

# Boisseau & Woods 2024 - Analyses of phasmid egg physiological dataset

Romain Boisseau & Art Woods

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## Load Packages and data

Load necessary packages to run script

```
library(readxl)
library(mgcv)
library(ggplot2)
library(lme4)
library(car)
# Display versions of loaded packages
installed.packages()[names(sessionInfo())$otherPkgs], "Version"]
```

```
##      car   carData    lme4   Matrix  ggplot2    mgcv    nlme    readxl
##  "3.1-2"  "3.0-5"  "1.1-33" "1.5-4.1"  "3.4.2"   "1.8-42" "3.1-162" "1.4.2"
##  formatR  tinytex    knitr
##    "1.14"   "0.49"   "1.43"
```

## Egg metabolic rate

Load metabolic rate data

```
data <- read_excel("Dataset_S1_eggs.xlsx", sheet = "Full_MR_data")
datah <- subset(data, data$hatched == "y") #Only takes hatched eggs

# correct baseline
datah$MR_corrected <- datah$MR - 0.39

# delete missing data
w <- !is.na(datah$MR_corrected)
d1 <- datah[w, ]
v <- !is.na(d1$mass)
d <- d1[v, ]
```

## Metabolic rate across embryonic development

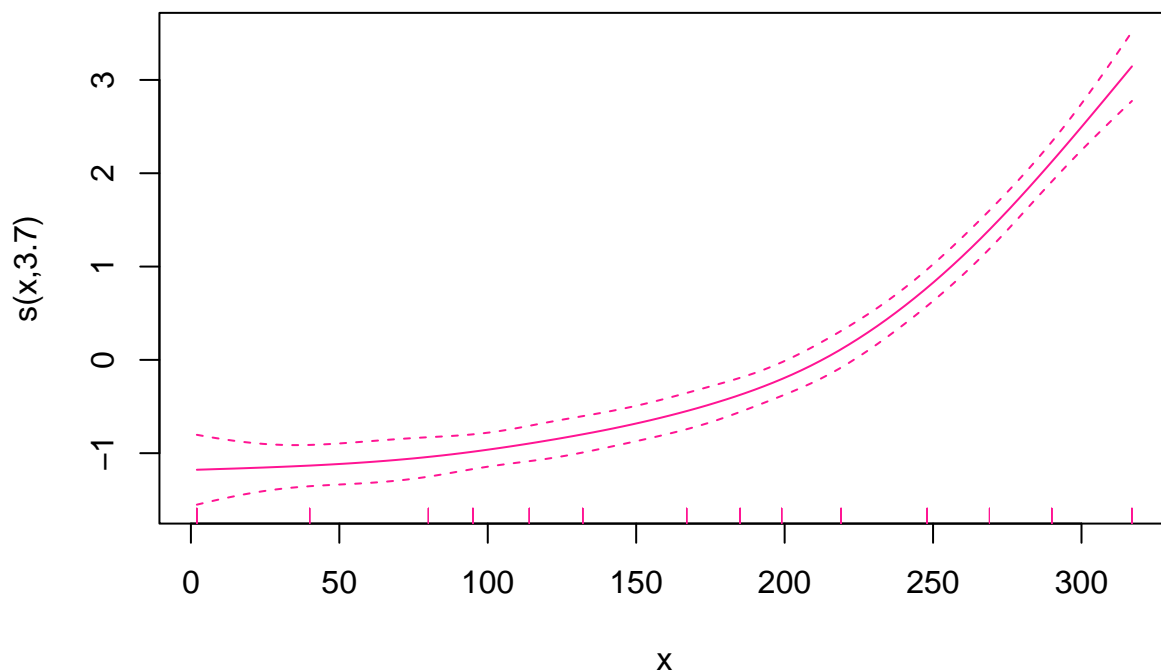
For each species: fit a generalized additive model to the metabolic rate trajectory over time.

### Species 1: *Heteropteryx dilatata*

```
d1 <- subset(d, d$species == "dilatata")

# Generalized additive model
y <- d1$MR_corrected # response
x <- d1$days_after_oviposition # predictor

g <- gam(y ~ s(x, k = 7, bs = "cr"))
plot(g, col = "deeppink")
```



```
mean(datah$incubation_time[datah$species == "dilatata"], na.rm = T) #341 days mean
↳ incubation for Heteropteryx dilatata
```

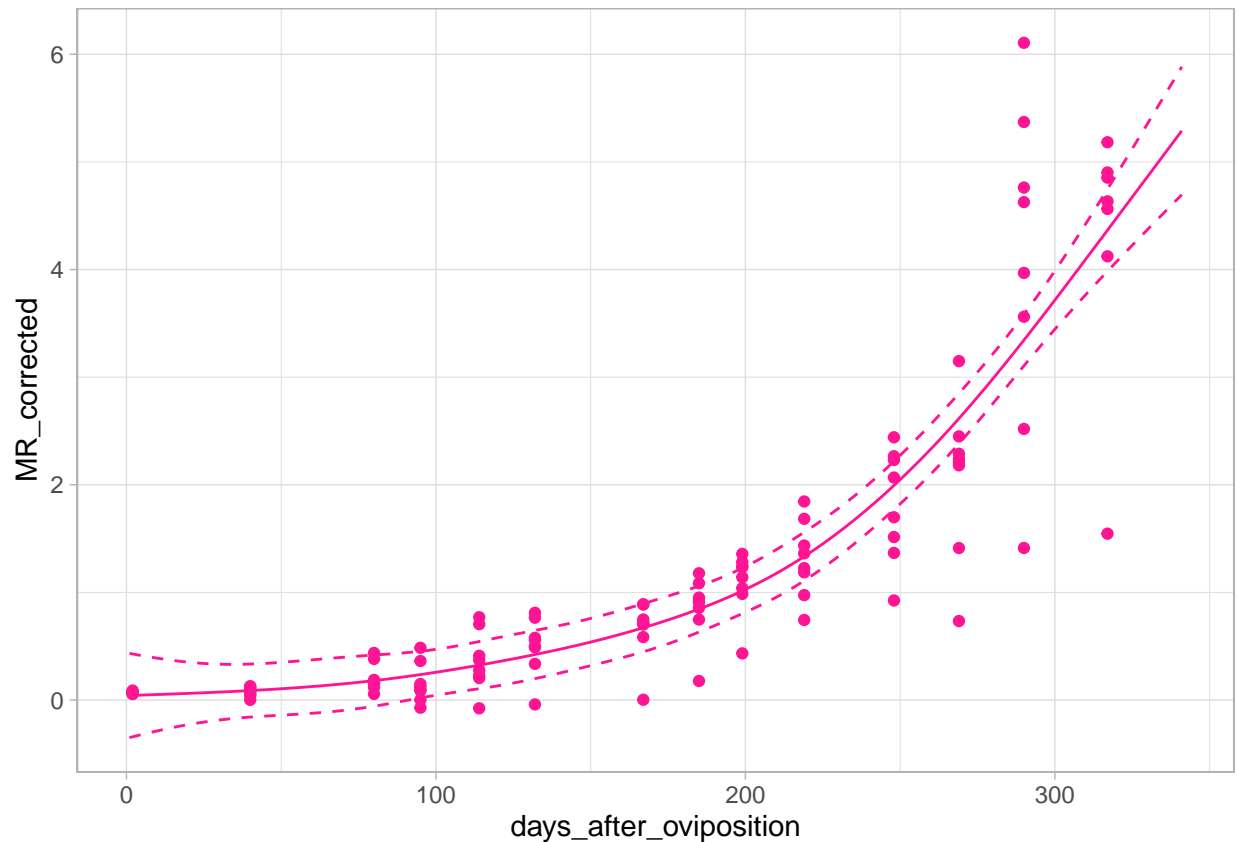
```
## [1] 340.625
```

```
pd <- data.frame(x = seq(1, 341, by = 1)) # fine grid of points
pr <- predict(g, newdata = pd, type = "response", se = TRUE) # get predicted response
↳ values from GAM
```

```

prr1 <- cbind(pd, pr)
ggplot(d1, aes(x = days_after_oviposition, y = MR_corrected)) +
  geom_point(color = "deeppink") + geom_line(data = prr1, aes(x,
fit), color = "deeppink") + geom_line(data = prr1, aes(x,
fit - qnorm(0.975) * se.fit), color = "deeppink", linetype = "dashed") +
  geom_line(data = prr1, aes(x, fit + qnorm(0.975) * se.fit),
color = "deeppink", linetype = "dashed") + theme_light()

```



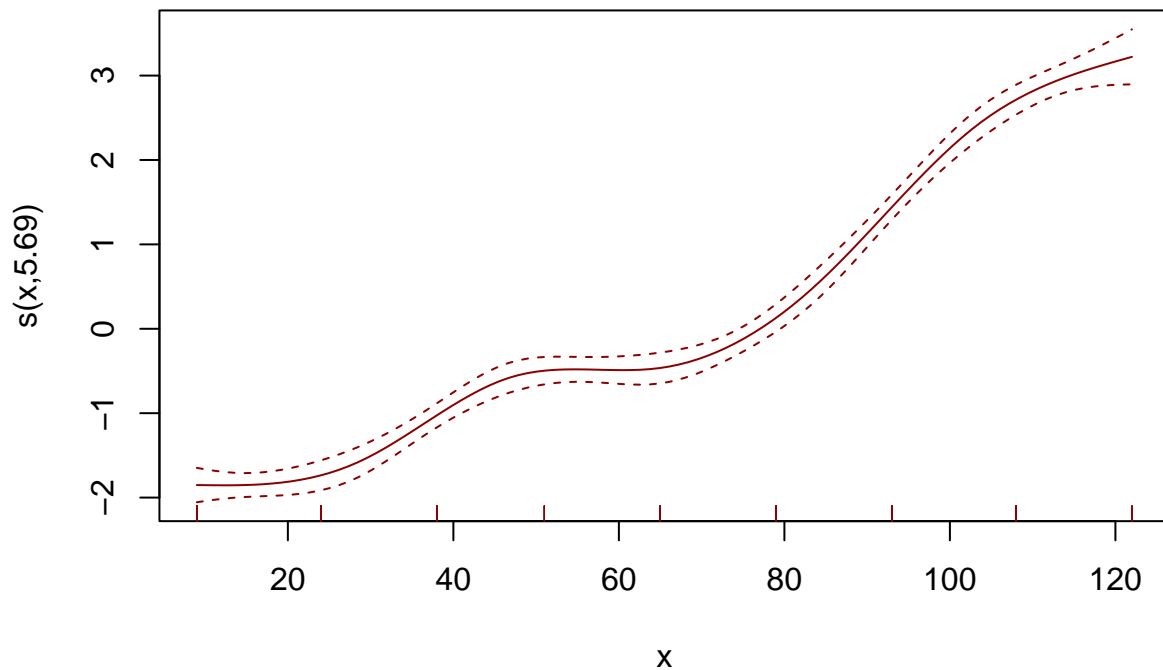
## Species 2: *Eurycantha calcarata*

```

d2 <- subset(d, d$species == "calcarata")

y <- d2$MR_corrected # response
x <- d2$days_after_oviposition # predictor
library(mgcv)
g <- gam(y ~ s(x, k = 7, bs = "cr"))
plot(g, col = "darkred")

```

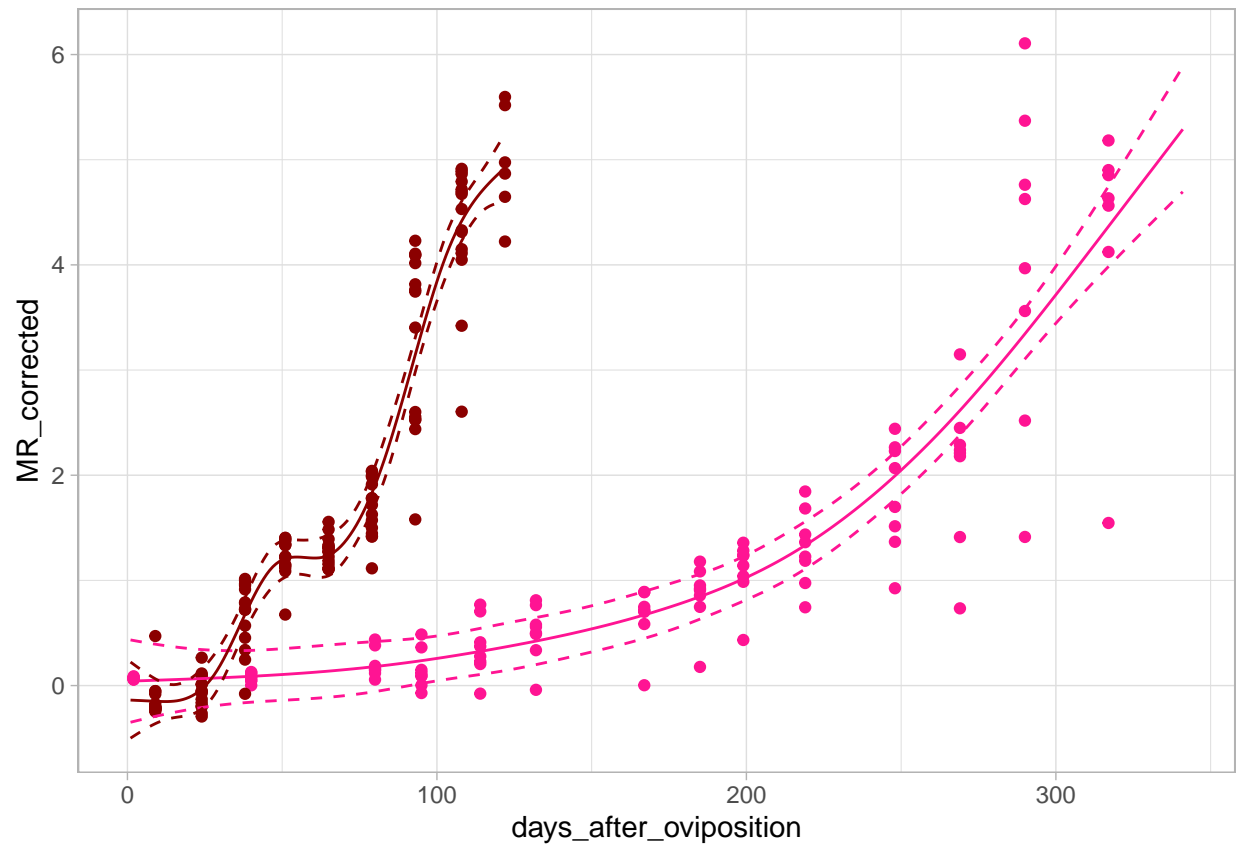


```
mean(datah$incubation_time[datah$species == "calcarata"], na.rm = T) #121 days mean
↳ incubation for Eurycantha calcarata
```

```
## [1] 120.7333
```

```
pd <- data.frame(x = seq(1, 121, by = 1)) # fine grid of points
pr <- predict(g, newdata = pd, type = "response", se = TRUE) # get predicted response
↳ values from GAM
```

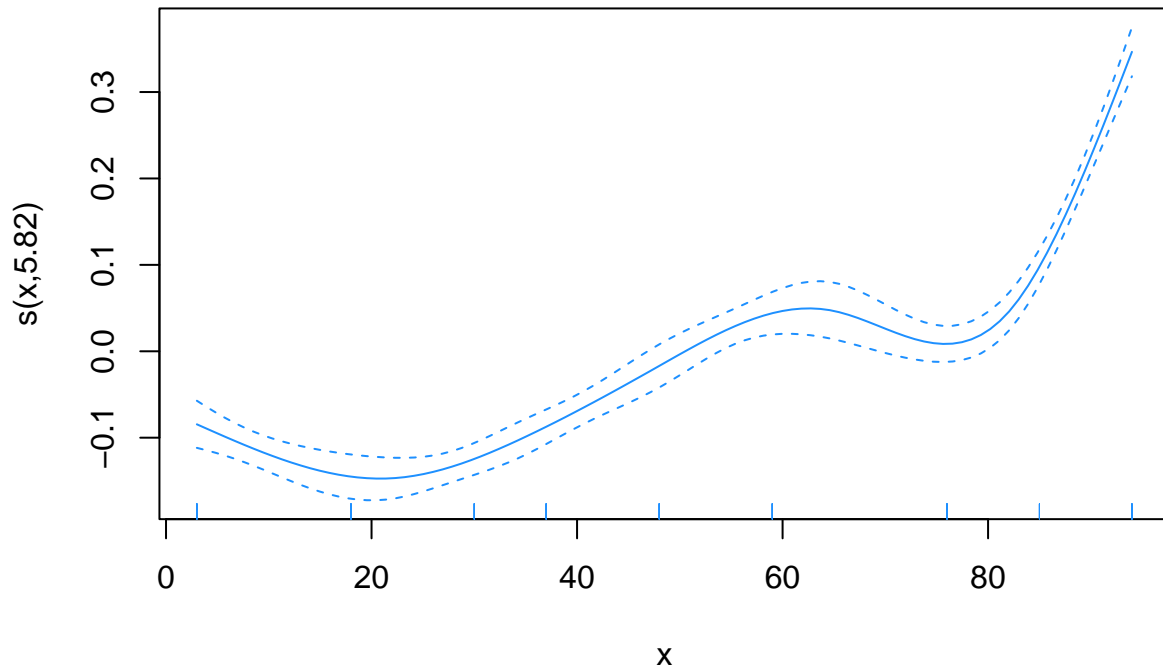
```
prr2 <- cbind(pd, pr)
ggplot(d1, aes(x = days_after_oviposition, y = MR_corrected)) +
  geom_point(color = "deeppink") + geom_point(data = d2, color = "darkred") +
  geom_line(data = prr1, aes(x, fit), color = "deeppink") +
  geom_line(data = prr1, aes(x, fit - qnorm(0.975) * se.fit),
    color = "deeppink", linetype = "dashed") + geom_line(data = prr1,
    aes(x, fit + qnorm(0.975) * se.fit), color = "deeppink",
    linetype = "dashed") + geom_line(data = prr2, aes(x, fit),
    color = "darkred") + geom_line(data = prr2, aes(x, fit -
    qnorm(0.975) * se.fit), color = "darkred", linetype = "dashed") +
  geom_line(data = prr2, aes(x, fit + qnorm(0.975) * se.fit),
    color = "darkred", linetype = "dashed") + theme_light()
```



