Project Number:	**USB #1830-352-0502**
Project Title:	**Combined ultrafiltration and reverse osmosis technologies to increase soy protein use in aquaculture feeds.**
Organization:	**Prairie AquaTech LLC**
Principal Investigator Name:	**H. William Harris M.D. Ph.D.**

Project Status - What key activities were undertaken and what were the key accomplishments during the life of this project? Please use this field to clearly and concisely report on project progress. The information included should reflect quantifiable results (expand upon the KPIs) that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be included in this section.

Background, Project Goals and Experimental Design: Protein production by global fish aquaculture has now surpassed that of world-wide production of beef. However, current conventional filtration technologies used in aquaculture have proven inadequate to meet the requirements of growing fish under high density conditions particularly when these fish are fed soy-based diets. As a result of this lack of appropriate water filtration technologies, soybean farmers are not able to benefit from the increased use of soybean protein products and meals into fish feeds that are used in such emerging land-based fish farming systems. Therefore, the central goal of this project was to quantify the benefits of commercially available ultrafiltration (UF) and reverse osmosis (RO) water treatment technologies in rainbow trout fed either fishmeal or soy-based diets in recirculating aquaculture systems (RAS). To accomplish this goal, the Subcontractor partnered with Suez Water Technologies & Solutions (SUEZ), formerly GE Water and Power Division, a global leader in the manufacture and use of highly efficient, commercially available UF and RO systems. To perform the project, we conducted two separate feeding trials using two matched tank-based RAS where one RAS system was used to rear fish at increasing stocking densities utilizing conventional water filtration technology while the other “sister” system utilized an identical rearing configuration but possessed a UF/RO system to treat rearing water.

In the first trial (Trial #1), juvenile rainbow trout (average weight 40+/-0.5 gm) were stocked in tanks at increasing stocking densities (25-125 fish; 10-50 kg/m^3/tank) in replicates of 4 tanks/stocking density and reared for 49 days on an extruded diet formulated and sold for rainbow trout commercial production, which contained 13% fishmeal and no soybean meal. In the second trial (Trial #2), juvenile trout (average weight of 20+/-1.2 gm) were stocked into tanks in the same two RAS systems at increasing densities (7.5-35 kg/m^3) and fed a high (40%) soybean meal diet for 42 days. This high soybean inclusion rate was designed to deliberately challenge the performance of the test trout to quantify the possible benefits of the UF/RO technology. A combination of fish performance characteristics (growth, survival, feed utilization), water quality and fish biological parameters were measured. Both trials and their accompanying data and analyses were completed within the contract period, used to estimate the financial impact of UF/RO technology on an RAS system and these results are summarized below. On the basis of these pilot scale data, we have attracted the attention of two candidate commercial scale RAS farms as commercial beta testing sites for these technologies.

<u>Trial #1 – Comparison of fish performance in Control vs. UF/RO RAS system fed a standard fishmeal-based diet</u> - Figure 1 shows that trout reared under UF/RO conditions achieved a higher final stocking density and larger average body weight vs. Control trout. While differences in stocking density were most apparent in the more densely stocked tanks, an increase in average body weight and growth were observed in all tanks regardless of stocking densities (Figure 1).

Figure 2 shows that trout stocked at higher densities (75-125 fish/tank) in Control tanks displayed significantly higher mortalities vs. fish reared under same stocking densities in the UF/RO system. As a result, the % biomass gain from the trial’s inception was 10-15% greater for UF/RO trout as compared to Control.

Trout reared under UF/RO conditions consumed more feed and displayed significantly reduced economic FCRs values as compared to trout reared under Control conditions (Figure 3).

