

**Genetic variability of yellow perch (*Perca flavescens*) in Lake Erie
from mitochondrial and nuclear DNA sequences**

Catherine M. Theisler

Principle Investigator: Carol A. Stepien

Department of Biology

Case Western Reserve University

Cleveland, OH 44106-7080

Abstract

The primary objective is to determine the levels of mitochondrial and nuclear DNA genetic variability and to assess the population genetic structure of the yellow perch *Perca flavescens* (Percidae: Teleostei) in Lake Erie. There have been few analyses of the population genetics of yellow perch and none have examined DNA sequences. The yellow perch is an environmentally important carnivore and comprises a top commercial and sport fishery. The second objective is to test whether there may be significant differences between yellow perch population groups in the western versus eastern basins of Lake Erie. We are sequencing the entire mitochondrial (mt)DNA control region (about 1100 bp) and the nuclear LDH-A6 (lactate dehydrogenase) intron (about 250 bp), which are both believed to be selectively neutral. Thirdly we are testing whether both the mtDNA control region and the LdhA6 intron reveal similar amounts of genetic variability, similar evolutionary rates, and if both are appropriate for addressing this problem. The data were analyzed with various evolutionary and population genetic statistics, including chi square tests to check for Hardy Weinberg equilibrium, heterozygosity tests, and nucleotide diversity, and divergence calculations. We found that DNA sequences from the mitochondrial DNA control region and the nuclear LdhA6 intron both reveal significant genetic variability in the yellow perch from Lake Erie. The data suggest that there are differences between yellow perch population groups in the western versus eastern basins of Lake Erie. The mtDNA revealed greater population variability in the western basin and the nuclear DNA was more variable in the eastern basin, suggesting possible sex differences.

Introduction

The yellow perch (*Perca flavescens*) (Fig. 1) has been native to the Great Lakes region since the early postglacial period, and is believed to have entered Lake Erie through the Maumee River from the Mississippi glacial

refugia about 10,000 years ago (Strittholt et al, 1988). The yellow perch today is widely ubiquitous to freshwater habitats in North America, and has been introduced in the United States as far west as California and as far south as the Florida panhandle. This spread of yellow perch is due to artificial stocking and illustrates their to thrive in a variety of temperatures and salinities (Scott and Crossman, 1973).

Yellow perch play an important role in the lake ecosystem food chain, comprising the diets of many larger fishes, including basses, sunfishes, crappies, walleye, sauger, and other yellow perch (Scott and Crossman, 1973). Recent invasions by exotic species, some of which are direct competitors (i.e., the ruffe *Gymnocephalus cernuus*) and others that detrimentally affect food chains (i.e., the dreissenid mussels *Dreissena polymorpha* and *D. bugensis* and the cladoceran *Bythotrephes*) have negatively affected yellow perch populations (Strittholt et al, 1988). The projected spread of the ruffe into the lower Great Lakes may threaten the abundant populations of yellow perch, rendering the present analysis of its genetic variation in Lake Erie critically timely and important (as a before-study).

The yellow perch provides a major economic asset to the Great Lakes community through commercial fisheries and sport fishing. Because of its size, schooling behavior, and migratory patterns during spawning, it is an easy catch for gill netters. The yellow perch catch is not seasonal and is the most abundant catch for ice fisherman. Over 3,628,720 metric tons of yellow perch are harvested from the Canadian border of Lake Erie, and restrictions on harvesting in the state of Ohio have restored populations for sport fishing (Bolsenga and Herdendorf, 1993).

In 1998 private anglers harvested 5.0 million yellow perch, which is a decrease of 5% in the charter boat harvest since 1997. Since 1994, sport harvests have shown a mean increase in the size of fish caught, indicating declining populations. Harvests by licensed trap net operators for Lake Erie yellow perch in 1998 totaled 436,207kg and their reported commercial harvest totaled 262,576 kg. The largest amounts of yellow perch were harvested in the spring, spawning months. These catches for 1998 show declines from previous years, suggesting that maintenance of yellow perch populations may become a concern for fishery management (Ohio Division of Wildlife, 1999).

