Protocol for Determining Bull Trout Presence

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Abstract

The Western Division of the American Fisheries Society was requested to develop protocols for determining presence/absence and potential habitat suitability for bull trout. The general approach adopted is similar to the process for the marbled murrelet, whereby interim guidelines are initially used, and the protocols are subsequently refined as data are collected. Current data were considered inadequate to precisely identify suitable habitat but could be useful in stratifying sampling units for presence/absence surveys. The presence/absence protocol builds on previous approaches (Hillman and Platts 1993; Bonar et al. 1997), except it uses the variation in observed bull trout densities instead of a minimum threshold density and adjusts for measured differences in sampling efficiency due to gear types and habitat characteristics. The protocol consists of: 1. recommended sample sizes with 80% and 95% detection probabilities for juvenile and resident adult bull trout for day and night snorkeling and electrofishing adjusted for varying habitat characteristics for 50m and 100m sampling units, 2. sampling design considerations, including possible habitat characteristics for stratification, 3. habitat variables to be measured in the sampling units, and 3. guidelines for training sampling crews. Criteria for habitat strata consist of coarse, watershed-scale characteristics (e.g., mean annual air temperature) and fine-scale, reach and habitat-specific features (e.g., water temperature, channel width). The protocols will be revised in the future using data from ongoing presence/absence surveys, additional research on sampling efficiencies, and development of models of habitat/species occurrence.

Introduction

As a result of federal Endangered Species Act requirements and other research and management concerns, state and federal agencies, tribes, universities, and private landowners continue to conduct surveys of bull trout populations and habitat. In 1998 Plum Creek Timber Company and Washington Department of Fish and Wildlife requested the Western Division American Fisheries Society (WDAFS) Bull Trout Committee to coordinate development of survey protocols for bull trout. Two types of protocols were requested: 1) to determine bull trout occurrence ("presence/absence") and 2) to determine potential or suitable bull trout habitat. The committee agreed to coordinate the protocol development and to use a process similar to that used by the Pacific Seabird Group to develop marbled murrelet protocols (Ralph et al. 1993). This is an iterative process in which the protocols are revised as new data become available.

We have chosen to focus this effort on refining a statistically rigorous sampling protocol for conducting detection or presence/absence surveys based on currently available information. Existing data on bull trout habitat relationships are inadequate to define suitable or potential habitat across the range of the species with a measure of precision, but they can be used to suggest strata for presence/absence sampling. Presence/absence surveys can be conducted to meet a variety of objectives, such as meeting Endangered Species Act requirements and planning management activities. They also involve considering acceptable levels of risk and uncertainty and weighing the costs and tradeoffs in conducting surveys. This protocol does not attempt to make those decisions. Rather we have concentrated on the technical aspects of the sampling protocol and related existing data and provide some flexibility in making those decisions. We recommend that biologists generally follow the methods of Bonar et al. (1997) as modified by the sample size requirements, design considerations, and procedures described in this document. In keeping with Ralph et al. (1993), we encourage those who conduct surveys of bull trout occurrence to follow this protocol and provide the resulting data to the Committee so the protocol can be strengthened in the future[1].

Limitations

The protocol applies to juvenile migratory bull trout and resident bull trout (i.e., early rearing habitat for fluvial, adfluvial, and anadromous forms and year-round habitat for resident forms). The data used to calculate sampling efficiencies for the interim guidelines were derived from sampling the habitat used by these life stages and life history forms. which are also likely to have less seasonal variability in the occurrence of the species. Habitat for juvenile and resident bull trout is essential for the persistence of the species, is likely to be used year-round, and is a logical place to begin development of these protocols. We acknowledge that this habitat represents only a portion of that used during the complete migratory life history. Additional work will be needed to develop protocols that address migratory distribution and habitat suitability.

The protocol is designed to detect bull trout occurrence within the sampling frame (i.e., the area from which sampling units are randomly selected). It was not designed to determine the extent of distribution of bull trout within the sampling frame.

<u>The sampling efficiencies and detection probabilities of this protocol are applicable to streams small enough to employ block nets for sampling (approximately #5 m. wetted width) during summer, low-flow conditions.[2]</u>

Despite these limitations, the process and concepts developed here should provide a useful basis for developing protocols to sample other life history stages and habitats of bull trout.

Evolution of Methods

Several methods have been used to survey fish in streams. Some are used to estimate fish abundance (e.g., Hankin and Reeves 1988), while others are used to detect presence of fish. Surveys designed to detect fish presence range from informal surveys with little statistical rigor to complex surveys where statistical designs are outlined in detail and efficiencies of various methods are considered (Bonar et al. 1997). As suggested by Bonar et al. (1997), informal surveys involving reduced effort and cost can be useful for initial surveys to detect bull trout presence and can be helpful in the design of more rigorous surveys. In fact, much of the current information on bull trout presence is the product of informal surveys or a by-product of sampling conducted for other purposes. The primary limitations of informal surveys are that, if no bull trout are found, they provide no estimate of certainty (i.e., measure of the probability of detection) and they may be inadequate for the densities and distribution of the population and sampling efficiency of the method used. Formal, standardized surveys can overcome these limitations and also reduce the uncertainty concerning whether the results of the surveys were due to differences in methods.

Hillman and Platts (1993) developed a statistically based bull trout survey methodology that incorporates random sampling of stream sections using day snorkeling and electrofishing. To detect the presence of bull trout with a specified degree of power, they used a sampling technique developed by Green and Young (1993), which assumed the distribution of any rare species could be approximated by the Poisson if the mean density was very low and the species was not highly aggregated. Green and Young arbitrarily defined rarity as mean densities (m) less than 0.1 individuals per sampling unit; however, $m < 0.4$ individuals per sampling unit was adequate for the riverine mollusc data they examined. When m/k approached 0.107 ($1/k$ is a measure of the excess variance or clumping of the population) Poisson, adequacy approached 95%. They reported that the number of samples n (i.e., stream sections or quadrats) required to detect a rare species with a specified power (1 - ∃) is

$$n = - \log(\beta)/m,$$

where log is the natural log. To determine n, Hillman and Platts (1993) set power at 80% and 95%, and selected a minimum expected value for mean density (m = 0.25 fish per 100 m) based on a review of available literature and interviews with experienced biologists. A 10 km section of stream was selected as the sampling frame.

The Hillman and Platts (1993) procedure assumed that all bull trout present are detected (i.e., sampling efficiency is 100%). However, sampling efficiency for bull trout rarely approaches 100% (Thurow and Schill 1996). Bonar et al. (1997) refined the Hillman and Platts (1993) and

Green and Young (1993) methods to incorporate the effects of reduced sampling efficiency on needed sample size and suggested that the number of samples (i.e. stream sections) required to detect a rare species with specified power (1-β) as:

$$n = -\log(\beta)/[q^*m],$$

where q is the estimated minimum sampling efficiency, m is the lower threshold density, and log is the natural log. In the following section, we will show that these values can be replaced with distributions instead of single values, which will allow us to incorporate existing information on a range of values for sampling efficiency and abundance of bull trout. This approach provides a more accurate means of determining presence of bull trout.

The sampling frame of Bonar et al. (1997) consisted of a discrete patch of bull trout rearing and spawning habitat separated from other patches by unsuitable habitat or barriers to migration (see Dunham et al., in press, for details on patch delineation). They recommended two sampling stages: informal and statistically rigorous. An informal survey lacks a valid statistical means to estimate probability of presence when bull trout are not detected. If such a determination is required, a formal survey is necessary. Sample sizes for a statistically rigorous design were calculated for both the 80% and 95% levels of confidence based on more recent reports of minimum bull trout density (m = 0.15 fish/100 m) than used by Hillman and Platts (1993). A minimum sampling efficiency of 25% assumed by Rieman and McIntyre (1995) was also incorporated into the design. An appendix of needed sample sizes was provided to reflect other values for minimum bull trout density and minimum sampling efficiency.

Additional information now available suggests that these protocols should be updated. This information includes the following:

- Existing protocols use the Poisson distribution to calculate sample sizes. However, recent information suggests that overdispersion of bull trout densities, caused by clumping, sampling error or other factors, is significant, even under conditions of extreme rarity. In fact, the variance to mean ratio, a measure of dispersion, actually *increased* as bull density declined. Therefore, the assumptions of Poisson distribution may be violated for bull trout.

- Bull trout densities have been observed as low as 0.02 fish per 100m. These values are well under the density thresholds previously used (Hillman and Platts 1993; Rieman and McIntyre 1995; Bonar et al. 1997; Watson and Hillman 1997).

- Sampling efficiencies for bull trout can vary substantially by habitat characteristics and sampling gear from those previously assumed (Rieman and McIntyre 1995; Bonar et al. 1997).

- Current data on bull trout densities and sampling efficiency can be used to more accurately and precisely estimate the probabilities of detection.

Estimating Sample Size Requirements for Detecting Bull Trout Presence

Detection of bull trout at a sampling site requires that bull trout have to be there and that at least 1 individual had to be captured or seen (e.g., during snorkeling). The probability of detecting these fish depends on 1) an estimate of the number of fish in a sampling site (i.e., the number of chances you get) and 2) an estimate of sampling efficiency (i.e., your ability to capture or count fish). In what follows, we discuss each component individually and explain the changes from the previous efforts (e.g., Hillman and Platts 1993; Bonar et al. 1997 discussed above). Bull trout density and sampling efficiency are then combined to estimate detection probabilities and sample size requirements. Details of the underlying statistical analyses are in Appendix IV.

Estimating the number of fish in a sampling site

Since fish are discrete units (i.e., a site cannot contain a fraction of a fish), abundance estimates should be modeled using a discrete statistical distribution (integer values) to estimate bull trout detection probabilities. Previous studies have modeled fish abundance using the Poisson distribution (discussed above). The Poisson distribution consists of integer values that can range from zero to infinity and has one parameter (m) that is both the mean and the variance. Hence, the Poisson distribution assumes that the mean and variance are equal. In biological terms, the Poisson assumes that individuals in a population are randomly distributed and spatially independent (e.g., not clumped). Thus, a key assumption for the protocol, identical to that for previous studies, is that bull trout are randomly distributed within a sampling frame.

To estimate the number of fish in a sampling site with a Poisson distribution requires an estimate of mean fish density (m), often referred to as a threshold density. Previous studies (cited above) used a somewhat arbitrary single low threshold value, based on literature reviews, which was assumed to be the minimum density for (presumably viable) bull trout populations. However, recent studies indicate that mean bull trout densities can be much lower (e.g., 0.02 per sampling unit, Appendix IV). Use of an arbitrary, single low threshold value can require additional sampling making it more time-consuming and expensive. For example, a threshold based on the minimum density observed in 50-m sample units in Idaho (Appendix IV) would require 21 times more samples than a threshold based on the mean density (assuming 80% power of detection). Similarly, a threshold of 1 fish in a sampling frame and sampling efficiencies of 10 and 25% would require sampling the entire sampling frame 16 and 6 times, respectively.

Choosing a single threshold based on an estimate of viable or effective population size is also problematic given the well-known difficulties with obtaining reliable, defensible estimates of viable population size (see Beissinger and Westphal 1998; White 2000), and even these are likely to have a considerable amount of uncertainty. An alternative statistically valid approach would be to use existing density data for areas occupied by bull trout. The variation of bull trout densities (m) then can be incorporated into the estimates of detection probabilities. The simplest means of modeling this variation is by fitting the empirical density data to a continuous statistical distribution known as the gamma (Berger 1985). This empirical gamma *distribution* of densities then replaces the single threshold density value for the Poisson mean (m). Additionally, the use of the gamma distribution of densities allows the incorporation of future sampling data via empirical Bayesian statistical methods.

To maintain some flexibility for field crews, we used existing bull trout sampling data collected using 2 different sized sampling units, 50-m and 100-m long. These data were collected in streams known to contain bull trout populations. The mean bull trout density estimates for all sampling frames were then fit to a gamma distribution to estimate the gamma shape (*a*) and scale (*b*) parameters (Appendix IV). Using these estimates, the probability (*p*) of a sampling site containing *i* individuals were estimated according to Pielou (1969) as:

$$p = \frac{b^i}{(1+b)^{a+i}} \frac{\Gamma(a+i)}{\Gamma(i+1)\Gamma(a)}, \qquad (1)$$

where Γ is the gamma function, which is similar to a factorial. The result is a function that descibes the distribution of bull trout densities.

