a smaller mesh. This mesh measured 11.8 cm, typically used to target male American Shad, which tend to be slightly smaller than females.

Gill nets were set parallel to the channel edge at depths varying between 7.0 and 14.0 m. Nets were set between 1730 and 2030 hours and were checked for catch after 10-20 minutes soak time.

Catch per unit effort is used as an index of relative abundance. Gill net catch per unit effort (CPUE) is established by dividing the number of fish caught per net, by the square footage of net fished per soak time. A hand tally counter (tallycounterstore.com) is used to keep accurate count of all American Shad and bycatch caught from each net. Although trends in overall American Shad catch rates can be monitored using CPUE, the use of non-standardized gear, and a change in fishing techniques through the years makes it difficult to establish an accurate relative abundance over time.

Egg Fertilization and Culture

Egg fertilization was conducted aboard the skiff. Ripe females and males were removed from gill nets and placed into separate water-filled holding tanks on the boat. Eggs were manually stripped into clean, dry, stainless-steel bowls and milt was deposited over the eggs using the dry method (Howey 1985). River water was added to activate the sperm. The eggs and milt were mixed with a turkey feather and set to rest for ten minutes. Afterwards, the fertilized eggs were rinsed clean of any blood and ovarian tissue, and carefully poured into an egg box. The egg box is a 114 L water filled (Rubbermaid™) container, made specifically for holding shad eggs. Eggs take one hour to water-harden in the egg box. Eggs must harden before transporting to the hatchery to prevent damage during transit. Once the eggs hardened, the egg box was secured to the deck of the boat and transported to Joseph Manning Hatchery (Brandywine, Maryland) for culture (Figure 1.4). Pure oxygen was supplied to the egg box during transport.

Upon arrival to the hatchery, eggs were removed from the egg box, and placed into modified McDonald hatching jars supplied by approximately 2.0 L/min water flow. Prophylactic treatments of formalin were administered in the morning and afternoon to control fungi. Eggs were exposed to a 600:1 treatment of formalin for approximately 17 min. Eggs were volumetrically measured at the hatchery and percent fertilization was determined 24 hours post-fertilization.

American Shad eggs hatch on day six at Joseph Manning Hatchery water temperatures. To stimulate a simultaneous hatch, jars from a common culture tank were removed from the egg bank, placed outdoors in sunlight for ten minutes and stirred vigorously. The increased temperature, lower oxygen content, concentrated hormonal influence and agitation stimulates simultaneous hatching. Jars were placed around 1.5 m circular, flow-through larval culture tanks. Water was again supplied at approximately 2.0 L/min. Larvae flowed out of the hatching jar into circular culture tanks after hatch.

Food was introduced to American Shad at day three. American Shad larvae were fed live *Artemia sp*. (www.brineshrimpdirect.com) and 100 µm AP100 larval fish food (Zeigler Bros, Gardners, PA) three times daily during daylight hours.

Eggs were volumetrically measured and counted while assessing fertilization rates prior to hatching for numeration.

Marking

All fish stocked into target tributaries were given an oxytetracycline (OTC) mark through larval immersion. Oxytetracycline marking is a valuable assessment tool to determine hatchery origin, larval survival, juvenile abundance, and mortality estimates. A current veterinarian-client-patient-relationship (VCPR) exists between Joseph Manning Hatchery and Dr. Cindy P. Driscoll, (State Fish & Wildlife Veterinarian, Fish & Wildlife Health Program, Maryland Department of Natural Resources, Cooperative Oxford Lab, 904 South Morris Street, Oxford, MD 21654).

Oxytetracycline is used under a prescription to mark all larvae that leave the hatchery. Larval marks were produced by immersion in a 300-ppm buffered OTC bath for six hours. Due to its low pH, OTC must be buffered with sodium bicarbonate (NaHCO3) to bring pH near neutral ~7.0. Dissolved oxygen (DO) content was monitored and regulated by a high pressure/low volume air stone (>5.0 ppm) connected to a liquid oxygen delivery system. All water used at Joseph Manning Hatchery for OTC marking was softened before use (Culligan™ ion exchange system). Reliable marking can only take place in water with hardness below 20 mg/L and well water hardness at Joseph Manning Hatchery routinely exceeds 200 mg/L.

A three-year rotating, year-specific mark for larval stocked American Shad was implemented in 2009 (Table 1.1). This procedure will validate current shad ageing protocols for adult, hatchery-origin American Shad collected. This research protocol was recommended by the Atlantic States Marine Fisheries Commission (ASMFC) American Shad and River Herring Technical Committee. All hatchery produced American Shad designated for stocking at the larval stage were given a day three mark in 2021. Larvae designated for early juvenile stocking were given a day three, six mark.

Larval Stocking

Stocking was accomplished by placing OTC-marked larvae into boxes designed for shipping tropical fish. These containers consisted of an outer shell cardboard box, an inner insulating foam box, a black plastic trash bag to reduce stress of bright sunlight and a double thickness plastic fish shipping bag. Larval culture tanks were drawn down to crowd the fish. Larvae were scooped out of the tanks using a modified milk jug and placed into the shipping bags/boxes, which were supplemented with approximately 1.0 ppt salt to mitigate stress. Each bag was filled with pure oxygen and sealed with electrical tape. Boxes were transported to either the Choptank River at Choptank River Park in Greensboro, MD, Tuckahoe Creek at Stoney Point, or the Patapsco River at the Rt. 648 Bridge or Southwest Area Park (SWAP) boat ramp (Figure 1.5; Figure 1.6).

The bags were placed in target tributary water long enough for temperature to acclimate. The bags were opened, and river water was slowly introduced to further acclimate larvae to river water conditions. Bags were emptied into flowing water to minimize predation.

Early Juvenile Stocking

Fish intended for early juvenile stocking were stocked into culture ponds as larvae after OTC marks were administered. Joseph Manning Hatchery, and University of Maryland Center for Environmental Science, Aquaculture and Restoration Ecology Laboratory (UMCES-AREL) provided grow out ponds to hold fish for the restoration effort (Figure 1.4). Larvae produced for stocking at UMCES-AREL culture ponds are treated differently than river stocked larvae. These culture ponds are filled with Choptank River water that have a salinity of 9-10 parts per thousand (ppt). For these fish, the salinity is raised to 2-4 ppt at the hatchery to better acclimate in the ponds. American Shad were grown in the ponds for approximately 50 days. The decision to take juveniles out of the ponds was based on zooplankton density. Juvenile shad are ready for stocking when zooplankton availability declines significantly, and fish begin foraging the edges of the pond in search of other food.

Juvenile shad tend to stress easily and direct netting from hatchery ponds into transport tanks results in massive, unnecessary mortality. To prevent loss, juvenile fish were concentrated within the grow out ponds, using a seine net 61.0 m long, 3.1 m deep, with 6.4 mm stretch mesh. Once the seine was pursed and the fish were concentrated, a one-horsepower water pump was used to create current within the seine net to orient shad into the water flow. This currently serves two purposes. Shad are concentrated in the flow, and it separates the fish from algae and detritus. Shad were effectively removed by scooping the concentrated schools of fish out with buckets and were transferred into the transport tanks. Early juvenile survival increased in recent years due to the reduction of algae and detritus in the transport tanks and indirect handling. Early juveniles were transported to Choptank and Patapsco river stocking locations in culture pond water with DO saturated to mitigate stress. Ponds at UMCES-AREL have elevated salinity of 9.0-10.0 ppt.

A one-horsepower trash pump was carried on the stocking truck to temper juvenile shad before stocking. Shad were tempered until temperature and salinity in the tank were within one degree Celsius (°C) and 1.0 ppt salinity of the river value. Although this adds a considerable amount of time that fish are aboard the transport tank, it is assumed that this procedure increases the survival of early juvenile stocked shad by reducing stress. Juvenile stocking was accomplished by quick dumping marked juveniles through a quick release drain hose with a diameter of 15.0 cm, directly from the transport vehicle into the river.

Stocking Goals

The project developed stocking goals of 2.75 million larvae and 450,000 early juveniles for the Choptank River (Table 1.2) and 200,000 larvae and 75,000 early juveniles for the Patapsco River (Table 1.3) based on experience with juvenile collections. Stocking multiple life stages gives fisheries managers the ability to assess larval survival and estimate juvenile mortality and abundance of each life stage.

Larval stocked fish can efficiently contribute large numbers of juveniles if larval survival is high. Shad stocked as early juveniles survive extremely well and are young enough to successfully imprint to the stocked tributary as larvae do. Stocking early juveniles can also mitigate the impacts of poor larval survival since post-stocking survival of this life stage is high. Early juvenile production is limited by grow out pond availability and space.

Results and Discussion

American Shad Strip Spawn Production Summary

American Shad were collected from the Potomac River spawning area when temperatures ranged from 17°C to 20°C. Maryland Department of Natural Resources collected 728 adult American Shad by gill net on the Potomac River in 2021. Two hundred and forty-nine ripe females produced 114.3 liters of eggs. Fertilization rate was 66.1%. The estimated number of fertilized eggs produced was 2,556,016.

Water temperature remained below optimal fishing temperatures until the last week in April, at which point collection began. Water temperatures were ideal for two weeks and egg collection was excellent. This was the second lowest egg collection year (114 L), with an average of 16.3L/night since the inception of the project in 2001 (Figure 1.7). Low egg collection is attributed to staffing constraints from COVID-19. Due to the lack of personnel, only seven nights were spent gill netting for American Shad during the 2021 spawning season. Although 2021 saw the lowest egg take of any other year, the average egg viability percentage was the second highest since the inception of the project. Egg viability for 2021 averaged 63.8%, second only to 2010, at 67.4% (Figure 1.8).

Stocking Summary

American Shad were stocked as larvae and early juveniles at historical locations in the Choptank River and Patapsco River (Figure 1.5, Figure 1.6).

Due to the COVID-19 staffing restraints, stocking rates were lower than expected, despite higher-than-average fertilization percentages. As a result, American Shad larval stocking goals and juvenile stocking goals for the Choptank River were not met (Table 1.2). Both larval and early juvenile stocking goals were met for the Patapsco River (Table 1.3). A summary of 2021 American Shad stocking production separated by event appears in Tables 1.4 and 1.5. Previous American Shad stocking production summaries for all years are contained in Tables 1.6 through 1.8.

2022 Sub-Project 1 – Preliminary Results – Work in Progress

Analysis of the data for 2022 is currently in progress. Adult American Shad were caught by gill net on the Potomac River from 11 April to 17 May 2022 for broodstock collection. A total of 1,068 American Shad were caught, with 200 being males and 463 ripe females. These shad produced 182 liters of eggs. Approximately 2.1 million larvae and 350,000 early juveniles were stocked into the Choptank River. Approximately 250,000 larvae and 90,000 early juveniles were stocked into Patapsco River.

The complete analyses and summary of the data collected in 2022 to produce, mark, and stock cultured American Shad in the Choptank, and Patapsco rivers will appear in the 2023 F-57-R progress report.

2021 Sub-project 2

Objectives

American Shad restorative stocking in the Choptank and Patapsco rivers began in 1996 and 2012, respectively. The Choptank River summer juvenile seine survey commenced in 1996 and the Patapsco River summer seine survey began in 2013. The survey goal is to collect juvenile American Shad to determine the success of the stocking program in each river. From 2013 to 2017, Maryland Port Authority (MPA) funded grant supported restoration activities in the Patapsco River and all fisheries monitoring was conducted by USFWS MDFWCO. The Patapsco River was added to the Maryland Department of Natural Resources' USFWS-Sportfish Restoration project in 2018 to continue the project for stocking and assessment.

Two quantifiable population variables were identified to evaluate restoration progression of juvenile American Shad in the targeted rivers.

A. "Assess the contribution of hatchery-produced fish to the resident/pre-migratory stock in the Choptank River and Patapsco River."

B. "Monitor the abundance and mortality of larval and juvenile shad using marked hatchery-produced fish".

Materials and Methods

Juvenile American Shad were collected by seine from the Choptank and Patapsco rivers. In the Choptank River, a seine 61.0 m long, 3.1 m deep, with 6.4 mm stretch mesh, was deployed by boat from shore into deep water and back to shore at established seine sites. The net was retrieved by hand. The Patapsco River was sampled with a seine 30.5 m long, 1.2 m deep, with 6.4 mm stretch mesh. The net was deployed by hand from shore into deep water and back to shore at established seine sites. Juvenile American Shad were collected from the seine, placed into plastic bags, labeled, and stored on ice. Upon return to the lab, the samples were frozen to -9 °C. All bycatch species data were recorded.

Sagittal otoliths were removed from each American Shad captured from the Choptank and Patapsco rivers. Otoliths were mounted on 76.2 mm x 25.4 mm glass slides with Crystalbond 509 (Aremco Products, Ossining, NY). Mounted otoliths were lightly ground on 600 grit silicon carbide wet sandpaper and viewed under an LED epifluorescent light at 400X magnification at 50-100 watts with a Zeiss Axioskop 20 microscope. The presence and location of OTC epifluorescence was recorded. Epifluorescence is a technique in which transmitted light in the wavelength of 490-515 nm is allowed to strike the specimen. The specimen then absorbs this light energy and reflects light of a longer wavelength back through the microscope objective.

CPUE and Geometric Mean

The juvenile index is described by calculation of a catch per unit effort (CPUE). It is defined as the number of captured juvenile American Shad divided by the number of seine hauls completed. Indices of relative abundance are presented as the geometric mean (GM) catch per haul. The GM has been adopted by the ASMFC as the preferred index of relative abundance. The GM is a more precise statistical tool for handling these data because it is not as sensitive to a single large sample value. American Shad are schooling fish and subject to these types of captures with a large seine net.

Mortality and Abundance Estimates

In addition to providing future broodstock, juvenile stocking is valuable as a pre-migratory stock assessment tool through use of a multiple marking technique. Hatchery stocking is also used to evaluate the efficacy of stocking different life stages and the eventual impact to the returning adult population.

There are assumptions made when using these types of estimates as described by (Ricker 1975):

- The marked fish suffer the same natural mortality as the unmarked fish.
- The marked fish are as vulnerable to capture as are the unmarked fish.
- The marked fish do not lose their mark.
- The marked fish become randomly mixed with the unmarked; or the distribution of fishing effort (in subsequent sampling) is proportional to the number of fish present in different parts of the body of water.
- All marks are recognized and reported on recovery.
- There is only a negligible amount of recruitment to the catchable population during the time of recoveries are being made.

Estimates of larval survival, instantaneous mortality, and abundance of juvenile shad were calculated for American Shad in the Choptank River and Patapsco River and were derived from the following formulas.

Larval survival to juvenile stocking was calculated by (Ricker 1975):

$$S_1 = \frac{(R_{12})\ M_2}{(M_1)\ R_{22}}$$

$$\text{Variance } S_1 = {S_1}^2 \{ \left(\frac{1}{R_{12}}\right) + \left(\frac{1}{R_{22}}\right) - \left(\frac{1}{M_1}\right) - \left(\frac{1}{M_2}\right) \}$$

where M_1 is the number of fish marked at the start of the first interval (larval stocking), M_2 is the number of fish marked at the start of the second interval (early juvenile stocking), R_{12} is recaptures of larval marked fish in the second interval (after early juvenile stocking), R_{22} is recaptures of early juvenile interval marked fish in the second interval or (after early juvenile stocking), and S_1 is the survival rate of larvae during interval one (from the time of marking larvae in interval one to time of marking early juveniles in interval two).

Instantaneous mortality is derived from survival estimates and is used in conjunction with stocking data to calculate juvenile abundance:

$$Z = \frac{-\ ln\ ln\ S_1}{interval}$$

Where Z is the instantaneous mortality rate and S_1 is the survival rate.

Abundance of juvenile shad prior to out migration was also calculated by Chapman's modification to the Peterson estimate (Ricker 1975):

$$N = \frac{\{(C+1)(M+1)\}}{R+1}$$

where N is the population estimate, M is the number of marked fish stocked, C is the number of fish examined for tags (total captures) and R is the number of marked fish that were recaptured (larval or early juveniles).

From Ricker (1975): Calculation of 95% confidence limits based on sampling error using the number of recaptures in conjunction with Poisson distribution approximation.

Chapman's modification (1951):

$$N^* = \frac{\{(C+1)(M+1)\}}{(R+1)}$$

Where R_1 is from Pearson's formula to calculate upper and lower limits:

$$R_1 = R + 1.92 \pm 1.960\sqrt{R + 1.0}$$

Results and Discussion

Choptank River

This seine survey collected 666 American Shad juveniles from the Choptank River in 2021 (Figure 1.1). The Choptank River was sampled weekly, ten times from 28 July through 30 September. Of the 666 collected samples, 659 were successfully analyzed for origin. In 2021, 74% were hatchery origin and 26% were wild origin. Of hatchery origin captures, 26% were day-3 marked larvae and 48% were day-3, 6 marked early juveniles (Table 2.1).

Juvenile American Shad were collected at all seven of the established seine sites on the Choptank River, and three additional established seine sites on the Tuckahoe Creek in 2021 (Table 2.2; Figure 2.1). The sample sites in the Choptank River and Tuckahoe Creek did not include the upper range of the juveniles in these tributaries. The area of greatest juvenile abundance was most likely sampled, but the lack of acceptable seining sites preclude collections upstream from Depue Landing on the Choptank River and Stoney Point on Tuckahoe Creek (Figure 2.1). Downstream juvenile habitat is historically limited by salinity. The salinity average during the 2021 summer seine survey was similar to the long-term averages (Figure 2.2).

Choptank River: Geometric Mean

Data were examined from the first year wild American Shad were detected on the Choptank River until the present (2004-2021). Only data for wild juvenile captures was used. The 2021 GM for wild American Shad was calculated to 0.90, which is above the 17-year average of 0.28 (Figure 2.3). The GM has increased significantly during the sample period from 2004 to 2021 with the low GM calculation of 0.05 in 2004 to a high of 0.90 in 2021 (R^2 = 0.50, $F_{(1,16)}$ = 15.84, p < 0.001, Figure 2.3). The GM in 2021 is the highest for wild American Shad in the sample period from 2004 to 2021.

Choptank River: Mortality and Abundance Estimates

Estimates of larval survival, instantaneous mortality, and juvenile abundance were calculated for Choptank River American Shad in 2021 (Table 2.3). Survival of day 3 marked larval stocked American Shad to early juvenile stocking in the Choptank River was calculated at 0.143 for the 47-day period, which is slightly below the average of the twenty-year data set (0.1996). Daily mortality of larval stocked shad to the time of early juvenile stocking was calculated to be Z = 0.041, (±2 SE = 0.027). Juvenile abundance of day 3 larval stocked American Shad was calculated to be 162,850 using survival estimates and stocking data. Traditionally, using early juvenile recaptures to estimate total juvenile abundance is a more accurate measure than larvae recaptured fish. Survey recapture rates were used to estimate the composition of the juvenile stock (Table 2.4). Total juvenile abundance in the Choptank River (1 July 2021) was calculated by Chapman's modification to the Peterson estimate at 635,018 (upper limit = 708,747 lower limit = 568,940). Early juvenile (stocked) abundance was estimated at 304,500 in 2021. Abundance of wild origin juveniles was estimated at 167,668 in 2021. The 2021 wild abundance estimate was the highest estimate in the twenty-year data set (Table 2.5; Figure 2.4).

Prior to 1996 stocking efforts, no American Shad were captured in this tributary in 35 years (sampling conducted by other department projects prior to 1996). Total juvenile abundance was calculated annually for the Choptank River since 1996 (Table 2.5). Minkkinen et al. (1997) estimated Choptank River total juvenile American Shad abundance in 1996 at 109,300. No wild fish were collected during that assessment. The population was comprised of 28,600 larval stocked fish and 80,700 fish that had been stocked as juveniles.

In comparison, estimated total abundance at the time of early juvenile stocking was 404,000 in 2002 and 349,800 in 2003. The 2005 and 2006 estimates indicated the highest levels of total juvenile abundance (more than 1 million) observed until 2015 in the Choptank River (Table 2.5). The 2021 total abundance estimate of 635,100 is similar to the 2002-2021 average of 668,000.

Survival of larval anadromous species can vary widely from year to year as observed in previous spawning seasons (Table 2.3). These larvae are sensitive to both biotic and abiotic factors during the first weeks of development (Crecco 1985). Larval-origin juvenile abundance is not correlated with larval stocking effort. This is due to variable larval survival from year to year (Table 2.3). Total juvenile abundance is variable according to the level of stocking effort and larval survival for each year and is positively correlated with larval stocked-origin juvenile abundance ($r^2 = 0.384$, $P = 0.0102$).

Based on past observations, recruitment to the juvenile population is set by approximately 40-60 days and mortality is very low past this point. Direct comparisons of Choptank River abundance to other target tributaries, such as the Patuxent River, are not appropriate without consideration of the quality and quantity of juvenile habitat available. Based on historical juvenile recaptures from this project, the Choptank River has much more juvenile habitat than the Patuxent River, so at this time it is unknown whether an abundance estimate of 1,000,000 is high for this river. Based on the amount of available juvenile nursery habitat, the Choptank River should be able to support at least four times the abundance of the Patuxent River. Considering past abundance estimates of more than 400,000 juveniles in the Patuxent River, it is possible that the Choptank River could support between 1.5 million and 2.0 million juveniles.

Natural recruitment is occurring in the Choptank River according to juvenile otolith analysis. No wild juveniles were captured in the first five years of the restoration effort. Total captures were low (1997-2000), and no wild juveniles were captured until 2001 (Table 2.1). Poor hatchery production in past years prevented stocking enough larvae in the Choptank River. At the inception of the project, it was estimated that a minimum of 2,750,000 larvae should be stocked into the Choptank River to ensure recaptures, juvenile recruitment, and subsequent sufficient adult recruitment.

The Choptank River wild juvenile abundance estimate was calculated using the time interval between larval and early juvenile stocking events. The wild juvenile abundance was calculated to 167,668 (26.4%) of the total juvenile population which was the highest since the inception of the restoration project.

In 2007 and 2008, wild origin juveniles accounted for 16% (54,800) and 19% (17,200) of the captures respectively, two of the higher recorded sample years for wild captures. The wild capture percentage substantially declined in 2009 to 7%, which initiated a percentage point decrease each year through 2011 (Table 2.1). Wild juvenile abundance estimates have increased since 2011 (Table 2.5; Figure 2.4). The wild origin captures averaged 10% per year from 2002-21.

Success of this program relies on natural recruitment from hatchery-produced adults. Fish stocked at larval and early juvenile size successfully imprint to their native rivers and return to spawn. Prior to the restoration effort, there had been no measurable American Shad recruitment in the Patuxent River or Choptank River since the 1970s (Maryland Juvenile Recruitment Survey). Increased angler participation is evident in the Patuxent River, Choptank River, and Tuckahoe Creek as anglers now routinely target these fish.

Patapsco River

The Patapsco River (Figure 1.2) was sampled weekly ten times from 29 July through 29 September. The study collected 28 American Shad juveniles, all of which were successfully analyzed for hatchery marks. Among 2021 samples, 86% were of hatchery origin, and 14% were wild origin (Table 2.6). Of hatchery origin captures 57% were day-3 marked larvae and 29% were day-3, 6 marked early juveniles. Juvenile American Shad were collected at six of the seven seine sites in the Patapsco River (Table 2.7; Figure 2.5).

Patapsco River: Geometric Mean

During the 2021 juvenile seine survey, 28 American Shad were captured from the Patapsco River (Table 2.7). The 2021 GM was calculated to 0.23. The GM calculated from a low of 0.16 in 2013 to a high of 0.42 in 2014 (Figure 2.6) with a mean of 0.271. The GM in 2018 has been omitted from these calculations due to the extreme rainfall events that flooded the Patapsco River in the summer. The GM has also been omitted in 2020. COVID-19 work restrictions precluded any stocking.

From 2013-2017 the Patapsco River seine survey work was subcontracted to USFWS-MDFWCO under a grant administered by the MPA. All efforts were made to sample historical seine sites for annual sampling surveys to maintain continuity. Project biologists observed changes in stream hydrology after the removal of Bloede Dam (2018), which has altered site quality and availability.

The project was able to collect juvenile shad at six of the seven sites in 2021 where they were collected in previous seasons. Additional assessment will be conducted in 2022 to find adequate seine sites below the Boat Ramp site.

Patapsco River: Mortality and Abundance Estimates

Estimates of larval survival, instantaneous mortality, and juvenile abundance were calculated for Patapsco River American Shad in 2021. Survival of day 3 marked larval stocked American Shad to early juvenile stocking in the Patapsco River was calculated at 0.75 for the 11-day period. Daily mortality of larval stocked shad to the time of early juvenile stocking was calculated to be Z = 0.026, (±2 SE = 0.65). Juvenile abundance of day 3 larval stocked American Shad was calculated to be 138,097 using survival estimates and stocking data. Traditionally, using early juvenile recaptures to estimate total juvenile abundance is a more accurate measure than larvae recaptured fish. Survey recapture rates were used to estimate the composition of the juvenile stock (Table 2.8). Total juvenile abundance in the Choptank River (2 June 2021) was calculated by Chapman's modification to the Peterson estimate at 241,670 (upper limit = 431,553 lower limit = 129,466). Early juvenile (stocked) abundance was estimated at 69,049 in 2021. Abundance of wild origin juveniles was estimated at 34,524 in 2021 (Table 2.8). The 2021 wild abundance estimate was below the average from 2013-2021 (45,700). The Patapsco River wild juvenile abundance estimate was calculated using the time interval between larval and early juvenile stocking events. This estimate calculated 34,524 wild juveniles for 2021, which accounted for 14.3% of the juvenile population. A steady increase in wild juveniles was observed from 2015 to 2017 (Figure 2.7).

Four out of the 28 juvenile seine samples were of wild origin in 2021, which indicates low natural spawning recruitment. Continued monitoring for the presence of wild American Shad juveniles will be an indicator for restoration progress within the Patapsco River. As the wild component increases, the hatchery contribution to the spawning population will decrease, which leads to a self-sustaining population. Continued stocking of larval and juvenile American Shad is expected to make positive impacts to the Patapsco River.

2022 Sub-Project 2 Preliminary Results - Work in Progress

Choptank River

Juvenile sampling on the Choptank River will resume in August 2022. A comprehensive analysis of GM and mortality and abundance estimates for 2022 will be conveyed in the 2023 F-57-R report.

Patapsco River

Juvenile sampling on the Patapsco River will resume in August 2022. A comprehensive analysis of GM and mortality and abundance estimates for 2022 will be conveyed in the 2023 F-57-R report.

2021 Sub-Project 3

Objectives

Patuxent River and Choptank River spawning ground surveys commenced in 1999 to collect adult American Shad. Restorative stocking of American Shad in these two target tributaries began in 1994 and 1996, respectively. The Patapsco River was considered for possible restoration in 2013 and added as a target tributary to restoration efforts in 2018. The 2018 removal of Bloede Dam opened the river at its most downstream blockage and reintroduced access for shad and herring for over 50 km of the Patapsco River and its tributaries.

To determine the success of this program the following objective was adopted: "*Analyze the contribution of hatchery origin American Shad to the adult spawning population and monitor the recovery of naturally produced stocks.*"

Three quantifiable population variables were identified to evaluate restoration progression of adult American Shad spawning stocks in the targeted rivers.

1) *"Estimate catch-per-unit effort (CPUE) in each targeted river using geometric mean (GM)."*
2) *"Estimate the contribution of hatchery produced fish to the adult spawning populations."*
3) *"Estimate the age composition and frequency of first-time and repeat-spawning."*

Materials and Methods

The following materials and methods indicate standard operating procedures during a typical American Shad production season.

Survey Locations

Sampling was conducted at historical American Shad spawning areas described by anecdotal data and concentrated in river reaches where shad were encountered during previous sampling efforts. Annual sampling events were scheduled to occur in the Choptank and Patapsco rivers in 2021 (Figure 1.1; Figure 1.2, respectively). The Patuxent River population was determined by project biologists to be recovered in 2014. Since recovery, the Patuxent River has been sampled on a three-year rotation to maintain trend data. Due to the COVID-19 pandemic, sampling scheduled for 2020 was postponed and was completed in 2021.

Anchored gill nets and electrofishing were utilized on the Choptank River. Two gill nets (12.7 cm stretch mesh), one 123 m and one 134 m in length, and 3.05 m deep, were deployed parallel to the current and to cover the entire water column. Gill netting was conducted one km upstream of the Daniel Crouse Memorial Park in Denton, MD to 6.0 km downstream near the Asbury community in Denton, MD (Figure 3.1). Gill nets remained stationary throughout the duration of the sample day and were set and pulled periodically to check for American Shad and bycatch. Gill netting was utilized on the Choptank River due to the difficulty of capturing American Shad in the historical electrofishing sampling area. The Patapsco River was not sampled by gill net. Electrofishing on the Choptank River was conducted in the historical electrofishing reach and started 1.78 km downstream of Christian Park and continued for 2.06 km to the Route 313 Bridge in Greensboro, MD (Table 3.1, Figure 3.2).

Electrofishing surveys were also conducted on the Patapsco and Patuxent rivers. On the Patapsco River, the electrofishing reach started at the wastewater treatment plant located just west of Rte. 648 (Baltimore Annapolis Road) and ended approximately 1.43 km downstream. (Table 3.1; Figure 3.3). On the Patuxent River, electrofishing occurred from just above the Patuxent River 4H Center to approximately 3.93 km downstream to the wastewater treatment plant located north of the intersection of Bayard Road and Sands Road (4500 block of Sands Road; Table 3.1, Figure 3.4)

In all rivers, electrofishing was conducted with a 7.5 GPP Smith-Root electrofishing boat model SR18-E (Vancouver, WA). Each survey was accomplished with one person piloting the boat and two people netting shad from the bow. The river was sampled in an upstream to downstream direction with constant voltage applied to the entire reach. Total pedal time (s) was recorded for calculating CPUE.

Water quality parameters were recorded at the end of each sampling event while still in the sampling reach. Water temperature (°C), dissolved oxygen (mg/L), salinity (ppt), and conductivity (µS/cm) were obtained using a YSI ProSolo water quality meter (Yellow Springs, OH). Secchi depth (cm) was also recorded.

It is likely that shad utilize tidal freshwater areas in each of the targeted rivers downstream of the electrofishing collection sites but increasing river width and depth reduces capture efficiency with electrofishing gear. Anecdotal evidence indicates that substantial spawning habitat and fish movement also exists upstream of currently sampled stream reaches, but sampling habitat is limited by boat access. The use of gill nets eliminate the depth and width challenges brought about by electrofishing by using specific mesh size and gill net length/height that targets American Shad and covers the entire water column.

A sub-sample of 20 American Shad was collected per sample trip for age, otolith collection, and spawning attempts analyses. All other observed shad were counted to calculate CPUE and released. Sampled fish were measured for total length (TL, mm), fork length (FL, mm), and sex was determined. Scale samples were taken for age analysis and spawning mark interpretations. Shad scales were cleaned and mounted between glass slides. Age was estimated and spawning attempts were counted using a microfiche reader. Two biologists interpreted the scales independently. In cases where readers disagreed on an age/spawning attempt analysis, a consensus age was used as the final age. Scales were analyzed using methods described by Cating (1953).

Sagittal otoliths were extracted from each American Shad sampled from the Choptank and Patapsco rivers to determine origin. All hatchery origin American Shad are marked with OTC, which permits analysis of hatchery contribution to the juvenile abundance estimate and the adult spawning stock composition. Otoliths were mounted on 76.2 mm x 25.4 mm glass slides with Crystalbond 509 (Aremco Products, Ossining, NY). Mounted otoliths were lightly ground on 400 grit silicon carbide wet sandpaper and viewed under an LED epifluorescent light at 400X magnification at 50-100 watts with a Zeiss Axioskop 20 microscope. The presence and location of OTC mark epifluorescence was recorded. Epifluorescence is a technique in which transmitted light in the wavelength of 490-515 nm is allowed to strike the specimen. The specimen then absorbs this light energy and reflects light of a longer wavelength back through the microscope objective.

Catch Per Unit Effort Analysis

Data were standardized using the number of shad encountered per day divided by the shock time in minutes applied to the river the day of sampling. Annual CPUE was calculated by finding the mean of the daily CPUEs. Adult sample data are unavailable prior to 1999 and any data prior to 2001 are deficient of the necessary catch and effort data to obtain a standard CPUE. Standardization of CPUE advanced in 2011 with the implementation of bracketing CPUE data. Before 2011, data were collected beginning the first week of April and lasting until the CPUE reached zero at the end of the spawning run. Protocol now calls for a CPUE zero at the beginning and end of the survey season to better understand how long fish remain in the spawning area each year and to ensure the entirety of the spawning run was sampled.

