

From: Kim, Eliah
Sent: Friday, April 1, 2022 8:07 PM
To: Jones, Simon **Provided in excel format**
Subject: sea lice study lab report and result package
Attachments: Observation report.docx; Productivity Observation report.docx; 1st Adult Study_Lice number_20211205.xlsx; Repeat Study_Lice number_20211216.xlsx; Sea Lice Fish Physical Data_20220125.xlsx; Sea lice larvae counting_FEB 2022.xlsx

Hello Simon,
Sorry about it taking so long to finish, as always something urgent one pop-up and also non-working days (holiday) part of delay cause as well. thank you for your understanding.
As you mentioned, I am sending a package so you can have all the necessary documents in one place.
Thank you
Eliah

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Sea Lice Observation note

Adult Lice Experiments: 1st Trial (Period Nov 27, 2021, to Dec 5, 2021)

TimeLine

- Sea Lice Collection: Nov 27, 2021
- Arrived at Station: Nov. 28
- Sea Lice Divide to 9 jugs of 5-liter: Nov 29 (3jugs and 3treatments)
- Peroxide treatment: Nov 29
- Exposure to Fish: Nov 30
- #_Tank_#_fish: 9 tanks and 20 fish per tank
- Sea Lice stage per tank: Used only Female lice without egg strings for tank 21days sample trial (3tanks), Used mixed sea lice (female and ovigerous female) for 3 & 5 days sample tanks (6tanks).
- Avg #_Lice per tank : 0ppm (4.833), 500ppm (5.367) & 1500ppm (5.133)
- Sampling date: Day 3 (Dec 3), Day 5 (Dec 5) – original experiment design is to terminate fish and count # lice from each fish as attached and unattached. Only Day 3 fish were euthanized and Day 5 fish were saved for later. Lice # counted as fish sedated with Aquacalm (0.025mg/l).

Methods

- Lice collection: In total over 2000 female and ovigerous females were collected from the harvesting boat. Lice were carefully removed with forceps and directly placed in aerated seawater. After lice collection, the bucket of water changed to cool/fresh seawater and aerated, and left in a cool area. The next day morning, the bucket of water was replaced with cool/fresh seawater and aerated. During the transportation, the lice bucket sat on ice to keep it cool. Once arrived at the station, the Lice bucket was left at T103 for the following day separation and peroxide treatment. Try not too much to handle the lice.
- By the following day, Lice divided nine 5-liter jugs randomly. Lost quite an amount of lice overnight. Afternoon prepared 9 of 500ml glass bottles; three for 0ppm, three for 500ppm, and 3 for 1500ppm of peroxide. All peroxide preparation had been performed under a fume hood to minimize the risk of peroxide and wore chemical resisted gloves as SDS recommended.
- Lice exposure to Peroxide : Each treatment had triplicates and worked from 0ppm -> 500ppm -> 1500ppm. Took five minutes intervals among the same treatment group of jugs. For example, add peroxide solution in one jug of 0ppm and increased air and then 5min later repeated to the second jug of 0ppm and 5min after for the third jug. Each exposure time was 20min and after 20min later, the jug of water dump into green dip nets, and captured any free unattached lice

were changed to fresh seawater and the lice jug was returned to the water bath. Repeated this until finish up all three jugs. Once finish all three jugs were finished in the same group, move to the next group. The same manner for 500ppm and 1500ppm. During peroxide exposure, jugs were covered with aluminum foil and kept in dark, water bath tank overlapped with a black garbage bag. For 24hrs cleansing time, jug covered aluminum foil and aerated in dark but no garbage bag over the bath.

