

Washington State
Cooperative Monitoring, Evaluation, and Research Committee
(CMER)

Study Plan
for the
**TYPE N Experimental Buffer Treatment Study:
Addressing Buffer Effectiveness on Stream-Associated
Amphibians, Riparian Inputs and Water Quality, and
Exports to and Fish in Downstream (Type F) Waters in
Basaltic Lithologies of the Coastal Areas and
the South Cascades of Washington State**

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Executive Summary

The TYPE N Experimental Buffer Treatment Study (hereafter the TYPE N Study) will evaluate the effectiveness of the TYPE N (non-fishbearing) stream buffers in addressing three overall Performance Goals of the Forests and Fish (FFR) Agreement: 1) maintain viable populations of stream-associated amphibians (SAAs), 2) meet water quality standards, and 3) provide harvestable levels of fish. Specifically, this study is aimed at understanding how timber harvest activities using different buffer configurations in relatively small TYPE N basins affect a suite of input processes (heat, litter, sediment, and wood) and how changes in those processes affect downstream fish-bearing waters. The study will also link changes in stream conditions and input processes to changes in abundance (or other responses, e.g., growth rate) of amphibians and fish.

The TYPE N Study uses a Before-After Control-Impact (BACI) design applied to 4 experimental treatments with 5 replicates per treatment for a total of 20 sites (i.e., TYPE N basins). The 4 treatments include: 1) no buffer (i.e., clearcut harvest throughout basin), 2) an FFR buffer (50% of the stream has a 50' buffer), 3) a 50' buffer along 100% of the stream, and 4) an unharvested reference site. All 20 sites will be surveyed 2 years pre-harvest and 2 years post-harvest, with opportunities for long-term monitoring not specifically covered in this proposal. BACI study designs are typically superior to alternative designs at revealing potential cause (forest practices) and effect (biotic responses and changes to inputs processes) relationships. Further, because this design includes harvest intensities that bracket an FFR prescription, it will provide important insights about thresholds of disturbance relative to FFR.

All studies, including this one, incorporate basic assumptions. By including amphibian (notably FFR-designated SAAs) response as a study objective, we have limited our study to areas with relatively competent geologies in western Washington (the range collectively occupied by FFR-designated SAAs). These geologies may not be as readily disturbed during harvest as other more easily weathered geologies such as sandstones. We limit our study to relatively small TYPE N basins that will be harvested across the entire TYPE N basin. This should serve as a “worst case scenario” in terms of impacts from timber harvest since larger basins will typically not be entirely harvested during a single operation. Lastly, we require that timber be felled away from streams in the unbuffered portions of the stream channel to allow instream sampling, a practice that represents only a segment of the range of activities across the landscape.

This proposal addresses funding for initial pre- and post-treatment periods, but the design could (and in our opinion should) serve as a laboratory for extended study. Opportunities for collaboration have not gone unnoticed. Currently, the project has multiple cooperators, including The Campbell Group, Green Crow; Hancock Forest Management; Port Blakely Tree Farms; the Makah Nation; Merrill & Ring; the Quinault Nation; Rayonier; the US Forest Service; Washington Departments of Ecology, Fish and Wildlife, and Natural Resources; Washington State University; Weyerhaeuser; and Longview Fibre, all of whom have brought unique expertise and/or in-kind support.

INTRODUCTION

This document describes an experimental study to evaluate the performance of a range of riparian management prescriptions for TYPE N (non-fishbearing) streams in western Washington. This study design was prepared for the Timber, Fish and Wildlife (TFW) Cooperative Monitoring, Evaluation and Research Committee (CMER), which was established by the Washington Forest Practice Board (WFPB) to “conduct research, validation and effectiveness monitoring to facilitate achieving the resource objectives” and to “advance the science needed to support adaptive management” for Washington State Forest Practices Rules (WFPB 2001).

The document provides 1) a brief description of the riparian prescriptions, 2) an overview of the scientific assumptions and uncertainties associated with the prescriptions, 3) justification for this study; and 4) detailed study design, and a budget.

Background on FFR Goals, Resource Objectives, and Riparian Prescriptions

In the spring of 2000, the WFPB adopted emergency rules designed to maintain and restore salmonid populations and meet the requirements of the federal Clean Water Act (WFPB 2000). These rules were based on the recommendations of the Forests and Fish Agreement (FFR), the product of negotiations between federal agencies (National Marine Fisheries Service, US Fish and Wildlife Service, US Environmental Protection Agency), timber landowners, state resource agencies (Washington Department of Ecology, Washington Department of Natural Resources, and Washington Department of Fish and Wildlife), and tribal and local governments (USFWS *et al.* 1999). A similar rule package was permanently adopted in May 2001 (WFPB 2001). These rules were drawn up around the three FFR Overall Performance Goals, which address avoiding impairment to the capacity of the aquatic habitat to:

- 1) meet or exceed water quality standards,
- 2) support harvestable levels of salmonids, and
- 3) support the long-term viability of other covered species, namely stream-associated amphibians (SAAS).

Riparian buffer prescriptions are a key element of the FFR strategy to achieve these goals. The FFR riparian prescriptions are designed to achieve the Performance Goals by maintaining important ecological functions provided by riparian forests, including large woody debris (LWD) recruitment, shade to control stream temperature, sediment filtering/bank stability, and litterfall.

The riparian prescription for westside TYPE N streams consists of a patch-buffer strategy that includes a 50' buffer along at least 50% of the perennial stream length and a 30' equipment exclusion zone along the entire (perennial and seasonal flow) stream channel. Fifty-foot buffers are also required around perennially saturated soils of headwall and sideslope seeps, headwater springs, stream junctions, and alluvial fans; the five sensitive sites categories designated in forest practices rules (WFPB 2001). Yarding corridors can comprise up to 20% of the channel length. Portions of riparian stands outside the prescribed buffers can be clear-cut to the stream. Overall, a minimum of 50% of the stream length is buffered with the distribution of buffered and harvested reaches

depending on the location of sensitive sites and other priority features. The TYPE N patch buffer strategy represents a negotiated reconciliation among amphibian conservation, uncertainties about how to implement conservation, and timber industry economics.

Purpose

At the time of FFR negotiations, almost no published studies existed either on the efficacy of buffers for headwater streams or on clear guidance addressing their design. Moreover, the few studies available are either correlative, retrospective (Bisson *et al.* 2002, Raphael *et al.* 2002) or lack power needed to interpret observed responses for specific FFR resource targets (e.g., O'Connell *et al.* [2000] and Jackson *et al.* [2003] for amphibians); all conditions that limit drawing conclusions about responses to different harvest treatments. Thus, the purpose of this study is to evaluate the relative effectiveness of alternative prescriptions in meeting FFR resource goals, which includes evaluating the response of SAAs to differing buffering strategies, within a design powerful enough to make drawing unambiguous conclusions likely.

Conceptual Models

Two conceptual models are useful for understanding the study design. The energy pathway model portrays the major pathways of potential effects on headwater amphibians and downstream export to fish-bearing streams (APPENDIX I). The landscape model describes the physical conditions in headwater basins and provides the basis for locating some sampling sites and understanding physical process pathways (APPENDIX II).

In brief, the energy pathway model shows that harvest can reduce stream shading and increase sedimentation inputs.

- Change in shade and sedimentation will translate into instream production responses. The relative degree of change in shade versus sedimentation is anticipated to structure the type of response obtained. Resulting secondary responses may alter downstream exports.
- Instream production and habitat changes, and alteration of downstream exports have the potential to alter SAAs (in the TYPE N) and fish (downstream).

For the landscape model:

- First- and 2nd-order valleys are less decoupled from their hillslopes than 3rd- and higher-order valleys, so hillslopes exert a greater influence on streams in lower-order than in higher-order valleys. Downstream effects are anticipated.
- Surface water chemistry and temperature are highly influenced by soil- and groundwater inflow to the channel. As groundwater is an important discharge component in lower-order streams, significant groundwater influence on water temperature and chemistry is anticipated.
- Within the channel, hyporheic flow between bedforms and channel reaches establish chemistry and temperature differences between hyporheic recharge and discharge zones; water sampling and temperature measurement stations should be consistently located relative to bedforms and hyporheic flow regimes.

- Sites are to be located in watersheds with competent, coarse clast-producing bedrock, which may be largely basalts. Streams in basalts tend to be groundwater dominated, which may limit their response to harvest and riparian treatments.

STUDY OBJECTIVES AND CRITICAL QUESTION

By comparing individual treatments to each of their pre-treatment (reference) conditions, this study seeks to assess the degree to which forestry practices may impact public resources. Comparison of individual treatments to reference treatments (basin with stand age between 30 and 80 years, but will not be harvested for this study) will help distinguish whether observed changes are attributable to environmental variation or forestry practices. Alignment of treatments along a gradient will permit identification of whether the potential impact of forestry practices may differ among treatments at the TYPE N basin scale for the three FFR resource goals (see previous section).

Thus, the primary objective of this study is to address the following critical question:

Critical Question: What is the magnitude, direction (positive or negative), and duration of change in riparian-related inputs (light, litterfall, sediment, and woody debris) and the response of instream (amphibians, water temperature, habitat) and downstream components (export of nutrients, organic matter, macroinvertebrates, and sediment; water temperature; and fish in the downstream [TYPE F¹] stream) associated with a range of timber harvest treatments that vary in buffer length relative to untreated reference conditions?

This question will be addressed by measuring a set of primary and secondary variables (TABLE 1). Primary variables, the study focus, reflect the FFR resources goals for evaluating treatment effects. Thus, measurement of amphibians, temperature, exports, and fish should reveal potential impacts to the three FFR resource goals. Importantly, harvest effects will be measured over a short duration relative to the periodicity of the disturbance (i.e., harvest). Results and conclusion about harvest effects must be viewed in this context. Through measurement of secondary variables (those measured to support primary variables) related to riparian inputs (light, litterfall, nutrients, sediment, and woody debris) and other instream habitat conditions, we hope to link potential differences in the primary variables between treatments to the secondary habitat factors that may be influencing them. These patterns can provide insights and produce hypotheses for how prescriptions can better achieve FFR resource goals.

Lastly, we hope to measure a limited set of covariates associated with temperature to minimize the effects of confounding factors for temperature.

STUDY DESIGN

A randomized block design will be used with four treatments (FIGURE 1); an unharvested reference basin from the timber harvest-managed landscape represents one treatment. Criteria for selecting TYPE N stream basins and description of the distribution of blocks, variables measured, and data analysis follow.

¹ TYPE F streams are fish-bearing in the new typing system and consist of a combination of the old TYPE 4 and 5 streams combined (WFPB 2001).

TABLE 1. VARIABLE LIST Covariates are only for the water temperature analysis (see APPENDIX XI); numbers indicate the relative importance rank of each covariate in ascending order.

VARIABLE OR VARIABLE GROUP		VARIABLE TYPE		
		Primary	Secondary	Covariates
In- or Near Channel Variables				
Amphibians	Occupancy	X		
	Density	X		
	Genetics	X		
Periphyton Standing Crop			X	
Temperature	Water	X		
	Air			1
	Soil			2
Channel Structure	Gross Morphology		X	
	LWD		X	
	Substrate		X	
	Bank erosion		X	
Downstream and Export Variables				
Fish	Density	X		
	Quality	X		
	Stable Isotopes		X	
Nutrients		X		
Macroinvertebrates		X		
Detritus		X		
Sediment			X	
Stream Flow			X	
Temperature	Water	X		
Riparian Input Variables				
Stand Growth/Survival and LWD recruitment			X	
Shade			X	
Litterfall			X	
Sediment			X	

Site Selection

Amphibian distribution and abundance are the primary drivers behind the selection criteria for geographic area of the study and the treatment unit size. Amphibians are a key FFR resource goal because they represent the vertebrate group that is thought to be most vulnerable to environmental change, and thus, most suitable for monitoring

environmental conditions (Wake 1991); they represent perhaps the largest segment of the resident vertebrate biota in headwater streams (Burton and Likens 1975, Bury *et al.* 1991); several headwater species, notably the seven amphibian listed as target species under FFR, have been suggested as being sensitive to forestry practices (Bull and Carter 1996, Corn and Bury 1989, Bury *et al.* 1991, Jackson *et al.* 2003, O'Connell *et al.* 2000, Steele *et al.* 2003); and five of the seven species are Washington State Species of Special Concern (Washington Department of Fish and Wildlife [WDFW] 2003)². The seven FFR amphibian species are: two tailed frogs [*Ascaphus* spp.], Dunn's salamander [*Plethodon dunnii*], Van Dyke's salamander [*P. vandykei*], and three torrent salamander species [*Rhyacotriton* spp.]. We cannot address all amphibian species in a single study because the species do not have overlapping distributions. In addition, two terrestrial FFR salamanders Dunn's and Van Dyke's are not addressed in this study because they are difficult to sample (see APPENDIX IV for a complete discussion)

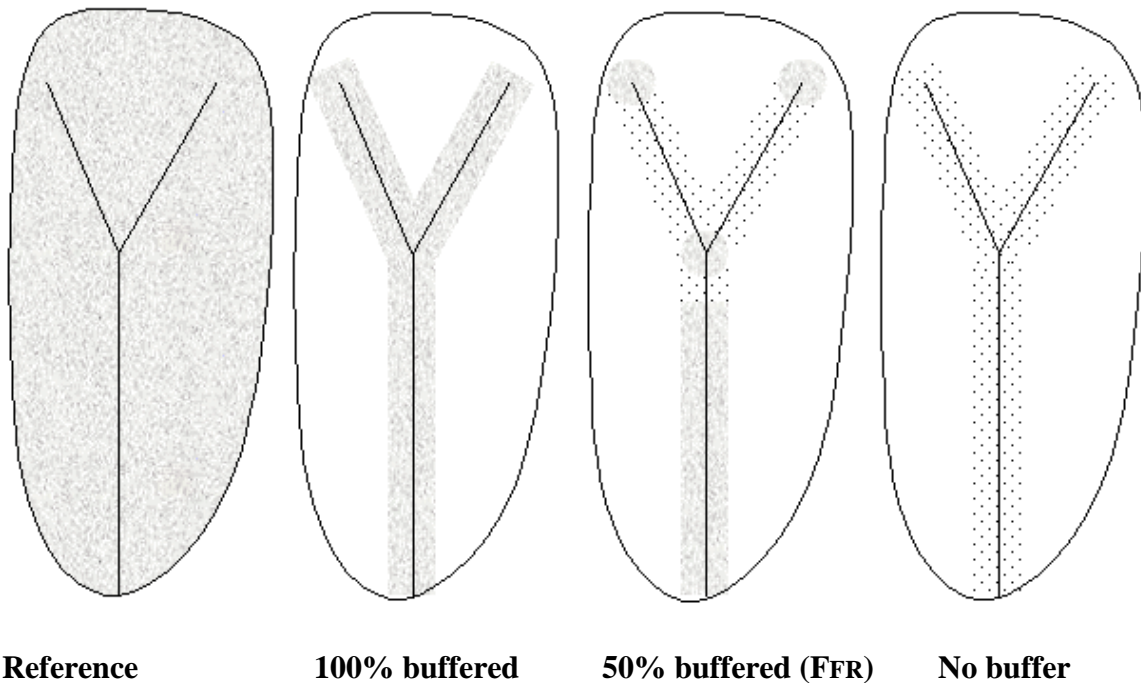


FIGURE 1. DIAGRAM OF THE FOUR TREATMENTS IN A BLOCK Harvested treatments (three on the right) vary in decreasing proportion of Np³ stream with a 50-ft riparian buffer. The textured gray areas are unharvested portions of a basin or buffers. Stippled areas on all unbuffered portions of treatments indicate a 30-ft equipment exclusion zone. Yarding corridors are not shown.

² Upon FFR finalization (2000), four of the six SAA species in the agreement were on the state candidate list: the Cascades and Columbia torrent salamanders, and Dunn's and Van Dyke's salamander. In 2001, Neilson *et al.* (2001) partitioned tailed frogs into two species, increasing the number of FFR amphibians to seven; in 2003, WDFW (WDFW 2003) placed one of the two species, the Rocky Mountain tailed frog on the state list of Species of Special Concern.

³ Np and Ns streams are the new subcategories of non-fishing bearing (TYPE N) stream that correspond to the TYPE 4 and 5 streams (WFPB 2001).

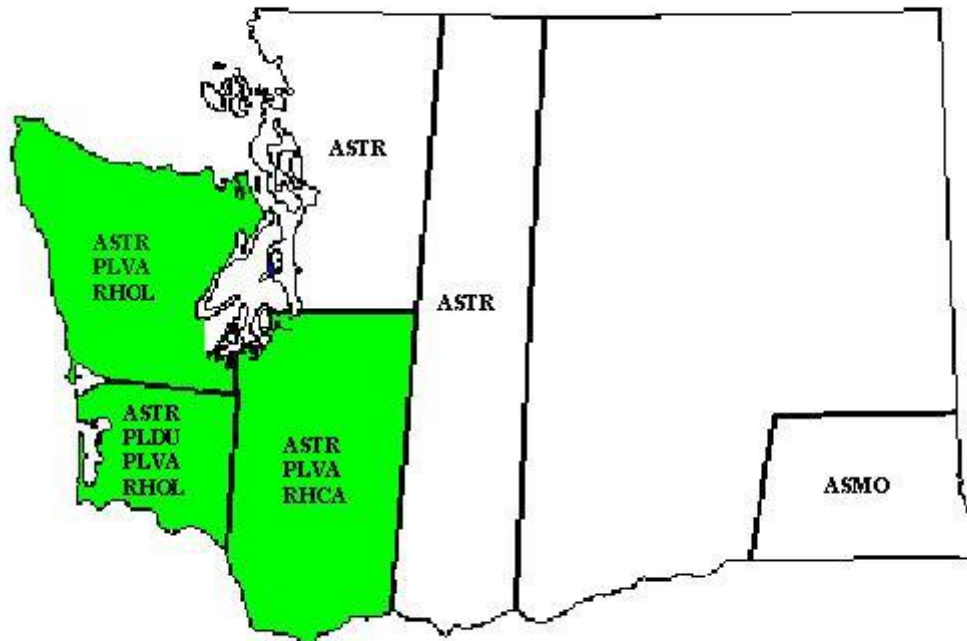


FIGURE 2. REGIONAL DISTRIBUTION OF FFR SPECIES IN WASHINGTON STATE AND SELECTED STUDY REGIONS. Green-highlighted physiographic regions encompass the area over which selection of sites (TYPE N basins) is based (see text). See APPENDIX V for species codes.

