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**Effectiveness of Riparian  
Management Zones in Providing  
Habitat for Wildlife: Resampling at  
Extended Post-treatment Interval**

**Revised Study Plan**

State of Washington Department of Natural Resources  
Olympia, Washington



**Contract No. PSC 02-097**

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## **Executive Summary**

This document provides information regarding LGL's proposed field and data analysis related to conducting a resampling study to evaluate the efficacy of riparian management zones in providing habitat for wildlife at post-treatment interval; a continuation of a study initiated in the early 1990's (O'Connell et al. (2000)). In this document we have outlined how data will be collected and analysed for each component. The executive summary provides a description of how LGL's methods deviate from those of O'Connell et al. (2000).

The study as designed is a split-plot repeated measures study with 36 sites, 18 in western Washington and 18 in eastern Washington. Within each half of the state are 18 sites comprised of 3 treatments: 6 unlogged controls; 6 logged sate buffers, and 6 logged modified buffers. The goal of this study is to compare abundance and diversity of select wildlife groups among and between sites and compare all data to the original study pre-harvest and post-harvest data. To facilitate this all field methods will be conducted such that data collection and study design mirrors that of O'Connell et a. (2000)/ Subsequent data analyses will also be conducted such that they are identical to O'Connell et al. (2000). To address some of the potential shortcomings of the initial data analyses we have proposed additional analyses that we believe are more appropriate for this kind of study. These analyses will be done in addition to the analyses done by O'Connell et al. (2000).

This study does vary in several aspects from the previous study:

1. We are not sampling for stream amphibians
2. We are not sampling for bats
3. We are not removing all captured terrestrial amphibians from the field. Rather, we are marking individuals and releasing them at the site of capture. Mark-recapture will enable us to collect abundance data for each species capture and it will enable population characterization by age-class, size structure, and number of individuals.
4. We are conducting additional analyses on the data set. This will be in addition to the analyses required to make direct comparisons with the original data sets.
5. In western Washington, 2 of the control sites require replacement due to recent logging at the sites which now precludes them from inclusion in the study. Data from the new sites will not be included in the repeated measures analyses but will be included in direct comparisons of all new data collected (LGL study only).

All other aspects of this study will mirror O'Connell et al. (2000) and comparative analyses will be conducted such that all data is directly comparable between data sets and years with the exception of the 2 new control sites.

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## **1.1 Work Plan**

### **1.2 Vegetation**

Vegetation will be sampled at each of the treatment and control sites in both western and eastern Washington for a total of 36 sites. To unify our data collection, our vegetation ecologist will collect vegetation data that are important for each species group. This will streamline vegetation data collection and will remove the requirement for several crews to sample the vegetation for each of the respective components of this project (birds, terrestrial amphibians, and small mammals).

Vegetation sampling will occur from late June to mid-July in the East and from mid July to mid August in the West. At each site the riparian and upland vegetation will be sampled for habitat features that are important to wildlife (snags, downed woody debris, canopy cover, etc.).

Vegetation data collection methods for the new RMZ work will be modeled on the original RMZ study (O'Connell et al. 2000).

#### **1.2.1 West Side**

Riparian and upland sampling will consist of the following at each site:

- 12 riparian quadrats; 6 on each side of the stream. Quadrats will be 10 X 8 m, with the long edge of each quadrat paralleling the stream. Riparian sites will be centered on bird point count stations and will be situated every 50 m starting at the mid-point between countpoints 1 and 2 and every 50 m to station 4. On the adjacent side of the stream, riparian vegetation samples will start at the mid-point between bird point count stations 6 and 7 and continue every 50 m to station 9 (Figure 1).
- 10 upland sites, 5 on each side of the stream. Upland sites will be approximately 100 m from the riparian sites and will be bounded by the bird point count stations. Quadrats will be situated in a similar manner to that described for the riparian zone; however 5 sites will be sampled rather than 6 (Figure 1).

#### **Ground cover measurements**

As with the original RMZ study, we will estimate the percent cover of herbaceous and woody vegetation, rock, litter, and bare soil at 1, 4, 7, and 10 m from the streamside edge of vegetation quadrats using 2 X 2 m and 1 X 1 m plots (Figure 2). Shrubs will be grouped into three categories:

1. berry-producing
2. evergreen
3. other deciduous

In each category, we will estimate the percent cover of shrubs > 1 m high, with the percent cover of taller shrubs being estimated in the larger quadrats.

In each 1 X 1 m plot we will measure the percent cover of herbs, ferns, moss, grass, *Lobaria* lichen, seedlings < 1 m tall, coarse woody debris >10 cm in diameter, litter, rock, and bare soil. Litter depth will be measured in millimeters at two positions from each 1 X 1 m plot (Figure 2).

**Tall shrubs**

Percent cover of tall shrubs (1 – 3 m) will be estimated in quadrats 2 and 3 at each site in the riparian and upland sampling areas. Again, shrubs will be grouped into the three categories identified above.

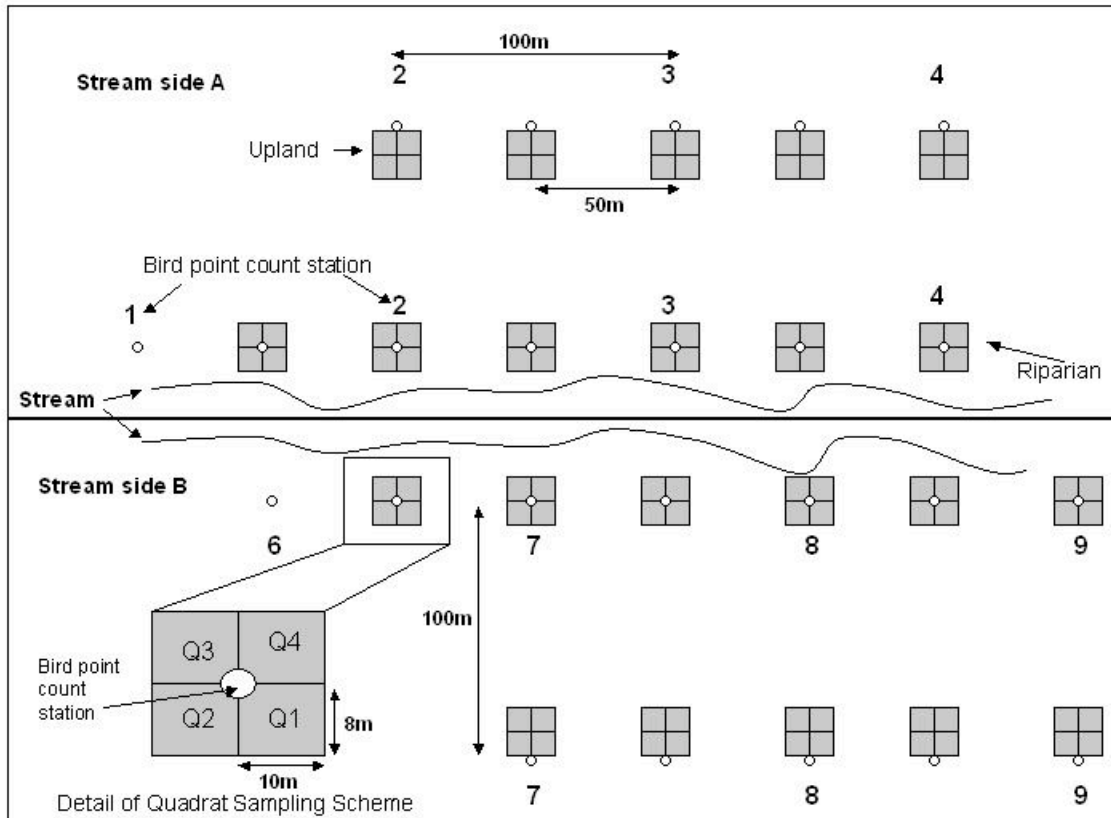


Figure 1. Riparian and upland vegetation sampling scheme using 10 X 8 m quadrats situated on each side of the stream at the 18 western and eastern study sites. Figure as shown is not to scale. Sampling is offset on each side of the stream.

**Down Wood**

Percentage of downed wood will be estimated from quadrats 2 and 3 at each survey site. Wood will be considered down if its angle of incidence with the ground is < 45°. Each piece of downed wood will be categorized by diameter (cm) (2 classes) and decay class (3 classes) (Table 1).

Table 1. Diameter and decay class categories to be used for downed wood measurements in quadrats 2 and 3.

Diameter Class (cm)		Decay Class	
1	10 – 30	1	Structurally sound wood with intact limbs
2	> 30	2	Reduced structural integrity and some limb loss
		3	Minimal structural integrity and presence of epiphytes

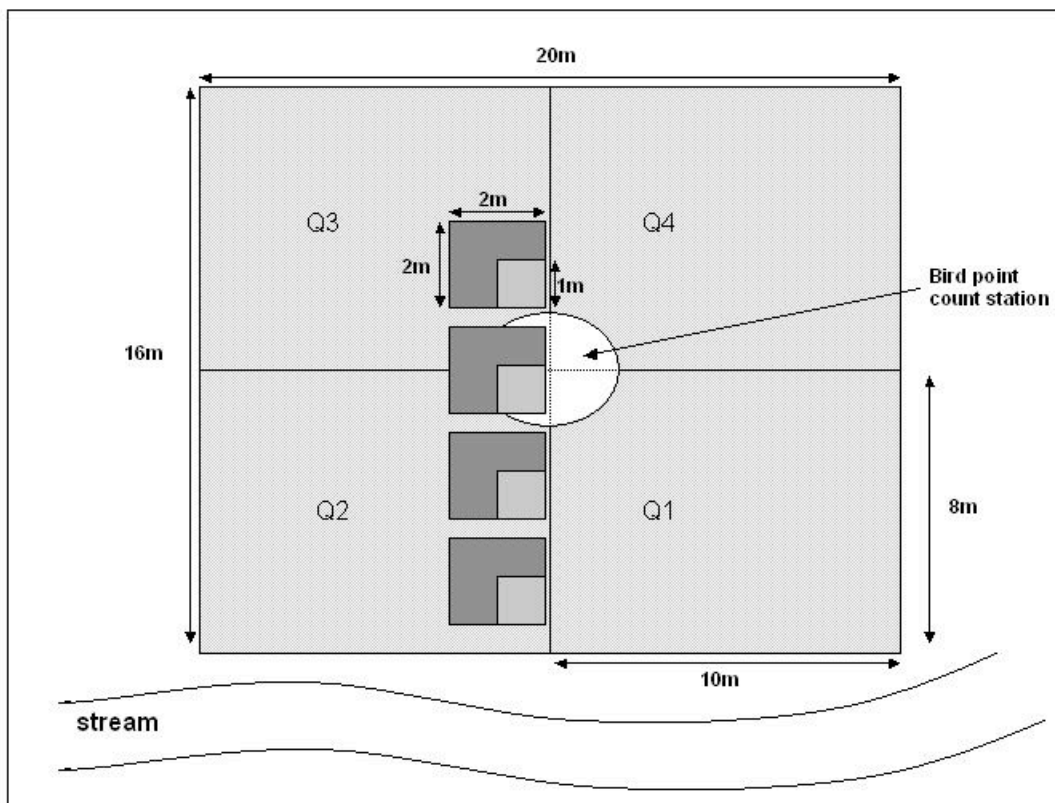


Figure 2. Vegetation sampling design (after O'Connell et al. 2000). Understory vegetation will be measured in 1 X 1 m and 2 X 2 m plots. Down wood, shrubs, snags, and trees will be measured in each 10 X 8 m quadrat.

### **Snags**

Snags will be counted in all four quadrats and grouped according to quadrat location. Snags will be counted as short (<1.5 m), medium (1.5 – 15 m) and tall (>15 m) in three diameter classes:

- Class 1 = all limbs attached and structurally sound
- Class 2 = losing limbs and showing reduces structural integrity
- Class 3 = about to fall down due to minimal structural integrity

### **Tree regeneration**

Percent cover of sapling trees will be estimated from quadrats 2 and 3. Saplings between 1 and 3 m in height will be included regardless if they originate from the ground, stump, or downed wood. Each sapling will be identified to species and we will obtain an estimate of percent cover for each species encountered in each quadrat. To be consistent with O'Connell et al. (2000) we will not include tree species in our analyses that are <1 m tall; however, to be thorough we will collect data on all tree species, regardless of height.

### **Tree counts**

Trees > 3 m in height will be counted in all four quadrats and grouped according to quadrat (1, 2, 3, or 4) and diameter size (10 cm, 10 – 50 cm, 50 – 100 cm, > 100 cm diameter at breast



height (DBH)). All trees with split boles will be counted as more than one tree, with the exception of vine maple, if the split occurs below breast height. Trees with more than half of the bole outside the quadrat will not be counted.

**Buffer width**

The buffer width will be measured from at least five locations on either side of the stream and will be measured from the normal high water mark to the outermost edge of forest. Slope distance rather than horizontal distance from the last tree to the normal high water mark will be measured.

**Canopy cover**

We will use a spherical, convex densiometer at the outer corners of each quadrat and at the center point where the four quadrats meet. At each of the five points, four readings will be taken and then averaged: 1) facing the stream, 2) away from the stream, 3) downstream, and 4) upstream (Figure 3).

