# POPULATION STRUCTURE OF WHITE PERCH (MORONE AMERICANA) IN DELAWARE BAY

by

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#### ABSTRACT

White perch (Morone americana) are one of the most common fish found in the waters of the eastern United States. In Delaware Bay, they are used to monitor contamination levels and set seafood safety consumption advisories and are therefore of ecologic and economic importance. This study sought to determine the genetic population structure of white perch in Delaware Bay. Fish were collected in the summer of 2013 from three separate locations. Five mitochondrial loci (cox2/ATP8/AT6/cox3, cyt-b, ND2, ND3/4L/4, ND5) and two nuclear loci (cx35, GH) were examined. Using PCR-RFLP analyses, polymorphisms were found in ND2, ND3/4L/4, ND5 and GH. An exact test performed on mitochondrial loci revealed no significant differentiation between the three locations, while a F<sub>st</sub> value for GH suggested slightly significant genetic differences between the three locations (P = 0.044). A two-way ANOVA with replication for size data, genotype and location revealed that the three locations sampled contained fish of significantly different sizes (P = 0.0112) but that genotype had no significant effect on fish size nor did location and genotype interact. Mitochondrial data was combined with previous data from Gaffney lab to allow for haplotype frequencies of fish to be compared between nine locations in Delaware Bay, which were significantly different (exact test, P < 0.0001). The results suggest at least three different populations of white perch exist in Delaware Bay. White perch have been found to have an upper salinity limit and salinity gradients in Delaware Bay may prevent white perch from migrating between

populations. The results of this study, in conjunction with future analyses, can allow fishery management practices to better coincide with the biology of white perch.

#### Chapter 1

#### **INTRODUCTION**

#### 1.1 White Perch

White perch, Morone americana, are one of the most ubiquitous fish found in coastal waters of eastern United States and are of great ecological and economic importance (Figure 1, Seltzer-Hamilton 1991). White perch are distributed from Nova Scotia to South Caroline and are typically found in coastal bays and estuaries, as well as freshwater and brackish lakes and ponds (Jones et al. 1978, Woolcot 1962). White perch are especially abundant in the New Jersey, Delaware and Maryland areas; in fact, in trawling surveys to classify fish in Delaware Bay, white perch were found to be the most abundant fish in the bay, comprising of 24.3% of the catch (Able 2001). Because of their abundance, white perch are used in Delaware Bay to monitor contamination levels in order to gauge the overall health of the bay and to set seafood consumption safety advisories (DNREC 2011a, Suk 2006). In addition, abundant white perch are often fished commercially and recreationally, which can increase local economies. In addition to economic value, white perch play an important ecologic role by providing food to larger predators and preving on smaller organisms, keeping a balanced ecosystem in terms of trophics (Stanley and Daine 1983). Because of their overall importance, the objective of this study was to determine the spatial distribution of white perch in Delaware Bay, particularly to see whether or not genetically distinct populations exist in the bay.



Figure 1. White perch, Morone americana.

#### **1.1.1** White Perch Life History and Ecology

White perch exhibit two unique characteristics that ultimately shape their distribution patterns: semi-anadromy and partial migration. White perch are semi-anadromous, meaning that they move upstream into fresh waters to spawn (Able and Fahay 1998). White perch exhibit habitat flexibility and are able to live under a variety of conditions, including both clear and turbid waters as well as in both slow and fast currents (Sutton et al. 1996). Despite this, white perch require specific environmental conditions for spawning, including salinity lower than 4 ppt (Able and Fahay 1998), waters less than 7 meters deep and temperatures greater than 7°C (Stanley and Daine 1983).

Due to these requirements, white perch return to shallow freshwater areas upstream to spawn from late spring to early summer (Able and Fahay 1998). In the nontidal Delaware River, spawning areas appear to occur in freshwater portions of streams and rivers from Morrisville to at least New Hope (Figure 2). Embryos are adhesive and hatch after one to seven days (Stanley and Daine 1983). Typically, over winter months, young of the year (less than 30 mm in fork length) white perch stay in low salinity nursery areas all year while adults (over two years old) retreat to deeper more saline waters (Stanley and Daine 1983, Able and Fahay 1998). Juvenile spatial distribution is, however, highly dependent on streamflow and salinity. For instance, in years of drought (i.e. restricted stream flow), white perch juveniles were found to remain upstream in the Patuxent River estuary of the Chesapeake Bay (Kerr et al. 2009). Salinity also acts as a barrier to where the juveniles can exist. For instance, due to an increasing salinity gradient toward the mouth of Delaware Bay, no young of the year white perch have been found below the Leipsic River in trawls in Delaware Bay for the past 30 years (Figure 2, Michels and Greco 2010).



Figure 2. Spawning regions for *M. americana*. For nontidal Delaware River (i.e. northern Delaware Bay), spawning occurs in freshwaters from Morrisville to New Hope. Salinity restricts juveniles to upper regions of the bay, as no young of the year juveniles have been found below the Leipsic River.

Adult white perch are thought to remain in the river system where they were spawned (Mansueti 1961, McGrath and Austin 2009). For instance, in a study conducted at the Patuxent River, white perch that were caught and tagged on the outskirts of the Patuxent River Estuary (i.e. in Chesapeake Bay waters) never entered the estuary but instead were found to have come from the upper parts of the neighboring Chesapeake Bay (Mansueti 1961). These results have suggested that white perch travel from lower salinities (i.e. born in freshwaters) to higher salinities and not vice versa, indicating that salinity could be a potential barrier between groups of white perch (Mansueti 1961, McGrath and Austin 2009). White perch are not found in salinities above 13-18 ppt (Mansueti and Scheltema 1953, Setzler-Hamilton 1991).