<u>Estimating sampling efficiency</u>

Sampling efficiency is a key issue because the Poisson-gamma model (above) requires that all individuals can be counted in a sampling unit. In practice, this is impossible for most fish collection efforts because fish sampling efficiency is rarely, if ever, 100%. Previous efforts have attempted to account for the influence of sampling efficiency by assuming that fish capture is a binomial process (Bonar et al.1997; Rieman and McIntyre 1995). That is, fish are either captured or missed (e.g., a fraction of a fish cannot be caught). Detection probabilities were then adjusted assuming a binomial distribution and using a point estimate of sampling efficiency (*q*). The binomial adjustment assumes that all individuals within a sampling unit have the same probability of capture and all respond independently. However, sampling efficiency is influenced by body size (Buttiker 1992; Dolloff et el. 1996) and thus, it can vary among individuals within a site. In addition, the prevalence of overdispersion (variance in excess of the binomial distribution) in many fish sampling efficiency and mark and recapture models suggests that fish do not respond independently (Bayley 1993). Independence means that the behavior of one fish doesn't affect that of another fish. The most obvious example of a non-independent response is fish schools. During sampling they can all swim away and avoid the sampler, so none are captured (0% efficiency), or they swim toward the sampler, so all are captured (100% efficiency). These violations of the binomial assumptions can result in the systematic underestimation of bull trout probability of detection.

Similar to the Poisson-gamma model discussed above, the extra variability in sampling efficiency (*q*) can be modeled with a continuous distribution known as the beta. The beta distribution of sampling efficiencies replaces a single mean estimate of sampling efficiency (*q*) in the binomial estimator, resulting in a beta-binomial distribution (Prentice 1986). The beta-binomial can explicitly account for extra variability (overdispersion) in sampling efficiency due to factors such as independence among individuals, which can improve the accuracy of the probability of detection estimates. Using the beta-binomial, the probability (p_c) of capturing at least 1 individual (detection) is estimated as:

$$\mathrm{p_c} = 1 - \left[\frac{\Gamma(i+1)\Gamma(a+x)\Gamma(a+b)\Gamma(i+b-x)}{\Gamma(x+1)\Gamma(i-x+1)\Gamma(a+b+i)\Gamma(a)\Gamma(b)} \right], \qquad (2)$$

where *a* and *b* are the beta shape parameters, *i* is the number of individuals in the sampling unit as defined above, Γ is the gamma function (Pielou 1969), and *x* is the number of individuals captured (0 in this case). The beta-binomial shape parameters are estimated using the mean sampling efficiency estimate (*q*) and dispersion parameter (γ) from a beta-binomial regression as $a = q/\gamma$ and $b = (1-q)/\gamma$. The dispersion parameter is a measure of the variability of sampling efficiency due a variety of sources including difference in catchability due to body size and non-independence of fish responses (e.g., the effect of the number of bull trout in a site on efficiency).

Sampling efficiency is also influenced by sampling method and the habitat characteristics of the sampling unit (Buttiker 1992; Bayley and Dowling 1993; Riley et al. 1993; Anderson 1995). Failure to account for differences in sampling efficiency introduces systematic error or bias into the data. "*Systematic error either in the imposition of treatments or in sampling or measurement procedures renders an experiment* [or observational study] *invalid or inconclusive*" (Hurlbert 1984). For instance, reliance on a single average estimate of sampling efficiency for all sampling methods would systematically overestimate detection probabilities (and underestimate sample size requirements) in situations where actual sampling efficiency was lower. In contrast, the use of the lowest estimated sampling efficiency would cause detection probabilities to be underestimated and sample sizes overestimated for some methods, resulting in the collection of too many samples, wasting limited time and funds. Therefore, gear and habitat-specific sampling efficiency estimates should be used for estimating detection probabilities and sample size requirements.

To develop a more efficient and effective approach to sampling bull trout, we developed sampling efficiency models using sampling efficiency data collected by USDAFS, Rocky Mountain Research Station personnel in 1999-2000. These data differed significantly from previous attempts to estimate sampling efficiency because they relied on the recapture or resighting (for snorkeling estimates) of a known number of individual bull trout. This method was chosen because removal estimates used to estimate sampling efficiency for the snorkeling and backpack electrofishing are generally biased low (Buttiker 1992; Riley et al. 1993; R. Thurow unpublished data; Peter Bayley, Oregon State University, pers. comm.) and, consequently, previous estimates of relative sampling efficiency were most likely biased high.

Sampling efficiencies and dispersion parameters were estimated for bull trout 70- 200 mm total length because we assume that the presence of these individuals is indicative of the presence of a local population (*sensu* Rieman and McIntyre 1995). The effects of habitat variables on sampling efficiency were estimated for day snorkeling, night snorkeling, and 1, 2, and 3-pass backpack electrofishing with beta-binomial regression (Appendix IV). Sampling efficiencies then were used, in combination with the Poisson-gamma model (above), to estimate detection probabilities and required sample sizes. In some cases the potential reduction of effort gained by incorporating the distribution of densities is counterbalanced by the greater effort required to detect bull trout when sampling efficiencies are lower than previously assumed.

Probability of detection and sample size estimates

Using the empirical estimates of fish density and sampling efficiency, the probability of detection is estimated as 1- the probability of capturing no (zero) fish. The probability of capturing no fish is estimated (in the general form) as:

Probability (No. of fish = 0)* Probability (catching 0 fish given 0 fish there) +
Probability (No. of fish = 1)* Probability (catching 0 fish given 1 fish there) +
Probability (No. of fish = 2)* Probability (catching 0 fish given 2 fish there) +

.

.

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Probability (No. of fish = x)* Probability(catching 0 fish given X fish there),

where *x* is summed to infinity (in theory). In practice, *x* is summed until the Probability (No. of fish = x) gets very, very small.

The probability of there being *x* fish in a sampling unit is estimated using the gamma shape and scale parameters and equation 1. For 50-m long sampling units, the probability that a unit contains 0 – 5 fish is:

Number of fish, *x*	0	1	2	3	4	5
Probability (Number of fish in unit = x)	0.8210	0.0842	0.0369	0.0203	0.0123	0.0079

The probability of catching no fish when there are x fish in the sampling unit is estimated using the beta-binomial mean sampling efficiency and dispersion parameter to estimate *a* and *b* and equation 2. For day snorkeling, the sampling efficiency is 0.11 and the beta-binomial dispersion parameter is 0.194. Thus,

a = 0.11/0.194 = 0.567

b = (1-0.11)/0.194= 4.588.

and the probability of collecting no fish when there are 0 – 5 fish in the sampling unit is:

Number of fish in unit, *x*	0	1	2	3	4	5
Probability (catching 0 fish given = *x* in unit)	1.000	0.890	0.8080	0.7440	0.6922	0.6494

Using the values above we then take the products:

Probability (No. of fish in unit = x)* Probability(catching 0 fish given *x* fish there),

Number of fish, *x*	Probability (No. of fish in unit = x)	Probability(catching 0 fish given *x* fish there)	Product
0	0.8210	1.0000	0.8210
1	0.0842	0.8900	0.0749
2	0.0369	0.8080	0.0298
3	0.0203	0.7440	0.0151
4	0.0123	0.6922	0.0085
5	0.0079	0.6494	0.0051
6	0.0052	0.6131	0.0032
7	0.0035	0.5819	0.0020
8	0.0024	0.5548	0.0013
9	0.0017	0.5309	0.0009
10	0.0012	0.5096	0.0006
11	0.0009	0.4906	0.0004
12	0.0006	0.4733	0.0003
13	0.0004	0.4577	0.0002
14	0.0003	0.4434	0.0001
15	0.0002	0.4303	0.0001
16	0.0002	0.4182	0.0001
17	0.0001	0.4070	0.0000
18	0.0001	0.3965	0.0000
19	0.0001	0.3868	0.0000
20	0.0001	0.3778	0.0000
	Probability of not detecting bull trout (the sum) =		0.9639
	Probability detecting bull trout (1 minus the sum)=		0.0361

The products are then summed to obtain the probability of catching no fish, and this value is subtracted from 1 to get the probability of collecting at least 1 fish (i.e., detection). Note that this value is identical to the single sample probability for 50 m sampling units with high visibility, high wood density, and temperatures greater than 9^0C in Appendix 1, Table 1.

Sample sizes requirements are easily estimated using the probability of catching no fish, from above, or subtracting 1 from the single sample probabilities of detection (Appendix 1, Tables 1-5). In the notation of Green and Young (1993), the number of sampling units (samples) needed to detect with 'power' 1- β is simply:

$$n = \log(\beta)/ \log[\text{Probability(catching no fish)}]$$

where log is the natural log. The resulting values for 16 combinations of habitat characteristics are calculated for day and night snorkeling and electrofishing in Appendix 1, Tables 1-3.

When using these tables, it is important to keep in mind that the sampling efficiency estimates are based on models developed under a limited range of habitat conditions (Appendix IV). Consequently, these estimates may not be applicable when physical habitat characteristics differ from those observed during the sampling gear calibrations. In some instances, sampling under conditions that are out of the range of those encountered during sampling efficiency calibration cannot be avoided. Therefore, crews should measure the core set of stream habitat characteristics as at each sampling unit as described in the **Field Sampling Procedures** (Appendix II) so that single sample probabilities can later be estimated with greater precision. Note also that estimates of fish densities vary slightly between 50 m and 100 m sites (see Appendix figure 1). The 100 m sites had slightly higher densities of bull trout; and, thus, detectability of bull trout is greater in 100 m sites, assuming equal sampling efficiencies. Collection of additional density data is needed to refine these estimates.

Interpretation of zero catch

A zero catch using the interim protocol in the sampling frame indicates that for a given power of detection bull trout densities are below the distribution of those observed in the occupied subwatersheds on which the sample sizes are based. For example, using the 80% detection level, there is still a 20% chance that bull trout were not detected at the densities modeled and up to a 100% chance that bull trout occur in the area at lower densities. Thus, a lack of detection does not necessarily mean that bull trout do not occur in the sampling frame, but that we are 80% confident that bull trout densities are lower than those observed in the Salmon, Clearwater and Boise river basins in Idaho, which were used to develop this protocol. Those basins contain relatively high quality habitat. Bull trout densities in other basins could differ. We will incorporate data from other basins as they become available to improve existing models.

Establishing bull trout absence with 100% confidence is not feasible using this or any other existing protocols. To estimate the probability that bull trout do not occur in the area would require a threshold of 1 fish/sampling frame, an assumption of no immigration, and correspondingly extremely high sample sizes unless the area was very small. For example, if the sampling frame contained 50 km of streams, it would require 6,000 50 m. sample units for an 80% probability of detection, assuming a 25% sampling efficiency. And there would still be a 20% chance that a bull trout was present but not detected. An alternative approach is to use empirical models to estimate the posterior probability of presence, given that bull trout were not detected (Bayley and Peterson 2001). These estimates could then be used to formally assess the risk of management actions to potential bull trout populations, but our current ability to develop those estimates is limited to areas, such as the Idaho basins with density data, where the data are adequate.

Temporal variability

A survey following the interim protocol provides a single point-in-time estimate of bull trout presence. Bull trout may move into areas that were not occupied at the time of sampling. Recent work (Ham and Pearsons 2000) suggests that temporal variation of the distribution of salmonids can be substantial. Rieman et al. (1997) reported recolonization of bull trout in streams apparently defaunated following an intense wildfire. Thus, bull trout occurrence may vary seasonally and at larger time scales. Current data are inadequate to incorporate temporal variability into sample size estimates.

Sampling Design Considerations

Basic terms, concepts, and assumptions

The goal of this protocol is to detect bull trout if they are present. Choice of sampling design is a key step in detecting presence. Here, we review some basic concepts and examples of sampling designs related to the protocol. We define basic terms following Thompson et al. (1998). Sampling of bull trout is assumed to occur within a reach of block-netted stream of 50 or 100m in length. These are the basic **sampling units** for surveys described herein. Sampling units are located within an area or sampling frame that an investigator wishes to make an inference about , in this case bull trout presence. The **sampling frame** is the complete collection of possible sampling units from which samples are drawn. For example, a sampling frame might be the total collection of potential stream reaches that could be sampled within a length of stream targeted for a bull trout survey. The sampling frame is the basis for which an inference can be made about occurrence of bull trout in a particular area (e.g., stream reach or basin). Strictly speaking, results from surveys within a sampling frame cannot be extrapolated to areas outside of its boundaries or in time. Extrapolation in time is particularly problematic because bull trout presence in some areas may vary on annual, decadal, or longer time scales (e.g., recolonization and extinction, range expansion and contraction). Because we are dealing only with presence of juvenile and resident bull trout, seasonal variation should not be a problem, because fish are assumed to be present year-round, except at relatively small scales (e.g., fish moving in and out of local stream reaches).