### Species 3: *Medauroidea extradentata*

```
d3 <- subset(d, d$species == "extradentata")

y <- d3$MR_corrected # response
x <- d3$days_after_oviposition # predictor
library(mgcv)
g <- gam(y ~ s(x, k = 7, bs = "cr"))
plot(g, col = "dodgerblue")
```



```
mean(datah$incubation_time[datah$species == "extradentata"],
      na.rm = T) #98 days mean incubation for Medauroidea extradentata
```

```
## [1] 97.66667
```

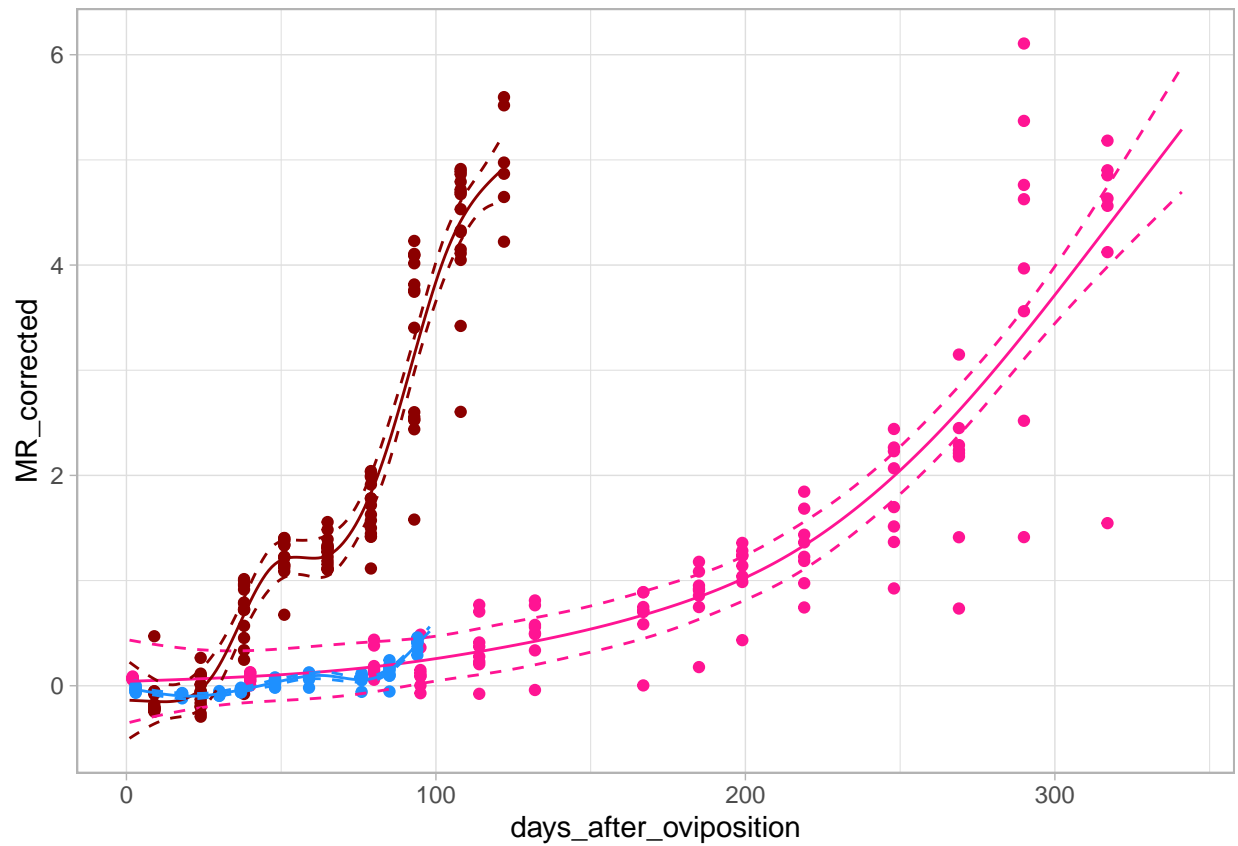
```
pd <- data.frame(x = seq(1, 98, by = 1)) # fine grid of points
pr <- predict(g, newdata = pd, type = "response", se = TRUE) # get predicted response
↪ values from GAM
```

```
prr3 <- cbind(pd, pr)
library(ggplot2)
ggplot(d1, aes(x = days_after_oviposition, y = MR_corrected)) +
  geom_point(color = "deeppink") + geom_point(data = d2, color = "darkred") +
  geom_point(data = d3, color = "dodgerblue") + geom_line(data = prr1,
  aes(x, fit), color = "deeppink") + geom_line(data = prr1,
  aes(x, fit - qnorm(0.975) * se.fit), color = "deeppink",
  linetype = "dashed") + geom_line(data = prr1, aes(x, fit +
  qnorm(0.975) * se.fit), color = "deeppink", linetype = "dashed") +
  geom_line(data = prr2, aes(x, fit), color = "darkred") +
  geom_line(data = prr2, aes(x, fit - qnorm(0.975) * se.fit),
  color = "darkred", linetype = "dashed") + geom_line(data = prr2,
  aes(x, fit + qnorm(0.975) * se.fit), color = "darkred", linetype = "dashed") +
  geom_line(data = prr3, aes(x, fit), color = "dodgerblue") +
  geom_line(data = prr3, aes(x, fit - qnorm(0.975) * se.fit),
  color = "dodgerblue", linetype = "dashed") + geom_line(data = prr3,
```

```

aes(x, fit + qnorm(0.975) * se.fit), color = "dodgerblue",
linetype = "dashed") + theme_light()

```



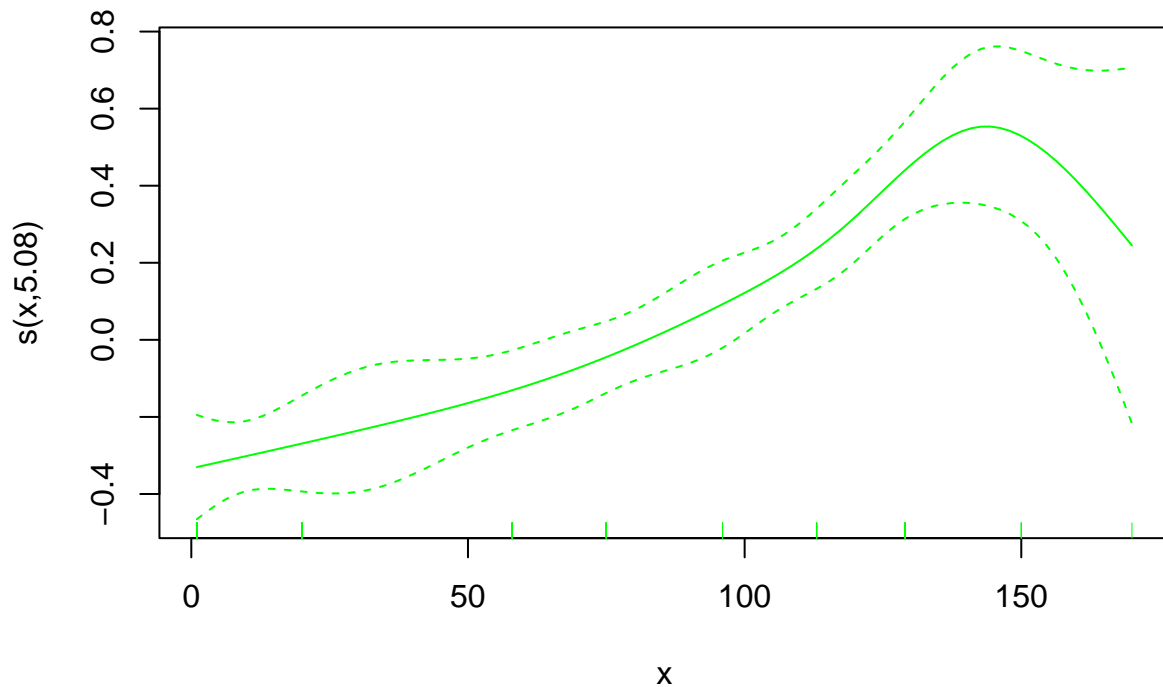
#### Species 4: *Phyllium philippinicum*

```

d4 <- subset(d, d$species == "philippinicum")

y <- d4$MR_corrected # response
x <- d4$days_after_oviposition # predictor
library(mgcv)
g <- gam(y ~ s(x, k = 7, bs = "cr"))
plot(g, col = "green")

```



```
mean(datah$incubation_time[datah$species == "philippinicum"],
      na.rm = T)  #139 days mean incubation for Phyllium philippinicum
```

```
## [1] 137.8615
```

```
pd <- data.frame(x = seq(1, 139, by = 1)) # fine grid of points
pr <- predict(g, newdata = pd, type = "response", se = TRUE) # get predicted response
↪ values from GAM
```

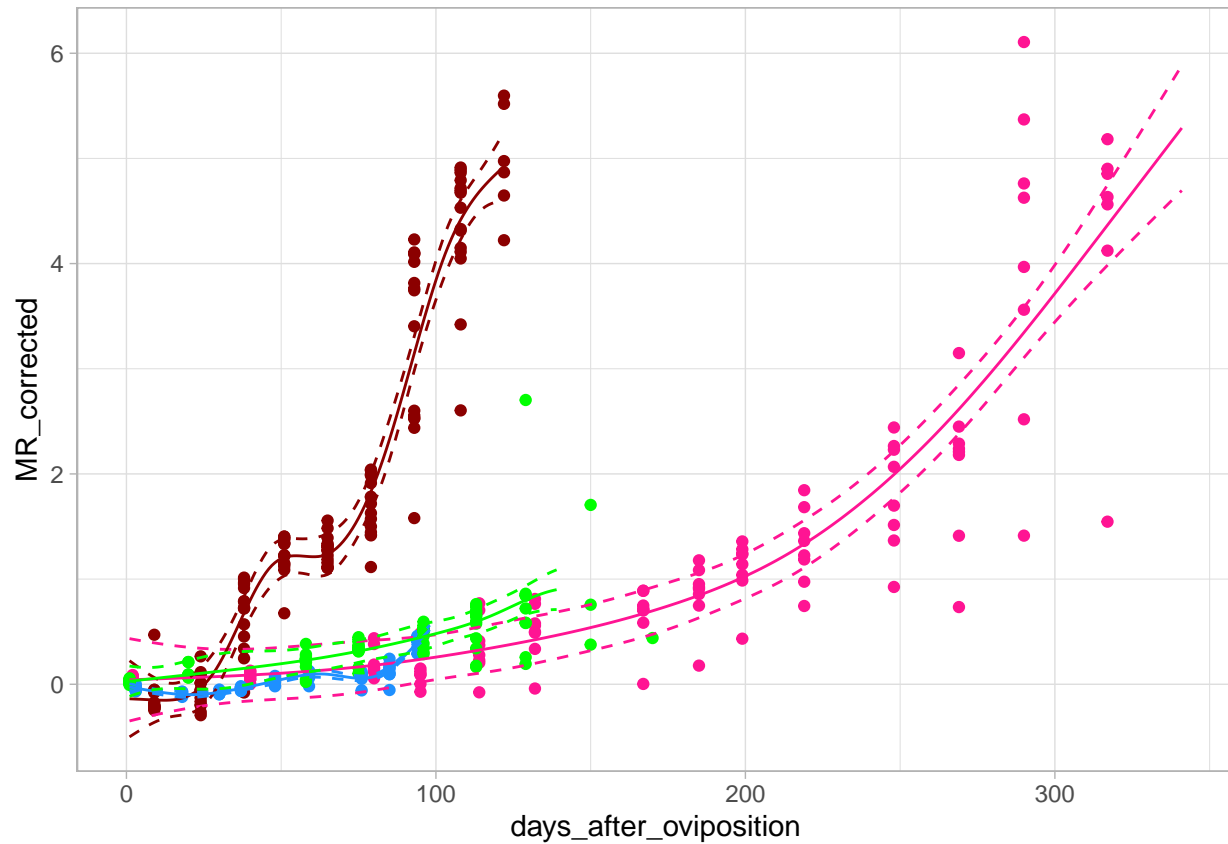
```
prr4 <- cbind(pd, pr)
library(ggplot2)
ggplot(d1, aes(x = days_after_oviposition, y = MR_corrected)) +
  geom_point(color = "deeppink") + geom_point(data = d2, color = "darkred") +
  geom_point(data = d3, color = "dodgerblue") + geom_point(data = d4,
  color = "green") + geom_line(data = prr1, aes(x, fit), color = "deeppink") +
  geom_line(data = prr1, aes(x, fit - qnorm(0.975) * se.fit),
  color = "deeppink", linetype = "dashed") + geom_line(data = prr1,
  aes(x, fit + qnorm(0.975) * se.fit), color = "deeppink",
  linetype = "dashed") + geom_line(data = prr2, aes(x, fit),
  color = "darkred") + geom_line(data = prr2, aes(x, fit -
  qnorm(0.975) * se.fit), color = "darkred", linetype = "dashed") +
  geom_line(data = prr2, aes(x, fit + qnorm(0.975) * se.fit),
  color = "darkred", linetype = "dashed") + geom_line(data = prr3,
  aes(x, fit), color = "dodgerblue") + geom_line(data = prr3,
  aes(x, fit - qnorm(0.975) * se.fit), color = "dodgerblue",
```



```

linetype = "dashed") + geom_line(data = prr3, aes(x, fit +
qnorm(0.975) * se.fit), color = "dodgerblue", linetype = "dashed") +
geom_line(data = prr4, aes(x, fit), color = "green") + geom_line(data = prr4,
aes(x, fit - qnorm(0.975) * se.fit), color = "green", linetype = "dashed") +
geom_line(data = prr4, aes(x, fit + qnorm(0.975) * se.fit),
color = "green", linetype = "dashed") + theme_light()

```



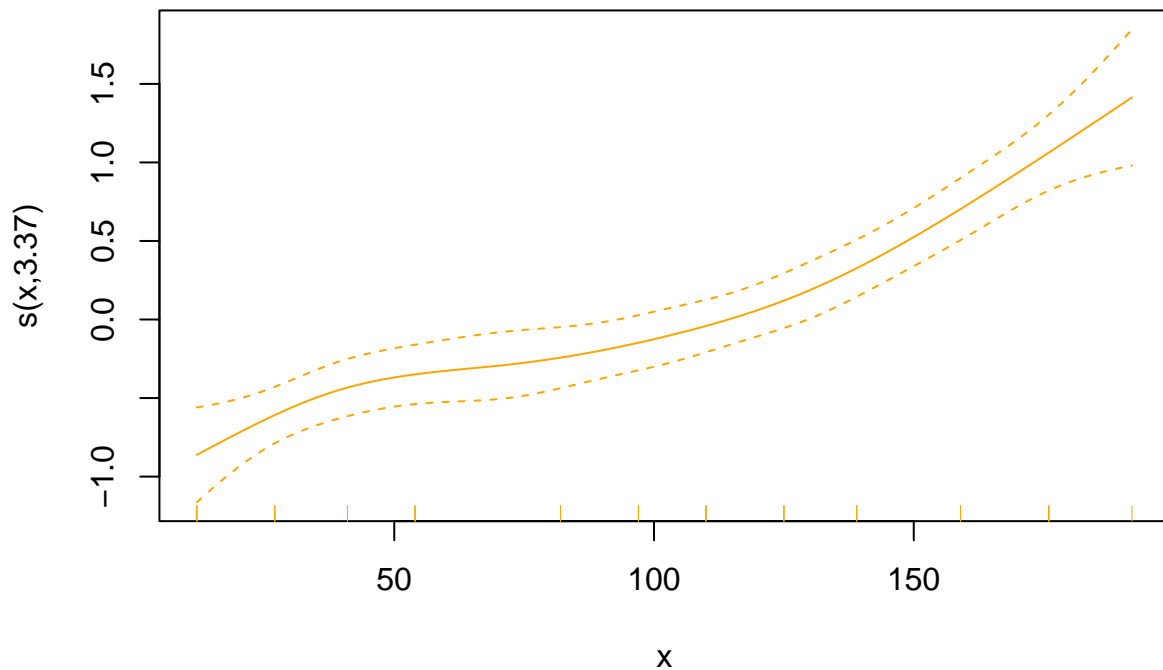
#### Species 5: *Extatosoma tiaratum*

```

d5 <- subset(d, d$species == "tiaratum")

y <- d5$MR_corrected # response
x <- d5$days_after_oviposition # predictor
library(mgcv)
g <- gam(y ~ s(x, k = 7, bs = "cr"))
plot(g, col = "orange")

