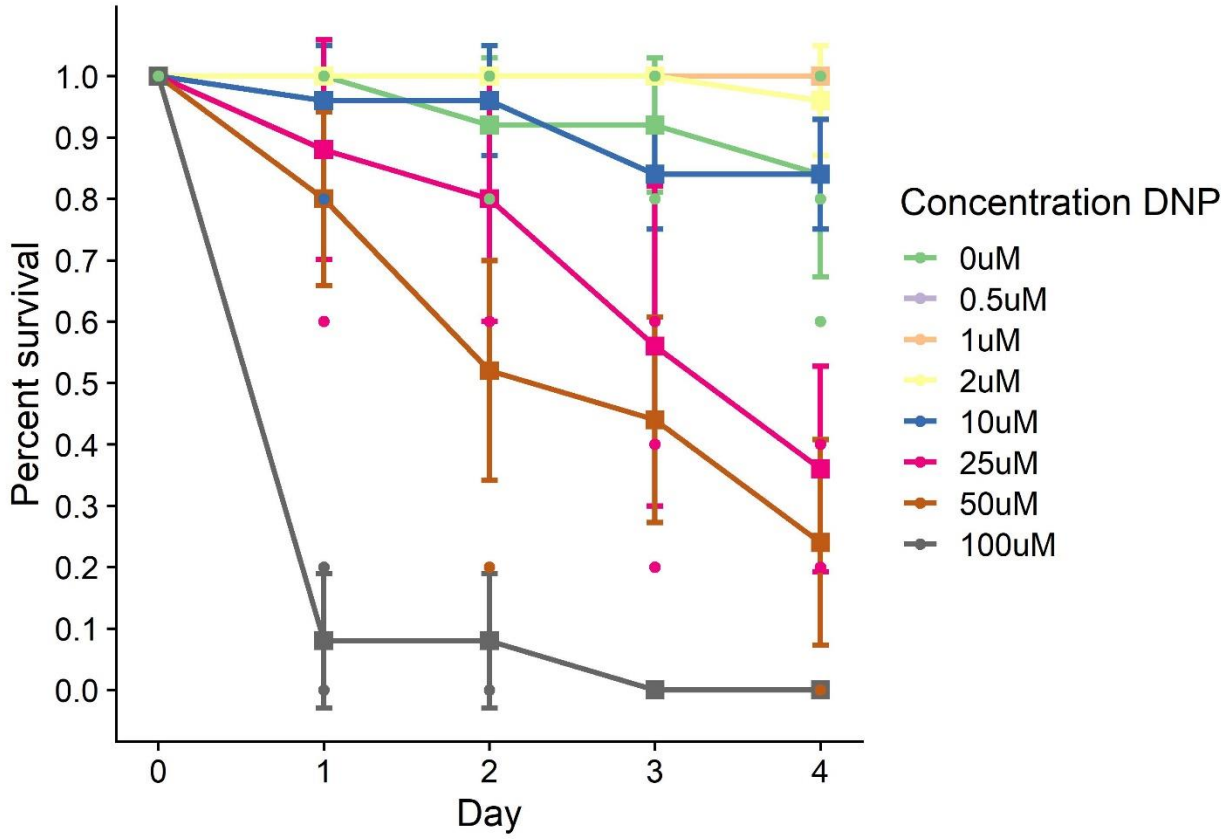

Fig. S1. Average survival out of 5 copepods over 4 days exposure time to different DNP concentrations. Each average value (indicated by the squares) represents the average of 5 trials (25 copepods total for each tested concentration). Bars represent standard errors around the averages for each day. Small circles indicate individual trials, with most falling on the average (thus, hidden visually). The lilac (0.5µM), orange (1µM), and yellow lines (2µM) overlap.

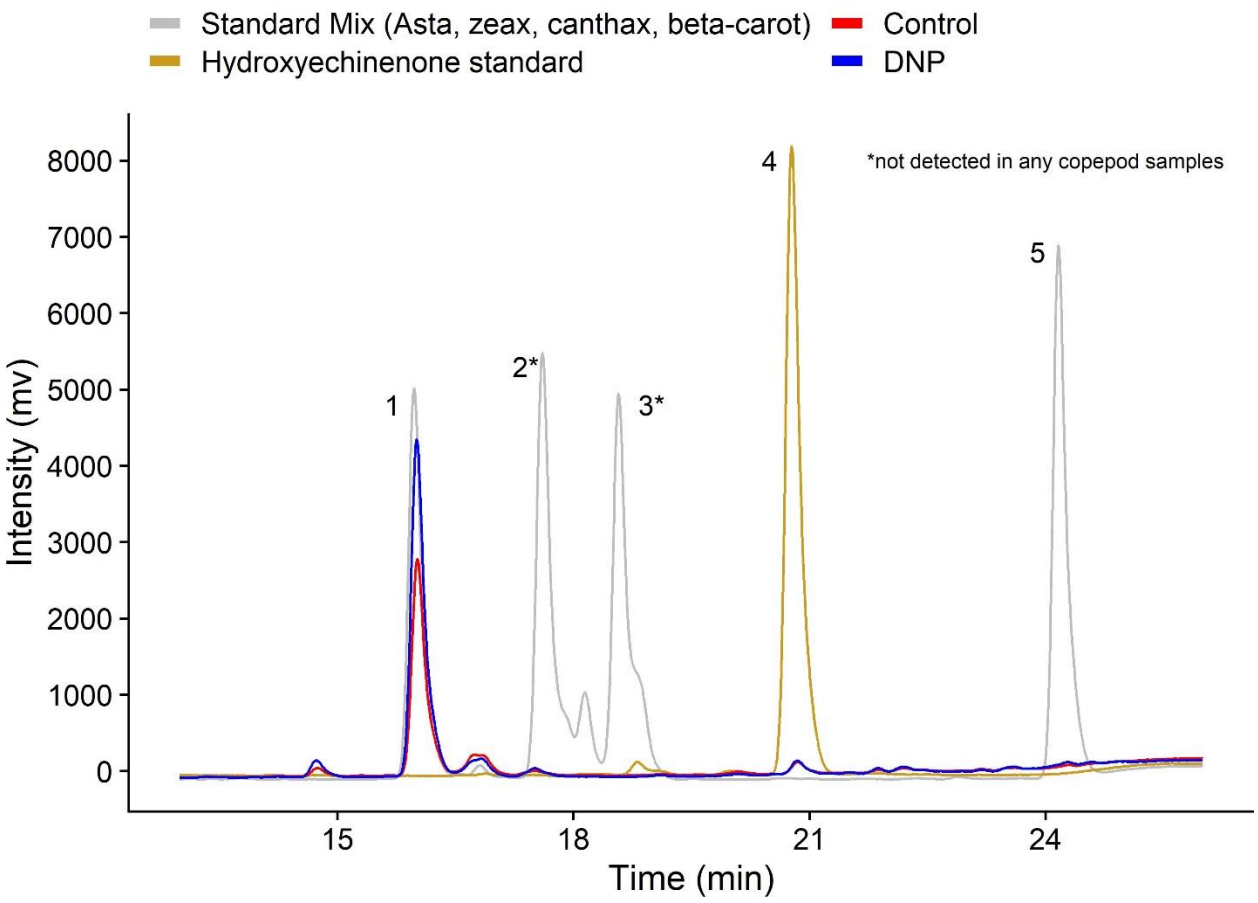

Fig. S2. Representative HPLC chromatogram showing carotenoid standards (grey and yellow), a DNP-treated copepod sample (blue), and a control copepod sample (red). Peaks are 1) astaxanthin, 2) zeaxanthin, 3) canthaxanthin, 4) hydroxyechinenone, 5) β-carotene. β-carotene and hydroxyechinenone are present in the *Tetraselmis* algae used to feed copepods. In many samples, like the representative graph shown here, β-carotene was only detectable in trace amounts.

