NRAC ANNUAL PROGRESS REPORT

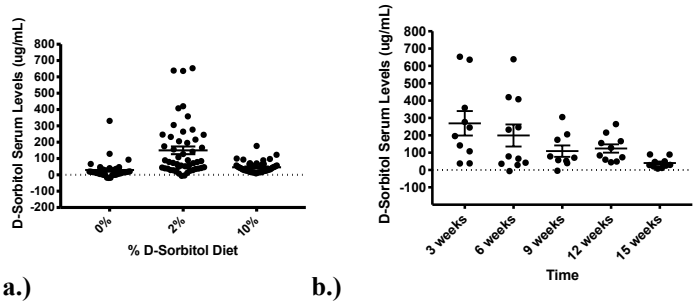
INSTRUCTIONS: An annual progress report for each funded project must be provided both as hard copy and electronically to the Director of NCRAC by October 31 of each year. Progress reports are to be an accumulation of activities through August 31 of the year of the report or up until the completion of a termination report (see **Appendix H)** which can serve in lieu of the annual progress report. Format for the report must adhere to the headings given below.

Project Title	A Novel Approach to Prevent Super Chill in Atlantic salmon
Reporting Period	6/29/18 through 6/18/19 (Final Report)
	Name of person(s) submitting this report. Ian Bricknell, Deborah Bouchard
Key Word	Atlantic salmon, super chill, osmopotentiator, simple sugars, sugar alcohols, freezing point depression
Funding Level	Total funds allocated for this project to date. *NOTE: This could be reported by Year. i.e.,* *Year One: FY 2017, $ amount* $86,451 *Year Two: FY 2018, $ amount* $79,123
Participants	*PD Ian Bricknell, PhD; Professor of Marine Science, School of Marine Science, University of Maine, 5735 Hitchner Hall, Orono, ME 04469 Business Phone/Fax: (207) 581-4380/4430 e-mail: Ian.bricknell@maine.edu *Co-PI Deborah A. Bouchard, University of Maine Animal Health Laboratory Manager, Director ARI University of Maine, Cooperative Extension, 17 Godfrey Dr, Orono, ME 04473 Business Phone/Fax: (207)581-2767/4430 e-mail: dbouchard@umext.maine.edu *Chris Bartlett, Marine Extension Associate, Marine Technology Center, City of Eastport, 16 Deep Cove Road, Eastport, ME 04631 Tel 207.853.2518 e-mail: cbartlett@maine.edu *Gary Burr PhD, Post-doctoral Research Scientist, National Cold Water Marine Aquaculture Center, National Cold Water Marine Aquaculture Center 25 Salmon Farm Road Franklin, Me, 04634 Business Phone/Fax: (207)422-2713/2723 e-mail: gary.burr@ars.usda.gov *Chong M. Lee, PhD, Professor Emeritus and Research, Department of Nutrition and Food Sciences, Food Science and Nutrition Research Center, University of Rhode Island, Kingston, RI Business Phone/Fax (401) 874-2862/2994; e-mail: chonglee@mail.uri.edu Leighanne Hawkins DVM, Cooke Aquaculture Maine P.O. Box 991 Calais , Maine 04619 Tel: (506) 755-1340 email: leighanne.hawkins@cookeaqua.com
Project Objectives	**Objective 1:** Investigate the concentrations of simple sugars and sugar alcohols (SSASA) in plasma of Atlantic salmon to achieve a higher freezing point depression limit. **Objective 2**: Investigate the uptake of simple sugars and sugar alcohols from the diet in both epithelia cells and the plasma of Atlantic salmon. **Objective 3:** Determine the efficacy of simple sugars and sugar alcohols in reducing the impact of a super chill event in Atlantic salmon under controlled