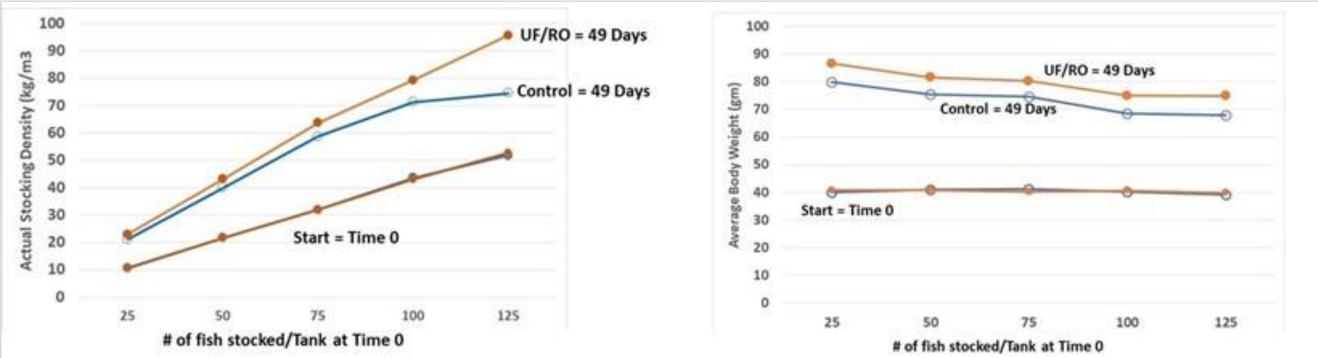

Figure 1: Comparison of average initial and final tank stocking densities (left panel) and body weights (right panel) for trout reared under Control (blue line, open circles) or UF/RO (orange line, solid circles) conditions at time 0 or after 49 days in trial #1. See text for details.

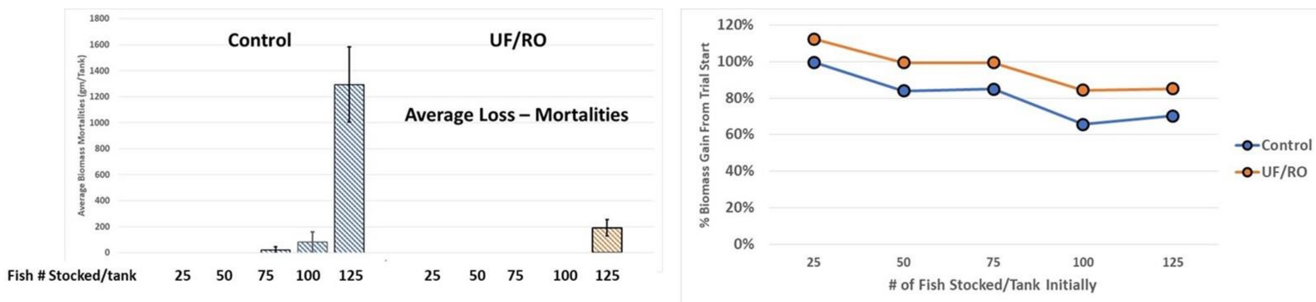

Figure 2: Comparison of average mortalities (left panel) and biomass gained (right panel) from trout reared under Control (blue line or crosshatched columns) or UF/RO (orange line or crosshatched columns) conditions.

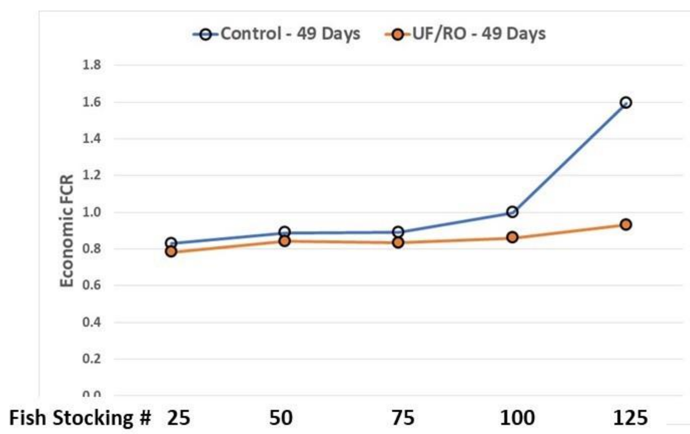

Figure 3: Comparison of economic FCR for trout reared under Control (blue line and open circles) vs. UF/RO (orange line and open circles). Note the larger economic FCR for trout reared under Control conditions. See text for details.

