Upper Yellow River Watershed Assessment Pilot Project Report

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Michael Miller, Lori Tate, Mark Hazuga, Ruth Person
Bureau of Water Quality – Monitoring Section

Wisconsin Department of Natural Resources
Box 7921
Madison, WI 53707-79212

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PILOT STUDY GOALS

The Wisconsin Department of Natural Resources (WDNR) Bureau of Water Quality with support from U.S. Environmental Protection Agency – Region 5 (USEPA) and the Midwest Biodiversity Institute (MBI), conducted a watershed assessment pilot study in the Upper Yellow River in central Wisconsin beginning in 2011.

The goals of the pilot were to:

- Apply and evaluate a watershed sampling design that systematically selects stream sampling sites based on the size of the watershed upstream of each sampling site.
- Use robust statistical methods and document their usefulness for assessing stream resources and identifying physical and chemical stressors impacting stream biology within watersheds.
- Evaluate how the various statistical methods used may be applied in future stream resources assessment and watershed management projects.
- Evaluate if the sampling design used can provide more complete information for a wider breadth of Water Division stream assessment and management activities than the Department's current stream assessment efforts.

SUMMARY of STUDY FINDINGS

The sampling design and statistical methods applied provided a rigorous assessment of the physical, chemical, and biological conditions of stream resources in the Upper Yellow River Watershed. The study results provided information to objectively determine which physical and chemical factors appeared to be most responsible for biological degradation at individual assessment sites and throughout the entire watershed, and documented the relative importance of these environmental stressors. Statistical analyses also provided precise estimates of the concentrations of individual pollutants or thresholds for various physical environmental stressors that caused the macroinvertebrate or fish assemblages to decline.

Overall, degraded in-stream and riparian habitat and stream bed sedimentation were the primary physical factors impacting aquatic life in the Upper Yellow River Watershed. The concentrations of total dissolved phosphorus in the Yellow River and its tributaries were some of the highest reported in the state and along with low dissolved oxygen concentrations contributed to biological degradation. The physical and chemical stressors measured throughout the watershed appear responsible for the predominance of environmentally-tolerant macroinvertebrate and fish species in Yellow River and its tributaries.

Riparian and Instream Physical Habitat Assessment Findings:

- Qualitative Habitat Evaluation Index (QHEI) scores, rated stream habitat conditions “good to excellent” at 20 percent of the assessment sites, “fair” at 47 percent of the sites and “poor” to “very poor” at 32 percent of the assessment sites.
- “Moderate” to “heavy” streambed siltation impacted 37 percent of the assessment sites.
- Based on Nonmetric Multidimensional Scaling (NMDS), Canonical Correspondence Analysis (CCA), and Classification and Regression Tree (CART) analyses; land cover: (percent forest or percent wetland) and land use: (percent agricultural land) within a 100m-wide riparian corridor upstream of each of the sampling sites strongly influenced the integrity of the fish and macroinvertebrate assemblages found at each site.
- Based on NMDS and CCA analytical results, overall habitat quality, and the individual habitat metrics of pool depth, stream width /depth ratio, and percent cover for fish were the most significant instream habitat measures influencing the fish or macroinvertebrates assemblages.
- Based on CART analyses, stream habitat quality (QHEI) was the second and stream gradient the third most influential factors affecting macroinvertebrates.
- Based on Quantile Regression (QR) analyses, “good” macroinvertebrate and fish Index of Biotic Integrity scores were strongly associated with “good” QHEI scores.

Streambed Sediment Chemistry Findings:

- While a number of different metals (Cr, Cu, Fe, Mn, Ni, Zn) were routinely detected in streambed sediment, only iron at one sampling site and manganese at another site were at concentrations thought to be toxic to benthic invertebrates based on WDNR sediment quality guidelines.
- Sediment samples collected downstream of 15 urban areas or wastewater treatment plant outfalls were analyzed for polycyclic aromatic hydrocarbons (PAHs). While several PAH compounds were detected at three sites, none of these compounds were at concentrations thought to toxic to benthic invertebrates based on WDNR sediment quality guidelines.

Water Column Chemistry Findings:

- Ten percent of the stream sampling sites had median instantaneous dissolved oxygen concentration measures below the state water quality criterion of five mg/L. Seventeen percent of all of the instantaneous measures of dissolved oxygen collected (n = 344) were below the state water quality criterion. Fifty-five percent of the sampling sites had at least one instantaneous dissolved oxygen concentration measure recorded that was below the state water quality criterion.

- Ninety percent of the sampling sites had median total phosphorus concentrations above the state water quality criterion.
- The median total phosphorus concentration for all of the Yellow River Watershed samples collected was more than twice the median concentration measured in streams statewide.
- The stream site with the highest mean concentration of water column total phosphorus was located downstream of a cheese factory wastewater outfall.
- Three of the five sampling sites with the highest mean total phosphorus concentrations in the Yellow River Watershed were located downstream of Concentrated Animal Feeding Operations (CAFOs).
- Elevated ammonia and total Kjeldahl nitrogen (TKN) levels were found at a number of the sampling sites and based on NMDS and CART analyses, higher concentrations of these chemicals were associated with degraded fish or macroinvertebrate assemblages.
- Three of the four stream sampling sites with the highest water column concentrations of TKN in the Yellow River Watershed were located downstream of CAFOs.
- Based on the results of quantile regression analyses, thirty-five percent of the survey sites' fish or macroinvertebrate populations were impacted by elevated total dissolved solids concentrations.
- Water column chlorophyll *a* concentrations were on average two times higher than what is typically found in "least-disturbed" reference streams in Wisconsin.

Biological Assessment Findings:

- Fifty-four percent of the sample sites had water column concentrations of *Escherichia coli* that exceeded USEPA's water quality criterion for "full body contact" recreational use.
- Sixty-nine different families of aquatic macroinvertebrates were identified from the watershed. The five most numerous invertebrate families in decreasing order of abundance included: Chironomidae (midges), Elmidae (riffle beetles), Aselidae (sow bugs) Pisidiidae (pouch snails), and Leptophlebiidae (prong-gilled mayflies), all of which (excluding the mayfly species) are relatively tolerant of environmental degradation.
- Based on Hilsenhoff's Biotic Index (HBI) scores of macroinvertebrate assemblage data, on average, stream sites throughout the watershed were determined to have a "significant degree of organic pollution" and were in "fairly poor" condition. Twenty-two percent of the sites were rated "good" to "excellent" based on HBI, sixty percent of the sites were rated "fair" to "fairly

poor” and eighteen percent of the sites were rated “poor” to “very poor”.

- Fifty-nine of the 60 watershed assessment sites were surveyed for fish. Ten percent of the sites had no or too few fish to calculate an index of biotic integrity (f-IBI) score.
- A total of 45 fish species were identified from the watershed.
- Central mudminnow had the highest frequency of occurrence of any species and were found at 85 percent of the sampling sites. Creek chub and green sunfish were the next most frequently occurring fish species; both were found at 75 percent of sites.
- The top five fish species most commonly captured in the watershed are all considered tolerant of environmental degradation.
- Fifty-nine percent of the sampling sites had f-IBI scores of “good”, 16 percent were rated “fair”, and 26 percent of the sites were rated “poor”.
- F-IBI scores compared with two macroinvertebrate indexes’ scores often gave conflicting assessments of stream quality, with f-IBI rating stream sites in better condition than the macroinvertebrate indexes at forty-three percent of the assessment sites.

Table 1. Upper Yellow River Watershed assessment summary results grouped by HUC12 watersheds. Sample sites where conditions did not meet state or federal water quality guidelines or standards, or sites with “poor” biological index or physical habitat ratings are highlighted in red. Sites-data colored yellow indicate “fair” condition and green indicates “good” condition. Site-specific physical or chemical stressors impacting fish or macroinvertebrate populations (based on quantile regression analyses) are listed in the far-right column.

Watershed Site ID	Watershed Area (sq. mi.)	Nat. Com. Class	Median TP Conc. mg/L (# samples)	D.O (mg/L)	Fish IBI	HBI	mIBI	QHEI	QHab	E. coli (col/100 ml)	Sediment Pollutants	Factors Limiting Fish or invertebrates
East Branch												
EBM	1.9	CWH	0.101 (2)	7.7	80	6.9	2.2	41	18	110		TP, Habitat
EBW	13.2	CWM	0.107 (4)	8.0	100	5.7	4.5	80	68	1400		TP, TDS
EBMC	7.8	CCH	0.135 (4)	8.1	80	6.0	4.1	72	72	490		TP, TDS
South Branch												
UC8	1.3	WH	0.353 (6)	2.8	0	7.9	3.5	40	42	1300		TP, DO, Habitat, Buffer, TDS
UC22	1.9	CWH	0.185 (2)	9.6	30	7.1	4.2	40	55	280		TP, Habitat
UC25	1.6	CCH	0.576 (2)	5.4	20	7.3	2.7	49	40	17		TP, DO, Habitat, Buffer, TDS
UC19	2.8	CCH	0.491 (3)	7.7	40	7.3	3.1	60	68	650		TP, Habitat, Buffer, TDS
SBM	2.0	CWH	0.771 (2)	3.9	30	7.3	3.6	31	35	520		TP, DO, Habitat, Buffer, TDS
UC23	2.3	CCH	0.347 (3)	5.4	60	8.5	1.8	35	35	120		TP, DO, Habitat, Buffer, TDS
UC4	1.5	CCH	0.281 (6)	6.6	70	7.7	2.3	34	30	99		TP,Habitat, Buffer, TDS
SBHR	8.3	CWH	0.286 (4)	9.7	80	6.9	1.7	72	68	140		TP
SBF	25.1	CWH	0.300 (6)	9.0	90	7.6	6.2	48	45	72		TP, Habitat, Buffer, TDS
SBHL	24.1	CWH	0.313 (6)	7.6	80	6.3	3.7	53	55	130		TP, Habitat, Buffer
SBP	20.5	CWH	0.302 (6)	7.0	90	7.0	2.2	45	47	70		TP, Habitat, Buffer
SBH	14.7	CWH	0.297 (4)	9.1	70	6.3	3.8	43	28	490		TP, Habitat, Buffer
Yellow River Mainstem												
UC27	0.1	CCH	0.084 (2)	7.0	70	7.2	2.7	23	15	119		TP, Habitat, Buffer
UC10	0.6	CCH	0.065 (6)	8.0	50	7.7	2.4	41	15	1120	Fe	TP, Habitat, Buffer
LOIC	3.6	CWH	0.112 (6)	5.1	20	ND	ND	45	40	272		TP, DO, Habitat
UC29	1.5	CCH	0.246 (2)	9.2	ND	6.4	3.6	51	35	1400		TP, Habitat, Buffer, TDS
UC9	0.9	CCH	3.285 (6)	3.6	30	ND	ND	55	62	1700		TP, DO, Habitat, Buffer, TDS
UC6	1.7	CWH	0.539 (5)	2.2	ND	6.9	3.1	28	50	1100		TP, DO, Habitat, Buffer, TDS
UC24	1.8	WH	0.390 (2)	6.7	ND	6.9	1.5	40	25	35		TP, Habitat
UC3	2.4	WH	0.505 (6)	3.5	0	6.9	3.1	35	52	56		TP, DO, Habitat
UC15	1.8	CWH	0.041 (2)	8.4	50	6.1	3.9	56	48	185		TP, Habitat
UC16	1.8	CWH	0.254 (2)	10.4	100	6.5	3.2	32	5	1700		TP, Habitat, Buffer, TDS
UC18	3.9	CCH	1.415 (4)	9.1	80	7.7	3.6	74	70	2400		TP, Buffer, TDS
YR26	17.8	CWH	0.268 (4)	8.3	100	6.3	4.1	70	65	1100		TP, Habitat
YRS	7.8	CWH	0.386 (4)	5.0	100	7.7	2.9	43	48	410		TP, DO, Habitat
YRY	60.7	CWM	0.213 (6)	7.6	80	5.5	4.9	72	57	200		TP, TDS
YRH	42.2	CWM	0.234 (6)	8.4	100	4.6	6.3	91	80	170		TP
YRHC	27.9	CWH	0.251 (6)	7.9	90	6.0	4.3	84	75	650		TP
YR80	212.6	WM	0.216 (6)	7.4	70	5.7	4.4	68	53	101		TP, Habitat
YR54	204.1	WM	0.246 (6)	8.6	72	8.4	3.5	72	48	2		TP
YR13	153.0	WM	0.286 (6)	9.5	77	4.6	6.5	79	63	130		TP
YRDL	144.0	WM	0.275 (6)	9.0	80	5.7	5.0	68	65	20		TP, Habitat
YRUL	131.0	WM	0.288 (6)	9.1	62	3.7	7.8	84	83	89		TP
YRN	128.0	WM	0.259 (6)	8.3	60	4.3	8.1	81	87	150		TP
YRL	109.1	CWM	0.231 (6)	8.7	80	7.2	4.0	80	55	190		TP, Buffer, TDS
YRR	100.3	CWM	0.231 (6)	7.6	100	5.0	4.2	83	53	520		TP, Buffer, TDS
YRE	185.0	WM	0.288 (6)	9.7	70	3.7	5.6	73	53	30		TP, Habitat
Beaver Creek												
BCBB	1.3	CWH	0.138 (2)	7.8	30	7.6	1.7	46	55	490		TP, Habitat, Buffer, TDS
BCM	3.1	CWH	0.159 (4)	5.6	30	7.1	2.3	39	42	1300		TP, DO, Habitat, Buffer, TDS
BC13	10.5	CWH	0.188 (4)	6.0	100	7.6	2.3	74	57	150		TP, DO
Rocky Creek												
UC28	1.5	CCH	0.047 (2)	7.7	80	3.7	4.2	56	62	580		TP, Habitat
RCB	13.5	CWM	0.076 (4)	8.4	80	4.6	5.8	83	77	190		TP
RCE	1.6	CWH	0.078 (2)	3.7	20	7.2	3.5	40	38	550		TP, DO, Habitat, Buffer
RCC	21.5	CWM	0.134 (6)	8.2	100	5.6	4.6	77	62	770		TP
RCR	6.5	CWH	0.068 (4)	6.3	0	6.7	4.1	29	45	99		TP, DO, Habitat
RCF	5.0	CWH	0.075 (6)	9.8	60	6.9	4.6	54	52	1300		TP, Habitat
Puff Creek												
UC21	1.2	CWH	0.129 (2)	8.9	10	7.5	2.5	36	35	1400		TP, TDS
PCP	13.1	CWH	0.145 (4)	7.4	100	4.8	4.2	87	80	118		TP
PCN	11.5	CWH	0.101 (4)	7.1	70	5.0	4.4	68	67	230		TP, Habitat
Unnamed Creek												
UC13	7.1	CCH	0.133 (4)	6.8	90	4.7	4.8	77	67	610		TP
UC1	4.3	CWH	0.141 (6)	6.7	70	6.0	4.3	54	52	650		TP, Habitat
UC7	1.5	CWH	0.298 (6)	8.0	90	5.1	4.3	53	55	920		TP, Habitat, TDS
UC17	1.5	CWH	0.067 (2)	7.4	70	6.3	4.6	55	62	1700		TP, Habitat
Cat Creek												
CCB	4.4	CWH	0.208 (6)	8.6	90	7.1	3.3	76	85	326	Mn	TP, TDS
CC13	3.4	CWH	0.286 (4)	6.0	60	6.8	4.0	59	48	613		TP, DO, Habitat, Buffer, TDS
Owl Creek												
OCL	1.0	WH	0.154 (2)	6.2	ND	6.3	3.2	32	20	ND		TP, Habitat, Buffer, TDS
OCUM	7.8	CWH	0.078 (5)	8.1	80	7.3	3.1	55	38	248		TP, Habitat

The sampling design applied provided a systematic survey and rigorous quantitative assessment of the health of the Upper Yellow River Watershed and objectively documented and ranked which specific environmental factors that based on statistical analyses were determined to be most responsible for biological degradation at each stream site. The information generated by this sampling design can be used by a variety of Department program areas including USEPA Clean Water Act section 305(b) reporting and Section 303(d) listing/delisting, Total Maximum Daily Load (TMDL) modeling, polluted runoff management (USEPA 319 Program) and Wisconsin Pollution Discharge Elimination System (WPDES) permit evaluation. The study also illustrated the utility of statistical tools that have application in a number of the Department's program areas.

The sampling design used in the Yellow River Pilot Project may be most effective for watershed management projects where it's important to accurately identify and quantify sources of environmental degradation with a high level of geographic precision for specific pollutants. This information would likely be most useful when targeted pollution control efforts are being planned, and site-specific implementation of best management practices (BMPs) are needed to cost-effectively control pollutant sources and constrain project and program costs.

INTRODUCTION

Stream monitoring data collected by WDNR can be used to assess resource conditions and help identify factors that cause environmental degradation. This information can also be used to direct and evaluate the effectiveness of watershed management activities, and inform the public, agencies, and legislature on the quality of Wisconsin's stream resources.

Assessment and management of stream resources in Wisconsin is challenging given there are over 40,000 miles of perennial streams and because pollution sources and impacts often vary geographically and over time. Also, there are limited regulatory tools, staff, and financial resources to address watershed management problems, and there is real and perceived competition among local, state, and federal programs needing information on water resource conditions.

The department's current stream monitoring strategy is primarily focused on determining the broad-scale status of stream and river resource conditions. The USEPA is encouraging the Bureau of Water Quality to develop a monitoring strategy that incorporates as many local, state, and federal watershed management program information needs as is practical into comprehensive, integrated, watershed assessment efforts.

It is USEPA's desire that watershed monitoring efforts focus on short–term (2-3 year), small-scale (200-300 square mile watersheds) projects that promote problem identification and direct management actions, versus having a number of different stream and river monitoring efforts that lack integration, and where direct stream and watershed management actions are primarily achieved through a variety of *ad hoc* special projects.

Physical Setting and Background on Watershed

The study was done in the Upper Yellow River Watershed located primarily in western Wood and east central Clark counties in north central Wisconsin (Figure 1).

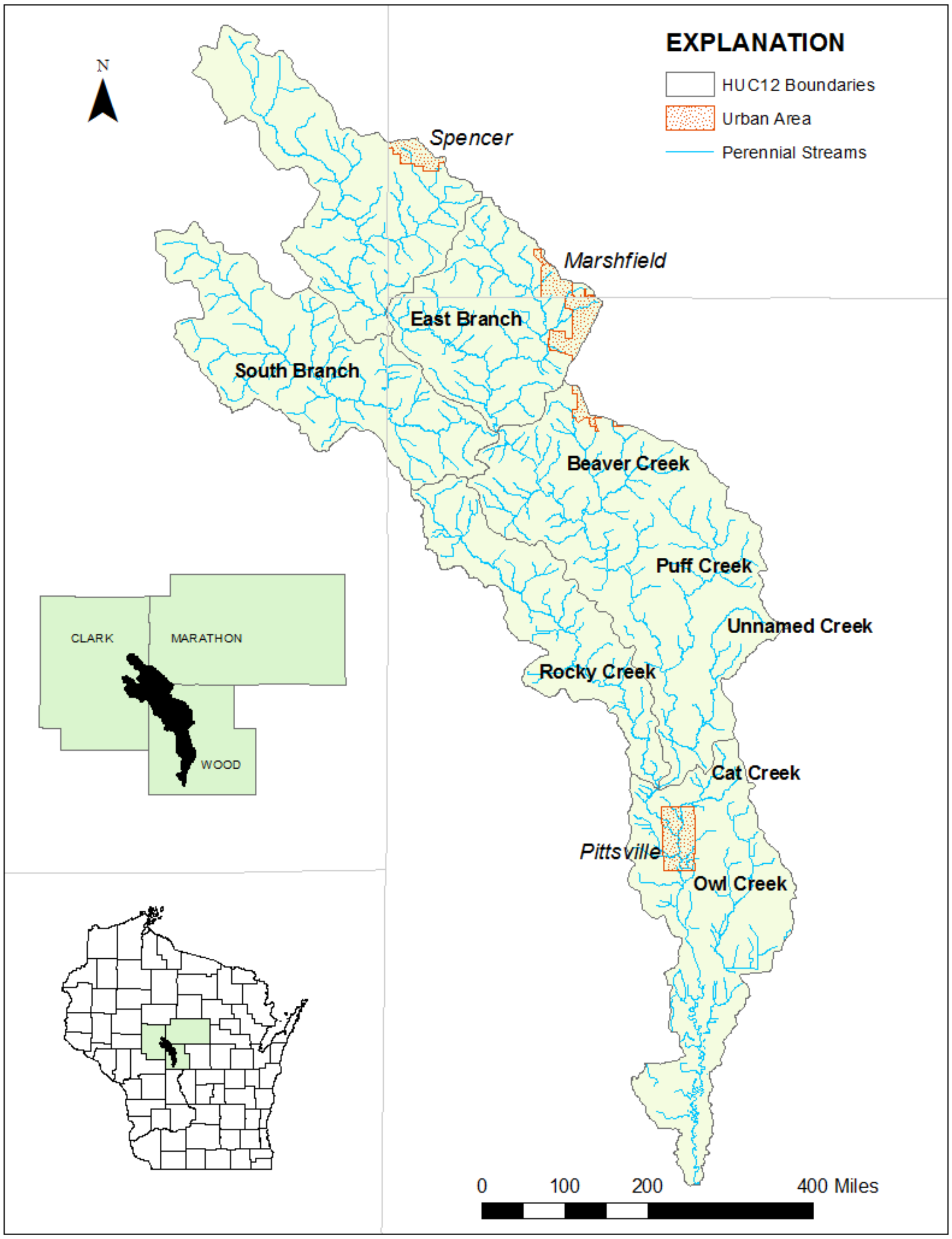


Figure 1. . Yellow River watershed map. Brown-stippled areas represent urban development. HUC12 boundaries within the HUC10 watershed are shown in grey.

The total land area of the Upper Yellow River Watershed is 224 square miles. There are 171 miles of streams most of which support a warm water fishery. The watershed ends at an impoundment of the Yellow River called Lake Dexter which is highly eutrophic and exhibits low oxygen or anoxic conditions in summer months. In addition to Dexter Lake there are two impoundments located in county parks farther upstream on the Yellow River: Lake Manakiki and Kaunewinne, both are shallow, eight and five acres impoundments, respectively. Land use in the watershed is dominated by agriculture. Streams in the watershed are characterized as having weak baseflow (lack of ground water inputs) and first and second order streams often flow intermittently. Streamflows rise and fall rapidly during runoff events as a result of poorly drained soils, and stream channelization, ditching, and tile drainage that accelerates rainfall conveyance. This type of streamflow degrades habitat as a result of channel scouring and streambank erosion and is a harsh environment for aquatic life. Runoff for agricultural land delivers significant sediment and nutrients loads to surface water during storm events.

Total phosphorus concentrations in the Yellow River are some of the highest measured in central Wisconsin and the state. Dexter Lake is listed as an Impaired Waterbody on the USEPA 303d List because of eutrophic conditions caused by excessive phosphorus loading from the watershed. WDNR is currently monitoring Dexter Lake for TMDL development. TMDL monitoring includes bi-weekly fixed period water chemistry sampling in the Yellow River upstream and downstream from Dexter Lake and bi-weekly summer in-lake water chemistry sampling. A long term continuous USGS gauging station at the Village of Babcock generated stream flow data that will be used to calculate pollutant loads for the TMDL project.

Watershed Pollution Sources

Nonpoint source pollution (NPS) contributes sediment, organic matter and nutrients to surface waters in the Upper Yellow Watershed. According to a 2001 Yellow River State of the Basin Report, significant animal waste runoff from barnyards and pastures occurs on the main watershed tributaries. Wood County ranked the watershed as highest priority for NPS erosion control practices. Smaller farms not required to have nutrient management plans dominate the watershed's land use and there are three permitted Concentrated Animal Feeding Operations (CAFOs) in the upper northwest portion of the watershed in Clark County.

Urban stormwater runoff from the west side of the City of Marshfield and the Village of Pittsville are sources of pollution to the East Branch of the Yellow River, the Yellow River mainstem, and Beaver Creek.

Four Point Sources (PS) discharge to streams in the watershed and include three municipal facilities and one cheese factory. The City of Pittsville discharges directly to the Yellow River above Dexter Lake, and the Village of Chili, Bethel Nursing Home and Nasonville Dairy Inc. discharge to small unnamed tributaries.

METHODS

Sampling Design Sites Selection

Data from both randomly and deliberately-selected (targeted) stream sampling sites were used to characterize site-specific and overall conditions of stream resources in the Yellow River Watershed.

