Survival of C.elegans with three different media

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

```
data <- read.table(here('data','Worm_4days.csv'), header = TRUE, sep = ";", dec = ",")
head(data)
```

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

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.

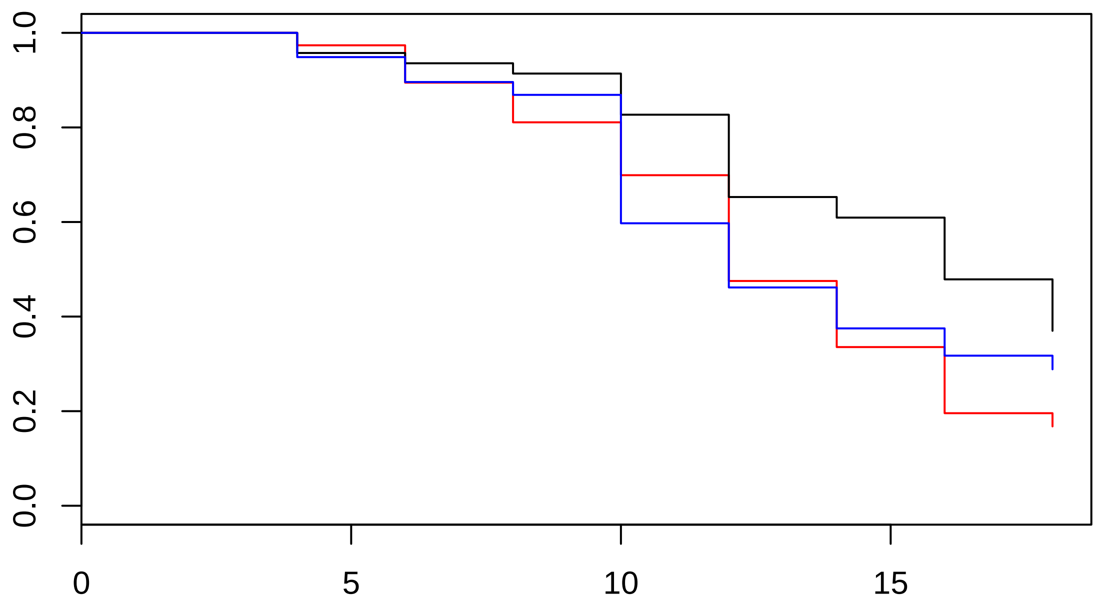
Or make a new .Rproj file in the folder 7.semester. There is a problem with the numbers of the worms so we change that with col.names()

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

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

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

```
data$Survobj <- with(data,Surv(data$Time, event = data$status))
km <- survfit(Survobj ~ media, data = data,conf.type = "log-log", error = "greenwood")
s_km <- summary(km)
plot(km, col = c("red","black",'blue'))
```



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.

```
df_fly_13 <- data.frame(c(0,s_km$time[1:8]),c(1,s_km$surv[1:8]),
                        c(0,s_km$std.err[1:8]))
colnames(df_fly_13) <- c('Time','Surv','Std.error')
df_fly_20 <- data.frame(c(0,s_km$time[9:16]),c(1,s_km$surv[9:16]),
                        c(0,s_km$std.err[9:16]))
colnames(df_fly_20) <- c('Time','Surv','Std.error')
df_OP50 <- data.frame(c(0,s_km$time[17:24]),c(1,s_km$surv[17:24]),
                        c(0,s_km$std.err[17:24]))
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
```

