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 2016

PROJECT WORK PERIOD: 1/5/2014-9/15/2016

AUTHOR: Benjamin Jennings Renquist

PARTICIPANTS: Benjamin Renquist*, University of Arizona
Kenneth Overturf, USDA, Hagerman Fish Culture Experiment Station
Gary Freitag, University of Alaska, Outreach Coordinator
Tark Rush, Desert Springs Tilapia, Industry Advisor
Chris Langdon*, Oregon State University
Matt Powell, University of Idaho, Project Monitor
Leo Ray, Fish Breeder of Idaho, Industry Advisor

REASON FOR TERMINATION: Objectives Complete

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

PRINCIPAL ACCOMPLISHMENTS: The progress and accomplishments to date are described in detail.

Major Procedural Accomplishments:

1. Developed collaborative arrangement with Chris Langdon to assess metabolic rate in oysters.
2. Submitted a patent cooperative treaty application 1 year after a provisional patent application to initiate commercialization of this assay.
3. Developed a funded USDA-NIFA proposal to extend this work with a focus on brood stock selection.
4. Journal of Visual Experimentation Video publication and video. The video has been placed on my university website.
5. Submitted a WAS 2017 Abstract
6. eXtension publication entitled "Applying a Test Developed to Combat Obesity and Diabetes to Improve Growth in Fish, Mollusks, and Crustaceans" and webinar presentation given on 9/15/16.

Major Scientific Accomplishments: Those underlined were not funded by WRAC funding, but are included as they extend the findings of the WRAC funding.

<u>Tilapia</u>

1. Established that embryos with a metabolic rate in the highest quartile maintain a growth advantage to harvest size/age over embryos with a metabolic rate in the lowest quartile.
    a. We showed a 29% increase in body weight at harvest in fish that were fed identical amounts of feed.
2. Extended application of the assay to include skeletal muscle and caudal fin explants.

   a. We found that within a fish as the explant mass increased so did the signal generated from this assay
   b. We also found that the signal generated from this assay increased with time.
   c. Additional studies with fin clips have been funded through USDA-NIFA yielding significant results. In fact, the embryonic metabolic rate is inversely related to the metabolic rate measured from fin clips. This suggests that selection of broodstock based on a low metabolic rate of the fin clip may be a valid selection criteria.
3. Initiated trials to use photographic analysis of a plate to perform a non-biased assessment of metabolic rate within a well by assessing the RGB color from the photograph.
4. Tested the effect of metabolic rate on feed efficiency. Initial results suggest a modest improvement in feed efficiency in fish that are selected have a high metabolic rate (4 ± 1.5%). Preliminary data collected in fish whose metabolic rate was assessed in February suggests a 23% improvement in feed efficiency when water temperature was maintained at 79º.
5. Developed studies to segregate aggression from growth. To complete preliminary data for these studies we first selected fish that have a high or low metabolic rate then at 2 months segregated the fast and slow growing individuals within a metabolic rate group. Thus we have 4 groups: 1) slow growing high metabolic rate, 2) fast growing high metabolic rate, 3) slow growing low metabolic rate, and 4) fast growing low metabolic rate.
   a. For these studies we segregated the largest and smallest fish from each tank that were previously selected based on metabolic rate.
      - As mentioned in point 4, fish with a high metabolic rate have improved feed efficiency.
   b. Our data shows that slow growing fish had similar feed efficiency to that of fast growing fish once segregated.
      - slow growing fish (1.16 ± 0.029) have similar feed efficiency to fast growing fish (1.14 ± 0.035); P = 0.18).
6. Developed at hypothesis that selection for metabolic rate will more robustly affect growth in fish reared below optimal temperatures for growth than in fish reared at optimal temperatures for growth.
   a. Rationale: At high water temperatures metabolic rate of all fish is elevated by water temperature. Thus, there is little difference in metabolic rate even amongst fish selected to have vastly different basal metabolic rates. Accordingly, in warm water all tilapia grow quickly. The current grow-out studies have been performed with water temperatures that have exceeded 85ºF
      - Preliminary trials conducted using a system filled with water from a cold water well (68ºF) at Desert Springs Tilapia, suggests that metabolic rate more robustly affects growth rates in fish reared in water temperatures below those ideal for growth.
   b. A final study of growth and feed efficiency will begin in the winter with water temperature held at 72 ºF. We expect embryonic metabolic rate to more robustly alter growth and feed efficiency in this cold water than in the warm water tests run over this past summer.

Oysters

1. Confirmed that this assay can be applied to measure metabolic rate in oyster D-larvae and spat.
   a. Showed that by increasing the number of D-larvae or spat within a well we could increase the signal
   b. Found that the signal generated by oysters increased with time of exposure to AlamarBlue.

   c. Confirmed that when spat oysters are sized the smaller oysters generate a lower signal than their larger siblings
   d. Showed that variability between families to allow for improvement based on family crosses
   e. Found that variability within a family was quite robust and that individual selection may allow for more rapid improvement of oyster stock.
2. Showed that temperature (14, 24, or 30ºC) and salinity (10-45 ppt) robustly alter oyster spat metabolic rate.
   a. Metabolic rate of oyster spat increases with temperature and with salinity.
3. Showed that when oyster spat (≈1 mm diameter) are segregated into high and low metabolic rate quartiles:
   a. High metabolic rate (top 25%) oyster spat survive better (82 ± 2.5%) over the next 2 months than low metabolic rate oyster spat (62 ± 4%; $P < 0.0001$).
   b. Average mass of high metabolic rate oyster spat did not differ from average mass of low metabolic rate oyster spat at the conclusion of 2 months.
   c. The increased survival resulted in an increased total mass of the surviving high metabolic rate oyster spat than low metabolic rate oyster spat ($P = 0.019$).
   d. Showed that metabolic rate of the 3-month oyster spat was highly correlated with body mass ($R^2 = 0.68$).
4. Performed metabolic rate assays in D-larvae and 8-day embryonic oysters from 53 family crosses. Growth was assessed in these same 53 families to 3 months. Data interpretation is awaiting oyster counts for each of over 1000 wells with an estimated 400 individuals/well.

Shrimp

1. Showed that this assay can be applied to measure metabolic rate in young shrimp and that increasing the number of shrimp/well increases the signal generated within a well.

