Quality Assurance Project Plan

Pacific Northwest Wetland Macroinvertebrate Monitoring: Protocols and Variability in Freshwater Riverine Impounding Wetlands

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Scott Hoffman Black date Sarina Jepsen date Project Manager and QA officer **Executive Director** The Xerces Society The Xerces Society Ralph Thomas Rogers Gretchen Hayslip date date Regional Wetland Ecologist US EPA **Project Officer** U.S. EPA

Prepared By:

Sarina Jepsen The Xerces Society 4828 S.E. Hawthorne Boulevard Portland, OR 97215

Submitted To:

Ralph Thomas Rogers and Gretchen Hayslip U.S. Environmental Protection Agency Region 10 1200 Sixth Avenue Seattle, WA 98101

Table of Contents	page
Title and approval page	1
Table of Contents	2
Distribution list	3
Project Organization	3
Problem background	5
Problem description	5
Project Timetable	5
Data quality objectives for measurement data	6
Training Requirements	7
Documentation and Records	7
Sampling Process Design	7
Sampling Methods Requirements	13
Sample Handling and Custody Requirements	13
Analytical Methods Requirements	
Quality Control Requirements	
Equipment Testing and Inspection and Maintenance Requirements	15
Instrument Calibration and Frequencies	16
Data Acquisition Requirements	
Data Management	
Assessments and Response Actions.	
Reports	
Data Review, Validation and Verification Requirements	
Validation and Verification Methods.	
Reconciliation with Data Quality Objectives	
References.	
Appendix A: QaQc procedures for ABA, Inc	
Appendix B: Field data sheet	
Appendix C: Table of study sites	
Appendix D: Topographical maps of sites	
Appendix E: Satellite photographs of study sites	
Appendix F: Modified Beaufort windspeed scale	
Appendix G: Sampling Standard Operating Procedures	
Appendix H: Analytical Standard Operating Procedures	58

Distribution list

Ralph Thomas Rogers Regional Wetland Ecologist U.S. Environmental Protection Agency Office of Ecosystems, Tribal & Public Affairs Aquatic Resources Unit (ECO-083) 1200 Sixth Avenue Seattle, WA 98101 Telephone: (206) 553-4012

Email: rogers.ralph@epa.gov

Gretchen Hayslip U.S. Environmental Protection Agency Unit (ECO-095) 1200 Sixth Avenue Seattle, WA 98101 Telephone: (206) 553-1685

Fax: (206) 553-0119

Email: Hayslip.Gretchen@epa.gov

Robert Wisseman Aquatic Biology Associates, Inc. 3490 NW Deer Run Road Corvallis, OR 97330

Telephone: (541) 752-1568 Fax: (541) 754-9605

Email: wisseman@aquaticbio.com

Project Organization

Key organizations involved in this project include: The Xerces Society for Invertebrate Conservation and The U.S. Environmental Protection Agency, Region 10.

Future data users include: scientists, tribes, stakeholders, and citizen monitoring groups.

The responsibilities of the Xerces Society include:

- 1. Conduct a literature review
- 2. Survey regional taxonomists and wetland specialists
- 3. Design and conduct a monitoring study based on existing protocols and advice from local experts
- 4. Analyze the results of the study
- 5. Write a report detailing the findings of our study

The EPA will be responsible for approving our QAPP and the final project results.

The agency responsible for ensuring that all aspects of the project are performed as described in the QAPP is The Xerces Society. The project manager and monitoring coordinator is Sarina Jepsen, Xerces. The laboratory work will be contracted with Robert Wisseman of Aquatic Biology Associates, Inc. (ABA).

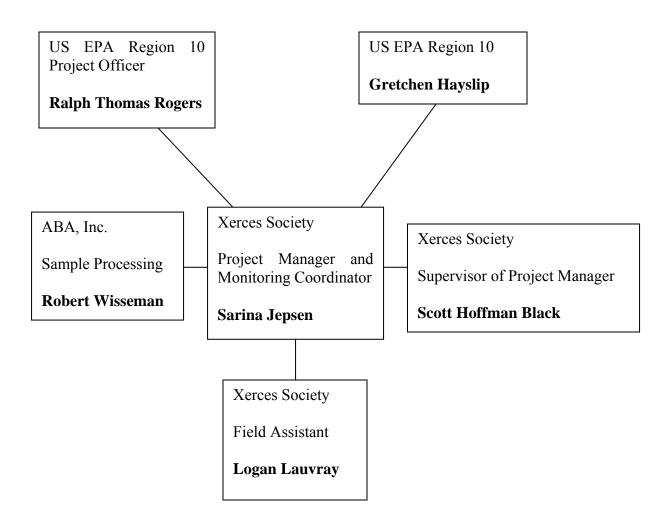


Figure 1. Wetland macroinvertebrate monitoring organizational chart

Project background

The Clean Water Act requires that each state assess and monitor the physical, chemical and biological condition of waters, including wetlands, within their jurisdiction. At present there are no consistent and cost-effective monitoring tools for certain biological attributes of Pacific Northwest (hereafter Northwest) wetlands. One such biological attribute is the macroinvertebrate assemblage; this project addresses the need to develop a protocol to monitor aquatic macroinvertebrates in Northwest wetlands. Macroinvertebrates can be excellent indicators of the biological integrity of water bodies and are used frequently in bioassessment (Rader and Shiozawa 2001). The use of macroinvertebrates in wetland bioassessment programs has been successfully implemented in numerous other states (US EPA 2003), but relatively little has been done in the Northwest. The data, protocols and descriptive information regarding local macroinvertebrate wetland taxa that emerge from this initial study will aid scientists and citizen monitoring groups in large scale wetland macroinvertebrate bioassessment programs in the Northwest.

Project Description

We plan to sample macroinvertebrates in Willamette Valley Riverine Impounding wetlands. Our study sites will include ten wetlands that are least-impacted and three wetlands that are most-impacted by human activity. The three most-impacted wetlands will be paired spatially and temporally with three of the least-impacted wetlands. Three composite samples will be taken from one strata of each wetland using aquatic D-frame sweep nets. Our critical measurements will be taxa richness, taxa diversity, taxonomic composition, and relative abundance. We will evaluate the responses of a variety of metrics in least-impacted and most-impacted wetlands to identify metrics that may eventually be used in an Index of Biological Integrity (Karr and Chu 1999) for macroinvertebrates in Riverine Impounding wetlands. This data will be useful in establishing a 'reference' condition for this particular subclass of wetlands in the Northwest

Project Timetable

110ject Timetable												
	20	06			20	07						
Milestones	S	0	N	D	J	F	M	A	M	J	J	A
Conduct literature review	*	*	*									
Design/ distribute surveys		*	*	*								
Write SOPs and QAPP		*	*									
Compile results of survey			*	*	*							
Select wetlands sites				*	*							
Do preliminary site visits					*			*				
Prepare for sampling					*	*	*					
Sample collection								*	*			
Sample identification								*	*	*	*	*
Data analysis/ review									*	*	*	*
Final report to EPA											*	*

Data Quality Objectives for Measurement Data

Our goal is to produce data of sufficient quality to address these project objectives:

- 1. Characterize the macroinvertebrate community in Willamette Valley Riverine Impounding wetlands.
- 2. Measure the variability in the invertebrate communities among ten least-impacted Riverine Impounding Willamette Valley wetlands.
- 3. Look for invertebrate metrics that vary predictably between least-impacted and most-impacted Riverine Impounding Willamette Valley wetlands.

We intend the data resulting from our study to be used for general education of scientists and citizens who may pursue wetland bioassessment using macroinvertebrates in this region. Some of the key elements of data quality include: completeness, representativeness, comparability, precision, accuracy, and measurement range.

Completeness

In order to gain an estimate of the taxa present in Northwest wetlands, we will collect a total of 42 samples from 13 wetland sites. Each site will be visited once; three composite samples will be collected from that site. One site will be visited a second time.

Representativeness

We chose to sample wetlands in one Hydrogeomorphic subclass in order to minimize the natural variability among different classes and subclasses of wetlands. Our study is sufficiently small that we could not sample multiple subclasses or classes without sacrificing a necessary amount of site replication. The results of this study will be most applicable to other Riverine Impounding wetlands in or near the Willamette Valley.

The location of collecting samples in the near shore emergent vegetation was chosen because other studies have found that this wetland stratum has a high abundance and diversity of macroinvertebrates (Driver 1977, Hanson and Swanson 1989, US EPA 2002b). Samples from this part of a wetland will not represent the entire wetland, but will represent a part of the wetland with a high abundance and diversity of macroinvertebrates. A desire to minimize natural variability within a wetland and have adequate replication of wetlands led us to choose a single stratum to sample; we judged the near shore emergent vegetation strata to be the best choice.

Comparability

Very few studies have been conducted that explore the macroinvertebrates in Northwest freshwater wetlands, which is one of the main reasons why we are conducting this study. Of the few studies that have been conducted, the researchers generally used benthic corers or emergence traps to sample the wetland (Richter et al. 1997, Harenda 1991, Hansen and Castelle 1999, Taft and Haig 2006). We are planning to use aquatic D-frame sweep nets, so the results of our study likely will not be very comparable to the results of these other studies in the region. One exception is a Master's thesis from Oregon State University (Beatty 2002) in which Odonata nymphs were sampled with aquatic D-frame sweepnets; since similar methodology was used, we would like to compare our Odonata results to his results. Our reasons for using aquatic D-frame sweepnets are discussed in

the 'Sampling Methods Requirements' section. We think that this sampling methodology will become the standard for use in wetland macroinvertebrate bioassessment, since it is extensively used in other parts of the country and it is the recommended sampler in a current text on bioassessment in wetlands (Batzer *et al.* 2001). Thus, we hope that our study will be very comparable to other studies conducted in this region in the future.

Precision

Three composite samples will be taken from one least-impacted wetland site a second time to gain an estimate of our sampling precision. For information on the precision of taxa identification, see Appendix A (QaQc procedures of ABA, Inc.).

Accuracy

See Appendix A for QaQc procedures of ABA, Inc.

Measurement Range

See Appendix A for QaQc procedures of ABA, Inc.

