Project Title: Develop Systems and Diet Strategies to Reduce Yellow Perch Larval Mortality Burst in Indoor Recirculating Aquaculture Systems [Termination Report]

Key Word(s): Yellow Perch

Total Funds Committed: $190,000

Initial Project Schedule: September 1, 2013 to August 31, 2015

Current Project Year: September 1, 2016 to August 31, 2017

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Project Objectives

1. Develop system(s) to address physical and behavioral barriers to enhance mass production and survival of yellow perch (YP) from onset of first feeding up to 70 days.
2. Develop strategies to increase survival of fry and larvae of yellow perch reared indoors using different feeding regimens.

Deliverables

1. Develop modules for self/group training for YP aquaculturists. Modules should be prepared at the initiation of the project and updated to include new procedures/protocols learned from the project.
2. Prepare an overall report of the findings including an executive summary.

Reason for Termination: Objectives completed

Project Summary

In culture conditions, there are several critical factors affecting survival of larval yellow perch, including small mouth gape, dependence on live food organisms, non-feeding behavior, non- inflation of the gas bladder, clinging behavior, and cannibalism. Despite the availability of high quality feeds for small larvae, mainly formulated for marine species, the acceptance, growth and survival of larval yellow perch fed formulated diets as starting food are still highly variable and rather unsatisfactory. This project is investigating the development of systems and strategies to enhance mass production and survival of yellow perch from onset of first feeding up to 70 days post hatch (dph), the critical period for yellow perch in recirculating aquaculture systems.

Technical Summary and Analysis:

Objective 1.1. (UWSP-NADF)— In year 1, non-feeding behavior, non- inflation of the gas bladder, clinging behavior, and cannibalism were examined using a series of methodical experiments evaluating the effects of: 1) Turbid water – three treatments of clear (0 NTU), slightly (50 NTU), and turbid (100 NTU) water using clay (L&R Specialties, Missouri); 2) Water surface spray – three treatments of no flow (0 L/min, 0 gal/min), moderate (0.4 L/min, 0.11 gal/min), and high (0.8 L/min, 0.21 gal/min) water flow (horticultural hanging basket flex misters; Hummert International, Missouri); 3) Tank color – three treatments of white, blue, and black interior colored tanks. Newly hatched fry were fed initially fed high quality dry diets and then trained to standard commercial yellow perch diets.

Differences ($p<0.05$) in growth, length and weight, were observed after 70 days for larval yellow perch raised in different color tanks (blue, white and black) with fish reared in black tanks showing the best growth. Significant differences ($p<0.05$) in growth, length and weight, were observed for larval yellow perch raised under different water surface spray. High spray velocity (0.8 L/min; 0.21 gal/min) resulted in the greatest growth, both length and weight, especially when comparing no flow, poorest length gain, to high flow. No flow and moderate flow (0.4 L/min; 0.11 gal/min) showed similar weight gain with high flow having the greatest weight gain. Significant differences ($p<0.05$) in growth, length and weight, were observed for larval yellow perch raised in different turbidity levels. Slightly turbid water (50 NTU) resulted in the greatest growth, both length and weight, when compared with clear water (0 NTU) and turbid water (100 NTU). There were no differences in growth of larval yellow perch between clear water and turbid water conditions.

Objective 1.2 (UWSP-NADF) — Based on year 1 results, culture conditions were optimized into a 3x3x1 factorial design with three replicates per treatment. The interior of all tanks were painted black, turbidity was narrowly defined as either 12.5, 25, or 37.5 NTU, and water surface spray was limited to 0.6 L/min (0.16 gal/min), 0.8 L/min (0.21 gal/min), or 1.0 L/min (0.26 gal/min); . A random sample of 25 fry per tank was collected every two weeks and measured for length and weight gain; mortalities were recorded as observed mortality and removed daily. At the end of the 70-day study, remaining fish were counted and measured for total length and an aggregate weight of 100 fry along with 100 fry/tank examined for the presence of food in the gut and gas bladder inflation. Percentages of unobserved mortality (cannibalism) and observed mortality were calculated.

