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## Genetic Analyses of Striped Bass in the Chesapeake Bay: an Investigation of Connectivity Among Virginia Subestuaries and an Evaluation of Close-Kinship Mark Recapture Methodology to Estimate Spawning Abundance.

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Genetic analyses of striped bass in the Chesapeake Bay: an investigation of connectivity among Virginia subestuaries and an evaluation of close-kinship mark recapture methodology to estimate spawning abundance.

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A Thesis  
Presented to

The Faculty of the School of Marine Science  
The College of William and Mary in Virginia

In Partial Fulfillment  
of the Requirements for the Degree of  
Master of Science

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by  
Savannah Ann Michaelsen  
August 2018

## APPROVAL PAGE

This thesis is submitted in partial fulfillment of  
the requirements for the degree of  
Master of Science

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Savannah Ann Michaelson

Approved by the Committee, July 2018

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## ABSTRACT

The striped bass (*Morone saxatilis*) is an anadromous fish distributed along the eastern coast of North America that currently supports one of the most lucrative and important commercial and recreational fisheries in the region. Since the recovery of the Atlantic stock after a collapse in the late 1970s, studies have focused on understanding the connectivity of major spawning grounds and improving methods of abundance estimation. Studies support strong site fidelity of striped bass to major estuaries along the Atlantic coast, but there has been disagreement about connectivity within the largest spawning ground, the Chesapeake Bay. Additionally, no estimates exist for striped bass abundance within the Chesapeake Bay. The objectives of my thesis were to examine the fine scale genetic population structure of striped bass within the lower Chesapeake Bay, and to test the feasibility of a novel, fishery-independent molecular methodology, close-kinship mark-recapture analysis (CKMR), to estimate spawning adult abundance within the Rappahannock River. Sampling of 1,132 adult striped bass and 389 young-of-year (YOY) striped bass was done during the 2016 and 2017 spawning seasons on major spawning grounds of the James, Mattaponi, and Rappahannock rivers. Twenty microsatellite loci were used to examine both the spatial genetic heterogeneity among the river systems and the temporal heterogeneity between sampling years within a river. Significant population pairwise  $F_{ST}$  values were recovered from 18 of the 21 pairwise comparisons. However, mean  $F_{ST}$  values between temporal comparisons were higher than those among spatial comparisons, suggesting a lack of biologically meaningful population structure among rivers. Additional analyses and a 30-year tagging data set also support a rate of connectivity among the major rivers high enough to maintain similar allele frequencies. Combined, the data support one genetic stock of striped bass within the lower Chesapeake Bay. The same suite of markers was then used to test the feasibility of CKMR to estimate adult abundance of striped bass within the Rappahannock River system. Using existing sampling programs, 371 spawning adults and 389 YOY were collected on the spawning and nursey grounds of the Rappahannock River in 2016. These samples yielded 2 parent-offspring pairs, resulting in an abundance estimate of 145,081 adult spawning striped bass. Additional analyses indicated that a relatively precise estimate (recovery of 50 POPs) would be made if sample sizes totaled 850 adults and 850 YOY. CKMR can be a feasible option of abundance estimation for striped bass. Overall, my study has provided the first estimate of abundance for Chesapeake Bay striped bass, and has provided strong support of a single, spawning stock of striped bass within the Chesapeake Bay.

Genetic analyses of striped bass in the Chesapeake Bay: an investigation of connectivity among Virginia subestuaries and an evaluation of close-kinship mark recapture methodology to estimate spawning abundance

CHAPTER I

GENERAL INTRODUCTION

## Introduction

Striped bass (*Morone saxatilis*) support one of the largest commercial and recreational fisheries of the United States Atlantic coast (ASMFC, 2016). The Atlantic stock of striped bass collapsed in the late 1970s due to overfishing and habitat degradation (ASMFC, 2013). Today the striped bass stock has rebounded, but there are many uncertainties associated with the assessment of the stock. This study examined two of these uncertainties: genetic connectivity of striped bass among the major sub-estuaries of Chesapeake Bay and the estimation of abundance of adult striped bass within a sub-estuary. A suite of highly polymorphic molecular markers was used to investigate genetic connectivity of striped bass among major Virginia sub-estuaries of the Chesapeake Bay. In addition, the applicability of a novel fishery independent method, close-kinship mark-recapture analysis (CKMR), was evaluated to provide an independent estimate of the size of the striped bass spawning stock within a major spawning ground, the Rappahannock River.

Striped bass are an anadromous species that undertake a seasonal migration from coastal marine waters, through estuaries, and into fresh water for spawning (Paramore and Rulifson, 2001). Anadromy is not displayed by all striped bass, and some individuals exhibit resident 'riverine' life histories (Secor et al., 2000; Paramore and Rulifson, 2001). Differences in life histories are influenced by fish size, sex, year class strength, and latitude (Setzler et al., 1980; Dunning et al., 2006; Ng et al., 2007; Callihan et al., 2014).

Striped bass exhibit sexual dimorphism in population dynamics and life histories including growth rates, movement patterns, and maturity schedules. Males mature much faster than females, reaching 50% maturity by age 1 (ASMFC 2013), and are thought to exhibit larger proportions exhibiting resident riverine life histories than females (Mansueti 1961; Hassin et al., 2000; Secor and Piccoli, 2004). Historically, females have been thought to reach 50% maturity between years 6 and 7 (ASMFC, 2016; Andrews et al., 2017). Female striped bass are thought to live longer than males, with >90% of fish caught over age 10 identified as female (ASMFC, 2016). The coastal migratory stock is comprised of up to 70% females of 2+ years of age (Secor and Piccoli, 2004), and these fish mix with the male dominated residential estuarine populations in the mid to upper freshwater reaches of major river systems during spawning season, which occurs in spring (late March to May) (Kohlenstein 1981; Chapman 1990).

The peak of striped bass spawning season is generally between April and May. Females are batch spawners capable of producing millions of pelagic eggs during spawning events (Secor and Houde, 1998). Survival of striped bass larvae is heavily affected by environmental conditions, such as freshwater flow and temperature (Ulanowicz and Polgar, 1980; Secor and Houde, 1995; Richards and Rago, 1999). The freshwater flow affects the location of the salt front and estuarine turbidity maximum (ETM), and this, in turn, influences the quantity and quality of food available for larval striped bass (North and Houde, 2003). Survival of striped bass larvae is greatest between 15°C and 19°C and temperatures outside of this range lead to higher rates of mortality (Secor and Houde, 1995; Secor, 2006). It is hypothesized that the striped bass life history strategy to spawn multiple batches over a long season increases the chances that some

larvae will be exposed to optimal environmental conditions including temperatures and freshwater flows (Secor and Houde, 1995). Within the Chesapeake Bay there are numerous areas that serve as spawning grounds for striped bass, and if one area has conditions not conducive to larval survival, another area may have conditions favoring high recruitment, ensuring the continuance of the population (Secor 2006).

The Chesapeake Bay is considered one of the most important spawning grounds for striped bass (Kernehhan 1981; Kohlenstein 1981; Richards and Rago, 1999). While contributions of the major spawning areas may vary annually depending on both the number of individuals spawning in the area and the impact of environmental variables, the Chesapeake Bay stock has been estimated to contribute as much as 84% of the total Atlantic stock in some years (Berggren and Lieberman, 1978; Waldman et al., 2012). A recent genetic study indicated that Chesapeake Bay striped bass have served as a large source of recruits supporting other Atlantic coastal regions (Gautier et al., 2013). Additional major spawning areas, in order of contribution to the Atlantic stock, include the Hudson River, the Delaware River, and the Roanoke River (Wirgin et al., 1993). Results of conventional tagging, acoustic tagging, and genetic studies demonstrate spawning site fidelity to the major spawning estuaries (Nichols and Miller, 1967; Ng et al., 2007; Gautier et al., 2013).

Striped bass have supported important commercial and recreational fisheries along the eastern US Atlantic coast since the 1600s (Richards and Rago, 1999). Recreational fisheries exist across the range of the Atlantic stock (Canada to South Carolina), while commercial fisheries operate in Massachusetts, Rhode Island, New York, Delaware, Maryland, Virginia, and North Carolina (ASMFC, 2013). In 2015, commercial fishery

landings of striped bass totaled 617,698 fish, primarily aged 3-10 years. Over 80% of the landings were from Chesapeake Bay, consisting of fish aged 3-8 years (ASMFC, 2016). The largest commercial fishery is currently in Maryland with landings of over 350,000 fish a year (Shepard et al., 2016). Recreational fisheries are larger, with 1.34 million fish landed in 2015 from Virginia, Maryland, New Jersey, and Massachusetts targeting fish aged 4-10 years (ASMFC 2016).

In the late 1970s and early 1980s, managers became alarmed by drastically decreasing catches of striped bass from commercial and recreational fisheries, as well as high rates of estimated fishing mortality for young striped bass (Richards and Deuel, 1987; Gibson, 1993). The fisheries did not experience an immediate collapse, but a slow decline as the successful year classes of older fish sustained the fisheries until 1983 (Richards and Rago, 1999). At that time, the majority of fish were less than 6 years of age (Gibson, 1993). The steady decline of the Atlantic stock biomass and changing environmental conditions resulted in a series of years with poor recruitment classes, and the spawning biomass was not able to replace itself (Goodyear 1985). As the number of older fish declined, there was increased fishing pressure on younger fish.

Due to the migratory nature of striped bass, independent (non-coordinated) state management measures were not effective in preventing overfishing. In several instances, measures to preserve and rebuild the spawning stock biomass adopted by one state were offset by less restrictive conservation measures in adjoining states (Richards and Rago, 1999). To ensure that all states participated equally in the management and conservation of striped bass, Congress enacted the Emergency Striped Bass Research Study in 1979. The measure allowed federal oversight of striped bass by the U.S. Fish and Wildlife



Service and the National Marine Fisheries Service (Richards and Deuel, 1987), and it provided research funding to federal agencies, state agencies, and academic institutions to determine the causes of the decline in the striped bass population with research published in annual reports (Shepard et al., 2005). The congressional measure also directed the Atlantic States Marine Fisheries Commission (ASMFC) to develop a striped bass fisheries management plan (FMP) which was implemented in 1981. Shortly thereafter, in 1984, Congress approved the Striped Bass Conservation Act (Field 1997; Richards and Rago, 1999). This legislation gave the ASMFC power to implement management policies across multiple states by allowing the secretaries of Commerce and Interior to place a moratorium on striped bass fishing for any state not complying with the FMP. The act was amended into the 1990s to provide continued federal oversight and funding to the recovery of striped bass stocks (Public Law 98-613).

The striped bass spawning stock decline during the late 1970s and 1980s in the Chesapeake Bay led to a decrease of the Atlantic Stock overall (Richards and Deuel, 1987). The primary cause of the population decline was a lack of strong recruitment from the Chesapeake Bay since 1970 that was combined with total mortality rates of 60-93% for males and 45% for females (Richards and Deuel, 1987). In addition, immature striped bass were experiencing high rates of fishing mortality from a lack of minimum size regulations and overfishing. Comparisons of fishing mortalities between the Hudson River, which did not experience a major stock collapse, and the Chesapeake Bay indicated that the Hudson River striped bass experienced about half the fishing mortality of those in the Chesapeake Bay (Richards and Rago, 1999). In addition to overfishing, the striped bass population was affected by degraded water quality (Hall 1989; Finger et

al., 1998), habitat impairment (Richards and Rago, 1999), nutritional stress (Setzler-Hamilton et al., 1981), and overall poor environmental conditions (Hall et al., 1993).

Prior to the stock crash, the only management measures for striped bass were minimum size limits that ranged from 10-14 inches total length (TL) depending on the state (ASMFC, 2013). The implementation of the Fisheries Management Plan for striped bass by the ASMFC in 1981 resulted in a minimum size limit for all states of 14 inches TL within bays and estuaries and 24 inches TL in coastal areas. Additionally, ASMFC recommended a fishing closure on spawning grounds during spawning season. ASMFC amended the FMP in 1984 and again in 1985, to set fishing mortality targets on a state by state basis (Richards and Rago, 1999). A third amendment adopted in 1985 included measures to protect a strong 1982-year class in the Chesapeake Bay. A minimum size of 38 inches TL was implemented to ensure that 95% of the females of the 1982-year class survived to have an opportunity to spawn. Rather than requiring the large minimum size, most states, with the exception of Massachusetts, implemented a moratorium on the striped bass fishery (Richards and Rago, 1999; ASMFC 2013). Mandatory monitoring surveys in subsequent years indicated that populations were recovering, and an amendment to the FMP reopened the fishery in 1990 with a target fishing mortality of  $F=0.25$ . Dual minimum size limits of 18 inches TL in bays and estuaries and 28 inches TL in coastal areas were implemented, as well as fishery restrictions including recreational trip limits and commercial fishing seasons (ASMFC 2013).

As a result of management efforts that reduced fishing mortality on striped bass and favorable environmental conditions that facilitated good recruitment, the striped bass spawning biomass within the Chesapeake Bay was restored to management thresholds by

1995 (Field 1997). An amendment to the striped bass FMP in 1995 eased some of the existing strict fishery regulations, but still allowed for the stock to continue to rebuild. Coastal states were also provided greater freedom to adjust regulations to fit their needs as long as ASMFC goals were being met (Richards and Rago, 1999). The population size of striped bass peaked in 1997 at 249 million fish, and the stock remained close to this size until 2005 when abundance began to decline (ASMFC 2013). Additional amendments to the striped bass FMP have since been adopted, the largest of which includes management triggers to protect female spawning biomass. Due to declines in the female SSB, management action was triggered in 2014 that resulted in 2015 harvest quotas being cut 25% for coastal states and 20.5% for Chesapeake Bay states (ASMFC 2015).

The 2016 striped bass assessment update indicated that the Atlantic stock of striped bass is not overfished nor experiencing overfishing; however, the stock is not considered fully rebuilt as the female spawning stock biomass (SSB) is estimated at 58,853 metric tons, just above the biomass threshold of 57,626 metric tons and well below the biomass target of 72,032 metric tons (ASMFC 2016). A lack of strong recruitment since 2012 has led to a further reduction in abundance of striped bass on the Atlantic Coast. Projections of the 2016 Atlantic Striped Bass Stock Assessment Update indicate that there is ~39% probability that female SSB will drop below the management biomass threshold over the next three years. Some studies have attributed this recent decline to an increase in the prevalence of *Mycobacteriosis*, a bacterial disease in striped bass shown to have large impacts on reproductive output and natural mortality (Gautier et al., 2008; Gervasi 2015; Hoenig et al., 2017), while others have attributed the decline to a

reduction in the availability of prey species (ASMFC 2013). Declines in the Atlantic stock often result from declines in Chesapeake Bay (Richard and Deuel, 1987). In 2015, estimates of the SSB within Virginia rivers for both males and females were both ~40% below the average SSB for the years 1987 to 2015, with survival estimates the lowest seen in recent years (Hoenig et al., 2016).

The recent declining trend in abundance of striped bass within the Chesapeake Bay has highlighted the need for an understating of the contributions of each subestuary to the Chesapeake Bay striped bass population. While connectivity and abundance of the Atlantic coastal stock is relatively well known, there has been little focus within Chesapeake Bay. The Chesapeake Bay is differentiated from other major striped bass spawning grounds because it is a large, complex estuary composed of several major subestuary river systems (Marshall and Alden, 1990; Boynton et al., 1995) while all other areas largely consist of one large river system with much smaller supporting tributaries (Chittenden 1971; Carmichael et al; 1998). Each of the subestuaries within Chesapeake Bay has the potential to host separate spawning populations of striped bass. Knowledge regarding the stock structure of striped bass within subestuaries of Chesapeake Bay, and estimates of the number of breeding individuals on each spawning ground can help fishery managers to address regionally the recent decline of striped bass spawning stock biomass.

Researchers have studied striped bass population structure within Chesapeake Bay since the early 20<sup>th</sup> century. Investigations of morphological characteristics (Lewis 1957; Lund 1957; Raney 1957; Murawski 1958) and tagging studies (Massmann and Pacheco, 1961; Nichols and Miller, 1967) supported the hypothesis of distinct stocks of

striped bass within Chesapeake Bay subestuaries. Modern molecular studies that examined genetic population structure using allozymes (Grove et al., 1976; Sidell et al., 1980), mitochondrial DNA (Chapman 1987, 1990; Wirgin et al., 1989, 1993), and nuclear DNA microsatellite loci (Laughlin and Turner, 1996; Diaz et al., 1997; Brown et al., 2005; Gauthier et al., 2013) have reached conflicting conclusions concerning the existence of separate striped bass stocks on the different subestuary spawning grounds within Chesapeake Bay. Additionally, there is little information regarding the abundance and spawning stock size of striped bass within subestuaries of Chesapeake Bay.

The research recommendations highlighted in the 2013 Striped Bass Benchmark Assessment included an in-depth analysis of stock composition and the need for the development of an independent estimate of abundance. The objective of this study was to address these two recommendations. Using a suite of highly polymorphic molecular markers, the second chapter of this thesis evaluated genetic connectivity of striped bass to determine if genetically distinct spawning populations exist within the Chesapeake Bay to provide an in-depth description of the stock composition. Using the same molecular markers, the third chapter of this thesis assessed the applicability of a novel fishery independent method, close-kinship mark-recapture analysis (CKMR), to estimate of the size of the striped bass spawning stock for striped bass within a well-known spawning river, the Rappahannock River.

## References

- Atlantic States Marine Fisheries Commission. 2013. Striped Bass Stock Assessment for 2013. Arlington, VA. Retrieved from [http://www.asmfc.org/uploads/file/529e5ca12013StripedBassBenchmarkStockAssessment\\_57SAWReport.pdf](http://www.asmfc.org/uploads/file/529e5ca12013StripedBassBenchmarkStockAssessment_57SAWReport.pdf).
- Atlantic States Marine Fisheries Commission. 2015. 2015 Atlantic Striped Bass Stock Assessment Update. Arlington, VA. Retrieved from [http://www.asmfc.org/uploads/file/564106f32015AtlStripedBassAssessmentUpdate\\_Nov2015.pdf](http://www.asmfc.org/uploads/file/564106f32015AtlStripedBassAssessmentUpdate_Nov2015.pdf).
- Atlantic States Marine Fisheries Commission. 2016. 2016 Atlantic Striped Bass Stock Assessment Update. Arlington, VA. Retrieved from [http://www.asmfc.org/uploads/file/581ba8f5AtlStripedBassTC\\_Report2016AssmtUpdate\\_Oct2016.pdf](http://www.asmfc.org/uploads/file/581ba8f5AtlStripedBassTC_Report2016AssmtUpdate_Oct2016.pdf).
- Andrews SN, Linnansaari T, Curry RA, Dadswell MJ. 2017. The misunderstood striped bass of the Saint John River, New Brunswick: Past, present, and future. *North American Journal of Fisheries Management* 37(1):235-254.
- Berggren TJ, Lieberman JT. 1978. Relative contribution of Hudson, Chesapeake and Roanoke striped bass, *Morone saxatilis*, stocks to the Atlantic coast fishery. *U. S. Fish. Bull.* 76: 335-345.
- Boynton WR, Garber JH, Summers R, Kemp WM. 1995. Inputs, transformations, and transport of Nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* 18(1): 285-314.

