Pecatonica River Watershed Assessment Pilot Project Report





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Michael Miller, Katherine Songer, Ronald Dolen, and Donald Barrette **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 Resource's Bureau of Water Quality, with support from U.S. Environmental Protection Agency's – Region 5 (USEPA) and the Midwest Biodiversity Institute (MBI), conducted a watershed assessment pilot study in the Upper East Branch Pecatonica Watershed in southwestern Wisconsin beginning in 2010.

The goals of the pilot were to:

- Apply and evaluate the effectiveness of a stream sampling design that systematically selects 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 quality and identifying physical and chemical stressors impacting stream biology.
- Evaluate how the applied statistical methods may be used by the Department for future stream resources assessment and watershed management projects.
- Evaluate how the pilot sampling design can effectively provide information for Water Division stream assessment and management activities compared to the Department's current stream assessment efforts.

SUMMARY of STUDY FINDINGS

The Pecatonica Watershed study area was 221 square miles. Stream sampling sites were systematically selected based on land area upstream of each sampling point, where stream sites draining progressively smaller and smaller watersheds were sampled. A total of 68 stream sites were sampled for habitat, macroinvertebrates, and fish. Water chemistry samples were collected at each of these sites between two and six times, with smaller watersheds being sampled fewer times relative to the larger catchments. Streambed sediment was collected at the 49 largest catchments and analyzed for metals. Sediment samples collected from nine sites located downstream of urban areas and wastewater treatment plants were also analyzed for polycyclic aromatic hydrocarbons (PAHs). The sampling design and statistical methods applied provided a rigorous assessment of stream physical, chemical, and biological conditions of the Pecatonica Watershed at precise spatial scales. This information allowed the determination of what proportions of the stream sampling sites (and by inference the total stream population) showed physical, chemical, or biological degradation. Statistical analysis results provided information to determine which environmental factors were most responsible for biological degradation and their relative importance. Statistical analyses also provided precise estimates of thresholds at which individual pollutants or other environmental stressors caused macroinvertebrate or fish populations to decline.

SUMMARY of STUDY FINDINGS CONTINUED

Overall Watershed Conditions:

- Thirty-two percent of the sampling sites had median total phosphorus concentrations exceeding the state water quality criterion.
- Eighteen percent of the stream sites were in poor condition based on fish Index of Biotic Integrity (IBI) scores.
- Six percent of the streams sites were in poor condition based on macroinvertebrate IBI scores.
- Seventy-six percent of the stream sites had *Escherichia coli* bacteria concentrations that exceeded the federal standard for recreational waters.
- Eighty-eight percent of the stream sites had physical habitat, water quality or chemical stressors that exceeded thresholds resulting in declines in the integrity of fish or macroinvertebrate populations.

Physical Habitat and Water Quality:

- Various statistical tests indicated that streambed sedimentation and embeddedness, water turbidity, suspended sediment, and low dissolved oxygen concentrations were the primary physical factors influencing stream biota.
- Streambed sedimentation and embeddedness degraded over 70 percent of the sampling sites.
- Overall habitat degradation at 38 percent of the sampling sites was correlated with poor fish or macroinvertebrate populations.
- Reduced dissolved oxygen concentrations at 38 percent of the sampling sites were associated with degraded biological conditions.
- Reduced water transparency at 25 percent of the sampling sites was associated with degraded biological conditions.

Streambed Sediment Chemistry:

- Streambed sediment was analyzed for metals at forty-nine of the larger catchments. Chromium, copper, lead, manganese, nickel, and zinc were detected at all of these sampling sites.
- Seven sites in the Dodge Branch Watershed (all downstream of the City of Dodgeville) had concentrations of various metals thought to be toxic to benthic organisms (macroinvertebrates) based on Department sediment quality guidelines.
- Nine stream sites downstream of urban areas or wastewater treatment plants were sampled for PAHs, all of these sites had detectable concentrations of these compounds, three of which had concentrations thought to be toxic to benthic organisms based on Department sediment quality guidelines.

Water Column Chemistry:

- Multiple statistical tests indicated that phosphorus and nitrogen concentrations were the primary water column nutrients impacting macroinvertebrate or fish populations.
- Quantile regression analysis results suggested that concentrations of total phosphorus above 0.05 mg/L impacted invertebrate or fish populations. This concentration was exceeded at 59 percent of the study sites.
- Fifty percent of the sampling sites had concentrations of total Kjeldahl nitrogen (TKN) that were associated with biological degradation.
- All of the sampling sites had detectable concentrations of atrazine or its metabolites in the water column.

Biological Samples:

- Eighty-seven percent of the sites were rated "good" to "excellent" based on Hilsenhoff's Biotic Index (HBI) scoring criteria of macroinvertebrate samples.
- All sites were surveyed for fish but 17 percent of sites had no fish or too few fish to calculate an index of biotic integrity (IBI) score.
- A total of 51 sites had fish IBI scores ranging from "good" to "excellent", 22 percent were rated "fair", and 10 percent of the sites were rated "poor" or "very poor".
- Overall 34 fish species were identified in the watershed.
- Brown trout had the highest frequency of occurrence of any fish species and were found at 71 percent of the sampling sites.

Statistical Modeling Results:

- Nonmetric multi-dimensional scaling analyses independently using fish and macroinvertebrate populations as biological measures of stream quality indicated that dissolved oxygen, suspended sediment, and chlorophyll concentrations, and quantities of fish cover and riffle habitat were key factors impacting these biological assemblages.
- Classification and regression tree analyses using fish and macroinvertebrate index scores independently as measures of environmental quality indicated that dissolved oxygen, total Kjeldahl nitrogen, and total phosphorus concentrations for fish, and dissolved oxygen, total dissolved solids, chlorophyll, and total phosphorus concentrations for macroinvertebrates had the strongest influence on stream biota.
- Structural equation modeling (SEM) indicated that 58 percent of the variability seen among fish IBI site scores and 47 percent of the macroinvertebrate HBI scores could be accounted for by various physical or chemical environmental factors.
- SEM results also indicated that overall "water quality" conditions at the sampling sites had the strongest influence on both fish and macroinvertebrates relative to stream physical habitat or other site or watershed-scale measures.

 Quantile regression analysis results indicate stream habitat features, water transparency, concentrations of total phosphorus, dissolved oxygen, suspended sediment, and total Kjeldahl nitrogen significantly influenced the fish assemblages found at the sampling sites. Dissolved oxygen, total dissolved solids, and total dissolved phosphorus concentrations were shown to be the major factors influencing the macroinvertebrate assemblages found at the stream sites.

The sampling design applied in this pilot study provided site and stream-specific information on physical, chemical and biological conditions and identified specific physical or chemical factors that were impacting aquatic life. This information can be used to direct land management actions at precise and economically feasible spatial scales, and can be used by a variety of Department program areas including Clean Water Act Section 305(b) reporting and Section 303(d) listing/delisting, Total Maximum Daily Load modeling (TMDL), polluted runoff management, and Wisconsin Pollution Discharge Elimination System (WPDES) permits evaluation.

The rigorous sampling design used in the Pecatonica Pilot may be most cost effective for watershed management projects where it is important to accurately identify specific pollutants and quantify sources of environmental degradation with a high level of geographic precision. This information will 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.

Finally, the watershed-based stream assessment strategy applied in the pilot study could provide a forum for local land and water resource management organizations to coordinate assessment and management actions. The size of the study area and duration of the assessment effort were at spatial and time scales at which management actions can effectively take place.

Table 1. Pecatonica Watershed assessment summary results by stream (denoted by blue bars). Sampling sites where water quality constituents exceeded state or federal standards or guidelines are highlighted in red, limiting factors were determined by quantile regression analyses of stressor thresholds.

		Watershed	Nat.	Median TP	T: ala		E aali		
Stream Name	011.10	Area sq.	Comm.		FISN	Invert	E. COII	De l'arrent De llester (-6	1 the little of F = 1 + 1 + 7
SWIMS Station ID	Site ID	mı.	Class	(No. Samples)	IBI	IBI.	(Col./100 ml)	Sediment Pollutants	Limiting Factors
1001/1310	1.7002	17	СН	0.03 (2)	50	5.5	3 030		TKN Transp
Brager Branch	1.7 wpz	1.7	OIT	0.03 (2)	50	5.5	3,330		Trin, Transp.
10031466	1 7a1	2.0	CCH	0.02 (2)	70	62	1 986		Hab Transp
Conley Lewis Cre	ek	2.0	0011	0.02 (2)	10	0.2	1,000		
10014163	3.5wp2	2.8	CCH	0.07 (4)	70	4.0	268		TP, TKN, TDS
10015180	13.5wp	13.5	CCM	0.08 (6)	80	5.5	549		TP, TDS, %DOmin.
Dodge Branch									
10031458	1.7wp4	0.7	CCH	0.12 (2)	0	8.3	5,120		Hab., TP, TKN, TDS, %DOmin.
									TKN, TDS, %DOmin. Metals,
253043	1p	0.9	CWH	0.02 (6)	20	2.7	488	Lead, Zinc, PAHs	PAHs
					_			Arsenic, Cadmium, Lead,	Hab., TP, TKN, TDS, %DOmin.
253044	1.12p	1.1	CWH	0.41 (6)	0	1.2	6,050	Zinc, PAHs	Metals, PAHs
40004407	4 0	4.0	0011	0.00 (0)	~		10, 100	Land Tree	Hab., IDS, %DOmin. Transp.,
10031467	1.6Wp12	1.3	CCH	0.30 (2)	10	4.1	10,460	Lead, Zinc	Metals
10031470	1.53wp11	1.5	CCH	0.67 (2)	40	4.5	4,350		TDS %DOmin Transp. Metals
253052	1 7wn5	4.4	ССН	0.04 (4)	90	47	613	Lead Zinc PAHs	
10031448	7.3wn	7.2	CCH	0.05 (4)	90	6.1	1 300	Lead, Zinc, 1 Allo	TKN TDS %DOmin Metals
10008143	13.8wp1	17.7	CWM	0.10 (6)	60	3.7	914	Lead Zinc	TP TKN TDS Transp Metals
10031445	28.7wp	25.9	CWM	0.10 (6)	60	5.6	1.046	Lead, Zinc	TDS. %DOmin. Transp., Metals
							,	,	TP, TKN, TDS, %DOmin.
10015258	38.6p	38.7	WM	0.06 (6)	20	4.7	1,300		Transp.
	· · · ·	ĺ					,		TP, TKN, TDS, %DOmin.
10031444	43p	43.3	WM	0.12 (6)	20	3.9	1,046		Transp.
									TP, TKN, TDS, %DOmin.
10015257	57.5wp1	58.2	WM	0.11 (6)	30	8.7	1,986		Transp.
253099	66.1p	66.0	WM	0.06 (6)	20	4.2	517		TP, TKN, TDS, Transp.
10031624	67.84p	67.8	WM	0.07 (4)	20	5.2	866		Hab., TP, TDS
Upper East Brance	n Pecator	nica			_				
									Hab., TP, TKN, %DOmin.
10031455	1.85up7	1.8	CH	0.24 (2)	ND	3.1	7,200		Transp.
10031463	1.7up2	2.0	CCH	0.11 (2)	0	2.8	27,550		Hab., TP, TKN, %DOmin.
10031454	1.92p	2.0	CH	0.02 (3)	ND	6.1	613		TKN, TDS, %DOmin.
10031459	1.7up6	2.4	CCH	0.02 (2)	80	6.2	196		Hab.
10021754	3.5up2	3.5	CH	0.06 (4)	70	7.8	1,120		IP, IKN, IDS
253094	6.5up	6.5	CH	0.04 (4)	50	4.9	2,419		Hab., IDS, %DOmin.
10031447	14.90p3	17.6	CCM	0.07 (6)	40	4.0	261		TP, TDS, %DOmin.
10031623	14.93p	17.7	CCM	0.11 (4)	70	2.3	9,330		IP, IDS
10031446	21p	24.9	CCM	0.10 (6)	60	6.9	617		TP, TEN
10014311	45.5p	40.3	CCM	0.07 (6)	00	4.9	301		IP, INN
10020046	120.4p1	122.0	CUM	0.05 (6)	90	4.0	479		TR TKN TDS
252100	120.4p1	122.0	CWW	0.09 (0)	40	2.0	900		TR TKN TDS
10031443	132 75n	135.4	CWM	0.09 (6)	50	3.9	222		Hab TP TKN TDS
10031443	136n	140.1	CWM	0.03 (0)	30	0.5	517		Hab TP TKN TDS
10031442	221	217.0	WM	0.07 (6)	25	6.4	649		Hab TP Transp
German Valley Br	anch	217.0		0.07 (0)	20	0.1	010		
10011872	1.706	2.1	CCH	0.02 (2)	80	8.0	201		TDS
10031449	7.3a	7.3	CM	0.02 (4)	60	4.2	276		TKN. TDS
Gordon Creek			•	0.02(1)	1 00				·····, ····
10031469	1.55q9	1.6	CCH	0.04 (2)	ND	7.2	770		Hab.
10016644	2.65g	2.5	CM	0.02 (4)	60	6.2	122		TKN, TDS
10011740	4.9g1	4.2	СМ	0.03 (4)	80	5.7	155		
10031450	6.9g1	6.8	CM	0.04 (2)	90	6.6	189		TDS
10008165	27.9g	27.8	CM	0.04 (6)	50	10.3	141		
253205	30g	30.9	CCM	0.04 (6)	50	6.8	214		TDS, %DOmin.
253101	66g	66.1	CCM	0.04 (6)	60	5.6	722		TDS
10021401	71g	71.0	CCM	0.05 (6)	80	4.9	326		
10029189	76.9g	76.7	CCM	0.05 (6)	70	5.4	326		Hab.

