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	Replic
10/26/2021	A1	20211026_resp_unfed.csv	-0.0113410	-0.0157749	-0.0152144	0.4	unfed	8.0	A
10/26/2021	A3	20211026_resp_unfed.csv	-0.0334605	-0.0314858	-0.0372399	0.4	unfed	7.5	A
10/26/2021	A5	20211026_resp_unfed.csv	-0.0206994	-0.0204531	-0.0204531	0.4	unfed	8.0	B
10/26/2021	A6	20211026_resp_unfed.csv	-0.0110909	-0.0114181	-0.0114181	0.4	unfed	7.5	D
10/26/2021	B1	20211026_resp_unfed.csv	-0.0091571	-0.0089935	-0.0089935	0.4	unfed	7.5	A
10/26/2021	B3	20211026_resp_unfed.csv	-0.0101901	-0.0110629	-0.0110521	0.4	unfed	8.0	C

Visual diagnostics of plots to correct poor data

- View on github: 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 = we reran this at the end of the LoLin script for 0-20 minutes and got an Lpc -0.0296, insert this

```
resp.data_merged[94,c(1:6)]  # C1 RR_9.30.21_AM_Plate_2_Run_1.csv # -0.02890813   -0.0608251  -0.0608
```

```
##         Date Channel                          Filename        Lpc        Leq
## 94 9/30/2021      C1 RR_9.30.21_AM_Plate_2_Run_1.csv -0.02890813 -0.0608251
##          Lz
## 94 -0.0608251
```

```r
resp.data_merged[110,c(1:6)] #  C5 RR_9.30.21_PM_Plate_2_Run_2.csv   0.029052351 -0.076034441    -0.07603
```

```
##         Date Channel                        Filename        Lpc        Leq
## 110 9/30/2021      C5 RR_9.30.21_PM_Plate_2_Run_2.csv 0.02905235 -0.07603444
##            Lz
## 110 -0.07603444
```

```r
resp.data_merged[95,c(1:6)]  #  C1 RR_9.30.21_PM_Plate_1_Run_2.csv   0.011656487 0.011656487 0.011656487
```

```
##        Date Channel                        Filename        Lpc        Leq
## 95 9/30/2021      C1 RR_9.30.21_PM_Plate_1_Run_2.csv 0.01165649 0.01165649
##           Lz
## 95 0.01165649
```

```r
resp.data_merged[94,4] <- resp.data_merged[94,5] # 20210930_Plate_2_Run_2_C5_regression - Lz and Leq ca
resp.data_merged[110,4] <- resp.data_merged[110,5] # 20210930_Plate_2_Run_1_C1_regression - Lz and Leq
resp.data_merged[95,4] <- -0.0296 # 20210930_Plate_1_Run_2_C1_regression - plot shows noise after the 2

resp.data_merged[94,c(1:6)]
```

```
##        Date Channel                        Filename       Lpc        Leq
## 94 9/30/2021      C1 RR_9.30.21_AM_Plate_2_Run_1.csv -0.0608251 -0.0608251
##          Lz
## 94 -0.0608251
```

```r
resp.data_merged[110,c(1:6)]
```

```
##         Date Channel                        Filename         Lpc         Leq
## 110 9/30/2021      C5 RR_9.30.21_PM_Plate_2_Run_2.csv -0.07603444 -0.07603444
##            Lz
## 110 -0.07603444
```

```r
resp.data_merged[95,c(1:6)]
```

```
##        Date Channel                        Filename     Lpc        Leq
## 95 9/30/2021      C1 RR_9.30.21_PM_Plate_1_Run_2.csv -0.0296 0.01165649
##           Lz
## 95 0.01165649
```

