Project completion report to the Oregon Watershed Enhancement Board

DEVELOPING A FRAMEWORK FOR THE OREGON WETLAND MONITORING AND ASSESSMENT PROGRAM:

Developing an Invertebrate-Based Monitoring Tool to Assess the Biological Integrity of Pacific Northwest Freshwater Wetlands

Wigeon Marsh, Ankeny NWR, OR

Delta Ponds, Eugene OR

Submitted to: Kyle Abraham
Effectiveness Monitoring Coordinator
Oregon Watershed Enhancement Board
775 Summer St., Suite 360
Salem, OR 97301

Submitted by: Celeste Mazzacano
Staff Scientist / Aquatic Program Director
The Xerces Society for Invertebrate Conservation
4828 SE Hawthorne Blvd.
Portland, OR 97215

THE XERCES SOCIETY FOR INVERTEBRATE CONSERVATION

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Project background & summary

There are currently no consistent cost-effective monitoring tools to assess biological integrity of Pacific Northwest wetlands. This project worked towards developing an invertebrate-based biological assessment tool that can be used reliably across wetlands in the Willamette Valley to assess wetland quality, detect responses to anthropogenic stressors, and evaluate restoration success. Invertebrate assemblages are effective biological indicators for wetlands in other states, but these communities are poorly characterized in Northwest wetlands. We collected macroinvertebrates from HGM-riverine and HGM-flats wetlands in the Willamette Valley to increase understanding of wetland invertebrates, identify community attributes that vary predictably in response to human stressors at natural wetlands in each HGM class, assess differences between macroinvertebrate community attributes at natural wetlands versus restored sites, and develop a preliminary invertebrate-based biological assessment tool.

Work done in wetlands is rarely accompanied by assessment of the biological health of these vulnerable habitats. Bioassessment tools evaluate the underlying health of a body of water by measuring the condition of its biological communities. If water quality is impaired by human activities, the structure of these communities changes in response. Aquatic invertebrates are excellent bioindicators: they are an important part of the food web, are confined to water for most or all of their life cycle, exhibit a range of responses to human-induced stressors, and have a short generation time that allows changes in community structure to be detected rapidly. Detailed knowledge about wetland invertebrate communities is lacking, and invertebrate-based bioassessment tools are not available for Pacific Northwest wetlands.

Detailed monitoring of wetland invertebrate communities and basic water chemistry parameters was done at 50 freshwater wetlands in the Willamette Valley. These sites included natural and restored wetlands representing a gradient of human impact as reference sites. These 50 wetlands included re-sampling about 24 riverine sites sampled by Xerces in 2007 & 2008 at earlier points in this study. Voluntary wetland restoration projects funded through OWEB's grant program were also included in the sample group. We analyzed invertebrate community composition to identify attributes that varied reliably across a gradient of disturbance and could be used as indicators of wetland biological quality. Major findings from this study include:

- The macroinvertebrate sampling protocols and the Human Disturbance Assessment (HDA) rubric used in this study are robust, reliable, and consistent among different trained practitioners. The HDA score provides a relevant reflection of the level of human impairment at a site.

- Multiple years of sampling at 50 wetlands of differing human impairment levels, HGM classes, and ecological types has expanded our knowledge of wetland taxa in the Willamette Valley and enabled us to begin building a larger ecoregion-specific dataset that may be used for reference purposes in the future.

- The macroinvertebrate community at natural flats sites is overall more restricted and comprised of more tolerant groups compared to the community at natural riverine sites. This resulted in a different suite of potential indicator attributes being identified for flats and riverine wetlands.

- Restored sites did not differ significantly from natural sites in water chemistry parameters or invertebrate community composition. However, restored wetlands lacked the higher proportions of rare species seen among natural riverine sites.

- The similarity of community composition among sites within the same class or category compared to sites in different categories rendered it difficult to pinpoint indicator taxa whose presence or abundance differs significantly at different types of sites.

- Variation in macroinvertebrate community composition at the same site across consecutive years indicates a high level of dynamism, which may ultimately be too great to allow a stable invertebrate bioassessment tool to be implemented.

- Changes in wetland invertebrate community characteristics are most apparent among natural wetlands experiencing different levels of human disturbance, especially when comparing least-disturbed to most-disturbed.

- Highly disturbed wetlands in both the riverine and flats classes have a more restricted and more stable macroinvertebrate community.

- The following invertebrate community attributes differed significantly among all least-impaired and most-impaired natural riverine sites sampled: abundance; #, relative diversity, and relative abundance of highly tolerant taxa; # of non-insect taxa; # taxa and relative diversity of (Crustacea + Mollusca); # taxa ECOT (Ephemeroptera, Coleoptera, Odonata, Trichoptera); relative diversity of Crustacea; % *Chironomus* of total Chironomidae; # taxa of Coleoptera; and relative abundance of Sphaeriidae.

- The following invertebrate community attributes differed significantly among all least-impaired and most-impaired natural flats sites sampled: # of taxa, relative diversity, and relative abundance ETSD (Ephemeroptera, Trichoptera, Sphaeriidae, dragonflies); relative abundance of Sphaeriidae; relative diversity of collector/gatherers; relative abundance of *Chironomus*; and relative abundance of taxa in the tribe Chironomini.

Methods

Site selection

Fifty wetlands in the Willamette Valley ecoregion were sampled per year in 2009 and 2010, consisting of 33 riverine and 19 flats-type wetlands, as determined by the hydrogeomorphic

(HGM) classification system (Brinson 1993, Adamus 2001). Roughly half the sites were riverine wetlands sampled by Xerces during previous studies on wetland invertebrates in 2007 & 2008; these sites had been selected to represent a gradient of human impact levels, from most-impaired to least-impaired by anthropogenic activity. The remaining sites were chosen to add the flats category of wetlands to the study, and to incorporate restored (10 total) and enhanced (24 total) wetlands into the study in addition to natural sites (18 total). Additional site characteristics such as ease of accessibility and willingness of owners and/or land managers to allow access were also considered in final site selection. A complete list of sites along with the geographic coordinates of the macroinvertebrate sampling location and a map of all sites within the Willamette Valley can be found in Appendix A.

It should be noted that HGM category (riverine vs. flats) and/or management status (natural vs. enhanced or restored) category was re-assigned for several sites during the course of the study, based on changing information from early assessments conducted by DSL, a wetlands assessment meeting conducted prior to the 2009 field season with an EPA wetland specialist (Mary Kentula), the OWEB contractor conducting ORWAP assessments (Paul Adamus), and project partners, as well as data from ORWAP assessments (Adamus *et al*. 2009) conducted in 2009 and 2010. This complicated data analysis to some extent, as it was necessary to examine different HGM types and management classes separately to ascertain differences in macroinvertebrate community characteristics that could be used to generate biological site assessment tools.

Habitat Assessment

Determining the range of anthropogenic stressors currently operating at a given wetland is problematic, particularly in an area with such extensive agricultural and urban development as the Willamette Valley. Rapid wetland assessment techniques have been developed for Oregon (ORWAP; Adamus *et al*. 2009), but these require trained professionals with specialized knowledge, and take several hours to complete. To render basic wetland assessment more accessible to a variety of users, we implemented a wetland Human Disturbance Assessment (HDA) form, modified from a rubric developed by Gernes & Helgen for wetland assessment in Minnesota (*in* U.S. EPA 2002). HDA components also follow recommendations of Rader & Shiozawa (2001) in developing criteria for defining reference conditions. The HDA assesses five site aspects:

- Buffer landscape disturbance (land use within 50 ft/15 m of wetland)
- Immediate landscape influence (500 ft/150 m of surrounding land)
- Habitat alteration, immediate landscape (500 ft/150 m of surrounding land)
- Hydrologic alteration, immediate landscape (500 ft/150 m of surrounding land)
- Chemical & Sediment Inputs

Each aspect can be rated as Excellent (0 points), Moderate (5 points), Fair (10 points), or Poor (15 points). Each section is accompanied by a checklist to guide the user rating, allowing notation of elements such as road density; industrial, agricultural, or residential development; proportion of non-native plant species; logging, grazing, construction, foot traffic and vehicle use; dams or culverts; etc. The site HDA score is calculated by summing the rating for each section. Thus, an utterly pristine site would receive an overall score of 0, while a completely disturbed site would receive 75 points. Because the Chemical & Sediment Inputs section includes nutrient levels, final scores for each site were not calculated until water chemistry data were returned by the contracted lab (see *Environmental data* below). Study sites were ultimately grouped into three classes, based on HDA scores: class 1 (least-disturbed, HDA score = 0-22), intermediate disturbance (HDA score = 22.1-42), and most-disturbed (HDA = 42.1-75). The lowest HDA score received by any site in any year was 5, and the highest was 65 points. The complete HDA form is presented in Appendix B.

Environmental data

The location of the sampling site within each wetland was recorded using a Garmin Rino 120 GPS unit (NAD 83 datum). The sampling transect was also photographed to allow sampling to be conducted in the same place in the wetland each year. Prior to macroinvertebrate sampling, water quality measurements were taken adjacent to the sampling region, to avoid trampling or disturbing the region from which macroinvertebrates would be netted. All water chemistry measurements were taken between 7:00 and 11:30 am to minimize the effects of normal daily fluctuations in dissolved oxygen (DO) levels. Water temperature, conductivity (μS), and pH were measured using a Hach SensIon 156 multiparameter meter. Dissolved oxygen (mg/L) was measured using the Hach multiparameter meter in 2009, and a Hach Winkler titration kit in 2010. Calibration of the pH and conductivity probes was checked at the beginning of each sampling day.

Additional water samples were taken for off-site determination of total Kjeldahl nitrogen, total phosphorus, and chloride. Samples to be analyzed for nitrogen and phosphorus were placed in acid-washed 1-liter containers, and a separate sample for chloride determination was taken in a 250 mL container. All samples were immediately placed in a cooler, and refrigerated afterwards until being delivered within 14 days to Alexin Analytical Laboratory (Portland, OR) for analysis.

Macroinvertebrate sampling

Two teams consisting of two people each conducted sampling all sites within the first three weeks of May each year. This index period was used because it is late enough in the spring that most macroinvertebrates will be mature enough to identify to genus and species, while being early enough in the season that there is less risk of losing sampling sites to dry down. One to three sites each year were too dry to sample by May, but sampling at pre-selected back-up sites enabled us to sample a total of 50 wetlands each year.

Macroinvertebrates were sampled using a D-frame dip net with 500 μm mesh in the near-shore zone of emergent vegetation, in water 1.6 – 3.2 ft. (0.5 to 1 m) deep. Sampling transects were 24-30 ft. (7-9 m) long, and were delineated using three 4-foot cedar stakes driven into the substrate. The water depth at each stake was measured and recorded. Two composite dip net samples were taken at each site. Each composite sample consisted of three sets of 1-meter sweeps taken through the top 1-3 in. (2.5-7.5 cm) of the benthos and up through the water column on one side of each of three cedar stakes ("shore" side and "open water" side). Thus, each composite sample was comprised of nine individual 1-meter sweeps, three sweeps each on one "side" of each cedar stake.

The volume of sediment in the net bag after three consecutive sweeps was often excessive. Sample volume was reduced by submerging the bottom of the net bag in the water in a region of the wetland away from the sampling site, and stirring the contents with one hand while gently swirling and bouncing the net in the water. This also allowed large pieces of debris to be rinsed and removed, along with any captured amphibians and fish. All nine sweeps comprising a single composite sample were pooled in a bucket. Any remaining fish and amphibians were removed, and larger pieces of debris were rinsed and discarded. The pooled material was then poured through a sieve with 500 μm mesh, and rinsed further to remove sediment. All rinse water was poured through a 500 μm mesh sieve prior to use, to avoid accidentally introducing additional invertebrates into the sample. Sample material was transferred to 1-L Nalgene jars and 80% ethanol was added as a preservative. For maximum preservation, sample volume comprised no more than 75% of the jar, and samples that contained large amounts of filamentous algae comprised no more than 50% of the jar volume. At the end of each day, the ethanol in each sample was poured off and replaced with fresh 80% ethanol. All samples were delivered to the taxonomic lab (ABR, Inc., Forest Grove, OR) by June 1st of each year for identification. Each composite sample was randomly subsampled to a target count of 500 organisms; if a sample contained fewer than 500 organisms, the entire sample was picked, counted, and identified. For samples with more than 500 organisms total, "large and rare" invertebrates were also picked and identified after the target subsample was reached. Organisms were identified to the lowest taxonomic level possible, usually genus.

Statistical methods

This study spanned only two field seasons (2009 & 2010), but it represented a continuation of wetland bioassessment work done by Xerces in 2007 and 2008, and almost half of the wetlands sampled in the course of this study were also sampled by Xerces in the previous field seasons. By the end of the 2010 field season, 23 sites had been sampled for two consecutive years, 17 sites had been sampled across three years, and seven sites had been sampled across four years. An important consideration in developing a bioassessment tool is the degree of annual variation that occurs in the target biological community, as this will affect the reliability and predictive power of indicator taxa and/or community composition metrics. Therefore, to better examine the variation in macroinvertebrate community composition at the same site in different years, and to select indicator taxa or attributes with the best predictive power, macroinvertebrate community data from the 2009 and 2010 field seasons was considered separately as well as in combination with data from previous years of study.

The PRIMER v6 software package (Clarke & Gorley 2006) was used to examine invertebrate community structure. Data from 2009 & 2010 were examined together, and in combination with data from sites also sampled in 2007 and 2008. Resemblance matrices were created for sites using fourth-root transformed data (Bray–Curtis distance measure). Patterns in taxa aggregations at each site were examined using CLUSTER analysis and non-metric multidimensional scaling (MDS). SIMPER analysis was done in PRIMER to reveal similarity in community composition within sites of the same category, dissimilarity in community composition between different categories of sites, and the relative contribution of each taxon to observed similarities or differences. Geometric class plots were drawn in PRIMER to investigate species abundance distributions among different wetland categories. This is a recommended method for detecting pollution-induced changes in sensitive species in benthic communities (Gray & Pearson 1982). The plots show the number of species represented by only 1 individual in the sample set (geometric class 1), 2-3 individuals (geometric class 2), 4-7 individuals (geometric class 3), 8-15 individuals (geometric class 4), etc.