Table 1 continued. Pecatonica Watershed assessment summary results by stream (denoted by blue bars). Sampling sites where water quality constituents exceeded state or federal standards or guidelines are highlighted in red, limiting factors were determined by quantile regression analyses of stressor thresholds.

		Wate web a d	Nat	Median TP					
Stream Name		watershed	Comm		Fich	Invert	E coli		
	CH4 1D	Area sq.	Class1		1131		(Cal. (100 m)) ⁵		Limiting Fraters
Swikis Station ID	Site ID		Class	(No. Samples)	IBI.	в	(Col./100 ml)	Sediment Pollutants	Limiting Factors
Gribble Branch	4.05	0.0	0011	0.00 (0)	00	6.4	1.000		T/NL TDC
	1.65wp9	2.2	ССП	0.02 (2)	00	0.4	1,900		IKN, IDS
Jegiuni valley Cre	1.7~2	2.1	CCH	0.09.(2)	0	6.0	1.096		Hab TR TKN TRS
10031465	1.7g2	2.1	LCH	0.08 (2)	0	6.0	1,986		Hab., IP, IKN, IDS
Kittleson valley C	4 7=4	2.0	011	0.44 (0)	00	0.4	4 700		U-F TR TKN TRC
10015426	1.794	3.0	CH	0.11(2)	60	8.1	1,733		Hab., IP, IKN, IDS
10009432	19.1g	19.2		0.02 (5)	80	5.5	179		Hat TDO
133444	29.89g	29.8	CCM	0.03 (6)	08	5.9	2/6		Hab., IDS
253058	33g	32.9	CCIVI	0.05 (6)	80	7.0	240		
Lee Greek	4.7-0	1.0	0011	0.40.(0)		5.0	1 000		TD T(N % DO
10029295	1.7g3	1.6	CCH	0.13 (2)	ND	5.9	1,300		IP, IKN, %DOmin. Iransp.
10031452	3.5g2	3.7	CH	0.02 (4)	70	8.7	250		Hab., IP, IKN, IDS
Ley Creek			0.011	0.05 (4)	=0		0.440		
253057	3.3wp3	3.2	CCH	0.05 (4)	70	1.1	2,419		Hab., IKN, IDS
Lynch Branch				(1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.					
10014320	1.54wp10	2.2	CCH	(0.02 (1)	70	7.9	365		IDS
Olson Creek			1						
10031456	1.7wp7	1.7	CCH	0.09 (2)	0	2.3	147		Hab., TP, TKN, TDS, %DOmin.
10029527	3.5wp1	3.1	CM	0.04 (4)	90	6.5	214		TDS
Pleasant Valley B	ranch		· · · · · · · · · · · · · · · · · · ·		_	·			
10031464	1.7g5	0.9	CCH	0.08 (2)	ND	4.9	222		TP, TDS, %DOmin.
10009781	3.5g3	3.8	CH	0.02 (4)	50	5.5	238		TDS, %DOmin.
10011636	7.5g	7.5	CM	0.02 (4)	60	5.7	172		TDS
Smith Conley Cree	ek			-					
10031762	0p5	0.8	CCH	0.07 (3)	40	4.1	5,560		Hab., TP, TDS, %DOmin.
10031462	1.7up3	1.7	CCH	0.47 (6)	0	1.1	3,730		Hab., TP, TKN, TDS, %DOmin.
10012856	9.68p	9.7	CM	0.07 (4)	60	4.8	792		TP, TKN, TDS
10016138	13.8up2	14.0	CM	0.03 (6)	80	7.8	57		
10008171	19.1p	19.0	CM	0.04 (7)	40	6.3	130		
Syfestad Creek									
10031453	3.19g3	3.2	CM	0.02 (4)	80	7.1	137		TKN, %DOmin.
Urnus Creek									
10031457	1.7wp6	2.0	CWH	0.03 (2)	30	2.9	1,046		Hab., %DOmin. Transp.
Williams-Barneve	ld Creek								
10012833	3.3un4	61	CM	0.04 (4)	40	35	461		TKN TDS %DOmin

¹Natural Community Classes (NCC) highlighted in blue are sites where the NCC model prediction for the stream differed from the fish assemblage found at the site and best professional judgment was used to assign a different NCC. ²Number of total phosphorus (TP) grab samples collected at each site are reported within parentheses, sites where the median TP concentrations exceeded the state water quality standard are colored red. ³Stream sites where too few fish to calculate an Index of Biotic Integrity (IBI) score, or no fish were captured are denoted by ND (no data); sites colored red indicate a "poor" fish IBI score. ⁴Sites with "poor" macroinvertebrate index scores are colored red. ⁵Sites colored red where single water column samples had Escherichia coli sample concentrations above the federal recreational water quality standard. ⁶Sample sites colored red had sediment concentrations of various metals that exceeded concentrations thought to be toxic to benthic organisms. ⁷Limiting factors were based on the results of quantile regression analyses where threshold values for the parameters listed for each site indicated macroinvertebrate or fish assemblages were impacted by specific stressors such as poor habitat, excess nutrients, turbidity, or low DO levels.

INTRODUCTION

Monitoring data can be used to assess stream 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 and agencies on the quality of Wisconsin's stream resources.

Assessment and management of stream resources in Wisconsin is challenging given that there are over 40,000 miles of perennial streams and pollution sources and impacts are often dynamic. 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 suggested by the USEPA that these 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

The study was done in the East Branch Pecatonica River Watershed located primarily in southeastern lowa and southwestern Dane counties in southwest Wisconsin (Figure 1).



Figure 1. Map of the Upper East Branch Pecatonica River Watershed and perennial streams.

The watershed is located in the Driftless Area ecoregion which is typified by having rolling hills, relatively deep valleys, and streams with higher than average slope. The relatively steep topography of the region promotes water infiltration on upland slopes and ridges and groundwater discharge to streams in the valley bottoms. This results in streams with a significant baseflow of groundwater and fish assemblages comprised of a number of "coolwater" and "coldwater" species. Land use is primarily agricultural, with valley bottoms used for corn, soybean, and alfalfa production, grazing, or grassland. Steeper hillsides are often forested and level uplands are also cropped or grazed. There is a small amount of urban or suburban land within the study area.

The northern edge of the Upper East Pecatonica watershed is bounded by the "Military Ridge" and encompasses portions of the villages of Mount Horeb, Blue Mounds, Barneveld, and Ridgeway. The City of Dodgeville is at the western edge of the watershed and contributes the largest amount of urban land to the study area. The village of Hollandale is south centrally located, and the Village of Blanchardville is situated at the southern extent of the watershed.

Three major streams drain the watershed:

- 1. The Dodge Branch of the Pecatonica River.
- 2. The Upper East Branch of the Pecatonica River.
- 3. Gordon Creek.

These streams join to form the East Branch of the Pecatonica River which flows out of the study watershed through the Village of Blanchardville. The East Branch of the Pecatonica joins the mainstem of the Pecatonica River in southeast Layfayette County and flows another 10 miles through southwest Green County before entering Illinois.

METHODS

Sampling Design - Sites Selection

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

The random sampling stratification was based on watershed land area. These sampling sites were systematically selected at the drainage outlet (pour point) of specifically sized watershed areas. This survey design is referred to as a "geometric" design, since the size of the watershed drainage areas selected for sampling was a geometric progression that depended on (with the exception of the initial catchment selected) the size of the most previously selected watershed area (Yoder, 2010). For example, the size of the Pecatonica Pilot Study watershed was 221.4 mi², and a sampling site was situated at the pour point of the watershed at Blanchardville (Figure 2). The next smallest watershed area sampled (110.7 mi.²) was half the size of the previous watershed; the size of the next watershed sampled is 55.5 mi², and so forth until the smallest watershed areas (1.7 mi.² for this study) were delineated and each pour point

and sampling site was identified for sampling (Figure 3).

The sampling locations of these geometric stream monitoring sites were moved to the nearest road crossing to help facilitate sampling, particularly since water chemistry samples were collected 2-6 times at each sampling site.

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



Figure 2. Examples of geometric site catchment sizes (blue areas) and numbers of catchment sampling sites per catchment size.

Location of Sampling Sites and Data Collected at Each Site

The stream sampling locations are shown in Figure 3. The location for the pour point for the entire watershed is indicated by a red "X". Dark-blue dots show the locations of the geometric sampling sites, light-green dots show gap site locations and red squares mark the upstream and downstream sampling locations of the WWTP outfalls that are represented by black triangles.



Figure 3. Locations and types of stream sampling sites in the Pecatonica River Watershed.

Data Collection Methods

Data Collected at Each Sampling Site:

Riparian and in-stream habitat, streamflow volume, water quality, bacteria, macroinvertebrate, and fish assemblage data were collected at each of the random and targeted sampling sites.