Coastal tailed frogs are the primary amphibian focus because they are the least frequently encountered instream FFR species in most physiographic regions richest in FFR species. Their presence is most often associated with the occurrence of other instream amphibian taxa (i.e., coastal tailed frogs were an instream amphibian species richness indicator; LWAG, unpubl. data), and their virtual disappearance from harvested units in a recent manipulative study (Jackson *et al.* 2003) suggests that forestry practices have the possibility of extirpating coastal tailed frog from TYPE N basins. Our study site selection attempts to maximize the number of FFR species included, which limits the potential area for site selection to the two coastal and South Cascades physiographic regions (FIGURE 2). Within the physiographic regions, distribution of competent bedrock lithologies, such as basalt, may be an important control on the distribution of SAAS. In other words, the occurrence of FFR amphibians on non-competent lithologies is too infrequent, especially for coastal tailed frog (e.g., Wilkins and Peterson 2000; LWAG, unpubl. data; see also Dupuis *et al.* 2000), to include them in this study.

Basin area is also a major constraint on site selection. Coastal tailed frogs appear to reproduce only in larger TYPE N basins and site selection is limited to the smallest basins in which tailed frogs are found to reproduce. Limiting basin size will ensure the maximum possible effect in treatment application (APPENDIX VI) and also keep basin size within the range of allowable harvest units (< 120 ac). The minimum size of basins in

which tailed frogs may reproduce is province-specific. In southwestern Washington, coastal tailed frogs reproduce in 2nd-order basins, however they may reproduce in some 1st-order basins in the South Cascades physiographic region. Because difference in basin area adds unwanted variability, we will attempt to select basins similar in size within and among blocks.

Stand age is also key constraint on site selection. As the basic study intent is to examine responses to different harvest treatments, treatment stands need to be something close to rotation age. Landscape processes do not seem to change significantly between the time a stand reaches 30 years of age and the point of harvest (40-80 years depending on landowner), thus treatment stands are constrained to between 30 and 80 years of age.

Experimental Treatments

The four proposed treatments are (FIGURE 1):

- 1) Reference basin that will not be harvested;
- 2) Clearcut basin with a 50' buffer along the entire Np stream length, except for yarding corridors as prescribed in the current rules;
- 3) Clearcut basin with the current FFR buffer: 50' buffer along 50% of the Np stream including buffers prescribed for the sensitive sites;
- 4) Clearcut basin with no riparian buffer, but with a 30' equipment exclusion zone along the entire Np stream length.

Treatments are arranged along a gradient reflecting increasing proportion of stream buffer while holding other aspects of the FFR riparian prescriptions as constant as conditions allow. Although forest practice rules do not explicitly prescribe it and it is not always typical of harvest operations on TYPE N streams, harvest and yarding operations will be done in a manner that minimizes harvest debris in the stream channel. Jackson *et al.* (2003) noted that substantial portions of headwater stream channels were covered with up to 2 m of larger organic debris (logs, branches) and sediment from felling, limbing and bucking in or near the stream channel. It is not possible to sample amphibians in channels buried by this type of debris. Moreover, minimizing harvest debris should reduce the effects of sedimentation, intentionally minimized in this study, and variability in the response variables. Consequently, the study is not intended to evaluate the entire range of FFR prescriptions applied in an operational setting.

As potential study sites are identified, they will be aggregated into blocks based on geology; stand age and time until harvest; basin size, dendritic stream network pattern, and stream length; channel morphology and hydrology (*fide* Montgomery and Buffington 1993, Montgomery and MacDonald 2002); aspect; gradient; number of road crossings and their locations; and proximity to other sites (in general order of descending importance). Each basin will be individually examined and may be excluded from specific blocks or from the entire study as needed. For example, high gradient channels are typically unresponsive to inputs of fine sediment in the absence of large woody debris as transport capacity typically exceeds sediment supply (Montgomery and MacDonald 2001); thus, treatment units will be screened during the selection process to select basins with similar channel morphologies and amounts of large woody debris. Each block will contain a replicate of each treatment. Treatments will be randomly assigned to sites

within a block wherever possible. A single block will not overlap regional boundaries that contain different FFR species compositions (see FIGURE 2). Ideally all sites within a block would come from within one or two adjacent Watershed Administrative Unit(s) (an area of ca. 20,000 ha).

Sampling methods

Data collection methods are categorized as follows:

- data collected from in or near the stream channel,
- upland measurements taken within the riparian buffer, and
- measurements taken to estimate downstream export from the non-fishbearing portion of the stream and to identify effects of downstream exports on fish-bearing reaches.

TABLE 1 identifies primary and secondary variables and variables measured as covariates to assist analysis or interpretation. A map will describe each study basin, and a longitudinal profile will be developed for each stream channel. These will be used to record the locations of monitoring stations, sampling sites, buffer boundaries, and harvest activities. The channel profile will be constructed from survey data (described below) and the site map will be constructed from aerial photointerpretation supplemented by field data collected by reconnaissance methods.

In- or Near- Channel Variables: In- or near-channel variable measurements focus on sampling amphibians with instream life stages and habitat features that are expected to change with treatments.

Amphibian Sampling: Amphibians will be sampled to identify potential treatment-specific changes in density over the short-term, and potential changes in genetic diversity and persistence over the longer term (for practical purposes, FFR defined the performance goal of “maintaining viability” as maintaining the occupancy of a species at the scale of TYPE N basin over time). Different sampling regimes are necessary to obtain each type of data.

Amphibian occupancy and density sampling will be conducted during all pre- and post-treatment years, but not during the year when harvest occurs (with a possible alternative, addressed in the Budget section, allowing for the possibility of excluding the year dedicated to harvest). Sampling will be with replacement; surveyors will replace all substrate material and release all amphibians following processing at the sample location. To minimize temporal variation, all treatments within a block will be sampled within a week; and all sampling will be performed during the low-flow period (mid July-early October), avoiding low temperatures that may depress amphibian activity. To minimize potential variation among survey crews and further minimize temporal variation, the sequence of sampling blocks and treatments within blocks will be randomly assigned.

Except for giant salamander identification, this study request is for genetic data obtained only for pre-treatment years; post-treatment sampling requires a delay of at least one generation for the sampled species (7-8 years in this case) and thus, will be addressed in an appropriately time-delayed request. Tissue (tail clips or mouth swabs) sampling to distinguish giant salamander species will be obtained from all giant salamanders collected during density and presence sampling over all sampling intervals (pre- and post-harvest). No evidence of detrimental effects from the small amounts of tissue obtained with single tail clips has been identified from similar studies (McCarthy and Parris 2004); mouth swab samples can be obtained from individuals the size of which a tail clip may be

deemed a risk. Tissues (tail or toe clips, or mouth swabs⁴) to identify potential genetic changes in treatments will be obtained from a minimum of 30 individuals per treatment unit. Additionally, a minimum of 30 individuals per treatment unit will be sampled from a minimum of 9 sites located at varying distances from two randomly selected treatments to obtain information on genetic neighborhood during one of the pre-treatment years.

Occupancy: A longitudinal light touch (LLT) approach will be used to determine occupancy; LLT sampling involves two surveyors searching upstream across the bankfull width and overturning any moveable surface objects to enhance detection. Sampling will be conducted along the entire length of non-fishbearing stream network and sensitive sites in all treatments. First encounters of each life stage of each new amphibian found will be recorded during LLT sampling, and vouchered using digital photography for independent verification. Body size (snout-vent length [SVL] for amphibians) and mass data will be recorded for all individuals representing first encounters. Locations of first encounter sites will be recorded on the site map and longitudinal channel profile.

Density: The length of the dominant stream thread in each stream will be segmented into 10-m reaches. Twenty-five 10-m reaches will be randomly selected for sampling; one 1-m segment targeted for sampling will be randomly selected from within each of these 10-m reaches. Each 1-m segment will be restricted with a block net at its upstream and downstream ends, and two surveyors will remove all material coarser than sand or small gravel in order to record and capture all amphibians. Once all coarser material and obvious amphibians are removed, remaining finer substrate material will be raked and sifted for any remaining individuals. The locations of these sites will be recorded on the site map and longitudinal channel profile.

Body length, mass, life stage, and condition will be recorded for each amphibian found. Condition factors will be derived from mass-body length regressions, and condition scoring will record missing or truncated limbs/digits, and other location-specific anomalies or injuries.

Genetics: Two species will be analyzed for the genetic portion of the study: coastal tailed frogs and Cope's giant salamanders. These species were chosen for two main reasons. First, they represent an important comparison representing the two possible extremes for amphibian dispersal abilities throughout the study area. Due to the fact that Cope's giant salamander is almost exclusively a neotenic species and often fail to metamorphose, they are restricted to streams and among stream dispersal should be low, relative to coastal tailed frog, which disperses overland. These data will provide critical information on the relative importance of migration and metapopulation dynamics for species persistence and/or recovery. Genetic analyses will be used in two key ways. First, genetic data are necessary to confidently distinguish giant salamander species during density sampling over all intervals to ensure proper determination of species composition and proper calculation of species richness and density metrics for both (Cope's and coastal) giant salamander species. Genetics are required to distinguish the two species because all amphibian sampling will occur with replacement, and the

⁴ A single toe clip or mouth swab will be needed from amphibian life stages with no tail (e.g., postmetamorphic tailed frogs).

smaller larval stages (< 55 mm SVL) of the two species are not distinguishable morphologically (e.g., Wilkins and Peterson 2000).

Second, genetic data provides insight into treatment effects in a way that no other segment of this study can. Genetic changes linked to the treatments may or may not be symmetric with potential demographic changes (Luikart *et al.* 1998a). Notably, genetic data can identify changes not manifest (i.e., cryptic declines in numbers of breeding individuals) from the demographic data collected in this study (APPENDIX VII). For example, although a population may appear stable in numbers after treatment, it may be that only a few breeding individuals survive and thus contribute their genes disproportionately to the next generation. This case would not be borne out with census surveys, but genetic data would show a decline in genetic variability and a possible concomitant increase in inbreeding. Thus, genetic data will augment demographic data to better identify the relative effects of treatments on amphibian population sizes and genetic variability.

Genetic variation of all individuals from each treatment unit will be compared at 15-20 microsatellite loci. A sample size of 30 is generally recommended to get an accurate estimate of the genetic variation in a population (Nei 1978; Goldstein and Schlotterer 1999), particularly with microsatellite markers, which are hypervariable (Pritchard *et al.* 2000). Simulation studies and existing empirical data indicate that this sample size range and number of loci should yield high power to detect genetic differences among treatments (Luikart *et al.* 1998a, 1999; Estoup *et al.* 2001; Leberg 2002). For example, 20 loci and 30 individuals were sufficient to detect population declines of fewer than 45 individuals 95% of the time using a variance test of changes in genetic diversity (Luikart *et al.* 1998a; APPENDIX VIII). Microsatellites are hypervariable, tandem nuclear DNA repeat motifs (e.g., GAGAGA...) that evolve rapidly (Goldstein and Schlotterer 1999). Microsatellites are increasingly used to study genetic variation, and their rapid evolution makes them particularly useful for detecting genetic changes over relatively short time scales (Luikart *et al.* 1998a; Hedrick 1999).

We propose to use several methods to estimate genetic changes associated with harvest treatments. First, we will estimate effective population size (N_e) using several methods, including estimates of linkage disequilibrium (Hill 1981; Bartley *et al.* 1992) and based on heterozygosity excess (see Pudovkin *et al.* 1996). Pre-harvest population sizes will be estimated using relative abundance index methods based on the two years of pre-harvest sampling as well as the genetic methods above. Using "direct" (census method) estimates of population sizes, we can estimate N_e/N ratios, which will provide valuable information on demographic parameters such as reproductive success, sex ratios and mortality. One potential problem with estimating N_e in amphibian studies comes from the fact that overlapping generations exist. To avoid this problem, we can sample juveniles and thus, the effective number of breeders in the previous generation can be estimated (*fide* Balloux 2004). To ensure non-relatedness of tadpoles and salamander larvae, animals will be collected through the broadest extent of the study basins possible. In the case of tailed frogs, different size classes of individuals can be collected to ensure breeding in different years, thus minimizing relatedness.

Several lines of evidence indicate that population bottlenecks or declines in population numbers can be detected using microsatellites, even after one generation (Cornuet and

Luikart 1996; Luikart *et al.* 1998a, 1999). First, because rare alleles are lost more easily than common ones, populations that undergo substantial population reductions will exhibit transient excesses in heterozygosity over expected levels (Luikart *et al.* 1998a). This method was used independently to correctly identify populations of natterjack toads (*Bufo calamita*) in Great Britain that were known to be declining or stable based on long-term census data (Beebee and Rowe 2001).

A second method is to detect whether shifts in allele frequency distributions exist (Luikart *et al.* 1998b). Using the sample sizes and number of loci proposed, computer simulations show that this method achieves greater than 80% power in detecting a bottleneck of fewer than 20 individuals (Luikart *et al.* 1998b). This method correctly identified bottlenecks in several species, including: mountain sheep, koalas, myna birds and galaxid fish (Luikart *et al.* 1998b).

A third method to detect population declines is by calculating the ratio of allele number to allele size range, or M (Garza and Williamson 2001). Again, because rare alleles are lost more easily than common ones in small populations, M is smaller in declining populations than stable ones (Garza and Williamson 2001). Declining populations such as Mediterranean monk seals, Northern elephant seals, and Northern wombats had M -ratios between 0.6 and 0.7, while stable populations such as black bears and honeybees had M -ratios close to 0.9. An endangered subspecies of tiger salamander, known to have suffered from several putative causes of bottlenecks, such as disease, had M -ratios close to 0.4 (Storfer *et al.*, *in review*). Changes in population size may not be necessary to show either heterozygote excesses or changes in M ratios, but we have obtained recent evidence that these methods are sensitive to detecting actual changes in the short term. Continued drought has occurred in Yellowstone National Park for the past 7 years; our microsatellite study of tiger salamanders showed evidence of significant heterozygote excesses, as well as M -ratios below estimated critical values in temporary ponds, but not in permanent ponds, across the region.

A fourth method for detecting changes in genetic diversity entails statistical comparisons of heterozygosity in a population through time (Luikart *et al.* 1998a). Several statistical tests exist to do this; traditionally a Chi-squared test for departures from Hardy-Weinberg equilibrium has been used. However, the most sensitive test appears to be the variance test, which has high power to detect population reductions to low numbers. Variance tests require more than one pre-harvest and one post harvest sample. For pre-harvest sampling, two ways exist that we can obtain multiple samples. One way is to treat samples collected from each of the two pre-harvest sampling years independently. The second is to obtain samples from different cohorts (a cohort consists of individuals of the same age, i.e., laid as eggs in the same year) that represent two adjacent generations with potentially different effective population sizes. For field sampling, this could consist of a larval cohort that would not metamorphose that year and a post-metamorphic cohort that had just metamorphosed; other combinations are possible.

A fifth method essentially re-draws the “family trees” of the individual genotypes present in a population (based on coalescent theory) to reconstruct effective population sizes (Beerli and Felsenstein 2001). Effective population size is essentially the number of breeding individuals in a population, which is almost always smaller than the census

population size. Declines in numbers of individuals through time will result in decreases in estimates of (genetic) effective population sizes.

Also important for continued management of amphibian species is an understanding of the spatial scale at which populations are exchanging migrants, or the spatial scale of gene flow. Once site selection is complete, we will be able to choose exact sites to be sampled around proposed treatment areas to estimate the “genetic neighborhood” (the distance at which gene flow drops off) of both Cope’s giant salamanders and tailed frogs in the study areas. Although site selection has not yet occurred, sampling will follow general methods for population genetics studies. That is, we will sample streams adjacent to at least 2 treatment streams in at 3-4 hierarchically distributed geographic distances (e.g., 3-4 streams within 1-2 km from the focal area, an additional 3-4 streams between 2-5 km from the focal area, 3-4 streams 5-10 km from the focal area and 3-4 streams 10-20 km). Exact site selection will allow us to determine how many streams are actually present (and occupied) within these hierarchical distances. Sampling in this fashion will allow determination of levels of gene flow as well as the “genetic neighborhood” using methods such as analysis of molecular variance (AMOVA) and assignment tests (using software such as STRUCTURE; Pritchard *et al.* 2000). Determination of the genetic neighborhood of each of the target species will allow us to elucidate the dispersal and consequent gene flow capabilities of each species. These data may provide insight into likely sources of possible immigrants or destinations of possible emigrants post-harvest. In addition, knowledge of the genetic neighborhoods of study species will be extremely valuable to assist in overall management of these species.

Using molecular data to infer population-level processes such as gene flow has been recently criticized because commonly used methods (i.e., F_{st}) assume equilibrium conditions in populations being surveyed (Whitlock and McCauley 1999; Latta 2004). Our study will address these potential problems in two ways. First, several methods have recently been developed for estimating population structure that do not require equilibrium conditions, such as the maximum likelihood-based methods proposed herein. Several software packages exist that have been developed to determine population structure using likelihood methods, including STRUCTURE which clusters individuals based on multilocus genotypes and BAYESASS+ (Wilson and Rannala 2003) that was created specifically for non-equilibrium populations. Second, our main interest is determining treatment-level differences before and after harvest. Because we will have paired samples, our data will not be subject to the pitfalls of inferring evolutionary history based on a single sample (as is commonly done). Rather, paired samples will allow us to determine *changes* in genetic variation and population structure, and given the rigorous controls proposed for this study, will allow inferences to be made regarding treatment-level effects.

Post-harvest genetic sampling will occur 7-8 years post-harvest (allowing approximately one generational turnover) due to relatively long generation times of the species being sampled. One generational turnover is necessary for detecting bottlenecks so that the individuals being sampled are not simply those that have survived since treatment application (Cornuet and Luikart 1996; Luikart *et al.* 1998b). Maximum likelihood assignment tests (Cornuet *et al.* 1999; Banks and Eichert 2000; Pritchard *et*

al. 2000) will be used post-harvest to determine the most likely source of animals captured in post-harvest surveys.

