

STUDY PROPOSAL

EFFECTS OF VEGETATION RETENTION ON THE DENSITY, BODY CONDITION, AND DISTRIBUTION OF STREAM-ASSOCIATED AMPHIBIANS

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Abstract – The 2 primary short-term effects of timber harvest on stream breeding amphibians appear to be: 1) reduction of shade, and 2) increased sediment inputs. This study will assess the effects of shade reduction on stream amphibians with a multiple treatment, before-after, control-impact design. Stream reaches will be randomly assigned to treatments (4 levels of shade retention) in 6-7 blocks (n = 24-28), following 2 years of data collection. An additional 2 years of post-treatment data will also be collected. Response variables include the abundance and body condition of free-ranging amphibians, and growth rates and spatial organization of larvae held in in-stream enclosures. In addition, water temperature and stream productivity will also be measured. The results should provide valuable information to 2 of CMER's top-ranked programs; Type N Buffer Characteristics and Integrity (#1) and Type N Amphibian Response (#3). The proposed study is validation research and also compliments CMER's effectiveness monitoring approach.

INTRODUCTION

Several factors confound estimating the effects of timber harvest on stream associated amphibians (SAA). Habitat is patchy and most sampling schemes generate large variances in abundance and density estimates. In addition, harvest appears to have 2 main effects – reduction in vegetation cover and increases in fine sediments – both of which geologic parent material, aspect, and stream gradient can either ameliorate or exacerbate.

Several studies have examined the effects of timber harvest on SAAs (Murphy and Hall 1981, Bury and Corn 1988, Corn and Bury 1989, Bury et al. 1991, Kelsey 1995, Bull and Carter 1996, Diller and Wallace 1996, 1999, Wahbe 1996, Wilkins and Peterson 2002, Steele et al. 2002, Russell et al. *in review*). Collectively, the results of these studies are contradictory and generate much uncertainty. Several factors likely contribute, including: 1) most studies were retrospective, lacked “controls” and replication, lacked randomization, and were confounded by interactions between the effects of harvest and abiotic factors, 2) most examined gradients in stand conditions, but analyzed the data in discrete categories, 3) most analyses were conducted with a null hypotheses framework despite violation of most of the statistical assumptions, and 4) a lack of attention to the range in scale at which the main effects may be operating. Some studies

suggested negative effects of timber harvest and others not. However, the importance of interactions among geologic parent material, substrate composition, and stream gradient has emerged as a general principle.

The productivity of headwater streams in most regions of the Pacific Northwest appears limited due to dense vegetation shading (Murphy 1998). A number of studies have indicated that vegetation removal increases stream productivity with positive effects on invertebrates and vertebrates (Murphy and Hall 1981, Hawkins et al. 1983, Bisson and Sedell 1984, Bilby and Bisson 1987, Holtby and Scrivener 1989, Murphy 1998, MacCracken 2002). Bisson and Bilby (1998:388) suggested that "trophic pathways that support salmonids have actually been enhanced by timber harvest in these headwater areas". Despite these findings and conclusions, most regulators and scientists suggest that headwater streams need full shade, at least on some portions.

Few studies have directly examined the effects of shading on SAAs (Hawkins et al. 1983, MacCracken 2002). Hawkins et al. (1983) found no effect of shading on the density and mass (gm/m^2) of *Dicamptodon ensatus* (= *D. tenebrosus*). MacCracken (2002) found lower densities, but greater mass/individual (i.e., body condition) for *Rhyacotriton kezeri* in streams with reduced vegetation cover. In addition, Wahbe (1996) reported intermediate densities and greater mass/individual for *Ascaphus truei* tadpoles in streams in clearcuts when compared to old growth, 2nd growth, and buffered streams in clearcuts. All of these studies were retrospective and not replicated.

Reduction of shade in light-limited headwater streams should have beneficial effects at all trophic levels. However, Murphy (1998) suggested that full sunlight might be detrimental and that an optimal level of shading may exist. In addition, MacCracken (2002) suggested that lower densities and better body condition of *R. kezeri* may be due to larger-bodied individuals displacing smaller conspecifics from food rich patches created by shade removal. The purpose of this study is to test those and other competing hypotheses (Chamberlin 1897) with a manipulative experiment. Four to 6 SAAs will be studied (*D. tenebrosus*, *D. copei*, *R. cascadae*, *R. kezeri*, *R. olympicus* and *Ascaphus truei*). The last 4 are FFR species. The response variables of primary interest are SAA abundance, SAA distribution, and SAA body condition. In addition, estimates of water temperature and stream productivity will be made to link SAA response to treatment effects on those variables. The hypotheses to be addressed (with predictions) include:

1) no effects of vegetation removal exist.

