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ILLINOIS NATURAL HISTORY SURVEY

Identification of Parental Strains of Lake Trout Eggs Captured During Spawning in Lake Michigan

Final Report
to
U.S. Fish and Wildlife Service Fishery Resources Office

Center for Aquatic Ecology

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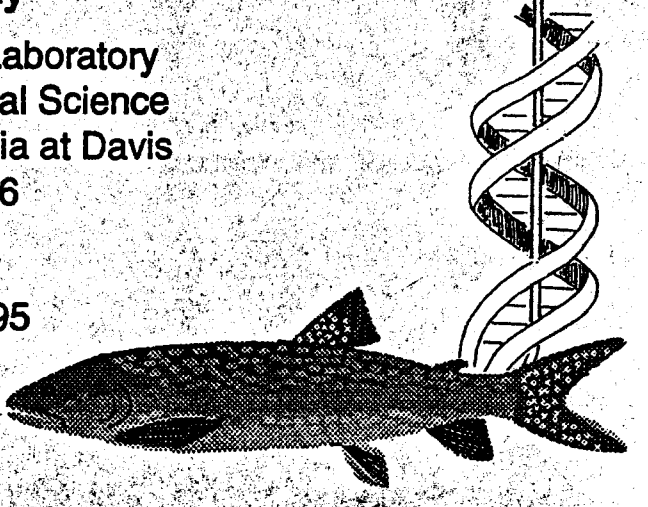
October, 1995



Aquatic Ecology Technical Report 95/12

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Introduction

Native lake trout (*Salvelinus namaycush*) were extirpated from Lake Michigan in the late 1950's. Prior to that time they provided a valuable sport and commercial fishing industry for the four states bordering the lake. The decline and eventual extinction of lake trout has been attributed to a combination of overfishing, predation by sea lamprey (*Petromyzon marinus*), and degradation of habitat. A program to restore a self-perpetuating population of lake trout to Lake Michigan has been underway since 1965. In the past decade, up to five million lake trout fingerlings and yearlings have been stocked in the lake each year. The survival of lake trout after stocking has been adequate to develop an important sport fishery in the lake. Assessment surveys conducted by the Illinois Department of Conservation, Wisconsin Department of Natural Resources, and the U. S. Fish and Wildlife Service have found aggregations of mature lake trout at several sites around the lake. The first evidence of spawning by stocked lake trout was documented in Grand Traverse Bay and the southeastern shoreline in the 1970s (Peck 1979, Jude 1981, Wagner 1981). However, since that time little evidence of spawning has been found in Lake Michigan, despite extensive efforts to find eggs at Clay Banks and Julian's Reef on the west side of the lake. In 1992 we collected eggs at several sites along the southwestern shore, and in the spring of 1993 through 1995 we collected hatched fry from a breakwall on the Indiana shore (Marsden 1994).

The goal of the fisheries agencies involved in lake trout management is to reestablish naturally reproducing lake trout populations. The optimal strategy to accomplish this goal would be to stock fish from the same genetic strain(s) that were once present in Lake Michigan. However, only two remnants of the original Lake Michigan genetic strains exist: the Lewis Lake strain apparently originated from gametes collected in northern Lake Michigan in the late 1800s (Krueger et al. 1983), and Green Lake strain contains genetic material from trout from southern Lake Michigan (Kincaid et al. 1993). Both of these strains have been stocked in Lake Michigan.

The key to successful stocking for rehabilitation is to identify the strains which successfully reproduce, and focus stocking effort on those strains. This objective requires acquisition of genetic data from potential parental strains and wild fry produced in Lake Michigan, and analysis of the data using second generation mixed stock analysis to identify the lineages of the fry (Marsden et al. 1989). In previous work in Lake Ontario, we determined that no detectable strain-specific selection takes place between the egg and fry stages in the wild, so eggs, which are much easier to collect than fry, can be used to obtain genetic data in place of fry (Grewe et al. 1994). The purpose of this study was to use the second-generation mixed-stock analysis method to determine the parental strain origins of wild-spawned lake trout eggs collected in southern Lake Michigan in 1994.

