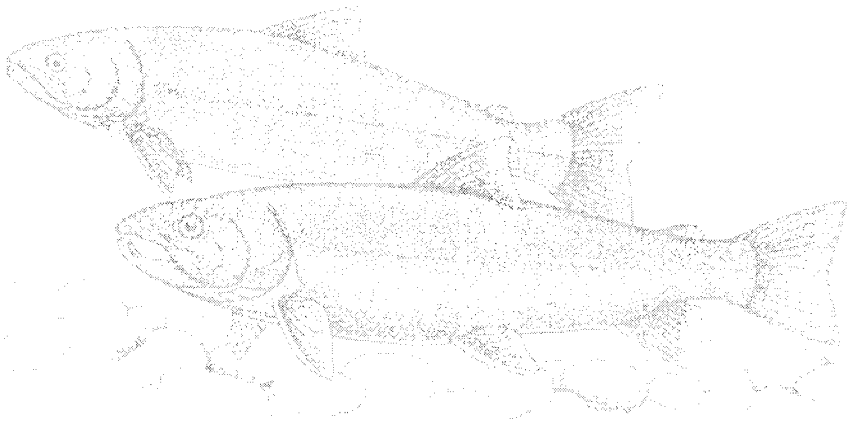
Bull Trout Recovery Planning: A review of the science associated with population structure and size



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PREFACE

Bull trout (*Salvelinus confluentus*) were listed as threatened in 1998. In 2002, the U.S. Fish and Wildlife Service published a Draft Recovery Plan for bull trout (USFWS 2002). As a result of internal, public and peer review of the Draft Recovery Plan several scientific questions were raised regarding the Draft Recovery Plan. These questions involved 1) attributes for the delineation of population units in the Draft Recovery Plan and 2) the number of bull trout necessary for recovery. The Management Oversight Team (MOT) of the U.S. Fish and Wildlife Service is responsible for guiding the recovery planning process. The MOT requested the Science Team of the U.S. Fish and Wildlife Service address the issues identified above by conducting a thorough review and analysis of the scientific information available. The results of that review are presented in this document.

REVIEW OF QUESTIONS

- Question 1a. With consideration for the varying levels of information available across the range of bull trout, is the definition below appropriate to identify panmictic groupings of bull trout? If not, what best describes groupings of bull trout representing local populations (panmictic populations)? What are the population attributes that are most useful for delineating these groupings?
    - *In the Draft Recovery Plan, local populations are defined as follows: "A group of bull trout that spawn within a particular stream or portion of a stream system. Multiple local populations may exist within a Core Area. A local population is considered to be the smallest group of fish that is known to represent an interacting reproductive unit. For most waters where specific information is lacking, a local population may be represented by a single headwater tributary or complex of headwater tributaries. Gene flow may occur between local populations (e.g. those within a core population), but is assumed to be infrequent compared with that among individuals within a local population."*

- Question 1b. Is there evidence to support that local populations are structured as a metapopulation? Does evidence for bull trout support a specific type of metapopulation structure (e.g. Levins model or Patch Model Hanski and Gilpin 1997)? Do these metapopulation units interbreed to a substantial degree and do they exhibit demographic independence (on a 100 year timescale)? Were the Core Areas in the Draft Recovery Plan delineated consistently with the prevailing evidence on metapopulation structure? If not, recommended criteria for adjusting delineations.

- Question 1c. What rationale best organizes groups of local populations into discrete units individually necessary to ensure evolutionary persistence? What are the population attributes that are most useful for delineating these groupings? Do these attributes support the conservation of genetic robustness, demographic robustness, important life history stages, or some other feature necessary for long-term sustainability?

- Question 1d. For the Columbia River Distinct Population Segment, use the attributes in the answer to question 1c to recommend delineation of areas that are discrete units individually necessary to ensure evolutionary persistence.

- Question 2. Were defensible abundance criteria selected in the Bull Trout Recovery Plan to address whether, a) In local populations, inbreeding depression is likely to occur when $N_e < 100$ (e.g. 0-100 years, generational time scale) and b) In related groups of local populations, or metapopulations, loss of genetic variation (e.g. drift) is likely to occur when $N_e < 1{,}000$ (e.g. 100-1000 years, ecological time scale)?

SUMMARY

Local Populations

The definition of local population in the Draft Recovery Plan describes units which closely approximate a panmictic group when compared to higher level population groupings defined in the plan. Panmixia is an ideal case of completely random breeding between individuals. Groups of animals, with a few exceptions, are unlikely to exhibit strict panmixia. Genetic, biogeographic and other evidence support the splitting of Core Areas into local populations. Some local populations of bull trout identified in the recovery plan, however, are isolated from other populations (for example, above a barrier) such that they have no opportunity for interbreeding or recolonization from other local populations. These small isolated populations do not belong to a core population, but may represent a significant component of the genetic legacy of the species. In defining local populations, the Draft Recovery Plan properly excludes attributes that may be misleading (such as life history form) or would be especially difficult to measure or estimate (such as extinction risk).

Metapopulations

Understanding the relevance of metapopulation theory to bull trout population structure is important in recovery planning. Evidence regarding whether local populations of bull trout tend to be structured as metapopulations is equivocal, but the weight of evidence from different sources suggests that generally, local populations within Core Areas do act (or historically have acted) to some extent as metapopulations, within a 100 year timescale. If different timescales were considered, the applicability of the metapopulation concept to bull trout Core Areas might be greater or less. It can be difficult in practice to determine how much current population structure is reflective of historical, natural organization and how much is due to anthropogenic effects such as habitat fragmentation. Available evidence does not point unambiguously to one model of metapopulation structure being universally relevant to bull trout. Authors studying bull trout population structure have suggested that island or patch models, where patch sizes are considered equal or migration rate is considered independent of distance between patches, are the least useful for bull trout. A number of authors have suggested that their genetic data and the biogeography and life history of salmonids in general and bull trout in particular imply that source-sink or mainland-island and particularly stepping stone (isolation by distance) metapopulation models are better supported and more useful for representing historical and current bull trout population dynamics. An understanding of the geological history of a given area may help determine which model is most appropriate for that case. Evidence with which to assess metapopulation structure for specific Core Areas in most Recovery Units is lacking, and it is beyond the scope of the Science Team to offer an opinion on the appropriateness of the demarcation of specific Core Areas to metapopulation theory. We do recommend that the following sentence in the recovery plan's definition of Core Area is too ambiguous to be useful and should be deleted: "A Core Area represents the closest approximation of a biologically functioning

unit for bull trout." Without reference to timescale or definition of what a "biologically functioning unit" is, the statement could apply to almost any aggregation of bull trout from an opposite sex pair of fish to the entire species.

Conservation Units

Biogeographical and genetic data suggest a divergence in coastal and inland populations of bull trout, likely occurring 10,000-15,000 years ago, with three separate lineages within the Columbia basin. Since then, significant genetic variation between populations has occurred which reflect, and influence, adaptations to the range of environmental conditions bull trout experience. These adaptations are shown in the diversity found in life histories, phenotype, behavior, temporal and spatial distribution, habitat selection and inter- and intra-specific relationships. The genetic variation observed has largely been influenced by short-term and long-term environmental changes, both natural and human-caused. It has occurred in interconnected as well as fragmented or isolated habitats. The evolutionary potential, represented by the genetic variability within and between populations must be conserved in order for the species to evolve, especially when environmental change is amplified by anthropogenic activities. A consensus has emerged that the population unit for conservation should focus on evolutionary lineages (see NRC 1995). The population, rather than the species, is the ecological and evolutionarily functional unit, since the population level is where genetic changes take place over generations and where local adaptations occur. Providing an interconnected continuum of complex habitats that support diverse life histories and life cycles is key to maintaining gene flow, genetic variation and facilitating metapopulation dynamics.

If a bull trout conservation unit is composed of a group of metapopulations and isolated local populations representing a discrete segment of biological diversity that shares evolutionary lineage and contains the potential for a unique evolutionary future, then long-term sustainability will be possible. Determinates of a bull trout conservation unit should include information on genotypic and phenotypic variation, ecological distinctiveness and importance, geographic location, temporal isolation, historic and prehistoric range changes and causes, behavior, and reproductive characteristics. Our assessment is that using these determinants to construct bull trout conservation units should provide the population attributes needed for long-term sustainability while exercising precautionary principles. Within a conservation unit is a complex and diverse environmental template that allows full expression of genotypic, phenotypic and spatial diversity among bull trout populations. Conserving this template will help ensure resilience and persistence when environmental changes occur.

Columbia River Distinct Population Segment

The genetic population structure of bull trout is complex. In the Draft Recovery Plan the species has been grouped into Distinct Population Segments. New information (Spruell et al. 2003) suggests that these groupings warrant further evaluation. Recovery Units (as

defined in the Draft Recovery Plan) provide a structure that considers both the genetic relationship of local populations and management options. Core Areas generally reflect metapopulation structure, which may be the functional demographic units in some circumstances (see Dunham and Rieman 1999). However, in other circumstances (see Spruell et al. 1999; Kanda and Allendorf 2001) strong local adaptation by bull trout may preclude them from functioning within a simple metapopulation structure. Most of the existing genetic data have been generated only within the past five years, and further understanding of the evolutionary history of bull trout throughout their natural range will undoubtedly be revealed as genetic studies continue.

The basic unit for ensuring long-term sustainability is the aggregation of local populations into Core Areas. The aggregation of Core Areas along evolutionary lineages should form the basis of conservation units in order to maintain the elements necessary for recovery. The integration of biological attributes associated with population identification as well as recent studies (Leary et al. 1993; Spruell et al. 2003) point toward organizing conservation units along Coastal-Puget Sound, Klamath River, Upper Columbia River, and Snake River lineages. However, at this time we do not have sufficient information to determine whether the extirpated bull trout population in the McCloud River of the Sacramento River basin should be a conservation unit or lumped with an existing conservation unit. Given the present biological information, we believe these conservation units are the best estimate for delineation of areas that are necessary to ensure evolutionary persistence of bull trout. For bull trout, recent genetic data and analyses and biogeographical analyses (Williams et al. 1997; Haas and McPhail 2001; Taylor et al. 2001; Costello et al. 2003; Spruell et al. 2003) are important information to guide the organization of bull trout populations into discrete units necessary for conservation and recovery. The bull trout populations that would be grouped to form these Conservation Units are not the same as those that were grouped to form the DPSs or Recovery Units identified in the Draft Recovery Plan.

Population Size

Many studies have described a relationship between the effective number of reproducing individuals in a population and the genetic risks to that population. Theoretical genetics and available empirical data for a variety of outcrossing organisms (see Franklin 1980; Lande 1995) suggest that, in general, closed populations will begin to show inbreeding depression effects after a few generations with an effective population size (N_e) < 50. Similarly, over ecological time scales, closed populations will begin to lose genetic variation due to the random effects of genetic drift when N_e drops below 500. It is reasonable to suggest that similar theory applies to bull trout populations (see Rieman and Allendorf 2001). Although information from certain populations suggests deviations from this theory do exist (e.g. Odell Lake, also see Rieman et al. 1997), departures from the 50/500 concept associated with N_e should be supported by empirical data that is robust and well described. N_e is typically believed to be smaller than the mean number of bull trout attempting to spawn annually (or census number, N) within a population. Rieman and Allendorf (2001) conservatively estimated that as few as 0.5 x N effectively

contribute to the breeding population. Hence, the N for a single population, or a group of populations that regularly exchange migrants via gene flow, should be greater than 100 (50/0.5) to avoid inbreeding depression. Similarly, the N for a single population or group of populations among which gene flow occurs, either regularly or irregularly, should be greater than 1000 (500/0.5) to avoid potential losses of genetic variation (e.g. rare alleles, heterozygosity) due to random genetic drift. Lande (1995) further proposed that, for the largest grouping of fish sharing an evolutionary trajectory, a $N_e > 5,000$ (following from Rieman and Allendorf 2001, $N > 10,000$ or 5,000/0.5) is required to maintain the genetic variation necessary to respond to changing environmental conditions. The Draft Bull Trout Recovery Plan (USFWS 2002) appears to confuse the concepts of N_e and N when suggesting that N_e be greater than 100 or 1,000 to avoid inbreeding depression or genetic drift, respectively. If the Draft Recovery Plan consistently applied 100 and 1,000 thresholds to N or the 50 and 500 thresholds to N_e, and recognized possible exceptions to these, then the criteria would be used in a scientifically defensible manner.

