

CHAPTER I

INTRODUCTION

Overview

Advancements in the field of molecular genetics since the mid-1960's have provided fisheries scientists with powerful investigative tools that can be used to answer questions related to the genetics of fish. Fisheries biologists no longer have to rely upon the uncertainty of phenotypic traits (length, weight, body condition, number of fin rays, timings of maturity and spawning, etc.), that can be dramatically influenced by the environment, to infer genetic relationships between and among fish populations. Genetic data provides information on an organism's genotype, the precise information encoded by its DNA that is transmitted from generation to generation. Innovative screening technologies using molecular genetic markers, that allow researchers to investigate the genetic composition and evolution of fish populations, are being applied to important fisheries issues such as conservation, domestication, forensics, phylogeography, reproductive success, stock identification, mixed-stock analysis, and taxonomy (Brown and Epifanio 2003).

The conservation of native fish stocks has become an increasingly important issue for fishery managers. The long-term survival of wild populations depends not only upon preserving their natural environment, but also maintaining their capacity to evolve in that

environment. Maintaining a population's genetic (allelic) diversity is considered a key factor in this evolutionary process (Frankham et al. 2002). The ultimate source of genetic variation is heritable mutations, that is, changes in DNA sequence resulting in different alleles, which are passed to offspring. Natural processes, such as random genetic drift, bottlenecks, and inbreeding, can diminish the genetic diversity of a population (Frankham et al. 2002). Hybridization and introgression of nonnative genes can also result in a loss in allelic diversity, which can disrupt locally adapted genotypes and affect population fitness (Ferguson 1990). All of these processes can increase the risk that a population will become extinct. Information about the amount and distribution of genetic variability within and among populations is important in the development of rational conservation strategies for a species (Ryman 1981).

Salmonid fisheries (salmon, trout, and charr) have been a particular focal point for population genetics investigations because of their commercial and sporting value, and their relative ease of culture. The brook trout, *Salvelinus fontinalis*, is a charr native to coldwater streams and lakes in eastern North America (MacCrimmon and Campbell 1969; Scott and Crossman 1973) (Figure 1) and is highly valued for its aesthetic and sport fish qualities. This salmonid species has been the subject of numerous ecological studies (see studies cited by Scott and Crossman 1973; Raleigh 1982; and Schmitt et al. 1993). More recently Hudy et al. (2005) have documented the range-wide decline of brook trout in the eastern United States as a result of anthropogenic landscape changes, pollution, and competition from stocked salmonids.

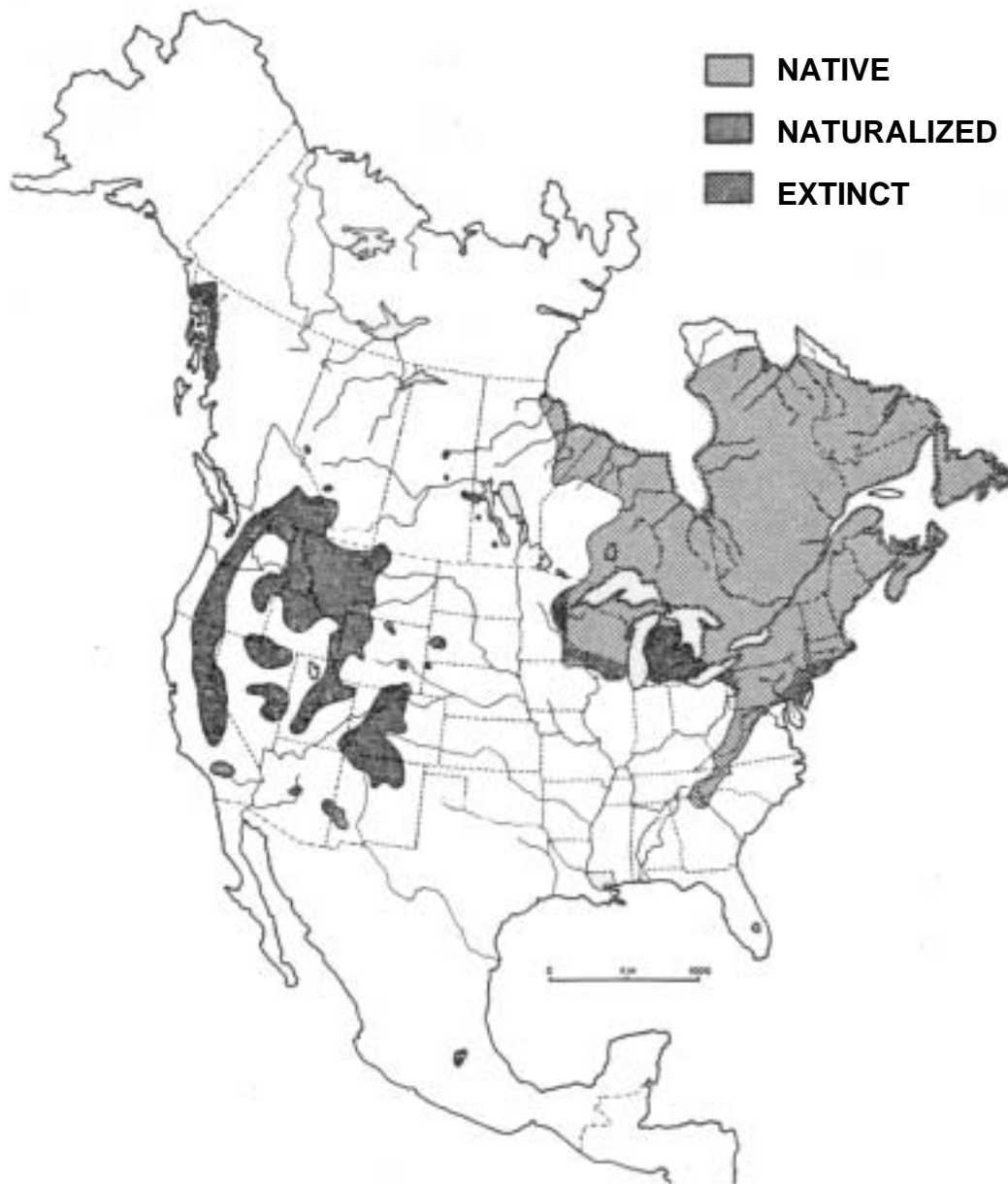


FIGURE 1.—Distribution of brook trout in North America (from MacCrimmon and Campbell 1969).

