Ecology of the Federally Threatened Aquatic Plant, *Howellia aquatilis*, and Implications for Northern Idaho Floodplain-Wetland Management

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Authorization to Submit Thesis

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Abstract

Howellia aquatilis, a Pacific Northwestern endemic, is highly dependent upon the ephemeral hydrology of the wetlands that it inhabits. This study aimed to understand more about the ecological needs and environmental correlates of *H.aquatilis*. Hydrologic, substrate, vegetative, and physical variables were assessed in 24 presence and absence ponds. Models comparing presence and absence ponds concluded that soil moisture and bulk density, habitat elevation to the river, and pond hydroperiod length best predict *H.aquatilis* presence or absence. Models assessing where *H.aquatilis* grows in presence ponds concluded that water depth and the percent cover of *Phalaris arundinacea* best predict *H.aquatilis* presence or absence. Our controlled germination experiments also indicate that soil moisture plays an important role in germination success rate. Overall, we find that local floodplain and wetland hydrology affect the soil and vegetation structure in *H.aquatilis* habitat, and may have important impacts on the inhabitability of different wetlands for this federally threatened species.

Dedication

I dedicate this master's thesis to my parents, who told me that whatever I decided to do as a career was my choice, but to find passion in it.
I did, so thank you for your love and support!

&

To my caring partner, Daniel Fleming- for countless encouraging words, soil cores, and pots of coffee. Thank you for believing in me!

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Introduction

World biodiversity is declining at an unprecedented rate, which gives biologists and managers the imperative role to create and implement conservation planning for thousands of species (Abell, 2002; Brussard, 1991; Schemske et al., 1994). While the earth's freshwater bodies contain only 0.1% of global water resources, these habitats comprise an astounding portion of the earth's biodiversity, and that biodiversity is declining at a extinction rate of 4% each year- five times the loss in terrestrial ecosystems (Abell, 2002; (Vaughn, 2010). Among those groups with diversity loss is vascular plants; there are nearly 9,000 *rare* plants in the U.S. (out of roughly 17,000 species of vascular plants), with only 800 listed under the Endangered Species Act (USDA, 2014). Currently, there are more additions to rare/threatened/endangered lists than subtractions (Havens, Kramer, & Guerrant, 2014; Schemske et al., 1994; USDA, 2014). Therefore, an increased research focus on the freshwater vascular plant species and their habitat is an important next step in biodiversity conservation. This focus should include up-to-date information on the species distribution, land use effects, and basic ecological and biological information needed for successful management (Abell, 2002; Brussard, 1991; Havens et al., 2014; Schemske et al., 1994). This study aims to narrow the knowledge gap for one of these imperiled species by assessing the environmental correlates of *Howellia aquatilis*, a rare and endemic wetland species.

Review of Howellia aquatilis

Howellia aquatilis (water howellia) is an aquatic annual of the Campanulaceae family (Figure 1), and is endemic to the Pacific Northwest region of the United States (Figure 2) (Lichthardt, Gray, & Pekas, 2012; Mincemoyer, 2005). In 1994, *H. aquatilis* was federally listed as threatened by the U.S. Fish and Wildlife Service (USFWS) under the Endangered Species Act with 107 known occurrences (Mincemoyer, 2005). As of 2005 (Mincemoyer), there were 214 occurrences; however, since then five more populations have been discovered in Latah and Benewah counties, Idaho (Lichthardt, Gray, & Pekas, 2012).

H.aquatilis' specific life cycle needs influence its rarity and ability to grow in various habitats. *H.aquatilis* is dependent on the wetting and drying cycles of the seasonally ephemeral ponds in which it occurs (Figure 3) (Lesica, 1992; Reeves & Woessner, 2004). Seeds germinate in the fall under dry conditions when seeds are exposed to aerobic conditions (Lesica, 1992). In the spring, when the ponds have filled, seedling growth resumes in early May and in June-July, maturation occurs and the small white flowers emerge from the water surface (Mincemoyer, 2005; Shelly & Gamon, 1996). Because of this tie to water body ephemerality, it is hypothesized that *H. aquatilis* population size in a given year is affected by the hydroperiod of that water body in the preceding year (Lesica, 1992; Mincemoyer, 2005). Annual production and viability is known to depend on local hydrologic conditions and water quality, but the further details of its ecologic and hydrologic preferences are unknown (Lichthardt et al., 2012; Shelly & Gamon, 1996). This *Callitriche heterophylla, Legenere limosa,* and *Downingia laeta*

The hydrology of *H. aquatilis* habitat varies across its Pacific Northwest range. They occur in a range of freshwater habitats that fill and dry annually including marshes, depressional ponds, and floodplain wetlands. In California, the six rediscovered populations grow in freshwater marshes and swamps (California Native Plant Society, 2014). *H. aquatilis* was thought to be extirpated from Oregon, but in 2002 was found in William Finley National Wildlife Refuge in vernal pools and oxbow sloughs (Oregon Department of Agriculture, 2014). Washington has many occurrences throughout Spokane and Clark County that mainly occur in depressional scabland wetlands that depend on precipitation as a water source (Mincemoyer, 2005; United States Forest Service, 1996). Montana's Swan River Valley has over 140 populations, which occur in glacially carved depressions that fill with rainwater and groundwater annually (Reeves & Woessner, 2004). In Idaho, the six known occurrences inhabit seasonal floodplain ponds in the Palouse River and Spokane River floodplain (Figure 4). The hydrology of the Idaho habitat is unique in that it's ephemerally is highly fluvially connected both through bank overflow, and fluvial groundwater.

Monitoring and various studies across *H. aquatilis*' range conclude that there are many potential and actual threats to populations, especially weed invasion potentially caused by alteration to floodplain hydrology due to land use, such as agriculture and manipulation of waterways (Lesica, 1992; Lichthardt et al., 2012). *Phalaris arundinacea* and *Acorus calamus* are two of the main problematic species that reportedly cause declines in *H. aquatilis* populations (Lesica, 1997). A nine-year study at a single marsh in Montana found that as *Phalaris arundinacea* (reed canary grass) monoculture stands rapidly increased, *H. aquatilis* numbers decreased (Lesica, 1997). *P. arundinacea* has

been evidenced to take over native wetland vegetation globally (Apfelbaum & Sams, 1987). Possible mechanisms for this competition is the over-shading caused by dense monocrops of *P. arundinacea*, as well as altercation of hydrologic regimes and substrate composition caused by production of thick litter layers in the ponds (Schooler, McEvoy, & Coombs, 2006). Idaho fish and wildlife biologists tested various weed control strategies such as clipping, laying down tin roof parcels on reed canary grass and completely excavating reed canary grass "islands" beginning in 2001 (Gray, Hill, & Mancuso, 2005). After the island removal, biologists observed *H.aquatilis* growth in the depression left by excavation on multiple occasions (Gray et al., 2005).

Land use in and around *H. aquatilis* wetlands is hypothesized to alter habitat, but little is understood about the particular impacts. It is hypothesized that the indirect hydrologic and substrate effects of timber harvest, such as increased erosion and altered hydro-graphs, could diminish *H. aquatilis* habitat (Shelly and Gamon 1996). The impact of grazing is unknown because while livestock do not directly graze *H.aquatilis*, their presence, depending on duration and intensity, could affect *H.aquatilis* habitat through sediment and seed bank disturbance and compaction (Shelly and Gamon 1996). However, low to moderate levels grazing may also be beneficial by controlling invasive plants, especially *P. arundinacea* (Pyke & Marty, 2005).

Although there are threat-related hypotheses garnered from field observation, there has been scarce work focused on *H. aquatilis* and its habitat beyond population monitoring and habitat description**.** In Idaho, the longest known occurrence at Harvard-Palouse River Flood Plain Conservation Site has been monitored annually since 1999 (Gray et al., 2005). At this location, between 1999 and 2004, depth of the pond was also

recorded from March until the ponds drained (Gray et al., 2005). Forest Service scientists working in Swan Valley, Montana conducted a large-scale monitoring project of 68 ponds from 1998 to 2007 (United States Forest Service, 1996). They did a non-peer reviewed study looking at the overall frequency of *H.aquatilis* with the annual precipitation to the water year prior to observation. In congruence with hypotheses by Lesica (1997) they found that *H.aquatilis* frequency is inversely related to precipitation (United States Forest Service, 1996).

Lesica's 1992 autecology study of *H.aquatilis* Swan valley populations is the most complete peer-reviewed *H.aquatilis* habitat study. Lesica discovered that a range of factors related to hydrology, water quality, and substrate type may influence *H.aquatilis* presence (Lesica, 1992). Timing of pond drying was a central driver in Lesica's regression models, as well as the type of substrate (organic or mineral). Lesica found that dense graminoid cover had an inverse affect on *H.aquatilis* populations, especially reed canary grass cover. Excessive land use, measured as levels of disturbance, from adjacent roads and logging were correlated with low levels of *H.aquatilis* (Lesica, 1992).

The aforementioned monitoring and peer-reviewed works established a solid basis for which further studies, like this one, can springboard from, and help to further inform *H.aquatilis* management. There is significant interest at the state and regional levels to restore and preserve *H.aquatilis*, but more in-depth studies and conclusions are required. The U.S. Fish and Wildlife Service recovery plan aims to secure an adequate level of conservation of current populations and their habitat to the extent that self-sustaining populations will be distributed throughout its historic range (Shelly

and Garmon 1996). Delisting will be considered once management has abated anthropogenic threats, conservation plants are implemented for Idaho populations, and a post-delisting monitoring plan is implemented (Shelley and Garmon 1996). In order to achieve the recovery plant goals, there needs to be an increased understanding of environmental requirements of *H.aquatilis.*

Research Goals

This study aims to fill the gap of *H.aquatilis* research on Idaho populations, and to identify key concepts for further research and restoration field testing. Idaho populations have been monitored via frequency surveys as they have been discovered over the past decade, but no study exists to describe ecological relationships between *H.aquatilis* and its environment in Idaho. The majority of the ecological studies have been performed in Montana and Washington, which are useful for Idaho management initiatives, but should not be depended on entirely to inform restoration in Latah and Benewah counties. As previously noted, the regional hydrology varies between populations; Washington and Montana's populations are groundwater and precipitation fed, whereas Idaho populations are exclusively fluvially dependent for their hydrology. This variation in hydrology between regions could yield dissimilar information related to the ecology of *H.aquatilis,* therefore separate habitat studies in Idaho are essential to understanding the floodplain populations and their unique wetland attributes.

Wetland plant community structure can be influenced by a variety of factors such as hydrology, surrounding land use, seed sources, and dispersal dynamics (Tsai,

Venne, McMurry, & Smith, 2012). One of the primary steps in assessing a plant species' relationship with its environment is measuring the key abiotic factors in the habitat, such as light, temperature, substrate nutrients, but especially hydrology in wetland environments (Bornette & Puijalon, 2010; Warwick & Brock, 2003). In wetlands, biotic factors such as competition interaction are also responsible for governing vegetation zonation and growth (Kennedy, Milne, Murphy, Maxim, & Holmberg, 2003). Intraspecific competition has been shown to decrease plant vigor and survival in greenhouse studies (Lentz, 2013). Hydroperiod is known to be a major determinant in the composition of wetland vegetation in many wetland ecosystems (Casanova & Brock, 2000; Correa-Araneda, Urrutia, Soto-Mora, Figueroa, & Hauenstein, 2012; Foti, Rinaldo, & Rodriguez-iturbe, 2012). For plants with limited ecological information, an overall habitat assessment is a well-accepted strategy for initiating restoration protocols (Lentz-Cipollini & Dunson, 2006; Lesica, 1992; Murphy, Boyd, & Boyd, 2014).

An important preliminary step in developing recovery efforts of a plant through biological inquiry is identifying which life history stage has the most impact on population growth and vigor (Schemske et al., 1994). This allows scientists to focus on the abiotic and biotic factors that impact those life stages, and to emphasize aspects of the species' biology that will more directly benefit conservation efforts (Schemske et al., 1994). This thesis research considers the specific biology of *H.aquatilis* and past research to choose appropriate environmental parameters to assess in *H. aquatilis* habitat that will reveal information about the species' habitat preferences and to inform restoration. This research also makes strides toward identifying which life stage is

critical for management by further exploring the establishment stage and seed germination dynamics.

From conclusions and methods gleaned from studies about *H.aquatilis* and wetland plant communities, we hypothesize that (1) pond hydroperiod plays a central role in *H. aquatilis* presence and frequency, specifically hydroperiod and spectrums of wetness, (2) land use, especially grazing may affect soil properties and HOAQ establishment, (3) there is a negative correlation between the presence of invasive plant *Pharlaris Arundinacea* and *H.aquatilis* frequency because of resource competition, (4) that light controlled by canopy cover may play an important role in *H.aquatilis* distribution and (5) *H.aquatilis* germination period may be the critical life stage that affects population health and vigor. We tested these hypotheses, through a habitat assessment in 24 temporary wetlands in ponds that contain *H.aquatilis* populations and ponds that do not in Northern Idaho. We also conducted a greenhouse germination experiment to assess *H.aquatilis* germination success under varying temperature and moisture environments. The overall goal of this study is to better understand the drivers of *H.aquatilis* on a floodplain (inter-site scale) and within a pond (intra-site scale). Specifically, we aim to (1) determine which environmental variables best predict *H.aquatilis* presence and absence, (2) identify a critical life stage for conservation, and (3) relate these findings to management and conservation protocols.

Methods

Study Area

The study was conducted in floodplain wetlands of the Palouse River and the Spokane River Basins in Benewah and Latah counties in Northern Idaho (Figure 4). The intermountain, warm-summer climate yields an average of 600 mm of precipitation annually with little rainfall in the summer months, and the majority of snowfall taking place in December, January, and February. Temperatures range from 33.4°F to 58.7°F (Western Regional Climate Center, 2014).

Temporary floodplain wetlands are a category of vernal pool wetlands that are seasonally ponded; the wetlands contain shallow water for variable periods during the winter and spring, and are dry for variable periods of summer months (Natural Resources Conservation Service, 2007). Ponds are filled from a combination of overbank flow, snowmelt and precipitation, and fluvial groundwater. Many of the wetlands are in abandoned channels that are relics of river movement over time. The Princeton property aerial image shows many examples of these abandoned channel ponds (Figure 5).

Ponds are often surrounded by riparian vegetation including deciduous shrubs black hawthorn (*Crataegus douglasii*), thin-leaf alder (*Alnus incana*), Pacific ninebark (*Physocarpus capitatus*), Bebb's willow (*Salix bebbiana*), and Drummond's willow (*Salix drummondiana*) (Lichthardt et al., 2012). Some sites are vegetated with conifers on pond's edges including grand fir (*Abies grandis*), Engelmann spruce (*Picea engelmannii*), Douglas fir (*Pseudotsuga menziesii*), and lodgepole pine *(Pinus contorta*) (Lichthardt et

al., 2012). There are a number of ponds lacking this surrounding vegetation cover due to land use and vegetation removal. Within the ponds, wetland vegetation includes common spikerush (*Eleocharis palustris*), reed canary grass (*Phalaris arundinacea*), simple stem bur-red (*Sparganium emersum*), short-awn foxtail (*Alopecurus aequalis*), water plantain (*Alima plantago-aquatica*), northerwestern mannagrass (*Glyceria occidentalis*), water butter-cup (*Ranunculs aquatilis*), water parsnip (*Sium suave*), inflated sedge (*Carex vesicaria*), duckweed (*Lemna minor*), liverwort (*Ricciocarpos natans*), pond lily (*Nuphar lutea*), climbing nightshade (*Solanum dulcamara*), cattail (*Typha latifolia*), water hemlock (*Cicuta douglasii*), and common rush (*Juncus effusus*) (Lichthardt et al., 2012).

The mean monthly discharge, as measured at United States Geologic Survey gauge location 13345000 on the Palouse River in Potlatch, ID, for 2012-2013 water year was lower than average for January-March, May-June, and December, with April and July-November close to average stream flow (Figure 6). Mean monthly discharge is calculated from stream gauge data from 1914-2014. Though annual precipitation, and therefore stream discharge have direct impacts on floodplain wetland hydroperiods, there are other factors, such as substrate type and condition, and pond geometry that ultimately dictate variation of hydrology in a populations of wetlands that are subjected to the same annual weather and climate conditions. Even though this study only reviewed one year of data, we were only interested in the difference between the ponds that contain *H.aquatilis* and those that do not when subjected to the same hydrologic and climatic conditions.

Field Methodology

H. aquatilis presence and absence wetlands were identified on aerial photographs and surveyed by the Idaho Heritage Program over the last 10 years (INHP 2013). We surveyed 24 sites 14 with HOAQ and 10 without. We selected 10 environmental variables hypothesized to influence HOAQ distribution related to resource competition, pond hydrology, land use and soil (Table 1). (C=Competition, H=Hydrology, S=Substrate, L=Land Use, D= Descriptive).

We collected vegetation and environmental data from March to November 2013 along three transects in each of the 26 ponds. The long axis of the pond was measured, and transects were placed perpendicular to this axis at the 1st, 2nd, and 3rd quarter marks. The transects spanned the width of the pond with the starting and ending points placed one meter past the edge on each side of the pond. The edge of the pond was indicated by drastic change in slope, vegetation, and substrate. We determined vegetation data, canopy cover, and water depth using quadrat sampling every 2 meters along the transects 1-2 times throughout the HOAQ growing season (June-July) depending on if *H. aquatilis* populations or conditions changed at the site.

Drawdown rate: To track the rate at which the water level dropped in the ponds (drawdown rate), staff gauges (7-8 foot pieces of rebar) were placed in the deepest point of each pond. Between April 2013 until dry date, the distance between to top of the rebar and the water surface was recorded every 7-14 days. The drawdown rate was calculated by finding the slope between the Julian date that each measurement was recorded and the depth of the water. The hydroperiod length is the Julian date at which

the pond was recorded as dry. For ponds that did not dry, the hydroperiod length is 365.

Canopy cover: We assessed the percent cover at each data point in the North, South, East and West using a densitometer, and multiplied this percent cover by 1.04 and divided by 100. Vegetation frequency was calculated by dividing the number of quadrats in which species occurred by the total number of quadrats sampled.

Vegetation cover: Cover of reed canary grass was the percent of the quadrat it covered at that location, and total cover of vegetation was the percentage of space that all vegetation (including graminoids, forbs and floating vegetation, but not overhead canopy cover) took up in the quadrat.

Solar access: To measure shading, solar access images were taken at the center of each transect using a Solmetric Suneye Solar access tool. The SunEye-210 is the world-leading shade measurement tool for solar site assessment. This hand-held electronic tool assesses the available solar energy by day, month, and year by measuring the shading patterns of a particular site, using a calibrated fisheye camera, electronic compass, tilt sensor, and optional GPS to give immediate measurements in the field (2011, Solmetric, Inc. ;http://www.solmetric.com/buy210.html).

Soil collection and metrics: Soil data collection was performed in the fall when the majority of ponds were dry. Soil cores were collected to 55 centimeters at two points along each transect; grab samples for soil bulk density and soil water content were take at 4 spots throughout the pond along transects using a metal cylinder specially design for soil bulk density and soil water content measurements. The goal of

soil sample placement was to capture the variability of ponds' edges, middle, and contrasting areas.

To prepare field samples for particle-size, pH, and nutrient analysis, the soil samples were air-dried, crushed with mortar and pestle, and sieved to <2 mm. Sample preparation included removing organic matter with NaOCl (Clorox Bleach), and removing excess ions, by washing and centrifuging with 95% methanol and 95% ethanol. Particle-size distribution and soil pH laboratory analysis were performed according to University of Idaho, and Soil Survey Laboratory Methods Manual written by United States Department of Agriculture (See specific methods in Appendix A,B,C). The lab manager in the University of Idaho Soil Science lab performed all nutrient analysis according to University of Idaho protocol. Soil nutrient measurements did not result as expected: three ponds soils samples (6 each) were analyzed for available nutrients (Nitrates, Ammonia, Phosphorus, and Potassium). Results fluctuated within and between ponds (Table 2). Due to this high fluctuation and inconsistency, as well as, high cost to finish nutrient analysis, soil nutrients were removed from further analysis.

Soil bulk density and soil water content measurements were measured from the same field sample. Field samples were obtained by pressing a metal cylinder into the top 0-12 centimeters of the pond substrate, and sample of known volume is extracted. All samples were taken from the central of three cylinders in order to control for consistency in depth. Plastic lids were placed on top and bottom of samples and kept in metal containers. To obtain soil water content measurements, samples were weighed (wet weight) with cylinders on a scale. Cores are oven dried at 110°C overnight or until weight was constant. Mass of dried sample with cylinder was recorded after drying.

