

## Project Completion Report to the Oregon Watershed Enhancement Board

Developing an Invertebrate Index of Biological Integrity in Pacific Northwest Wetlands

OWEB Grant # 208-3046



*McDonald Forest Ponds, Corvallis OR*

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## **Abstract**

There are currently no reliable, cost-effective tools for assessing the biological quality of Pacific Northwest wetlands. This project worked towards developing an invertebrate-based Index of Biological Integrity (I-IBI) that could be used reliably across riverine wetlands in the Willamette Valley. Macroinvertebrates were sampled in May at 24 HGM-riverine class wetlands in the Willamette Valley, using D-frame aquatic dip nets and activity traps. A Human Disturbance Assessment was completed to rank sites as least-disturbed, intermediate disturbance, or most-disturbed. Multivariate analysis was used to examine relationships between species composition and environmental variables; analyze differences in community composition between sites with different levels of human disturbance and in different HGM subclasses (riverine-flowthrough vs. riverine-impounding); test for differences between sampling techniques; and examine the similarity of site community structure across two consecutive sampling years. Linear regression was used to examine relationships between 69 invertebrate community attributes and the level of human disturbance at wetland sites. We achieved all the major goals of this project:

*Determine effective wetland sampling methods for macroinvertebrates:* Wetlands were sampled using both D-frame dip nets and activity traps. The data indicate that a D-frame dip net provides a standard, low-cost sampling technique for wetland sampling.

*Develop a more extensive database of macroinvertebrate taxa in riverine wetlands of the Willamette Valley:* Compiling data from 2007 and 2008, Xerces has identified 169 macroinvertebrate taxa in Willamette Valley wetlands.

*Identify biological attributes of the wetland macroinvertebrate community that can be used towards developing an Index of Biological Integrity and create a draft invertebrate Index of Biological Integrity accessible to a variety of users that can be used to monitor wetland health:* We examined 69 different attributes of the wetland macroinvertebrate community assemblages and generated a preliminary IBI consisting of six metrics that varied reliably with the level of human disturbance and were applicable to both riverine-flowthrough and riverine-impounding wetlands. Our analysis indicates that the wetland community at an individual site should be consistent enough from year to year for assessment based on a subset of community attributes to reliably reveal useful information about the site's biological condition. Our analysis also revealed that site impairment level accounted for the greatest dissimilarity between sites, while HGM subclass was of minimal importance. These findings suggest that the same set of community attributes can be used to assess both riverine-impounding and riverine-flowthrough wetlands in the Willamette Valley, and that a single IBI will serve for the HGM riverine class.

Water chemistry data from this project was entered into the LASAR database spreadsheet and submitted via e-mail to the DEQ Volunteer Monitoring Specialist (Steve Hanson). A copy of the Project Completion Report was e-mailed to the OWEB Monitoring & Reporting Section (Courtney Shaff).

## **Project Background**

The purpose of this study was to develop an invertebrate-based biological assessment tool that could be used across riverine wetlands in the Willamette Valley to assess wetland quality and detect responses to anthropogenic stressors. This project extends and expands the work of an EPA-funded pilot study conducted by the Xerces Society in 2007.

Wetlands are important components of watersheds, providing valuable ecological services such as flood control, water filtration, erosion control, and plant and wildlife habitat. The 2006 Oregon State of the Environment Report estimated that Oregon lost over one-third of the wetlands that were present prior to European colonization, and almost two-thirds of the wetlands in the Willamette Valley have been lost. The wetlands that remain are understudied and little information is available on their level of biological function. Pacific Northwest wetlands are affected by a variety of anthropogenic stressors, including nutrient enrichment, heavy metal contamination, thermal alteration, invasive species and increased salinization. This project was conducted because there are currently no consistent and cost-effective tools to assess biological integrity of Pacific Northwest wetlands. An invertebrate-based biological assessment tool holds the potential to effectively monitor wetland condition and evaluate wetland restoration or mitigation success, and has been successfully used in other parts of the country.

## **Project Summary**

The goals of this study were to:

- Identify biological attributes of the wetland macroinvertebrate community that can be used to develop an Index of Biological Integrity.
- Implement a reliable, rapid, on-the-ground rubric for scoring the level of human disturbance of wetlands that is accessible to a variety of users.
- Create a preliminary invertebrate IBI that can be used to reflect wetland biological quality and is accessible to a variety of users.
- Determine effective wetland sampling methods for macroinvertebrates.
- Develop a more complete database of the macroinvertebrate taxa in riverine wetlands of the Willamette Valley.
- Increase outreach and collaboration in wetland monitoring and assessment projects with regional watershed councils, related nonprofits, and state and city agencies.

## **Methods**

### *Site selection*

The wetlands sampled in this study included riverine-impounding and riverine-flowthrough sites, as determined by the hydrogeomorphic (HGM) classification system (Brinson 1993, Shaffer *et al.* 1999, Adamus 2001). All riverine impounding sites sampled during the 2007 Xerces pilot study were sampled again in 2008, with the exception of a single site (Anderson Park), as disputes about park boundaries and public access arose. The remaining sites were chosen based on data collected in previous work by the Division of State Lands (DSL) (Adamus & Field, 2001) to apply the HGM classification system to Willamette Valley wetlands. We prioritized sites classified in the DSL study as HGM-riverine flowthrough in order to investigate potential differences in the macroinvertebrate communities at riverine impounding versus flowthrough sites. Potential new sites were also selected to differ in the degree of human disturbance, such

that all sites sampled represented a gradient of anthropogenic disturbance. Our initial site selection based on these qualities was followed up with on-site reconnaissance visits and consultation with site managers and local wetland experts. All sampling took place from May 16 to June 5, 2008.

### *Habitat Assessment*

To develop an index of biological integrity, a gradient of human disturbance must be established among wetland sampling sites, and the response of specific attributes of the biological assemblage(s) to that gradient is determined (Karr & Chu 1999; Barbour *et al.*, 1999).

Determining the range of anthropogenic stressors currently operating at a given wetland site 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 (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 2002a). 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 may 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, a completely pristine site would receive an overall score of 0, while a severely 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 Variables* below). The complete HDA form is presented in Appendix A.

Study sites were ultimately grouped into three categories, based on HDA scores: least-disturbed (HDA = 5-15), intermediate disturbance (HDA = 20-40), and most-disturbed (HDA = 45-65). Although it is not possible at this point to establish a clear gradient of impairment among wetlands experiencing intermediate levels of disturbance, we are confident that wetlands ranked as most-disturbed are experiencing significantly greater anthropogenic stress compared to those ranked as least-disturbed.

### *Environmental data*

The location of the sampling site within each wetland was recorded using a Garmin Rino 120 GPS unit (NAD 83 datum). 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 7:30 and 11:30 am to minimize the effects of normal daily fluctuations in dissolved oxygen (DO) levels. Water temperature and conductivity ( $\mu\text{S}$ ) were measured using a YSI 30 conductivity meter. pH was measured using an Orion Model 210A pH meter. The pH probe was calibrated at the beginning of each sampling day, and calibration was checked at each site sampled during a single day. DO ( $\text{mg/L}$ ) was measured using a Hach Winkler titration kit. Turbidity (NTU) was assessed using a turbidity tube with attached Secchi disk.

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

#### *Macroinvertebrate sampling*

Macroinvertebrates were sampled at each site on the same day water chemistry measurements were taken. Sampling was done using a D-frame dip net with 500  $\mu\text{m}$  mesh in the near-shore zone of emergent vegetation, in water 0.5 to 2.5 ft (0.15 to 0.8 m) deep. Sampling transects were 16-20 ft (5-6 m) long, and were delineated using five cedar stakes driven into the substrate at 4-5 ft (1.2-1.5 m) intervals. 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 surface 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 the 1<sup>st</sup>, 3<sup>rd</sup>, and 5<sup>th</sup> cedar stake.