The present study analyzes the amount and distribution of DNA sequence variability in yellow perch populations from Lake Erie. Data include sequences of the mitochondrial DNA control region (1100bp) and the nuclear Ldh-A6 (lactate dehydrogenase) intron (250bp). The present genetic study will test whether stock structure of the yellow perch in Lake Erie can be discerned and whether there is genetic variability among the lake basins. This study will also allow for fisheries management agencies to interpret population fluctuations and whether

habitats housing specific genotypes occur. This study may provide information about the relative reproductive success and genetic contribution of areas across Lake Erie. Appreciable amounts of variability in adult populations may suggest a history of continued reproductive success for many individuals. If success is concentrated in particular areas, studies of genetic differences among the sites may lead to insight about their historical success. It may be possible to augment the stocks of less genetic variability with genetically variable stocks by fishery management. It may also be possible to protect some key spawning populations.

Little or no allozymic variability was found in the yellow perch in studies by Leary and Boone (1982) and Strittholt et al.(1988). Studies on mtDNA using restriction endonucleases were successful in revealing some genetic variability (Billington, 1993). Comparison of previous results with our ongoing research, and data from the nuclear Ldh-A6 intron will provide a more accurate assessment of the levels and distribution of genetic variability in the yellow perch of Lake Erie.

Materials and Methods

Yellow perch specimens for our DNA sequencing study were collected by management agency contacts from eight different sites in Lake Erie (Fig. 2). The Lake Erie sites included South Bass Island, Sandusky, Fairport Harbor, and Geneva, in Ohio; Long Point Bay in Ontario; Erie and Presque Isle in Pennsylvania; and Dunkirk in New York. The samples were stored frozen at -80°C .

Samples were deep frozen in liquid nitrogen and ground with a cylindrical mortar and pestle. The DNA was extracted in guanidine thiocyanate buffer, and purified with proteinase K, RNase, PCI (phenol: chloroform: isoamyl alcohol), and chloroform. The DNA was precipitated in 95% ethanol and centrifuged into a pellet that was then isolated and dried. The dried precipitate was rehydrated in STE (sodium chloride Tris, EDTA) buffer, and run through a 1% agarose mini-gel to check for DNA quality, following standard protocols described in Stepien (1995).

The mtDNA control region (approximately 1150 bp) was PCR amplified in three sections, including the 3' or "right" domain and 5' or "left" domain, and the central conserved region. The right domain of the mtDNA was amplified with the light primer L16498 (5'-CAT-CTG-GTT-CCT-ACT-TCA-GG-3') and the heavy primer 12SARH (5'-ATA-GTG-GGG-TAT-CTA-ATC-CCA-GTT-3')(Faber and Stepien, 1997). The right domain of the control region was amplified using 40 cycles of 94°C for 45s, 50°C for 50s, and 72°C for 90s. The left domain was amplified with the light primer K16007 (5'CCC-AAA-GCT-AAA-ATT-CTA-A-3') and the heavy primer H16498 (5'-CCT-GAA-GTA-GGA-ACC-AGA-TG-3'). The amplification program for the left domain of the control region

was 38 cycles of 92°C for 40s, 49°C for 60s, and 72°C for 90s. The central conserved region was amplified with the light primer CCRL (5'-AAT-GTA-GTA-AGA-GCC-TA-3') and the heavy primer CCRH (5'-GGG-TAA-CGA-GGA-GTA-TG-3'). The central conserved region was amplified using 35 cycles of 94°C for 45s, 54°C for 30s, and 72°C for 90s.

The Ldh-A6 intron (about 250bp) was amplified using two primers, LDHA6F1 (5'-TAC-ACT-TCC-TGG-GCS-ATY-GGB-ATG-3'; S=G,C; Y=C,T; B=A,T,G) for the light chain and LDHA6R (5'-GCT-SAG-GAA-SAC-CTC-RTC-CTT-CAC-3'; S=G,C; R=A,G) for the heavy chain (J. Quattro, unpublished, pers. commun.). The Ldh-A6 intron was amplified with 38 cycles of 94°C for 45s, 51°C for 45s, and 72°C for 60s. All of the amplification programs ended with an extension step of 72°C for 5 min to ensure full polymerization of the chains (Stepien *et al.*, 1998).