The distribution and population genetics of brook trout, and indeed many other freshwater fish faunas in North America, is deeply rooted in geological changes related to glaciation events. Repeated glacial advances and retreats during the Pleistocene Epoch, which commenced about 2.5 – 3.0 million years ago, profoundly affected the dispersal of northern temperate fishes and other freshwater organisms (Briggs 1986; Bernatchez and Wilson 1998). As glaciers advanced and receded, the distributional patterns of fishes were disrupted. Some populations were eliminated and those that were isolated lost genetic variation due to a reduced gene pool and genetic drift. Some fish populations occupying areas of refugia were able to re-invade glaciated areas where they could potentially differentiate (Briggs 1986). The last ice sheet retreated from the northern United States during the Wisconsinan glacial stage, 10,000 – 15,000 years ago.

The differentiation of evolutionary lineages and determination of native brook trout populations has been confounded by events far more recent than glaciers, and is directly related to the its popularity as a sport fish. In the United States, brook trout have been cultivated in hatcheries for more than a century, and both cultured and wild fish have been used to augment existing populations and establish new ones. The potential for introductions of nonnative brook trout strains to compromise the genetic integrity and fitness of wild populations through interbreeding is a major concern of fisheries managers (Perkins et al. 1993).

Prompted by questions regarding the phylogeography of brook trout populations across their native range, and the genetic hazards imposed by hatchery and transplantation programs, scientists began investigating the genetic structure and variation of wild brook trout populations in the 1970's. Over the last two decades,

advances in laboratory techniques and computing technology have resulted in the development of new classes of genetic markers and a rapid expansion in the power of these markers to address a myriad of ecological questions (Selkoe and Toonen 2006; DeYoung and Honeycutt 2005). Molecular markers used to assess genetic variation of brook trout at the population level have been developed for proteins and also mitochondrial and nuclear DNA. An overview of these molecular markers, the results of investigations relevant to brook trout population genetics, the distribution and status of brook trout in New Jersey, and the rationale and research objective for this study, are presented in this chapter.

Molecular Genetics Approaches Used to Investigate Populations

One of the first molecular techniques developed for quantifying genetic variation was protein electrophoresis, which can detect genetically different forms of proteins encoded at the same locus (Avisé 2004). However, the electrophoretic expressions of proteins can be strongly affected by the length and conditions of sample storage (May 2003), and although proteins reflect differences at the DNA level, they are nonetheless two steps removed from the gene itself and only a fraction of the genome codes for these soluble enzymes (Avisé 2004). Despite these shortcomings, protein electrophoresis remains a viable tool for examining genetic diversity because the procedures are relatively easy and inexpensive, large quantities of data can be produced quickly, and for many species there are large baseline datasets (May 2003). However, more genetic variation can be found at the DNA level, and in recent years molecular procedures have

been developed that can examine mitochondrial and nuclear DNA at the nucleotide level and provide a finer level of genetic resolution.

By the 1980's, technological advancements in molecular genetics gave scientists the ability to investigate the mitochondrial genomes of fish. Mitochondrial DNA (mtDNA) is a useful genetic marker, thanks to many of its unique attributes, such as the uniparental and nonrecombining mode of inheritance, simplicity of genomic organization, and relatively high point mutation rates compared to nuclear genomes (Moritz et al. 1987). The analysis of mtDNA sequence variation has proven most useful in defining major phylogenetic assemblages within species that were often undetected by allozymes and other genetic methods (Angers and Bernatchez 1998). Although many copies are present in each cell, early studies involving mtDNA often required the sacrifice of the fish so that purified mtDNA for whole-molecule analysis could be extracted from fresh or frozen tissue (liver or gonads).

The development of the polymerase chain reaction-based (PCR) method in 1986 allowed scientists to employ nonlethal sampling techniques to obtain minute amounts of mitochondrial and nuclear DNA from blood and fresh, frozen, alcohol-preserved, or dry tissue (fins, barbels, scales, muscle biopsy). Nuclear DNA (nDNA) contains most of the functional, protein-encoding DNA that provides instructions for making and, for the most part, maintaining an organism, as well as non-coding ("junk") DNA (Avisé 2004).

Microsatellites, discovered in 1989, have become an increasingly popular and versatile means of assessing contemporary genetic variability. The term microsatellites refers to a class of co-dominant DNA markers that are inherited in a Mendelian fashion (DeWoody and Avisé 2000). These markers are blocks of repetitive DNA, involving

tandem repeats of 1-6 nucleotides (such as (AC)_n or (GATA)_n, where n lies between 5 and 50), that are scattered abundantly throughout the nuclear genome of most taxa. A pair of oligonucleotide primers, designed to bind to the regions flanking the microsatellite, guide the amplification of the microsatellite locus during PCR.

Microsatellites typically far surpass allozyme loci in heterozygosity and number of alleles per locus (Avisé 2004) and increase the probability that isolated populations diverge rapidly at these loci (Angers and Bernatchez 1998). For genetic studies of processes acting on ecological time scales, high levels of allelic diversity are necessary and microsatellites are one of the few molecular markers that researchers can use to answer fine-scale ecological questions (Selkoe and Toonen 2006).

Studies Describing Genetic Variation in Populations of Brook Trout

Genetic studies of brook trout have employed a range of molecular markers, from allozymes and mtDNA to nuclear sequences and microsatellite DNA. Since the 1960's, researchers have used protein electrophoresis to analyze protein polymorphisms and compare the genetic diversity of brook trout populations. Building on earlier studies on protein polymorphisms in other fish species, Wright and Atherton (1970) surveyed allele frequencies at two protein loci, transferrin (*Tf*) and eye-specific lactate dehydrogenase (*LDH*), for seven northeast hatchery populations and eight wild brook trout populations. With only two loci, they were able to distinguish all hatchery strains, and some of the wild populations, and found the degree of variations of allele frequencies and the amount of heterozygosity was generally greater among hatchery fish than natural populations. Other early studies that examined protein polymorphisms in hatchery and wild trout also

found that some natural and hatchery populations brook trout were distinguishable from each other (Eckroat 1971; Eckroat 1973).

These early electrophoretic studies generally found that allele frequencies were often quite different among wild and hatchery populations of brook trout. They also provided limited biochemical evidence of possible genetic interchange between wild and hatchery brook trout stocks. However, study results were contradictory and the data interpretation was clouded by difficulties associated with the genetic interpretation of the isozyme banding patterns. In addition, the data could not be used to evaluate the genetic impact of stocking because stocking history information was lacking. Electrophoretic studies that included stocking histories soon followed and began to resolve lingering questions about the genetic relationships of wild brook trout populations over a broad geographical range, as well as the genetic effects of stocking.

