Semi-Annual Progress Report
Subaward # Z54060
Grant # 2010-38500-21074

PROJECT CODE: **SUBCONTRACT/ACCOUNT NO: 10-15**

PROJECT TITLE: Breeding Resistance to Sea Lice and ISAV in Atlantic Salmon
REPORTING PERIOD: August 2011 to October 15th 2012
FUNDING LEVEL: Total allocated to date. $131,134
PARTICIPANTS:

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PROJECT OBJECTIVES: List objectives as written in approved proposal.
1.) To evaluate genetic variation for sea lice and ISAV resistance in North American Atlantic salmon. Specifically our project will answer what is the best approach to incorporate selective breeding for sea lice and ISAV resistance into the USDA breeding program.
2.) To determine the potential risks of sea lice transmitting the serious fish pathogen infectious salmon anemia virus (ISAV) to wild and cultured Atlantic salmon populations. Specifically our project will answer:

a. Do sea lice take ISAV up from infected hosts?
b. Is it carried internally or externally by sea lice?
c. Do sea lice remain infectious following re-assortment (*i.e.* transfer to new hosts)?
d. Are Atlantic salmon infected with sea lice more susceptible to viral diseases or *vice versa*?

3.) Provide industry outreach by preparing information for distribution *i.e.* extension fact sheet publications (web postings) and organizing a special one day session at an appropriate workshop (*e.g*. NACE)
Milestone 1: ***ISAV transmission from ISAV-infected salmon (host) to sea lice (vector)***.
Milestone 2: ***ISAV transmission from sea lice (vector) to naïve salmon (host)***.
Milestone 3: ***Duration of pathogen association with sea lice, and virus viability in lice.***
Milestone 4: ***Effects of ISAV infection on sea lice resistance and sea lice infection on ISAV resistance.***
Milestone 5: ***Evaluate different families of Atlantic salmon for genetic variation to sea lice resistance and ISAV in laboratory trials and field trials***.
Milestone 6: ***Extension component***

ANTICIPATED BENEFITS: State how the project will benefit the aquaculture industry either directly or indirectly.

Products: This project will result in direct answers to industry's concern about sea lice (*L. salmonis*) in transmitting diseases, as well as demonstrating the potential benefits of using disease resistant families in reducing disease impacts. An evaluation on the feasibility of using Atlantic salmon families with improved resistance to both sea lice and ISAV will be produced.

End-users and beneficiaries of the project results: Marine finfish growers will benefit through the ability to employ new disease management strategies and lower the impact of their operation on the environment. State and federal resource management personnel will also benefit by having a better understanding of disease interactions and having the needed knowledge to establish useful and effective regulations to protect fish health. Disease-resistant traits identified in this work may also be applied to hatchery-reared families to identify more resistant progeny for release. Finally the general public will benefit by having a steady supply of sustainably farmed seafood products and reduced impact of those farming operations on the natural ecosystems we all share.

Measurable economic benefits: Measurable benefits of this project will include the capacity to reduce the frequency of disease outbreaks on salmon farms, in the long term by the use of disease resistant families, not only

permitting higher yield from the fish farm but the potential for disease interactions between endangered wild and farmed fish will be greatly reduced. Secondly, it provides growers with a new tool for managing diseases on their farms, possibly allowing a reduction in costly treatments.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Milestone 4: Determine whether sea lice infection impairs the immune response of the fish in terms of affecting the ability of Atlantic salmon to produce a specific antibody response.

Naïve Atlantic salmon smolts (~200g) from 6 families represented in the families used in the familial resistance trials (3 Penobscot-derived and 3 St John-derived) were infected on 07/29/2011 with *L. salmonis* copepodids by a dip method and all sham-infected control uninfected fish went through the same procedure without copepodids. Then 20 days post-infection with *L. salmonis* (08/18/2011), salmon were vaccinated with 100µl of 50µg BSA in Freud's incomplete adjuvant (Difco) or the vaccination controls were vaccinated with PBS in adjuvant. Therefore, the following treatment groups in triplicates were included in the trial:

Lice infected with PBS control vaccination
Lice infected with BSA vaccination
Sham-infected control with PBS control vaccination
Sham-infected control with BSA vaccination

All fish were euthanized at 6 weeks post-vaccination (09/29/2011), blood serum samples taken and lice counts performed on all fish. The specific antibody response post-vaccination will be determined by ELISA on the blood serum samples collected. The ELISA for salmon anti-BSA antibody response was optimized during February and March 2012 using an anti-trout/salmon monoclonal antibody produced by Dr Erin Bromage. Therefore, the frozen serum samples will be run through the optimized ELISA in November 2012.