Allocation of samples within a sampling frame can be determined through a variety of nonrandom sampling methods (e.g., purposive, haphazard, and convenience), or through random sampling. We consider only random sampling because it provides valid, unbiased estimates of parameters, such as probability of bull trout presence. We focus on two classes of random sampling: simple and stratified random sampling. In **simple random sampling** every possible sampling unit within a frame has an equal chance of being selected for the survey. Typically, random sampling involves sampling without replacement. This means the same unit cannot be selected twice. **Stratification** refers to the process of dividing the sampling frame into two or more relatively homogeneous units. An example would be to stratify the stream based on habitat characteristics, such as stream temperature, or stream size. **Stratified random sampling** refers to random allocation of samples within each stratum. Stratification can produce more precise parameter estimates by more efficiently distributing sampling units in space or time. Sometimes simple random sampling can produce clumped distributions of sampling units, especially when sample sizes are small. Unintentional clumping introduces potential for bias (Hurlbert 1984). Stratification can reduce this problem. The simplest method for allocation of sampling units among strata is proportional allocation. For the purposes of this document, we define **proportional allocation** as the allocation of sampling units in proportion to the size of the strata, such that larger strata receive more sampling units. Other more complex procedures are available, but they are beyond the scope of this protocol (see Schaeffer et al. 1990).

Fisheries biologists often deal with two typical sampling situations based on the aerial extent of the sampling frame: one for a large-scale (e.g., watershed) and the other for smaller-scale (e.g., reach) surveys. For instance, if the goal of a study was to estimate bull trout occurrence in a specific stream, the frame of inference (sampling frame) would be the *stream* and a potential survey design could include sampling for fish in randomly selected sampling units within that stream. However, this design (sampling in a single stream) would be inadequate if the goal was to estimate bull trout occurrence in an entire watershed. For this goal, the sampling frame would be the *watershed* and a more appropriate design could include the random selection of streams within the watershed and sampling for fish in randomly selected sampling units within each of these. The spatial dimensions of these different areas of interest relative to sampling unit size can influence the sampling design.

We define **large-scale surveys** as those for which the area of the sampling frame exceeds the total area sampled by the required number of samples. For example, assuming 50 m sampling units and 10 required samples, a large-scale study would be one for which the sampling frame (total stream length) exceeded 500 m (i.e., 50 * 10 = 500). This could include one or several 6th code HUCs (hydrologic units). The interim detection probabilities and sample size requirements were estimated using existing data collected in known occupied subwatersheds (6th code HUC) and represent the (spatial) variability among subwatersheds and among sampling units within subwatersheds.

We consider **small-scale surveys** to be those for which the total length of the reaches sampled by the required number of samples exceeds the total length of the sampling frame. In these instances, the entire sampling frame could be sampled and the power of detection would be limited to the sum of the probabilities of detection for the individual sampling units. Thus, it is possible to sample an entire sampling frame without finding bull trout and still not be certain regarding presence of bull trout. A more efficient sampling method (e.g., night snorkeling) would help maximize the power possible. Alternatively, a sampling design could include repeated measurements of sampling units through time for a large enough sample size to reach a desired power of 80% or 95%. However, this introduces a temporal variance component into the sampling design, whereas the interim estimates of detection probabilities and required sample sizes more accurately represent the variation in spatial distribution. Consequently, we advise cautious interpretation of probability of detection estimates and required sample sizes for repeated measure sampling designs.

Ideally, the sequence of surveys of individual units should be randomized. However, this may not be logistically feasible or efficient. A non-random sequence should not alter the results *if it can be assumed that* the sequence of sampling in space or time does not affect detection of fish in other units. Thus, the most suitable or most accessible habitats could be surveyed first, or units could be surveyed proceeding in an up or downstream direction. In some cases, this may allow for more efficient detection of bull trout. For example, if the surveys start in what are believed to be the “best” *randomly* selected sampling units first and bull trout are found quickly, then some effort may have been saved. However, if bull trout are not found in the “best” sites, sampling must continue either until bull trout are detected, or if not, until the entire collection of sampling units is surveyed in order to make valid inferences about bull trout presence in the entirety of the original sampling frame.

Sampling design alternatives

Given the diverse areas likely to be of interest, there are many possible kinds of sampling designs, sampling frames, possible strata, and allocation of units within sampling frames. We provide three simple examples to illustrate.

Simple random sample--Consider a watershed in which a biologist is interested in sampling to determine presence of bull trout. If information on habitat is limited, one option is to use a simple random design to locate sampling units. An example with 20 sampling units is shown in Figure 1.

Stratified random sample with proportional allocation--If the biologist in the above example had some information on stream or habitat characteristics, a stratified sampling design could be used. For example, habitats can be stratified based on stream order, and sampling units can be allocated in proportion to length of streams in each stratum (=stream order class). In Figure 1, middle, the 20 sampling units are allocated within a basin having 50% first-order streams (narrowest lines), 30% second-order streams (lines of medium width), and 20% third order streams (line of heaviest width). Stream order is presented here as a simple example, but other factors, especially stream temperature, may be useful for stratification of sampling (Appendix 2).

Discontinuous and "reduced" sampling frames--A biologist is often faced with logistical constraints that prevent sampling of an entire stream or basin that may be of interest. For example, road access, land ownership, or safety concerns may be limiting. In such cases, the biologist may limit the sampling frame to a subset of habitats within the area of interest (e.g., areas delineated in black in Figure 1, bottom). Selection of a reduced or discontinuous sampling frame may be warranted under many circumstances, but we emphasize that inferences about bull trout presence cannot be extended beyond the sampling frame. Thus, a biologist must weigh the logistical advantages of this approach versus the cost of limited inference.

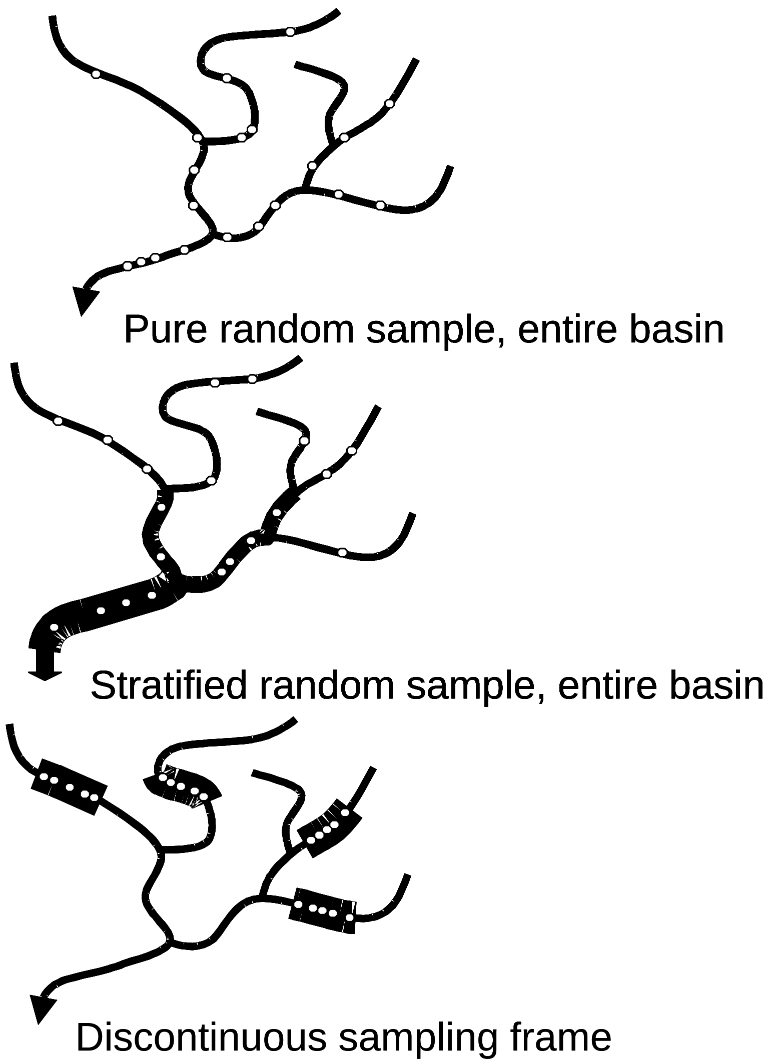

Figure 1. Three alternative sampling designs for a single watershed or stream (stream flow indicated by arrows). *Top*: watershed with simple random sampling and 20 sampling units (unfilled circles). *Center*: stratified random sample with proportional allocation of 20 sampling units based on stream order (order ranges from 1 to 3 and corresponds to line thickness). *Bottom*: sampling design in a basin with a spatially discontinuous sampling frame (indicated by heavy black lines) and 20 (total) sampling units.

Example

A natural resource management agency has proposed a new land-use activity for a watershed and has asked agency biologists to determine if the watershed contains bull trout. The team's chosen frame of inference is the entire *watershed*, which contains 100 km of perennial streams with a mix of habitats. The team decides that they will use a completely randomized sampling design, sample units 50 m in length, and day snorkeling to sample bull trout. To obtain preliminary estimates of required sample sizes, they consult Appendix 3, Table 1. Based on previous surveys, they believe that streams in the watershed are, on average, cold (low temp) with high visibility and high wood densities; hence they need to sample approximately 152 units in the watershed to be 80% confident of detecting bull trout at densities within the interim design thresholds. The biologists select these sampling units using topographic maps.

Because habitat characteristics in the sampling units will vary from unit to unit, single sample unit probabilities based habitat characteristics measured during the survey are used for the final calculation of detection probabilities. For this example, assume that the first 3 units are sampled, no bull trout are detected, and that they have habitat characteristics and single sample detection estimates of 0.007, 0.004, and 0.020 (the first 3 lines from Appendix 1, Table 1). The probability of detection after sampling these 3 units would be:

$$
\begin{aligned}
\text{Prob(detect)} &= 1-[(1-0.007)*(1-0.004)*(1-0.020)], \\
&= 1-[(0.993)*(0.996)*(0.980)], \\
&= 1-[0.969], \\
&= 0.031.
\end{aligned}
$$

These estimates would continue to be made sequentially as the team proceeds with sampling until the desired level of detection (80% power) is reached. This can potentially occur before reaching the preliminary sample size estimate (152). Conversely, the measured habitat characteristics could indicate lower sampling efficiencies than assumed based on the average conditions. In these instances, additional sampling units should be randomly selected and samples collected to reach the desired level of detection.

We have presented a range of sampling designs that provide biologists with a great deal of flexibility in terms of delineating sampling frames, strata, units, and method of sampling. Ultimately the selection of sampling design and methods should be based on a number of considerations, such as desired levels of certainty (i.e., detection probability), logistics, crew safety, risk to the species, sampling efficiency, and costs. It is critical to carefully document the sampling design used for any survey. A sampling design places limits on exactly where (and when) an inference about bull trout presence can and *cannot* be made. For example, a biologist cannot simply sample the sites where she or he may believe bull trout ought to be if they are anywhere in a basin, sample only those areas, and then conclude bull trout are not present in the basin if they are not found in those sample sites. This kind of approach is informal and cannot be used for probabilistic inferences. Due to the large amount of effort and rigor involved with formal sampling, we urge biologists to carefully consider the costs and benefits of an informal versus formal sampling approach. Either may be sufficient, depending on the circumstances (Bonar et al. 1997).