Interest in brook trout population genetics was fueled by speculation that southern Appalachian brook trout (SABT) populations were taxonomically different from northern populations. This was based in part upon a limited amount of morphological data, such as smaller and more numerous red spots on the sides and different relative sizes of body parts (Lennon 1967). Researchers initially employed electrophoretic techniques to obtain genetic data that could be used to explore the taxonomic distinctness of SABT.

Stoneking et al. (1981) compared allozyme variation among five wild northeastern populations and three wild southeastern populations with known stocking histories. The pattern of genetic variation observed suggested the existence of separate northern and southern phylogenetic lineages.

In a later study, stocked and unstocked populations of wild brook trout in the Great Smoky Mountains National Park (GSMNP), and brook trout from two northeastern U.S. hatcheries, were examined for variation in protein products encoded by 34 presumptive gene loci using starch-gel electrophoresis (McCracken et al. 1993). Putative native southeastern populations and northeast hatchery strains stocks were found to have substantial genetic divergence as a consequence of fixed genetic differences at one locus and allele frequency differences at nine loci. The CK-A2 locus, which codes for creatine kinase enzymes, was diagnostic for northern-derived and southern Appalachian strains of brook trout. Their data also showed relatively low average heterozygosity and polymorphism in all five native populations, relatively high variability in all three hatchery populations, and intermediate values of heterozygosity and polymorphism in all three of the populations comprised of mixed native and hatchery fish. These results were consistent with previous studies suggesting that native brook trout in the southeastern U.S. are taxonomically distinct from northeastern brook trout. Subsequent investigations involving allozyme analyses (Kriegler et al. 1995; Hayes et al. 1996; Guffey 1998, cited by Habera and Moore 2005; Galbreath et al. 2001) and molecular analyses that directly assayed DNA (discussed later in this chapter) support these earlier findings that northern-derived hatchery strains are genetically distinct from southeastern populations of brook trout. Protein electrophoresis has become the method of choice among fisheries management agencies to identify the genetic origin of brook trout populations in the southern Appalachians because of the existing large data set and relative ease of use.

As a result of these genetic and other ecological studies, fisheries managers in southeastern states began recognizing that brook trout populations in the southern

Appalachians had special management needs, which might include protecting and preserving their genetic integrity (Habera and Strange 1993). Kriegler et al. (1995) recommended that management programs that attempt to expand the current distribution of SABT should take into account the presence of hybrid and nonnative brook trout populations. They also cautioned that the genetic identity of brook trout populations can not be reliably inferred from stocking records, and genetic analyses are necessary to determine whether recorded or unrecorded stocking has affected the genetic composition of southern Appalachian brook trout populations. Continuing concern regarding distribution shrinkage and the long-term survival of SABT prompted the American Fisheries Society's Southern Division Trout Committee to release a position statement on managing SABT (Habera and Moore 2005). The authors indicated that the genetic identity of brook trout within this region is known for approximately 37% of the 3,000 km of stream length they inhabit, and of this, 47% supports SABT.

Investigators have also used protein electrophoresis to probe the genetic diversity of brook trout in other geographic regions. In Wisconsin, the long-term genetic impact of maintenance stocking upon wild brook trout populations was evaluated using blood and whole-eye proteins at several loci (Krueger and Menzel 1979). Hatchery stocks were genetically distinct from most wild populations at both loci, and reduced genetic variability was observed in the hatchery stock. Although significant correlation between allelic frequencies and stocking histories was found, the data did not provide compelling evidence of interbreeding between hatchery and wild stocks. The authors suggested that the study data indicated alteration of selective pressures induced by ecological interactions between the two stocks.

In New York and Pennsylvania, the genetic variability of wild brook trout populations was found to be organized by river basin, suggesting colonization of river basins by genetically different groups of brook trout at different times (Perkins et al. 1993). A high level of genetic differentiation was found, even within the same minor river drainage, for wild populations. Other allozyme studies have also found that high levels of population differentiation exist among brook trout populations located close to one another (Eckroat 1971; Krueger and Menzel 1979; Jones et al. 1996). Perkins et al. (1993) suggest that management strategies for conserving the genetic variability of wild brook trout should focus on individual lake and stream populations within river basins as the primary management units.

In summary, allelic protein data sets obtained through electrophoresis have provided convincing evidence that (1) demonstrates substantial genetic differentiation between northeastern and southeastern brook trout, (2) shows native gene pools have been altered through interbreeding of wild and hatchery fish, and (3) high genetic variability is present among local populations. Although protein electrophoresis will continue to be a useful tool in fishery management, technical advances in molecular genetics over the last two decades has prompted many researchers to shift from this traditional approach to direct assays of DNA.

Mitochondrial DNA analysis of population structure has been a useful method to ascertain the postglacial dispersal routes and phylogeographical structuring in many freshwater fishes (Danzmann et al. 1998). In the 1990's, researchers began using mtDNA markers to probe the genetic variability and phylogeographic patterns of brook trout. Quattro et al. (1990), using RFLP analysis of mtDNA from ten brook trout

populations inhabiting two major drainages in western Maryland, found two distinct matriarchal lineages that fell on either side of a major geographical feature – the eastern continental divide. Mitochondrial DNA variability in 49 populations of brook trout from the Algonquin Park region suggested that fish from two different glacial refugia colonized the southern and northern regions of the park (Danzmann and Ihssen 1995). In eastern Canada, mitochondrial DNA variation of brook trout showed low divergence among mtDNA haplotypes, which suggested a single glacial refugium for the trout that recolonized that region (Jones et al. 1996).

In a large-scale phylogeographic survey, Danzmann et al. (1998) examined 155 brook trout populations from eastern North America using RFLP analysis of mtDNA and identified six major phylogenetic clades (evolutionarily divergent lineages) of brook trout. Large phylogenetic differences between northern and southern populations were found. Populations outside the zone of glaciation were the most genetically heterogeneous, while low mtDNA diversity was found in northern brook trout populations inhabiting recently deglaciated regions of Canada and northeastern United States. The phylogenetic patterning suggests that the extent of mtDNA variation found in brook trout is related to geological events. The least amount of divergence was found in northern populations and the greatest divergence occurred in populations from a southern, unglaciated region. The patterning also lends support to an earlier hypothesis that brook trout recolonizing deglaciated areas originated from different refugial zones. Danzmann et al. (1998) recommended that certain lineages/populations be recognized as evolutionary significant units and managed as such.

