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DNA Markers for Discriminating Lake Erie Walleye Stocks

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web sites (include additional information on this project):

<http://www.csuohio.edu/cestp/glegl> & <http://bgesweb.artscipub.csuohio.edu>

Summary of Project Results

The research study (1) evaluated genetic stock structure and its geographic patterning for the walleye *Stizostedion vitreum* (family Percidae), which comprises the most important sport fishery in Lake Erie, and (2) developed genetic markers and a baseline data set enabling rapid discrimination of stocks. This research project addressed the question of what populations comprise genetically meaningful management units, i.e. stocks, which is a critical problem in fisheries science today. How to delineate stocks of walleye also is of stated primary interest to fishery managers in Lake Erie. Throughout the project we worked closely with the Lake Erie Walleye Task Group and the Ohio Division of Wildlife, in order to disseminate the results and enable their utilization. We also consulted with the Michigan Department of Natural Resources, the Ontario Ministry of Natural Resources, the Pennsylvania Fish and Boat Commission, and the New York Department of Environmental Conservation throughout the course of the project. We held a day-long workshop for 20 Ohio Division of Wildlife managers and personnel and guest researchers from the Ohio State University in order to present the results of this funded project and develop plans for the future (November 20, 2002 at Cleveland State University). We are meeting with the Lake Erie Walleye Task Group on February 19, 2003.

This research project specifically developed a baseline data base comprising 6 microsatellite DNA markers and sequences for the entire mtDNA control region (about 1150 bp) for 500 walleye. We conducted a comparative analysis of spawning site compositions in Sandusky

River, Sandusky Bay, the Maumee River, and western basin reef sites. We compared these data with spawning sites in the central basin at the Grand River, Ohio and in the eastern basin at the Van Buren Bay, Cattaraugus Creek, and Grand River, Ontario. We also tested outgroup populations from Lake Ontario, Lake Michigan, and Lake Superior, in order to discern the genetic history of the genotypes in light of colonization patterns from historic glacial refugium populations.

Our findings reveal greater statistical differences among spawning populations of walleye than was previously known. Our previous studies, for example, did not detect statistical differences between spawning groups in the Sandusky and Maumee Rivers – but the increased sample sizes in the present study showed that they are statistically divergent, using both mitochondrial (maternally inherited) DNA as well as nuclear (biparentally inherited) microsatellite DNA markers. The results of this project support the hypothesis that walleye home to natal spawning sites and return from generation to generation, with apparent high fidelity under natural conditions. Both reef and river spawning populations and both males and females reveal similar patterns. These results make it clear that we need to protect and maintain our walleye spawning habitat in order to safeguard the fishery for generations to come.

The good news is that walleye spawning groups in Lake Erie, at the present time, appear to be quite genetically diverse. Such appreciable genetic diversity is important for maintaining disease resistance and withstanding natural climatic fluctuations, as well combating the ongoing anthropogenic influences posed by nonindigenous species introductions, exploitation, pollution, global warming, and habitat degradation. During non-spawning months, walleye apparently may forage far from spawning areas and what is a mixed stock in the summer, is not so in the spring. Since fishing effort is mostly in the summer, the fishing stocks are of mixed genetic composition and are presumably not concentrated on given spawning groups. Thus, exploitation appears even enough to maintain genetic diversity of the overall population and that of individual spawning groups.

At the request of New York Department of Natural Resources and the Lake Erie Walleye Task Force (including representatives from Ohio Division of Wildlife, Michigan DNR, Pennsylvania Fish and Boat Commission, New York Department of Environmental Conservation, and the Ontario Ministry of Natural Resources), we conducted additional analyses of a walleye stocking problem in Cattaraugus Creek, New York, eastern basin of Lake Erie, in order to determine the effects of stocking Maumee Bay hatchery fingerlings and fry into a riverine area thought to be devoid of spawning walleye. After stocking, a native walleye run was discovered in the Seneca Nation region of Cattaraugus Creek, which our genetic analyses show is very genetically diverse. This project is resulting in a published paper and several research presentations have been made.

In consultation with the Ohio Division of Wildlife, we also worked towards completing a study of the extinct “blue pike” *Stizostedion vitreum glaucum* as part of the project, which is resulting in another peer-reviewed scientific publication. Several research presentations have been made on those results. We sequenced museum specimens of the historic blue pike *S. glaucum* in comparison to the walleye *S. vitreum* for the mtDNA control region, the nuclear LdhA6 intron, and presently are assaying microsatellite variability. We also sequenced 30 blue-colored walleye from various areas in the Great Lakes and Canada, in order to test their relationship to yellow

walleye from the Great Lakes and to the historic blue pike. We conducted a morphological study of variation in walleye and the blue pike with Drs. Miles Coburn of John Carroll University and Ted Cavender of Ohio State University. This study is being completed and will be submitted for publication soon.

Publications on Research Results from this Funding (N=4)

Stepien, C.A. and C.D. Taylor. Fine-scale genetic divergence of spawning populations of walleye in Lake Erie: A comparative analysis of mtDNA sequence and nuclear microsatellite data. In prep. (for 2003 submission)

Stepien, C.A., M. M. Coburn, T. M. Cavender, and C. D. Taylor. Genetic and morphological identity of the “extinct” blue pike *Stizostedion glaucum*: Endemism, speciation, and divergence. In prep. (for 2003 submission)

Stepien, C.A., C.D. Taylor, M.A. Tumeo, and D.W. Einhouse. Analysis of genetic hybridization risk posed by fish stocking to a historic walleye spawning group using mtDNA control region sequences and nuclear DNA microsatellites.

Stepien, Carol A. and Joseph E. Faber. 1998. Population genetic structure, phylogeography, and spawning philopatry in walleye (*Stizostedion vitreum*) from mtDNA control region sequences. *Molecular Ecology*. 7(12): 1757-1769.

Students Supported with this Funding

Alex M. Ford. Graduate student, Ph.D. Department of Biological, Geological and Environmental Sciences, Cleveland State University
Dissertation Title: Population genetic structure, phylogenetic history, and phylogeographic patterns of percid fishes in the Great Lakes.
Began 1-01, Ph.D. projected 2004.

Clifford D. Taylor. Undergraduate, B.S., Department of Chemistry. Cleveland State University. Worked in our laboratory on this project since fall 2000. Graduated with B.S. in summer 2002. Is now employed full-time as the research technician in our laboratory and has continued work on the walleye project. Will work on the new study (beginning March 2003) on Walleye population genetics and stock structure, funded by the Great Lakes Fishery Commission Restoration Funds to identify 200 spawning walleye unknowns to eastern versus western Lake Erie basin.

Presentations on Research Results from this Funding (N=14)

- Stepien, C.A., M. Coburn, and T. Cavender. 1999.** Evolutionary significant units and the genetic identity of the "extinct" blue pike: Fact or fiction? At the International Association for Great Lakes Research (IAGLR) Annual Meeting. May 1999.
- Stepien, C.A. 2000.** Genetic and morphological identity of the "extinct" blue pike *Stizostedion glaucum*: Endemism, speciation, and divergence in the lower Great Lakes" by Stepien, C.A., M. Coburn, and T. Cavender. Invited research presentation at the annual Lake Erie Protection Fund meeting held in Willoughby, Ohio in September 2000.
- Stepien, C.A. 2001.** Phylogeographic, population genetics, and systematic relationship patterns in percid fishes using nuclear and mitochondrial DNA sequences. Seminar at John Carroll University, October 2001.
- Stepien, C.A. 2002.** Genetic and morphological relationships of the extinct blue pike to walleye. Invited Ecology lunch 45-minute seminar presentation, Department of Biology, University of Akron, March 13, 2002.
- Stepien, C.A., M. M. Coburn, T. M. Cavender, and C. D. Taylor. 2002.** Genetic and morphological identity of the "extinct" blue pike *Stizostedion glaucum* versus walleye *S. vitreum*. Society of Ichthyologists and Herpetologists held in Kansas City July 4, 2002, oral research presentation.
- Stepien, C.A. 2002.** Unlocking the mysteries of Lake Erie fishes and invasive species using DNA clues. Stone Laboratory, Ohio State University seminar. 45 minute seminar oral research presentation, August 1, 2002.
- Stepien, C.A. 2002.** Unlocking the mysteries of Great Lakes fishes and exotic invasions: DNA clues. Beckman Sequencing Conference, University of Pittsburgh, Pa., August 28, 2002. 30 minute oral research presentation.
- Stepien, C.A., C.D. Taylor, and D.W. Einhouse. 2002.** Analysis of genetic hybridization risk posed by fish stocking to a historic walleye Group. Woodlake Environmental Field Station Annual Conference, Cleveland State University, oral research presentation, October 8, 2002.
- Stepien, C.A. 2002.** Using DNA data to understand fish stock structure in Lake Erie" presented to the Ohio Division of Wildlife and held at CSU, 30 minute seminar presentation followed by demonstration workshops. November 20, 2002.
- Stepien, C.A., and C.D. Taylor. 2002.** Population genetics and stock structure of walleye in Lake Erie. Workshop and seminar conference presentation on "Using DNA data to understand fish stock structure in Lake Erie" presented to the Ohio Division of Wildlife and held at CSU, November 20, 2002.