Then calculate the internal volume of the metal cylinder in cubic centimeters from its dimensions using calipers. Soil Bulk density is calculated by Equation A (See below), and gravimetric soil water content is calculated from Equation B (See below):

Equation A: Soil bulk density

$$BD\,(Mg/m^3) = \frac{Mass\ of\ dry\ soil\ (g)}{Volume\ of\ core\ (cm^3)}$$

Equation B: Gravimetric water content

$$WCg\ (g/g) = \frac{Mass\ of\ wet\ soil\ -\ Mass\ of\ dry\ soil\ (g)}{Mass\ of\ dry\ soil\ (g)}$$

Statistical Analysis

All analyses were run using R statistical software version 2.14.2 (R Development Core Team; http://www.r-project.org/) and significance was evaluated at $\alpha = 0.01$. Prior to statistical analysis, data were scaled and tested for compliance with basic assumptions of normality and equal variance. Data met tests of normality and showed normality in QQ –plots and Histogram plots.

Principal components analysis (PCA) was performed on scaled and centered inter-site environmental variables to examine the structure of the data and reduce dimensionality in the environmental characteristics (Figure 7; Table 3). The first three

principal components were retained in order to capture at least 60% variability. The first axis (PC1=41.1%) was strongly correlated to several substrate variables (Soil Bulk Density and Soil water content), and Canopy Cover as well as cross-correlated with *H.aquatilis* presence/absence. The second axis (PC2=14.6%) was correlated to variables related pond hydrology and water quality (Water temperature, distance to river and elevation to river). The third axis (PC3 = 10.7%) was correlated to variables related to vegetation dynamics (Canopy cover and solar access), and soil bulk density.

A Hotelling-Lawley MANOVA test was used to test environmental differences between presence and absence occurrences. Further investigation included running Unpaired Welch Two Sample t-tests on all environmental variables to assess significance individually. The importance of explanatory variables was also assessed using a classification random forest model, which is a powerful statistical classifier (Cutler et al., 2007). Random forest is robust with non-parametric data and for data sets that contain a high quantity of predictor variables (Cutler et al., 2007). Relative importance of variables was evaluated by rescaling the percent decrease in mean square error from 0 to 100%.

Variables were selected for intra-site and inter-site models based on the outputs of the combination of previously mentioned statistical procedures. Because of high levels of cross-correlation, single variables were chosen to represent different groups of PCA axes. Soil bulk density, date of drying, the elevation difference between the pond and the river, and canopy cover were chosen to include in subsequent inter-site regression analyses based on their t-test significance, eigenvalues, relative importance in the random forest, and on their interpretability. For the intra-site scale regression

analyses, all variables were included in the global model because of the low number and interpretability of variables.

Logistic regression mixed models were created to evaluate the relative importance of environmental variables in predicting *H.aquatilis* presence or absence in (1) a floodplain (inter-site) and (2) a pond (intra-site). Mixed models incorporate both fixed-effects parameters, which represent an entire population, and random effects, which apply to various experimental units in a study (Bates, 2011). Mixed models are appropriate to account for potential spatial similarities (at site, pond, and transect scales), and to avoid committing pseudoreplication (Bolker et al., 2009). Substrate (soil bulk density), hydrologic (date of drying and elevation difference) and light dynamics (canopy cover) characteristics were used in the intra-site scale model. Site and pond were held as random effect parameters. Hydrologic (water depth), resource competition (PHAR presense, and PHAR percent cover), and lighting dynamics (canopy cover) characteristics were used in the inter-site models. Pond and transect were held as random effect parameters.

Akaike information criterion was used for model selection, and stepwise model selection function. Best fit and candidate models were identified using AIC weights (w_i) and considered that there was substantial evidence for use of a competitor model if the $\Delta AIC < 1$.

Seed Germination Experiment Design

We collected HOAQ seeds beween 19 July 2013 by at the Ownbey Palouse Land Trust, Latah County, Idaho. Due to federally threatened status and rare nature of

H.aquatilis, a limited number of seeds were collected (~150). Thus, due to the low number of seeds available for the study, only six seeds each were used for each treatment replicate.

Plants with seeds still attached were kept moist in a 100 liter aquarium filled with water until the seeds naturally dropped to the bottom. Seeds were transported to the Idaho Agricultural Experiment Greenhouse at the University of Idaho, where all specimens were stored in a 12°C chamber in moist native soil for 3 weeks to act as a dormancy period until greenhouse space was available. Seeds were collected at the end of this control dormancy period by sieving the soil and removing *H.aquatilis* seed capsules.

For the germination trial, seeds were incubated in 3-1/2" square by 2 7/8" high square plastic planting containers at daily cycles of 14 hours of light and 10 hours of dark in three different temperature controlled chambers/rooms (Figure 8A shows chamber). Temperature was held constant in all three treatment chambers due to available and types of resources (not altered at night time). Treatments consisted of three temperatures levels (5°C, 12°C, 18°C) (18 replicates of each), three soil moisture levels (10%, 20%, 30%) (18 replicates of each), and two planting depths (1 mm and 8 mm below the surface) (27 replicates of each (Figure 8B shows tray layout). Seeds were placed randomly in trays within chambers to account for any uncontrolled effects. Statistical analysis consisted of two-way and three-way ANOVA's assessing the effects of moisture, temperature, and planting depth.

Results

Summary of Pond Characteristics

In the 14 ponds that contained *H. aquatilis* populations, the average frequency of HOAQ occurrence was 51%. Howellia was found in all transects (3) for 9 ponds, in 2 out of 3 transects in 3 ponds, and only a trace amount of howellia was found in 2 ponds. The majority of howellia populations were large and had relatively consistent growth throughout ponds. *Pharlaris arundinacea* has invaded all but one of the 24 ponds with a mean frequency of 49.66, and a mean cover of 28.43%. Mean total vegetation cover in the sample quadrats was 49.7%, including *H. aquatilis* and *P. arundinacea*.

Environmental variables varied between the 24 ponds (Table 4 includes all environmental variable results). Pond area ranged from 22.77 m^2 to 8305.01 m^2 ($\bar{x}$= 836.43), pond perimeter from 22.20 m to 815.64 m ($\bar{x}$= 170.09), pond distance to the river from 15.11 m to 328.97 m ($\bar{x}$=170.64), and the difference in elevation from the pond to the river ranged from 1.22 m to 4.88 m ($\bar{x}$= 1.68). The shallowest pond depth was 3 cm while the deepest was 79 cm, with a mean depth of 26.82 cm during howellia monitoring (June-July), but ponds reached depths of 142 cm in the spring months.

Water quality varied by pond, and changed consistently with certain environmental factors. Conductivity ranged from 55 µS/ cm^3 to 452 µS/ cm^3 ($\bar{x}$= 20.84), and is positively correlated with soil pH (r= 0.431) . The water pH ranged from 6.4 to 9.3 ($\bar{x}$=7.26), and is negatively correlated with the drawdown rate of the pond (r= -0.646). Water temperature ranged from 15.9°C to 27.92°C ($\bar{x}$=20.84), and negatively correlated with percent canopy cover (r= -0.609). Dissolved oxygen ranged from 3.57

mg/L to 13.12 mg/L ($\bar{x}$=6.65), and was positively related to water temperature (r= 0.533).

Pond substrate ranged from loam to clay, with the majority of ponds containing silty clay, silty clay loam, or silt loam with some sites containing loam, clay loam and clay (Figure 9). The amount of clay was the main factor that dictated soil type, and is also negatively correlated with soil bulk density (r= -0.447).

Inter-site Scale

The Hotelling-Lawley MANOVA confirmed that the presence and absence sites were significantly different among the 31 measured variables ($F_{(1,24)}$=5.61, p < 0.0001). Thirteen of the 31 environmental factors tested significantly different between those ponds containing *H. aquatilis* and those not containing *H. aquatilis* (p < .01) (Table 5) unpaired Welch Two Sample t-test. (To eliminate redundancy and variables, only the October solar access variable was chosen to move forward with tests (eliminating April, May, Aug., Sept.) bringing the significant variables down to 10). These variables were canopy cover, soil bulk density, soil water content, October solar access, elevation to river, distance to river, clay content, soil pH, water temperature, maximum pond depth, and hydro-period length. It is important to note that many of these variables are cross-correlated as shown in the PCA figure and loadings.

To further examine how variables relate to *H.aquatilis* presence and absence and to each other, a graphic correlation matrix was produced in R (Figure 10). There are many notable relationships shown in this figure: *H.aquatilis* presence/absence is shown to be most strongly correlated to canopy cover, soil bulk density, soil water

content, solar access, elevation, distance to river, and date of drying (hydroperiod length). The strongest explanatory variable relationships are between soil bulk density and soil water content (r=∼-0.8), solar access and canopy cover (r=∼-0.65), canopy cover and water temperature (r=∼-0.65), solar access and water temperature and maximum pond depth (r=∼0.4), pond elevation and distance to the river (r=∼0.65), and maximum pond depth and date of drying (r=∼-0.7). There are other relationships, but had Pearson's r of less than absolute 0.5.

Random Forest output showed soil bulk density, soil water content, and water temperature at the factors consistently turned up as the most strongest classifiers of *H.aquatilis* presence or absence in 3,000 random forest classification trees. (Figure 11) However, there is strong cross-correlation between soil water content and soil bulk density, and water temperature is strongly cross-correlated with canopy cover and October solar access.

Boxplots of the 11 significantly different environmental parameters according to the t-tests reveal differences between presence and absence ponds (Figure 12,13,14). Hydrologically, presence ponds had shorter hydroperiod lengths (Presence: $\bar{x}$= 196.3 days ± 3.5; Absence: $\bar{x}$= 240.9 days ± 11.6), shallower maximum pond depths (Presence: $\bar{x}$= 61.45 cm. ± 3.2; Absence: $\bar{x}$= 78.7 cm. ± 4.6), and cooler water temperatures (Presence: $\bar{x}$= 19.8°C ± 0.4; Absence: $\bar{x}$= 21.7°C ± 0.5) than absence ponds (Figure 12). Presence ponds are further from the river (Presence: $\bar{x}$= 199.92 m. ± 13.3; Absence: $\bar{x}$= 143.67 m. ± 16.0), and higher elevation from the river (Presence: $\bar{x}$= 2.50 m. ± 0.3; Absence: $\bar{x}$= 0.9 m. s ±0.2) than absence ponds (Figure 12). Substrate boxplots reveal that presence ponds have lower soil bulk densities (Presence: $\bar{x}$= 0.87 g/cm^3 ± 0.04;

Absence: $\bar{x}$= 1.16 g/cm^3 ± 0.04), higher soil water content during germination period (Presence: $\bar{x}$= 0.72 g/g ± 0.05; Absence: $\bar{x}$= 0.56 g/g ± 0.03), higher soil pH (Presence: $\bar{x}$= 5.6 ± 0.17; Absence: $\bar{x}$= 5.26 ± 0.05), and higher clay content (Presence: $\bar{x}$= 33.8% ± 01.9; Absence: $\bar{x}$= 25.5 % ± 1.9) (Figure 13). Presence ponds had higher canopy cover (Presence: $\bar{x}$= 42.9 % ± 3.3; Absence: $\bar{x}$= 22.9 % ± 3.4), and lower October solar access (Presence: $\bar{x}$= 49.1 % ± 5.9; Absence: $\bar{x}$= 73.4 % ± 4.4) than absence ponds (Figure 14). Table 6 also summarizes the explanatory variable's measurements for presence and absence sites.

For mixed model analysis, the most important random effect parameter was *pond* number, which accounted for 99% of the total variance of random effect. Less than 1% of the total variance in random effect was attributed to the *site* component, therefore site was eliminated in the models, and AIC scores improved. The most important pond-scale environmental characteristics (fixed effects) in predicting *Howellia aquatilis* presence or absence ponds as indicated by stepwise model selection were soil bulk density, hydro-period length (date of drying), and the elevation difference between the pond and the river (AIC: 28.3). The second and third place models (AIC: 29.6) included 1) global model containing soil bulk density, date of drying, canopy cover, and the elevation difference and 2) date of drying, soil bulk density, and canopy cover. (See model selection in Table 7 and final model details in Table 8)

The model captures the majority of the concepts proposed in our hypothesis including substrate and hydrology dynamics, and resource competition dynamics. To further examine the relationships between the model predictor variables, and *H.aquatilis*, the variables were graphed against *H.aquatilis* frequency, and Pearson's r

was calculated for each variable (Figure 15). Canopy cover and elevation from the river are positively correlated with *H.aquatilis* frequency with medium strength (r=0.46, r=0.45 respectively, p< 0.01). Soil bulk density and hydroperiod length were negatively associated with *H.aquatilis* frequency (r= -0.53, r= -0.38, respectively, p< 0.01).

Soil bulk density and soil water content (r=-0.48, p< 0.01) consistently came up as important predictors of *H.aquatilis*. Soil bulk density was chosen for the model to eliminate redundancy and maximize model sufficiency, but both are highly related to *H.aquatilis* presence and absence and *H.aquatilis* frequency. As substrate characteristics, they are highly correlated because as a soil becomes more compacted (higher soil bulk density), the less water it will hold (lower soil water content) (Figure 16). Soil water content is strongly related to *H.aquatilis* frequency (r=0.42, p< 0.01) (Figure 17).

Intra-site Scale

The Hotelling-Lawley MANOVA confirmed that the presence and absence sites within ponds were significantly different among the 5 measured variables ($F_{(1,5)}$=14.9, p< 0.01). Three of the 5 environmental factors tested significantly different between those locations within ponds containing *H. aquatilis* and those not containing *H. aquatilis* (p< 0.01) according to unpaired Welch Two Sample t-test (Table 9). Pond depth, canopy cover and pond number are the factors that consistently turned out as the strongest classifiers of howellia presence or absence in 3,000 random forest classification trees. (Figure 18).

Boxplots of the 3 significantly different environmental parameters according to the t-tests reveal differences between presence and absence quadrats within ponds (Figure 19). Hydrologically, *H.aquatilis* grows in deeper areas of the pond (Presence: $\bar{x}$= 21.1 cm. ± 0.96; Absence: $\bar{x}$= 10.5 cm. ± 1.1). *H.aquatilis* presence quadrats contain a lower total vegetation cover (graminoids and forbs and floating vegetation) that absence quadrats (Presence: $\bar{x}$= 40.6% ± 3.2; Absence: $\bar{x}$= 51.5% ± 3.1). Total vegetation cover is influenced by the amount of reed canary grass (*P. arundinacea*), and *H.aquatilis* presence quadrats also contain lower *P. arundinacea* cover than sample points without *H.aquatilis* (Presence: $\bar{x}$= 11.6% ± 2.2; Absence: $\bar{x}$= 31.7% ± 3.3). (Table 10 summarizes the differences between presence and absence sites of the statistically significant environmental parameters).

Seed Germination

The average germination rate for moisture levels 10%, 20%, and 30% were 24.1, 22.2, and 30.6 % respectively. The average germination rates for the temperatures levels 5°C, 12°C, and 18°C were 37.0, 39.8, and 0 % respectively (Figure 20). The average germination for planting depth for 2 millimeters and 8 millimeters was 23.4 and 27.8 respectively. (Table 12) There was a low level of statistically significant information gleaned from seed germination experiments. Temperature was significant ($F_{(2,53)}$= 24.23, p < 0.01), while moisture ($F_{(2,53)}$=0.49, *p=0.61*) and depth ($F_{(2,53)}$=0.36, p=0.55) were not (Table 13).

The two highest germination treatments were with 30% moisture levels for the at 5°C and 12°C. However, the moisture level is not statistically significant so no

concrete conclusions can be drawn about moisture effects. Since the 18°C had no germination, we focused on the other two cooler temperature levels for further analysis. The 5°C temperature treatment had a mean of 33.3% germination at 10% moisture, 33.3% at 20% moisture, and 44.4 % at 30% moisture (Table 14; Figure 21). The 12°C temperature treatment had a mean of 38.9% germination at 10% moisture, 33.3% at 20% moisture, and 47.2 % at 30% moisture (Table 14; Figure 21).

Further analysis of two-way and three ANOVA's show that the only combination of treatments that is statically, significant is the temperature treatment (Table 15).

Discussion

Understanding the specific abiotic and biotic drivers of an organism's biology, ecology, and occurrence can be a challenge for biologists. This study design attempted to quantitatively address this challenge for *Howellia aquatilis* with a comprehensive presence/absence habitat study design in northern Idaho populations using a single year's weather and population conditions as a control, as well as a simple germination experiment. Here we discuss the dominant drivers of *H.aquatilis* occurrence, how they compare to related studies, and make recommendations for restoration and management, as well as future investigations.

Literature investigation and partnership with Pacific Northwest biologists and federal natural resource management agencies helped to identify the potentially important abiotic and biotic variables to assess, as well as important life stages to target. The original assessment included over 35 environmental parameters (including soil nutrients) to capture environmental variation and processes that may be important for *H.aquatilis* habitat suitability. Similar studies have assessed some of these variables, but to our knowledge, this study is the most comprehensive habitat assessment performed on *H.aquatilis* wetlands, and the only study performed on northern Idaho populations.

Inter-site Scale

Inter-site scale model revealed important differences between ponds that hosted *H.aquatilis* populations and those that did not in the 2013 growing season: 1) Ponds

that contained *H.aquatilis* were 1-2 meters higher in elevation from the river than those that do not. 2) Ponds that contained *H.aquatilis* had shorter hydroperiods by an average of 30 days then those that do not. 3) *H.aquatilis* pond substrate has lower soil bulk densities that non- *H.aquatilis* ponds. If the global model is considered, which had an AIC value only 1.3 higher than the best model, then canopy cover is also an important predictor variable. 4) Canopy cover was between 20-40% more for *H.aquatilis* ponds than non- *H.aquatilis* ponds. These parameters are not isolated from each other, and have important interactions that create conditions predicted to be suitable for *H.aquatilis* growth. Here we discuss how they variables relate to other measured variables, as well as compare our results to related studies. Also, the relationship between the best predictor variables and those significant to *H.aquatilis* presence and absence, but not in the best-fit model, will be discussed.

We will examine results from a similar experiment performed on Montana's *H.aquatilis* populations to understand the similarities or dissimilarities between *H.aquatilis* habitats in adjacent states. Similar to Montana findings, hydroperiod length (date of drying) was highly significant in regression models for *H.aquatilis* presence or absence (Lesica, 1992). Hydroperiod is known to be a major determinant in the composition of wetland vegetation in many wetland ecosystems (Casanova & Brock, 2000; Correa-Araneda et al., 2012; Foti et al., 2012; Walker & Coupland, 1970). Wetland vegetation clearly have a integral tie to the hydrologic regime, but *H.aquatilis* is especially unique because of its requirement for complete inundation to grow and mature, as well as periods with no standing water during germination season. Our results indicated that the majority of *H.aquatilis* wetlands had dry dates ranging

between Julian day 193 (July 12) to 206 (July 25). Ponds that have longer hydroperiods than this range do not often provide suitable hydrologic regimes for *H.aquatilis* growth. The Montana study did not specify drying regime dates.

Drawdowns directly affect aquatic plants by exposing them to aerobic conditions, and indirectly by transforming substrate features (Bornette & Puijalon, 2010). They are also considered to increase plant biodiversity, not only because they increase emergent aquatic plant communities, but limit competitive interactions between plants (Bornette & Puijalon, 2010). In *H.aquatilis* ponds, the *rate* of the drawdown was not as important as *when* the ponds reached dryness. We expected the rate of drawdown and hydroperiod length to be directly linked, but parameters such as pond size, and location relative to their alluvial water source seem to be more important.

The elevation difference from the pond to the river was on average 2.5 meters for ponds containing *H.aquatilis* populations and 0.92 meters for ponds without *H.aquatilis* populations. It was expected that this variable would have a strong relationship with hydroperiod length, but the relationship is of medium strength (pearson's $r = -0.38$, $p < 0.01$). This correlation indicates that the higher in elevation ponds are from the river, the less connected they are to the alluvial water inputs, and the shorter the hydroperiod will be. However, the difference in elevation is also positively correlated to canopy cover (pearson's $r = 0.40$, $p < 0.01$). There are a few hypotheses for why this relationship may exist. First, higher elevation ponds are likely to be closer to forested areas. In the Palouse and Hangman Creek watersheds, due to

land use and ecology, forests do not often border the streams. Second, larger canopy trees may establish more successfully and permanently without high water stress.

Similar to Lesica's autecological study of *H.aquatilis*, pond water chemistry was not shown to be a dominate driver in *H.aquatilis* occurrence, but some parameter measurements differ between studies (Lesica, 1992). Lesica found conductivity to be negatively correlated with *H. aquatilis* cover, whereas we saw high fluctuation of conductivity between ponds, but without an impact on *H.aquatilis* presence or frequency. It is possible that the Montana populations have mineral deposits that create higher dissolved solids during various parts of the year and impact pond environment. Montana ponds had overall cooler temperatures whether ponds had *H.aquatilis* or not, ranging from 12°C to 17°C, whereas the northern Idaho pond temperatures ranged from 16°C to 28°C. Also, the Idaho study showed water temperature to be on average 2°C cooler in ponds that contain *H.aquatilis* (t-value=2.4, $p < 0.01$). The most probable explanation for these disparate temperature findings is that many of the Montana populations have wetland buffer management in place, or populations exist on non-private and/or protected lands, whereas a number of the Idaho pond habitats lacked riparian tree and shrub cover to create shading wetlands (Mincemoyer, 2005).