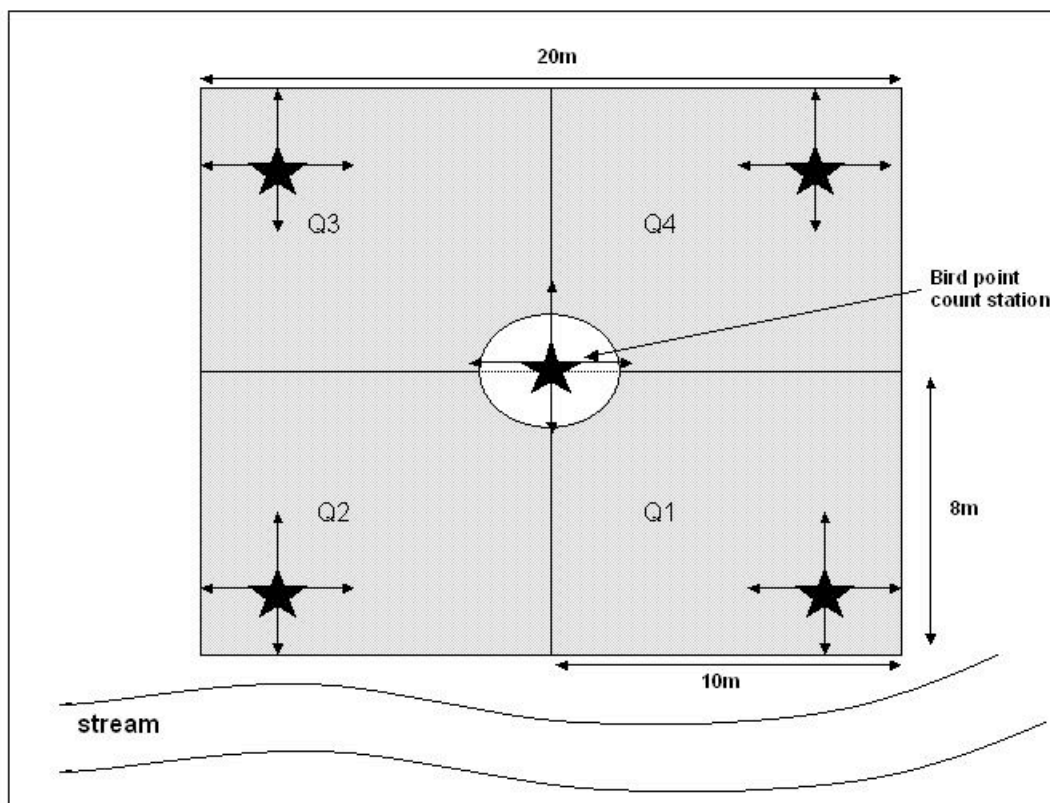


Figure 3. Location of canopy cover measurements using a spherical convex densiometer at each vegetation sample site. Four measurements will be collected from each of the five locations. Measurements at each site will be averaged.

**1.2.2 East Side Vegetation**

Vegetation sampling for the eastern sites will be similar to that of the western sites. However, to be consistent with the original RMZ study, the data collection parameters from East

to West will vary slightly. For example rather than 12 riparian and 10 upland sample areas, we will establish 15 riparian and 15 upland sample areas in each of the eastern Washington sites.

Other data collection that will differ from western WA includes:

- The measurement of the composition and dispersion of shrubs >0.5 m tall
- The distance to the nearest shrub in each of the 4 quadrats will be measured and the area (length X width) will be measured
- In two opposite quadrats, the number and decay class of woody debris and stumps will be recorded. Size classes used will be:
  1. > 5 m long and < 15 cm diameter (d)
  2. > 5 m long and 16 – 24 cm d
  3. > 5 m long and > 25 cm d
  4. < 5 m long and > 25 cm d
- Decay classes will be defined as:
  1. Freshly fallen tree with bark essentially intact, wood solid, no decomposition
  2. Bark beginning to slough or almost completely gone, decomposition beginning with sapwood partially softened but log generally firm
  3. Decomposition progresses to a point where wood is soft and breaks into blocks; each block with structural integrity
  4. Essentially no integrity to log, wood decomposed to soil-like texture

Stumps will be assigned as “natural” or “cut” and will also be assigned to one of the decay classes identified above. Stumps will be differentiated from snags by height, with stumps being < 1.37 m in height.

- Trees will be counted within each plot and identified to species and assigned to one of four DBH categories:
  1. 4 – 10 cm
  2. 11 – 25 cm
  3. 26 – 50 cm
  4. > 50 cm

All snags will be counted in each plot and assigned to one of two conditions:

- Condition 1 = bark basically intact
- Condition 2 = bark peeling off or absent
- Four average DBH trees and one average DBH snag will be randomly selected and their height estimated using a clinometer. Canopy cover (understory and overstory) will be estimated using a spherical convex densiometer at five locations at each sample area (Figure 4). Measurements will be averaged for each plot.
- Tree regeneration will be recorded from two opposite quadrats for coniferous trees only (>0.5 m high, < 4 cm dbh)
- Floristic diversity will be evaluated by establishing 30 m point intercept transects between each of the 15 sample plots for a total of 14 upland and 14 riparian transects per site (Figure 5).

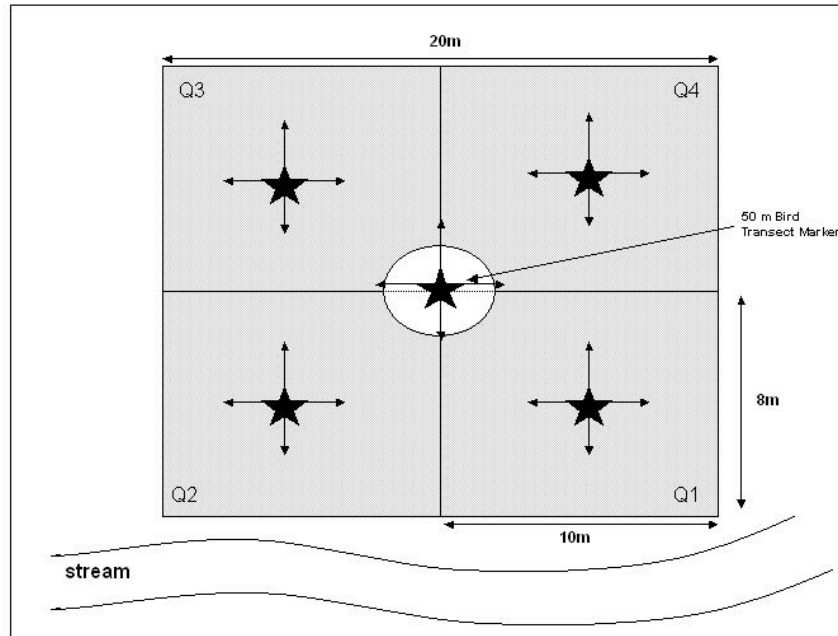


Figure 4. Location of canopy cover measurements using a spherical convex densiometer at each vegetation sample site.

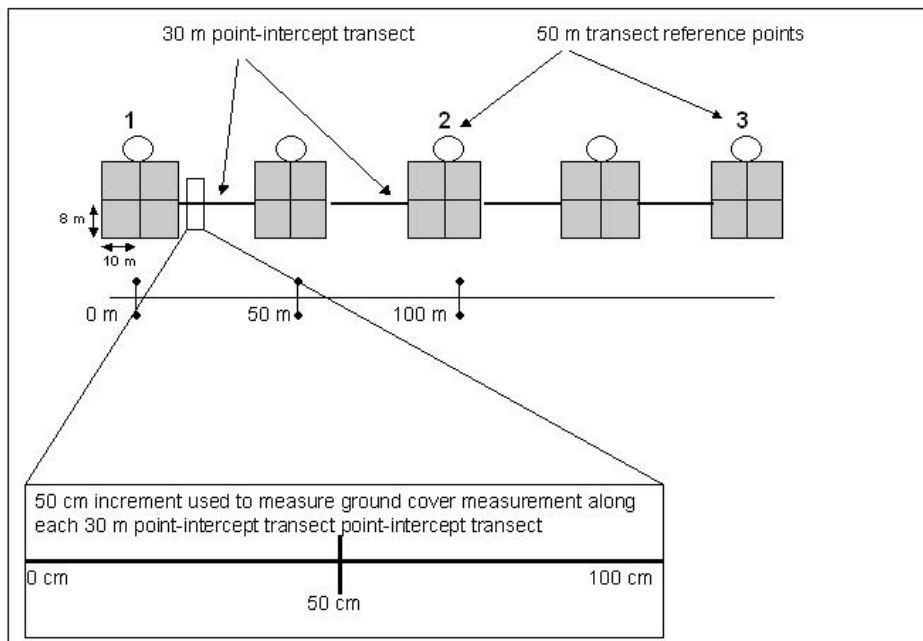


Figure 5. Location of point-intercept transects between each quadrat. Along each 30 m point-intercept transect, 50-cm increments will be delineated and ground cover vegetation measured.

Using a point-intercept rod (50-cm) lowered perpendicular to the ground; we will measure all vegetation, woody debris and substrate that come in contact with the rod. All vegetation, woody

debris and substrate will be categorized into the following height class: 15 m, 1.0 m, 0.5 m, 0.25 m, and 0 m. Herbaceous plants, shrubs, ferns, and trees will be recorded to species and grasses will be recorded as present or absent and not identified to species.

Table 2 defines the size and decay classes that will be assigned to logs and woody debris encountered in each point-intercept transect.

Table 2. Size and decay class categories for logs and woody debris to be use during point-intercept transects used to conduct vegetation surveys in eastern WA.

<b>Size Class</b>	<b>Decay Class</b>
<b>1</b> < 5 m long and <15 cm d	<b>1</b> Freshly fallen tree with bark essentially intact, wood solid, no decomposition
<b>2</b> < 5 m long and 16- 24 cm d	<b>2</b> Bark beginning to slough or almost completely gone, decomposition beginning with sapwood partially softened but log generally firm
<b>3</b> > 5 m long and < 15 cm d	<b>3</b> Decomposition progresses to a point where wood is soft and breaks into blocks; each block with structural integrity.
<b>4</b> > 5 m long and > 16- 24 cm d	<b>4</b> Essentially no integrity to log, wood decomposed to soil-like texture
<b>5</b> > 5 cm long and > 25 cm d	
<b>6</b> < 5 m long and > 25 cm d	

- Stumps will be delineated as natural or cut and only if they are less than 1.37 m in height, otherwise they will be included in the snag category.
- Litter depth will be measured every 5 m on each point-intercept transect for a total of seven measurements per transect.
- At each plot slope will be determined using a clinometer and aspect of the slope will be measured with a compass.
- The buffer width of each site will be measured as the perpendicular distance from the stream to the edge of the riparian harvest unit. The buffer measurements will be taken from 17 points at 50 m intervals at each site on each side of the stream.

### **1.2.3 Personnel**

Sergei Yazvenko, Ph.D., will supervise the design, logistic planning, and data analysis for the vegetation sampling portion of the study. Dr. Yazvenko is a professional botanist with experience and competency in identification and classification of plants and plant communities, as well as with forest stand attributes. Dr. Yazvenko has conducted a number of vegetation and habitat studies throughout the Northern Hemisphere during the course of his career. Prior to initiating major inventory surveys, a short workshop with field crews will be conducted to clarify field methods and field identification and to update personnel on survey procedures and requirements.

### **1.3 Stream Classification**

Physical stream classification measurements will be collected at each 400 m stream reach to be sampled and measurements will be taken at 100 m intervals to correspond to the riparian bird point count stations and to the original RMZ study. We will collect this information from 5 sites at each stream for each of the 36 sites (18 West and 18 East). The information collected will include:

1. **Channel width or bankfull width ( $W_b$ )**. This is the width of the bankfull flood stage of the stream channel. We will use the following criteria to determine the  $W_b$ :
  - A change in vegetation (>2 years old) from bare ground, with no trees, to vegetated ground with trees, from moss to no moss covered ground, or from bare ground to grass-covered ground;
  - A topographic break from vertical to flat floodplain;
  - A topographic break from steep bank to more gentle slope;
  - The highest elevation below which no fine woody debris (needles, leaves, cones, or seeds) occurs; and,
  - A change in texture of deposited sediment (e.g., from clay to sand, sand to pebbles, or boulders to pebbles).
2. **Channel width** measurements will include:
  - All unvegetated gravel bars will be included in the measurement.
  - If multiple channels are present that are separated by more than one vegetated island, the width will be the sum of all the separate channels.
3. **Wetted width**. This is the width of the wetted portion of the stream.
4. **Residual pool depth**. This is the difference between the maximum pool depth and the riffle crest depth (or pool outlet depth). Water depths will be measured to the horizontal plane of the ordinary high water width at three points: \_\_, \_\_, and \_\_ of the distance between shorelines. Average depth will then be calculated by dividing the sum of these three measurements by four to account for the starting point at the bank where the water surface and the bank meet.
5. **Gradient**. Using a clinometer, we will measure gradient along the longest sighting within the site. Preferably, the sighting will be at least 60 m long; however, due to the constraints associated with thick riparian vegetation, we will attempt to use a minimum sighting of 30 m. If visibility is limited (i.e. < 30 m) we will sight both upstream and downstream to maximize the length of stream used to calculate gradient. Right and left bank gradient will also be recorded.
6. **Stage**. This is the stage of current discharge and is defined as the amount of water passing through the channel at the time of survey. Water height will be visually estimated as a percentage of  $W_b$  at the same site where  $W_b$  was determined. Stage will be classified as follows:
  - Low flow: 0 –30% of  $W_b$  depth
  - Medium flow: 30 – 90% of  $W_b$  depth
  - High flow: >90% of  $W_b$  depth

Flow will be assessed using the following criteria:

**Low flow:** indicators such as riffles and pools or steps and pools, high bank tops, wetted width significantly smaller than channel width, dry, unvegetated gravel bars, and/or debris jams well above water surface.

**High Flow:** indicators such as difficulty distinguishing between riffles and pools or steps and pools, water level at or above bank tops, wetted width similar to or greater than channel width, no bars or bank sides visible, debris movement in channel, submerged riparian vegetation, and/or flood channels full of water.

7. **Stream cover.** Cover is any structure in the wetted channel or within 1 m above the water surface that provides hiding, resting, or feeding places for stream amphibians. Stream cover estimates are visual assessments of the type and amount of in-channel cover available to amphibians (Table 3).

Table 3. Stream cover descriptions.

Type	Description
Small organic debris (SOD)	Woody debris smaller than LOD
Large organic debris (LOD)	LOD is any large piece of relatively stable woody material having a minimum diameter > 20 cm. Root wads are included.
Boulder (B)	Stream substrate particles > 25.6 cm diameter that block stream flow and provide surface turbulence, shade, and escape from higher velocity and predation.
Undercut (U)	Cover that consists of stream bank that has had its base cut away by the water or has been man-made and overhangs part of the stream.
Deep pool (DP)	A portion of the stream with reduced current velocity at low flow, deeper than the surrounding area.
Overstream vegetation (OV)	Vegetation that projects over the stream that is <1 m above the water surface.
Instream vegetation (IV)	Vegetative materials such as attached, filamentous algae or other aquatic plants.

As visual estimates can be subjective, each stream cover type will be ranked as follows:

- None = no cover of this type exists at the site
- Trace = a small amount of cover (3 – 5%)
- Moderate = cover of 5 – 20% of site
- Abundant = more than 20% of site is considered covered.

8. **Crown closure.** The stream-side riparian vegetation that projects over the stream channel and that is higher than 1 m above the water surface. The percentage of channel area covered by crown closure will be visually assessed.

