Survival of C.elegans with three different media

Jonas Gehrlein

12 nov 2018

code for fitting Kaplan-Meier and log-rank test and for displaying survival curves for each type of media and mutant.

```
data <- read.table(here('data','Worm_mutant.csv'), header = TRUE, sep = ";", dec = ",")
head(data)
```

```
##   ï..Time Status Replicate Group Mutant
## 1       4      1        NA  FG13    PMK
## 2       6      1        NA  FG13    PMK
## 3       8      1        NA  FG13    PMK
## 4       8      1        NA  FG13    PMK
## 5      10      1        NA  FG13    PMK
## 6      10      1        NA  FG13    PMK
```

here() should show that your position is in the folder 7.semester else select the active project to be 7.semester in the upper right corner.

Or make a new .Rproj file in the folder 7.semester. There is a problem with the numbers of the worms so we change that with col.names()

```
colnames(data)<-c("Time","status",'Replicate', 'media',
                  'mutant')
head(data)
```

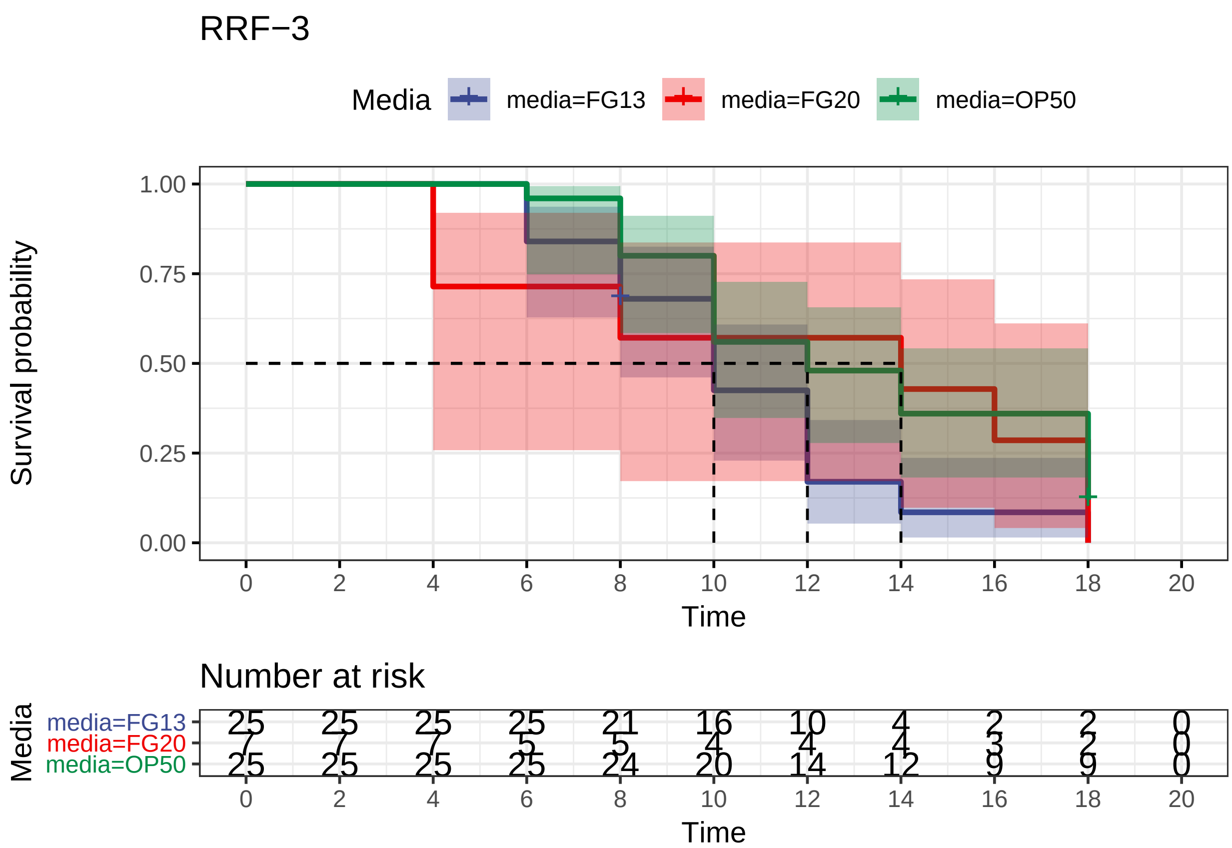
```
##   Time status Replicate media mutant
## 1    4      1        NA  FG13    PMK
## 2    6      1        NA  FG13    PMK
## 3    8      1        NA  FG13    PMK
## 4    8      1        NA  FG13    PMK
## 5   10      1        NA  FG13    PMK
## 6   10      1        NA  FG13    PMK
```

Then we create a survival object with the survival package and make a kaplan-meier curve

First we see for each mutant

```
d_RRF <- subset(data, mutant == 'RRF')
d_PMK <- subset(data,mutant == 'PMK')
d_DAF <- subset(data, mutant == 'DAF')
```

```
d_RRF$Survobj <- with(d_RRF,Surv(d_RRF$Time, event = d_RRF$status))
km_RRF <- survfit(Survobj ~ media, data = d_RRF,conf.type = "log-log", error = "greenwood")
s_km_RRF <- summary(km_RRF)
ggsurvplot(km_RRF,data = d_RRF, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'RRF-3')
```




```
df_fly_13 <- data.frame(c(0,s_km_RRF$time[1:6]),c(1,s_km_RRF$surv[1:6]),            c(0,s_km
colnames(df_fly_13) <- c('Time','Surv','Std.error')
df_fly_20 <- data.frame(c(0,s_km_RRF$time[7:11])
                        ,c(1,s_km_RRF$surv[7:11]),    c(0,s_km_RRF$std.err[7:11]))
colnames(df_fly_20) <- c('Time','Surv','Std.error')
df_OP50 <- data.frame(c(0,s_km_RRF$time[12:17])
                      ,c(1,s_km_RRF$surv[12:17]),
                        c(0,s_km_RRF$std.err[12:17]))
colnames(df_OP50) <- c('Time','Surv','Std.error')

plot(df_fly_13$Time[2:7],df_fly_13$Surv[2:7], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13$Time,df_fly_13$Surv)
arrows(df_fly_13$Time, df_fly_13$Surv-df_fly_13$Std.error, df_fly_13$Time,
       df_fly_13$Surv+df_fly_13$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_13$Time, df_fly_13$Surv - df_fly_13$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 2)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
```

