ILLINOIS

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

# PRODUCTION NOTE

University of Illinois at
Urbana-Champaign Library
Large-scale Digitization Project, 2007.

Illinois Natural History Survey
Center For Aquatic Ecology

Final Report

# Genetic Assessment of Two *Stizostedion* Species and Their Hybrid in the Ohio River

**T. W. Kassler and D. P. Philipp**

Submitted to Ohio Division of Wildlife

November 2000

Aquatic Ecology Technical Report 00/9

# Final Report

## Genetic Assessment of Two *Stizostedion* Species and Their Hybrid in the Ohio River

**T. W. Kassler and D. P. Philipp**

Submitted to Ohio Division of Wildlife

Todd W. Kassler
Principal Investigator
Center for Aquatic Ecology

David P. Philipp
Principal Investigator
Center for Aquatic Ecology

David H. Wahl
Director, Center for Aquatic Ecology

Problem Statement:

Walleye and sauger were collected from tailwaters along the Ohio River extending from river mile 54 in Ohio to river mile 918 in Illinois (Table 1) to address two objectives. The first objective was to determine the accuracy of field identification of individual fish to species and to assess the extent of hybridization between walleye and sauger in any or all of the tailwater areas. This objective also included analysis of any saugeye detected to determine if the saugeye were F1 hybrids or backcrossed Fx hybrids. The second objective was to assess genetic variation in both species from tailwater areas on the Ohio River where samples could be collected.

Table 1. Tailwater location, River mile, and number of walleye, saugeye, and sauger that were analyzed.

| Tailwater | River Mile | # Walleye | # Saugeye | # Sauger |
|---|---|---|---|---|
| New Cumberland, OH | 54 | 61* | 107 | 100 |
| Pike Island, WV | 84 | 29 | 23 | 100 |
| Willow Island, OH | 162 | 65 | 20 | 100 |
| Belleville, OH | 204 | 0 | 7 | 0 |
| Racine, OH | 238 | 5 | 6 | 0 |
| Greenup, OH | 342 | 60 | 47 | 100 |
| Meldahl, OH | 436 | 19 | 0 | 0 |
| Markland, KY | 606 | 23 | 0 | 100 |
| McAlpine , IN | 720 | 8 | 0 | 43 |
| Uniontown, IL | 918 | 0 | 0 | 71 |

* = 16 of these walleye were analyzed using only fin clips, whereas the remaining 45 were analyzed using tissue sampled from muscle, eye, and liver.

Methods:

For the first objective a total of 1,078 *Stizostedion* individuals were collected from the Ohio River study sites. Of those, 614 fish were identified as sauger from seven tailwaters, 210 as saugeye from six tailwaters, and 254 as walleye from eight tailwaters. For the first objective, species status, including F1 and Fx hybrids was determined using three allozyme loci (AAT-M, MDH-M, and PGM-A) that have a fixed difference between walleye and sauger. An individual was scored as an F1 saugeye if all three loci were heterozygous. A fish was scored as an Fx saugeye if one or two of the three-allozyme loci were heterozygous. Individuals homozygous for all three diagnostic species loci were categorized as pure species.

For the second objective, genetic variation was assessed within sauger using fin tissue from seven tailwater areas and within walleye using fin, muscle, eye, and liver tissue from eight tailwater areas. An initial run of eight enzyme systems (12 loci) was screened to detect polymorphism within sauger and walleye populations using fin tissue. These enzyme systems were selected because previous analyses had identified them to be polymorphic within these species or diagnostic between them. Some enzymes were both diagnostic between species and polymorphic within species as shown below.

AAT-M*, AAT-A*, ADH-1**(***), CBP-1**(***), IDHP-A**, IDHP-B***, LDH-1**,
MDH-M*(**), MDH-A***, MDH-B**(***), PGM-A*(**)(***), SOD-1***

* = Diagnostic locus between walleye and sauger
** = Known polymorphic locus in sauger
*** = Known polymorphic locus in walleye

After the initial screen of 12 loci, one locus (AAT-A) was excluded from further analyses because it could not be resolved. After completion of the second run two additional loci (ADH and CBP) were dropped because we were not able to resolve adequate activity from all fin tissue samples.

Analysis of whole fish was completed using 15 loci that could be resolved. An additional six loci could be screened for this analysis because of the muscle and liver tissues that were used.

Results:

I. Analysis of sauger populations (fin clips):

This analysis was conducted on a total of 614 fish identified as sauger from seven tailwater areas. All fish, but one were correctly identified as a pure sauger (Table 2). One individual from the New Cumberland population was an F1 saugeye and, therefore dropped from the allele frequency table for pure sauger populations.

