Determining the Contribution of Osmotic Stress to the Antibacterial Properties of Honey

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ABSTARCT

Researchers have repeatedly shown that honey possesses distinctive antimicrobial properties; however, there is uncertainty over which compounds in honey are responsible for these properties. In this research study, we sought to quantify the role of osmotic stress in honey and determine the efficacy of two types of honey: Manuka and raw pasture honey. Bacteria were sequentially cultured in sublethal concentrations of Manuka and raw pasture honey for five days. The role of osmotic stress as a contributor to the antibacterial properties of Manuka and raw pasture honey was quantified in the first culture and over five serial cultures. The growth levels of bacteria in honey were compared to growth levels in glucose, an osmotic control, to quantify the role of osmotic stress in the two types of honey. The results of this study indicate that in the first culture, the antibacterial impacts of Manuka and raw pasture honey were primarily attributable to osmotic stress. However, over five days of sequential transfers, both raw pasture honey and Manuka honey showed significant antibacterial properties beyond osmotic strength. It was established that the antibacterial properties of honey cannot be investigated based solely on the first culture. Serial transfers over several days should be employed to investigate the efficacy of honey as an antibacterial substance.

Read in Data

```
honeyGlu.d1 <- read.csv("Data/honeyGlucoseCd1.csv", header = T)
honeyGlu.d5 <- read.csv("Data/honeyGlucosetoCd5.csv", header = T)
```

Day 1 Analysis

Combined effect Day 1

Default: control as reference

```
table (honeyGlu.d1$Culture)
```

```
##
##  1
## 57
```

```
honeyGlu.d1$Day <- as.numeric(as.character(honeyGlu.d1$Day))
honeyGlu.d1$StrCon <- factor(honeyGlu.d1$StrCon)

honeyGlu.d1$StrCon <- relevel(honeyGlu.d1$StrCon, ref = "Control")
od.lm.control.1 <- lm(OD ~ Day + StrCon, data = honeyGlu.d1)
summary(od.lm.control.1)
```

```
##
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.15969 -0.04097  0.00200  0.05200  0.20333
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      1.82331    0.04173  43.695  < 2e-16 ***
## Day             -0.03931    0.01391  -2.826  0.00706 **
## StrConGlu16     -0.92469    0.06573 -14.067  < 2e-16 ***
## StrConGlu6      -0.66802    0.06573 -10.163 4.05e-13 ***
## StrConLocal140  -0.30450    0.13342  -2.282  0.02736 *
## StrConLocal160  -0.38881    0.12206  -3.186  0.00266 **
## StrConLocal180  -0.38450    0.13342  -2.882  0.00609 **
## StrConLocal210  -0.57420    0.09440  -6.082 2.55e-07 ***
## StrConLocal260  -0.89303    0.05251 -17.008  < 2e-16 ***
## StrConManuka50  -0.23312    0.11128  -2.095  0.04196 *
## StrConManuka60  -0.24612    0.10030  -2.454  0.01816 *
## StrConManuka70  -0.33875    0.07868  -4.305 9.18e-05 ***
## StrConManuka80  -0.71569    0.05015 -14.271  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.08797 on 44 degrees of freedom
## Multiple R-squared:  0.9213, Adjusted R-squared:  0.8999
## F-statistic: 42.95 on 12 and 44 DF,  p-value: < 2.2e-16
```

Re-leveling the data with Glu6 as reference

```
honeyGlu.d1$StrCon <- relevel(honeyGlu.d1$StrCon, ref = "Glu6")
od.lm.Glu6.1 <- lm(OD ~ StrCon, data = honeyGlu.d1)
summary(od.lm.Glu6.1)
```

```
##
## Call:
## lm(formula = OD ~ StrCon, data = honeyGlu.d1)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.1990 -0.0420  0.0020  0.0510  0.2033
##
## Coefficients:
```

```
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)     1.076667   0.054592  19.722  < 2e-16 ***
## StrConControl   0.707333   0.069054  10.243 2.44e-13 ***
## StrConGlu16    -0.256667   0.077204  -3.325 0.001768 **
## StrConLocal140  0.088333   0.086317   1.023 0.311608
## StrConLocal160  0.043333   0.086317   0.502 0.618097
## StrConLocal180  0.008333   0.086317   0.097 0.923518
## StrConLocal210 -0.092917   0.064014  -1.451 0.153578
## StrConLocal260 -0.244667   0.062244  -3.931 0.000289 ***
## StrConManuka50  0.238333   0.086317   2.761 0.008307 **
## StrConManuka60  0.225333   0.069054   3.263 0.002107 **
## StrConManuka70  0.211333   0.069054   3.060 0.003719 **
## StrConManuka80 -0.047667   0.062244  -0.766 0.447792
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.09456 on 45 degrees of freedom
## Multiple R-squared:  0.9071, Adjusted R-squared:  0.8843
## F-statistic: 39.93 on 11 and 45 DF,  p-value: < 2.2e-16
```

Re-leveling the data with Glu16 as reference

```
honeyGlu.d1$StrCon <- relevel(honeyGlu.d1$StrCon, ref = "Glu16")
od.lm.Glu16.1 <- lm(OD ~ Day + StrCon, data = honeyGlu.d1)
summary(od.lm.Glu16.1)
```

```
##
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.15969 -0.04097  0.00200  0.05200  0.20333
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      0.89862    0.05791  15.518  < 2e-16 ***
## Day             -0.03931    0.01391  -2.826 0.007057 **
## StrConGlu6       0.25667    0.07183   3.573 0.000869 ***
## StrConControl    0.92469    0.06573  14.067  < 2e-16 ***
## StrConLocal140   0.62019    0.12621   4.914 1.28e-05 ***
## StrConLocal160   0.53588    0.11582   4.627 3.27e-05 ***
## StrConLocal180   0.54019    0.12621   4.280 9.95e-05 ***
## StrConLocal210   0.35048    0.08895   3.940 0.000287 ***
## StrConLocal260   0.03166    0.05833   0.543 0.590043
## StrConManuka50   0.69156    0.10624   6.510 6.00e-08 ***
## StrConManuka60   0.67856    0.09468   7.167 6.51e-09 ***
## StrConManuka70   0.58594    0.07661   7.649 1.30e-09 ***
## StrConManuka80   0.20900    0.05791   3.609 0.000782 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.08797 on 44 degrees of freedom
```

```
## Multiple R-squared:  0.9213, Adjusted R-squared:  0.8999
## F-statistic: 42.95 on 12 and 44 DF,  p-value: < 2.2e-16
```