The GM has been adopted by this project as the preferred index of relative abundance to evaluate stock status and restoration progress. The GM is calculated from the $\log_e(x+1)$ transformation, where x is the number of American Shad encountered per shock time (min). Beginning and ending zeros are omitted from the analysis. The number one is added to all catches to transform zero catches, because the log of zero does not exist (Ricker 1975). Since the $\log_e$-transformation stabilizes the variance of catches (Richards 1992), the GM estimate is more precise than the arithmetic mean (AM) and is not as sensitive to a single large sample value. It is almost always lower than the AM (Ricker 1975).

The traditional method of calculating electrofishing CPUE for Choptank River relative abundance was not applicable beginning with implementation of gill net sampling in 2015. Two identical mesh size gill nets were used. The nets only differed in length by 11.0 m. The CPUE for Choptank River gill net sampling was calculated by dividing the number of fish caught in each net by the hours fished for that specific net. That created a CPUE for both nets fished each day. The data were then averaged to create a CPUE for each sample day. The length of each net was not considered when calculating CPUE.

Origin Composition (Hatchery vs. Wild)

The percentage of hatchery versus wild origin American Shad adults sampled on the spawning grounds provides insight into the impact to the adult population of stocking larval and juvenile shad. The presence of adult hatchery origin fish on the spawning grounds early in restoration may stimulate annual natural reproduction, something that had not occurred in decades prior to the restoration efforts. As restoration efforts continue, a transition from a high proportion of hatchery origin fish to a high proportion of wild fish year after year indicates natural reproduction events, which results in successful recruitment to adulthood. Documenting shifts from predominantly hatchery origin adults to a wild origin population indicates a substantial effect upon the adult spawning stock population. This variable is sensitive to small sample sizes.

First-time and Repeat-Spawning Compositions

A third estimator uses analysis of first-time and repeat-spawning composition. The number of times a fish embarks on an annual spawning run during its lifetime can be determined through the examination of American Shad scales. The composition of first-time and repeat-spawn frequency observed on the spawning grounds provides additional insight to population stability and recruitment. Low levels of first time-spawners may indicate problems associated with juvenile recruitment to the adult stock or poor spawning success. Conversely, a high level of first time-spawners usually indicates successful recruitment of individual year classes to the adult spawning stock. A substantial contribution of first time-spawners and several repeat-spawning classes utilizing the spawning grounds year after year is indicative of a stable spawning stock.

Results and Discussion

Choptank River Adult American Shad Spawning Stock

Thirteen American Shad adults were captured by gill net on the Choptank River during the 10 weeks from 31 March to 1 June 2021 (Figure 3.5). The water temperature ranged from 15.0°C to 23.4°C. All American Shad encountered during this survey were sampled for scale analysis and spawning stock composition. The fish were captured in the Denton, MD area using 12.7 cm stretch mesh gill net. The goal of the study was to determine Choptank River American Shad habitat preference for spawning and to capture adult American Shad for population assessment.

In addition, eight adult American Shad were captured in the historical Choptank River electrofishing area. This reach is sampled every three years to maintain trend data for Hickory

Shad on the Choptank River. American Shad encountered during this survey were collected to increase sample size. The electrofishing reach was sampled for thirteen weeks from 4 March to 1 June and water temperatures ranged from 7.6°C to 19.6°C. (Figure 3.6). Beginning and ending zeros were obtained for this sample period to ensure the entirety of the spawning run was sampled. Beginning and ending zeros were eliminated from analysis.

Choptank River American Shad CPUE

The average American Shad CPUE was 0.13 fish/h during gill net sampling on the Choptank River in 2021 (Table 3.2; Figure 3.7). This CPUE is the highest in the two most recent sample years (2018 and 2019) but still lower than average CPUE in 2016 and 2017 and the 5-year average (0.19 fish/h). Beginning and ending zeros were obtained for this sample period to ensure the entirety of the spawning run was sampled. Beginning and ending zeros were eliminated from analysis.

Choptank River American Shad Origin Composition (Hatchery vs. Wild)

Twenty-one adult American Shad from the Choptank River were retained for origin composition analysis using otolith OTC mark interpretations. Twenty otoliths were successfully analyzed, and origin was determined (Table 3.3). The samples were comprised of 13 larval origin (65%), five early juvenile origin (25%) and two wild origin (10%). A larger data set with more captures is needed for robust analysis using origin composition.

Choptank River American Shad First-Time and Repeat-Spawning Compositions

Twenty-one adult American Shad scale samples were collected in 2021. All but one of the collected samples were successfully analyzed and used to determine the annual spawning attempt composition. The 2021 sample population consisted of 24% first-time spawners, 24% second-time spawners, 38% third-time spawners and 14% fourth-time spawners (Table 3.4).

Choptank River American Shad Spawning Stock Discussion

The gill net survey has indicated mixed success in five years. Thirteen American Shad were captured in 2021. This number is relatively low compared to the 35 adults captured in 2017 and 45 captured in 2016 but higher than the eight fish captured in both 2018 and 2019 (Figure

3.8). The program sampled with boat electrofishing downriver of the historical electrofishing reach in search of Choptank River American Shad adults in the past with limited success and has not been adopted as a viable alternative to capturing large numbers of spawning adult American Shad.

In previous years, the historical electrofishing sampling area of the Patuxent River yielded both American Shad and Hickory Shad in sufficient quantities to calculate a GM. The historical electrofishing sampling area on the Choptank River produced sufficient numbers of Hickory Shad to calculate a GM, while very few American Shad adult samples were collected. Traditional analyses (GM, origin, and spawning attempt composition) from electrofishing collections do not permit robust assessment of the spawning stock population dynamics due to small sample size. It is possible that a more detailed population analysis can be performed in future years if American Shad gill net capture rates return to the trend from 2016 and 2017.

A trend is emerging that depicts that male and female American Shad could be utilizing different portions of the Choptank River. In 2017 and again in 2021, when both electrofishing and gill net sampling occurred on the Choptank River, the sexes were captured in disproportionate numbers depending on the area that was sampled (Table 3.5). Males tend to be captured more frequently in the electrofishing reach while females were encountered more often in the gill nets downstream. Adult American Shad are either using different sections of the river or there is a gear bias. Project staff are considering utilizing a smaller mesh gill net to capture more male American Shad for a more robust sample analysis.

Project staff will continue to sample different sections of the Denton, MD area of the Choptank River using gill nets to determine where American Shad stage and spawn. Juvenile American Shad analysis will continue to serve as the component to estimate Choptank River progress. This is due to sufficient captures of juvenile American Shad, which are used to calculate wild juvenile abundance estimates described in sub-project two. The data collected from increased adult recaptures will further aid in Choptank River assessment of restoration success.

Patapsco River Adult American Shad Spawning Stock

American Shad adults were sampled on the Patapsco River for 13 weeks in 2021 from 18 March to 9 June by electrofishing (Figure 3.9). Temperature ranged from 11.3°C to 27.1°C. A total of 199 adult American Shad were encountered and 114 fish were retained for sample collection. This is the fifth year that hatchery stocked American Shad adults have returned to the Patapsco River. Beginning and ending zeros were obtained for this sample period to ensure the entirety of the spawning run was sampled. Beginning and ending zeros were eliminated from analysis.

Patapsco River American Shad CPUE

The mean relative abundance (GM) was calculated to 0.42 fish/min (Figure 3.10). Preliminary evaluations indicate the abundance of American Shad in the Patapsco River is increasing. As stocking and sampling continues, more conclusions can be made as to the health of this population based on the GM.

Patapsco River American Shad Origin Composition (Hatchery vs. Wild)

In 2021, 114 adult American Shad from the Patapsco River were retained for origin composition analysis using otolith OTC mark interpretations (Table 3.6). Otoliths were analyzed and origin was successfully determined on 110 otolith samples. The samples comprised 67 (61%) larval origin, 38 (35%) early juvenile origin and five (5%) wild origin. Additional years of data collection combined with higher catch rates will provide a more robust analysis using origin composition.

Patapsco River American Shad First-Time and Repeat-Spawning Compositions

All 114 American Shad scale samples collected from the Patapsco River in 2021 were successfully analyzed to determine the annual spawning attempt composition. The 2021 sample population consisted of 27% first-time spawners, 39% second-time spawners, 30% third-time spawners and 4% fourth-time spawners (Table 3.7).

Patapsco River American Shad Spawning Stock Discussion

Hatchery stockings have been successful. Larvae and early juvenile stocked American Shad are returning to the Patapsco River as adults. Of the 110 American Shad otoliths successfully analyzed from the Patapsco River in 2021, 95% were of hatchery origin. This marks the fifth year that hatchery stocked American Shad returned to the Patapsco River as adults and the first year that wild offspring of hatchery produced adults are expected to return to the spawning grounds. Five of the American Shad caught in 2021 were of wild origin (5%). As stocking continues more wild spawned shad are expected to return to the Patapsco River and trends in wild spawning success will emerge.

The American Shad repeat-spawning composition for 2021 is appropriate at this stage in recovery efforts. The proportion of repeat spawners in the population is increasing as well as the number of repeat spawning runs an individual fish embarks on. This was the first year that an American Shad was captured that made four spawning runs in the Patapsco River. While the number of first time-spawners is lower than expected, Patapsco River American Shad population appears to be trending towards recovery.

Few inferences to the health of the American Shad population can be made with only four years of sample data. More conclusions can be made regarding the success of recovery efforts as sampling continues.

Patuxent River Adult American Shad Spawning Stock

Sampling occurred on the Patuxent River in 2021 from 8 March to 9 June, on 14 sampling trips (Figure 3.11). A total of 109 American Shad were encountered and were retained for sample analysis. Temperature ranged from 9.6°C to 25.5°C. Beginning and ending zeros were obtained for this sample period to ensure the entirety of the spawning run was sampled. Beginning and ending zeros were eliminated from analysis.

Patuxent River American Shad CPUE

The mean relative abundance (GM) for the Patuxent River in 2021 was calculated at 0.16 fish/min (Figure 3.12). Although the GM is slightly below the 16-year average (0.18 fish/min), this value falls within the typical inter-annual variation that has been observed over the course of the time series.

Patuxent River American Shad Origin Composition (Hatchery vs. Wild)

American Shad have not been stocked into the Patuxent River since 2009. Hatchery origin shad are not expected to remain in the system, so it was not necessary to complete origin composition analysis. All American shad were returned to the river once non-lethal metrics were collected.

Patuxent River American Shad First-Time and Repeat-Spawning Compositions

Sixty-two American Shad scale samples were collected in 2021. Sixty-one of the scale samples collected were successfully analyzed to determine the annual spawning attempt composition. The 2021 sample population consisted of 34% first time-spawners, 36% second-time spawners and 30% third-time spawners (Table 3.8). The percentage of first time-spawners is similar to that of second and third time-spawners. Second- and third-time spawners remain at high numbers, but fourth time spawners were absent in 2021. One American Shad scale could not be analyzed for age and spawn analysis because all the collected scales were regenerated.

Patuxent River American Shad Spawning Stock Discussion

The Patuxent River American Shad population appears to be stable. The GM observed in 2021 (0.16 fish/min) was close to the long-term average over the time series (0.18 fish/min) and higher than the observed GM in 2014 (0.03 fish/min) and 2017 (0.06 fish/min; Figure 3.12). Continued monitoring of this population will determine if this population will remain at sustainable levels without hatchery inputs.

Examination of the first-time and repeat-spawning data can be used to evaluate stability or instability in a spawning stock and can aid in the prediction of a stock decline or expansion. A stable American Shad spawning stock consists of a substantial contribution from several spawning classes. However, there are several factors that can impart variability in these distributions, including maturity schedules of males (3-4 years) and females (5-6 years), timing of the spawning run, inter-annual spawning events, annual recruitment of wild fish, number of fish stocked annually, and recruitment of wild fish. It may be possible to remove some of the variability from these distributions by evaluating male and female distributions separately, but there are already small sample size concerns when combining the males and females in these distributions. This is especially true when assessing fish making their fifth and sixth spawning attempt. There are rarely sample sizes of five fish in these age categories, which is required to evaluate these distributions statistically (i.e. chi-square analysis relies on sample sizes of 5 individuals or more per bin). Years where small sample sizes of fish are collected leads to uninformative gaps in the time series. Small sample sizes of American Shad were collected from the Patuxent River in most years over the time series. This resulted in distributions that appeared uniform (2010 and 2014) or spawning attempt classes that contained very few individuals (2003-

2004, 2007-2011, 2014, 2017), making these distributions uninformative at current capture levels (Table 3.8).

2022 Sub-Project 3 – Preliminary Results – Work in Progress

Choptank River

Normal gill net surveys were conducted on the Choptank River for 10 weeks from 6 April to 1 June 2022. Ten adult American Shad were captured. Electrofishing surveys were also conducted on the Choptank River to locate additional American Shad for eight weeks from 14 April to 1 June 2022. Two adult American Shad were captured as part of this survey. All captured American Shad were retained for scale age and otolith analysis. A complete analysis of CPUE, origin composition, and repeat spawning analysis will be presented in the 2023 F-57-R report.

Patapsco River

Electrofishing surveys were conducted on the Patapsco River for 14 weeks from 10 March to 7 June 2022. A total of 142 adult American Shad were encountered and 87 were retained for scale age and otolith analysis. A complete analysis of CPUE, origin composition, and repeat spawning analysis will be presented in the 2023 F-57-R report.

2021 Overall Restoration Progress

Choptank River

Determining the progression of the American Shad restoration program through time has been difficult due to low numbers of adult American Shad captures. The GM for wild juvenile American Shad has increased significantly during the sample period from 2002 to 2021 with the low GM calculation of 0.06 in 2002 to a high of 3.51 in 2019 (R^2 = 0.53, $p < 0.01$). The GM in 2021 is fourth highest for the wild American Shad in the sample period from 2002 to 2021.

Adult American Shad samples in the vicinity of the Denton, MD boat ramp continue to be dominated by female fish (85%). In 2017, the Maryland Department of Natural Resources caught 15 American Shad in the historical electrofishing area, 12 of which were male. This trend continued in 2021 with six of eight captured American Shad being male. This suggests that males and females use different staging areas of the river, presumptively prior to spawning or there is a gear bias. Project biologists will continue to evaluate staging or spawning areas in the Choptank River in 2022. Project biologists will sample with a smaller mesh gill net in an attempt to catch more males in the gill net survey.

Patapsco River

At this time, the only conclusions that can be made are that stockings have been successful for juvenile recruitment and that increasing numbers of hatchery-raised American Shad are beginning to return to the Patapsco River as adults to spawn. The Geometric mean during the spring adult recapture survey has increased yearly from a low of 0.06 in 2017 to 0.2 in 2021. Hatchery produced adults are currently dominating the spawning population and as stocking continues, those hatchery produced adults will return to produce wild juveniles. As years progress, wild adults should become the dominant producers in the spawning population. Additional years of stocking and sampling will lead to a more refined analysis of the recovery efforts.

Patuxent River

Post-recovery monitoring was not conducted in 2020 due to the COVID-19 pandemic but monitoring continued in 2021. The population currently appears to be stable with a GM near the long-term average for the time series. The American Shad population in the Patuxent River will be sampled one more time in 2024 to maintain trend data. If data remain stable, we will discontinue sampling for American Shad in the Patuxent River.

Project-wide Observations 2022

Project wide observations for the 2022 calendar year will be discussed in the next reporting cycle. Sub-project two will be completed for 2022 and all data will be analyzed to give a more complete picture of restoration efforts in the Choptank and Patapsco rivers. These data will be presented in the 2023 reporting period.

Literature Cited

Cating, J.P. 1953. Determining age of Atlantic Shad from their scales. Fishery Bulletin 85, Fishery Bulletin of the Fish and Wildlife Service, Volume 54.

Hildebrand, S.F. and W.C. Schroeder. 1928. Fishes of Chesapeake Bay. Bulletin of the U.S. Bureau of Fisheries. 43:99.

Howey, R.G. 1985. Intensive culture of juvenile American shad. The Progressive Fish-Culturist 47 (4): 203-212.

Klauda, R.J., S.A. Fischer, L.W. Hall and J.A Sullivan. 1991. American shad and Hickory Shad in Habitat Requirements for Chesapeake Bay Living Resources, editors Steven L. Funderburk ... [et al.]; prepared for Living Resources Subcommittee, Chesapeake Bay Program; prepared by Habitat Objectives Workgroup, Living Resources Subcommittee [and] Chesapeake Research Consortium. Second edition, 1991 rev. ed., Annapolis, Maryland.

Mansueti, R.J. and H. Kolb. 1953. A historical review of the shad fisheries of North America. Chesapeake Biological Laboratory, Publication No. 97. Solomons, MD.: State of Maryland, Board of Natural Resources, Department of Research and Education.

Richards, A.R.1992. Incorporating Precision into Management Trigger Based on Maryland's Juvenile Index. National Marine Fisheries Service, Woods Hole, MA 02543.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191:382 p.

Table 1.1. *Three-year rotating, year-specific mark for larval stocked American Shad.*

Year	Mark
2009	3
2010	3,9
2011	3,6,10
2012	3
2013	3,9
2014	3,6,10
2015	3
2016	3,9
2017	3,6,10
2018	3
2019	3,9
2020	3,6,10
2021	3

Table 1.2. *Maryland Department of Natural Resources American Shad stocking goals and total amount stocked for the Choptank River in 2021.*

Stocking Phase	Stocking Goal	Total Stocked
Larvae	2,750,000	1,140,000
Early Juvenile	450,000	305,000

Table 1.3. *Maryland Department of Natural Resources American Shad stocking goals and total amount stocked for the Patapsco River in 2021.*

Stocking Phase	Stocking Goal	Total Stocked
Larvae	200,000	200,000
Early Juvenile	75,000	75,000

Table 1.4. *Historical stocking summary for larval and juvenile American Shad in the Choptank River (1996-2021).*

Choptank River American Shad			
Year	**Larvae**	**Early Juveniles**	**Late Juveniles**
1996	626,000		115,110
1997	1,245,000		32,612
1998	136,000		16,885
1999	442,000		64,956
2000	357,000		64,369
2001	0	15,000	32,483
2002	1,020,000	100,000	23,118
2003	1,322,000	167,500	0
2004	675,000	125,000	28,898
2005	1,930,000	170,000	41,483
2006	1,720,000	199,800	0
2007	980,000	139,000	0
2008	‡ 985,000	35,000	0
2009	980,000	139,000	0
2010	3,725,000	530,000	34,272
2011	1,621,922	269,500	45,000
2012	3,692,956	548,000	69,900
2013	3,120,000	441,000	40,000
2014	1,390,000	421,000	50,000
2015	635,000	456,000	38,000
2016	1,905,000	537,000	25,000
2017	2,530,000	403,000	0
2018	1,550,000	535,000	0
2019	2,340,000	465,000	0
2020	0	0	0
2021	1,140,000	305,000	0
Total	**35,082,878**	**6,000,800**	**722,086**

‡ *Stockings include 495,000 day 3,6,9 marked larvae. Only day 3 marked larvae were used in abundance estimates. No eggs were collected in 2020 due to COVID-19 sampling restrictions.*

Table 1.5. *Historical stocking summary for larval and juvenile American Shad in the Patapsco River (including fish passage work 2012-2021). No eggs were collected in 2020 due to COVID-19 sampling restrictions.*

Patapsco River American Shad		
Year	**Larvae**	**Early Juveniles**
2012	925,000	0
2013	200,000	95,000
2014	90,000	70,000
2015	220,000	90,000
2016	215,000	75,000
2017	225,000	75,000
2018	260,000	75,000
2019	200,000	135,000
2020	0	0
2021	200,000	75,000
Total	**2,535,000**	**690,000**

Table 1.6. *Historical stocking summary for larval and juvenile American Shad in the Patuxent River (1994-2009).*

Patuxent River American Shad			
Year	**Larvae**	**Early Juveniles**	**Late Juveniles**
1994	14,000		89,760
1995	346,000		121,124
1996	655,000		173,994
1997	1,345,000		60,040
1998	61,000		16,726
1999	526,000		60,377
2000	349,000	37,250	26,765
2001	364,000	77,500	21,903
2002	472,000	124,750	24,968
2003	717,000	108,000	31,061
2004	537,000	93,000	36,571
2005	708,000	93,000	40,873
2006	720,000	222,300	93,808
2007	431,000	170,500	34,382
2008	490,000	150,000	0
2009	758,000	130,000	25,954
Total	**8,493,000**	**1,206,300**	**832,352**

Table 1.7. *Historical stocking summary for larval and juvenile American Shad in Marshyhope Creek (2002-2009).*

Marshyhope Creek American Shad			
Year	**Larvae**	**Early Juveniles**	**Late Juveniles**
2002	100,000	39,000	9,074
2003	243,000	50,000	0
2004	238,000	33,000	0
2005	205,000	40,000	0
2006	500,000	100,000	0
2007	0	137,000	0
2008	‡ 335,000	119,500	0
2009	330,000	78,000	0
Total	**1,951,000**	**596,500**	**9,074**

‡ Stockings include 85,000 day 3,6,9 marked larvae. Only day 3 larvae are used in abundance estimates.

Table 1.8. *Historical stocking summary for larval and juvenile American Shad in the Nanticoke River (1995-2006). Only fish raised and stocked by Maryland Department of Natural Resources are included. The state of Delaware also raises and stocks shad for the mainstem Nanticoke River and those figures are not included in these data.*

Nanticoke River American Shad

Year	**Larvae**	**Early Juveniles**	**Late Juveniles**
1995	34,000		8,400
1996	0		0
1997	152,000		0
1998	0		0
1999	0		0
2000	0		0
2001	40,000		0
2002	90,000	20,000	13,347
2003	324,000	73,500	0
2004	100,000	60,000	0
2005	275,000	60,000	0
2006	0	40,500	0
Total	**1,015,000**	**254,000**	**21,747**

Table 2.1. *Juvenile American Shad recaptures in Choptank River from Maryland Department of Natural Resources summer seine survey since inception of the restoration effort, 1996-2021. Data are percentage of origin composition of all juveniles collected by the survey. n=number of captured juvenile American Shad that were successfully analyzed for origin*.

Sample Year	n	Larval Stocked Origin	Early Juvenile Stocked Origin	Late Juvenile Stocked Origin	Wild Fish
1996	99	37%	NA	63%	0%
1997‡	NA	NA	NA	NA	NA
1998	1	100%	NA	0%	0%
1999	13	36%	NA	62%	0%
2000	8	0%	NA	100%	0%
2001	41	0%	32%	51%	17%
2002	200	58%	25%	8%	9%
2003	188	36%	48%	NA	16%
2004	145	52%	41%	1%	5%
2005	213	76%	14%	1%	9%
2006	290	72%	19%	NA	9%
2007	263	43%	41%	NA	16%
2008	94	43%	38%	NA	19%
2009	151	66%	26%	NA	7%
2010	551	31%	62%	1%	6%
2011	341	19%	75%	2%	5%
2012	550	20%	70%	3%	8%
2013	299	18%	60%	15%	6%
2014	443	21%	66%	5%	8%
2015	531	37%	43%	10%	10%
2016	300	35%	54%	3%	8%
2017	489	46%	44%	NA	10%
2018	490	44%	47%	NA	9%
2019	762	22%	57%	NA	21%
2020*	66	NA	NA	NA	100%
2021	659	26%	48%	NA	26%

*‡There are no data available for 1997. *No American Shad were stocked in 2020 due to COVID-19 work restrictions, only wild juveniles were collected.*

Table 2.2. *Number and location of American Shad juveniles collected in the Maryland Department of Natural Resources 2021 Choptank River seine survey. * Indicates Tuckahoe Creek sites.*

Site	7/28	8/3	8/11	8/17	8/24	9/2	9/9	9/16	9/22	9/30	Total
Depue Landing	22	7	4	5	10	5	4	4	0	0	**61**
High School	5	2	13	15	13	3	6	11	8	6	**82**
Railroad Bridge	0	2	3	1	7	8	0	1	0	4	**26**
Guano Company	6	41	9	64	9	30	35	55	4	5	**258**
Martinak State Park	29	5	3	6	1	3	5	0	2	4	**58**
*Medfield Lane	3	12	2	0	1	7	0	0	2	1	**28**
*Fallen Trees	16	6	10	20	7	4	0	0	5	3	**71**
*Stoney Point	14	1	2	7	3	2	17	11	2	0	**59**
Dover Bridge	0	0	1	0	4	5	9	1	0	0	**20**
Fossil Cliff	1	1	0	0	0	0	1	0	0	0	**3**
Grand Total	**96**	**77**	**47**	**118**	**55**	**67**	**77**	**83**	**23**	**23**	**666**

Table 2.3. *Estimates of stocked American Shad larval survival and instantaneous mortality to the date of early juvenile stocking in the Choptank River, 2002-2021*

Year	Instantaneous Mortality (Z)	±2 S.E.	Survival	Interval (days)
2002	0.0677	0.0015	0.2255	22
2003	0.1243	0.0304	0.0943	19
2004	0.0690	0.0810	0.2346	21
2005	0.0290	0.2007	0.4757	24
2006	0.0440	0.1305	0.4335	19
2007	0.0652	0.0407	0.1511	29
2008	0.0459	0.0383	0.0800	55
2009	0.0571	0.1066	0.2850	22
2010	0.0975	0.0135	0.0720	27
2011	0.1444	0.0117	0.0417	22
2012	0.0691	0.0091	0.0416	46
2013	0.1571	0.0133	0.0432	20
2014	0.1380	0.0228	0.0958	17
2015	0.0280	0.1204	0.6209	17
2016	0.0391	0.0464	0.1856	43
2017	0.0418	0.0316	0.1659	43
2018	0.0321	0.0618	0.3256	35
2019	0.0728	0.0141	0.0781	35
2020*	N/A	N/A	N/A	N/A
2021	0.0414	0.0273	0.143	47

**No American Shad were stocked in 2020 due to COVID-19 work restrictions.*

Table 2.4. *Estimates of American Shad juvenile abundance in the Choptank River on 1 July 2021. Estimates were calculated using Chapman's modification to the Peterson equation (95% confidence interval).*

Life Stage	Peterson Estimate	Upper Limit	Lower Limit
Larval Stocked	162,850	181,758	145,904
Early Juvenile Stocked	304,500	339,855	272,815
Wild Juveniles	167,668	187,135	150,221
Totals	**635,018**	**708,747**	**568,940**

Table 2.5. *1996-2021 American Shad summer juvenile abundance estimates in the Choptank River. Estimates were calculated using Chapman's modification to the Peterson equation (95% confidence interval, numbers may not add up due to rounding).*

Year	Larval Stocked Origin	Early juvenile Stocked Origin	Late Juvenile Stocked Origin	Wild Origin	Total Juveniles
1996	28,600	80,700	0	0	109,300
1997‡	NA	NA	0	NA	NA
1998‡	NA	NA	0	NA	NA
1999‡	NA	NA	0	NA	NA
2000‡	NA	NA	0	NA	NA
2001‡	NA	NA	0	NA	NA
2002	231,200	100,500	36,200	36,200	404,000
2003	124,000	168,400	0	57,300	349,800
2004	159,400	125,900	4,200	14,700	304,200
2005	922,300	170,800	11,400	108,200	1,212,700
2006	748,300	200,500	0	89,500	1,038,300
2007	148,700	139,500	0	54,800	343,000
2008	48,200	35,400	0	17,200	100,800
2009	377,500	151,000	0	41,500	570,000
2010	268,600	531,000	11,000	50,000	860,400
2011	68,000	270,300	7,400	15,900	361,500
2012	154,000	549,000	20,000	61,300	784,300
2013	135,200	442,500	113,100	44,200	735,000
2014	133,000	420,500	31,500	48,650	633,600
2015	393,300	455,000	103,000	103,000	1,054,300
2016	353,000	535,000	33,000	76,500	998,000
2017	418,700	402,000	NA	89,300	910,000
2018	503,500	533,800	NA	104,900	1,142,200
2019	182,600	464,500	NA	166,600	813,800
2020*	NA	NA	NA	NA	NA
2021	162,900	304,500	NA	167,700	635,100

*‡Insufficient sample size to calculate estimate. *No American Shad were stocked in 2020 due to COVID-19 work restrictions.*

Table 2.6. *Juvenile American Shad recaptures in Patapsco River from Maryland Department of Natural Resources summer seine survey. Data are percentage of origin composition of all juveniles collected by the survey. n=number of captured juvenile American Shad that were successfully analyzed for origin*.

