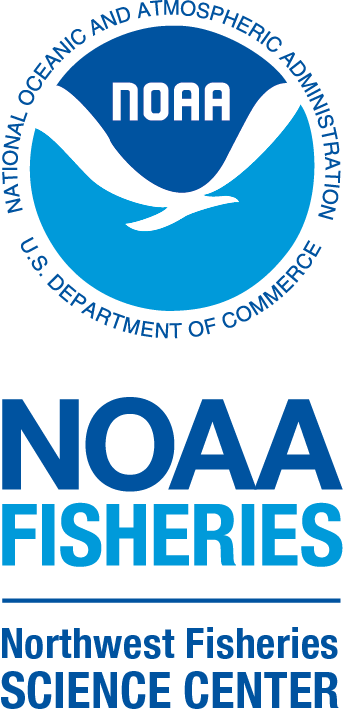
|  |  |
| --- | --- |
| From | Kate’s understanding of the Meeting |
| To | Shallin Busch Paul McElhany, Mike Maher, Danielle Perez |
| Date | Wednesday 2021.04.05 (Current Draft) |
| SUBJ | Respirometry Narrative |
| Drive | https://drive.google.com/drive/folders/1sMiXNW6YCKHZoMop1wlQl-i1HPmBZEEW?usp=sharing |
| REPO | https://github.com/KROVINSKI/  .MUK\_KRL2019respirometry90min |
| Study | 2019 Krill Exposure Cross Study |

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Description automatically generatedA picture containing text, clipart

Description automatically generated

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**Conditions prior to Respirometry**

**Dates of Respirometry Rounds:** Two rounds of respirometry were conducted on Monday October 28th with two subsequent rounds on Tuesday October 29th, 2019.

**Fasting Period Actions:** To achieve a 24hour fast for all animals MOATs 2, 6, 8, and 12 were not fed and EZ larva and Artemia cysts remaining were siphoned away/vacuumed out on Sunday 27OCT19.

On Sunday 27OCT19, remaining MOATs were fed per routine after 1230 local. Diet inputs were introduced following the 1230 shut off.

EZ larva remnants and Artemia cysts for MOATs 1, 7, 10, 11, and 13 were siphoned away/vacuumed out the morning of 28OCT21 prior to 1230.

**Assumptions:** All krill were removed during the night period when water chemistry pH conditions drifted to their higher conditions and dissolved oxygen returned to 100%.

All animals’ oxygen uptake was measured in post-adsorptive state following a 24 fasting period. Cannibalism was the only feeding opportunity for 24 hours prior to the 90minute observation window. All krill, Euphausia pacifica, were considered to be young adult krill in the same life stage. Sex determination was inconclusive for live animals. The 28mL vial left little room for krill movement; minimum movement was assumed.

**2.0 Water Conditions Throughout Respirometry**

All krill were removed during the night period when water chemistry pH conditions drifted to their higher conditions and dissolved oxygen returned to 100%.

MOATs 12 “system water”, water outside the animal box, was exclusively used for respirometry. Water was pulled during nighttime conditions to pre-fill the 250mL jars, 28mL, and catch basins.

Sorting jars were kept in a chilling water bath prior to loading a krill into a vial. The chiller maintained a water temperature between 11.0 ~ 11.4°C. The PreSens DO probe was set to HUMID, mg/L, with a manual temperature set to 11°C, and pressure set to 1atmosphere.

**3.0 Respirometry Project Information** “MUK\_KRL2019respirometry90min”

R scripts and documents inside the project listed above was generated to create slopes, intercepts, and apply linear/mixed effects models to the slopes. Only observations included in a 90minute observation were included.

**Overall Link to Respirometry Repository:** <https://github.com/KROVINSKI/NWFSC.MUK_KRL2019respirometry90min>

**Two Rnotebooks for database creation & slope statistics:**

1. “KRL\_ADLT\_EXPOcross\_RESPRSlopeGen\_2019\_(2021.04.30).Rmd”
2. KRL\_ADLT\_EXPOcross\_RespirometryDataframeCreation\_Summer2019\_(2021.04.30).Rmd

**4.0 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”.

|  |  |  |
| --- | --- | --- |
| **4.1a 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> | | |

|  |
| --- |
| **4.1b Merged database dRESPmsr**  **Contains all Animal Specific Information and Probe Measurements** |
| A picture containing text, cup  Description automatically generated |

**5.0 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.

|  |
| --- |
| **5.1a DO Corrections and Oxygen Units**  **Following DO correction “oxygen” units is mg** |
| Diagram  Description automatically generated |
| Vial volume was .02806. PRESENS probe measured Dissolved Oxygen as mg/L.  Volume of water displaced for tissue was substracting using an assumed krill density of 1 ml/g |