White perch are also known to show partial migration, in which there is a coexistence of resident and migratory fish within the same population (Figure 3, Kerr and Secor 2009, Kerr et al. 2009). In a study performed by Kerr and Secor (2009) in the Patuxent River, white perch were shown to have two contingent types: resident contingents (i.e. fish that spend their lives residing in freshwater habitats where born) and dispersive contingents (i.e. fish that spend their lives migrating downstream into brackish habitats). Those dispersive contingents were shown to have increased growth rates and increased consumption rates, overall allowing them to be bigger (Figure 4); in addition, salinity also showed a positive relationship with growth and metabolism (Kerr and Secor 2009, Kerr et al. 2009).



Figure 3. Partial migration of white perch, where white arrows represent residence in freshwater and gray arrows represent movements and migration in brackish waters (Kerr et al. 2009).



Figure 4. Length of both *M. americana* contingents (i.e. resident and migratory) in Patuxent River. Numbers above each box correspond to sample size; center vertical line represents the median; the length of each box represents range where 50% of the values fall; asterisks are datapoints outside of the range (Kerr et al. 2009).

With semi-anadromy and partial migration, it is apparent that the distribution of white perch is complex. These unique characteristics are thought to be evolutionary advantageous to white perch. For instance, semi-anadromy, in which fish can migrate to more saline waters, is thought to be associated with benefits including increased growth potential, increased feeding opportunities, more favorable temperature and osmoregulatory conditions (Kerr and Secor 2009, McDowall 2001). Similarly, though the exact mechanisms are not known, partial migration is thought to occur due to conditions during development, where the two morphs exhibit phenotypic plasticity (Kerr and Secor 2009). In addition, in terms of population dynamics, having migrating contingents allows for genetic exchange and occupation of greater niches while resident contingents allow for a simultaneous protection and stability of the genetic stock (Kerr and Secor 2009). Though currently poorly understood, these characteristics nonetheless affect the spatial distribution of white in a bay system, shaping the overall population structure of white perch.

#### 1.1.2 White Perch Distribution in Chesapeake and Delaware Bays

Because of their unique life history, white perch often occur in distinct genetic populations in which hydrographic containment is thought to occur because saline waters act as a barrier to migration and subsequent gene flow (Mulligan & Chapman 1989, Woolcot 1962, Kerr et al. 2009). Genetic analyses of mitochondrial DNA have revealed that white perch in the Chesapeake Bay exist as reproductively isolated populations, where more than 50% of the variation observed attributable to interpopulation variation (Mulligan & Chapman 1989). Chesapeake Bay data from Mulligan & Chapman (1989) reveal that in the lower and more saline waters of the bay, genetic differences between populations were more distinct; whereas, in upper and less saline waters more gene exchange appeared to occur. This further supports the claim that salinity acts as a key barrier to gene flow and that it may in fact be a key determining factor of population structure for white perch in the Chesapeake Bay.

It is currently unclear as to whether or not this phenomenon is also observed for white perch in Delaware Bay. In a study conducted for the Delaware Department of Natural Resources and Environmental Control (DNREC), initial analyses of mitochondrial DNA from white perch reveal a similar trend in Delaware Bay (Gaffney lab, unpublished). Significant haplotype differences between locations sampled in Delaware Bay were seen, where white perch seem to be of two genetic classes – upper and lower bay (Figure 5). Further, analyses between proximate locations showed significant differences in genetic composition (Gaffney lab, unpublished).



Figure 5. Composite mt-DNA haplotype frequencies of white perch in Delaware Bay, where N = 30-50 white perch/site (Gaffney lab, unpublished).

#### **1.2 Understanding Population Genetics**

Molecular genetic analyses provide a powerful tool to determine population structure. A population of white perch would be defined as a group of reproductively isolated individuals – that is, fish that are more likely to mate with each other than from those from other locations (Waples and Gaggiotti 2006, Hartl and Clark 2007, Beebee and Rowe 2008). Genetic drift occurs via the transmission of genetic material with generations, resulting in natural fluctuations of allele frequency, assuming a large sized population and little selection acting on the group (Hartl & Clark 2007). When a group of organisms are isolated (i.e. by spatial or temporal barriers) and can therefore not reproduce with each other, divergence occurs and the populations begin to become genetically distinct, as can be reflected by their allele frequencies (Waples and Gaggiotti 2006). In contrast, migration allows for populations to mix, ultimately leading to a genetically homogenized population with similar allele frequencies (Waples and Gaggiotti 2006). In examining population structure of white perch, analyses of both mitochondrial and nuclear DNA allow for the determination and comparison of the genetic compositions of groups of white perch.

#### **1.2.1 Mitochondrial DNA**

Examining variation in mitochondrial DNA (mt-DNA) is a popular technique in population genetics because the maternally inherited mitochondrial genome is small, non-recombining and quickly evolving (Meyer 1994). In fact, mt-DNA is said to evolve five to ten times faster than nuclear DNA (Meyer 1994). Because of these characteristics, genetic differences between mt-DNA molecules of isolated populations within a species can easily be detected using a variety of techniques that look at sequence variation through both direct and indirect means. For instance, DNA sequencing allows for direct comparison of individuals while polymerase chain reaction and restriction length fragment polymorphism (PCR-RFLP) allow for an indirect comparison of individuals via resolution of banding patterns from digested PCR products (Innis et al. 1990). Denaturing gradient electrophoresis (DGGE), in which a chemical gradient is used to denature DNA samples as they move across a gel, is another means of indirect determination of variation in the mitochondrial genome (Innis et al. 1990). Overall, these methods provide haplotypes for individuals and allow overall inter and intrapopulation variation to be determined (Innis et al. 1990).

