Survival Analysis – Handout

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Goal

We have absorbance (optical density, OD) measurements of algal cultures over time for three treatments. This R Markdown walks through an analysis that **uses a survival-analysis approach**: we define the event as *time to reach a event* (i.e. reach end of experiment milestone). This allows handling of cultures that never reach the threshold within the experiment (right-censored observations) and comparing groups using Kaplan–Meier curves, log-rank tests, and Cox proportional hazards models.

Justification!

Why We Are Using Survival Analysis

Alright Korey and Maria, here is an explanation of why I think you should and CAN use this approach.

We are not looking for a specific growth threshold. The issue is that the experiment has a hard stop. Some algae cultures get close to something interesting, and others do not, but everything ends at the same final time point because the experiment has to end.

So the real question becomes:

"How long does each culture continue growing before the experiment forces us to stop?"

This is exactly the kind of situation survival analysis is made for. Here is why it fits what we are doing.

1. Our event is the end of the experiment

Instead of waiting for the algae to reach some specific level, the "event" in our case is that the experiment ends. Some cultures are still growing. Some appear to have slowed down. Others might be about to separate from the pack but never get the chance because the experiment stops.

Survival analysis lets us model this without pretending the algae hit a threshold that they did not actually reach.

2. All cultures are censored by design

Normally, censoring happens when something fails to happen in time. But here, censoring is simply the structure of the experiment. Every culture that does not finish whatever process we care about by the final measurement is censored at the end-of-experiment time.

This is not a problem for survival analysis. In fact, survival analysis is built to handle exactly this type of "we stopped watching before the event happened" data.

3. It lets us compare treatments without assuming perfect growth curves

The algae do not follow perfect smooth logistic curves. They wiggle, plateau, slow down, and then take off again. If we tried to fit nonlinear models, we would be forcing the data into shapes it does not actually follow.

Survival analysis does not care about the exact shape of the growth curve. It only cares about time until the event or censoring. That makes it realistic and robust for the type of noisy, real-world growth dynamics we have.

4. It gives us simple comparisons between treatments

Kaplan Meier curves show how much "growth potential" is still left in each group at each time point. Even though the event is the experiment ending, the KM curves still tell us who is ahead or behind as time goes on.

Cox models give hazard ratios. In our case, the hazard ratio tells us whether one treatment is "progressing toward the cutoff" faster or slower than another. This becomes a practical way to compare treatments even when none of them reach a traditional endpoint.

5. We do not lose data by only looking at the final OD measurement

If we just looked at the final absorbance for each treatment, we would miss all the differences in how fast each culture was growing over time. Survival analysis uses all the data: - every time point, - every culture, - all the differences in growth speed, - all the censored cases.

Nothing gets thrown away.

6. It fits the story of the experiment

The goal is not "Did it reach some number?"
The goal is "How long did it keep progressing until we had to stop the experiment?"

Survival analysis is exactly the framework that treats our forced stop correctly and lets us compare treatments in a statistically meaningful way.

Summary

We are using survival analysis because the experiment does not run long enough for the cultures to reach a common endpoint. Instead, everyone gets cut off at the end of the experiment, and survival analysis is designed to handle that kind of censoring. It uses all of our data across time, does not require perfect growth curves, and gives us straightforward comparisons between treatments.

In short:

Survival analysis matches the way the experiment actually works, and it gives us the cleanest and most meaningful way to compare how the treatments influence growth over time.

1. Packages & session info

We load tidyverse for data wrangling and plotting, survival for survival analysis, and survminer for nice plotting functions.

```r
library(tidyverse)
library(survival)
library(survminer)
```

2. Data: two options

For Maria and Korey, I have created simulated example data below (Option B). You can also use your own data (Option A) if you have it in the suggested format. Hopefully, this handout will give you a sense of what this approach looks like and how to implement it!