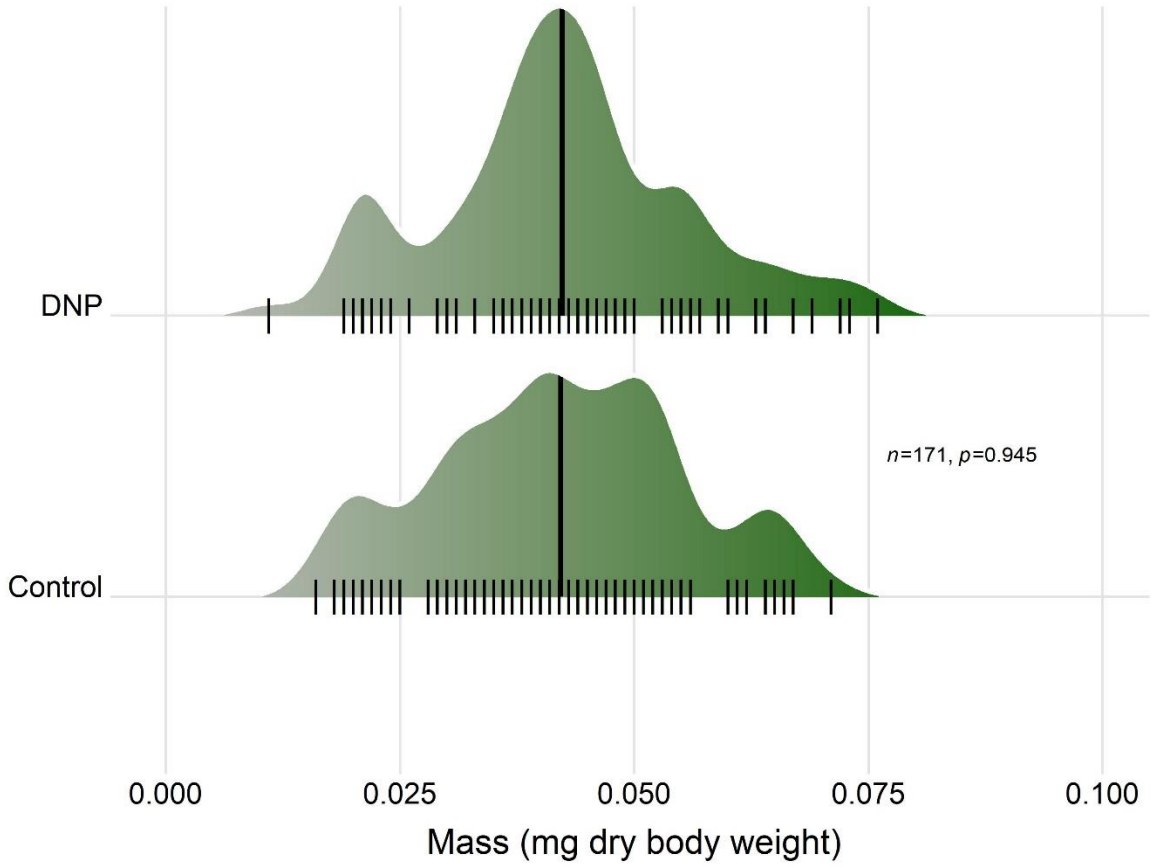

Fig. S3. Ridgeline plot showing the distribution of dry mass measurements for DNP-treated and control copepods. The tall black line represents the group average. Small black lines indicate individual trial replicates of 3 copepods each. The p-value shown is reported from a linear mixed effects model comparing the two treatment groups that included a fixed effect of sex and a random effect of diet (i.e., red stock or color-restored). The samples sizes indicated by *n* represents the number of experimental replicates analyzed in the model, with each replicate containing 3 individual copepods.

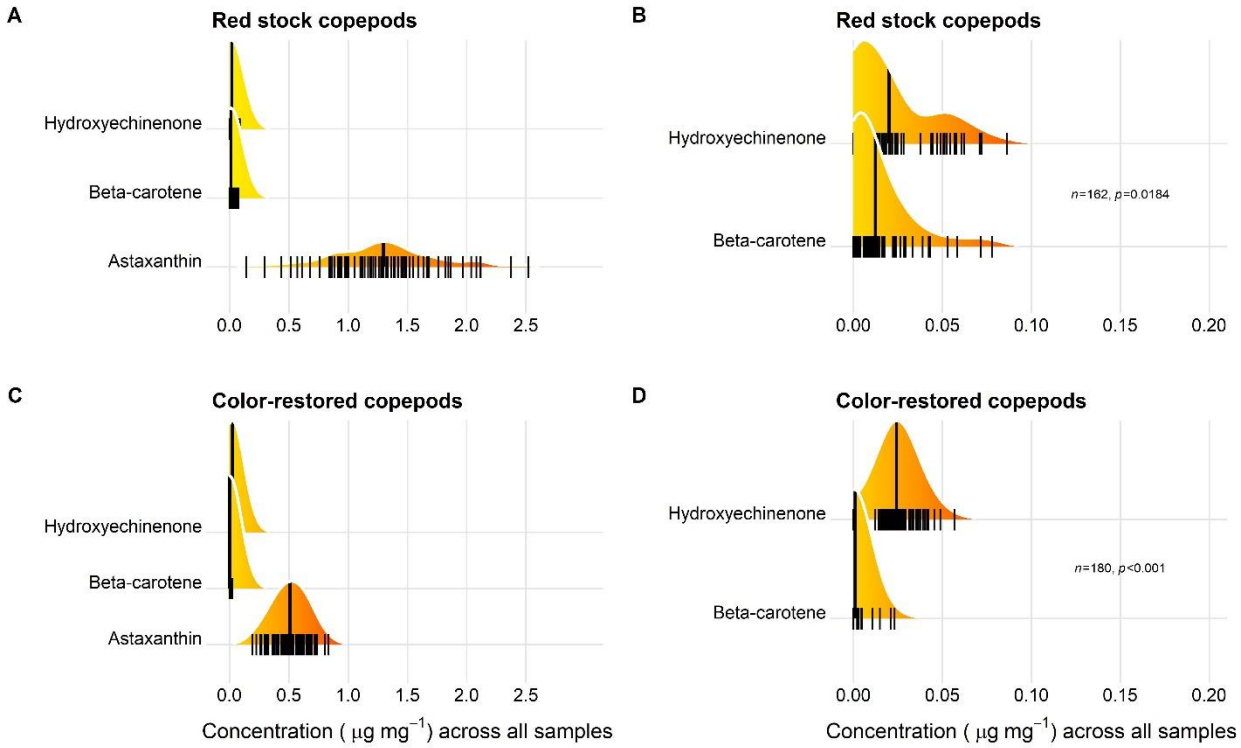

Fig. S4. Ridgeline plots showing the distribution of carotenoid concentrations for all copepod samples in red stock copepods (AB) and color-restored copepods (CD). The same data is shown twice: panels C and D are the same hydroxyechinenone and β-carotene data shown without the swamping effect of plotting astaxanthin on the same graph. The concentration of astaxanthin was significantly higher than either hydroxyechinenone or β-carotene (p<0.0001 for each comparison). The tall black line represents the average for each carotenoid. Small black lines indicate individual trial replicates of 3 copepods each.

