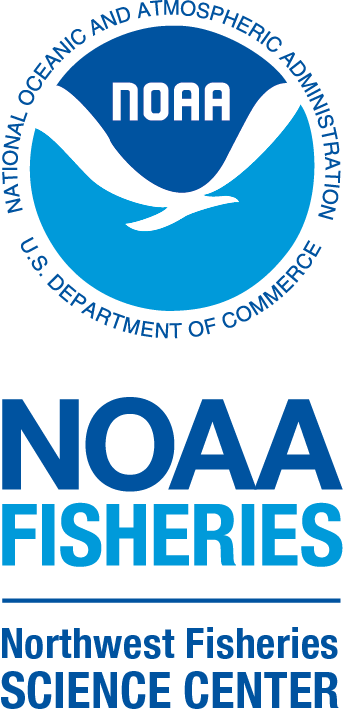
|  |  |
| --- | --- |
| From | Kate’s understanding of the Meeting |
| To | Shallin Busch Paul McElhany, Mike Maher, Danielle Perez |
| Date | Wednesday 2021.02.24 (Last Draft) |
| SUBJ | Respirometry Narrative |
| Drive | https://drive.google.com/drive/folders/1sMiXNW6YCKHZoMop1wlQl-i1HPmBZEEW?usp=sharing |
| REPO |  |
| Study | 2019 Krill Exposure Cross Study |

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**1.0 Respirometry Protocol Description & Refresher**

**1.1.a Respirometry and Project End Goals.** Four lipid samples from each MOAT at the end of the study. Whole body “MORTs” were measured to get a sense of the size for the animals as the study progressed. The termination of the study was biomass dependent.

**Other Samples Collected for Lipids Analysis.**

A wild cohort was measured for both their size and wet weight.

72 animals were selected for respirometry

112 other animals were prepared for lipids analysis (including wild cohort)

184 total number of animals were sent for lipids analysis

|  |  |
| --- | --- |
| Google Drive | Lipids Documents (Louise Copeland Lab) |
| https://drive.google.com/drive/folders/1ViSaymvzDdrf1Xu2ZRz79vqYLQRvC6lp?usp=sharing | |
| Google Drive | Lipids Documents (NWFSC prepared documents) |
| <https://drive.google.com/drive/folders/1yDILSlUahFfrmPkOjkfjW2OcTXI-o0p1?usp=sharing> | |
| GitHUB | (McElhany Repository) |
| https://github.com/pmcelhany/krill\_lipids.git | |

**1.1.b Project’s End Respirometry Trials** **Determination**

19 vials (volume 28.06mL) were available

Four trials were conducted over two day

Two blank vials were included per trial

“Krill Doctrine” was employed to determine how best to distribute krill across trials

**Krill Doctrine**

Fast krill for 24hours prior to respirometry and lipids analysis preparation

Choose MOATs with the lowest number of animals remaining for the first round(s) of respirometry and follow on preparation for lipids analysis

Balance between MOATs across days of respirometry

Balance between Treatments when choosing which respirometry round

Must achieve 4 vials per MOATs

Achieve 15-20 animals across treatments for respirometry

Wait until Night Time Conditions begin for the Krill to start Respirometry Trials. The night period for krill began at 1230pm.

**1.1.c Project’s End Respirometry Trials** **Description**

16 Animals per “Ambient Treatment”

16 Animals per “All Change Treatment”

16 Animals per “High Temperature Treatment”

1. nimals per “Current Treatment”

**2.0 Respirometry Project** ““NWFSC.MUK\_KRL2019respirometrySLOPES”

R scripts and documents inside the project listed above was generated to create slopes, intercepts, and apply linear/mixed effects models to the slopes.