No statistical differences ($p>0.05$) in growth, length and wet weight, were observed every two weeks nor were differences observed after 70 days for larval yellow perch raised in any of the treatments. Both length and weight gain were greatest, though minimal gain, for larval yellow perch raised with water surface spray of 1.0 L/min (0.26 gal/min) with turbidity set at 25 and 37.5 NTU. After the first week, 100% of the larval yellow perch sampled contained food in their gut (feed acceptance), yet after three weeks only 50% of the fish had food in their gut. From week five to eight, 75-100% of the remaining larval yellow perch had food in their gut. From week one through week eight, 96% of the larval yellow perch had inflated their swim bladder. Mortality rates were high throughout the experiment and among all treatments. Approximately 3% of the larval yellow perch survived until the end of the 70-day experiment regardless of treatment. Minimal cannibalism was observed but developmental deformities were common including poor opercula development, spinal deformities, and mouth structural abnormalities.

Objective 2.1 (OSU). – Commercial-scale marine rotifer and *Artemia* production systems were further improved and the 24/7 auto-feeders were optimized starting the first year of the project. Marine rotifers (hereafter referred to as simply rotifers) were harvested and fed twice rather than once in consideration of their rapid life cycle. Live feed production systems were improved either through reducing feeding times for rotifers or reducing production cost, e.g., decreasing salinity for *Artemia* hatching from 25-30% to 12-15%, without any compensation of output. The regular air stones in the stocking buckets of live feeds (rotifer/*Artemia*) in the auto-feeders were replaced by round, flat air stones because researchers found a considerable proportion of live feed sink on the bottom and were not pumped out to fry tanks last year. This improvement allows more than 90% of live feed to be delivered to fry tanks.

Feeding interval for 24/7 auto-feeders was adjusted to 1 hour because *Artemia* survive up to about 1 hour when they were dispersed into freshwater rearing tanks. In addition, researchers found that contamination between two production systems, rotifers and *Artemia*, should be avoided because even a few *Artemia* will considerably reduce rotifer production.

Objective 2.2 (OSU).— Researchers tested and identified the two best feeding regimes (Table 1) The co-feeding strategy that consists of mixing rotifers and *Artemia* into one container, feeding formula feed before hand-feeding live feed, and gradually reducing live feed, significantly increased larvae survival to about 25% at 31 days post-hatching (dph) These two feeding regimes provided yellow perch larvae with a combination of live and formula feeds, a wide range of feed sizes that , as larvae grow. During 25 to 31 dph, OSU researchers found that perch fry were aggressive to formula feeds and could digest formula feed without any observable problem. Unfortunately, a technique issue (heavy aeration caused violent agitation of water for about an hour) resulted in totally loss of fry. It is suggested that live feed not be completely replaced until fry reach 40 dph.

Table 1. Two best feeding regimes for culturing yellow perch larvae using marine rotifers, *Artemia*, and commercial diets (Otohime, B1 200-360 µm and B2 360-600 µm; Zeigler 150-250 µm and 250-400 µm; and Purina® AquaMax® Fry Starter 100), Ohio State University.

Feeding Regime	**Schedule (days post hatch – dph)**						
1	3-7 dph	5-10 dph	8-20 dph	21-30 dph	30-45 dph	45-55 dph	55 dph-
	Rotifer	small *Artemia*	Regular *Artemia* +	Regular *Artemia* +	Regular *Artemia* + B2 + AP250-450 + Starter	B2 + AP250-450 + Starter	Starter
	160 µm	428 µm	Otohime B1 + AP100-150	Otohime B2 + AP100-150 + AP150-250			
2	3-10 dph	8-20 dph	21-30 dph	30-45 dph	45-55 dph	55 dph-	
	Small *Artemia* 428 µm	Regular *Artemia* + Otohime B1 + AP100-150	Regular *Artemia* + Otohime B2 + AP100-150 + AP150-250	Regular *Artemia* + B2 + AP250-450 + Starter	B2 + AP250-450 + Starter	Starter	

Several related studies were completed: 1) by monitoring egg size produced by different strains/families, we have identified some strains/families that produced significantly larger-mouth gape progeny and larger eggs than others; 2) variation of egg size is dramatically different among strains of our genetically improved fish, indicating there is a large range of selection for large eggs; and 3) we found predation and ingestion of prey at the beginning of feeding is limited by the mouth gape in fish larvae which determine larvae survival.