Water temperature was not in the final inter-scale regression model, but a cross-correlate, canopy cover, was in the global model. Logically, water temperature and canopy cover are inversely related. Water temperature and pond size can be correlated, but show no relationship in our study. Ponds that lack sufficient tree and shrub shading were easily warmed by hot conditions, and more subject to outside effects of land cover such as cow trampling, runoff, etc., which may all lead to less

suitable *H.aquatilis* habitat. For forested wetlands, adjacent and overhead vegetation cover is a commonly assessed character to signify a healthy wetland system, and an important predictor of wetland surface temperature (Fennessy, Jacobs, Kentula, & Control, 2004; Smesrud, Boyd, Cuenca, & Eisner, 2014). Causation cannot be claimed that warm water inhibits *H.aquatilis* growth from our results, but water temperature and canopy cover likely play an important role in creating and maintaining suitable *H.aquatilis* habitat. Mechanistically, the soil and water do not stay cool enough to maintain soil moisture, and soil temperature and moisture are key characteristics for plant germination (Lesica, 1992).

Soil bulk density was consistently revealed as an important predictor of *H.aquatilis* presence and absence throughout data analysis (Figure 22). Soil bulk density is a common measure of substrate compaction, and is calculated as the dry weight of soil divided by its volume. Soil bulk density reflects a soil's ability to function for structural support, water and solute movement, and soil aeration. Different soil textures will have different expected compaction measurements (Table 16). Loamy soils become too compact around 1.4 g/cm^3, whereas sandy clays become too compact around 1.10 g/cm^3 . When they come close to or surpass these thresholds, they begin to affect and/or prevent root growth. The establishment and germination of a small annual like *H. aquatilis* could be hindered by over-compacted soils.

Over-compaction can often be cause by cattle trampling, so we assessed the soil bulk density in ponds currently being grazed or grazed within the last 3 years, and those not grazed, or had not been for over 3 years (Figure 23). There was a significant difference in the grazed (SBD $\bar{x}$= 1.28 g/cm^3) and un-grazed ($\bar{x}$= 0.87 g/cm^3) ponds (t=-

4.1, p< 0.01). Seventy-five percent of the ponds containing *H.aquatilis* fell into the un-grazed category. There were three outliers in the un-grazed group that have contained *H.aquatilis* in the past, but had no populations in 2012, and had very high compaction. These ponds are in the Hangman Creek watershed on wildlife land. In the past they were grazed heavily, but cattle have not been on them in 7 years. It is possible that the compaction impacts from grazing are still in effect, or that they are compacted for other reasons.

Soil water content is correlated with soil bulk density (Pearson's r=-0.48, p< 0.01) likely because more compact soils do not transport water or absorb water very readily. Soil water content is high correlated to *H.aquatilis* frequency (r= 0.72, p< 0.01). As mentioned previously, all soil measurements were taken during the *H.aquatilis* germination period (late October). These measurements could be strong indicators of *H.aquatilis'* germination needs are. The majority of *H.aquatilis* ponds had fall soil water content between 51-90%. Not only does this imply that howellia germination requires a high amount of soil moisture (but without standing water), but that this requirement is largely why *H.aquatilis* requires such a specific drying regime. It is possible that only with the ideal drying regime does the soil moisture sync with *H.aquatilis'* germination timing.

Intra-site Scale

There are specific micro-habitats within *H.aquatilis* wetlands that that are best suited for *H.aquatilis* growth. Of the 7 parameters measured every two meters on the pond transects, the model chose two factors that were dominate in predicting which

micro-habitats within ponds will contain *H.aquatilis*: (1)Percent cover of reed canary grass (*P. arundinacea*), and (2) depth of water. The predictive power of these variables aligns with hypotheses, but we expected to see different values of the parameters for presence versus absence locations in the ponds.

We expected reed canary grass to have a severe effect on the occurrence and robustness of *H.aquatilis* populations, since invasive species are one of the major proposed threats to *H.aquatilis* populations. Our results show that the *density* of *P. arundinacea* stands in a given area is more important than the actual presence or absence of *P. arundinacea.* It appears that *H.aquatilis* is able to compete with *P. arundinacea* until a certain threshold of density, and at this point, *H.aquatilis* is outcompeted. In the short-term, possible mechanisms of this competition are the over-shading caused by dense mono-crops of *P. arundinacea*, and competition for spatial resources (Schooler et al., 2006). In the long term, *P. arundinacea* can cause altercation of hydrologic regimes and substrate composition caused by production of thick litter layers in the ponds.

In congruence with Lesica's nine-year study at a single marsh in Montana, as *P. arundinacea* stands rapidly increased, *H.aquatilis* numbers decreased (Lesica, 1997). This is not a surprising conclusion as *P. arundinacea* has been evidenced to take over native wetland vegetation globally (Apfelbaum & Sams, 1987). It is believed that *P. arundinacea* originated from nonnative cultivars, but has hybridized with native plants making discrimination difficult, and giving the plant more ecological advantage (Apfelbaum & Sams, 1987; Galatowitsch, Anderson, Ascher, & Hall, 1999). *P. arundinacea* is not only a threat to *H.aquatilis*, but to all wetland biodiversity in North

America, and shown to reduce plant community diversity in many wetland studies (Galatowitsch et al., 1999; Schooler et al., 2006; Souza, Bunn, Simberloff, Lawton, & Sanders, 2011).

Water depth was also a dominant predictor of *H.aquatilis* occurrence at the intra-site scale. It has been observed in Fish and Wildlife reports that *H.aquatilis* grows in moderate to shallow depths, but not very deep waters (Gray et al., 2005; Lichthardt et al., 2012). We observed that *H.aquatilis* grew in a wide range of depths ranging from very saturated soil with no standing water to 45 centimeters. *H.aquatilis* grows at a time of year that there is not high pond area that has deeper waters than 50 centimeters, but this depends on the pond and it's location, substrate, and geometry. From the wide range of growth we saw, *H.aquatilis* does not seem to be limited by water depth, but rather by spatial, light, and nutrient resources from *P. arundinacea*. Figure 26 illustrates this idea and shows *H.aquatilis* and *P. arundinacea* proportion of frequency to be practically inverse of each other. *P. arundinacea* dominates at shallower depths (0-10cm.), whereas *H.aquatilis* dominates at deeper depths (16-45 cm.). Between 10-15 cm., there seems to be a transitional zone, where *H.aquatilis* and phalaris compete for resources the most. What these results show is that water depth is not as limitied to *H.aquatilis* as *P.arundinacea* related competition. *H.aquatilis* is capitalizing on resources and space that *P. arundinacea* has not inhabited. It seems that *H.aquatilis* is ecologically taking advantage of *P.arundinacea*'s limitations, by inhabiting the niche that *P. arundinacea* cannot

Seed Germination

Unfortunately, attempted seed germination trials did not prove that soil moisture is integral in germination, but this could have been at the fault of experimental design (For example: lack of fine range of soil moistures, lack of maintaining soil moisture in treatments, too few *H.aquatilis* seeds used in design, or other variables). The most germination did occur at the highest (30% by mass) moisture level, but was statistically significant. This can weakly back-up the above stated hypothesis that high moisture level in soils is key for germination. Two temperature and moisture probes were placed in the soil of Princeton Pond 9 (a pond with very robust *H.aquatilis* populations) between 10 Oct 2014 to 2 Nov 2014, which is the prime *H.aquatilis* germination period. The results showed soil moisture between 18 and 30% at the site, and temperatures of between around 3-4°C at night and 11-12°C during the day (Figure 24). This data was not used in analysis due to lack of repetition, but just as a supplement to germination data. (See Figure 25 for images of *H.aquatilis* germination in the field).

Overall conclusions

As expected, hydrologic, substrate, vegetation, and landscape variables played a role in predicting *H.aquatilis* presence or absence at either the floodplain (inter-site) scale and pond (intra-site) scale. We predicted that ponds that were more connected to the river and their fluvial actions would have more *H.aquatilis* presence, but the opposite was found. Ponds that are higher and further from the river host shorter hydroperiods, which fosters a hydrologic dynamic best for *H.aquatilis* presence. We

conclude that the hydrologic needs of *H.aquatilis* are extremely specific and require moisture during germination, but a long enough period of dry conditions preceding the fall germination period in order for seeds to develop.

We did not expect to see soil bulk density as such a significant driver in our models. There a few hypotheses related to why this may be: 1) Compaction driven from cattle tramping has an inverse affect on *H.aquatilis* presence 2) Soil texture influences soil bulk density 3) Soil bulk density is cross-correlated with soil moisture during germination period or 4) A combination of these hypotheses.

As hypothesized, canopy cover was a significant driver in our analyses. Loss of surrounding riparian vegetation decreases wetland quality, by increasing surface water temperatures, and increasing solar access at the soil level. It can be concluded that higher water temperature and increased soil dryness are negatively associated with *H.aquatilis* presence.

We expected to see *P. arundinacea* have significant effects at the floodplain scale, but actually found that *P.arundinacea* is a driver at finer scales, within the ponds, rather than at coarse, floodplain scales. The percent cover of *P.arundinacea* at any given point within a pond is what drives *H. aquatilis,* not whether it occurs in a wetland. We expected to see *H. aquatilis* grow in a specific water depth due to its physical growth preferences, but our final results suggest that *H. aquatilis* is capable of growing in a wider range of water depth, but is driven by competition to a narrower range.

For the seed germination portion of the thesis, we were expecting to see stronger results related to soil moisture, but only saw significance in the temperature of the environment at which seeds grew. This could be due to research design short-

comings related to how seeds were collected and stored, or how soil moisture was quantified and administered in the greenhouse. We still believe that soil moisture plays an important role in germination, as patterns in our results displayed, but further analysis is needed.

Implications for management will be discussed in the next chapter.

Chapter 2: Implications for floodplain management

Wetlands provide more ecosystem services per hectare than any other ecosystem type (Mitsch & Gosselink, 2000). This study is mainly concerned with the habitat provision that wetlands provide. Historic wetland destruction has altered the ecosystem function of wetlands worldwide (Ballantine, Schneider, Groffman, & Lehmann, 2012). Restoring and managing wetlands is a critical tool for restoring ecosystem functions (Ballantine et al., 2012). I will discuss how floodplain wetlands can be managed for *H.aquatilis* habitat in northern Idaho.

Overall there are two main options for *H. aquatilis* managers: 1) Maintain current populations through landscape and pond management OR 2) Transplant *H.aquatilis* into absence ponds that meet environmental characteristics preferred by *H. aquatilis* as outlined in this study. I will recommend strategies related to these options in this chapter.

To maintain current *H.aquatilis* populations and thereby their habitat, there are four main variables that must be considered as garnered from this research: 1) riparian vegetation, 2) soil compaction, 3) floodplain connectivity, and 4) reed canarygrass. This study found that for ponds that historically contained *H.aquatilis* but now do not, and for ponds that have not historically document *H.aquatilis* populations, these variables were highly related to whether or not *H.aquatilis* was present in that habitat. In order for current populations to be maintained or historic populations to return, I posit that all if not most of these variables must meet certain criterion suitable for *H.aquatilis* growth.

The loss of riparian buffer zones surround the study sites due to agriculture, development, and grazing leaves the wetlands vulnerable to several negative effects. There was an inverse relationship between the amount of canopy cover around a wetland, and the water temperature. Scientists find that vegetation buffer zones maintain healthy and cool temperatures in many types of freshwater habitats including wetlands (Haycock, Burt, Goulding, & Pinay, 1996). Vegetation buffers also help to mitigate potential negative effects of runoff through their ability to act as catchments before these inputs reach the water source (Haycock et al., 1996). Another important role of these riparian zones is to maintain soil moisture during *H.aquatilis* germination season. As noted in Chapter 1, high levels of soil moisture in the fall are potentially critical for *H.aquatilis* seed recruitment. Without shading from a canopy of trees and shrubs, soils may dry up too quickly leading to little to no *H.aquatilis* establishment. Managers should work with landowners to maintain riparian buffer zones around *H.aquatilis* habitat, or work to replant vegetation in areas that have already lost their vegetation envelopes.

In ponds that had healthy *H.aquatilis* populations we found that soil bulk densities were on average lower than 1.1 g/cm ^{3}, and had high soil moisture in the fall. We also found that high soil bulk densities may be associated with cattle trampling. The potential for cattle traffic to compact soils over time is high. With a lack of management, cattle often walk directly in and around wetlands on the properties they graze. This compaction makes it difficult for a small annual like *H. aquatilis* to establish its roots. We recommend diverting cattle traffic via fence implementation around pond

edges. It may also be necessary to perform soil remediation through tillage, to restore soil bulk density levels back to normal, as compaction affects can last for decades.

As outlines in chapter 1, *H. aquatilis* has extremely specific hydrologic needs including requiring ponds to dry out early enough that seeds can go through dormancy, but not being so dry in the fall that seeds experience desiccation. Because ponds need to dry out earlier rather than later, it is necessary to avoid any practices that bring more water into the wetlands, as well as practices that deplete the wetlands of their water. Development and agricultural-based management of water over the years has altered the connectivity of rivers to their floodplains. It is very possible that is alteration has created *H. aquatilis* habitat over time, by creating wetlands where the river used to be. But, maintaining some connectivity between wetlands and their fluvial water sources is essential for the continuing existence of healthy *H. aquatilis* populations.

Lastly results indicated that as the density of *P.arundinacea* increases in ponds, the amount of *H. aquatilis* decreases. Any land manager who has tried to abate *P.arundinacea* knows it can be extremely difficult, but this study actually find that *H. aquatilis* can persist with certain levels of *P. arundinacea* in the same wetland. Therefore is *P. arundinacea* populations can be maintained so they do not overtake entire populations and ponds as seen in Idaho and Montana, then it does not need to be eradicated entirely (Lesica, 1997). *P. arundinacea* is also known to create thick islands from the accumulation of its own live and dead organic matter over time, and the island can have a negative impact to *H. aquatilis* growth by altering the hydrology of *H. aquatilis* ponds (Gray et al., 2005). Excavating these dense island every few years will likely have a positive impact on the persistence *of H. aquatilis* populations.

The second option, besides managing for existing populations, is to establish new populations in ponds that have many of the required environmental characteristics for *H. aquatilis* habitat. Many of the wetlands that represented "absence" ponds in this study actually met many of environmental requirements we found in the "presence" ponds. Therefore, these ponds may be a suitable area to attempt to transplant *H. aquatilis* seeds or plants (Noël et al., 2011). Many studies find that moving already mature plants, rather than seeds to new wetlands is the most successful strategy for transplantation (Godefroid et al., 2011). However, scientists have grown mature *H. aquatilis* plants from seed in controlled settings, so establishment from seeds may a viable option. This study did not focus on transplanting or reestablishing *H. aquatilis* populations so management agencies may have to implement adaptive restoration strategies to test out the best replanting options *for H. aquatilis.*

Tables

Variable	Hypothesis	Field Method	Spatial	Temporal
Vegetation Measurements				
HOAQ Presence/Absence	N/A	Frequency	Transects	1-2 X Growing Season
Cover in water of all veg. (%)	C	Quadrat cover	Transects	1-2 X Growing Season
Frequency *P. Arundinacea*	C, H	Frequency	Transects	1-2 X Growing Season
Cover of *P. Arundinacea(%)*	C, H	Quadrat cover	Transects	1-2 X Growing Season
Canopy Cover (%)	C, L	Densiometer	Transects	1-2 X Growing Season
April-October Solar Access (%)		Suneye Tool	Transects	1-2 X Growing Season
Substrate Measurements				
Soil Bulk Density (g/cm^3)	S, L, H	Bulk Density cylinder	Transects	1 X Fall
Soil Water Content (v/v; %)	S, L, H	Bulk Density cylinder	Transects	1 X Fall
Sand percentage	S	Soil core	Transects	1 X Fall
Silt percentage	S	Soil core	Transects	1 X Fall
Clay percentage	S	Soil core	Transects	1 X Fall
Texture type	S	Soil core	Transects	1 X Fall
Soil Nutrients (K, NO3−, NO2−, P)	S, H	Soil core	Transects	1 X Fall
Soil pH	S, H	Soil core	Transects	1 X Fall
Hydrology Measurements				
Elevation to River (m)	H	Aerial Map Measures	N/A	N/A
Distance to River (m)	H	Aerial Map Measures	N/A	N/A
Area of Pond (m)	D	Aerial Map Measures	N/A	N/A
Perimeter of Pond (m)	D	Aerial Map Measures	N/A	N/A
Water pH	H	YSI-556 Multimeter	Transects	2 X Growing Season
Water Conductivity (µS)	H	YSI-556 Multimeter	Transects	3 X Growing Season
Water Temperature (°C)	H	YSI-556 Multimeter	Transects	4 X Growing Season
Dissolved Oxygen	H	YSI-556 Multimeter	Transects	5 X Growing Season
Drawdown Rate (m/day)	H	Pond Gauge	Deepest Point	March- Dry date
Max. Depth at Transect (m)	H	Meter sticks	Transects	1-2 X Growing Season
Maximum Depth in Pond (m)	H	Pond Gauge	Deepest Point	March- Dry date
Date of Drying (Julian Day)	H	Pond Gauge	Deepest Point	March- Dry date

Table 1: Complete list of environmental (abiotic and biotic) variables measured in *Howellia aquatilis* presence and absence habitat. Hypothesis column relates to ecological topic that the specific variable helps to quantify (C=Competition, H=Hydrology, S=Substrate, L=Land Use, D= Descriptive). Field method summarizes how the variable was collected. The spatial column describes where the measurements were taken with the pond, and the temporal column describes how often and when the measurement was taken.

Pond	Sample #	*Amount of soil tested*	*Millimeter of solution*	*NO3-N (m/kg)*	*NH4-N (mg/kg)*	*Available Soil K (mg/kg)*	*Available Soil P (mg/kg)*
Absence Pond 1	1	5	40	5.03	2.1	1.466	0.086
	2	5	40	23.45	6.95	1.235	0.0799
	3	5	40	21.3	6.1	0.8766	0.0889
	4	5	40	26.53	3.45	0.9935	0.0831
	5	5	40	22.72	1.33	1.048	0.0784
	6	5	40	9.76	2.68	1.378	0.0582
Presence Pond 1	1	5	40	5.03	3.31	0.2135	0.0407
	2	5	40	2.98	1.52	1.199	0.1625
	3	5	40	0.87	8.06	1.05	0.1453
	4	5	40	3.9	7.69	1.716	0.1882
Presence Pond 2	1	5	40	2.41	1.67	0.4321	0.117
	2	5	40	28.59	3.31	0.8103	0.1669
	3	5	40	15.53	5.91	1.249	0.0938
	4	5	40	6.4	0.74	1.347	0.09
	5	5	40	11.23	5.56	0.3642	0.0889
	6	5	40	21.52	6.21	1.1	0.093

Table 2: Results of Nitrates, Amonia, Phosphours, and Potassium soil nutrients for three sites that were used to assess if soil nutrients should be included in final analysis. Results fluctuated dramatically within and between ponds, and it was decided that because of this inconsistent data, and high cost to finish nutrient analysis, that soil nutrients be removed from further analysis.

Variable	PC1	PC2	PC3
Vegetation Measurements			
H. aquatilis Pres./Abs.	**-0.786**	-0.084	-0.252
Canopy Cover (%)	*-0.682*	-0.255	**0.410**
October Solar Access (%)	*-0.675*	-0.490	**-0.490**
Substrate Measurements			
Soil Bulk Density (g/cm^3)	**-0.737**	0.103	**0.549**
Soil Water Content (v/v; %)	**0.742**	-0.203	-0.359
Clay content	0.579	0.266	-0.309
Hydrology Measurements			
Elevation to River (m)	0.622	**0.561**	0.059
Distance to River (m)	0.354	**0.752**	0.085
Water Temperature (°C)	-0.637	**0.563**	-0.100
Maximum Depth in Pond (m)	-0.619	0.056	-0.350
Date of Drying (Julian Day)	0.497	*0.518*	-0.184
Eigenvalue	4.517	1.609	1.174
Variability (%)	41.059	14.629	10.676
Cumulative %	41.059	55.689	66.365

Table 3: Principal component analysis factor loadings for the 10 environmental variables. Three PC axes were included in order to capture ~60% cumulative variability of data. Bolded coefficients indicate the three highest loadings for each PC axis; italicized coefficients have marginally smaller values than the top three.

