

GREAT LAKES FISHERY COMMISSION

2000 Project Completion Report¹

**DNA-Based Markers for the Assessment of Genetic Population
Structure in Yellow Perch**

by:

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DNA-based markers for the assessment of genetic population structure in yellow perch

A Completion Report to the Great Lakes Fishery Commission by

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Abstract

We have identified the first genetic markers that resolve significant variation within and among populations of yellow perch in the north-central United States. Eight microsatellite DNA markers each resolved 4-34 alleles in samples from the Milwaukee area of Lake Michigan, Green Bay, and Lake Winnibigoshish, in northern Minnesota. The markers could be roughly grouped according to their levels of variation. Three had high variation (observed heterozygosities, H , ranging from 0.91 - 0.99), four had moderate variation ($H = 0.35-0.71$), and only one had low variation ($H = 0.03$). Exact tests indicated significant differentiation in allele frequencies ($P < 0.001$) among all three populations. Five additional, but smaller, samples were included from populations throughout the region. A UPGMA diagram based on Nei 1978 genetic distance showed population clusters consistent with the geographical locations of the sampling sites. Within Lake Michigan, Green Bay was distinct from three main-body populations. Genetic structure among the main-body populations suggests reproductive isolation by distance. Adding more populations with increased sample sizes will be necessary to draw more definitive conclusions about population structure within Lake Michigan and throughout the region.

Background

The recent declines in Lake Michigan's yellow perch (*Perca flavescens*) has prompted interest in the possible genetic structuring of perch populations within the lake. Maintenance of genetic variation between and within exploited fish populations, a product of past evolution in different environmental conditions of the Great Lakes, is essential to increase the chances that depleted populations will rebuild and persist over the long-term. Conservation of discrete genetic populations of yellow perch -- where they actually exist -- is a building block of conservation of biological diversity of fish communities. Baseline information about genetic population structure is needed to design and implement effective rehabilitation strategies, particularly any possible hatchery stocking efforts.

Little is known definitively about the population structure of yellow perch. On the one hand, the existence of distinct breeding groups (demes) within single lakes has been proposed based on tagging studies, comparative growth and behavior studies, and patterns of egg mass deposition (Aalto and Newsome 1990). On the other hand, prior genetic studies detected very little population structure across broad geographic regions (e.g., Todd and Hatcher 1993, Billington 1996). Low variation within and between populations for the types of genetic markers tested in prior studies (allozymes and mitochondrial DNA) has limited the use of these markers for discriminating populations.

In this report we describe the identification and characterization of eight polymorphic microsatellite DNA markers in yellow perch. Microsatellites are regions of repetitive nuclear DNA (e.g. ACACAC...) subject to high rates of mutation, which often leads to high levels of variation. These markers are evaluated using primers for the polymerase chain reaction (PCR), a DNA amplifying technique that allows the use of minute amounts of tissue, including scales, spines, fins clips, etc. We tested microsatellite primers developed for walleye (*Stizostedion vitreum*), a related species in the family Percidae, for their ability to amplify perch DNA. Markers that amplified were assessed for genetic variation in perch in order to develop a panel of genetic markers suitable for evaluating population structure among Lake Michigan perch populations.

