

Respiration Analysis F1 Scallops 2021

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10/6/2021

merge master resp data frame

Date	Channel	Filename	Lpc	Leq	Lz	alpha	Food	pH	Replicate
9/14/2021	CH1	Run_1_restart_raw.txt	-0.0105879	-0.0090352	-0.0108642	0.4	NA	8	A
9/14/2021	CH1	Run_2_raw.txt	0.0009955	0.0020145	0.0020145	0.4	NA	8	D
9/14/2021	CH1	Run_3_raw.txt	-0.0106760	-0.0106760	-0.0106760	0.4	NA	8	C
9/14/2021	CH1	Run_4_raw.txt	-0.0233471	-0.0241120	-0.0241120	0.4	NA	8	B
9/14/2021	CH2	Run_1_restart_raw.txt	-0.0202747	-0.0206863	-0.0208296	0.4	NA	8	B
9/14/2021	CH2	Run_2_raw.txt	-0.0143181	-0.0144040	-0.0225380	0.4	NA	8	A

Visual diagnostics of plots to correct poor data

- View on github: [Airradians_OA/RAnalysis/Output/Respiration/plots_alpha0.4_increm15sec/](https://github.com/SamGurr/Airradians_OA/RAnalysis/Output/Respiration/plots_alpha0.4_increm15sec/)

found three calls that inaccurately represent the rates of oxygen consumption

- (1) https://github.com/SamGurr/Airradians_OA/blob/master/RAnalysis/Output/Respiration/plots_alpha0.4_increm15sec/20210930_Plate_2_Run_2_C5_regression.pdf

- solution = call 'Lz' instead of the default Leq

- (2) https://github.com/SamGurr/Airradians_OA/blob/master/RAnalysis/Output/Respiration/plots_alpha0.4_increm15sec/20210930_Plate_2_Run_1_C1_regression.pdf

- solution = call 'Lz' instead of the default Leq

- (3) https://github.com/SamGurr/Airradians_OA/blob/master/RAnalysis/Output/Respiration/plots_alpha0.4_increm15sec/20210930_Plate_1_Run_2_C1_regression.pdf

- solution = omit

```
resp.data_merged[77,c(1:6)]
```

```
##           Date Channel           Filename           Lpc           Leq
## 77 9/30/2021      C5 RR_9.30.21_PM_Plate_2_Run_2.csv 0.02905235 -0.07603444
##           Lz
## 77 -0.07603444
```

```
resp.data_merged[61,c(1:6)]
```

```
##           Date Channel           Filename           Lpc           Leq
## 61 9/30/2021      C1 RR_9.30.21_AM_Plate_2_Run_1.csv -0.02890813 -0.0608251
##           Lz
## 61 -0.0608251
```

```
resp.data_merged[62,c(1:6)]
```

```
##           Date Channel           Filename           Lpc           Leq
## 62 9/30/2021      C1 RR_9.30.21_PM_Plate_1_Run_2.csv 0.01165649 0.01165649
##           Lz
## 62 0.01165649
```

```
resp.data_merged[77,4] <- resp.data_merged[77,5] # 20210930_Plate_2_Run_2_C5_regression - Lz and Leq ca
resp.data_merged[61,4] <- resp.data_merged[61,5] # 20210930_Plate_2_Run_1_C1_regression - Lz and Leq ca
resp.data_merged[62,4] <- NA # 20210930_Plate_1_Run_2_C1_regression - Lz and Leq call better regression
```

```
resp.data_merged[77,c(1:6)]
```

```
##           Date Channel           Filename           Lpc           Leq
## 77 9/30/2021      C5 RR_9.30.21_PM_Plate_2_Run_2.csv -0.07603444 -0.07603444
##           Lz
## 77 -0.07603444
```

```
resp.data_merged[61,c(1:6)]
```

```
##           Date Channel           Filename           Lpc           Leq
## 61 9/30/2021      C1 RR_9.30.21_AM_Plate_2_Run_1.csv -0.0608251 -0.0608251
##           Lz
## 61 -0.0608251
```

```
resp.data_merged[62,c(1:6)]
```

```
##           Date Channel           Filename Lpc           Leq           Lz
## 62 9/30/2021      C1 RR_9.30.21_PM_Plate_1_Run_2.csv  NA 0.01165649 0.01165649
```

call blanks

```
## `summarise()` has grouped output by 'Date'. You can override using the `.groups` argument.
```

