CHAPTER IV
DISCUSSION

This study represents the first evaluation of genetic diversity and population structure for spawning brook trout populations in New Jersey. The brook trout is the only salmonid species native to New Jersey, with spawning populations found primarily across the northern tier of the state, in the headwater and tributary streams within four Atlantic slope drainages. The 13 microsatellites used produced a data set that contained sufficient allelic diversity to reveal unique multilocus genotypes for all individuals sampled, identified moderately high levels of genetic diversity, and provided insight on the fine-scale genetic relationships within New Jersey's wild brook trout populations.

The geographical distribution of genetic variability among the 22 wild brook trout populations in this study suggests remnants of ancestral brook trout exist in New Jersey and that the stocking of hatchery trout has minimally influenced the gene pools of most of these populations.

Genetic Diversity within Populations

Allelic diversity is often used to characterize the extent of genetic diversity within and across populations. Moderately high genetic diversity, 133 alleles at 13 loci (10.2 alleles per loci), with 2 to 24 alleles per locus, was observed among 218 individuals

collected from 22 small streams in New Jersey. Genetic studies involving brook trout populations from elsewhere in North America have revealed comparable levels of genetic variation using microsatellite DNA markers. These other studies typically had larger sample sizes, which generally yielded more alleles per locus. A study using 5 microsatellites to survey 496 individuals from 8 ponds within a watershed in Newfoundland found the number of alleles per locus averaged 11 (67 total), and a range of 2 to 25 alleles per locus (Adams and Hutchings 2003). Higher levels of polymorphism (10 to 43 alleles per locus, average 18.8 per locus, 94 alleles total) were found in 779 individuals representing 26 populations in a national park in Quebec using 5 microsatellites (Angers and Bernatchez 1998). In Maryland, 100 alleles were observed across 8 microsatellite DNA loci (12.5 average per locus) for 325 brook trout from 9 locations (King and Jullian 2000). A study of 30 populations (771 individuals) representing 6 major river drainages in Maine found 10 to 57 alleles per locus (average 27.3) and 164 total, using 6 microsatellite loci (Castric et al. 2001). Samples from 12 sites (441 individuals total) in the Miramichi River drainage, New Brunswick, assessed using 6 microsatellites detected 8 to 48 alleles per locus (Rogers and Curry 2004). King (2006) has found very high genetic diversity (247 alleles) using the same 13 microsatellites used in this study, in more than 7,000 brook trout from 125 separate collection sites across the eastern Unites States and Canada, with much of the diversity represented in the mid-Atlantic region.

Heterozygosity is often used to characterize genetic diversity at the population level. In the 22 wild populations included in this study, observed heterozygosities ranged from 0.342 to 0.734. Comparable levels of heterozygosity have also been found in other

studies of brook trout using microsatellites (0.594–0.766, King and Jullian 2000; 0.36–0.72, Castric et al. 2001; 0.17–0.79, Angers and Bernatchez 1998). Allelic richness is another important diversity measure because populations subjected to bottlenecks or to prolonged periods of low effective population size may retain high levels of heterozygosity while losing large numbers of alleles (Petit et al. 1998). Allelic richness for the wild populations in my study varied from 1.7 to 4.4; other microsatellite studies did not report allelic richness.

A relationship between levels of genetic diversity and population size was observed in this study. Low levels of heterozygosity and allelic richness generally coincided with field observations of low population abundance (i.e. inferred by difficulty obtaining individuals by electrofishing). Small, isolated populations in Mason's Run, Preakness Brook, Crooked Brook tributary, and Oakdale Creek had the lowest levels of heterozygosity (0.342, 0.350, 0.353, and 0.391 respectively) and allelic richness (1.7, 2.0, 2.2, and 2.1 respectively), whereas robust populations such as Cooley's Brook and Turkey Brook had the highest levels (0.734 and 0.684, and 4.5 and 4.9, respectively).

