Supplementary Material S4: R code

Experimental heatwaves negatively impact sperm quality in the zebra finch

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R Markdown

This is an R Markdown output. It includes all the R code to generate the main results in the paper by Hurley et al. on the impact of experimental heatwaves on zebra finch sperm quality.

Main data file for all data analysis

```
dat <- read.csv("Heatwave/Data_Analysis”)
```

Recovery Comparison Analysis

Data preperation for looking at recovery

```
#Proportion Motile transfomation
dat$propMotile <- (dat$Motile + 1)/(dat$NumberSperm + 1)
dat$logit.propMotile <- log(dat$propMotile/(1-dat$propMotile))

#Proportion Normal Transfomation
dat$propNormal <- (dat$CountNormal)/(dat$TotalCount)
dat$logit.propNormal <- log(dat$propNormal/(1-dat$propNormal))

#Subsetting data to only the 23ºC samples
dat2 <- dat[dat$SamplingTemp<=24,]
```

Proportion Normal (transformed) recovery comparisons

```
#Remove NAs
dat2Prop <- dat2 %>% drop_na(PropNormal)

#Comparisons of birds that went from 30ºC in period 1 to 40ºC in period
2
datA <- dat2Prop[dat2Prop$Part=="P1"&dat2Prop$Timept=="Day01"&dat2Prop$
Order=="LH",]
datB <- dat2Prop[dat2Prop$Part=="P1"&dat2Prop$Timept=="Day26"&dat2Prop$
```

```
Order=="LH",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datA$logit.propNormal, datB$logit.propNormal, paired = TRUE )

datC <- dat2Prop[dat2Prop$Part=="P2"&dat2Prop$Timept=="Day01"&dat2Prop$
Order=="LH",]
datD <- dat2Prop[dat2Prop$Part=="P2"&dat2Prop$Timept=="Day21"&dat2Prop$
Order=="LH",]
datE <- dat2Prop[dat2Prop$Part=="P2"&dat2Prop$Timept=="Day26"&dat2Prop$
Order=="LH",]

#Period2 Day01-Day21
t.test(datC$logit.propNormal, datD$logit.propNormal, paired = TRUE )
#Period2 Day01-Day26
t.test(datC$logit.propNormal, datE$logit.propNormal, paired = TRUE )
#Period2 Day21-Day26
t.test(datD$logit.propNormal, datE$logit.propNormal, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datA$logit.propNormal, datE$logit.propNormal, paired = TRUE )

#Comparisons of birds that went from 40ºC in period 1 to 30ºC in period
2
datF <- dat2Prop[dat2Prop$Part=="P1"&dat2Prop$Timept=="Day01"&dat2Prop$
Order=="HL",]
datG <- dat2Prop[dat2Prop$Part=="P1"&dat2Prop$Timept=="Day26"&dat2Prop$
Order=="HL",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datF$logit.propNormal, datG$logit.propNormal, paired = TRUE )

datH <- dat2Prop[dat2Prop$Part=="P2"&dat2Prop$Timept=="Day01"&dat2Prop$
Order=="HL",]
datI <- dat2Prop[dat2Prop$Part=="P2"&dat2Prop$Timept=="Day21"&dat2Prop$
Order=="HL",]
datJ <- dat2Prop[dat2Prop$Part=="P2"&dat2Prop$Timept=="Day26"&dat2Prop$
Order=="HL",]

#Period2 Day01-Day21
t.test(datH$logit.propNormal, datI$logit.propNormal, paired = TRUE )
#Period2 Day01-Day26
t.test(datH$logit.propNormal, datJ$logit.propNormal, paired = TRUE )
#Period2 Day21-Day26
t.test(datI$logit.propNormal, datJ$logit.propNormal, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datF$logit.propNormal, datJ$logit.propNormal, paired = TRUE )
```