- Brown KM, Baltazar GA, Hamilton MB. 2005. Reconciling nuclear microsatellite and mitochondrial marker estimates of population structure: breeding population structure of Chesapeake Bay striped bass (*Morone saxatilis*). *Heredity* 94: 606-615.
- Callihan, JL. Godwin CH, Buckel JA. 2014. Effect of demography on spatial distribution: movement patterns of the Albemarle Sound-Roanoke River stock of Striped Bass (*Morone saxatilis*) in relation to their recovery. *Fishery Bulletin* 112: 131-143.
- Carmichael JT, Haeseker SL, Hightower JE. 1998. Spawning migration of telemetered striped bass in the Roanoke River, North Carolina. *Transactions of the American Fisheries Society* 127(2): 286-297.
- Chapman RW. 1987. Changes in the population structure of male striped bass, *Morone saxatilis*, spawning in three areas of Chesapeake Bay from 1984 to 1986. *Fishery Bulletin* 85: 167-171.
- Chapman RW. 1990. Mitochondrial DNA analysis of striped bass populations in the Chesapeake Bay. *Copeia*, 1990: 355-366.
- Chittenden ME. 1971. Status of the striped bass, *Morone saxatilis*, in the Delaware River. *Chesapeake Science* 12(3): 131-136.
- Diaz M, Leclerc GM, Fishtec BE. 1997. Nuclear DNA markers reveal low levels of genetic divergence among Atlantic and Gulf of Mexico populations of striped bass. *Transactions of the American Fisheries Society* 126(1): 163-165.

- Dunning DJ, Ross QE, Blumberg AF, Heimbuch DG. 2006. Transport of striped bass larvae out of the lower Hudson River estuary. *American Fisheries Society Symposium* 51: 273.
- Field J. 1997. Atlantic striped bass management: where did we go right? *Fisheries* 22(7):6–8.
- Finger SE, Livingstone AC, Olson SJ. 1998. Influence of contaminants on survival of striped bass in Chesapeake Bay tributaries. In *Second US–USSR symposium on reproduction, rearing, and management of anadromous fishes* Edited by: Weaver, J. E. 77–86. Seattle: U.S. Geological Survey, Biological Resources Division.
- Gautier DT, Latour RJ, Heisey DM, Bonzek CF, Gartland J, Burge EJ, Vogelbein WK. 2008. *Mycobacteriosis* associated mortality in wild striped bass (*Morone saxatilis*) from Chesapeake Bay, USA. *Ecological Applications* 18: 1718-1727.
- Gauthier DT, Audemard CA, Carlsson JEL, Darden TL, Denson MR, Reece KS, Carlsson J. 2013. Genetic population structure of US Atlantic coastal striped bass (*Morone saxatilis*). *Journal of Heredity* 104: 510-520.
- Gervasi CL. 2015. The reproductive biology of striped bass (*Morone saxatilis*) in Chesapeake Bay (MS Thesis). [Williamsburg, (VA)]: College of William & Mary.
- Gibson MR. 1993. Historical estimates of fishing mortality on the Chesapeake Bay striped bass stock using Separable Virtual Population Analysis applied to market class catch data. Report of Rhode Island Division of Fish and Wildlife to the Atlantic States Marine Fisheries Commission, West Kingston.



- Goodyear CP, Cohen JE, Christensen SW. 1985. Maryland striped bass: recruitment declining below replacement. *Transactions of the American Fisheries Society*, 114: 146–151.
- Grove TL, Berggren TS, Powers DA. 1976. The use of innate genetic tags to segregate spawning stocks of striped bass (*Morone saxatilis*). *Estuarine Processes* 1: 166-176.
- Hall LW Jr., Ziegenfuss MC, Bushong SJ, Unger MA, Herman RL. 1989. Studies of contaminant and water quality effects on striped bass prolarvae and yearlings in the Potomac River and upper Chesapeake Bay in 1988. *Transactions of the American Fisheries Society*. 118(6): 619-629.
- Hall LW, Finger SE, Ziegenfuss MC. 1993. A review of in situ and on-site striped bass contaminant and water-quality studies in Maryland waters of the Chesapeake Bay watershed. In *Water quality and the early life stages of fishes, Symposium 14* Edited by: Fuiman, L. A. 3–15. Bethesda, Maryland: American Fisheries Society.
- Hassin S, Claire M, Holland H, Zohar Y. 2000. Early maturity in the male striped bass, *Morone saxatilis*: follicle-stimulating hormones and luteinizing hormone gene expression and their regulation by gonadotropin-releasing hormone analogue and testosterone. *Biology of Reproduction* 63(6): 1691-1697.
- Hoenig JM, Sadler PW, Michaelsen S, Goins LM, Harris RE (2016) Evaluation of striped bass stocks in Virginia: monitoring and tagging studies, 2015-2019, Progress Report.VMRC.
- Hoenig JM, Groner ML, Smith MW, Vogelbein WK, Taylor DM, Landers Jr DF, Swenarton JT, Gauthier DT, Sadler P, Matsche MA, Haines AN. (2017) Impact of

- disease on the survival of three commercially fished species. *Ecological Applications* 27: 2116-2127.
- Kernehan RJ, Headrick MR, Smith RE. 1981. Early life history of Striped Bass in the Chesapeake and Delaware Canal and vicinity. *Transactions of the American Fisheries Society* 110: 137-150.
- Kohlenstein LC. 1981. On the proportion of the Chesapeake Bay stock of Striped Bass that migrates into the coastal fishery. *Transactions of the American Fisheries Society* 110: 168-179.
- Laughlin TF, Turner BJ. 1996. Hypervariable DNA markers reveal high genetic variability within striped bass populations of the lower Chesapeake Bay. *Transactions of the American Fisheries Society* 125: 49-55.
- Lewis RM. 1957. Comparative study of the populations of the striped bass. U.S. Fish and Wildlife Service Species Science Report, 204.
- Lund WA Jr. 1957. Morphometric study of the striped bass, *Roccus saxatilis*. U.S. Fish and Wildlife Service Species Science Report, 216.
- Mansueti RJ. 1961. Age, growth, and movements of the striped bass, *Roccus saxatilis*, taken in size selective fishing gear in Maryland. *Chesapeake Science* 2: 9-36.
- Marshall HG, Alden RW. 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine rivers of the lower Chesapeake Bay. *Estuaries* 13(3): 287-300.
- Massmann WH, Pacheco AL. 1961. Movements of striped bass tagged in Virginia waters of the Chesapeake Bay. 2(1): 3-44.

- Murawski WS. 1958. Comparative study of populations of striped bass, *Roccus saxatilis*, based on lateral line scale counts. (MS Thesis), [Ithaca (NY)]: Cornell University.
- Ng CL., Able KW, Grothues TM. 2007. Habitat use, site fidelity, and movement of adult Striped Bass in a southern New Jersey estuary based on mobile acoustic telemetry. *Transactions of the American Fisheries Society* 136:1344-1355.
- Nichols PR, Miller RV. 1967. Seasonal movements of striped bass, *Roccus saxatilis* (Walbaum), tagged and released in the Potomac River, Maryland, 1959–1961. *Chesapeake Science*, 8: 102–124.
- North EW, Houde ED. 2003. Linking ETM physics, zooplankton prey, and fish early-life histories to striped bass, *Morone saxatilis*, and white perch, *M. Americana*, recruitment. *Marine Ecology Progress Series* 260:219-236.
- Paramore LM, Rulifson RA. 2001. Dorsal coloration as an indicator of different life history patterns for Striped Bass within a single watershed of Atlantic Canada. *Transactions of the American Fisheries Society* 130: 663-674.
- Raney, EC. 1957. Subpopulations of the Striped Bass *Roccus saxatilis* (Walbaum) in Tributaries of Chesapeake Bay. US Fish and Wildlife Service.
- Richards RA, Deuel DG. 1987. Atlantic striped bass: stock status and the recreational fishery. U.S. National Marine Fisheries Service *Marine Fisheries Review*, 49(2): 58–66.
- Richards RA, Rago PJ. 1999. A case history of effective fishery management: Chesapeake Bay striped bass. *North American Journal of Fisheries Management* 19(2): 356-375.
- Secor DH. 2006. The year-class phenomenon and the storage effect in marine fishes. *Journal of Sea Research* 57: 91-103.

- Secor DH, Houde ED. 1995. Temperature effects on the timing of Striped Bass egg production, larval viability, and recruitment potential in the Patuxent River (Chesapeake Bay). *Estuaries*, 18: 527-544.
- Secor DH, Houde ED. 1998. Use of larval stocking in restoration of Chesapeake Bay striped bass. *ICES Journal of Marine Science* 55(2): 228-239.
- Secor DH, Piccoli PM. 2004. Oceanic migration rates of upper Chesapeake Bay striped bass (*Morone saxatilis*) determined by otolith microchemical analysis. *Fishery Bulletin* 105: 62-73.
- Secor DH, Gunderson TE, Karlsson K. 2000. Effect of temperature and salinity on growth performance in anadromous (Chesapeake Bay) and nonanadromous (Santee-Cooper) strains of Striped Bass *Morone saxatilis*. *Copeia* 2000: 291-296.
- Setzler EM, Boynton WR, Wood KV, Zion HH, Lubbers L, Mountford NK, Frere P, Tucker L, Mihursky JA. 1980. Synopsis of biological data on Striped Bass, *Morone saxatilis* (Walbaum). NOAA technical report NMFS circular. FAW Fisheries Synopses (FAO) no. 121.
- Setzler-Hamilton EM, Boynton WR, Mihursky JA, Polgar TT, Wood KV. 1981. Spatial and temporal distribution of striped bass eggs, larvae, and juveniles in the Potomac estuary. *Transactions of the American Fisheries Society*, 110: 121–136.
- Shepherd GR, Laney RW, Appelman M, Honabarger D, Wright CL. 2016. Biennial Report to Congress on the Progress and Findings of Studies of Striped Bass Populations-2015, 13 p. National Marine Fisheries Service, Silver Spring, MD. Shepherd GR, Laney RW, Meyer T, Beal R. 2005. Atlantic Striped Bass Studies 2005 Biennial Report to Congress, 39 p. National Marine Fisheries Service, Silver Spring, MD.

- Sidell, BD, Otto RG, Powers DA, Kerweit M, Smith J. 1980. A reevaluation of the occurrence of subpopulations of striped bass (*Morone saxatilis*, Walbaum) in the Upper Chesapeake Bay. Transactions of the American Fisheries Society 109:99-107.
- Striped Bass Conservation Act of 1984, Public Law No. 98-613, § 98 STAT 3187 (1984).
- Ulanowicz, RE. Polgar TT. 1980. Influences of anadromous spawning behavior and optimal environmental conditions upon striped bass (*Morone saxatilis*) year-class success. Canadian Journal of Fisheries and Aquatic Sciences 37: 143-154.
- Waldman J, Maceda L, Wirgin I. 2012. Mixed-stock analysis of wintertime aggregations of Striped Bass along the Mid-Atlantic coast. Journal of Applied Ichthyology 28:2012.
- Wirgin I, Procenca R, Grossfield J. 1989. Mitochondrial DNA diversity among populations of striped bass in the Southeastern United States. Canadian Journal of Zoology 67: 891-907.
- Wirgin I, Maceda L, Waldman JR, Crittenden RN. 1993. Use of mitochondrial DNA polymorphisms to estimate the relative contributions of the Hudson River and the Chesapeake Bay striped bass stocks to the mixed fishery on the Atlantic Coast. Transactions of the American Fisheries Society 122: 669-684.

CHAPTER II  
ASSESSMENT OF STRIPED BASS GENETIC STRUCTURE AND  
CONNECTIVITY OF THE LOWER CHESAPEAKE BAY

## **Introduction**

Striped bass are distributed along the Atlantic coast of North America from Canada to northern Florida and support important recreational and commercial fisheries throughout their range (Kirkley et al., 2000). The species exhibits multiple life history strategies including riverine residential populations in the southern portion of the range and coastal, more migratory populations in the northern portion of the range (Boreman and Lewis, 1987; Greene et al., 2009; Wingate et al., 2011). Striped bass spawning occurs in the freshwater reaches of major river and estuary systems (Paramore and Rulifson, 2001), and both tagging and genetic studies have indicated significant spatial population structuring among the major spawning grounds of the Atlantic stock which include the Hudson River, Delaware Bay, Chesapeake Bay, and the Roanoke River (Wirgin et al., 1993; Gautier et al., 2013). Of these systems, the Chesapeake Bay is considered the largest and most productive spawning ground (Kohlenstein 1981), contributing upwards of 90% of the stock composition in some years (Waldman et al., 2012).

Striped bass are managed as a single stock but results from tagging and molecular genetic studies suggest the presence of distinct spawning populations in major estuary systems along the Atlantic Coast (Merriman, 1941; Kohlenstein 1981; Fabrizio, 1987; Dorazio et al., 1994; Lindley et al., 2011). Conventional tagging studies (Mansueti,

1961; Nichols and Miller; 1967; McLaren et al., 1981) and acoustic tagging studies (Ng et al., 2007; Gahagen et al., 2015; Callihan et al., 2015) have demonstrated that striped bass exhibit a high rate of spawning site fidelity during the spawning season to major spawning grounds. Genetic analyses of striped bass of both mitochondrial DNA (mtDNA) and nuclear DNA markers have reported significant genetic heterogeneity among striped bass from the major Atlantic coast spawning grounds (Wirgin et al., 1993; Gautier et al., 2013).

While genetic and tagging studies of striped bass indicate that there is spawning site fidelity to the major spawning grounds, spawning site fidelity to river systems, or subestuaries, within the major spawning grounds may also exist. For the Chesapeake Bay, the largest and most diverse estuary that striped bass inhabit (Marshall and Alden, 1990; Boynton et al., 1995), tagging studies have demonstrated high spawning site fidelity to specific subestuaries within the Chesapeake Bay. Studies in which fish were tagged on riverine spawning grounds during the spawning season and ultimately recaptured on spawning grounds during the spawning season in subsequent years reported a fidelity to the spawning ground on which they were tagged in excess of 70% (Vladykov and Wallace, 1938; Massmann and Pacheco, 1961; Nichols and Miller, 1967). Based on the results, these authors hypothesized that separate spawning populations of striped bass exist in each river system, and their hypothesis receives support from subsequent tagging studies (Wingate et al., 2011).

In contrast to conventional tagging, results of genetic studies of striped bass population structure within Chesapeake Bay have been conflicting (Wirgin et al., 1987, 1990; Chapman 1990; Laughlin and Turner, 1996; Brown et al., 2005). Early allozyme



studies found little electrophoretic variation and no significant heterogeneity among striped bass populations sampled during one year within the upper- and mid-Chesapeake Bay (Grove et al., 1976; Sidell et al., 1980). Restriction fragment length polymorphism analyses of striped bass mtDNA found significant heterogeneity among collection locations within Chesapeake Bay (Chapman et al., 1987; Wirgin et al., 1993). The studies sampled spawning adults, with Wirgin et al. (1993) sampling spawning males and Chapman (1990) sampling spawning adults as well as immature fish collected in prior years. Genetic studies analyzing nuclear DNA loci from Chesapeake Bay striped bass have reported conflicting results. Laughlin and Turner (1996) reported no significant genetic differentiation among samples of young-of-year (YOY) striped bass collected during one year from the major subestuaries of the lower Chesapeake Bay. Similarly, Brown et al. (2005) found no significant heterogeneity among pooled samples of YOY striped bass collected from both Virginia and Maryland subestuaries of Chesapeake Bay during two, non-consecutive years. In contrast to Laughlin and Turner (1996) and Brown et al. (2005), Gautier et al. (2013) reported weak, but statistically significant genetic population structuring among collections of YOY striped bass taken from different sampling periods throughout the entire Chesapeake Bay.

The inferences of genetic studies for striped bass population structure within Chesapeake Bay have varied depending on the types of molecular markers analyzed and the sampling framework employed. Resolution of genetic differences for Chesapeake Bay striped bass may be facilitated by using a biologically informed sampling design that includes individuals collected on the spawning grounds during spawning season (or early life history stages that could not have moved far from the spawning grounds), temporal

replicates to assess interannual variation, and large sample sizes of both individuals and molecular markers (Hedgecock, 1991; Ruzzante 1998; Kalinowski 2005). The objective of this study was to closely examine the population genetic structure of striped bass within the lower Chesapeake Bay using a biologically informed sample design that incorporated large numbers of adult striped bass captured on spawning grounds during two consecutive spawning seasons in the James River, the Rappahannock River, and the York River, screened with a panel of 20 highly variable microsatellite loci. Specifically, I was testing the null hypothesis that my collections were drawn from a single genetic population, and that significant population structure would be signaled by higher levels of variation between collections from different rivers in the same year than the variation between collections taken from the same river in consecutive years.

## Materials and Methods

### *Sample Collection*

Caudal fin clips were taken from adult striped bass captured on the spawning grounds of the James, Mattaponi, and Rappahannock rivers during the spawning season (March to May) in 2016 and 2017 (Figure 1). Striped bass were sampled from three commercial pound nets on the Rappahannock River (river miles 46, 47, 55), with samples collected twice a week at all sampling sites from 4 April to 26 May 2016 and 10 April to 27 April 2017. Fish from the James River (river miles 43-62) and Mattaponi River (river miles 30-42) were sampled using multiple-meshed anchored gill nets (3 in-10 in) and a drift gill net (4.5"-8"). The gill nets were fished from 30 min to 60 min, with shorter deployments at higher water temperatures. Only fish with a total length equal to or greater than 458 mm were sampled to ensure that all fish were mature (Mansueti, 1961). Fish were released alive after being tagged. For each individual, fork length and total length were measured, and a sample of scales taken for aging. Sex was determined in the field by expressing reproductive products. Fin clips were stored in 95% ethanol.

Young-of-year striped bass were sampled from the Rappahannock River (rivers miles 28 to 75) from June to September 2016 using a 100 ft long, 4ft deep, 0.25 in mesh beach seine net and had fork lengths of 22 mm to 73 mm, well below 118mm reported mean size for YOY striped bass prior to their first winter (Hurst and Conover, 2003). Genetic samples were taken as fin clips or white muscle tissue and stored in 95% ethanol.