Smaller and more isolated populations are predicted to lose genetic diversity at a greater rate, and are more at risk of interbreeding, reduced fitness, and localized extinctions (Frankham et al. 2002). Since wild brook trout populations in New Jersey occur primarily in small streams, and often in small numbers (personal observation, based upon 28 years of electrofishing small New Jersey trout streams), they may be more vulnerable to extinction. Mason's Run, an isolated stream in south Jersey, had the lowest observed heterozygosity (0.342), allelic richness (1.7) and polymorphism (61.5%), and no unique alleles, though surprisingly inbreeding was not detected ($F_{IS} < 0$). The reason

for the low genetic diversity observed in this population is unclear, however, judging from the difficulty obtaining just nine fish >10 cm over a considerable distance (in comparison to other streams sampled) the population in Mason's Run is small. Processes that diminish genetic diversity (genetic bottlenecks, random genetic drift, and inbreeding) can be problematic in small populations. A founder event (the arrival of a few individuals to a new area, either naturally or via stocking, that can result in a reduced gene pool) is a plausible explanation, given the absence of other wild brook trout populations in central and south Jersey. Whatever the explanation for the apparent low genetic diversity, small populations such as Mason's Run, and others in New Jersey may be at greater risk for extirpation.

Interestingly, the cultured trout from the Pequest Trout Hatchery collection had the second highest heterozygosity (0.677) and the highest allelic richness (4.7). Surveys of electrophoretic variation in brook trout have shown a similar pattern of relatively high variability in hatchery populations and generally low variability in wild populations in the southeastern United States (Wright and Atherton 1970; McGlade and MacCrimmon 1979; summarized in Stoneking et al. 1981). Similar results were found in a genetic study of wild brook trout populations in the Great Smoky Mountains, some of which were established through stocking (McCracken et al. 1993). The authors speculated that the relatively high variability found in populations founded by hatchery strains could reflect (1) the higher variability that is apparently carried in northeastern brook trout populations, (2) the possible founding of the hatchery strains with fish from several locations, and (3) possible interbreeding of hatchery fish with other hatchery stains or wild populations. Stoneking et al. (1981) speculated that low variability in unstocked

wild populations could result from isolation and small population size that promote genetic drift and inbreeding. The differences in heterozygosity observed between wild populations and the hatchery population in this study are likely due to differences in effective population size. The Pequest Trout Hatchery typically takes eggs from 400 to 450 females annually, and three times as many males as females are used to fertilize the eggs (W. Martka, NJDFW – Pequest Trout Hatchery, personal communication). Although the effective population size for wild brook trout in small New Jersey streams is not known, the degree of difficulty in obtaining fish greater than 10 cm in many of the streams sampled suggests that the effective population size is much smaller in small streams than in the hatchery. It is also possible that the Nashua strain in the Pequest Trout Hatchery is more genetically diverse than wild trout populations.

Inbreeding ($F_{IS} > 0$) was detected in eight stream collections and the Pequest Trout Hatchery collection (Table 3). Sampling bias may explain why inbreeding was detected in some wild populations, where suitable specimens were abundant and typically collected over a shorter distance, thereby increasing the likelihood of relatedness. Small sample sizes may also contribute to the inbreeding detected.

Genetic Diversity among Populations and Genetic Structure

All of the statistical methods used in this study reject the null hypothesis of no genetic differentiation among brook trout from different streams and drainages in New Jersey. The microsatellite data in this study revealed a strong pattern of population subdivision among drainages, which suggests that geographic factors have played a major role in determining patterns of genetic structure among brook trout collections from New

Jersey. F_{ST} values measure population divergence and typically an F_{ST} above about 0.15 is considered to be an indication of significant differentiation among populations (Frankham et al. 2002). In my study, many pairwise F_{ST} estimates, and to a lesser extent R_{ST} estimates, for collections differed greatly from zero indicating divergence among populations. The AMOVA test also showed the highest variance at the population level (50.8%) rather than the drainage level. The general pattern of population uniqueness was further supported by the multilocus assignment tests, which correctly assigned individuals to their source population with a high level of accuracy (94.5%). The presence of 22 private alleles, 10 of which occurred at high frequencies, also indicates that populations have differentiated. Although the sample sizes and number of populations surveyed may limit the ability of the analyses to provide conclusive results, the results from my study agree with those from earlier genetic surveys involving microsatellite studies of brook trout, which have also shown a strong pattern of population sub-division (Castric et al. 2001, Angers et al. 1995, Adams and Hutchings 2003). The ability of the suite of 13 microsatellite loci to provide high resolution with small sample sizes was notable.