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

|  |
| --- |
| **6.1a 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 ~1.5hrs.  Simple slopes of krill respiration are recorded as μg / hr.  Slopes will later be corrected for background noise (blank slopes) and krill wet weight. |

**7.0 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 the distribution of animals between treatments. The Ambient Treatment was initially included but ultimately excluded due to a lack of consistent monitoring and equipment logs from both MOATs 04 and MOATs 05. 60 vials in all are included in analysis. With two blanks per trial, 52 animals, across three treatments comprise the respirometry analysis.

**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.  # [1] “Trial01\_KrilLR12”  # [2] “Trial01\_KrilLR19” | | | | | |
| Trail 1 of 4 Loading Start Time | | | 1407 | | |
| Trail 1 of 4 Loading Stop Time | | | 1419 | | |
| Trail 1 of 4 Acclimation Start Time | | | 1419 | | |
| Trail 1 of 4 Acclimation Stop Time | | | 1424 | | |
| Trail 1 of 4 First Measurement Time | | | "2019-10-28 14:25:03" | | |

**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  # [1] “Trial02\_KriLR224”  # [2] “Trial02\_KriLR230” | | | | | |
| Trail 2 of 4 Loading Start Time | | | 1810 | | |
| Trail 2 of 4 Loading Stop Time | | | 1824 | | |
| Trail 2 of 4 Acclimation Start Time | | | 1828 | | |
| Notes about how loading went wrong/awry:  A new loading map needed to be made on Monday following continued water chemistry problems on the MOATs following the 18-19OCT21 winter storm event. Barcodes for the first round of respirometry were printed prior to 1230 but not the second round. There was an error with barcodes and that batch of 19 needed to be reprinted. Loading began on the second trial with only one person and without jars already filled with source water. This led to a longer loading time in Trail 02. 250mL jars of source water were submerged in the 11-11.4°C chilled water bath.  *This problem with loading and barcodes, along with lipid preparation organization was a lesson learned the project. Four people is the minimum number of people to work simultaneous respirometry and lipids prep. Allow two people to be dedicated vial loaders (follow on lipids prep) and two people to be dedicated PreSens operators.* | | | | | |
| Trail 2 of 4 Acclimation Stop Time | | | 1849 (due to error with the labels) | | |
| Trail 2 of 4 First Measurement Time | | | “2019-10-28 18:55:23” | | |

**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.  # [1] “Trial03\_KRLr3\_43”  # [2] “Trial03\_KRLr3\_52” | | | | | |
| Trail 3 of 4 Loading Start Time | | | 1349 | | |
| Trail 3 of 4 Loading Stop Time | | | 1400 | | |
| Trail 3 of 4 Acclimation Start Time | | | 1400 | | |
| Trail 3 of 4 Acclimation Stop Time | | | 1410 | | |
| Trail 3 of 4 First Measurement Time | | | “2019-10-29 14:06:23” | | |

|  |  |
| --- | --- |
|  |  |
| Noticed a 4 minute difference between acclimation stop time and first measurement by the PreSens. This observations were removed.  **obstrial3 <- subset(dRESPmsr, dRESPmsr$TrialID == "Trial03")**  926 observations of 65 variables  **obstrial3 <- filter(obstrial3,**  **Time >= as.POSIXct("2019-10-29 14:10:00", tz = "UTC"))**  844 observations of 65 variables  82 observations were removed | |

**Trail 4 of 4**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trial4Animals** | **Trial4\_CHG** | **Trial4\_CUR** | | **Trial4\_TMP** | **Trial4\_Blanks** |
| **10** | **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  # [1] "Trial04\_KRLr4\_59"  # [2] "Trial04\_KRLr4\_68" | | | | | |
| Trail 4 of 4 Loading Start Time | | | 1700 | | |
| Trail 4 of 4 Loading Stop Time | | | 1713 | | |
| Trail 4 of 4 Acclimation Start Time | | | 1713 | | |
| Trail 4 of 4 Acclimation Stop Time | | | 1723 | | |
| Trail 4 of 4 First Measurement Time | | | "2019-10-29 17:20:23" | | |
| Noticed a three minute difference in measurement time  **obstrial4 <- subset(dRESPmsr, dRESPmsr$TrialID == "Trial04")**  477 observations of 65 variables  **obstrial4 <- filter(obstrial4,**  **Time >= as.POSIXct("2019-10-29 17:23:00", tz = "UTC"))**  448 observations of 65 variables  29 observations removed | | | | | |

