Appendix D – Genetic Conservation Considerations (excerpt taken from Whitesel et al. 2004)

Measures of genetic diversity within and between populations are principal attributes by which to infer population (breeding) structure. Genetic data can provide an indication of the extent of reproductive isolation among groups. Molecular genetic markers such as allozymes and nuclear or mitochondrial DNA can be used to statistically describe a species population structure based on measures of genetic similarity between groups. Although inference about population structure from data on genetic characters requires various assumptions, there is a growing body of literature from genetic studies of bull trout that allows for general conclusions to be made. Most research, using allozymes, mitochondrial DNA, and microsatellite DNA has found that bull trout exhibit relatively low levels of intrapopulation variation, but high levels of interpopulation variation (Costello et al. 2003; Kanda and Allendorf 2001; Neraas and Spruell 2001; Spruell et al. 1999; Taylor et al. 1999; Whiteley et al. 2003; Williams et al. 1995). Even in the case where bull trout populations are connected by suitable habitat, reproductive isolation appears to occur between adjacent drainages (Kanda and Allendorf 2001) and within the same tributary (Spruell et al. 1999).

In a study across a broad geographic range using mitochondrial DNA, Taylor et al. (1999) found that significant variation did exist within individual sample sites, but that most of the molecular variation resides at the inter-population and inter-region levels, with greater variation between regions considered at greater scales. Spruell et al. (2003) collected and examined data on four microsatellite loci from 65 bull trout populations in the northwest United States. Their findings concurred with previous work that bull trout have relatively low levels of genetic variation within populations compared to other salmonids. They found that population-specific levels of heterozygosity varied substantially among the different regions, perhaps reflecting historic isolation due to geography. Systems with large natural lakes were found to have above average heterozygosities. Spruell et al. (2003) also caution that genetic drift and low levels of variation appear to have influenced the relationships inferred from their data.

The degree of population differentiation in bull trout tends to be higher than among other salmonids. A commonly used indicator of degree of population subdivision is Wright's fixation index (F_{st}), which characterizes the reduction in heterozygosity of a subpopulation due to genetic drift (Hartl 1988), and can be used as an indicator of relative levels of gene flow in different species. It provides a measure of the proportion of genetic variation that lies between subpopulations within the total population. Values of F_{st} can range between 0 and 1, with higher values indicating greater genetic difference between populations. The mechanisms influencing genetic variation among and within populations include historical processes of glacial refugia, colonization and gene flow, natal stream fidelity, life history form, natural and anthropogenic barriers, patch occupancy, habitat complexity, spatial connectivity, and effective population size (Costello et al. 2003; McPhail and Baxter 1996; Neraas and Spruell 2001; Rieman and Allendorf 2001; Spruell et al. 2003; Spruell et al. 1999).

The genetic variation between and within bull trout populations represents their evolutionary potential (Laikre 1999). Their evolutionary lineages provide the basic genetic template for that to occur. Laikre (1999) concurs with the majority of authors who suggest that conservation efforts should focus on evolutionary lineages within the species. Doing so will preserve the genetic legacy from which bull trout evolved. When available, genetic data for bull trout is critical in trying to discern population structure and identify evolutionary lineages, however it is not necessarily sufficient.

Allendorf and Leary (1986) show that the evolutionary potential of any species depends upon the amount of genetic variation it contains. Once genetic variation is lost, it must be replaced by the slow process of genetic mutation, which can take many generations. Genetic variation needs to be preserved in order to increase the likelihood of a species survival. Genetic variation is the raw material from which populations adapt to changing environments and is critical to evolutionary change (Meffe and Carroll 1997). The concept that connects evolutionary potential to genetic variation was first formulated by Fisher (1930) in his 'fundamental theorem of natural selection.' Fisher (1949) rephrased this theorem as: 'The rate of increase in average fitness of a population is equal to the genetic variance of fitness of that population.' Loss of genetic variation may occur at low population levels through genetic drift and inbreeding depression (Fisher 1949). Wang et al. (2002) found that inbreeding in salmonids is often associated with a reduction in mean phenotypic value of one or more traits with respect to fitness. They believe that although experimental studies detected inbreeding depression in salmonids, its genetic basis has rarely been addressed or demonstrated in the wild (Wang et al. 2002).

