GENETIC IMPACTS OF BROODSTOCK SELECTION STRATEGIES FOR WISCONSIN'S WILD BROOK TROUT STOCKING PROGRAM

by

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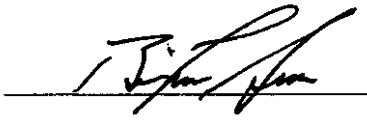

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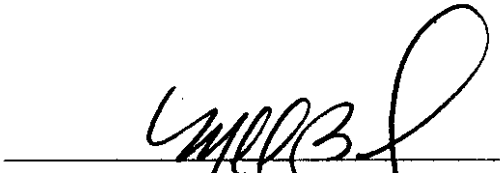

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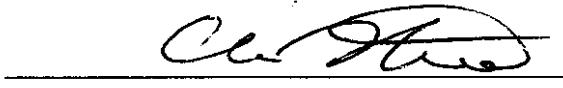

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ABSTRACT

In 1995, the Wisconsin Department of Natural Resources began a wild trout propagation program to improve the efficacy of wild trout stocking. The program captures broodstock from a wild source population, spawns these fish in a hatchery, returns the broodstock to their source population, and stocks the offspring in other streams. Two critical decisions in designing supplemental propagation programs that rely upon wild broodstock are: 1) selecting a genetically appropriate brood source, and 2) determining a sustainable proportion of fish collected from the source population. Since 2000, Ash Creek (Richland Co., WI) has served as the sole source of broodstock for brook trout (*Salvelinus fontinalis*) in southwestern Wisconsin. Questions exist regarding 1) the genetic suitability of Ash Creek as a broodstock source, and 2) the genetic impact of annual removals approaching 50% or more of the adult brook trout in the population. To determine the suitability of Ash Creek as a wild source of broodstock, the genetic diversity of Ash Creek and 13 other southwestern Wisconsin brook trout populations were characterized using 12 microsatellite loci. High levels of genetic divergence were found among populations; however, the majority of divergence appeared to be unrelated to the populations' geographical proximities to one another. The results suggest the surveyed brook trout populations are 1) small and reproductively isolated and strongly influenced by genetic drift, and/or 2) have been impacted by historical stocking events through the introduction of exogenous genes into the native gene pool. As a consequence, the determination of Ash Creek's population as a genetically appropriate and regionally representative broodstock is difficult. However, the degree of genetic differentiation observed among all populations studied would suggest a single source of

brook trout would be insufficient for propagation throughout the southwestern region of Wisconsin. To determine if annual broodstock removals have resulted in any realized or potential detrimental impacts to levels of genetic diversity in Ash Creek's population, genetic and morphological (length and weight) measurements were taken from the in-hatchery (wild hatchery broodstock) and in-stream adults (post-broodstock removals), and their in-hatchery and in-stream young-of-year (YOY) counterparts over two years. Levels of genetic diversity among adult components (in-hatchery and in-stream trout) did not differ over two years indicating that despite the large proportion of adult fish removed (50 to >80% per year), the yearly in-stream adults maintained levels of genetic diversity. However, a size bias was observed with larger fish being used as broodfish. The broodstock removals resulted in no reductions in levels of genetic diversity within or among the yearly in-stream YOY components. Levels of genetic diversity among the in-hatchery and in-stream YOY components were similar, although, the effective number of breeders that produced the in-hatchery YOY components was approximately seven times larger than that of the in-stream YOY components. This skew likely reflects a combination of the reduction in the size of the number of in-stream breeders and a lower variance in family size within the hatchery. These data suggest current broodstock selection strategies have had no detectable short-term impacts on genetic diversity levels within Ash Creek. Several results of this study raise concerns that the long-term impact of such strategies will be detrimental. The removal of larger brook trout and subsequent reductions in the mean body size of Ash Creek's in-stream breeding population will have negative consequences to the population's reproductive potential (lower mean fecundity) and genetic integrity (lower mean number of breeders).

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This thesis is dedicated to Abel for the hours he spent patiently waiting for me to rise from my desk. You were a miraculous friend whose companionship is sorely missed.

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INTRODUCTION

The brook trout (*Salvelinus fontinalis*) is the only salmonid native to Wisconsin's inland streams (Becker 1983). They inhabit cool, clear, headwater streams throughout the state. In northern Wisconsin, brook trout are widespread, residing in numerous small and medium sized streams. In southern Wisconsin, brook trout are less numerous with the exception of streams in Richland, Columbia, Dane and Sauk Counties. However, in these counties, brook trout populations have decreased or, in some cases, have been extirpated (Becker 1983). In Wisconsin, brook trout are a highly sought after sportfish. In 2001, it was estimated from trout stamp sales that approximately 223,000 anglers directed their efforts toward brook trout and other inland trout species (WDNR 2004). This popularity of brook trout among Wisconsin's anglers has led the Wisconsin Department of Natural Resources (WDNR) to develop an extensive management program (see Epifanio and Lindloff 1999).

The WDNR classifies Wisconsin's trout streams into one of three classes based on the reproductive capacity and stocking requirements of their trout populations (WDNR 2002). Class I waters (40% of total trout waters) are the highest quality trout waters in the state and support naturally reproducing trout populations at or near perceived carrying capacities; therefore, supplemental stocking of hatchery-reared trout is not required. Class II waters (45%) contain trout populations that lack sufficient natural reproduction to reach the perceived carrying capacity; therefore they receive supplemental stocking to accommodate angling pressure. Class III waters (15%) represent marginal trout habitat supporting no natural reproduction and are solely dependent on stocking to maintain a trout fishery.

The WDNR and public support the use of hatchery supplementation (i.e., stocking) as a management tool for Wisconsin's brook trout resource. Since the late 1800s, the state of Wisconsin has stocked brook trout produced from domestic broodstocks (i.e., fish maintained in a hatchery setting for two or more generations; Krueger et al. 1979). Two domestic brook trout strains have been documented as being predominately used in Wisconsin since the late 1800s: 1) the Osceola strain, whose origin is largely unknown but is thought to have been indigenous to Wisconsin (Callen 1983; Fields and Philipp 1998; Avery 1999) and 2) the St. Croix strain, which replaced the Osceola strain in 1973 and originated from eggs acquired from the Nashua National Fish Hatchery in New Hampshire (Claggett and Dehring 1983; Avery 1999), as such, this strain is considered a non-native source. The St. Croix strain continues to be used for maintaining brook trout populations within Class II and III waters throughout a majority of the state (WDNR Stocking Records available at http://infotrek.er.usgs.gov/wdnr_public).

Numerous studies examining the performance of domestic trout strains have shown poor survival of hatchery fish in the wild resulting in a failure to enhance the carrying capacity or reproductive potential of the fisheries (Flick and Webster 1964; Mason et al. 1967; Hunt 1979; Fraser 1989; Avery et al. 2001). In some situations, this failure has been attributed to a reduction of genetic variation within domestic strains compared to wild populations (Allendorf and Phelps 1980; Vuorinen 1984) and artificial selection to hatchery conditions resulting in fish that are maladapted to natural environments (Busack and Currens 1995; Campton 1995; Reisenbichler and Rubin 1999; Waples 1999). Allendorf and Phelps (1980) found hatchery stocks of west-slope

cutthroat trout (*Oncorhynchus clarki lewisi*) had a 57% reduction in genetic diversity when compared to their wild, ancestral populations. The authors questioned the use of these stocks for conservation purposes as this loss of diversity would make the strains less adaptive and could negatively impact ecological efficiency.

The use of domestic strains, especially strains of non-native origin in supplemental stocking programs, may hinder the sustainability and viability of declining native populations as a consequence of reductions in population fitness. Outbreeding depression is the loss of fitness in a population when genetically divergent fish are stocked and introgress with the locally adapted fish population (Ryhmer and Simberloff 1996; Allendorf et al. 2001; Hallerman 2003a). This loss of fitness is attributed to the breakdown of locally adapted gene complexes and non-complementary adaptations in the wild and domestic strains. The reliance on domesticated brook trout coupled with some non-native brook trout sources when supplementing trout populations represents a distinct risk to population fitness due to outbreeding depression.

Numerous studies have shown wild-origin, hatchery-reared trout are superior to domesticated strains in performance and long-term survival (Flick and Webster 1964; Fraser 1989; Lachance and Magnan 1990; Avery et al. 2001; Mitro 2004). For instance, Avery et al. (2001) found wild-origin, hatchery-reared brown trout (*Salmo trutta*) stocked into two central Wisconsin streams had survival rates 1.3–4.5 times higher than domestic strains after one year and 4–42 times higher after two years. In Wisconsin, interest in propagating wild-origin, hatchery-reared fish became prominent during the early 1990s when it was shown that stocked domestic trout exhibited low survival rates and poor longevity despite a prohibition on angler harvest (Avery et al. 2001; Mitro 2004).

In 1995, the WDNR began a wild trout stocking program (Mitro 2004); a supplemental stocking program using wild broodstock for hatchery propagation. The goal of this program was to improve the quality of hatchery-reared trout used to augment self-sustaining Class II brook trout populations in the southwestern region of Wisconsin (Mitro 2004). In this program, wild adults are captured from a selected stream in the fall, transferred to a hatchery, spawned, and returned to their home stream in the late fall/early winter. To maintain their wild characteristics, the resulting progeny are reared under conditions attempting to simulate their natural environment. These conditions include rearing at a lower density in partially shaded tanks and the use of automatic feeders to limit human contact. These fish are ultimately stocked out into Class II streams as spring or fall fingerlings (Epifanio and Lindloff 1999; WDNR 1999; Mitro 2004).

The conservation of genetic diversity is vital to the recovery and long-term sustainability of Wisconsin's brook trout populations because genetic variation is the raw material of adaptive change in populations and has been positively correlated with population fitness (Reed and Frankham 2003). Two of the most critical decisions toward conserving genetic diversity, within and among hatchery and wild populations in wild broodstock programs, are the choice of broodstock and the proportion of wild fish used for propagation purposes (Miller and Kapuscinski 2003). The selection of an appropriate broodstock for Wisconsin's wild trout stocking program should be a critical initial decision to ensure adequate levels of genetic diversity in propagated fish, and to conserve the overall genetic integrity of native populations (Miller and Kapuscinski 2003). Several principles and guidelines have been established and recognized for making initial decisions about the broodstock selection and the number of individuals to use in a

supplementation program (Miller and Kapuscinski 2003). A key principle is that selected broodstocks should contain similar genetic characteristics and life history patterns as the recipient populations to minimize the risk of outbreeding depression (Krueger et al. 1981; Waples 1991; Busack and Currens 1995; Miller and Kapuscinski 2003). Therefore, an *a priori* understanding of the distribution of genetic diversity within and among brook trout populations in southwestern Wisconsin should be obtained to successfully select an appropriate broodstock.

Several studies have examined the spatial distribution of genetic diversity among brook trout populations in Wisconsin (Kreuger and Menzel 1979; Callen 1983; Fields and Philipp 1998). Specifically, Fields and Philipp (1998) evaluated potential brood sources and attempted to identify stock structure for Wisconsin's brook trout. This study identified seven broad genetic management zones (GMZ's) of brook trout populations throughout Wisconsin (Figure I) which approximated watershed boundaries. These findings were consistent with other studies of warmwater species in Wisconsin (Fields et al. 1997). As a result, the suggested management zones were adopted by the WDNR and the wild trout stocking program was implemented within the southwest GMZ (Fields and Philipp 1998; WDNR 1999).

Since 1999, Ash Creek (Richland County) has served as the single source of broodstock for the entire wild brook trout stocking program (Mitro 2004). The WDNR selected Ash Creek as a brood source for this management region because it contains a naturally self-sustaining brook trout population cleared for use after an extensive health check and it was believed the population was of sufficient size to provide the broodstock necessary to meet production requirements of the wild trout stocking program (Mitro

2004). However, no *a priori* data was evaluated to determine the appropriateness of Ash Creek's brook trout population as a genetically representative broodstock either in terms of similarity of genetic characteristics or similarity of life history characteristics with other trout populations in the southwest GMZ.

Ideally, the source population for Wisconsin's wild trout stocking program would be free of non-native, stocked genes and representative of historical endemic brook trout (i.e., heritage brook trout). Unfortunately in Wisconsin, widespread and systematic stocking from the late 1800s to present has resulted in few brook trout populations that have not been stocked. The earliest known stocking events in Ash Creek are in the early 1970s when Osceola and St. Croix brook trout strains were stocked (WDNR Stocking Records available at http://infotrek.er.usgs.gov/wdnr_public). The effect of these stocking events on the genetic integrity of Ash Creek's brook trout has never been examined. Moreover, Fields and Philipp's (1998) found inconsistencies in the patterns of relatedness of brook trout populations within the southwest GMZ and recommended further genetic research. Because Ash Creek's brook trout population was never included in previous studies of spatial genetic diversity, determining the genetic characteristics of Ash Creek's population, the spatial distribution of genetic variation among brook trout populations in the southwest GMZ, and whether Ash Creek is genetically representative of the region are essential if the goals of the wild trout stocking program are to be attained.

A second critical decision when establishing wild trout propagation programs is to determine the proportion of a wild population to collect for hatchery production (Miller and Kapuscinski 2003). This decision is critical because it can impact the genetic

composition of not only the hatchery-reared fish, but also the brood source itself. The proportion of fish collected should represent the overall distribution of genetic diversity and life history characteristics (i.e., spawning time, size, age, sex ratio, etc.) found within a source population to optimize levels of genetic variation in hatchery propagated fish. Likewise, it is important to ensure that the remaining in-stream source population maintains its original level and distribution of genetic diversity and life history characteristics or future use of the brood source will result in lowered benefits. As a guideline, Miller and Kapuscinski (2003) recommended removing ≤ 50% of a population's individuals for hatchery production to ensure that a representative sample remains in the source population.

Concerns exist that >50% of Ash Creek's spawning population are annually removed to meet required egg quotas (~198,000 eggs in 2002) for Wisconsin's wild trout program. On average, the wild trout stocking program annually removes ~700 adult fish from Ash Creek. However, efforts have only recently been initiated to establish and monitor the standing census size of adult brook trout in Ash Creek (Mitro 2004). Despite the fact that spent broodstock are returned to Ash Creek, thereby maintaining a relatively stable annual adult population size, the current strategy likely restricts the overall reproductive efforts. Current collection strategies may also be size-selective toward larger breeding adults as a consequence of the gear bias associated with back-pack electrofishing (Anderson 1995) which could alter Ash Creek's mating system, overall population fecundity, and adult size structure in subsequent years. Theoretically, returning spent trout back to Ash Creek, thereby allowing them to contribute to following breeding seasons, may reduce the loss of reproductive potential in subsequent years.

However, this is based on two assumptions: 1) reproductive potential of a brook trout is equal across all breeding seasons, and 2) recruitment is consistent in any given year. Both of these assumptions are unrealistic for most fish species, especially brook trout. Adult male brook trout have been shown to not spawn every year (Vladykov 1956) and brook trout, in general, often have fluctuating recruitment as a consequence of the unstable environmental characteristics associated with the streams they reside in (Titus and Mosegaard 1992).

The genetic consequences of annually removing a large proportion of Ash Creek's breeding population include increased rates of genetic drift and/or inbreeding in the remaining in-stream spawning population. Genetic drift results in random changes in allele frequencies from one generation to the next as a result of sampling errors associated with a finite number of breeders (Hallerman 2003b). Genetic drift within a population will eventually lead to the fixation of a single allele at individual loci resulting in the loss of all alternative alleles; a net loss of genetic variation (Hallerman 2003b). The rate at which this variation is lost from a population is inversely proportional to its effective population size (N_e) or, in general terms, the number of individuals contributing genetic material to the next generation (Hallerman 2003b). As a consequence of broodstock removals, Ash Creek's N_e may be drastically reduced (i.e., $\leq$ 50% of its census size) rendering it more vulnerable to genetic drift and the loss of genetic diversity, changing the dynamics and characteristics of the population, and, ultimately, threatening the long-term viability of this population.

Increased rates of inbreeding in Ash Creek could negatively impact the diversity of genotypes within the population and subsequently the population's fitness. Inbreeding

results in a decrease in heterozygosity within progeny and overall reductions in genetic variation within populations by reducing heterozygous combinations (Wang et al. 2002a). The subsequent increase in homozygosity can lead to a greater expression of deleterious alleles that would otherwise be hidden (i.e., recessive) within a heterozygous state (Frankham 2003; Frankham et al. 2004). Inbreeding eventually results in losses of genetic diversity with negative impacts on a population's fitness (reproductive success) and performance (survival and/or growth rates). This resulting reduction in fitness and performance (i.e., inbreeding depression) has been recognized as one of the greatest threats to the viability of small populations because inbreeding arises as an inevitable consequence of small population sizes (Amos and Balmford 2001; Wang et al. 2002b; Frankham et al. 2004).

Since all genetic diversity is ultimately the result of random mutation events, any reductions in genetic diversity are permanent and will eventually jeopardize the sustainability of Ash Creek's population. Subsequently, gains observed from using Ash Creek's population for restoring brook trout populations in the southwest GMZ could be lost as a consequence of propagating genetically depauperate and ecologically inferior brook trout. This would be a gradual process as the impacts of genetic drift and inbreeding would manifest cumulatively as the loss of diversity increases with time. Eventually, the original goals of the program would be unattainable due to systemic problems in the current broodstock collection strategies.

Attempts to restore or rehabilitate fish populations must recognize the importance of self-sustaining, naturally reproducing populations and their subsequent value to recovery efforts. These populations represent the source of genetic diversity that will

provide the basis for adaptation and long-term sustainability of local populations (Bowles 1995). Therefore, Ash Creek's brook trout population was examined to assess the potential impact of current broodstock collection strategies. To ensure the protection of genetic integrity within naturally reproducing brook trout populations, genetic evaluations of brook trout populations in the southwest region were conducted to determine the appropriateness of Ash Creek as a brood source.

The goal of this research was to identify the genetic consequences of Wisconsin's wild trout stocking program. The two objectives of this study were:

1. To determine whether Ash Creek's brook trout population is a representative broodstock for the southwest GMZ by characterizing regional patterns of genetic variation within Wisconsin's southwest genetic management zone.

2. To evaluate whether Ash Creek has experienced any discernible genetic and/or demographic impacts as a result of current broodstock selection strategies by conducting a series of four comparisons among Ash Creek's population components:
    a. The in-stream adults were compared to the in-hatchery adults to determine if the proportion of adult trout removed resulted in a bias in genetic or size (total length and weight) diversity.
    b. The in-stream adults were compared to their respective in-stream young-of-year (YOY) counterparts to determine if the reductions to the size Ash Creek's in-stream population resulted in reductions to levels of genetic diversity within the in-stream YOY populations.

c. Genetic diversity levels within the in-stream YOY in 2005 and 2006 were compared to determine if the difference in the proportion of fish removed from Ash Creek (44% and 84%, respectively) resulted in differential reproductive contribution to the in-stream YOY populations.

d. Genetic diversity levels within the in-stream and in-hatchery YOY components were compared to determine if differential reproductive contributions existed among these YOY populations.

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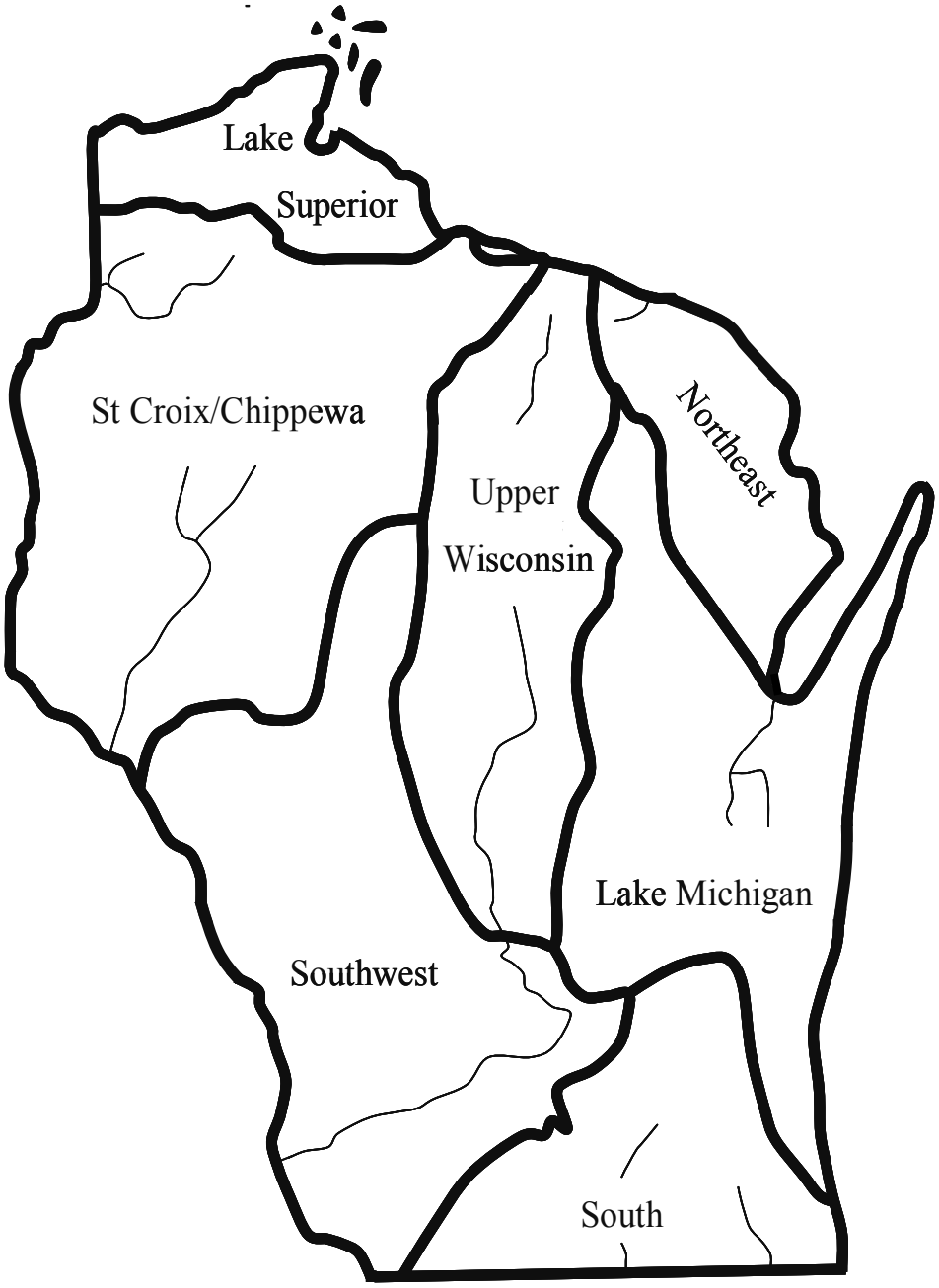

Figure I. Seven genetic management zones recommended by Fields and Philipp (1998) to conserve genetic structure of brook trout in Wisconsin.

Chapter I:

Genetic Diversity of Wisconsin Brook Trout in the Driftless Region: The Suitability of Ash Creek's Brook Trout Population as a Genetically Appropriate and Genetically Representative Source of Wild Broodstock

Abstract- In 1995, the Wisconsin Department of Natural Resources began a wild trout stocking program. The program captures broodstock from a wild source, spawn these fish in a hatchery, return the broodstock to their source, and stock the offspring in other streams in southwestern Wisconsin. Selecting a genetically appropriate and representative source of broodstock is a critical decision toward maintaining the genetic diversity within and among managed populations. Since 2000, Ash Creek (Richland Co., WI) has served as the sole source of broodstock for brook trout (*Salvelinus fontinalis*) in southwestern Wisconsin. It was selected because it contains a healthy, naturally reproducing population. The genetic characteristics of Ash Creek's population and its relationship to other brook trout population in southwestern Wisconsin are unknown. To determine the appropriateness of Ash Creek as a brood source, the genetic diversity of Ash Creek brook trout and 13 other southwestern Wisconsin brook trout populations were characterized using 12 microsatellite loci. High levels of population divergence were found among populations; however, the majority of divergence appeared to be unrelated to the populations' geographical proximities to one another. The failure to observe geographical patterns in the genetic structure of brook trout is most likely attributed to two factors: 1) populations are small and reproductively isolated with very limited gene flow between them and, therefore, highly influenced by the stochastic effects of genetic drift, and, 2) populations have been impacted to varying degrees by historical stocking across streams, thus introducing exogenous genes into the native gene

pool. As a consequence, the determination of Ash Creek's population as a genetically appropriate and regionally representative broodstock is difficult. Given the complexity of the mechanisms driving population genetic structure in the southwest region, it is vital that restoration efforts attempt to minimize adverse genetic changes to recipient wild populations. The most genetically conservative stocking approach would be to manage supplemental stocking efforts in accordance with watershed drainage boundaries within the southwest GMZ. While such a strategy still carries some genetic risks, it reduces the overall risks across Wisconsin's southwestern region as a whole and ensures the likelihood that a sufficient number of populations will remain viable to enable brook trout to persist in the short-term and diversify in the future.