Date	Channel	Filename	pH	Chamber_tank	Lpc	Leq	
9/14/2021	CH4	Run_1_restart_raw.txt	8.0	blank	-0.0014879	-0.0016125	0.00
9/14/2021	CH4	Run_2_raw.txt	8.0	blank	-0.0024339	-0.0024339	-0.00
9/14/2021	CH4	Run_3_raw.txt	8.0	blank	-0.0042119	-0.0042201	-0.00
9/14/2021	CH4	Run_4_raw.txt	8.0	blank	-0.0064080	-0.0064596	-0.00
9/14/2021	CH8	Run_2_raw.txt	7.5	blank	-0.0029619	-0.0030399	-0.00
9/14/2021	CH8	Run_3_raw.txt	7.5	blank	-0.0037013	-0.0041097	-0.00
9/14/2021	CH8	Run_4_raw.txt	7.5	blank	-0.0044681	-0.0045407	-0.00
9/30/2021	A1	RR_9.30.21_PM_Plate_1_Run_2.csv	7.5	blank	-0.0118063	-0.0124888	-0.01
9/30/2021	A2	RR_9.30.21_PM_Plate_2_Run_2.csv	8.0	blank	-0.0116445	-0.0148425	-0.01
9/30/2021	A3	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0154144	-0.0154144	-0.01
9/30/2021	A4	RR_9.30.21_PM_Plate_1_Run_2.csv	7.5	blank	-0.0103471	-0.0117755	-0.01
9/30/2021	A5	RR_9.30.21_PM_Plate_2_Run_2.csv	8.0	blank	-0.0086168	-0.0121027	-0.01
9/30/2021	A6	RR_9.30.21_AM_Plate_2_Run_1.csv	7.5	blank	-0.0038102	-0.0041451	-0.00
9/30/2021	A6	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0058170	-0.0136642	-0.01
9/30/2021	B1	RR_9.30.21_PM_Plate_2_Run_2.csv	8.0	blank	-0.0134095	-0.0095129	-0.00
9/30/2021	B2	RR_9.30.21_AM_Plate_1_Run_1.csv	7.5	blank	-0.0236932	-0.0226223	0.00
9/30/2021	B3	RR_9.30.21_AM_Plate_2_Run_1.csv	8.0	blank	-0.0132592	-0.0138365	-0.01
9/30/2021	B5	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0217783	-0.0195525	-0.01
9/30/2021	B6	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0066049	-0.0038889	-0.00
9/30/2021	C2	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0219181	-0.0226875	-0.02
9/30/2021	C6	RR_9.30.21_PM_Plate_1_Run_2.csv	7.5	blank	-0.0137323	-0.0186497	-0.01
9/30/2021	C6	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0205009	-0.0200935	-0.02
9/30/2021	D1	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0079405	-0.0077582	-0.00
9/30/2021	D5	RR_9.30.21_AM_Plate_1_Run_1.csv	8.0	blank	-0.0024248	-0.0020850	-0.00
9/30/2021	D5	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0098883	-0.0115005	-0.01

Date	pH	BLANK.mean_Lpc	BLANK.mean_Leq	BLANK.mean_Lz	n
9/14/2021	7.5	0.0037104	0.0038968	0.0037690	3
9/14/2021	8.0	0.0036354	0.0036815	0.0034417	4
9/30/2021	7.5	0.0135890	0.0142058	0.0117710	9
9/30/2021	8.0	0.0111450	0.0120854	0.0122455	9

```
## [1] Date           pH           Channel      Filename      Lpc
## [6] Leq             Lz           alpha         Food          Replicate
## [11] Chamber_tank    Number       Plate         Run           Fed_Unfed
## [16] Center          Length.um.   Instrument     Notes         BLANK.mean_Lpc
## [21] BLANK.mean_Leq BLANK.mean_Lz n             resp_norm
## <0 rows> (or 0-length row.names)
```

Calculate rates as ng L-1 O2 um shell length-1 hr-1

note: this chunk calls the data that used the 8-channel loligo system and the SDR system to correct for total respiration chamber (vial/vessel, well, etc.) volume separately