Physical Habitat:

Visual estimates of in-stream and riparian habitat were surveyed once at all sites using both WDNR qualitative stream habitat assessment and MBI's Qualitative Habitat Evaluation Index (QHEI) protocols.

Sediment:

Streambed sediment samples were collected at a subset of the larger watershed area sampling sites (n = 49). Surficial sediment samples from instream depositional areas were analyzed for:

- Total organic carbon
- o Nutrients
 - total phosphorus
 - Kjeldahl nitrogen
 - ammonia
 - nitrate and nitrite
- o Metals
 - cadmium
 - copper
 - iron
 - lead
 - magnesium
 - zinc
- Pesticides
 - 4,4-DDD
 - 4,4-DDE
 - 4,4-DDT
 - total DDT
 - Aldrin
 - alpha-BHC
 - beta-BHC
 - Dieldrin
 - Endrin
 - Lindane
 - gamma-Chlordane
 - Heptachlor epoxide
 - Methoxychlor
 - Alachlor ESA
 - Acetochlor
 - Acetochlor ESA
 - Acetochlor OA
 - Atrazine

- Metolachlor ESA
- Metolachlor OA
- Simazine
- Polychlorinated biphenyls (PCBs)
- Polycyclic aromatic hydrocarbons (PAHs)

Water Quality:

Water clarity (transparency tube readings) and water chemistry grab samples were collected at all sites. Electronic meters were used to collect instantaneous measures of:

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

at all sites in conjunction with fish surveys and each time water chemistry grab samples were collected.

Water-Column Chemistry:

Grab samples were collected May through October during "baseflow" conditions. The smallest (1.7 mi.²) watershed area pour points were sampled twice during the field season, larger watershed pour points $(3 - 7 \text{ mi}^2)$ were sampled four times, and the largest watershed area pour points (14 mi.² - 221 mi.²) were sampled six times over the course of the field season (Figure 4, Table1).

Laboratory-analyzed parameters included:

- o total phosphorus
- o total dissolved phosphorus
- nitrate and nitrite
- o **ammonia**
- o total Kjeldahl nitrogen
- \circ chlorides
- o sulfates
- o biological oxygen demand
- total suspended solids
- total dissolved solids
- o suspended sediment concentration

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

Biological Measures were collected at all sites and included:

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

Fish assemblage data were interpreted using the appropriate fish index of biotic integrity (fIBI) for the site based on the stream's thermal regime and size. Sampling sites were classified using a statewide natural community classification scheme. If, based on the fish assemblage data collected at the stream site and knowledge of the area water quality the classification model appeared to be in error, best professional judgment was used to assign a different stream classification to the site and apply the appropriate fish IBI.

Quality Control Sampling:

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



Figure 4. Number of repeat sampling visits per site for each of the three sample populations (panel) sizes.

Panel	Area (square miles)	Site Type ^ª	No. of Sites	No. of Sample Events: Water Nutrients, Solids
1	1.7	Targeted	6	6
1	221.0	Geometric	1	6
1	110.0	Geometric	4	6
1	55.0	Geometric	8	6
1	27.7	Geometric	7	6
1	13.8	Geometric	6	6
2	6.9	Geometric	6	4
2	3.5	Geometric	10	4
3	1.7	Geometric	20	2
Total			68	294

Table 1. Number of sites and sample events by panel.

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

STATISTICAL METHODS

A series of statistical tests including: Bray-Curtis Ordination, Nonmetric Multidimensional Scaling, Canonical Correspondence Analysis, Classification and Regression Trees, Structural Equation Modeling, and Quantile Analyses were applied independently to both the macroinvertebrate and fish assemblage datasets. The primary goal of using these statistical tests was to determine which watershed land use, stream physical habitat, or water quality or chemistry factors had the greatest influence on the biological quality of stream resources in the Pecatonica Watershed. This information was used to help determine which watershed land uses and other human activities were most responsible for stream degradation. The tests can provide information to evaluate and direct watershed and water resource management actions.

Bray-Curtis Ordination (BC)

BC is an ordering method that was used to group stream sampling sites that had similar fish or macroinvertebrate assemblages (McCune and Grace, 2002). Stream sites most similar (in terms of numbers of individuals of a species, total number of species, and total number of individuals collected at each sampling site), are closer to one another along a continuum axis and sites more dissimilar are farther away from one another. The BC plots also produce a hierarchy of clusters (groups), showing (usually small) clusters of stream sites most similar to each other and larger aggregates of sites that are less similar to each other. 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 – only that similar groups of similar stream sites exist.

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 assemblages are clustered more closely together and dissimilar sites are plotted farther apart. This specific NMDS test is "unconstrained", meaning only the fish or macroinvertebrate data influence the clustering in the data plots, and clusters are not influenced (constrained) by physical or chemical data collected at the sampling sites. NMDS results were compared with the clustering of the sites seen in the BC plots. If BC and NMDS analyses result in similar groupings, it provides strong evidence that the site clusters observed are real and 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 Pecatonica watershed. Over 130 candidate watershed land cover, stream physical conditions and chemical response variables were regressed upon the NMDS clusters with the stream sites as independent variables and stream sites' physical and chemical characteristics as the dependent variables. Those variables most strongly correlated with the stream site clusters were plotted as vectors; arrow direction shows increasing magnitude of the environmental variable values, 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 Pecatonica 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 the biota (ter Braak, 1995). Similar to NMDS, CCA plots have vector arrows whose direction indicate increasing magnitude of the environmental variable values, and the longer the arrow length, the greater the relative effect the factor has in influencing the fish or macroinvertebrate populations.

Major assumptions of CCA 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 size or stream flow volume) was subjectively removed from the CCA input parameter dataset.

Classification and Regression Tree Analysis (CART)

CART is a statistical method (De'ath and Fabricius, 2000) that was used to group stream sites that were most similar to each other in terms of the fish or macroinvertebrate metric scores and identifies which physical or chemical factors are most influence differences between the groups. In statistical terms CART explains observed variation in individual response variable scores (macroinvertebrate and fish metric indexes) caused by one or more explanatory variables (e.g. dissolved oxygen concentrations, stream habitat quality, etc.).

A regression "tree" is constructed that repeatedly splits the response variable data (fish or macroinvertebrate index scores) into two groups that maximizes between group differences and reduces within group differences. The regression tree identifies the key explanatory variables, and their relative influence, that are most significant in causing variation in the response variable scores. 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" or "cold" streams) and continuous (e.g. water temperature) explanatory variables can be included in this statistical test.

Structural Equation Modeling (SEM)

SEM is a statistical model that was used to determine which individual watershed characteristics, stream habitat features, or water chemistry parameters, as well as the interactions between and among these factors, most strongly influenced the biological assemblages (macroinvertebrates and fish) in the Pecatonica watershed (McCune and Grace, 2002). The key explanatory factors included in the initial model analyses were based on general scientific understanding of environmental factors that affect stream quality and the physical and chemical factors found to be significant explanatory variables in the previous statistical analyses done in this study (Bray-Curtis, Non-metric Multi-dimensional Scaling, Canonical Correspondence Analysis, and Regression Tree Analysis). Multiple iterations of the SEM model were run until only environmental factors that were most statistically significant to the biological metric scores remained in the model.

The SEM output reports how well the model explains the overall variability observed in the fish and macroinvertebrate metric scores (sample variance), and how strong the influence of each individual explanatory variable is, or interactions among variables are, in affecting the biological metric scores. The greater the amount of variance explained by the model, the greater the likelihood that the model is accurately identifying and ranking key environmental factors that are influencing the biological condition of streams in the Pecatonica watershed. The SEM model also grouped physical and chemical parameters into more general variables, labeled (e.g., "WQ," for water quality degradation) to show that, while each of these individual water chemistry (or watershed characteristic or physical habitat measure) variables were shown to have a strong influence on the fish or invertebrate assemblages, there is often significant measurement error for any one predictor variable and that the correlations and interactions between and among these measures are not always clearly understood.

Quantile Regression Analysis (QA)

QA is a modification of linear regression (Cade and Noon, 2003). Linear regression is used to evaluate relationships between response (e.g. biological index scores) and explanatory variables (e.g. water quality measures). The correlation between the two variables needs to be relatively linear (i.e. a 1:1 response) if the relationship is to be shown to be statistically significant. Many ecological cause and effect relationships are not linear. While there may be strong underlying causal relationships are often not detected with simple linear regression models, because of their non-linear responses and the confounding influences of other explanatory variables. QA allows for the detection of more than one slope by breaking the data into quantiles within an x-y plot, and as a result, is more sensitive in detecting correlations between the response and explanatory variables than simple linear regression.

QA was used in this study to look for various environmental stressor or other explanatory variable thresholds (e.g. in turbidity, nutrient concentrations, dissolved oxygen concentrations, etc.) that, once exceeded, resulted in significant degradation in the biological assemblages. These threshold evaluations can then be used to: 1) determine which physical or chemical stressors are primarily responsible for causing biological degradation; 2) determine at what concentration or value of the stressor the biological degradation occurs; 3) measure how far stressor thresholds have exceeded the point where biological degradation occurs; and 4) estimate what degree of environmental remediation (reduction of the stressor) is needed to bring the streams back to a healthy state.

STUDY RESULTS

Agricultural Land Use and Soil Erosion Potential in the Pecatonica Watershed U.S. Dept. of Agriculture (USDA) 2010 land use data indicates that approximately 70 percent of the watershed is agricultural land (Figure 5). Corn and soybeans were the dominant row crops (16% of land area) with extensive areas used for livestock pasture, alfalfa production, and grassland (57%); steeper slopes are forested (20%) and some developed land (farmsteads, suburban and urban land) exists within the watershed (6%). USDA National Agriculture Statistics Service (NASS) data were used to identify catchments with high proportions of field corn and soybean acreage since these crop fields have high potential to deliver sediment and nutrients to surface waters (Figure 6). USDA estimates of Highly Erodible Land were also mapped to provide information on geographic areas that also may be of concern because of high potential to deliver sediment and nutrients to surface waters (Figure 7).



Figure 5. Land use types in the Pecatonica Watershed.



Figure 6. Percentage of soybean and field corn cropland in Pecatonica watershed catchments.



Figure 7. Percentage of U.S. Department of Agriculture – defined Highly Erodible Land in Pecatonica watershed catchments.

Livestock Densities in the Pecatonica Watershed

Dane County Land and Water Conservation Department information indicates that livestock production is primarily beef and dairy cattle with herd sizes ranging from 5 to just over 200 animal units. The total number of livestock in the Dane Country portion of the watershed is estimated to be 1,400 animal units. Similar estimates for Iowa County were not readily available.

Physical Habitat, Water Chemistry, and Biological Summary Statistics

A total of 68 randomly-selected and targeted stream sites were sampled in May through October 2010 to assess the physical, chemical, and biological conditions of stream resources in the Pecatonica watershed. Summary statistics of a subset of the key field sampling results are presented in Table 2. For samples with analyte concentrations below the laboratory detection limit, values were reported at a concentration of one-half the detection limit.