Nevertheless, Wang et al. (2002) feel this reinforces the importance of maintaining genetic variation within populations as a primary goal of conservation and management. Loss of genetic variation can have deleterious effects on the development, growth, fertility, and disease resistance of fishes, among other processes important to survival and reproduction (Danzmann et al. 1985; Kincaid 1983; Kripichinikov 1981; Leary et al. 1985; Leary and Booke 1990). This loss of variation may also negatively affect fitness and preclude adaptive change in populations (Frankham 1995).

Deciding what needs to be conserved in order for a species to perpetuate is the basic issue for any conservation activity. Recognizing that there can be considerable biological diversity within a species, an approach that focuses on just conserving species is not enough. The evolutionary potential, represented by the genetic variability within and between populations of a species must also be conserved in order for the species to evolve in response to short-term and long-term environmental changes (Frankel and Soulé 1981). This is particularly important for a species like bull trout where distinct genetic differences have been observed between populations and where within population variation is low (Neraas and Spruell 2001; Spruell et al. 2003; Spruell et al. 1999; Taylor et al. 1999).

Since the 1998 ESA listing, DNA analyses have suggested that bull trout may be organized on a finer scale than previously thought. In the past 10 years a tremendous volume of genetic information about bull trout has been developed. Much of what is known about the evolutionary process and bull trout genetics has been developed in the last few years. Mitochondrial DNA data has revealed genetic differences between coastal populations of bull trout, including the lower Columbia and Fraser rivers, and inland populations in the upper Columbia and Fraser river drainages, east of the Cascade and Coast (Taylor et al. 1999; Williams et al. 1995). Nuclear DNA allele frequencies at microsatellite loci have revealed an apparent genetic differentiation between inland populations within the Columbia

River Basin. This differentiation occurs between (a) mid-Columbia (John Day, Umatilla, Walla Walla), lower Snake River (Clearwater, Grande Ronde, Imnaha rivers, etc) populations and (b) upper Columbia (Methow, Clark Fork, Flathead River, etc.), upper Snake River (Boise River, Malheur River, Jarbidge River, etc.) populations (Spruell et al. 2003). Allozyme, mtDNA, and nDNA data indicate bull trout inhabiting the Deschutes River drainage of Oregon are derived evolutionarily from coastal populations and not from inland populations in the Columbia River Basin (Leary et al. 1993; Spruell and Allendorf 1997; Taylor et al. 1999; Williams et al. 1995).

Although there are multiple resources that contribute to the subject, Spruell et al. (2003) best summarized genetic information on bull trout population structure. Spruell et al. (2003) analyzed 1,847 bull trout from 65 sampling locations, four located in three coastal drainages (Klamath, Queets, and Skagit Rivers), one in the Saskatchewan River drainage (Belly River), and 60 scattered throughout the Columbia River Basin. They concluded that there is a consistent pattern among genetic studies of bull trout, regardless of whether examining allozymes, mitochondrial DNA, or most recently microsatellite loci. Typically, the genetic pattern shows relatively little genetic variation within populations, but substantial divergence between populations. Microsatellite loci analysis supports the existence of at least three major genetically differentiated groups (or lineages) of bull trout (Spruell et al. 2003). They are characterized as:

- "Coastal," including the Deschutes River and all of the Columbia River drainage downstream, as well as most coastal streams in Washington, Oregon, and British Columbia. A compelling case also exists that the Klamath River Basin represents a unique evolutionary lineage within the coastal group.

- "Snake River," which includes the John Day, Umatilla, and Walla Walla rivers. Despite close proximity of the John Day and Deschutes rivers, a striking level of divergence between bull trout in these two systems was observed.