Option A — use your own CSV: a suggested format is long: one row per sample per timepoint. Columns:

- `sample_id` (unique for each biological replicate)
- `treatment` (factor with 3 levels, e.g. `A`, `B`, `C`)
- `time` (time in hours or days; numeric)
- `absorbance` (OD or fluorescence reading; numeric)

Read it with:

```r
# df_raw <- read.csv("your_algae_data.csv")
# glimpse(df_raw)
```

Option B — simulated example data (used in this report so the analysis runs end-to-end). I thinnk this simulation creates realistic growth curves with noise, i.e. variation needed for a statistical analysis!

More over, I created 10 replicates – where some replicates that never hit the threshold.

```r
set.seed(2025)
# parameters
n_rep <- 10   # replicates per treatment
times <- seq(0, 72, by = 8) # sample every 8 hours for 72 hours

make_growth <- function(n, treatment_label, mu_time_to_mid = 30, sd_time = 6, maxOD = 1.2, prop_no_reach
  tibble(sample = paste0(treatment_label, "_", seq_len(n))) %>%
    rowwise() %>%
    mutate(
      # latent time to reach midpoint (control over growth speed)
      t_mid = rnorm(1, mu_time_to_mid, sd_time) |> pmax(6),
      slope = (maxOD) / (t_mid + 0.1),
      # for a small proportion, set very slow growth so they won't reach threshold
      never = runif(1) < prop_no_reach
    ) %>%
    ungroup() %>%
    select(-t_mid, -slope, -never) -> samples

  expand_grid(sample = samples$sample, time = times) %>%
    left_join(samples, by = "sample") %>%
    rowwise() %>%
    mutate(
      # generate an underlying logistic-like growth curve with noise
      mu = plogis((time - rnorm(1, mu_time_to_mid, sd_time))/7) * maxOD,
      absorbance = mu + rnorm(1, 0, 0.03)
    ) %>%
    ungroup() %>%
    mutate(treatment = treatment_label)
```

```
}

# create three treatments with different typical growth rates
df_A <- make_growth(n_rep, "A", mu_time_to_mid = 28, sd_time = 6, maxOD = 1.1, prop_no_reach = 0.05)
df_B <- make_growth(n_rep, "B", mu_time_to_mid = 34, sd_time = 7, maxOD = 1.15, prop_no_reach = 0.12)
df_C <- make_growth(n_rep, "C", mu_time_to_mid = 22, sd_time = 5, maxOD = 1.0, prop_no_reach = 0.08)

df_raw <- bind_rows(df_A, df_B, df_C) %>%
  mutate(treatment = factor(treatment))

# inspect a handful
df_raw %>% group_by(treatment) %>% slice_head(n = 6)
```

```
## # A tibble: 18 x 5
## # Groups:   treatment [3]
##    sample  time      mu absorbance treatment
##    <chr>  <dbl>   <dbl>      <dbl> <fct>
##  1 A_1        0 0.0233      0.0581 A
##  2 A_1        8 0.0623      0.0175 A
##  3 A_1       16 0.123       0.122  A
##  4 A_1       24 0.699       0.692  A
##  5 A_1       32 0.199       0.189  A
##  6 A_1       40 0.960       0.941  A
##  7 B_1        0 0.00495    -0.0645 B
##  8 B_1        8 0.213       0.199  B
##  9 B_1       16 0.0448      0.0604 B
## 10 B_1       24 0.365       0.342  B
## 11 B_1       32 0.793       0.842  B
## 12 B_1       40 0.960       0.968  B
## 13 C_1        0 0.0587      0.0488 C
## 14 C_1        8 0.104       0.0963 C
## 15 C_1       16 0.233       0.217  C
## 16 C_1       24 0.432       0.420  C
## 17 C_1       32 0.482       0.503  C
## 18 C_1       40 0.959       1.02   C
```