POPULATION ATTRIBUTES

A population can be defined in biological terms as a reproductive community of individuals that share in a common gene pool (Dobzhansky 1950). Populations are often structured in a hierarchical manner to reflect the degree to which they are related, interact, and share evolutionary histories and trajectories. Although groups smaller than a local population (subpopulations) can sometimes be defined, the smallest functional unit of biological interest is generally the local population (or stock, see Ricker 1972). Multiple local populations may interact to form metapopulations (Hanski and Gilpin 1991). A single local population, single metapopulation, or multiple interacting metapopulations may, in turn, form distinct population segments (sometimes referred to as evolutionary units, evolutionarily significant units, or conservation units) within a species (see Waples 1995; Meffe and Carroll 1997). Taken together, all distinct population segments may be combined to account for a species (see Haig 1998). Various attributes have been used to evaluate the degree to which groups, in this case of bull trout, are related. These attributes consider information on phenotype, genetics, biogeography, habitat, life history, behavior, ecology, and distribution. Ultimately, attributes useful for delineating groups of populations all relate to reproduction and genetic exchange.

Phenotypic variation

Phenotype can be defined as the genetically and environmentally determined physical appearance of an organism. It is essentially the expression of genetic attributes (usually through environmental mediation) such as color, size, shape, etc. that can be quantified by osteological, morphometric, and meristic measurements or other classifiable characteristics. In the publication that formed the rationale for separating bull trout and Dolly Varden into two species, Cavender (1978) analyzed information on the size and shape of the head and jaws, head length, number of branchiostegal rays, morphology of the gill rakers, and the shape of certain bones in the skull. He stated that these characters remained relatively constant throughout the distributional range of bull trout. He explained that the observed characters were consistent with the direction of evolution of bull trout, away from a diverse predator (such as anadromous Dolly Varden) and toward a more specialized piscivore living mostly in fresh water. He further suggested that lake trout (*Salvelinus namaycush*) have undergone parallel evolutionary changes. Haas and McPhail (2001) revealed that bull trout groups differentiated by historical morphometric analysis matched groupings identified by molecular genetic patterns (Bellerud et al. 1997; Spruell and Allendorf 1997; Taylor et al. 1999).

Some unique or unusual bull trout populations have been described in the literature. Frequently, these populations are described from isolated headwater lakes, often in isolation from other fish species, and some are purported to exhibit dwarfism, nonpiscivory, brilliant spawning coloration, or other characteristics. For example, Carl et al. (1989) described the population in Pinto Lake, Alberta in detail. Where these populations are described, they are generally not compared to other regional populations

and while these cases represent the expression of important phenotypic extremes, there has been no comprehensive evaluation to indicate their particular genetic uniqueness. Similarly, some bull trout stocks express spawning or migratory patterns that, to date, have not been shown to have a genetic basis. In Montana, outlet spawning bull trout occur in the Flathead River basin in Upper Kintla Lake in Glacier National Park. An isolated adfluvial bull trout population in Bull Lake, in the Kootenai River drainage, migrates several kilometers downstream in Lake Creek before ascending Keeler Creek to spawn. Undoubtedly, there are bull trout populations with additional life history patterns, food habits, migratory patterns, and perhaps morphological or osteological differences that have not been quantified. Such populations merit further study, particularly if any of the variants can be linked to specific genetic patterns.

Bull trout and other species of char (e.g. brook trout, arctic char, Dolly Varden, white-spotted char) are nearly circumpolar and exhibit considerable complexity and variability in life history and migratory patterns as well as in phenotypic and genotypic attributes. The adaptive capabilities and plasticity exhibited within the char complex is well-documented though not thoroughly discussed in this document. A more thorough examination of literature for arctic char (Balon 1984), white-spotted char (Yamamoto and Morita 1999; Morita et al. 2000; Morita et al. 2002) and Dolly Varden would be necessary to fully explore these subjects. By necessity, this discussion is confined to observed variability in bull trout within the ESA-listed entity~~ies~~ in five northwest states, primarily within the Columbia River Basin. The relative usefulness of phenotypic variability in describing and categorizing local populations, metapopulations, or discrete evolutionary units is limited. The large amount that remains unknown, combined with the relative difficulty of classifying such information limits the present use of this information. However, phenotypic variability may be a useful modifier to consider when such classifications are made, and particularly when it comes to decisions about prioritizing stock conservation (see Allendorf et al. 1997).

Genetic variation

Measures of genetic diversity within and between populations are a principal attribute 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 (Williams et al. 1995; Spruell et al. 1999; Taylor et al. 1999; Kanda and Allendorf 2001; Neraas and Spruell 2001; Whitely et al. 2003; Costello et al. 2003). 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. Taylor et al. contrasted 12 watershed regions, each consisting of several pooled sample localities, and also compared drainages west of the Coast or Cascade mountain ranges with those east of the mountain ranges. Taylor et al. (2001), using microsatellite DNA, concluded that bull trout exhibit relatively low levels of within-population variation. However, they estimated that exchange among populations within watersheds they examined ranged from one to three individuals per generation, and that the most variation resided with populations, followed closely by variation among populations within watersheds. They concluded that the data suggest that bull trout populations are highly isolated from each other genetically and demographically within watersheds.

Spruell et al. (2003) collected and examined data on four microsatellite loci from 65 bull trout populations in the northwest U.S. 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}). F_{st} is 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's 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. Average F_{st} values from a number of studies of different salmonid species are shown in Table 1. The studies don't all use identical methods of quantifying genetic variation. However, while F_{ST} values are sensitive to the numbers of polymorphic loci, they are not sensitive to the technique used in their estimation (Allendorf and Seeb 2000). The comparison suggests that local bull trout populations tend to be more genetically distinct from each other than local populations of other salmonid species are from each other.

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 (McPhail and Baxter 1996; Spruell et al. 1999; Nerass and Spruell 2001; Rieman and Allendorf 2001; Costello et al.

The timing of migration and the timing and the frequency of spawning may add another dimension to the life history diversity (Rieman and McIntyre 1993). For instance, as with other salmonids, there may be multiple runs of bull trout within the same spawning tributary. In some instances genetic samples may show that geographically close populations are relatively distinct genetically. Telemetry in the Wenatchee River basin is showing that there are fish migrating from geographically different overwinter areas to spawn in the same tributaries, and some adults are returning at different times (USFWS 2000, 2001). This may be a form of multiple runs to the same natal stream. Information being gathered through ongoing telemetry studies suggests that some migrations may be for reasons other than spawning. Population structures may be influenced by all the mechanisms driving migration.

It has been suggested that maintaining variability in life history strategies and dispersal over many habitats may be as important to bull trout conservation as maintaining genetic variability (Rieman and Allendorf 2001). Maintenance of independent local populations requires maintaining conditions that lead to local adaptation and homing preferences. Should local populations interact to form larger, interacting groups with relatively predictable gene flow (metapopulations), then it is vital to maintain the opportunity for gene flow and the life history characteristics that influence dispersal of individuals. Adequate gene flow is heavily affected by demographic parameters such as reproductive rate and expression of different life history strategies. Salmonids exhibit many kinds of migratory life history strategies, ranging from resident or non-migratory to fully anadromous, which complicates predictions of gene flow between local populations. This is especially true when life history strategies expressed in a local population have been demonstrated to depend on local geographic and environmental conditions (Nelson et al. 2002).

Anadromous, fluvial, adfluvial, and resident forms of bull trout populations are somewhat artificial constructs. This is especially true when fish size is used as delineating criteria. While the majority of fish in a given population unit may conform to a specific life history strategy, there is increasing evidence from radio telemetry research and other studies that much of the life history behavior exhibited by bull trout may be facultative (Northcote 1992; Nelson et al. 2002). Life history form may be a conditional strategy, influenced by local environmental conditions that mediate migratory tendency, whereby both migratory and resident individuals may exist in a population of bull trout (Jakober et al. 1998, Nelson et al. 2002). Consequently, where they overlap, life history forms would not seem to be a definitve indicator of population structure. For example, in other char species (e.g. Arctic char – *S. alpinus*) the expression of feeding and life-history morphs can be influenced by exposing the developing juveniles to different feeding or other stimuli (Nordeng 1983; Hindar & Jonsson 1993; Mclaughlin & Grant 1994; Vøllestad & L'Abee-Lund 1994; Jonsson & Jonsson 2001; Boula et al. 2002; Adams et al. 2003; Klemetsen et al. 2003). Life history form is an important population attribute, deserving of continuing study and evaluation. The continued emphasis on the preservation of migratory life history forms is especially critical. However, the existing organization of the various life history forms as we understand them is of limited value as a tool for determining population structure. The presence or absence of particular life history

forms should not be used as a primary factor upon which to base the organization of units of evolutionary persistence. However, the presence of multiple life-history types in a population is likely a sign of population health and genetic variability.

The organizational opportunity presented by the linearity and natural partitioning of aquatic ecosystems must play a key role in advising the decision making process. Resident forms that represent populations would, by definition, be confined to a particular stream or stream reach and, if isolated, may function independently of other populations. Where this is a natural situation, such populations may also be considered evolutionary units. Where the resident population is the remnant of a fragmented migratory population, the conservation unit may be represented by the range of the former migratory population. Adfluvial forms are relatively easy to characterize, with the lake typically forming subadult and adult foraging, migrating, and overwintering habitat and portions of the interconnected river and tributaries functioning as the primary spawning habitat and rearing habitat for juvenile fish. In most cases, all the available genetic evidence indicates that such adfluvial systems function as biological units isolated from other lake systems, with limited genetic interchange, particularly with downstream waters (Spruell et al. 2002). Fluvial forms present the most difficult challenge on how to compartmentalize and organize populations, metapopulations and evolutionary units. Individual bull trout may stray far from their natal stream, and in many systems have the potential to return to these streams. In extreme cases, individual bull trout could perhaps range several hundred miles from their natal stream. Thus, while the range of the population must include a common sense evaluation, it must also be defined fairly broadly. Judgment calls will be required when defining the population structure associated with fluvial forms of bull trout and precautionary principles should be employed.

Behavioral evidence also sheds some light on the question of bull trout population structure. For example, telemetry studies of adult migratory bull trout have demonstrated that local populations (as described by the USFWS Draft Bull Trout Recovery Plan) overwinter in downstream areas such as lakes, reservoirs or river mainstems (Hemmingsen et al. 2000; USFWS 2000; USFWS 2001). Bull trout have been observed returning to local population spawning tributaries from multiple overwintering areas. Fish have also been observed migrating both within the same spawning season or within the same year, and in multiple years to multiple local population spawning tributaries. Data from telemetry projects indicate that there are some populations that appear to use smaller home ranges than other populations. These telemetry projects have demonstrated that the Columbia and Snake rivers can be important as overwinter areas and are within the home range for multiple populations (see Chelan County Public Utility District 2002, 2003).

Geographic location and temporal isolation

Along with genetic data, information on distribution should be a primary factor when evaluating how populations, and particularly evolutionary units, are structured. Bull trout

distribution data is relatively well-developed and well-documented in some areas, particularly in the upper portions of the Columbia River Basin (i.e. Montana, parts of Idaho). While historic distribution is typically less well-known, in a number of areas in the contiguous U.S. it is believed that current distribution essentially represents the historic distribution pattern of bull trout The historic distribution is important in defining how evolutionary units should be considered. See, for example, the earlier reference to biogeography by Haas and McPhail (2001) and genetic analysis by Taylor et al. (2001) and Costello et al. (2003). For areas where distribution is poorly known (both current and historic) a greater emphasis on habitat suitability modeling and monitoring and evaluation may be required.