Calculate blanks, print all and means

Date	Channel	Filename	pH	Chamber_tank	Lpc	Leq	
10/26/2021	A2	20211026_resp_unfed.csv	8.0	blank	-0.0070228	-0.0068196	-0.0
10/26/2021	A4	20211026_resp_unfed.csv	8.0	blank	-0.0075575	-0.0072676	-0.0
10/26/2021	B2	20211026_resp_unfed.csv	7.5	blank	-0.0077631	-0.0080538	-0.0
10/26/2021	B5	20211026_resp_unfed.csv	7.5	blank	-0.0081600	-0.0107801	-0.0
10/26/2021	C5	20211026_resp_unfed.csv	8.0	blank	-0.0089772	-0.0108088	-0.0
10/26/2021	CH3	Run_3_raw.txt	8.0	blank	-0.0063271	-0.0063328	-0.0
10/26/2021	CH4	Run_1_raw.txt	8.0	blank	-0.0012052	-0.0011750	-0.0
10/26/2021	CH4	Run_2_raw.txt	8.0	blank	-0.0022487	-0.0021095	-0.0
10/26/2021	CH8	Run_1_raw.txt	7.5	blank	-0.0022893	-0.0027042	-0.0
10/26/2021	CH8	Run_3_raw.txt	7.5	blank	-0.0089182	-0.0089103	-0.0
10/26/2021	D3	20211026_resp_unfed.csv	8.0	blank	-0.0063181	-0.0062967	-0.0
10/26/2021	D4	20211026_resp_unfed.csv	7.5	blank	-0.0049751	-0.0051825	-0.0
10/26/2021	D6	20211026_resp_unfed.csv	7.5	blank	-0.0057702	-0.0059470	-0.0
9/14/2021	CH4	Run_1_restart_raw.txt	8.0	blank	-0.0014879	-0.0016125	0.0
9/14/2021	CH4	Run_2_raw.txt	8.0	blank	-0.0024339	-0.0024339	-0.0
9/14/2021	CH4	Run_3_raw.txt	8.0	blank	-0.0042119	-0.0042201	-0.0
9/14/2021	CH4	Run_4_raw.txt	8.0	blank	-0.0064080	-0.0064596	-0.0
9/14/2021	CH8	Run_2_raw.txt	7.5	blank	-0.0029619	-0.0030399	-0.0
9/14/2021	CH8	Run_3_raw.txt	7.5	blank	-0.0037013	-0.0041097	-0.0
9/14/2021	CH8	Run_4_raw.txt	7.5	blank	-0.0044681	-0.0045407	-0.0
9/30/2021	A1	RR_9.30.21_PM_Plate_1_Run_2.csv	7.5	blank	-0.0118063	-0.0124888	-0.0
9/30/2021	A2	RR_9.30.21_PM_Plate_2_Run_2.csv	8.0	blank	-0.0116445	-0.0148425	-0.0
9/30/2021	A3	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0154144	-0.0154144	-0.0
9/30/2021	A4	RR_9.30.21_PM_Plate_1_Run_2.csv	7.5	blank	-0.0103471	-0.0117755	-0.0
9/30/2021	A5	RR_9.30.21_PM_Plate_2_Run_2.csv	8.0	blank	-0.0086168	-0.0121027	-0.0
9/30/2021	A6	RR_9.30.21_AM_Plate_2_Run_1.csv	7.5	blank	-0.0038102	-0.0041451	-0.0
9/30/2021	A6	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0058170	-0.0136642	-0.0
9/30/2021	B1	RR_9.30.21_PM_Plate_2_Run_2.csv	8.0	blank	-0.0134095	-0.0095129	-0.0
9/30/2021	B2	RR_9.30.21_AM_Plate_1_Run_1.csv	7.5	blank	-0.0236932	-0.0226223	0.0
9/30/2021	B3	RR_9.30.21_AM_Plate_2_Run_1.csv	8.0	blank	-0.0132592	-0.0138365	-0.0
9/30/2021	B5	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0217783	-0.0195525	-0.0
9/30/2021	B6	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0066049	-0.0038889	-0.0
9/30/2021	C2	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0219181	-0.0226875	-0.0
9/30/2021	C6	RR_9.30.21_PM_Plate_1_Run_2.csv	7.5	blank	-0.0137323	-0.0186497	-0.0
9/30/2021	C6	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0205009	-0.0200935	-0.0
9/30/2021	D1	RR_9.30.21_PM_Plate_1_Run_2.csv	8.0	blank	-0.0079405	-0.0077582	-0.0
9/30/2021	D5	RR_9.30.21_AM_Plate_1_Run_1.csv	8.0	blank	-0.0024248	-0.0020850	-0.0
9/30/2021	D5	RR_9.30.21_PM_Plate_2_Run_2.csv	7.5	blank	-0.0098883	-0.0115005	-0.0