Periphyton Standing Crop: The periphyton biomass variable will be sampled and assessed against mass-size amphibian regressions by treatment to determine whether changes in standing crop translate to changes in individual quality as a function of differences among treatments. This variable is an important measure given that canopy removal is likely to induce significant, at least short term, changes in primary production. Changes in productivity can have substantial impacts on grazers (invertebrate and amphibian) within the stream and on the export of organic matter and invertebrates to fishbearing waters. A change in productivity during amphibian larval stages may extend through an individual's lifetime, but isolating this effect may be beyond the scope of this effort.

Periphyton standing crop will be measured using the clay tiles method (Rosemond *et al.* 1993, Kiffney and Richardson 2001, Wipfli *et al.* 1998) within each TYPE N unit for 2 years pre-harvest, the harvest year, and 2 years post-harvest (with a possible alternative, addressed in the Budget section, allowing for the possibility of excluding the year dedicated to harvest). Two clay tile station pairs will be systematically centered in each of the buffered and unbuffered reaches of the FFR stream. Four clay tile station pairs will be placed at equivalent positions in the other treatments and the reference sites. Tiles will be exchanged monthly, dried and ashed to get ash-free dry mass (AFDM) estimates of net primary productivity. The locations of these stations will be recorded on the site map and longitudinal channel profile.

Temperature: Stream temperature will be recorded at 30-minute intervals at fixed stations within each TYPE N unit through 2 years pre-harvest, the harvest year, and 2 years post-harvest (with a possible alternative, addressed in the Budget section, allowing for the possibility of excluding the year dedicated to harvest). Measurement of temperature changes during the harvest is important, since changes in temperature, if they occur, are expected to be rapid and attenuation following over a relatively short time. In the FFR buffer treatments, temperature stations will be placed:

- 1) in pools near the highest point of perennial flow (Np/Ns break);
- 2) near the upstream end of the TYPE N buffer boundary;
- 3) at the interface between buffered and unbuffered stream reaches; and
- 4) at the TYPE F/TYPE N confluence.

Data loggers will be placed at comparable locations in the reference stream and other treatment streams within the block, allowing direct comparison to potential changes in the FFR treatment. Air temperature and soil temperature will also be recorded at 30-min intervals at each water temperature site. The locations of these stations will be recorded on the site map and longitudinal channel profile.

Channel Structure: Channel structure will be evaluated to assess its importance as amphibian habitat and its potential influence on stream processes. All variables relating to channel structure will be evaluated or measured within each TYPE N unit for 2 years pre-harvest, the harvest year, and 2 years post-harvest (but see also alternative in Budget section addressing the possibility of reducing the entire the year dedicated to harvest).

A longitudinal profile will be used to collect and map bed elevation changes, tributary junctions, and other important geomorphological information within each treatment. The profile will follow only the dominant stream thread (based on flow) along its channel thalweg. This information will be used to identify differences in the local geomorphology of study streams, and can help interpret potential differences among treatments (both pre- and post-treatment). The profile will begin 20 feet downstream of the TYPE F/N break and continue upstream to 20 feet past the channel head (where possible). The profile will include all-important elevational changes, record the basis of the change (e.g. LWD, bedrock ledge, boulders, etc), and visually estimate the substrate in morphological unit (e.g. pool, riffle, etc).

Gross Morphology: This variable will also be sampled to identify differences in the local geomorphology of study streams, and can help interpret potential differences among treatments (pre- or post-harvest). Channel morphology will be classified following the schema of Montgomery and Buffington (1997) shown in APPENDIX FIGURE II and recorded on the longitudinal profile with their stream axis dimension measured. Channel widths will be measured at 10-m intervals.

Large Woody Debris: This variable will be sampled to assess differences in the large woody debris (LWD) loading patterns among study streams, and can help interpret differences that may arise among treatments (pre- or post-harvest). Large woody debris associated with stream channels (intruding into the vertical plane of the bankfull channel) will be tallied following a modified TFW protocol (Roorbach and Schuett-Hames 2003) using a minimum 10 cm large end diameter and minimum 50 cm length. The qualitative positioning of downed trees and snags will also be scored (see Robison and Beschta 1990), particularly as influencing habitat units of gross channel morphology (see next variable). Each piece will be identified to species (if possible) and the level of decay (decay class; McCullough 1948, Söderström 1988, Sollings 1982) will be scored. The location of the LWD will be shown on the longitudinal profile.

Substrate: This variable will be sampled to assess differences in substrate structure among study streams, and can help interpret differences that may arise among treatments (pre- or post-harvest). Besides the visual estimates made during profile construction, the composition of channel substrates will be assessed by two methods. High-resolution photography will record particle size and embeddedness at a series of 20 monumented points in which leveled photographs are taken at 50 cm from the stream surface to encompass an undistorted area 0.5 m² (Whitman *et al.* 2003). Placement of monumented points will be within a randomly located pool within representative reaches above the N/F boundary in the dominant stream thread of each treatment unit. Photographs, which would be retaken in each pre- and post-harvest year, can be quantified for analysis using a superimposed grid to obtain estimates of areas in standard substrate categories. Pebble counts and particle size distribution of the matrix will be taken for calibration purposes at five photographic sites. The location of substrate stations will be shown on the longitudinal profile.

Bank erosion: This variable will be sampled to assess important inputs resulting from bank erosion that could result in differences among treatments. Because of the difficulty in evaluating sediment streambank erosion of colluvium in low-order

channels (Reid and Dunne 1996), only major bank failures will be recorded with this variable. Location of failures will be shown on the longitudinal profile. Bank erosion by discrete bank failures will be evaluated by the size of landslide. Landslide volume will be expressed as tons per unit length (m) of each channel order to account for differences in channel form and flow erosiveness. The Sediment variable in the Riparian Input Variables section will capture important sediment input points resulting from other than bank failures and the Sediment variable in Export Variables Section addresses sediment exports.

Export (to Fish-bearing Streams) and Downstream Effects Variables: All variables involving riparian inputs will be evaluated or measured within each TYPE N unit for two years pre-harvest, the harvest year, and two years post-harvest (with a possible alternative, addressed in the Budget section, allowing for the possibility of excluding the year dedicated to harvest). Export variables will be measured at a V-notch weir constructed within 5 m of the Type F/Type N boundary point using sandbags; the pool behind the weir will be lined with plastic to minimize seepage.

Fish: This set of variables will be sampled to assess potential treatments effects on fishes in downstream waters. Small headwater streams transport nutrients, organic matter and invertebrates downstream to areas that support fish populations (Wipfli and Gregovich 2002). However, the importance of transported material in supporting fish populations and the extent to which alterations in type and amount of material transported might affect fish is largely unknown (e.g., Sullivan *et al.* 1986). This experiment offers special opportunity to evaluate this response for treatments that are directly manipulated.

Some of the proposed treatments (see FIGURE 1) are likely to reduce the input of terrestrial litter to the stream, and consequently its export downstream. Treatments also may enhance algal growth in the headwater channel due to increased sunlight and elevated nutrient input following harvest. Therefore, the amount and type of export may change as a result of different treatments. Alterations in export may influence food availability and water temperature in the downstream reaches that support fish. If so, the transport changes should be reflected in alterations in fish density, size and biomass after treatments are applied. In addition, alterations in diet can be evaluated by examining changes in the nitrogen (N) and carbon (C) stable isotope ratios in the tissues of the fish and their potential food sources.

Evaluation of fish response will be limited to those sites where flow from the treated stream contributes at least 50% of the flow to the reach supporting fish. Fish populations will be sampled in reaches immediately downstream from the treated, headwater streams (i.e., uppermost TYPE F). Fish will be sampled by isolating at least 75-m of stream channel immediately downstream from the upper extent of fish distribution. Samples will be collected three times annually, in spring, late summer and mid-winter.

Density: Densities will be measured to assess potential differences in fish abundance in waters immediately downstream from study streams that may result from differences among treatments (pre- or post-harvest). Fish densities will be estimated using a 3-pass removal summation estimator, modified for small populations (Carle and Strub 1978). All fish captured will be identified to species, so species-specific densities can be obtained.

Quality: Fish quality will be measured to assess whether qualitative differences exist in fish condition in study streams that may result from differences among treatments (pre- or post-harvest). Fishes will be measured (as fork length; L) and weighed (W; to nearest 0.01 g). Fulton's condition index (W/L^3) will then be calculated as an index of individual quality (Ricker 1975).

Stable Isotopes: This variable will be sampled to assess fish trophic position and predominant links in the food web leading to fish. Stable N and C isotope analysis has been used for the last 20 years to examine trophic dynamics in aquatic and terrestrial ecosystems (Peterson and Fry 1987). This technique is particularly well suited to distinguish between trophic support provided by terrestrial plant litter and that provided by in-channel algal growth, the type of change that may occur as a result of treatments applied to the headwater stream reaches.

Samples for stable isotope analysis will be collected in conjunction with the fish sampling. Small pieces of fin tissue will be collected from five fish of each of the two most abundant species captured at the study sites. These samples will be composited by species into one sample for each stream. To interpret stable isotope data for fish, stable isotope values for several other items are required. Samples of organic matter transported from the TYPE N stream and the two most common invertebrate taxa captured in the drift samples will be collected. In addition, samples of terrestrial plant litter and algae collected from the streambed of the TYPE N stream will be analyzed to provide an indication of the isotopic signature of the primary types of organic matter that contribute to the transported material. Seven total samples for each stream on each sample date will be collected: two fish species, two invertebrate taxa, algae, terrestrial litter and transported organic matter.

Samples for stable isotope analysis will be cooled in the field and frozen within a few hours of collection. The frozen samples will be sent to the isotope lab at the NMFS, Northwest Fisheries Science Center in Seattle for analysis. Samples will be freeze-dried, ground to a fine powder and 1.0 to 1.5 mg will be combusted. The evolved N_2 and CO_2 gas will be introduced into a continuous flow isotope ratio mass spectrometer to determine $\delta^{15}N$ and $\delta^{13}C$ values.

Nutrients: This composite variable will be sampled to assess potential differences in nutrients exported to downstream waters pre- and post-harvest and among treatments. Water samples will be collected at monthly intervals at the weir using acid-washed sampling bottles plus three to six high flow events per year, and analyzed for three forms of nitrogen (NO_2+NO_3-N , NH_3-N , Total N), total and soluble reactive phosphorus (TP, SRP), and total organic carbon (TOC).

Macroinvertebrates: This variable will be sampled to assess potential differences in macroinvertebrate export among treatments. Export of macroinvertebrates from headwaters will be measured at a weir using modified 250 μm mesh drift nets over a 24-hr period (Wipfli and Gregovich 2002). Samples will be collected at 3-4 week intervals. Each sample will be sorted into detritus and invertebrate components. The monthly invertebrate samples will be combined into four samples per year. These samples will be sorted; the invertebrates will counted and sorted into functional groups, measured, and

their masses estimated using mass-length regression equations. Invertebrate transport will be reported as numbers per day and dry mass (mg) per day.

Detritus: This variable will be sampled to assess the export of coarse detritus from TYPE N basins. Export of coarse detritus from headwaters will be measured at a weir using modified 250 μm mesh drift nets over a 24-hr period (Wipfli and Gregovich 2002). Samples will be collected at 3-4 week intervals. Each sample will be sorted into detritus and invertebrate components. Detritus will be dried at 55 C, weighed, then ashed at 550 C to obtain dry and ash-free dry weights. Debris transport will be reported as dry mass (g) per m^3 water and dry mass (g) per day.

Sediment: This variable will be sampled to assess variation in sediment input, including that potentially exportable from the TYPE N system. This study is designed to limit sediment input, but its measurement is needed to determine what actually occurs. Water samples will be collected monthly (with the nutrient samples) and analyzed for suspended sediment concentration. In addition, a continuously recording *in situ* turbidity sensor will be installed at each weir and programmed to record at 15-minute intervals. An automatic pump sampler, activated at a specific turbidity threshold value, will collect discrete samples during high turbidity events similar to the Turbidity Threshold Sampling method described in Lewis (1996). The SSC concentration of all water samples will be used to develop a regression model to estimate SSC from the continuous turbidity record. The product of the SSC and associated flow will be summed to estimate total annual suspended sediment load. Loads may also be evaluated over shorter time intervals to describe suspended sediment transport seasonally or as a function of specific events.

Bedload will be measured using a portable bedload trap with 3.5 mm mesh similar to that describe in Bunte (2004). A single trap will be installed at when water samples are collected (monthly plus high flows) for approximately one hour per sample event. The traps will be constructed so that approximately 50% of the streambed is sampled.

Stream Flow: This variable will be sampled to assess variation in discharge among treatments. A stage height recorder will be installed at the weir near the bottom of each basin and its location recorded on the site map and longitudinal profile. Stage height measurements will be correlated with stream flow measurements collected across the range of flow conditions and used to estimate mean daily flows.

Temperature: See Temperature subsection in the In- or Near-Channel Variables section.

Riparian Input Variables: Frequency of measurement of riparian inputs will be variable-specific.

Stand Growth/Survival and LWD Recruitment: This variable will be sampled to assess the potential differences that may result in stand characteristics among treatments. Data on riparian vegetation will be collected to determine the effects of treatments on stand composition, tree growth and mortality, large woody debris (LWD) recruitment rates, and understory vegetation composition. Data will be collected at a series of plots that will be used to sample longitudinal (stream axis direction) and lateral (upland direction) variation in vegetation in a manner similar to that used in the field methods for the NF buffer integrity, characteristics and function study (Washington Department of Natural Resources [WDNR] 1996, Roorbach and Schuett-Hames 2003). Data will be collected during the second pre-treatment year as close to the interval during which treatments will occur as possible, immediately post-harvest, and during the second post-treatment year as far from the application of the treatment in time as possible (i.e., as late as possible). This will enable bracketing the treatment within the narrowest time interval possible and measuring post-treatment as late as possible within this initial study interval to identify potential short-term changes.

The sampling scheme is designed to sample the range of post-harvest conditions occurring at each site, by delineating buffered stream reaches, harvested (i.e., unbuffered) stream reaches and sensitive sites. Not all treatment units will have both harvested and buffer reaches, but all will have at least one sensitive site plot associated with the headwater spring on the dominant stream thread and at least one sensitive site plot associated with the tributary junction(s) present. The location of the headwater spring may move between years (Hunter *et al.*, 2005). Should that occur, a sensitive site plot will be associated with the original position of the spring in subsequent years, and a second plot will be associated with its new position. This will enable both an analysis from the origin position among years and an analysis at the spring position in each year. Additional sensitive site plots will be included if headwall seeps, side-slope seeps, or alluvial fans are present within the units. Adequate analysis of sensitive site conditions in seeps may require an addendum to this design or a separate study (APPENDIX IX).

Two vegetation plots will be placed at randomly selected points along the stream in each buffered reach and each harvested reach in the FFR buffer treatment. In each of the other three treatments, two vegetation plots will be at randomly selected points along the stream selected from areas geographically equivalent to each of the buffered and harvested reaches in the FFR buffer treatment. Each plot will be 60 horizontal ft in length and extend out 50 horizontal ft in either direction from the stream. For sensitive sites, the vegetation plots will encompass the entire FFR buffer, irrespective of shape.

Data will be collected on all trees (\geq 4-in dia breast height [DBH]) including the species, DBH, condition, canopy class (live trees only), crown type, and crown ratio of individual standing trees or snags; and the landform on which each tree or snag is located. Decay class of snags will also be scored (Sollings 1982). Each tree will be individually marked for recognition during re-sampling, and a stem map will be created to determine distance from the stream. Height and age data will be collected on a sub-sample of trees from each plot. These data will be used to determine changes in stand composition, density, basal

area and volume over time. Additional data will be collected on trees that fall in the course of the study (see also APPENDIX X), including mortality agent, fall direction, and wood recruited to the stream channel. Low-elevation aerial photography for a permanent quantification record of tree data of the aforementioned categories is currently being evaluated in an ongoing CMER-sponsored pilot study. If results of that study show the technique to be useful, it will be considered for use here.

The shrub and herbaceous layers will be assessed through a series of circular plots placed along the center transect of the plot perpendicular to the stream. Three circular plots will be placed on either side of the stream at distances of 10, 25, and 40 feet as described in the NF effectiveness study (Roorbach and Schuett-Hames 2003). For each plot, the shrub and herb species will be recorded and percent shrub or herb cover will be estimated for each species. Mosses will not be identified to species, but the percentage of area in moss mat will be estimated.

Shade: This variable will be sampled to assess differences in the degree of shading that will exist among treatments. Riparian canopy cover will be recorded at 50-m intervals from the TYPE F/N junction to the uppermost point of perennial flow using hemispheric canopy photographs taken along the center of the stream channel with a digital camera and a fish-eye lens. Positional accuracy of photopoints will be ensured with a pair of individually identifiable streambank monuments. Except for immediately post-harvest, photographs will be obtained annually during June-July. Harvest may occur anywhere between 15 March and late summer, depending on study design options (see Budget section); therefore, a post-harvest photographs would also be obtained immediately after whenever the harvest interval falls. The locations of these stations will be recorded on the site map and longitudinal channel profile. Photographs will be analyzed using Hemiview software (Delta-T Devices 1999) to calculate canopy cover. Densimeter measurements will be taken at the same locations for comparison.

Litterfall: This variable will be sampled to assess potential differences in overall riparian litterfall among treatments. Litterfall traps will be placed over the stream at bankfull height at 6 randomly selected sampling points (three each in the buffered and non-buffered reaches of the FFR stream) along the stream channel. Litter from the traps will be collected at monthly intervals and combined into one composite sample per stream per month. Litterfall measurements will be “continuous” over the study period, so pre- and post-harvest years will be summarized as annual means or totals with appropriate pre- and post-harvest partitioning for the year in which harvest occurs. The material will be sorted into deciduous leaves, coniferous needles, woody material, and miscellaneous, dried at 55 C for 96 hours, and weighed to obtain dry mass, then placed in a muffle furnace at 550 C and weighed to obtain ash free dry mass (AFDM).

