North Central Regional Aquaculture Center

Project Title: Development of an All-Female Yellow Perch Population: A Strategic Approach Using Thermal Manipulation, Sperm Selection, and Genomic Data Analysis [Progress Report]
Key Word(S): Yellow Perch
Total Funds Committed: $162,261
Initial Project Schedule: July 1, 2017 to June 30, 2019
Current Project Year: July1, 2-2017 to June 30, 2018
Participants: Sepulveda Villet, O.J., University of Wisconsin-Milwaukee; Dabrowski, K.E., The Ohio State University
Extension Liaison: Jim Held (replaced by Joseph E. Morris)
Industry Liaison: Stinton, A., RDM Shrimp, Fowler, Indiana

Project Objectives

1. To determine the influence of temperature on gonadal differentiation in Yellow Perch of Ohio origin raised at low 14oC (57oF) or high 24oC (75oF) water temperature from fertilization until completion of sex differentiation.
2. Examine the sex ratio and growth rate of progenies sired by potentially sex reversed males (obtained from objective 1) reared in parallel groups (OSU) and separately in a "common garden" design by factorial crossing (UW-Milwaukee). Additionally, outcross performance (fertilization, survival, growth rates at 30 and 90 days, feed efficiency) will be evaluated among crosses of Ohio strain and hybrids between UW-MILWAUKEE genetically improved yellow perch x Ohio perch sperm.
3. To determine if the use of a flow-cytometry-based cell sorting method will correctly identify and segregate "Y"- and "X"-sperm, using a fluorescent nuclear tag and differential fluorescence as separation criteria (UW-Milwaukee).
4. To characterize DNA from "X"-sperm and utilize a novel yellow perch genome to identify putative sex-linked markers that can be used to increase efficiency of cell-sorting or other molecular-based sperm selection methods (UW-Milwaukee).
5. To optimize high-throughput cryopreservation methods for yellow perch sperm and develop a pilot cryo-bank of sex-reversed ("XX") male yellow perch sperm, which will be immediately available for use by fish farmers in the North-Central region to produce all-female progenies for grow-out (OSU).

Deliverables

1. The development of standardized methods for collection, extension, cryopreservation and distribution of yellow perch semen.
2. A technique of thermal manipulation that will result in sex-reversed male yellow perch, which produce all-female progenies when crossed to female yellow perch.
3. The identification of putative sex-determining gene(s) for yellow perch.
4. A method to screen and select sperm, as a strategy to produce monosex lines.
5. Primary, peer-reviewed literature highlighting our research products.
6. Technical white paper(s) on collection techniques and use of cryopreserved semen in commercial fish farms.
7. A web-based outreach and training program for the use of cryopreserved semen in commercial farms.

Project Summary

Strong consumer demand and high fillet value support the development of yellow perch aquaculture. However, commercial perch production yields fish with highly variable sizes, due to females growing larger and faster than males. Collaborative efforts have resulted in genetically-improved yellow perch broodstocks selected for faster growth, but while mean growth to market size has been reduced from 26 to eight months, selection has not reduced gender-size variability. One approach to eliminating this variability is mono-sex culture. Current methods are inefficient and typically involve the controversial application of steroids. We propose thermal manipulation techniques to develop putative sex-reversed males, then to test their progeny sex ratio and identify neo-males ("XX"-males). Sperm from these males will then be analyzed using flow-cytometry to verify the exclusive production of "X" sperm. DNA from "X" sperm will be analyzed against a new yellow perch genome,to characterize sex-determining genes. Finally, these selected sperm samples will be cryopreserved and stored in a pilot cryo-bank. The ability to culture all-female yellow perch will directly benefit commercial producers and increase profitability.

Project Progress

Objective 1. — To determine the effect of temperature on gonad differentiation in Yellow Perch, 11 full-sibling progenies were produced at OSU in April 2018. Sibling groups were divided to individual tanks in \two separate recirculation systems and reared at low (14°C; 57°F) and high (27°C; 81°F) water temperature from the start of exogenous feeding until presumed sex differentiation was completed (mean total length of individuals reached approximately 30mm [1.3 inch]). Growth and survival were monitored throughout (56 days high temperature, 144 days low temperature) and samples were taken at the end of target temperature rearing for histological analysis of gonad formation. Temperatures were then adjusted to follow seasonal variation for continued grow-out of the experimental groups for future external determination of sex ratio and comparison between temperature groups.