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

Date	pH	filetype	BLANK.mean_Lpc	BLANK.sd.Lpc	BLANK.mean_Leq	BLANK.mean_Lz	n
10/26/2021	7.5	LoLigo_data	0.0056037	0.0046874	0.0058072	0.0056232	2
10/26/2021	7.5	SDR_data	0.0066671	0.0015381	0.0074908	0.0077362	4
10/26/2021	8.0	LoLigo_data	0.0032603	0.0027067	0.0032058	0.0035334	3
10/26/2021	8.0	SDR_data	0.0074689	0.0011264	0.0077981	0.0078353	4
9/14/2021	7.5	LoLigo_data	0.0037104	0.0007531	0.0038968	0.0037690	3
9/14/2021	8.0	LoLigo_data	0.0036354	0.0021660	0.0036815	0.0034417	4
9/30/2021	7.5	SDR_data	0.0135890	0.0069915	0.0142058	0.0117710	9
9/30/2021	8.0	SDR_data	0.0111450	0.0057253	0.0120854	0.0122455	9

Merge blanks with the master file by DAte, pCO2 treatment, and filetype

- 'filteype' is only important here on 20211026 when both Loligo and SDR were used due to difference in size for fed (larger) and unfed animals

Master REsp file

dplyr::mutate for the following:

- 'volume' of the different vessels throughout the fed*OA challenge
- 'Age' 8 'Fed_unfed'
- 'pCO2'

```
##  [1] Date                pH                  filetype            Channel
##  [5] Filename            Lpc                 Leq                 Lz
##  [9] alpha               Food                Replicate           Chamber_tank
## [13] Number              Plate               Run                 Fed_Unfed
## [17] Center              Length_um           Shell_tin_weight    tin_plus_shell
## [21] Tissue_tin_.weight  tin_plus_tissue     Dry_Shell_weight    Dry_Tissue_weight
## [25] Condition_Index     Instrument          Notes               BLANK.mean_Lpc
## [29] BLANK.sd.Lpc        BLANK.mean_Leq      BLANK.mean_Lz       n
## [33] 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

```
Resp.Master_OM <- Resp.Master_OM[!is.na(Resp.Master_OM$Length_um),]

# NORMALIZED for Shell Size (Length)
 Resp.Master_OM$resp_ng_L_m_Length_hr <- (
        ( ( (abs(Resp.Master_OM$resp_norm)*1000000) * # call absolute value of resp in mg per minute
```

```
                  (Resp.Master_OM$volume/1000) ) / # vial volume for loligo 8 channel runs was 2.2 mL!!!
              Resp.Master_OM$Length_um) * # normalize by individual or larvae count - as to ng L-1 indiv
          (60)) # correct for the time; final value is ng O2 Liter-1 um shell length-1 hour-1




# NORMALIZED for Dry Tissue Weight
 Resp.Master_OM$resp_mg_L_g_hr <- (
          ( ( (abs(Resp.Master_OM$resp_norm)) * # call absolute value of resp in mg per minute -  mg O2
                (Resp.Master_OM$volume/1000) ) / # vial volume for loligo 8 channel runs was 2.2 mL!!!
              (Resp.Master_OM$Dry_Tissue_weight) ) * # normalize by Dry tissue weight  as to mg O2 L-1
          (60)) # correct for the time; final value is ug O2 Liter -1 mg dry tissue weight-1 hour-1
```

Mean standard error plot of all data