Though mt-DNA is a powerful tool to determine molecular diversity between individuals and between species, recent studies have shown that it may in fact be a poor reflection of population history (Galtier et al. 2009). For instance, in using mt-DNA to determine species delineation and identification, it is assumed that the mitochondrial genome is clonal (i.e. non-recombinant), neutral (i.e. not selected upon) and has a constant mutation rate (Galtier et al. 2009). These presumably inherent mt-DNA properties, however, are being challenged and studies have found that mt-DNA may in fact be recombinant, nearly neutral (i.e. selected upon) and have a sporadic mutation rate (Galtier et al. 2009). Nonetheless, using mt-DNA provides a framework for population structure in which further genetic analyses (i.e. analyses of the nuclear genome) can better resolve.

#### 1.2.2 Nuclear DNA

Nuclear DNA (n-DNA) allow for additional genetic information in determining population structure of an organism. An advantage to using the nuclear genome is that it is biparentally inherited and therefore can give an indication as to

whether males and females have similar evolutionary histories. Two types of nuclear markers are typically used: microsatellites and single-copy nDNA (sc-nDNA) polymorphisms (Balloux and Lugon-Moulin 2002, Kuhn and Gaffney 2008). Microsatellites are regions of nDNA that are highly polymorphic and therefore allow variation of organisms within a species to be determined (Balloux and Lugon-Moulin 2002, Lui and Ely 2009, Couch et al. 2006). In addition, sc-nDNA polymorphisms can be determined via PCR-RFLP and are often found in highly variable intron sequences of genes (Kuhn and Gaffney 2008). Restriction digests of amplified sc-nDNA targets can be run on a gel and the resulting banding patterns can be scored for single nucleotide polymorphisms (SNPs) or indels (insertion/deletions) affecting the restriction enzyme recognition site. These can fine tune and add to the mt-DNA and microsatellite findings. Overall, all of the techniques discussed can be employed to examine allele frequencies in isolated populations and allow for the determination of the population structure of an organism, such as white perch in Delaware Bay.

#### **1.3** Sustainable Fisheries

The determination of white perch population structure in Delaware Bay has considerable applications in sustainable fisheries. Sustainable management of fisheries sets regulations based on the ecological and biological characteristics of an organism (Charles 2008). Fishery management becomes especially difficult when waters are polluted and the consequent contaminated organisms are deemed unsafe to eat, making the stock limited. When this occurs, regulations are generally made to harvest the maximum catch in the most economical way possible, which often means having to neglect the biological features of the organism. However, when fishery boundaries

do not coincide with biological boundaries, the maximum output of the fishery is ultimately hindered (Charles 2008).

#### **1.3.1** Delaware Bay Fisheries

Delaware Bay is a unique case where management has to consider toxic contaminants in the water while maintaining important recreational and commercial fisheries. The Delaware River Basin Commission (DRBC) sets different management zones based on water type and concentration levels (Figure 6, Suk 2006). These zones are set via the use of indicator species, in which that the tissues of a particular organism are tested for various chemical contaminants (DNREC 2011). In Delaware Bay, white perch are used as an indicator species because of their abundance and life history (i.e. they do not leave Delaware Bay). This allows fishery zonation and safe consumption advisories to be constructed, which give an overall indication of the health of the bay (DNREC).



Figure 6. Management boundaries in Delaware Bay based on various contamination levels, as set by Delaware River Basin Commission (Suk 2006).

Delaware Bay contains a number of various contaminants, most notably polychlorinated biphenyls (PCBs) but also dioxins, mercury, chlorinated pesticides and furans (DNREC 2011b). Polychlroinated biphenyl (PCB) levels in white perch in the Delaware Bay have been kept on record since 1969 (Figure 7, Suk 2006). While the data shows a decrease in PCB concentrations in fish tissue over the course of about 50 years, the concentration of the contaminant are still too high for safe consumption (Suk 2006, Fikslin et al. 2005). Therefore, consuming white perch remains a great health risk. PCBs are thought to be a likely human carcinogen causing defects in human development, immune response and hormone function (Carpenter 1998, Johnson et. al 1999). PCBs also have deleterious effects on organisms that accumulate the chemical; for instance, studies have shown that fish containing significant PCBs in their tissues have decreased abilities to reproduce, less viable offspring, defective hormone levels, skeletal deformities and detrimental effects on the heart and circulatory system (Monosson, et al. 1994, MacGillivary et al. 2011). PCBs in Delaware Bay are higher in the upper parts of the bay and lower near the mouth of the bay due to less terrestrial run off and more mixing with less contaminated Atlantic Ocean waters in the lower bay (MacGillivary et al. 2011). PCBs found throughout the entire Delaware Bay region (i.e. zones 2-6 as determined by the DRBC, however, are deemed above the safe limits for consumption (Figure 7, Suk 2006, Fikslin et al. 2005).



Figure 7. PCB concentrations of filet samples of white perch from 1969 to 2002 in zones 2 to 6 of Delaware Bay (according to DBRC zones). Units in ppb; bars are mean values with lines representing standard error. (Graph from Suk 2006 via DNREC).

To truly understand indicator species and the significance of the magnitude of contamination levels, it is important to note how fish like white perch accumulate such pollutants. Fish can either accumulate pollutants via diffusion (i.e. diffusion of dissolved chemicals in the water) or via bioaccumulation (i.e. through the consumption of their prey) (Monosson 1994, Bickham et al. 2000). Both of these ways of accumulating pollutants are a direct result of the location in which fish like white perch live; therefore, population structure of white perch is valuable information in fisheries management and seafood consumption advisories as it is deeply intertwined with contamination levels in fish tissues. Based on these contamination levels, the DNREC Division of Fish & Wildlife constructs a Delaware Fish Advisory Chart which sets seafood consumption advisories, providing information to the public about lowering the health risks of consumption of fish occupying contaminated waters (Figure 8, DNREC 2011). The chart shows consumption boundaries based on areas with certain levels of contaminants (PCBs, dioxins, mercury, dioxins). Currently, consumers are advised not to consume any finfish (including white perch) caught from the Delaware River to the Chesapeake and Delaware Canal (C&D Canal). From the C&D Canal to the mouth of the bay, women of child-bearing age and children are not recommended to eat any finfish, while one meal of finfish per year is recommended for all others (DNREC 2011). These boundaries are set without regard to the potential spatial distribution of white perch populations. Zone 6 of DRBC's zonation particularly offers a large area where knowledge of population structure of white perch can allow improvements can to be made.