Methods

Sample collections

Naturally spawned lake trout eggs were collected from the outer west side of the Port of Indiana breakwall using egg bags (Perkins and Krueger 1994). A total of 742 eggs were collected in 9 egg bags. 502 of the eggs were still viable after collection and were incubated in the hatchery facility at the Lake Michigan Biological Station of the Illinois Natural History Survey. Hatching rates and fry survival were reduced by two uncontrolled water shut-downs; 135 fry were eventually frozen in liquid nitrogen for genetic analysis. All samples were shipped on dry ice to the Cornell Genome Variation Analysis Facility and stored at -80^{o}C.

Allozyme analysis

Genetic analysis was performed using horizontal starch-gel electrophoresis of allozymes (May 1992). An initial screening of 102 loci revealed polymorphisms at 18 loci in lake trout; the tissues, enzymes, and buffers used are described in Krueger et al. (1989). All fish in this study were examined at these 18 polymorphic loci. The loci examined were aspartate aminotransferase 2.6.1.1 (AAT-1,2*), acid phosphatase 3.1.3.2 (ACP-1*), fumarase 4.2.1.2 (FH-1,2*), glucose-6-phosphate isomerase 5.3.1.9 (GPI-1*), glycerol-3-phosphate dehydrogenase 1.1.1.8 (G3PDH-1*), L-lactate dehydrogenase 1.1.1.27 (LDH-B3*), malate dehydrogenase 1.1.1.37 (sMDH-B3,4*), malic enzyme 1.1.1.40 (mMEP-2*), peptidase with phenyl-alanyl-proline 3.4.11-13 (PEP-PAP-1,2*), phosphoglycerate kinase 2.7.2.3 (PGK-1*), phosphoglucomutase 5.4.2.2 (PGM-2* and PGM-3,4*), and superoxide dismutase 1.15.1.1 (sSOD-2*). Allozyme nomenclature follows the system of Shaklee et al. (1990). Allozyme analysis of samples of lake trout from each of the strains stocked into Lake Michigan had previously been conducted in the same lab (Krueger et al. 1989, Kincaid et al. 1993).

Parental strain identification

Parental strains of the fry were identified using second-generation mixed-stock analysis (Marsden et al. 1989). This analysis employs the method developed by Grant et al. (1980). Second-generation analysis estimates the strain composition (i.e., proportion of pure-strain and hybrid fry types) in a mixture of F_1 individuals. The strain composition of the parental population can then be derived from these estimates (Marsden et al. 1989). The earliest maturing lake trout are usually 4-year old males, although mature 3-year old males are occasionally seen. If we disregard the minimal contribution from 3-year old mature males, then a given year class of eggs or fry would primarily be produced by fish stocked as yearlings at least three or more years previously. Thus, fish stocked through 1990 could have produced fry year classes prior to 1995. Strains which could have contributed to the fry were the Clearwater, Superior, Seneca, Manitou, Jenny, Jenny x Lewis, Lewis, and Green Lake strains (Table 1). The Lake Michigan "strain", which was produced using gametes from feral fish and is therefore a mixture of strains, was first stocked in 1983 and comprised 0.08% of the fish stocked through 1990. We did not include this "strain" in the baseline data because it is likely genetically similar to one or more of the pure strains and would confound the model. Any contribution of this "strain" to the fry will most likely be attributed to the strain or strains which were primary contributors to the "Lake Michigan strain". For the same reason we did not include the Lake Ontario "strain", which was stocked first in 1990 and comprised 0.29% of the total stockings through 1990. We did not have baseline genetic data from the pure Jenny and Lewis lake strains, but only the Jenny x Lewis lake cross. Contributions from the Jenny and Lewis pure strains will likely be clustered with the Jenny x Lewis cross. The Green Lake strain has had a checkered history, described in detail by Kincaid et al. (1993). Fish stocked prior to 1977 were progeny of the original Green Lake strain, of which the 84-DOM group (Kincaid et al. 1993) is the closest remaining descendent. Kincaid et al. also sampled four groups of Green Lake strain fish (86A-WILD, 86B-WILD, 87-WILD, and 88-WILD) which were hatchery-reared progeny of feral fish which bore fin clips identifying them as Green Lake strain. These fish are most closely related to the Green Lake strain fish stocked after 1988. We ran the mixed-stock analysis twice, once using the 84-DOM group and once using the combined WILD groups in the baseline samples.