IMPACT STATEMENT:

1. Tilapia: Problem: Selection of broodstock based on growth results in selection for dominance. Action: Developed a tool that can be used to identify tilapia with an improved genetic potential for growth and improved feed efficiency. Impact: This tool will allow for informed brood stock selection that is independent of aggression and feed intake. Moreover, we propose future studies that will prove that this tool can be used to select against aggression. Selection against aggression will allow for fish domestication, which will improve feed efficiency and decrease morbidity and mortality. The Renquist laboratory (PI: Benjamin Renquist) is responsible for this finding. Contact: bjrenquist@email.arizona.edu
2. Oysters: Problem: Through these studies we have established that the variability in oyster metabolic rate is greater within a family than between families. Thus, family cross based selection does not allow for the most rapid advancements in genetic selection. Action: We have validated that we can quantify the effects of temperature and salinity on metabolic rate in oysters. Additionally, we established that metabolic rate of oyster spat is related to survival rate. Initiated studies in D-larvae and 8-day embryonic oysters that will allow us to address the potential of this assay to inform crossing strategies. Impact: These studies recommend that selection of individuals with low metabolic rates will improve oyster production. The Renquist laboratory is responsible for this finding. Contact: bjrenquist@email.arizona.edu
3. Shrimp: Showed that this assay could be applied to crustaceans. The Renquist laboratory is responsible for this finding. Contact: bjrenquist@email.arizona.edu

This project has provided the aquaculture industry a tool to inform selection of individuals that are superior for growth and feed conversion.

RECOMMENDED FOLLOW-UP ACTIVITIES:

Will Be Performed with Current Funding (Expires 7/30/17)

1. Finish current grow-out study in Tilapia comparing fish with a high and low metabolic rate (Warm water; logged 3 times daily).
2. Perform cool water grow-out study in Tilapia comparing the growth and feed efficiency of fish that have a high and lower metabolic rate as embryos (Water will be maintained at 76ºF)
3. Publish Tilapia research to date.
4. Count D-larvae and 8-day old oyster larvae to normalize data from 53 families, measure DNA and protein from these same samples for additional normalization. Compare metabolic rate/oyster larvae to growth rate measured by Chris Langdon's group at Hatfield Marine Science Center
5. Publish Oyster research

Funded by USDA-NIFA Award:

1. Compare metabolic rate of skeletal muscle biopsies from fish selected to have a high or low metabolic rate as embryonic fish (Allows for comparison against fin biopsies, which showed a metabolic rate that was lower in fish that were previously selected to have a high metabolic rate).
2. Compare broodstock selected based on the metabolic rate of a fin or skeletal muscle biopsy.
3. Perform RGB analyses to compare against fluorescent measures (Pictures and Fluorescence have already been collected. Only computer analysis remains)

Proposed New Studies:

1. Perform studies that aim to employ this assay to eliminate aggression in fish, through behavioral selection against aggression
2. Address the potential to apply this assay to assess the response to altering broodstock management.

SUPPORT:

YEAR	WRAC-USDA Funding	OTHER SUPPORT					Total Support
		University	Industry	Other Federal	Other	Total	
2014	60,000	3562.50	2,000				$65,562.00
2015		3562.50	2,000	35,625			$41,187.50
2016	60,000	3562.50	2,000	142,500			$208,062.50
2017		3562.50	2,000	106,875			$112,437.50
Total	120,000	14,250	8,000	285,000			$427,250.00

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

- Renquist, B.J. 2016. Applying a Test Developed to Combat Obesity and Diabetes to Improve Growth in Fish, Mollusks, and Crustaceans. eXtension https://learn.extension.org/events/2803
- Kentch, K., C. Foy, V. Maxwell, and B. Renquist. 2017. Measuring Metabolic Rate of Embryonic Fish to Predict Growth Rate. World Aquaculture Society: Aquaculture America 2017. San Antonio, TX. Submitted.

- Williams, S.Y. and B.J. Renquist. 2016. High Throughput Danio Rerio Energy Expenditure Assay. JoVE. Jan 27;(107):e53297.

SUBMITTED BY: _____ **9/13/2016**

Title: (PI and Work Group Chair) Date

APPROVED: _____ **9/15/2016**

Project Monitor Date

PART II:

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 2016

PROJECT WORK PERIOD: 1/5/2014-9/15/2016

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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

TECHNICAL SUMMARY AND ANALYSIS

In this research project we set out to apply an assay that monitors metabolic rate in aquatic species to improve production aquaculture. Prior to initiation of this study, the positive relationship between energy expenditure and growth in fish had been well established. This link between metabolic and growth rates suggested to us that metabolic rate may be an ideal measure to improve brood stock selection for growth.

Maximizing growth rates of aquaculture species minimizes the time to achieve a marketable size and decreases the investment time and expense of rearing slow growing individuals or families. By increasing growth rate we will decrease the proportion of total dietary energy that goes to meet maintenance energy demands and increase the proportion that goes toward growth. As such, selection for increased growth rate is expected to improve feed conversion ratios and profitability.

Problem: Genetic improvement in aquaculture has been slow, despite advantages associated with high fecundity and, in some cases, short generation intervals.

The high fecundity of fish, which allows for strict selection criteria, should result in robust genetic improvements for any trait. However, this is dependent on the selection criteria being an accurate representation of the genetic potential for a given attribute. Social hierarchy robustly influences fish growth, with dominant fish eating more and growing more quickly than subordinate fish. Thus selection of fast growing individuals is selection for dominance, not growth potential. Accordingly, brood stock selection for growth has yielded little improvement in growth rate of fish species except under the most stringent of conditions designed to limit aggression.

Production aquaculture has been limited by brood stock selection based on dominance driven growth, not the genetic potential for growth. Within this project, we aimed to identify a phenotypic variable that better assessed the genetic potential for growth.

In poikilotherms metabolic rate is directly related to the synthesis of protein and DNA for growth. Oxygen consumption, the gold standard for measuring metabolic rate/energy expenditure, is indicative of growth rate. Oxygen consumption per unit of body weight is higher in fast growing, growth hormone overexpressing salmon than in wildtype salmon. Despite the knowledge linking energy expenditure to growth, application of this information to select brood stock has been limited by the difficulty in large scale assessment of oxygen consumption. Oxygen consumption systems that depend on a closed chamber circulating system are limited in throughput by the number of chambers available. We have developed and validated an assay focused on measuring the production of $NADH_2$, which is tightly linked to O_2 consumption. Over the 3 years of this project we have conducted research aimed at 1) Confirming that the AlamarBlue® assay can be applied to tilapia and 2) Testing the applicability of the AlamarBlue® assay in predicting growth rate of oysters.