**2.1 Merging.** The database is created by merging two datafiles that brought together observer data collected data about individual krill (NIKON SMZ715T with Sony Camera manufactured by Imaging Source (DFK 33UX226c) and IC capture and measure software packages) and the PRESENS (PreSens optical oxygen Fitbox4 sensor). The merged dataframe was referred to as “DRESPmsr”.

|  |  |  |
| --- | --- | --- |
| **2.1a Creating the database** | | |
| Google Drive | dRESP <- read.csv(file = "**KRILL\_Resp\_alltrials.csv**", stringsAsFactors = FALSE)  dim(dRESP) | dRESP |
| https://drive.google.com/file/d/19veU1VIWJSrzlMEoVsmDicNNEKG-kWpW/view?usp=sharing | | |
| Google Drive | dRESPanimal <- read.csv(file = "RespirometryTrials\_all.Animal.Info.csv")  dim(dRESPanimal) | dRESPanimal |
| https://drive.google.com/file/d/1vdumqXO0O-3-htxKjtagctW3YLOZoGj3/view?usp=sharing | | |
| Google Drive | dRESPmsr <- merge(dRESP, dRESPanimal, by="SensorName") | dRESPmsr |
| https://drive.google.com/file/d/1pWiqUsxNBWs5nYindWgORBDG9eMeg4sB/view?usp=sharing | | |

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| --- |
| **2.1b Merged database dRESPmsr**  **Contains all Animal Specific Information and Probe Measurements** |
| **A picture containing text, cup  Description automatically generated** |

**2.2 DO Correction.** On Trail 03 of Respirometry Day 02 the PRESENS default temperature of 14°C was entered. All krill under respirometry trials were kept in a water bath between 11.4-12℃. To correct for this temperature correction, Salinity constant was taken from the water supply MOATs 12 (30.3PSU) and modified with the package “wql”. Table below details the units of dissolved of oxygen, including volume inside the incubation vial.

|  |
| --- |
| **2.2a DO Corrections and Oxygen Units**  **Following DO correction “oxygen” units is mg** |
| Diagram  Description automatically generated |
| Vial volumne was .02806. PRESENS probe measured Dissolved Oxygen as mg/L. |

**2.3 Units of Time.** The PRESENS probe initially recording in minutes with decimal time.

|  |
| --- |
| **2.3a Units of Time “delta\_t”**  **Cumulative Amount of Time (.00hr)** |
| Graphical user interface  Description automatically generated |
| Observations were collected from start (T00) to 90minute completion (T90).  The “delta\_t” value will increase until an approximate value of ~1.3hrs.  Simple slopes of krill respiration are recorded as μg / hr.  Slopes will later be corrected for background noise and krill wet weight. |

**2.4 Animals Per Trial, Per Treatment Tables.** The tables below detail how many animals have been included in each trial 01-04. The tables break down between treatments. The Ambient Treatment was initially included. The Respirometry 90 minute dataframe excludes the Ambient Treatment due a lack of consistent monitoring and equipment logs from both MOATs 04 and MOATs 05.

**Trial 1 of 4**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trial1Animals** | **Trial1\_CHG** | **Trial1\_CUR** | **Trial1\_TMP** | **Trial1\_Blanks** |
| **19** | **5** | **4** | **8** | **2** |
| All Change  Conditions | Current  Conditions | | High Temperature  Conditions | |
| 1. "Trial01\_KrilLR16" 2. "Trial01\_KrilLR17" 3. "Trial01\_KrILR110" 4. "Trial01\_KriLR116" 5. "Trial01\_KriLR117" | 1. "Trial01\_KrilLR11" 2. "Trial01\_KrilLR13" 3. "Trial01\_KrilLR18" 4. "Trial01\_KriLR114" | | 1. "Trial01\_KrilLR14" 2. "Trial01\_KrilLR15" 3. "Trial01\_KriLR111" 4. "Trial01\_KriLR112" 5. "Trial01\_KriLR113" 6. "Trial01\_KriLR115" 7. "Trial01\_KriLR118" 8. "Trial01\_KriLR119" | |
| Two blank vials included to complete a 19 vial respirometry test. | | | | |

**Trial 2 of 4**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trial2Animals** | **Trial2\_CHG** | **Trial2\_CUR** | **Trial2\_TMP** | **Trial2\_Blanks** |
| **11** | **5** | **4** | **0** | **2** |
| All Change  Conditions | Current  Conditions | | High Temperature  Conditions | |
| 1. "Trial02\_KriLR223" 2. "Trial02\_KriLR227" 3. "Trial02\_KriLR229" 4. "Trial02\_KriLR233" 5. "Trial02\_KriLR235" | 1. "Trial02\_KriLR220" 2. "Trial02\_KriLR226" 3. "Trial02\_KriLR232" 4. "Trial02\_KriLR234" | | character(0) | |
| Two blank vials included to complete a 11 vial respirometry test.  Eight vials were originally collected from the “Ambient Treatment”,  all from MOATs 04 for the second round of respirometry | | | | |