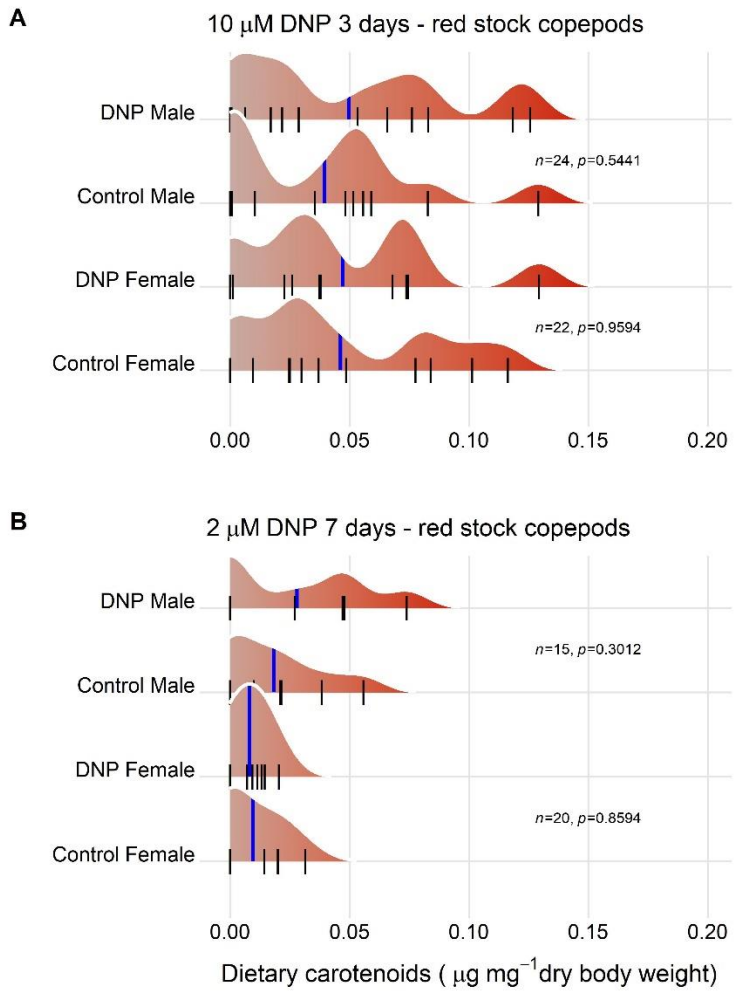

Fig. S5. Ridgeline plot showing the distribution of dietary carotenoid concentration measurements for DNP-treated and control red stock copepods, separated by sex. Panels are separated by A) the dietary carotenoid concentrations after 3 days exposure to 10μM DNP and B) dietary carotenoid concentrations after 7 days exposure to 2μM DNP. The label at the top of each panel indicates the concentration of DNP tested and the exposure time. The blue line represents the group average. Small black lines indicate individual trial replicates of 3 copepods each. The p-value shown is reported from a linear model comparing the two treatment groups that included the interaction with sex. The samples sizes indicated by *n* represents the number of experimental replicates analyzed in each model, with each replicate containing 3 individual copepods.

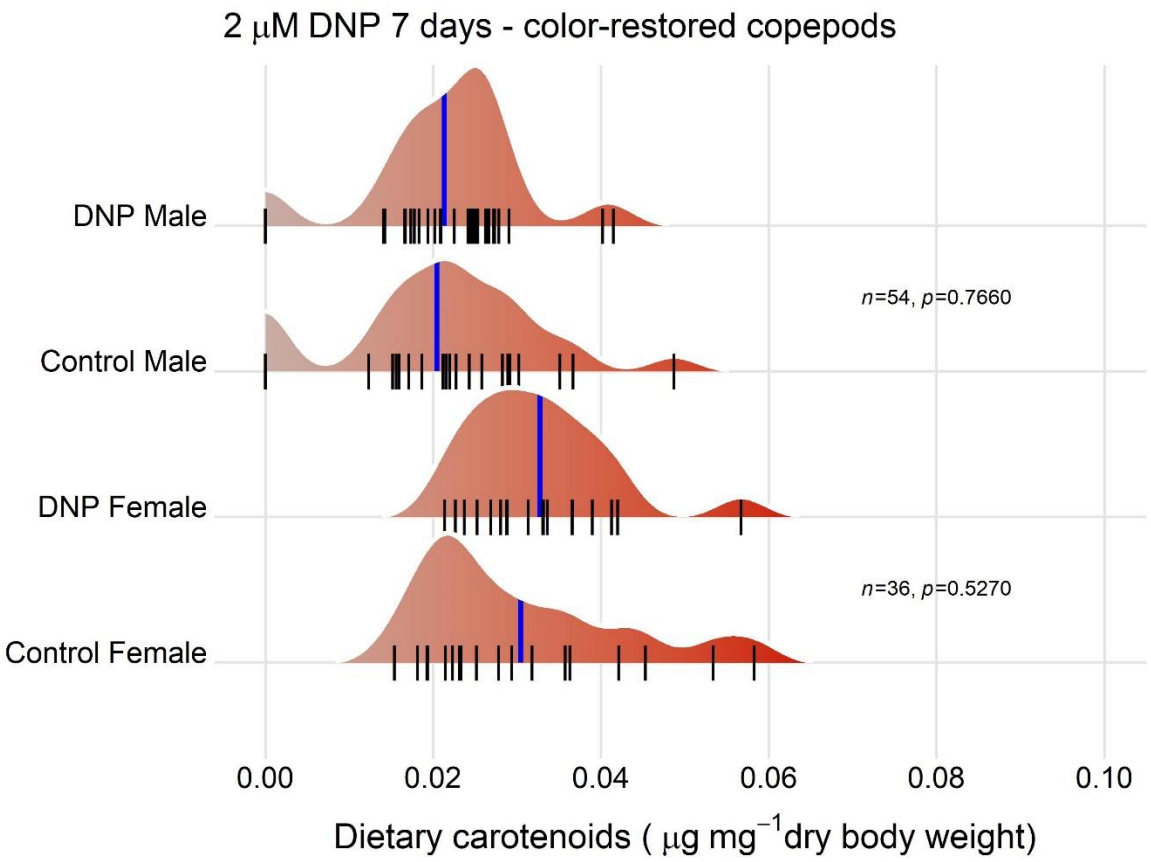

Fig. S6. Ridgeline plot showing the distribution of dietary carotenoid concentration measurements for DNP-treated and control color-restored copepods, separated by sex. The blue line represents the group average. Small black lines indicate individual trial replicates of 3 copepods each. The p-value shown is reported from a linear model comparing the two treatment groups that included the interaction with sex. The samples sizes indicated by *n* represents the number of experimental replicates analyzed in each model, with each replicate containing 3 individual copepods. We detected no dietary carotenoids in the tissues of colorless males and females never given access to algae to restore their coloration.

Supplementary Materials and Methods. Annotated R code.