Bull trout are known to exhibit patchy distribution, even in unroaded and unmanaged watersheds (see Rieman and McIntyre 1995). This characteristic, undoubtedly with strong historical correlation to habitat suitability, makes it difficult to predict with certainty the current or historical distribution of bull trout, especially in watersheds where water temperature and other parameters are not documented. In addition, portions of a watershed that are currently unsuitable for bull trout may have been historically suitable, or in fewer cases the opposite may apply. In part, for these reasons, distribution should be used in a general sense to define bull trout habitat patches, but there should not be an overemphasis on defining distribution at a fine scale. In perfectly healthy bull trout ecosystems, stochastic events such as fire, flood, and debris jams may cause distribution of bull trout in a particular watershed to vary over time.

While the issues of past and present population distribution and abundance are integral to determination of how to best organize groups of local populations into discrete units, it is a given that all of the information we might desire will not be available to decision makers in this process. For many populations, no information is available. The best data sets extend 10-20 years and are primarily for adfluvial populations in Flathead Lake, Swan Lake, and Lake Pend Oreille. While we can use these data sets to inform our decision-making process as to the relative abundance and annual variation in some of the more studied systems, ultimately some professional judgment will also be necessary.

LOCAL POPULATIONS

What is a panmictic grouping?

In short, panmixia refers to random mating of individuals where all individuals have equal probability of interbreeding. A panmictic unit or population is one in which all members randomly interbreed. Development of terminology referring to population structure was originally motivated to aid description of population genetic processes relevant to evolution. The smallest Mendelian population (or deme) is defined by Dobzhansky (1950): "A Mendelian population is a reproductive community of sexual and cross-fertilizing individuals which share in a common gene pool. The smallest Mendelian populations are panmictic units (Wright, 1943), which are groups of individuals any two of which have equal probability of mating and producing offspring." For sexually reproducing species, more precisely, a group is said to be panmictic if a mature individual is equally likely to mate with any mature member of the opposite sex in that group. Dobzhansky envisioned larger Mendelian populations to be groups that engaged in gene flow, the largest of which would be at the level of species. These larger groups would presumably include groups corresponding to what now are referred to as metapopulations.

It is difficult, if not impossible, to divide larger assemblages of animals into groups where the stated criterion for panmixia holds exactly. Most populations are spatially and temporally structured; individuals tend to cluster in areas of suitable habitat at appropriate times. In practice it is likely that any group of more than two adults would not strictly satisfy the condition of equal likelihood, given variation in microhabitat and differences in homing fidelity, breeding habitat preference, and mate preference. Dobzhansky's description of Mendelian populations consists of nested levels of gene flow, beginning with high gene flow and panmixia at the finest scale, with progressively less frequent gene flow as smaller units are accreted to form larger groups. Mating would not be completely random within the smallest groups, and reproductive isolation would be neither absolute nor permanent among groups at the highest level. However, as long as the trend for gene flow to decrease with hierarchical level is true, this model of population structure could still be useful in describing biological relationships between groups, as long as the potential for a common gene pool is present even at higher levels of organization.

The attributes of local populations (or subpopulations) are often defined concurrently with defining the attributes and behavior of groups of interacting populations, or metapopulations. Historically, metapopulation theory has focused on non-migratory species. Esler (2000) explored ways to apply metapopulation theory to the conservation of migratory birds. Esler suggested that the term "demographic panmixia" be applied to groups which cannot be divided into smaller groups (subpopulations) with independent extinction probabilities. Accordingly, Esler used extinction probability to define a subpopulation: "subpopulations must be sufficiently independent that extinction of a subpopulation can occur irrespective of the demographics of other subpopulations." Like

migratory populations of bull trout, groups of migratory birds that are geographically separate at one part of the life (or annual) cycle may occur together at others without implying panmixia, thus resulting in demographic independence of subpopulations. Similarly, Harrison and Taylor (1997), in the process of defining local extinction, imply an extinction probability criterion is useful for distinguishing local populations: "Local extinction may be defined as the extirpation of any population segment sufficiently closed to immigration that, once extinct, typically remains so for several generations or more. For local extinction to occur, populations on separate patches must be reasonably isolated from one another, with most recruitment coming from within the patch rather than from immigration."

Application of panmictic group to salmonids

In McElhany et al. (2000), NOAA Fisheries supplied the rationale for their delineation of population structure of anadromous Pacific salmonids for use in conservation assessment. NOAA Fisheries' guidance is tailored to their responsibility to conserve Evolutionary Significant Units (ESUs) of anadromous salmonids. Extinction risk and time scale are key to the population structure proposed in McElhany et al. They define an independent population as any collection of one or more local breeding units whose population dynamics or extinction risk over a 100-year period is not substantially altered by exchanges of individuals with other populations. McElhany et al. liken an independent population to Ricker's (1972) definition of a "stock": "an independent population is a group of fish of the same species that spawns in a particular lake or stream (or portion thereof) at a particular season and which, to a substantial degree, does not interbreed with fish from any other group spawning in a different place or in the same place at a different season." McElhany et al. also indicate that an independent population, while likely to be smaller than a whole ESU, is likely to inhabit a geographic range on the scale of an entire river basin or major subbasin.

McElhany et al. (2000) acknowledge that degree of reproductive isolation is a relative measure that increases from pairs of fish up to pairs of species, providing the rationale for the description of units below the independent population level: "Within a single population, for example, individual groups of fish are often reproductively isolated to some degree from other groups but not sufficiently isolated to be considered independent by the criteria adopted here. These groups of fish are termed 'subpopulations'." McElhany et al. liken spawning habitat to discrete patches, and add that if spawning among patches is limited, the independent population may be divided into subpopulations. While 'limited' is not defined in McElhany et al. (2000), the concept of local population in the Draft Bull Trout Recovery Plan (USFWS 2002) would seem to correspond closely to NOAA Fisheries' subpopulation, as long as exchange of individuals among local populations can affect extinction probabilities of those populations over a 100 year period. In any event, local populations or subpopulations would most closely approximate the property of panmixia.

Panmictic groups and local populations of bull trout

The attributes that have been used to define panmictic groups also have application to bull trout. Attributes described in the literature include: random mating, random genetic mixing, equal probability of mating, individuals that are not reproductively isolated, contiguous suitable habitat (i.e. relevant range of habitat is sufficiently connected so that dispersal is possible over the extent), consistent homing fidelity based on microhabitat and genetic similarities, similar breeding habitats, and similar mate preference. Random genetic mixing, individuals which are not reproductively isolated and similar breeding habitats are also attributes defined in the Draft Recovery Plan for bull trout (USFWS 2002). In addition, the Draft Recovery Plan defines local populations as those with the attributes of contiguous spawning habitat, interacting reproductive units, high rates of gene flow within local populations and gene flow among local populations. Although the Draft Recovery Plan definition of local populations is generally consistent with the literature descriptions of panmictic groups, it is not completely inclusive. However, as noted earlier, it is difficult to find an assemblage of animals where the stated criterion for panmixia holds exactly. A direct outcome of panmixia, or random mating, is that populations conform to Hardy-Weinberg genotypic proportions. In most cases, random samples of individuals from a population almost always reveal statistical conformance to panmixia. This would be evidence that local populations are indeed panmictic with respect to genetic markers used in population genetic surveys.

A panmictic group and local population as defined in the Draft Recovery Plan may be considered analogous to the smallest Mendelian population as described by Dobzhansky (1950). Also, Dobzhansky's portrayal of nested levels of gene flow, beginning with high gene flow and panmixia at the lower limit, with progressively less frequent gene flow as smaller units are accreted to form larger units, is similar in concept to the recovery plan's population hierarchy. Bull trout in at least some local populations likely have the potential to mate with individuals outside the local population but within a group of local populations (e.g. metapopulation or Core Area). Fish populations might be expected to be subdivided in a manner mirroring the structure of the basin they inhabit since gene flow among neighboring populations should be constrained by the linear nature of riverine systems (Whiteley et al. 2002). Spawners in some local populations may have the opportunity to mate only within the local population due to geographic or manmade structures such as barriers falls, debris slides, or dams. However, these barriers may allow passage downstream, while blocking upstream passage. Hence, gene flow may be present, but unidirectional, in some cases.

The population attributes discussed earlier are useful for delineating panmictic groupings of bull trout. However, a subset of the attributes may be more manageable to develop a practical definition of local population. While assessing the utility of any attribute it would be prudent to determine how well it can be quantified. For example, confirmation of true panmixia in a population is exceedingly difficult and probably unnecessary for practical purposes. On the other hand, it seems vital to determine population distribution based on spatial and temporal scales that may enable random mating. The most useful and manageable attributes for identifying local populations of bull trout include

individuals which: are not reproductively isolated, exist in connected and suitable habitat, share similar breeding habitats (space and time), have independent extinction probability from other groups, and whose risk of extinction is on a similar time frame (e.g. 100 years).

METAPOPULATIONS

What is a metapopulation?

Hanski and Gilpin (1991) provide a general definition of a metapopulation: a "[s]et of local populations which interact via individuals moving among populations." However, Hanski and Simberloff (1997) suggest a definition which seems even more inclusive, since it requires only the potential for interaction: a "[s]et of local populations within some larger area, where typically migration from one local population to at least some other patches is possible." Implicit in the concept of a metapopulation is the idea of turnover, which Hanski and Simberloff (1997) define as extinction of local populations and establishment of new local populations in empty habitat patches by migrants from existing local populations. Hanski and Gilpin (1997) contend that in the broad definition of a metapopulation, the rate of population turnover is irrelevant in determining whether some group of local populations is a metapopulation. Hanski and Simberloff (1997), however, write that one characteristic of a metapopulation is that local dynamics occur on a fast time scale in comparison with metapopulation dynamics.

Hanski (1997) suggests that if a system satisfies four conditions then a metapopulation approach based on Levins' (1969) original concept is likely to be helpful. Condition 1 is that the suitable habitat occurs in discrete patches that may be occupied by local breeding populations. Condition 2 is that even the largest local populations have a substantial risk of extinction. If this were not the case then the metapopulation would persist simply because of the persistence of the largest population(s), and would be an example of mainland-island metapopulation. Condition 3 is that habitat patches must not be too isolated to prevent recolonization. If they were, this suggests a nonequilibrium metapopulation heading toward global extinction. Condition 4 is that local populations do not have completely synchronous dynamics. If they do, the metapopulation would not persist for much longer than the local population with the smallest risk of extinction. At least some of these conditions would apply to any type of metapopulation (specific metapopulation models are defined later). In a review of theory relevant to and evidence for metapopulation structure in salmonids, Rieman and Dunham (2000) specified three conditions defining a metapopulation. Condition 1 is that habitat consists of discrete patches or collections of habitats capable of supporting local breeding populations. Condition 2 is that the dynamics of occupied patches are not perfectly synchronous. Condition 3 is that dispersal among the component populations influences the dynamics or the persistence of the metapopulation, or at least some of the local populations. Structuring and at least partial independence of local populations are the fundamental

concepts that distinguish a metapopulation from a simple panmictic group in a patchy environment.

In general, then, a metapopulation can be defined as a collection of relatively isolated, spatially distributed, local populations bound together by the potential for dispersal between populations. The timescale on which local populations must exchange migrants is unspecified. These dispersal events may be infrequent, but must be possible if recolonization of populations that have suffered local extinction is necessary. A metapopulation would have the potential to persist in the face of local extinctions precisely because of sufficient dispersal among populations. However, if dispersal among populations is so frequent that local extinctions never occur, the concept of a metapopulation may be inappropriate. There is no clear, universal demarcation for distinguishing between a metapopulation and one large population, but Hanski and Simberloff (1997) suggest that the metapopulation concept may be useful even when ten percent of individuals per local population per generation leave their natal patch. Harrison and Taylor (1997) assert that the critical level of migration will depend greatly on the exact type of metapopulation behavior.