INTRODUCTION

The Wisconsin Department of Natural Resources (WDNR) currently uses a wild trout stocking program to ensure high-quality, hatchery-reared trout and subsequently strengthen rehabilitation efforts of brook trout populations in the southwestern region of Wisconsin (Mitro 2004). In this program, wild adults are captured from a selected stream in the fall, transferred to a hatchery, spawned, and returned to their home stream in the late fall/early winter. To maintain their wild characteristics, the resulting progeny are reared under hatchery conditions attempting to simulate their natural environment. These conditions include rearing at a lower density in partially shaded tanks and the use of automatic feeders to limit human contact. These fish are eventually stocked out as spring or fall fingerlings into brook trout streams that are perceived to have limited natural reproduction (Epifanio and Lindloff 1999; WDNR 1999; Mitro 2004).

The conservation of genetic diversity has become one of the principal goals in the management and restoration of wild populations (Frankham et al. 2004). Genetic diversity represents the raw material of adaptive change and evolutionary potential in populations and has been positively correlated with population fitness (Reed and Frankham 2003). For Wisconsin's wild trout stocking program, the selection of an appropriate source of broodstock is the first and most critical decision toward conserving genetic diversity (Miller and Kapuscinski 2003) within and among brook trout populations in the southwest genetic management zone (GMZ; Figure 1).

The selection of an appropriate source of broodstock for restoration efforts should be based on two genetic criteria: 1) broodstock(s) should be representative of regional patterns of genetic variation (Miller and Kapuscinski 2003) to ensure they represent

historical, endemic lineages that are free of maladapted, exogenous genes, and 2) broodstock(s) should exhibit high levels of genetic diversity to provide recipient populations with a greater ability to maintain optimum fitness levels and conserve their evolutionary adaptive potential (Busack and Currens 1995). To determine whether potential source populations meet the above criteria, it is necessary to have *a priori* understanding of the distribution of genetic diversity within and among populations.

Several studies have examined the spatial distribution of genetic diversity among brook trout populations in Wisconsin (Kreuger and Menzel 1979; Callen 1983; Fields and Philipp 1998). Specifically, Fields and Philipp (1998) attempted to identify stock structure for Wisconsin's brook trout and identified seven broad genetic management zones (GMZ's) of brook trout populations throughout the State that approximated watershed boundaries (see Figure 1). These findings were consistent with findings in various warmwater species in Wisconsin (Fields et al. 1997). The suggested management zones were adopted by the WDNR and the wild trout stocking program was implemented within the southwest GMZ (Fields and Philipp 1998; WDNR 1999).

The southwest GMZ presents a unique problem because Fields and Philipp (1998) were unable to resolve patterns of genetic variation among brook trout populations in the southwestern region and instead recommended this region warranted separate management as a consequence of its ecological distinctiveness from other regions in the state. This region largely contains Wisconsin's 'driftless region', a geologically distinct area in the upper Midwest that was bypassed by the last continental glacial advance (Martin 1965). Because of our limited knowledge of the population genetic resources

among brook trout in this region, the identification of potential brood sources representative of regional genetic variation is difficult.

Currently, the WDNR uses Ash Creek (Richland County) as its source of broodstock for the wild brook trout stocking program in the southwestern GMZ (Mitro 2004). This stream was selected because it contains a naturally self-sustaining brook trout population cleared for use after an extensive health check and believed to be of sufficient size to provide the broodstock necessary to meet production required from the wild trout stocking program (Mitro 2004). However, the genetic characteristics of this population have never been examined. Historical stocking records show Ash Creek was stocked in the early 1970s with Osceola and St. Croix brook trout strains (WDNR Stocking Records available at http://infotrek. er.usgs.gov /wdnr_public). The Osceola strain's origin is largely unknown but is thought to have been indigenous to Wisconsin (Avery 1999). However, the St. Croix strain, which replaced the Osceola strain in 1973, originated from eggs acquired from the Nashua National Fish Hatchery in New Hampshire (Claggett and Dehring 1983; Avery 1999). As a consequence of these past stocking events, the potential exists that the wild trout stocking program is propagating brook trout from a population that has been genetically compromised by the introduction of exogenous genes from domestic trout.

If the restoration goals of the wild trout stocking program are to be attained, it is essential to determine the genetic characteristics of Ash Creek's population and the patterns of genetic variation among brook trout populations (i.e., population structure) in the southwest GMZ. The objective of this study was to characterize regional patterns of brook trout genetic variation within and among populations in the southwest GMZ to

determine whether Ash Creek's population is a genetically appropriate and representative brood source.

METHODS

Study Site

The southwest GMZ (Figure 1.1) encompasses a major portion of the driftless area, a distinct topographic region of Wisconsin bypassed by the last continental glacier. This region is comprised of a vast number of spring-fed coldwater streams that drain into the Mississippi and Wisconsin Rivers. The Mississippi River, below the Chippewa River confluence (River Mile 763.4), forms the western boundary of this management zone and serves as an outlet for the Buffalo-Trempealeau River, and the Black River, Bad Axe-Lacrosse River drainage basins. Along the southern edge, the Wisconsin River, from the Castle Rock Flowage dam to its confluence with the Mississippi River (River Mile 630.6) near Prairie du Chien, WI (~ 165 miles), serves as the southern most drainage basin for this GMZ (Figure 1.2; Martin 1965; Fields and Philipp 1998; WDNR 1999).

Sample Collection

To compare and contrast the genetic diversity within Ash Creek's brook trout population to other regional brook trout populations, Ash Creek and 13 additional brook trout populations throughout the southwest GMZ were sampled. The selection of brook trout populations focused on populations with either no history or limited history of recent stocking events (post-1970s) and current status as a Class I stream (i.e., sufficient natural reproduction to preclude stocking by the WDNR). At least two populations were selected from each of the four major drainage basins in this region that contain self-sustaining, naturally occurring populations (Table 1.1; Figure 1.2). Stocking histories from 1972 to 2006 varied for each selected populations (Table 1.2).

Sample collection occurred in the spring of 2005 and 2006. From each stream, brook trout were sampled using a Smith-Root 15-D electrofishing backpack unit (Smith-Root, Inc., Vancouver, WA). Pelvic fin clips (>25 mg) were taken from each fish to be used for DNA extraction and analysis. Each fin clip was stored in an individually labeled tube containing 95–100% non-denatured ethanol. Based on the recommendations of Ruzzante (1998), ≥ 50 fish were collected from each population to ensure accurate and precise estimates of genetic diversity measures and accurate delineation of genetic structure among populations in the southwest GMZ.

DNA Extraction

Total genomic DNA was extracted from collected tissue samples using Promega Wizard® Genomic DNA purification kit in accordance with the manufacturer's suggested protocol (Promega Corp., Madison, WI). The final step of the protocol was modified by re-hydrating the DNA in 200 µl of Tris-low-EDTA buffer (TLE; 10 mM NaCl, 0.1 mM EDTA, pH 8.0) instead of the manufacturer's supplied buffer. The quality (molecular weight) of each DNA sample was evaluated via electrophoresis in a 0.7% agarose gel and compared to a known molecular weight ladder (Hyperladder™ I, Bioline USA Inc., Randolph, MA). The quantity of DNA (ng/µL) for each sample was analyzed using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). All DNA samples were normalized to a consistent DNA quantity (25 ng/uL) prior to microsatellite analysis to ensure consistent results.

Microsatellite Genotyping

A standardized set of 12 microsatellite DNA markers used in Lake Superior brook trout studies was used for this study (Table 1.3; King and Burnham-Curtis, unpublished; Angers et al. 1995; Wilson et al. 2005). The extracted DNA samples were amplified by the using the polymerase chain reaction (PCR). PCR conditions for multiplex and singlet reactions as well as thermal profiles are shown in Table 1.4. Samples were genotyped on an ABI Prism^{TM} 377XL automated DNA sequencer (Applied Biosystems, Inc. Foster City, CA). A known size standard (GeneFlo^{TM} 625 DNA Ladder, Chimerx Corp., Milwaukee, WI) was included with each sample to facilitate accurate sizing of alleles. The resulting data consisted of direct-count, multi-locus genotype data.

Data Analysis

Hardy-Weinberg equilibrium and gametic disequilibrium.—Tests for deviations from Hardy-Weinberg Equilibrium (HWE; Hardy 1908; Weinberg 1908) were conducted for each locus/population combination using exact tests that employ the Markov Chain method to estimate p-values and calculated in Genepop v3.4 (1000 dememorization steps, 100 batches and 1000 iterations; Guo and Thompson 1992). To ensure all loci were independently segregating within sampled populations, tests of gametic disequilibrium (i.e., linkage) were conducted for all combinations of locus pairs within populations using a Markov Chain method calculated in Genepop 3.4 (1000 dememorization steps, 100 batches and 1000 iterations; Raymond and Rousset 1995). For both tests of HWE and gametic disequilibrium, significance values for multiple tests were adjusted with a sequential Bonferroni correction (Rice 1989).

Measure of intrapopulation genetic diversity.— The levels of genetic diversity within brook trout populations in the southwest GMZ were estimated from allelic diversity (A), observed heterozygosity (H_o), and expected heterozygosity (H_e), calculated using Microsatellite Toolkit v3.1 (Parks 2001). In addition, estimates of allelic richness (A_r) were estimated for each population using a rarefraction method implemented in HP-RARE 1.0 (Kalinowski 2005) to account for potential bias in A due to unequal sample sizes (Leberg 2002, Kalinowski 2005)

Interpopulation comparisons of genetic diversity.—To assess whether Ash Creek's brook trout population contained levels of genetic diversity similar to other southwestern GMZ populations, pairwise comparisons of A_r and H_e were conducted. Genetic diversity comparisons among all populations were performed using an analysis of variance (ANOVA) of ranked data in SPSS 14.0 (SPSS Inc., Chicago, IL). Because of the non-parametric nature of genetic data, locus-specific data were ranked across populations prior to the analysis. *Post hoc* comparisons followed Dunnett's procedure (Dunnett 1955) with Ash Creek's population serving as a single control to which all other sampled populations were compared.

Effective population size and inbreeding coefficients.—To assess potential impacts on the maintenance of genetic variation, the effective number of breeders (N_e) and inbreeding coefficient (F_{IS}) for all southwestern GMZ populations were estimated. The N_e is a measure roughly equating to the number of reproducing individuals in an ideal population (i.e., one that meets all the Hardy-Weinberg assumptions) that loses genetic variation (via genetic drift and inbreeding) at the same rate as the number of reproducing individuals in the observed population (Hallerman 2003). The N_e for individual

populations was estimated based on the linkage disequilibrium estimator of Hill (1981) and Bartley et al. (1992) using NeEstimator 1.3 (Peel et al. 2004). This estimation assumes associations among alleles are produced by the effects of genetic drift within small panmictic population (Bartley et al. 1992). Confidence intervals (95%) were calculated according to Waples (1991). The inbreeding coefficient (F_{IS}; Wright 1931) was used to estimate inbreeding within each sampled population. Computations of population-specific inbreeding coefficients and their significance level (h_o = no deviation from zero) were calculated in Arlequin 3.10 (Excoffier et al. 2005) with significance based on 1000 permutations.

Spatial structuring of genetic diversity.—To assess if the genetic characteristics of Ash Creek's population were representative of the southwest GMZ, the genetic structure of brook trout populations in the management zone was evaluated with a variety of tests. A global test of genic differentiation was used to test for panmixia by testing each locus for significant differences in allele frequencies among populations. In the absence of panmixia, a series of population pairwise tests was run to allow for more fine scale assessment of genic differentiation. The global and pairwise tests of genic differentiation were conducted in Genepop 3.4 (Guo and Thompson 1992; Raymond and Rousset 1995), using exact tests with a Markov Chain method (1000 dememorization steps, 100 batches and 1000 iterations) to generate an exact probability distribution under the null hypothesis that allelic frequency distributions do not differ between populations (Raymond and Rousset 1995). Significance values of pairwise tests were corrected for multiple tests using a sequential Bonferroni correction (Rice 1989).

To further examine variation genetic variation among brook trout populations in the southwest GMZ, genetic differentiation (F_{ST}) was measured using Weir and Cockerham's (1984) θ (theta), analogous with Wright's (1951) F_{ST}. F_{ST} is a measure of population differentiation ranging from zero (no difference) to one (completely different genetic characteristics). A global F_{ST} was calculated using FSTAT 2.93 (Goudet 2001) and significance (h_o = no deviation from zero) was assessed by bootstrapping over loci to estimate the 95% confidence intervals. Population pairwise values of F_{ST} were calculated in Arlequin 3.10 with the estimation of significance values based on 1000 permutations (Excoffier et al. 2005). Significant values were corrected for multiple tests through sequential Bonferroni correction (Rice 1989).

A combination of genetic distances, analysis of molecular variance, and isolation by distance measures were used to further examine the partitioning and patterns of genetic variation among brook trout populations in the southwest GMZ. To quantify the level of genetic similarity between southwestern GMZ populations, allele frequency data was used to calculate pairwise estimates of Cavalli-Sforza and Edward's (1967) chord distance (D_c). This distance measure was selected because it makes no assumptions about the particular mode of microsatellite evolution (Takezaki and Nei 1996). To visualize population relationships, an unrooted neighbor-joining dendrogram (NJ; Saitou and Nei 1987) was constructed from estimated values of D_c. All distance analyses were conducted in POPULATIONS 1.2.14 (Langella 2001). Node support of tree topology was constructed using 1000 bootstrap pseudoreplicates of the data across all loci. Individual nodes were considered resolved if bootstrap support was > 50%. Trees were visualized and edited using TREEVIEW 1.6 (Page 1996).

Populations are often structured in larger groups (e.g., stocks, evolutionary significant units, etc.) such that individuals within a specific group are more similar than individuals from different groups (Allendorf and Luikart 2007). For example, brook trout populations could potentially be grouped by major drainage basins and then subsequently, the basins could be grouped by major geographical regions. To determine if hierarchical structuring of genetic variation among brook trout in the southwest GMZ was present, an analysis of molecular variance (AMOVA) was performed. The AMOVA test calculates the percentage of total genetic variance explained by differences within populations (V_c), among populations within groups (V_b), and by differences between groups (V_a) defined from several geographical scales (Excoffier et al. 1992; Allendorf and Luikart 2007). AMOVA tests were used to determine if the partitioning of brook trout populations by major drainage basins explained a significant proportion of the genetic variance among populations within the southwest GMZ. AMOVA tests were conducted using Arlequin 3.10 with significance values based on 1000 permutations (Excoffier et al. 2005).

Geographical patterns of genetic divergence between populations can provide insight into the influences of genetic drift and gene flow (Slatkins 1987). Gene flow is expected to be greater among neighboring populations, reducing genetic differences between them. As geographical distances increase, gene flow is expected to decrease and the influence of genetic drift to increase, resulting in greater genetic divergence among populations (Relethford 1996). An isolation by distance (IBD) analysis was performed to determine if there was a significant correlation between geographical (fluvial) distances and genetic differentiation (F_{ST}) using a Mantel test (Mantel 1967) conducted in IBD 3.03

(Bohonak 2002). The significance and the strength of any correlation was calculated based on 1000 randomizations of the data. Geographic distances between populations were determined using the Geographic Information System (GIS) program, ArcView 9.1® (Environmental Systems Research Institute, Redlands CA). To aid in ecological interpretation of the resulting relations, a scatter plot of genetic and geographical population pairwise distances was constructed in SPSS 14.0 (SPSS 1999) and evaluated according to the suggested patterns associated with the relative influences of genetic drift and gene flow on the regional population structure (see Figure 1.3; Hutchison and Templeton 1999; Koizumi et al. 2006).

Population assignments.—Assignment tests were performed to assess the overall genetic distinctiveness of populations surveyed in this study by determining population membership of sampled individuals or groups of individuals. If individuals assigned to their population of origin with a high confidence that population was deemed a genetically distinct population (Paetkau and Strobeck 1994; Bernatchez and Duchesne 2000). Assignment tests were conducted in GeneClass 2.0 (Piry et al. 2004) and employed the "leave-one out" method (Efron 1983). The distribution of genotype likelihoods were calculated for all sampled populations and individuals from these baseline reference populations were then treated as 'unknowns' and assigned back to a reference population. For each individual assignment, a score was calculated as follows:

$$Score_{i,l} = \frac{L_{i,l}}{\sum_{j=1}^{k} L_{i,j}}$$

where, given k reference populations, $L_{i,l}$ is the likelihood that individual i belongs to population l and $L_{i,j}$ is the likelihood of individual i belongs to population j (Piry et al.

2004). Individual assignments were determined to be classified correctly if individuals assigned back to their population of origin with a score greater than or equal to 80 (Sloss et al. in press). To assess the confidence of individual population assignments, the genotype log likelihood ratio (LOD) was calculated. The LOD is the ratio of the highest assignment log likelihood value to the second highest value. LOD scores ≥ 1 mean the individual population assignment was at least 10 times more likely than to any other population, while an LOD score ≥ 2 was at least 100 times more likely (Banks and Eichert 2000). If populations are highly differentiated, an individual would be expected to assign back to its population of origin with high confidence. However, wide-scale stocking impacts or migration could result in a low confidence of self-assignment.

RESULTS

A total of 802 brook trout were sampled from 14 target populations in the southwest GMZ (Table 5). Sample size ranged from 50 (Grinsell Branch and King Creek) to 71 (Elk Creek). A total of 132 total alleles were observed across the twelve loci and the overall numbers of alleles per locus ranged from 4 (Sfo 38) to 21 (Sfo115 and Sfo 91). Allele sizes and their population frequencies are shown in Appendix 1.

Measure of Intrapopulation Genetic Diversity

Relatively high levels of genetic diversity were observed within populations (Table 5). The mean number of alleles detected across loci within populations ranged from 3.42 (Parfrey's Glen) to 7.75 (Pine Creek). When unequal sample size was taken into account, Parfrey's Glen and Pine Creek samples still demonstrated the lowest (3.37) and highest (7.60) allelic richness, respectively. The mean H_o values across all loci ranged from 0.43 in North Branch of Chipmunk Coulee Creek and 0.75 in Pine Creek. Likewise, mean values of H_e ranged from 0.48 (North Branch of Chipmunk Coulee Creek) to 0.74 (Pine Creek).

Hardy-Weinberg Equilibrium and Linkage Disequilibrium

Initially, tests for HWE showed 28 of 168 (16%) locus/population comparisons deviated from HWE expectations based on a 0.05 alpha level. Following sequential Bonferroni correction only five (3%) locus/population comparisons significantly deviated from HWE expectations. The distribution of deviations did not indicate a specific population or locus issue, therefore, all populations were considered in HWE for

subsequent analyses. Tests of gametic disequilibrium initially showed 159 of 924 (17%) locus comparisons were significant at a 0.05 nominal alpha. Following sequential Bonferroni correction, significant gametic disequilibrium was observed in 31 (3%) pairwise locus comparisons. The significant locus comparisons revealed no consistent pattern among any of the 12 loci, indicating the significant values were most likely an artifact of sampling error resulting from small population sizes and genetic drift (Ohta 1982; Allendorf and Luikart 2007). Therefore, all loci and populations were considered to be in gametic equilibrium for subsequent analyses.

Interpopulation Comparisons of Genetic Diversity

When Ash Creek was used as a reference population for genetic diversity comparisons, numerous differences were observed. Ash Creek exhibited a total of 53 alleles across the 12 surveyed loci. The observed number of alleles per locus ranged from 2 (Sfo 24) to 6 (Sfo 52 and Sfo 91) with a mean of 4.30 alleles/locus and mean H_e of 0.59 (Appendix 1). Genetic diversity comparisons among all populations showed significant population differences in mean A_r (ANOVA: $df = 13$, $F = 4.64$, $p < 0.001$) and mean H_e (ANOVA: $df = 13$, $F = 3.72$, $p < 0.001$). *Post hoc* comparisons showed Ash Creek contained significantly lower A_r diversity than King Creek ($p = 0.012$), Pine Creek ($p = 0.002$), Soper Creek ($p = 0.008$), and West Branch of Mill Creek populations ($p = 0.024$). Furthermore, Ash Creek was found to contain significantly lower H_e diversity than Pine Creek ($p = 0.041$).

Effective Population Size and Inbreeding Coefficients

Effective population sizes varied greatly across populations. The mean N_e across all 14 populations was 78.18. However, population-specific N_e ranged from a low of 15.30 (North Brach of Chipmunk Coulee Creek) to a high of 179.10 (John Coulee Creek); although, many 95% confidence intervals overlapped among populations (Table 5). The F_{IS} values for individual populations ranged from -0.21 (West Branch of Mill Creek) to 0.11 (North Branch of Chipmunk Coulee (Table 5) and four populations showed significant signs of inbreeding. Of these four populations, three (Big Springs Branch, Elk Creek, and North Branch of Chipmunk Coulee Creek) demonstrated relatively small effective sizes ($N_e < 50$). Alternatively, the fourth population, Soper Creek, had a relatively high N_e (154.5) but exhibited significant inbreeding.

Spatial Structuring of Genetic Diversity

Initial tests of spatial genetic structuring demonstrated an overall lack of panmixia among populations in the southwest GMZ. Exact tests of global population genic differentiation showed all populations were significantly differentiated from one another ($p < 0.001$) and the global F_{ST} (0.144) was significantly different than zero (95% confidence intervals of 0.129 and 0.159) indicating the presence of genetic divergence and structuring among brook trout populations. Pairwise tests of population differentiation found all populations in the southwest GMZ to be highly divergent from one another (Table 6). Population pairwise F_{ST} values ranged from 0.0304 to 0.3221 and all comparisons were significantly different than zero ($p < 0.001$).

The genetic relationships among populations in the southwest GMZ was found to be unrelated to the geographical distributions of populations. The unrooted NJ dendrogram, constructed from Cavalli-Sforza and Edwards (1967) chord distance, showed low node support (i.e., majority of node support <50%) and populations appear to show only moderate affinity to other within-basin populations (Figure 4). For example, the populations within the lower Wisconsin basin (Ash Creek, Melancthon Creek, Grinsell Branch, Big Springs Branch, Elk Creek, Parfrey's Glen, and Fancy Creek) reside throughout the tree with no relation to one another. Populations from the remaining three basins appeared to cluster, however, there was little support for these relationships. Overall, the NJ tree showed a high amount of genetic distance/diversity within populations (i.e., length of terminal nodes) compared to between populations (i.e., internal nodes).

No hierarchical structuring based on major drainage basins was detected among populations in the southwest GMZ (Table 7). An AMOVA showed the percentage of total genetic variance in the region attributed to differences between basins was small (2.02%) and not significant ($\sigma^2 = 0.083$, $p = 0.143$). Rather, the majority of the total variance was attributed to within-population variation (82.70%, $\sigma^2 = 3.41$, $p < 0.0001$) and, to a lesser extent, between-populations within basins (15.28%, $\sigma^2 = 0.630$, $p < 0.0001$).

The divergence among populations (F_{ST}) was found to be unrelated to their geographical distances from one another. A Mantel test showed no significant correlation between pairwise F_{ST} values and geographical distances ($p = 0.97$). The scatter plot exhibited a weak negative association between genetic and geographic

distances (Figure 5). Qualitatively, this relationship most closely resembled Hutchison and Templeton's (1999) Case III scenario (Figure 3) suggesting a lack of regional drift-migration equilibrium.