Table 2. Tailwater location, River mile, number of individuals collected and identified as pure sauger, and number of each type of *Stizostedion* identified based upon genetic analysis.

| Tailwater | River Mile | # Identified as Sauger | Results of Genetic Analysis | | | |
|---|---|---|---|---|---|---|
| | | | Sauger | F1 | Fx | Walleye |
| New Cumberland, OH | 54 | 100 | 99 | 1 | 0 | 0 |
| Pike Island, WV | 84 | 100 | 100 | 0 | 0 | 0 |
| Willow Island, OH | 162 | 100 | 100 | 0 | 0 | 0 |
| Greenup, OH | 342 | 100 | 100 | 0 | 0 | 0 |
| Markland, KY | 606 | 100 | 100 | 0 | 0 | 0 |
| McAlpine , IN | 720 | 43 | 43 | 0 | 0 | 0 |
| Uniontown, IL | 918 | 71 | 71 | 0 | 0 | 0 |

Analysis was completed on all individuals using the nine loci that could be resolved. Allele frequencies have been calculated for those loci in seven populations of sauger and one population of walleye (Table 3).

Table 3. Allele frequencies of sauger from seven tailwater populations. One walleye population is included to show allelic differences.

| | Sauger - Populations listed by River Mile | | | | | | | WAE |
|---|---|---|---|---|---|---|---|---|
| | 54 | 84 | 162 | 342 | 606 | 720 | 918 | 54 |
| | N=99 | N=100 | N=100 | N=100 | N=100 | N=43 | N=71 | N=15 |
| AAT-M | | | | | | | | |
| 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| 2 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 |
| IDHP-A | | | | | | | | |
| 1 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| IDHP-B | | | | | | | | |
| 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.567 |
| 2 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.993 | 0.433 |
| 3 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.007 | 0.000 |
| LDH-1 | | | | | | | | |
| 1 | 0.000 | 0.000 | 0.005 | 0.000 | 0.000 | 0.000 | 0.007 | 0.000 |
| 2 | 0.995 | 1.000 | 0.995 | 0.995 | 1.000 | 1.000 | 0.986 | 1.000 |
| 3 | 0.005 | 0.000 | 0.000 | 0.005 | 0.000 | 0.000 | 0.007 | 0.000 |
| MDH-M | | | | | | | | |
| 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| 2 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 |
| MDH-A | | | | | | | | |
| 1 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.012 | 0.007 | 0.000 |
| 2 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.988 | 0.993 | 1.000 |
| MDH-B | | | | | | | | |
| 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.400 |
| 3 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.600 |
| PGM-A | | | | | | | | |
| 1 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 |
| 2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| SOD-1 | | | | | | | | |
| 1 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Although, three loci were found to be polymorphic (IDHP-B, LDH-1, and MDH-A) in sauger the variant alleles occurred in only one or two individuals. The low level of variability detected in sauger did not provide enough information to conduct further analyses on the distribution of genetic variation as it might relate to stock structure.

II. Analysis of walleye populations (whole fish)

Table 4 lists the number of individuals that were visually identified as walleye and the actual species status based upon genetic analysis.

| Tailwater | River Mile | # Identified as Walleye | Results of Genetic Analysis | | | |
|---|---|---|---|---|---|---|
| | | | Sauger | F1 | Fx | Walleye |
| New Cumberland, OH | 54 | 61 | 0 | 3 | 0 | 58 |
| Pike Island, WV | 84 | 29 | 0 | 3 | 4 | 22 |
| Willow Island, OH | 162 | 65 | 14 | 5 | 7 | 39 |
| Racine, OH | 238 | 5 | 0 | 0 | 1 | 4 |
| Greenup, OH | 342 | 60 | 1 | 13 | 6 | 40 |
| Meldahl, OH | 436 | 19 | 0 | 1 | 9 | 9 |
| Markland, KY | 606 | 23 | 0 | 1 | 6 | 16 |
| McAlpine, IN | 720 | 8 | 1 | 1 | 0 | 6 |

Genetic analysis revealed hybrid individuals in every population and misidentified pure sauger in three populations; indicating the ability to identify pure walleye in the field can be low.

Sixteen walleye (fin clips) were analyzed from New Cumberland, OH and 15 of those individuals were pure walleye and one an F1 saugeye. These 16 individuals were not included with the analysis of whole fish because six loci were not scored.

For the assessment of genetic variation in whole walleye, an additional six loci were screened for this analysis because of the muscle and liver tissues. Allele frequencies are calculated for those loci in eight populations of walleye (Table 5).