```



```
mean(datah$incubation_time[datah$species == "tiaratum"], na.rm = T) #193 days mean
↪ incubation for Extatosoma tiaratum
```

```
## [1] 192.6667
```

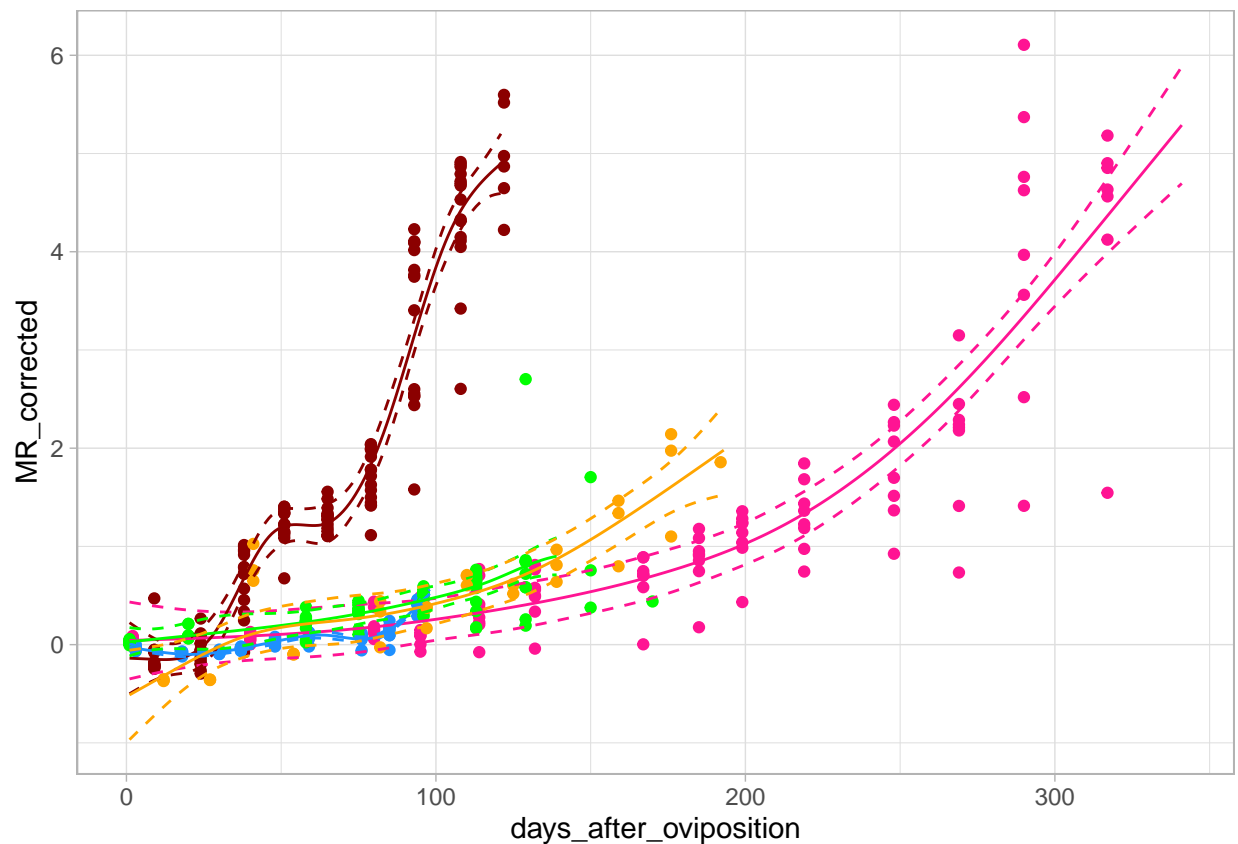
```
pd <- data.frame(x = seq(1, 193, by = 1)) # fine grid of points
pr <- predict(g, newdata = pd, type = "response", se = TRUE) # get predicted response
↪ values from GAM
```

```
prp5 <- cbind(pd, pr)
library(ggplot2)
ggplot(d1, aes(x = days_after_oviposition, y = MR_corrected)) +
  geom_point(color = "deeppink") + geom_point(data = d2, color = "darkred") +
  geom_point(data = d3, color = "dodgerblue") + geom_point(data = d4,
  color = "green") + geom_point(data = d5, color = "orange") +
  geom_line(data = prr1, aes(x, fit), color = "deeppink") +
  geom_line(data = prr1, aes(x, fit - qnorm(0.975) * se.fit),
  color = "deeppink", linetype = "dashed") + geom_line(data = prr1,
  aes(x, fit + qnorm(0.975) * se.fit), color = "deeppink",
  linetype = "dashed") + geom_line(data = prr2, aes(x, fit),
  color = "darkred") + geom_line(data = prr2, aes(x, fit -
  qnorm(0.975) * se.fit), color = "darkred", linetype = "dashed") +
  geom_line(data = prr2, aes(x, fit + qnorm(0.975) * se.fit),
  color = "darkred", linetype = "dashed") + geom_line(data = prr3,
  aes(x, fit), color = "dodgerblue") + geom_line(data = prr3,
```

```

aes(x, fit - qnorm(0.975) * se.fit), color = "dodgerblue",
linetype = "dashed") + geom_line(data = prr3, aes(x, fit +
qnorm(0.975) * se.fit), color = "dodgerblue", linetype = "dashed") +
geom_line(data = prr4, aes(x, fit), color = "green") + geom_line(data = prr4,
aes(x, fit - qnorm(0.975) * se.fit), color = "green", linetype = "dashed") +
geom_line(data = prr4, aes(x, fit + qnorm(0.975) * se.fit),
color = "green", linetype = "dashed") + geom_line(data = prr5,
aes(x, fit), color = "orange") + geom_line(data = prr5, aes(x,
fit - qnorm(0.975) * se.fit), color = "orange", linetype = "dashed") +
geom_line(data = prr5, aes(x, fit + qnorm(0.975) * se.fit),
color = "orange", linetype = "dashed") + theme_light()

```

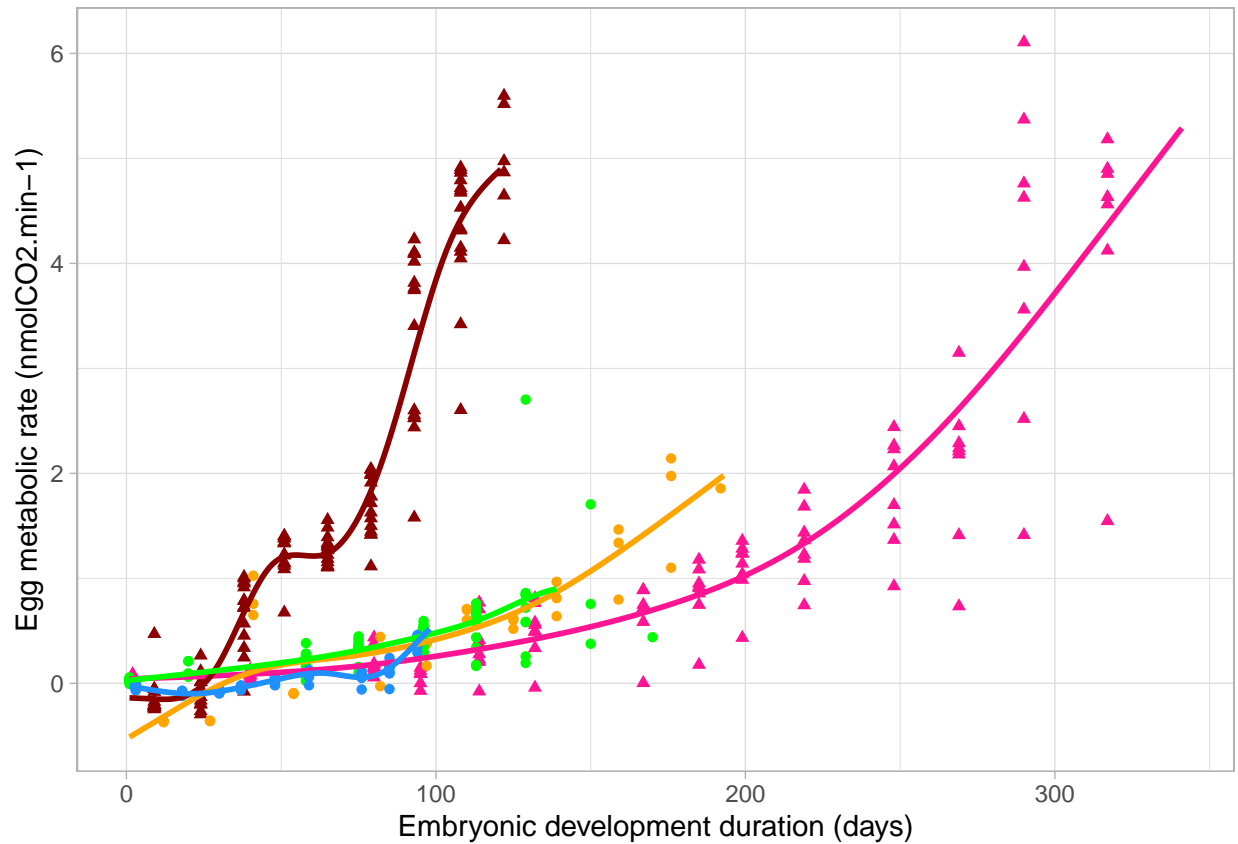


```

p0 <- ggplot(d1, aes(x = days_after_oviposition, y = MR_corrected)) +
geom_point(color = "deeppink", size = 1.5, shape = 17) +
geom_point(data = d2, color = "darkred", size = 1.5, shape = 17) +
geom_point(data = d5, color = "orange", size = 1.5, shape = 16) +
geom_point(data = d4, color = "green", size = 1.5, shape = 16) +
geom_point(data = d3, color = "dodgerblue", size = 1.5, shape = 16) +
geom_line(data = prr1, aes(x, fit), color = "deeppink", size = 1) +
geom_line(data = prr2, aes(x, fit), color = "darkred", size = 1) +
geom_line(data = prr5, aes(x, fit), color = "orange", size = 1) +
geom_line(data = prr4, aes(x, fit), color = "green", size = 1) +
geom_line(data = prr3, aes(x, fit), color = "dodgerblue",
size = 1) + xlab("Embryonic development duration (days)") +
ylab("Egg metabolic rate (nmolCO2.min-1)") + theme_light()

```

p0



## Metabolic rate summary data

Reconstruct each individual egg's CO<sub>2</sub> production and extract summary data

```
tot <- data.frame(matrix(nrow = 80, ncol = 9))
colnames(tot) <- c("species", "egg_ID", "mass", "mass_shell",
  "total_energy", "chamber", "incubation_time", "MR_mid", "max_MR")
```

For each species and eggs reconstruct metabolic trajectory across development

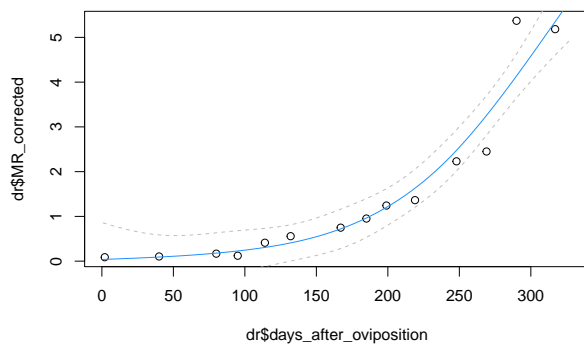
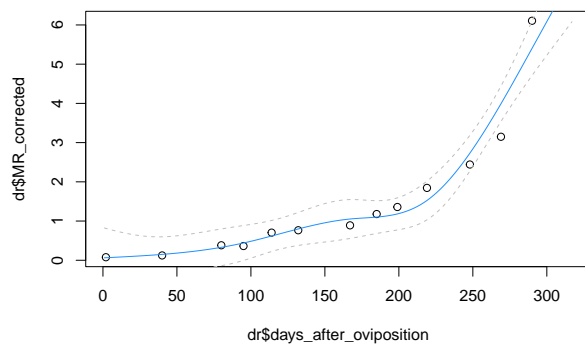
### Heteropteryx dilatata

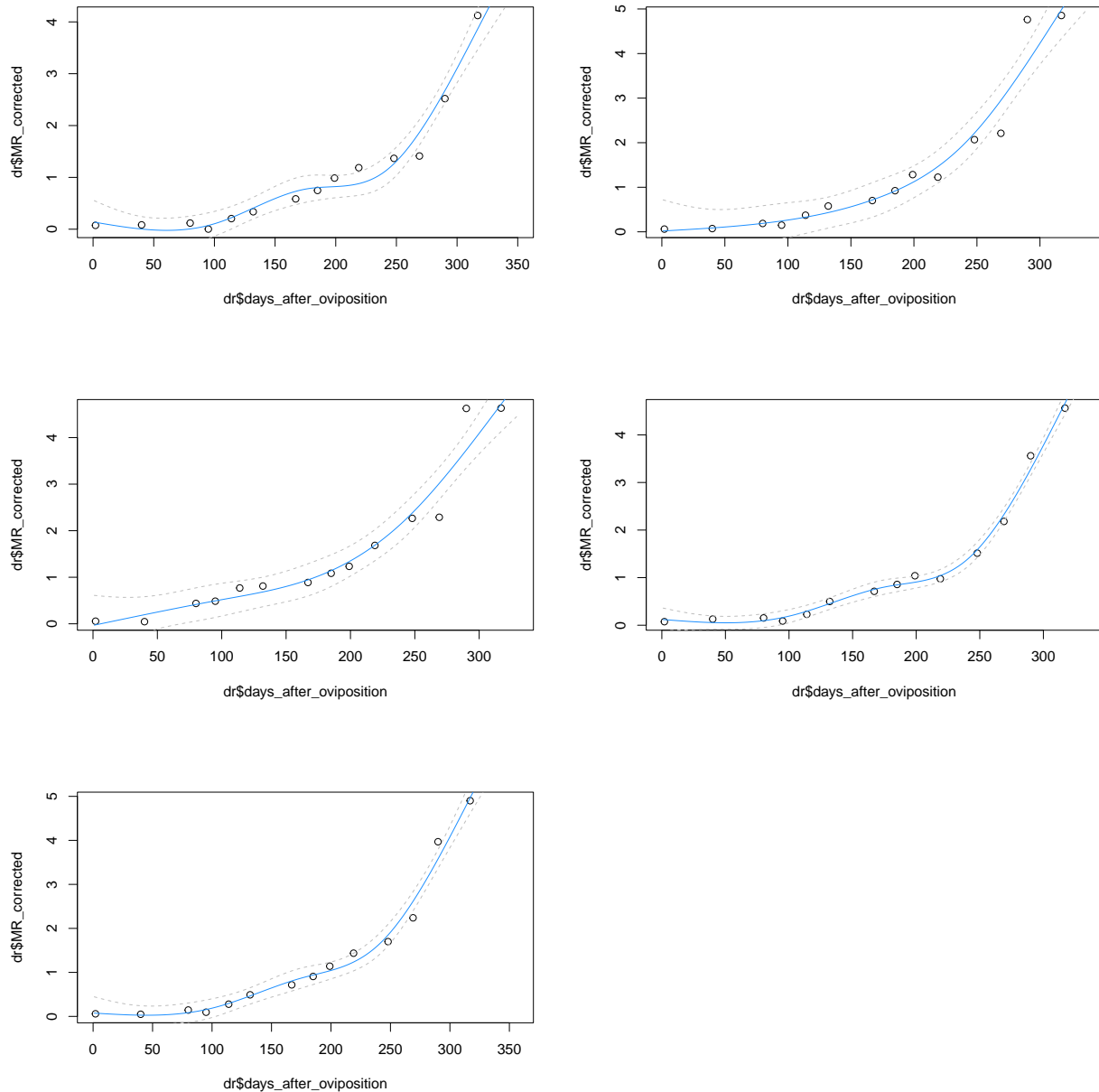
```
dd <- d1
j <- 1
for (i in unique(dd$egg_ID)) {
  if (i != 61) {
    dr <- subset(dd, dd$egg_ID == i)
    y <- dr$MR_corrected # response
    x <- dr$days_after_oviposition # predictor
```

```

    ga <- gam(y ~ s(x, k = 5, bs = "cr")) #the number of knots k is reduced from 7
    to 5 because we are fitting a different gam to each individual egg which reduces
    sample size.
    inc <- mean(dr$incubation_time, na.rm = T)
    pd <- data.frame(x = seq(1, round(inc), by = 1))
    pr <- predict(ga, newdata = pd, type = "response", se = TRUE) # get predicted
    response values from GAM
    plot(dr$days_after_oviposition, dr$MR_corrected, xlim = c(1,
    round(inc)))
    lines(pd$x, pr$fit, col = "dodgerblue") # plot predicted fit
    lines(pd$x, pr$fit - qnorm(0.975) * pr$se.fit, lty = 2,
    col = "gray") # plot lower 95% CI endpoint
    lines(pd$x, pr$fit + qnorm(0.975) * pr$se.fit, lty = 2,
    col = "gray") # plot upper 95% CI endpoint
    sum(pr$fit * 60 * 24, na.rm = na.omit)
    tot$species[j] = "dilatatata"
    tot$egg_ID[j] = i
    tot$mass[j] = median(dr$mass, na.rm = T)
    tot$mass_shell[j] = median(dr$egg_shell_mass, na.rm = T)
    tot$incubation_time[j] = median(dr$incubation_time, na.rm = T)
    # tot$total_energy[j]=sum(pr$fit*60*24,
    # na.rm=na.omit)
    tot$max_MR[j] <- max(dr$MR_corrected, na.rm = T)
    tot$MR_mid[j] <- pr$fit[round(median(dr$incubation_time,
    na.rm = T)/2)]
    tot$total_energy[j] = sum(subset(pr$fit, pr$fit > 0) *
    60 * 24, na.rm = na.omit)
    tot$chamber[j] = mean(dr$chamber, na.rm = T)
    j <- j + 1
  } else {
  }
}