Subsequent studies have yielded similar phylogenetic results. A large-scale analysis using allozymes and mtDNA revealed that the majority of genetic variance in brook trout populations was partitioned along major drainages or regions associated with distinct glacial refugia (Hébert et al. 2000). The evolutionary genetic relationships among mid-Atlantic brook trout populations from Maryland drainages, augmented with data from previously studied populations in Virginia, West Virginia, and Tennessee, was examined using RFLP analysis of mtDNA (Hall et al. 2002). Genetic diversity among these populations was considered high, when compared with results from northern populations analyzed previously. The mosaic patterning of mtDNA variation observed in these mid-Atlantic brook trout populations suggests that the region may be a transitional zone between major historical lineages - the genetically diverse southern populations and the relatively homogenous northern groups.

Mitochondrial DNA studies also support the findings of earlier allozyme studies that indicated that Appalachian brook trout are distinct evolutionary entities. Comparisons of mtDNA have also been used to discriminate hatchery and wild stocks, by using mtDNA haplotype variation to determine the level of introgression of nonnative genes in wild brook trout populations. A high degree of genetic differentiation between two hatchery stocks and two wild brook populations in Ontario was detected through RFLP analysis using 51 restriction enzymes (Danzmann et al. 1991). This survey showed that by sampling a high number of restriction enzymes, unique clonal variants might be discovered that can unambiguously discriminate hatchery and wild fish. While the sharing of mtDNA haplotypes by both wild and hatchery brook trout does not indicate

that the wild fish are of hatchery origin, the presence of unique haplotypes in wild fish does preclude their being of hatchery origin.

A subsequent study showed no or very low frequencies of mtDNA 'hatchery' haplotypes in wild populations in Algonquin Park, Ontario despite extensive plantings of hatchery reared trout (Danzmann and Ihssen 1995). Comparisons of mtDNA haplotypic distributions in hatchery and wild fish also suggested that hatchery females had minimal spawning success and/or their progeny survived poorly in the wild. In the southern Appalachians a comparison of the genetic diversity of native, stocked, and hybrid brook trout populations showed that native fish were genetically distinct from hatchery-derived fish and could be distinguished using three restriction enzyme sites (Hayes et al. 1996).

Although protein electrophoresis and mtDNA analyses still have utility in the exploration of genetic variability in organisms, the development of newer screening technologies that allow direct assessment of nuclear DNA sequence variation are gaining in popularity. Researchers are increasingly utilizing more recently developed PCR-based methods, particularly microsatellite analysis, which allows direct assessment of nuclear DNA variation.

The development of microsatellite primers for brook trout has lagged in comparison to other commercially important salmonid species, and much of the molecular work in this genus has relied upon cross-familial amplification of microsatellites from other salmonid species (Perry et al. 2005). Limited success in applying microsatellite primers developed for other salmonids to brook trout prompted efforts to isolate specific microsatellite loci from a partial genomic library brook trout. Angers et al. (1995) successfully isolated seven microsatellite loci and used them to

examine brook trout populations in five geographically proximal lakes in Quebec. Four of the microsatellites were moderately to highly polymorphic (5 – 18 alleles detected) and this contrasted with the low mtDNA variation generally observed in this species for the region surveyed. The results of this study suggested that microsatellite loci could be valuable in addressing fine scale population genetics structuring in brook trout.

In an expanded study, involving 26 brook trout populations in a National Park in Quebec, microsatellite and mtDNA variation was characterized and compared by Angers and Bernatchez (1998). Their analysis of microsatellite variation revealed extensive polymorphism, which resolved a finer population structuring than mtDNA. These results lent additional support to the authors' hypothesis that microsatellites may be more appropriate than mtDNA for inferring relationships among closely related populations.

Microsatellite studies have been used to analyze relationships between intrapopulational genetic diversity of brook trout and landscape features such as hydrogeography and habitat types. The relationship of hydrography and population genetic structure of brook trout from eastern Canada was explored using six microsatellites (Hébert et al. 2000). Each of the 24 populations examined represented distinct, nonrandomly mating populations, even when found in the same drainage over short distances (less than five kilometers). Riverine populations of brook trout have been shown to have consistently higher levels of allelic diversity than lacustrine populations (Hébert et al. 2000; Angers and Bernatchez 1998; Castric et al. 2001). No correlation was found between habitat size and intrapopulational genetic diversity (Hébert et al. 2000; Angers et al. 1999; Castric et al. 2001). However, altitude has been shown to strongly influence genetic variability among brook trout populations, with lower

heterozygosity observed in higher elevation populations, presumably constrained by physical barriers that influence dispersal and gene flow processes (Angers et al. 1999; Castric et al. 2001).

A suite of 13 microsatellite markers for brook trout, developed by the U.S. Geological Survey (USGS) - Leetown Science Center, Kearneysville, West Virginia (T. King, personal communication), has been used to investigate the amount and patterns of genetic diversity of brook trout from 125 collection sites in Canada and the U.S. King (2006) found high levels of genetic diversity among brook trout and demonstrated genetic differences at scales ranging from local streams to river basins, including differences among regions, major drainages, watersheds, streams, and specific locations within streams. Much of the genetic diversity was found in the mid-Atlantic region, with differences associated with the geographical separation of major drainages (Atlantic slope and Ohio River), while very low levels of diversity were found in certain southern Appalachian populations. In some of the populations studied, the impacts of stocking were discernable. This, and previously mentioned research, has demonstrated the ability of microsatellite DNA analysis to reveal fine-scale population structure and patterns of genetic divergence that may prove useful in developing a conservation roadmap for this species.

A variety of molecular screening techniques have been used to obtain genetic data sets to investigate the genetic variability within and among brook trout populations in many geographic areas of their native range. These studies contribute to greater knowledge and understanding of wild brook trout resources and aid resource managers in the development of conservation strategies for indigenous populations. For example,

existing populations of trout that have been determined to be remnants of fish that originally colonized an area after deglaciation have been termed “heritage” trout (Perkins et al. 1993). Efforts to identify and preserve the gene pools of genetically distinct southern Appalachian brook trout populations have been undertaken by state fish and wildlife agencies, most notably in North Carolina, Virginia, and Tennessee (Habera and Strange 1993). With interest in brook trout conservation growing, molecular genetics is poised to play an increasingly key role in management decisions that will affect the short and long-term survival of this fish species.