- loligo 8-channel on 9/14/21 = 2.2 ml vessels
- SDR 24-channel on 9/3-/21 = 1.7 ml wells

```
for (i in 1:nrow(Resp.Master_OM)) {
  if (Resp.Master_OM$Date[i] == '9/14/2021') {
    Resp.Master_OM$resp_ng_L_umLength_hr[i] <- (
      ( (abs(Resp.Master_OM$resp_norm[i])*1000000) * # call absolute value of resp in mg per minu
```

```

        (2.2/1000) ) / # vial volume for loligo 8 channel runs was 2.2 mL!!! - correct ng minut
        Resp.Master_OM$Length.um.[i]) * # normalize by individual or larvae count - as to ng L-1
        (60)) # correct for the time; final value is ng Liter-1 individual-1 hour-1
    } else {
    Resp.Master_OM$resp_ng_L_umlLength_hr[i] <- (
        ( ( abs(Resp.Master_OM$resp_norm[i])*1000000) * # call absolute value of resp in mg per mi
            (1.7/1000) ) / # vial volume for loligo 8 channel runs was 1.7 mL!!! - correct ng min
        Resp.Master_OM$Length.um.[i]) * # normalize by individual or larvae count - as to ng L-1
        (60)) # correct for the time; final value is ng Liter-1 individual-1 hour-1
    }
}

```

Analysis

9/14/2021 F1 juvenile scallops

About - two pCO₂ treatments - **before** we culled and started the food limitation trials

number of replicates for analysis...

```

## # A tibble: 8 x 2
##   Chamber_tank `n()`
##   <chr>        <int>
## 1 7.5_A         3
## 2 7.5_B         3
## 3 7.5_C         3
## 4 7.5_D         3
## 5 8_A          3
## 6 8_B          3
## 7 8_C          3
## 8 8_D          3

```

```

LMmod_0914 <- aov(lm(resp_ng_L_umlLength_hr~pCO2,data=Resp_0914))
pander(summary(LMmod_0914), style='rmarkdown')

```

linear model, diagnostics, and plots

Table 1: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
pCO₂	1	0.1162	0.1162	0.6327	0.4348
Residuals	22	4.041	0.1837	NA	NA

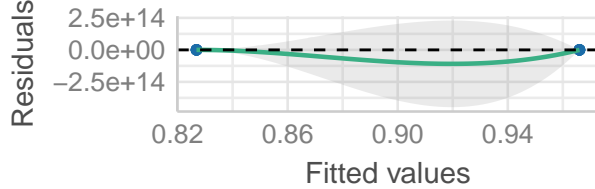
```

check_model(LMmod_0914) # observe the diagnostics of the model

