

*S Cook*

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***Biology 3103 - Ecology  
Laboratory***

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"Shouldn't you be working on your dissertation?" - Katherine Hooker

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## **Preface**

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Filler.

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### **Why read this book**

More filler.

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### **Structure of the book**

Chapters ?? introduces a new topic, and ...

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### **Software information and conventions**

I used the **knitr** package ([Xie, 2015](#)) and the **bookdown** package ([Xie, 2018](#)) to compile my book. My R session information is shown below:

```
sessionInfo()

## R version 3.4.4 (2018-03-15)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 17134)
##
## Matrix products: default
##
## locale:
```

```
## [1] LC_COLLATE=English_United States.1252
## [2] LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] stats      graphics   grDevices utils      datasets
## [6] methods    base
##
## loaded via a namespace (and not attached):
## [1] compiler_3.4.4 backports_1.1.2 bookdown_0.7.11
## [4] magrittr_1.5   rprojroot_1.3-2 tools_3.4.4
## [7] htmltools_0.3.6 rstudioapi_0.7  yaml_2.1.19
## [10] Rcpp_0.12.16   stringi_1.1.7  rmarkdown_1.9
## [13] knitr_1.20.3   xfun_0.1     stringr_1.3.1
## [16] digest_0.6.15  evaluate_0.10.1
```

Package names are in bold text (e.g., **rmarkdown**), and inline code and filenames are formatted in a typewriter font (e.g., `knitr::knit('foo.Rmd')`). Function names are followed by parentheses (e.g., `bookdown::render_book()`).

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## Acknowledgments

There are lots.

# 1

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## *Population Ecology*

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### 1.1 Background information

Organisms have evolved different life history strategies which differ in their methods of reproduction, care of offspring, timing of growth, means of resource acquisition, and prey avoidance. While these factors and how they interact can be highly complex, **survivorship** offers a simple means to quantify how a particular population ensures their reproductive success. For example, humans devote an enormous amount of energy and resources to offspring care, which results in low mortality rates among their young. On the other hand, most insects produce a massive number of offspring that have extremely high rates of mortality.

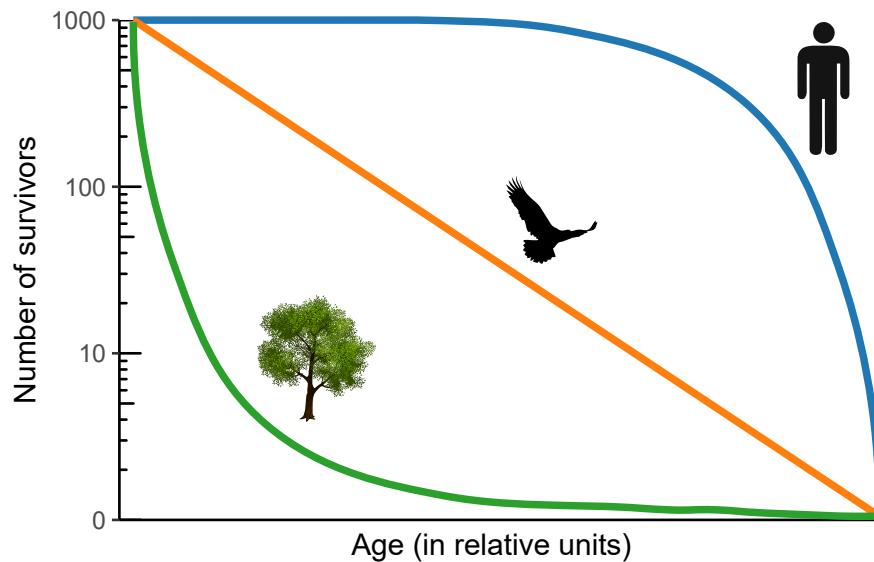
Plotting the number of survivors against age yields what is called a ‘survivorship-curve’ (Fig. 1.1), which is a visual way to assess how various organisms differ in their **life-history strategies** (number of offspring, number of reproductive cycles, degree of parental care, etc.). Scientists can use these plots to examine differences in organisms, or assess changes within subsets of a population.

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### 1.2 Objectives

You will use the cemetery data, as well as data generated by the U.S. Fish & Wildlife Service ([Milsap et al., 2016](#)), to address hypotheses about different populations. We can use birth and death years on gravestones, as well as names (to infer gender), to collect simple but useful information to collect demographic data for the local human population. The survivorship curve you will generate from this data will inform some ideas about the life history strategy of humans.