Sperm swimming speed (VCL) recovery comparisons

```
More10 <- dat2[dat2$Motile>=10,] #for VCL
dat2VCL <- More10 %>% drop_na(VCL)

##Comparisons of birds that went from 30ºC in period 1 to 40ºC in perio
d 2
datAv <- dat2VCL[dat2VCL$Part=="P1"&dat2VCL$Timept=="Day01"&dat2VCL$Ord
er=="LH",]
datBv <- dat2VCL[dat2VCL$Part=="P1"&dat2VCL$Timept=="Day26"&dat2VCL$Ord
er=="LH",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datA$VCL, datB$VCL, paired = TRUE )

datCv <- dat2VCL[dat2VCL$Part=="P2"&dat2VCL$Timept=="Day01"&dat2VCL$Ord
er=="LH",]
datDv <- dat2VCL[dat2VCL$Part=="P2"&dat2VCL$Timept=="Day21"&dat2VCL$Ord
er=="LH",]
datEv <- dat2VCL[dat2VCL$Part=="P2"&dat2VCL$Timept=="Day26"&dat2VCL$Ord
er=="LH",]

#Period2 Day01-Day21
t.test(datCv$VCL, datDv$VCL, paired = TRUE )
#Period2 Day01-Day26
t.test(datCv$VCL, datEv$VCL, paired = TRUE )
#Period2 Day21-Day26
t.test(datDv$VCL, datEv$VCL, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datA$VCL, datE$VCL, paired = TRUE )

##Comparisons of birds that went from 40ºC in period 1 to 30ºC in perio
d 2
datFv <- dat2VCL[dat2VCL$Part=="P1"&dat2VCL$Timept=="Day01"&dat2VCL$Ord
er=="HL",]
datGv <- dat2VCL[dat2VCL$Part=="P1"&dat2VCL$Timept=="Day26"&dat2VCL$Ord
er=="HL",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datFv$VCL, datGv$VCL, paired = TRUE )

datHv <- dat2VCL[dat2VCL$Part=="P2"&dat2VCL$Timept=="Day01"&dat2VCL$Ord
er=="HL",]
datIv <- dat2VCL[dat2VCL$Part=="P2"&dat2VCL$Timept=="Day21"&dat2VCL$Ord
er=="HL",]
datJv <- dat2VCL[dat2VCL$Part=="P2"&dat2VCL$Timept=="Day26"&dat2VCL$Ord
er=="HL",]

#Period2 Day01-Day21
t.test(datHv$VCL, datIv$VCL, paired = TRUE )
```

```
#Period2 Day01-Day26
t.test(datHv$VCL, datJv$VCL, paired = TRUE )
#Period2 Day21-Day26
t.test(datIv$VCL, datJv$VCL, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datFv$VCL, datJv$VCL, paired = TRUE )
```

Proportion Motile (transformed) recovery comparisons

```
#Remove NAs
dat2Motile <- dat2 %>% drop_na(PropMotile)

##Comparisons of birds that went from 30ºC in period 1 to 40ºC in perio
d 2
datAm <- dat2Motile[dat2Motile$Part=="P1"&dat2Motile$Timept=="Day01"&da
t2Motile$Order=="LH",]
datBm <- dat2Motile[dat2Motile$Part=="P1"&dat2Motile$Timept=="Day26"&da
t2Motile$Order=="LH",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datAm$logit.propMotile, datBm$logit.propMotile, paired = TRUE )

datCm <- dat2Motile[dat2Motile$Part=="P2"&dat2Motile$Timept=="Day01"&da
t2Motile$Order=="LH",]
datDm <- dat2Motile[dat2Motile$Part=="P2"&dat2Motile$Timept=="Day21"&da
t2Motile$Order=="LH",]
datEm <- dat2Motile[dat2Motile$Part=="P2"&dat2Motile$Timept=="Day26"&da
t2Motile$Order=="LH",]

#Period2 Day01-Day21
t.test(datCm$logit.propMotile, datDm$logit.propMotile, paired = TRUE )
#Period2 Day01-Day26
t.test(datCm$logit.propMotile, datEm$logit.propMotile, paired = TRUE )
#Period2 Day21-Day26
t.test(datDm$logit.propMotile, datEm$logit.propMotile, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datAm$logit.propMotile, datEm$logit.propMotile, paired = TRUE )

##Comparisons of birds that went from 40ºC in period 1 to 30ºC in perio
d 2
datFm <- dat2Motile[dat2Motile$Part=="P1"&dat2Motile$Timept=="Day01"&da
t2Motile$Order=="HL",]
datGm <- dat2Motile[dat2Motile$Part=="P1"&dat2Motile$Timept=="Day26"&da
t2Motile$Order=="HL",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datFm$logit.propMotile, datGm$logit.propMotile, paired = TRUE )

datHm <- dat2Motile[dat2Motile$Part=="P2"&dat2Motile$Timept=="Day01"&da
```

```
t2Motile$Order=="HL",]
datIm <- dat2Motile[dat2Motile$Part=="P2"&dat2Motile$Timept=="Day21"&da
t2Motile$Order=="HL",]
datJm <- dat2Motile[dat2Motile$Part=="P2"&dat2Motile$Timept=="Day26"&da
t2Motile$Order=="HL",]

#Period2 Day01-Day21
t.test(datHm$logit.propMotile, datIm$logit.propMotile, paired = TRUE )
#Period2 Day01-Day26
t.test(datHm$logit.propMotile, datJm$logit.propMotile, paired = TRUE )
#Period2 Day21-Day26
t.test(datIm$logit.propMotile, datJm$logit.propMotile, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datFm$logit.propMotile, datJm$logit.propMotile, paired = TRUE )
```

