

# Respiration Analysis F1 Scallops 2021

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## 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/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](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](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](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-
          (60)) # correct for the time; final value is ng Liter-1 individual-1 hour-1
    }
  }
}

```

## Analysis

### 9/14/2021 F1 juvenile scallops

About - two pCO<sub>2</sub> 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)
<b>pCO<sub>2</sub></b>	1	0.1162	0.1162	0.6327	0.4348
<b>Residuals</b>	22	4.041	0.1837	NA	NA

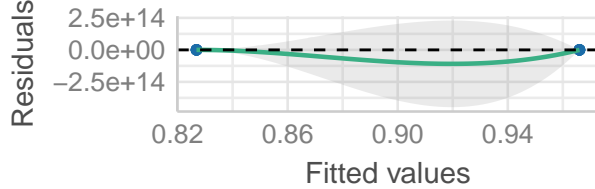
```

check_model(LMmod_0914) # observe the diagnostics of the model

```

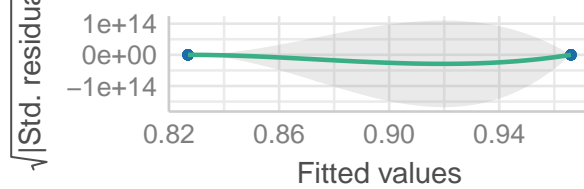
### Linearity

Reference line should be flat and horizontal



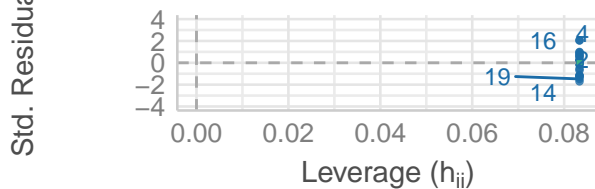
### Homogeneity of Variance

Reference line should be flat and horizontal



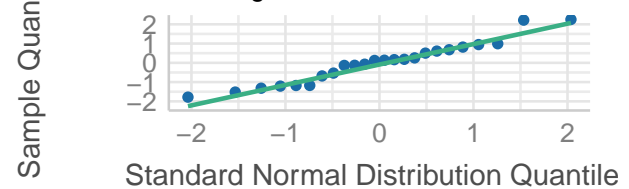
### Influential Observations

Points should be inside the contour lines



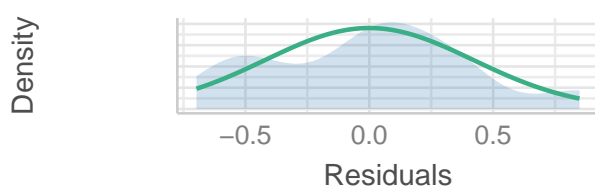
### Normality of Residuals

Dots 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
```

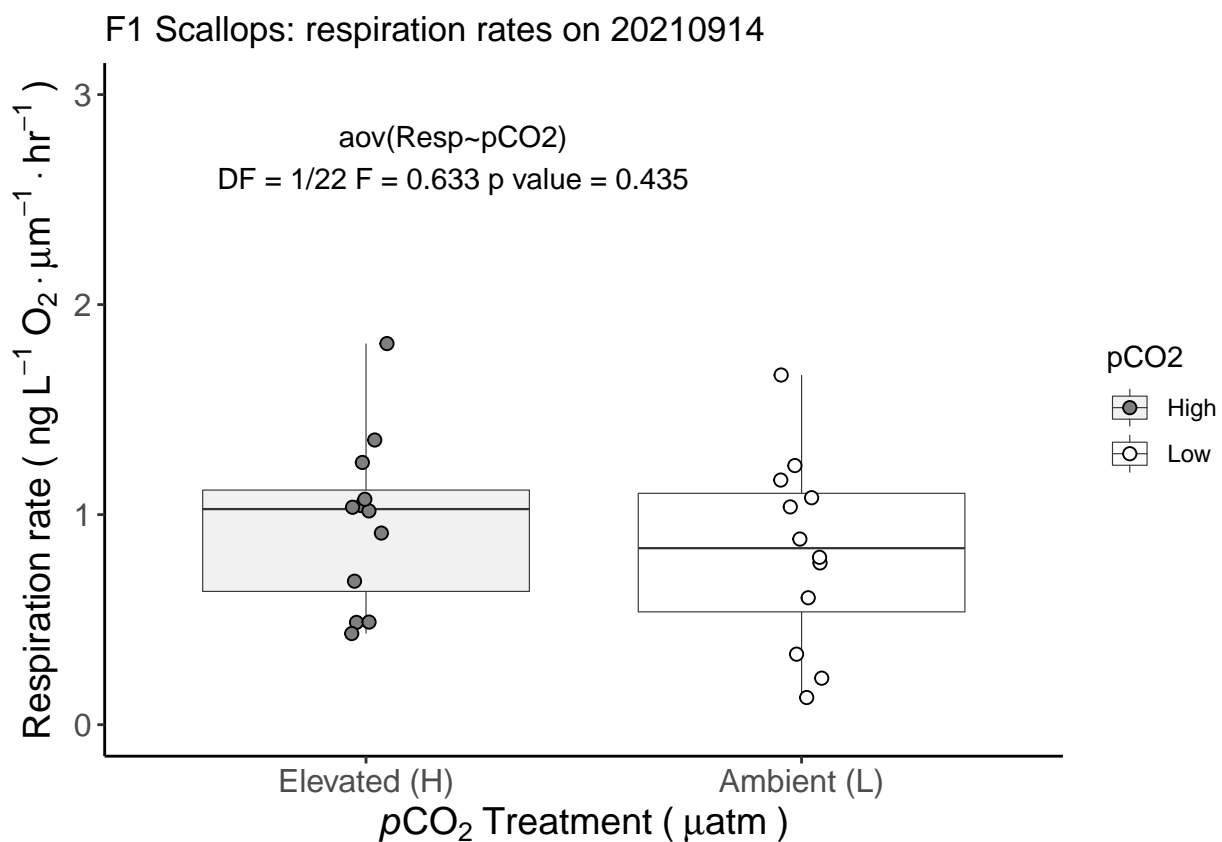
```
DF <- paste( (summary(LMmod_0914)[[1]][["Df"]])[1], (summary(LMmod_0914)[[1]][["Df"]])[2], sep = '/')
Fval <- (summary(LMmod_0914)[[1]][["F value"]])[1]
pval <- (summary(LMmod_0914)[[1]][["Pr(>F)"]])[1]

ggplot(Resp_0914, aes(pCO2 , resp_ng_L_umLength_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('Elevated (H)', 'Ambient (L)')) +
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}~O_2~\mu m^{-1}~hr^{-1}~")"),
     x = expression(italic(p)*CO[2]~Treatment~"(" ~mu*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 val'))