Sample Year	n	Larval Stocked Origin	Early Juvenile Stocked Origin	Wild Fish
2013	66	0%	98%	2%
2014	108	37%	60%	3%
2015	81	70%	15%	15%
2016	47	24%	57%	19%
2017	56	7%	59%	34%
2018‡	0	0%	0%	0%
2019	40	0%	92%	8%
2020*	0	0%	0%	0%
2021	28	57%	29%	14%

*‡Insufficient sample size to calculate estimate. *No American Shad were collected in 2020 due to COVID-19 work restrictions.*

Table 2.7. *Number and location of American Shad juveniles collected in the Maryland Department of Natural Resources 2021 Patapsco River seine survey.*

Site	7/29	8/4	8/9	8/16	8/23	8/30	9/8	9/13	9/20	9/29	Total
Light Rail	0	0	0	0	0	0	0	0	0	0	**0**
Borrow Pit	1	1	0	0	0	0	0	0	0	0	**2**
Back Island Point	2	0	1	0	4	0	0	0	0	0	**7**
Back Island	0	0	2	1	0	0	0	2	0	0	**5**
Fisherman's Point	1	0	2	2	0	0	0	2	0	0	**7**
Goose Island Point	0	2	0	0	0	0	0	0	0	0	**2**
Boat Ramp	1	0	2	0	0	1	0	0	1	0	**5**
Grand Total	**5**	**3**	**7**	**3**	**4**	**1**	**0**	**4**	**1**	**0**	**28**

Table 2.8. *Estimates of American Shad juvenile abundance in the Patapsco River on 2 June 2021. Estimates were calculated using Chapman's modification to the Peterson equation (95% confidence interval).*

Life Stage	Peterson Estimate	Upper Limit	Lower Limit
Larval Stocked	138,097	246,602	73,981
Early Juvenile Stocked	69,049	123,301	36,990
Wild Juveniles	34,524	61,650	18,495
Totals	**241,670**	**431,553**	**129,466**

Table 3.1. The *Maryland Department of Natural Resources adult American Shad electrofishing survey starting and ending coordinates for target rivers.*

River	Starting latitude/longitude	Ending latitude/longitude
Choptank River	38.984728° N -075.788325° W	38.977021° N -075.801606° W
Patapsco River	39.224738° N -076.640593° W	39.225713° N -076.629978° W
Patuxent River	38.885666° N -076.674890° W	38.855692° N -076.691094° W

Table 3.2. *Maryland Department of Natural Resources 2021 American Shad gill net sets and captures for American Shad on the Choptank River with associated CPUE.*

Date	Set # (1 net only)	Set Time	Pull Time	# of Fish Caught	Hours Fished	CPUE (Fish/Hour)	Mean CPUE	
3/31/2021	1	9:52	15:14	0	5:22	0.0000	3/31/2021	0.000
3/31/2021	2	10:03	15:23	0	5:20	0.0000		
4/7/2021	1	9:25	15:00	1	5:35	0.1791	4/7/2021	0.090
4/7/2021	2	9:31	15:16	0	5:45	0.0000		
4/14/2021	1	9:53	14:12	0	4:19	0.0000	4/14/2021	0.108
4/14/2021	2	9:47	14:24	1	4:37	0.2166		
4/20/2021	1	10:00	15:38	3	5:38	0.5325	4/20/2021	0.582
4/20/2021	2	10:07	13:17	2	3:10	0.6316		
4/27/2021	1	9:38	15:22	0	5:44	0.0000	4/27/2021	0.00
4/27/2021	2	9:32	15:13	0	5:41	0.0000		
5/3/2021	1	9:57	15:15	3	5:18	0.5660	5/3/2021	0.283
5/3/2021	2	10:03	15:25	0	5:22	0.0000		
5/11/2021	1	9:22	15:28	1	6:06	0.1639	5/11/2021	0.168
5/11/2021	2	9:28	15:17	1	5:49	0.1719		
5/17/2021	1	7:31	12:37	0	5:06	0.0000	5/17/2021	0.096
5/17/2021	2	7:37	12:51	1	5:14	0.1911		
5/26/2021	1	9:30	14:42	0	5:12	0.0000	5/26/2021	0.000
5/26/2021	2	9:37	14:51	0	5:14	0.0000		
6/1/2021	1	8:43	13:51	0	5:08	0.0000	6/1/2021	0.000
6/1/2021	2	8:48	13:38	0	4:50	0.0000		

Table 3.3. *Summary of American Shad adults caught by Maryland Department of Natural Resources from the Choptank River in 2021. Data from the electrofishing and gill netting surveys were combined.*

			Male			
Year Class	Spawns	N	Larvae	Early Juvenile	Wild	No Sample
2013	3	1	1			
	4	1			1	
2015	3	3	2			1
2016	2	1	1			
2017	1	1	1			
	2	1	1			
Total		8	6	0	1	1
			Female			
Year Class	Spawns	N	Larvae	Early Juvenile	Wild	No Sample
2012	4	1	1			
2014	3	2	1	1		
	4	1	1			
2015	1	2		2		
	2	3	3			
	3	2		1	1	
2016	1	1		1		
2017	1	1	1			
Total		13	7	5	1	0

Table 3.4. *2016-2021 Maryland Department of Natural Resources American Shad adult recapture survey spawning composition on the Choptank River. In 2017and 2021 adults were sampled with both electrofishing and gillnetting gear. Values may not add up to 100% due to rounding. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

		Spawning Attempts				
Year	**Sample Size (n)**	**First-Time Spawner**	**2**	**3**	**4**	**5**
2001	0					
2002	14	1 (7%)	6 (43%)	6 (43%)	1 (7%)	
2003	1			1 (100%)		
2004	0					
2005	0					
2006	0					
2007	0					
2008	18	1 (6%)	3 (17%)	8 (44%)	6 (33%)	
2009	13	2 (15%)	5 (38%)	3 (23%)	2 (15%)	1 (8%)
2010	0					
2011	7		1 (14%)	6 (86%)		
2012	0					
2013	1				1 (100%)	
2014	3		1 (33%	1 (33%)	1 (33%)	
2015	3			3 (100%)		
2016	46		16 (36%)	18 (39%)	11 (24%)	
2017	50	32 (64%)	7 (14%)	9 (18%)	2 (4%)	
2018	8		4 (50%)	4 (50%)		
2019	8	3 (38%)	2 (25%)	3 (38%)		
2020	0	n/a	n/a	n/a	n/a	n/a
2021	21	5 (24%)	5 (24%)	8 (38%)	3 (14%)	

Table 3.5. *The proportion of male and female American Shad captured in the two sampling gears on the Choptank River in 2017 and 2021.*

	Electrofishing		Gill Netting	
	2017	2021	2017	2021
Male	12 (80%)	6 (75%)	1 (3%)	2 (15%)
Female	3 (20%)	2 (25%)	34 (97%)	11 (85%)

Table 3.6 *Summary of American Shad adults caught by Maryland Department of Natural Resources from the Patapsco River during electrofishing surveys in 2021.*

			Male			
Year Class	Return Spawn	N	Larvae	Early Juvenile	Wild	No Sample
2013	4	1		1		
2014	1	1	1			
	2	1		1		
	3	4		4		
	4	1		1		
2015	1	1	1			
	2	27	26	1		
	3	24	18	5	1	
	4	1	1			
2016	1	10	2	5	2	1
	2	4	1	1	1	1
	3	1			1	
2017	1	10		9		1
Total		86	50	28	5	3
			Female			
Year Class	Return Spawn	N	Larvae	Early Juvenile	Wild	No Sample
2013	2	1		1		
2014	2	2		1		1
	3	2		2		
2015	1	8	7	1		
	2	11	7	4		
	3	3	2	1		
2016	1	1	1			
Total		28	17	10	0	1

Table 3.7. *Patapsco River American Shad spawning attempt composition from 2013-2021 as determined from a subsample of fish. The United States Fish and Wildlife Service Maryland Fish and Wildlife Conservation Office determined spawning composition from 2013-2017. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

		Spawning Attempts			
Year	**Sample Size (n)**	**First-Time Spawners**	**2**	**3**	**4**
2013	1		1 (100%)		
2014	1		1 (100%)		
2015	0				
2016	1	1 (100%)			
2017	22	20 (91%)	2 (9%)		
2018	25	4 (16%)	17 (68%)	4 (16%)	
2019	38	20 (53%)	10 (26%)	8 (21%)	
2020	0*				
2021	114	31 (27%)	45 (39%)	34 (30%)	4 (4%)

Table 3.8. *Patuxent River American Shad spawning attempt composition from 2002-2021 as determined from a subsample of fish. This population of American Shad was considered recovered in 2014 and is now sampled on a three-year rotating basis. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

		Spawning Attempts					
Year	**Sample Size (n)**	**First-Time Spawners**	**2**	**3**	**4**	**5**	**6**
2002	103	12 (12%)	50 (49%)	31 (30%)	10 (10%)		
2003	35	1 (3%)	7 (20%)	16 (46%)	9 (26%)	2 (6%)	
2004	28	4 (14%)	7 (25%)	5 (18%)	8 (29%)	2 (7%)	2 (7%)
2005	82	33 (40%)	23 (28%)	17 (21%)	9 (11%)		
2006	87	27 (31%)	26 (30%)	17 (20%)	8 (9%)	7 (8%)	2 (2%)
2007	23	1 (4%)	8 (35%)	8 (35%)	4 (17%)	2 (9%)	
2008	39	5 (13%)	7 (18%)	20 (51%)	3 (8%)	4 (10%)	
2009	19	1 (5%)	3 (16%)	9 (47%)	6 (32%)		
2010	32	7 (22%)	9 (28%)	7 (22%)	9 (28%)		
2011	69	31 (45%)	29 (42%)	9 (13%)			
2012	152	33 (22%)	39 (26%)	59 (39%)	20 (13%)	1 (1%)	
2013	117	20 (17%)	33 (28%)	41 (35%)	20 (17%)	3 (3%)	
2014	20		6 (30%)	9 (45%)	5 (25%)		
2017	30	10 (33%)	7 (23%)	10 (33%)	2 (7%)		
2021	61	21 (34%)	22 (36%)	18 (30%)			

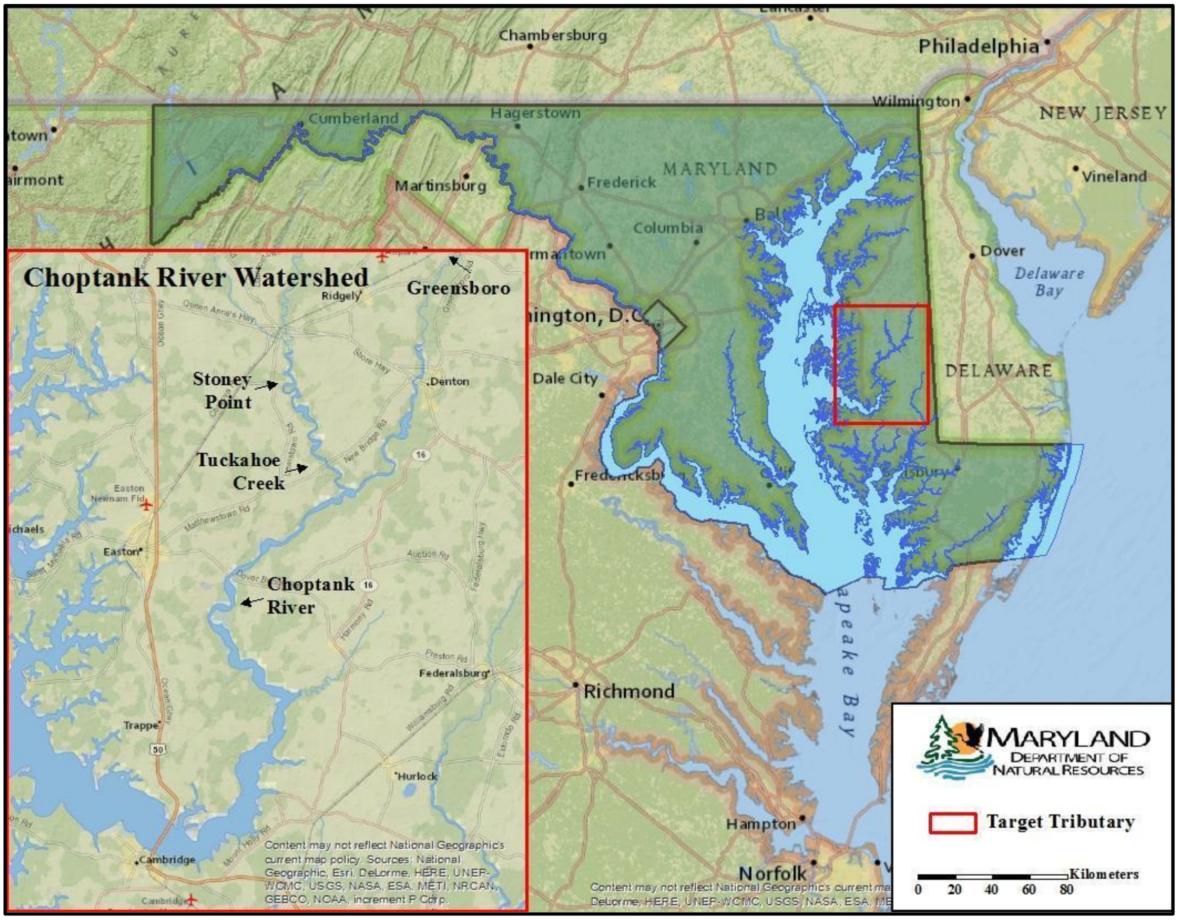

Figure 1.1. *Choptank River target tributary for the Maryland Department of Natural Resources American Shad restoration project.*

Figure 1.2 *Patapsco River target tributary for Maryland Department of Natural Resources American Shad restoration project.*

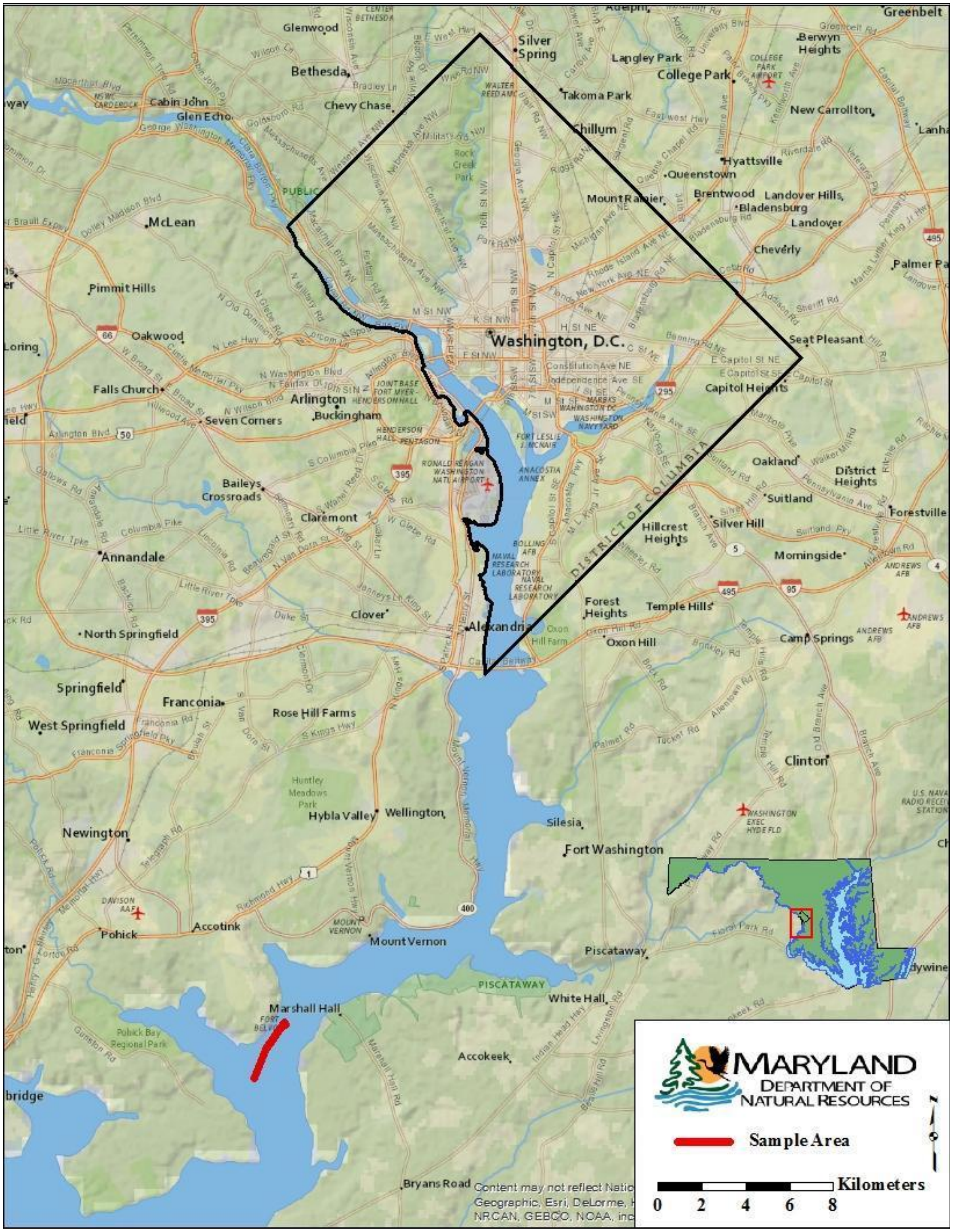

Figure 1.3. *Maryland Department of Natural Resources American Shad broodstock collection site on the Potomac River.*

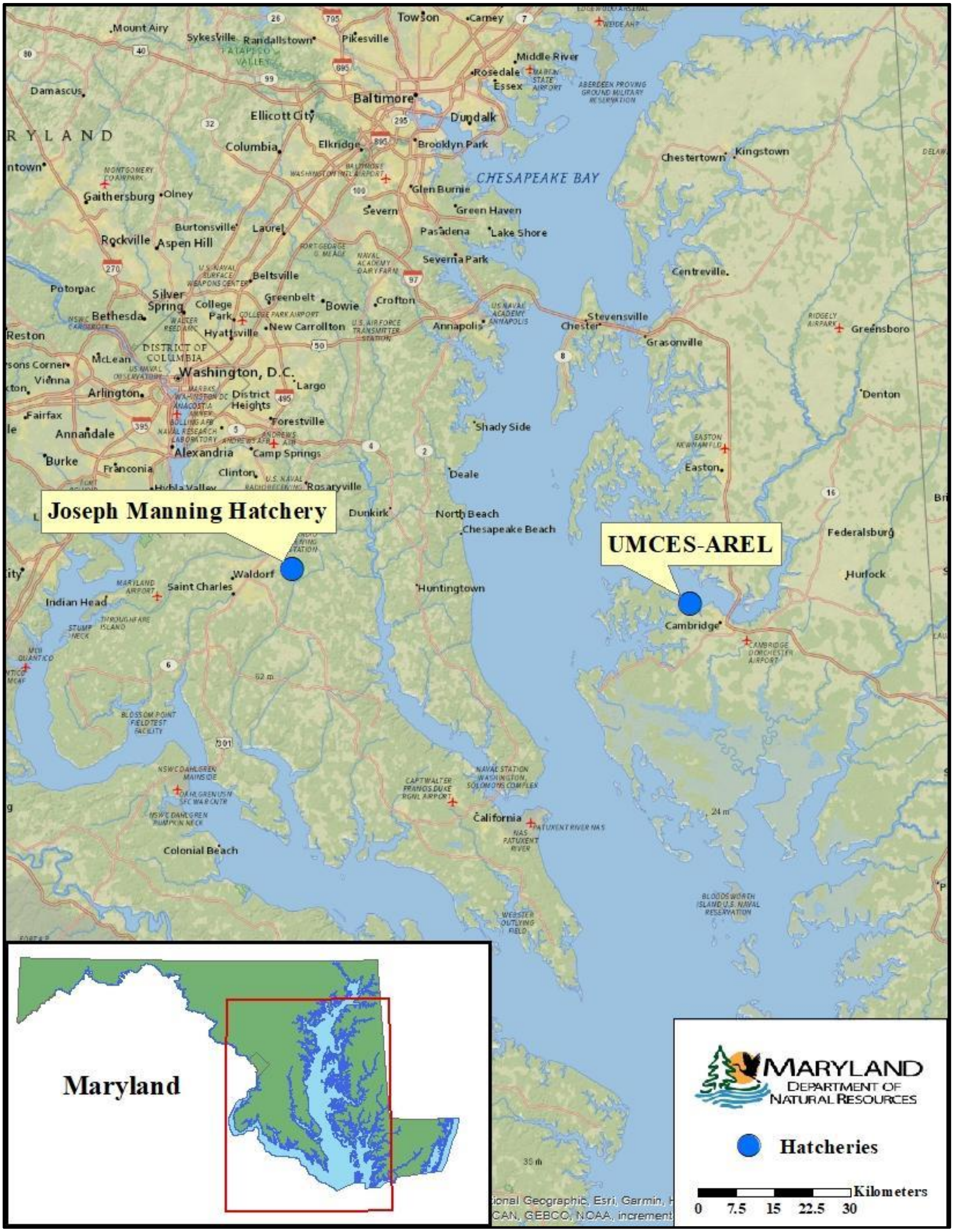

Figure 1.4. *Participating fish culture facilities in the Maryland Department of Natural Resources restoration project. The University of Maryland Center for Environmental Science (UMCES) Horn Point Aquaculture and Restoration Ecology laboratory (AREL) is a facility that supplies culture ponds for the restoration effort.*

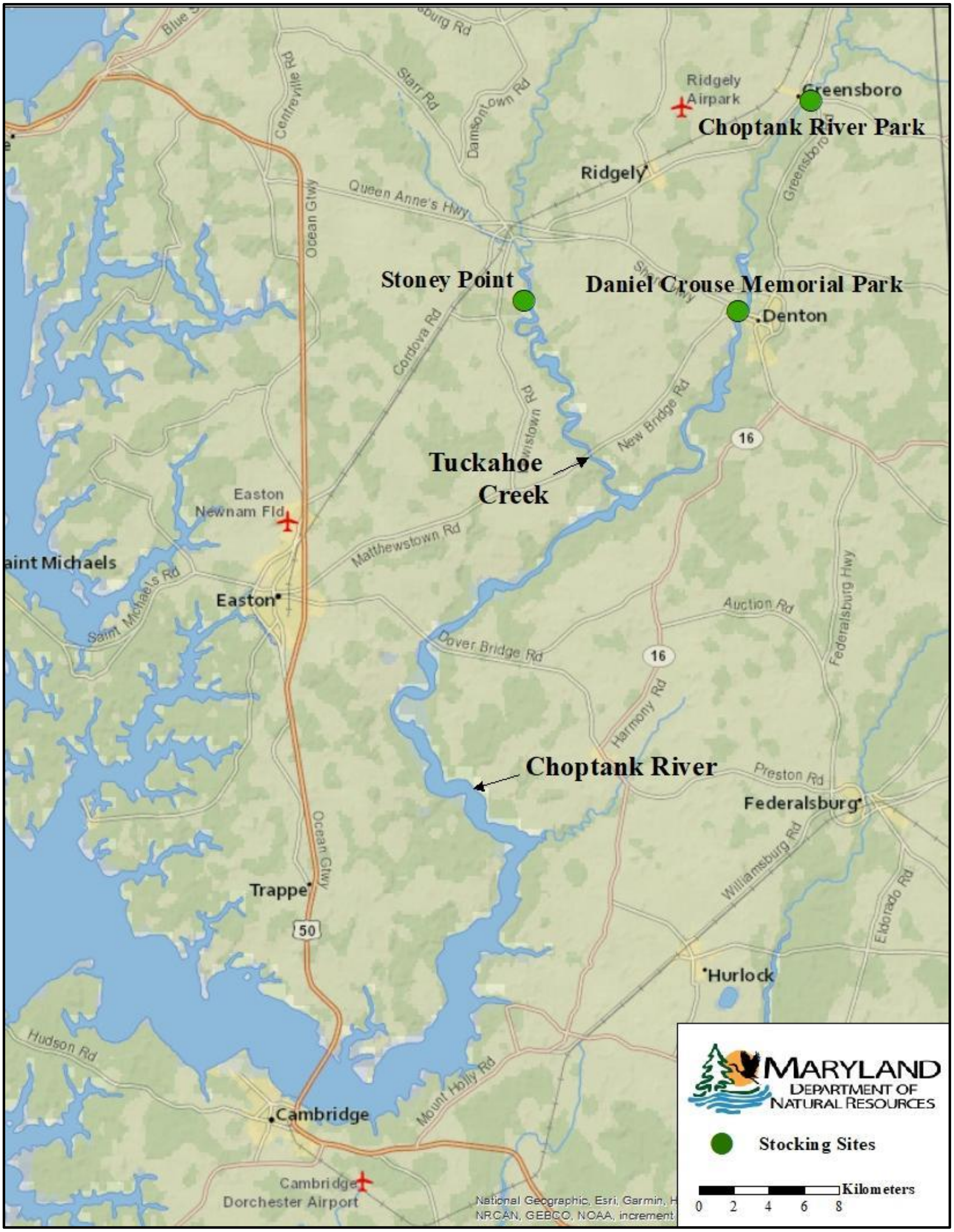

Figure 1.5 *Maryland Department of Natural Resources Choptank River American Shad stocking sites.*

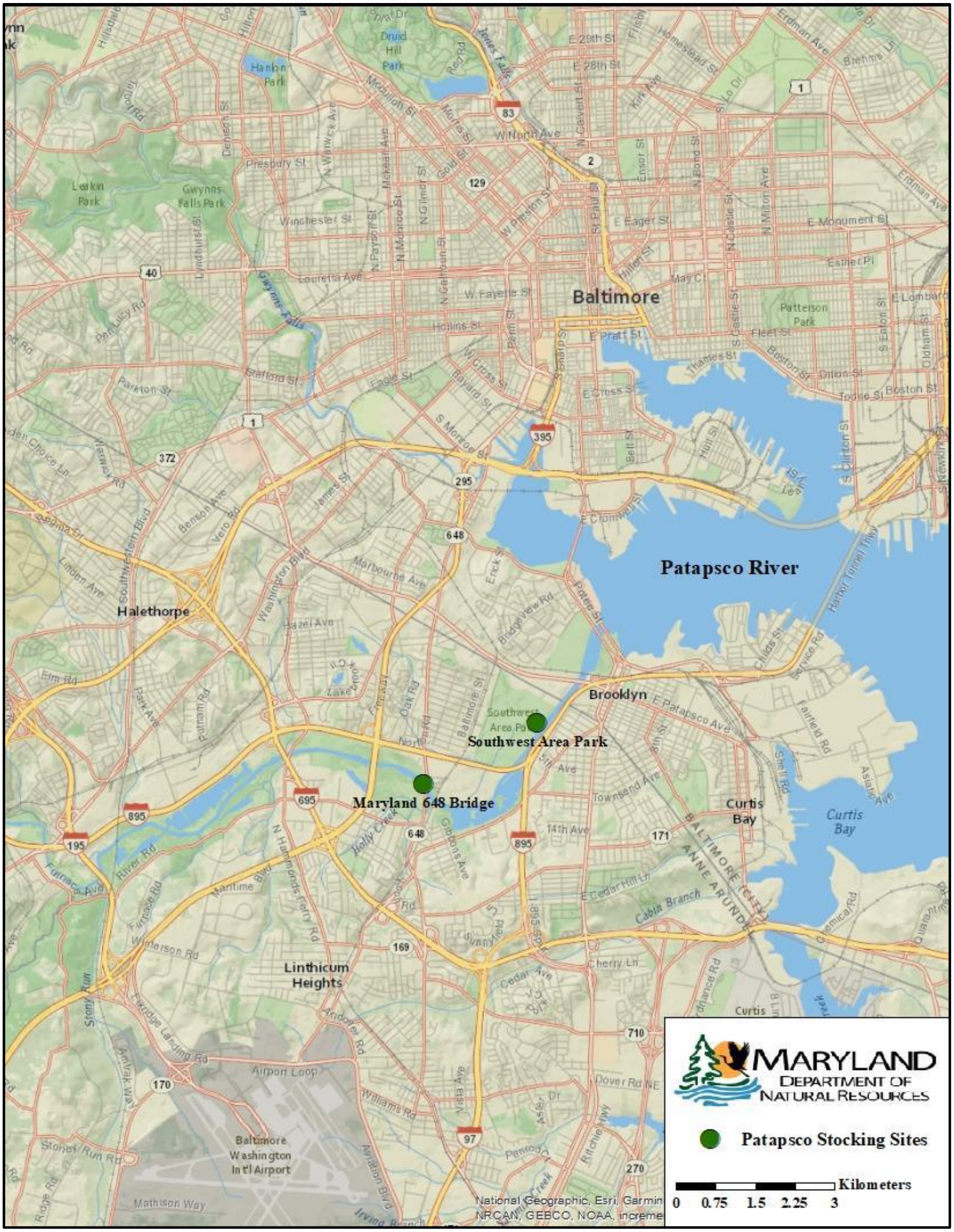

Figure 1.6 *Maryland Department of Natural Resources Patapsco River stocking sites.*

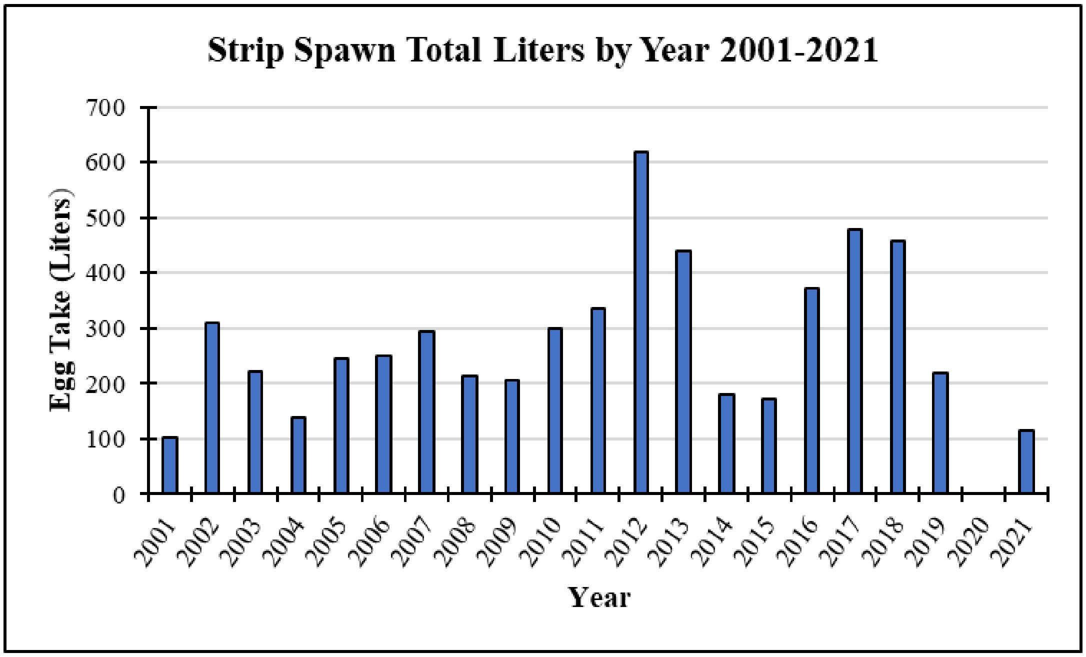

Figure 1.7 *Historical egg take (in liters) from the Potomac River Strip Spawn survey from 2001-2021.* No eggs were collected in 2020 due to COVID-19 sampling restrictions.

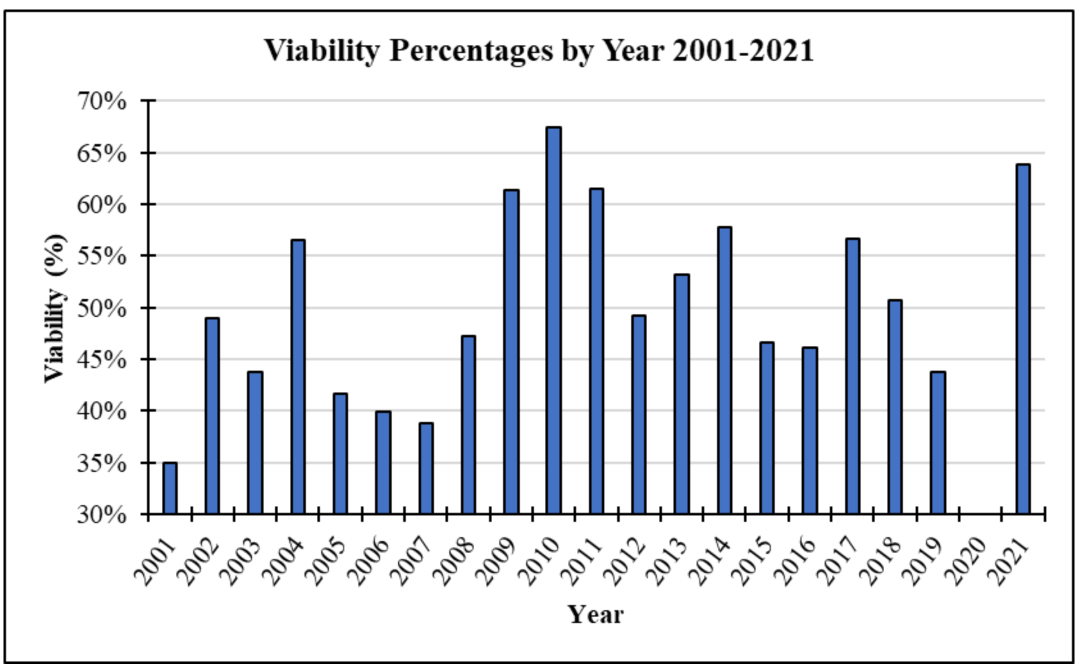

Figure 1.8 *American Shad Potomac River Strip Spawn viabilities (%) since the project's inception (2001-2021).* No eggs were collected in 2020 due to COVID-19 sampling restrictions.