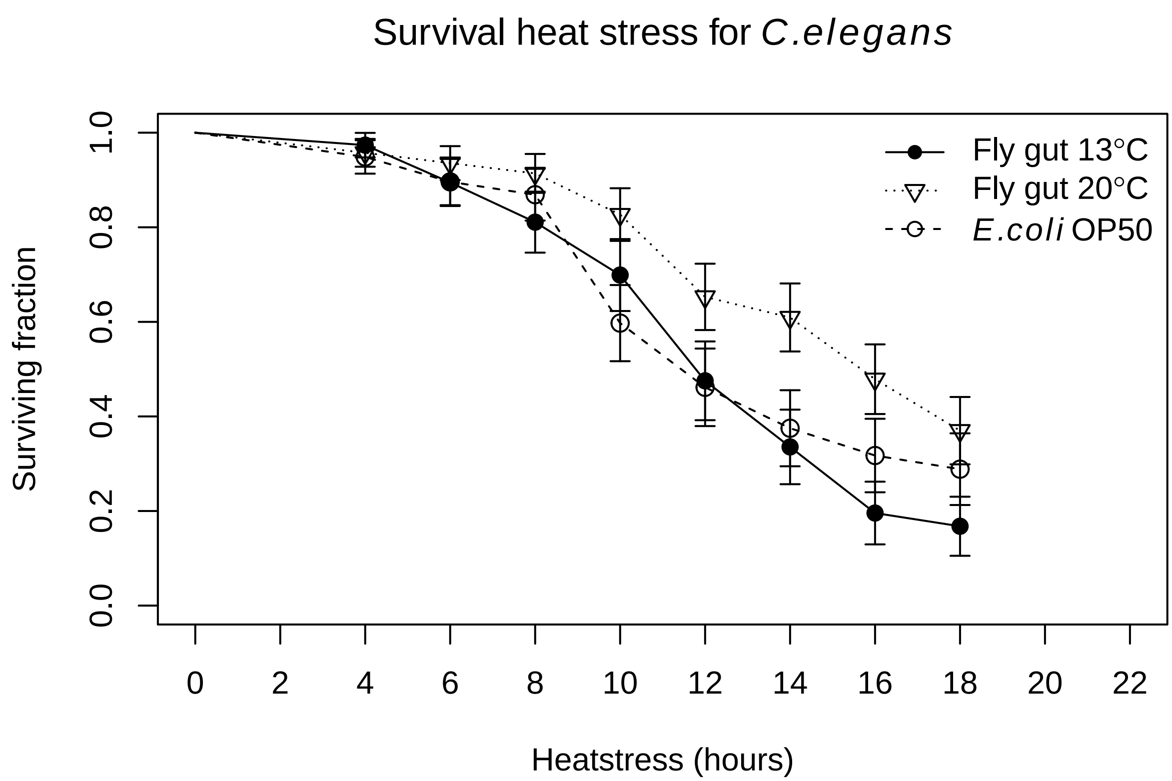
```r
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 2)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## 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')
```