Rieman and Dunham (2000) note that migratory behavior of salmonids can influence the degree to which local dynamics covary within a metapopulation. They point out that spawning and early rearing habitats may be distributed as discrete patches throughout a basin, though juveniles may migrate downstream. The common rearing habitats and migratory areas may act to link the dynamics of local breeding populations. Conversely, independent environmental variation and disturbance in spawning and rearing habitats may act to decrease the extent to which local populations covary (Rieman and Dunham 2000). McElhany et al. (2000) note that homing provides a mechanism for maintaining a hierarchy of reproduction isolation among groups of salmonids, while straying can offset the demographic and genetic isolation of those groups. The frequency and effect of straying are likely population-size dependent, however. Further, bull trout are iteroparous and exhibit relatively broad life-history variation when compared to the mostly anadromous salmonids that are the focus of McElhany et al. (2000).

Models of metapopulations

Various metapopulation schemes have been proposed in the scientific literature. These spatial patterns all assume that habitat is to some extent divided into discrete patches, surrounded by unsuitable habitats. For stream-dwelling animals, dry land would be an obvious example of unsuitable habitat. However, if the watershed alone is considered as the relevant geographic template, stream segments with suitable spawning habitat might be considered as patches of suitable habitat for local populations (breeding units), as suggested by Dunham et al. (2002), while stream reaches used only for migration could be considered "unsuitable" habitat, for the purposes of reproduction. Metapopulation models fall into several categories, not necessarily mutually exclusive, and the same model may go by multiple names in the literature. Several of these metapopulation models are illustrated in Figure 1.

1. Levins metapopulation (classical metapopulation)
First proposed by Levin (1969). A large network of similar small patches, with local dynamics occurring at a much faster time scale than metapopulation dynamics; sometimes used to describe a system in which all local populations have a high risk of extinction. Local populations are "rescued" from extinction by migrants, but straying is insufficient to create a patchy panmictic population (McElhany et al. 2000).

2. Mainland-island metapopulation (Boorman-Levitt metapopulation)
One or a few large stable populations ("mainlands") are surrounded by smaller local populations ("islands") that are prone to extinction (McElhany et al. 2000).

3. Patch model (Occupancy model, presence/absence model)
A metapopulation model in which local population size is ignored and the number (or fraction) of occupied habitat patches is modeled (Hanski and Simberloff 1997). Levin's model is an occupancy model (Hanski and Gilpin 1991).

4. Island (spatially implicit)
Any model in which all local populations are equally connected; patch models and structured metapopulation models are spatially implicit models (Hanski and Simberloff 1997).

5. Source-sink metapopulation
System where there are subpopulations with negative growth rates (in absence of dispersal) and those with positive growth rates (source populations) that support the sink populations with emigration (Hanski and Gilpin 1991). Source-sink metapopulations are similar to mainland-island metapopulations in that migration is nonreciprocal; however, unlike mainland-island, the difference between source and sink populations is due by definition to deterministic habitat differences, and source populations need not be larger than sink populations (Hanski and Gilpin 1991).

6. Nonequilibrium metapopulation
A metapopulation in which (long-term) extinction rate exceeds colonization rate or vice versa; an extreme case is where local populations are located so far from each other that there is no migration between them and hence no possibility for recolonization (Hanski and Simberloff 1997). A nonequilibrium metapopulation situation is described by Hanski and Simberloff as one in which a previously more continuous population becomes divided into smaller units, with consequent local extinctions, without the formation of a functional metapopulation. Instead, the group would merely be an assemblage of populations all slowly declining to extinction. It's possible to conceive of the converse situation as well, where population expansion into available habitat is rapid enough to obliterate all functional distinction between local populations; hence the apparent "metapopulation" would be a transitory phase.

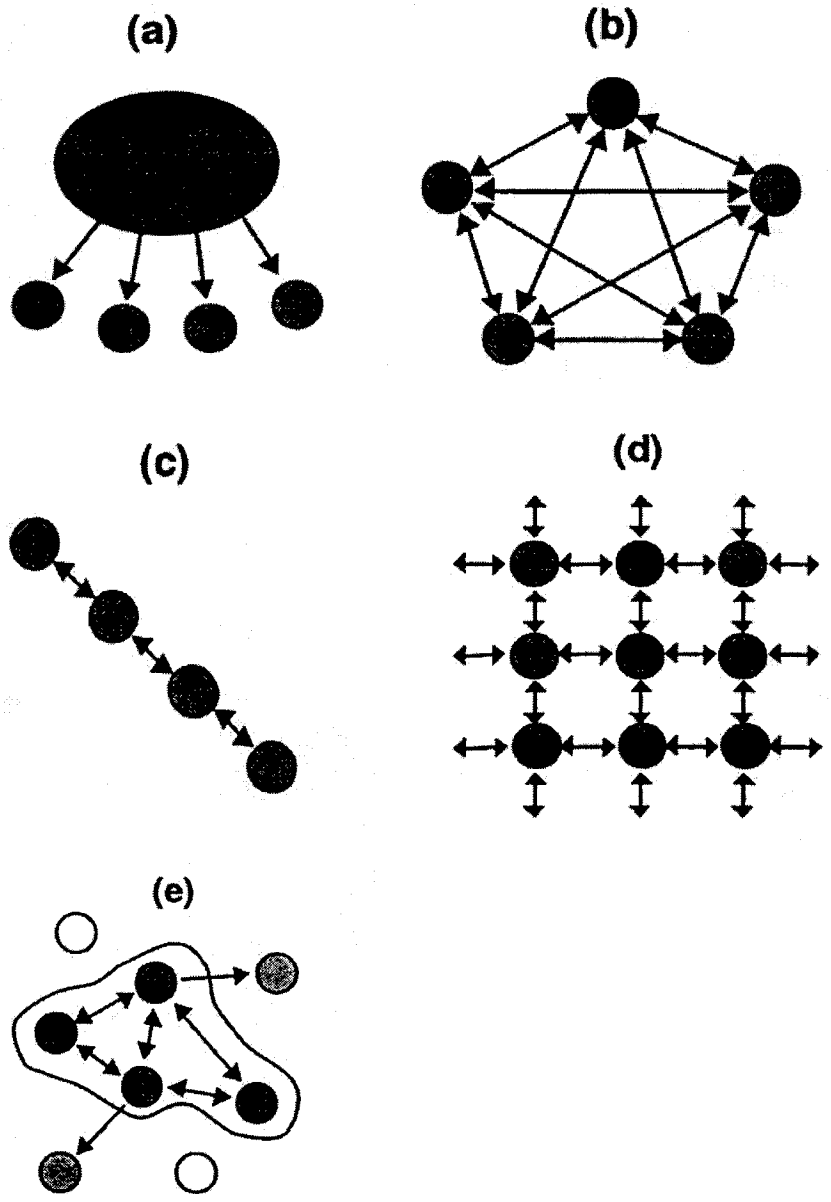


Figure 1. Five models of interacting groups of local populations (metapopulations): a) Mainland – Island: mainland provides all input to islands; b) Island model: migration equal among equally sized islands; c) Linear stepping stone: only neighboring populations exchange migrants; d) Two dimensional stepping stone (lattice): only surrounding populations exchange migrants; e) Levins-type metapopulation: regular extinction and recolonization (figure adapted from Harrison and Taylor 1997).

7. Stepping stone / isolation by distance / spatially explicit (linear or lattice [two-dimensional])

A population model in which migration is distance-dependent, often restricted to the nearest habitat patches; the patches are typically identical cells on a regular grid, and only presence or absence of the species in a cell is considered (Hanski and Simberloff 1997). This model has its origin in population genetics theory (Kimura & Weiss 1964), where it describes the case where gene flow primarily occurs between neighboring populations.

Evidence regarding metapopulation structure in fish

Several investigators have considered the question of which, if any, of the well known metapopulation models are most useful for describing the population structure of salmonids or other fish species. Gotelli and Taylor (1999) used data on 41 species native to the Cimarron River, Oklahoma, to assess the ability of metapopulation models to predict patterns of local extinctions and recolonizations. They found that the fraction of sites occupied did not affect extinction, and that for most species, position in the linear stream gradient was more important than site occupancy in determining colonization and extinction patterns. They concluded that a metapopulation model that incorporates spatial variability in extinction and colonization probabilities may be more appropriate for the system than classic Levins-style models. McElhany et al. (2000) note that many essential features of salmonid biology, such as discrete, dynamic habitat patches and limited straying, are captured in the models. They provide examples, using some empirical data, of where a source-sink model might apply to species of Pacific salmon. McElhany et al. (2000) also point out that patch spacing affects the probability that colonization can occur should empty, suitable habitat become available, and therefore that the spacing of habitat patches that rarely contain fish may affect population viability. This suggests that features of a stepping stone model might be relevant to salmonid population structure. Hansen et al. (2002), citing Adkison (1995), state that stepping stone models appear to be most realistic in accounting for patterns of gene flow in anadromous salmonids. Hansen et al. imply this holds for anadromous brown trout (*Salmo trutta*). They cite several recent studies, some dealing with trout populations elsewhere and some dealing with the same Danish trout populations, as having demonstrated isolation by distance. Rieman and Dunham (2000), however, contend that though the basic elements of spatial structuring and dispersal are present in many systems, dynamics of inland salmonids are not simple or consistent enough to support a generalizeable model of metapopulation dynamics.

Evidence regarding metapopulation structure in bull trout

Rieman and McIntyre (1993) assert that the characteristics of bull trout populations are consistent with the metapopulation concept, but that bull trout metapopulation structure is not well understood. Their viability analysis supports the conclusion that regional persistence of bull trout depends on the maintenance of multiple local populations. They mention that it is unlikely that most local populations are actually independent of others

and that climatic events would cause changes in adjacent local populations which in turn would exchange individuals to support or refound each other. Populations close to each other would likely decline together, and those too far apart would likely exchange individuals too rarely to rescue each other.

Recent research suggests that there may be more complexity to these metapopulations than originally thought. Dunham et al. (2002), reviewing data from bull trout and Lahontan cutthroat trout, imply that simple metapopulation models, such as patch or island (including Levins-type) models, are often inappropriate for freshwater salmonids. Studies Dunham et al. cite have found that patch area appears to be a significant correlate of species occurrence. Patch size tends to be related to fish occurrence, perhaps because habitats in larger patches may be more complex and resilient to disturbance and should generally support larger populations. Rieman and McIntyre (1995), using multiple logistic regression, found patch area to be the strongest predictor of occurrence of bull trout in the Boise River Basin. Other significant factors included patch isolation (stream distance between occupied patches) and road density within patches, both inversely related to occurrence (Dunham and Rieman 1999). Dunham and Rieman (1999) concluded that patterns of occurrence in the Boise River are not consistent with Levins-type models, but that the configuration of Boise River bull trout populations lies somewhere between nonequilibrium and mainland-island metapopulations. They suggest that some recolonization occurs at broad scales, and that the few large patches in the Boise River basin may serve as important sources of bull trout for recolonization of unoccupied habitat. Parts of the basin may therefore function as mainland-island or patchy metapopulations.

Several studies of genetic structure of bull trout populations have suggested substantial divergence among breeding populations and relatively little within-population variability (Dunham and Rieman 1999). These studies provide support for the idea that a drainage scale aggregation of local populations is unlikely to be a panmictic group, thus meeting one key criterion for being a metapopulation. Leary et al. (1993) investigated 51 isozyme loci from populations in the Columbia and Klamath River basins, and concluded that their genetic structure was typical of salmonids inhabiting interior waters. Local populations contained little variation; significant variation was found between local populations at numerous loci. Leary et al. (1993) remarked that the divergence could be the result of founder effects or drift, but could also reflect import adaptive differences between populations. They did not discuss the implications of their study for metapopulation structure within either river drainage.