**8.0 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.

|  |
| --- |
| **8.1a Trial 01 Percent Dissolved Oxygen Reduction Timeseries** |
| A picture containing chart  Description automatically generated  A picture containing timeline  Description automatically generated |
|  |

|  |
| --- |
| **8.2 Trial 02 Percent Dissolved Oxygen Reduction Timeseries** |
| Chart, line chart  Description automatically generatedChart, line chart  Description automatically generated |
| Notice that there are some flat and slightly positive oxygen consumption trends  It’s possible bacteria could be generating oxygen, perhaps a leak is more likely |

|  |
| --- |
| **8.3 Trial 03 Oxygen (μg) Reduction Timeseries** |
| Graphical user interface  Description automatically generated with low confidenceChart  Description automatically generated with medium confidence |
|  |

|  |
| --- |
| **8.4 Trial 04 Oxygen (μg) Reduction Timeseries** |
| Chart, line chart  Description automatically generatedChart, line chart  Description automatically generated |
|  |

|  |  |
| --- | --- |
| **8.5 All Trials, Across Treatments Oxygen (μg) Reduction Timeseries** | |
| Chart  Description automatically generated | |
| All trails are included. 52 animals and 8 blank vials are included in the figures above.  Trail 02 experienced the longest loading and acclimation time due to only person loading. | |
| Trail 01 Loading Time | 12minutes |
| Trial 01 Acclimation Time | 10minutes |
| Total Trial 01(before observation recorded) | **22 min** |
| Trial 02 Loading Time | 14minutes |
| Trial 02 Acclimation Time | 21minutes |
| Total Trial 02 (before observation recorded) | **35 min** |
| Trial 03 Loading Time | 13minutes |
| Trial 03 Acclimation Time | 10minutes |
| Total Trial 03 (before observation recorded) | **23 min** |
| Trial 04 Loading Time | 11minutes |
| Trial 04 Acclimation Time | 10minutes |
| Total Trial 04 (before observation recorded) | **21 min** |

|  |
| --- |
| **8.6 All Trials, Across Treatments Actual Dissolved Oxygen (mg) Reduction Timeseries** |
| Chart  Description automatically generated |

**9.0 Outlier Removal.** Removing outliers was a six step process to remove observations that had a Cook’s Distance above 4/n. All slopes were included. There was no minimum reduction in oxygen consumption. Positive Slopes were later removed.

The following table details steps to remove outlier observations but not completed slopes:

|  |  |  |
| --- | --- | --- |
| # | 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 |  |
| Number of observations removed 108.  Original dataframe: 1898 observations (across 52 krill (animals) & eight blank vials)  Filtered dataframe: 1790 observations  Outliers represent 5.69% of original dataframe’s observations. | | |

**10.0 Blank Slope Averages & Correcting Slopes** based on blank vials and animal wet weight

|  |  |  |
| --- | --- | --- |
| 1 | Sub setting only the blank vials |  |
| 2 | Creating four different averages based on Trial No. |  |
| 3 | Setting mean values to new variable names |  |
| Blank Mean  Slope Trial 01 | | -12.9572 |
| Blank Mean  Slope Trial 02 | | -6.7701 |
| Blank Mean  Slope Trial 03 | | -8.58243 |
| Blank Mean  Slope Trial 04 | | -8.91736 |
| 4 | CorrSlope  Creating  (only subtracting the blank mean values) |  |
| 5 | Correcting for wet animal weight |  |
| “CorrWeightSlope” value factors in both the blank mean (specific to Trial) and the animal wet weight | | |

**11.0 Slopes Plots**

|  |
| --- |
| 11.1 Slopes Prior to Blank Correction by Treatment (Vial Oxygen (μg)/hr) |
|  |
| Notice that positive slopes were excluded. These slopes represent oxygen consumption rates prior to the blank vial background respiration rate correction. Used expression “oxygen consumption” however, standard metabolic rate or MO2 (oxygen uptake) might be more appropriate for the Y axis  These values aren’t corrected for the krill wet weight:  (μg O2 h -1) Oxygen Consumption units |