Milestone 5: Identify North American Atlantic salmon families, originating from the Penobscot river and the St John river, which are more resistant to sea lice infection.

Lice counts have now been completed on all 3182 Atlantic salmon smolts (~100-200g) from 136 families produced at the ARS-USDA coldwater facility, Franklin that were infected in the laboratory with *Lepeophtheirus salmonis* using a bath method. Dr. W. Wolters at the National Cold Water Marine Aquaculture Center, Franklin, ME have performed pedigree analysis to determine heritability of sea lice resistance in the families using data collected from the smolts placed out in sea cages the summer of 2011 as well as that from the laboratory infection. This data is also being used to determine if any families were more resistant or susceptible to sea lice infection. A meeting will take place between the PIs in November to discuss the pedigree analysis and implications for the salmon farming industry and breeding program.

Milestone 1, 3 and 4: Determine whether a) sea lice infection increases the susceptibility of different families of Atlantic salmon to ISAV infection; and b) *vice versa* whether ISAV infection increases the susceptibility of different families to sea lice infection:

A) Naïve Atlantic salmon smolts (~100g) from 6 families represented in the families used in the familial resistance trials (3 Penobscot-derived and 3 St John-derived) were infected in the laboratory with infective copepodids of *L. salmonis* using a bath method on 07/29/2011. The infection was allowed to progress for 18 days, at which point the lice were molting into the more detrimental motile stages (figure 3), and the fish were infected with ISAV by a cohabitation method. Ten percent of the tank populations were injected with $10^{4.4}$ $TCID_{50}$ of ISAV in order to naturally infect the remaining fish in the tank. The fish were sampled prior to lice infection and at 0, 3, 16 and 37d.p.ISAV infection in order to test for viral load and viable ISAV presence as well as the immune response of the fish as the disease(s) developed. The trial was continued until 7.5 weeks post-ISAV infection (10/06/20211) in order to monitor mortality rates in the different treatment groups. The treatment groups were (6 replicates each): 1) No lice, no ISAV control; 2) No lice, ISAV only; 3) Lice plus ISAV infection

Kaplan Meyer analysis followed by log rank analysis demonstrated that salmon with a prior *L. salmonis* infection were found to possess a higher end-point mortality as well as to start dying faster due to ISAV infection compared to ISAV infected salmon without *L. salmonis* infection ($p < 0.0001$; Figure 1).

The fish showed clinical signs of ISAV infection (figure 2) with blood-tinged or yellow ascites and petechial hemorrhaging of the viscera. All sampled fish and mortalities were tested for the presence of viable ISAV by cell culture on ASK cells, and viral load determined by QRT-PCR. Dr Mark Fast's laboratory has performed the QRT-PCR for ISAV and immune gene expression and will shortly be sending the data to Dr. Ian Bricknells laboratory for data analysis and report writing. The salmon infected with lice and ISAV were found to be positive for viable ISAV from 16 days post-ISAV cohab-infection compared to the ISAV only group of salmon, which did not possess viable ISAV at 16 d.p.i. ISAV infection. At 37 d.p.i. ISAV-infection, viable ISAV

was detected in the ISAV only group however, a significantly lower proportion of the salmon sampled at 37d.p.ISAV infection were positive for viable ISAV compared to the lice plus ISAV group.