Distribution and Status of Brook Trout in New Jersey

Brook trout is the only salmonid species native to New Jersey, but unfortunately the distribution of this species in New Jersey prior to the late 1960’s is poorly documented. Using available data dating back to 1862, Fowler (1920) published a list of the fishes of New Jersey, in which 16 (of 21) counties and a handful of localities therein were named where brook trout were known to occur. More than half the localities (21) were in central and southern counties, while only 10 were given for counties in north Jersey. In relation to his list for brook trout, Fowler stated “In many localities formerly, now largely introduced”, but did not differentiate between wild or stocked trout for localities listed. Fowler’s list does not appear to be particularly comprehensive, judging from the paucity of localities given for other, more ubiquitous native freshwater fishes, most notably cyprinids (minnows), catostomids (suckers), and ictalurids (catfishes).

Unpublished records kept by the NJDFW, including stream assessments conducted in the late 1800’s, and surveys conducted from 1918 –1920 under the direction

of four biologists (W.T. Foster, F.N. Miller, H.E. Schradieck, and H.M. Spandau), suggest brook trout were more widespread. However, the lack of detail (trout species not identified, no indication of wild vs. stocked trout, survey location not specified, etc.) limits the usefulness of these and other data in describing the distribution of brook trout in New Jersey prior to stocking activities. In a comprehensive range-wide review of the worldwide distribution of brook trout (MacCrimmon and Campbell 1969), a brief description of the brook trout's occurrence in New Jersey is given. Relying upon a personal communication with Charles Hayford, then the Director of the New Jersey Division of Fish, Game, and Shellfisheries, the authors stated that "in New Jersey, where the species was found in nearly all counties, native brook trout populations now exist only in headwater streams of the northwestern counties of Sussex, Warren, Morris, and Passaic." Their map depicting the North American distribution of brook trout (Figure 1) conveys the false impression that brook trout had been extirpated from New Jersey. The present day occurrence of brook trout in New Jersey is more widespread than previously reported in the literature. In addition to those counties cited by MacCrimmon and Campbell (1969), fish surveys conducted by the New Jersey Division of Fish and Wildlife (NJDFW) from 1968 to 2003 have documented wild populations in the counties of Hunterdon, Somerset, Bergen, and Camden (Hamilton and Barno 2005). During this period, wild brook trout populations were found in 120 streams scattered across forested hills and mountains in the northern tier of the state, and also in one south Jersey stream. These streams are located in the freshwaters of four major river systems (Delaware, Hudson, Passaic-Hackensack, and Raritan) within the Atlantic Slope drainage (Figure 2). No anadromous populations have been documented in rivers where access to marine

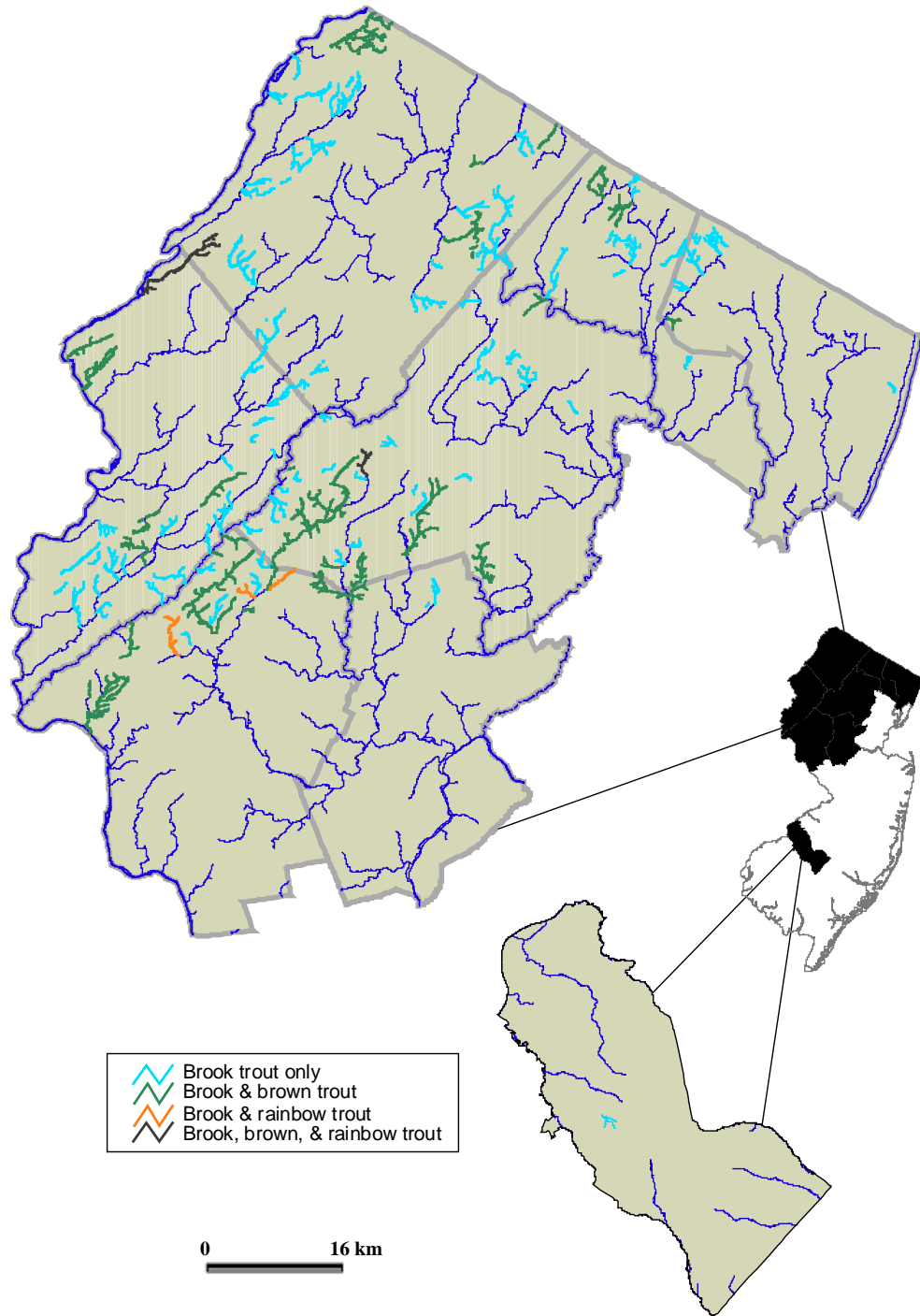


FIGURE 2.—Distribution of wild (spawning) brook trout populations in New Jersey as documented by stream surveys conducted by the New Jersey Division of Fish and Wildlife from 1968 through 2003 (from Hamilton and Barno 2005).

environments exists. Differences in coloration and markings on brook trout residing in different streams in New Jersey has also been observed (Figure 3).