Sediment: This variable will be sampled to assess potential major sources of sediment that could result in differences among treatments. Upland sediment sources include landslides (see Bank Erosion variable in previous section), road surface sediment, and sediment derived from erosion of disturbed areas will be evaluated in each year of the study. Locations of these and other sediment entryways to the channel will be shown on the site map and longitudinal profile.

ANALYSES

This study measures initial impact of harvest and is intended to follow the expected recovery over time. The focus of these analyses is to estimate temporal changes within the three buffer treatments and the reference site following harvest application to each of the former, and to compare treatments and the unharvested reference basins to each other. Thus, analysis will evaluate the generalized null hypothesis:

$$\Delta T_i = \Delta T_{i+1} = \Delta T_{i+2} = \Delta T_{i+3}$$

where ΔT_i is the change (pre-harvest – post-harvest) in the reference basin, and ΔT_{i+1} and ΔT_{i+2} and ΔT_{i+3} are the changes in treatments $i+1$ and $i+2$ and $i+3$, respectively.

General Model: The general model used to evaluate this hypothesis is a mixed-effects model within a repeated-measures analysis of variance (ANOVA). The repeated-measures approach will allow distinguishing whether post-treatment trajectories differ among treatments (Underwood 1994, Winer 1971), a pattern of focal interest in this study. The mixed-effects approach will allow entering the directly manipulated buffer length treatments (the effect of interest) as fixed; and the effects of different years and blocks (the effects that constitute a large segment of undesired variation) as random.

TABLES 2A and 2B display the sources, degrees of freedom, and origins of the F and expected mean squares statistics for the general model. Period and Treatment are the terms of interest in evaluating the generalized null hypothesis. Significance in Period would indicate a difference between the pre- versus post-harvest condition, and will allow identifying the rapid-change trajectory anticipated for some response variables following harvest (see Jackson *et al.* 2003, MacCracken 2002). Significance in Treatment would indicate a difference among treatments. Significance in the Treatment term would not identify specifically which treatments differ from which, so a post-hoc analysis would be used to reveal which treatments really differ. Dunnett's is the post hoc test of choice for this analysis, where a control does not exist for each separate treatment (K. Ryding, WDFW, pers. comm.). Significance in the interaction term (Period \times Treatment) would indicate that pre- and post-harvest variation is confounded with treatment variation, and

TABLE 2A. ANALYSIS OF VARIANCE (ANOVA) TABLE FOR GENERAL MODEL

Source	Units	df (model)	df	Sum of Squares	F
Periods (p): Before-After (BA)	2	p – 1	1	SS _{BA}	SS _{BA} /SS _{BACI}
Treatments (t): Control-Impact (CI)	4	t – 1	3	SS _{CI}	SS _{CI} /SS _{BACI}
Treatment x Period: BA x CI		(p – 1)*(t – 1)	3	SS _{BACI}	SS _{BACI} /SS _{Blocks}
Blocks (B) (Treatment x Period)	5	(B – 1)*t*p	32	SS _{Blocks}	SS _{BACI} /SS _{Res}
Residual (n)	2	(n – 1)B*t*p	40	SS _{Res}	
Total		(n*t*p*B)-1	79	SS _{Total}	

TABLE 2B. ANALYSIS OF VARIANCE (ANOVA) TABLE FOR GENERAL MODEL (CONTINUED)

Source	Expected (Mean Squares)
Periods (p): Before-After (BA)	$\sigma^2_{\text{Res}} + n\sigma^2_{\text{Blocks}} + nB\sigma^2_{\text{BACI}} + nBt\sigma^2_{\text{BA}}$
Treatments (t): Control-Impact (CI)	$\sigma^2_{\text{Res}} + n\sigma^2_{\text{Blocks}} + nB\sigma^2_{\text{BACI}} + nBp\sigma^2_{\text{CI}}$
Treatment x Period: BA x CI	$\sigma^2_{\text{Res}} + n\sigma^2_{\text{Blocks}} + nB\sigma^2_{\text{BACI}}$
Block (B) (Treatment x Period)	$\sigma^2_{\text{Res}} + n\sigma^2_{\text{Blocks}}$
Replicates (n)	σ^2_{Res}
Total	

significance in the Block term would indicate that differences exist among blocks.

The likelihood of between-year differences is high (Holtby and Scrivener 1989, Limpasuvan and Hartman 1999, Pfaff *et al.* 1999) so variation between years in each of pre- and post-treatment periods is addressed by the Residual term.

In the event that non-normal data prevent direct use of the repeated measures ANOVA, data will be normalized using an appropriate transformation (Zar 1996). If the data cannot be normalized or inhomogeneous variances prevent using the repeated measure ANOVA, alternative methods will be explored (Siegel and Castellan 1998).

As effect size needed to detect a difference among treatments for the amphibian density data is large (see APPENDIX VI), interest lies in distinguishing among treatments, and balance between Type I and Type II errors is desired, α and β are set at 0.1 (e.g., Underwood 1997, Welsh and Ollivier 1998). The approach of examining p values to directly gauge differences in standardized effect sizes will also be employed (see MacCracken 2002). This progressive approach has the advantage of directly assessing potential biological differences, but both approaches will be used to allow direct comparisons between this research and other CMER-sponsored research.

If the null hypothesis that the treatments are the same is rejected, then conducting *post hoc* regression type analyses or analyses of covariance (ANCOVAs) will be considered as long as those analyses are exclusively treated as exploratory or hypothesis-generating. In choosing a BACI study design, we create by default a design structure that renders the regression approach as less valuable (i.e., only 20 replicates). Had we *a priori* chosen the regression approach, we could have maximized the number of replicates (i.e., no categorical treatment *per se*) and designed across a wide range of conditions for many independent variables. For example, if canopy closure was focal, we could have chosen at least 50 replicates across a wide range of canopy closure, then asked about fish or amphibian response as canopy changed, and using ANCOVA, other key variables like stand age or geology could have been controlled. This represents the classic multiple regression approach extensively used historically that has proven limited in revealing cause and effect relationships. Moreover, the complexity of our kind of landscape-level analysis strongly constrains such an approach because of the inability to expand the sample size beyond a moderate number of replicates (i.e., individual replicates carry a high cost).

Treatment of Variables: Analysis of most variables will be addressed using the repeated measures ANOVA as described above, but some response variables will require different types of analyses:

In- or Near- Stream Variables: Amphibian occupancy data are descriptive and will not be used in the general model. Amphibian density data will be entered in the ANOVA as the means of the 25 1-m sampling plots in each treatment. Net primary productivity will be entered as the means of AFDM from each treatment unit. See Genetics and Temperature sections for handling of genetic and temperature data.

Riparian Input Variables: Stand growth, stand survival, LWD recruitment, shade, litterfall, and sediment data will be entered in the ANOVA as the means of the respective values in each treatment.

Downstream and Export Variables: Annual nutrient loads (nitrogen and phosphorus) and total organic carbon will be calculated using the Cohn *et al.* (1992) minimum variance unbiased estimator and a ‘smearing’ correction for bias, if concentration data require log transformation (Helsel and Hirsch 2002). Detritus macroinvertebrate export data will be summed for each entire year and analyzed by each detritus category (deciduous, conifer, woody material, and miscellaneous), dominant macroinvertebrate taxa, and total detritus mass and total macroinvertebrate mass and numbers.

Sediment: Annual suspended sediment loads will be calculated by first developing a regression model to estimate SSC using the measured SSC in the water samples and the associated *in situ* turbidity measurements. The product of the estimated SSC (mass/volume) value and associated stream flow (volume/time) will be summed to get annual, seasonal, or event-specific loads. Annual sediment loads will be analyzed using the repeated measures ANOVA. The seasonal and event specific loads will be used to evaluate the effects of site specific conditions or events on suspended sediment transport and the effects of changes in suspended sediment transport on instream condition within the TYPE N basin and downstream.

Bedload transport rating curves will be constructed using the bedload trap data and compared across treatments. Annual bedload transport will be calculated but will depend upon our ability to sample high flow events on short notice at remotes sites.

Temperature: Temperature, for which many measurements are obtained year-round, requires a different approach to evaluate temporal changes within each treatment basin. Temperature metrics are calculated from the monitor at the downstream end of the TYPE N treatment stream (near the weir). In addition, a temperature metric based on changes in the treatment stream temperature relative to reference stream temperature will be calculated as:

$$T_{\text{pre}, 15\text{C}} - T_{\text{post}, 15\text{C}}$$

where $T_{\text{pre}, 15\text{C}}$ is the predicted pre-harvest treatment stream temperature evaluated at a reference stream temperature = 15 C; and

$T_{\text{post}, 15\text{C}}$ is the predicted post-harvest treatment stream temperature evaluated at a reference stream temperature = 15 C.

$T_{\text{pre}, 15\text{C}}$ and $T_{\text{post}, 15\text{C}}$ will be estimated using the multiple linear regression model below:

$$T_{\text{treatment}} = b_0 + b_1 * T_{\text{reference}} + b_2 * \sin(\text{time}) + b_3 * \cos(\text{time}) + b_4 * (\text{pre vs post}) + b_5 * T_{\text{reference}}$$

where $T_{\text{treatment}}$ = temperature leaving the treatment site,

$T_{\text{reference}}$ = temperature at the reference basin

$\sin(\text{time})$ and $\cos(\text{time})$ = terms to account of seasonal temperature variation,

pre vs post = dummy variable (pre = 0, post = 1) designating pre- or post-harvest

b_0 , b_1 , b_2 , b_3 , b_4 , and b_5 are the regression coefficients.

The reference stream temperature at which treatment stream temperature is evaluated may need to be adjusted so that the temperature is within the range of stream temperatures recorded (i.e. to avoid extrapolating beyond the range of data in the regression).

Serially collected (time series) data are often auto-correlated, violating the independent observations assumption, so seasonality terms are included in the model and the interval length between observations may have to be increased (Helsel and Hirsch 1992; APPENDIX XI). Typically, a sampling frequency of one to two observations per week will minimize autocorrelation.

Covariates: Air and substrate temperature may be used as covariates in the temperature analysis. Use of covariates in this analysis will not affect the degrees of freedom in the ANOVA for the general model as the covariates will be applied in the regression analysis to determine the likely cause of temperature changes.

Genetics: The two giant salamander species will be distinguished with a real-time PCR assay of a portion of the control region of the mitochondrial DNA. The (Storfer) lab has found a fixed single nucleotide polymorphism (SNP) that identifies the two species. In short, two fluorescent probes have been developed, and only one probe will bind to DNA from each species. Each probe fluoresces at a different wavelength, and thus, species can be identified by estimating the fluorescence of one probe relative to the other in each sample. The methodology follows a two-step process. First, DNA will be extracted from all giant salamander tissue samples, and real-time PCR will be performed in an ABI 3700 PCR machine to amplify the segment of the mitochondrial control region with diagnostic base pairs. Species identification is accomplished by using ABI 7300 analysis software and determining relative fluorescence units of the two probes. This method has proven extremely effective for identifying samples correctly. Of 282 samples presently analyzed, field identification was either not possible or incorrect 41.8% (118 samples) of the time.

Several microsatellite loci have already been developed for *Dicamptodon* (Curtis and Taylor 2000) and some of these loci work for *D. tenebrous* and *D. copei*. Additional microsatellites will be developed for coastal tailed frog and Cope's giant salamander to test for differences in genetic diversity using standard methods (e.g., see Mech *et al.* 2003). In summary, these methods include: 1) generating a genomic library for the species under study; 2) using selective amplification methods to enrich the library for microsatellites; 3) cloning the enriched library into *E. coli*; 4) screening the library for microsatellites using fluorescent techniques that detect hybridization; 5) sequencing clones to confirm presence of microsatellites; 6) developing primers for clones confirmed to have microsatellites; and, 7) using these primers to screen natural

populations for variation (Goldstein and Schlotterer 1999). When 15-20 loci are developed per species under study, we will genotype all individuals for which tissue is collected. First, DNA is extracted from each tissue sample and purified. Then, PCR is used to amplify each microsatellite for each sample. PCR products will be run on an Applied Biosystems 3730 automated DNA sequencer to genotype individuals at each locus and analyses will be performed on a PC computer using GENEMAPPER 3.7 (Applied Biosystems Inc.).

Initial studies will collect individuals from neighboring streams within the likely study area of the 2 species to be used for genetic analyses. The focus of the proximate sampling is to determine the genetic neighborhoods of the two target species. This will be accomplished by calculating standard estimates of gene flow among sites by calculating F-statistics using FSTAT software (Goudet 2001). Additional analyses will include using STRUCTURE (Pritchard *et al.* 2000) and BAYESASS+ (Wilson and Rannala 2003) to determine genetic neighborhoods of populations under study. Migration rate and individual dispersers can also be identified using assignment tests (Berry *et al.* 2004). Overall population genetic structure will be analyzed using AMOVA in hierarchical fashion to determine the genetic neighborhood (Excoffier *et al.* 1992) in ARLEQUIN 2.0 (Schneider *et al.* 2000). Delineation of breeding population is important for detecting changes in population sizes or bottlenecks (below) because these methods rely on correct identification of populations. If populations are not properly sampled, then phenomena such as Wahlund effects can affect estimates of genetic variability simply due to sampling error, as opposed to actual changes in effective population size. Mantel tests will be used to test for a significant isolation-by-distance relationship, as expected for amphibian species.

To detect whether a significant change in genetic variation exists within a treatment, several methods will be used. First, GENEPOP 3.4 (Raymond and Rousset 1995) will be used to calculate allelic diversity, overall observed and expected heterozygosity, and departures from Hardy-Weinberg equilibrium using Fisher's exact tests in each paired sample. If departures from Hardy-Weinberg are manifest as deficiencies in heterozygosity relative to expected values under a random-mating model, inbreeding is suggested. Change in genetic variation will be averaged within each treatment and compared through time within treatments using variance tests (Luikart *et al.* 1998a). Change in average allelic diversity and heterozygosity will be calculated within treatments (across blocks) and then compared among treatments using ANOVA. Fisher's Least-Significant-Difference (LSD) tests will be used to test individual contrasts among treatment means.

To test further for population declines, the computer program BOTTLENECK (Piry *et al.* 1999) will be employed. Specifically, BOTTLENECK will be used to test whether a significant number of loci (relative to the total number of loci examined) are in heterozygote excess; transient excesses in heterozygosity are expected due to the loss of rare alleles more quickly than common ones due to genetic drift in small populations (Cornuet and Luikart 1996). Significance of allele frequency shifts will also be examined using BOTTLENECK. To compare treatment types, mean values of numbers of loci in heterozygote excess will be averaged within each treatment across blocks and Wilcoxon-signed rank tests will be employed to assess overall treatment effects.

Magnitude of change in numbers of allele in heterozygote excess will be averaged within each treatment and compared among treatments using hierarchical AMOVA and LSD independent contrasts.

One potential issue is whether these methods have the sensitivity to detect bottlenecks after a single generation. If the bottleneck is small enough ($N_e < 50$), then simulation studies suggest that we will have relatively high power (Cornuet and Luikart 1996; Luikart *et al.* 1998). However, reductions in population size that are not as severe may not be as easily detected. As a potential remedy to this problem, we are also conducting a correlative study (not part of the funding requested herein) of *Ascapthus* genetic diversity and genetic population structure in harvested versus unharvested areas on the Olympic peninsula. Unharvested areas with the appropriate scale (i.e., a size similar to the harvest treatments), hydrogeomorphic position (i.e., TYPE N headwaters), and landscape vicinity conditions will serve as additional reference sites for the genetic portion of the present study.

M -ratios will be calculated for each sampling site before and after harvest treatment application. To test significance of M -values generated for each treatment within each block, Critical_M.exe software (Garza 2001), will be used to generate a critical M value (that which 5% of simulations were below) based on number of individuals sampled and number of loci using 10,000 replicates. Thus, observed M values below the critical value suggest significant bottlenecks. Within each treatment type, changes in M will be tested with paired t-tests. To compare M values among treatments, change in M will be averaged among blocks for each treatment and compared among treatments using ANOVA. Pairwise comparison of treatment means will use LSD tests.

In addition, reconstruction of effective population sizes using coalescent methods will be employed using MIGRATE 1.7.6 (Beerli and Felsenstein 2001, Beerli 2002). Specifically, a parameter (Θ) equal to $4N_e\mu$ (where N_e = effective population size and μ = mutation rate), is generated by MIGRATE using a Monte Carlo Markov Chain approach. Effective population size is then calculated by using the established mutation rates for microsatellites (10^{-3} mutations per locus; Goldstein and Schlotterer 1999). Paired t-tests will be used to compare effective population sizes within each treatment mean before and after the harvest treatment has been applied. AMOVA and independent contrasts will be used to compare means among treatments.

Finally, to determine whether individuals collected in post-harvest treatment conditions are residents or immigrants, maximum likelihood assignment tests will be employed using WHICHRUN (Banks and Eichert 2000). With 15-20 loci and sampling of 30 individuals per population, power should be well above 99% to include or exclude individuals from putative parental populations (Cornuet *et al.* 1999; Banks and Eichert 2000). Changes in population structure will be estimated in a subset of treatments using STRUCTURE (Pritchard *et al.* 2000), which clusters groups of individuals together based on multilocus genotypes. If large enough proportions of the populations can be sampled, then it may be possible to assign individuals to proper parentage, a better estimate of effective population size and more accurate quantifications of inbreeding and reproductive success than with smaller samples.

Initial conditions may vary among blocks, thus potentially confounding any genetic differences that may exist among treatments. Therefore, genetic data will be analyzed separately for each treatment within each block, unless data are consistent among blocks. We will use randomization methods (such as bootstrapping) to determine confidence intervals for genetic estimates (such as F_{st} analogs) within blocks so as to determine whether there are significant differences in the reference data among blocks.

BUDGET

The proposed budget encompasses setup, sampling, and analysis through an initial pre- and post-sampling interval extending over 7 years. The first year is site selection and set-up, now ongoing. Pre-harvest sampling is 2 years, treatment application and analysis of pre-harvest data is 1 year, post-harvest sampling is 2 years, and overall analysis and write-up is 1 year.