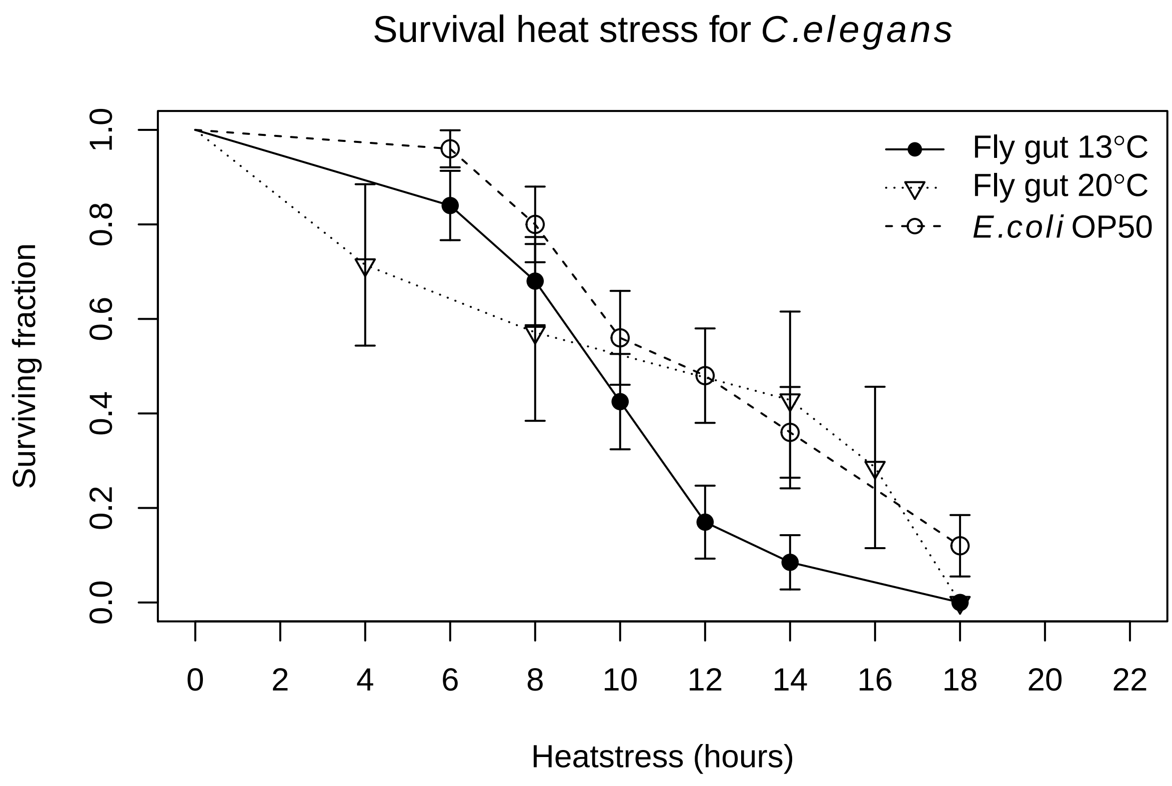
```
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_OP50$Time, df_OP50$Surv - df_OP50$Std.error,
## df_OP50$Time, : zero-length arrow is of indeterminate angle and so skipped
```

```
points(df_fly_20$Time[2:9],df_fly_20$Surv[2:9], pch = 6)
lines(df_fly_20$Time,df_fly_20$Surv,lty = 3)
arrows(df_fly_20$Time, df_fly_20$Surv-df_fly_20$Std.error, df_fly_20$Time,
       df_fly_20$Surv+df_fly_20$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

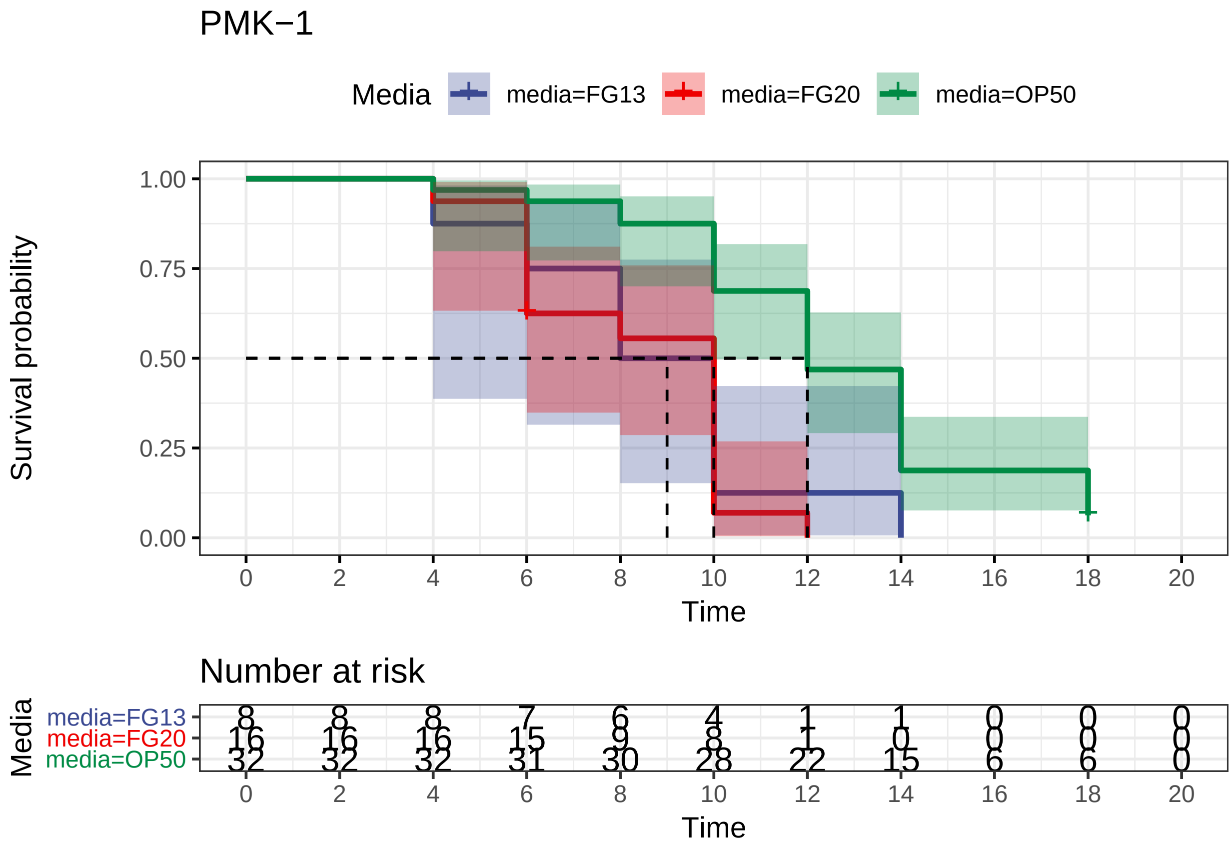
```
## Warning in arrows(df_fly_20$Time, df_fly_20$Surv - df_fly_20$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
legend('topright', pch = c(16,6,1),lty = c(1,3,2),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression('Fly gut 20'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```




```
d_PMK$Survobj <- with(d_PMK,Surv(d_PMK$Time, event = d_PMK$status))
km_PMK <- survfit(Survobj ~ media, data = d_PMK,conf.type = "log-log", error = "greenwood")
s_km_PMK <- summary(km_PMK)
ggsurvplot(km_PMK,data = d_PMK, conf.int = TRUE,
```

```r
          ggtheme = theme_bw(),risk.table = 0.25,
          palette ='aaas', surv.median.line = 'hv',
          legend.title = 'Media',break.x.by = 2,
          title = 'PMK-1')
```




```r
df_fly_13 <- data.frame(c(0,s_km_PMK$time[1:5]),
                        c(1,s_km_PMK$surv[1:5]),
                        c(0,s_km_PMK$std.err[1:5]))
colnames(df_fly_13) <- c('Time','Surv','Std.error')
df_fly_20 <- data.frame(c(0,s_km_PMK$time[6:10]),
                        c(1,s_km_PMK$surv[6:10]),
                        c(0,s_km_PMK$std.err[6:10]))
colnames(df_fly_20) <- c('Time','Surv','Std.error')
df_OP50 <- data.frame(c(0,s_km_PMK$time[11:17]),
                      c(1,s_km_PMK$surv[11:17]),
                        c(0,s_km_PMK$std.err[11:17]))
colnames(df_OP50) <- c('Time','Surv','Std.error')

plot(df_fly_13$Time[2:9],df_fly_13$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13$Time,df_fly_13$Surv)
arrows(df_fly_13$Time, df_fly_13$Surv-df_fly_13$Std.error, df_fly_13$Time,
       df_fly_13$Surv+df_fly_13$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_13$Time, df_fly_13$Surv - df_fly_13$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

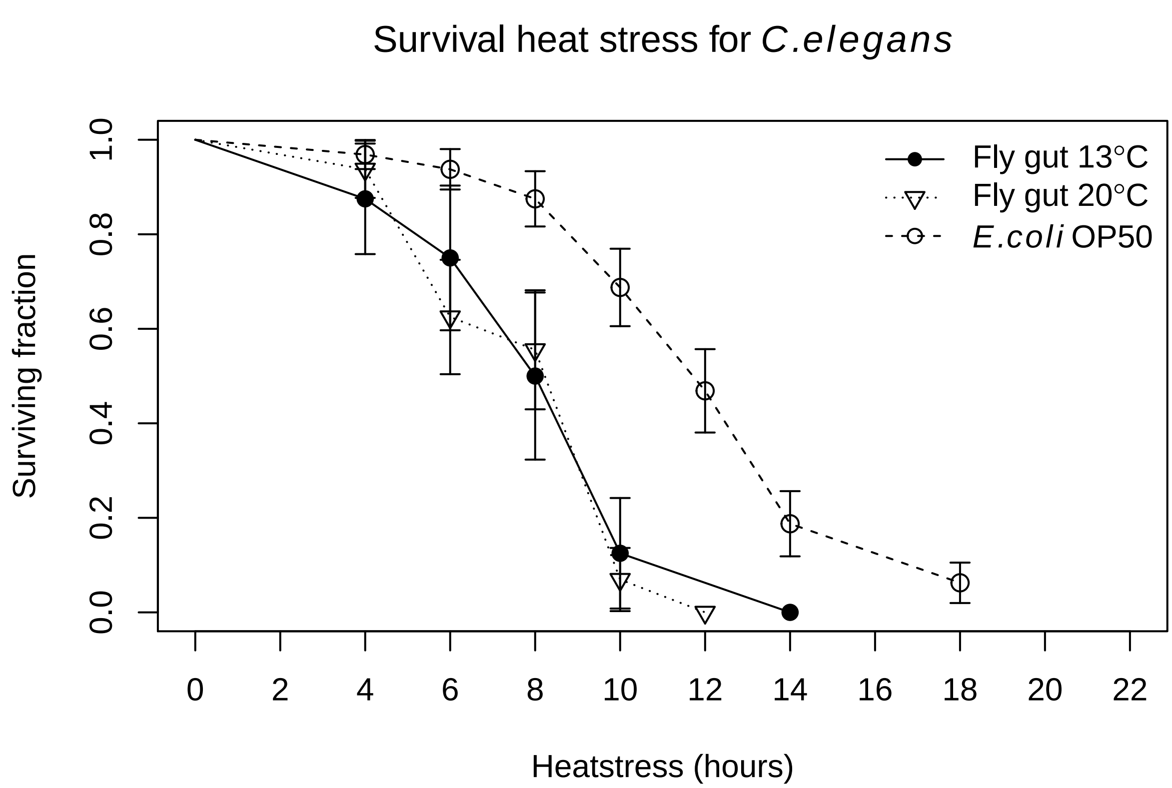
```r