```





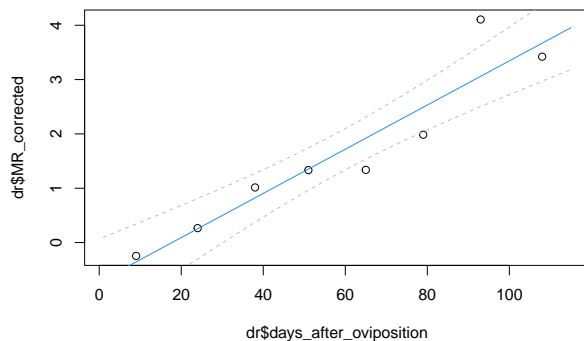
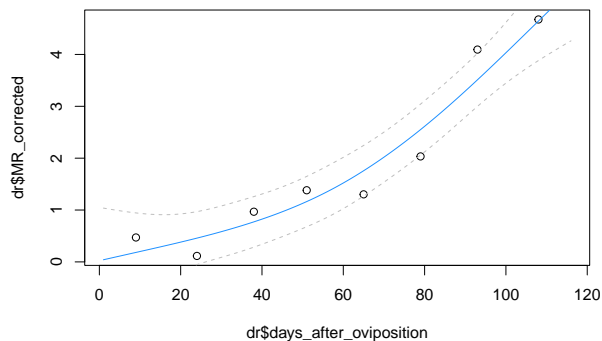
## Eurycantha calcarata

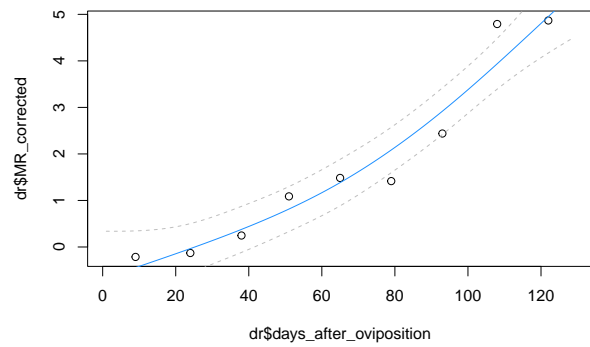
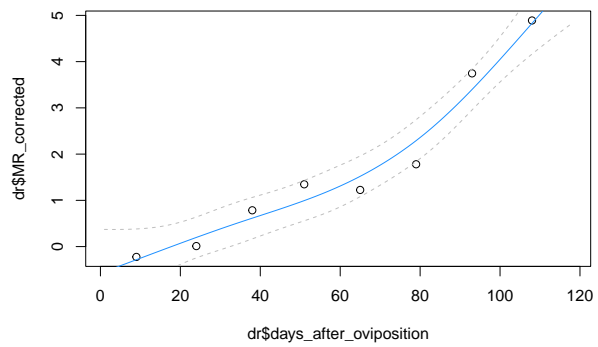
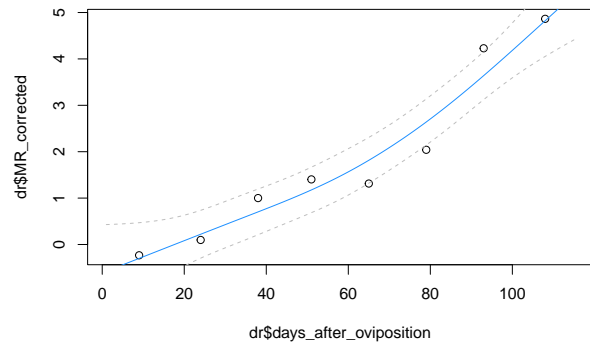
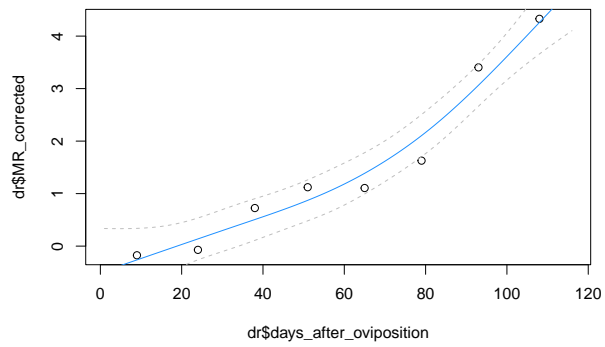
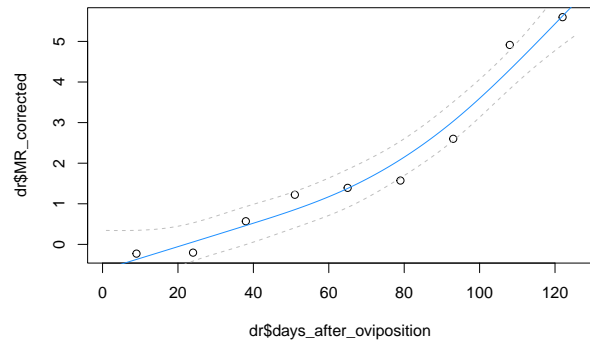
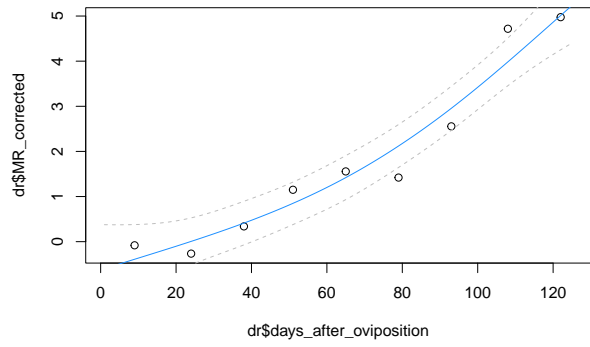
```
dd <- d2
for (i in unique(dd$egg_ID)) {
  dr <- subset(dd, dd$egg_ID == i)
  y <- dr$MR_corrected # response
  x <- dr$days_after_oviposition # predictor
  ga <- gam(y ~ s(x, k = 5, bs = "cr")) #the number of knots k is reduced from 7 to 5
  ↪ because we are fitting a different gam to each individual egg which reduces sample
  ↪ size.
```

```

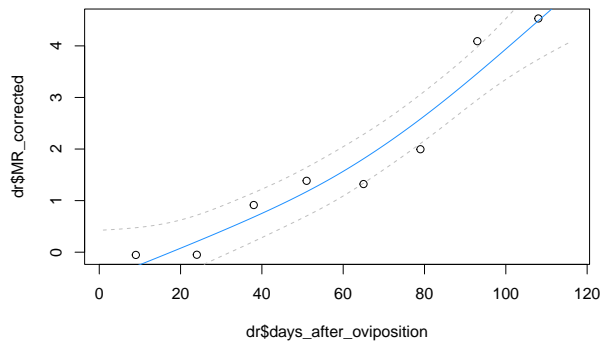
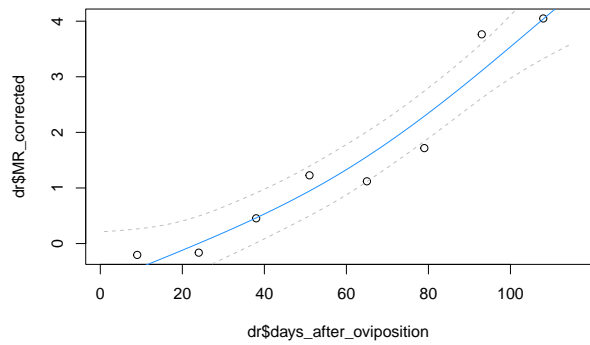
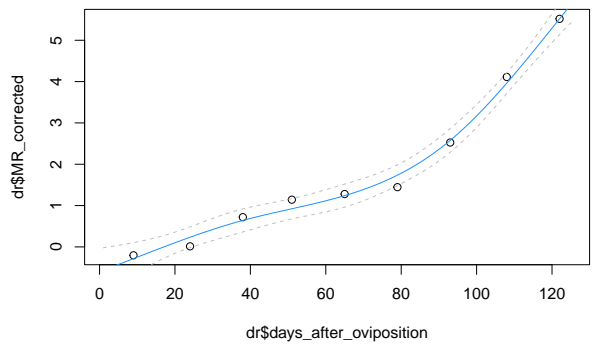
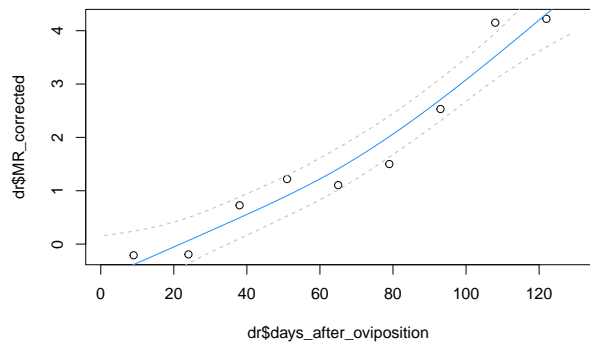
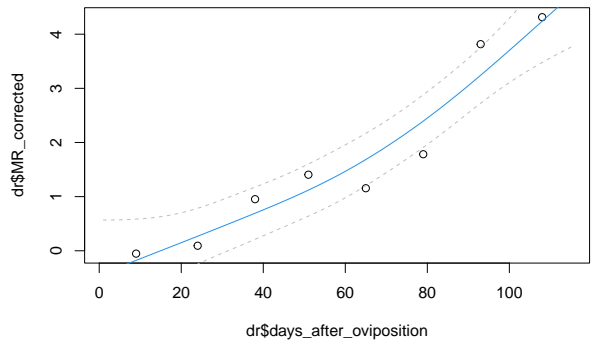
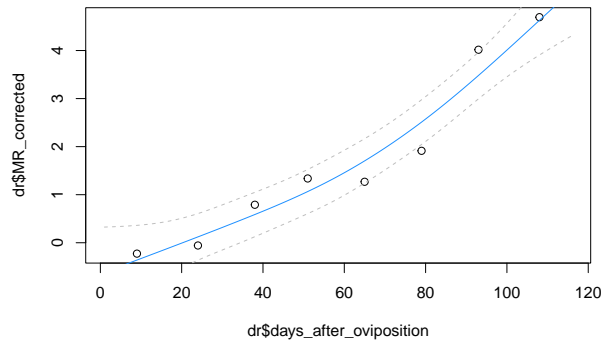
inc <- mean(dr$incubation_time, na.rm = T)
pd <- data.frame(x = seq(1, round(inc), by = 1))
pr <- predict(ga, newdata = pd, type = "response", se = TRUE) # get predicted
↪ response values from GAM
plot(dr$days_after_oviposition, dr$MR_corrected, xlim = c(1,
  round(inc)))
lines(pd$x, pr$fit, col = "dodgerblue") # plot predicted fit
lines(pd$x, pr$fit - qnorm(0.975) * pr$se.fit, lty = 2, col = "gray") # plot lower
↪ 95% CI endpoint
lines(pd$x, pr$fit + qnorm(0.975) * pr$se.fit, lty = 2, col = "gray") # plot upper
↪ 95% CI endpoint
sum(pr$fit * 60 * 24, na.rm = na.omit)
tot$species[j] = "calcarata"
tot$egg_ID[j] = i
tot$mass[j] = median(dr$mass, na.rm = T)
tot$mass_shell[j] = median(dr$egg_shell_mass, na.rm = T)
tot$incubation_time[j] = median(dr$incubation_time, na.rm = T)
# tot$total_energy[j]=sum(pr$fit*60*24, na.rm=na.omit)
tot$max_MR[j] <- max(dr$MR_corrected, na.rm = T)
tot$MR_mid[j] <- pr$fit[round(median(dr$incubation_time,
  na.rm = T)/2)]
tot$total_energy[j] = sum(subset(pr$fit, pr$fit > 0) * 60 *
  24, na.rm = na.omit)
tot$chamber[j] = mean(dr$chamber, na.rm = T)
j <- j + 1
}

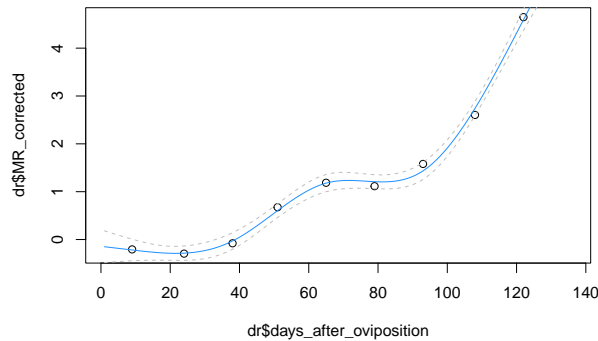
```