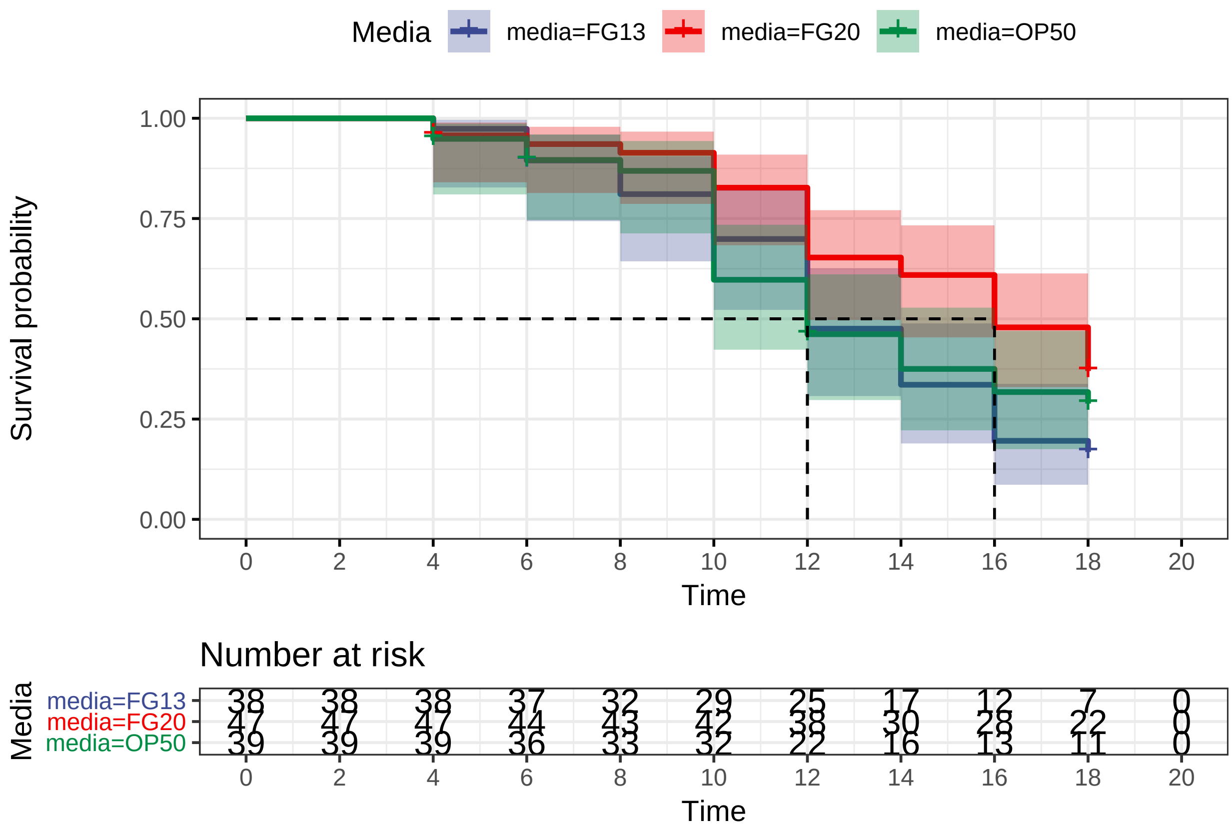
```
## Warning in arrows(df_fly_20$Time, df_fly_20$Surv - df_fly_20$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```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')
```



Then we create a normal Kaplan-Meier curve

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



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

```r
survdiff(Survobj ~media, data = data, rho = 0)
```

```
## Call:
## survdiff(formula = Survobj ~ media, data = data, rho = 0)
##
##                N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 38       30     23.0     2.115     3.498
## media=FG20 47       29     38.4     2.280     5.052
## media=OP50 39       26     23.6     0.238     0.396
##
##  Chisq= 5.6  on 2 degrees of freedom, p= 0.06
```

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

```
## Call:
## survdiff(formula = Survobj ~ media, data = data, rho = 1)
##
##                N Observed Expected (O-E)^2/E (O-E)^2/V
```

```
## media=FG13 38     21.1     16.7     1.149      2.42
## media=FG20 47     18.6     26.0     2.101      5.80
## media=OP50 39     20.0     17.0     0.531      1.13
##
##  Chisq= 5.9  on 2 degrees of freedom, p= 0.05
```

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

```
## Call:
## coxph(formula = Survobj ~ media, data = data)
##
##   n= 124, number of events= 85
##
##              coef exp(coef) se(coef)      z Pr(>|z|)
## mediaFG20 -0.6010    0.5482   0.2622 -2.293   0.0219 *
## mediaOP50 -0.1943    0.8234   0.2685 -0.724   0.4693
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20    0.5482      1.824    0.3280    0.9165
## mediaOP50    0.8234      1.214    0.4865    1.3936
##
## Concordance= 0.576  (se = 0.037 )
## Rsquare= 0.044   (max possible= 0.997 )
## Likelihood ratio test= 5.57  on 2 df,   p=0.06
## Wald test            = 5.44  on 2 df,   p=0.07
## Score (logrank) test = 5.57  on 2 df,   p=0.06
```