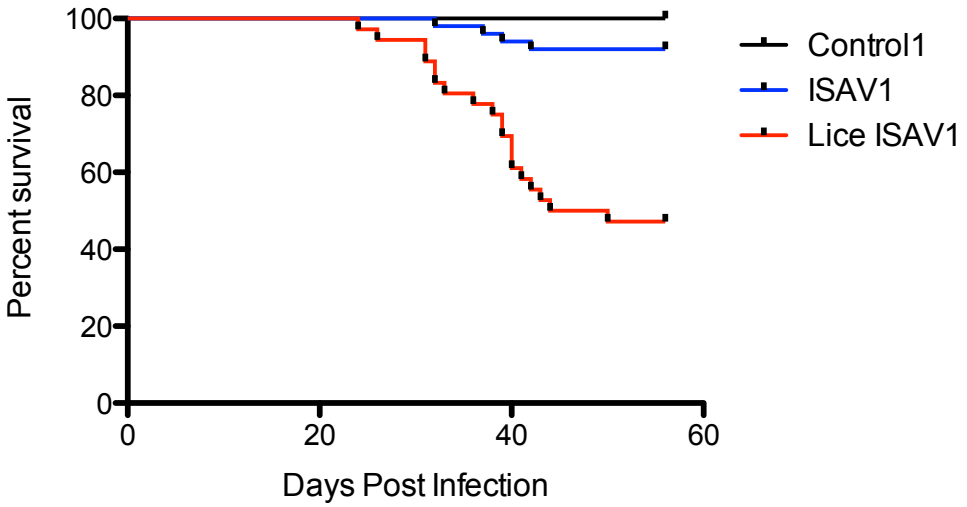

Figure 1: Percent survival of salmon in control group compared to that of salmon infected with ISAV only or *L. salmonis* plus ISAV.

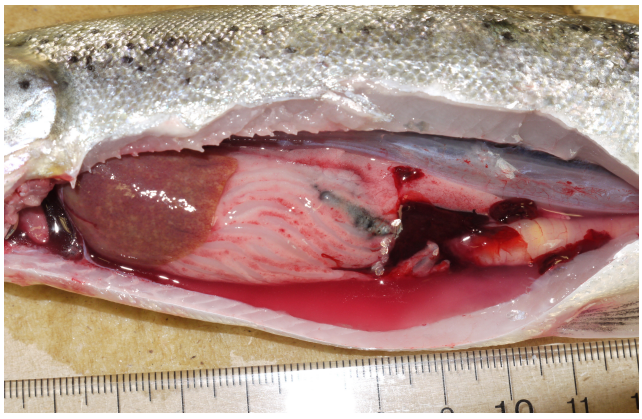

Figure 2: Clinical signs of ISA detected in the Atlantic salmon post-ISAV cohab-challenge.

This data therefore, suggests that salmon infected with *L. salmonis* molting into the motile stages are more susceptible to ISAV infection. However, the lice load with which the fish were infected during this trial was heavy with approximately 75 lice total per fish on average at the point of ISAV cohab-infection (figure 3). Therefore, we wanted to determine if the same effect would be observed in salmon infected with a low intensity of lice. It was decided to pursue this avenue of research instead of milestone part b: whether ISAV infection increases the susceptibility of different families to sea lice infection, as once ISAV is identified on a farm the animals are euthanized. This trial was conducted this summer commencing 08/03/2012 at the UMaine Orono campus. The trial consisted of six treatment groups in triplicate: 1) Low lice no ISAV; 2) Low lice plus ISAV; 3) High lice no ISAV; 4) High lice ISAV; 5) No lice no ISAV; 6) No lice plus ISAV.

The fish were cohab-infected with ISAV at 16 days post-lice infection, followed by sampling at 0, 6, 14 and 28 d.p.i. ISAV cohab-challenge for viable ISAV presence, viral load and immune gene expression. Mortalities were monitored for 7 weeks post-ISAV infection and all survivor fish sampled for ISAV presence and load. This trial finished on 10/09/2012. To this date, all of the cell culture and lice counts have been performed and the samples will be sent to Dr Mark Fast for QRT-PCR analysis of ISAV load and immune gene expression.

Lice were also collected from the salmon during both of these trials at all sampling points in order to fulfill milestone 1: ISAV transmission from ISAV-infected salmon (host) to sea lice (vector). Lice were processed for cell culture on ASK cells in order to determine if the lice were positive for viable virus when taken from ISAV infected fish. During the first trial lice (results from the 2nd trial are currently being analyzed) infecting ISAV infected salmon were positive for viable ISAV virus from 16 days post–ISAV infection, and this was observed in all four replicate tanks. At 37 d.p.i ISAV infection there were only enough lice sampled from one of the four replicate tanks, however, 50% of the lice sampled in this tank were positive for viable ISAV. Therefore, ISAV

can transfer from infected salmon to *L. salmonis* motile stages, however, the location of the virus will be determined using immunohistochemistry with an anti-ISAV antibody, and whether these lice are capable of transmitting ISAV infection during host re-assortment is currently being determined in a trial commencing 10/17/12.