This study seeks to determine the population structure of white perch in Delaware Bay. Based on their life history and on previous findings (Mulligan and Chapman 1989 and Gaffney lab, unpublished), genetic analyses are expected to reveal distinct populations of white perch in Delaware Bay. These findings will allow for a better understanding of the spatial dynamics of whiter perch inhabiting Delaware Bay and could have important applications in fishery management, allowing overall for both ecologically and economically sustainable use.



#### 2010 - 2011 DELAWARE SPORT FISH CONSUMPTION ADVISORY

\* Women of childbearing age and children should not eat any amount of these fish.
\*\* Women of childbearing age and children should not eat more than one meal per month

Figure 8. Fish consumption advisories, from Fish Smart, Eat Smart brochure by DNREC, available online at:

http://www.dnrec.delaware.gov/fw/Fisheries/Documents/fishsmarteatsma rt\_2010-11.pdf

#### Chapter 2

#### METHODOLOGY

#### 2.1 Sample Collection

White perch were collected from May to August 2012 in three separate trawls performed by DNREC (Figure 9). The first sample was obtained from DNREC Trawl Station 92 (GPS decimal coordinates: -75.540733, 39.652400). The second collection of fish occurred near the Delaware Memorial Bridge in a nontidal region of the Delaware Bay (exact GPS coordinates unknown at this time). Lastly, the final collection was from the Nanticoke River /Broad Creek, a tributary from the Chesapeake Bay Watershed (GPS decimal coordinates: -75.672430, 38.564000). After collection, fish were frozen at -80°C and a 20-30 mg tissue sample for each fish was stored at room temperature in 95% ethanol. In total, 94 fish (31 from DNREC Trawl Station 92, 32 from Nontidal Delaware River and 31 from Nanticoke River) were used for analysis of white perch population structure in Delaware Bay. These results were also combined with previous data from fish sampled in other parts of Delaware Bay (i.e. Appoquinimink River, Alloway Creek, St. Jones River, Broadkill River and Lower New Jersey Bay, refer to Figure 9).

DNA was extracted from muscle tissue of each fish directly below the dorsal fin using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA) in accordance with the manufacturer's instructions. Fork length and weight of each fish were also recorded. DNA extraction was verified using universal mitochondrial 16S rRNA primers, which showed strong amplification and therefore successful extraction.



Figure 9. Location of sample sites in Delaware Bay. Larger numbered stations (i.e. 1,2,3) represent those new locations tested in this study, while smaller unnumbered stations are those that have been previously tested in Gaffney lab.

#### 2.2 Genetic Analyses

Primers were designed by Patrick M. Gaffney using GenBank sequences (refer to Table 1 for Accession numbers) of white perch (*M. americana*) and in some cases the congener striped bass (*M. saxatlis*) to allow for amplification of targeted genes (Table 1). Five mitochondrial genes were targeted: (1) cytochrome c oxidase subunit 2, ATPase subunit 8, ATPase subunit 6, cytochrome oxidase subunit 3 (cox2/ATP8/ATP6/cox3); (2) cytochrome-b (cyt-b); (3) NADH dehydrogenase subunit 2 (ND2); (4) NADH dehydrogenase subunits 3/4L/4 (ND3/4L/4); and (5) NADH dehydrogenase subunit 5 (ND5). In addition, two nuclear genes containing introns were amplified (1) connexin 35 (cx35); and (2) growth hormone (GH).

Table 1. White perch loci, forward (F) and reverse (R) primer sequences, annealing temperatures, approximate product size and GenBank accession numbers, divided into groups for mt-DNA and n-DNA.

MTDNA							
Loci	Primers	Ta	Size	GenBan k Acc. No.			
cox2/ATP8/ ATP6/cox3	mt-400/401 F: CTATTAGAAGCCGACCATCGAA R: ACATGTAAGCCGTGAAAGCC	50°C	1850 bp	HM447585			
cyt-b	mt-406/407 F: CACTATACCTCGGACATTGCCACA R: CGAATTAGAAAACAAAGCTCTAGGG	50°C	975 bp	AF240744			
ND2	mt-25/62 F: AAGCTATCGGGGCCCATACCC R: CTGAGGGCTTTGAAGGCCC	57°C	1150 bp	HM447585			
ND3/ND4L/ ND4	mt-18/19 F: AGTATAAGTGACTTCCAATCAC R: TTAGAATCACAATCTAATGTTTT	48°C	2100 bp	HM447585			
ND5	mt-50/51 F: AATAGTTTATCCRTTGGTCTTAGG R: TTACAACGTGGTTTTTCATRTCA	48°C +0.5mM MgCl	2075 bp	HM447585			
	NDNA						
Loci	Primers	Ta	Size	GenBan k Acc. No.			
cx35	N-346/347 F:CGCTTTGGAGACTGAGAACAACGAG R:TCTTATGTGTGAAATGGGAAATGCC	54°C	1150 bp	AF059183			
GH	N-344/345 F: AGCGGTCCTCCTATTATCGG R:TAGGTTCGTCTCAGCGACTC	57°C	1250 bp	HM245611			

