PROJECT COMPLETION REPORT

98-4A **"Determination of Optimal Conditions for Swimbladder Inflation**
98-4CE **in Striped Bass Larvae Reared in Intensive Systems"**

Termination Report Period: March 1, 1999 through August 15, 2000

NRAC Total Funding: $84,642 (March 1, 1998 – Febuary 28, 2000)
(No-Cost Ext'n through August 15, 2000)

Principal Investigator: William F. Van Heukelem, University of Maryland, Center for Environmental Science, Horn Point Laboratory, P.O. Box 775, Cambridge, MD 21613

Participating Investigator/ Cooperative Agencies:

Reginal M. Harrell	University of Maryland	Maryland
Steven G. Hughes	University of Maryland	Maryland
Scott Lindell	AquaFuture, Inc. (now Fins Technology)	Massachusetts
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REASON FOR TERMINATION

Objectives were completed and funds were spent for project.

PROJECT OBJECTIVES:

The overall objective of this project was to determine the optimal conditions for increasing numbers of striped bass larvae with inflated swimbladders in intensive culture systems and to disseminate the information obtained to producers in the industry. Specific objectives for the two year study at the three sites were as follows:

Year 1, In 70 liter tanks at Horn Point Laboratory (HPL), Maryland.

1. Determine optimum light intensity for swimbladder inflation.
2. Determine optimum water turbidity and light level for swimbladder inflation.
3. Determine effect of spray bars on swimbladder inflation.
4. Present results at a national Aquaculture meeting.

Year 1, In beakers at University of Maryland Eastern Shore (UMES).

1. Determine effects of three surfactants on swimbladder inflation.

Year 2, HPL.

Using a combination of optimal conditions of light intensity, turbidity and spray bars found during the first year of the study at Horn Point:

1. Determine if the best surfactant found in year 1 studies in beakers at UMES is of added benefit in 70 liter tanks.
2. Determine if the best combination of factors found in the preceding experiments are equally effective when used in production scale (900 and 2,000 liter) tanks.
3. Verify that optimal conditions determined in prior experiments work with larvae from five additional broods of larvae.
4. Present results at a national aquaculture meeting
5. Prepare a fact sheet on optimal conditions for swimbladder inflation
6. Prepare results for publication in a scientific journal.

Year 2, UMES.

1. Determine effects of antibiotics on swimbladder inflation.
2. Determine effects of water hardness on swimbladder inflation.

Year 2, AquaFuture, Massachusetts:

1. Determine effectiveness of techniques developed at HPL and UMES under "real world" production conditions at a different facility.

Year 3, HPL (Drs William Van Heukelem and Reginal Harrell)

1. Determine maximum stocking density that will still produce high rates of swimbladder inflation.
2. Determine optimum water hardness for swimbladder inflation based on additional research to be performed at UMES.
3. Determine if new surfactants pre-tested at UMES in 2 liter beakers can increase swimbladder inflation in our larger tanks.

Year 3,UMES (Dr. Steven Hughes)

1. Expand previous experiments to determine optimum water hardness for swimbladder inflation and survival including the interaction of hardness with chlorides or magnesium.
2. Test new surfactants and concentrations for effects on swimbladder inflation and survival. Medical surfactants which are used with respiratory patients and premature infants will be tested.

Year 3, Fins Technology (Scott Lindell)

1. Test best conditions for swimbladder inflation as found at HPL and UMES under "real world conditions" at a commercial facility. They will use three broods of larvae and compare results with those obtained using their normal rearing conditions.

Year 3 Outreach

We will present results at Aquaculture '01 as well as in a publication and will prepare a new fact sheet on optimal conditions for swimbladder inflation

Results obtained from this project will be presented at a national aquaculture meeting at the end of each year of study. Results will also be published in a scientific journal at the end of the study. In addition, a fact sheet will be developed and distributed and made available for publication on the NRAC Home Page on the World Wide Web.