3. Visualize raw growth curves

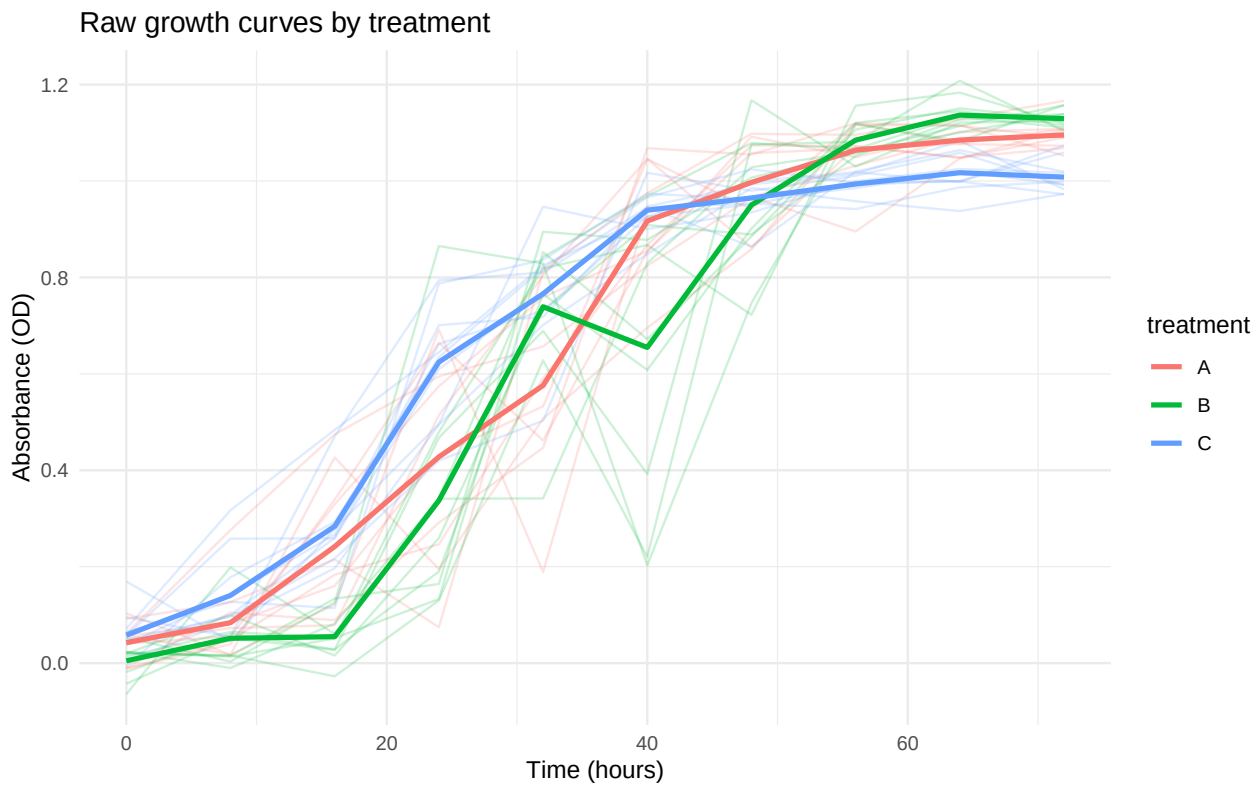
Plotting raw absorbance over time for each replicate is important to see shapes, noise, and whether a single threshold makes sense.

```
# spaghetti plot: individual curves (light) + treatment mean (bold)
df_means <- df_raw %>%
  group_by(treatment, time) %>%
  summarise(mean_abs = mean(absorbance), .groups = "drop")

p_raw <- ggplot() +
  geom_line(data = df_raw,
            aes(time, absorbance, group = sample, color = treatment),
            alpha = 0.2, show.legend = FALSE) +
  geom_line(data = df_means,
            aes(time, mean_abs, color = treatment),
            size = 1.1) +
```

```
  labs(x = "Time (hours)",
       y = "Absorbance (OD)",
       title = "Raw growth curves by treatment") +
  theme_minimal()

p_raw
```