The random-sampling sites selection stratification was based on watershed land area. Sampling sites were systematically selected at the drainage outlet (pour point) of specifically-sized watershed land areas. This survey design is referred to as a “geometric” design, since the sizes of the watershed drainage areas selected for sampling were a geometric progression that depended on (with the exception of the initial catchment selected) the size of the most-previously selected watershed land area (Yoder, 2010). For example, the size for the Yellow River Pilot Study watershed was 212.6 mi^2, and a sampling site was situated at the pour point of the watershed at a USGS gauging station site slightly upstream of the terminus of the watershed (Figure 1). The next smallest watershed area sampled (106.3 mi.^{2}) was half the size of the previous watershed land area; the size of the next watershed sampled was 53.2 mi^2, and so forth until the smallest watershed areas (approximately 1.7 mi.^{2} for this study) were delineated and the pour point for each catchment was identified for sampling (Figure 2).

The stream sampling locations of the geometric monitoring sites were moved upstream or down to the nearest road crossing to help facilitate sampling, particularly since water chemistry samples were collected up to six times at each sampling site. Moving the stream sampling sites changed the watershed area of the geometric sites assessed by each pour point by less than ± 10 percent.

Targeted sampling sites were also selected and situated upstream and downstream of known point source discharges, primarily wastewater treatment plant (WWTP) outfalls. Also, in areas of the watershed where stream resources were thought to be underrepresented by the geometric sampling design, best professional judgment was used to place additional “gap” sampling sites (Figure 3).

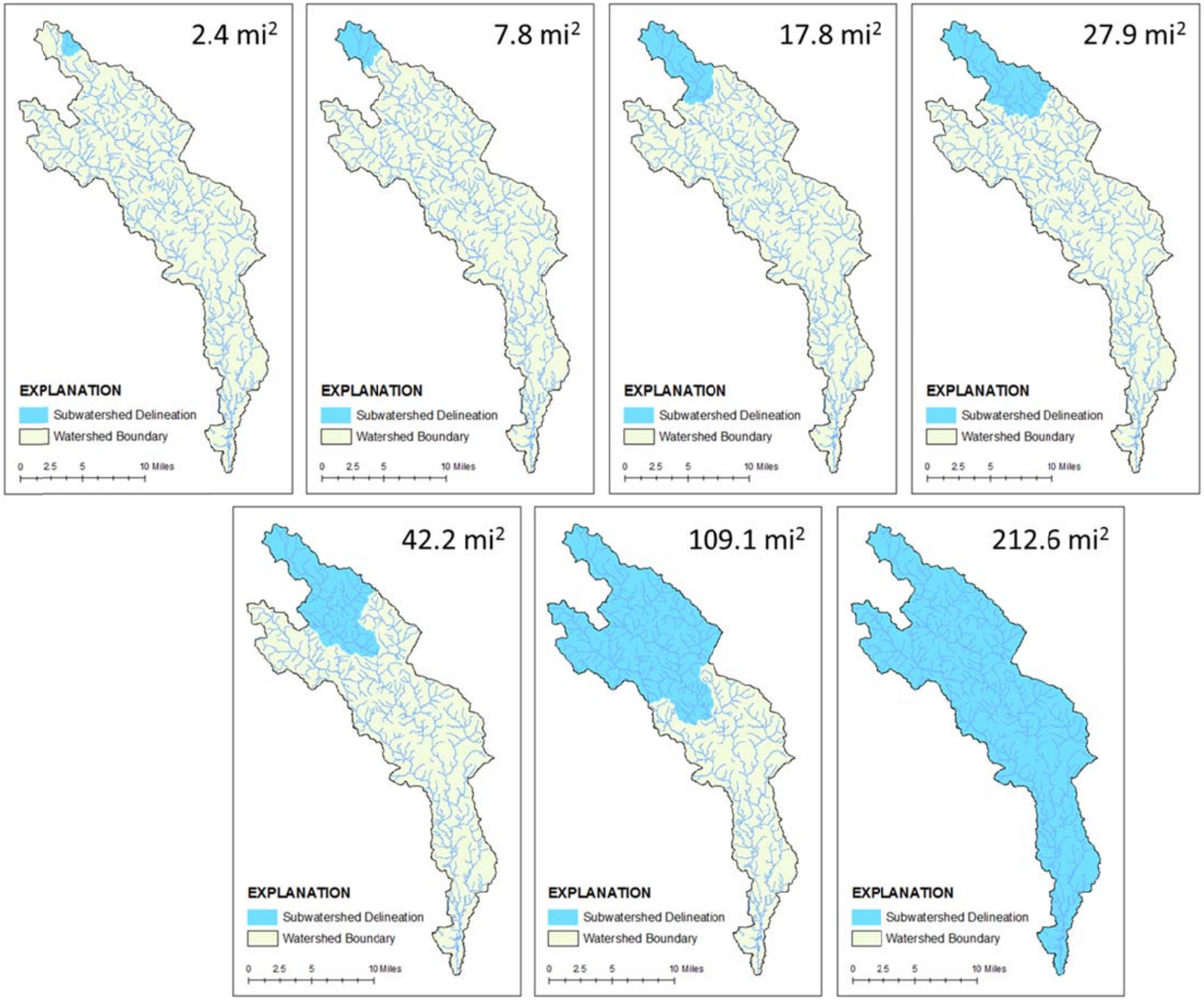


Figure 2. Example showing catchment sizes sampled based on the geometric sampling design. Sites are selected such that the drainage area upstream of each site is approximately half that of the previously-selected sites' drainage area.

Location of Sampling Sites and Data Collected at Each Site

The stream sampling site locations are shown in Figure 3. The location for the pour point draining the entire study area watershed is indicated by a red “X”. Blue dots show the locations of the geometric sampling sites, red triangles show gap-site locations, and green stars mark the targeted sites, including upstream and downstream sampling locations of the WWTP outfalls that are represented by the green municipal symbols. Figure 4 provides the sample site identification numbers that correspond to the data in Table 1.

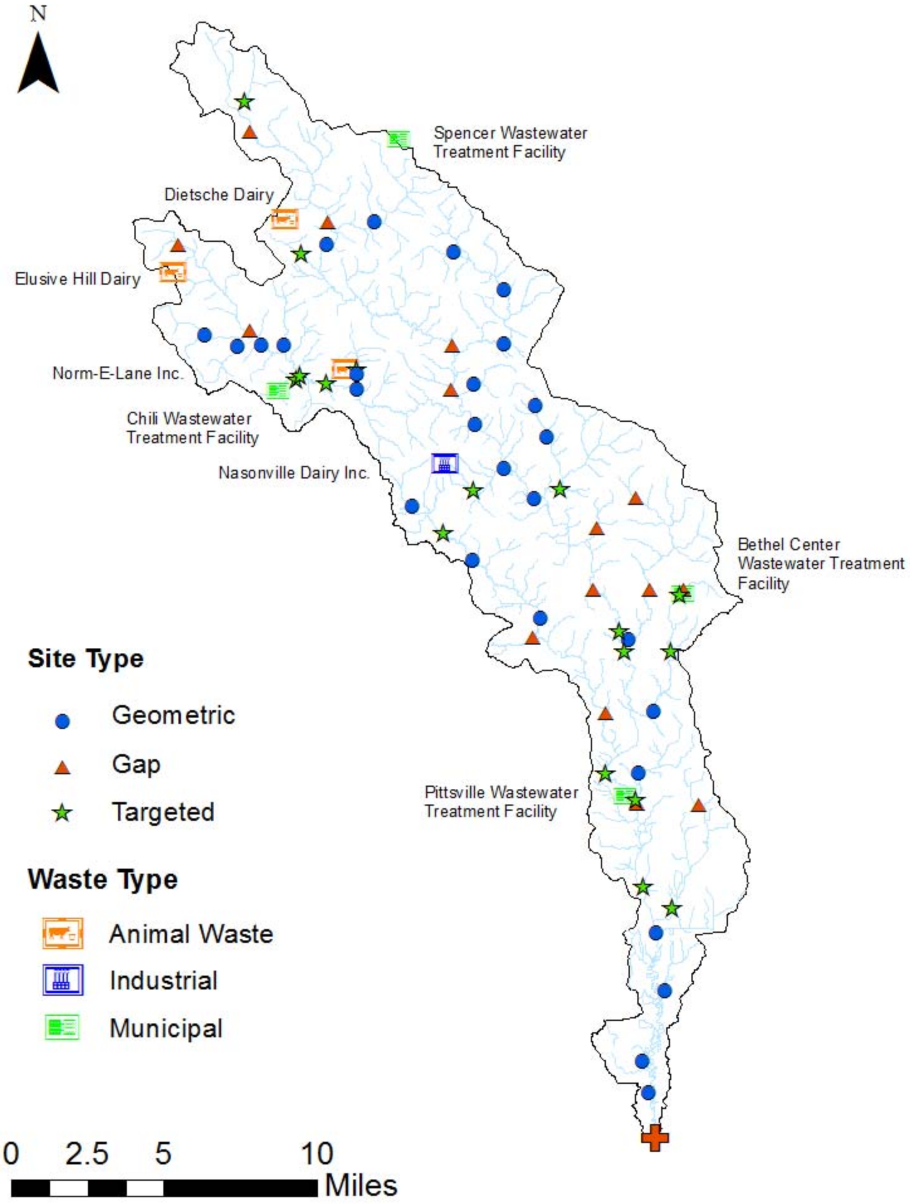


Figure 3. Map of sample-site types, and agricultural, industrial and municipal pollutant sources in the Yellow River Watershed. Red cross identifies the watershed pour point.

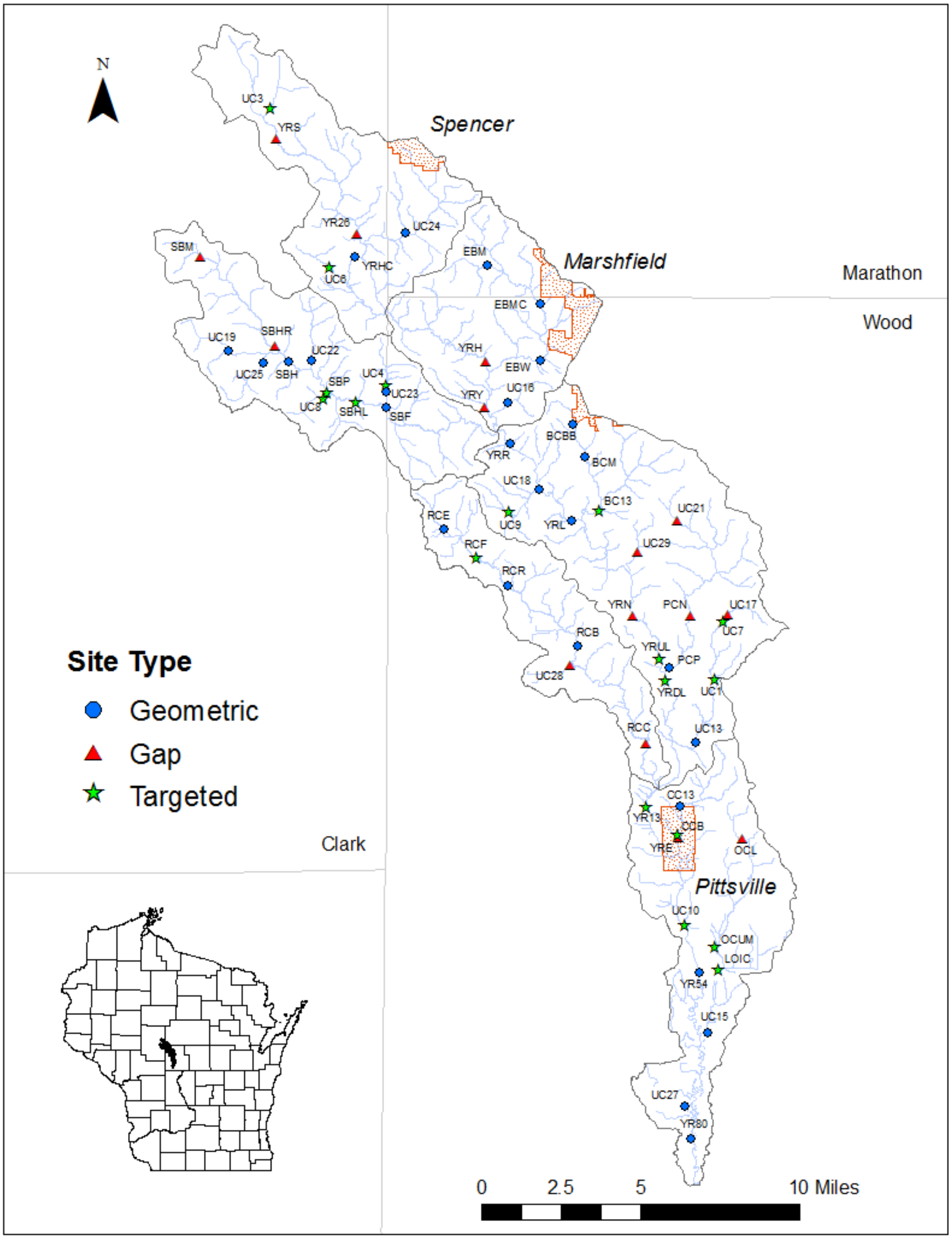


Figure 4. Map showing site ID numbers for Upper Yellow River Watershed sampling locations.

Field Data Collection Methods

Data Collected at Each Sampling Site:
Riparian and in-stream habitat, streamflow volume, water quality, bacteria concentrations, macroinvertebrate, and fish assemblage data were collected at each of the random and targeted sampling sites (n = 60).

Physical Habitat:
Visual estimates of in-stream and riparian habitat were surveyed once at all sites using both WDNR qualitative stream habitat assessment (QHAB) and MBI's Qualitative Habitat Evaluation Index (QHEI) protocols. Watershed and riparian land cover and land uses were quantified using geographic information system (GIS) software and federal land cover information data.

Sediment:
Composite streambed sediment samples were collected at a subset (n = 46) of the larger watershed-area sampling sites. Surficial sediment collected from 4 depositional areas within each study stream reach was composited (500 mL) and were analyzed for:

- Total organic carbon
- Nutrients
    - total phosphorus
    - total Kjeldahl nitrogen
    - ammonia
    - nitrate and nitrite
- Metals
    - cadmium
    - copper
    - iron
    - lead
    - magnesium
    - zinc
- Pesticides
    - 4,4-DDD
    - 4,4-DDE
    - 4,4-DDT
    - Aldrin
    - Alpha-BHC
    - Alpha-Chlordane
    - Beta-BHC
    - Decachlorobiphenyl
    - Dieldrin
    - Endosulfan I
    - Endosulfan II
    - Endosulfan Sulfate
    - Endrin
    - Endrin aldehyde
    - Endrin ketone

    - Gamma-BHC
    - Gamma-Chlordane
    - Heptachlor
    - Heptachlor epoxide
    - Methoxychlor
    - Atrazine
  - Polychlorinated biphenyls (PCBs)
  - Polycyclic aromatic hydrocarbons (PAHs) at 15 sites only
    - 2-Fluorobiphenyl
    - 2-Methylnaphthalene
    - Acenaphthene
    - Acenaphthylene
    - Anthracene
    - Benzo(a)anthracene
    - Benzo(a)pyrene
    - Benzo(b)fluoranthene
    - Benzo(g,h,i)perylene
    - Benzo(k)fluoranthene
    - Chrysene
    - Dibenz(a,h)anthracene
    - Fluoranthene
    - Fluorene
    - Indeno(1,2,3-cd)pyrene
    - Naphthalene
    - Nitrobenzene-d5
    - Phenanthrene
    - Pyrene
    - Terphenyl-d14

Water Quality and Quantity Measures:
Instantaneous measures of water clarity (transparency tube readings), and water quality (measured with electronic meters) were collected in conjunction with the fish surveys and each time water chemistry grab samples were collected. These parameters included:

- water temperature
- dissolved oxygen (DO) concentration
- DO percent saturation
- conductivity
- pH

Stream flow volume was measured once at each stream survey site using an electronic flow meter.

Water Chemistry Grab Samples:
Grab samples were collected May through October during “base flow” conditions.

The smallest (~ 1.7 mi.^{2}) watershed-area pour points were sampled twice during the study, larger watershed pour points (3 - 7 mi^2) were sampled four times, and the largest watershed area pour points (14 mi^2 - 213 mi^2) were sampled six times over the course of the field season (Figure 5, Table 2).

Laboratory-analyzed water chemistry parameters included:

- total phosphorus (TP)
- total dissolved phosphorus
- nitrate and nitrite
- ammonia (NH_3)
- total Kjeldahl nitrogen (TKN)
- chlorides
- sulfates
- biological oxygen demand (BOD)
- total suspended solids (TSS)
- total dissolved solids (TDS)
- suspended sediment concentration (SSC)

One water column grab sample collected from each site was analyzed for chlorophyll *a* concentrations, as well as the same metal and pesticide analytes measured in the sediment samples.

Biological Measures or samples were collected at all sites included:

- fish assemblage data
- macroinvertebrate samples
- *Escherichia coli* samples

Fish assemblage data were interpreted using the appropriate fish index of biotic integrity (f-IBI) for each sampling site, based on the stream's thermal regime and flow volume. Sampling sites were classified using a statewide fish assemblage classification scheme (natural community classification). If the fish assemblage data collected at the stream site and the knowledge of the local water quality biologist suggested the classification model was in error, best professional judgment was used to assign a different stream classification to the site to determine which f-IBI to apply.

Quality Control Sampling:
Ten percent (n=6) of the sample sites were resampled for water chemistry (including sample "blanks"), bacteria, physical habitat, macroinvertebrates, and fish within a few weeks of the initial sampling to evaluate both sampling method and temporal variability.

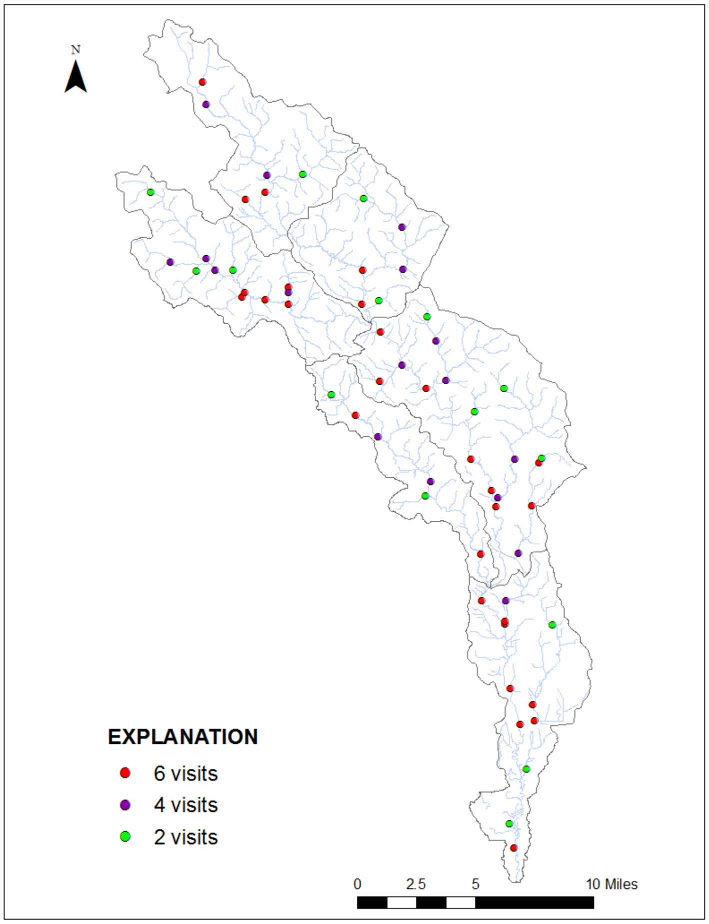


Figure 5. Number of sampling visits per stream site.

Table 2. Number of Yellow River sites and sample events by subwatershed size (panel) groupings.

Panel	**Area (square miles)**	**Site Type** [a]	**No. of Sites**	**No. of Sample Events**	**Mean Drainage Area (range) (sq. mi.)**
1	various	Targeted	18	6	28.8 (0.6-153.0)
1	212.6	Geometric	3	6	200.6 (185.0-212.6)
1	106.3	Geometric	3	6	112.5 (100.3-128.0)
1	53.2	Geometric	2	6	51.5 (42.2-60.7)
1	26.6	Geometric	3	6	24.8 (21.5-27.9)
2	13.3	Geometric	6	4	14.0 (11.5-17.8)
2	6.6	Geometric	5	4	7.5 (6.5-8.3)
2	3.3	Geometric	4	4	3.3 (2.8-3.9)
3	1.7	Geometric	16	2	1.5 (0.1-2.3)
Total			**60**	**262***	

[a] For simplicity, the term "geometric" includes both geometric and gap sites.

*Some targeted sites were added after the first samples were taken (total number of sample events would otherwise be 266).

STATISTICAL METHODS

A series of statistical tests including: Bray–Curtis ordination, Nonmetric Multi-dimensional Scaling, Canonical Correspondence Analysis, Regression Trees, and Quantile Regression were applied independently to both the macroinvertebrate and fish assemblage datasets. The primary goal of using this battery of tests was to determine which watershed land uses, stream physical habitat features, and water quality and chemistry factors had the greatest influence on the biological integrity of individual stream sites and overall stream resources quality in the Yellow River Watershed. This information was then used to help determine which watershed land uses and other human activities appeared to be most responsible for stream degradation. The tests helped provide objective information to evaluate resource conditions, information that can be used direct watershed land use and water resource management actions.

Bray-Curtis Ordination (BC)

BC is a statistical method that simply arranges items along an axis (McCune and Grace, 2002). It was used to group stream sampling sites that had similar fish or macroinvertebrate assemblages. Stream sites most similar (in terms of numbers of species, total numbers of individuals of a species and total number of individuals collected at each sampling site), are closer to one another and sites more dissimilar are farther away from one another along a continuum axis. The BC plots also produce a hierarchy of clusters (groups), showing (usually small) clusters of stream sites very similar to each other and larger aggregates of sites that are less similar. The graphic representation of the data produced by BC ordination is known as a dendrogram or tree that shows the hierarchical clustering and ordering of the dataset. Environmental factors that may explain why some stream sites are similar (cluster) and why other sites are different and placed in different groups, are not identified by BC analysis.

Nonmetric Multi-Dimensional Scaling (NMDS)

NMDS is a type of classification (grouping) process similar to BC ordination (Gauch, 1995). NMDS transforms relatedness among sampling sites’ fish or macroinvertebrate data into a visual representation of distance; sampling sites with similar biotic assemblages are clustered more closely together and dissimilar sites are plotted farther apart. This specific NMDS test is “unconstrained”, meaning that only the fish or macroinvertebrate data influenced the clustering in the data plots, and clusters were not influenced (constrained) by physical or chemical data collected at the sampling sites. The fish or macroinvertebrate taxa most closely associated with the site symbols are plotted on the NMDS diagrams. Some taxa names (particularly macroinvertebrates since there were large numbers of species) were omitted so that the taxa names plotted were legible. Symbols in the NMDS plots used the color coding results from the BC plots. If BC and NMDS analyses result in similar groupings, it provides strong evidence that the site clusters observed are ecologically meaningful.

Constrained NMDS was then used to investigate which environmental characteristics were most strongly correlated with the clustering or dispersion of the stream sites in the unconstrained NMDS cluster analysis, and therefore thought to have the strongest influence on the stream biota within the Yellow River Watershed. Over 130 candidate physical and chemical response variables were regressed upon the NMDS clusters with the stream sites as independent (explanatory) variables and stream sites' physical and chemical characteristics as the dependent (response) variables. Those explanatory variables most strongly correlated with the stream site clusters were plotted as vectors; arrow direction shows increasing magnitude of the environmental variable value, and the longer the arrow length, the greater the relative importance of the variable in influencing the fish or macroinvertebrate site clusters.

Canonical Correspondence Analysis (CCA)

CCA was used in addition to constrained NMDS to further identify the most important physical and chemical factors influencing the fish or macroinvertebrate populations in the Yellow River Watershed. Constrained NMDS relates macroinvertebrate and fish data (numbers of species, individuals within species, and total numbers of individuals collected at each site) to environmental factors that influence these biota (ter Braak, 1995). Similar to NMDS, CCA plots have vector arrows whose direction indicate an increasing magnitude of the environmental variable value, and the longer the arrow length, the greater the relative affect the factor has in influencing the fish or macroinvertebrate populations.

Major assumptions of CCA statistics are that the environmental variables and the biological metric values analyzed have linear responses, and that there is no collinearity between the environmental variables used in the analyses. To address these assumptions, data used in the CCA were transformed (when necessary) to improve linearity, and physical and chemical variable data-pairs were analyzed using linear regression to identify environmental variables that were collinear. One parameter from each collinear-variable pair (e.g. watershed area and stream flow volume) was subjectively removed from the CCA input parameter dataset (e.g. watershed size and stream flow volume are highly correlated, so stream flow volume was removed from the CCA analyses).

