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Port Angeles, WA

Predicting seed germination in the sediments of Lake Mills after removal of the Glines Canyon Dam on the Elwha River

Natural Resource Report NPS/MWR/HTLN/NRR—2007/001

ON THE COVER
Seedling emerges from the Lake Mills sediments, June 2005
Photograph by: Jennifer Nicole Chenoweth

Predicting seed germination in the sediments of Lake Mills after removal of the Glines Canyon Dam on the Elwha River

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Abstract

Plant succession in the reservoir basins after dam removal on the Elwha River will depend on the species available to colonize and thrive in the lacustrine sediments that have accumulated in the reservoirs for the last 80+ years. The first species to successfully germinate and establish on newly exposed soil surfaces have a significant impact on the trajectory of ecosystem succession. This report studied the seed bank and the species performance of two native species in the fine sediments from Lake Mills.

The first species available to colonize disturbed areas often come from a soil seed bank. To determine if the reservoir sediments have a readily germinable, persistent seed bank, sediments from Lake Mills were collected at 91 sites from 5 different transects bisecting the lake. Sites were designed to sample different lake depths to determine the relationship between water depth and germinable seed density. An additional 10 sites along a stretch of shallow shoreline were sampled six times over a period of 8 months to determine if there was a seasonal fluctuation in seed bank densities (referred to as a transient seed bank). Overall, the sediments did contain a seed bank of mostly native species, but the mean densities of germinable seed were low and decreased significantly with water depth. There was significant spatial heterogeneity of seed densities. The overall mean density was 144 ±24 seeds/m^2. The transient seed bank samples revealed no statistically significant seasonal fluctuations in seed densities. The transient samples had a mean of 480 ±74 m^{-2}. These results appear to be typical for lentic seed banks, where viable seed densities peak in shallow areas and decrease with depth. Lakes with seasonally fluctuating shorelines often contain a diversity of emergent and submersed plant species that contribute to high density seed banks. Lake Mills does not fluctuate significantly or with any seasonal regularity, and the shoreline does not contain a diversity of herbaceous, emergent plant species. Large numbers of seeds are likely falling into the reservoir, but the seed rain is probably dominated by forest species with short-lived seeds not adapted to anaerobic conditions.

There is over 13 million cubic yards of sediment that has accumulated in Lake Mills since the Glines Canyon Dam was installed (USDI, 1995). Nearly 5.3 million cubic yards of the sediments are fine textured, and it is anticipated that the physical characteristics of the fine sediments will "present inherent challenges and limitations to revegetation efforts" (USDI, 1994). When fine textured soil surfaces dry up, a crust is formed which resists water uptake. Sediment size may also dictate the life forms likely to establish on the surface. Woody plants may not establish readily, while the literature suggests grasses will have no difficultly establishing on fine sediment substrates (Grubb, 1977). The ability of seeds to germinate and establish on fine textured, smooth surfaces is enhanced by safe sites (Harper, 1977; del Moral and Wood, 1993; Walker and del Moral, 2003). I tested the effects of the fine sediments from Lake Mills on the germination rates of a native grass, *Elymus glaucus,* and a native woody plant, *Alnus rubra.* Four sediment treatments were created to test the effects of moisture and surface roughness on seed germination and establishment rates. Treatment one, the dry/smooth treatment, consisted of dried sediments with a smooth, homogeneous surface. Treatment two, the dry/safe site treatment, contained dried sediments with a surface indented with safe sites: 36 small depressions, one for each seed. Treatment three, the moist/smooth treatment, consisted of flats containing moist sediments (sediments not allowed to dry out prior to seeding). The surface of this treatment was smooth. Treatment four, the moist/safe site treatment, consisted of moist sediments with the same safe

site depressions as treatment two. Across all treatments, *Alnus rubra* germination rates were low, with a mean of 6%. This may be the result of the overall effect of the fine textured sediments on germination rates. The fine sediments never dried out during the three month study, and water from the misting bench would often pool on the surface of the sediments, creating temporary anaerobic conditions that are known to limit *Alnus rubra* establishment (Harrington, 1994; Dobkowski, 1994). *Elymus glaucus* had no difficulty germinating in any of the treatments, and had a mean germination rate of 62%. These results support the theory that grasses will out-perform woody seedlings in fine sediment substrates, regardless of initial moisture or surface roughness conditions.

Introduction

Two dams on the Elwha River in Olympic National Park (ONP), the Glines Canyon Dam and the Elwha Dam, will be removed to "restore, protect, and enhance the ecosystem, fisheries, and wildlife of the Elwha River Basin" (Elwha River Ecosystem and Fisheries Restoration Act, Public Law 102-495, 1992). Standing at 82 meters tall, the Glines Canyon Dam, a hydroelectric dam, will be the largest dam ever removed (Gregory et al, 2002). Although many smaller dams have been removed throughout the world, there are no empirical studies that can prepare the park for the ecological consequences of removing these two large dams.

Prior to dam construction, the land beneath Lake Mills was referred to as Smokey Bottom. The disturbance to Smokey Bottom created by the installation and subsequent removal of the Glines Canyon Dam after more than 80 years of operation will be severe. Dam removal will expose a landscape devoid of vegetation and covered by more than 10.6 million cubic meters of lacustrine sediments that have accumulated at the lake's bottom (USDI, 1995). Few natural disturbances, aside from volcanic eruptions, create such a denuded landscape. Until vegetation establishes in Smokey Bottom, ecosystem processes such as resistance to erosion, nutrient cycling, and water retention will be severely degraded. Erosion of fine sediments into the river may delay salmon restoration efforts, since excess fine sediments in rivers are known to clog critical spawning gravels and can directly kill salmonoids (Wood and Armitage, 1997; Shafroth *et al*, 2002). Ecosystem processes will remain degraded until vegetation reestablishes in the basin. Vegetation stabilizes ecosystem processes by reducing erosion and retaining water and nutrient resources on the landscape (Whisenant, 2003).

The rate of ecosystem recovery is directly related to the size of the disturbance (Pickett *et al.*, 1987; Walker and del Moral, 2003). Plant succession is slow after large disturbances, because fewer species are available to colonize the post-disturbance landscape relative to small disturbances. Pickett *et al* (1987) described three general causes of succession. The first cause, site availability, is the creation of open space for plants to establish following ecosystem disturbance. The size, severity, and the timing of the disturbance determine the availability of a site for particular species (Pickett *et al.*, 1987). The second cause, differential species availability, is the ability of species to disperse and colonize a landscape after disturbance. Diaspore dispersal depends on disturbance size and severity, and "species may be made available through persistence in the soil seed bank" (Pickett *et al.*, 1987). Soil seed banks provide an *in situ* source of species to colonize a site after disturbance, accelerating plant succession. The third cause of succession, differential species performance, concerns the eco-physiological ability of species to colonize a newly disturbed site (Pickett *et al.*, 1987). Differential species performance is determined by physiological adaptations such as germination requirements, life history traits, and the ability to endure environmental stress common to severely disturbed landscapes. Succession in the denuded Lake Mills basin is not expected be limited by site availability (Chenoweth *et al.*, 2007), so the rate and trajectory of primary succession will be determined by species availability and species performance.

Most large-scale disturbances do not have a homogeneous effect on the landscape (Turner *et al*, 1998; Walker and del Moral, 2003). The spatial variability of catastrophic disturbance often leads to a heterogeneous landscape with surviving patches of organisms within a denuded matrix

(Turner *et al*, 1998). Surviving organisms, or biological legacies, include remnant trees, woody debris, seed banks and viable root systems (Walker and del Moral, 2003). These biological legacies directly influence the rates and trajectories of succession. Sites with biological legacies recover more quickly than sites with few biological legacies left on the landscape (Halpern and Harmon, 1983; del Moral and Wood, 1993; Fastie, 1995; Turner *et al.*, 1998; Walker and del Moral, 2003; Keeton and Franklin, 2005). The only biological legacies that may be present beneath Lake Mills are dead standing trees, stumps and logging slash left *in situ* prior to damming of the river in 1927 (Winters, pers. comm.). The structure created by remnant stumps, snags and downed woody debris at Mount St. Helens after the 1980 eruption influenced geomorphic processes and provided safe sites for species to establish (Halpern and Harmon, 1983; del Moral and Wood, 1993; Franklin and MacMahon, 2000). Woody debris left in Smokey Bottom may provide the same function. It is not known if there is a viable seed bank within the accumulated sediments in Lake Mills. The lake basin is 4 km long and as much as 0.8 km wide; so much of the basin will be far from seed sources after dam removal. Unless viable seeds are present in the sediments, colonization of isolated sites will rely on long-distance seed dispersal and may be slow (Halpern and Harmon, 1983; del Moral and Wood, 1993). The presence of seed banks would provide an *in situ* source of species to colonize the basin, and could determine species composition and rates of early succession in the Lake Mills basin.