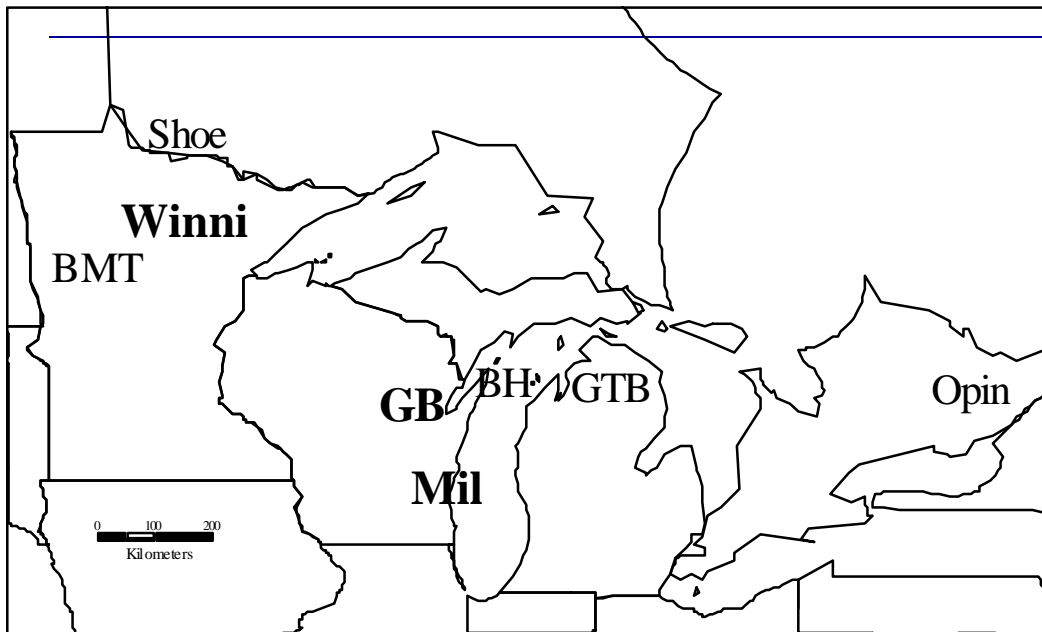
Methods

Population Samples

We analyzed samples of 57-58 individuals from each of three primary populations: Green Bay, Wisconsin, the Milwaukee area of Lake Michigan, and Lake Winnibigoshish in northern Minnesota (Figure 1, in bold). The samples were provided as scales (Milwaukee and Winnibigoshish) or spines (Green Bay), which were collected as part of routine assessment netting by the Wisconsin and Minnesota Departments of Natural Resources. The Lake Michigan fish ranged in size from 150-310 mm and were all mature and the Winnibigoshish fish were 125-280 mm with < 5 immature individuals. The three populations provided the base for evaluating genetic variability in populations within and outside of the Lake Michigan basin. In addition, we obtained 15 samples each from Bailey's Harbor in Lake Michigan, Shoepack Lake, MN, in the Hudson drainage, Big Mantrap Lake, MN, in the Mississippi drainage, and Lake Opinicon, ONT, which flows into Lake Ontario, and 30 samples from Grand Traverse Bay in Lake Michigan

(Figure 1). These samples were all provided as extracted DNA from David Philipp [Illinois Natural History Survey (INHS), Champaign]. Philipp's laboratory found little genetic variability in these samples using allozymes, mtDNA, and randomly amplified polymorphic DNA (RAPDs) (D. Philipp, INHS, personal communication).

Figure 1. Yellow perch sampling sites. The three primary study sites (in bold) were the Milwaukee area of Lake Michigan (Mil), Green Bay (GB), and Lake Winnibigoshish (Winni). These secondary sites had smaller sample sizes: Big ManTrap Lake (BMT), Shoepack Lake (Shoe), Grand Traverse Bay (GTB), Bailey's Harbor (BH) and Lake Opinicon, (Opin).



Genetic Marker Evaluation

We attempted to amplify perch DNA via PCR using 13 microsatellite loci primers developed for walleye (Borer et al 1999, Eldridge et al. submitted). We used techniques reported in Miller and Kapuscinski (1996). Briefly, samples were prepared by extracting DNA from individual dried fish scales or 5 mm pieces of spine using 5% Chelex (Sigma Chemical) in water. This DNA was added to a PCR reaction mix and thermal cycling was initially conducted at an annealing temperature of 50 °C for all primers. If a product was not produced, the temperature was reduced to 48 °C. Reaction products were resolved on 8% non-denaturing polyacrylamide mini-gels stained with ethidium bromide. Initially, we ran 10 samples total from the eight populations. If no polymorphism was evident for a marker, we ran 10-30 additional samples before discontinuing its use. If polymorphism was detected, further PCRs were conducted using one fluorescently-labelled primer per locus. Products were then evaluated on an ABI Prism 377 DNA Sequencer and scored for alleles according to the number of base pairs.