The accuracy of mixed-stock analysis is directly related to the magnitude of genetic differences between the baseline samples (heterogeneity of stocks). To predict how well the mixed-stock analysis model should be able to differentiate component fry types, we used allozyme data with Nei's index (Nei 1972) to calculate genetic distances between the parental strains. A dendrogram was constructed based on the Unweighted Pair Group Method of Analysis (UPGMA) cluster analysis using the 18 polymorphic loci (Figure 1).

Results

Three of the 18 loci examined were monomorphic in the wild fry: malate dehydrogenase (MDH-1), phosphoglucokinase (PGK-1), and superoxide dismutase (SOD-1). Allelic frequencies for the 15 polymorphic loci are given in Table 2.

Mixed stock analysis using both the domestic and wild Green Lake strains indicated that almost 50% of the parental population was Superior strain, 22-26% was Seneca strain, and the remaining sizable contribution (20-27% was from the Green Lake strain (Table 3). However, the actual composition of the fry population was different in each analysis. The primary fry types when the wild Green Lake strain was used in the baseline were Green x Superior and Seneca x Superior hybrids, whereas when the domestic Green Lake strain was used, the primary fry types were pure-strain Superior, Green x Seneca, and Seneca x Superior hybrids; the contribution of the latter hybrid was not significantly different from zero.

The UPGMA analysis indicated that, of the potential parental strains, the wild Green Lake and Superior strains were the most genetically similar (genetic distance = 0.003; Figure 1; see discussion in Kincaid et al. 1993). Therefore, we can predict that the mixed-stock analysis model will have the most difficulty resolving fry produced by these strains. This similarity likely accounts for the large proportion of fry attributed to Green x Superior hybrids in the analysis using wild Green Lake strain, which were attributed to pure-strain Superior in the analysis using domestic Green Lake strain.

Discussion

The majority of lake trout stocked into Lake Michigan prior to 1990 were from the Lake Superior strain - at least 82% (the strain composition of the fish stocked by the University of Wisconsin - Milwaukee is unknown, and may have contained Superior strain). Six percent of the remaining trout were Green Lake strain, Seneca and Clearwater strains comprised 1.3 and 1.4%, and Jenny, Jenny x Lewis, and Manitou strains each comprised less than 0.5% (Table 1). The sizable contribution of the Green and Seneca Lake strains to the fry is therefore somewhat surprising. However, the Seneca strain has a history of successful reproduction in Lake Ontario which is out of proportion with the numbers stocked (Marsden et al. 1989, Grewe et al. 1994). A priori, we would also predict that the Green Lake strain might be unusually successful in Lake Michigan, as this strain contains the only surviving remnant of the original Lake Michigan lake trout genome (Kincaid et al. 1993).

Before these results can be used to dictate changes in the stocking ratios of lake trout strains in Lake Michigan, it would be wise to examine wild-spawned eggs or fry from either different locations, subsequent years, or both, to confirm that the reproductive success of the Green and Seneca Lake strains is consistent over time and at different spawning locations (Grewe et al. 1994). Unfortunately, the parental origins of the fry will become increasingly difficult to resolve as the contribution of the Lake Michigan "strain" to the stocked population increases.

Acknowledgments

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Table 1. Lake trout strains stocked into Lake Michigan prior to 1990. Lake Michigan strain fish were the progeny of fish stocked in the lake, and were likely a mixture of strains. WI-M fish were trout stocked by University of Wisconsin-Madison, and were presumably a mixture of strains.