The third cause of succession described by Pickett *et al.* (1987), differential species performance, describes the ability of species to germinate, establish and thrive on a site. Substrate is a modifying factor of species performance and the unique substrate in Smokey Bottom will influence succession. Dam removal will reveal a valley covered in an estimated 10.6 million cubic meters of fluvial sediments (USDI, 1995). Most of the sediments are sands and gravels deposited into a dynamic complex of deltas where the Elwha River, Cat Creek and Boulder Creek converge at the southern end of Lake Mills. The deltas already support native upland plant communities and will not be actively rehabilitated by the park service (Chenoweth *et al.*, 2007). The inundated reservoir, over 142 hectares, is currently covered in at least 4 million cubic meters of fine sediments (USDI, 1995). The fine sediments are 6-12 meters deep in the southern reaches of the reservoir basin and are progressively thinner in the northern sections and towards the margins of the lake (USDI, 1995). These fine sediments are relatively uniform, with nearly 95% of the sediment clay and silt sized and a 5% component of fine sands (USDI, 1995). A chemical analysis of the fine sediments revealed a nutrient-poor substrate with little organic matter (Chenoweth *et al.*, 2007).

Substrate texture can influence successional trajectories. Several studies have demonstrated a relationship between substrate texture and plant species performance (Harper *et al.*, 1965; Keddy and Constabel, 1986; Smith *et al.*, 1995; Walker and del Moral, 2003; Naiman *et al.*, 2005). Grubb (1986) suggested that fine textured substrates favor colonization by graminoids over forbs or woody species. If the fine-textured sediments in Smokey Bottom favor grasses, rates of succession and the return of ecosystem processes to pre-dam levels may be delayed by slow colonization of woody species into the basin. Although grasses are known to provide some erosion control, an important ecosystem process, woody species have a greater influence over ecosystem processes in riparian ecosystems (Naiman *et al.*, 2005). Large woody debris provides key structural foundations that influence geomorphic landforms (Abbe & Montgomery, 2003), riparian forest formation (Montgomery & Abbe, 2005), and aquatic habitat diversity. Woody debris creates important in-channel features including pools, riffles, eddies, side channels, meanders and areas of cover for aquatic fauna (Apostol & Rae Berg, 2006). Aquatic fauna depend on riparian litterfall from woody species for external sources of energy (Naiman *et al.*, 2005). Without significant litter inputs in the river, the abundance and diversity of macroinvertebrates decreases and directly impacts riparian food webs, including top consumers such as salmonoids (Naiman *et al.*, 2005). It is vital to restoration efforts to understand the affects of sediment texture on succession and the differential establishment of plant life forms.

This report consists of two parts that will provide vital information on species availability and performance in the sediments from Lake Mills. The seed bank analysis will address species availability in the succession of Smokey Bottom. The germination experiment will address the species performance question by examining the germination rates and seedling mortality rates of two different growth forms, a grass and a tree species, in the fine sediments. The two species studied are potentially important species native to the Elwha watershed; *Alnus rubra* Bong., a seral woody species, and *Elymus glaucus* Buckl., a grass species.

PART I: Lake Mills Seed Bank

Methods

Study Design

To determine if there is a persistent, readily-germinable seed bank in the Lake Mills sediments, five transects were established across the lake from west to east in the summer of 2005. Each transect sampled the sediments at pre-defined depth categories. Depth categories were defined as (1) shallow sites sampled at a water depth of 0.8 meters (2) middle sites ranging from 1.5 to 4.5 meters (3) lower sites ranging from 5.5 to 10.6 meters and (4) deep sites ranging from 13.5-34 meters deep. Transects 1, 3 and 4 were sampled at all four depth categories. Transect two was sampled from the middle, lower and deep depth categories. Transect five was sampled only from the middle and lower depth categories from the western shores. The steep bathymetry made it unsafe to collect on the eastern shores. Depths greater than 1 meter were measured with a weighted Keson® Fiberglass Depth Gauge. Shallow-sample depths were measured with a wooden yardstick.

Each site was sampled at three points to capture variability at each site and to increase overall replication. This design provides samples from four depth categories to determine the relationship between germinable seed densities and water depth. The five transects also provide samples representing different distances from the Elwha River Delta.

Ten additional samples were collected directly from sediments exposed on the Elwha River delta. The Elwha River delta is estimated to contain 62% sand, 5% clay and silt, 31% gravel and 1% cobbles (USDI Bureau of Reclamation, 1995). Samples were randomly selected, and only one of the samples was collected beneath standing water at a depth of 0.3 meters. The remaining nine sites were likely inundated only seasonally. A total of 91 persistent seed bank samples were collected from the four depth categories, including samples collected from the exposed delta.

To determine if the sediments from Lake Mills contain a transient seed bank, sediment samples were collected along a 200-meter stretch of shoreline that had been sampled for the persistent seed bank study (Transect 3, site 1). This stretch of shoreline was far removed from the human impacted boat launch and it was accessible from the West Lake Mills Trail. Accessibility was an important quality since sampling dates occurred throughout the winter months. The site was sampled in ten locations parallel to the shoreline once every five weeks from October 2005 to April 2006. This produced six sampling events with ten samples each for a total of 60 samples. Samples were collected at a constant depth of 0.8 meters. Sample sites were spaced six meters apart. Each site was marked on the shoreline by a small flag. Repeat sampling of a single site occurred within a block, an area defined by a radius of one meter from the first sample taken in October. This design prevented re-sampling of the sediment from a single point, thus reducing the possibility of depleting viable seed in any one location. The design provides a dataset appropriate for analysis using Repeated Measures statistics where n = site and k = month collected. Each month there were 10 samples (n=10, N=60) and samples were collected 6 separate months (k=6).

To compare lacustrine and terrestrial seed banks, the forest soils were sampled at ten sites perpendicular to the lacustrine sediment sites. Sites close to the lacustrine samples are exposed to similar terrestrial seed rain, and may reveal similar species in the seed bank. Soil seed bank samples were collected on October 30th, 2005.

Collection Methods

Numerous collection methods are employed to study persistent and transient seed banks (Thompson and Grime, 1979; Leck *et al*, 1989; Gross, 1990; Brown, 1992; Ter Heerdt *et al*, 1996). Most use coring devices designed to preserve soil stratigraphy. Preserving soil stratigraphy has obvious advantages; viable seeds are generally concentrated in the top layers of soil and litter (Harper, 1977; Nicholson and Keddy, 1983; Leck, 1989; Hills and Morris, 1992; Baker, 1994; Abernethy and Willby, 1999; de Winton *et al.*, 2000; McGowen, 2004). Preserving soil stratigraphy also allows the researcher to investigate the history of seed input patterns into the soil (Leck and Graveline, 1979; Nicholson and Keddy, 1983; de Winton *et al.*, 2000). Preliminary attempts with a coring device failed, since saturated sediments dominated by high contents of silts and clays were difficult to trap and contain (Olson, pers comm.). Therefore, soil-coring devices were not used to collect samples for this study.

Three different collection methods were used to sample the various lake depths. Each method was designed to capture the top 12 cm of sediment. The upper 12 cm of soils usually contains most of the viable seed in soil seed banks (Harper, 1977; Nicholson and Keddy, 1983; Leck, 1989; Hills and Morris, 1992; Baker, 1994; Abernethy and Willby, 1999; de Winton *et al.*, 2000; McGowen, 2004).

Sediment samples from the shallow depths were collected using an 46 cm sharpshooter shovel. The sharpshooter shovel has a narrow blade ideal for collecting sediment trapped between obstructions such as woody debris, which was abundant at shallow sites. Samples from the western shore of Lake Mills were collected on June 24th, 2005. Samples from the eastern shore of the lake were collected on July 3rd, 2005. Shallow samples were collected from only 3 of the 5 transects due to the limited greenhouse space. A total of 18 samples were collected from 6 shallow sites.

National Park Service certified scuba divers collected sediment samples from depths between 1.5 and 10.6 meters. The method was developed by Rich Olson of Olympic National Park, Natural Resource Management Division. At each site, three buckets weighted by rocks were attached to buoys approximately 7.6-9 meters apart. The wide bucket spacing was necessary to avoid low visibility problems created by sediment turbidity during sampling. The divers filled each bucket with sediment by shallowly dragging the lip of the bucket no deeper than 12 cm into the sediment. Once a bucket was filled, it was raised to the surface using a crab-pot puller attached to a boat. Buckets were then immediately covered to prevent contamination by wind-blown seed. Transects 1-3 were sampled on May 16th, 2005. Transects 4 and 5 were sampled on May 18th, 2005. Divers collected a total of 55 samples from the middle depths.

The deep sites were below safe diving levels (according to NPS policies). To sample from depths greater than 13 meters, a deep-lake dredge was dropped into the sediments three or four times per site to acquire five cups of sediment. This method has previously been used to sample lentic seed banks (Van der Valk, 1978). These sites were collected on May 26th, 2005. A total of 8 samples were collected from deep sites.

Ten coarse sediment sites were collected directly from the Elwha Delta (downstream delta). Sample collection methods from the delta were the same as those used at the shallow sites.