Variable	*Mean*	*SE*	*Minimum*	*Maximum*
Vegetation Measurements				
Cover graminoids, forbs (%)	50.8	2.9	0.0	100.0
Frequency *P. Arundinacea*	49.7	3.7	0.0	100.0
Cover of *P. Arundinacea (%)*	28.4	3.0	0.0	100.0
Canopy Cover (%)	33.0	2.8	0.2	97.2
May Solar Access (%)	83.8	2.1	25.4	99.7
October Solar Access (%)	61.8	3.9	0.0	98.5
Substrate Measurements				
Soil Bulk Density (g/cm^3)	1.0	0.0	0.4	1.6
Soil Water Content (g/g; %)	0.6	0.0	0.2	1.5
Sand content	13.1	1.3	0.2	52.5
Silt content	57.4	1.4	35.9	79.8
Clay content	10.0	6.8	29.5	12.5
Soil pH	5.4	0.1	4.8	11.0
Hydrology Measurements				
Elevation to River (m)	1.7	0.2	-1.2	4.9
Distance to River (m)	170.6	10.9	15.1	329.0
Area of Pond (m)	836.4	189.3	22.8	8305.0
Perimeter of Pond (m)	170.1	18.9	22.2	815.6
Water pH	7.26	0.8	6.4	9.3
Water Conductivity (µS)	143.6	10.0	55.0	452.0
Water Temperature (°C)	20.8	0.4	15.9	27.9
Dissolved Oxygen	6.7	0.3	3.6	13.1
Drawdown Rate (m/day)	-0.6	0.0	-1.3	-0.3
Maximum Depth at Transect (m)	26.8	1.9	3.0	79.0
Maximum Depth in Pond (m)	70.4	3.0	38.0	142.0
Hydroperiod length (Julian day)	219.5	6.8	137.0	365.0

Table 4: List of all environmental variables measured split up into vegetation, substrate and hydrologic measurements. Table includes the mean value, standard error, minimum and maximum for the environmental variable from the result of all data including presence and absence pond data.

Explanatory Variables	*Degree of Freedom*	*t-value*	*P-value*	*Sig. level*
Max Depth Transect	67.75	1.15	0.2530	
Cover All Vegetation	69.91	1.30	0.1987	
Frequency P.arundinacea	70.85	-0.56	0.5756	
Cover *P.arundinacea*	65.67	-0.24	0.8118	
Canopy Cover	69.60	-3.74	0.0004	***
Soil bulk density	70.88	5.61	0.0000	***
Soil water content	63.89	-5.61	0.0000	***
April solar access	61.52	2.31	0.0242	*
May solar access	65.22	1.87	0.0659	.
June solar access	68.40	1.68	0.0971	.
July solar access	66.40	1.64	0.1062	.
August solar access	60.98	2.12	0.0383	*
September solar access	65.57	2.44	0.0175	*
October solar access	70.08	3.00	0.0038	**
Elevation to river	69.65	-5.23	0.0000	***
Distance to river	65.93	-2.47	0.0161	*
Pond area	36.01	1.64	0.1091	
Pond perimeter	49.19	1.44	0.1570	
Substrate sand content	44.92	0.88	0.3849	
Substrate silt content	65.52	2.07	0.0421	.
Substrate clay content	69.23	-2.74	0.0079	**
Texture type	70.34	-2.08	0.0416	.
Soil pH	44.64	-1.96	0.0563	
Water pH	67.56	0.15	0.8805	
Water conductivity	55.51	0.03	0.9751	
Water temperature	66.64	2.36	0.0210	*
Dissolved oxygen content	63.32	1.10	0.2750	.
Drawdown rate	60.25	-1.11	0.2726	.
Maximum depth pond	58.33	2.86	0.0058	*
Drying date (Julian day)	38.62	3.80	0.0005	***

*Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 5: Results of Unpaired Welch Two Sample t-tests on all environmental variables to assess significance individually. Thirteen of the 31 environmental variables' measurements were significantly different between ponds containing *H.aquatilis* populations and those not containing *H.aquatilis* populations.

	Presence		Absence	
Variable	*Mean*	*Std. Error*	*Mean*	*Std. Error*
Resource Competition				
Canopy Cover (%)	**42.96**	3.929	**22.91**	3.44
Solar access (Oct.)	**49.14**	5.868	**73.40**	4.42
Hydrology				
Water temperature (°C)	**19.82**	0.414	**21.72**	0.53
Elevation (m)	**2.50**	0.263	**0.92**	0.21
Distance to River (m)	**199.92**	13.318	**143.67**	16.03
Max. pond depth (m)	**61.45**	3.197	**78.68**	4.60
Date of drying (Julian)	**196.26**	3.498	**240.92**	11.59
Substrate				
Soil pH	**5.59**	0.174	**5.26**	0.05
Clay (%)	**33.79**	1.929	**25.53**	1.98
Soil bulk density (g/cm^3)	**0.87**	0.039	**1.16**	0.04
Soil water content (g/g)	**0.72**	0.051	**0.56**	0.03

Table 6: Summary of mean and standard error of environmental/explanatory variable measurement between presence and absence sites at an inter-site scale. Only the variables that tested significant for the t-tests were included.

Model	*AIC*	*Δ AIC*	*Model likelihood*	w_i
Inter-site scale				
DE+ JDD+ SBD+ CC	29.60	1.300	0.522	0.230
DE +JDD +SBD	28.30	0.000	1.000	0.441
JDD+ SBD+ CC	29.60	1.300	0.452	0.199
DE+SBD+CC	33.70	5.400	0.058	0.026
JDD+ CC+ DE	32.50	4.200	0.289	0.127
JDD+CC	37.50	9.200	0.024	0.010
JDD+ SBD	35.60	7.300	0.062	0.027

Table 7: Table showing potential inter-scale models starting with the global model. (DE=Difference in elevation, JDD= Julian dry date (hydroperiod length), SBD= Soil bulk density, CC= Canopy cover). Table includes model AIC score, difference in AIC score from best model, model likelihood with best model as 1.0, and model weight with best model having a model weight of 0.441.

Scale	*Best-fit model*	*Fixed effects*	*Coefficient*	*p-value*
Inter	$ln = 18.5x_1 - 47.6x_2 - 29.0x_3 - 24.9$	Elevation to river	18.461	0.00338
		Hydro-period length	-47.588	0.01102
		Soil bulk density	-29.048	0.00102
Intra	$ln = 0.85x_1 - 0.61x_2 - 0.32$	Water depth	0.1993	1.86E-05
		P. arundinacea cover	0.2316	0.00835

Table 8: Best fit model for inter-scale and intra-scale analysis. Best models contain at least an AIC value >1.0 difference of other models. The complete model, fixed effects names, fixed effects coefficients, and fixed effects p-value are described here.

Environmental Variables	*Degree of Freedom*	*t-value*	*p-value*	*Sig. level*
P. arundinacea Pres./Abs.	260.01	1.59	0.1121	
P. arundinacea cover	263.90	5.11	6.20E-07	***
Water depth	279.00	-7.22	5.05E-12	***
Canopy cover	270.86	-1.79	0.07444	.
Total vegetation cover	269.45	2.44	0.01554	*

Table 9: Results of Unpaired Welch Two Sample t-tests on all environmental variables measured at each point along pond transects (intra-site scale) to assess significance individually. Three of the 5 environmental variables' measurements were significantly different between ponds containing howellia populations and those not containing howellia populations.

	Presence		Absence	
Variable	*Mean*	*Std. Error*	*Mean*	*Std. Error*
Resource Competition				
Canopy cover (%)	**37.14**	2.31	**31.36**	2.25
Total veg. cover (%)	**40.60**	3.23	**51.50**	3.10
P.arundinacea cover (%)	**11.60**	2.18	**31.68**	3.27
Hydrology				
Water depth (cm)	**21.07**	0.96	**10.52**	1.11

Table 10: Summary of mean and standard error of environmental/explanatory variable measurement between presence and absence sites at an intra-site scale. Only the variables that tested significant for the t-tests were included.

Model	AIC	Δ AIC	Model likelihood	w_i
Intra-site scale				
PHAR + PHARcov + Depth + CC + Veg. Cover	318.90	3.300	0.192	0.109
PHARcov + Depth + CC + Veg. Cover	319.30	3.700	0.157	0.089
Depth + CC	323.10	7.500	0.024	0.013
Depth + PHARcov	315.60	0.000	1.000	0.568
Depth + PHARcov + CC	317.50	1.900	0.387	0.220

Table 11. Table showing potential intra-scale models starting with the global model. (PHAR= Presence and absence of *P. arundinacea*, PHARcov= Percent cover of *P. arundinacea,* Depth= Water depth, CC=Canopy cover, Veg.Cover= Total present cover of all graminoids and forbs). Table includes model AIC score, difference in AIC score from best model, model likelihood with best model as 1.0, and model weight with best model having a model weight of 0.568.

Parameter	*Level*	*N*	*Mean germination (%)*
Moisture (%)	10	18	24.074
	20	18	22.222
	30	18	30.556
Temp. (Celsius)	5	18	37.037
	12	18	39.815
	18	18	0
Depth (mm)	2	27	23.457
	8	27	27.778

Table 12: Summary of germination rates for the various moisture, temperature, and depth treatment level with the number of replicates listed for each (N).

Parameter	*DF*	*Sum of squares*	*Mean Square*	*F-value*	*Pr >F*
Moisture					
Model	2	0.069	0.034	0.49	0.615
Error	51	3.582	0.070		
Corrected Total	53	3.651			
Temperature					
Model	2	1.779	0.889	24.23	< .0001*
Error	51	1.872	0.037		
Corrected Total	53	3.651			
Depth					
Model	2	0.025	0.025	0.36	0.5503
Error	51	3.626	0.070		
Corrected Total	53	3.651			

Table 13: Three-way and two-way ANOVA output of seed germination data including Moisture, Temperature, and Depth analysis. Table includes degrees of freedom, sum of squares, mean square, f-value and p-value. The only significant treatment was temperature.

Level of Temp.	Level of Moist.	N	Mean	Std. Dev.
5	10	6	0.33333333	0.27888668
5	20	6	0.33333333	0.21081851
5	30	6	0.44444444	0.25092422
12	10	6	0.38888889	0.0860663
12	20	6	0.33333333	0.27888668
12	30	6	0.47222222	0.2870669
18	10	6	0	0
18	20	6	0	0
18	30	6	0	0

Table 14: Mean and standard deviation of germination rates (last two columns) filtered into temperature and moisture intervals.

Parameters	*DF*	Anova	*Sum of squares*	*F-value*	*Pr >F*
Temp	2	1.77880658	0.88940329	24.35	<.0001
Mois	2	0.06893004	0.03446502	0.94	0.3986
Temp*Mois	4	0.03909465	0.00977366	0.27	0.8969
Dep	1	0.02520576	0.02520576	0.69	0.4116
Temp*Dep	2	0.35288066	0.17644033	4.83	0.0139
Mois*Dep	2	0.02572016	0.01286008	0.35	0.7056
Temp*Mois*Dep	4	0.04526749	0.01131687	0.31	0.8695

Table 15: ANOVA results for seed germination study filtered by treatments. Only temperature is significant.

Soil Texture	Ideal bulk densities for plant growth (grams/cm^3)	Bulk densities that affect root growth (grams/cm^3)	Bulk densities that restrict root growth (grams/cm^3)
Sands, loamy sands	< 1.60	1.69	> 1.80
Sandy loams, loams	< 1.40	1.63	> 1.80
Sandy clay loams, clay loams	< 1.40	1.60	> 1.75
Silts, silt loams	< 1.40	1.60	> 1.75
Silt loams, silty clay loams	< 1.40	1.55	> 1.65
Sandy clays, silty clays, clay loams	< 1.10	1.49	> 1.58
Clays (> 45% clay)	< 1.10	1.39	> 1.47

Table 16: Table from United States Department of Agriculture delineating the estimation of bulk density for various soil textures. The ideal bulk densities as well as bulk densities that affect and restrict root growth are included.

Figures

Figure 1: (Upper) Image of *Howellia aquatilis* inflorescence at maturity (Taken by Dubois, K. from Oregon Department of Agriculture web page) (Lower) Image of Howellia showing habit, and spreading branches at maturity.

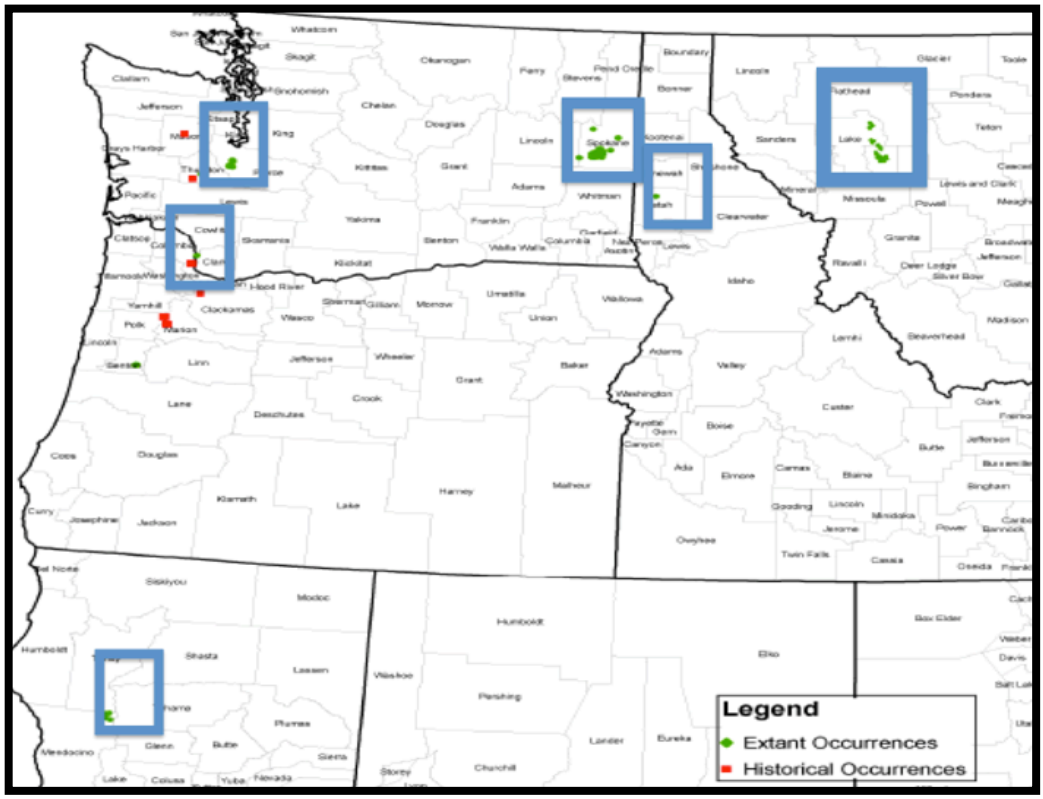

Figure 2: Map of extant howellia occurrences (green dots with blue square), and historical occurrences (red dot). This study focused on the northern Idaho population.

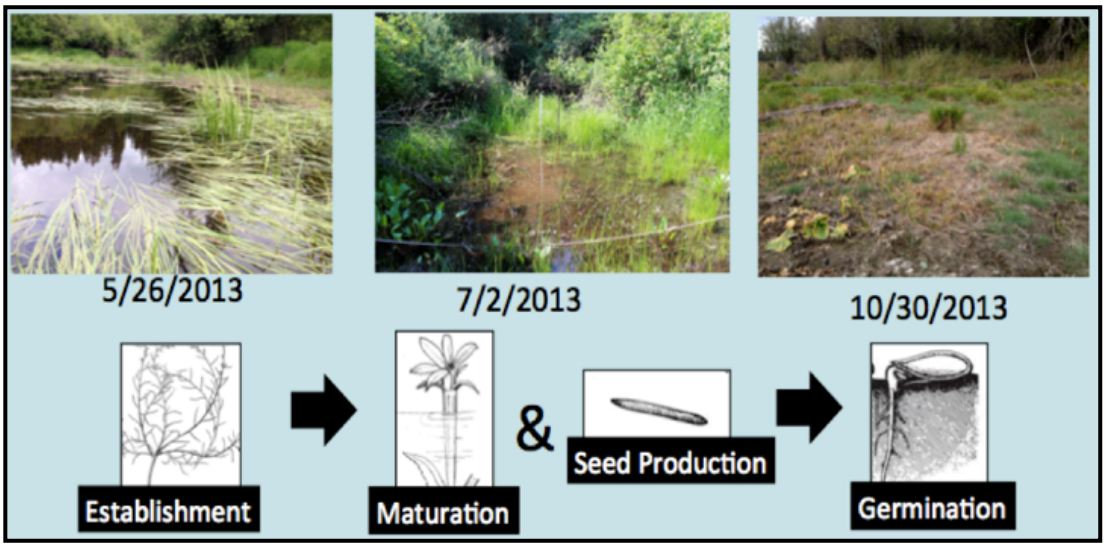

Figure 3: Graphic illustrating the relationship with pond wetting and drying, and Howellia aquatilis life cycle. Plants establish in spring when ponds are full, and lacking in vegetation throughout. Plants mature, flower, and produce seed in mid-summer, when ponds have drawn down markedly. Seeds germination in the fall, when ponds are void of standing water, and seeds are exposed to aerobic conditions.

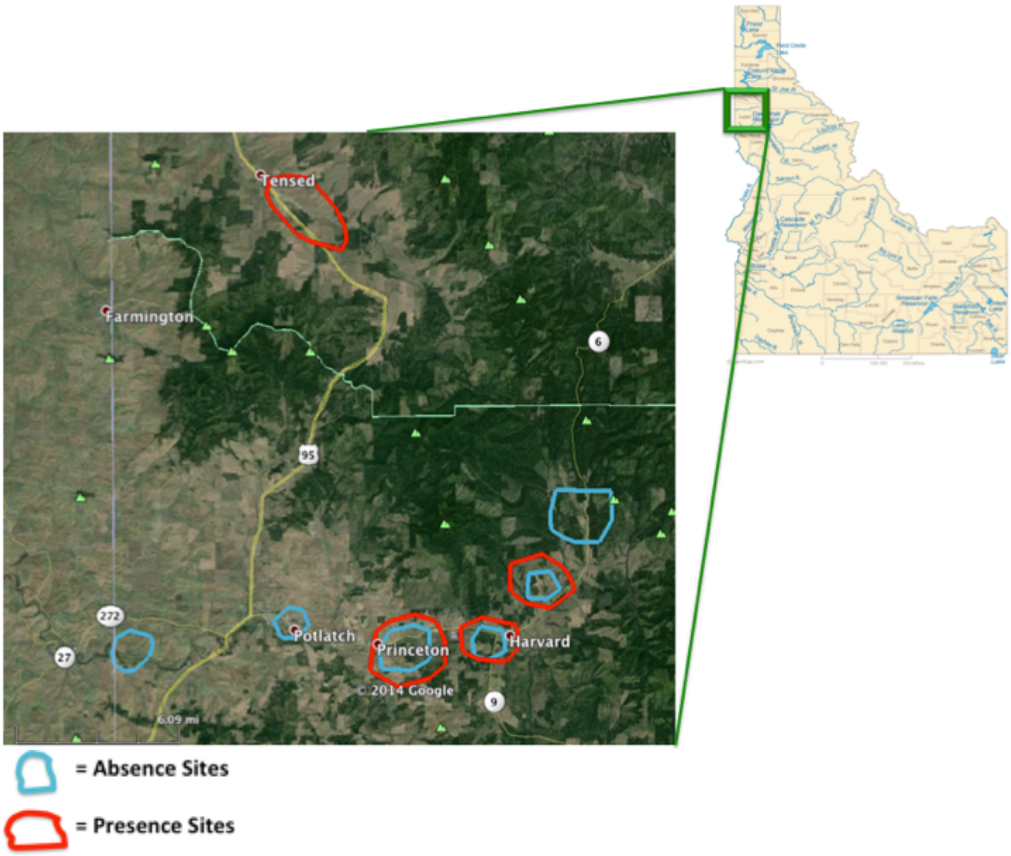

Figure 4: Howellia populations and study sites in the Palouse River and Hangman Creek watersheds in Latah and Benewah county, Idaho. Red circles represent ponds used as absence sites, and blue circles represent ponds used as presence sites. The majority of sites contain more than one pond.