Population Assignments

Assignment tests accurately showed strong delineation and accuracy of assignment among the 14 populations in the southwest GMZ (Table 8). Overall accuracy of self-assignment was 768/802 fish (95.9%) correctly assigned to their true population of origin using a minimum assignment score of 80. The mean value of individual assignment scores (Piry et al. 2004; Sloss et al. in press) was high (98.1%). The overall confidence in individual assignment was moderately high with a mean LOD score of 1.57 (SD = 0.43). Out of the 802 total samples, 118 (14.7%) fish assigned to their population of origin with an LOD ≥ 2. The remaining 684 fish were assigned to their population of origin with an LOD ≥ 1.

DISCUSSION

A suitable broodstock candidate for rehabilitation/propagation programs efforts should exhibit at least two genetic characteristics: 1) it should contain appropriate levels of genetic diversity, and 2) it should be representative of regional patterns of genetic variation. The population structure of brook trout within the southwest GMZ and levels of genetic diversity within populations were evaluated to assess the suitability of Ash Creek's brook trout population as a source of broodstock for Wisconsin's wild trout stocking program.

Levels of genetic diversity displayed within the sampled brook trout populations were similar to those seen in other studies assessing microsatellite diversity in brook trout (Castric et al. 2001; Castric and Bernatchez 2003; Fraser et al. 2004; Sloss et al. in press). For instance, Castric and Bernatchez (2003) examined 30 brook trout populations in Maine and observed levels of H_e ranging from 0.41–0.79 similar to the range found in this study (0.48–0.74). Direct comparisons with these studies should be cautiously interpreted due to differences among studies in both the number and type of loci utilized. However, Sloss et al. (in press) found similar levels of H_e (0.44–0.67) among six brook trout populations along the south shore of Lake Superior using the same 12 loci used in the present study.

Brook trout within the southwest GMZ are highly structured with a complex pattern of genetic variation among populations in the southwest GMZ. As a species, brook trout have been identified as being one the more highly structured freshwater fish species (Ward et al. 1994; Anger et al 1995). Therefore, significant genic and genetic divergence among all populations was not unexpected and was consistent with other

studies of brook trout, (e.g., Herbert et al. 2000; Castric et al. 2001; Castric and Bernatchez 2003; Castric and Bernatchez 2004; Poissant et al. 2005; Sloss et al. in press). However, studies of salmonid species generally show a strong geographic component in relation to the divergence among populations; where populations within the same drainage show greater similarities to one another than to populations in adjacent drainages (Allendorf and Waples 1996). In the present study, the divergence between brook trout populations appeared to be unrelated to their geographical proximities to one another. For instance, Ash Creek resides approximately 72 river km from Big Springs Branch and 296 river km from Soper Creek, however, differentiation estimates indicated Ash Creek's population was more divergent from Big Springs Branch's (F_{ST} = 0.204) than from Soper Creek's (F_{ST} = 0.148). Furthermore, the lack of geographical structuring among brook trout populations was evident by the highly unresolved NJ tree, the failure to detect hierarchical structuring between populations based on drainage basin groupings (i.e., AMOVA), and the failure to detect an IBD signature between populations.

Failure to observe geographical patterns within the genetic structure of populations is not uncommon in studies of nonanadromous salmonids and has been attributed to the effects of reproductive isolation (Ryman 1983; Crozier and Ferguson 1986; Campos et al. 2006), stocking impacts (Machordom et al. 1999; Castric et al. 2001), and hydrographic changes (Castric et al. 2001; Poissant et al. 2005). In the present study, the lack of a geographical pattern within the geographical structuring of brook trout is most likely attributed to two factors: 1) populations are small and reproductively isolated with very limited gene flow between them and, therefore, highly

influenced by the stochastic nature of genetic drift, and 2) populations have been impacted by historical stocking events and the introduction of exogenous genes into the native gene pool.

Reproductive Isolation

Brook trout have been shown to be naturally subdivided into numerous reproductively isolated and genetically distinct populations (Herbert et al. 2000; Castric et al. 2001; Castric and Bernatchez 2003; Castric and Bernatchez 2004; Poissant et al. 2005). This reproductive isolation can be attributed, in part, to the life history characteristics of brook trout. Salmonids typically exhibit some degree of reproductive isolation as a consequence of natal stream fidelity or homing behavior (Taylor 1991). O'Conner and Power (1973) found that displaced brook trout returned to their home stream with a high proportion of accuracy. Studies have also shown that a high percentage of stream-dwelling fish, such as brook trout, are sedentary; rarely leaving a particular pool or stream stretch (Smithson and Johnston 1999; Knouft and Spotila 2002; Rodriguez 2002). The affinity brook trout exhibit for their home streams may cause populations to become isolated as a consequence of limited dispersal of individuals and exchange of genetic material between brook trout populations.

Anthropogenic activities likely contributed to the isolation of brook trout populations through habitat fragmentation. Anthropogenic disturbance can cause or contribute to the isolation of populations due to the alteration or degradation of suitable stream habitats. Within southwestern Wisconsin, historical farming, grazing and land-use practices have severely degraded the quality of stream habitats in this region (e.g.,

sedimentation, reduced stream flows, and increased temperatures) resulting in the decline of brook trout in both their numbers and their range (Thorn et al. 1997). The widespread introduction of brown trout throughout the region has further contributed to an apparent restriction of brook trout to headwater stream sections (Krueger and May 1991; Thorn et al. 1997). These anthropogenic activities likely further contribute to the isolation of brook trout populations through habitat fragmentation.

Restricted gene flow among brook trout populations in the southwest GMZ likely resulted in populations diverging from one another as a consequence of genetic drift, resulting in random allele frequency changes within populations. Over time, the individual differences have resulted in large divergences between populations similar to that observed in this study. Additionally, small population sizes (i.e., populations with small effective sizes) can further contribute to the strong effects of genetic drift and lead to the stochastic divergence of populations (Hansen and Mensberg 1998). In the case of the southwest GMZ, it appears that the strong effects of genetic drift among brook trout populations have obscured the relationships between populations. Several results of this study support this hypothesis. First, the overall level of genetic divergence (Global F_{ST} = 0.144) and distinctiveness (mean confidence of individual population assignment = 95.5%) among populations in this region provide strong evidence of reproductive isolation among brook trout populations in this region. The strong influence of genetic drift can be seen even between geographically proximate and genetically related populations. For example, Melancthon Creek and Grinsell Branch, which reside less than 3 km apart, are significantly divergent from one another (F_{ST} = 0.03, $p < 0.01$). This is a strong indicator that even at the microgeographical level (i.e., within watersheds), the life

history characteristics and/or anthropogenic disruptions have led to reproductive isolation and genetic divergence among populations in this region. Second, the lack of an IBD relationship coupled with the wide degree of observed scatter (see Case III, Figure 2) when geographic distances were plotted against genetic distances (Figure 3) indicated genetic drift is more influential than gene flow in shaping patterns of genetic variation in this region. This is supported by the relatively small N_e's and significant inbreeding estimates in a subset of the populations. The values of within populations in this region were relatively small (15.3–179.1) considering it is often suggested that an N_e of 50 is needed to maintain the short-term genetic diversity within a population (Frankham et al. 2004). Likewise, the significant inbreeding observed in four populations (Big Springs Branch, Soper, Elk and North Chipmunk Coulee Creeks) was indicative of small isolated populations whereby restricted mating schemes within populations resulted in a greater tendency for related individuals to breed compared to random expectations (Mills and Smouse 1994).

Historical Stocking Impacts

While there are apparent signs that the genetic structuring of brook trout within the southwest GMZ may have been influenced to varying degrees by the reproductive isolation of populations, it is also likely that the long and prolific history of brook trout stocking in this region has contributed to the lack of geographical patterns within the genetic structure of brook trout populations. Historically, trout waters in Wisconsin were stocked as early as 1873. Prior to the 1950s, the State of Wisconsin emphasized stocking large numbers of trout. For example, in 1940 an estimated 5 million brook trout were

stocked throughout Wisconsin. Since the 1960s, the WDNR has shifted its fisheries management efforts toward habitat restoration and protection with a gradual reduction in stocking efforts. However, in 1995 ~1 million brook trout were stocked into state waters (WLAB 1997). While the majority of sampled populations in this study have known stocking histories or reside in heavily stocked watersheds (Table 1.2), the effects of these stockings are unknown and may have disrupted the native gene pool.

The reproductive success of stocked domestic fish may have influenced the genetic composition and genetic structure of southwest GMZ brook trout. Past stocking events within the southwest region potentially altered patterns of genetic variation among native populations, whereby introduced exogenous genes diluted the natural patterns of genetic variation and altered levels of genetic differentiation between brook trout populations (Hindar et al. 1991; Mork 1991; Busack and Currens 1995; Campton 1995; Araguas et al. 2004). As a consequence of disrupting the natural evolutionary divergence among native brook trout populations, the genetic structure of populations in this region may have been altered by stocking, obscuring the genetic relationships among native populations (Mork 1991; Araguas et al. 2004). Even a minimal level of introgression from previous stockings could have long term consequences on the genetic structure of brook trout in the southwest GMZ. Araguas et al. (2004) suggested that low rates of introgression per year (1%) over a short time span could severely alter wild populations and eventually obscure historical genetic relationships between native populations (see also Mork 1991).

Several results from this study suggested that past stocking activities have contributed to the lack of resolved genetic structure among southwest GMZ brook trout.

First, the unresolved structure patterns observed in this study resemble the patterns seen in other salmonid studies where stocking impacts were present. For example, Koljonen et al. (1999) examined IBD in Baltic Sea salmon populations and found IBD in self-sustaining naturally reproducing populations but failed to find a correlation between geographic and genetic distances in populations maintained by stocking. Likewise, Machordom et al. (1999) found similar inconsistencies among genetic and geographical relationships at allozyme markers for wild brown trout populations in central Spain and attributed it to introgression based on the presence of exogenous domestic alleles in wild populations. Second, the overall level of differentiation observed among brook trout (F_{ST} = 0.144) in relation to the spatial scale of this study (maximum distance = 408 km) is modest compared to other studies of brook trout (Angers and Bernatchez 1998; Castric et al 2001; Fraser and Bernatchez 2005). For example, the overall F_{ST} value was 54% lower than that reported by Angers and Bernatchez (1998; F_{ST} = 0.37), who examined 26 brook trout populations over a smaller geographical distance (42 km) within the La Mauricie National Park, Quebec, Canada. Furthermore, the overall confidence of assignment testing ($\bar{x}$ LOD = 1.57) was lower than a comparable study of brook trout in northern Wisconsin streams ($\bar{x}$ LOD = 5.44; Sloss et al. in press). The lower divergence and lower confidence in assignment tests could indicate signs that introgression of domestic fish has shifted patterns of regional genetic variation. This introgression may have reduced the genetic divergence among populations in this region, although, not to the point where the individual identity of brook trout populations was compromised.

Management Implications and Future Research

The results of this study show the genetic structure among southwest GMZ brook trout has likely been influenced by strong effects of genetic drift and/or introgression with stocked domestic trout. As a result, the determination of Ash Creek's population as a regionally representative broodstock is difficult. Ash Creek's population exhibits levels of genetic diversity similar to the majority of sampled populations, and therefore, could be considered an appropriate and viable source candidate to maintain levels of diversity in supplemented populations. However, the failure to resolve patterns of population structure raises concerns about the representative nature of Ash Creek's population as a sole source of broodstock for the entire southwest GMZ.

The influence of past stocking events on the contemporary genetic structure of brook trout in this region is a challenge to identifying an appropriate broodsource. Ash Creek's stocking records indicate the population was stocked in the early 1970's with both the Osceola and the St. Croix brook trout strains. It is important to note that prior to 1970 stocking activities in Ash Creek are poorly documented. The degree to which stocking may have contributed to the genetic diversity within Ash Creek is difficult to quantify without some knowledge of the genetic composition of this population before stocking or the genetic characteristic of the historical domestic strains stocked into it (Hansen et al. 2001b). As recently as 1970, Ash Creek was listed as a Class III trout stream (Ball et al. 1970), meaning it showed limited or no natural reproduction and was therefore largely sustained through stocking efforts. Based on this finding alone, the representative nature of Ash Creek's brook trout population is questionable.

Even if it is assumed that historical stocking events failed to genetically contribute to Ash Creek's population, the reproductive isolation observed among the brook trout populations sampled makes it questionable to use a sole representative population for augmentation efforts in the southwest GMZ. For instance, if the isolation and genetic divergence between brook trout populations reflects mostly natural adaptive differences in relation to local stream environments (Taylor 1991; Allendorf and Waples 1996), stocking fish from a genetically divergent brood source could potentially result in outbreeding depression (i.e., reduction in population fitness); a consequence of disruptions to local adaptations and/or coadapted gene complexes (Gharrett and Smoker 1991; Allendorf and Waples 1996). Alternatively, if limited gene flow and the isolation of populations is strictly a consequence of life history characteristics and/or anthropogenic disruptions resulting from habitat fragmentation or decreased population connectivity (i.e., non-adaptive change), the subsequent stocking of a genetically distinct intra-regional population, such as Ash Creek, may benefit recipient populations by reestablishing gene flow and counteracting the negative effects of genetic drift and inbreeding.

Given the complexity of the mechanisms driving population genetic structure in the southwest GMZ, restoration efforts should attempt to minimize adverse genetic changes to recipient wild populations. Conservative approaches should be undertaken to restore or supplement natural reproduction in wild populations while also attempting to minimize the risk of disrupting local adaptations. One approach would be to limit stocking, specifically the use of domestic strains or sources of unknown origin, as a means of rehabilitating populations. Efforts should alternatively be aimed at habitat

restoration to improve habitat connectivity and promote natural processes of gene flow between populations. The advantage of this approach is that it carries little risk of long-term genetic impacts. The use of stocking as a primary restoration tool should be limited to cases where there are evident signs that populations are at risk because of small population sizes or isolation. When stocking is necessary, brook trout supplementation efforts need to weigh the potential costs of admixture (i.e., mixing of distinct strains) against those of population isolation. In such cases, the most conservative approach would be to select wild brood sources that are geographically proximate to recipient populations or use gametes from the population of concern. Reisenbichler (1988) found the success of translocated populations (i.e., recovery rate and survival) decreased as distances from their natal stream increased. Therefore, the use of geographically proximate broodstock would increase the likelihood that stocked fish would successfully contribute to the next generation and also increase the recipient population's probability of maintaining their local adaptive capabilities.

The most conservative stocking approach would be to manage supplementation efforts in accordance with drainage boundaries because the majority of previous studies of brook trout have found genetic variation partitioned in relation to drainages (Perkins et al. 1993; Angers et al. 1995; Jones et al. 1996; Castric and Bernatchez 2003; Castric and Bernatchez 2004). Ideally, source populations would be selected, and their subsequent hatchery offspring would be released, within their "drainage of origin." While such an approach may offer a greater opportunity to preserve all brook trout populations within the southwest GMZ, it is likely not economically or logistically feasible. Alternatively, the southwest GMZ is naturally divided into two major domains: 1) those rivers that drain

into the Mississippi river, and 2) those rivers that drain into the lower Wisconsin basin. Therefore, a more economically feasible and sound alternative biogeographical approach would be to create two genetic management units within this region based on tributaries of these two major river systems. While such a strategy still carries some genetic risks, it reduces the overall risks across the southwest GMZ as a whole and ensures the likelihood that a sufficient number of populations will remain viable to enable brook trout to persist in the short-term and diversify in the future. With such an approach, the use of Ash Creek's population as a source of wild broodstock would be limited strictly to the lower Wisconsin River basin (Lower Wisconsin Genetic Management Unit). Selection of source populations for the Mississippi Genetic Management Unit should consist of selecting populations with no or very limited documentation of stocking, and high levels of genetic variation (i.e., high effective population size and no evidence of population bottlenecks or inbreeding). It is important to note that this approach assumes that all populations reflect native lineages unaltered or at least not significantly altered by previous historical stocking events.

This study demonstrates a need for further investigation into the genetic structure of brook trout in the southwest GMZ. The sampling design used in this study was geared toward a broad-scale (i.e., major drainage basins) regional assessment and the majority of populations sampled were more than 25 km apart. It is possible that the genetic structure of brook trout in this region has evolved on a finer scale. Based on preliminary results collected from additional analyses, it appears that brook trout populations in the southwest GMZ could be structured on a microgeographical scale based on river systems within basins (Table 9). For example, populations within the lower Wisconsin basin

show indications that brook trout are structured below the basin level and in association with regional river systems. However, the current study design limits the delineation of definitive geographical boundaries and, therefore, necessitates a critical need for additional sampling. Future studies need to encompass a sampling design focused on assessing genetic variation among brook trout at the level of major rivers that are tributaries to the Mississippi and Wisconsin Rivers. This would entail sampling multiple brook trout populations along all the major river systems in this region. For example, within the lower Wisconsin basin, brook trout populations from the Kickapoo, Green, Fennimore, Knapp Mill, Otter, and Pine rivers should be genetically characterized to assess if patterns of genetic variation occur on a finer microgeographical scale within this basin.

It is critical that future studies attempt to assess the impacts of historical stockings on the genetic structure of brook trout in this region. This would require a thorough stocking review and the genetic characterization of all domestic source populations that have been historically used for restoration efforts in this region. The feasibility of such a study would be dependent on the success of identifying all potential domestic brood sources and obtaining archived scale samples for genetic analyses.

Finally, microsatellite DNA marker results must be interpreted cautiously because the manner in which they evolve is poorly understood (Phillipe and Lagoda 1996; Morin et al. 2004). The inclusion of an additional molecular marker(s) would be beneficial to future genetic analyses. One such potential marker that may be useful in clarifying brook trout relationships in this region is single nucleotide polymorphisms (SNP's). Single nucleotide polymorphisms refer to single base mutations at specific sites within nuclear

DNA, whereby, some individuals will have a single base pair difference when compared to the most common form found in that species. Variation at SNP loci is biallelic rather than multiallelic and, hence, is less variable than microsatellites. However, SNP's are the most prevalent form of genetic variation in the nuclear genome and allow for a substantial increase in the number of loci that can be surveyed (Morin et al. 2004; Van Straalen and Roelofs 2006). Furthermore, SNP's provide a simpler mutational model that, unlike microsatellites, is less susceptible to homoplasy (i.e., alleles shared between populations but not derived from a common ancestor; Morin et al. 2004; Sprowles et al. 2006; Van Straalen and Roelofs 2006). Future genetic analyses enlisting SNP's would provide a complementary approach to microsatellites for assessing patterns of genetic variation among brook trout in the southwest GMZ (Morin et al. 2004; Smith et al. 2005).

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Table 1.1. Streams used to assess the genetic structure of brook trout in the southwest GMZ with their abbreviated name, respective basin, and watershed locations.

Stream	Abbreviation	Basin	Watershed
Elk	ELK	Lower Wisconsin River	Middle Kickapoo River
Melancthon	MEL	Lower Wisconsin River	Upper Pine River
Grinsell Branch	GRN	Lower Wisconsin River	Upper Pine River
Fancy	FCY	Lower Wisconsin River	Upper Pine River
West Branch Mill	WBM	Lower Wisconsin River	Mill/Indian Creeks
Big Springs Branch	BSB	Lower Wisconsin River	Blue River
Parfrey's Glen	PFG	Lower Wisconsin River	Lake Wisconsin
Soper	SOP	Black River	Big/Douglas Creeks
Joe Coulee	JNC	Black River	Beaver Creek/Lake Marinuka
King	KNG	Buffalo/Trempealeau River	Buffalo River
Pine	PNE	Buffalo/Trempealeau River	Trempealeau River
North Branch Chipmunk Coulee	NCC	Bad Axe/La Crosse River	Coon Creek
Johns Coulee	JOC	Bad Axe/La Crosse River	Coon Creek

Table 1.2. Stocking records from 1972-2007 for all populations sampled in the southwest GMZ including the year and the hatchery source.

Population	Year	# of Fish Stocked	Hatchery Source
ASH	1972	500	Osceola
	1973	500	Osceola
	1974	500	St. Croix
BSB	1972	2000	Osceola
	1973	2000	Osceola
	1974	2000	St. Croix
	1991	150	Field Transfer
	1991	700	Nevin
	1992	700	Nevin
	1993	700	Nevin
	1994	4700	Nevin
	1995	4700	Nevin
ELK	1972	300	Viola
	1974	500	St. Croix
FCY	1972	1500	Osceola
	1973	1500	Osceola
	1974	1500	St. Croix
	1976	1000	Osceola
GRN	1973	500	Osceola
	1974	500	St. Croix
JNC	No Record of Stocking between 1972-2006		
JOC	No Record of Stocking between 1972-2006		
KNG	No Record of Stocking between 1972-2006		
MEL	No Record of Stocking between 1972-2006		
NCC	No Record of Stocking between 1972-2006		

Table 2. Continued.

Population	Year	# of Fish Stocked	Hatchery Source
PNE	1972	2500	Osceola
	1973	1000	Pinnacle Rock Pond
	1973	1500	Osceola
	1974	500	Osceola
PFG	No Record of Stocking between 1972-2006		
SOP	1972	500	Osceola
	1973	1500	Pinnacle Rock Pond
	1974	5000	Osceola
	1975	700	Osceola
	1976	1200	Osceola
	1977	2200	St. Croix
	1978	1200	St. Croix
	1980	1000	St. Croix
	1981	1000	St. Croix
	1982	500	St. Croix
	1983	2500	St. Croix
	1984	2500	St. Croix
	1985	1500	St. Croix
	1988	121	Federal Hatchery/WC District
WBM	1978	1500	St. Croix
	1979	1500	St. Croix
	1980	1500	St. Croix
	1981	1500	Nevin
	1982	500	Nevin
	1983	500	Nevin

Table 1.3. Microsatellite loci used in current study and description of primer sequence, allele size range in base pairs, and number of alleles per locus (*A*). Sfo 12 and Sfo 18 are from Angers et al. 1995 and the remaining loci are from King and Burnham-Curtis, (unpublished).

Locus	Forward and Reverse Primer (5'-3')	Range	*A*	Motif
Sfo 52	GCACACGAAACCAGTATATTTC	187-239	18	tetra/dinucleotide
	TTGTCTTGGTGATTTCAGAGC			
Sfo 24	GCTACTGTTGGATTTCATCTCAG	110-186	13	trinucleotide
	ATCACAGAGATGGGGTGATG			
Sfo 28	CAGTTGAAGTGATTGGGTTAGC	167-201	18	trinucleotide
	TCATCCTTAAAGCAGAATACCAC			
Sfo 38	GTTGTGTTGCTTTGGTTTCAG	140-152	5	trinucleotide
	TTACTGATTACAATTTTGGACTGG			
Sfo 86	ACCGATGGCCTTCAACAC	101-125	8	trinucleotide
	ATAGGCCCCTACCTCAAACC			
Sfo 88	TAGTCTCTGGTGGGGAATAATG	178-213	11	trinucleotide
	ATATCAGCCATAAGAGCTGGAG			
Sfo 113	GGAGCCCAGACTATATTGACG	114-169	16	trinucleotide
	CCTTGAAGTCTTGCCAGATG			
Sfo 115	CAGTTTCTATCTCCAGGCAATC	217-367	46	tetra/dinucleotide
	TTCTGAAAGCACTCAACATGG			
Sfo 75	GTAGTGCCAAAACAGGTAGAGC	168-248	21	tetranucleotide
	CATCCTTATTCCAACCTCAATC			
Sfo 91	AAATAACAACAATATGTGAGAAC	204-340	27	tetranucleotide
	TATGCTGATATTGACTTTGG			
Sfo 12	CCCGTTTCACAATCAGAG	249-275	5	dinucleotide
	GGTTTTGAAGAGTGACAG			
Sfo 18	TGGTGTATCCTGCTCCTG	173-225	12	dinucleotide
	TGGATTGTGTGTCTGTTTTCT			

Table 1.4. PCR conditions for four multiplex and two simplex reactions used in this study, including loci, locus-specific primers and labels, 10x buffer, dNTPs, $MgCl_2$ and *Taq* concentrations, and thermal profiles.