```
RespSumTab <- summarySE(Resp.Master_OM, measurevar="resp_ng_L_ m_Length_hr", groupvars=c("Age", "pCO2",
RespSumTab$pCO2 <- factor(RespSumTab$pCO2, c("500  atm","800  atm"))
## Use geom_line and geom_point to plot over time
Resp_geomline<-ggplot(data=RespSumTab, aes(x=Age, y=resp_ng_L_ m_Length_hr, color=pCO2)) +
  geom_line(aes(linetype = factor(Fed_Unfed)), size = 0.5) +
  geom_point()+
  scale_color_manual(values=c("forestgreen","darkorange2"))+
  geom_errorbar(aes(ymin=resp_ng_L_ m_Length_hr-se, ymax=resp_ng_L_ m_Length_hr+se), width=.2,
                position=position_dodge(.1))+
  theme_bw() +  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+
  scale_x_continuous(name ="Age (days)") +
  labs(title = "F1 Scallops: Food x OA Experiment",
       y = expression(Respiration~rate~"("~ng~L^{-1}~O[2]%.% *m^{-1}%.% hr^{-1}~")")) +
  scale_linetype_manual(values = c("High food" = "solid", "Low food" = "dashed"))  +
  theme(legend.position="none") +
  # facet_wrap(~factor(Fed_Unfed, level = c('Low food', 'High food')))
  facet_wrap(~pCO2)


#pdf(paste0("C:/Users/samjg/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respiration/Ju
pdf(paste0("C:/Users/samjg/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respiration/Juv

Resp_geomline
dev.off()


## pdf
##   2
```
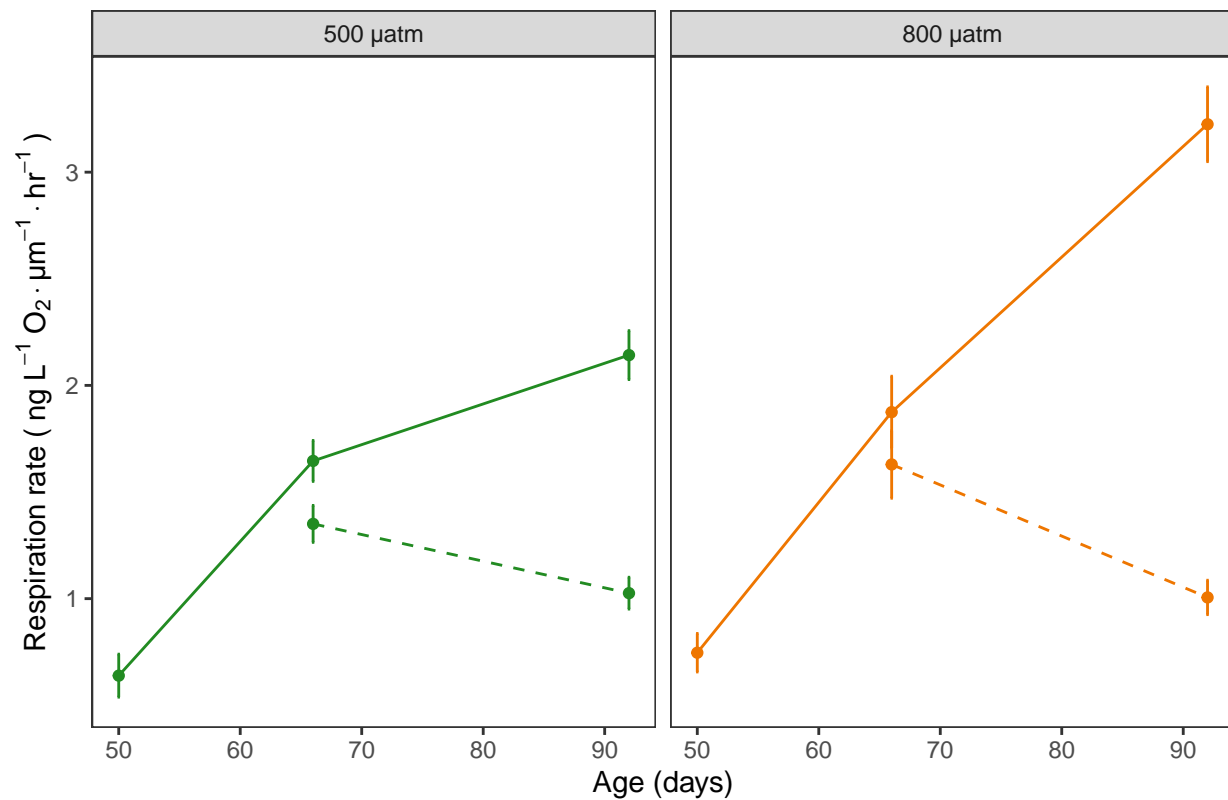
F1 Scallops: Food x OA Experiment

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
```