Cloacal temperature recovery comparisons

```
##Comparisons of birds that went from 30ºC in period 1 to 40ºC in perio
d 2
datAc <- dat2[dat2$Part=="P1"&dat2$Timept=="Day01"&dat2$Order=="LH",]
datBc <- dat2[dat2$Part=="P1"&dat2$Timept=="Day26"&dat2$Order=="LH",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datAc$CloacalTemp, datBc$CloacalTemp, paired = TRUE )

datCc <- dat2[dat2$Part=="P2"&dat2$Timept=="Day01"&dat2$Order=="LH",]
datDc <- dat2[dat2$Part=="P2"&dat2$Timept=="Day21"&dat2$Order=="LH",]
datEc <- dat2[dat2$Part=="P2"&dat2$Timept=="Day26"&dat2$Order=="LH",]

#Period2 Day01-Day21
t.test(datCc$CloacalTemp, datDc$CloacalTemp, paired = TRUE )
#Period2 Day01-Day26
t.test(datCc$CloacalTemp, datEc$CloacalTemp, paired = TRUE )
#Period2 Day21-Day26
t.test(datDc$CloacalTemp, datEc$CloacalTemp, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datAc$CloacalTemp, datEc$CloacalTemp, paired = TRUE )

##Comparisons of birds that went from 40ºC in period 1 to 30ºC in perio
d 2
datFc <- dat2[dat2$Part=="P1"&dat2$Timept=="Day01"&dat2$Order=="HL",]
datGc <- dat2[dat2$Part=="P1"&dat2$Timept=="Day26"&dat2$Order=="HL",]

#Period1 Day01-Day26 (also Period 1-Period2 Day01)
t.test(datFc$CloacalTemp, datGc$CloacalTemp, paired = TRUE )

datHc <- dat2[dat2$Part=="P2"&dat2$Timept=="Day01"&dat2$Order=="HL",]
datIc <- dat2[dat2$Part=="P2"&dat2$Timept=="Day21"&dat2$Order=="HL",]
datJc <- dat2[dat2$Part=="P2"&dat2$Timept=="Day26"&dat2$Order=="HL",]
```

```
#Period2 Day01-Day21
t.test(datHc$CloacalTemp, datIc$CloacalTemp, paired = TRUE )
#Period2 Day01-Day26
t.test(datHc$CloacalTemp, datJc$CloacalTemp, paired = TRUE )
#Period2 Day21-Day26
t.test(datIc$CloacalTemp, datJc$CloacalTemp, paired = TRUE )
#Period1 Day01-Period2 Day26
t.test(datFc$CloacalTemp, datJc$CloacalTemp, paired = TRUE )
```

Comparisons between groups at start of experiment

```
#Proportion Normal
t.test(datA$logit.propNormal, datF$logit.propNormal, paired = TRUE )
#Sperm swimming speed (VCL)
t.test(datAv$VCL, datFv$VCL, paired = TRUE )
#Proportion Motile
t.test(datAm$logit.propMotile, datFm$logit.propMotile, paired = TRUE )
#Cloacal Temperature
t.test(datAc$CloacalTemp, datFc$CloacalTemp, paired = TRUE )
#Body Conditon (BCI)
t.test(datAc$BCI, datFc$BCI, paired = TRUE )
```

Change Data Analysis

Data preparation looking at the change in trait value (i.e. value at dayX – value at day 1, where X is day 3, 7, 11, or 14)

```
library(lme4)
library(lsmeans)
library(tidyr)
library(MuMIn)
#remove all the 23ºC samples from dataframe
dat3 <- dat[dat$SamplingTemp>=24,]
#Drop lines with < 10 sperm swimming: for use with CngVCL only
dat4 <- dat3[dat3$Motile>=10,]
#remove NAs just form CngVCL column
dat5 <- dat4 %>% drop_na(CngVCL)
#removes NAs just from CngPropNormal
dat6 <- dat3 %>% drop_na(CngPropNormal)
#gets rid of row with INF issue in last case that causes issue w logit
transformation
dat7 <- dat[-nrow(dat),]
#removes NAs just from CngPropMotile
dat8 <- dat3 %>% drop_na(CngPropMotile)
```