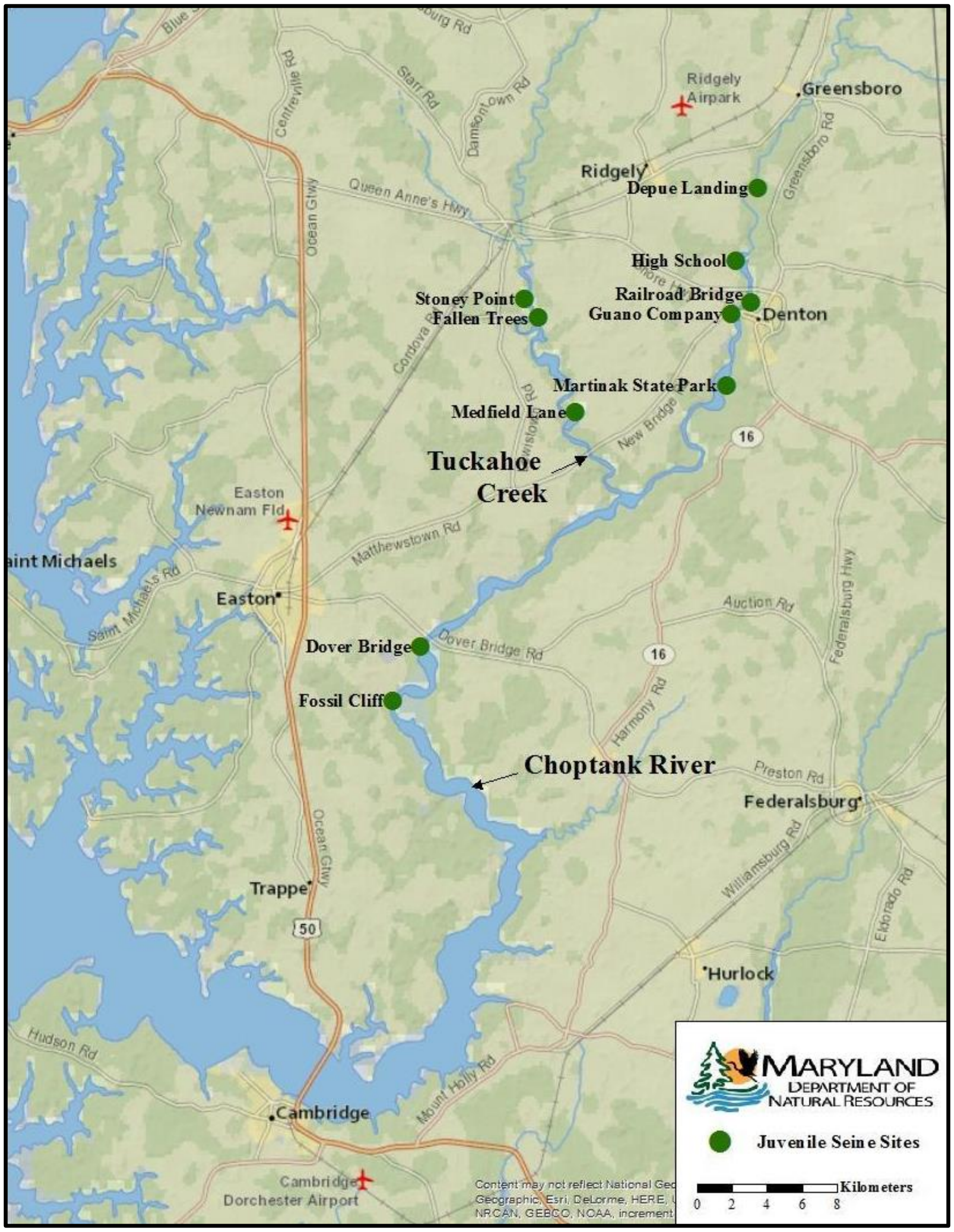

Figure 2.1. *Maryland Department of Natural Resources Choptank River juvenile American Shad survey seine sites sampled in 2021.*

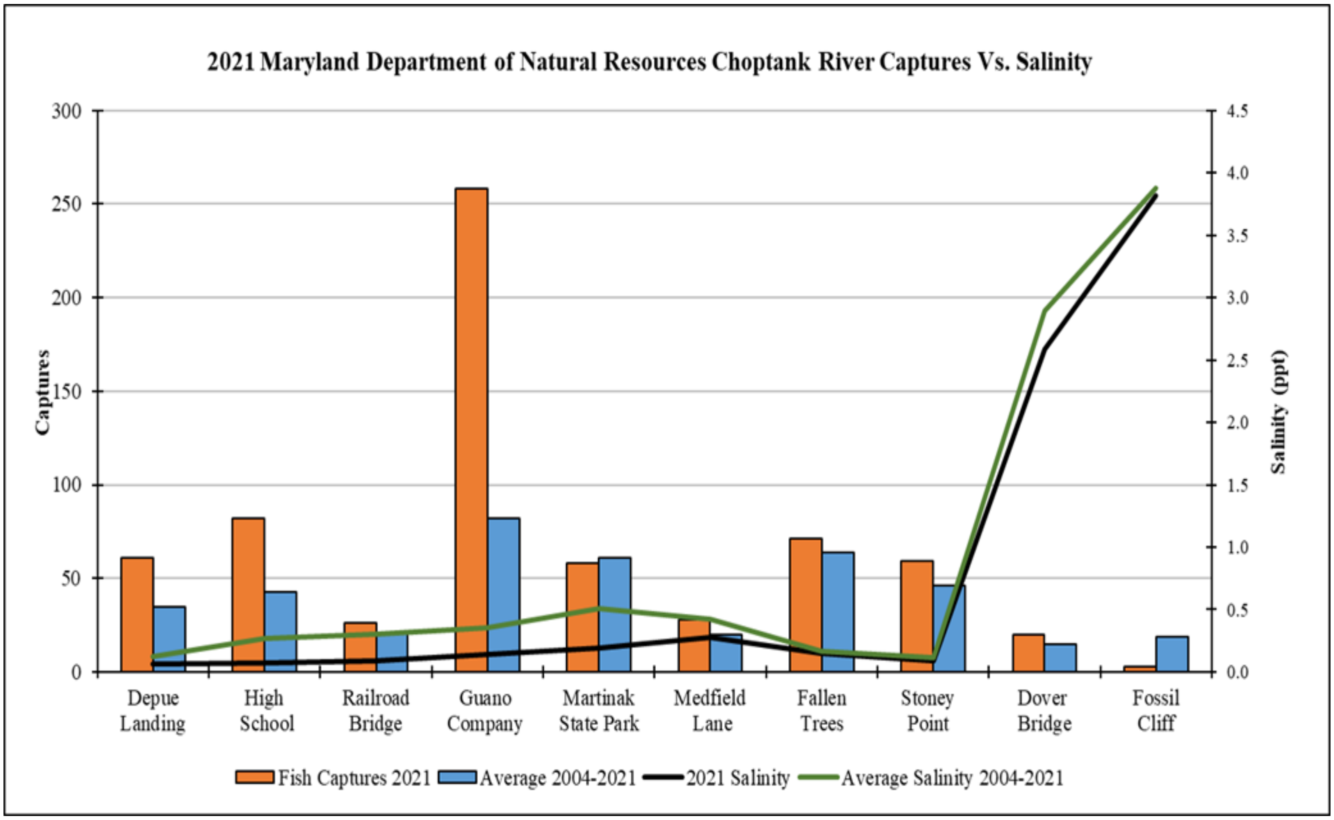

Figure 2.2. *2004-2021 Maryland Department Natural Resources summer seine survey wild juvenile American Shad catch composition and salinity by site in the Choptank River and Tuckahoe Creek. *Stoney Point, Fallen Trees and Medfield Lane are sample sites on Tuckahoe Creek.*

.

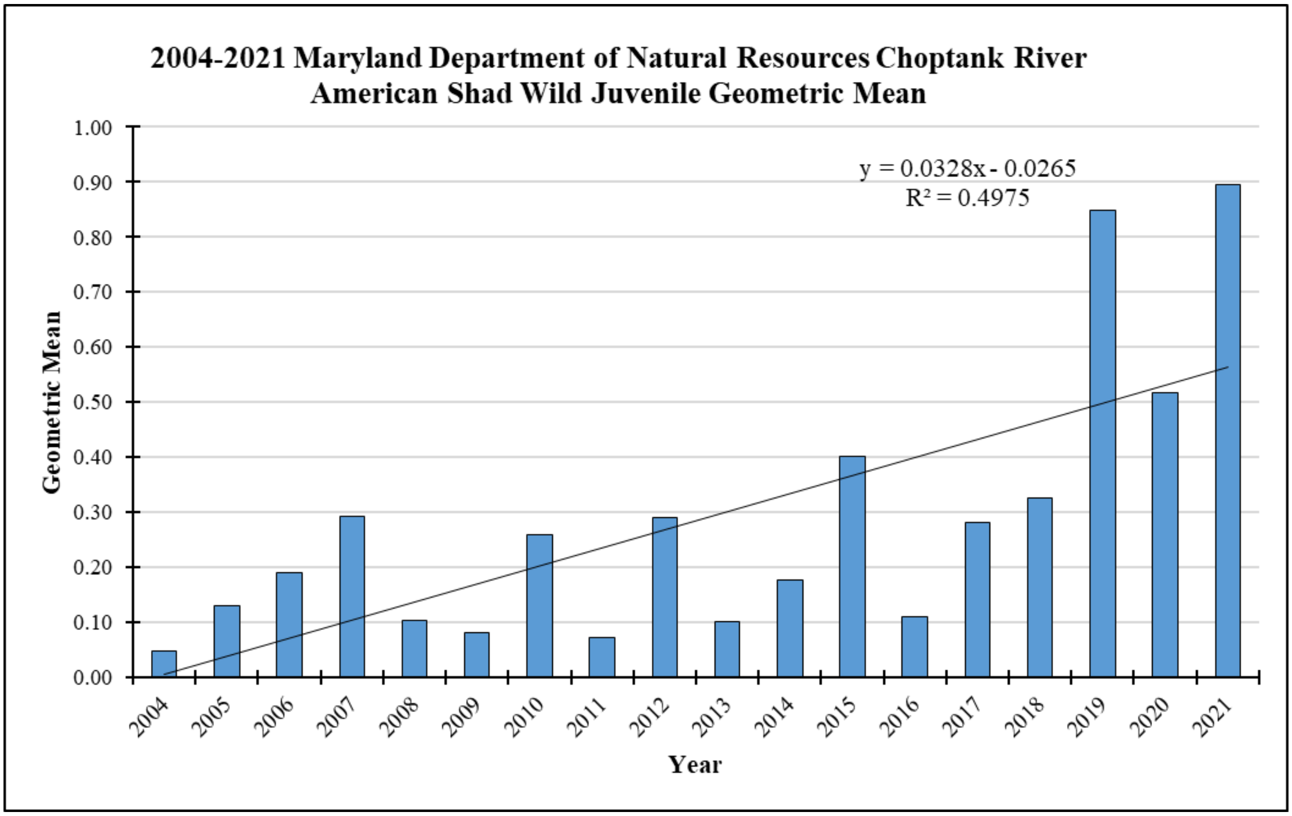

Figure 2.3. *Maryland Department of Natural Resources Choptank River wild juvenile American Shad historical geometric mean (GM). Data were generated from the permanent summer seine survey sites conducted on the Choptank River from 2004-2021.*

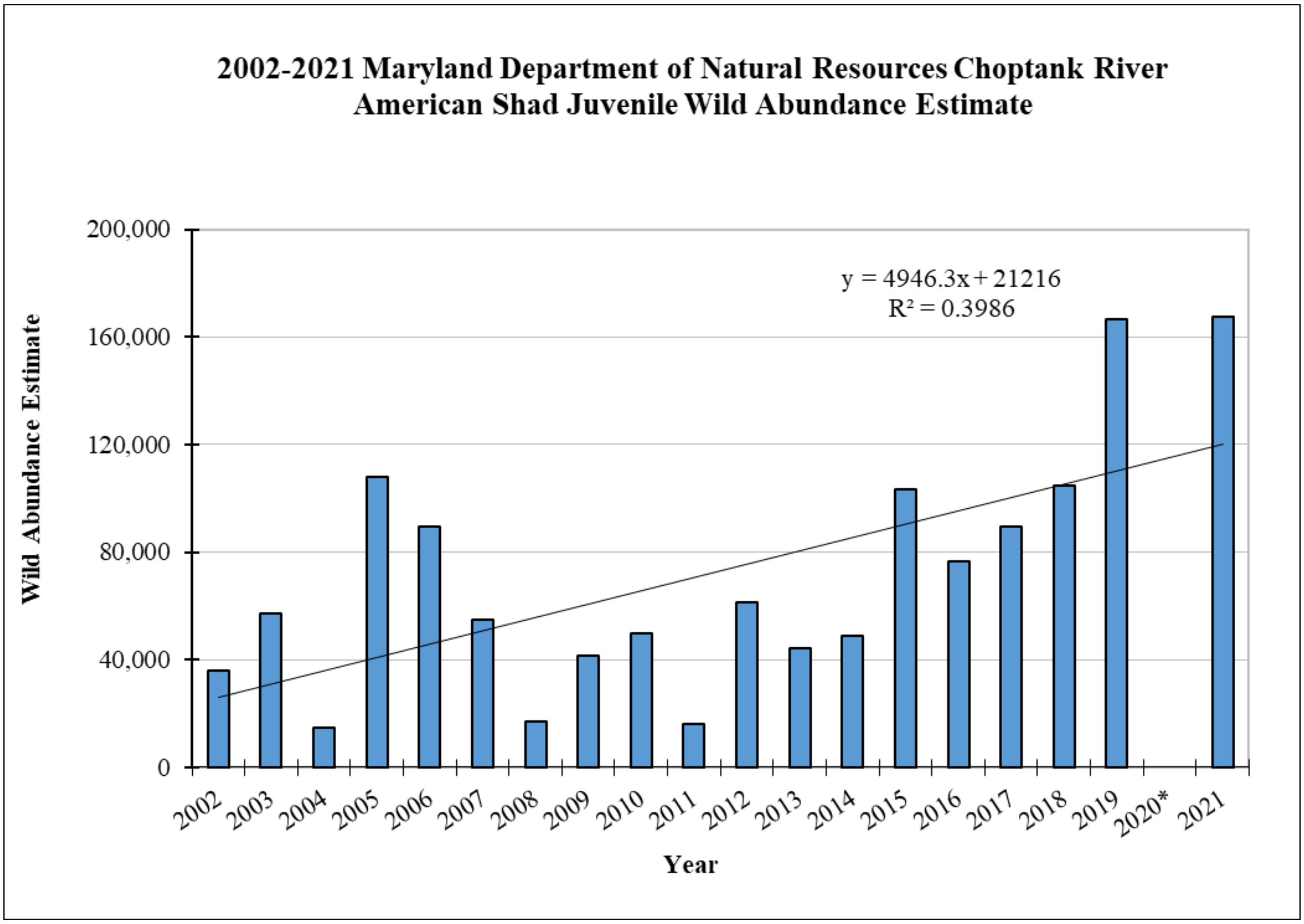

Figure 2.4. *2002-2021 Maryland Department of Natural Resources American Shad juvenile wild abundance estimates in the Choptank River. Estimates were calculated using Chapman's modification to the Peterson equation (95% confidence interval). *No American Shad were stocked in 2020 due to COVID-19 work restrictions.*

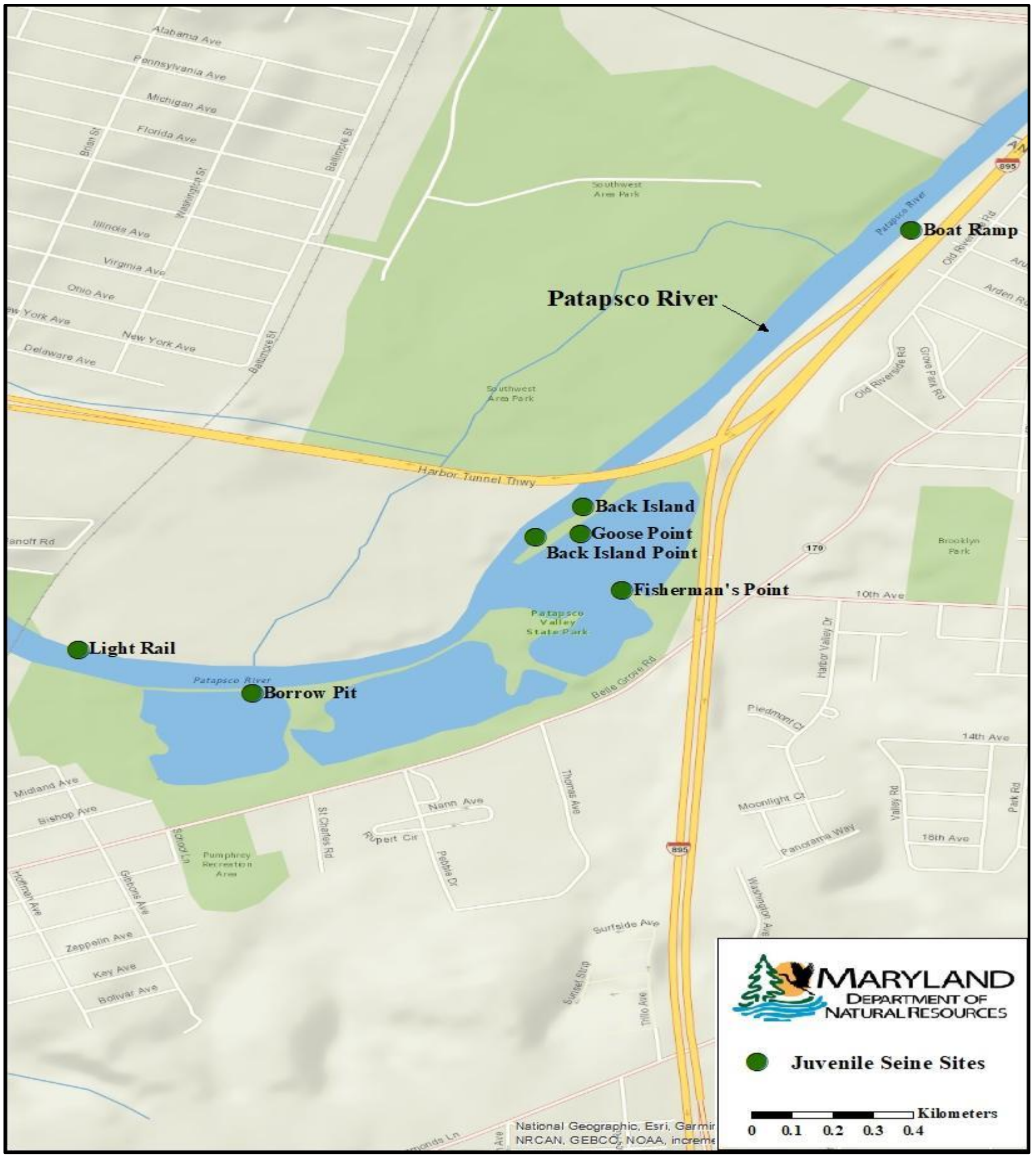

Figure 2.5. *Maryland Department of Natural Resources Patapsco River juvenile American Shad survey seine sites sampled in 2021.*

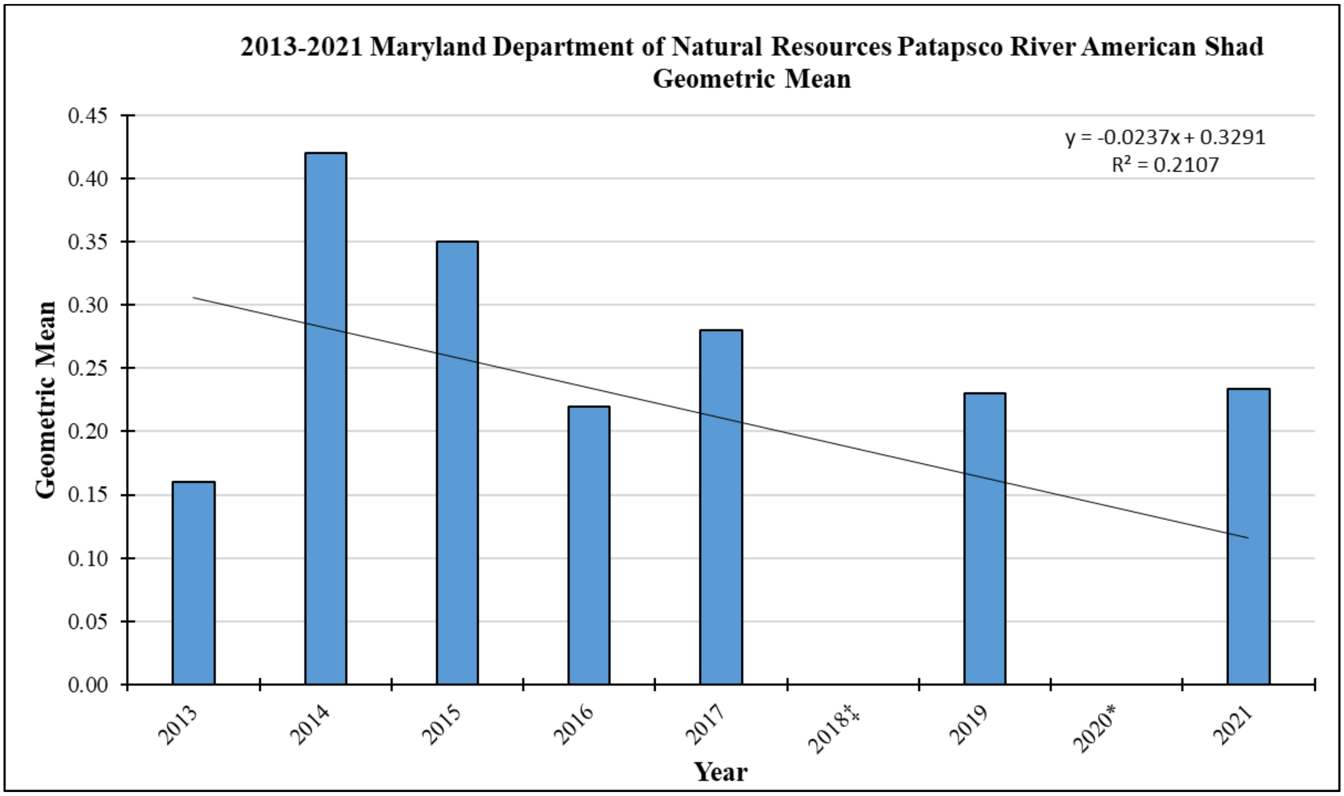

Figure 2.6. *Maryland Department of Natural Resources Patapsco River juvenile American Shad historical geometric mean (GM). Data were generated from the permanent summer seine survey sites conducted on the Choptank River from 2013-2021. ‡Insufficient sample size to calculate estimate. *No American Shad were stocked in 2020 due to COVID-19 work restrictions.*

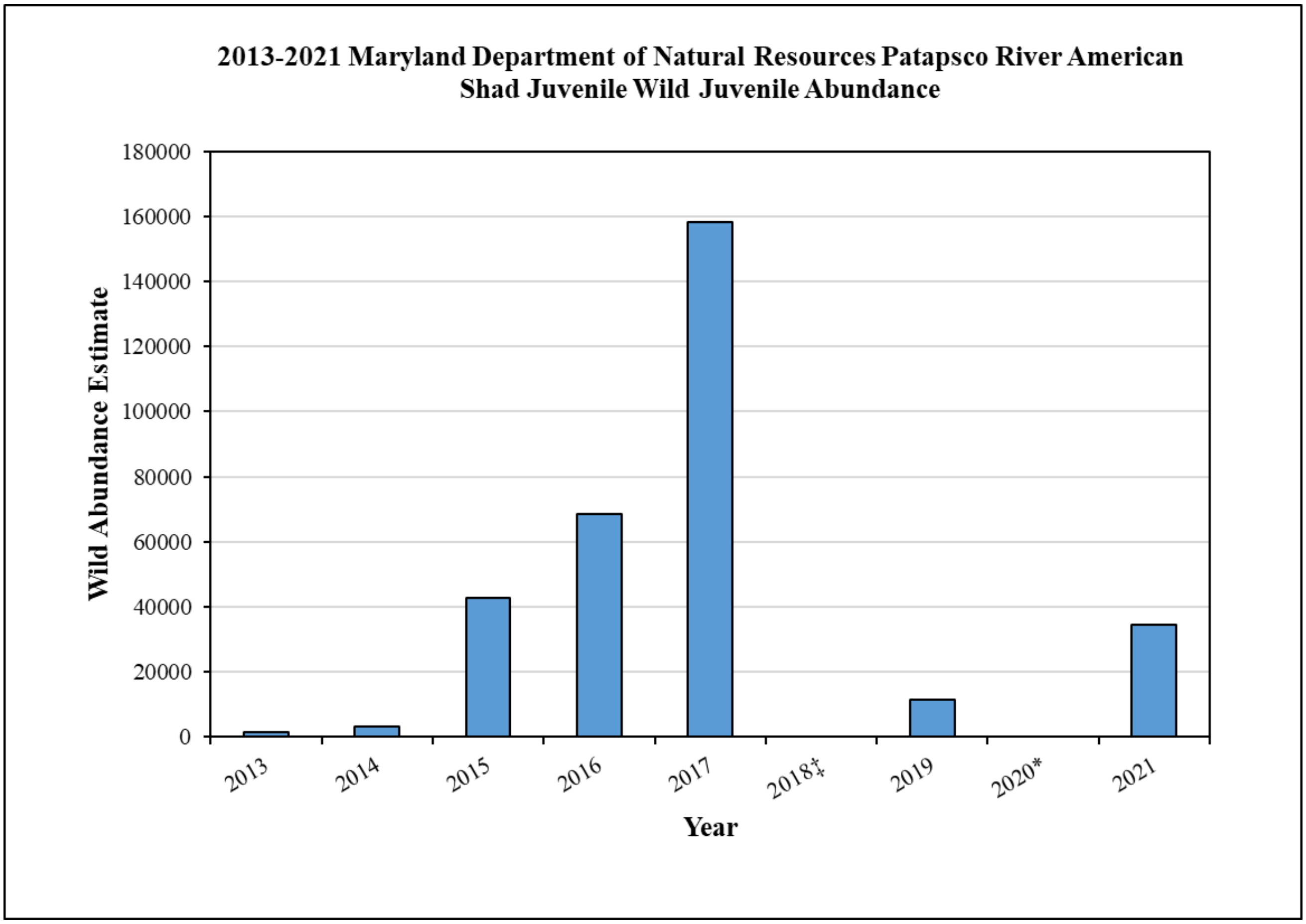

Figure 2.7. *Maryland Department of Natural Resources American Shad juvenile wild abundance estimates in the Patapsco River from 2013-2021. Estimates were calculated using Chapman's modification to the Peterson equation (95% confidence interval). ‡Insufficient sample size to calculate estimate. *No American Shad were stocked in 2020 due to COVID-19 work restrictions.*

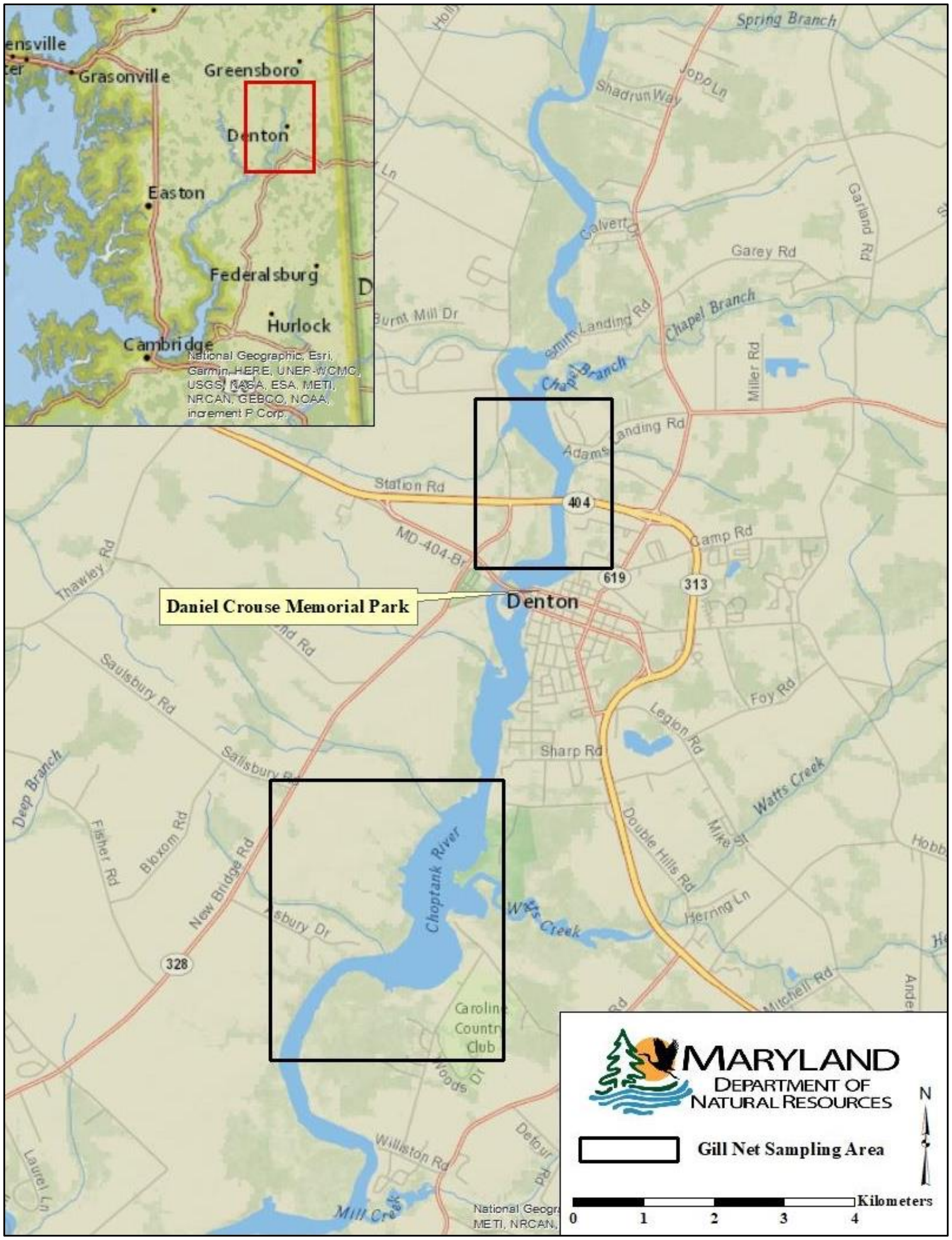

Figure 3.1. *2021 Maryland Department of Natural Resources gill net areas for adult American Shad on the Choptank River.*

Figure 3.2. *Maryland Department of Natural Resources historical electrofishing area for adult American Shad on the Choptank River.*

Figure 3.3. *Maryland Department of Natural Resources historical electrofishing area for adult American Shad on the Patapsco River.*

Figure 3.4. *Maryland Department of Natural Resources historical electrofishing area for adult American Shad on the Patuxent River.*

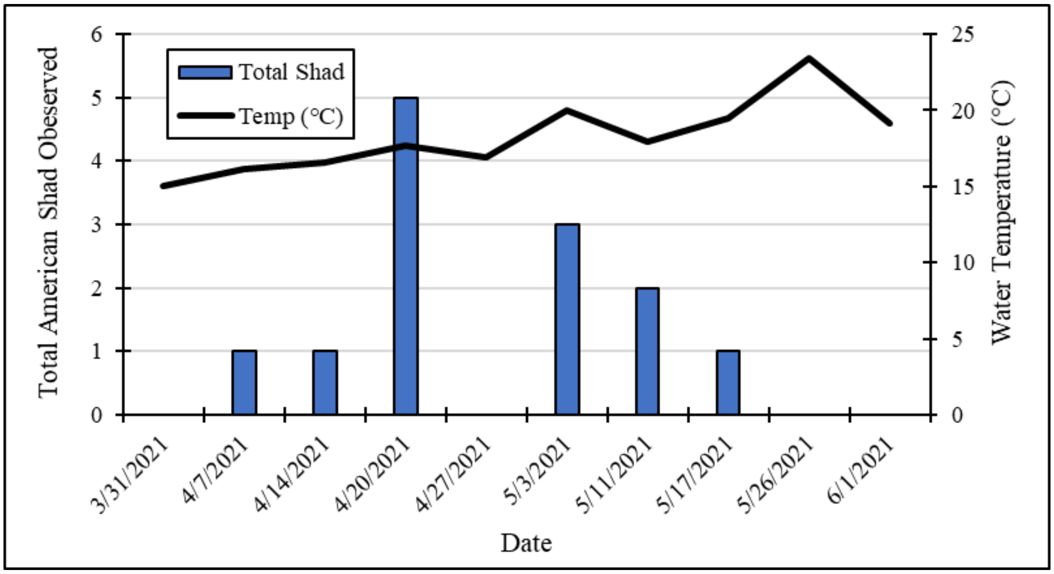

Figure 3.5. *2021 Maryland Department of Natural Resources gill net collections and observations of adult American Shad on the Choptank River in relation to water temperature.*

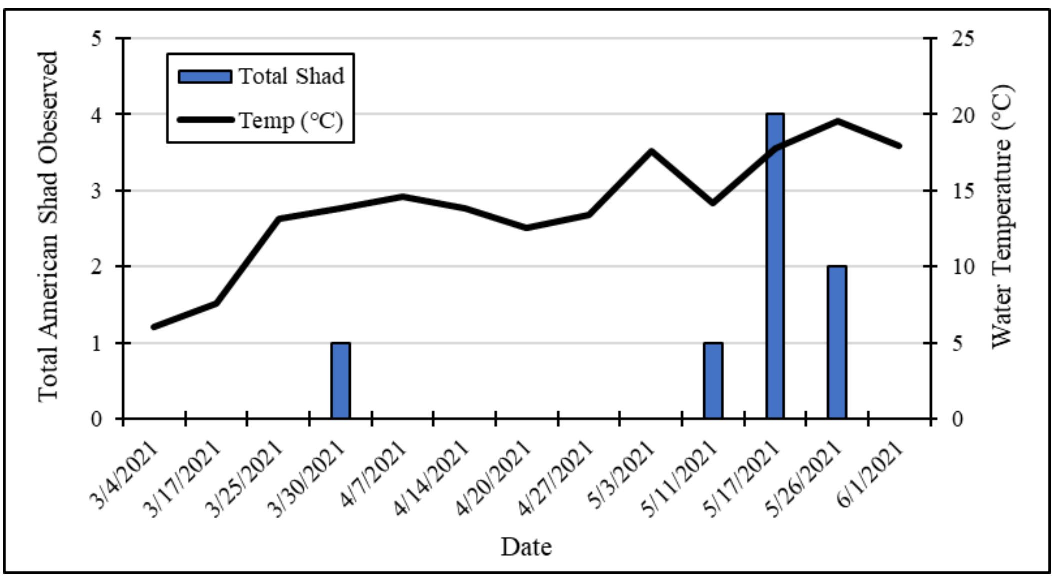

Figure 3.6. *2021 Maryland Department of Natural Resources electrofishing collections and observations of adult American Shad on the Choptank River in relation to water temperature.*