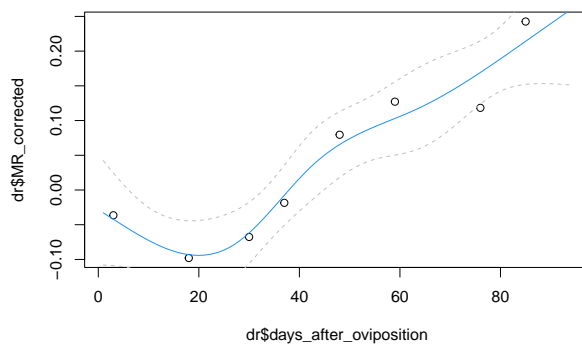
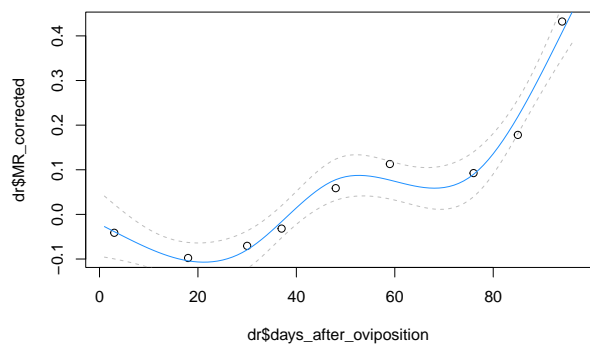
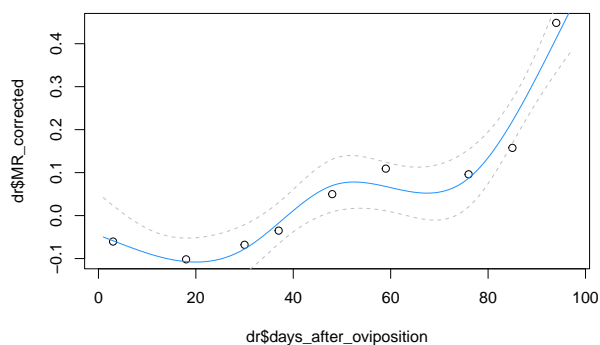
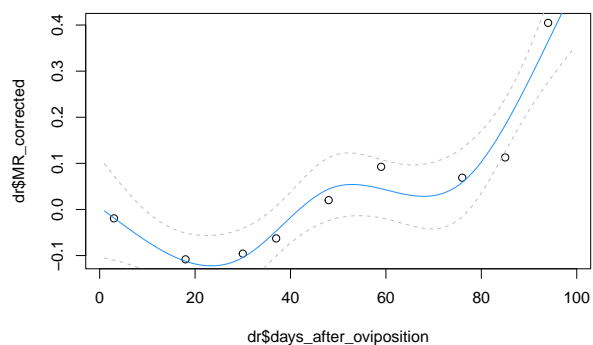
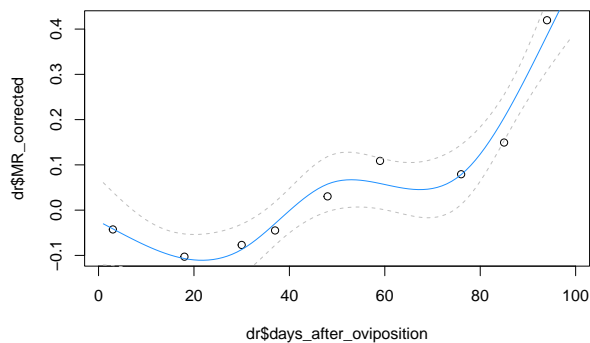
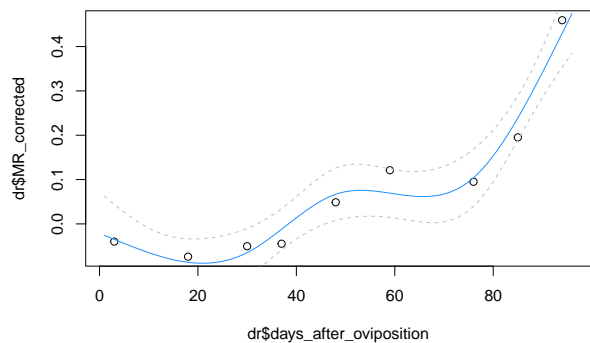
### Medauroidea extradentata

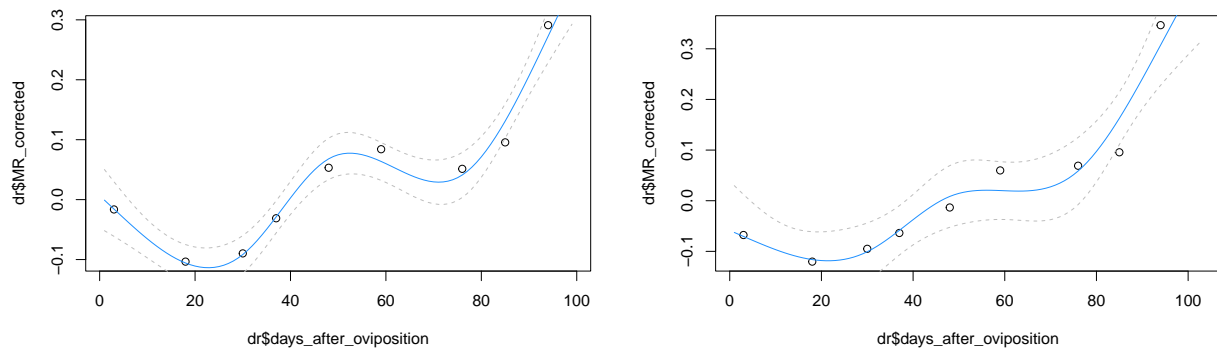
```
dd <- d3
for (i in unique(dd$egg_ID)) {
  if (i != 76 & i != 211 & i != 183) {
    dr <- subset(dd, dd$egg_ID == i)
    y <- dr$MR_corrected # response
    x <- dr$days_after_oviposition # predictor
    ga <- gam(y ~ s(x, k = 5, bs = "cr")) #the number of knots k is reduced from 7
    ↪ to 5 because we are fitting a different gam to each individual egg which reduces
    ↪ sample size.
    inc <- mean(dr$incubation_time, na.rm = T)
    pd <- data.frame(x = seq(1, round(inc), by = 1))
    pr <- predict(ga, newdata = pd, type = "response", se = TRUE) # get predicted
    ↪ response values from GAM
    plot(dr$days_after_oviposition, dr$MR_corrected, xlim = c(1,
      round(inc)))
    lines(pd$x, pr$fit, col = "dodgerblue") # plot predicted fit
    lines(pd$x, pr$fit - qnorm(0.975) * pr$se.fit, lty = 2,
      col = "gray") # plot lower 95% CI endpoint
    lines(pd$x, pr$fit + qnorm(0.975) * pr$se.fit, lty = 2,
      col = "gray") # plot upper 95% CI endpoint
    sum(pr$fit * 60 * 24, na.rm = na.omit)
    tot$species[j] = "extradentata"
    tot$egg_ID[j] = i
    tot$mass[j] = median(dr$mass, na.rm = T)
    tot$mass_shell[j] = median(dr$egg_shell_mass, na.rm = T)
    tot$incubation_time[j] = median(dr$incubation_time, na.rm = T)
    # tot$total_energy[j]=sum(pr$fit*60*24,
    # na.rm=na.omit)
    tot$max_MR[j] <- max(dr$MR_corrected, na.rm = T)
    tot$MR_mid[j] <- pr$fit[round(median(dr$incubation_time,
      na.rm = T)/2)]
    tot$total_energy[j] = sum(subset(pr$fit, pr$fit > 0) *
      60 * 24, na.rm = na.omit)
    tot$chamber[j] = mean(dr$chamber, na.rm = T)
    j <- j + 1
  }
}
```

```

    } else {
    }
}

```





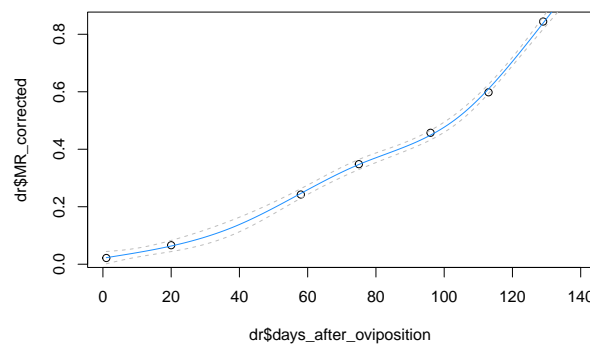
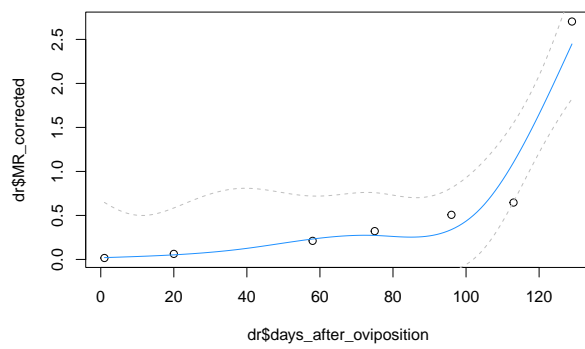
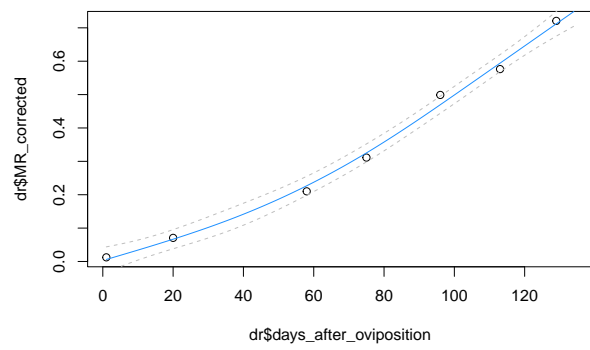
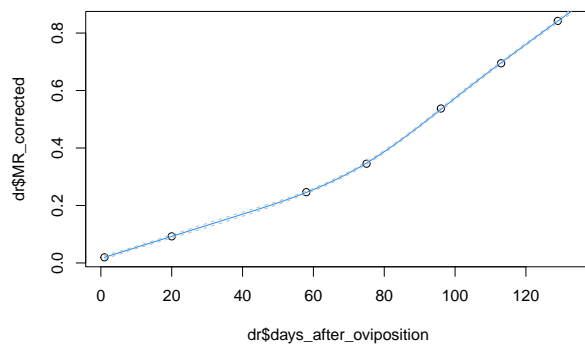
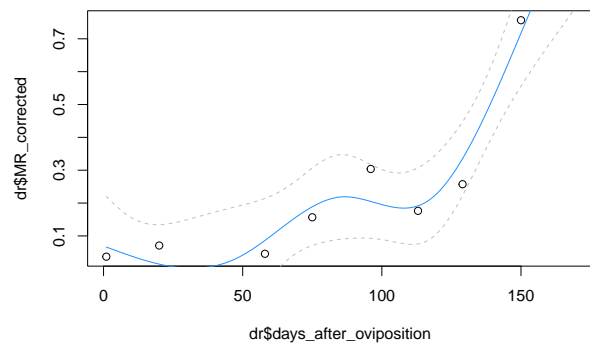
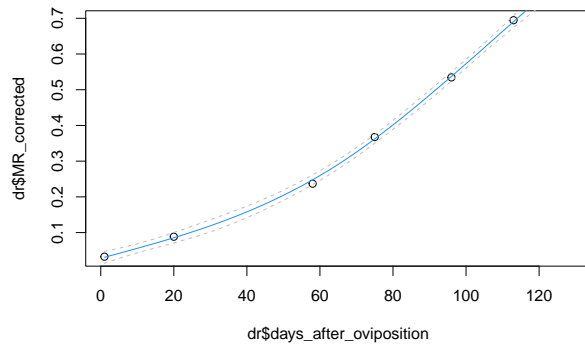
## Phyllium philippinicum

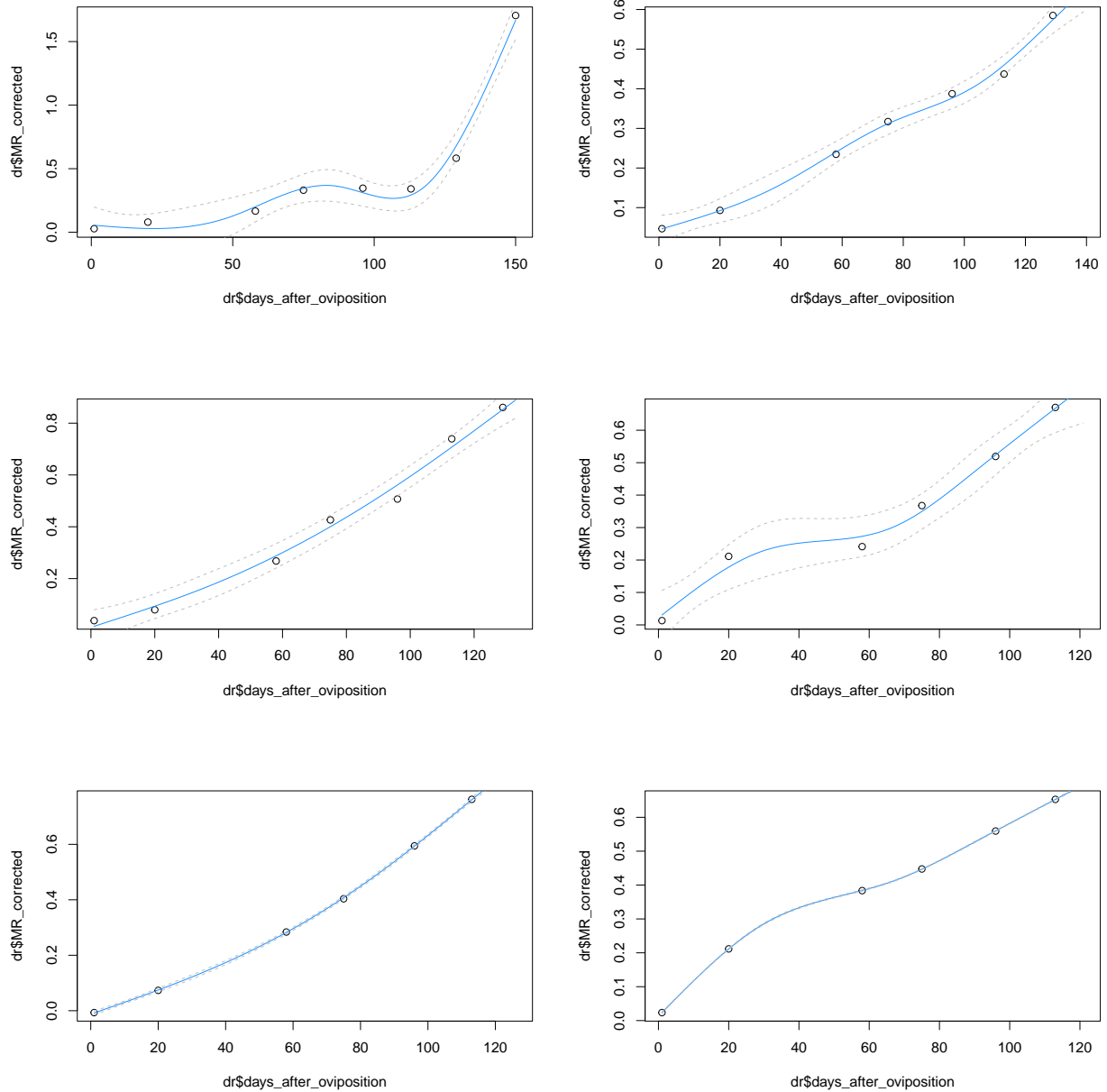
```
dd <- d4
for (i in unique(dd$egg_ID)) {
  if (i != 33) {
    dr <- subset(dd, dd$egg_ID == i)
    y <- dr$MR_corrected # response
    x <- dr$days_after_oviposition # predictor
    ga <- gam(y ~ s(x, k = 5, bs = "cr")) #the number of knots k is reduced from 7
    ↪ to 5 because we are fitting a different gam to each individual egg which reduces
    ↪ sample size.
    inc <- mean(dr$incubation_time, na.rm = T)
    pd <- data.frame(x = seq(1, round(inc), by = 1))
    pr <- predict(ga, newdata = pd, type = "response", se = TRUE) # get predicted
    ↪ response values from GAM
    plot(dr$days_after_oviposition, dr$MR_corrected, xlim = c(1,
      round(inc)))
    lines(pd$x, pr$fit, col = "dodgerblue") # plot predicted fit
    lines(pd$x, pr$fit - qnorm(0.975) * pr$se.fit, lty = 2,
      col = "gray") # plot lower 95% CI endpoint
    lines(pd$x, pr$fit + qnorm(0.975) * pr$se.fit, lty = 2,
      col = "gray") # plot upper 95% CI endpoint
    sum(pr$fit * 60 * 24, na.rm = na.omit)
    tot$species[j] = "philippinicum"
    tot$egg_ID[j] = i
    tot$mass[j] = median(dr$mass, na.rm = T)
    tot$mass_shell[j] = median(dr$egg_shell_mass, na.rm = T)
    tot$incubation_time[j] = median(dr$incubation_time, na.rm = T)
    # tot$total_energy[j]=sum(pr$fit*60*24,
    # na.rm=na.omit)
    tot$max_MR[j] <- max(dr$MR_corrected, na.rm = T)
    tot$MR_mid[j] <- pr$fit[round(median(dr$incubation_time,
      na.rm = T)/2)]
    tot$total_energy[j] = sum(subset(pr$fit, pr$fit > 0) *
      60 * 24, na.rm = na.omit)
    tot$chamber[j] = mean(dr$chamber, na.rm = T)
    j <- j + 1
  }
}
```

```

    } else {
    }
}