4. Define event: time to reach a threshold

Choose a threshold that represents a biologically meaningful growth milestone. For example, `threshold <- 0.6` (you can change this). We compute, per sample, the **first time** the absorbance is >= threshold. If a sample never reaches the threshold during the recorded times, we treat it as **right-censored** at the last observed timepoint.

```
threshold <- 0.6

time_to_event <- df_raw %>%
  group_by(sample, treatment) %>%
  arrange(time) %>%
  summarise(
    event_time = {
      hit_rows <- which(absorbance >= threshold)
      if(length(hit_rows) == 0) NA_real_ else time[min(hit_rows)]
    },
    last_time = max(time),
    .groups = "drop"
  ) %>%
```

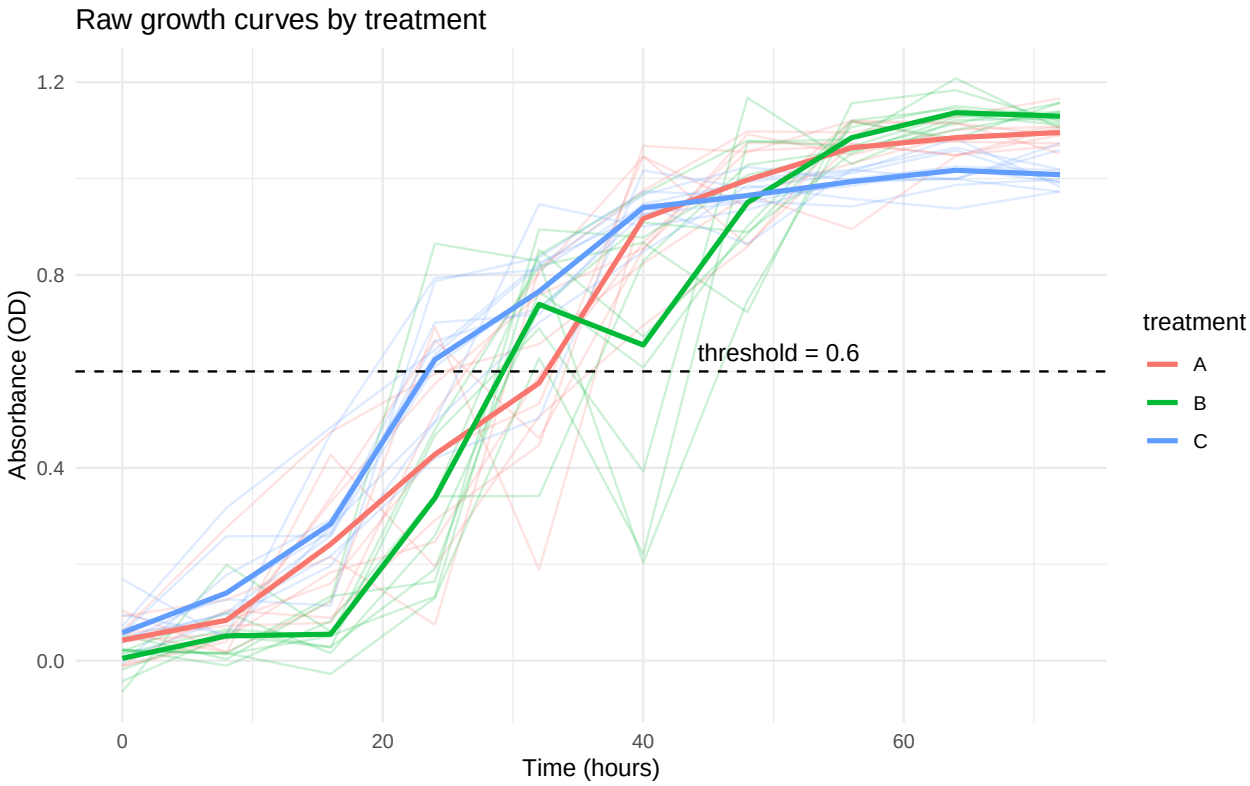
```
  mutate(
    status = if_else(is.na(event_time), 0L, 1L),        # 1 = event observed, 0 = censored
    time = if_else(is.na(event_time), last_time, event_time)
  )

# show table
time_to_event %>% count(treatment, status)
```

```
## # A tibble: 3 x 3
##   treatment status     n
##   <fct>      <int> <int>
## 1 A              1    10
## 2 B              1    10
## 3 C              1    10
```