### *Molecular Marker Selection*

Candidate nuclear loci were selected from the more than 500 potential striped bass microsatellite markers available in the literature (Couch et al., 2006; Rexroad et al., 2006; Fountain et al., 2009; Gauthier et al., 2013). Microsatellite loci were selected based on allelic diversity, chromosome location (linkage map from Liu et al., 2011), estimated heterozygosity, and repeat length. Each candidate microsatellite locus was amplified over a thermal gradient to verify optimum annealing temperatures and subsequently assembled into multiplexes using Multiplex Manager (Holleley and Geerts, 2009). Each multiplex was evaluated on a temperature gradient to determine an optimal annealing temperature for the combination of loci. The final selection was of 20 microsatellite loci comprised of four multiplex panels consisting of five loci each (Supplementary Table 1).

### *Extraction and Amplification*

Total genomic DNA was extracted from each fin clip using Machary Nagel NucleoSpin® DNA tissue kits. Extractions were performed in 96-well plates following the manufacturer's protocol on the Tecan Freedom EVO® 75 liquid handling system. DNA quantity and quality was assessed using both a NanoDrop spectrophotometer and Qubit fluorometric quantitation. Microsatellite multiplexes were amplified using the polymerase chain reaction (PCR) with locus-specific fluorescent labels in 10 ul reactions. Following amplification, 2 ul of product was combined with 8 ul of formamide and 0.2 ul 500 LIZ Gene Scan Size standard (Applied Biosystems), and denatured for 10 minutes at 95°C before sequencing on a 36 cm 3130xl Capillary Genetic Analyzer (Applied Biosystems, Inc.). The chromatic peaks representing each microsatellite locus were scored using GeneMarker v2.6.0 (SoftGenetics, LLC). After scoring, evidence of scoring

errors and the presence of null alleles were evaluated using MicroChecker 2.2.3 (Van Oosterhout et al., 2004). Accuracy of allele calling was verified by scoring all electropherograms from the initial year of data twice to ensure consistency in allele calls. To ensure reliability in amplification and allele calling, 5% of all samples were re-analyzed (PCR amplification through allele scoring).

### *Descriptive Statistics*

Observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) were calculated using GenePop v4.0 (10,000 iterations; Rousset 2008), and the conformation of genotypic distributions to expectations of Hardy Weinberg Equilibrium was evaluated using probability tests (10,000 iterations; Guo and Thompson, 1992) with significance values adjusted using the sequential Bonferroni correction for multiple comparisons (Rice, 1990). PopGenReport (Gruber and Adamack, 2014) was used within the statistical language R (R Core Team, 2017) to calculate allele frequencies, number of alleles per locus, mean allelic richness (following El Mousadik and Petit, 1996), genetic distances, private alleles (alleles observed in only one population; Kalinowski 2004), and  $F_{IT}$ ,  $F_{ST}$ , and  $F_{IS}$  values for each locus. Effective population size was estimated using NeEstimator v2.01 (Do et al., 2014) using the linkage disequilibrium method by jackknifing over loci with random mating and a  $P_{crit}$  value of 0.02.

Previous studies have reported that male striped bass tend to be more resident than females (Mansueti, 1961), and genetic results suggest an asymmetrical homing of adults (Chapman et al., 1987; Wirgin et al., 1993; Laughlin and Turner, 1996; Brown et al., 2005). To compare sex-specific connectivity in this study, all analyses were also performed on datasets consisting of only adult males and of only adult females.

### *Population Structure*

Arlequin v 3.5.1.2 (Excoffier and Lischer, 2010) was used to calculate population pairwise  $F_{ST}$  values and significance was assessed based on 10,000 permutations of the data and a critical value based on a modified false discovery rate (Narum 2006) was used that corrected for multiple comparisons. A hierarchical analysis of molecular variance (AMOVA) was performed in Arlequin to examine spatial differences (between rivers), temporal differences (between years), and sex differences over 1,000 permutations of the data. A Mantel Test (Smouse et al., 1986) was used to assess isolation by distance, by using the coordinates of central sampling locality for each river. STRUCTURE v2.3.4 (Pritchard et al., 2000, Falush et al., 2003; Falush et al., 2007) was used to recover any potential population subdivision and genotypic clusters using the R package ParallelStructure run on CIPRES (Miller et al., 2010), a high-performance computing cluster. The simulations utilized the loci prior option (Hubisz et al., 2009), consisted of five iterations of K of 1-7 with a 1,000,000 MCMC after the burn-in of 100,000, and utilized an admixture ancestry model. A principal component analysis was performed to examine the relationships between collections in multivariate space using custom R code.

## Results

### *Population Genetic Statistics*

A total of 2,197 adult and young of year (YOY) striped bass were sampled during 2016 and 2017 from the Rappahannock, James, and Mattaponi rivers. The samples comprised seven collections: six adult striped bass collections consisting of individuals caught on the spawning grounds during the spawning season of each of the three rivers in 2016 and 2017, and a single collection of YOY striped bass caught in the Rappahannock River during 2016. All 2,197 individuals were genotyped for 20 microsatellite markers; however, 676 individuals were removed from the dataset because 10% or more of the loci could not be used due to problems with amplification or sizing of alleles (Table 1). The greatest numbers of samples were removed from the Rappahannock 2016 collection (50%) due to inconsistent amplifications and trouble getting correct allele size calls across all loci. The fraction of individuals removed from the other collections ranged from 12% (Mattaponi 2016) to 30% (YOY Rappahannock). In an attempt to include samples with inconsistent amplifications and troubleshoot the amplification problem, subsets of individuals from each river were re-isolated and amplified using different protocols. No methodology yielded consistent amplifications for the problematic samples. Additionally, the 5% of the samples that were redone to assess error included individuals with inconsistent amplifications and consistent amplifications to confirm the protocol. Of these, samples from individuals with the original inconsistent amplifications remained inconsistent, and the samples from individuals with consistent amplifications remained consistent. Prior to removing loci from the analysis, the samples with inconsistent amplification were removed to ensure that no bias was introduced. The 1,521 samples used in these analyses included 1,132 adults collected in 2016 and 472 from

2017. Males (840, overall; 487 in 2016, 354 in 2017) were much more abundant in adult collections than females (overall 202 overall; 173 in 2016, 119 in 2017). The YOY collection consisted of 389 individuals.

Tests for conformation of genotypic distributions to Hardy-Weinberg equilibrium expectations indicated three loci, 1322, 1437, and 1491, had significant deviations in two or more collections after correction for multiple comparisons. Additionally, reanalysis of 5% of all samples (PCR amplification through scoring) indicated these three loci did not have consistent amplification and had issues assessing allele sizes; they were removed from further analyses. Reanalysis of the 17 remaining loci demonstrated an error rate of less than 1% due to peak shifts of 1 to 3 repeat motifs. MicroChecker indicated that the genotypic distributions of one locus, 1559, showed evidence of the presence of a null allele in five of seven collections; however, due to the low impact of null alleles on  $F_{ST}$  estimates (Carlsson 2008), and no other foreseen issues, the marker was included in subsequent analyses. In total, 17 loci were used for the analyses.

Pairwise tests for linkage disequilibrium between the 17 loci revealed significant disequilibrium between 65 of 952 pairwise comparisons after correction for multiple comparisons, indicating a non-random association of the alleles at some loci (Bartley et al., 1992). Of these, 43 were pairwise comparisons of loci of adult Rappahannock River collections from 2016 and 2017. No pairwise comparisons between loci demonstrated significant disequilibrium in more than one collection, indicating independent assortment of loci indicating that loci were not physically linked. These results are consistent with a linkage map produced by Li (2009) that indicated that loci are on different chromosomes. No loci were excluded due to linkage disequilibrium.



Levels of genetic variation were comparable among collections, regardless of collection year or river (Table 2). The total number of alleles for all loci for each collection ranged from 248 (James 2017) to 278 (YOY Rappahannock). The number of private alleles varied across collections, with the largest collection YOY Rappahannock having 10 private alleles, and all other adult collections having 0-5 private alleles. No private allele occurred in more than two individuals in a collection. Values of mean allelic richness pooled across all loci for all collections were similar, ranging from 13.931 (Mattaponi 2016) to 14.605 (Mattaponi 2017). The average number of alleles per locus for pooled collections was 18, and the total number of alleles per locus ranged from 6 (*I271*) to 30 (*I556*) (Table 3). The mean observed heterozygosity across all loci for the pooled collections was 0.834 and ranged from 0.518 (*SI271*) to 0.926 (*SI273*). Observed heterozygosities were similar among collections pooled across loci and ranged from 0.827 (James 2016) to 0.883 (Rappahannock 2017). For rivers pooled over years, observed heterozygosities were 0.862 in the Rappahannock, 0.848 in the James, and 0.841 in the Mattaponi. For years pooled over rivers, the observed heterozygosities were 0.835 in 2016 and 0.865 in 2017. Inbreeding coefficients ( $F_{IS}$ ) for each collection ranged from -0.064 to 0.031, with a mean of 0.002.

Levels of variation between male and female striped bass pooled across loci and pooled across collections were comparable (Table 4). Male striped bass had a similar number of alleles for pooled loci ranging from 237 (James 2017) to 257 (Rappahannock 2016). Female striped bass had slightly lower numbers of alleles relative to males across collections for all loci combined from 200 (James 2016 & Mattaponi 2017) to 233 (Rappahannock 2016); the slightly lower values could be the result of the smaller number

of females sampled compared to male fish (850 compared to 292). Mean allelic richness for males was similar among rivers, ranging from 13.835 (Mattaponi 2017) to 13.277 (Rappahannock 2017). Female mean allelic richness was slightly lower than males, ranging from 11.046 (James 2017) to 10.539 (James 2016). The average number of alleles per locus across all male collections was 17.29, with the total number of alleles ranging from 26 (*I591*) to 6 (*I271*). The average number of alleles per locus across all female populations was 16.11, with the total number of alleles ranging from 26 (*I556*) to 6 (*I271*). Mean inbreeding coefficients ( $F_{IS}$ ) for male collections was -0.010, while the mean  $F_{IS}$  for females was 0.01. Mean observed heterozygosity for males across all loci was 0.853 and ranged from 0.518 (*SI271*) to 0.936 (*SI556*), and for females mean observed heterozygosity across all loci was 0.840. For both male and female fish, observed heterozygosities were similar when pooled across both years and among rivers.

### *Genetic Structure*

The global  $F_{ST}$  among the seven collections was 0.003 ( $P < 0.05$ ) and single locus  $F_{ST}$  values ranged from 0.001 (*I598*) to 0.014 (*I602*) (Table 3). Locus *I602* produced the highest individual locus  $F_{ST}$  value (0.0135). Removal of this locus from subsequent analyses did not result in changes of significance of the results. Pairwise multi-locus  $F_{ST}$  values were calculated between all collections among rivers in each year. The two lowest values were between the juvenile Rappahannock and the sampled parents in Rappahannock 2016 (-0.0002) and between juvenile Rappahannock and Mattaponi 2016 (-0.0005). The highest  $F_{ST}$  value was 0.007 between James 2016 and James 2017. Out of 21 pairwise comparisons, 18 were significant ( $P < 0.05$ ) after corrections for multiple comparisons. The three non-significant comparisons ( $P > 0.05$ ) were between collections

from different rivers in different years (Table 6). For all three rivers, the  $F_{ST}$  values between years for the same river were comparable to values among rivers in the same year. For the same river between years, all  $F_{ST}$  values were statistically significant ( $P < 0.05$ ) and ranged from 0.00725 for the James, 0.00293 for the Mattaponi, and 0.00498 for the Rappahannock. When adults were pooled together across years from each of the three rivers (Table 5), the Mattaponi River was not significantly different from the Rappahannock River ( $F_{ST} = 0.0003$ ,  $P > 0.05$ ), but was significantly different from the James River ( $F_{ST} = 0.002$ ,  $P < 0.05$ ). The Rappahannock had a significant pairwise comparison between the James River ( $F_{ST} = 0.003$ ,  $P < 0.05$ ).

When males and females were analyzed separately, population pairwise  $F_{ST}$  values among males and females were also low across river collections and temporal replicates. Population pairwise  $F_{ST}$  comparisons among male collections had 14 out of 15 pairwise comparisons significant after corrections for multiple comparisons (Table 7), with values ranging from 0.002 between the Mattaponi and James rivers in 2016 to 0.006 between the James and Rappahannock rivers in 2017. Population pairwise  $F_{ST}$  comparisons of female collections had with 3 out of 15 comparisons being significant after corrections for multiple comparisons (Table 8) with values ranging from 0.001 between the James and Rappahannock rivers in 2016 to 0.006 between the Mattaponi and Rappahannock rivers in 2017.

A variety of AMOVA analyses found no significant population genetic variation between sample years pooled over river collections (2016 vs. 2017) or in sampling location (between rivers pooled over years). AMOVA analysis indicated that temporal differences between 2016 and 2017 pooled across rivers accounted for 0.27% of the

overall genetic variation for all river collections with a small, but significant  $F_{CT}$  (0.003,  $P < 0.05$ ). AMOVA analysis for spatial differences among rivers pooled over years was non-significant ( $F_{CT} = -0.001$ ,  $P > 0.05$ ), and genetic variation among rivers accounted for 0.04% of the total variation. For both AMOVA analyses, the largest partitioning of genetic variance was found within samples (99.6%). Tests for isolation by distance indicated no significant relationship between three geographic distance points (one for each river) and pairwise  $F_{ST}$  values ( $P > 0.05$ ,  $R^2 = 0.035$ ).

STRUCTURE analyses using proposed genetic clusters (K) of 1 to 7 revealed that the most likely K was 1, even with the Evanno correction (Evanno et al., 2005). The mean likelihood value was  $\ln(P) = -113946.46$ . The STRUCTURE output indicated that only one genetic cluster of individuals exists over temporal replicates and river collections. A principal component analysis (PCA) for all collections showed no patterns of correlation between the geographical groups or temporal replicates (Figure 2). For all sample groups combined, the first PC explained 2.51% of total variability in the data set, and PC2 explained 2.40% of the data set. The PC1 for males explained 3.11% of variability in the data set, and PC2 explained 2.95% variability. For females, PC1 explained 3.91% of variability in the data set, and PC2 explained 3.82% variability.

Contemporary effective population sizes were variable, ranging from 194.5 (2017 Rappahannock) to 1187 (Mattaponi 2017) (Table 9). Both Rappahannock year samples had the lowest effective population sizes for each year, and the Mattaponi had the highest effective population size for both yearly collections.

## Discussion

The objective of this study was to investigate regional genetic connectivity of striped bass within the lower Chesapeake Bay using a biologically informative sampling protocol with large sample sizes, temporal replicates, and highly polymorphic microsatellite markers. A total of 1,521 striped bass samples, comprising 1,132 adults and 389 YOY and, were collected from the spawning and nursery grounds of the James, York, and Rappahannock rivers over two years (2016 and 2017). Samples were analyzed at 20 polymorphic microsatellite loci selected based on their availability for striped bass and, in some cases, documented utility for resolving population structure in this species (Abdul-Muneer, 2014; Putman and Carbone, 2014; Gautier et al., 2013).

Difficulties were encountered with the analysis of some of the microsatellite loci including problems with amplification of all loci in some samples that resulted in unreliable sizing of alleles, as well as a few loci that failed to amplify consistently over all samples. Several DNA isolations, most notably for individuals in the Rappahannock River collections, either failed to amplify any loci or amplified inconsistently for most loci. These included some samples that produced high molecular weight DNA extractions. Various attempts were made to improve amplification success, including adjusting primer binding conditions, re-extracting DNA, and concentrating DNA. Microsatellite allele electropherograms exhibited stuttering artifacts for a large proportion of loci for some individuals. These artifacts may have resulted from slipped-strand mispairing (O'Reilly and Wright, 1995), annealing of truncated products (Hauge and Litt, 1993), an addition of a 3' nucleotide to the end of some strands (Weber 1989; O'Reilly et al 1995), or the generation of extra electrophoresis products on the ABI during

sequencing reactions (Fernando et al., 2001). Microsatellite loci with tri- and tetra-nucleotide repeats were selected to avoid problems with stuttering that can lead to incorrect genotyping, but an unknown external factor or factors resulted in poor amplification success across most loci for some samples. Those individuals with amplification issues that resulted in unreliable scoring for more than 2 loci were removed from further analysis. Due to the large sample sizes of most collections, removal of these individuals did not significantly reduce the power of subsequent analyses.

Initial analyses of genotypic data indicated significant deviations from the expectations of Hardy-Weinberg equilibrium for three loci in two or more collections. In each case the deviations resulted from a lack of heterozygotes. Re-amplification and analysis of these three markers revealed one to three repeat motif peak shifts, and many samples failed to amplify again. Previous studies have shown that the mobility of some microsatellite loci may be sensitive to temperature shifts during electrophoresis (Applied Biosystems, 2002; Davison and Chiba, 2003), and temperature control has been an issue in the Fisheries Genetics Laboratory space. Microsatellite locus panels were optimized during summer months, with the majority of 2016 collection samples run in the fall/winter months. The presence of null alleles (which arise from mutations in the primer binding sites) and/or allelic dropouts may have also contributed to deviations from Hardy-Weinberg equilibrium (Pompanon et al., 2005). Because all three loci had significant deviations in multiple collections, they were removed to ensure that subsequent analyses, such as calculations of  $F_{ST}$ , were not biased (Morin et al., 2009).

The 17 loci selected for analysis revealed considerable variation within all collections, levels that were comparable to prior microsatellite-based studies of striped

bass population structure. The mean number of alleles per locus was 18, higher than values reported by both Brown et al. (2005), with a mean of 10 alleles per locus, and Gautier et al. (2013), with an average of 15 alleles per locus. The mean observed heterozygosity across all sample collections was 0.851, with a value of 0.833 in 2016 and 0.869 in 2017. Brown et al. (2005) had a mean observed heterozygosity at ten microsatellite loci of 0.505 (range of 0.255 to 0.893) and Gautier et al. (2013) had a mean observed heterozygosity of 0.757 (range of 0.610 to 0.910).

### ***Population Structure***

Previous genetic studies of striped bass within Chesapeake Bay have reached differing conclusions regarding the presence of population structure. Some investigations have revealed statistically significant genetic heterogeneity, whereas others have not. However, in all of the studies, the magnitude of genetic differences between samples was very low, leaving the biological significance of the observed results in question. In an effort to better understand population structure of striped bass in the lower Chesapeake Bay, this study employed a sampling design that incorporated temporal replicates for each of the three river systems sampled. The expectation was that variation among temporal replicates from the same river system in different years would reflect the baseline stochastic “noise” in the system, and that if there was significant population structuring, levels of variation between collections from different rivers would be greater than the differences between collections taken on the same river in different years.