Predictive model for osm

11/22/18: no longer used due to actual measure of all concentration osm

```r
OsmVsMgperml <- read.csv("Data/OsmVsMgpermlData.csv", header = T)
OsmVsMgperml <- rbind(OsmVsMgperml[1:2,], OsmVsMgperml)
OsmVsMgperml$Solution <- as.character(OsmVsMgperml$Solution)
OsmVsMgperml$Solution[1:2] <- "Manuka honey"
OsmVsMgperml$Solution[3:4] <- "Local honey"
lm.OsmVsMgperml <- lm(mmol.per.kg ~ mgperml + Solution, data = OsmVsMgperml)
summary(lm.OsmVsMgperml)
```

```
##
## Call:
## lm(formula = mmol.per.kg ~ mgperml + Solution, data = OsmVsMgperml)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -38.333  -1.125  -0.250   1.000  47.667
##
## Coefficients: (1 not defined because of singularities)
##                      Estimate Std. Error t value Pr(>|t|)
## (Intercept)          4.200e+02  1.656e+01  25.368 3.78e-08 ***
## mgperml              4.641e+00  2.535e-01  18.309 3.59e-07 ***
## SolutionLocal Honey -5.123e+01  5.494e+01  -0.933    0.382
## SolutionManuka honey -4.676e-13  2.341e+01   0.000    1.000
## SolutionManuka Honey       NA         NA      NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 23.41 on 7 degrees of freedom
## Multiple R-squared:  0.9984, Adjusted R-squared:  0.9976
## F-statistic:  1415 on 3 and 7 DF,  p-value: 4.211e-10
```

Assigning Osmolality

```r
OsmVsMgperml$predicted.osm <- 430.46878 + 4.41526 * OsmVsMgperml$mgperml
predict.osm <- function(x) {430.46878 + 4.41526 * x}
honeyGlu.d1$predicted.osm <- 0
honeyGlu.d1[honeyGlu.d1$Conc >16 ,"predicted.osm"] <- predict.osm(honeyGlu.d1[honeyGlu.d1$Conc >16 ,"Con
honeyGlu.d1[honeyGlu.d1$Conc ==16 ,"predicted.osm"] <- 1494.5997
honeyGlu.d1[honeyGlu.d1$Conc ==6 ,"predicted.osm"] <- 802
honeyGlu.d1[honeyGlu.d1$Conc ==0 ,"predicted.osm"] <- 420
```

Osmalality within Treatment Analysis d1

- No Control
- No lethals

4

- ref = glu

```
honeyGlu.d1.no.control <- honeyGlu.d1[honeyGlu.d1$Trt != 'Control',]
lm.osm.within.trt.d1 <- lm(OD ~ Day + Trt + predicted.osm%in%Trt, data = honeyGlu.d1.no.control)
summary(lm.osm.within.trt.d1)
```

```
##
## Call:
## lm(formula = OD ~ Day + Trt + predicted.osm %in% Trt, data = honeyGlu.d1.no.control)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.268515 -0.050153 -0.001465  0.060218  0.203333
##
## Coefficients:
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)              1.3953424  0.1477112   9.446    3e-12 ***
## Day                     -0.0107335  0.0128907  -0.833 0.409438
## TrtLocal                 0.7377006  0.3662986   2.014 0.050019 .
## TrtManuka                2.1409106  0.6143579   3.485 0.001110 **
## TrtGlu:predicted.osm    -0.0003706  0.0001213  -3.056 0.003765 **
## TrtLocal:predicted.osm  -0.0008025  0.0002132  -3.764 0.000482 ***
## TrtManuka:predicted.osm -0.0031212  0.0007733  -4.036 0.000208 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1029 on 45 degrees of freedom
## Multiple R-squared:  0.7423, Adjusted R-squared:  0.7079
## F-statistic:  21.6 on 6 and 45 DF,  p-value: 9.192e-12
```

Separating the effect of osmotic strength and antimicrobials d1

```
honeyGlu.d1.no.control <- honeyGlu.d1[honeyGlu.d1$Trt != 'Control',]
lm.osm.trt.d1 <- lm(OD ~ Day + Trt + predicted.osm, data = honeyGlu.d1.no.control)
summary(lm.osm.trt.d1)
```

```
##
## Call:
## lm(formula = OD ~ Day + Trt + predicted.osm, data = honeyGlu.d1.no.control)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.301606 -0.046706 -0.001185  0.076096  0.199156
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)   1.3627153  0.1478311   9.218 4.14e-12 ***
## Day           0.0216131  0.0080011   2.701 0.009577 **
## TrtLocal      0.0304380  0.0718505   0.424 0.673769
## TrtManuka     0.0163590  0.0650426   0.252 0.802514
## predicted.osm -0.0003985  0.0001135  -3.512 0.000994 ***
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.114 on 47 degrees of freedom
## Multiple R-squared:  0.6694, Adjusted R-squared:  0.6413
## F-statistic: 23.79 on 4 and 47 DF,  p-value: 8.438e-11
```