The volume of sediment in the net bag after three consecutive sweeps was frequently excessive. Sample volume was reduced by taking the net to a different part of the wetland, submerging the bottom of the net bag in the water, and stirring the contents with one hand while gently swirling and bouncing the net in the water. This also allowed any large pieces of debris to be rinsed and removed, as well as 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  $\mu\text{m}$  mesh, and rinsed further to remove sediment. All rinse water was poured through a 500  $\mu\text{m}$  mesh sieve prior to use, to avoid introducing additional invertebrates into the sample. Sample material was transferred to 1-L Nalgene jars and 95% ethanol was added as a preservative, to a final concentration of ~80%. 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.

#### *Activity Traps*

To optimize wetland sampling technique, we investigated whether using activity traps allowed us to capture any taxa that were not represented in dip net samples, particularly active swimmers such as beetles and true bugs that might be better able to escape the nets. Prior to field season we conducted trials of four different types of activity trap construction: clear 2-L plastic bottles with a funnel at each end; clear 2-L plastic bottles with a funnel at one end and the other end

closed; aluminum mesh cylinders with a funnel at each end; and aluminum mesh cylinders with a funnel at one end and the other end closed. The bottle and mesh trap designs were modified from the Minnesota and Ohio EPA, respectively (U. S. EPA, 2002b). Traps were placed in a Portland-area wetland and collected the following morning. Subsequent counts and identification done by the Project Manager indicated that mesh traps with a funnel at each end captured the greatest diversity of macroinvertebrates, and this design was selected for field use.

Each activity trap consisted of fine-mesh aluminum window screen stapled into a 2-foot long open-ended cylinder. A funnel made of flexible mesh with a 0.5 inch opening was stapled securely at each end of the cylinder. After dip net sampling was completed at each site, five activity traps were deployed and left in place overnight. Each trap was submerged ~1 inch below the level of the water in a horizontal orientation and secured to a cedar stake with wire. Traps were retrieved the following morning and organisms from all five traps were pooled into a single sample. The flexible mesh of the funnels facilitated removal of captured organisms; each trap was held vertically over a bucket, both funnels everted, and the entire trap was rinsed down using a squirt bottle. The traps were examined after rinsing and any clinging organisms were removed using forceps. Sample material was concentrated by pouring through a 500  $\mu\text{m}$  mesh sieve, and the pooled sample was preserved in 80% ethanol. In the evening, the ethanol in each sample jar was poured off and replaced with fresh 80% ethanol.

Samples were delivered to Aquatic Biology Associates, Inc. (Corvallis, OR) for identification. All dip net and activity trap samples were randomly subsampled to a count of 500 organisms; if a sample contained fewer than 500 organisms, the entire sample was picked, counted, and identified. Organisms were identified to the lowest possible taxonomic level, usually genus.

### *Statistical methods*

Following a landscape profiling meeting conducted with Mary Kentula (U.S. EPA) and Paul Adamus (Adamus Resource Assessment, Inc.) prior to the 2009 field season, it was determined that several sites sampled in 2007/2008 that were previously classified by DSL as riverine (Adamus & Field 2001) were actually in the HGM flats class: Coyote Creek, EE Wilson north ponds, Finley Brown Swamp, and Pascuzzi. Thus, although 24 sites were sampled in 2008, only 20 were truly HGM-riverine class, and except where noted, all subsequent statistical analysis was conducted on these riverine sites.

### Multivariate Analyses

Except for the collection method analyses (described below), abundance values from dip net and activity trap samples were combined to give cumulative abundance values for each taxon at each site. Resemblance matrices were created for the sites using square-root transformed species abundance data (Bray–Curtis distance measure) and normalized environmental data (Euclidean distance measure) using PRIMER V.6 and PC-ORD5 (Clarke & Gorley 2006, McCune & Mefford 2006). The relationships between species composition and environmental variables were examined using the Mantel test, which tests the hypothesis that species and environmental similarity matrices are not related in multivariate structure using the Pearson correlation method and Mantel's asymptotic approximation for test-statistic evaluation (McCune & Grace 2002). The BEST analysis (BIOENV algorithm, PRIMER V.6) was used to select the environmental variables best explaining species community pattern by maximizing a rank correlation between

their respective resemblance matrices (Clarke & Gorley 2006). Community structure (species by sites) was analyzed with non-metric multi-dimensional scaling (NMS) in PC-ORD5 (McCune & Mefford, 2006). NMS avoids the assumption of linear relationships among variables (species abundances) and is recommended for datasets with many zeros (McCune & Grace 2002). Correlation of species data with the NMS axes indicated the species most important in determining community structure. Correlation of environmental variables with the NMS axes indicated which variables were candidate drivers of community structure. The environmental variables examined included HDA score, pH, conductivity, DO, chloride, total Kjeldahl nitrogen, total phosphorus, water temperature, and average water depth. For visualization, the joint biplot generated by the ordination was rotated to align the first axis with the taxon causing the largest (Bray–Curtis) differences between sites.

SIMPER analysis (PRIMER V.6) was used to reveal dissimilarity in community composition between different types of sites, as well as the contribution of each taxon to observed dissimilarities. Sites were grouped by level of human disturbance (least (1), intermediate (2), and most (3)) and HGM subclass (riverine-impounding and riverine-flowthrough). The additional HGM class (flats) represented in this study did not occur with enough replication to warrant examination of interclass community structure differences.

ANOSIM (Analysis of Similarities, PRIMER V.6) was used to test for differences between activity trap and dip net samples using permutation/randomizations methods on the Bray-Curtis resemblance matrix of species community structure. Presence-absence data was used to determine whether taxa differed between activity traps and dip net samples. Because the Global Test didn't regard the pairwise nature of the data, an additional "proportion" approach was used, in which the number of times a taxon was collected in activity traps but not dip nets at that same site was compared to the total number of times the taxon was collected by either method across all sites. Since the more mobile Odonata (dragonflies & damselflies), Heteroptera (true bugs), and Coleoptera (beetles) were expected to occur more frequently in activity traps, the "proportion" approach was reserved for these taxonomic groups.

Reliability of the sampling technique was examined by comparing species variation between replicates at sites both within a given year and between years. The taxa list was restricted to the fifty species accounting for > 3% of the total abundance score in any one sample, and a single resemblance matrix was created using the 20 samples from both years of data (2007 and 2008) (Clarke & Gorley, 2006). Hierarchical cluster analysis of site assemblages based on species presence-absence community data (Group Average Linkage method, Bray-Curtis similarity, PRIMER V.6) allowed visualization of the tendencies of paired sites to cluster. The SIMPROF permutation procedure was used to test for significance (5% level) of the resulting clades (Clarke & Gorley, 2006).

#### Univariate Analyses

Linear regression analysis was done in Excel to assess the relationship between selected invertebrate community attributes and site disturbance levels. Data from 2007 and 2008 were analyzed separately and also as a pooled dataset. Community attributes were plotted against individual site HDA scores and the  $R^2$  value was determined. The same attributes were also plotted against disturbance category (class 1= least-disturbed, HDA score 5-15; class 2 =

intermediate disturbance, HDA score 20-40; class 3 = most-disturbed, HDA score 45-65). An unpaired t-test was done to see if the attribute mean values differed significantly ( $P < 0.05$ ) between class 1 and class 3 sites. Community attributes were also plotted against environmental variables (pH, water temperature, conductivity, dissolved oxygen, total Kjeldahl nitrogen, and total phosphorus) to examine potential correlations. This was only possible for the 2008 dataset, as water chemistry measurements for 2007 were less extensive.