20210914 linear model, diagnostics, and plots

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

Table 1: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
pCO2	1	0.0694	0.0694	0.6327	0.4348
Residuals	22	2.413	0.1097	NA	NA

```
check_model(LMmod_0914) # observe the diagnostics of the model
```

Linearity
Reference line should be flat and horizontal
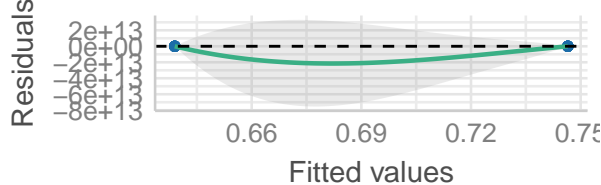
Homogeneity of Variance
Reference line should be flat and horizontal
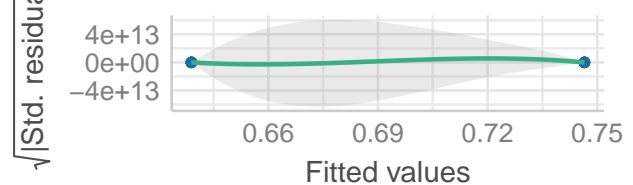
Influential Observations
Points should be inside the contour lines
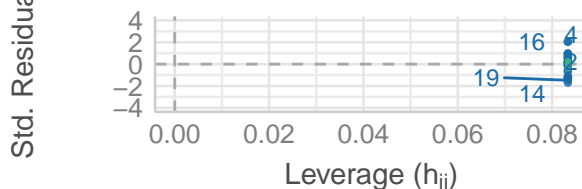
Normality of Residuals
Dots should fall along the line
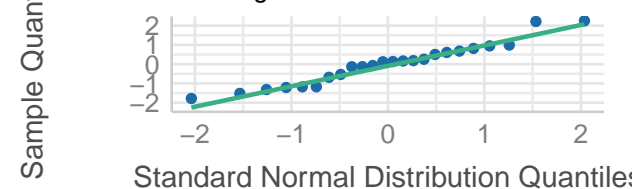
Normality of Residuals
Distribution should be close to the normal curve
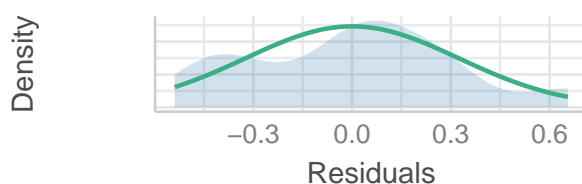
```
shapiro.test(residuals(LMmod_0914)) # normal - 0.3875
```

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

```
leveneTest(LMmod_0914) # good - 0.6186
```

```
## 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/20
pdf(paste0("C:/Users/samjg/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respiration/202

ggplot(Resp_0914, aes(x = factor(pCO2, level = c('500 atm', '800 atm')), resp_ng_L_ m_Length_hr , fill
  theme(panel.grid=element_blank()) +
  geom_boxplot(size=0.2, width=0.5, alpha=0.1, aes(fill=pCO2)) +
  scale_fill_manual(values=c("white","grey50")) +
  geom_point(shape = 21, size = 2, position = position_jitterdodge(jitter.width = 0.1)) +
  theme_classic() +
  scale_x_discrete(labels= c('~500 atm', '~800 atm')) +
  scale_y_continuous(expand = c(0, 0), limits = c(0, 4.25)) +
  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}~O[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>        <chr> <int>
##  1 7.5_A        fed       4
##  2 7.5_A        unfed     5
##  3 7.5_B        fed       5
##  4 7.5_B        unfed     4
##  5 7.5_C        fed       5
##  6 7.5_C        unfed     4
##  7 7.5_D        fed       5
##  8 7.5_D        unfed     2
##  9 8_A          fed       5
## 10 8_A          unfed     5
## 11 8_B          fed       5
## 12 8_B          unfed     6
## 13 8_C          fed       4
## 14 8_C          unfed     4
## 15 8_D          fed       5
## 16 8_D          unfed     4
```