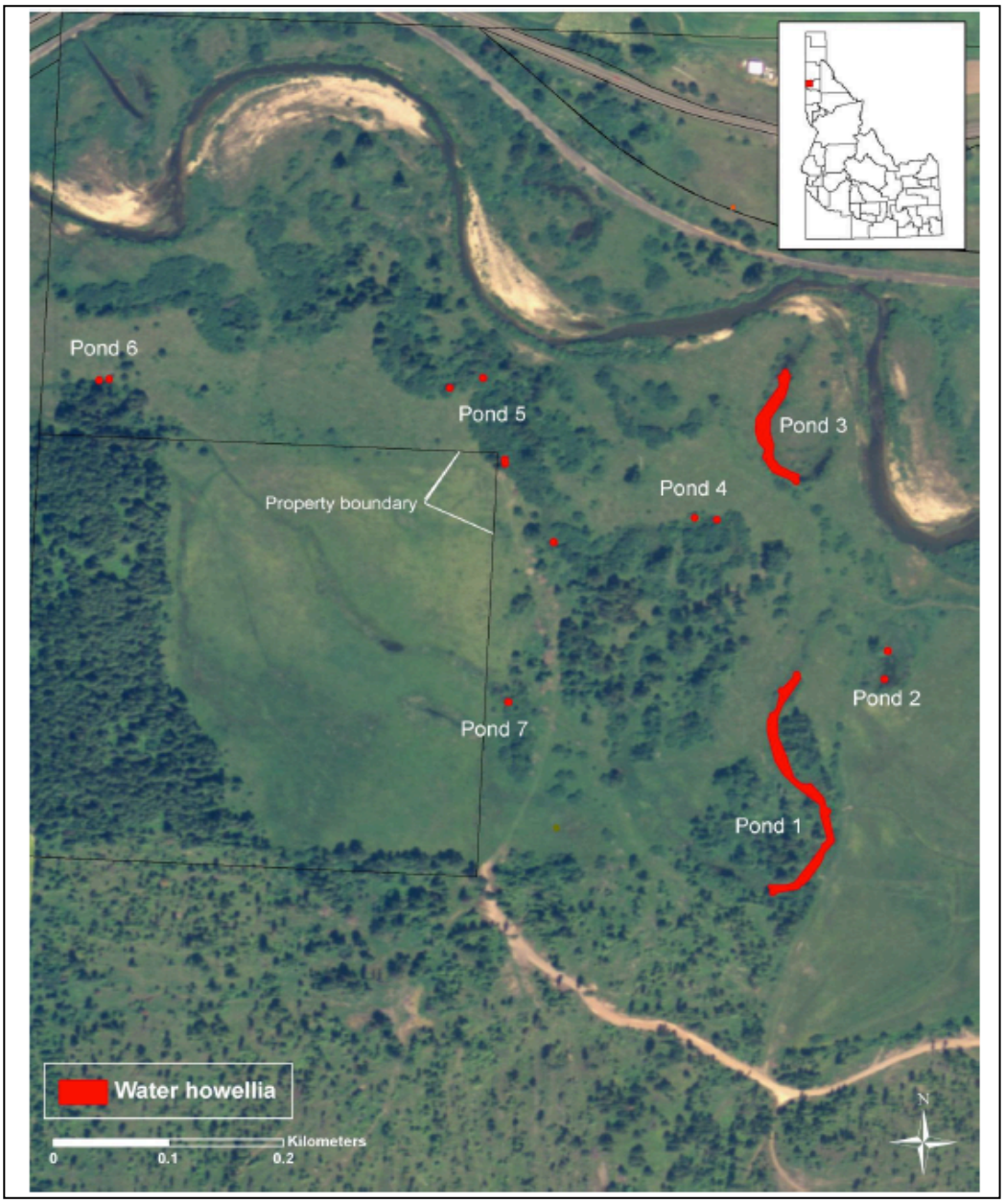

Figure 5: Aerial image of the Princeton howellia occurrence with 7 of the ponds highlighted. This image is from Juanita Lichthardt, Idaho natural heritage program botanist. Many of these ponds are channel relics that now are temporarily flooded sloughs providing high quality howellia habitat.

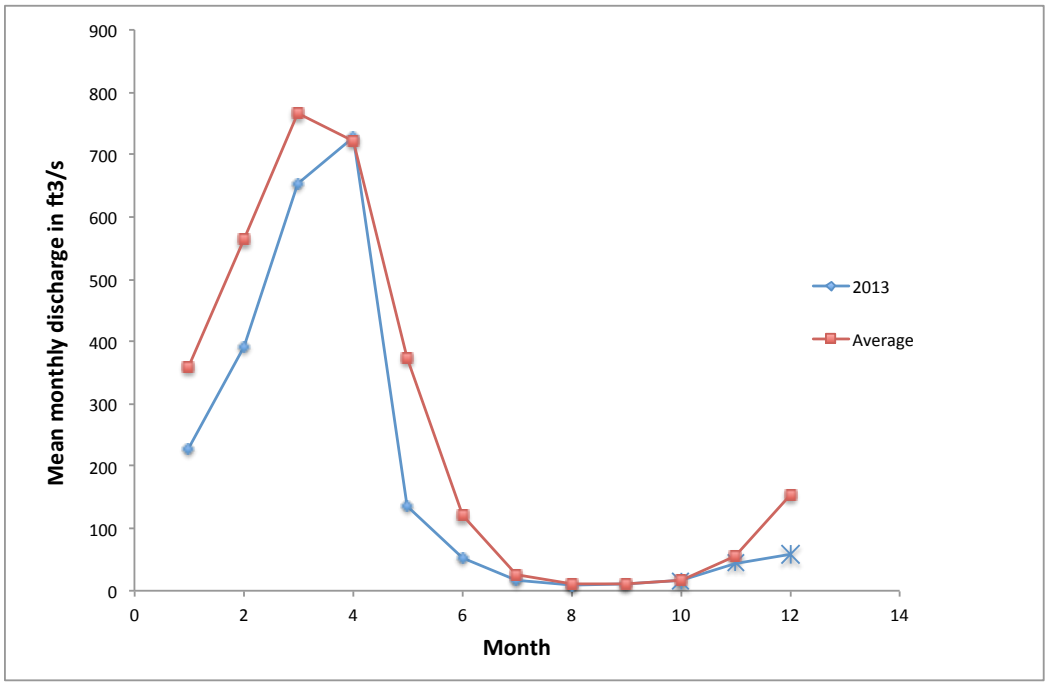

Figure 6: (Upper graphic) Mean monthly discharge from January 2013 to December 2013 (Blue line), and average mean monthly discharge on the Palouse River as calculated by the United States Geologic Survey from 1914-2014.

Figure 7: Plot of Principle component axis 1 and principle component axis two which comprise 40% of the data variability. Principles components analysis was run with all 30 measured environmental variables to assess relationships between variables and layout of the high-quantity of variables.

Figure 8: (A; upper) Image of one type of temperature chamber used at the University of Idaho Sixth Street Experimental greenhouse. (B; Lower) Image of tray of replicates used for germination experiments.

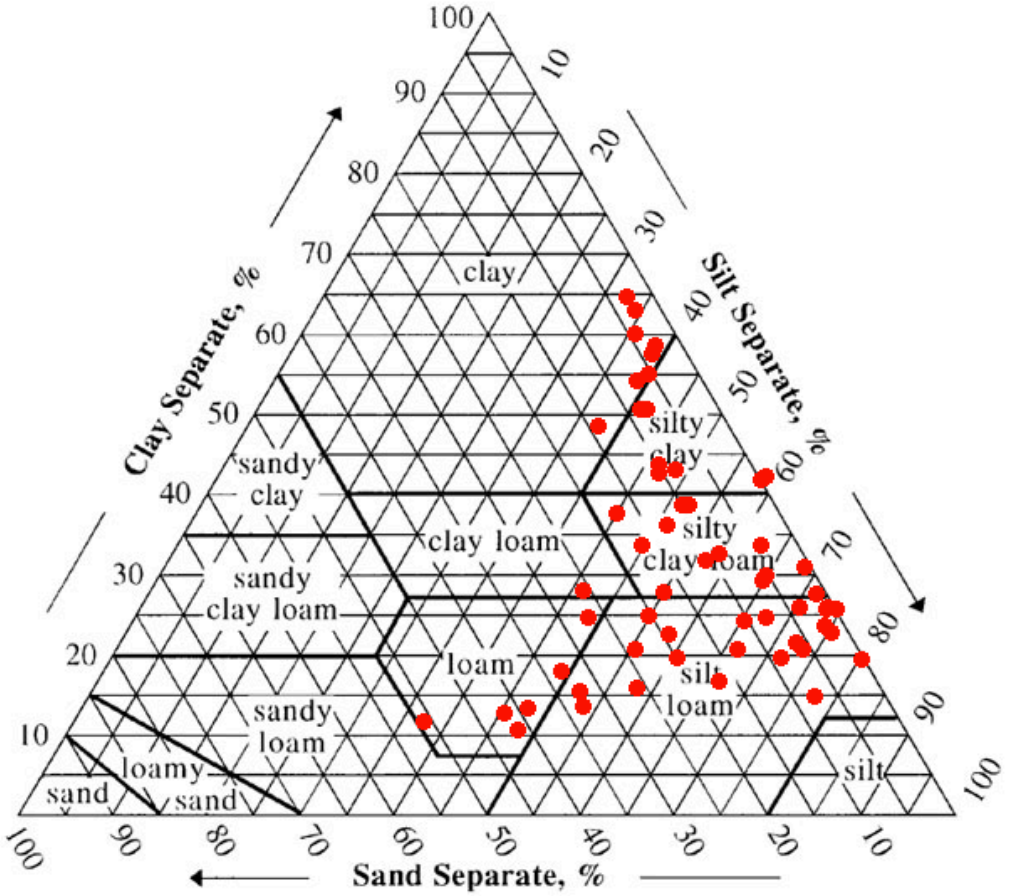

Figure 9: Soil texture triangle with red dots representing a pond transect and overall, showing the range of soil textures the experiment sites comprised. The majority of the sites were silty clay loam, silt loam, and silty clay, with some points falling into loam, clay loam and clay.

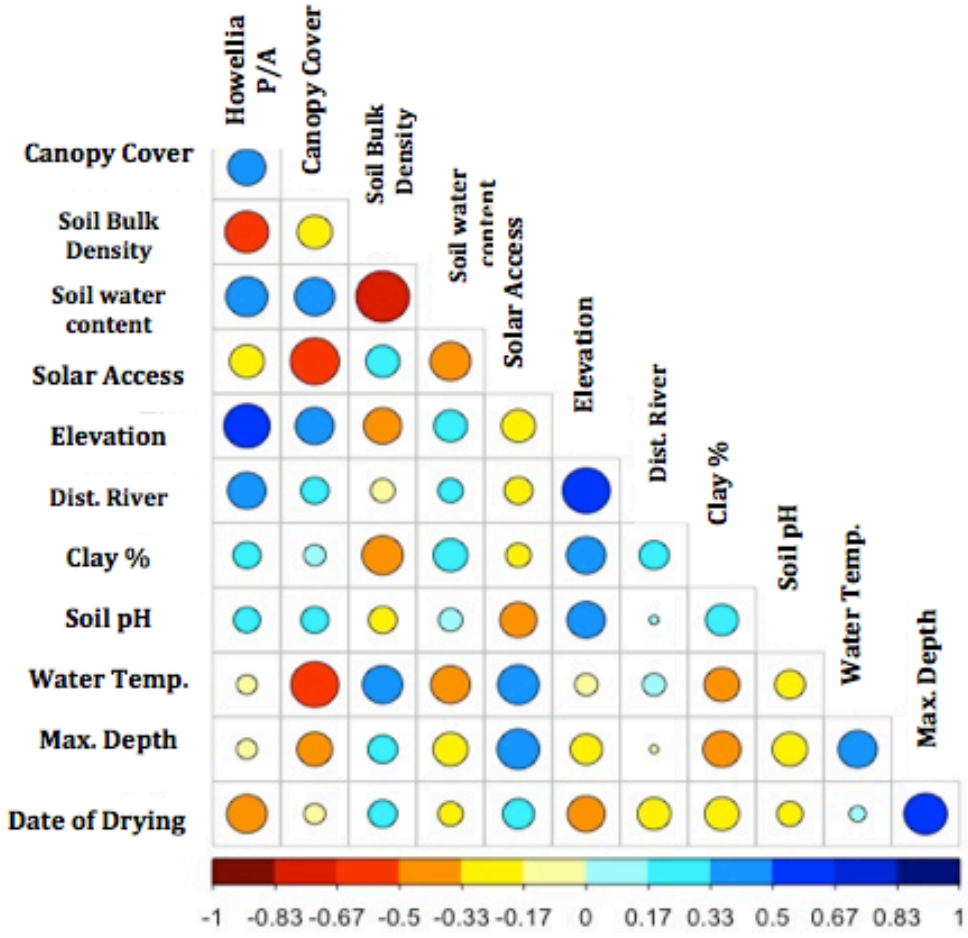

Figure 10: A graphic correlation matrix was produced in R to further examine how variables relate to howellia presence and absence and to each other. These are pearson's are correlation coefficients, and the color and size of the dots relates to the strength and direction of correlation. The warmer colors are negative correlations and the darker and bigger the dot is signifies a stronger the correlation. The cooler colors are positive correlations and the darker and bigger the dot is signifies a stronger the correlation.

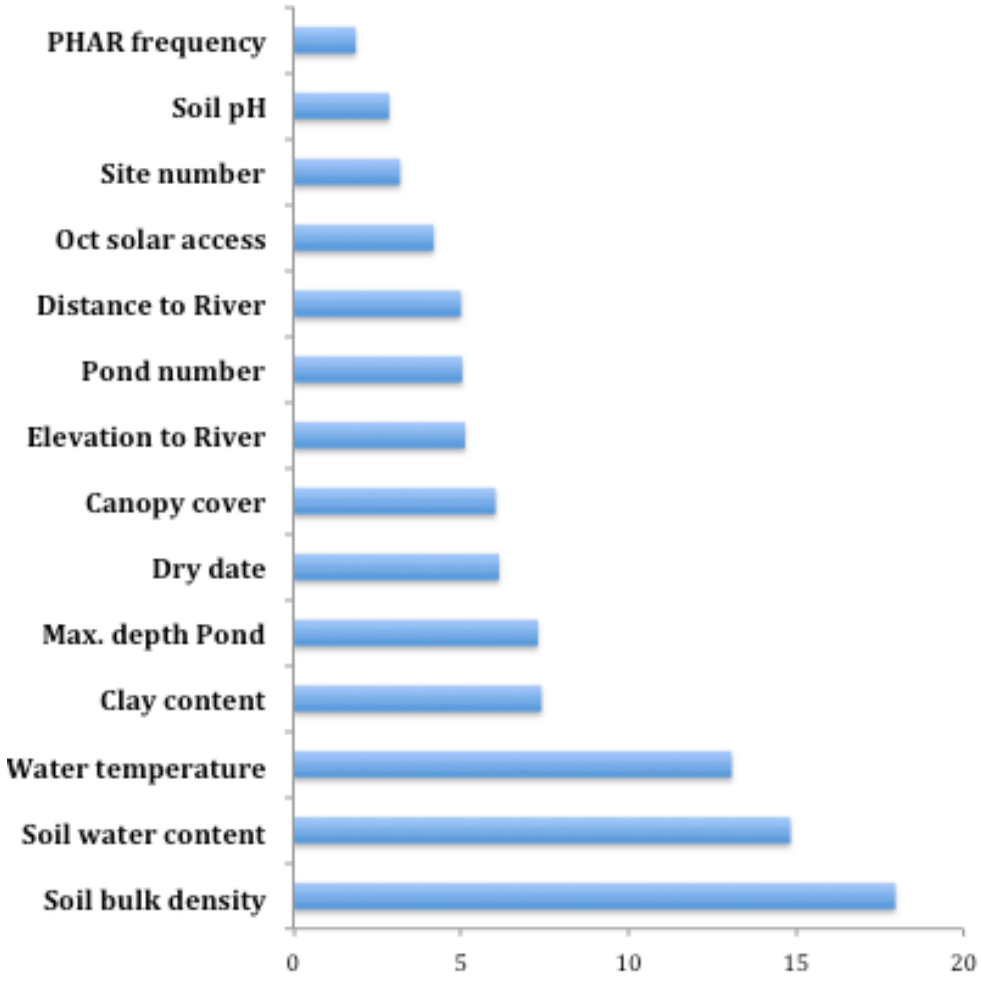

Figure 11: Graphical output of relative importance of environmental factors from random forest classification at the inter-site scale. Soil bulk density, soil water content, and water temperature are the factors that consistently turned out as the strongest classifiers of howellia presence or absence in 3,000 random forest classification trees.

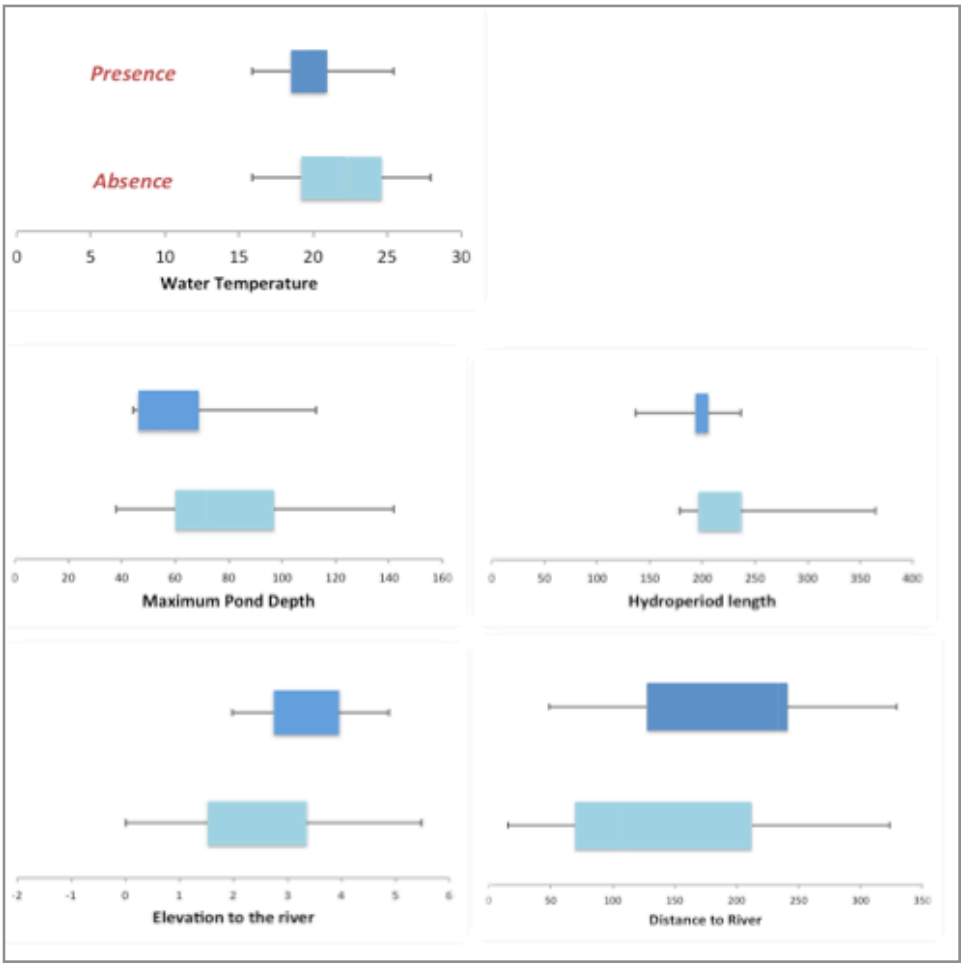

Figure 12: Boxplots for each hydrology related environmental variable that tested significant in the t-test showing the range of values for presence and absence sites.