Training Requirements

All samples will be collected by Sarina Jepsen (project manager) and an assistant. Sarina Jepsen has training in collecting invertebrates and will receive additional training from Scott Hoffman Black who has sampled invertebrates in wetlands. Sarina Jepsen will be present at every sampling location. The assistant will be trained at the Xerces Society by Sarina Jepsen and Scott Hoffman Black and will make pre-sampling site visits in January and April 2007, at which point both Sarina and the assistant will practice sampling and make sure that their sampling techniques are consistent with each other.

Documentation and Records

Field information that is needed includes: maps and descriptions of each site and field data sheets (see Appendix B for a copy of field data sheets, see Appendix D for topographical maps and Appendix E for satellite photographs of each site). All records will be stored in a three ring binder in the Xerces Society office for a minimum of one year after the completion of this project.

Sampling Process Design

Collection of samples

Three composite samples will be taken within the emergent vegetation zone of each wetland. Composite samples will be taken in order to reduce time and costs associated with processing multiple samples. Additionally, since each wetland site is the basic replicate, no additional statistical information would be gained by keeping samples separate (Rader and Shiozawa 2001). Each composite sample will be comprised of two sweep-netting efforts. Each effort will probably be comprised of five sweep samples in which the net is swept through the vegetated water column in a 180 degree arc, so that most of the aquatic community is sampled (surface water, water column, vegetation surfaces, top of the benthos). When sites are visited prior to sampling (in January and April 2007), we will practice the sampling methodology and determine how many sweep samples are needed to comprise a composite sample, as recommended by the ninth EPA module on methods for evaluating wetland condition (US EPA 2002b). We will aim to obtain at least 600 organisms in each composite sample. If five sweeps turns out to be an

inappropriate number, we will adjust our methods accordingly. Nets will be rinsed out and clinging macroinvertebrates will be removed between each composite sample.

Wetlands will be sampled in the shallow areas with emergent vegetation (shore to 0.5 m water depth), as this is the area that generally contains a high abundance and richness of macroinvertebrates (Driver 1977, Hanson and Swanson 1989, US EPA 2002b). The three sampling locations within the emergent vegetation zone of each wetland will be selected by placing a transect from North to South. Composite samples will be taken every 3 m along the transect. The dominant vegetation type(s) that corresponds to each composite sample will be recorded on the field data sheet.

Indicators

Since this is a relatively small study, we will only be collecting macroinvertebrates, and will not measure additional physical parameters. Ostracods and other smaller microinvertebrates that are not retained by a 500 micron sieve will not be identified from our samples.

Sampling frequency and period

Two wetlands will be sampled per day during a two week period. First we will sample our six paired wetlands (three least-impacted paired with three most-impacted), sampling one least-impaired and one most-impaired wetland each day, for three days. Then, we will sample the remaining seven least-impacted wetlands. Finally, we will re-sample one of the previously sampled least-impacted wetlands in order to obtain an estimate of our sampling precision. In total, ten least-impacted wetlands and three most-impacted wetlands will be sampled. The three most-impacted wetlands will be spatially and temporally paired with the three least-impacted wetlands. The sampling scheme will be as follows:

Week 1

Monday-least-impaired site 1 and **most-impaired** site 1 Tuesday- least-impaired site 2 and **most-impaired** site 2 Wednesday- least-impaired site 3 and **most-impaired** site 3 Thursday- least-impaired site 4 and least-impaired site 5 Friday- least-impaired site 6 and least-impaired site 7

Week 2

Monday- least-impaired site 8 and least-impaired site 9 Tuesday- least-impaired site 10 and duplicate sample from least-impaired site 3

With this sampling scheme, we are trying to establish a standard time period that minimizes variation caused by seasonal changes in community composition. There may be daily or weekly variation in weather conditions during our sampling period, which may influence water surface roughness or water temperature. To account for this potential source of variation, we will measure the air temperature and water temperature with a thermometer and record the data on our field data sheets. We will use a modified Beaufort scale to assess wind speed (see Appendix F), and we will record the categorical

wind rating on our data sheets. The sampling period will be in late April and May, based on advice from local wetland and macroinvertebrate specialists and evidence from a volunteer study of Willamette Valley wetland macroinvertebrates (N. Engelfried, unpublished data). This index period is when we are likely to find invertebrates sufficiently developed to be identifiable, yet before the wetlands are heavily colonized by invertebrates from other water bodies, dry down or are choked by vegetation. All wetlands will be sampled between 9:00h and 13:00h, in order to minimize variation in invertebrate species active at different times during a 24 hour period.

Site selection

Selection of sample sites will be based on a targeted sampling design, which is recommended by the EPA's Biological Assessment of Wetlands Work Group when developing wetland bioassessment methods (US EPA 2002a), since random sampling often does not identify enough least-impacted or severely impacted wetland sites.

Previous characterization of sites

Sites were chosen based on previous studies done by Paul Adamus to apply the Hydrogeomorphic (HGM) classification system (Brinson 1993, Shaffer *et al* 1999) to Riverine Impounding and Slope/Flats wetlands in the Willamette Valley ecoregion (Adamus and Field 2001, Adamus 2001a, b). HGM classification is primarily based on the geomorphic setting of a wetland, which is used to deduce the hydrology of individual sites.

We chose to sample wetlands previously studied by Adamus and others because an extensive amount of information has already been gathered on their hydrology, vegetation, surrounding land use patterns and types and levels of human disturbance. The EPA recommends using at least 2 assemblages in an Index of Biological Integrity (IBI) (Karr and Chu 1999); macroinvertebrate data from these same sites will assist one in eventually developing an IBI that includes both macroinvertebrates and vegetation.

Choice of sample size and level of human impairment

We chose to study 10 relatively unimpacted wetlands and 3 wetlands that are impacted by human disturbance. The sample size for the least-impacted wetlands was based on findings from a study of benthic macroinvertebrates in streams by Reynoldson and Rosenberg (1996); they found that 10 is the minimum number of reference sites that should be sampled in a given class to adequately represent that class. We selected 3 most-impacted wetlands from the same class and paired them both spatially and temporally with 3 of 10 least-impacted wetlands. Three is the minimum number of wetlands that should be sampled to do a statistical analysis (Detenbeck *et al* 1996). The data collected from the paired wetland sites (3 least-impacted wetlands and 3 most-impacted wetlands) may allow us to identify preliminary metrics that can eventually be used to develop an invertebrate Index of Biological Integrity (IBI) for Willamette Valley wetlands.

Choice of subclass

In the Adamus 2001 study, 109 wetlands from one HGM *subclass* (Riverine Impounding) and one combined HGM *class* (Slope/Flats) of wetlands in the Willamette Valley were extensively characterized. Some of the wetland attributes that were studied include: percent of native vegetation, level of human visitation, water flow (whether or not water level is controlled), proximity of surrounding roads, land use of surrounding areas, and soil compaction and leveling, among others. We chose to study wetlands belonging to a single subclass so that we could minimize sources of variation from hydrological and geomorphological factors. Thus, differences we see between least-impacted and most-impacted wetlands will more likely be due to variable levels of human impairment, rather than due to differences in hydrology or geology.

We chose to study Riverine Impounding subclass for these reasons:

- 1. Riverine Impounding wetlands belong to the only subclass of wetlands in the Willamette Valley ecoregion that has been extensively characterized using the HGM classification system. By choosing wetlands within a subclass, rather than a class, we can minimize the natural variability among the wetlands we sample.
- 2. All of the wetlands in the Riverine Impounding subclass categorized as 'least altered' (Adamus 2001a) also received scores of greater than 0.75 for the Invertebrate Habitat Support function.
- 3. According to the EPA module #1 on Methods for evaluating wetland condition (US EPA 2002), the current sampling methods developed for other regions focus on wetlands with standing water. Wetlands in the Riverine Impounding subclass have much more standing water than wetlands in the Slope/Flats class. By choosing wetlands in the Riverine Impounding subclass, we will more easily be able to apply already developed methodology to our study.

The Riverine Impounding subclass includes wetlands with the following traits: most of the surface water during 2-year flood events is substantially delayed, water flow is mostly unidirectional, delay of water is caused by channel or floodplain constrictions, water passes more slowly through the sites than in upstream or downstream areas, and some inputs may be from groundwater during non-flood seasons. Often RI sites are depressions in a 2-year floodplain. Most Riverine Impounding sites have a fluctuating water regime. This subclass encompasses a diversity of sites, including: seasonally flooded gravel pits in floodplains, headwater channels with functioning beaver dams, oxbows on major rivers, wet meadows on alluvium, intermittent desert channels that are restricted just before entering narrow canyons or road culverts, waterfowl impoundments fed mostly by piped in river water, and backwater swamps behind natural levees that adjoin low-gradient channels (Adamus 2001b).

Choice of sites

We chose to use 10 least-impacted sites and 3 sites that are most-impacted by human activity. All sites are listed in Appendix C. Please note that an additional less-impacted site is listed, in case, for some unforeseen reason, we are unable to sample one of our other sites. If this situation occurs, we will instead sample the additional site (Minto-Brown slough 1).

The criteria for choosing the ten least-impacted sites were twofold. First, we chose the 23 Riverine Impounding wetland sites that received scores of 0.75 of greater for invertebrate habitat support in the 2001 Adamus study. Then, we chose 10 wetland sites from these 23 sites that were considered to be less altered for their hydrology, soils, level of human visitation, surrounding land uses, proximity to roads, and composition of native vs. non-native plant species. Five of the 10 chosen wetlands were categorized as overall 'least-altered' sites for the Adamus study and the remaining 5 sites had many attributes of being 'least altered'. All ten of the less-impacted sites were qualitatively assessed in the initial Adamus study as "apparently one of the less obviously degraded sites." The initial 2001 Adamus study scored all of the sites for a variety of ecological functions, including: water storage and delay, sediment stabilization and phosphorus retention, nitrogen removal, primary production, invertebrate habitat support, and support of characteristic vegetation. The range of scores given to our 10 least-impacted sites is higher than the range of scores given to our 3 most-impacted sites for each of these functions (see Table 1).

To choose our three sites as most-impacted, we first examined the sites that were qualitatively assessed in the initial Adamus study as "apparently one of the more obviously degraded sites." Only five sites were labeled as such in the Adamus study, and of those five sites, there was only data on function for three of the sites. We noted that those three sites received relatively low scores for each of the wetland functions, and that they all received scores of below 0.55 for Invertebrate Habitat Support. We paired those three sites spatially with three of the less-impacted sites (see Appendix C).