- Observation during the cleansing period: Right after exposure to peroxide solution, after 20min, most of the lice lost their attachment from 500ppm (few attached on wall-roughly 20%?) and from 1500ppm group' lice 100% lost its attachment. There were no indication lice were alive, basically, lice were floating inside the jug as depended on the aeration current for the first two hours. After 3hrs 500ppm group show the recovery, more likely swimming on its own and more lice started attached to the jug wall. Not much improving in 1500ppm. About 5hrs after expose, 1500ppm group' lice started to recover as like 500ppm. The next morning, still the recovering in progress especially 1500ppm group. By the time passed 24hrs, all 1500ppm group' lice were completely recovered vs 500ppm groups were the next morning so about 18hrs.
- After 24hrs, jugs of lice were transferred to Brett building for fish attachment. The order of lice expose to fish from 0ppm -> 500ppm -> 1500ppm. Fish were sedated with Aquacalm (0.25mg/liter) and after 20min, all 20fish from a tank move to a temporary holding bucket (0.25mg/liter) with aeration. During exposure time both lice and fish are kept in under aeration. For lice exposure, prepared an aerated tote with 20liter of Aquacalm (0.50 mg/liter level) and put 5fish at a time, and added lice. Waited for 5-10min lice attached on naturally, after 10min later tried to put lice on fish to get aiming lice number per fish. Surprisingly Lice had a very specific preference for fish and position. Seemed like lice were also sensitive to Aquacalm. Noticed lice movement slowed down and attached to the wall of the tote. Fish got at least 5 attached lice, immediately scooped with a green dip net, and transferred to its own tank. Repeat this until all five fish were out and added another five fish. Recorded attached lice # when returning the fish into the tank. Even 15 minutes after in the tote, very rare chance only three lice were attached to the fish, and in this case return the fish to the original tank and recorded the attached lice number.
- During lice fish exposure, noticed a preference from lice to fish. Not only a fish preference and also even the same fish it had a location preference as well. Some of the fish had fewer lice as planned so manually grabbed a louse and put it down on a fish. Often lice swung away from fish once drop off on fish and attached to either the bottom or sidewall of the tote. Some lice tried to the glid surface of fish until found a preference spot and stayed on.
- The average attached sea lice # is 4.88 lice (0ppm), 5.37 lice (500ppm), and 5.13 lice (1500ppm).

Sampling Period:

- 3days after sea lice implant: ten fish per each tank and two tanks per each treatment in total 60 fish were euthanized with TMS and each fish was put into an individual 5liter bucket until fully euthanized and recorded attached and unattached lice #. Worked on five fish at a time and

repeated until the finish. The sample order was the same as previous 0ppm -> 500ppm -> 1500ppm treatment group.

- Noticed lice # is significantly lower than exposure day, average sea lice # per fish 1.15 lice (0ppm), 1.1 lice (500ppm), and 0.25 lice (1500ppm).
- 5days after sea lice implant: remain ten fish per each tank and two tanks per each treatment previously sampled, sedated with Aquacalm (0.25mg/liter), and checked the lice #. Same as before, each individual fish was kept in individual 5liter sedated water and worked on five fish at a time and 0ppm -> 500ppm -> 1500ppm. Once fish had been counted lice number returned to the original tank. The average sea lice # per fish is 0.2 lice (0ppm), 0.2 lice (500ppm) and 0 lice (1500ppm)
- Very minimum skin damage found during sampling.

Some Events (meanwhile)

- All tank drain pipes were covered with a 100um filter bag to catch free unattached sea lice and on Dec. 8, 2021 (8days after lice implant days), all socks were collected and counted sea lice number. Most sea lice lost their shape and only partial skeleton remained.
- Dec. 9, 2021: Combined fish together for the second challenge. And also separated fish with skin damage. Sedated fish with Aquacalm (0.25mg/liter) and scoop out fish one at a time not only to check for possible remaining sea lice and also for skin damage. End up 6tanks with most of 19fish and one tank with 20fish.