Additionally, survey data collected by state and federal agencies provide valuable information about natural population. We can use ancillary data about



**FIGURE 1.1:** Idealized examples of Types I, II, and III survivorship curves overlaid with example organisms. Type I survivorship is characterized by high probability of survival early in life, followed by a rapid decline as individuals reach older age. Type II survivorship displays roughly constant mortality throughout the lifespan of the organism, and Type III exhibits high mortality among young offspring.

individuals within a population to examine how different forces influence demographics within a population. Golden Eagles are federally protected in the United States, and Fish & Wildlife collects detailed information from tagged individuals (Fig. 1.2).

We will use this data to test hypotheses addressing the following questions:

1. Do humans and eagles display different life history strategies?
2. Does gender affect survivorship in human populations?
  - And if so, how?
3. Does human impact affect survivorship in eagle populations?
  - And if so, how?

Please form testable null hypotheses to address question number 1 and **either** question 2 or 3 (pick one). If you want, you may also substitute question 2 or 3 to address a hypothesis using the extra data we generated from the gravestones (height of gravestones as a proxy of material wealth).

To evaluate your hypotheses, you will...



**FIGURE 1.2:** Migratory Golden eagle in Denali National Park and Preserve. Mating pairs return each year to northern nesting territory in the spring, and most new fledglings leave the nest by mid-August. During winter their range extends from southern Canada to south of the Rocky Mountains(Brown et al., 2017).

1. Statistically address differences in survivorship between groups using a **t-test**, and display that information using a **bar-graph**
2. Unpack question 1 by **computing** and **displaying survivorship** (no statistical test needed for this part).

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### 1.3 Data analysis

1. Calculate the age at death of every individuals in both data sets
2. If necessary, use Excel to sort (Google it) the data based on your column of interest (i.e. gender).
  - Sorting the data easily splits the population into groups that you can then run the calculations (below) on. If you are doing the entire population, you will not need to split the population, but for within-population questions this step will come in handy. Keep in mind that every time you split the data based on a categorical variable, you will normalize to a hypothetical population of 1000 for the survivorship plots below.
3. Calculate mean age at death, as well as a measure of variation around that mean for use in the **bar-graphs**.
  - The **bar-graph** is just a visual representation of the data. You

will perform a **t-test** on this data and report the results to determine if the population means are actually different.

4. Create a survivorship table
  - Create “bins” of individuals
    - 0 to 1, 1 to 2, 2 to 3, etc... for Golden Eagles
    - 0-9, 10-19, 20-29, etc... for humans
  - Calculate the number of individuals surviving to that age class (the ‘countif’ function in Excel will come in handy here). Keep in mind that for the first group you will want to count **all** of the observations in the data set, so your condition will be ‘ $>=0$ ’.
  - Normalize survivors to a hypothetical population of 1000
    - This will make comparisons possible between unequal sample – so if you have 1250 observations in the data set, your normalized number for the first age class will be  $1250/1250 = 1.0$ , which is a proportion you can multiply by 1000. For the second age class (if you have some mortality), it might be  $975/1250 = 0.78$ , which you can then multiply by 1000 which equals 780.
5. Plot the number of survivors (y-axis values) against age class (x-axis values) to construct the survivorship curves. You may plot the data from the survivorship table either as normalized survivors, or on a logarithmic x-axis (typically how this data is displayed, as in Fig. 1.1).

The procedure above will ultimately yield survivorship (number of surviving individuals at a particular age class), which you may plot to visually explore differences between groups of interest to you.

## 1.4 Lab Report Specifics

Below are some specific guidelines for this lab report, but you should also utilize the general grading rubric in the Syllabus!

- **Participation** (1 pts)
- **Introduction** (3 pts)
  - General information about population ecology / life history strategies
  - How are survivorship curves used in population ecology?
  - Build up rationale to lead into your objectives/hypotheses statements.
- **Methods** (3 pts)
  - Explanation of data collection and analysis
- **Results** (6 pts)
  - Summary statistics in the text
  - Bar-plots and associated t-tests for each question
  - Survivorship curves for each question
- **Discussion** (3 pts)
  - Explain your results in light of your hypotheses
  - What are some plausible explanations for differences (or lack thereof) between groups?
  - Place your results in an evolutionary context.