Heavy chain primers were end-labeled with biotin (Hultman *et al.* 1989) and the DNA strands were separated using Dynabeads M-180 streptavidin (DynaL Corp., Oslo, Norway) for single strand sequencing (Hultman *et al.* 1989; Uhlen, 1989). Both strands were sequenced separately using diluted PCR primers with Sanger di-deoxy chain termination sequencing (Sanger *et al.* 1977), an S35 label and Sequenase vers. 2.0 kits (Amersham/U.S. Biochemical Corp., Cleveland, OH). Sequencing reactions were run on 6% polyacrylamide gels with a 1X glycerol tolerant gel buffer for two, five and eight hours in order to resolve approximately 600 bp from the primer. Gels were fixed in a 5% acetic acid/15% methanol solution and transferred to chromatography paper (Whatman International Ltd., Maidstone, England). Gels were vacuum dried at 80°C for 2 h and autoradiographed using BioWorld X-ray film (#0110114, Dublin, OH) for 72 h or longer.

Mitochondrial sequences were aligned with those of other yellow perch from Faber (1997). The *P. fluviatilis* mtDNA sequence from GenBank (Nesbo *et al.*, 1997) was aligned with that of *P. flavescens* from Faber (1997) to identify possible ancestral polymorphisms. The nuclear LDHA6 intron sequences were aligned with other yellow perch LDHA6 sequences from Dillon (1998). These alignments facilitated the identification of structural elements in the mitochondrial and nuclear DNA of yellow perch.

The haplotypic frequency was calculated for each haplotype of the mtDNA control region for all locations and then separately for the western and eastern basins. The proportion of polymorphic nucleotides (p_n), nucleotide diversity, and nucleotide divergence were calculated for both the mtDNA and nuclear LDHA6 intron for all samples, and then separately for the western and eastern basins. Haplotypic diversity(h) was calculated for the

mtDNA control region for all individuals in the population and for each basin. The LDHA6 intron was analyzed for heterozygosity (h)=gene diversity and nucleotide diversity (π) for the entire population and for the western and eastern basins. Conformance to Hardy-Weinberg equilibrium expectations was checked for the polymorphic alleles using a chi square test. All statistical methods followed equations in Nei (1987).

Results and Conclusions

Sequences from the mitochondrial DNA control region and the nuclear LdhA6 intron both reveal significant genetic variability in the yellow perch from Lake Erie. Six different haplotypes were found with nine polymorphic sites in the mtDNA control region sequence for the 17 individuals sampled (862 bp; Table 1). There were 9 polymorphic sites in the nuclear LdhA6 intron (194 bp; Table 4) for the nine individuals sampled. All polymorphisms in the nuclear DNA significantly conformed to Hardy-Weinberg equilibrium expectations, with $p < .05$ values (Table 4) allowing the assumption of neutrality (Nei, 1987). The variability discerned with DNA sequencing was significantly greater than that revealed by previous studies by other investigators using allozymes and whole mtDNA RFLPs (Billington, 1993). These findings indicate that this pilot study should continue with the sequencing of other individuals, in order to test for differences among sites, lake basins, lakes, and geographic areas in North America.

The mtDNA control region and nuclear LdhA6 intron data suggest that there are differences between yellow perch population groups in the western versus eastern basins of Lake Erie. Five of the 6 total mtDNA control region haplotypes occurred in the western basin. Four of the five haplotypes were common only in the western basin (Table 2). There were two mtDNA control region haplotypes in the eastern basin, of which one was only common in the east (Table 2). This haplotype distribution needs to be tested with greater sample sizes. There were two polymorphisms in samples from the western basin for the nuclear LdhA6 intron sequences, one of which was unique (Table 5). There were seven polymorphisms in samples from the eastern basin for the nuclear LdhA6 intron, six of which were restricted to the east (Table 5). These findings indicate possible stock structure differences between the western and eastern basins of Lake Erie, knowledge of which will provide important information for fisheries management. Additional tests, such as phylogenetic and stock structure, among other sites and with larger sample sizes will be done, based on these preliminary data sets.

The mtDNA control region and the LdhA6 intron reveal appreciable amounts of genetic variability, and further tests with the same samples and sizes are needed to compare differences among sites, shown in this pilot study. The

mtDNA revealed greater variability in the western basin and the nuclear DNA was more variable in the eastern basin (Tables 3 and 6). This may indicate a difference between the sexes and migration patterns. Use of both data sets together provides a robust approach for analyzing and comparing population genetic variability as contributed by both sexes since mtDNA control region is maternally inherited while the nuclear LdhA6 intron is biparentally inherited. These results show that further sampling and study of the genetics of yellow perch is worthwhile both for molecular biologists in studying genetic patterns across biogeographic areas, and also for fisheries management agencies studying exploitation pressures and historical parameters that may decrease genetic variability.