Collection methods for the transient seed bank study followed the same procedure as described above for shallow site. Only shallow samples (0.8 m) were collected for this phase of the study for several reasons. Preliminary data from the persistent seed bank study showed that germinable seed densities were higher in the shallow sites. This is consistent with a study by Keddy and Reznicek (1982), which found peak densities of germinable seeds between 0.6 and 0.9 meters deep in Matchedash Lake, Ontario Canada. Therefore, the shallow sites provided the best chance of observing a transient seed bank. The shallow sites also did not require the use of divers or boats and dredges, both of which would have been difficult on Lake Mills in the winter months.

Forest soil samples were collected using a small trowel. The soil was sampled from the top 12 cm of soil. Large litter (recognizable duff layer) was removed before sampling. Sample volume was 946 cm^3 resulting in an area per sample of 79 cm^2 (0.0079 m^2).

Germination Methods

Two general methods to estimate seed bank composition have been extensively studied; physical extraction (flotation, sieving, and air flow separation) and greenhouse seedling emergence (Simpson *et al*, 1989; Gross, 1990; Brown, 1991; Ter Heerdt, 1996). Brown (1991) compared extraction methods directly to greenhouse emergence and found that extraction detected more seeds but generally overestimated the viable seed bank, since seeds tallied using the extraction methods included dormant and dead seed as well as viable seeds. The study also found extraction produced highly variable results, requiring large sample sizes (Brown, 1991). Gross (1990) compared the two methods and found that the presence of large numbers of unviable seeds detected by extraction complicates conclusions about seed banks. This study concerns the role of seed banks in restoration of Smokey Bottom. Therefore only readily germinable seeds are of interest. The greenhouse emergence method is the best method to achieve this project's goals.

Species of seeds often require many different treatments in order to germinate. Cold stratification is a common sample treatment in seed bank studies if collection times occur in the summer months. The persistent seed bank samples were not cold stratified for this study. Collecting in May ensured that most seeds within the sediments had at least three months of exposure to temperatures necessary for cold stratification. River temperatures measured just upstream of Lake Mills ranged from a mean low of 3.9 C in February 2005, to a monthly high of 6.2 C in April 2005 (USDI, 2005). Typical cold stratification protocols are temperatures of 5° C for twelve weeks (Baskin and Baskin, 2001). Temperatures within the lake were not measured, but were unlikely to be more than a few degrees warmer than river flow during the winter months.

All samples were processed in the greenhouse within 48 hours of collection. Volume of sediment was kept constant. Each sample was 0.95 liters of sediment thinly spread into two 25.4 X 25.4 cm pots pre-filled with a 4 cm deep layer of a sterile, soil-less mixture. The soil-less mixture was

70-80% aged Douglas fir bark, 10-20% sphagnum peat, 10% Oregon white pumice and small amounts of bone meal. Spreading the sediment was difficult due to the highly cohesive nature of the clay-silt sediments. Consolidated lumps of clay were not unusual. The target thickness for the samples was 4-5mm. The average thickness was 7.3 mm (0.95 liters/1290 cm^2). Variability in thickness was due to consolidated clumps of silt/clay that were difficult to spread.
Ten control flats filled with the soil-less mixture were scattered randomly among the flats to test for potential greenhouse contaminants. Samples were randomly re-distributed every two weeks throughout the study to minimize the possible influence of spatial variability on moisture or light in the greenhouse. All flats were watered from below periodically by flooding the benches to a depth of 2 cm and allowing the water to slowly drain off the bench. This method of watering prevented further burying of seed beneath the fine-textured sediments that may occur by watering from above. Samples were kept moist for the duration of the study, which required a flexible watering schedule to adapt to the changing heat conditions in the greenhouse (the park service greenhouse had no cooling system). There was no supplementary light treatment, so the samples were subjected to ambient light only.

Literature suggests a wide range of exposing samples in the greenhouse from six weeks to two years (Brown, 1991). Most studies found that seeds germinated within the first six weeks of treatment (Gross, 1990; Baker, 1994; McGowen, 2004). Brown (1991) suggested a four-month treatment. I opted for a six-month treatment period. The study began in June and the last samples were kept moist until January.

As seeds germinated, they were marked with color-coded wire based on seedling morphology until they could be identified (Gross, 1990; Baker, 1994; McGowan, 2004). Once identified, the seedlings were removed from the flat and either destroyed or re-potted by ONP greenhouse staff for use in future Elwha revegetation efforts. Plants were identified using Hitchcock and Conquest (1973) and Kozloff (2005). Botanical names were updated based on Kartez (Integrated Taxonomic Identification System, 2007). Grasses were identified at least to genus by using a vegetative key for grasses (Hitchcock *et al.*, 1969). Plant nativeness was determined for each species using Buckingham *et al.* (1995).

The methods used to germinate seeds from transient seed bank samples were the same as those described above. This part of the study began after results from the persistent seed bank study were known. The results from the persistent seed bank produced low mean seed densities, so some transient samples were cold stratified. Cold stratification may increase the number of readily germinable seeds in the samples (Thompson and Grime, 1979; Haag, 1983; Gross, 1991). Baskin and Baskin (2001) suggest a cold stratification of 5° C. To test the effect of cold-stratification on the transient seed bank samples, the 0.95-liter samples were split into two 0.47-liter samples. One was cold stratified for four weeks at 5-6° C; the other was immediately processed in the greenhouse within 48 hours of collection. I decided to limit the cold stratification treatment to the October, November and January samples due to limited greenhouse space.

The terrestrial seed bank samples were treated the same as the transient seed bank samples. The samples were split into a cold stratification treatment and a non-stratified treatment. All other germination methods were the same as those already described.

Persistent Seed Bank Statistical Analysis

To determine if there were discernible spatial patterns in germinable seed densities in the sediments of Lake Mills, the relationship between measured sample depth and germinable seedling densities was analyzed using linear regression in SPSS 11.5 (SPSS 2003). The water depth and seedling data were heteroscedastic and not normally distributed (Figure 1), so sample depth was log transformed, which strongly improved the distribution as assessed by residual plots and normal quantile plots. The relationship between sample distance from the Elwha River delta and germinable seedling density was also analyzed using linear regression in SPSS. No data transformations were necessary. The measuring tool in ArcGIS (ESRI 2004) was used to determine the distance of each site from the Elwha River Delta based on collected GPS points.

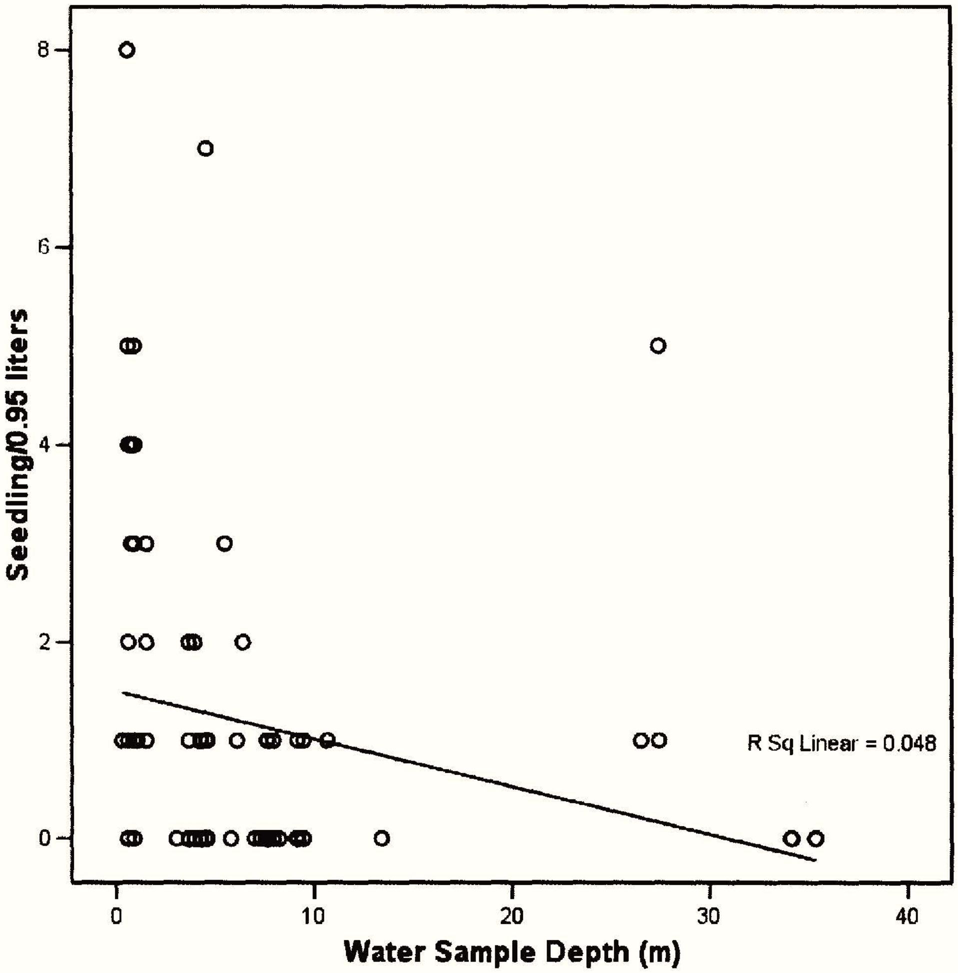

Figure 1. Scatterplot of untransformed seed bank data.