Year	Superior	Green	Clear-water	Seneca	Jenny Lake	Jenny x Lewis	Lewis	Manitou	Lake Michigan	WI-M	Ontario
1960		-	112,778	-	-	-	-	-	-	-	-
1961	-	-	94,823	-	-	-	-	-	-	-	-
1962	-	-	72,936	-	-	-	-	-	-	-	-
1963	-	-	-	-	-	-	-	-	-	-	-
1964	-	-	-	-	-	-	-	-	-	-	-
1965	1,273,878	-	-	-	-	-	-	-	-	-	-
1966	1,551,800	164,990	-	-	-	-	-	-	-	-	-
1967	1,705,660	177,805	-	-	-	-	-	-	-	-	-
1968	1,430,710	445,190	-	-	-	-	-	-	-	-	-
1969	1,760,590	239,215	-	-	-	-	-	-	-	-	-
1970	1,640,000	320,000	-	-	-	-	-	-	-	-	-
1971	2,128,145	115,400	-	-	-	-	-	-	-	-	-
1972	2,656,160	220,000	-	-	-	-	-	-	-	-	-
1973	2,110,450	293,700	-	-	-	-	-	-	-	-	-
1974	1,397,100	515,000	260,250	-	-	-	-	-	-	-	-
1975	1,305,374	886,000	235,713	-	-	-	-	-	-	-	-
1976	1,694,500	693,300	-	-	-	-	-	-	-	-	-
1977	2,327,000	-	47,500	-	-	-	-	-	-	-	-
1978	2,539,400	-	-	-	-	-	-	-	-	-	-
1979	2,321,173	-	13,900	-	-	-	-	-	-	-	-
1980	2,791,300	-	-	-	-	-	-	-	-	-	-
1981	2,395,020	-	-	-	33,000	-	-	-	-	720,000	-
1982	2,644,720	-	-	-	-	-	-	-	-	210,000	-
1983	2,130,090	-	31,480	-	-	-	-	-	31,480	300,000	-
1984	1,544,620	-	20,440	-	-	-	-	-	20,440	630,000	-
1985	3,126,339	-	-	453,704	-	-	-	-	-	1,300,000	-
1986	2,476,832	-	-	349,786	-	234,388	-	-	-	1,052,000	-
1987	1,871,400	-	-	24,984	-	-	-	-	-	1,300,000	-
1988	2,477,550	-	-	20,800	49,417	-	-	-	-	-	-
1989	4,622,296	522	-	27,223	175,091	-	-	287,965	-	-	-
1990	310,332	-	-	149,473	423,565	-	237,346	-	-	-	196,378
total	54,232,439	4,071,122	889,820	1,025,970	681,073	234,388	237,346	287,965	51,920	5,512,000	196,378
percent	80.44	6.04	1.32	1.52	1.01	0.70	0.35	0.43	0.08	8.18	0.29
percent*	88.29	6.63	1.45	1.67	1.11	0.38	0.38	0.47	-	-	-

*percent of total not including WI-M, Lake Michigan, and Lake Ontario fish.

Table 2. Allelic frequencies for 15 polymorphic protein loci in 135 wild lake trout fry hatched from eggs collected in Lake Michigan in 1994.

