* Pilot experiment
* June 1 2016 – Trial 1
  + 13 PAL lice collected from juvenile pink &chum salmon between 8 AM – 3 PM
  + stored in container with sea water in cooler with ice packs
  + Returned to station – lice kept in cooler overnight
  + Made stock solution
    - Weighed 40 mg of EMB on scale on petri dish (**this will be weighed at Hakai)**
    - Transferred 40 mL of methanol onto petri dish with EMB
    - Mixed with pipette
    - Transferred methanol/EMB into stock jar
    - Added 360 ml of methanol to stock jar using 100mL cylinder
    - Stored 400 mL of stock solution overnight
* June 2 2016
  + Checked on lice – all alive
  + Made working solution
    - 99 ml of sea water, 1 mL of stock solution
    - Collected bucket of sea water
    - Used 10 mL cylinder to measure 99mL into working solution jar
    - Used 1 mL pipette to transfer from stock into work jar

Made concentrations for petri dishes

|  |  |  |
| --- | --- | --- |
| Concentration of EMB | Working solution | Sea water |
| 0 ppb | 0 mL | 100mL |
| **15ppb** | **1.5mL** | **98.5mL** |
| **30ppb** | **3mL** | **97mL** |
| **60ppb** | **6mL** | **94mL** |
| 90ppb | 9 mL | 91 mL |
| 180 ppb | 18 mL | 82 mL |

* **Prepare a control working solution that is 99mL sea water, 1ml methanol. Then prepare another control with 18ml control working solution and 82mL sea water.**
* 0 – 2 PAL II male, PAL II female, PAL I male (PAL II male blood spot)
* 90 - 2 PAL II male, PAL II female, 2 PAL I female
* 180 – 3 PAL II males, PAL I female (PAL II male algae on tail, PAL II male poor suction)
  + stored in container & cooler with ice packs for 24 hours

June 3 2016

Temperature Monitoring

|  |  |  |
| --- | --- | --- |
| Time | Concentration of EMB (ppb) | Temperature (degrees Celsius) |
| 12:00 | 0 | 8 |
| 12:00 | 90 | 8 |
| 12:00 | 180 | 11 |
| 18:00 | 0 | 14 |
| 18:00 | 90 | 11 |
| 18:00 | 180 | 13 |
| 0:00 | 0 | 11 |
| 0:00 | 90 | 9 |
| 0:00 | 180 | 8.5 |
| 6:00 | 0 | 10 |
| 6:00 | 90 | 7 |
| * 6:00 | * 180 | * 8 |

* + Temperature monitored every six hours – lice stored in cooler with two ice packs
  + Hand held thermometer placed in each petri dish and waited until temperature stabilized
  + Cleaned thermometer between each dish
  + Changed 1 ice pack at 0:00 temperature check

EMB Reaction – Trial 1

Data

|  |  |  |  |
| --- | --- | --- | --- |
| Louse # | Concentration of EMB (ppb) | Sex | Status (live/moribund/dead) |
| 1 | 0 | PAL II male | Live – blood spot guy |
| 2 | 0 | PAL II female | Live |
| 3 | 0 | PAL II male | Live |
| 4 | 0 | PAL I male | Live |
| 5 | 90 | PAL II female | Moribund |
| 6 | 90 | PAL I female | Dead |
| 7 | 90 | PAL II male | Moribund |
| * 8 | * 90 | * PAL I male | * Dead |
| * 9 | * 90 | * PAL II male | * Dead |
| * 10 | * 180 | * PAL II male | * Moribund – barely moving – some movement ear tail with algae |
| * 11 | * 180 | * PAL II male | * Live |
| * 12 | * 180 | * PAL II male | * Moribund |
| * 13 | * 180 | * PAL I female | * Moribund |

* + lice stored in different concentrations for 24 hours
  + Status: - adapted from Westcott et al 2008 – element of subjectivity – trying to replicate their system in order to create consistency
    - Live
      * Normal swimming ability – active darting
      * Can suction onto petri dish (bottom, side, spoon)
      * Movement of extremities
    - Moribund
      * Weak movement
      * Poor suction ability
      * Minimal movement of extremities
    - Dead
      * Floating – no suction
      * No movement or swimming
  + SLICE paralyzes lice – to deter suction? Morbidity resulting from paralysis?
* June 8 – Trial 2
  + Collected 9 PALs from juvenile chum & pink salmon
  + Stored in cooler overnight with ice packs
* June 9
  + Took temperature of lice after being stored overnight
  + Made working solution
    - 99 ml seawater
    - 1 ml stock solution
  + Made concentrations for lice
    - 0 ppb – control – 100 ml sea water
    - 90 ppb – 9 ml working solution – 91 mL seawater
  + ID’ed PALs under microscope and placed in petri dishes
    - 0 ppb – PAL II male, PAL I male, PAL I male
    - 90 ppb – PAL I male, PAL II female, PAL II male, PAL I male (slightly moribund)