20210930 linear model, diagnostics, and plots

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

Table 2: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
pCO2	1	1.291	1.291	4.247	0.04314
Fed_Unfed	1	1.322	1.322	4.348	0.04081
pCO2:Fed_Unfed	1	0.01103	0.01103	0.0363	0.8495
Residuals	68	20.67	0.304	NA	NA

```
check_model(LMmod_0930) # observe the diagnostics of the model
```
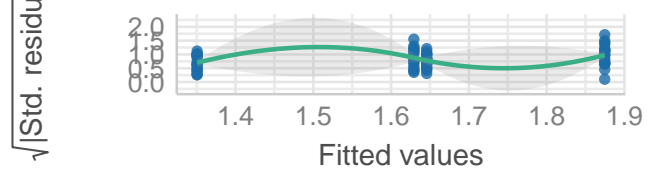
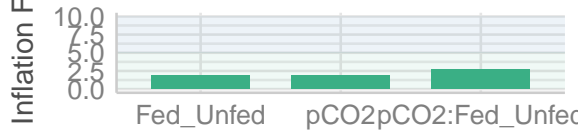## Linearity
Reference line should be flat and horizontal

Homogeneity of Variance
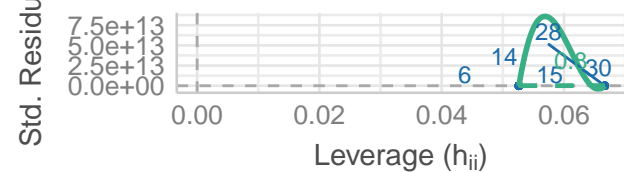Reference line should be flat and horizontal

Collinearity
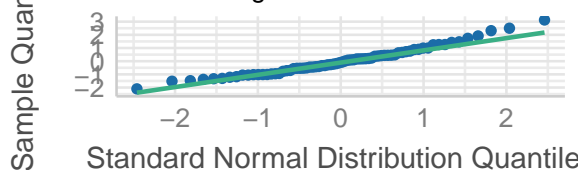Higher bars (>5) indicate potential collinearity iss

Influential Observations
Points should be inside the contour lines

low (< 5) moderate (< 10) high (>

Normality of Residuals
Dots should fall along the line

Normality of Residuals
Distribution should be close to the normal curve


```
shapiro.test(residuals(LMmod_0930)) #  normal - 0.07641
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(LMmod_0930)
## W = 0.97334, p-value = 0.1295
```

```
leveneTest(LMmod_0930) # good - 0.1648
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.8365 0.1488
##       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/20.
pdf(paste0("C:/Users/samjg/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respiration/202

ggplot(Resp_0930, aes(x = factor(pCO2, level = c('500 atm', '800 atm')), resp_ng_L_ m_Length_hr , fill
  theme(panel.grid=element_blank()) +
  geom_boxplot(size=0.2, alpha=0.1, aes(fill=pCO2)) +
  scale_fill_manual(values=c("white","grey50")) +
  geom_point(shape = 21, size = 2, position = position_jitterdodge(jitter.width = 0.1)) +
  theme_classic() +
  scale_x_discrete(labels= c('~500 atm', '~800 atm')) +
  scale_y_continuous(expand = c(0, 0), limits = c(0, 4.25)) +
  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
```

10/26/2021 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>        <chr> <int>
## 1 7.5_A        fed       2
## 2 7.5_A        unfed     2
## 3 7.5_B        fed       3
## 4 7.5_B        unfed     2
## 5 7.5_C        fed       3
```

```
##  6 7.5_C      unfed     2
##  7 7.5_D      fed       2
##  8 7.5_D      unfed     2
##  9 8_A        fed       2
## 10 8_A        unfed     2
## 11 8_B        fed       2
## 12 8_B        unfed     2
## 13 8_C        fed       2
## 14 8_C        unfed     2
## 15 8_D        fed       2
## 16 8_D        unfed     2
```

20211026 linear model, diagnostics, and plots