Because most studies were simple retrospective sample surveys in small areas over a short time period, it is quite possible that their results are spurious, e.g., conditions found post-harvest were the same that existed pre-harvest. In addition, most studies did not control for aspect, substrate composition, stream gradient, and parent geology, thus confounding those factors with vegetation removal.

Prediction: variation in the response variables are not related to shade levels.

2) shade removal increases or decreases habitat quality (i.e., food abundance) for SAAs.

Because of the above, and the conflicting results of some studies, statements about the direction and magnitude of the effect of shade removal can not be made with much certainty. This hypothesis will be assessed by sampling SAAs for abundance, growth rates, and body condition in treated and reference reaches.

Prediction: SAA metrics are negatively or positively related to shade levels.

3) an optimum level of shading exists.

Murphy (1998), one of the leading experts on stream productivity in the Pacific Northwest, proposed this hypothesis. However, he did not speculate as to what an optimum level may be. This will be estimated by randomly assigning 3 levels of shade retention to experimental stream reaches.

Prediction: response variables will exhibit a non-linear (e.g., asymptotic or curvilinear) relationship to shade levels.

4) shade levels and food resources for SAAs are inversely related and larger individuals exclude smaller conspecifics from more open patches.

MacCracken (2002) proposed this as a potential mechanism. This will be assessed by maintaining individuals in in-stream enclosures in both treatment and control reaches and monitoring body condition, growth rates, and spatial distribution within the enclosures.

Predictions: spatial segregation as proposed will occur only in the enclosures in the treatment reaches and may also exhibit a non-linear relationship with shade. Based on the pilot study (see Appendix), more individuals will occur in the upstream portion of the enclosures in the control reaches, but there will be no differences in mass/individual between individuals in enclosure halves.

Sampling of free ranging populations in the study reaches may also provide information pertinent to the distribution-body condition relationship and serve as controls for any potential effects of the enclosures.

Predictions: SAA abundance will decline in the treated reaches, but the body condition index will be greater than in the control reaches. SAA abundance will not change in the control reaches and the body condition index will be lower than in the treatment reaches.

STUDY AREA

The study area includes Longview Fibre Company lands in Clatsop County, Oregon and Skamania and Wahkiakum counties and DNR properties in Mason county, Washington. Sampling on Longview Fibre lands began in 2004. Sampling on the DNR lands in Mason county began in 2006. *D. tenebrosus* occur in Clatsop, Skamania, and Wahkiakum, Counties. *D. copei* are found in Clatsop, Skamania, Wahkiakum, and portions of Mason counties. *R. cascadae*,

are found only in Skamania county, *R. kezeri* in Clatsop and and Wahkiakum counties, and *R. olympicus* in Mason county. *Ascaphus truei* may be found in all areas. Animals for introduction to the enclosures will first be taken from the stream reach between the treatment and reference reaches, then from below the treatment reach, or from the nearest stream not used in the study. Each stream reach will have at a minimum, enclosures with, *A. truei*, one species of *Dicamptodon*, and one species of *Rhyacotriton*.

METHODS

Treatments

The study will use a randomized block, before-after, control-impact (BACI) design. Blocks are distinct areas within the range of the 3 species of *Rhyacotriton* that occur in Washington. Blocking factors include elevation, forest type, disturbance history, and management regime. Four streams will be sampled in 6-7 blocks ($n = 24-28$, 1 block in each County, except Skamania which has 2). Each stream will be divided into a 50-m treatment reach and an upstream 50-m control reach. Control and treatment reaches will be ≥ 50 m apart. Reach size is based on the desire to control for aspect, gradient, and substrate; SAA life history characteristics; the feasibility of treatment application and maintenance; and forest practices permitting requirements (Class 1, slash control). Each reach in each stream will be sampled in summers 2004, and 2005 (pre-treatment), 1 of 4 treatments (0%, 25%, 50%, and 75% shade retention) will be randomly assigned to a treatment reach in each block (6-7 replicates) and applied during fall, winter, and spring 2005-2006. Each reach will then be sampled again in summers 2006, and 2007 (post-treatment).

Streams selected for study will have southerly aspects (between 135° - 235° , where the effect of shade removal is expected to be greatest), cobble-gravel dominated substrates, $\geq 10\%$ gradient, and on basalt or competent sandstone parent material. Shade (canopy cover) will be estimated with a spherical densiometer at the water level and breast height (1.4 m). Cover at both levels will be reduced to specified levels by removing the appropriate amount of shrubs and trees based on densiometer estimates. Densiometer readings will be taken at 5-10-m intervals along each stream reach. Light intensity in each reach at the waters surface will also be measured with a light meter at the same points as densiometer readings. In addition, canopy photographs will also be taken at each point and analyzed for shade with software developed by Frazer et al. (1999).