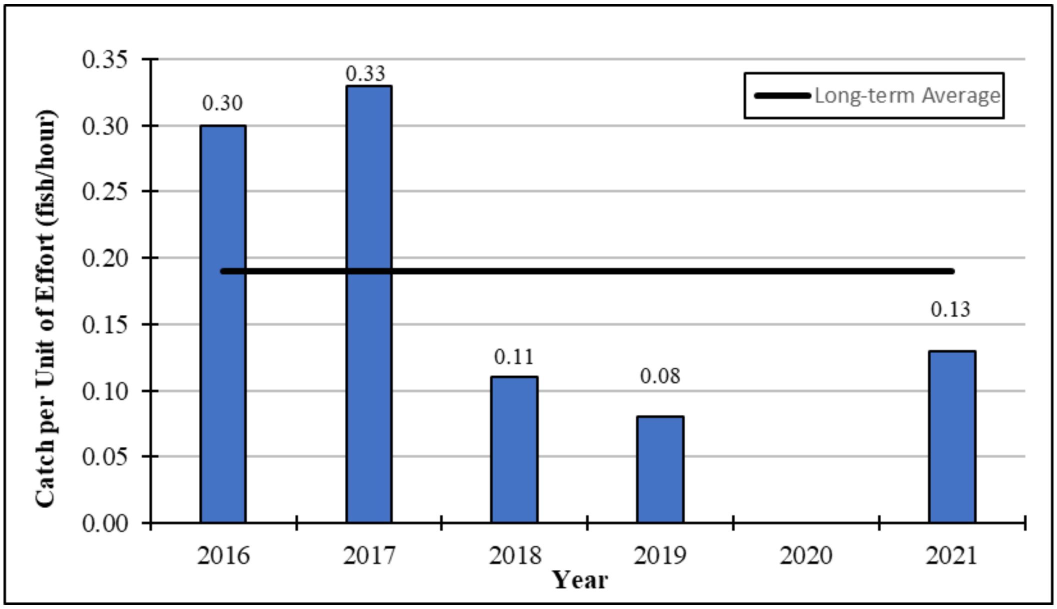

Figure 3.7. *Maryland Department of Natural Resources observed catch per unit of effort (fish/h) during adult American Shad gill net surveys on the Choptank River from 2016-2021. Long-term average = 0.19 fish/hour. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

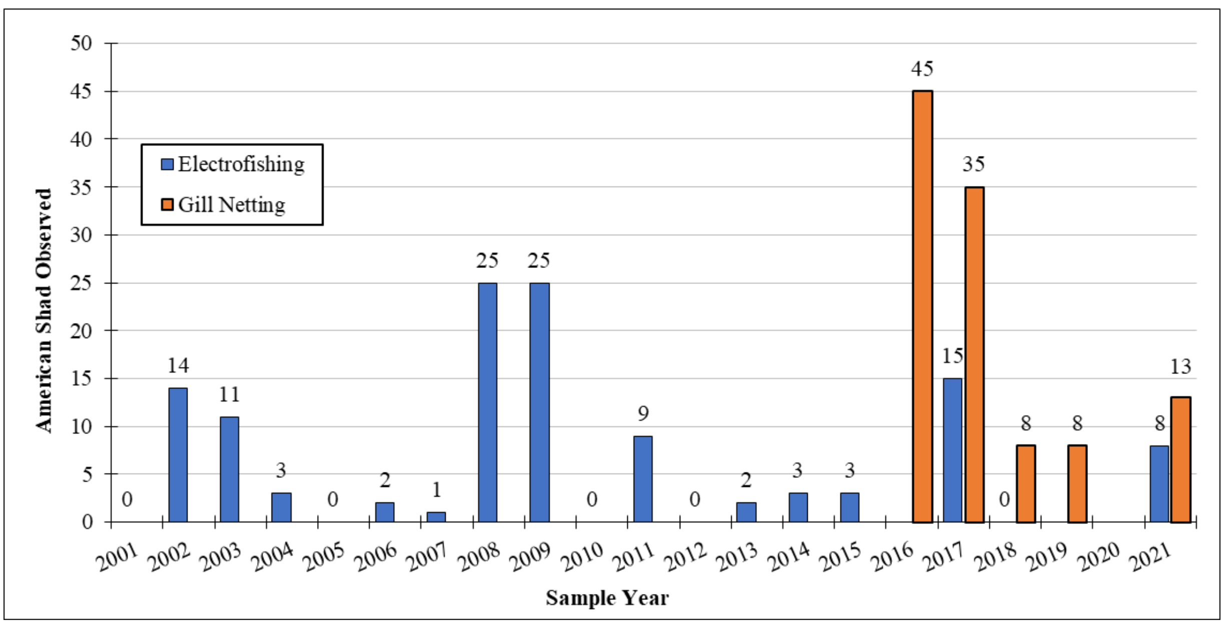

Figure 3.8. *Maryland Department of Natural Resources observations of adult American Shad in Choptank River 2001-2021. Gill netting for American Shad on the Choptank River began in 2016. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

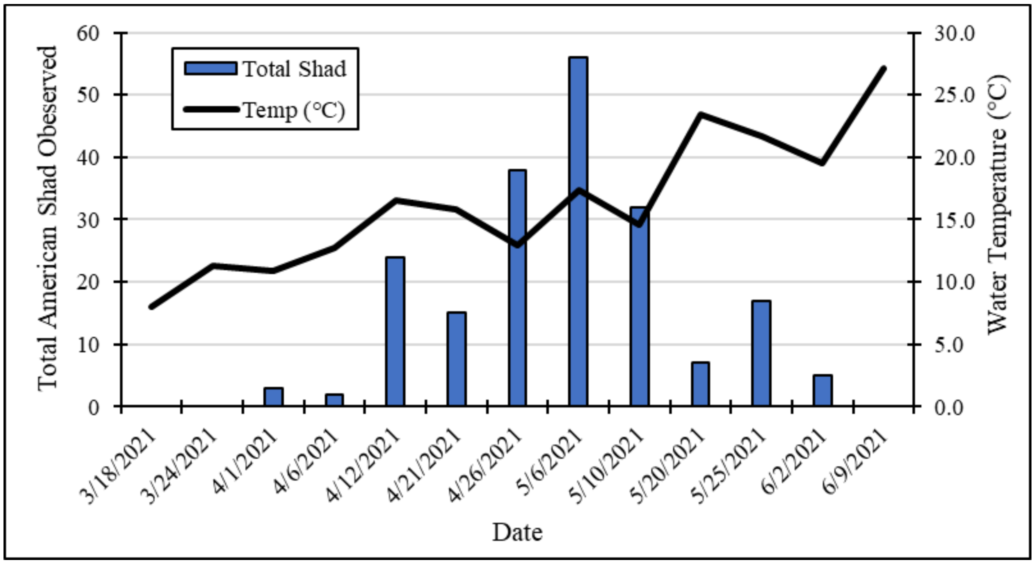

Figure 3.9. *2021 Maryland Department of Natural Resources electrofishing collections and observations of adult American Shad on the Patapsco River in relation to water temperature.*

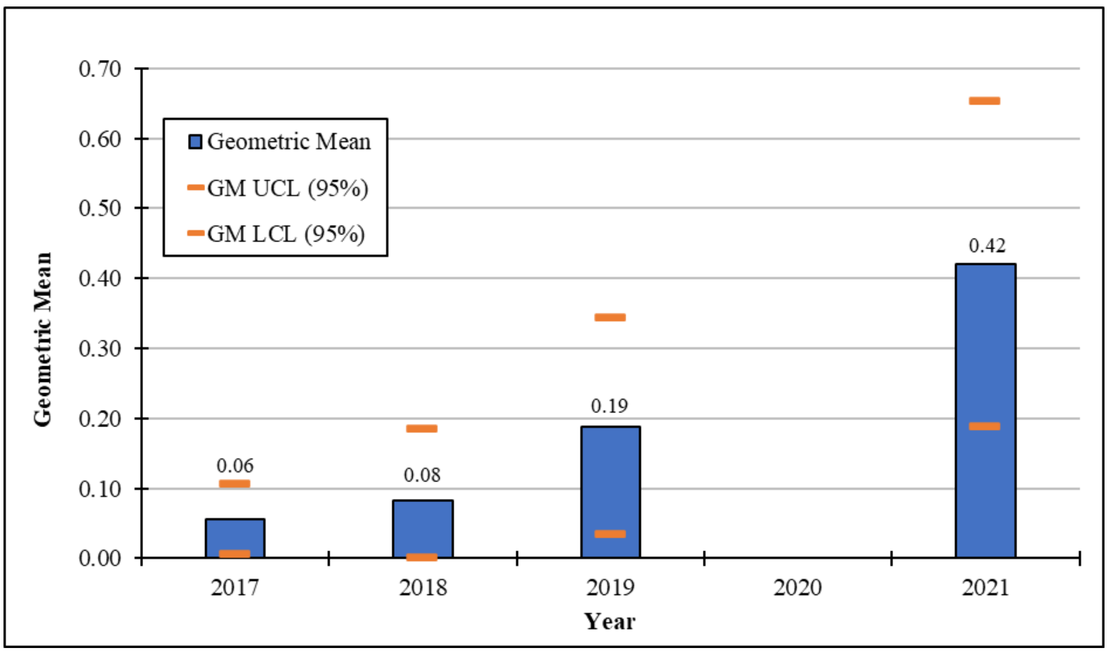

Figure 3.10. *Maryland Department of Natural Resources observed Geometric Mean (GM with 95% confidence intervals) for adult American Shad on the Patapsco River from 2017-2021. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

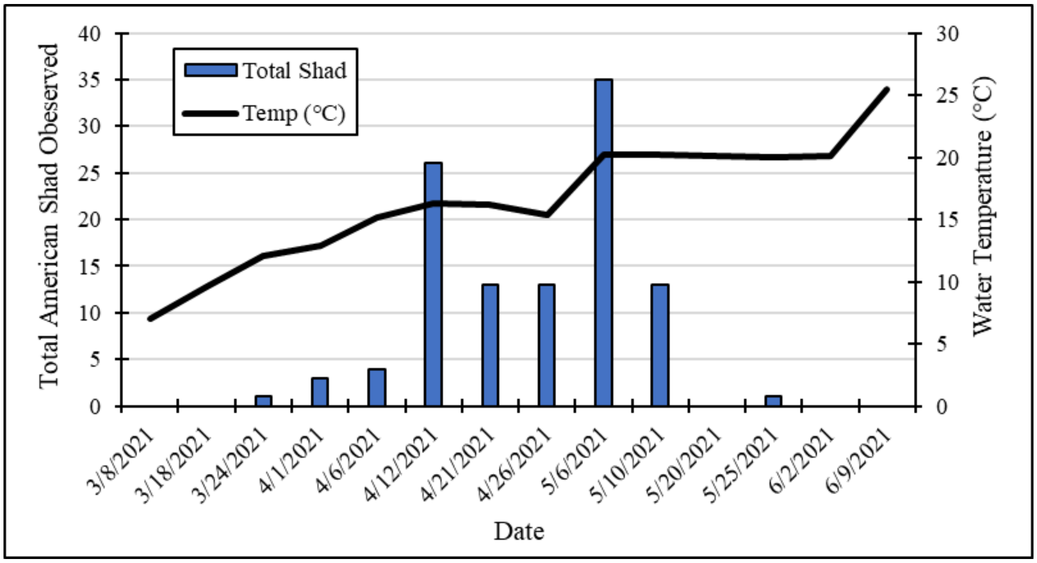

Figure 3.11. *2021 Maryland Department of Natural Resources electrofishing collections and observations of adult American Shad in Patuxent River in relation to temperature.*

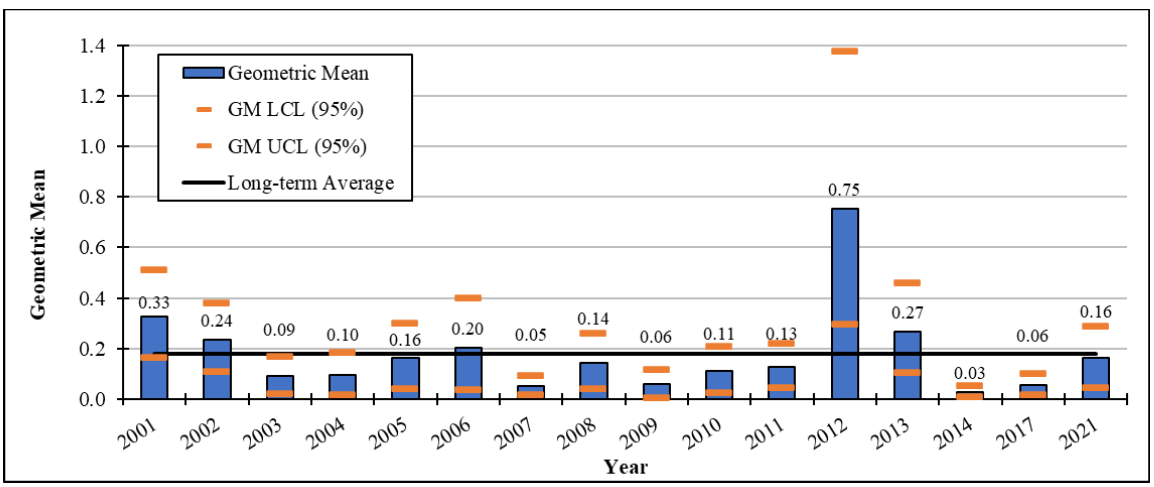

Figure 3.12. *Maryland Department of Natural Resources electrofishing survey, Patuxent River adult American Shad geometric mean (GM) with 95% confidence intervals of CPUE for sample years 2001-2021. Long-term average = 0.18 fish/min. Beginning and ending zero CPUEs were omitted from analysis.*

Hickory Shad Restoration in Three Maryland Rivers

F-57-R Segment 22 Progress Report
Reporting period January 1, 2021 to December 31, 2021

Charles P. Stence*
Ashlee N. Horne
Matthew W. Baldwin
William T. Stoker

Maryland Department of Natural Resources
Fishing and Boating Services
301 Marine Academy Drive
Stevensville, MD 21666

**Corresponding author: chuck.stence@maryland.gov*

Reporting Timeline*

This progress report will cover calendar year 2021 sub-projects one, two, three, and overall restoration progress. Elements of the project initiated in 2022 will be briefly reported on. A comprehensive progress report for 2022 will be submitted in 2023.

<u>OUTLINE</u>

1. **Need**
2. **Objective**
3. **Expected Results and Benefits**
4. **Approach**
5. **Justification to amend approach 2017**
6. **Location**
7. **2021 Sub-Project 1-** *Produce, mark and stock cultured Hickory Shad*.
    a. **Objectives**
    b. **Materials and Methods**
        i. ***Broodstock Collection***
        ii. ***Hormone Induced Ovulation***
        iii. ***Egg Culture***
        iv. ***Marking***
        v. ***Larval Stocking***
        vi. ***Early Juvenile Stocking***
        vii. ***Stocking Goals***
    c. **Results and Discussion**
        i. ***Hickory Shad Tank Spawn Production Summary***
        ii. ***Stocking Summary***
    d. **2022 Sub-Project 1 - Preliminary Results - Work In Progress**
8. **2021 Sub-Project 2** - *A. Assess the contribution of hatchery-produced fish to the resident/pre-migratory stock in the Patapsco River. B. Monitor the abundance and mortality of larval and juvenile shad using marked hatchery-produced fish.*
    a. **Objectives**
    b. **Materials and Methods**
        i. ***Catch Per Unit Effort (CPUE) and Geometric Mean***
        ii. ***Mortality and Abundance Estimates***
    c. **Results and Discussion**
        i. ***Patapsco River***
    d. **2022 Sub-Project 2 - Preliminary Results - Work In Progress**

9. **2021 Sub-Project 3 -** *Estimate the contribution of hatchery origin Hickory Shad to the adult spawning population and monitor recovery of naturally produced stocks.*
    a. **Objectives**
    b. **Materials and Methods**
        i. ***Survey Locations***
        ii. ***CPUE Analysis***
        iii. ***Origin Composition (Hatchery vs. Wild)***
        iv. ***First-time and Repeat-Spawning Compositions***
    c. **Results and Discussion**
        i. ***Patapsco River Adult Hickory Shad Spawning Stock***
            a. *Patapsco River Hickory Shad CPUE*
            b. *Patapsco River Hickory Shad Origin Composition (Hatchery vs. Wild)*
            c. *Patapsco River Hickory Shad First-Time and Repeat-Spawning Compositions*
        ii. ***Patapsco River Hickory Shad Spawning Stock Discussion***
        iii. ***Choptank River Adult Hickory Shad Spawning Stock***
            a. *Choptank River Hickory Shad CPUE*
            b. *Choptank River Hickory Shad Origin Composition (Hatchery vs. Wild)*
            c. *Choptank River Hickory Shad First-Time and Repeat-Spawning Compositions*
        iv. ***Choptank River Hickory Shad Spawning Stock Discussion***
        v. ***Patuxent River Adult Hickory Shad Spawning Stock***
            a. *Patuxent River Hickory Shad CPUE*
            b. *Patuxent River Hickory Shad Origin Composition (Hatchery vs. Wild)*
            c. *Patuxent River Hickory Shad First-Time and Repeat-Spawning Compositions*
        vi. ***Patuxent River Hickory Shad Spawning Stock Discussion***

vii. Susquehanna River (Brood Source) Hickory Shad Spawning Stock

d. 2021 Overall Restoration Progress

i. Patapsco River

ii. Choptank River

iii. Patuxent River

e. 2022 Sub-Project 3 – Preliminary Results – Work in Progress

i. Patapsco River

ii. Choptank River

iii. Patuxent River

iv. Project-wide Observations 2022

10. Literature Cited

Need

Hickory Shad *Alosa mediocris* were historically abundant in many Chesapeake Bay tributaries (O'Dell et al. 1975, 1978). Populations declined similarly to other Clupeid species during the 1970s (Minkkinen 1999). A moratorium was enacted on all Maryland Hickory Shad harvest in 1981. Some upper Chesapeake Bay tributaries have experienced a mild resurgence in Hickory Shad runs. The increased availability of Hickory Shad broodstock provided the opportunity to culture and stock this species. Few studies have been conducted on Hickory Shad. Funding obtained through Sportfish Restoration Act (F-57-R) has supported the Maryland Department of Natural Resources (department) restoration project since 1999.

Previous work conducted under F-57-R yielded new Hickory Shad spawning strategy and life history information. Many Chesapeake Bay tributaries had historical Hickory Shad runs equal to or greater than that of American Shad *Alosa sapidissima.* Since shad populations indicate evidence of density dependent spawning behavior, self-sustaining shad populations are not likely to return to tributaries without hatchery stocking. Since 1999 the Maryland Department of Natural Resources has been developing spawning, culture, marking, and stocking techniques that could restore spawning populations of Hickory Shad to target tributaries. These techniques have been continuously refined, and reintroduction of Hickory Shad to target tributaries has progressed similar to the department's American Shad restoration projects.

Objective

The overall objective for this proposed scope of work is to reintroduce self-sustaining Hickory Shad populations to the Patapsco River and continue to monitor populations in the previously stocked Choptank and Patuxent rivers. Stocking larval and juvenile hatchery-origin fish should produce adult stock that will return to spawn upon maturity. The depressed native stocks do not optimally utilize the tributary. This tributary has historically supported spawning runs.

Expected Results and Benefits

Hatchery inputs are intended to provide adult spawning stock that will produce self-sustaining populations in the target tributary. These fish have tremendous value for stock assessment purposes at the larval, juvenile, and adult life stages, since all stocked shad receive an otolith mark. Larval and early juvenile otolith marking is the primary identification method for hatchery reared Hickory Shad. Natural spawn culture techniques allow for the production of large numbers of larval and juvenile shad for stocking and assessment efforts.

Upper Bay shad populations, specifically the Susquehanna River, currently support Hickory Shad populations that sustain active catch and release recreational fishing. Restoring Hickory Shad stocks to tributaries that historically supported runs will increase fishing opportunities for anglers. An indirect benefit of restoring shad populations to self-sustainable levels is the increased prey availability provided by both juvenile and adult shad for larger, more economically important recreational species such as Striped Bass *Morone saxatilis*, Bluefish *Pomatomus saltatrix*, and Weakfish *Cynoscion regalis*.

Approach

The Maryland Department of Natural Resources American Shad hatchery-based restoration project incorporated Hickory Shad into the project in 1996. The project continued over the next three years through various short-term funding sources. In 1998 it was determined that a long-term funding source would be required, since it would take years of additional stocking and assessment to successfully support restoration. Federal Aid in Sport Fish Restoration funds were utilized to conduct this long-term effort.

The project consists of three sub-projects:

1. *Produce, mark, and stock cultured Hickory Shad.*
2. *A. Assess the contribution of hatchery-produced fish on the resident/pre-migratory stock in the Patapsco River.*
   B. Monitor the abundance and mortality of larval and juvenile shad using marked hatchery-produced fish.
3. *Analyze the contribution of hatchery-origin Hickory Shad to the adult spawning population and monitor the recovery of naturally produced stocks.*

Justification to amend approach 2017

Hickory Shad populations are considered restored when wild fish comprise 75% of the population for three consecutive years. Hickory Shad populations in the Patuxent River were deemed restored in 2007, and the Choptank River was deemed self-sustaining in 2014. Both rivers were original target tributaries with stocking beginning in 1996. The Patuxent River watershed is heavily urban-impacted but has been the subject of numerous mitigation efforts due to its designation as a targeted watershed (e.g., sewage treatment upgrades). The Choptank River watershed is rural-impacted by agricultural activities and low urban development. Choptank River efforts included the tributary Tuckahoe Creek. Both rivers will be sampled on a three-year rotation to maintain adult trend data.

Hickory Shad were stocked into the Choptank River for the final time in 2014 and a tributary needed to be identified for redirected stocking efforts. The program shifted focus in 2015 and conducted exploratory surveys on the Pocomoke River, Marshyhope Creek, Chester River, Sassafras River, Elk River, Northeast River, and the Patapsco River for baseline Hickory Shad data to assess restoration needs. Future target tributaries would be chosen based on historical data, angler access, and suitable Hickory Shad spawning and nursery habitat. Restoration efforts are now directed to the Patapsco River because of this assessment. The main-stem Patapsco River is 63 km long and heavily urban impacted. With its origins in Carroll County and Howard County farmland, the Patapsco River quickly transitions to urban sprawl and the tidal portion creates the Baltimore Harbor. The river is considered impacted by industry and dams. A Baltimore City drinking reservoir is in its north branch. Union Dam and Simkins Dam were removed in 2010 and 2011 respectively, and the lowermost dam, Bloede Dam, was removed in 2018. Once the Bloede Dam was removed, anadromous species had access to more than 100 km of the river and its many tributaries. Only one dam (Daniels Dam) remains in the main-stem as of 2018.

The Patapsco River was the subject of a separate restoration effort from 2012-2017 known as the Patapsco River Shad and Herring Restoration Project. American Shad and Hickory Shad were stocked by the Maryland Department of Natural Resources. The United States Fish and Wildlife Service Maryland Fish and Wildlife Conservation Office (USFWS-MDFWCO) was subcontracted to perform assessments. This project was funded by a six-year grant from the Maryland Department of Transportation (MDOT) through the Maryland Port Authority (MPA).

Recent Patapsco River fish passage implementation has reopened historical spawning habitat for anadromous species such as American Shad, Hickory Shad, Blueback Herring *Alosa aestivalis* and Alewife *Alosa pseudoharengus*. This tributary historically supported spawning stocks, and reintroduction through hatchery inputs could indicate positive impacts. Restoring Hickory Shad populations takes many years, and it was determined by project biologists that it would be advantageous to continue the MPA funded project, given the head start the river had received. Several years of additional inputs would be necessary to determine whether hatchery stockings were successful.

The USFWS Wildlife and Sport Fish Restoration F-57-R grant proposal was amended in 2017 to continue stocking and assessment on the Patapsco River that was previously funded through the MPA grant.

Location

Stocking restoration efforts will focus on the Patapsco River (Figure 1.1). The Patapsco River watershed is heavily impacted by urban, commercial, and industrial development, but has been the subject of numerous mitigation efforts (e.g., sewage treatment upgrades, habit improvements, fish passage, and dam removal).

Previously stocked tributaries will continue to be monitored periodically. The Choptank River watershed (Figure 1.2) is rural-impacted by agricultural activities and low urban development. The Patuxent River watershed (Figure 1.3) is heavily urban-impacted but has been the subject of numerous mitigation efforts due to its designation as a targeted watershed (e.g., sewage treatment upgrades).

2021 Sub-Project 1.

Objectives

"Produce, mark, and stock cultured Hickory Shad."

Materials and Methods

The following materials and methods indicate standard operating procedures during a typical Hickory Shad production season.

Broodstock Collection

Prior to 2005, Hickory Shad broodstock were collected by hook and line, either immediately downstream of Deer Creek or at Shure's Landing, near the base of Conowingo Dam (Figure 1.4). The Maryland Department of Natural Resources staff transitioned to boat electrofishing in 2005 to collect Hickory Shad broodstock. The sample area was located along the western shore of the Susquehanna River, from just downstream of Deer Creek at Rock Run Mill down to Lapidum boat ramp in the Susquehanna State Park (Figure 1.4). Electrofishing was used for its ability to more efficiently collect Hickory Shad compared to hook and line. Electrofishing for Hickory Shad broodstock requires less project staff and reduces handling stress. During broodstock collection, immobilized Hickory Shad were netted and placed into the electrofishing boat's live well (220 L). The live well water was flow-through and oxygenated.

Hormone Induced Ovulation

Injections of a synthetic peptide analogue of salmon gonadotropin-releasing hormone (sGnRHa) were used to stimulate the pituitary to induce sexual maturation. The use of sGnRHa induces gonadal maturation, ovulation, and spawning (Mylonas et al. 1995). In accordance with the Investigational New Animal Drug (INAD) Permit (INAD #13-298-21-006), Maryland Department of Natural Resources purchased vials of liquid hormone for ovulation induction. The hormone is sold under the product name Ovaplant-L® and is produced by Syndel (Ferndale, WA).

After capture, Hickory Shad were transferred from the electroshocking boat live well to a 385 L holding tank, which was set up on land at the collection site ramp. Hickory Shad were temporarily anesthetized within the holding tank to minimize handling stress. AQUI-S20E (Aqua

Tactics, Kirkland, WA 98034 USA) was administered to the tank water as the sedative, in accordance with the INAD Permit (INAD #11-741-21-062H). Once anesthetized, lengths and weights were recorded for all fish, and both males and females received an intramuscular (IM) injection of Ovaplant-L®. Ovaplant-L® was drawn into a 1 ml Luer-lock syringe with an 18 ga needle. Once the Ovaplant-L® was in the syringe, the needle was switched to a smaller 22 ga needle for injection into the dorsal musculature. Smaller syringes were used to prevent the hormone from seeping out of the injection site.

After injection, Hickory Shad were revived in the circular flow, insulated 3,785 L tanks at 4.0-6.0 ppt salinity, onboard the transport vehicle and transported to Joseph Manning Hatchery (Figure 1.5). Dissolved oxygen (DO) was continuously monitored and regulated to saturation (approximately 10.0 mg/L) with a Point Four oxygen monitoring system (Coquitlam, BC, V3K 6X9, Canada).

Egg Culture

At Joseph Manning Hatchery, broodstock were netted from the transport tanks and transferred into 3.05 m diameter natural spawn tank systems. A sex ratio of approximately 3:2 males: females is preferable in natural spawn systems, but there are times when males are not sufficiently available to meet this ratio. Salinity was maintained at 2.0 ppt. A 25% water exchange was performed each day to maintain adequate water quality. Fish spawned naturally and eggs flowed into an egg collection box through an airlift system.

Eggs were volumetrically measured (ml) and fertilization was determined 24 hours post spawn. Eggs were placed into modified McDonald hatching jars supplied by approximately 2.0 L/min water flow. Prophylactic treatments of formalin were administered in the morning and afternoon to control fungi. Eggs were exposed to a 600:1 treatment of formalin for approximately 17 min. Hickory Shad eggs began hatching at day four. To stimulate a simultaneous hatch, jars were removed from the egg bank, placed outdoors in sunlight for ten minutes and stirred vigorously. The rapid temperature change, lower oxygen content, concentrated hormonal influence and agitation stimulated simultaneous hatching. Hatching jars were placed on benches beside 1.5 m (1,800 L) circular flow-through larval tanks that allowed water and larvae to flow from the hatching jars to the flow-through tanks. Water was supplied at approximately 2.0 L/min.

Hickory Shad feed on rotifers that are difficult to culture in the hatchery. Therefore, Hickory Shad larvae were marked and stocked into hatchery ponds or target tributaries prior to first feeding (<six days age). Eggs were volumetrically measured and counted while assessing fertilization rates prior to hatching for numeration.

Marking

All fish stocked into the target tributary were given an oxytetracycline (OTC) mark to identify recaptured fish as hatchery origin that will still be visible as adults. Oxytetracycline marking is a valuable assessment tool to determine hatchery origin, larval survival, and juvenile abundance and mortality estimates. A current veterinarian-client-patient-relationship (VCPR) exists between Joseph Manning Hatchery and Dr. Cindy P. Driscoll, (State Fish & Wildlife Veterinarian, Fish & Wildlife Health Program, Maryland Department of Natural Resources, Cooperative Oxford Lab, 904 South Morris Street, Oxford, MD 21654). Oxytetracycline is used under a prescription to mark all larvae that leave the hatchery. Larval marks were produced by immersion in a 300-ppm buffered OTC bath for six hours. Due to its low pH, OTC must be buffered with sodium bicarbonate (NaHCO3) to minimize mortality. Dissolved oxygen content was monitored and regulated (>5.0 ppm) by a carbon air stone connected to a liquid oxygen delivery system. All water used at Joseph Manning Hatchery for OTC marking was softened before use (Culligan ion exchange system). Reliable marking can only take place in water with hardness below 20 mg/L and water hardness at Joseph Manning Hatchery routinely exceeds 200 mg/L. Samples analyzed from each group of OTC marked fish indicated that all fish stocked were successfully marked. Marks were verified by viewing larval otoliths with an ultraviolet microscope (Zeiss Axioskop 20). Hickory Shad intended for larval stocking were given an immersion OTC mark at day one after hatch and Hickory Shad intended for early juvenile stocking were given OTC immersion marks at day one and day three after hatch.

Larval Stocking

Larval stocking was accomplished by placing marked larvae into boxes designed for shipping tropical fish. These containers consisted of a cardboard box outer shell, an inner insulating foam box, a black plastic trash bag to reduce stress of bright sunlight, and a double thickness plastic fish transport bag. Larval culture tanks were drawn down to crowd the fish. Larvae were scooped out of the tanks and placed into the shipping bags/boxes, which were supplemented with salt (1.0 ppt) to mitigate stress. Each bag was filled with pure oxygen and sealed with electrical tape. Boxes were transported to the Patapsco River and the bags were placed into the water to temperature acclimate. Once acclimated, the bags were opened, and river water was slowly introduced to further acclimate larvae to river water chemistry. Bags were emptied into flowing water to minimize predation.

Early Juvenile Stocking

After marking, larvae were stocked into hatchery ponds and cultured for approximately thirty days. Joseph Manning Hatchery provided grow out ponds to hold fish for the restoration effort (Figure 1.5). The decision to harvest juveniles from the pond was based on zooplankton density. Food availability was evaluated with a plankton net. Early juveniles were removed from culture ponds when food availability declined substantially.