Classification and Regression Tree (CART)

CART is a statistical method (De'ath and Fabricius, 2000) that was also used to explore which watershed land use features, stream habitat measures, or water quality or chemistry parameters were most important in influencing the differences seen among macroinvertebrate or fish index scores at the watershed's stream sampling sites.

A "tree" is constructed that repeatedly splits the response variable data (fish or macroinvertebrate index scores) into two groups that maximizes within-group homogeneity and between-group heterogeneity. The regression tree identifies the key explanatory variables (and their relative influence), that are most significant in causing variability seen in biotic index score observed among the sampling sites, which in essences are the key environmental factors influencing the fish and macroinvertebrate populations. The tree can be allowed to continue to split until all of the sample variation is explained which usually results in an overly-large tree that is then “pruned” so that only the most statistically significant explanatory variables are included in the results. Both categorical (e.g. “warm” streams and “cold”), or continuous (e.g. water temperature) explanatory variable data can be included in this statistical test.

Random Forests (RF)

RF is a refinement of CART (Breiman, 2001), that was used to further explore which watershed land use features, physical habitat, or chemical parameters most strongly influenced the variability in macroinvertebrate index scores observed throughout the watershed and therefore were most likely the key explanatory variables influencing the stream biota in the Yellow River Watershed. The RF statistical routine repeatedly runs regression tree analyses (in this study 500 independent repeated iterations of the regression tree analysis were done for on Hilsenhoff’s Biotic index scores) with slightly different subsets of the sample population data used in each iteration. Each statistical run can result in slightly different factors (or orders of importance) being identified as most strongly influencing macroinvertebrate or fish index scores. The watershed land use, habitat or chemical variables that were determined to most strongly influence the variability in biological metric scores most often over the 500 iterations of the RF routine were thought to be the most important factors influencing the stream biota.

Quantile Regression (QR)

QR is a modification of linear regression (Cade and Noon, 2003). Linear regression analysis is often used to evaluate relationships between response variables (e.g. for this study: fish or macroinvertebrate metric scores) and explanatory variables (e.g. water or stream habitat quality or stream habitat measures). The range of the mean value of the response variable (y) is some function of the explanatory variable (x); or y = f(x). A regression model is developed and a straight line is fitted to the y = f(x) equation. The correlation between the cause and effect variables needs to be relatively linear (1:1 response) if the relationship is to be determined to be statistically significant. Ecological cause and effect relationships are often non- linear responses for a variety of reasons. Some cause and effect relationships have a threshold that must be reached by an explanatory variable before the biota will respond (e.g. decreasing dissolved oxygen concentrations). Also, often there are multiple factors influencing the response variable of interest (e.g. macroinvertebrate populations); while there may be strong underlying causal relationships between a single explanatory variable and the response variable, these relationships are often not detected with simple linear regression models, because of the confounding influences of other contributing explanatory variables. QR allows for the detection of more than one slope within an x-y relationship by breaking the data into quantiles, and as a result is more sensitive in detecting the influence of individual explanatory variables.

QR was used in this study to determine whether various land cover or stream habitat features important to supporting healthy populations of macroinvertebrates or fish, and environmental factors (e.g. chemical pollutants, dissolved oxygen, nutrient concentrations) stressful to the stream biota, had specific threshold that once exceeded resulted in improving of declining biotic index scores. These threshold evaluations were then be used to: 1) determine which environmental factors primarily responsible for promoting ecological health or causing biological degradation; 2) determine at what concentration or value of the stressor biological degradation occurs; 3) measure how far stressor thresholds have exceeded the point where biological degradation occurs at individual stream sites; and 4) and discussed how this information can be used to estimate what degree of environmental remediation (reduction of the stressor) is needed to bring the streams back to a healthy state.

STUDY RESULTS

Land Cover and Agricultural Land Use in the Yellow River Watershed

U.S. Department of Agriculture (USDA) 2010 land use data shows that approximately 54 percent of the watershed is agricultural land (Figure 6). Corn and soybeans were the dominant row crops (28% of land area) with extensive areas used for livestock pasture, alfalfa production, and grassland (25%); steeper slopes and some riparian areas are forested (36%), and some developed land (farmsteads, suburban and urban land) also occur within the watershed (6%). Forest and wetlands are more dominant is the southern part of the watershed.

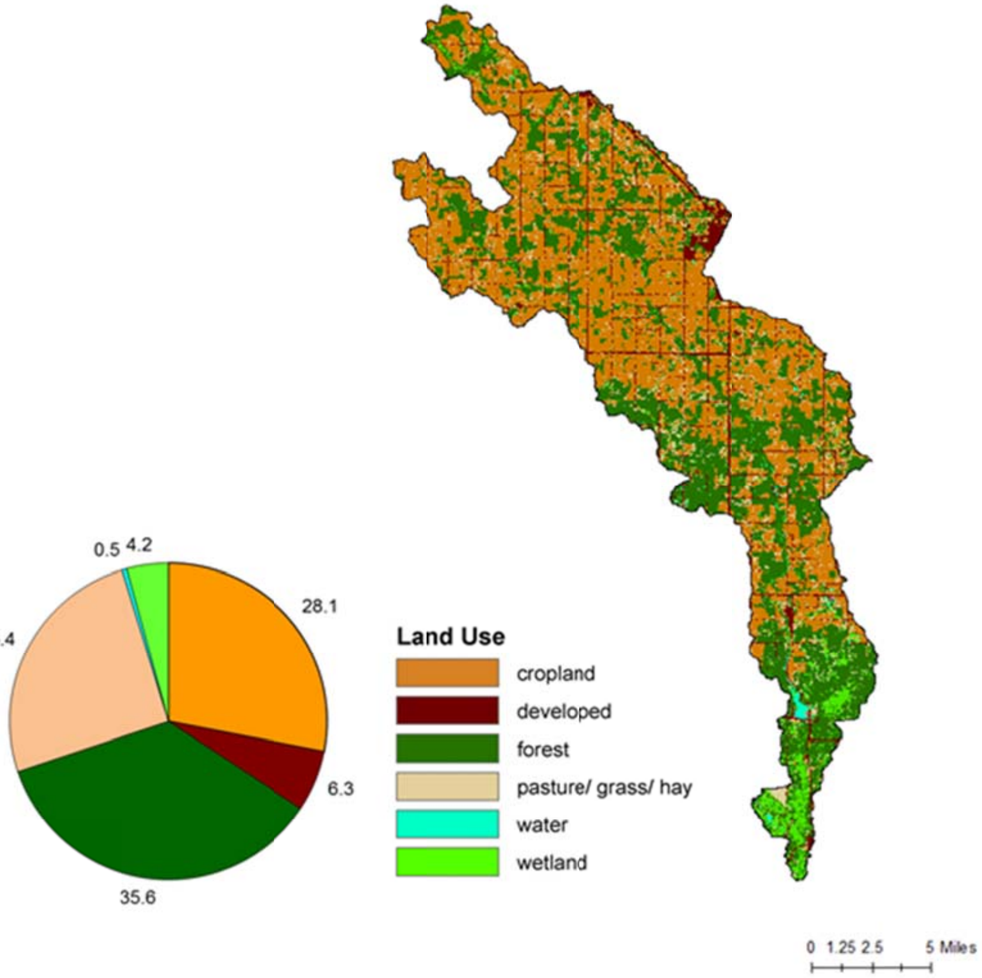


Figure 6. Land use pie chart and map of Yellow River Watershed showing proportions and distribution of land use types.

Physical Habitat, Water Chemistry, and Biological Summary Statistics

A total of 60 randomly-selected and targeted stream sites were sampled in May through October 2011 to assess the physical, chemical, and biological conditions of stream resources in the Upper Yellow River watershed. Summary statistics of a sub-set of the key field sampling results are presented in Table 3. For samples with sediment or water chemistry analyte concentrations below the laboratory detection limits, values were reported at a concentration of one-half the lab’s detection limit, and these values were also used in the computation of the summary statistics and in all other statistical analyses.

Throughout the Results sections “stoplight” colors (red, yellow, green) and weighted dots (big and smaller) are used to distinguish sample site conditions; red is “poor”, yellow is “fair”, and green is “good”, on weighted dot maps large dots indicate “poor” conditions and smaller dots indicate “better” conditions.

Table 3 . Summary statistics for physical, chemical, and biological measures collected from sampling sites in the Yellow River watershed.

	Detection Limit[1]	WI Criteria or Guidance[2,3]	Sample Count	% Non-detect	% Exceed Criteria	Min.[4]	Max.	Mean	SD	Median
Physical Measures										
Drainage Area (mi^2)			60			0.10	212.60	29.90	54.81	4.71
Flow volume (m^3/s)			47			0.00	2.10	0.21	0.45	0.02
Stream gradient (ft/mi)			60			2.33	26.30	11.83	6.49	11.18
Water temperature (° C)			343			6.00	27.70	15.96	5.08	15.20
pH			344			5.92	9.52	7.56	0.46	7.59
Conductivity (µS/cm)			331			27.90	1665.00	235.37	179.90	198.45
Transparency (cm)			335			8.00	120.00	83.34	30.58	90.00
Dissolved O_2 conc (mg/L)		5	344		17%	0.06	16.98	7.63	2.83	7.69
QHEI			60			22.50	91.00	56.87	18.42	54.75
WI Qualitative habitat			60			5.00	87.00	51.78	18.43	53.00
Water Column Chemistry Measures										
TP (mg/L)	0.005	0.075	262	0%	90%	0.03	27.7	0.4	1.8	0.2
TKN (mg/L)	0.14		262	0%		0.2	8.6	1.3	0.8	1.1
NH3 (mg/L)	0.015		262	8%	0%	0.0	1.5	0.1	0.2	0.0
NO_3NO_2-N (mg\L)	0.019		262	16%		0.0	5.2	0.5	0.7	0.2
BOD (mg\L)	n/a		262	n/a		0.05	19.90	1.72	2.32	0.97
TSS (mg/L)	2.0		262	4%		1.00	152.00	10.61	17.40	5.00
TDS (mg\L)	50		262	0%		52.00	970.00	176.76	123.59	146.00
SSC (mg\L)	2.0		262	7%		1.00	159.00	11.62	21.69	5.00
Chloride (mg\L)	1.0	757[3]	262	0%	0%	1.40	308.00	31.48	42.77	20.95
SO_4 (mg\L)	4.5		262	1%		2.25	107.00	10.91	11.40	8.20
Chlorophyll-a (µg/L)	0.26		60	5%		0.13	104.00	10.25	18.73	3.31
Aluminum (mg/L)	0.02		60	67%		0.01	42.70	0.83	5.50	0.01
Manganese (mg/L)	0.0008		60	0%		0.01	22.30	1.27	3.75	0.13
Atrazine (µg/L)	0.25		60	83%		0.13	2.74	0.28	0.43	0.13
Decachlorobiphenyl (DCB) (µg/L)	0.25		60	n/a		0.13	0.20	0.16	0.02	0.15

	Detection Limit[1]	WI Criteria or Guidance[2,3]	Sample Count	% Non-detect	% Exceed Criteria	Min.[4]	Max.	Mean	SD	Median
Streambed Sediment Chemistry Measures										
Arsenic (mg/kg)	8.4-24		46	100%		4.20	12.00	5.03	1.10	4.93
Cadmium (mg/kg)	0.25-0.73		46	65%		0.13	2.60	0.66	0.78	0.15
Chromium (mg/kg)	0.27-0.73		46	0%		2.50	44.00	11.98	7.18	11.50
Copper (mg/kg)	0.18-0.48		46	4%		0.10	25.00	6.77	4.85	5.60
Iron (mg/kg)	0.006-0.08		46	0%		680	54,000	9028	8,024	7,250
Lead (mg/kg)	2.5-7.3		46	87%		1.25	12.00	2.65	2.97	1.50
Manganese (mg/kg)	0.084-0.48		46	0%		5.00	1300.00	247.02	238.45	180.00
Nickel (mg/kg)	0.73-1.9		46	28%		0.38	26.00	6.78	5.81	6.30
Zinc (mg/kg)	4.2-12.0		46	15%		2.25	100.00	38.89	24.69	38.00
Total PAHs (mg/kg)	45.3-458		15	79%		0.02	2.00	0.21	0.41	0.04
Biological Measures										
Hilsenhoff's Biotic Index			59			3.67	8.45	6.42	1.22	6.76
Macroinvertebrate Biotic Index			59			1.53	8.10	3.83	1.41	3.80
Percent EPT Taxa[5]			59			0.00	73.00	20.07	21.37	12.00
Fish Index of Biotic Integrity			56			0.00	100.00	65.91	29.13	71.00
E. coli (colonies/100mL)			59			2.00	2400.00	542.05	557.04	280.00

[1] Laboratory detection limits for individual sediment chemistry analytes and BOD varied based on sample dilution necessary to process individual samples. [2] Criterion values for ammonia vary based on water pH and stream classification and cannot be reported as a single value. [3] Acute toxicity criterion is listed for chloride concentration. [4] Minimum values reported for some analytes were ½ the laboratories' detection limit for those analytes of individual samples that were below detection, these values were also used in all statistical computations and reporting. [5] Percent of individual samples comprised of **E**phemeroptera, **P**lecoptera, and **T**richoptera insect taxa

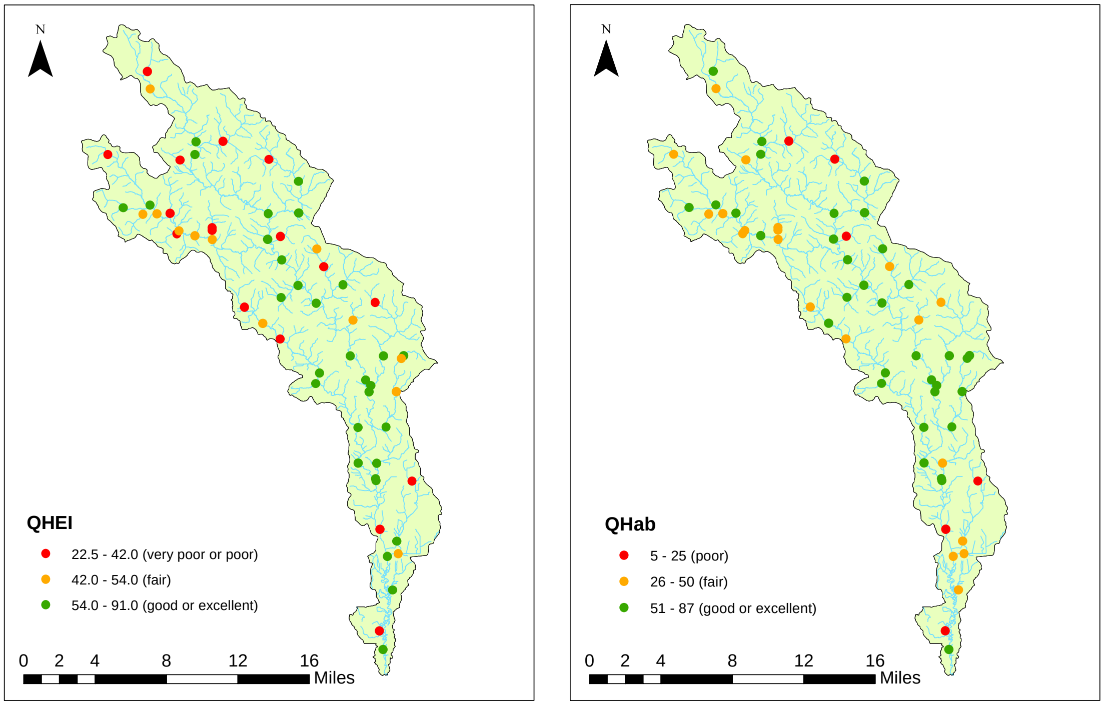


Figure 7. Yellow River watershed stream habitat assessments sites color coded by MBI's QHEI and WDNR's Qualitative Habitat (QHab) scores.

Physical Habitat

Instream and riparian physical habitat was visually assessed at all stream sites using both the MBI's QHEI and the WDNR's QHAB methods and scoring criteria. QHEI scoring ranked more sites as "poor" (29 percent) compared to QHAB (10 percent), both methods had similar numbers of sites rated as "good" (Table 1, Fig. 7). Individual QHEI scoring metrics indicated that "heavy/moderate" silt cover (37% of sites), "high/moderate" overall embeddedness" (27% of sites), and "high/moderate" riffle embeddedness (20% of sites) were the most significant physical factors degrading stream habitat.

Water Quality Measures

Instantaneous measures of water quality (temperature, pH, conductivity, dissolved oxygen concentration and saturation, and water transparency) were collected each time a study site was sampled for habitat, water chemistry, or biology. Instantaneously-measured water quality parameters can be temporally-dynamic over the course of the day and the seven-month study period, and may be strongly influenced by current and antecedent meteorological and hydrologic conditions. But, given the large number of repeated measures taken at each site, spatial patterns in water temperature, transparency, and dissolved oxygen concentrations were discernible. A series of "weighted dot" maps are presented illustrating the results of select water quality and water chemistry measures collected.

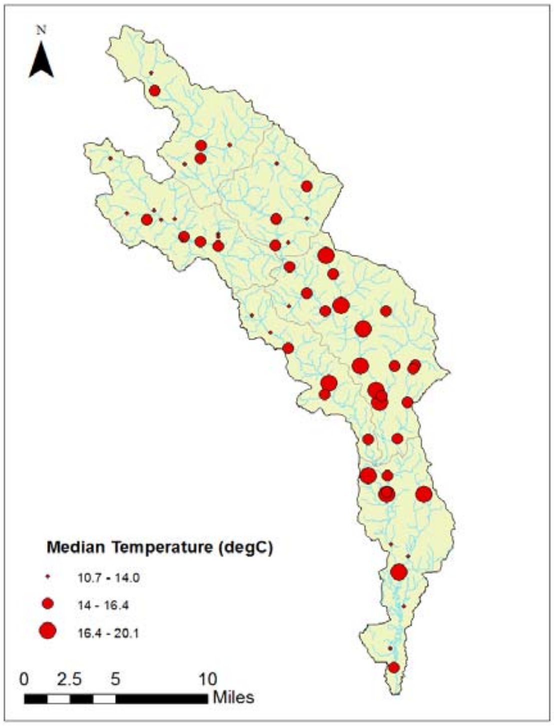


Figure 8. Median instantaneous water temperature measures for Yellow River Watershed stream assessment sites.

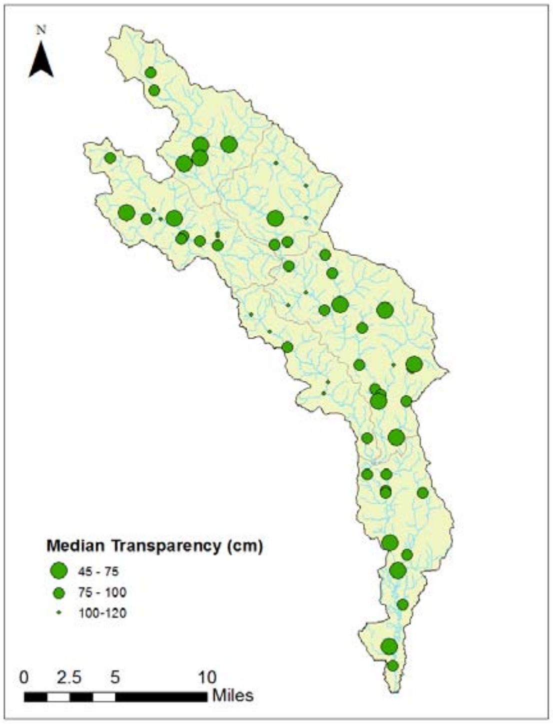


Figure 9. Median instantaneous transparency tube measures for Yellow River Watershed stream assessment sites.

The mean water transparency in the Yellow River Watershed was 83 cm with a standard deviation of ± 31 cm (Table 3), indicating high levels of turbidity and variability in turbidity levels within the watershed. Figure 9 does not show obvious geographic differences in water column transparency within the watershed.

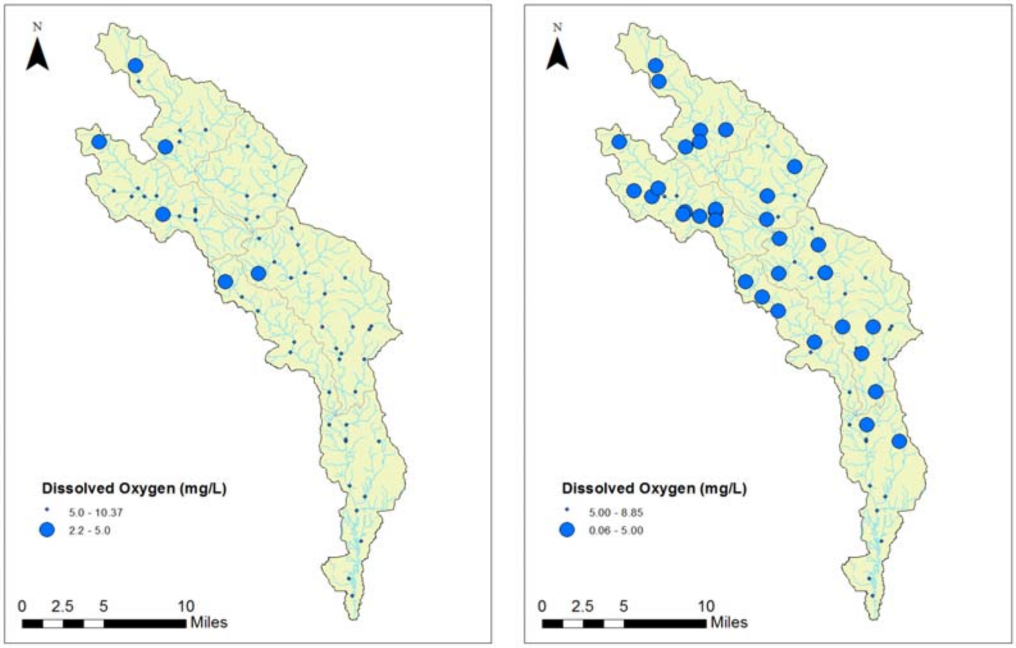


Figure 10. Median (figure on left) and minimum (figure on right) instantaneous dissolved oxygen concentration values at Yellow River Watershed stream sampling sites. Large dots show values that fall below the state dissolved oxygen concentration criterion of 5.0 mg/L.

On average six instantaneous measures of dissolved oxygen concentration were collected at each sampling site. The mean DO concentration of all samples was 8.00 ppm with a standard deviation of 3 mg/L (Table 3). While the average DO concentration at the sampling sites rarely was below the state water quality standard of 5.00 mg/L, over half of the sampling sites had one or more DO measures that were below the state standard (Fig. 10).

Water chemistry sampling results

Nearly 300 water chemistry grab samples from the Yellow River Watershed were analyzed. Small catchment-area sampling sites were sampled twice, mid-sized catchments four times, and the largest catchment areas were sampled six times during the study period (Figure 5).

Water Column Total Phosphorus Concentrations:

Ninety percent of the 262 water column grab samples collected throughout the watershed and analyzed for total phosphorus were above State of Wisconsin’s Water Quality Criterion concentration of 0.075 mg/L. Mean and median total phosphorus concentrations for the sample population were 0.44 mg/L and 0.22 mg/L respectively, indicating a smaller number of very high phosphorus concentration values skewed the population data resulting in a high mean concentration.

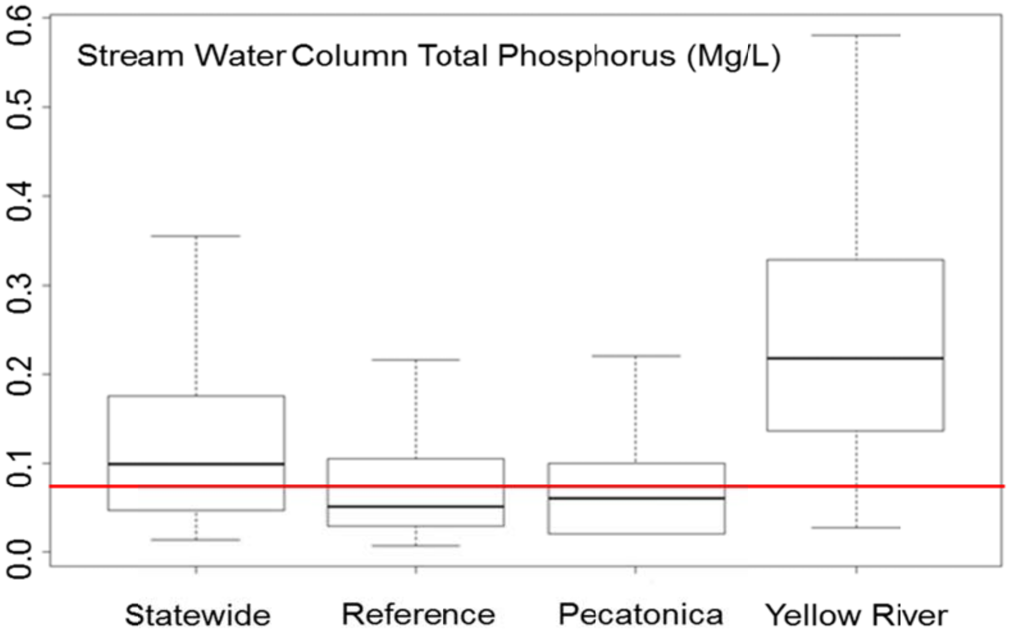


Figure 11. Comparison of total phosphorus concentrations for various stream populations from across the state.