Response variables

Amphibian abundance

Amphibian abundance in each reach will be estimated during low flow periods (August-October) each year. Each reach will be divided into 25 2-m long plots (width = the wetted channel). Ten plots will be randomly picked for sampling and sampled in the upstream direction. Plots will be sampled by blocking the lower edge with fine mesh screen and removing all wood and

cobble, then raking the gravel and fines with a potato rake while holding a dip net below during the search. All material removed from the stream will be replaced and the banks searched for SAAs that may have left the channel.

All individuals captured will be marked with a fluorescent elastomer injection in order to distinguish between treatment and control reaches. It will not be necessary to provide individual recognition for this part of the study, but marking may allow for estimates of possible movements between reaches.

Amphibian body condition

Amphibian body condition will be indexed by the residuals of a regression of mass on snout-vent or total length (Green 2001). Each individual captured during the above sampling will be weighed with an electronic balance to the nearest 0.01 g and snout-vent and total length measured to the nearest 0.1 mm with calipers. All amphibians will be released at the point of capture.

Amphibian growth rates, development, and spatial distribution.

Growth rates, development, and spatial organization will be estimated by introducing 4-6 individuals of each species to separate in-stream enclosures in both treatment and control reaches. These individuals will receive a unique fluorescent elastomer mark. Enclosures will be made of plastic boxes (54 x 48 x 13 cm, 0.24 m²) placed in the stream in June. Water flow and detritus input will be maintained using 2.5 cm diameter PVC pipe, 2 upstream for inputs and 2 as outlets, and numerous holes (1 mm) drilled in the bottom and sides. Each enclosure will have enough gravel/cobble from the immediate stream location to completely cover the enclosure bottom. Measurements of mass and length for each individual will be made every 7-14 days as well as development stage. The spatial organization of individuals will be assessed by dividing each enclosure into quarters and noting the position of each individual prior to taking the physical measurements (see Appendix). At the end of the season, all detritus in the enclosures will be collected by quarter and ash-free dry mass (AFDM) estimated.

Water temperature

Water temperature will be monitored in each reach in each stream from June – October each year. Onset Stowaway data loggers will be programmed to record water temperature every 30 minutes. Stowaways will be placed at the bottom of each treatment and reference reach. Daily maximum, daily minimum, and absolute daily temperature change are the primary variables of interest. These data will estimate the effects of shading on water temperature and the effects of water temperature on SAA growth and body condition.

Stream productivity

Reach productivity will be estimated with 2 methods. Eight unglazed ceramic tiles will be placed in both reaches and scraped of algae and other organisms twice/month from June-October (Wipfli et al. 1998, Rosemond et al. 2000, Kiffney and Richardson 2001). In addition, drift weirs (Wipfli and Gregovich 2002) will be established at the top and bottom of each reach and operated for a 24 hr period each month from June-October. Samples will be divided into plant, invertebrate and vertebrate material and weighed to the nearest 0.01 g, dry weight.

Enclosure controls

The enclosures alone may also have an effect on water temperature and stream productivity. To assess this possibility, 1 enclosure/reach will be established as described above, but amphibians will not be introduced. However, 4 unglazed tiles and a stowaway temperature logger will be added to each and sampled as described above. In addition, detritus will be collected at the end of the season and analyzed as described above.

Hypotheses evaluation and data analysis

The replicated, randomized block BACI design should resolve issues associated with sampling artifacts and possible spurious results. In addition, sampling streams with the aspect, substrate, gradient, and parent material specifications should eliminate confounding the effect of shade removal with those abiotic factors.

Dr. Howard Stauffer, Humboldt State University, is the consulting statistician for this project. The data analysis will consist of 3 corroborative approaches: 1) ANOVA as described by Underwood (1994) for BACI designs with an emphasis on the interaction terms, 2) mixed-effects modeling with treatments (shade levels) as fixed effects, blocking factors as random effects, and covariates (e.g., water temperature), and 3) Bayesian modeling based on the mixed-effects approach, but relaxation of the normality assumption, incorporation of priors, and a Bayesian interpretation of the results. In addition, the multiple treatment approach (Steury et al. 2002), as applied in this study, has the potential to uncover threshold responses that should be evident in plots of the data as well as the fit of regression models, e.g., linear, asymptotic, or curvilinear.