Major Procedural Accomplishments:

1. Submitted a patent cooperative treaty application 1 year after a provisional patent application to initiate commercialization of this assay.
2. Developed a funded USDA-NIFA proposal to extend this work with a focus on brood stock selection.
3. Journal of Visual Experimentation Video publication and video. The video has been placed on my university website.
4. Submitted a WAS 2017 Abstract
5. eXtension publication entitled "Applying a Test Developed to Combat Obesity and Diabetes to Improve Growth in Fish, Mollusks, and Crustaceans" and webinar presentation given on 9/15/16.
6. Developed collaborative arrangement with Chris Langdon to assess metabolic rate in oysters.

Major Scientific Accomplishments:

<u>Tilapia</u>

1. Established that Tilapia embryos with a metabolic rate in the highest quartile maintain a growth advantage to harvest over embryos with a metabolic rate in the lowest quartile. A primary concern based on our preliminary data was that differences in body weight would not be maintained through maturation. Herein, we

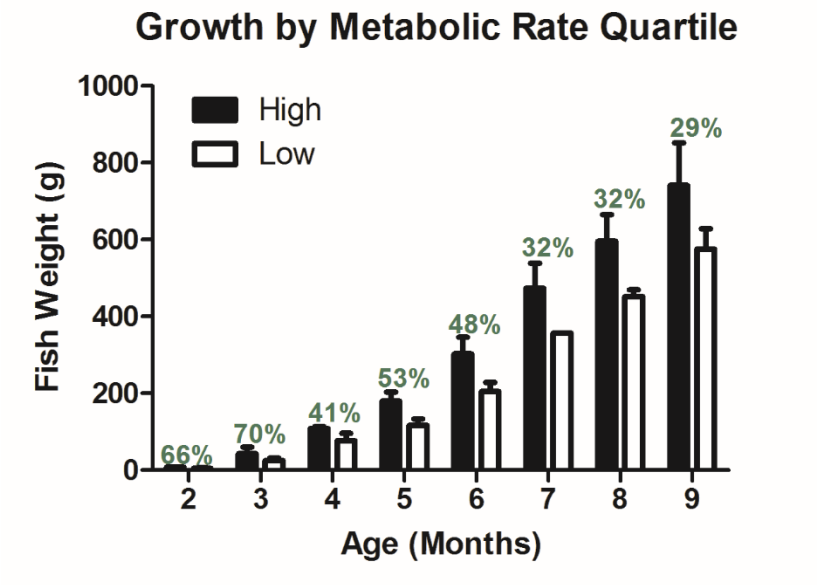


Figure 1. Embryonic tilapia with a high metabolic rate grow more quickly than embryonic tilapia with a low metabolic rate.

show a 30% increase in body weight at harvest in fish that were fed identical amounts of feed (Figure 1).

2. Extended application of the assay to include skeletal muscle and caudal fin explants. To validate the application of this assay in explants we tested the effect of varying time and of varying explant size on signal generated. In support of using this assay to test explants, we found that within a fish as the explant mass increased so did the signal generated from this assay (Figures 2B and 2D). We also found that the signal generated from this assay increased with time (Figures 2A and 2C). These data were integral to the development of a funded USDA NIFA grant, which is examining the potential to select broodstock based on fin or skeletal muscle biopsy metabolic rate.

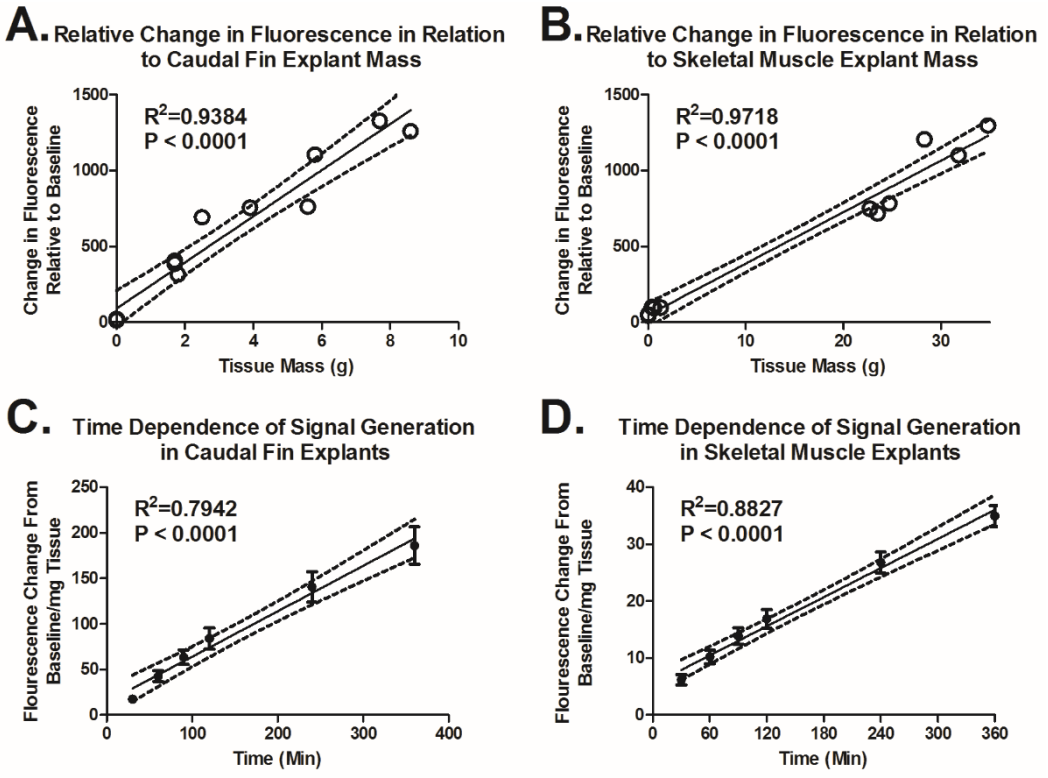
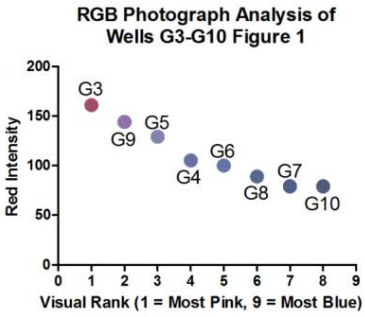


Figure 2. Change in Fluorescence is highly correlated with A) caudal fin and B) skeletal muscle explant mass. Change in fluorescence increases with duration of incubation with C) caudal fin and D) skeletal muscle explants.