Plotting day 1 concentrations of local and Manuka (and glucose/LB)

```r
honeyGlu.d1.zeros <- read.csv("Data/honeyGlucoseCd1zeros.csv") # Adding lethals
library("Hmisc")

#tiff("./Final\ Figures/Fig.1.300.tiff", units = "in", width = 8, height = 5, res = 300)
par(mar = c(5,5,2,2))
Mconc <- honeyGlu.d1.zeros[honeyGlu.d1.zeros$Trt == "Manuka",]
plot(Mconc$Conc, Mconc$OD, ylim = c(-0.1,2), xaxt = "n", xlim = c(50, 270), pch=16, cex = 1.5, col= "da
axis(1, at = seq(50, 270, by = 20), cex.lab = 1.2, cex.axis = 1)
minor.tick(nx=5, ny=4, tick.ratio=0.75)

Mconc.line <- tapply(Mconc$OD, Mconc$Conc, mean)
lines(as.numeric(names(Mconc.line)), Mconc.line, type='l', col="dark orange", lwd = 2, lty = 2)

# Using linear model instead of avg
# lm.Mconc <- lm(OD ~ Conc, data = Mconc)
# lines(Mconc$Conc, predict(lm.Mconc), type='l', col="dark orange", lwd = 3, lty = 3)

Lconc <- honeyGlu.d1.zeros[honeyGlu.d1.zeros$Trt == "Local",]
points(Lconc$Conc, Lconc$OD, pch=16, cex = 1.5, col= "dark green")
lm.Lconc <- lm(OD ~ Conc, data = Lconc)
lines(Lconc$Conc, predict(lm.Lconc), type='l', col= "dark green", lwd = 2, lty = 2)

avgControl.d1 <- mean(honeyGlu.d1.zeros[honeyGlu.d1.zeros$Trt == "Control", "OD"])
lines(seq(40, 270, 10), rep(avgControl.d1, 24), type='l', col= "black", lwd = 2, lty = 2)

glu.lm <- lm(OD ~ Conc, data = honeyGlu.d1.zeros[honeyGlu.d1.zeros$Trt == "Glu",])
avgGlu6.d1 <- mean(honeyGlu.d1.zeros[honeyGlu.d1.zeros$StrCon == "Glu6", "OD"])
avgGlu16.d1 <- mean(honeyGlu.d1.zeros[honeyGlu.d1.zeros$StrCon == "Glu16", "OD"])
points(10*(4.5:27), predict(glu.lm, newdata = data.frame(Conc = 4.5:27)), type='l', col= "blue", lwd = 3

symbols(x=c(90,100, 110), y=c(0,0,0), circles=c(3.5,3.5,3.5), add=T, inches=F, fg = "dark orange", lwd

legend(210, .4, pch = c(NA,NA,16,16), lty = c(2,1,2,2), lwd = 1.5, col = c("black","blue", "dark orange
       legend=c("LB Control","Glucose Control", "Manuka Honey", "Raw Pasture Honey"), cex = 0.8, pt.cex
```
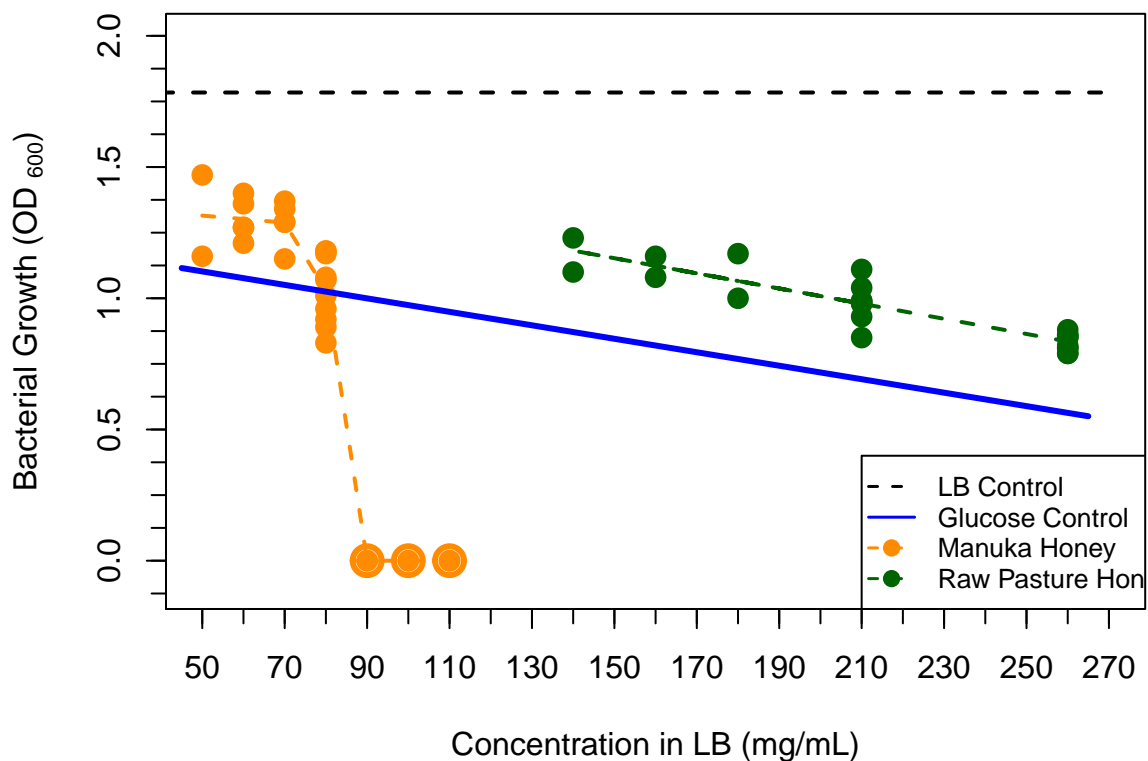
Day 1-5 Analysis (without 48 hr strain)

Combined effect 5 days

Default: control as reference

```
table (honeyGlu.d5$Culture)
```

```
##
##  1  2  3  4  5
## 57 53 20 21 22
```

```
honeyGlu.d5.no.48 <- honeyGlu.d5
honeyGlu.d5 <- honeyGlu.d5[honeyGlu.d5$OD.Hrs == 24 & honeyGlu.d5$Comments == "",]
honeyGlu.d5$Day <- as.numeric(as.character(honeyGlu.d5$Day))
honeyGlu.d5$StrCon <- factor(honeyGlu.d5$StrCon)
#honeyGlu.d5$Day <- factor(honeyGlu.d5$Day)
honeyGlu.d5$StrCon <- relevel(honeyGlu.d5$StrCon, ref = "Control")
od.lm.control.5 <- lm(OD ~ Day + StrCon, data = honeyGlu.d5)
summary(od.lm.control.5)
```

```
##
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d5)
```

```
## 
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.9602 -0.1553  0.0369  0.2175  0.7116
## 
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      1.87866    0.10625  17.682  < 2e-16 ***
## Day             -0.02888    0.02141  -1.349  0.17949
## StrConGlu16     -0.85715    0.13155  -6.516 1.03e-09 ***
## StrConGlu6      -0.66849    0.13155  -5.082 1.10e-06 ***
## StrConLocal140  -0.62965    0.22412  -2.809  0.00562 **
## StrConLocal160  -0.66828    0.21222  -3.149  0.00198 **
## StrConLocal180  -0.53377    0.30294  -1.762  0.08012 .
## StrConLocal210  -1.07558    0.16000  -6.722 3.50e-10 ***
## StrConLocal260  -1.37603    0.12011 -11.457  < 2e-16 ***
## StrConManuka50  -0.32677    0.19708  -1.658  0.09940 .
## StrConManuka60  -0.34792    0.17909  -1.943  0.05393 .
## StrConManuka70  -1.07584    0.15636  -6.881 1.51e-10 ***
## StrConManuka80  -1.29197    0.12014 -10.754  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 0.3638 on 150 degrees of freedom
## Multiple R-squared:  0.5836, Adjusted R-squared:  0.5503
## F-statistic: 17.52 on 12 and 150 DF,  p-value: < 2.2e-16
```

Re-leveling the data with Glu6 as reference

```
honeyGlu.d5$StrCon <- relevel(honeyGlu.d5$StrCon, ref = "Glu6")
od.lm.Glu6.5 <- lm(OD ~ Day + StrCon, data = honeyGlu.d5)
summary(od.lm.Glu6.5)
```

```
## 
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d5)
## 
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.9602 -0.1553  0.0369  0.2175  0.7116
## 
## Coefficients:
##                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)     1.2101733  0.1271121   9.521  < 2e-16 ***
## Day            -0.0288767  0.0214118  -1.349  0.17949
## StrConControl   0.6684857  0.1315515   5.082 1.10e-06 ***
## StrConGlu16    -0.1886667  0.1328311  -1.420  0.15758
## StrConLocal140  0.0388357  0.2060690   0.188  0.85077
## StrConLocal160  0.0002073  0.1958885   0.001  0.99916
## StrConLocal180  0.1347167  0.2940222   0.458  0.64748
## StrConLocal210 -0.4070946  0.1507155  -2.701  0.00771 **
## StrConLocal260 -0.7075433  0.1260835  -5.612 9.41e-08 ***
## StrConManuka50  0.3417167  0.1830758   1.867  0.06392 .
```

```
## StrConManuka60  0.3205692  0.1643608   1.950  0.05299 .
## StrConManuka70 -0.4073517  0.1519430  -2.681  0.00816 **
## StrConManuka80 -0.6234817  0.1283360  -4.858 2.95e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3638 on 150 degrees of freedom
## Multiple R-squared:  0.5836, Adjusted R-squared:  0.5503
## F-statistic: 17.52 on 12 and 150 DF,  p-value: < 2.2e-16
```