Spruell et al. (1999), using microsatellite loci, investigated a potential metapopulation system of bull trout from five spawning sites in a drainage tributary to Lake Pend Oreille, Idaho. They concluded that there was significant genetic differentiation within the Lightning Creek drainage. However, they also concluded that bull trout populations within the drainage have not historically behaved as a metapopulation with frequent extinction and recolonization or demographic support among tributary populations. An alternative possibility noted by the authors is that the populations are remnants of large, isolated populations that have undergone recent declines, and they conclude that an island

model with migration averaging about one individual per year is consistent with their data.

Some investigators have cautioned against taking literally absolute migration rate estimates extrapolated from genetic frequency data, because the model used requires assumptions that are often biologically unrealistic (Whitlock and McCauley 1999). Further, after the Spruell et al. (1999) results were published, an apparent recolonization event was discovered in a small tributary of the system (B. Rieman, personal communication), suggesting that extinction/recolonization processes may be operating on a relatively short timescale (decades) within the Lightning Creek basin. In any event, the overall rate of gene flow does not specify the pattern of gene flow, which is important for understanding how populations or metapopulations function. A migration rate estimate from measures of genetic variance among populations is a long-term average since the time two populations diverged. Even where such estimates are accurate, they do not reveal whether migration historically occurred at a relatively small, constant rate or instead consisted of episodic large migration events interspersed with long periods with negligible gene flow. Evidence regarding patterns of gene flow is generally lacking in bull trout, but patterns of disturbance and evidence of recolonization (e.g., in the Lightning Creek system) suggest episodic gene flow occurs in at least some cases.

Kanda and Allendorf (2001) used samples of mitochondrial and microsatellite DNA from juvenile bull trout to examine the population structure of bull trout populations in the Flathead River basin of Montana and British Columbia. They found that both types of DNA revealed substantial genetic differences among local populations and that most of these were attributable to differentiation within drainages. They also found no relationships between relative genetic similarity of the samples and geographic proximity. Kanda and Allendorf (2001) contend that their work suggests that little gene flow has occurred among the populations they examined, even over short geographic distances, and that the populations have been highly isolated reproductively. However, they also note that recent demographic declines may lead to loss of genetic variation through drift, which may either increase or decrease population differentiation and hence mask the effects of historical gene flow among the populations. They assert that the within-population levels of DNA diversity suggest that a metapopulation model with frequent local extinctions and recolonizations does not seem applicable to the populations in their study area, but that long-term persistence of this species requires maintenance of existing local population structure.

Costello et al. (2003) examined patterns of variation in microsatellite DNA in British Columbia and gathered information on environmental variables at watershed and site-specific scales to test if any were associated with differences among sites in genetic variation. They found that barriers segregating habitats within individual streams are important factors in organizing localized patterns of genetic barriers in bull trout. In all regions, the smallest genetic distances were between populations isolated above a common migration barrier, and the greatest distances were between populations isolated above different barriers. Combined analysis of environmental and spatial components indicated that the spatial component was the more powerful descriptor of the genetic

variation they observed. Costello et al. (2003) suggest the combination of low within-population variation and high levels of inter-population differentiation observed in bull trout is consistent with what would be expected if alleles are drawn from one (or a few) of a series of possible source populations during recolonization of vacant (recently deglaciated) habitats rather than as independent samples from all possible source populations. Costello et al. (2003) contend that this would enhance the loss of intrapopulation variation while increasing inter-population differentiation, particular if recolonization occurs in a stepping-stone manner. They suggest that while intrapopulation diversity appears to be largely a product of historical factors accumulating over time, inter-population diversity is more influenced by the degree of spatial connectivity between sites and by contemporary factors affecting dispersal and gene flow. They indicate that established models of gene flow, such as the isolation-by-distance model, may be too simplistic for hierarchically-structured or dendritic habitats such as are inhabited by bull trout.

In an analysis of microsatellite DNA from bull trout populations in the Boise River basin, Idaho, Whiteley et al. (2003) found evidence of structuring consistent with a stepping stone model. They did not find evidence that the genetic structure in the Boise system parallels the stream hierarchy, nor that distribution of genetic variation is shaped by patch structure. Whiteley et al. (2003) asserted that they found evidence for stronger patterns of isolation by distance than the more northern populations examined by Costello et al. (2003), in addition to evidence for higher overall levels of gene flow. Bull trout are long-lived, late maturing species with small effective populations sizes, which makes them particularly sensitive to the genetic effects of bottlenecks and founder events that likely accompanied postglacial dispersal (Costello et al. 2003).

Application of metapopulation to bull trout recovery planning

The question of whether bull trout are structured as metapopulations is difficult to answer conclusively. Different models of metapopulations exist, and in each model only qualitative conditions which should hold for a group of populations to fit the model are specified. Nevertheless, at least two of the criteria in Hanski (1997) and Rieman and Dunham (2000) would seem to hold for many bull trout Core Areas: habitat consists of discrete patches or collections of habitats capable of supporting local breeding populations, and habitat patches are not too isolated to preclude recolonization of at least some local populations. There is ample genetic evidence to indicate that local populations of bull trout are often highly divergent from other nearby local populations. This implies that populations in many river basins are not panmictic at the level of Core Area, therefore meeting one condition for a metapopulation. The evidence is much more ambiguous about the level of migration between local populations, except in the case of single local populations isolated above a migration barrier. These local populations would not be characterized as components of a standard metapopulation model and could only represent a modified stepping stone (downstream migration). We do not know the current status of many Core Areas, or to what extent evidence indicating a high level of population subdivision reflects historical versus recent (or natural versus anthropogenic)

events. Aggregations of bull trout populations that once may have acted as metapopulations may now be too fragmented, depressed, or contracted to be recognized as metapopulations today. Where there is evidence in the literature of a particular metapopulation structure, it is often ambiguous and sometimes contradictory. Several investigators, citing physiographic features of salmonid habitat or empirical evidence, have suggested that an isolation-by-distance model is most appropriate for salmonids in general and bull trout in particular. Some bull trout populations are structured somewhere between nonequilibrium and mainland-island metapopulations, or as source-sink metapopulations. Some investigators have assumed an island model in order to estimate migration rate, but there is little support to date for either an island or a classical, Levins-type metapopulation structure in bull trout.

The question of whether every Core Area identified in the Draft Recovery Plan was delineated consistently based on metapopulation theory is beyond the scope of the Science Team. In lieu of this evaluation, we provide the following series of questions that may be useful in distinguishing the presence or kind of metapopulation structure in bull trout and how well this corresponds with the Core Areas identified in the Draft Recovery Plan. In identifying these questions we assumed that patch is defined as a stream segment with suitable spawning habitat (as suggested by Dunham et al. 2002). However, in most Core Areas, there is little available evidence, other than considerations of geography, with which to determine with certainty to what degree the local populations within a Core Area act as a metapopulation, or which of the models of metapopulation structure is the best approximation of the behavior of the Core Area. This situation is not unique to bull trout; there exists little data that firmly demonstrates the existence of any of the commonly proposed metapopulations strictly applying to any animal group (Hanski and Simberloff 1997) and detailed study is required before any natural system can be confidently classified as a metapopulation (Harrison and Taylor 1997). Harrison and Taylor (1997) further suggest that many real metapopulations have combined features of the more classically defined structures.

Questions to aid in determining whether Core Areas reflect metapopulation structure

1. Is there evidence for subdivided habitat? If so, is habitat subdivision a result of continuously varying environmental conditions (e.g. temperature) or abrupt discontinuities (e.g. passage barriers)? For what kind of biological response are habitats patches defined?

2. What is the spatial distribution and connectivity of habitats including how are they positioned on the landscape and within the stream network?

3. Is there empirical evidence for genetic structuring or population subdivision? In addition to gene frequencies, evidence may come from mark-recapture experiments, telemetry, or other data.

4. How do fish move around the habitat? Is there evidence for source-sink or other processes? What is the role of dispersal (i.e. fish born in one area breed in another)?

5. Is there evidence for correlated population dynamics? Is there synchrony in population behavior or, for example, correlated changes in population abundance, demographic parameters, or patterns of persistence?

Metapopulation theory suggests the importance of paying attention to landscapes and networks (as opposed to individual reserves in isolation). Ideally, in order to classify metapopulation structure solid, defensible data would be gathered and assessed for: 1) movement rates among local populations; 2) relative reproduction rates within and among local populations; 3) relative mortality rates within and among local populations; 4) extinction rates of local populations; and 5) how migration and extinction rate are affected by patch size and isolation. However, there are potentially serious and detrimental consequences to management and monitoring of incorrect assumptions about metapopulation structure. For listed salmonids, ignoring metapopulation structure if it exists has several potential risks (Cooper and Mangel 1999). For instance, when metapopulation structure exists (especially source-sink dynamics), the abundance of a species in an area can be disconnected from the specific survivorship and fecundity rates of that area owing to the effects of immigration. This could lead to lack of detection of detrimental impacts in the sink areas until the sink areas go extinct. Another problem could arise if deme abundance is no longer a good indicator of habitat quality, and managers are thus led into wasting resources by conserving the wrong type of habitat. Conversely, if metapopulation structure is assumed where it doesn't actually exist, it could result in insufficient attention to the fate of relatively distinct local populations, under the assumption that they will be rescued by migrants from healthier, nearby populations.

CONSERVATION UNITS

What should be conserved?

According to Meffe and Carroll (1997) a conservation biology program is successful when a system retains the diversity of its structure and function over long periods of time, and when the process of evolutionary adaptation and ecological change are permitted to continue. Their first guiding principle for conservation biology is that evolution is the basic axiom that unites all of biology. Strategies for biological conservation need to be developed within an evolutionary framework. Here we develop a framework for identifying discrete conservation units necessary to ensure evolutionary persistence of bull trout. In the process of developing this framework, we will focus on the biological meaning and evolutionary importance of these units.

As amended in 1978 (16 U.S.C. 1532[16], the U.S. Endangered Species Act (ESA) allows listing of "any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature." The authority to list a "species" as endangered or threatened is not restricted to a species as recognized in taxonomic terms. The unit of conservation is therefore extended to a subspecies, and for vertebrate taxa to a Distinct Population Segment (DPS). However, in original ESA legislation how distinctness would be evaluated was not explained.

There has been considerable work on the topic of what should be appropriate units for conservation to ensure genetic variation for short-term fitness, continued adaptation, and the speciation process (see Meffe and Carroll 1997). Meffe and Carroll feel that the population, rather than the species, is the ecological and evolutionarily functional unit. The population level is where genetic changes take place over generations and where local adaptations occur. Accepting the population as the unit of conservation, the problem then becomes how to define the population unit.

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. Their feeling is 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 (1941) 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.

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 (Kirpichinikov 1981; Kincaid 1983; Danzman et al. 1985; 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 Soule 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 (Spruell et al. 1999; Taylor et al. 1999; Neraas and Spruell 2001; Spruell et al. 2003). When we discuss populations it may mean anything from a single clearly isolated unit to a complex network of units with some degree of gene flow (see previous discussion on local populations and metapopulations). The discussion of conservation units below will elucidate our rationale for including such a range of conditions that could define a population of bull trout.