3. Initiated trials to use photographic analysis of a plate to perform a non-biased assessment of metabolic rate within a well by assessing the RGB color from the photograph. With these studies we showed that signal change can be assessed from a photograph (Figure 3). This data is now being further applied as a part of the USDA-NIFA grant.



Well (Figure 1)	Visual Rank	R	G	B
G3	1	161	77	105
G9	2	144	113	172
G5	3	129	132	175
G4	4	105	112	161
G6	5	100	116	165
G8	6	89	104	144
G7	7	79	93	134
G10	8	79	93	122

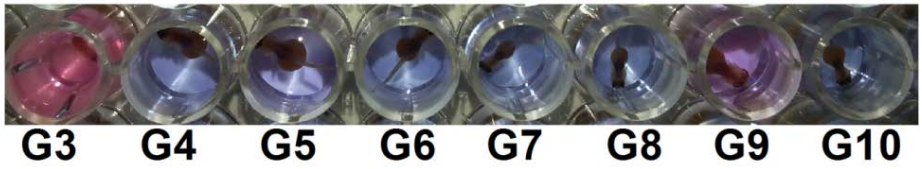
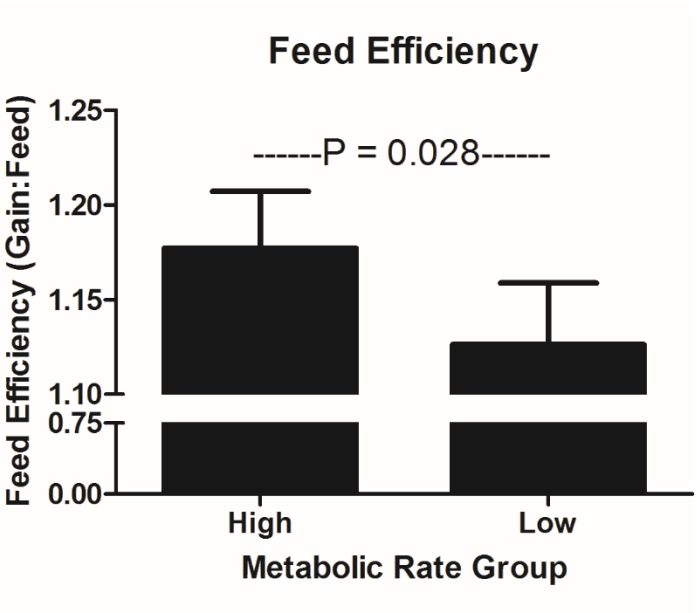


Figure 3. RGB Analysis of Results

4. Tested the effect of metabolic rate on feed efficiency. Initial results suggest a modest improvement in feed efficiency in fish that are selected have a high metabolic rate (4 ± 1.5%). We suspect a more robust response in fish reared in the winter when water temperatures are less ideal for growth. In fact, feed efficiency has been shown to be lower in tilapia raised at 24 ºC than in tilapia raised at 30ºC. Preliminary data collected in fish whose metabolic rate was assessed in February suggests a 23% improvement in feed efficiency when water temperature was maintained at 79º. Thus, we hypothesize that at 24ºC (76ºF) our high metabolic rate fish will have a much higher feed efficiency than low metabolic rate fish. This is because our fish are selected to have a high metabolic rate at 24-26ºC. The trial presented in Figure 1 was performed in a production system that averages 78 ºF water temperatures. Since heating water is cost prohibitive to tilapia production, identifying fish that have improved performance at cool temperatures may vastly improve production while keeping down costs.



5. Developed studies to segregate aggression from growth. To complete preliminary data for these studies we first selected fish that have a high or low metabolic rate then at 2 months segregated the fast and slow growing individuals within a metabolic rate group. Thus we have 4 groups: 1) slow growing high metabolic rate, 2) fast growing high metabolic rate, 3) slow growing low metabolic rate, and 4) fast growing low metabolic rate. For these studies we segregated the largest and smallest fish from each tank that were previously selected based on metabolic rate. As shown in Figure 4, fish with a high metabolic rate have improved feed efficiency. When comparing the feed efficiency of slow growing and fast growing fish within each metabolic rate group we can see that although high metabolic rate fish had a higher feed efficiency there was no difference in feed efficiency between fast and slow growing fish. When data from both metabolic rate groups slow growing fish (1.16 ± 0.029) have similar feed efficiency to fast growing fish (1.14 ± 0.035); P = 0.18).

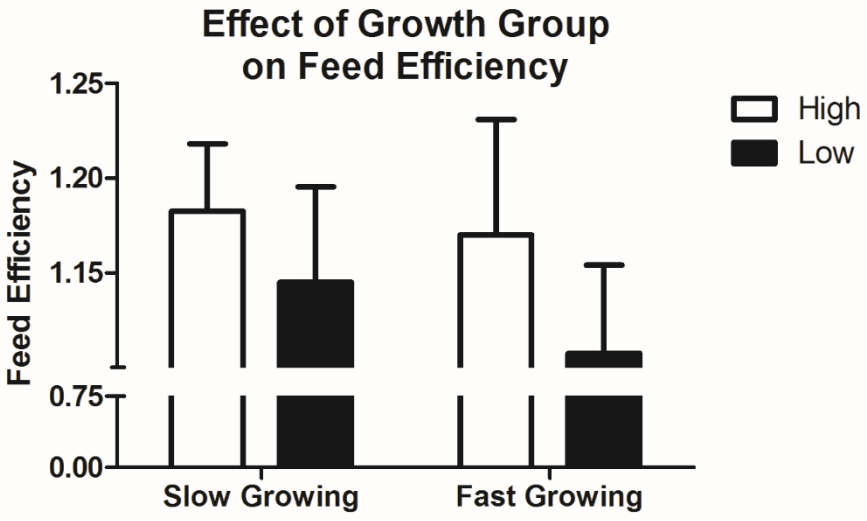


Figure 5. After segregation, feed efficiency is similar in fish that grew fast or slow when co-cultured.