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 2)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_OP50$Time, df_OP50$Surv - df_OP50$Std.error,
## df_OP50$Time, : zero-length arrow is of indeterminate angle and so skipped
```

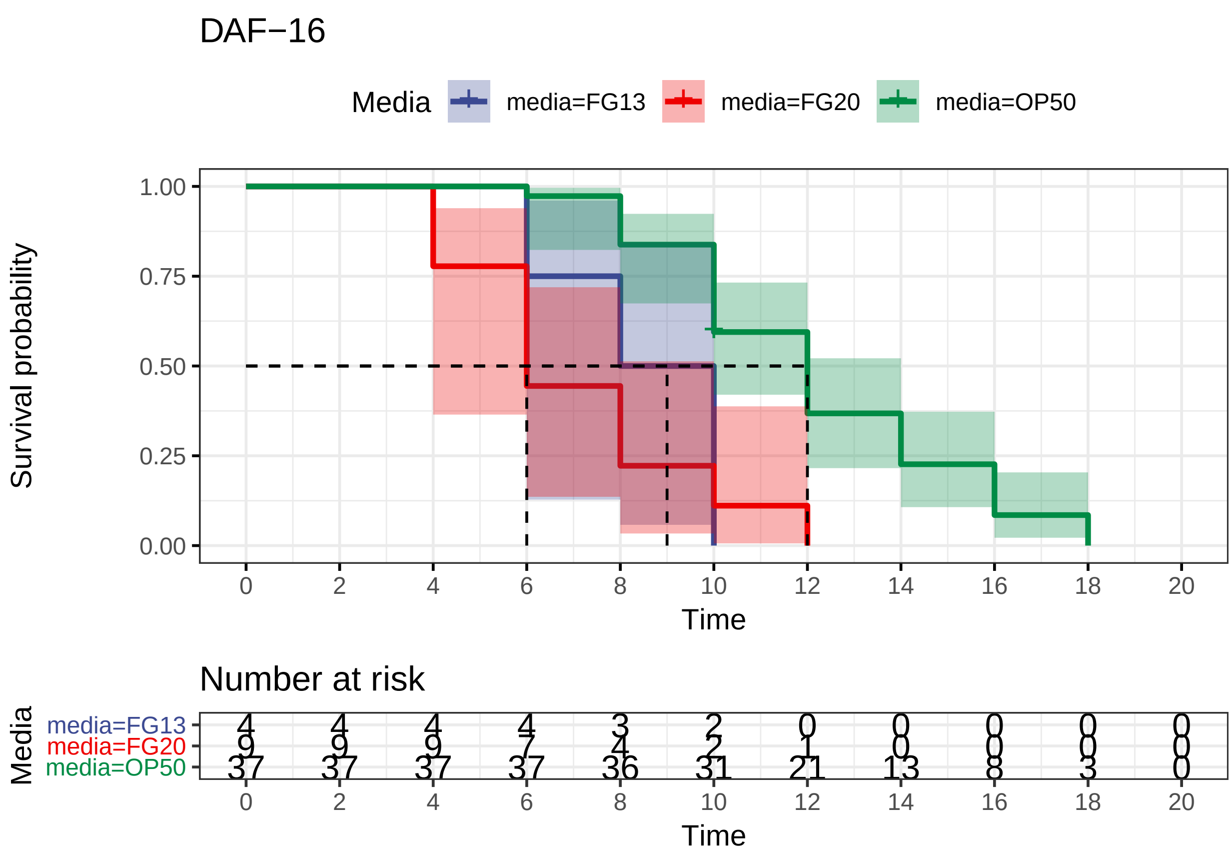
```r
points(df_fly_20$Time[2:9],df_fly_20$Surv[2:9], pch = 6)
lines(df_fly_20$Time,df_fly_20$Surv,lty = 3)
arrows(df_fly_20$Time, df_fly_20$Surv-df_fly_20$Std.error, df_fly_20$Time,
       df_fly_20$Surv+df_fly_20$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_20$Time, df_fly_20$Surv - df_fly_20$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```r
legend('topright', pch = c(16,6,1),lty = c(1,3,2),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression('Fly gut 20'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```



```r
d_DAF$Survobj <- with(d_DAF,Surv(d_DAF$Time, event = d_DAF$status))
km_DAF <- survfit(Survobj ~ media, data = d_DAF,conf.type = "log-log", error = "greenwood")
s_km_DAF <- summary(km_DAF)
ggsurvplot(km_DAF,data = d_DAF, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'DAF-16')
```




```r
df_fly_13 <- data.frame(c(0,s_km_DAF$time[1:3]),
                        c(1,s_km_DAF$surv[1:3]),
                        c(0,s_km_DAF$std.err[1:3]))
colnames(df_fly_13) <- c('Time','Surv','Std.error')
df_fly_20 <- data.frame(c(0,s_km_DAF$time[4:8]),
                        c(1,s_km_DAF$surv[4:8]),
                        c(0,s_km_DAF$std.err[4:8]))
colnames(df_fly_20) <- c('Time','Surv','Std.error')
df_OP50 <- data.frame(c(0,s_km_DAF$time[9:15]),
                      c(1,s_km_DAF$surv[9:15]),
                        c(0,s_km_DAF$std.err[9:15]))
colnames(df_OP50) <- c('Time','Surv','Std.error')

plot(df_fly_13$Time[2:9],df_fly_13$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
```

```r
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13$Time,df_fly_13$Surv)
arrows(df_fly_13$Time, df_fly_13$Surv-df_fly_13$Std.error, df_fly_13$Time,
       df_fly_13$Surv+df_fly_13$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_13$Time, df_fly_13$Surv - df_fly_13$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

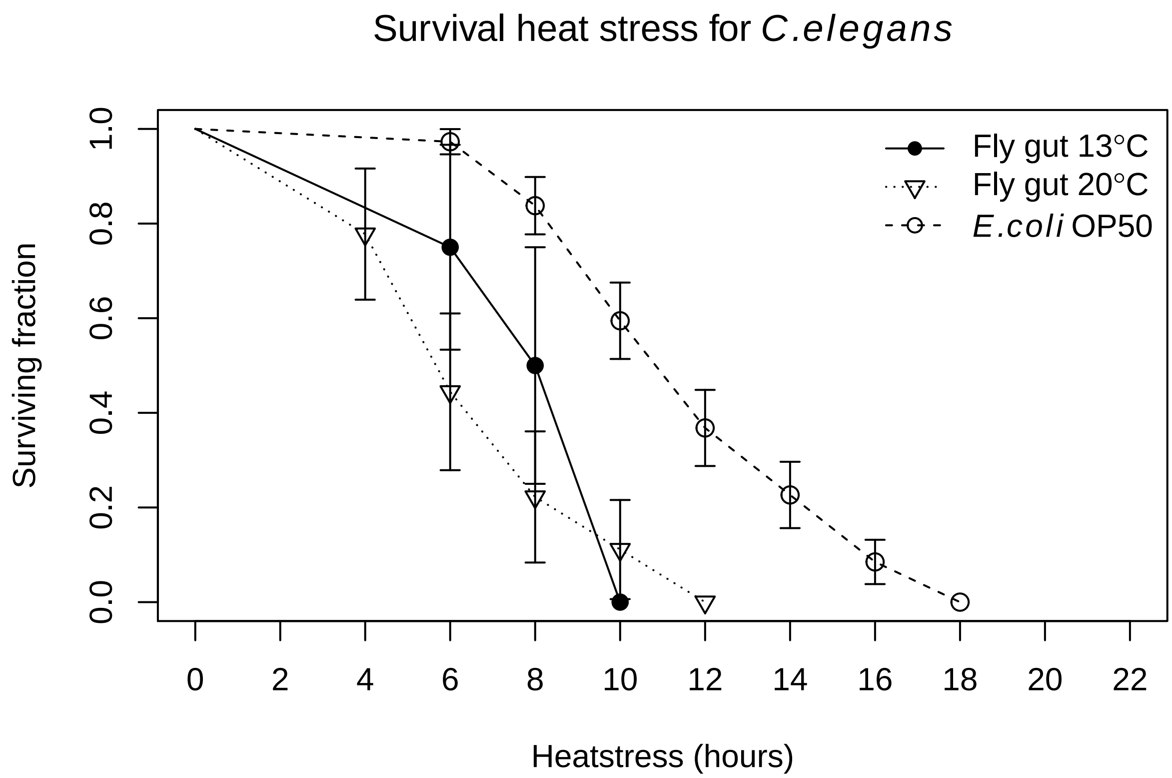
```r
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 2)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_OP50$Time, df_OP50$Surv - df_OP50$Std.error,
## df_OP50$Time, : zero-length arrow is of indeterminate angle and so skipped
```