A total of 21 pairwise comparisons were made between the collections, 5 of which were comparisons of temporal replicates from the same river, and 16 were between collections from different rivers. Overall, 18 of the 21 pairwise comparisons resulted in

statistically significant  $F_{ST}$  values, although the magnitude of the significant  $F_{ST}$  values was quite low (0.00124 to 0.00725). Analysis indicated that the significant  $F_{ST}$  values were not driven by a single locus or group of loci. Interestingly, and contrary to expectations, the mean  $F_{ST}$  value for pairwise comparisons of temporal replicates was larger (0.005) than the mean  $F_{ST}$  value for pairwise comparisons of samples from different rivers (0.003 for 2016 collections and 0.002 for 2017 collections). Two of the non-significant  $F_{ST}$  values were between collections from different rivers within the same year, and the third was a comparison of YOY and adults from the same river in the same year. Based on the difference in the magnitude of the  $F_{ST}$  values between comparisons of temporal replicates and collections from different rivers, I conclude that the statistical significance of the low magnitude  $F_{ST}$  values reflects sampling error or biological noise rather than true population structuring.

The results of several other analyses support the null hypothesis of a single genetic stock of striped bass within the lower Chesapeake Bay. The genetic clustering analysis software STRUCTURE failed to recover multiple clusters on spatial or temporal scales, supporting the existence of a single genetic population. Principal component analysis also produced a single cluster and did not detect significant genetic heterogeneity among collections. AMOVA analyses found no significant partitioning of genetic variance among temporal collections. Finally, a Mantel test did not detect a significant relationship between geographic distances of  $F_{ST}$  values, consistent with a lack of spatial genetic structure. Together, these analyses provide no support to reject the null hypothesis of a lack of a single genetic population of striped bass within the lower Chesapeake Bay.



In a previous study using analysis of microsatellite loci to evaluate population structuring of the striped bass population in the lower Chesapeake Bay, Brown et al. (2005) found a nonsignificant global analog to the  $F_{ST}$  value of -0.007, and the recovery of one genetic grouping of YOY striped bass in a STRUCTURE analysis. In contrast, Gautier et al. (2013) found a significant global  $F_{ST}$  of 0.001 between YOY striped bass pooled into upper and lower Chesapeake Bay groups over sample years, and the study concluded that shallow population genetic structure existed within the Chesapeake Bay. The magnitude of the indices of genetic differentiation reported in Brown et al. (2005), Gautier et al. (2013), and the current study are very similar, ranging from 0.000 to 0.0072. The current study, by employing temporal replicates, was able to evaluate the biological significance of spatial structure over time, a comparison that supports the existence of a single homogenous population of striped bass in the lower Chesapeake Bay.

Striped bass exhibit less genetic population structuring relative to other anadromous species along the U.S. the Atlantic coast. A population genetic study of alewife and Blueback herring, anadromous fishes with distributions along the U.S. Atlantic coast and with spawning grounds in the Chesapeake Bay, showed strong support for genetic structuring within the Bay (Ogburn et al., 2017). Population pairwise  $F_{ST}$  values for blueback herring were more than an order of magnitude higher than values recovered in this study (0.018 to 0.156) and STRUCTURE analyses indicated multiple genetic clusters for both species. American shad populations showed genetic differentiation among eastern and western Chesapeake Bay shores, with significant  $F_{ST}$  values ranging from 0.081 to 0.468 (Hasselman et al., 2013). For striped bass, straying

during spawning runs may prevent the establishment of differentiated spawning areas on an evolutionary time-scale, and the amount of straying can be evaluated using traditional tagging studies (Secor, 2000a).

### *Evaluation of Connectivity*

The lack of robust genetic spatial structuring among spawning striped bass from the major rivers of the lower Chesapeake Bay suggests that there is either some level of connectivity between the river systems, or the populations are sufficiently recent that there has not been enough time for genetic differences to accrue. Since the Chesapeake Bay is a geologically young estuary, having reached the current shoreline position only 7,000 to 6,000 years ago (Colman and Mixon, 1988), enough generational time may not have elapsed for striped bass genetic divergence to occur in the major river systems of Chesapeake Bay (Begg and Waldman, 1999). Based on generation time of 9 years (Ginzburg et al. 1982), there have been fewer than 800 generations of striped bass since the Chesapeake Bay arrived at its current configuration.

Conventional tagging studies of striped bass have supported the existence of separate spawning aggregations in each of the larger Chesapeake Bay rivers, documenting high rates of fidelity by adult fish to a particular river (Vladykov and Wallace, 1938; Massmann and Pacheco, 1961; Nichols and Miller, 1967; Winegate et al. 2011). Many of these studies reported more than 90% of adult striped bass returning to spawn in the same river in a subsequent year. The other tag returns during the spawning season suggested the animals were spawning in other spawning grounds or not spawning that year. As low levels of migration may be sufficient to prevent the accumulation of

significant genetic differences, I analyzed a 30-year conventional tagging dataset of striped bass from the James and Rappahannock rivers to evaluate levels of connectivity.

From 1987 to present day, the VIMS Striped Bass Tagging Program has tagged 99,694 adult fish on spawning grounds of the Chesapeake Bay, and at the time of this study there have been 23,495 tag returns reported. Filtering the tag return data to include only those fish tagged on the Rappahannock and James rivers during the spawning season (February to May), at large for at least one year, and subsequently recaptured during the spawning season, resulted in a total of 1,118 recaptures between 1988 and 2016 (Supplementary Table 2). Of these, 90% occurred within Chesapeake Bay waters, and 10% were collected outside of the Bay (Table 10). For fish tagged in the James River during the spawning season and recaptured during a subsequent spawning seasons, 76% were recaptured in the James River, 6% on spawning grounds of other rivers within the Chesapeake Bay, 5% in the main stem of the Bay, and 13% outside of the Chesapeake Bay. For fish tagged initially in the Rappahannock River during the spawning season and recaptured during a subsequent spawning season, 74% were recaptured in the Rappahannock River during the spawning season, 8% were recaptured on spawning grounds of other rivers within Chesapeake Bay, 7% in the main stem of the Bay, and 11% outside of the Chesapeake Bay.

Although these tagging data demonstrate high fidelity of striped bass to specific rivers within the lower Chesapeake Bay, but they also show movement of fish between the major rivers during the spawning season (straying). At least 6% of striped bass recovered during the spawning season were caught on the spawning grounds of a different river from the one on which they were originally tagged. As only a handful of

migrants per generation (~10) are needed to negate the impacts of genetic drift between local populations of striped bass to maintain similar allele frequencies (Lowe and Allendorf, 2010), the conventional tagging data support the genetic results of this study. For striped bass in the lower Chesapeake Bay, genetic and conventional tagging data support the hypothesis that there is sufficient straying of adult fish to spawning grounds in different rivers to homogenize a gene pool.

The collections of spawning adult and YOY striped bass from the Rappahannock River in the same year provide an opportunity to evaluate differential reproductive success for striped bass. For many highly fecund species that have a high census population size low effective population sizes have been estimated when small but statistically significant differences between spawning adults and their progeny are seen with reduced genetic variation within progeny relative to adults and increased linkage disequilibrium within progeny. These factors have been attributed to the “sweepstakes effect” in which a few spawning individuals have extremely high reproductive success relative to their conspecifics (Hedgecock 1994; Flowers et al 2002; Hedgecock 2011). It has been suggested that older, larger striped bass may have disproportionately high reproductive success (Rago and Goodyear, 1987; Secor 2000a, 2000b). A comparison of the 2016 adult Rappahannock collection with the 2016 YOY Rappahannock collection, did not reveal significant genetic heterogeneity between the collections, or reduced variation in the YOY collection. In fact, the YOY had a higher number of alleles than the adults and had the lowest linkage disequilibrium observed. Therefore, realizing that sample sizes were not huge, no evidence was found to support the existence of a sweepstakes effect in the Rappahannock River.

## *Conclusions and Management Implications*

Ecological stocks and evolutionary stocks are both important concepts for the management of striped bass, as each type considers the stock on a different time scale. Ecological stocks are mainly concerned with the demographics of the stock and the co-occurrence of individuals over time and space for ecological interactions (Waples and Gaggiotti, 2006). In contrast, evolutionary stocks have a genetic basis, and are impacted by forces such as genetic drift and natural selection (Carvalho and Hauser, 1994). For striped bass, the Atlantic stock was primarily managed as one ecological and evolutionary stock until Amendment 6 to the Atlantic Striped Bass Fisheries Management Plan in 2003. This amendment delineated the Chesapeake Bay as a distinct management area due to the demography of smaller, male fish relative to the Atlantic stock. This allowed managers to set separate biological reference points for the Chesapeake Bay to allow for a more ecologically managed species. The recognition of a Chesapeake Bay stock is supported by genetic data (e.g. Gautier et al. 2013) and conventional tagging data (e.g. Nichols and Miller 1967; Wingate et al. 2011).

While separate evolutionary and ecological stocks have been identified for striped bass along the Atlantic coast, there is controversy regarding the stock structure within Chesapeake Bay. For the lower Chesapeake Bay, this study observed small, but statistically significant genetic heterogeneity among collections of striped bass on different spawning grounds, but the heterogeneity between collections from the same river in consecutive years was greater than that among river systems in the same year, suggesting that differences between rivers were not biologically (evolutionarily) meaningful. While the straying of adults may be sufficient to prevent the development of

evolutionary stocks, the observed fidelity to spawning areas of 75% or more clearly suggests the existence of ecological stocks. Known areas of site fidelity may allow fisheries managers to apply area-specific management measures to conserve well-documented spawning stocks and grounds (Thorrold et al., 2001). Evidence indicates that while faithful to the Chesapeake Bay and generally to a river system, striped bass may choose different breeding grounds in different years. Straying may occur for a variety of reasons including prey availability, water temperature, and river flow, and it has been suggested that females may be more likely to stray among rivers compared to males (Secor 2000a).

This study aimed to improve prior attempts to determine if significant temporally stable heterogeneity existed among striped bass sampled from major spawning grounds in the lower Chesapeake Bay. The results of this study support the presence of a single genetic stock of striped bass in the lower Chesapeake Bay using an improved sampling design and increased power with large sample sizes and many microsatellite markers. Connectivity among striped bass spawning grounds within Chesapeake Bay is high enough to overcome genetic forces that create evolutionary stocks. The examination of tagging data indicates that a large majority of striped bass are faithful to their spawning grounds potentially creating ecological stocks. Further investigation including the analysis of additional spawning grounds within the Chesapeake Bay may provide some evidence of genetic heterogeneity, but the tagging data show that straying of individuals from two major rivers across the entire Chesapeake Bay during spawning season may be large enough to prevent the detection of significant heterogeneity.

Table 1: Sample sizes of striped bass collections from each river and year, and the number of samples that were used in the analyses after removal of problematic individuals. The values shown include original samples, overall numbers, males, and females.

	Original Sample Size	Analyzed Sample Size	Male	Female
<b>2016</b>				
James	164	139	105	34
Mattaponi	171	150	100	50
Rappahannock	755	371	282	89
Juvenile Rappahannock	555	389	-	-
<b>2017</b>				
James	172	143	80	63
Mattaponi	138	118	92	26
Rappahannock	243	211	181	30

Table 2: The number of alleles, private alleles, mean allelic richness adjusted for sample size, and the inbreeding coefficient for each collection of striped bass pooled over all loci.

	Alleles	Private Alleles	Mean Allelic Richness	$F_{IS}$
<b>2016</b>				
James	248	1	13.951	0.004
Mattaponi	250	2	13.931	0.02
Rappahannock	261	5	13.995	0.031
Juvenile Rappahannock	278	9	14.334	0.013
<b>2017</b>				
James	249	0	13.999	-0.024
Mattaponi	258	2	14.605	0.003
Rappahannock	257	1	13.997	-0.064

Table 3: Number of alleles, expected and observed heterozygosities,  $F_{ST}$ , and the inbreeding coefficient for each locus pooled over all striped bass collections.

	Number of Alleles	Expected Heterozygosity	Observed Heterozygosity	$F_{ST}$	$F_{IS}$
S1598	12	0.858	0.883	0.0005	-0.0206
S1568	19	0.910	0.905	0.0011	0.0066
S1584	21	0.892	0.902	0.0011	-0.0086
S1617	22	0.902	0.913	0.0012	-0.0083
S1628	13	0.867	0.854	0.0014	0.0204
S1290	9	0.663	0.689	0.0015	-0.0366
S1587	15	0.846	0.871	0.0015	-0.0286
S1556	30	0.939	0.912	0.0018	0.0291
S1559	23	0.909	0.865	0.002	0.0491
S1271	6	0.499	0.518	0.0021	-0.0094
S1603	12	0.750	0.720	0.0021	0.0424
S1591	26	0.912	0.913	0.0032	-0.0023
S1273	26	0.925	0.926	0.0037	-0.0024
S1592	26	0.922	0.910	0.004	0.0111
S1577	16	0.860	0.690	0.0044	-0.0118
S1638	14	0.819	0.821	0.0044	-0.0034
S1602	16	0.910	0.891	0.0135	0.0115
<i>Mean</i>	<i>18</i>	<i>0.846</i>	<i>0.834</i>	<i>0.003</i>	<i>0.002</i>



Table 4: Number of alleles, private alleles, and mean allelic richness for each for all samples, and by sex, for all striped bass collections.

<b>Males</b>			
	Alleles	Private Alleles	Mean Allelic Richness
<b>2016</b>			
James	240	6	13.458
Mattaponi	240	5	13.294
Rappahannock	257	8	13.38
<b>2017</b>			
James	237	0	13.398
Mattaponi	249	3	13.835
Rappahannock	253	5	13.277
<b>Females</b>			
	Alleles	Private Alleles	Mean Allelic Richness
<b>2016</b>			
James	200	5	10.539
Mattaponi	217	5	10.868
Rappahannock	233	5	10.907
<b>2017</b>			
James	226	1	11.046
Mattaponi	200	2	10.861
Rappahannock	209	3	10.906

Table5: Population pairwise  $F_{ST}$  values for each striped bass collection on the lower diagonal with p-values on the upper diagonal. Statistically significant pairs are shown in bold and with a \*.

	Rappahannock 2016	Juvenile Rappahannock	Rappahannock 2017	James 2016	James 2017	Mattaponi 2016	Mattaponi 2017
Rappahannock 2016	-	0.8447	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.1318
Juvenile Rappahannock	-0.0002	-	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.5371
Rappahannock 2017	<b>0.00498*</b>	<b>0.00534*</b>	-	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>
James 2016	<b>0.00452*</b>	<b>0.00392*</b>	<b>0.00409*</b>	-	<b>0.0000</b>	<b>0.0020</b>	<b>0.0000</b>
James 2017	<b>0.00235*</b>	<b>0.00186*</b>	<b>0.00263*</b>	<b>0.00725*</b>	-	<b>0.0098</b>	<b>0.0000</b>
Mattaponi 2016	<b>0.00268*</b>	<b>0.0034*</b>	<b>0.00288*</b>	<b>0.00158*</b>	<b>0.00534*</b>	-	<b>0.0000</b>
Mattaponi 2017	0.00029	-0.0005	<b>0.00412*</b>	<b>0.00386*</b>	<b>0.00124*</b>	<b>0.00293*</b>	-

Table 6: Population pairwise  $F_{ST}$  values between striped bass collections from each river pooled over years on the lower diagonal with p-values on the upper diagonal. Statistically significant pairs are shown in bold and with a \*.

	Rappahannock	Mattaponi	James
Rappahannock	-	0.0684	0.0000
Mattaponi	0.0002	-	0.0000
James	<b>0.0030*</b>	<b>0.0020*</b>	-

Table 7: Population pairwise  $F_{ST}$  values for male striped bass in each collection on the lower diagonal with p-values on the upper diagonal. Statistically significant pairs are shown in bold and with a \*.

	Rappahannock 2016	Rappahannock 2017	James 2016	James 2017	Mattaponi 2016	Mattaponi 2017
Rappahannock 2016	-	0.0000	0.0000	0.0000	0.1855	0.0000
Rappahannock 2017	<b>0.00496*</b>	-	0.0000	0.0000	0.0000	0.0010
James 2016	<b>0.00247*</b>	<b>0.0081*</b>	-	0.0010	0.0156	0.0000
James 2017	<b>0.00562*</b>	<b>0.00577*</b>	<b>0.0029*</b>	-	0.0000	0.0039
Mattaponi 2016	<b>0.004*</b>	<b>0.00538*</b>	<b>0.00155*</b>	<b>0.0046*</b>	-	0.0000
Mattaponi 2017	<b>0.00307*</b>	<b>0.00184*</b>	<b>0.00543*</b>	0.00284	<b>0.00335*</b>	-

Table 8: Population pairwise  $F_{ST}$  values for female striped bass in each collection on the lower diagonal with p-values on the upper diagonal. Statistically significant pairs are shown in bold and with a \*.

	Rappahannock 2016	Rappahannock 2017	James 2016	James 2017	Mattaponi 2016	Mattaponi 2017
Rappahannock 2016	-	0.1201	0.1221	0.0000	0.0195	0.0957
Rappahannock 2017	0.00185	-	0.2471	0.0664	0.0020	0.0283
James 2016	0.00123	0.00138	-	0.0020	0.0547	0.0713
James 2017	<b>0.00529*</b>	0.0023	<b>0.00374*</b>	-	0.0147	0.0225
Mattaponi 2016	0.00348	<b>0.00644*</b>	0.00288	0.00384	-	0.1201
Mattaponi 2017	0.00223	0.00505	0.0029	0.00405	0.0022	-

Table 9: Effective population size for each striped bass collection from the adult population is shown with 95% confidence intervals.

	$N_e$	95% CI
2016		
James	869.4	574.6-1716.4
Mattaponi	888.5	608.3-1598.3
Rappahannock	507.6	451.1-577.4
2017		
James	882.5	593.7-1660.2
Mattaponi	1186.7	677.7-4301.3
Rappahannock	194.5	176.3-215.9

Table 10: Recapture locations of striped bass tagged on spawning grounds of the James and Rappahannock rivers during the spawning season and subsequently recaptured during the spawning season after being at large for at least one year.