Linear regression analysis was done in Excel to assess the relationship between selected invertebrate community attributes and site disturbance levels. Data from 2009 and 2010 were analyzed separately, as a pooled dataset, and in conjunction with site data from 2007 and 2008, where possible. Community attributes were plotted against individual site HDA scores and the R^2 value was determined. The same attributes were also plotted against disturbance class (class 1= least-disturbed, class 2 = intermediate disturbance, class 3 = most-disturbed) and ORWAP stressor score. Unpaired t-tests were done to assess whether attribute mean values differed significantly ($P < 0.05$) between class 1 and class 3 natural wetland sites. Table 1 lists all invertebrate community attributes calculated for each site.

Our goal was to identify community characteristics and/or taxa that differed substantially and reliably between: 1. natural wetlands experiencing different levels of human disturbance, especially class 1 vs. class 3; and 2. wetlands experiencing different management techniques, especially natural vs. restored.

Table 1. Wetland invertebrate community attributes, 2009 & 2010

abundance	richness (total # of taxa)
# of highly tolerant taxa (MHBI 8-10)[a]	# of predator taxa
% diversity highly tolerant (MHBI 8-10) [a]	% diversity predator
% abundance highly tolerant (MHBI 8-10) [a]	% abundance predator
# genera in Chironomini	# genera Gastropoda
% diversity Chironomini	% diversity Gastropoda
% abundance Chironomini	% abundance Gastropoda
# taxa Tanytarsini	# taxa collector/gatherers
% div Tanytarsini of Chironomidae	% diversity collector/gatherers
% abundance Tanytarsini of Chironomidae	% abundance collector/gatherers
# taxa ETSD[b]	% diversity ET[c]
% diversity ETSD[b]	# taxa ET[c]
% abundance ETSD[b]	% abundance ET[c]
# taxa Coleoptera	# taxa Orthocladiinae
% diversity Coleoptera	% diversity Orthocladiinae
% abundance Coleoptera	% abundance Orthocladiinae
# taxa Chironomidae	% abundance top dominant taxon
% abundance Chironomus	% abundance top 3 dominant taxa
% Chironomus of total Chironomidae	% abundance mites
MHBI [a], unweighted mean	# of non-insect taxa
MHBI [a], weighted mean	# rare taxa (<1% abundance at site)
# taxa (Crustacea + Mollusca)	% diversity rare taxa (<1% abundance at site)
% diversity (Crustacea + Mollusca)	% diversity Crustacea
% abundance (Crustacea + Mollusca)	% abundance Crustacea
# taxa sensitive (MHBI 1-4) [a]	% abundance microcrustacea
% diversity sensitive (MHBI 1-4) [a]	% abundance *Caecidotea*
% abundance sensitive (MHBI 1-4) [a]	% abundance (Amphipoda + Isopoda)
% abundance Sphaeriidae	% abundance (Amphipoda + Isopoda) of total Crustacea

[a] MHBI = modified Hilsenhoff Biotic Index
[b] ETSD = Ephemeroptera, Trichoptera, Sphaeriidae, dragonflies
[c] ET = Ephemeroptera, Trichoptera

Results & Discussion

Consistency and reproducibility

Because one goal of this project was to create a biological wetland assessment tool accessible to a variety of users, it was important for us to determine the consistency and reliability of both the macroinvertebrate sampling technique and the HDA assessment form. Even with standardized protocols and trained users, practitioner-related differences are a matter of concern in bioassessment studies, and we were interested in examining the robustness of our technique. To determine the consistency of our invertebrate sampling technique, duplicate samples were taken at two sites each year. In each year, hierarchical cluster analysis of site assemblages (CLUSTER routine, PRIMER v6) showed the greatest degree of similarity between duplicate sample pairs, indicating reliability in sampling technique among the three different team leaders and four different teams involved in the course of this study. Overall, the sampling protocol was very consistent among users.

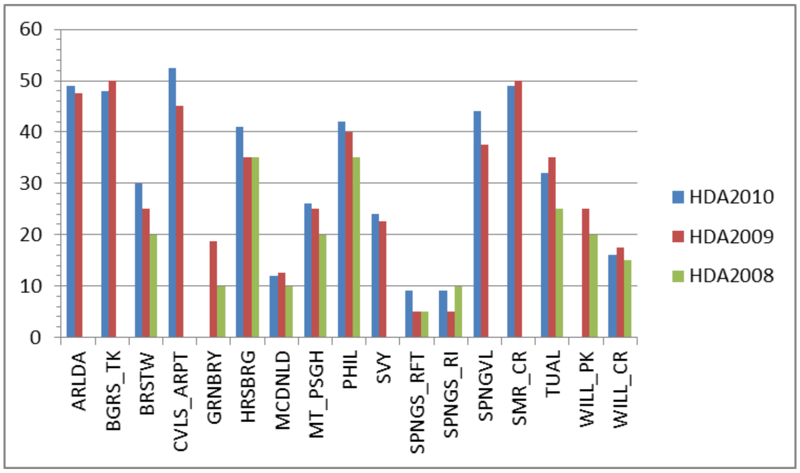
Development of a bioassessment tool requires identifying biological community attributes that change predictably along a gradient of human impairment. Therefore, a critical part of this study was the performance of the simple rubric used to assess human-induced site disturbance. The Human Disturbance Assessment (HDA) form was designed to be accessible to users with little experience in wetland assessment, and may be subject to a degree of subjective judgment by different users. We wanted to assess the reliability of the HDA score in reflecting the degree of anthropogenic impact at a site, and to determine the consistency of site HDA scoring among different users of the rubric.

HDA assessment was implemented at existing sites (25 riverine wetlands) in 2008, and it was conducted at all sites in 2009 and 2010. About half of the sites each year were sampled by a different trained sampling team, and HDA assessment was done on each site each year at sampling time. Linear regression analysis showed a positive correlation between site HDA score and the more comprehensive ORWAP stressor score ($R^2 = 0.4734$ for all natural sites sampled in 2010; $R^2 = 0.428$ across all sites sampled); this relationship was slightly stronger for riverine vs. flats wetlands ($R^2 = 0.5939$ for all riverine natural sites; $R^2 = 0.4787$ for all flat natural sites). A similar relationship was seen for site HDA scores in 2009, although less strong for natural flat sites ($R^2 = 0.3424$ across all sites; $R^2 = 0.5585$ for natural riverine sites; $R^2 = 0.2822$ for all natural flat sites). This consistent correlation with the much more detailed stressor score, which is comprised of a wide range of different site aspects evaluated during ORWAP assessment, indicates that raw HDA score and the associated scaled HDA classes (least-impaired (1), intermediate-impaired (2), or most-impaired (3)) provide a realistic reflection of the level of anthropogenic impact at a site.

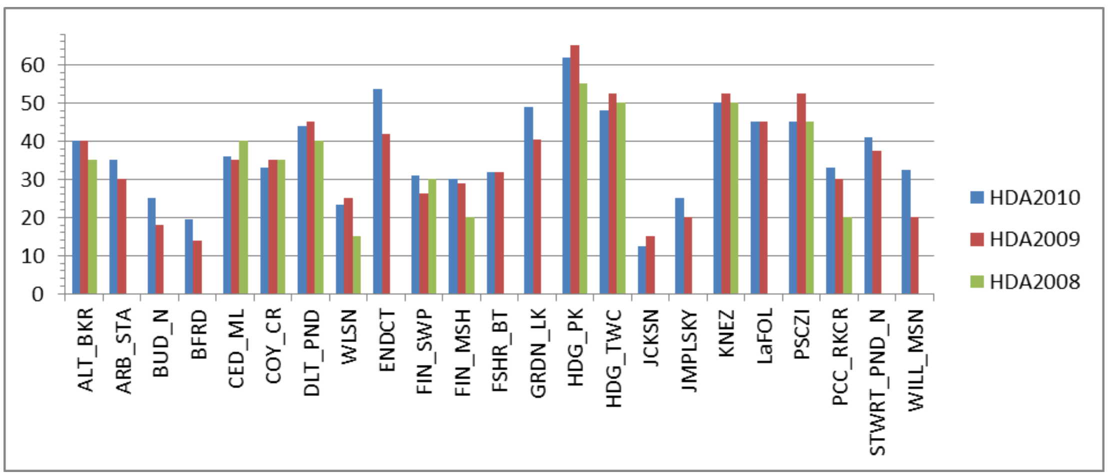
For sites that were sampled across multiple years, the overall HDA score was generally not identical for the same site in different years. However, the magnitude of the change was small enough that the overall classification of a site as least-impaired, intermediate-impaired, or most-impaired changed for only four sites, which were at the upper or lower score limit for a given impairment class. These results indicate that the parameters of the HDA assessment process are laid out clearly enough that consistent results regarding site impairment level can be obtained by different trained users.

Figure 1. Annual variation in HDA rubric scoring. See Appendix A for site abbreviations.

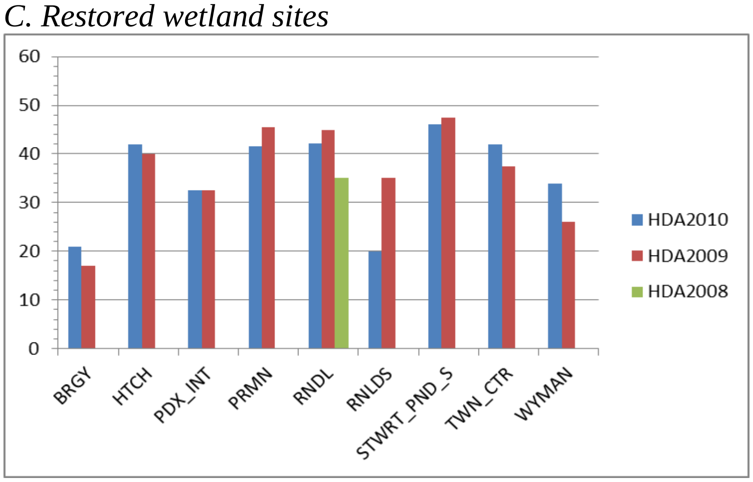
A. Natural wetland sites



B. Enhanced wetland sites



C. Restored wetland sites



Wetland macroinvertebrate taxa

Additional taxa were collected among the complete set of sampling sites in each year this project was conducted. The 2008 dataset contained 169 wetland macroinvertebrate taxa collected among 25 riverine sites, expanding the list considerably from 2007, when 92 taxa were found among 11 riverine sites. The complete taxa list, comprised of organisms sampled at 50 wetlands in both the riverine and flats HGM categories, increased to 231 taxa following the 2009 and 2010 field seasons. This increase was due both to sampling at greater number of additional types of wetlands, as well as the fact that the taxonomic lab that performed specimen identification in 2009 and 2010 identified many groups to genus or species level that had been left at family or genus in previous years, including aquatic mites and multiple families of aquatic beetles. The complete 2007-2010 wetland macroinvertebrate taxa list is presented in Appendix C.

The number of unique taxa per site among all riverine wetlands ranged from 17 to 51 in 2010 (mean richness = 30.3 $\pm$ 7.76), and from 12 to 42 in 2009 (mean richness = 28.6 $\pm$ 6.96). The most abundant, ubiquitous taxa among all riverine sites were common and tolerant groups including nematodes, oligochaete worms, chironomid midges, fingernail clams, snails, microcrustacea, and crustaceans (mainly scuds and aquatic sowbugs). Corixid bugs and ceratopogonid midges were also common among riverine sites.

The number of unique taxa per site at all flats wetlands was slightly lower than for riverine sites, ranging from 10 to 43 in 2010 (mean richness = 27.5 $\pm$ 7.97), and from 12 to 40 in 2009 (mean richness = 25.9 $\pm$ 8.61). Many of the common taxa groups at riverine sites were also among the most common at flats sites, including oligochaete worms, chironomid midges, snails, microcrustacea, and crustaceans. Other common groups at flats sites included corixid bugs and dytiscid beetles.

Eleven taxa in the complete taxa list were completely absent from all riverine sites in all years; of these, eight were represented by other genera in the same family (aeshnid dragonflies, planorbid snails, dytiscid beetles, haliplid beetles, and tipulid flies), and only three (Conchostraca <clam shrimp>, Microveliidae <short-legged water striders>, and Polycentropidae <trumpetnet caddisflies>) were completely unrepresented among all riverine sites. Flat sites had a much more restricted community, with 91 of the taxa found among all sites during all years absent from any flats site. Over half of these absent taxa (51) were represented by other genera in the same families (tipulid flies, chironomid midges, haliplid beetles); the remaining missing taxa were comprised largely of groups that require colder, faster-moving water, including all stoneflies, elmid beetles, and blackflies; and the majority of mayflies and caddisflies. This difference is not unexpected, as the nature of ephemeral flats wetlands is such that the water levels are generally lower, warmer, contain less dissolved oxygen, and are much more lentic compared to riverine wetlands. It is interesting to note that while the community at all flats sites included abundant numbers of multiple taxa of chironomid midges, 33 of the 66 genera of

Chironomidae found among all sites did not occur at any flats site; in contrast, none of the chironomid genera were lacking among all of the riverine sites in this study.

Environmental data

Basic water chemistry data were collected each year prior to sampling. Conductivity, pH, and DO (dissolved oxygen) were measured from 2007 through 2010; total Kjeldahl nitrogen (N), total phosphorus (P), and chloride (Cl) were measured in 2008-2010. Natural riverine and flats wetlands exhibited a similar range of values across all years for pH, P, Cl, conductivity, and DO (Table 2). The range for N levels was about three times higher among natural flats sites compared to natural riverine sites.

Values for all water chemistry parameters sampled varied substantially from year to year at the same site for both riverine and flats sites. For riverine wetlands, the magnitude of this change from year to year did not appear to be related to the level of human disturbance (HDA class) at the site; among flats sites, the magnitude of annual change in N and P levels was lowest at least-impaired (class 1) sites.

