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Source, Abundance, and Fate of Lake Trout Eggs on Julian's Reef

Aquatic Biology Section
Technical Report

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Principal Investigator

Final Report
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Federal Aid Project F-64-R

Source, Abundance, and Fate of Lake Trout Eggs
on Julian's Reef

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submitted by
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to
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# Table of Contents

Summary ......................................................................................................................... 1  
Statement of Problem ................................................................................................. 1  
Objectives ..................................................................................................................... 2  
Approach ....................................................................................................................... 3  
Findings .......................................................................................................................... 4  
References ...................................................................................................................... 7
Summary

This study was designed to address three questions: 1) Which, if any, of the strains of lake trout that have been stocked in Lake Michigan deposit eggs on Julian’s Reef? 2) How many eggs are deposited there? 3) How many of those eggs survive to emerge? We determined that at least two strains of lake trout aggregated over Julian’s Reef during the 1986 and 1987 spawning seasons: Green Lake and Lake Superior. A third strain, Seneca Lake, has been stocked over Julian’s Reef since 1985 and is expected to produce a significant proportion of spawners in future years. Our attempts to estimate numbers of eggs deposited on Julian’s Reef are described in detail in the attached manuscript, "An inexpensive method for quantitative assessment of lake trout egg deposition in the Great Lakes". We explored the reef using side-scan sonar and underwater video camera. Results of that work allowed the Illinois State Geological Survey to develop a substrate map of the reef. We developed a new method for protecting and recovering spawned lake trout eggs, but were unsuccessful in recovering any from Julian’s Reef even though we confirmed using underwater video that the collection devices were placed in the areas that we believe would have provided the most suitable spawning substrate. We conducted mitochondrial DNA analyses with adults of the Lake Superior strain captured on Julian’s Reef. The mitochondrial phenotypes of those fish were not consistent with mitochondrial phenotypes found in Seneca and Green Lake fish by other investigators. Among the fish examined we found genetic variants not previously reported in the literature.

Statement of Problem

Over 30 million lake trout, representing six strains, have been stocked in Lake Michigan since 1965 (Brown et al. 1981). Although this program has resulted in the establishment of an adult lake trout population large enough to sustain a sport fishery, the stated goal of re-establishing a self-sustaining population has not been met; no offspring of the stocked fish are known to have survived to reach sexual maturity. There is a clear need for better information about the source (i.e., parental strain), abundance, and fate of eggs spawned by the present adult population.

Stocked fish may not be genetically adapted to the conditions they find in Lake Michigan. The importance of genetic adaptations in rehabilitating lake trout populations in Lake Michigan, was recognized by Krueger et al. (1983) and Ihssen (Eshenroder et al. 1984). Ihssen et al. (1988) have shown a high degree of genetic segregation among Lake Superior stocks. This suggests a high degree of adaptation to local conditions. Most of the lake trout that have been stocked in Lake Michigan have been derived from Lake Superior or Lake Michigan (the so-called Green Lake strain). In recent years substantial numbers of fish derived from Seneca Lake, New York, have been stocked over Julian’s Reef. The strains may not do equally well in Lake Michigan. There is a need to know which, if any, of the strains that have been stocked in Lake Michigan are producing viable eggs.
Stocked lake trout may not spawn in areas appropriate for reproduction. There is a need to assess spawning activity in areas, such as Julian's Reef, that may provide suitable spawning substrate. Lake trout do not prepare redds nor do they protect their eggs after spawning (Royce 1951). Thus, if spawning does not occur over substrate that is sheltered from wave action and that provides natural shelter from predators, the eggs will perish (Horns and Magnuson 1981). Evidence for spawning in suitable locations in Lake Michigan is extremely limited (Wagner 1981, Jude et al. 1981, Dorr et al. 1981). Anecdotal reports of lake trout eggs washed onto beaches suggest that inappropriate spawning site selection has been a problem in the past. The recent common practice of stocking lake trout offshore has probably alleviated that problem to some degree, but it is still not known whether suitable offshore spawning locations are being selected. Since Julian's Reef is believed to have been used for spawning by native lake trout (Coberly and Horrall 1982), we assumed that it provides suitable substrate. One reason for the lack of needed information about spawning activity on Julian's Reef was the lack of an economical method for quantitative estimation of egg deposition.