Change model proportion normal

```
CngPropNormal<-lm(CngPropNormal ~SamplingTempF*Part*TimePtN+BCI, data=d
at6)
CngPropNormal1<-lmer(CngPropNormal ~SamplingTempF*Part*TimePtN+BCI+(1|B
andNumber), data=dat6, REML=FALSE) #Use this one
CngPropNormal2<-lmer(CngPropNormal ~SamplingTempF*Part*TimePtN+BCI+(1|B
andNumber)+(1|Cage)+(1|Mom), data=dat6, REML=FALSE)
anova(CngPropNormal1,CngPropNormal) #bandnumber - no random
anova(CngPropNormal2,CngPropNormal1) #all random - band number
summary(CngPropNormal1) #drop three way
AIC(CngPropNormal1)

CngPropNormal1drop1<-lmer(CngPropNormal~SamplingTempF*TimePtN+SamplingT
empF*Part+Part*TimePtN+BCI+(1|BandNumber), data=dat6, REML=FALSE)
anova(CngPropNormal1drop1, CngPropNormal1)
summary(CngPropNormal1drop1) #all significant but BCI.
AIC(CngPropNormal1drop1)

CngPropNormalTRUE<-lmer(CngPropNormal~SamplingTempF*TimePtN+SamplingTem
pF*Part+Part*TimePtN+BCI+(1|BandNumber), data=dat6, REML=TRUE)
summary(CngPropNormalTRUE) #30-P1
AIC(CngPropNormalTRUE)
r.squaredGLMM(CngPropNormalTRUE)

samplingPN <- relevel(dat6$SamplingTempF, ref="40C")
CngPropNormalTRUEr1<-lmer(CngPropNormal~samplingPN*TimePtN+samplingPN*P
art+Part*TimePtN+BCI+(1|BandNumber), data=dat6, REML=TRUE)
summary(CngPropNormalTRUEr1) #40-P1

PartPN <- relevel(dat6$Part, ref="P2")
CngPropNormalTRUEr2<-lmer(CngPropNormal~SamplingTempF*TimePtN+SamplingT
empF*PartPN+PartPN*TimePtN+BCI+(1|BandNumber), data=dat6, REML=TRUE)
summary(CngPropNormalTRUEr2) #30-P2

CngPropNormalTRUEr3<-lmer(CngPropNormal~samplingPN*TimePtN+samplingPN*P
artPN+PartPN*TimePtN+BCI+(1|BandNumber), data=dat6, REML=TRUE)
summary(CngPropNormalTRUEr3) #40-P2


anova(CngPropNormalTRUE)
```

Change model sperm swimming speed (VCL)

```
CngVCL<-lm(CngVCL~SamplingTempF*Part*TimePtN+BCI, data=dat5)
CngVCL1<-lmer(CngVCL~SamplingTempF*Part*TimePtN+BCI+(1|BandNumber), dat
a=dat5, REML=FALSE) #Use this one
CngVCL2<-lmer(CngVCL~SamplingTempF*Part*TimePtN+BCI+(1|BandNumber)+(1|C
age)+(1|Mom), data=dat5, REML=FALSE)
```

```
anova(CngVCL1,CngVCL) #bandnumber - no random
anova(CngVCL2,CngVCL1) #all random - band number
summary(CngVCL1) #no sig interactions
AIC(CngVCL1)

CngVCL1drop1<-lmer(CngVCL~SamplingTempF*Part+SamplingTempF*TimePtN+Part
*TimePtN+BCI+(1|BandNumber), data=dat5, REML=FALSE) #dropping threeway
anova(CngVCL1drop1,CngVCL1)
summary(CngVCL1drop1) #drop Temp:Part
AIC(CngVCL1drop1)

CngVCL1drop2<-lmer(CngVCL~SamplingTempF*Part+Part*TimePtN+BCI+(1|BandNu
mber), data=dat5, REML=FALSE) #dropping threeway
anova(CngVCL1drop2, CngVCL1drop1)
summary(CngVCL1drop2) #drop Part:Time
AIC(CngVCL1drop2)

CngVCL1drop3<-lmer(CngVCL~SamplingTempF*Part+TimePtN+BCI+(1|BandNumber)
, data=dat5, REML=FALSE) #dropping threeway
anova(CngVCL1drop3,CngVCL1drop2)
summary(CngVCL1drop3) #drop Temp:Part
AIC(CngVCL1drop3)

CngVCL1drop4<-lmer(CngVCL~SamplingTempF+Part+TimePtN+BCI+(1|BandNumber)
, data=dat5, REML=FALSE)
anova(CngVCL1drop4, CngVCL1drop3)
summary(CngVCL1drop4)
AIC(CngVCL1drop4)

CngVCL1TRUE<-lmer(CngVCL~SamplingTempF+Part+TimePtN+BCI+(1|BandNumber),
data=dat5, REML=TRUE)
summary(CngVCL1TRUE) #30-P1
AIC(CngVCL1TRUE)
r.squaredGLMM(CngVCL1TRUE)

samplingVCL <- relevel(dat5$SamplingTempF, ref="40C")
CngVCL1TRUEr1<-lmer(CngVCL~samplingVCL+Part+TimePtN+BCI+(1|BandNumber),
data=dat5, REML=TRUE)
summary(CngVCL1TRUEr1) #40-P1

PartVCL <- relevel(dat5$Part, ref="P2")
CngVCL1TRUEr2<-lmer(CngVCL~SamplingTempF+PartVCL+TimePtN+BCI+(1|BandNum
ber), data=dat5, REML=TRUE)
summary(CngVCL1TRUEr2) #30-P2

CngVCL1TRUEr3<-lmer(CngVCL~samplingVCL+PartVCL+TimePtN+BCI+(1|BandNumbe
r), data=dat5, REML=TRUE)
summary(CngVCL1TRUEr3) #40-P2
```

```r
anova(CngVCL1TRUE)
```