		% Non-	% Exceed							
	Count	detect	criteria	Mean	Median	Min	Мах	SD		
		Physic	al Measures	5						
Flow volume (ft [°] /s)	68	0		24	7	0	194	37		
Stream gradient (ft/mi)	68	0		22	17	0	104	21		
Water temperature (°C)	460	0		14	15	4.9	23	4		
Conductivity (µS/cm)	461	0		696	637	357	1900	216		
Transparency (cm)	391	0		87	100	5	123+	34		
Diss. O ₂ Sat. (%)	461	0		97	96	54	145	12		
Diss. O ₂ Sat. Min. (%)	68	0		84	86	54	100	9		
QHEI	68	0		60	59	33	91	14		
QHEI substrate metric	68	0		10	9	1	20	5		
WI Qualitative Habitat	68	0		50	48	18	92	20		
	Water	Column	Chemistry M	Measures						
TP (mg/L)	295	34	35	0.10	0.06	0.02	1.05	0.13		
TKN (mg/L)	295	13		0.61	0.53	0.15	6.66	0.56		
NH3 (mg/L)	294	97	0	0.03	0.03	0.03	0.15	0.01		
NO₃NO₂-N (mg\L)	294	0		5.12	4.47	0.22	18.5	2.09		
BOD (mg\L)	177	54		2.1	1	1	12.70	1.59		
TSS (mg\L)	297	28		22.5	10	2.5	469	49		
TDS (mg\L)	297	0		382.6	356	42	1060	135		
SSC (mg\L)	182	25		24.8	9	2.5	484	56		
SSC max (mg\L)	68	15		48.1	18	2.5	484	86		
Chloride (mg\L)	297	0	0	36.5	17.7	3.7	366	59		
SO₄ (mg\L)	297	0		17.8	16.10	8.3	50.7	7		
Chlorophyll-a (µg/L)	68	0		1.5	0.98	0.13	11.4	2		
PCB-209 (µg/L)	69	0		0.19	0.19	0.15	0.25	0.02		
Organochlorine Pest. (µg/L)	69	0		0.19	0.19	0.10	0.26	0.02		
	Stre	ambed S	ediment Cho	emistry						
Arsenic (mg\Kg)	49	98	2	6.4	5.5	4.6	64	8.4		
	49	88	2	0.6	0.16	0.14	8.8	1.4		
Chromium (mg\Kg)	49	0	0	13	13	7.8	19	2.7		
Copper (mg\Kg)	49	0	0	12	11	5.7	41	6.2		
	49	0	14	102	18	1.4	1700	280		
	49	0	24	690	670	390	1400	191		
	49	0	0	13	12	7.4	36	4.3		
	49	0	12	230	75	32	3600	536		
Iotal PAHS (mg\Kg)	g	0	11	2760	605	509	13343	4531		
Biological Measures										
Masseinon S Biolic Index	60 60	0		4	4.Z	1.4	9 10	1.4		
WIACI UNIVERTEDIALE IDI	00	0		C A A	5.5 26 F	0.5	10	1.9		
/0 LF I Fish Index of Ristic Integrity	00	0		41 50	30.3	2	90	20 07		
Brown trout (No $m > 0^{\circ}$)	03	40		2C 11	00 דד ר	0	30	21		
E coli (Colonies / 100ml)	00	49	75	41	Z.11 501	0 57	204	2010		
	00	U	10	1003	100	57	2100	2019		

Table 2. Summary statistics for physical, chemical, and biological data collectedfrom sampling sites in the East Branch Pecatonica River watershed.



Figure 8. Qualitative Habitat Evaluation Index scores for Pecatonica Watershed stream assessment sites.



Figure 9. WDNR Qualitative Habitat Assessment scores for Pecatonica Watershed stream assessment sites.

Physical Habitat

Instream and riparian physical habitat were visually assessed and evaluated using both the MBI's QHEI protocol and the WDNR's qualitative stream habitat field assessment methods and scoring criteria. Both metrics rated individual sites and overall watershedwide stream habitat conditions similarly, although the WDNR methods tended to rate sites' habitat as being of lower quality relative to the QHEI. QHEI scoring indicated that "Heavy/Moderate Silt Cover" (75% of sites), "High/Moderate Overall Embeddedness" (72% of sites), and "High/Moderate Riffle Embeddedness" (68% of sites) were the most significant physical factors degrading stream habitat. There were spatial differences in habitat quality among the three subwatersheds (West Branch, Upper East Branch, Gordon Creek), but none of the sub-watersheds had a significantly higher proportion of "poor" habitat relative to the others (Figures 8 and 9).

Water Quality Measures

Instantaneous measures of water quality (pH, conductivity, dissolved oxygen concentration and saturation) and water transparency were measured each time a stream site was sampled for habitat, water chemistry, or biology. These measures are often temporally dynamic over the course of the day and the seven-month study period, but, given the large number of measures taken, spatial patterns in water temperature, transparency, and dissolved oxygen concentrations and correlations with biological quality at stream sites were detected.



Figure 10. Median instantaneous water temperature measures for Pecatonica Watershed stream assessment sites.

Median water temperatures were highest in the Dodge Branch sub-watershed (Figure 10). Most of the stream reaches sampled in the Pecatonica watershed appeared to be groundwater-dominated based on the presence of obligate "coldwater" fish species such as brown trout (*Salmo trutta*) and mottled sculpin (*Cottus bairdi*), and coldwater invertebrate taxa such as *Gammarus* spp. Warmer water temperatures in the Dodge Branch sub-watershed suggests this catchment had the higher proportions of surface runoff relative to the rest of the Pecatonica watershed. In addition to influencing water temperature, water source has a number of significant physical, chemical, and biological ramifications, including increased delivery of sediment and nutrients to the streams receiving substantial surface runoff.

Higher median water temperatures associated with surface runoff in the Dodge Branch subwatershed help explain the lower water transparency and dissolved oxygen concentrations, and higher total phosphorus, TKN, and suspended sediment measured at the Dodge Branch sampling sites (Figures 11–15).



Figure 11. Median water transparency tube readings for Pecatonica Watershed stream assessment sites.



Figure 12. Instantaneous measures of minimum dissolved oxygen for Pecatonica Watershed stream assessment sites.

Water Column Chemistry Measures:

Nearly 300 water chemistry grab samples from the Pecatonica 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 13. Total phosphorus concentration values for Pecatonica Watershed stream assessment sites.

Total Phosphorus Concentrations:

The U.S. EPA Region 5 Laboratory that processed the water chemistry samples had a relatively high detection limit for total phosphorus (0.04 mg/L as compared to 0.005 mg/L at the Wisconsin State Laboratory of Hygiene), and as a result, a significant proportion (34%) of water column samples had total phosphorus concentrations below detection. Thirty-five percent of the 296 water chemistry samples analyzed were above Wisconsin's Water Quality Criterion for total phosphorus (0.075 mg/L).

Medium sized dots in Figure 13 show sites where total phosphorus concentrations were above the concentration threshold thought to impact fish or macroinvertebrates based on this study's quantile regression analyses. Large dots are sites where the biota were thought to be affected by phosphorus and the state water quality criterion were exceeded. The Dodge Branch subwatershed had the highest within subwatershed percentage of sample sites with total phosphorus concentrations above the State's criterion (64%), followed by the Upper East Branch Pecatonica subwatershed (43%), and the Gordon Creek subwatershed (18%). Sampling sites downstream of municipal wastewater treatment facilities had some of the highest total phosphorus concentrations measured in the watershed.



Figure 14. Total Kjeldahl nitrogen concentrations for Pecatonica Watershed stream assessment sites.

Total Kjeldahl Nitrogen (TKN):

TKN is the concentration of organic nitrogen (~ 40% of the total) and ammonia (~%60) in water. There are no state or federal criterion for TKN, but data from "least-disturbed" stream sites in Wisconsin suggest a concentration between 0.40 and 0.50 mg/L is typical for streams with "good" water quality. Table 2 shows the average value for TKN in the Pecatonica was 0.61 mg/L. Similar to the total phosphorus findings, the Dodge Branch had the greatest within subwatershed percentage of sample sites with "high" (1.02 – 4.14 mg/L) TKN values (64%), followed by the Upper East Branch of the Pecatonica sub-watershed (50%), and the Gordon Creek sub-watershed (43%).



Figure 15. Maximum suspended sediment concentration values for Pecatonica Watershed stream assessment sites.

Suspended Sediment Concentration (SSC):

SSC is the concentration of inorganic material suspended in water. The Dodge Branch subwatershed had the greatest proportion of sampling sites (50%) with "moderate" to "high" (57.5 – 484 mg/L) SSC, followed by the Upper East Branch Pecatonica subwatershed with 20% of sampling sites having medium to high SSC, and lastly the Gordon Creek sub-watershed with 4% of the sample sites having high SSC (Figure 15).



Figure 16. Streambed sediment sampling sites in Pecatonica Watershed. Large dots denote sites where metals and/or PAHs were at concentrations thought to affect benthic organisms.

Streambed Sediment Chemistry Analyses

Forty-nine of 68 sample sites had sediment samples collected for the analysis of metals and PAHs (Figure16). The majority of sites not sampled were headwater streams with small (1.7 sq. mi.) watersheds. Most sites had detectable levels of metals and PAHs (Table 2). Manganese (24% of sites), lead (14%), and zinc (12%) most often exceeded concentrations thought to have probable negative effects on aquatic life (WDNR, 2003). Sample sites located below municipal wastewater outfalls had the highest concentrations of metals. Total PAHs were sampled at 9 sites. The highest concentrations of PAHs were found at the two urban sites within the City of Dodgeville, upstream and downstream of the city's WWTP. The sample site below the Dodgeville WWTP exceeded "probable effects concentrations" for PAHs (WDNR, 2003).



Figure 17. Numbers of physical and/or chemical factors thought to limit fish or macroinvertebrate populations at Pecatonica Watershed sampling sites.

Numbers of stressors of fish or macroinvertebrates at sampling sites

Based on quantile regression analyses and water and sediment quality criteria, a number of different physical or chemical factors were suspected of impacting stream biota. Figure 17 shows how individual monitoring sites and subwatersheds varied in the number factors impacting the fish or macroinvertebrate populations at each assessment site. The Dodge Branch had the greatest proportion (86 percent) of sites within a subwatershed with three or more stressors per stream site, and Gordon Creek the least (23 percent).

Biological Measures

Escherichia coli (*E. coli*) bacteria and chlorophyll *a* concentrations, macroinvertebrate samples, and fish survey data were used to assess the biological condition of stream sites in the Pecatonica Watershed.



Figure 18. *Escherichia coli* bacteria concentrations (colonies per 100 mL water) for Pecatonica Watershed stream assessment sites.

E. coli concentrations were measured once at each sampling site. Bacteria concentrations often have high spatial and temporal variability relative to other instantaneous water quality measures. While the limited sampling effort in the Pecatonica 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 Pecatonica Watershed, bacteria concentrations routinely exceeded the federal water quality criterion. The USEPA water quality standard for *E. coli* for protecting human health during recreational contact in surface waters is 235 colony forming units (CFUs) per 100 ml of water. The average CFU concentration for Pecatonica sampling sites was 1803/mL. Seventy-five percent of the sampling sites had *E. coli* concentrations above the federal water quality standard (Figure 18).