A consensus has emerged among a number of studies that the population unit for conservation should focus on evolutionary lineages (Utter 1981; Ryder 1986; Waples 1991, 1995; Moritz 1994, 1999; Moritz et al. 1995; Allendorf et al. 1997; Fraser and Bernatchez 2001). A National Research Council (NRC) panel that examined science aspects of the Endangered Species Act developed the concept of an evolutionary unit (EU) to aid in identifying DPSs. An EU is defined as a group of organisms representing a segment of biological diversity that shares evolutionary lineage and contains the potential for a unique evolutionary future (NRC 1995). The defining characteristic of an EU is that it must be distinct from other EUs. The NRC's recommendation is that distinctiveness should be based on genetic, molecular, behavorial, morphological, or ecological characteristics. They stress that the identification of an EU (that is, to provide compelling evidence of distinctiveness) requires the careful integration of these several lines of evidence. Others have reached similar conclusions regarding evidence of distinctiveness (Utter 1981; Ryder 1986; Waples 1991, 1995; Moritz 1994, 1999; Moritz et al. 1995; Allendorf et al. 1997; Fraser and Bernatchez 2001).

The National Marine Fisheries Service (NMFS) developed a species policy to guide ESA listing determinations for Pacific salmon species (Waples 1995). The measure NMFS used to determine distinctness of a population (or group of populations) was if it represented an evolutionarily significant unit (ESU) of a biological species. Waples (1991) defined an ESU as a population (or group of populations), that (1) is substantially reproductively isolated from other conspecific population units, and (2) represents an important component in the evolutionary legacy of the species. The evolutionary legacy

of a species is the genetic variability that is a product of the past evolutionary events and that represents the reservoir on which future evolutionary potential depends. The methods employed to identify ESUs includes genetic, molecular, behavioral, morphological, and ecological data, and analysis of these data to determine hierarchical genetic/ecological relationships among populations (Waples 1991, 1995; Nielson 1995, Laikre et al. 2003).

Allendorf et al. (1997) proposed a biologically based process for prioritizing Pacific salmon populations for conservation. The system they identified prioritizes populations based on: (1) correctly identifying the relative risk of extinction faced by different populations, and (2) correctly evaluating the biological/evolutionary significance of the different populations. After the level of threat and the priority for conservation has been determined, the information can be displayed to identify populations with high risk and where the extinction has serious consequences to evolutionary potential. One drawback is that in many cases the data for bull trout is not available to formally estimate extinction risk. However, as Currens et al. (1998) point out this system does not require waiting for perfect data for a full evaluation. This can be an advantage for listed populations that are presently threatened.

The nature and origins of phylogenetic divisions within a species are important in identifying units of conservation (Moritz 1994; Bernatchez 1995). A hierarchal gene diversity approach can not only outline the overall genetic structure of a species and estimate historical rates of gene flow, but can also help to discriminate deeper (and older) evolutionary separation from shallower or more recent divergences that have lesser components of a species "bioheritage" or evolutionary significance (Bowen 2000). This assessment is possible because the degree of genetic separation is thought to be correlated with the time since physical separation, and could be used as a sort of molecular genetic clock (Meffe and Carroll 1997). Avise (1994) argued that, because we cannot save every variant of a species, the older lineages, all else being equal, should receive conservation priority, as they represent major branches.

Biogeographical and genetic data suggest a divergence in coastal and inland populations of bull trout, likely occurring 10,000-15,000 years ago, with three separate lineages within the Columbia basin. Since then, significant genetic variation between populations has occurred which reflect, and influence, adaptations to the range of environmental conditions bull trout experience. These adaptations are shown in the diversity found in life histories, phenotype, behavior, temporal and spatial distribution, habitat selection and inter- and intra-specific relationships. The genetic variation observed has largely been influenced by short-term and long-term environmental changes, both natural and human-caused. It has occurred in interconnected as well as fragmented or isolated habitats. The evolutionary potential, represented by the genetic variability within and between populations must be conserved in order for the species to evolve, especially when environmental change is amplified by anthropogenic activities. A consensus has emerged that the population unit for conservation should focus on evolutionary lineages. The population, rather than the species, is the ecological and evolutionarily functional unit, since the population level is where genetic changes take place over generations and

where local adaptations occur. Providing an interconnected continuum of complex habitats which support diverse life histories and life cycles is key to maintaining gene flow, genetic variation and facilitating metapopulation dynamics.

In bull trout, a Conservation Unit should represent a complex and diverse environmental template that allows full expression of genotypic, phenotypic and spatial diversity among bull trout populations. Providing this template will help ensure resilience and persistence when environmental changes occur. In the majority of cases, a bull trout Conservation Unit will likely be a group of metapopulations and isolated local populations representing a separate segment of biological diversity that shares evolutionary lineage and contains the potential for a unique evolutionary future.

In the foregoing discussion we have attempted to partially resolve several questions. First, what information is available upon which to base a determination of how local populations should be organized into discrete units individually necessary to ensure evolutionary persistence? Secondly, what is the relative rank of importance for this information? The process to identify a bull trout Conservation Unit should include analysis of information on genotypic and phenotypic variation, ecological distinctiveness and importance, geographic location, temporal isolation, historic and prehistoric range changes and causes, behavior, and reproductive characteristics (NRC 1995). Delineation of bull trout Conservation Units needs to be based on evidence from as many of these determinants as possible. Clearly, two primary sources, genetic and population (distribution and abundance) information are the most useful for identifying conservation units. Data on ecological distinctiveness and phenotypic characteristics help shape genetic makeup and population parameters, but do not in themselves define conservation units. They will, however, be useful in informing the decision process, in conjunction with the genetic information. These factors, along with a temporal evaluation may provide clues as to the speed and direction of evolutionary changes in the species, and their ability to adapt and exploit habitat and connectivity improvements through recovery efforts.

COLUMBIA RIVER DISTINCT POPULATION SEGMENT

Groupings of bull trout

Bull trout have been defined as a distinct species (Cavender 1978; Haas and McPhail 1991), however, the relationship between various groups of bull trout within the species can be complex (see Rieman and Allendorf 2001). According to genetic population theory, bull trout can be grouped into Conservation Units (or population units that share an evolutionary legacy). With respect to the Draft Recovery Plan, bull trout have been grouped into Distinct Population Segments, Recovery Units, Core Areas and local populations (see Appendix A for definitions). Core Areas are composed of one or more local populations, Recovery Units are composed of one or more Core Areas, and a DPS is composed of one or more Recovery Units. The manner in which bull trout were grouped in the Draft Recovery Plan appears to represent a juxtaposition of biological population structure and management considerations. In the following discussion we have attempted to use the attributes of Conservation Units and recommend delineation of areas for bull trout that contain populations, which are individually needed to ensure evolutionary persistence.

Local populations

Local population, as defined in the recovery plan, may be considered analogous to the smallest Mendelian population as described by Dobzhansky (1950). Local populations appear to be the smallest practical level of bull trout population groupings that could be aggregated to form Conservation Units. However, a local population typically does not possess the biological attributes that would warrant its designation as a Conservation Unit.

Core Areas and metapopulations

The intent of the Draft Recovery Plan was to use Core Areas to protect the integrity of possibly existing metapopulation structure, whether we can currently recognize it or not. Also, Core Areas were used to protect isolated populations, which may be important to the evolutionary legacy of the species (Costello et al. 2003). Within a metapopulation, local populations are expected to exhibit relatively frequent local extinctions and recolonizations (Hanski and Gilpin 1997). In order to classify population structure, solid defensible data would be gathered and assessed for various attributes. These include movement rates among local populations, relative reproduction rates within and among local populations, relative mortality rates within and among local populations, local extinction rates of local populations and how migration and extinction rate are affected by patch size and isolation. In the majority of cases, a metapopulation (or Core Area populations) does not possess the biological attributes that would warrant its designation as a Conservation Unit (although there are exceptions, e.g. Klamath River).

Recovery Units

Bull trout may be grouped so that they share genetic characteristics as well as management jurisdictions (see Dunham and Rieman 1999; Rieman and Allendorf 2001). Such groups have been classified as Recovery Units. They can range from one local population to multiple Core Areas. The Recovery Units identified in the Draft Recovery Plan are the units at which recovery efforts are specified and evaluated. Most Recovery Units do not cross state lines nor do they include mainstem areas of the Snake or Columbia rivers. As such, these units do not necessarily reflect a biological grouping of bull trout but, rather, appeared to be designed to facilitate local management decisions.

DPS structure and the evolutionary legacy of population units

Population units of bull trout exist in which all fish share an evolutionary legacy and which are significant from an evolutionary perspective (Spruell et al. 1999; Spruell et al. 2003). These population units can range from a few local populations to multiple local and metapopulations. Although such Conservation Units can be difficult to characterize, genetic markers such as allozyme, mtDNA, and nDNA data have provided useful information on bull trout population structure (see Appendix B). For example, allozyme frequencies first revealed genetic differences between Klamath and Columbia river populations of bull trout (Leary et al. 1993). Based largely on this 1993 information and the lack of additional information, from a biological perspective, the current DPS structure of bull trout was developed for the listing in 1998. At that time, the current DPS structure reflected the best biological information available about population units that shared an evolutionary legacy.

In the existing Draft Recovery Plan five DPSs were identified in the coterminous United States: 1) Klamath River; 2) Columbia River, 3) Coastal-Puget Sound; 4) Jarbidge River; and St. Mary Belly River. The following is the rationale for the determination of the five DPSs based on the best available scientific and commercial information at the time of listing (Federal Register Vol. 63, no. 111 1998). The Klamath River DPS was found discrete because of the physical isolation from other bull trout by the Pacific Ocean and several small mountain ranges. The Klamath River unit was found significant because it differs markedly in genetic characteristics from bull trout in the Columbia River basin (Leary et al. 1991; Williams et al. 1995). The Columbia River DPS occurs throughout the entire Columbia River basin within the United States and its tributaries. Although Williams et al. (1995) identified two distinct clades in the Columbia River based on genetic diversity patterns; a discrete geographical boundary between the two clades was not documented. The Columbia River unit was found significant because the overall range of the species would be substantially reduced if this segment was lost. The Coastal –Puget Sound DPS was found discrete because it is geographically segregated from other subpopulations by the Pacific Ocean and the Crest of the Cascade Mountain Range. The Coastal –Puget Sound unit was found to be significant because it is thought to contain the

only anadromous forms of bull trout in the coterminous United States, thus, occurring in a unique ecological setting. In addition, the loss of the population segment would significantly reduce the range of the taxon. The Jarbidge River DPS was found to be discrete because it is segregated from other bull trout in the Snake River basin by a large gap in suitable habitat. The Jarbidge River unit is considered significant because it occupies a unique ecological setting and its loss would result in a substantial modification of the species range. The St. Mary Belly River DPS was found discrete because it is segregated from other bull trout by the Continental Divide and it is the only bull trout population found east of the Continental Divide in the coterminous United States. The St. Mary Belly River unit was found significant because its loss would result in a significant reduction in range of the taxon. The Draft Recovery Plan (USFWS 2002) did not consider recovery of bull trout in the McCloud River basin. Bull trout were extirpated from the southernmost portion of their historic range, the McCloud River in California, around 1975 (Moyle 1976; Rode 1990). The extirpation of bull trout in the McCloud River basin, California, has been attributed primarily to construction and operation of McCloud Dam, which began operation in 1965 (Rode 1990). McCloud Dam flooded bull trout spawning, rearing, and migratory habitats. The dam also resulted in elevated water temperatures. Brown trout may have been a contributing factor in the decline and eventual extirpation of bull trout in the McCloud River, California, after dam construction altered bull trout habitat (Rode 1990).

Since the 1998 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 we now know 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 Mountains (Williams et al. 1997; Taylor et al. 1999). 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. 2000; 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; Williams et al. 1997; Spruell and Allendorf 1997; Taylor et al. 1999; Spruell et al. 2000). Bull trout in the Klickitat and Hood rivers in the Columbia River Gorge area have nDNA allele frequencies characteristic of the coastal group (Spruell et al. 2000, personal communication). However, the Hood River population has mtDNA more similar to the inland group (Taylor et al. 1999). Both mtDNA and nDNA place Klamath River populations in the coastal group (Taylor et al. 1999; Spruell et al. 2000).