The observed variation across time at the same sites for water chemistry parameters may explain the overall lack of strong correlation between individual water chemistry parameters and site HDA scores. In 2009, higher N and P levels showed a moderate to weak correlation with increased impairment at natural riverine sites (R^2 = 0.294 and 0.2187, respectively). Data for the same sites in 2010 showed weak correlation with increased site impairment and higher levels of N (R^2 = 0.2169), P (R^2 = 0.107) and higher conductivity (R^2 = 0.1606). The water chemistry dataset among all years of sampling for natural riverine sites revealed a weak correlation between site impairment and higher levels of N (R^2 = 0.1773), P (R^2 = 0.1057), and higher conductivity (R^2 = 0.1196).

More and slightly stronger correlations between water chemistry and site impairment were seen for natural flats sites, which may be a reflection of the lower magnitude of annual variation among sites observed for some parameters. In 2009, higher N levels and increased conductivity showed a moderate to strong correlation with increased impairment at natural flats sites (R^2=0.4641 and 0.3797, respectively). In 2010, data from these same sites indicated correlation with increasing site impairment for higher pH (R^2=0.108), Cl (R^2=0.4848), N (R^2=0.1539), P (R^2=0.2502), conductivity (R^2= 0.9147) and DO (R^2=0.3389). When water chemistry data was for natural flats sites considered across all years, however, many of these relationships disappeared, and weaker correlations with increasing site impairment were seen only for conductivity (R^2=0.1778) and DO (R^2=0.2705).

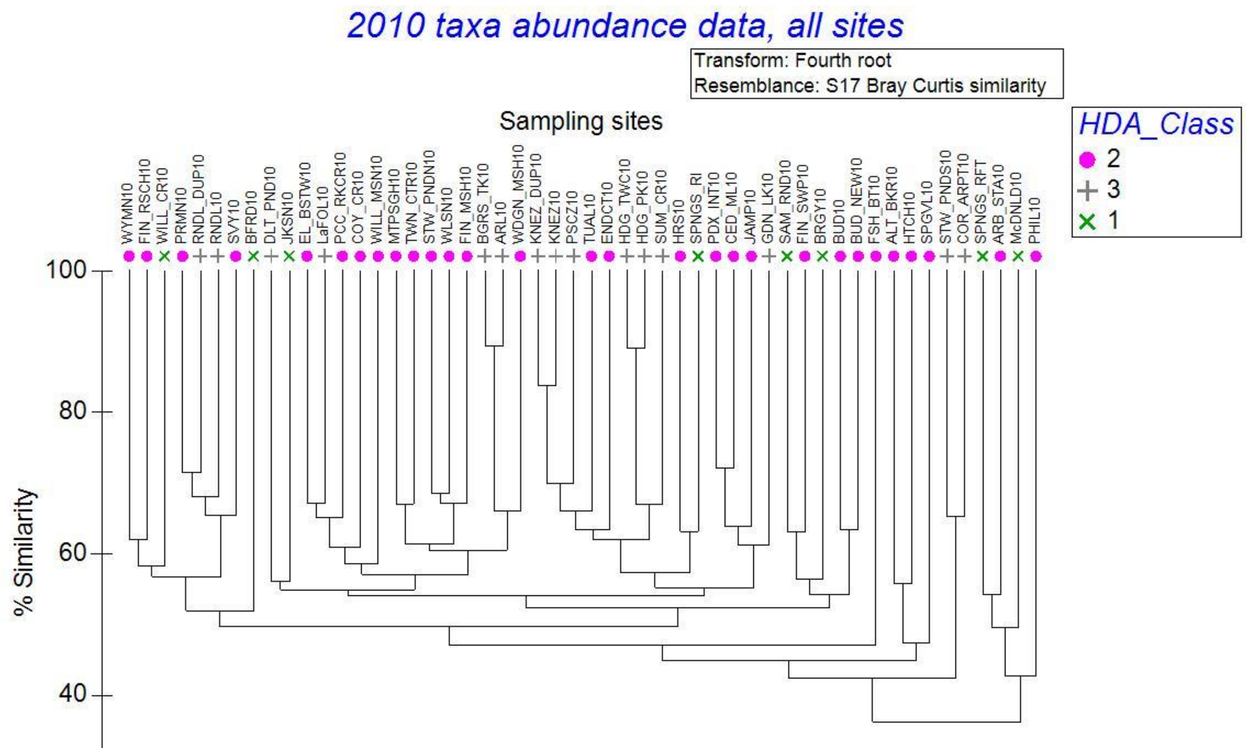
Water chemistry parameters among restored sites did not differ significantly from those seen at natural sites. T-tests comparing the mean water chemistry values at natural and restored wetlands for riverine and flats sites indicated no significant differences, with the single exception that total N values were significantly higher among restored riverine sites compared to natural riverine sites. See Appendix D for a table of water chemistry measures at natural and restored sites.

Macroinvertebrate community structure

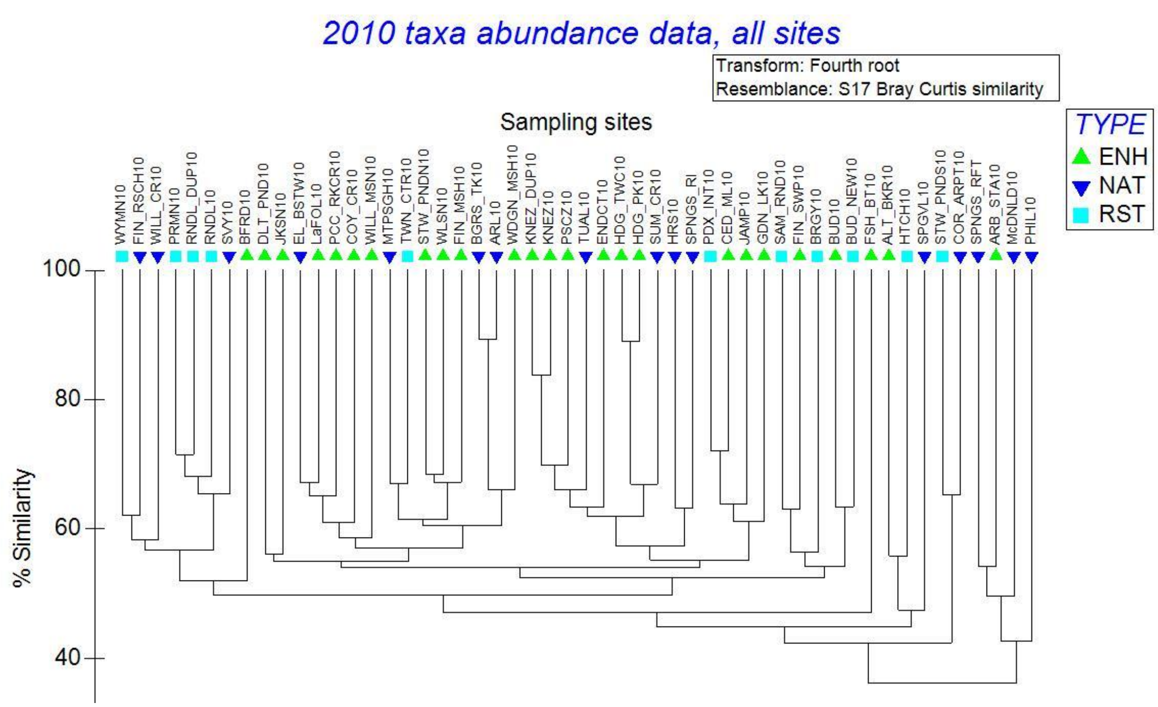
Patterns in community composition were examined in PRIMER using the CLUSTER and SIMPER routines and MDS ordination, with the goal of identifying taxa and/or taxa groups that merited further investigation for their utility in discriminating between most- vs. least-impaired sites, and/or restored vs. natural wetlands.

CLUSTER analyses run on Bray-Curtis similarity matrices of fourth-root transformed data revealed limited association among sites based on level of human disturbance (HDA class), ecological condition (natural, enhanced, or restored), or HGM class (flats vs. riverine). The most consistent similarity was seen in duplicate site samples, which always clustered together as a pair (see Fig. 2 for illustration). Most-disturbed sites (class 3) separated out most frequently into the same clades, although this same level of association was not observed among least-disturbed (class 1) sites (Fig. 2A). Limited and inconsistent association was observed among sites in different ecological classes, with a slight suggestion of clustering among restored wetland sites (Fig. 2B). Flats sites also appeared slightly more likely to cluster together (Fig. 2C). This pattern was true for the data from 2009 and 2010 considered separately and in combination, and for the entire pooled datset across all years of sampling (2007-2010).

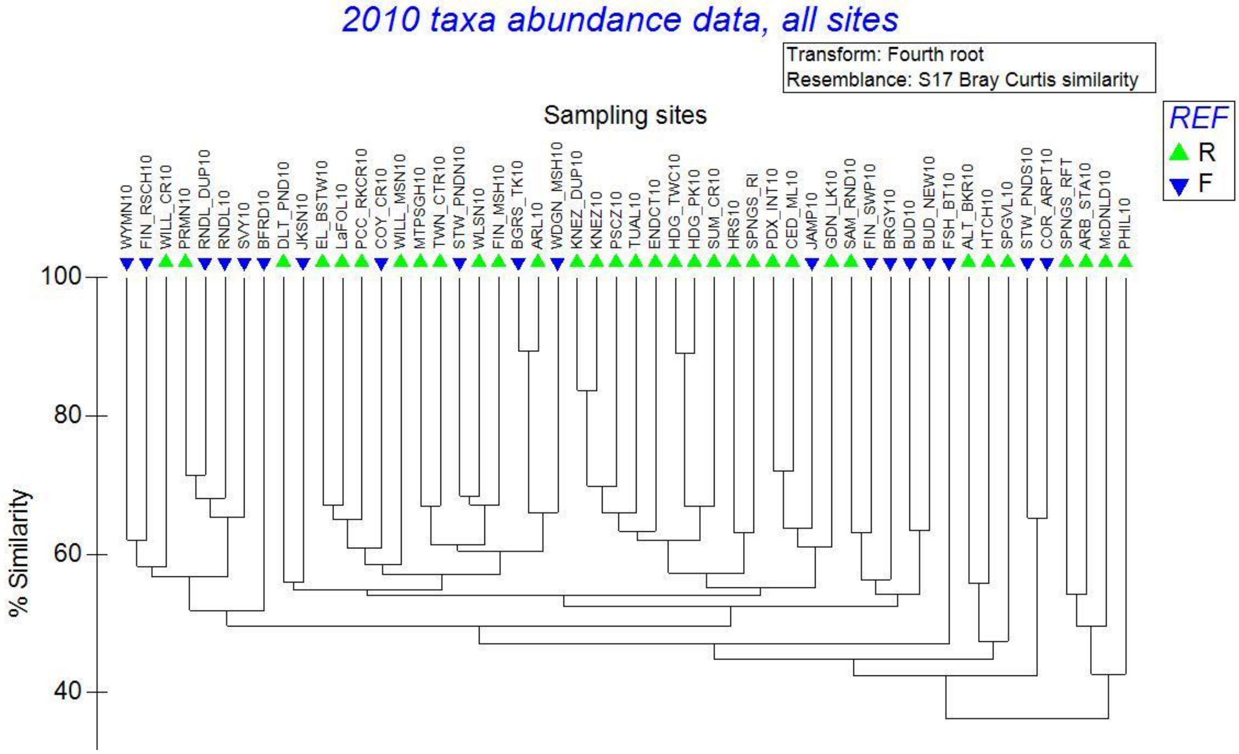
Figure 2. CLUSTER analysis of macroinvertebrate taxa for all sites sampled in 2010.
A. Analysis of sampling sites by human disturbance level. Class 1 = least-disturbed, class 2 = intermediate disturbance, class 3 = most disturbed.



B. Analysis of sampling sites by type (ENH = enhanced; NAT = natural; RST = restored)



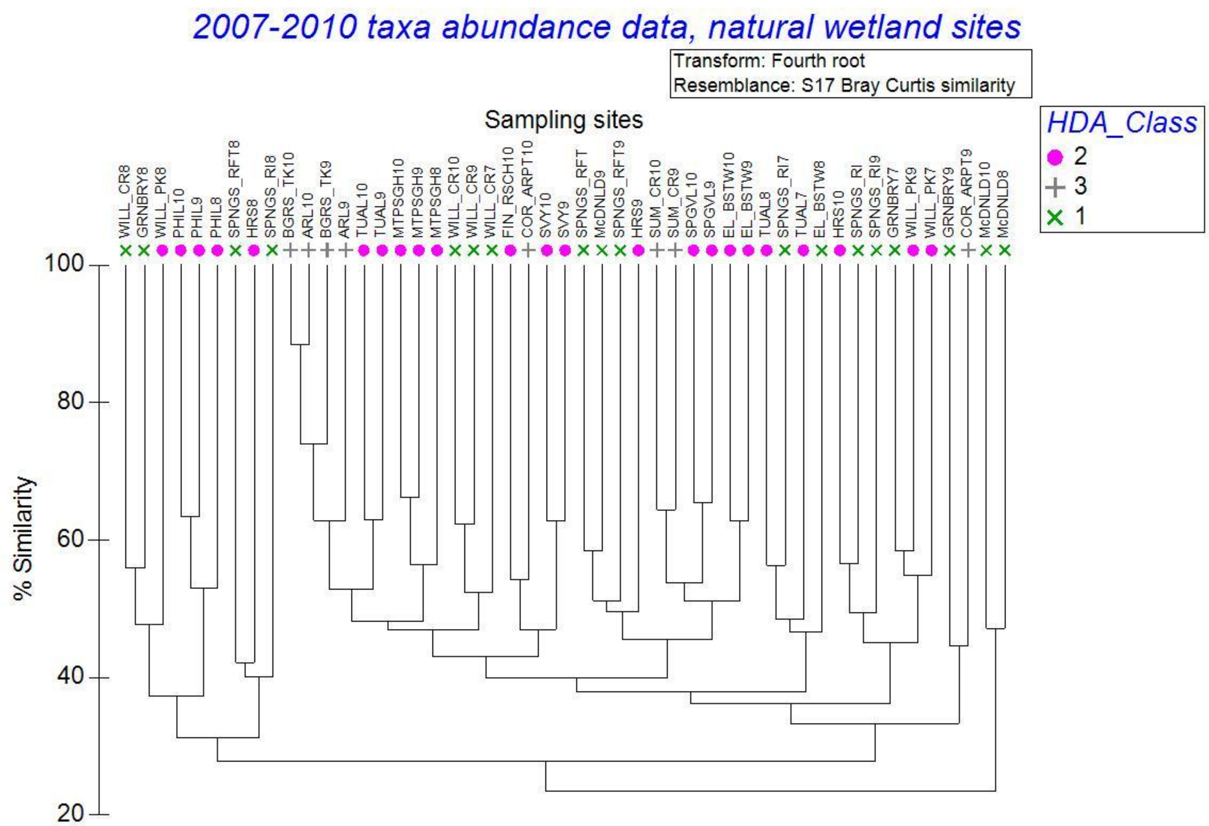
C. Analysis of sampling sites by HGM class (R = riverine, F = flats)