#### *Preliminary I-IBI development*

We used a variety of criteria to select community attributes with the potential to serve as individual metrics in the developing Index of Biological Integrity. We examined attributes that represented different categories, including taxonomic richness, taxonomic composition, tolerance/intolerance, and feeding group (Barbour *et al.* 1999), paying particular attention to taxa shown to contribute to community differences in multivariate analyses. Final metric selection was based additionally on attributes that discriminated between most-disturbed and least-disturbed sites, and also had a sufficient range of values among sites to be useful in scoring (Karr & Chu 1999). Attributes that were redundant (measured the same community aspect) or represented by a large number of zero's in the dataset were not included (Barbour *et al.* 1999, Karr & Chu, 1999). Attributes that were found to correlate strongly with wetland quality in the Xerces 2007 pilot study were retested; additional community attributes tested were drawn from other studies addressing wetland I-IBI development, including Burton *et al.* (1999), Blocksom *et al.* (2002), and Gernes & Helgen (2002). Community Tolerance Index (CTI) values for taxa reported here are based on the modified Hilsenhoff Biotic Index (Hilsenhoff 1987) and the best professional judgment and regional expertise of the Aquatic Biology Associates, Inc. taxonomic specialist (Robert Wisseman, personal communication) who identified all the samples in this study. We examined the 69 community attributes listed below for each site:

- Total abundance of organisms
- Richness (total # of taxa)
- Margalef's Index  $d$  (a measure of the number of species present for a given number of individuals; sensitive to sample size; calculated in PRIMER V.6)
- Shannon Index  $H'$  (considers both the number and the evenness of the species, sensitive to sample size; better for larger sample sizes; calculated in PRIMER V.6)
- Pielou's Evenness Index  $J'$  (ratio of observed diversity to maximum possible diversity of a community with the same species richness; calculated in PRIMER V.6)
- Simpson Index (a measure of dominance, *i.e.* probability that two randomly selected individuals from the same community will belong to the same species; insensitive to rare species; calculated in PRIMER v6)
- Gastropods: richness, % diversity, and % abundance
- Crustacea + Mollusca: richness, % diversity, and % abundance
- Odonata: richness, % diversity, and % abundance
- Chironomidae: richness, % diversity, and % abundance
- Chironomini richness, % diversity, and % abundance
- Tanytarsini: richness, % diversity, and % abundance
- ETSD (Ephemeroptera, Trichoptera, Sphaeriidae, dragonflies): richness, % diversity, and % abundance
- ECOT (Ephemeroptera, Coleoptera, Odonata, Trichoptera): richness, % diversity, and % abundance

- Sphaeriidae: % abundance
- Hirudinea: % abundance
- Dominance: % abundance of top three and top five most dominant taxa
- Corixidae: % abundance of Heteroptera, % abundance of Heteroptera + Coleoptera, % total abundance
- Tolerant taxa (CTI = 7-8): richness, % diversity, and % abundance
- Highly tolerant taxa (CTI = 9-11): richness, % diversity, and % abundance
- Predators: richness, % diversity, and % abundance
- Collector-gatherers: richness, % diversity, and % abundance
- Non-chironomid Diptera: richness, % diversity, and % abundance
- Coenagrionidae: % abundance of Odonata and % total abundance
- *Caecidotea*: % abundance
- *Chironomus*: % abundance
- *Hyalella*: % abundance
- *Crangonyx*: % abundance
- Oligochaeta: % abundance
- *Hyalella* + *Caecidotea*: % abundance
- Crustacea: richness, % diversity, and % abundance
- Non-insect taxa: richness, % diversity, and % abundance
- Sensitive taxa (CTI 3-5): richness, % diversity, % abundance

Following univariate analyses on the above attributes, those with potential to serve as IBI metrics were selected. Potential metrics were divided into three tiers: Tier 1 consisted of attributes that had both an  $R^2$  value  $>0.25$  when plotted against site HDA scores and a significant difference between the means for least-disturbed (class 1) and most-disturbed sites (class 3), for the 2007 and 2008 datasets analyzed separately and together; Tier 2 consisted of attributes that had either an  $R^2$  value  $>0.25$  when plotted against site HDA scores or a significant difference between the means for least and most impaired sites, for the 2007 and 2008 datasets analyzed separately and together; and Tier 3 attributes had either an  $R^2 > 0.25$  or a significant difference between the means for class 1 and class 3 sites for a single year dataset. For this study, Tier 1 and 2 attributes were examined for their suitability as potential IBI metrics; Tier 3 attributes were noted for additional attention as this work continues in 2009 and 2010.

Each Tier 1 and Tier 2 attribute was further assessed to determine whether the relationship to site impairment could be explained, and if the data range was sufficient to assign values for a preliminary IBI. Because sites were grouped into three main classes (most, intermediate, and least disturbed), potential metric values from 0 to the 95th percentile were trisected (Karr *et al.* 1986). Values in the top one-third received a 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.

## Results & Discussion

### *Sampling method*

ANOSIM was used to test for differences between activity trap and dip net samples. The Global Test comparing within and between variance of groups revealed that the presence-absence community structure of activity traps was not different than that of dip net samples (Global R: 0.013,  $p = .347$ ). Taxa that contributed most to the similarity between the two collection methods were the ramshorn snail *Menetus opercularis* (2.13%), Ceratopogoninae (2.10%), Corixidae (2.09%), and the chironomid *Psectrocladius* (2.06%). However, the Global Test didn't regard the pairwise nature of the data, and wasn't targeted to investigate the specific taxonomic groups we considered most likely to be under-represented in dip nets. Therefore, a "proportion" approach was also designed to draw out potential differences between the two methods that the Global Test may not have detected. This approach examined the proportion of times a taxonomic group of interest was collected by an activity trap but not by dip net at that same site. Odonata, Heteroptera, and Coleoptera were specifically investigated, as these taxa are highly mobile and difficult to capture, and thus had the potential for substantially higher representation (pre-defined to be  $\geq 30\%$ ) in activity traps. This analysis revealed that, when present at a site, Coleoptera, Heteroptera, and Odonata were collected by both methods 98% of the time, 80.6% of the time, and 71.5% of the time, respectively. Although Odonata were most underrepresented of these three in dip net collections, they were also the most underrepresented of these groups in wetland samples in general. Odonata were absent from eight of 20 sites, as opposed to Coleoptera and Heteroptera, which were only absent from either two or three sites.

Since the Global Test revealed that the presence-absence community structure of activity traps did not differ from dip net samples, and that none of the three groups of interest were substantially under-represented in dip net samples, it was concluded that with this study design, a standardized dip net technique captures a representative sample of the wetland macroinvertebrate community, including highly mobile taxa. A single, straightforward sampling method will render this bioassessment technique more accessible to potential users, although it should be noted that the sampling techniques compared in this study are only usable when sufficient standing water is available, and are not suitable in other types of wetlands, such as wet meadows.

### *Consistency and reproducibility*

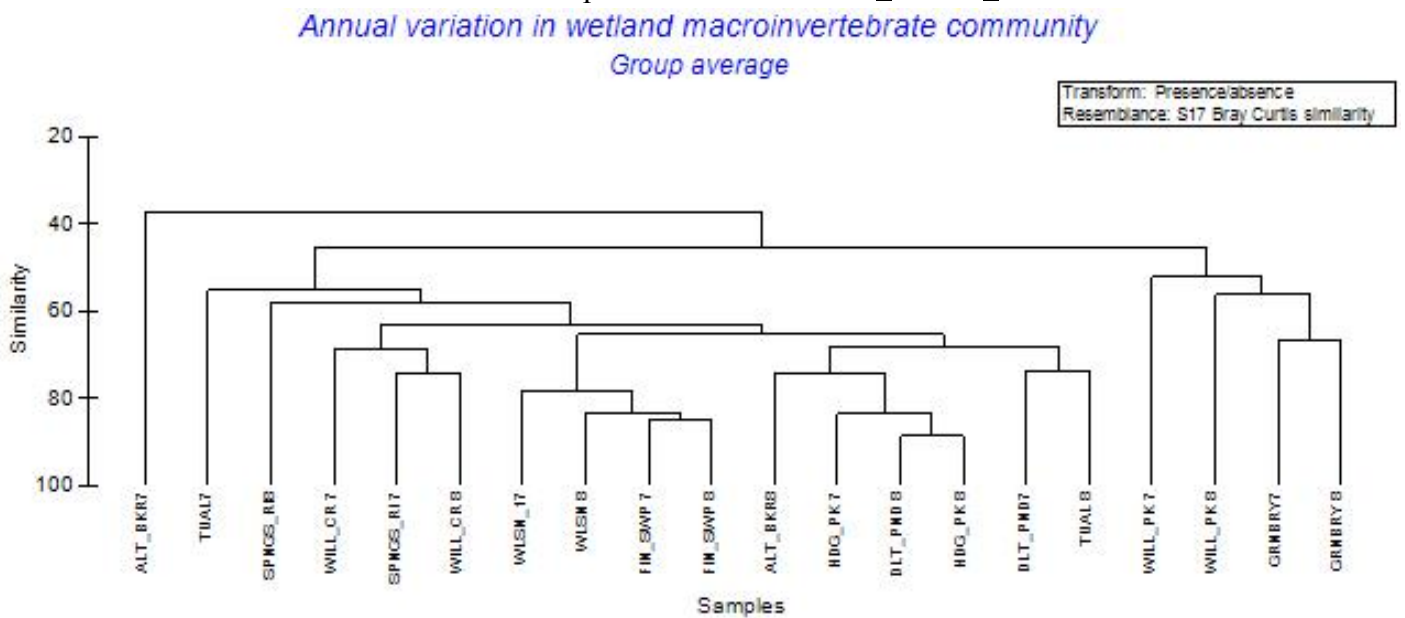
All sampling was conducted according to Xerces' established QAQC. Replicate sampling was done at Hedges Creek TWC to assess sampling precision. Apart from DO and total phosphorus, the results were extremely similar for every chemical aspect measured, as well as for the 69 invertebrate community attributes. Although the overall abundance was almost three times as great in the replicate sample, the majority of community attributes were almost identical, with the greatest differences seen in % abundance Chironomidae (72% difference), % abundance Tanytarsini (76% difference), and % Sphaeriidae (67% difference).