This budget excludes post-harvest genetic sampling, which necessarily requires a delay because of the generation time of the amphibian species sampled (i.e., coastal tailed frog and Cope's giant salamander) to, at minimum, 7-8 years post-harvest (see Genetics subsection under the In- or Near-Stream Sampling section).

Additional funding would also be required for post-harvest sampling over longer timelines. The intent of this study design is to extend sampling into the next harvest rotation as a function of the amphibian viability criterion that LWAG established for TYPE N basins. So, the structure of this study provides an unparalleled opportunity for study that is clearly longer than this initial interval. In other words, to redo a separate study encompassing a similarly long timeline would require the same high cost of set up to reach the same point where this study would be following the initial pre- and post-harvest sampling, so the value of this opportunity should not be underestimated.

That this study will provide substantial return even if it only extends through the proposed post-harvest sampling interval also needs emphasis. In particular, the proposed post-harvest sampling will either show or not show that prescription alternatives differ over the short timeline, and show or not show that if differences exist, they may be linked to forestry practices. A demonstration of either an effect or no-effect linked to forestry practices, or differences or no differences among treatments will be strong inducements for many investigators to seek funding to support studies on longer timelines because of the questions that each raise. Demonstration of an effect immediately poses the question of the ability for recovery during the harvest rotation if one sampled on an extended timeline; demonstration of no effect immediately poses the question of whether any lag effects might be manifest under a longer timeline. Even with only the initial post-harvest sampling, the genetic data from this study would make significant contributions with genetic neighborhood information and pre-treatment population assessment. The former would enable identifying the relative spatial scales at which the sampled amphibians move, providing management insights into the scale of habitat use, data that are currently unavailable for any stream-associated amphibians in managed landscapes. The latter would identify whether any of the treatment site populations might have undergone historical bottlenecks, legacy data for which no information currently exists.

Budget Options: The Type N Experimental Buffer Treatment Study has been in development for nearly four years. It represents the only CMER study that tests alternative prescriptions for meeting resource objectives as well as integrating information across multiple resource issues including wood, nutrient, heat inputs, and to a lesser extent, sediment; the effects of TYPE N basin harvest on fish-bearing waters, and harvest effects on amphibian populations. At the request of Douglas Martin (CMER Co-chair), study authors have been asked to present a range of lower cost alternatives to the original study in terms of cost/benefits. LWAG and RSAG have devoted considerable effort

to presenting CMER with the best study design, the preferred design from a scientific perspective, a view that the SRC reviewers supported when they were queried on how the cost of the project might be reduced. To the reviewer, they recommended not reducing either the number of treatments or number of replicates (blocks); they did recommend eliminating instream macroinvertebrate sampling (but not the exports) and reducing the level of litter sampling to something that would provide a per-treatment gauge. Both these suggestions have been implemented in the revision, but no cost savings was realized because of a substantial miscalculation involving indirect costs. Regardless, LWAG and RSAG made special effort to find alternatives that could substantially lower study cost; the description of these follow.

The first option to reduce costs is to narrow the window when harvest activities could take place. Originally designed to allow a full year for landowners to harvest their sites, reducing the period of harvest from a year to a four-month interval (15 March-15 July) will save over \$500,000. This change will not affect study results, rather it reflects savings associated with keeping critical personnel on staff during this period. Initial discussion with a few industry representatives suggests that this option could work but will require additional landowner coordination.

The next opportunity for savings is to cut funding for the 2nd year post-harvest sampling (i.e, fund only year 1 of post harvest sampling) for a savings of approximately \$400,000. While we do not believe that sampling should only occur in one year after treatments are applied, we feel confident that we will be able fund the second year post-harvest sample with outside sources. This belief is based on our experience with funding agencies like EPA, USDA, and NSF who often limit their support to **ongoing** multiple partnership studies.

The final option for reducing cost is to eliminate whole study segments (e.g., amphibian demographics, genetics, or water quality). Based on peer-reviewers' comments and the need for these elements as part of adaptive management, this option flies in the face of CMER's attempt to efficiently bundle projects across many disciplines, limits our ability to interpret cause and effect mechanisms of the study, and thus diminishes the value of the overall study.

In-Kind Support: Development of this project would not have been possible without substantial in-kind support. This support includes personnel time for study development, contribution of information and resources for site selection, contributions of personnel assisting in study site evaluation, and other miscellaneous tasks related to the project. To date, study authors and co-operators have conservatively contributed over \$120,000 of their time in the development of this study; Marc Hayes and Tim Quinn have donated time that represents over half of this contribution. Notably, the Longview Fibre Company, Rayonier, the WDFW, the WDNR, the Weyerhaeuser Company contributed critical landscape data and GIS and other map data essential for site selection; including personnel time required by these agencies to develop or retrieve this information, this collective support to date is conservatively estimated at \$30,000. A suite of co-operators and personnel have assisted evaluation of study sites in the field to date that conservatively estimated to have cost \$15,000. Thus, overall in-kind contributions to date are estimate to be in the vicinity of \$170,000.

For the duration of the portion of this project for which funding is requested from CMER, in-kind contributions are expected to be even more substantial. These include, but are not limited to, contributions of personnel time from WDFW (Marc Hayes [55%; \$39,000 annually], Tim Quinn [10%; \$9,000 annually], Mark Hunter [100%; \$65,000 annually]) for execution of the shade and selected physical characteristics of the study; co-operator contribution for harvest coordination and implementation according to the study design, conservatively estimated at over \$300,000; and miscellaneous assistance from other authors and entities for fieldwork, ca. \$60,000 annually. Thus, if implemented under the reduced harvest year option (see above), in-kind support for the projected duration of this study is conservatively estimated at \$992,000; it would be well over \$1,100,000 if the full-year harvest option was chosen.

TABLE 3. Budget

Study Piece	Budget Category	2005	2006	2007	2008	2009	2010	2011	Total
Amphibian Demography	Personnel	10,890	127,456	130,467	95,251	136,786	139,498	49,366	689,714
	Equipment	-	-	-	-	-	-	-	-
	Travel	2,000	22,500	23,500	1,467	25,500	26,500	1,603	103,070
	Lab & Suppl	5,002	933	978	3,538	1,037	1,069	200	12,757
	Indirect	4,990	43,441	44,609	28,864	47,021	48,099	14,732	231,754
	Subtotal	22,882	194,330	199,554	129,120	210,344	215,166	65,901	1,037,295
Amphibian Genetics	Personnel	46,648	74,488	78,196	75,437				274,769
	Equipment	12,000	-	-	-	-	-	-	12,000
	Travel	500	1,500	1,500	500	-	-	-	4,000
	Lab & Suppl	57,000	10,000	10,000	11,800	-	-	-	88,800
	Indirect	48,741	38,790	40,453	39,459	-	-	-	167,443
	Subtotal	164,889	124,778	130,148	127,196	-	-	-	547,011
Vegetation, Productivity, Stream Profile, Woody Debris	Personnel	5,494	43,578	46,589	34,630	49,024	50,132	-	229,447
	Equipment	-	-	-	-	-	-	-	-
	Travel	2,000	13,000	14,000	733	15,000	16,000	-	60,733
	Lab & Suppl	4,967	467	489	1,769	519	534	-	8,745
	Indirect	3,651	16,423	17,584	10,690	18,582	19,193	-	86,124
	Subtotal	16,112	73,468	78,662	47,822	83,125	85,859	-	385,049
Fish and Stable Isotopes	Personnel	-	13,265	13,565	13,867	14,167	14,468	-	69,332
	Equipment	-	-	-	-	-	-	-	-
	Travel	-	3,721	3,907	4,098	4,292	4,492	-	20,510
	Lab & Suppl	-	2,100	2,150	2,200	2,250	2,300	-	11,000
	Indirect	-	4,147	4,559	4,975	5,395	5,821	-	24,897
	Subtotal	-	23,333	24,181	25,140	26,104	27,081	-	125,739
Exports & Instream Litter & Shade	Personnel	32,838	98,513	98,513	98,513	98,513	98,513	37,308	562,711
	Equipment	60,700	600	600	35,100	600	600	300	98,500
	Travel	11,200	30,600	31,600	32,600	33,600	34,600	2,400	176,600
	Laboratory	-	49,900	49,900	49,900	49,900	49,900	-	249,500
	Indirect	13,036	39,109	39,109	39,109	39,109	39,109	13,223	221,804
	Subtotal	117,774	218,722	219,722	255,222	221,722	222,722	53,231	1,309,115
	Annual Totals	321,657	634,631	652,267	584,500	541,295	550,828	119,132	\$3,404,209

SCHEDULE OF ACTIVITIES

- Year 1 – a) contact landowners and select sites for screening
b) sample to confirm amphibian occupancy
c) assign sites to blocks
d) establish sampling monuments, plots, and monitoring locations
e) organize data collection and entry logistics
f) organize structure among co-operators and co-investigators
g) develop genetic markers
- Year 2 – a) collect 1st year of pre-treatment data for entire variable suite
b) enter 1st year of pre-treatment data
c) develop end of year progress report summarizing results
- Year 3 – a) collect 2nd year of pre-treatment data for entire variable suite
b) enter 2nd year of pre-treatment data
c) develop end of year progress report summarizing results
- Year 4 – a) coordinate and monitor application of treatments
b) analyze pre-treatment data; provide neighbor analysis for genetic data
c) develop end of year progress report summarizing results
- Year 5 – a) collect 1st year of post-treatment data for entire variable suite except genetics
b) enter 1st year of post-treatment data
c) develop end of year progress report summarizing results
- Year 6 – a) collect 2nd year of post-treatment data for entire variable suite except genetics
b) enter 2nd year of post-treatment data
c) develop end of year progress report summarizing results
- Year 7 – a) compare and analyze pre- versus post-treatment data
b) develop report outlining and interpreting results

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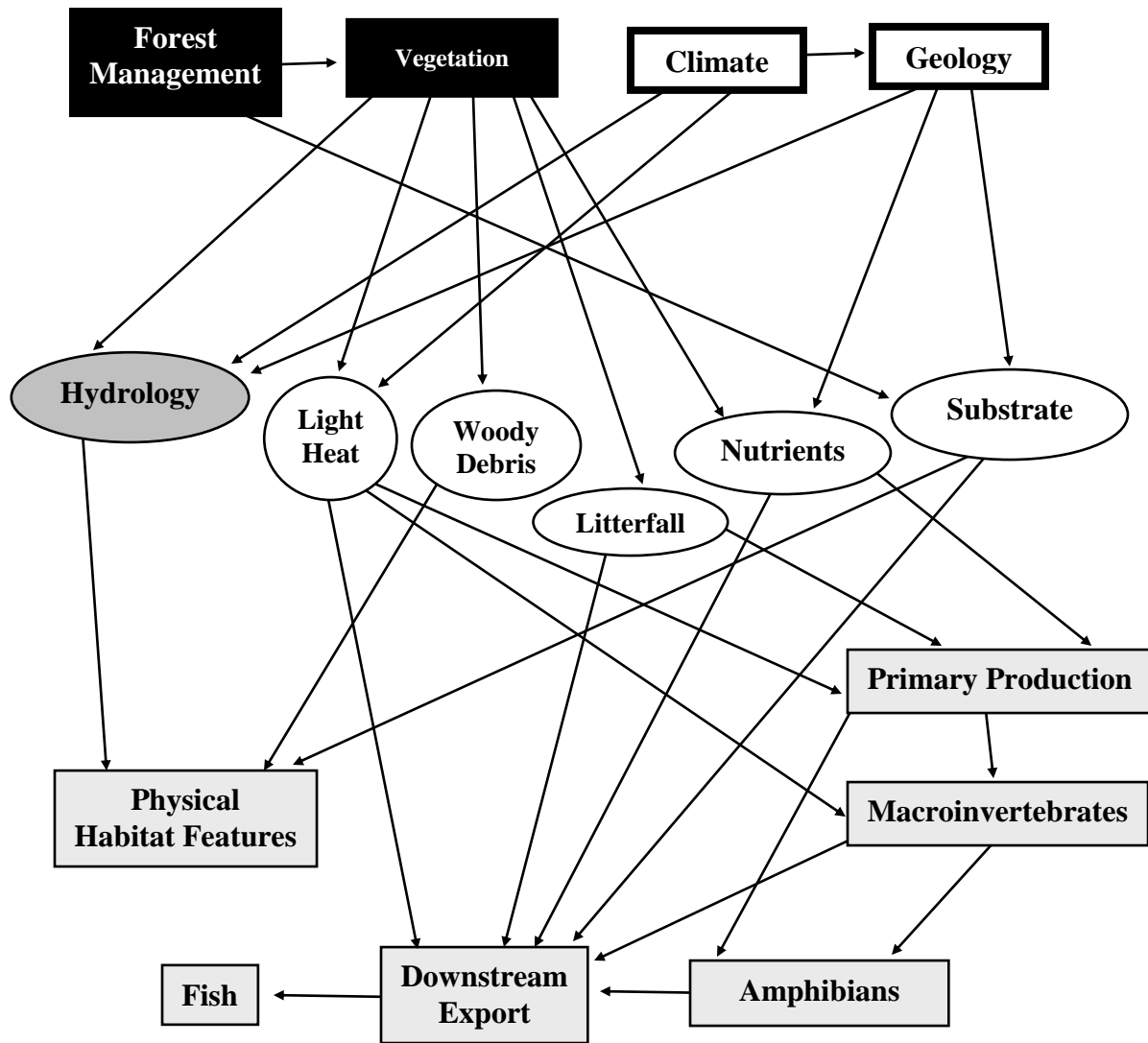
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APPENDIX I

The Energy Pathway Conceptual Model

The *energy pathway conceptual model* illustrates the major pathways of potential effects on headwater amphibians and downstream export to fish-bearing streams (APPENDIX FIGURE 1). In general, harvest will decrease the riparian canopy cover, thereby allowing more light and heat to reach the stream, which may increase stream temperature (Johnson and Jones 2000), reduce the long-term rate of LWD recruitment to the stream, and possibly reduce the input of litterfall. Reduced canopy effects on shading may result in elevated stream temperatures for 15 years in selected landscapes (Johnson and Jones 2000). A relatively brief interval of increased light may increase primary production (Murphy 1998), favoring instream grazers (e.g., selected macroinvertebrates [Hawkins 1988, Hawkins *et al.* 1982] and larval tailed frogs [Hawkins *et al.* 1988]), and may translate into a positive response by consumers at higher levels in the food web (e.g., salamanders: Hawkins *et al.* 1983; fishes: Bisson and Sedell 1984, Bilby and Bisson 1987, Hartman *et al.* 1987). If sedimentation patterns are changed, these levels of primary and consumer production may be altered (Murphy and Hall 1981, Hawkins *et al.* 1983). In conjunction with decreased litterfall, this could change both the quantity and quality of organic matter transported downstream. Basin-scale harvest can increase the concentration and export of nutrients from the basin, which could affect productivity both within headwater streams and downstream. Long-term changes in LWD recruitment may result in changes to physical habitat and sediment transport. This study will explicitly compare the response of amphibians; stream temperature; downstream export of nutrients, sediment, detritus, and macroinvertebrates; and downstream fish from three buffer treatments with an unharvested reference stream.

APPENDIX FIGURE I. ENERGY PATHWAY CONCEPTUAL MODEL. Forest management has the potential to impact amphibians and downstream exports through changes to physical habitat, primary productivity, or invertebrate composition or abundance. Likewise, downstream export of heat, nutrients, and organic matter may be affected by changes in light penetration to the stream and allochthonous inputs may be affected by changes in the riparian vegetation. In this study, forest management will apply different treatments by manipulating vegetation (independent variables; black squares), which influence system features or processes (white or gray ovals). Amphibians, selected habitat and export variables, primary production, and macroinvertebrates will be measured as response variables (dependent variables; gray squares) that may be influenced if system features or processes are altered.



APPENDIX II

The Landscape Conceptual Model

The *landscape model* describes headwater basins and the interactions between landforms, physical processes and pathways. Streams begin within headwater basins where surface runoff, soilwater and groundwater from hillslopes converge into a surface flow with enough power to scour a channel as it moves downslope. The smallest “finger-tip” streams converge downgradient to form progressively larger streams. This progressive convergence gives rise to streams of different **order**⁵. Streams of higher order draining larger basins, having greater discharge, and flowing down lower gradients in larger channels, often change systematically in morphology. Headwater basins, though small, are numerous, occupying ca. 80% of the landscape (Leopold *et al.* 1992, Wondzell 1994).

A major change that occurs in the downstream direction is the relationship between hillslope and channel. In lower-order basins (about 3rd-order and lower) the channel lies in a narrow V-shaped valley wherein the hillslope is directly connected (coupled) to the channel. In higher-order valleys, the valley floor becomes wider as the floodplain develops and hillslopes become separated (decoupled) from the channel (Church 2002, Gomi *et al.* 2002). The valley floor and sediments underlying it buffer the channel from direct input of sediment, organic debris, and water from adjacent hillslopes by storing the input (delaying its delivery), and providing opportunity for mixing inputs from various sources and events and for modification by organisms. Valley floor buffering results in a changed delivery rate, quantity, and physical and chemical characteristics (e.g., temperature, composition) of hillslope input (McGlynn, *in press*; McGlynn and McDonald 2003; McDonnell *et al.* 1998). Thus, the landscape model is best divided into two intimately connected components – the hillslope and valley floor-river systems.

The hillslope system consists of an upland and inclined slope connecting the upland with the adjacent channel or valley floor. This upland-slope unit occurs in different configurations affecting the flow of water and sediment toward the valley floor and channel. Convergent slopes progressively concentrates water and sediment at their junctions; divergent slopes (“noses”) at valley confluences tend to disperse water and sediments; and the intervening channel-parallel sideslopes concentrate inputs only in the downslope direction. The hillslope system includes the land surface; underlying soil/regolith, bedrock, soilwater⁶ and groundwater; surface and subsurface biota, and overlying atmosphere (Winter 2001). The hillslope interactions important to this study are between vegetation, soil, and water.