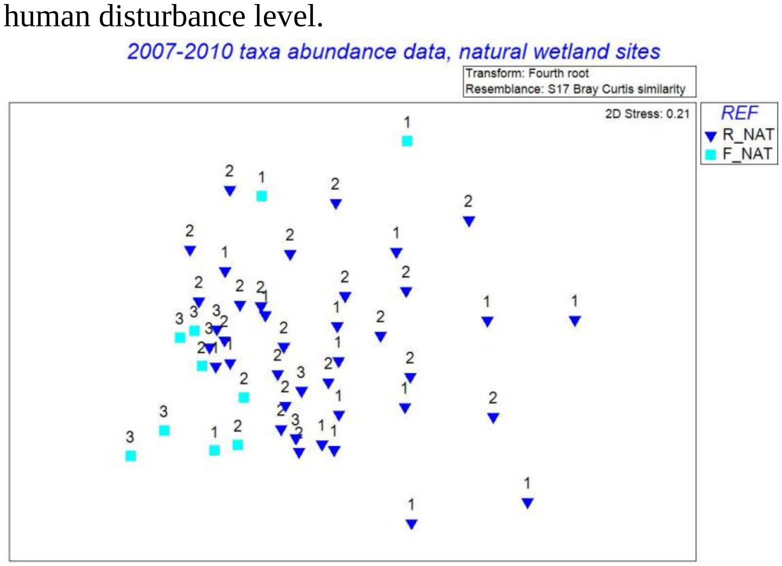
For the purposes of establishing a macroinvertebrate-based assessment tool, it is necessary to determine whether the macroinvertebrate community composition differs among sites according to a gradient of human disturbance. When the macroinvertebrate community was examined across all years among all natural sites, clustering among sites of different HDA classes became more apparent (Figure 3A), due in part to the fact that the datasets from the same site sampled across multiple years tended to segregate into the same clade for most of the natural sites. However, the macroinvertebrate community at each site sampled across multiple years differed enough annually that not all of the sampling years for a single site cluster together. Clustering based on HGM class proved less revealing, as there were far fewer natural flats than natural riverine sites (5 natural flats vs. 13 natural riverine), although MDS ordination of the same data suggested some association among flats sites, even those in different HDA classes, when compared to natural riverine sites (Figure 3B).

Figure 3. Analysis of macroinvertebrate taxa for all natural wetland sites (2007-2010). The numeral at the end of the site name abbreviation indicates sampling year.

A. Cluster analysis of natural wetland sampling sites. Numbers indicate disturbance level (class 1 = least-disturbed; class 2 = intermediate disturbance; class 3 = most-disturbed).



B. MDS ordination of natural flats and riverine wetland sampling sites. Numbers indicate human disturbance level.



SIMPER analysis of average community similarity of all sites across all years sampled supported the site separation based on HDA class. Although within-group similarity among the different classes was low overall, average similarity in invertebrate community composition increased from least-impaired (32.5% average similarity) through intermediate-impaired (37.1% average similarity) and most-impaired (44.5% average similarity) sites. The taxa that contributed the most to the observed average similarity within each HDA class were the same among all three classes: oligochaete worms, *Chironomus* midges, and copepods. The between-group dissimilarities were similar among all pairwise comparisons (class 1 vs. 2, class 2 vs. 3, class 1 vs. 3), ranging from 62.7% to 68% average dissimilarity, but was highest for class 1 vs. class 3. Each individual taxon contributed only a small percentage to the observed average dissimilarities (3-5%), but the taxa that contributed the most to differences between site classes were the microcrustacea (Cladocera, Copepoda, Ostracoda).

When natural riverine sites sampled across all years were considered separately, most-impaired (HDA class 3) sites had greater average abundance of *Orthocladius* (chironomid midge genus), *Crangonyx* (scud), and corixid bugs (water boatmen), while least-impaired (HDA class 1) sites had a greater average abundance of Ephemeroptera (mayflies) and non-dytiscid beetles (*i.e.* aquatic beetle taxa other than the predaceous diving beetle family). Among natural flats wetlands considered across all years, most-impaired (class 3) sites had greater average abundances of Culicidae (mosquitoes), *Pseudosmittia*, *Corynoneura*, and *Psectrocladius* (all three are chironomid midge genera), while least-impaired (HDA class 1) sites had five taxa groups that were completely absent from class 3 sites: Ephemeroptera, Sphaeriidae (fingernail clams), *Menetus* (planorbid snail genus), *Siphlonurus columbianus* (mayfly), and *Chaoborus* (phantom midge).

Community composition did not differ greatly between flat vs. riverine sites, or between natural vs. restored sites. For the dataset across all four years of sampling, restored sites had a higher within-group similarity (40% average similarity) than did natural sites (33% average similarity), while natural and restored sites had 68% average dissimilarity, due primarily to differences in mean abundance of microcrustacea, snails (both of which were more abundant at restored sites), and aquatic earthworms (more abundant at natural sites). When considered according to both HGM class and ecological type, restored riverine sites showed slightly higher within-group similarity than was seen among all natural riverine sites (39% vs. 33% average similarity); when the two site types were compared, natural riverine sites had a greater average abundance of mites and *Microspectra* (a chironomid midge genus), while restored riverine sites had a higher average abundance of Hirudinea (leeches). A similar trend was seen for sites in the flats class, with restored flats having a higher within-group similarity than natural flats sites (38% vs. 33% average similarity, respectively). Overall community composition differed little among different groups, but restored flats had a greater average abundance of odonates and corixid bugs, while natural flats had more non-chironomid Diptera.

Annual variation in macroinvertebrate communities

The overall level of variation in the macroinvertebrate community at the same site across multiple years was similar among all the sites examined as a group (mean community similarity across years for all sites = 56.3% ± 9.4). This mean was virtually identical when the average similarity across years for all sites was examined in relationship to different HGM classes or ecological type. In addition, the mean % similarity across years in community composition was virtually identical for riverine vs. flats wetlands, and for natural vs. enhanced. However, the mean % similarity across years in community composition differed significantly when sites were examined according to HDA class ($p = 0.0282$), with most-disturbed sites (class 3) exhibiting a greater % similarity in community composition from year to year (mean similarity = 59.5% ± 9.4) than least-disturbed sites (class 1; mean similarity = 50.0% ± 8.0).

The same relationship was observed when natural wetlands were considered as a separate group. Among all natural riverine sites sampled across multiple years, the mean % change annually in community composition was the same as that seen for all sites together (56.6% ± 10.8). However, when the mean % similarity in community composition was examined for natural riverine sites in different HDA classes, the mean annual % similarity at least-disturbed sites was significantly lower than that seen at most-disturbed sites (class 1 mean community similarity across years = 47.4% ± 7.7; class 3 mean = 69.3% ± 4.7; $p = 0.0395$). Similar calculations were not possible for natural flats sites sampled across multiple years, as this subset consisted of only a single least-disturbed and two most-disturbed sites.

Restored sites examined according to level of human disturbance not did exhibit the same difference in mean community similarity across years. The mean similarity across years for all restored sites was again similar to that seen for all sites in general (57.7% ± 8.7), and while the mean annual community similarity was greater at most-disturbed sites (61.1% ± 11.1) vs. least-disturbed (55.0% ± 2.8), this difference was not significant ($p = 0.5270$).

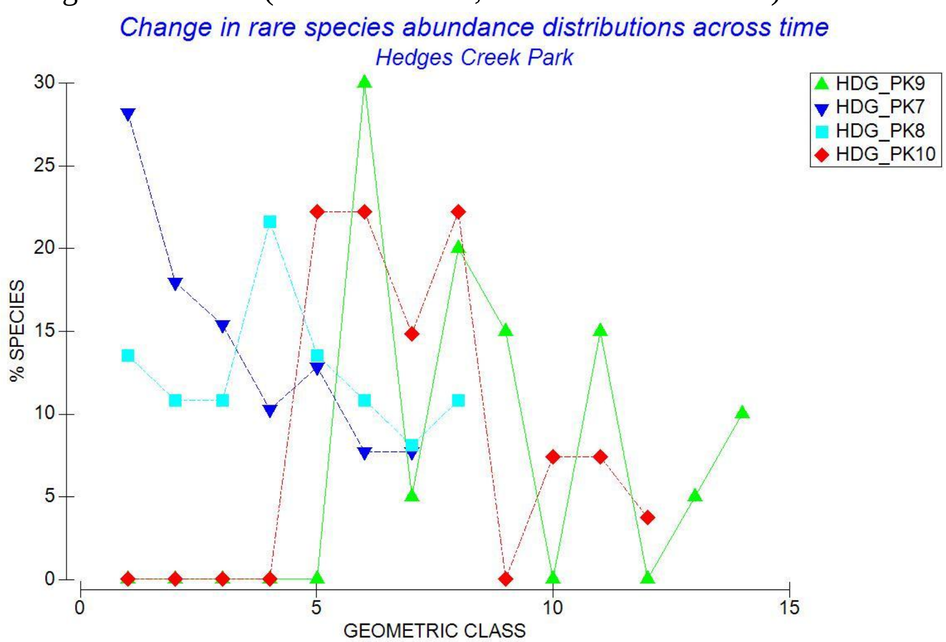
Geometric class plots

The higher degree of community similarity from year to year at HDA class 3 sites described above suggests that these highly disturbed wetlands support a more restricted macroinvertebrate community, one likely more tolerant of disturbance and/or pollution. The higher degree of change annually at class 1 sites, combined with the lower degree of community similarity overall among class 1 as compared to class 3 sites, suggested that least-disturbed sites may support a greater diversity of rare species. Because taxa that contributed the most to observed within-site similarity among class 1 sites were common and abundant, it is likely that the rare species these sites support occur at low abundance, and the rare species present may change from year to year. We investigated this further using geometric class plots drawn in PRIMER, to show species abundance distributions among different wetland categories (i.e. HDA class, natural vs. restored, and HGM class).

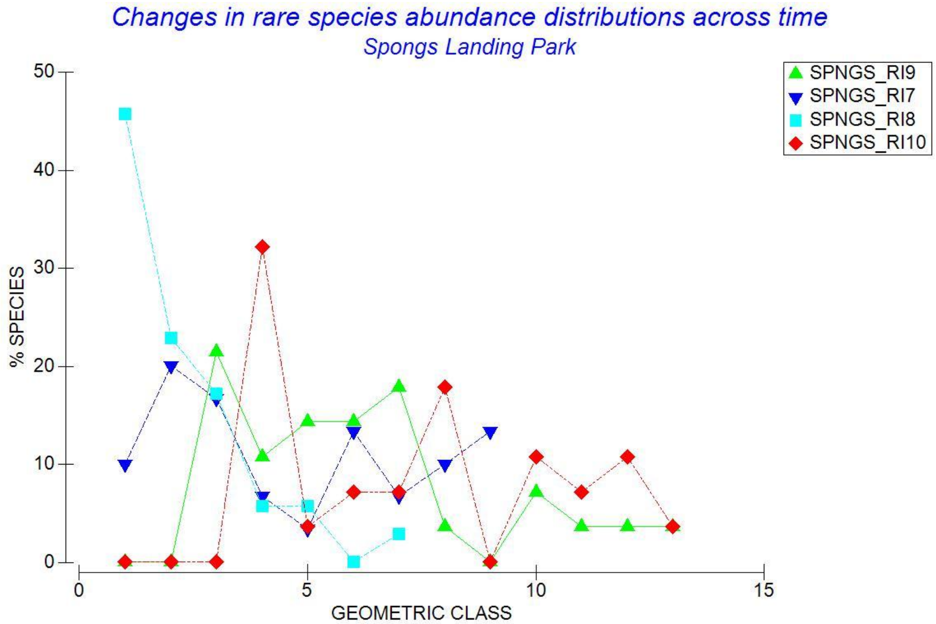
Geometric class plots of individual sites sampled across multiple years reflected the annual changes in community composition seen using SIMPER analysis, with wide variation each year in the proportion of species represented by fewer than 15 individuals (geometric classes 1-4). This change in the distribution of geometric abundance classes from year to year at the same site appears to be true at both least-disturbed and most-disturbed sites (Figure 4). Multiple sites that had been sampled across several years were examined and the same pattern was seen; the graphs in Figure 4 are shown as illustrations. These plots also reflect the greater similarity between macroinvertebrate community composition in 2007-2008 compared to 2009-2010, as the curves for 2007 and 2008 follow each other more closely than the curves for the later years, while the curves for 2009 and 2010 follow a similar pattern together.

Figure 4. Annual within-site variation in rare species distribution among sampling sites. Plots show the proportion of total species at the sites represented by 1 individual in the sample (geometric class 1), 2-3 individuals (geometric class 2), 4-7 individuals (geometric class 3), 8-15 individuals (geometric class 4), etc.