Function	Less-Impacted sites	More-Impacted sites				
	(range of scores)	(range of scores)				
Water Storage & Delay	1.00 - 0.14	0.1 - 0.06				
Sediment Stabilization &	0.87 - 0.54	0.68 - 0.41				
Phosphorus Retention						
Nitrogen Removal	0.97 - 0.74	0.86 - 0.63				
Primary Production	0.98 - 0.71	0.82 - 0.52				
Invertebrate Habitat	1.00 - 0.75	0.53 - 0.44				
Support						
Support of Characteristic	0.97 - 0.66	0.51 - 0.28				
Vegetation						

Table 1. Ranges of scores received by least-impacted sites versus most-impacted sites for six wetland functions.

The specific criteria used by Adamus to give a site a score for 'invertebrate habitat support' includes:

- 1. Percent of the site that is inundated permanently and contains herbs
- 2. Percent of site that is inundated only seasonally

- 3. Type of connection to associated channel
- 4. Predominant depth category during biennial low water
- 5. Percent and distribution of pools at biennial high water
- 6. Percent of site affected by soil leveling
- 7. Percent of site currently affected by soil compaction
- 8. Mapped soil series is hydric
- 9. Number and distribution of vegetation forms
- 10. Percent of surrounding land cover within 200 feet of the site that is not cropland, lawn, buildings, or pavement
- 11. Percent of land cover in contributing watershed and within 200 feet of the site that is not cropland, lawns, pavement, or buildings

The criteria listed above represent attributes that contribute to an individual wetland's ability to support the life requirements of many invertebrate species characteristic of the site's location within an ecoregion (Adamus and Field 2001). Criteria used to give scores for other wetland functions can be found in the HGM guidebook for the Willamette Valley Ecoregion (Adamus and Field 2001).

Caveats

Our study wetlands are highly variable in size (ranging from 0.3 acres to 87 acres), which could add a degree of variability to the study; a previous study showed that species richness and number of functional feeding groups increased as the size of a temporary pool increased (Ferrella and Bass 1995). We are still choosing to use wetlands of variable sizes in our study because this set of wetlands holds many other advantages. However, we will examine whether 'wetland acreage' or 'acreage of permanent water' influenced each of our invertebrate variables (richness, diversity, composition, relative abundance) by running an Analysis of Variance (ANOVA).

Sites will be visited in January and April of 2007 to practice our sampling methodology and to ensure that the sites have not drastically changed since the Adamus study was conducted in 1999 and 2000 (Adamus 2001a). We will be looking for changes that may have occurred that would indicate that the wetlands currently belong to a different HGM subclass, or that there are greater levels of human impairment at our less impaired sites. If this is found, we will reevaluate our choice of sites.

Weather conditions

Sampling will take place regardless of inclement weather conditions, although weather conditions will be recorded on field data sheets.

Site safety

In order to ensure maximum levels of safety while conducting field work, we will take the following steps:

- 1. A person will never visit a site alone (all samples will be taken by Sarina Jepsen and one field assistant)
- 2. Cell phones will be carried to the field to be used in case of an emergency

- 3. A first aid kit will be carried to the field
- 4. Each person will carry a set of dry clothes to the field, to avoid hypothermia
- 5. Extra water will be taken to the field to avoid dehydration

Sampling Methods Requirements

Sampling methods

Samples will be taken with aquatic D-frame sweep nets with 500-micron mesh similar to the protocol used in Minnesota to collect macroinvertebrates in the emergent vegetation zone of depressional wetlands (US EPA 2003). The choice of this device was based on the recommendation of Batzer, Shurtleff and Rader (Batzer *et al.* 2001); they surveyed wetland macroinvertebrate researchers and concluded that the aquatic D-frame sweep net should become the sampler of choice for most bioassessment efforts that use wetland macroinvertebrates. In the past, Minnesota has used a combination of aquatic D-frame sweep nets and activity traps to monitor invertebrates in wetlands; recent studies on the importance of activity traps in adding taxa richness to invertebrate metrics led them to conclude that activity traps should not be used in future studies. In 2007, Minnesota will begin sampling protocols using only aquatic D-frame sweep nets (Mark Gernes, personal communication).

Preservation of samples

The contents of the aquatic D-frame sweep net will be put in sample jars (a 1-L plastic Nalge Nunc International straight-side wide-mouth jar), labeled and preserved in 95% ethanol. The ethanol will be decanted and replaced with 80% ethanol after 2 days to ensure sample preservation. This method is similar to that used by researchers in Minnesota (US EPA 2003), except that invertebrates will be separated from vegetation in the laboratory instead of the field (recommended by Batzer *et al.* 2001). Preliminary site visits in April 2007 will determine the most appropriate number of sweeps per sampling effort; we will aim to collect at least 600 organisms per composite sample.

Samples preserved in ethanol will be held for a maximum of 21 days before they are delivered to Aquatic Biology Associates, Inc. to be processed. Samples will be separated from debris by water flotation, then sieved through 500 micron mesh. A random subsample of 300 organisms will be taken from each composite sample, as recommended by Batzer *et al.* (2001). Samples will be searched for large, rare, adult and mature larvae as well. Chironomid midges will be identified to genus or, if possible, species group. Oligochaete worms will be identified to class. All other organisms will be identified to the lowest practical taxonomic level.

Sample Handling and Custody Requirements

Samples will be collected and labeled in the field by Sarina Jepsen and a field assistant. The labels will include the following information: sample location, sample number, date and time of collection, sample type, sampler's name, and method used to preserve sample. Samples will be held at the Xerces Society office in Portland for a maximum of 21 days in an area that is not exposed to direct sunlight. Sarina Jepsen will deliver all samples to ABA laboratory in Corvallis, Oregon to be processed. After they are processed, samples will be archived for one year at ABA, Inc.

Analytical Methods Requirements

Treatment of data from 10 least-impacted wetland sites

We will assess the following traits from each of the ten least-impacted wetlands: richness (number of taxa present at a site), taxonomic composition (identification of taxa present in a community), relative abundance (percent representation of various taxa), and diversity (Shannon's diversity index, H'). We will also look at tolerance (tolerance levels will be taken from genus-level tolerance values published for stream invertebrates by the US EPA, Green 1990, and from the database developed by Adamus and Gonyaw for tolerance values of wetland macroinvertebrates, 2001). Each of the above attributes can be assessed with a qualitative or semi-quantitative sampling methodology (i.e. aquatic D-frame sweep netting).

Treatment of data from paired sites

To examine overall differences between least-impacted and most-impacted sites, we will calculate community similarity between each pair of sites (i.e. calculate similarity between least-impacted site 1 and most-impacted site 1, then between least-impacted site 2 and most-impacted site 2, etc.). We will identify abundant, common and rare taxa and examine qualitative differences between our least-impacted and most-impacted wetland sites.

To look for changes in individual metrics between least-impacted and most-impacted sites, we will create boxplots for each metric to show the means and variances of each attribute, following the approach by Mundahl and Simon (1999). Metrics that are most different between most-impacted and least-impacted sites will be retained; paired *t*-tests will be used to examine whether significant differences exist for each metric between least-impacted and most-impacted sites. If we find numerous (>8) metrics that differ significantly between least-impacted and most-impacted sites, we will begin to develop a preliminary invertebrate Index of Biological Integrity (IBI, Karr and Chu 1999) for Willamette Valley Riverine Impounding wetlands. However, given our small sample size of least-impacted and most-impacted wetlands, it is unlikely that we will be able to fully develop an invertebrate IBI. We expect to identify only a few metrics that ultimately will contribute to the development of an IBI.

Some of the metrics that we will examine, based on metrics that have been successfully used in wetland invertebrate IBIs in Minnesota and other regions of the United States, include:

Richness

Total # of genera

Taxonomic Composition

of Crustacea + Mollusca genera

of Crustacea genera

of Mollusca genera

of Odonata genera

of Chironomid genera

- # of Hirudinidae genera
- # of species of snails
- # of genera mayflies, caddisflies, presence of fingernail clams, dragonflies (ETSD metric)

Relative Abundance

- % Gastropoda of total abundance
- % Odonata of total abundance
- % Spheariidae of total abundance
- % Erpobdella of total abundance
- % top 3 dominant taxa of total abundance
- % Corixidae of Hemiptera

Diversity

Shannon's index of diversity (H')

Tolerance

of intolerant taxa

% intolerant taxa of total sample

Quality Control Requirements

Field sampling training

Sarina Jepsen has extensive experience in insect field sampling. She has done extensive research on the methods used in wetland macroinvertebrate collection and has consulted with local wetland and macroinvertebrate professionals. Scott Hoffman Black will be present and provide assistance in some of the preliminary site visits to practice sampling; he has a background sampling invertebrates in wetlands and has sampled macroinvertebrates extensively in streams. Both Sarina Jepsen and Scott Hoffman Black will train the field assistant. Sampling methods will be practiced in the field during preliminary site visits in January and April 2007.

Field replication

Field data sheets will be used each time sampling is conducted (Appendix B). Data sheets will then be taken to the Xerces office, entered into an Excel spreadsheet, then stored in a three ring binder. One wetland site will be sampled a second time to gain an estimate of our sampling precision.

Equipment Testing and Inspection and Maintenance Requirements

All field equipment (aquatic D-frame sweep nets, stop watches, squirt bottles, sample bottles, permanent pens, ethanol, waders, field notebook, measuring tape, meter sticks and compass) will be inspected in December 2006. Equipment will be tested in the field during our preliminary site visits in January and early April 2007. Any damaged equipment will be fixed, or if that is not possible, replaced before we begin collecting samples. We will carry extra netting with us for the aquatic D-frame sweep nets when we sample, in case netting gets torn.

Instrument Calibration and Frequencies

All equipment will be inspected prior to sampling to ensure that it is clean and in working order. We will not be using any instruments that require routine calibration.

Data Acquisition Requirements

We have used data from a previous study by Adamus and Field (2001) to select our wetland sites. For a complete description of how wetlands were classified along a range of human disturbance, see (Adamus 2001a). We are confident that the data taken is of high quality from the description of how the data was taken, the reputation of the principal investigator, and the fact that the project was overseen by the Oregon Department of State Lands.