```
#load packages
library(Hmisc)
library(tidyverse)
library(lme4)
library(cowplot)
library(lmerTest)
library(agricolae)
library(MASS)
library(emmeans)
library(ggpubr)
library(dplyr)
library(RColorBrewer)
library(reshape2)
library(car)
library(sf)
library(ggjoy)
library(MuMIn)
library(plotrix)
library(HH)
library(bestNormalize)
#Set theme globally
theme_set(theme_cowplot())

#####Assessing survival####

##Lethal dose analysis
datum = read.csv(file = "DNP_survival.csv")

datum2 = melt(datum[,c(2, 7, 8, 9, 10, 11)], id="ID")

datum2.5 <- as.data.frame(str_split_fixed(datum2$ID, "_", 2))
datum2$dose = as.factor(datum2.5$V1)
datum2$variable = as.numeric(datum2$variable)
datum2$variable = as.numeric(datum2$variable-1)

mod.1 = lm(value~dose*variable, data = datum2)
mod.1.comp <- emmeans(mod.1, pairwise~dose | variable)

datum2$dose <- factor(datum2$dose, levels = c("0uM", "0.5uM", "1uM", "2uM", "10uM", "25uM",
"50uM", "100uM"))

jpeg(file = "survival curves.jpg", units = "in", width = 6.5, height = 4.5, res = 500)
ggplot(data=datum2, aes(x=variable, y=value, col = dose)) +

 stat_summary(fun.data = mean_sdl, fun.args = list(mult = 1), geom = "errorbar", size =1, width=0.1)
+   stat_summary(fun = mean, geom = "line", size =1) +
 stat_summary(fun = mean, geom ="point", size = 3, shape =15, show.legend = FALSE)+
 geom_point()+
 scale_x_continuous(name="Day", breaks = seq(0, 4, by = 1))+
```

```
 scale_y_continuous(name="Percent survival", breaks = seq(0, 1, by = 0.1))+
 guides(color = guide_legend(title="Concentration DNP"))+
 scale_color_brewer(palette = "Accent")
dev.off()



############### DNP oxygen consumption  ##############################

####10uM 3 day trials
datum3 = read.csv(file = "InhibitorData10uM.csv")

 datum3$ID <- factor(datum3$ID, levels = c("Control Female", "DNP Female", "Control Male", "DNP
Male"))


mod.2 = lm(abs(Slope_ppm_per_min)~Group * Sex + Weight_mg, data = datum3)
emmeans(mod.2, pairwise ~ Group | Sex)
confint(emmeans(mod.2, pairwise ~ Group | Sex))

#Ridgeline plot
resp.10uM.3days <- ggplot(datum3, aes(x=abs(Slope_ppm_per_min), y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.7, bandwidth = 0.3, rel_min_height =0.01,
   jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "red3", color =
'white')+
  scale_x_continuous(breaks = seq(0,6, by =1), limits = c(0,6)) +
  theme_ridges(center_axis_labels = TRUE)+
  xlab("") +ylab("")+
  ggtitle(label =  bquote('10'~mu*'M'~'DNP 3 days - red stock copepods'))+
  theme(legend.position = "non")+
  annotate("text", x = 5, y = 3.5, label = expression(italic(n)*"=36,"~italic(p)*"=0.0359"), size = 3)+
  annotate("text", x = 5, y = 1.5, label = expression(italic(n)*"=27,"~italic(p)*"=0.0011"), size = 3)

jpeg(file = "Resp_10uM_3day.jpg", units = "in", width = 7, height = 5, res = 500)
resp.10uM.3days
dev.off()


####10uM 7 day trials
datum4 = read.csv(file = "InhibitorData7Day10uM.csv")

datum4$ID <- factor(datum4$ID, levels = c("Control Female", "DNP Female", "Control Male", "DNP
Male"))


mod.3 = lm(abs(Slope_ppm_per_min)~Group * Sex + Weight_mg, data = datum4)
```

```
emmeans(mod.3, pairwise ~ Group | Sex)

#Ridgeline plot
resp.10uM.7days <- ggplot(datum4, aes(x=abs(Slope_ppm_per_min), y=ID, fill = ..x..)) +
 geom_density_ridges_gradient(scale=1.2, bandwidth = 0.2, rel_min_height =0.01,
   jittered_points = TRUE, position = position_points_jitter(width = 0.00000101, height = 0), point_shape
= "|", point_size = 5, point_color = "black",
   quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "red3", color = 'white')+
 scale_x_continuous(breaks = seq(0,6, by =1), limits = c(0,6)) +
 theme_ridges(center_axis_labels = TRUE)+
 xlab("") +ylab("")+
 ggtitle(label =  bquote('10'~mu*'M'~'DNP 7 days - red stock copepods'))+
 theme(legend.position = "non")+
 annotate("text", x = 5, y = 3.5, label = expression(italic(n)*"=18,"~italic(p)*"=0.1416"), size = 3)+
 annotate("text", x = 5, y = 1.5, label = expression(italic(n)*"=18,"~italic(p)*"=0.9646"), size = 3)

jpeg(file = "Resp_10uM_7day.jpg", units = "in", width = 7, height = 5, res = 500)
resp.10uM.7days
dev.off()


###2uM analysis
datum5 = read.csv(file = "InhibitorData7Day2uM.csv")

datum5$ID <- factor(datum5$ID, levels = c("Control Female", "DNP Female", "Control Male", "DNP
Male"))

mod.4 = lm(abs(Slope_ppm_per_min)~Group * Sex + Weight_mg, data = datum5)
emmeans(mod.4, pairwise ~ Group | Sex)
confint(emmeans(mod.4, pairwise ~ Group | Sex))

#Ridgeline plot

 resp.2uM.7day<- ggplot(datum5, aes(x=abs(Slope_ppm_per_min), y=ID, fill = ..x..)) +
   geom_density_ridges_gradient(scale=1.3, bandwidth = 0.2, rel_min_height =0.01,
    jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                        quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "red3", color =
'white')+
  scale_x_continuous(breaks = seq(0,6, by =1), limits = c(0,6)) +
   theme_ridges(center_axis_labels = TRUE)+
   xlab(bquote('Respiration rate (mmol O2'~min^-1*')')) +ylab("")+
   ggtitle(label =  bquote('2'~mu*'M'~'DNP 7 days - red stock copepods'))+
   theme(legend.position = "non")+
   annotate("text", x = 5, y = 3.5, label = expression(italic(n)*"=22,"~italic(p)*"=0.0346"), size = 3)+
   annotate("text", x = 5, y = 1.5, label = expression(italic(n)*"=28,"~italic(p)*"=0.0357"), size = 3)

 jpeg(file = "Resp_2uM_7day.jpg", units = "in", width = 7, height = 5, res = 500)
```

```
  resp.2uM.7day
  dev.off()



#####Yeast 2 um 7 day #####
datum5yeast = read.csv(file = "Yeast.data.csv")
str(datum5yeast)
#Remove erroneous data point
datum5yeast = datum5yeast[-c(18),]

#Make respiration data absolute
datum5yeast$resp.final = abs(datum5yeast$resp.final)

#order ID factor
datum5yeast$ID <- factor(datum5yeast$ID, levels = c("Control Female", "DNP Female", "Control Male",
"DNP Male"))

#Run model and check results
mod.5 = lm(abs(Slope_mmol_per_min)~Group * Sex +Weight_mg, data = datum5yeast)
emmeans(mod.5, pairwise ~ Group | Sex)
confint(emmeans(mod.5, pairwise ~ Group | Sex))

#Ridgeline plot

resp.2uM.yeast <- ggplot(datum5yeast, aes(x=abs(Slope_mmol_per_min), y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.3, bandwidth = 0.1, rel_min_height =0.01,
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "red3", color =
'white')+
  theme_ridges(center_axis_labels = TRUE)+
  ggtitle(label = bquote('2'~mu*'M'~'DNP 7 days - color-restored copepods'))+
  scale_x_continuous(breaks = seq(0,3, by =0.5), limits = c(0,3)) +
  xlab(bquote('Respiration rate (mmol O2'~min^-1*')')) +ylab("")+
  theme(legend.position = "non")+
  annotate("text", x = 2, y = 3.5, label = expression(italic(n)*"=54,"~italic(p)*"=0.0353"), size = 3)+