	conditions. **Objective 4:** Measure the physiological parameters of the fish subjected to Objective 3 and compare to control. **Objective 5:** Liaise with Atlantic salmon aquaculture industry, extension and technology transfer
Anticipated Benefits	This project will benefit the salmon aquaculture industry directly and will deliver critical research exploring a novel approach to mitigating super chill in cultured Atlantic salmon. The Northeastern US (Maine) salmon aquaculture industry has stated that super chill risk is the major limiting factor for growth of the industry. Successful completion of this project would provide the groundwork to mitigate current losses resulting from super chill, and it would also provide the industry with the opportunity for considerable expansion. The Maine salmon industry has stated that if super chill risk was eliminated, production potential could reach three times the current levels in Maine.
Project Progress	This update report covers the extended period of the project from June 29th 2018 through June 18, 2019. Delays in the production of the super chill diets required a no cost extension to complete objectives 3 and 4. **Objective 1**: Investigate the concentrations of simple sugars and sugar alcohols (SSASA) in plasma of Atlantic salmon to achieve a higher freezing point depression (FPD) limit. *Hypothesis: The addition of SSASA to the plasma of Atlantic salmon will elevate the freezing point depression in vitro.* An *in-vitro* experiment was designed to determine the levels of SSASA in plasma required to depress the FPD point below -0.7°C which is the point at which Atlantic salmon (ATS) experience physiological collapse and mortality. Each SSASA was added at concentrations of 0.1,1,10 and 100mM and the FPD of the SSASA enhanced plasma was determined. The SSASA plasma mixture was subjected to a FPD determination using a 6002 Touch Micro OSMETTE (PSI Precision Systems Inc.) and osmolality and the freezing point determined. This experiment did provide the basal levels required of each SSASA tested *in-vitro* to decrease the FPD value of the plasma compared against the FPD value established for the normal plasma collected from the fish. The following SSASA presented in Table 1 were tested. [redacted]

Objective 2: Investigate the uptake of simple sugars and sugar alcohols from the diet in both epithelia cells and the plasma of Atlantic salmon. *Hypothesis: Both epithelial cells and plasma will uptake SSASA from the diet of Atlantic salmon.*

Objective 2 was to assess uptake of the SSASA added to feed formulations and its duration *in vivo*. A preliminary pilot experiment was performed. As determined in objective 1, *in-vitro* all SSASA added to the plasma were able to lower the FPD relatively equally. For this pilot trial two SSASA were selected; trehalose (large molecular weight) and sorbitol (low molecular weight). A standardized formulation of salmon diet was made as the base diet. Four test diets were made using the base diet with added trehalose or sorbitol at two concentrations, 2% and 10%. A 15-week feeding trial was conducted using the two SSASA diets and the control diet and the trial was also run with fish at two temperatures 14±1°C and 4±1°C. Fish sampling occurred at 0, 3,6, 9, 12 weeks post feeding. Feeding was stopped at week 12 and a final sampling was done at week 15 to determine duration of SSASA in the fish. Samples of blood and skin were taken at each time point to determine the FPD, and update and duration of trehalose and sorbitol in plasma and skin cells. Assays for determining the levels of trehalose and sorbitol in collected samples were optimized using Megazyme's© highly sensitive and specific enzyme colorimetric assay kits.
The following figures summarize the results from fish plasma:



a.) **b.)**

Figure 1. a.) Levels of sorbitol in salmon plasma in diets containing 0, 2 and 10% sorbitol and b.) the decrease of sorbitol over time after being fed a 2% sorbitol diet over 15 weeks

Results from the pilot study demonstrated that the optimal concentration of sorbitol in the diet was 2% and that sorbitol concentrations in the plasma decreased over time with the highest sorbitol concentrations being at 3 weeks. There was very little sorbitol detected in the plasma of the 10% diet group. This was unexpected.

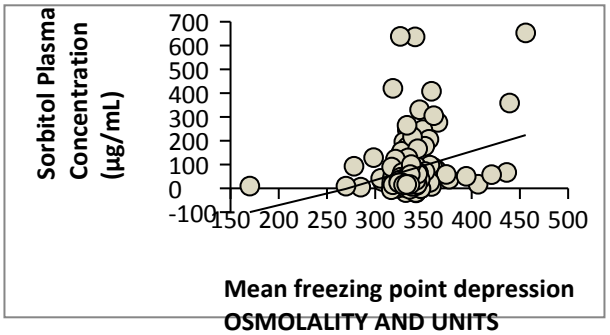
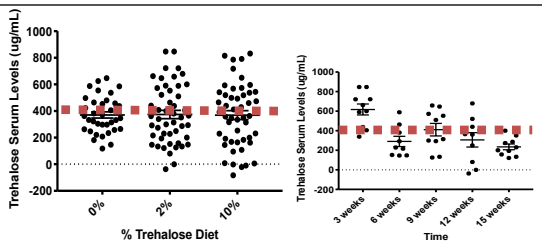


Figure 2 Relationship between sorbitol plasma concentrations (µg/mL) and mean plasma osmolality (mOsM).