We then try to split up in replicates to see if there are any differences between scorers

```
d1 <- data[data$Replicate == 1,]
d2 <- data[data$Replicate == 2,]
d1$Survobj <- with(d1,Surv(d1$Time, event = d1$status))
d2$Survobj <- with(d2,Surv(d2$Time, event = d2$status))
```

Then we make kaplan-meier curves and dose response curves

```
km1 <- survfit(Survobj ~ media, data = d1,conf.type = "log-log")
s_km1 <- summary(km1)
km2 <- survfit(Survobj ~ media, data = d2,conf.type = "log-log")
s_km2 <- summary(km2)
df_fly_13 <- data.frame(c(0,s_km1$time[1:7]),c(1,s_km1$surv[1:7]),
                        c(0,s_km1$std.err[1:7]))
colnames(df_fly_13) <- c('Time','Surv','Std.error')
df_fly_20 <- data.frame(c(0,s_km1$time[8:15]),c(1,s_km1$surv[8:15]),
                        c(0,s_km1$std.err[8:15]))
colnames(df_fly_20) <- c('Time','Surv','Std.error')
df_OP50 <- data.frame(c(0,s_km1$time[16:24]),c(1,s_km1$surv[16:24]),
                        c(0,s_km1$std.err[16:24]))
colnames(df_OP50) <- c('Time','Surv','Std.error')
```

```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
```

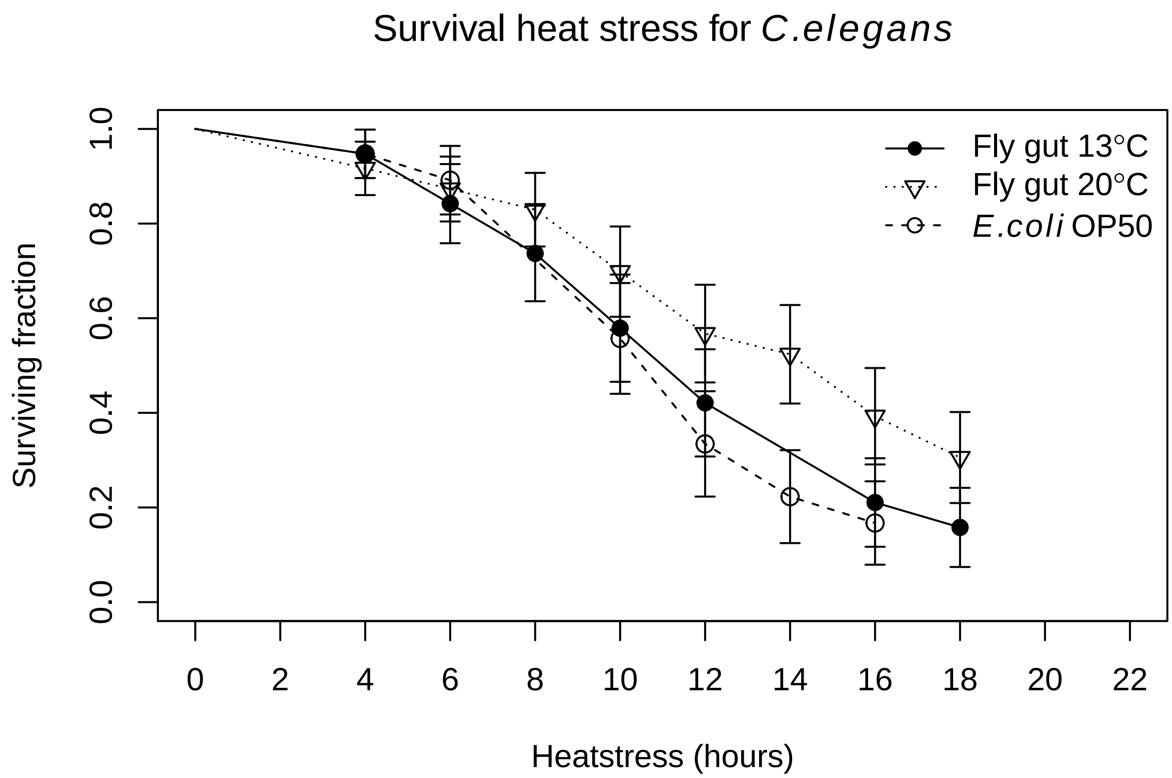
```r
points(df_OP50$Time[2:9],df_OP50$Surv[2:9], pch = 1, cex = 1.2)
lines(df_OP50$Time,df_OP50$Surv, lty = 2)
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## 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')
```




```
df_fly_13 <- data.frame(c(0,s_km2$time[1:6],18),
                        c(1,s_km2$surv[1:6],s_km2$surv[6]),
                        c(0,s_km2$std.err[1:6],s_km2$std.err[6]))
colnames(df_fly_13) <- c('Time','Surv','Std.error')
df_fly_20 <- data.frame(c(0,s_km2$time[7:11]),c(1,s_km2$surv[7:11]),
                        c(0,s_km2$std.err[7:11]))
colnames(df_fly_20) <- c('Time','Surv','Std.error')
df_OP50 <- data.frame(c(0,s_km2$time[12:19]),c(1,s_km2$surv[12:19]),
                        c(0,s_km2$std.err[12:19]))
colnames(df_OP50) <- c('Time','Surv','Std.error')

plot(df_fly_13$Time[2:8],df_fly_13$Surv[2:8],
     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)
```

