

Great Lakes Fish and Wildlife Restoration Act

FINAL Project Report Template

*Indicates required content

** Note that the GLFWRA legislation mandates that this report be made available on a public website once USFWS and Sponsor Review has been completed and the Report has been officially accepted.

***Project Title:** Quantifying Genetic, Phenotypic, and Reproductive Differences of Siscowet and Lean Lake Trout Reared in a Controlled Environment

***Project Sponsor:** Board of Regents of the University of Wisconsin

***FWS Agreement Number:** 30181AG153

***Principal Investigator(s):** Frederick William Goetz, Shawn Sitar

***Report Author(s):** Frederick William Goetz

***Period Covered:** 10/01/2010 – 09/30/2012

***Date Submitted:** 01/31/2013

Study Objectives:

1. Determine differences in length, weight, morphometry, lipid levels and lipid composition in cultured leans and siscowets.
2. Determine the timing of first reproduction and fecundity in cultured leans and siscowets.
3. Determine differences in gene expression in muscle and liver between cultured leans and siscowets and develop antibody to C1q-protein 2.

Description of Tasks:

Objective 1: All cultured leans and siscowets were measured for length and weight in October, 2010 and 2011 and the lipid levels assessed in all fish using a fatmeter (Distell). Further, the fatty acid composition of lean and siscowet muscle was analyzed biochemically for fish from 2010. Digital photographs from cultured leans and siscowets taken at years 3 and 4 were analyzed using Truss analysis to determine differences in morphometry. Lengths, weights, lipid levels and morphometry were assessed in October, 2010 and 2011 as proposed.

Objective 2: A blood sample was obtained from all cultured leans and siscowets during the October, 2010 sampling and all blood samples were analyzed for the level of estradiol and 11-ketotestosterone. All fish were investigated at October, 2010 and again at a November, 2010 sample to determine if females had ovulated or males were running ripe. In October and November, 2011 >95% of all fish were reproductively mature. The following crosses were made from gametes obtained in the Fall, 2011: siscowet X siscowet; lean X lean; siscowet (F) x lean (M); lean (F) x siscowet (M). Progeny of these crosses are being reared at the Northern Aquaculture Demonstration Facility in Ashland, WI. In September, 2012, 220 of each cross

were PIT tagged and length and weights were recorded. Lipid levels were assayed on 20 individuals of each cross using the Soxhlet method.

Objective 3: The transcript expression of the C1q protein in liver and muscle at year 3 was determined by QPCR and the full-length cDNA for the C1q was generated from both leans and siscowets. The genomic sequence for C1q was determined for both leans and siscowets and compared with transcript sequence. Expression of two C1q variants (lean-like and siscowet-like) was determined in 20 leans and siscowet livers from year 3.

Executive Summary/Abstract for Project: In Lake Superior, there is both a shallow and deepwater form of lake trout. The shallow water form is the lean lake trout that occupies waters less than 80 m, while the siscowet is the deepwater form most common in waters 80 m to the maximum depths of Lake Superior (~ 405 m). Emphasis in the past has been on stocking lean lake trout for restoration, but recently there has been interest in the stocking deepwater forms. Little is known about the basic biology of siscowets, and their performance under controlled rearing conditions as found in hatcheries is limited. Therefore, as a first empirical step in evaluating deepwater lake trout as a candidate for recovery programs, it is important to define their basic physiology in relation to other lake trout morphotypes under identical, controlled conditions. This was accomplished in the present study. Results show that siscowets reared under identical conditions as leans in the lab have a higher condition factor and muscle lipid levels as observed in the wild. Further, differences in growth and lipid levels persist in the F1 generation indicating that these are genetic traits of the two morphotypes and not a result of environmental plasticity. Finally, the expression of a gene (C1q) in the liver was found to be highly different between morphotypes and the difference persists as the fish aged. The difference appears to be a result of a different splice variant of the gene between leans and siscowets and could be used as a marker for discriminating the morphotypes.