Polymerase chain reaction (PCR) was used in order to amplify these mt-DNA and n-DNA genes, of which a detailed explanation of can be found in Innis et al. (1990). Genes were amplified using the following reagents (per reaction): 13.3 µl dH2O, 0.4 µl dNTP mix, 4.0 µl 5X Crimson *Taq* Reaction buffer, 0.4 µl forward primer (10mM), µl reverse primer (10mM), 0.10 Crimson *Taq* polymerase (New England Biolabs), and 1 µl DNA template. These products were amplified using Pheix Applied Biosystems's 2720 and Verti 96 well thermal cyclers with the proper PCR protocol (based on the NEB PCR protocol for Crimson *Taq* Polymerase). This routine consisted of: initial denaturing at 95°C for 1 min; 35 cycles of 1 min at 95°C, 45 s at the gene-specific annealing temperate (Table 1), and 1-2 mins (depending on the product size) at 68°C, followed by a final extension at 68°C for 5 min. Successful amplification was checked by running PCR products on 1 and 2% agarose gels.

Restriction Fragment Length Polymorphism (RFLP) analysis was then performed on the mitochondrial and nuclear amplicons. For ND2, ND3/4L/4 and ND5, restriction enzymes were chosen based on previous haplotype detection (Gaffney lab, unpublished). For the rest of the genes (cox2/ATP8/ATP6/cox3, cyt-b, cx35, GH), 4 and 5 cutter enzymes (i.e. enzymes that have a 4 or 5 cut recognition site) were randomly tested with 24 individuals in order to detect possible polymorphisms. In addition, known sequences were put into NEBcutter and restriction enzymes were chosen to test for possible polymorphisms. Each amplicon was surveyed with a battery of restriction enzymes (~8-10 enzymes/gene). RFLP reactions were conducted using dH<sub>2</sub>O, BSA (enzyme-dependent), buffer (enzyme-dependent) and NEB restriction enzymes (with values according to manufacturer's

recommendations). Digests were loaded onto 1% or 2% Eppendorf premade agarose gels or handmade gels and electrophoresed at ~80-110V. Banding patterns of the digests were stained in ethidium bromide if handmade and then scored and photographed. Gene:restriction enzyme pairs that were tested can be seen in Table 2.

Amplicons	Restriction Enzymes
cox2/ATP8/ ATP6/cox3	AluI, BfaI, Crf13I, DraI, DdeI, EcoRI, HaeII, Hpy188I, NlaIII, TaqαI
cyt-b	AciI, CviQI, DdeI, HhaI, Hpy188III, Sau96I, StyI, TaqαI
ND2	BsmAI
ND3/ND4L/ND4	NlaIII
ND5	DpnII
cx35	ApaLI, BamHI, BsrBI, CviQI, Hpy188III, NsiI, ScaI, StyI, TaqαI
GH	AseI, HhaI, HpaI, NgoMIV, ScaI, SspI, StyI, TaqαI

Table 2. Restriction enzymes tested on white perch amplicons.

Procedures for PCR, RFLP analysis and gel loading (when N=48 or greater) were performed by the Eppendorf epMotion 5075 robot, which has precise volume measuring and pipetting capabilities.

#### 2.3 Data Analyses

Data were analyzed using GENALEX 6.5 software (Peakall and Smouse 2012). For each of the three populations examined, the haplotype diversity (h) was calculated for the composite haplotype data for mitochondrial amplicons ND2, ND3/4L/4 and ND5 (given by the equation  $h = \frac{N}{N-1}(1 - \sum x_i^2)$ , where N is the sample size and x is the haplotype frequency of each haplotype in the sample, Nei 1987). These mitochondrial regions were used because detectable polymorphisms were observed and previous data existed for these regions to allow for comparisons, while cox2/ATP8/ATP6/cox3 and cyt-b showed no variation and thus provided no further resolution. Exact tests of differentiation for mitochondrial data were conducted to determine differentiation between the three collections (i.e. fish from Trawl Station 92, Nontidal DE River, and Nanticoke River). Further, for these three locations, the phylogenetic relationship between the observed haplotypes was determined to show the most parsimonious relationship and a neighbor joining phylogram was constructed based on these findings (Hartl and Clarke 2007). Lastly, mitochondrial haplotype frequencies for each population were determined and added to previous data to obtain a picture of distribution of white perch in Delaware Bay, with an overall exact test of differentiation performed on combined data to identify differences.

For nuclear data, allele frequencies for the polymorphic GH were tested against Hardy Weinberg equilibrium and mapped to show spatial relationship between genotypes and populations. To identify the relationship between the three locations sampled, exact tests of differentiation were conducted and Fst values for nuclear data were determined using the GENALEX software (Peakall and Smouse 2012).

In order to determine variation between fish size between locations, the fork lengths and weights of fish were averaged for each site and examined for normality and homoscedasticity. Because fork length was normal but heteroscedastic, Welch's ANOVA was employed to determine if variation between sites were significant. In

examining fish weight, the data was nonnormal and heteroscedastic and because of this, common statistical tests were not appropriate to allow for a comparison of weight across locations. Nonetheless, fork length allowed for an indication of overall size of fish at each location.

In order to determine variation between fish size between locations, the forklengths and weights of fish were averaged for each site and examined for normality and homoscedasticity. Because forklength was normal but heteroscedastic, Welch's ANOVA was employed to determine if variation between sites were significant (where data was considered significant below the 0.05 level). In examining fish weight, the data was nonnormal and heteroscedastic and because of this, common statistical tests were not appropriate to allow for a comparison of weight across locations. Nonetheless, forklength allowed for an indication of overall size of fish at each location.