Costs of sampling design alternatives

The cost of sampling can be broken down into several components. There are costs associated with preparing and evaluating sampling design alternatives, the "pre-sampling" costs, and those associated with the actual field operations. For field operations, the largest cost is usually associated with travel to and from sample sites. Once at the site, it may be cost efficient to sample more intensively, rather than to travel to more sites. In other words, if the cost of sampling 100 vs. 50 m sites is relatively small, it would be more cost efficient to sample 100 m sites. Detectability for bull trout is actually higher in 100 m sites (Appendix 1), but this difference is relatively small. Among the three methods of sampling we consider, day snorkeling might be initially the least expensive, at least on a per-unit-sampled basis. However, night snorkeling or electrofishing could be more cost-efficient overall to attain a given detection probability because of their higher sampling efficiencies. The key is getting to an acceptable detection probability with the least cost. Cost estimates must be made on a case-by-case basis. Cost is only one of many factors (e.g., safety, permitting, access) that may dictate selection of sampling design alternatives and gear types.

Field Sampling Procedures

The interim sample size requirements and bull trout detection probabilities provided in this document were estimated using sampling efficiency models that were fit with the sampling efficiency data previously discussed. Because sampling procedures likely influence sampling efficiency, deviations from those used to collect that data could affect the applicability of the interim sample size requirements and bull trout detection probabilities. Therefore, we recommend that all crews using the interim sample size recommendations follow the same sampling procedures used, as outlined below.

Methods

Sampling Unit Selection

Sample units should be either 50 or 100 m in length (Appendix 1). Standardized lengths of sampling units will also assist us in developing more robust sampling efficiency models. Pace the unit and select upper and lower boundaries where block net can be set, if possible at hydraulic controls. Site selection should also be in accordance with the goals of the study or survey (see **Sampling Design Considerations**).

Sampling Unit Location

Record sampling unit locations (geographic coordinates) and elevations via a topographic map or a global positioning system (GPS) unit.

Block Nets

Install block nets at the upper and lower unit boundaries to help insure they are barriers to movement. Clean nets regularly and keep them in place until sampling is completed. Do not place block nets in areas of high flows or in places where fish may be entrained. Fish attempting to escape or emigrate from the site may be caught in the net mesh and killed.

Day Snorkeling

Inspect the sample unit and select the number of snorkelers necessary to survey the unit in a single pass [See Thurow (1994)]. Only snorkelers who have been trained in species identification and size estimation should complete counts. Snorkel the reach by moving slowly upstream. To improve underwater visibility, biologists should strive to conduct daytime snorkel surveys during periods of maximum light. Depending on the latitude, this typically coincides with about 1000 and 1700 during the summer months. We recognize that day snorkel surveys will sometimes need to be conducted during overcast conditions with limited direct sunlight. Count the total number of salmonids by species and estimate size classes to the nearest 100 mm size group. Use a small halogen light to spot fish hidden in shaded locations. Record the fish species by size class and the presence of amphibian adults or juveniles by species.

Measure the underwater visibility of a salmonid silhouette at three locations using a secchi disk-like approach as follows. The silhouette can be cut out of a blue plastic sheet and spots and other features added with an indelible black marker. One crew member suspends the silhouette in the water column and a snorkeler moves away until the marks on the object cannot be distinguished. The snorkeler moves back toward the object until it reappears clearly. Measure that distance and record. Measure visibility in the longest and deepest habitats (i.e., pools or runs) where a diver has the furthest unobstructed underwater view. Also record whether a snorkeler can see from bank to bank underwater

Night Snorkeling

Inspect the unit during the day and complete the survey with a halogen light during the period of darkness (i.e., avoid dusk and dawn) using the identical technique for day snorkeling.

Electrofishing[4]

Use unpulsed direct current (DC) where feasible (see Bonar et al. 1997, p. 46) to reduce the potential for injuring fish. Record the waveform, voltage, and frequency. Make individual upstream passes through the unit, capture all salmonids, and place them in live wells along the stream margins. Sample slowly and deliberately, especially near cover. Inspect captured fish and adjust voltages and/or frequency to reduce injury to fish. Bull trout may be increasingly susceptible to handling stress as water temperatures increase above 16^0C. During warm days, you may need to conduct electrofishing surveys in the early morning and late evening to reduce the risk of injury. After completing a pass, anesthetize, measure, and record the species and total lengths. Record the data by individual pass. Complete 3 passes until bull trout are detected.

If available, use a conductivity meter, adjusted for temperature to measure conductivity. Otherwise, collect a water sample by submerging and overflowing a plastic bottle in the stream. Label the sample, fix with two drops of $CHCl_3$, place in a cooler, and measure conductivity at a lab.

Habitat Variables

Gradient

Estimate gradient from a topographic map or digital elevation map (DEM), and measure unit gradient in the field with a hand level and stadia rod using the following procedure: crew member #1 stands along the bank at the start of the unit and holds a stadia rod vertically at the waters surface (using a rock to steady the rod). Crew member #2 stands near the middle of the unit level with the water surface and uses the level to shoot an elevation on the stadia rod. After recording the elevation, crew member #1 moves along the same bank to the end of the unit and crew member #2 shoots an elevation to that location. These elevations and the unit length are used to calculate gradient.

Temperature

When feasible, install a thermograph at the lowermost block net in the reach and record hourly temperatures during the sampling period. Also, use a calibrated hand-held thermometer to record the starting and ending water temperatures and times for the snorkeling or electrofishing.

Channel Dimensions

Measure the channel dimensions along transects at regular intervals (e.g., at 20m intervals in a 100m long sampling unit and at 10m intervals in a 50m unit). To establish transects, use a string machine or tape to measure the unit along the centerline of the stream. At each transect, measure wetted channel width perpendicular to the flow, mean depth, and maximum depth. Mean depth will be calculated by measuring the depth at approximately 1/4, 1/2, and 3/4 the channel width and dividing the sum by four to account for zero depth at each bank. Also record the total length of the unit.

Woody Debris

In the stream segment between each transect, count the number of pieces of woody debris. Woody debris is defined as a piece of wood, lying above or within the active channel, at least 3 m long by 10 cm in diameter. Also record the number of large aggregates (more than four single pieces acting as a single component) and rootwads.

Undercut Banks

Undercut bank cover will be related to the length and surface area of the sampling unit. Measure the length and average width of the undercut along each bank and record it. Undercuts are defined as areas beneath stream banks, boulders, bedrock, or wood that are solid portions of the stream bank. Undercut banks must be at least 5 cm wide and within 0.5 m above the water surface (or below the surface) to be included (see Platts et al. 1983 for illustrations).

<u>Field Crew Training</u>[5]

All crew members (novice and experienced) will improve the accuracy and precision of the data collected with annual training and review of methods. We recommend a consistent training approach that addresses all aspects of the methods to be used (site selection, block net installation, snorkeling and/or electrofishing, habitat surveys, recording data) and includes office orientation and practicing methods in a controlled environment, if possible, and in the field.

Office Orientation

This portion of the training can include an overview of the purpose and importance of the work, an introduction to the approaches to be used, and an introduction to fish identification and handling. For each method to be employed, the training can be structured to address equipment, safety, techniques, data collection, and ethics. Pertinent literature can also be provided to crews for review and discussion.

Practice in a Controlled Environment

Hatchery raceways, artificial stream channels, etc. work well for this portion of the training. If crews are to snorkel, for example, a hatchery raceway can be used to practice fish identification, fish size estimation, and fish enumeration. There are several options for testing snorkelers abilities to differentiate species underwater. One method is to capture fish of several species and place them in temporary live cages. Snorkelers independently view each fish and report their results to an instructor. As another method, an instructor in the water points out fish for a snorkeler to identify and record. In either case, results are reviewed with the snorkeler and training continues until all snorkelers accurately identify the target species.

Accurate estimates of fish size require snorkelers to practice prior to initiating counts. Objects viewed underwater are magnified about 1.3 times. A useful technique for estimating fish size underwater is to approach the fish, associate its snout and tail position with adjacent objects, and measure that distance with a rule. Snorkelers can carry a rule, mark one on their counting sleeve, or use a known distance (index finger to thumb, for example). Snorkelers can also practice estimating fish sizes by viewing objects of known sizes underwater. Calibrated wooden dowels or cut-outs of fish of various sizes can be attached to weights with monofilament line and distributed through a raceway or stream channel. Snorkelers approach each object and estimate its size. Live fish of known size can also be used. One method is to individually mark fish of known sizes in a raceway. Snorkelers approach each marked fish and estimate its size. Another method is to capture fish of several size classes and place them in temporary live cages. Snorkelers independently view each fish and report their results to an instructor.

Controlled environments can also provide snorkelers with an opportunity to count the total number of salmonids and record them by species and size class. Snorkelers can be tested on their ability to make precise counts of fish by replicating counts and comparing them. Sampling units can contain a known number of fish of known sizes and snorkelers test their abilities to complete precise and accurate counts.

Field Training

It is useful to select locations for training that provide crews with opportunities to practice the selected technique under field conditions that mimic those that will be encountered during surveys. It is imperative that crews practice the methods to be employed under actual field conditions. For example, if snorkeling is to be used, crews should practice identifying, estimating the size of, and counting the principal fish species to be encountered. Crews can also practice collecting habitat information under field conditions.

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Appendix 1. Probability of detection and sample size tables.

Table 1. Habitat-specific estimated mean sampling efficiencies for single-pass day snorkeling, single sample probabilities of detection, and number of samples required to detect bull trout (<200mm TL) with 80% and 95% probabilities of detection in 50 and 100m long sampling units.

			50 m sampling units			100m sampling units		
				Number of samples for desired power			Number of samples for desired power	
Visibility	Wood Density	Estimated sampling efficiency	Single sample prob. of detection	80%	95%	Single sample prob. of detection	80%	95%
Water temperature less than or equal to 9^0C								
low	low	2%	0.007	226	421	0.009	180	334
low	high	1%	0.004	449	836	0.005	355	661
high	low	6%	0.020	78	145	0.025	62	116
high	high	3%	0.011	152	283	0.013	121	225
Water temperature greater than 9^0C								
low	low	7%	0.024	67	125	0.029	54	101
low	high	3%	0.011	152	283	0.013	121	225
high	low	22%	0.065	24	45	0.078	20	37
high	high	11%	0.036	44	82	0.044	36	67

Visibility classes:

low- visibility less than or equal to 50% of the mean channel width

high- visibility greater than 50% of the mean channel width

Wood density classes

low – less than or equal to 0.07 pieces per square meter

high – greater than 0.07 pieces per square meter

Table 2. Habitat-specific estimated mean sampling efficiencies for single-pass night snorkeling, single sample probabilities of detection, and number of samples required to detect bull trout (<200mm TL) with 80% and 95% probabilities of detection in 50 and 100m long sampling units.

			50 m sampling units			100m sampling units		
			Single sample prob. of detection	Number of samples for desired power		Single sample prob. of detection	Number of samples for desired power	
Visibility	Gradient	Estimated sampling efficiency		80%	95%		80%	95%
low	low	14%	0.046	34	64	0.055	28	53
low	high	22%	0.066	24	44	0.078	20	37
high	low	28%	0.081	19	35	0.095	16	30
high	high	40%	0.105	15	27	0.120	13	23

Visibility classes:

low- visibility less than or equal to 50% of the mean channel width

high- visibility greater than 50% of the mean channel width

Gradient classes:

low- less than or equal to 4.5%

high- greater than 4.5%

Table 3. Habitat-specific estimated mean sampling efficiencies for backpack electrofishing (1-3 passes), single sample probabilities of detection, and number of samples required to detect bull trout (<200mm TL) with 80% and 95% probabilities of detection in 50 and 100m long sampling units.

			50 m sampling units			100m sampling units		
			Single sample prob. of detection	Number of samples for desired power		Single sample prob. of detection	Number of samples for desired power	
Depth	Undercut banks	Estimated sampling efficiency		80%	95%		80%	95%
One electrofishing pass								
Mean channel width less than or equal to 3.5 m								
low	low	31%	0.087	18	33	0.101	15	28
low	high	18%	0.056	28	52	0.066	24	44
high	low	22%	0.066	24	44	0.078	20	37
high	high	12%	0.039	40	75	0.047	34	62
Mean channel width greater than 3.5 m								
low	low	25%	0.073	21	40	0.085	18	34
low	high	14%	0.044	36	66	0.053	30	55
high	low	17%	0.053	29	55	0.063	25	46
high	high	9%	0.030	52	98	0.037	43	80
Two electrofishing passes								
Mean channel width less than or equal to 3.5 m								
low	low	46%	0.115	13	24	0.131	11	21
low	high	32%	0.089	17	32	0.103	15	28
high	low	36%	0.097	16	29	0.111	14	25
high	high	23%	0.070	22	41	0.083	19	35
Mean channel width greater than 3.5 m								
low	low	34%	0.093	17	31	0.107	14	26
low	high	22%	0.066	23	44	0.078	20	37
high	low	25%	0.074	21	39	0.087	18	33
high	high	15%	0.050	32	59	0.059	26	49

Depth classes:

low- mean maximum depth less than 0.25 m
high- mean maximum depth greater than 0.25 m

Undercut banks

low – less than or equal to 30% of streambank
high – greater than 30% of streambank

Table 3 (cont).