In trial #1, the UF/RO system used ~60% of the daily make up water vs. Control. Despite reduced water consumption, the average UF/RO water turbidity was 40% lower as compared to values in the Control system.

<u>Trial #2 – Comparison of fish performance in Control vs. UF/RO RAS System fed a high soybean meal diet</u> – Figure 4 shows that final stocking densities for Trial #2 Control trout declined dramatically when fish were

stocked at >100 fish/tank. By contrast, both Control and UF/RO trout achieved similar final average body weights. Table I shows that Control Trial #2 trout reared at high stocking densities had ~ 50% cumulative mortality whereas UF/RO fish displayed a 33.4% mortality rate as compared to data obtained in Trial #1.

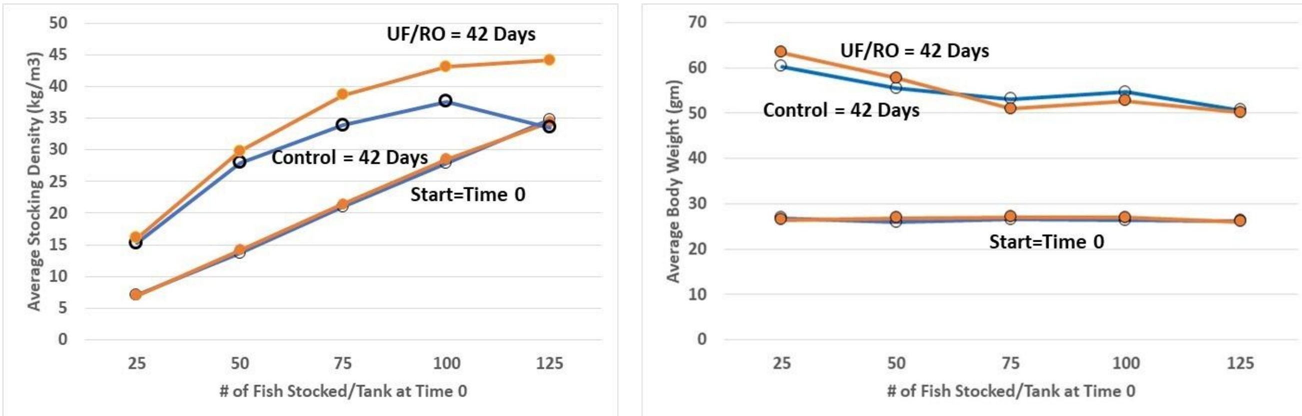

Figure 4: Comparison of average initial and final tank stocking densities (left panel) and body weights (right panel) for trout reared under Control (blue line, open circles) or UF/RO (orange line, solid circles) conditions at time 0 or after 49 days in trial #2. See text for details.

As a result of these mortalities, Control trout showed a negative % biomass gain after stocking at high densities (125 fish/tank) whereas UF/RO fish displayed a 28% biomass gain and 33.4% cumulative mortality. Similarly, the economic FCR of Control Trial #2 trout increased dramatically at higher stocking densities due to losses whereas the economic FCR of UF/RO trout also increased but achieved a value that was nearly 40X lower. Of interest, UF/RO trout consumed 10-15% less feed vs. trout in the Control system.