The pattern of genetic variation revealed by the 13 microsatellites indicates that population differentiation has occurred on a hydrogeographic scale in New Jersey. Populations within the Passaic and Raritan drainages showed the strongest genetic groupings, perhaps because these drainages are considerably smaller than the Delaware drainage and confined, for the most part to New Jersey. Furthermore, within these two drainages the streams that were geographically closest (i.e. connected by the shortest fluvial distance) were consistently shown in the tree topology to be most closely related.

For example, in the Raritan drainage, Oakdale Creek (OAK) and Hacklebarney Brook (HAC) in the Lamington River sub-drainage formed a subgroup, and Flanders Brook (FLA), Kruegers Creek (KRU), and Turkey Brook (TUR), which are located in the headwaters of the S/Br. Raritan River sub-drainage, also grouped together. Similarly in the Passaic drainage, Crooked Brook tributary (CBT) and Hibernia Brook (HIB) in the Rockaway River sub-drainage paired, as did Burnt Meadow Brook (BMB) and Havemeyer Brook (HAV) in the Wanaque-Ramapo subdrainage. Another striking feature of the NJ tree is that none of the populations from the Delaware and Hudson drainages, and the Hackensack subdrainage, grouped with populations from the Raritan or Passaic drainages. The tree topology suggests that the Hackensack River system, where Cresskill Brook (CRE) is found, should be considered a separate drainage from the Passaic, or alternatively, perhaps stocking has influenced the gene pool of this stream. Collectively, these patterns of gene diversity appear to reflect colonization of different drainages by genetically distinct fish and populations within drainages subsequently became further differentiated due to geographic isolation.

The grouping of populations by drainage or major basin has also been found in New York (Perkins et al. 1993), Tennessee (Kriegler et al. 1995), eastern Canada (Jones et al. 1996), and Maryland (Quattro et al. 1990; Hall et al. 2002). In contrast, a genetic study of brook trout populations inhabiting an open water system (Miramichi River drainage, New Brunswick) found that geographical factors play only a minor role in determining the patterns of genetic structure among drainages within a large river system (Rogers and Curry 2004). In open-river systems the potential for brook trout to disperse is much greater than in more closed systems having natural or manmade barriers. In New Jersey,

natural conditions and manmade barriers result in relatively closed river systems that separate populations of brook trout inhabiting small streams and inhibit their dispersal within the same drainage. This separation can effectively restrict or limit gene flow among these populations. Over time, this reproductive isolation, in concert with genetic drift and local mutations, has apparently resulted in sufficiently different allelic frequencies among populations, such that individuals can be correctly assigned to their population of origin with remarkable accuracy. Yet despite this divergence, many populations within drainages have retained sufficient genetic similarity, which allows them to form distinct groupings by drainage. The pattern of genetic structuring observed in this study suggests that a single panmictic population may have initially colonized each drainage. If true, then the presumed historical genetic relationships of populations within several New Jersey drainages may be relatively intact.

The Delaware drainage populations did not form a strong group compared to those from within the Passaic and Raritan drainages. Only three of the six Delaware drainage populations grouped together, and of these, two (Van Campens Brook, VCB, and Forked Brook, FOR), were proximate hydrogeographically while the third population (Kurtenbach's Brook, KUR) was much more distant. Of the three remaining populations from the Delaware drainage, a close genetic relationship was observed between two populations from Halfway House Brook (HWH) and Mason's Run (MAS), the isolated south Jersey stream, while Independence Brook (IND) grouped with two populations from two separate drainages. The failure of the Delaware drainage populations to group as a unit may be more a reflection of the sheer size of the Delaware drainage, and its more linear nature, when compared to the Passaic and Raritan drainages. Perhaps the

stocking of nonnative brook trout strains has impacted these Delaware drainage populations.