Plot the threshold on the raw curves so readers see what the event means.

```
p_raw + geom_hline(yintercept = threshold, linetype = "dashed") +
  annotate("text", x = max(df_raw$time)*0.7, y = threshold + 0.04, label = paste0("threshold = ", thresh
```



5. Kaplan–Meier curves by treatment

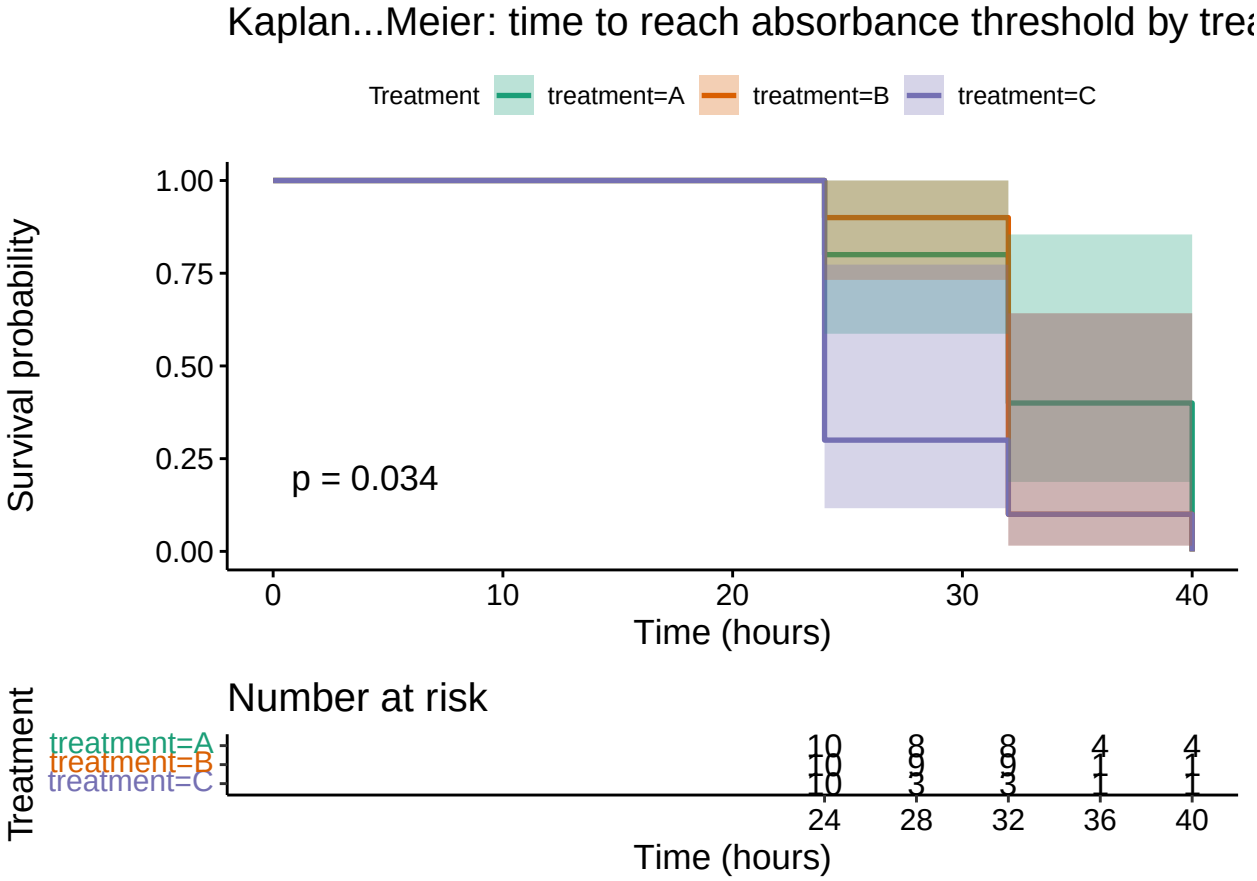
Create a `Surv` object and fit Kaplan–Meier curves. We will visualize them and report median time-to-event where available.

```
km_fit <- survfit(Surv(time, status) ~ treatment, data = time_to_event)
summary(km_fit)
```

```
## Call: survfit(formula = Surv(time, status) ~ treatment, data = time_to_event)
```

```
##
##                 treatment=A
##  time n.risk n.event survival std.err lower 95% CI upper 95% CI
##    24     10       2      0.8   0.126        0.587        1.000
##    32      8       4      0.4   0.155        0.187        0.855
##    40      4       4      0.0     NaN           NA           NA
##
##                 treatment=B
##  time n.risk n.event survival std.err lower 95% CI upper 95% CI
##    24     10       1      0.9  0.0949       0.7320        1.000
##    32      9       8      0.1  0.0949       0.0156        0.642
##    40      1       1      0.0     NaN           NA           NA
##
##                 treatment=C
##  time n.risk n.event survival std.err lower 95% CI upper 95% CI
##    24     10       7      0.3  0.1449       0.1164        0.773
##    32      3       2      0.1  0.0949       0.0156        0.642
##    40      1       1      0.0     NaN           NA           NA
```

```
ggsurvplot(km_fit, data = time_to_event, risk.table = TRUE, pval = TRUE,
           conf.int = TRUE, palette = "Dark2",
           title = "Kaplan-Meier: time to reach absorbance threshold by treatment",
           xlab = "Time (hours)", legend.title = "Treatment")
```