Transient Seed Bank Statistical Analysis

If there is a transient seed bank in the Lake Mills sediments, there should be a significant change in germinable seed densities with time (season). To ensure differences within the seed bank are an effect of time of collection and not a variation between the ten sites (block effect). Repeated

Measures Analysis was performed on the data using SPSS 11.5 (SPSS, 2003). Repeated Measures Analysis is a statistical method designed to measure data from a repeatedly visited plot or subject. It was designed to overcome the problems associated with the risk of temporal non-independence inherent in re-sampling one plot (Underwood, 1998). The test produces two measurements of significance. The Tests of Between-Subjects Effects measures the variation of the dependent variable (seedlings) between sites within a month. If the Test of Between-Subject Effects is significant, then there is no significant effect of the independent variable (time) on the dependent variable. The test was conducted at a 0.05 significance level (DF=9). To test for the effect of time on seedling variation, Repeated Measures Analysis conducts a multivariate test. The effect of time on the dependent variable can be linear or non-linear.

The data required data transformation due to their distribution. Data from the months of February and March were normally distributed, but October was not normally distributed due to the significant number of samples that did not have any germinable seeds (seven samples produced zero seedlings). To deal with the zeros in the data, I transformed all the data: log (x + 1). This improved the distribution, so I performed the Repeated Measures Analysis with the transformed data.

A paired t-test of means was used to determine the effect of cold stratification on germinable-seed densities.

Terrestrial Seed Bank Statistical Analysis

A paired t-test of means was used to determine the effect of cold stratification on germinable seed densities from the terrestrial soil samples.

A Mann-Whitney Test was used to compare the mean number of seeds in the terrestrial seed bank mean and the October lacustrine seed bank mean. Samples from the cold stratified and non-stratified samples were combined for the analysis.

Results

Results

The persistent seed bank samples produced few seedlings. A total of 104 seedlings germinated from the 91 samples collected from Lake Mills, a mean of 1.1 germinable seeds per sample (0.95 liters of sediment). Individual samples had as few as 0 to as many as 8 germinants. Over 52% of the samples (47 of 91) had no germinants. No seeds germinated in the control flats. There were 17 different species to germinate in the samples (Table 1). Graminoids were the dominate life form to germinate, with over 52 seedlings (50%) represented by 5 species. There were 42 forb seedlings (40%) represented by 10 species, and 10 seedlings (10%) of woody plants, all of which were *Alnus rubra.*

Plants in the genera *Juncus, Agrostis, Carex* (with the exception of *C. deweyana*), and *Equisetum* were not identified to species since they did not flower. There are no exotic species from the genera *Carex* or *Equisetum* known to occur on the Olympic Peninsula (Buckingham *et al*, 1995), so they were considered native. The foliage of *Carex* sp. 1 resembled *Carex obnupta* Bailey, an obligate wetland (OBL) sedge (USFWF, 1988). This species was commonly observed around the lake's edge. The foliage of *Carex* sp. 2 strongly resembled the species *Carex hendersonii* Bailey, a facultative upland sedge (FACU). *C. hendersonii* is a common understory plant in the riparian and upland forests of the lower Elwha. The foliage of the *Juncus* species resembled *Juncus balticus* var. *balticus* Willd., a facultative wetland rush (FACW+) (USFWS, 1988), but some may have been *Juncus effusus,* which has very similar foliage. In a trial sample collected from Lake Mills in February 2005, several *Juncus* seedlings emerged, flowered and were identified as *J. balticus. J. balticus* and *J. effuses* are present along the shoreline of Lake Mills and are common on the Elwha Delta.

Three of the seedlings (15%) identified were not native to the Olympic Peninsula. These seedlings were identified as *Mycelis muralis* (L.) Dormort, *Sagina procumbens* L. and *Senecio vulgaris* L. (Buckingham *et al*, 1995). *Mycelis muralis* was the most abundant exotic species to germinate in the samples and represented 10% of all the seedlings observed during the study.

Native species represented at least 61% of all seedlings that germinated from the sediments. There were 20 (~19%) sedge seedlings, all belonging to the genus *Carex. Alnus rubra* made up 10% of the total species, while the pioneering forb *Epilobium ciliatum* ssp. *ciliatum* Raf. represented 9% of the total. The most abundant germinable seeds were from the genus *Juncus*, producing ~24% of all germinable seeds. Facultative or obligate wetland species were 56% of the seedlings

Table 1: Persistent seed bank results. Origin was determined using Buckingham *et al* (1995). Wetland status was determined using USFWS (1988).

Species	Count	Origin	Wetland status
Juncus sp.	25	Unknown	
Carex deweyana var. *deweyana*	18	Native	
Mycelis muralis	11	Exotic	Not listed
Alnus rubra	10	Native	FAC
Epilobium ciliatum ssp. *ciliatum*	9	Native	FACW-
Equisetum sp.	7	Native	
Agrostis sp. 1	5	Unknown	
Sagina procumbens	3	Exotic	FAC
Mimulus lewisii	3	Native	FACW+
Senecio vulgaris	2	Exotic	FACU
Stellaria crispa	2	Native	FAC+
Galium trifidum	2	Native	FACW+
Carex sp. 1	3	Native	FACU
Unknown forb 1	1	Unknown	
Unknown forb 2	1	Unknown	
Veronica americana	1	Native	OBL
Carex sp. 2	1	Native	
SEEDLING TOTAL	104		

Seed Densities and Sample Depth

Log-transforming sample depth variable improved the distribution of data and allowed me to compare seedling density and water depth of samples (Figure 2). Despite the small numbers of germinable seeds found during the study, there was a significant relationship between seed densities and sample water depth (p-value < .001, Table 2). The adjusted R^2 from this regression was low (0.21), leaving significant unexplained variation.

Table 2. Seed Density and Sample Depth Coefficients table. The regression model was seed density per sample = $\beta_0 + \beta_1$/depth.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.233	.296		7.535	.000
	Log(depth)	-.757	.165	-.459	-4.588	.000

a Dependent Variable: Seedlings

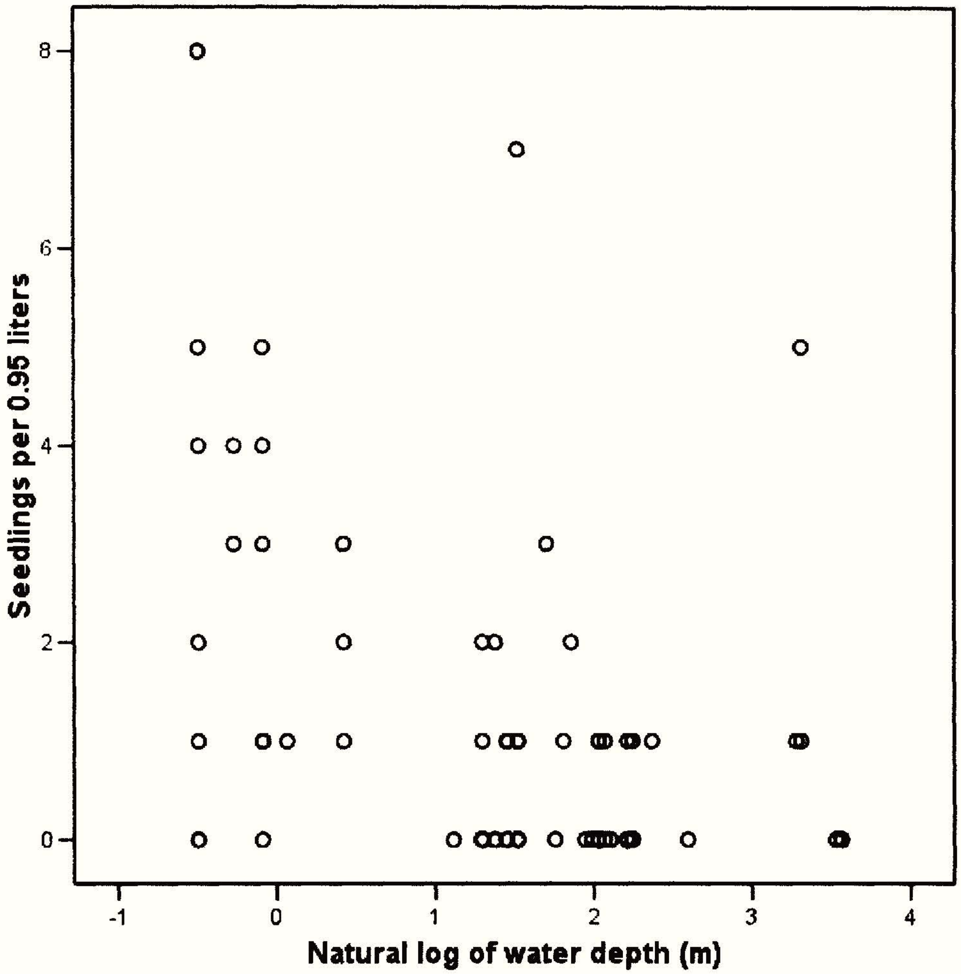

Figure 2. Scatterplot of transformed seed bank data.