Acknowledgements

I would like to thank Dr. Carol Stepien for her expertise and advice in performing this research and giving me the opportunity to study with her. I would also like to thank Dan Dorosheff and Jennifer Skidmore for their time and help in showing me lab procedure and skills. Special thanks to Kevin Kayle of the Fairport Fish Station Ohio Division of Wildlife, Roger Knight of the Sandusky Fish Research Unit Ohio Division of Wildlife, and Roger Kenyon of the Pennsylvania Fish and Boat Commission for collecting the specimens. This study was supported by a grant award from the Lake Erie Protection Fund to Dr. Carol Stepien.

References

- Billington, N. 1993. Genetic variation in Lake Erie yellow perch (*Perca flavescens*) demonstrated by mitochondrial DNA analysis. *J. of Fish Biology*. 43:1-3.
- Bolsenga, S. J., Herdendorf, C. E. 1993. *Lake Erie and Lake St. Clair Handbook*. Detroit, Michigan: Wayne State University Press.
- Dillon, A. K. 1998. A genetic comparison of two exotic fishes-the ruffe (*Gymnocephalus cernus*) and the round goby (*Neogobius melanostomus*)-from Great Lakes versus Eurasian populations, M.S. thesis, Case Western Reserve Univ., Cleveland, OH.
- Faber, J. E. 1997. Population genetics, biogeography, and phylogenetics of walleye (*Stizostedion vitreum*), M. S. thesis, Case Western Reserve Univ., Cleveland, OH.
- Faber, J., Stepien, C. A. 1997. The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the percidae. In *Molecular systematics of fishes*, eds. T. D. Kocher and C. A. Stepien, pp.129-143. San Diego, CA: Academic Press.
- Hultman, T., Stahl, S., Hornes, E., and Uhlen, M. 1989. Direct and solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. *Nucleic Acids Research* 17: 4937-4946.
- Nei, M. 1987. *Molecular evolutionary genetics*. New York, NY: Columbia Univ. Press..
- Nesbo, C.L., Arab, M. and Jakobsen, K.S. 1997. Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid species *Perca fluviatilis*, *Acerina cernua*, and *Stizostedion lucioperca*. unpublished.
- Ohio Department of Natural resources, Division of Wildlife. 1998. *Ohio's Lake Erie fisheries, 1998*. Sport Fish Restoration Project, Sandusky, OH and Fairport Harbor, OH. F-69-P.
- Sanger, F., Nicklen, S., and Coulson, A. R. 1977. *DNA sequencing with chain-terminating inhibitors*. Proceedings of the National Academy of Sciences of the USA 74: 5463-5467.
- Scott, W.B., Crossman, E.J. 1973. *Freshwater Fishes of Canada*. Ottawa, Canada: Canadian Government Publishing Centre.
- Stepien, C. A., Dillon, A. K., and Chandler, M. D. 1998. Genetic identity, phylogeography, and systematics of ruffe *Gymnocephalus* in the North American Great Lakes and Eurasia. *J. Great Lakes Res.* 24(2): 361-378.
- Stepien, C.A. 1995. Population genetic divergence and geographic patterns from DNA sequences: examples from marine and freshwater fishes. In *Evolution and the aquatic ecosystem: defining unique units in population conservation*, ed. J. L. Nielson, pp. 263-287. Bethesda, MD: American Fisheries Society Symposium 17.
- Strittholt, J. R., Sheldon, I. G., and Wissing, T. E. 1988. Low levels of genetic variability of yellow perch (*Perca flavescens*) in Lake Erie and selected impoundments. In *The biogeography of the island region of western Lake Erie*, ed. J. Downhower, pp. 246-257. Columbus, OH: Ohio State University Press.
- Uhlen, M. 1989. Magnetic separation of DNA. *Nature* 340: 733-734.