```
LMmod_01026 <- aov(lm(resp_ng_L_ m_Length_hr~pCO2*Fed_Unfed ,data=Resp_01026))
pander(summary(LMmod_01026), style='rmarkdown')
```
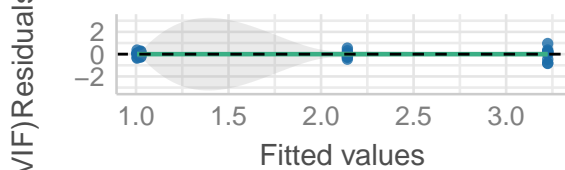
Table 3: Analysis of Variance Model

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
pCO2	1	3.629	3.629	26.07	1.729e-05
Fed_Unfed	1	24.31	24.31	174.6	4.845e-14
pCO2:Fed_Unfed	1	2.559	2.559	18.38	0.0001725
Residuals	30	4.177	0.1392	NA	NA

```
check_model(LMmod_01026) # observe the diagnostics of the model
```
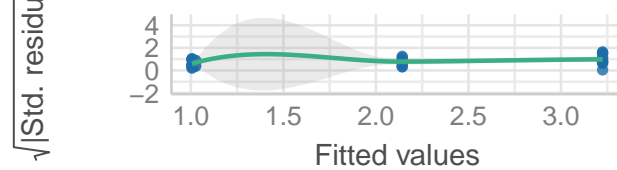
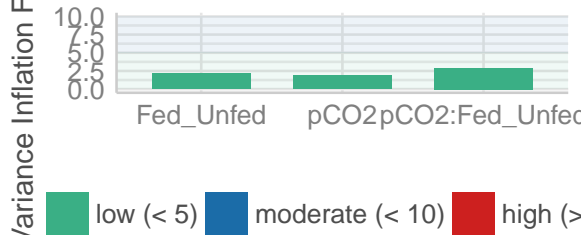## Linearity
Reference line should be flat and horizontal

Homogeneity of Variance
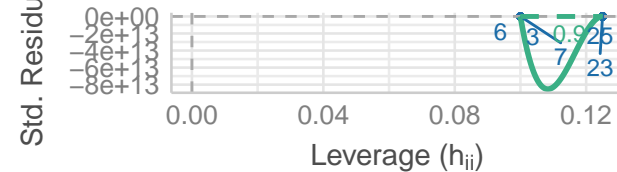Reference line should be flat and horizontal

Collinearity
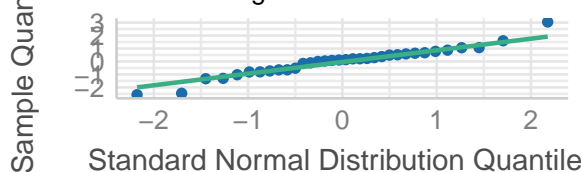Higher bars (>5) indicate potential collinearity iss

- low (< 5)
- moderate (< 10)
- high (>

Influential Observations
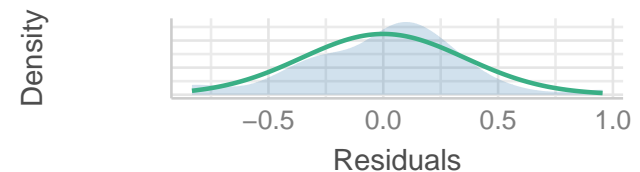Points should be inside the contour lines

Normality of Residuals
Dots should fall along the line

Normality of Residuals
Distribution should be close to the normal curve


```
shapiro.test(residuals(LMmod_01026)) #  normal - 0.3096
```

```
##
##  Shapiro-Wilk normality test
##
## data:  residuals(LMmod_01026)
## W = 0.96365, p-value = 0.3096
```

```
leveneTest(LMmod_01026) # 0.1998
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3  1.6456 0.1998
##       30
```

```
TukeyHSD(LMmod_01026)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = lm(resp_ng_L_µm_Length_hr ~ pCO2 * Fed_Unfed, data = Resp_01026))
##
## $pCO2
##                         diff       lwr       upr    p adj
```