Finally, several populations failed to exhibit any affinity to their drainage of origin. Given the strong grouping of five Passaic drainage populations, the failure of Lake Stockholm Brook (LSO) and Cooley's Brook (COO) to group in this drainage suggests other forces have affected these populations. In the case of Lake Stockholm Brook, the presence of a null allele at one locus may have caused this aberration. With others, it is possible that the legacy of widespread stocking of cultured brook trout in New Jersey over the last century has left a lasting footprint on the native gene pools of some wild populations. This may be particularly true in Cooley's Brook, which was routinely stocked with trout prior to 1990. Rocky Run (ROC) in the Raritan drainage may also have been affected, as trout have been stocked downstream in Spruce Run Creek. Several wild brown trout were encountered when sampling for brook trout in this stream, indicating trout have been stocked in the stream or that stocked fish have migrated from downstream areas. Yet, other streams having a history of trout stocking (Flanders Brook and Van Campens Brook) do not show evidence of having been affected by stocking. Therefore, a history of stocking does not necessarily indicate that the genetic integrity of a wild population has been compromised by introgression of non-native genes.

Land use practices and widespread stocking of cultured salmonids over the last century have likely influenced the current distribution of this species and may have affected some native gene pools. This study provides evidence of genetic structure among wild brook trout populations in New Jersey and suggests that the stocking of hatchery-reared brook trout or transference of brook trout between drainages has likely

affected the genetic integrity of some native gene pools. I emphasize that small sample sizes for each collection were used in this study and single populations were used to represent entire drainages or large sub-drainages, which may limit the ability of the analyses to provide conclusive results. However, the data suggests that the detection of population structure is possible with a small sample size (10 individuals per population) when 12-13 microsatellite loci are used. Additional studies using a larger sample size and more collection sites are recommended to reinforce the inference gained in this study.

Management and Conservation Implications

As concerns increase for brook trout across its native range, the distribution and pattern of genetic variation in brook trout populations have emerged as important considerations in conservation of the species. Several pieces of evidence in this study suggest that brook trout in New Jersey drainages should be considered a conservation priority. The study revealed (1) distinct genetic structuring of brook trout in two drainages, (2) genetically distinguishable populations in all four drainages, and (3) the influence of hatchery stock on the Cooley's Brook population and possibly others in this study. These results have important implications for managing and conserving New Jersey's wild brook trout populations and the natural ecosystems they depend upon.

First, the pattern of fine-scale genetic variation, as indicated by the genetic distance tree structure, the distribution of genetic variation, as measured by pairwise F_{ST} values, and hierarchical gene diversity analysis, suggests that local populations of wild brook trout in small streams should be treated as separate management units in order to preserve their genetic integrity. However, separate management of every stream in New Jersey

suspected of containing ancestral brook might not be feasible due to economic, legal, and sociocultural limitations. As suggested by Perkins et al. (1993), conservation efforts may therefore have to focus on a subset of populations that at a minimum maintains the genetic differentiation observed at two fundamental levels – among populations within drainages and among drainages.

Second, the low level of genetic diversity observed in small populations emphasizes the importance of restoring habitat connectivity and quality. Habitat loss and fragmentation are among the biggest threats to the long-term survival of brook trout populations in New Jersey (Hudy et al. 2005). Unfortunately, the restoration of historically connected streams may be impossible in much of New Jersey, given the realities of water development (streams and wetlands dammed by property owners to create permanent impoundments). Fisheries managers may have more success restoring physical habitat rather than re-connecting stream fragments.