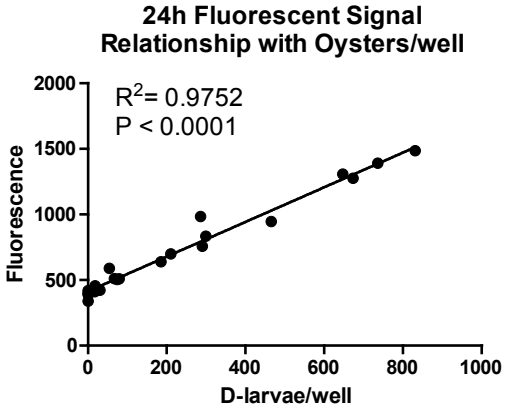


Figure 6. Increasing the number of D-larvae increases signal

Oysters

1. Confirmed that this assay can be applied to measure metabolic rate in oyster D-larvae and spat. In our first study with D-larvae we tested the response to varying the number of individuals within a well. After determining fluorescence a blinded investigator counted the number of larvae in each well. As the number of D-larvae within a well increases so does the fluorescent signal generated by 24h exposure to AlamarBlue (Figure 6. R^2 = 0.9752). This tight relationship is indicative of the assay precision. Of note, as few as 25 D-larvae/well generated a signal significantly different from that observed in the absence of oysters.

2. In our next set of studies we focused on application in spat. By the time oysters have reached the spat stage many differences in size exist. Accordingly, we decided to compare within a family the signal of oysters > 100 µM in size to those that fell through this screen (runts). We initiated these studies by comparing the number of normal and runt spat that would generate a signal and the timeline of that signal generation. In Figure 7A and 7C we show that signal generated by normal spat

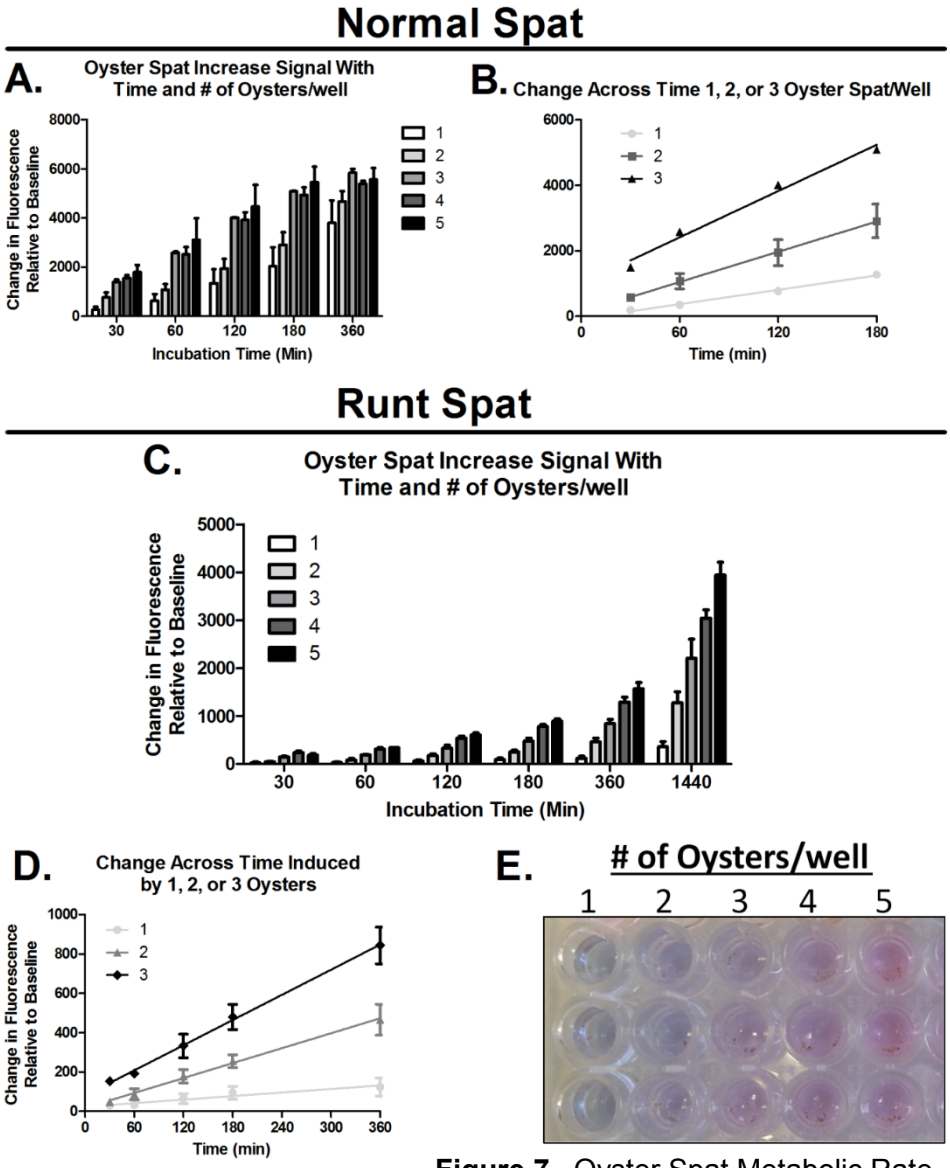


Figure 7. Oyster Spat Metabolic Rate

and runt spat increases with both the number of spat/well and time. Figures 7B and 7D show that for both groups that increase across time is linear. Figure 7E is a visual representation of results.

3. We compared the normal and runt spat across a number of families. In every family we found that runt spat had a lower signal than that observed with normal spat (Figure 8A). Figure 6B shows an image of the signal generated by normal oysters (2/well; Rows A, C, E, G) and the signal generated by runt oysters (4/well; Rows B, D, F, and H). We look forward to wet lab work that will allow us to correct these change in fluorescence values to adjust for differences in oyster size. Specifically, we have devised a method to measure the small levels of both protein and DNA within the wells to specifically correct for total protein and the number of cells within a well.