```r
points(df_fly_20$Time[2:9],df_fly_20$Surv[2:9], pch = 6)
lines(df_fly_20$Time,df_fly_20$Surv,lty = 3)
arrows(df_fly_20$Time, df_fly_20$Surv-df_fly_20$Std.error, df_fly_20$Time,
       df_fly_20$Surv+df_fly_20$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_20$Time, df_fly_20$Surv - df_fly_20$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

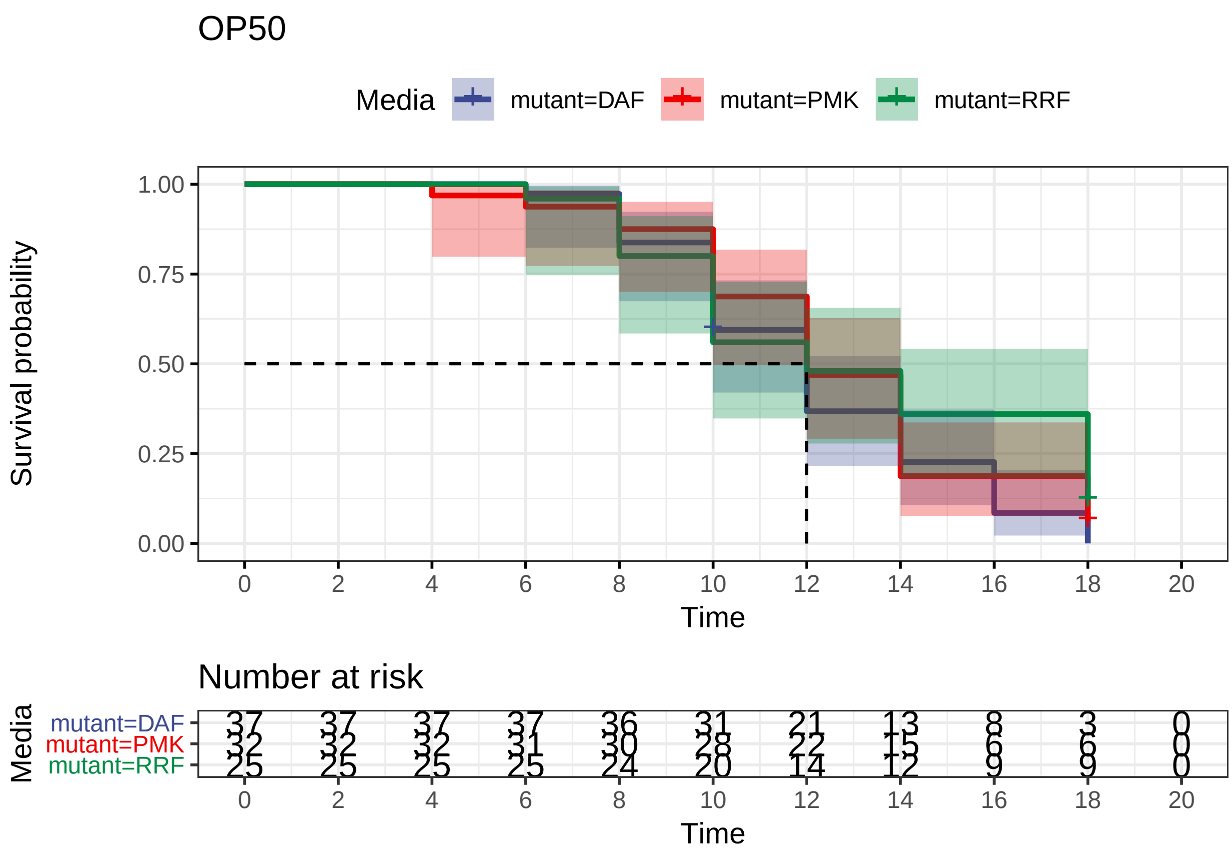
```r
legend('topright', pch = c(16,6,1),lty = c(1,3,2),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression('Fly gut 20'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```



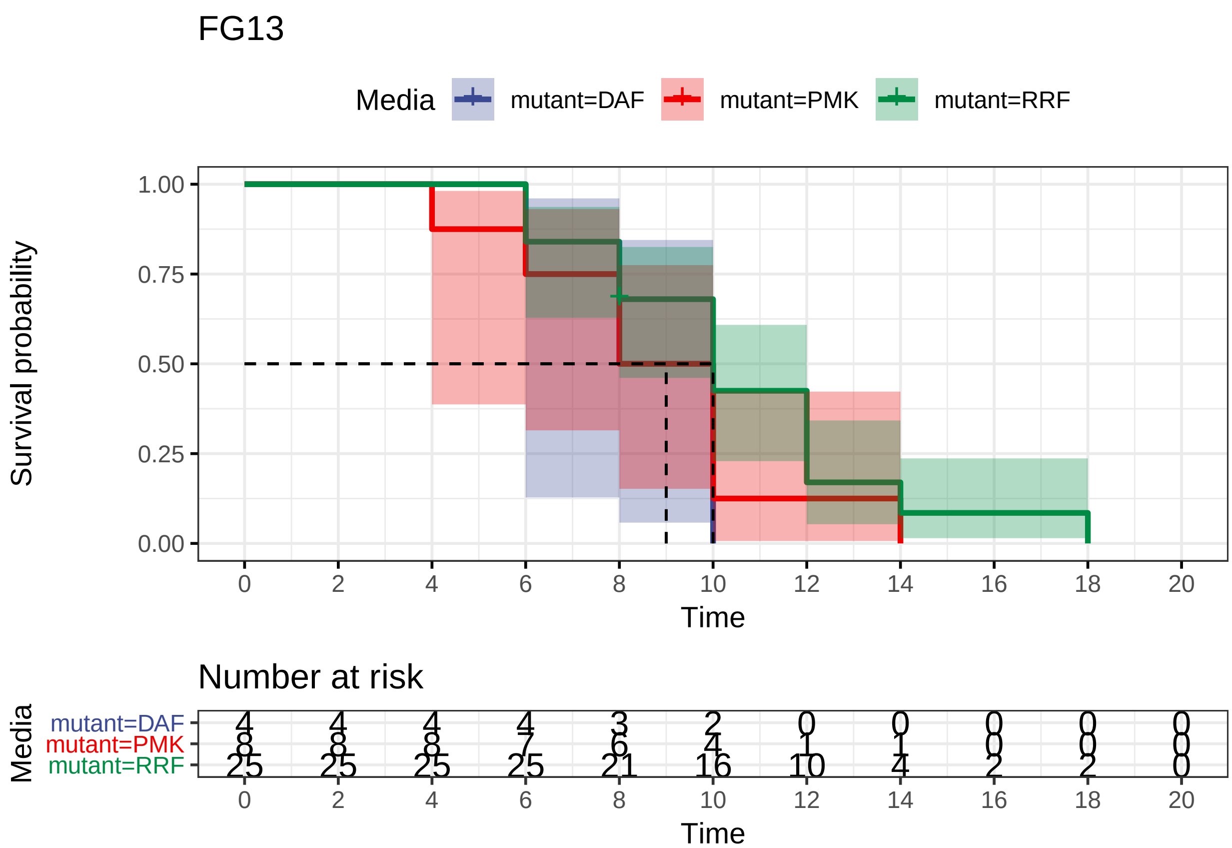
Then we compare by medium

```
data_OP50 <- subset(data, media == 'OP50')
data_FG13 <- subset(data, media == 'FG13')
data_FG20 <- subset(data, media == 'FG20')
```

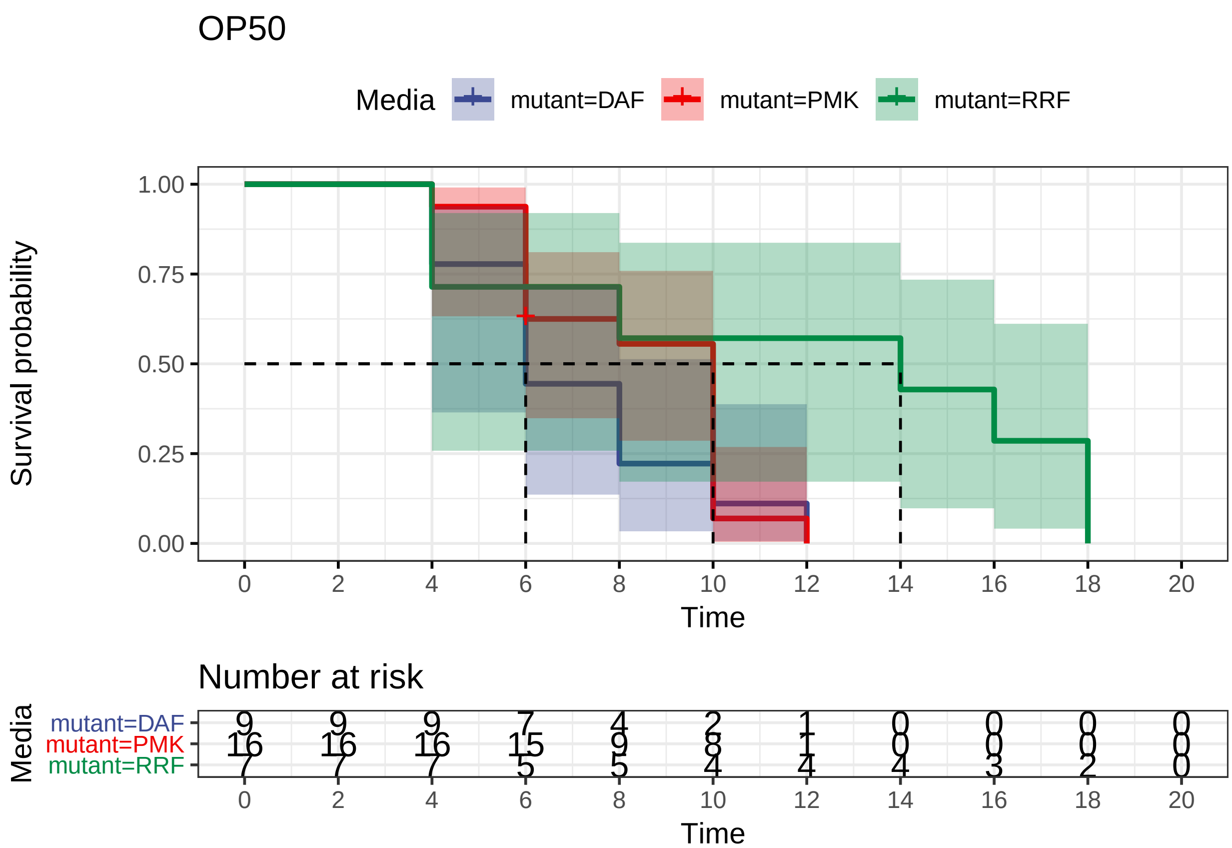
```
data_OP50$Survobj <- with(data_OP50,Surv(data_OP50$Time, event = data_OP50$status))
km_OP50 <- survfit(Survobj ~ mutant, data = data_OP50,conf.type = "log-log", error = "greenwood")
s_km_OP50 <- summary(km_OP50)
ggsurvplot(km_OP50,data = data_OP50, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'OP50')
```