Data Management

Field data sheets will be filled out in the field, then taken to the Xerces Society office. All data will be transferred to an Excel spreadsheet on the computer. All data sheets will be examined for completeness and accuracy at the end of each field sampling day; any mistakes will be fixed, or noted if it is not possible to fix them. Inaccuracies that often result with many different individuals collecting data and filling out data sheets will be minimized since the same two people will collect all data and fill out all data sheets. Data that comes from ABA, Inc. from the laboratory processing of field samples will be entered into an Excel file. All data in the Excel spreadsheets will be double checked with paper data sheets to ensure accurate transfer to the computer, then critically reviewed for reasonableness and correspondence with data quality objectives. Any suspect data will be flagged. Paper data will be stored in a three ring binder in the Xerces office. Electronic data will be backed up on a CD and stored in another location. Copies will be made of the paper data sheets, which will also be stored in another location.

Assessments and Response Actions

Evaluation of field, lab and data management activities will be conducted by Sarina Jepsen and occur throughout the duration of the project. The performance of the lab that processes the samples will be evaluated; problems are not expected because the lab (ABA, Inc.) has a good reputation for producing quality data.

Because this is a small project, the same two people will go to every site to collect macroinvertebrate samples. Sarina Jepsen and an assistant will work in close proximity to each other, which will ensure that all data is collected in a consistent manner. Scott Hoffman Black will evaluate the performance of both of the field samplers.

Any problems identified through these assessments will be corrected if possible. For example, if either of the two people sampling macroinvertebrates is found to be sampling incorrectly, protocols will be reviewed and he or she will be given further training. If other problems are discovered that cannot be corrected, the problems will be noted and their potential impact on the results of the project will be detailed in the final report.

Reports

We will create a final report stating our findings, the results of our internal assessments, and how any QA problems that may have come up were resolved. In addition, our findings will be detailed on an interactive CD-ROM, which will be distributed to citizen groups, scientists and other interested individuals.

Data Review, Validation and Verification Requirements

Once data is received from the lab that processes the samples, it will be thoroughly reviewed by Sarina Jepsen. All data will be accepted unless errors are found or suspected. Any errors or suspected errors will be evaluated further, and, if possible, the data will be re-taken. If it is not possible to re-take the data, that data will be rejected.

Validation and Verification Methods

Computer entries will be compared to field and lab data sheets to make sure that there are no mistakes. All calculations will be double-checked (i.e. Shannon's index calculations). To identify outliers, we will visually inspect the data for data recording or entry errors. Extreme values will be double-checked. We will then arrange the data in scatter plots and visually examine the data for outliers. We will create box plots and compute the inter-quartile range (IQR), then use a multiple of the IQR as a number that defines what values are considered outliers. We will flag observations as potential outliers that lie outside of quartile1-(1.5* IQR) and quartile3+(1.5* IQR), and we will flag observations as problematic outliers that lie outside of quartile1-(3* IQR) and quartile3+(3* IQR). If we find outliers, we will first try to find out if they are due to mistakes. If they are mistakes, we will correct the mistakes if possible, or if not possible, delete the values. If the values appear to be legitimate, we will transform the data. As a last resort, we will run the statistical tests both with and without the outlier values and report both results.

Reconciliation with Data Quality Objectives

Once the data is collected, we will evaluate the level of precision, accuracy, completeness and representativeness of the data. If the data quality indicators do not meet our standards, we will exclude that data and re-sample if possible. If it is not possible to resample, we will exclude that portion of the data from the final analysis or we will include it and set limits on the use of the data in our final report.

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Appendix A: Quality Assurance/ Quality Control Laboratory Guidelines for ABA, Inc.

The following quality assurance/quality control (QA/QC) procedures are routinely followed at Aquatic Biology Associates, Inc. in processing benthic macroinvertebrate samples. Procedures will be altered to fit the needs of the client for specific projects. Alterations in QA/QC procedure may add to the per sample cost.

- 1. Samples are unpacked upon receipt and preservative levels checked. Labels are checked to make sure they are intelligible and that the experimental design is understandable (e.g. sites & replicates). Non-smear labels are made that go on the inside of sample jars. The client is called if samples have been damaged in shipping and/or if the labeling system is not understandable.
- 2. The entire sample is floated in water in a white plastic tray. Large debris is rinsed and removed. The sample is then elutriated until all organic matter and invertebrates are floated off the mineral residue. Sieves of a pore size specified by the client are used in this process (500 micron is the most common). The mineral residue remaining in the white pan after elutriation is searched for stone-cased caddisflies and molluscs that have not floated off.
- 3. Unless otherwise specified by the client, a portion of the sample will be sorted that contains 500-600 organisms. The Caton Tray is normally used to randomly obtain a fraction of the total sample containing 500-600 organisms. Sample data is converted to a full sample basis. Other methodologies may be used to split some sample types, such as lake benthic samples. If densities are low, Surber and Hess samples are usually processed in their entirety. If a sample is subsampled, our normal procedure is to archive the unused sample portion until the project is completed. Unused sample fractions will be returned to the client if requested (shipping charges will be billed to the client). If requested, Aquatic Biology Associates, Inc. will archive unused sample fractions for 1 year at no charge.
- 4. Experienced technicians are used to remove all invertebrates from the sample residue using dissecting scopes at 6X or 12X power. For small projects, a single technician is assigned. For larger projects, several technicians are given the responsibility for sorting. All invertebrates removed from a sample are placed in a single sorting vial and given directly to Robert W. Wisseman, Senior Scientist of Aquatic Biology Associates, Inc. Logs are kept by each technician to record label data, fraction sorted, hours required to complete sorting, and any comments on sample matrix or problems. Our sorting efficacy is well above EPA requirements, as has been determined by an independent lab. Detailed sorting procedures followed by Aquatic Biology Associates, Inc. can be sent upon request.
- 5. The entire sample residue is saved after sorting to check for sorting efficacy. Sorting efficacy of 95% or better is required on all samples. A 20% aliquot of each residue is thoroughly re-sorted to determine efficacy. The entire residue is re-sorted if 95% or better sorting efficacy has not been achieved, as estimated from the 20% aliquot re-sort. All

sample residues can be returned to the client for independent checks. The client will be charged for shipping and sample containers.

6. Invertebrate identifications are performed by Robert W. Wisseman and associates.

For standard level identifications, Robert W. Wisseman performs the initial identifications and counts on all samples, and then determines which specialists will be required to assure accurate identifications to levels specified for a project. He has over 15 years of experience in the identification of freshwater invertebrates. Aquatic Biology Associates, Inc. uses specialists from throughout North America for performing more detailed taxonomy, or to verify questionable identifications.

- 7. The choices for archiving invertebrate material for QA/QC checks by other experts, are as follows: 1) You can trust Aquatic Biology Associates, Inc. to do a competent job, and let us pull out material that we think is significant...e.g. for verification by specialists, to be incorporated into museum collections, or to save for educational purposes. This is our preferred method of operating. 2) Save a reference/synoptic series of specimens of each taxa identified. There will be nominal charge for this service. 3) All invertebrate material can be saved by each individual sample for archiving or QA/QC checks by another lab. An additional charge per sample will be added for this service, since it greatly slows sample processing. 4)The client can request that specific taxonomic groups be archived by individual sample for possible future taxonomic analysis (e.g. all the oligochaete worms). There is usually no charge if one or a few groups are involved. 5)Aquatic Biology Associates, Inc. requests permission to remove material from samples that may be of interest to specialists or that we feel would be a valuable addition to museum collections.
- 8. Identifications and counts are recorded on bench-sheets and then transferred to electronic files. Standardized bench-sheets reduce data entry errors. Robert W. Wisseman and Mary Jo Wevers (Aquatic Biology Associates, Inc. senior scientists) perform all data entry and analysis.

Appendix B: Wetland macroinvertebrate monitoring field data sheet

Wetland site name:	Wetland Bottom (circle one)					
	Firm					
	Soft					
Date:/	EMERGENT VEGETATION					
Wetland Location	Emergent (circle one)					
County:	None					
Latitude:	Sparse					
Longitude:	Moderate					
	Dense					
Time:	Shoreline vegetation (circle one)					
Air temperature:	Grassy					
Water temperature:	Shrubs					
Modified Beaufort Wind speed rating:	Wooded					
	Other					
None Low Moderate High						
Sample #: Sampler:	Sample #: Dominant vegetation:					
W 27 2 1	***					
# of Jars per Sample:	Weather:					
	Class Doubles alouds					
	Clear Partly-cloudy					
	Overcast Precipitation					
	Overeast Trecipitation					
☐ Photographic documentation of site	Additional Comments:					
☐ Identify direction of view in photo on						
sketch of site						
☐ Sketch on close-up topo map exactly						
where samples were taken						
•	OF WETLAND SITE					
	ad samples taken, and any other relevant information.					
N W+E						
S						

Appendix C: Table of sites

Sites in **bold text** indicate that they were selected as 'least-altered' in the previous study by Paul Adamus (Adamus 2001a).

MI = More Impacted site

LI = Less Impacted site

PN = Pair Number

 $\mathbf{Ac} = \mathbf{Acreage}$ of site

AcPerm = Acreage of permanent water

River = Wetland is associated with a large river (L), or associated with a smaller river or stream (S)

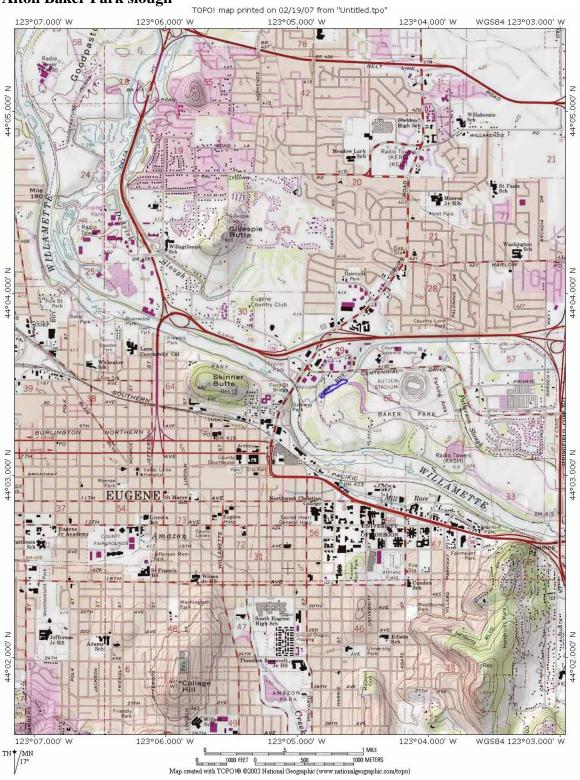
Veg = site is dominated by herbaceous vegetation (H), or site is dominated by woody vegetation (W)

Invert = Invertebrate Habitat support score given by the Adamus 1999-2000 study (pages 121-122, Adamus and Field 2001).