Interpretation: The survival curve here shows the probability that a culture has *not yet* reached the threshold at each timepoint. Lower curves correspond to faster attainment of the threshold.

6. Log-rank test (overall)

The log-rank test (implemented as `survdiff`) checks for differences in survival functions across groups.

```
logrank <- survdiff(Surv(time, status) ~ treatment, data = time_to_event)
logrank
```

```
## Call:
## survdiff(formula = Surv(time, status) ~ treatment, data = time_to_event)
##
##              N Observed Expected (O-E)^2/E (O-E)^2/V
## treatment=A 10       10    12.93    0.6653     3.318
## treatment=B 10       10    10.63    0.0377     0.153
## treatment=C 10       10     6.43    1.9774     6.069
##
##  Chisq= 6.7  on 2 degrees of freedom, p= 0.03
```

```
# compute approximate p-value
p_val <- 1 - pchisq(logrank$chisq, df = length(logrank$n) - 1)
cat("Omnibus log-rank p-value:", signif(p_val, 3), "\n")
```

```
## Omnibus log-rank p-value: 0.0345
```

7. Pairwise comparisons (adjusted)

If the omnibus test is significant, we may want pairwise comparisons. We'll compute pairwise log-rank tests and adjust p-values using the Benjamini-Hochberg method.

```
# pairwise_survdiff is available via survminer
pairwise_res <- pairwise_survdiff(Surv(time, status) ~ treatment, data = time_to_event)
pairwise_res
```

```
##
##  Pairwise comparisons using Log-Rank test
##
## data:  time_to_event and treatment
##
##   A     B
## B 0.365 -
## C 0.073 0.073
##
## P value adjustment method: BH
```

```
# extract p-values and adjust
pw <- pairwise_res$p.value
pw_vec <- na.omit(as.vector(pw))
adj <- p.adjust(pw_vec, method = "BH")
adj
```

```
## [1] 0.3652911 0.1088803 0.1088803
```