The box and whisker plots (Fig. 11) show the range and most frequent water column concentrations of total phosphorus found in various Wisconsin stream populations. The end of the bottom whiskers represent the lowest phosphorus concentration values reported, boxes show the second and third quartiles of the data range (50 percent of the data), bold line within each box is the median concentration value and end of upper whisker the highest reported phosphorus concentration for each stream population. Statistical outliers were excluded from the top quartile of each sample population. Red line denotes the state water quality criterion threshold for total phosphorus of 0.075 mg/L. “Statewide” data in Figure 11 are from 185 randomly-selected stream sites from across the state sampled in 2010 – 2011. “Reference” data were from targeted sites on streams thought to have least amounts of human disturbance in the state based on land use modeling, or best professional judgment by water quality biologists, sampled from 2004 – 2010 (n = 327). “Pecatonica” sites were a total of 295 grab samples collected from 68 stream sites in 2010 for the East Branch Pecatonica River Watershed Assessment Pilot Project (Miller et al. 2013). The Yellow River data are from the 60 stream sites sampled a total of 262 times with 21 samples excluded as statistical outliers.

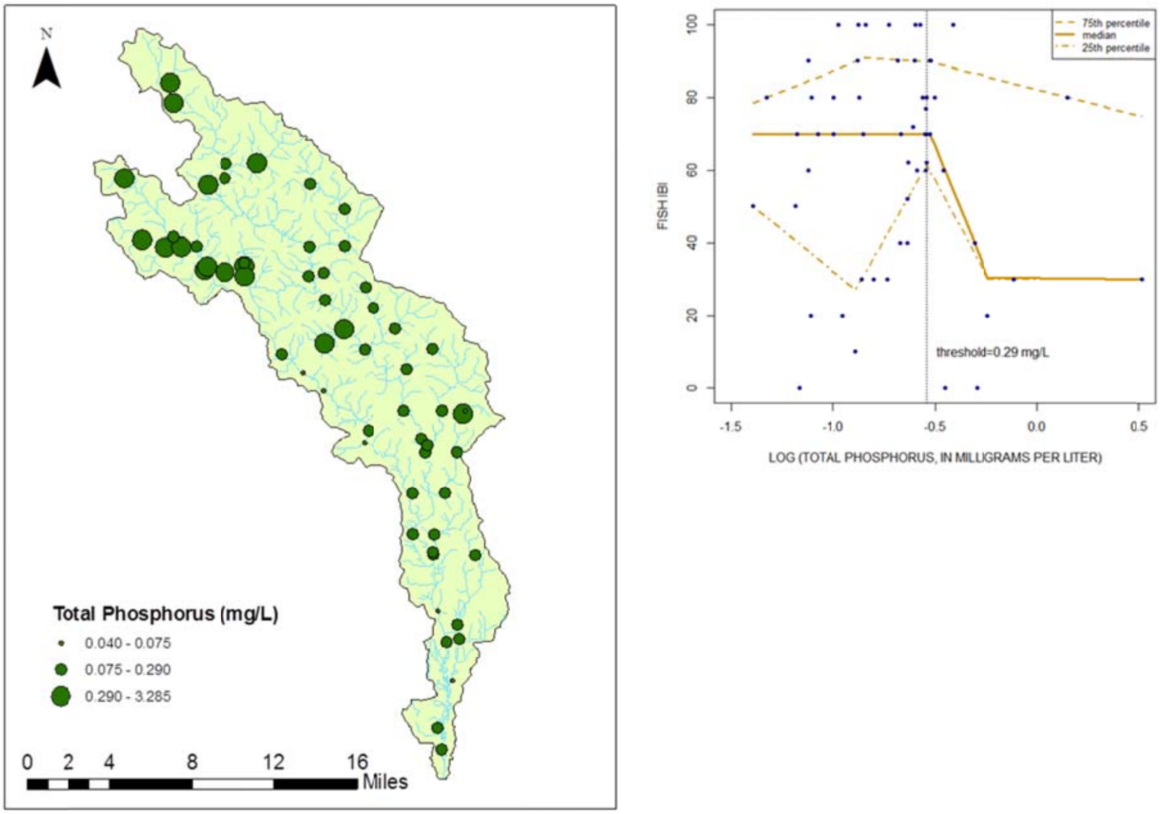


Figure 12. Map showing median water column total phosphorus concentrations in the Yellow River Watershed sampling sites and quantile regression plot showing response of f-IBI to increasing water column total phosphorus concentrations.

Geographic differences in Water Column Total Phosphorus Concentrations
The South Branch subwatershed (Fig. 1) had the highest proportion of sampling sites with median total phosphorus concentrations above 0.29 mg/L, which in addition to being nearly four times greater than the State of Wisconsin Water Quality Criterion, QR analyses suggested this phosphorus concentration was a threshold that once exceeded resulted in degraded fish assemblages at these sites (Fig. 11). The South Branch subwatershed is the location of two of the three CAFOs (Elusive Hill Dairy and Norm E Lane Inc.) located in the Yellow River Watershed (Fig. 3). Site UC7 (Fig. 4) located below the Bethel Nursing Home wastewater treatment outfall had a median total phosphorus concentration of 0.30 mg/L. The highest concentrations of total phosphorus were recorded from a small (Strahler stream order 2) unnamed tributary (Fig. 4, site UC9) sampled below the Nasonville Dairy Inc. cheese factory wastewater outfall. Site UC9 was sampled six times in May – October 2011 with a maximum total phosphorus concentration of 27.7 mg/L, and a mean of 7.04 mg/L (a concentration nearly 100 times greater than the state water quality criterion).

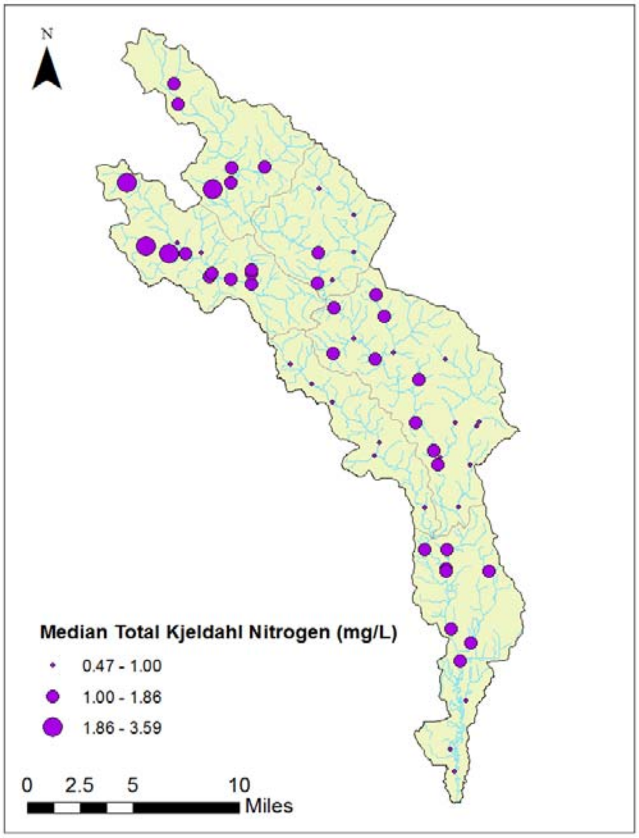


Figure 13. Median Total Kjeldahl nitrogen concentrations for Yellow River Watershed stream assessment sites.

Total Kjeldahl Nitrogen (TKN):
TKN is the concentration of organic nitrogen (approximately 40% of the total concentration) and ammonia (approximately 60% of the total) that occurs in flowing water. While there are no state or federal water quality criteria for TKN concentration, data from “least-disturbed” stream sites throughout Wisconsin (WDNR unpublished data) suggests a concentration between 0.40 and 0.50 mg/L is typical for streams with “good” water quality. The Yellow River summary data in Table 2 shows the average TKN concentration value for the Yellow River was 1.3 mg/L. Similar to the findings for total phosphorus, the South Branch sub-watershed had the greatest proportion of sample sites with “high” (1.86 – 3.59 mg/L) TKN concentration values. Three of the four sampling sites with the highest median concentrations of TKN in the Yellow River Watershed were located downstream of CAFOs.

Water column concentrations of chlorophyll *a* provide a measure of benthic and sestonic algal productivity. Algal productivity is influenced by a number of factors including nutrient concentrations, turbidity, water velocity, stream shading and streambed scouring by storms. The median value of chlorophyll concentrations in the Yellow River watershed was 3.3 µg/L. Robertson et al. (2006) suggest that stream water column concentrations of chlorophyll *a* between 1.2 – 1.7µg/L are indicative of healthy streams in Wisconsin.

Streambed Sediment Chemistry Analyses

Forty-six of 60 stream assessment sites had sediment samples collected for metals analyses. The majority of sites not sampled were headwater streams with small (~1.7 sq. mi.) watersheds. Chromium, copper, iron, manganese, and zinc were routinely detected in streambed sediment (Table 2). Based on WDNR sediment quality guidelines only two samples had metal concentrations thought to be toxic to benthic invertebrates: the iron concentration at targeted site UC10 located downstream of a cranberry farm, and the manganese concentration at targeted site CCB which receives urban runoff from the Village of Pittsville (Table 1).

Fifteen stream sites were sampled for polycyclic aromatic hydrocarbons (PAHs). While three sites (including an urban site receiving runoff from Pittsville) had detectable concentrations of PAHs in the sediment, none reached concentrations thought to be toxic to benthic invertebrates based on WDNR sediment quality guidelines (Table 2).

Bacteria Concentrations

Escherichia coli (*E. coli*) bacteria concentrations data were used to assess the condition of stream sites in the Yellow River Watershed.

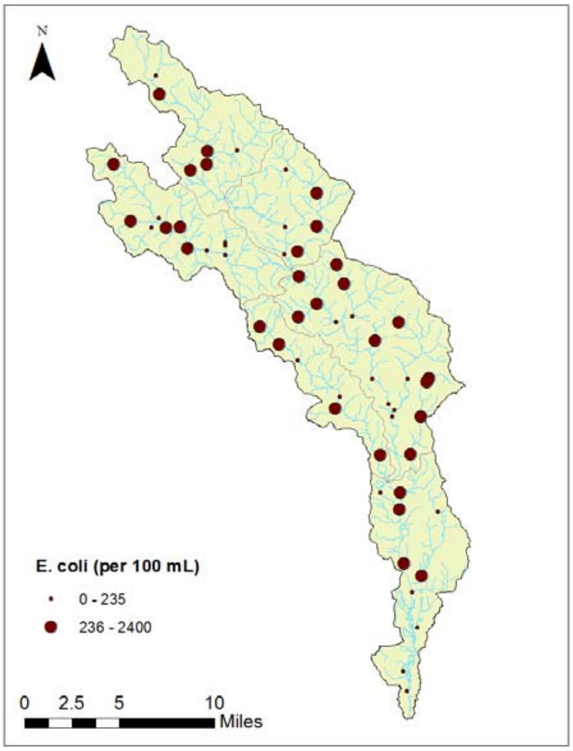


Figure 14. *Escherichia coli* bacteria concentrations (colonies per 100 mL water) for Yellow River Watershed stream assessment sites.

E. coli concentrations were measured once at each sampling site. Bacteria concentrations often have high temporal variability relative to other instantaneous water quality measures. While the limited sampling effort in the Yellow River may restrict the ability to draw strong conclusions about the sources of human and animal feces, or the threats to human or environmental health in the Yellow River Watershed, bacteria concentrations routinely exceeded the federal water quality criterion for full body contact of 235 colony forming units (CFUs) per 100 ml of water. The average CFU concentration for Yellow River sampling sites was 542 CFUs/100mL water. Fifty-four percent of the sampling sites had *E. coli* concentrations above the federal water quality criterion (Figure 14).

Macroinvertebrate Samples

Macroinvertebrate samples were collected at all of the monitoring sites in the fall of 2011. Both Hilsenhoff's Biotic Index (HBI) and a macroinvertebrate Index of Biotic Integrity (mIBI) were used to evaluate the sample results (Fig. 15).

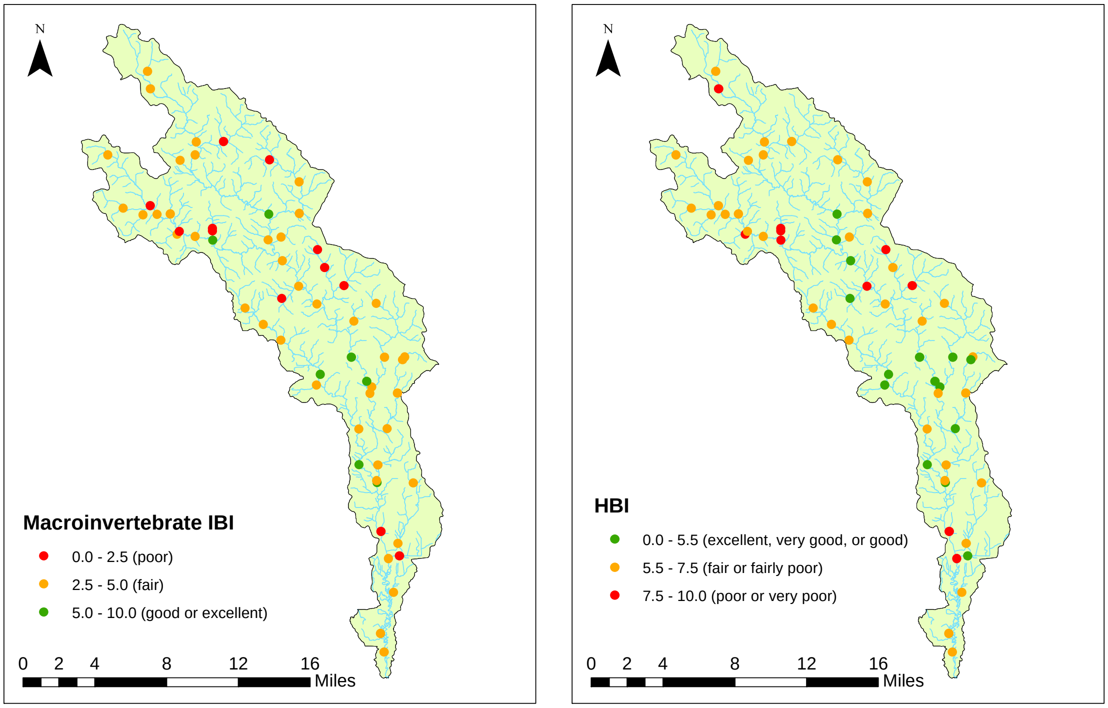


Figure 15. Yellow River watershed assessment sites color coded by macroinvertebrate IBI (m-IBI) and Hilsenhoff's biotic index (HBI) scores.

Fish Assemblage Data

Fish surveys were conducted at 59 sampling sites. No fish were captured at four headwater sites. A total of 45 fish species and 12,890 individuals were captured for the study. Gamefish represented less than one percent of the total fish catch and included: largemouth bass (Micropterus salmoides) n = 8, smallmouth bass (Micropterus dolomieu) n = 6 as well as various panfish (Lepomis spp.) n = 53. Common shiner (Luxilus cornutus) was the most numerically dominant species (n =2,479), and was found at 56% of the sampling sites. Central mudminnow (Umbra limi) n = 1,949, had the highest frequency of occurrence, being found at 85% of the sampling sites. The top five most frequently encountered fish species are all thought to be "environmentally-tolerant" (Fig. 16).

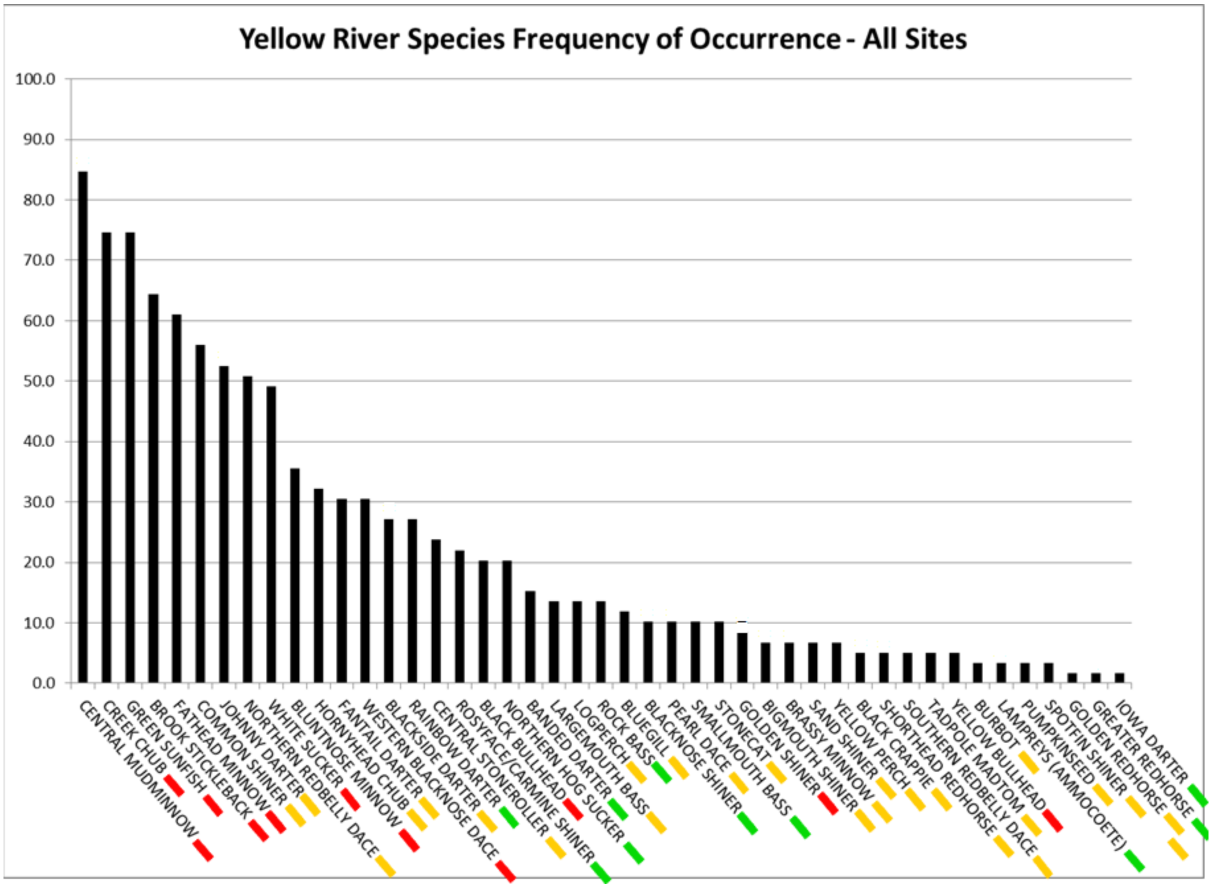


Figure 16. Frequency of occurrence of fish at 59 Yellow River Watershed stream assessment sites. Red color following species common name indicates an environmentally-tolerant species, yellow moderately-tolerant, and green indicates an environmentally-sensitive species.

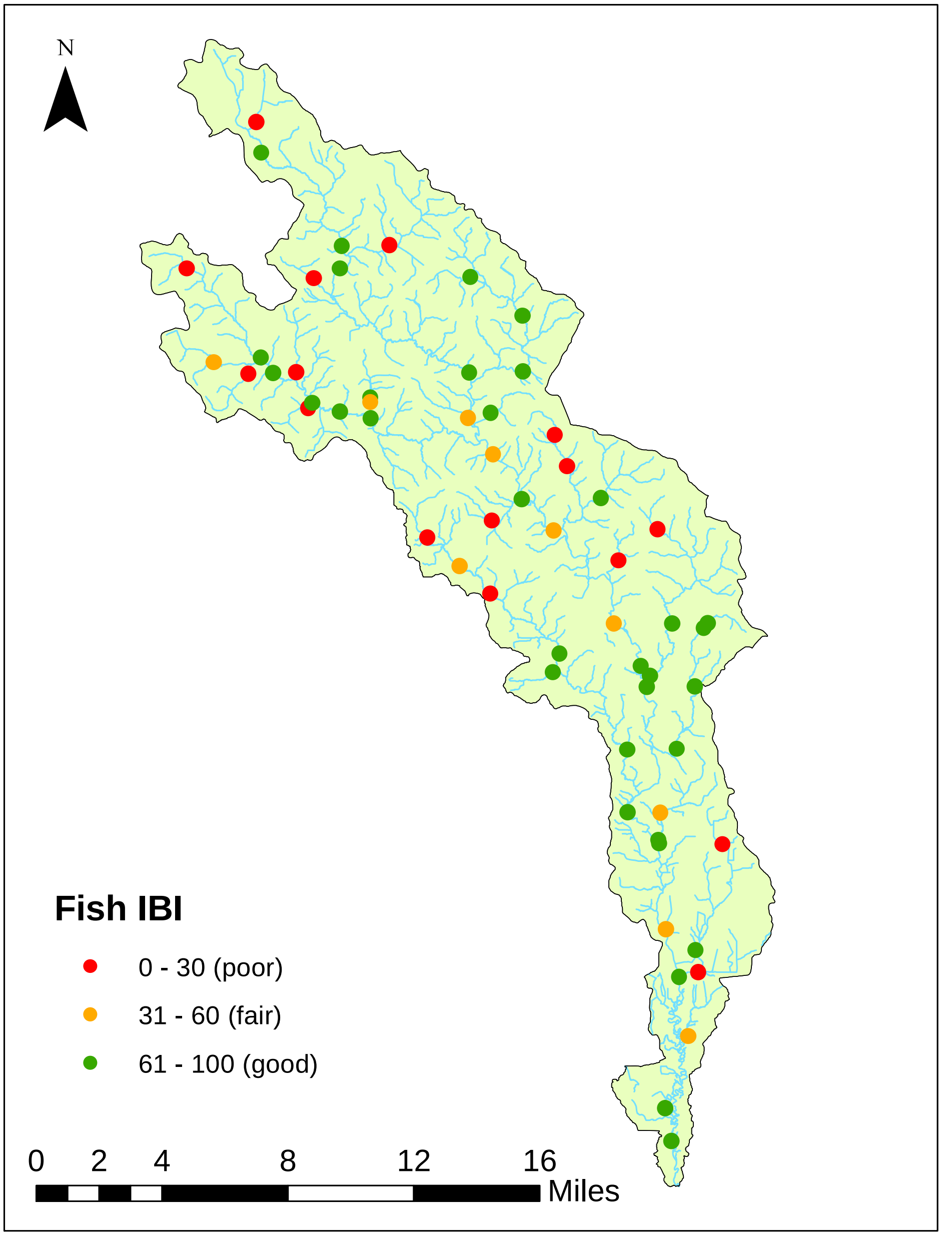


Figure 17. Fish Index of Biotic Intergrity (f-IBI) scores for Yellow River Watershed assessment sites.

Fish Indexes of Biotic Integrity (f-IBI) were used to evaluate the environmental quality of the stream sampling sites (Figure 17). No fish were captured at four survey sites and a f-IBI score were not computed for these sites. Six sites had fewer than 25 individual fish captured per site and a f-IBI scores were not computed but the sites were assigned a rating of “poor”. A total of 26 percent of the sampling sites in the Yellow River Watershed were rated “poor”, 16 percent were rated “fair” and 59 percent of the sites were rated “good”.