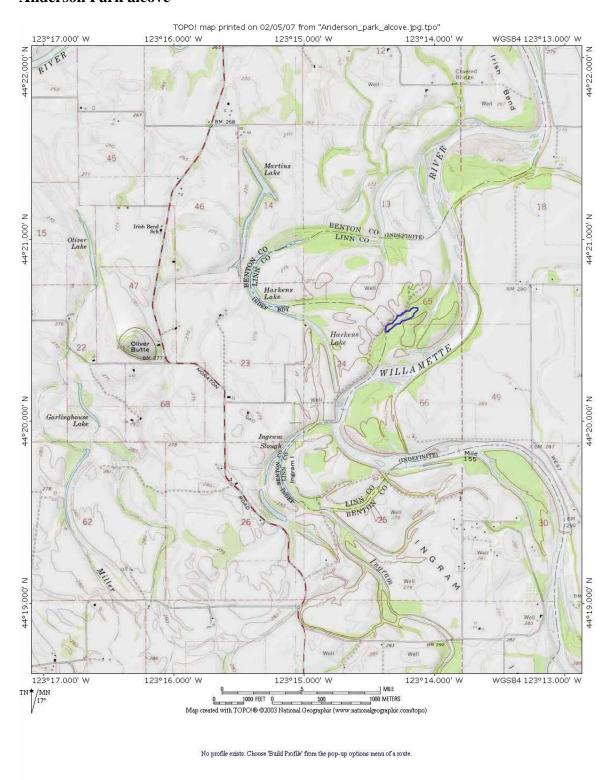
	Site	MI	LI	PN	Ac	AcPerm	County	River	Veg	Invert
1	Wilson Wildlife		X		13.2	0.6	Benton	S	Н	1.00
	Area north									
2	Willow Creek		X	3	3.4	0.3	Lane	S	W	0.99
	riverine									
3	Willamette Park		X		2.1	0.1	Benton	L	W	0.98
	slough									
4	Anderson Park		X		6.4	1.2	Benton	L	W	0.90
	alcove									
5	Greenberry		X		32.1	0.3	Benton	S	W	0.88
	floodplain									
6	Buford West		X	1	4.4	3.0	Lane	L	W	0.85
	slough							~		
7	Tualatin Hills		X	2	1.6	0.4	Washington	S	Н	0.81
	marsh						_	~		
8	Finley		X		18	0	Benton	S	W	0.78
	floodplain							_		
9	Spongs		X		26	1.3	Marion	L	W	0.77
	Landing									
	slough							_		
10	Willamette		X		87	34.8	Marion	L	Н	0.77
	Mission slough							_		
11	Minto-Brown		X		6.7	1.3	Marion	L	W	0.75
	slough 1									
10	(alternate site)					• • •	-	<u> </u>	**	0.70
12	Delta Ponds	X		1	22	20.9	Lane	L	Н	0.50
13	Hedges Creek	X		2	0.3	0.1	Washington	S	Н	0.44
	duck ponds				0.6		-	-		0.72
14	Alton Baker	X		3	0.9	0.8	Lane	L	Н	0.53
	Park slough									

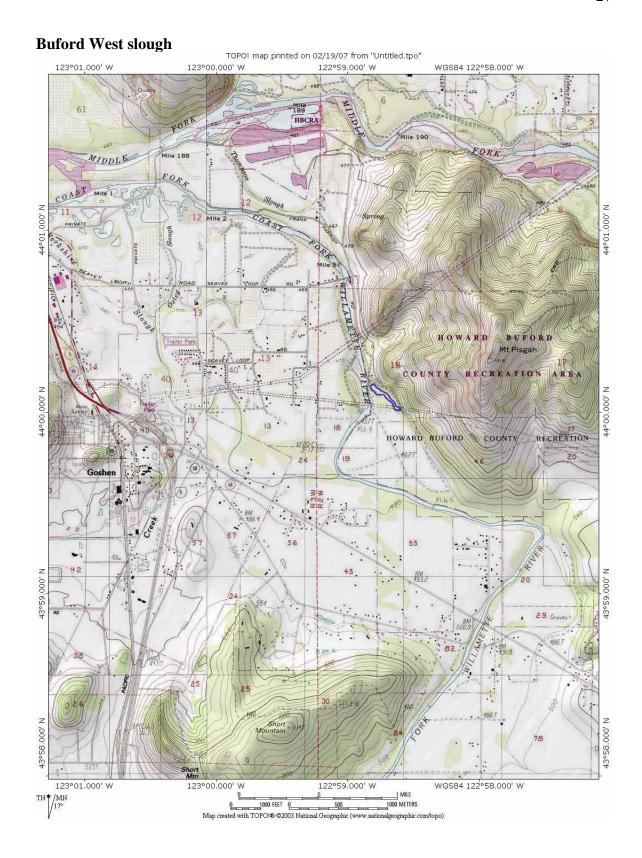
Appendix D: Topographical maps of sites Sampling locations are outlined in blue on maps.



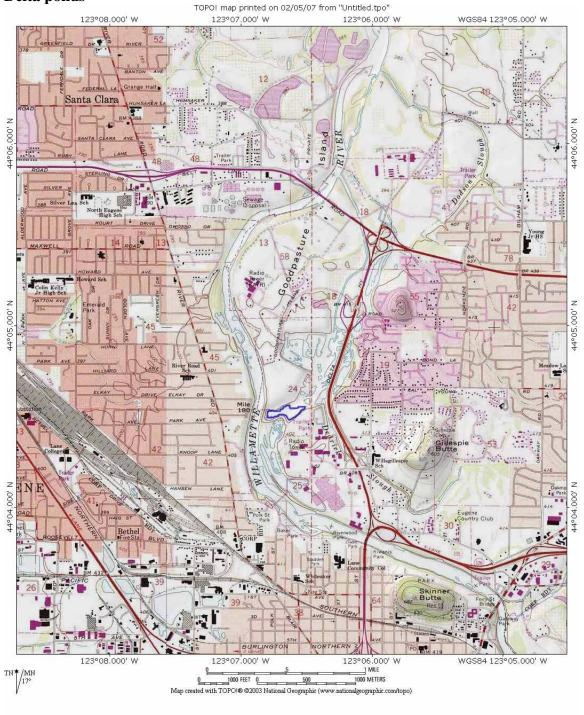


Anderson Park alcove



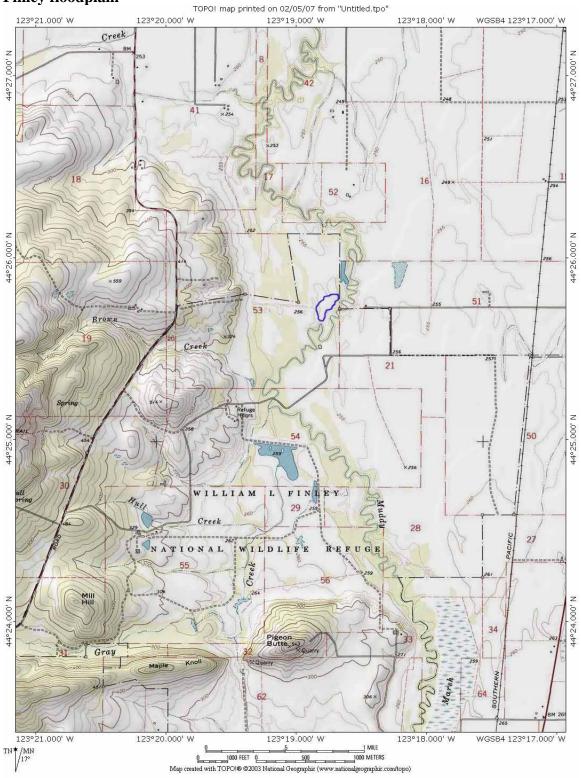


Delta ponds

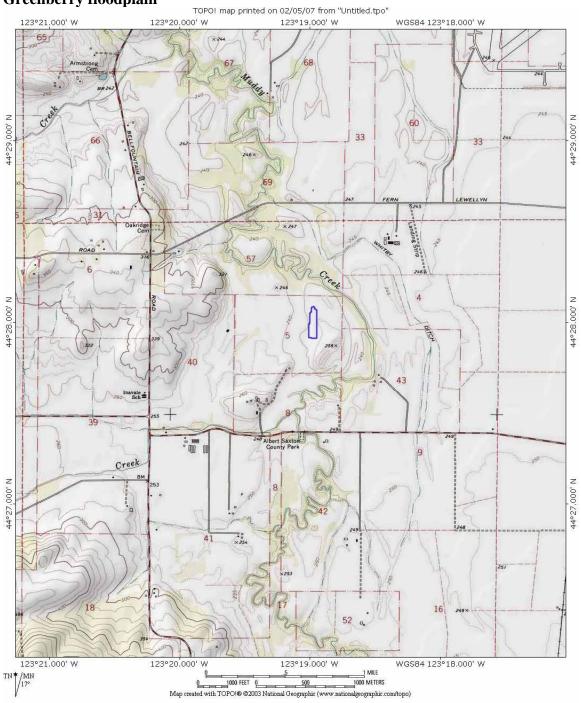


No profile exists. Choose 'Build Profile' from the pop-up options menu of a route.