9. **Large organic debris (LOD).** Visual estimates of the percentage of LWD within the channel (bankfull width) will be recorded. All LOD that is >10 cm diameter that crosses each transect or occurs within 5 m upstream or downstream of each transect will be measured (length and diameter). The following items will be assessed as LWD:

- Rootwads embedded in the stream or bank; and
- Large pieces of LOD that create pools or scour.

The abundance of LOD will be assessed as follows:

- None

- Few = fewer than one piece per  $W_b$ , or
- Abundant = more than one piece of LOD per  $W_b$ .

Location of LOD will be recorded as:

- 1) all of the piece occurs within the high water width that would be covered by water during high flow,
  - 2) > 50% of length occurs within stream channel,
  - 3) < 50% of the length occurs within stream channel, and
  - 4) the piece does not enter the channel but is suspended above the channel.
10. **Rock embeddedness.** Embeddedness of a rock within the streambed indirectly measures the amount of fine sediment in the stream channel. We will examine a minimum of five rocks in riffles and estimate the average percent of the rocks vertical dimension buried in the bottom substrate. Rocks sitting on top of the substrate will be relatively free from embeddedness and will be recorded as 15%. If 5.25% of the vertical dimension is below the substrate surface it will be recorded as 25%. Likewise, 25-50%, 50-75%, and 75-100% embeddedness will be recorded as 50%, 75%, and 100%, respectively.
11. **Instream habitat.** To measure the amount of habitat in pools, we will estimate the percentage of pool habitat that intersects each transect.
12. **Water temperature.** Temperature will be measured at each transect and will represent average temperatures for those streams for the season in which they are sampled (likely summer).
13. **Bank stability.** Stability will be assessed using the classes developed in O’Connell et al. (2000) (Table 4).

Table 4. Bank stability classification scheme.

Class	Vegetation and Rock Cover (%)
1	0 – 25
2	25 – 50
3	50 – 75
4	75 - 100

Estimates will be made considering vegetation and rock cover from both sides of the stream. A higher classification ranking will indicate a more stable slope or bank and this classification scheme will also be applied in circumstances where a stream bank is actually a valley side.

14. **Soil alteration rating.** To evaluate the amount of stream bank area previously altered by stream processes we will apply the soil alteration rating described in O’Connell et al. (2000) (Table 5).

Table 5. Soil alteration classification scheme.

Rating	% of bank altered / eroded
4	0 – 25
3	25 – 50
2	50 – 75
1	75 - 100

15. **Buffer width.** The buffer width at each site will be measured on both sides of the stream from the high ordinary high water mark to the line of trees at the outer edge of the buffer. Note that this parameter is measured by the vegetation sampling component (see 0)

16. **Shape.** Shape refers to the shape or form of the identified channel bank using the following definitions:
- **V** = v-shaped (steep sloping or vertical)
  - **S** = sloping (gradual or shallow slope)
  - **O** = overhanging bank
  - **U** = undercut (similar to overhanging, but undercut has water or wetted channel underneath the overhanging portion of the bank)
17. **Bed Material.** The dominant and subdominant groups of materials will be estimated visually and will be categorized as per Table 6.
18. **Trapsite sampling.** At trapping locations, 10 m lengths of stream will be flagged at 1 m intervals. At each 1 m interval, the following microhabitat features will be measured:
- Estimate of percent pool habitat
  - Dominate substrate class
  - Width of stream and depth at \_\_, \_\_, and \_\_ of the width of the stream (i.e. wetted width) at time of sampling
  - A map of the 10 m length of stream being sampled will be produced that includes locations of pools, riffles, boulders, undercut banks, LOD, and other prominent habitat features

All microhabitat assessments will be done without disturbing the site. A Plexiglas frame may be required for viewing the substrate in deep pools or fast flowing water.

Table 6. Bed material (substrate) size classification.

<b>Class</b>	<b>Size</b>	<b>Description*</b>
Fines (F)	< 2 mm	Smaller than ladybug size
Gravels (G)	2 – 64 mm	Ladybug to tennis ball size
Cobbles (C)	64 – 256 mm	Tennis ball to basketball size
Boulders (B)	> 256 mm	Larger than a basketball
Rock (R)	> 4000 m	Includes boulders and blocks larger than 4 m, and bedrock



## **1.4 Birds**

Birds play critical roles in the functioning of forested ecosystems in the Pacific Northwest. Many of those same forests sustain local and regional economies through their yield of high-quality timber. The loss and alteration of wildlife habitat present serious concerns to wildlife conservation and the healthy functioning of ecosystems. Because industrial forestry operates on such a large scale and can alter the landscape considerably, it has considerable potential to adversely affect bird populations. Because of the potentially competing interests of forestry and wildlife conservation, considerable efforts have been expended to integrate the habitat needs of forest-dwelling birds (and other organisms) with the economic opportunities offered by the forests they occupy.

Resource managers faced with conserving wildlife in managed forests face three broad issues (Bunnell et al. 1999): (1) establishing goals, (2) developing tactics, and (3) monitoring success. This research program is designed to fulfil the needs of the third issue by furnishing accurate information on the success of conservation tactics and identifying any corrective actions necessary in a timely manner.

O'Connell et al. (2000) present the results of springtime breeding bird surveys before and shortly after the various forest harvesting regimes (treatments) were applied to the landscape in two study areas in Washington State. We propose to continue monitoring the effect of riparian buffer width on habitat suitability for breeding birds in those same two areas: western Washington and eastern Washington.

This component of the study will have two primary objectives: 1) to determine the species that are associated with riparian habitats in two study areas in Washington; and 2) to assess the effect of riparian buffer width on the breeding bird community as ascertained through several measures. To accomplish these objectives we will compare the breeding bird community in riparian and upland habitats before and after harvest. We will examine the riparian breeding bird community in unharvested stands (Controls) with stands where the upland had been clearcut leaving either a wide (Modified) or narrow (State) unharvested buffer along the stream. Our analysis will incorporate the effects of not only the various treatments, but time and location as well.

### **1.4.1 Methods**

There are three basic methods for estimating songbird numbers: encounter transects, point counts and spot mapping. Spot mapping is the most labour intensive and is only appropriate for relatively small areas when absolute abundance data are required. As such, it is not the optimal method for the present study. Encounter transects and point counts can cover much greater areas with far less effort. Encounter transects should theoretically sample the greatest number of birds, but point counts are more conducive to standardization. This is because variation in the speed travelled between any two transects, or even within transects, can contribute an undetectable bias; whereas with point counts, the effort spent actually counting birds can be precisely controlled by standardizing the duration of the counts. In addition, point counts are a more efficient method for obtaining large sample sizes than either encounter transects or spot mapping. For these reasons, point counts are usually recommended for estimating relative bird abundance, both for long term trends and for comparing abundances between habitats. For western Washington sites we will conduct song-bird point counts and for eastern Washington sites we will use encounter transects to develop a species list. These methods are modeled on O'Connell et al. (2000).

### **1.4.2 Pre-survey Work**

- Delineate the East and West Study Area boundaries on 1:250000 scale maps to indicate the area to which the results of each study apply.
- For each study area, locations of sampling sites (Controls, State, Modified) will be identified using information in O'Connell et al. (2000) and in consultation with the proponent. We anticipate that 18 sample sites (comprising control and treatment areas) will be identified in each of the East and West study areas.
- Compile a list which includes all potential terrestrial birds for the East and West study areas.
- Ensure that the bird identification skills of all observers are current. Among other field equipment, each observer will possess a current field guide, list of potential species, and high-quality binoculars.
- Ensure that observers are competent at estimating distances in representative habitats by establishing a series of standardized distances from an observation point and running trials to familiarize observers with the visual nature of such distances.
- Riparian sampling sites (Control, State and Modified) will be delineated as stretches of riparian habitat, within which, either point-counting stations spaced at 100 m will be established (West) or 30-m-wide by 800-m-long transects will be laid out (East). In the West we will conduct 10 countpoints within each riparian strip and will locate them on both sides of the watercourse (Figure 6). Countpoint locations will maximize the area of riparian habitat within a 15-m radius. For transects, the transect line will parallel the stream bank at a distance of 8 m and include that 8 m swath as well as 22 m upland from the transect (Figure 7). Transects will be broken down (flagged) into 50 m intervals for reference and to assist with survey pace.
- Upland sampling sites will be delineated as stretches of habitat that run upslope of and parallel to the riparian zone. Ten countpoints spaced at 100 m will be identified along each upland stretch in the west, and a 30-m-wide by 800 m long transect (with flagged 50-m intervals) will be established at sites in the east.
- All countpoints and transects will be marked with bright flagging tape to indicate: (1) the location of the countpoint/transect, and (2) key distance intervals to assist observers in determining the distance to birds.
- In addition, a GPS position for all countpoints and for the start, 50 m intervals, and endpoints of transects will be taken to facilitate returning to them within and among years.

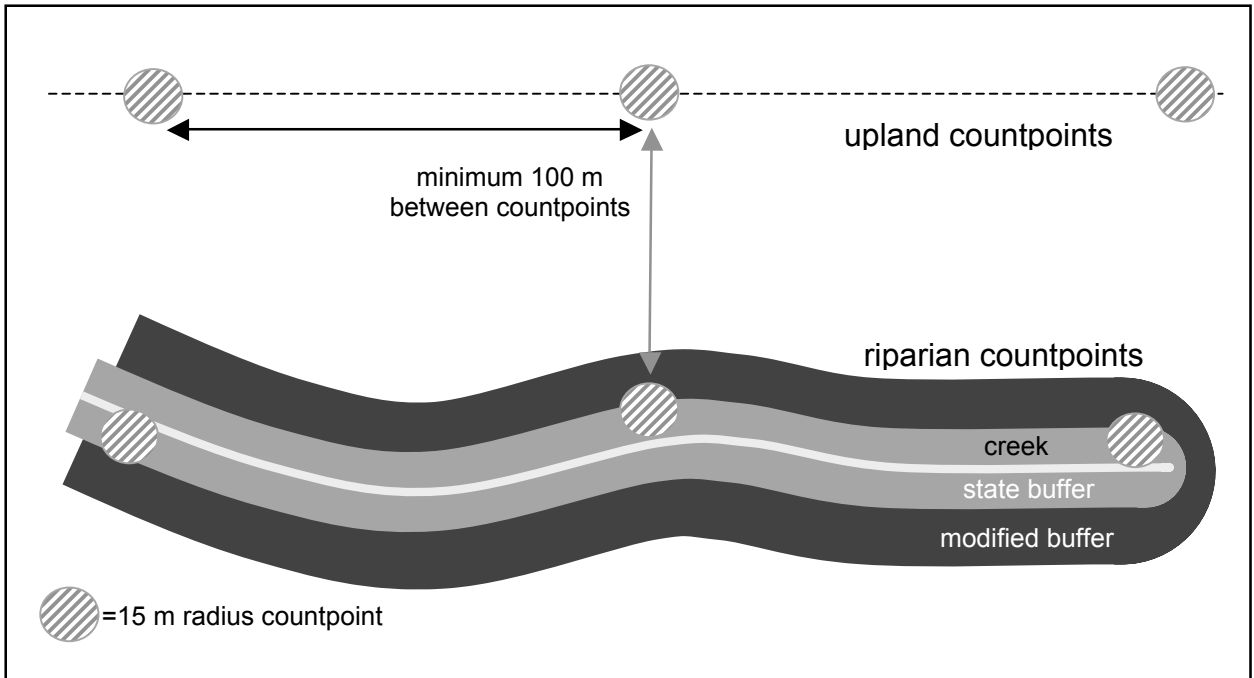


Figure 6. Conceptualized version of a bird countpoint layout for a portion of a sampling site in the western study area. Key countpoint sample areas are hatched. Note that sites will have either no buffer (control), State buffer, or Modified buffer. Upland countpoints will be in either forested (control) or harvested (treatment) habitats.

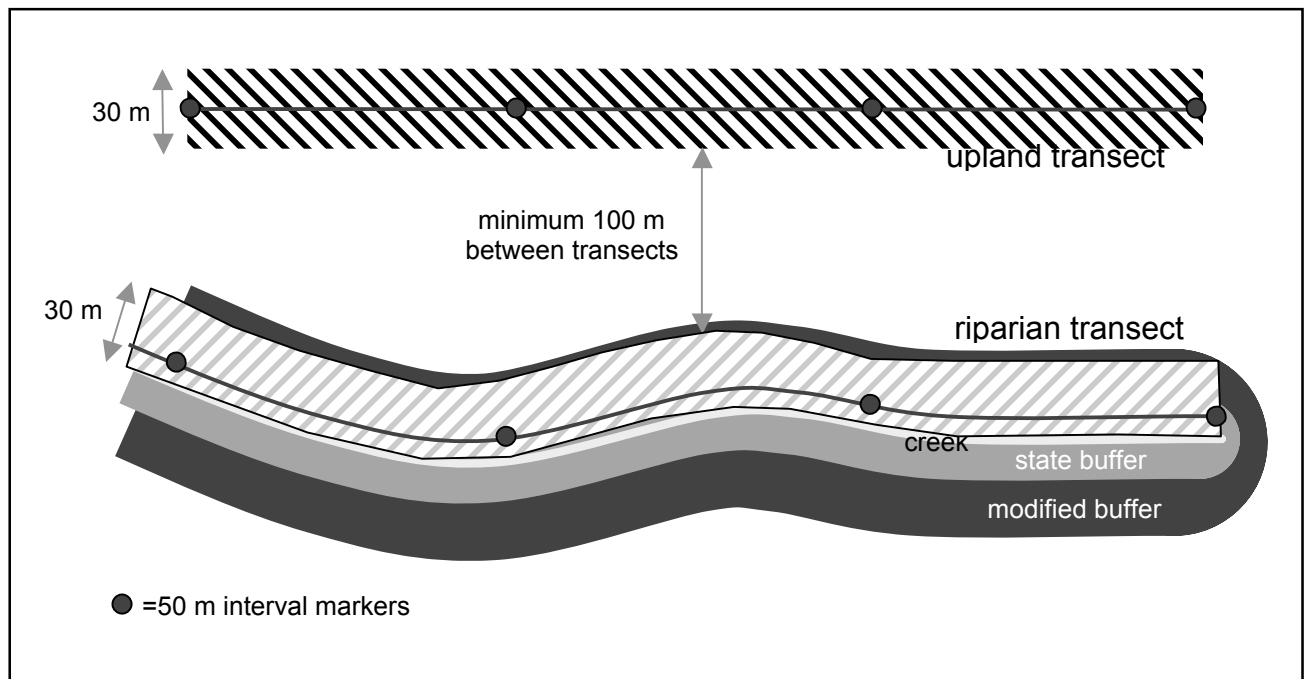


Figure 7. Conceptualized version of a bird sampling transect layout for a portion of a sampling site in the eastern study area. Surveyed areas are hatched. Note that sites will have either no

buffer (control), State buffer, or Modified buffer. Upland countpoints will be in either forested (control) or harvested (treatment) habitats.

