PART I:

PROJECT TITLE: Efficient, Rapid Assay for Predicting the Growth Rate of Aquaculture Species Based on Metabolic Rate of the Fertilized Egg

REPORT GIVEN IN YEAR 2014

REPORTING PERIOD: 1/15/2014-9/5/2014

AUTHOR: Benjamin Jennings Renquist

FUNDING LEVEL: $60,000/year for 2 years

The investigators had initially requested $120,000/year over 4 years to conduct studies in Tilapia and Trout, as well as the role of polyploidy and methyl-testosterone hormone treatment on assay performance. As all of the preliminary data was collected in Zebrafish, the board asked that the scope of the study be limited to Tilapia in year 1 and Oysters in year 2, with a primary focus on assay development and fish growth.

PARTICIPANTS: Benjamin Renquist*, University of Arizona
Kenneth Overturf, USDA, Hagerman Fish Culture Experiment Station
Gary Freitag, University of Alaska

PROJECT OBJECTIVES:

1. Confirm that the AlamarBlue® assay, developed in zebrafish, can be applied to tilapia.
2. Test the applicability of the AlamarBlue® assay in predicting growth rate of oysters

ANTICIPATED BENEFITS: We anticipate that this research will establish the validity of measuring metabolic rate of the embryonic fish to predict the genetic potential for growth. Growth of fish depends on many conditions including dominance, feed availability, water quality, and water temperature. Thus, selection of breeding fish based on growth isn't based on the genetic potential for growth. This assay can be employed to 1) identify brood stock that pass along the highest genetic potential for growth to their offspring 2) select future brood stock that have the highest genetic potential for growth 2) identify crosses that best improve genetic potential for growth and 3) evaluate treatments that may modify the epi-genetic potential for growth (e.g. nutrition, water temperature, salinity).

The genetic potential for milk production increased rapidly in the dairy industry as individual data on milk production was collected and the cows with the most potential to produce milk were identified and selectively bred. We expect that selection of fish that have the most genetic potential for growth could have more robust and rapid effects in aquaculture as the number of offspring/breeding is much larger and the generation interval for some species much shorter.
PROGRESS AND PRINCIPAL ACCOMPLISHMENTS: The progress and accomplishments to date are described in detail.

Major Procedural Accomplishments:
1. Developed collaborative arrangement with Desert Springs Tilapia to perform grow-out studies at production level.
2. Developed a secondary study to be funded by Desert Springs Tilapia that will compare the selection of breeders based on fish growth versus selection based on metabolic rate of the embryo.
   a. Of note, selection based on the embryo will limit the costs of rearing large groups of non-sex determined fish.
3. Developed streamlined method to assay 1000s of fish/day (4,000 eggs in a single day).

Major Scientific Accomplishments:
1. Confirmed that metabolic rate measured by AlamarBlue® assay corresponded with measures based on oxygen consumption.
   a. By varying the number of fish/well and regressing the results obtained using an assay that measured oxygen concentration to results obtained using the AlamarBlue® assay, we can confidently state that the signal generated by each of these assays is correlated.
2. Established that assay could measure changes in metabolic rate in Tilapia.
   a. We have shown that AlamarBlue® signal increases as we 1) increase the number of tilapia within a well and 2) increase the incubation time.
3. Established that assay could measure changes in metabolic rate in Trout Eggs.
   a. We have shown in Trout that AlamarBlue® signal increases as we increase the number of trout within a well.
4. Showed that relative metabolic rate amongst embryos was maintained across days.
   a. We measured metabolic rate of eggs at 3 days post-fertilization. We subsequently segregated these eggs into quartiles and measured metabolic rate of the same eggs on day 4 post-fertilization. Eggs with the highest metabolic rate on day 3 had the highest metabolic rate on day 4. This suggests that relative metabolic rate does not vary across days.
5. Established that embryos display significant variation in metabolic rate.
   a. For selection based on this assay to improve genetic stock there must be sufficient variability in the metabolic rate of fish. We see that the fluorescence change induced by embryos varies 25 fold. Suggesting that variation in metabolic rate is considerable.
6. Found that fish selected to have metabolic rates in the top quartile have increased growth (24%) relative to fish selected to have low metabolic rates.
   a. Embryos in the highest quartile for metabolic rate grow 24% faster than embryos in the lowest quartile for metabolic rate. Growth to harvest size and time to reach harvest size will be determined.