Finley floodplain

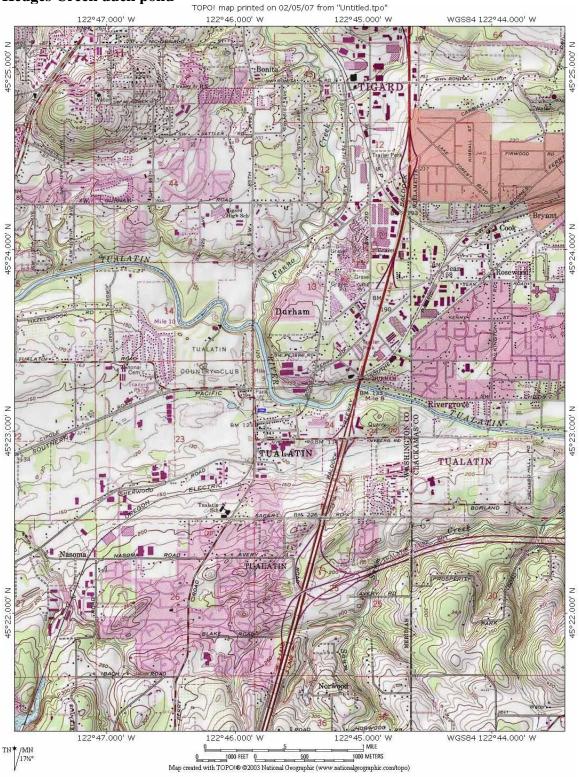


Greenberry floodplain



No profile exists. Choose 'Build Profile' from the pop-up options menu of a route.

Hedges Creek duck pond



Minto-Brown slough 1 (alternate site) TOPO! map printed on 02/19/07 from "Untitled.tpo" 123°06,000' W 123°05,000' W 123°04,000' W WGS84 123°03,000' W WILLAMETTE-Browns Gravet 32 0 44°55,000' N GOLF COURSE 44°54,000' N

123°06.000' W

123°05.000' W

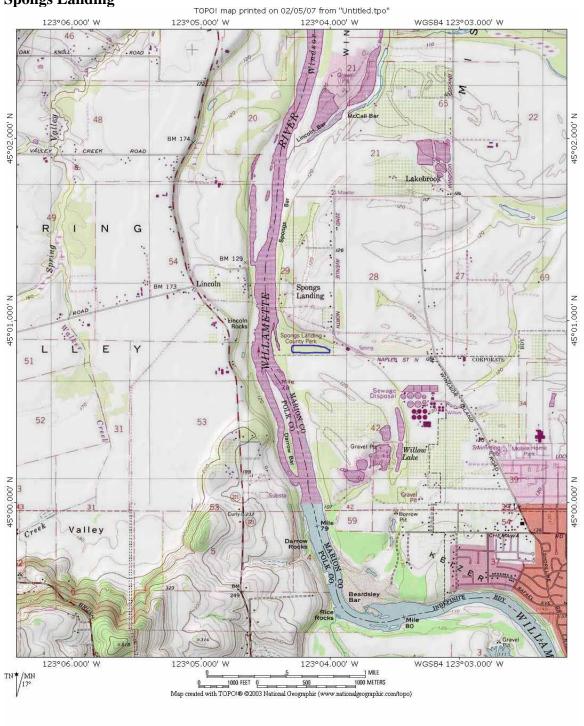
0 1000 FEET 0 500 1000 METERS

Map created with TOPOI® ©2003 National Geographic (www.nationalgeographic.com/topo)

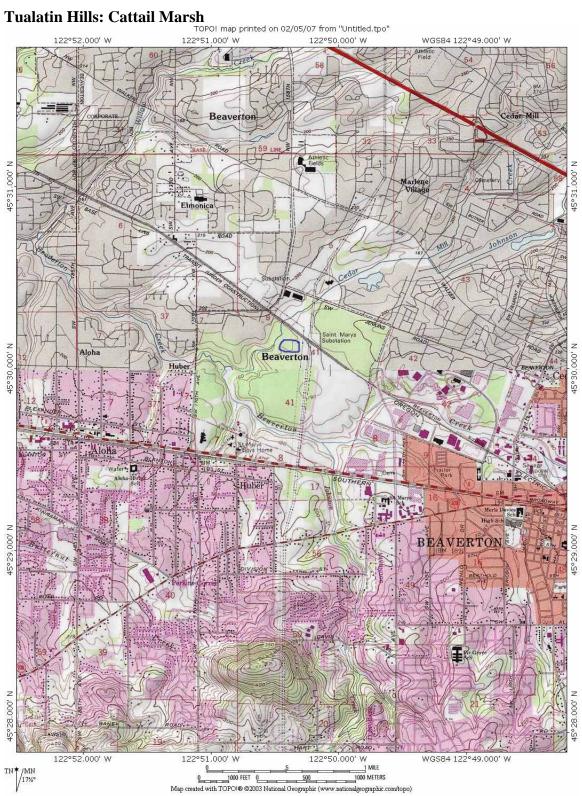
123°04,000' W _____1 MILE _____1000 METERS

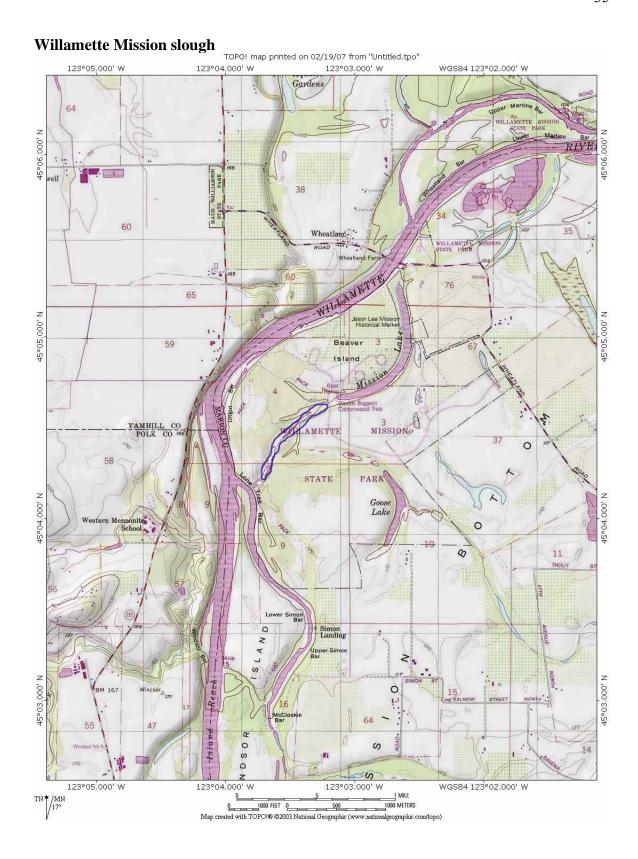
WGS84 123°03.000' W

Spongs Landing

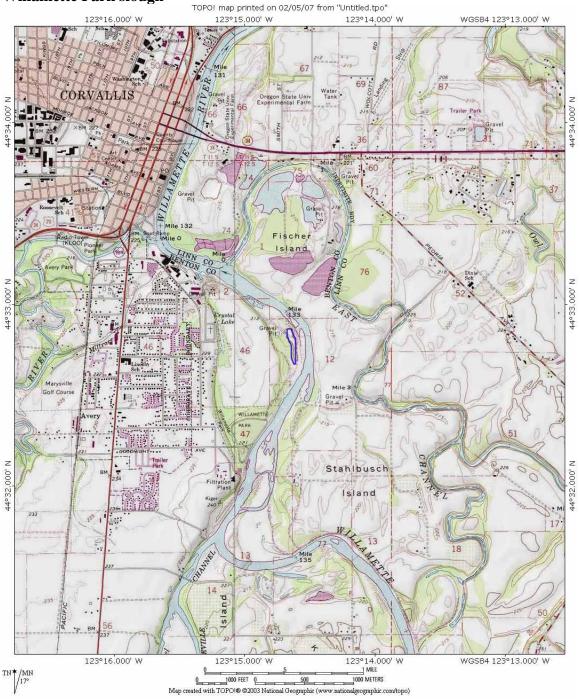


No profile exists. Choose 'Build Profile' from the pop-up options menu of a route.



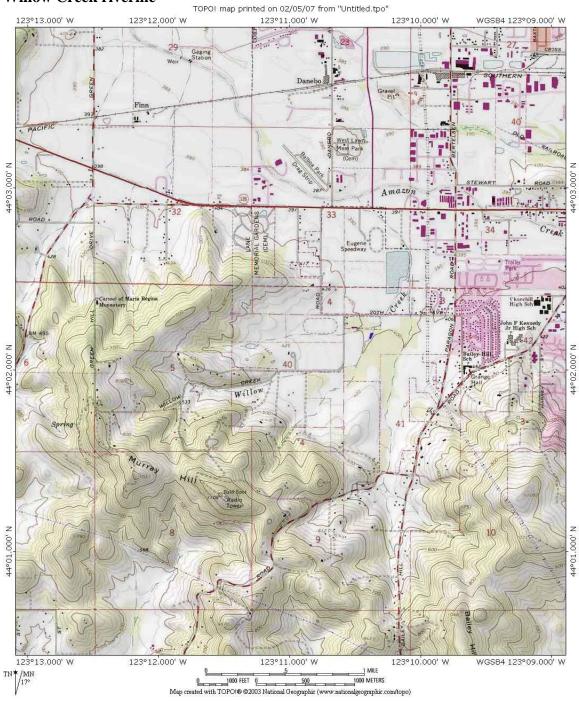


Willamette Park slough

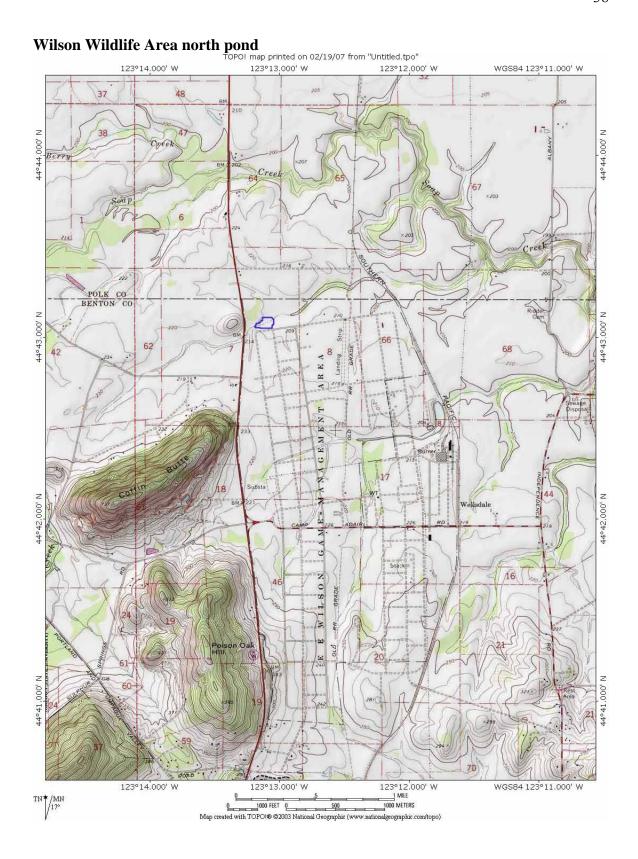


No profile exists. Choose 'Build Profile' from the pop-up options menu of a route.