Examination of food in digestive tract, combining with mouth size as well as total length data proved that the yellow perch larvae were able to ingest live feed, either rotifers or *Artemia*, when only total length reached 6 mm and mouth gape reached 0.6 mm, regardless the age of larvae, which was usual from 1 to 7 dph. Swim bladders started to inflate as early as 4 dph, and were correlated with the size of fish. When pooling all families together, we found that the swim bladders inflate when larvae the size reached 6.7 - 9.7 mm, regardless the age of fish. Yolk disappeared between 7 and 11 dph for most of larvae. For one family, yolk was still observed in 70% of larvae at 11 dph.

Principal Accomplishments

Non-feeding behavior, non- inflation of the gas bladder, clinging behavior, and cannibalism were examined using combinations of selected turbidity, water surface spray and tank color in husbandry tanks. Differences ($p<0.05$) in growth, length and weight, were observed after 70 days for larval yellow perch raised in different color tanks (blue, white and black) with fish reared in black tanks showing the best growth. Significant differences ($p<0.05$) in growth, length and weight, were observed for larval yellow perch raised under different water surface spray. High spray velocity (0.8 L/min; 0.21 gal/min) resulted in the greatest growth, both length and weight, especially when comparing no flow, poorest length gain, to high flow. No flow and moderate flow (0.4 L/min; 0.11 gal/min) showed similar weight gain with high flow having the greatest weight gain. Significant differences ($p<0.05$) in growth, length and weight, were observed for larval yellow perch raised in different turbidity levels. Slightly turbid water (50 NTU) resulted in the greatest growth, both length and weight, when compared with clear water (0 NTU) and turbid water (100 NTU).

Based on the broad husbandry conditions a second year of larval yellow perch culture was completed with narrower tank conditions that included only black tanks and water surface spray and turbidity that centered on the optimal conditions identified in the first year. No differences in growth, length and weight, were observed every two weeks nor were differences observed after 70 days for larval yellow perch raised under these more stringently defined husbandry conditions. In contrast to similar percid studies culturing Walleye (*Sander vitrus)* larvae, modifications in culture operations to address similar physical and behavioral barriers were not effective in improving yellow perch larvae survival. Approximately 3% of the larval yellow perch survived until the end of the 70-day experiment regardless of treatment.

Two commercial-scale algae auto feeders and rotifer production systems were constructed that allow us to culture and concentrate the needed number of rotifers to feed at a rate and concentration deemed necessary for the amount of fry in each tank. Eight rotifer/*Artemia* auto-feeders were constructed and paired with sixteen yellow perch fry production tanks. Twelve feeding regimes or diets were tested for newly hatched yellow perch larvae starting from day 3 dph for improve survival rate. Two effective regimes were identified.

Several other related studies was completed: 1) by monitoring egg size produced by different strains/families, we have identified some strains/families that produced significantly larger-mouth gape progeny and larger eggs than others; 2) variation of egg size is dramatically different among strains of our genetically improved fish, indicating there is a large range of selection for large eggs; 3) we found predation and ingestion of prey at the beginning of feeding is limited by the mouth gape in fish larvae which determine larvae survival. Examination of food in digestive tract, combining with mouth size as well as total length data proved that the yellow perch larvae were able to ingest live feed, either rotifer or *Artemia*, when only total length reached 6 mm and mouth gape reached 0.6 mm, regardless the age of larvae, which was usual from 1 to 7 dph. Swim bladders started to inflate as early as 4 dph, and were correlated with the size of fish. When pooling all families together, we found that the swim bladder would inflate when larvae the size reached 6.7 - 9.7 mm, regardless the age of fish. Yolk disappeared between 7 and 11 dph for most of larvae. For one family, yolk was still observed in 70% of larvae at 11 dph.