|  |  |  |
| --- | --- | --- |
| * Time | * Concentration of EMB (ppb) | * Temperature (degrees Celsius) |
| * 9:30 | * 0 | * 14 |
| * 9:30 | * 90 | 14 |
| * 15:30 | * 0 | * 11.5 |
| * 15:30 | * 90 | 12 |
| * 3:30 | * 0 | 11 |
| 3:30 | * 90 | * 10 |
| * 9:30 | * 0 | 10 |
| * 9:30 | * 90 | * 9 |

* June 10, 2016
  + Checked lice under microscope to determine alive/moribund/dead

|  |  |  |
| --- | --- | --- |
| Concentration (ppb) | Sex/Stage | Status |
| 0 | PAL II male | Alive |
| 0 | PAL I male | Alive |
| 0 | PAL I Male | Alive |
| 90 | PAL II male | Moribund |
| 90 | PAL II female | Moribund |
| 90 | PAL I male | Dead |
| 90 | PAL I female | Dead |

* June 12 - Trial 3
  + Collected 20 PALs from sockeye, chum, pink salmon (Bauza Cove)
  + Prepared stock solution with 40 mg of EMB – measured with scale – analytical balance with glass window at Quadra
  + Prepared working solution
  + Prepared concentrations of EMB
    - 0, 30, 60, 90

|  |  |  |
| --- | --- | --- |
| * Time | * Concentration of EMB (ppb) | * Temperature (degrees Celsius) |
| * 18:30 | * 0 | * 10 |
| * 18:30 | * 30 | * 10 |
| * 18:30 | * 60 | * 9 |
| * 18:30 | * 90 | * 12 |
| * 1:00 | * 0 | * 8 |
| * 1:00 | * 30 | * 8 |
| * 1:00 | * 60 | * 8 |
| * 1:00 | * 90 | * 9 |
| * 8:00 | * 0 | * 8 |
| * 8:00 | * 30 | * 9 |
| * 8:00 | * 60 | * 7 |
| * 8:00 | * 90 | * 7 |
| * 13:00 | * 0 | * 9 |
| * 13:00 | * 30 | * 13 |
| * 13:00 | * 60 | * 11 |
| * 13:00 | * 90 | * 9 |

|  |  |  |
| --- | --- | --- |
| Concentration | Sex & Stage | Status |
| 0 | PAL I female | Alive |
| 0 | PALII male | Alive |
| 0 | PAL I female | Moribund |
| 0 | PAL II female | Alive |
| 0 | PALII male | Alive |
| 30 | PAL II female | Alive |
| 30 | PAL I female | Moribund |
| 30 | PAL II male | Alive |
| 30 | PAL II male | Moribund |
| 30 | PAL I male | Alive |
| 60 | PAL II male | Moribund |
| 60 | PAL II female | Moribund |
| 60 | PAL II female | Moribund |
| 6 | PAL II male | Alive |
| 60 | PAL I female | Moribund |
| 90 | PAL II male | Moribund |
| 90 | PAL II male | Dead |
| 90 | PAL II male | Alive |
| 90 | PAL I female | Dead |
| 90 | PAL II male | Alive |

* Trial 4 – June 16
  + Collected sea lice from Wednesday monitoring
  + Made new working solution with stock solution
  + Concentrations of 0, 60, 90

|  |  |  |
| --- | --- | --- |
| * Concentration | * Sex & Stage | * Status |
| * 0 | * PAL II male | * Alive |
| * 0 | * PALII male | * Alive |
| * 0 | * PAL II female | * Alive |
| * 0 | * PAL II female | * Alive |
| * 60 | * PALII female | * Moribund |
| * 60 | * PAL II male | * Moribund |
| * 60 | * PAL I male | * Dead |
| * 60 | * PAL II male | * Alive |
| * 60 | * PAL I female | * Moribund |
| * 90 | * PAL I female | * Dead |
| * 90 | * PAL II female | * Moribund |
| * 90 | * PAL II male | * Dead |
| * 90 | * PAL I female | * Dead |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| * year | * Concentration | | | | | |
|  | * 0 | * 15 | * 30 | * 60 | * 90 | * 180 |
| * 2012 | * 0.964285714 |  | * 0.333333333 |  | * 0.178571429 | * 0.2 |
| * 2015 | * 0.863636364 | * 0.875 | * 0.541666667 | * 0.458333333 | * 0.333333333 | * 0.047619048 |
| * 2016 | * 0.9375 | * 0.6 | * 0.2 | * 0.2 | * 0.111111111 | * 0.25 |