Longer-term responses

Long-term responses to any treatment by SAAs are of concern due to life history characteristics that include several years to metamorphosis from aquatic to semi-aquatic/terrestrial stages and sexual maturity. Following 2 years of post treatment data collection and analyses, an assessment of the critical questions associated with longer term effects will be made to determine if continuing to maintain the treatments and additional data collection would be warranted. Four-6 years post-treatment would be long enough for most individuals that were sampled pre-treatment to have metamorphosed and reached sexual maturity (Nussbaum et al. 1983).

RELEVANCE TO CMER PROGRAMS

The Type N Buffer Characteristics, Integrity, and Function Program was given the highest priority by CMER in December 2002. The Type N Amphibian Response Program was ranked third. The proposed research will provide valuable information to both programs. The current direction of the Buffer Characteristics and Amphibian Response Programs is effectiveness monitoring of Type N buffers. Shade reduction due to blowdown or other sources of tree mortality is one of the hypothesized changes that may affect water temperature and amphibian viability. The CMER effort at this point in time is largely a passive monitoring approach. Only by coincidence will that effort be able to provide information on the effects of those hypothesized changes, which will largely be unpredictable in space and time and highly variable. However, the proposed study will directly address the effects of changes in levels of shading and bridge the gap between passive monitoring and the implications of the monitoring results.

The Monitoring Design Team of CMER proposed that most validation research will occur in the intensive monitoring program. The current scenario is that the intensive monitoring program will be a collaboration among CMER and other entities sharing the cost of such a program. The proposed project is validation research that addresses some of the assumptions behind the Type N buffer strategy as related to water temperature and amphibian viability. It will be a collaborative effort among Longview Fibre Co., CMER, WDFW, and potentially other landowners. In addition, this study coupled with the results of other CMER intensive monitoring will provide valuable insight on the effects of the spatial extent of change on the response variables. The reach length of this study is similar to that of the Type N patch buffers.

Management implications

Shading provided by Type N patch buffers may change through tree blowdown and mortality associated with edges (Chen et al. 1995). The proposed project will provide information on the effects of such change on amphibians, stream productivity, and water temperature. Identification of thresholds for those variables in response to shade levels will provide some of the basis for developing and testing alternative buffer designs and may suggest more efficient and effective buffer prescriptions.

A number of studies on several taxa have shown that relative abundance and density estimates are often not related to habitat quality (Van Horne 1983). The enclosure experiments will test relationships between some aspects of habitat quality (i.e., irradiance and food resources) and species spatial organization and whether abundance estimates are positively related to habitat quality, which has implications for the interpretation of past research and the design of future studies.

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APPENDIX

Pilot study results

A pilot study was conducted from 11 July – 4 October 2002. The primary purpose of the study was to test the feasibility of maintaining SAAs in in-stream enclosures. A headwater stream in Skamania County at about 732 m elevation that contained both *Ascaphus truei* and *Dicamptodon* spp. with a north-east aspect (28°), cobble-gravel substrate, and 15% gradient was selected. Two enclosures each were placed in both an open stream reach (3m long, 49% canopy cover,) and a shaded reach (7m long, 94% cover). Gravel and small cobble were added to each enclosure to completely cover the bottom. Five-7 first and second year *A. truei* tadpoles (Metter 1967) were introduced into each enclosure on 11 July, plus 1 adult frog. In addition, 1-3 *Dicamptodon* spp., of various sizes (snout-vent length [SVL] 29-53 mm), were also introduced to each enclosure. All individuals were weighed and SVL and total length (TL) measured. A water temperature logger was set at the bottom of each reach on 11 July.

The maximum and minimum water temperatures recorded between 11 July and 3 October were the same for the shaded and open reach. The maximum was 14 C and the minimum was 7 C. In addition, the average daily maximum was 12(0.1) C and the average daily minimum was 11(0.1) C for both reaches. These results are not surprising given the small reaches sampled and their close proximity.

Enclosures were visited every 7-10 days and the position of individual animals recorded in each quarter (i.e., upstream left, upstream right, downstream left, etc.). In addition, individuals were weighed and measured on every other visit.

Individual *A. truei* were missing from the enclosures on 49% of the visits (21% was the loss of 1 individual), and 1 or 2 individuals were gained 8% of the time. All the adult frogs apparently jumped out of the enclosures. Tadpoles entered the enclosures when screening over the intake pipe was temporarily removed.

There was no change in *Dicamptodon* spp. numbers in the enclosures on 79% of the visits. The largest individual escaped between the 1st and 2nd visit. One small individual was found dead with $\frac{3}{4}$ of it's tail missing – it was in an enclosure with another individual twice it's size.