ANTICIPATED BENEFITS:

This project will increase our understanding of conditions that must be provided to produce batches of striped bass larvae with a high percentage of inflated swimbladders. Results should be applicable to hybrid striped bass also as well as other species cultured in intensive systems. Using the techniques learned from our studies, researchers and producers will be able to produce more larvae from each spawning that are viable, grow well and are free from lordosis that is caused by uninflated swimbladders.

PRINCIPAL ACCOMPLISHMENTS:

Year 1 (1998)

Three experiments were performed in April 1998 in five independent recirculating systems each containing ten 70 liter tanks. Five replicate tanks were used to test the effects of each variable in the first two experiments.

In the first experiment the effects of light intensities of 0, 10, 25, 50, 100, 250, 500, 1000 and 2000 lux were studied. The percentage of larvae with inflated swimbladders was highest in the two lowest light intensities but there was a block effect among the different systems that clouded the results to some extent.

In the second experiment effects of Aquashade concentrations 0, 2, 4, 8 and 16 ppm were tested at two light intensities (1 and 10 lux). There was no difference in the percentage of larvae with inflated swimbladders at any of the tested concentrations of Aquashade or at either light level. The percentage of larvae with inflated swimbladders averaged 98% in all treatments.

Although we planned to study the effects of spray bars in the third experiment, upon reviewing our preliminary results from 1997 (funded by HPL) it was clear that spray bars did not increase the percentage of larvae with inflated swimbladders. Therefore, instead of repeating that experiment we decided to examine the effects of direct verses indirect light at a low level of intensity.

In the third experiment the effects of direct verses indirect light were studied. Light intensity at the surface of each tank was maintained at 15 - 20 lux. A total of 40 tanks were used and half of the tanks received direct lighting whereas the other half received indirect light bounced off the ceiling. There was no difference in swimbladder inflation between tanks receiving direct vs indirect light.

Three surfactants (Span 20, Witconol 14, and Glucopon) were tested as surface sprays in two-liter beakers using stock solutions of 10, 100 and 500 ppm. Only Witconol 14 showed promise as an aid to increasing swimbladder inflation in striped bass larvae and only at the lowest concentration tested. Survival in the 10 ppm Witconol treatment was slightly lower than in the control treatment and swimbaldder inflation showed a significant ($P<0.05$) increase.

Results were presented at Aquaculture Americas '99 in Tampa, FL, January 1999.

Year 2 (1999)

Three experiments were performed at HPL in April 1999 in five independent recirculating systems each containing ten 70 liter tanks. Larvae were stocked in tanks at a density of 30 /L (2,100 per tank) at three days post hatch and were reared at 18^{o}C and 3 ppt salinity. The percentage of larvae with inflated swimbladders was determined on day 8 post hatch.

In experiment one, the effects of ten light intensities from 0 to 2000 lux were studied in five replicate tanks per light level. Light intensities were held constant during this experiment. The percentage of larvae with inflated swimbladders was highest in constant dark (0 lux). Similar results were obtained at AquaFuture, Inc. (Turners Falls, Massachusetts) in covered vs uncovered 125 gal tanks.

In experiment two, effects of two surfactants and a 12:12 light cycle were tested. The use of surfactants (Witconol 14 and Glucopon) did not increase swimbladder inflation and the 12:12 light cycle was detrimental to swimbladder inflation when compared to constant dark.

In experiment three, swimbladder inflation was studied in 5 different broods of larvae using the best conditions found during the previous experiments. All tanks remained constantly dark (0 lux) and a total of 40 tanks were used. Half of the tanks were sprayed with a surfactant solution (Glucopon at 5 ppm) twice daily. The other 20 tanks served as controls (no surfactant). There was no difference in swimbladder inflation between the surfactant treatment and control. One brood of larvae had significantly lower swimbladder inflation than the others.

Experiments at the University of Maryland Eastern Shore in 2 L beakers showed that the surfactant Witconol 14 enhances swimbladder inflation. The effects of three antibiotics on swimbladder inflation were also studied and none were beneficial. Increasing calcium content of rearing water to 250 ppm resulted in higher swimbladder inflation rates.

Swimbladder inflation in production scale tanks (2000 L) at HPL was poor in comparison to that obtained in the 70 L experimental tanks. We believe that the poor inflation in these tanks was due to high densities of eggs and larvae (resulting in heavy oil films) and low water hardness.