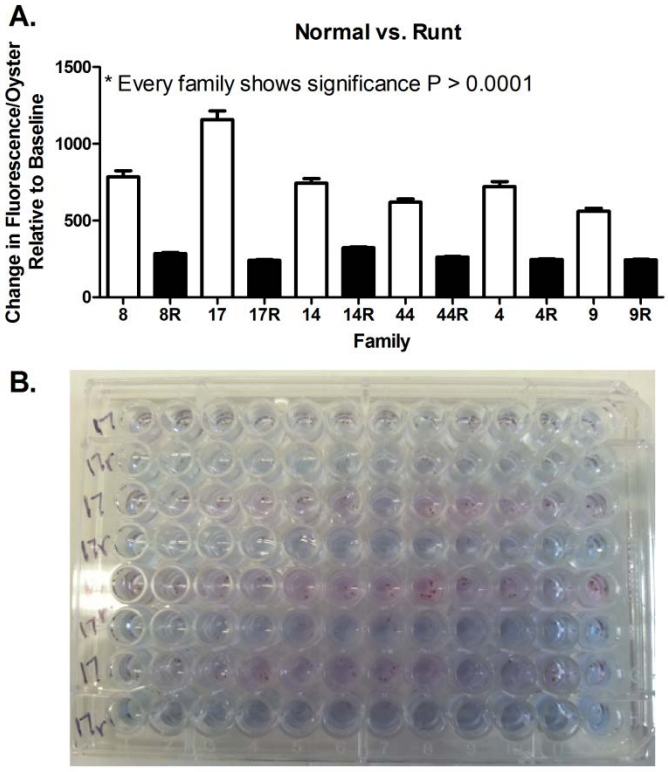


Figure 8. Comparison of Normal and Runt Spat within a Family.

4. We further went on to show that metabolic rate of spat corrected for DNA has a great deal of variation. In fact, the variation within a family exceeds variation between families. This recommends against family selection practices that are common in genetic selection of oysters and proposes that more significant responses may be gained by selecting individuals based on metabolic rate. Of note, by correcting for DNA we have corrected for signal/cell.

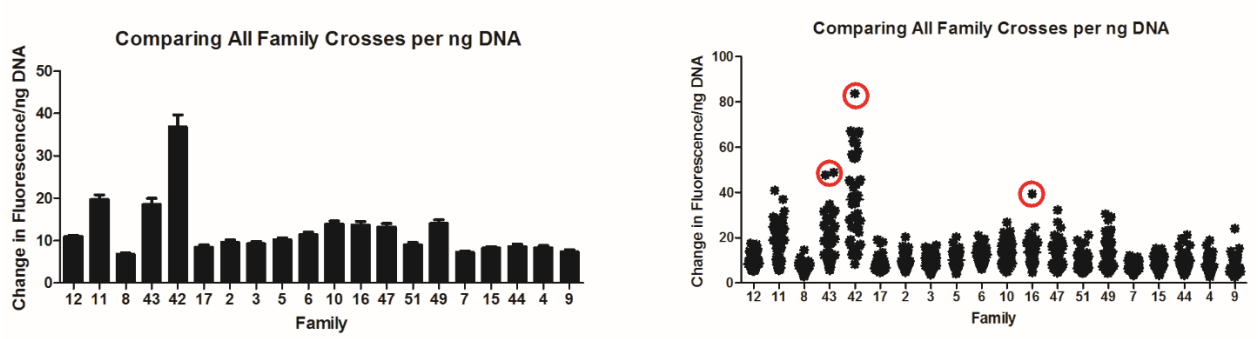


Figure 9. With this figure we can see that certain families do have an average metabolic rate well above other families. When we look at the individuals we can observe the variability within a family and see that certain individuals (red circles) have a much higher metabolic rate than their average family mates.

5. As was discussed at the last IAC/TC meeting we conducted studies this year that are focused on understanding the role of temperature and salinity on oyster spat metabolic rate. With these studies we have shown that as temperature increases so does metabolic rate. In poikilotherms metabolic rate is known to increase with temperature. Oysters become less active as the salt concentration decreases, accordingly, we show here that as salinity increases metabolic rate increases. Figure 10 is showing data from the 6 h incubation to prevent saturation of signal generated by oysters incubated in water with salinity of 30 parts per thousand. At lower salinities the incubation temperature had more robust and significant effects when measured at 16 h. Thus, by altering incubation time we are able to increase sensitivity. This is a primary advantage of this assay relative to a typical oxygen consumption assay. The change in color associated with the different temperatures and salinities as observed at 16 h is displayed as figure 11.

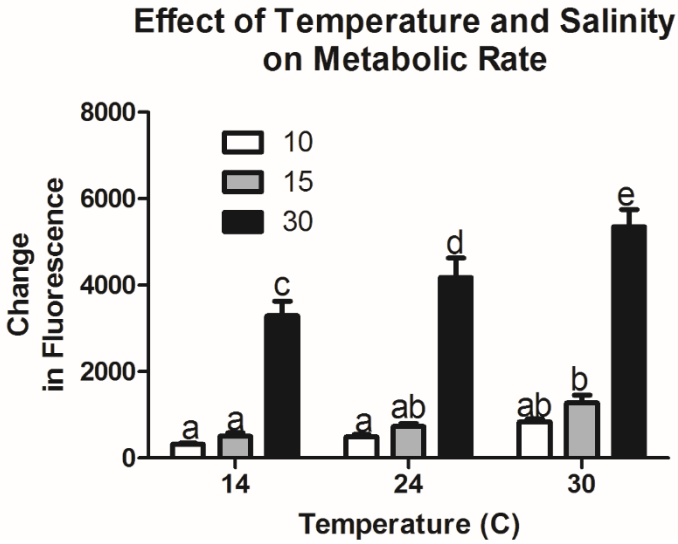


Figure 10. Metabolic Rate in Oyster Spat Increases with Salinity and Incubation Temperature (6h Timepoint). 10, 15, and 30 signify salinity in parts per thousand. [a,b,c,d,e] bars with differing superscripts differ significantly ($P < 0.05$).