```



## 9/14/2021 F1 juvenile scallops

### About

- two pCO<sub>2</sub> 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_umlLength_hr~pCO2*Fed_Unfed ,data=Resp_0930))
pander(summary(LMmod_0930), style='rmarkdown')
```

## linear model, diagnostics, and plots

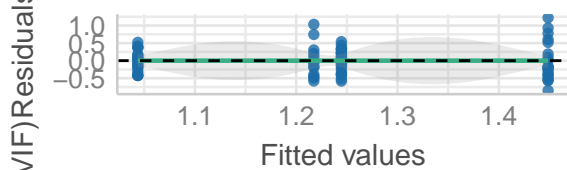
Table 2: Analysis of Variance Model

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

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

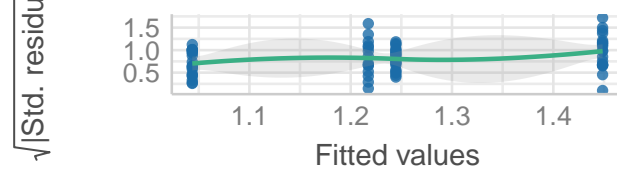
### Linearity

Reference line should be flat and horizontal



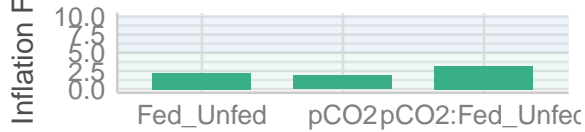
### Homogeneity of Variance

Reference line should be flat and horizontal



### Collinearity

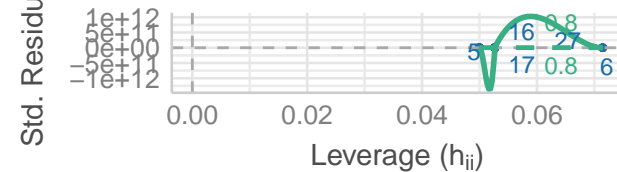
Higher bars (>5) indicate potential collinearity issues



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

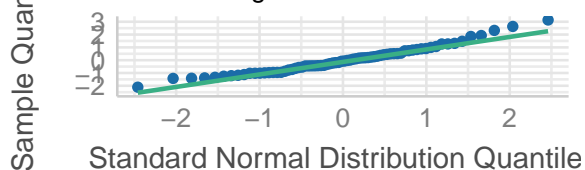
### Influential Observations

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]
```

```
arr <- list('High food'=TeX("x_1"), "Low food"=TeX("x_2"))
```



```

mylabel <- function(val) { return(lapply(val, function(x) arr[x])) }

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

levels(Resp_0930$Fed_Unfed) <- c("High food", "Low food")
ggplot(Resp_0930, aes(pCO2 , resp_ng_L_umLength_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('Elevated (H)', 'Ambient (L)')) +
  theme(axis.text=element_text(size=12),
        axis.title=element_text(size=14,face="bold")) +
  labs(title = "F1 Scallops: respiration rates on 20210930",
       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(~Fed_Unfed) # +

```