Data Analysis

We evaluated genetic variation within and among populations for all polymorphic markers. To assess within-population genetic variation, we determined the number of alleles and heterozygosity for each locus in the three primary populations with large sample sizes. We also tested markers for agreement with Hardy-Weinberg expectations in these three populations. To assess among-population variation, we performed exact tests for population differentiation (Raymond and Rousset 1995), which determine if allele frequency distributions differ between pairs of populations. We then developed UPGMA dendrograms based on Nei's 1978 unbiased genetic distance among the three populations. To better evaluate the use of these microsatellites for describing population structure, we repeated the among population analysis including five additional populations from within and without Lake Michigan. All analyses were conducted using Tools For Population Genetic Analysis (Mark Miller, Northern Arizona University, Flagstaff, <http://herb.bio.nau.edu/~miller/tfpga.htm>).

Results and Discussion

PCR amplification

We successfully amplified perch DNA using 12 of 13 walleye microsatellite markers (Table 1). This high level of conservation of microsatellite loci within a family is similar to that found within the family salmonidae (e.g., Condrey and Bentzen 1998). Of the 12 markers that amplified, three were monomorphic in our initial testing and were no longer used. The remaining nine were scored for genetic variation in the three primary populations. During this evaluation we had difficulties consistently amplifying Svi3 and dropped it from our analysis.

Table 1. Amplification of yellow perch DNA via PCR using microsatellite markers developed for walleye.

Marker	Amplification? ^a	Polymorphic? ^b	Annealing Temp. (°C)
Svi2	yes	yes	50
Svi3	yes	yes	48
Svi4	yes	yes	48
Svi5	yes	yes	50
Svi6	yes	yes	48
Svi7	yes	yes	48
Svi14	yes	yes	48
Svi16	yes	no	50
Svi17	yes	yes	48
Svi18	yes	no	50
Svi20	yes	no	50
Svi26	no	-	48
Svi33	yes	yes	48

^a Was a product produced by PCR?

^b Was genetic variation (multiple alleles) detected?

Genetic Variation

Eight microsatellite markers could be roughly grouped according to their levels of variation in the three primary populations (Table 2). Three (Svi33, Svi6, Svi4) had high variation (observed heterozygosities, H , ranging from 0.91 - 0.99), four (Svi5, Svi17, Svi7, Svi2) had moderate variation ($H = 0.35$ - 0.71), and only one (Svi14) had low variation ($H = 0.03$). Within each population, H over all loci was similar (Milwaukee = 0.59, Green Bay = 0.71, Winnibigoshish = 0.54). Average H for Winnibigoshish was particularly decreased at Svi2, with a value of 0.04 compared to 0.47 and 0.54 for Milwaukee and Green Bay, respectively. Numbers of alleles were high (21-34) for all high variation markers. Within the moderate and low variation groups, allele numbers were similar (4-11). Heterozygosity did not increase linearly with allele number because of different allele frequencies among markers. For example, Svi14 had four alleles but the common allele was present at a frequency > 0.95 in all three populations. Genotypes were generally consistent with Hardy-Weinberg equilibrium. Single-locus tests were significant ($P < 0.05$) for Svi2 and Svi5 in the Green Bay sample but neither test was significant after Bonferroni correction for multiple testing (24 tests; eight markers x three populations).

Table 2. Observed heterozygosities and numbers of alleles for eight variable microsatellite markers in three yellow perch populations.

Locus	Heterozygosity				Total
	Milwaukee	Green Bay	Winnibigoshish	Average	Alleles
Svi33	1.00	0.98	1.00	0.99	34
Svi6	0.82	0.96	1.00	0.93	25
Svi4	0.94	0.91	0.88	0.91	21
Svi5	0.71	0.93	0.62	0.75	11
Svi17	0.47	0.75	0.35	0.52	9
Svi7	0.22	0.57	0.45	0.41	6
Svi2	0.47	0.54	0.04	0.35	4
Svi14	0.06	0.04	0.00	0.03	4
Average	0.59	0.71	0.54	0.61	14.6

These microsatellites represent the first set of genetic markers that resolve moderate to high variation across populations in the north-central United States. This degree of variation makes them suitable for examining population structure among populations in Lake Michigan and throughout the region. In addition, the high variation at three of the markers makes them very useful for identification of individuals or parentage, as we have done for walleye with these markers (Eldridge et al., submitted).