```r
data_FG13$Survobj <- with(data_FG13,Surv(data_FG13$Time, event = data_FG13$status))
km_FG13 <- survfit(Survobj ~ mutant, data = data_FG13,conf.type = "log-log", error = "greenwood")
s_km_FG13 <- summary(km_FG13)
ggsurvplot(km_FG13,data = data_FG13, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'FG13')
```




```r
data_FG20$Survobj <- with(data_FG20,Surv(data_FG20$Time, event = data_FG20$status))
km_FG20 <- survfit(Survobj ~ mutant, data = data_FG20,conf.type = "log-log", error = "greenwood")
s_km_FG20 <- summary(km_FG20)
ggsurvplot(km_FG20,data = data_FG20, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2,
           title = 'OP50')
```



Shows errorbars and lineplot based on one of Anders papers https://onlinelibrary.wiley.com/doi/full/10.1111/acel.12165 This is done by extracting the surviving proportion, standard errors and time from survfit(). and adding a startpoint where the survival is 100% at 0 hours.

Now we test for difference between the curves with both log-rank and gehan-wilcoxon and with an cox proportional hazard model

```
data$Survobj <- with(data,Surv(data$Time, event = data$status))
survdiff(Survobj ~media + mutant, data = data, rho = 0)
```

```
## Call:
## survdiff(formula = Survobj ~ media + mutant, data = data, rho = 0)
##
##                            N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13, mutant=DAF  4        4     1.78     2.762     3.545
## media=FG13, mutant=PMK  8        8     4.22     3.384     4.615
## media=FG13, mutant=RRF 25       24    19.78     0.901     1.483
## media=FG20, mutant=DAF  9        9     2.82    13.539    17.336
## media=FG20, mutant=PMK 16       15     6.88     9.595    13.238
## media=FG20, mutant=RRF  7        7     8.29     0.200     0.341
## media=OP50, mutant=DAF 37       36    38.84     0.208     0.404
## media=OP50, mutant=PMK 32       30    39.06     2.100     4.263
## media=OP50, mutant=RRF 25       22    33.34     3.855     7.900
##
##  Chisq= 51.7  on 8 degrees of freedom, p= 2e-08
```

```r
survdiff(Survobj ~media + mutant, data = data, rho = 1)
```

```
## Call:
## survdiff(formula = Survobj ~ media + mutant, data = data, rho = 1)
##
##                            N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13, mutant=DAF  4     3.24     1.50    2.0432    2.9683
## media=FG13, mutant=PMK  8     6.12     3.18    2.7304    4.3317
## media=FG13, mutant=RRF 25    15.24    12.93    0.4151    0.8063
## media=FG20, mutant=DAF  9     7.76     2.32   12.7917   18.3933
## media=FG20, mutant=PMK 16    12.12     5.63    7.4975   11.8513
## media=FG20, mutant=RRF  7     3.62     3.99    0.0348    0.0719
## media=OP50, mutant=DAF 37    18.26    23.74    1.2635    2.9998
## media=OP50, mutant=PMK 32    14.58    22.00    2.5059    6.0333
## media=OP50, mutant=RRF 25    11.37    17.04    1.8896    4.3076
##
##  Chisq= 48  on 8 degrees of freedom, p= 1e-07
```

```r
fit_both<- coxph(Survobj ~media + mutant, data = data)
summary(fit_both)
```

```
## Call:
## coxph(formula = Survobj ~ media + mutant, data = data)
##
##   n= 163, number of events= 155
##
##                 coef exp(coef)  se(coef)      z Pr(>|z|)
## mediaFG20  0.005922  1.005940  0.250871  0.024 0.981166
## mediaOP50 -1.147199  0.317525  0.231637 -4.953 7.32e-07 ***
## mutantPMK -0.253909  0.775762  0.202013 -1.257 0.208791
## mutantRRF -0.870655  0.418677  0.229717 -3.790 0.000151 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20    1.0059     0.9941    0.6152    1.6448
## mediaOP50    0.3175     3.1494    0.2017    0.5000
## mutantPMK    0.7758     1.2891    0.5221    1.1526
## mutantRRF    0.4187     2.3885    0.2669    0.6568
##
## Concordance= 0.67  (se = 0.028 )
## Rsquare= 0.211   (max possible= 1 )
## Likelihood ratio test= 38.69  on 4 df,   p=8e-08
## Wald test            = 38.5  on 4 df,   p=9e-08
## Score (logrank) test = 39.91  on 4 df,   p=5e-08
```

```r
fit_int <- coxph(Survobj ~media + mutant + mutant:media, data = data)
AIC(fit_both)
```

```
## [1] 1273.218
```

```r
fit_med <- coxph(Survobj ~media, data = data)
summary(fit_med)
```

```
## Call:
## coxph(formula = Survobj ~ media, data = data)
##
##   n= 163, number of events= 155
##
##                coef exp(coef) se(coef)      z Pr(>|z|)
## mediaFG20  0.1971    1.2178   0.2456  0.802 0.422369
## mediaOP50 -0.7195    0.4870   0.2019 -3.564 0.000365 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20     1.218     0.8211    0.7525    1.9709
## mediaOP50     0.487     2.0535    0.3278    0.7234
##
## Concordance= 0.649  (se = 0.025 )
## Rsquare= 0.131   (max possible= 1 )
## Likelihood ratio test= 22.98  on 2 df,   p=1e-05
## Wald test            = 24.52  on 2 df,   p=5e-06
## Score (logrank) test = 25.84  on 2 df,   p=2e-06
```

```r
AIC(fit_med)
```

```
## [1] 1284.932
```

```r
fit_mut <- coxph(Survobj ~mutant, data = data)
summary(fit_mut)
```

```
## Call:
## coxph(formula = Survobj ~ mutant, data = data)
##
##   n= 163, number of events= 155
##
##                 coef exp(coef) se(coef)      z Pr(>|z|)
## mutantPMK -0.08361   0.91979  0.19910 -0.420    0.675
## mutantRRF -0.33095   0.71824  0.20139 -1.643    0.100
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mutantPMK    0.9198      1.087    0.6226     1.359
## mutantRRF    0.7182      1.392    0.4840     1.066
##
## Concordance= 0.526  (se = 0.029 )
## Rsquare= 0.018   (max possible= 1 )
## Likelihood ratio test= 3.03  on 2 df,   p=0.2
## Wald test            = 2.97  on 2 df,   p=0.2
## Score (logrank) test = 2.99  on 2 df,   p=0.2
```

```r
AIC(fit_mut)
```

```
## [1] 1304.882
```

Then we compare the different groups individually

```r
d_OP50 <- data[data$media == 'OP50',]
d_13 <- data[data$media == "FG13",]
d_20 <- data[data$media == "FG20",]
d_fly <- rbind(d_13, d_20)
d_2050 <- rbind(d_20, d_OP50)
d_1350 <- rbind(d_13, d_OP50)
```

First tests for each combination first fly media

```r
survdiff(Surv(Time,status) ~ media, data = d_fly, rho = 0)
```

```
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_fly,
##     rho = 0)
##
##              N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 37       36     39.5     0.310       1.2
## media=FG20 32       31     27.5     0.446       1.2
##
##  Chisq= 1.2  on 1 degrees of freedom, p= 0.3
```