Depth	Undercut banks	Estimated sampling efficiency	50 m sampling units: Single sample prob. of detection	50 m: Number of samples for desired power 80%	50 m: 95%	100m sampling units: Single sample prob. of detection	100m: Number of samples for desired power 80%	100m: 95%
Three electrofishing passes								
Mean channel width less than or equal to 3.5 m								
low	low	53%	0.127	12	22	0.143	10	19
low	high	36%	0.097	16	29	0.112	14	25
high	low	41%	0.107	14	26	0.123	12	23
high	high	26%	0.076	20	38	0.089	17	32
Mean channel width greater than 3.5 m								
low	low	42%	0.109	14	26	0.124	12	23
low	high	26%	0.078	20	37	0.091	17	31
high	low	31%	0.088	18	33	0.102	15	28
high	high	18%	0.058	27	50	0.069	23	42

Depth classes:

low- mean maximum depth less than 0.25 m
high- mean maximum depth greater than 0.25 m

Undercut banks

low – less than or equal to 30% of streambank
high – greater than 30% of streambank

Appendix 2.

Useful Strata for Bull Trout Sampling Designs

At large scales ("landscape," see Allen 1998), bull trout are more likely to occur in thermally suitable habitats large enough to support local populations (Rieman and McIntyre 1995; Rieman et al. 1997). Other factors, such as habitat isolation and human disturbance also appear to be relevant (Lee et al. 1997; Rieman et al. 1997; Dunham and Rieman 1999). At a smaller scale, the occurrence of bull trout within streams of suitable temperature has been shown to be a function of habitat size (Rich 1996; Dunham and Rieman 1999). In relatively simplistic terms, fish distributions can be viewed as constrained on coarse (geographic) and fine (local) scales (e.g. Dunham et al. 1999).

Geographic variation in distributions is thought to result largely from the influence of regional climatic gradients. Geographic variation in salmonid distributions has been modeled from variation in groundwater, air, and surface water temperatures, and elevation gradients (which presumably are correlated with temperature). Distribution models based on these variables can be used to delineate strata on a coarse scale (Dunham et al., in press) without intensive field surveys. This advantage is countered by the degree to which the influence of local factors limits the predictive utility of geographic models (i.e., Are local or geographic factors most relevant?). Consequently, geographic models must be viewed as a "coarse" filter for delineating strata.

Local factors that may influence the distribution of fish within streams include influences of native and nonnative fishes, localized groundwater influence, proximity to lakes, large streams, marine, or other migratory habitats, dispersal barriers, and stream size. Variation in distribution limits of fish within streams not explained by geographic models may be the result of such localized influences. An understanding of localized factors requires more detailed information (e.g., distribution of temperatures within a stream, location of barriers) but also provides more detailed insights for developing sampling strata. Information on the local factors within streams can be viewed as part of a "fine" filter for delineating strata.

<u>Coarse scale--subwatersheds</u>

Models of bull trout occurrence are available at a coarse scale for much of the range of bull trout in the U. S. (Lee et al. 1997; Rieman et al. 1997). Predictions based on these models should serve as the basis for preliminary stratification and prioritization of sampling to detect bull trout. Rieman et al. (1997) reported that bull trout in the interior Columbia River basin were less likely to occur in subwatersheds (6^{th} code HUCs; Maxwell et al. 1995) with mean annual air temperatures greater than 5^{o} C. (Note: This does not mean that bull trout do not occur in subwatersheds with air temperatures greater than 5^{o} C, simply that occurrence is less likely.) Since groundwater temperatures closely parallel mean annual air temperatures (e.g., Meisner 1990), this may indicate the influence of climate and water temperatures on the regional distribution of bull trout.

Subwatersheds to be sampled should be stratified according to predicted probabilities of occurrence reported by Rieman et al. (1997) and Lee et al. (1997). Predictive models reported in those studies incorporate effects of other important variables. Outside of the interior Columbia basin, sampling should be stratified by mean annual air temperature isoclines.

Fine scale

Streams and stream reaches

Habitat data within a stream basin may also be used to delineate potentially suitable strata for bull trout sampling. Recent analysis of stream temperatures and bull trout occurrence indicates juvenile bull trout are unlikely to be found in stream sites with maximum summer temperatures of 18-19° C (Rieman and Chandler 1999). Data used in these studies are not representative of the entire range of bull trout within the coterminous United States, however. For example, streams from Washington State are not well represented (Rieman and Chandler 1999).

Studies in western Montana (Rich 1996) and southwest Idaho (Rieman and McIntyre 1995; Dunham and Rieman 1999) show bull trout are less likely to occur in streams less than two meters in width. Temperature is viewed as the primary constraint on bull trout distribution, but within suitably thermal habitats, habitat size may be an additional constraint.

Sampling of bull trout in subwatersheds with suitable habitat may be stratified by temperature and stream size. The nature of stratification will depend on study objectives, and logistical and financial constraints. If the objective is simply to determine occurrence of bull trout within a given area, sampling strata could be based on stream temperature and size with initial sampling occurring in streams or stream reaches with larger (e.g., > 2 m) wetted widths and cooler temperatures. However, if bull trout are not detected, less suitable strata would also have to be sampled to make inferences about occurrence in the sampling frame.

Habitat types within streams and stream reaches

It is widely recognized that bull trout are more likely to be found in deeper (e.g. pool) habitats with access to cover (e.g. large wood, undercut banks) and reduced fine sediment (Rieman and McIntyre 1993; Rich 1996; Dambacher et al. 1997; Watson and Hillman 1997). Therefore, strata could be developed using characteristics of habitat units, and sampling within reaches could be focused on strata with these characteristics.

Context

Recent work on bull trout “metapopulations” (Dunham and Rieman 1999) has shown that more isolated habitats are less likely to support local populations. Sampling to detect bull trout may be stratified by isolation, depending on survey objectives. If the model proposed by Dunham and Rieman (1999) applies to other areas, habitats close to others already supporting bull trout may be more likely to support populations.

Key Questions for Developing Bull Trout Sampling Strata

Stratification of subwatersheds

Is mean annual air temperature in a subwatershed less than 5° C?

Stratification of streams or stream reaches within subwatersheds

Is the maximum summer water temperature in a particular location within the subwatershed less than 19° C?

If so, is stream width larger than 2 m?

Stratification of sites within streams or stream reaches

Do the sample sites contain deeper habitat with cover and low embeddedness?

Context

Do bull trout occupy adjacent reaches/watersheds with similar habitat?

Exceptions

Is there any evidence for the potential influence of (especially local) factors that should receive additional consideration? For example, resident bull trout populations may occur in streams less than 2 meters in width (e.g., Little Lost River basin in Idaho, Bart Gamett, Salmon and Challis National Forests, personal communication; Jarbidge basin in Nevada, Gary Johnson, Nevada Division of Wildlife, personal communication).

The final question is perhaps the most difficult to address. While much of the variation in bull trout distribution and density can be addressed by asking the specific key questions posed above, there will always be locally important influences or patterns. Exceptions should be expected as better information on bull trout habitat requirements becomes available in different areas. Each level of resolution (subwatershed, stream or stream reach, habitats with streams or stream reaches) requires an increasing amount of information. Different goals may require different sampling strategies and strata.

Appendix 3. Sample data forms

SNORKEL DATA FORM

UNIT # ______ DATE ______
STREAM ______ DIVERS INITIALS: ______
BASIN ______ VISIBILITY(m): ______ / ______ / ______
BANK TO BANK VISIBILITY? Yes or No
TIME SINCE MARKING: ______
COMMENTS ______

SPECIES BY SIZE CLASS (mm)	DAY COUNT Time: Temp:		start		NIGHT COUNT Time: Temp:		start	finish
	Diver___		Diver___		Diver___		Diver___	
BULL TROUT	Unmarked	Marked	Unmarked	Marked	Unmarked	Marked	Unmarked	Marked
YOY								
<100								
100-199								
200-299								
300-399								
>400								
TOTAL								
RBW TROUT								
YOY								
<100								
100-199								
200-299								
>300								
TOTAL								
OTHER: Ct=cutthroat Bk=brook Ck=chinook Co=coho								
YOY								
<100								
100-199								
200-299								
>300								
TOTAL								
PRESENCE(+)/ ABSENCE JUVENILLE(j) ADULT(a)								
Mt. Whitefish								
Sucker								
Sculpin								
Squawfish								
Tailed Frog								
Spotted Frog								
Western Toad								
OTHER?								

ELECTROFISH DATA FORM

UNIT # ________ DATE ________ SHOCKER WAVE, VOLTAGE, FREQ: ________

STREAM ________ Start ________ Finish ________

BASIN ________ Time: ________

Temp: ________

CREW INITIALS: ________

COMMENTS: ________

LENGTH CLASS (mm)	CATCH BY PASS AND SPECIES Bt=bull trout; Ct=cutthroat; Rb=rainbow/steelhead; Bk=brook trout; Ck=chinook; Co=coho;									
	PASS 1		PASS 2		PASS 3		PASS 4		PASS 5	
	Unmarked	Marked	Unmarked	Marked	Unmarked	Marked	Unmarked	Marked	Unmarked	Marked
30-39										
40-49										
50-59										
60-69										
70-79										
80-89										
90-99										
100-109										
110-119										
120-129										
130-139										
140-149										
150-159										
160-169										
170-179										
180-189										
190-199										
200-209										
210-219										
220-229										
230-239										
240-249										
250-259										
260-269										
270-279										
280-289										
290-299										
300-309										
310-319										
320-329										
330-339										
340-349										
350-359										
360-369										
370-379										
380-389										
390-399										
400-449										
450-499										
>500										
TOTAL:										
ASSOCIATED SPECIES: Present (+); Absent (-); Juvenile (J), Adult (A)										
Mt. Whitefish										
Sucker										
Sculpin										
Squawfish										
Tailed Frog										
Spotted Frog										
Western Toad										
Other?										

	River system	WRIA	State	County	Township	Range	Section	UTM E	UTM N	Snorkeler	Recorder	Affiliation	Contact info

		Width/depth transects								Gradient							Undercut		
Stream		Transect	Spacing	0.25 chan. depth (cm)	0.50 chan. depth)	0.75 chan. depth	max depth	wet. width (m)	length (m)	Up1 (cm)	Down1 (cm)	Topo. (%)	DEM (%)	#LWD	#Aggregates	#Rootwads	Length (m)	Avg. wid.	Date

Appendix 4.

Statistical Analyses

Density estimates (Poisson-gamma model)

Study areas and sampling data-- To include the greatest geographical range possible, estimates of bull trout densities were obtained from fish survey data collected by USDA Forest Service (FS) biologists during 1991- 96 (Peterson and Wollrab 1999) and fish monitoring data collected by Idaho Department of Fish and Game, FS, Nez Perce Tribe and Shoshone-Bannock Tribes during 1984-97 (Rieman et al. 1999). All of the sampling locations were within the known range of bull trout, and most were in the Salmon, Clearwater, and Boise River Basins in Idaho. In all applications, trained personnel collected fish with standardized protocols (day-snorkeling) from various stream types. For bull trout survey or monitoring conducted under similar circumstances, these data were considered fairly typical. Note that these data represent the best currently available data on bull trout densities over a relatively large geographical area. However, they were collected on low-order streams (2^{nd} -4^{th} order) in areas of relatively good habitat in Idaho. Consequently, estimates generated from this data should be considered to be first approximations that will be adjusted as new data are collected.