Subsurface flow of water and nutrients dominates forested hillslopes. Precipitation infiltrates the thick organic layer on the soil surface and slowly percolates downward through the underlying soil until lower permeability material is encountered and flow of soilwater or groundwater is diverted downslope (Asano *et al.* 2002, McGlynn *et al.* 2003, Montgomery and Dietrich 1995). During this movement, interactions between soilwater,

⁵ Various approaches to stream ordering exist. The most common, and that used here, is that of Strahler (1952) in which the smallest finger-tip channels are first order, two first-order channels converge to form a second-order stream, two second-order channels converge to form a third-order stream and so on.

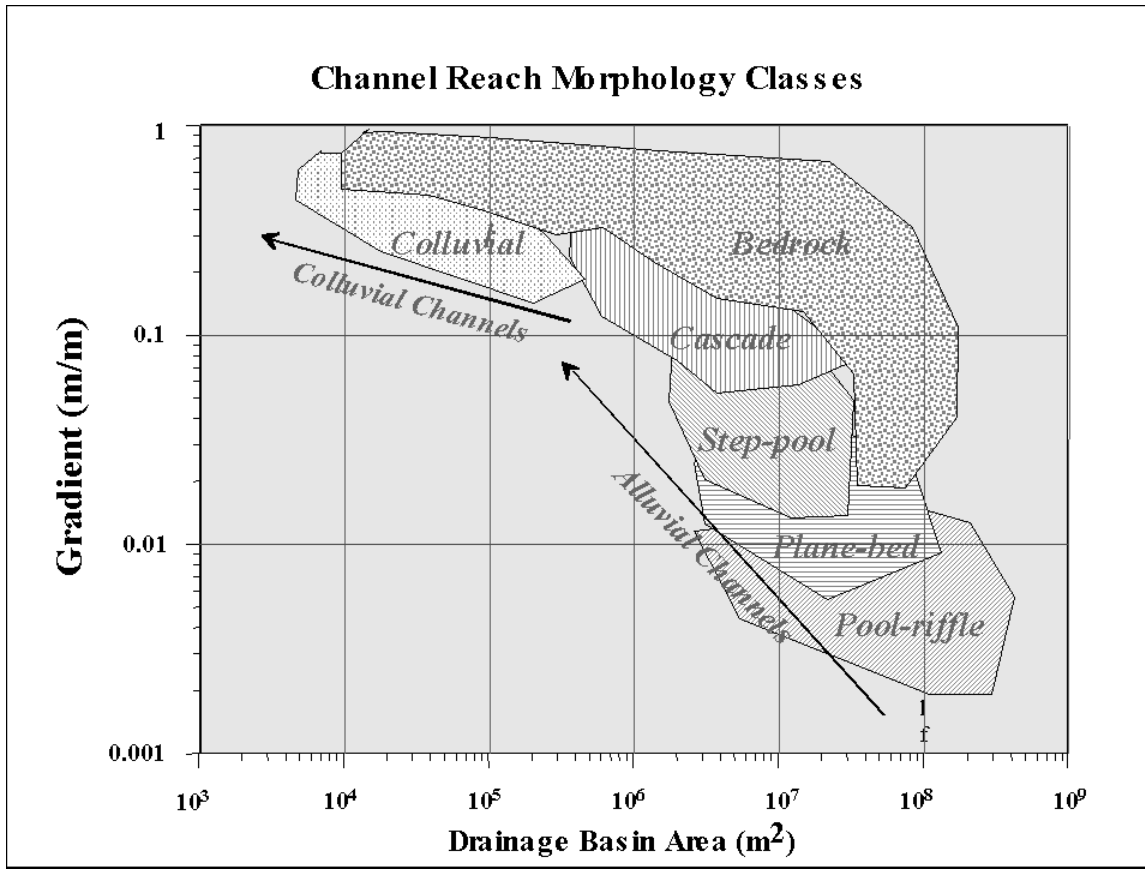
⁶ Soilwater is that subsurface water occurring in unsaturated soils above the water table, below which groundwater occurs (Asano *et al.* 2002). At times of complete soil saturation, this distinction is blurred.

organic and mineral matter change water composition (Clement *et al.* 2003). Analysis of water ages indicates that vertical percolation of soilwater can take weeks to months, and its downslope movement can take months to a year or more (Asano *et al.* 2002). The composition of the subsurface discharge to a channel changes seasonally as water from different parts of the hillslope-valley floor reaches the channel (Clement and others 2003). Water discharged to the stream during the early phases of a storm event can be several weeks to months old as new storm water displaces stored soilwater (McGlynn, in press). Surface flow of water down a hillslope, and thus the potential for surface erosion, is limited to lower slopes and convergent slopes when the soil is saturated to the surface and the rate of precipitation exceeds the rate of infiltration.

Complexity of valley floor-channel system increases with size of the valley floor as the system passes through a series of thresholds (Church 2002). In low-order valleys without a valley floor, the system is simple and consists of the channel and scattered patches of bedrock and sediments. As the valley floor increases in width with increasing order, the extent of the sediments increases, as does the quantity of water flowing between the channel and adjacent sediments. With increasing valley floor width, the **hyporheic**⁷ flow system becomes a more important control on water chemistry and temperature (Olsen and Townsend 2003, Kasahara and Wondzell 2003). Likewise, channel morphology systematically changes with valley gradient, basin area, and sediment supply and caliber as shown in APPENDIX FIGURE II (Montgomery and Buffington 1997), although channel forms can be “forced” into a higher-gradient category by the presence of large obstacles (LWD, boulders, etc) in the channel. Substrate characteristics and mobility is controlled by the shear stresses within these channel forms as described in APPENDIX III. Within the channel, downgradient hyporheic flow occurs within the alluvium and between channel features (Boulton *et al.* 1998, Malard *et al.* 2002) and as the valley floor becomes wider and more complex with side channels, wetlands, and tributaries, the quantity and length of hyporheic flow beneath the valley floor increases (Kashari and Wondzell 2002). The complex flow within the hyporheic-channel system may be the best buffer to stream temperature modifications resulting from human modifications (Poole and Berman 2000).

Groundwater inflow to the channel and hyporheic zone further enhances the complexity of the valley floor-channel system. Flow in low-order channels is comprised primarily of inputs from soil- and groundwater sources, with the exception of direct inputs from precipitation and surface runoff during the later stages of large storm events (Pearce *et al.* 1986, Stewart and McDonnell 1991, McGlynn *et al.* 2003). In intermittent (seasonal) reaches, discharge is dominated by soilwater inputs and as flow becomes more continuous in the perennial reach, groundwater inputs become more important and remain important until overwhelmed by surface water inputs from tributaries. Groundwater inputs to low-order channels are usually at least one year old and the age of the direct groundwater inflow to the channel increases in a downstream direction.

⁷ Hyporheic system is the area wherein surface water flows through adjacent sediments to return to the surface (Boulton *et al.* 1998).



APPENDIX FIGURE II. RELATIONSHIP AMONG CHANNEL MORPHOLOGY, BASIN SIZE AND GRADIENT (adapted from Montgomery and Buffington 1997).

A controlling factor on characteristics of headwater valleys is mass wasting (Dunne 1998), which is one of numerous disturbance processes that exert distinct influences on lotic and riparian ecosystems (Montgomery 1999). Debris flows originate in bedrock hollows, which tend to be located in the convergent valley head, and flow down lower-order valleys until they are deposited at the junction with a higher-order stream. Debris-flows scour the upper valley, frequently to bedrock, and deposit coarse sediment and woody debris in the higher-order valleys. Scoured valleys recover slowly as woody debris captures sediment (May and Cresswell 2003). Most headwater valleys in high relief areas lie within the debris flow process domain that lies upstream of the fluvial process domain, which is characteristic of the higher-order fish-bearing streams (Montgomery 1999).

APPENDIX III

CHANNEL CHARACTERISTICS

TYPE N streams, or non-fishbearing headwater streams, are a product of hydrologic, geomorphic, and biological processes (Gomi *et al.* 2002). The TYPE N experimental study design must adequately characterize biological and hydrogeomorphic processes in order to effectively link changes in biological assemblages (e.g., amphibians and macro-invertebrates) to land use activities. Low-order channels that amphibians such as the tailed frog (*Ascaphus truei*) and torrent salamanders (*Rhyacotriton spp.*) use are characterized by:

APPENDIX TABLE I. CHARACTERISTICS OF MOUNTAIN RIVERS (adapted from Wohl 2000) .

- Steep (≥ 10 % slope) average channel gradient;
- High channel-boundary resistance and high boundary roughness from the bedrock and a greater likelihood of the presence of coarse clasts along these channels than along low gradient channels;
- Highly turbulent flow and stochastic sediment movement resulting from the steep gradient, rough channel boundaries, and limited sediment supply;
- A strongly seasonal discharge regime, whether driven by glacial meltwater, snowmelt or rainfall, with high spatial and temporal discharge variability resulting from the effect of changes in precipitation with elevation and basin orientation;
- Channel morphology that has high spatial variability because of the external control of geology (lithology, tectonics, structure, glaciation, sediment supply), but low temporal variability because only infrequent floods or debris flows are able to exceed channel-boundary resistance;
- The potential for extraordinarily high sediment yields over a period of a few years following watershed-scale disturbance (e.g. forest fire, timber harvest); and
- A longitudinal zonation of aquatic and riparian biota that is influenced both by stream characteristics and elevation, which influences the local temperature and precipitation regime.

These characteristics may have important implications for the distribution and abundance of amphibian species. Additionally, land use impacts in the form of increased mass-wasting, modification of flow regimes, chronic fine sedimentation, and riparian denudation can alter processes in headwater streams.

Physical Controls on Channel Morphology and Substrate Size

Channel morphology and substrate size is a function of sediment transport capacity, resisting forces (e.g., boundary roughness, form roughness) of the stream channel, and sediment supply (Whiting and Bradley 1993, Montgomery and Buffington 1997).

Sediment transport capacity (Q_s) can be defined using the following two models:

$$Q_s = k \tau (\tau - \tau_c) \quad (1)$$

$$Q_s = k(\Omega - \Omega_c) \quad (2)$$

where k is an index of the mobility of the sediment, τ is shear stress, τ_c is the critical shear stress for incipient motion (Knighton 1998), Ω is the stream power, Ω_c is the critical stream power for incipient motion (Bagnold 1977). Shear stress and stream power are given by

$$\tau = \rho_w g d S \quad (3)$$

$$\Omega = \rho_w g q S \quad (4)$$

where ρ_w is the density of water, g is gravity, d is the depth of flow or hydraulic radius, q is flow per unit width, and S is water or bed surface slope.

Flow resistance is a primary element of stream behavior because it influences bed substrate properties, sediment transport, and the way a stream loses its energy (Knighton 1998). Flow resistance in channels is commonly calculated using the Darcy-Weisbach equation:

$$ff = 8 g R S v^{-2} \quad (5)$$

where ff is the friction factor, g is gravity, R is hydraulic radius (i.e., roughly equivalent to mean depth), S is slope, and v is mean velocity. Energy loss is in the form of grain resistance, undulating bedforms (step-pools), and channel obstructions such as large-woody debris (Montgomery and Buffington 1998). Energy loss due to resistance decreases the shear stress, thereby reducing sediment transport capacity. Montgomery and Buffington (1997) distinguish the roughness corrected sediment transport capacity as the effective sediment transport capacity.

Bathurst (1993) noted that boulder form drag dominated flow resistance in cascade channels, whereas spill resistance (e.g., from plunge pools) dominated flow resistance in step-pool channels. Darcy-Weisbach friction factor has been correlated with the ratio of hydraulic radius to D_{84} (i.e., relative submergence; Bathurst 1985, Ugarte and Madrid 1994). Curran and Wohl (2003) estimated that resistance from step-pool bedforms provided over 90% of the total channel roughness for small step-pool streams in western Washington. Friction factor was significantly correlated with the ratio of reach-averaged step height and the reach-averaged length between steps (i.e., H/L), indicating flow resistance increased as step height increased and length between steps decreased (MacFarlane and Wohl 2003). Large woody debris (LWD) was indirectly related to flow resistance because LWD was associated with higher steps (Curran and Wohl 2003), which in turn provided the most resistance. Additionally, channels with abundant LWD had significantly higher flow resistance than debris flow scoured channel with no LWD (MacFarlane and Wohl 2003). Large woody debris that obstructs the channel also exerts strong form drag on water velocity, providing roughly half of the flow resistance in larger rivers (Manga and Kirchner 2000). Large woody debris in smaller channels may provide flow resistance greater than values for larger rivers, but the pattern is dependent on larger woody debris loading.

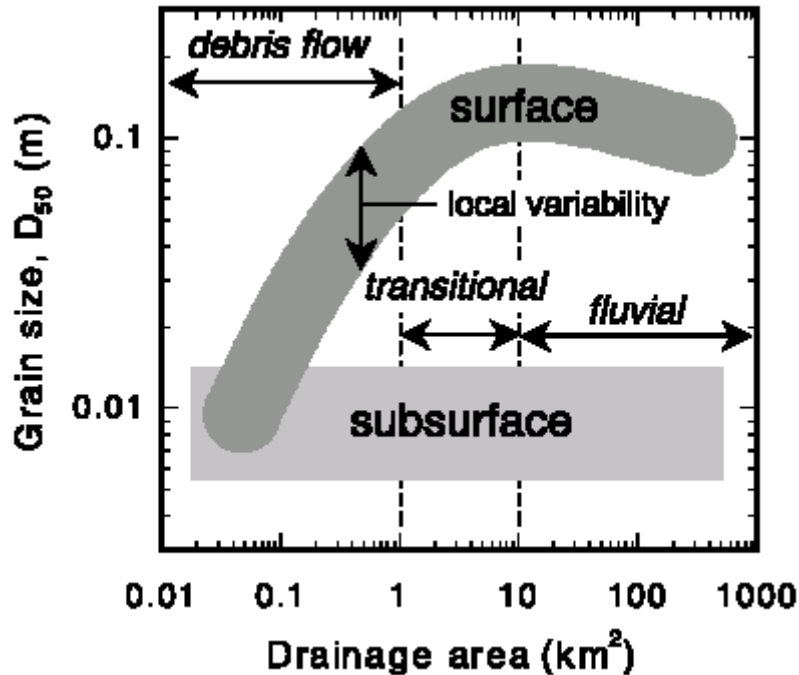
Hydraulic roughness from LWD can affect channel substrate size and habitat complexity in gravel-bedded rivers (Montgomery and Dietrich 1995; Buffington and Montgomery 1999). In headwater channels, LWD promotes sediment deposition (May and Greswell 2003, Faustini and Jones 2003), creates large plunge pools that dissipate energy (Curran and Wohl 2003), promotes textural heterogeneity in surface sediments (MacFarlane and Wohl 2003), and encourages channel stability (Faustini and Jones 2003). Furthermore, LWD can force step-pool morphologies in otherwise bedrock-dominated reaches (Montgomery and Buffington 1997). Recent work indicates that flow resistance controls the drift rate of nutrients and macroinvertebrates in low-order streams (D. Wilcox, personal communication).

Montgomery and Buffington (1999) hypothesized that channel morphology reflects the magnitude of effective sediment transport capacity (i.e., shear stress corrected for flow resistance) to sediment supply. Supply-limited channels are channels where effective transport capacity exceeds sediment supply. Headwater channels typically reflect these supply-limited conditions due to coarse substrate size and stochastic inputs of sediment. The effect of sediment supply on channel morphology and bed material characteristics is dependent upon the magnitude and temporal distribution of sediment inputs to the channel. Chronic fine sedimentation (e.g., road surface erosion) can lead to a fining of pool bed material in step-pool sequences (Madsen 1995). Sediment supply can also affect the size and distribution of roughness elements in the channel. Coarse clasts from debris flow lag deposits can increase grain roughness in headwater channels (Brummer and Montgomery 2003). Debris flows can also provide LWD, which are important in the formation of step-pool sequences, and in turn provide spill resistance (Lancaster *et al.* 2001, Curran and Wohl 2003). Conversely, debris flows can scour channels to bedrock. Debris flow scour can remove roughness elements, thereby maximizing the sediment transport capacity of the channel (May and Greswell 2003, Montgomery and Buffington 1997). Variability in grain and form roughness may be a function of recovery time following debris flow (Brummer and MacDonald 2003, MacFarlane and Wohl 2003). Large woody debris recruitment increases linearly with post debris flow recovery time. This has important implications for riparian harvesting because without sufficient wood recruitment debris flow scoured channels can remain in a bedrock state for prolonged periods (May and Greswell 2003).

Hydrogeomorphic Controls on Amphibian Distribution

Based on data from Oregon, Altig and Brodie (1972) placed optimum substrate size for tailed frogs at 55-125 mm (gravel-cobble range). Diller and Wallace (1999) found that coastal tailed frog larvae in California were positively associated with cobble, boulder, and gravel substrates, and negatively associated with fine substrates. Southern torrent salamanders, also in California, occupy a wider range of substrates, varying from 2-256 mm (Welsh and Lind 1996). The preferred size class of substrate suggests that tailed frogs occupy transitions between debris flow-dominated channels and fluvial dominated channels (APPENDIX FIGURE I). These transition zones have the highest surface D_{50} , and occur coincident with maximum unit stream power (APPENDIX FIGURE III; Brummer and Montgomery 2003). Prior disturbance is another factor on surface grain size because debris flows can form lag deposits with large clasts that cannot be mobilized except during low frequency flow events. This is consistent with the hypothesis that tailed frogs

prefer steep gradient streams that are less prone to channel scour (NCASI 1999, 2001). Typically these zones are cascade or step-pool channel reaches (Brummer and Montgomery 2003). Torrent salamanders are thought to occupy a wider variety of geomorphic niches, ranging from colluvial channels to the wetted margin of larger streams (Anderson 1968, Diller and Wallace 1996, Welsh and Lind 1996).



APPENDIX FIGURE III. SCHEMATIC ILLUSTRATION SHOWING RELATIONS BETWEEN PROCESS DOMAINS AND SYSTEMATIC TRENDS IN SURFACE AND SUBSURFACE GRAIN SIZE (from Brummer and Montgomery 2003).