We also examined differences in macroinvertebrate community composition at sites sampled in both 2007 and 2008. Wetlands are dynamic; almost all of the sites in this study dry down by late summer, and must be re-colonized each year by invertebrates that are carried in by floodwaters, fly in from nearby areas that retain water, or persist through the dry season via a drought-tolerant life stage. Large annual dissimilarities in community composition at the same wetland could affect the performance of IBI metrics. Also, while sampling was done at the same time of year

in 2007 and 2008, and in the same portion of the wetlands (with one exception, noted below), a different Xerces staff member conducted the sampling each year. 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.

Hierarchical cluster analysis of site assemblages (presence/absence data) revealed a high degree of similarity in community structure between sites sampled in both 2007 and 2008 (Figure 1). Because the goal of this analysis was to determine annual site variation, we included all wetlands sampled during consecutive years, even though two (Finley Brown Swamp and EE Wilson north ponds) were later determined to be in the HGM flats class, not riverine. Eight of the ten sites sampled in 2007 occur in the same “clade” as the 2008 sample of that same site, with clades defined as having similarities of greater than 60% (Figure 1). The greater annual variation at Alton Baker (Alt\_Bkr) may be due to the fact that a different region of the wetland was sampled in 2008, as the area sampled in 2007 was affected by strong outflow from a large culvert in 2008 and was thus no longer suitable for sampling. The greater variation at Tualatin Hills (Tual) may be because this site is choked with large woody debris and tends to dry down very early, and the thick, debris-filled mud hampers consistent dip net use. Overall, annual variation in the macroinvertebrate community at individual wetlands does not appear to be drastic across the two years measured thus far, even with two different practitioners conducting sampling. This suggests that community attributes used as metrics in the developing IBI should apply consistently from year to year, and that the techniques will be robust for different users.

Figure 1. Comparison of wetland sites sampled in two consecutive years. The numeral at the end of each site name indicates whether it represents data from 2007 or 2008.



### *Descriptive Statistics*

The 2008 dataset contained 169 wetland macroinvertebrate taxa, expanding list considerably from 2007, when 92 taxa were found. Additional taxa in the 2008 dataset included two crayfish, six odonate, nine mayfly, four stonefly, three true bug, six caddisfly, seven beetle, 29 chironomid midge, and 11 non-chironomid Diptera taxa. Ten of the taxa present at sites in 2007 were not

found in 2008; however, all of these taxa had been present at both very low frequency and low abundance in 2007 (at either one or two sites, with 1-3 individuals present) and may not be a regular component of the wetland macroinvertebrate community. The complete 2007-2008 macroinvertebrate taxa list is in Appendix B.

The number of taxa per site at all HGM-riverine wetlands sampled in 2008 ranged from 13 to 51, with a mean richness of 35 taxa. The most abundant taxa were non-biting midges in the genus *Chironomus* (13.2% of total abundance), the aquatic isopod *Caecidotea occidentalis* (12.6% of total abundance), *Crangonyx* amphipods (9.3% of total abundance), corixid bugs (8.1% of total abundance), and oligochaete worms (5.4% of total abundance).

Twenty taxa found in 2008 had only a single occurrence among all riverine sites sampled (*i.e.* one individual at one site), including *Erythemis* dragonflies, *Seratella tibialis* (mayfly), *Pteronarcella* (stonefly), the caddisflies *Hydroptila*, *Mystacides*, *Oecetis*, *Glyphopsyche irrorata*, and *Rhyacophila narvae*, *Zaitzevia* (riffle beetle), *Gyrinus* (whirligig beetle), *Dixa* (dixid midge), Mycetophilidae (fungus gnat), Muscidae (higher flies), *Odontomyia* (soldier fly), and the chironomid midge genera *Macropelopia*, *Paracladopelma*, *Pentaneura*, *Radotanypus*, *Stempellina*, and *Tvetenia bavarica* group. As indicated in Appendix B, some of these taxa are either semi-aquatic or more commonly associated with lotic (flowing water) systems, and are thus expected to occur at lower frequency in lentic (still water) wetlands.

#### *Environmental data*

The 20 HGM-riverine sites varied in HDA scores, physical variables, and water chemistry (Table 1). pH values among all riverine sites ranged from 6.4 to 7.8 (mean =  $7.0 \pm 0.4$ ). Conductivity showed similar between-site variation, ranging from 47 to 405  $\mu\text{S}$  (mean =  $179.7 \pm 86.2$ ). Sites with the lowest conductivity values tended to be more recently flooded; the site with the highest conductivity, Tualatin Hills, had one of the lowest water levels of all the sites at sampling time. Dissolved oxygen levels also varied, ranging from 1.6 to 10.7 mg/L (mean =  $5.6 \pm 3.1$  mg/L). Turbidity was routinely low and varied little among all sites, with the exception of a single outlier (Finley McFadden Marsh). Total Kjeldahl nitrogen was below detection levels (laboratory reporting limit = 0.5 mg/L) at 2 sites; levels at the remaining sites ranged from 0.5-2.8 mg/L (mean =  $1.3 \pm 0.6$  mg/L). Total phosphorus tended to be extremely low; it was below detection limits (0.05 mg/L) at six of the 20 sites, and ranged from 0.08 to 0.86 mg/L (mean =  $0.30 \pm 0.27$  mg/L) at the remaining sites. Chloride levels were below detection limits (1 mg/L) at one site; levels at the remaining sites ranged from 2 to 42 mg/L (mean =  $9.4 \pm 8.6$  mg/L).

The relationship between environmental variables and site HDA score was examined using linear regression analysis. Potential differences between these values for most-disturbed (class 3) and least-disturbed (class 1) sites were examined using an unpaired t-test. Linear regression did not reveal a clear relationship between HDA score and any of the water chemistry parameters examined, although there was a trend towards higher pH at more disturbed sites ( $R^2 = 0.1119$ ). However, pH varied greatly among class 2 (intermediate disturbance) sites (range = 6.4 – 7.7), and mean pH values were not significantly different between class 1 and class 3 sites ( $p = 0.0777$ ). The single exception was water temperature, which showed a strong positive correlation with site HDA score ( $R^2 = 0.4884$ ), as well as significantly different means between class 1 and class 3 sites ( $p < 0.0001$ ). Although lentic systems may be expected to have overall

warmer temperatures than lotic systems, due to the presence of shallower standing water, thermal pollution is a known factor in stream degradation, and may play a role in wetland impairment.