Statistical analyses-- To maintain some flexibility for field crews, data collected using two different sized sampling units were selected, approximately 50-m and 100-m long. Only density data from subwatersheds (USGS 6^{th} code hydrologic units) known to contain bull trout populations (Lee et al. 1997) were used. The influence of sampling error (within subwatershed variation) was minimized by averaging density estimates for all sample units within each subwatershed (mean number of units = 10.7, range 8-36). The resulting subwatershed means were then fit to a gamma distribution and goodness-of-fit was assessed via chi-square tests (Sokal and Rohlf 1995).

Results--Fifty-meter units were sampled in 40 subwatersheds, and 100-m units were sampled in 101 subwatersheds. Bull trout density (fish per sampling unit) varied across subwatersheds and averaged 0.44 and 0.61 in the 50-m and 100-m long sites, respectively. The distribution of densities for the 100-m units was strongly influenced by values that were similar the thresholds used by previous studies (Appendix figure 1). Goodness-of fit tests indicated that the distribution of bull trout densities could be adequately represented by a gamma for both the 50-m (χ^2 = 8.23, 7 df, P= 0.312) and 100-m (χ^2 = 8.93, 6 df, P= 0.177) sampling units. The gamma shape (a) and scale parameters (b) were estimated for the 50-m units as: a = 0.13, b = 3.43, and the 100-m units as: a = 0.12, b = 5.13 (Appendix figure 1).

Sampling Efficiency

Field methods-- Sampling efficiency calibrations were conducted during the summer and fall 1999-2000 in 43 streams in central and northern Idaho (R. Thurow, unpublished data). The sampling gear evaluation procedure consisted of blocking off stream sections (henceforth, sampled units) with 6-mm opening mesh nets that were secured to the streambed. During the 2000 field season, a second set of blocknets was placed approximately 3-m immediately above and below the up- and downstream blocknets, respectively, to evaluate potential fish escape from the sample units (see *Evaluation of Bias*, below). Bull trout were collected within the sample unit via a backpack electrofisher with unpulsed direct current (DC) and 2 passes, one up- and one downstream. Captured fish were held in live wells, total length measured, and the dorsal or caudal fin received either a hole from a paper punch or was notched in a manner that would be visible to snorkelers. All fish were released back into the unit and allowed to recover and disperse for a randomly assigned period of time: 24, 48, or 72 hours. Following the recovery period, fishes in the unit were sampled between the hours of 1000 and 1700, via snorkeling (hereafter, day snorkeling) that began at the downstream end of each unit and completed on a single pass. Bull trout were then sampled the following night, between 2230 and 0230 hours via snorkeling (hereafter, night snorkeling) and identical procedure as used during the day, except that night counts were completed with the aid of an underwater halogen light. Snorkeling counts consisted of all bull trout >70 mm TL (Age 1+) and divers took great care to distinguish marked individuals. Bull trout were then sampled in the unit the following day (between 1000 and 1700 hours) with a DC backpack electrofisher and 3 passes, each in an upstream direction. During the 2000 field season, the blocked off areas outside of the unit then were sampled with the backpack electrofisher to capture marked individuals that might have escaped the sample unit. Following all sampling, fish were measured for total length, the presence or absence of a mark recorded, and released back into the site.

The physical features of sample units believed to affect the efficiency of bull trout sampling, were measured or estimated prior to or immediately following fish sampling. To examine the repeatability of measurement procedures, habitat variables were re-measured within units during the 2000 field season.

Statistical analysis--Imprecise or inaccurate habitat measurements could reduce our ability to detect important influences on sampling efficiency and could affect the adequacy of sampling efficiency models and probability of detection estimates. Thus, we estimated the precision (P) of stream habitat measures for each site using the formula (Snedecor and Cochran 1967):

$$P = \sqrt{\frac{t^2 CV^2}{N}} ,$$

where P is the precision expressed as a percentage of the mean, CV is the within site coefficient of variation for a habitat measure, t is a constant that varies with confidence level (t = 1.96 at the 95% confidence level), and N is the number of measurements taken within a unit (e.g., N = 3 for visibility estimates). Precision at the 95% confidence level was only calculated for habitat variables that were estimated via several measurements within a unit (e.g., transect measurements). The repeatability of habitat measurements was assessed by calculating the difference between the original and remeasured estimates and expressing these as a percentage of the original estimates.

Beta-binomial regression (Prentice 1986) was used to examine the influence of physical and chemical variables (Appendix table 1) on sampling efficiency for day and night snorkeling, and 1, 2, and 3-pass backpack electrofishing for bull trout 70- 200 mm total length. Beta-binomial regression is similar to logistic regression in that it uses dichotomous dependent variables, here the number of marked and recaptured individuals (resighted during snorkeling). However, beta-binomial regression models differ from logistic regression in that they model variance as a beta distribution and can automatically account for extra variance (Prentice 1986). Note that marked individuals captured outside of the sampling unit (i.e., between the second set of blocknets, described above) were not used for the sampling efficiency modeling (see *Evaluation of Bias*, below).

The goal of our sampling efficiency modeling was to obtain the simplest, best fitting (predicting) models, given our data. Thus, we used the information-theoretic approach, described by Burnham and Anderson (1998), to evaluate the relative plausibility of models relating habitat characteristics to sampling efficiency. To select a set of variables for inclusion in our global models (i.e., models containing all of the predictors), Pearson correlations were run on all pairs of predictor variables (i.e., site characteristics). Uncorrelated predictor variables ($r^2 < 0.25$) then were selected for inclusion in our candidate models on the basis of the effect they represent and their relative precision and repeatability. For example, stream substrate measures were significantly intercorrelated. Among the substrate measures, cobble estimates were the most precise and repeatable (Appendix table 1); hence, cobble was the only substrate measure (i.e., substrate effect) included in our set of candidate models. The subset of uncorrelated variables was used to construct the global model for each sampling method. From these models, we constructed a subset of 20 candidate models per method that contained various combinations of the predictors contained in the global model that we considered biologically relevant. The candidate models were then fit via beta-binomial regression. To assess the fit of each candidate model, we calculated Akaike's Information Criteria (AIC; Akaike 1973) with the small-sample bias adjustment (AIC_c; Hurvich and Tsai 1989). AIC is an entropy-based measure used to compare candidate models for the same data (Burnham and Anderson 1998), with the best fitting model having the lowest AIC_c. The relative plausibility of each candidate model was assessed by calculating ΔAIC_c weights as described in Burnham and Anderson (1998). These weights can range from 0 to 1, with the most plausible candidate model having the greatest ΔAIC_c weight.

We incorporated model-selection uncertainty by computing model-averaged estimates of the model coefficients and their standard errors as described by Buckland et al. (1997) and Burnham and Anderson (1998). To assess the statistical significance of these model-averaged predictors, we calculated 90% confidence intervals for each. The 0.1 significance level was chosen because of the relatively small sample size and the inherently noisy (highly variable) nature of sampling efficiency data (e.g., Peterson and Rabeni *in press*). Predictors were considered statistically significant if the confidence intervals did not contain 0.

To allow for ease of use in the field, the habitat characteristics were separated into 2 categories (high, low) prior to model fitting and assigned dummy values (0 = low, 1= high). Category breaks were based, in part, on the range of values during the calibration (Appendix table 1). Beta-binomial regression dispersion parameters were estimated for each method using the global model (Burnham and Anderson 1997). Goodness-of-fit was assessed for each global model by examining residual and normal probability plots (Agresti 1990).

Relative bias of the best fitting models for each method, as indicated by the ΔAIC_c weights (above), was assessed via leave-one-out cross validation. Cross validation estimates are nearly unbiased estimators of out-of-sample model performance (Funkunaga and Kessel 1971) and provide a measure of overall predictive ability without excessive variance (Efron 1983). Hence, they should provide an adequate estimate of the relative bias of the efficiency models. During this procedure, one observation was left out of the data, the beta-binomial model was fit with the remaining *n*-1 observations, and the sampling efficiency for the left out observation was predicted. Error was then estimated as the difference between the predicted and measured (i.e., number recaptured/ number marked) efficiency.

Results--Examination of residual plots for the 3 backpack electrofishing global models indicated that the fit of the beta-binomial model was adequate for each. Consequently, we assumed that the fit was adequate for all candidate electrofishing efficiency models (i.e., the subsets of the global model). Model selection results of the 1, 2, and 3 pass electrofishing efficiency models were virtually identical (i.e., same best fitting candidate model and statistically significant coefficients). Hence, we only report the results of the 3-pass model. The 3-pass efficiency model containing mean wetted width, mean maximum depth, and percent undercut banks was the most plausible, given the data (Appendix table 2). Three other candidate models containing different combinations of these three variables and wood density, conductivity, and map reach gradient were somewhat plausible and had ΔAIC_c weights that were at least 0.1 of the greatest weight, 0.56. The model-averaged coefficients, however, indicated that the 3 habitat variables contained in the most plausible model were the only statistically significant predictors (Appendix table 2). These 3 habitat variables were negatively related to electrofishing efficiency and among these, undercut banks had the greatest influence (i.e., largest coefficient).

Leave one out cross-validation procedure indicated a slight tendency of the 1 and 2-pass electrofishing models to underestimate and the 3-pass models to overestimate sampling efficiency with average cross validation errors of -0.0005 (standard error, 0.021), -0.005 (0.0241), and 0.0017 (0.0246), respectively. However, none were statistically significant from zero (i.e., the standard errors greatly exceeded the means) suggesting that the models were relatively unbiased.

Similar to backpack electrofishing global models, examination of the day snorkeling residuals indicated that the fit of the beta-binomial model was adequate and we assumed that the fit was adequate for all candidate models (i.e., the subsets of the global model). The day snorkeling efficiency model containing visibility, mean water temperature, and wood density was the most plausible, given the data (Appendix table 3). Two other candidate models containing different combinations of these 3 variables were also plausible with ΔAIC_c weights that were at least 0.1 of the greatest weight, 0.53. Model-averaged coefficients also indicated that these variables were also the only statistically significant predictors (Appendix table 3). Among these, mean water temperature had the greatest (and positive) influence on day snorkeling efficiency. Visibility also had a relatively strong, positive influence on efficiency, whereas wood density had a negative influence.

Leave one out cross-validation procedure indicated a slight tendency of the day snorkeling model to underestimate sampling efficiency with an average cross validation error of -0.0028 (standard error, 0.0166), but this difference was not significant.

Inspection of the night snorkeling residuals suggested that the fit of the beta-binomial model was also adequate. The night snorkeling efficiency model containing visibility, and map reach gradient was the most plausible, given the data (Appendix table 4). Only one other candidate model containing these two variables and mean water temperature was also plausible with a ΔAIC_c weight that was at least 0.1 of the greatest weight, 0.56. Model-averaged coefficients, however, indicated that water temperature was not a statistically significant predictor (Appendix table 4), whereas mean visibility and reach gradient were positively and significantly related to night snorkeling efficiency.

Similar to day snorkeling efficiency, leave one out cross-validation procedure indicated a slight, but nonsignificant, negative bias in the night snorkeling efficiency model with an average cross validation error of -0.0097 (standard error, 0.0242).

Evaluation of potential bias in calibration procedures –We were concerned that the some of the methods could have affected fish behavior, potentially biasing the estimates of bull trout sampling efficiency. Thus, we evaluated 2 potential sources of bias: 1) the effect of capture and handling of bull trout during the marking process on fish vulnerability during sampling and 2) the escape of marked fish from the sample units.

Presumably, the effect of handling and marking on fish vulnerability would diminish with time. For example, plasma cortisol (stress hormone) levels in fish generally return to normal within 24 hours after handling (Robert Reinert, University of Georgia, *personal communication*). Thus, we randomly varied recovery times (outlined above) at 24, 48, and 72 hours. The relationship between recovery time and sampling efficiency was then examined for each method via quasi-likelihood logistic regression (Bayley 1993). The results of the each regression indicated no statistically significant relationship between recovery times and sampling efficiency (Appendix table 5).

As discussed above, areas were blocked with 2 sets of blocknets during the 2000 field season to estimate the escape rate of marked fish. In general, the number marked bull trout escaping from the sample units was low and averaged 0.55 per unit. It also appeared to be related to recovery time with only 1 escapee in the 11 calibrations with 24-hour recovery times. To examine this relationship, we fit a quasi-likelihood logistic regression model relating escape rate to recovery time. The resulting regression indicated a statistically significant (P= 0.042), positive relationship between recovery time and escape rate. The model predicted that, on average, the escape rates at 24, 48 and 72 hours are 0.7%, 2.0% and 5.0%, respectively, which translates to less than 1 marked fish in the 24 (0.03 individuals) and 48 (0.62) hour recovery times and approximately 1 in the 72 hour period. Thus, it is unlikely that escaping individuals had a large effect on the sampling efficiency estimates, especially considering that marked individuals that had escaped were not included in the sampling efficiency modeling (above).