Table I: Comparison of mortalities and survival of trout reared in either Control or UF/RO Systems in Trial #2

	Control System		UF/RO System		% Increase in Survival
Fish/Tank Stocked	Average #	% Mortality	Average #	% Mortality	UF/RO Fish vs. Control
25	1.0	4.0%	1.0	4.0%	0.0%
50	2.3	4.5%	1.3	2.5%	2.0%
75	14.0	18.7%	3.3	4.3%	14.3%
100	34.3	34.3%	22.3	22.3%	12.0%
125	62.3	49.8%	41.8	33.4%	16.4%

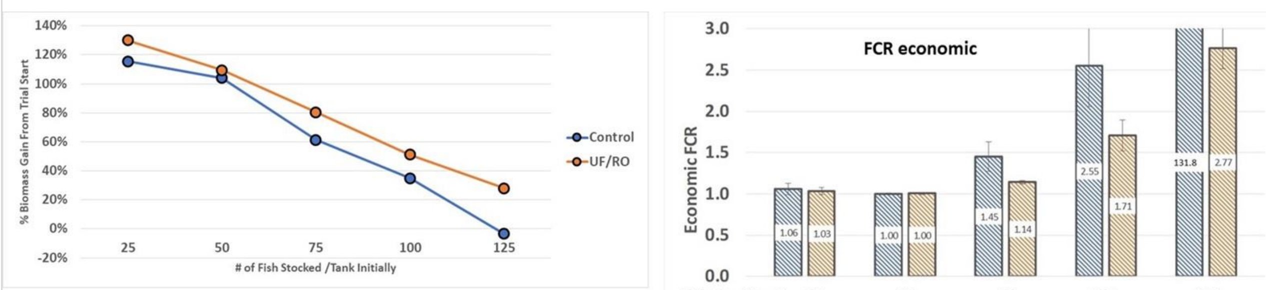

Figure 5: Comparison of % biomass gain (left panel) and economic FCR (right panel) for trout stocked at various initial densities and reared under Control (blue line or crosshatched columns) or UF/RO (orange line or crosshatched columns) in Trial #2. See text for details.

In trial #2, UF/RO system used ~65% of the daily make up water vs. the Control system. Despite the reduced water consumption, the average water turbidity of the UF/RO system was 35.3% lower as compared to values for the Control system.

Histological examination of various tissues including gills, liver, spleen, kidney and intestine was performed on trout reared at either low or high stocking density conditions in both Control and UF/RO systems. Other than indications of early distal intestinal enteritis consist with their high soybean diet, all tissues were within normal limits and no significant differences were observed between them.

Comparison of Financials and Fish Performance in Control vs. UF/RO Systems – Data from Trials #1 and #2 provide a wealth of performance metrics upon which to estimate the potential value(s) of implementation of UF/RO technology in RAS trout rearing systems. Due to final report space limitations, our estimates will focus on value calculations for a UF/RO application in a juvenile rearing system designed to supply 75-80gm trout (corresponds to Trial #1 final trout size) for larger production tanks to grow fish to market size (~500-600gm). The financial estimate summarized below is focused solely on expanding the production of an existing juvenile rearing unit via increasing the amount of juvenile trout that could be supplied to a production system.

Data from Trial #1 shows that UF/RO technology increased production of test RAS systems by 25 kg/m3 of biomass as compared to rearing of fish in the same system under Control conditions. This increased productivity corresponds to 333 fish/m3 (13 fish/kg) and if valued at $1.50/fish a total of $500/m3 increased production value attributable to use of the UF/RO system. Multiple commercial trout farms possess juvenile rearing units of 20,000 gallons tank rearing space (75 m3) that are stocked and emptied a total of four times (4 turns)/year. Thus, the increased value provided by the UF/RO system for such a system is $37,879/turn or $151,515/year. This increased productivity does NOT include the potential increased output of the final production step to rearing trout to market size fish utilizing these technologies.