```

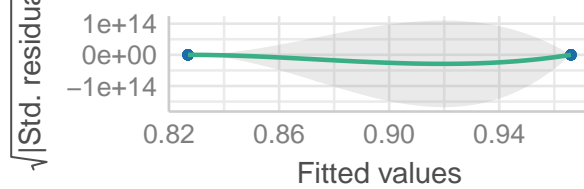
Linearity

Reference line should be flat and horizontal



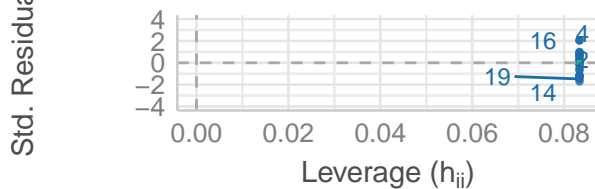
Homogeneity of Variance

Reference line should be flat and horizontal



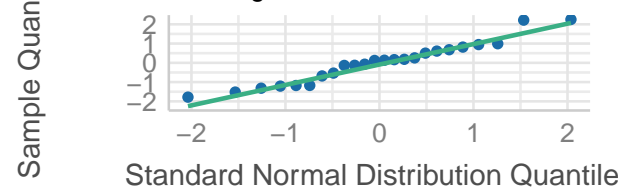
Influential Observations

Points should be inside the contour lines



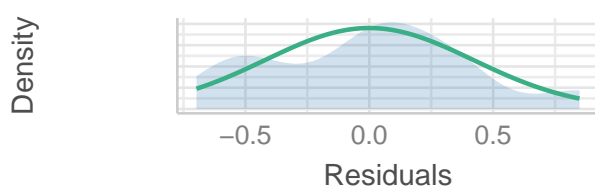
Normality of Residuals

Points should fall along the line



Normality of Residuals

Distribution should be close to the normal curve



```
shapiro.test(residuals(LMmod_0914)) # non normal
```

```
##
## Shapiro-Wilk normality test
##
## data: residuals(LMmod_0914)
## W = 0.95735, p-value = 0.3875
```

```
leveneTest(LMmod_0914) # good
```

```
## Warning in leveneTest.default(y = y, group = group, ...): group coerced to
## factor.
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 1  0.2549 0.6186
##      22
```

```
DF    <- paste( (summary(LMmod_0914)[[1]][["Df"]])[1], (summary(LMmod_0914)[[1]][["Df"]])[2], sep = '/')
Fval  <- (summary(LMmod_0914)[[1]][["F value"]])[1]
pval  <- (summary(LMmod_0914)[[1]][["Pr(>F)"]])[1]
```

```
# pdf(paste0("C:/Users/samuel.gurr/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respira
pdf(paste0("C:/Users/samjg/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respiration/202
```

```
ggplot(Resp_0914, aes(x = factor(pCO2, level = c('Low', 'High')), resp_ng_L_umlLength_hr , fill = pCO2))
  theme(panel.grid=element_blank()) +
  geom_boxplot(size=0.2, alpha=0.1, aes(fill=pCO2)) +
  scale_fill_manual(values=c("grey50","white")) +
  geom_point(shape = 21, size = 2, position = position_jitterdodge(jitter.width = 0.1)) +
  theme_classic() +
  scale_x_discrete(labels= c('~500 atm', '~800 atm')) +
  ylim(0, 3) +
  theme(axis.text=element_text(size=12),
        axis.title=element_text(size=14,face="bold")) +
  labs(title = "F1 Scallops: respiration rates on 20210914",
       y = expression(Respiration~rate~("("~ng~L~{-1}~0[2]~.%~*m~{-1}~.%~hr~{-1}~)~"),
       x = expression(italic(p)*CO[2]~Treatment~("("~*atm~)~")) # +
  # annotate("text", x=1.2, y=2.8, size = 4, label = "aov(Resp~pCO2)") +
  # annotate("text", x=1.2, y=2.6, size = 4, label= paste('DF =',DF,'F =', signif(Fval, digits=3), 'p v
dev.off()
```

```
## pdf
## 2
```

9/14/2021 F1 juvenile scallops

About

- two pCO2 treatments
- *before* we culled and started the food limitation trials

number of replicates for analysis...

`summarise()` has grouped output by 'Chamber_tank'. You can override using the `.groups` argument.

```
## # A tibble: 16 x 3
## # Groups:   Chamber_tank [8]
##   Chamber_tank Food `n()`
##   <chr>         <int> <int>
## 1 7.5_A         1     4
## 2 7.5_A         2     5
## 3 7.5_B         1     5
## 4 7.5_B         2     4
## 5 7.5_C         1     5
## 6 7.5_C         2     3
## 7 7.5_D         1     5
## 8 7.5_D         2     2
## 9 8_A          1     5
## 10 8_A          2     5
## 11 8_B          1     5
## 12 8_B          2     6
## 13 8_C          1     5
## 14 8_C          2     4
## 15 8_D          1     5
## 16 8_D          2     4
```

```
LMmod_0930 <- aov(lm(resp_ng_L_umLength_hr~pCO2*Fed_Unfed ,data=Resp_0930))
pander(summary(LMmod_0930), style='rmarkdown')
```

linear model, diagnostics, and plots

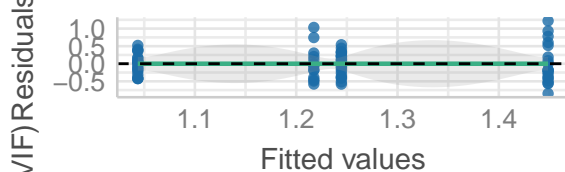
Table 2: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
pCO2	1	0.7427	0.7427	4.12	0.0463
Fed_Unfed	1	0.8203	0.8203	4.55	0.03653
pCO2:Fed_Unfed	1	0.00405	0.00405	0.02247	0.8813
Residuals	68	12.26	0.1803	NA	NA

```
check_model(LMmod_0930) # observe the diagnostics of the model
```

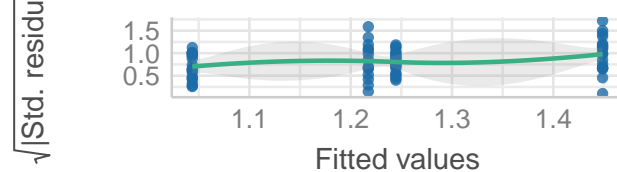
Linearity

Reference line should be flat and horizontal



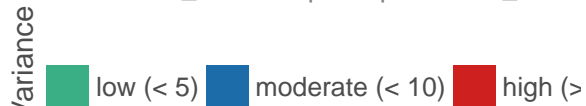
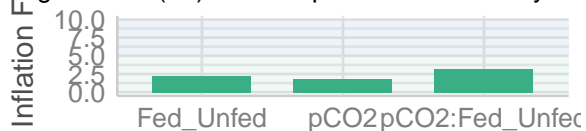
Homogeneity of Variance