  annotate("text", x = 2, y = 1.5, label = expression(italic(n)*"=36,"~italic(p)*"=0.1298"), size = 3)

jpeg(file = "Resp_Yeast_7day_2uM.jpg", units = "in", width = 7, height = 5, res = 500)
resp.2uM.yeast
dev.off()



#Combine algae respiration figures into one three-panel figure
Fig.resp.ridgelines <- ggarrange(resp.10uM.3days, resp.10uM.7days, resp.2uM.7day,
                                 labels = c("A", "B", "C"),
```

```
                    ncol=1, nrow=3, common.legend = FALSE, legend = "none")

jpeg(file="Algae resp ridgelines.jpg", units="in", width=6, height=10, res=500)
Fig.resp.ridgelines
dev.off()

############ HPLC data analysis ############

carotenoid.datum = read.csv(file = "carotenoid.data.csv")

carotenoid.datum$log.resp.min = log(abs(carotenoid.datum$resp.min))

#Split data set into separate data frames
#Plot data and run models for each group comparing across males and females
#algae fed copepods
carotenoid.datum.algae = subset(carotenoid.datum, diet == "algae" )
carotenoid.datum.algae = droplevels(carotenoid.datum.algae)

#Compare all identifiable carotenoids in algae dataset
#Subset desired samples from data frame
datum.algae_sub = carotenoid.datum.algae[,c(1,13,14,15)]
##Then rearrange your data frame
dd.algae = melt(datum.algae_sub, id=c("HPLC.ID"))

mod.carot.comparison <- lm(value~variable, data = dd.algae)
summary(mod.carot.comparison)
emmeans(mod.carot.comparison, pairwise ~ variable)

#Repeat for just beta-carot vs hydroxy
datum.algae_sub.2 = carotenoid.datum.algae[,c(1,14,15)]
##Then rearrange your data frame
dd.algae.2 = melt(datum.algae_sub.2, id=c("HPLC.ID"))

mod.carot.comparison.2 <- lm(value~variable, data = dd.algae.2)
summary(mod.carot.comparison.2)
confint(mod.carot.comparison.2)


#Ridgeline plot for all carots
all.carots.algae <- ggplot(dd.algae, aes(x=value, y=variable, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.3, bandwidth = 0.1, rel_min_height =0.01,
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "black", color =
'white')+
  scale_x_continuous(breaks = seq(0,2.5, by =0.5), limits = c(0,3)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="yellow", high="red")+
```

```
  xlab(bquote('')) +ylab("")+
  ggtitle(label = "Red stock copepods")+
  scale_y_discrete(labels = c('Astaxanthin','Beta-carotene', 'Hydroxyechinenone'))+
  theme(legend.position = "non")

jpeg(file = "carotenoid comparison ridgeline.jpg", units = "in", width = 6, height = 4, res = 500)
all.carots.algae
dev.off()

#Ridgeline plot for just beta carot and hydroxy
dietary.carots.algae <- ggplot(dd.algae.2, aes(x=value, y=variable, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.3, bandwidth = 0.01, rel_min_height =0.01,
                               jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0),
point_shape = '|', point_size = 5, point_color = "black",
                               quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "black", color =
'white')+
  scale_x_continuous(breaks = seq(0,0.2, by =0.05), limits = c(0,0.2)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="yellow", high="red")+
  xlab(bquote('')) +ylab("")+
  ggtitle(label = "Red stock copepods")+
  scale_y_discrete(labels = c('Beta-carotene', 'Hydroxyechinenone'))+
  theme(legend.position = "non")+
  annotate("text", x = 0.15, y = 1.5, label = expression(italic(n)*"=162,"~italic(p)*"=0.0184"), size = 3)




####Yeast fed copepods
carotenoid.datum.yeast = subset(carotenoid.datum, diet == "yeast" )
carotenoid.datum.yeast = droplevels(carotenoid.datum.yeast)
carotenoid.datum.yeast$ID <- factor(carotenoid.datum.yeast$ID, levels = c("Colorless Female",
"Colorless Male","Control Female", "DNP Female", "Control Male", "DNP Male"))

#Compare all identifiable carotenoids in color restored copepods only (subset out colorless)
#Subset desired samples from data frame
datum.yeast_sub = carotenoid.datum.yeast[c(1:90),c(1,13,14,15)]
##Then rearrange your data frame
dd.yeast = melt(datum.yeast_sub, id=c("HPLC.ID"))

mod.carot.comparison.yeast <- lm(value~variable, data = dd.yeast)
summary(mod.carot.comparison.yeast)
emmeans(mod.carot.comparison.yeast, pairwise ~ variable)

#Repeat for just beta-carot and hydroxy
datum.yeast_sub.2 = carotenoid.datum.yeast[c(1:90),c(1,14,15)]
##Then rearrange your data frame
```

```
dd.yeast.2 = melt(datum.yeast_sub.2, id=c("HPLC.ID"))

mod.carot.comparison.yeast.2 <- lm(value~variable, data = dd.yeast.2)
summary(mod.carot.comparison.yeast.2)
confint(mod.carot.comparison.yeast.2)

#Ridgeline plot of all carotenoids
all.carots.yeast <- ggplot(dd.yeast, aes(x=value, y=variable, fill = ..x..)) +
  geom_density_ridges_gradient(scale=2, bandwidth = 0.1, rel_min_height =0.01,
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "black", color =
'white')+
  scale_x_continuous(breaks = seq(0,2.5, by =0.5), limits = c(0,3)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="yellow", high="red")+
  xlab(bquote('Concentration ('~mu*'g'~'mg'^-1*') across all samples')) +ylab("")+
  ggtitle(label = "Color-restored copepods")+
  scale_y_discrete(labels = c('Astaxanthin','Beta-carotene', 'Hydroxyechinenone'))+
  theme(legend.position = "non")

jpeg(file = "carotenoid comparison ridgeline yeast.jpg", units = "in", width = 6, height = 4, res = 500)
all.carots.yeast
dev.off()

#Ridgeline plot for just beta carot and hydroxy
dietary.carots.yeast <- ggplot(dd.yeast.2, aes(x=value, y=variable, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.3, bandwidth = 0.01, rel_min_height =0.01,
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "black", color =
'white')+
  scale_x_continuous(breaks = seq(0,0.2, by =0.05), limits = c(0,0.2)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="yellow", high="red")+
  xlab(bquote('Concentration ('~mu*'g'~'mg'^-1*') across all samples')) +ylab("")+
  ggtitle(label = "Color-restored copepods")+
  scale_y_discrete(labels = c('Beta-carotene', 'Hydroxyechinenone'))+
  theme(legend.position = "non")+
  annotate("text", x = 0.15, y = 1.5, label = expression(italic(n)*"=180,"~italic(p)*"<0.001"), size = 3)

#Combine yeast and algae carotenoids data into one figure
Fig.all.carots <- ggarrange(all.carots.algae,  dietary.carots.algae, all.carots.yeast, dietary.carots.yeast,
                            labels = c("A", "B", "C", "D"),
                            ncol=2, nrow=2, common.legend = FALSE, legend = "none")

jpeg(file="All carots.jpg", units="in", width=11.5, height=7, res=500)
Fig.all.carots
```

```
dev.off()



#Comparing astaxanthin concentration (subset out colorless copepods)
mod.yeast = lm(asta.conc~treatment * sex, data = carotenoid.datum.yeast[1:90,])
summary(mod.yeast)
emmeans(mod.yeast, pairwise ~ treatment | sex)