Figure 2 demonstrates that there is a relationship between plasma sorbitol levels and osmolality.

A different picture emerged with Trehalose. There was no significant difference between the control fish and the 2% and 10% trehalose diet groups. (Figure 3a)



a.) **b.)**

Figure 3. a.) Levels of trehalose in salmon plasma in diets containing 0, 2 and 10% trehalose and b.) the decrease of trehalose being fed 2% over 15 weeks. There was no significant difference seen between the control and test diets. Dotted line is the limit of detection in the assay.

The results of the pilot study revealed interesting data that warranted further investigation as to optimal length of time for feeding the diets for peak SSASA plasma concentrations. The research team decided on performing a 2nd larger uptake study using five SSASA combinations.

The efficacy of SSASAs in reducing super chill

The *in vivo* uptake and retention study was repeated as described for the pilot study in objective 2. Five SSASA diets were prepared containing 2% xylose, 2% sucrose, 2% trehalose, 2% sorbitol and 10% sorbitol. **Objectives 3 and** 4 were combined into the uptake study. The experiment included a larger number of salmon and samples to increase the statistical power of the study. Due to the delay in the commercial manufacturing of the diets, the trial was run from April 2017 to July 19, 2018. **(Objective 3**). Over 800 plasma samples were analyzed for freezing point depression. Figure 4 represents the mean plasma osmolality (mOsM) of the plasma from Atlantic salmon fed the SSASA diets compared to the plasma from Atlantic salmon fed the base feed diet by week of the trial. For reference, the osmopotential of the plasma needs to reach approximately 403 mOsM for assumed prevention of super chill in Atlantic salmon. No plasma samples with any of the diets achieved the osmopotential level required.

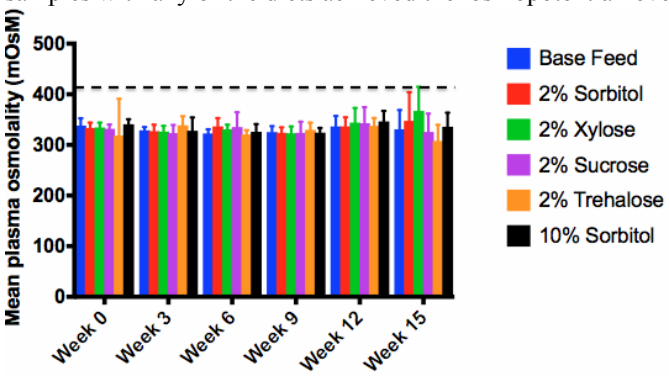


Figure 4. Two-way ANOVA of mean plasma osmolarity (mOsM) by diet and week of trial. Significant differences are observed in plasma osmolarity in the 2% Xylose diet between week 0 compared to week 15 (P=0.0183), between week 3 compared to week 15 (P= 0.0002), week 6 compared to week 15 (P=0.0032), and between week 9 compared to week 15 (P<0.0001). Additionally, significant differences are observed in the 2% Trehalose diet between week 3 and week 15 (P=0.0413). The dotted line is approximately at 403 mOsM, which is the osmopotential the plasma would need to reach to prevent super chill. Error bars are standard error of the mean.