Objective 2. — Extended samples of OSU Yellow Perch semen were received by UW-Milwaukee, to establish a common protocol of cross-strain fertilization. Semen was collected from OSU normal males, OSU staff macerated testes from putative neomales, and from UW-Milwaukee Choptank strain males. Semen samples were distributed to small sections of freshly spawned eggs (UW-Milwaukee choptank strain) to allow for fertilization. Number on non-viable eggs, eggs per 1-inch grid, and fertilized eggs after 2 hours were collected. Fertilization was low for both the OSU normal males, and OSU putative neomale semen samples, ranging from 0-10.35% fertilization. Use of fresh semen resulted in almost complete fertilization (81.86-93.08%), underscoring a need to improve methods and protocols for the transportation of cryopreserved and extended semen samples.

Objective 3. — We have identified a compatible dye that allows in vivo sorting of yellow perch sperm using flow cytometry. The dye is able to be used in both fresh and cryopreserved viable sperm cells. We are working to optimize the cell sorting method using the FACsaria flow cytometer with cell sorter at UWM's main campus. That trial will occur in the next three weeks. We anticipate that we will be able to sort viable and non-viable (dead) sperm cells, and that from those viable cells, we will segregate at least two populations (putative male and putative female). The cell sorting will occur under immobilization solution as to preserve the viability of the sperm cells for fertilization trials which will happen mid-March.

Objective 4. — A high resolution nuclear genome of the Yellow Perch has been completed, using a number of resources, including existing transcriptome data from USDA-ARS, as well as new data generated in Illumina Hi-Seq and Pacific Biosciences RSII analyzers. Total read coverage of the new genome exceeds 87x coverage, with a putative size of 1.1Gbp. Additionally, a histone-based spatial scaffold has been constructed using Dovetail Genomics' HiC method. This resulted in an annotated scaffold containing 24 likely chromosomes, matching already known karyotypes for yellow perch. Predicted protein transcripts identified at least seven likely sex-determination genes also observed in other teleost fishes. Further analyses will determine if these genes are concentrated in a specific putative chromosome.

Objective 5. — UW-Milwaukee staff have collected sperm from March-spawning male broodstock, and have processed it as described in Miller et al. (2018). A portion of these samples for long term storage (beginning the cryobank at UWM, 25 individuals collected from two strains, (50 individuals total), and another portion will be used for the fertilization trials in March.

OSU staff are currently conducting a literature review on cryopreservation of percid sperm. This literature review is part of the currently funded PhD student's dissertation and is expected to be completed by mid-summer. During 2018 spawning season the amount of sperm from "XX-neomales" was not sufficient due to their size to establish the depository. OSU staff will address this objective in Spring 2019.

Targeted Audiences

We intend to share these results with fisheries and aquaculture researchers, state and federal agencies involved in stocking and monitoring of yellow perch stocks, members of the industry, and other stakeholders that could benefit from the establishment of these resources. To date, we have identified at least one fish producer with prior experience in cryopreserved semen handling and use, and he has expressed great interest in collaborating on a future field-based evaluation of cryopreserved semen for yellow perch broodstocks in his facility. We will continue to search and identify other stakeholders that can participate in evaluations, or that can benefit from having a cryo-bank of yellow perch gametes.

North Central Regional Aquaculture Center

Outreach Overview

Mr. James Held was our extension liaison (formerly of UW-Extension). Our goal is to co-develop media (including an instructional video on how to properly use cryopreserved semen to fertilize yellow perch eggs at a hatchery facility) and outreach information products, present at the 2019 Wisconsin Aquaculture association or World Aquaculture Society meeting, and at the 2019 NCRAC regional meeting. Other information products will also be incorporated into the class curriculum of the Applied Urban Aquaculture undergraduate certificate at UW-Milwaukee. Other primary literature products (journal articles, posters and/or oral presentations) will be produced by the PIs and their graduate students. Additional interaction with NCRAC, Wisconsin Aquaculture Association, Wisconsin Sea Grant personnel, and with UW-MILWAUKEE and OSU personnel will increase our dissemination of the material to stakeholders. To date, the Yellow Perch genome is in the process of final annotation, and a consortium has been developed among researchers from UW-Milwaukee, USDA- ARS, Mississippi State University, and with contract work from Dovetail Genomics. This high resolution genome comprises annotated gene sequences, predicted protein transcripts, as well as spatial and likely chromosome-level information (derived from Dovetail Genomics' HiC method). This genomic resource will be made available to other researchers as an online resource once completed. Other outreach will take place in year 2, as planned. Our outreach coordinator, Jim Held, passed away early last year, thus we will work with Dr. Morris to establish an alternate outreach liaison and coordinator.