Lastly, in order to determine the relationship between fish size, genotype and locations for the three populations surveyed, two-way ANOVAs with replication were performed between fork length, genotype and location to test the null hypotheses that: mean fork lengths were the same among locations, mean fork lengths were the same among genotypes, and that there was no interaction between genotype and location. This was done for both nuclear data (GH) and mitochondrial data (composite ND2, ND3/4L/4 and ND5). Five of the 94 fish were missing information for the ND2 region and ND5 only contained three variants that were difficult to resolve; therefore, a two-way ANOVA with replication was also performed on only the ND3/ND4L/ND4 region, which provided the most complete data set. All calculated P-values were considered significant at the 0.05 level.

# Chapter 3 RESULTS

#### 3.1 Mitochondrial DNA

#### 3.1.1 cox2/ATP8/ATP6/cox3

Amplification with primers mt-400/401 revealed a product about 1850 bp in size. Samples of 24 fish representative from all locations were screened with 4 and 5 cutter restriction enzymes to detect any variation. The following enzymes were tested on cox2/ATP8/ATP6/cox3, with the number of visible bands indicated in parentheses: AluI (5), BfaI (2), Crf13I (2), DdeI (4), HaeII (3), Hpy188I (2), NlaIII (2) and TaqαI (5). With these restriction enzymes, no detectable polymorphisms were observed. For restriction enzymes DraI and EcoRI, 48 white perch were tested and both restriction enzymes revealed either one or two resolvable bands, though there was poor amplification and overall scoring was difficult.

#### 3.1.2 cyt-b

Primers mt-406/407 amplified a product of about 975 bp. Samples of 24 fish (with ~8 fish per location) were digested with 4 and 5 cutter restriction enzymes. The following restriction enzymes were tested, with the number of cut sites indicated in

parentheses: AciI (2), CviQI (1), HhaI (1), Sau96I (1), StyI (1) and TaqαI (1). With these restriction enzymes, no detectable variation was observed between fish. For Hpy188III and DdeI, 48 fish were tested. Hyp188III revealed 2 cut sites for all 48 fish. DdeI also revealed 2 cut sites; however, a rare polymorphism was observed in which one of the 48 fish was missing one of the two cut sites.

#### 3.1.3 ND2, ND3/4L/4, ND5

The ND2 region was amplified using primers mt-25/62 and revealed a product of about 1150 bp in size. The ND2 region of all fish (N = 94) was digested with BsmAI and revealed one polymorphic site that resulted in either an uncut product (1150 bp) or a product cut once (~950 bp and ~200bp). ND2 polymorphisms were observed in the Nontidal Delaware River population as well as the Nanticoke River population, while the Trawl Station 92 site contained fish with only the uncut genotype.

The ND3/4L/4 region revealed a 2100 bp product with primers mt-18/19 and showed three different haplotypes when digested with NlaIII. One haplotype was rare (i.e. seen once) and was only observed in the Nanticoke River population. Sizing data of the banding patterns were known from previous findings (Gaffney lab unpublished) and allowed for resolution of two main haplotypes observed. The main haplotype had three cut sites (~820 bp, ~600 bp, ~530 bp, ~160 bp) while the second haplotype contained an additional cut site that cut the ~530 bp piece into a ~360 and a ~170 bp piece. The rare haplotype that was observed in the Nanticoke River population had three cut sites where the final cut to produce the ~160bp piece was not cut with NlaIII, resulting in the following pieces: ~820 bp, ~700 bp, ~600 bp, ~160bp.

The ND5 region was amplified using primers mt-50/51 and resulted in a product of about 2075 bp. ND5 was digested with DpnII and showed four different haplotypes, with three of them occurring only once. The main haplotype consisted of six bands (~560 bp, ~470 bp, ~400 bp, ~280 bp, ~205 bp, ~160 bp, sizing based on previous data). The sizing of the other rare haplotypes was difficult to resolve on the gel though one haplotype showed a cut site on the ~470bp piece to make a ~400bp and a ~70 bp piece. The Trawl Station 92 population exhibited one of the rare haplotypes, while the Nanticoke River population exhibited the other two rare haplotypes.

Composite haplotype data were complied and overall, seven different compositie haplotypes were observed for the three populations surveyed. Of these seven, five haplotypes were found in only one individual fish. The northern Delaware Bay populations (i.e. DNREC Trawl Station 92 and Nontidal Delaware River) showed the most haplotype diversity while the Nanticoke River showed less haplotype diversity (Table 3). A haplotype network of the composite haplotypes (Figure 10) was constructed and revealed one common haplotype present among the three locations (those unresolved haplotypes were excluded in the analyses). An exact test of differentiation revealed no significant difference between the haplotype composition of the three locations (P = 0.124,  $X^2 = 17.7$ , df = 12). Table 3. Haplotype diversity calculated for each population of *Morone americana* sampled in the Delaware Bay, where N is sample size,  $N_h$  is number of haplotypes, and h is haplotype diversity.

Population	<b>Collection Date</b>	N	$N_h$	h
DNREC Trawl Station 92	May 2012	31	3	0.443
Nontidal Delaware River, near the Delaware Memorial Bridge	June 2012	32	4	0.385
Nanticoke River, Broad Creek	August 2012	31	3	0.145



Figure 10. Median joining network of haplotypes observed for the three sampled collections of *Morone americana* (Nontidal DE River, Nanticoke River, DNREC Trawl Station 92). Each circle represents a different haplotype, with the segments representing the frequency of the haplotype found in each population. The size of each circle is proportional to the number of individuals displaying that haplotype. Haplotype numbers correspond to haplotypes found throughout Delaware Bay, shown in Figure 11. Thick black lines represent a single mutation leading to a new haplotype in the most parsimonious fashion, whereas the grey thin lines represent two different but equally likely path of evolution.