Two videos were produced on "Feeding Yellow Perch Fry" and "Growing & Maintaining Natural Feeds for Larval Fish" by Iowa State University. They are available on the NCRAC website under the "videos" tab and on the NCRAC vimeo channel at https://vimeo.com/channels/958980 . Associated with this project is the video "Develop systems and diet strategies to reduce yellow perch larval mortality burst in indoor RAS" is at https://www.ncrac.org/video/develop-systems-and-diet-strategies-reduce-yellow-perch-larval-mortality-burst-indoor-ras. An addition video entitled "Developing Systems and Diet Strategies to Reduce Larval Yellow Perch Burst Mortality in Indoor RAS" is also available on the NCRAC website https://www.ncrac.org/video/develop-systems-and-diet-strategies-reduce-yellow-perch-larval-mortality-burst-indoor-ras . These materials discuss the results and conclusions drawn from the 2-year study on husbandry and culture conditions that resulted in the best survival of larval yellow perch under variable culture conditions including intensive tank rearing at the UW-Stevens Point Northern Aquaculture Demonstration Facility.

Impacts

The impacts of this proposed project will be primarily through the development of systems and strategies to enhance mass production and survival of yellow perch from onset of first feeding up to 70 days. However, data garnered from this project indicates that early rearing of yellow perch using modified culture conditions or feeding regimes using commercial diets is not yet practical to the commercial yellow perch aquaculture community. Information garnered from this include recirculating system components, feeding strategies and feed types for successfully reducing the early-life stage bottlenecks, and online training modules available through the NCRAC website for aquaculturists in the NCR. The online training modules will provide direct information to yellow perch culturists on current and updated procedures and protocols for reducing yellow perch larval mortality burst in indoor recirculating aquaculture systems.

Recommended Follow-Up Activities

NCRAC-funded researchers have concluded that it is extremely difficult to improve survival rate through either culture conditions modification, i.e., tank color, water turbidity or surface spray, or different feeding regimes. One key reason for poor indoor survival of yellow perch larvae is the mouth gape limitation of newly hatched. Predation and ingestion of prey at the beginning of feeding is limited by the mouth gape in larvae, which determine larvae survival. Yellow perch larvae are not able to ingest small size *Artemia* (~428 um) until their total length reaches >6 mm and mouth gape >0.6 mm. Even though researchers provided an adequate number of rotifers small enough to be consumed by the fish, there were still high levels of deformities and larvae survival rates were still poor, as reported by others. These findings suggest that digestive enzymes may not establish in perch larvae until they reach >6 mm in length.

Future projects should investigate the role of digestive enzymes and their relation with larvae survival of yellow perch. As with other fish species with small larvae, there continues to be a need to further develop larval diets that are both small enough to be consumed and processed by the limited digestive enzymes present early in the development of the larvae.

In addition to studies investigating the perceived limited number of digestive enzymes in yellow perch larvae, a project to select yellow perch strains that have a larger mouth gape to improve egg quality and survival is suggested. To date, researchers have identified some strains/families that produced significantly larger-mouth gape progeny and larger eggs than others, indicating there is a large range of selection for larger-mouth gape traits. Therefore, developing yellow perch broodstock (using identified strains/families) with larger-mouth gape and larger egg size to increase indoor survival of larvae and fry has the potential to enhance yellow perch industry development.

At the same time, there is a need to identify more strains and families that genetically produce larger-mouth gape progeny and larger eggs, using genetically improved broodfish from different families/strains with different weights and ages. For both parts, broodfish can be selected, tagged and genotyped. Molecular pedigrees can be determined and a genetic relatedness chart developed. At least 60 pairs of related fish, with the highest breeding value can be selected for production of progeny. Newly hatched larvae (1^{st} day dph) from each family can be subject to measurement of total length and the size of mouth gape. The parents that produced the largest mouth and fastest growing progeny can be selected as next generation of large-mouth broodfish,

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Yellow Perch activities.