Tailed frogs are positively associated with LWD (Welsh 1993). However, it is unclear whether this is because LWD promotes optimal hydraulic environments (e.g., high roughness and reduced shear stress/stream power) for amphibian habitat, promotes channel stability, or provides cover from predation (NCASI 2001). Tailed frogs and torrent salamanders prefer stream gradients in excess of 9% (Wallace and Diller 1998, Diller and Wallace 1996), and flow resistance is positively correlated with channel slope (equation 5). These amphibians are also associated with shallow flow (Nussbaum *et al.* 1983, Welsh and Lind 1992, Welsh 1993) and relatively large substrate, suggesting that they occupy channels with low relative submergence (R/D_{84}), and high flow resistance.

How extensively amphibians occupy bedrock channels is unclear. Bedrock channels contain little alluvial bed material or valley fill, and have higher channel gradient than alluvial channel types with similar drainage areas (Montgomery and Buffington 1998). These conditions reflect a state where transport capacity exceeds sediment supply (Montgomery *et al.* 1996). Literature on tailed frogs and torrent salamanders suggest that they require instream substrate with numerous interstitial spaces (NCASI 2001), thereby precluding them from occupying many bedrock reaches.

Implications for the TYPE N Experimental Study Design:

The literature suggests that certain process domains create optimum habitat for amphibians. Amphibians may prefer certain hydraulic environments based on a balance of driving forces (e.g., shear stress and stream power), resisting forces (e.g., flow resistance due to wood induced bedforms, large-scale grain roughness, and form drag on LWD), and sediment supply (i.e., a function of disturbance magnitude and history). To successfully characterize hydrogeomorphic processes for the study, driving forces, flow resistance, and sediment supply should be characterized on a reach-scale basis. A reach is a segment of common channel morphology typically 10-20 channel widths in length. Reach delineation can be done either using physical criteria (e.g., uniform slope) or a process-based channel classification scheme. Montgomery and Buffington (1997) offer a stream classification scheme based on channel bedforms. Whiting and Bradley's (1993) channel classification scheme is based upon: 1) channel gradient relative to sediment supply; 2) confinement; and 3) sediment size relative to hydraulic forces.

Characterization of transport capacity should include:

1. Ten cross-section measurements to approximate reach averaged mean flow depth at the water surface and estimated bankfull stage;
2. Reach-averaged channel gradient at the thalweg, water surface, and estimated bankfull water surface;
3. Estimates of water velocity using an empirical approach such as Manning's formula;
4. Measure water velocity and discharge using salt tracer methods or flow meters.

This will result in independent variables such as reach-averaged shear stress, stream power, stream gradient, water velocity, and discharge for low-flow and bankfull flow conditions.

Characterization of flow resistance should include:

1. A longitudinal profile to measure bed elevation changes due to vertical bedforms such as channel steps and pools;
2. Pebble counts to determine grain roughness;
3. LWD inventory including:
 - a. Number of pieces per channel length;
 - b. Volume of pieces per channel length;
 - c. Area flow blockage at low-flow and bankfull stage;
 - d. Orientation of LWD relative to flow
4. A calculation of total flow resistance using empirical methods such as Darcy-Weisbach friction. This will require estimates of water velocity (see above).

This will result in independent variables such as reach-averaged height to length ratio for channel steps (H/L by LWD, clasts, or combination), grain roughness, relative submergence (R/D_{84}), form roughness due to wood, and total roughness.

Characterization of sediment supply should include using sediment budget techniques to estimate inputs due to bank erosion, mass-wasting, and surface erosion (Reid and Dunne 1996). In addition, LWAG should characterize subsurface particle size distribution in pools, and the ratio of surface particle size to subsurface particle size for pools (D^*_{50}).

This will provide a relative index of sediment supply since high D_{50}^* values (e.g. armoring) reflect supply-limited conditions, and low D_{50}^* values represent high sediment supply (Dietrich *et al.* 1989).

Study Design Limitations: Representation of Basalt Terrain for Effectiveness Monitoring

TYPE N effectiveness is proposed in basalt terrain streams, or streams on terrains that have hydrological processes similar to those on basalt. Detecting management-induced changes in bed substrate size and composition (e.g., embeddedness and fining) is notably difficult in channels with basalt lithology. Lisle and Hilton (1999) did not find a correlation between sediment supply and V^* for drainage underlain by basalt and andesite lithologies. They also found no relationship between sediment supply and pool D_{50} for the same drainages. In contrast, drainages in sedimentary and granitic lithologies showed a positive correlation between sediment supply and V^* and pool D_{50} (Lisle and Hilton 1999).

Lack of channel response in volcanic lithologies has been shown in several unpublished studies (Sable and Wohl 2002; Kaufmann *et al.* 2003; MacDonald *et al.* 2003). Sable and Wohl (2002) showed that in the Oregon coast range, low-gradient streams (<1%) in marine volcanic lithology had significantly lower amounts of fine sediment in pools relative to fine-grained sandstones. Kaufmann and others (2003) and Faustini and Kaufmann (2003) found that Pacific Northwest streams draining basalt lithologies showed no increase in fine sediments due to anthropogenic disturbance, whereas streams draining sedimentary lithology displayed significant fining in relation to increased road density and land disturbance. MacDonald and others (2003) showed that V^* and pool D_{50} increased with road density and modeled road sediment production in granitic lithology, whereas stream channels draining andesitic parent materials showed no relationship between disturbance and channel response.

Amphibians such as tailed frogs and torrent salamanders require cold water, ranging between 5-18 C (De Vlaming and Bury 1970, Diller and Wallace 1996; Marshall *et al.* 1996). Temperature change is directly proportional to the surface area of the stream and inversely proportional to stream discharge (Beschta *et al.* 1987). Stream temperatures are highest during the summer and lowest during the winter. An exception to this cyclical pattern occurs when springs or groundwater sources feed streams. Streams dominated by groundwater and seepage sources can often display little seasonal variability (Minkley 1963). Basalt lava plateaus of the northwestern United States are good examples of groundwater-dominated systems (Dunne and Leopold 1978). Groundwater-dominated TYPE N streams may be less sensitive to biologically adverse solar radiation inputs than streams dominated by near-surface flowpaths.

Basalt and similar lithologies are representative of only a portion of the FFR landscape. Previous research suggests that basalt is more resilient to increased inputs of sediment and solar radiation. Thus, the results of TYPE N effectiveness experimental study on basalt terrain may not apply to other lithologies. [developed largely by Drew Coe]

APPENDIX IV

Near-stream Sampling for Terrestrial FFR Species

Amphibian sampling in this proposal uses instream sampling methods, which are unable to effectively sample two of the FFR SAAs, Dunn's and Van Dyke's salamanders, both of which occupy terrestrial habitats that are near-stream. Because addressing both species requires a near-stream terrestrial sampling approach, we considered the possibility of adding a separate sampling piece that would address both these species. We found this possibility untenable for the following reasons:

Effective (density-reliable) near-stream sampling requires excavation of 10 2-m wide belt transects between the stream edge and the stream valley wall break to a depth of 30-cm in each treatment unit. Because of this disturbance, near-stream sample plots cannot be resampled at the same point in consecutive years. Trenches this deep will intercept water (groundwater and surface flow) and route it directly to the stream along with the exposed sediment picked up as water flows through the trench. Moreover, each pre- and post-harvest year sampled will add 10 new trenches. This sampling method would alter sedimentation and instream amphibian habitat in a manner undesirable for its inclusion in this field experiment.

Stream selection based on coastal tailed frog occupancy is an excellent indicator of other instream taxa, but it is uncorrelated to the presence of either terrestrial FFR salamanders. This means that stream selection based on coastal tailed frog occupancy cannot guarantee the presence of either terrestrial FFR salamander in treatment units selected. Therefore, besides the aforementioned habitat-modification risk, it would not be guaranteed that either of the terrestrial FFR salamander species would be present in the units selected.

Lastly, the sampling required to adequately detect these two species would represent a costly addition to this study. Terrestrial sampling for these two taxa would cost as much as instream sampling for all other amphibians combined.

APPENDIX V

Amphibian Species in FFR Landscapes in Washington State

Species in **blue** occur only in eastern Washington, species in **red** occur in at least some of eastern and western Washington, and all other species (black) occur only in parts of western Washington.

Appendix Table II - Amphibian Species in FFR Landscapes in Washington State

Species Name		Code	FFR Target
Common	Scientific		
Northwestern salamander	<i>Ambystoma gracile</i>	AMGR	
Long-toed salamander	<i>Ambystoma macrodactylum</i>	AMMA	
Coastal tailed frog	<i>Ascaphus truei</i>	ASTR	yes
Rocky Mountain tailed frog	<i>Ascaphus montanus</i>	ASMO	yes
Boreal (western) toad	<i>Bufo boreas</i>	BUFO	
Cope's giant salamander	<i>Dicamptodon copei</i>	DICO	
Coastal giant salamander	<i>Dicamptodon tenebrosus</i>	DITE	
Ensatina	<i>Ensatina eschscholtzii</i>	ENES	
Pacific treefrog (chorus frog)	<i>Hyla regilla</i>	HYRE	
Dunn's salamander	<i>Plethodon dunni</i>	PLDU	yes
Larch Mountain salamander	<i>Plethodon larselli</i>	PLLA	
Van Dyke's salamander	<i>Plethodon vandykei</i>	PLVA	yes
Western red-backed salamander	<i>Plethodon vehiculum</i>	PLVE	
Northern red-legged frog	<i>Rana aurora</i>	RAAU	
Cascades frog	<i>Rana cascade</i>	RACA	
Columbia spotted frog	<i>Rana luteiventris</i>	RALU	
Cascades torrent salamander	<i>Rhyacotriton cascadae</i>	RHCA	yes
Columbia torrent salamander	<i>Rhyacotriton kezeri</i>	RHKE	yes
Olympic torrent salamander	<i>Rhyacotriton olympicus</i>	RHOL	yes
Rough-skinned newt	<i>Taricha granulosa</i>	TAGR	

APPENDIX VI

Power To Detect Changes in Amphibian Density

The power ($1-\beta$) to detect changes in amphibian density, based here on coastal tailed frog (ASTR) density, among treatments is a function of within treatment (also referred to as within cell) variability, the effect size, the number of samples (replicates) per treatment, and levels of Type I error. Within treatment variability in a before/after design is the variability of the difference in density before and after treatment. In order to better understand the relationship between the power of our study design to detect changes in ASTR density and the other variables listed above, we used data from Kelsey (1993) to estimate within treatment variability in ASTR density, ASTR density, and a plausible range of effect sizes.

The data set from which we drew our estimates is small. Kelsey's (1993) before/after study design contained 5 sites, 3 replicates of a control treatment (i.e, no management action) and 2 replicates of a clearcut harvest treatments. Since Kelsey (1993) found that ASTR densities were lognormally distributed, we used log-transformed data. We calculated within treatment variability (the input into the power calculation) as the standard deviation of the $\log(\text{density}_{\text{before}}) - \log(\text{density}_{\text{after}})$ or alternatively, the $\log(\text{density}_{\text{before}}/\text{density}_{\text{after}})$ across the five sites. We believe pooling the replicates across 2 treatments (control and clearcut) was appropriate based on the fact that that we had no other data from which to estimate within treatment variability. Pooling replicates across treatments should not be a problem since the variance between treatments is either equal (an ANOVA assumption) or unequal in which case pooling should produce a more conservative (higher) estimate of sample sizes all else being equal.

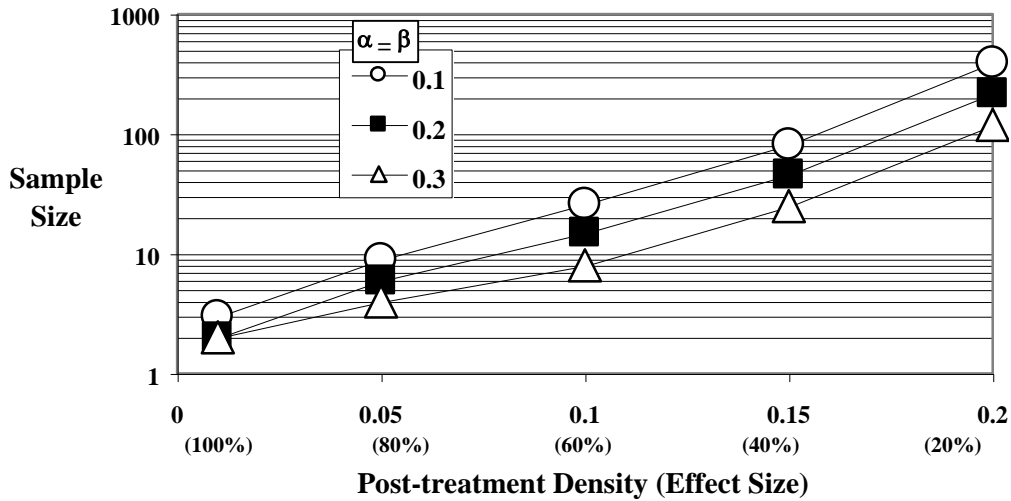
We conducted our power analysis based on a single-factor ANOVA design using SYSTAT (1993) despite the fact that our study design is more complicated. Power analysis based on a single-factor ANOVA should provide a more conservative (higher) estimate of the sample sizes needed to meet a given level of power because the repeated measures design controls for site-to-site variability by considering differences within sites. Subsequently, precision of the estimated pre- and post-treatment effects is improved (Kris Ryding, WDFW, personal communication). The power analysis provides estimates of needed sample sizes for testing the null hypothesis for a one-way ANOVA that no difference exists among treatments as opposed to individual treatment comparisons.

A consequence of using log-transformed data is that ΔT (i.e., $\log(\text{density}_{\text{before}}/\text{density}_{\text{after}})$) becomes a function of the magnitude of density even when the difference between $\text{density}_{\text{pre}} - \text{density}_{\text{post}}$ remains the same. In order to understand how the magnitude of pre- and post-density (but not the difference between $\text{density}_{\text{before}} - \text{density}_{\text{after}}$) affects sample size, we determined ΔT at 2 pre-treatment densities (0.5 and 0.75 ASTR/m²) and post-treatment densities ranging from 0.1 to 0.5 simulating different treatment effects in the density of ASTR. Again, we used a range of effect sizes in the analysis because we do not exactly know what to expect. We then calculated sample size at three combinations of confidence and power, $\alpha = \beta = 0.10$, $\alpha = \beta = 0.20$, and $\alpha = \beta = 0.30$ (APPENDIX FIGURES IV AND V). Sample size varies depending upon the effect size, the magnitude of densities pre and post, and the degree of uncertainty tolerated. For example, to detect a change of

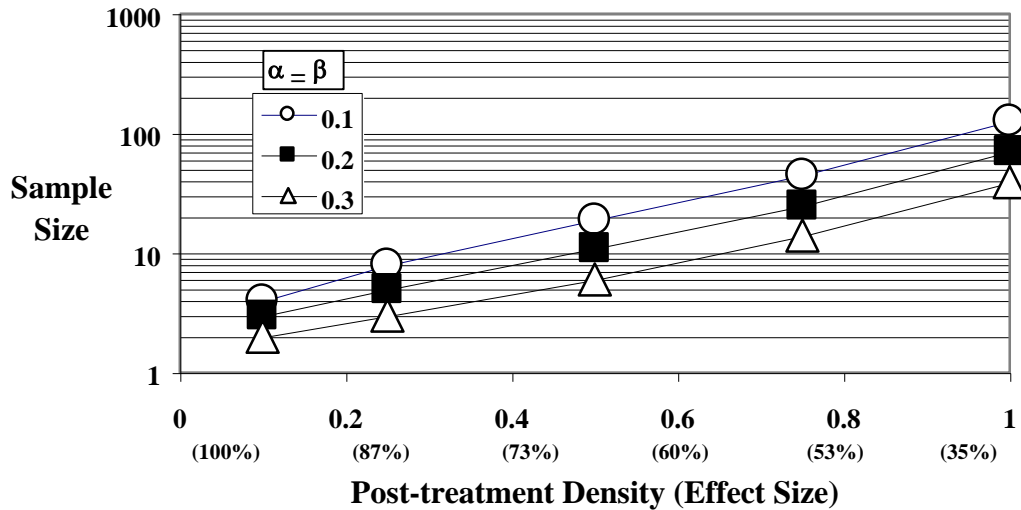
ASTR density from 0.75 m² to 0.20 m² would require a sample size of 4 replicates per treatment at $\alpha = \beta = 0.30$.

Estimating sample size for equal levels of confidence and power was based on a need to balance these two conditions, and in particular, not underestimating Type II errors (not identifying an effect when an effect is actually present), a crucial, but frequently underestimated condition in this kind of landscape study (Schradet-Frechette and McCoy 1993). Setting α above 0.05 reflects feasibility for this kind of landscape experiment related to sample size (Schradet-Frechette and McCoy 1993, Toft and Shea 1983, Toft 1991); α and β could not be made equal at a value of 0.05 and have a sample size small enough to make a landscape study feasible.

Given the power analysis results (APPENDIX FIGURES IV AND V), a large effect (ca. 80%) will be necessary to detect differences among treatments, as it is unfeasible for the total number of blocks available for this design to be very large. The number of blocks that will be feasible to implement is in the 4-10 range. Thus, treatments should be selected in a manner that will maximize the potential of an effect resulting in differences among treatments. Several factors are important to maximize the likelihood of this occurring, but a critically important one is that harvest unit size be equal to or approach treatment unit size in order to maximize the influence of the treatment on treated units. This need would exclude the selection of blocks from eastern Washington.



APPENDIX FIGURE IV. SAMPLE SIZES FOR ONE-FACTOR ANOVA WITH A PRE-TREATMENT DENSITY OF 0.25 ASTR/M², 0.43 WITHIN-CELL STANDARD DEVIATION, AND DIFFERENT POST-TREATMENT DENSITIES, α_s , AND β_s .