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, 4 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 were 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 Basin represents a unique evolutionary lineage within the coastal group (see attached discussion on "Molecular genetic markers used in population biology").

- "**Snake River**", which also included 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 reflected the existence of two glacial refugia, consistent with the conclusions of Spruell 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.

There have been arguments made that Coastal/Puget Sound group should be a separate lineage from Lower Columbia River group based, on the lack of anadromy expressed in the Lower Columbia River bull trout. However, the remnant populations in the lower Columbia River are primarily above man-made blockages and would have difficulty expressing an anadromous behavoir. There have been historic reports of Dolly Varden (bull trout) that were caught in fishwheels operated on the mainstem Columbia in the late 1800s (Donaldson and Cramer 1971) and bull trout were captured in the lower Columbia River near Jones Beach (NMFS Hammond Laboratory Catch Card records 1961-1981). This information provides indirect evidence that bull trout in the lower Columbia River may have expressed anadromy.

Species

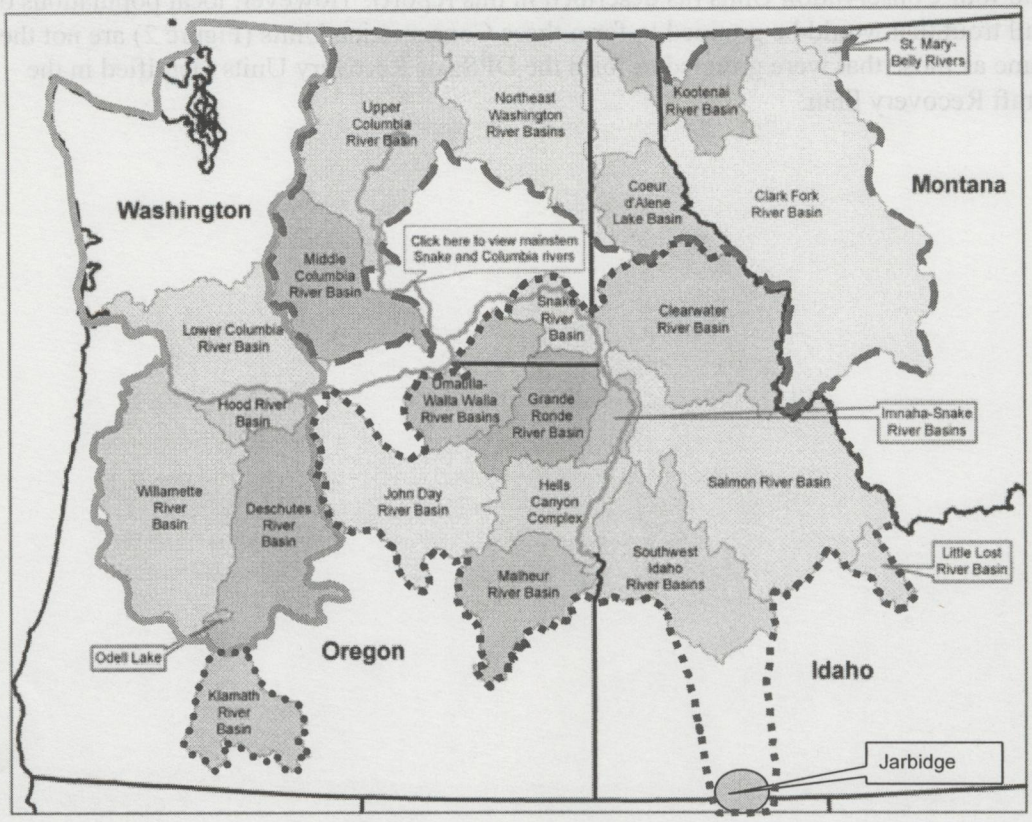
Based on morphological characters, bull trout were described formally as a distinct species of *Salvelinus* in 1978 (Cavender 1978). Biologists had previously confused bull trout with Dolly Varden (*S. malma*), due largely to their external similarity of appearance and the previous unavailability of adequate specimens of both species to any one systematist. Multivariate analyses have confirmed the morphological distinctiveness of the two species and their different, but overlapping, geographic distributions (Haas and McPhail 1991). Several genetic studies have subsequently confirmed the species distinction of bull trout and Dolly Varden. Chromosomal differences exist between the two species (Phillips et al. 1989) as do fixed, or nearly fixed, allelic differences at several allozyme loci (Crane et al. 1994). Both species occur sympatrically in western Washington, for example, with little or no interbreeding (Leary and Allendorf 1997). Analyis by Taylor et al. (2001) documented hybridization and some introgression between Dolly Varden and bull trout at areas of contact. They presume this hybridization and some gene flow between these species have occurred at least since post-glacial times. Despite the gene flow, Dolly Varden and bull trout are clearly distinct gene pools diagnosable over their ranges and, in particular, in areas of sympatry. However, Taylor et al. (2001) state that it is also clear that the two species' evolutionary histories have not been completely independent. Lastly, bull trout and Dolly Varden each appear to be more closely related phylogenetically to other species of *Salvelinus* than they are to each other (Phillips et al. 1989; Grewe et al. 1990; Phillips et al. 1991; Phillips et al. 1992; Pleyte et al. 1992; Phillips et al. 1994). Based on a combination of chromosome, allozyme, mitochondrial DNA (mtDNA), and nuclear DNA (nDNA) data, bull trout are most closely related to Japanese char (*S. leucomaenis*) whereas Dolly Varden are most closely related to Arctic char (*S. alpinus*). As a group, those four species appear to represent an evolutionary lineage distinct from brook trout (*S. fontinalis*) and lake trout (*S. namaycush*).

The evolutionary potential, represented by the genetic variability within and between populations must be conserved in order for the species to evolve. A consensus has emerged that the population unit for conservation should focus on evolutionary lineages (see NRC 1995). The population, rather than the species, is the ecologically and evolutionarily functional unit, since the population level is where genetic changes take place over generations and where local adaptations occur. Therefore, in the case of bull trout, currently available scientific information suggests that Conservation Units should be identified at a hierarchical level below a species.

Conservation Units of Bull Trout

Bull trout in Washington, Oregon, Nevada, Idaho and Montana have been listed as threatened under the Endangered Species Act. The Draft Recovery Plan (USFWS 2002) divided these bull trout into five DPSs and 27 Recovery Units. Based on our review of conservation and biological literature, it also appears that these bull trout are best grouped

into four Conservation Units (as described in this report). However, local populations of bull trout that would be grouped to form these Conservation Units (Figure 2) are not the same as those that were grouped to form the DPSs or Recovery Units identified in the Draft Recovery Plan.



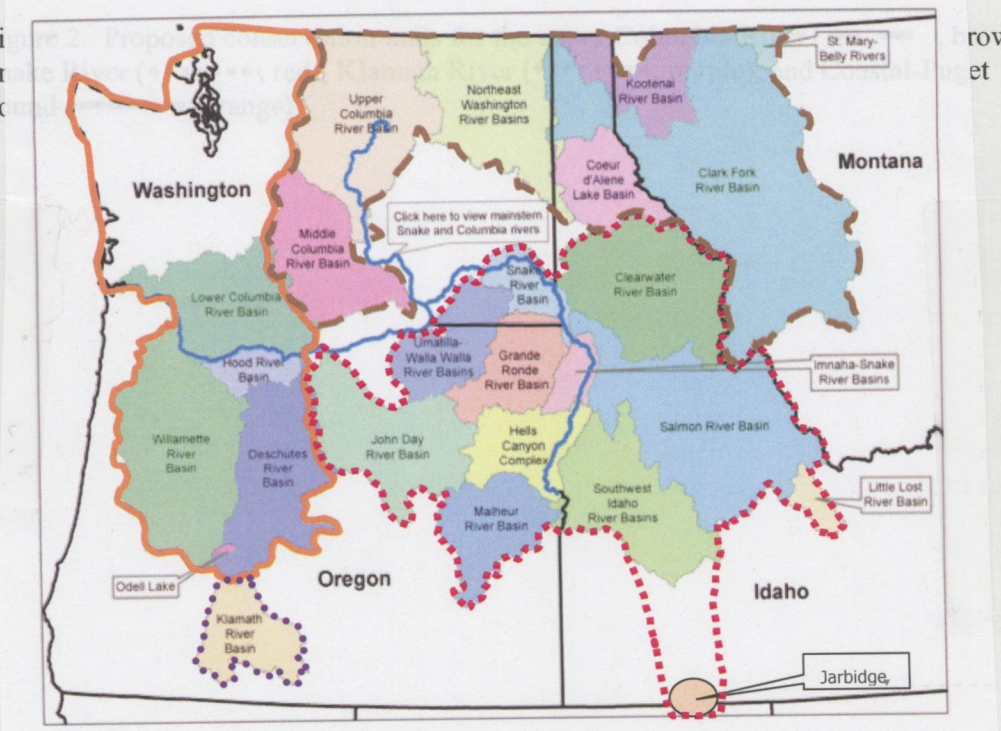


Figure 2. Proposed conservation units for the upper Columbia River (— — —, brown), Snake River (▪▪▪▪▪▪▪▪, red), Klamath River (••••••••, purple), and Coastal-Puget Sound (————, orange).

POPULATION SIZE

How is population size described?

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 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 (see 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 (see 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 (see 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) (see Thompson 1991; Lande 1995; Allendorf et al. 1997).

Genetic Theory

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 in 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, 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 (see and 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 (see 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, $N_e > 5{,}000$ 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 the retention of evolutionary potential an $N_e > 5{,}000$ or (following from Rieman and Allendorf 2001) $N > 10{,}000$ (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 $N_e<50$ for an isolated population, inbreeding depression may be expected to occur over relatively few generations (e.g. 2-5 generations). When $N_e<500$ 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 $N_e<5000$ 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

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 1000 adults (i.e., 500/0.5 = 1000) is necessary to prevent loss of genetic variation due to genetic drift. This later value of 1000 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. These types of connection of local populations, linked by migration, is termed a metapopulation (see Hanski and Gilpin 1991). 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 1000 annual spawners.

Yakima River

As populations decrease in size, genetic diversity is lost at a higher rate, the rate of inbreeding increases, and the risk of stochastic extirpation increases. For example, in the Yakima River subbasin, we calculated the expected reduction in genetic diversity (measured as the loss of heterozygosity) within current bull trout populations using the following equation:

$$1 - (1 - \frac{1}{2N_e})^t$$

where N_e is the effective population size and t is the number of generations under consideration (Wright 1931). We examined each population over one, five, and ten generation time periods. The number of spawners returning annually to a population was estimated by multiplying the average redd count in the population by 2.0 (Dunham et al. 2001; Hemmingsen et al. 2001). Rieman and Allendorf (2001) suggest the best estimate of N_e is between 0.5 and 1.0 times the mean number of adults observed annually. We

therefore calculated two values of N_e for each population: 0.5 and 1.0 times the mean number of adults spawning annually.

We made the following assumptions in performing this analysis:

1. Local populations of bull trout above dams are isolated and no new genetic material is being introduced from below the dam (i.e., via migration).
2. Mutation rates are low and did not contribute to genetic variability within the isolated sub-populations in the time periods examined.
3. Redd counts accurately reflect the number of redds produced by a local population.
4. Generation time is equal to 5 years.

Three of the six bull trout populations had N_e values below 50. The results of this analysis yield predicted reductions in genetic variation over the next 50 years ranging from 0.7 percent within the Rimrock Lake population to 19.7 percent within the Kachess Lake population. In other words, we would expect a 0.7 to 19.7% reduction in genetic variation, as measured by mean heterozygosity, within individual local populations over the next 50 years due to random changes in allele frequencies and potential losses of alleles. Such effects are expected to lead to an increased level of inbreeding relative to the metapopulation as a whole.