Table 5. Allele frequencies for eight tailwater areas where walleye were collected as part of this study and L. Erie, OH, and L. Winnebago, WI for comparison.

| | Populations listed by River Mile | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 54 | 84 | 162 | 238 | 342 | 436 | 606 | 720 | Erie | Winnebago |
| | N=43 | N=22 | N=39 | N=4 | N=40 | N=9 | N=16 | N=6 | N=30 | N=30 |
| *AAT-M* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *AAT-B* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.944 | 1.000 | 1.000 | 1.000 | 1.000 |
| 115 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.056 | 0.000 | 0.000 | 0.000 | 0.000 |
| *ADH-1* | | | | | | | | | | |
| -60 | 0.023 | 0.090 | 0.077 | 0.000 | 0.083 | 0.056 | 0.062 | 0.167 | 0.259 | 0.167 |
| -100 | 0.977 | 0.910 | 0.923 | 1.000 | 0.917 | 0.944 | 0.938 | 0.833 | 0.741 | 0.833 |
| *AK-1* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *CBP-1* | | | | | | | | | | |
| 100 | 0.430 | 0.410 | 0.551 | 0.625 | 0.575 | 0.500 | 0.719 | 0.833 | 0.552 | 0.833 |
| 160 | 0.570 | 0.590 | 0.449 | 0.375 | 0.425 | 0.500 | 0.281 | 0.167 | 0.448 | 0.167 |
| *FBP-1* | | | | | | | | | | |
| 85 | 0.000 | 0.000 | 0.000 | 0.125 | 0.000 | 0.000 | 0.062 | 0.000 | 0.000 | 0.000 |
| 100 | 1.000 | 1.000 | 1.000 | 0.875 | 1.000 | 1.000 | 0.938 | 1.000 | 1.000 | 1.000 |
| *IDHP-A* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *IDHP-B* | | | | | | | | | | |
| 75 | 0.640 | 0.705 | 0.590 | 0.500 | 0.444 | 0.278 | 0.375 | 0.333 | 0.310 | 0.483 |
| 100 | 0.360 | 0.295 | 0.410 | 0.500 | 0.556 | 0.722 | 0.625 | 0.667 | 0.690 | 0.517 |
| *LDH-1* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *MDH-M* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *MDH-A* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *MDH-B* | | | | | | | | | | |
| 100 | 0.465 | 0.500 | 0.577 | 0.125 | 0.662 | 0.778 | 0.750 | 0.417 | 0.741 | 0.883 |
| 120 | 0.535 | 0.500 | 0.423 | 0.875 | 0.338 | 0.222 | 0.250 | 0.583 | 0.259 | 0.117 |
| *PGDH-1* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *PGM-A* | | | | | | | | | | |
| 75 | 0.011 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 100 | 0.988 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| *SOD-1* | | | | | | | | | | |
| 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

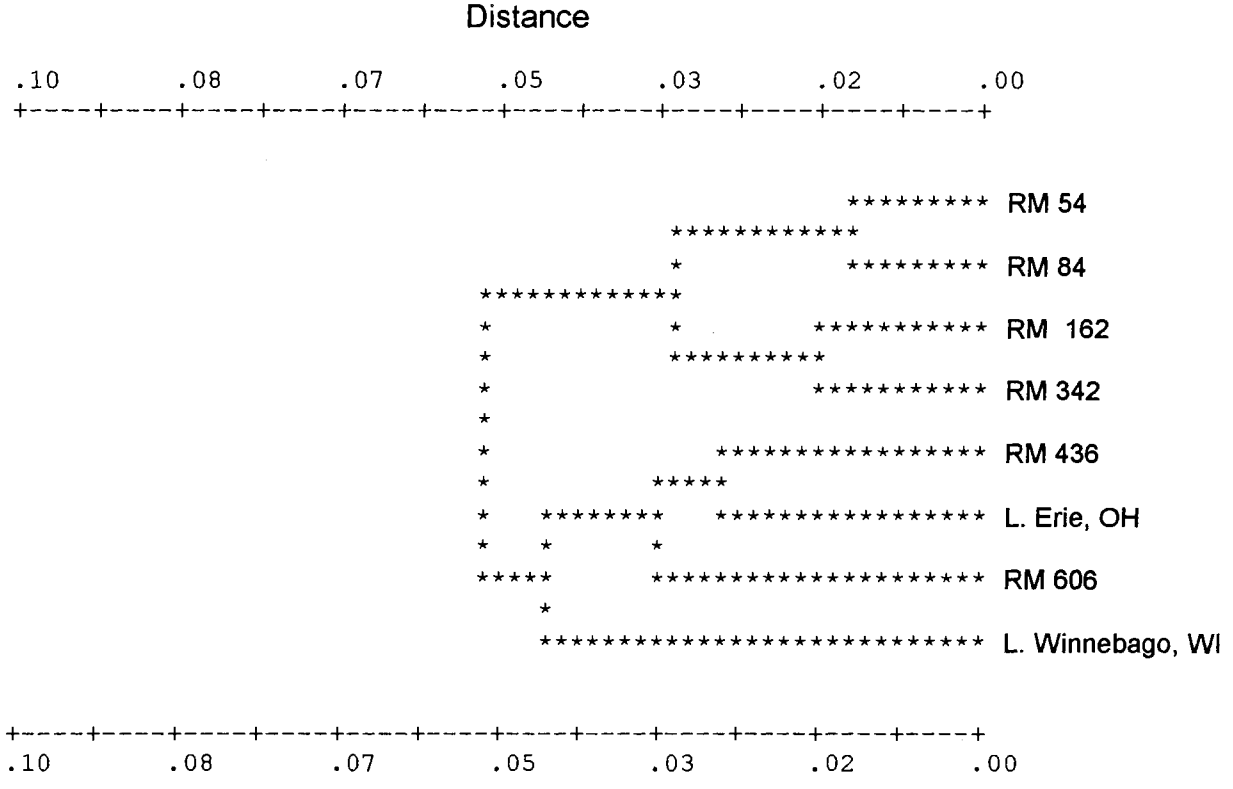
A total of 15 loci were used to assess the genetic variation in walleye from eight locations on the Ohio River, one location from Lake Erie, and one location from Lake