Third, the genetic integrity of many of the wild populations in this study appears to be relatively unaffected by past stocking practices, allowing for potentially successful restoration efforts using locally adapted wild stock. The pattern of population structuring by drainage indicates that drainage and geographic proximity appear to be effective surrogate indicators of genetic relationships between populations. A restoration program should, therefore, rely upon transfers of wild stock from adjacent areas within the same drainage, preferably ones with no history of stocking. Translocation of fish among major drainages and stocking with cultured trout is not recommended because of large genetic differences observed among drainages and between wild fish and fish from the Pequest Trout Hatchery.

Fourth, since stocking appears to have affected the genetic integrity of at least one of three brook trout populations that has been stocked in the past, resource managers should consider strategies to avoid or minimize further genetic interactions between cultured and wild brook trout. Hybridization between native and hatchery-produced salmonids is considered a serious threat to the long-term persistence and genetic integrity of native stocks (Allendorf and Leary 1988). If a stocking program is widespread and interbreeding frequent, locally adapted native stocks will be replaced by larger more homogeneous populations (Krueger and May 1991). Therefore, genetic diversity should be an important consideration when stocking hatchery-reared trout in drainages where wild brook trout occur.

In New Jersey, the annual stocking of catchable-size hatchery-reared brook, brown, and rainbow trout has led to strong public support and high demand for trout. In recent years, NJDFW has instituted changes that address the ecological and genetic impacts of stocking while minimally impacting harvest-oriented anglers. Since the mid-1980's the stocking of cultured brook trout in small streams having wild brook trout has been, for the most part, discontinued, though non-native cultured salmonids were often substituted. When the *Wild Trout Stream* fishing regulation was adopted in 1990, stocking was discontinued on 29 designated streams, and some of those had spawning brook trout populations. Since then, nearly all small streams having wild brook trout have been removed from the stocking program, and a policy implemented in 2005 prevents stocking in most streams having reproducing trout populations (Hamilton and Barno 2005). An increase in the statewide minimum harvestable size for trout, from seven to nine inches,

has been proposed for 2008 which would further curtail the harvest of larger, potentially sexually mature trout, by anglers.

To protect the genetic integrity of New Jersey's native brook trout populations it may be prudent to consider additional strategies. For example, developing a sterile (triploid) trout program would allow for the continued stocking of brook trout at existing stocking locations while preventing introgression of non-native genes. Sterile trout, when stocked as catchables in streams, may provide recreational fisheries that are equal or superior to normal diploid fish (Kozfkay et al. 2006). The use of sterile trout would have the added benefit of further limiting the establishment of non-native salmonids in existing or potential brook trout habitat. Technical and economic considerations (equipment and manpower costs) associated with the production of sterile trout may limit the ability of an agency to undertake such a program.

Another strategy to preserve the genetic integrity would be to restrict the use of hatchery-produced (nonnative) brook trout in drainages where spawning brook trout populations occur or stock exclusively non-native trout species at existing stocking locations wirhin these drainages. Because brook trout declines in New Jersey have been attributed in part to the intrusion of non-native brown trout (Hudy et al. 2005), rainbow trout may be the preferred non-native species to stock in this situation. Although ecological hazards are still associated with sterile and non-native trout, these strategies would allow the stocking of hatchery-reared trout for harvest-oriented anglers to continue, with quantities of trout that anglers are accustomed to receiving. Perhaps combination of these and other options that capitalize on the flexibility of the existing

stocking program, while striking a better balance between ecological, economic, and social needs, have the best chance of succeeding.

Clearly, the recreational and intrinsic value of brook trout, coupled with an alarming decline in its distribution in parts of the eastern U.S., has triggered a concerted effort to manage and conserve the species. To protect the long-term viability of wild brook trout, management decisions regarding stewardship of this valuable resource must be based upon the best biological information available. Ancestral brook trout populations represent an irreplaceable part of the natural resources in New Jersey, and indeed elsewhere in its native range. Management agencies should make a concerted effort to identify native populations and safeguard their gene pools to preserve their genetic variability and potential to evolve in response to environmental change.