Figure 19. Water column chlorophyll *a* concentrations for Pecatonica Watershed stream assessment sites.

Water column concentrations of chlorophyll provide a measure of benthic and sestonic algal productivity. Algal productivity is influenced by a number of factors including nutrient concentrations, turbidity, streamflow conditions, and stream shading. The median value of chlorophyll *a* concentrations in the Pecatonica watershed was $1.5 \mu g/L$. No strong spatial patterns were observed in the concentrations of chlorophyll *a* within or among the three sub-watersheds (Figure 19).



Figure 20. Hilsenhoff Biotic Index scores for Pecatonica Wastershed stream assessment sites.



Figure 21. Macroinvertebrate Index of Biotic Integrity scores for Pecatonica Watershed stream assessment sites.

Macroinvertebrate Samples

Macroinvertebrate samples were collected in the fall of 2010. Both Hilsenhoff's Biotic Index (HBI) and a macroinvertebrate Index of Biotic Integrity (mIBI) were used to evaluate the sample results. The mIBI showed a lower proportion of "good" to "excellent" sites (57%) compared to the HBI (87%). Both the HBI and mIBI results indicated that the Dodge Branch had the highest number of degraded sites relative to the Upper East Pecatonica and Gordon Creek sub-watersheds (Figures 20 and 21).



Figure 22. Fish Index of Biotic Integrity scores for Pecatonica Watershed stream assessment sites.
Fish Assemblage Data

Fish surveys were conducted at all 69 sampling sites. No fish were captured at 5 headwater sites. A total of 34 fish species and 5,010 individuals were captured during the study. Brown trout (Salmo trutta) n = 1,069, had the highest frequency of occurrence, being found at 71% of the sampling sites. Brown trout of harvestable size (\geq 9.0") were found at 51% of the sites. For sampling sites with harvestable-sized brown trout, the catch rate averaged 80 harvestable trout per mile, with a range of 1-264 harvestable trout per mile. Low numbers of other gamefish were also captured, including: brook trout (Salvelinus fontinalis) n = 28, northern pike (Esox lucius) n = 3, largemouth bass (Micropterus salmoides) n = 4, walleye (Sander vitreus) n = 3, and various panfish (Lepomis spp.) n = 43. White sucker (Catostomus commersoni) was the most numerically dominant species (n =1,450) being found at 52% of the sampling sites, followed by mottled sculpin (Cottus bairdi) n = 1,437, found at 68% of the sites.

Fish Indexes of Biotic Integrity (fIBI) were used to evaluate the environmental quality of the stream sampling sites (Figure 22). For the 5 "fishless" sites, a fIBI score could not be computed. Also, 7 sites had fewer than 25 fish captured per site, and, according to fIBI protocols, an index score should not be computed. These sites were given a rating of "poor". A total of 22% of the sampling sites in the Pecatonica Watershed were rated as "poor" or "very poor". The Dodge Branch subwatershed had the highest proportion of "poor" or "very poor" fIBI scores (40%), followed by the Upper East Branch of Pecatonica sub-watershed (24%), and the Gordon Creek sub-watershed (10%).

Statistical Ordination Results

Bray-Curtis ordination was used to group stream sites that had similar fish or macroinvertebrate species assemblages (Figures 23 and 24). Site groups are thought to have similar environmental characteristics that influence the fish or macroinvertebrates found at these within-group sites. The fish BC ordination analysis shows 5 main groups of stream sites with between 2 to 33 stream sampling sites per group.



Figure 23. Bray-Curtis ordination plot of Pecatonica Watershed fish assemblage groupings.



Figure 24. Bray-Curtis ordination plot of Pecatonica Watershed macroinvertebrate assemblage data.

The Bray-Curtis ordination (Figure 24) shows two different populations of stream sites in the Pecatonica watershed based on the macroinvertebrate assemblages found at each of the sampling sites .



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Figure 25. Nonmetric Multi-dimensional Scaling (NMDS) plot of Pecatonica sampling sites fish assemblage data color-coded by Bray Curtis analysis groups (Figure 19).

Nonmetric Multi-Dimensional Scaling (NMDS) translates similarities or dissimilarities among stream sampling sites (based on fish or macroinvertebrate populations) into a visual representation of distance (Figure 25). Stream sites are represented by colored triangles: site triangles closer together are more similar in terms of numbers of fish species, individuals per species, and total numbers of fish present at each site, and those site triangles farther apart have less similar fish populations. Triangles are colorcoded to represent the five groups identified in the Bray-Curtis (BC) ordination analysis Figure 19). The BC groups in the NMDS plot provides corroborating evidence of distinctly different groups of stream sites in the Pecatonica Watershed. The locations of the fish names on the plot show a species common to the nearby site triangles. Knowledge of the environmental requirements of each fish species allows one to infer what physical or chemical conditions of the watershed and stream sites result in the clustering or dispersion of the sites, providing insights into the major environmental "drivers" (explanatory variables) that most strongly influence the fish populations in the Pecatonica Watershed. Three site clusters are evident; a "warmwater/somewhat degraded" streams site cluster towards the middle-right of the plot, a "coldwater/clean" stream" sites cluster in the upper left, and "small and/or degraded" sites with few fish species or only brook stickleback clustering in the lower left of the NMDS plot.





Figure 26. Non-metric Multi-dimensional Scaling (NMDS) plot of Pecatonica Watershed macroinvertebrate assemblage data.

Similar to the NMDS plot for fish, the red triangles in the NMDS plot for macroinvertebrates (Figure 26) represent the environmental relationships among stream sites based on the macroinvertebrate populations found at each sampling site. The presence of a number of warmwater taxa (e.g., *Iswaeon anoka, Baetisca lacustris, Stenelmis, Caenis, Hyalella azteca*) on the left side of the plot and coldwater taxa on the right side (e.g., *Amphinemura, Brachycentrus americanus, Sialis, Pericoma, Paracapnia, Hybiosoma seriatum, Heteroissocladius, and Pseudorthocladius*) suggest a water temperature gradient differentiating stream sites and most strongly influencing the distribution of macroinvertebrate taxa in the watershed.



Figure 27. Correlation of key environmental explanatory variables of fish species distributions with NMDS site clusters using the Bray-Curtis fish populations groups.

In Figure 27, the site locations are the same as in the previous fish NMDS plot (Figure 23) and are again color-coded by their respective Bray-Curtis groups. The NMDS site clusters were treated as potential explanatory (x) variables, and all 135 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 135 candidate response variable pairs. For example, watershed drainage area, streamflow volume, and stream width are strongly correlated to each other and 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 analysis. A subset of key explanatory variables thought to most strongly influence fish (those with correlation coefficients \geq 0.4) were used in the plot environmental factors shown in Figure 27. The directionality of the vector arrows indicates a positive value for the variable shown. The longer the length of the arrow, the stronger the correlation between the respective response and explanatory variables. Various measures of stream size, direct or surrogate measures of water quality (e.g., chlorophyll *a* concentration), and physical habitat measures such

as stream gradient (labeled as QHEIgradv), were all strong correlates of the fish assemblage data. 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.



AXIS 1

Figure 28. Correlation of key environmental explanatory variables with NMDS macroinvertebrate site clusters. Sites are color-coded according to the Bray-Curtis macroinvertebrate site groups.

Similar to the correlation analyses of fish sites and environmental variables, NMDS (x,y) coordinates for stream sites macroinvertebrate data were treated as explanatory variables and incorporated into regression analyses with all 135 physical and chemical variables measured at each stream site (Figure 28). Only the most significant response variables (parameters with correlation coefficients \geq 0.3 in this analysis) were plotted to determine which watershed characteristics, and physical and chemical measures from the stream sites, had the greatest influence on the macroinvertebrate populations. Environmental factors associated with larger stream sites such as increasing flow-volume, water temperature, percent pool habitat and greater percent fish cover appeared to influence the macroinvertebrate taxa, based on the vector arrows in the upper-left of Figure 28.



AXIS 1

Figure 29. Canonical Correspondence Analysis plot of fish assemblage and most strongly correlated environmental parameters data.

Canonical correspondence analysis (CCA) is another type of cluster analysis that was used to determine which watershed characteristics, stream habitat features, or water chemistry measures were most influential in structuring the biological assemblages in the Pecatonica Watershed. Similar to NMDS, those explanatory variables with the longest vector arrows are most strongly correlated with fish assemblage attributes and are thought to have the strongest influence on the fish populations (Figure 29). For fish assemblages in the Pecatonica Watershed, water temperature, flow volume, stream habitat features (WDNR Qualitative Habitat Index and Qualitative Habitat Evaluation Index (QHEI) scores), and various measures of water quality (total phosphorus, chlorophyll *a*, total dissolved solids, and *E. coli* concentrations) appeared to have the greatest influence on the fish populations.



Figure 30. Canonical Correspondence Analysis (CCA) plot of macroinvertebrate assemblage data.

CCA was also used to determine which watershed land use, stream habitat features, or water chemistry measures were most influential in structuring the macroinvertebrate assemblages (Figure 30). For macroinvertebrates (similar to the fish assemblage CCA results), water temperature, streamflow volume, and stream and habitat features (WDNR qualitative habitat index and QHEI measures) were most influential in structuring the macroinvertebrate assemblages in the Pecatonica Watershed.

Regression tree for fish IBI scores



Error : 0.373 CV Error : 1.26 SE : 0.241

Figure 31. Regression tree plot of most significant environmental parameters influencing fish index of biotic integrity (fIBI) scores and resulting groups of similar stream sites based on fIBI scores.

Figure 31 is a regression tree plot illustrating the most statistically significant environmental parameters influencing the fIBI scores, and resulting site groupings based on fIBI scores. Regression trees statistically create groups with low within-group variability (in this case fIBI scores) and high between-group variability (groups "apples with apples, oranges with oranges"), and determines what environmental factors are most influential in creating the 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.

Regression tree output is rich in information (e.g. Fig. 31). The regression tree divided the stream population into four distinct groups based on fIBI scores. The first fork or split in the regression tree limbs is based on the minimum percent dissolved oxygen concentration (DOperMin) observed at each stream site. Sites with minimum dissolved oxygen concentrations less than 81.8 percent had significantly lower (poorer) fIBI scores than sites with concentrations higher than 81.8 percent. The relative length of the vertical lines (limbs) of the regression tree show how important each individual explanatory variable is in explaining the amount of overall variation in the response variable values (in this example fIBI scores) observed across all of the stream sites. So minimum dissolved oxygen concentrations at each stream site was a much stronger predictor of fIBI scores at each site than TKN (total Kjeldahl Nitrogen) or TP (total phosphorus) concentrations which have much shorter vertical limbs. At the bottom terminal nodes (leaves) the mean fIBI score and the number of stream sites in each group are reported. Error statistics are also reported, the first (farthest left) term shown

is the residual error (RE) - a measure of how well the statistical model fits the data. The number one minus the RE equals the correlation coefficient (R²) which in the example from Figure 31 is 0.63, which indicates 63 percent of the variability seen among the fIBI scores at the Pecatonica stream sites site can be explained by the minimum dissolved oxygen and average nutrient (TKN and TP) concentrations measured at each of the sampling sites.