Re-leveling the data with Glu16 as reference

```
honeyGlu.d5$StrCon <- relevel(honeyGlu.d5$StrCon, ref = "Glu16")
od.lm.Glu16.5 <- lm(OD ~ Day + StrCon, data = honeyGlu.d5)
summary(od.lm.Glu16.5)
```

```
##
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d5)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.9602 -0.1553  0.0369  0.2175  0.7116
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)      1.02151    0.12711   8.036 2.54e-13 ***
## Day             -0.02888    0.02141  -1.349 0.179487
## StrConGlu6       0.18867    0.13283   1.420 0.157581
## StrConControl    0.85715    0.13155   6.516 1.03e-09 ***
## StrConLocal140   0.22750    0.20607   1.104 0.271357
## StrConLocal160   0.18887    0.19589   0.964 0.336502
## StrConLocal180   0.32338    0.29402   1.100 0.273155
## StrConLocal210  -0.21843    0.15072  -1.449 0.149349
## StrConLocal260  -0.51888    0.12608  -4.115 6.36e-05 ***
## StrConManuka50   0.53038    0.18308   2.897 0.004331 **
## StrConManuka60   0.50924    0.16436   3.098 0.002324 **
## StrConManuka70  -0.21868    0.15194  -1.439 0.152161
## StrConManuka80  -0.43482    0.12834  -3.388 0.000899 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3638 on 150 degrees of freedom
## Multiple R-squared:  0.5836, Adjusted R-squared:  0.5503
## F-statistic: 17.52 on 12 and 150 DF,  p-value: < 2.2e-16
```

Predictive model for osm

```
OsmVsMgperml <- read.csv("Data/OsmVsMgpermlData.csv", header = T)
lm.OsmVsMgperml <- lm(mmol.per.kg ~ mgperml, data = OsmVsMgperml)
summary(lm.OsmVsMgperml)
```

```
##
## Call:
## lm(formula = mmol.per.kg ~ mgperml, data = OsmVsMgperml)
##
## Residuals:
##     Min     1Q  Median     3Q     Max
## -41.435 -11.469   6.311   7.311  44.565
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 430.46878   12.89920   33.37 5.62e-09 ***
## mgperml       4.41526    0.08097   54.53 1.83e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 24.82 on 7 degrees of freedom
## Multiple R-squared:  0.9977, Adjusted R-squared:  0.9973
## F-statistic:  2973 on 1 and 7 DF,  p-value: 1.829e-10
```

Assigning Osmolality

```
OsmVsMgperml$predicted.osm <- 430.46878 + 4.41526 * OsmVsMgperml$mgperml
predict.osm <- function(x) {430.46878 + 4.41526 * x}
honeyGlu.d5$predicted.osm <- 0
honeyGlu.d5[honeyGlu.d5$Conc >16 ,"predicted.osm"] <- predict.osm(honeyGlu.d5[honeyGlu.d5$Conc >16 ,"Co
honeyGlu.d5[honeyGlu.d5$Conc ==16 ,"predicted.osm"] <- 1494.5997
honeyGlu.d5[honeyGlu.d5$Conc ==6 ,"predicted.osm"] <- 802
honeyGlu.d5[honeyGlu.d5$Conc ==0 ,"predicted.osm"] <- 420
```

Osmalality within Treatment Analysis d5

- No Control
- No zeros
- ref = glu

```
x <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Comments != "",]
x$predicted.osm = 1136.91
honeyGlu.d5.no.48 <- rbind(honeyGlu.d5, x)
honeyGlu.d5.no.control <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt != 'Control' & honeyGlu.d5.no.48$Comme
lm.osm.within.trt.d5 <- lm(OD ~ Day + Trt + predicted.osm%in%Trt, data = honeyGlu.d5.no.control)
summary(lm.osm.within.trt.d5)
```

```
##
## Call:
## lm(formula = OD ~ Day + Trt + predicted.osm %in% Trt, data = honeyGlu.d5.no.control)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.99181 -0.29212  0.09338  0.28699  0.64505
##
## Coefficients:
##                        Estimate Std. Error t value Pr(>|t|)
```

```
## (Intercept)                 1.3776851   0.2626582    5.245 5.69e-07 ***
## Day                         -0.0161377   0.0229022   -0.705  0.48222
## TrtLocal                     1.1376774   0.6767248    1.681  0.09498 .
## TrtManuka                    5.2920851   1.2552243    4.216 4.45e-05 ***
## TrtGlu:predicted.osm        -0.0002724   0.0002052   -1.327  0.18661
## TrtLocal:predicted.osm      -0.0012990   0.0004293   -3.026  0.00296 **
## TrtManuka:predicted.osm     -0.0078075   0.0016429   -4.752 4.96e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3893 on 139 degrees of freedom
## Multiple R-squared:   0.37,  Adjusted R-squared:  0.3428
## F-statistic: 13.61 on 6 and 139 DF,  p-value: 4.103e-12
```

```
averageOD.day.trt <- tapply(honeyGlu.d5.no.control$OD, list (honeyGlu.d5.no.control$Trt, honeyGlu.d5.no
```

Osmalality within Treatment Analysis d2-5

- No Control
- No lethals
- ref = glu

```
honeyGlu.d25.no.control <- honeyGlu.d5.no.control[honeyGlu.d5.no.control$Culture > 1,]
lm.osm.within.trt.d25 <- lm(OD ~ Trt + predicted.osm%in%Trt, data = honeyGlu.d25.no.control)
summary(lm.osm.within.trt.d25)
```

```
##
## Call:
## lm(formula = OD ~ Trt + predicted.osm %in% Trt, data = honeyGlu.d25.no.control)
##
## Residuals:
##      Min        1Q   Median        3Q       Max
## -0.90768 -0.08601  0.03887  0.15871  0.45658
##
## Coefficients:
##                           Estimate Std. Error t value Pr(>|t|)
## (Intercept)              1.2979491  0.1947234    6.666 2.22e-09 ***
## TrtLocal                 1.5603197  0.3349835    4.658 1.13e-05 ***
## TrtManuka                7.6586759  0.6978598   10.975  < 2e-16 ***
## TrtGlu:predicted.osm    -0.0002479  0.0001624   -1.527     0.13
## TrtLocal:predicted.osm  -0.0018602  0.0002120   -8.774 1.20e-13 ***
## TrtManuka:predicted.osm -0.0114784  0.0009326  -12.308  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.2754 on 88 degrees of freedom
## Multiple R-squared:  0.7609, Adjusted R-squared:  0.7473
## F-statistic: 56.01 on 5 and 88 DF,  p-value: < 2.2e-16
```

Separating the effect of osmotic strength and antimicrobials d5

```
honeyGlu.d5.no.control <- honeyGlu.d5[honeyGlu.d5$Trt != 'Control',]
lm.osm.trt.d5<- lm(OD ~ Day + Trt + predicted.osm, data = honeyGlu.d5.no.control)
summary(lm.osm.trt.d5)
```

```
##
## Call:
## lm(formula = OD ~ Day + Trt + predicted.osm, data = honeyGlu.d5.no.control)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.9272 -0.1715  0.1025  0.2907  0.6226
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)    1.1203079  0.2647635   4.231 4.16e-05 ***
## Day            0.0566569  0.0131837   4.298 3.20e-05 ***
## TrtLocal      -0.4431446  0.1139828  -3.888 0.000155 ***
## TrtManuka     -0.3417624  0.1175659  -2.907 0.004240 **
## predicted.osm -0.0003018  0.0001931  -1.563 0.120276
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4154 on 141 degrees of freedom
## Multiple R-squared:  0.2722, Adjusted R-squared:  0.2516
## F-statistic: 13.19 on 4 and 141 DF,  p-value: 3.76e-09
```