Stepien, C.A., C.D. Taylor, and D.W. Einhouse. **2003.** Analysis of genetic hybridization risk posed by fish stocking to a historic walleye spawning group. Ohio Division of Wildlife Poster presentation at annual meeting, Columbus, Ohio. Feb. 7, 2003.

Stepien, C.A. 2003. Population genetics of walleye in Lake Erie: Using DNA data for fishery Management. Presentation scheduled for Wed. Feb. 19, Lake Erie Walleye Task Force.

Stepien, C.A. 2003. Population genetics of walleye and stock structure using nuclear and mtDNA. Percis Symposium (Percid fishes), Madison, Wisconsin, July 2003.

Stepien, C.A., C.D. Taylor, Mark A. Tumeo, and D.W. Einhouse. **2003.** Analysis of genetic hybridization risk posed by fish stocking to a historic walleye spawning group. Invited symposium oral presentation for “Use of genetic markers for management and conservation”. American Fisheries Society annual meeting, Quebec City, Quebec. August 2003.

New Research Funding Stemming from this Project

U.S. Fish and Wildlife Service Restoration Act, Great Lakes Fishery Commission. Walleye population genetics and stock structure. (with Dr. Tim Johnson and Dr. Christopher Wilson, Ontario Ministry of Natural Resources and Dr. Brian Dixon, University of Waterloo), \$63,000. March 2003-4. *Collaborative fishes grant with Great Lakes federal and State Agencies.* U.S. Fish and Wildlife Service Restoration Act, Great Lakes Fishery Commission.

Grant in Review: Great Lakes Fishery Commission. Development and implementation of a high-resolution DNA data base for fishery management: Walleye and yellow perch stock structure. \$300,000/3 years.

Benefits to Lake Erie and the State of Ohio

This investigation provided fundamental data on the genetic variability and stock structure of walleye in Lake Erie, key to maintaining diversity for successful fisheries and ecological management. Preserving genetic variability of stocks and maintaining their habitats is important for ensuring diverse and resilient species for sustainable fisheries (Allendorf et al. 1987). Genetic diversity is believed to enable native species to inhabit a variety of environments and withstand perturbations, such as exploitation, habitat degradation, and effects of invading species. It is essential that we continue these efforts to analyze genetic diversity of the walleye at the present time and incorporate the results in our fisheries and environmental policies, including stocking programs, potential protection of some spawning areas, and procedures that help to maintain genetic variation. For example, our results to date show that the Ohio River native stock of walleye has been separated from populations in the Great Lakes for over a million years (Stepien and Faber 1998). Any future stocking of the Ohio River should thus comprise the native type.

The project provided data directly utilizable by the Ohio Division of Wildlife, the other Great Lakes State Departments of Natural Resources, the Walleye Task Group, the Great Lakes Fishery Commission, the Ontario Ministry of Natural Resources, the U.S. Fish and Wildlife Service, and the EPA. This research directly met the need and strategies the Ohio Division of Wildlife Strategic Plan 1995-2000, for managing Lake Erie walleye, to “Identify discrete spawning stocks and their relative contribution to the overall population”. It also met the primary goals of the Great Lakes Fishery Commission (Colby et al., 1994) “Walleye-Rehabilitation guidelines for the Great Lakes area”, specifically by defining and delineating stocks, developing stock-status indicators (i.e., genetic diversity), and providing data for evaluating stock recruitment (via contribution of spawning populations to open lake stocks). This investigation directly addressed the needs for defining stock structure in the annual reports of the Walleye Task Group (1998) of the Great Lakes Fishery Commission.

The results are valuable to management agencies for interpreting population fluctuations and delineating potential critical areas of genetic diversity in order to sustain fisheries. This research specifically produced data of the number and description of genetic types, their distributions, and their relative abundances in Lake Erie. It is also providing the link between contribution of spawning populations and fisheries.

This project benefited the public through providing data for maintaining a sustainable walleye fishery and determining which habitats are important to conserve. For example, our results have suggested that the spawning population of walleye in the Sandusky River tributary of Lake Erie houses a high number of unique genotypes of walleye (Stepien and Faber, 1998), indicating that it may be an especially important spawning habitat. Likewise Cattaraugus Creek spawning walleye in the Seneca Nation territory of New York is highly diverse and should be maintained for generations to come.

Abstract for ASIH talk presented July 2002

Genetic and Morphological Identity of the “Extinct” Blue Pike *Stizostedion glaucum*
versus walleye *S. vitreum*

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It has long been questioned in fisheries biology whether the blue pike *Stizostedion glaucum* was a separable species or an ecophenotypic variant of the yellow walleye *S. vitreum*. Morphological characters included the larger eyes and the smaller interorbital of *S. glaucum*. The blue pike was endemic to deeper areas of Lake Erie (and Lake Ontario) and supported a large commercial fishery. The fishery crashed, presumably due to pollution and/or overexploitation, and the blue pike was declared extirpated by the 1970s. The Stepien laboratory obtained the last known blue pike specimen caught by a commercial fisherman, Jim Anthony of Conneaut, Ohio, which has been stored frozen since 1962. We sequenced its mitochondrial control region (about 1200 bp) and the nuclear lactate dehydrogenase A6 intron (about 200 bp) and made comparisons with preserved specimens of the blue pike, possible relatives that were stocked in other lakes, and over 300 walleye. We also analyzed morphological characters, finding evidence for significant divergence between walleye and blue pike and indications that the Anthony specimen was aberrant (a possible hybrid). Sequence data also suggest that the blue pike was a separate species and that the Anthony specimen is significantly different from walleye. The Anthony specimen appears to have had a blue pike mother and possibly a walleye father. Modern specimens of blue-colored walleye from the Great Lakes and Canada are within the normal range of variation for walleye, and are not *S. glaucum*.

Abstract submitted and accepted for Symposium Presentation in August 2003 for the coming annual American Fisheries Society meeting in Quebec City

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Analysis of genetic hybridization risk posed by fish stocking to a historic walleye spawning group, using mtDNA control region sequences and nuclear microsatellites

Prior studies showed that walleye spawning groups are genetically distinguishable, apparently due to spawning site philopatry. This study investigates the genetics of a newly discovered spawning group (estimated as 2 to 4,000 individuals) in Cattaraugus Creek, a tributary in eastern Lake Erie. Unfortunately, its genetic composition may be altered by the 1995 through 2000 artificial stocking of 2.2 million fry and 44,000 fingerlings per year from Maumee River broodstock (introducing genotypes from western Lake Erie). We tested older spawning individuals (both males and females, whose ages pre-date the stocking) in comparison with younger individuals (who may represent returns of stocked individuals as well as offspring of the original genotypes). We sequenced the mitochondrial DNA control region (~1150 bp) and analyzed variation at 6 microsatellite loci. Results indicate that the historic Cattaraugus Creek spawning group is genetically divergent from other walleye in Lake Erie. Results to date suggest that younger individuals in Cattaraugus Creek are not significantly different from the older ones, indicating that the stocking had little overall effect. Hybridization among the original genotypes and the introduced Maumee River types thus does not appear to constitute a significant risk to the genetic integrity of the Cattaraugus Creek walleye spawning population.

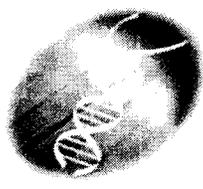
August 1, 2002
Seminar Presentation at
Stone Laboratory
Ohio State University

Unlocking the mysteries of Lake Erie fishes and invasive species using DNA clues



Great Lakes Environmental Genetics Lab
Center for Environmental Science,
Technology & Policy
Cleveland State University

Why DNA?



- The sequences of DNA in living organisms (and in some cases, even fossils) contain a record of the history of life.
- The instructions for the organism's structure, function, and reproduction are contained in DNA.
- These instructions have been modified over time.
- When we examine the code for organisms and compare it to their relatives, we can understand how it and the organisms have changed.

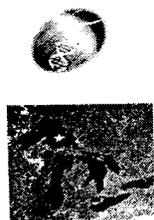
Usefulness of "Junk" DNA



- There is up to 95% of other code – sometimes called "junk" DNA – that is not currently used by that organism
- However, the junk DNA is inherited too – and some may be used again. Much of this junk DNA is freer to change/mutate
- Population geneticists usually find a better trail to track recent genetic history using this "garbage" DNA
- An example is DNA fingerprints – can be used to tell all persons apart easily with 100% accuracy

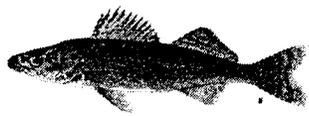
Today's Examples from Lake Erie

- 1. What does DNA tell us about our native fish? *How have their populations changed due to man's influences?*
- 2. What does DNA tell us about exotic species invasions? *Where did they come from and how are they changing?*



Mystery #1

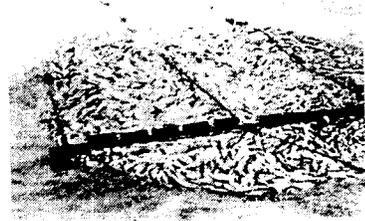
What was the Blue Pike?
Did it disappear? Are there any left?