Change model proportion motile

```r
CngPropMotile<-lm(CngPropMotile~SamplingTempF*Part*TimePtN+BCI, data=da
t8)
CngPropMotile1<-lmer(CngPropMotile~SamplingTempF*Part*TimePtN+BCI+(1|Ba
ndNumber), data=dat8, REML=F) #Use this one
CngPropMotile2<-lmer(CngPropMotile~SamplingTempF*Part*TimePtN+BCI+(1|Ba
ndNumber)+(1|Cage)+(1|Mom), data=dat8, REML=FALSE)
anova(CngPropMotile1,CngPropMotile) #bandnumber
anova(CngPropMotile2,CngPropMotile1) #all random - band number
summary(CngPropMotile1) #all non significant - drop three way
AIC(CngPropMotile1)

CngPropMotile1drop1<-lmer(CngPropMotile~SamplingTempF*Part+SamplingTemp
F*TimePtN+Part*TimePtN+BCI+(1|BandNumber), data=dat8, REML=FALSE)
anova(CngPropMotile1drop1, CngPropMotile1)
summary(CngPropMotile1drop1) #all non significant dropt part:time
AIC(CngPropMotile1drop1)

CngPropMotile1drop2<-lmer(CngPropMotile~SamplingTempF*Part+SamplingTemp
F*TimePtN+BCI+(1|BandNumber), data=dat8, REML=FALSE)
anova(CngPropMotile1drop2, CngPropMotile1drop1)
summary(CngPropMotile1drop2) #all non significant drop temp:part
AIC(CngPropMotile1drop2)

CngPropMotile1drop3<-lmer(CngPropMotile~SamplingTempF*TimePtN+Part+BCI+
(1|BandNumber), data=dat8, REML=FALSE)
anova(CngPropMotile1drop3,CngPropMotile1drop2)
summary(CngPropMotile1drop3) #all non significant drop temp:time
AIC(CngPropMotile1drop3)

CngPropMotile1drop4<-lmer(CngPropMotile~SamplingTempF+TimePtN+Part+BCI+
(1|BandNumber), data=dat8, REML=FALSE)
anova(CngPropMotile1drop4, CngPropMotile1drop3)
summary(CngPropMotile1drop4)
AIC(CngPropMotile1drop4)

CngPropMotile1TRUE<-lmer(CngPropMotile~SamplingTempF+TimePtN+Part+BCI+(
1|BandNumber), data=dat8, REML=TRUE)
summary(CngPropMotile1TRUE) #30-P1
AIC(CngPropMotile1TRUE)
r.squaredGLMM(CngPropMotile1TRUE)

samplingPM <- relevel(dat8$SamplingTempF, ref="40C")
CngPropMotile1TRUEr1<-lmer(CngPropMotile~samplingPM+TimePtN+Part+BCI+(1
|BandNumber), data=dat8, REML=TRUE)
```

```r
summary(CngPropMotile1TRUEr1) #40-P1

PartPM <- relevel(dat8$Part, ref="P2")
CngPropMotile1TRUEr2<-lmer(CngPropMotile~SamplingTempF+TimePtN+PartPM+B
CI+(1|BandNumber), data=dat8, REML=TRUE)
summary(CngPropMotile1TRUEr2) #30-P2

CngPropMotile1TRUEr1<-lmer(CngPropMotile~samplingPM+TimePtN+PartPM+BCI+
(1|BandNumber), data=dat8, REML=TRUE)
summary(CngPropMotile1TRUEr1) #40-P2

anova(CngPropMotile1TRUE)
```