```r
survdiff(Surv(Time,status) ~ media, data = d_fly, rho = 1)
```

```
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_fly,
##     rho = 1)
##
##              N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 37     19.6     23.6     0.679      2.96
## media=FG20 32     20.4     16.4     0.977      2.96
##
##  Chisq= 3  on 1 degrees of freedom, p= 0.09
```

```r
fit <- coxph(Surv(Time,status) ~media, data = d_fly)
summary(fit)
```

```
## Call:
## coxph(formula = Surv(Time, status) ~ media, data = d_fly)
##
##   n= 69, number of events= 67
##
##              coef exp(coef) se(coef)     z Pr(>|z|)
## mediaFG20 0.2228    1.2496   0.2462 0.905    0.365
## mediaOP50     NA        NA   0.0000    NA       NA
```

```
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20      1.25     0.8003    0.7713     2.025
## mediaOP50        NA         NA        NA        NA
##
## Concordance= 0.573  (se = 0.041 )
## Rsquare= 0.012   (max possible= 0.998 )
## Likelihood ratio test= 0.81  on 1 df,   p=0.4
## Wald test            = 0.82  on 1 df,   p=0.4
## Score (logrank) test = 0.82  on 1 df,   p=0.4
```

Then 20 degrees and OP50

```r
survdiff(Surv(Time,status) ~ media, data = d_2050, rho = 0)
```

```
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_2050,
##     rho = 0)
##
##               N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG20 32       31     16.5     12.74      20.7
## media=OP50 94       88    102.5      2.05      20.7
##
##  Chisq= 20.7  on 1 degrees of freedom, p= 5e-06
```

```r
survdiff(Surv(Time,status) ~ media, data = d_2050, rho = 1)
```

```
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_2050,
##     rho = 1)
##
##               N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG20 32       24     11.2     14.54      27.5
## media=OP50 94       47     59.8      2.72      27.5
##
##  Chisq= 27.5  on 1 degrees of freedom, p= 2e-07
```

```r
fit <- coxph(Surv(Time,status) ~media, data = d_2050)
summary(fit)
```

```
## Call:
## coxph(formula = Surv(Time, status) ~ media, data = d_2050)
##
##   n= 126, number of events= 119
##
##              coef exp(coef) se(coef)     z Pr(>|z|)
## mediaFG20 0.9076    2.4784   0.2122 4.276  1.9e-05 ***
## mediaOP50     NA        NA   0.0000    NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
```

```
## mediaFG20     2.478     0.4035     1.635     3.757
## mediaOP50        NA         NA        NA        NA
##
## Concordance= 0.62  (se = 0.025 )
## Rsquare= 0.117   (max possible= 0.999 )
## Likelihood ratio test= 15.71  on 1 df,   p=7e-05
## Wald test            = 18.29  on 1 df,   p=2e-05
## Score (logrank) test = 19.51  on 1 df,   p=1e-05
```

Then for 13 degrees and OP50

```
survdiff(Surv(Time,status) ~ media, data = d_1350, rho = 0)
```

```
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_1350,
##     rho = 0)
##
##              N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 37       36     22.4      8.27      14.7
## media=OP50 94       88    101.6      1.82      14.7
##
##  Chisq= 14.7  on 1 degrees of freedom, p= 1e-04
```

```
survdiff(Surv(Time,status) ~ media, data = d_1350, rho = 1)
```

```
## Call:
## survdiff(formula = Surv(Time, status) ~ media, data = d_1350,
##     rho = 1)
##
##              N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 37     26.4     15.7      7.43      15.5
## media=OP50 94     48.4     59.2      1.96      15.5
##
##  Chisq= 15.5  on 1 degrees of freedom, p= 8e-05
```

```
fit <- coxph(Surv(Time,status) ~media, data = d_1350)
summary(fit)
```

```
## Call:
## coxph(formula = Surv(Time, status) ~ media, data = d_1350)
##
##   n= 131, number of events= 124
##
##               coef exp(coef) se(coef)      z Pr(>|z|)
## mediaFG20       NA        NA   0.0000     NA       NA
## mediaOP50 -0.7709    0.4626   0.2028 -3.802 0.000143 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20        NA         NA        NA        NA
## mediaOP50    0.4626      2.162    0.3109    0.6883
```

```
##
## Concordance= 0.599  (se = 0.024 )
## Rsquare= 0.094   (max possible= 0.999 )
## Likelihood ratio test= 12.94  on 1 df,   p=3e-04
## Wald test            = 14.46  on 1 df,   p=1e-04
## Score (logrank) test = 15.13  on 1 df,   p=1e-04
```

Where only 20 degrees and 13 degrees are significantly different from each other. But there is also some difference between 20 degrees and OP50. Where there isn´t a big difference between 13 degrees and OP50.

Then dose response curves with first the two fly media.

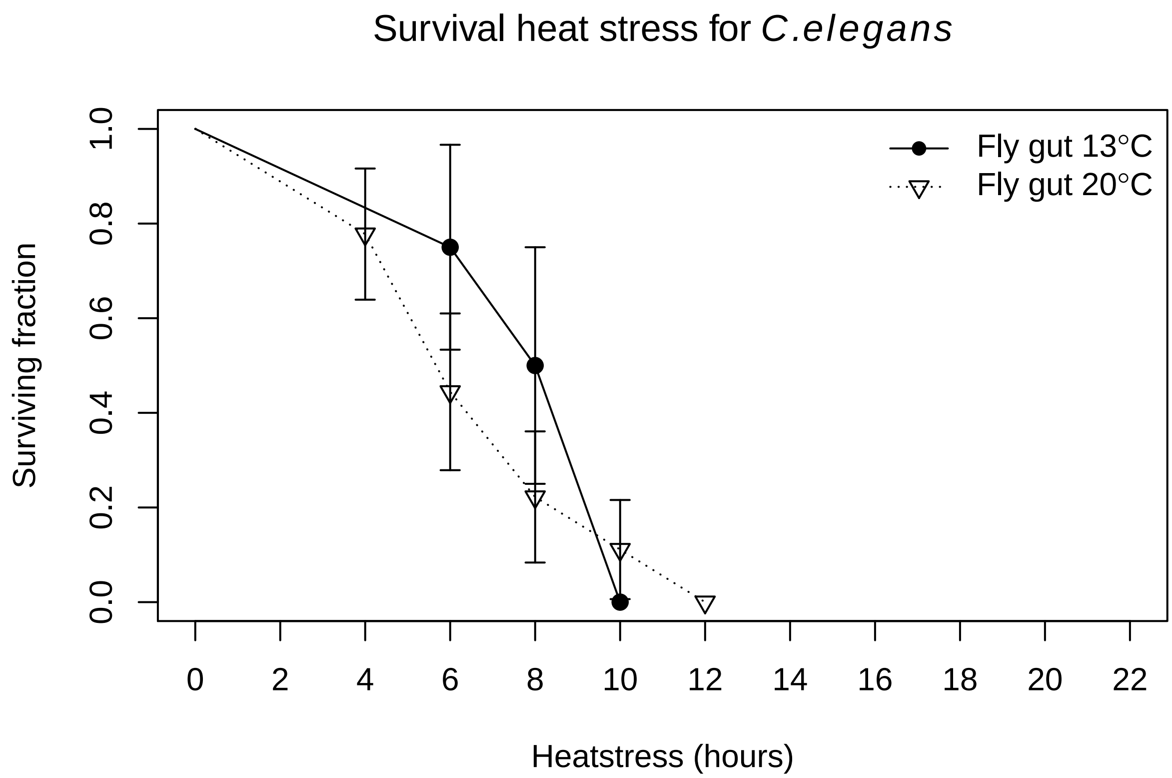
```r
plot(df_fly_13$Time[2:9],df_fly_13$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13$Time,df_fly_13$Surv)
arrows(df_fly_13$Time, df_fly_13$Surv-df_fly_13$Std.error, df_fly_13$Time,
       df_fly_13$Surv+df_fly_13$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_13$Time, df_fly_13$Surv - df_fly_13$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```r
points(df_fly_20$Time[2:9],df_fly_20$Surv[2:9], pch = 6)
lines(df_fly_20$Time,df_fly_20$Surv,lty = 3)
arrows(df_fly_20$Time, df_fly_20$Surv-df_fly_20$Std.error, df_fly_20$Time,
       df_fly_20$Surv+df_fly_20$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_20$Time, df_fly_20$Surv - df_fly_20$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```r
legend('topright', pch = c(16,6),lty = c(1,3),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression('Fly gut 20'*degree*C)), bty = 'n')
```



Than 20 degrees and OP50

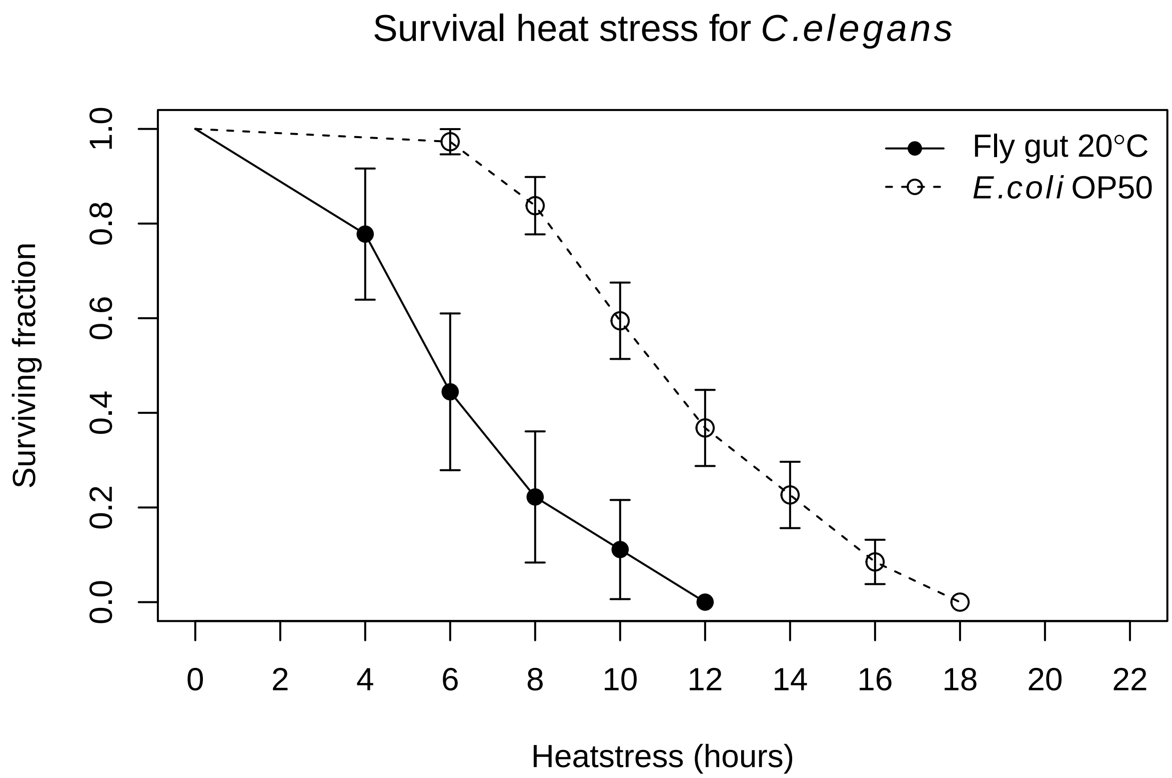
```
plot(df_fly_20$Time[2:9],df_fly_20$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 2)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_OP50$Time, df_OP50$Surv - df_OP50$Std.error,
## df_OP50$Time, : zero-length arrow is of indeterminate angle and so skipped
```