The known distribution of brook trout in New Jersey, as documented by NJDFW over a 35-year period (1968 – 2003), appears to be strongly related to geomorphology. The majority of New Jersey's wild brook trout populations can be found in streams located within two physiographic provinces, the Valley and Ridge and the Highlands, and to a much lesser extent in the Piedmont province along its northern and western fringes (Figure 4). These three provinces are located within the Appalachian Rise and lie to the north and west of the Fall Line. The Fall Line separates the hard metamorphic rocks of these provinces from the older, unconsolidated sediments of the Coastal Plain provinces (Dalton 2003).

Phylogenetic studies of brook trout across its native range have demonstrated the importance of glacial events in shaping the distribution and genetic diversity of this species. New Jersey has undergone at least three glaciations during the last one and half million years of the Pleistocene Epoch (Witte 1998). The last ice sheet, which occurred during the late Wisconsinan advance, began to recede from its maximum extent roughly 17,000 – 18,000 years ago (Briggs 1986). In New Jersey, the furthest advance of the Wisconsinan ice mass is marked in most places by a terminal moraine known as the Ronkonkoma moraine (Figure 5). This moraine forms a nearly continuous low ridge, from Belvidere eastward through Perth Amboy to New York, and effectively delineates glaciated and unglaciated regions that resulted from this last glacial stage (Witte 1998).



FIGURE 3.—Examples of color variation in wild brook trout from New Jersey streams. (A) Burnt Meadow Brook (Passaic drainage), (B) Turkey Brook (Raritan drainage), (C) Cooley's Brook (Passaic drainage), and (D) Lake Stockholm Brook (Passaic drainage).

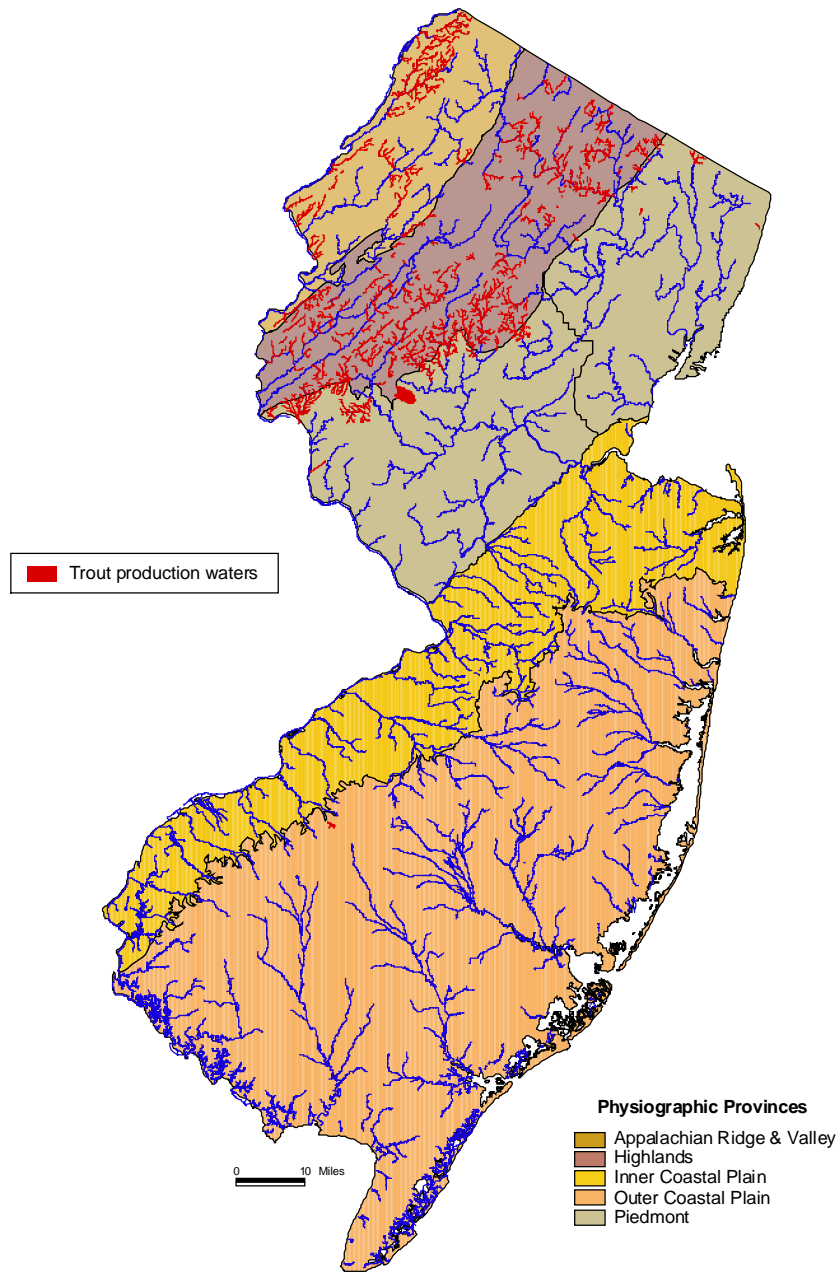


FIGURE 4.—New Jersey’s physiographic provinces and freshwaters having self-sustaining salmonid populations (trout production waters), as documented through NJDFW surveys conducted from 1968 through 2003 (Hamilton and Barno 2005).