Juvenile fish tend to stress easily and direct netting from hatchery ponds into transport tanks results in unnecessary mortality. To prevent losses, juvenile fish were concentrated within the grow out ponds, using a seine net 61.0 m long, 3.1 m deep, with 6.4 mm stretch mesh. They were effectively removed by scooping the concentrated schools of fish out with buckets and were poured into the transport tanks. A small one-horsepower water pump was used to create current within the seine net to orient shad into the water flow. This currently serves two purposes. Shad concentrate into dense schools at the water's surface, which allows them to be easily bucketed, and it separates the fish from algae and detritus. The use of the pump has resulted in high early juvenile survival rates, due to the reduction of algae and detritus in the transport tanks. Early juveniles were transported in fish hauling tanks at 3.0-5.0 ppt salinity and saturated DO to mitigate stress.

A one-horsepower trash pump was carried on the stocking truck to temper juvenile shad before stocking. Fish were tempered until temperature and salinity in the tank were within one degree Celsius and 1.0 ppt salinity of the river value. Although this adds a considerable amount of time that fish are aboard the transport truck, this procedure increases the survival of early juvenile stocked shad by reducing stress. Juvenile stocking was accomplished by quick-dumping marked juveniles through a quick release drain hose, with a diameter of 15.0 cm, directly from the transport vehicle into the river.

Stocking Goals

The project developed stocking goals for the Patapsco River are based on previous experience with larval survival. Stocking multiple life stages gives fisheries managers the ability to assess larval survival and estimate juvenile mortality and abundance of each life stage. Larval stocked fish can efficiently contribute large numbers of juveniles if survival is high. The stocking goal for the Patapsco River was set at 500,000 larvae (Table 1.2).

Fish stocked as early juveniles survive extremely well and are young enough to successfully imprint to the target tributary. Stocking early juveniles can also mitigate the impacts of poor larval survival since post-stocking survival of this life stage is high. The project developed stocking goals based on past experience with juvenile survival. The stocking goal for the Patapsco River was set at 75,000 early juveniles (Table 1.2).

Results and Discussion

Hickory Shad Strip Spawn Production Summary

Hickory Shad were collected from the Susquehanna River spawning area when temperatures reached optimal spawning temperature, around 17°C. Maryland Department of Natural Resources electrofished for Hickory Shad broodstock on 04/19/2021 and 04/22/2021. During these two visits, biologists captured 68 adult Hickory Shad, consisting of 32 males and 36 females (Table 1.1). These fish produced 2,615,140 eggs, with an overall fertilization rate of 34.5%, resulting in 902,223 viable eggs (Table 1.3, figure 1.6). Lower than average egg collection is attributed to staffing constraints from COVID-19. Lack of personnel only allowed for two trips to the Susquehanna River for Hickory Shad broodstock collection.

Stocking Summary

In 2021, Hickory Shad larval and juvenile stocking goals for the Patapsco River were not met. In total, 435,000 larval stage and 50,000 early juvenile stage Hickory Shad were stocked (Table 1.2). Lack of personnel due to COVID-19 restraints on Hickory Shad broodstock collection and low fertilization rates were attributed to not meeting stocking goals.

Hickory Shad were stocked as early juveniles at the Rt. 648 Bridge and Southwest Area Park (SWAP) boat ramp on the Patapsco River (Figure 1.7). Stocking occurred three times throughout May of 2021, with 485,000 Hickory Shad released (Table 1.6).

A summary of all Hickory Shad stocked into the Patapsco River can be found in Table 1.5. Previous Hickory Shad stocking production summaries for all tributaries are contained in Tables 1.7 through 1.11, for reference.

In the early years of restoration efforts, larvae and late juveniles (90-day old) were the only life stages stocked into the target tributaries. In 2001, early juveniles (30-day old) were cultured in hatchery ponds and stocked into the Patuxent River. In 2002, juveniles were cultured and stocked into the Patuxent River, Choptank River, and Marshyhope Creek. Hickory Shad stocking was suspended in the Patuxent River in 2008 and Marshyhope Creek in 2010 to focus project resources towards stocking the Choptank River. In 2014, stocking Hickory Shad concluded on the Choptank River. The stocking effort resulted in the formation of a stable population.

In 2017, it was determined that Hickory Shad stocking efforts would focus on the Patapsco River. The Patapsco River Shad and Herring Restoration Project grant funded by the Maryland Port Administration (MPA) from 2012-2017 had ended. The Maryland Department of Natural Resources had been providing juvenile Hickory Shad for this short-term project. Also included in the decision was the removal of Bloede Dam in 2018. The removed dam opened miles of potential spawning habitat for returning Hickory Shad adults. This coupled with recent survey results, historic data, angling opportunities, nursery habitat, and suitable habitat for Hickory Shad spawning, it was determined it would be advantageous to continue the project efforts, given the head start the river had received.

2022 Sub-Project 1 – Preliminary Results – Work in Progress

Analysis of the data for 2022 is currently in progress. Adult Hickory Shad used as broodstock were caught via electrofishing on the Susquehanna River on 26 April and 4 May 2022. A total of 259 Hickory Shad (113 females and 146 males) were used in spawning culture tanks at Joseph Manning Hatchery. In 2022, approximately 515,000 larvae and 50,000 early juveniles were stocked into the Patapsco River. A complete analysis and summary of the data collected in 2022 to produce, mark, and stock cultured Hickory Shad in the Patapsco River will appear in the next F-57-R Segment 2023 Progress Report.

2021 Sub-Project 2

Objectives

Restoration stocking of Hickory Shad in the Patapsco River began in 2012 and the summer juvenile seine survey was initiated in 2013. The survey's goal is to collect juvenile Hickory Shad to determine the success of the stocking program. From 2013 to 2017, the MPA funded grant supported restoration activities in the Patapsco River and all fisheries monitoring was conducted by USFWS Maryland Fish and Wildlife Conservation Office (MDFWCO). In 2018, the Patapsco River was added to the Maryland Department of Natural Resources' Wildlife and Sport Fish Restoration grant to continue the project for stocking and assessment.

Two quantifiable population variables were identified to evaluate restoration progression of juvenile Hickory Shad in the targeted rivers.

A. "Assess the contribution of hatchery-produced fish to the resident/pre-migratory stock in the Choptank River and Patapsco River."

B. "Monitor the abundance and mortality of larval and juvenile shad using marked hatchery-produced fish".

Survey Location

The Patapsco River watershed is agriculturally impacted in headwaters and heavily urban-impacted in the lower reaches but has been the subject of numerous mitigation efforts due to its designation as a targeted watershed (e.g., sewage treatment upgrades, dam removal). It is likely that shad utilize tidal areas downstream of the seine sites, but increasing river width and depth reduces capture efficiency with a 30.4 m seine net. Anecdotal evidence indicates that substantial spawning habitat and fish movement also exists upstream of currently sampled stream reaches, but sampling habitat is limited by boat access. Juvenile seine survey sampling is conducted upstream from the Light Rail bridge downstream to just below the SouthWest area Park boat ramp (Figure 2.1).

Materials and Methods

The Patapsco River was sampled with a seine 30.5 m long, 1.2 m deep, with 6.4 mm stretch mesh. The net was deployed by hand from shore into deep water and back to shore at established seine sites. Juvenile Hickory Shad were collected from the seine, placed into plastic bags, labeled, and stored on ice. Upon return to the lab, the samples were frozen to -9 °C. All bycatch species data were recorded.

Sagittal otoliths are removed from each Hickory Shad captured from the Patapsco River. Otoliths are mounted on 76.2 mm x 25.4 mm glass slides with Crystalbond 509 (Aremco Products, Ossining, NY).

Mounted otoliths are lightly ground on 600 grit silicon carbide wet sandpaper and viewed under an LED epifluorescent light at 400X magnification at 50-100 watts with a Zeiss Axioskop 20 microscope. The presence and location of an OTC mark epifluorescence is recorded. Epifluorescence is a technique in which transmitted light in the wavelength of 490-515 nm is allowed to strike the specimen. The specimen then absorbs this light energy and reflects light of a longer wavelength back through the microscope objective.

Catch Per Unit Effort and Geometric Mean

The juvenile index is described by calculation of a catch per unit effort (CPUE). It is defined as the number of captured juvenile Hickory Shad divided by the number of seine hauls completed. Indices of relative abundance are presented as the geometric mean (GM) catch per haul. The GM has been adopted by the Atlantic States Marine Fisheries Commission (ASMFC) as the preferred index of relative abundance. The GM is a more precise statistical tool for handling these data because it is not as sensitive to a single large sample value. Hickory Shad, like American Shad, are schooling fish and subject to these types of captures with a large seine net.

Mortality and Abundance Estimates

In addition to providing future broodstock, juvenile stocking is valuable as a pre-migratory stock assessment tool through use of a multiple marking technique. Hatchery stocking is also used to evaluate the efficacy of stocking different life stages and the eventual impact to the returning adult population.

There are assumptions made when using these types of estimates as described by (Ricker 1975):

- The marked fish suffer the same natural mortality as the unmarked fish.
- The marked fish are as vulnerable to capture as are the unmarked fish.
- The marked fish do not lose their mark.
- The marked fish become randomly mixed with the unmarked; or the distribution of fishing effort (in subsequent sampling) is proportional to the number of fish present in different parts of the body of water.
- All marks are recognized and reported on recovery.
- There is only a negligible amount of recruitment to the catchable population during the time recoveries are being made.

Estimates of juvenile abundance, mortality, and survival were derived from the following formulas.

Larval survival to juvenile stocking was calculated by (Ricker 1975):

$$S_1 = \frac{(R_{12})\, M_2}{(M_1)\, R_{22}}$$

$$\text{Variance } S_1 = {S_1}^2\{\left(\frac{1}{R_{12}}\right) + \left(\frac{1}{R_{22}}\right) - \left(\frac{1}{M_1}\right) - \left(\frac{1}{M_2}\right)\}$$

where M_1 is the number of fish marked at the start of the first interval (larval stocking), M_2 is the number of fish marked at the start of the second interval (early juvenile stocking), R_{12} is recaptures of larval marked fish in the second interval (after early juvenile stocking), R_{22} is recaptures of early juvenile interval marked fish in the second interval or (after early juvenile stocking), and S_1 is the survival rate of larvae during interval one (from the time of marking larvae in interval one to time of marking early juveniles in interval two).

Instantaneous mortality is derived from survival estimates and is used in conjunction with stocking data to calculate juvenile abundance:

$$Z = \frac{-\ ln\ ln\ S_1}{interval}$$

Where Z is the instantaneous mortality rate and S_1 is the survival rate.

Abundance of juvenile shad prior to out migration was also calculated by Chapman's modification to the Peterson estimate (Ricker 1975):

$$N = \frac{\{(C+1)(M+1)\}}{R+1}$$

where N is the population estimate, M is the number of marked fish stocked, C is the number of fish examined for tags (total captures), and R is the number of marked fish that were recaptured (larval or early juveniles).

From Ricker (1975): Calculation of 95% confidence limits based on sampling error using the number of recaptures in conjunction with Poisson distribution approximation.

Chapman's modification (1951):

$$N^* = \frac{\{(C+1)(M+1)\}}{(R+1)}$$

Where R_1 is from Pearson's formula to calculate upper and lower limits:

$$R_1 = R + 1.92 \pm 1.960\sqrt{R + 1.0}$$

Results and Discussion

Patapsco River

No juvenile Hickory Shad were collected by seine from the Patapsco River in 2021. Juvenile Hickory Shad are difficult to recapture with seine gear. Very few Hickory Shad have been captured over the years in previous restoration work. Juveniles are generally larger in size than American Shad, well developed, and have the ability to avoid sampling gear. Until juvenile Hickory Shad are captured in sufficient numbers, calculation of geometric mean and mortality and abundance estimates are not possible. Currently, adult Hickory Shad assessment is a better indicator of restoration progress (Sub-Project 3).

2022 Sub-Project 2 - Preliminary Results - Work In Progress

Juvenile Hickory Shad sampling on the Patapsco River will resume in August 2022. A comprehensive analysis of GM and mortality and abundance estimates for 2022 will be conveyed in the 2023 F-57-R report.

2021 Sub-Project 3

Objectives

Choptank River and Patuxent River spawning ground surveys commenced in 1999 to collect adult Hickory Shad. Restorative stocking of Hickory Shad began in 1996 on these targeted rivers. Patuxent River composition of wild adults in 2008 indicated a stable population and wild adults exceeded 80% for three consecutive years, so hatchery stocking was suspended. The composition of wild adults in the Choptank River was also stable and exceeded 75% for three consecutive years, so hatchery stocking was suspended in 2015. Both the Choptank and Patuxent rivers are monitored on a three-year rotation to ensure that the populations remain stable. The Patapsco River was considered for possible restoration in 2013 and added as a target tributary to restoration efforts in 2018. The 2018 removal of Bloede Dam opened the river at its most downstream blockage and reopened access for shad and herring for 104 km of the Patapsco River and its tributaries.

Three quantifiable population variables were identified to evaluate restoration progression and relative abundance of adult Hickory Shad spawning stocks in the targeted tributaries. A fourth objective is to evaluate the population status of Hickory Shad spawning stocks from brood source tributaries.

1) *Estimate CPUE in each target river using geometric mean.*
2) *Estimate the contribution of hatchery produced fish to the adult spawning populations.*
3) *Estimate the frequency of first-time and repeat spawning.*
4) *Monitor the viability of the Susquehanna River as a Hickory Shad brood source through analysis of first-time and repeat spawning compositions.*

Methods and Materials

Survey Locations

Electrofishing surveys were conducted on the Patapsco, Choptank, and Patuxent rivers in 2021 (Figure 1.1; Figure 1.2; Figure 1.3). Electrofishing surveys on the Patapsco River were conducted by both this project and the Maryland Department of Natural Resources Maryland Biological Stream Survey (MBSS). This project sampled the electrofishing reach in the tidal portion of the Patapsco River starting at the wastewater treatment plant located just west of Rte. 648 (Baltimore Annapolis Road) and ending approximately 1.43 km downstream (Table 3.1; Figure 3.1). The MBSS staff sampled the Patapsco River by electrofishing in the upper, non-tidal portion of the study area (Table 3.1, Figure 3.2). Site 591 is from 200 m above Deep Run to 200 m below Stony Run, 0.63 km long. Site 592 runs from Route 1, 0.59 km downstream to the Elkridge Furnace Inn. Site 595 starts 0.30 km upstream of the Ilchester Road bridge crossing and runs downstream to below the bridge and ends at a riffle. Site 596 starts 0.50 km upstream of the

Route 144 bridge crossing and continues downstream for 0.25 km. Site 597 is in the tailrace to Daniel's Dam.

Electrofishing on the Choptank River started 1.80 km upstream from the Route 313 Bridge in Greensboro, Maryland and continued downstream to the bridge crossing (Table 3.1, Figure 3.3). In the Patuxent River, electrofishing sampling started 0.20 km upstream of the canoe launch near the Patuxent River 4-H Center and continued downstream 4.00 km to the wastewater treatment plant located north of the intersection of Bayard Road and Sands Road (4500 block of Sands Road; Table 3.1, Figure 3.4).

Patapsco River adult sampling was conducted by the MBSS using a 4.2 m inflatable raft with a 2,000-watt generator powering a Smith-Root 1.5 KVA electrofisher in the upper, non-tidal portion of the study area with one person netting and one person piloting the boat. This project used a Smith-Root electrofishing boat SR18-E (Vancouver, WA) and accomplished each survey with one person piloting the boat and two people netting from the bow.

Each river section was sampled in an upstream to downstream direction with constant voltage applied to the entire reach. Total pedal time (s) was recorded for calculating CPUE. Water temperature (°C), dissolved oxygen (mg/L), and conductivity (µS/cm) were obtained using a YSI ProSolo water quality meter (Yellow Springs, OH). Secchi depth (cm) was also recorded.

It is likely that shad utilize tidal freshwater areas downstream of our collection sites, but increasing river width, depth, and increased conductivity reduces capture efficiency with electrofishing gear. Additionally, anecdotal evidence suggests that substantial spawning habitat and fish movement also exists upstream of currently sampled stream reaches, but sampling upstream habitat is limited by electrofishing boat access (Figure 3.1).

A sub-sample of 20 Hickory Shad per day was collected for age, origin composition, and spawning attempt analysis. All other observed shad were counted to calculate CPUE. Fish collected were measured for total length (TL; mm), fork length (FL; mm) and sex was determined. Scale samples were taken for age estimation and spawning mark interpretations. Otoliths were extracted to identify hatchery OTC marks. Shad scales were cleaned and mounted between glass slides. Age was estimated, and spawning attempts were counted using a microfiche reader. Two biologists interpreted the scales independently. In cases where readers disagreed on an age estimate, a consensus age was used as the final age. Scales were aged using methods described by Cating (1953). Otoliths were processed as described in Section 2.

CPUE Analysis

To standardize data collection, bracketing CPUE data began in 2011. With this methodology, sampling begins each year before Hickory Shad return and continue until zero Hickory Shad are captured at the end of the spawning run. This ensures that biologists sampled the area throughout the entire duration of the spawning run and reduces sampling bias.

The GM has been adopted by this project as the preferred index of relative abundance to evaluate stock status and restoration progress. The GM is calculated from the $\log_e(x+1)$ transformation, where x is the number of Hickory Shad encountered per shock time (min). Beginning and ending zeros are omitted from the analysis. One is added to all catches in order to transform zero catches, because the log of zero does not exist (Ricker 1975). Since the $\log_e$-transformation stabilizes the variance of catches (Richards 1992), the GM estimate is more precise than the arithmetic mean (AM) and is not as sensitive to a single large sample value. The GM is almost always lower than the AM (Ricker 1975). The GM was calculated independently for each of the five sample sites.

Origin Composition (Hatchery vs. Wild)

The percentage of hatchery versus wild-origin Hickory Shad adults sampled on the spawning grounds provides insight into the impact to the adult population of stocking larval and juvenile shad. The presence of adult hatchery-origin fish on the spawning grounds early in restoration may stimulate annual natural production. As restoration efforts continue, a transition from a high proportion of hatchery origin fish to a high proportion of wild fish year after year indicates natural reproduction events leading to successful recruitment to the spawning population. Observation of changes from mostly hatchery contribution to a population dominated by wild origin adults is a good indication of whether hatchery contributions are having a substantial effect upon the adult spawning stock population.

First-time and Repeat-Spawning Compositions

A third estimator uses analysis of first-time and repeat-spawning compositions. The number of spawning migrations by an individual fish can be determined through examination of

Hickory Shad scales. The composition of first-time and repeat-spawn frequency observed on the spawning grounds provides additional insight into population stability and recruitment. Low levels of first-time spawners may indicate problems associated with juvenile recruitment to the adult stock, or poor spawning success. Conversely, a high level of first-time spawners usually indicates successful recruitment of individual year classes to the adult spawning stock. A substantial contribution of first-time spawners and several repeat-spawning classes utilizing the spawning grounds year after year is indicative of a stable spawning stock.

Results and Discussion

Patapsco River Adult Hickory Shad Spawning Stock

The Patapsco River was sampled by this project for 13 weeks from 18 March to 9 June 2021. A total of 140 adult Hickory Shad were encountered and 114 were retained for length, sex, otolith, and scale analysis. Water temperature ranged from 10.9°C to 27.1°C (Figure 3.5). Maryland Biological Stream Survey sampled five sites in the upper non-tidal portion of the Patapsco River for 11 weeks from 11 March to 19 March 2021. A total of seventeen Hickory Shad were encountered with four captured at Site 591 and 13 at Site 592. Four fish were retained for length, sex, otolith, and scale analysis. No Hickory Shad were encountered at Sites 595, 596 or 597.

Patapsco River Hickory Shad CPUE

Preliminary calculations indicate that the abundance of Hickory Shad in the Patapsco River is increasing (Figure 3.6A). The mean relative abundance (GM) for Hickory Shad in the wastewater treatment plant to I-895 reach was calculated to be 0.40, which is the highest calculated since the project's inception in 2013. The GM for Sites 591 and 592 were calculated at 0.02 and 0.11, respectively. These GMs are the lowest calculated for these sites since 2013. Site 593 has not been sampled since 2018 when Bloede Dam was removed (Figure 3.6B-D). To date, no Hickory Shad have been captured at the sites upstream of the old Bloede Dam site.

Patapsco River Hickory Shad Origin Composition (Hatchery vs. Wild)

A total of 114 Hickory Shad samples were collected from the 2021 electrofishing survey on the Patapsco River. Origin was determined on 110 of the samples by examining OTC marks (Table 3.2). Male Hickory Shad were comprised of 18 larval origin (28%), nine early juvenile origin (14%) and 38 wild origin (58%). Among female Hickory Shad, the samples comprised 5 larval origin (11%), five early juvenile origin (11%) and 35 wild origin (78%). Overall, the percentage of wild Hickory Shad captured in 2021 (66%) has increased from 2018 and 2019 in which wild fish comprised of 47% and 50%, respectively.

Patapsco River Hickory Shad First-Time and Repeat-Spawning Compositions

All 114 Hickory Shad scale samples collected were successfully analyzed and used to determine the annual spawning attempt composition. The 2021 sample population consisted of 83% first-time spawners, 11% second-time spawners, and 6% third-time spawners (Table 3.3).

Patapsco River Hickory Shad Spawning Stock Discussion

Survey results for 2021 indicate that the Patapsco River supports a wild Hickory Shad population and hatchery inputs are supplementing the stock. Overall, wild Hickory Shad make up 66% of the population on the Patapsco River (Figure 3.7) an increase over 2018 and 2019 wild populations. Additional years of sampling will determine if this trend continues. For the Hickory Shad population to be considered recovered on the Patapsco River, the proportion of wild fish must exceed 75% for three consecutive years.

The majority of Hickory Shad sampled in 2021 were first-time spawners (83%; Table 3.3). Ideally, wild populations would have more fish returning from additional repeat spawning groups. The 2017 and 2018-year classes dominated the sampled Hickory Shad with most of them being first-time spawners (Table 3.2). This influx of young fish may dilute the percentage of older repeat spawners. As the 2017 and 2018-year classes continue to return, the percentage of fish having spawned multiple times should increase.

Hatchery stocking success is highly variable between stocking years. Gear avoidance exhibited by the fast-growing juvenile Hickory Shad eliminates the ability to evaluate stocking success the summer following stocking. By examining adult Hickory Shad otoliths and scales, stocking success can be determined when stocking phases (larval, early juvenile) from individual age classes are present or absent. All stocking phases were present in 2021 for each age class.

This evidence of successful stocking is positive when considering the Patapsco River is prone to rapidly rising and falling water level during spring rains when larval and young Hickory Shad are present in the river system.

The Hickory Shad GM on the Patapsco River continues to increase at the Rt. 648 to I895 site while the MBSS sites saw a decline in 2021 (Figure 3.6). Annual variations in GM analysis make it difficult to determine how much the stock is increasing. With the removal of Bloede Dam in September 2018, the morphology of the river and consequently the preferred habitat of Hickory Shad could change. Additional years of hatchery inputs and stock assessments on the Patapsco River will present a clearer representation of the population recovery.

Choptank River Adult Hickory Shad Spawning Stock

Three hundred thirty-seven Hickory Shad were observed in the Choptank River in 2021, of which 142 were retained for length, sex, and scale analysis. Surveys were conducted from 4 March to 1 June when water temperatures were between 7.6°C and 19.6°C (Figure 3.8). The majority (66%) of the Hickory Shad were observed in the month of April. Once water temperatures started rising in May, observations of Hickory Shad fell.

Starting in 2011, the department implemented a protocol that requires CPUE zeros at the beginning and end of the Hickory Shad survey season to better understand how long fish remain on the spawning grounds each year. In 2021, beginning and ending zeros were established and the entire spawning run was sampled.

Choptank River Hickory Shad CPUE

During the eleven weeks from 4 March to 1 June 2021 when Hickory Shad were surveyed on the Choptank River, the mean relative abundance (GM) was calculated at 0.65 fish/min (Figure 3.9). The long-term average for the time series (2001-2021) is 0.78 fish/min average.

Choptank River Hickory Shad Origin Composition (Hatchery vs. Wild)

Hickory Shad captured in the 2021 electrofishing survey on the Choptank River were not examined for hatchery or wild origin. Since Hickory Shad are considered restored in the Choptank River, no stocking occurs and there is no need to examine otoliths.

Choptank River Hickory Shad First-Time and Repeat-Spawning Compositions

One hundred forty-two Hickory Shad scale samples were collected in 2021 and all of them were successfully analyzed to determine the annual spawning attempt composition. The 2021 sample population consisted of 58% first-time spawners, 16% second-time spawners, 18% third-time spawners, 6% fourth-time spawners and 1% fifth-time spawners (Table 3.4).

Choptank River Hickory Shad Spawning Stock Discussion

Data analysis from the Hickory Shad adult recapture survey on the Choptank River indicates that wild contributions steadily increased each year, from a low of 26% in 2001 to a high of 75% in 2014. Wild contribution exceeded 75% since 2011. First-time spawners now substantially contribute to the spawning population (Table 3.4) and the relative abundance estimates vary without trend since CPUE was standardized (Figure 3.9). This static pattern of relative abundance and spawning attempt data has continued since 2010, therefore the Choptank River is considered a self-sustaining population.

Examination of the first-time and repeat-spawning data can be used to evaluate stability or instability in a spawning population and can aid in the prediction of a stock decline or expansion. A stable Hickory Shad spawning population consists of a substantial contribution from several spawning classes. However, there are several factors that can impart variability in these distributions, including maturity schedules of males (3-4 years) and females (4-5 years), timing of the spawning run, skipped spawning (non-annual), annual recruitment of wild fish, number of fish stocked annually, and recruitment of stocked fish. It may be possible to remove some of the variability from these distributions by evaluating male and female distributions separately, but there are already small sample size concerns when combining the males and females in these distributions. This is especially true when assessing fish making their fifth and sixth spawning attempt. There are rarely sample sizes of five fish in these categories, which is required to evaluate these distributions statistically (i.e., chi-square analysis). Although the sample sizes of Hickory Shad collected from the Choptank River are larger than those collected from the Patuxent River, they are still too small, which leads to uninformative gaps in the time series.

Patuxent River Adult Hickory Shad Spawning Stock

Two hundred one Hickory Shad were observed on the Patuxent River in 2021 of which 79 were retained for length, sex, and scale analysis. Surveys were conducted from 8 March to 9 June, when water temperatures ranged from 9.6° to 25.5°C (Figure 3.10). A majority (81%) of the Hickory Shad were observed in the month of April. Sightings of Hickory Shad diminished as water temperatures rose above 15°C.

The first and the last sampling dates of the 2021 season yielded no adult Hickory Shad, successfully bracketing the beginning and the end of the spawning run per the new protocol. In years prior to 2012 the end data were bracketed, but the beginning of the sampling season typically started when shad were already present in the river.

Patuxent River Hickory Shad CPUE

During the twelve weeks from 8 March to 9 June 2021 when Hickory Shad were surveyed on the Patuxent River, the mean relative abundance (GM) was calculated as 0.21 fish/min (Figure 3.11). The long-term average (2001-2021) for the Patuxent River is 0.43 fish/min.

Patuxent River Hickory Shad Origin Composition (Hatchery vs. Wild)

Hickory Shad captured in the 2017 electrofishing survey on the Patuxent River were not examined for hatchery or wild origin. Since Hickory Shad have not been stocked in the Patuxent River since 2009, project biologists determined that capture of hatchery origin adults was unlikely.

Patuxent River Hickory Shad First-Time and Repeat-Spawning Compositions

Seventy-nine Hickory Shad scale samples were collected in 2021. All collected samples were successfully analyzed and used to determine the annual spawning attempt composition. The 2021 sample population consisted of 85% first-time spawners, 9% second-time spawners, 5% third-time spawners and 1% fourth-time spawners (Table 3.5).

Patuxent River Hickory Shad Spawning Stock Discussion

Survey results for 2021 indicate a Patuxent River spawning stock is exhibiting a stable population pattern. Prior to 2007, while stocking was occurring, the GM values varied without trend at an average of 0.59 fish/min (2001-2007; Figure 3.11). This was followed by a post-stocking adjustment period from 2008-2009. Starting in 2010 (2010-2021), three years post-stocking, the GM values continued to vary without trend, but at a much lower level (0.20 fish/min). Project biologists believe that this decline in CPUE was potentially associated with increased turbidity levels, which led to lower catch rates. However, correlation analysis indicated no correlation between CPUE and Secchi values. Available data indicate that this population is self-sustaining, although at a lower abundance than when stocking was occurring. This observation is supported by the origin contribution data, which was initially used to deem this population restored.

Statistical examination of the first-time and repeat-spawning data can be used to evaluate stability or instability in a spawning stock and can aid in the prediction of a stock decline or expansion. A stable Hickory Shad spawning population consists of a substantial contribution from several spawning year classes. However, there are several factors that can impart variability in these distributions, including maturity schedules of males (3-4 years) and females (4-5 years), timing of the spawning run, annual recruitment of wild fish, number of fish stocked annually, and recruitment of stocked fish. It may be possible to remove some of the variability from these distributions by evaluating male and female distributions separately, but there are already small sample size concerns when combining the males and females in these distributions. This is especially true when assessing fish making their fifth and sixth spawning attempt. There are rarely sample sizes of five fish in these categories, which is required to evaluate these distributions statistically (i.e., chi-square analysis). These small sample sizes lead to uninformative gaps in the time series (2011-2021).

Susquehanna River (Brood Source) Hickory Shad Spawning Stock

The Susquehanna River Hickory Shad population has been the sole brood source for restoration efforts (Figure 1.4). This population declined along with other Chesapeake Bay Hickory Shad stocks during the 1970s, but experienced resurgence during the 1990s as a dominant year class appeared in 1993. This year class provided a sufficient source of brood stock adults when they began to return as spawning adults in 1996 (Minkkinen et.al. 2000). Strong and stable Hickory Shad spawning runs have occurred since 1996 and have been sufficient to support broodstock collection and a large catch-and-release recreational fishery.

Analysis of spawning attempt data indicates a spawning population that naturally recruits several year classes to the spawning grounds annually (Table 3.6). In 2021, 63 Hickory Shad scale samples were collected for spawning composition analysis and all 63 samples were analyzed successfully. Three year classes were present among 2021 samples which is the fewest number of year classes encountered since broodstock collections began in 2004. The percentage of first-time spawning encountered in 2021 was the second-highest recorded (76%). The highest recorded was 80% in 2019. The mean percentage of first-time spawners over the 17-year time series is 35%.

Reduced stocking goals results in fewer sampling days (two days each in 2018, 2019, and 2021) to collect broodstock. As a result, fewer samples are being collected than in the past, potentially causing a less-robust age and repeat spawn analysis. An attempt was made in 2021 to collect additional samples, however all samples were collected in a single week instead of the entire spawning run. Sampling only during a discrete window within the spawning run results in uncertainty in the data. We will attempt to obtain samples throughout the spawning season in future years to obtain a more accurate representation of Susquehanna River Hickory Shad spawning stock.

2021 Overall Restoration Progress:

Patapsco River

The Patapsco River is in the early stages of the restoration effort. It is encouraging to see a greater proportion of wild-origin adults spawning in 2021. Starting in 2020, these fish could be from wild offspring of hatchery stocked adults or from a remnant population remaining prior to the initiation of stocking. The composition of the adult population will be monitored in the future until the wild portion of the adult population comprises greater than 75% of the population for three consecutive years. At that point we may be able to say that the Hickory Shad population in the Patapsco River is restored to a point that further stocking is not needed.

Choptank River

From 2010-2012, all of the restoration efforts focused on the Choptank River. This was to permit maximum stocking impact and more detailed analysis of assessment activities. The lack of Hickory Shad juvenile recaptures hinders a complete assessment of the restoration effort, but trend data using adult electrofishing surveys demonstrate a stable population of adult Hickory Shad. Several year-classes of repeat spawners were observed from a robust sample size (n=142) in 2021. The 2021 GM (0.65 fish/min) was similar to the long-term average (0.78 fish/min) on the Choptank River. This is a positive indicator of a stable population. This population is scheduled to be sampled again in 2024.

Future comparison of the relative abundance estimate (GM) trends will be invaluable to evaluate the success of the restoration progress on both the Patuxent and Choptank rivers. These rivers will continue to be surveyed on a three-year rotational basis. Success of this program relies on future generations continuing to naturally spawn in these rivers when hatchery inputs no longer supplement natural populations.