Reaction	Loci	Label	Primer (μM)	10x Buffer	dNTPs (mM)	$MgCl_2$ (μM)	*Taq* (units)
Multiplex A[1]	Sfo 86	HEX™	0.07	1x	0.50	1.20	0.15
	Sfo 88	HEX™	0.09				
	Sfo 28	NED™	0.23				
Multiplex B[2]	Sfo 18	NED™	0.30	1x	1.20	0.80	0.16
	Sfo 115	6FAM™	0.21				
	Sfo 113	6FAM™	0.09				
Multiplex C[1]	Sfo 52	6FAM™	0.07	1x	1.20	1.50	0.24
	Sfo 75	NED™	0.10				
Multiplex D[1]	Sfo 24	6FAM™	0.08	1x	0.50	1.50	0.15
	Sfo 38	NED™	0.07				
Singlet 1[1]	Sfo 91	HEX™	0.40	1x	0.40	0.60	0.12
Singlet 2[2]	Sfo 12	6FAM™	0.40	1x	0.40	0.60	0.15

[1] 94°C for 2 min, followed by 35 cycles of 92°C for 45 s, 53°C for 45 s, and 70°C for 90 seconds and ending with a final elongation of 68°C for 30 min.

[2] 94°C for 2 min, followed by 35 cycles of 92°C for 45 s, 58°C for 45 s, and 72°C for 90 s and ending with a final elongation of 68°C for 30 min.

Table 1.5. Genetic diversity values for the 14 populations sampled within the southwest GMZ. Sample size (n), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), allelic richness (A_r), inbreeding coefficient (F_{IS}), and effective population size (N_e) with lower and upper 95% confidence intervals are given for each population. Significant F_{IS} values ($p < 0.05$) are indicated in bold.

							95% C.I.'s	
Population	n	H_o	H_e	A_r	F_{IS}	N_e	Lower	Upper
ASH	60	0.58	0.59	4.31	0.02	93.1	63.5	158.9
BSB	62	0.67	0.69	5.99	**0.04**	41.6	35.3	49.7
ELK	71	0.64	0.67	5.72	**0.05**	18.7	16.8	20.8
FCY	60	0.64	0.65	5.61	0.01	62.0	49.2	81.2
GRN	50	0.67	0.67	4.92	-0.01	42.3	33.5	55.3
JNC	51	0.52	0.54	4.82	0.04	179.1	106.0	488.9
JOC	52	0.62	0.63	5.91	0.01	26.1	22.0	31.3
KNG	50	0.69	0.70	6.84	0.01	125.9	85.6	223.4
MEL	61	0.68	0.64	5.16	-0.07	39.9	32.9	49.4
NCC	51	0.43	0.48	4.43	**0.11**	15.3	13.3	17.8
PFG	60	0.52	0.51	3.37	-0.01	92.6	57.3	197.3
PNE	51	0.75	0.74	7.59	-0.01	87.2	68.0	118.7
SOP	63	0.68	0.72	7.14	**0.05**	154.5	110.0	248.0
WBM	60	0.73	0.71	6.55	-0.02	116.2	85.4	174.6

Table 1.6. Genic and genetic differentiation (F_{ST}) population pairwise comparison for all sampled populations. Significance values of pairwise genic differentiation comparisons are shown above the diagonal. Pairwise F_{ST} comparisons are shown below the diagonal. All F_{ST} comparisons were found to be significant following sequential Bonferroni correction ($\alpha_{adjusted} = 0.0005$).

	ASH	BSB	ELK	FCY	GRN	JNC	JOC	KNG	MEL	NCC	PFG	PNE	SOP	WBM
ASH	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BSB	0.205	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ELK	0.187	0.140	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
FCY	0.189	0.080	0.140	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GRN	0.189	0.092	0.183	0.125	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
JNC	0.234	0.234	0.142	0.257	0.267	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
JOC	0.185	0.113	0.163	0.110	0.119	0.279	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
KNG	0.131	0.102	0.097	0.096	0.107	0.148	0.120	---	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MEL	0.203	0.082	0.190	0.121	0.030	0.276	0.122	0.112	---	<0.001	<0.001	<0.001	<0.001	<0.001
NCC	0.208	0.289	0.165	0.284	0.300	0.176	0.285	0.184	0.322	---	<0.001	<0.001	<0.001	<0.001
PFG	0.272	0.306	0.223	0.300	0.299	0.247	0.282	0.225	0.322	0.211	---	<0.001	<0.001	<0.001
PNE	0.174	0.081	0.100	0.080	0.095	0.168	0.095	0.046	0.089	0.215	0.209	---	<0.001	<0.001
SOP	0.148	0.067	0.131	0.090	0.085	0.215	0.083	0.066	0.092	0.248	0.262	0.065	---	<0.001
WBM	0.162	0.117	0.064	0.126	0.153	0.138	0.155	0.058	0.162	0.172	0.223	0.076	0.116	---

Table 1.7. Analysis of molecular variance based on major basin groupings with significance values based on 5,000 permutations (Excoffier et al. 2005). Sum of squares, percentage of variation and p-values associated with selected grouping is included for each source of variation.

AMOVA Groups	Populations	Source of Variation	Sum of Squares	% of Variation	p-value
(1) Lower Wisconsin	ASH	Among Basins	292.521	2.02	0.14272
	BSB				
	ELK	Among Populations within Basins	765.834	15.28	< 0.0001
	FCY				
	GRN	Within Populations	5414.896	82.70	< 0.0001
	WBM				
	PFG				
	MEL				
(2) Black	JOC				
	SOP				
(3) Bad Axe/Lacrosse	NCC				
	JNC				
(4) Buffalo/Trempealeau	KNG				
	PNE				

Table 1.8. Results of individual assignment testing to population of origin conducted using GeneClass 2.0 (Piry et al. 2004).

Population	*n*	# Correctly Assigned to Population	% Correctly Assigned to Population	# Correctly Assigned to Population with a Score ≤80	% Correctly Assigned to Population with a Score of≤80
ASH	60	60	100.00	60	100.00
BSB	62	62	100.00	62	100.00
ELK	71	66	92.96	66	92.96
FCY	60	60	100.00	60	100.00
GRN	50	45	90.00	43	86.00
JNC	51	51	100.00	51	100.00
JOC	52	51	98.08	51	98.08
KNG	50	50	100.00	49	98.00
MEL	61	56	91.80	50	81.97
NCC	51	51	100.00	51	100.00
PFG	60	60	100.00	60	100.00
PNE	51	51	100.00	50	98.00
SOP	63	58	92.06	55	87.30
WBM	60	60	100.00	60	100.00
Total	802	781	97.49	768	95.88

Table 1.9. Analysis of molecular variance based on 'within basin' regional population groupings with significance values based on 5,000 permutations (Excoffier et al. 2005). Sum of squares, percentage of variation and p-values associated with selected grouping is included for each source of variation.

AMOVA Groups	Populations	Source of Variation	Sum of Squares	% of Variation	p-value
(1) Western Wisconsin Basin	ELK	Among Basins	663.598	6.03	0.00218
	BSB				
	WBM	Among Populations within Basins	505.094	11.41	< 0.0001
(2) Central Wisconsin Basin	FCY	Within Populations	2997.000	82.49	< 0.0001
	GRN				
	ASH				
	MEL				
(3) Eastern Wisconsin Basin	PFG				
(4) Black	JOC				
	SOP				
(5) Bad Axe/Lacrosse	NCC				
	JNC				
(6) Buffalo/Trempealeau	KNG				
	PNE				

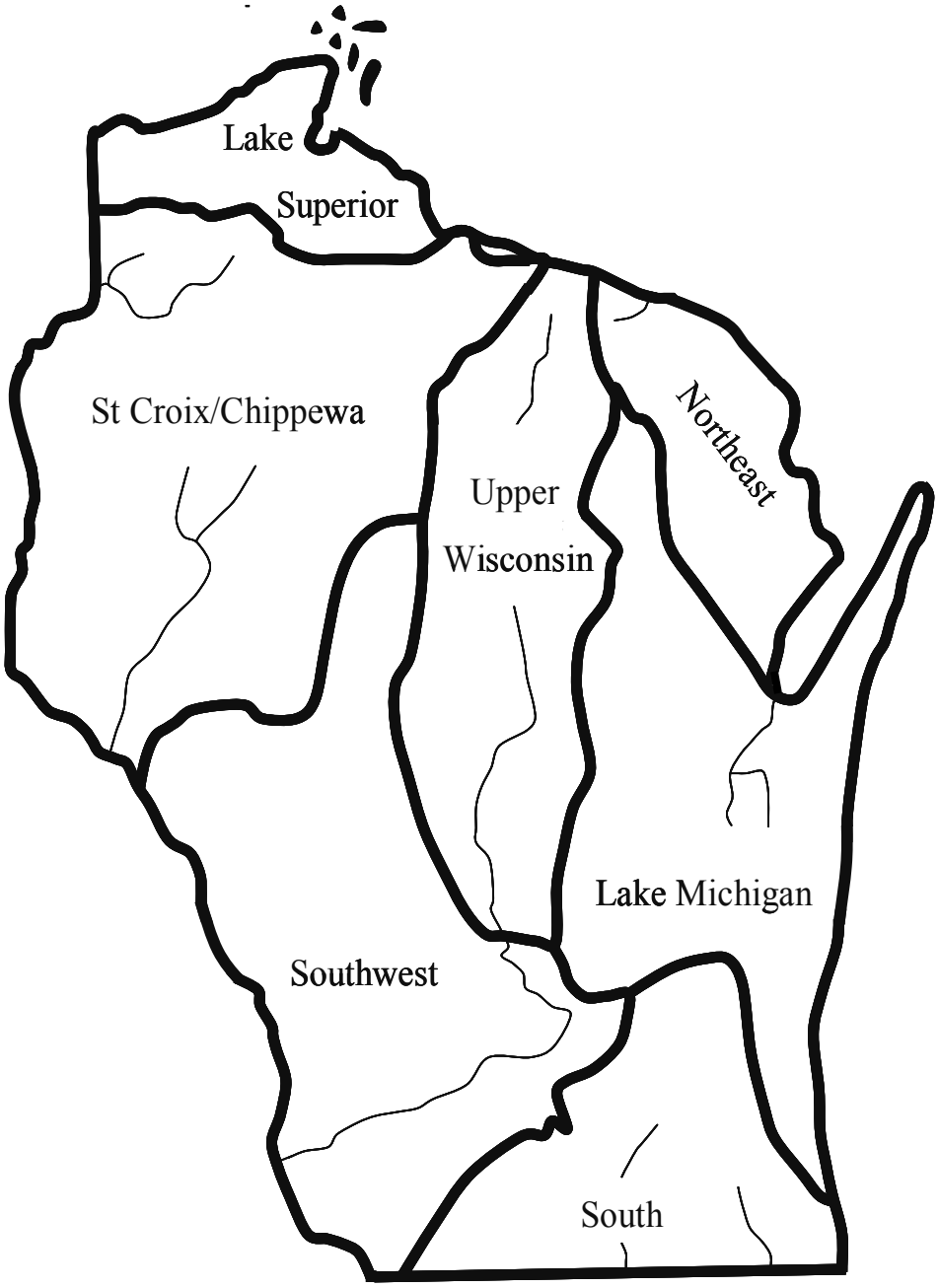

Figure 1.1. Seven genetic management zones recommended by Fields and Philipp (1998) to conserve genetic structure of brook trout in Wisconsin.

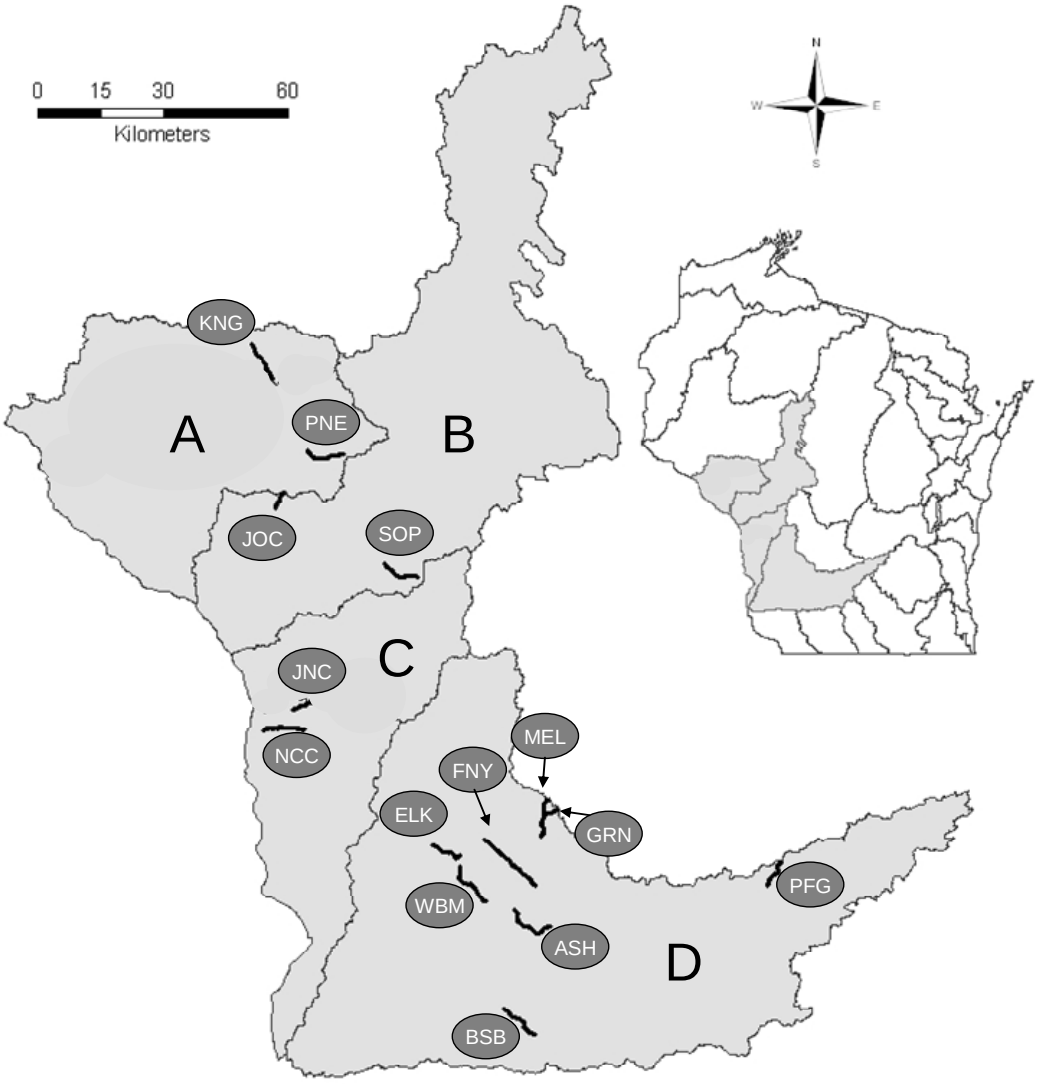

Figure 1.2. Map of Wisconsin's southwest GMZ for brook trout. The 14 populations sampled for this study region and the boundaries of the four major drainage basins they reside in are shown. The major drainage basins are identified as A = Buffalo-Trempealeau Rivers, B = Black River, C = Bad Axe-La Crosse Rivers, and D = Lower Wisconsin River.

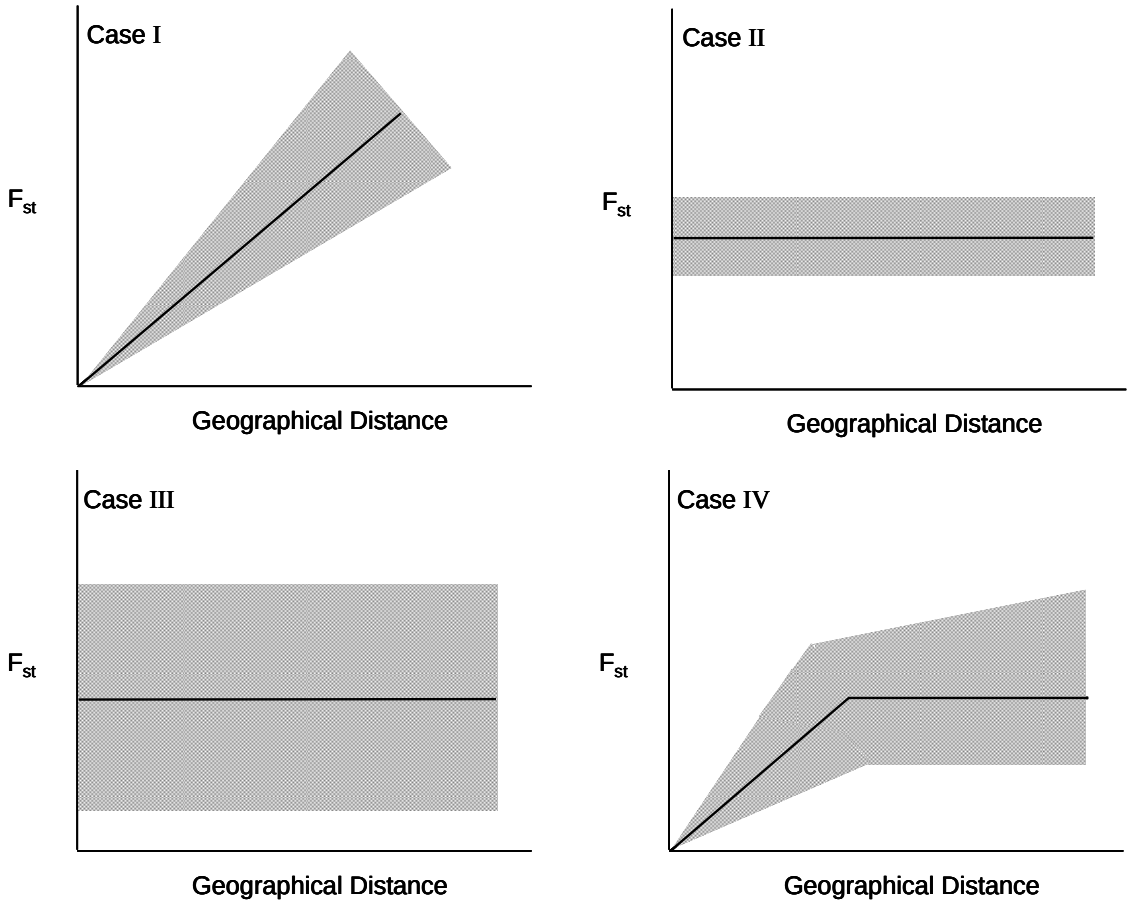

Figure 1.3. Potential relationships between genetic differentiation and geographical distances based on regional effects of genetic drift and gene flow. Shaded areas indicate the degree of scatter anticipated in plotted points and the bold line within the shaded area represents the relationship between genetic and geographic distances. Case I represents a regional equilibrium between gene flow and drift. Case II shows the expected pattern if gene flow is more influential than genetic drift throughout the region. Case III represents a situation where genetic drift is more influential than gene flow within the region. Case IV illustrates the expected results if gene flow is more influential at shorter distances and genetic drift more influential at greater geographical distances. Redrawn from Hutchison and Templeton (1999).

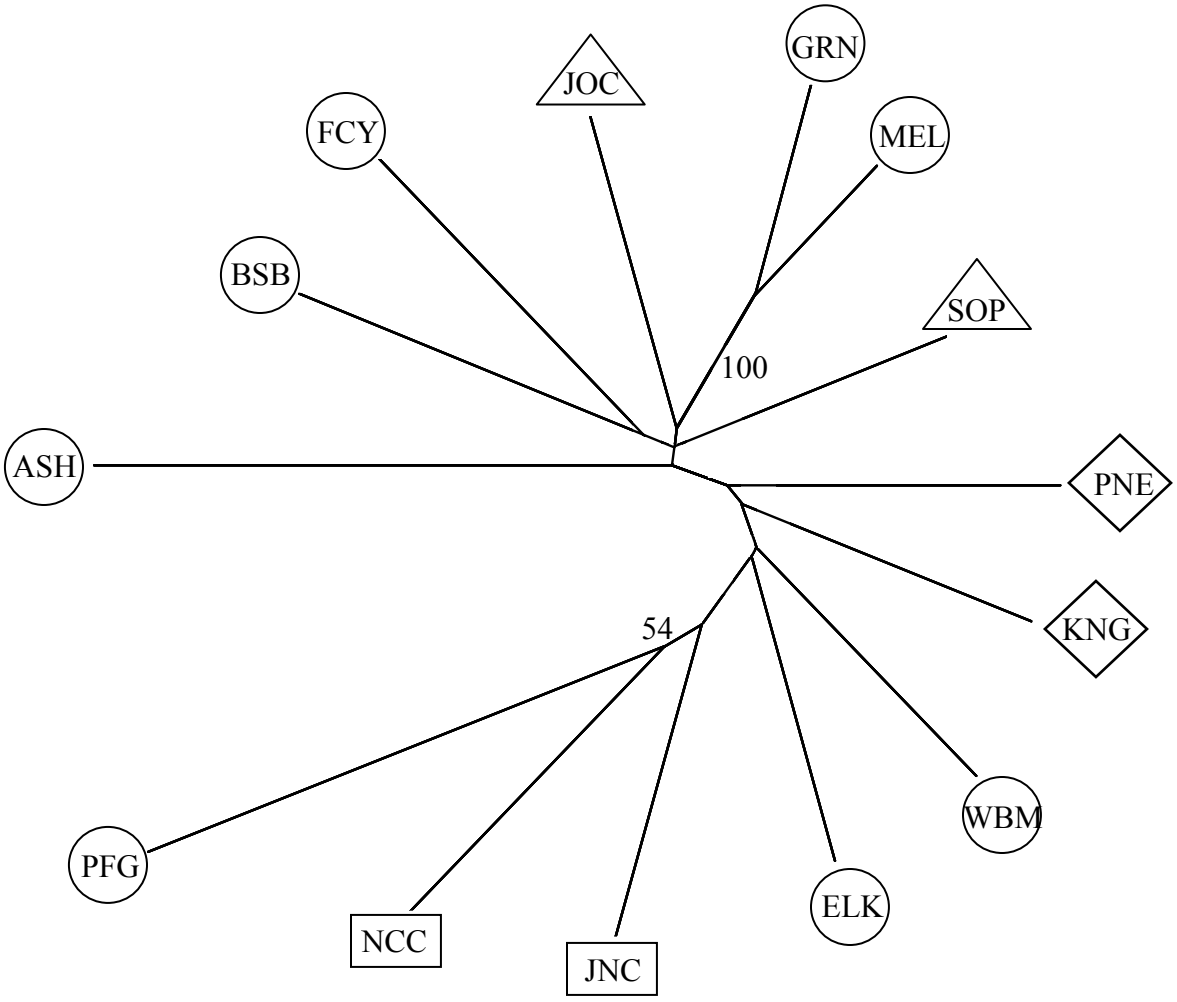

Figure 1.4. Neighbour-joining tree constructed using the Cavalli-Sforza and Edwards' (1967) chord distance showing the genetic relationships of the 14 populations sampled in the southwest GMZ. Bootstrap values are based on 1000 bootstrap pseudo replicates with support greater than 50% shown. The drainage basins that populations were sampled in are indicated by different shapes: Lower Wisconsin River basin = circle; Bad Axe/La Crosse Rivers Basin = square; Buffalo/Trempealeau Rivers Basin = diamond; Black River Basin = triangle.