None of the local populations had N_e values above 500 indicating none of the populations were likely to have gained additive genetic variation or heterozygosity through mutation during these time periods. However, for the aggregation of all the local populations in the geographic area, if they were interconnected by migration and gene flow, N_e may exceed 500. Fragmentation of the bull trout metapopulation into genetically isolated subpopulations (i.e. via dams) is expected to increase the long-term probability of local extinction.

Perspective and Alternatives

Bull trout are currently distributed throughout much of the Pacific Northwest and found mostly in inland watersheds from the northern regions of California and Nevada to at least the Yukon and Northwest Territories (see Baxter et al. 1997; Taylor et al. 2001). Relatively recent genetic information has suggested that bull trout are divided into coastal and interior lineages (see Taylor et al. 1999, Spruell et al. 1999). Most of the genetic variation found in bull trout appears to occur between geographical regions or populations, with very low levels of genetic variation found within populations (see Taylor et al. 2001, Spruell et al. 2003). Taylor et al. (2001) have suggested that this pattern of genetic variation is consistent with the post-glacial dispersal of bull trout after recently being isolated in (probably two) glacial refugia. As bull trout recolonize areas, particularly at the extremes of their current distribution, founder effects and subsequent effective population sizes may be influenced by population bottlenecks (Taylor et al. 2001).

Small populations of bull trout do exist and appear to have persisted for many generations. For example, bull trout spawning annually in both Odell Lake and the Little Minam River (both in Oregon) appear to number fewer than 1,000 fish while many of the bull trout populations to Lake Pend Oreille (Idaho) appear to number fewer than 100 annual spawners (USFWS 2002). Some of these populations (e.g. Odell Lake) are closed and it is not possible for new genetic material to enter the population through immigration. Furthermore, under current conditions and possibly for numerous generations the habitat available to some of these populations may never have supported hundreds or thousands of bull trout. Despite the habitat conditions and lack of connectivity during this period, each of these populations appears to have persisted at low numbers for many generations. Although these populations may be relatively small in size, they often exist at the extremes of bull trout distribution and their persistence can be very important to the conservation of bull trout.

Small populations of bull trout may be able to persist over long periods of time. For example, relatively small population size of bull trout in Odell Lake, Lake Pend Oreille and the Little Minam River has not resulted in these populations becoming extinct. It is possible that these populations have experienced significant losses of genetic or phenotypic variation but that habitat conditions (or selective pressures) are extremely benign. In this case the loss of variation may not be detectable or expressed phenotypically. Alternatively, circumstances in these populations may allow for spawning bull trout to adhere relatively closely to Hardy-Weinberg equilibrium dynamics (so that N more closely approximates N_e). In this case, fewer fish would be required to minimize genetic risks to the populations. Finally, a wide variation in the effects of inbreeding has been observed in numerous vertebrates (Wang et al. 2002). These examples suggest there may be circumstances where bull trout populations persist without adhering to the theoretical 50, 500 or 5,000 guidelines for N_e.

Conclusions

Guidelines on effective population size appear to apply reasonably well to bull trout (see Rieman and Allendorf 2001 for review). 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>500 (see 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 $N_e < 50$

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. Although a prudent first step, a minimum N_e is not sufficient to conserve bull trout populations (see Rieman and Allendorf 2001). Spawning bull trout and their progeny are components of the ecosystem in which they evolve and as such, interact with the environment. Without a properly functioning environment, achieving a minimum numbers of effective breeders may not result in the population being conserved.

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APPENDIX A: Molecular Genetic Markers used in Population Biology

Introduction

Information about intraspecific heterogeneity in fish has been gathered even before the approaches and methods of population genetics were applied to fish (Altukhov et al. 2000). This led to the formation of concepts that species are characteristically subdivided into groups confined to different geographic localities and diverging from one another morphologically and ecologically. Given the accumulation of such information, theories concerning the adaptive nature of such differences and their hereditary basis emerged (Ricker 1972; Altukhov et al. 2000). Many researchers have provided evidence that natural local salmonid populations are far from being panmictic but represent populations systems organized hierarchically. The hierarchy of population structure of salmonid populations is typically specified by the natural history of a species range. Population systems are characterized by distinct intra and intersystemic relationships, specific territorial distribution (range), and a certain level of genetic diversity, which is maintained over long periods of time, and was initially characteristic of ancestral populations that progressively differentiated in a series of generations over the range. Spatial genetic differentiation in all salmonid species has exhibited a regional geographic pattern. This differentiation has been shown to be largely determined by natural history of recent populations, which is directly associated with the ice ages (McPhail and Lindsey 1970; Haas and McPhail 2001). That is during the last Pleistocene glaciation large areas were covered with ice, and only a few populations survived in ice free refugia. These populations isolated in various refugia led to intraspecific genetic divergence through adaptive and neutral evolutionary processes. Once the retreat of the ice sheets took place, these populations' colonized appropriate ecological niches, and new populations formed from one or several refugia.

Advances in molecular technology have allowed for detailed assessments related to species conservation and associated population biology. Genetic markers allow for the identification of parentage, more distant relatives, founders to new populations, unidentified individuals, population structure, effective population size, metapopulation structure, and delineation of species, subspecies, and races, all of which aid in setting species recovery priorities (Haig 1998). The fate of an individual genetic variant in time and space will be influenced by the biology and circumstances of individuals through which it passes, including reproductive success, migration, population size, natural selection, and historical events. Processes that effect individuals ultimately accumulate into effects on populations and their structure, which, in turn, influence speciation. Information can be obtained about almost any population and their evolutionary process, by evaluating genetic markers with appropriate rates of change (Sunnucks 2000). The rate of change of the distribution of different genetic markers varies owing to differential action of fundamental processes, including recombination, mutation and selective constraints. Sunnucks (2000) suggest considering three levels of molecular change that would provide information at different levels of population boiology.

- Genotypic – most sensitive genetic signals useful for detecting the finest scale population processes, such as, tracking movement of individuals, parentage and relatedness of interacting individuals. Also, population level assessments including N_e estimation, understanding metapopulation dynamics, and identifying recent colonization and introductions.
- Genic – evaluation of gene frequencies and geographic distribution. These properties change on larger spatial and temporal scales than genotypic arrays, and are effective markers for gene flow and population history, even in species with limited genetic variation. This can be used to estimate gene flow and population subdivision.
- Gene genealogies - new alleles are created slowly by mutation, therefore the analysis of their evolutionary relationships (allele genealogies, or phylogenies) is informative about the longer-term processes of phylogeography, speciation, and deeper taxonomic phylogenetic reconstruction.

The following is a description of the genetic marker information available for determination of bull trout conservation units:

Randomly Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), and Paired Interspersed Nuclear Elements (PINES). These techniques are similar. They allow for quick and efficient screening of populations for between-population differences, usually indicated by the presence or absence of a particular, anonymous fragment. However, these markers are "dominant markers" that are scored simply as the presence or absence of a particular fragment (i.e. band on a gel). As a result, homozygotes for a particular "band" or marker cannot be distinguished from heterozygotes. This "dominant" property significantly inhibits the application of these markers to general surveys of natural populations because the resulting data cannot be analyzed statistically, or interpreted, according to the basic principles of theoretical population genetics. These markers provide information at the genotypic level. However, the non-repeatability of RAPDS as a dominant makers is problematic.

Microsatellite nuclear DNA. Microsatellite DNA is comprised of highly repetitive base sequences of a core sequence consisting of 2-4 base pairs. For example, $(CA)_n$, $(CAT)_n$, and $(CATA)_n$ each represent "n" repeats of a di, tri, and tetranucleotide repeat core sequence at microsatellite loci. Genetic variation is represented by variation in "n", the number of repeats of the core unit. Short, nucleotide PCR "primers" are developed which flank a particular microsatellite locus. PCR amplification of a particular microsatellite DNA locus will thus detect only a single amplified sequence of one size in homozygotes but two PCR products of different length in heterozygotes. Automated DNA sequencers can be programmed to detect and record the fragment lengths and, hence, genotype of an individual at each of several microsatellite DNA loci. These are codominant markers that are particular useful for fine-scale, genetic studies of natural populations and reconstruction of pedigree relationships among individuals in natural populations. This use of the marker would provide information at the genotypic level. However, Sunnucks (2000) found that the most sensitive genetic signals are genotypic arrays, most commonly

encountered in the form of multiple microsatellite loci scored in samples of individuals. These provide sensitive, connectible data from individual identification through shallow phylogeny. Loci have a wide range of evolutionary rates, thus examine different time scales. Sunnucks feels microsatellites are the mainstay of modern population genetics other than systematics. Therefore, microsatellites analyzed in this manner operate at the genic level. Also, microsatellites can be used to infer recent bottlenecks without pre-bottleneck data, by examining observed relationships between allelic diversity and heterozygosity compared with molecular genetic models.

Mitochondrial DNA (mtDNA). The first DNA-based, population genetic surveys of natural populations used mtDNA as a molecular marker (Avise et al. 1979). Mitochondrial DNA has some unique properties that make it particularly useful for tracking phylogenetic relationships among populations over relatively large geographic areas (termed "phylogeography"). Some of the unique properties of mtDNA are the following. (1) mtDNA in most vertebrates is a closed circular molecule of only approximately 16,000 base pairs (or 16 kilobases or kb). It is a relatively small molecule (relative to nuclear DNA) that can be isolated and purified from soft tissues in relatively large quantities, a necessity prior to the development of the polymerase chain reaction (PCR) in the late 1980's. (2) mtDNA is inherited clonally from the maternal parent only via the cytoplasm of the egg cell without any genetic recombination. Hence, over evolutionary time scales, the nucleotide sequences of mtDNA "evolve" as new mutations become incorporated randomly and are then propagated by chance into subsequent mtDNA lineages. As a consequence, one can track the evolutionary relationships of mtDNA molecules within a species and among closely related taxa (a gene geneology) independent of the evolutionary relationships of the taxa in which those mtDNA lineages occur. (3) In most cases, an individual has only one type of mtDNA (i.e. haplotype) which is the same haplotype as the individual's mother. The paternal parent contributes little if any detectable mtDNA. Because of all these unique genetic transmission properties, mtDNA is particularly useful for complementing codominantly expressed, nuclear markers (e.g. allozymes, microsatellite nuclear DNA markers) in population genetic surveys. For example, mtDNA is particularly useful in studies of natural hybridization because genetic differences in mtDNA between species can be used to determine the female parent of the original hybridizing crosses if codominant nuclear markers are used to track the incidence of hybridization. In general, genetic variation in mtDNA is detected by one of two methods: (1) restriction fragment lengthy polymorphisms detected by breaking mtDNA into a number of digested fragments with a battery of restriction endonucleases, and running those fragments out on an electrophoresis gel to identify them by size or (2) direct sequencing of a particular portion of the mtDNA molecule. The mtDNA markers provide information at the genic and gene genealogy levels.

Allozymes. Allozymes represent allelic variants of enzymes (proteins that perform catalytic functions) and non-enzymatic proteins. The amino acid sequence proteins reflect the nucleotide DNA sequences of the genes coding for those enzymes or proteins. At a single loci heterozygotes and homozygotes can be distinguished by allozyme markers. Many of the enzymes used in population genetic surveys are respiratory

enzymes involved in glycolysis, Krebs cycle, or oxidative phosphorylation. These markers change relatively slowly and are, most likely, constrained by natural selection over evolutionary time spans but are generally believed to behave genetically as neutral markers over time spans involving only multiple generations. Major allele frequency differences between major, geographic groups (e.g. Klamath River basin versus Columbia River) may represent the product of genetic drift over evolutionary time scales but potentially influenced by stabilizing or divergent natural selection. For example, allozymes for lactate dehyrogenase have been shown to have different enzyme kinetics in fishes, and those kinetic differences are often correlated with mean environmental temperature and geographic location of the source population. The allozyme markers provide information at the genotypic and genic levels.