Table 1. Environmental data for HGM-riverine wetlands sampled in 2008. ND = not detectable

Site name	HDA score	pH	Conductivity (uS)	DO (mg/L)	Turbidity (NTU)	Air temp (°C)	Water temp. (°C)	Total Kjeldahl N (mg/L)	Total P (mg/L)	Cl (mg/L)
Alton Baker	35	7.7	51.1	10.2	<10	12.5	14.2	ND	ND	ND
Delta Ponds	40	7.1	159	7.1	<10	22	18.9	0.5	ND	3
Greenberry floodplain	10	6.9	135	1.7	<10	Missing value	12	1.6	0.51	13
Hedges Creek Park	55	7.1	209	3.6	14.5	26	19	2.1	0.58	4
Mt. Pisgah	20	6.9	194	3.5	<10	17.5	14.6	1.3	0.31	4
Randall east Spongs	35	7.8	243	7.3	10	27.2	22.2	1.6	0.1	13
Impounding	10	6.7	155	2.9	<10	16.7	12.6	1.4	0.1	5
Tualatin Hills Willamette	25	7	405	2	21	16.7	17.2	2.8	0.86	12
Park	20	6.4	187	9.7	<10	15.5	13.1	0.9	0.1	13
Willow Creek TNC	15	6.4	147	4.9	<10	14.5	12.8	1	0.08	13
Cedar Mill TWC	40	7.5	202	1.8	<10	25.5	15.8	1.6	0.26	8
Elijah Bristow State Park	20	6.6	118	7.1	<10	15	13.1	0.6	ND	2
Finley McFadden Marsh	20	6.7	94	2.8	90	15.5	13.3	1.7	0.84	9
Harrisburg Riverfront Park	35	6.6	72	5	<10	13	10.4	1.4	0.22	11
Hedges TWC	50	7.1	244	3.9	22	25.5	20	1.4	0.24	5
Knez TWC	50	7.1	106	4.5	12	17.8	18.2	1.4	0.18	8
McDonald Forest Ponds	10	7.5	230	10.2	<10	12.2	9.6	ND	ND	5
PCC Rock Creek	20	7.1	247	6.4	<10	25.5	18	1.1	0.08	42
Philomath Industrial	35	7	274	9.7	<10	21.1	13.7	0.7	ND	10
Spongs Landing RFT	5	7.1	47	10.7	11	15	11	0.7	ND	2

### *Community Structure*

Non-metric multi-dimensional scaling (MDS) revealed patterns of species assemblages, and agreement was found between rank dissimilarities in the Bray–Curtis matrix and distances among sites in ordination space (stress = 0.14). The correlation between environment and species variables in multidimensional space was validated by the RELATE test (Rho= 0.269, p = 0.012).

Human disturbance, pH, water temperature, dissolved oxygen, and total Kjeldahl nitrogen were identified by the BEST test (PRIMER V.6) as the main contributors to the correlation between

species and environmental structures. Together, these five variables did the best job correlating the resemblances of sites based on their community structure and environmental structure (correlation = 0.431). Chloride and water depth contributed very little to the correlation between multivariate species and environmental structures, while conductivity, total phosphorus and turbidity contributed nothing.

SIMPER analysis examined differences in community composition of sites when grouped by either level of human disturbance or HGM subclass. The greatest average dissimilarity between any two groups occurred between most-disturbed and least-disturbed sites, which had an average dissimilarity of 78.90. The average dissimilarity between least- and intermediate-impaired sites was 74.00, while that between most- and intermediate-impaired sites was 60.34. In contrast, differences in community structure were not apparent between wetland subclasses; neither the within-group similarity of riverine-flowthrough sites (35.28) or riverine-impounding sites (35.59) was substantially greater than the similarity of flowthrough and impounding sites (35.18). The same test was performed on the four wetland sites that were re-designated as HGM flats. Although the sample number was extremely small, the within-group similarity of flats (35.66) was not substantially greater than the similarity of flats and riverine impounding (34.31) or flats and riverine flow-through sites (33.00). These results suggest that a single set of IBI metrics will be robust across the HGM riverine wetland class in the Willamette Valley, and that a different set of metrics will not be needed for flowthrough and impounding classes. Additional studies incorporating greater numbers of HGM-flats wetlands will reveal whether a single IBI can be used across different HGM classes.

This analysis also revealed interesting within-group similarities for the different human disturbance classes. Most-disturbed (class 3) sites were the most self-similar in community structure (average similarity = 47.67), while least-disturbed sites were much less similar to each other in community structure (average similarity = 28.05). This suggests that a wider range of more sensitive taxa may utilize minimally disturbed sites, resulting in greater within-group community differences, while only a more limited subset of taxa can survive in more severely disturbed wetlands.

SIMPER also revealed potential indicator taxa for highly disturbed and minimally disturbed sites. Potential indicator taxa should account for much of the multivariate dissimilarity between sites with different disturbances, and exhibit a substantial difference in average abundance between these sites (Keleher & Radar 2008). *Chironomus*, *Caecidotea occidentalis*, and Corixidae each accounted for at least 5% of the dissimilarity between most- and least-impaired sites (cumulative contribution = 18.71%), and exhibited increased abundance at disturbed sites. A list of species that accounted for at least 3% of the dissimilarity between highly and minimally disturbed sites is shown in Table 2.

Table 2. Contribution of taxa to differences in community structure between most-disturbed (class 3) and least-disturbed (class 1) sites.

<u>Species</u>	<i>Class 3</i>	<i>Class 1</i>	Av.Diss	Diss/SD	Contrib (%)	Cum. (%)
	Av. Abund	Av. Abund				
<i>Chironomus</i>	17.10	1.16	5.63	2.19	7.14	7.14
<i>Caecidotea occidentalis</i>	15.63	1.29	5.01	1.36	6.34	13.48
Corixidae	14.79	3.88	4.13	1.26	5.23	18.71
<i>Crangonyx</i>	6.81	6.44	3.76	0.97	4.76	23.48
Oligochaeta	13.07	4.80	3.29	1.71	4.17	27.65
<i>Hyalella</i>	9.78	1.16	3.27	1.04	4.14	31.79
<i>Physa</i>	9.89	0.25	3.06	1.98	3.88	35.67
Dytiscidae	9.27	2.05	2.97	1.25	3.77	39.44
<i>Tanypus</i>	8.46	0.00	2.71	0.75	3.43	42.87
<i>Paratanytarsus</i>	7.69	1.29	2.61	1.37	3.31	46.19
<i>Menetus opercularis</i>	8.98	0.96	2.47	0.90	3.13	49.31

#### *Metric selection and IBI development*

Sixty-nine macroinvertebrate community attributes were selected as described in the Methods and assessed for their relationship to human disturbance and potential for use as metrics in the developing IBI. All community attributes assessed in 2007 were examined again with the 2008 and pooled 2007/2008 datasets. Additional metrics were selected based on the results of multivariate analysis, with particular attention paid to taxa that contributed most substantially to community differences between least-disturbed and most-disturbed sites (Table 2).

Tier 1, Tier 2, and Tier 3 attributes were identified as described in the Methods section (ten, sixteen, and eight attributes, respectively). Metrics for the developing IBI were selected from Tier 1 and Tier 2 attributes only, with Tier 3 attributes noted for continuing assessment as more wetlands in additional HGM classes are sampled in the future. Several Tier 3 attributes related to richness and/or abundance of taxa determined by multivariate analysis to contribute the most to community differences between most- and least-disturbed sites, especially *Hyalella*, *Caecidotea*, and *Crangonyx*. Six of the seven community attributes shown to respond strongly to wetland quality in the Xerces 2007 study of 13 riverine-impounding wetlands were also Tier 1 attributes in this study, and two were incorporated as metrics into the IBI (# of highly tolerant taxa and # genera in Chironomini). This suggests that the IBI metrics will be robust for wetlands across different years and different riverine subclasses.