Results were presented at Aquaculture Americas 2000, in New Orleans, LA, February, 2000.

A fact sheet (NRAC Publication No. 00-006) was produced in May, 2000.

Year 3 (2000)

Several experiments were performed at HPL in April 2000 in five independent recirculating systems each containing ten 70 liter tanks. Separate experiments also were performed in 1000 L and 2000 L tanks to determine if results obtained in small tanks could be obtained in larger tanks. Larvae were stocked in tanks at a density of 30 /L and 60/L at three days post hatch and were reared at 18 - 20^{o}C and 0 or 3 ppt salinity. The percentage of larvae with inflated swimbladders was determined on day 8 post hatch.

Effects of water hardness were studied by rearing larvae in fresh well water. Calcium hardness was increased by adding calcium chloride to obtain hardness of 20, 70, 120 and 170 mg/L. Swimbladder inflation was somewhat better at higher hardness levels (95 % at 170 mg/L versus 91 % at 20 mg/L). Larvae did equally well at densities of 30 and 60 /L in 70 L black cones but swimbladder inflation was better at the lower density in 1000 L tanks.

Results in 1000 and 2000 liter tanks were mixed. By covering tanks with black plastic we were able to obtain better swimbladder inflation than we had in previous years, but we could not consistently obtain the high inflation rates (> 90%) in our large tanks that we obtained in 70 L black cones.

Results will be presented at Aquaculture '01 in Orlando, FL in January, 2001.

IMPACTS:

Using the techniques learned from our studies, researchers and producers will be able to produce more larvae from each spawning that are viable, grow well, and are free from lordosis that is caused by uninflated swimbladders.

RECOMMENDED FOLLOW-UP ACTIVITIES:

Because oil on the surface of the water (from decomposing eggs and larvae) prevents some larvae from penetrating the surface of the water to obtain air for initial swimbladder inflation, the search for effective, non-toxic surfactants should continue as these hold the most promise for increasing the percentage of larvae with inflated swimbladders in high density culture. Turbidity, produced by adding clay to the water, has proved to be an aid in swimbladder inflation in walleye larvae but has not been investigated in striped bass larvae, and this should be done. More research should also be done on the relationship of water hardness, both calcium and magnesium, to swimbladder inflation. Our research has focused on striped bass but we think that the techniques should also apply to hybrid striped bass larvae as well as unrelated species. Experiments should also be performed to verify our belief that the techniques we used will work with other species.

PUBLICATIONS:

Van Heukelem, W.F., J.M. Jacobs, R.M. Harrell, S.G. Hughes, S. Lindell and B. Friedmann. Optimal conditions for swimbladder inflation in striped bass larvae reared in intensive systems. Northeastern Regional Aquaculture Center Publication No. 00-006 (May, 2000).

PAPERS PRESENTED:

William F. Van Heukelem, John M. Jacobs, Reginal M. Harrell, and Steven G. Hughes. 1999. Effects of Light Intensity, Aquashade, and Surfactants on Swimbladder Inflation in Larval Striped Bass. Aquaculture America '99, January 27 - 30, 1999, Tampa, Florida

William F. Van Heukelem, John M. Jacobs, Pilantana Anderson, Megan O'Connor, Steven G. Hughes, Scott Lindell, Bruce R. Friedmann, and Reginal M. Harrell. Effects of Light Intensity, Photoperiod, Water Hardness, and Surfactants on Swimbladder Inflation in Larval Striped Bass. Aquaculture Americas 2000, February 2 - 5, 2000, New Orleans, Louisiana.

Paper to be presented at Aquaculture '01 January 21-25, 2001, Orlando, Florida: William F. Van Heukelem, John M. Jacobs, Pilantana Anderson, Megan O'Connor, Steven G. Hughes, Scott Lindell, and Reginal M. Harrell. Swimbladder Inflation in Larval Striped Bass: Effects of Water Hardness, Larval Density and Tank Size.

The Full Report with all the data, graphs and tables is available at the NRAC office upon request.