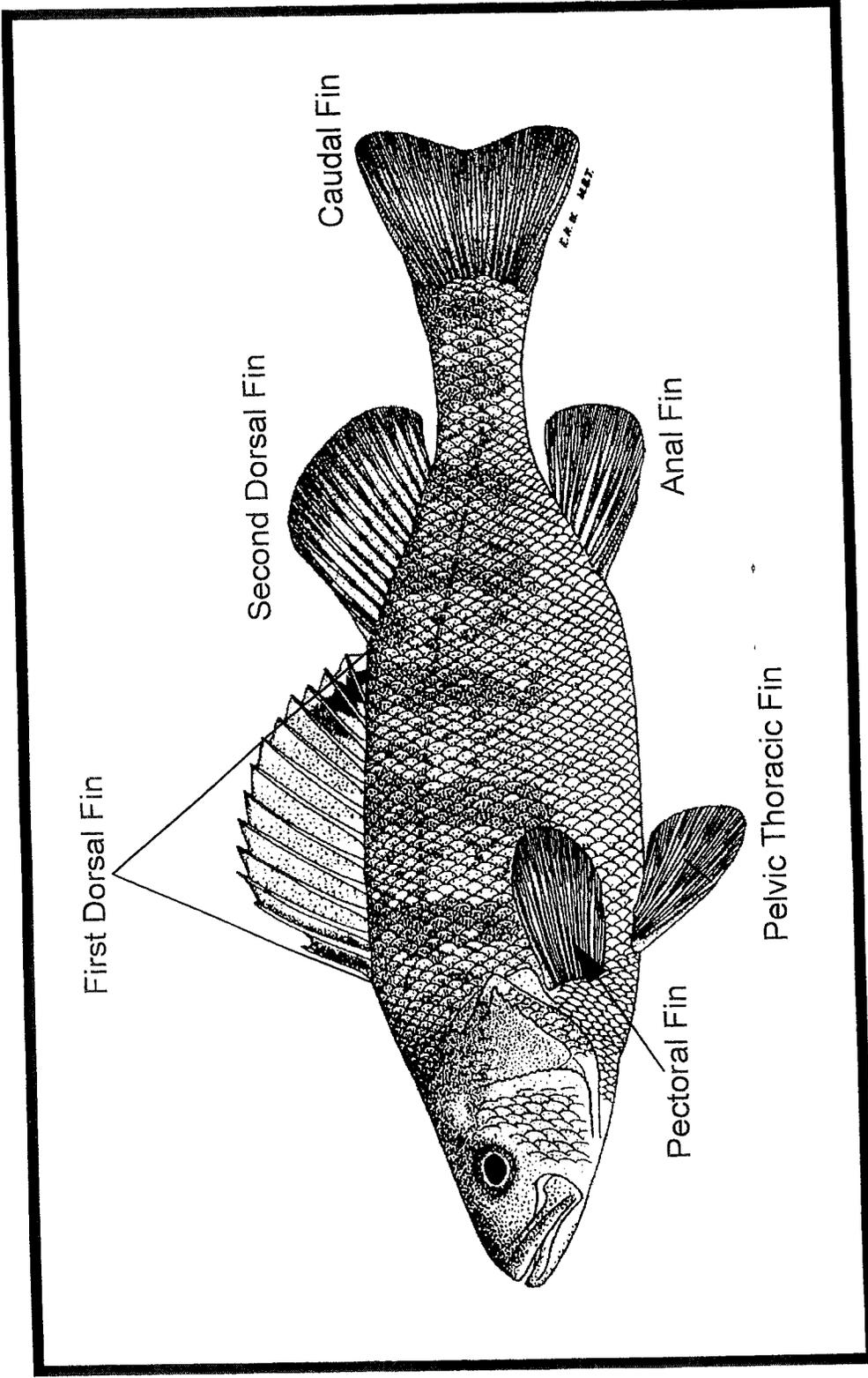


FIG. 1 Morphological characteristics of yellow perch

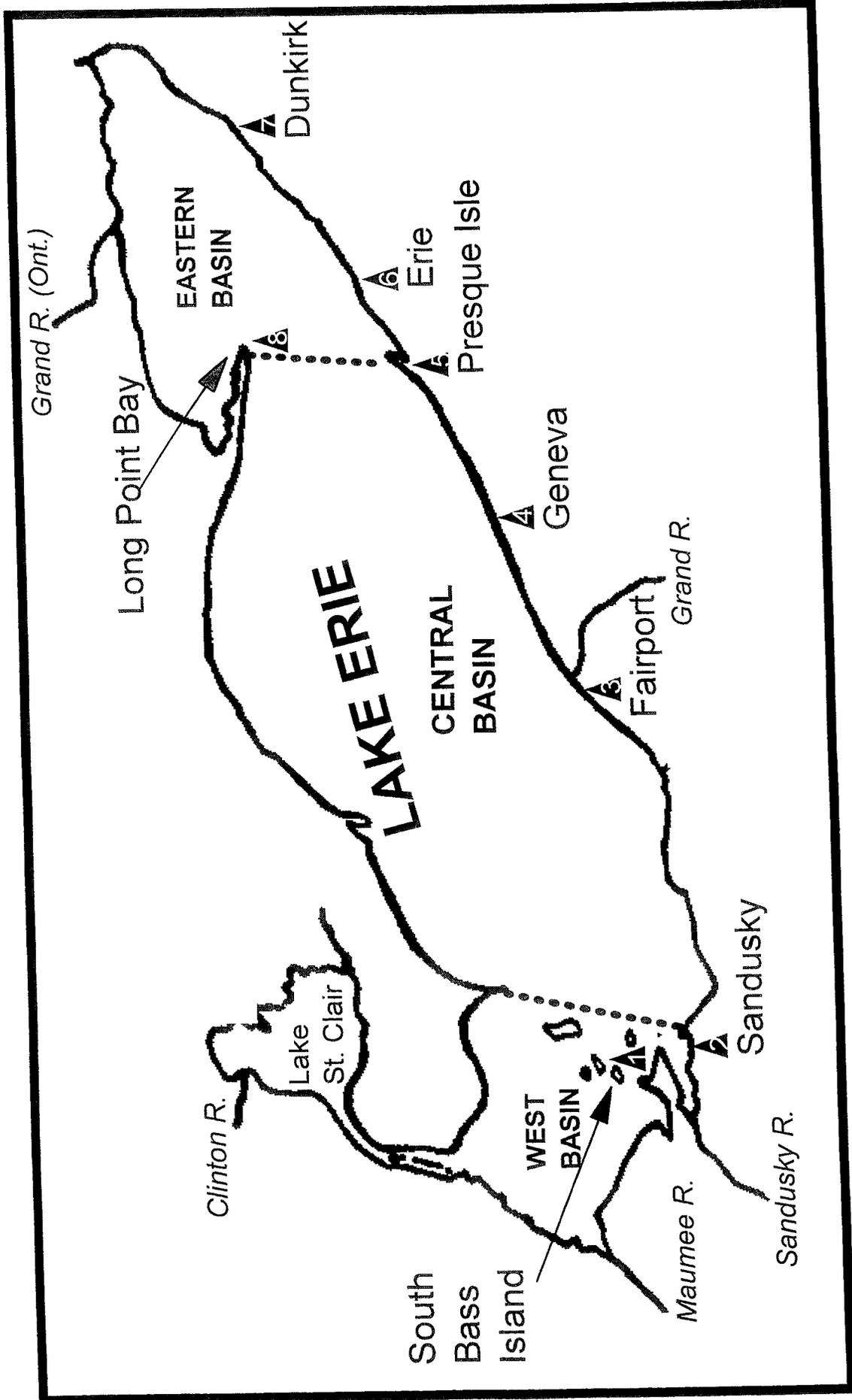


FIG. 2 Location of *Perca flavescens* sampling sites from Lake Erie

Table 1. Mitochondrial DNA Control Region Haplotypes of *Perca flavescens* in Lake Erie
(N=17 individuals).

Haplotype	Nucleotide Position										Total N	Haplotype Frequency
	448	453	459	520	524	593	713	721	723			
1	T	A	C	C	T	T	-	A	C		10	0.59
2	A	.	.		3	0.18
3	.	C		1	0.06
4	.	.	.	A	.	A	A	G	T		1	0.06
5	.	.	T		1	0.06
6	C		1	0.06
Ancestral (<i>P. fluviatilis</i>)*	T	A	C	C	T	T	A	C	C		109*	-

(*Resfeth et al, 1998)

Table 2.
Distribution of MtDNA Control Region Haplotypes in the Western
Versus Eastern Basin Populations of *Perca flavescens*

Haplotype	Western Basin	Eastern Basin	Total	Haplotype Frequency Western Basin	Haplotype Frequency Eastern Basin
1	7	3	10	0.54	0.75
2	3	0	3	0.23	0.00
3	1	0	1	0.08	0.00
4	1	0	1	0.08	0.00
5	1	0	1	0.08	0.00
6	0	1	1	0.08	0.25
Total N	13	4	17	1.00	1.00

Table 3.

Genetic Diversity Measures for mitochondrial DNA sequence variability in *Perca flavescens* from Lake Erie.