IBI metrics were selected from among the Tier 1 and Tier 2 attributes based on whether the attribute relationship to site impairment could be explained, and if the data range was sufficient to assign values for a preliminary IBI. If two attributes both performed well but were redundant (i.e. measured the same community aspect, such as both richness and % diversity of collector-gatherers), the Tier 1 attribute was used in the IBI. Metrics, ranges, and corresponding IBI values are presented in Table 3. The BEST test (PRIMER V.6) had identified pH, water temperature, dissolved oxygen, and total Kjeldahl nitrogen as the main contributors to the correlation between species and environmental structures. Half of the six IBI metrics selected in this study correlated with at least two of these environmental variables ( $R^2 \geq 0.25$ ), with both # of highly tolerant species and # of predator taxa showing a positive correlation with pH and water temperature, and # of non-insect taxa showing a negative correlation with DO and a positive correlation with water temperature.

Table 3. Preliminary invertebrate-based IBI for Willamette Valley riverine wetlands. For each metric, the range corresponding to least-disturbed sites is given the lowest possible score (1).

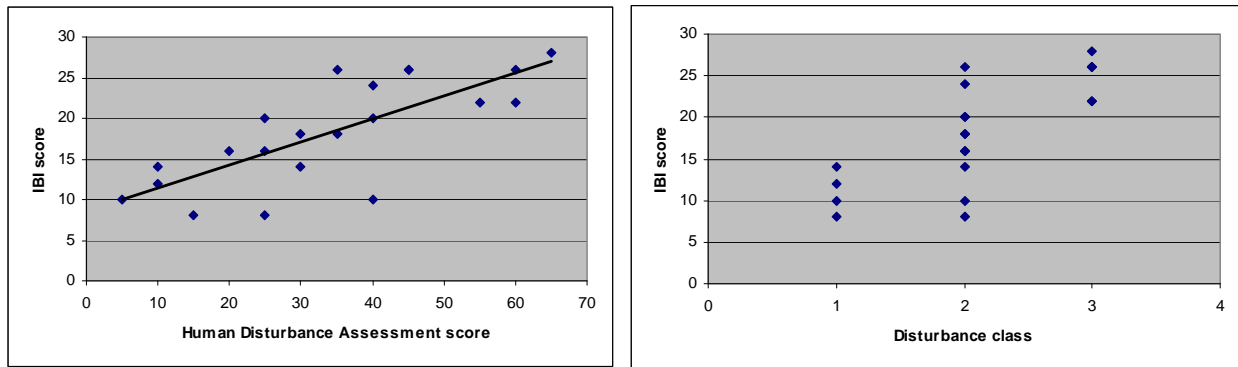
<b>Attribute</b>	<b>Metric range</b>	<b>Score</b>	<b>Rationale</b>
# of highly tolerant taxa* data range 4-20	0-6	1	Increases with site disturbance
	7-12	3	
	13-21	5	
# of predator taxa* data range 0-18	0-5	1	Increases with site disturbance
	6-11	3	
	12-18	5	
# genera in Chironomini* data range 1-8	0-2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

\* indicates Tier 1 attribute

<sup>1</sup> % diversity = # of taxa collector/gatherer / total number of taxa at each site

This preliminary IBI was applied to the 20 HGM-riverine sites sampled in 2008. The four sites classified as least-disturbed received total IBI scores ranging from 8-14 (mean =  $11.0 \pm 2.6$ ); the 11 sites with intermediate disturbance received total IBI scores ranging from 8-26 (mean =  $17.3 \pm 5.4$ ), and the six most-disturbed sites scored from 22-28 (mean  $25 \pm 2.5$ ). Total IBI scores correlated strongly with HDA score ( $R^2 = 0.5743$ ), and separation between overall impairment classes (class 1, 2, or 3) was clearly evident, indicating that these metrics distinguish between most- and least-disturbed sites (Figure 2). Only a single site, Willamette Park, exhibited substantial disparity between HDA and IBI score. Willamette Park was rated as class 2 (intermediate disturbance), with an HDA score near the lower end of the range for this class (HDA = 25). However, this site scored a 1 for five of the six IBI metrics, suggesting that the level of impairment at Willamette Park, an urban greenspace in Corvallis, is likely to have been mis-calculated. If Willamette Park is omitted, the IBI score for the remaining 10 intermediate disturbance sites ranges from 10-16 (mean  $18.2 \pm 4.7$ ).

Figure 2. Relationship between wetland site impairment and IBI score



## Conclusions

This project successfully achieved the stated goals:

### 1. Determine effective wetland sampling methods for macroinvertebrates:

Wetlands were sampled using D-frame dip nets and activity traps. Multivariate analysis of composite dip net and activity trap samples did not reveal significant community differences between the two sampling methods. This was true for the entire pooled dataset, as well as for direct comparison of activity trap versus dip net samples at each individual wetland. With the caveat that users must be properly trained in dip net sampling techniques, these data indicate that a single, standard, low-cost sampling technique can attain a representative sample of the benthos surface and water column of wetlands with sufficient standing water, such as riverine wetlands.

2. *Develop a more extensive database of macroinvertebrate taxa in riverine wetlands of the Willamette Valley:* In 2007, Xerces sampled at 12 riverine-impounding wetlands in the Willamette Valley and found 92 macroinvertebrate taxa. This project, which expanded both the number and the HGM subclass of sampling sites, increased the existing Willamette Valley wetland macroinvertebrate database to 169 taxa.

3. *Identify biological attributes of the wetland macroinvertebrate community that can be used towards developing an Index of Biological Integrity:* We examined 69 different attributes of the wetland macroinvertebrate community assemblages and identified those that varied reliably with the level of human disturbance. These attributes were also applicable to both HGM riverine-flowthrough and riverine-impounding wetlands.

Hierarchical cluster analysis of site assemblages resulted in pairing of sites sampled in both 2007 and 2008. This indicates that the wetland community at a site should be consistent enough from year to year for assessment based on a subset of community attributes to consistently reveal useful information about the site's biological condition. In addition, SIMPER analysis of community composition revealed that site impairment level accounted for the greatest dissimilarity between sites, while HGM subclass was of minimal importance, as riverine impounding and flowthrough sites exhibited similar within-group and between-group similarities. These findings suggest that the same set of community attributes can be used to

assess both riverine-impounding and riverine-flowthrough wetlands in the Willamette Valley, and that a single IBI will serve for the HGM riverine class. This will render the IBI more useful and accessible, especially as the distinction between the two riverine subclasses can be difficult to determine for users not specifically trained in wetland delineation and HGM methodology.

Some macroinvertebrate community attributes that varied strongly with human disturbance level were not selected as potential metrics. Several diversity-related attributes, including total # of taxa per site and Shannon Index of Diversity, showed a strong positive correlation with the level of disturbance. These findings run counter to the commonly accepted premise that increasing impairment and greater anthropogenic stressors result in a decrease in macroinvertebrate community diversity. However, in both 2007 and 2008, sites ranked as most-disturbed consistently exhibited the highest overall diversity. It may be possible that the more severely impaired sites have a consistently high enough level of environmental disturbance that organisms are constantly being removed and re-introduced and that many populations, including those that may otherwise be competitively inferior, are being maintained at lower levels (Cornell 1978). This pattern of overall greater diversity at most-disturbed sites could affect other diversity-related community attributes. We will continue to examine patterns of taxonomic diversity at our sampling sites, and be cognizant of the potential confounding effect of this phenomenon on other diversity-related metrics.

4. *Create a draft invertebrate Index of Biological Integrity accessible to a variety of users that can be used to monitor wetland health:* As a result of this study, Xerces generated a preliminary IBI consisting of 6 metrics that address different aspects of taxonomic richness, taxonomic composition, stress tolerance, and feeding group: # of highly tolerant taxa, # of predator taxa, # of genera in Chironomina, % diversity collector/gatherers, # of non-insect taxa, and Simpson Index  $(1-\lambda)$ .

5. *Create a reliable, rapid, on-the-ground rubric for scoring the level of human impairment of wetlands accessible to a variety of users:* With the exception of a single site (Willamette Park), the Human Disturbance Assessment form adapted here appears to provide a rapid, straightforward, on-the-ground technique to determine the type and degree of anthropogenic impact surrounding the wetland sampling site. Although much less detailed than the Oregon Rapid Wetlands Assessment Protocol (ORWAP, Adamus *et al.* 2009), the HDA rubric can be used easily by individuals who are not specifically trained in wetland assessment, rendering this bioassessment tool more accessible to a variety of users.