	<b>River of Origin</b>	
	<i>James River</i>	<i>Rappahannock River</i>
<b>Area of Recapture</b>		
Out of Chesapeake Bay	33	82
In Chesapeake Bay	222	773
On Spawning Grounds		
<i>James River</i>	194	13
<i>Rappahannock River</i>	7	645
<i>Potomac River</i>	3	22
<i>Other Rivers</i>	6	34
Non-Spawning Grounds	45	151
<b>Total</b>	255	865

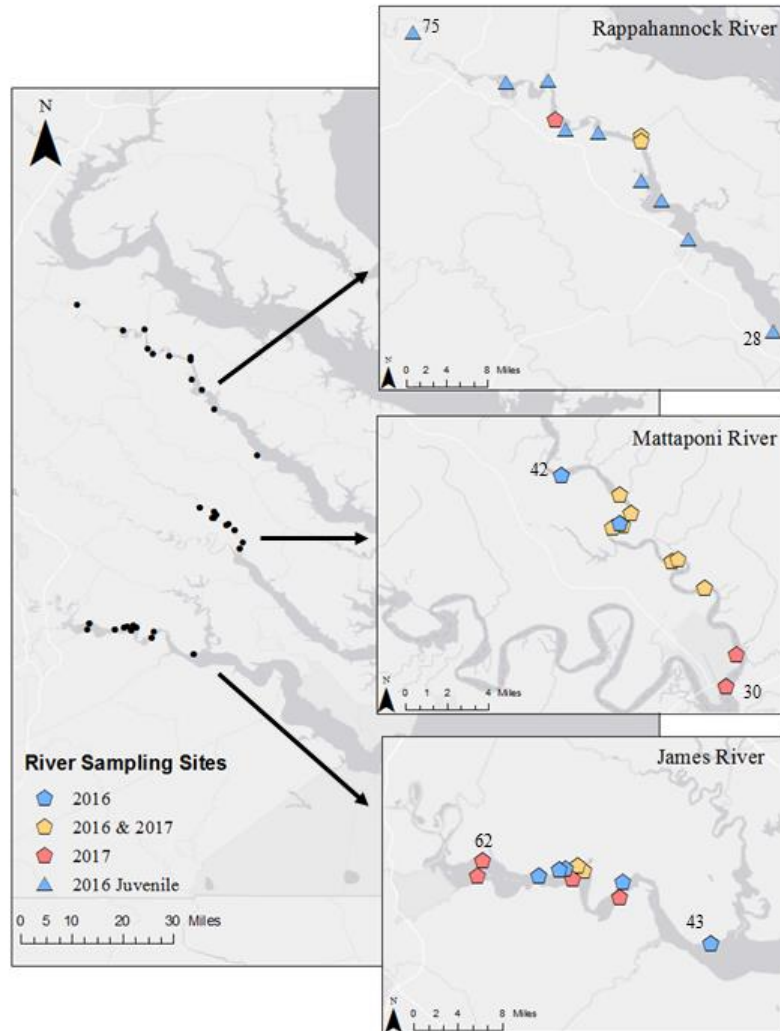


Figure 1: Map showing the sampling locations on the Rappahannock, Mattaponi, and James Rivers over the two sampling years. The black dots indicate sampling sites. Within the smaller boxes, blue indicates 2016 sampling, red indicates 2017 sampling, and yellow indicates both years. Triangles indicate juvenile samplings and pentagons indicate adult samplings. Mile markers at each end of sampling are shown with black numbers.

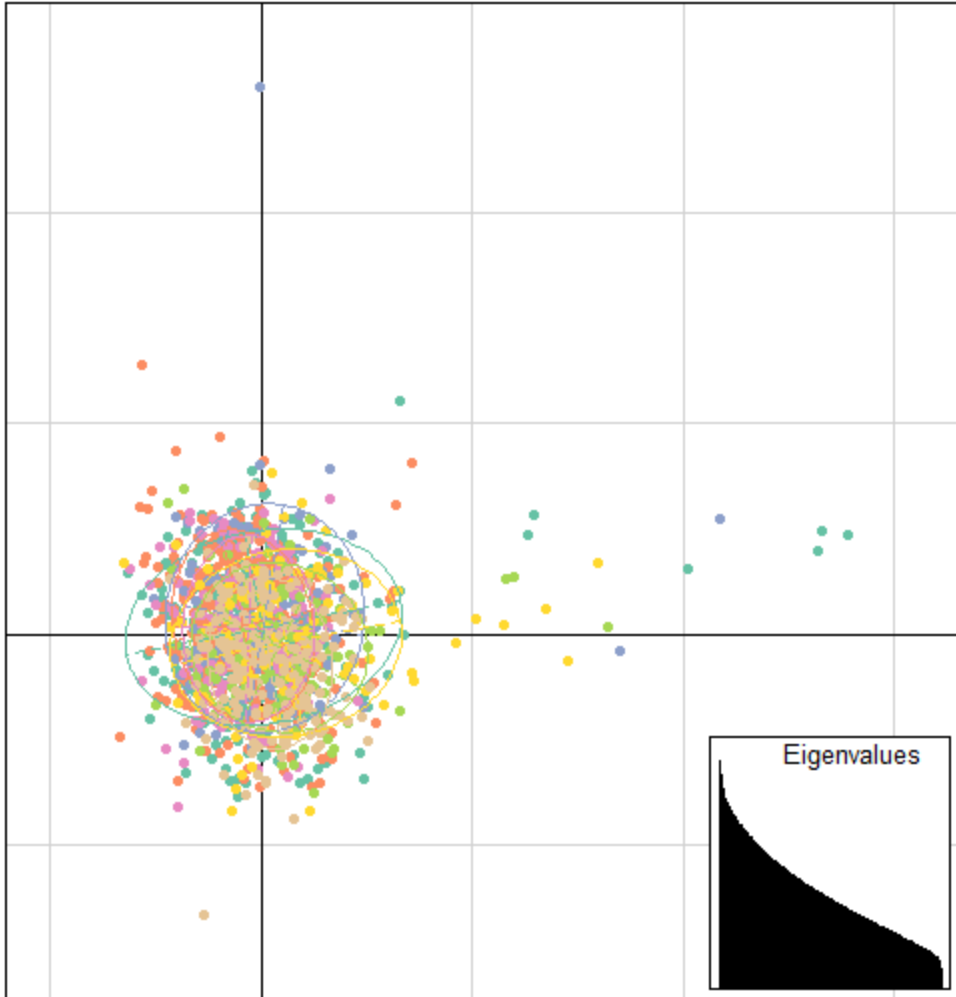


Figure 2: PCA of all populations showing principal components 1 and 2. Rappahannock 2016 is blue, Rappahannock 2017 is brown, Juvenile Rappahannock is orange, James 2016 is purple, James 2017 is green, Mattaponi 2016 is pink, and Mattaponi 2017 is yellow. Eigenvalues are presented in the lower right-hand corner.

## References

- Abdul-Muneer, PM. 2014. Application of microsatellite markers in conservation genetics and fisheries management: recent advances in population structure analysis and conservation strategies. *Genetics Research International* 2014: 691759.
- Adamack, AT, Gruber, B. 2014. PopGenReport: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution*, 5: 384-387.
- Allendorf, FW, Phelps, SR. 1981. Use of allelic frequencies to describe population structure. *Canadian Journal of Fisheries and Aquatic Sciences* 38: 1507-1514.
- Atlantic States Marine Fisheries Commission (2013) *Striped Bass Stock Assessment for 2013*. Arlington, VA. Retrieved from [http://www.asmfc.org/uploads/file/529e5ca12013StripedBassBenchmarkStockAssessment\\_57SAWReport.pdf](http://www.asmfc.org/uploads/file/529e5ca12013StripedBassBenchmarkStockAssessment_57SAWReport.pdf).
- Balloux, F, Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. *Molecular Ecology* 11: 155-165.
- Bartley, D, Bagley, M, Gall, G, Bentley, B. 1992. Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. *Conservation Biology* 6: 365-375.
- Beacham, TD, Candy, JR, Supernault, KJ, Wetklo, M, Deagle, B, Labaree, K, Irvine, JR, Miller, KM, Nelson, RJ, Withler, RE. 2003. Evaluation and application of microsatellites for population identification of Fraser River Chinook salmon (*Oncorhynchus tshawytscha*). *Fishery Bulletin* 101: 243-259.
- Begg, GA, Waldman, JR. 1999. An holistic approach to fish stock identification. *Fisheries Research* 43: 35-44.

- Boreman, J, Lewis, RR. 1987. Atlantic coastal migration of striped bass. In *American Fisheries Society Symposium* 1:331-339.
- Boynton WR, Garber JH, Summers R, Kemp WM. 1995. Inputs, transformations, and transport of Nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* 18: 285-314.
- Brown KM, Baltazar GA, Hamilton MB. 2005. Reconciling nuclear microsatellite and mitochondrial marker estimates of population structure: breeding population structure of Chesapeake Bay striped bass (*Morone saxatilis*). *Heredity* 94: 606-615.
- Callihan, JL. Godwin CH, Buckel JA. 2014. Effect of demography on spatial distribution: movement patterns of the Albemarle Sound-Roanoke River stock of Striped Bass (*Morone saxatilis*) in relation to their recovery. *Fishery Bulletin* 112: 131-143.
- Carvalho, GR, Hauser, L. 1994. Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* 4: 326-350.
- Chambers, RC., Waiwood, KG. 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 1986-2003.
- Chapman RW. 1990. Mitochondrial DNA analysis of striped bass populations in the Chesapeake Bay. *Copeia*, 1990: 355-366.
- Colman, SM, Mixon, RB. 1988. The record of major Quaternary sea-level changes in a large coastal plain estuary, Chesapeake Bay, eastern United States: *Palaeogeography, Palaeoclimatology, and Palaeoecology* 68: 99-116.



- Couch, CR, Garber, AF, Rexroad, CE, Abrams, JM, Stannard, JA, Westerman, ME, Sullivan, CV. 2006. Isolation and characterization of 149 novel microsatellite DNA markers for striped bass, *Morone saxatilis*, and cross-species amplification in white bass, *Morone chrysops*, and their hybrid. *Molecular Ecology Resources* 6: 667-669.
- Davison, A, Chiba, S. 2003. Laboratory temperature variation is a previously unrecognized source of genotyping error during capillary electrophoresis. *Molecular Ecology Resources* 3: 321-323.
- Diaz M. Leclerc GM, Fishtec BE. 1997. Nuclear DNA markers reveal low levels of genetic divergence among Atlantic and Gulf of Mexico populations of striped bass. *Transactions of the American Fisheries Society* 126(1): 163-165.
- Do, C, Waples, RS, Peel, D, Macbeth, GM, Tillett, BJ, Ovenden, JR. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size ( $N_e$ ) from genetic data. *Molecular Ecology Resources*, 14: 209-214.
- Dorazio, RM, Hattala, KA, McCollough, CB, Skjveland, JE. 1994. Tag recovery estimates of migration of striped bass from spawning areas of the Chesapeake Bay. *Transactions of the American Fisheries Society* 123: 950-963.
- El Mousadik, A, Petit, RJ. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) *Skeels*] endemic to Morocco. *Theoretical and Applied Genetics* 92: 832-839.
- Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.

- Fabrizio, MC. 1987. Growth-invariant discrimination and classification of striped bass stocks by morphometric and electrophoretic methods. *Transactions of the American Fisheries Society* 116: 728-736.
- Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 4:1567-1587.
- Falush D, Stephens M, Pritchard JK. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 5:1322-1332.
- Fernando, P, Evans, BJ, Morales, JC, Melnick, DJ. 2001. Electrophoresis artefacts—a previously unrecognized cause of error in microsatellite analysis. *Molecular Ecology Resources* 1: 325-328.
- Flowers, JM, Schroeter, SC, Burton, RS. 2002. The recruitment sweepstakes has many winners: genetic evidence from the sea urchin *Strongylocentrotus purpuratus*. *Evolution* 56: 1445-1453.
- Fountain J, Darden T, Jenkins W, Denson M. 2009. Three multiplexed microsatellite panels for striped bass. *Southeastern Naturalist* 8: 671-676.
- Gahagan, BI, Fox, DA, Secor, DH. 2015. Partial migration of striped bass: revisiting the contingent hypothesis. *Marine Ecology Progress Series* 525: 185-197.
- Gauthier DT, Audemard CA, Carlsson JEL, Darden TL, Denson MR, Reece KS, Carlsson J. 2013. Genetic population structure of US Atlantic coastal striped bass (*Morone saxatilis*). *Journal of Heredity* 104: 510-520.

- Ginzburg, LR, Slobodkin, LB, Johnson, K, Bindman, AG. 1982. Quasiextinction probabilities as a measure of impact on population growth. *Risk Analysis* 2: 171-181.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversity and fixation indices (v 2.9.3). <http://www2.unil.ch/popgen/softwares/fstat.htm>.
- Greene, KE, Zimmerman, JL, Laney, RW, Thomas-Blate, JC, 2009. Atlantic coast diadromous fish habitat: a review of utilization, threats, recommendations for conservation, and research needs. Atlantic States Marine Fisheries Commission Habitat Management Series: 464.
- Grove TL, Berggren TS, Powers DA. 1976. The use of innate genetic tags to segregate spawning stocks of striped bass (*Morone saxatilis*). *Estuarine Processes* 1: 166-176.
- Guo, SW, Thompson, EA. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 361-372.
- Hasselman, DJ, Ricard, D, Bentzen, P. 2013. Genetic diversity and differentiation in a wide ranging anadromous fish, American shad (*Alosa sapidissima*), is correlated with latitude. *Molecular Ecology* 22: 1558-1573.
- Hauge, XY, Litt, M. 1993. A study of the origin of 'shadow bands' seen when typing dinucleotide repeat polymorphisms by the PCR. *Human Molecular Genetics* 2: 411-415.
- Hedgecock, D. 1991. Contrasting population genetic structures of pelagic clupeoids in the California current. Long term variability of pelagic fish populations and their environment. Pergamon Press, Oxford, 199-207.

- Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In *Genetics and Evolution of Aquatic Organisms*, London: Chapman & Hall: 122-134.
- Hedgecock, D, Pudovkin, AI. 2011. Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. *Bulletin of Marine Science* 87: 971-1002.
- Holleley CE and Geerts PG. 2009. Multiplex Manager 1.0: a crossplatform computer program that plans and optimizes multiplex PCR. *BioTechniques* 7: 511-517.
- Hurst, TP, Conover, DO. 2003. Seasonal and interannual variation in the allometry of energy allocation in juvenile striped bass. *Ecology* 84: 3360-3369.
- Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 19, 1099-1106.
- Kirkley JE, McConnell KE, Ryan W. 2000. Economic aspects of allocating striped bass among competing user groups in Virginia. *Virginia Marine Resources Report*, No. 2000-05, Newport News, Virginia, US
- Kohlenstein LC. 1981. On the proportion of the Chesapeake Bay stock of Striped Bass that migrates into the coastal fishery. *Transactions of the American Fisheries Society* 110: 168-179.
- Laughlin TF, Turner BJ. 1996. Hypervariable DNA markers reveal high genetic variability within striped bass populations of the lower Chesapeake Bay. *Transactions of the American Fisheries Society* 125: 49-55.

- Lindley, ST, Erickson, DL, Moser, ML, Williams, G, Langness, OP, McCovey, BW, Belchik, M., Vogel, D, Pinnix, W, Kelly, JT, Heublein, JC. 2011. Electronic tagging of green sturgeon reveals population structure and movement among estuaries. *Transactions of the American Fisheries Society* 140: 108-122.
- Liu, JX, Ely, B. 2009. Sibship reconstruction demonstrates the extremely low effective population size of striped bass *Morone saxatilis* in the Santee–Cooper system, South Carolina, USA. *Molecular Ecology* 18: 4112-4120.
- Liu S, Rexroad CE 3rd, Couch CR, Cordes JF, Reece KS, Sullivan CVA. 2011. Microsatellite linkage map of striped bass (*Morone saxatilis*) reveals conserved synteny with the three-spined stickleback (*Gasterosteus aculeatus*). *Marine Biotechnology* 14:237–244.
- Lowe, WH, Allendorf, FW. 2010. What can genetics tell us about population connectivity? *Molecular Ecology* 19: 3038-3051.
- Mamoozadeh, NR, McDowell, JR, Rooker, JR., Graves, JE. 2017. Genetic evaluation of population structure in white marlin (*Kajikia albida*): the importance of statistical power. *ICES Journal of Marine Science*: fsx047.
- Mansueti, RJ. 1961 Age, growth, and movements of the striped bass, *Roccus saxatilis*, taken in size selective fishing gear in Maryland. *Chesapeake Science*, 2, 9-36.
- Marshall HG, Alden RW. 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine rivers of the lower Chesapeake Bay. *Estuaries* 13: 287-300.
- Massmann WH, Pacheco AL. 1961. Movements of striped bass tagged in Virginia waters of the Chesapeake Bay. 2(1): 3-44.

- McLaren, JB, Cooper, JC, Hoff, TB, Lander, V. 1981. Movements of Hudson River striped bass. *Transactions of the American Fisheries Society* 110: 158-167.
- Merriman, D. 1941. Studies on the striped bass (*Roccus saxatilis*) of the Atlantic coast. US Government Printing Office.
- Miller, MA, Pfeiffer, W, Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Gateway Computing Environments Workshop (GCE), 2010*: 1-8.
- Morin, PA, Leduc, RG, Archer, FI, Martien, KK, Huebinger, R, Bickham, JW, Taylor, BL. 2009. Significant deviations from Hardy–Weinberg equilibrium caused by low levels of microsatellite genotyping errors. *Molecular Ecology Resources* 9: 498-504.
- Narum, S. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation Genetics*, 7: 783–787.
- Ng CL, Able KW, Grothues TM. 2007. Habitat use, site fidelity, and movement of adult Striped Bass in a southern New Jersey estuary based on mobile acoustic telemetry. *Transactions of the American Fisheries Society* 136:1344-1355.
- Nichols, PR, Miller, RV. 1967. Seasonal movements of striped bass, *Roccus saxatilis* (Walbaum), tagged and released in the Potomac River, Maryland, 1959–61. *Chesapeake Science* 8: 102-124.
- Ogburn, MB, Hasselman, DJ, Schultz, TF, Palkovacs, EP. 2017. Genetics and Juvenile Abundance Dynamics Show Congruent Patterns of Population Structure for Depleted River Herring Populations in the Upper Chesapeake Bay. *North American Journal of Fisheries Management* 37: 1083-1092.