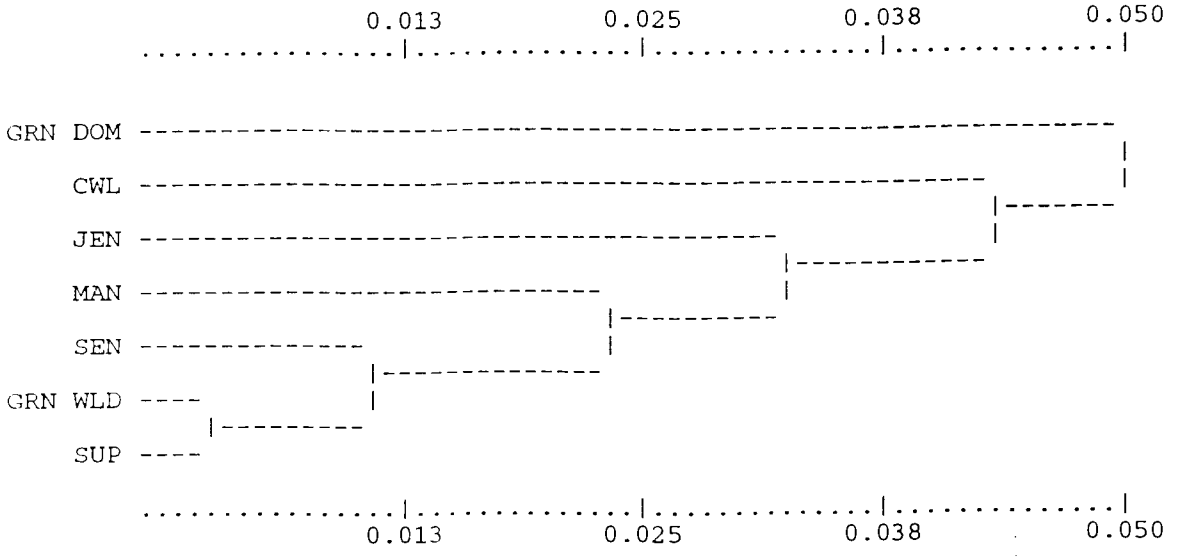
Locus	Allele	Frequency	Locus	Allele	Frequency
AAT-1	85	0.810	ME-2	100	0.989
	100	0.190		115	0.011
AAT-2	100	0.173	MUP-1	-100	0.993
	85	0.827		-140	0.007
G3P-1	100	0.966	PAP-1	100	0.295
	35	0.034		179	0.675
				138	0.030
GPI-1	100	0.985			
	200	0.015	PAP-2	100	0.000
				179	1.000
FH-1	100	0.996			
	90	0.004	PGM-2	100	0.989
				150	0.011
FH-2	100	0.831			
	90	0.169	PGM-3	94	0.762
				100	0.238
LDH-3	100	0.996			
	78	0.004	PGM-4	100	0.138
				94	0.783
MDH-4	100	0.970		91	0.079
	144	0.030			

Avg. Hs	0.127
std err	0.038
Avg. Ho	0.113
std err	0.034

Table 3. Estimated parental strain contributions to 135 wild lake trout fry from the 1995 year class in Lake Michigan. Wild = analysis using the wild Green Lake strain in the baseline; Domestic = analysis using the domestic Green Lake strain in the baseline. A dash indicates absence of a strain contribution; an asterisk indicates estimates which were more that two standard deviations from zero. CWL= Clearwater Lake, JEN = Jenny Lake, GRN = Green Lake, MAN = Lake Manitou, SEN = Seneca, SUP = Superior.

	Wild		Domestic	
Population	Contrib.	S.D.	Contrib.	S.D.
CWL x CWL	-	-	-	-
JEN x JEN	-	-	-	-
GRN x GRN	0.005	0.015	-	-
MAN x MAN	-	-	-	-
SEN x EN	-	-	0.014	0.071
SUP x SUP	0.001	0.001	0.366*	0.134
CWL x JEN	-	-	-	-
CWL x GRN	-	-	-	-
CWL x MAN	0.029	0.023	-	-
CWL x SEN	-	-	-	-
CWL x SUP	-	-	-	-
JEN x GRN	-	-	-	-
JEN x MAN	-	-	-	-
JEN x SEN	-	-	-	-
JEN x SUP	-	-	0.013	0.033
GRN x MAN	-	-	0.108	0.069
GRN x SEN	-	-	0.288*	0.088
GRN x SUP	0.531*	0.095	-	-
MAN x SEN	-	-	-	-
MAN x SUP	-	-	0.008	0.043
SEN x SUP	0.434*	0.091	0.204	0.193
Proportion of each strain in parental population				
CWL	0.015	-		
JEN	-	0.006		
GRN	0.270	0.198		
MAN	0.015	0.058		
SEN	0.217	0.260		
SUP	0.483	0.478		

Figure 1. Dendrogram generated by UPGMA cluster analysis of Nei's (1972) genetic distance coefficients based on 18 protein loci in lake trout. Abbreviations and sample sizes (in parentheses) are: CWL= Clearwater Lake (78); JEN = Jenny Lake (80); GRN-DOM = Green Lake domestic strains (41); GRN-WLD = Green Lake wild strains (4 popns, 162); MAN = Lake Manitou (80); SEN = Seneca (294); SUP = Superior 1983 (64).



NEI'S GENETIC DISTANCE