Carol A. Stepien
Miles M. Coburn
Ted M. Cavender
Clifford D. Taylor

Conneaut, Ohio Blue Pike Fishery in the 1950's

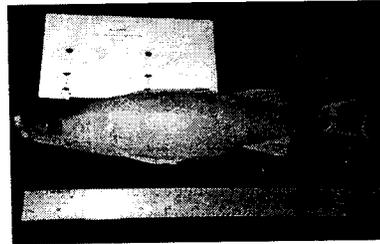
(courtesy of Jim Anthony)



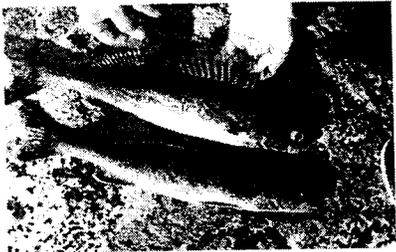
A blue pike processing plant in Conneaut, Ohio in the early 1950's.
(courtesy of Jim Anthony)



Preserved Blue Pike *Stizostedion glaucum*
Cleveland Museum of Natural History
Collected from Ashtabula, Ohio Lake Erie in 1896.
This specimen is being sequenced by the Stepien laboratory.



Comparison of blue and yellow walleye
McKim Lake, Ontario (Dr. Wayne Schaefer)



Frozen Asset

Ohio banker Jim Anthony gives a gift to Columbus — a rare blue pike he had kept in his freezer for 20 years.

When Jim Anthony gave a gift to Columbus — a rare blue pike he had kept in his freezer for 20 years — he was giving more than just a fish. He was giving a piece of history.

The pike, which was collected in Ashtabula, Ohio, in 1896, is now being sequenced by the Stepien laboratory at the Cleveland Museum of Natural History.

Anthony, a banker and avid fisherman, had kept the pike in his freezer since he caught it. He had never seen it before, and he was sure it was a new species.

The pike is now being kept at the Cleveland Museum of Natural History, where it will be studied and preserved for future generations.

Measurements of Anthony Blue Walleye/Blue Pike Specimen

1. Eye diameter: Interorbital width
Anthony specimen: 1.37
Mean for blue pike: 1.56 +/- 0.058
Mean for walleye: 1.32 +/- 0.057

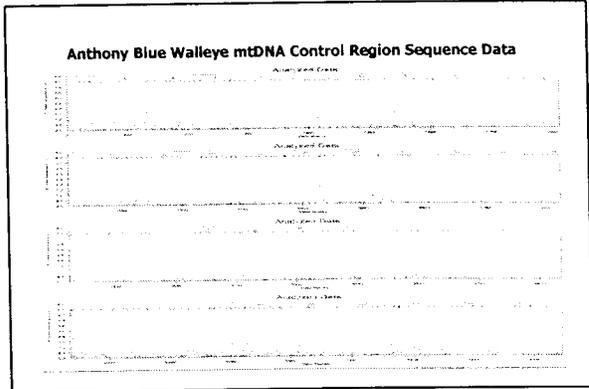
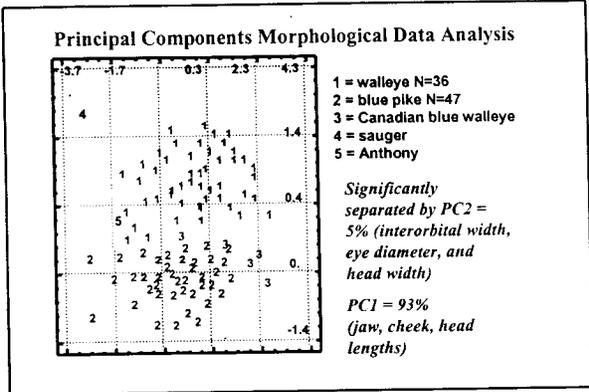
2. Upper jaw: lower jaw
Anthony specimen: 0.755
Mean for blue pike: 0.772 +/- 0.006
Mean for walleye: 0.792 +/- 0.005

3. *N* Vertebrae
Anthony specimen: 48 (high for both walleye and blue pike)
Walleye 44-48

Approach and Methods

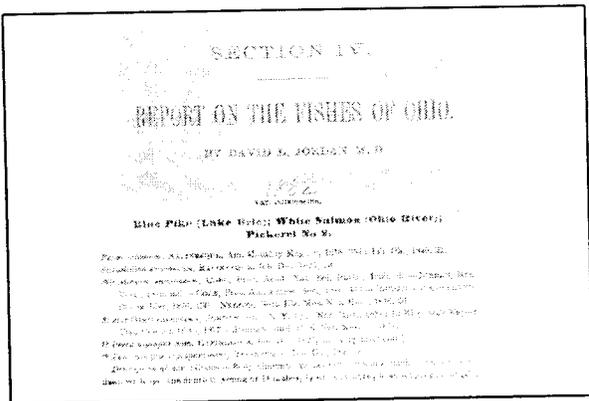
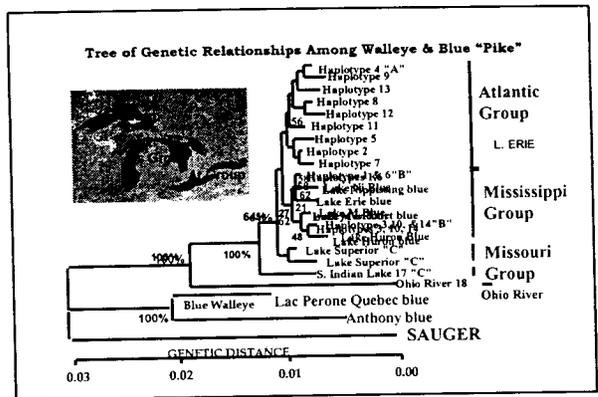


- Test Anthony blue walleye specimen and other blue walleyes for morphological and genetic comparisons
- Test known blue pike specimens (historic material – scales, old specimens in alcohol)
- Compare above with morphological and genetic data bases for yellow walleye and relatives



Distinguishing mtDNA Characters of some Blue Walleyes

Base Position	r0	r0	r0	r0	r1	1	2	4	5	5	5	7	8
Walleye	C	C	A	A	T	C	A	C	C	G	A	A	G
Anthony	G	G	G	G	C	A	G	G	T	A	T	T	C
Lac Perone	G	G	A	A	T	C	A	G	C	G	T	A	G



What does DNA say about the mystery of the Blue "Pike"?

1. The historic blue pike *Stizostedion vitreum glaucum* appears to have had some slight but significant morphological divergence from walleye *S. vitreum vitreum*.
2. Modern Blue-colored walleye are morphologically walleye, and not blue pike.
3. The Anthony specimen was not morphologically a blue pike, but appears to have been a hybrid. It was genetically quite different from walleye.
4. Historic samples of blue pike are being sequenced.

Other Fish Mysteries Solved Using DNA

- Fish "detective" work – unknown fillets for potential illegal import cases identified (imports from Europe of European pike perch and from Canada of sauger)
- Stocking problems – Separate out native walleye runs from stocked individuals (Cattaraugus Creek, New York)
- Identify spawning groups and their contribution to the open lake stocks



Mystery #2

Where do exotic species come from?
Why can't we get rid of them?
Why are they so successful?

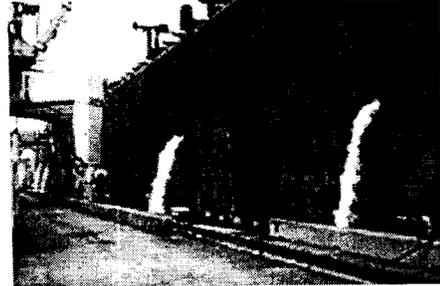


Carol A. Stepien
Clifford D. Taylor
Kora Dabrowska
Toriano Bownes

What's so Bad about Exotics?

- If they are successful, they usually spread uncontrollably.
- They compete with native species for food, space, & other resources.
- They disrupt & alter food chains, and may change the entire structure of the physical & biological habitats.
- They often carry parasites & pathogens.
- They often result in economic & habitat damage.

Aquatic Ballast Water Introductions



Are the Great Lakes Experiencing an Invasional Meltdown?

Invasions may be aided by:



1. An ecologically "young" system: Lots of vacant niches
2. Ecological disturbance: Loss of habitat, pollution
3. Facilitative interactions among invaders
(*ex: round goby eats dreissenid mussels*)

Genetic Structure of a Successful Invasive Population is Dependent on:

- 1. Number of individuals introduced
- 2. The genetic diversity of the source population(s)
- 3. The number of founding sources



Objectives

- 1. To develop species- and population-specific DNA markers to allow rapid specimen typing
- 2. To determine possible number and source locations for the nonindigenous populations.
- 3. To analyze variation within and among population sites, to test for a founder effect, mixing from several founding sources, etc.
- 4. To test for genetic changes over the time course of the invasions.