Willow Creek riverine

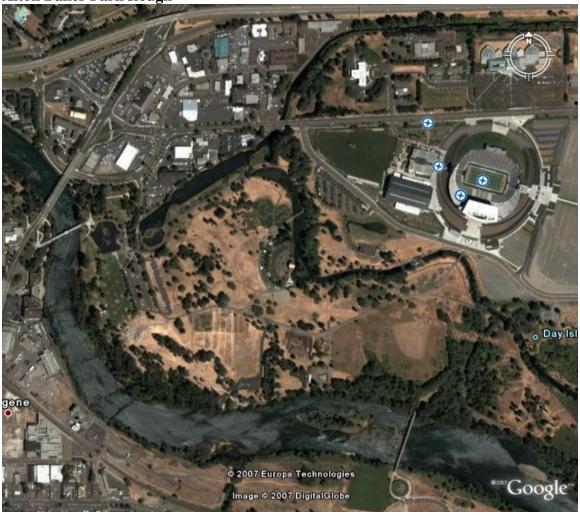


No profile exists. Choose 'Build Profile' from the pop-up options menu of a route.

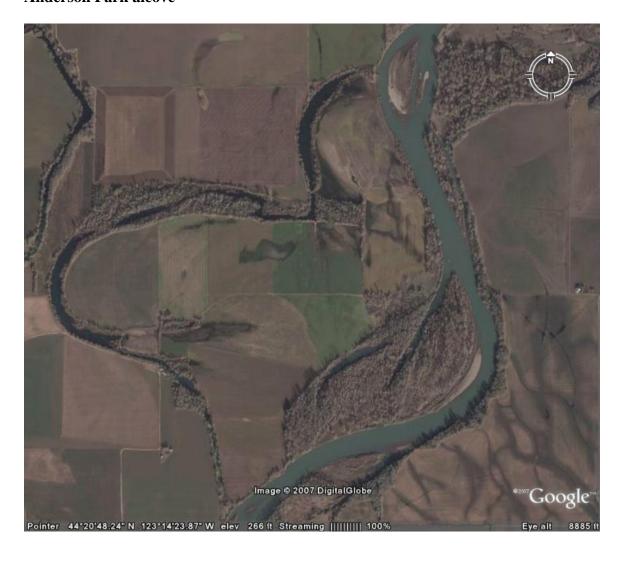


Appendix E: Satellite photographs of study sites

Alton Baker Park slough



Anderson Park alcove



Buford West slough



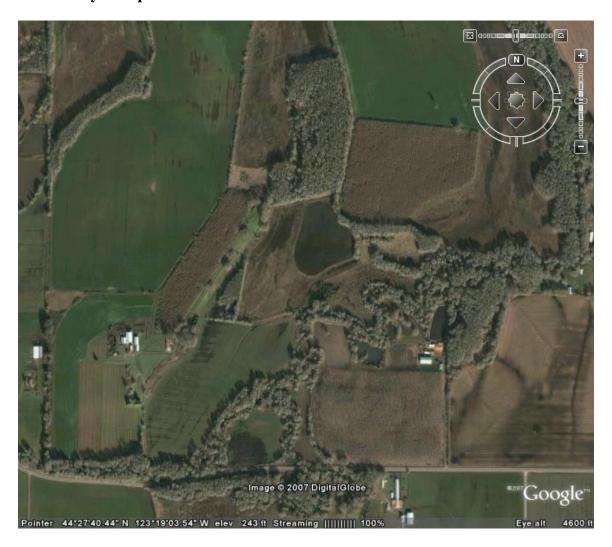
Delta ponds



Finley floodplain



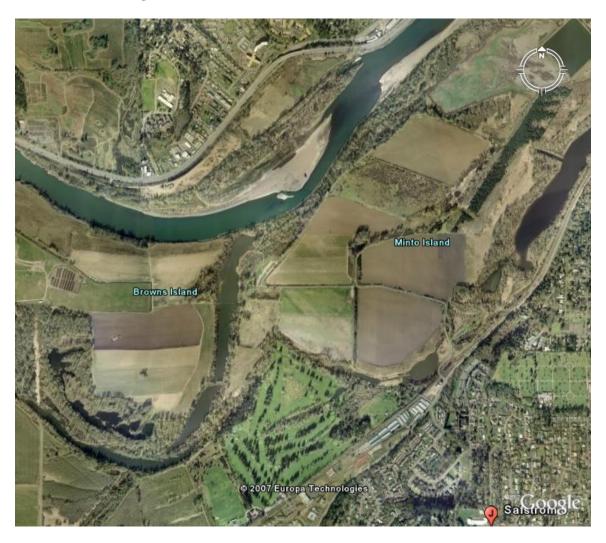
Greenberry floodplain



Hedges Creek duck pond



Minto-Brown slough 1



Spongs Landing



Tualatin Hills: Cattail Marsh



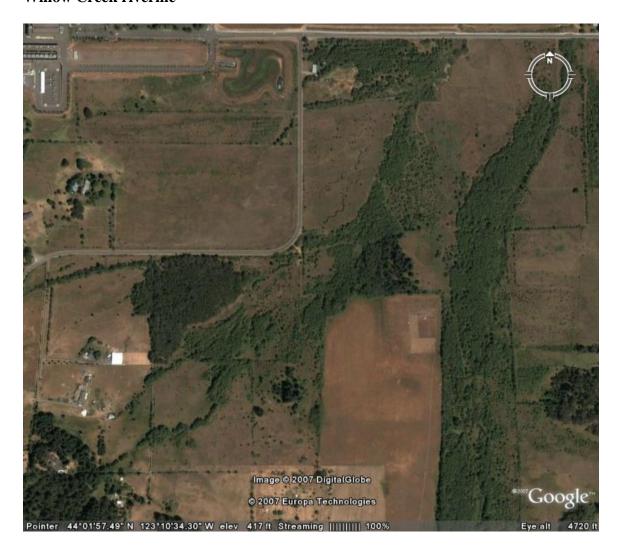
Willamette Mission slough



Willamette Park slough



Willow Creek riverine



Wilson Wildlife Area north pond



Appendix F: Modified Beaufort Wind Speed Scale

	Calm
None	Smoke rises vertically
	Light air to gentle breeze
Low	Smoke drifts slowly downwind to leaves rustling or in
(Beaufort scale 1-3)	Motion
	Moderate to fresh breeze
Moderate	Small branches to small trees are swaying
(Beaufort scale 4-5)	
	Strong breeze and more
High	Large branches to whole trees are swaying, or stronger
(Beaufort scale 6+)	

The Xerces Society **Standard Operating Procedures**

Sampling Macroinvertebrates in Willamette Valley Riverine-Impounding wetlands

Summary:

We plan to sample macroinvertebrates in 13 Willamette Valley Riverine-Impounding wetlands. Our study sites will include ten wetlands that are least-impacted and three wetlands that are most-impacted by human activity. The three most-impacted wetlands will be paired spatially and temporally with three of the least-impacted wetlands. Three composite samples will be taken from one strata of each wetland using aquatic D-frame sweep nets. Our critical measurements will be taxa richness, taxa diversity, taxonomic composition, and relative abundance. We will evaluate the responses of a variety of metrics in least-impacted and most-impacted wetlands to identify metrics that may eventually be used in an Index of Biological Integrity (Karr and Chu 1999) for macroinvertebrates in Riverine Impounding wetlands. This data will be useful in establishing a 'reference' condition for this particular subclass of wetlands in the Northwest.

Our goal is to produce data of sufficient quality to address these project objectives:

- 1. Characterize the macroinvertebrate community in Willamette Valley Riverine Impounding wetlands.
- 2. Measure the variability in the invertebrate communities among ten least-impacted Riverine Impounding Willamette Valley wetlands.
- 3. Look for invertebrate metrics that vary predictably between least-impacted and most-impacted Riverine Impounding Willamette Valley wetlands.

Equipment needed:

Aquatic D-frame nets
Sample jars
Sample jar labels
Pencils
Permits
Maps
Field notebook
Squirt bottles with ethanol
Measuring tape
Compass
5 gallon buckets
Ethanol

Waders
Digital camera
Extra batteries for camera
Thermometer

Sampling procedures:

Number and types of samples

Three composite samples will be taken within the emergent vegetation zone of each wetland. Composite samples will be taken in order to reduce time and costs associated with processing multiple samples. Additionally, since each wetland site is the basic replicate, no additional statistical information would be gained by keeping samples separate (Rader and Shiozawa 2001). Each composite sample will be comprised of two sweep-netting efforts. Each effort will probably be comprised of five sweep samples in which the net is swept through the vegetated water column in a 180 degree arc, so that most of the aquatic community is sampled (surface water, water column, vegetation surfaces, top of the benthos). When sites are visited prior to sampling (in January and April 2007), we will practice the sampling methodology and determine how many sweep samples are needed to comprise a composite sample, as recommended by the ninth EPA module on methods for evaluating wetland condition (US EPA 2002b). We will aim to obtain at least 600 organisms in each composite sample. If five sweeps turns out to be an inappropriate number, we will adjust our methods accordingly. Nets will be rinsed out and clinging macroinvertebrates will be removed between each composite sample.