- "Upper Columbia River," which includes the entire basin in Montana and northern Idaho. A tentative assignment was made by Spruell et al. (2003) of the Saskatchewan River drainage populations (east of the continental divide), grouping them with the Upper Columbia River group.

Spruell et al. (2003) noted that within the major assemblages, populations were further subdivided, primarily at the level of major river basins. Taylor et al. (1999) surveyed bull trout populations, primarily from Canada, and found a major divergence between inland and coastal populations. Costello et al. (2003) suggested the patterns reflect the existence of two glacial refugia, consistent with the conclusions of Spruell et al. (2003) and the biogeographic analysis of Haas and McPhail (2001). Both Taylor et al. (1999) and Spruell et al. (2003) concluded that the Deschutes River represented the most upstream limit of the Coastal lineage in the Columbia River Basin.

A number of different definitions and parameters have been used to describe populations and their size. From a theoretical perspective, an ideal population is a discrete population in which all adults mate randomly and reproduce at the same age, once in their life (Frankham 1995). Ideal populations also have an equal sex ratio and all individuals have an equal probability of contributing offspring to subsequent generations (Frankham 1995). Few, if any, natural populations conform to these ideal conditions. Thus, within a population, the census number of sexually mature individuals per generation (N) is not necessarily a measure of how many individuals reproduce effectively, and thus, the amount of genetic variation transmitted between parental and progeny generations (Allendorf and Ryman 1987). The effective population size (N_e) has been defined as the size of the ideal population that will result in the same amount of genetic drift as in the actual population being considered (Wright 1969) or as the number of individuals per generation that actually spawn and produce offspring in the next generation (Crow and Kimura 1970; Lynch 1990). The effective breeding population size (N_b) has been defined as the number of individuals per year that actually spawn and contribute offspring the next generation assuming the number of progeny per spawner follows a Poisson probability distribution (Waples 1990). For semelparous species, N_e can be estimated by multiplying N_b and generation length (g), or the average age of spawners (Waples 1990). Although the relationship is complicated by multiple spawning events, N_e for iteroparous species can also be approximated by the mean number of first time spawners multiplied by generation length (Hill 1972).

The likelihood that a population will persist (or go extinct) over time depends on both its demographic size and genetic effective size. The ability of a population to persist is, in part, a function of stochastic events as well as demographic and genetic risks. The impacts to a population of stochastic events are difficult to predict. For demographic risks to be minimized, it has been shown that the variance in population abundance over a time period covering two or more generations needs to be less than the mean abundance during that period. In general, however, unless population sizes are very small, demographic risks can be difficult to quantify. Alternatively, various size thresholds have been identified that are associated with the genetic risk to populations. Theoretical models of genetic characteristics have suggested that the effective size (N_e) of a population (or group of populations) needed to minimize genetic risk typically range from 50 (to prevent inbreeding depression in closed populations) to 5,000 (for entire species to have sufficient genetic variation to respond to changing, or stochastically variable, environmental conditions) (Allendorf et al. 1997; Lande 1995; Thompson 1991).

Genetic variation is the raw material that allows organisms to adapt evolutionarily to changing environments. Significant reductions and fragmentation of habitat and associated reductions in population sizes have the potential to rapidly change the genetic composition of populations due to both random genetic drift in isolates and altered selection regimes. The amount of genetic variation in a population is a balance between (a) losses due to random genetic drift and directed natural selection and (b) gains due to mutation and migration from other populations (Wright 1931). Loss of genetic variation can influence the dynamics and persistence of populations through three mechanisms: inbreeding depression, loss of phenotypic variation, and loss of evolutionary potential (Allendorf and Ryman 2002). The loss of genetic variation in a population is directly influenced by N_e (Ryman et al. 1995).