Dreissena polymorpha vs. *D. bugensis*

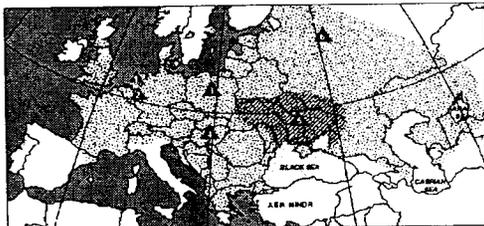


Zebra Mussel



Quagga Mussel

Eurasian Distribution & Sampling Sites



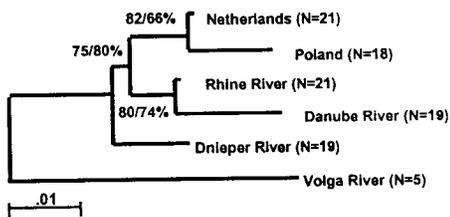
Distribution of *Dreissena polymorpha*
 Sympatric distribution of *D. polymorpha* and *D. bugensis*
 Collection Sites

Genetic Divergence of Zebra Mussel Populations

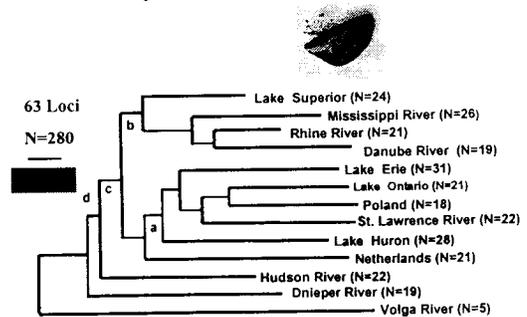
Variation	N. Amer.	Eurasia	Between N. A. & Eurasia	Overall
Ht	0.207	0.211	0.198	0.211
Hc	-----	-----	0.197	0.180
Gst	0.095	0.189	0.065	0.070
Nm	4.78	2.15	3.46	5.66
N sig. diff. loci	20	20	11	20
Mean Nei's D	0.029	0.060	0.032	0.032

Relationships Among European Zebra Mussels:

% = 1000 bootstrap replications (Parsimony/ NJ)



Relationships of Zebra Mussel Populations



What does DNA tell us about Zebra Mussels?

- 1. Exotic populations of zebra mussels have surprisingly high levels of genetic variability, suggesting large numbers of founding individuals and consistent with multiple colonizations.
- 2. Zebra mussels in North America likely were founded by multiple source populations from northwestern and northcentral Europe, but not southcentral or eastern Europe.
- 3. Sampling areas within North America were significantly divergent, with levels of gene flow and migration about twice that separating long-established Eurasian populations.

Other Mussels Mysteries We Solved Using DNA

- The quagga mussel was found to also have very high genetic variability
- The deep water profundal variant was determined a quagga and not a separate type or species
- Identification markers were developed for rapidly distinguishing species at all life history stages
- We examined the genetics of a living fossil relative – thought to be extinct for 10 million years – that was found alive in underground caverns in the former Yugoslavia.

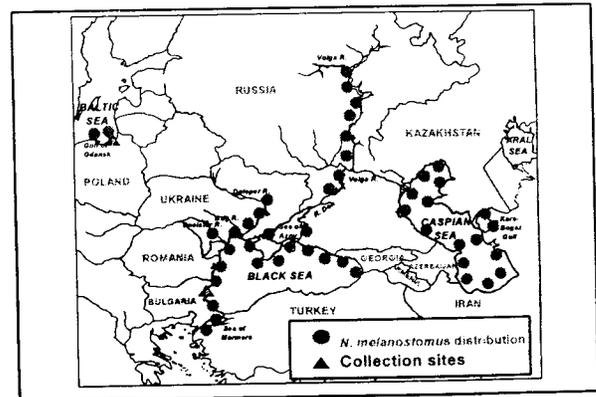
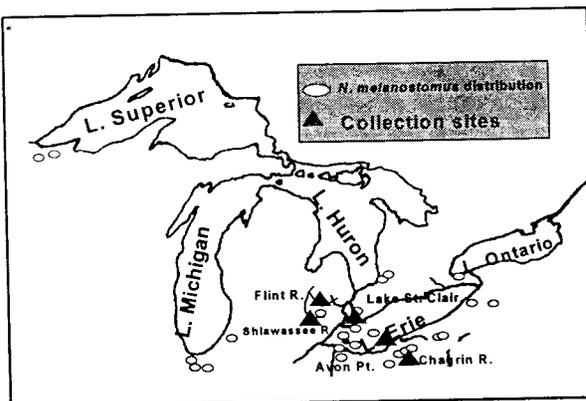


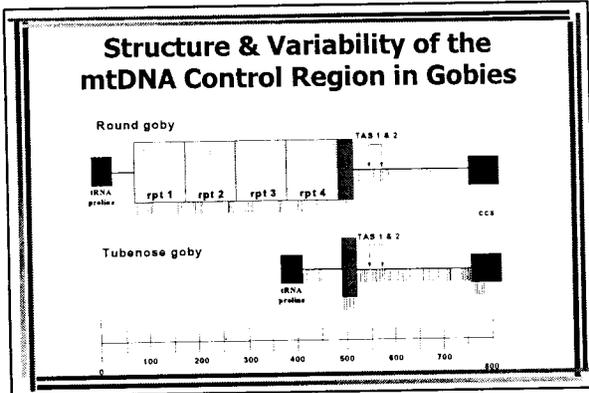
Another Mystery: Origin of Invasive Gobies

Round goby *Neogobius melanostomus*



Tubenose Goby *Proterorhinus marmoratus*



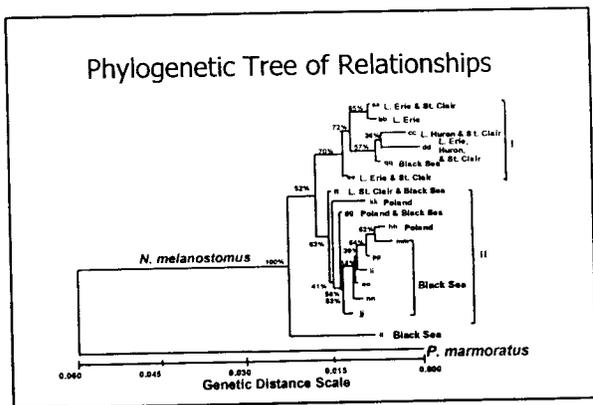


- ### Goby Species Comparisons
- No population genetic variation in North American samples of tubenose gobies- but some in Europe.
 - The round goby *Neogobius melanostomus* and the tubenose goby differ by about 5.2 mya.
 - We are developing quick identification markers for other related species that may invade

- ### Round goby Genetic Diversity Comparison
- Genetic diversity in the Black Sea polymorphism = 0.046
h = 0.96 +/- 0.04
 - Genetic diversity in North America polymorphism = 0.025
h = 0.84 +/- 0.08
 - Genetic diversity in the Gulf of Gdansk, Poland polymorphism = 0.014
h = 0.56 +/- 0.13
-

Distribution of Round Goby Haplotypes

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	N	
Lake Erie	9	3		2	6														20
Lake St. Clair	1		3	3	1	2													10
Lake Huron			5	5															10
Poland							7	3			1								11
Black Sea					2	1		2	2		1	1	1	1	1	1	1	1	13
N	10	3	8	10	7	4	8	3	2	2	1	64							



Modified Chi-Square Monte Carlo Tests for Differences Among Sampling Sites

	Lake Erie (N=20)	Lake St. Clair (N=10)
Lake St. Clair	$\chi^2 = 16.5$ $P < 0.001^*$	
Lake Huron (N=10)	$\chi^2 = 22.6$ $P < 0.001^*$	$\chi^2 = 5.0$ $P < 0.09$

~~What does DNA tell us about~~

Gobies

1. The round goby has relatively high variability in the mtDNA control region in both native and invasive populations. There is some new evidence that the invasive populations have become more variable over time (indicating new introductions)
2. There were marked differences among populations from the Black Sea, the Gulf of Gdansk, and the Great Lakes.
3. Neither the Great Lakes nor the Gulf of Gdansk populations appeared to have been introduced from the northern Black Sea. The Great Lakes and the Gulf of Gdansk populations were founded by different sources. We are now testing other possible founding sources and additional populations.
4. The tubenose goby is less variable than the round goby.

Thanks!



Grants:

- NOAA Sea Grant
- Ohio Sea Grant
- Lake Erie Protection Fund
- National Fish & Wildlife Foundation
- U.S. EPA

Samples:

- | | |
|---------------------|-------------------|
| Colin Adams | Vadim Panov |
| John Clay Bruner | Jeffrey Ram |
| Mary Burnham-Curtis | Gary Rosenberg |
| Thomas Busiahn | Svetlana Rudnicka |
| Renata Claudi | Mariusz Sapoto |
| Lynnie Corkum | James Selgeby |
| Ronald Dermott | Fredrig |
| David Garton | Simonovich |
| Jeffrey Gunderson | George Spangler |
| Bob Haas | Adrian Spidle III |
| John Hageman | David Stein |
| Anjie Hintz | David Strayer |
| Henk Jenner | Mike Thomas |
| Douglas Jensen | Bruce Thompson |
| David Jude | Christian Wiesner |
| Kevin Kayle | Ian Winfield |
| Carey Knight | Timothy Zak |
| Vladimir Kovac | |
| Yuriy Kvach | |
| J. Ellen Marsden | |
| Bernie May | |

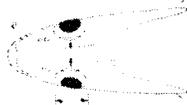
July 1, 2002
 ASIH Presentation
 Kansas City, MO.