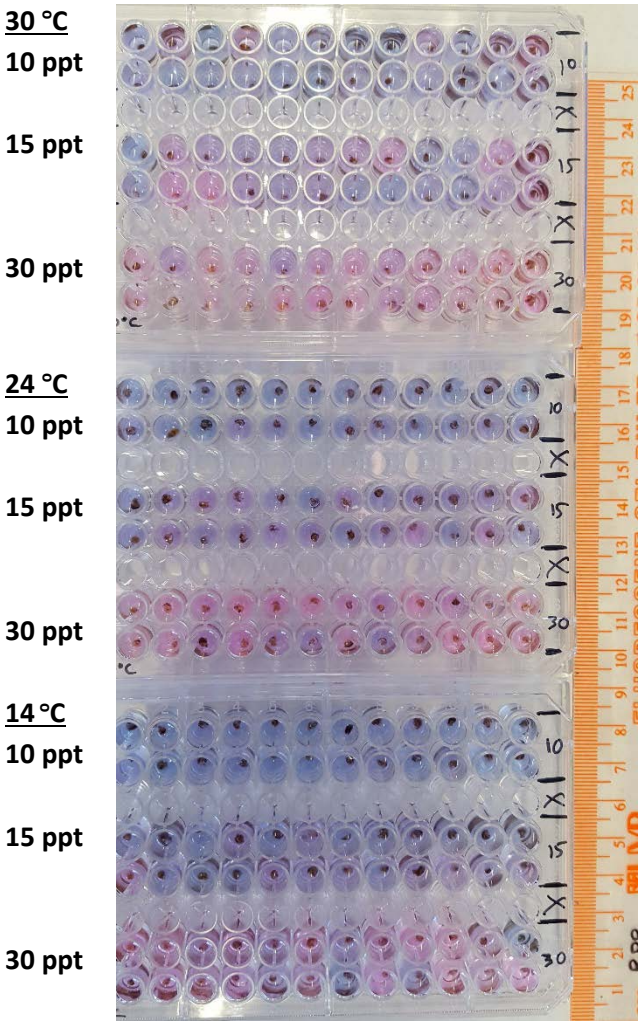


Figure 11. Picture of change in color induced by oyster spat that were exposed to varying incubation temperatures (30, 24, and 14 C) and salinities (10, 15, and 30 parts per thousand).

6. We assessed the metabolic rate of 96 randomly selected oyster spat from each of 24 family crosses that were reared by Chris Langdon's laboratory at Hatfield Marine Science Center. We segregated those oysters with a metabolic rate in the top quartile from those with a metabolic rate in the lowest quartile and Chris reared these oyster in an upwelling system for 2 months. At the end of two months the oysters were sent to Tucson where body weight and metabolic rate were again assessed. This data suggests that our oysters with a low metabolic rate had a lower survival (Figure 12A) which resulted in a lower total weight of the group (P = 0.019; Figure 12C). The difference in apparent survival may be explained by the possibility that when selecting oysters with a low metabolic rate, we selected some oysters that were dead. Obviously dead oysters would have a low metabolic rate. Interestingly, average oyster weight tended to be higher in oysters with a low metabolic rate (P = 0.10; Figure 12B). This would fall in line with our previous results that showed that D-larvae of inbred oysters, which grow more slowly had a higher metabolic rate than outcrossed oysters (Figure 12D).

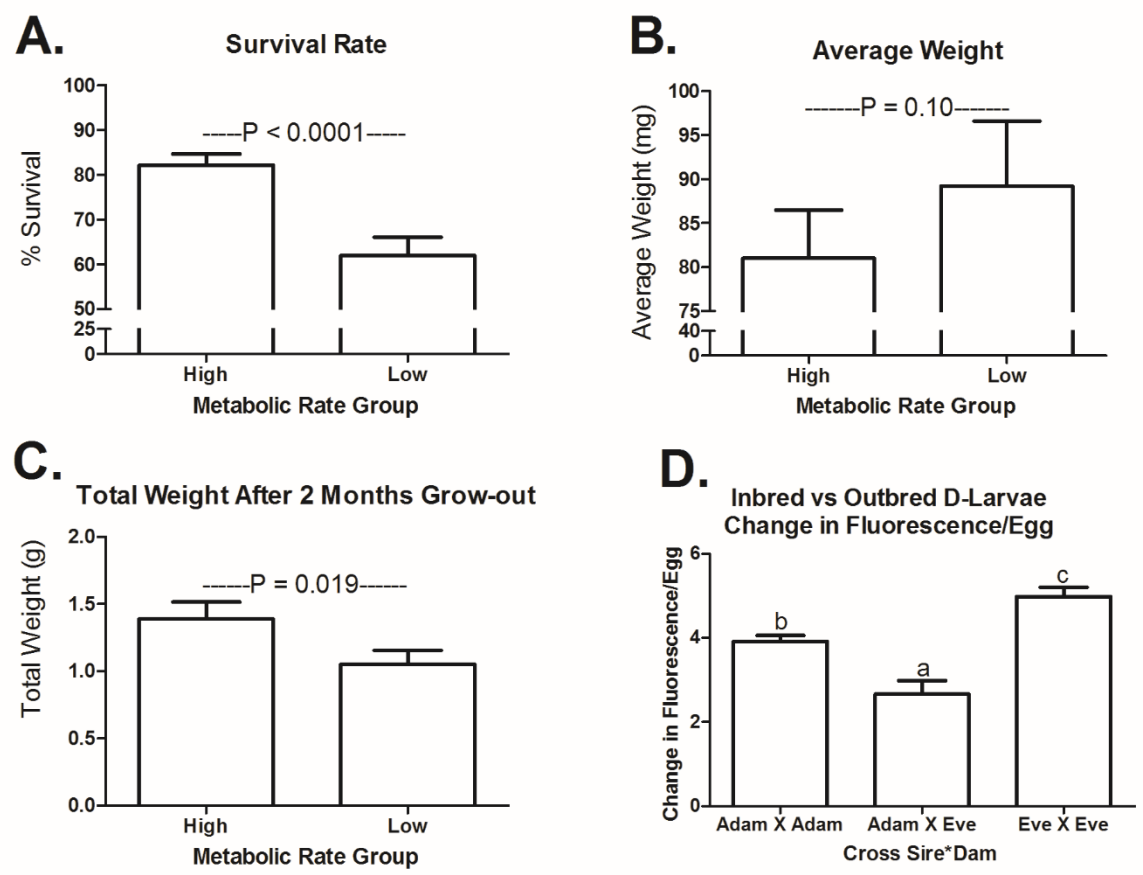


Figure 12. Comparing A) Survival, B) Average Weight, and C) Total Group Weight of Oyster spat segregated by metabolic rate and reared for 2 months. D) Shows that D-larvae from inbred lines have a higher metabolic rate than D-larvae from outcrossed lines.