8. Cox proportional hazards model

A Cox model estimates hazard ratios between treatments, which can be interpreted as relative rates of reaching the threshold.

```
# encode treatment as factor; choose reference
time_to_event <- time_to_event %>% mutate(treatment = relevel(treatment, ref = "A"))
cox1 <- coxph(Surv(time, status) ~ treatment, data = time_to_event)
summary(cox1)
```

```
## Call:
## coxph(formula = Surv(time, status) ~ treatment, data = time_to_event)
##
##   n= 30, number of events= 30
##
##              coef exp(coef) se(coef)     z Pr(>|z|)
## treatmentB 0.3785    1.4601   0.4586 0.825   0.4092
## treatmentC 0.9301    2.5347   0.4554 2.042   0.0411 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##            exp(coef) exp(-coef) lower .95 upper .95
## treatmentB     1.460     0.6849    0.5943     3.587
## treatmentC     2.535     0.3945    1.0382     6.189
##
## Concordance= 0.722  (se = 0.085 )
## Likelihood ratio test= 4.04  on 2 df,   p=0.1
## Wald test            = 4.23  on 2 df,   p=0.1
## Score (logrank) test = 4.45  on 2 df,   p=0.1
```

Notes: the hazard ratio (HR) > 1 means faster attainment of the threshold relative to the reference.

9. Check proportional hazards assumption

```
ph_test <- cox.zph(cox1)
ph_test
```

```
##           chisq df    p
## treatment  3.61  2 0.16
## GLOBAL     3.61  2 0.16
```

```
plot(ph_test)
```

If the proportional hazards assumption fails for treatment, consider alternatives: stratified Cox, time-varying covariates, or non-parametric comparisons.

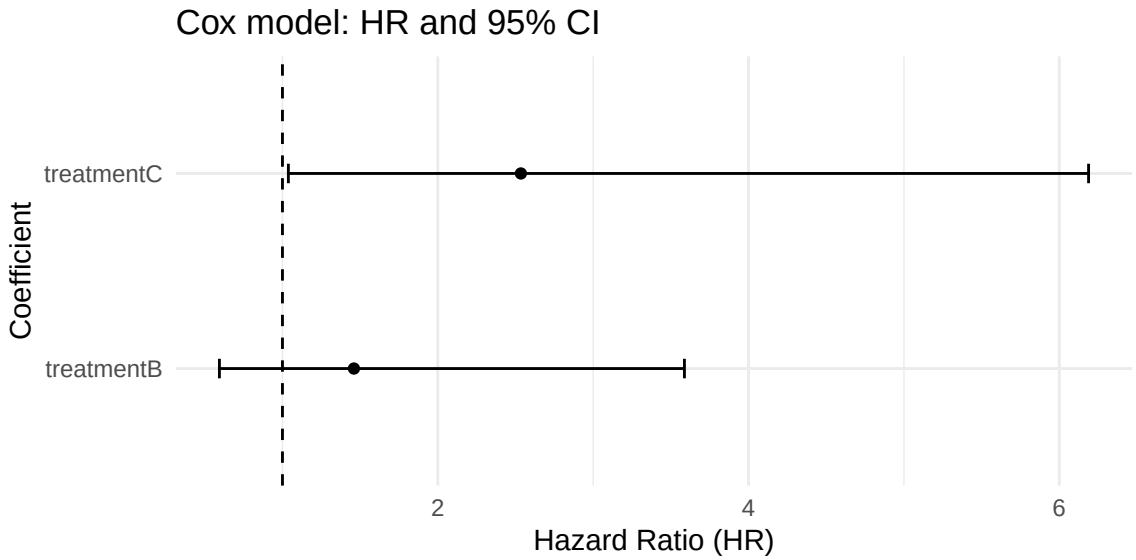
10. Visual diagnostics and alternative visualizations