- O'Reilly, P, Wright, JM. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *Journal of Fish Biology*, 47: 29-55.
- Paramore LM, Rulifson RA. 2001. Dorsal coloration as an indicator of different life history patterns for Striped Bass within a single watershed of Atlantic Canada. *Transactions of the American Fisheries Society* 130: 663-674.
- Pompanon, F, Bonin, A, Bellemain, E, Taberlet, P. 2005. Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics* 6: 847.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.
- Putman, AI, Carbone, I. 2014. Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecology and Evolution* 4: 4399-4428.
- Rago PJ, Goodyear CP. 1987. Recruitment mechanisms of striped bass and Atlantic salmon: comparative liabilities of alternative life histories. *American Fisheries Society Symposium* 1: 402-416.
- Rexroad C, Vallejo R, Coulibaly I, Couch C, Garber A, Westerman M, Sullivan C. 2006. Identification and characterization of microsatellites for striped bass from repeat-enriched libraries. *Conservation Genetics* 7:971-982.
- Rice, WR. 1990. A consensus combined P-value test and the family-wide significance of component tests. *Biometrics* 46: 303-308.
- Rousset, F. 2008. Genepop'007: a complete reimplementaion of the Genepop software for Windows and Linux. *Molecular Ecology Resources* 8: 103-106.

- Ruzzante, DE. 1998. A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. *Canadian Journal of Fisheries and Aquatic Sciences* 55: 1-14.
- Secor, DH. 2000a. Longevity and resilience of Chesapeake Bay striped bass. *ICES Journal of Marine Science* 57: 808-815.
- Secor, DH. 2000b. Spawning in the nick of time? Effect of adult demographics on spawning behaviour and recruitment in Chesapeake Bay striped bass. *ICES Journal of Marine Science* 57: 403-411.
- Sidell, BD, Otto RG, Powers DA, Kerweit M, Smith J. 1980. A re-evaluation of the occurrence of subpopulations of striped bass (*Morone saxatilis*, Walbaum) in the Upper Chesapeake Bay. *Transactions of the American Fisheries Society* 109:99-107.
- Smouse PE, Long JC, Sokal RR. (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, 35: 627-632.
- Thorrold, S. R., Latkoczy, C., Swart, P. K., & Jones, C. M. (2001). Natal homing in a marine fish metapopulation. *Science*, 291(5502), 297-299.
- Van Oosterhout, C, Hutchinson, WF, Wills, DP, Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Resources* 4: 535-538.
- Vladykov, VD, Wallace, DH. 1938. Is the striped bass (*Roccus lineatus*) of Chesapeake Bay a migratory fish? *Transactions of the American Fisheries Society* 67: 67-86.
- Waldman J, Maceda L, Wirgin I (2012) Mixed-stock analysis of wintertime aggregations of Striped Bass along the Mid-Atlantic coast. *Journal of Applied Ichthyology* 28:2012.



- Waples, RS. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* 89: 438-450.
- Waples, RS., Teel, DJ. 1990. Conservation genetics of Pacific salmon I. Temporal changes in allele frequency. *Conservation Biology* 4: 144-156.
- Waples, RS, Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* 15: 1419-1439.
- Weber, JL., May, PE. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* 44: 388.
- Wingate, RL, Secor, DH, Kraus, RT. 2011. Seasonal patterns of movement and residency by Striped Bass within a subestuary of the Chesapeake Bay. *Transactions of the American Fisheries Society* 140: 1441-1450.
- Wirgin I, Proenca R, Grossfield J. 1987 Mitochondrial DNA diversity among populations of striped bass in the Southeastern United States. *Canadian Journal of Zoology* 67: 891-907.
- Wirgin, II, Grunwald, C, Garte, SJ, Mesing, C. 1991. Use of DNA fingerprinting in the identification and management of a striped bass population in the southeastern United States. *Transactions of the American Fisheries Society* 120: 273-282.
- Wirgin, I, Maceda, L, Waldman, JR, Crittenden, RN. 1993. Use of mitochondrial DNA polymorphisms to estimate the relative contributions of the Hudson River and Chesapeake Bay striped bass stocks to the mixed fishery on the Atlantic coast. *Transactions of the American Fisheries Society* 122: 669-684.

CHAPTER III  
FEASIBILITY OF CLOSE-KNISHIP MARK-RECAPTURE METHODOLOGY TO  
ESTIMATE STRIPED BASS ADULT ABUNDANCE

## **Introduction**

Striped bass support important recreational and commercial fisheries along the Atlantic Coast of North America from Canada to northern Florida (Boreman and Lewis, 1987; Kirkley et al., 2000; Greene et al., 2009; Wingate et al., 2011). Striped bass are anadromous with spawning occurring each spring in the freshwater reaches of major river and estuary systems (Paramore and Rulifson, 2001), including the Chesapeake Bay, the Hudson River, Delaware Bay, and the Roanoke River. Of these systems, the Chesapeake Bay is considered the largest and most productive spawning ground (Kohlenstein 1981), contributing upwards of 90% of young-of-the-year (YOY) individuals in some years (Waldman et al., 2012). The Chesapeake Bay differs from other major spawning grounds because it is a large estuary composed of several major sub-estuary river systems (Marshall and Alden, 1990; Boynton et al., 1995), and each of these systems is thought to host separate spawning populations of striped bass (Nichols and Miller, 1967; Wirgin et al., 1993, Ng et al., 2007).

Recent assessment of the status of the Atlantic striped bass stock indicates that it is not overfished nor experiencing overfishing; however, the female spawning stock biomass (SSB) is just above the Atlantic States Marine Fisheries Commission (ASMFC) biomass threshold of 67,626 metric tons (ASMFC 2016). Since 2012, annual recruitment levels of striped bass have been average to low, with no evidence of a strong year class during that time. The Atlantic Striped Bass Stock Assessment Update

2016 model projections indicate that there is a 39% probability that female SSB will drop below the management biomass threshold over the next three years. While several hypotheses have been put forward to explain the recent decline of striped bass SSB, no single factor or factors have been identified with certainty (ASMFC 2016). Some studies indicate that *Mycobacteriosis*, a bacterial disease in striped bass shown to have large impacts on reproductive output and natural mortality, may be a large contributor (Gautier et al., 2008; Gervasi 2015; Hoenig et al., 2017). The ASMFC (2013) attributed the decline in SSB to a reduction in the availability of prey species in combination with *Mycobacteriosis*.

The indices of relative abundance and SSB for the Atlantic stock of striped bass are derived from fishery independent and fishery dependent catch-per-unit-effort (CPUE) data (ASMFC 2013). In addition to a coast wide tagging program, annual spawning stock and juvenile index surveys are conducted in the major subestuaries of the Chesapeake Bay by both the Maryland Department of Natural Resources and the Virginia Institute of Marine Science (ASMFC, 2016). The Maryland Department of Natural Resources striped bass spawning stock survey uses a CPUE-based method similar to the Virginia survey to determine an index of adult spawning abundance, as well as an index of spawning potential and an age-independent measure for female striped bass (Maryland Department of Natural Resources, Personal Communication). The Virginia Pound Net Survey targets spawning striped bass on the Rappahannock River. This survey calculates an annual abundance index (number, sex, and age of fish captured) and a spawning stock biomass index (SSBI), defined as the catch-per-unit-effort, or CPUE, (kg/net day) of mature male and female fish sampled during the spawning season (Hoenig et al, 2016). Per

Amendment 6 of the Striped Bass Fisheries Management Plan (FMP), both states conduct juvenile striped bass recruitment surveys to develop annual recruitment indices. Data from these surveys are assumed to reflect relative abundance trends of the fishery, and supply data to all striped bass assessments and assessment updates (ASMFC 2016).

Striped bass are assessed as a single Atlantic coastal stock (Maine to South Carolina), and there is little information regarding spawning stock abundance for the major spawning grounds that contribute to the stock. The exception to this is the Chesapeake Bay, which for some analyses is considered a separate management unit within the Atlantic stock (ASMFC 2003; Gautier et al., 2013); however, there are no estimates of striped bass abundance for Chesapeake Bay as a whole or its subestuaries. Annual recruitment or year class strength is known to vary among the subestuaries of Chesapeake Bay (Davis et al., 2016), but lacking good estimates of the number of individuals spawning in each subestuary annually, it is not possible to confidently determine spawning stock/recruitment relationships within subestuaries.

The 2013 striped bass benchmark assessment highlighted the need for new, fishery independent methodologies to estimate adult striped bass abundance. Recently, novel molecular approaches have provided researchers with an alternative method to estimate abundance of spawning adults (Bravington et al., 2014a). Close-kinship mark-recapture analysis (CKMR) is one such genetic approach for abundance estimates that has been applied to Minke whales (Skaug 2001), southern bluefin tuna (Bravington et al., 2014a; Bravington et al., 2016a), and Antarctic blue whales (Bravington et al., 2014b), and may be applicable to striped bass.

CKMR analyses are similar to traditional mark-recapture studies in which individuals are marked with a physical tag (e.g. a spaghetti tag); however, in CKMR studies, individuals are marked by a ‘genetic tag’. These genetic tags are identified using molecular markers, which must be sufficiently numerous and variable to allow for unambiguous individual identification. In essence, spawning adults are ‘marked’ by the genotypes of the YOY. Each YOY receives an allele from each parent at each nuclear locus, effectively ‘marking’ two adults. The genetic tags of the YOY can be ‘recaptured’ (essentially, before the YOY exist) in random samples of spawning adults as genetically identified parent-offspring pairs (POPs). If the population of adults is large, then the number of POPs will be low, whereas if many POPs are recovered, the population is small. The probability that a captured adult is one of the parents for a selected YOY is  $2/N_a$  where  $N_a$  represents the total number of adults alive when the YOY was spawned. Comparing all sampled adults ( $m_a$ ) to a selected YOY, the expected number of POPs is  $m_a * 2/N_a$ . Comparing all sampled YOY ( $m_j$ ) to all sampled adults ( $m_a$ ), the expected number of POPs ( $E[h]$ ) is  $m_j m_a * 2/N_a$  (Bravington et al., 2014a). Rearranging the formula to include the actual number of POPs, or hits ( $h$ ):

$$\widehat{N}_a = \frac{2m_j m_a}{h}$$

This closely resembles the estimate of abundance using the Lincoln-Peterson abundance estimator, a conventional tag-based estimator (Bravington et al., 2016a). While the relationship is useful, a more explicit statistical mark-recapture model is required to accommodate several life-history characters of striped bass, including age-

dependent sampling probability, and non-equilibrium conditions in the spawning population (Bravington et al., 2014a).

A more explicit statistical model used for a CKMR analysis incorporates a length, sex, and age-structured population dynamics model in which demographic parameters are estimated from a maximum likelihood model that combines POP likelihood values from pairwise comparisons of genotypes from all juveniles and adults, length and age compositions of adults, and life history parameters, such as growth and size- or age-specific fecundity (Bravington et al., 2016b). Assumptions of this model include: 1) adults and offspring are independently sampled 2) the von Bertalanffy growth curve closely models fish growth, 3) genetic markers are in Hardy-Weinberg and linkage equilibria, 4) random mating of adults, and 5) individuals are sampled from a ‘closed population’ wherein sampled YOYs are the result of the adults that spawned in the area during the year of the YOY’s birth. A pairwise comparison of every sampled juvenile genotype to every sampled adult genotype provides an estimate of the number of POPs. To qualify as a POP, the putative parent must contain at least one of the YOY’s alleles at each locus. Bravington et al. (2016a) recommend a minimum of 50 confidently assigned parent-offspring pairs (POPs) to reduce the coefficient of variation and increase precision of CKMR estimates.

The purpose of this study was to evaluate the utility of CKMR to estimate abundance of striped bass in a model river system, the Rappahannock River. Specifically, the main objective was to determine if a large enough number of POPs could be recovered using ongoing sampling programs for spawning adults and YOY to develop and implement CKMR to estimate the population size of spawning adults. Based on the

number of POPs recovered, a secondary objective was to estimate the sample sizes required to obtain 50 POPs, the number recommended by Bravington et al. (2016a) to provide precise estimates of abundance for CKMR analyses with a coefficient of variation (CV) of 15%. Testing the feasibility of this novel methodology is an important first step in the application of a new abundance model, and it has been noted that the testing of new methodologies on a smaller scale is an important, but often overlooked step (Teijlingen and Hundley, 2001).



## Materials and Methods

### *Sample Collection*

Adult striped bass were sampled on the spawning grounds of the Rappahannock River during the spawning season (March to May) in 2016 (Figure 1). Striped bass were captured using two commercial pound nets (river miles 46, 47) checked twice a week from 4 April to 26 May 2016. Genetic samples were taken in the form of caudal fin clips stored in 95% ethanol, and only fish with a total length of 458 mm were clipped to ensure that all fish sampled were mature (Mansueti, 1961). Sex was determined by the expression of reproductive products and scales were removed for aging. Following sampling, fish were released alive.

From June to September 2016, young-of-year (YOY) striped bass were collected from the Rappahannock River (river miles 75 to 37) using a 100 ft long, 4ft deep, 0.25 inch mesh beach seine net. YOY fish ranged in size from 22 mm to 73 mm, well below 118mm, the reported mean size of YOY striped bass prior to their first winter (Hurst and Conover, 2003). Genetic samples were taken in the form of fin clips or muscle tissue and stored in 95% ethanol.

### *Marker Selection, DNA Extraction, and Amplification*

Candidate microsatellite loci were selected from more than 500 potential striped bass microsatellite markers available in the literature (Couch et al., 2006; Rexroad et al., 2006; Fountain et al., 2009; Gauthier et al., 2013), based on allelic diversity, chromosome location (linkage map from Liu et al., 2011), and estimated heterozygosity. Candidate microsatellite loci were evaluated to ensure proper amplification. Optimum annealing

temperatures were determined using thermal gradients, and loci were subsequently assembled into multiplexes using Multiplex Manager (Holleley and Geerts, 2009). To ensure each multiplex amplified consistently, each was run on a temperature gradient to ensure an optimal annealing temperature. In total, the 20 microsatellite markers used comprised four multiplex panels consisting of five markers each (Supplementary Table 1).

Total genomic DNA was extracted using Machary Nagel NucleoSpin® DNA tissue kits on the Tecan Freedom EVO® 75 liquid handling system. Microsatellite multiplexes were amplified for plates of 92-95 samples at a time, with negative controls for each step to check for possible contamination. Polymerase chain reaction (PCR) amplifications were performed in 10 ul reactions with locus-specific fluorescent probes. In order to visualize the PCR product, 2 ul of product was combined with 8 ul of formamide and 0.2 ul 500 LIZ Gene Scan Size standard (Applied Biosystems), denatured for 10 minutes at 95°C, and sequenced on a 36 cm 3130xl Capillary Genetic Analyzer (Applied Biosystems, Inc.). The output consisted of electropherograms with different chromatic peaks representing the alleles at each microsatellite locus. The electropherograms were scored using GeneMarker v2.6.0 (SoftGenetics, LLC). To check for evidence of scoring errors and null allele presence, scores were evaluated using MicroChecker 2.2.3 (Van Oosterhout et al., 2004). To ensure consistency in amplification and allele calling, 5% of samples were re-analyzed from PCR reaction to allele scoring. To ensure accuracy of allele calling, the entire data set was scored twice.

### *Recovery of POPs*

The conformation of genotypic distributions to expectations of HWE was evaluated using probability tests (Guo and Thompson, 1992) within GenePop v4.0 (Rousset 2008), with significance values corrected using the sequential Bonferroni correction for multiple comparisons (Rice, 1990).

The presence of POPs among all sampled adults and juveniles was investigated using the parentage analysis program COLONY (Jones and Wang, 2010). The software allows for the assignment of parents to offspring based on genotypes while accounting for allelic dropout and mistyping. The settings for the analyses in COLONY used the full likelihood run, a medium run length, a high likelihood precision, and with a 5% error rate. The relatively high error rate was allowed to ensure that no false negatives would be missed in the analyses. Additionally, inbreeding and polygamy were allowed. All potential POPs that COLONY identified were screened by eye within GeneMarker to confirm that the pair had at least one allele in common at every locus.

COLONY was also used to infer sib-ship relations between sampled YOY. Effective population size ( $N_e$ ) was estimated using the full likelihood method within COLONY, and the analysis was run with the assumption of random mating (Wang 2009).

### *Estimation of Population Size*

To provide a rough estimate of population size, a modified version of the Lincoln-Petersen model was used combining Bravington et al. (2014a) and the Chapman modification (Chapman, 1951) to account for a small sample size as follows:

$$\widehat{N}_a = \frac{2(m_j+1)(m_a+1)}{(h+1)} = 2\widehat{N}_a \quad (1)$$

Here  $\widehat{N}_a$  is the estimated number of adults,  $m_j$  is the number of juveniles (or originally marked individuals),  $m_a$  is the number of adults (or size of second sample), and  $h$  is the number of POPs (or number of marked individuals in the second sample) (Chapman 1951; Pollock et al., 1990, Bravington et al 2014a).

Confidence intervals (95% CI) for the estimates of adult abundance were calculated by first determining the unbiased estimate of variance wherein  $var(2\widehat{N}_a) = 4var(\widehat{N}_a)$ , given by equation 2, and then equation 3 was used to estimate 95% CI where  $z$  is the standard normal variable (Seber 1970, 1982; Pollock et al., 1990)

$$var = 4 \frac{(m_j+1)(m_a+1)(m_j-h)(m_a-h)}{(h+1)^2(2+2)} \quad (2)$$

$$CI = N_a \pm z * \sqrt{var} \quad (3)$$

The coefficient of variation was calculated using the estimated adult abundance,  $\widehat{N}_a$ , in equation 4 taken from Bravington et al. (2014a).

$$CV = \frac{\sqrt{var}}{\widehat{N}_a} \quad (4)$$

If more than 1 but less than 50 POPs are recovered in this study, the number of samples expected to result in 50 POPs will be estimated using the CKMR population size estimate. Bravington et al (2014a) and Bravington et al (2016b) provide methodology that allows for a crude estimation of the number of samples needed for relatively precise estimates using the metric of 50 POPs ( $h_{target}$ ) and/or a CV of 15%. Formula 5 and Formula 6 were both used to estimate the sample sizes needed where  $\widehat{N}_a$  the estimated

adult abundance size is, and  $c$  is a variable representing the fraction of the overall sample comprised of YOY. For these purposes, it was assumed that the sample size was half YOY and half adults. Formula 6 is an analog to formula 5, and the two were compared to determine the appropriate sample size.

$$n_{target} \approx \sqrt{\widehat{N}_a h_{target} / c} \quad (5)$$

$$10\sqrt{\widehat{N}_a} \quad (6)$$

## Results

Adult and young-of-year (YOY) samples were collected and analyzed from 2016 from the Rappahannock. All samples were genotyped for 20 microsatellite markers. Individuals that were unable to be accurately scored or failed to amplify for 3 or more loci were removed from the dataset. A total of 755 adults and 555 YOY were collected from spawning and nursery grounds on the Rappahannock River. However, 384 adult and 166 juvenile samples were removed from the dataset due to problems with amplification. The remaining 760 samples, comprising 371 adults and 389 YOY, were used for CKMR analysis (Table 1). The adult samples consisted of 282 males and 89 females.