confint(emmeans(mod.yeast, pairwise ~ treatment | sex))

#Comparing astaxanthin concentration in colorless males and females
mod.yeast.b = lm(asta.conc~ID, data = carotenoid.datum.yeast[91:103,])
summary(mod.yeast.b)

#Compare astaxanthin concentration in colorless to control and DNP copepods
mod.yeast.c = lm(asta.conc~ID * sex, data = carotenoid.datum.yeast)
emmeans(mod.yeast.c, pairwise ~ ID | sex)


#Ridgeline plot
asta.2uM.yeast <- ggplot(carotenoid.datum.yeast, aes(x=asta.conc, y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.7, bandwidth = 0.03, rel_min_height =0.01,
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "blue", color =
'white')+
  scale_x_continuous(breaks = seq(0,1, by =0.2), limits = c(0,1)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="grey", high="red3")+
  xlab(bquote('Astaxanthin ('~mu*'g'~'mg'^-1*'dry body weight)')) +ylab("")+
  ggtitle(label = bquote('2'~mu*'M'~'DNP 7 days - color-restored copepods'))+
  theme(legend.position = "non")+
  annotate("text", x = 0.88, y = 3.5, label = expression(italic(n)*"=36,"~italic(p)*"=0.0312"), size = 3)+
  annotate("text", x = 0.88, y = 5.5, label = expression(italic(n)*"=54,"~italic(p)*"=0.5836"), size = 3)+
  annotate("text", x = 0.35, y = 1.5, label = expression(italic(n)*"=12"), size = 3)

jpeg(file = "Asta_Yeast_7day_2uM.jpg", units = "in", width = 7, height = 5, res = 500)
asta.2uM.yeast
dev.off()



#Combine 2uM yeast respiration figure and asta figure into one
Fig.2uM.yeast.resp.and.asta <- ggarrange(resp.2uM.yeast, asta.2uM.yeast,
                       labels = c("A", "B"),
                       ncol=1, nrow=2, common.legend = FALSE, legend = "none")

jpeg(file="Resp and asta figure 2uM yeast.jpg", units="in", width=6, height=9, res=500)
```

```
Fig.2uM.yeast.resp.and.asta
dev.off()



#Comparing dietary carotenoid concentration
mod.yeast.dietary = lm(dietary.conc~treatment * sex, data = carotenoid.datum.yeast)
summary(mod.yeast.dietary)
emmeans(mod.yeast.dietary, pairwise ~ treatment | sex)
#Ridgeline plot

dietary.2uM.yeast<- ggplot(carotenoid.datum.yeast[1:90,], aes(x=dietary.conc, y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.1, bandwidth = 0.003, rel_min_height =0.01,
   jittered_points = TRUE, position = position_points_jitter(width = 0.00000001, height = 0), point_shape
= "|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "blue", color =
'white')+
  scale_x_continuous(breaks = seq(0,0.1, by =0.02), limits = c(0,0.1)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="grey", high="red3")+
  xlab(bquote('Dietary carotenoids ('~mu*'g'~'mg'^-1*'dry body weight)')) +ylab("")+
  ggtitle(label = bquote('2'~mu*'M'~'DNP 7 days - color-restored copepods'))+
  theme(legend.position = "non")+
  annotate("text", x = 0.08, y = 1.5, label = expression(italic(n)*"=36,"~italic(p)*"=0.5270"), size = 3)+
  annotate("text", x = 0.08, y = 3.5, label = expression(italic(n)*"=54,"~italic(p)*"=0.7660"), size = 3)

jpeg(file = "Dietary_Yeast_7day_2uM.jpg", units = "in", width = 7, height = 5, res = 500)
dietary.2uM.yeast
dev.off()



####10uM copepods for 3 days
carotenoid.datum.10uM = subset(carotenoid.datum.algae, conc.dnp == "10uM")
carotenoid.datum.10uM = droplevels(carotenoid.datum.10uM)
carotenoid.datum.10uM$ID <- factor(carotenoid.datum.10uM$ID, levels = c("Control Female", "DNP
Female", "Control Male", "DNP Male"))

#Comparing astaxanthin concentration
mod.10uM = lm(asta.conc~treatment * sex , data = carotenoid.datum.10uM)
summary(mod.10uM)
emmeans(mod.10uM, pairwise ~ treatment | sex)
#Ridgeline plot

asta.10uM.3day <- ggplot(carotenoid.datum.10uM, aes(x=asta.conc, y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.1, bandwidth = 0.15, rel_min_height =0.01,
```

```
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                      quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "blue", color =
'white')+
  scale_x_continuous(breaks = seq(0,3, by =0.5), limits = c(0,3)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="grey", high="red3")+
  xlab(bquote('')) +ylab("")+
  ggtitle(label = bquote('10'~mu*'M'~'DNP 3 days - red stock copepods'))+
  theme(legend.position = "non")+
  annotate("text", x = 2.5, y = 1.5, label = expression(italic(n)*"=22,"~italic(p)*"=0.5159"), size = 3)+
  annotate("text", x = 2.5, y = 3.5, label = expression(italic(n)*"=24,"~italic(p)*"=0.9071"), size = 3)


jpeg(file = "Asta_10uM_3day.jpg", units = "in", width = 7, height = 5, res = 500)
asta.10uM.3day
dev.off()

#Comparing dietary carotenoid concentration
mod.10uM.dietary = lm(dietary.conc~treatment * sex, data = carotenoid.datum.10uM)
summary(mod.10uM.dietary)
emmeans(mod.10uM.dietary, pairwise ~ treatment | sex)
#Ridgeline plot

dietary.10uM.3day <- ggplot(carotenoid.datum.10uM, aes(x=dietary.conc, y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.1, bandwidth = 0.009, rel_min_height =0.01,
   jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                      quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "blue", color =
'white')+
  scale_x_continuous(breaks = seq(0,0.2, by =0.05), limits = c(0,0.2)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="grey", high="red3")+
  xlab(bquote('')) +ylab("")+
  ggtitle(label = bquote('10'~mu*'M'~'DNP 3 days - red stock copepods'))+
  theme(legend.position = "non")+
  annotate("text", x = 0.15, y = 1.5, label = expression(italic(n)*"=22,"~italic(p)*"=0.9594"), size = 3)+
  annotate("text", x = 0.15, y = 3.5, label = expression(italic(n)*"=24,"~italic(p)*"=0.5441"), size = 3)

jpeg(file = "Dietary_10uM_3day.jpg", units = "in", width = 7, height = 5, res = 500)
dietary.10uM.3day
dev.off()
```

```
#2uM copepods for 7 days
carotenoid.datum.2uM = subset(carotenoid.datum.algae, conc.dnp == "2uM")
carotenoid.datum.2uM = droplevels(carotenoid.datum.2uM)
carotenoid.datum.2uM$ID <- factor(carotenoid.datum.2uM$ID, levels = c("Control Female", "DNP
Female", "Control Male", "DNP Male"))

#Comparing astaxanthin concentration
mod.2uM = lm(asta.conc~treatment * sex, data = carotenoid.datum.2uM)
summary(mod.2uM)
emmeans(mod.2uM, pairwise ~ treatment | sex)
confint(emmeans(mod.2uM, pairwise ~ treatment | sex))
#Ridgeline plot

asta.2uM.7days <- ggplot(carotenoid.datum.2uM, aes(x=asta.conc, y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.3, bandwidth = 0.15, rel_min_height =0.01,
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "blue", color =
'white')+
  scale_x_continuous(breaks = seq(0,3, by =0.5), limits = c(0,3)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="grey", high="red3")+
  xlab(bquote('Astaxanthin ('~mu*'g'~'mg'^-1*'dry body weight)')) +ylab("")+