Adult Lice Experiments: 2nd Trial (Period Dec 12, 2021, to Dec 19, 2021)

TimeLine

- Sea Lice Collection: Dec. 13, 2021
- Arrived Station: Dec. 14, 2021
- Sea Lice Divide to 6 jugs of 5-liter: Dec. 14 (2jugs and 3treatments)
- Peroxide treatment: Dec. 15
- Exposure to Fish: Dec. 16
- #_Tank_#_fish: 6 tanks and 19 fish per tank except one (500ppm) 20fish
- Sea Lice stage per tank: Used mix of female and ovigerous female.
- Avg #_Lice per tank : 0ppm (5.05), 500ppm (5.38), 1500ppm (5.17)
- Sampling date: Post Day 1(Dec 17), Day 3 (Dec 19) – Euthanized ten fish on PD 1 and Euthanized rest of fish on 3 PD

Methods

- Lice collection: about 1000 female and ovigerous females were collected from a harvesting boat. Lice were carefully removed with forceps and directly placed in aerated seawater. After lice collection, the bucket of water changed to cool/fresh seawater and aerated, and left in a cool area. The next morning, the water in the bucket changed again to fresh and cool seawater. During the transportation, the lice bucket was aerated and sat on a cooler with ice to keep it cool. Once arrived at the station, Lice divided six 5liter jugs randomly and left them in the water bath with aeration. The lice divided immediately once arrived at the station due to possible overnight mortality as in the first time period. In the first time period, discovered few mortality overnight.
- The next day, Lice exposure to Peroxide accordingly. 0ppm -> 500ppm -> 1500ppm. Note that there was no significant mortality observed overnight like in the first lice collection. When added prepared peroxide solution was, the air increased trying to minimize the hot zone. Prior to peroxide exposure, the peroxide solution had been prepared in 500ml of a glass bottle. The bottles were kept in dark until use and as same as likely during the first trial.
- Once marked on 20min exposure, the lice water is dumped into green dip nets and changed to fresh cold seawater, and returned to the water bath. Any lice recovered from dip nets also returned to the original jug.
- The next day, after 24hrs exposure, the lice jug was transferred to Brett building for fish exposure. Again the process did as same as the first trial.
- Observation during the cleansing period: Right after exposure to peroxide solution, after 20min, half of the lice lost their attachment from 500ppm and from 1500ppm group' lice 100% lost their attachment as same as the first trial. There is no movement from lice, basically, lice were floating inside the jug as depended on the aerated current for the first two hours. After 3hrs 500ppm group started its recovery, more likely swung its own and more lice started attached to the jug wall. Not much improvement in 1500ppm was found. About 5hrs after expose, 1500ppm group' lice started to recover as like 500ppm. The next morning, still the recovering in progress especially 1500ppm group. By the time passed 24hrs, all 1500ppm group' lice completely recovered vs 500ppm groups was the next morning so about 18hrs.
- The average attached sea lice # is 5.05 lice (0ppm), 5.35 lice (500ppm), and 5.15 lice (1500ppm).

Sampling Period:

- 1day after sea lice implant: ten fish per each tank and two tanks per each treatment in total 60 fish were euthanized with TMS and each fish was put into an individual 5liter bucket until fully euthanized and count attached and unattached lice #. Worked on five fish at a time and

repeated until the finish. The sample performed an order 0ppm -> 500ppm -> 1500ppm treatment group.

- Noticed lice # was significantly lower than exposure day, average sea lice # per fish was 2.8 lice (0ppm), 1.5 lice (500ppm), and 2.1 lice (1500ppm).
- 3days after sea lice implant: remain ten fish per each tank and two tanks per each treatment previously sampled, sedated with Aquacalm (0.25mg/liter), and checked the lice #. Same as before, each individual fish was kept in individual 5liter sedated water and worked on five fish at a time and 0ppm -> 500ppm -> 1500ppm. The average sea lice # per fish is 0.45 lice (0ppm), 0.7 lice (500ppm) and 0.25 lice (1500ppm)
- Very minimum skin damage found during the lice counting.