Regression tree for HBI scores

Error : 0.527 CV Error : 1.55 SE : 0.346

Figure 32. Regression tree plot of most significant environmental parameters influencing Hilsenhoff's Biotic index (HBI) scores and resulting groups of similar stream sites based on HBI.

Figure 32 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 HBI scores. Total dissolved solids (TDS) concentration was shown to be the most significant factor influencing HBI scores. Those stream sites with higher TDS concentrations had higher (poorer) HBI scores. The next most significant factors influencing HBI scores were water column TP concentrations and water temperature. Stream sites with higher TP concentrations or higher water temperatures were in poorer condition based on the HBI scoring.

Regression tree for mIBI scores



Error : 0.6 CV Error : 1.21 SE : 0.224

Figure 33. Regression tree plot of most significant environmental parameters influencing macroinvertebrate index of biotic integrity (mIBI) scores and resulting groups of similar stream sites based on mIBI.

Minimum DO concentrations were shown to be the most significant factor influencing mIBI scores; those sites with higher DO concentrations had higher (better) mIBI scores (Figure 33). The next strongest factor influencing mIBI scores was water column chlorophyll *a* concentrations. Stream sites with lower chlorophyll concentrations had better mIBI scores. The amount of variability in mIBI scores seen among stream sites explained by the model in Fig. 33 is 40 percent, indicating other important factors influencing mIBI scores are not accounted for by this statistical model.

Structural Equation Modeling (SEM)

The SEM diagram in Figure 34 illustrates physical and chemical factors shown to have statistically significant influences on fIBI scores. Arrows pointing directly to the "Fish IBI" box are factors that directly affect the fish IBI scores. Arrows pointing from the "WQ" (Water Quality) oval to various chemical parameter boxes indicate those parameters that affect the fIBI indirectly through complex interactions between and among these various chemistry parameters, and are therefore grouped in the broad category labeled "WQ" (more accurately, it should be thought of as water quality degradation).

The most significant findings of the SEM analysis of environmental factors influencing the Pecatonica fish populations are that: 1) 58% of the variability in fIBI scores (sample variance) can be explained by the model (0.58 value lower left of Fish IBI box), indicating that the model was useful in identifying and ranking key environmental parameters influencing fish populations in the Pecatonica Watershed; 2) water quality degradation had a slightly stronger influence on the fish populations (- 0.43 value next

to arrow between WQ oval and Fish IBI box) than the other direct-influence environmental factors, which included stream physical habitat quality (0.33 value next to arrow between QHEI and Fish IBI boxes), and minimum dissolved oxygen concentrations (0.29 value next to arrow); 3) "Water Quality" was influenced by a number of water chemistry parameters including, in order of decreasing influence: water column total phosphorus concentrations, *E. coli* concentrations, suspended sediment (maximum concentrations), chlorophyll *a* concentrations, water transparency, water temperature, dissolved oxygen (minimum concentrations), and total dissolved solids concentrations; 4) streamflow volume directly influenced a number of water chemistry measures and several key habitat features that ultimately affected the fish populations; and 5) a number of water chemistry parameters had interactions between and among these parameters that affected the overall influence that water quality had on the fish populations.



Figure 34. Structural Equation Model (SEM) diagram representing the relationships among key watershed, stream habitat, and water chemistry variables influencing fish populations (Fish IBI scores) in the Pecatonica Watershed.



Figure 35. Structural Equation Model (SEM) diagram representing the relationships between key watershed, stream habitat and water chemistry factors influencing macroinvertebrate populations (Hilsenhoff's Biotic Index scores) in the Pecatonica Watershed.

The detailed SEM shown in Figure 35 illustrates physical and chemical factors shown to influence macroinvertebrate populations in the Pecatonica Watershed. The model explained 47% of the variability observed among HBI scores for the stream sites. In order of decreasing influence, WQ (water quality), total dissolved solids, Pas100 (the percentage of livestock pastureland within a 100m-wide riparian corridor for the entire stream reach upstream of each sampling site), water column chlorophyll *a* concentrations, and percent substrate fines (estimated with qualitative habitat survey methods) had the greatest direct influence on the macroinvertebrate populations.

Total phosphorus concentrations, minimum dissolved oxygen concentrations, qualitative habitat, chlorophyll *a* concentrations, water temperature, and water transparency had the greatest overall influence on water quality degradation, which had the greatest overall influence on the macroinvertebrate populations.

Quantile Regression Analysis

Quantile regression analyses were used to determine whether the environmental factors that were determined by previous statistical tests to influence the stream biota, had linear responses or specific thresholds that, once exceeded, resulted in significant biological degradation. Stressor threshold information can be used to determine how severely a stream site is impacted by a particular stressor, and to help develop objective, quantifiable in-stream and watershed restoration goals.



Figures 36 and 37. Quantile regression plots showing the response of fish Index of Biotic Integrity (fIBI) scores to changes in Qualitative Habitat Evaluation Index (QHEI) scores and instantaneous measures of median water temperature respectively, in the Pecatonica Watershed.

Higher QHEI scores indicate better habitat quality (Figure 36). A threshold and subsequent asymptote (plateau) in fish IBI scores is evident once a QHEI score of 53 was reached. Fish were also influenced by median instantaneous water temperatures collected over the course of the May-October sampling season. The results indicate that once a threshold of 15.9 Celsius (58 degrees Fahrenheit) is reached, the fish IBI scores declined (Figure 37).



Figures 38 and 39. Quantile regression plots showing the response of fish Index of Biotic Integrity (fIBI) scores to streamflow volume and minimum concentrations of dissolved oxygen respectively, in the Pecatonica Watershed.

Streamflow volume data showed a unimodal distribution where very low streamflow volumes (<0.5 CFS) were associated with poorer fish IBI scores. An asymptote was reached at 2.8 cubic feet per second, after which higher flow volumes were also associated with poorer fish IBI scores (Figure 38).

Minimum dissolved oxygen concentrations (below 84%) were shown to have a strong negative effect on fish IBI scores (Figure 39).



Figures 40 and 41. Quantile regression plots showing the response of fish Index of Biotic Integrity (fIBI) scores to maximum values of suspended sediment (SSC) and total dissolved solids (TDS) concentration respectively, in the Pecatonica Watershed.

Increasing water column suspended sediment concentrations had a negative effect on fIBI scores (Figure 40).

The concentration of total dissolved solids showed a threshold at 328 mg/L, past which there was a significant decline in fIBI scores (Figure 41). Conversely, as water transparency increased to a water column height of 65 cm (measured with a transparency tube), there was a significant increase in fIBI scores (Figure 42).



Figures 42 and 43. Quantile regression plots showing the response of fish Index of Biotic Integrity scores to water transparency and concentrations of total dissolved phosphorus respectively, in the Pecatonica Watershed.

Nutrients had a significant influence on fIBI scores. At a threshold of approximately 0.05 mg/L of total dissolved phosphorus, there was a significant decline in fIBI scores (Figure 43).

Similarly for total Kjeldahl nitrogen (TKN), when the water column concentration reached 0.5 mg/L, there was a significant decline in fIBI scores (Figure 44). Fish IBI scores declined with increasing water column chlorophyll and *E. coli* concentrations. A threshold is seen for chlorophyll *a*, where concentrations above 0.69 μ g/L resulted in declining fIBI scores (Figure 45). Fish IBI scores showed a general decline with increasing bacteria concentrations, but no threshold was evident (Figure 46).



Figures 44 and 45. Quantile regression plots showing the response of fish Index of Biotic Integrity scores to total Kjeldahl nitrogen and chlorophyll *a* concentrations respectively, in the Pecatonica Watershed.



Figure 46. Quantile regression plot showing the response of fish Index of Biotic Integrity scores to *Escherichia coli* bacteria concentrations in the Pecatonica Watershed.

Quantile Regression (QR) analysis of macroinvertebrate metric data

Macroinvertebrates' responses to environmental stressors were also evaluated using Hilsenhoff's Biotic Index (HBI) and a macroinvertebrate index of Biotic Integrity (mIBI) metric scores. Lower HBI scores and higher mIBI scores indicate better macroinvertebrate populations. Similar to the fish quantile regression analyses, the explanatory variables that were shown to be statistically significant in previous statistical analyses were evaluated using quantile analysis.



Figures 47 and 48. Quantile regression plots showing the response of macroinvertebrate metrics scores to stream flow volume in the Pecatonica Watershed.

Both HBI and mIBI scores showed decreasing biological integrity with increasing streamflow volume. A threshold of 2.4 cubic feet per second (CFS) was seen with the HBI that, once exceeded, showed poorer invertebrate populations, while mIBI scores indicated reduced invertebrate assemblage health once a threshold of 8.5 CFS was exceeded (Figures 47 and 48).



Figures 49 and 50. Quantile regression plots showing the response of macroinvertebrate metrics scores to stream water temperature in the Pecatonica Watershed.

Mean water temperature was shown to influence HBI and MIBI scores with declines in 57

the biological integrity of macroinvertebrate populations once a temperature threshold of 18 degrees Celsius was reached (Figures 49 and 50).



Figure 51 and 52. Quantile plots showing the response of macroinvertebrate metrics scores to habitat quality in the Pecatonica Watershed.

Stream habitat quality measured with both the Qualitative Habitat Evaluation Index (QHEI) and the Department's Qualitative Habitat assessment 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 MIBI (Figures 51 and 52).



Figure 53 and 54. Quantile plots showing the response of macroinvertebrate metrics scores to water transparency in the Pecatonica Watershed.

Water transparency appeared to have a weak influence on the macroinvertebrate populations (Figures 53 and 54).



Figure 55 and 56. Quantile plots showing the response of macroinvertebrate metrics scores to minimum concentrations of dissolved oxygen in the Pecatonica Watershed.

The minimum dissolved oxygen concentrations measured at the stream sites strongly influenced the macroinvertebrate populations. Higher dissolved oxygen concentrations were correlated with better macroinvertebrate populations (Figures 55 and 56).



LOG (TOTAL PHOSPHORUS. N MILLIGRAMS PER LITER) LOG (TOTAL PHOSPHORUS. N MILLIGRAMS PER LITER) Figure 57 and 58. Quantile plots showing the response of macroinvertebrate metrics scores to total phosphorus concentrations in the Pecatonica Watershed.

Higher concentrations of water column total phosphorus concentrations were associated with a trend of poorer macroinvertebrate metric scores, but no thresholds were evident (Figures 57 and 58).



Figure 59 and 60. Quantile plots showing the response of macroinvertebrate metrics scores to total dissolved solids concentrations in the Pecatonica Watershed.

Increasing concentrations of total dissolved solids in water had a negative effect on the macroinvertebrate populations. A threshold of 316 mg/L was noted with both HBI and mIBI that, once exceeded, resulted in a significant decline in the integrity of the macroinvertebrate populations (Figures 59 and 60).



Figure 61 and 62. Quantile plots showing the response of macroinvertebrate metrics scores to chlorophyll *a* concentrations in the Pecatonica Watershed.

Increasing water column chlorophyll concentrations negatively influenced the macroinvertebrate populations with a more definitive inflection point in mIBI at a concentration of 0.63 μ g/L (Figures 61 and 62).