A. Hedges Creek Park (most-disturbed, enhanced riverine site)



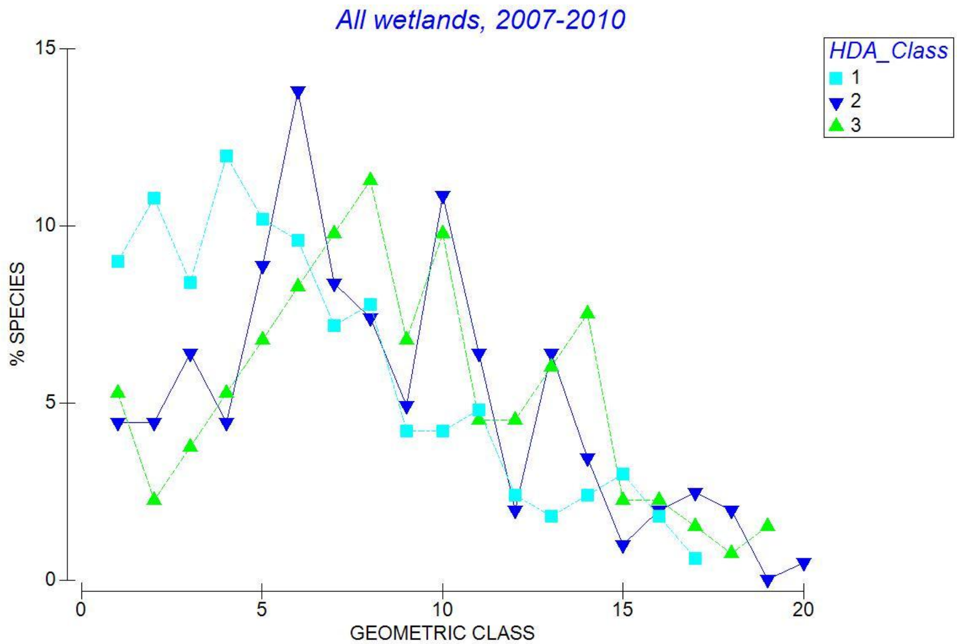
B. Spongs Landing Park, riverine-impounding (a least-disturbed, natural riverine site)



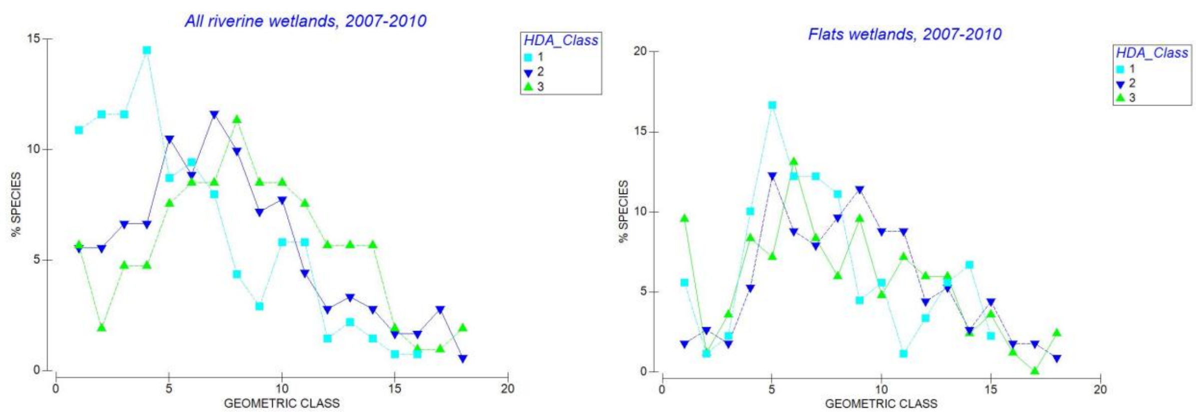
Analysis of all sites sampled across all years showed a greater proportion of species in low geometric classes for least-impaired sites compared to most-impaired sites (Figure 5A). This same pattern was seen when all riverine sites were examined as a group, with class 1 sites exhibiting greater proportions of rare species; however, little to no relationship between rare species abundance and HDA class was observed among all flats sites sampled, and the geometric abundance plot curves were similar for all three HDA classes (Figure 5B).

Figure 5. Difference in rare species abundance distributions among wetlands experiencing different levels of human disturbance. Class 1 = least-disturbed; class 2 = intermediate disturbance; class 3 = most-disturbed.

A. All sites, all years (2007-2010)



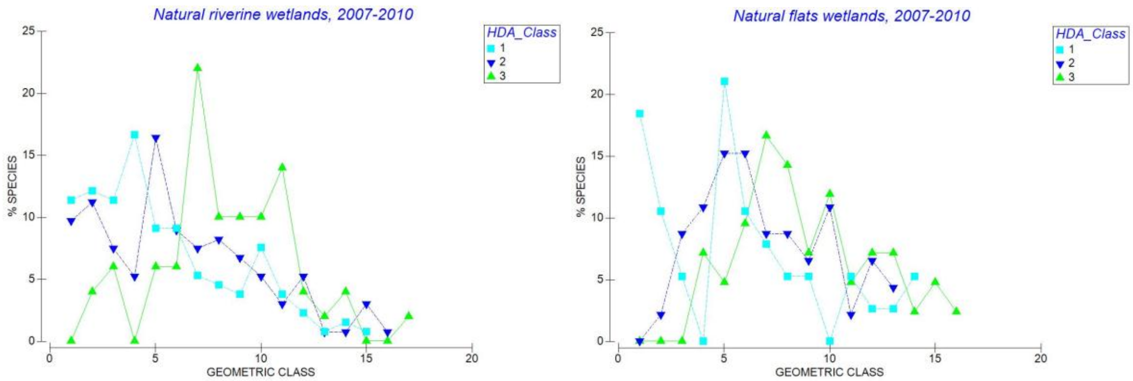
B. Riverine vs. flats sites



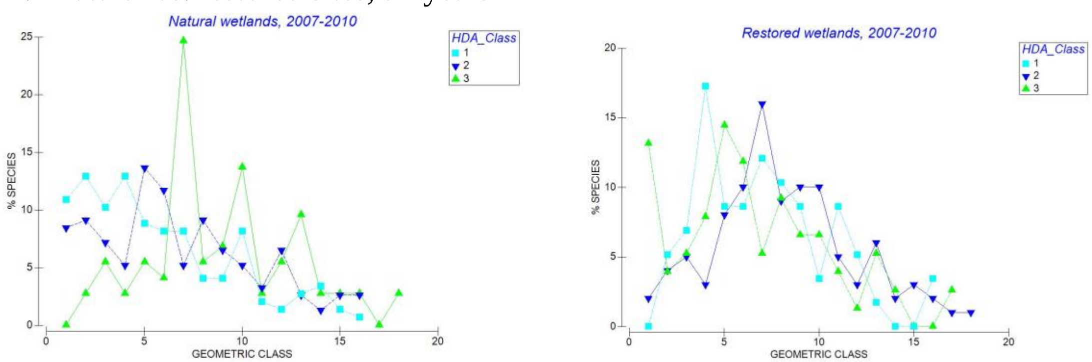
To investigate whether the reduced proportions of rare species among flats wetlands was related to ecological type, we examined rare species distributions for both natural riverine and natural flats sites (Figure 6A), and compared rare species distributions between natural and restored sites (Figure 6B). Higher proportions of rare species were present among least-disturbed sites for both natural riverine and flats wetlands, while rare species were either absent or present at a much lower proportion of the total species among most-disturbed sites. The situation was quite different among restored wetlands, however, with most-disturbed sites exhibiting the highest proportion of species in the lowest abundance classes.

Figure 6. Relationship between rare species abundance and human disturbance levels among natural and restored wetlands.

A. Natural riverine and natural flats sites, all years



B. Natural vs. restored sites, all years



Regression analysis of macroinvertebrate community attributes

To develop an invertebrate-based bioassessment toolI, it is necessary to identify macroinvertebrate community attributes that vary consistently and predictably at reference wetlands according to a gradient of human disturbance. Community attributes used as biotic index metrics generally represent different categories including taxonomic richness, taxonomic composition, tolerance/intolerance, and functional feeding group (Barbour *et al*. 1999). The choice of macroinvertebrate community attributes examined in this study (Table 1) was guided by past work done to develop wetland and lake invertebrate IBIs in other states, including New Jersey (Blocksum *et al*. 2002), Michigan (Burton *et al*. 1999, Uzarski *et al*. 2004), and Minnesota (Galatowitsh *et al*. 1999, Helgen & Gernes 2001), and analyses (described above) of wetland invertebrate community data collected by Xerces in 2007-2008 as well as in 2009-2010. Wetlands designated as "natural" served as reference sites throughout the study.

Despite the differences in community characteristics suggested by PRIMER analysis of taxa abundance data, such as taxa contributing to differences between different wetland categories, and differences of rare taxa abundances, strong correlations between wetland site disturbance

(HDA) score and invertebrate community attributes were generally absent to weak; R^2 values for attributes that showed correlation ranged only from ~0.1 to 0.4. In addition, attributes that correlated with site disturbance among sites from one year of sampling data frequently showed no correlation with data from a different year. The attributes that exhibited consistent correlation with impairment gradient across consecutive years at both riverine and flats reference wetlands included % abundance of highly tolerant taxa, # of predator taxa, and # of Coleoptera (beetle) taxa. Attributes that exhibited consistent correlation with impairment gradient across consecutive years in only one HGM class of reference sites included % diversity of highly tolerant taxa (riverine); % abundance Gastropoda (flats). However, the strength of correlation for all of these attributes was low to moderate, based on R^2 values.

Regression analysis conducted on all natural riverine and natural flats wetlands across all years of sampling revealed almost no consistent relationships between community attributes and site impairment gradient among riverine sites. R^2 >0.1 was seen for only a single community attribute (abundance), and the strength of the correlation was very weak (R^2 = 0.1072). Total nitrogen, phosphorus, and conductivity also showed a weak correlation with site impairment, and were higher at more impaired sites (R^2 = 0.1773, 0.1057, and 0.1196, respectively). In contrast, many more and much stronger correlations were seen for attribute values among natural flats sites, with 28 macroinvertebrate community attributes varying with site impairment gradient, at R^2 values ranging from 0.1164 to 0.5618. Conductivity also showed a weak correlation with site impairment gradient, and was higher at more impaired sites (R^2 = 0.1778).

An important caveat should be considered when comparing the differences in the results of regression analysis across years for natural riverine versus natural flats sites. Due to unanticipated difficulties in finding true natural flats wetlands representing a range of human impairment levels in the Willamette Valley, only about one-third as many natural flats sites were sampled compared to natural riverine sites. In addition, Xerces' pilot studies in wetland bioassessment were conducted initially in riverine wetlands (2007-2008), and the flats category was added only as the study expanded in 2009-2010. As a result, almost all of the natural riverine wetlands in this study were sampled across three or four consecutive years, while each flat site was sampled across one or two years. We noted earlier that for sites sampled across multiple years, macroinvertebrate community composition is most similar between 2007 & 2008, and between 2009 & 2010. Most natural riverine wetlands were sampled across a longer period of time, and thus the calculated community attributes will be more affected by annual within-site variation. Most natural flat sites were sampled only in 2009 and 2010, and the results of regression analysis do not reflect the level of within-site community variation that is likely to occur across a longer span.

One explanation for the overall poor discriminatory ability of regression analysis lies in the extremely high levels of variation seen among all reference sites experiencing intermediate levels of disturbance (class 2). This variation is not unexpected and may be explained by the intermediate disturbance hypothesis (Connell 1978), which suggests that taxa diversity is greater in systems that experience a moderate level of disturbance than in systems with high or low degrees of disturbance. It is possible that while most-disturbed sites experience a level of stress such that more sensitive or intolerant taxa can no longer survive and perhaps do not attempt to colonize, sites experiencing intermediate disturbance may have differential survival of different taxa groups, opening up new niches repeatedly to colonization. PRIMER analyses described above revealed that class 3 sites had the highest degree of internal community similarity, and that differences in community composition were most pronounced between class 1 and class 3 sites. Therefore, we examined whether there was a significant difference between the mean values of attributes for class 1 and class 3 reference wetlands across all years sampled (2007-2010, but note that some reference sites were only sampled for a subset of that period). Due to the observed differences in invertebrate community composition, we examined riverine and flats reference wetlands separately (Table 3).

Among natural riverine wetlands, 12 community attributes were significantly different between class 1 and class 3 sites, although three were redundant as they measure aspects of the same group (highly tolerant taxa). Two of these 12 attributes (# of highly tolerant taxa, # of non-insect taxa) were among those selected as potential IBI metrics following analysis of the 2007-2008 dataset. Seven attributes were found to be significantly different among most-impaired and least-impaired natural flats sites, three of which were redundant (all measuring aspects of ETSD taxa). Five of these seven attributes also correlated with site impairment gradient in regression analysis, and one was among those selected as potential IBI metrics based on the 2007-2008 data (% diversity collector/gatherers). Only one attribute differed significantly between most- and least-impaired sites among both natural riverine and flats wetlands (% abundance Sphaeriidae).

Table 3. Significant differences in means of macroinvertebrate community attributes between least- and most-impaired natural wetlands (*p value <0.05; **p value between 0.05 and 0.1).