  ggtitle(label = bquote('2'~mu*'M'~'DNP 7 days - red stock copepods'))+
  theme(legend.position = "non")+
  annotate("text", x = 2.5, y = 1.5, label = expression(italic(n)*"=20,"~italic(p)*"=0.3948"), size = 3)+
  annotate("text", x = 2.5, y = 3.5, label = expression(italic(n)*"=15,"~italic(p)*"=0.0042"), size = 3)

jpeg(file = "Asta_2uM_7day.jpg", units = "in", width = 7, height = 5, res = 500)
asta.2uM.7days
dev.off()

#Comparing dietary carotenoid concentration
mod.2uM.dietary = lm(dietary.conc~treatment * sex, data = carotenoid.datum.2uM)
summary(mod.2uM.dietary)
emmeans(mod.2uM.dietary, pairwise ~ treatment | sex)
#Ridgeline plot

dietary.2uM.7days <- ggplot(carotenoid.datum.2uM, aes(x=dietary.conc, y=ID, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.1, bandwidth = 0.009, rel_min_height =0.01,
  jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                       quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "blue", color =
'white')+
  scale_x_continuous(breaks = seq(0,0.2, by =0.05), limits = c(0,0.2)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="grey", high="red3")+
  xlab(bquote('Dietary carotenoids ('~mu*'g'~'mg'^-1*'dry body weight)')) +ylab("")+
```

```
  ggtitle(label = bquote('2'~mu*'M'~'DNP 7 days - red stock copepods'))+
  theme(legend.position = "non")+
  annotate("text", x = 0.15, y = 1.5, label = expression(italic(n)*"=20,"~italic(p)*"=0.8594"), size = 3)+
  annotate("text", x = 0.15, y = 3.5, label = expression(italic(n)*"=15,"~italic(p)*"=0.3012"), size = 3)

jpeg(file = "Dietary_2uM_7day.jpg", units = "in", width = 7, height = 5, res = 500)
dietary.2uM.7days
dev.off()



#Combine astaxanthin figures for 10uM 3day and 2uM 7day into one
Fig.asta.10uM.and.2uM <- ggarrange(asta.10uM.3day, asta.2uM.7days,
                          labels = c("A", "B"),
                          ncol=1, nrow=2, common.legend = FALSE, legend = "none")

jpeg(file="Asta 10uM and 2uM.jpg", units="in", width=6, height=8, res=500)
Fig.asta.10uM.and.2uM
dev.off()

#Combine dietary carotenoids figures for 10uM 3day and 2uM 7day into one
Fig.dietary.10uM.and.2uM <- ggarrange(dietary.10uM.3day, dietary.2uM.7days,
                          labels = c("A", "B"),
                          ncol=1, nrow=2, common.legend = FALSE, legend = "none")

jpeg(file="Dietary 10uM and 2uM.jpg", units="in", width=6, height=8, res=500)
Fig.dietary.10uM.and.2uM
dev.off()



####Comparing astaxanthin vs respiration controlling for effect of diet and sex####
#DNP copepods
mod.asta.vs.resp.DNP = lmer(asta.conc~abs(resp.min.mg) + sex + (1|diet),
                    data = subset(carotenoid.datum, treatment == "DNP"))
summary(mod.asta.vs.resp.DNP)
confint(mod.asta.vs.resp.DNP)
r.squaredGLMM(mod.asta.vs.resp.DNP)
#plot asta vs resp
asta.vs.resp.dnp <- subset(carotenoid.datum, treatment == "DNP") %>%
  ggplot(aes(x = abs(resp.min.mg), y=asta.conc)) +
  geom_point(aes(y = asta.conc),size = 5, alpha =0.9)+
  scale_x_continuous(breaks = seq(0, 80, by =20))+
  geom_smooth(method='lm')+
  xlab(bquote('Respiration rate (mmol O2'~min^-1~'mg'^-1*')'))+
  ylab(bquote('Astaxanthin ('~mu*'g'~'mg'^-1*')'))  +
```

```
  ggtitle(label = "DNP treated")+
  theme(axis.text = element_text(size = 16),
        axis.title = element_text(size = 18))+
  annotate("text", 70, 0.5, label =  bquote('p=0.029, R'^2*'=0.754'), size=4)

#Control copepods
mod.asta.vs.resp.control = lmer(asta.conc~abs(resp.min.mg)+ sex+(1|diet),
                        data = subset(carotenoid.datum, treatment == "Control"))
summary(mod.asta.vs.resp.control)
r.squaredGLMM(mod.asta.vs.resp.control)
#plot asta vs resp
asta.vs.resp.control <- subset(carotenoid.datum, treatment == "Control") %>%
  ggplot(aes(x = abs(resp.min.mg), y=asta.conc)) +
  geom_point(aes(y = asta.conc),size = 5, alpha =0.9)+
  geom_smooth(method='lm')+
  xlab(bquote('Respiration rate (mmol O2'~min^-1~'mm'^-1*')'))+
  ylab(bquote('Astaxanthin ('~mu*'g'~'mg'^-1*')'))  +
  ggtitle(label = "Control")+
  theme(axis.text = element_text(size = 16),
        axis.title = element_text(size = 18))+
  annotate("text", 70, 0.5, label =  bquote('p=0.565, R'^2*'=0.768'), size=4)


###Comparing dietary carotenoids vs respiration controlling for effect of diet
xtrans <- bestNormalize(carotenoid.datum$dietary.conc)
carotenoid.datum$tf.dietary = xtrans$x.t

#DNP copepods
mod.dietary.vs.resp.DNP = lmer(tf.dietary~abs(resp.min.mg) + sex + (1|diet),
                        data = subset(carotenoid.datum, treatment == "DNP"))
summary(mod.dietary.vs.resp.DNP)
confint(mod.dietary.vs.resp.DNP)
r.squaredGLMM(mod.dietary.vs.resp.DNP)
#plot asta vs resp
dietary.vs.resp.dnp <- subset(carotenoid.datum, treatment == "DNP") %>%
  ggplot(aes(x = abs(resp.min.mg), y=tf.dietary)) +
  geom_point(aes(y = tf.dietary),size = 5, alpha =0.9)+
  scale_x_continuous(breaks = seq(0, 80, by =20))+
  geom_smooth(method='lm')+
  xlab(bquote('Respiration rate (mmol O2'~min^-1~'mg'^-1*')'))+
  ylab(bquote('Dietary carotenoids concentration (transformed)'))  +
  ggtitle(label = "DNP treated")+
  theme(axis.text = element_text(size = 15),
        axis.title = element_text(size = 14))+
  annotate("text", 75, -1, label =  bquote('p=0.016, R'^2*'=0.134'), size=4)

#Control copepods
mod.dietary.vs.resp.control = lmer(tf.dietary~abs(resp.min.mg)+ sex+(1|diet),
```

```
                        data = subset(carotenoid.datum, treatment == "Control"))
summary(mod.dietary.vs.resp.control)
confint(mod.dietary.vs.resp.control)
r.squaredGLMM(mod.dietary.vs.resp.control)
#plot asta vs resp
dietary.vs.resp.control <- subset(carotenoid.datum, treatment == "Control") %>%
  ggplot(aes(x = abs(resp.min.mg), y=tf.dietary)) +
  geom_point(aes(y = tf.dietary),size = 5, alpha =0.9)+
  geom_smooth(method='lm')+
  xlab(bquote('Respiration rate (mmol O2'~'min^-1~'mg'^-1*')'))+
  ylab(bquote('Dietary carotenoids concentration (transformed)'))  +
  ggtitle(label = "Control")+
  theme(axis.text = element_text(size = 15),
        axis.title = element_text(size = 14))+
  annotate("text", 70, -1, label =  bquote('p=0.608, R'^2*'=0.013'), size=4)


#Combine ALL scatterplots (astaxanthin and dietary) into one four-panel figure
Fig.carotenoids.vs.resp.overall <- ggarrange(asta.vs.resp.control, asta.vs.resp.dnp,
dietary.vs.resp.control, dietary.vs.resp.dnp,
                                    labels = c("A", "B", "C", "D"),
                                  ncol=2, nrow=2, common.legend = TRUE, legend = "top")

jpeg(file="carotenoids.vs.resp.scatters.jpg", units="in", width=11, height=11, res=500)
Fig.carotenoids.vs.resp.overall
dev.off()



##Is there a relationship between astaxanthin and dietary carotenoids?