Major findings and accomplishments:

1. Lean and siscowet lake trout reared under identical environmental conditions exhibit vastly different skeletal muscle lipid levels; siscowets have approximately twice the amount of lipid compared to leans. This difference was observed in samples from year 1 to year 5.
2. Lean and siscowet lake trout reared under identical environmental conditions have different condition factors. Siscowet lake trout are significantly heavier at a given age than leans and since the two morphotypes are similar in length, siscowets have a significantly higher condition factor than leans at all ages.
3. Greater than 95% of the lean and siscowet lake trout reared under identical environmental conditions reproduced by year 5 and the timing was the same for each morphotype; occurring in October and November. Ovulated eggs and sperm were obtained from both morphotypes and crosses were made (siscowet X siscowet; lean X lean; siscowet (F) x lean (M); lean (F) x siscowet (M)) and reared to the juvenile stage. Data collected at year 1 showed that progeny of the siscowet x siscowet cross were the heaviest and the lightest were from the lean x lean cross. Chemical analysis of lipid levels also indicated that progeny of the siscowet x siscowet cross had the highest and lean x lean the lowest lipid levels. Interestingly the reciprocal crosses were

intermediate, with the order being siscowet x siscowet > siscowet (female) x lean (male) > siscowet (male) x lean (female) > lean x lean. This strongly suggests that lipid levels are associated with the female siscowet.

4. We found that the expression level of a gene, C1q, in the liver was significantly higher in leans than in siscowets. The difference in expression between morphotypes increased with age. We cloned the transcript for C1q in both leans and siscowets and found them to be different with leans having a 27 bp fragment that was lacking in the siscowet transcript. However, when we sequenced the C1q gene from genomic DNA we found the same sequence for both leans and siscowets, suggesting that the difference between leans and siscowets is a result of a splice variant. The 27 bp fragment is at the beginning of an intron suggesting that more of the intron is present in leans than in siscowets and that constitutes the splice variant.

Management implications of your work: Stocking of hatchery lake trout continues to be a major management tool in lake trout recovery programs for the Great Lakes. Recently, there has been interest by fisheries agencies to assess the possibility of introducing lake trout in deeper habitats in other Great Lakes. However, little is known about the basic biology of deepwater forms such as the siscowet, and their performance under controlled rearing conditions as found in hatcheries is limited. It is unclear whether the differences observed in the wild are genetic traits or a result of environmental plasticity. One approach to determine this is to rear lean and siscowets from birth to adults under identical environmental conditions and determine if differences that are observed in the wild persist. Our results from these rearing experiments show that some of these characteristics appear to be genetic and persist even in the F1 generation as would be expected in hatchery conditions. Results from these studies provide managers with information on key physiological attributes of deepwater lake trout that are useful in selecting optimum hatchery products to support lake trout recovery efforts. From a basic viewpoint, understanding their physiology would provide key insights into adaptive mechanisms of lake trout diversity in the Great Lakes, and laboratory lines of siscowets could be used for many future experiments benefiting basic and applied science; particularly determining if depth selection is a genetic trait.

Additional restoration work needed and/or areas for future research: Given the results of this study, it is clear that some differences between wild lean and siscowet lake trout have a genetic basis. However, at this point, the most important question is whether the drive to inhabit deep water by siscowets is a genetic trait. While it may be assumed that depth selection in lake trout is genetically controlled, this has never been tested and would be extremely important in restoration programs that assume that stocked fish would occupy a specific habitat.

List of presentations delivered and outreach activities:

1. *A Genetic Basis for the Phenotypic Differentiation Between Lean and Siscowet Lake Trout (Salvelinus namaycush)*. Annual Meeting of the American Fisheries Society, Seattle, WA, September 4-8, 2011. This presentation was part of special symposium titled: "Conservation Genetics and Genomics In Fisheries."
2. *The Contribution of Next Generation Sequencing to Help Define Differences in Lake Trout Morphotypes in Lake Superior*. Annual Meeting of the American Fisheries Society, St. Paul, MN, August 19-23, 2012. This presentation was part of special symposium titled: This

presentation was part of special symposium titled: “The Role of Molecular Genetics in Fisheries Management in the Great Lakes Region”

3. *Lake trout morphotypes exhibit wide differences in lipid physiology that are genetically determined.* Aquaculture America, Las Vegas, NV, February 29 - March 2, 2012.

***Include relevant pictures or images associated with the project:** See attached files

Geographic region project occurred in or effects: Milwaukee and Bayfield, Wisconsin.

***List of reports and peer-reviewed papers completed or in-progress:**

Annual report for the GLFWS: Quantifying Genetic, Phenotypic, and Reproductive Differences of Siscowet and Lean Lake Trout Reared in a Controlled Environment. FWS Agreement Number: 30181AG153

Goetz, F., Johnson, R., Biga, P. 2013. Physiological differences between lean and siscowet lake trout: Are these metabolotypes as well as morphotypes? (in preparation).