**Trail 3 of 4**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trial3Animals** | **Trial3\_CHG** | **Trial3\_CUR** | **Trial3\_TMP** | **Trial3\_Blanks** |
| **19** | **5** | **4** | **8** | **2** |
| All Change  Conditions | Current  Conditions | | High Temperature  Conditions | |
| 1. "Trial03\_KRLr3\_42" 2. "Trial03\_KRLr3\_46" 3. "Trial03\_KRLr3\_48" 4. "Trial03\_KRLr3\_53" 5. "Trial03\_KRLr3\_56" | 1. "Trial03\_KRLr3\_41" 2. "Trial03\_KRLr3\_47" 3. "Trial03\_KRLr3\_51" 4. "Trial03\_KRLr3\_57" | | 1. "Trial03\_KRLr3\_39" 2. "Trial03\_KRLr3\_40" 3. "Trial03\_KRLr3\_44" 4. "Trial03\_KRLr3\_45" 5. "Trial03\_KRLr3\_49" 6. "Trial03\_KRLr3\_50" 7. "Trial03\_KRLr3\_54" 8. "Trial03\_KRLr3\_55" | |
| Two blank vials included to complete a 19 vial respirometry test. | | | | |

**Trail 4 of 4**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trial4Animals** | **Trial4\_CHG** | **Trial4\_CUR** | **Trial4\_TMP** | **Trial4\_Blanks** |
| **11** | **1** | **7** | **0** | **2** |
| All Change  Conditions | Current  Conditions | | High Temperature  Conditions | |
| 1. "Trial04\_KRLr4\_70" | 1. "Trial04\_KRLr4\_58" 2. "Trial04\_KRLr4\_62" 3. "Trial04\_KRLr4\_65" 4. "Trial04\_KRLr4\_67" 5. "Trial04\_KRLr4\_71" 6. "Trial04\_KRLr4\_75" 7. "Trial04\_KRLr4\_76" | | character(0) | |
| Two blank vials included to complete a 11 vial respirometry test.  Eight vials were originally collected from the “Ambient Treatment”,  all from MOATs 05 for the second round of respirometry | | | | |

**3.0 Timeseries Plots (prior to slope function).** The figures below detail the decrease in oxygen per vial over time in each trial. The figures are broken apart by treatment. The “n/a” treatment refers to blank vials, filled only with filtered seawater from MOATs 12.

|  |
| --- |
| **3.1a Trial 01 Percent Dissolved Oxygen Reduction Timeseries** |
| A picture containing chart  Description automatically generated  A picture containing timeline  Description automatically generated |
|  |

|  |
| --- |
| **3.2 Trial 02 Percent Dissolved Oxygen Reduction Timeseries** |
| A picture containing chart  Description automatically generated  A picture containing timeline  Description automatically generated |
|  |

|  |
| --- |
| **3.3 Trial 03 Oxygen (μg) Reduction Timeseries** |
| Graphical user interface  Description automatically generated with low confidenceChart  Description automatically generated with medium confidence |
|  |

|  |
| --- |
| **3.4 Trial 04 Oxygen (μg) Reduction Timeseries** |
| Chart, line chart  Description automatically generatedChart, line chart  Description automatically generated |
|  |

4.0 Outlier Removal. Removing outliers was a six step process to remove observations that had a Cook’s Distance above 4/n. The following table details those steps below:

|  |  |  |
| --- | --- | --- |
| Step | Line Command | Example of the Line Command |
| 1 | Use the unique tool to get names for each krill |  |
| 2 | Create a linear model object for all observations of one krill |  |
| 3 | Using the fortify function, search residuals for cook.sd score for each observation |  |
| 4 | Identify observations with cook.sd score above 4/n |  |
| 5 | Use one dataframe to sort another  (removing the outliers) |  |
| 6 | Rbind back the filtered dataframes |  |

Placeholder