#Supplementary information to: Effects of anthropogenic sounds on the behaviour and physiology of the Eastern oyster (Crassostrea virginica)

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#### Get packages ####

library(lmerTest)

library(lme4)

library(Matrix)

library(car)

library(rcompanion)

library(multcomp)

library(ggthemr)

library(ggplot2)

library(ggpubr)

#### Upload dataset for analysis (Noise&Oystersdata.csv) ####

dataset <-read.table("/Users/tam/Desktop/Noise&Oystersdata.csv", header = T,sep = ",",dec = ";")

#Note that this entry will be different when you upload the dataset from your computer

#See dataset information below

#Exp: # of trial

#ValvoID: Individual oyster identification during a trial

#Treatment: 1-Control 2-Boat 3-Drilling 4-Pile Driving

#Relative: data for the relative change in valve gaping response

#Totdist: data for the total distance moved response

#Glycogen: data for the glycogen content

#TG: data for triglycerides content

# variable modifications

dataset$Exp<-as.factor(dataset$Exp)

dataset$Treatment<-as.factor(dataset$Treatment)

dataset$Relative<-as.numeric(dataset$Relative)

dataset$Totdist<-as.numeric(dataset$Totdist)

dataset$Glycogen<-as.numeric(dataset$Glycogen)

dataset$TG<-as.numeric(dataset$TG)

#### Relative change in valve gaping ####

#build LMER model

modRelative<-lmer(Relative~Treatment+(1|Exp),data=dataset)

#Assumptions

plot(modRelative)

#Homogeneity of variance

leveneTest(Relative~Treatment,data=dataset)

#Normality

shapiro.test(dataset$Relative)

#Run ANOVA on model to test for the effect of treatment (type = 3 due to random effect in model)

Anova(modRelative, type = 3)

#p = 0.0150 \* significant difference between treatments

#Tukey HSD post hoc comparisons to determine pairwise treatment differences

summary(glht(modRelative,linfct=mcp(Treatment="Tukey")))

#4 - 1, p = 0.00858 \*\* significant difference between Control and Pile driving

#### Total distance moved ####

#build LMER model

modtotdistance<-lmer(Totdist~Treatment+(1|Exp),data=dataset)

#Assumptions

plot(modtotdistance)

#Homogeneity of variance

leveneTest(Totdist~Treatment,data=dataset)

#Normality

shapiro.test(dataset$Totdist)

#Run ANOVA on model to test for the effect of treatment (type = 3 due to random effect in model)

Anova(modtotdistance, type = 3)

# p = 0.2385, no significant difference between treatment

#### Glycogen content ####

#build LMER model

modGlycogen<-lmer(Glycogen~Treatment+(1|Exp),data=dataset)

#Assumptions

plot(modGlycogen)

#Homogeneity of variance

leveneTest(Glycogen~Treatment,data=dataset)

#Normality

shapiro.test(dataset$Glycogen)

#log transformation for glycogen content variable

l\_Glycogen<-log(dataset$Glycogen)

#build LMER model with the log glycogen content variable

modGlycogenlog<-lmer(l\_Glycogen~Treatment+(1|Exp),data=dataset)

#Run ANOVA on model to test for the effect of treatment (type = 3 due to random effect in model)

Anova(modGlycogenlog, type = 3)

#p = 0.009752 \*\* significant difference between treatments

#Tukey HSD post hoc comparisons to determine pairwise treatment differences

summary(glht(modGlycogenlog,linfct=mcp(Treatment="Tukey")))

#4 - 1, p = 0.00651 \*\* significant difference between Control and Pile driving

#### Triglycerides content ####

#build LMER model

modTG<-lmer(TG~Treatment+(1|Exp),data=dataset)

#Assumptions

plot(modTG)

#Homogeneity of variance

leveneTest(TG~Treatment,data=dataset)

#Normality

shapiro.test(dataset$TG)

#Run ANOVA on model to test for the effect of treatment (type = 3 due to random effect in model)

Anova(modTG, type = 3)

# p = 0.9308, no significant difference between treatment

#### Create boxplots to visualize the data ####

#Relative change in valve opening

bpRelative <- ggplot(dataset, aes(x=Treatment, y=Relative, fill=Treatment)) +

geom\_boxplot(color="black", outlier.shape = NA) +geom\_jitter(position=position\_jitter(0.2), cex=2.8, color="black") + theme(legend.position = "none") + scale\_fill\_brewer(palette="Blues")

bpRelative

#Total distance moved

bptotdistance <- ggplot(dataset, aes(x=Treatment, y=Totdist, fill=Treatment)) +

geom\_boxplot(color="black", outlier.shape = NA) +geom\_jitter(position=position\_jitter(0.2), cex=2.8, color="black") + theme(legend.position = "none") + scale\_fill\_brewer(palette="Blues")

bptotdistance

#Build Figure 3 (side by side)

figure3 <- ggarrange(bpRelative,bptotdistance, ncol = 2, nrow = 1)

figure3

#Glycogen content

bpGlycogen <- ggplot(dataset, aes(x=Treatment, y=Glycogen, fill=Treatment)) +

geom\_boxplot(color="black", outlier.shape = NA) +geom\_jitter(position=position\_jitter(0.2), cex=2.8, color="black") + theme(legend.position = "none") + scale\_fill\_brewer(palette="Blues")

bpGlycogen

#Triglycerides content

bpTG <- ggplot(dataset, aes(x=Treatment, y=TG, fill=Treatment)) +

geom\_boxplot(color="black", outlier.shape = NA) +geom\_jitter(position=position\_jitter(0.2), cex=2.8, color="black") + theme(legend.position = "none") + scale\_fill\_brewer(palette="Blues")

bpTG

#Build Figure 4 (side by side)

figure4 <- ggarrange(bpGlycogen,bpTG, ncol = 2, nrow = 1)

figure4

#### LINEAR REGRESSION (relation between physiological energetics and valve gaping responses) ####

#Relative change in valve gaping

#with Glycogen content

#build LM model

modLRG.R <- lm(Glycogen~Relative, data=dataset)

summary(modLRG.R)

#with triglycerides

#build LM model

modLRT.R <- lm(TG~Relative, data=dataset)

summary(modLRT.R)

#Total distance moved

#with Glycogen content

#build LM model

modLRG.Tot <- lm(Glycogen~Totdist, data=dataset)

summary(modLRG.Tot)

#with triglycerides

#build LM model

modLRT.Tot <- lm(TG~Totdist, data=dataset)

summary(modLRT.Tot)

#no relationships between physiological energetics and valve gaping responses