6. *Increase outreach and collaboration in wetland monitoring and assessment projects with regional watershed councils, related nonprofits, and state and city agencies:* During this project, Xerces worked with individuals from a variety of agencies, including Oregon Parks and Recreation Department, Portland Metro Parks, U. S. Fish and Wildlife Service, The Nature Conservancy, The Wetlands Conservancy, West Eugene Wetlands, and several city- and county-level parks and recreation departments. Project data was shared with all site managers, who exhibited a strong interest in the wetland macroinvertebrate communities and the implications for site quality and management practices. Monitoring protocols and taxa lists have been shared with many regional watershed councils, several of which are planning floodplain restoration projects in the future and have expressed an interest in biological monitoring techniques for the

wetlands that will be restored in the process. Most watershed councils are already familiar with the practice of stream macroinvertebrate monitoring, so the application of wetland invertebrate-based IBIs will be a logical extension of their monitoring practices. Xerces Society staff has also been asked to participate in different wetland outreach and education events hosted by the West Eugene Wetlands project, and by the US Army Corps of Engineers & Oregon Department of Fish and Wildlife.

### **Next steps**

- Sample the same riverine wetlands that were assessed in 2007-2008 in 2009 and 2010 to continue to examine annual variation in community composition across a longer time period and test the robustness of the preliminary IBI.
- Sample at additional HGM class of wetlands (flats) to determine if IBI can be used across different HGM classes as well as different subclasses; determine whether there are any major differences in community composition between flats and riverine wetlands, and what taxa contribute the most to any identified differences.
- Continue to expand the database of wetland macroinvertebrate taxa in Willamette Valley wetlands.
- With the expanded dataset, examine Tier 1-3 attributes identified in this study for their performance and potential addition to IBI, and investigate whether different IBI metrics will be required for HGM-riverine versus HGM-flats wetlands.
- Compare wetland disturbance/impairment ratings generated via the HDA rubric to ORWAP results to determine how well the methods agree, and if ORWAP assessment generates a different impairment ranking level for existing wetland sites. High levels of development and agriculture in the Willamette Valley make it difficult to find true reference-quality wetlands. We feel confident that both our HDA rankings and IBI metrics differentiate between most- and least-disturbed sites, but it is an ongoing challenge to determine where along the overall gradient of disturbance each site ranked as having intermediate levels of impairment falls. By comparing HDA with ORWAP, we hope to determine which method gives the most reliable ranking for human disturbance level, and potentially use elements of ORWAP to refine the HDA rubric.
- Revise and amend preliminary IBI as work in this area continues. With continuing sampling at existing wetland sites and the addition of a new HGM wetlands class (flats) in 2009 and 2010, we will be able to test the robustness of the existing metrics and their applicability across different HGM classes in the same ecoregion. We also hope to incorporate additional attributes into the IBI. A multimetric IBI should consist of at least five different metrics, with 8-12 metrics being desirable (Karr & Chu 1999, US EPA 2002a). With additional data, more of the Tier 1 and Tier 2 attributes identified in this study may be revealed a suitable for inclusion in the IBI, and some Tier 3 attributes may become stronger candidate metrics.

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## Appendix A. Wetland Human Disturbance Assessment (HDA) 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 2 <sup>nd</sup> 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 2 <sup>nd</sup> 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:**

**Appendix B. Macroinvertebrate taxa in Willamette Valley riverine wetlands, 2007-2008.**

<b>Taxon</b>	<b>Phylum: Class</b>	<b>Order</b>	<b>Family</b>	<b>Common name</b>
Porifera	Porifera			sponge
<i>Hydra</i>	Cnidaria: Hydrozoa	Hydroida	Hydridae	hydra
Turbellaria	Turbellaria			flatworm
Nematoda	Nematoda			round worms
Oligochaeta	Annelida: Oligochaeta			segmented worms
Erpobdellidae	Annelida: Hirudinea		Erpobdellidae	leech
<i>Helobdella</i>	Annelida: Hirudinea		Glossiphoniidae	leech
<i>Musculium</i>	Mollusca: Bivalvia		Sphaeriidae	fingernail clams
<i>Pisidium</i>	Mollusca: Bivalvia		Sphaeriidae	pea clams
<i>Sphaerium</i>	Mollusca: Bivalvia		Sphaeriidae	fingernail clams
<i>Ferrissia</i>	Mollusca: Gastropoda		Ancylidae	limpets
<i>Lymnaea</i>	Mollusca: Gastropoda		Lymnaeidae	pond snails
<i>Physa</i>	Mollusca: Gastropoda		Physidae	tadpole snails
<i>Gyraulus</i>	Mollusca: Gastropoda		Planorbidae	ramshorn snails
<i>Menetus opercularis</i>	Mollusca: Gastropoda		Planorbidae	ramshorn snails
<i>Helisoma trivolvis</i>	Mollusca: Gastropoda		Planorbidae	ramshorn snails
<i>Crangonyx</i>	Arthropoda: Crustacea	Amphipoda	Crangonyctidae	scuds
<i>Hyalella</i>	Arthropoda: Crustacea	Amphipoda	Hyalellidae	scuds
<i>Caecidotea occidentalis</i>	Arthropoda: Crustacea	Isopoda	Asellidae	aquatic sow bugs
<i>Orconectes</i>	Arthropoda: Crustacea	Decapoda	Cambaridae	crayfish
<i>Orconectes virilis</i>	Arthropoda: Crustacea	Decapoda	Cambaridae	crayfish
<i>Pacifasticus</i> <sup>a</sup>	Arthropoda: Crustacea	Decapoda	Astacidae	crayfish
Acari	Arthropoda: Hydrachnida	Acariformes		mites
<i>Aeshna</i>	Arthropoda: Insecta	Odonata	Aeshnidae	dragonflies
<i>Anax</i>	Arthropoda: Insecta	Odonata	Aeshnidae	dragonflies
<i>Somatochlora</i>	Arthropoda: Insecta	Odonata	Corduliidae	dragonflies
Libellulidae	Arthropoda: Insecta	Odonata	Libellulidae	dragonflies
<i>Erythemis</i>	Arthropoda: Insecta	Odonata	Libellulidae	dragonflies
<i>Sympetrum</i>	Arthropoda: Insecta	Odonata	Libellulidae	dragonflies
<i>Tramea</i>	Arthropoda: Insecta	Odonata	Libellulidae	dragonflies
<i>Coenagrion/Enallagma</i>	Arthropoda: Insecta	Odonata	Coenagrionidae	damsel flies
<i>Ischnura</i>	Arthropoda: Insecta	Odonata	Coenagrionidae	damsel flies
<i>Lestes</i>	Arthropoda: Insecta	Odonata	Lestidae	damsel flies
<i>Acentrella insignificans</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Baetidae	mayflies
<i>Baetis tricaudatus</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Baetidae	mayflies
<i>Callibaetis</i>	Arthropoda: Insecta	Ephemeroptera	Baetidae	mayflies
<i>Centroptilum</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Baetidae	mayflies
<i>Procloeon</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Baetidae	mayflies
<i>Pseudocloeon</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Baetidae	mayflies