Seed Density and Distance from Delta

The relationship between germinable seed densities and distance for the Elwha River delta was not statistically significant ($R^2 = 0.01$, $t = -0.71$, $P = 0.48$). (Table 3).

Table 3. Seed density and distance coefficients table.

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.714	1	1.714	.507	.479
	Residual	267.199	79	3.382		
	Total	268.914	80			

a Predictors: (Constant), Distance
b Dependent Variable: Seedlings

Transient Seed Bank

The transient seed bank samples produced more seeds than the persistent seed bank samples. A total of 216 seeds germinated in the 90 transient seed bank samples, a mean of 2.4 seeds per sample. Individual samples germinated as few as 0 to as many as 18 seeds. There were 20 different species to germinate. Germinants included 142 forbs (66%) representing 10 species, 57 graminoids (26%) representing 9 species, and 12 woody seedlings (6%) all *Alnus rubra* (Table 4). The un-stratified samples germinated 153 of the 216 seeds, a mean of 2.6 seeds per sample. A total of 63 seeds representing 11 species germinated from the 30 cold stratified samples, a mean of 2.1 seeds per sample.

Epilobium ciliatum ssp. *ciliatum* was the dominant species to germinate in the samples, producing 127 seedlings, 59% of the total. *Alnus rubra* represented 6% of the germinable seeds. Of the seedlings successfully identified, only 8 (3.7%) representing 5 species were not native to the Olympic Peninsula (Buckingham *et al*, 1995). The five exotic species were *Cortaderia jubata* (purple pampas grass), *Echinochloa crus-galli* L. Beauv., *Senecio vulgaris* (common groundsel), *Taraxacum officinale* (common dandelion) and *Cirsium vulgare* (bull thistle).

Table 4. Transient Seed Bank Results. Origin was determined using Buckingham *et al* (1995). Wetland status was determined using USFWS (1988).

Species	Non-strat samples (60)	Cold-strat samples (30)	Origin	Wetland status
Epilobium ciliatum ssp. *ciliatum*	88	39	Native	FACW-
Agrostis	19	5	Unknown	
Carex deweyana var. *deweyana*	13	6	Native	
Alnus rubra	10	2	Native	FAC
Equisetum sp.	2	3	Native	
Chamerion angustifolium spp. *angustifolium*	2	2	Native	FAC
Myosotis laxa	2	1	Native	OBL
Cirsium vulgare	2	1	Exotic	FACU
Carex sp. 2 (*obnupta?)*	4	2	Native	FACU
Juncus sp. 1	1	1	Unknown	
Rorippa sp.	1	0	Native	
Senecio vulgaris	1	0	Exotic	FACU
Galium trifidum	0	1	Native	FACW+
Echinochloa crus-galli	2	0	Exotic	FACW
Unknown forb	1	0	Unknown	
Cortaderia jubata	1	0	Exotic	Not listed
Unknown poaceae sp. 4	1	0	Unknown	
Carex stipata	1	0	Native	
Taraxacum officinale	1	0	Exotic	FACU
Juncus sp. 2	1	0	Unknown	
SEEDLING TOTAL	153	63		

Two species, *Oxalis corniculata* L. and a *Salix* species, appeared in the control flats and the study flats. These species were also observed growing in the cracks and in other study flats in the University of Washington greenhouse, so they were removed from the analysis.

There was significant variability of germinants between months and between sites. February had the most seeds germinate with 42 (mean 4.2 ±1.5 SE). Samples from October had the fewest with only 7 (mean 0.7 ±0.42 SE) (Figure 3).

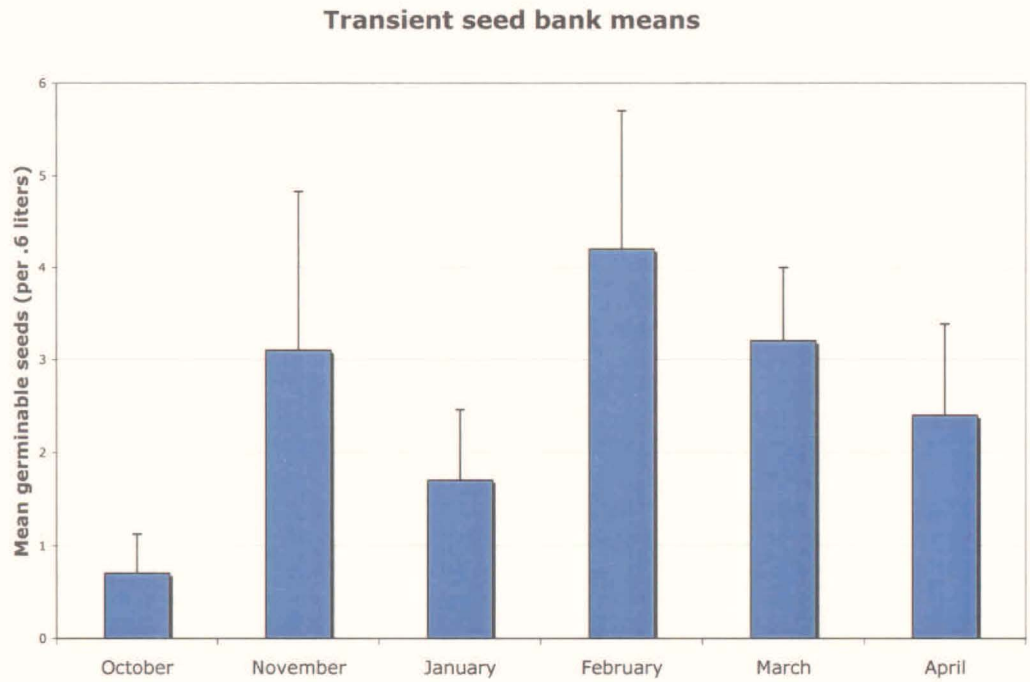

Figure 3. Monthly Seedling Means

Repeated Measures Analysis

Repeated Measures Analysis revealed significant spatial heterogeneity in the readily-germinable seed densities along the stretch of shore sampled. The multivariate test resulted in a significance level of 0.112, indicating no significant differences in mean germinable seed density between months. The Tests of Between-Subjects Effects resulted in a p-value of < 0.01. This indicates that the mean germinable seed density differed significantly between sample sites (Figure 4).

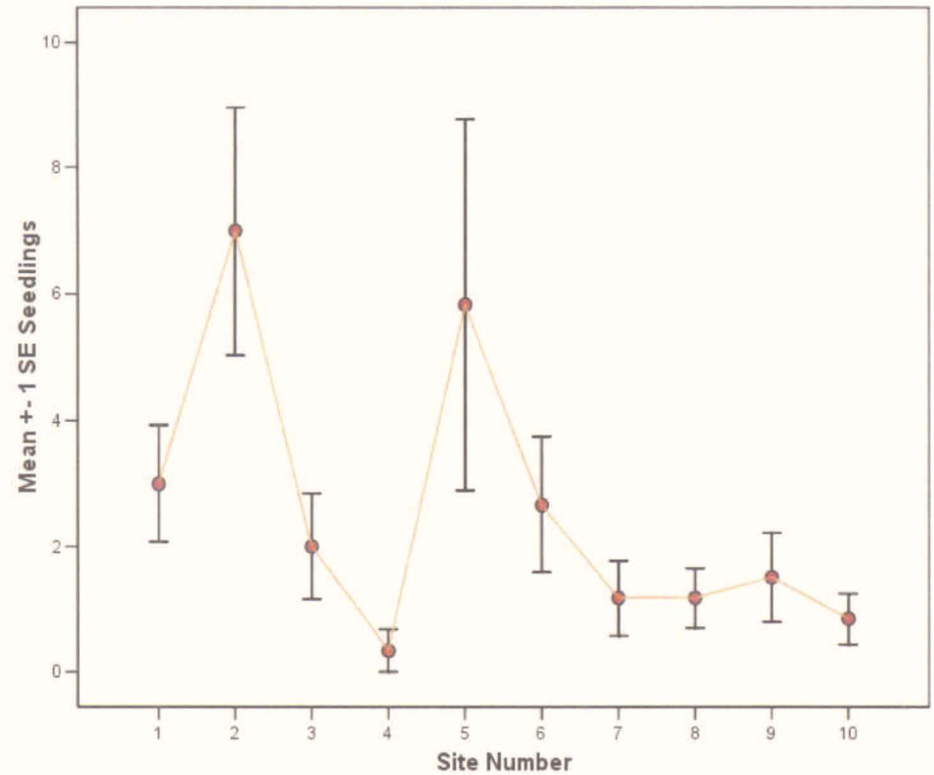

Figure 4. Site means across all months. Each site was sampled once every 5 weeks for a total of 6 samples (*n*=6). There was significant variation of seedling means between sites.