Even when spawning occurs in suitable locations, survival to emergence may be insignificant. There are two concerns: 1) Exotic predators or chemical contaminants may kill embryos and sac-fry prior to emergence. 2) Stocking densities may have been too low to produce measurable recruitment.

Objectives

Strain Identification.

To determine what strains of lake trout are presently aggregating on Julian's Reef. To obtain livers and ovaries for subsequent genetic analyses.

Egg Collection.

To capture lake trout eggs spawned on Julian's Reef in such a way that a) the eggs can be retrieved from the lake alive and b) quantitative estimates of egg deposition are possible.

Fry Collection.

To capture lake trout fry as they emerge from Julian's Reef.

Genetic Markers.

To determine genetic markers which identify adult strains captured on Julian's Reef and which can be used later to determine the parentage of eggs and fry collected on the reef.
Approach

Strain identification.

Adult lake trout were collected during the spawning seasons of 1986 and 1987 by the Illinois Department of Conservation. Collections were made with graded mesh gill nets (2.5 to 6.0 inch, stretch measure) deployed and retrieved from a commercial fishing boat. Hatchery origins and stocking dates were determined from fin clips by Rich Hess using stocking records maintained by the Great Lakes Fishery Commission. Those stocking records also contain strain designations. Since the lineages of some brood stocks are unclear and since the wild populations from which they were derived may have been composed of two or more distinct genetic strains, it is appropriate to think of these as presumptive strain designations. We use the term strain here to refer to these presumptive strain designations. Tissues were shipped frozen to Dr. David Philipp in Champaign, Illinois, for genetic work.

Egg Collection.

Egg collection was attempted through the use of specially designed collection devices (egg nets). The nets, details of our procedures, and results are described in an attached manuscript (Horns et al. 1988). During the spawning seasons of 1986 and 1987, 1180 egg nets were deployed on Julian's Reef – 280 in 1986 and 900 in 1987. The placement of egg nets was guided by surveys of the reef using side-scan sonar and underwater video camera. Holm et al. (1987), working under Federal Aid in Sport Fish Restoration Project No. F-54-R, used data from these surveys to develop a substrate map of the reef.

Fry Collection.

No collection of fry was attempted. In consultation with DOC biologist Rich Hess, we decided that it was not appropriate to attempt to collect fry on Julian's Reef until spawning there had been documented. Accordingly we devoted all of our resources to the other objectives.

Genetic Markers.

This work was conducted in the laboratory of Dr. David Philipp (Aquatic Biology Section, Illinois Natural History Survey). The approach was to look for strain-specific patterns in mitochondrial DNA, an approach that has also been attempted by Grewe and Hebert (1987). They examined fish from nine brood stocks and four natural populations and reported the following: "Seven mitochondrial clones were unique to a particular stock. In addition there were dramatic shifts in the relative proportions of the six remaining mitochondrial clones among the brood stocks. These results indicate that mt-DNA markers have great potential for the identification and management of lake trout strains."

The following method was employed in Dr. Philipp's laboratory:

Livers were extracted from fish obtained from Julian's Reef by DOC biologist Rich Hess. Individual livers were divided into aliquots of three grams each. The tissues were subjected to gentle grinding in the presence of a standard grinding buffer. These homogenized samples were centrifuged (700 x g; 10 min.; 4 C) to separate whole mitochondria from cellular debris. The
supernatants containing mitochondria were then centrifuged (12,000 x g; 20 min; 4°C) and the resulting pellets resuspended in fresh buffer three subsequent times to further purify the whole mitochondria. At this point the mitochondria were present as a white pellet. The pellet was resuspended in buffer and treated with a mild detergent (10% SDS). This detergent acts to disrupt the mitochondrial membrane, thus exposing the whole closed circular strands of mt-DNA. The mt-DNA and organelle debris were separated via centrifugation (12,000 x g; 30 min.; 4°C). All DNA-bound and soluble proteins were removed with a standard phenol/chloroform extraction. The mt-DNA were then precipitated into a solid form with 95% ethanol. A small amount of storage buffer was used to redissolve the mt-DNA. The samples could then be measured for mt-DNA content or used for fragment analysis.