As a final examination of the possible effects of marking and handling, we cross-compared sampling efficiency estimates for each method as follows.

<u>Step 1</u>: Estimate sampling efficiency (π_m) for each method (m), site combination using the beta-binomial efficiency models.

<u>Step 2</u>: Estimate the number of unmarked fish in a site using the method- specific estimates of the number of unmarked fish and the sampling efficiency as:

$$T_m = N_m / \pi_m \qquad (3)$$

where: T_m = efficiency adjusted estimate of the number of unmarked bull trout, π_m = predicted sampling efficiency as a fraction, and N_m = the number of unmarked bull trout collected or counted with method *m*.

Step 3: Cross-calculate estimates of the sampling efficiency of each method by dividing the raw (actual) count /catch of unmarked fish by the efficiency adjusted estimates of unmarked fish of the other sampling methods. This results in 3 sampling efficiency estimates per method: (1) the efficiency estimate which is the ratio of recaptured (resighted) to marked individuals and (2-3) two cross-calculated efficiency estimates which are the ratio of the number of unmarked fish counted with that method to the efficiency adjusted estimates of the number of unmarked fish for the other 2 methods. For example, the 2 cross-calculated estimates for day snorkeling would be calculated by dividing N_d by T_n and T_e, where N_d = the number of unmarked bull trout counted during day snorkeling, T_n = efficiency adjusted estimate of the number of unmarked bull trout for night snorkeling, and T_e = the efficiency adjusted estimate of the number of unmarked bull trout for 3-pass electrofishing.

Each of the 3 estimates then was averaged across sites and 95% log-based confidence intervals (Burnham et. al. 1987) calculated to assess the relative accuracy of the methods. Large differences among the 3 efficiency estimates for each method would indicate that marking/ handling affected the vulnerability of marked fishes differently among methods or through time. The cross-comparison of the 3 sampling efficiency estimates for all methods indicated that they were similar to the mark-recapture (resight) estimates (Appendix figure 2). In fact, all were well within the 95% CI of the all of the mark-recapture based estimates. This suggested that either there was no detectable effect of marking on bull trout vulnerability or that this effect did not differ among methods or through time.

Appendix table 1. Mean, range, and standard deviation (SD) of habitat characteristics (n= 43), mean precision of estimates, and mean difference between 2 successive measurements at a site expressed as a percent of the original measurement. Variables with abbreviation codes were included in candidate models for predicting sampling efficiency.

Variable	Code	Mean	SD	Range	Mean precision	Remeasured difference	Cutoff value
Site length (m)		81.97	21.54	36.1 - 134		3	
Mean depth (m)		0.14	0.04	0.05 - 0.21	24	21	
Mean maximum depth (m)	MMAXD	0.27	0.08	0.11 - 0.41	20	21	0.25
Maximum unit depth (m)		0.45	0.10	0.3 - 0.65		10	
Map reach gradient (%)	REACH_GR	4.67	2.25	2 - 9.9		0	4.50
Field measured gradient (%)		4.32	1.57	2.2 - 7.3		9	
Mean wetted width (m)	MWID	3.44	1.00	2.33 - 7.42	11	13	3.50
Bankfull width (m)		5.24	1.64	2.95 - 10.5	20	13	
Wood density (no./m2)	DENLWD	0.09	0.07	0.01 - 0.3		40	0.07
Pool composition (%)		8.18	8.59	0 - 33.8		57	
Undercut banks (%)	PCTUCT	27.96	20.89	3.2 - 93.43		25	30.00
Overhanging vegetation (%)		45.78	26.37	5.1 - 97.84		31	
Surface turbulence (%)		21.21	9.47	2 - 40		48	
Submerged cover (%)		25.16	14.48	5 - 80		39	
Water temperature (^{0}C)	MWT						
backpack electrofishing		9.17	2.40	3 - 13.5			9.00
day snorkeling		10.66	2.99	4-15.5			9.00
night snorkeling		9.35	1.82	3.5-13.5			9.00
Conductivity (μohms)	CONDUCT	57.96	48.22	16 - 203		8	55.00

Appendix table 1 (cont).

Variable	Code	Mean	SD	Range	Mean precision	Remeasured difference	Cutoff value
Visibility (proportion of mean wetted width)							
day snorkeling	MVSBD	0.67	0.18	0.33 - 1.0	6		0.50
night snorkeling	MVSBN	0.70	0.15	0.40 - 1.0	4		0.50
Substrate (percent of substrate composition)							
Fines		16.82	9.77	1.7 - 36	75	46	
Gravel		21.85	12.16	6.3 - 60.8	48	50	
Cobble	PCTCOBB	25.37	8.53	5 - 44.2	40	38	25.00
Rubble		35.96	18.34	1 - 78.3	52	33	
Wollman counts							
< 8 mm		12.88	7.91	0 - 23.3		85	
8-15 mm		5.27	5.23	0 - 22.5		131	
16-32 mm		12.60	12.50	2 - 52		63	
33-63 mm		11.34	6.21	2 - 23.3		85	
64-127 mm		24.65	7.92	6.8 - 40		43	
128-255 mm		19.58	11.98	0 - 45		39	
> 256 mm		13.68	9.53	0 - 31.7		35	
Recovery time after marking (hrs.)		31.81	14.55	24 - 72			

Appendix table 2. Predictor variables, AIC_c, ΔAIC_c, ΔAIC_c weights, *w*, for the set of candidate models, *i*, (top) and model-averaged estimates of beta-binomial regression coefficients, standard errors (SE), and upper and lower 90% confidence limits (bottom) for 3-pass electrofishing sampling efficiency for bull trout. ΔAIC_c weights are interpreted as relative plausibility of candidate models. Predictor variable abbreviations can be found in Appendix table 1.

Candidate Model	AIC_c	ΔAIC_c	w_i
MWID MMAXD PCTUCT	559.92	0.00	0.56
MWID MMAXD PCTUCT DENLWD	561.97	2.05	0.20
MMAXD CONDUCT PCTUCT	563.66	3.74	0.09
MWID MMAXD PCTUCT REACH_GR DENLWD	564.04	4.12	0.07
PCTUCT PCTCOBB DENLWD	566.68	6.76	0.02
MMAXD PCTUCT PCTCOBB REACH_GR DENLWD	566.80	6.88	0.02
REACH_GR PCTUCT DENLWD	567.35	7.43	0.01
MWID MMAXD REACH_GR	568.78	8.86	0.01
MWID MMAXD	569.75	9.83	0.00
MMAXD CONDUCT	570.56	10.64	0.00
MWID MMAXD CONDUCT	570.64	10.72	0.00
MWID MMAXD DENLWD	570.68	10.76	0.00
MMAXD DENLWD	570.70	10.78	0.00
Global Model	571.25	11.33	0.00
MWID MMAXD PCTCOBB	571.66	11.74	0.00
MWID DENLWD	572.03	12.11	0.00
MMAXD MWT CONDUCT	572.58	12.66	0.00
MWID MMAXD MWT CONDUCT	572.92	13.00	0.00
MWT CONDUCT	574.07	14.15	0.00
CONDUCT DENLWD	574.19	14.27	0.00

Parameter	Estimate	SE	Upper 90%	Lower 90%
INTERCEPT*	0.118	0.215	0.468	-0.232
MWID*	-0.443	0.203	-0.112	-0.773
MMAXD*	-0.476	0.198	-0.154	-0.799
PCTUCT*	-0.707	0.195	-0.389	-1.025
DENLWD	-0.084	0.213	0.263	-0.430
REACH_GR	0.219	0.268	0.656	-0.217
CONDUCT	0.094	0.202	0.423	-0.235
MWT	0.010	0.205	0.344	-0.324
PCTCOBB	0.281	0.220	0.640	-0.078
Dispersion*	0.158			

* Included in final model for estimating detection probabilities.

Appendix table 3. Predictor variables, AIC_c, ΔAIC_c, ΔAIC_c weights, *w*, for the set of candidate models, *i*, (top) and model-averaged estimates of beta-binomial regression coefficients, standard errors (SE), and upper and lower 90% confidence limits (bottom) for day snorkeling sampling efficiency for bull trout. ΔAIC_c weights are interpreted as relative plausibility of candidate models. Predictor variable abbreviations can be found in Appendix table 1.

Candidate Model	AIC_c	ΔAIC_c	w_i
MVSBD MWT DENLWD	157.58	0.00	0.53
MVSBD MWT PCTUCT	160.15	2.57	0.15
MWT DENLWD	160.61	3.03	0.12
MWT PCTUCT	163.06	5.48	0.03
MWT	163.14	5.55	0.03
MVSBD MWT	163.54	5.96	0.03
MWID MVSBD MWT	163.66	6.08	0.03
MVSBD MWT PCTCOBB DENLWD	164.19	6.61	0.02
MWT PCTUCT DENLWD	164.57	6.98	0.02
MVSBD MWT PCTCOBB	165.40	7.81	0.01
MVSBD REACH_GR MWT	165.42	7.84	0.01
MWID MVSBD REACH_GR MWT	165.69	8.11	0.01
MVSBD REACH_GR MWT PCTUCT DENLWD	165.83	8.25	0.01
MWT PCTUCT PCTCOBB DENLWD	166.33	8.74	0.01
PCTUCT DENLWD	167.67	10.09	0.00
MWID MWT PCTUCT PCTCOBB DENLWD	168.09	10.51	0.00
Global Model	168.80	11.21	0.00
MVSBD REACH_GR DENLWD	169.25	11.67	0.00
PCTUCT PCTCOBB DENLWD	169.30	11.72	0.00
REACH_GR PCTUCT PCTCOBB DENLWD	169.83	12.25	0.00

Parameter	Estimate	SE	Upper 90%	Lower 90%
INTERCEPT*	-4.176	1.066	-2.428	-5.924
MVSBD*	1.379	0.833	2.745	0.013
MWT*	1.524	0.564	2.448	0.599
DENLWD*	-0.772	0.407	-0.104	-1.440
PCTUCT	-0.670	0.412	0.006	-1.346
MWID	0.609	0.384	1.238	-0.021
REACH_GR	-0.117	0.506	0.713	-0.947
PCTCOBB	0.156	0.388	0.793	-0.480
Dispersion*	0.194			

* Included in final model for estimating detection probabilities.

Appendix table 4. Predictor variables, AIC_c, ΔAIC_c, ΔAIC_c weights, *w*, for the set of candidate models, *i*, (top) and model-averaged estimates of beta-binomial regression coefficients, standard errors (SE), and upper and lower 90% confidence limits (bottom) for night snorkeling sampling efficiency for bull trout. ΔAIC_c weights are interpreted as relative plausibility of candidate models. Predictor variable abbreviations can be found in Appendix table 1.