Genetic and Morphological Identity of the "Extinct" Blue Pike versus Walleye



Carol A. Stepien
 Miles M. Coburn
 Ted M. Cavender
 Clifford D. Taylor

Morphological Features of Walleye vs. Blue Pike *The Fishes of Ohio (Trautman 1981)*

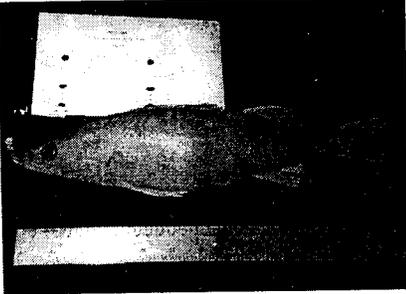
<p>Walleye <i>Stizostedion vitreum vitreum</i></p>	<p>BLUE PIKE <small><i>Stizostedion vitreum glaucum</i> (Hubbs)</small> <small><i>v. glaucum</i></small></p>	<p>Blue pike <i>S. v. glaucum</i></p>
		
		

Distinguishing characters of the blue pike (*Hubbs & Lagler 1964*)

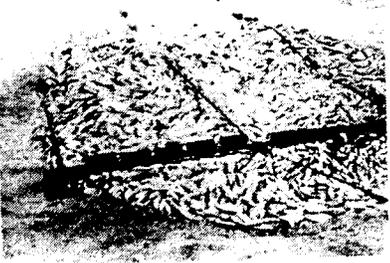


1. Body in life grayish blue, without brassy yellow mottlings.
2. Lower fins bluish white.
3. Eyes larger and set more closely (*bony interorbital width 1.4 to 2.0 X in length of the orbit*).
4. Dusky blotch on webbing between three last dorsal spines.

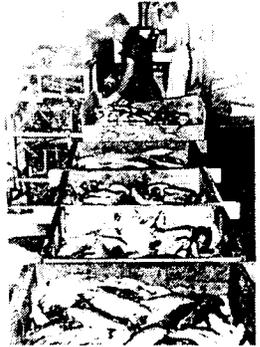
Preserved Blue Pike *Stizostedion glaucum* Cleveland Museum of Natural History Collected from Ashtabula, Ohio Lake Erie in 1896. *This specimen is being sequenced by the Stepien laboratory.*



Conneaut, Ohio Blue Pike Fishery in the 1950's (courtesy of Jim Anthony)



A blue pike processing plant in Conneaut, Ohio in the early 1950's (courtesy of Jim Anthony)



Blue walleye caught in McKim Lake, Ontario
Summer 2001
(Dr. Wayne Schaefer)



Comparison of blue and yellow walleye
caught in McKim Lake, Ontario (Dr. Wayne Schaefer)



The New York Times **ALEX**
MONDAY, MARCH 19, 2001

**In Angler's Freezer Since '62,
Fish May Retain 'Extinction'**

**Scientific bonanza
in family's freezer**

DNA of frozen blue pike from Lake Erie
studied to learn if species really is extinct

INSIDE

Disaster looms in Turkey

An Unusual Case of Angling

Wetland Leaps Off

Frozen Asset

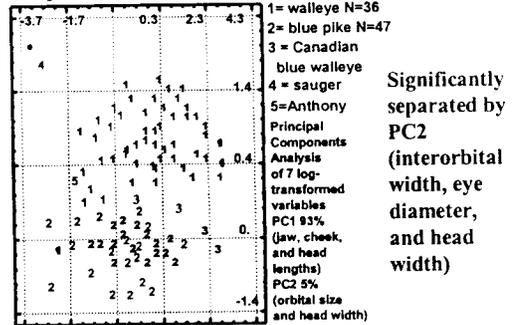
One member Jim Anthony spent a gift to science—
a blue pike he had kept in his freezer for 37 years

When Jim Anthony caught a blue pike in 1962, he had no idea that the fish would become a scientific treasure. The fish, which he kept in his freezer for 37 years, was recently found to be a unique genetic specimen, possibly representing a new subspecies of blue pike. The fish was found to have a unique combination of genetic markers that are not found in any other blue pike specimens that have been studied.

**Measurements of Anthony Blue
Walleye/Blue Pike Specimen**

1. Eye diameter: Interorbital width
Anthony specimen: 1.37
Mean for blue pike: 1.56 +/- 0.058
Mean for walleye: 1.32 +/- 0.057
2. Upper jaw: lower jaw
Anthony specimen: 0.755
Mean for blue pike: 0.772 +/- 0.006
Mean for walleye: 0.792 +/- 0.005
3. N Vertebrae
Anthony specimen: 48 (high for both walleye and blue pike)

Principal Components Morphological Data Analysis



**Genetic results from nuclear DNA
Ldh-A6 intron sequences**

- Anthony blue pike is more closely related to the blue walleye samples from Lac Perone, Quebec, but they are very genetically different from each other.
- The other blue walleyes tested are more closely related to yellow walleyes from Lake Erie and other areas.
- The degree of genetic divergence is less in the nuclear DNA than in the mtDNA, but both show similar relationships.



• What do we need to know? •

- 1. How different was “our” “extinct” blue pike in Lake Erie from yellow walleyes and the extant blue walleyes? •
- 2. Is it evolutionary convergence or close genetic relationship that regulates blue color/big eyes in some habitats? •
- 3. Are blue and yellow walleyes genetically and/or ecologically distinct?
- 4. Do blue and yellow walleyes hybridize? If so, under what conditions?

Analysis of Genetic Hybridization Risk Posed by Fish Stocking to a Historic Walleye Spawning Group



Carol A. Stepien and Clifford D. Taylor
 Great Lakes Environmental Genetics Lab, CESTP, CSU
 and Donald W. Einhouse
 NY Dept. of Environmental Conservation

What is Fish Stocking?

- Fish stocking is the artificial supplementation of hatchery-reared young to the population.
- One problem is that if the original population is not used as the source of the stock, foreign genotypes are introduced to the natural population.
- This mistake was made by stocking in Cattaraugus Creek, an eastern Lake Erie tributary.
- Our assignment is to assess the genetic characters of the native stock in comparison with returns of the stocked individuals (unknowns) and, in the future, with possible hybrid offspring.

Genetics of the Walleye *Stizostedion vitreum*



- Our work to date, as well as tagging data and ecological data, have indicated that there are significant differences in genotypic composition among spawning locations.
- These data appear to support spawning site philopatry (i.e., natal homing).
- Differences among populations in the Great Lakes (including sites in Lake Erie) have been maintained by this behavior since their founding after the Ice Ages.

Significant Differences Among Walleye Spawning Groups

Analysis of Molecular Variance (AMOVA) tests
 (Arlequin 2.0, 2001)

- Between Lakes St. Clair & Erie
 Variance=14.3% , $\Phi_{CT} = 0.131$
 $P < 0.001^*$
- Among Lake Erie Basins
 Variance= 7.0% , $\Phi_{ST} = 0.025$
 $P < 0.047^*$
- Among Spawning Sites within Basins in Lake Erie
 Variance= 7.5% , $\Phi_{ST} = 0.031$
 $P < 0.001^*$

Comparison: Significant Differences Among Walleye Spawning Groups

Modified χ^2 contingency table tests, with Bonferroni corrections for multiple post-hoc tests

Tests Among Spawning Locations:

- χ^2 between Lakes St. Clair & Erie = 17.3, $p < 0.001^*$
- χ^2 among 3 basins in Lake Erie = 21.4, $p < 0.001^*$
- χ^2 among Lake Erie sites = 18.7, $p < 0.001^*$

Controls:

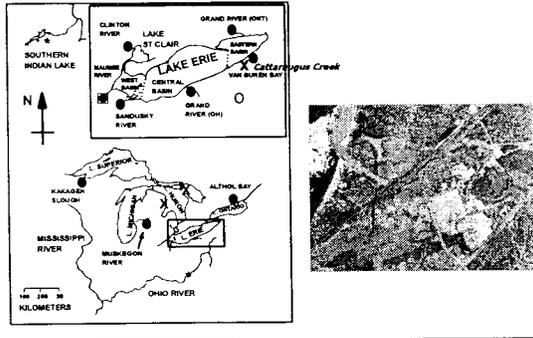
- χ^2 between males & females = 2.0, $p < 0.73$, N.S.
- χ^2 between years = 5.8, $p < 0.23$, N.S.