```
lines(df_fly_20$Time,df_fly_20$Surv,lty = 1)
arrows(df_fly_20$Time, df_fly_20$Surv-df_fly_20$Std.error, df_fly_20$Time,
       df_fly_20$Surv+df_fly_20$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_20$Time, df_fly_20$Surv - df_fly_20$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
legend('topright', pch = c(16,1),lty = c(1,2),
       legend = c(expression('Fly gut 20'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```



Then 13 degrees and OP50

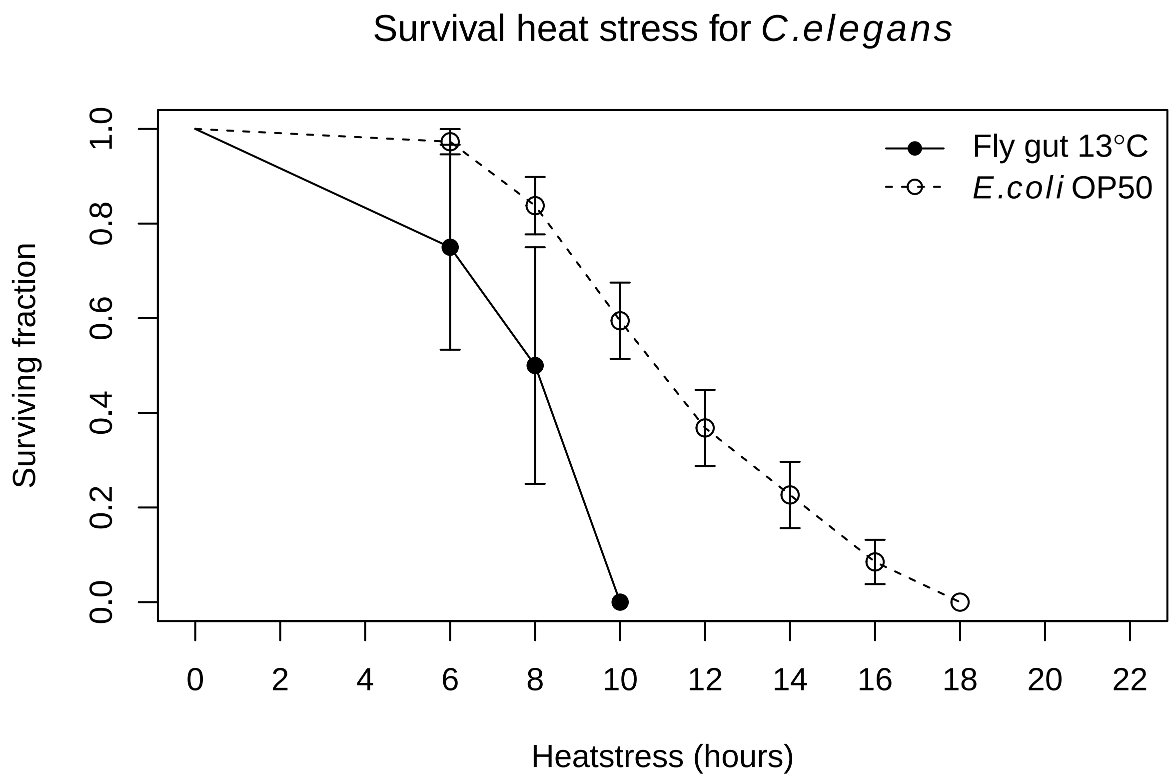
```
plot(df_fly_13$Time[2:9],df_fly_13$Surv[2:9], pch = 16, cex = 1.2, xlab = 'Heatstress (hours)',
     ylab = 'Surviving fraction',
     main = expression('Survival heat stress for'~italic(C.elegans)),
     xlim = c(0,22), ylim = c(0,1), xaxp = c(0,22,11))
lines(df_fly_13$Time,df_fly_13$Surv)
arrows(df_fly_13$Time, df_fly_13$Surv-df_fly_13$Std.error, df_fly_13$Time,
       df_fly_13$Surv+df_fly_13$Std.error, length=0.05, angle=90, code=3, col = 'black')
```

```
## Warning in arrows(df_fly_13$Time, df_fly_13$Surv - df_fly_13$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 2)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

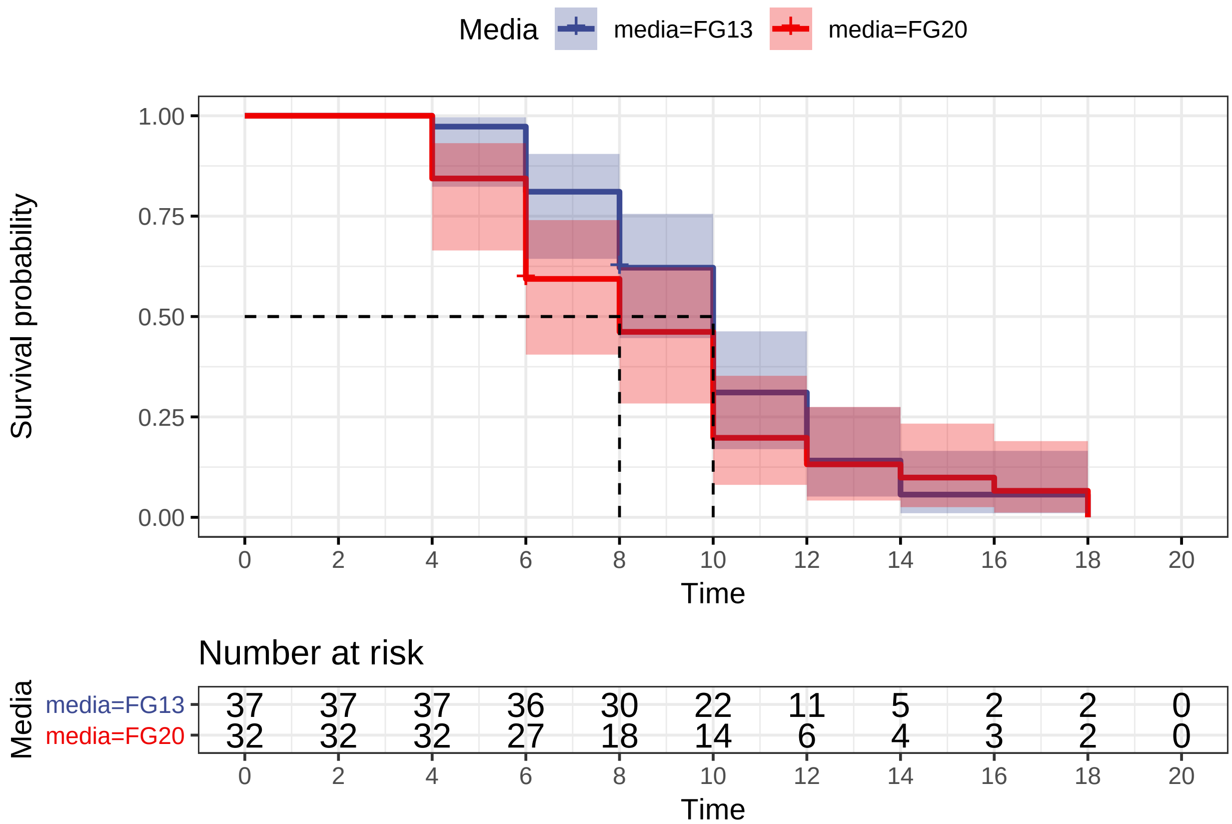
```
## Warning in arrows(df_OP50$Time, df_OP50$Surv - df_OP50$Std.error,
## df_OP50$Time, : zero-length arrow is of indeterminate angle and so skipped
```