Effective population size is a parameter that incorporates relevant demographic information and influences the evolutionary consequences of members in a population (Wright 1931). When prioritizing populations for conservation, N_e is an important parameter. In a population that is finite but otherwise randomly mating, the rate of loss of genetic variation and the rate of increase in inbreeding is inversely related to N_e (Waples 2002). Within a population, N and N_e are the same when the following conditions are met: constant and large population size, variance in reproductive success is binomial (number of progeny per parent follows a Poisson distribution), and sex ratio is equal. Because most populations do not conform to these conditions, the N_e to N ratio is usually below 1.0 (Frankham 1995). For example, in a population that has 20 mature females and 30 mature males (i.e., differing sex ratios), N=50. Based on the formula $N_e = 4 N_m N_f / N_m + N_f$ (where N_m = the number of males and N_f = the number of females) random mating among these individual would yield $N_e = 48$. In this case the N_e to N ratio would be 0.96 (48/50). The N_e to N ratio for most bull trout populations is thought to be between 0.15 and 0.27 (Rieman and Allendorf 2001).

Effective sizes of more than 50 have been considered a minimum requirement to ensure the short-term persistence of a local population (Allendorf and Ryman 2002). Effective population sizes smaller than 50 are subject to the effects of inbreeding (Franklin 1980). Over very few generations, inbreeding can reduce the amount of potentially adaptive genetic variation within local populations (Lande 1995). Increased homozygosity of deleterious recessive alleles is thought to be the main mechanism by which inbreeding depression decreases the fitness of individuals within local populations and viability of these populations (Allendorf and Ryman 2002). Deleterious recessive alleles are introduced into the genome via random mutations, and natural selection is slow to purge them because they are usually found in the heterozygous form where they are often not detrimental. When local populations become small, heterozygosity decreases at the rate of $0.5N_e$ per generation which in turn causes an increase in the frequency of homozygosity of all alleles, including those that are deleterious recessive (Lande 1995). Hedrick and Kalinowski (2000) provide a review of studies demonstrating inbreeding depression in wild populations (also see Wang et al. 2002).

By preventing significant loss of genetic variation from genetic drift, effective population sizes of 500 have been considered a minimum requirement to ensure the long-term persistence of local populations or metapopulations (Allendorf and Ryman 2002). Over ecological time scales, or centuries, effective populations larger than 500 may be necessary to avoid the risks from random genetic drift (Franklin 1980; Soulé 1980). In effective populations smaller than 500, the loss of genetic variation from drift is likely to exceed the increase in genetic variation from mutation (Lande 1995). When the lost genetic variation is associated with heritable traits (such as age at maturity), a population can also lose genetic variation for quantitative traits. Although phenotypic differences may have little effect on individual fitness, the loss of life-history variability among individuals may reduce the likelihood of a population being viable (Allendorf and Ryman 2002). Maintaining an effective population size large enough to prevent the erosion of quantitative traits may require gene flow from neighboring populations or within a metapopulation (Allendorf and Ryman 2002).

To be able to adapt over evolutionary time periods, N_e greater than 5,000 has been recommended for entire species or discrete groups that share an evolutionary legacy within a species (Lande 1995). When the persistence of a species, taxon, or phylogenetic lineage is of concern, it is important to consider the amount of genetic variation necessary to uphold the evolutionary potential that is needed for that taxon to adapt to a changing environment. A large amount of genetic variation may be selectively neutral under present environmental conditions (i.e., during the time when new mutations underlying that genetic variation arose). However, some of this variation may be at a selective advantage when environmental conditions change and a species must adapt to those changes or potentially face extinction. Thus, for retention of evolutionary potential, an N_e greater than 5,000 or (following from (Rieman and Allendorf 2001)) N greater than 10,000 (5,000/0.5) would apply to the largest grouping of fish that share an evolutionary trajectory (Franklin and Frankham 1998; Lynch and Lande 1998). Populations of this size are able to retain additive genetic variation for fitness-related traits gained via neutral-mutations at the time of their origin (Franklin 1980).