Loci were removed that exhibited consistent amplification issues and deviations from Hardy Weinberg Equilibrium. Tests for HWE indicated that the genotypic distributions among individuals for three loci, 1322, 1437, and 1491, were significantly out of equilibrium in two or more sample groups after Bonferroni correction for multiple comparisons. Visual inspection of the multiple electropherograms showed large peak shifts at these three loci and they were not included in further analyses. The remaining loci showed a less than 1% error rate in amplification due to peak shifts of 1 to 3 repeat motifs. MicroChecker indicated that one locus, 1559, showed evidence of a null allele presence in sampled groups. To reduce the effect of possible null alleles, a slight increase in the error rate was allowed to be 5% in COLONY. Matches identified by COLONY as parent-offspring pairs were then checked by eye to confirm a 100% POP match and no null allele impacts at 1559. The use of likelihood scores aided in determining if mismatches at this marker were due to null alleles, and this would be detected by comparing alleles by eye to see if one allele was a homozygote where there should be a

heterozygote (Bravington, personal communication). No issues were detected with the use of this locus either by eye or by likelihood scores. In total, 17 loci were used for the analyses.

#### *POP Assignment*

COLONY identified 18 potential POPs from the dataset. After checking the genotypes of each POP by eye in GeneMarker, it was found that the dataset contained 2 true POPs with 100% matches of at least one allele at each locus. One potential POP had a mismatch at one locus, but with the likelihood values indicated 70% support and both alleles mismatching, it was considered a false positive. For the two recovered POPs, one parent was a female 4 years of age with a total length of 498 mm. The other POP parent was a male 5 years of age with a total length 512 mm.

Analysis of the offspring dataset revealed 14 pairs of potential full siblings, and of these, 9 pairs had probability values of >90%. There were 405 pairs of potential half siblings recovered in the offspring dataset, of which 121 pairs had a probability >90%. COLONY estimated the effective population size of adults at 823, with 95% confidence intervals of 718-946.

#### *Adult Abundance Estimates*

Using the modified Lincoln-Petersen estimator, the estimated adult abundance of spawning adult striped bass in the Rappahannock River in 2016 was 145,081 individuals. The 95% confidence intervals around this estimate were 51,042 on the lower boundary and 239,119 on the upper boundary, with a CV of 0.33. Based on the recovery of 2 POPs with a sample of 371 adults and 389 juveniles, a total (adults and juveniles) of 1,703

individuals would be required to recover 50 POPs, resulting in an abundance estimate with a 15% CV.



## Discussion

The primary objective of this work was to test the feasibility of a novel molecular abundance model, close-kinship mark-recapture (CKMR), to estimate striped bass spawning stock abundance for a major sub-estuary in the Chesapeake Bay, the Rappahannock River. Specifically, this study first sought to determine if a relatively precise estimate of adult abundance could be made using the number of adult and juvenile samples typically available from ongoing surveys. If a sufficiently precise estimate could not be made, I wished to determine how many additional samples would be needed to achieve a desired level of precision. Genetic screening of 371 spawning adults and 389 YOY collected from the Rappahannock River in 2016 resulted in the identification of two parent-offspring pairs (POPs) and an estimated adult population size of 145,081 individuals. The recovery of 2 POPs was well below the target of 50 POPs recommended by Bravington et al. (2014a) for adequate precision. Further calculations showed that a minimum sample size of 1,703 individuals comprising equal numbers of adults and YOY would be required to produce a sufficiently precise estimate of adult abundance. These preliminary results suggest that CKMR analysis is a practical, fishery independent method to estimate the population size of adult striped bass.

This study represents the first attempt to estimate abundance of spawning striped bass within a subestuary of the Chesapeake Bay. Using CKMR, the estimated abundance of adult striped bass in the Rappahannock River during the 2016 spawning season was 145,081 fish (51,042 – 239,119 individuals, 95% CI). The current striped bass assessment is for the entire Atlantic stock, and there are no estimates of population sizes within estuaries or subestuaries with which to compare the CKRM estimate. The 2016 Striped

Bass Stock Assessment Update estimated over 30 million age 4+ fish in the Atlantic stock (ASMFC 2016), and Berggren and Lieberman (1978) suggested that 75% of the Atlantic stock may be of Chesapeake Bay origin. For the Chesapeake Bay, 90% of all the freshwater discharge comes from five major rivers, with the Susquehanna providing 50% of the freshwater discharge (Environmental Protection Agency, 2003). The Potomac, James, Rappahannock, and York rivers account for the remaining 40%, with the Rappahannock River contributing 2.2% of the freshwater discharge (Brush, personal communication). If one assumes that the number of spawning striped bass within a river is proportional to freshwater discharge, it is estimated that 501,332 age 4+ fish in the Rappahannock River, or about three times more than that estimated by CKMR in this study.

While the estimated number of adult striped bass in the Rappahannock River based on a division of the ASMFC coastwide assessment is much larger than the number estimated using CKMR, the numbers might not be as disparate as they seem. The majority of female striped bass do not start spawning until age 7 or 8 (Cowan et al., 1993), and the ASMFC assessment number includes several year classes that may not be spawning, and these individuals would not be detected by CKMR. Alternately, one can compare the CKMR estimate of 145,081 adults to the 2016 commercial catch from the Rappahannock River of 9,554 adults and an exploitation rate of 7% (exploitation rate = catch/abundance) (VMRC, data request, May 2018).

The estimate of striped bass abundance made in this study using CKMR was for adults within the Rappahannock River during the 2016 spawning season. Future estimates using the CKMR theory should be cautious in combining samples from multiple years.

CKMR theory assumes individuals are randomly sampled from a ‘closed population’, and that YOYs are the result of the adults that spawned in the area during the year of the YOY’s birth. Previous work (see Chapter II) suggests that some adult fish may stray to spawn in other rivers in subsequent years. Pooling collections across years to obtain larger sample sizes can bias CKMR estimates, as individuals may spawn in different rivers in different years, and some spawners may be lost due to natural and fishing mortality. These aspects can be addressed in a full implementation of the CKMR model, and Bravington et al (2016b) provide recommendations for addressing various population scenarios. CKMR provides a methodology to estimate adult spawning abundances on an annual basis and over a time series if certain assumptions are considered and sample sizes are appropriate.

For the single year estimate made in this study, 2 POPS were recovered from one sampling season. Recovering less than 50 POPS resulted in a relatively imprecise estimate, with a CV of 33% well above the target CV of 15%. To provide a relatively precise estimate (50 POPS), a total sample size of at least 3,807 individuals comprising an equal number of adults and YOY fish would be needed. The second estimate for total sample size was 3,809 individuals. Both formulas give approximately the same estimated sample size. Annual collections of 1900 adult and 1900 YOY fish may possibly be achieved with the current sampling scheme, but additional sampling will be required in some years. It is important to note that the target of 50 POPS and a CV of 15% was proposed for southern bluefin tuna and may not be an appropriate target for striped bass. However, more samples and research will be needed to determine an appropriate number of POPS and CV for striped bass

The samples for this study were collected using existing VIMS surveys of adult and juvenile striped bass. In the Rappahannock River, the adult tagging survey has averaged 362 adult live releases per year over the last 8 years (Sadler et al., 2017), while the juvenile seine survey has averaged 804 YOY per year over the last 7 years (Gallagher et al., 2017). Both surveys have had annual sample sizes that have ranged from 100 fish to over 1,000 fish. In 2016, the adult survey averaged about 13 fish per day from two pound nets in the Rappahannock, and the juvenile striped bass survey averaged 10 YOY fish per seine haul from a total of 50 hauls. The peak sampling time for adults was mid to late April, and YOY peak sampling time was June to early July. Additional sampling during these time periods could increase samples for both adults and YOY. For adult striped bass, the sampling of four pound nets instead of the two or three pound nets historically sampled would provide additional samples needed in the Rappahannock. Gill nets are used to sample adult striped bass on other rivers, and this gear type could be used to augment pound net samples in the Rappahannock. To sample additional YOY striped bass, more sample sites can be added near documented peak sampling areas between river mile 44 and river mile 55 (Gallagher et al., 2017). Additionally, more frequent visits to peak areas as well as extra tows during June and early July would bolster sample sizes; repeating one or two tows per visit to the three highest catch areas during the first or second round of sampling would provide the study the samples it needs. A few modifications to the sampling schemes in place would provide sufficiently large sample sizes of striped bass for future applications of CKMR.

In addition to increasing sampling efforts, improvements in molecular methodologies could result in greater efficiencies to match parents and offspring.

Original sample sizes collected for this project approached a fraction of the estimated sample sizes with 755 adult samples and 555 YOY samples collected. However, problems with the DNA isolations and amplifications occurred and resulted in the loss of adult and YOY samples. No specific cause for the isolation and amplification issues was determined, despite several attempts to salvage the samples. The use of a robotic methodology for DNA isolations may have contributed to the high failure rate, either with protocol errors or with contamination. While controls were used to test for contamination, there was no way to exclude the possibility of contamination in all DNA isolations. The robot was in a shared space with other research, and while every effort was made to eliminate cross contamination, the high rate of failure may indicate more stringent protocols are needed. For future studies, increased care in sample collection and improvements to DNA isolation could result in a higher success rate for collected samples.

Microsatellite loci were used to determine parentage in this analysis, and they were also surveyed in the original applications of CKRM analysis to other species. Microsatellite markers were selected for this study based on their availability and proven utility in previous genetic analyses of striped bass (Brown et al., 2005; Gautier et al., 2013), and parentage analysis studies (O'Reilly et al., 2002; Castro et al., 2006; Bravington et al., 2016a). Stringent quality control measures were implemented in this study to ensure consistent and reliable sizing of microsatellite alleles. However, due to problems with amplifications, it was necessary to re-amplify and resize several loci to ensure accurate allele sizing. As correct allele calling is necessary to provide absolute certainty that a pair were a parent and offspring match (Selkoe and Toonen, 2006;

Bravington et al, 2014a), and the use of microsatellite alleles has many challenges, the application of a newer molecular marker, single nucleotide polymorphisms, may be of interest to future studies.

Single nucleotide polymorphisms, SNPs, are becoming the preferred molecular marker for genetic fisheries studies (Anderson and Garza, 2006; Hauser et al., 2011). These are bi-allelic markers that are not only abundant in the genome but have been shown to have low error rates and high replicability between labs (Anderson and Garza, 2006). Because SNPs only have two alleles, whereas the microsatellite loci used in this study had as many as 30 alleles, a greater number of SNP loci will be needed to provide unambiguous individual identification and identify POPs in CKMR analysis. However, the reliability and consistency of SNPs outweighs the cost of development, which would also be offset with decreasing high-throughput costs (Glover et. al, 2010; Hauser et al., 2011; Bravington et al., 2016a; Feutry et al., 2017).

In future studies, the combined use of SNPs and the implementation of a full CKMR model may allow for abundance estimates of not only the Rappahannock River, but other major Chesapeake Bay tributaries. A full CKMR model is needed over the basic Lincoln-Peterson estimation used here because of the non-equilibrium sampling that occurs in spawning populations, likelihood estimation adjustments, and the variation in sampling probability of different ages (Bravington et al., 2014). For this full operating model, an integration of statistical-catch-at-age models is needed to address the unique life history parameters for striped bass by way of covariates for age, growth rate, residence, selectivity, fecundity, length, and sex identification. For striped bass, the incorporation of the life history parameters, including growth curves and fecundity, must

be separated by sex because of compelling evidence that supports different growth rates, movement patterns, and maturity schedules for male and female striped bass (Mansueti 1961, Setzler et al., 1980, Secor and Piccoli, 2004; Bravington et al., 2014; Bravington et al., 2016a). Additionally, the post-release mortality of striped bass, which can introduce a bias into the abundance estimation, can vary depending on gear type, age, and water temperature (Dunning et al., 1989; Hopkins and Cech, 1992; Brick and Cech, 2002). The full CKMR model has provided reliable estimates of abundance for other marine species, including southern bluefin tuna, white sharks (Hillary et al., 2018), and Antarctic blue whales (Bravington et al., 2016b).

The application of CKMR provides a potential fishery independent methodology to recover relatively precise abundance estimates of spawning striped bass in the Rappahannock River utilizing sample sizes of adults and YOY collected by ongoing sampling programs. Though crude, this study was able to provide the first estimate of adult abundance for a local, spawning population of striped bass in the Chesapeake Bay. With the addition of increased sampling, improved molecular methodologies, and the use of SNP markers, CKMR for striped bass may yield relatively precise estimates of adult abundance that are specific to spawning grounds within the Chesapeake Bay. The development of a full CKMR model is feasible with the incorporation of striped bass-specific biological parameters. A relatively precise estimate of the number of spawning adults present in a subestuary during specific years, combined with the annual juvenile index, would allow for managers to determine how recruitment varies over time and space, and to better identify those factors that may be directly influencing adult abundance and recruitment.

Table 1: Sample sizes of adult and young-of-year (YOY) striped bass collected in the Rappahannock River in 2016 used in this study from each river and year used in the analyses after removal of problematic individuals. Values shown for overall numbers, males, and females.

	Overall	Male	Female
<b>2016</b>			
Adult Rappahannock	371	282	89
YOY Rappahannock	389	-	-

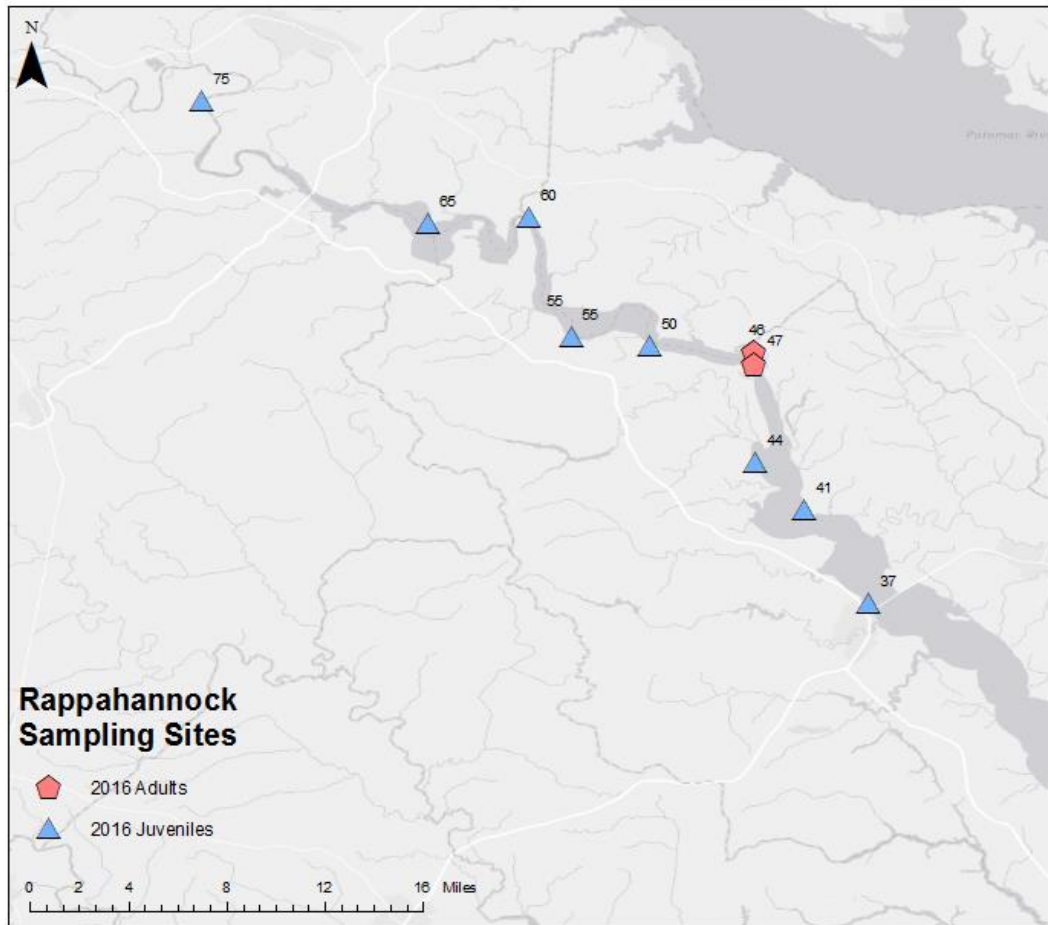


Figure 1: Map of sample locations for adult and young-of-year striped bass samples taken in the Rappahannock River in 2016. Blue triangles indicate juvenile sampling sites, and red pentagons indicate adult sampling sites. River mile is indicated by black numbers.



## References

- Anderson EC, Garza JC. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. *Genetics Society of America* 172: 2567-2582.
- Atlantic States Marine Fisheries Commission (2016) *2016 Atlantic Striped Bass Stock Assessment Update*. Arlington, VA. Retrieved from [http://www.asmfc.org/uploads/file/581ba8f5AtlStripedBassTC\\_Report2016AssmtUpdate\\_Oct2016.pdf](http://www.asmfc.org/uploads/file/581ba8f5AtlStripedBassTC_Report2016AssmtUpdate_Oct2016.pdf).
- Atlantic States Marine Fisheries Commission. 2015. *2015 Atlantic Striped Bass Stock Assessment Update*. Arlington, VA. Retrieved from [http://www.asmfc.org/uploads/file/564106f32015AtlStripedBassAssessmentUpdate\\_Nov2015.pdf](http://www.asmfc.org/uploads/file/564106f32015AtlStripedBassAssessmentUpdate_Nov2015.pdf).
- Berggren TJ, Lieberman JT. 1978. Relative contribution of Hudson, Chesapeake and Roanoke striped bass, *Morone saxatilis*, stocks to the Atlantic coast fishery. *U. S. Fish. Bull.* 76: 335-345.
- Boreman, J, Lewis, RR. 1987. Atlantic coastal migration of striped bass. *American Fisheries Society Symposium* 1:331-339.
- Boynton WR, Garber JH, Summers R, Kemp WM. 1995. Inputs, transformations, and transport of Nitrogen and phosphorus in Chesapeake Bay and selected tributaries. *Estuaries* 18: 285-314.
- Bravington MV, Grewe PM, Davies CR. 2014a. Fishery-independent estimate of spawning biomass of Southern Bluefin Tuna through identification of close-kin using genetic markers. FRDC Report 2007/034. CSIRO, Australia.