Given that the UF/RO system uses 35-40% less daily make up water vs. Control systems, our initial estimates indicate that all of the additional operational costs incurred by the UF/RO technology (cleaning reagents, electricity, etc.) are covered within the cost of pumping, heating and treating this additional water requirement for the Control system to operate without the extra biomass of trout. Thus, all operating costs of the UF/RO system are estimated to be accounted for in cost savings in water usage and treatment. The UF/RO system used in Trial #1 provided a total of 4 turnovers of rearing water volume per day that were filtered through the UF component. This would correspond to a UF/RO unit capable of ~50-100 gallons per minute of water flow. While the capex cost of such a unit does vary significantly, a value of $150,000 can be assigned for purposes of this report. Thus, the ROI for a UF/RO unit capex cost is estimated to be an attractive one-year payback investment.

Data from Trial #2 show that the UF/RO technology possessed benefits when applied to trout reared on high soybean meal inclusion diets. As expected, trout fed a high soybean meal diet grew less well vs. fish fed a fishmeal diet under the same Control conditions, although the diets utilized in the current trials had an extremely high level of soybean meal inclusion of 40% of the dry diet. Similar to data obtained in Trial #1, the benefits of UF/RO technology were most apparent at higher stocking densities where Control tanks fed the soybean meal diet produced no biomass gains due to massive mortalities whereas trout reared under UF/RO conditions grew and achieved a significant net biomass gain albeit with substantial mortalities. This ability of UF/RO technology to “push the envelope” to increase the productivity of ultra high inclusion of soy-fed trout under high density RAS rearing conditions deserves more study and development. In addition, it is likely that testing of additional diets containing 20-30% soybean meal will yield a better understanding of UF/RO benefits.

Follow-On Commercial Trial Beta Test – As reported previously, Prairie AquaTech and SUEZ management have visited the large RAS farm, Vero Blue Farms, Inc. of Webster City, IA and discussed the implementation of a larger beta commercial trial to further validate the possible advantages of using UF/RO technology for rearing fish in RAS tank-based systems. Based on current discussion, such a trial is being scheduled for Q1 of 2019. In addition, Prairie AquaTech and SUEZ are also in early stage discussions with an Ohio-based RAS farm, Hanilu Farms LLC for a similar beta testing trial. It is anticipated that for each of these beta tests (one involving hatchery water reuse and improved quality) and the other (improvements in production water rearing conditions) that current farm financial metrics will be applied to the beta testing protocol. Moreover, since both farms involve growing barramundi (Australian seabass) using soy containing diets, these commercial trials will provide additional information on the combination of UF/RO technology and commercial soy based diets.

Did this project meet the intended Key Performance Indicators (KPIs)? List each KPI and describe progress made (or not made) toward addressing it, including metrics where appropriate.

This project fulfilled all of the three KPI's that were projected for completion and provided the foundation for the other 3 KPIs that are now the subject of the current efforts of Prairie AquaTech and SUEZ. The following 3 KPI's were completed:

1. **Establishment of experimental systems consisting of replicate Industry Standard (Control) and Modified System using SUEZ UF and RO equipment packages** – A laboratory scale tank-based experimental system was designed, built and commissioned that enabled direct comparison between the rearing of fish (rainbow trout for initial trials) using either Control conditions widely utilized in RAS aquaculture vs. the same system provided with UF/RO equipment for conditioning rearing water. Importantly, the experimental design included stocking fish at various stocking densities to determine the effects of UF/RO on fish grown under different biomass densities. This system can now provide metrics to quantify changes in a variety of important parameters influencing fish growth as well as quantification of water usage, quality, etc. Prairie AquaTech and SUEZ plan additional testing efforts using this system.

2. **Fish performance in either Control or Experimental System containing UF/RO fed a standard diet or soy containing diet was compared using this experimental system** –Two independent trials (the first consisting of a total of 49 days and the second spanned 42 days) were performed and completed. Data collected from each trial where trout were fed either a fishmeal-based or soy-based diet were used to compared fish performance under different rearing conditions (Control vs. UF/RO) at different rearing fish biomass densities. These fish were further analyzed using a variety of metrics summarized in the Technical Report Section of this Final Report.