Composite haplotype frequencies were obtained and combined with previous findings to allow for the haplotype distribution for the entire Delaware Bay (Figure 11). Unresolvable haplotypes were excluded in the analyses. Qualitative observations reveal that the northern, middle and southern bay each appear to exhibit a different haplotype construction (i.e. each had a different most common haplotype). An exact test revealed significant differences between all sample sites (P < 0.0001,  $X^2 = 470$ , df = 56).



Figure 11. Mitochondrial DNA haplotype distribution for samples of *Morone americana* collected in different areas of Delaware Bay. The colors of the pie charts represent different haplotypes.

#### 3.2 Nuclear DNA

#### 3.2.1 cx35

Amplification with primers N-346/347 revealed a product of about 1150 bp, of which included two introns. Samples of 24 fish representative of all three locations were digested with a variety of restriction enzymes to search for polymorphisms in the population. The following restriction enzymes were tested, with the number of cut sites indicated in parentheses: CviQI (1), Hyp188III (4) and Taq $\alpha$ I (1). No cut sites were observed when digested with ApaLI, BamHI, BsrBI, NsiI, ScaI or StyI. Overall, no detectable variation was observed between individuals in cx35.

#### 3.2.2 GH

Primers N-344/345 amplified a product of about 1250 bp, which included two introns. Samples of 24 fish (~8 fish per location) were screened with 4 and 5 cutter enzymes to detect variation between individual fish. The following restriction enzymes were used for digestion, with the number of cut sites indicated in the parentheses: AseI (1), HhaI (1), ScaI (1) and StyI (1). When digested with SspI, no cut sites were observed. HpaI revealed multiple polymorphic cut sites (2, 3, 4) for a sample of 24 fish; to better resolve this, NgoMIV was tested as it contains the cut site of HpaI with an additional base. Digestion with NgoMIV revealed what appeared to be one cut site, though scoring of any polymorphisms was difficult due to the size similarities in fragments.

When GH was digested with TaqaI, polymorphisms were detected and data for all 96 fish revealed three genotypes (i.e. two homozygous, AA/BB and one heterozygous AB) in the population (Figure 13). A chi-square test of goodness of fit for revealed that these genotypes were in Hardy-Weinberg equilibrium for all 94 fish

(P = 0.193,  $X^2 = 1.692$ , df = 1). The individual locations were also tested for Hardy-Weinberg equilibrium and all showed genotype frequencies that were in Hardy-Weinberg proportions (for Trawl Station 92, P = 0.686,  $X^2 = 0.164$ , df = 1; for Nontidal DE River, P = 0.837,  $X^2 = 0.042$ , df = 1; for Nanticoke River, P = 0.122,  $X^2$ = 2.391, df = 1). The F<sub>ST</sub> value was found to be slightly significant and suggestive of population differentiation between the three sampled locations (P = 0.044, F<sub>ST</sub> = 0.039). Allele frequencies for GH for each location can be seen in Figure 12.



Figure 12. Allele frequencies for GH in *Morone americana* for the three locations sampled, where white is the A allele and black is the B allele.



Figure 13. RFLP analysis of GH with TaqαI for white perch (N = 94), where the left most lanes show 1 kb ladder (NEB), the right most lanes show 100 bp ladder (NEB) and lanes 1 and 96 have distilled water (control). Three scorable genotypes are observed: AA, AB and BB, as marked.

#### 3.3 Fish Size and Genetic Composition across Sample Locations

Size and weight data showed most fish to be between about 5 to 20 centimeters and 5 to 100 grams (Figure 14). The mean fish length for all locations was 9.7 cm (SD  $= \pm 3.28$  cm) while the mean weight was 29.73 g (SD  $= \pm 37.90$ ). For the Nontidal Delaware River population, qualitative observation shows two different size groups in terms of length, though these were not shown to be significantly different.



Figure 14. Weight (left) and length (right) of white perch at three different sample sites, where A = DNREC Trawl Site 92, B = Nontidal Delaware River and C = Nanticoke River. Dots represent individual fish. Weight is in grams while length is in centimeters.

In looking at the fork length of white perch sampled between locations, variation between sites A, B and C, where A had the longest fish and C had the shortest (Figure 16). Because the fork length data showed normality but was heteroscedastic (via Bartlett's test, P = 4.29E-09), Welch's ANOVA was employed and revealed a significant difference in average fork length between the three locations ( $F_{2,91} = 7.26$ , P = 0.0012).



Figure 16. Mean fork lengths (in centimeters) of white perch at DNREC Trawl Station 92, Nontidal Delaware River, and Nanticoke River, where error bars represent standard error of the mean.

A two-way ANOVA with replication comparing mean fork lengths to both GH genotypes and to sample locations revealed only a significant difference in fork length among locations (P = 0.0011, F = 7.38), while no significant difference in length occurred between GH genotypes (P = 0.5324, F = 0.29). Further, no significant

interaction term between genotypes of GH and location was observed (P = 0.1176, F = 1.91).

For the mitochondrial DNA, a two-way ANOVA with replication was conducted for the composite haplotypes. Here, a significant difference in fork length was observed across locations (P = 0.00989, F = 7.553), while no differences were found between length and haplotype (P = 0.135, F = 1.910) and no interaction was found between location and haplotype (P = 0.266, F = 1.344). Looking at just the ND3/4L/4 region, the effect of location on fork length was again found to be significant (P = 0.000936, F = 7.564). The effect of ND3/ND4L/ND4 genotype was not found to be significant (P = 0.275, F = 1.206). The interaction between location and genotype, however, was found to be slightly significant (P = 0.048930, F = 3.124). A paired box plot for ND3/ND4L/ND4 shows this relationship in Figure 17.