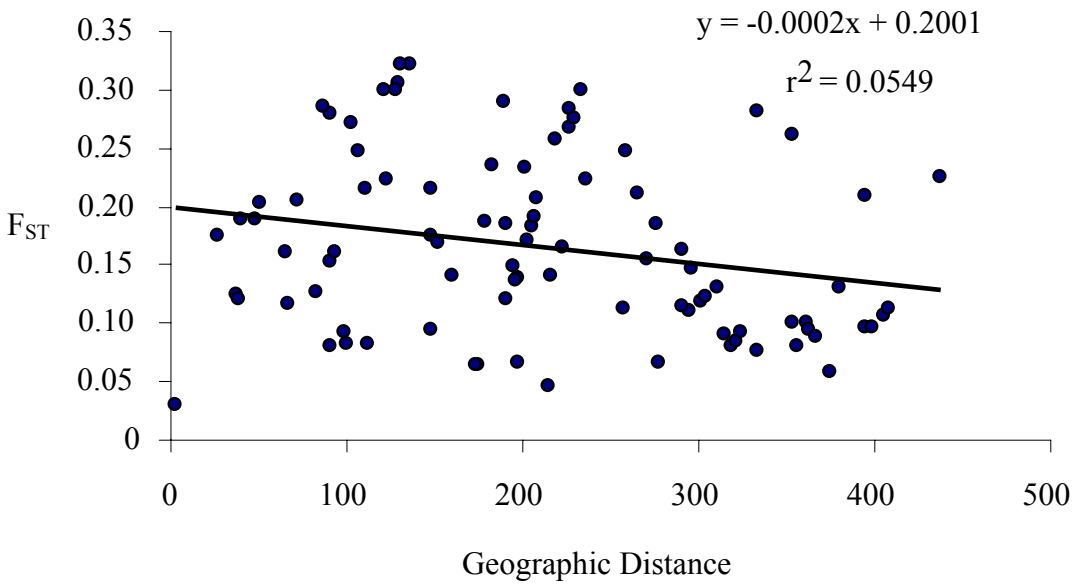

Figure 1.5. Test of isolation by distance (IBD) pairwise F_{ST} values plotted against pairwise geographical distances (km) for all sampled brook trout populations within the southwest GMZ. A Mantel test showed no significant correlation between genetic divergences and geographical distances (p = 0.97).

Appendix 1.1. Locus-specific genetic diversity measures for 14 sampled populations within the southwest GMZ including sample size at each locus (*n*), allelic diversity (*A*), allelic richness (A_r), most frequently observed allele (*S*) and its frequency (*F*), observed heterozygosity (H_o), and expected heterozygosity (H_e). Overall sample sizes are in parentheses under population abbreviations.

Locus		ASH (60)	BSB (62)	ELK (71)	FCY (60)	GRN (50)	JNC (51)	JOC (52)	KNG (50)	MEL (61)	NCC (51)	PFG (60)	PNE (51)	SOP (63)	WBM (60)
Sfo 18	*n*	60	62	71	60	50	51	51	49	60	51	60	50	63	60
	A	5.00	6.00	6.00	5.00	5.00	5.00	3.00	6.00	5.00	4.00	3.00	7.00	5.00	6.00
	A_r	5.00	5.71	5.62	5.00	4.88	4.73	2.86	5.90	4.73	3.98	3.00	6.87	4.97	5.73
	S	183	183	177	177	181	183	177	183	181	183	173	177	177	183
	F	0.26	0.40	0.32	0.44	0.34	0.85	0.96	0.48	0.35	0.77	0.39	0.41	0.47	0.58
	H_o	0.73	0.73	0.76	0.75	0.68	0.27	0.08	0.67	0.8	0.43	0.62	0.78	0.71	0.67
	H_e	0.79	0.74	0.76	0.7	0.74	0.27	0.08	0.71	0.73	0.38	0.66	0.73	0.67	0.62
Sfo 28	*n*	60	61	66	59	48	51	52	49	61	49	59	51	60	59
	A	4.00	6.00	6.00	7.00	4.00	4.00	9.00	6.00	4.00	4.00	2.00	10.00	7.00	6.00
	A_r	4.00	5.98	6.00	6.43	3.92	4.00	8.84	5.89	3.92	3.80	1.98	9.94	6.91	6.00
	S	178	178	182	178	190	170	178	190	190	182	182	190	190	178
	F	0.51	0.39	0.42	0.31	0.64	0.35	0.30	0.51	0.75	0.89	0.98	0.38	0.36	0.37
	H_o	0.58	0.69	0.71	0.8	0.63	0.71	0.9	0.73	0.44	0.18	0.05	0.75	0.72	0.76
	H_e	0.65	0.75	0.75	0.75	0.53	0.74	0.82	0.66	0.41	0.21	0.05	0.8	0.75	0.76
Sfo 52	*n*	60	62	71	60	50	51	51	44	60	51	60	51	63	60
	A	6.00	7.00	7.00	7.00	4.00	4.00	6.00	6.00	4.00	4.00	4.00	7.00	8.00	8.00
	A_r	5.46	6.97	6.99	6.73	4.00	3.86	5.85	6.00	4.00	4.00	4.00	6.71	7.31	7.64
	S	225	219	219	225	225	219	225	225	225	219/225	225	219	219	227
	F	0.35	0.36	0.39	0.48	0.59	0.74	0.46	0.53	0.50	0.35	0.48	0.35	0.41	0.33
	H_o	0.68	0.73	0.75	0.6	0.56	0.47	0.71	0.55	0.67	0.53	0.67	0.73	0.67	0.7
	H_e	0.68	0.75	0.75	0.71	0.58	0.43	0.69	0.61	0.67	0.7	0.64	0.74	0.73	0.71

Appendix 1.1. Continued.

Locus		ASH (60)	BSB (62)	ELK (71)	FCY (60)	GRN (50)	JNC (51)	JOC (52)	KNG (50)	MEL (61)	NCC (51)	PFG (60)	PNE (51)	SOP (63)	WBM (60)
Sfo 75	n	60	62	71	60	49	51	51	45	60	51	60	51	63	60
	A	4.00	8.00	9.00	8.00	6.00	7.00	9.00	10.00	6.00	7.00	5.00	10.00	12.00	11.00
	A_r	3.93	7.99	8.22	7.73	5.99	6.83	8.43	10.00	5.73	6.70	5.00	9.84	11.19	9.86
	S	179	179	179	223	179	203	179	179	203	179	203	203	203	179
	F	0.67	0.29	0.63	0.28	0.35	0.34	0.48	0.36	0.39	0.78	0.67	0.34	0.27	0.45
	H_o	0.50	0.76	0.51	0.87	0.78	0.63	0.73	0.78	0.82	0.27	0.47	0.82	0.81	0.78
	H_e	0.50	0.81	0.59	0.81	0.75	0.72	0.66	0.82	0.71	0.37	0.52	0.82	0.83	0.75
Sfo 86	n	60	62	69	60	50	51	52	49	61	49	59	51	63	60
	A	5.00	4.00	5.00	4.00	5.00	5.00	5.00	5.00	5.00	3.00	3.00	5.00	5.00	5.00
	A_r	4.96	4.00	5.00	4.00	5.00	4.86	4.85	4.89	5.00	3.00	3.00	5.00	5.00	5.00
	S	104	116	104	104	122	104	116	104	116	104	104	104/116	116	104
	F	0.79	0.42	0.65	0.48	0.39	0.82	0.34	0.56	0.37	0.78	0.64	0.33	0.48	0.68
	H_o	0.35	0.52	0.58	0.73	0.80	0.29	0.77	0.73	0.72	0.31	0.58	0.73	0.57	0.53
	H_e	0.37	0.69	0.55	0.68	0.75	0.31	0.75	0.59	0.75	0.38	0.52	0.74	0.70	0.52
Sfo 88	n	60	61	66	59	48	51	52	49	61	49	59	51	60	59
	A	4.00	6.00	5.00	5.00	4.00	5.00	4.00	6.00	5.00	5.00	3.00	6.00	6.00	6.00
	A_r	3.98	5.97	5.00	4.92	2.92	4.86	2.98	6.00	4.65	4.90	2.75	6.00	5.65	6.00
	S	186	192	183	192	192	186	192	186	192	186	186	186/192	186	192
	F	0.64	0.73	0.33	0.75	0.77	0.54	0.59	0.41	0.69	0.78	0.89	0.39	0.42	0.25
	H_o	0.57	0.46	0.76	0.42	0.38	0.61	0.40	0.76	0.43	0.33	0.14	0.65	0.55	0.85
	H_e	0.53	0.45	0.76	0.41	0.36	0.61	0.51	0.73	0.47	0.38	0.20	0.68	0.67	0.81

Appendix 1.1. Continued.

Locus		ASH (60)	BSB (62)	ELK (71)	FCY (60)	GRN (50)	JNC (51)	JOC (52)	KNG (50)	MEL (61)	NCC (51)	PFG (60)	PNE (51)	SOP (63)	WBM (60)
Sfo 91	n	60	62	62	57	49	51	49	46	49	50	60	51	62	60
	A	6.00	8.00	7.00	9.00	8.00	5.00	10.00	9.00	9.00	5.00	5.00	13.00	10.00	11.00
	A_r	5.66	7.71	6.89	8.98	7.98	5.00	9.86	8.87	8.79	4.99	4.73	12.45	9.53	10.84
	S	237	261	241	225	237	241	237	237	237	237	265	233	225	233
	F	0.46	0.36	0.36	0.34	0.39	0.76	0.44	0.37	0.47	0.46	0.48	0.22	0.36	0.38
	H_o	0.70	0.79	0.44	0.74	0.73	0.41	0.71	0.70	0.80	0.64	0.72	0.78	0.68	0.78
	H_e	0.69	0.76	0.76	0.81	0.78	0.42	0.75	0.78	0.71	0.67	0.66	0.87	0.77	0.81
Sfo 113	n	60	62	71	60	50	51	51	50	58	51	60	50	63	60
	A	5.00	7.00	7.00	4.00	4.00	5.00	7.00	7.00	6.00	5.00	3.00	6.00	8.00	5.00
	A_r	5.00	6.89	7.00	4.00	4.00	4.85	6.69	6.87	5.94	5.00	3.00	6.00	7.52	5.00
	S	146	146	134	146	140	155	146	146	146	155	155	146	146	146
	F	0.31	0.48	0.30	0.73	0.46	1.55	0.59	0.60	0.41	0.62	0.50	0.54	0.50	0.40
	H_o	0.73	0.73	0.80	0.43	0.72	0.55	0.53	0.60	0.78	0.57	0.63	0.72	0.68	0.75
	H_e	0.76	0.71	0.80	0.44	0.70	0.53	0.56	0.60	0.72	0.58	0.62	0.66	0.70	0.73
Sfo 115	n	60	62	71	60	50	51	51	50	60	51	60	50	63	60
	A	5.00	7.00	6.00	7.00	10.00	7.00	8.00	13.00	8.00	7.00	5.00	12.00	13.00	8.00
	A_r	4.73	7.00	5.89	6.71	9.40	6.85	7.73	12.72	7.51	6.84	5.00	12.49	11.97	7.86
	S	309	241	241	309	241	241	313	241	233	309	309	233	247	241
	F	0.44	0.24	0.32	0.43	0.36	0.34	0.30	0.27	0.28	0.47	0.25	0.30	0.51	0.36
	H_o	0.72	0.85	0.73	0.67	0.72	0.71	0.80	0.78	0.73	0.51	0.78	0.88	0.67	0.77
	H_e	0.71	0.83	0.75	0.73	0.77	0.74	0.80	0.86	0.64	0.70	0.78	0.81	0.72	0.78

Appendix 1.1. Continued.

Locus		ASH (60)	BSB (62)	ELK (71)	FCY (60)	GRN (50)	JNC (51)	JOC (52)	KNG (50)	MEL (61)	NCC (51)	PFG (60)	PNE (51)	SOP (63)	WBM (60)
Sfo 24	n	60	62	69	60	49	51	52	49	61	51	59	51	62	60
	A	2.00	4.00	3.00	4.00	3.00	3.00	3.00	4.00	4.00	3.00	3.00	4.00	4.00	3.00
	A_r	2.00	4.00	3.00	4.00	3.00	3.00	3.00	3.99	3.72	3.00	3.00	4.00	3.92	3.98
	S	121	118	118	118	121	121	121	121	121	121	121	118	118	118
	F	0.71	0.69	0.60	0.57	0.44	0.65	0.59	0.50	0.45	0.83	0.69	0.50	0.49	0.43
	H_o	0.48	0.44	0.48	0.62	0.69	0.45	0.48	0.57	0.67	0.20	0.51	0.71	0.60	0.68
	H_e	0.42	0.49	0.51	0.61	0.66	0.48	0.57	0.57	0.63	0.29	0.48	0.61	0.61	0.66
Sfo 38	n	60	62	69	60	49	51	52	49	61	51	59	51	63	60
	A	3.00	4.00	3.00	3.00	4.00	3.00	4.00	4.00	4.00	3.00	2.00	4.00	4.00	4.00
	A_r	3.00	3.98	3.00	3.00	4.00	3.00	4.00	4.00	4.00	3.00	2.00	4.00	4.00	4.00
	S	149	149	146	146	149	146	149	146	149	146	146	146	149	146
	F	0.79	0.46	0.83	0.58	0.46	0.70	0.50	0.32	0.54	0.65	0.65	0.37	0.38	0.48
	H_o	0.32	0.68	0.29	0.55	0.69	0.41	0.58	0.65	0.67	0.65	0.42	0.76	0.84	0.73
	H_e	0.35	0.64	0.29	0.52	0.69	0.46	0.57	0.70	0.63	0.50	0.46	0.72	0.72	0.64
Sfo 12	n	59	61	68	59	49	51	51	49	61	50	59	51	62	60
	A	4.00	7.00	6.00	6.00	4.00	6.00	6.00	7.00	4.00	4.00	3.00	8.00	8.00	7.00
	A_r	3.98	5.65	6.00	5.87	4.00	6.00	5.86	7.00	3.98	4.00	3.00	7.83	7.70	6.73
	S	271	273	197	273	273	275	273	253	273	271	253	273	197	253
	F	0.51	0.46	0.29	0.48	0.44	0.36	0.32	0.34	0.61	0.59	0.64	0.37	0.44	0.41
	H_o	0.59	0.64	0.85	0.56	0.69	0.75	0.71	0.78	0.66	0.50	0.61	0.73	0.68	0.72
	H_e	0.63	0.68	0.82	0.64	0.70	0.78	0.75	0.79	0.56	0.58	0.53	0.73	0.72	0.76

Appendix 1.2. Allele frequencies and sample sizes (*n*) for all 12 microsatellites loci within each brook trout population sampled in southwest GMZ populations.

Locus	Size (bp)	ASH	BSB	ELK	FCY	GRN	JNC	JOC	KNG	MEL	NCC	PFG	PNE	SOP	WBM
Sfo 18	(*n*)	60	62	71	60	50	51	51	49	60	51	60	50	63	60
	173	0.183	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.392	0.000	0.000	0.075
	175	0.000	0.000	0.000	0.000	0.220	0.039	0.000	0.010	0.250	0.029	0.000	0.040	0.024	0.000
	177	0.233	0.242	0.317	0.442	0.280	0.000	0.961	0.184	0.292	0.000	0.000	0.410	0.468	0.175
	179	0.000	0.000	0.148	0.000	0.000	0.000	0.000	0.102	0.000	0.186	0.000	0.020	0.000	0.000
	181	0.083	0.145	0.049	0.250	0.340	0.010	0.000	0.082	0.350	0.000	0.000	0.080	0.238	0.000
	183	0.258	0.403	0.296	0.217	0.150	0.853	0.029	0.480	0.100	0.765	0.250	0.250	0.238	0.583
	185	0.000	0.089	0.183	0.058	0.000	0.000	0.010	0.000	0.008	0.000	0.358	0.190	0.032	0.008
	189	0.000	0.113	0.000	0.033	0.010	0.088	0.000	0.143	0.000	0.020	0.000	0.010	0.000	0.075
	191	0.242	0.008	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083
Sfo 28	(*n*)	60	61	66	59	48	51	52	49	61	49	59	51	60	59
	170	0.000	0.025	0.114	0.000	0.000	0.353	0.010	0.092	0.000	0.092	0.000	0.069	0.000	0.034
	178	0.508	0.393	0.114	0.305	0.240	0.176	0.298	0.245	0.189	0.010	0.025	0.029	0.258	0.373
	180	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.000	0.000	0.000	0.000	0.176	0.000	0.000
	182	0.208	0.230	0.417	0.119	0.000	0.265	0.240	0.122	0.000	0.888	0.975	0.098	0.225	0.203
	186	0.000	0.000	0.098	0.246	0.000	0.000	0.135	0.020	0.016	0.010	0.000	0.108	0.042	0.229
	190	0.050	0.180	0.220	0.297	0.635	0.206	0.125	0.510	0.746	0.000	0.000	0.382	0.358	0.102
	194	0.000	0.000	0.000	0.008	0.010	0.000	0.058	0.000	0.000	0.000	0.000	0.078	0.025	0.000
	198	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.020	0.000	0.000
	202	0.233	0.074	0.038	0.008	0.115	0.000	0.029	0.000	0.049	0.000	0.000	0.020	0.075	0.059
	206	0.000	0.098	0.000	0.017	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.020	0.017	0.000

Appendix 1.2. Continued.

Locus	Size (bp)	ASH	BSB	ELK	FCY	GRN	JNC	JOC	KNG	MEL	NCC	PFG	PNE	SOP	WBM
Sfo 12	(*n*)	59	61	68	59	49	51	51	49	61	50	59	51	62	60
	197	0.280	0.295	0.294	0.356	0.245	0.000	0.265	0.031	0.230	0.000	0.000	0.020	0.444	0.083
	249	0.000	0.000	0.000	0.000	0.000	0.039	0.000	0.092	0.000	0.040	0.000	0.049	0.040	0.000
	253	0.186	0.008	0.125	0.076	0.000	0.196	0.029	0.337	0.000	0.260	0.644	0.324	0.032	0.408
	265	0.000	0.098	0.000	0.017	0.194	0.000	0.000	0.000	0.139	0.000	0.000	0.000	0.008	0.008
	269	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
	271	0.508	0.016	0.169	0.051	0.122	0.157	0.108	0.173	0.025	0.590	0.000	0.176	0.258	0.075
	273	0.025	0.459	0.169	0.483	0.439	0.088	0.324	0.092	0.607	0.110	0.000	0.373	0.105	0.192
	275	0.000	0.123	0.125	0.000	0.000	0.363	0.265	0.235	0.000	0.000	0.169	0.029	0.073	0.100
	277	0.000	0.000	0.118	0.017	0.000	0.157	0.010	0.041	0.000	0.000	0.000	0.000	0.040	0.133
	279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
	295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.186	0.000	0.000	0.000
Sfo 75	(*n*)	60	62	71	60	49	51	51	45	60	51	60	51	63	60
	175	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000
	179	0.667	0.290	0.627	0.250	0.347	0.245	0.480	0.356	0.342	0.784	0.067	0.088	0.238	0.450
	183	0.000	0.000	0.007	0.058	0.000	0.039	0.010	0.000	0.000	0.029	0.000	0.000	0.024	0.008
	187	0.000	0.032	0.063	0.125	0.000	0.020	0.069	0.022	0.000	0.118	0.000	0.000	0.087	0.058
	191	0.000	0.000	0.000	0.000	0.020	0.000	0.010	0.000	0.008	0.000	0.000	0.020	0.056	0.008
	199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.088	0.000	0.108
	203	0.017	0.242	0.056	0.142	0.316	0.343	0.324	0.133	0.392	0.010	0.667	0.343	0.270	0.100
	207	0.000	0.113	0.042	0.033	0.092	0.000	0.069	0.067	0.150	0.010	0.000	0.000	0.159	0.108
	211	0.000	0.177	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.039	0.071	0.000
	215	0.000	0.000	0.000	0.000	0.173	0.010	0.010	0.156	0.042	0.020	0.075	0.137	0.016	0.125
	219	0.000	0.000	0.000	0.008	0.000	0.324	0.000	0.000	0.000	0.000	0.158	0.147	0.000	0.000
	223	0.000	0.032	0.106	0.283	0.000	0.020	0.020	0.056	0.000	0.029	0.000	0.078	0.008	0.008
	227	0.242	0.048	0.028	0.000	0.000	0.000	0.010	0.033	0.067	0.000	0.000	0.000	0.048	0.008
	231	0.000	0.000	0.007	0.000	0.051	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.017
	235	0.075	0.065	0.000	0.100	0.000	0.000	0.000	0.089	0.000	0.000	0.000	0.049	0.016	0.000
	239	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000
	247	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000

Appendix 1.2. Continued.

Locus	Size (bp)	ASH	BSB	ELK	FCY	GRN	JNC	JOC	KNG	MEL	NCC	PFG	PNE	SOP	WBM
Sfo 86	(*n*)	60	62	69	60	50	51	52	49	61	49	59	51	63	60
	104	0.783	0.056	0.645	0.475	0.210	0.824	0.212	0.561	0.246	0.776	0.636	0.333	0.214	0.675
	113	0.025	0.234	0.138	0.258	0.050	0.078	0.010	0.010	0.172	0.122	0.000	0.059	0.095	0.033
	116	0.050	0.419	0.051	0.150	0.210	0.049	0.337	0.286	0.369	0.000	0.000	0.333	0.476	0.142
	119	0.117	0.290	0.051	0.117	0.140	0.010	0.221	0.020	0.172	0.000	0.263	0.078	0.056	0.058
	122	0.025	0.000	0.116	0.000	0.390	0.039	0.221	0.122	0.041	0.102	0.102	0.196	0.159	0.092
Sfo 88	(*n*)	60	61	66	59	48	51	52	49	61	49	59	51	60	59
	174	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	183	0.025	0.082	0.326	0.000	0.000	0.010	0.000	0.041	0.057	0.031	0.000	0.029	0.025	0.203
	186	0.642	0.033	0.295	0.178	0.219	0.539	0.394	0.408	0.230	0.776	0.890	0.392	0.358	0.220
	189	0.225	0.057	0.083	0.000	0.010	0.294	0.019	0.051	0.016	0.010	0.102	0.049	0.175	0.153
	192	0.108	0.730	0.167	0.746	0.771	0.127	0.587	0.276	0.689	0.153	0.008	0.392	0.417	0.254
	195	0.000	0.025	0.129	0.025	0.000	0.029	0.000	0.163	0.008	0.031	0.000	0.098	0.008	0.102
	198	0.000	0.074	0.000	0.017	0.000	0.000	0.000	0.061	0.000	0.000	0.000	0.039	0.017	0.068
Sfo 113	(*n*)	60	62	71	60	50	51	51	50	58	51	60	50	63	60
	128	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.010	0.000	0.000	0.000	0.000	0.000	0.000
	134	0.000	0.153	0.303	0.058	0.000	0.020	0.010	0.040	0.017	0.167	0.000	0.130	0.087	0.083
	137	0.225	0.194	0.070	0.142	0.160	0.284	0.049	0.180	0.060	0.127	0.192	0.150	0.159	0.183
	140	0.250	0.024	0.042	0.067	0.460	0.000	0.000	0.000	0.103	0.000	0.308	0.050	0.071	0.000
	143	0.000	0.000	0.049	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.008	0.000
	146	0.308	0.476	0.254	0.733	0.180	0.010	0.588	0.600	0.414	0.059	0.000	0.540	0.500	0.400
	149	0.033	0.097	0.106	0.000	0.200	0.059	0.304	0.060	0.302	0.029	0.000	0.070	0.016	0.275
	152	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	155	0.183	0.040	0.176	0.000	0.000	0.627	0.020	0.090	0.103	0.618	0.500	0.060	0.143	0.058
	158	0.000	0.016	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.016	0.000

Appendix 1.2. Continued.

Locus	Size (bp)	ASH	BSB	ELK	FCY	GRN	JNC	JOC	KNG	MEL	NCC	PFG	PNE	SOP	WBM
Sfo 91	(*n*)	60	62	62	57	49	51	49	46	49	50	60	51	62	60
	217	0.000	0.000	0.000	0.061	0.000	0.000	0.000	0.011	0.000	0.020	0.042	0.010	0.000	0.025
	221	0.000	0.089	0.000	0.070	0.020	0.000	0.000	0.043	0.000	0.030	0.000	0.010	0.000	0.000
	225	0.217	0.000	0.016	0.342	0.020	0.000	0.051	0.141	0.041	0.000	0.008	0.127	0.290	0.017
	229	0.000	0.000	0.000	0.000	0.051	0.000	0.000	0.054	0.010	0.000	0.000	0.000	0.000	0.017
	233	0.000	0.048	0.153	0.000	0.143	0.088	0.020	0.207	0.031	0.290	0.000	0.216	0.016	0.375
	237	0.458	0.298	0.105	0.158	0.388	0.049	0.439	0.370	0.469	0.460	0.267	0.167	0.355	0.142
	241	0.233	0.056	0.355	0.000	0.000	0.755	0.082	0.152	0.041	0.200	0.000	0.147	0.032	0.117
	245	0.008	0.008	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.075
	249	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	253	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	257	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.033
	261	0.067	0.363	0.073	0.079	0.184	0.078	0.143	0.011	0.194	0.000	0.000	0.118	0.153	0.042
	265	0.000	0.000	0.274	0.000	0.133	0.000	0.194	0.000	0.184	0.000	0.475	0.029	0.056	0.075
	269	0.000	0.040	0.000	0.175	0.000	0.000	0.000	0.000	0.010	0.000	0.208	0.069	0.016	0.000
	273	0.000	0.097	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.040	0.000
	277	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	281	0.000	0.000	0.000	0.061	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	283	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	285	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.039	0.032	0.083
	289	0.000	0.000	0.000	0.000	0.061	0.000	0.020	0.000	0.020	0.000	0.000	0.010	0.000	0.000
	293	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.049	0.000	0.000
Sfo 38	(*n*)	60	62	69	60	49	51	52	49	61	51	59	51	63	60
	143	0.167	0.024	0.051	0.042	0.102	0.000	0.038	0.143	0.230	0.275	0.347	0.294	0.119	0.042
	146	0.042	0.347	0.833	0.583	0.194	0.696	0.423	0.418	0.074	0.647	0.653	0.373	0.198	0.475
	149	0.792	0.460	0.116	0.375	0.459	0.225	0.500	0.316	0.541	0.078	0.000	0.216	0.381	0.150
	152	0.000	0.169	0.000	0.000	0.245	0.078	0.038	0.122	0.156	0.000	0.000	0.118	0.302	0.333

Appendix 1.2. Continued.