Time Trends

Plotting time trends

```
# tiff("./Final\ Figures/Fig.3.300.tiff", units = "in", width = 8, height = 3, res = 300)

# Plotting both honey time trends together
par(mfrow=c(1, 2))

#plotting Manuka honey time trend
Manuka.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Manuka",]
M.lethal2 <- Manuka.ttrend[Manuka.ttrend$Conc == 80 | Manuka.ttrend$Conc == 70,]
M.sublethal2 <- Manuka.ttrend[Manuka.ttrend$Conc == 60 | Manuka.ttrend$Conc == 50,]

plot(M.lethal2$Culture, M.lethal2$OD, ylim=c(-0.1,2), xlim = c(1,5), pch=16, col = "dark red",cex = 1.5
minor.tick(nx = 0, ny=4, tick.ratio=0.75)
M.lethal2.trend <- lm(OD ~ Culture, data = M.lethal2)
lines(M.lethal2$Culture, predict(M.lethal2.trend), lwd=2, lty=2, col ="dark red")

points(M.sublethal2$Culture, M.sublethal2$OD, pch=16, col = "orange", cex=1.5)
M.sublethal2.trend <- lm(OD ~ Culture, data = M.sublethal2)
lines(M.sublethal2$Culture, predict(M.sublethal2.trend), lwd=2, lty=2, col = "dark orange")
```

```r
control.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Control",]
points(control.ttrend$Culture, control.ttrend$OD, pch=16, col = "black", cex=1.5)
lm.control.ttrend <- lm(OD ~ Culture, data = control.ttrend)
lines(control.ttrend$Culture, predict(lm.control.ttrend), lwd=2, lty = 2, col = "black")

symbols(x=2, y=0, circles=c(0.09), add=T, inches=F, fg = "dark red", lwd = 3)

legend(3.65,.6, pch = 16, col = c("black","dark red", "orange"),
        legend=c("LB Control",expression("">="70 mg/mL"), " < 70 mg/mL"), cex = 1, pt.cex = 1.5)


# Plotting Local honey time trend
local.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Local",]
L.lethal2 <- local.ttrend[local.ttrend$Conc > 160 & local.ttrend$Conc != 180 ,]
L.sublethal2 <-local.ttrend[local.ttrend$Conc <= 160 & local.ttrend$Comments == "",]

plot(L.lethal2$Culture, L.lethal2$OD, ylim=c(-0.1,2), xlim = c(1,5), pch=16, col = "blue",cex = 1.5, yla
minor.tick(nx = 0, ny=4, tick.ratio=0.75)
L.lethal2.trend <- lm(OD ~ Culture, data = L.lethal2)
lines(L.lethal2$Culture, predict(L.lethal2.trend), lwd=2, lty = 2, col ="blue")

points(L.sublethal2$Culture, L.sublethal2$OD, pch=16, col = "green", cex=1.5)
L.sublethal2.avg <- tapply(L.sublethal2$OD, L.sublethal2$Culture, mean)
lines(c(1:5), L.sublethal2.avg, lwd=2, lty=2, col = "green")

legend(3.65,.6, pch = 16, col = c("black", "blue", "green"),
        legend=c("LB Control", expression("">="210 mg/mL"), "<210 mg/mL"), cex = 1, pt.cex = 1.5)

control.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Control",]
points(control.ttrend$Culture, control.ttrend$OD, pch=16, col = "black", cex=1.5)
lm.control.ttrend <- lm(OD ~ Culture, data = control.ttrend)
lines(control.ttrend$Culture, predict(lm.control.ttrend), lwd=2, lty = 2, col = "black")
```
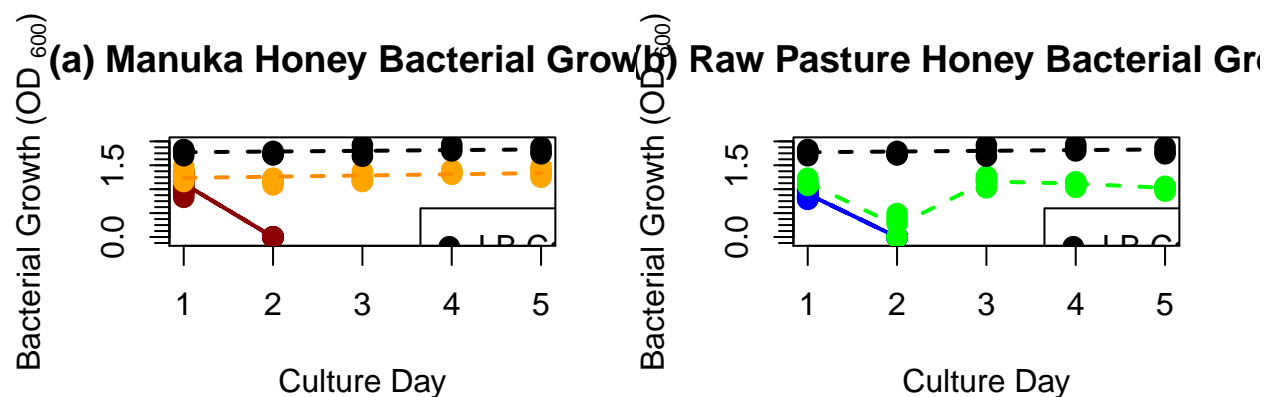