Shrimp

The University of Arizona has a world renowned shrimp pathology laboratory that regularly gets shipments of post-larval shrimp. To extend our findings to crustaceans we performed the AlamarBlue based assay across time while varying the number of shrimp/well.

The change in fluorescence increases with both time (1-6 hours) and # of shrimp/well.

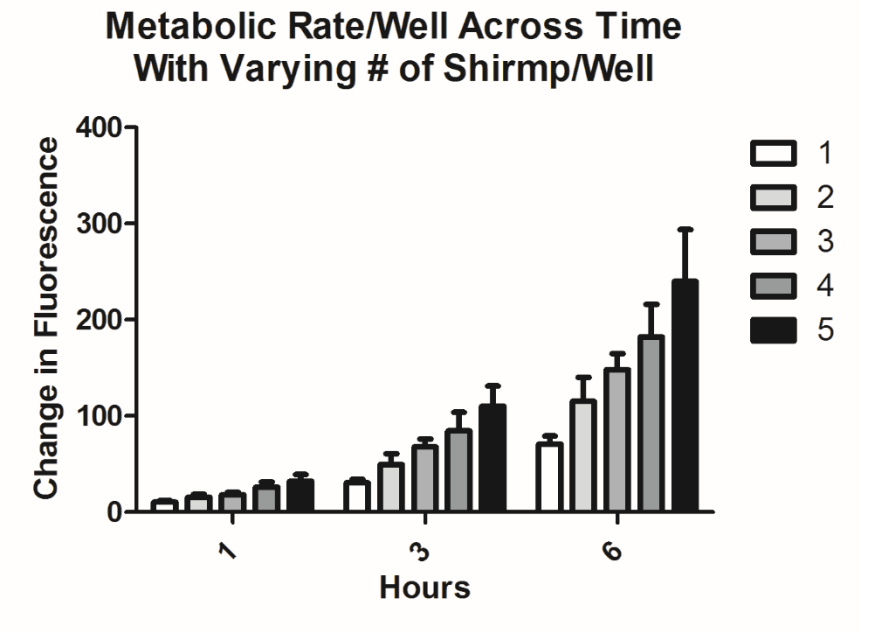


Figure 13. Metabolic rate/well increases with the number of oysters/well and time.

We have a number of studies planned to complete this work. First, with the Tilapia we will continue the growth and feed efficiency study with the fish that are now 3 months of age. We will start a second group in the winter and run the same study at 76ºF. We expect to find that selection for metabolic rate more robustly affects growth and feed efficiency at this low temperature than at the 85-95ºF water temperatures in which the current fish are being reared. With the oysters, we have measured metabolic rate on 54 families at both D-larvae and 8 day larvae stages. Chris Langdon measured average oyster weights at 1 month. We intend to see if our measures of metabolic rate can indicate average oyster weight at 1 month. We are currently counting oysters from over 1400 samples each containing approximately 400 oyster larvae. Upon completion of counting we will measure protein and DNA from these samples to correct for larvae number, total DNA, and total protein. This will allow for a complete understanding of the relationship between metabolic rate and growth to 1 month in oyster larvae. Some of these families are inbred and outcrossed oysters, which will provide an internal control for these studies.

The data generated from this grant:

1. Establishes the validity of this AlamarBlue based assay in measuring the metabolic rate of teleost, mollusk, and crustaceans.
2. Extends the relationship between metabolic rate and growth rate in teleosts.
3. Initiates research that suggests a high metabolic rate in the embryonic fish improves feed efficiency.
4. Recommends that metabolic rate is inversely related to growth in oysters.
5. Proposes that genetic selection of oysters through family crosses is less efficient than selection of individuals.

IMPACT STATEMENT:

4. Tilapia: Problem: Selection of broodstock based on growth results in selection for dominance. Action: Developed a tool that can be used to identify tilapia with an improved genetic potential for growth and improved feed efficiency. Impact: This tool will allow for informed brood stock selection that is independent of aggression and feed intake. Moreover, we propose future studies that will prove that this tool can be used to select against aggression. Selection against aggression will allow for fish domestication, which will improve feed efficiency and decrease morbidity and mortality. The Renquist laboratory (PI: Benjamin Renquist) is responsible for this finding. Contact: bjrenquist@email.arizona.edu
5. Oysters: Problem: Through these studies we have established that the variability in oyster metabolic rate is greater within a family than between families. Thus, family cross based selection does not allow for the most rapid advancements in genetic selection. Action: We have validated that we can quantify the effects of temperature and salinity on metabolic rate in oysters. Additionally, we established that metabolic rate of oyster spat is related to survival rate. Initiated studies in D-larvae and 8-day embryonic oysters that will allow us to address the potential of this assay to inform crossing strategies. Impact: These studies recommend that selection of individuals with low metabolic rates will improve oyster production. The Renquist laboratory is responsible for this finding. Contact: bjrenquist@email.arizona.edu
6. Shrimp: Showed that this assay could be applied to crustaceans. The Renquist laboratory is responsible for this finding. Contact: bjrenquist@email.arizona.edu

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

- Renquist, B.J. 2016. Applying a Test Developed to Combat Obesity and Diabetes to Improve Growth in Fish, Mollusks, and Crustaceans. eXtension https://learn.extension.org/events/2803
- Kentch, K., C. Foy, V. Maxwell, and B. Renquist. 2017. Measuring Metabolic Rate of Embryonic Fish to Predict Growth Rate. World Aquaculture Society: Aquaculture America 2017. San Antonio, TX. Submitted.
- Williams, S.Y. and B.J. Renquist. 2016. High Throughput Danio Rerio Energy Expenditure Assay. JoVE. Jan 27;(107):e53297.

SUBMITTED BY: _____ **9/13/2016**
Title: (PI and Work Group Chair) Date

APPROVED: _____ **9/15/2016**
Project Monitor Date