Locus	Size (bp)	ASH	BSB	ELK	FCY	GRN	JNC	JOC	KNG	MEL	NCC	PFG	PNE	SOP	WBM
Sfo 115	(*n*)	60	62	71	60	50	51	51	50	60	51	60	50	63	60
	233	0.000	0.226	0.000	0.008	0.190	0.157	0.245	0.000	0.283	0.000	0.000	0.300	0.071	0.000
	239	0.167	0.000	0.000	0.000	0.000	0.010	0.000	0.110	0.017	0.000	0.242	0.010	0.024	0.000
	241	0.167	0.242	0.317	0.075	0.360	0.343	0.010	0.270	0.517	0.049	0.000	0.150	0.071	0.358
	243	0.000	0.000	0.204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
	245	0.000	0.056	0.303	0.042	0.080	0.088	0.000	0.060	0.008	0.196	0.217	0.060	0.032	0.200
	247	0.217	0.161	0.021	0.233	0.250	0.000	0.167	0.200	0.117	0.000	0.000	0.040	0.508	0.017
	253	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.008	0.000
	289	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	301	0.000	0.000	0.000	0.000	0.000	0.049	0.000	0.020	0.000	0.216	0.000	0.000	0.000	0.067
	305	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.008	0.000
	309	0.442	0.097	0.134	0.425	0.010	0.333	0.088	0.090	0.017	0.471	0.250	0.270	0.032	0.208
	313	0.008	0.000	0.021	0.192	0.000	0.000	0.304	0.080	0.000	0.039	0.225	0.070	0.056	0.042
	317	0.000	0.000	0.000	0.000	0.010	0.000	0.078	0.020	0.000	0.000	0.000	0.020	0.063	0.000
	321	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.103	0.092
	325	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.010	0.000	0.000	0.000	0.000
	329	0.000	0.129	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.010	0.000	0.000
	333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000
	337	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.067	0.010	0.008	0.000
	341	0.000	0.089	0.000	0.000	0.070	0.020	0.098	0.010	0.025	0.000	0.000	0.040	0.000	0.000
	353	0.000	0.000	0.000	0.025	0.010	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
	357	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sfo 24	(*n*)	60	62	69	60	49	51	52	49	61	51	59	51	62	60
	115	0.000	0.145	0.000	0.058	0.276	0.000	0.144	0.020	0.156	0.000	0.186	0.088	0.129	0.025
	118	0.292	0.694	0.601	0.567	0.286	0.324	0.269	0.429	0.385	0.049	0.127	0.500	0.492	0.433
	121	0.708	0.113	0.355	0.217	0.439	0.647	0.587	0.500	0.451	0.833	0.686	0.363	0.363	0.333
	124	0.000	0.048	0.043	0.158	0.000	0.029	0.000	0.051	0.008	0.118	0.000	0.049	0.016	0.208

Appendix 1.2. Continued.

Locus	Size (bp)	ASH	BSB	ELK	FCY	GRN	JNC	JOC	KNG	MEL	NCC	PFG	PNE	SOP	WBM
Sfo 52	(*n*)	60	62	71	60	50	51	51	44	60	51	60	51	63	60
	195	0.192	0.000	0.035	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025
	203	0.000	0.000	0.000	0.200	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.008	0.008
	207	0.408	0.161	0.000	0.108	0.190	0.000	0.000	0.000	0.208	0.000	0.000	0.020	0.048	0.017
	211	0.000	0.000	0.141	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.098	0.016	0.000
	215	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.042
	219	0.000	0.355	0.394	0.133	0.190	0.735	0.078	0.330	0.192	0.353	0.108	0.353	0.413	0.333
	221	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
	223	0.000	0.024	0.000	0.000	0.000	0.000	0.010	0.034	0.000	0.059	0.000	0.010	0.008	0.000
	225	0.350	0.306	0.077	0.475	0.590	0.127	0.461	0.534	0.500	0.353	0.483	0.304	0.262	0.142
	227	0.008	0.056	0.268	0.042	0.000	0.127	0.167	0.023	0.100	0.235	0.333	0.206	0.071	0.400
	229	0.000	0.032	0.049	0.033	0.000	0.000	0.020	0.068	0.000	0.000	0.075	0.000	0.000	0.000
	231	0.033	0.065	0.035	0.000	0.030	0.010	0.265	0.000	0.000	0.000	0.000	0.000	0.175	0.000
	235	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Chapter II:

The Genetic Impacts of Broodstock Sampling Strategies on a wild brook trout population.

Abstract-In 1995, the Wisconsin Department of Natural Resources began a wild brook trout stocking program to improve the quality of hatchery-reared trout and subsequently supplement brook trout populations in the southwestern region of Wisconsin. In this program, wild adults are captured from Ash Creek (Richland Co.) in the fall, transferred to a hatchery, spawned, and returned to their home stream in the late fall/early winter. The number of wild fish collected for hatchery propagation is a critical decision that can influence the levels of genetic diversity and the demographic characteristics of the propagated fish and Ash Creek's remaining in-stream population. Since ≥50% of Ash Creek's adult population is removed annually to meet hatchery production goals. The removal of this many fish could have genetic (i.e., increased rates of genetic drift and/or inbreeding), and demographic (i.e., reduction in the population's fecundity and adult size structure) consequences that would threaten the viability and sustainability of Ash Creek's population. To evaluate whether Ash Creek has experienced any discernible genetic and/or demographic impacts, levels of genetic diversity within Ash Creek were characterized using 12 microsatellite loci and size data (length and weight) was recorded for collected adult brook trout. From this data, a series of four comparisons were conducted: 1) the in-stream adults were compared to the in-hatchery adults to determine if the proportion of adult trout removed resulted in a bias in genetic or size (total length and weight) diversity, 2) the in-stream adults were compared to their respective in-stream young-of-year (YOY) counterparts to determine if the reductions to the size Ash Creek's in-stream population resulted in reductions to levels of genetic diversity with in the in-

stream YOY populations, 3) the in-stream YOY in 2005 and 2006 were compared to determine if the difference in the proportion of fish removed from Ash Creek resulted in differential reproductive contribution to the in-stream YOY populations, and 4) the in-stream and in-hatchery YOY components were compared to determine if differential reproductive contributions existed among these YOY populations. To determine if annual broodstock removals have resulted in any realized or potential detrimental impacts to levels of genetic diversity in Ash Creek's population, genetic and morphological (length and weight) measurements were taken from the in-hatchery (wild hatchery broodstock) and in-stream adults (post-broodstock removals), and their in-hatchery and in-stream young-of-year (YOY) counterparts over two years. The genetic characteristics within each population component were characterized and compared using 12 microsatellite DNA loci. Levels of genetic diversity among adult components (in-hatchery and in-stream trout) did not differ over two years indicating that despite the large proportion of adult fish removed (50 to >80% per year), the yearly in-stream adults maintained levels of genetic diversity. However, a size bias was observed with larger fish being used as broodfish. The broodstock removals resulted in no reductions in levels of genetic diversity within or among the yearly in-stream YOY components. Levels of genetic diversity among the in-hatchery and in-stream YOY components were similar, although, the effective number of breeders that produced the in-hatchery YOY components was approximately seven times larger than that of the in-stream YOY components. This skew likely reflects a combination of the reduction in the size of the number of in-stream breeders and a lower variance in family size within the hatchery. These data suggest that current broodstock selection strategies have had no detectable

short-term impacts on genetic diversity levels within Ash Creek. Several results of this study raise concerns that the long-term impact of such strategies will be detrimental. The removal of larger brook trout and subsequent reductions in the mean body size of Ash Creek's in-stream breeding population will have negative consequences to the reproductive potential (lower mean fecundity) and genetic integrity (lower mean number of breeders).

INTRODUCTION

In 1995, the Wisconsin Department of Natural Resources (WDNR) began a wild brook trout (*Salvelinus fontinalis*) stocking program to improve the quality of hatchery-reared trout and, subsequently, strengthen rehabilitation efforts of brook trout populations in the southwestern region of Wisconsin (Figure 2.1; Mitro 2004). In this program, wild adults are captured from a selected stream in the fall, transferred to a hatchery, spawned, and returned to their home stream in late fall/early winter. The resulting progeny are reared under hatchery conditions simulating their natural environment to maintain their wild characteristics. These conditions include lower rearing density, partially shaded tanks, and automatic feeders to limit human contact. These fish are stocked out as spring or fall fingerlings into brook trout streams that are perceived to have limited natural reproduction (Epifanio and Lindloff 1999; WDNR 1999; Mitro 2004).

Since 2000, Ash Creek (Richland County; Figure 2.1) has served as the sole source of broodstock for the wild brook trout stocking program in southwestern Wisconsin (Mitro 2004). This brook trout population was selected because it is a healthy, naturally self-sustaining population and was assumed to be of sufficient size to provide the gamete production required for the wild trout stocking program (Mitro 2004). The WDNR annually removes ~700 adult brook trout from Ash Creek to provide enough eggs to meet yearly quotas (~198,000 eggs in 2002; Mitro 2004).

The number of fish collected from Ash Creek's population is a critical decision for the wild trout stocking program that can drastically influence the levels of genetic diversity of both the propagated fish and Ash Creek's remaining in-stream population (Miller and Kapuscinski 2003). The conservation and maintenance of genetic diversity

within a managed species is an important consideration in any restoration effort (Frankham et al. 2004). Genetic diversity represents the raw material of adaptive change and evolutionary potential in populations and is positively correlated with population fitness (Reed and Frankham 2003). The sampled broodfish should annually represent the distribution of genetic diversity and life history characteristics (i.e., spawning time, size, age, sex ratio, etc.) found within the source population to optimize levels of genetic variation in hatchery propagated fish. Likewise, it is important to ensure that the remaining source population maintains its original level and distribution of genetic diversity and life history characteristics for its own viability and the long-term sustainability of the current propagation program.

The wild brook trout stocking program's current broodstock collection strategy annually removes close to or greater than 50% of Ash Creek's spawning population. In 2004, an estimated 44% of the adult population was removed for hatchery production but in 2005, it was estimated that 84% of the adult population was removed (Matthew Mitro, WDNR personal communication). As a guideline to conserve genetic diversity levels within brood source populations, Miller and Kapuscinski (2003) recommended removing no more than 50% of the individuals for hatchery production. Despite the return of spent broodfish to Ash Creek, which helps maintain a relatively stable annual adult population size, the current strategy exceeds this limit in some years and is likely restricting the overall reproductive efforts of the in-stream population.

Domestication and inadvertent hatchery selection is a major concern in feral programs such as Wisconsin's wild trout program. Current collection strategies may be size selective toward larger breeding adults as a consequence of the gear bias associated

with backpack electrofishing (Anderson 1995) which could alter Ash Creek population's mating system, overall population fecundity, and adult size structure in subsequent years. Theoretically, returning spent trout back to Ash Creek allows them to contribute to future breeding seasons; alleviating the loss of reproductive potential in any given year. However, this assumes that the reproductive potential of a brook trout is equal across all breeding seasons, and natural recruitment is consistent in any given year. Both of these assumptions are unrealistic for most fish species, including brook trout. Adult male brook trout have been shown to not spawn every year (Vladykov 1956). Furthermore, brook trout are generally found in headwater streams consisting of highly unstable environments resulting demographic fluctuations (Titus and Mosegaard 1992).

As a consequence of the current broodstock strategies, Ash Creek's effective population size (N_e) may be drastically reduced (i.e., ≤50% of its census size) rendering it more vulnerable to genetic drift and the loss of genetic diversity (Hallerman 2003), changing the dynamics and characteristics of the population, and, ultimately, threatening the long-term viability of this population. The genetic consequences of annually removing large proportions of a breeding population include increased rates of genetic drift and/or inbreeding, both of which will eventually lead to a loss of genetic diversity. Genetic drift occurs when progeny fail to exhibit a representative sampling of the overall population's allele frequencies usually due to a small number of breeding individuals. Allele frequencies within a population fluctuate from one generation to the next because of these reproductive sampling errors. The eventual result of genetic drift is the fixation of a single allele at an individual locus and a net loss of genetic variation.

The annual reduction in Ash Creek's adult population size could also result in greater rates of inbreeding. Increased inbreeding in Ash Creek would negatively impact the diversity of genotypes within the population and, subsequently, the population's fitness. Inbreeding results in a decrease in heterozygosity within progeny and overall reductions in genetic variation within a population by reducing and ultimately eliminating heterozygous combinations (Wang et al. 2002a). The subsequent increased homozygosity can lead to a greater expression of deleterious alleles that would otherwise be hidden (i.e., recessive) within a heterozygous state (Frankham 2003; Frankham et al. 2004). Overtime, inbreeding results in loss of genetic diversity with negative impacts on a population's fitness (i.e., reproductive success) and performance (i.e., survival and/or growth rates). This resulting reduction in fitness and performance, referred to as inbreeding depression, is recognized as a great threat to the viability of small populations (Amos and Balmford 2001; Wang et al. 2002b; Frankham et al. 2004).

Since all genetic diversity is ultimately the result of random mutation events (Allendorf and Luikart 2007), any reductions in genetic diversity are permanent and can eventually jeopardize the sustainability of Ash Creek's population. Subsequently, any gains observed from using Ash Creek's population for restoring brook trout populations in the southwest GMZ could be lost as a consequence of propagating genetically depauperate and ecologically inferior brook trout. This would be a gradual process as the impacts of genetic drift and inbreeding would accumulate as the loss of diversity increases with time. Over time, the original goals of the program would be unattainable due to specific fitness and performance issues within the source population.

The goal of this study was to evaluate the genetic and demographic impacts of Wisconsin's current wild broodstock collection strategy. To evaluate whether Ash Creek has experienced any discernible genetic and/or demographic impacts as a result of current broodstock strategies by conducting a series of four comparisons among Ash Creek's population components:

1. The in-stream adults were compared to the in-hatchery adults to determine if the proportion of adult trout removed resulted in a bias in genetic or size (total length and weight) diversity.
2. The in-stream adults were compared to their respective in-stream young-of-year (YOY) counterparts to determine if the reductions to the size Ash Creek's in-stream population resulted in reductions to levels of genetic diversity with in the in-stream YOY populations.
3. Genetic diversity levels within the in-stream YOY in 2005 and 2006 were compared to determine if the difference in the proportion of fish removed from Ash Creek (44% and 84%, respectively) resulted in differential reproductive contribution to the in-stream YOY populations.
4. Genetic diversity levels within the in-stream and in-hatchery YOY components were compared to determine if differential reproductive contributions existed among these YOY populations.

METHODS

Study Site

Ash Creek is located approximately three miles southwest of Richland Center (Richland Co.), WI and resides in the Willow Creek watershed within the lower Wisconsin River drainage basin. Ash Creek is a spring and seepage fed stream, approximately 12.55 km in length and flows into the Pine River, Richland Co. (Ball et al. 1970; WDNR 2002). The stream supports natural reproduction of brook trout and brown trout (*Salmo trutta*). The WDNR has designed a fisheries management plan to improve the health of the brook trout fishery in Ash Creek that includes removing obstructions (e.g., beaver dams and deadfall), stream bank habitat improvements, in-stream habitat work (i.e., creation of greater pool space) and a catch-and-release fishing regulation. Baseline monitoring has been prescribed (every five years) to assess the success and health of the brook trout fishery (WDNR 2002). The adult fish removed from Ash Creek for hatchery production are transferred approximately 130 km to Nevin Fish Hatchery (Monona, WI); the oldest and second largest fish hatchery in the state of Wisconsin.

Sample Collection

Four population components of Ash Creek's brook trout population were sampled over two consecutive years: 1) adult trout removed for spawning at the hatchery (in-hatchery adults), 2) Ash Creek's remaining adult population after broodstock removals (in-stream adults), 3) hatchery-reared young-of-year (in-hatchery YOY), and 4) naturally-spawned in-stream young-of-year (in-hatchery YOY). The in-hatchery and in-stream adults were sampled during fall of 2004 and 2005 to determine if inadvertent sampling

bias (both genetic and size selective) was occurring. In-hatchery adults were sampled in the hatchery after they completed spawning on a weekly basis over their entire spawning period (October through November) to ensure a representative sample. The in-stream adults were sampled in Ash Creek via Smith-Root 15-D electrofishing backpack unit (Smith-Root, Inc., WA) in the fall after broodstock collections had taken place. Pelvic fin clips (>25 mg) were taken from all adult brook trout collected and stored in individually labeled tubes containing 95–100% non-denatured ethanol. In addition, all collected adult brook trout were measured for total length (mm) and weight (g). The in-stream YOY (collected backpack electrofishing) and in-hatchery YOY were sampled in the spring of 2005 and 2006 to assess the impacts of differential reproductive contributions. Collected YOY samples were stored in pre-labeled tubes containing 95–100% non-denatured ethanol. A target sample size of ≥50 fish was collected from each population component to ensure accuracy and precision while estimating levels of genetic diversity (Ruzzante 1998).

DNA Extraction

Total genomic DNA was extracted from collected tissue samples using Promega Wizard® Genomic DNA purification kit in accordance with the manufacturer's suggested protocol (Promega Corp., Madison WI). The final step of the protocol was modified by re-hydrating the DNA in 200 µl of Tris-low-EDTA buffer (TLE; 10 mM NaCl, 0.1 mM EDTA, pH 8.0) instead of the manufacturer's supplied buffer. The quality (molecular weight) of each DNA sample was evaluated via electrophoresis in a 0.7% agarose gel and compared to a known molecular weight ladder (Hyperladder™ I, Bioline USA Inc.,

Randolph, MA). The quantity of DNA (ng/µL) for each sample was determined using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). All DNA samples were normalized to 25 ng DNA/uL prior to microsatellite analysis to ensure consistent results.

Micosatellite Genotyping

The high variability of microsatellite loci makes them ideal markers to assess genetic structure of populations and detect differences between closely related samples (O'Connell and Wright 1997). Previous studies have shown microsatellite markers contain greater levels of variability than other molecular markers such as the allozymes and mtDNA used by Fields and Philipp (1998; Brunner et al. 1998; Angers and Bernatchez 1998). Furthermore, only small amounts of DNA are required to assess microsatellite genetic diversity making it possible to conduct non-lethal sampling (e.g., fin clip, scales, etc.).

A standardized set of 12 microsatellite DNA markers used in Lake Superior brook trout studies was used for this study (Table 2.1; King and Burnham-Curtis, unpublished; Angers et al. 1995; Wilson et al. 2005). The extracted DNA samples were amplified using the polymerase chain reaction (PCR). PCR conditions for multiplex and singlet reactions as well as thermal profiles are shown in Table 2.2. Samples were genotyped on an ABI Prism™ 377XL automated DNA sequencer (Applied Biosystems, Inc. Foster City, CA). A known size standard (GeneFlo™ 625 DNA Ladder, Chimerx Corp., Milwaukee, WI) was included with each sample to facilitate accurate sizing of alleles. The resulting data consisted of direct-count, multi-locus genotype data.

Data Analysis

Hardy-Weinberg equilibrium and gametic disequilibrium.—Tests for deviations from Hardy-Weinberg Equilibrium (HWE; Hardy 1908; Weinberg 1908) were conducted for each locus/population combination using exact tests that employ a Markov Chain method in Genepop v3.4 (1000 dememorization steps, 100 batches and 1000 iterations; Guo and Thompson 1992). To ensure all loci were independently segregating within Ash Creek's population components, tests of gametic disequilibrium were conducted for all combinations of locus pairs within components using a Markov Chain method in Genepop 3.4 (1000 dememorization steps, 100 batches and 1000 iterations; Raymond and Rousset 1995). For both tests of HWE and gametic disequilibrium, significance levels for multiple tests were adjusted with a sequential Bonferroni correction to account for multiple pairwise tests (Rice 1989).

Measure of intrapopulation genetic diversity.— The levels of genetic diversity within each of Ash Creek's population components over two years was estimated from allelic diversity (A), observed heterozygosity (H_o), and expected heterozygosity (H_e) using Microsatellite Toolkit v3.1 (Parks 2001). In addition, estimates of allelic richness (A_r) were calculated using a rarefaction method to account for potential bias in A, due to unequal sample sizes for each sampled component (Leberg 2002, Kalinowski 2005), in HP-RARE 1.0 (Kalinowski 2005).

Study Design for Interpopulation Comparisons

Four sets of comparisons were conducted to assess if a realized or potential genetic impact has occurred or is occurring in Ash Creek. The sets of comparisons

focused on the assumption that the annual removal of large numbers of brook trout and more importantly the subsequent loss of reproductive contributions from Ash Creek would have genetic impacts on the Ash Creek's in-stream population.

Bias of genetic and/or demographic diversity due to broodstock removals.—A series of comparisons were conducted under the assumption that the annual removal of a large number of brook trout from Ash Creek would result in a negative impact on levels of genetic diversity within the in-stream adult population. The first analysis was to compare levels of genetic diversity between the two adult population components (in-stream and in-hatchery). The two measures used to assess genetic diversity were allelic richness (A_r), calculated using HP-RARE 1.0 (Kalinowski 2005), and expected heterozygosity (H_e), calculated using Microsatellite Toolkit 3.1 (Parks 2001). Because of the non-parametric nature of genetic data, genetic diversity levels (A_r and H_e) within each component was ranked across loci and rankings were used in an analysis of variance (ANOVA) in SPSS 14.0 (SPSS Inc., Chicago, IL). The second analysis was to compare the allele frequency distributions of the two components performing a genic differentiation test of allele frequency distributions using Genepop 3.4 (1000 dememorization steps, 100 batches and 1000 iterations; Guo and Thompson 1992). This tests the null hypothesis that the recovered allele frequencies represent independent samples from a single, homogeneous mixture. If the test shows significant differences, it is interpreted as the two components not having the same levels and distributions of genetic diversity and, thus, they represent differential or biased samples.

In addition to the genetic analysis, size comparisons were conducted to determine if the current broodstock sampling strategy resulted in size bias selection. To account for

known size differences between the brook trout sexes (Power 1980), total length (mm) and weight (g) comparisons were conducted separately for male and female brook trout. Comparisons consisted of paired, year-to-year t-tests conducted in SPSS 14.0 (SPSS Inc., Chicago, IL).

Breeding population size restrictions within years .—A series of comparisons were performed to determine if restrictions to Ash Creek's in-stream adult population size has resulted in reductions of genetic diversity within the in-stream YOY when compared to their parental counterparts (i.e., a bottleneck effect). This was tested using the same genetic measures and approaches described for the first series of comparisons but testing for differences between the in-stream adults and the in-stream YOY.

In-stream differential reproductive contributions between years .—A series of comparisons were performed to determine if the proportion of adult fish removed from Ash Creek's population (44% of adult fish removed in 2004 versus 84% removed in 2005) resulted in differential reproductive contributions to the in-stream YOY population components. The first approach was to compare levels of genetic diversity between the in-stream YOY in 2005 and 2006. This was tested using the same genetic measures described previously, however, because examining levels of genetic diversity between years consisted of a pairwise comparison, a Mann-Whitney test was conducted in SPSS 14.0 (SPSS Inc., Chicago, USA).