Sampling and Stocking Cattaraugus Creek by New York Department of Environmental Conservation



- Between 1995 and 2000, about 2.2 million 1-3 day-old fry and 44,000 fingerlings per year from Maumee River origin hatchery fish were stocked in Cattaraugus Creek.
- The objective of our study is to determine the impact (if any) on the genetics of the native fish spawning in Cattaraugus Creek. The native spawning population is estimated as 2-4,000.
- How unique are the originals? Have the genotypes changed? What is the risk of hybridization?

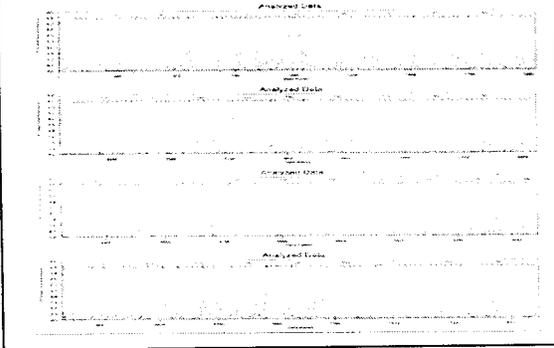
Walleye Spawning Population Study Sites



Methods

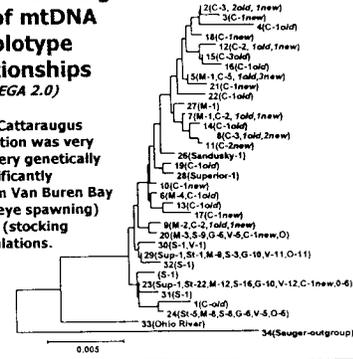
- Analyzed 1200 bp of the mtDNA control region in comparison with our data base of 300 walleye from the Great Lakes.
- Analyzed 3 nuclear microsatellite loci.
- Compared 20 old (pre-stocking) versus 20 young (stocked and non-stocked individuals). Also compared a nearby population (Van Buren Bay) and the stocking source population (Maumee).

Cattaugus Creek Walleye mtDNA Control Region Sequence Data

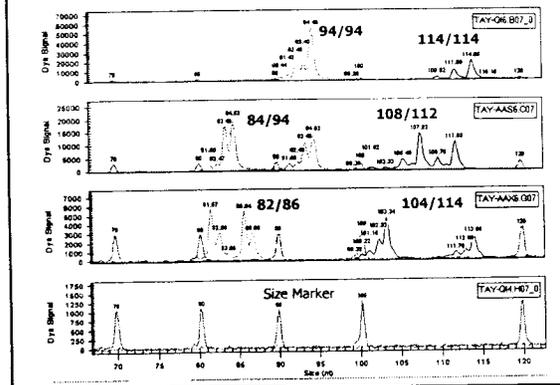


Neighbor-Joining Tree of mtDNA Haplotype Relationships (MEGA 2.0)

Conclusion: Cattaugus Creek population was very unique and very genetically diverse, significantly different from Van Buren Bay (nearby walleye spawning) and Maumee (stocking source) populations.



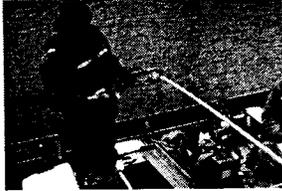
Two Microsatellite Loci (SVI33-green and SVI-4-black) in Walleye



Data from SVI33 microsatellite locus

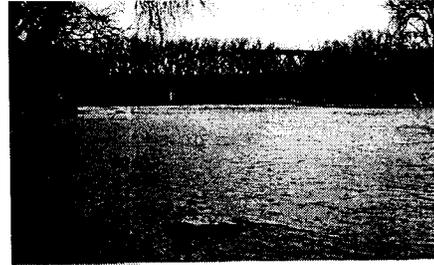
	80	82	84	86	88	90	92	94	96	98	100
Cattaugus Creek	1	5	7	10	1	3	13	18	2	4	3
<i>CC Old</i>	.015	.073	.103	.147	.015	.044	.191	.265	.023	.059	.044
N=34											
CC New	1	4	6	4	1	3	4	6	0	2	1
<i>CC New</i>	.031	.125	.188	.125	.031	.094	.125	.188		.031	.031
N=16											
Maumee River	0	1	1	6	0	0	9	13	2	2	2
<i>Maumee River</i>	.056	.028	.167				.250	.361	.056	.056	.056
N=18											
VanBuren Bay	0	1	2	1	1	2	5	5	0	1	2
<i>VanBuren Bay</i>	.050	.100	.050	.050	.100	.250	.250	.250		.050	.100
N=10											
VanBuren Bay	2	0	0	3	2	0	6	8	0	11	0
<i>VanBuren Bay</i>	.063			.094	.063		.188	.250		.306	
N=16											

**Conclusions to
Date:**
Cattaraugus Creek
Walleye



- The native Cattaraugus Creek spawning stock is very genetically diverse and should be maintained.
- The introduced Maumee spawning stock population is less genetically diverse.
- There is some preliminary indication that there are now some differences between the older fish and the younger ones (the latter which now represent Cattaraugus Creek and stocked Maumee origin walleye).
- Possible differences in the mtDNA versus the microsatellite data may be due to differential input of spawning males in the latter, rather than egg-laying females.

***Funded by the Ohio Sea Grant NOAA Program,
the Lake Erie Protection Fund,
the NY Department of Environmental Conservation,
and the Risk Analysis Program of CSU.***



Ingredients for PCR

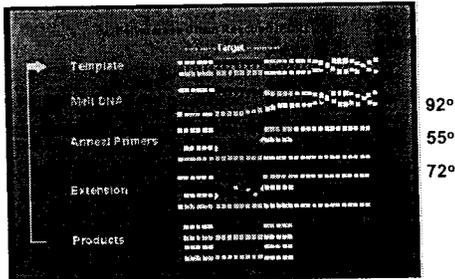
- 1. Template DNA
- 2. 2 primers (forward and reverse) that are about 18-24 bases long, made from conserved known DNA areas that border the target sequence
- 3. four deoxynucleotide triphosphates (dATP, dCTP, dGTP, dTTP) to build the chains
- 4. Taq DNA polymerase
- 5. A buffer

PCR: The Polymerase Chain Reaction Thermalcycler



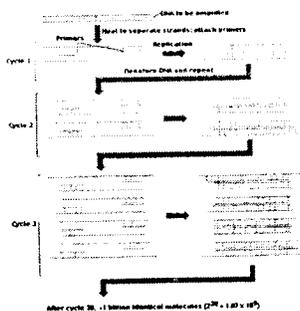
Changes temperature of the PCR reaction tubes according to the program.

PCR thermalcycler: repeats sets of three different temperatures for 35 to 40 cycles



A template sequence with 5 ATT repeats

PCR: makes multiple copies of target DNA



Microsatellite DNA

Microsatellites (or VNTRs = variable number of tandem repeats) are short segments of DNA that have a repeated sequence such as CACACACA, which occur in non-coding DNA.

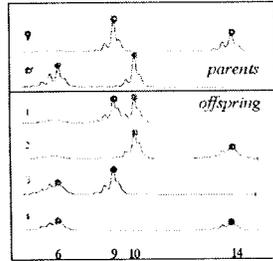
Microsatellites mutate rapidly and have no known function = "junk DNA".

These mutations are in the form of losses or gains of repeats. Individuals in a population typically possess microsatellite alleles of different numbers of repeat copies, having variable lengths.

CACACACACACACACACACA 10
CACACACACACACACACACA 11
CACACACACACACACACACACA 12
CACACACACACACACACACACA 13
CACACACACACACACACACACA 14

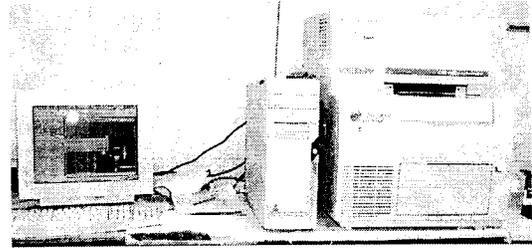
Inheritance of Microsatellites

CACACACACACACACA 9
 CACACACACACACACACACACA 14
 CACACACACACA 4
 CACACACACACACACACA 10
 CACACACACACACACACA 9
 CACACACACACACACACACA 10
 CACACACACACACACACACACA 14
 CACACACACACA 4
 CACACACACACACACACA 9
 CACACACACACA 4
 CACACACACACACACACACACA 14

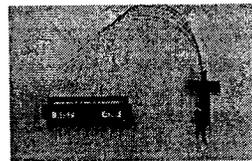
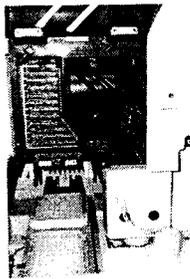


Diploid organisms (such as walleye and humans) each have 2 copies.