To analyze fragment length variability, a small amount of mt-DNA from each fish was removed and cut with the following restriction endonucleases (RE): Ava I, Bam HI, Hind III, Hinf I, Nci I, Nco I (two fish only), Bcl I, and Eco RI. Of these, only Eco RI and Bcl I were not used by Grewe and Hebert. Grewe and Hebert used twelve additional REs, seven of which revealed no polymorphisms among the fish examined. Digestion with the REs required 3-4 hours at 37°C for completion. Upon completion, another digestion, this time with RNAse, was used to remove the influences of RNA. The cut mt-DNA was then placed on a horizontal agarose gel and electrophoresed to separate the fragments. The fragments migrated on the gel in proportion to their molecular weight. A molecular weight standard was included to allow molecular weight determination of the fragments. The gel was stained with ethidium bromide and visualized with transilluminated 302 nanometer UV light. A photograph was taken as a permanent record. Restriction patterns obtained for each RE were assigned a letter consistent with those assigned by Grewe and Hebert. For each fish the sequence of RE patterns (or profiles) describes a phenotype.

Findings

Strain identification.

Fish of several distinct origins were collected by gill nets on Julian’s Reef during 1986 and 1987. The presumptive strains of those fish are indicated in Table 1. In some case fin clips were ambiguous. The following strains appeared in the catch: Green Lake, Lake Superior, and (possibly) Clearwater Lake, Manitoba. Fish of the Seneca Lake strain had not appeared in collections in either of these years, but began to appear in 1988.

<table>
<thead>
<tr>
<th>Collection Year</th>
<th>Total Number Captured</th>
<th>GL</th>
<th>LS</th>
<th>GL or LS</th>
<th>CW or LS</th>
<th>unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>364</td>
<td>20</td>
<td>319</td>
<td>11</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5%)</td>
<td>(88%)</td>
<td>(3%)</td>
<td>(2%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>1987</td>
<td>240</td>
<td>4</td>
<td>224</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2%)</td>
<td>(93%)</td>
<td>(2%)</td>
<td>(1%)</td>
<td>(3%)</td>
</tr>
</tbody>
</table>
**Egg Collection.**

Our egg collection work is described in detail in a manuscript (Horns et al. 1988) submitted for publication by the North American Journal of Fish Management. A copy of that manuscript accompanies this report. Reprints will be sent to IDOC upon publication. No eggs were collected from Julian's Reef.

**Fry Collection.**

No fry collection was attempted.

**Genetic Markers.**

Nine fish collected from Julian's Reef were subject to mt-DNA analysis as described above. All were probably derived from the Lake Superior strain, although two may have been of the Green Lake strain. The following results were obtained in the laboratory of Dr. David Philipp. Most of this information has also been reported in annual and quarterly reports for Federal Aid in Sport Fish Restoration Project No F-53-R.

Table 2 illustrates the phenotypes obtained. The phenotype names follow those used by Grewe and Hebert, even though a smaller set of REs was used here than by those authors. For reference, the two phenotypes found in Seneca fish and the three phenotypes found in Green Lake fish by Grewe and Hebert also shown (Table 3).

Grewe and Hebert (1987) used several restriction endonucleases not used in this work, and did not use Eco RI or Bcl I. Therefore, assignment of fish to phenotypes defined by those authors is somewhat ambiguous. For example, while our fish 2, 3, and 4 show RE patterns consistent with phenotypes A2 of Grewe and Hebert, we cannot be certain that had the other five restriction endonucleases used by Grewe and Hebert also been used here the RE patterns would have still been consistent with their phenotype A2. Nor can we be sure that had Grewe and Hebert also used Eco RI they would have seen our pattern A for the fish they classified in phenotype A2. Pattern E for Hinf I and pattern C for Nco I were not reported by Grewe and Hebert. The phenotypes revealed by fish 1, 5, 6, and 9 were not found by Grewe and Hebert.