#DNP copepods
mod.dietary.vs.asta = lmer(asta.conc~tf.dietary+ sex+(1|diet),
                           data = subset(carotenoid.datum, treatment == "DNP"))
summary(mod.dietary.vs.asta)
r.squaredGLMM(mod.dietary.vs.asta)

#plot asta vs dietary
dietary.vs.asta.dnp <- subset(carotenoid.datum, treatment == "DNP") %>%
  ggplot(aes(x = tf.dietary, y=asta.conc)) +
  geom_point(aes(y = asta.conc),size = 5, alpha =0.9)+
  geom_smooth(method='lm')+
  xlab(bquote('Dietary carotenoids concentration (transformed'))+
  ylab(bquote('Astaxanthin ('~mu*'g'~'mg'^-1*')'))  +
  ggtitle(label = "DNP")+
  theme(axis.text = element_text(size = 14),
        axis.title = element_text(size = 15))+
  annotate("text", 2, 0, label =  bquote('p=0.894, R'^2*'=0.777'), size=4)
```

```
#Control copepods
mod.dietary.vs.asta.control = lmer(asta.conc~tf.dietary+ sex+(1|diet),
                    data = subset(carotenoid.datum, treatment == "Control"))
summary(mod.dietary.vs.asta.control)
confint(mod.dietary.vs.asta.control)
r.squaredGLMM(mod.dietary.vs.asta.control)

#plot asta vs dietary
dietary.vs.asta.control <- subset(carotenoid.datum, treatment == "DNP") %>%
 ggplot(aes(x = tf.dietary, y=asta.conc)) +
 geom_point(aes(y = asta.conc),size = 5, alpha =0.9)+
 geom_smooth(method='lm')+
 xlab(bquote('Dietary carotenoids concentration (transformed)'))+
 ylab(bquote('Astaxanthin ('~mu*'g'~'mg'^-1*')'))  +
 ggtitle(label = "Control")+
 theme(axis.text = element_text(size = 14),
     axis.title = element_text(size = 15))+
 annotate("text", 1.5, 0, label =  bquote('p=0.001, R'^2*'=0.774'), size=4)

#Combine scatterplots into one figure
Fig.dietary.vs.asta <- ggarrange(dietary.vs.asta.control, dietary.vs.asta.dnp,  labels = c("A", "B"),
                          ncol=2, nrow=1, common.legend = TRUE, legend = "top")

jpeg(file="dietary.vs.asta.scatters.jpg", units="in", width=11, height=6, res=500)
Fig.dietary.vs.asta
dev.off()



#Is there a significant difference in mass between DNP-treated and control copepods?
mod.mass = lmer(mass~treatment + sex + (1|diet), data = carotenoid.datum[1:171,])
summary(mod.mass)
emmeans(mod.mass, pairwise~ treatment)

#Try with fixed effect of diet to see if difference between diets
mod.mass2 = lm(mass~treatment + sex + diet, data = carotenoid.datum[1:171,])
summary(mod.mass2)


#Ridgeline plot (subset out colorless data, irrelevant)
jpeg(file = "Mass_vs_treatment.jpg", units = "in", width = 7, height = 5, res = 500)
ggplot(carotenoid.datum[1:171,], aes(x=mass, y=treatment, fill = ..x..)) +
 geom_density_ridges_gradient(scale=1.1, bandwidth = 0.003, rel_min_height =0.01,
 jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0), point_shape =
"|", point_size = 5, point_color = "black",
                    quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "black", color =
'white')+
 scale_x_continuous(breaks = seq(0,0.1, by =0.025), limits = c(0,0.1)) +
```

```
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="grey", high="darkgreen")+
  xlab(bquote('Mass (mg dry body weight)')) +ylab("")+
  theme(legend.position = "non")+
  annotate("text", x = 0.085, y = 1.5, label = expression(italic(n)*"=171,"~italic(p)*"=0.945"), size = 3)
dev.off()


#Is there a significant difference in respiration rate between algae raised and yeast raised copepods?
mod.resp.diet = lm(abs(resp.min)~diet + mass + sex, data = carotenoid.datum)
summary(mod.resp.diet)
emmeans(mod.resp.diet, pairwise~ treatment)


#Ridgeline plot (subset out colorless data, irrelevant)
jpeg(file = "resp.vs.diet.jpg", units = "in", width = 7, height = 5, res = 500)
ggplot(carotenoid.datum, aes(x=abs(resp.min.mg), y=diet, fill = ..x..)) +
  geom_density_ridges_gradient(scale=1.7, bandwidth = 5, rel_min_height =0.01,
                               jittered_points = TRUE, position = position_points_jitter(width = 0.000001, height = 0),
point_shape = "|", point_size = 5, point_color = "black",
                               quantile_lines = TRUE, quantile_fun = mean, size =1, vline_color = "black", color =
'white')+
  scale_x_continuous(breaks = seq(0,100, by =20), limits = c(0,100)) +
  theme_ridges(center_axis_labels = TRUE)+
  scale_fill_gradient(low="lightblue", high="darkblue")+
  xlab(bquote('Respiration rate (mmol O2'~'min^-1~'mg'^-1*')')) +ylab("")+
  theme(legend.position = "non")+
  annotate("text", x = 80, y = 1.8, label = expression(italic(n)*"=183,"~italic(p)*"<0.001"), size = 3.5)
dev.off()



####Chromatogram figure####
datumhplc = read.csv(file = "hplc.chromatograms.csv")

#Trim first 13 minutes off to remove solvent front peak and reduce white space of graph
#trim off last 5 minutes to remove equilibration period and reduce white space of graph
datumhplctrim = subset(datumhplc, time >=13 & time <=26)

#Subset desired samples from data frame
dd_sub = datumhplctrim[,c(1,3,4,7,8)]
dd_subtetra = datumhplctrim[,c(1,2,3,4)]

#You will notice numbers after the Y variable call for the standard mix. This is just an adjustment to
#all of the values of the intensity column for that standard to correct for the baseline drift
#of the HPLC system

#Adjust y values for baseline drift during hplc
```

```
dd_sub$X3HE = dd_sub$X3HE+200
dd_subtetra$X3HE = dd_subtetra$X3HE+200

##Then rearrange your data frame
dd = melt(dd_sub, id=c("time"))
ddtetra = melt(dd_subtetra, id=c("time"))

#Make plot comparing DNP and control copepod chromatograms representatives
chromatogram.yeast <- ggplot(dd) + geom_line(aes(x=time, y=value, colour=variable)) +
  scale_colour_manual(name="",values=c("grey","goldenrod3", "red", "blue"),
                      labels=c("Standard Mix (Asta, zeax, canthax, beta-carot)","Hydroxyechinenone standard",
"Control", "DNP"))+
  scale_y_continuous(breaks=seq(0,8000,1000))+
  scale_x_continuous(breaks=seq(15,27,3))+
  ylab("Intensity (mv)")+ xlab("Time (min)")+
  annotate("text", x = 15.7, y = 4800, label = "1",size = 4, color ="black")+
  annotate("text", x = 17.4, y = 5300, label = "2*", size = 4, color ="black")+
  annotate("text", x = 19, y = 4800, label = "3*", size = 4, color ="black")+
  annotate("text", x = 20.5, y = 8000, label = "4", size = 4, color ="black")+
  annotate("text", x = 23.9, y = 6800, label = "5",  size = 4, color ="black")+
  annotate("text", x = 24, y = 8000, label = "*not detected in any copepod samples",  size = 3, color
="black")+
  guides(colour = guide_legend(override.aes = list(size=2), nrow = 2, ncol = 2))+
  theme(legend.position = "top", legend.direction = "horizontal")+
  theme(plot.margin = unit(c(0,0,0,0), "cm"))

#Print plot
jpeg(filename = "representative chromatogram.jpg", units = "in", width = 7, height = 5, res=500)
chromatogram.yeast
dev.off()
```