Figure 17. Fork length of white perch in each sampled location, with A = Trawl Station 92, B = Nontidal Delaware River and C = Nanticoke River. Boxes represent 50% of the samples (i.e. the first and third quartiles) while the median value is represented by the horizontal line contained in the box. Vertical lines from each box represent the range. Length is in centimeters. The dark boxes represent one haplotype while the white boxes represent the other haplotype observed in ND3/4L/4.

#### Chapter 4

#### DISCUSSION

Analyses of mitochondrial DNA among the three locations sampled revealed little genetic differentiation between fish in these locations. One haplotype (haplotype 1, refer to Figure 10) was common in all of the locations. Haplotype 2 (Figure 10) was common only in those northern Delaware Bay locations (i.e. DNREC Trawl Station 92 and Nontidal Delaware River) and not observed in the Nanticoke River populations. Further, the Nanticoke River contained a haplotype that was not observed in the other locations. All three locations have similar characteristics in that they occur in upper portions of a river or tributary and have less influence from tidal fluctuations. The similar genetic construction and lack of differentiation between the samples could suggest adaptations to similar environments. A geographical cline analysis between similar nontidal regions (i.e. upper tributary, upper stream, upper river regions) in the Chesapeake Bay and Delaware Bay may better resolve this hypothesis. For instance, if populations of fish across these similar locations exhibit similar allele frequencies compared to other regions, a pattern between genotype and environment may become detectable. Future work in this area is certainly needed to further validate this claim.

After the mitochondrial haplotype data from the three locations was combined with previous data, the resulting distribution of haplotypes suggests the possibility of spatially distinct populations of white perch throughout Delaware Bay. Three different haplotype kinds were observed, with white perch seeming to exhibit distinct populations in the upper, middle and lower bay. Though the exact mechanism for population differentiation is unclear in Delaware Bay, the salinity structure of the bay

may provide some insight. White perch have an upper salinity limit of 13-18 ppt (Mansueti and Scheltema 1953, Setzler-Hamilton 1991) and salinity gradients shaped by currents and tidal fluctuations may prevent white perch from migrating between populations. Future work could compare the salinity structure of the bay to the population structure of white perch to better resolve this hypothesis.

Recent studies have revealed that mitochondrial DNA may not be the best marker for population structure (Galtier et al. 2009). Nuclear DNA was also used in this study to obtain a clearer picture of population structure and to compare findings to mitochondrial DNA data. Growth hormone (GH) was the only nuclear marker to show significant variation. The  $F_{ST}$  value suggested population differentiation, though allele frequencies were similar between populations (refer to Figure 12). Random drift could account for differences, especially in a small population, where random drift has a greater affect in altering allele frequencies. It remains unclear if nuclear data follow the same trends as mitochondrial data for the three locations sampled. More nuclear markers should be analyzed to better determine the relationship between the samples. Further, future analyses should look at nuclear markers for fish at other sites in Delaware Bay to allow for a map showing spatial distribution of fish throughout the entire bay. This could be compared to the haplotype distribution (Figure 11) and would allow for a comparison of mitochondrial and nuclear markers.

Size data reveal a complex relationship between fish length, location and genotype. All three locations had showed significantly different fish lengths. Fish size has been shown to be associated with salinity, where higher salinity waters are known to support increased growth potential and feeding opportunities (Kerr and Secor 2009, McDowall 2001). Perhaps among the different locations surveyed, different salinities

result in different metabolic costs, resulting in various sizes of fish. For instance, the Nontidal Delaware River location would be expected to have a lower salinity than the DNREC Trawl Station 92 location as it is more upstream; the fish found in the Nontidal Delaware River were found to be smaller than those from the DNREC Trawl Station 92, perhaps a reflection of different growth potentials associated with salinity differences. Salinity measures of the sample sites would permit a comparison between size and salinity, allowing this hypothesis to be better resolved.

White perch are shown to exist as two contingents, a smaller resident and a larger migrant contingent (Kerr et al. 2009). Two distinct groupings of sizes in each location, however, were only observed in the Nontidal Delaware River population and this was relationship was not significant (Figure 14). Small sample sizes for each location could have limited these analyses. Further, contingent sizes may be overlapping and not detectable through superficial comparison, instead only evident through genetic data. To determine this, a two-way ANOVA was performed to look at the effect of genotype on size in addition to location on size. A pattern between genotypes and size (i.e. one genotype was consistently smaller) may better suggest distinct morphs of fish. A two-way ANOVA using GH and composite mitochondrial data showed that genotype had no effect on fish size. When only the ND3/4L/4 region was examined, an interaction between genotype and location was found; this effect, however, was only slightly significant and may be the result of a small sample size as this effect was not observed for the composite haplotypes or GH. It remains evident that our data about the size structure of white perch in Delaware Bay is incomplete and more analyses are needed in order to gain a better understanding.

#### Chapter 5

#### CONCLUSION

Results from the three locations surveyed reveal a main haplotype common to all three locations surveyed. Mitochondrial data revealed no differences between sample sites while nuclear data showed a slightly significant differentiation across locations. These results call for the need of future studies to use additional markers to allow for a better resolution of the complex spatial distribution of white perch in Delaware Bay. After combining our mitochondrial data with previous data obtained in Gaffney lab, haplotype frequencies of white perch from nine different locations in Delaware Bay suggest that Morone americana exist in at least three separate populations. Future studies focusing on salinity information will allow for resolution of the hypotheses suggesting that spatial distribution of white perch populations is a function of the salinity structure of Delaware Bay. Future studies are also needed to better classify contingent classes of white perch and to determine whether or not genetic differences are correlated with size differences. This may put our all of results into a better context and allow for a greater understanding of white perch. It remains clear that this seemingly ubiquitous fish has a complex life history and distribution that still remains to be completely understood. Our present study, in combination with future studies, has implications for safe seafood management and can allow for fishery management practices to better match the biology of white perch.

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