Deliverables

A draft of the nuclear yellow perch genome will be made available this year as an online resource. Links to this resource will be included in UW-Milwaukee School of Freshwater Sciences' website, and information will also be disseminated at WAS 2019 in New Orleans.

Outcomes-Impacts

We have identified at least one commercial producer with experience handling cryopreserved gametes, working with yellow perch, and interested in doing a field evaluation of cryopreserved gamete resources under development at UWM and OSU. We hope that having at least one stakeholder already participating will allow members of the aquaculture industry to become more aware of the possibility and availability of these resources for their own needs in their farms.

Impacts Summary

Issue. — Objective 1- There is a lack of analytical and research tools needed for development of a sustainable method to produce all-female yellow perch fingerlings. Temperature-dependent sex determination (TSD) is a promising sustainable method to produce sex-reversed phenotypes of yellow perch males which could be used as broodstock to produce all-female progenies. However, TSD has not been studied in yellow perch experimentally or other closely related species due to difficulties with larval growth in captivity. Objective 2- Although genetically improved yellow perch strains have been developed to assist the industry, there is little information on the viability of using cryopreserved gametes to assist in reducing broodstock size, and increase fertilization success during fish spawning. Additionally, it is not known how these genetically improved strains will perform if outcrosses take place, and it these outcrosses would affect the proportion of female progeny, if potential all-female semen from neomales is used. Objective 4- little is known about the gene systems that govern sex-determination in most fishes, as is the case for yellow perch. One potential strategy to obtain all-female yellow perch strains is to identify these genes, and use them as markers to select sperm cells that are specific to produce female offspring. Additionally, the identified sex-determination genes can be targeted to regulate gene expression, as an alternative mechanism for all-female lines.

Response. — Objective 1 - Following yolk-sac absorption, yellow perch larvae were reared at low (14°C) and high (27°C) water temperatures in order to determine the effect of temperature on gonad differentiation. Objective 2- egg ribbons from UWM strain yellow perch were cross fertilized with extended semen obtained from OSU strain males, and putative neomale testes. A comparison to fresh semen from UWM strain yellow perch males was done to determine if extended semen was similar in performance to fresh semen. Objective 4- An annotated genome of the yellow perch was constructed using high resolution sequence data from two platforms (Illumina and Pacific Biosystems), and further organized into coherent scaffolds using a proprietary approach (Dovetail Genomics HiC). Sequence data from this genome has allowed for the identification of at least seven sex-related genes present in other teleosts. These genes will assist in identify and separate sperm cells through flow cytometry, and will help identify sex-linked chromosomes, or chromosome regions.

Results.— Objective 1 - The effect of temperature on gonad differentiation is still unknown as fish are currently being grown-out so that sex ratio can be determined externally (spermiation). We anticipate collection of these results in January 2019. Objective 2- while fertilization was poor during this first attempt, we were able to observe fertilized eggs from all three semen samples; extended OSU male, extended OSU neomale, and UWM fresh semen, therefore confirming the viability of cold shipping processed semen to labs, hatcheries, and potentially producers in the North Central region. Further work will aim to refine these techniques. Objective 4- the completion of a high resolution, annotated genome marks a watershed point for development of improved strains of yellow perch, as marker assisted selection can now take place at an accelerated rate. By having this genomic resource, gene families associated with desirable traits, such as growth rate, disease resistance, sex-determination, etc., can now be more easily identified. This type of effort and product has resulted in improved strain development in other species, such as catfish, tilapia, and rainbow trout.

Recap.— While more work is pending during year 2, our results indicate that semen obtained from neomale yellow perch, whether fresh or extended and stored in wet ice, can successfully fertilize freshly spawned eggs of OSU and UWM genetically improved strains, which can then be selected using genomic-derived markers to develop all-female strains of yellow perch.

Publications, Manuscripts, Workshops, and Conferences
See the Appendix for a cumulative output for all NCRAC-funded Yellow Perch activities.