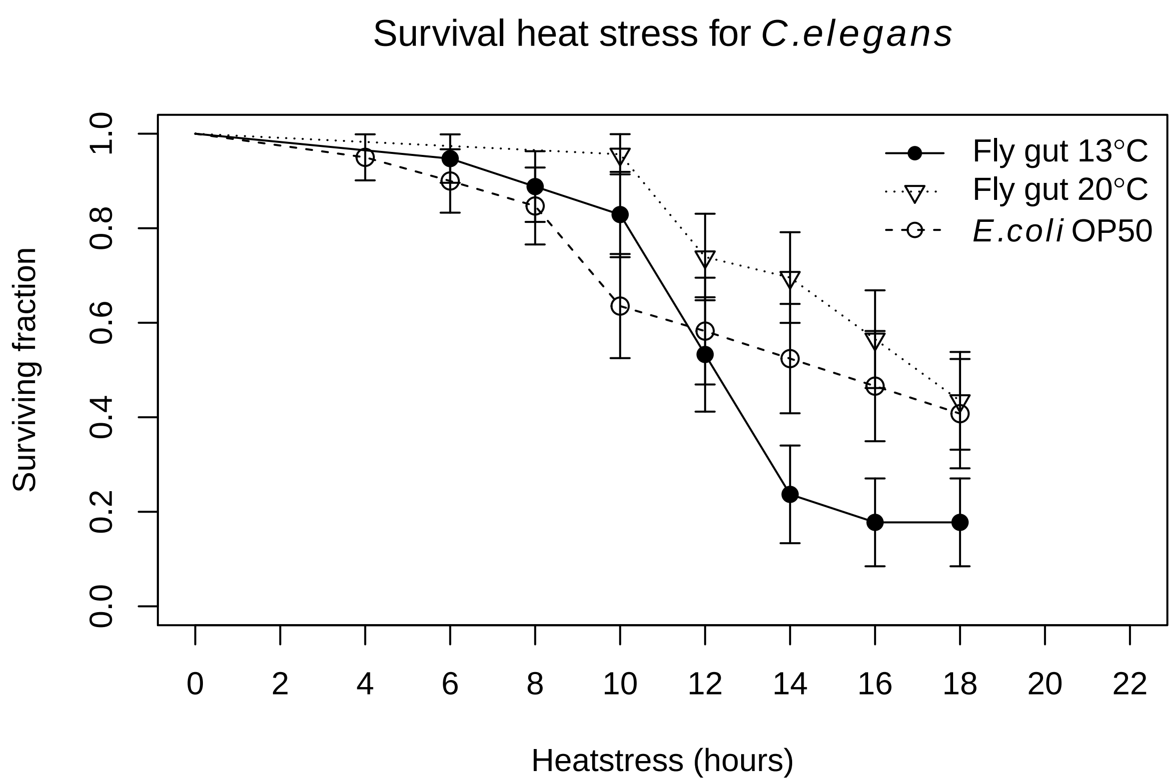
```
arrows(df_OP50$Time, df_OP50$Surv-df_OP50$Std.error,
      df_OP50$Time, df_OP50$Surv+df_OP50$Std.error,
      length=0.05, angle=90, code=3, col = 'black')
```

```
## 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')
```