Cold-stratification Effect on Seed Density

There was no difference between the mean germinable seed density of the cold-stratified samples and the un-stratified samples collected in November and January (Table 5).

The October data set was not normally distributed. A Mann-Whitney Test of means was used to test the difference between means of the cold stratified and non-stratified samples. The tests showed no significance difference in means (Exact significance value = 0.280).

Table 5. Cold Stratification Results. T-test: Paired Two Sample for Means. Ho: There is no difference between means. October was not included, since the data was not normally distributed.

	Mean seed densities not stratified	*Mean seed densities cold stratified*	*t-stat (one-tailed)*	*t-crit (one-tailed)*
November	3.1	3	0.190	1.833
January	1.7	1.8	-0.122	1.833

Terrestrial Seed Bank Results

Soil samples from the terrestrial sites collected in October did not produce an abundance of seeds. The overall germinant mean for terrestrial samples (cold stratified combined with un-stratified) was 2.8 ±0.6. This mean was higher than the October samples collected from the lake, but was lower than three of the lacustrine monthly means (November, February, and March). Germinants included 36 seeds representing 12 different species in the non-stratified terrestrial seed bank samples, while only 20 seeds representing 12 different species germinated in the cold-stratified samples (Table 6). Individual samples germinated as few as 0 to as many as 13 seeds.

Forbs represented 75% of the seedlings from all the samples, 16% were woody species and 9% were graminoids. *Tolmiea menziesii* was the most abundant species to germinate in the samples, representing 23% of the total seeds germinated. Only four known exotic species germinated in the terrestrial seed bank samples, *Echinochloa crus-galli* (barnyard grass), *Cirsium vulgare* (bull thistle) and *Senecio vulgaris* (common groundsel). These species represented only 7% of the total seedlings to germinate in the samples.

Table 6. Terrestrial Seed Bank Results. Origin was determined using Buckingham *et al* (1995). Wetland status was determined using USFWS (1997).

Species	Non strat samples	Cold strat samples	Origin
Tolmiea menzeisii	8	5	Native
Epilobium ciliatum ssp. *ciliatum*	6	3	Native
Trientalis borealis	6	2	Native
Rubus parviflorus	4	1	Native
Chamerion angustifolium ssp. *angustifolium*	3	1	Native
Campanula scouleri	2	1	Native
Rubus ursinus	2	0	Native
Echinochloa crusgalli	1	1	Exotic
Sambucus racemosa	1	0	Native
Luzula species	1	2	Native
Cirsium vulgare	1	0	Exotic
Unknown forb 1	1	1	Unknown
Linnaea borealis	0	1	Native
Senecio vulgaris	0	1	Exotic
Claytonia siberica	0	1	Native
SEEDLING TOTAL	36	20	

Cold Stratification Effect on Seed Density

The terrestrial seed bank data distribution was not normal, so the non-parametric Mann-Whitney Test of means was used to compare means of the cold stratified samples and the non-stratified cold samples. There was no statistically significant difference between the means at a 0.05 confidence level (p-value = .218).

Comparing Terrestrial and Lacustrine Seed Banks

The terrestrial seed bank mean was significantly higher than the October lacustrine seed bank (p-value = 0.020) (Figure 5).

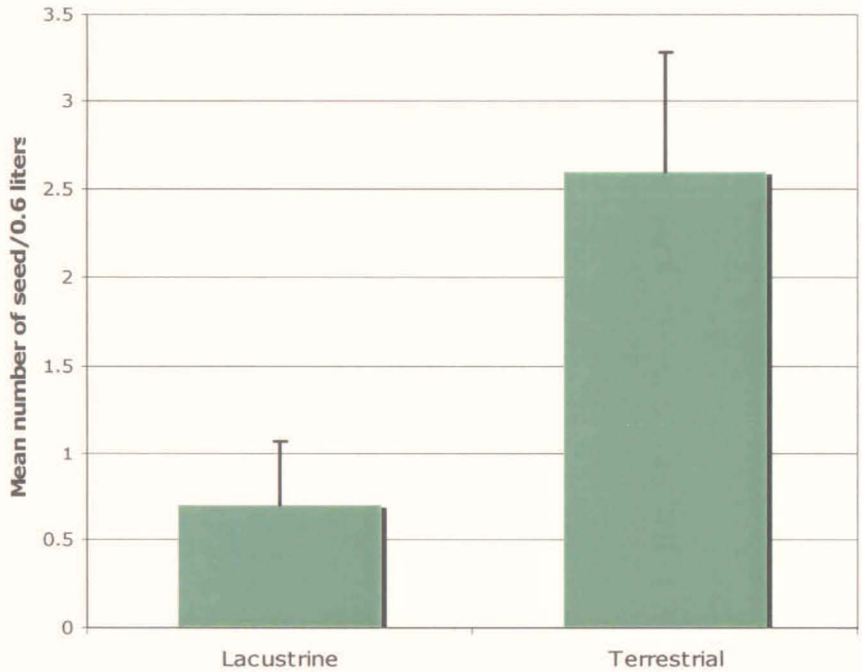

Figure 5. Terrestrial versus Lacustrine Seedling Means. Terrestrial samples were collected in October only. Lacustrine samples are from October. Data combined cold-stratified and un-stratified samples.

Discussion

Readily Germinable Seed Densities

After the removal of Glines Canyon Dam, plant species available to colonize the basin will not be enhanced by seed banks at most sites in Smokey Bottom. Mean densities of germinable seed in the sediments from Lake Mills were low and decreased significantly with water depth. Germinable-seed densities along stable shorelines are often low and decrease with depth (Haag, 1983; Nicholson and Keddy, 1983; Poiani and Johnson, 1989; Collins and Wein, 1995; Abernethy and Willby, 1999). Haag (1983) found a mean density of 4.01 seeds per liter of sediment from the stable shorelines of Lake Wabamun in Ontario, Canada. This number is similar to the mean of 5.1 seeds per liter of sediment I found in the transient study. Although these numbers are low compared to terrestrial seed banks, shallow shoreline areas will generate some early colonizing species from the seed bank after dam removal. The decline in viable seed densities with water depth means a decline in seeds with increasing distance from the forest surrounding Lake Mills. The implications for the revegetation of the basin after dam removal are that the seed rain and not the seed bank will likely determine species availability at sites far from the matrix. To facilitate comparison to published studies, I will estimate the number of seeds per area for Lake Mills. I did not use a coring device; so all per-area estimates are based on the assumption that samples came from the upper 12 cm of lacustrine sediments, the goal of all sampling methods. Based on these assumptions, the mean density for the persistent seed bank study was 144 ±24 seeds/m2. The shallow sites from the transient seed bank study had a mean of 480 ±74 m-2. Peak densities were found in the February 2006 samples, with a mean of 840±300 seeds/m2. These numbers are similar to other lentic seed bank studies. Grelsson and Nilsson (1991) estimated a mean germinable seed density of 464 seeds/m2 in Storvideln Lake on the Vindal River in Sweden. Haag (1983) estimated a range of 0-542 seeds/m2 from areas of Lake Wabamun (in Alta, Canada). Seed banks of prairie marshes had seed densities ranging from 145 to 1879 seeds/m2 with a mean of 442 seeds/m2 (van der Valk and Davis, 1976). In the second study of the marshes, seed densities were much higher, with a mean of 19,930 seeds/m2 (van der Valk and Davis, 1978). One of the explanations they proposed for the differences in seed densities between the studies was a drawdown event prior to the second study that "replenished the seed bank with large quantities of mudflat seed" (van der Valk and Davis, 1978). The seed banks within the Lake Mills' sediments appear to be within the range of other studies of stable lakeshores, and have lower densities than shorelines with fluctuating water levels.