Change model cloacal temperature

```r
CngCloacalTemp<-lm(CngCloacalTemp~SamplingTempF*Part*TimePtN+BCI, data=
dat3)
CngCloacalTemp1<-lmer(CngCloacalTemp~SamplingTempF*Part*TimePtN+BCI+(1|
BandNumber), data=dat3, REML=FALSE) #Use this one
CngCloacalTemp2<-lmer(CngCloacalTemp~SamplingTempF*Part*TimePtN+BCI+(1|
BandNumber)+(1|Cage)+(1|Mom), data=dat3, REML=FALSE)
anova(CngCloacalTemp1,CngCloacalTemp) #bandnumber - no random
anova(CngCloacalTemp2,CngCloacalTemp1) #all random - band number
summary(CngCloacalTemp1) #Temp:Part and Part:time sig, drop 3 way first
AIC(CngCloacalTemp1)

CngCloacalTemp1drop1<-lmer(CngCloacalTemp~SamplingTempF*Part+SamplingTe
mpF*TimePtN+Part*TimePtN+BCI+(1|BandNumber), data=dat3, REML=FALSE)
anova(CngCloacalTemp1drop1, CngCloacalTemp1)
summary(CngCloacalTemp1drop1) #drop Temp:time
AIC(CngCloacalTemp1drop1)

CngCloacalTemp1drop2<-lmer(CngCloacalTemp~SamplingTempF*Part+Part*TimeP
tN+BCI+(1|BandNumber), data=dat3, REML=FALSE)
anova(CngCloacalTemp1drop2, CngCloacalTemp1drop1)
summary(CngCloacalTemp1drop2) #interactions both significant
AIC(CngCloacalTemp1drop2)


CngCloacalTempTRUE<-lmer(CngCloacalTemp~SamplingTempF*Part+Part*TimePtN
+BCI+(1|BandNumber), data=dat3, REML=TRUE)
summary(CngCloacalTempTRUE) #30-P1
AIC(CngCloacalTempTRUE)
r.squaredGLMM(CngCloacalTempTRUE)

samplingCT <- relevel(dat3$SamplingTempF, ref="40C")
CngCloacalTempTRUEr1<-lmer(CngCloacalTemp~samplingCT*Part+Part*TimePtN+
BCI+(1|BandNumber), data=dat3, REML=TRUE)
summary(CngCloacalTempTRUEr1) #40-P1
```

```
PartCT <- relevel(dat3$Part, ref="P2")
CngCloacalTempTRUEr2<-lmer(CngCloacalTemp~SamplingTempF*PartCT+PartCT*TimePtN+BCI+(1|BandNumber), data=dat3, REML=TRUE)
summary(CngCloacalTempTRUEr2) #30-P2

CngCloacalTempTRUEr3<-lmer(CngCloacalTemp~samplingCT*PartCT+PartCT*TimePtN+BCI+(1|BandNumber), data=dat3, REML=TRUE)
summary(CngCloacalTempTRUEr3) #40-P2

anova(CngCloacalTempTRUE)
```

Figures Code

Library and code needed for all main and supplemental figures

```
library(ggplot2)

#code for calculating SE
summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
                      conf.interval=.95, .drop=TRUE) {
  library(plyr)

  # New version of length which can handle NA's: if na.rm==T, don't count them
  length2 <- function (x, na.rm=FALSE) {
    if (na.rm) sum(!is.na(x))
    else       length(x)
  }

  # This does the summary. For each group's data frame, return a vector with
  # N, mean, and sd
  datac <- ddply(data, groupvars, .drop=.drop,
                 .fun = function(xx, col) {
                   c(N    = length2(xx[[col]], na.rm=na.rm),
                     mean = mean   (xx[[col]], na.rm=na.rm),
                     sd   = sd     (xx[[col]], na.rm=na.rm)
                   )
                 },
                 measurevar
  )

  # Rename the "mean" column
  datac <- rename(datac, c("mean" = measurevar))

  datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean
```

```
  # Confidence interval multiplier for standard error
  # Calculate t-statistic for confidence interval:
  # e.g., if conf.interval is .95, use .975 (above/below), and use df=N
-1
  ciMult <- qt(conf.interval/2 + .5, datac$N-1)
  datac$ci <- datac$se * ciMult

  return(datac)
}
```