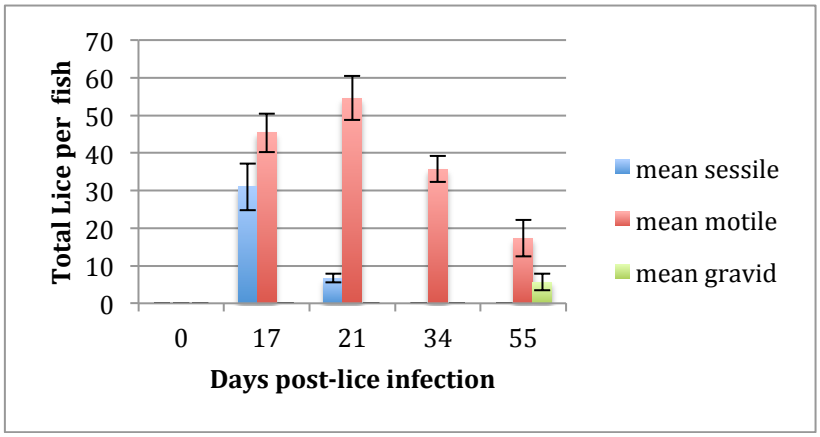

Figure 3: Mean number of sessile, motile and gravid lice per fish at 0, 17, 21, 34 and 55 days post-lice infection.

WORK PLANNED:

Milestone 2: Determine the potential role of sea lice as vectors for ISAV.

This trial will commence 10/17/12. Adult lice will be collected from i) ISAV-free salmon (negative control), ii) ISAV-infected salmon and iii) ISAV-free salmon followed by bathing lice in ISAV (positive control). These lice will then be used to infect naïve salmon. This will allow us to determine whether the lice are capable of transmitting ISAV to naïve salmon. Immunohistochemistry on lice collected during trial 1 and 2 of milestone 4 outlined above will be used to determine whether the lice carry the viral particles on the surface or internally.

Milestone 6: Extension component

At the end of this grant the PI's will give presentations and discussion groups at an extension meeting with stakeholders such as Atlantic salmon farmers.

IMPACTS: In concise statements (possibly a bulleted list) indicate how the project has benefited the aquaculture industry either directly or indirectly and resulting economic values gained (where appropriate).

- The analyses from Milestone 5 will provide information on the susceptibility of North American Atlantic salmon to sea lice infection and if selective breeding in North American strains of Atlantic salmon for sea lice resistance is possible.
- Milestones 1 through 4 investigate the interactions of ISAV and sea lice and will highlight the potential risks of lice-infected salmon contracting or transmitting ISAV.

SUPPORT:

YEAR	NRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1	$131,134						$131,134
2	X						X
TOTAL	$131,134						$131,134

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Paper presented:

Barker, S.E., Bouchard, D., Wolters, W., Fast, M., Bricknell. (**2012**) I. Are 'lousy' fish more susceptible to ISAV infection? Eastern Fish Health Workshop 2011, USA.

Barker, S.E. (**2012**) Delving into the amazing world of two economically important parasitic copepods: *Lepeophtheirus salmonis* & *Lernaeocera branchialis*! SMS seminar series University of Maine, ME, USA.

Barker, S.E. (**2012**) Delving into the amazing world of two economically important parasitic copepods: *Lepeophtheirus salmonis* & *Lernaeocera branchialis*! AVS seminar series University of Prince Edward Island, PEI, Canada.

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SIGNATURE PAGE

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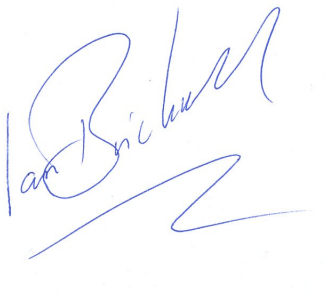
PREPARED BY:



_____ 15th October 2012_____
Project Coordinator of Subawardee Date