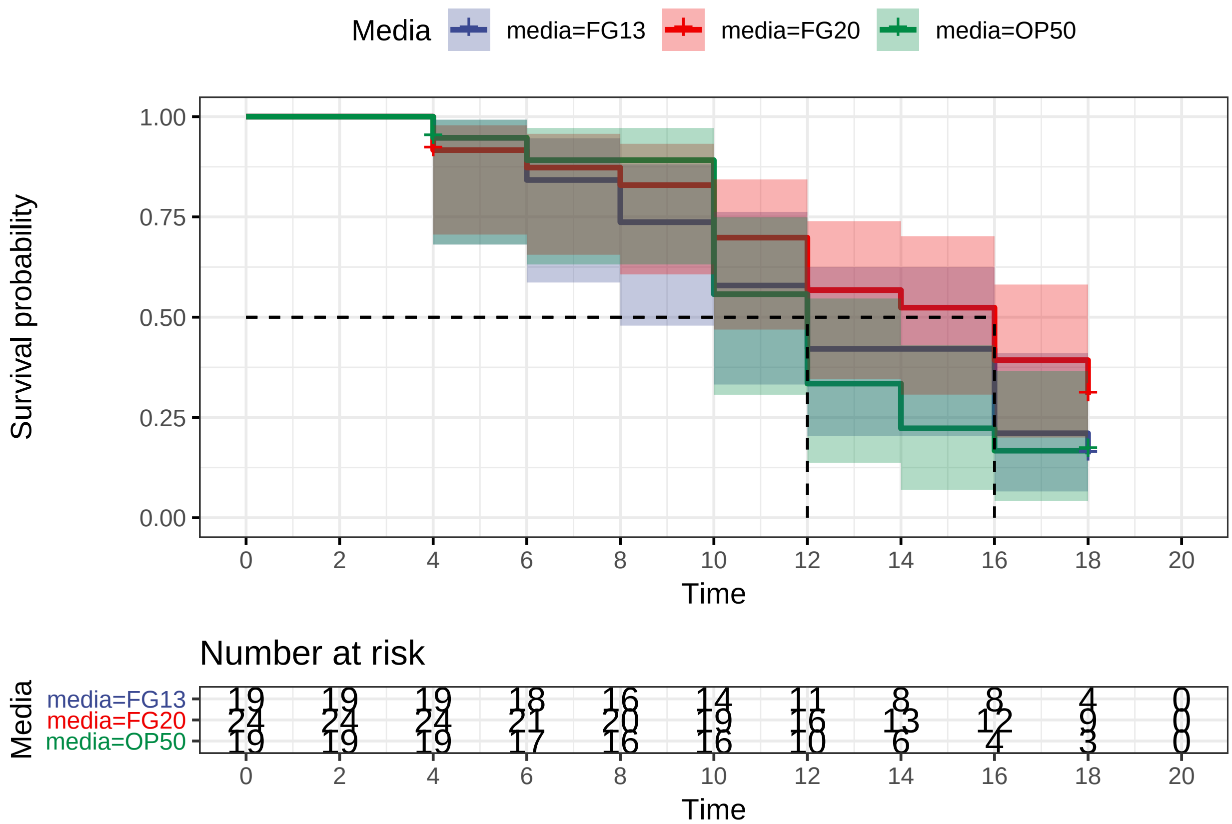
```
## Warning in arrows(df_fly_20$Time, df_fly_20$Surv - df_fly_20$Std.error, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
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')
```