3. **Project financials and fish performance in Control vs. UF/RO Systems were analyzed and** compared – Based on data collected from both fishmeal and soy-based feeding trials, financial analysis of the cost-benefit of the commercial use of UF/RO technology was significantly positive and appears to be cost effective with regards to both Cap Ex and Op Ex costs vs. increased fish production benefits and lower makeup water requirements. These data serve as the basis for comparisons in larger commercial scale beta testing trials.

The foundation for three other project KPI's was successfully completed and planning for one future KPI is underway.

4. **Follow-on commercial trial beta test**- Using data obtained from both fishmeal and soy-based trials, Prairie AquaTech and SUEZ have attracted the interest of two commercial fish producers that currently utilizing soy-based feeds. One producer is based in Iowa while the other is located in Ohio. Current planning involves possible trials in 2019.

5. **Existing farms switch to high soy diets after UF/RO retrofits**- This project KPI will follow on from the results of the commercial beta testing results achieved by completion of KPI #4

6. **UF/RO & high soy diets become viable technologies for new and existing aquaculture facilities** – Successful beta tests on farms already using soy-based diets will provide the basis for more widespread use of UF/RO systems that can be retrofitted on existing commercial RAS farms. Further success with

these retrofits will drive incorporation of UF/RO technology together with soy-based diets into future designs for RAS facilities in the United States and elsewhere.

Expected Outputs/Deliverables - List each deliverable identified in the project, indicate whether or not it was supplied and if not supplied, please provide an explanation as to why.

The previous KPI section together with the technical report lists the three major expected outputs/deliverables that were completed in the Project period. All expected outputs/deliverables were delivered as described above. It is anticipated that 3 other KPI's will be delivered in the 1-4 years after completion of USB subcontract in a sequential manner as summarized in the future KPI section above.

Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.)

There were no major unforeseen events or circumstances that affected the proposed project timeline or its costs or deliverables. There were several unanticipated observations during trials #1 and #2 that are very interesting and will be the focus of future work to gain further insights into these data. These are: 1) interactions with bacterial-based biofilter and UF/RO system – The influence of UF/RO on the physiology and numbers of denitrifying bacterial species deserve further study; 2) Determination of positive growth influences of UF/RO even at lower stocking densities of trout; 3) Relationship between UF/RO rearing water clearance rates and soybean meal inclusion rates in extruded diets.

What, if any, follow-up steps are required to capture benefits for all US soybean farmers? Describe in a few sentences how the results of this project will be or should be used.

The data obtained in this project has provided two important elements to increasing the benefits that US soybean farmers will receive. The first element is obtaining a project data set that provides proof of concept for testing a combination of UF/RO technology in conjunction with soy-based feeds. To enable these benefits to expand and reach their full potential, a concerted effort must be undertaken to conduct such beta testing trials and make results available to interested US-based RAS facilities so as to increase their competitiveness in the global marketplace. A second important element is to provide a proof of concept study to the highly experienced engineering, manufacturing and deployment teams at SUEZ so as to enable them to utilize their experience and expertise to focus on how to drive these technology advances into current RAS wastewater treatment systems so as to improve and expand both US RAS aquaculture and its use of soy-based extruded feeds.

List any relevant performance metrics not captured in KPI's.

Prairie Aquatech LLC has independently developed a fermented soy-based protein aquaculture feed ingredient called ME-PRO® possessing a 70% protein content that is highly digestible and low in the plant phosphate sequestering compound phytate. In separate work (a portion of which has been funded by USB, SAA and other US-funded soybean initiatives), feeding trials using ME-PRO as compared to soybean meal has shown significant improvements in fish growth and economic FCR. Based on these efforts, Prairie Aquatech is currently constructing a large ME-PRO® manufacturing plant possessing a maximum production capacity of 30,000 tons annually that is projected to be completed in 2019. Use of diets containing ME-PRO® in conjunction with UF/RO water treatment technology is likely to result in further improvements in both fish growth and RAS rearing water quality that will advance the metrics set via testing of the combination of UF/RO methods and standard soybean meal extruded diets.