Seed bank species richness and density is directly related to vegetation history (autogenic seed inputs) and influx of seeds from surrounding vegetation communities (allogenic seed inputs) (Leck and Simpson, 1987). Seed banks from fluctuating shorelines are abundant, species rich and diverse, a product of frequent autogenic and allogenic inputs of seeds (Leck and Graveline, 1979; Keddy and Reznicek, 1982; Poaini and Johnson, 1989; Abernethy and Willby, 1999; DeBerry and Perry, 2005; Liu *et al.*, 2006). The shorelines of Lake Mills are stable and do not support large populations of mudflat species, limiting the inputs of seed adapted to remain viable in anaerobic conditions. Emergent vegetation also enhances lentic seed banks by physically trapping seed that is wind-dispersed or floats into a site (Poiani and Johnson, 1989). Without emergent vegetation, seed inputs to lentic seed banks are primarily allogenic and dispersal dependent, and tend to reflect the local vegetation (Haag, 1983). Stable water levels in reservoirs narrow the width of habitable shoreline and promote the establishment of woody vegetation

(Toner and Keddy, 1997). The water level of Lake Mills does not fluctuate significantly, and is generally close to or at the erosion scarp (the boundary between the terrestrial forest and the eroding lacustrine shoreline). Woody plants (*Pseudotsuga menziesii, Alnus rubra, Tsuga heterophylla* and *Thuja plicata*, to name a few) dominate the surrounding vegetation and are well established up to the shoreline of Lake Mills. The forests are late-seral, and the understory is dominated by shade-tolerant species with seeds that generally do not persist in seed banks (Halpern *et al.*, 1999). In the tranquil bays common along the lake, the tree canopy extends more than 10 meters over the water, shading any shallow or exposed shoreline. Additionally, shallow water depths are rare in Lake Mills, due to the steep bathymetry of the lake. In the tranquil bays water depths generally exceed two meters in less than eight meters distance from the erosion scarp. Shade from the surrounding forest, stable water levels, and limited shallow or exposed areas discourage the establishment of any ruderals, helophytes or hydrophytes along the Lake Mills shoreline, thereby limiting germinable seed bank densities in the lacustrine sediments. Large numbers of seeds are likely falling into the reservoir, but the seed rain is probably dominated by species with short-lived seeds not adapted to anaerobic conditions.

The Transient Seed Bank and Spatial Heterogeneity

Seasonal variation in the density of germinable seed was not statistically significant, but there was some suggestion in the data of higher densities in winter months. The highest seedling means for the study were from November samples (3.1 per sample), February (4.2 per sample), and March (3.2 per sample). Repeated Measures Analysis did not reveal any seasonal trends. Repeated Measures Analysis may not have been the ideal statistical test for my data, because it is best suited to measure a single object or space over time and not for measuring samples separated spatially. Seed banks are spatially variable, and this variability may have influenced differences in seasonal densities of viable seeds. Since no other statistical tests (such as a two-way ANOVA) are better suited to detect trends over time, Repeated Measures Analysis was the best option to for this study. More samples per month combined with more sampling months may reveal statistically significant trends.

Consistent with terrestrial seed bank studies (van der Valk and Davis, 1978; Haag, 1983; Leck and Simpson, 1987; Leck *et al.*, 1989; Abernethy and Willby, 1999; Halpern *et al.*, 1999; de Winton *et al.*, 2000), there was tremendous spatial heterogeneity in viable seeds in Lake Mills. Samples from the transient study produced between 0-18 seedlings, which translate to an estimate of 0-3,600 seeds per m2. The pilot study site produced even more seedlings, with over 400 seeds/1.8 liters (~ 30,000 seeds/m-2) germinating from a sample collected in February 2005. The pilot site was sampled a second time in April 2006 and produced 62 seedlings (~12,400 seeds/m-2). The site was located close to the boat launch, a sunny disturbed area with several helophytes (*Juncus balticus* and *Juncus effusus*) along the exposed shoreline. Since the seed bank at the site were dominated by *Juncus* sp., it is likely that the seeds came from these plants, and contributed to the dense seed bank found at that site. *Juncus* species are known to produce prodigious amounts of small seeds that can remain viable in hypoxic conditions for up to 80 years, and several studies found large allogenic inputs of *Juncus* seed in lentic seed banks (Keddy and Reznicek, 1982; Abernethy and Willby, 1999).

The spatial heterogeneity at the transient seed bank study site may be due in part to the differential seed deposition based on the buoyancy of seeds. Nilsson *et al.* (2002) found that species with short-floating seeds are poorly represented in lake shorelines, and that species with long-floating seeds are well represented and are preferentially distributed in tranquil areas of the shoreline over turbid areas. The low seed bank densities of exposed shoreline with coarse sediments, such as samples 8, 9 and 10 from site 3WS, are likely due to turbulent waters (Keddy, 1985; Nilsson *et al.,* 2002). These three samples were located on a point extending out into the lake, and were characterized by coarse textured sediments (sands and gravels) without any organic matter. The samples produced low seed densities relative to the sample sites located in the tranquil bay. Samples 1 thru 7 were all located in tranquil waters and the sediments were a mix of fines (clay and silt) and organic material. Interestingly, 75% of the *Alnus rubra* seedlings to germinate from the transient samples were from the coarse sediment sites. Seedlings of *A. rubra* are known to preferentially establish in coarse textured substrates (Dobowski *et al,* 1994; Shafroth *et al.,* 2002), and despite their Site means across all months seed being small and light, significantly more seed settled out in the turbulent waters in this study. Due to the strong spatial heterogeneity of the Lake Mills seed banks, the limited number of samples collected for this study may not have been adequate to detect all the species that may be present in the sediments. Bossuyt *et al.* (2007) found that seed bank studies using methods similar to mine did not successfully detect rare species that may occur in the seed bank. The methods do successfully predict viable seed densities (Bossuyt *et al.*, 2007). My results predict seed bank densities are likely higher in areas close to populations of helophytes or ruderal species such as *Juncus*, *Carex,* or *Epilobium.* The presence of just a few plants of these species could locally add significant numbers of seed to lacustrine sediments.

Species Composition of Lake Mills Seed Bank

Species to germinate in the sediments from Lake Mills were predominantly forbs, representing nearly 58% of all seedlings from the transient and persistent study samples. Graminoids were also a significant proportion of my results, representing over 35%. *Carex* was the most well represented genus of graminoids. *Alnus rubra,* the only woody species in the samples, was approximately 7% of the seedlings. The species that dominated my samples was the forb *Epilobium ciliatum* ssp. *ciliatum.* This species accounted for almost 60% of all observed seedlings in the transient study and nearly 43% of all seedlings observed. It germinated from samples collected at all depth categories in the persistent seed bank study. *E. ciliatum* ssp. *ciliatum* is a facultative wetland species (FACW-) native to the area (USFWS, 1988). It is a fast growing perennial of open, disturbed areas and is particularly common along streams, rivers, wetlands and lakeshores (Pojar and MacKinnon, 1994). During the study, it flowered within weeks of germinating, a ruderal trait common to facultative annuals. Its small seeds (<1mm) are likely persistent in seed banks, a trait shared by its close relative *Epilobium hirsutum* L. (Thompson *et al.*, 1993). *Epilobium* species are well known to dominate disturbed ecosystems in the Pacific Northwest, and are considered difficult to eradicate in horticulture fields in Oregon (Altland, 2006). Species common to disturbed systems are well suited to colonize denuded ecosystems. A close relative to *Epilobium ciliatum* ssp. *ciliatum* is *E. ciliatum* ssp. *watsonii.* After the eruption of Mt. St Helens, *E. ciliatum* ssp. *watsonii* dominated some wetlands within the blast zone (Titus and del Moral, 1999). The seeds are adapted to wind dispersal and traveled several kilometers to colonize blast zone (Wood and del Moral, 2000). The significant presence of *E. ciliatum* in the Lake Mills sediments and its life history traits suggests it may play an important role in the colonization of the denuded Lake Mills basin. Successful plant establishment in the Lake Mills basin may be strongly influenced by the texture of the sediments.

Sluis and Tandarich (2004) studied the influence of silt deposition on succession in riparian wetlands, and found that high silt deposition suppressed seed germination (buried the seed). Silt has small pore space, and even thin layers can bury seeds, especially small seed, preventing germination (Walker *et al.*, 1986). Small viable seeds buried in the highly uniform Lake Mills silts may not readily germinate. Seeds that do germinate may find it difficult to establish in the silts. The small pore space in silts reduces root penetration, and the substrate can behave like an anaerobic wetland soil (Mussman, 2006). Initial water infiltration and percolation rates are slow in dry, silty loams, and decrease further as the silt becomes saturated (Brady and Weil, 2004). Therefore, silty loams tend to favor species with fine root systems, a common trait of graminoids. Graminoids are known to dominate primary succession on fine textured substrates (Grubb, 1986). Many of the graminoids were facultative wetland sedges and rushes, and may not persist in the basin once the water table subsides. Another plant trait that will influence primary succession on fine substrates is the ability to spread laterally by stolons, turions and rhizomes (Shaojun *et al.*, 2001; Sluis and Tandarich, 2004; Naiman *et al.*, 2005). The seed bank included several species with the ability to spread vegetatively and these species may persist and dominate succession in the silts. Of these, *Agrostis* sp. Is the most likely to successfully spread. The genus was 12% of all seedlings from the transient seed bank study, while ~5% of the seedlings from the persistent study were *Agrostis*. *Agrostis* species spread laterally via stolons and are known to colonize severely disturbed sites. The grass did not flower, so it is not yet known which species of *Agrostis* is present.

Lentic seed banks rarely contain many seeds of woody species (Leck and Graveline, 1979; Nicholson and Keddy, 1983; Leck and Simpson, 1987; Leck, 1989; Brown, 1991). The presence of *Alnus rubra* in the Lake Mills sediments is unusual. Seeds of *Alnus* were present in samples from water depths of 0.8-4.5 meters. Seedlings of *Alnus rubra* represented 7% of all germinable seeds observed throughout the study. The seeds of *A. rubra* appear to be short-floaters, as evidenced by the significant presence of the seedlings in the coarse-textured, turbulent shoreline. *A. rubra* is not likely to perform well in the silt, since the seedlings of the species do not establish well in poorly drained, fine substrates (Harrington *et al.*, 1994; Dobowski *et al.*, 1994; Chenoweth *et al.*, 2007).