These results suggest that only 1st year *A. truei* should be used in the study and that it may be necessary to occasionally augment the enclosures with new individuals. This should not hinder the assessment of the spatial distribution of *A. truei* and it will be possible to account for this in the analysis of growth rates and body condition. In addition, it will be necessary to use *Dicamptodon* spp. and *Rhyacotriton* spp. of the same approximate size in any enclosure. Also, species should probably not be mixed in an enclosure.

Due to the small number of *Dicamptodon* spp., the loss of adult *A. truei*, and the fact that metamorphosing tadpoles decline in weight and size, the following analyses were conducted only on the 1st year *A. truei*. The spatial

distribution of tadpoles differed ($\chi^2_3 = 20.02$, $P = 0.002$) by reach. In the shaded reach, more tadpoles were counted at the upstream (inlet) portion of the enclosures than at the downstream portions. However, tadpole distribution in the open enclosures was more uniform (Table 1).

Table 1. Counts and mean mass/individual (g) of first year *Ascaphus truei* tadpoles in enclosures in shaded and open reaches of a stream in southwest Washington.

Enclosure quarter	Shaded		Open	
	Number	Mass (SE)	Number	Mass (SE)
Upstream left	37	0.4(0.04)	18	0.5(0.05)
Upstream right	30	0.5(0.04)	34	0.6(0.04)
Downstream left	8	0.4(0.08)	30	0.7(0.04)
Downstream right	8	0.5(0.07)	13	0.6(0.07)

The mean mass/individual was greater (ANOVA_{1, 78}; $P = 0.007$) in the open reach (0.6 g) than the shaded reach (0.4 g). However, mean mass was similar (ANOVA_{3, 78}; $P = 0.3$) among enclosure quarters (0.5 g). In addition, there was no reach x quarter interaction (ANOVA_{3, 78}; $P = 0.3$).

In general, the residuals (body condition index) of a regression of mass on total length ($r = 0.70$, $r^2 = 0.48$, $P = 0.0001$, $\beta_0 = -0.92[0.16]$, $\beta_1 = 0.04[0.004]$) were consistent with the above analyses. The mean (SE) of the residuals for the open area was 0.02(0.02) and for the shaded reach was $-0.03(0.01)$. This indicates that when body size is accounted for, tadpoles in the open reach had greater mass than those in the shaded reach. If greater mass is indicative of greater energy stores and greater energy stores is related to individual fitness (i.e., lifetime reproductive output), then the open reach provided *A. truei* tadpoles with higher quality habitat.

The predicted response that individuals would be spatially segregated in the open enclosures with larger individuals displacing smaller individuals does not appear to be supported by the data. However, if the data are summarized by upstream and downstream halves of the enclosures and the mean mass/individual is also considered, the results are consistent with the prediction for the open area (Table 2), i.e., the downstream ½ contained fewer, but larger individuals. Due to water flow characteristics in the enclosures, more detritus accumulated in the downstream ½ (this was much more apparent for open enclosures) suggesting that the downstream ½ would have greater food resources. In addition, algal growth was noticeably more abundant in the enclosures in the open reach.

Table 2. Counts and mean mass/individual (g) of first year *Ascaphus truei* tadpoles in enclosures in heavily and moderately shaded reaches of a stream in southwest Washington.

Enclosure half	Shaded		Open	
	Number	Mass (SE)	Number	Mass (SE)
Upstream	67	0.5(0.03)	57	0.5(0.04)
Downstream	16	0.4(0.03)	43	0.6(0.03)

No prediction about the spatial distribution of tadpoles in the shaded reach was made. The distribution observed indicates spatial segregation, but the mean mass/individual did not differ between the 2 groups. The higher numbers in the upstream portion suggest that organic inputs to the enclosures may be important, particularly if algae were limited throughout the shaded enclosures. There was a noticeable difference in algae abundance in the shaded and open enclosures by 1 August, with rocks in the open enclosures supporting more algae.

Growth rates (g/14 days) for the shaded reach averaged 0.0 and for the open reach 0.1 ($t = -1.0$, $p = 0.4$). Mean mass/individual in shaded enclosures was 0.4 g on 26 July, increased to 0.5 g on 22 August, then declined to 0.4 g on 18 September. For the open reach, mean mass/individual was 0.5 g on 26 July and increased to 0.6 g by 4 September and to 0.7 g on 4 October. These data also suggest that the open reach was more productive.

The results of this pilot study are limited. The stream reaches studied were small, and only 2 enclosures/reach were sampled. In addition, the effects of reduced shade would be expected to be minimal given the aspect and elevation of the stream. However, the pilot study indicates that it is possible to keep SAAs in the enclosures and that the probability of obtaining meaningful results is high.