<i>Caenis youngi</i>	Arthropoda: Insecta	Ephemeroptera	Caenidae	mayflies
<i>Atennella soquele</i>	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	mayflies
<i>Ephemerella excrucians</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	mayflies
<i>Eurylophella lodi</i>	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	mayflies
<i>Seratella tibialis</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Ephemerellidae	mayflies
<i>Hexagenia</i>	Arthropoda: Insecta	Ephemeroptera	Ephemeridae	mayflies
<i>Rhithrogena</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Heptageniidae	mayflies
<i>Tricorythodes minutus</i> <sup>a</sup>	Arthropoda: Insecta	Ephemeroptera	Leptohiphidae	mayflies
Leptophlebiidae	Arthropoda: Insecta	Ephemeroptera	Leptophlebiidae	mayflies
<i>Paraleptophlebia</i>	Arthropoda: Insecta	Ephemeroptera	Leptophlebiidae	mayflies
<i>Siphonurus columbianus</i>	Arthropoda: Insecta	Ephemeroptera	Siphonuridae	mayflies
<i>Siphonurus occidentalis</i>	Arthropoda: Insecta	Ephemeroptera	Siphonuridae	mayflies
<i>Malenka</i> <sup>a</sup>	Arthropoda: Insecta	Plecoptera	Nemouridae	stoneflies
<i>Zapada cinctipes</i> <sup>a</sup>	Arthropoda: Insecta	Plecoptera	Nemouridae	stoneflies
<i>Isoperla</i> <sup>a</sup>	Arthropoda: Insecta	Plecoptera	Perlodidae	stoneflies
<i>Pteronarcella</i> <sup>a</sup>	Arthropoda: Insecta	Plecoptera	Pteronarcyidae	stoneflies
<i>Belostoma</i>	Arthropoda: Insecta	Heteroptera	Belostomatidae	giant water bugs
Corixidae	Arthropoda: Insecta	Heteroptera	Corixidae	water boatmen
<i>Gerris</i>	Arthropoda: Insecta	Heteroptera	Gerridae	water striders
<i>Ranatra</i>	Arthropoda: Insecta	Heteroptera	Nepidae	water scorpions
<i>Notonecta</i>	Arthropoda: Insecta	Heteroptera	Notonectidae	back swimmers
Saldidae <sup>b</sup>	Arthropoda: Insecta	Heteroptera	Saldidae	shore bugs
<i>Sialis</i>	Arthropoda: Insecta	Megaloptera	Sialidae	alder flies
<i>Brachycentrus occidentalis</i> <sup>a</sup>	Arthropoda: Insecta	Trichoptera	Brachycentridae	caddisflies
<i>Cheumatopsyche</i> <sup>a</sup>	Arthropoda: Insecta	Trichoptera	Hydropsychidae	caddisflies
<i>Agraylea</i>	Arthropoda: Insecta	Trichoptera	Hydroptilidae	caddisflies
<i>Hydroptila</i>	Arthropoda: Insecta	Trichoptera	Hydroptilidae	caddisflies
<i>Oxyethira</i>	Arthropoda: Insecta	Trichoptera	Hydroptilidae	caddisflies
<i>Lepidostoma</i>	Arthropoda: Insecta	Trichoptera	Lepidostomatidae	caddisflies
<i>Mystacides</i>	Arthropoda: Insecta	Trichoptera	Leptoceridae	caddisflies
<i>Oecetis</i>	Arthropoda: Insecta	Trichoptera	Leptoceridae	caddisflies
<i>Glyphopsyche irrorata</i>	Arthropoda: Insecta	Trichoptera	Limnephilidae	caddisflies
<i>Grammotaulius</i>	Arthropoda: Insecta	Trichoptera	Limnephilidae	caddisflies
<i>Limnephilus</i>	Arthropoda: Insecta	Trichoptera	Limnephilidae	caddisflies
<i>Onocosmoecus unicolor</i> <sup>a</sup>	Arthropoda: Insecta	Trichoptera	Limnephilidae	caddisflies

<i>Rhyacophila narvae</i> <sup>a</sup>	Arthropoda: Insecta	Trichoptera	Rhyacophilidae	caddisflies
Dytiscidae	Arthropoda: Insecta	Coleoptera	Dytiscidae	predaceous diving beetles
<i>Optioservus</i> <sup>a</sup>	Arthropoda: Insecta	Coleoptera	Elmidae	riffle beetles
<i>Zaitzevia</i> <sup>a</sup>	Arthropoda: Insecta	Coleoptera	Elmidae	riffle beetles
<i>Gyrinus</i>	Arthropoda: Insecta	Coleoptera	Gyrinidae	whirligig beetles
<i>Brychius</i>	Arthropoda: Insecta	Coleoptera	Haliplidae	crawling water beetles
<i>Haliphus</i>	Arthropoda: Insecta	Coleoptera	Haliplidae	crawling water beetles
<i>Peltodytes</i>	Arthropoda: Insecta	Coleoptera	Haliplidae	crawling water beetles
Hydrophilidae	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetles
<i>Berosus</i>	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetles
<i>Helophorus</i>	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetles
<i>Hydrophilus</i>	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetles
<i>Tropisternus</i>	Arthropoda: Insecta	Coleoptera	Hydrophilidae	water scavenger beetles
Brachycera	Arthropoda: Insecta	Diptera		Higher flies
Ceratopogoninae	Arthropoda: Insecta	Diptera	Ceratopogonidae	biting midges
<i>Dasyhelea</i>	Arthropoda: Insecta	Diptera	Ceratopogonidae	biting midges
<i>Chaoborus</i>	Arthropoda: Insecta	Diptera	Chaoboridae	phantom midges
Culicidae	Arthropoda: Insecta	Diptera	Culicidae	mosquitoes
<i>Dixa</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Dixidae	dixid midges
<i>Dixella</i>	Arthropoda: Insecta	Diptera	Dixidae	dixid midges
Ephydriidae	Arthropoda: Insecta	Diptera	Ephydriidae	shore/brine flies
Muscidae	Arthropoda: Insecta	Diptera	Muscidae	flies
Mycetophilidae <sup>b</sup>	Arthropoda: Insecta	Diptera	Mycetophilidae	fungus gnats
Sciomyzidae	Arthropoda: Insecta	Diptera	Sciomyzidae	marsh flies
<i>Simulium</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Simuliidae	black flies
<i>Odontomyia</i>	Arthropoda: Insecta	Diptera	Stratiomyidae	soldier flies
Tabanidae	Arthropoda: Insecta	Diptera	Tabanidae	horse & deer flies
Tipulidae	Arthropoda: Insecta	Diptera	Tipulidae	crane flies
<i>Arctoconopa</i>	Arthropoda: Insecta	Diptera	Tipulidae	crane flies
<i>Limnophila</i>	Arthropoda: Insecta	Diptera	Tipulidae	crane flies
<i>Limonia</i>	Arthropoda: Insecta	Diptera	Tipulidae	crane flies
<i>Pilaria</i>	Arthropoda: Insecta	Diptera	Tipulidae	crane flies
<i>Tipula</i>	Arthropoda: Insecta	Diptera	Tipulidae	crane flies
<i>Tipula (Angarotipula)</i>	Arthropoda: Insecta	Diptera	Tipulidae	crane flies
Chironomidae pupae	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Ablabesmyia</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Acricotopus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges

<i>Apedilum</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Brillia</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Chaetocladius</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Chironomus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Cladopelma</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Cladotanytarsus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Clinotanypus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Corynoneura</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Cricotopus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Cricotopus</i> <i>Bicinctus Group</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Cryptochironomus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Dicrotendipes</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Diplocladius</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Endochironomus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Eukiefferiella</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Glyptotendipes</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Guttipelopia</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Heterotrissocladius</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Hydrobaenus</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Labrundinia</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Limnophyes</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Macropelopia</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Metriocnemus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Microchironomus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Micropsectra</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Microtendipes</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Nanocladius</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Odontomesa</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Orthocladius</i> <i>Complex</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Parachironomus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Paracladopelma</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Parakiefferiella</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Paralauterborniella</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Paramerina</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Parametriocnemus</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Paraphaenocladius</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Paratanytarsus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Paratendipes</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Pentaneura</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Phaenopsectra</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Polypedilum</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Procladius</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Prodiamesa</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Psectrocladius</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Psectrotanypus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Pseudochironomus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Pseudosmittia</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges

<i>Radotanypus</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Rheocricotopus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Smittia</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Stempellina</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Stempellinella</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Tanypus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Tanytarsus</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Thienemanniella</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Thienmannimyia</i> <i>Complex</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Tvetenia Bavarica</i> <i>Group</i> <sup>a</sup>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges
<i>Zavreliomyia</i>	Arthropoda: Insecta	Diptera	Chironomidae	nonbiting midges

<sup>a</sup> stream taxa, generally rare in samples; may have been washed into wetland sites

<sup>b</sup> semi-aquatic taxa, generally rare in samples