4. Run Microsatellite PCR Products on Auto Sequencer to determine their lengths



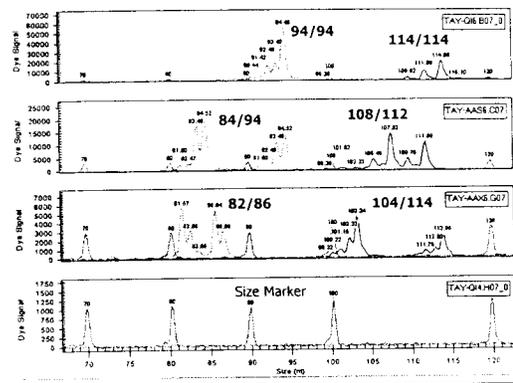
Capillary Auto Sequencer



Capillary Array

The autosequencer detects the length of the microsatellite repeat, using a laser to detect the dye-labelled primer.

Two Microsatellite Loci (SVI33-green and SVI-4-black) in Walleye



Populations (stocks) that are isolated diverge in microsatellite frequency lengths over time

	Pop A:	Pop B:
9	10%	1%
10	80%	35%
11	8%	47%
12	2%	17%

We assay several different microsatellite loci to test this hypothesis independently and statistically.

Another type of DNA Data is Sequence

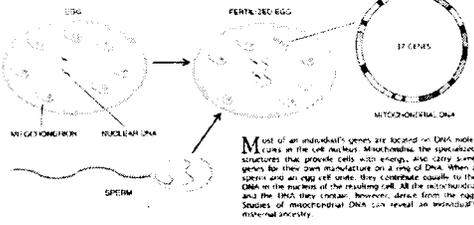
- Sequences are easiest to analyze with haploid (single copy) DNA
- Examples: Mitochondrial (mt) DNA, genes on X and Y chromosomes in males
- We use the same beginning steps: DNA extraction & PCR
- But now we determine the genetic code
- Mutations in the code are usually point mutations called Single Nucleotide Polymorphisms (SNPs)


```
GATCAAATCTA
GACCAAATCAA
```
- Other changes are insertions or deletions (Indels)


```
GATCAAATCTA
GATCAA- TTCTA
```

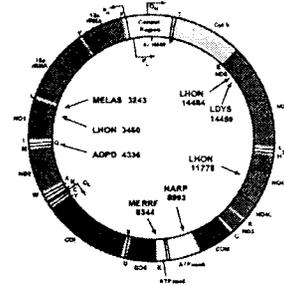
Mitochondrial DNA

The Inheritance of Mitochondrial DNA

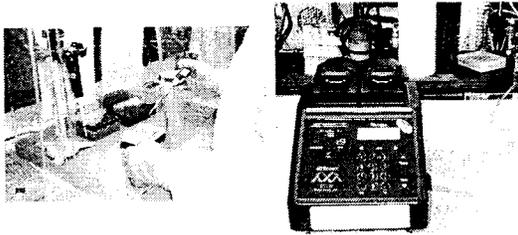


MtDNA

Maternally inherited
Circular
Independent from nuclear DNA
High mutation rate



3. PCR Amplify DNA using conserved primers that flank the target sequence



4. DNA sequencing Steps:

- Purify PCR product to get DNA template
- Set-up Cycle Sequencing Reaction
- Perform cycle sequencing reaction on thermalcycler
- Prepare the sequencer sample plate
- Run sequencing products on autosequencer

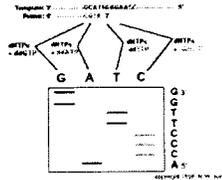


b. Set up Cycle Sequencing

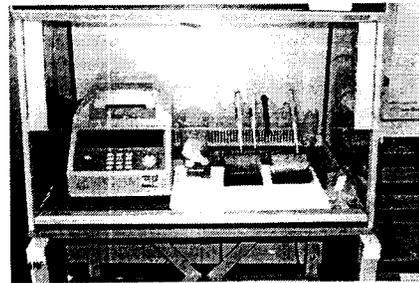


Add:
Purified template DNA
primer
dNTPs
Dye-labeled ddNTPs that will randomly terminate the reaction
DNA polymerase

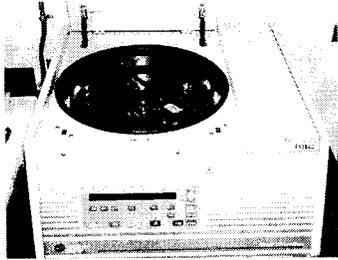
Sanger dNTP Chain Termination Sequencing



c. Do Cycle Sequencing Reaction on the Thermalcycler

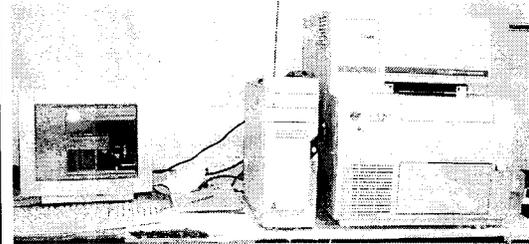


d. Preparing the DNA Template

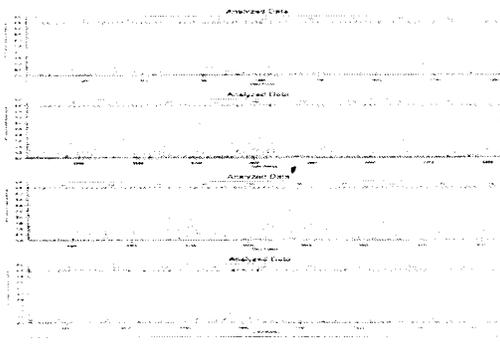


Precipitate and clean the Sequencing Products; Resuspend in buffer

e. Run Sequencing Products on Capillary Auto Sequencer



Walleye mtDNA Control Region Sequence Data



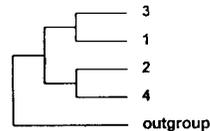
SNPs (Single Nucleotide Polymorphisms)

In this example, there are 5 different haplotypes:

- 1. GAATCGTTCACTGGGACATGATTACT
- 2. GAATTGTTCACTGGGACATGATTACT
- 3. GAATCGTTCACTAGGACATGATTACT
- 4. TAATCGTTCACTGGGACATGATTACT
- 5. GAATCGTTCACTGGGACATGACTACT

Ways in which DNA haplotypes yield information

- We can analyze the evolutionary pattern of the relationships among haplotypes, yielding a phylogenetic tree



- We can compare the frequencies of the haplotypes within and among populations

	Pop A:	Pop B:
1	10%	1%
2	80%	38%
3	8%	47%
4	2%	17%

5. Read and Analyze Data

- Two Primary Ways:
 1. Measure amount of variation within and among populations
 - a. calculate *F_{st}* values (measure of genetic divergence)
 - b. calculate genetic distances among populations
 2. Test Phylogenetic (evolutionary) relationships among genotypes (which ones are ancestral, which ones are more recently derived)

Population Genetics and Stock Structure of Walleye in Lake Erie

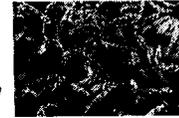
By Carol A. Stepien
 and
 Clifford D. Taylor



Great Lakes Environmental Genetics Lab

Center for Environmental Science, Technology and Policy
 Cleveland State University

Genetics of the Walleye *Stizostedion vitreum*



- Our work to date, as well as tagging data and ecological data, have indicated that there are significant differences in genotypic composition among spawning locations.
- These data appear to support spawning site philopatry (i.e., natal homing).
- Differences among populations in the Great Lakes (including sites in Lake Erie) have been maintained by this behavior since their founding after the Ice Ages.

What maintains these genetic differences?

Philopatry: Spawning Site Faithfulness

- Natal homing examples from studies of salmon, sea turtles, rainbow trout, many marine and freshwater fishes.
- They appear to track back to the sites using olfactory cues imprinted as larvae.
- Similar patterns in other Great Lakes fishes (glacial recolonizations, spawning site/group fidelity)
- Jennings *et al.* (1996): Evidence for heritable preference in spawning habitat among walleye groups (river vs. reef spawners)



Objectives

1. Delineate spawning & commingling lake stocks
2. Determine which spawning groups/locations are the most critical (rivers & reefs)
3. Develop baseline data sets to measure changes in genetic diversity over time, in the face of exploitation and irregular year classes and recruitment
4. Predict where individuals originated (spawning group)



Testing design



- Among Lake Erie Spawning sites (rivers, reefs)
- Among spawning years
- Among size classes (ages)
- Between the sexes
- Between Lake Erie and other Great Lakes (Lakes St. Clair, Michigan, Superior, Ontario) and also Ohio & Mississippi River Systems

Applied Example: Sampling and Stocking Cattaraugus Creek by New York Department of Environmental Conservation

Between 1995 and 2000, about 2.2 million 1-3 day-old fry and 44,000 fingerlings per year from Maumee River origin hatchery fish were stocked in Cattaraugus Creek.

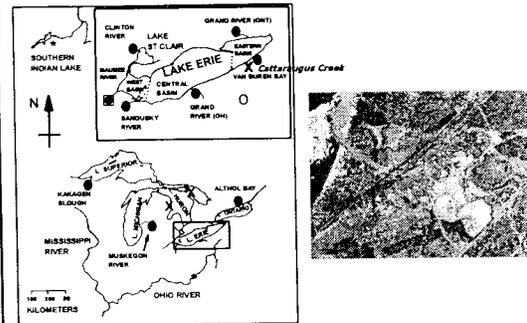
- The objective of our study is to determine the impact (if any) on the genetics of the native fish spawning in Cattaraugus Creek. The native spawning population is estimated as 2-4,000.
- How unique are the originals? Have the genotypes changed? What is the risk of hybridization?