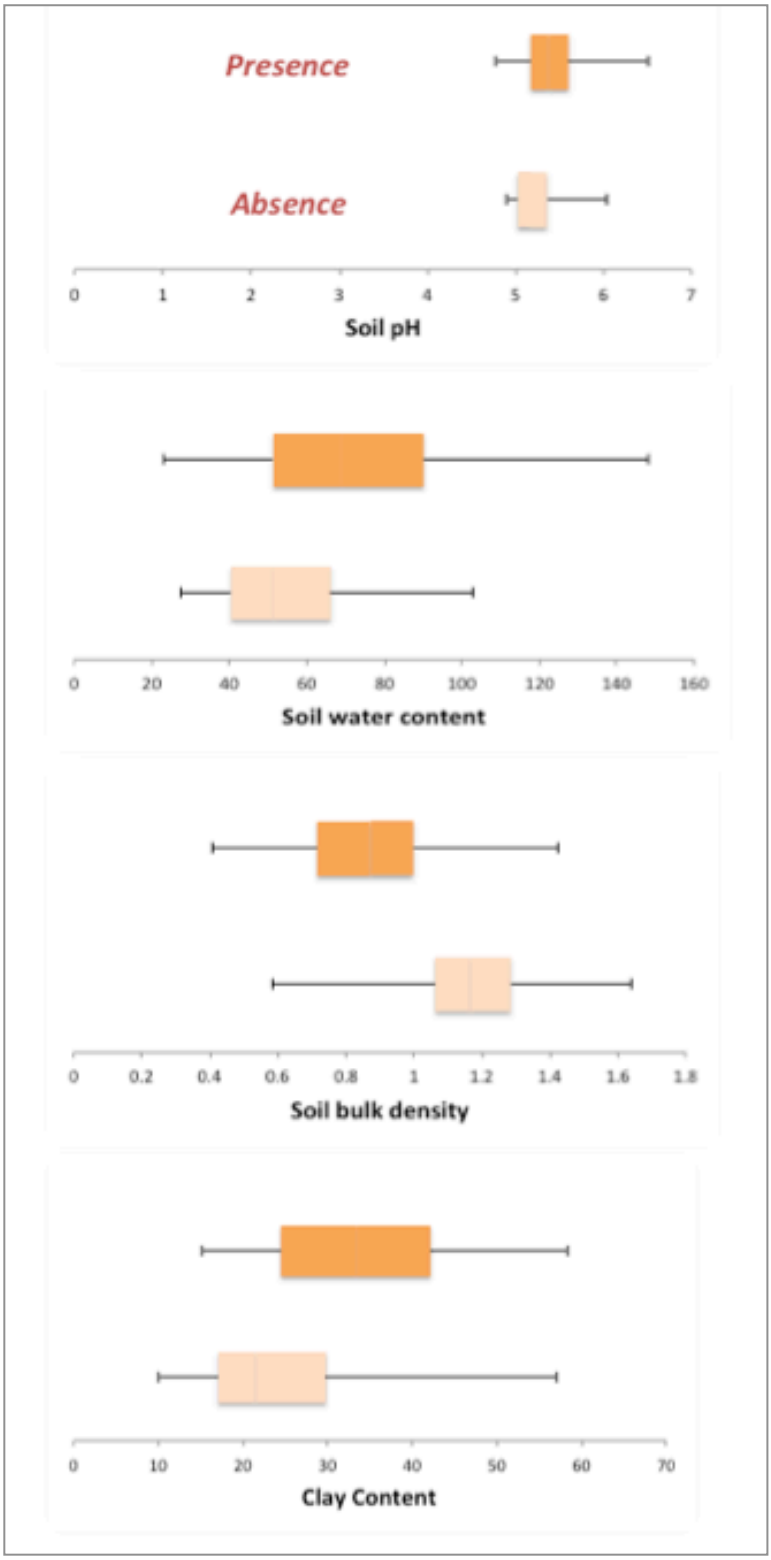

Figure 13: Boxplots for each substrate related environmental variable that tested significant in the t-test showing the range of values for presence and absence sites.

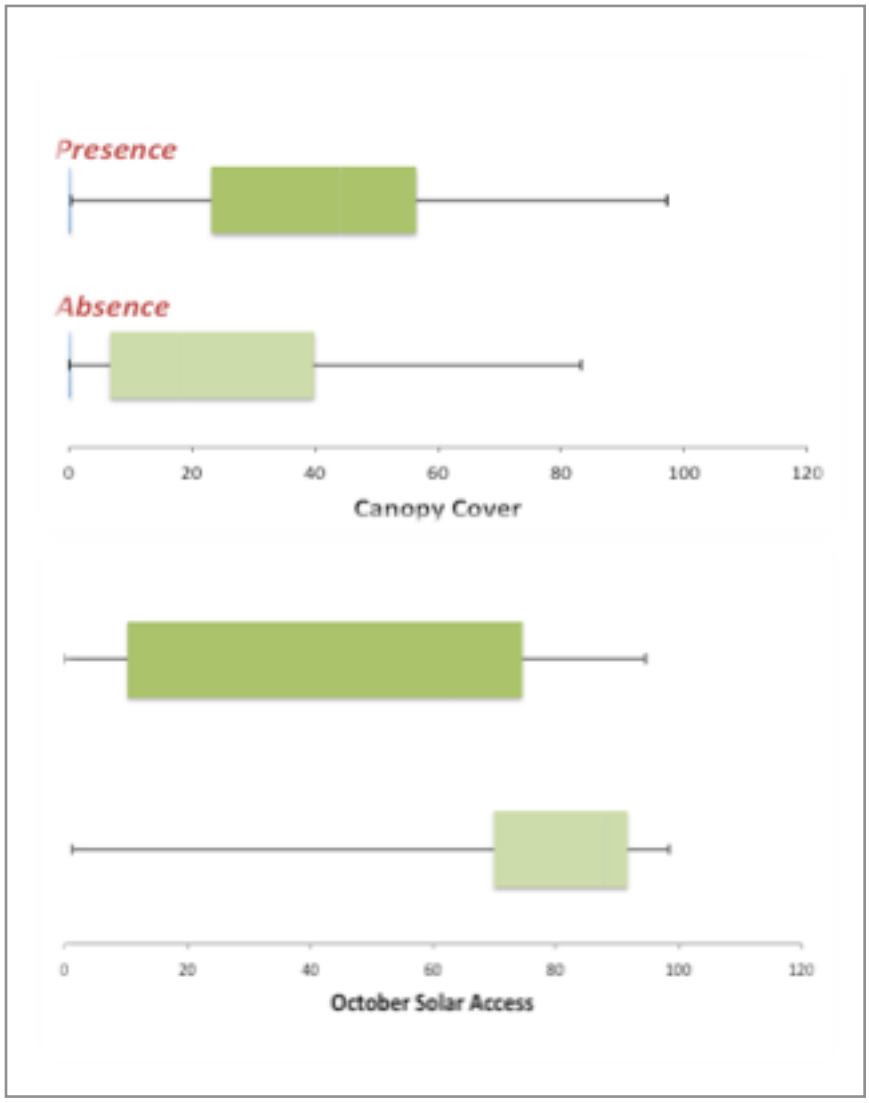

Figure 14: Boxplots for each vegetation related environmental variable that tested significant in the t-test showing the range of values for presence and absence sites.

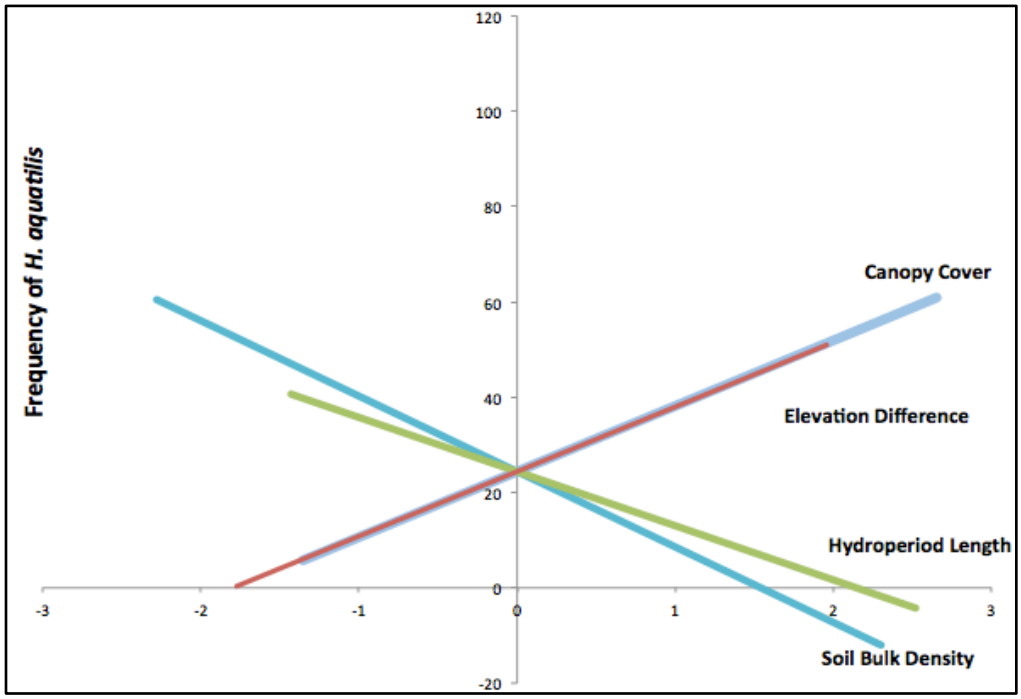

Figure 15: The best-fit model predictors (canopy cover from global model also included here) were graphed against howellia frequency to further examine relationships and patterns. Canopy cover and elevation from the river are positively correlated with howellia frequency with medium strength (r=0.46, r=0.45 respectively, p<.0001). Soil bulk density and hydroperiod length were negatively associated with howellia frequency (r= -0.53, r= -0.38, respectively, p <.0001).

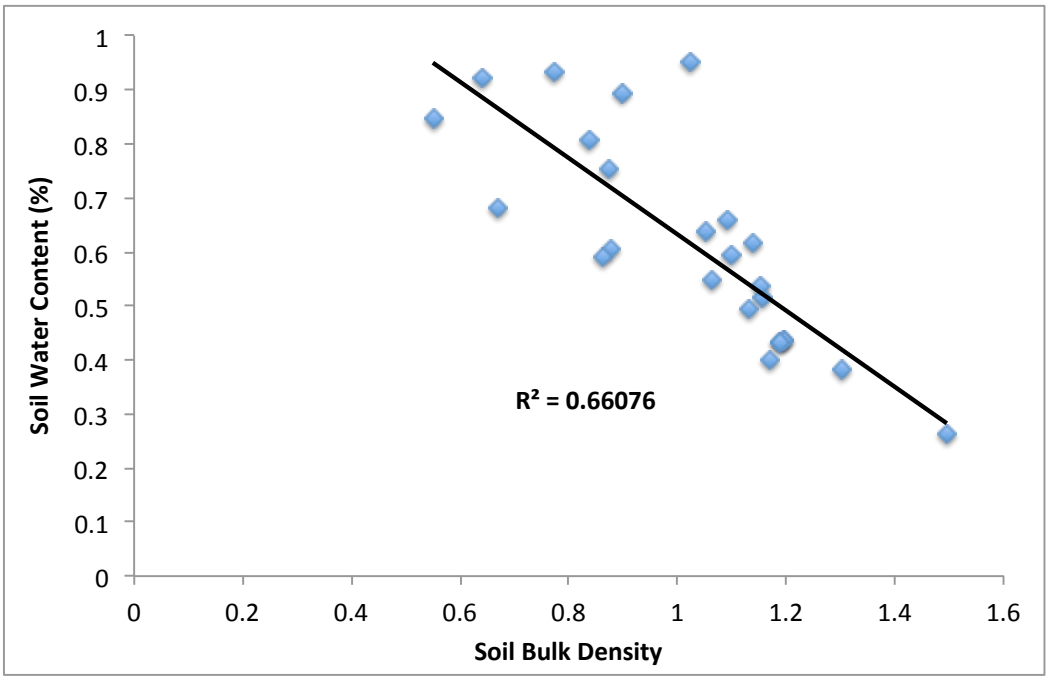

Figure 16: Soil bulk density and soil water content measurements from all presence and absence sites are graphed here to show their strongly inverse correlation ($r=-0.48$, $p<.0001$).

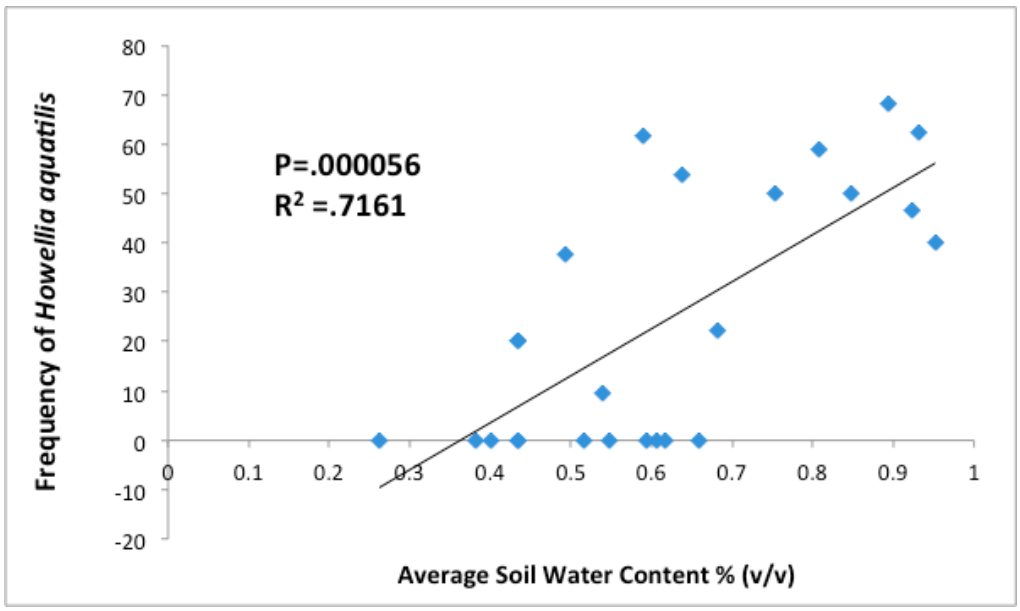

Figure 17: Relationship of average soil water content for a pond and the frequency of howellia aquatilis in that pond (r=0.42, p<.001).

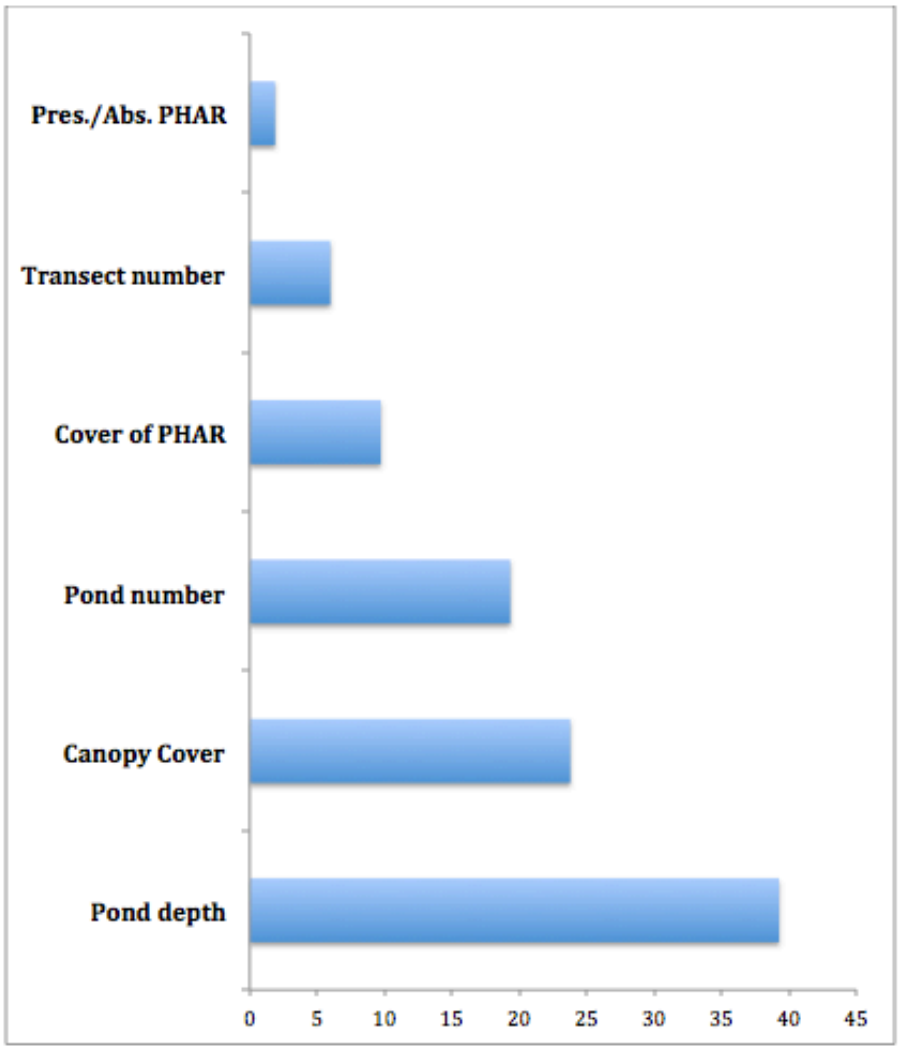

Figure 18: Graphical output of relative importance of environmental factors from random forest classification at the intra-site scale. Pond depth, canopy cover and pond number are the factors that consistently turned out as the strongest classifiers of howellia presence or absence in 3,000 random forest classification trees.

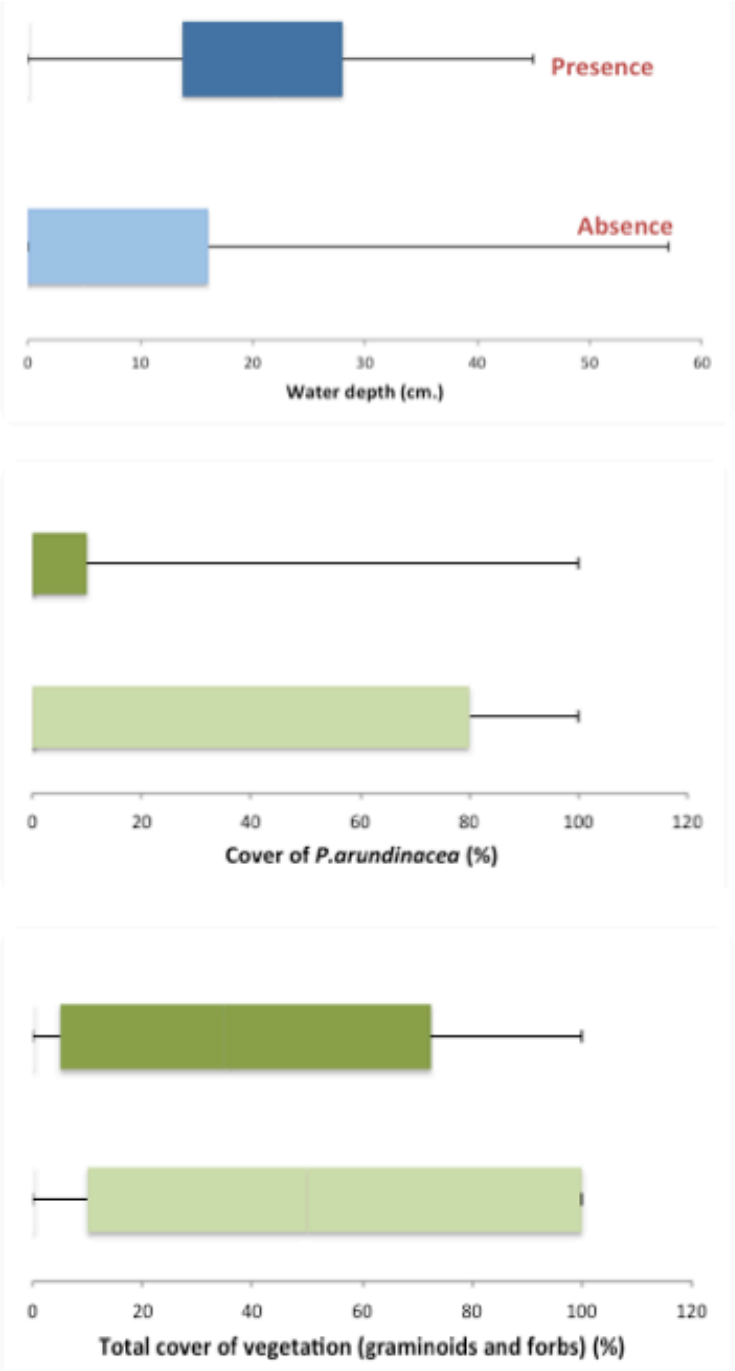

Figure 19: Boxplots for all intra-scale environmental variable that tested significant according to Welch's t-test. Boxplot shows the range of values for presence and absence sites.