Natural riverine wetlands	
Attribute	**Mean greater at**:
Abundance	class 3*
# of highly tolerant taxa (MHBI 8-10)[a]	class 3*
# of non-insect taxa	class 3**
# genera (Crustacea + Mollusca)	class 3**
# genera ECOT[b]	class 1*
% diversity highly tolerant (MHBI 8-10) [a]	class 3*
% abundance highly tolerant (MHBI 8-10) [a]	class 3*
% div. Crustacea	class 3*
% diversity (Crustacea + Mollusca)	class 3*
% Chironomus[c] of total Chironomidae	class 3**
# taxa Coleoptera	class 1*
% abundance Sphaeriidae[d]	class 3*

Natural flats wetlands	
Attribute	**Mean greater at**:
% diversity collector/gatherers	Class 3*
% abundance Chironomini[e]	Class 3*
# taxa ETSD[f]	Class 1*
% diversity ETSD	Class 1*
% abundance ETSD	Class 1*
% abundance Chironomus[c]	Class 3*
% abundance Sphaeriidae[d]	Class 1*

a MHBI = modified Hilsenhoff Biotic Index

b ECOT = Ephemeroptera, Coleoptera, Odonata, and Trichoptera

c tolerant genus of chironomid midge

d fingernail clams

e tribe of chironomid midges with many tolerant genera

f ETSD = Ephemeroptera, Trichoptera, Sphaeriidae, and dragonflies

Invertebrate-based assessment of wetlands

In 2009, Xerces developed set of preliminary IBI metrics, according to data from riverine wetland reference sites sampled in 2007 and 2008. These preliminary metrics were selected based on three criteria:

1. Linear regression against site HDA scores with an R^2 value >0.25
2. Significant difference between the means of class 1 vs. class 3 sites ($p < 0.05$)
3. Sufficient range within the attribute values that a scoring system could be devised

Because sites were grouped into three main impairment classes, potential metric values from 0 to the 95th percentile were trisected (Karr *et al*. 1986). Values in the top one-third received a score of 1, values in the middle third received a 3, and values in the bottom third received a 5. The trisection method is thought to be best for scoring in regions where conditions are such that nearly all reference sites are thought to be impacted (Gerritsen *et al*., 1988), which is true of wetlands in the Willamette Valley. The trisection system was also used by the Minnesota Pollution Control Agency in developing biological IBIs for wetland assessment (Gernes & Helgen 2002). To be consistent with our HDA score ranking, attribute ranges corresponding to least-disturbed condition were assigned an IBI score of 1, and ranges corresponding to more severely disturbed conditions were scored as 5.

Table 4. Preliminary invertebrate-based IBI for Willamette Valley riverine wetlands (based on sampling data from 2007-2008). For each metric, the range corresponding to least-disturbed sites is given the lowest possible score (1).

Attribute	Metric range	Score	Rationale
# of highly tolerant taxa data range 4-20	0-6 7-12 13-21	1 3 5	Increases with site disturbance
# of predator taxa data range 0-18	0-5 6-11 12-18	1 3 5	Increases with site disturbance
# genera in Chironomini data range 1-8	0-2 3-5 6-8	1 3 5	Increases with site disturbance
% diversity collector/gatherers data range 37-100%	>48% 25.1-48% 0-25%	1 3 5	Decreases with site disturbance
# of non-insect taxa Data range 3-13	0-4 5-9 10-13	1 3 5	Increases with site disturbance
Simpson Index ($1-\lambda$) Data range 0.31-0.93	0-0.31 0.32-0.63 0.64-0.93	1 3 5	probability that 2 randomly chosen individuals will belong to same taxon; increases with site disturbance
Total possible IBI scores			Near-pristine = 6 Severely impaired = 36

In 2009, the macroinvertebrate community composition at all sites differed enough from previous years that the preliminary IBI metrics performed very badly, losing nearly all predictive power. At that point, we did not know whether the data from the 2009 field season represented an anomaly, due to differences in winter and spring weather, or if it reflected a true normal level of annual variation in community composition. The preliminary IBI metrics also performed very poorly for the 2010 dataset, which again was shown to be more similar to that of 2009 than to previous years. The data ranges for each attribute in the metric (shown in Table 4) had changed slightly in 2009 and 2010, which may have been responsible for the poor IBI performance in those years. To investigate this, we recalculated the scoring for each metric by determining the 95^{th} percentile of each metric based on the 2009-2010 data and trisecting that value. However, the entire IBI still performed badly under the revised scoring system.

By the end of this study, with up to four years of consecutive sampling data from some sites, we hoped to identify community attributes that were robust across time, maintaining a predictive value unaffected by annual macroinvertebrate community variation at each site. Because linear regression analysis was largely unrevealing, and because PRIMER analysis did not pinpoint significant taxa differences, we used comparison of community attribute means across all years for most- and least-disturbed sites to find those that were significantly different (see Table 3 above). All of these attributes have values that span a sufficient range across all sites to allow a scoring rubric to be developed. However, we feel that all the attributes shown to be significantly different between class 1 and class 3 sites at natural wetlands should be investigated further before being incorporated into a formal IBI, for the several reasons:

1. As described above, several attributes that are significant for both riverine and flats wetlands measure different aspects of the same taxa group. Redundant metrics in an IBI must be avoided, as they artificially weight the final score. Therefore, it would be necessary to arbitrarily select a single attribute relating either to highly tolerant taxa for riverine wetlands, or to ETSD taxa for flats wetlands; however, we lack sufficient data at this point to judge which among those redundant attributes will have the best predictive value. By selecting one and discarding the rest, we lose the opportunity to examine multiple attributes further for their robustness and predictive power.

2. The reliability of the potential indicator attributes developed from natural flats wetlands is even more uncertain at this point, due to the fact that only a small number of reference flats wetlands could be identified for inclusion in this study, thereby resulting in a much smaller pool of reference flats compared to riverine sites, as well as a smaller subset of class 1 and class 3 sites for comparison. In addition, sampling data for flats sites covers only two years, as opposed to three or four years of data from the majority of reference riverine sites, so the continued significance of these attributes in the face of expected annual variation among reference site invertebrate communities is unknown.

3. The wide range of values for all attributes among sites experiencing intermediate levels of impairment (class 2) renders use of an IBI problematic, as these sites are more likely to receive an artificially high or low IBI score that does not truly reflect their biological condition.

We therefore recommend that these attributes be considered as a suite of potential indicators of human-induced wetland impairment, and that continued targeted testing (described in *Conclusions and next steps* below) be done to determine their robustness and predictive capabilities.

Conclusions and next steps

- The macroinvertebrate sampling protocols and Human Site Disturbance Assessment (HDA) rubric used in this study are robust, reliable, and consistent among different trained practitioners.
- Based on consistent correlation with ORWAP stressor scores, HDA score provides a relevant reflection of the level of human impairment at a site.
- Multiple years of sampling at 50 wetlands of differing human impairment levels, HGM classes, and ecological types has expanded our knowledge of wetland taxa in the Willamette Valley and enabled us to begin building a larger ecoregion-specific dataset that may be used for reference purposes in the future.
- General differences in macroinvertebrate community composition were observed between riverine and flats wetlands.
    - Flats sites exhibit less annual variation in water chemistry parameters than riverine sites.
    - The macroinvertebrate community at flats sites overall is more restricted and composed of more tolerant groups.
    - A different suite of macroinvertebrate community attributes was significantly different among most- vs. least-impaired sites in natural flats and natural riverine wetlands.
- General differences between restored and natural wetlands:
    - Restored sites do not differ in water chemistry parameters.
    - Macroinvertebrate community composition is very similar among restored and natural wetlands sites in the same HGM class.
    - Least-impaired restored wetlands do not have the higher proportions of rare species seen at least-impaired natural wetlands.
- The similarity of community composition among sites within the same class or category compared to sites in different categories renders it difficult to pinpoint indicator taxa whose presence or abundance differs significantly at different types of sites.
- Variation in macroinvertebrate community composition at the same site across consecutive years indicates a high level of dynamism. Because this level of annual variation occurred at sites across all impairment classes, it suggests that even least-impaired sites in the Willamette Valley are experiencing constant anthropogenic stressors that impact invertebrate

communities, and that annual variation in invertebrates may naturally be high. It remains to be seen whether the annual variation in invertebrate community composition across time is too great to allow a stable invertebrate bioassessment tool to be implemented.

- Changes in wetland invertebrate community characteristics are most apparent among natural wetlands experiencing different levels of human disturbance. These differences are mainly apparent when comparing natural wetlands that are highly versus minimally disturbed, with most-disturbed wetlands having a more restricted and more stable macroinvertebrate community. These differences are apparent in both riverine and flats wetlands.
    - Most-impaired sites have higher levels of total Kjeldahl nitrogen, phosphorus, and higher conductivity than least-impaired.
    - Most-impaired sites have higher within-group invertebrate community similarity, lower annual variation in invertebrate community composition, and a lower proportion of rare (and possibly more sensitive) species compared to least-disturbed sites. While community attributes that focus on rare or sensitive species have not shown any predictive power (i.e. # or relative diversity of rare taxa; #, relative diversity, or relative abundance of sensitive taxa), attributes relating to highly tolerant taxa have consistently differed significantly between least- and most-impaired sites, especially at riverine wetlands.

Next steps

Data analysis across up to four years of wetland sampling revealed two different suites of attributes that are significantly different at most-impaired versus least-impaired sites at natural riverine and natural flats wetlands. However, our data also indicate that there is substantial community similarity among natural wetlands at all levels of human disturbance, but that the community composition may change by as much as 50% from year to year at a single site. Therefore, in order to determine the most consistent and predictive indicators for wetland biological condition, and to better investigate the effects of restoration activities on wetland invertebrate communities, we recommend the following:

- Investigate differences in macroinvertebrate communities among restored wetlands by sampling at a targeted selection of restored sites across more years. The most effective way to do this could be to focus on only most-impaired and least-impaired natural riverine and natural flats wetlands as reference groups, and attempt to pair reference with restored sites in the same area that are as similar as possible. The restored wetlands should also encompass a more limited post-recovery period, and be monitored for several years; the restored sites used in this study were at a different number of years port-restoration.

- Investigate the minor taxonomic differences seen between natural and restored sites to determine whether they persist across time and have predictive value regarding the biological condition of restored wetlands.

- Incorporate a greater number of natural flats wetlands into the reference group, as many fewer natural flats than natural riverine sites were able to be identified for this study. It would be most effective to use only sites that are assessed as least-impaired and most-impaired.

- Obtain additional sampling data at all natural wetland sites to further assess the magnitude of annual variation in macroinvertebrate community composition at the same site sampled across consecutive years; this is especially important for the flats sites, for which only two years of sampling data was obtained.

- Continue to evaluate the consistency, reliability, and predictive value of the macroinvertebrate community attributes that were identified in this study as being significantly different between most-impaired and least-impaired natural wetlands. If these attributes retain their significance across several years in the face of annual macroinvertebrate community variation, they may be considered reliable enough to be incorporated into an IBI.

- Depending on the results of continued testing of the above-mentioned attributes, it may ultimately be deemed more effective to use this expanded reference site dataset to generate a predictive model instead of a set IBI, to compare identified attributes at a test site to those in a reference group of similar sites in the same ecoregion.

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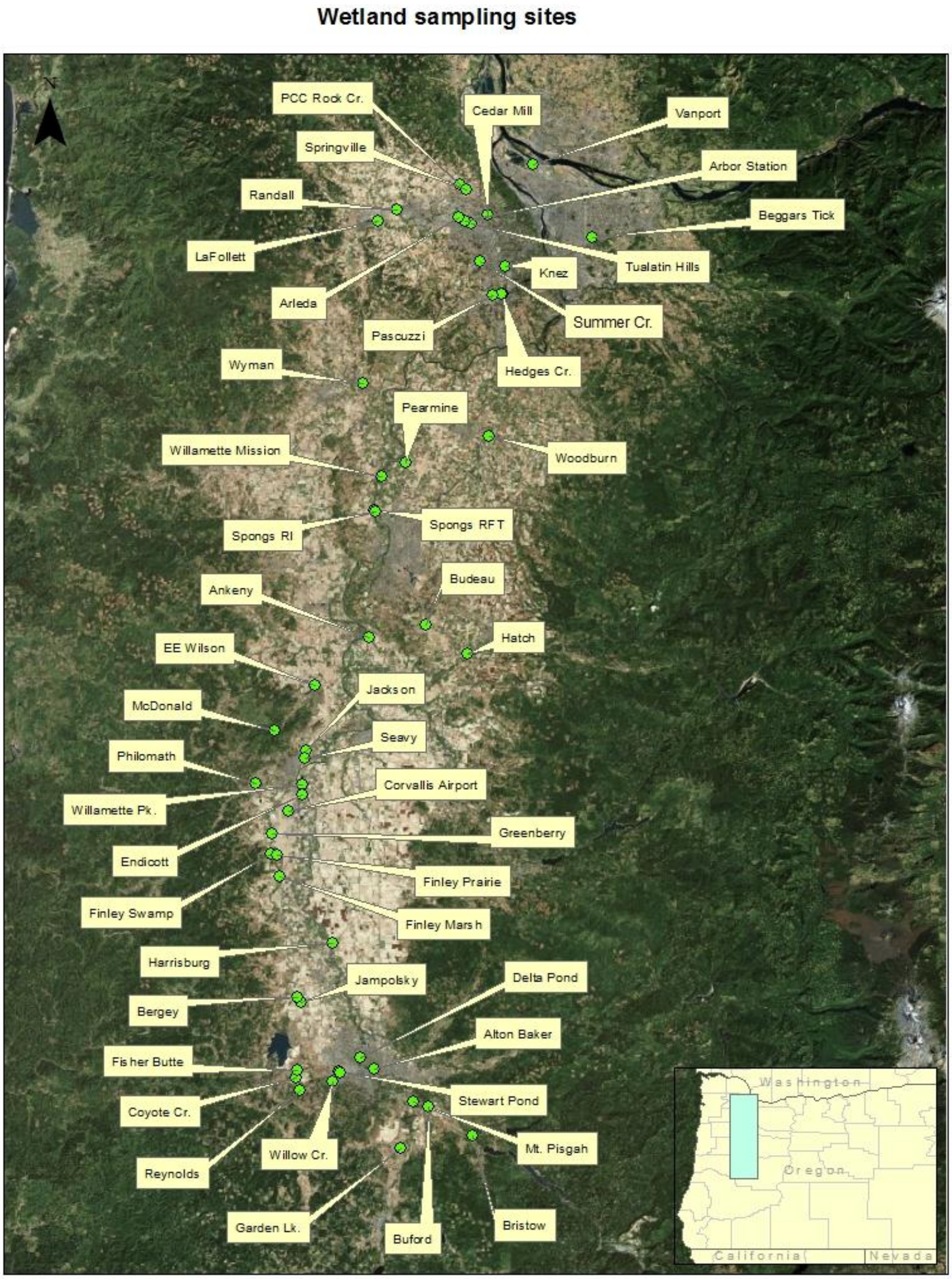
Appendix A. Wetland sampling sites in the Willamette Valley