APPENDIX FIGURE V. SAMPLE SIZES FOR ONE-FACTOR ANOVA WITH A PRE-TREATMENT DENSITY OF 1.5 ASTR/M², 0.43 WITHIN-CELL STANDARD DEVIATION, AND DIFFERENT POST-TREATMENT DENSITIES, α_s , AND β_s

APPENDIX VII

IMPORTANCE OF GENETIC DATA

Genetic data are increasingly used in making current management and conservation decisions (Hedrick 2001, Frankham 2003). For example, genetic data are used to delineate “distinct population segments” as provisioned for protection under the Endangered Species Act (ESA)(Waples 1992, Moritz 1994). Notably, genetic studies were an essential part of listing separate Pacific Northwest salmonid populations under ESA, a major part of the impetus for this study and FFR. Recently, genetic data were used to evaluate coastal giant salamander genetic population structure in managed landscapes in British Columbia (Curtis and Taylor 2003). While demographic data are used to assess the immediate health of a population, maintenance of genetic variability and avoidance of inbreeding are critical to ensure continued survival of a species (Schrader-Frechette and McCoy 1993, Frankham 2003). Moreover, maintenance of genetic variability was a fundamental part of the L-2 Schedule that addressed the basic questions within FFR.

Estimating genetic variation is important because it is genetic diversity that ensures the long-term ability of a species to respond to environmental change (Franklin 1980). Short-term persistence of a population can also be reduced due to fixation of deleterious alleles and inbreeding depression in cases where effective population size becomes bottlenecked (Leberg 1990, Lande 1994, Frankham 1995).

Reductions in genetic diversity (“cryptic bottlenecks”) can occur that may be undetectable with demographic studies (Luikart *et al.* 1998a). Census population size is almost always larger than breeding effective population size (Lande and Barrowclough 1987), particularly in species such as amphibians that often have high variance in family sizes or skewed sex ratios (Luikart *et al.* 1998a). Imagine a fragmentation event that results in a handful of breeding individuals in the following generation. Census population size may be not statistically reduced, but (genetic) effective population size will be substantially reduced. Cryptic bottlenecks or reductions in genetic effective population size can thus occur in absence of a demographic bottleneck. In addition, although numbers of individuals may remain high after a habitat fragmentation event, gene flow among populations may be restricted, consequently limiting the genetic effective population size.

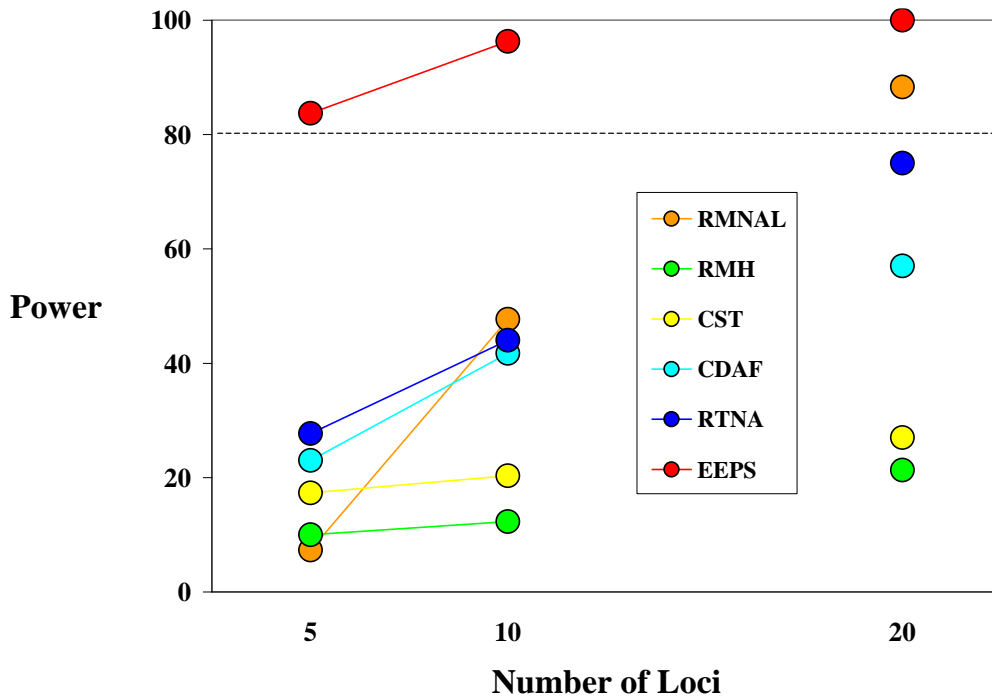
That amphibian populations fluctuate widely in numbers from one year to the next, and that long-term data are necessary to detect a decline is well known (Pechmann *et al.* 1991). Recent empirical and theoretical work suggests that, in the short term, genetic modeling may be more powerful than demographic modeling for detecting declines, particularly with a high number of genetic loci that increase statistical power (Hoyle *et al.* 1995; Luikart *et al.* 1998a; Garza and Williamson 2001).

Thus, genetic diversity estimates, in addition to demographic population size estimates, give a more rigorous basis for making predictions about short and long-term survival of species in response to land use change.

APPENDIX VIII

POWER ANALYSES ADDRESSING GENETIC DATA SAMPLE SIZES

Cornuet and Luikart (1996) and Luikart *et al.* (1998a) performed a series of analyses with different approaches to identify the levels of power (β) obtained with varying sample sizes of microsatellite loci. Mode of evolution of microsatellites is a basic assumption made in each set of analyses. APPENDIX FIGURE VI illustrates the power of six tests for detecting a bottleneck with the effective population size (N_e) of 10 when monitoring five, 10, or 20 microsatellite loci assuming a stepwise mutation model (SMM), and sampling 30 individuals both before and one generation after the bottleneck.

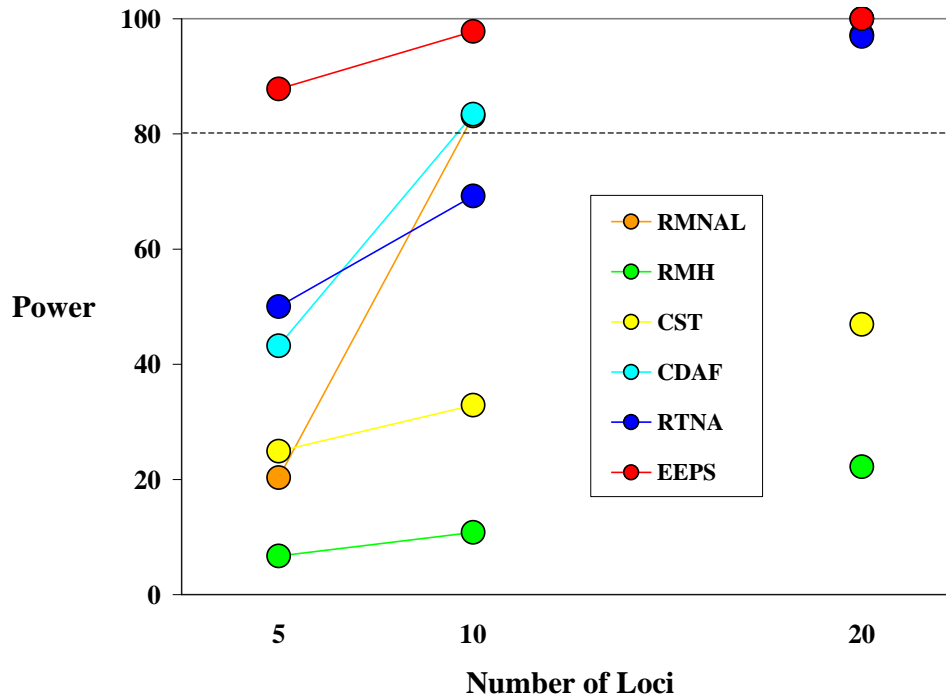


APPENDIX FIGURE VI – ESTIMATED POWER (β) TO DETECT GENETIC CHANGE USING DIFFERENT NUMBERS OF MICROSATELLITE LOCI WITH A STEPWISE MUTATION MODEL. This analysis assumes an effective population size (N_e) of 10. The six modes of analysis are: a Wilcoxon signed-rank test for **R**eduction of the **M**ean **N**umber of **A**lleles per **L**ocus (**RMNAL**), a Wilcoxon signed-rank test for **R**educed **M**ean **H**eterozygosity (**RMH**), **C**hi-**S**quare **T**est (**CST**), a Kolmogorov-Smirnov test for **C**hange in the **D**istribution of **A**llele **F**requencies (**CDAF**), a resampling test for **R**eduction of the **T**otal **N**umber of **A**lleles (**RTNA**), and a variance test for **E**stimating **E**ffective **P**opulation **S**ize (**EEPS**). Adapted from Luikart *et al.* (1998a).

The stepwise mutation model (SMM) was originally proposed because microsatellite loci evolve via slippage mutations and it is thought that they are much more likely to slip one base away (either by a deletion or an insertion) than by insertions or deletions that are larger. The SMM assumption is thought to be too restrictive, and represents the most

conservative among these analyses. A greater number of loci are needed to achieve high power if population sizes are reduced to numbers that are larger than 10 individuals (see APPENDIX TABLE III).

APPENDIX FIGURE VII illustrates the parallel analysis assuming an infinite allele model (IAM). The IAM, the first model used to generate estimates, is mathematically highly tractable, but is unrealistic because it assumes each mutation results in a new allele. Thus, IAM lacks restrictions and is too liberal. Comparison of the two extremes



APPENDIX FIGURE VII – ESTIMATED POWER (β) TO DETECT GENETIC CHANGE USING DIFFERENT NUMBERS OF MICROSATELLITE LOCI WITH AN INFINITE ALLELE MODEL. This analysis assumes an effective population size (N_e) of 10. Modes of analysis are: a Wilcoxon signed-rank test for **Reduction of the Mean Number of Alleles per Locus (RMNAL)**, a Wilcoxon signed-rank test for **Reduced Mean Heterozygosity (RMH)**, **Chi-Square Test (CST)**, a Kolmogorov-Smirnov test for **Change in the Distribution of Allele Frequencies (CDAF)**, a resampling test for **Reduction of the Total Number of Alleles (RTNA)**, and a variance test for **Estimating Effective Population Size (EEPS)**. Adapted from Luikart *et al.* (1998a).

represented by the IAM and SMM, respectively, provides an indication of what sample sizes of loci are necessary to achieve high power due to the fact that microsatellites evolution likely falls in between the two models (Di Rienzo *et al.* 1994). Both analyses indicate that the variance test for estimating effective population size is the test of choice and that a sample size of 20 loci ensures 100% power to detect a population bottleneck of 10 individuals under this test. Because the harvest conditions in this study are likely to result in larger effective population sizes, some reduction in power is anticipated.

APPENDIX TABLE III. THRESHOLD CRITICAL VALUES OF EFFECTIVE POPULATION SIZE THAT PROVIDE A 5% TYPE I ERROR RATE WHEN USING THE VARIANCE TEST TO DETECT GENETIC BOTTLENECKS VIA MONITORING (from Luikart *et al.* 1998a). For example, when sampling 60 individuals and 10 microsatellite loci, the variance test gives an effective population size estimate of 72 in 5% of simulations in which no bottleneck has occurred (Luikart *et al.* 1998a). In other words, with the recommended 30 individuals sampled before and after harvest and 20 microsatellite loci, there is a 95% chance of correctly identifying a bottleneck in effective population size of 45 or fewer.

Number of Loci Monitored	Number of Individuals Sampled (pre- and post-event)		
	15	30	60
Microsatellite SMM loci			
5	12	22	52
10	15	28	72
20	22	45	100
Allozyme IAM loci			
5	10	15	26
10	13	21	40
20	20	35	63

Thus, the sample size of 30 individuals and 15-20 microsatellite loci represents a good threshold point where power to detect changes in population size are relatively high, while minimizing the logistical difficulty of dealing with extremely large sample sizes. Sample sizes of 30 individuals accurately sample the extant genetic variation in a population, and the combination of 30 individuals and 15-20 loci maximizes power to detect changes in population structure in migration rates (Pritchard *et al.* 2000; Wilson and Rannala 2003).

APPENDIX IX

SENSITIVE SITE VARIATION

Forest practice rules address five types of sensitive sites (WFPB 2001). Two of these, headwater springs and TYPE N_p intersections, occur in all TYPE N basins 2nd-order or larger (APPENDIX TABLE IV). As variation in their occurrence is a function of basin complexity, they provide another reason for blocking on TYPE N basins of the same order (see BLOCKING section) as their variation within stream order groups is reduced. Part of site matching criteria for placing TYPE N_p basins in a block will be whether basins have similar numbers of 1st-order segments and tributaries. Both these sensitive site categories are part of the instream channel network and will be sampled with instream methods.

APPENDIX TABLE IV. SENSITIVE SITE CHARACTERISTICS

Sensitive Site	In All TYPE N Basins 2 nd -order or larger?	Basis of Variability
Alluvial Fan	No	Channel confinement patterns Unconsolidated bedload
Headwall Seep	No	Groundwater patterns Local geomorphology
Headwater Spring	Yes	Number of 1 st -order segments
Side-slope Seep	No	Groundwater patterns Local geomorphology
TYPE N _p Intersection	Yes	Number of tributaries

The remaining three sensitive site categories are variable in their occurrence in TYPE N basins based on several characteristics (APPENDIX TABLE IV). One of these, alluvial fan, is rare, so incorporating it into this study design is not feasible. The two types of seeps might be addressed if they are common enough, if their numbers are near parallel, and if their individual characteristics allow creating sufficiently similar groupings that one could perform systematic comparisons. Such a possibility is unlikely, but if seeps are found in some treatment units, a manipulative study addendum could address seeps.

An addendum addressing seeps would be less complex than treatments in the main study. Sensitive sites represent small landscape areas, so the only configuration options are varying buffer presence or its width. A simple manipulation could be done across paired seeps that would leave a buffer in one of the pair and completely remove it from the other; directional felling away from the seep similar to the equipment exclusion zone treatment in the main study would be used in the latter. Only sensitive sites outside the stream buffer would be used in such a manipulation. Additional seeps outside the main study treatment blocks with appropriate characteristics (based on site matching) could be included in another comparison. Problems exist with detecting the life stages of post-metamorphic life stages of amphibians when sampling seeps non-destructively, but LWAG has developed a repeatable non-destructive sampling method for seeps that could be applied to such a manipulation.

APPENDIX X

OTHER CONSIDERATIONS

Distribution of FFR lands: Western Washington has the greatest proportion of FFR lands, and in western Washington, the greatest proportion of FFR landscape is in the two coastal physiographic regions and the southern half of the west slope of the Cascades. The Blue Mountains of southeastern Washington have a relatively small proportion of FFR land, and most of the FFR landscape in the Blue Mountains and on the east slope of the Cascades lacks any FFR target species because the distribution of the only two FFR target taxa present there (coastal tailed frog on the east slope of the Cascades and Rocky mountain tailed frog in the Blue Mountains) is largely on federal, state, and tribal lands. Thus, this study has a westside focus in part because much of eastside FFR lands are unoccupied by FFR amphibians.

Blowdown: Blowdown is a common occurrence with buffers (e.g., Jackson *et al.* 2001 2003), especially narrow ones (Grizzell and Wolf 1998), and can be anticipated in treatments with any kind of buffer configuration. Because susceptibility to blowdown is site-specific, some ability to select sites for their similarity in blowdown susceptibility is possible, but blowdown will have to be measured as a co-variate and its amount and pattern will be characterized.

APPENDIX XI

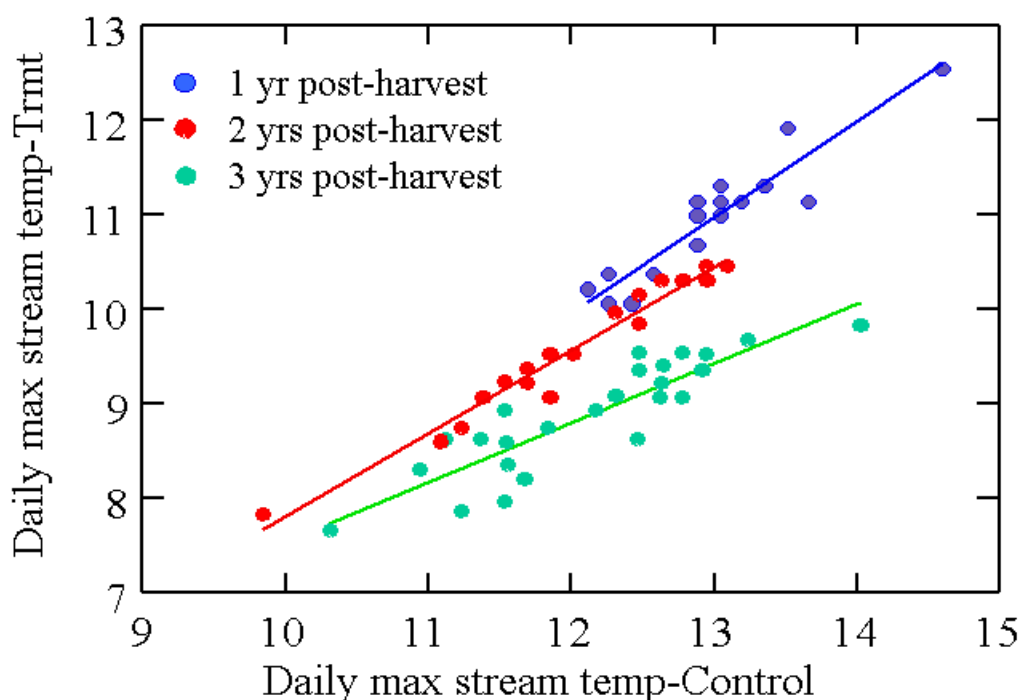
Power Analysis Addressing Temperature Measurement

A power analysis was performed using small stream data provided by Weyerhaeuser to estimate the minimum detectable change in temperature between years; APPENDIX FIGURE VIII illustrates this method. The linear model described in the text with sampling twice a week was used and the variance of the regression residuals was calculated for each of seven sites and three years each. The minimum detectable difference was calculated as:

$$\Delta T = \sqrt{\frac{2s^2(t_{1-\alpha/2} + t_{1-\beta})^2}{n}}$$

where ΔT = detectable change, s^2 = variance of residuals, n = sample size, and α and $\beta = 0.05$.

Estimates of ΔT ranged from 0.1 to 1.7 C ($n = 21$) with median and mean values of 0.3 and 0.4 C, respectively. Mean and median values are well within the range of expected change and are near the operational limits of the temperature monitors.



APPENDIX FIGURE VIII. CHANGES IN POST-HARVEST TEMPERATURE IN TREATMENT REACHES VS. REFERENCE REACHES USING A REGRESSION APPROACH.