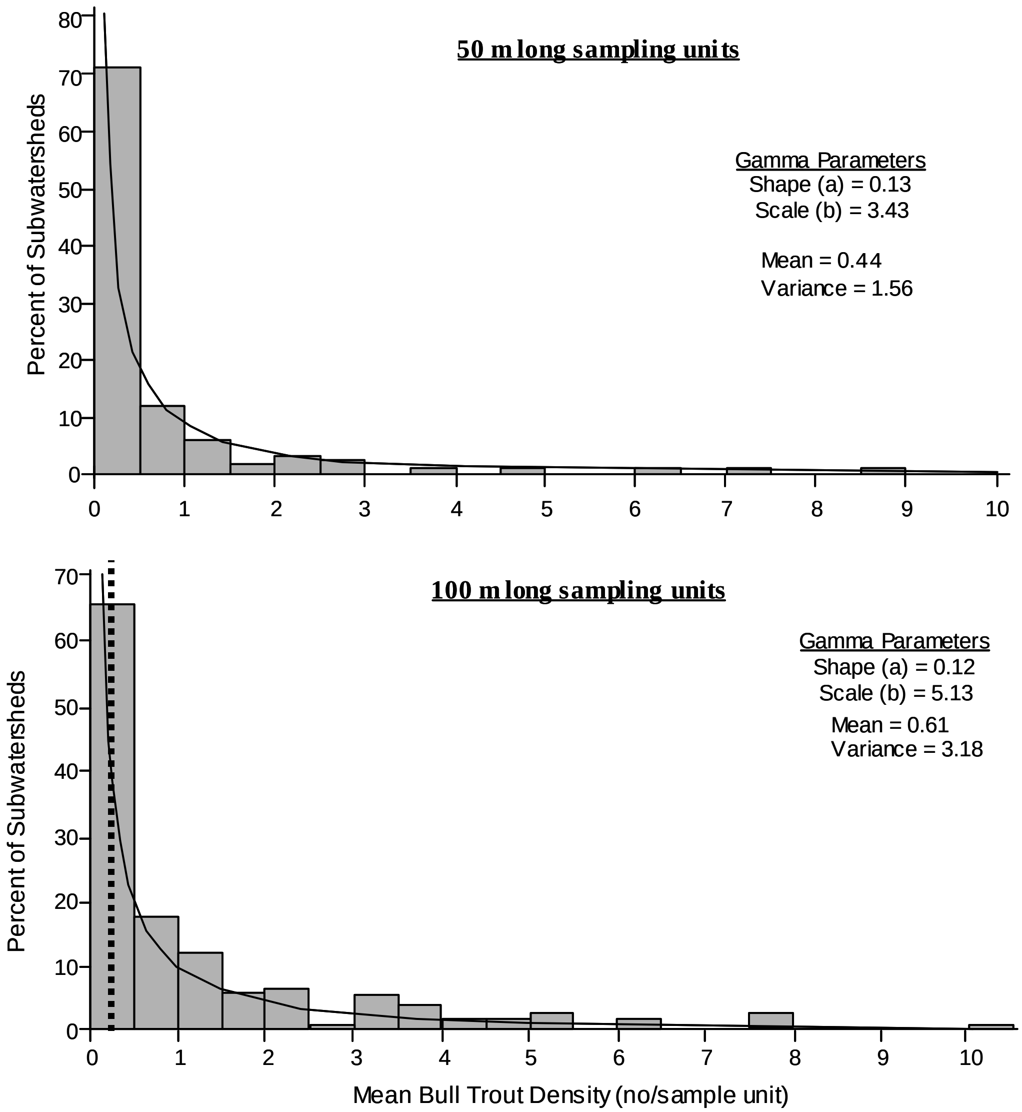
Candidate Model	AIC_c	ΔAIC_c	w_i
MVSBN REACH_GR	277.42	0.00	0.56
MVSBN MWT REACH_GR	278.96	1.55	0.26
MVSBN REACH_GR DENLWD	283.17	5.75	0.03
MVSBN MWT	283.51	6.09	0.03
MWT	284.26	6.84	0.02
MWT REACH_GR	284.44	7.02	0.02
MWID MVSBN REACH_GR MWT	284.78	7.37	0.01
MVSBN MWT PCTCOBB	285.02	7.60	0.01
MVSBN MWT DENLWD	285.29	7.87	0.01
MWID MVSBN MWT	285.35	7.93	0.01
MWT DENLWD	285.73	8.32	0.01
MWT PCTUCT	285.93	8.51	0.01
PCTUCT DENLWD	286.35	8.94	0.01
MVSBN MWT PCTCOBB DENLWD	286.93	9.52	0.00
MVSBN REACH_GR MWT PCTUCT DENLWD	287.14	9.72	0.00
REACH_GR PCTUCT PCTCOBB DENLWD	287.89	10.48	0.00
PCTUCT PCTCOBB DENLWD	288.17	10.75	0.00
MWT PCTUCT PCTCOBB DENLWD	289.45	12.03	0.00
Global Model	291.58	14.17	0.00
MWID MWT PCTUCT PCTCOBB DENLWD	291.61	14.19	0.00

Parameter	Estimate	SE	Upper 90%	Lower 90%
INTERCEPT*	-1.810	0.513	-0.969	-2.651
MVSBN*	0.881	0.443	1.607	0.155
REACH_GR*	0.524	0.291	1.001	0.047
MWT	0.110	0.319	0.633	-0.413
MWID	0.127	0.276	0.579	-0.325
PCTUCT	0.041	0.274	0.491	-0.408
PCTCOBB	0.139	0.275	0.591	-0.312
DENLWD	0.125	0.267	0.563	-0.313
Dispersion*	0.170			

* Included in final model for estimating detection probabilities.

Appendix table 5. Quasi-likelihood regression coefficients, standard errors (SE), and chi-square test statistics of the relationship between recovery time and bull trout sampling efficiency.

Parameter	Estimate	SE	Chi-Square	P-value
3-pass electrofishing				
Intercept	-0.848	0.289	8.610	0.003
Recovery time	0.012	0.013	0.880	0.348
Day snorkeling				
Intercept	-2.513	0.464	29.320	0.001
Recovery time	0.010	0.013	0.630	0.426
Night snorkeling				
Intercept	-0.580	0.284	4.180	0.041
Recovery time	-0.008	0.009	0.790	0.374



Appendix figure 1. Distributions of mean bull trout densities for 50 m and 100m long stream sampling units in subwatersheds (USGS 6th code hydrologic units) containing know populations of bull trout (shaded bars). Thin lines represent gamma distributions fit to the mean density data and heavy broken line (below) approximates threshold values of Hillman and Platts (1993) (0.25/100 m) and Bonar et al. (1997) (0.15/100 m).
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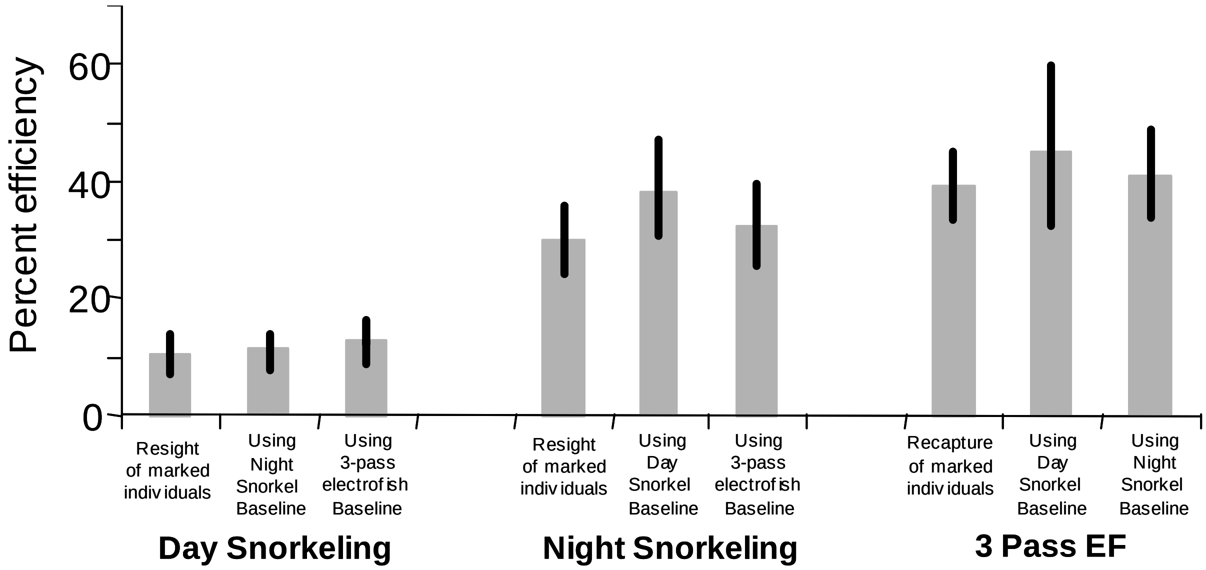

Appendix figure 2. Cross-comparison of estimated average sampling efficiency, with log-based 95% confidence limits (black bars), of day and night snorkeling and 3-pass backpack electrofishing (EF) for bull trout 70-200 mm total length. Method-specific resight (snorkeling) or recapture efficiencies were estimated as the ratio of the number of recaptured or resighted individuals to number of marked individuals. Method-specific baselines were based on efficiency-adjusted estimates of the number of unmarked fish. See text for a complete explanation.

Appendix 5.

Research Needs

A variety of information could be collected to advance our understanding of sampling methods for bull trout. We identified what we view to be the five highest priorities, based on developing this protocol and feedback from numerous biologists working with bull trout.

- Bull trout sampling efficiencies and habitat associations throughout the range of the species, particularly Montana, Oregon, and Nevada. Work is currently underway in Washington.

- Estimates of the influence of block nets on sampling efficiency

- Protocols for detecting presence and habitat associations of migratory forms

- Examination of temporal variability in bull trout presence

- Decision support tools to aid in managing for bull trout when presence is not certain

Notes

1. Use of data for protocol revision

Information from agency surveys can be used to compare to predicted levels of effort predicted by our models. In essence, agency survey data can provide us with the opportunity to test the predictive ability of our models. If there is a discrepancy, we may need to revise our models. For example, if agency surveys consistently detect bull trout with significantly less effort than predicted by the models, we might need to revisit our sampling efficiency models or fish density models.

The bull trout densities used in determining sample sizes for the protocol are based on data collected from a relatively limited number of sampling frames within the range of the species. Incorporating a greater number of samples collected over a greater proportion of the bull trout range would more closely approximate a random sample of all occupied sample frames. Furthermore, data specific to a particular area or basin may provide a more realistic distribution of densities.

Previously collected data on bull trout densities can also be incorporated directly into the fitting of the gamma distribution. Additionally, these data can be used to parameterize models for estimating the probability that a particular area contains bull trout given that they were not detected (*sensu* Bayley and Peterson in press).

2. Block Nets

We used block nets to restrict emigration or immigration of bull trout in an attempt to meet assumptions for sampling closed populations. Unfortunately, we have very little information to assess the effectiveness of block nets in bull trout streams. During the 2000 field season Idaho sampling crews evaluated bull trout movements through block nets and found that, in some cases, a small number of fish moved past nets. Since we have not compared fish movements in sites with and without block nets, we are unsure how effective block nets are. Although block nets may not always be 100% barriers to movement, they likely prevent some movement. Consequently, because the estimated efficiencies in the protocol are based on surveys of block netted sites, we encourage biologists who apply this protocol to also use block nets. We recognize that it may not be feasible to block net many streams because of stream size or physical characteristics. In those cases, we encourage biologists to sample without block net and to apply the remainder of the protocol; however, we caution that the actual sampling efficiencies without block nets may be less than values we present in our tables. The number of fish that move and the distance they move during sampling will likely determine the effect of not using block nets. We recognize that additional research is needed to evaluate sampling efficiencies without block nets.

3. Sampling methods

Our intent is to provide protocols for various sampling methods rather than to recommend a specific method. We believe those conducting the surveys should consider a variety of factors before choosing the most appropriate method. While the relative sampling efficiency of the proposed method is a critical consideration, it should not be the only consideration. Other factors may include: the survey objectives, the physical characteristics of the area to be sampled, risks to aquatic taxa, human safety concerns, and time and budgetary considerations. For example, to reduce potential injury to listed species a biologist may select snorkeling over electrofishing. If stream access is difficult or sampling potentially hazardous, the biologist may choose day snorkeling over night snorkeling.

4. Electrofishing

Smith-Root gas and battery powered backpack electrofishers were used for the surveys on which the protocol sample sizes are based. Depending on the conductivity of the water, a variety of waveform settings and voltages were used at individual sampling sites. Prior to the survey, the effectiveness of the settings was tested in areas outside of our sample sites. The selected settings were retained until that site was completely surveyed.

5. Training

Inter-observer variation is inevitable, in spite of the most well-intentioned training. We developed our database with a wide variety of observers that have all had a minimum amount of training and experience. Training involved laboratory and field sessions that spanned at least two days. Additional hands-on training was provided in the field, along with close supervision by experienced personnel. Ultimately, training should strive for a minimum degree of proficiency, rather than time logged in the field or classroom. This could be achieved with standardized evaluations. For example, test the ability of different observers to correctly identify, measure, and capture (or observe) fish in the field. Require a minimum level of proficiency for observers participating in surveys. This should be periodically tested over time. Both experience *and* training with periodic evaluations are key.