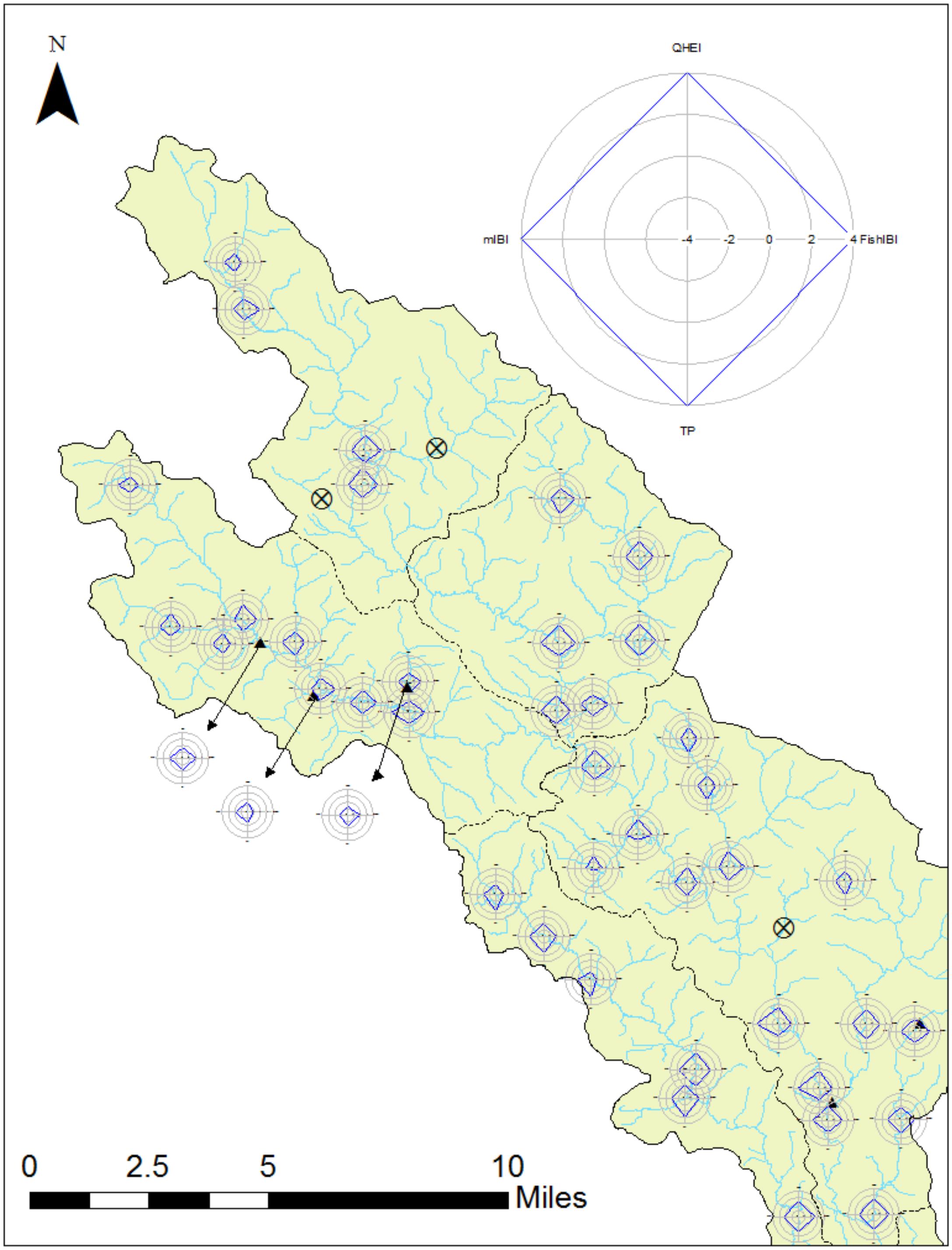


Figure 18. Upper half of Yellow River watershed showing radar plots for individual sample sites; axis arms closer to the center of the plot (smaller diamond shapes) indicate poorer conditions with respect to each variable axis (e.g. high phosphorus, poor habitat and IBI scores). Dashed lines show HUC12 boundaries within the HUC10 watershed. Circles with “Xs”-symbols are sites lacking fish or macroinvertebrate IBI data used in the radar plots.

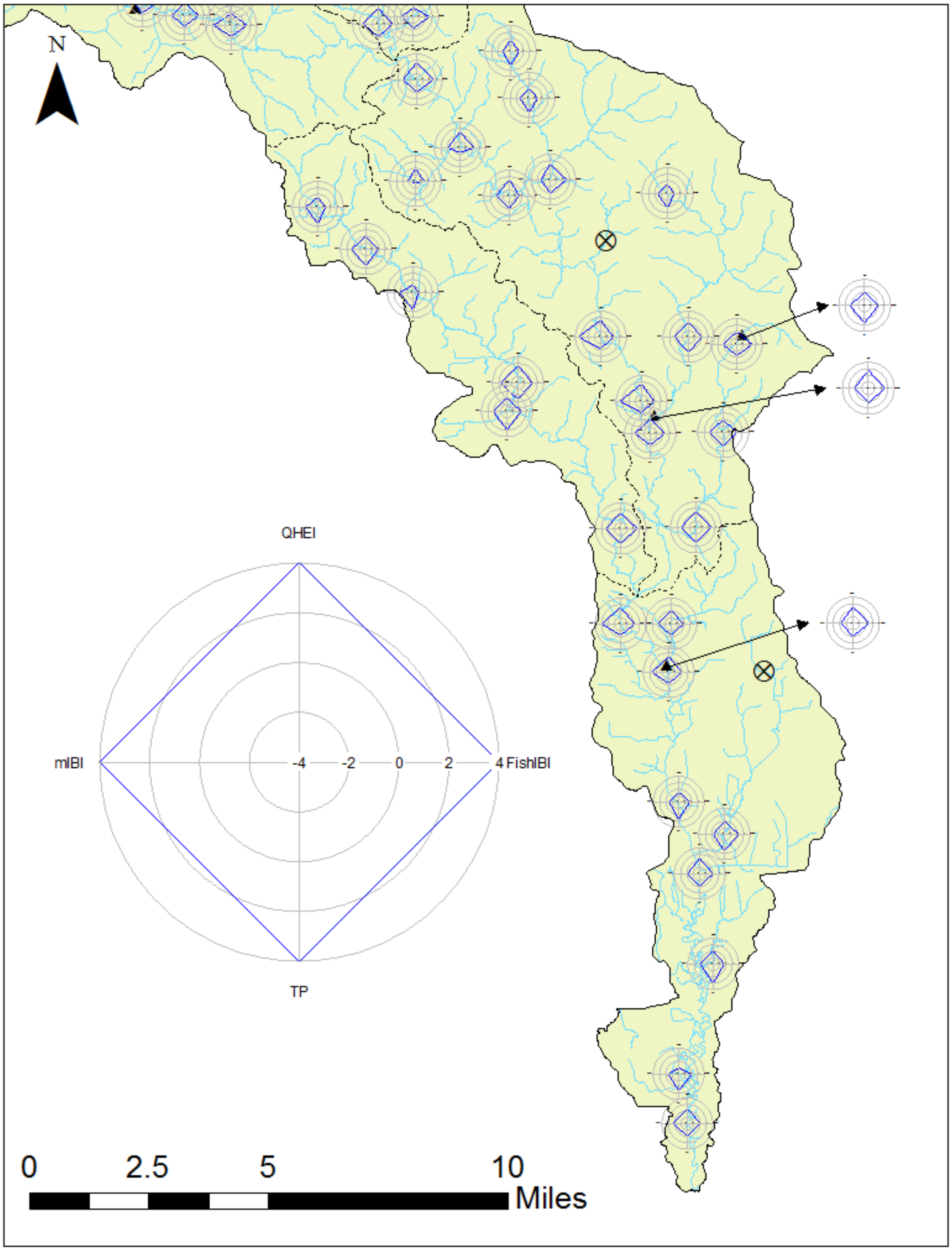


Figure 19. Lower half of Yellow River watershed showing radar plots for individual sample sites; axis values closer to the center of the plot (smaller diamonds) indicate poorer conditions with respect to each variable axis (e.g. high phosphorus, poor habitat and IBI scores). Dashed lines show HUC12 boundaries within the HUC10 watershed. Black circles with “Xs”-symbols are sites lacking fish IBI data used in the radar plots.

“Radar” plots, allow the presentation of multiple types of data in map symbols to characterize the conditions of individual sampling sites. The symbols in this report have four axes, one representing habitat quality based on QHEI scores, fish assemblage integrity based on f-IBI scores, mean total phosphorus concentrations, and macroinvertebrate IBI scores. Each data type was standardized to the same axis scale. The longer the axis for any individual parameter the “better” the environmental quality at the stream site for that parameter. A “longer” axis for total phosphorus would indicate a lower mean phosphorus concentration at the respective stream site. The range of phosphorus concentrations used to scale the phosphorus axis was based on the range of all phosphorus concentrations measured in the Yellow River Watershed. Similarly, the “longer” IBI axis would indicate a higher “better” biological assemblage index score (fish or macroinvertebrates) at that respective stream site. Radar plots allow the viewer to assimilate a lot of information quickly for individual stream sites and the watershed overall. For example, smaller “diamonds” (area created by the four axes) indicate “poorer” overall conditions at a stream site; sites with axes of unequal lengths indicate some parameters are in poorer or better condition than other parameters at a site and often provide insights into whether habitat or water chemistry is limiting the “biology” at a stream site (Figs. 18 and 19).

STATISTICAL RESULTS

Statistical Ordination

Bray-Curtis ordination is a statistical cluster analysis technique that was used to group stream sites that had similar macroinvertebrate or fish species assemblages (Figures 20 and 21, respectively). Site groups are thought to have similar environmental characteristics influencing the macroinvertebrates or fish assemblages found at these within-group sites.

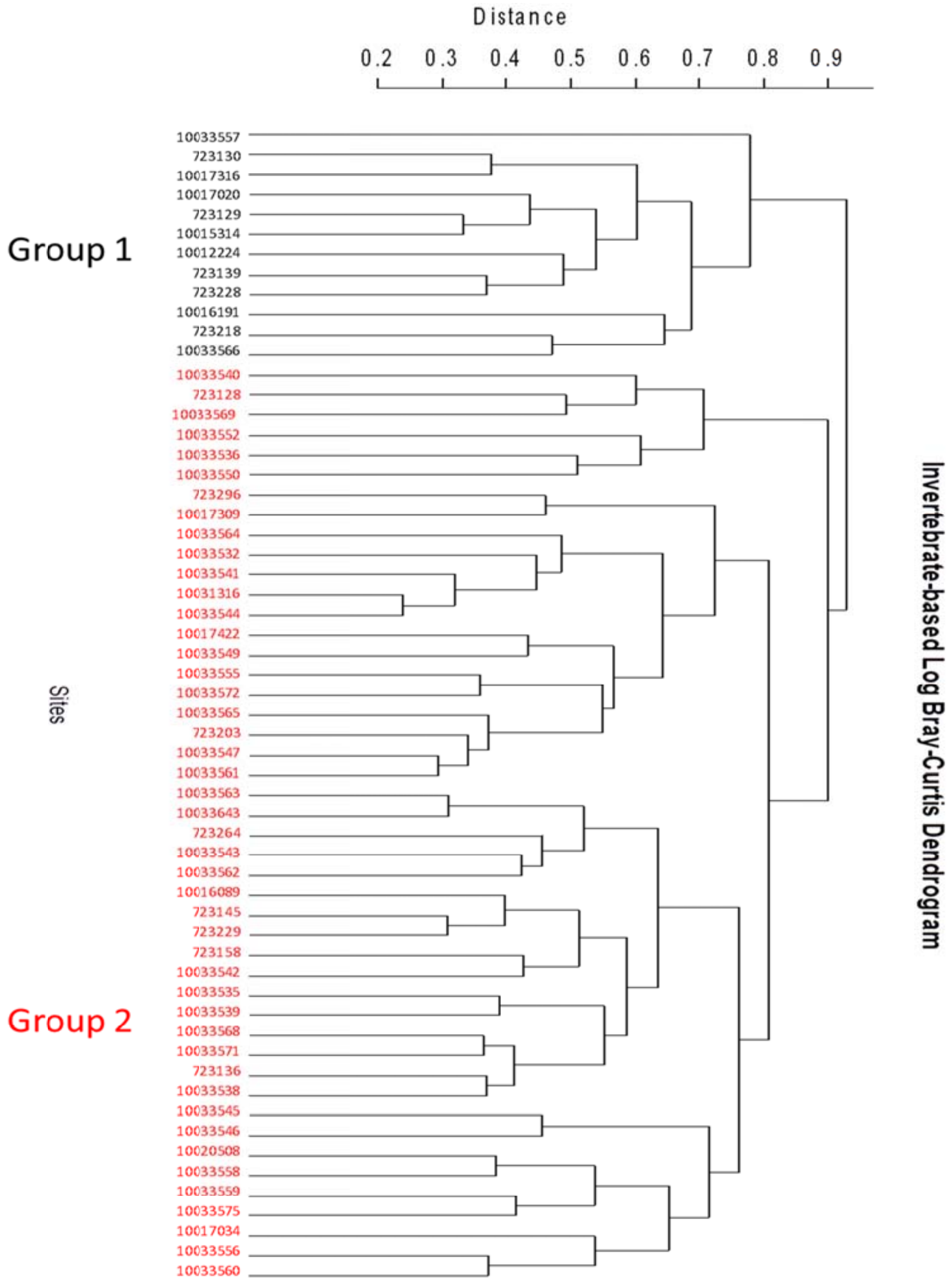


Figure 20. Bray-Curtis ordination plot of Yellow River Watershed macroinvertebrate assemblage data.

The macroinvertebrate BC ordination plot (Fig. 20) shows 2 main groups of stream sites based on the total number of species, the total number of individuals of each species, and the total number of individual specimens found at each stream site. There were 12 sampling sites in one group and 46 sites in the other group. BC results do not provide insights into why there appears to be two distinct groups of stream sites – only that two site groups exist within the Upper

Yellow River Watershed sampling sites population.

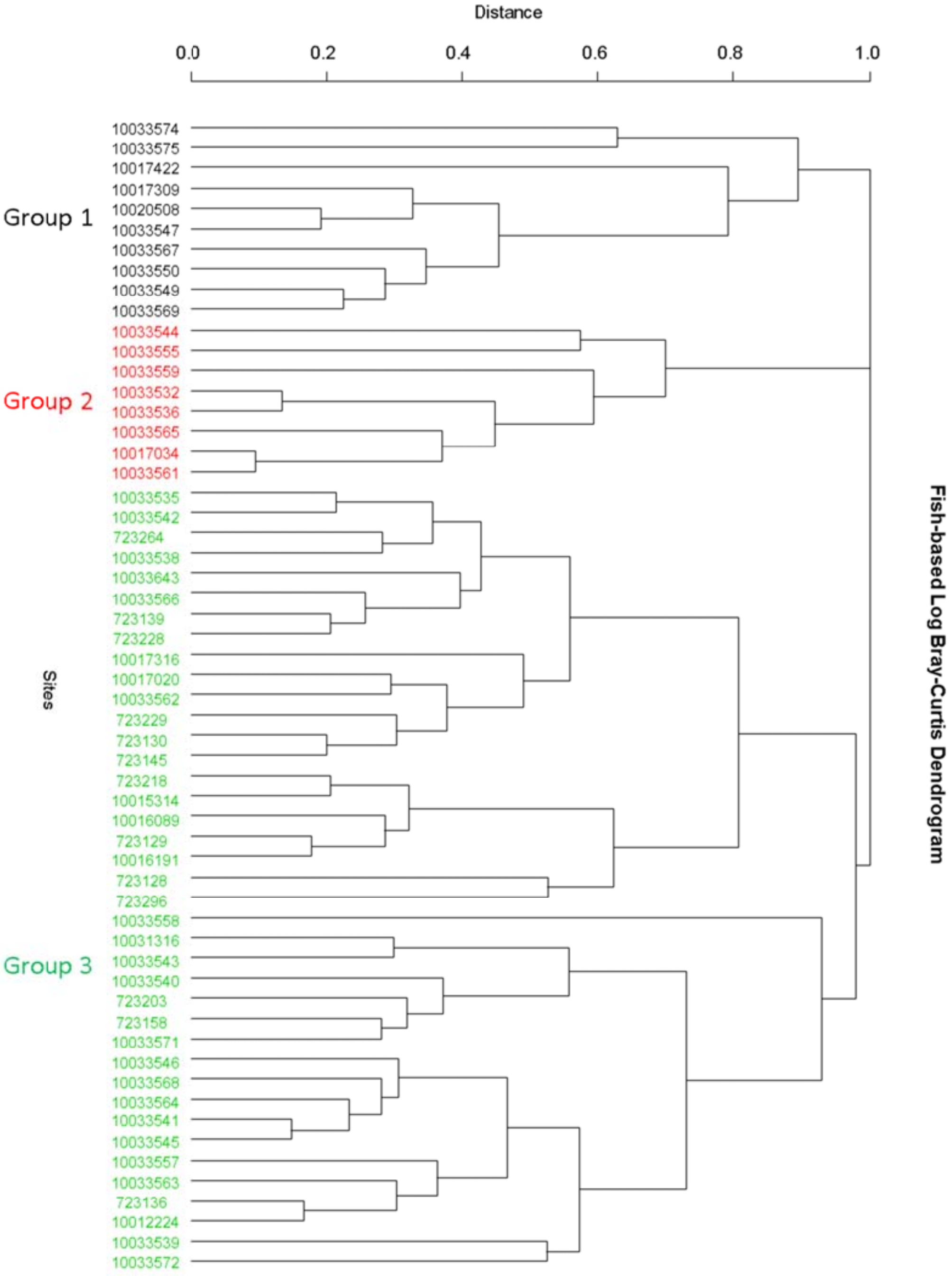


Figure 21. Bray Curtis ordination plot of Yellow River Watershed fish assemblage groupings.

The Bray-Curtis ordination of the fish assemblage data (Fig. 21) shows three distinct populations of stream sites, with 10, 8, and 39 sampling sites within each group respectively. As with the macroinvertebrate BC analysis, the results do not provide insights into what factors may have caused the fish site groupings, only that these groups existed. Subsequent statistical analyses (NMDS and CCA) were used to attempt to validate the groupings observed in the macroinvertebrate and fish BC plots. These latter statistical methods provided some insights into what physical and chemical environmental factors may have resulted in the macroinvertebrate and fish stream groups observed and were likely important factors influencing the overall biological integrity of individual stream sites and entire watershed.

Unconstrained Nonmetric Multi-Dimensional Scaling

NMDS translates similarities or dissimilarities among stream sampling sites (based on macroinvertebrate or fish populations in this study, Figures 22 and 23 respectfully) into a visual representation of distance. This specific NMDS analysis presented in Figures 22 and 23 are referred to as “unconstrained” since the only factors influencing the clustering or dispersion of the site triangles in these NMDS plots were the similarities or dissimilarities of the macroinvertebrate or fish assemblages found at each stream survey site – not any of the environmental variables measured at each of the sites. For the unconstrained macroinvertebrate NMDS plot (Figure 22), stream sampling sites are represented by triangles; those triangles/sites closer together are more similar in terms of numbers of macroinvertebrates species, individuals of a species at each stream site, and total numbers of macroinvertebrates present at each site, and those sites farther apart have more dissimilar macroinvertebrate assemblages.

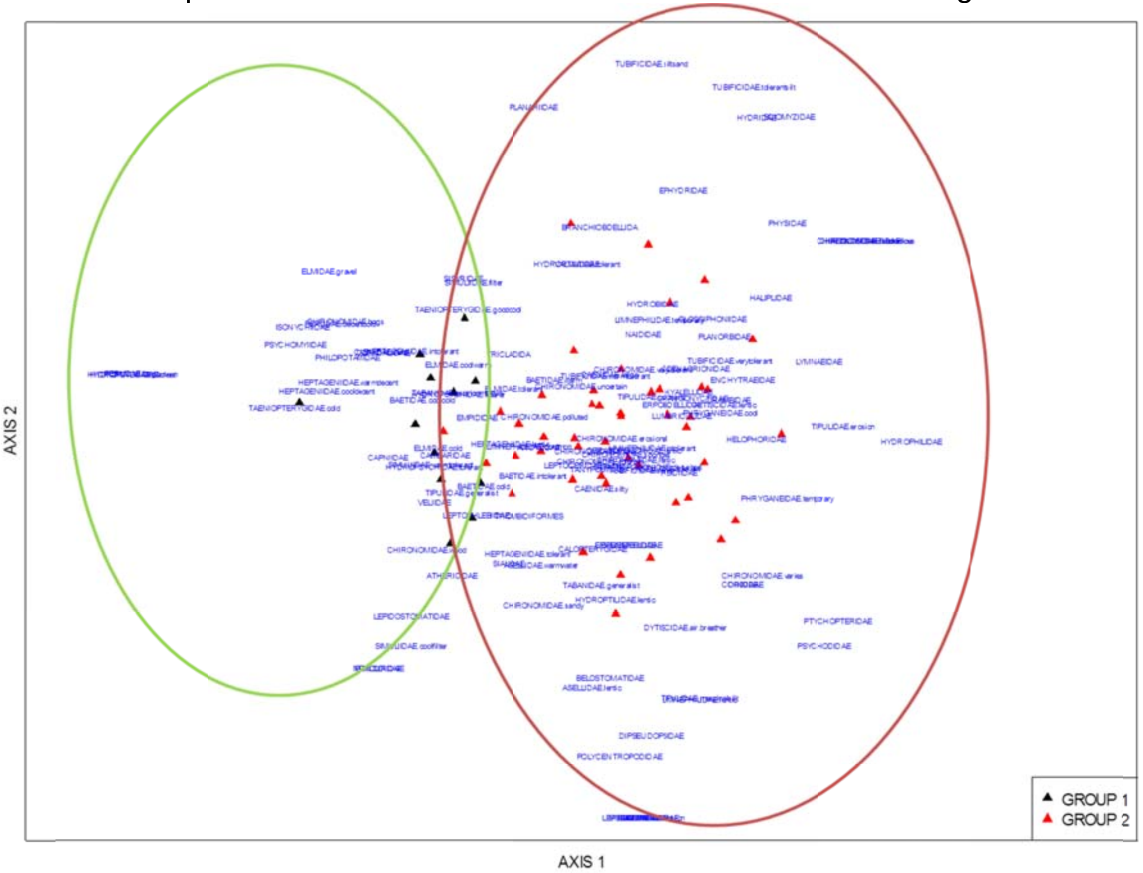


Figure 22. Unconstrained Non-metric Multi-dimensional Scaling (NMDS) plot of Yellow River Watershed macroinvertebrate assemblage data. Black and red triangle colors represent the two groups of stream sites identified in the BC cluster analysis. The green circle encompasses taxa that generally require "cooler" summer water temperatures, the red circle encompasses taxa that in general tolerate wider ranges of water temperatures and degraded physical habitat and/or "poorer" water quality conditions.

Triangles in the macroinvertebrate NMDS plot are color-coded to represent the two macroinvertebrate groups that were identified by the Bray-Curtis (BC) ordination analysis. The same two BC groups appear to be clustering in the NMDS plot, providing corroborating evidence that two distinctly different groups of stream sites (based on the macroinvertebrate assemblages found at each site) truly existed in the Yellow River Watershed.

The locations of the macroinvertebrate taxonomic names on the plot show the species that were common to the nearby stream sampling sites (triangles). Knowledge of the environmental requirements of individual macroinvertebrate species allows one to make inferences about what physical or chemical conditions of the watershed and stream sites may be causing the clustering or dispersion of the sites in the NMDS plot, providing insights into the major environmental "drivers" (explanatory variables) that most strongly influenced the macroinvertebrate populations in the Yellow River Watershed.

Evaluating the groupings of the macroinvertebrate taxa found in the NMDS plot (Fig. 22) and discussions with entomologist Prof. Kurt Schmude of UW-Superior regarding the environmental requirements of the individual invertebrate taxa collected, revealed a cluster of macroinvertebrate taxa that require "cooler" summer water temperatures and/or prefer coarse substrate located on the left side of the plot (circled with a green line). Many of the taxa found within the red circle are known to be tolerant of low dissolved oxygen concentrations, sediment - covered substrate, highly erosional habitat, ephemeral stream-flow, lentic environments, or are taxa that breath atmospheric oxygen instead of relying on dissolved oxygen.