(a) Manuka Honey Bacterial Growth (b) Raw Pasture Honey Bacterial Growth

Plotting glucose time trend

```r
glu.ttrend <- honeyGlu.d5[honeyGlu.d5$Trt == "Glu",]
glu6 <- glu.ttrend[glu.ttrend$Conc == 6,]
glu16 <- glu.ttrend[glu.ttrend$Conc == 16,]
```

```
plot(glu6$Culture, glu6$OD, ylim=c(-0.1,2), xlim = c(1,5), pch=16, col = "navyblue",cex = 2, ylab = "Ba
glu6.trend <- lm(OD ~ Culture, data = glu6)
lines(glu6$Culture, predict(glu6.trend), lwd=3, lty = 3, col ="navyblue")

points(glu16$Culture, glu16$OD, pch=16, col = "blue", cex=2)
glu16.trend <- lm(OD ~ Culture, data = glu16)
lines(glu16$Culture, predict(glu16.trend), lwd=3, lty = 3, col ="blue")

control.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Control",]
points(control.ttrend$Culture, control.ttrend$OD, pch=16, col = "black", cex=2)
lm.control.ttrend <- lm(OD ~ Culture, data = control.ttrend)
lines(control.ttrend$Culture, predict(lm.control.ttrend), lwd=3, lty = 3, col = "black")

legend(3.5,.6, pch = 16, col = c("black", "navyblue", "blue"),
       legend=c("LB Control","6% Glucose", "16% Glucose"), cex = 1, pt.cex = 2)
```
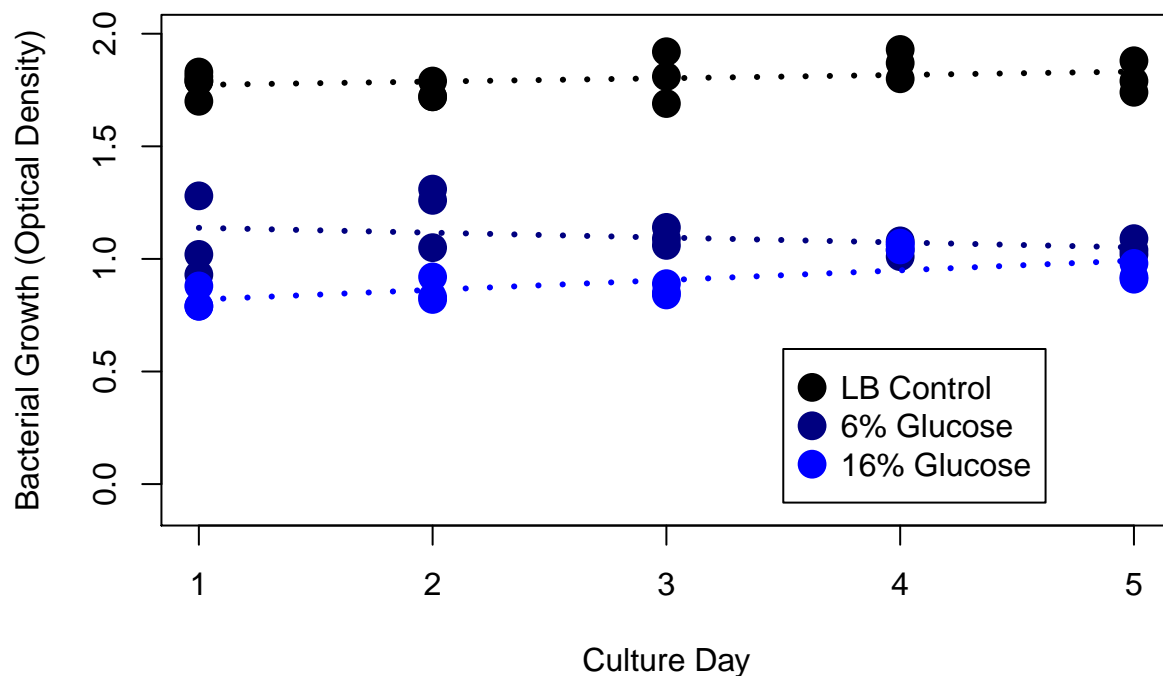

Day 1-5 Analysis (but with control on all 13 days and no L180)

```
alldata <- read.csv("Data/RawData.csv")
c13 <- alldata[alldata$Trt == "Control" & alldata$Solvent == "LB" & alldata$Day < 14,]
c13$OD.Hrs <- 24
c13[c13$Conc ==0 ,"predicted.osm"] <- 420
c13$Strain <- "Control"
```

```r
c13$StrCon <- "Control"
c13$Trt <- c13$Trt[,drop=T]
honeyGlu.d5c13 <- honeyGlu.d5
honeyGlu.d5c13 <- honeyGlu.d5c13[honeyGlu.d5c13$Trt != "Control",]
honeyGlu.d5c13 <- rbind(honeyGlu.d5c13, c13)
honeyGlu.d5c13 <- honeyGlu.d5c13[honeyGlu.d5c13$StrCon != "Local180",]

tapply(honeyGlu.d5c13$OD, list(honeyGlu.d5c13$StrCon, honeyGlu.d5c13$Day), mean)
```

```
##                  1        2         3        4         5         6         7         8
## Glu16           NA 0.820000 0.8566667 0.860000 1.056667 0.9366667        NA        NA
## Glu6            NA 1.076667 1.2066667 1.096667 1.043333 1.0500000        NA        NA
## Control      1.784 1.743333 1.8066667 1.866667 1.803333 1.7900000 1.166667 1.733333
## Local140        NA       NA        NA       NA       NA        NA        NA        NA
## Local160        NA       NA        NA       NA       NA        NA        NA 1.120000
## Local180        NA       NA        NA       NA       NA        NA        NA        NA
## Local210        NA       NA        NA       NA       NA 1.0100000 0.000000 0.940000
## Local260     0.848 0.000000        NA 0.816000 0.000000        NA        NA        NA
## Manuka50        NA       NA        NA       NA       NA        NA 1.315000 1.175000
## Manuka60        NA       NA        NA       NA       NA        NA 1.302000 1.156667
## Manuka70        NA       NA        NA       NA 1.288000 0.0000000        NA        NA
## Manuka80     1.136 0.000000 0.9220000 0.000000       NA        NA        NA        NA
##                  9        10       11       12       13
## Glu16           NA        NA       NA       NA       NA
## Glu6            NA        NA       NA       NA       NA
## Control   1.686667 1.7766667 1.696667 1.726667 1.706667
## Local140  1.165000 0.2933333 1.210000 1.093333 1.046667
## Local160  0.145000 1.1150000 1.150000 1.006667       NA
## Local180        NA        NA       NA       NA       NA
## Local210        NA        NA       NA       NA       NA
## Local260        NA        NA       NA       NA       NA
## Manuka50  1.165000 1.3650000 1.440000       NA       NA
## Manuka60  1.256667 1.3400000 1.316667       NA       NA
## Manuka70        NA        NA       NA       NA       NA
## Manuka80        NA        NA       NA       NA       NA
```

Combined Effect Analysis

Default: control as reference

```r
honeyGlu.d5c13$Day <- as.numeric(as.character(honeyGlu.d5c13$Day))
honeyGlu.d5c13$StrCon <- factor(honeyGlu.d5c13$StrCon)
honeyGlu.d5c13$StrCon <- relevel(honeyGlu.d5c13$StrCon, ref = "Control")
od.lm.control.5c13 <- lm(OD ~ Day + StrCon, data = honeyGlu.d5c13)
summary(od.lm.control.5c13)
```

```
##
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d5c13)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
```

```
## -1.71297 -0.11215  0.04316  0.17356  0.71773
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)     1.82868    0.10075  18.150  < 2e-16 ***
## Day            -0.01653    0.01239  -1.334 0.183943
## StrConGlu16    -0.85656    0.11504  -7.446 4.36e-12 ***
## StrConGlu6     -0.66789    0.11504  -5.806 3.00e-08 ***
## StrConLocal140 -0.71738    0.12849  -5.583 8.99e-08 ***
## StrConLocal160 -0.74401    0.13110  -5.675 5.73e-08 ***
## StrConLocal210 -1.11013    0.11608  -9.563  < 2e-16 ***
## StrConLocal260 -1.36309    0.10956 -12.442  < 2e-16 ***
## StrConManuka50 -0.38791    0.13171  -2.945 0.003673 **
## StrConManuka60 -0.40615    0.10824  -3.752 0.000239 ***
## StrConManuka70 -1.09376    0.12948  -8.447 1.18e-14 ***
## StrConManuka80 -1.27285    0.11230 -11.335  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3647 on 173 degrees of freedom
## Multiple R-squared:  0.6267, Adjusted R-squared:  0.603
## F-statistic:  26.4 on 11 and 173 DF,  p-value: < 2.2e-16
```