Patuxent River

The last year of hatchery stocking in the Patuxent River was 2007. Sampling in 2017 indicated a population that appeared to be in decline with the lowest GM calculated since the inception of the project, leading to concerns among project biologists. In 2021, the GM was calculated at 0.21 fish/min. While this is lower than the long-term average (0.61 fish/min), it is similar to the post-stocking (2010-2021) average (0.20 fish/min). Geometric mean numbers could have been artificially inflated during supplemental stocking and once stocking ceased, population numbers stabilized at lower levels. Sampling in 2021 resulted in a larger number of adult Hickory Shad being collected and allowed for a more robust sample analysis and reduced numbers in 2017 were determined to be the product of interannual variation. Periodic sampling on the Patuxent River will continue to maintain trend data and is scheduled to be sampled again in 2024.

2022 Sub-Project 3 – Preliminary Results – Work in Progress

Patapsco River

Electrofishing surveys were conducted on the Patapsco River for 13 weeks from 10 March to 31 May 2022. A total of 60 adult Hickory Shad were encountered and 36 were retained for scale age and otolith analysis. A complete analysis of CPUE, origin composition, and repeat spawning analysis will be presented in the 2023 F-57-R report.

Choptank River

The Choptank River Hickory Shad population was not sampled in 2022. The adult Hickory Shad population on this river will be re-evaluated again in 2024.

Patuxent River

The Patuxent River Hickory Shad population was not sampled in 2022. The adult Hickory Shad population on this river will be re-evaluated again in 2024.

Project-wide Observations 2022

Project wide observations for the 2022 calendar year will be discussed in the next reporting cycle. Sub-project two will be completed for 2022 and all data will be analyzed to give a more complete picture of restoration efforts in the Patapsco River. These data will be presented in the 2023 reporting period.

Literature Cited

Cating, J. P. 1953. Determining age of Atlantic shad from their scales. Fishery Bulletin 85, Fishery Bulletin of the Fish and Wildlife Service, Volume 54.

Minkkinen, S. P., B. Richardson and R. Morin. 1999. Assessment of alosid stocking and natural reproduction in a restoration setting, 1998 report pursuant to Federal Aid #NA86FA0330.

Minkkinen, S. P., B. Richardson and R. Morin. 2000. American Shad and Hickory Shad restoration, 1999 report to Federal Aid in Sport Fish Restoration Act grant #F-57- R.

Mylonas, C., Y. Zohar, B. Richardson and S. Minkkinen. 1995. Induced spawning of wild American Shad Alosa sapidissima using sustained administration of gonadotropin-releasing hormone analog (GnRHa). Journal of the World Aquaculture Society 26(3):240-251.

O'Dell, J., J. Gabor and J. Mowrer. 1975. Survey of anadromous fish spawning areas for Chester River drainage in Queen Anne's County. Project AFC-9-2. Maryland Department of Natural Resources. Annapolis, Maryland.

O'Dell, J., J. Gabor and J. Mowrer. 1978. Survey of anadromous fish spawning areas for Potomac River drainage, Upper Chesapeake Bay drainage. Project AFC-9-1. Maryland Department of Natural Resources. Annapolis, Maryland.

Table 1.1. *Maryland Department of Natural Resources 2021 Hickory Shad broodstock collection data.*

Date	Females(n)	Males(n)
4/19/2021	26	19
4/22/2021	10	13

Table 1.2. *Maryland Department of Natural Resources Hickory Shad stocking goals and total amount stocked for the Patapsco River in 2021.*

Stocking Phase	Stocking Goal	Total Stocked
Larvae	500,000	435,000
Early Juvenile	75,000	50,000

Table 1.3. *Maryland Department of Natural Resources 2021 tank spawn Hickory Shad egg production summary.*

Total eggs produced	2,615,140
Overall fertilization	34.5%
Fertilized eggs produced	902,223
Total larvae stocked	435,000
Total juveniles stocked	50,000

Table 1.4. *Maryland Department Natural Resources annual Hickory Shad stocking production in all tributaries, 1996-2021. The juvenile category includes fish stocked as early juveniles (late June) and late juveniles (July/August). *No eggs were collected in 2020 due to COVID-19 sampling restrictions.*

Year	Larvae Stocked	Juvenile Stocked
1996	871,000	20,622
1997	10,689,000	35,982
1998	11,466,000	31,979
1999	16,825,000	4,601
2000	13,869,000	66,944
2001	4,999,679	93,645
2002	2,675,000	119,606
2003	2,310,000	118,551
2004	9,757,000	130,332
2005	4,410,000	403,000
2006	4,095,000	382,000
2007	1,700,000	211,500
2008	6,238,581	364,500
2009	2,737,636	242,000
2010	4,260,000	117,000
2011	4,399,000	143,750
2012	2,503,203	380,100
2013	560,000	471,000
2014	520,000	240,500
2015	1,045,000	82,500
2016	795,000	35,000
2017	750,000	87,000
2018	915,000	85,000
2019	1,725,000	5,000
2020	N/A	N/A
2021	435,000	50,000
Total	**110,550,099**	**3,922,112**

Table 1.5. *Historical stocking summary for larval and juvenile Hickory Shad in the Patapsco River since the inception of Hickory Shad restoration efforts (including fish passage work 1997-2004). *No eggs were collected in 2020 due to COVID-19 sampling restrictions.*

Patapsco River Hickory Shad

Year	Larvae	Early Juveniles
1997	1,695,000	0
1998	250,000	0
1999	825,700	0
2000	500,000	0
2001	0	0
2002	0	0
2003	0	0
2004	542,000	0
2005	0	0
2006	0	0
2007	0	0
2008	0	0
2009	0	0
2010	0	0
2011	0	0
2012	0	0
2013	561,000	77,000
2014	538,500	73,000
2015	1,045,000	82,500
2016	580,000	35,000
2017	750,000	82,000
2018	915,000	85,000
2019	1,475,000	5,000
2020	0	0
2021	435,000	50,000
Total	**10,112,200**	**489,500**

Table 1.6. *Maryland Department of Natural Resources 2021 Hickory Shad stocking events in the Patapsco River.*

Date	Life stage	Mark	Number
5/2/2021	Larvae	Day 1	400,000
5/4/2021	Larvae	Day 1	35,000
5/25/2021	Early Juvenile	Day 1, 3	50,000

Table 1.7. *Historical stocking summary for larval and juvenile Hickory Shad in the Chester River (2003-2008).*

Chester River Hickory Shad	
Year	**Larvae**
2003	90,000
2004	200,000
2005	0
2006	0
2007	0
2008	602,000
Total	**892,000**

Table 1.8 *Historical stocking summary for larval and juvenile Hickory Shad in the Patuxent River (2001-2013).*

Patuxent Hickory Shad

Year	Larvae	Early Juveniles	Late Juveniles
2001	1,380,776	53,500	20,238
2002	350,000	40,000	0
2003	395,000	37,000	0
2004	3,425,000	68,500	0
2005	1,160,000	120,000	0
2006	1,350,000	70,000	0
2007	520,000	36,500	0
2008	0	0	0
2009	0	0	0
2010	0	0	0
2011	0	0	0
2012	0	0	0
2013	0	0	0
2014	0	0	0
2015	0	0	0
2016	0	0	0
2017	0	0	0
2018	0	0	0
2019	250,000	0	0
Total	**8,830,776**	**425,500**	**20,238**

Table 1.9. *Historical stocking summary for larval and juvenile Hickory Shad in the Choptank River (1996-2014).*

Choptank River Hickory Shad

Year	Larvae	Early Juveniles	Late Juveniles
1996	125,000	0	7,963
1997	5,571,000	0	0
1998	4,991,000	0	0
1999	8,719,000	0	0
2000	5,634,000	0	38,508
2001	1,158,800	0	19,907
2002	1,050,000	25,000	0
2003	700,000	34,500	0
2004	4,090,000	42,350	0
2005	2,430,000	177,000	0
2006	1,770,000	220,000	0
2007	1,080,000	149,500	0
2008	3,028,000	225,000	0
2009	1,953,000	120,000	0
2010	4,260,000	117,000	0
2011	4,399,000	143,750	0
2012	2,503,000	380,100	0
2013	560,000	471,000	0
2014	520,000	240,500	0
Total	**54,541,800**	**2,345,700**	**66,378**

Table 1.10. *Historical stocking summary for larval and juvenile Hickory Shad in Marshyhope Creek (2001-2009).*

Marshyhope Creek Hickory Shad			
Year	**Larvae**	**Early Juveniles**	**Late Juveniles**
2001	1,230,000	0	0
2002	300,000	26,000	17,247
2003	500,000	17,000	18,551
2004	500,000	14,000	5,482
2005	370,000	66,000	0
2006	750,000	70,000	0
2007	100,000	25,500	0
2008	2,209,000	140,000	0
2009	785,000	122,000	0
Total	**6,744,000**	**480,500**	**41,280**

Table 1.11. *Historical stocking summary for larval and juvenile Hickory Shad in the Nanticoke River (2001-2006).*

Nanticoke River Hickory Shad			
Year	**Larvae**	**Early Juveniles**	**Late Juveniles**
2001	1,230,000	0	0
2002	975,000	0	11,058
2003	625,000	11,500	0
2004	1,000,000	0	0
2005	450,000	40,000	0
2006	225,000	22,000	0
Total	**4,505,000**	**73,500**	**11,058**

Table 3.1. *Maryland Department of Natural Resources and Maryland Biological Stream Survey 2021 adult Hickory Shad electrofishing survey starting and ending coordinates for the Patapsco River. *Site 597 has no ending latitude/longitude because the site was at the base of Daniel's Dam.*

River	***Site Name***	***Starting latitude/longitude***	***Ending latitude/longitude***
Patapsco River	Maryland 648 to I895	39.224738° N -076.640593° W	39.228271° N -076.627297° W
Patapsco River	MBSS Site 591	39.212930° N -076.699898° W	39.216105° N -076.696884° W
Patapsco River	MBSS Site 592	39.216443° N -076.705498° W	39.213893° N -076.702091° W
Patapsco River	MBSS Site 595	39.251901° N -076.766736° W	39.250976° N -076.763750° W
Patapsco River	MBSS Site 596	39.271320° N -076.792447° W	39.269792° N -076.794531° W
Patapsco River	MBSS Site 597	39.314848° N -076.816555° W	*
Choptank River	Dream Property to Rt. 313 Bridge	38.984728° N -075.788325° W	38.977021° N -075.801606° W
Patuxent River	4H Park to Sewage Treatment Plant	38.885666° N -076.674890° W	38.855692° N -076.691094° W

Table 3.2. *Summary of Hickory Shad adults caught by Maryland Department of Natural Resources from the Patapsco River during electrofishing surveys in 2021.*

			Male			
Year Class	Return Spawn	N	Larvae	Early Juvenile	Wild	No Sample
2015	0	2	1		1	
	2	1	1			
2016	0	6	1	3	2	
	1	4		2	2	
2017	0	33	6	3	23	1
	1	3	1		2	
2018	0	19	8	1	8	2
Total		68	18	9	38	3
			Female			
Year Class	Return Spawn	N	Larvae	Early Juvenile	Wild	No Sample
2015	2	5	1	1	2	1
2016	0	13	1	1	11	
	1	4	1	1	2	
	2	1	1			
2017	0	22	1	1	20	
	1	1		1		
Total		46	5	5	35	1

Table 3.3. *Patapsco River Hickory Shad spawning attempt composition from 2013-2021 as determined from a subsample of fish. The United States Fish and Wildlife Service Maryland Fish and Wildlife Conservation Office determined spawning composition from 2013-2017. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

		Spawning Attempts			
Year	**Sample Size (n)**	**First-Time Spawners**	**2**	**3**	**4**
2013	37	18 (49%)	16 (43%)	3 (8%)	
2014	20	18 (90%)	2 (10%)		
2015	23	21 (91%)	2 (9%)		
2016	114	106 (93%)	7 (6%)	1 (1%)	
2017	68	63 (93%)	5 (7%)		
2018	36	11 (31%)	7 (19%)	18 (50%)	
2019	30	18 (60%)	8 (27%)	4 (13%)	
2020	0*				
2021	114	95 (83%)	12 (11%)	7 (6%)	

Table 3.4. *Choptank River Hickory Shad spawning attempt composition from 2002-2021 as determined from a subsample of fish. This population of Hickory Shad was considered recovered in 2014 and is now sampled on a three-year rotating basis. Percentages may not add up to 100 due to rounding. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

		Spawning Attempts					
Sample Year	**Sample Size (n)**	**First-Time Spawners**	**2**	**3**	**4**	**5**	**6**
2002	217	73 (34%)	41 (19%)	84 (39%)	17 (8%)	2 (1%)	
2003	92	19 (21%)	13 (14%)	37 (40%)	20 (22%)	2 (2%)	1 (1%)
2004	83	29 (35%)	16 (19%)	27 (33%)	8 (10%)	3 (4%)	
2005	64	30 (47%)	11 (17%)	7 (11%)	7 (11%)	9 (14%)	
2006	80	49 (61%)	14 (18%)	13 (16%)	1 (1%)	2 (3%)	1 (1%)
2007	80	31 (39%)	25 (31%)	19 (24%)	4 (5%)	1 (1%)	
2008	131	53 (40%)	49 (37%)	23 (18%)	4 (3%)	2 (2%)	
2009	62	9 (15%)	15 (24%)	27 (44%)	11 (18%)		
2010	122	50 (41%)	42 (34%)	21 (17%)	9 (7%)		
2011	137	65 (47%)	19 (14%)	27 (20%)	21 (15%)	4 (3%)	1 (1%)
2012	166	70 (42%)	62 (37%)	30 (18%)	4 (2%)		
2013	123	50 (41%)	43 (35%)	21 (17%)	7 (6%)	2 (2%)	
2014	84	35 (42%)	21 (25%)	22 (26%)	6 (7%)		
2017	137	40 (29%)	67 (49%)	24 (18%)	3 (2%)	1 (0.7%)	
2021	142	83 (58%)	23 (16%)	26 (18%)	9 (6%)	1 (1%)	

Table 3.5. *Patuxent River Hickory Shad spawning attempt composition from 2002-2021 as determined from a subsample of fish. This population of Hickory Shad was considered recovered in 2014 and is now sampled on a three-year rotating basis. Percentages may not add up to 100 due to rounding. *Sampling could not be completed in 2020 due to COVID-19 pandemic restrictions.*

		Spawning Attempts					
Sample Year	**Sample Size (n)**	**First-Time Spawners**	**2**	**3**	**4**	**5**	**6**
2002	204	87 (43%)	26 (13%)	71 (35%)	17 (8%)	3 (1%)	
2003	85	28 (33%)	11 (13%)	26 (31%)	19 (22%)	1 (1%)	
2004	59	24 (41%)	6 (10%)	15 (25%)	11 (19%)	3 (5%)	
2005	103	66 (64%)	2 (2%)	18 (17%)	13 (13%)	4 (4%)	
2006	93	41 (44%)	27 (29%)	17 (18%)	2 (2%)	4 (4%)	2 (2%)
2007	99	48 (48%)	14 (14%)	20 (20%)	11 (11%)	5 (5%)	1 (1%)
2008	127	30 (24%)	43 (34%)	35 (28%)	13 (10%)	6 (3%)	
2009	65	7 (11%)	20 (31%)	26 (40%)	10 (15%)	2 (3%)	
2010	55	17 (31%)	12 (22%)	15 (27%)	11 (20%)		
2011	38	8 (21%)	8 (21%)	8 (21%)	12 (32%)	2 (5%)	
2012	88	44 (50%)	26 (30%)	16 (18%)	2 (2%)		
2013	87	56 (64%)	27 (31%)	1 (1%)	2 (2%)	1 (1%)	
2014	58	21 (36%)	23 (40%)	12 (21%)	1 (2%)	1 (2%)	
2017	26	10 (38%)	11 (42%)	5 (19%)			
2021	79	67 (85%)	7 (9%)	4 (5%)	1 (1%)		

Table 3.6. *Maryland Department of Natural Resources Hickory Shad Susquehanna River broodstock collection spawning attempt composition for sample years 2004-2021.*

		Spawning Attempts						
Sample Year	**Sample Size (n)**	**First-Time Spawners**	**2**	**3**	**4**	**5**	**6**	**7**
2004	80	25 (31%)	11 (14%)	17 (21%)	20 (25%)	6 (8%)	1 (1%)	
2005	80	14 (18%)	10 (13%)	22 (28%)	25 (31%)	7 (9%)	2 (3%)	
2006	178	58 (33%)	29 (16%)	48 (27%)	29 (16%)	11 (6%)	3 (2%)	
2007	139	29 (21%)	26 (19%)	40 (29%)	23 (17%)	17 (12%)	3 (2%)	1 (1%)
2008	149	24 (16%)	37 (25%)	50 (34%)	29 (19%)	7 (5%)	2 (1%)	
2009	118	13 (11%)	19 (16%)	54 (46%)	20 (17%)	11 (9%)	1 (1%)	
2010	240	59 (25%)	72 (30%)	73 (30%)	25 (10%)	10 (4%)		1 (0.4%)
2011	216	67 (31%)	65 (30%)	57 (26%)	19 (9%)	6 (3%)	2 (1%)	
2012	200	72 (36%)	64 (32%)	45 (23%)	15 (8%)	4 (2%)		
2013	193	73 (38%)	62 (32%)	41 (21%)	15 (8%)	2 (1%)		
2014	100	41 (41%)	19 (19%)	30 (30%)	10 (10%)			
2015	113	46 (41%)	41 (36%)	21 (19%)	5 (4%)			
2016	120	35 (29%)	38 (32%)	36 (30%)	10 (8%)	1 (1%)		
2017	60	15 (25%)	19 (32%)	20 (33%)	5 (8%)			
2018	40	19 (48%)	13 (33%)	5 (13%)	3 (8%)			
2019	99	79 (80%)	8 (8%)	10 (10%)	2 (2%)			
2020	0*							
2021	63	48 (76%)	11 (17%)	4 (6%)				

Figure 1.1. *Maryland Department of Natural Resources target tributary for the Hickory Shad restoration project.*

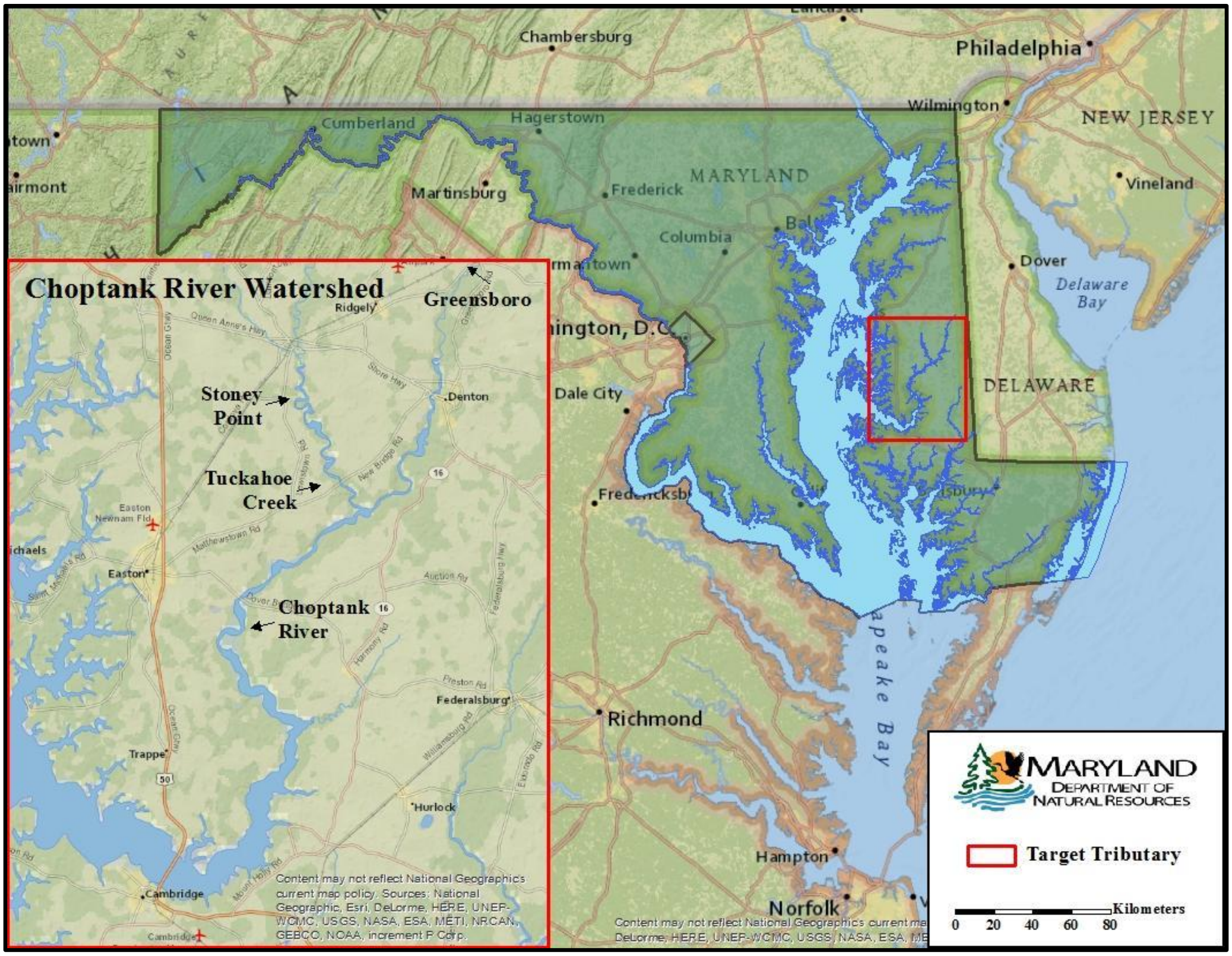

Figure 1.2. *Maryland Department of Natural Resources target tributary for the Hickory Shad restoration project.*

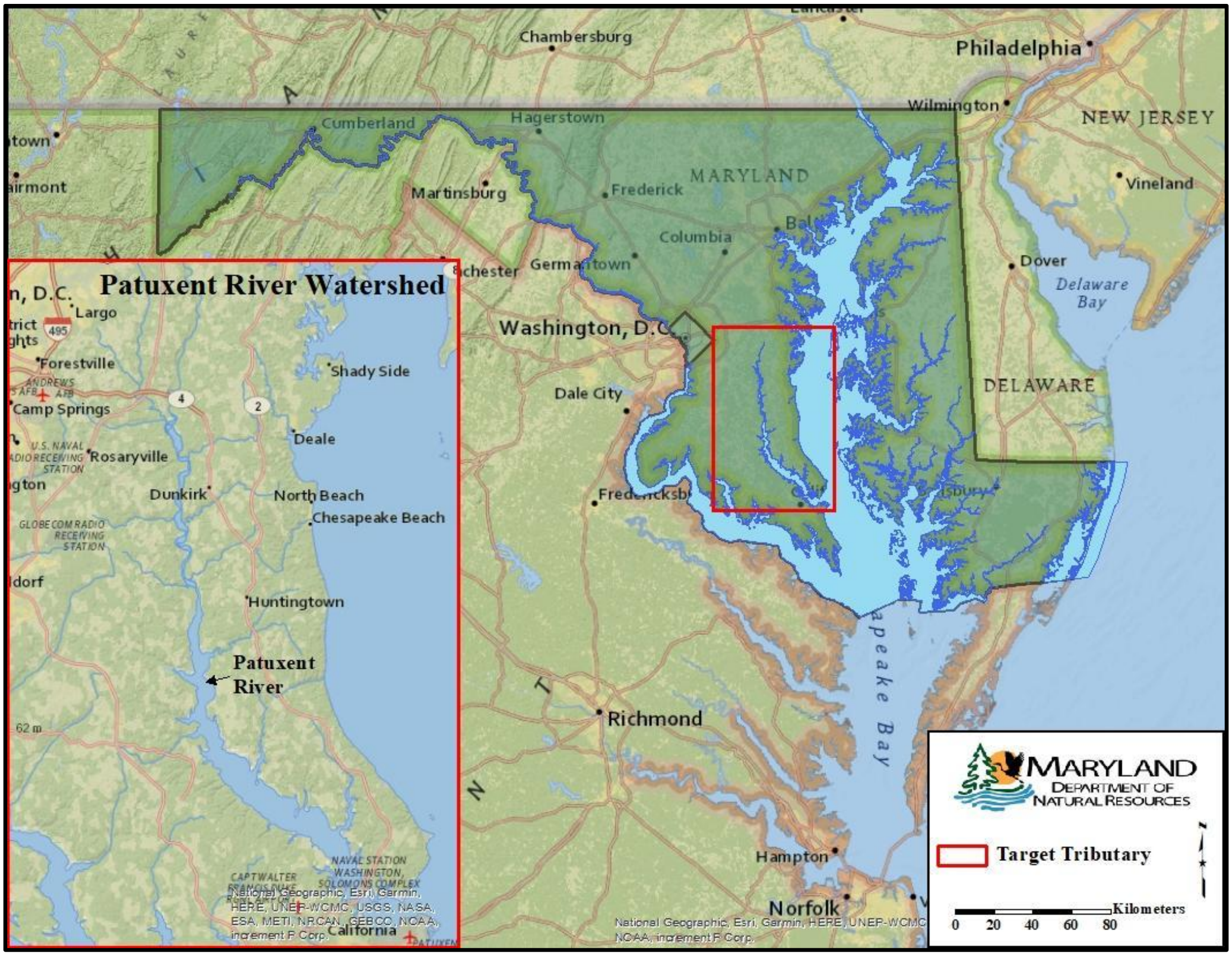

Figure 1.3. *Maryland Department of Natural Resources target tributary for the Hickory Shad restoration project.*

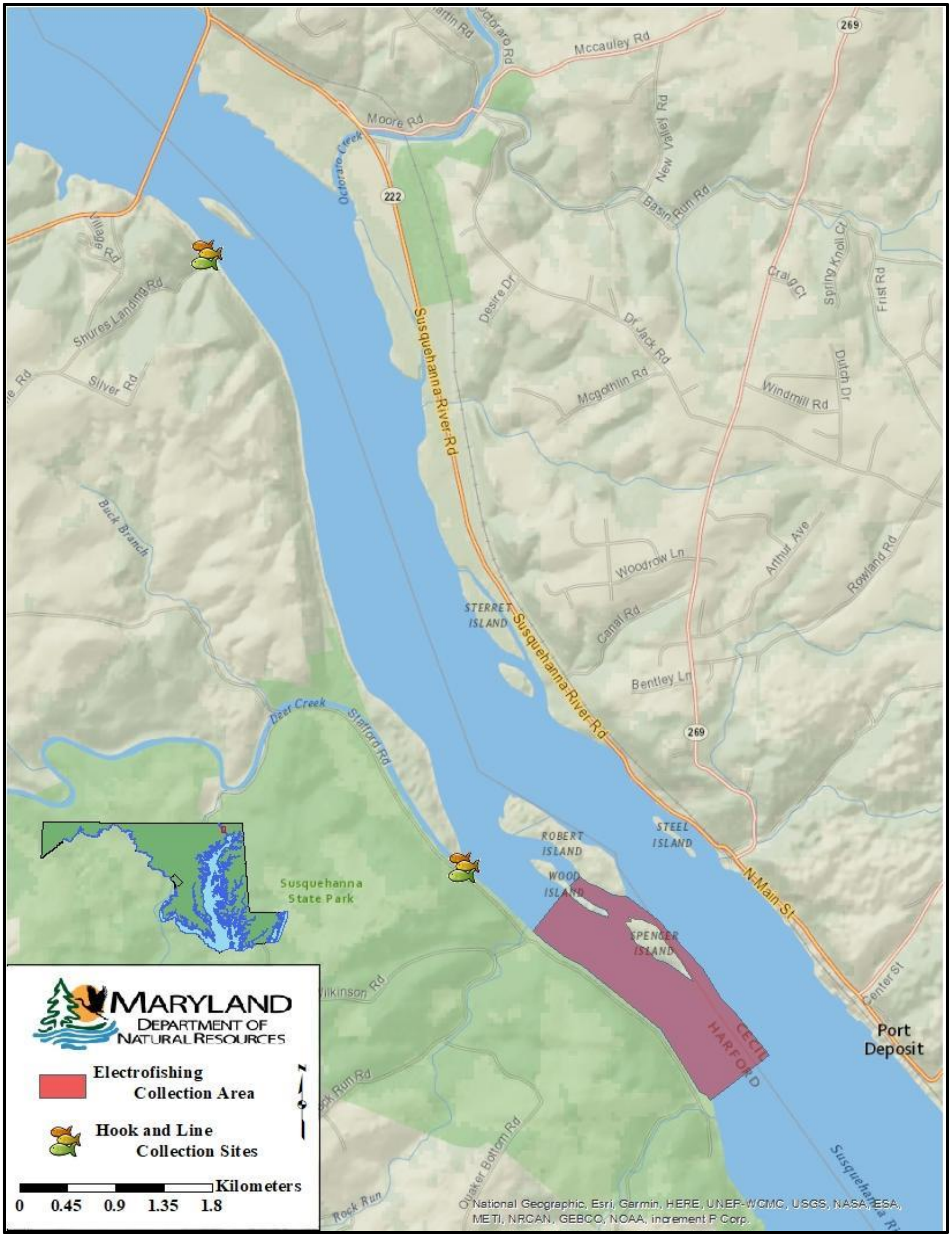

Figure 1.4. *Maryland Department of Natural Resources Hickory Shad broodstock collection site on the Susquehanna River*.