```





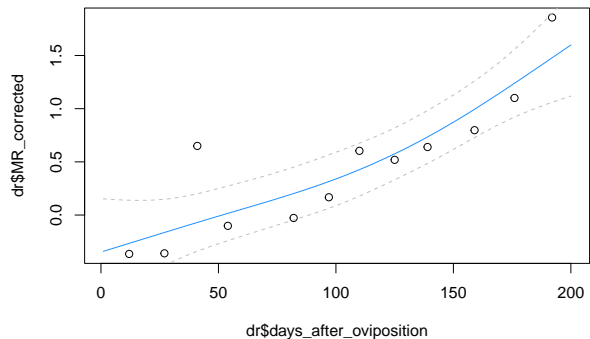
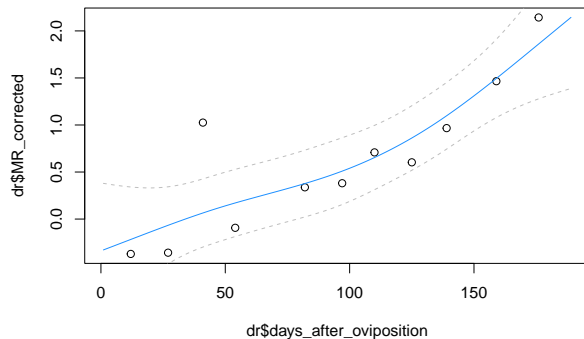
## Extatosoma tiaratum

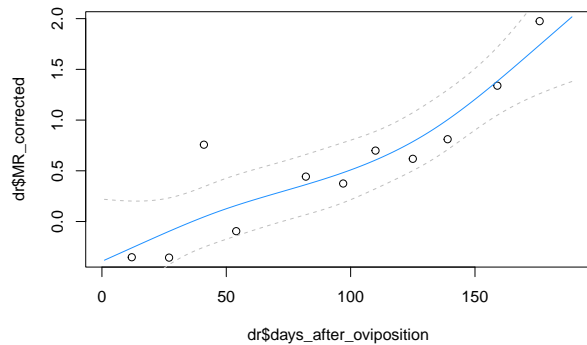
```
dd <- d5
for (i in unique(dd$egg_ID)) {
  dr <- subset(dd, dd$egg_ID == i)
  y <- dr$MR_corrected # response
  x <- dr$days_after_oviposition # predictor
  ga <- gam(y ~ s(x, k = 5, bs = "cr")) #the number of knots k is reduced from 7 to 5
  ↪ because we are fitting a different gam to each individual egg which reduces sample
  ↪ size.
```

```

inc <- mean(dr$incubation_time, na.rm = T)
pd <- data.frame(x = seq(1, round(inc), by = 1))
pr <- predict(ga, newdata = pd, type = "response", se = TRUE) # get predicted
↪ response values from GAM
plot(dr$days_after_oviposition, dr$MR_corrected, xlim = c(1,
  round(inc)))
lines(pd$x, pr$fit, col = "dodgerblue") # plot predicted fit
lines(pd$x, pr$fit - qnorm(0.975) * pr$se.fit, lty = 2, col = "gray") # plot lower
↪ 95% CI endpoint
lines(pd$x, pr$fit + qnorm(0.975) * pr$se.fit, lty = 2, col = "gray") # plot upper
↪ 95% CI endpoint
sum(pr$fit * 60 * 24, na.rm = na.omit)
tot$species[j] = "tiaratum"
tot$egg_ID[j] = i
tot$mass[j] = median(dr$mass, na.rm = T)
tot$mass_shell[j] = median(dr$egg_shell_mass, na.rm = T)
tot$incubation_time[j] = median(dr$incubation_time, na.rm = T)
# tot$total_energy[j]=sum(pr$fit*60*24, na.rm=na.omit)
tot$max_MR[j] <- max(dr$MR_corrected, na.rm = T)
tot$MR_mid[j] <- pr$fit[round(median(dr$incubation_time,
  na.rm = T)/2)]
tot$total_energy[j] = sum(subset(pr$fit, pr$fit > 0) * 60 *
  24, na.rm = na.omit)
tot$chamber[j] = mean(dr$chamber, na.rm = T)
j <- j + 1
}

```





## Summary

```
tot <- na.omit(tot)
```

## Metabolic rate allometry

```
# Load dataset (same as tot which was just generated)
tot <- read_excel("Dataset_S1_eggs.xlsx", sheet = "summary_MR_data")
```

## Allometry of Mid-development metabolic rate

```
# without oviposition term
mm1 <- lmer(log10(MR_mid) ~ log10(egg_mass) + (1 | species),
  data = tot)
summary(mm1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(MR_mid) ~ log10(egg_mass) + (1 | species)
## Data: tot
##
## REML criterion at convergence: -55.8
##
## Scaled residuals:
## Min      1Q  Median      3Q      Max
## -4.8724 -0.3240  0.1434  0.4704  1.6540
##
## Random effects:
## Groups Name Variance Std.Dev.
## species (Intercept) 0.04214 0.2053
## Residual 0.01084 0.1041
## Number of obs: 45, groups: species, 5
##
```



```
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept)   -1.4413    0.2464  -5.849
## log10(egg_mass)  0.6953    0.1557   4.464
##
## Correlation of Fixed Effects:
##           (Intr)
## lg10(egg_ms) -0.925
```

```
Anova(mm1)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(MR_mid)
##           Chisq Df Pr(>Chisq)
## log10(egg_mass) 19.93  1  8.032e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm1)
```

```
##           2.5 %    97.5 %
## .sig01      0.08728812 0.3683713
## .sigma      0.08488792 0.1320461
## (Intercept) -1.89375644 -0.9181037
## log10(egg_mass) 0.36092664 0.9828517
```

```
# with oviposition term
mm2 <- lmer(log10(MR_mid) ~ log10(egg_mass) + oviposition + (1 |
  species), data = tot)
summary(mm2)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(MR_mid) ~ log10(egg_mass) + oviposition + (1 | species)
## Data: tot
##
## REML criterion at convergence: -56
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -4.8752 -0.2957  0.1683  0.4717  1.4929
##
## Random effects:
## Groups Name Variance Std.Dev.
## species (Intercept) 0.07863 0.2804
## Residual 0.01057 0.1028
## Number of obs: 45, groups: species, 5
##
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept)   -0.9157    0.6844  -1.338
```

```
## log10(egg_mass)    0.4542    0.3187    1.425
## ovipositiondrop   -0.2896    0.4071   -0.711
##
## Correlation of Fixed Effects:
##           (Intr) l10(_)
## lg10(gg_ms) -0.956
## ovipostndrp -0.882  0.773
```

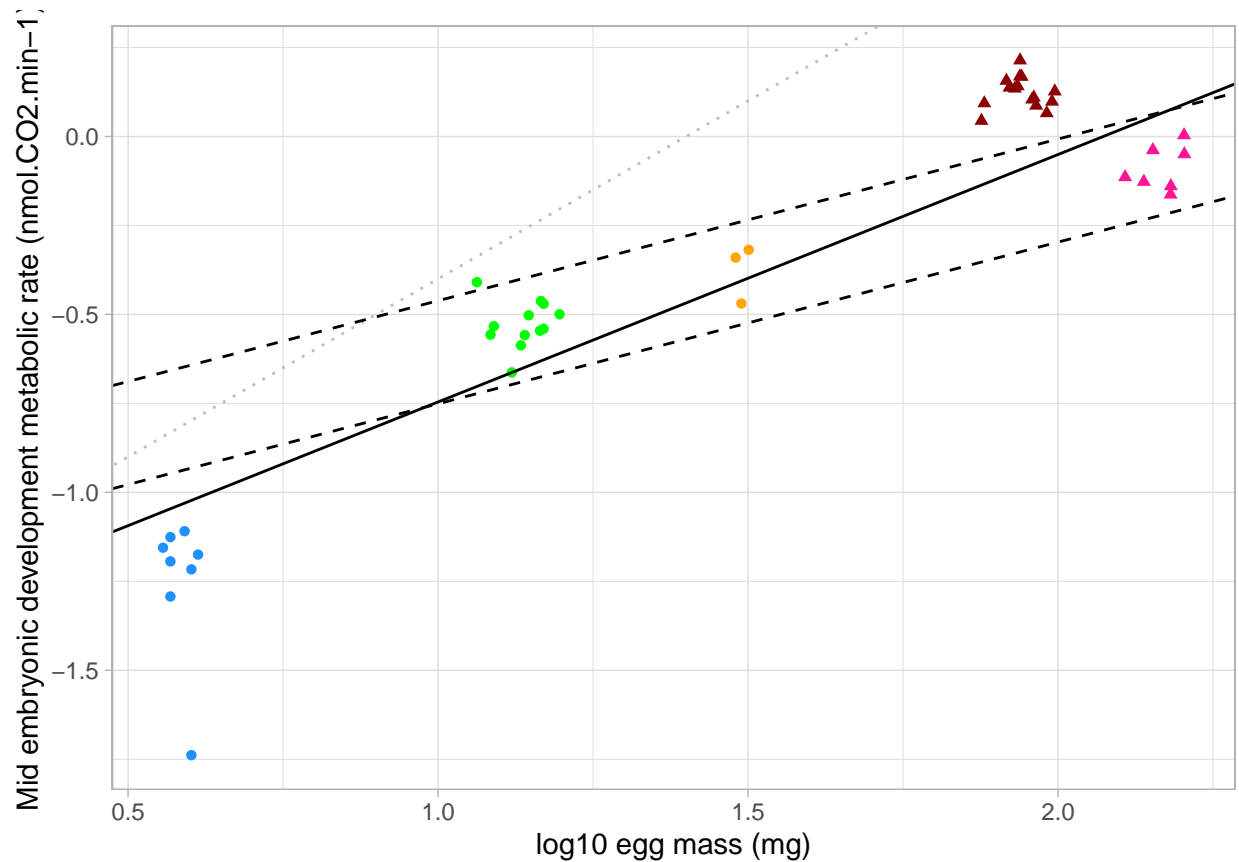
```
Anova(mm2)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(MR_mid)
##               Chisq Df Pr(>Chisq)
## log10(egg_mass) 2.0316  1    0.1541
## oviposition      0.5059  1    0.4769
```

```
confint(mm2)
```

```
##                2.5 %    97.5 %
## .sig01          0.08703484 0.4088018
## .sigma          0.08430618 0.1317896
## (Intercept)     -2.32079796 0.1277774
## log10(egg_mass) -0.03197816 1.1257470
## ovipositiondrop -0.90700526 0.4684382
```

```
p1 <- ggplot(tot, aes(x = log10(egg_mass), y = log10(MR_mid),
  color = species, shape = oviposition)) + geom_point() + scale_shape_manual(values =
  ↪ c(17,
  16)) + scale_color_manual(values = c("darkred", "deeppink",
  "dodgerblue", "green", "orange")) + geom_abline(slope = fixef(mm1)[2],
  intercept = fixef(mm1)[1], color = "black") + geom_abline(slope = 1,
  intercept = -1.4, linetype = "dotted", color = "grey") +
  geom_abline(slope = fixef(mm2)[2], intercept = fixef(mm2)[1],
    color = "black", linetype = "dashed") + geom_abline(slope = fixef(mm2)[2],
  intercept = fixef(mm2)[1] + fixef(mm2)[3], color = "black",
  linetype = "dashed") + xlab("log10 egg mass (mg)") + ylab("Mid embryonic development
  ↪ metabolic rate (nmol.CO2.min-1)") +
  guides(color = FALSE, shape = FALSE) + theme_light()
p1
```



### Allometry of Mean metabolic rate

```
tot$mean_MR <- tot$total_energy/(tot$incubation_time * 24 * 60)

# without oviposition term
mm1 <- lmer(log10(mean_MR) ~ log10(egg_mass) + (1 | species),
  data = tot)
summary(mm1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(mean_MR) ~ log10(egg_mass) + (1 | species)
## Data: tot
##
## REML criterion at convergence: -123.4
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.37406 -0.49350 -0.03846  0.56086  2.50756
##
## Random effects:
## Groups Name Variance Std.Dev.
## species (Intercept) 0.019532 0.1398
## Residual 0.002116 0.0460
```

```
## Number of obs: 45, groups: species, 5
##
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept)   -1.42176    0.15742  -9.031
## log10(egg_mass) 0.78162    0.09855   7.931
##
## Correlation of Fixed Effects:
##           (Intr)
## lg10(gg_ms) -0.916
```

```
Anova(mm1)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(mean_MR)
##           Chisq Df Pr(>Chisq)
## log10(egg_mass) 62.903  1 2.171e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm1)
```

```
##           2.5 %      97.5 %
## .sig01      0.06230046 0.25256555
## .sigma      0.03746786 0.05834365
## (Intercept) -1.71520231 -1.09313689
## log10(egg_mass) 0.57512951 0.96701603
```

```
# with oviposition term
mm2 <- lmer(log10(mean_MR) ~ log10(egg_mass) + oviposition +
  (1 | species), data = tot)
summary(mm2)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(mean_MR) ~ log10(egg_mass) + oviposition + (1 | species)
## Data: tot
##
## REML criterion at convergence: -122.3
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.42090 -0.41288 -0.07286  0.54649  2.37765
##
## Random effects:
## Groups Name Variance Std.Dev.
## species (Intercept) 0.029184 0.17083
## Residual 0.002097 0.04579
## Number of obs: 45, groups: species, 5
##
## Fixed effects:
```

```
##               Estimate Std. Error t value
## (Intercept)    -1.2254    0.3590  -3.413
## log10(egg_mass)  0.6959    0.1645   4.230
## ovipositiondrop -0.1185    0.2255  -0.525
##
## Correlation of Fixed Effects:
##      (Intr) l10(_)
## lg10(gg_ms) -0.941
## ovipostndrp -0.859  0.719
```

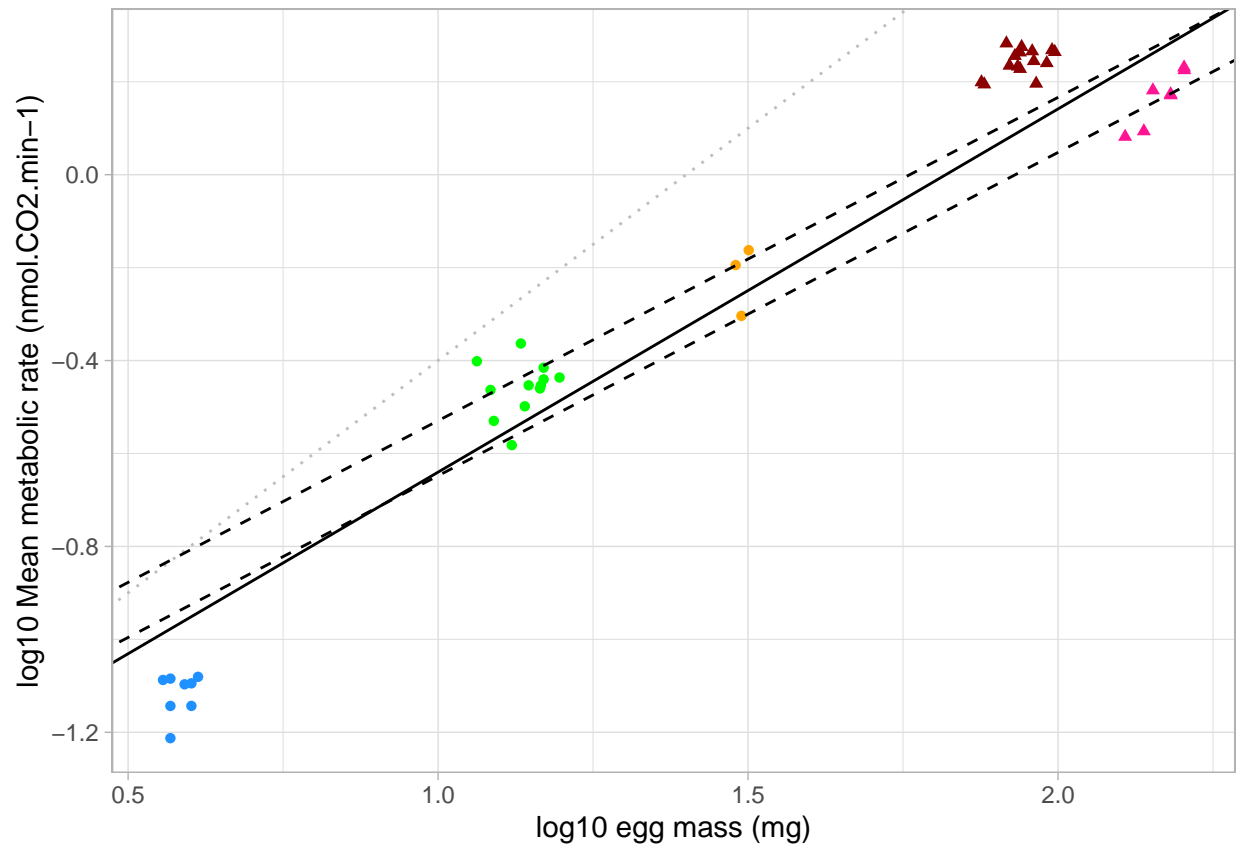
```
Anova(mm2)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(mean_MR)
##               Chisq Df Pr(>Chisq)
## log10(egg_mass) 17.8937  1  2.336e-05 ***
## oviposition      0.2758  1    0.5995
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm2)
```

```
##               2.5 %      97.5 %
## .sig01         0.06179203  0.26502674
## .sigma         0.03726802  0.05826012
## (Intercept)    -1.96533840 -0.63046248
## log10(egg_mass)  0.42386391  1.04654397
## ovipositiondrop -0.49429553  0.29621282
```

```
p12 <- ggplot(tot, aes(x = log10(egg_mass), y = log10(mean_MR),
  color = species, shape = oviposition)) + geom_point() + scale_shape_manual(values =
  ↪ c(17,
  16)) + scale_color_manual(values = c("darkred", "deeppink",
  "dodgerblue", "green", "orange")) + geom_abline(slope = fixef(mm1)[2],
  intercept = fixef(mm1)[1], color = "black") + geom_abline(slope = 1,
  intercept = -1.4, linetype = "dotted", color = "grey") +
  geom_abline(slope = fixef(mm2)[2], intercept = fixef(mm2)[1],
    color = "black", linetype = "dashed") + geom_abline(slope = fixef(mm2)[2],
  intercept = fixef(mm2)[1] + fixef(mm2)[3], color = "black",
  linetype = "dashed") + xlab("log10 egg mass (mg)") + ylab("log10 Mean metabolic rate
  ↪ (nmol.CO2.min-1)") +
  guides(color = FALSE, shape = FALSE) + theme_light()
p12
```