Population Genetic Measure	Overall (N=17)	Western Basin (N=13)	Eastern Basin (N=4)
% Polymorphism	0.010	0.008	0.001
Haplotypic Diversity (h)	0.64 +/- 0.01 s.e.	0.42 +/- 0.01 s.e.	0.43 +/- 0.03 s.e.
Nucleotide Diversity	0.064	0.061	0.035
Nucleotide Divergence	0.0007	0.0006	0.0001

Table 4.
Nucleotide Polymorphisms, Frequencies, and Variability in LdhA6 Intron Sequences
from *Perca flavescens* in Lake Erie.

Nucleotide Position	Number and Frequency of Homozygotes	Number and Frequency of Heterozygotes	Allelic Frequencies	Heterozygosity +/- s.e.	Nucleotide Diversity +/- s.e.	Hardy-Weinberg Equilibrium
061	8 TT 0.89	1 TA 0.11	0.94 T 0.06 A	0.116 +/- .010	.0145 +/- .0059	.033 .5<p<.9 (N.S.)
065	8 AA 0.89	1 AT 0.11	0.94 A 0.06 T	0.116 +/- .0098	.0145 +/- .00538	.033 .5<p<.9 (N.S.)
066	7 GG 0.78	2 GA 0.22	0.89 G 0.11 A	0.207 +/- .045	.0518 +/- .00242	.143 .5<p<.9 (N.S.)
068	8 TT 0.89	1 TG 0.11	0.94 T 0.06 G	0.116 +/- .0098	.0145 +/- .00538	.033 .5<p<.9 (N.S.)
078	7 TT 0.78	1 TG 0.22	0.89 T 0.11 G	0.207 +/- .045	.0518 +/- .00242	.143 .5<p<.9 (N.S.)
080	8 TT 0.89	1 TA 0.11	0.94 T 0.06 A	0.116 +/- .0098	.0145 +/- .00538	.033 .5<p<.9 (N.S.)
087	8 TT 0.89	1 TC 0.11	0.94 T 0.06 C	0.116 +/- .0098	.0145 +/- .00538	.033 .5<p<.9 (N.S.)
194	7 AA 0.78	1 AC 0.22	0.89 A 0.11 C	0.207 +/- .045	.0518 +/- .00242	.143 .5<p<.9 (N.S.)

Table 5.
Distribution of polymorphisms for the nuclear LdhA6 intron
Between the western and eastern basins of Lake Erie for *Perca flavescens*.

Nucleotide Position	Genotype Frequencies		Allelic Frequencies		Heterozygosities	
	Western Basin N=5	Eastern Basin N=4	Western Basin N=5	Eastern Basin N=4	Western Basin N=5	Eastern Basin N=4
061	4 TT 1 TA	4 TT 0 TA	0.90 T 0.10 A	01.0 T 0.00 A	0.24 +/- 0.06	0.00
065	5 AA 0 AT	3 AA 1 AT	01.0 A 0.00 T	0.88 A 0.12 T	0.00	0.23 +/- 0.02
066	4 GG 1 GA	3 GG 1 GA	0.90 G 0.10 A	0.88 G 0.12 A	0.24 +/- 0.06	0.23 +/- 0.02
068	5 TT 0 TG	3 TT 1 TG	01.0 T 0.00 G	0.88 T 0.12 G	0.00	0.23 +/- 0.02
078	5 TT 0 TG	2 TT 2 TG	01.0 T 0.00 G	0.75 T 0.25 G	0.00	0.40 +/- 0.01
080	5 TT 0 TA	3 TT 1 TA	01.0 T 0.00 A	0.88 T 0.12 A	0.00	0.23 +/- 0.02
087	5 TT 0 TC	3 TT 1 TC	01.0 T 0.00 C	0.88 T 0.12 C	0.00	0.23 +/- 0.02
194	5 AA 0 AC	2 AA 2 AC	01.0 A 0.00 C	0.75 A 0.25 C	0.00	0.40 +/- 0.01

Table 6.

Genetic Diversity Measures for nuclear LdhA6 sequence variability in *Perca flavescens* from Lake Erie.

Population Genetic Measure	Overall (N=9)	Western Basin (N=5)	Eastern Basin (N=4)
% Polymorphism	0.046	0.010	0.036
Heterozygosity (h) = Gene Diversity	0.006 +/- 0.001	0.002 +/- 0.001	0.010 +/- 0.001
Nucleotide Diversity	0.017	0.007	0.015
Nucleotide Divergence	0.0002	0.0001	0.0002