DISCUSSION

This watershed assessment pilot study was undertaken to:

- Apply and evaluate the effectiveness of a stream sampling design that systematically selects 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 quality and identifying physical and chemical stressors impacting stream biology.
- Evaluate how the applied statistical methods may be used for future stream resources assessment and watershed management projects.
- Evaluate how the sampling design can effectively provide information for Water Division stream assessment and management activities compared to the Department's current stream assessment efforts.

Sampling Design Applicability to Department Water Resources Assessment and Management Efforts

Sampling intensity

A total of 68 stream sites, selected in a systematic fashion were sampled for the Pecatonica Watershed Pilot Study. At each site a large number of physical, chemical and biological parameters were measured. This spatial and parameter-intensive effort provided a robust dataset to rigorously assess the overall condition of the stream resources in the watershed over a 1-year timeframe and objectively determine what physical and chemical factors most significantly impacted the stream biota, and when possible identify numeric thresholds of various stressors that once exceeded resulted in degraded fish or macroinvertebrate populations. Prior to the pilot study, well over 100 stream sites had been sampled in the Pecatonica Watershed over a 15 year period for various projects. However, these projects had varying goals, differences in parameters, methods, and sampling frequency, and the sampling sites were not well dispersed throughout the watershed (Figure 63). While much has been learned at sub-watershed or stream resources in the watershed or quantitatively determine what factors most strongly affect stream quality.



Figure 63. Historic stream sampling sites in the Pecatonica Watershed.



Figure 64. Proportions of stream resource conditions in Pecatonica Watershed by Aquatic Life Use designations.

Information reported from the Water Assessment Tracking Electronic Reporting System (WATERS) indicates that over 80% of the water resources in the Upper East Branch and Gordon Creek Watershed were unassessed prior to the Pilot study (Figure 64).

The spatial intensity of this pilot study sampling effort provided reach-specific information on specific factors influencing the physical, chemical and biological condition of stream resources. This information can allow land management agencies to prioritize the location of restoration efforts, target specific riparian or instream habitat limitations or pollutants and work with individual landowners for strategically implementing farm or stream reach-specific Best Management Practice (BMPs).

Pollutant types and source determinations

It is generally understood that excess sediment and nutrients in surface runoff are the major pollutants impacting stream in Wisconsin and across the US. Determining which specific pollutants are most detrimental may be less important if the BMPs being applied control multiple pollutant types (e.g., minimum till plowing 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 implementation is not targeted to specific areas, but are being broadly applied.

However, geographically targeting pollutant-specific control practices within subwatersheds that are most significantly impaired may be a more pragmatic, costeffective strategy. The Department's Targeted Runoff Management Program recognizes that with limited funding, focusing BMP implementation on key geographic problem areas is more cost-effective and likely to achieve the desired goals of restoring and protecting aquatic resources than broadly applying BMPs and perhaps missing key pollutants sources within a watershed or applying BMPs to areas where little benefit is gained. Similarly, it is helpful to know which types of habitat degradation and/or pollutants are most responsible for loss of biological integrity versus making assumptions about their relative importance. If pollution control efforts include costly infrastructure that for example control nutrients only (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.

Previous BMP implementation costs in the Pecatonica Watershed have been significant. Data from the Department's Bureau of Community Financial Assistance shows that within the last 10 years nearly \$2.5 million dollars have been spent by Dane County in the Gordon Creek Watershed alone (which represents about 1/3 of the study area) on upland and riparian BMPs and stream habitat restoration work. The total cost of land management efforts in the entire Pecatonica watershed is undoubtedly much higher particularly if Iowa County Land Conservation Department and private organizations such as Trout Unlimited, The Nature Conservancy spending on land and water resources management efforts and the various programs staff time were accounted for.

Economic shifts towards larger livestock herd sizes and increasing numbers of concentrated animal feeding operations (CAFOs) are creating geographically-concentrated areas of manure spreading. Knowing whether animal waste management regulations and individual operators' implementation of nutrient management plans are protective of surface waters is fundamentally important in protecting water resources and relies on spatially precise monitoring data to effectively address these issues.

Biotic responses to pollutants and threshold determination

The ability to document relationships between specific pollutants and degradation of aquatic life is important. Data obtained the pilot study was used to determine whether there were pollutant concentration thresholds that once exceeded, caused stream biology to decline. Estimates of the magnitude of biological degradation within a stream reach or watershed and the level of stressor or pollutant reduction necessary to restore the biological integrity of a stream can be estimated. 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 Pecatonica Pilot project generated data and information of use to a number of Department programs and other agencies.

- The project generated data to rigorously characterize stream resource conditions in the Upper East Branch Pecatonica River Watershed for U.S. EPA 305(b) reporting.
- Thirty-two of 68 stream sites (47%) 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 provided 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 situated upstream and down of each of the WWTPs within the watershed provided information on the influence of point source discharges on water quality and biological integrity of the streams. This information can be used as a screening tool for discharges that may be impacting stream quality and warranting additional sampling to evaluate the efficacy of WPDES permits for these facilities.
- Water chemistry data collected from several streams receiving urban runoff provided data to determine the concentrations of metals and PAHs flowing off of urban developments and indicated that metals and PAHs were likely impacting benthic life and may warrant follow-up investigations.

- 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.
- The gamefish data was sufficient to characterize the quality of the fishery throughout the watershed, in terms of sizes and numbers of trout per stream mile.

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 Pecatonica 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 staff interest in specific streams or stream reaches.

Spatial scale and sampling intensity

Given the relatively small size (221 sq. mi.) of the study area and large number of sampling sites (n = 69), 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 Pecatonica 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 project 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 three major subwatersheds' water quality and resulting biological conditions were evident, and this information can be used to inform Best Management Practices within the Pecatonica 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 Pecatonica 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.

Analyses of water column and stream bed sediment samples for metals and PAHs provided site-specific information to show that stormwater flowing off developed areas in the City of Dodgeville and discharge from the Dodgeville WWTPs were high in metals and PAHs, and that all of the WWTP discharges contained elevated levels of metals.

Key Findings of the Statistical Analyses

The statistical techniques applied to the Pecatonica River dataset were rigorous and reflect a significant advancement over data analytical methods currently used by 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 to which stream sites are degraded, and to 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 macroinvertetbrate assemblage data), and results were validated with unconstrained Nonmetric Multi-dimensional Scaling. Constrained Nonmetric Multidimensional 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 centralized computer 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 by using the tools provided.

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 Pecatonica 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. These data can be used to refine existing water quality standards and help develop new standards

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Appendix I. Map of Pecatonica Watershed with Site Names and Subwatershed Delineations.



Appendix II. Drainage area size and water chemistry summary statistics for each sampling site.

	Site	River	Drain				SSC	DO %				
SWIMS ID	Name	mile	Area	ΤР	TKN	TDS	max	min	Temp	Trans	Chl-a	E. coli
Blotz Branch												
10014319	1.7wp2	1.5	1.7	0.03	0.68	319	16	90	14.2	63	0.54	3930
Brager Bra	nch											
10031466	1.7g1	2.0	2	0.02	0.35	318	2.5	87.7	15.1	50	0.52	1986
Conley Lew	vis Creek											
10014163	3.5wp2	6.1	2.8	0.07	0.51	398	27	90.4	14.85	120	0.97	268
10015180	13.5wp	0.3	13.5	0.08	0.47	343	98	73.3	15.25	95	0.88	549
Dodge Branch												
253043	1p	21.8	0.9	0.02	0.64	839	20	62	17.05	120	1.17	488
253044	1.12p	21.4	1.1	0.41	0.8	979	2.5	66	18.15	120	0.13	6050
253099	66.1p	1.7	66	0.06	0.66	378	72	95.9	19.4	57.5	2.85	517
10008143	13.8wp1	17.7	14.4	0.1	0.65	500	27	89.1	16	57.5	3.97	914
10015257	57.5wp1	6.0	58.2	0.11	0.7	409	375	80.6	17.3	40	2.48	1986
10015258	38.6p	9.0	38.7	0.06	0.62	402	484	74.8	18.8	54	2.35	1300
10031444	43p	6.5	43.3	0.12	0.69	423	360	79.3	18.7	38.5	2.16	1046
10031445	28.7wp	14.4	25.9	0.1	0.42	443	160	80.1	18.1	62.5	1.32	1046
10031624	67.84p	0.6	67.8	0.07	0.46	394	76	92.7	16.1	76.5	3.02	866
Upper East	Branch Pe	catonica	I									
253094	6.5up	55.1	6.5	0.04	0.48	390	16	83.4	18.1	90	2.59	2419
253100	124.2p	39.9	127.2	0.09	0.64	350	80	91.1	14.2	102.5	2.37	222
253128	51.4up1	45.7	53	0.05	0.49	324	69	85.6	17.3	100	1.52	479
10014311	45.5p	49.3	48.3	0.07	0.54	322	84	85.5	15.1	99	1.55	361
10020046	120.4p1	43.6	122.8	0.09	0.63	360	68	89.4	20.4	72	5.01	980
10021754	3.5up2	59.4	3.5	0.06	0.54	361	6	90.1	16	110	0.32	1120
10031441	221	33.1	217	0.07	0.49	339	42	89.3	13.7	56	1.81	649
10031442	136p	33.6	140.1	0.1	0.65	361	45	80.4	14.75	85	1.56	517
10031443	132.75p	36.6	135.4	0.09	0.64	361	79	86.1	15.2	70	1.33	222
10031446	21p	52.9	24.9	0.1	0.26	366	21	85.6	16.5	72.5	0.94	617
10031447	14.9up3	54.9	17.6	0.07	0.46	370	19	83.1	18	82.5	0.27	261.3
10031455	1.85up7	61.0	1.8	0.24	0.92	243	68	79.3	14.5	55	1.98	7200
10031623	14.93p	54.8	17.7	0.11	0.4	366	22	84.6	15.9	80	1.26	9330
German Va	lley Branch	1										
10011872	1.7g6	5.0	2.1	0.02	0.49	383	2.5	86.6	13.5	117.5	0.13	201
10031449	7.3g	1.5	7.3	0.02	0.58	393	6	93.9	16.1	120	0.13	276
Gordon Cre	eek											
253101	66g	4.5	66.1	0.04	0.52	343	22	86	16.2	90	1.23	722
253205	30g	6.8	30.9	0.04	0.33	331	12	74	16.3	120	0.56	214
10008165	27.9g	9.4	27.8	0.04	0.47	314	17	90.3	16	114	0.93	141
10011740	4.9g1	18.9	4.2	0.03	0.54	378	7	86.3	12.3	120	0.13	155
10021401	71g	1.9	71	0.05	0.45	333	25	87.9	15.15	85	0.98	326
10029189	76.9g	0.04	76.7	0.05	0.48	332	37	85.1	14.75	70	1.72	326
10031450	6.9g1	17.1	6.8	0.02	0.43	363	5	88.8	12.8	120	0.13	189

Appendix II. cont. Drainage area size and water chemistry summary statistics for each sampling site.