### Allometry of Maximum metabolic rate

```
# without oviposition term
mm1 <- lmer(log10(max_MR) ~ log10(egg_mass) + (1 | species),
  data = tot)
summary(mm1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(max_MR) ~ log10(egg_mass) + (1 | species)
## Data: tot
##
## REML criterion at convergence: -58.3
##
## Scaled residuals:
## Min      1Q  Median      3Q      Max
## -1.8136 -0.4726 -0.0620  0.2957  4.4224
##
## Random effects:
## Groups Name Variance Std.Dev.
## species (Intercept) 0.003883 0.06231
## Residual 0.011916 0.10916
## Number of obs: 45, groups: species, 5
##
```

```
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept)   -0.88139    0.08984  -9.811
## log10(egg_mass) 0.76150    0.05702  13.355
##
## Correlation of Fixed Effects:
##           (Intr)
## lg10(egg_ms) -0.930
```

```
Anova(mm1)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(max_MR)
##           Chisq Df Pr(>Chisq)
## log10(egg_mass) 178.35  1 < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm1)
```

```
##           2.5 %    97.5 %
## .sig01      0.00000000 0.1177358
## .sigma      0.08892158 0.1375963
## (Intercept) -1.04897284 -0.7130069
## log10(egg_mass) 0.65457951 0.8678890
```

```
# with oviposition term
mm2 <- lmer(log10(max_MR) ~ log10(egg_mass) + oviposition + (1 |
  species), data = tot)
summary(mm2)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(max_MR) ~ log10(egg_mass) + oviposition + (1 | species)
## Data: tot
##
## REML criterion at convergence: -56.2
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.8165 -0.4165 -0.0943  0.2593  4.4321
##
## Random effects:
## Groups Name Variance Std.Dev.
## species (Intercept) 0.005797 0.07614
## Residual 0.011989 0.10949
## Number of obs: 45, groups: species, 5
##
## Fixed effects:
##           Estimate Std. Error t value
## (Intercept)   -0.865699    0.275178  -3.146
```

```
## log10(egg_mass) 0.754444 0.131254 5.748
## ovipositiondrop -0.009275 0.154249 -0.060
##
## Correlation of Fixed Effects:
## (Intr) l10(_)
## lg10(gg_ms) -0.977
## ovipostndrp -0.925 0.862
```

```
Anova(mm2)
```

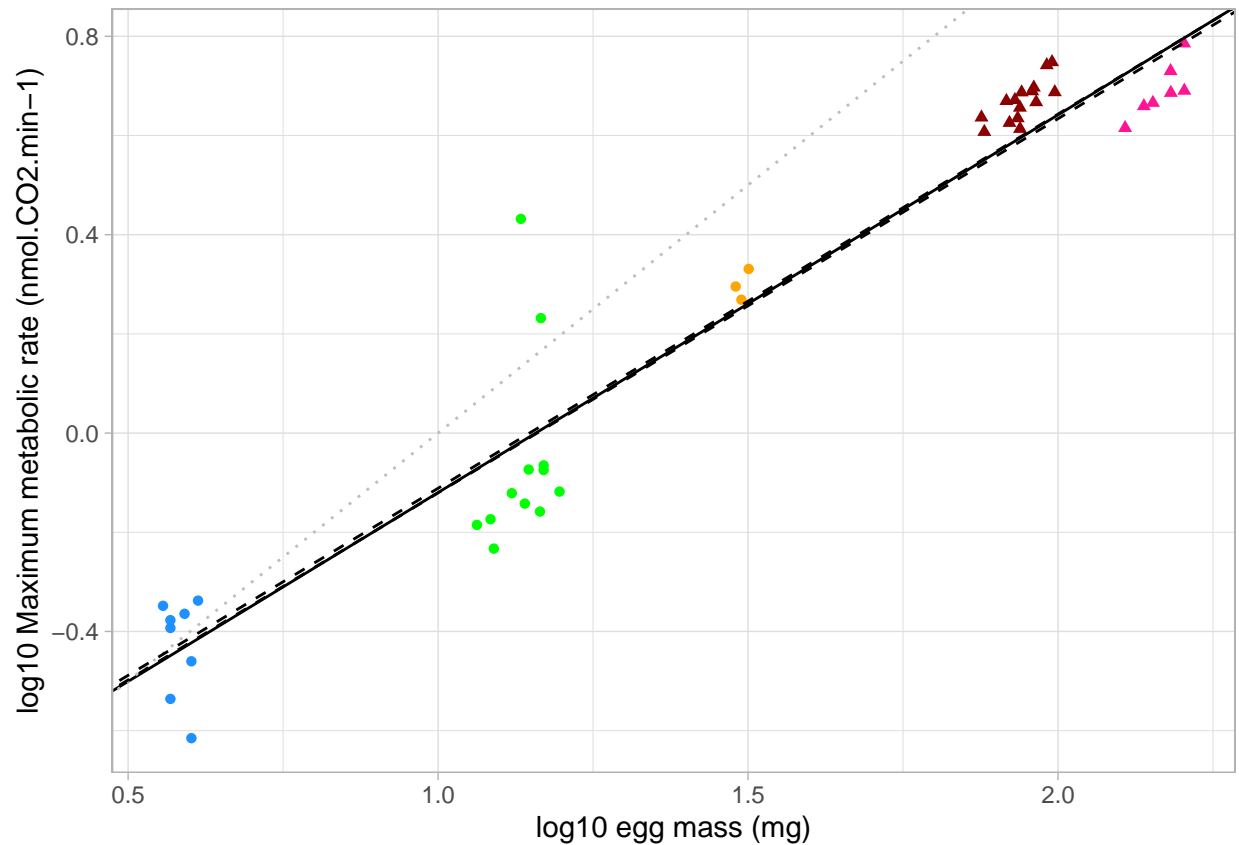
```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(max_MR)
##           Chisq Df Pr(>Chisq)
## log10(egg_mass) 33.0391 1 9.033e-09 ***
## oviposition      0.0036 1 0.9521
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm2)
```

```
##           2.5 %      97.5 %
## .sig01      0.00000000 0.1144356
## .sigma      0.08921734 0.1390509
## (Intercept) -1.28919727 -0.4136728
## log10(egg_mass) 0.53905945 0.9564737
## ovipositiondrop -0.26565331 0.2281214
```

```
p13 <- ggplot(tot, aes(x = log10(egg_mass), y = log10(max_MR),
  color = species, shape = oviposition)) + geom_point() + scale_shape_manual(values =
  ↪ c(17,
  16)) + scale_color_manual(values = c("darkred", "deeppink",
  "dodgerblue", "green", "orange")) + geom_abline(slope = fixef(mm1)[2],
  intercept = fixef(mm1)[1], color = "black") + geom_abline(slope = 1,
  intercept = -1, linetype = "dotted", color = "grey") + geom_abline(slope =
  ↪ fixef(mm2)[2],
  intercept = fixef(mm2)[1], color = "black", linetype = "dashed") +
  geom_abline(slope = fixef(mm2)[2], intercept = fixef(mm2)[1] +
  fixef(mm2)[3], color = "black", linetype = "dashed") +
  xlab("log10 egg mass (mg)") + ylab("log10 Maximum metabolic rate (nmol.CO2.min-1)") +
  guides(color = FALSE, shape = FALSE) + theme_light()
p13
```





### Allometry of Total CO2 produced

```
mm <- lmer(log10(total_energy) ~ log10(egg_mass) + (1 | species),
  data = tot)
summary(mm)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(total_energy) ~ log10(egg_mass) + (1 | species)
## Data: tot
##
## REML criterion at convergence: -141
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.06893 -0.46112 -0.03454  0.32623  2.25280
##
## Random effects:
## Groups Name Variance Std.Dev.
## species (Intercept) 0.019836 0.14084
## Residual 0.001359 0.03686
## Number of obs: 45, groups: species, 5
##
## Fixed effects:
```

```
##               Estimate Std. Error t value
## (Intercept)    3.65692    0.15089   24.24
## log10(egg_mass) 0.97380    0.09356   10.41
##
## Correlation of Fixed Effects:
##           (Intr)
## lg10(egg_ms) -0.908
```

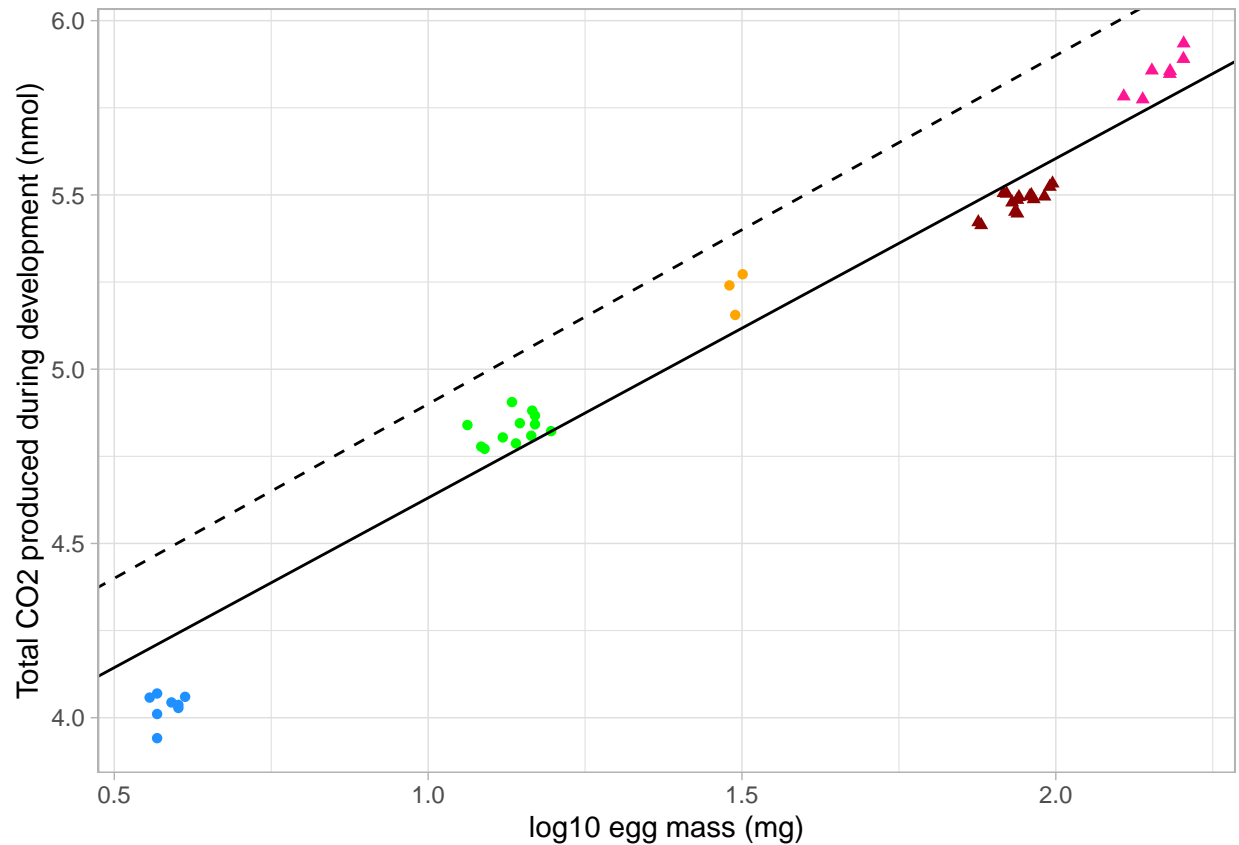
```
Anova(mm)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(total_energy)
##               Chisq Df Pr(>Chisq)
## log10(egg_mass) 108.34  1 < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm)
```

```
##               2.5 %      97.5 %
## .sig01         0.05720983 0.26861711
## .sigma         0.03010464 0.04717297
## (Intercept)    3.35863780 4.00269783
## log10(egg_mass) 0.75751817 1.16432535
```

```
p2 <- ggplot(tot, aes(x = log10(egg_mass), y = log10(total_energy),
  shape = oviposition, color = species)) + geom_point() + scale_shape_manual(values =
  ↪ c(17,
  16)) + scale_color_manual(values = c("darkred", "deeppink",
  "dodgerblue", "green", "orange")) + geom_abline(slope = fixef(mm)[2],
  intercept = fixef(mm)[1], color = "black") + geom_abline(slope = 1,
  intercept = 3.9, linetype = "dashed") + xlab("log10 egg mass (mg)") +
  ylab("Total CO2 produced during development (nmol)") + guides(color = FALSE,
  shape = FALSE) + theme_light()
p2
```



## Egg water loss

### Raw water loss over development

#### Load water loss data

```
library(readxl)
data <- read_excel("Dataset_S1_eggs.xlsx", sheet = "Full_water_loss_data_75RH")
```