Plot the cumulative hazard or complementary log-log plots if helpful. We can also overlay median times and forest plots of HRs.

```r
# simple forest plot for Cox HRs
hr <- broom::tidy(cox1, exponentiate = TRUE, conf.int = TRUE)
hr
```

```
## # A tibble: 2 x 7
##   term       estimate std.error statistic p.value conf.low conf.high
##   <chr>         <dbl>     <dbl>     <dbl>   <dbl>    <dbl>     <dbl>
## 1 treatmentB     1.46     0.459     0.825  0.409     0.594      3.59
## 2 treatmentC     2.53     0.455     2.04   0.0411    1.04       6.19
```

```r
ggplot(hr, aes(x = term, y = estimate)) +
  geom_point() +
  geom_errorbar(aes(ymin = conf.low, ymax = conf.high), width = 0.1) +
  geom_hline(yintercept = 1, linetype = "dashed") +
  coord_flip() +
  labs(y = "Hazard Ratio (HR)", x = "Coefficient", title = "Cox model: HR and 95% CI") +
  theme_minimal()
```



11. Reporting recommended outputs

A minimal reproducible report should include:

- Raw growth curve figure with threshold annotated (Step 3 & 4).
- Table of `time_to_event` with `sample`, `treatment`, `time`, `status` (observed/censored).
- Kaplan–Meier plot with risk table and p-value (Step 5).
- Omnibus log-rank test results and pairwise p-values with multiple-testing corrections (Steps 6–7).
- Cox model coefficients, hazard ratios and 95% CI (Step 8) and asses

12. Rationale Notes for Marc

Rationale for Using Survival Analysis

Survival analysis is a powerful and appropriate statistical framework for analyzing algae growth when the primary question concerns *how long* it takes cultures to reach a biologically meaningful milestone, such as exceeding a specified absorbance threshold. This section explains why survival methods are preferred in such situations.

1. Focus on Time-to-Threshold Outcomes

Many algal growth studies aim to compare how long cultures under different treatments require to reach: - a specified absorbance (OD) level, - the onset of exponential growth, or - a density indicating establishment or competitive success.

Survival analysis directly models **time-to-event** outcomes, aligning the statistical method with the biological question.

2. Natural Handling of Right-Censoring

Not every culture reaches the absorbance threshold during the experimental window. Traditional approaches (e.g., ANOVA on final OD or curve-fitting) often discard these observations or treat them incorrectly.
Survival analysis handles such cases as **right-censored** data: - the event did not occur during the observation period, - but the culture still provides valid information up to its last measurement time.

This prevents bias and maximizes data use.

3. Minimal Assumptions About Growth Curves

Kaplan–Meier curves and log-rank tests compare groups **without requiring a specific growth model**. This is helpful because real algal populations may deviate from ideal logistic or Gompertz growth curves due to: - environmental fluctuations, - physiological acclimation, - measurement noise, and - treatment-specific growth dynamics.

Survival methods remain robust under these conditions.

4. Interpretable Effect Sizes via Cox Models

The Cox proportional hazards model provides hazard ratios that quantify how treatments affect the **rate at which cultures reach the absorbance threshold**: - hazard ratio (HR) > 1 indicates faster growth, - HR < 1 indicates delayed growth.

These effect sizes are intuitive and directly comparable across treatments.

5. Avoiding Information Loss

Analyzing only end-point absorbance values ignores valuable temporal data. Survival analysis incorporates: - timing of all measurements up to the event, - differences in growth trajectories, - censored observations.

This increases statistical power and provides a more complete view of growth dynamics.

6. Flexibility for Complex Experiments

Survival frameworks easily extend to: - multiple covariates, - time-varying predictors, - stratified models, - non-proportional hazards scenarios, - mixed-effects survival models.

This flexibility allows the approach to scale with more sophisticated experimental designs.

Summary

Survival analysis is an ideal approach for algae growth experiments where the goal is to compare the **time required to reach a growth milestone**, especially when: - treatments create different growth rates, - some cultures never reach the threshold, - the full growth curves are noisy or irregular, or - time-based comparisons provide the most biological insight.

It allows full use of the data, handles censoring