Population structure is often complicated and dynamic. Isolated local populations function autonomously, demographically independent of other local populations. Local populations that are not isolated may exchange genetic material on a regular basis and be structured as part of a larger metapopulation. In addition, relatively large groups of local populations or groups of metapopulations that share an evolutionary trajectory may be structured as evolutionary (or conservation) units. Effective population size is associated with the population unit being considered and has both a temporal and spatial element (Allendorf and Ryman 2002; Waples 2002). When N_e less than 50 for an isolated population, inbreeding depression may be expected to occur over relatively few generations (e.g., 2-5 generations). When N_e less than 500 for an isolated population or single metapopulation, loss of genetic variation due to genetic drift may be expected to occur over tens of generations. When N_e less than 5,000 for an entire species or evolutionary lineage within which some gene flow occurs, loss of evolutionary potential may be expected to occur over hundreds of generations.

Bull trout specific benchmarks have been developed concerning the minimum N_e necessary to maintain genetic variation important for short-term fitness and long-term evolutionary potential. These benchmarks are based on the results of a generalized, age structured, simulation model, VORTEX (Miller and Lacy 1999), used to relate N_e to the number of adult bull trout spawning annually under a range of life histories and environmental conditions (Rieman and Allendorf 2001). In this study, the authors estimated N_e for bull trout to be between 0.5 and 1.0 times the mean number of adults spawning annually. Rieman and Allendorf (2001) concluded that an average of 100 (i.e., 50/0.5 = 100) adults spawning each year would be required to minimize risks of inbreeding in a population and that 1,000 adults (i.e., 500/0.5 = 1,000) are necessary to prevent loss of genetic variation due to genetic drift. This later value of 1,000 spawners may also be reached with a collection of local populations among which gene flow occurs.

The combination of resident forms completing their entire life cycle within a stream and the homing behavior of the migratory forms returning to the streams where they hatched to spawn can promote reproductive isolation among local bull trout populations. This reproductive isolation creates the opportunity for genetic differentiation and local adaptations to occur. However, migratory behavior and straying from natal streams also provide a mechanism to maintain genetic continuity among breeding units (local populations) located in different streams or tributaries. This type of connection of local populations, linked by migration, is termed a metapopulation (Hanski and Gilpin 1997). Where local populations cannot support the minimum N_e necessary to maintain genetic variation important for long-term evolutionary potential, managers should attempt to conserve a metapopulation that is at least large enough to meet the minimum of 1,000 annual spawners.

Guidelines on effective population size appear to apply reasonably well to bull trout (Rieman and Allendorf 2001). The recommendation that N_e exceed 50 to avoid inbreeding depression appears to be most closely related to the short-term genetic viability of local bull trout populations. The recommendation that N_e exceed 500 to avoid the loss of genetic and phenotypic variation through drift appears to be most closely related to the long-term persistence of groups of local populations among which gene flow occurs to form a metapopulation of bull trout. Since few local populations may support a N_e greater than 500 (Rieman and Allendorf 2001), effective populations of this size may often require the possibility of gene flow between local populations. It also appears reasonable that effective population sizes that exceed 5,000 may be required to ensure the evolutionary persistence of bull trout conservation units.

The risk of extinction for a population is clearly related to its size and its variance in abundance relative to its mean size over time. More specifically, theoretical evidence suggests that inbreeding and genetic drift are likely to occur in populations when N_e less than 50 and 500, respectively. When detailed information is lacking for bull trout populations, these guidelines would be the most useful tool for managers to apply for avoiding loss of genetic variation and trying to ensure population persistence. These numbers represent relatively straightforward and defensible, theoretical minimums. While theoretical N_e can reflect the minimum number necessary to alleviate certain genetic risks, it does not necessarily reflect the most appropriate population size. Detailed information for a population may allow the justification of effective population sizes larger or smaller than 50 or 500. If possible, when estimating the population size necessary for persistence, managers should consider, for example, demographic risks and selective pressures as well as stochastic and historical events in addition to genetic risks.

It is clear that a sufficient N_e is a necessary consideration for conserving bull trout populations. Except for well-documented exceptions, the 50, 500, and 5,000 values should be considered necessary minimums and viewed as generalizations. For any given population the specific N_e necessary for conservation purposes will depend on characteristics of the population such as the ratio of $N:N_e$, the dominant life history form present, and the frequency of spawning.