II. Coordinates of wetland sampling sites. Each site name is followed by the database abbreviation used.

Site	Longitude	Latitude		Site	Longitude	Latitude
Philomath Industrial PHIL	-123.359	44.54973		Beggars Tick BGRS_TK	-122.55	45.48065
Alton Baker ALT_BKR	-123.074	44.0571		Buford BUF	-122.944	43.99328
Cedar Mill CED_ML	-122.801	45.51818		Budeau North BUD_N	-122.95	44.82076
Coyote Creek COY_CR	-123.261	44.04227		Budeau South BUD_S	-122.9496	44.81918
Delta Pond DLT-PND	-123.107	44.07788		Corvallis airport CRVLS_ARPT	-123.279	44.50333
EE Wilson WLSN	-123.215	44.71843		Fisher Butte FSHR_BT	-123.257	44.05477
Elijah Bristow BRSTW	-122.837	43.94153		Garden Lake GRDN_LK	-123.011	43.9209
Finley Brown Swamp FIN_SWP	-123.321	44.42912		Pearmine PRMN	-122.997	45.09837
Finley McFadden Marsh FIN_MSH	-123.3	44.3895		Jampolsky JMP	-123.248	44.17303
Greenberry GRNBRY	-123.318	44.46333		Wyman WYMN	-123.101	45.23223
Harrisburg HRS	-123.174	44.27607		Bergey BRGY	-123.259	44.1825
Hedges Park HDG_PK	-122.763	45.38512		Hatch airstrip HTCH	-122.849	44.7731
Hedges Cr. TWC HDG_TWC	-122.767	45.38432		Sam Reynolds RNLD	-123.253	44.02157
Knez KNEZ	-122.759	45.4299		Summer Creek SUM_CR	-122.818	45.43958
McDonald MCDNLD	-123.313	44.64032		LaFollett LAFOL	-123.063	45.50648
Mt. Pisgah MT_PSGH	-122.979	44.00243		Springville SPGVL	-122.853	45.5602
Pascuzzi PSCZI	-122.788	45.38128		Arbor Station ARB_STA	-122.856	45.5083
PCC Rock Creek PCC_RKCR	-122.867	45.5684		Vanport (Portland International) PDX_INT	-122.69	45.6034
Randall RNDL	-123.018	45.5269		Arleda ARL	-122.87	45.51372
Spongs Landing flowthrough SPNGS_RFT	-123.073	45.01633		Seavy SVY	-123.24	44.59393
Spongs Landing	-123.07	45.01437		Stewart Pond	-123.157	44.0537

impounding SPNGS_RI				North STWRT_PNDN		
Tualatin Hills TUAL	-122.84	45.50268		Willamette Mission WILL_MSN	-123.057	45.07563
Willamette Park WILL_PK	-123.247	44.54855		Endicott ENDCT	-123.247	44.53155
Willow Creek WILL_CR	-123.173	44.03678		Stewart Pond South STWRT_PNDS	-123.155	44.05042
Jackson-Frazier JCKSN	-123.239	44.6053		Finley Prairie FIN_RSCH	-123.306	44.42723
Ankeny Wigeon Marsh WGN_MSH	-123.087	44.79963		Woodburn (Town Ctr.) TWN_CTR	-122.799	45.1435

Appendix B. Wetland Human Disturbance Assessment form

Site name: **Date:** **County/City:** **Rated by:**

Total HDA score (75 possible) =

1. Buffer landscape disturbance (land use within 50 ft/15 m of wetland): _______ points

Excellent: reference-quality; little to no evidence of disturbance in buffer	(0)	
Mod.: mainly undisturbed, some evidence of human use in buffer	(5)	
Fair: significant human influence; large proportion of buffer filled with human use	(10)	
Poor: intense human influence; all or almost all of buffer filled with human use	(15)	

Use the checklist below to guide your rating:

Excellent		Moderate	
	Mature woodlot (>20 yr.), forested	Old field, rangeland, conservation reserve	
	Mature prairie	Restored prairie (>10 yr)	
	Other wetlands	Young 2nd growth woodlot (<20 yr)	
	Other long-recovered area	Shrubland	
Fair		Poor	
	Residential with unmowed areas	Urban development	
	Active pasture/grazing	Industrial development	
	Less intensive agriculture	Intensive residential, mowed	
	Park turf or golf course	Intensive agriculture or grazing	
	Newly fallowed agricultural fields	Mining in/adjacent to wetland	
	High road density/other impervious surface	Active construction activity	

Comments:

2. Immediate landscape influence (500 ft/150 m of surrounding land): _______ points

Excellent: reference-quality; natural landscape; little/no evidence of human use	(0)	
Mod.: mainly undisturbed, some evidence of human use influence	(5)	
Fair: significant human influence; large proportion of landscape filled with human use	(10)	
Poor: all or most of landscape area filled with human use, isolating the wetland	(15)	

Use the checklist below to guide your rating:

Excellent		Moderate	
	Mature woodlot (>20 yr.), forested	Old field, rangeland, conservation reserve	
	Mature prairie	Restored prairie (>10 yr)	
	Other wetlands	Young 2nd growth woodlot (<20 yr)	
	Other long-recovered area	Shrubland	
Fair		Poor	
	Residential with unmowed areas	Urban development	

	Active pasture/grazing	Industrial development	
	Less intensive agriculture	Intensive residential, mowed	
	Park turf or golf course	Intensive agriculture or grazing	
	Newly fallowed agricultural fields	Mining in/adjacent to wetland	
	High road density/other impervious surface	Active construction activity	

Comments:

3. Habitat alteration, immediate landscape (500 ft/150 m of surrounding land): ______ points

Excellent: reference-quality; natural landscape; no evidence of alteration (0)	
Mod.: low intensity alteration or past alteration not currently affecting wetland (5)	
Fair: highly altered but with some recovery from previous alterations (10)	
Poor: little natural habitat present, highly altered habitat (15)	

Use the checklist below to guide your rating:

Vegetation removal/disturbances present			
	Excessive mowing	Shrub removal	
	Tree plantations	Woody debris removal	
	Tree removal/logging/clearcutting	Emergent vegetation/aquatic bed removal	
	Low spp diversity and/or predominance of nonnative or disturbance-tolerant native spp	Excessive grazing/herbivory	
	Livestock hooves	Vehicle use	
	Cultivation	Other:	

Comments:

4. Hydrologic alteration, immediate landscape (500 ft/150 m of surrounding land): ______ points

Excellent: reference-quality; natural landscape; no evidence of alteration (0)	
Mod.: low intensity alteration or past alteration not currently affecting wetland (5)	
Fair: current or active alteration at significant levels (10)	
Poor: current or active alterations with major hydrologic disturbance (15)	

Use the checklist below to guide your rating:

	Ditch inlet/outlet	Berm, levee or dike	
	Tile drain	Road or railroad bed	
	Point source input	Drainage	
	Weir or dam	Unnatural connection to other waters	
	Dredging	Dewatering in/near wetland	
	Grading or filling in/near wetland	Source water alteration	
	Other:		

Comments:

5. Chemical & Sediment Inputs: ______ points

Excellent: as expected for natural site, little/no evidence of additional human-related input 0)	
Mod.: inputs in low range, little/slight evidence of additional human-related input (5)	
Fair: inputs in mid-range, significant evidence of additional human-related input (10)	
Poor: high levels of human-related inputs, high potential for biological harm (15)	

Use the checklist below to guide your rating:

	High [Cl]	High conductivity	
	High [total P]	Unnaturally high or low pH	
	High [total N]	High turbidity reading	
	Excessive algal growth/density	Soil disturbance in immediate buffer	
	Eroding banks/slopes	Other:	

Comments:

Appendic C. Wetland macroinvertebrate taxa list 2007-2010. This is a cumulative list representing all taxa found at any site sampled across four years of surveys.

Taxon	Phylum:Class or other	Order	Family	Common name
Porifera	Porifera			sponge
Hydra	Cnidaria: Hydrozoa	Hydroida	Hydridae	hydra
Turbellaria	Turbellaria			flatworm
Nematoda	Nematoda			round worm
Oligochaeta	Annelida: Oligochaeta			segmented worm
Erpobdellidae	Annelida: Hirudinea		Erpobdellidae	leech
Helobdella stagnalis	Annelida: Hirudinea		Glossiphoniidae	leech
Theromyzon	Annelida: Hirudinea		Glossiphoniidae	leech
Corbicula	Mollusca: Bivalvia		Corbiculidae	fingernail clam
Musculium	Mollusca: Bivalvia		Sphaeriidae	fingernail clam
Pisidium	Mollusca: Bivalvia		Sphaeriidae	pea clam
Sphaerium	Mollusca: Bivalvia		Sphaeriidae	pea clam
Ferrissia	Mollusca: Gastropoda		Ancylidae	limpets
Fluminicola	Mollusca: Gastropoda		Hydrobiidae	pebblesnail
Lymnaea	Mollusca: Gastropoda		Lymnaeidae	pond snail
Physa	Mollusca: Gastropoda		Physidae	tadpole snail
Gyraulus	Mollusca: Gastropoda		Planorbidae	ramshorn snail
Menetus opercularis	Mollusca: Gastropoda		Planorbidae	ramshorn snail
Helisoma trivolvis	Mollusca: Gastropoda		Planorbidae	ramshorn snail
Promenetus exacuous	Mollusca: Gastropoda		Planorbidae	ramshorn snail
Juga	Mollusca: Gastropod		Pleuroceridae	pleurocerid snail
Chydoridae	Crustacea	Cladocera	Chydoridae	waterflea
Ostracoda	Crustacea	Ostracoda		seed shrimp
Copepoda	Crustacea	Copepoda		copepod
Lynceus	Crustacea	Conchostraca	Lynceidae	clam shrimp
Crangonyx	Arthropoda: Crustacea	Amphipoda	Crangonyctidae	scuds
Hyalella	Arthropoda: Crustacea	Amphipoda	Hyalellidae	scuds

Caecidotea occidentalis	Arthropoda: Crustacea	Isopoda	Asellidae	aquatic sow bugs
Orconectes virilis	Arthropoda: Crustacea	Decapoda	Cambaridae	crayfish
Pacifasticus[a]	Arthropoda: Crustacea	Decapoda	Astacidae	crayfish
Oribatida	Arthropoda: Arachnida	Oribatida		aquatic mite
Arrenurus	Arthropoda: Arachnida	Trombidiformes	Arrenuridae	aquatic mite
Eylais	Arthropoda: Arachnida	Trombidiformes	Eylaidae	aquatic mite
Hydrachna	Arthropoda: Arachnida	Trombidiformes	Hydrachnidae	aquatic mite
Lebertia	Arthropoda: Arachnida	Trombidiformes	Lebertiidae	aquatic mite
Limnesia	Arthropoda: Arachnida	Trombidiformes	Limnesiidae	aquatic mite
Mesobates (Hygrobates)	Arthropoda: Arachnida	Trombidiformes	Hygrobatidae	aquatic mite
Mideopsis	Arthropoda: Arachnida	Trombidiformes	Mideopsidae	aquatic mite
Piona	Arthropoda: Arachnida	Trombidiformes	Pionidae	aquatic mite
Thyas	Arthropoda: Arachnida	Trombidiformes	Thyasidae	aquatic mite
Unionicola	Arthropoda: Arachnida	Trombidiformes	Unionicolidae	aquatic mite
Aeshna	Arthropoda: Insecta	Odonata	Aeshnidae	darner dragonfly
Anax	Arthropoda: Insecta	Odonata	Aeshnidae	darner dragonfly
Epitheca	Arthropoda: Insecta	Odonata	Corduliidae	emerald dragonfly
Somatochlora	Arthropoda: Insecta	Odonata	Corduliidae	emerald dragonfly
Gomphidae	Arthropoda: Insecta	Odonata	Gomphidae	clubtail dragonfly
Libellula	Arthropoda: Insecta	Odonata	Libellulidae	skimmer dragonfly
Erythemis	Arthropoda: Insecta	Odonata	Libellulidae	skimmer dragonfly
Plathemis	Arthropoda: Insecta	Odonata	Libellulidae	skimmer dragonfly
Sympetrum	Arthropoda: Insecta	Odonata	Libellulidae	skimmer dragonfly
Tramea	Arthropoda: Insecta	Odonata	Libellulidae	skimmer dragonfly
Argia	Arthropoda: Insecta	Odonata	Coenagrionidae	pond damselfly
Coenagrion/Enallagma	Arthropoda: Insecta	Odonata	Coenagrionidae	pond damselfly
Ischnura	Arthropoda:	Odonata	Coenagrionidae	pond damselfly