Genetic Structure

Exact tests for population differentiation indicated that allele frequencies differed ($P < 0.0001$) among the three primary populations (Appendix 1 provides a table of allele frequencies for all eight populations). A UPGMA dendrogram based on Nei's unbiased genetic distance

showed that Milwaukee and Green Bay populations clustered separately from Winnibigoshish (Figure 2), as would be expected. Using just three populations does not provide much guidance as to how well the markers might discriminate among numerous populations with varying degrees of isolation. To better evaluate the use of these microsatellites for describing population structure, we determined allele frequencies for five additional populations from within and without Lake Michigan (Appendix 1). Exact tests indicated differentiation for all pair-wise population comparisons ($P < 0.01$) except for Milwaukee and Bailey's Harbor and Bailey's Harbor and Grand Traverse Bay ($P > 0.05$). Although sample sizes were too small to make definitive statements, the resulting groupings of populations are consistent with expectations based on drainage geography (Figure 3). A major division separated the Minnesota populations (Hudson Bay and Mississippi drainages) from the Great Lakes populations. Within the Great Lakes, Opinicon, which connects to Lake Ontario, was separated from the Lake Michigan populations. Green Bay stands out as quite distinct from populations in the main body of Lake Michigan, even though two of the main-body populations were from the north, closer to the entrance of Green Bay than to Milwaukee. The preliminary indication is that populations from the main body may be only slightly distinct from one another, at least relative to Green Bay and other populations outside of Lake Michigan. However, there may be reproductive isolation by distance within the main body. Bailey's Harbor, which was undifferentiated from the other main-body populations, is located between Milwaukee and Grand Traverse Bay. Milwaukee and Grand Traverse Bay, separated by a greater distance, were differentiated ($P = 0.004$). Adding more populations with increased sample sizes will be necessary to draw more definitive conclusions about population structure within Lake Michigan.

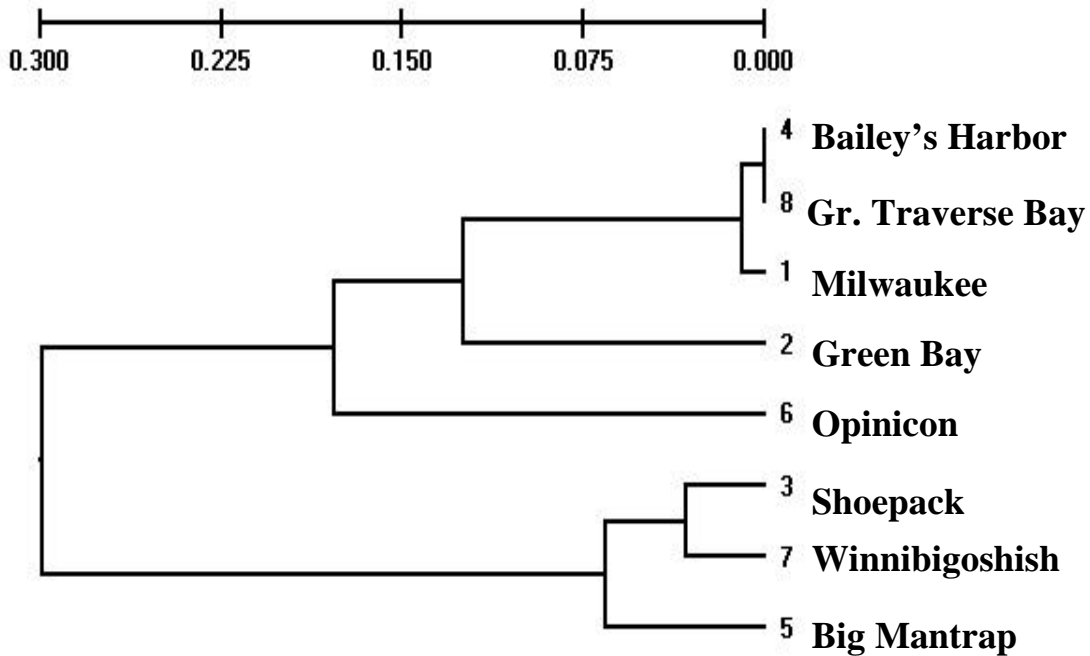
Figure 2. UPGMA dendrogram of three Lake Michigan yellow perch populations using Nei's 1978 unbiased genetic distance. Milwaukee and Green Bay samples are from Lake Michigan and Winnibigoshish is in the Mississippi drainage in northern Minnesota.