Reference line should be flat and horizontal



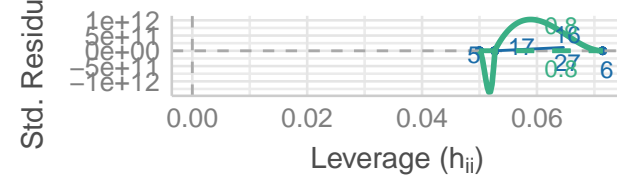
Collinearity

Higher bars (>5) indicate potential collinearity issues



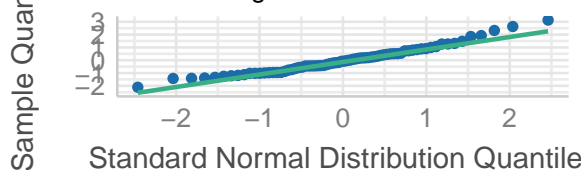
Influential Observations

Points should be inside the contour lines



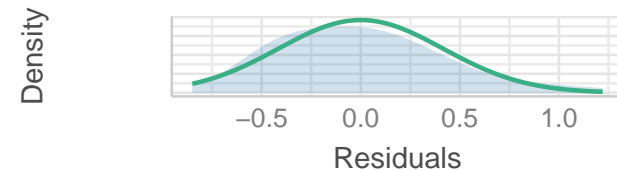
Normality of Residuals

Points should fall along the line



Normality of Residuals

Distribution should be close to the normal curve



```
shapiro.test(residuals(LMmod_0930)) # non normal
```

```
##
## Shapiro-Wilk normality test
##
## data: residuals(LMmod_0930)
## W = 0.96927, p-value = 0.0752
```

```
leveneTest(LMmod_0930) # good
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  1.6481 0.1865
##      68
```

```
DF_pCO2 <- paste( (summary(LMmod_0930)[[1]][["Df"]])[1], (summary(LMmod_0930)[[1]][["Df"]])[4], sep =
Fval_pCO2 <- (summary(LMmod_0930)[[1]][["F value"]])[1]
pval_pCO2 <- (summary(LMmod_0930)[[1]][["Pr(>F)"]])[1]
```

```
DF_Fed <- paste( (summary(LMmod_0930)[[1]][["Df"]])[2], (summary(LMmod_0930)[[1]][["Df"]])[4], sep =
Fval_Fed <- (summary(LMmod_0930)[[1]][["F value"]])[2]
pval_Fed <- (summary(LMmod_0930)[[1]][["Pr(>F)"]])[2]
```

```
Resp_0930$Fed_Unfed <- as.factor(Resp_0930$Fed_Unfed)
```

```
levels(Resp_0930$Fed_Unfed) <- c("High food", "Low food")
```

```
# pdf(paste0("C:/Users/samuel.gurr/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respira
pdf(paste0("C:/Users/samjg/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respiration/202
ggplot(Resp_0930, aes(x = factor(pCO2, level = c('Low', 'High')), resp_ng_L_uumlLength_hr , fill = pCO2))
  theme(panel.grid=element_blank()) +
  geom_boxplot(size=0.2, alpha=0.1, aes(fill=pCO2)) +
  scale_fill_manual(values=c("grey50","white")) +
  geom_point(shape = 21, size = 2, position = position_jitterdodge(jitter.width = 0.1)) +
  theme_classic() +
  ylim(0, 3) +
  scale_x_discrete(labels=c('~500 atm', '~800 atm')) +
  theme(axis.text=element_text(size=12),
        axis.title=element_text(size=14,face="bold")) +
  stat_summary(fun.y=mean, geom="point", shape=18, size=4, color="black", fill="white") +
  labs(title = "Respiration rate; F1 Scallops (juveniles) 66 days post-fertilization",
        y = expression(Respiration~rate~("("~ng~L~^{-1}~O[2]~.%mu*m^{-1}~.% hr^{-1}~")"),
        x = expression(italic(p)*CO[2]~Treatment~("("~mu*atm~")")) +
  facet_wrap(~factor(Fed_Unfed, level = c('Low food', 'High food')) # +
```

```
## Warning: `fun.y` is deprecated. Use `fun` instead.
```

```
dev.off()
```

```
## pdf
## 2
```