```
## 800 μatm-500 μatm 0.6545763 0.3927428 0.9164097 1.73e-05
##
## $Fed_Unfed
##                         diff       lwr        upr p adj
## Low food-High food -1.691433 -1.953266 -1.429599     0
##
## $`pCO2:Fed_Unfed`
##                                       diff        lwr        upr
## 800 μatm:High food-500 μatm:High food  1.08257128  0.6013042  1.5638383
## 500 μatm:Low food-500 μatm:High food  -1.11638666 -1.6236867 -0.6090866
## 800 μatm:Low food-500 μatm:High food  -1.13635249 -1.6436525 -0.6290525
## 500 μatm:Low food-800 μatm:High food  -2.19895794 -2.6802250 -1.7176909
## 800 μatm:Low food-800 μatm:High food  -2.21892377 -2.7001908 -1.7376567
## 800 μatm:Low food-500 μatm:Low food   -0.01996583 -0.5272658  0.4873342
##                                          p adj
## 800 μatm:High food-500 μatm:High food 0.0000058
## 500 μatm:Low food-500 μatm:High food  0.0000084
## 800 μatm:Low food-500 μatm:High food  0.0000062
## 500 μatm:Low food-800 μatm:High food  0.0000000
## 800 μatm:Low food-800 μatm:High food  0.0000000
## 800 μatm:Low food-500 μatm:Low food   0.9995510
```

```r
DF_pCO2   <- paste( (summary(LMmod_01026)[[1]][["Df"]])[1], (summary(LMmod_01026)[[1]][["Df"]])[4], sep
Fval_pCO2 <- (summary(LMmod_01026)[[1]][["F value"]])[1]
pval_pCO2 <- (summary(LMmod_01026)[[1]][["Pr(>F)"]])[1]

DF_Fed    <- paste( (summary(LMmod_01026)[[1]][["Df"]])[2], (summary(LMmod_01026)[[1]][["Df"]])[4], sep
Fval_Fed  <- (summary(LMmod_01026)[[1]][["F value"]])[2]
pval_Fed  <- (summary(LMmod_01026)[[1]][["Pr(>F)"]])[2]


DF_Int    <- paste( (summary(LMmod_01026)[[1]][["Df"]])[3], (summary(LMmod_01026)[[1]][["Df"]])[4], sep
Fval_Int  <- (summary(LMmod_01026)[[1]][["F value"]])[3]
pval_Int  <- (summary(LMmod_01026)[[1]][["Pr(>F)"]])[3]


Resp_01026$Fed_Unfed <- as.factor(Resp_01026$Fed_Unfed)

levels(Resp_01026$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/20
pdf(paste0("C:/Users/samjg/Documents/Github_repositories/Airradians_OA/RAnalysis/Output/Respiration/202

ggplot(Resp_01026, aes(x = factor(pCO2, level = c('500 atm', '800 atm')), resp_ng_L_ m_Length_hr , fill
  theme(panel.grid=element_blank()) +
  geom_boxplot(size=0.2, alpha=0.1, aes(fill=pCO2)) +
  scale_fill_manual(values=c("white","grey50")) +
  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')) +
  scale_y_continuous(expand = c(0, 0), limits = c(0, 4.25)) +
  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) 92 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

```
# see what the dry tissue weight looks like for the data we have so far.... ( only have it for the fed (
Resp_fed_drywt <- Resp_01026 %>% dplyr::filter(Food %in% "fed") %>%
                  ggplot(aes(x = factor(pCO2, level = c('500 atm', '800 atm')), resp_mg_L_g_hr, fill = p(
                  theme(panel.grid=element_blank()) +
                  geom_boxplot(size=0.2, alpha=0.1, aes(fill=pCO2)) +
                  scale_fill_manual(values=c("white","grey50")) +
                  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')) +
                  #scale_y_continuous(expand = c(0, 0), limits = c(0, 4.25)) +
                  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) 92 days post-fertilization",
                       y = expression(Respiration~rate~"("~mg~L^{-1}~O[2]%.%g^{-1}%.% hr^{-1}~")"),
                       x = expression(italic(p)*CO[2]~Treatment~"("~mu*atm~")"))
```

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

```
Resp_fed_drywt # the effect with normalization to shell size persists here...
```

Respiration rate; F1 Scallops (juveniles) 92 days post–fertilization