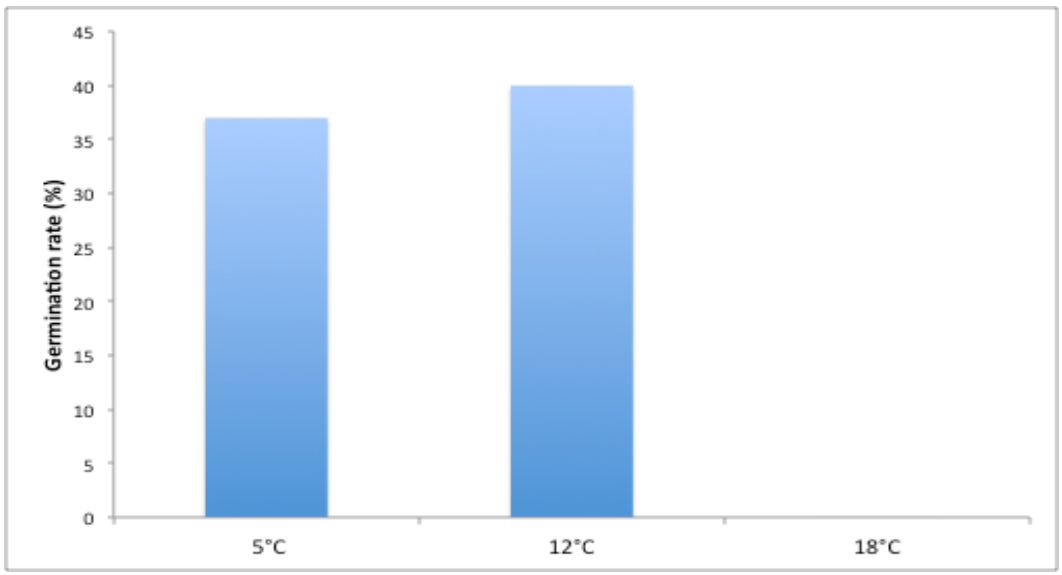

Figure 20: Graphic of germination rate difference between the three temperature levels: 5°C,12°C, and 18°C. . The average germination rates were 37.0, 39.8, and 0 % respectively

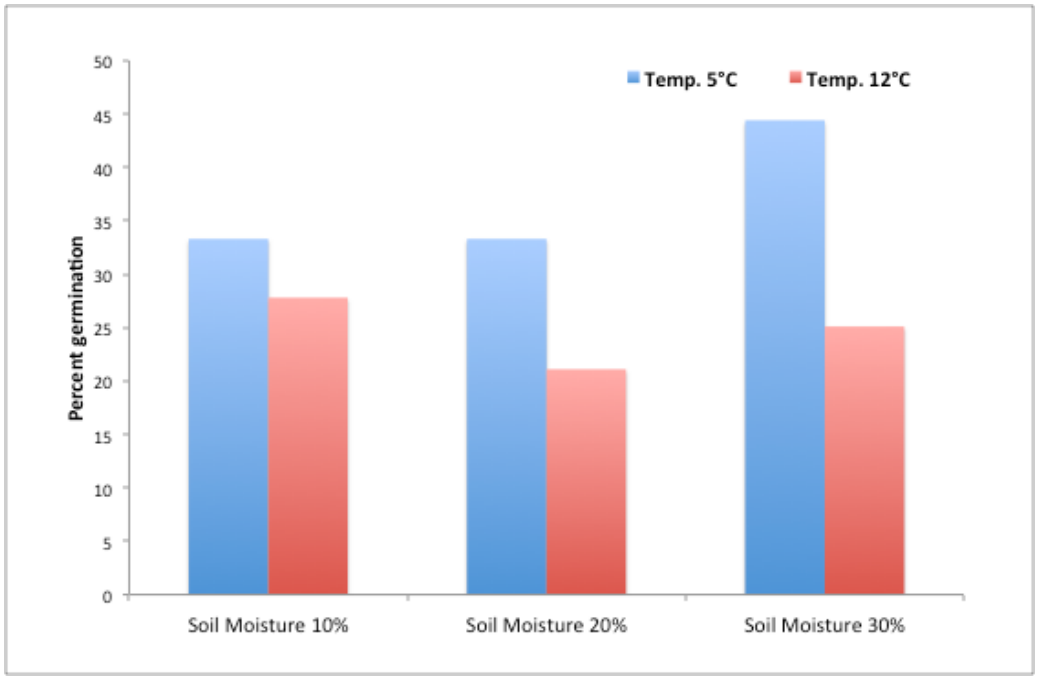

Figure 21: Graphic of germination rate for temperatures 5°C (blue bars) and 12°C (red bars), at each soil moisture treatment level (10, 20, 30%).

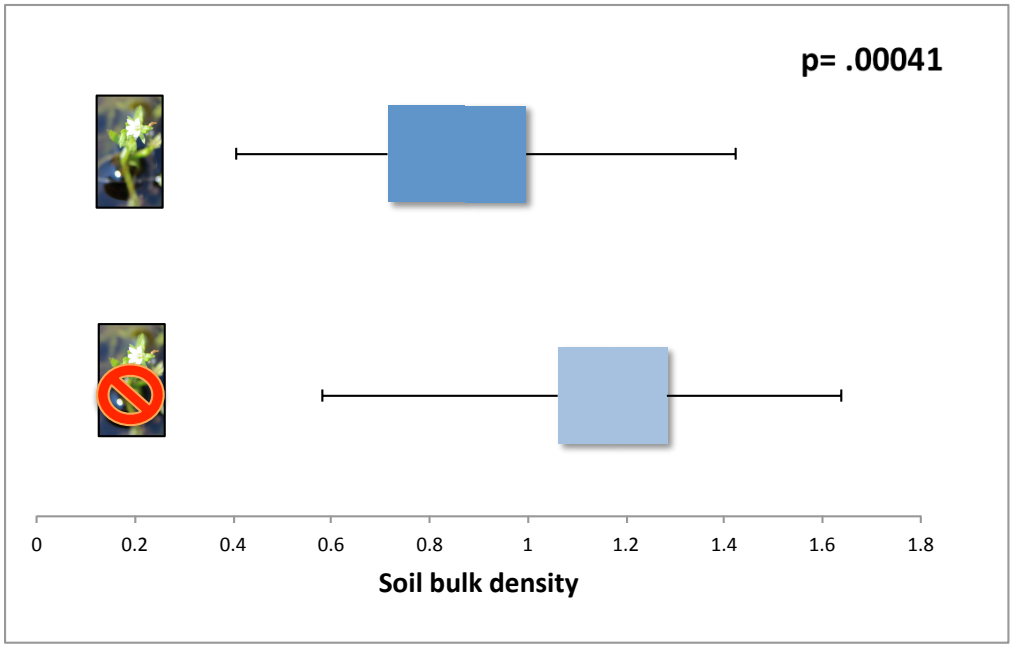

Figure 22: Reiteration of soil bulk density boxplot graphic comparing soil bulk density range for howellia-inhabited and uninhabited ponds.

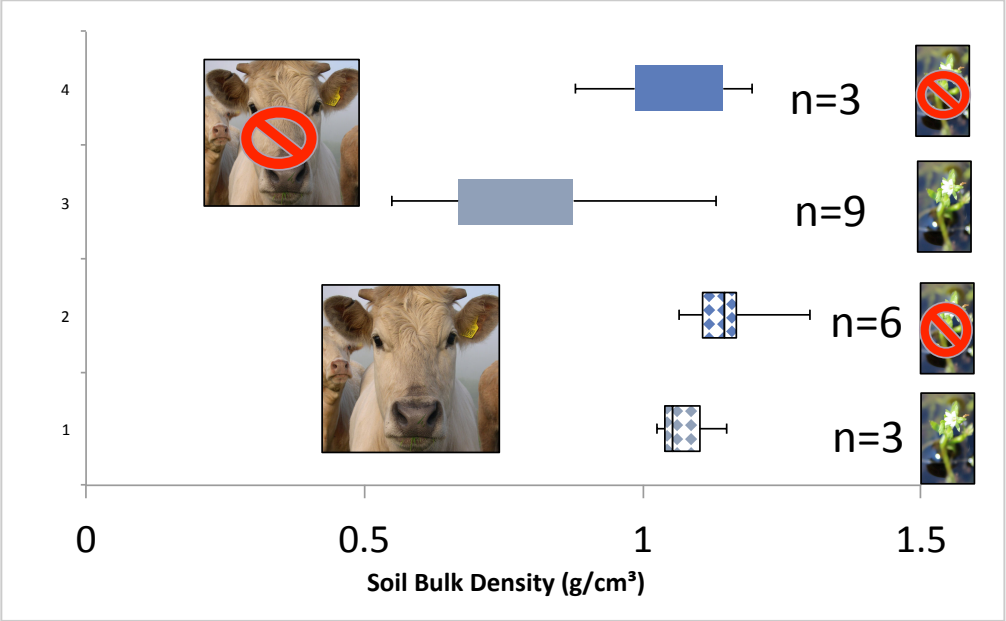

Figure 23: Figure showing range of soil bulk densities between ponds with howellia and grazing, ponds with howellia and without grazing, ponds without howellia and with grazing and ponds without howellia or grazing.

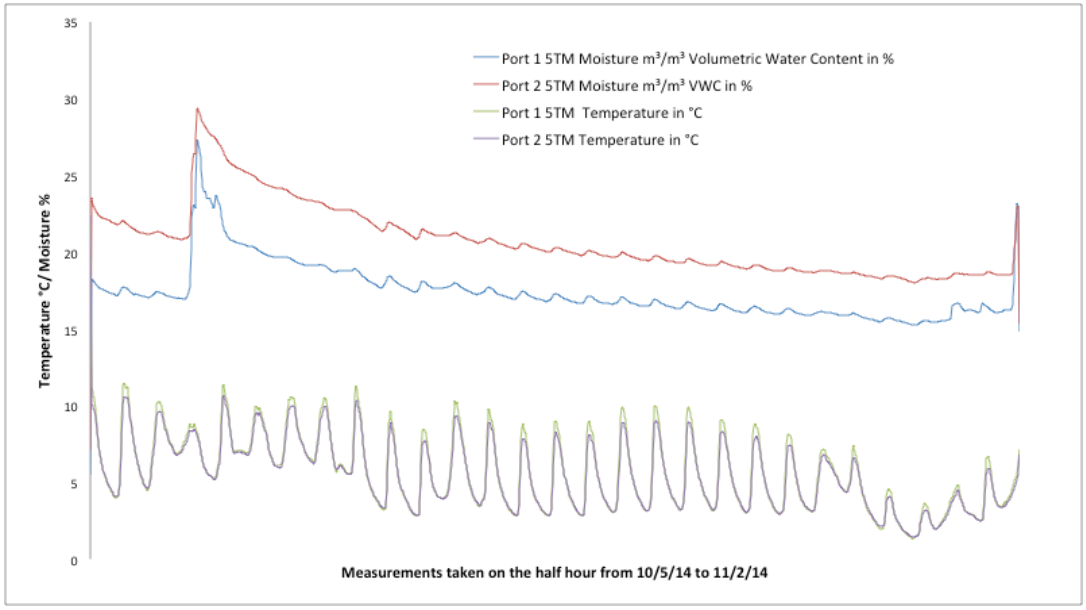

Figure 24: Graphic of results of soil and moisture probes placed in Princeton Pond 9 from 10 Oct 2014 to 2 Nov 2014, which is prime howellia germination season. Soil moisture ranged between 18 and 30% at the site, and temperatures ranged between around 3-4°C at night and 11-12°C during the day.

Figure 25: (A, Upper) Image showing what germinating howellia looks like and the condition of soil at this time. (B, Lower) Image showing that high levels of duff and organic litter do not hinder germination. Taken by Catherine Wiechmann at Princeton howellia site, Pond 9.

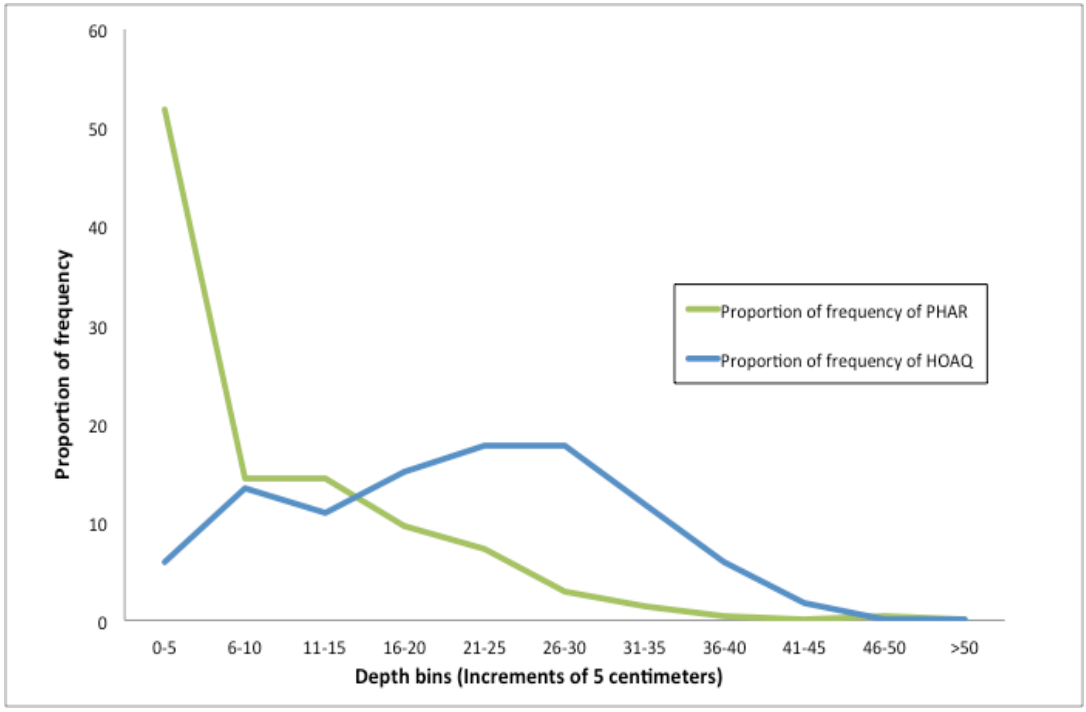

Figure 26: Graphic illustrating the relationship between *Howellia aquatilis* and *Phalaris arundinacea* and water depth. Phalaris dominates at shallower depths (0-10cm.), whereas howellia dominates at deeper depths (16-45 cm.). Between 10-15 cm., there seems to be a transitional zone, where howellia and phalaris compete for resources the most.

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Appendix A: Soil sample preparation methodology

Sample Preparation

Equipment

- mortar and rubber-tipped pestle
- 2-mm sieve (No. 10)
- weighing balances
- 400-mL beakers and watch glasses
- 100-mL plastic tubes w/caps or #6.5 rubber stoppers
- wash bottles
- Rainin automatic pipette w/macro-tips
- double beam balance
- centrifuge
- steam table
- drying oven and desiccators
- triple-distilled water (TDW) system

Reagents

1 N HCl (hydrochloric acid) - Measure 900 mL TDW into a graduated cylinder. Add 100 mL concentrated HCl to TDW and make to 1000 mL. Use care in addition, wear gloves, eye protection, lab coat and perform in a fume hood.

1 M $AgNO_3$ (silver nitrate) - Weigh 17.0 grams $AgNO_3$ and add to a 100-mL volumetric flask. Make the $AgNO_3$ to volume with TDW.

95% ethanol - Use Baker or Fisher analyzed reagent-grade stock.

Methanol - Use Baker or Fisher analyzed reagent-grade stock.

1 N $NaC_2H_3O_2$ (sodium acetate) - Dissolve 1224 grams $NaC_2H_3O_2$ in 8 L TDW. Add ~ 300 mL concentrated $HC_2H_3O_2$ (acetic acid) to adjust the pH to 5.0. Dilute to 9 L with TDW and check pH again to make sure it is 5.0.

Saturated NaCl (sodium chloride) - In a 500-mL plastic bottle add NaCl to TDW until saturated. It does not matter if crystals are on the bottom of the bottle.

0.5 M $MgCl_2$ (magnesium chloride) - Weigh 102 grams $MgCl_2 \bullet 6H_20$, dissolve, and make to a volume of one liter volumetrically using TDW.

NaOCl (sodium hypochlorite), pH 9.5 - Use NaOCl (Clorox bleach or other brand) from a retail grocery store or reagent grade hypochlorite. Adjust pH using 1N HCl or dilute NaOH. Make reagent in a 500-mL plastic bottle daily or as needed. Do not adjust the pH of the entire gallon of bleach or pour unused bleach back into the bottle. Discard bleach that is old and not yellow in color.

Comments

Wear eye protection when working around steam table and in the hood. Wear rubber gloves when making the additions of methanol. Check with laboratory supervisor on the proper operation of the centrifuge, balances, and the pH meter. Calibrate the pH meter each day. Keep equipment covered when not in use. Heavy items should not be placed on the weighing table. Check grease in centrifuge once a month and clean daily.

Procedure

A. Crushing and sieving

1. All samples should be air-dried to a constant weight.
2. Lightly crush or grind soils using a rubber-tipped pestle and sieve sample through a 2-mm sieve. Do not do grinding in the Pedology Laboratory! The dust is not good for the computers and laboratory instruments. Use the teaching lab, room 133, or stay in the hallway. Use the well-mixed <2mm soil sample for particle-size determination and fractionation procedures.
3. If gravel percentage is desired, save the >2-mm fraction. Make sure >2-mm fraction consists of rock fragments and not soil aggregates; further crushing/grinding may be necessary. Weigh gravel and calculate % gravel as follows:

$$\% \text{ gravel} = [\text{weight of gravel}/(\text{weight of soil} + \text{weight of gravel})] \times 100$$

This calculated number for % gravel will be used as an adjective to the textural class name. If gravel less than 15%, no adjective; 15-35% gravely; 35-60% very gravely; >60% extremely gravely.

4. Test a small amount of dry bulk soil sample with a few drops of 1N HCl (hydrochloric acid). If the soil sample fizzes or froths, it contains calcium carbonate and Part B of the procedure (Calcium Carbonate Removal) should be followed. Otherwise, proceed to Part C (Organic Matter Removal).

B. Calcium Carbonate Extraction

1. If the soil sample contains calcium carbonate that must be removed, weigh 12 grams of soil into a labeled 400-mL beaker and start extracting calcium carbonate. If the soil sample does not contain calcium carbonate or does not need to be removed, weigh 12 grams of soil and proceed with the organic matter removal procedure.
2. Add 100 mL of 1N NaOAC (sodium acetate) buffered at pH 5 to the soil in 400-mL beakers.
3. Mix samples slowly, cover beakers with watch glasses, and place the beakers on the steam table. Maintain a temperature of 70-80 ^{o}C for 15 minutes. Stir samples occasionally.
4. When the CO_2 bubbles are no longer evident, let the samples cool. Extract clear liquid with an automatic pipette after solution has cooled and soil settled. If there is not any further frothing after testing a small portion of the soil, then proceed with step 5. If the soil continues to froth, then repeat step 2-4 until all the calcium carbonate has been removed.
5. Add ~50 mL of TDW to samples. Stir and let soil suspension settle until clear. Use an automatic pipette to extract clear liquid. If the liquid is not clear, the samples will need to be centrifuged to promote settling.
6. Transfer soil suspension to labeled 100-mL plastic tubes using a wash bottle filled with TDW. Balance each set of two centrifuge cups and tubes on a double-beam balance by adding water to the cups. Do not add water to the tubes. Centrifuge the samples for 10 minutes at ~1200 rpm (setting 20). Decant and discard clear liquid. If the soil suspension stays cloudy, add 1-5 drops of saturated NaCl solution, wait 10 minutes, recentrifuge, discard the clear liquid or repeat, if necessary.
7. Transfer soil samples back to labeled 400-mL beakers and proceed to organic matter removal procedure.

C. Organic Matter Removal

1. Add enough pH 9.5 NaOCl (Clorox bleach) to cover the soil. Depending on the amount of soil, usually 10-15 g, 50 mL is sufficient.
2. Let the soil/bleach mixture sit for 1 h. Turn on the steam table or hot plate, using a low heat setting. Depending on the amount of soil and amount of organic matter (OM) present, let the soil/bleach mixture heat with frequent stirring until the reaction has subsided (~15 min). If violent frothing takes place, use a squirt of ethanol to calm the reaction.

3. Using an automatic pipette, remove the particle-free liquid off the top of the soil. Be careful to not disturb the settled soil.
4. Add more pH 9.5 bleach to the soil. Repeat step 2 using a 15-min reaction time and then step 3. The supernatant should be discolored (brown, black, yellow, or pink). The pink liquid can indicate the sample is done as well as the presence of manganese oxides.
5. Repeat step 4. Three total treatments should be sufficient, except for soils having large amounts of OM. In this case more treatments may be needed.
6. Repeat step 3. Transfer soil suspension to labeled 100-mL plastic tubes using TDW in a wash bottle. Balance each set of two centrifuge cups and tubes on a double beam balance by adding water to the cups. Do not add water to the tubes. Usually water will cause the soils to disperse. Centrifuge the samples for 10 minutes at ~1200 rpm (setting 20). Decant and discard clear liquid. If the soil suspension stays cloudy, add 1-5 drops of saturated NaCl solution, wait 10 minutes, recentrifuge, discard the clear liquid or repeat, if necessary. Decanted liquid can be tested for the presence of excess ions (Procedure D, step 1).
8. If the samples are being used for particle-size analysis or other quantitative analyses, proceed to section for removal of excess ions.