In addition, three core population measures were evaluated and compared to determine if differential reproductive contributions to Ash Creek's in-stream YOY production existed over the two years of this study. The effective number of breeders (N_b) that produced each cohort (2005 and 2006) was estimated using the linkage

disequilibrium estimator of Hill (1981) and Bartley et al. (1992) as implemented in NeEstimator 1.3 (Peel et al.2004). To assess the accuracy of N_b estimates, 95% confidence intervals were calculated according to Waples (1991) using NeEstimator 1.3 (Peel et al. 2004). To estimate levels of inbreeding, computations of inbreeding coefficients (F_{IS}) and their significance levels (h_o = no deviation from zero) were estimated for the in-stream YOY population components in Arlequin 3.10 (Excoffier et al. 2005) with significance based on a permutation process. The degree of relatedness among individuals within each of the in-stream YOY population components was quantified using the Queller and Goodnight's (1989) coefficient of relatedness (r_{xy}). The overall relatedness (R) and proportion of pairwise comparisons among individuals belonging to different relatedness categories (unrelated, half-siblings, full-siblings) was calculated for each in-stream YOY population component using Kinship 1.2 (Goodnight and Queller 1999). A chi-square test was conducted to determine if the proportion of fish in each relatedness category differed between the in-stream YOY samples in 2005 and 2006. This test was conducted using the proportions of related individuals from the 2005 in-stream YOY samples as the expected values.

Differential reproductive contributions between Ash Creek's in- hatchery YOY and in-stream YOY.—A series of comparisons were performed to determine if broodstock removals resulted in functional differences between the broodstock and Ash Creeks' production (i.e., reproduction). This was tested using the same genetic measures and approaches described for the previous series of YOY population component comparisons but testing for differences in levels of genetic diversity between the in-hatchery YOY and in-stream YOY within each year. In addition, comparisons of genetic diversity (H_e and

A_r) were conducted using an ANOVA to account for multiple population components comparisons. Again, the data was ranked across the population components and these rankings were used in an ANOVA conducted in SPSS 14.0.

RESULTS

A total of 683 brook trout were sampled from all population components over the course of this study (Table 2.3). The overall number of alleles per locus ranged from three (Sfo 24) to nine (Sfo 91). Allele sizes and their population component frequencies for all 12 sampled loci are shown in Appendix 2.1.

Hardy-Weinberg Equilibrium and Linkage Disequilibrium

Initial tests for HWE showed 9 of 96 (9%) locus/population comparisons deviated from HWE expectations based on a 0.05 alpha level. Following a sequential Bonferroni correction only two (2%) locus/population component comparisons significantly deviated from HWE expectations. Therefore, all population components were considered in HWE for subsequent analyses. Initial tests of gametic disequilibrium showed significant disequilibrium ($\alpha = 0.05$) in 66 of 528 (13%) locus/population component comparisons. Following a sequential Bonferroni correction ($a_{djusted} = 0.0008$), gametic linkage remained significant in 14 (3%) pairwise comparisons. Six of the 14 significant locus/population component comparisons included the Sfo 88/Sfo 91 locus combination. This locus pair was also found to be significant ($P < 0.0008$) in global tests across all population components. Because Sfo 91 was observed in three additional significant locus/population component combinations (Sfo 91/Sfo 86, Sfo 91/Sfo 18 and Sfo 91/Sfo 115), this locus was removed from further genetic analyses due to concerns over independence.

Measures of Intrapopulation Genetic Diversity

Relatively moderate levels of genetic diversity were observed within Ash Creek's population components over the course of this study (Table 2.3). The mean *A* within Ash Creek's population components ranged from 4.45 (in-stream adults 2005) to 4.73 (in-hatchery adults 2004 and in-stream YOY 2005). When rarefaction was employed to account for unequal sample sizes, in-stream adults 2004 and in-stream YOY 2005 showed the lowest (4.22) and highest (4.71) rarefacted number of alleles, respectively. Mean H_o across loci within Ash Creek's population components ranged from 0.55 (in-hatchery adults 2004 and in-hatchery YOY 2005) to 0.59 (in-stream YOY 2005). The mean H_e levels across all loci ranged from 0.56 (in-stream adults 2004, in-hatchery YOY 2005 and in-stream YOY 2006) to 0.57 (all addition population components). All of Ash Creek's adult population components contained similar levels of genetic diversity over the two years of this study.

Bias of Genetic and/or Demographic Diversity Due to Broodstock Removals

Annually, the Ash Creek's in-stream and in-hatchery adults exhibited similar levels and distributions of genetic diversity. Genetic diversity comparisons conducted using an ANOVA of ranked population data showed H_e (ANOVA; df = 3, p = 1.00) and A_r (ANOVA; df = 3, p = 0.90) were not different among the adult population components. Tests of genic differentiation failed to show a significant allele distribution difference between the in-hatchery and in-stream adults in both 2004 (p = 0.53) and 2005 (p = 0.33).

Significant size differences (lengths and weights) were observed between Ash Creek's in-stream and in-hatchery adults (Table 2.4 and 2.5). In both 2005 and 2006, the in-hatchery adults consisted of larger size (length and weight) males and females when compared to in-stream adults.

Breeding Population Size Restrictions within Years

Ash Creek's in-stream adult and in-stream YOY population components contained similar levels of genetic diversity (Table 2.3). Genetic diversity comparisons conducted using an ANOVA of ranked population data indicated that H_e (ANOVA; df = 3, p = 1.00) and A_r (ANOVA; df = 3, p = 0.82) were not different among population component. However, genic differentiation tests revealed a significant shift in allele frequencies distributions between the in-stream adults 2004 adults and in-stream YOY 2005 ($p < 0.001$) and in-stream adults 2005 and in-stream YOY 2006 ($p < 0.01$).

In-stream Differential Reproductive Contributions between Years

Ash Creek's in-stream YOY in 2005 and 2006 contained similar levels of genetic diversity (Table 2.3) but maintained different allele distributions. Genetic diversity comparisons conducted using Mann-Whitney tests indicated that H_e (p = 0.90) and A_r (p = 0.85) were not different between the two in-stream YOY population components. Tests of genic differentiation between the in-stream YOY components showed a significant shift in allele distributions over the two years ($p < 0.01$). The N_b's for in-stream YOY were relatively similar from 2005 to 2006 (33.20 and 43.50, respectively; Table 2.6).

No detectable inbreeding was observed in the in-stream YOY in either 2005 or 2006 (Table 2.6). Values of F_{IS} for the in-stream YOY were -0.01 in 2005 and -0.02 in 2006. These values were not significantly different from zero ($\alpha = 0.05$) indicating there was no detectable inbreeding in the two surveyed years. Individuals within each of Ash Creek's in-stream YOY population components exhibited low levels of relatedness (Table 2.7). The overall *R* value within the in-stream YOY was 0.016 in 2005 and 0.035 in 2006. However, based on the 95% confidence intervals, none of these estimates were significantly different from zero. The proportions of pairwise r_{xy} comparisons with a relatedness value equal to or greater than half-siblings within each population component were similar over the two years (29% in 2005 and 30% in 2006). A chi-square test found the proportion of individuals within each relatedness category did not significantly differ between the two in-stream YOY components ($\chi^2 = 4.74$, df = 2, p = 0.11).

Differential Reproductive Contributions between Ash Creek's In-hatchery YOY and In-stream YOY

No differences in the levels of genetic diversity were observed between in-hatchery YOY and in-stream YOY. An ANOVA of ranked population data indicated that H_e (ANOVA; df = 3, p = 0.99) and A_r (ANOVA; df = 3, p = 0.90) did not significantly differ among in-hatchery YOY and in-stream YOY. Tests of genic differentiation showed a significant difference between the allele distributions of the in-stream YOY and in-hatchery YOY during both 2005 ($p < 0.01$) and 2006 ($p < 0.01$).

A strong difference was apparent in the N_b estimates of the in-hatchery YOY versus the in-stream YOY (Table 2.6). In both 2005 and 2006, the in-hatchery YOY

exhibited a higher N_b (279.30 and 274.70, respectively) than their in-stream counterparts (33.20 and 43.50, respectively). No detectable differences in the levels of inbreeding or relatedness were observed among the in-stream and in-hatchery YOY population components (Table 2.6 and Table 2.7, respectively). Values of F_{IS} for the two in-hatchery YOY population components were –0.02 in 2005 and 0.01 in 2006. The inbreeding values did not significantly differ from zero ($\alpha = 0.05$). In both years, the in-hatchery YOY exhibited low levels of relatedness. The overall R value within in-hatchery YOY were 0.015 in 2005 and 0.001 in 2006. Based on the 95% confidence intervals, none of these estimates significantly differed from zero. The proportion of pairwise r_{xy} comparisons with a relatedness value equal to or greater than half-siblings was also similar among the in-stream and in-hatchery YOY over the two years (30% in 2005 and 29% in 2006). A chi-square test showed the proportion of individuals within each relatedness category did not significantly differ between the in-stream and in-hatchery YOY components within each year ($\chi^2 = 4.74$, df = 2, p = 0.11).

DISCUSSION

For rehabilitation programs, the removal of wild fish for hatchery production can generate a series of genetic and/or ecological risks that could adversely effect brood source populations. Currently, Ash Creek's population is the sole source of brood fish for Wisconsin's wild brook trout stocking program. This study attempted to better understand the impacts of broodstock removals on Ash Creek's brook trout population. To evaluate the potential or realized genetic risks/hazards associated with Wisconsin wild trout stocking program's broodstock collection practices, a series of comparisons were conducted to evaluate levels of genetic diversity within Ash Creek's brook trout population and the subsequent reproductive contributions to YOY production.

The overall levels of genetic diversity within Ash Creek's population were comparable to other Class I brook trout populations in the southwest GMZ (Table 2.8). Cautiously interpreted, this finding suggests that the utilization of Ash Creek's population over the last eight years has not resulted in the drastic depletion of this population's genetic resources. However, in the absence of historical genetic data (i.e., data pre-dating the use of Ash Creek's population as a source of broodstock), the degree to which broodstock removals have affected the genetic integrity of Ash Creek's population is difficult to assess. Within the short time frame of this study, there were no detectable reductions in genetic diversity, but several findings warrant concern about the long-term implications of the current broodstock collection practices.

Bias of Genetic and/or Demographic Diversity due to Broodstock Removals

While the proportion of fish removed annually from Ash Creek was near or exceeded the 50% threshold, the distribution and content of genetic diversity (A_r and H_e) among Ash Creek's adult population components (in-hatchery and in-stream) remained similar over the two years. For instance, even though an estimated 80% of Ash Creek's adult population was removed in 2006 for hatchery production (Matthew Mitro, WDNR, personal communication), levels of A_r and H_e within the remaining 20% of Ash Creek's in-stream adults were found to be equivalent to the in-hatchery adults. These results suggest that while even a large proportion of adult fish are removed, the remaining annual in-stream adult sizes have remained large enough to sufficiently retain levels of genetic diversity comparable to their broodstock counterpart.

While there was no detectable sampling bias in the levels of genetic diversity, the results of this study showed a consistent bias in the size of fish being selected for hatchery propagation. Broodstock collections in 2005 and 2006 resulted in larger male and female fish (both length and weight) being selected for hatchery propagation. This unintentional selection for larger fish being taken into the hatchery could have long-term adverse effects, such as reducing spawning success and/or decreasing the size structure of Ash Creek's adult population. Because of the heritable nature of size related traits, alterations to Ash Creek's adult size structure could eventually result in alteration of Ash Creek's genetic diversity as well.

Breeding Population Size Restrictions within Years

The apparent reduction of Ash Creek's in-stream adult population size resulted in no observable reductions in levels of genetic diversity within the in-stream YOY in either 2005 or 2006. In both years, the in-stream YOY maintained levels of genetic diversity similar to their parental counterparts. However, there was evidence that the allele frequency distributions between the in-stream adults and in-stream YOY within each year differed. While these shifts may, in part, be associated with restrictions to Ash Creek's in-stream adult breeding population size and/or alterations to its breeding structure, it is not uncommon to see annual fluctuations in allele frequencies in trout as a consequence of their complex life history patterns (Waples 1990; Palm et al. 2003).

Random shifts in allele frequencies are often expected to occur within/among individual cohorts in age structured populations where generations overlap (Ryman 1997). In Ash Creek's population, it is likely that the age at maturity varies among individual brook trout, and once mature, not all individuals will spawn or reproductively contribute equally in a given year (Power 1980, Waples 1990, Blanchfield et al. 2003). As a consequence, the in-stream YOY components are not representative of the entire in-stream adult components in each spawning season (Ryman 1997). However, without a temporal perspective (i.e., over several generations), identifying the central cause (breeding restrictions or natural shifts) or degree of these shifts in allele frequencies are difficult. Nevertheless, this fluctuating pattern of allele frequencies could also be influenced by the large scale removals of adults for propagation purposes.

In-stream Differential Reproductive Contributions between Years

Over the two years of this study (2005–2006) it was estimated that 44% and 84% of Ash Creek's adult population was removed annually for hatchery production (Matthew Mitro, WDNR, personal communication). The proportion of fish removed from Ash Creek's in-stream adult population appeared to have no observable detrimental effect on the levels of genetic diversity within the in-stream YOY. Furthermore, over the course of two years, no inbreeding was detected within the in-stream YOY population components and the levels of relatedness and N_b remained relatively similar over the course of both years. These results indicate that in the short-term, the proportion of broodstock removed had no detectable differential influence on the reproductive contributions to Ash Creek's in-stream YOY. However, significant shifts in allele frequencies were detected between the in-stream YOY population components, again though, without a temporal perspective identifying the central cause or degree of these shifts is difficult.

Differential Reproductive Contributions between In-hatchery YOY and In-stream YOY

While levels of genetic diversity, inbreeding and relatedness among Ash Creek's in-hatchery and in-stream YOY components were similar, the N_b within the in-hatchery YOY was approximately seven times larger than that of their in-stream counterparts in both 2005 and 2006. In part, the smaller N_b of the in-stream YOY may be attributable to breeding restrictions within Ash Creek; however it is likely that the spawning procedures within Nevin's Hatchery played a role in elevating the N_b of the in-hatchery YOY. Typically, during spawning, the N_b can be reduced as a result of small numbers of brood fish, skewed sex ratios, and large variance in family sizes (Hallerman 2003). Currently at

Nevin's Hatchery, spawning procedures attempt to equalize the numbers of females and males spawned by using a 'one male to one female' mating scheme. In addition, brood fish are allowed to naturally ripen in the hatchery, and therefore spawned fish are representative of the entire spawning season for Ash Creek's population. This mating scheme allows all breeding fish to have approximately equal reproductive contributions to the YOY production in the hatchery (Miller and Kapuscinski 2003). The predicted consequence of this strategy would be an increased N_b in the in-hatchery YOY versus the in-stream YOY. The results suggest that the hatchery practices have been successful at capturing a large proportion of the genetic variability found in the wild broodstock, which increases the likelihood that Ash Creek's diversity will be passed on to recipient populations and be beneficial to genetically depauperate populations. Alternatively, the small N_b observed within the in-stream YOY could be associated with breeding restrictions (small number of breeding fish) as a result of the large proportion of spawning fish from the population. However, in light of the severe differences of hatchery spawning practices versus natural spawning, this increased N_b is likely due to the propagation program's spawning strategies.

Management Implications and Future Research

The evidence collected in this study indicates that current broodstock collections have had no detectable impacts on genetic diversity levels within Ash Creek. However, several results of this study raise concerns that current collection practices (i.e., number of fish collected and observed size bias) may not be sustainable in the long-term and could potentially jeopardize the viability of Ash Creek's brook trout. In the short-term

(i.e., a single generation), it appears that Ash Creek's population has been capable of buffering the genetic effects associated with losing a large proportion of its reproductive effort associated with broodstock removals; the long-term implications of the current collection practices are difficult to predict and could pose serious genetic risks. During the duration of this study, the observed allele frequency shifts between the in-stream adults and their in-stream YOY counterparts were not unexpected due to the complex life history of brook trout. However, in light of the fact that approximately 50% or more of Ash Creek's population is annually removed, it is probable that these frequency shifts are associated with and/or influenced by the large annual losses of reproductive effort because of the current broodstock collection strategy. To assess the magnitude of genetic change occurring within Ash Creek's population, it would be necessary to examine shifts within the population as a whole over future generations.

A second concern revolves around the selection of larger fish for hatchery production because it will result in an overrepresentation of smaller adult brook trout in Ash Creek and potentially greater reproductively contributions to future generations. Accumulating evidence has indicated that the reproductive potential in salmonids (i.e., brook trout) may be strongly affected by parental body size, specifically in females (Bagenal 1969; Beacham et al. 1985; van den Berghe and Gross 1989; Smoker et al. 2000; Wilson et al. 2002). For example, there is typically a positive relationship between female size and fecundity whereby smaller females produce fewer and smaller eggs (van den Berghe and Gross 1989; Smoker et al. 2000; Wilson et al. 2002). In addition, when compared to larger individuals, smaller females have been shown to select poorer quality nesting areas and dig shallower redds which can lead to higher embryo mortality (van den

Berghe and Gross 1989). They may also produce smaller alevins and fry which have lower growth and survival rates (Bagenal 1969; Beacham et al. 1985). The unintentional removal of Ash Creek's larger brook trout could decrease the mean body size of Ash Creek's in-stream breeding population and subsequently result in a reduction in the population's annual reproductive potential, ultimately affecting the number of fish recruiting to the spawning population in subsequent years.

The continued removal of the largest adults could eventually lead to the alteration of Ash Creek adult population's size structure. In part, the phenotypic variation of body sizes within Ash Creek's adult population is attributable to genetic difference between individuals. Several studies have demonstrated that variation in body sizes of fish arise from a combination of environmental and genetic factors (Nilsson 1992, Smoker et al. 1994). The ability of brook trout in Ash Creek to grow to larger sizes is partially a heritable genetic trait that is influenced to certain degrees by environmental factors (e.g., population density and/or habitat and resource availability, genotype-environment interactions; Allendorf and Luikart 2007). Because of the genetic component, reductions to the annual mean adult size within this population will result in the overall population's size structure shifting toward fish sexually mature at smaller sizes. A similar trend was observed among pink salmon (*Oncorhynchus* spp.) on North America's Pacific coast, where individuals have become smaller at sexual maturity during the past 25 years. The shift in size at maturity has been attributed, in part, to genetic changes resulting from a size-selective fishery where larger fish have a higher possibility of being harvested (Ricker 1981, Law 2000, Smoker et al. 2000).

If smaller fish equate to lower fecundity, the proportion of fish collected from Ash Creek would have to be increased to meet yearly egg quotas. At the same time, the decrease in the overall population fecundity would mean fewer fish were recruiting to the adult spawning population. The potential reduction of Ash Creek's size structure could not only result in genetic changes in Ash Creek's population, but also likely reduce current levels of natural recruitment within it, ultimately threatening the sustainability of the wild brook trout stocking program (based on Ash Creek as a sole brood source) and, more importantly, the long-term viability of Ash Creek's population.

As a consequence of these demographic and genetic risks, future collection strategies should strive to collect broodstock samples that adequately represent an unbiased sample (both length and weight) of Ash Creek's population to limit the under representation of larger adults remaining in the stream. To limit the demographic bias associated with the collection of larger adults, a stratified random sampling approach should be used, whereby, all potential brood fish could be separated into large, medium, and small male and female size categories. During collections, an equal number of fish should be sampled from each of these six categories ensuring that all size ranges of adult fish have an equal opportunity of being collected. This would subsequently increase the likelihood that larger adults are reproductively represented within the in-stream population (Miller and Kapuscinski 2003)

To further limit the long-term pressures on Ash Creek's population, several additional collection strategy modifications could be implemented to minimize the risks associated with large broodstock removal practices, yet still provide sufficient resources to meet egg quota demands. For instance, a supportive breeding approach could be

implemented, where a proportion of the progeny from the in-hatchery adults are annually returned to Ash Creek to minimize the population's overall loss of reproductive potential. An additional alternative would be to select and use multiple sources of broodstock, alternating use between years, thereby reducing the long-term reliance on any one population. Such an approach would allow a satisfactory level of genetic diversity to be sampled in any given year yet minimize accumulative reproductive losses to any single population over multiple years; largely increasing the sustainability and the viability of all selected source populations.

With the continued use of Ash Creek's brook trout population as a source of wild broodstock for restoration efforts in the southwest GMZ, it is highly recommended that future management efforts be conducted with a strong commitment to adaptive management. Ash Creek's population should be periodically monitored and genetically evaluated with broodstock collection efforts adjusted in subsequent generations based on the findings of these evaluations. Currently, Ash Creek's fishery is monitored every five years to assess habitat restoration efforts and fishing regulation changes (WDNR 2002). These assessments should include genetic sampling (i.e., collection of tissue samples) to support a long-term genetic assessment of Ash Creek's brook trout population. The strength of this current study is that it serves as a baseline from which future evaluations can compare to examine the effects of broodstock removals over multiple generations.

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Table 2.1. Microsatellite loci used in current study and description of primer sequence, allele size range in base pairs, and number of alleles per locus (*A*). Sfo 12 and Sfo 18 are from Angers et al. 1995 and the remaining loci are from King and Burnham-Curtis, unpublished.

Locus	Forward and Reverse Primer (5'–3')	Range	*A*	Motif
Sfo 52	GCACACGAAACCAGTATATTTC	187–239	18	tetra/dinucleotide
	TTGTCTTGGTGATTTCAGAGC			
Sfo 24	GCTACTGTTGGATTTCATCTCAG	110–186	13	trinucleotide
	ATCACAGAGATGGGGTGATG			
Sfo 28	CAGTTGAAGTGATTGGGTTAGC	167–201	18	trinucleotide
	TCATCCTTAAAGCAGAATACCAC			
Sfo 38	GTTGTGTTGCTTTGGTTTCAG	140–152	5	trinucleotide
	TTACTGATTACAATTTTGGACTGG			
Sfo 86	ACCGATGGCCTTCAACAC	101–125	8	trinucleotide
	ATAGGCCCCTACCTCAAACC			
Sfo 88	TAGTCTCTGGTGGGGAATAATG	178–213	11	trinucleotide
	ATATCAGCCATAAGAGCTGGAG			
Sfo 113	GGAGCCCAGACTATATTGACG	114–169	16	trinucleotide
	CCTTGAAGTCTTGCCAGATG			
Sfo 115	CAGTTTCTATCTCCAGGCAATC	217–367	46	tetra/dinucleotide
	TTCTGAAAGCACTCAACATGG			
Sfo 75	GTAGTGCCAAAACAGGTAGAGC	168–248	21	tetranucleotide
	CATCCTTATTCCAACCTCAATC			
Sfo 91	AAATAACAACAATATGTGAGAAC	204–340	27	tetranucleotide
	TATGCTGATATTGACTTTGG			
Sfo 12	CCCGTTTCACAATCAGAG	249–275	5	dinucleotide
	GGTTTTGAAGAGTGACAG			
Sfo 18	TGGTGTATCCTGCTCCTG	173–225	12	dinucleotide
	TGGATTGTGTGTCTGTTTTCT			

Table 2.2. PCR conditions for four multiplex and two simplex reactions used in this study, including loci, locus-specific primers and labels, 10x buffer, dNTPs, $MgCl_2$ and *Taq* concentrations, and thermal profiles.

Reaction	Loci	Label	Primer (μM)	10x Buffer	dNTPs (mM)	$MgCl_2$ (μM)	*Taq* (units)
Multiplex A[1]	Sfo 86	HEX™	0.07	1x	0.50	1.20	0.15
	Sfo 88	HEX™	0.09				
	Sfo 28	NED™	0.23				
Multiplex B[2]	Sfo 18	NED™	0.30	1x	1.20	0.80	0.16
	Sfo 115	6FAM™	0.21				
	Sfo 113	6FAM™	0.09				
Multiplex C[1]	Sfo 52	6FAM™	0.07	1x	1.20	1.50	0.24
	Sfo 75	NED™	0.10				
Multiplex D[1]	Sfo 24	6FAM™	0.08	1x	0.50	1.50	0.15
	Sfo 38	NED™	0.07				
Singlet 1[1]	Sfo 91	HEX™	0.40	1x	0.40	0.60	0.12
Singlet 2[2]	Sfo 12	6FAM™	0.40	1x	0.40	0.60	0.15

[1] 94°C for 2 min, followed by 35 cycles of 92°C for 45 s, 53°C for 45 s, and 70°C for 90 seconds and ending with a final elongation of 68°C for 30 min.