### **1.4.3 Bird Surveys**

- A sampling schedule will be developed and followed. Each sampling site and its associated countpoints (West)/transect (East) will be visited a minimum of 6 times. The starting order of sampling sites will be rotated and the sampling period will be spread out to ensure that each site is sampled near the beginning, middle and end of the survey period. Observers will be rotated among sites to homogenize observer-caused biases.
- Survey data will be collected by two observers in each of the East and West study areas as per the forms presented in Appendix A.
- As recommended by Huff et al. (2000) for Washington State, surveys will span the period of mid May through June. This timing window maximizes the probability of detecting breeding adults while minimizing the probability that migrants are still passing through the area.
- Surveys will commence 15 minutes before sunrise (about 04:45 a.m. in the East and 05:00 a.m. in the west) and end around 10:00 a.m.
- Surveys will not be conducted during periods of rain, cold drizzle, sleet, snow, heavy ground fog or wind >32 km/h.
- After arriving at a countpoint, 1 minute will pass before counting begins to allow bird activity to settle down if there was any disturbance due to the arriving observer. Following that, we will begin counting birds for a period of 6 minutes that will be divided into an initial 3-minute period (to allow for comparison with other state-wide breeding bird surveys that use 3-minute point counts), followed by a 2-minute interval (to allow for comparisons with surveys that use a standard 5-minute period), and a final 1-minute period (to allow for direct comparisons with data collected by O'Connell et al 2000). Transects will be sampled at an observer movement rate of 50 m per 5 min according to the original study in the East (O'Connell et al. 2000).
- In the west, observers will focus on birds within 15 m of the countpoint (to keep consistent with past surveys and to optimize bird detections in steep terrain subject to stream noise). Birds seen or heard (i.e., detected) outside the 15-m radius during the observation period will be tallied separately. In the east, observers will focus on all birds within the 30 m transect, but will separately record birds seen or heard outside the transect as well.
- Detections will be noted as primary, juvenile, and supplemental. Primary detections comprise adult birds, juveniles are young of the year, and supplemental are detections that do not conform to the main survey protocol. For example, birds flushed while moving between countpoints (either within or outside the countpoint radius).
- For riparian countpoints and transects, the location of each bird will also be noted (i.e., within riparian buffer/outside riparian buffer). For transects (riparian and upland), birds outside the buffer will be identified as streamside or upland. Flyovers (i.e., birds seen or heard flying over the tree canopy at a countpoint/transect but not directly associated with the terrestrial environment at that site) will also be identified as such.
- Observers will be familiar with the status of birds in the study areas, and special attention will be paid to sightings of State Listed (currently 7 species) and State Candidate Species (currently 23 species). Note: not all of those species occur in habitats that characterize either of the study areas.

- As the effect of riparian buffer width on the vulnerability of bird nests to depredation was not an original study objective, we have not proposed to study nesting success either by monitoring natural nests (it is practically impossible to obtain adequate sample sizes from an adequate number of species in each of the sample areas to make meaningful conclusions about habitat-based predation rates) or by monitoring depredation of eggs in artificial nests (a method that we feel is of limited inferential value). However, we will document observations of nesting birds and fledged young.

#### **1.4.4 Pre-Analysis Data Handling**

All data will be analysed as per O'Connell et al. (2000). Specifically, we will perform the following tests and analyses:

- Species richness;
- Species turnover;
- Extinction probability;
- Index of abundance t-test;
- Repeated measures ANOVA;
- Tukey HSD;
- Stepwise multiple regression

All data will be entered into a customized database (MS Access compatible) on a Palm Pilot type field computer and downloaded daily throughout the study. We will obtain all raw digital data as collected by O'Connell et al. (2000) and subject that data to our analyses.

The statistical approaches to data analysis are detailed in Section 1.7 but before those analyses can proceed, the data must be prepared by either transforming them (e.g., to meet assumptions of the statistical tests) or by calculating ecological indices that integrate factors such as number of species and numbers of individuals. The data that will be collected are appropriate for addressing a number of research hypotheses that range from questions about individual species to questions about ecological communities. We propose to examine the more abundant and high-profile species individually. For example, we can compare the number of Purple Martins detected in the different sites and infer the effects of riparian management prescriptions on the species. We will also compare the similarity of ecological communities (treatments) using Morisita's Index of Similarity (Krebs 1999). Wolda (1981) indicated that this was the best ecological measure of similarity for ecological use. In addition to examining the entire ecological communities, we will examine different guilds among sites using Morisita's Index. Such guilds can be based on a number of different ecological criteria, but perhaps most useful will be the general location of nesting (e.g., ground nesters, shrub nesters, external tree nesters, cavity nesters) within groups of resident, short distance migrants, and neotropical migrants (as classified by O'Connell et al. 2000). By calculating Morisita's Index for pairs of habitats, we will be able to identify the degree of similarity between habitats as displayed by specific attributes of the avian community (i.e., entire community and specific guilds).

O'Connell et al. (2000) did not calculate Morisita's Index of Similarity, but rather, for the East side they used the Shannon-Wiener Index of ecological diversity. We will also calculate the Shannon-Wiener Index

O'Connell et al. (2000) also reported extinction probabilities in the West, but their methods section did not describe in sufficient detail, their approach to calculating such values. We do not feel that it is appropriate to speak in terms of extinction probabilities at the scale at which the present study is occurring. While it is meaningful to examine how the bird community changes in the different treatments over time, the long-term nature of this study will allow the collection of empirical data that will show any changes without having to resort to vague and likely

misleading statistics like “extinction probabilities”. However, should someone wish to conduct such analyses for reasons that we are unaware of, the standardized way in which we will collect data will permit them to.

### **1.4.5 Personnel**

Mike Demarchi, M.Sc., will supervise the design, logistic planning, and data analysis for the bird surveys. Mr. Demarchi conducted his Master’s research on forest-wildlife ecology and has conducted a number of bird studies during the course of his career. Prior to initiating major inventory surveys, a short workshop with all field crews will be conducted to clarify field methods and field identification and to update personnel on survey procedures and requirements. Fieldwork will be led by Dr. Martin McNicholl. Dr. McNicholl is a professional ornithologist with considerable experience and competency in identification of birds.

### **1.4.6 Permits and Animal Welfare Protocol**

During our field investigations, we will abide by the document: Guidelines to the use of Wild Birds in Research (Gaunt and Oring 1999). The possession, capture, handling, collecting, marking, or disturbing of native wild birds, their nests, or their eggs requires some kind of special license or permit. However, because we will only be passively observing birds, there are no special considerations to follow while conducting this research, and no permits are expected to be required. As a formality and courtesy, we will inform both state and federal wildlife officials as to the nature and locations of our field activities.

## **1.5 Terrestrial Amphibians**

Only in recent years have non-game species of wildlife, including amphibians and reptiles, come to be widely recognized as important elements in the fauna of the Pacific Northwest. Consequently, little is known about the effects of habitat-altering activities such as logging on the populations of these species. As with other states and provinces in the Pacific Northwest, state and provincial governments have attempted to design riparian management zones (RMZ) that will continue to provide habitat that fulfills the life requisites of stream and terrestrial amphibians. The Washington State Department of Natural Resources has taken the implementation and design of RMZ further by implementing and testing the suitability and capability of two different RMZ treatments (current regulations and modified buffer width) when compared to an untreated control.

This component of the proposed RMZ research will evaluate the diversity and richness of terrestrial amphibians between the two treatments and the control. The results will be used to determine if the current or modified RMZ treatments continue to provide suitable habitat for stream and terrestrial amphibians, and if not, to determine statistically what habitat variables have been modified such that the riparian area no longer provides suitable habitat. Comparisons of community similarity will be made between the riparian and upland habitats of 18 sites in western Washington and 18 sites in eastern Washington.

Most terrestrial amphibians are fairly adaptable to changes in their immediate habitat provided that the changes do not result in a net loss of habitat within an individual's home range. An indication of treatment effects on a population of terrestrial amphibians will be evident by a net reduction in density in the absence of other compounding factors (e.g., increased predation or disease). However, if common species are not encountered at either of the treatments, or the density of common species such as *Hyla regilla* and *Ensatina eschscholtzii* is lower than densities observed during the original RMZ study, then there may be reason to infer treatment effects on a particular population of amphibians.

Terrestrial amphibian surveys will be conducted following the same protocol for both the western and eastern portions of Washington State. Methods used will be modeled on the previous RMZ study and on methods described in Bury and Corn (1991) and will consist of the following:

1. Surveys will be conducted during fall at the onset of the rainy season, most likely during late September and throughout October.

A modified pitfall sampling grid will be used to sample for terrestrial amphibians in both the riparian and upland sites. The grid will consist of 36 pitfall traps running parallel to the stream. Eighteen Riparian traps will be situated approximately 5 m from the stream's ordinary high water mark, with the 18 upland traps situated approximately 100 m from the riparian grid. The riparian grid will follow the same transect as small mammal snap traps and the upland transect will follow the bird count stations and small mammal snap trap locations. The pitfall traps will be placed every 15 m centered along the same route as the small mammal transects. (Figure 8).

2. Traps will be constructed from 2 No. 10 cans. The bottom of one can will be cut out to make a tube. This can will be placed over the opening of the 2<sup>nd</sup> can and the two will be joined with duct tape.
3. Traps will be filled with approximately 2.5 cm of water so that any captured amphibians do not dry out.
4. Traps will remain open for approximately 4 weeks in both the western and eastern parts of the study area and will be checked at least every 4-5 days.

5. All live animals will be identified, measured and weighed. Measurements to be taken from all amphibians include:

- Total Length (cm)
- Snout-vent Length (cm)
- Weight (grams)

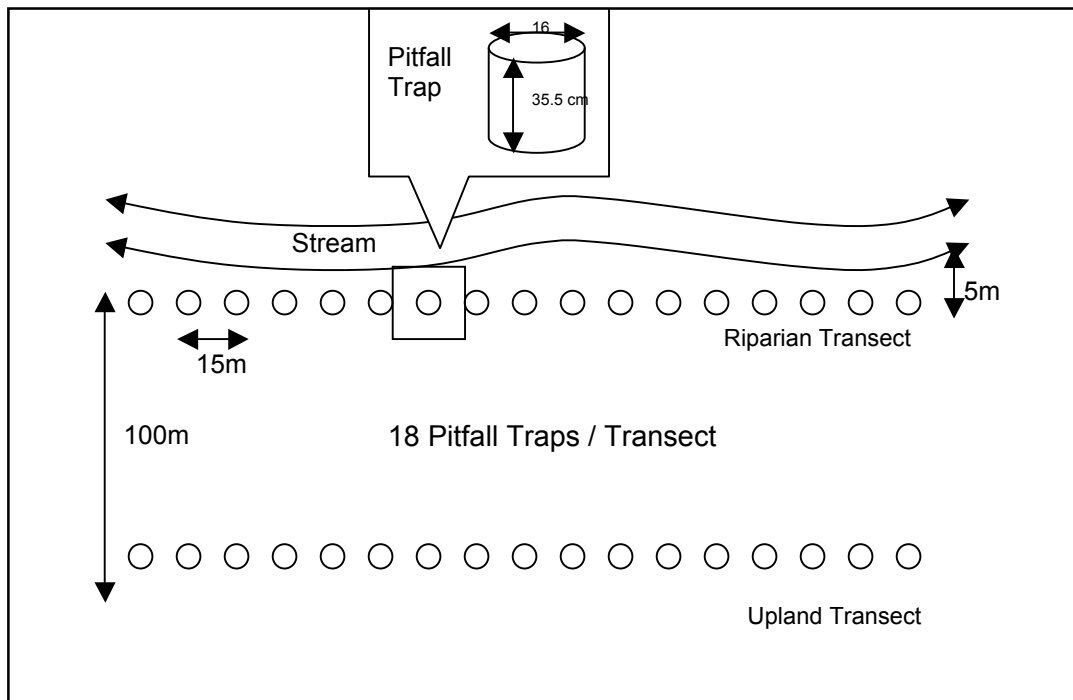


Figure 8. Pitfall trap placement in both the riparian and upland sample zones at each of the 36 sites (West and East) to be sampled for terrestrial amphibians.

The original RMZ study removed all live-captured terrestrial amphibians from each sample site and housed them off-site until the close of the trapping period. Presumably, this was done to avoid re-captures of the same animals that could artificially inflate the total number of animals present in a given treatment or control area. An alternative to removing all live-captured animals and housing them off-site, is to mark individuals captured and release them at the site of capture. This has the advantage of not disrupting the ecological community by temporarily removing large numbers of predators and prey. Suitable marking techniques include toe clipping and subcutaneous injections of fluorescent-elastomer markers (Donnelly et al. 1994; Davis and Ovaska 2001). Generally speaking, toe clipping is the least expensive and easiest way to mark a large number of individuals in a short period of time. By toe clipping individuals, animals that are recaptured can be identified and not 'double-counted'.

Therefore, we propose to mark animals using toe clipping and/or fluorescent-elastomer marking rather than removing animals from their natural habitat and storing them in a laboratory for approximately one month for each year of research. Not only would it add to the overall cost associated with this work, but there is some risk of mortality associated with transporting amphibians from the field to the laboratory and then back to the field. We also do not know what removing animals may do to the social structure of each amphibian population.



6. Any amphibian and/or reptile mortalities will be offered as museum specimens and submitted to an appropriate organization (e.g., Burke Museum).

Expected forest amphibian species are listed in Table 7.

Table 7. Expected terrestrial amphibian species for forests in eastern and western Washington State.