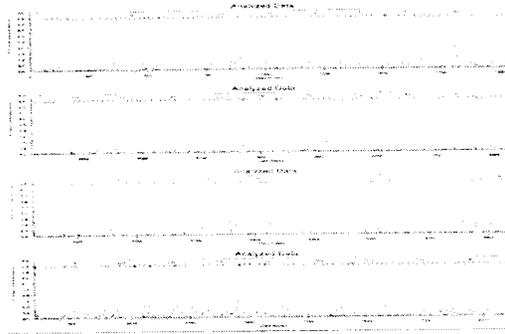
**Applied Example:
Methods for Catt Creek Study**

- Analyzed 1200 bp of the mtDNA control region in comparison with our data base of 300 walleye from the Great Lakes.
- Analyzed 2 nuclear microsatellite loci.
- Compared 20 old (pre-stocking) versus 20 young (stocked and non-stocked individuals). Also compared a nearby population (Van Buren Bay) and the stocking source population (Maumee).

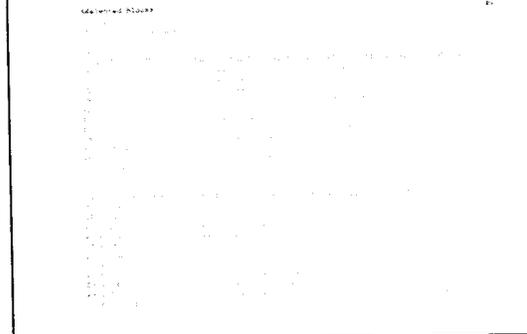
Walleye Spawning Population Study Sites



**Cattaraugus Creek Walleye
mtDNA Control Region Sequence Data**



Walleye Haplotype Relationship Data



**Significant Differences Among Walleye
Spawning Groups
with mtDNA sequences
Analysis of Molecular Variance (AMOVA) tests
(Arlequin 2.0, 2001)**

- Between Lakes St. Clair & Erie
Variance=16.7% , $\Phi_{CT} = 0.131$
 $P < 0.001^*$
- Among Lake Erie Basins
Variance= 8.7% , $\Phi_{ST} = 0.055$
 $P < 0.001^*$
- Among Spawning Sites within Basins
in Lake Erie
Variance= 7.5% , $\Phi_{ST} = 0.031$
 $P < 0.001^*$

**Comparison: Significant Differences Among
Walleye Spawning Groups
with mtDNA sequences**

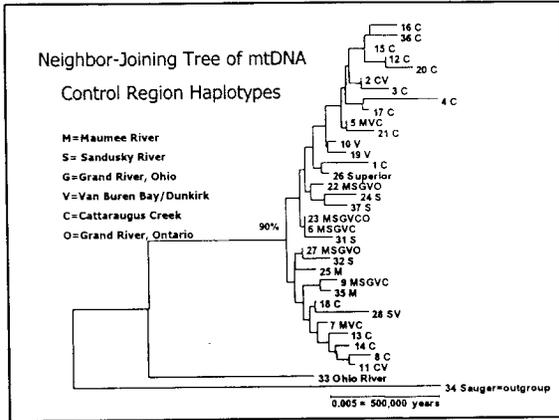
Modified χ^2 contingency table tests, with Bonferroni corrections for multiple post-hoc tests

Tests Among Spawning Locations:

- χ^2 between Lakes St. Clair & Erie = 17.3, $p < 0.001^*$
- χ^2 among 3 basins in Lake Erie = 21.4, $p < 0.001^*$
- χ^2 among Lake Erie sites = 18.7, $p < 0.001^*$

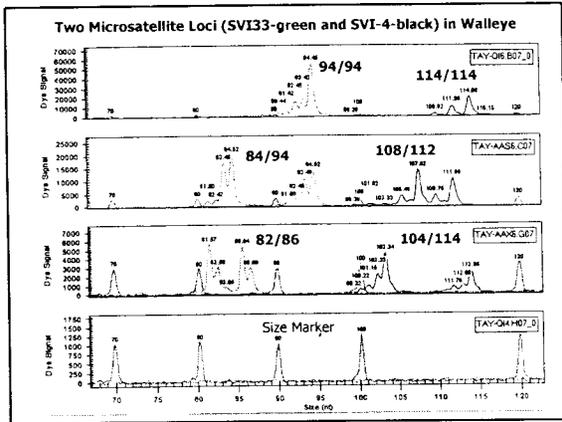
Controls:

- χ^2 between males & females = 2.0, $p < 0.73$, N.S.
- χ^2 between years = 5.8, $p < 0.23$, N.S.



MtDNA haplotype Frequencies per Site

Spawning Site	Haplotype									
	6	9	22	26	4	6	27	24,29 32,5	1,3,8, 12	2, 10, 11, 19
Catt Creek Total	5	3						5	15	9
Catt Creek Old	2	2						4	5	5
Catt Creek New	3	1						1	10	4
Maumee River	35%	6%	17%	19%	13%	4%		6%		
Van Buren Bay	32%	12%	8%	21%	7%	4%	2%			14%



Data from SVI-4 Microsatellite Locus in Spawning Populations of Walleye

	100	102	104	106	108	110	112	114	116
Maumee River N=31	1 .016	0	1 .016	2 .032	13 .209	2 .032	11 .178	25 .403	7 .113
Sandusky River N=32	1 .016	1 .016	2 .031	0	7 .109	5 .078	15 .234	26 .406	7 .109
VanBuren Bay N=28	0	1 .018	12 .214	0	12 .214	3 .054	11 .196	13 .232	4 .071
Cattaraugus Creek Old N=13	0	0	1 .038	1 .038	3 .115	4 .154	9 .346	8 .308	0

Yellow Vs. White= frequency differences between western & eastern basin

Data from SVI-4 Microsatellite Locus

	100	102	104	106	108	110	112	114	116	118
Cattaraugus Creek N=30	1 .017	0	7 .117	3 .043	9 .150	7 .117	13 .217	14 .233	4 .067	2 .033
CC Old N=13	0	0	1 .038	1 .038	3 .115	4 .154	9 .346	8 .308	0	0
CC New N=17	1 .029	0	6 .162	2 .059	6 .176	3 .088	4 .118	6 .176	4 .118	2 .059
Maumee River N=31	1 .016	0	1 .016	2 .032	13 .209	2 .032	11 .178	25 .403	7 .113	0

Yellow=most common alleles in each population
Catt Creek old, Catt Creek new, and Maumee River populations are all significantly different.

Data from SVI133 microsatellite locus

	80	82	84	86	88	90	92	94	96	98	100
Maumee River N=36	0	1 .014	7 .097	7 .097	5 .069	5 .069	15 .208	18 .250	1 .014	7 .097	6 .083
Sandusky River N=28	1 .017	3 .054	6 .107	13 .232	3 .054	5 .089	8 .143	6 .107	2 .036	6 .107	3 .054
Van Buren Bay N=35	3 .043	0	5 .071	8 .114	5 .071	1 .014	10 .143	18 .257	1 .014	16 .228	2 .029
Cattaraugus Creek Old N=15	1 .033	3 .100	6 .200	4 .133	1 .033	2 .067	4 .133	6 .200	0	2 .067	1 .033

Yellow=most common alleles in each population

Data from SVI33 microsatellite locus

	80	82	84	86	88	90	92	94	96	98	100
<i>Cattaraugus Creek</i> N=34	1 .015	5 .073	7 .103	10 .147	1 .015	3 .044	12 .176	20 .294	2 .029	4 .059	3 .044
<i>CC Old</i> N=15	1 .033	3 .100	6 .200	4 .133	1 .033	2 .067	4 .133	6 .200	0	2 .067	1 .033
<i>CC New</i> N=19	0	2 .053	1 .026	6 .157	0	1 .026	8 .211	14 .368	2 .053	2 .053	2 .053
<i>Maumee River</i> N=36	0	1 .014	7 .097	7 .097	5 .069	5 .069	15 .208	18 .250	1 .014	7 .097	6 .083

Yellow=most common alleles in each population

Conclusions to Date:
Cattaraugus Creek Walleye



- The native Cattaraugus Creek spawning stock is very genetically diverse and should be maintained.
- The introduced Maumee spawning stock was significantly different from the native Cattaraugus Creek stock. The Cattaraugus Creek stock is also significantly different from the Van Buren Bay reef spawning population.
- There is indication that there are some differences between the older fish and the younger ones (the latter which now represent Cattaraugus Creek and stocked Maumee origin walleye).

Where do we go?

- Large baseline data sets
- Use in monitoring
- What is most important?
- Cost/time
- Fastest/cheapest= ms analyses Goal=400 samples/ 2 weeks, 12 loci, Cost=\$25/fish Feasibility= within 1 year