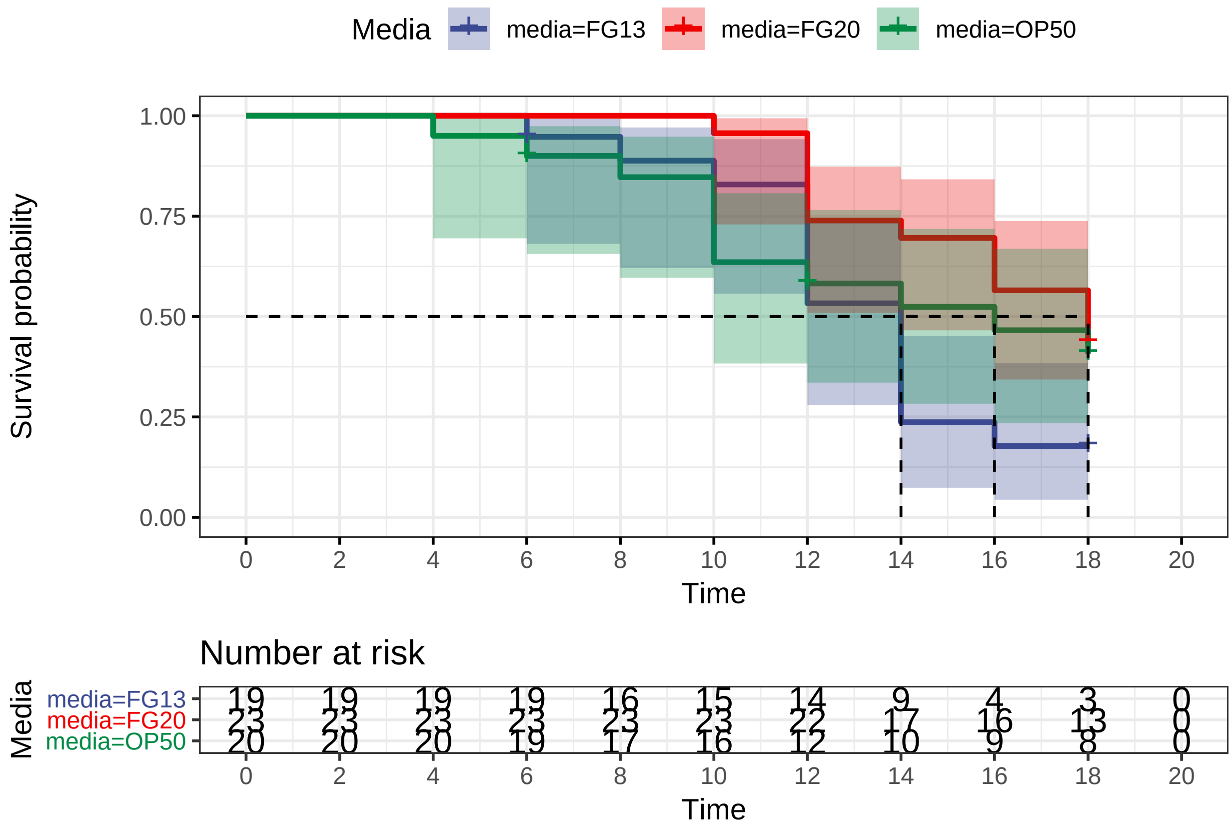


Then kaplan meier

```r
ggsurvplot(km1,data = data, 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(km2,data = data, conf.int = TRUE, ggtheme = theme_bw(),
           risk.table = 0.25, palette ='aaas', surv.median.line = 'hv',
           legend.title = 'Media',break.x.by = 2)
```