	(**Objective 4**) Subsamples of fish from the update study were exposed to super chill conditions (lowest temperature -1˚C targeted) as the diet study progressed. Three fish from each tank (6 fish per treatment) were transferred into 2 insulated tanks attached to a glycol chiller. Fish were transferred starting at week 7 and concluded at week 10. The insulated tanks were at 2˚C and the temperature was lowered 1˚C per day until a temperature of -1˚C was obtained. In the first replicate the water temperature only reached -0.7˚C due to a malfunction with the chiller, and only one mortality was observed. Subsequent replicates the water temperature reached -1˚C for 24 hours. All the fish died after 24 hours at -1˚C in the other three replicates. Keeping the water temperature at -1˚C was challenging with the system design as the room temperature warmed the water slightly and the temperature hovering between -0.7˚C and -1˚C. It does not appear, however, that any of the SSASA diets increased the cold tolerance in Atlantic salmon in this study and using these aquaria systems. **Objective 5:** Liaise with Atlantic salmon aquaculture industry, extension and technology transfer The proposed project involves direct participation of the salmon aquaculture industry. Cooke Aquaculture's lead veterinarian, Leighanne Hawkins and US marine production manager, David Morang are current with all research progress to date. The project's first year was primarily groundwork that involved *in-vitro* testing, formulating the ATS feed and a preliminary uptake study. Cooke Aquaculture's production managers and lead research and development personnel traveled to the USDA/ARS for formal discussions on the project's research progress in May and July of 2017. Although occurring later than planned due in the delay in the final larger study, a half day workshop is scheduled with Cooke Aquaculture for late summer 2019.
Accomplishments:	
Outreach Overview	The project's first year was primarily groundwork that involved in-vitro testing and formulating the feed. However, Cooke Aquaculture's production managers and lead research and development personnel traveled to the USDA/ARS to discuss the project's research progress in May and July of 2017. A formal half day workshop is planned for late summer 2019.
Targeted Audiences	The target audiences were University researchers and students, USDA ARS scientists and the Atlantic salmon aquaculture industry in Maine and aquaculture feed production companies. This project had direct involvement from all listed and with Cooke Aquaculture, Maine's major salmon aquaculture producer and a company that is a global leader in feed production.
Outputs:	Outputs are tangible, measurable products (website, events, workshops, products [AV, curricula, models, software, technology, methods, websites, patents, etc.], trainees, etc.). Three undergraduate student interns gained research experience.
Outcomes/Impacts:	Describe how findings, results, techniques, or other products that were developed or extended from the project generated or contributed to an outcome/impact. **Outcomes/impacts are defined as changes in Knowledge, Action, or Condition.** Completion of Year 1, knowledge on SSASA osmopotentiators for ATS diet formulation were identified and diets were formulated. Communication between researchers at UMaine, the USDA ARS and the ATS industry increased.

<table>
<tr><td></td><td>Knowledge on SSASA osmopotentiators in diet formulations and performance in fish trials was gained.</td></tr>
<tr><td>Impacts Summary</td><td>Provide short statements (2-3 sentences) about each of the following:
(pre-established fields for Researchers to complete short statement answers)
1. Relevance: Issue – what was the problem?
Super chill is a physiological collapse in salmon occurring during periods of extreme cold weather in the Northeastern US. The Maine salmon industry has stated that super chill is a limiting factor for growth of the industry in Maine
2. Response: What was done?
Simple sugar and sugar alcohol (SSASA) osmopotentiators for diet formulations were identified and diets for mitigating super chill in Atlantic salmon were formulated. Studies to determine effectiveness of SSASA diets were performed. The Atlantic salmon industry was informed of all research and progress.
3. Results: How did your work make a difference (change in knowledge, actions, or conditions) to the target audiences?
Extensive knowledge was gained on the use of SSASA diets and the potential mitigating effect on super chill in Atlantic salmon. Although the diets did not prevent super chill, diet formulation and results obtained provided valuable information on the use of osmopotentiators in salmon diets.
4. Recap: One- sentence summary
The primary product/outcome was the development of diet formulations with simple sugars and/or sugar alcohols to reduce the impacts of super chill in cultured Atlantic salmon and allow for expansion of the ATS salmon aquaculture industry in the northeastern US.</td></tr>
<tr><td>Publications</td><td>Follow the format to list publications in the following categories:
We anticipate submitting a manuscript to Aquaculture within the next 12 months</td></tr>
<tr><td>Students/Participants:</td><td>Provide the following information for every student that worked with you during the reporting period:
Emily Tarr; BS in Marine Sciences, Yr 4 undergraduate Student Served as a 2017/18 summer laboratory intern for the project. Anticipated degree date Dec 2019
Laurel Anderson; BS in Biology, Yr 4 undergraduate Student Served as a summer 2017/18 laboratory intern for the project. Anticipated degree date Dec 2019
Emma Blackdeer; BS in Marine Sciences, Yr 4 undergraduate Student Served as a 2018/19 school year laboratory intern for the project. Graduated May 2019</td></tr>
<tr><td>Partnerships</td><td>List any partners that you worked with on your project. Provide the following information for each Partner:
<table><tr><td>Partner
Cooke Aquaculture</td><td>Specific Type
Atlantic salmon aquaculture industry</td><td>Level
Non-funded</td><td>Nature of Partnership
Participates in all</td></tr></table></td></tr>
</table>

				findings and results