[2] 94°C for 2 min, followed by 35 cycles of 92°C for 45 s, 58°C for 45 s, and 72°C for 90 s and ending with a final elongation of 68°C for 30 min.

Table 2.3. Genetic diversity values for the eight population components of Ash Creek's population. Sample size (n), mean observed (H_o) and mean expected heterozygosity (H_e), allelic diversity (A) and allelic richness (A_r) are presented for each component over two years.

Ash Creek's Population Component	n	H_o	H_e	A	A_r
		2004			
In-stream Adults	119	0.57	0.56	4.55	4.22
In-hatchery Adults	86	0.55	0.57	4.73	4.50
		2005			
In-stream Adults	90	0.57	0.57	4.45	4.34
In-hatchery Adults	102	0.58	0.57	4.64	4.46
In-stream YOY	64	0.59	0.57	4.73	4.71
In-hatchery YOY	74	0.55	0.56	4.55	4.55
		2006			
In-stream YOY	75	0.56	0.56	4.64	4.64
In-hatchery YOY	73	0.57	0.57	4.55	4.61

Table 2.4. Annual sex-specific weight and total length comparisons between Ash Creek's in-hatchery and in-stream adults in 2005 and 2006. The standard error for each mean, degrees of freedom (df) and significance value for each comparison are given.

Year	Sex	In-stream Adults	In-hatchery Adults	df	p-value
			Weight (g)		
2004	males	115.46 ± 4.40	131.19 ± 4.04	351.00	0.019
	females	105.51 ± 4.08	132.36 ± 4.20	377.08	< 0.001
2005	males	103.14 ± 9.09	146.85 ± 4.88	32.81	< 0.001
	females	114.07 ± 6.46	147.58 ± 4.70	200.00	< 0.001
			Total Length (mm)		
2004	males	223.18 ± 1.75	233.92 ± 1.77	528.00	< 0.001
	females	211.63 ± 1.75	228.37 ± 2.48	515.16	< 0.001
2005	males	220.00 ± 3.80	242.60 ± 3.01	165.00	0.008
	females	226.67 ± 6.72	238.72 ± 3.03	200.00	0.017

Table 2.5. Levels of inbreeding (F_{IS}) and the effective number of breeders (N_b) with lower and upper 95% confidence intervals (*C.I.*) for Ash Creek's in-stream and in-hatchery YOY population components over the two years of this study.

Ash Creek's Population Component	F_{IS}	N_b	Lower 95% *C.I.* Lower	Upper
		2005		
In-stream YOY	-0.01	33.20	27.10	41.40
In-hatchery YOY	-0.02	279.30	133.70	7,342.20
		2006		
In-stream YOY	-0.02	43.50	35.20	55.10
In-hatchery YOY	0.13	274.70	129.60	26,626.80

Table 2.6. Relatedness within each of Ash Creek's YOY population components including the overall mean relatedness (*R*) and the 95% confidence intervals (*C.I.*), and the proportion of pairwise coefficients of relatedness (r_{xy}) that were assigned to one of three relatedness categories. Threshold values for the relatedness categories are shown in parentheses.

Ash Creek's Population Components	*R*	*C.I.*	r_{xy} Unrelated (≤0.1250)	r_{xy} Half siblings (0.1251–0.3750)	r_{xy} Full siblings (≥0.3751)
			2005		
In-stream YOY	0.016	0.099	0.71	0.23	0.06
In-hatchery YOY	0.015	0.048	0.70	0.22	0.08
			2006		
In-stream YOY	0.035	0.056	0.70	0.22	0.08
In-hatchery YOY	0.001	0.036	0.71	0.23	0.06

Table 2.7. Genetic diversity values for Ash Creek (2005) and 13 additional brook trout populations sampled within the southwest GMZ. Sample size (n), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), and allelic richness (A_r) are presented for each population.

Population	n	H_o	H_e	A_r
Ash (2005)	60	0.58	0.59	4.31
Big Spring Branch	62	0.67	0.69	5.99
Elk	71	0.64	0.67	5.72
Fancy	60	0.64	0.65	5.61
Grinsell	50	0.67	0.67	4.92
John Coulee	51	0.52	0.54	4.82
Joe Coulee	52	0.62	0.63	5.91
King	50	0.69	0.70	6.84
Melancthon	61	0.68	0.64	5.16
North Branch Chipmunk Coulee	51	0.43	0.48	4.43
Parfrey's Glen	60	0.52	0.51	3.37
Pine	51	0.75	0.74	7.59
Soper	63	0.68	0.72	7.14
West Branch Mill	60	0.73	0.71	6.55

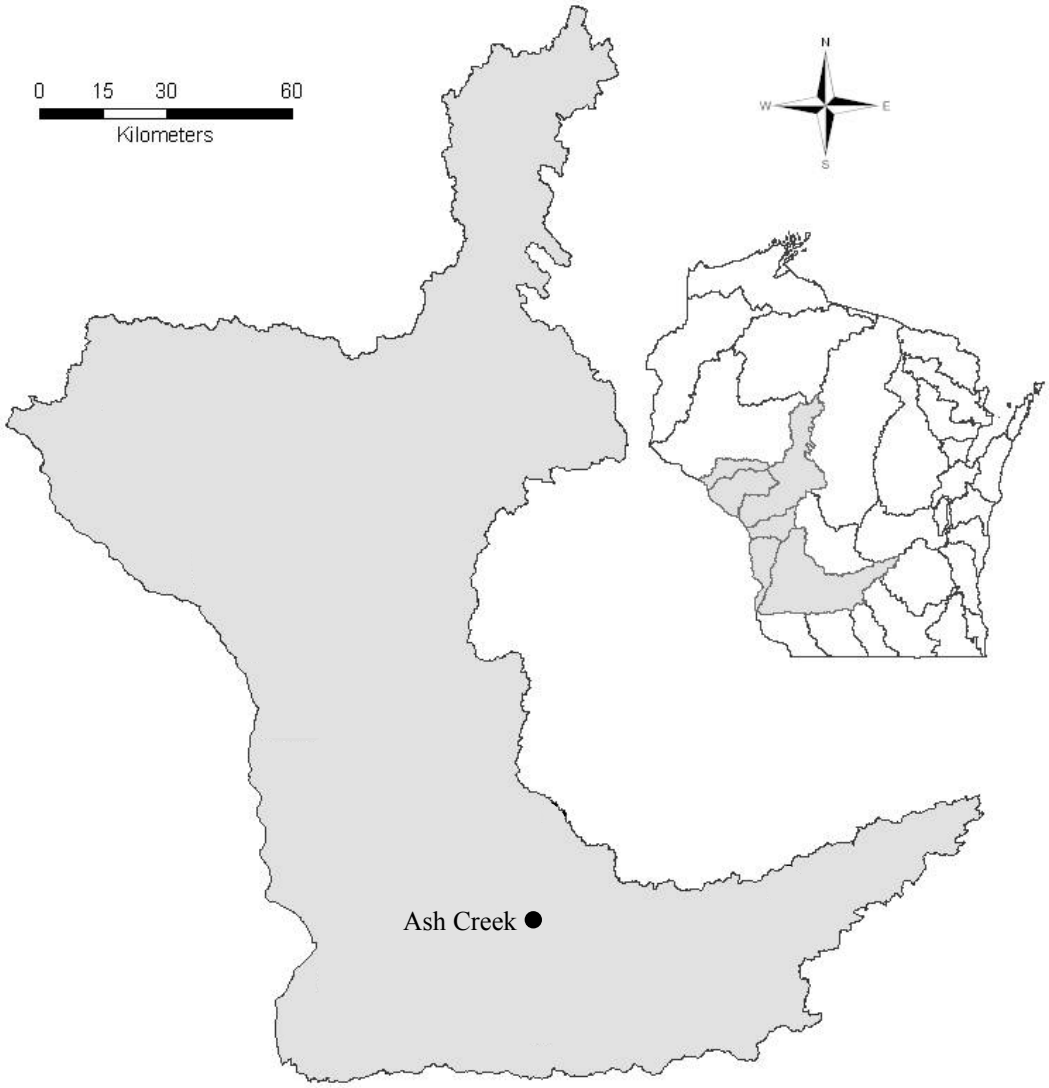

Figure 2.1. Map indicating the approximate location of Ash Creek (circle) and the area that its progeny are used for supplemental stocking purposes (gray).

Appendix 2.1. Locus-specific genetic diversity measures for the eight sampled components of Ash Creek's populations including sample size for each locus (n), allelic diversity (A), allelic richness (A_r), most frequently observed allele (S) and its frequency (F), observed heterozygosity (H_o), and expected heterozygosity (H_e). Overall sample sizes are in parentheses.

Locus		In-stream Adults 2004 (119)	In-hatchery Adults 2004 (86)	In-stream Adults 2005 (90)	In-hatchery Adults 2005 (102)	In-stream YOY 2005 (64)	In-hatchery YOY 2005 (74)	In-Stream YOY 2006 (75)	In-hatchery YOY 2006 (73)
Sfo 18	n	117	86	89	102	63	74	75	70
	A	6.00	7.00	5.00	6.00	6.00	6.00	6.00	6.00
	A_r	5.50	6.64	5.00	5.57	5.99	5.95	5.95	5.83
	S	177	177	191	177;183	177	177	183	177
	F	0.34	0.32	0.26	0.27	0.33	0.37	0.39	0.34
	H	0.80	0. 71	0.79	0.71	0.70	0.70	0.71	0.71
	H_e	0.76	0.76	0.79	0.77	0.76	0.72	0.73	0.77
Sfo 28	n	119	86	86	102	63	74	75	72
	A	4.00	4.00	4.00	4.00	4.00	5.00	4.00	5.00
	A_r	4.00	4.00	4.00	4.00	4.00	4.78	4.00	4.96
	S	178	178	178	178	178	178	178	178
	F	0.48	0.53	0.52	0.54	0.50	0.51	0.44	0.51
	H	0.61	0.60	0.63	0.62	0.57	0.62	0.65	0.64
	H_e	0.65	0.63	0.64	0.62	0.62	0.64	0.70	0.66
Sfo 52	n	119	85	90	102	60	73	75	72
	A	6.00	6.00	7.00	6.00	7.00	7.00	6.00	6.00
	A_r	5.73	5.58	6.27	5.55	6.93	6.58	5.90	5.77
	S	225	225	225	225	225	225	225	207
	F	0.34	0.42	0.37	0.35	0.54	0.40	0.45	0.38
	H	0.75	0.68	0.72	0.74	0.58	0.59	0.64	0.79
	H_e	0.73	0.69	0.71	0.72	0.64	0.70	0.66	0.69

Appendix 2.1. Continued.

Locus		In-stream Adults 2004 (119)	In-hatchery Adults 2004 (86)	In-stream Adults 2005 (90)	In-hatchery Adults 2005 (102)	In-stream YOY 2005 (64)	In-hatchery YOY 2005 (74)	In-Stream YOY 2006 (75)	In-hatchery YOY 2006 (73)
Sfo 75	n	119	85	87	102	60	73	75	73
	A	4.00	5.00	4.00	6.00	7.00	4.00	5.00	4.00
	A_r	3.49	4.68	4.00	5.13	7.00	3.79	4.76	4.00
	S	179	179	179	179	179	179	179	179
	F	0.70	0.74	0.70	0.67	0.62	0.69	0.73	0.74
	H	0.50	0.45	0.49	0.53	0.62	0.49	0.44	0.42
	H_e	0.46	0.44	0.47	0.50	0.57	0.47	0.44	0.42
Sfo 86	n	119	86	87	102	63	74	75	72
	A	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
	A_r	4.87	4.88	4.96	4.98	4.92	4.77	4.95	4.80
	S	104	104	104	104	104	104	104	104
	F	0.80	0.83	0.83	0.78	0.71	0.86	0.85	0.84
	H	0.39	0.29	0.30	0.40	0.49	0.22	0.29	0.29
	H_e	0.35	0.30	0.31	0.38	0.48	0.26	0.28	0.29
Sfo 88	n	119	86	86	102	63	74	75	72
	A	4.00	4.00	4.00	4.00	3.00	4.00	3.00	5.00
	A_r	3.97	3.97	4.00	3.99	3.00	3.78	3.00	4.77
	S	186	186	186	186	186	186	186	186
	F	0.58	0.56	0.58	0.60	0.66	0.48	0.63	0.47
	H	0.55	0.49	0.62	0.54	0.57	0.64	0.53	0.67
	H_e	0.58	0.56	0.57	0.56	0.50	0.62	0.53	0.63

Appendix 2.1. Continued.

Locus		In-stream Adults 2004 (119)	In-hatchery Adults 2004 (86)	In-stream Adults 2005 (90)	In-hatchery Adults 2005 (102)	In-stream YOY 2005 (64)	In-hatchery YOY 2005 (74)	In-Stream YOY 2006 (75)	In-hatchery YOY 2006 (73)
Sfo 91	n	117	81	88	102	58	70	74	72
	A	7.00	7.00	6.00	6.00	5.00	7.00	6.00	7.00
	A_r	6.37	6.71	5.96	5.97	5.00	6.83	6.00	6.81
	S	237	237	237	237	237	237	237	237
	F	0.51	0.41	0.43	0.47	0.44	0.39	0.37	0.46
	H	0.63	0.59	0.72	0.70	0.71	0.73	0.69	0.68
	H_e	0.66	0.70	0.71	0.69	0.70	0.77	0.72	0.72
Sfo 113	n	118	86	89	102	63	74	75	71
	A	6.00	5.00	6.00	5.00	5.00	5.00	5.00	5.00
	A_r	4.98	5.00	5.64	4.97	4.92	4.99	4.99	4.99
	S	140	140	137	137	140	140	140	140
	F	0.35	0.29	0.28	0.27	0.32	0.34	0.34	0.32
	H	0.79	0.73	0.75	0.75	0.84	0.74	0.63	0.69
	H_e	0.74	0.76	0.76	0.76	0.75	0.75	0.73	0.75
Sfo 115	n	117	86	89	102	64	74	75	70
	A	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.00
	A_r	4.50	4.67	4.96	4.92	5.00	4.78	4.95	4.00
	S	309	309	309	309	309	309	309	309
	F	0.56	0.53	0.49	0.49	0.66	0.50	0.61	0.51
	H	0.60	0.67	0.67	0.75	0.58	0.64	0.63	0.64
	H_e	0.62	0.64	0.66	0.68	0.53	0.67	0.58	0.65

Appendix 2.1. Continued.

Locus		In-stream Adults 2004 (119)	In-hatchery Adults 2004 (86)	In-stream Adults 2005 (90)	In-hatchery Adults 2005 (102)	In-stream YOY 2005 (64)	In-hatchery YOY 2005 (74)	In-Stream YOY 2006 (75)	In-hatchery YOY 2006 (73)
Sfo 24	n	117	83	90	102	61	74	74	72
	A	3.00	3.00	2.00	3.00	3.00	3.00	3.00	3.00
	A_r	2.50	2.70	2.00	2.97	2.95	2.78	2.95	2.81
	S	121	121	121	121	121	121	121	121
	F	0.80	0.71	0.72	0.78	0.75	0.71	0.75	0.75
	H	0.34	0.41	0.42	0.37	0.43	0.42	0.45	0.35
	H_e	0.33	0.42	0.40	0.35	0.38	0.42	0.38	0.38
Sfo 38	n	117	83	90	102	62	74	74	72
	A	3.00	4.00	3.00	3.00	3.00	3.00	4.00	3.00
	A_r	3.00	3.70	2.96	3.00	3.00	3.00	3.78	2.99
	S	149	149	149	149	149	149	149	149
	F	0.79	0.74	0.81	0.79	0.74	0.80	0.67	0.76
	H	0.30	0.43	0.32	0.32	0.42	0.35	0.55	0.35
	H_e	0.35	0.41	0.31	0.35	0.41	0.35	0.47	0.37
Sfo 12	n	117	80	89	101	61	74	73	69
	A	4.00	4.00	4.00	4.00	4.00	3.00	5.00	4.00
	A_r	3.87	3.73	3.99	3.97	4.00	3.00	4.79	4.00
	S	271	271	271	271	197	271	271	271
	F	0.50	0.51	0.48	0.55	0.45	0.53	0.46	0.46
	H	0.63	0.60	0.58	0.64	0.69	0.68	0.66	0.67
	H_e	0.63	0.62	0.64	0.60	0.66	0.61	0.63	0.66

Appendix 2.2. Allele frequencies and sample sizes (*n*) within each of Ash Creek's sampled population components.

Locus	Size (bp)	In-stream Adults 2004	In-hatchery Adults 2004	In-stream Adults 2005	In-hatchery Adults 2005	In-stream YOY 2005	In-hatchery YOY 2005	In-Stream YOY 2006	In-hatchery YOY 2006
Sfo 18	(*n*)	117	86	89	102	63	74	75	70
	173	0.124	0.140	0.157	0.186	0.183	0.054	0.060	0.186
	177	0.338	0.320	0.236	0.270	0.333	0.372	0.287	0.343
	179	0.004	0.006	0.000	0.000	0.000	0.000	0.000	0.000
	181	0.064	0.035	0.101	0.054	0.024	0.047	0.113	0.071
	183	0.269	0.244	0.247	0.270	0.246	0.324	0.393	0.214
	189	0.000	0.017	0.000	0.005	0.016	0.014	0.013	0.007
	191	0.201	0.238	0.258	0.216	0.198	0.189	0.133	0.179
Sfo 28	(*n*)	119	86	86	102	63	74	75	72
	178	0.483	0.529	0.523	0.544	0.500	0.507	0.440	0.507
	182	0.210	0.221	0.186	0.191	0.135	0.155	0.233	0.160
	186	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.014
	190	0.042	0.035	0.058	0.054	0.024	0.047	0.107	0.076
	202	0.265	0.215	0.233	0.211	0.341	0.284	0.220	0.243
Sfo 52	(*n*)	119	85	90	102	60	73	75	72
	195	0.189	0.218	0.233	0.235	0.142	0.185	0.147	0.243
	207	0.336	0.306	0.328	0.319	0.233	0.322	0.333	0.382
	215	0.000	0.000	0.006	0.005	0.000	0.007	0.013	0.000
	223	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000
	225	0.340	0.418	0.367	0.353	0.542	0.404	0.453	0.326
	227	0.013	0.006	0.006	0.000	0.008	0.021	0.013	0.007
	231	0.109	0.041	0.039	0.064	0.033	0.055	0.040	0.028
	235	0.013	0.012	0.022	0.025	0.033	0.007	0.000	0.014

Appendix 2.2. Continued.

Locus	Size (bp)	In-stream Adults 2004	In-hatchery Adults 2004	In-stream Adults 2005	In-hatchery Adults 2005	In-stream YOY 2005	In-hatchery YOY 2005	In-Stream YOY 2006	In-hatchery YOY 2006
Sfo 75	(*n*)	119	85	87	102	60	73	75	73
	179	0.702	0.735	0.695	0.672	0.617	0.692	0.727	0.740
	203	0.004	0.041	0.029	0.029	0.033	0.007	0.020	0.041
	207	0.000	0.000	0.000	0.005	0.000	0.000	0.007	0.000
	215	0.000	0.006	0.000	0.000	0.017	0.000	0.000	0.000
	225	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
	227	0.197	0.124	0.190	0.211	0.192	0.205	0.147	0.164
	231	0.000	0.000	0.000	0.005	0.017	0.000	0.000	0.000
	235	0.097	0.094	0.086	0.078	0.108	0.096	0.100	0.055
Sfo 86	(*n*)	119	86	87	102	63	74	75	72
	104	0.798	0.831	0.828	0.779	0.706	0.858	0.847	0.840
	113	0.017	0.012	0.029	0.025	0.008	0.034	0.027	0.007
	116	0.067	0.041	0.057	0.083	0.103	0.007	0.040	0.056
	119	0.101	0.093	0.069	0.083	0.071	0.081	0.073	0.076
	122	0.017	0.023	0.017	0.029	0.111	0.020	0.013	0.021
Sfo 88	(*n*)	119	86	86	102	63	74	75	72
	183	0.021	0.017	0.029	0.029	0.000	0.000	0.000	0.014
	186	0.576	0.558	0.581	0.603	0.659	0.480	0.627	0.472
	189	0.282	0.349	0.291	0.260	0.230	0.372	0.273	0.368
	192	0.122	0.076	0.099	0.108	0.111	0.142	0.100	0.139
	198	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.007
Sfo 24	(*n*)	117	83	90	102	61	74	74	72
	118	0.201	0.283	0.278	0.201	0.238	0.284	0.236	0.243
	121	0.795	0.711	0.722	0.779	0.754	0.709	0.750	0.750
	124	0.004	0.006	0.000	0.020	0.008	0.007	0.014	0.007

Appendix 2.2. Continued.

Locus	Size (bp)	In-stream Adults 2004	In-hatchery Adults 2004	In-stream Adults 2005	In-hatchery Adults 2005	In-stream YOY 2005	In-hatchery YOY 2005	In-Stream YOY 2006	In-hatchery YOY 2006
Sfo 91	(*n*)	117	81	88	102	58	70	74	72
	225	0.145	0.111	0.210	0.132	0.267	0.143	0.223	0.174
	231	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	233	0.000	0.006	0.000	0.000	0.000	0.007	0.000	0.000
	237	0.513	0.407	0.426	0.471	0.440	0.386	0.372	0.458
	241	0.231	0.346	0.244	0.250	0.190	0.186	0.304	0.167
	245	0.043	0.062	0.057	0.054	0.060	0.143	0.047	0.083
	249	0.013	0.025	0.017	0.020	0.000	0.029	0.027	0.035
	261	0.051	0.043	0.045	0.074	0.043	0.107	0.027	0.076
	269	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007
Sfo 113	(*n*)	118	86	89	102	63	74	75	71
	137	0.246	0.279	0.275	0.265	0.246	0.236	0.320	0.289
	140	0.352	0.285	0.258	0.250	0.317	0.338	0.340	0.324
	146	0.220	0.244	0.264	0.240	0.262	0.236	0.207	0.169
	149	0.004	0.041	0.022	0.020	0.008	0.020	0.020	0.021
	155	0.174	0.151	0.174	0.225	0.167	0.169	0.113	0.197
	158	0.004	0.000	0.006	0.000	0.000	0.000	0.000	0.000
Sfo 115	(*n*)	117	86	89	102	64	74	75	70
	239	0.128	0.110	0.124	0.162	0.070	0.162	0.067	0.171
	241	0.094	0.134	0.096	0.176	0.086	0.115	0.167	0.086
	243	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000
	247	0.209	0.221	0.270	0.162	0.148	0.216	0.147	0.229
	309	0.564	0.529	0.494	0.485	0.664	0.500	0.607	0.514
	313	0.004	0.006	0.017	0.015	0.000	0.007	0.013	0.000

Appendix 2.2. Continued.

Locus	Size (bp)	In-stream Adults 2004	In-hatchery Adults 2004	In-stream Adults 2005	In-hatchery Adults 2005	In-stream YOY 2005	In-hatchery YOY 2005	In-Stream YOY 2006	In-hatchery YOY 2006
Sfo 38	(*n*)	117	83	90	102	62	74	74	72
	143	0.150	0.199	0.172	0.152	0.185	0.101	0.284	0.215
	146	0.060	0.054	0.017	0.059	0.073	0.101	0.041	0.021
	149	0.791	0.741	0.811	0.789	0.742	0.797	0.669	0.764
	152	0.000	0.006	0.000	0.000	0.000	0.000	0.007	0.000
Sfo 12	(*n*)	117	80	89	101	61	74	73	69
	197	0.235	0.300	0.287	0.297	0.451	0.297	0.390	0.275
	253	0.248	0.181	0.208	0.139	0.172	0.176	0.103	0.217
	271	0.504	0.513	0.483	0.545	0.336	0.527	0.459	0.464
	273	0.013	0.006	0.022	0.020	0.041	0.000	0.041	0.043
	275	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000