Milwaukee

Green Bay

Winnibigoshish

Figure 3. UPGMA dendrogram of eight yellow perch populations using Nei's 1978 unbiased genetic distance. Bailey's Harbor, Grand Traverse Bay, and Milwaukee are within the main body of Lake Michigan, Green Bay connects to the main body, Opinicon connects to Lake Ontario, and Shoepack, Winnibigoshish, and Big Mantrap are all in Minnesota, outside of the Great Lakes drainage.



Conclusion

We have identified the first genetic markers that resolve significant variation in populations of yellow perch in the north-central United States. Our results indicate that these markers will give us high power to describe population structure within Lake Michigan, if it exists. Preliminary results indicate that a Green Bay population is quite distinct from main body populations and that isolation by distance may occur within the lake. Ongoing sampling of numerous spawning sites within the lake and of inland lakes in the surrounding states will allow us to thoroughly assess the degree of genetic population structure among perch populations in the Lake Michigan region.

Acknowledgements

Rich Hess, Illinois Department of Natural Resources, coordinated sample collection and the Wisconsin and Minnesota Departments of Natural Resources provided scale and spine samples. David Philipp, Illinois Natural History Survey, provided DNA samples. Steve Rees and Brice Adams, technicians in our laboratory, processed many of the samples. We thank the Great Lakes Fishery Commission for providing funding for this project.

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Appendix 1. Allele frequencies for eight microsatellite markers in eight yellow perch populations.

Population	Alleles (base pairs)																	
Marker: Svi2																		
	204	214	216	218														
Milwaukee	0.01	0.42	0.53	0.04														
Green Bay	0.00	0.28	0.58	0.14														
Winnibigoshish	0.00	0.00	0.98	0.02														
Bailey's Harbor	0.00	0.63	0.37	0.00														
Big Mantrap	0.00	0.00	1.00	0.00														
Opinicon	0.00	0.00	0.96	0.04														
Shoepack	0.00	0.07	0.93	0.00														
Gr. Traverse Bay	0.02	0.62	0.29	0.08														
Marker: Svi4																		
	112	114	118	124	126	136	137	139	144	146	149	151	153	155	157	159	161	163
Milwaukee	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.05	0.00	0.02	0.03	0.27	0.18
Green Bay	0.05	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.01	0.02	0.01	0.01	0.13	0.05	0.04	0.20	0.14	0.16
Winnibigoshish	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.11	0.04	0.37	0.05	0.23	0.06	0.07
Bailey's Harbor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.07	0.00	0.00	0.10	0.20	0.17
Big Mantrap	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.10	0.03	0.10	0.07	0.30	0.13	0.10
Opinicon	0.00	0.39	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.11	0.29
Shoepack	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.04	0.00	0.11	0.32	0.07
Gr. Traverse Bay	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.02	0.06	0.08	0.02	0.08	0.23	0.15
	165	167	169	171	173	175	177											
Milwaukee	0.13	0.15	0.03	0.03	0.00	0.08	0.00											
Green Bay	0.05	0.02	0.04	0.01	0.02	0.00	0.00											
Winnibigoshish	0.01	0.02	0.01	0.00	0.00	0.00	0.00											
Bailey's Harbor	0.10	0.10	0.03	0.03	0.03	0.07	0.03											
Big Mantrap	0.03	0.03	0.03	0.00	0.00	0.00	0.00											
Opinicon	0.07	0.00	0.04	0.00	0.00	0.00	0.00											
Shoepack	0.07	0.14	0.04	0.00	0.00	0.00	0.00											
Gr. Traverse Bay	0.06	0.00	0.04	0.06	0.04	0.06	0.00											