Location	Scientific Name	Common Name
<b>Western Washington</b>		
<b>Salamanders</b>	<i>Ambystoma gracile</i>	Northwestern Salamander
	<i>Ambystoma macrodactylum</i>	Long-toed Salamander
	<i>Dicamptodon copei</i>	Cope's Giant Salamander
	<i>Dicamptodon tenebrosus</i>	Pacific Giant Salamander
	<i>Rhyacotriton olympicus</i>	Olympic Torrent Salamander
	<i>Rhyacotriton kezeri</i>	Columbia Torrent Salamander
	<i>Rhyacotriton cascadae</i>	Cascade Torrent Salamander
	<i>Taricha granulosa</i>	Rough-skinned Newt
	<i>Plethodon dunni</i>	Dunn's Salamander
	<i>Plethodon larselli</i>	Larch Mountain Salamander
	<i>Plethodon vandykei</i>	Van Dyke's Salamander
	<i>Plethodon vehiculum</i>	Western Red-backed Salamander
	<i>Ensatina eschscholtzii</i>	Ensatina
<b>Frogs</b>	<i>Ascaphus truei</i>	Tailed Frog
	<i>Hyla regilla</i>	Pacific Treefrog
	<i>Rana aurora</i>	Red-legged Frog
	<i>Rana cascadae</i>	Cascades Frog
	<i>Rana pretiosa</i>	Spotted Frog
<b>Toads</b>	<i>Bufo boreas</i>	Western Toad
<b>Eastern Washington</b>		
<b>Salamanders</b>	<i>Ambystoma macrodactylum</i>	Long-toed Salamander
<b>Frogs</b>	<i>Hyla regilla</i>	Pacific Treefrog
	<i>Rana luteiventris</i>	Spotted Frog
<b>Toads</b>	<i>Bufo boreas</i>	Western Toad

### 1.5.1 Pre-Analysis Data Handling

All data will be analysed as per O'Connell et al. (2000). Specifically, we will perform the following tests and analyses:

- Species richness;
- Species turnover;
- Extinction probability;
- Index of abundance t-test;
- Repeated measures ANOVA;
- Tukey HSD;
- Stepwise multiple regression

All data will be entered into a customized database (MS Access compatible) on a Palm Pilot type field computer and downloaded daily throughout the study. We will obtain all raw digital data as collected by O'Connell et al. (2000) and subject that data to our analyses.

Comparisons of species richness will be made for each site for riparian and upland trapping results. This will be done by comparing total numbers of amphibian species captured during each trapping session. Details of our statistical approach can be found in Section 1.7. Our statistical approach will enable us to identify differences in species richness among treatment types and between sites. We will also compare snout-vent length of salamanders and tailed frogs between and within sites and we will evaluate habitat relationships at several spatial scales (e.g., microsite and stand-level).

Data used to compare the similarity of ecological communities will utilize Morisita's Index of similarity. We plan to use logistic and Poisson regression to identify key habitat attributes and temporal effects that may be important in determining the observed distribution and abundance of birds in the various habitats. Refer to Section 1.4.4 for a discussion of Morisita's Index and of the limitations of the Shannon-Wiener Index. To be consistent with the original study all data will be analysed using identical methods and the one's presented above.

### **1.5.2 Personnel**

Virgil Hawkes will supervise the design, logistic planning, fieldwork, and data analysis for the amphibian sampling portion of the study. Mr. Hawkes has conducted a number of amphibian studies during the course of his career. Prior to initiating major inventory surveys, a short workshop with all field crews will be conducted to clarify field methods and field identification and to update inexperienced personnel on survey procedures and requirements.

### **1.5.3 Permits and Animal Welfare Protocol**

The following sections have been extracted from *GUIDELINES FOR USE OF LIVE AMPHIBIANS AND REPTILES IN FIELD RESEARCH* compiled by American Society of Ichthyologists and Herpetologists (ASIH) The Herpetologists' League (HL) Society for the Study of Amphibians and Reptiles (SSAR):

#### **Field Activities with Wild Amphibians and Reptiles**

##### **Collecting**

Field research with amphibians and reptiles frequently involves capture of specimens, whether for preservation, data recording, marking, temporary confinement, or relocation. While certain of these activities are treated separately below, they form a continuum of potential field uses of amphibians and reptiles.

The collection of samples for museum preparation from natural populations is critical to: 1) understanding the biology of animals throughout their ranges and over time; 2) recording the biotic diversity, over time and/or in different habitats; and 3) establishing and maintaining taxonomic reference material essential to understanding the evolution and phylogenetic relationships of amphibians and reptiles. The number of specimens collected should be kept to the minimum the investigator determines necessary to accomplish the goal of a study. Some studies (e.g., diversity over geographic range or delineation of variation of new species) require relatively large samples.

*Museum Specimens and Other Killed Specimens.* - The collection of live animals and their preparation as museum specimens is necessary for research and teaching activities in Systematic zoology, and for many other types of studies. Such collections should further our understanding of these animals in their natural state and do not serve merely as tools for teaching specimen preparation techniques. Herpetological collecting techniques and representative practices of collection management have been compiled (5), as have references to field techniques (32). Whenever amphibians or reptiles are collected for museum deposition, specimens should be fixed and preserved according to accepted methods (6, 7) to assure the maximum utility of each animal and to minimize the need for duplicate collecting. In principle, each animal collected should serve as a source of information on many levels of organization from behavior to DNA

sequence. Whenever practical, blood and other tissues should be collected for karyotypic and molecular study prior to formalin fixation of the specimen.

Formalin fixation of dead specimens is acceptable practice; however, killing unanesthetized specimens by immersion in a formalin solution is unacceptable, unless justified for scientific reasons. Formalin immersion of unanesthetized animals may, however, be the only way to adequately fix certain details of morphology critical to the successful completion of research. Adult amphibians (A) and reptiles (R) may be painlessly killed by use of a chemical anesthetic such as sodium pentobarbital (R), hydrous chlorobutanol (A), MS-222 (A) (Tricaine methane sulfonate, marketed as Fiquel(tm) by Ayerst, Inc.), urethane-ethyl-carbamate (A) (referred to hereafter as urethane), 10% ethanol (A) or similar anesthetics. The euthanasia agent T-61 (National Laboratories) is very effective on reptiles (27). Use of such chemicals requires little additional time and effort, adds little to the bulk or weight of collecting equipment, and allows for preparation of better quality specimens. Urethane is carcinogenic, and caution should be observed with its use and field disposal. Other anesthetics may also be acceptable, especially since new agents are frequently developed. Gunshot is an acceptable and often necessary collecting technique, and is also recognized for euthanasia (13).

When special circumstances require that specimens (very small or larval animals, for example) be formalin-fixed without prior anesthetic killing, prior light anesthetization with an anesthetic such as MS-222 is recommended (31).

*Live Capture.* - Investigators should be familiar with herpetological capture techniques (5) and should choose a method suited to both the species and the study. Live-capture techniques should prevent or minimize damage to the animal.

*Trapping.* - Traps of various kinds are often necessary to obtain unbiased samples of secretive, nocturnal or infrequently active species. The interval between visits to traps should be as short as possible, although it may vary with species, weather, objectives of the study, and the type of trap. Traps should be checked at least daily when weather conditions threaten survival of trapped animals. Investigators must make every effort to prevent trap deaths from exposure, drowning, cardiogenic shock, or capture myopathy (1). Traps should be sheltered from direct sunlight, and care should be taken to reduce predation in pitfall traps (29). Pitfall traps set during extremely dry periods should have some moisture provided to prevent desiccation of captured amphibians. Traps should be tightly covered between sampling periods and removed at conclusion of a study.

*Habitat and Population Considerations.* - Whether collecting for future release or for museum preparation, each investigator should observe and pass on to students and co-workers a strict ethic of habitat conservation. Because many essential details of life history will remain unknown until a study is well along, collecting always should be conducted so as to leave habitat as undisturbed as possible. Permanent removal of more than 50% of the animals from any breeding or hibernation aggregation should be avoided unless justified in writing for scientific reasons by the investigator. Similarly, relatively large collections of gravid females from any population for destructive sampling should be avoided unless justified for scientific reasons. When permanent, destructive human alteration of habitat is imminent (construction, water impoundment, etc.), removal of entire populations

may be justified. Systematists should investigate extant collections for suitable specimens before conducting field work.

## **Restraint and Handling**

*General Principles.* - The decision to use physical or chemical restraint of wild amphibians or reptiles should be based upon design of the experiment, knowledge of behavior of the animals, and availability of facilities. Investigators should determine and use the least amount of restraint necessary to do the job in a humane manner. Because amphibians or reptiles, especially venomous species (including those with toxic skin secretions), may be capable of inflicting serious injury either on themselves or those handling them, some form of restraint often is prudent. Species should not be confined with others (other than food prey) that they may injure. The well-being of the animal under study is of paramount importance; improper restraint, especially of frightened animals, can lead to major physiological disturbances that can result in deleterious or even fatal consequences.

Animals are best handled quietly and with the minimum personnel necessary. Darkened conditions tend to alleviate stress and quiet the animals and are recommended whenever appropriate. When handling large reptiles, netting, or maneuvering or dropping them into a bag via hook, tongs, etc., is preferable inasmuch as they may suffer disproportionately great damage during struggling.

Administration of a tranquilizer to an animal that is restrained in a body squeeze may prevent injury to the animal and/or persons working with it. A brief review of restraint techniques for venomous snakes is available (15). Techniques often vary with size and species of the animal being handled.

In some cases, administration of general anesthesia for restraint in the field may be advisable. If so, the anesthetic chosen should be a low-risk one that permits rapid return to normal physiological and behavioral state. The animal must be kept under observation until complete recovery occurs. The relatively unpredictable and potentially delayed response of some ectotherms to immobilants or anesthetics may contraindicate use of these chemicals under field conditions. Investigators must understand the specific action of restraint chemicals on the taxa studied.

*Hazardous Species.* - Venomous snakes and lizards, certain large non-venomous lizards and snakes, some colubrid snakes (35), highly poisonous frogs, crocodylians, and some large turtles potentially are dangerous, and require special methods of restraint as a compromise between potential injury to handlers and injurious restraint of the animal. The particular method chosen will vary with species and the purpose of the project. Adherence to the following general guidelines is recommended when working with hazardous species (36):

- a. Procedures chosen should minimize the amount of handling time required, and reduce or eliminate contact between handler and animal.
- b. Those handling venomous snakes or lizards should be knowledgeable concerning the proper method of handling those animals. They should be aware of emergency procedures to be instituted in case of accidental envenomation. Location of a reasonably nearby supply of antivenin and of a physician with knowledge of envenomation treatment should be ascertained in advance.

c. One should avoid working alone. A second person, knowledgeable of capture/handling techniques and emergency measures, should be present whenever possible.

d. Prior consultation with workers experienced with these species, and review of the relevant literature, is of particular importance here since much of the information on handling dangerous species is not published, but is passed simply from one investigator to another.

### **Animal Marking**

Marking animals for field recognition is an essential technique in biological research. Important considerations in choosing a marking technique concern effects on behavior, physiology, and survival of the animal. The utility of any technique varies with the species under study; tissue-removal techniques may pose less long-term survival threat to some species than certain tagging methods. Marking techniques for amphibians and reptiles have been reviewed extensively (12). Although field observation indicates that individual wild animals can survive extensive tissue damage from natural causes (30), the effect of most tissue-removal marking techniques on survival and fitness is not adequately known and is a topic worth investigating.

When choosing an acceptable marking technique, investigators must consider the nature and duration of restraint, the amount of tissue affected, whether pain is momentary or prolonged, whether the animal will be at greater than normal predation risk, whether the animal's ability to mate is reduced, and whether the risk of infection is minimal. Careful testing of marking techniques on captive animals before use on free-ranging animals may reveal potential problems and is recommended. It may be desirable to use redundant techniques to assure accuracy during a study.

*Toe Clipping.* - Toe clipping should be used only for general marking of free-ranging animals when toe removal is not judged (by observation of captives or of a closely-related species) to impair the normal activities of the marked animal. Toes essential to animals for activities such as burrowing, climbing, amplexus, or nest excavation, should never be removed. No more than two non-adjacent toes per foot should ever be removed. If behavior or survival of the animal is likely to be seriously impaired, alternate marking techniques should be used. Clarke (24) reported adverse effect of toe-clipping on survival of *Bufo woodhousei*. Critical study of the effects of this technique on fitness would be a valuable contribution.

*Tattoos and Dye Markers.* - Tattooing has been used with success on both amphibians and reptiles. Two potential problems should be resolved prior to tattooing: 1) selection of a dye which will contrast with the normal skin pigmentation; and 2) loss of legibility due to diffusion or ultraviolet degradation of the dye.

Paint should not be used to mark the moist and permeable skin of amphibians. Various vital stains are more suitable. Reptile skin permeability is quite variable, and paint or paint solvents may be absorbed and cause death of the animal. Paints with non-toxic pigments, bases, and solvents must be used. When toxicity is unknown, laboratory trials, even if limited, should be done before field use. Very tenacious paints may, if applied across shell sutures, severely distort the normal

shell growth of turtles, especially sub-adults. Paint should not be applied to sutures of turtle shells.

## **1.6 Small Mammals**

### **1.6.1 Introduction**

Given that the objective of the TFW Agreement is to assure the greatest diversity of species in the greatest diversity of habitats and to ensure the survival and reproduction of enough individuals to maintain those species, it is imperative that the studies conducted for each taxonomic group result in verifying whether this objective is being met and, if not, ensure that sufficient data are collected to assess which habitats adversely affect species assemblages and how RMZ guidelines can be improved to achieve the TFW goals.

To accomplish these goals, the focus of the small mammal studies will be to assess community similarity and relative abundance in treatment and control areas. It will also be necessary to assess, to the extent possible, habitat components that contribute to or detract from those measures.

O'Connell et al. (2000) reported on the original RMZ study in eastern and western Washington which monitored small mammal communities in riparian and upland forests before and immediately after various forest harvesting practices were implemented. As such, they laid the groundwork for all future monitoring that would be done and set the choice of survey methods by their selection of a particular study design. We propose to continue monitoring the effect of riparian buffer width on community structure and habitat suitability for small mammals in the treatment and control areas in Washington State.

### **1.6.2 Methods**

The secretive nature and small size of shrews, rodents and other small mammals make them difficult to inventory by direct counts or sign surveys. For the most part, determining the relative abundance of these species requires capture of animals. For this reason, trapping is the most efficient means to inventory small mammal species and communities. Several methods of trapping are available and the choice of method and sampling design depends on the objectives, the targeted species and the available time. We will sample terrestrial small mammals in the same manner as O'Connell et al. (2000) using a combination of Museum Special snap traps and pitfall traps. Pitfall traps capture insectivores and non-jumping rodents well, but are less effective at capturing deer mice, chipmunks, and jumping mice. The opposite is true for snap traps, which capture large-bodied, agile rodents much more effectively than pitfall traps.