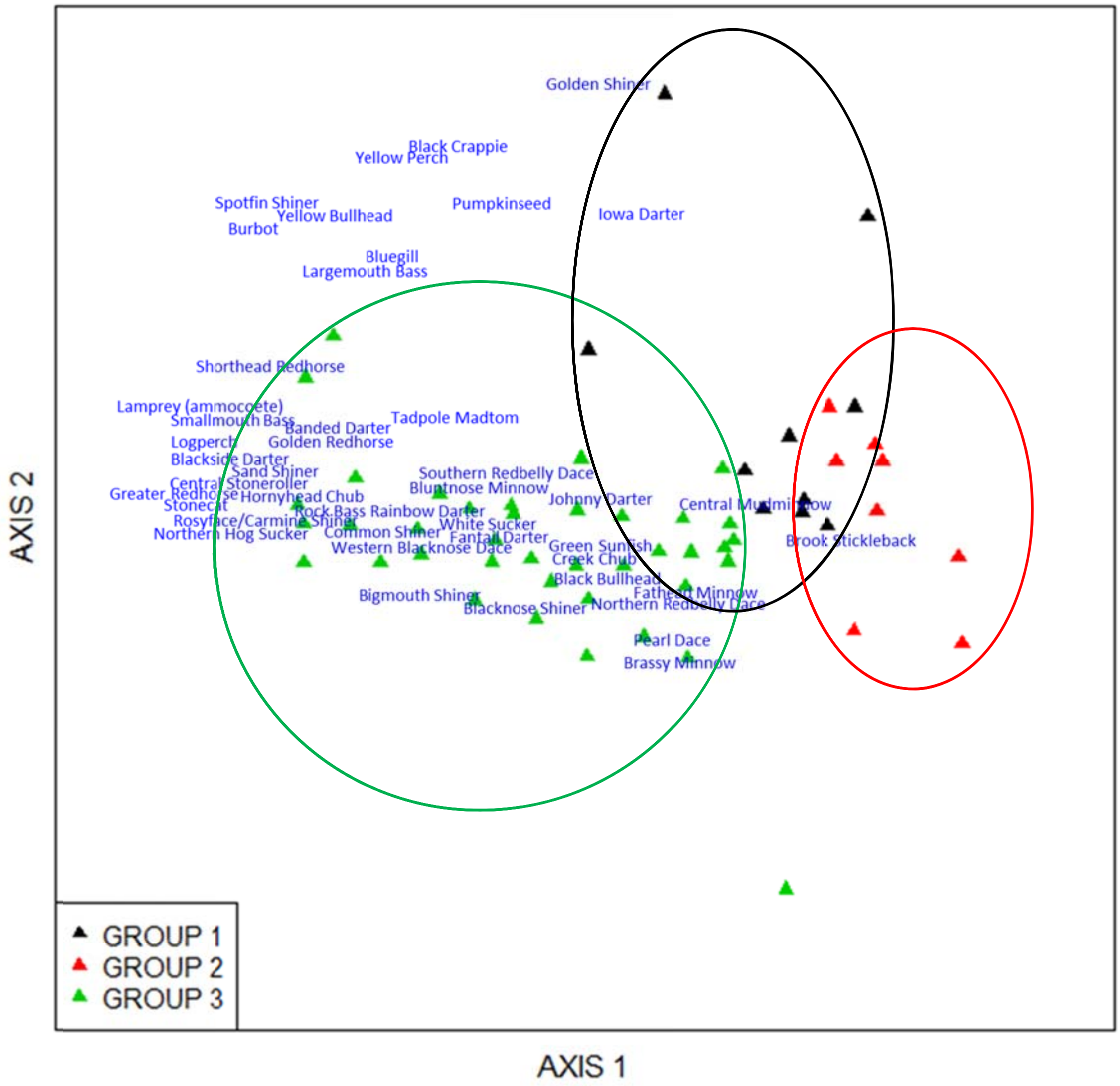


Figure 23. Unconstrained Nonmetric Multi-dimensional Scaling (NMDS) plot of Yellow River sampling sites' fish assemblages. Site symbols are color-coded by the site groups identified in the Bray-Curtis cluster analysis shown in Figure 21.

Similar to the unconstrained NMDS plot for macroinvertebrates, the stream sampling sites fish assemblage data are represented by colored triangles (Fig. 23). The triangles in the NMDS plot that are closer together represent stream sites with fish assemblages most similar to other stream sites, and triangles (sites) farther away from each other have fish assemblages that are more dissimilar among stream sites. The three different triangle colors correspond to the three different groups of stream sites identified in the Bray-Curtis cluster analysis of the fish data. The clustering of the individual Bray-Curtis groups in the fish NMDS plot provides corroborating evidence that the three groups of stream sites are ecologically meaningful. The stream sites represented by the red triangles tended to have low numbers of fish were and comprised of a small number of environmentally-tolerant species, suggesting degraded environmental conditions at these sites. Sites represented by the black triangles had more fish species at each site and these species were slightly more sensitive to environmental degradation than the red triangle sites, but much more tolerant to environmental degradation than the fish species found at the stream sites represented by the green-colored triangles.

Constrained NMDS
The stream sites' macroinvertebrate and fish assemblages were also analyzed using constrained NMDS (Figure 24 and 25 respectively). In this analysis NMDS (x, y) coordinates for stream sites macroinvertebrate and fish assemblage data were treated as explanatory variables and incorporated into individual linear regression analyses with all of the 135 physical and chemical variables measured at each stream site being treated as response variables. Only the most significant response variables (parameters with correlation coefficients ≥ 0.3 in this analysis) were plotted in the constrained NMDS analyses to determine which watershed land cover and land use, and physical and chemical measures from the stream sites had the greatest influence on the macroinvertebrate and fish assemblages.

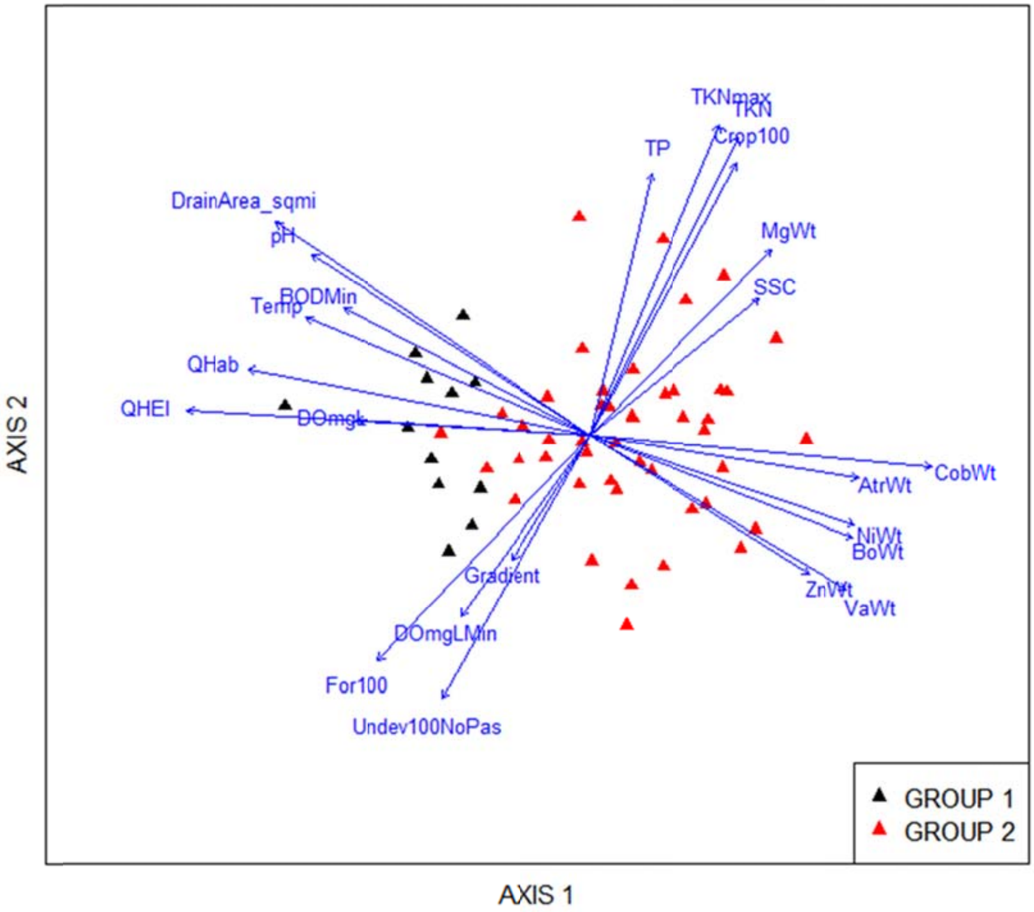


Figure 24. Correlation of key environmental explanatory variables with constrained NMDS macroinvertebrate site clusters in the Yellow River. Site

triangles are color-coded according to the Bray-Curtis macroinvertebrate site groups.

Environmental factors such as higher dissolved oxygen concentrations, improving habitat quality, higher pH, higher proportions of forest or no development or agricultural land within a 100m buffer along and upstream of the stream sampling sites (For100 and Undev100NoPas, respectively) were key explanatory variables of the sites with more sensitive macroinvertebrate taxa (black triangles). Increasing water column total phosphorus and TKN concentrations, increasing proportions of cropland within 100m corridors along and upstream of the stream sampling sites, increasing suspended sediment and water column metals concentrations were associated with stream sites with poorer macroinvertebrate assemblages (red triangles).

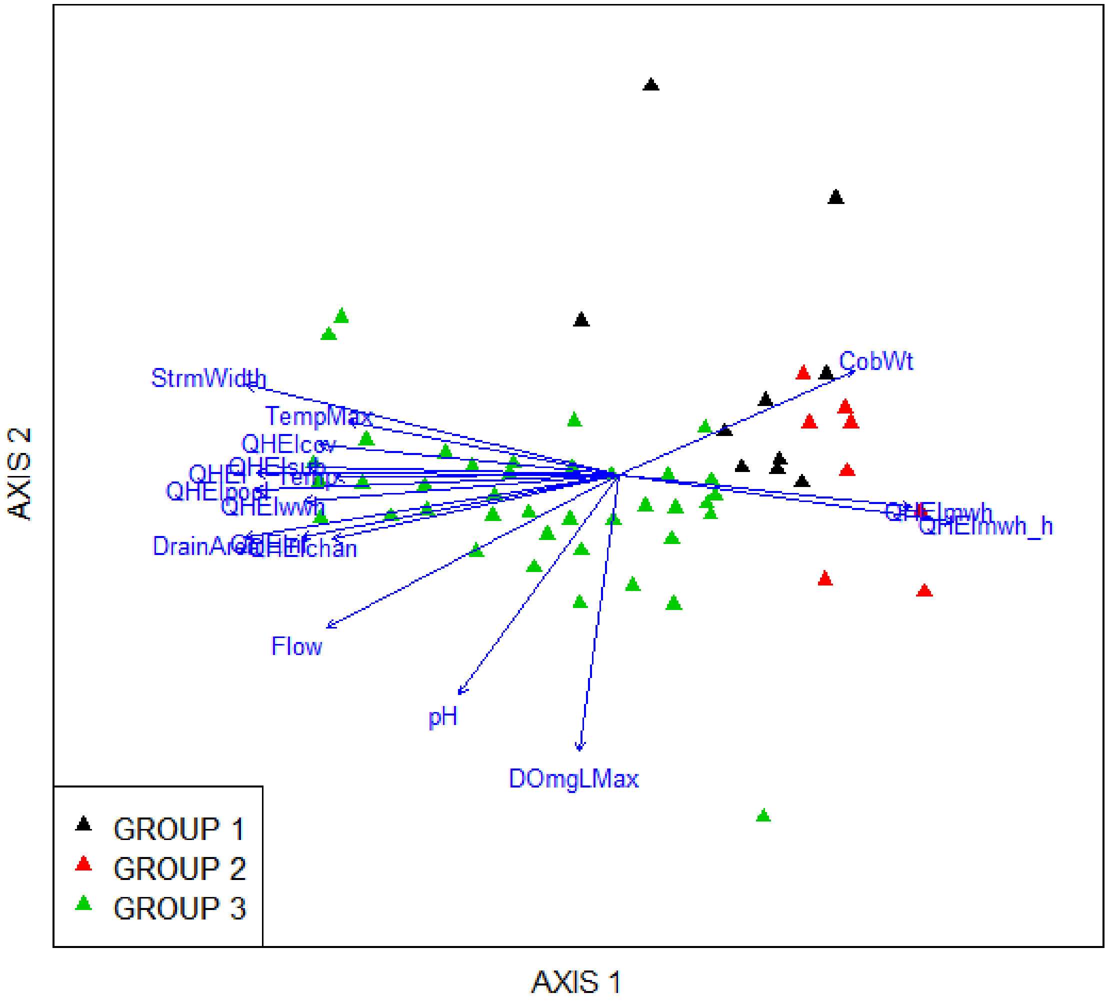


Figure 25. Correlation of key environmental explanatory variables of Yellow River fish species distributions with constrained NMDS site-clusters using the Bray-Curtis fish populations groups (colored triangles).

In Figure 25, the site locations are the same as in the previous fish NMDS plot (Fig. 23) and are again color-coded by their respective Bray-Curtis groups. The NMDS sites were treated as potential explanatory (x) variables, and all watershed, stream habitat, and water chemistry variables quantified in this study were treated as response (y) variables. Correlation analyses were run to determine which environmental factors were most strongly related to (and presumably influenced) the clustering of the stream sites based on fish assemblage data.

Since the test was a simple correlation of each environmental factor regressed against the NMDS coordinates, the test was not sensitive to collinearity between any of the candidate response variable – pairs. For example, watershed drainage area, and streamflow volume are strongly correlated to each other and likely influence the fish assemblages similarly. Only one factor of each of the candidate response variable pairs (as in the previous example) was kept in the constrained NMDS analysis. A subset of key explanatory variables thought to most strongly influence fish, (those with correlation coefficients ≥ 0.4) were used in the plot environmental factors shown in Figure 25. The direction the vector arrow points indicates an increasing positive value for the variable shown. The longer the length of the vector arrow, the stronger the correlation between the respective response (environmental) and explanatory (NMDS X-Y coordinates) variables. Various measures of stream size, direct or surrogate measures of water quality (e.g., chlorophyll *a* concentration), and physical habitat features such as stream gradient (labeled as QHEIgradv), were all strong correlates of the fish assemblages found. The data suggests these physical and chemical factors were highly significant in influencing the fish populations found in the individual stream sites and in the watershed overall.

Canonical Correspondence Analysis

CCA is another type of cluster analysis that was used to determine which watershed land cover or land use characteristics, stream habitat features, or water chemistry measures were most influential in structuring the biological assemblages in the Yellow River Watershed. Similar to NMDS, those explanatory variables with the longest vector arrows are most strongly correlated with macroinvertebrate or fish assemblage responses to environmental conditions and thought to have the strongest influence on these animal populations (Figures 26 and 27 respectively).

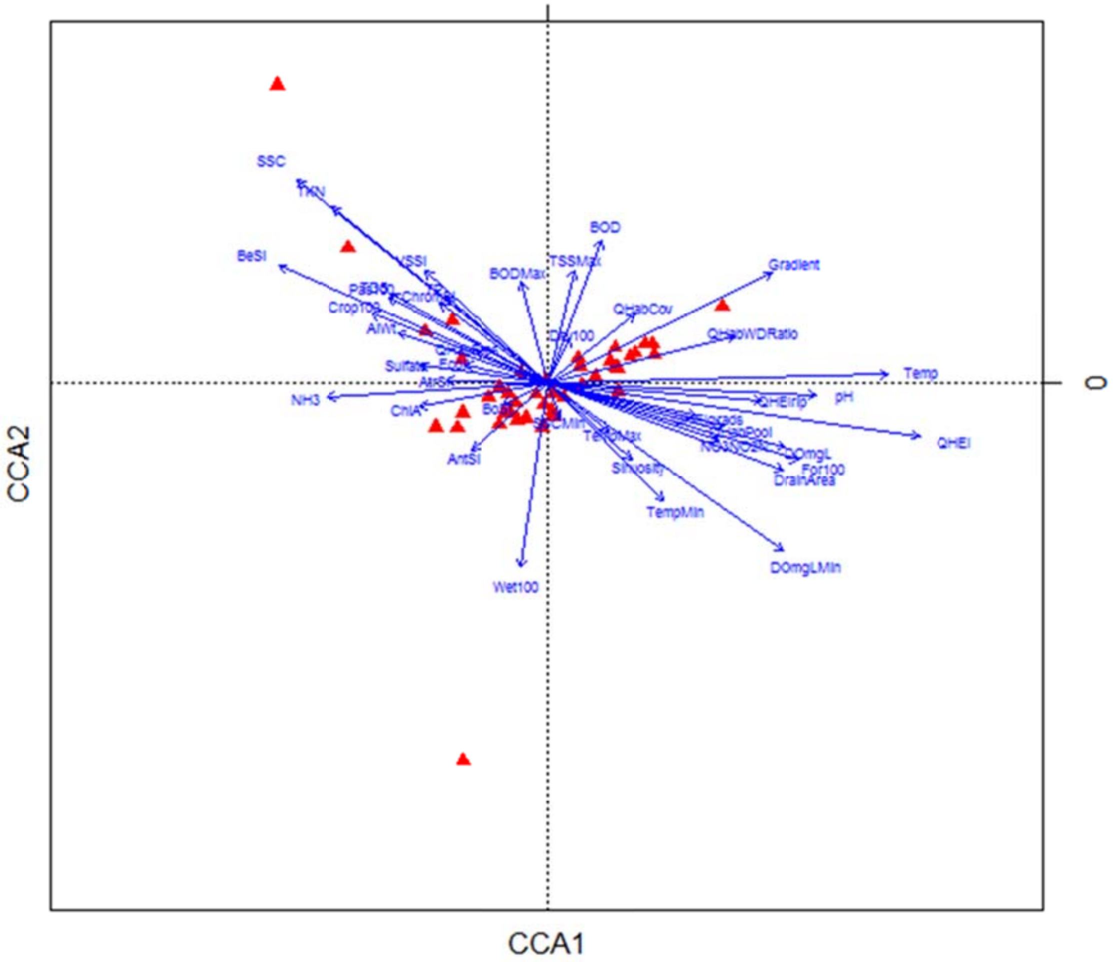


Figure 26. Canonical Correspondence Analysis (CCA) plot of Yellow River macroinvertebrate assemblage data and environmental factors most strongly correlated with the macroinvertebrate taxa found at individual stream sampling sites.

For macroinvertebrates, SSC, TKN, gradient, water temperature, percent wetland within a 100m-wide corridor next to and upstream of the sampling site (Wet100), and QHEI were some of the environmental factors most strongly correlated with the differing macroinvertebrate assemblages found in the Yellow River Watershed based on CCA results.

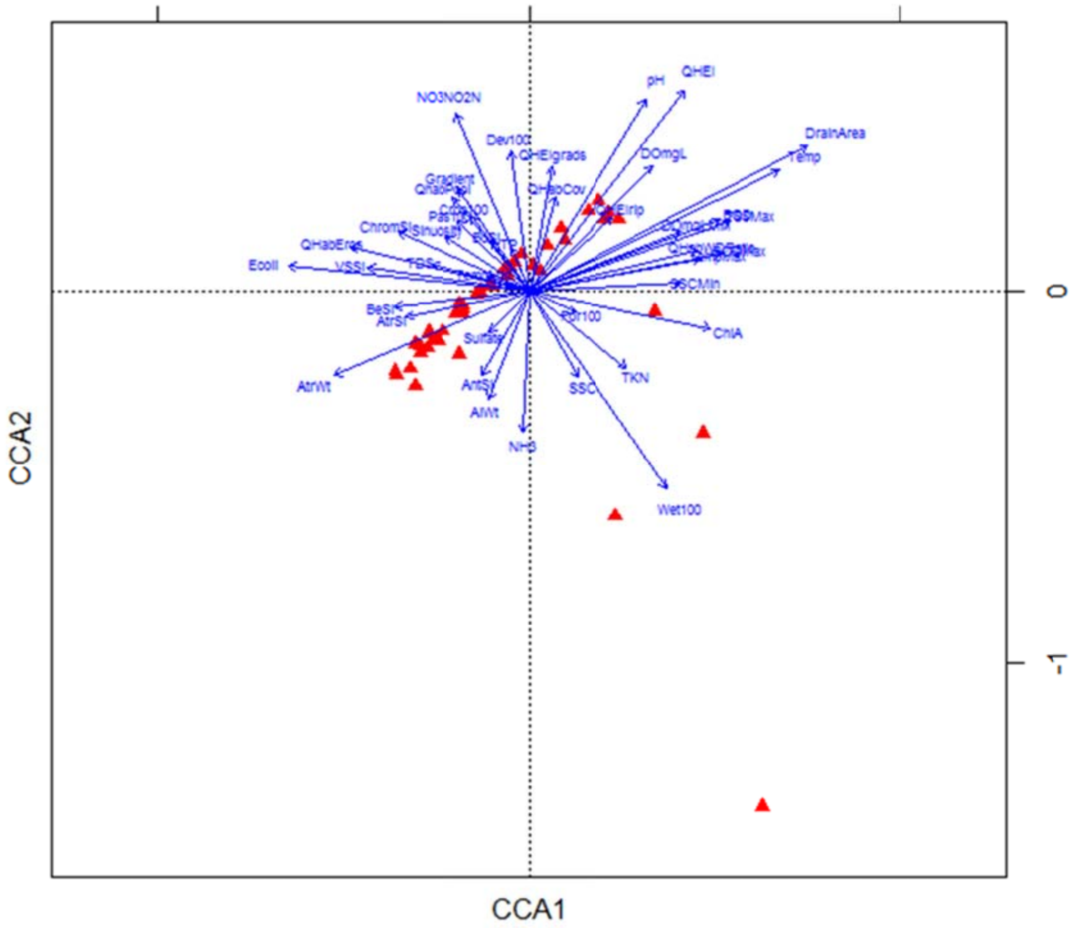


Figure 27. Canonical Correspondence Analysis (CCA) plot of Yellow River fish assemblage data and environmental factors most strongly correlated with the fish taxa found at individual stream sampling sites.

For fish assemblages, watershed drainage area, water temperature, percentage of wetland in a 100m-wide corridor at and upstream of the sampling site, QHEI, pH, and water column nitrate and nitrite concentrations appeared to have the greatest influence on the fish populations based on the results of the CCA analysis (Fig. 27).

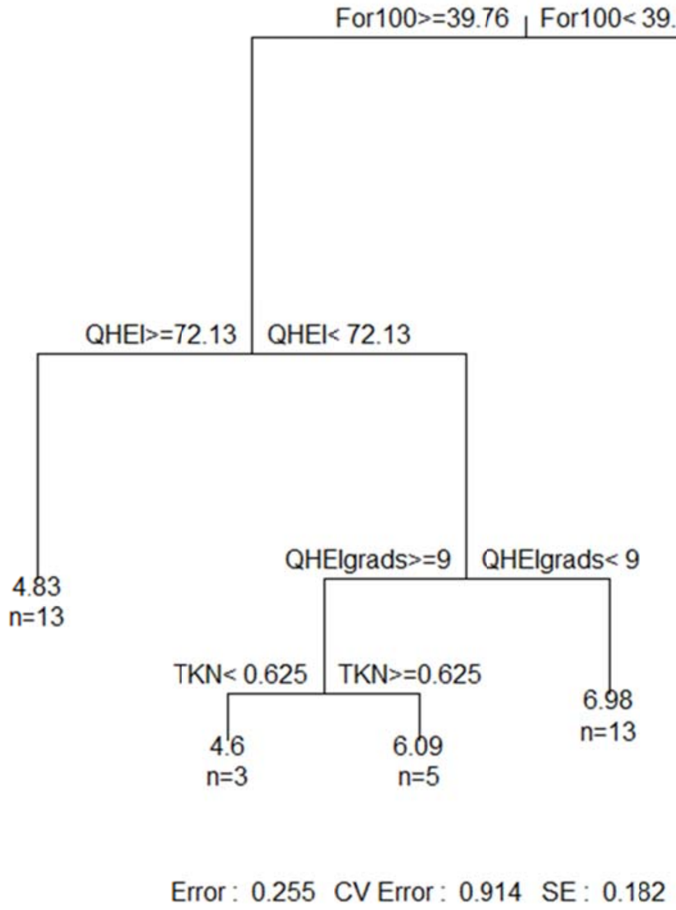


Figure 28. Regression tree plot of most significant environmental parameters influencing Hilsenhoff's Biotic Index (HBI) scores of macroinvertebrate taxonomic data for the Yellow River and resulting groups of similar stream sites based on HBI scores.

Figure 28 is a regression tree plot illustrating the most statistically significant environmental parameters influencing the Hilsenhoff's Biotic Index (HBI) scores and resulting site groupings based on the HBI scores. Amount of forested land within 100m of the stream was shown to be the most significant factor influencing HBI scores. The next most significant factor influencing HBI scores was the overall QHEI score.