Marker: Svi5

	175	178	182	184	186	188	190	192	194	196	198	200	202
Milwaukee	0.00	0.00	0.03	0.00	0.35	0.06	0.12	0.06	0.35	0.03	0.00	0.00	0.00
Green Bay	0.01	0.00	0.01	0.03	0.11	0.06	0.26	0.15	0.24	0.09	0.02	0.01	0.00
Winnibigoshish	0.00	0.00	0.03	0.00	0.65	0.03	0.05	0.13	0.10	0.00	0.00	0.01	0.00
Bailey's Harbor	0.00	0.03	0.00	0.03	0.33	0.03	0.07	0.03	0.33	0.10	0.00	0.00	0.03
Big Mantrap	0.00	0.00	0.11	0.14	0.18	0.07	0.00	0.18	0.25	0.07	0.00	0.00	0.00
Opinicon	0.00	0.00	0.07	0.00	0.43	0.07	0.07	0.00	0.29	0.07	0.00	0.00	0.00
Shoepack	0.00	0.00	0.04	0.00	0.61	0.00	0.07	0.18	0.11	0.00	0.00	0.00	0.00
Gr. Traverse Bay	0.00	0.00	0.00	0.00	0.36	0.03	0.00	0.08	0.36	0.17	0.00	0.00	0.00

Marker: Svi6

	162	169	171	173	175	177	179	181	183	185	187	189	190	192	194	196	198	200
Milwaukee	0.00	0.01	0.00	0.00	0.00	0.05	0.42	0.10	0.04	0.00	0.00	0.03	0.03	0.05	0.05	0.00	0.01	0.00
Green Bay	0.00	0.02	0.00	0.00	0.01	0.00	0.08	0.01	0.05	0.01	0.01	0.06	0.05	0.32	0.16	0.08	0.04	0.03
Winnibigoshish	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.06	0.10	0.21	0.27	0.14	0.05	0.08
Bailey's Harbor	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.13	0.03	0.00	0.03	0.00	0.00	0.10	0.07	0.00	0.00	0.00
Big Mantrap	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03	0.10	0.00	0.30	0.23	0.17	0.07	0.03
Opinicon	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.07	0.32	0.21	0.11	0.04	0.11
Shoepack	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.25	0.00	0.00	0.00	0.00	0.25	0.32	0.04	0.04	0.04
Gr. Traverse Bay	0.02	0.00	0.00	0.02	0.00	0.04	0.46	0.02	0.06	0.02	0.00	0.02	0.00	0.06	0.02	0.00	0.02	0.02

	202	204	208	210	212	214	216	218	220	222	226
Milwaukee	0.00	0.03	0.03	0.01	0.01	0.04	0.01	0.04	0.01	0.00	0.00
Green Bay	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.02	0.00
Winnibigoshish	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bailey's Harbor	0.00	0.00	0.00	0.00	0.00	0.20	0.07	0.03	0.03	0.00	0.00
Big Mantrap	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Opinicon	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shoepack	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gr. Traverse Bay	0.00	0.02	0.00	0.00	0.02	0.08	0.04	0.02	0.00	0.00	0.02

Marker: Svi7

	174	178	180	184	186	188	190	192					
Milwaukee	0.00	0.00	0.01	0.00	0.89	0.06	0.04	0.00					
Green Bay	0.00	0.01	0.00	0.00	0.62	0.35	0.02	0.01					
Winnibigoshish	0.00	0.00	0.01	0.00	0.50	0.48	0.01	0.00					
Bailey's Harbor	0.00	0.00	0.00	0.03	0.93	0.03	0.00	0.00					
Big Mantrap	0.00	0.00	0.00	0.00	0.47	0.53	0.00	0.00					
Opinicon	0.00	0.00	0.00	0.00	0.96	0.04	0.00	0.00					
Shoepack	0.00	0.00	0.00	0.00	0.54	0.46	0.00	0.00					
Gr. Traverse Bay	0.03	0.00	0.00	0.00	0.86	0.05	0.05	0.00					