and at last some tests

```
survdiff(Survobj ~media, data = d1, rho = 0)
```

```
## Call:
## survdiff(formula = Survobj ~ media, data = d1, rho = 0)
##
##              N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 19       16     14.1     0.244     0.431
## media=FG20 24       16     20.3     0.915     2.014
## media=OP50 19       15     12.5     0.480     0.815
##
##  Chisq= 2.1  on 2 degrees of freedom, p= 0.4
```

```
survdiff(Survobj ~media, data = d1, rho = 1)
```

```
## Call:
## survdiff(formula = Survobj ~ media, data = d1, rho = 1)
##
##              N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 19     10.8     9.54     0.162     0.368
## media=FG20 24     10.3    13.14     0.613     1.687
## media=OP50 19     10.6     9.03     0.282     0.613
##
##  Chisq= 1.7  on 2 degrees of freedom, p= 0.4
```

```r
fit <- coxph(Survobj ~media, data = d1)
summary(fit)
```

```
## Call:
## coxph(formula = Survobj ~ media, data = d1)
##
##   n= 62, number of events= 47
##
##                coef exp(coef) se(coef)      z Pr(>|z|)
## mediaFG20 -0.41066   0.66321  0.35443 -1.159    0.247
## mediaOP50  0.07651   1.07951  0.36076  0.212    0.832
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20    0.6632     1.5078    0.3311     1.328
## mediaOP50    1.0795     0.9263    0.5323     2.189
##
## Concordance= 0.557  (se = 0.052 )
## Rsquare= 0.035   (max possible= 0.995 )
## Likelihood ratio test= 2.2  on 2 df,   p=0.3
## Wald test            = 2.13  on 2 df,   p=0.3
## Score (logrank) test = 2.16  on 2 df,   p=0.3
```

```r
survdiff(Survobj ~media, data = d2, rho = 0)
```

```
## Call:
## survdiff(formula = Survobj ~ media, data = d2, rho = 0)
##
##             N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 19       14     9.32  2.355719   3.72463
## media=FG20 23       13    17.77  1.282531   2.84312
## media=OP50 20       11    10.91  0.000743   0.00122
##
##  Chisq= 4.3  on 2 degrees of freedom, p= 0.1
```

```r
survdiff(Survobj ~media, data = d2, rho = 1)
```

```
## Call:
## survdiff(formula = Survobj ~ media, data = d2, rho = 1)
##
##             N Observed Expected (O-E)^2/E (O-E)^2/V
## media=FG13 19    10.58     7.27     1.510      2.95
## media=FG20 23     8.40    12.74     1.475      4.04
## media=OP50 20     8.98     7.96     0.131      0.27
##
##  Chisq= 4.6  on 2 degrees of freedom, p= 0.1
```

```r
fit <- coxph(Survobj ~media, data = d2)
summary(fit)
```

```
## Call:
## coxph(formula = Survobj ~ media, data = d2)
```

```
##
##   n= 62, number of events= 38
##
##              coef exp(coef) se(coef)      z Pr(>|z|)
## mediaFG20 -0.8083    0.4456   0.3905 -2.070   0.0385 *
## mediaOP50 -0.4944    0.6099   0.4073 -1.214   0.2248
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##           exp(coef) exp(-coef) lower .95 upper .95
## mediaFG20    0.4456      2.244    0.2073     0.958
## mediaOP50    0.6099      1.639    0.2746     1.355
##
## Concordance= 0.603  (se = 0.053 )
## Rsquare= 0.066   (max possible= 0.989 )
## Likelihood ratio test= 4.23  on 2 df,   p=0.1
## Wald test            = 4.36  on 2 df,   p=0.1
## Score (logrank) test = 4.53  on 2 df,   p=0.1
```