Trapping will be conducted in 2003 and 2004 at all study sites. Sampling effort will be limited to one period per year. In the east, it will be conducted during May and June. Sampling in the west will begin after the onset of fall rains. The timing of the trapping will be set by the need to sample when amphibians are surface-active, generally during October and November. We will sample on the existing paired traplines established by O'Connell et al. (2000).

In eastern Washington, parallel transects 720 m in length are located 8 m from the stream and 100 m upslope. A total of 72 snap-trapping stations are spaced at 10-m intervals along each transect. Two snap-traps will be placed within 3 m of each station, baited with a mixture of oats and peanut butter, and checked for 4 consecutive days each year. Eighteen pitfall traps, constructed of double deep #10 cans buried in the soil, are placed at 15-m intervals on each transect. Pitfall traps will be checked every other day for 2 weeks. Captured animals will be

weighed, measured, numbered, labeled, and frozen. Any living animals will be marked and released.

In western Washington, two traplines are located within the riparian zone on either side of the creek, and another two transects are well outside the zone about 100 m from, and on either side of, the stream. Each trapline consists of 36 stations set 10 m apart (350 m total length) which will be set with two Museum Special traps per station. Traplines are centered on the 500-m stream study sites. Traps will be baited with peanut butter and whole oats and operated for 4 consecutive days and nights (4 trapnights). Pitfall traps (double deep #10 cans) will be operated for 2 continuous weeks. Traps will be checked weekly. Eighteen pitfall traps are placed at 15-m intervals on the central portion of one snap trapping transect on one side of the creek (riparian and upland). The snap and pitfall trapping will occur simultaneously. Animals will be frozen for later species identification and measurement.

The sex of captured animals will be determined through examination of genitalia (sizes and relative positions of the genital papillae) and mammarys. Sex is particularly difficult to determine with young animals and shrews outside of the breeding season.

The age of trapped animals can often be assessed by size, weight, pelage condition and colour. Sexual maturity can be determined by palpation of testes (scrotal or abdominal) for males and the condition of the vaginal opening (perforate or not) and mammarys (large or small), and whether or not obviously pregnant for females.

### **1.6.3 Pre-Analysis Data Handling**

Capture data will be expressed as the number of individuals captured per 100 trapnights. For pitfall traps only the number of days and nights the trap arrays were operated will be necessary to compute these values because pitfall traps are multiple capture traps. Snap trap data will be corrected to yield traps available per 100 trap nights recognizing that previously snapped traps cannot catch animals. Trapping totals for each technique will be slumped to give an overall catch per unit effort index. The overall indices will be used in statistical testing.

All data will be analysed as per O'Connell et al. (2000). Specifically, we will perform the following tests and analyses:

- Species richness;
- Species turnover;
- Extinction probability;
- Index of abundance t-test;
- Repeated measures ANOVA;
- Tukey HSD;
- Stepwise multiple regression

All data will be entered into a customized database (MS Access compatible) on a Palm Pilot type field computer and downloaded daily throughout the study. We will obtain all raw digital data as collected by O'Connell et al. (2000) and subject that data to our analyses.

Interspecific density comparisons will not be conducted because capture and detection probabilities (species trapability) are not constant between species. For example, although it is possible to compare the relative abundance of the same species in two different habitat types, it is not possible to conclude that one species is twice as abundant as another in a single trap line because we are not able to correct for differential trapability. Similarly, it is not particularly useful to conduct temporal comparisons within areas because of the typically large fluctuations in small mammal populations. Thus, the analysis of small mammal data will focus on treatment effects on relative abundance of individual species and ecological measures of the small mammal

community. Even measures of richness, evenness and diversity often are biased due to the differential probabilities of capture of small mammal species.

Before we begin our analysis, we will examine the distribution of the data we have collected. Small mammals tend to have clumped populations and often do not fit normal distributions, but rather conform better to negative binomial distributions. It may be necessary to either transform the data or adjust the analysis approach to match the observed distribution.

We propose to use Moritita's Index of Similarity (Krebs 1999) as a measure of the small mammal community. This measure replaces the individual analysis of richness and evenness conducted by O'Connell et al. (2000) and circumvents the problems associated with the Shannon-Wiener Index of ecological diversity used by them. It is a more powerful and meaningful tool and we believe it is a superior approach to this aspect of data analysis.

Through logistic and Poisson regression we will also examine the components of habitat in riparian and upland areas that appear to be contributing to relative abundance of small mammals that have an adequate number of captures.

Details of the statistical analysis that will be performed on the data are contained in Section 1.7. The data analysis and presentation will include the entire dataset, including those data collected from 1992-1998.

As with all other components of this study, all data for small mammals will be analysed using the methods described in O'Connell et al. (2000) in addition to the methods described herein.

#### **1.6.4 Personnel**

Gary F. Searing, M.Sc., will supervise the design, logistic planning, and data analysis for the small mammal portion of the study. Mr. Searing conducted his Master's research on small mammals and has conducted a number of small mammal trapping studies during the course of his 25 year career. Prior to initiating major inventory surveys, a short workshop with all field crews will be conducted to clarify field methods and field identification and to update personnel on survey procedures and requirements.

Each field crew will contain a qualified biologist with experience and competency in identification of small mammals. Juveniles of some species are particularly difficult to identify. When necessary, additional small mammal specialists will be consulted to ensure accuracy. Identification manuals and field guides will be provided to each field crew. A customized identification key based on external features will be developed for species expected in the study area.

#### **1.6.5 Permits and Animal Welfare Protocol**

Permits for the trapping and possession of mammals caught during this study will be obtained from DNR and any other agencies that may require permits for this work.

The following excerpt from the Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists (Animal Care and Use Committee 1998) summarizes the relevant requirements for this study.

Some types of research in mammalogy require the killing of individuals, either by use of traps or firearms. Investigators must endeavor to ensure that such collecting does not adversely affect the populations being sampled. In such collecting, it is essential to employ methods of trapping ... that will ensure that death occurs as quickly and painlessly as possible... Some species may be taken effectively only by use of specialized traps such as snap or break-back traps (e.g.,



Victor or McGill traps for rat-sized mammals and Museum Special traps for smaller species); pitfalls for shrews or other small terrestrial mammals.... These latter traps are preferable to leg-hold traps where appropriate. Kill traps must be positioned with care so as to ensure the highest probability of capture of "target" species and the lowest probability of capture of other animals. Traps must be secured well and marked conspicuously to prevent loss. Traps must be checked at least once each day to remove captured mammals. If a captured animal not already dead, it should be killed immediately and humanely. Pitfalls may be used as kill traps only when no other effective method of kill-trapping is available.

When live-caught animals are retained as voucher specimens or when specimens are injured or distressed and cannot be released, they must be euthanized humanely. Field methods used to euthanize mammals should be quick, as painless as possible, and compatible with both the design of the investigation, and the size and behavior of the species of mammal under investigation.... For euthanizing small mammals, cervical dislocation and thoracic compression are commonly used methods because they are quick and impart little pain, thus meeting the criteria for euthanasia methods of the United States Department of Agriculture's Animal and Plant Health Service (APHIS)... Regardless of method used, death of the animal should be confirmed.

The use of Museum Special traps and live trapping using pitfall traps are the recommended methods. Any animals that are not able to be released alive, will be killed by cervical dislocation. All methods used during this study will conform to the ASM Guidelines.

## **1.7 Study Design and Data Analysis**

### **1.7.1 Study Design**

The study as laid out by the previous investigators is a split-plot repeated measures design for each side of the state. Eighteen sites within either the West or East sides of Washington state were selected according to a number of criteria (see section 1.3). They will be considered to provide adequate representation to other similar sites throughout either the West or East side of Washington state<sup>1</sup>. The 18 sites are the mainplots and were assigned<sup>2</sup> one of three site treatments: Control (no upland harvesting), State (RMZ as per older state regulations), and Modified (wider RMZ). Subsampling was conducted within either or both of two habitat types: the RMZ around the stream and the upland area harvested (or area that would have been harvested in the case of the control). These two habitat levels<sup>3</sup> are the split-plot treatments and the areas within each site belonging to one of these habitat levels are the split-plot experimental units. Each of the habitat areas within each site has been and will be subsampled for a variety of response variables. Analyses of all response variables will be conducted on a summary statistic (often the mean) of the subsampling responses within each split-plot and sampling year.

The overall design for this study is shown in Table 8. ANOVA will be a suitable statistical analysis method for many response variables and the suitable error terms for this method are included in the table. These error terms are based on the assumptions that the treatment factors, site, habitat, and time are fixed effects while sites and split-plots are random effects. Count variables may be analysed after a log transformation if the counts are large and the transformation improves the approximation to normality. If counts are low then a Poisson linear regression with a log link may be used instead. The random effects listed in the table may not be included in the Poisson model.

### **1.7.2 Combining the East and West Sides**

While many variables would be best analyzed separately for the East and West sides of Washington state, there may be some that can be suitably analyzed in a combined design. This would necessitate adding another mainplot factor to the design in Table 1: the side of the state and consequent interactions throughout the table. The design would now have 36 sites nested within two factors: side and treatment. It is hard to say whether the sites are the experimental units for side or are subsamples (and thus pseudo-replicates). In any case, the between site variability is the only available error term for the test between sides. The more interesting tests will be the interactions of treatment and habitat with side. For instance, if any of the interactions including treatment and side are significant, then treatment effects are not similar between the two sides of the state with the result that separate RMZ rules may be required.

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<sup>1</sup> Ideally, these sites would have been randomly selected from a population of suitable sites thus providing the statistical foundation for inferring the results to other sites. Practically, of course, this is usually difficult to do.

<sup>2</sup> Ideally, the treatment assignment would have been done randomly. No clear statement can be found in TFW about this. Treatment assignment should have been practically possible – if it was not done, then we must keep a sharp lookout for possible confounding of treatment with site attributes which might invalidate treatment responses.

<sup>3</sup> This is an observed factor since random assignment of habitat type to parts of the site are not possible.

Table 8. Basic design with ANOVA error terms for one side of Washington state.

Source of Variation		DF	Error Term
<u>Mainplot:</u>			
Site Treatment (Control, State, Modified)	T	2	S(T)
Sites nested within T	S(T)	15	--
<u>Split-plot:</u>			
Habitat type (Riparian Vs Upland)	H	1	HS(T)
Interaction: H x T	HT	2	HS(T)
Split-plot Error: H x S(T)	HS(T)	15	--
<u>Repeated Measures (split-split-plot in time)</u>			
Sampling times I	I	7	IHS(T)
Interactions: I x T	IT	14	IHS(T)
I x H	IH	7	IHS(T)
I x H x T	IHT	14	IHS(T)
Error: (Remaining I interactions)	IHS(T)	210	--
Total		287	

### 1.7.3 Repeated Measures Analysis

Repeated measures can be considered a split-plot in time and treated as another level in a split-plot analysis. This requires an additional assumption to the usual set for ANOVA, namely that the covariance matrix satisfy the Huhn-Feldt condition. If this condition is not satisfied then one can:

1. Treat the response as a set of variables, one variable for each time, and do a multivariate analysis. This is not as powerful a test.
2. Look at the extreme results using the original df's in the test vs the df's reduced according to Box's correction (for the design in Table 8, this means dividing the df's for the split-split plot in time portion by the df for I. For example, calculate two p-values for the IH test using df = 14, 210 and df = 7, 30). If both p-values are consistent then declare the test result accordingly. If not, then adjust the df's according to the Greenhouse-Geisser correction (see Milliken and Johnson, page 359) and use the resulting p-value. Many statistical packages do these calculations automatically if programmed properly.

Given the large gap in timed data points, autocorrelation or the repeated measures will be difficult to assess, although we may try running models with and without an AR(1) component to see if it helps the fit any.

### 1.7.4 Checking Assumptions of Data Analysis

After the appropriate split-plot repeated measures model has been fit to the data the resulting residuals (differences between the observed and fitted values) will be checked by examining a variety of plots: probability plots for the appropriate distribution (normal for ANOVA and Poisson for Poisson linear models); boxplots to look for outliers and distributional properties, including heterogeneity of variance; and scatterplots of the residuals against the fitted values and time to look for outliers, unexpected patterns and heterogeneity of variance. Hartley's F-max procedure will be used to test for heterogeneity of variance. Appropriate remedial action will be taken if necessary. Temporal autocorrelation between adjacent observations will be looked for but is expected to be hard to detect because of the large gap in the middle of the data.

### 1.7.5 Contrasts and Multiple Range Tests

Most of the interesting questions would be better answered using contrasts instead of a multiple range test. While it is generally best if the contrasts are orthogonal, this is not strictly necessary if the number of contrasts is about the same as the df for that source of variation. Contrasts will be calculated either within the context of ANOVA, or other statistical method (e.g. Poisson linear models). Not all contrasts will be of the same interest for all response variables.

While contrasts are usually determined for main effects and possibly some of their interactions, in this case, specific tests that cross these 'boundaries' will provide answers to the more interesting questions. Since these are somewhat unusual, these questions and their contrasts will be described in some detail below (strictly speaking, of course, the questions should be stated as null hypotheses, but the question form is more specific and appealing). The numbers in the tables are the weights to use when calculating the sum for the contrast (see, <http://www.for.gov.bc.ca/research/biopamph/pamp16.pdf>). Further, since many of the contrasts 'cross' the split-plot and repeated measures boundaries, great care will be taken that the correct errors terms are used (see, for example, Milliken and Johnson, section 24.4, page 306). This is most easily accomplished by using proper programming with the MIXED procedure within SAS.

The contrasts referred to above have been grouped into several sets of similar questions. In summary the question sets answer the following types of questions:

**Question Set 1:** Are the pre-treatment and control means all the same? We might expect this if a) there are no differences in the pre-treatment values and b) the controls do not change over time.

**Question Set 2:** Do the treated sites show linear trends with time after harvest of the upland areas? And, are there differences in these trends between the RMZ treatments and the two habitat types?

**Question Set 3:** Overall, are the two groups of means from sets 1 and 2 different?

**Question Set 4:** Do the control sites show different linear trends than the treated sites in question set 2? (resulting contrasts are not orthogonal to the contrasts Q1.12 to Q1.21).