Main manuscript figures

Note: different data set needed

Figure 1. Proportion Normal

```
RawHeatwave <- read.csv("Heatwave/Data_Figures_Main”)

HeatwaveNormal <- RawHeatwave[-c(105,125,235),]

HNormal <- summarySE(HeatwaveNormal, measurevar="PropNormal", groupvars
=c("Order","TimeCode","RmTempF"),na.rm=TRUE) #Need to run code for summ
arySE for this to work!!
HNormalgg <- ggplot(HNormal, aes(x=TimeCode, y=PropNormal, group=Order)
)
pd <- position_dodge(0.2) # to jitter them .2 to the left and right

HNormalgg +
  theme_bw() + #gets rid of grey background / inverts grey and white.
  annotate("rect", xmin=0, xmax=1, ymin=0.5, ymax=0.92, fill="grey", al
pha=0.2,) + #add shaded bar1
  annotate("rect", xmin=5.2, xmax=7, ymin=0.5, ymax=0.92, fill="grey",
alpha=0.2) + #add shaded bar2
  annotate("rect", xmin=11.2, xmax=13.5, ymin=0.5, ymax=0.92, fill="gre
y", alpha=0.2) + #add shaded bar3
  geom_vline(xintercept=7, colour= "grey45", linetype="longdash") +
  geom_errorbar(data=HNormal[-c(6,19),], aes(ymin=PropNormal-se, ymax=P
ropNormal+se), width=.25,position=pd) + #se bars
  geom_line(aes(linetype=c(rep("dashed",13),rep("solid",13))), position
=pd) + #lines (group=Order) and jitter
  geom_point(data=HNormal[-c(6,19),], aes(shape=RmTempF),size = 2,posit
ion=pd) + # point size and jitter
  scale_y_continuous("Proportion normal sperm", limits=c(0.5,0.92), exp
and=c(0,0)) + #label y axis
  scale_x_discrete("Sampling time point ", labels = c("T01"="Day1","T02
"="Day3","T03"="Day7","T04"="Day11","T05"="Day14", "T06"="","T07"="Day2
```

```
6/Day1",  "T08"="Day3","T09"="Day7","T10"="Day11","T11"="Day14","T12"="D
ay21","T13"="Day26")) + #relabel x-axis
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) + #
tilt the labels
  scale_colour_manual(values=c("black","#666666"))+
  theme(legend.position = "none")
```

Figure 2. Cloacal Temperature

```
HTemp <- summarySE(RawHeatwave, measurevar="CloacalTemp", groupvars=c("
Order","TimeCode","RmTempF"),na.rm=TRUE)
HTempgg <- ggplot(HTemp, aes(x=TimeCode, y=CloacalTemp, group=Order))
pd <- position_dodge(0.2)

HTempgg +
  theme_bw() +
  annotate("rect", xmin=0, xmax=1, ymin=38, ymax=43.25, fill="grey", al
pha=0.2,) +
  annotate("rect", xmin=5.2, xmax=7, ymin=38, ymax=43.25, fill="grey",
alpha=0.2) +
  annotate("rect", xmin=11.2, xmax=13.5, ymin=38, ymax=43.25, fill="gre
y", alpha=0.2) +
  geom_errorbar(data=HTemp[-c(6,19),], aes(ymin=CloacalTemp-se, ymax=Cl
oacalTemp+se), width=.25,position=pd) +
  geom_line(aes(linetype=Order),position=pd) +
  geom_point(data=HTemp[-c(6,19),], aes(shape=RmTempF), size = 2,positi
on=pd) +
  geom_vline(xintercept=7, colour= "grey45", linetype="longdash") +
  scale_y_continuous("Male cloacal temperature (ºC)", limits=c(38,43.25
), expand=c(0,0)) +
  scale_x_discrete("Sampling time point ", labels = c("T01"="Day1","T02
"="Day3","T03"="Day7","T04"="Day11","T05"="Day14",  "T06"="","T07"="Day2
6/Day1",  "T08"="Day3","T09"="Day7","T10"="Day11","T11"="Day14","T12"="D
ay21","T13"="Day26")) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  scale_colour_manual(values=c("black","#666666"))+
  theme(legend.position = "none")
```

Supplemental 2 Figures

Need summarySE code from above!

Data upload and transformations

```
Change <- read.csv("Heatwave/Data_Figures_S2”)

#subset to only 30º and 40ºC samples
Change2 <- Change[Change$SamplingTemp>=24,]
```

```r
#Removing <10 motile sperm samples for use with CngVCL
Change3 <-  Change2[Change2$Motile>=10,]
```

Figure S3. Change Proportion Normal

```r
Normal2 <- Change2[-c(95,120,130,160),] #remove NAs by hand, so leave ones to create spaces

HCngNormal <- summarySE(Normal2, measurevar="CngPropNormal", groupvars=c("Order","TimeCode","RmTempF"),na.rm=TRUE)
HCngNormalgg <- ggplot(HCngNormal, aes(x=TimeCode, y=CngPropNormal, group=Order, shape=RmTempF))
pd <- position_dodge(0.2)

HCngNormalgg +
  theme_bw() +
  geom_errorbar(aes(ymin=CngPropNormal-se, ymax=CngPropNormal+se), width=.25,position=pd) +
  geom_line(aes(linetype=Order),position=pd) +
  geom_point(size = 2,position=pd) +
  geom_vline(xintercept=5, colour= "grey45", linetype="longdash") +
  scale_y_continuous("Change in proportion normal sperm", limits=c(-0.4,0.1), expand=c(0,0)) +
  scale_x_discrete("Sampling time point ", labels = c("T02"="Day3","T03"="Day7","T04"="Day11","T05"="Day14","T06"="", "T07"="12 day post/Day1", "T08"="Day3","T09"="Day7","T10"="Day11","T11"="Day14","T12"="7 days post","T13"="12 days post")) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  scale_colour_manual(values=c("black","#666666"))+ scale_shape_manual(values=c(16,17))+
  theme(legend.position = "none")
```