- Bravington MV, Jarman SN, Skaug HJ. 2014b. Antarctic blue whale surveys: augmenting via genetics for close-kin and ordinal age. International Whaling Commission Background Paper.
- Bravington, MV, Grewe, PM., Davies, CR. 2016a. Absolute abundance of southern bluefin tuna estimated by close-kin mark-recapture. *Nature Communications* 7: 13162.
- Bravington, M. V., Skaug, H. J., & Anderson, E. C. (2016b). Close-kin mark-recapture. *Statistical Science* 31: 259-274.
- Brick, M. E., & Cech, J. J. (2002). Metabolic responses of juvenile striped bass to exercise and handling stress with various recovery environments. *Transactions of the American Fisheries Society* 131: 855-864.
- Brown KM, Baltazar GA, Hamilton MB. 2005. Reconciling nuclear microsatellite and mitochondrial marker estimates of population structure: breeding population structure of Chesapeake Bay striped bass (*Morone saxatilis*). *Heredity* 94: 606-615.
- Castro, J., Pino, A., Hermida, M., Bouza, C., Riaza, A., Ferreiro, I., Sánchez, L. and Martínez, P., 2006. A microsatellite marker tool for parentage analysis in Senegal sole (*Solea senegalensis*): Genotyping errors, null alleles and conformance to theoretical assumptions. *Aquaculture*, 261(4), pp.1194-1203.
- Chapman, D. G. 1951. Some properties of the hypergeometric distribution, with applications to zoological sample censuses. *University of California Publications in Statistics* 1:131–160.

- Couch, CR, Garber, AF, Rexroad, CE, Abrams, JM, Stannard, JA, Westerman, ME, Sullivan, CV. 2006. Isolation and characterization of 149 novel microsatellite DNA markers for striped bass, *Morone saxatilis*, and cross-species amplification in white bass, *Morone chrysops*, and their hybrid. *Molecular Ecology Resources* 6: 667-669.
- Cowan, J. H., Jr, Rose, K. A., Rutherford, E. S., and Houde, E. D. 1993. Individual-based model of young-of-the-year striped bass population dynamics. II. Factors affecting recruitment in the Potomac River, Maryland. *Transactions of the American Fisheries Society*, 122: 439–458
- Davis, CD, Fabrizio, MC, Tuckey, TD. 2016. Estimation of juvenile striped bass relative abundance in the Virginia portion of Chesapeake Bay: annual progress report: 2015-2016. Virginia Institute of Marine Science, College of William and Mary. <https://scholarworks.wm.edu/reports/1157>
- Dunning DJ, Ross QE, Blumberg AF, Heimbuch DG. 2006. Transport of striped bass larvae out of the lower Hudson River estuary. *American Fisheries Society Symposium* 51: 273.
- Feutry, P, Berry, O, Kyne, PM, Pillans, RD, Hillary, RM, Grewe, PM, Marthick, JR, Johnson, G, Gunasekera, RM, Bax, NJ Bravington, M. 2017. Inferring contemporary and historical genetic connectivity from juveniles. *Molecular Ecology*, 26: 444-456.
- Fountain J, Darden T, Jenkins W, Denson M. 2009. Three multiplexed microsatellite panels for striped bass. *Southeastern Naturalist* 8: 671-676.

- Gallagher, BK, Fabrizio, MC, Tuckey, TD. 2017 Estimation of Juvenile Striped Bass Relative Abundance in the Virginia Portion of Chesapeake Bay: Annual Progress Report 2016-2017. Virginia Institute of Marine Science, College of William and Mary. <https://doi.org/10.21220/V5NC83>
- Gautier DT, Latour RJ, Heisey DM, Bonzek CF, Gartland J, Burge EJ, Vogelbein WK. 2008. *Mycobacteriosis* associated mortality in wild striped bass (*Morone saxatilis*) from Chesapeake Bay, USA. *Ecological Applications* 18: 1718-1727.
- Gauthier DT, Audemard CA, Carlsson JEL, Darden TL, Denson MR, Reece KS, Carlsson J. 2013. Genetic population structure of US Atlantic coastal striped bass (*Morone saxatilis*). *Journal of Heredity* 104: 510-520.
- Gervasi CL. 2015. The reproductive biology of striped bass (*Morone saxatilis*) in Chesapeake Bay (MS Thesis). [Williamsburg, (VA)]: College of William & Mary.
- Glover, KA, Hansen, MM, Lien, S, Als, TD, Høyheim, B, Skaala, Ø. 2010. A comparison of SNP and STR loci for delineating population structure and performing individual genetic assignment. *BMC Genetics* 11: 2.
- Greene, KE, Zimmerman, JL, Laney, RW, Thomas-Blate, JC, 2009. Atlantic coast diadromous fish habitat: a review of utilization, threats, recommendations for conservation, and research needs. Atlantic States Marine Fisheries Commission Habitat Management Series: 464.
- Guo, SW, Thompson, EA. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*: 361-372.

- Hauser, L, Baird, M, Hilborn, RAY, Seeb, LW, Seeb, JE. 2011. An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (*Oncorhynchus nerka*) population. *Molecular Ecology Resources* 11: 150-161.
- Hillary RM, Bravington MV, Patterson TA, Greme P, Bradford R, Feutry P, Gunasekera R, Peddemors V, Werry J, Francis MP, Duffy CAJ, Bruce BD. Genetic relatedness reveals total population size of white sharks in eastern Australia and New Zealand. *Scientific Reports*, 8: 2661.
- Hoenig JM, Sadler PW, Michaelsen S, Goins LM, Harris RE. 2016. Evaluation of striped bass stocks in Virginia: monitoring and tagging studies, 2015-2019, Progress Report.VMRC.
- Holleley CE and Geerts PG. 2009. Multiplex Manager 1.0: a cross platform computer program that plans and optimizes multiplex PCR. *BioTechniques* 7: 511-517.
- Hopkins, TE, & Cech Jr, JJ. 1992. Physiological effects of capturing striped bass in gill nets and fyke traps. *Transactions of the American Fisheries Society* 121: 819-822.
- Hurst, TP, Conover, DO. 2003. Seasonal and interannual variation in the allometry of energy allocation in juvenile striped bass. *Ecology* 84: 3360-3369.
- Jones, OR, Wang, J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, 10: 551-555.
- Kohlenstein LC. 1981. On the proportion of the Chesapeake Bay stock of Striped Bass that migrates into the coastal fishery. *Transactions of the American Fisheries Society* 110: 168-179.

- Kirkley JE, McConnell KE, Ryan W. 2000. Economic aspects of allocating striped bass among competing user groups in Virginia. Virginia Marine Resources Report, No. 2000-05, Newport News, Virginia, US.
- Liu S, Rexroad CE 3rd, Couch CR, Cordes JF, Reece KS, Sullivan CVA. 2011. Microsatellite linkage map of striped bass (*Morone saxatilis*) reveals conserved synteny with the three-spined stickleback (*Gasterosteus aculeatus*). Marine Biotechnology 14:237–244.
- Mansueti RJ. 1961. Age, growth, and movements of the striped bass, *Roccus saxatilis*, taken in size selective fishing gear in Maryland. Chesapeake Science 2: 9-36.
- Marshall HG, Alden RW. 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine rivers of the lower Chesapeake Bay. Estuaries 13: 287-300.
- Nichols PR, Miller RV. 1967. Seasonal movements of striped bass, *Roccus saxatilis* (Walbaum), tagged and released in the Potomac River, Maryland, 1959–1961. Chesapeake Science, 8: 102–124.
- Ng CL, Able KW, Grothues TM. 2007. Habitat use, site fidelity, and movement of adult Striped Bass in a southern New Jersey estuary based on mobile acoustic telemetry. Transactions of the American Fisheries Society 136:1344-1355.
- O'Reilly, P, Wright, JM. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. Journal of Fish Biology 47: 29-55.
- Paramore LM, Rulifson RA. 2001. Dorsal coloration as an indicator of different life history patterns for Striped Bass within a single watershed of Atlantic Canada. Transactions of the American Fisheries Society 130: 663-674.

- Pollock, KH, Nichols, JD, Brownie, C, Hines, JE. 1990. Statistical inference for capture-recapture experiments. *Wildlife Monographs*, 3-97.
- Rexroad C, Vallejo R, Coulibaly I, Couch C, Garber A, Westerman M, Sullivan C. 2006. Identification and characterization of microsatellites for striped bass from repeat-enriched libraries. *Conservation Genetics* 7: 971-982.
- Rice, WR. 1990. A consensus combined P-value test and the family-wide significance of component tests. *Biometrics* 46: 303-308.
- Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* 8: 103-106.
- Sadler, PW, Goins, LM, Hoenig, JM, Michaelsen, S, Groner, ML, Harris, RE. 2017. Evaluation of Striped Bass Stocks in Virginia: Monitoring and Tagging Studies, 2015-2019 Progress Report. Virginia Institute of Marine Science, College of William and Mary. <https://doi.org/10.21220/V5SF00>
- Seber, GAF. 1970. The effects of trap response on tag recapture estimates. *Biometrics*: 13-22.
- Seber, GAF. 1982. The estimation of animal abundance and related parameters.
- Secor DH, Piccoli PM. 2004. Oceanic migration rates of upper Chesapeake Bay striped bass (*Morone saxatilis*) determined by otolith microchemical analysis. *Fishery Bulletin* 105: 62-73.
- Selkoe, KA, Toonen, RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters*, 9: 615-629.
- Setzler, EM., Boynton, WR., Wood, KV, Zion, HH, Lubbers, L, Mountford, NK, Frere, P, Tucker, L, Mihursky, JA, 1980. Synopsis of biological data on striped bass,

*Morone saxatilis* (Walbaum). NOAA technical report NMFS circular 433. *FAO Fisheries Synopses (FAO). no. 121.*

Skaug, H. 2001. Allele-Sharing Methods for Estimation of Population Size. *Biometrics*, 57: 750-756.

Teijlingen, ER, Hundley, V. 2001. The importance of pilot studies. *Social research Update*, 35: 1-4.

United States Environmental Protection Agency. 2003. *Technical Support Document for Identification of Chesapeake Bay Designated Uses and Attainability*. Region III Chesapeake Bay Program Office. Retrieved from [https://www.chesapeakebay.net/content/publications/cbp\\_13218.pdf](https://www.chesapeakebay.net/content/publications/cbp_13218.pdf)[http://www.asmfc.org/uploads/file/581ba8f5AtlStripedBassTC\\_Report2016AssmtUpdate\\_Oct2016.pdf](http://www.asmfc.org/uploads/file/581ba8f5AtlStripedBassTC_Report2016AssmtUpdate_Oct2016.pdf).

Van Oosterhout, C, Hutchinson, WF, Wills, DP, Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Resources* 4: 535-538.

Waldman J, Maceda L, Wirgin I. 2012. Mixed-stock analysis of wintertime aggregations of Striped Bass along the Mid-Atlantic coast. *Journal of Applied Ichthyology* 28:2012.

Wang, J. and Santure, A. W. (2009) Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics* 181: 1579–1594.

Wingate, RL, Secor, DH, Kraus, RT. 2011. Seasonal patterns of movement and residency by Striped Bass within a subestuary of the Chesapeake Bay. *Transactions of the American Fisheries Society* 140: 1441-1450.



Wirgin I, Maceda L, Waldman JR, Crittenden RN. 1993. Use of mitochondrial DNA polymorphisms to estimate the relative contributions of the Hudson River and the Chesapeake Bay striped bass stocks to the mixed fishery on the Atlantic Coast. *Transactions of the American Fisheries Society* 122: 669-684.

CHAPTER IV

CONCLUSION

## Conclusion

The objectives of this study were to 1) examine the genetic population structure of striped bass within the subestuaries of lower Chesapeake Bay, and 2) to determine the feasibility of a new methodology, close-kinship mark-recapture (CKMR), to estimate the adult abundance within a subestuary. Knowledge of the genetic population structure of striped bass and the development of fishery independent estimates of adult abundance have been identified as critical management needs for this important resource.

In Chapter II, I was unable to reject the null hypothesis of a single genetic population of striped bass in the Virginia portion of the Chesapeake Bay. Prior studies of striped bass population structure along the Atlantic coast concluded that striped bass within the major estuaries of the Atlantic coast comprised genetically distinct spawning populations, but within the Chesapeake Bay conflicting conclusions have been drawn regarding the existence of intra-estuary genetic structure. My study found low, but significant population pairwise  $F_{ST}$  values between the James, Mattaponi, and Rappahannock rivers; however, the pairwise  $F_{ST}$  values between years (2016 and 2017) for collections from the same river were higher than those among rivers in the same year. Additional genetic analyses showed no support for significant genetic structuring among subestuaries, providing support for genetic connectivity among subestuaries within the lower Chesapeake Bay. A long-term striped bass tagging data set was reviewed to determine if there is straying of adults among major river systems between different years. While the majority of adult striped bass return to the same

spawning grounds in subsequent years, the tagging data demonstrate sufficient straying to prevent the accumulation of genetic differences between spawning grounds. In effect, each subestuary represents an ecological stock, but not an evolutionary stock.

In Chapter III, the feasibility of a new methodology to estimate spawning stock abundance using CKMR was tested within a model river system, the Rappahannock River in 2016. This method has been successfully applied to estimate adult abundance for a few pelagic species and provides a new fishery independent methodology for stock assessment. Using samples of spawning adults and young-of-year collected by on-going surveys, this study recovered 2 parent-offspring pairs (POPs), resulting in an estimate of 148,081 striped bass adults in the Rappahannock River. There are no fishery-dependent abundance estimates with which to compare this number with, however the total commercial catches from the Rappahannock River number less than 10,000 individuals in 2016. Additional calculations show that with extra sampling, the 50 POPs recommended for a relatively precise estimate can be obtained. This study represents a preliminary use of the CKMR method and theory. For management purposes, a larger number of POPs as well as a full, empirical model that accounts for sampling biases, life history parameters, and sex differences among striped bass should be employed.

Improvements on the work done in this study include the expansion of the sampling range, the addition of more samples of a longer period of time, and the use of a newer molecular marker. Both parts of this study focused on Virginia

river systems. The tagging data analyzed in Chapter II indicate that straying is occurring among Maryland and Virginia river systems. Expansion of sampling to include more rivers can provide a more complete picture of striped bass genetic population structure throughout the Bay. Additionally, for both studies, incorporating additional years of sampling would allow evaluation of temporal stability of samples. For CKMR, a longer time series would allow for an investigation into how abundance estimates change between spawning years. Both studies complement each other in that understanding where striped bass spawn impacts how inferences of adult spawning abundance can be made.

For both studies, future genetic analyses incorporating single-nucleotide polymorphisms (SNPs) over microsatellite are recommended. Recent studies show that the use of SNP markers not only match, but can greatly exceed, the power of microsatellite markers to resolve genetic population structure, and the cost of SNPs has come down to allow for the high-throughput needed for CKMR estimations. The complications encountered in this study may have been avoided with the development of SNP markers, which show greater consistency in scoring within and among laboratories, but these markers have yet to be developed for striped bass.

Overall, the results from my study will improve the understanding of striped bass habitat use for spawning, spawning patterns, and spawning abundance within Chesapeake Bay to better address key management concerns. Currently, management is primarily focused on the Atlantic stock, with little local focus on the stock characteristics within the Chesapeake Bay management area.

This is important because the Chesapeake Bay is the largest striped bass spawning ground, and, in most years, provides the most recruits to the Atlantic stock. A decline in recruitment in the Chesapeake Bay was one of the driving forces behind the striped bass stock collapse in the late 1970s and early 1980s. Understanding how striped bass recruitment is impacted on a more local, river-by-river level can provide managers with the ability to allocate management resources more efficiently to prevent negative impacts to the striped bass Atlantic stock.

## Appendix I

Supplementary Table 1: Table of the multiplexes, microsatellite loci, and protocols used for both Ch. II and Ch. III

Multiplex	Protocol	Locus	Repeat Motif	Marker Dye	Color	Source
1	<i>Denature: 95°C for 5 minutes</i>	1584	Tetra	FAM	Blue	Rexroad et al 2006
	<i>Annealing: 28 Cycles at 63°C</i>	1556	Tetra	VIC	Green	Rexroad et al 2006
		1568	Tetra	NED	Black	Rexroad et al 2006
		1271	Tri	VIC	Green	Rexroad et al 2006
	<i>Final Extension at 60°C for 30 minutes</i>	1617	Tetra	PET	Red	Rexroad et al 2006
2	<i>Denature: 95°C for 5 minutes</i>	1322	Tetra	FAM	Blue	Rexroad et al 2006
	<i>Annealing: 28 Cycles at 63°C</i>	1591	Tri	FAM	Blue	Rexroad et al 2006
		1437	Tri	NED	Black	Rexroad et al 2006
		1491	Tri	PET	Red	Rexroad et al 2006
	<i>Final Extension at 60°C for 30 minutes</i>	1559	Tetra	VIC	Green	Rexroad et al 2006
3	<i>Denature: 95°C for 5 minutes</i>	1577	Tetra	VIC	Green	Rexroad et al 2006
	<i>Annealing: 28 Cycles at 56°C</i>	1587	Tetra	FAM	Blue	Rexroad et al 2006
		1592	Tetra	FAM	Blue	Rexroad et al 2006
		1628	Tetra	PET	Red	Rexroad et al 2006
	<i>Final Extension at 60°C for 30 minutes</i>	1273	Tetra	PET	Red	Rexroad et al 2006
4	<i>Denature: 95°C for 5 minutes</i>	1602	Tetra	NED	Black	Rexroad et al 2006
	<i>Annealing: 28 Cycles at 56°C</i>	1603	Tetra	PET	Red	Rexroad et al 2006
		1290	Tetra	NED	Black	Rexroad et al 2006
		1598	Tetra	MED	Black	Rexroad et al 2006
	<i>Final Extension at 60°C for 30 minutes</i>	1638	Tetra	VIC	Green	Rexroad et al 2006

Supplementary Table 2: Table representing tagging recapture rates. The columns represent the river in which fish were originally tagged. The area of recapture on each row shows the percentage of fish originally tagged in a river that were recaptured in a specific area. The last two rows indicate what percentages of fish from a river were recaptured within Chesapeake Bay and outside the Chesapeake Bay.

<b>Area of Recapture</b>	<b>River of Origin</b>	
	<i>James River</i>	<i>Rappahannock River</i>
Atlantic VA	0	7
Choptank	2	2
DC	0	1
DE	0	4
Elk River	0	1
James	194	13
MA	17	20
Main Stem MD	8	38
Main Stem VA	4	24
ME	2	0
NC	2	11
NJ	6	27
NY	3	16
Patapsco	0	1
Patuxent	0	4
Piankatank	0	2
Potomac	3	21
Rappahannock	7	645
RI	3	4
Severn	1	6
Susquehanna	0	1
VA Wimico	0	2
York	3	15
In Chesapeake Bay	222	776
Out of Chesapeake Bay	33	89