**Question Set 5:** If temporal effects are expected to be erratic and meaningless, except possibly for the pre- and post-harvest comparison, then the main treatment effects could be "averaged" over time using two contrasts. These would compare the state and modified treatments to each other and the average of these two RMZ treatments against the control as a) a main effect, averaged over habitats and all the times (2df); b) tested separately for each habitat and averaged over time (4 df); c) tested separately for each habitat and pre vs. post (8 df); d) conducted separately for each habitat and time (32 df). This is a reasonable approach if very few other contrasts are to be run (e.g. habitat x treatment interaction, 2 df).

### 1.7.6 Question Sets

**Question Set 1:** The means of the cells checked below should all be the same if i) there are no differences in the pre-treatment values and ii) the controls do not change over time. Twenty-four of the 42 means are involved here and so 23 orthogonal contrasts are required to answer the main question. Some suggestions are listed below.

Question Set 1		Pre-treatment		1 <sup>st</sup> Post-treatment		2 <sup>nd</sup> Post-treatment			
Habitat:	Site:	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8
Riparian	Control:								
	State:								
	Modified:								
Upland	Control:								
	State:								
	Modified:								

The first five questions within the Riparian pre-treatment data would be (contrast coefficients shown in table below):

**Q1.1:** Is the response to the control different from the two RMZ treatments?

**Q1.2:** Is there a difference in response between the two RMZ treatments?

**Q1.3:** Is there a difference between the two times?

**Q1.4:** Is the difference between the control and RMZ treatments the same for both times?

**Q1.5:** Is the difference between the two RMZ treatments the same for the two times?

These five questions will also be asked of the Upland pre-treatment means (Q1.6 to Q1.10).

Riparian Means Only (all Upland means have a weight of zero):						
Site Treatment:		Q1.1:	Q1.2	Q1.3:	Q1.4:	Q1.5:
Control:	Time 1	2	0	1	-2	0
	Time 2	2	0	-1	2	0
State:	Time 1	-1	-1	1	1	-1
	Time 2	-1	-1	-1	-1	1
Modified:	Time 1	-1	1	1	1	1
	Time 2	-1	1	-1	-1	-1
Habitat Means Only (all Riparian means have a weight of zero):						
Site Treatment:		Q1.6:	Q1.7:	Q1.8:	Q1.9:	Q1.10:
Control:	Time 1	-2	0	-1	2	0
	Time 2	-2	0	1	-2	0
State:	Time 1	1	1	-1	-1	1
	Time 2	1	1	1	1	-1
Modified:	Time 1	1	-1	-1	-1	-1
	Time 2	1	-1	1	1	1

The eleventh question will involve all 12 pre-treatment means:

**Q1.11:** Is the response different between the riparian and upland pre-treatment areas? This would use weights of one (1) for the 6 riparian pre-treatment means and negative one (-1) for the 6 upland pre-treatment means.

The next ten questions compare the post-treatments, again separately for the Riparian and Upland areas:

**Q1.12 & 1.16:** Is the response different between the first two post-treatments?

**Q1.13 & 1.18:** Is there a difference between the 5<sup>th</sup> and 6<sup>th</sup> post-treatment times?

**Q1.14 & 1.19:** Is the response for the 7<sup>th</sup> post-treatment mean different from the 5<sup>th</sup> and 6<sup>th</sup> times?

**Q1.15 & 1.20:** Is the response for the 8<sup>th</sup> post-treatment mean different from the 5<sup>th</sup>, 6<sup>th</sup>, and 7<sup>th</sup> times?

**Q1.16 & 1.21:** Is there a difference between the first set of post-treatments and the second set of post-treatment means?

Riparian Means Only (all Upland means have a weight of zero):							
Site Treatment:		Q1.12:	Q1.13:	Q1.14:	Q1.15:	Q1.16:	
Control:	Time 3	1	0	0	0	2	
	Time 4	-1	0	0	0	2	
	Time 5	0	1	1	1	-1	
	Time 6	0	-1	1	1	-1	
	Time 7	0	0	-2	1	-1	
	Time 8	0	0	0	-3	-1	
	Habitat Means Only (all Riparian means have a weight of zero):						
	Site Treatment:		Q1.17:	Q1.18:	Q1.19:	Q1.20:	Q1.21:
Control:	Time 3	1	0	0	0	2	
	Time 4	-1	0	0	0	2	
	Time 5	0	1	1	1	-1	
	Time 6	0	-1	1	1	-1	
	Time 7	0	0	-2	1	-1	
	Time 8	0	0	0	-3	-1	

The next two questions complete an orthogonal set of contrasts for these 24 means:

**Q1.22:** Is there a difference between the overall post-treatment means for the Riparian and Upland habitats? This would use weights of one (1) for the Riparian post-treatment means and negative one (-1) for the Upland post-treatment means.

**Q1.23:** Is there a difference between all of the pre-treatment means and the post-harvest control means? This would use weights of one (1) for the pre-treatment means and negative one (-1) for the control post-treatment means.

The main overall question of whether these twenty-four means are the same will be answered by an F-value for all the contrasts lumped together and it will have 23 numerator degrees of freedom.

One more test of interest (Q1.24) that is not orthogonal to the above set is the question of whether, overall, the twelve Riparian means are different from the twelve Upland means. This is easily tested by using weights of one (1) and negative one (-1) for the respective sets of means.

**Question Set 2:** Responses to treatment will be shown by the means of the twenty-four cells checked below that are different from the means in question set 1.

Question Set 2		Pre-treatment		1 <sup>st</sup> Post-treatment		2 <sup>nd</sup> Post-treatment			
Habitat:	Site:	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8
Riparian	Control:								
	State:								
	Modified:								
Upland	Control:								
	State:								
	Modified:								

Linear trends for the post-harvest data, whether increasing or decreasing, will be tested by the linear contrasts listed below. The remaining 15 df will be pooled into a lack of fit test for the linear trend. The addition of a quadratic component to the model for trend might be considered if warranted (that is, if the linear models do not appear to provide adequate fits).

**Q2.1:** Is there an overall linear response to time for the RMZ treatments and habitat type combinations? The values given in the table below assume that sampling occurred in years 1995, 1996, 2002, 2003, 2004, and 2005. If the actual dates are different then they would be adjusted accordingly.

**Q2.2:** Do the two riparian lines for state and modified have the same slope?

**Q2.3:** Do the two riparian lines have means at the same height? If the lines are parallel, then this is also a test for coincidence of the lines and that they have the same intercept.

**Q2.4:** Do the two upland lines for state and modified have the same slope?

**Q2.5:** Do these two upland lines have means at the same height? If the lines are parallel, then this is also a test for coincidence of the lines and that they have the same intercept.

**Q2.6:** Do the average lines for riparian and upland have the same slope?

**Q2.7:** Do the average lines for riparian and upland have the same height? Again, if lines are parallel then this is a test for same intercept.

**Q2.8 to Q2.23:** Pooled together for a lack of fit test.

Riparian Means:									
Site Treatment:		Q2.1:	Q2.2:	Q2.3:	Q2.4:	Q2.5:	Q2.6:	Q2.7:	
State:	Time 3	-35	-35	1	0	0	-35	1	
	Time 4	-29	-29	1	0	0	-29	1	
	Time 5	7	7	1	0	0	7	1	
	Time 6	13	13	1	0	0	13	1	
	Time 7	19	19	1	0	0	19	1	
	Time 8	25	25	1	0	0	25	1	
	Modified:	Time 3	-35	35	-1	0	0	-35	1
		Time 4	-29	29	-1	0	0	-29	1
Time 5		7	-7	-1	0	0	7	1	
Time 6		13	-13	-1	0	0	13	1	
Time 7		19	-19	-1	0	0	19	1	
Time 8		25	-25	-1	0	0	25	1	
Upland Means:									
State:		Time 3	-35	0	0	-35	1	35	-1
	Time 4	-29	0	0	-29	1	29	-1	
	Time 5	7	0	0	7	1	-7	-1	
	Time 6	13	0	0	13	1	-13	-1	
	Time 7	19	0	0	19	1	-19	-1	
	Time 8	25	0	0	25	1	-25	-1	
	Modified:	Time 3	-35	0	0	35	-1	35	-1
		Time 4	-29	0	0	29	-1	29	-1
Time 5		7	0	0	-7	-1	-7	-1	
Time 6		13	0	0	-13	-1	-13	-1	
Time 7		19	0	0	-19	-1	-19	-1	
Time 8		25	0	0	-25	-1	-25	-1	

**Question Set 3:** There is one orthogonal degree of freedom left. This can be used to test the two groups of means in sets 1 and 2 against each other (Q3.1). The weights for this are easy to determine: a weight of 1 would be used for all the means from set 1 while a weight of -1 used for the means from 2.

Question Sets 3 & 4		Pre-treatment		1 <sup>st</sup> Post-treatment		2 <sup>nd</sup> Post-treatment			
Habitat:	Site:	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8
Riparian	Control:	X	X	X	X	X	X	X	X
	State:	X	X						
	Modified:	X	X						
Upland	Control:	X	X	X	X	X	X	X	X
	State:	X	X						
	Modified:	X	X						

This overall test may not be of much interest.

Should there be a linear trend with time for the state and modified treatments after harvest, comparisons of these trends with that of the control will be of considerable interest. Instead of Q1.12 through Q1.21 the following might be of greater biological relevance.

**Q4.1:** Is there a linear trend for the control sites?

**Q4.2:** Is there a difference in the linear trends between the riparian and upland controls?



**Q4.3:** Do the two upland lines have means at the same height? If the lines are parallel, then this is also a test for coincidence of the lines and that they have the same intercept.

**Q4.4:** Is the trend for the riparian control different than the combined riparian state and modified lines?

**Q4.5:** Is the trend for the upland control different than the combined upland state and modified lines?

**Q4.6:** Is the difference between the riparian control and the combined riparian RMZ treatments the same as the difference between the upland control and combined upland RMZ treatments?

**Q4.7 to Q4.11:** Pooled together for a lack of fit test.

Riparian Means:								
Site Treatment:		Q4.1:	Q4.2:	Q4.3:	Q4.4:	Q4.5:	Q4.6:	
Control:	Time 3	-35	-35	1	70	0	70	
	Time 4	-29	-29	1	58	0	58	
	Time 5	7	7	1	-14	0	-14	
	Time 6	13	13	1	-26	0	-26	
	Time 7	19	19	1	-38	0	-38	
State:	Time 8	25	25	1	-25	0	-25	
	Time 3	0	0	0	-35	0	-35	
	Time 4	0	0	0	-29	0	-29	
	Time 5	0	0	0	7	0	7	
	Time 6	0	0	0	13	0	13	
Modified:	Time 7	0	0	0	19	0	19	
	Time 8	0	0	0	25	0	25	
	Time 3	0	0	0	-35	0	-35	
	Time 4	0	0	0	-29	0	-29	
	Time 5	0	0	0	7	0	7	
	Time 6	0	0	0	13	0	13	
	Time 7	0	0	0	19	0	19	
	Time 8	0	0	0	25	0	25	
	Upland Means:							
	Control:	Time 3	-35	35	-1	0	70	-70
Time 4		-29	29	-1	0	58	-58	
Time 5		7	-7	-1	0	-14	14	
Time 6		13	-13	-1	0	-26	26	
Time 7		19	-19	-1	0	-38	38	
State:	Time 8	25	-25	-1	0	-25	25	
	Time 3	0	0	0	0	-35	35	
	Time 4	0	0	0	0	-29	29	
	Time 5	0	0	0	0	7	-7	
	Time 6	0	0	0	0	13	-13	
Modified:	Time 7	0	0	0	0	19	-19	
	Time 8	0	0	0	0	25	-25	
	Time 3	0	0	0	0	-35	35	
	Time 4	0	0	0	0	-29	29	
	Time 5	0	0	0	0	7	-7	
	Time 6	0	0	0	0	13	-13	
	Time 7	0	0	0	0	19	-19	
	Time 8	0	0	0	0	25	-25	

## 1.8 Power Analysis

### 1.8.1 Introduction

The potential for Type II error, the probability of not rejecting the null hypothesis when it is false, is often high with biological observational studies. This is generally true because there are a large number of variables, many of which are not only difficult to control but may also be unknown. The purpose of controlling these variables is to:

1. Reduce or eliminate *bias* in the observed treatment means and particularly in the differences and contrasts between treatment means; and
2. Reduce the *background variability* so that smaller treatment differences and contrasts can be detected (i.e. reducing the Type II error with the same resources).

Ideally these extraneous variables can be controlled by:

1. *randomization*, both during the selection of (sub)sampling units and during assignment of treatments to experimental units. This achieves control by ‘averaging out’ the effects of these extraneous variables. This can work well for unknown variables but significant replication may be required for this to be truly effective;
2. *inclusion* in the study either as a factor nested or crossed with other factors, or as a continuous independent variable or covariate. Blocks are a common design technique used to “explain” and remove variability due to extraneous variables; and
3. *confounding* with other variables of little interest. For instance, extraneous variables are often confounded with the blocks in randomized block and Latin square designs.

In observational studies, though, it is usually not possible to control all of the extraneous variables with either of these three approaches. Large well-replicated studies are often difficult to set up not only because of the limited availability of time, money and people, but also because of the limited availability of suitable biological material. For example, there are not likely to be available many sites with a type 3 or 4 stream running through the middle and which may or may not be logged two years from now. But even when large studies can be established, many of the variables in biological systems are highly correlated, some by chance and some by the nature of the subject matter, so that separation of effects may be quite difficult. Knowledge of the subject matter and research/statistical principles here is very important so that careful consideration can be given to balancing all of these complications when drawing conclusions.

### 1.8.2 Discussion

Mathematical examination of the potential for making Type II errors during hypothesis testing and the study’s ability to detect changes through time and due to treatment effects is a complicated procedure which requires choosing the following values:

1. The probability of making a Type I error (also known as the  $\alpha$ -level). By convention this is usually set at  $\alpha = 0.05$ .
2. An acceptable probability of making a Type II error (also known as  $\beta$ ). This is often set at  $\beta = 0.20$  for a power of 80%. Other choices are possible.
3. For each statistical test, determine the minimum biological or practical size for the treatment difference or contrast that is desired to be detected. This may require considerable thought. One approach would be to ask questions like: “When comparing the state and modified RMZ treatments, what is the minimum amount by which variable x must increase (or

decrease) for it to be worthwhile that we switch from the state to the modified treatment?”. Another example would be: “Assuming a linear trend, what minimum rate of change (slope of line) for variable x would be of concern?”. It is often useful to produce graphs of power as a function of these treatment contrasts.