Figure 1.5. *Maryland Department of Natural Resources participating fish culture facilities in the restoration project.*

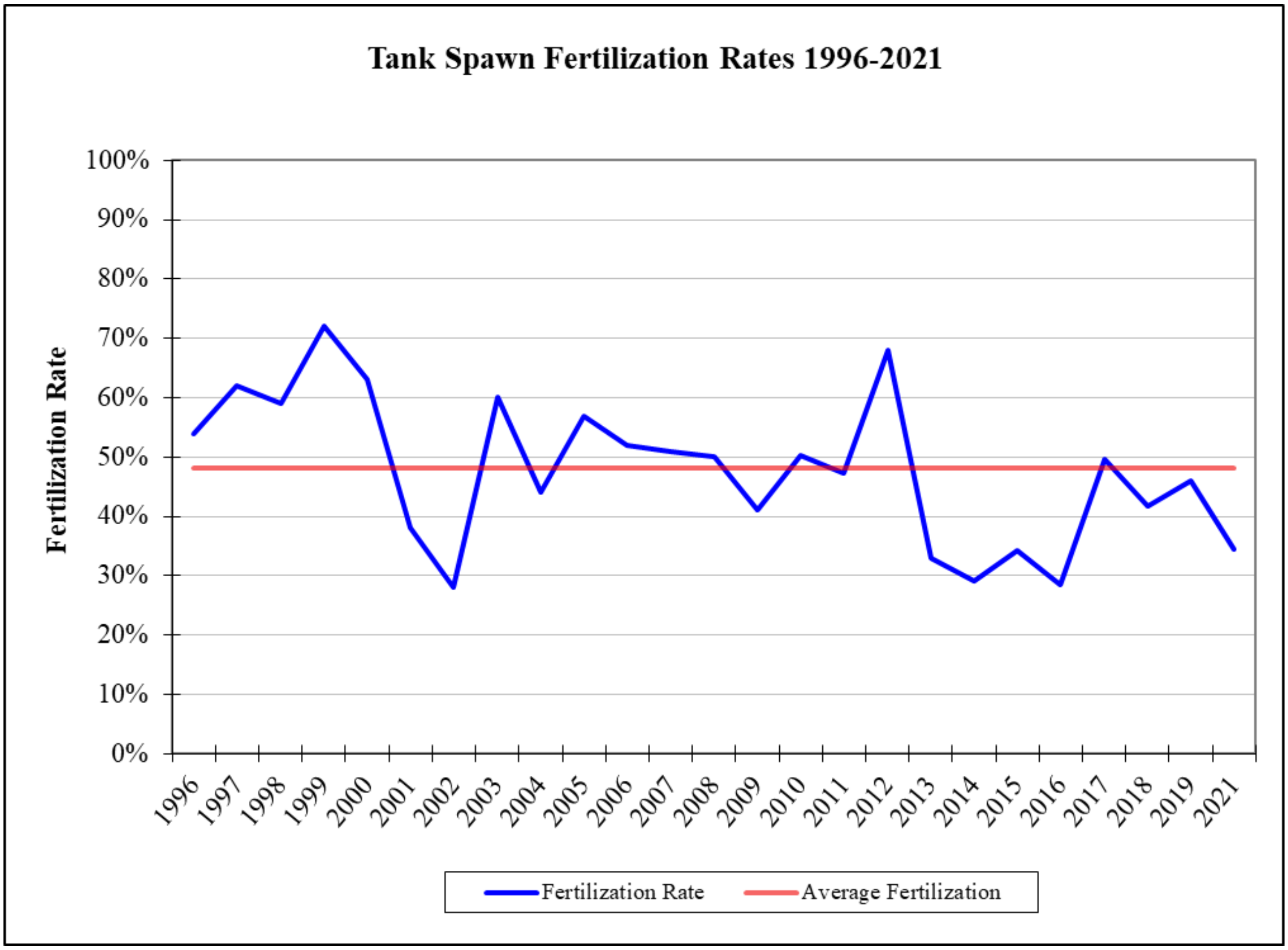

Figure 1.6. *Maryland Department of Natural Resources tank spawn fertilization rates for Hickory Shad, 1996-2021.*

Figure 1.7. *Maryland Department of Natural Resources Patapsco River stocking sites in 2021.*

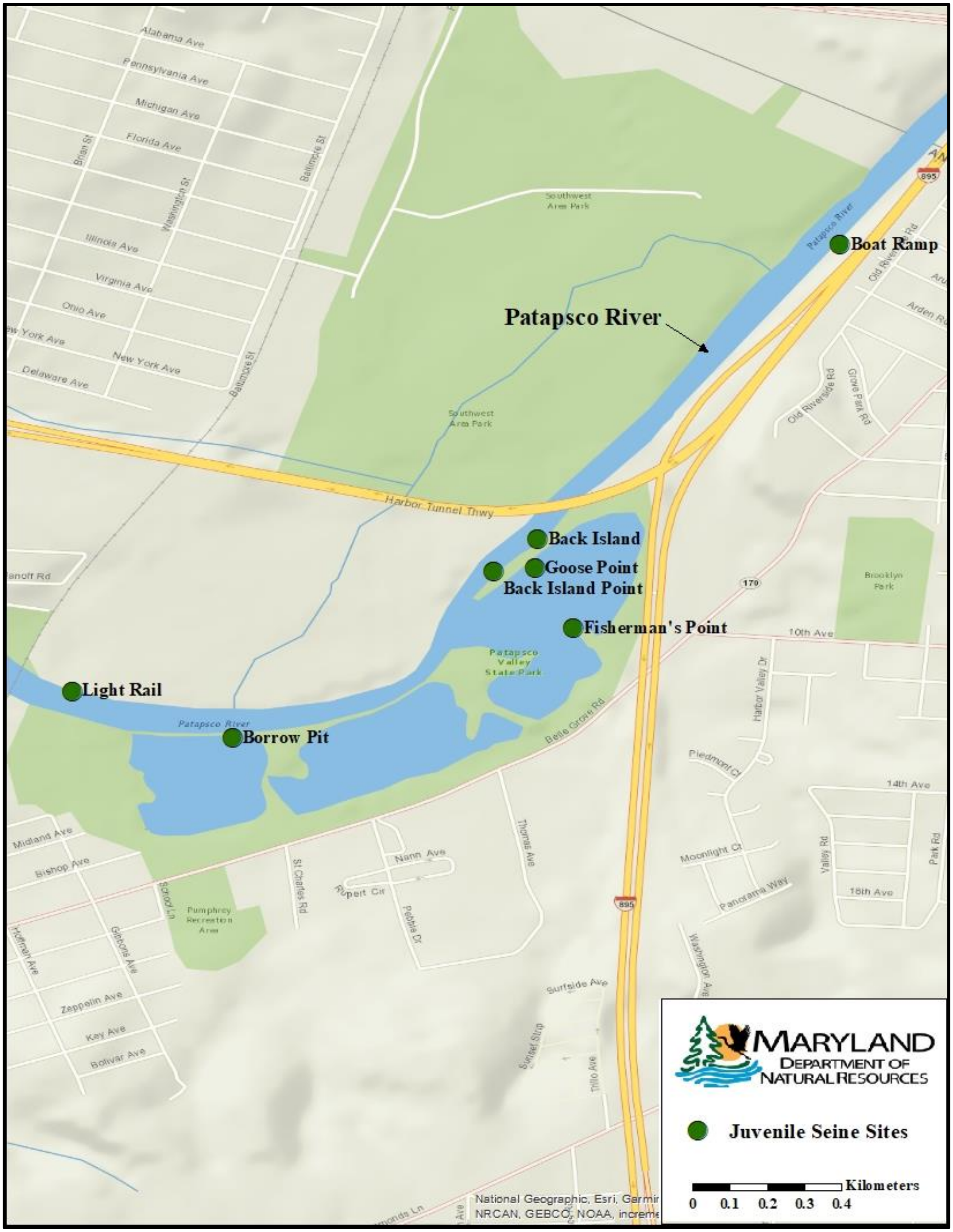

Figure 2.1 *Maryland Department of Natural Resources Patapsco River juvenile seine survey sites in 2021.*

Figure 3.1. *Maryland Department of Natural Resources 2021 electrofishing survey site on the lower tidal freshwater portion of the Patapsco River.*

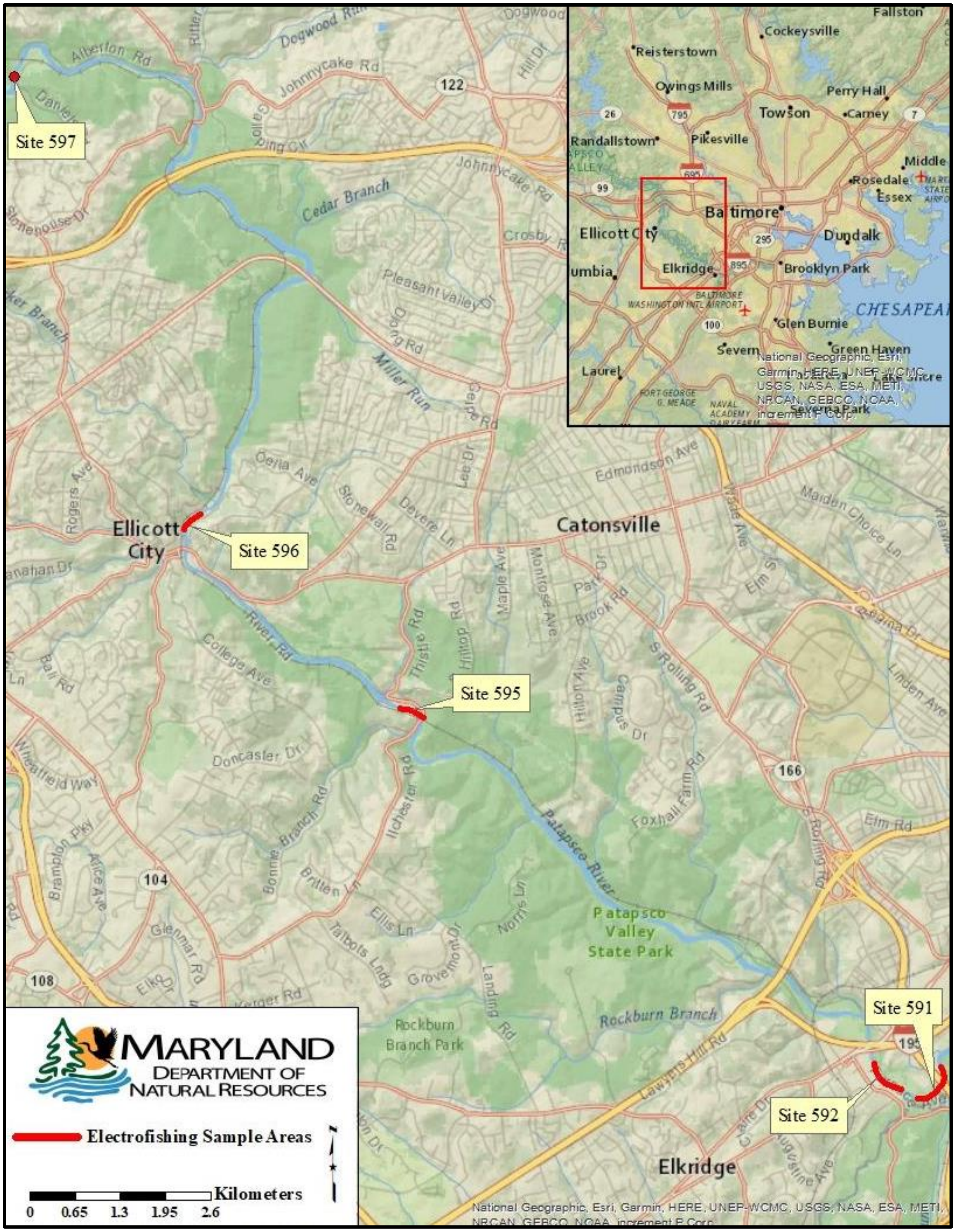

Figure 3.2. *Maryland Biological Stream Survey 2021 electrofishing survey sites on the upper non-tidal freshwater portion of the Patapsco River.*

Figure 3.3. *Maryland Department of Natural Resources 2021 electrofishing survey site on Choptank River.*

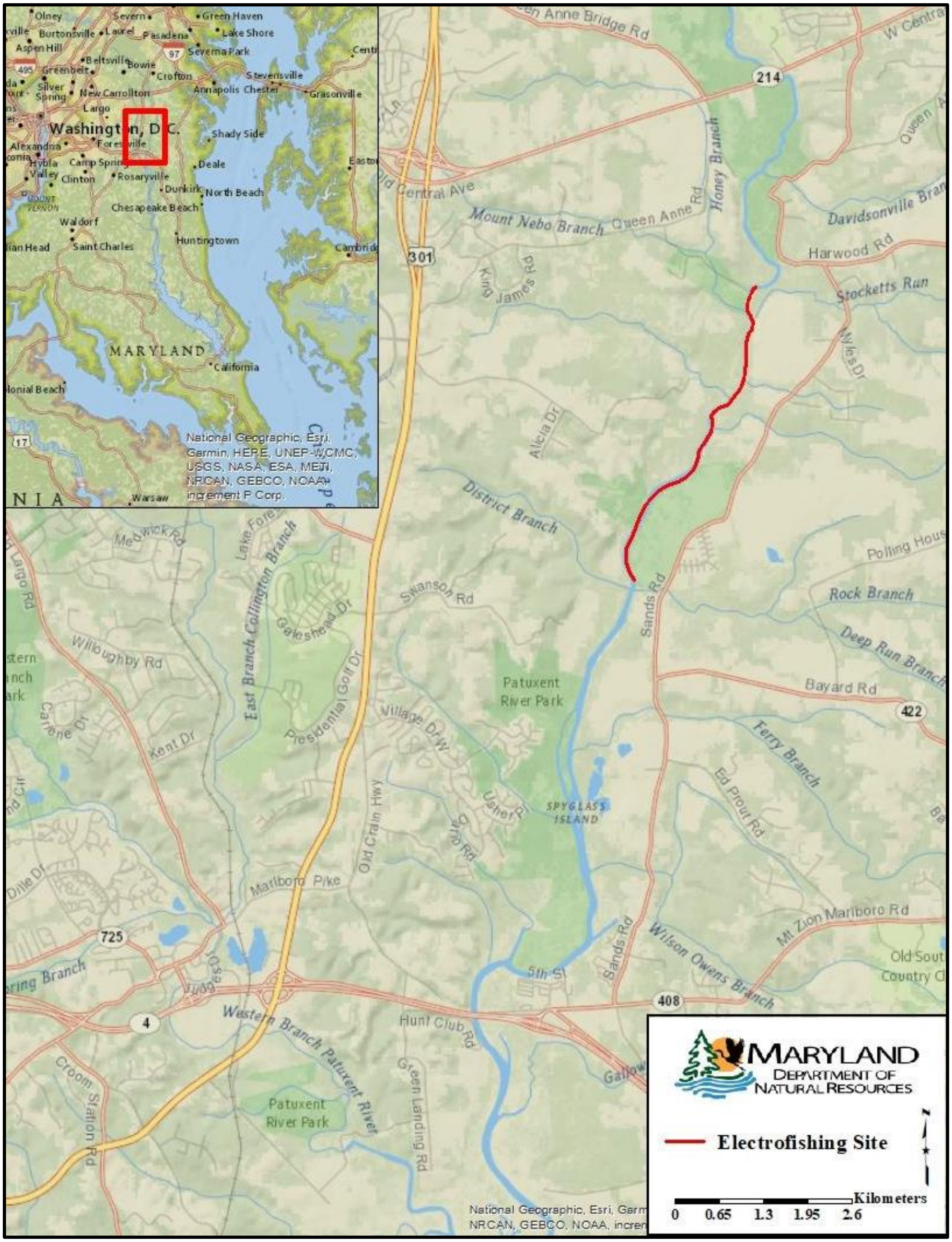

Figure 3.4. *Maryland Department of Natural Resources 2021 electrofishing survey site on Patuxent River.*

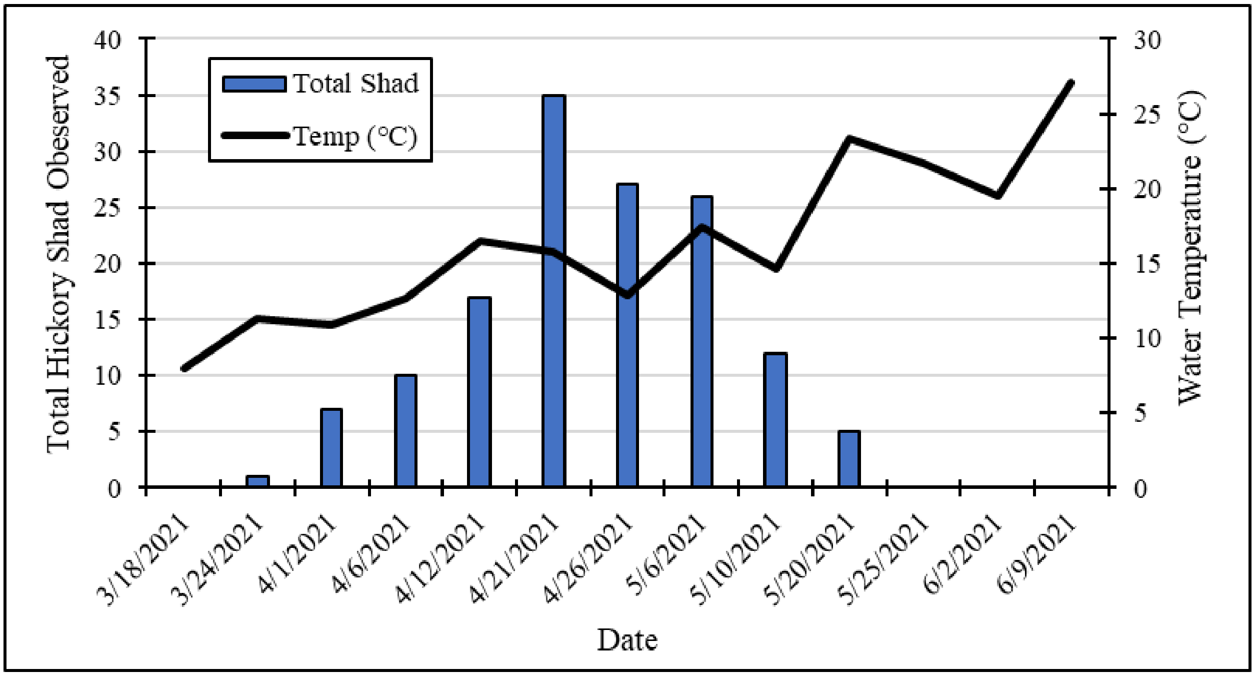

Figure 3.5. *2021 Maryland Department of Natural Resources electrofishing collections and observations of adult Hickory Shad in the Patapsco River in relation to water temperature*.

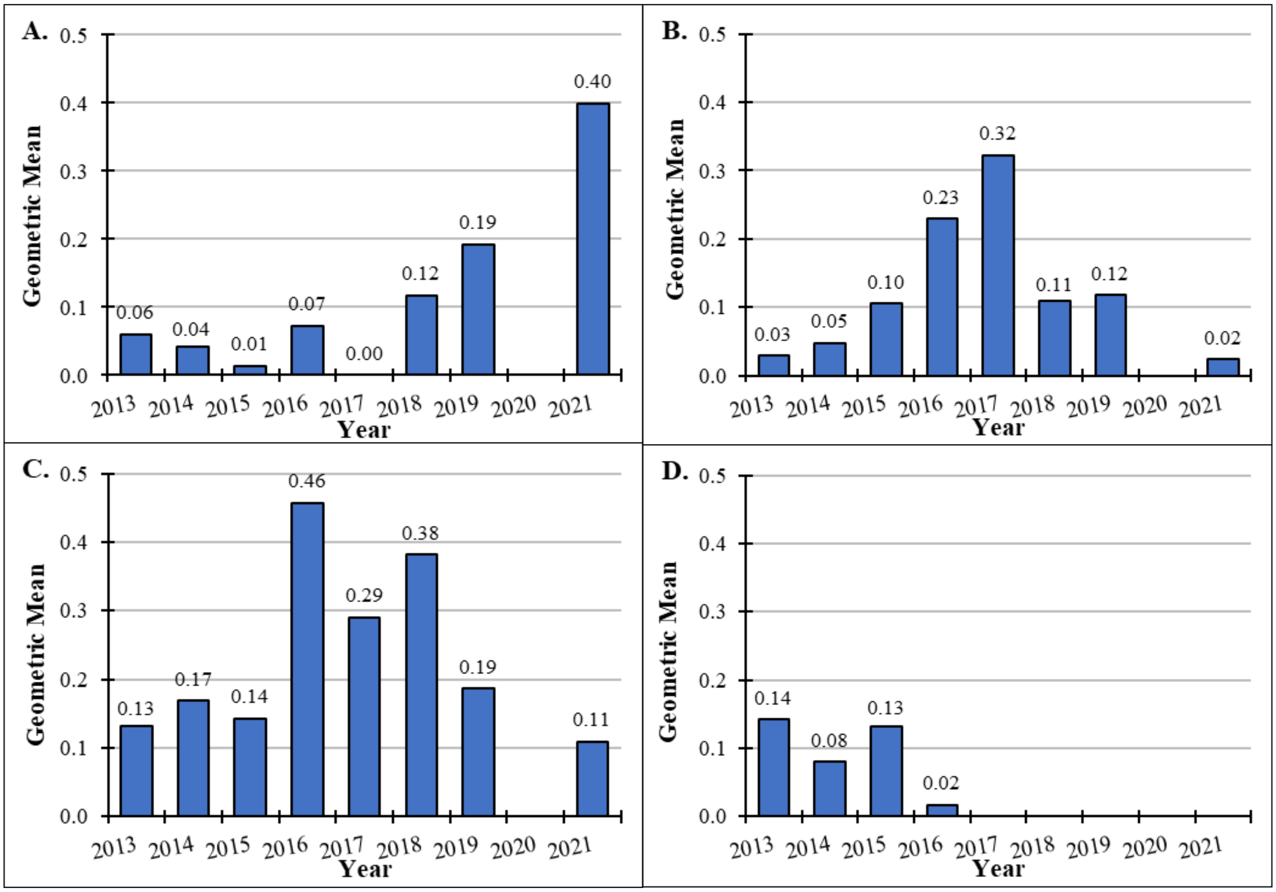

Figure 3.6. *Geometric Mean (GM) calculated at all sites sampled by Fishing and Boating Services and Maryland Biological Stream Survey on the Patapsco River from 2013-2021. Sites without a bar indicate zero Hickory Shad were sampled during that sample season. A. Rt. 648 to I895. B. MBSS Site 591. C. MBSS Site 592. D. MBSS Site 593.*

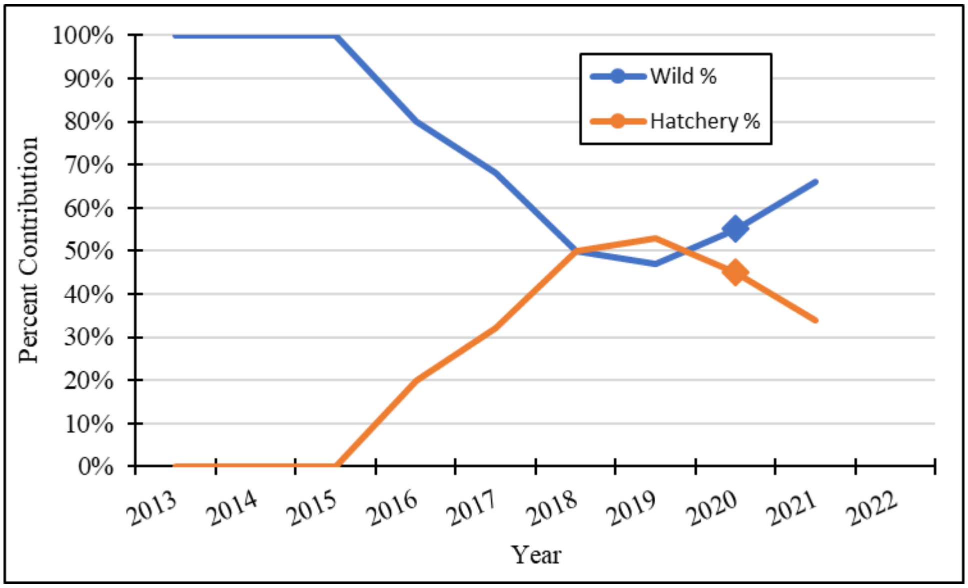

Figure 3.7. Percent of wild versus hatchery contribution of sampled adult Hickory Shad on the Patapsco River 2013-2021. ♦ indicates when percent contribution was estimated.

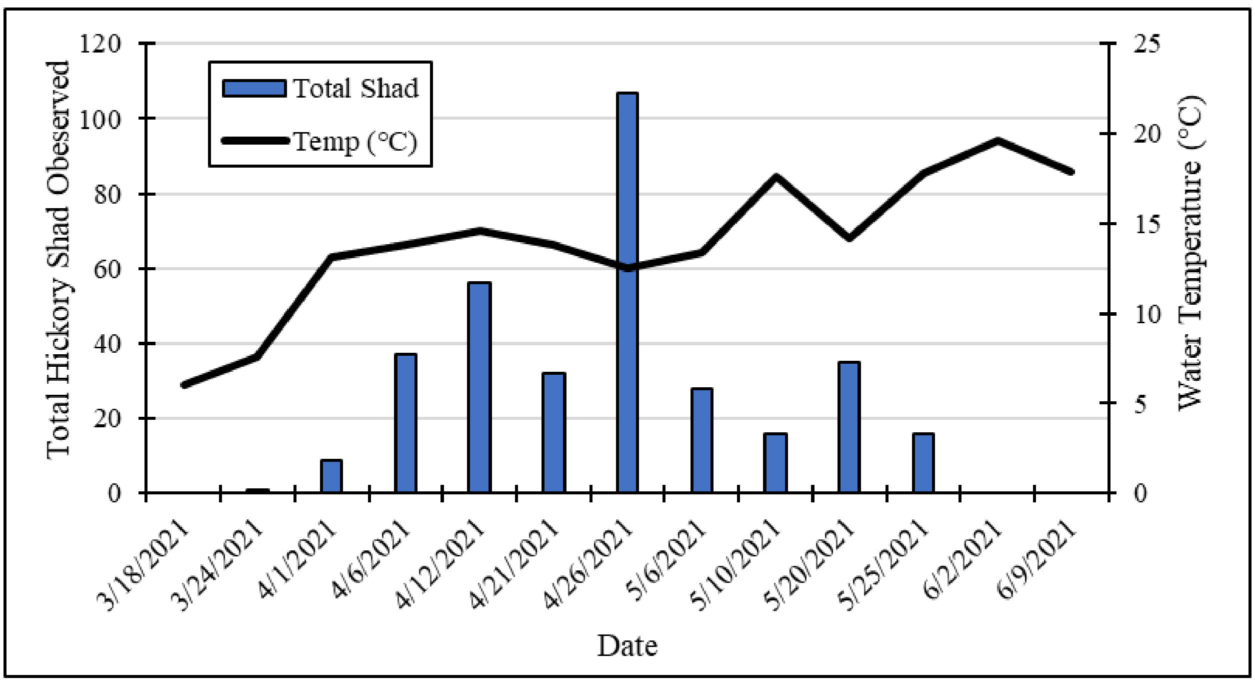

Figure 3.8. *2021 Maryland Department of Natural Resources electrofishing collections and observations of adult Hickory Shad in the Choptank River in relation to water temperature*.

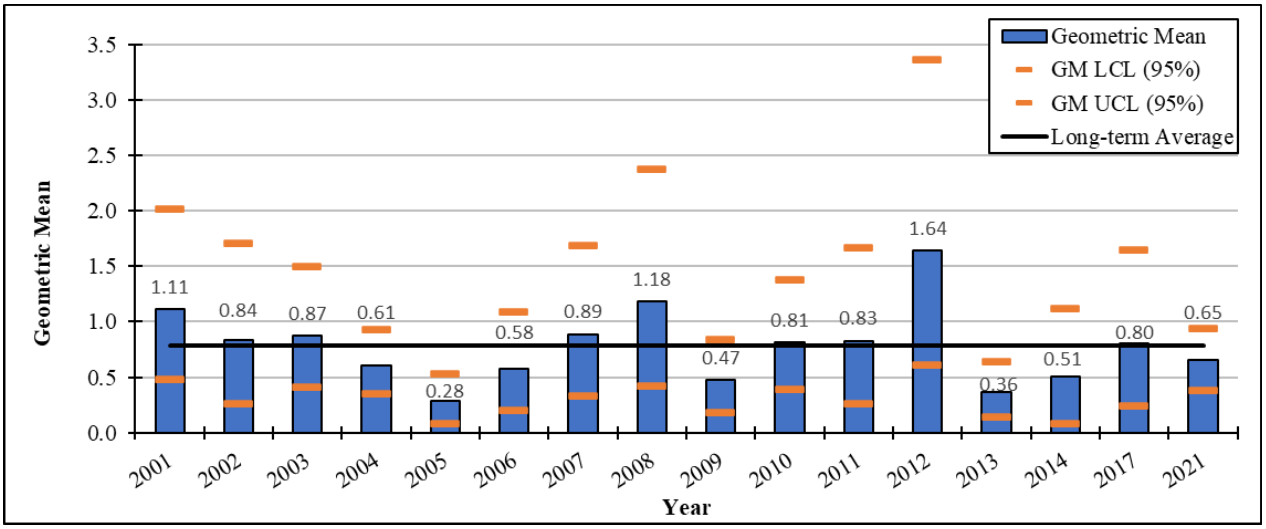

Figure 3.9. *Maryland Department of Natural Resources electrofishing survey, Choptank River adult Hickory Shad geometric mean (GM) with 95% confidence intervals of CPUE for sample years 2001-2021. Long-term average = 0.78 fish/min. Beginning and ending zero CPUEs were omitted from analysis.*

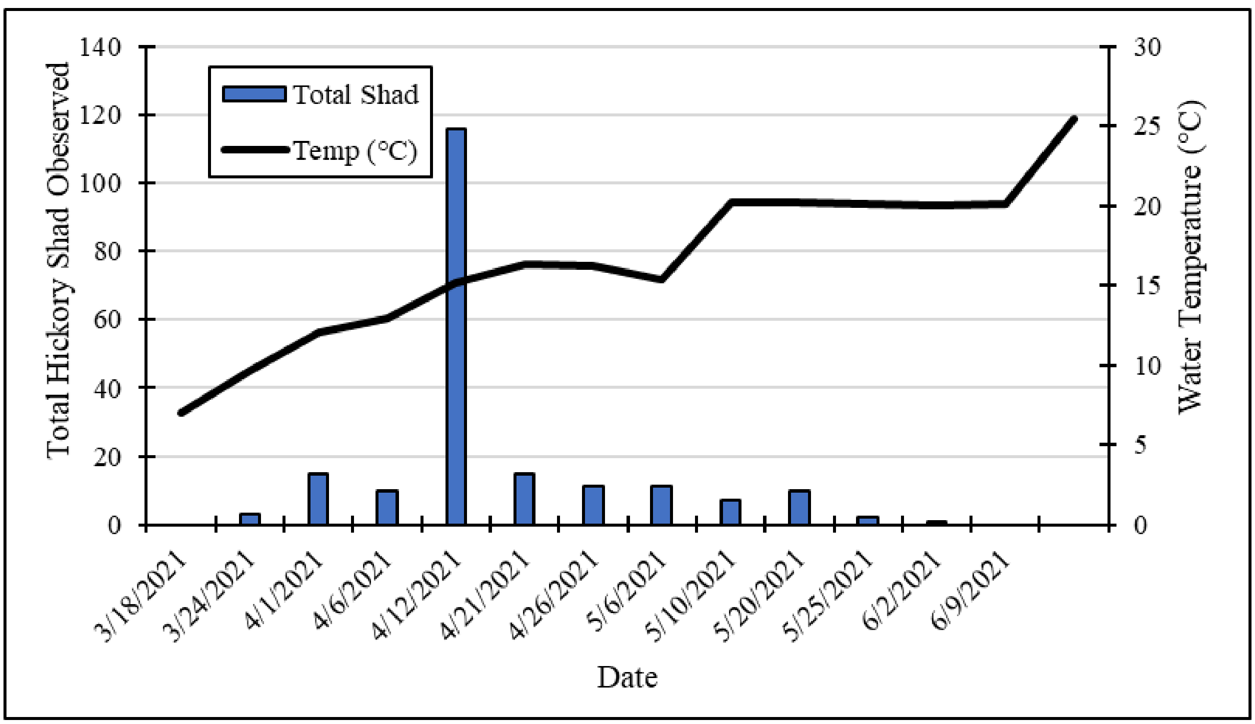

Figure 3.10. *2021 Maryland Department of Natural Resources electrofishing collections and observations of adult Hickory Shad in the Patuxent River in relation to water temperature*.

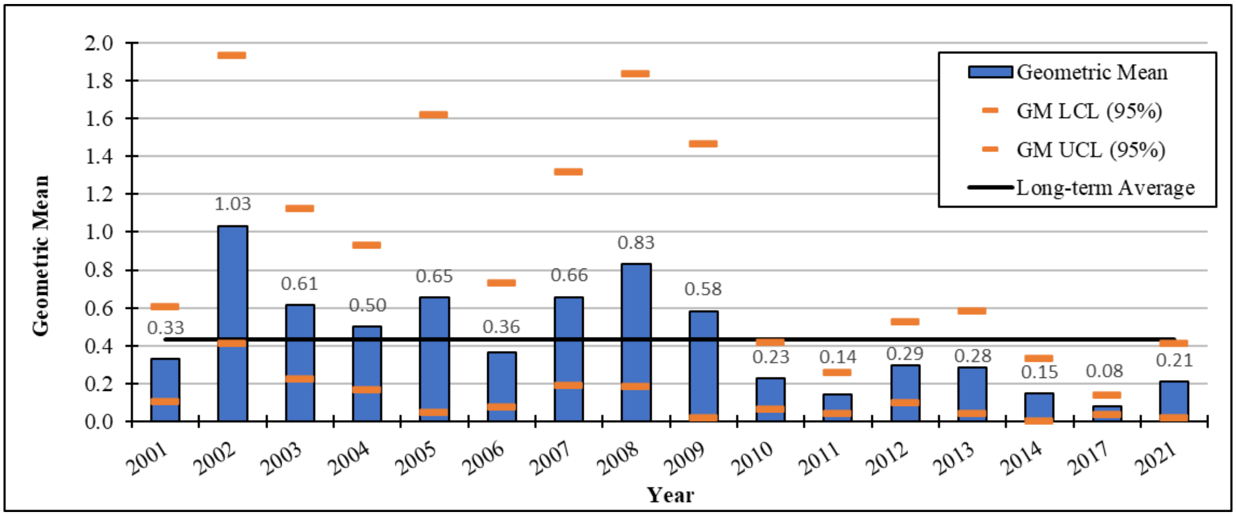

Figure 3.11. *Maryland Department of Natural Resources electrofishing survey, Patuxent River adult Hickory Shad geometric mean (GM) with 95% confidence intervals of CPUE for sample years 2001-2021. Beginning and ending zero CPUEs were omitted from analysis.*