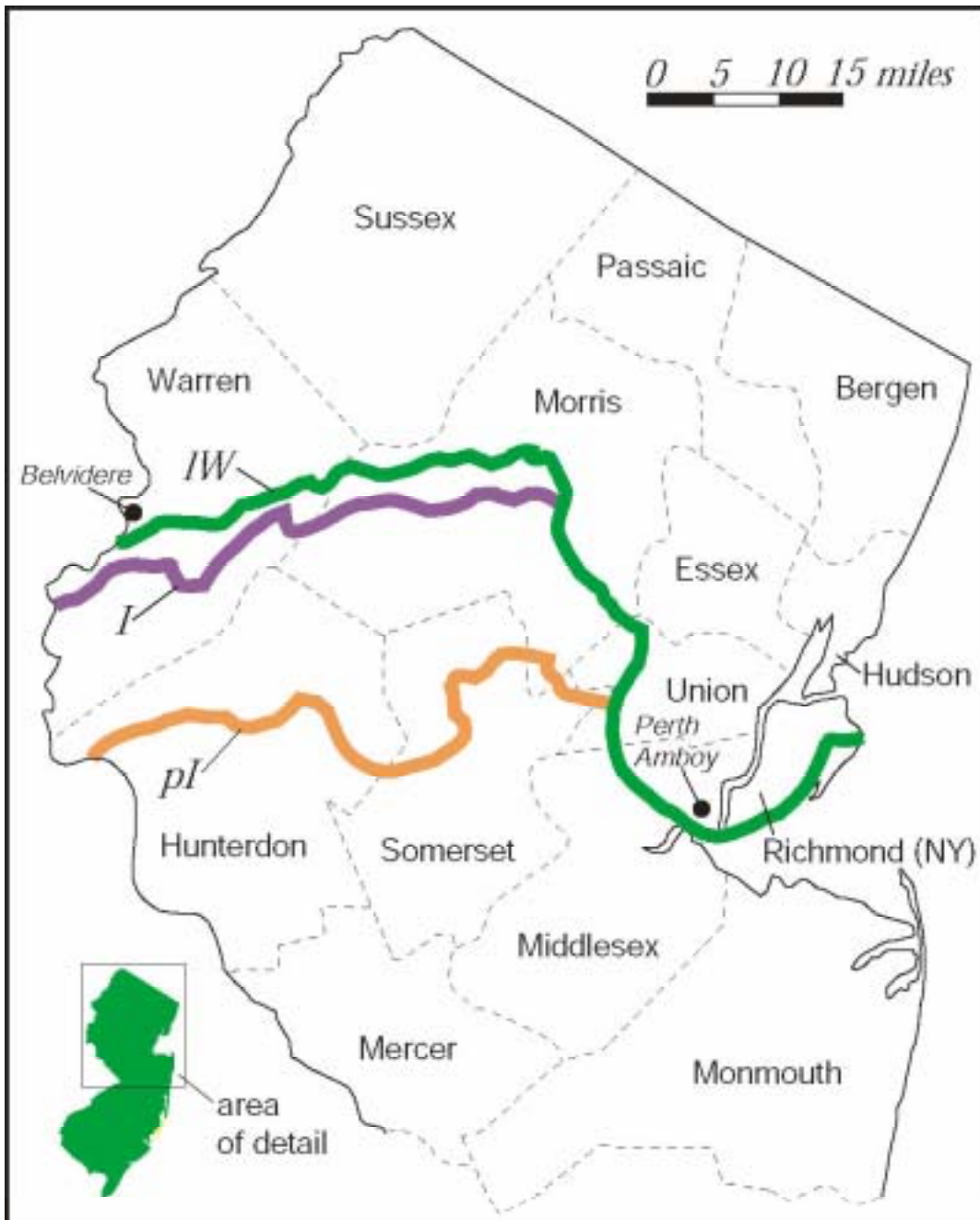


FIGURE 5.—Limits of glaciation in New Jersey and nearby New York. The trace of the *IW* limit generally marks the position of the Terminal (Ronkonkoma) Moraine. *IW* – late Wisconsinan, *I* – Illinoian, and *pI* – pre-Illinoian (modified from Witte 1998).

Although glacial events have likely shaped the distribution and genetic structure of brook trout populations in New Jersey, this relationship has not been confirmed. Events far more recent than glaciers, beginning with European colonization of North America, have likely impacted brook trout populations in New Jersey and throughout their native range. A recent range-wide assessment of brook trout in the eastern United States, based upon the professional opinion of experts from state and federal agencies, identified where wild brook trout populations remain strong, where they are struggling, and where they have vanished (Hudy et al. 2005; Figure 6a).

This assessment also categorized a variety of threats to brook trout and their habitats. In New Jersey, it was estimated that brook trout persist in less than half their original range (Figure 6b). The five most pervasive impacts considered to have affected New Jersey's native brook trout were sedimentation (roads), urbanization, dam inundation/fragmentation, high water temperature, stream fragmentation (roads), and one or more non-native fish species (trout). Man-made dams have not only contributed to the demise of many of New Jersey's brook trout populations, through elimination or degradation of habitat, but also fragmented their habitat, which has resulted in reproductive isolation of brook trout populations. Some wild brook trout populations may have benefited from habitat fragmentation, if artificial barriers successfully prevented interbreeding with cultured brook trout or intrusion and colonization by competing cultured trout species stocked in downstream waters.

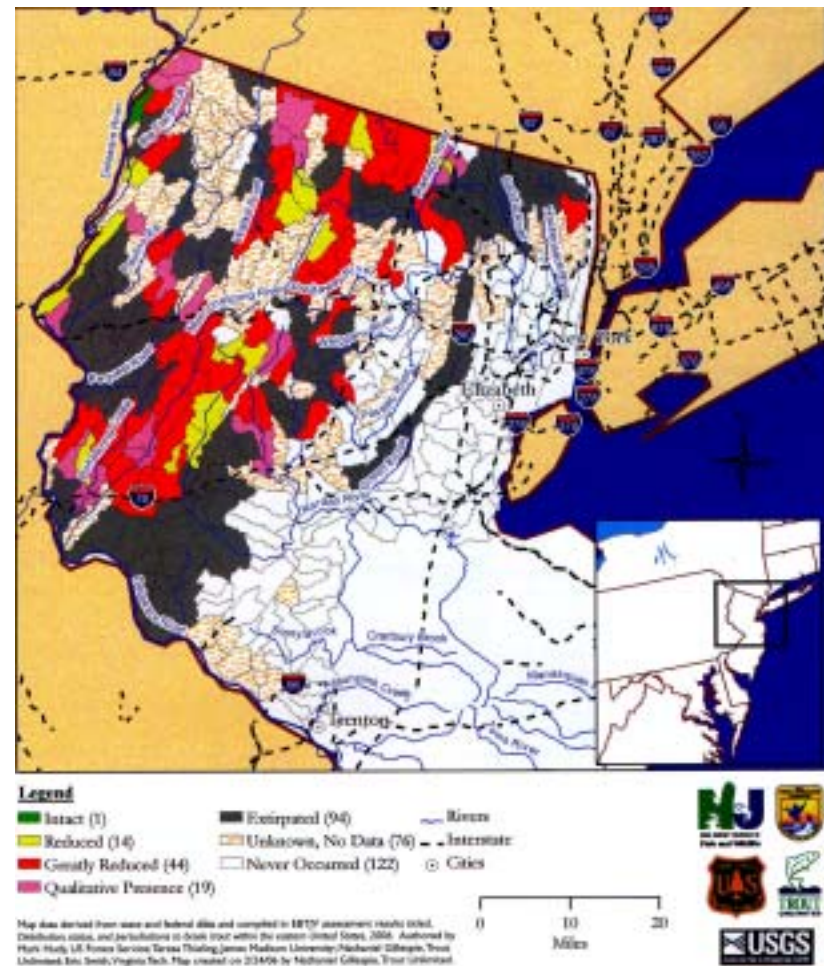
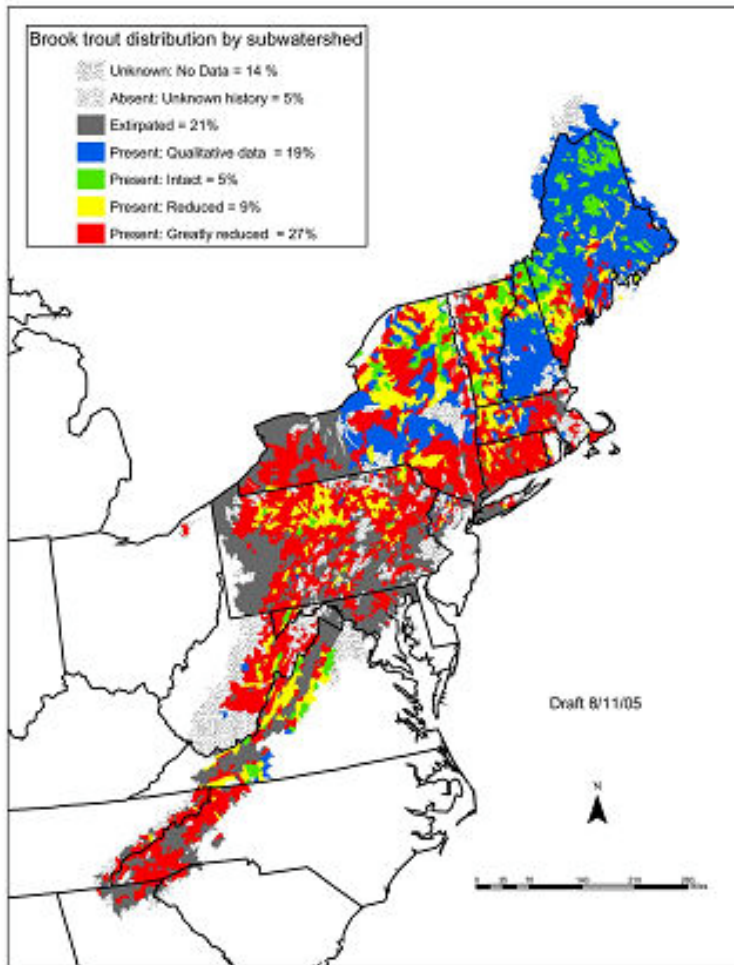