Grewe and Hebert dealt with six brood stocks relevant to the situation on Julian's Reef; one was classified in the Seneca strain, two were classified in the Green Lake strain, and three were classified in the Marquette (Lake Superior) strain. Their results suggest that in its present stage of development, mitochondrial DNA analysis may be useful in distinguishing one of the strains that has been stocked on Julian's Reef (Seneca) from the others; the mitochondrial phenotypes found among fish of the Seneca strain, were not found by those authors in fish of either of the other two strains. The results of Grewe and Hebert provide less hope, however, for distinguishing Lake Superior and Green Lake fish, although two phenotypes found among Green Lake fish were not found among the Lake Superior fish and several phenotypes (including A2) found among Lake Superior fish were not found among Green Lake fish.
All of the nine fish analyzed here may have been derived from Lake Superior ancestors, so these results are consistent with those of Grewe and Hebert; none of the phenotypes found in these nine fish were found in Seneca or Green Lake fish by those authors. Also, five of the nine fish carried mitochondrial phenotypes that were consistent with A1, A2, or C1, three of the Lake Superior phenotypes found by Grewe and Hebert.

Our finding of genetic variants not reported by Grewe and Hebert implies that the phenotypes seen so far do not include all existing variants, and suggests that considerable caution should be exercised in the use of mitochondrial genotypes for strain identification.

Table 2. Restriction patterns obtained for fish obtained from Julian's Reef (all probably Lake Superior strain, except 7 and 8 which may be Green Lake strain). Phenotype designations follow Grewe and Hebert (1987) and are only probable, for reasons given in the text.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Ava</th>
<th>Bam</th>
<th>Hind</th>
<th>Nco</th>
<th>Hinf</th>
<th>Nci</th>
<th>Bcl</th>
<th>Eco</th>
<th>RI</th>
<th>Pheno-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>***</td>
<td>E*</td>
<td>A,B,orD</td>
<td>***</td>
<td>A</td>
<td></td>
<td>C4**</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>***</td>
<td>D</td>
<td>A,B,orD</td>
<td>***</td>
<td>A</td>
<td></td>
<td>A2</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>***</td>
<td>D</td>
<td>A,B,orD</td>
<td>***</td>
<td>A</td>
<td></td>
<td>A2</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>***</td>
<td>D</td>
<td>A,B,orD</td>
<td>***</td>
<td>A</td>
<td></td>
<td>A2</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>***</td>
<td>D</td>
<td>A,B,orD</td>
<td>***</td>
<td>A</td>
<td></td>
<td>B2**</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>***</td>
<td>E*</td>
<td>A,B,orD</td>
<td>***</td>
<td>A</td>
<td></td>
<td>C4**</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>***</td>
<td>A</td>
<td>A,B,orD</td>
<td>A</td>
<td>A</td>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>C</td>
<td>A,B,orD</td>
<td>A</td>
<td>A</td>
<td></td>
<td>C1</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>C*</td>
<td>A</td>
<td>A,B,orD</td>
<td>A</td>
<td>A</td>
<td></td>
<td>A10**</td>
</tr>
</tbody>
</table>

* Pattern not observed by Grewe and Hebert (1987).
** Probable new phenotype, not observed by Grewe and Hebert.
*** Not tested or tested with inconclusive results.
Table 3. Restriction patterns found by Grewe and Hebert (1987) in Seneca and Green Lake fish.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ava</th>
<th>Bam</th>
<th>Hind</th>
<th>Hco</th>
<th>Hinf</th>
<th>Nci</th>
<th>Bcl</th>
<th>Eco</th>
<th>RI</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seneca (found in 1 fish)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>D</td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Seneca (found in 14 fish)</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Gr.Lake (found in 10 fish)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Gr.Lake (found in 4 fish)</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Gr.Lake (found in 1 fish)</td>
<td>A</td>
<td>D</td>
<td>A</td>
<td>A</td>
<td>D</td>
<td>A</td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

*** Not tested.

References