Winnebago, WI. Seven of 15 loci were polymorphic (AAT-B, ADH-1, CBP-1, FBP-1, IDHP-B, MDH-B, and PGM-A) within individuals identified as pure walleye. Four of the loci (ADH-1, CBP-1, IDHP-B, MDH-B) were polymorphic in all populations except for ADH-1 in Racine, OH. Genetic distance among populations was calculated with Rogers (1972) coefficient using the program BIOSYS-1 (Swofford and Selander, 1981). The resulting distance matrix was used to determine phenetic relationships among populations using a UPGMA cluster analysis (Sneath and Sokal, 1973).

The resulting dendrogram reveals two main groups: the four tailwaters closest to the headwaters (RM 54, 84, 162, and 342); the remaining two (most downstream) tailwaters sampled (RM 436 and 606) plus the Lake Erie walleye and somewhat more distant population from L. Winnebago, WI (Figure 1). Walleye from Racine, OH (RM 238) and McAlpine, IN (RM 720) were not included in the analysis because of the low sample sizes analyzed from those sites. These results suggest that walleye from Lake Erie are more similar to walleye from the lower portions of the Ohio River. This may be the result of L. Erie walleye being used to stock the lower reaches of the Ohio River and not the headwater reaches. Secondly, because the L. Winnebago, WI population grouped with the L. Erie population it suggests that walleye within the Great Lakes drainage are more similar to each other than they are to walleye native in the Ohio River.

These results are based on frequency differences of allozyme loci and not fixed differences. Completing work on the mitochondrial genome may help us detect more distinct differences between walleye in the upper reaches of the Ohio River to walleye in the lower reaches of the Ohio River and L. Erie.

Figure 1. Phenogram of UPGMA cluster analysis of protein electrophoretic data generated with Rogers' (1972) genetic distance for eight populations of walleye.



III. Analysis of saugeye (fin clips and whole fish)

Table 6 lists the number of individuals that were visually identified as saugeye and the actual species status based upon genetic analysis. The numbers of individuals visually identified in the field as saugeye were broken down into categories of pure walleye, F1 saugeye, Fx saugeye, and pure sauger based on the protein electrophoretic analysis.

| Tailwater | River Mile | # Identified as Saugeye | Results of Genetic Analysis | | | |
|---|---|---|---|---|---|---|
| | | | Sauger | F1 | Fx | Walleye |
| New Cumberland, OH | 54 | 107 | 55 | 43 | 3 | 6 |
| Pike Island, WV | 84 | 23 | 15 | 5 | 0 | 3 |
| Willow Island, OH | 162 | 20 | 2 | 18 | 0 | 0 |
| Belleville, OH | 204 | 7 | 3 | 0 | 0 | 4 |
| Racine, OH | 238 | 6 | 1 | 3 | 2 | 0 |
| Greenup, OH | 342 | 47 | 39 | 6 | 2 | 0 |

The assessment of hybridization revealed that a majority of the individuals identified as saugeye were in most cases pure sauger. The twenty individuals sampled from Willow Island, Ohio were the exception with 18 individuals being F1 saugeye and 2 pure sauger. Further hybridization of saugeye or backcrossing of saugeye individuals with pure walleye or sauger was detected in the three populations (New Cumberland, OH; Racine, OH; and Greenup, OH) and identified as Fx saugeye. It is clear based on the genetic results that visual identification of *Stizostedion* can be difficult with mistakes in all possible directions.