	Site	River	Drain				SSC	DO %				
SWIMS ID	Name	mile	Area	TP	TKN	TDS	max	min	Temp	Trans	Chl-a	E. coli
Gribble Branch												
10031468	1.65wp9	0.9	2.2	0.02	0.58	363	11	93.3	13.8	93.5	1.35	1986
Jeglum Valley Creek												
10031465	1.7g2	0.04	2.1	0.08	0.58	365	56	87.8	14.1	37.5	1.22	1986
Kittleson Valley Creek												
133444	29.89g	3.5	29.8	0.03	0.47	333	33	85.3	15.5	110	0.86	276
253058	33g	0.3	32.9	0.05	0.44	329	27	89.6	16	110	0.66	240
10009432	19.1g	5.4	19.2	0.02	0.37	328	2.5	95.7	14.7	120	0.13	179
10015426	1.7g4	7.9	3	0.11	0.75	330	10	88.2	13.9	105	0.13	1733
Lee Creek												
10029295	1.7g3	2.4	1.6	0.13	0.63	217	76	82	14	47.5	1.67	1300
10031452	3.5g2	1.3	3.7	0.06	0.71	348	18	89.7	13.3	85	1.15	250
Ley Creek												
253057	3.3wp3	1.2	3.2	0.05	0.57	386	16	90	16.15	100	0.13	2419
Lynch Brand	ch											
10014320	1.54wp10	1.4	2.2	0.02	0.15	408	2.5	99.5	12.65	120	0.52	365
Olson Creek	Σ.											
10029527	3.5wp1	2.5	3.1	0.04	0.5	345	8	91.3	14.95	120	0.13	214
10031456	1.7wp7	3.0	1.7	0.09	0.67	344	2.5	64.2	14.3	110	0.13	147
Pleasant Va	lley Branch											
10009781	3.5g3	2.1	3.8	0.02	0.47	337	2.5	84	13.35	120	0.13	238
10011636	7.5g	1.5	7.5	0.02	0.44	357	2.5	85.3	14	120	0.13	172
10031464	1.7g5	4.8	0.9	0.08	0.46	350	2.5	78	14	120	0.13	222
Smith Conle	y Creek											
10008171	19.1p	0.1	19	0.04	0.49	322	9	85	15.35	120	0.58	130
10012856	9.68p	3.3	9.7	0.07	0.58	359	15	90.3	14.6	110	11.4	792
10016138	13.8up2	0.5	14	0.03	0.15	322	5	86.7	13.9	120	0.5	57
10031462	1.7up3	6.8	1.7	0.47	0.67	534	13	57.1	18.5	117.5	8.08	3730
10031762	0p5	7.2	0.8	0.07	0.4	476	13	78.1	12.4	73	0.13	5560
Syfestad Cr	eek											
10031453	3.19g3	2.0	3.2	0.02	0.58	316	2.5	83	13.9	120	0.13	137
Unnamed												
253052	1.7wp5	2.4	4.4	0.04	0.39	403	18	84.2	14.25	60	0.69	613
10016644	2.65g	1.1	2.5	0.02	0.52	345	6	88	13.8	120	0.13	122
10031448	7.3wp	1.6	7.2	0.05	0.51	355	17	83.8	16.4	65	2.91	1300
10031454	1.92p	0.8	2	0.02	0.6	344	14	80.1	14.8	85	0.8	613
10031458	1.7wp4	2.9	0.7	0.12	0.8	479	62	72	14.5	23.5	1.57	5120
10031459	1.7up6	0.2	2.4	0.02	0.32	302	8	95	13.95	65	0.58	196
10031463	1.7up2	0.2	2	0.11	1.02	276	23	54	14.9	77.5	2.69	27550
10031467	1.6wp12	1.0	1.3	0.3	2.2	385	136	75	17.1	21.5	4.96	10460
10031469	1.55g9	0.02	1.6	0.04	0.5	289	7	90.3	14.6	95	0.63	770
10031470	1.53wp11	0.02	1.5	0.67	4.14	271	139	86.1	16.4	15	3.24	4350
Urnus Creek	ζ.											
10031457	1.7wp6	0.2	2	0.03	0.32	211	11	73.8	17.2	45	2.36	1046
Williams-Barneveld Creek												
10012833	3.3up4	3.5	6.1	0.04	0.53	362	33	83.7	16.35	75	1.45	461

Units for the parameters above (in parentheses): River mile (mile), Drain Area (square mile), TP (mg/L), TKN (mg/L), TDS (mg/L), SSC max (mg/L), DO % min (%), Temp (° C), Trans (cm), Chl-a (µg/L), *E. coli* (per 100 ml).
			Drain						
	Site	River	Area						
SWIMS ID	Name	mile	(sq. mi)	HBI	MIBI	FIBI	QHab	QHEI	
Blotz Branch									
10014319	1.7wp2	1.5	1.7	4.51	5.45	50	42	60.5	
Brager Bran	ch								
10031466	1.7g1	2.0	2.0	2.64	6.22	70	35	40.5	
Conley Lew	is Creek								
10014163	3.5wp2	6.1	2.8	4.05	4.03	70	58	58.5	
10015180	13.5wp	0.3	13.5	4.77	5.46	80	58	77.5	
Dodge Bran	ch								
253043	1p	21.8	0.9	5.70	2.68	20	37	57.5	
253044	1.12p	21.4	1.1	5.66	1.18	0	25	51	
10008143	13.8wp1	17.7	14.4	4.64	3.71	60	62	87	
10031445	28.7wp	14.4	25.9	6.47	5.62	60	50	75.25	
10015258	38.6p	9.0	38.7	5.11	4.72	20	53	60.5	
10031444	43p	6.5	43.3	5.21	3.85	20	53	75.5	
10015257	57.5wp1	6.0	58.2	8.53	8.71	30	23	58.5	
253099	66.1p	1.7	66.0	3.98	4.19	20	28	64	
10031624	67.84p	0.6	67.8	4.44	5.23	20	42	50.75	
Upper East Branch Pecatonica									
10031455	1.85up7	61.0	1.8	5.02	3.07		53	50	
10021754	3.5up2	59.4	3.5	2.37	7.76	70	72	67	
253094	6.5up	55.1	6.5	5.17	4.86	50	38	43	
10031447	14.9up3	54.9	17.6	5.17	4.01	40	33	61	
10031623	14.93p	54.8	17.7	5.03	2.25	70	33	57	
10031446	21p	52.9	24.9	4.32	6.93	60	38	59	
10014311	45.5p	49.3	48.3	4.70	4.89	50	57	69.25	
253128	51.4up1	45.7	53.0	3.80	4.58	90	48	73.75	
10020046	120.4p1	43.6	122.8	4.10	5.70	60	48	68.5	
253100	124.2p	39.9	127.2	4.42	3.87	40	28	58.5	
10031443	132.75p	36.6	135.4	4.28	4.44	50	24	37.5	
10031442	136p	33.6	140.1	6.92	0.50	30	29	40.5	
10031441	221	33.1	217.0	3.99	6.39	25	29	44.5	
German Val	ley Branch								
10011872	1.7g6	5.0	2.1	3.22	7.99	80	87	58	
10031449	7.3g	1.5	7.3	4.85	4.21	60	77	78.5	
Gordon Cre	ek								
10011740	4.9g1	18.9	4.2	2.59	5.71	80	57	50.5	
10031450	6.9g1	17.1	6.8	2.25	6.55	90	53	66.5	
10008165	27.9g	9.4	27.8	1.62	10.27	50	80	90.5	
253205	30g	6.8	30.9	2.19	6.82	50	85	85.5	
253101	66g	4.5	66.1	2.93	5.60	60	55	70.5	
10021401	71g	1.9	71.0	4.20	4.88	80	48	59.25	
10029189	76.9g	0.0	76.7	4.16	5.41	70	33	50.5	
Gribble Branch									
10031468	1.65wp9	0.9	2.2	1.44	6.37	80	68	78.25	
Jeglum Valle	ey Creek								
10031465	1.7g2	0.0	2.1	4.51	6.04	0	40	38.5	

Appendix III. Drainage area and summary statistics for biotic and physical habitat index scores for Pecatonica Watershed sampling site locations.

	Sito	Divor	Drain							
SWIMS ID	Name	mile	(sq. mi)	нві	MIBI	FIBI	QHab	QHEI		
Kittleson Valley Creek										
10015426	1.7a4	7.9	3.0	3.06	8.10	60	20	42.5		
10009432	19.1a	5.4	19.2	4.02	5.49	80	87	83.5		
133444	29.89a	3.5	29.8	4.33	5.90	80	28	50		
253058	33a	0.3	32.9	2.39	6.96	80	28	59		
Lee Creek	9									
10029295	1.7a3	2.4	1.6	3.82	5.90		53	53.25		
10031452	3.5a2	1.3	3.7	3.25	8.67	70	43	46.5		
Lev Creek	<u>9</u>		•							
253057	3.3wp3	1.2	3.2	9.46	7.71	70	47	52.5		
Lynch Bran	ch									
10014320	1.54wp10	1.4	2.2	2.46	7.94	70	82	64		
Olson Creel	۰ ۲									
10031456	1.7wp7	3.0	1.7	6.58	2.32	0	52	45.5		
10029527	3.5wp1	2.5	3.1	2.95	6.50	90	63	62.5		
Pleasant Valley Branch										
10031464	1.7g5	4.8	0.9	4.34	4.88		58	56		
10009781	3.5g3	2.1	3.8	2.81	5.50	50	92	83.5		
10011636	7.5g	1.5	7.5	5.89	5.70	60	77	83.5		
Smith Conley Creek										
10031762	0p5	7.2	0.8	4.49	4.15	40	43	48		
10031462	1.7up3	6.8	1.7	6.31	1.09	0	38	46.5		
10012856	9.68p	3.3	9.7	4.11	4.83	60	27	57.5		
10016138	13.8up2	0.5	14.0	3.59	7.84	80	63	62.5		
10008171	19.1p	0.1	19.0	4.24	6.25	40	52	65		
Syfestad Cr	eek									
10031453	3.19g3	2.0	3.2	3.16	7.13	80	63	67.5		
Unnamed										
10031458	1.7wp4	2.9	0.7	5.16	8.27	0	33	48.5		
253052	1.7wp5	2.4	4.4	3.35	4.70	90	47	58.5		
10031448	7.3wp	1.6	7.2	4.71	6.07	90	87	82		
10016644	2.65g	1.1	2.5	4.05	6.17	60	48	60		
10031467	1.6wp12	1.0	1.3	5.20	4.10	0	28	42.5		
10031454	1.92p	0.8	2.0	2.56	6.08		82	59.5		
10031459	1.7up6	0.2	2.4	4.20	6.17	80	43	53.25		
10031463	1.7up2	0.2	2.0	4.63	2.78	0	23	46		
10031469	1.55g9	0.0	1.6	2.39	7.25		48	42		
10031470	1.53wp11	0.0	1.5	3.98	4.52	40	42	46		
Urnus Creel	k				_	_				
10031457	1.7wp6	0.2	2.0	4.97	2.90	30	18	32.5		
Williams-Barneveld Creek										
10012833	3.3up4	3.5	6.1	4.16	3.55	40	85	82		

Appendix III. Drainage area and summary statistics for biotic and physical habitat index scores for Pecatonica Watershed sampling site locations.

Index names: HBI (Hilsenhoff's biotic index); MBI (Macroinvertebrate index of biotic integrity); fIBI (Fish index of biotic integrity); QHab (Qualitative fish habitat rating, Wisconsin DNR); QHEI (Qualitative habitat evaluation index, Ohio EPA).