Marker: Svi14

	134	136	138	140									
Milwaukee	0.01	0.97	0.01	0.01									
Green Bay	0.00	0.98	0.01	0.01									
Winnibigoshish	0.00	1.00	0.00	0.00									
Bailey's Harbor	0.00	0.96	0.04	0.00									
Big Mantrap	0.00	1.00	0.00	0.00									
Opinicon	0.04	0.96	0.00	0.00									
Shoepack	0.00	1.00	0.00	0.00									
Gr. Traverse Bay	0.00	0.93	0.05	0.02									

Marker: Svi17

	148	150	152	154	156	158	160	162	164	166	168	170	180
Milwaukee	0.00	0.00	0.05	0.02	0.69	0.13	0.05	0.03	0.00	0.00	0.02	0.00	0.00
Green Bay	0.01	0.00	0.08	0.01	0.37	0.07	0.36	0.08	0.02	0.00	0.00	0.00	0.00
Winnibigoshish	0.00	0.00	0.00	0.00	0.03	0.07	0.78	0.08	0.04	0.00	0.00	0.00	0.00
Bailey's Harbor	0.00	0.00	0.06	0.00	0.44	0.22	0.17	0.06	0.00	0.00	0.00	0.06	0.00
Big Mantrap	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.11	0.46	0.04	0.00	0.00	0.00
Opinicon	0.00	0.00	0.00	0.00	0.41	0.14	0.00	0.32	0.05	0.00	0.00	0.00	0.09
Shoepack	0.00	0.00	0.00	0.00	0.00	0.08	0.67	0.00	0.25	0.00	0.00	0.00	0.00
Gr. Traverse Bay	0.00	0.04	0.14	0.00	0.66	0.04	0.12	0.00	0.00	0.00	0.00	0.00	0.00

Marker: Svi33

	109	113	115	117	119	121	123	125	127	129	131	133	135	137	139	141	143	145	
Milwaukee	0.00	0.02	0.01	0.00	0.01	0.02	0.01	0.03	0.01	0.01	0.02	0.08	0.05	0.03	0.04	0.04	0.08	0.08	
Green Bay	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.02	0.04	0.04	0.07	0.04	0.04	0.07	0.06	0.13	0.08	
Winnibigoshish	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.02	0.03	0.00	0.08	0.04	0.11	0.13	0.11	0.11	0.09	
Bailey's Harbor	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.03	0.07	0.07	0.07	0.07	0.00	0.00	0.07	
Big Mantrap	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.03	0.00	0.20	0.17	0.17	0.07	
Opinicon	0.04	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.07	
Shoepack	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.07	0.07	0.11	0.07	0.18	0.14	
Gr. Traverse Bay	0.00	0.03	0.05	0.02	0.02	0.00	0.00	0.00	0.03	0.02	0.03	0.05	0.05	0.05	0.05	0.02	0.14	0.02	
	147	149	151	153	155	157	159	161	163	165	167	169	171	173	175	176	180	182	184
Milwaukee	0.05	0.11	0.05	0.07	0.03	0.03	0.03	0.03	0.03	0.02	0.04	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Green Bay	0.04	0.07	0.02	0.06	0.03	0.02	0.04	0.01	0.00	0.01	0.02	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.01
Winnibigoshish	0.08	0.06	0.05	0.04	0.03	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bailey's Harbor	0.13	0.03	0.10	0.07	0.03	0.00	0.03	0.03	0.03	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Big Mantrap	0.10	0.07	0.03	0.00	0.03	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Opinicon	0.04	0.00	0.11	0.11	0.14	0.04	0.07	0.00	0.07	0.04	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.04	0.00
Shoepack	0.14	0.00	0.00	0.07	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gr. Traverse Bay	0.12	0.03	0.12	0.02	0.03	0.00	0.03	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00