	Insecta			
Lestes	Arthropoda: Insecta	Odonata	Lestidae	spreadwing damselfly
Ameletus	Arthropoda: Insecta	Ephemeroptera	Ameletidae	comb-mouthed minnow mayfly
Acentrella insignificans[a]	Arthropoda: Insecta	Ephemeroptera	Baetidae	small minnow mayfly
Acentrella turbida[a]	Arthropoda: Insecta	Ephemeroptera	Baetidae	small minnow mayfly
Baetis tricaudatus[a]	Arthropoda: Insecta	Ephemeroptera	Baetidae	small minnow mayfly
Callibaetis	Arthropoda: Insecta	Ephemeroptera	Baetidae	small minnow mayfly
Centroptilum[a]	Arthropoda: Insecta	Ephemeroptera	Baetidae	small minnow mayfly
Procloeon[a]	Arthropoda: Insecta	Ephemeroptera	Baetidae	small minnow mayfly
Pseudocloeon[a]	Arthropoda: Insecta	Ephemeroptera	Baetidae	small minnow mayfly
Caenis youngi	Arthropoda: Insecta	Ephemeroptera	Caenidae	small squaregill mayfly
Atennella soquele	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	spiny crawler mayfly
Ephemerella excrusians[a]	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	spiny crawler mayfly
Eurylophella lodi	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	spiny crawler mayfly
Seratella tibialis[a]	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	spiny crawler mayfly
Hexagenia limbata	Arthropoda: Insecta	Ephemeroptera	Ephemeridae	common burrower mayfly
Rhithrogena[a]	Arthropoda: Insecta	Ephemeroptera	Heptageniidae	flatheaded mayfly
Tricorythodes minutus[a]	Arthropoda: Insecta	Ephemeroptera	Leptohyphidae	little stout crawler mayfly
Paraleptophlebia	Arthropoda: Insecta	Ephemeroptera	Leptophlebiidae	pronggill mayfly
Siphlonurus columbianus	Arthropoda: Insecta	Ephemeroptera	Siphlonuridae	primitive minnow mayfly
Siphlonurus occidentalis	Arthropoda: Insecta	Ephemeroptera	Siphlonuridae	primitive minnow mayfly
Malenka[a]	Arthropoda: Insecta	Plecoptera	Nemouridae	little brown stonefly
Soyedina	Arthropoda: Insecta	Plecoptera	Nemouridae	little brown stonefly
Zapada cinctipes[a]	Arthropoda: Insecta	Plecoptera	Nemouridae	little brown stonefly
Isoperla[a]	Arthropoda: Insecta	Plecoptera	Perlodidae	stripetail stonefly
Pteronarcella[a]	Arthropoda: Insecta	Plecoptera	Pteronarcyidae	giant stonefly
Belostoma	Arthropoda: Insecta	Heteroptera	Belostomatidae	giant water bug

Corixidae	Arthropoda: Insecta	Heteroptera	Corixidae	water boatman
Gerris	Arthropoda: Insecta	Heteroptera	Gerridae	water strider
Ranatra	Arthropoda: Insecta	Heteroptera	Nepidae	water scorpion
Buenoa	Arthropoda: Insecta	Heteroptera	Notonectidae	backswimmer
Notonecta	Arthropoda: Insecta	Heteroptera	Notonectidae	backswimmer
Saldidae	Arthropoda: Insecta	Heteroptera	Saldidae	shore bug
Microvelia	Arthropoda: Insecta	Heteroptera	Veliidae	shortlegged strider
Sialis	Arthropoda: Insecta	Megaloptera	Sialidae	alderfly
Amiocentrus aspilus	Arthropoda: Insecta	Trichoptera	Brachycentridae	humpless casemaker caddisfly
Brachycentrus occidentalis[a]	Arthropoda: Insecta	Trichoptera	Brachycentridae	humpless casemaker caddisfly
Micrasema	Arthropoda: Insecta	Trichoptera	Brachycentridae	humpless casemaker caddisfly
Cheumatopsyche[a]	Arthropoda: Insecta	Trichoptera	Hydropsychidae	common netspinner caddisfly
Agraylea	Arthropoda: Insecta	Trichoptera	Hydroptilidae	purse-making caddisfly
Hydroptila	Arthropoda: Insecta	Trichoptera	Hydroptilidae	purse-making caddisfly
Oxyethira	Arthropoda: Insecta	Trichoptera	Hydroptilidae	purse-making caddisfly
Lepidostoma	Arthropoda: Insecta	Trichoptera	Lepidostomatidae	casemaking caddisfly
Mystacides	Arthropoda: Insecta	Trichoptera	Leptoceridae	long-horned caddisfly
Oecetis	Arthropoda: Insecta	Trichoptera	Leptoceridae	long-horned caddisfly
Triaenodes	Arthropoda: Insecta	Trichoptera	Leptoceridae	long-horned caddisfly
Glyphopsyche irrorata	Arthropoda: Insecta	Trichoptera	Limnephilidae	northern caddisfly
Grammotaulius	Arthropoda: Insecta	Trichoptera	Limnephilidae	northern caddisfly
Limnephilus	Arthropoda: Insecta	Trichoptera	Limnephilidae	northern caddisfly
Onocosmoecus unicolor[a]	Arthropoda: Insecta	Trichoptera	Limnephilidae	northern caddisfly
Polycentropus	Arthropoda: Insecta	Trichoptera	Polycentropodidae	trumpetnet caddisfly
Rhyacophila narvae[a]	Arthropoda:	Trichoptera	Rhyacophilidae	free-living

	Insecta			caddisfly
Acilius	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Agabus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Dytiscus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Graphoderus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Hydaticus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Hydroporus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Hygrotus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Neoporus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Rhantus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Nebrioporus	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetle
Lara avara[a]	Arthropoda: Insecta	Coleoptera	Elmidae	riffle beetle
Cleptelmis	Arthropoda: Insecta	Coleoptera	Elmidae	riffle beetle
Optioservus[a]	Arthropoda: Insecta	Coleoptera	Elmidae	riffle beetle
Zaitzevia[a]	Arthropoda: Insecta	Coleoptera	Elmidae	riffle beetle
Gyrinus	Arthropoda: Insecta	Coleoptera	Gyrinidae	whirligig beetle
Apteraliplus	Arthropoda: Insecta	Coleoptera	Haliplidae	crawling water beetle
Brychius	Arthropoda: Insecta	Coleoptera	Haliplidae	crawling water beetle
Haliplus	Arthropoda: Insecta	Coleoptera	Haliplidae	crawling water beetle
Peltodytes	Arthropoda: Insecta	Coleoptera	Haliplidae	crawling water beetle
Hydraena	Arthropoda: Insecta	Coleoptera	Hydraenidae	minute moss beetle
Ochthebius	Arthropoda: Insecta	Coleoptera	Hydraenidae	minute moss beetle
Ametor	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Anacaena	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Berosus	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Crenitis	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Cymbiodyta	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle

Enochrus	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Helophorus	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Hydrobius	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Hydrophilus	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Laccobius	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Tropisternus	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetle
Brachycera	Arthropoda: Insecta	Diptera		higher flies
Ceratopogoninae	Arthropoda: Insecta	Diptera	Ceratopogonidae	biting midge
Dasyhelea	Arthropoda: Insecta	Diptera	Ceratopogonidae	biting midge
Chaoborus	Arthropoda: Insecta	Diptera	Chaoboridae	phantom midge
Culicidae	Arthropoda: Insecta	Diptera	Culicidae	mosquito
Dixa[a]	Arthropoda: Insecta	Diptera	Dixidae	dixid midge
Dixella	Arthropoda: Insecta	Diptera	Dixidae	dixid midge
Meringodixa	Arthropoda: Insecta	Diptera	Dixidae	dixid midge
Dolichopodidae	Arthropoda: Insecta	Diptera	Dolichopodidae	longlegged fly
Neoplasta	Arthropoda: Insecta	Diptera	Empididae	dance fly
Trichoclinocera	Arthropoda: Insecta	Diptera	Empididae	dance fly
Ephydridae	Arthropoda: Insecta	Diptera	Ephydridae	shore fly
Muscidae	Arthropoda: Insecta	Diptera	Muscidae	aquatic kin of house fly
Mycetophilidae	Arthropoda: Insecta	Diptera	Mycetophilidae	fungus gnat
Pericoma/Telmatoscopus	Arthropoda: Insecta	Diptera	Psychodidae	moth fly
Psychoda	Arthropoda: Insecta	Diptera	Empididae	dance fly
Sciomyzidae	Arthropoda: Insecta	Diptera	Sciomyzidae	marsh fly
Simulium[a]	Arthropoda: Insecta	Diptera	Simuliidae	black fly
Odontomyia	Arthropoda: Insecta	Diptera	Stratiomyidae	soldier fly
Tabanidae	Arthropoda: Insecta	Diptera	Tabanidae	horse & deer fly
Tipulidae	Arthropoda:	Diptera	Tipulidae	crane fly

	Insecta			
Arctoconopa	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Dicranota	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Erioptera	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Holorusia	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Limnophila	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Limonia	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Pilaria	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Tipula	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Tipula (Angarotipula)	Arthropoda: Insecta	Diptera	Tipulidae	crane fly
Chironomidae pupae	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Ablabesmyia	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Acricotopus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Apedilum	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Brillia[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Chaetocladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Chironomus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Cladopelma	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Cladotanytarsus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Clinotanypus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Constempinella	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Corynoneura	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Cricotopus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Cricotopus Bicinctus Group	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Cryptochironomus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Cryptotendipes	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Diamesa	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge

Dicrotendipes	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Diplocladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Endochironomus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Eukiefferiella[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Glyptotendipes	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Guttipelopia	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Heterotrissocladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Hydrobaenus[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Labrundinia	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Lauterborniella	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Limnophyes	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Macropelopia	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Metriocnemus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Micropsectra	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Microtendipes	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Nanocladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Odontomesa	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Orthocladius Complex[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Parachironomus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Paracladopelma	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Parakiefferiella	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Paralauterborniella	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Paramerina	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Parametriocnemus[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Paraphaenocladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Paratanytarsus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Paratendipes	Arthropoda:	Diptera	Chironomidae	nonbiting midge

	Insecta			
Pentaneura	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Phaenopsectra	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Polypedilum	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Potthastia Gaedii group	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Procladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Prodiamesaa	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Psectrocladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Psectrotanypus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Pseudochironomus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Pseudosmittia	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Radotanypusa	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Rheocricotopus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Smittia	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Stempellina[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Stempellinella[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Synorthocladius	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Tanypus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Tanytarsus	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Thienemanniella[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Thienmannimyia Complex	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Tribelos	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Tvetenia Bavarica Group[a]	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge
Zavrelimyia	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midge

[a] denotes stream taxa, generally rare in samples; some may have been washed in to wetland sites

Appendix D. Water chemistry measures at wetland sampling sites. Unpaired t-tests were done to assess whether mean values differed significantly ($p < 0.05$) between different classes or types of sites. R_NAT = natural riverine; F_NAT = natural flats; R_ RST = restored riverine; F_RST = restored flats.

pH	Range	Mean	HDA class 1 vs. class 3 mean	NAT vs. RST mean
R_NAT, all yrs	5.8 to 8.4	7.0 ± 0.6	class 1 = 7.2+0.97 class 3 = 7.0+0.54 not sig. diff.	not sig. diff.
R_RST, all yrs	5.3 to 7.8	6.8 ± 0.8	N/A	
F_NAT, all yrs	3.8 to 8.6	6.7 ± 1.4	class 1 = 6.6 ± 0.4 class 3 = 7.1 ± 1.1 not sig. diff.	not sig. diff.
F_RST, all yrs	6.2 to 7.8	7.0 ± 0.5	N/A	
N (mg/L)	Range	Mean	HDA class 1 vs. class 3 mean	NAT vs. RST mean
R_NAT, all yrs	ND to 2.8	0.92 ± 0.72	class 1 = 0.43 ± 0.50 class 3 = 1.13 ± 0.79 not sig. diff.	sig. diff. (p=0.0382)
R_RST, all yrs	ND to 4.8	1.7 ± 1.5	N/A	
F_NAT, all yrs	0.9 to 9.1	3.0 ± 2.6	class 1 = 1.55 ± 0.07 class 3 = 3.8 ± 3.66 not sig. diff.	not sig diff.
F_RST, all yrs	1.0 to 20.8	6.1 ± 8.4	N/A	
P (mg/L)	Range	Mean	HDA class 1 vs. class 3 mean	NAT vs. RST mean
R_NAT, all yrs	ND to 1.08	0.20 ± 0.28	class 1 = 0.05 ± 0.05 class 3 = 0.35 ± 0.49 not sig. diff.	not sig. diff.
R_RST, all yrs	ND to 1.6	0.4 ± 0.5	N/A	
F_NAT, all yrs	0.16 to 1.33	0.53 ± 0.37	class 1 = 0.66 ± 0.21 class 3 = 0.59 ± 0.51 not sig. diff.	not sig diff
F_RST, all yrs	0.1 to 1.8	0.5 ± 0.6	N/A	
Cl (mg/L)	Range	Mean	HDA class 1 vs. class 3 mean	NAT vs. RST mean
R_NAT, all yrs	1.0 to 13.0	5.46 ± 3.72	class 1 = 6.0 ± 3.46 class 3 = 4.3 ± 1.71 not sig. diff.	not sig. diff.
R_RST, all yrs	ND to 9.0	3.9 ± 2.9	N/A	
F_NAT, all yrs	1.0 to 13	4.3 ± 4.0	class 1 = 10.5 + 3.5 class 3 = 3.5 + 1.7 sig. diff. (p = 0.0252)	not sig. diff.
F_RST, all yrs	2 to 30	11.3 ± 10.1	N/A	

Conductivity (µS)	Range	Mean	HDA class 1 vs. class 3 mean	NAT vs. RST mean
R_NAT, all yrs	48.5 to 405	181.1 ± 80.9	class 1 = 144.01 ± 55.6 class 3 = 192.1 ± 67.0 not sig. diff.	not sig. diff.
R_RST, all yrs	35 to 267	150.3 ± 91.3	N/A	
F_NAT, all yrs	23.6 to 151.1	103.6 ± 45.6	class 1 = 121.8 ± 12.4 class 3 = 136.7 ± 12.9 not sig. diff.	not sig. diff.
F_RST, all yrs	45.6 to 360.7	165.1 ± 102.3	N/A	
DO (mg/L)	Range	Mean	HDA class 1 vs. class 3 mean	NAT vs. RST mean
R_NAT, all yrs	2 to 13	6.2 ± 3.3	class 1 = 8.2+3.4 class 3 = 6.1+2.4 not sig. diff.	not sig. diff.
R_RST, all yrs	3.1 to 9.6	5.7 ± 2.4	N/A	
F_NAT, all yrs	1.7 to 13	5.6 ± 4.0	class 1 = 2.8 = 1.4 class 3 = 8.08 + 5.2 not sig. diff.	not sig. diff.
F_RST, all yrs	3 to 10	6.2 ± 2.5	N/A	