D. Removal of excess ions

1. Place a small drop of clear liquid on a small watch glass from OM removal procedure, step 7, and add a drop of 1N silver nitrate. If the liquid turns white, proceed with the following steps (2-5) to remove excess ions, otherwise, proceed to step 6.
2. Do duplicate washings of soil suspension with 95% methanol. Fill 100-mL plastic tubes to ~60 mL mark with methanol, mix, balance tubes, centrifuge for 10 minutes at ~1200 rpm (setting 20), and discard the clear liquid. Use labeled waste container for discarded liquid.
3. Add 95% ethanol to ~60 mL mark, mix, balance tubes, centrifuge for 10 minutes at ~1200 rpm, and discard clear liquid to labeled waste container.
4. If at any time during these washes the liquid remains cloudy, then add 1-5 drops of 1N HCl or saturated NaCl, stir and wait 5 minutes, recentrifuge, discard the clear liquid to labeled waste containers or repeat if necessary. Adding HCl or NaCl will add ions so at least two washes will be required.
5. Check the clear liquid for complete removal of excess ions by placing a drop of the liquid on a small watch glass and adding a drop of silver nitrate. It the liquid turns white, repeat washing with ethanol until all the excess ions have been removed.
6. Place 100-mL centrifuge tubes plus soil in a 105 ^{o}C oven and let dry overnight.
7. Remove tubes from the oven and cool in a desiccator for at least one hour. Plastic tubes will become brittle and break if subjected to longer than 24 hour heat periods.
8. Weigh tubes from desiccator on a 4-place Mettler balance (example: 50.9988). Record the weights under the heading entitled “Original Sample Weight” of worksheet No. 1.

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Appendix B: Soil texture analysis methodology

Particle Size Distribution - Pipette

Equipment

- weighing balances and weighing boats
- 100-mL beakers
- watch glasses
- 50 and 100-mL plastic tubes w/caps or #6.5 rubber stoppers
- 1000-mL graduated cylinders
- metal stirring rods
- wash bottles
- Rainin automatic pipette w/macro-tips
- double beam balance
- sonifier (dismembrator)
- centrifuge
- powder funnels
- ceramic evaporating dishes
- drying oven and desiccators
- mechanical shaker
- 325-mesh sieves
- USDA sieve nest
- # 6 paint brushes and metal probe
- sieve shaker
- stopwatch and timers
- refrigerator
- storage vials/bags - sand and silt fractions
- triple-distilled water (TDW) system
- disk, computer and spreadsheet software

Reagents

5% $Na_6P_6O_{12}$ (sodium hexametaphosphate) - Weigh 50 grams $Na_6P_6O_{12}$ (calgon) and make to 1000-mL with TDW. Store in a liter plastic bottle.

Acetone - Use Baker or Fisher analyzed reagent-grade stock.

Comments

Pipette method is used when clay and silt are not separated for XRD or a faster less expensive method for determining particle size analysis is needed. With some modification the pipette method can be used for XRD.

In this method the weighing, temperature and transferring are very important. Keep equipment covered when not in use. Leave balances and instruments on all the time to decrease start-up impact on electronic systems and calibrate daily. Heavy items should not be placed on the weighing table. Oil shakers and check grease in centrifuge once a week, and clean the balance daily.

When shaking sands, check the 140 and 300 mesh screens of USDA sieve nests for breaks along the edges. If an unusual amount of material is found in any of the sieves, use a small hand lens to locate the break. If a break is found, reshake and weigh separates again using a different sieve. A small bead of liquid solder applied with plastic syringe around edges of the 1 and 0.5 mm, and 300 mesh screens will decrease time extracting sands from edges and prolong the life of the delicate 300 mesh sieve.

When transferring very coarse and coarse sands to weighing boat, it may be necessary to loosen sands in sieve holes with a probe. Be careful not to enlarge holes with probe. The 325 mesh used in silt-sand separating sieves should be replaced with new mesh every 6 months depending on their use. Sieves should only be left in the drying oven for one hour because water/drying will change sieve hole size and shrink gaskets.

It is recommended that soils from Costa Rica or other tropical countries and/or contaminated soils high in iron and aluminum use other methods for dispersion instead of using the standard addition of sodium hexametaphosphate (dispersant). These soils usually have a low pH. Sodium hexametaphosphate can cause the soil solutions to flocculate, not disperse. The pH can be adjusted to pH 4 or pH 10 to obtain better dispersion along with sonification. Dispersion of these soils can be enhanced by removing iron, etc. by the CBD method. Calculations will need to be adjusted, if sodium hexametaphosphate is not used as the dispersion agent.

Procedure

A. Dispersion of Samples

1. Weigh tubes from desiccator on a 4-place electronic balance (example: 50.9988) from the sample preparation procedure. Record the weights on worksheet No. 1 under the heading “Original Sample Weight”. Add 10-mL 5% sodium hexametaphosphate (dispersant) to weighed samples. If soils are possibly from volcanic ash origin or contaminated with high amounts of iron, etc., the addition of hexametaphosphate may cause flocculation instead of dispersion. See comment section.

2. Fill 100-mL plastic tube to ~ 60-mL with TDW. Place a #6.5 rubber stopper in the top of the 100-mL tube, twist stopper to tighten it, lay tubes on their sides by alternating ends in a mechanical shaker for over night shaking (at least 16 hours) and dispersion. Start the shaker on high speed and then put it on low speed once soil mixture is thoroughly mixed.
3. Add 10-mL 5% sodium hexametaphosphate to 2-tared 100-mL beakers to be oven dried, cooled, weighed, and used later in calculations. Remember all weights in this procedure will be done on the 4-place electronic balance after samples have been oven-dried and cooled in a desiccator.
4. Record weights of dried hexametaphosphate under the heading entitled "Dispersant Weight" of worksheet No. 1. The average dispersant weight is divided by 100 (based on 1000-mL volume and a 10-mL sample). This number will be subtracted from the silt - clay fraction and the clay fraction.

B. Separating Sands from Silt and Clay

1. Remove samples carefully from the shaker. If the samples are high in clay, 30 seconds of sonification with the dismembrator might make sure the clay is dispersed. Check with lab supervisor for proper operation of the dismembrator.
2. Allow the samples to settle for 30 seconds, pour suspensions through 325 mesh sieves that tilt in powder funnels, and deliver into 1000-mL graduated cylinders. Use a fine tip water bottle to transfer the sample into the sieves and to wash the silts from the sands. There should be less than 2 grams silt in the sands, if washing was complete.
3. Wash sands with a small amount of acetone to decrease drying time. Place the sieves into tared labeled evaporating dishes and then into a drying oven at 105^{o}C. Leave the sands in the oven for ~one hour, remove the dishes, place into a desiccator, and cool. Only leave sieves in oven for short periods because the sieve opening will change size and gaskets to hold screens will shrink (refer to comments section).
4. Transfer the sands from the sieves into the evaporating dishes very carefully using a paint brush. Place sands in the oven overnight or until the sands can be sieved into five separates using a mechanical shaker.
5. Make the silt and clay suspensions in the 1000-mL graduated cylinder to volume, cover, and set aside until pipettings are made. Place the labeled tubes in the drying oven at 105^{o}C overnight. Transfer tubes to a desiccator, cool, and weigh using a 4-place electronic balance. Record weights on the worksheet entitled original sample weight (worksheet No. 1). Obtain the original sample weight by subtracting the tube weight from the tube plus sample weight. The original weight is used to determine the % error for the procedure and accuracy of separations. Try to obtain a 2% or less error.

C. Sieving and Weighing the Sand Fraction

1. Remove sands from oven, cool, and weigh to 4 places on an electronic balance. Record weight on worksheet No. 1 under heading entitled, "Total Sand Weight". Subtract the evaporating dish weight from the evaporating dish plus sand to give

the total sand weight. This is actually not total sand because there is always silt left in the sand fraction no matter how carefully the sands are washed.

2. To obtain actual total sand if 5 sand separates are not needed, add sand using a #6 paint brush to the 300-mesh sieve in a USDA-sieve nest. Place sieve nest on sieve shaker for 15 minutes.
3. Weigh only the silt in bottom pan to 4 places and record on worksheet No. 1 under heading entitled, "Total Sand Weight". Subtract silt weight from sand weight obtained in step 1 to secure actual total sand fraction. If a computer spreadsheet is used, enter appropriate weights to obtain actual total sand fraction.
4. If sands need to be divided into five USDA standard sizes or 23 1/4-phi separates, then follow the steps below.
5. Using a #6 paint brush transfer the total sand to the top sieve of the sieve nest desired. Place the sieve nest on sieve shaker for 15 minutes.
6. Tare a small plastic weighing dish, add all the first sieve contents to dish, weigh to 4-places, and record weight on worksheet No. 2 under heading "Sand Fraction". Tare weighing dish and contents of first sieve. Repeat step 6 with next sieve until all sieves have been weighed and recorded.
7. The last or bottom sieve is silt. After this separate is weighed and recorded, it could be added to the corresponding 1000-mL graduated cylinder of silt + clay or weight added to total silt fraction located on worksheet No.2.
8. Save any or all sand separates in small labeled plastic vials or small plastic bags for future examinations or work. The addition of sand separates can be made with a calculator or in the spreadsheet prepared for the pipette procedure.

D. Silt - Clay Fraction

1. Using a long metal stirring rod, stir the silt - clay sample in the graduate cylinder at least 10 times. If samples have set for a few days, make sure the cylinder is plunged several times to get everything off the bottom.
2. Using an automatic pipette take a 10-mL aliquot using the 10-cm mark on the pipette tip. Place contents into a tared labeled 100-mL beaker. Stir sample again 10 times and take another 10-mL aliquot and place it into the same 100-mL beaker. Add a 10-mL pipetting of TDW to wash the pipette tip into the 100-mL beaker.
3. Put beakers into a drying oven overnight at 105^{o}C. Place beakers in a desiccator for at least 30 minutes to cool. Weigh beakers using a 4-place electronic balance and record weights on the silt - clay section of worksheet No. 2.
4. The samples contain silt, clay and dispersant, if added. Subtract the adjusted average dispersant weight from worksheet No. 1 from the silt - clay weight to obtain the silt - clay weight. Multiply this number by 50 (20-mL aliquot from 1000-mL) to obtain the actual silt - clay in the 1000-mL cylinder.

E. Clay Fraction

1. Determine the temperature of the samples in the cylinders. Use the time table below and calculate time lapse for pipetting clay from the 10-cm or 5-cm mark.

If you have plenty of time use the 10-cm pipetting time; otherwise, use the 5-cm pipetting time. Data will be the same regardless of the time used.

2. Stir samples in cylinders in one minute intervals at a designated start time. Calculate the stop time using the time table below. Pipette clay using an automatic pipette at the 10-cm or 5-cm mark on the pipette tip. Do not stir the samples or disturb them while setting the required time.
3. Using an automatic pipette take a 10-mL aliquot using the 10-cm or 5-cm mark on the pipette tip. Pipette clay from samples in one minute intervals to keep the exact time calculated from the time table. Place contents into a tared labeled 100-mL beaker. Pipette another 10-mL aliquot and place it into the same 100-mL beaker. Add a 10-mL pipetting of TDW to wash the pipette tip into the 100-mL beaker.
4. Put beakers into a drying oven overnight at 105^{o}C. Place beakers in a desiccator for at least 30 minutes to cool. Weigh beakers using a 4-place electronic balance and record weights on clay section of worksheet No. 2.
5. Subtract the adjusted average dispersant weight from worksheet No. 1 from the clay weight to obtain the clay weight. Multiply this number by 50 (20-mL aliquot from 1000-mL) to obtain the actual clay in the 1000-mL cylinder. Subtract the clay weight from the silt - clay weight to obtain actual silt weight.
6. If clay is needed for X-ray analysis, let the silt + clay suspension settle covered in the cylinders for 2-days undisturbed. Assume silt has settled to the middle or bottom of the cylinders, so clay can be pipetted off in the top of the cylinder or pour some of the suspension into a 600-mL beaker. Add 3-mL of 1M $MgCl_2$ to the beaker. The clay suspension will flocculate. Transfer clay to a 50-mL plastic tube for refrigerator storage, until needed.

Time Table for Pipetting Clay from the Silt - Clay Fraction

Temp. ^{o}C	10 cm depth		5 cm depth	
	Hour	Minute	Hour	Minute
15.0	9	03		
15.5	8	56		
16.0	8	49		
16.5	8	41		
17.0	8	35		
17.5	8	28		
18.0	8	22		
18.5	8	16		
19.0	8	10		
19.5	8	04		
20.0	7	59		
20.5	7	52		
21.0	7	46		
21.5	7	41		
22.0	7	35	4	01

22.5	7	30	3	56
23.0	7	25	3	51
23.5	7	20	3	46
24.0	7	15	3	41
24.5	7	10	3	36
25.0	7	05	3	31
25.5	7	00	3	26
26.0	6	55	3	20
26.5	6	50	3	15
27.0	6	46	3	10
27.5	6	40	3	05
28.0	6	36	3	00
28.5	6	32		
29.0	6	29		
29.5	6	24		
30.0	6	20		

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Soil Survey Division Staff. 1993. Soil Survey Manual. U. S. Dept. Ag. Handbook No. 18, U. S. Govt. Print. Office, Washington, D. C.

Calculations

1. Place total clay weight, total silt weight, sand fraction weights, and original sample weights on worksheet No. 3 under the heading of "Final". Sum all the sand fractions to give a total sand weight.
2. Add total sand, silt and clay of each sample to obtain the component weight. Subtract either/or component weight from original weight to give loss/gain of sample.
3. Divide the loss or gain by the original weight times 100 to give the percent error. Two percent or less is acceptable. If the percent error is above 5 percent, then redo the sample.
4. Divide each sand fraction weight, total sand weight, total silt weight, total clay weight by the component weight times 100 to obtain percent sand, silt, and clay in samples.

5. Calculate % gravel, if any, from "Sample Preparation" procedure by dividing gravel weights by soil plus gravel weights times 100. This number will be used as an adjective to the textural class name. See Soil Survey Manual pages 142-143. If gravel less than 15%, no adjective; 15-35% gravelly; 35-60% very gravelly; >60% extremely gravelly, etc.
7. Determine textural class from Soil Survey Manual pages 137-140. The computer spreadsheet will compute the percentages and print worksheet and final data.
8. Refer to other calculations throughout procedure according to specific section. The following equations are a summary of the most important calculations.

original wt. =	(100-mL tube + soil sample wt.) – 100-mL tube wt.
dispersant wt. =	(dispersant wt + beaker wt.) - beaker wt.
clay wt. =	(clay + dispersant wt.) - beaker wt.
	[(clay + dispersant wt.) - dispersant wt.] X 50
silt wt. =	(silt + clay beaker wt.) – beaker wt.
	silt + clay wt. – clay wt.
	silt + silt from sand fraction
sand wt. =	very coarse + coarse + medium + fine + very fine sand wt.
component wt. =	total sand wt. + total silt wt. + total clay wt.
% error =	[(original wt. - component wt.)/original wt.] X 100
% sand =	(sand wt./component wt.) X 100
% silt =	(silt wt./component wt.) X 100
% clay =	(clay wt./component wt.) X 100
% very coarse sand =	[(very coarse sand wt.)/component wt.] X 100

Appendix C: Soil pH Methods

1:1 pH and 1:2 $CaCl_2$ pH

Equipment

- 3-place weighing balance
- 100-mL plastic beakers or cups
- pH meter
- automatic stirrer or stir rods
- large & small magnets for stirring
- 25, 50, 100-mL graduated cylinder
- automatic pipette
- colored marking tape and pen
- triple distilled water (TDW) system
- water bottle for rinsing
- timer
- protective gloves
- disk, computer and spreadsheet software

Safety

Follow standard laboratory safety practices. Wear eye protection in the laboratory at all times. Wear rubber gloves when making standards, diluting samples and working around concentrated hazardous elemental standards and reagents. Read the labels on all standards and reagents before using them.

Reagents

Triple Distilled water (TDW) – Each laboratory usually has water purification systems that produce DW or TDW. Check with lab supervisor on correct TDW system operation. Use water directly from systems.

pH buffers 4.0 and 7.0 – Use ready made buffers for standardizing the pH meter or put a capsule of each buffer into a 100-mL volumetric flask. Make to volume with TDW and stir until dissolved. Follow pH meter instructions for standardization.

0.02 M calcium chloride ($CaCl_2$) – Weigh and dissolve 23.52 grams reagent grade $CaCl_2$ in 8-L of TDW. Mix solution thoroughly using a magnetic stirrer.

Comments

Check with laboratory supervisor on the proper operation of the pH meter and standardization. Do not wipe or rub the pH electrode dry with a cloth, laboratory tissue, or similar material because it may cause electrode polarization. Only dab the excess liquid with a tissue on the end. Keep working area very clean.

Using $CaCl_2$ or KCl solutions are popular methods for masking seasonal variation in soil pH readings. The pH readings are usually less with dilute salt solutions than with distilled water but may be equal to or greater in highly weathered tropical soils. When the pH values of various soils are compared, determination by the same method is important (Foth and Ellis, 1988).

The $CaCl_2$ soil pH is generally less than the 1:1 water pH. The combination of exchange and hydrolysis in salt solutions (0.1 to 1 M) can lower the measured pH from 0.5 to 1.5 units, compared to the pH measured in RO water (Foth and Ellis, 1988). For convenience, the pH is initially measured in water and then measured in $CaCl_2$. With the addition of an equal volume of 0.02M $CaCl_2$ to soil suspension that was prepared for the water pH, the final soil-solution ratio is 1:2 0.01M $CaCl_2$.

When making interpretations about the soil, the saturated paste pH is usually compared to the 1:1 water pH and the 1:2 $CaCl_2$ pH. The usual sequenced is as follows: 1:1 water pH > 1:2 $CaCl_2$ pH > saturated pH. If saturated paste pH is > 1:2 $CaCl_2$ pH, the soil is not saline. If the saturated paste pH ≥ 1:1 pH, the soil may be Na saturated and does not have free carbonates. Because of the interrelations that exist among the various soil chemical determinations, the saturated paste pH value may be used as a means of cross-checking salinity data for internal consistency and reliability (U. S. Salinity Laboratory Staff, 1954). The difference in the sediment and supernatant pH is called the suspension effect (McLean, 1982).

To maintain uniformity in pH determinations, measure the pH just beneath the surface of saturated paste or 1:1 water or $CaCl_2$ pH. Clays may clog the KCl junction and slow the electrode response. Clean the electrode by rinsing with TDW and patting it dry with tissue. When doing pH, raise and lower electrode to make sure it is not clogged. Watch the meter response.

Procedure

1. Plastic cups are labeled with numbers for samples. Soil samples are mixed with weighing
   device before adding to the cup. Plastic cup placed on balance and tared.
2. Crushed soil sample is added to the tared plastic cup. The exact weight is recorded on the worksheet, example 10 grams (+ or - 0.02). A reference soil (Palouse) is weighed with each batch of 1:1 pH determinations. The Lincoln Lab uses a calibrated spoon to add sample to their cups. In the case of Andisols or Spodosols, possibly others, the weight might not be very accurate because of the density of the samples.
3. The worksheet is used to determine the amount of TDW to add to the cup. This amount is precisely measured with the Rainin pipette by pedon. The soil sample + water are mixed for 30 seconds. If the soil is too dry, then an additional amount is added. If the weight was 10 grams, 10-mL TDW was added. If the soil is dry, then another 10-mLs TDW was added and recorded on the worksheet.
4. In one-half hour the samples are stirred again for ~30 seconds or 30 stirs.
5. The pH meter is turned on at least one-hour before readings are to be taken. This helps make sure the instrument is warmed up to work. The pH meter is calibrated using known pH 4 and 7 buffers purchased from Chem. Stores. The pH meter has to have a good slope in order to proceed. Our tolerance is 97-99% for the slope.
6. After one hour the soil sample is stirred for ~30 seconds using a small magnet on a stirring plate making sure all the soil is in suspension, while electrode is equilibrating to the side. Turn off the stirrer and read the pH at 30 seconds or a total of 1 minute. In most cases with 10 grams soil the electrode will be in the reading area of the tip. If less soil is used like 5 grams, sometimes they must be tipped to insure good contact with the electrode. A stop watch is used to time the 30-seconds and 1 minute to read the pH. The pH is recorded on the worksheet to the nearest one-hundredth, but reported to one-tenth.
7. The electrode is left in the soil sample. To the same 1:1 (soil/H20) sample 10-mL 0.02 M calcium chloride ($CaCl_2$) solution is added to the soil suspension, stirred for 30-seconds and the 1:2 $CaCl_2$ pH read at 1-minute. Raise and lower electrode slightly to make sure it is not clogged. The pH reading is recorded to the nearest one-hundredth, but reported to one-tenth.
8. The electrode is rinsed thoroughly and steps 6-7 are repeated for the next sample.
9. The pH 4 or 7 buffer is checked after each pedon (at least every 5-10 samples) is read to make sure it is still reading pH 4 or 7. If it does not read 4 or 7, then the pH meter is recalibrated. The end of the electrode is touched with a Kimwipe to remove excess water. Never rub the electrode because it will cause it to fail or be ruined.

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Calculations

There are not any calculations required for this procedure.