```
legend('topright', pch = c(16,1),lty = c(1,2),
       legend = c(expression('Fly gut 13'*degree*C),
                  expression(italic(E.coli) ~ OP50)), bty = 'n')
```

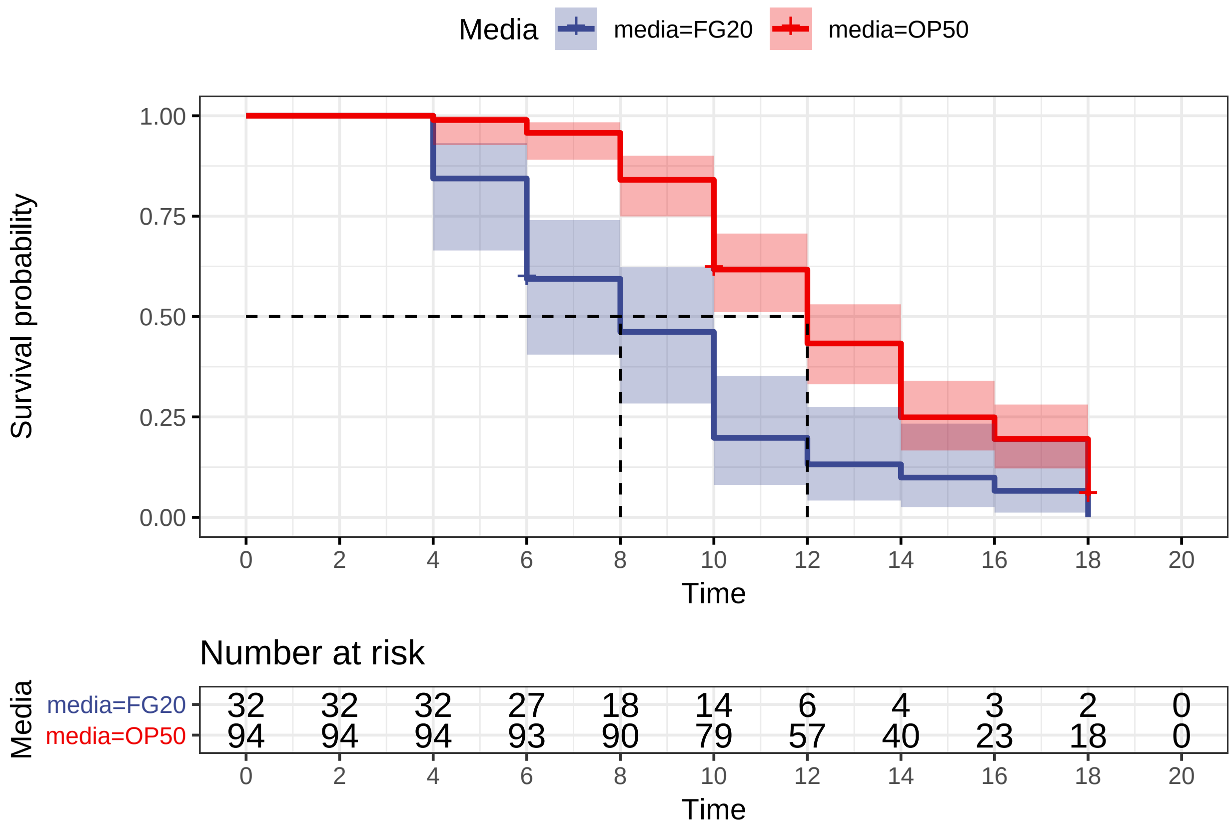


Then Kaplan meier curves

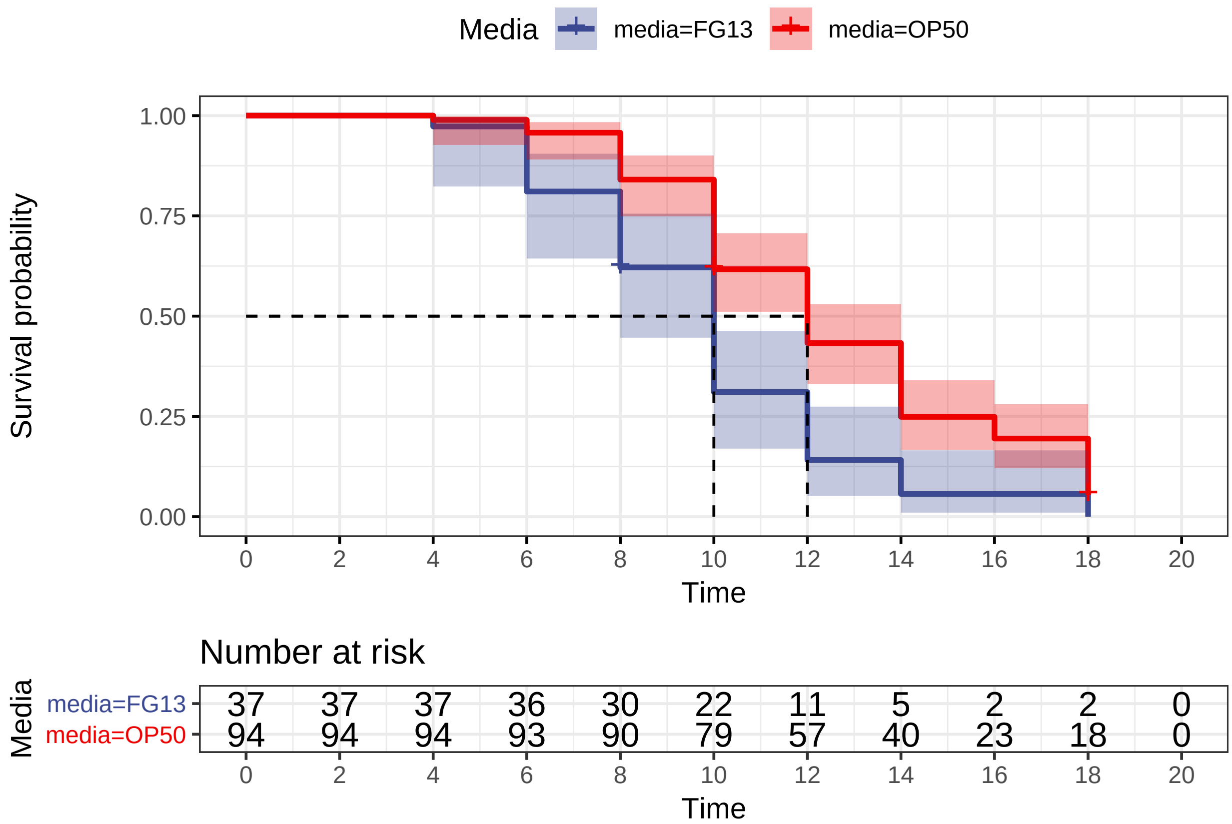
```
km_fly <- survfit(Surv(Time,status) ~ media, data = d_fly,conf.type = "log-log", error = "greenwood")
km_2050 <- survfit(Surv(Time,status) ~ media, data = d_2050,conf.type = "log-log", error = "greenwood")
km_1350 <- survfit(Surv(Time,status) ~ media, data = d_1350,conf.type = "log-log", error = "greenwood")
ggsurvplot(km_fly,data = d_fly, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2)
```




```r
ggsurvplot(km_2050,data = d_2050, conf.int = TRUE,
           ggtheme = theme_bw(), risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2)
```




```r
ggsurvplot(km_1350,data = d_1350, conf.int = TRUE,
           ggtheme = theme_bw(),risk.table = 0.25,
           palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media', break.x.by = 2)
```


Media
media=FG13
media=OP50
Survival probability
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
14
16
18
20
Time
Number at risk
Media
media=FG13
media=OP50
37 37 37 36 30 22 11 5 2 2 0
94 94 94 93 90 79 57 40 23 18 0
Time