Exotic Species in the Lake Mills Seed Bank

Exotic species were not present in abundance in the seed bank of Lake Mills. The exotic species that did appear are not considered invasive. Olympic National Park identified exotic species of concern in the lower Elwha watershed in 2001 (Olson *et al.*, 2001). Of the exotic species to appear in the seed bank, none are listed in the Olson report nor are they listed under the 2000 federal noxious weed list (USDA website, 2000). One species, *Senecio vulgaris* (common groundsel) is listed as a noxious weed in the state of Washington. The Washington State Noxious Weed Control Board (WSNWCB) lists *S. vulgaris* as a Class C noxious weed in 2007. Eradication of Class C weeds is not required by state authorities. *Senecio vulgaris* is a winter or summer annual, sometimes acts as a biennial, and flowers from April to October (WSNWCB, 2004). These ruderal traits may enhance the ability of *S. vulgaris* to invade the denuded basin if the plant proves capable of thriving in the silty substrate. A close relative to *Senecio vulgaris*, *Senecio sylvaticus* is known to invade forests in western Oregon after clear cutting (Halpern, *et al.*, 1997). After a few years of high density and cover, *S. sylvaticus* populations have been shown to decline precipitously (Halpern *et al.*, 1997). Changes in the physical environment may have caused this decline (Halpern *et al.*, 1997), and a similar pattern of increase and decline may occur in the Lake Mills' basin after dam removal. Exotic species invasions in denuded sites are enhanced by high-nutrient substrates typical of former reservoir bottoms (Shafroth *et al.*, 2002; Green and Galatowitsch, 2002; Orr and Stanley, 2006). Orr and Stanley (2006) surveyed former reservoirs after dam removals in Wisconsin, and found several sites had dense populations of exotic species, predominantly *Phalaris arundinacea*, which appear to be inhibiting the colonization of the former reservoirs by native woody species. These sites had nutrient-rich substrates, probably the result of years of accumulations of organic material. Much of the land surrounding these reservoirs was not protected wilderness, but was under mixed use, including agricultural lands which often contribute nutrients to local watersheds. Lake Mills' sediments differ from the Wisconsin reservoir sediments. They are not nutrient-rich, but are exceedingly nutrient-poor (Chenoweth *et al.*, 2007). The primary inputs of sediment to the lake are weathered inorganic bedrock material and alpine and continental glacial deposits weathered by fluvial processes (USDI, 1995). Most native northwest species are adapted to nutrient-poor substrates, which may help limit the invasion of problematic exotic species in the denuded basin. The invasion of problematic exotic species into the Lake Mills' basin after dam removal is unlikely to initiate from a seed bank in the sediments. The few exotic species detected in this study do not represent a threat to natural succession, and the low nutrient levels of the sediment may help to limit the invasion of noxious weeds into the basin after dam removal.

Hydrochory and the Lake Mills Seed Bank

The overall low seed densities suggest that the Elwha River is not supplying a significant source of long-lived seeds to the Lake Mills sediment seed bank. There was no trend of readily germinable seed densities in the seed bank relative to distance from the Elwha River Delta. The Elwha River does transport hydrochorous seeds (Brown, 2006) but any seed that is settling into the lake from the river does not appear to remain viable. Many riparian plant species, such as cottonwoods and willows, are adapted to set seed in conjunction with seasonal river flows and do not remain viable for long once the seed becomes wet (Naiman *et al.*, 2005). Over 60% of all species to germinate from the Lake Mills sediments were wind-dispersed species. *Epilobium ciliatum* ssp. *ciliatum*, a wind dispersed species, dominated a tranquil bay along the shoreline of Lake Mills. Seeds adapted to wind dispersal may also readily float in water. *Populus balsamifera* ssp. *trichocarpa* disperses its wind-blown seed at low spring water flows, and likely combines the ability to disperse in wind and water to effectively colonize new substrates (Rood *et al.*, 2003; Naiman *et al.*, 2005).

Hydraulic patterns in lakes are simple relative to rivers, and patterns of hydrochorous seed dispersal in lakes is largely governed by surface wind patterns (Nilsson *et al.*, 2002). Therefore, the dispersal of floating seeds entering the lake from river currents is likely diverted by wind patterns. Some hydrochorous seeds floating in river currents are deeply submersed (Brown and Chenoweth, in press), and likely escape the influence of surface winds. Submersed seeds are likely to end up in the deeper sections of the lake, and may not disperse far from the river.

PART II: Germination Experiment on the Fine Sediments

Methods

Study Design

Four sediment treatments were created to test the effects of moisture and surface roughness on seed germination and establishment rates (see Appendix B). Each treatment had ten replicates (per species). Each replicate contained 645 cm^2 of fine sediment from Lake Mills. Treatment one, the dry/smooth treatment, consisted of dried sediments with a smooth, homogeneous surface. Treatment two, the dry/safe site treatment, contained dried sediments with a surface indented with safe sites: 36 small depressions, one for each seed. Treatment three, the moist/smooth treatment, consisted of flats containing moist sediments (sediments not allowed to dry out prior to seeding). The surface of this treatment was smooth. Treatment four, the moist/safe site treatment, consisted of moist sediments with the same safe site depressions as treatment two.

Each sample (replicate) was sown with 36 seeds of one of two species, *Alnus rubra* or *Elymus glaucus*. A total of 2880 seeds (1440 per species) were sown in 80 different flats (40 per species). For the safe site surface treatment (dry and moist), sediments were spread evenly into the flats and safe sites were created by using the heads of six nails attached in a single row to a 25.4 cm board. The row of nails (head down) was depressed into the sediment six times to create six rows of safe sites for a total of 36 per flat. The nail heads created a 2-3 mm depression in the surface of the sediments. These depressions created small resource-capturing surface undulations intended to mimic safe sites conditions in denuded landscapes (Walker and del Moral, 2003; Whisenant, 2003). To create the smooth surface treatment, the surface of the sediments was leveled with a spatula, reducing surface heterogeneity. All sediment treatments were prepared while the sediments were wet and pliable. The dry treatment was placed in the greenhouse to thoroughly dry for four weeks before seeding. Moist treatments were prepared 48 hours before sowing the seeds and were not allowed to dry out. All flats were filled with sediment to a depth of 4 cm.

Flats were sown by hand. All seeds were hand-picked to select viable seeds and avoid empty seeds and chaff. Seeds were sown onto the surface of the sediments in even rows and columns to ensure easy data collection and prevent the influence of clumping on germination rates (Baskin and Baskin, 2001).

Once the flats were sown, they were randomly distributed in the Olympic National Park greenhouse to reduce the effect of light or moisture variability on the samples. The flats were randomly re-arranged in the greenhouse every week for the length of the study. Samples were kept moist by a Mist-O-Matic misting machine. The mister was routinely checked to ensure the system did not fail during the study. The study relied exclusively on ambient light, since there was no supplemental lighting in the park greenhouse. Flats were checked once every two or three days for newly germinated seedlings and seedling mortality. Emergence of the radicle was the criteria for germination (Baskin and Baskin, 2001). When seeds germinated, they were marked with a small piece of wire. Seedlings of *Alnus rubra* were considered established when two true leaves opened at more than 45° (Keddy and Constabel, 1986). Seedlings of *Elymus glaucus* were considered established when the first true leaf appeared (Keddy and Constabel, 1986). Seedlings that died after germination were counted at the end of the study.

To characterize alder growth in the sediments, the number of true leaves on all *Alnus rubra* seedlings was counted at the end of the study.

The seeds were obtained from Inside Passage Seed (Port Townsend, Washington), and were collected from the Olympic Peninsula. Inside Passage advertised the seed as "germination ready" not requiring stratification. Fine sediments from Lake Mills were obtained in May 2005 during the sediment collection for the persistent seed bank study.

Statistical Analysis

The *Alnus rubra* and *Elymus glaucus* germination data were normally distributed and there were no significant differences in the variance (Table 8 and 9). A two-way factorial analysis of variance (2-way ANOVA) was used to reveal treatment effects at a significance level of 0.05. The two factors were moisture (moist or dry sediments) and surface (safe site or smooth) treatment. The dependent variable was germinants. The interaction term was moisture * surface treatment.

Percent seedling mortality data (p') was arcsin transformed ($p'=\arcsin(\mathrm{sqrt}(p))$) to deal with the binomial distribution (Zar, 1999). The *Elymus* arcsin transformed data was still not normally distributed, but the variance was equal (Table 10), so the nonparametric Kruskal-Wallis Test was conducted on the data to test treatment effects. The *Alnus* study germinated few seeds, so there was not enough data to statistically analyze seedling mortality rates.