Figure S4. Change VCL

```r
VCL2 <- Change3[-c(1,6,11,19,27,28,38,43,45,55,60,65,75,76,93,94,113,115,117,118,121,124),] #remove NAs by hand, so leave ones to create spaces

HCngVCL <- summarySE(VCL2, measurevar="CngVCL", groupvars=c("Order","TimeCode","RmTempF"),na.rm=TRUE)
HCngVCLgg <- ggplot(HCngVCL, aes(x=TimeCode, y=CngVCL, group=Order, shape=RmTempF))
pd <- position_dodge(0.2)

HCngVCLgg +
  theme_bw() +
  geom_errorbar(aes(ymin=CngVCL-se, ymax=CngVCL+se), width=.25, position=pd) +
  geom_line(aes(linetype=Order),position=pd) +
```

```
  geom_point(position=pd, size =2) +
  geom_vline(xintercept=5, colour= "grey45", linetype="longdash") +
  scale_y_continuous("Change in sperm swimming speed (VCL)" ~ (mu ~ m ~ s^{-1}), limits=c(-16,10), expand=c(0,0)) +
  scale_x_discrete("Sampling time point ", labels = c("T01"="Day1","T02"="Day3","T03"="Day7", "T04"="Day11","T05"="Day14","T06"="","T07"="12 day post/Day1", "T08"="Day3","T09"="Day7","T10"="Day11","T11"="Day14","T12"="7 days post","T13"="12 days post")) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) + scale_shape_manual(values=c(16,17))+
  scale_colour_manual(values=c("black","#666666"))+
  theme(legend.position = "none")
```

Figure S5. Change Proportion Motile

```
Motilel2 <- Change2[-c(1,22,44,62,88,91,110,111,139,141,149,152),] #remove NAs by hand, so leave ones to create spaces

HCngPropMotile <- summarySE(Motilel2, measurevar="CngPropMotile", groupvars=c("Order","TimeCode","RmTempF"),na.rm=TRUE)
HCngPropMotilegg <- ggplot(HCngPropMotile, aes(x=TimeCode, y=CngPropMotile, group=Order, shape=RmTempF))
pd <- position_dodge(0.2)

HCngPropMotilegg +
  theme_bw() +
  geom_errorbar(aes(ymin=CngPropMotile-se, ymax=CngPropMotile+se), width=.25, position=pd) +
  geom_line(aes(linetype=Order),position=pd) +
  geom_point(position=pd, size =2) +
  geom_vline(xintercept=5, colour= "grey45", linetype="longdash") +
  scale_y_continuous("Change proportion motile sperm", limits=c(-0.4,0.35), expand=c(0,0)) +
  scale_x_discrete("Sampling time point ", labels = c("T01"="Day1","T02"="Day3","T03"="Day7","T04"="Day11","T05"="Day14","T06"="","T07"="12 day post/Day1", "T08"="Day3","T09"="Day7","T10"="Day11","T11"="Day14","T12"="7 days post","T13"="12 days post")) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  scale_colour_manual(values=c("black","#666666"))+ scale_shape_manual(values=c(16,17))+
  theme(legend.position = "none")
```

Figure S6. Change Cloacal Temperature

```
HCngTemp <- summarySE(Change2, measurevar="CngCloacalTemp", groupvars=c("Order","TimeCode","RmTempF"),na.rm=TRUE)
HCngTempgg <- ggplot(HCngTemp, aes(x=TimeCode, y=CngCloacalTemp, group=Order, shape=RmTempF))
pd <- position_dodge(0.2)
```

```r
HCngTempgg +
  theme_bw() +
  geom_errorbar(aes(ymin=CngCloacalTemp-se, ymax=CngCloacalTemp+se), width=.25,position=pd) +
  geom_line(aes(linetype=Order),position=pd) +
  geom_point(size = 2,position=pd) +
  geom_vline(xintercept=5, colour= "grey45", linetype="longdash") +
  scale_y_continuous("Change in cloacal temperature (ºC)", limits=c(-0.5,4.25), expand=c(0,0)) +
  scale_x_discrete("Sampling time point ", labels = c("T01"="Day1","T02"="Day3","T03"="Day7","T04"="Day11","T05"="Day14","T06"="","T07"="12 day post/Day1", "T08"="Day3","T09"="Day7","T10"="Day11","T11"="Day14","T12"="7 days post","T13"="12 days post")) +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1)) +
  scale_colour_manual(values=c("black","#666666"))+   scale_shape_manual(values=c(16,17))+
  theme(legend.position = "none")
```