4. Estimates of the variability expected for the data to be collected. Previously collected data can be a great help here if it is expected that the variability will be similar. Determination of which variance estimates will be needed requires the expected mean squares (EMS's) for the design at hand. This is presented in Table 9 while the individual components of the terms in the EMS's are described in Table 10.

There are at least four methods available to obtain the required estimates:

- Re-analysis of the raw data collected in the previous study; or they could be available from directly from the report by O’Connell et al. (2000) if these variability estimates, either as
- individual variance components; or as
- the corresponding mean squares from the ANOVA’s, were presented anywhere. While mean square errors should be reported they are often not presented. But we would expect to see
- standard errors for the marginal means of site treatment, habitat type, site, and/or time presented in the report could also provide suitable estimates. But in O’Connell et al. (2000) these are rarely presented in a multi-way table and most values that are displayed are calculated incorrectly. Table 3 on page 12-20 comes closest to providing the power calculation requirements but is for a variable we don’t plan to collect. A close second is Table 2 on page 6-37 which lists the means for mean number of species pre- and post-harvest. But individual site/habitat means are not included and, while the df are appropriately presented, they suggest that the subsampling error was inappropriately pooled with the other error terms. At most, the df should be 1, 10, but instead the denominator df’s were substantially larger.

Table 9. Basic design with ANOVA error terms<sup>†</sup> for one side of Washington state.

Source of Variation		df	Expected Mean Squares
<u>Mainplot:</u>	T	2	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 80 \sigma_{HST}^2 + 160 \sigma_{ST}^2 + 960 \sigma_T^2$
Error:	S(T)	15	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 80 \sigma_{HST}^2 + 160 \sigma_{ST}^2$
<u>Split-plot:</u>	H	1	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 80 \sigma_{HST}^2 + 1440 \sigma_H^2$
	HT	2	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 80 \sigma_{HST}^2 + 480 \sigma_{HT}^2$
Error:	HS(T)	15	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 80 \sigma_{HST}^2$
<u>Repeated Measures:</u>	I	7	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 360 \sigma_I^2$
	IT	14	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 120 \sigma_{IT}^2$
	IH	7	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 180 \sigma_{IH}^2$
	IHT	14	$\sigma_e^2 + 10 \sigma_{IHST}^2 + 60 \sigma_{IHT}^2$
Error:	IHS(T)	210	$\sigma_e^2 + 10 \sigma_{IHST}^2$
Subsampling error:		2592	$\sigma_e^2$
Total		2879	

<sup>†</sup> For illustration purposes, calculations were done assuming 10 subsamples per cell.

### 1.8.3 Power Analysis Example

We used the pre-harvest data from Table 2 on page 6-37 of the original RMZ study as the basis for the power analysis shown in Figure 9. See the next section for a discussion of the approximations and calculations used to obtain this plot.

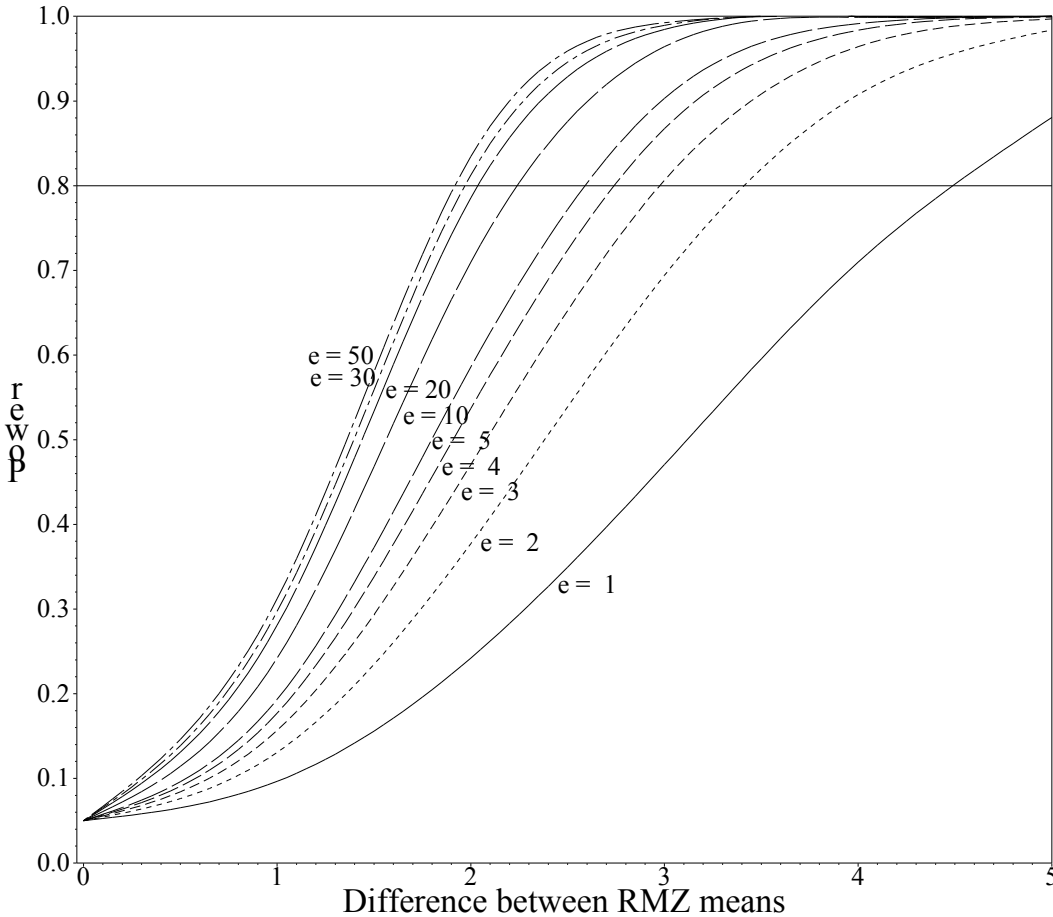


Figure 9. Plot of power as a function of difference between the RMZ means and for subsample sizes of  $e = 1, 2, 3, 4, 5, 10, 20, 30,$  and  $50$ .

For this study, the number of treatments, sites per treatment and habitats have already been determined. The only real choice left for controlling power is the number of subsamples placed within each site/habitat area. While this figure was determined for one specific response variable, power figures for the other variables will be similar. The main difference will be the horizontal scale showing the size of the treatment effect and the location of the minimum biologically significant effect.

From Figure 9 we can see that we will be able to detect a difference of around 4 easily and do this with more than sufficient power with only 5 subsamples within each site and habitat type. On the other hand, a difference of 2 will require at least 30 subsamples to get 80% power. Detecting a difference of 2 with greater power will be quite difficult since even 50 subsamples would not be sufficient. Differences much less than 2 cannot be detected with at least 80% power even with quite large numbers of subsamples.

Table 10. Descriptions of components within the Expected Mean Squares.

EMS Component	Description
Variance Components:	
$\sigma_e^2$	Variability between (sub)samples
$\sigma_{IHST}^2$	Additional variability due to repeated measurements
$\sigma_{HST}^2$	Additional variability due to split-plot differences
$\sigma_{ST}^2$	Additional variability due to main-plot differences
Fixed Treatment Effects:	Functions of the sums of squares for the respective means. When the null hypothesis is true, this parameter is expected to be zero for that effect. E.g. $\sigma_T$ is a function of the three overall (that is, marginal) means for the control, state and modified treatments.
$\sigma_T, \sigma_H, \sigma_{HT}, \sigma_I, \sigma_{IT}, \sigma_{IH}, \sigma_{IHT}$	

### 1.8.4 Calculation methods for power figure

The pre-harvest data from Table 2 on page 6-37 was:

Site Treatment:	Riparian	Upland	df of F-test	Sample size
Control	12.6 ± 0.9	13.2 ± 0.9	1, 30	16
Modified	13.3 ± 1.0	15.2 ± 0.8	1, 22	12
State	13.5 ± 1.2	16.3 ± 1.5	1, 20	11

The following power calculation was rather involved since a number of assumptions were required. It would have been much simpler to do with the original data.

Assumptions and calculation steps:

- 1) The denominator df of the F-test were evenly split between the two habitat types so that sample sizes for each mean are as shown above.
- 2) The authors appear to have treated this as a simple factorial design. In any case, only one error MS is available from the table of means and standard errors. This one error MS can be reconstituted by pooling the weighted squares of the standard errors:

$$SS = 16(15)[0.9^2 + 0.9^2] + 12(11)[1.0^2 + 0.8^2] + 11(10)[1.2^2 + 1.5^2] = 1011.18$$

with df = total no. of observations – df for factorial tests = 78 – 5 = 73.

Thus the MS = SS/df = 1011.18/73 = 13.85 with df = 73.

- 3) The third factor, the two pre-harvest data collection times will be ignored, treated as subsamples and pooled with the subsampling error. The resulting ANOVA table should have had three error terms instead of one:

Source of Variation		df	Expected Mean Squares	Mean Square
Mainplot:	T	t-1 = 2	$\sigma_e^2 + e \sigma_{HST}^2 + eh \sigma_{ST}^2 + ?? \sigma_T^2$	MS <sub>T</sub>
Error:	S(T)	t(s-1) = 15	$\sigma_e^2 + e \sigma_{HST}^2 + eh \sigma_{ST}^2$	MS <sub>S<sub>T</sub></sub>
Split-plot:	H	h-1 = 1	$\sigma_e^2 + e \sigma_{HST}^2 + ?? \sigma_H^2$	MS <sub>H</sub>
	HT	(h-1)(t-1) = 2	$\sigma_e^2 + e \sigma_{HST}^2 + ?? \sigma_{HT}^2$	MS <sub>HT</sub>
Error:	HS(T)	hs(t-1) = 15	$\sigma_e^2 + e \sigma_{HST}^2$	MS <sub>HS<sub>T</sub></sub>
Subsampling error:		hst(e-1) = 43	$\sigma_e^2$	MSE
Total		hste-1 = 77		

- 4) Since the subsampling error df = h\*s\*t\*(e-1) = 2\*6\*3\*(e-1) = 43 then the average subsample size used was  $e_{used} = 43/(2*6*3) + 1 = 2.2$ .
- 5) The reconstituted error MS is a weighted sum of  $\sigma_e^2$ ,  $\sigma_{HST}^2$ ,  $\sigma_{ST}^2$  but with unknown weights. Since we might expect that MS<sub>S<sub>T</sub></sub> > MS<sub>HS<sub>T</sub></sub> and MS<sub>HS<sub>T</sub></sub> > MSE let us suppose that MS<sub>S<sub>T</sub></sub> = R3\* MS<sub>HS<sub>T</sub></sub> and that MS<sub>HS<sub>T</sub></sub> = R2\*MSE where the ratios R2 and R3 are greater than one. Further, let us suppose that MSE = R1\*MS with R1 less than one since the subsampling error should be less than the average pooled variance. Then we can estimate the variance components by:

$$\sigma_e^2 = R1 * MS$$

$$\sigma_{HST}^2 = (R2-1) * R1 * MS / e_{used}$$

$$\sigma_{ST}^2 = (R3-1) * R2 * R1 * MS / (e_{used} * h)$$

For sample size calculations, these estimates can be plugged into the expected means squares for the error terms in the ANOVA table above to get an estimate of the mean square for the error term.

Suppose that we are interested in the power for a simple contrast or planned comparison between two of the mainplot treatments: the state and modified riparian management zones. The error term for the corresponding F-test will be a mean square estimated by  $\sigma_e^2 + e \sigma_{HST}^2 + e h \sigma_{ST}^2$ . The numerator for the contrast F-test is:  $\{(Difference\ between\ the\ State\ and\ Modified\ Means) * (e * h * s / 2)\}^2$ .

A plot of the power against this difference for a range of sample sizes is presented in Figure 9. The ratios used were R1 = 0.8, R2 = 1.0, and R3 = 1.6.

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### **1.10 Schedule**

The following charts represent LGL's proposed work plan for this project. The amount of time allocated for field work and report preparation will allow adequate time for the collection and analysis of all data, preparation of all required reports, and the development of manuscripts for publication in peer-reviewed journals.

2001 / 2002 Component	2001												2002														
	December		January		February		March		April		May		June		July		August		September		October		November		December		
	15-31	1-15	16-31	1-15	16-28	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-31		
Contract Award																											
Project Initiation																											
Revised Study Plan																											
Study Site Field Trip (if requested)																											
Field Setup																											
Vegetation (east / west)																											
Breeding Birds (east / west)																											
Stream Amphibians (west)																											
Terrestrial Amphibians (east / west)																											
Small Mammals (east)																											
Small Mammals (west)																											
Bats (east and west)																											
Quarterly Reports																											
Annual Report																											

Figure 10. Proposed work plan for year 1 (2001) and year 2 (2002). There will be no annual report for 2001 & 2002 as most of field work comes after 30 June.

2003 Component	2003																										
	December		January		February		March		April		May		June		July		August		September		October		November		December		
	15-31	1-15	16-31	1-15	16-28	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-31		
Vegetation (east / west)																											
Breeding Birds (east / west)																											
Stream Amphibians (west)																											
Terrestrial Amphibians (east / west)																											
Small Mammals (east)																											
Small Mammals (west)																											
Bats (east and west)																											
Quarterly Reports																											
Annual Report																											

Figure 11. Proposed work plan for year 3 (2003). The annual report will be submitted on or before 30 June 2003 and will report on 2002 field work.

2004 Component	2004																										
	December		January		February		March		April		May		June		July		August		September		October		November		December		
	15-31	1-15	16-31	1-15	16-28	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-31		
Vegetation (east / west)																											
Breeding Birds (east / west)																											
Stream Amphibians (west)																											
Terrestrial Amphibians (east / west)																											
Small Mammals (east)																											
Small Mammals (west)																											
Bats (east and west)																											
Quarterly Reports																											
Annual Reports																											

Figure 12. Proposed work plan for year 4 (2004). The annual report will be submitted on or before 30 June 2004 and will report on 2003 field work.

2005 Component	2005																										
	December		January		February		March		April		May		June		July		August		September		October		November		December		
	15-31	1-15	16-31	1-15	16-28	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-30	1-15	16-31	1-15	16-31	1-15	16-31		
Quarterly Reports																											
Annual Reports																											
Draft Final Report																											
Manuscripts																					*						
Workshop																											
Final Report																											

Figure 13. Proposed work plan for year 5 (2005) and project completion. The annual report will report on the 2004 field season and the draft final report will report on all years of this study

\*Submission of manuscripts suitable for publication in peer-reviewed journals will accompany the final report and will also be developed after acceptance of the final report by DNR.