USEFULNESS OF FINDINGS: The work completed thus far, suggests that by selecting embryos with a high metabolic rate we are selecting fish with an increased potential for growth. This could prove extremely useful in the selection of brood stock. Current selection practices based on growth are not only indicative of the genetic potential for growth, but also social hierarchy, water conditions, and feed seeking behavior. Moreover, as females must be selected based on growth in mixed sex tanks, female selection is further affected by sexual behavior. By selecting embryos that are still dependent on the yolk for nutrition, we hypothesize that we are selecting fish with the most genetic potential for growth. This selection criterion appears to hold similar power to the individual data collected on milk production in dairy cows and is expected to allow improvements of the same or greater magnitude.
WORK PLANNED FOR NEXT YEAR:

Tilapia Studies: In the next year, we will continue the growth studies at Desert Springs Tilapia. These studies will include monthly growth monitoring to harvest size and proximate analysis fish body composition.

Additionally, we plan to perform a privately funded study to compare breeder selection using the AlamarBlue assay to breeder selection based on the more traditional growth trajectory.

Oyster Study: We expect to begin and complete studies at the Hatfield Marine Science Center in collaboration with Chris Langfield on the effect of metabolic rate in growth of oysters. Since social influences likely play a lesser role in oyster growth than in tilapia growth, we expect that selection based on growth potential would have an even more robust effect in oysters, where social interactions are likely to have little effect on growth. The first test of this assay in oysters will compare the metabolic rate of spat from a fast growth line to that of a slow growth line. Subsequent tests will be performed similarly to the studies in Tilapia.

Studies to Ease Assay Application: The goal of this work is to develop an assay that can be used by producers on a large scale. As such, we would like make the assay simpler to implement by developing a 96-well microplate insert that can be used to expose and remove fish from Alamar Blue solution. Different inserts will be developed and tested throughout the year.

SUPPORT:

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BUDGET:

INSTITUTION: University of Arizona
PRINCIPAL INVESTIGATOR: Benjamin Renquist

SALARIES $25,500.00

- Research Specialist Senior (50%) $23,500.00
- Hourly Student $2,000.00

BENEFITS $11,364.54

- Research Specialist Senior $11,280.00
- Hourly Student $84.54

TRAVEL $6,966.00

- Mileage (Desert Springs Tilapia) 348 miles RT 8 trips $0.45/mile $1,566.00
- 4 Flights from Tucson, AZ to Portland, OR $2,400.00
- Rental Car and Gas for 2 Trips to Oregon $1,000.00
- Hotel (10 nights, 2 rooms) $2,000.00

SUPPLIES $4,000.00

- AlamarBlue $700.00
- Assay Plates $300.00
- General Laboratory Reagents $3,000.00

EQUIPMENT $0.00

OTHER DIRECT COSTS $11,850.00

- 3D Printer Costs $1,250.00
- Publications (8 pages at $75/page) $600.00
- Contract rearing/growth assessment (Oysters) $10,000.00

TOTAL: $59,680.54
BUDGET NARRATIVE:
Salaries: This includes 50% of salary for Mark Higgins, M.S. Mark has been integral to the successful implementation of the work presented herein. He will perform metabolic rate assays in oysters, monitor growth in Tilapia, measure body composition in tilapia and oysters, and test assay plates designed to ease implementation. This also includes hourly wages for undergraduate student help. We expect to hire 1 student at 10 hours/week for 25 weeks.
Benefits: Calculated based on ERE rates of 48% for Research Specialist Senior and 3.8% for hourly students.
Travel: Costs to travel to Desert Springs Tilapia and to make 2 trips to Oregon to set up studies at Hatfield Marine Science Center and to collect samples for analysis thereafter. Duration of Stay for both trips - 10 days.
Supplies: Cost of AlamarBlue®, 96 well plates, and general lab supplies (sterile filters, pipette tips, paper towels, sodium bicarbonate, test tubes, reagents for proximate analysis etc.)
Other Direct Costs: 3-D printer costs – include costs develop and print model inserts for 96 well plates, whose application will be tested in tilapia. Publication costs – we expect this work to yield one publication that will be approximately 8 pages in length. Contract oyster rearing and growth monitoring – This is the cost to perform the oyster studies with Chris Langford at Hatfield Marine Science Center.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED: This work has not yet been presented or published. However, we did present the findings in the zebrafish at the World Aquaculture Conference, Nashville, TN 2013.

SUBMITTED BY: ___________________________ Benjamin J. Renquist
Title: PI Date: 9/5/2014

APPROVED: ____________________________________________________________
Project Manager Date: 9/8/2014