Sample location within wetland

Wetlands will be sampled in the shallow areas with emergent vegetation (shore to 0.5 m water depth), as this is the area that generally contains a high abundance and richness of macroinvertebrates (Driver 1977, Hanson and Swanson 1989, US EPA 2002b). The three sampling locations within the emergent vegetation zone of each wetland will be selected by placing a transect from North to South. Composite samples will be taken every 3 m along the transect. The dominant vegetation type(s) that corresponds to each composite sample will be recorded on the field data sheet.

Sample Collection

Samples will be taken with aquatic D-frame sweep nets with 500-micron mesh similar to the protocol used in Minnesota to collect macroinvertebrates in the emergent vegetation zone of depressional wetlands (US EPA 2003). The choice of this device was based on the recommendation of Batzer, Shurtleff and Rader (Batzer *et al.* 2001); they surveyed wetland macroinvertebrate researchers and concluded that the aquatic D-frame sweep net should become the sampler of choice for most bioassessment efforts that use wetland macroinvertebrates. In the past, Minnesota has used a combination of aquatic D-frame sweep nets and activity traps to monitor invertebrates in wetlands; recent studies on the importance of activity traps in adding taxa richness to invertebrate metrics led them to conclude that activity traps should not be used in future studies. In 2007, Minnesota will begin sampling protocols using only aquatic D-frame sweep nets (Mark Gernes, personal communication).

Preservation of samples

The contents of the aquatic D-frame sweep net will be put in sample jars (a 1-L plastic Nalge Nunc International straight-side wide-mouth jar), labeled and preserved in 95% ethanol. The ethanol will be decanted and replaced with 80% ethanol after 2 days to ensure sample preservation. This method is similar to that used by researchers in Minnesota (US EPA 2003), except that invertebrates will be separated from vegetation in the laboratory instead of the field (recommended by Batzer *et al.* 2001). Preliminary site visits in April 2007 will determine the most appropriate number of sweeps per sampling effort; we will aim to collect at least 600 organisms per composite sample.

Sampling period and scheme

The sampling period will be in late April and May, based on advice from local wetland and macroinvertebrate specialists and evidence from a volunteer study of Willamette Valley wetland macroinvertebrates (N. Engelfried, unpublished data). This index period is when we are likely to find invertebrates sufficiently developed to be identifiable, yet before the wetlands are heavily colonized by invertebrates from other water bodies, dry down or are choked by vegetation. All wetlands will be sampled between 9:00h and 13:00h, in order to minimize variation in invertebrate species active at different times during a 24 hour period.

Two wetlands will be sampled per day during a two week period. First we will sample our six paired wetlands (three least-impacted paired with three most-impacted), sampling one least-impaired and one most-impaired wetland each day, for three days. Then, we will sample the remaining seven least-impacted wetlands. Finally, we will re-sample one of the previously sampled least-impacted wetlands in order to obtain an estimate of our sampling precision. In total, ten least-impacted wetlands and three most-impacted wetlands will be sampled. The three most-impacted wetlands will be spatially and temporally paired with the three least-impacted wetlands. The sampling scheme will be as follows:

Week 1

Monday-least-impaired site 1 and **most-impaired** site 1 Tuesday- least-impaired site 2 and **most-impaired** site 2 Wednesday- least-impaired site 3 and **most-impaired** site 3 Thursday- least-impaired site 4 and least-impaired site 5 Friday- least-impaired site 6 and least-impaired site 7

Week 2

Monday- least-impaired site 8 and least-impaired site 9 Tuesday- least-impaired site 10 and duplicate sample from least-impaired site 3

With this sampling scheme, we are trying to establish a standard time period that minimizes variation caused by seasonal changes in community composition.

Site reconnaissance

Sites will be visited in January and April of 2007 to practice our sampling methodology and to ensure that the sites have not drastically changed since the Adamus study was conducted in 1999 and 2000 (Adamus 2001). We will be looking for changes that may have occurred that would indicate changes in the water regime or changes in the level of human impairment at each site. If this is found, we will reevaluate our choice of sites.

Other variables

There may be daily or weekly variation in weather conditions during our sampling period, which may influence water surface roughness or water temperature. To account for this potential source of variation, we will measure the air temperature and water temperature with a thermometer and record the data on our field data sheets. We will use a modified Beaufort scale to assess wind speed and we will record the categorical wind rating on our data sheets.

Sampling will take place regardless of inclement weather conditions.

Sample preservation and holding

Samples will be collected and labeled in the field by Sarina Jepsen and a field assistant. The labels will include the following information: sample location, sample number, date and time of collection, sample type, sampler's name, and method used to preserve sample. Samples will be preserved in ethanol and held at the Xerces Society office in Portland for a maximum of 21 days in an area that is not exposed to direct sunlight. Sarina Jepsen will deliver all samples to ABA laboratory in Corvallis, Oregon to be processed. After they are processed, samples will be archived for one year at ABA, Inc.

At ABA, Inc, samples will be separated from debris by water flotation, then sieved through 500 micron mesh. A random subsample of 300 organisms will be taken from each composite sample, as recommended by Batzer *et al.* (2001). Samples will be searched for large, rare, adult and mature larvae as well. Chironomid midges will be identified to genus or, if possible, species group. Oligochaete worms will be identified to class. All other organisms will be identified to the lowest practical taxonomic level.

The Xerces Society **Standard Operating Procedures**

Analysis of macroinvertebrate data from Willamette Valley Riverine-Impounding wetlands

Summary

We plan to sample macroinvertebrates in 13 Willamette Valley Riverine-Impounding wetlands. Our study sites will include ten wetlands that have been previously characterized as least-impacted by human activity and three wetlands that have been previously characterized as most-impacted by human activity (Adamus 1999). One least-impacted wetland will be sampled a second time to gain an estimate of our sampling precision. The three most-impacted wetlands will be paired spatially and temporally with three of the least-impacted wetlands. Three composite samples will be taken from one strata of each wetland using aquatic D-frame sweep nets. Our critical measurements will be taxa richness, taxa diversity, taxonomic composition, and relative abundance. We will evaluate the responses of a variety of metrics in least-impacted and most-impacted wetlands to identify metrics that may eventually be used in an Index of Biological Integrity (Karr and Chu 1999) for macroinvertebrates in Riverine Impounding wetlands. This data will be useful in establishing a 'reference' condition for this particular subclass of wetlands in the Northwest.

Our goal is to produce data of sufficient quality to address these project objectives:

- 1. Characterize the macroinvertebrate community in Willamette Valley Riverine Impounding wetlands.
- 2. Measure the variability in the invertebrate communities among ten least-impacted Riverine-Impounding Willamette Valley wetlands.
- 3. Look for invertebrate metrics that vary predictably between least-impacted and most-impacted Riverine-Impounding Willamette Valley wetlands.

Our study wetlands are highly variable in size (ranging from 0.3 acres to 77 acres), which could add a degree of variability to the study; a previous study showed that species richness and number of functional feeding groups increased as the size of a temporary pool increased (Ferrella and Bass 1995). We are still choosing to use wetlands of variable sizes in our study because this set of previously studied wetlands holds many other advantages. However, we will examine whether 'wetland acreage' or 'acreage of permanent water' influenced each of our invertebrate variables (richness, diversity, composition, relative abundance) by running an Analysis of Variance (ANOVA). Additionally, we have paired three least-impacted wetlands with three most-impacted wetlands based partially on size, which should help control for size variability.

Treatment of data from 10 least-impacted wetland sites

We will assess the following traits from each of the ten least-impacted wetlands: richness (number of taxa present at a site), taxonomic composition (identification of taxa present in a community), relative abundance (percent representation of various taxa), and diversity (Shannon's diversity index, H'). We will also look at tolerance (tolerance levels will be taken from genus-level tolerance values published for stream invertebrates by the US EPA, Green 1990, and from the database developed by Adamus and Gonyaw for tolerance values of wetland macroinvertebrates, 2001). Each of the above attributes can be assessed with a qualitative or semi-quantitative sampling methodology (i.e. aquatic D-frame sweep netting).

Treatment of data from paired sites

To examine overall differences between least-impacted and most-impacted sites, we will calculate community similarity between each pair of sites (i.e. calculate similarity between least-impacted site 1 and most-impacted site 1, then between least-impacted site 2 and most-impacted site 2, etc.). We will identify abundant, common and rare taxa and examine qualitative differences between our least-impacted and most-impacted wetland sites.

To look for changes in individual metrics between least-impacted and most-impacted sites, we will create boxplots for each metric to show the means and variances of each attribute, following the approach by Mundahl and Simon (1999). Metrics that are most different between most-impacted and least-impacted sites will be retained; paired *t*-tests will be used to examine whether significant differences exist for each metric between least-impacted and most-impacted sites. If we find numerous (>8) metrics that differ significantly between least-impacted and most-impacted sites, we will begin to develop a preliminary invertebrate Index of Biological Integrity (IBI, Karr and Chu 1999) for Willamette Valley Riverine Impounding wetlands. However, given our small sample size of least-impacted and most-impacted wetlands, it is unlikely that we will be able to fully develop an invertebrate IBI. We expect to identify only a few metrics that ultimately will contribute to the development of an invertebrate IBI.

Some of the metrics that we will examine, based on metrics that have been successfully used in wetland invertebrate IBIs in Minnesota and other regions of the United States, include:

Richness

Total # of genera

Taxonomic Composition

of Crustacea + Mollusca genera

of Crustacea genera

of Mollusca genera

of Odonata genera

of Chironomid genera

of Hirudinidae genera

of species of snails

of genera mayflies, caddisflies, presence of fingernail clams, dragonflies (ETSD metric)

Relative Abundance

- % Gastropoda of total abundance
- % Odonata of total abundance
- % Spheariidae of total abundance
- % Erpobdella of total abundance
- % top 3 dominant taxa of total abundance
- % Corixidae of Hemiptera

Diversity

Shannon's index of diversity (H')

Tolerance

of intolerant taxa

% intolerant taxa of total sample

Field data sheets will be used each time sampling is conducted. Data sheets will then be taken to the Xerces office, entered into an Excel spreadsheet, then stored in a three ring binder. Computer entries will be compared to field and lab data sheets to make sure that there are no mistakes. All calculations will be double-checked (i.e. Shannon's index calculations). Data will be arranged in scatter plots and examined visually for outliers; outliers will be excluded from the final analysis.