#### Water lost over time

```
data <- subset(data, data$age != 0) #delete first time point
data$id <- NA

# Make egg IDs unique
for (i in c(1:length(data$ID))) {
  if (data$Species[i] == "calcarata") {
    data$id[i] <- paste("c", data$ID[i], sep = "")
  }
  if (data$Species[i] == "dilatata") {
```

```

    data$id[i] <- paste("d", data$ID[i], sep = "")
  }
  if (data$Species[i] == "extradentata") {
    data$id[i] <- paste("e", data$ID[i], sep = "")
  }
  if (data$Species[i] == "philippinicum") {
    data$id[i] <- paste("p", data$ID[i], sep = "")
  }
  if (data$Species[i] == "tiaratum") {
    data$id[i] <- paste("t", data$ID[i], sep = "")
  }
}

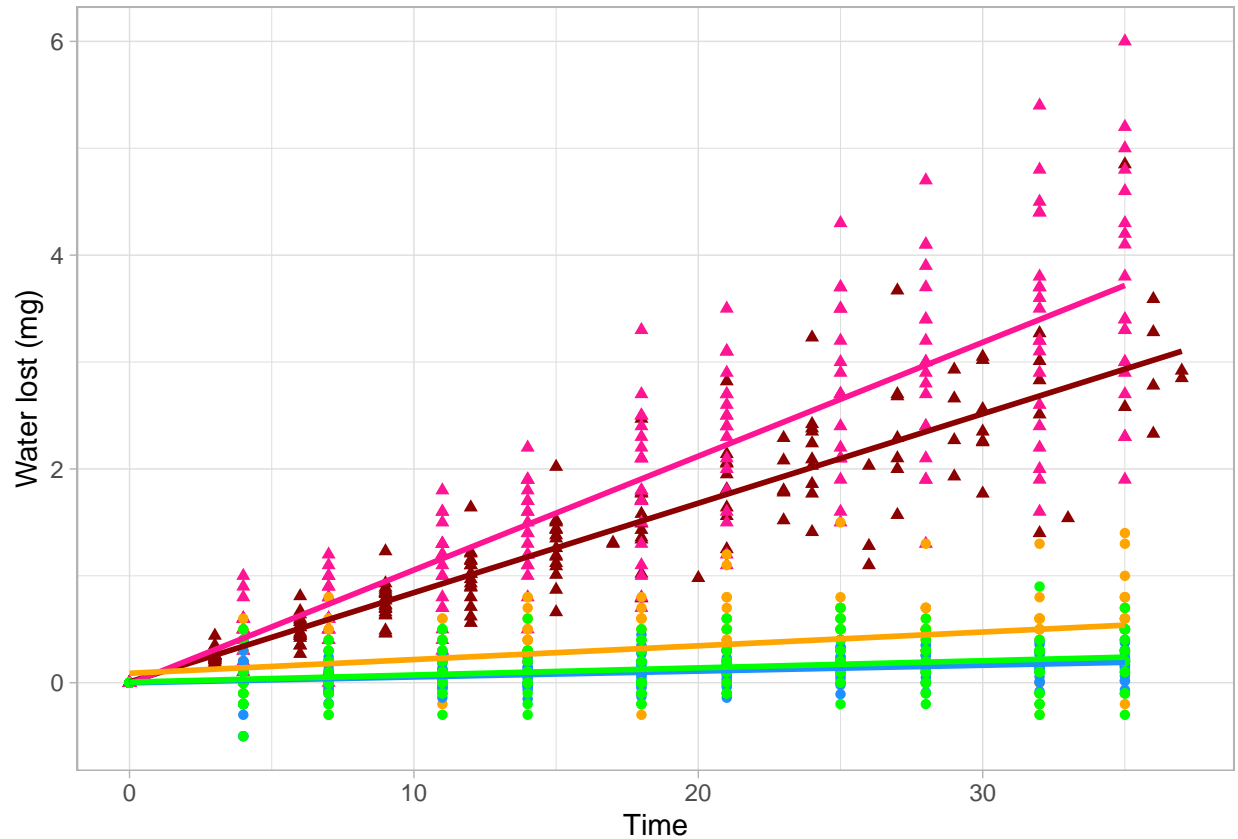
# Make day 3 be the starting point (to avoid variation in
# initial weight due to varying amounts water on outside of
# the egg)
data$weight_3days <- NA
for (i in c(1:length(data$ID))) {
  sp <- data$Species[i]
  id <- data$ID[i]
  data$weight_3days[i] <- data$weight[data$Species == sp &
    data$ID == id & data$age == 3]
}

data$weight2 <- (data$weight_3days - data$weight)
data$age2 <- data$age - 3

# delete cracked eggs
data <- subset(data, data$id != "e23")
data <- subset(data, data$id != "e10")

p6 <- ggplot(data, aes(age2, weight2, color = Species, shape = Species)) +
  geom_point() + scale_shape_manual(values = c(17, 17, 16,
16, 16)) + scale_color_manual(values = c("darkred", "deeppink",
"dodgerblue", "green", "orange")) + xlab(label = "Time") +
ylab(label = "Water lost (mg)") + geom_smooth(method = lm,
se = FALSE) + xlim(0, 37) + ylim(-0.5, 6) + guides(color = FALSE,
shape = FALSE) + theme_light()
p6

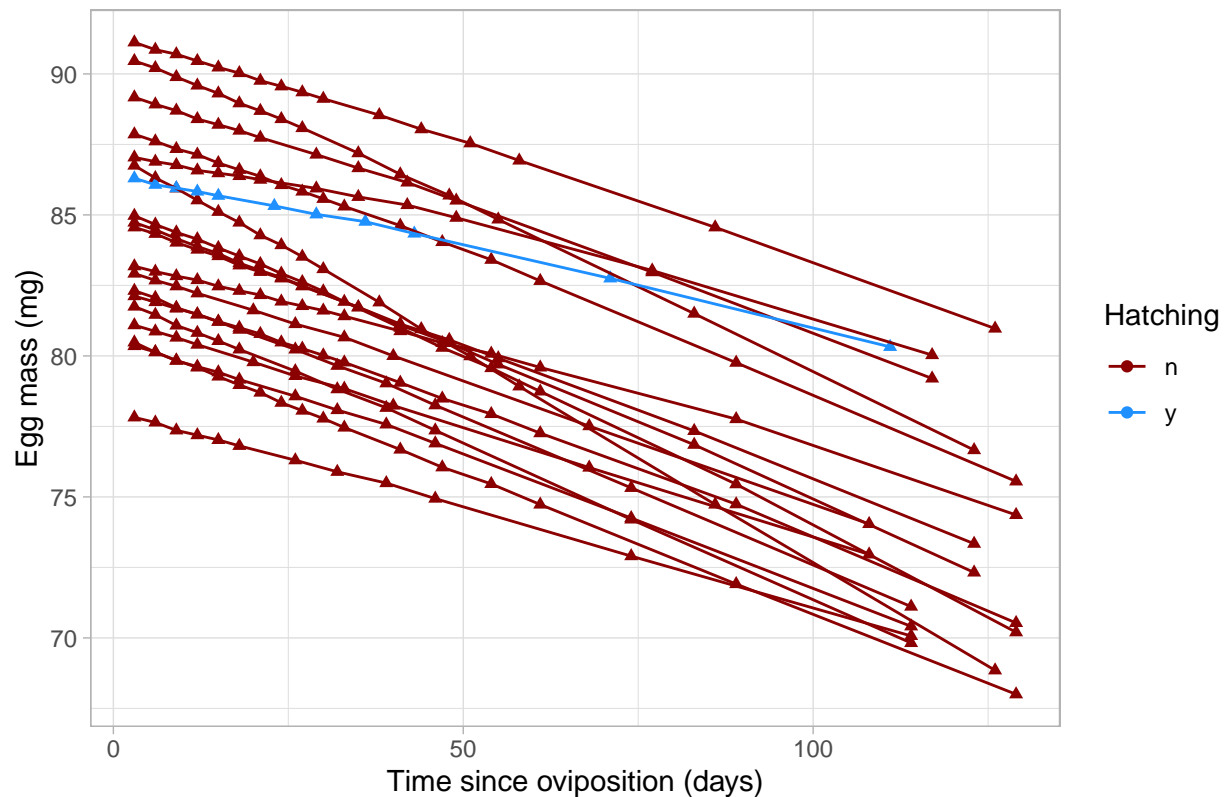
```



Water loss throughout whole development in *Eurycantha calcarata*

```
datae <- subset(data, data$Species == "calcarata")
# egg c18 hatched while the others did not after being held
# constantly at 75%RH.
datae$hatched <- NULL
for (i in c(1:length(datae$id))) {
  if (datae$id[i] == "c18") {
    datae$hatched[i] <- "y"
  } else {
    datae$hatched[i] <- "n"
  }
}
ggplot(datae, aes(age, weight, color = hatched, group = ID, shape = Species)) +
  geom_point() + geom_line() + scale_shape_manual(values = c(17)) +
  scale_color_manual(values = c("darkred", "dodgerblue")) +
  labs(x = "Time since oviposition (days)", y = "Egg mass (mg)",
       color = "Hatching", title = "Eurycantha calcarata eggs - 75RH") +
  guides(shape = FALSE) + theme_light()
```

## Eurycantha calcarata eggs – 75RH



### Extract water loss rate for each egg

Water loss rate is calculated for each egg as the slope of a linear regression between egg mass and time.

```
matrix <- NULL
matrix <- matrix(, nrow = 0, ncol = 4)
colnames(matrix) <- c("Species", "ID", "Initial_weight", "water_loss_rate(mg/day)")
for (i in c(1:length(unique(data$Species)))) {
  d <- subset(data, data$Species == unique(data$Species)[i])
  for (j in c(1:length(unique(d$ID)))) {
    d1 <- subset(d, d$ID == d$ID[j])
    m <- lm(weight ~ age, data = d1)
    matrix <- rbind(matrix, c(d1$Species[1], d1$ID[1], d1$weight[1],
                             m$coefficients[2])) #initial weight = weight after second weighing (to
    ↪ standardize for shell)
  }
}
matrix <- as.data.frame(matrix)
```

### Water loss allometry

#### Load water loss rate data

These are the same water loss rates as we just calculated

```
data <- read_excel("Dataset_S1_eggs.xlsx", sheet = "summary_water_loss_data")
data <- data[-c(47, 56, 75), ] #remove outlier (water gain)
```

## Allometry of total water content

```
mm <- lmer(log10(Total_water_mass) ~ log10(egg_mass) + (1 | Species),
  data = data)
summary(mm)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(Total_water_mass) ~ log10(egg_mass) + (1 | Species)
## Data: data
##
## REML criterion at convergence: -476.1
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.3045 -0.5097  0.0797  0.4975  2.8278
##
## Random effects:
## Groups Name Variance Std.Dev.
## Species (Intercept) 0.0005836 0.02416
## Residual 0.0003963 0.01991
## Number of obs: 101, groups: Species, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)   -0.29622    0.02953  -10.03
## log10(egg_mass)  0.99300    0.01854   53.55
##
## Correlation of Fixed Effects:
##              (Intr)
## lg10(gg_ms) -0.928
```

```
Anova(mm)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(Total_water_mass)
##              Chisq Df Pr(>Chisq)
## log10(egg_mass) 2867.5  1 < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

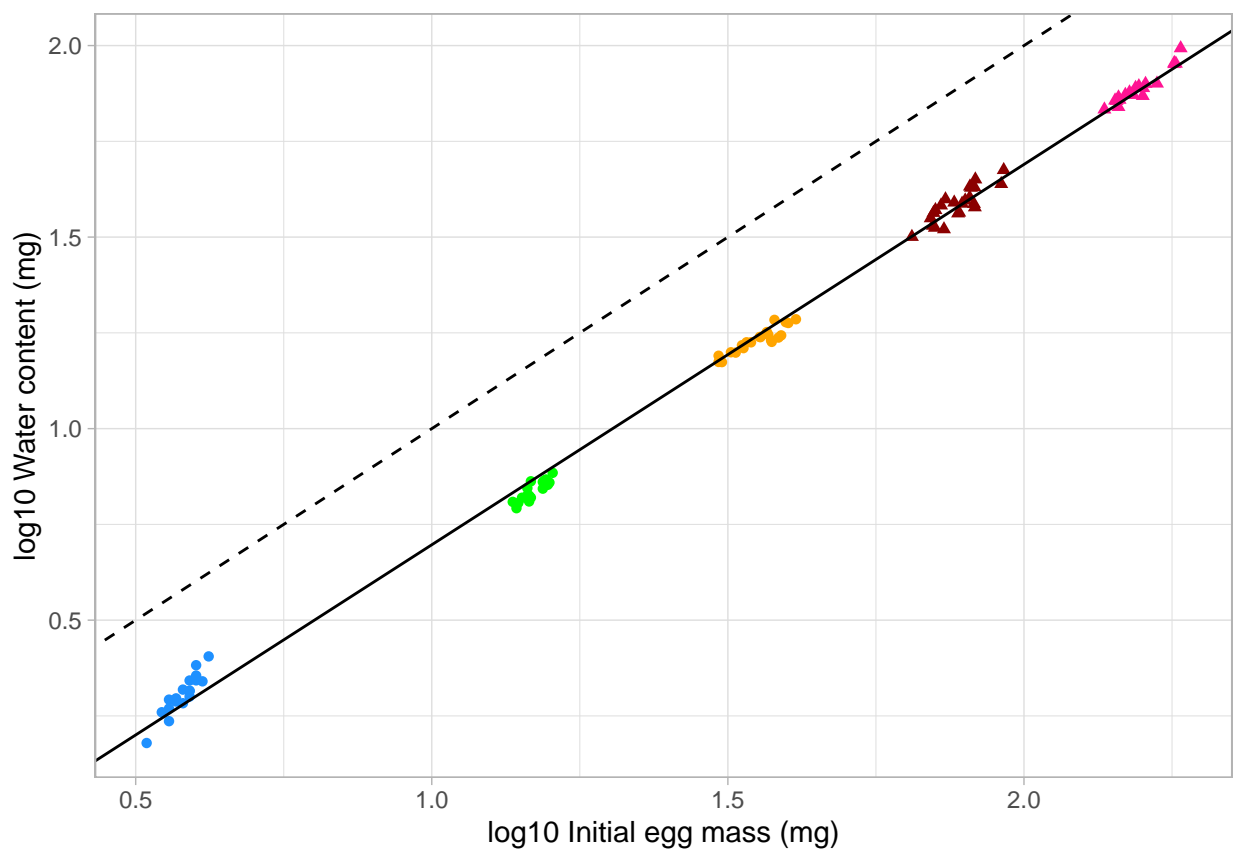
```
confint(mm)
```

```
##              2.5 %      97.5 %
## .sig01      0.01047493 0.04219331
```

```
## .sigma          0.01738764  0.02309187
## (Intercept)     -0.35378600 -0.24220013
## log10(egg_mass)  0.95909997  1.02920760
```

```
p3 <- ggplot(data, aes(x = log10(egg_mass), y = log10(Total_water_mass),
  color = Species, shape = oviposition)) + geom_point() + scale_shape_manual(values =
  ↪ c(17,
  16)) + scale_color_manual(values = c("darkred", "deeppink",
  "dodgerblue", "green", "orange")) + geom_abline(slope = fixef(mm)[2],
  intercept = fixef(mm)[1], color = "black") + geom_abline(slope = 1,
  intercept = 0, linetype = "dashed") + xlab("log10 Initial egg mass (mg)") +
  ylab("log10 Water content (mg)") + guides(color = FALSE,
  shape = FALSE) + theme_light()
```

p3



### Allometry of water loss rate

```
# Without oviposition strategy
mm1 <- lmer(log10(water_loss_rate) ~ log10(egg_mass) + (1 | Species),
  data = data)
summary(mm1)
```

```
## Linear mixed model fit by REML ['lmerMod']
```



```
## Formula: log10(water_loss_rate) ~ log10(egg_mass) + (1 | Species)
## Data: data
##
## REML criterion at convergence: 14.5
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.0795 -0.4463  0.1141  0.5901  3.0756
##
## Random effects:
## Groups Name Variance Std.Dev.
## Species (Intercept) 0.09949 0.3154
## Residual 0.05595 0.2365
## Number of obs: 97, groups: Species, 5
##
## Fixed effects:
## Estimate Std. Error t value
## (Intercept) -2.6517 0.3844 -6.899
## log10(egg_mass) 0.6590 0.2402 2.743
##
## Correlation of Fixed Effects:
## (Intr)
## lg10(gg_ms) -0.928
```

```
Anova(mm1)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(water_loss_rate)
## Chisq Df Pr(>Chisq)
## log10(egg_mass) 7.5246 1 0.006086 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm1)
```

```
##           2.5 %      97.5 %
## .sig01      0.12287776 0.6019063
## .sigma      0.20655614 0.2763329
## (Intercept) -3.36785043 -1.7022050
## log10(egg_mass) 0.04844999 1.1122966
```

```
# With oviposition strategy
```

```
mm2 <- lmer(log10(water_loss_rate) ~ log10(egg_mass) + oviposition +
  (1 | Species), data = data)
summary(mm2)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: log10(water_loss_rate) ~ log10(egg_mass) + oviposition + (1 |
## Species)
## Data: data
```

```
##
## REML criterion at convergence: 1.5
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.01244 -0.45737  0.08976  0.64427  3.01229
##
## Random effects:
##   Groups   Name      Variance Std.Dev.
##   Species (Intercept) 0.00000  0.0000
##   Residual              0.05346  0.2312
## Number of obs: 97, groups: Species, 5
##
## Fixed effects:
##              Estimate Std. Error t value
## (Intercept)   -1.61743    0.15407 -10.498
## log10(egg_mass)  0.28991    0.07249   3.999
## ovipositiondrop -0.80971    0.08277  -9.783
##
## Correlation of Fixed Effects:
##              (Intr) l10(_)
## lg10(gg_ms) -0.971
## ovipostndrp -0.899  0.816
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
Anova(mm2)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: log10(water_loss_rate)
##              Chisq Df Pr(>Chisq)
## log10(egg_mass) 15.993  1  6.358e-05 ***
## oviposition      95.698  1  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
confint(mm2)
```

```
##              2.5 %      97.5 %
## .sig01        0.0000000  0.05732294
## .sigma        0.1989801  0.26381765
## (Intercept)   -1.9176287 -1.31702057
## log10(egg_mass) 0.1485554  0.43115013
## ovipositiondrop -0.9711092 -0.64845479
```

```
p4 <- ggplot(data, aes(x = log10(egg_mass), y = log10(water_loss_rate),
  color = Species, shape = oviposition)) + geom_point() + scale_shape_manual(values =
  ↪ c(17,
  16)) + scale_color_manual(values = c("darkred", "deeppink",
  "dodgerblue", "green", "orange")) + geom_abline(slope = fixef(mm2)[2],
  intercept = fixef(mm2)[1], color = "black", linetype = "dashed") +
```

```

geom_abline(slope = fixef(mm2)[2], intercept = fixef(mm2)[1] +
  fixef(mm2)[3], color = "black", linetype = "dashed") +
geom_abline(slope = 2/3, intercept = -2.5, color = "grey",
  linetype = "dotted") + geom_abline(slope = fixef(mm1)[2],
  intercept = fixef(mm1)[1], color = "black") + xlab("log10 Initial egg mass (mg)") +
ylab("log10 Water loss rate (mg.day-1)") + guides(color = FALSE,
  shape = FALSE) + theme_light()

```

p4