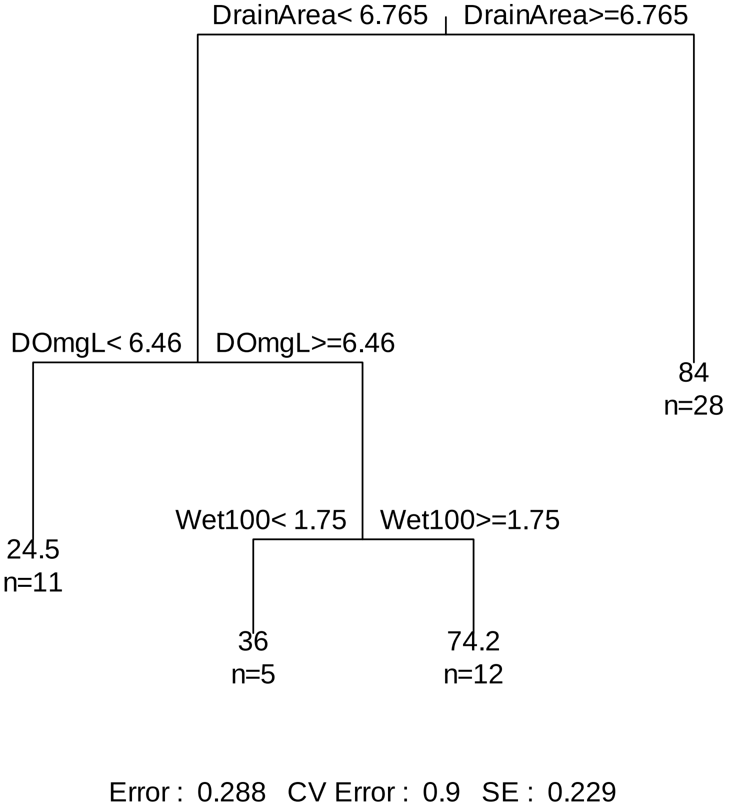


Figure 29. Regression tree plot of most significant environmental parameters influencing Yellow River fish index of biotic integrity (f-IBI) scores and resulting groups of similar stream sites based on f-IBI scores.

Figure 29 is a regression tree plot illustrating the most statistically significant environmental parameters influencing the f-IBI scores, and resulting site groupings based on f-IBI scores. Regression trees statistically create groups with low within-group variability and high between-group variability (groups “apples with apples” and “oranges with oranges”), and determine what factors are most influential in creating these groupings. Similar to the NMDS and CCA analyses, all 135 physical and chemical variables reported for each sampling site were included in the regression tree analyses as potential explanatory variables influencing stream sites’ fish IBI scores.

The regression tree divided the entire stream site sample population into four statistically distinct groups based on f-IBI scores. Drainage area (our proxy for flow in these analyses) was shown to be the strongest predictor of the f-IBI scores. The next strongest predictors were dissolved oxygen (DO) and percentage of wetland within 100m-wide corridor at and upstream of the assessment sites. The respective mean f-IBI scores and number of stream sites within each group are reported at the bottom end of the terminal “branches” of the tree; for example, the farthest left branch are stream sites with small drainage area and low minimum DO concentrations, these sites (n = 11) had an average f-IBI score of 24.5 which is “Poor” on the f-IBI stream rating scale. Conversely, stream sites with larger drainage areas had an average f-IBI score of 84 which is “Very Good.”

Random Forest Analysis

A RF statistical routine was applied to the HBI and f-IBI scores to re-evaluate what environmental factors were determined to most strongly influence the macroinvertebrate populations found at each stream survey site. Briefly stated RF runs numerous (in this study 500) permutations of the potential explanatory variables used in the CART analyses to reduce any influence the order in which input variables are entered into the CART analysis may have on determining which environmental variables may have on the HBI or f-IBI scores. Overall, stream habitat quality, water column ammonia concentrations, and the percent forest cover within a 100m-wide riparian corridor at and upstream of the sampling site were most influential in affecting HBI scores and therefore are thought to be the most significant factors influencing macroinvertebrates in the Yellow River Watershed.

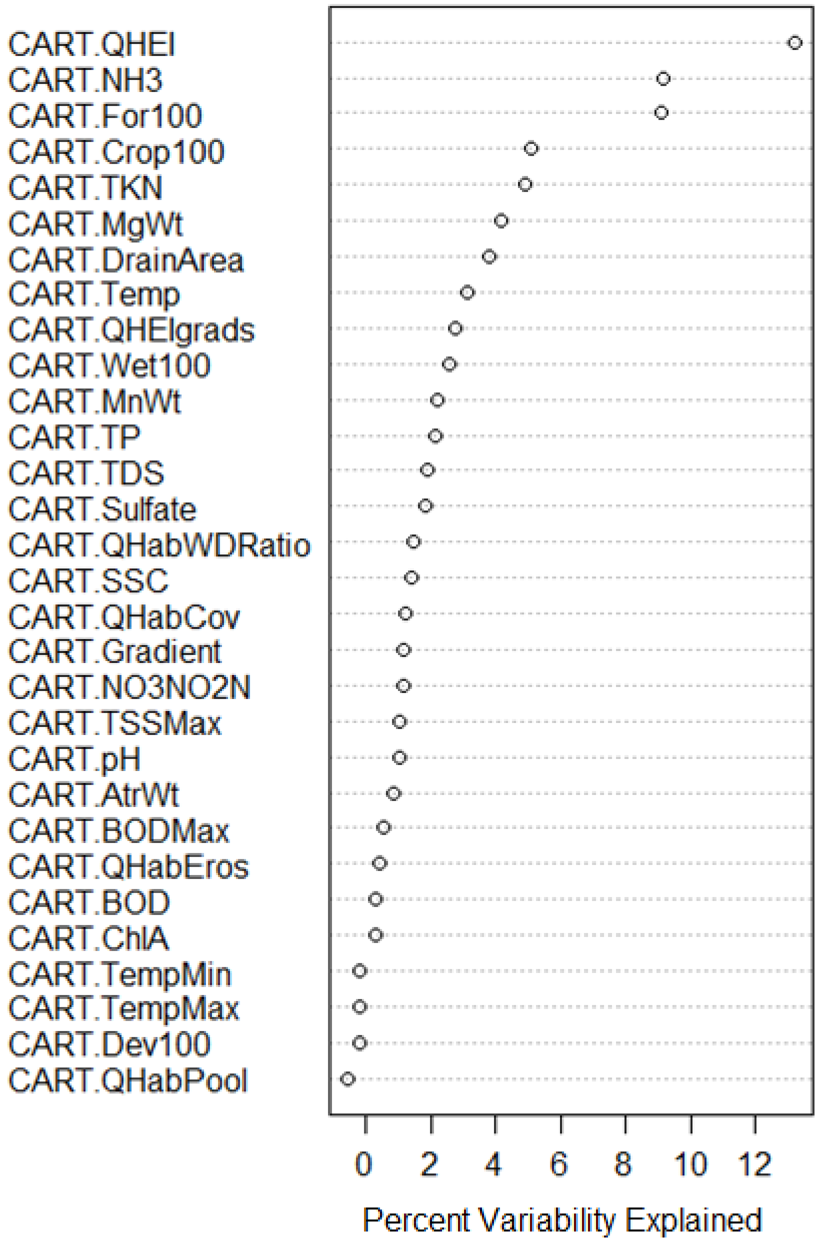


Figure 30. Plot of Random Forest analysis of environmental factors (Y axis) influencing the variability in Hilsenhoff's Biotic Index scores of macroinvertbrate data from all sampling sites.

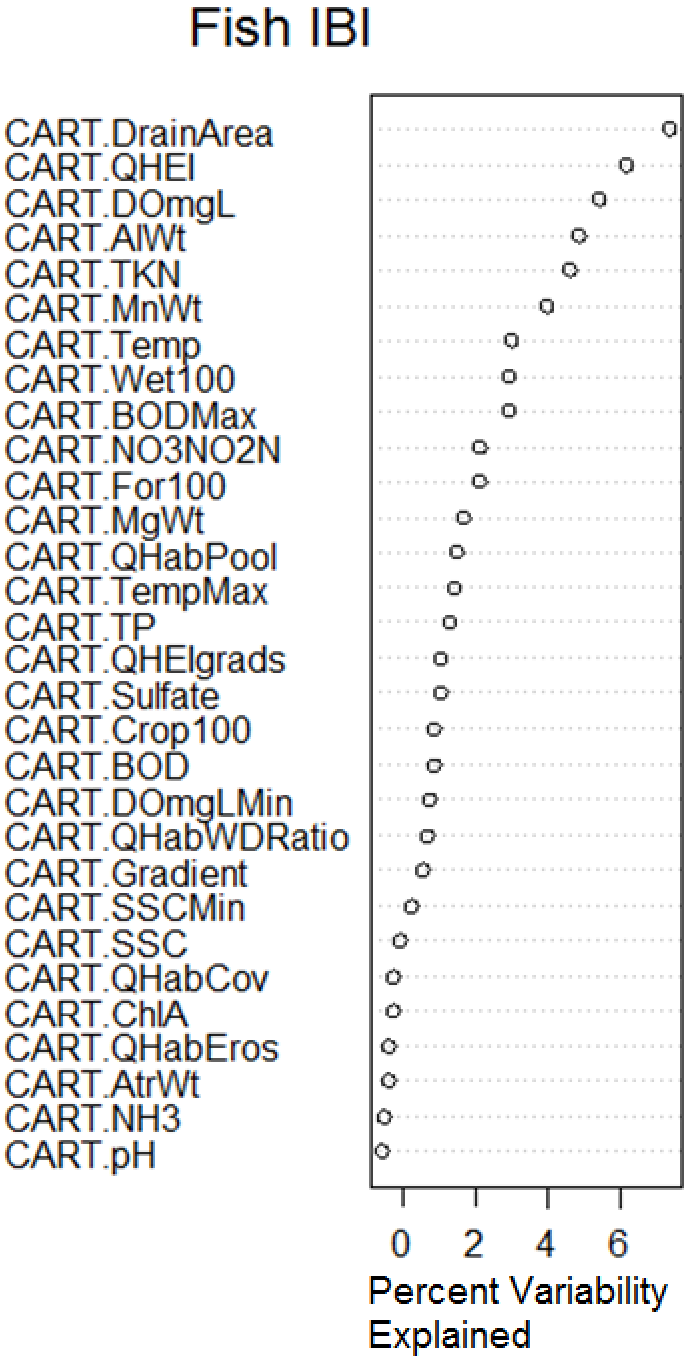


Figure 31. Plot of Random Forest analysis of environmental factors (Y axis) influencing the variability of fish index of biotic integrity scores from all sampling sites.

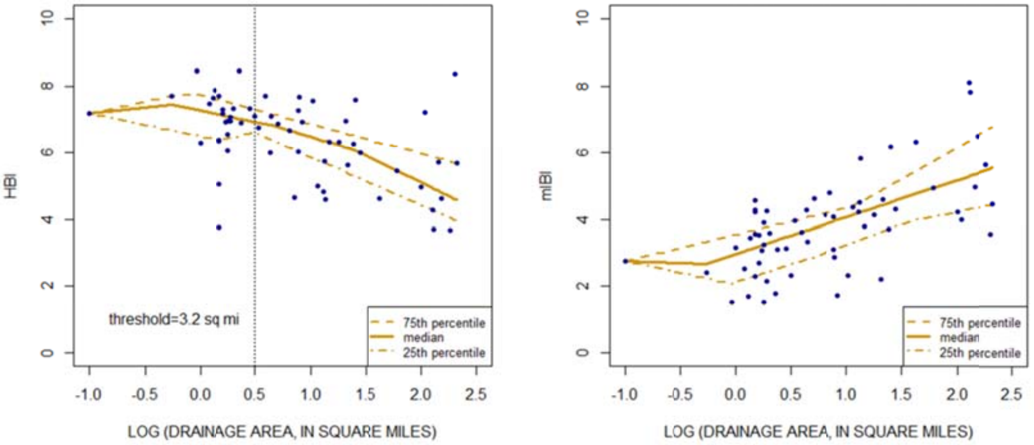
Results of Random Forests analysis of the f-IBI scores provided evidence of which environmental factors most strongly influenced the variability in f-IBI scores at all of the watershed assessment sites. Overall drainage area upstream of the sampling sites, habitat quality (as measured by QHEI) and various water chemistry measures appeared most influential.

The four most important individual explanatory variables influencing HBI scores explained five, eight, eight, and thirteen percent of the overall HBI score sample variance whereas the top four explanatory variables in the f-IBI analyses explained five, six, six, and seven percent of the overall f-IBI variability respectively indicating the top macroinvertebrate explanatory variables were nearly twice as influential (on macroinvertebrates) than the fish explanatory variables were in influencing f-IBI scores.

Quantile Regression Analysis

Quantile regression analyses were used to evaluate whether environmental factors that were shown in earlier tests to influence macroinvertebrate or fish assemblages, had linear responses where increasing or decreasing values of various environmental factors resulted in a decline in biological condition of stream sites in the Yellow River Watershed or whether there were specific thresholds that, once exceeded, resulted in significant biological degradation. Linear response and threshold information can be used to determine how severely a stream site is impacted by a particular stressor, and to develop objective, quantifiable, watershed restoration goals to improve conditions for stream biota.

Macroinvertebrates' responses to environmental stressors were evaluated using Hilsenhoff's Biotic Index (HBI) and macroinvertebrate index of Biotic Integrity (mIBI) metric scores. Lower HBI scores and higher mIBI scores indicate "better" macroinvertebrate populations and presumably higher quality environmental conditions. The explanatory variable results reported below were for environmental factors shown to be statistically significant in previous statistical analyses were evaluated using quantile analysis.



Figures 32. Quantile plots showing the response of macroinvertebrate metrics scores (HBI and m-IBI) to drainage area size in the Yellow River Watershed.

Both HBI and mIBI scores showed improved biological integrity at stream sites with greater watershed land area (Fig. 32).

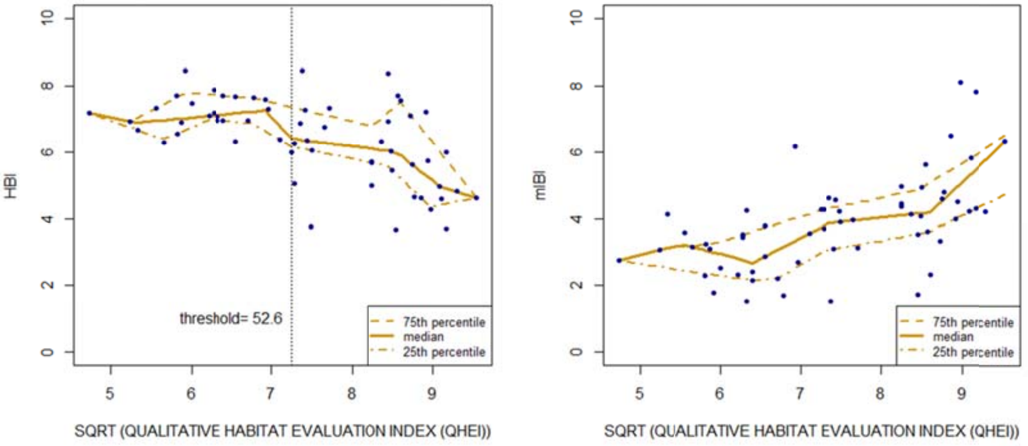


Figure 33. Quantile plots showing the response of macroinvertebrate metrics scores (HBI and m-IBI) to habitat quality in the Yellow River Watershed.

Stream habitat quality as measured with the Qualitative Habitat Evaluation Index (QHEI) was shown to influence the macroinvertebrate populations. As habitat quality improved, there was a trend of improving macroinvertebrate index scores for both the HBI and m-IBI (Fig. 33).

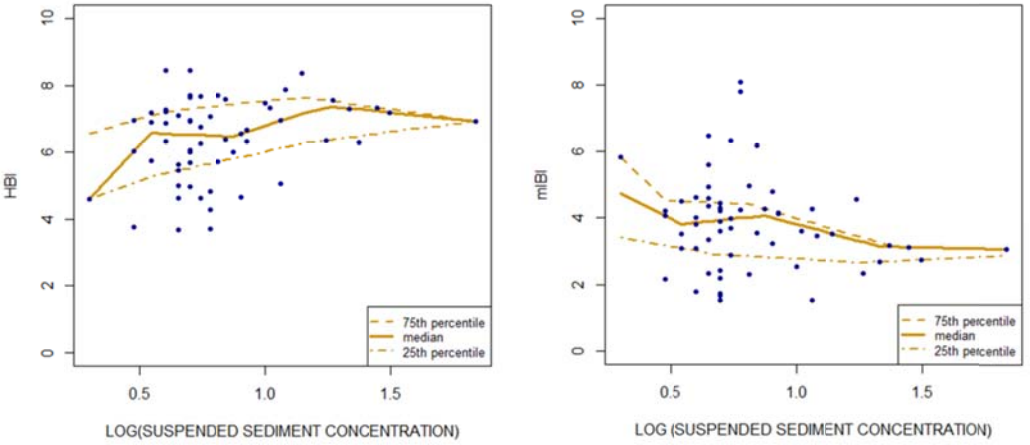


Figure 34. Quantile plots showing the response of macroinvertebrate metrics scores to suspended sediment concentration in the Yellow River Watershed.

Increasing suspended sediment concentration had a weak negative influence on the macroinvertebrate populations based on both HBI and m-IBI results (Fig. 34).

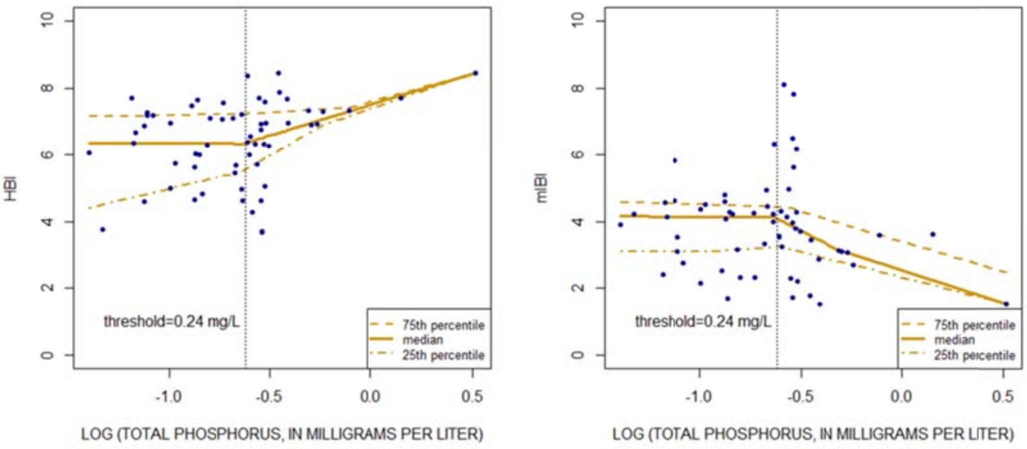


Figure 35. Quantile plots showing the response of macroinvertebrate metrics scores to total phosphorus concentrations in the Yellow River Watershed.

Higher concentrations of water column total phosphorus concentrations were associated with a trend of poorer macroinvertebrate metric scores. There appeared to be a threshold for decline in biotic integrity for both index scores when water column total phosphorus scores reached a concentration of 0.24 mg/L. (Figure 35).

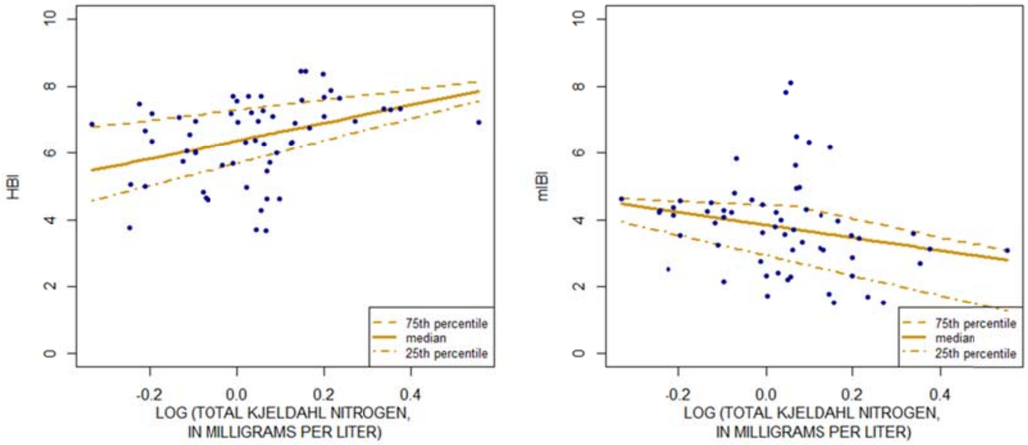


Figure 36. Quantile plots showing the response of macroinvertebrate metrics scores to total Kjeldahl nitrogen concentrations in the Yellow River Watershed.

Increasing total Kjeldahl nitrogen concentrations in water had a negative linear effect on the macroinvertebrate populations in the Yellow River Watershed (Figures 36).

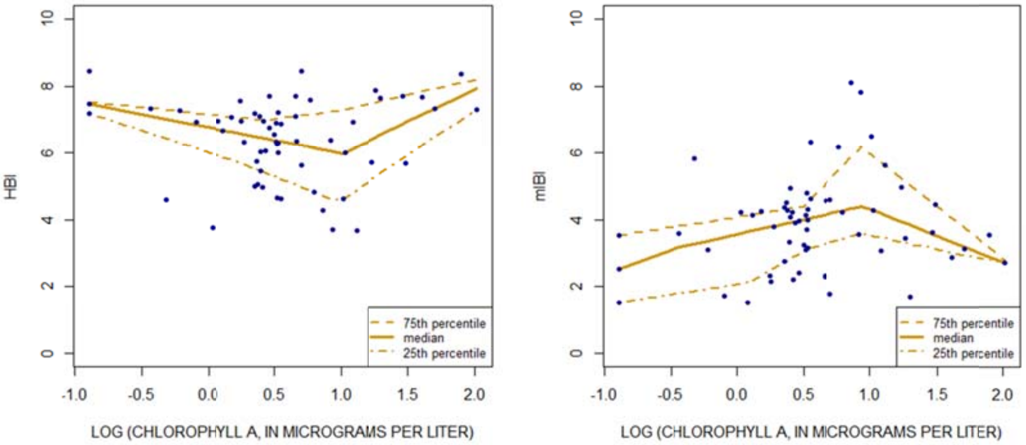
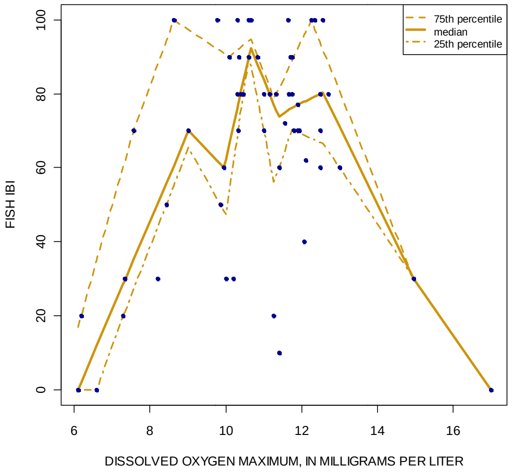


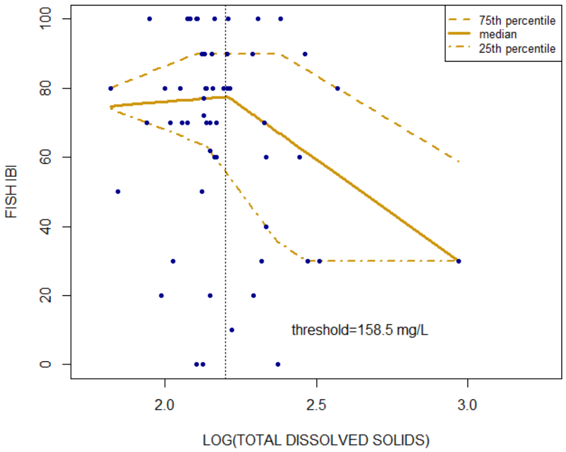
Figure 37. Quantile plots showing the response of macroinvertebrate metrics scores to chlorophyll *a* concentrations in the Yellow River Watershed.

Increasing water column chlorophyll *a* concentrations had a weak influence on the macroinvertebrate populations based on the responses of HBI and m-IBI scores (Fig. 37).



Figures 38. Quantile plot showing the response of fish Index of Biotic Integrity (f-IBI) scores to maximum concentrations of dissolved oxygen, in the Yellow River Watershed.

F-IBI scores showed a unimodal response to changing dissolved oxygen (DO) concentrations, with low (poor) f-IBI scores associated with both very low and very high DO concentrations (Fig. 38).



Figures 39. Quantile plot showing the response of fish Index of Biotic Integrity (f-IBI) scores to total dissolved solids (TDS) concentrations, in the Yellow River Watershed.

The concentration of total dissolved solids showed a threshold at 158 mg/L, that once exceeded resulted in a significant decline in f-IBI scores (Figure 35).

Discussion

Both the watershed assessment pilot study done in the Upper East Branch Pecatonica and Upper Yellow River watersheds were undertaken to evaluate the utility of the geometric sampling design and statistical tools applied. This report describes how well the sampling design characterized stream resources and sources of stream degradation in the Yellow River Watershed, and whether the sampling design provides more complete information for a wider breadth of Water Division stream assessment and management activities than the Departments current stream assessment efforts.

Three main aspects of the Yellow River Watershed Pilot Study are discussed:

- The overall assessment of the Yellow River Watershed and environmental factors that appear most responsible for degraded stream conditions.