Re-leveling the data with Glu6 as reference

```
honeyGlu.d5c13$StrCon <- relevel(honeyGlu.d5c13$StrCon, ref = "Glu6")
od.lm.Glu6.5c13 <- lm(OD ~ Day + StrCon, data = honeyGlu.d5c13)
summary(od.lm.Glu6.5c13)
```

```
##
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d5c13)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.71297 -0.11215  0.04316  0.17356  0.71773
##
## Coefficients:
##                Estimate Std. Error t value Pr(>|t|)
## (Intercept)     1.16079    0.10641  10.909  < 2e-16 ***
## Day            -0.01653    0.01239  -1.334  0.18394
## StrConControl   0.66789    0.11504   5.806 3.00e-08 ***
## StrConGlu16    -0.18867    0.13316  -1.417  0.15832
## StrConLocal140 -0.04949    0.16417  -0.301  0.76345
## StrConLocal160 -0.07611    0.16377  -0.465  0.64269
## StrConLocal210 -0.44223    0.14261  -3.101  0.00225 **
## StrConLocal260 -0.69520    0.12517  -5.554 1.04e-07 ***
## StrConManuka50  0.27999    0.16125   1.736  0.08428 .
## StrConManuka60  0.26174    0.14203   1.843  0.06707 .
## StrConManuka70 -0.42587    0.15003  -2.839  0.00507 **
## StrConManuka80 -0.60496    0.12594  -4.804 3.36e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 0.3647 on 173 degrees of freedom
## Multiple R-squared:  0.6267, Adjusted R-squared:  0.603
## F-statistic:  26.4 on 11 and 173 DF,  p-value: < 2.2e-16
```

Re-leveling the data with Glu16 as reference

```
honeyGlu.d5c13$StrCon <- relevel(honeyGlu.d5c13$StrCon, ref = "Glu16")
od.lm.Glu16.5c13 <- lm(OD ~ Day + StrCon, data = honeyGlu.d5c13)
summary(od.lm.Glu16.5c13)
```

```
##
## Call:
## lm(formula = OD ~ Day + StrCon, data = honeyGlu.d5c13)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.71297 -0.11215  0.04316  0.17356  0.71773
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.97212    0.10641   9.136  < 2e-16 ***
## Day           -0.01653    0.01239  -1.334  0.18394
## StrConGlu6     0.18867    0.13316   1.417  0.15832
## StrConControl  0.85656    0.11504   7.446 4.36e-12 ***
## StrConLocal140 0.13918    0.16417   0.848  0.39774
## StrConLocal160 0.11255    0.16377   0.687  0.49285
## StrConLocal210 -0.25357   0.14261  -1.778  0.07716 .
## StrConLocal260 -0.50653   0.12517  -4.047 7.82e-05 ***
## StrConManuka50 0.46865    0.16125   2.906  0.00414 **
## StrConManuka60 0.45041    0.14203   3.171  0.00180 **
## StrConManuka70 -0.23720   0.15003  -1.581  0.11569
## StrConManuka80 -0.41630   0.12594  -3.306  0.00115 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.3647 on 173 degrees of freedom
## Multiple R-squared:  0.6267, Adjusted R-squared:  0.603
## F-statistic:  26.4 on 11 and 173 DF,  p-value: < 2.2e-16
```

Separating the effect of osmotic strength and antimicrobials d5

```
honeyGlu.d5c13.no.control <- honeyGlu.d5c13[honeyGlu.d5c13$Trt != 'Control',]
lm.osm.trt.d5c13<- lm(OD ~ Day + Trt + predicted.osm, data = honeyGlu.d5c13.no.control)
summary(lm.osm.trt.d5c13)
```

```
##
## Call:
## lm(formula = OD ~ Day + Trt + predicted.osm, data = honeyGlu.d5c13.no.control)
##
## Residuals:
```

```
##       Min       1Q   Median       3Q      Max
## -0.91515 -0.18079  0.09394  0.29401  0.62741
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## (Intercept)    1.1188888  0.2657677   4.210 4.56e-05 ***
## Day            0.0560467  0.0132498   4.230 4.22e-05 ***
## TrtLocal      -0.4512176  0.1147447  -3.932 0.000132 ***
## TrtManuka     -0.3391024  0.1180448  -2.873 0.004709 **
## predicted.osm -0.0002985  0.0001939  -1.540 0.125935
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.417 on 139 degrees of freedom
## Multiple R-squared:  0.2741, Adjusted R-squared:  0.2532
## F-statistic: 13.12 on 4 and 139 DF,  p-value: 4.309e-09
```

Collected osmolality measures in lab... updated analysis

Inputing osmolality measures into data

```
newD5.osm <- honeyGlu.d5c13.no.control
newD5.osm$predicted.osm <- NULL
newD5.osm[newD5.osm$StrCon == "Manuka50" ,"osm"] <- 605
newD5.osm[newD5.osm$StrCon == "Manuka60" ,"osm"] <- 671
newD5.osm[newD5.osm$StrCon == "Manuka70" ,"osm"] <- 699.67
newD5.osm[newD5.osm$StrCon == "Manuka80" ,"osm"] <- 765.33
newD5.osm[newD5.osm$StrCon == "Local140" ,"osm"] <- 992.67
newD5.osm[newD5.osm$StrCon == "Local160" ,"osm"] <- 1090
newD5.osm[newD5.osm$StrCon == "Local180" ,"osm"] <- 1167.33
newD5.osm[newD5.osm$StrCon == "Local210" ,"osm"] <- 1323.33
newD5.osm[newD5.osm$StrCon == "Local260" ,"osm"] <- 1577.67
newD5.osm[newD5.osm$StrCon == "Glu6" ,"osm"] <- 802
newD5.osm[newD5.osm$StrCon == "Glu16" ,"osm"] <- 1494.5997


newD1.osm <- honeyGlu.d1.no.control
newD1.osm$predicted.osm <- NULL
newD1.osm[newD1.osm$StrCon == "Manuka50" ,"osm"] <- 605
newD1.osm[newD1.osm$StrCon == "Manuka60" ,"osm"] <- 671
newD1.osm[newD1.osm$StrCon == "Manuka70" ,"osm"] <- 699.67
newD1.osm[newD1.osm$StrCon == "Manuka80" ,"osm"] <- 765.33
newD1.osm[newD1.osm$StrCon == "Local140" ,"osm"] <- 992.67
newD1.osm[newD1.osm$StrCon == "Local160" ,"osm"] <- 1090
newD1.osm[newD1.osm$StrCon == "Local180" ,"osm"] <- 1167.33
newD1.osm[newD1.osm$StrCon == "Local210" ,"osm"] <- 1323.33
newD1.osm[newD1.osm$StrCon == "Local260" ,"osm"] <- 1577.67
newD1.osm[newD1.osm$StrCon == "Glu6" ,"osm"] <- 802
newD1.osm[newD1.osm$StrCon == "Glu16" ,"osm"] <- 1494.5997
```