FIGURE 6.—Distribution and assessment of the status of wild brook trout in the eastern United States (left), with detail provided for New Jersey (right) (Hudy et al. 2005).

For many years, stocking hatchery-reared fish has been the most common way to meet the demand for recreational angling and to restore declining fish stocks, with little regard to the ecological and genetic consequences for native stocks (Nielson 1993). In New Jersey, a catastrophic drought in 1875 triggered the first stocking of hatchery-reared trout (fingerling brook trout) to re-establish trout populations in streams where they had been depleted. Soon after, in 1882, rainbow trout (*Oncorhynchus gairdneri*) were introduced and brown trout (*Salmo trutta*) followed in 1908 (Hamilton and Barno 2005). As rearing techniques were refined, and hatchery facilities expanded to meet angler demand for trout, the production and stocking of trout increased. The state's Hackettstown State Fish Hatchery, one of the oldest trout hatcheries in the U.S., discontinued production of approximately 500,000 brook, brown, and rainbow trout in 1985 after more than 70 years of operation (Hamilton and Barno 2005). The origin of the strain of brook trout cultured at this hatchery is not known.

In 1984, NJDFW began stocking trout reared at a newly constructed, disease-free facility, the Pequest Trout Hatchery. The brook trout at this facility originated from eggs obtained from North Attleboro National Fish Hatchery in Massachusetts (Nashua strain – Atlantic Slope origin). Currently, NJDFW produces and stocks more than 600,000 brook, brown, and rainbow trout in nearly 200 waters statewide to enhance recreational angling (Hamilton and Barno 2005). Of these trout, approximately 250,000 are catchable-sized brook trout that average 26 cm. Much smaller numbers of trout, purchased by local fishing clubs from privately owned fish hatcheries in New Jersey and surrounding states, are also stocked annually in New Jersey waters.

Repeated annual stockings of salmonids for nearly a century has resulted in the establishment of spawning populations of non-native salmonids in New Jersey. Stream surveys conducted by NJDFW from 1968 through 2003 documented 183 self-sustaining trout populations, and of these, barely half (94) were comprised solely of brook trout (Hamilton and Barno 2005). Of the remaining 89 streams, brook trout occurred in sympatry with naturalized populations of brown and/or rainbow trout in 27 streams (16% overall), and 62 streams (34% overall) had wild trout populations consisting exclusively of brown and/or rainbow trout. Hybridization between brook and brown trout has also been documented in two streams where wild populations of both species occur (Dunnfield Creek and the S/Br. Raritan River; NJDFW electrofishing surveys). These patterns suggest that hatchery supplementation with all three species, and perhaps translocations by well-intentioned managers and anglers, has caused displacement of native brook trout and facilitated potential interbreeding of non-native strains of brook trout with native brook trout populations.

Study Rationale and Research Objective

Brook trout are valued for their beauty, sport fish qualities, and as indicators of good water quality and a healthy ecosystem. Over much of their historic range in the eastern United States, wild populations of brook trout have declined due to a combination of land and water practices, and competition with non-native fishes (Hudy et al. 2005). Previous studies have described levels of genetic diversity in brook trout across their native range and demonstrated that geologic events, landscape features, and stocking of non-native salmonid species and brook trout strains have affected the occurrence and

genetic structuring of brook trout populations. However, no genetic studies have evaluated brook trout from New Jersey waters.

The objective of this study was to characterize genetic variation within and among wild brook trout the populations in New Jersey, and evaluate patterns of fine-scale genetic variation to resolve questions regarding their genetic ancestry and integrity. Thirteen polymorphic microsatellite DNA markers were used to examine the genetic diversity of a subset of spawning brook trout populations in New Jersey. A hierarchy consisting of river drainages, subdrainages and individual populations was used to examine the distribution of gene diversity. The wild populations, some having a history of trout stocking and others suspected of being genetically “pure”, were also compared with stock collected from a hatchery. In gathering this baseline information I hope to provide insight into the genetic variation of brook trout that will prove useful in shaping management strategies to ensure the long-term viability of wild brook trout populations in New Jersey and elsewhere in their native range.