- The overall application and utility of the sampling design for WDNR watershed assessment and management efforts, and

- Technical details of the sampling design, data analyses, findings, and their relevance to future WDNR water resource and watershed assessment efforts.

Overall Condition of Stream Resources in the Upper Yellow River Watershed

- Stream habitat degradation and poor water quality conditions are pervasive throughout the Upper Yellow River watershed and study results indicate these factors are primarily responsible for the poor biological condition of the stream resources in the watershed.

- Nearly 70 percent of the stream assessment sites had benthic or riparian habitat conditions that were detrimental to the macroinvertebrate or fish assemblages at these sites. Historic channelization of stream reaches, streambank erosion resulting from storm runoff, and lack of groundwater inputs result in sediment-covered benthic habitat, eroded stream banks and intermittent, ephemeral or extended low-flow conditions. Each of these physical factors singly and in concert result in harsh environmental conditions for stream biota. Land management practices that protect or restore riparian habitat, reduce sediment delivery to streams, and promote groundwater infiltration can help improve the physical stream and riparian habitat in the Yellow River Watershed.

- Study results show very high concentrations of water column total dissolved phosphorus in the Yellow River Watershed. The median concentration was 2.0mg/L and the mean was 0.40mg/L. The large difference between the median and mean concentrations indicate a very skewed data set where sites with extremely high phosphorus concentrations are creating a very high mean. The mean concentration of total dissolved phosphorus is nearly six times the state's water quality standard. A primary source of instream phosphorus is surface runoff and tile drainage of cropland manure and chemical fertilizers. Cropland total phosphorus concentration trend data from the UW-Madison Soils Laboratory suggest a slight increase in soil phosphorus in Wood County since the mid-1980s and very slight decrease in Clark County during this same time period. Improved nutrient management will be critical to help reduce the extremely high phosphorus, particularly with economic shifts towards farm operations with increased herd sizes.

- Based on macroinvertebrate samples evaluated using Hilsenhoff's Biotic Index, sixty percent of the stream sites were rated "fair" to "fairly poor." fish index of biotic integrity results indicate only 20% of the stream survey sites were in "poor" condition. Further analysis of the discrepancies between the biological two biological indexes are warranted, but based on the physical and chemical data collected it's likely the macroinvertbrate indicators may be a better measure of the biological conditions found in this watershed. The aforementioned physical habitat and water quality conditions will need to improve to expect marked improvement in biological conditions in the Upper Yellow River Watershed.

Geometric Sampling Design Applicability to Department Stream Assessment and Watershed Management Efforts

Stream site and subwatershed-specific assessments

The sampling effort in the Yellow River Watershed was spatially intensive with 60 sites monitored within a 213 square mile HUC10 watershed, a relatively large number of sites given the size of the watershed, many more sites than what are routinely sampled in most WDNR watershed assessment studies. This level of sampling intensity and dispersal of sites among catchments of varying size provided information to identify geographic differences in land impacts at individual streams sites and in smaller (HUC 12) watersheds. Additional targeted-sites sampling provided data to assess potential impacts of point source pollution from municipal and industrial waste dischargers and from areas with urban runoff. The spatial scale and distribution of the assessment sites would allow WDNR and agencies such as county land conservation departments identify specific land uses, geographic areas, land owners, or point sources that may be causing degradation in stream quality. The sampling design provided WDNR and land management agencies sufficient information to work with individual or small numbers of landowners to address localized cropland or stream bank erosion or nutrient management problems.

The rigorous sampling design used in the Yellow River Pilot may be most cost-effective for watershed management projects where it is important:

- To accurately identify and quantify sources of environmental degradation with a high level of geographic precision and confidence in the concentrations and ecological effects of anthropogenic stressors. This information would likely be most useful when targeted pollution control efforts are being planned, site-specific implementation of BMPs are needed to cost-effectively control pollutant sources, or for studies evaluating the effectiveness of watershed management practices or programs.

Pollutant types and source determinations

It is generally understood that excess sediment and nutrients in polluted runoff negatively impact Wisconsin streams. Determining which specific pollutants are most damaging may not be needed if the BMPs being applied control multiple pollutant types (e.g., minimum till plowing that reduces both sediment and nutrient delivery to surface waters). Similarly, determining the specific geographic sources of polluted run-off within a watershed may be less critical if the BMPs being employed are not targeted to specific areas, but are being broadly applied voluntarily by landowners.

Conversely, geographically targeting pollutant-specific BMPs within a subwatershed that is in relatively poor condition may be more cost-effective. If pollution control efforts include costly infrastructure that controls nutrients and not sediment (e.g., manure storage or barnyard runoff control structures), then it is desirable to be confident that the installation expenditures are necessary and cost-effectively applied. Economic shifts towards larger livestock herd and flock sizes and increasing numbers of concentrated animal feeding operations (CAFOs), are creating geographically-concentrated areas of manure spreading. It is important to know whether animal waste management regulations and individual operators' implementation of nutrient management plans are protective of surface waters.

Biotic responses to pollutants and threshold determination

The ability to document relationships between specific pollutants and degradation of aquatic life is of significant value. Data can be used to determine whether there are pollutant concentration thresholds that once exceeded, cause stream biology to significantly decline. Estimates of the magnitude of biological degradation within a stream or stream sites and the level of stressor or pollutant reduction necessary to restore the biological integrity of a stream can be assessed. If this information is coupled with watershed pollutant loading estimates and knowledge of BMP effectiveness in reducing pollutant loads, objective and quantifiable watershed land management goals can be set. These data can be used to develop realistic, cost-effective, and quantifiable watershed management goals.

Data applications to multiple program areas

Cost effectiveness of watershed assessment projects can be increased by generating data that can be used by a wider breadth of Department and other agencies' watershed management programs than previous monitoring efforts. The Yellow Pilot project could have provided more useful information if additional discussions and planning among Department programs and county land and water conservation departments had occurred prior to the start of the project. The relatively short time frame for implementing the study, reduced the ability to meet more frequently with stakeholders than would have been desirable.

The Yellow River Pilot project did, however, generate data and information of use to a number of Department programs and other agencies.

- The project generated data to adequately characterize stream resource conditions in the Yellow River Watershed for U.S. EPA 305(b) reporting.
- Thirty-two of 6o stream sites had sufficient (six per field season) water chemistry grab samples collected to allow determination of U.S. EPA Impaired Waters (303(d)) Listing or Delisting under the current Wisconsin Comprehensive Assessment and Listing Methodology (WisCALM) guidelines.
- While the study was not designed as a TMDL project, the geometric design and a streamflow gaging station at the pour point of the watershed, and the intensity of sampling sites and parameters measured, would provide adequate information for assessing stream resource conditions. The sampling design and statistical tools used identified key pollutants and thresholds for degradation. Streamflow data coupled with water column pollutant concentrations, would allow the estimation of pollutant loading.
- Stream sampling sites were situated upstream and downstream of each of the WWTPs within the watershed to provide some information on the influences these point source discharges had on water quality and biological integrity of the streams. This information can be used to evaluate the efficacy of WPDES permit program.
- Limited water chemistry data, collected from several streams receiving urban runoff, provided sample data to determine concentrations of metals and PAHs flowing off of urban developments.
- The sampling effort provided sufficient spatial resolution to direct Department water quality biologists and county land conservation staff to specific areas with high pollutant levels and degraded biological conditions.

Stakeholder involvement

A key benefit of small-scale watershed-based projects is that they provide an opportunity for various government agencies and other stakeholder groups involved in localized watershed and point source management issues to collaborate on a focused effort. Projects where all key stakeholders are engaged, from planning through implementation, with defined goals, milestones, and timelines, are more likely to achieve positive cost-effective results.

Technical Aspects of the Geometric Sampling Design, Data Collected, and Statistical Techniques

Site selection method

Stream quality is often strongly influenced by adjacent riparian and upstream watershed land use. The Yellow River watershed, like much of Wisconsin, is a patchwork of land cover types and uses. As a result, factors that degrade stream quality often have high spatial and temporal variability. Since only a relatively small proportion of stream miles can be assessed in any given watershed each year, selecting sampling sites in an unbiased fashion is important if the survey goals are to accurately characterize overall stream resource conditions and identify and quantify sources of environmental degradation. The geometric sampling design reduces the potential for site selection bias, since sites are selected in a systematic fashion and not by land use activities, perceived or known stream quality conditions, or by interest in specific streams or stream reaches.

Spatial scale and sampling intensity

Given the relatively small size (213 sq. mi.) of the study area and large number of sampling sites (n = 60), the study design allowed accurate characterization of stream resource conditions at precise spatial scales. This detailed information allows the targeting of pollutant-specific watershed management actions at small catchment or farm scales, which would allow the Department and other land management agencies to target the application of site and pollutant-specific best management practices.

Sampling upstream and downstream of WWTP point sources of pollution provided information that can be used to determine the impacts of these discharges to stream resources and evaluate WPDES Program efforts. Sampling runoff from urban areas provided information on the effects of developed lands on stream quality, information that can be used to determine whether urban stormwater runoff is a source of pollutants of concern within a watershed.

High numbers of stream samples and analytes

A relatively high number of chemical parameters (some with high lab analytical costs) were sampled for the Yellow River Pilot project. It was recognized during the planning of the pilot that the Department cannot routinely afford this level of sampling intensity (numbers and types of analytes, and numbers of repeated sampling efforts per site). A goal of the pilot was to measure a wide array of chemical parameters to help ensure that all key factors likely impacting the biological integrity of the streams in this watershed were adequately sampled. In addition, testing for chemical analytes not routinely monitored by the Department also provided information on pollutants that may be of concern, but where general information is lacking. Based on the results of the pilot study, it is possible to shorten parameter lists for future studies to decrease project costs and increase cost effectiveness.

Instantaneous measures of water quality are both spatially and temporally dynamic, but repeated measurements of these parameters (up to six times) at each of the stream sites over the course of the sampling season provided data that was sufficiently robust to characterize site-specific water quality conditions. Instantaneous measures of water temperature, water transparency, and dissolved oxygen concentrations were shown to be strong predictors of the condition of the macroinvertebrate and fish assemblages.

Lab-analyzed water column grab samples were collected twice at each small watershed pour point, four times at intermediate-sized sites, and six times at the pour points of the largest catchments. This level of repeat sampling provided fairly robust characterization of stream sites, streams, and watersheds. The chemistry data can be aggregated to the individual stream scale for relatively robust characterization of individual streams and their watersheds. Differences among the major subwatersheds' water quality and resulting biological conditions were evident, and this information can be used to target Best Management Practices within the Yellow River watershed.

The repeat sampling of water quality (electronic meter readings) and grab samples provided sufficient data to determine which environmental parameters influenced the biological assemblages at individual stream sites within the Yellow River watershed, and to document significant differences among the three subwatersheds. Similarly, assessing stream habitat provided site-specific habitat characterization and data to demonstrate that physical habitat quality was a key factor influencing the macroinvertebrates and fish assemblages at site-specific, subwatershed, and watershed scales.

Analysis of water column and stream bed sediment samples for metals and PAHs provided site-specific information to show that stormwater flowing off developed areas influenced stream sediment quality.

Key Findings of the Statistical Analyses

The statistical techniques applied to the Yellow River dataset are a significant advancement over data analytical methods currently used by most Department staff. It would be advantageous for staff to become familiar with at least some of these tools and apply them in future monitoring and assessment projects in order to objectively identify and rank environmental factors impacting aquatic resources, better determine the extent of stream degradation, and estimate the level of corrective actions needed to restore degraded streams or stream sites.

Bray-Curtis analysis was used to determine distinct groupings of streams (based on fish and macroinvertebrate assemblage data), and results were validated with unconstrained Nonmetric Multi-dimensional Scaling. Constrained Nonmetric Multi-dimensional Scaling, Canonical Correspondence Analysis, and Regression Tree analyses are statistical tools that were used to identify different groupings of streams based on their biology. Ultimately, these tools determined what environmental factors were most significant in causing these groupings. Once key “drivers” of biological condition were identified with NMDS, CCA, and RT, this information was then incorporated into Structural Equation Models for both fish and macroinvertebrates to validate the previous statistical findings, and rank the relative influence of these key environmental drivers in influencing the fish or macroinvertebrates. Lastly, Quantile Analyses were used to determine whether correlations could be seen between individual stressors and biotic condition, and identify thresholds for the various stressors that, once exceeded, resulted in biological degradation.

Future Use of Statistical Tools in R Programming Language

A number of statistical methods unfamiliar to most Department monitoring staff were applied for the first time, and were run using “R” software (R Development Core Team, 2006). R software is an open source computer programming software that is relatively new and used by few Department staff. The rationale for using R for the pilot project was that this free software had scripts written for most of the statistical analyses to be used, and helpful “on-line” guidance on running scripts and interpreting results.

It took Department staff some time to understand the applications of the statistical tools and how to run them in R. While it is unlikely that a majority of field biologists have sufficient training to apply many of these statistical routines, most field biologists and some Bureau staff would benefit from a greater understanding and application of at least some of these statistical tools in their work. Since the R scripts have been developed for the tests that were run in the pilot study, and there are now specific examples of how these statistical routines can be used and how results are interpreted for watershed assessments. Future applications of these tools should be more efficient and their value more evident. A folder will be placed on a central server containing the R code, an overview of how to run the statistical routines and guidelines to interpret the results to provide staff the opportunity to advance their data analytical skills.

Key findings of this study

Water quality measures including dissolved oxygen, nutrients, and suspended sediment concentrations, as well as measures of stream physical habitat quality, were shown to be the most significant factors influencing the biological conditions of the Yellow River Watershed stream sites. While numerous studies have shown that physical habitat and water quality influence stream biota, the results of this study help identify specific chemical parameters and habitat features that are most influential, the relative importance of each measure, and at what threshold (if a threshold exists) these individual factors begin to significantly affect biological quality of a stream.

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Appendix 1. Study variables and their abbreviations grouped and color-coded by parameter types.

Variable	**R Variable Name**	**Explanatory**	**Response**	**Category**
conductivity median	Cond	y		field chem
DO mg/L median	DOmgL	y		field chem
pH	pH	y		field chem
temperature median	Temp	y		field chem
transparency	Trans	y		field chem
Fish IBI integrity rating	FishInteg		y	fish
Fish IBI score (based on Lyons' IBIs for cold, cool, & warm water; IBI was chosen based on stream natural community)	FishIBI		y	fish
Fish IBI used (cold/cool/warm--simpler proxy for natural community)	FishIBIUsed	y		fish
Bank Erosion Score	QHabEros	y		habitat
Fine Sediments Score	QHabFines	y		habitat
Fish Cover Score	QHabCov	y		habitat
Flow (baseflow)	Flow	y		habitat
Gradient	Gradient	y		habitat
Natural Community (cold/cool/warm mainstem/headwater)	NatComm	y		habitat
Pool Area Score	QHabPool	y		habitat
QHEI Channel metric: sinuosity, development, condition (recovering, etc.), stability	QHEIchan	y		habitat
QHEI Cover metric: types, amount (extensive, sparse, etc.)	QHEIcov	y		habitat
QHEI Gradient metric: map gradient, drainage area	QHEIgrads	y		habitat
QHEI high & moderate influence modified habitat attributes (?)	QHEImwh	y		habitat
QHEI high influence modified habitat attributes (?)	QHEImwh_h	y		habitat
QHEI map gradient (DON'T INCLUDE--ALREADY IN QHEIgrads)	QHEIgradv	y		habitat
QHEI Pool metric: maximum depth, current types, pool-riffle width ratio	QHEIpool	y		habitat
QHEI Riffle-Run metric: riffle-run depth, substrate size, stability, embeddedness	QHEIrif	y		habitat
QHEI Riparian metric: width, type, bank erosion	QHEIrip	y		habitat
QHEI Score	QHEI	y		habitat
QHEI Substrate metric: 2 dominant types, number "high quality", origin, cover, embeddedness	QHEIsub	y		habitat
QHEI warmwater habitat attributes	QHEIwwh	y		habitat
Qualitative Habitat Rating (excellent/good/fair/poor/very poor)	QHabRating	y		habitat
Qualitative Habitat Score	QHab	y		habitat
Riffle Riffle Ratio Score	QHabRif	y		habitat
Riparian Buffer Score	QHabBuff	y		habitat

Small stream or large stream (large: > 10m average width)	SmallLarge	y		habitat
Stream Width	StrmWidth	y		habitat
Width Depth Score	QHabWDRatio	y		habitat
Inverts - HIBI	HBI		y	inverts
Inverts - MIBI	mIBI		y	inverts
Drainage area	DrainArea	y		land use
%Cropland within 100 m of the stream channel	Crop100	y		land use
%Cropland within 500 m of the stream channel	Crop500	y		land use
Total %Cropland (subwatershed)	CropSub	y		land use
%Developed within 100 m of the stream channel	Dev100	y		land use
%Developed within 500 m of the stream channel	Dev500	y		land use
Total %Developed (subwatershed)	DevSub	y		land use
%Forest within 100 m of the stream channel	For100	y		land use
%Forest within 500 m of the stream channel	For500	y		land use
Total %Forest (subwatershed)	ForSub	y		land use
%Pasture/ Grass/ Hay within 100 m of the stream channel	Pas100	y		land use
%Pasture/ Grass/ Hay within 500 m of the stream channel	Pas500	y		land use
Total %Pasture/ Grass/ Hay (subwatershed)	PasSub	y		land use
%Water within 100 m of the stream channel	Wat100	y		land use
%Water within 500 m of the stream channel	Wat500	y		land use
Total %Water (subwatershed)	WatSub	y		land use
%Wetland within 100 m of the stream channel	Wet100	y		land use
%Wetland within 500 m of the stream channel	Wet500	y		land use
Total %Wetland (subwatershed)	WetSub	y		land use
%Undeveloped land within 100 m (forest, pas/grass/hay, wetland, open water)	Undev100	y		land use
%Undeveloped land within 500 m (forest, pas/grass/hay, wetland, open water)	Undev500	y		land use
%Undeveloped land (subwatershed) (forest, pas/grass/hay, wetland, open water)	UndevTotal	y		land use
%Undeveloped land within 100 m (forest, wetland, open water)	Undev100NoPas	y		land use
%Undeveloped land within 500 m (forest, wetland, open water)	Undev500NoPas	y		land use
%Undeveloped land (subwatershed) (forest, wetland, open water)	UndevTotNopas	y		land use
% Dry Solids 105C	DS105C	y		soil chem
% Solids 60C	L60C	y		soil chem
% Volatile Solids	VS	y		soil chem
2-Fluorobiphenyl	2Fluor	y		soil chem
2-Methylnaphthalene	2Meth	y		soil chem
4,4'-DDD	44DDD	y		soil chem
4,4'-DDE	44DDE	y		soil chem
4,4'-DDT	44DDT	y		soil chem
Acenaphthene	Anap	y		soil chem
Acenaphthylene	Athy	y		soil chem

Aldrin	Ald	y		soil chem
alpha-BHC	ABHC	y		soil chem
Alpha-Chlordane	Achl	y		soil chem
Aluminum	Al	y		soil chem
Ammonia	NH3	y		soil chem
Anthracene	Anth	y		soil chem
Antimony	Ant	y		soil chem
Arsenic	Ar	y		soil chem
Atrazine	Atr	y		soil chem
Barium	Ba	y		soil chem
Benzo (a) anthracene	BenzA	y		soil chem
Benzo(a)pyrene	BenzP	y		soil chem
Benzo(b)fluoranthene	BenzbF	y		soil chem
Benzo(g,h,i)perylene	Benzpe	y		soil chem
Benzo(k)fluoranthene	BenzkF	y		soil chem
Beryllium	Be	y		soil chem
beta-BHC	BHC	y		soil chem
Boron	Bo	y		soil chem
Cadmium	Cad	y		soil chem
Calcium	Ca	y		soil chem
Chromium	Chrom	y		soil chem
Chrysene	Chry	y		soil chem
Cobalt	Cob	y		soil chem
Copper	Cop	y		soil chem
Decachlorobiphenyl	DCB	y		soil chem
delta-BHC	DBHC	y		soil chem
Dibenz(a,h)anthracene	Dbenz	y		soil chem
Dieldrin	Diel	y		soil chem
Endosulfan I	End1	y		soil chem
Endosulfan II	End2	y		soil chem
Endosulfan Sulfate	ESS	y		soil chem
Endrin	Endrin	y		soil chem
Endrin aldehyde	EA	y		soil chem
Endrin ketone	EndK	y		soil chem
Fluoranthene	FloA	y		soil chem
Fluorene	Fluor	y		soil chem
gamma-BHC	GBHC	y		soil chem
gamma-Chlordane	GC	y		soil chem
Heptachlor	Hept	y		soil chem
Heptachlor epoxide	HepE	y		soil chem
Indeno(1,2,3-cd)pyrene	Ind123	y		soil chem
Iron	Fe	y		soil chem
Lead	Pb	y		soil chem
Lithium	Li	y		soil chem
Magnesium	Mg	y		soil chem
Manganese	Mn	y		soil chem
Methoxychlor	Meth	y		soil chem
Molybdenum	Mb	y		soil chem

Naphthalene	Nap	y		soil chem
Nickel	Ni	y		soil chem
Nitrobenzene-d5	Nitro	y		soil chem
Phenanthrene	Phen	y		soil chem
Potassium	Pot	y		soil chem
Pyrene	Pyr	y		soil chem
Selenium	Sln	y		soil chem
Silver	Ag	y		soil chem
Sodium	Na	y		soil chem
Strontium	St	y		soil chem
Terphenyl-d14	Terp	y		soil chem
Tetrachloro-meta-xylene	TMX	y		soil chem
Thallium	Thal	y		soil chem
Tin	Tin	y		soil chem
Titanium	Ti	y		soil chem
Total Kjeldahl Nitrogen	TKN	y		soil chem
Total Organic Carbon	TOC	y		soil chem
Total Phosphorus	TP	y		soil chem
Vanadium	Va	y		soil chem
Zinc	Zn	y		soil chem
4,4'-DDD	44DDD	y		water chem
4,4'-DDE	44DDE	y		water chem
4,4'-DDT	44DDT	y		water chem
Aldrin	Ald	y		water chem
Alpha-BHC	ABHC	y		water chem
Alpha-Chlordane	Achl	y		water chem
Aluminum	Al	y		water chem
Ammonia as N median	NH3	y		water chem
Ammonia as N min	NH3Min	y		water chem
Antimony	Ant	y		water chem
Arsenic	Ar	y		water chem
Atrazine	Atr	y		water chem
Barium	Ba	y		water chem
Beta-BHC	BHC	y		water chem
Biochemical Oxygen Demand median	BOD	y		water chem
Boron	Bo	y		water chem
Cadmium	Cad	y		water chem
Calcium	Ca	y		water chem
Chloride	Chloride	y		water chem
Chlorophyll-a	ChlA	y		water chem
Chromium	Chrom	y		water chem
Cobalt	Cob	y		water chem
Copper	Cop	y		water chem
Decachlorobiphenyl	DCB	y		water chem
delta-BHC	DBHC	y		water chem
Dieldrin	Diel	y		water chem
E. coli	Ecoli	y		water chem
Endosulfan I	End1	y		water chem

Endosulfan II	End2	y		water chem
Endosulfan Sulfate	ESS	y		water chem
Endrin	Endrin	y		water chem
Endrin aldehyde	EA	y		water chem
Endrin ketone	EndK	y		water chem
gamma-BHC	GBHC	y		water chem
gamma-Chlordane	GC	y		water chem
Heptachlor	Hept	y		water chem
Heptachlor epoxide	HepE	y		water chem
Iron	Fe	y		water chem
Lead	Pb	y		water chem
Lithium	Li	y		water chem
Magnesium	Mg	y		water chem
Manganese	Mn	y		water chem
Methoxychlor	Meth	y		water chem
Molybdenum	Mb	y		water chem
Nickel	Ni	y		water chem
Nitrate-Nitrite Nitrogen as N	NO3NO2N	y		water chem
Potassium	Pot	y		water chem
Selenium	Sln	y		water chem
Silver	Ag	y		water chem
Sodium	Na	y		water chem
Strontium	St	y		water chem
Sulfate as SO4	Sulfate	y		water chem
Suspended Sediment Concentration max	SSCMax	y		water chem
Suspended Sediment Concentration median	SSC	y		water chem
Tetrachloro-meta-xylene	TMX	y		water chem
Thallium	Thal	y		water chem
Tin	Tin	y		water chem
Titanium	Ti	y		water chem
Total Dissolved Solids median	TDS	y		water chem
Total Kjeldahl Nitrogen median	TKN	y		water chem
Total Phosphorus median	TP	y		water chem
Total Suspended Solids median	TSS	y		water chem
Vanadium	Va	y		water chem
Zinc	Zn	y		water chem