|  |
| --- |
| 11.2 Slopes with Blank Correction by Treatment (Vial Oxygen (μg)/hr per gram of Krill) |
| Units of the Measurement - µg O2 h-1 g-1  All slopes represent only negatives slopes as their absolute values  Krill Respirometry Literature (Units)  Oiller et al 2018 = μg O2 h -1 g -1  when referencing a resting metabolic rate.  General Respirometry Literature (Units)  Chabot et al 2016 MO2 = μmol O2 min -1 kg -1  Zimmerman 2001 MO2 = mg O2 h -1 & mg O2 h -1 kgWM -1  I don’t know that we need to spell out wet mass but we can |
| Below is a diagram of the units of measurement (krill slopes):  A picture containing graphical user interface  Description automatically generated |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **12.0 “dSlopes.summary” Table (Corrected Slopes ~ Treatment)** | | | | | | | |
| Treatment | sd | mean | median | IQR | n | se | ci |
| CUR | 115.012569 | 146.914898 | 102.677129 | 145.34494 | 16 | 28.7531422 | 56.3561587 |
| CHG | 149.271134 | 218.594784 | 201.092119 | 144.569066 | 16 | 37.3177835 | 73.1428556 |
| TMP | 220.521013 | 242.121902 | 178.991221 | 339.592918 | 15 | 56.9382808 | 111.59903 |

**13.) Corrected Slope Summary Plot**

|  |
| --- |
| **13.1) Corrected Slope Summary Plot**  **((Slope is μg of Vial Oxygen)/hr per Grams of Krill (wet weight in grams))** |
|  |
| There appears to be no significant difference between treatments when corrected against blanks and the individual’s krill wet weight. |
| The purple dots represent the mean- all trials included  Green boxplots show from the 25th percentile to the 75th percentile  error bars +/- SD shown in blue  Confidence Intervals set to 95%  error bars(CI) +/- our confidence intervals (95%) - shown in red |

**14.0) Mixed Effects Model to Account for Possible MOATs Effect**

|  |
| --- |
| **14.0) Mixed Effects Model to Account for Possible MOATs Effect with the lmer4 package and “lmer” function** |
|  |
| Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']  Formula: CorrWeightSlope ~ Treatment + (1 | MOATS)  Data: dSlopes  REML criterion at convergence: 583  Scaled residuals:  Min 1Q Median 3Q Max  -1.3820 -0.6070 -0.2275 0.4717 2.8817  Random effects:  Groups Name Variance Std.Dev.  MOATS (Intercept) 0 0.0  Residual 27579 166.1  Number of obs: 47, groups: MOATS, 8  Fixed effects:  Estimate Std. Error df t value Pr(>|t|)  (Intercept) 146.91 41.52 44.00 3.539 0.000963 \*\*\*  TreatmentCUR 71.68 58.71 44.00 1.221 0.228652  TreatmentTMP 95.21 59.68 44.00 1.595 0.117833  ---  Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1  Correlation of Fixed Effects:  (Intr) TrtCUR  TreatmntCUR -0.707  TreatmntTMP -0.696 0.492  optimizer (nloptwrap) convergence code: 0 (OK)  boundary (singular) fit: see is Singular |
| This test ordered All Change Conditions against current conditions, followed by High Temperature Conditions.  No Treatment Effect Found; All Change Treatment compared to Current Conditions (p =0.23) and Current to High Temperature Conditions (p = .12).  Random Effects Model large variance (27579) within-treatment level indicates a MOATs effect is relatively small.  **No treatment effect observed, no MOATs effect observed** |

|  |
| --- |
| **14.1) Mixed Effects Model to Account for Possible MOATs Effect with the lmer4 package and “lmer” function**  Reordered for Current Conditions Comparison |
|  |
| [1] Linear mixed model fit by REML. t-tests use Satterthwaite's method ['lmerModLmerTest']  Formula: CorrWeightSlope ~ Treatment + (1 | MOATS)  Data: dSlopes  REML criterion at convergence: 583  Scaled residuals:  Min 1Q Median 3Q Max  -1.3820 -0.6070 -0.2275 0.4717 2.8817  Random effects:  Groups Name Variance Std.Dev.  MOATS (Intercept) 0 0.0  Residual 27579 166.1  Number of obs: 47, groups: MOATS, 8  Fixed effects:  Estimate Std. Error df t value Pr(>|t|)  (Intercept) 218.59 41.52 44.00 5.265 4e-06 \*\*\*  TreatmentCHG -71.68 58.71 44.00 -1.221 0.229  TreatmentTMP 23.53 59.68 44.00 0.394 0.695  ---  Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1  Correlation of Fixed Effects:  (Intr) TrtCHG  TreatmntCHG -0.707  TreatmntTMP -0.696 0.492  optimizer (nloptwrap) convergence code: 0 (OK)  boundary (singular) fit: see is Singular |
| Direct Comparison of Current Conditions to High Temperature Corrected Slope values (p = .70) doesn’t reveal a significant relationship. |