(Updated) Separation of the effect of osmotic strength and antimicrobials d5

```r
lm.newosm.trt.d5 <- lm(OD ~ Day + Trt + osm, data = newD5.osm)
summary(lm.newosm.trt.d5)
```

```
##
## Call:
## lm(formula = OD ~ Day + Trt + osm, data = newD5.osm)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.91761 -0.17561  0.09174  0.29640  0.62860
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  1.1094038  0.2630851   4.217 4.44e-05 ***
## Day          0.0553233  0.0135622   4.079 7.58e-05 ***
## TrtLocal    -0.4594371  0.1125830  -4.081 7.53e-05 ***
## TrtManuka   -0.3413733  0.1194571  -2.858  0.00492 **
## osm         -0.0002877  0.0001894  -1.519  0.13103
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4171 on 139 degrees of freedom
## Multiple R-squared:  0.2737, Adjusted R-squared:  0.2528
## F-statistic:  13.1 on 4 and 139 DF,  p-value: 4.439e-09
```

(Updated) Separation of the effect of osmotic strength and antimicrobials d1

```r
lm.newosm.trt.d1 <- lm(OD ~ Day + Trt + osm, data = newD1.osm)
summary(lm.newosm.trt.d1)
```

```
##
## Call:
## lm(formula = OD ~ Day + Trt + osm, data = newD1.osm)
##
## Residuals:
##        Min        1Q    Median        3Q       Max
## -0.300830 -0.048042  0.000058  0.075335  0.197397
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)  1.3622929  0.1444425   9.431 2.05e-12 ***
## Day          0.0196236  0.0082406   2.381 0.021353 *
## TrtLocal     0.0264444  0.0702943   0.376 0.708465
## TrtManuka    0.0117241  0.0650415   0.180 0.857727
## osm         -0.0003947  0.0001097  -3.599 0.000766 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
```

```
## Residual standard error: 0.1134 on 47 degrees of freedom
## Multiple R-squared:  0.6728, Adjusted R-squared:  0.645
## F-statistic: 24.17 on 4 and 47 DF,  p-value: 6.641e-11
```

Updated Time Trends

```r
#tiff("./Final\ Figures/Fig.3.300.tiff", units = "in", width = 12, height = 3, res = 300)
par(mfrow = c(1,2))

Manuka.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Manuka",]
M80 <- Manuka.ttrend[Manuka.ttrend$Conc == 80,]
M70 <- Manuka.ttrend[Manuka.ttrend$Conc == 70,]
M60 <- Manuka.ttrend[Manuka.ttrend$Conc == 60,]
M50 <- Manuka.ttrend[Manuka.ttrend$Conc == 50,]

plot(M80$Culture, M80$OD, ylim=c(-0.1,2), xlim = c(1,5), pch=16, col = "darkorange4",cex = 1.5, ylab = e
minor.tick(nx = 0, ny=4, tick.ratio=0.75)
M80.trend <- lm(OD ~ Culture, data = M80)
lines(M80$Culture, predict(M80.trend), lwd=2, lty=2, col ="darkorange4")

points(M70$Culture, M70$OD, pch=16, col = "darkorange", cex=1.5)
M70.trend <- lm(OD ~ Culture, data = M70)
lines(M70$Culture, predict(M70.trend), lwd=2, lty=2, col = "darkorange")

points(M60$Culture, M60$OD, pch=16, col = "firebrick2", cex=1.5)
M60.trend <- lm(OD ~ Culture, data = M60)
lines(M60$Culture, predict(M60.trend), lwd=2, lty=2, col = "firebrick2")

points(M50$Culture, M50$OD, pch=16, col = "goldenrod2", cex=1.5)
M50.trend <- lm(OD ~ Culture, data = M50)
lines(M50$Culture, predict(M50.trend), lwd=2, lty=2, col = "goldenrod2")

control.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Control",]
points(control.ttrend$Culture, control.ttrend$OD, pch=16, col = "black", cex=1.5)
lm.control.ttrend <- lm(OD ~ Culture, data = control.ttrend)
lines(control.ttrend$Culture, predict(lm.control.ttrend), lwd=2, lty = 2, col = "black")

symbols(x=2, y=0, circles=c(0.09), add=T, inches=F, fg = "darkorange4", lwd = 3)

legend(4,.7, pch = 16, col = c("black", "darkorange4","darkorange", "firebrick2", "goldenrod2"),
       legend=c("LB Control", "80 mg/mL", "70 mg/mL", "60 mg/mL", "50 mg/mL"), cex = 1, pt.cex = 1.5)


#Local
local.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Local",]
L260 <- local.ttrend[local.ttrend$Conc == 260 & local.ttrend$Comments == "",]
L210 <- local.ttrend[local.ttrend$Conc == 210 & local.ttrend$Comments == "",]
L160 <- local.ttrend[local.ttrend$Conc == 160 & local.ttrend$Comments == "",]
L140 <- local.ttrend[local.ttrend$Conc == 140 & local.ttrend$Comments == "",]

plot(L260$Culture, L260$OD, ylim=c(-0.1,2), xlim = c(1,5), pch=16, col = "khaki4",cex = 1.5, ylab = exp
minor.tick(nx = 0, ny=4, tick.ratio=0.75)
```

```
L260.trend <- lm(OD ~ Culture, data = L260)
lines(L260$Culture, predict(L260.trend), lwd=2, lty = 2, col ="khaki4")

points(L210$Culture, L210$OD, pch=16, col = "khaki", cex=1.5)
L210.trend <- lm(OD ~ Culture, data = L210)
lines(L210$Culture, predict(L210.trend), lwd=2, lty = 2, col ="khaki")

points(L160$Culture, L160$OD, pch=16, col = "lightseagreen", cex=1.5)
L160.avg <- tapply(L160$OD, L160$Culture, mean)
lines(c(1:5), L160.avg, lwd=2, lty=2, col = "lightseagreen")

points(L140$Culture, L140$OD, pch=16, col = "darkgreen", cex=1.5)
L140.avg <- tapply(L140$OD, L140$Culture, mean)
lines(c(1:5), L140.avg, lwd=2, lty=2, col = "darkgreen")

symbols(x=2, y=0, circles=c(0.09), add=T, inches=F, fg = "khaki", lwd = 3)

legend(4,.7, pch = 16, col = c("black", "khaki4", "khaki", "lightseagreen", "darkgreen"),
       legend=c("LB Control", "260 mg/mL", "210 mg/mL", "160 mg/mL", "140 mg/mL"), cex = 1, pt.cex = 1.5

control.ttrend <- honeyGlu.d5.no.48[honeyGlu.d5.no.48$Trt == "Control",]
points(control.ttrend$Culture, control.ttrend$OD, pch=16, col = "black", cex=1.5)
lm.control.ttrend <- lm(OD ~ Culture, data = control.ttrend)
lines(control.ttrend$Culture, predict(lm.control.ttrend), lwd=2, lty = 2, col = "black")
```


(a) Manuka Honey

(b) Raw Pasture Honey