# 2

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## *Physiological Ecology*

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### 2.1 Background information

In the presence of light, photosynthetic organisms can utilize light and carbon dioxide ( $\text{CO}_2$ ) to make sugars - the process of photosynthesis. The sugars made are used by these organisms (and organisms that eat them) as a source of energy. A by-product of the photosynthetic process is the liberation of oxygen ( $\text{O}_2$ ).

At the same time, these organisms are consuming oxygen via respiration. Although respiration and photosynthesis both take place in the light, in the dark only respiration occurs (since photosynthesis is a light-dependent process). Though it is impossible to directly measure gross photosynthesis (or the total amount of  $\text{O}_2$  produced, (Wohlfahrt and Gu, 2015), we *can* measure respiration ( $R$ ) as the **rate of  $\text{O}_2$  decrease** in the dark, and the **rate of oxygen increase** in the presence of light as a measure of net photosynthesis ( $P_{\text{net}}$ ). Combining these direct measurements, we can estimate gross photosynthesis ( $P_{\text{gross}}$ ).

$$P_{\text{net}} = P_{\text{gross}} - R \quad (2.1)$$

Using equation (2.1) can directly measure the terms in blue , and may use them to calculate  $P_{\text{gross}}$  .

Organisms capable of photosynthesis span an incredible range of phylogeny, from *unicellular algae* to *vascular plants* (Fig. 2.1). While every algae cell is photosynthetic, vascular aquatic plants (i.e. aquatic macrophytes) have large amounts of specialized tissue devoted to the transportation of resources and structural support. As Chapter 1 mentioned, every adaptation is a trade-off.



**FIGURE 2.1:** Aquatic algae (left pane, both underwater and floating mats) and aquatic macrophytes (right pane) are photosynthetic organisms that evolved in aquatic ecosystems.

## 2.2 Objectives

We will form hypotheses to test questions about photosynthesis in 2 aquatic organisms - a community of algae and a common aquatic macrophyte.

1. Which aquatic organism, algae or aquatic macrophyte, has the highest rate of *biomass specific* gross photosynthesis ( $P_{gross}$ )?
2. Which organism has the highest rate of *biomass-specific* respiration?
3. Assuming these organisms photosynthesize at  $P_{gross}$  for 10 hours a day and respire for 24 hours a day, which organism has the highest rate of net primary production ( $P_{net}$ ) per day?

## 2.3 Materials & methods

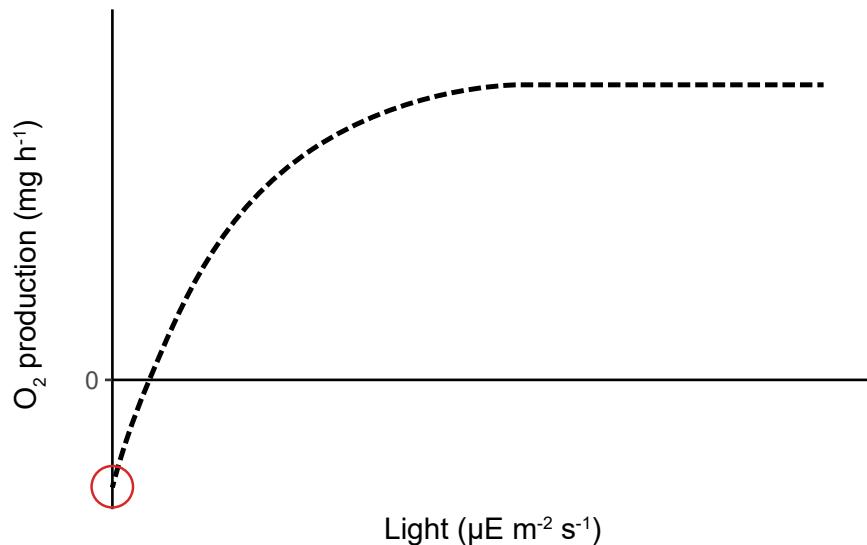
- 10 L photosynthesis solution<sup>1</sup>  
(40% air saturation<sup>2</sup>)
- Samples of algae and macrophytes
- 330 mL BOD bottles
- Dissolved O<sub>2</sub> meter (D.O. Meter)
- Light source (> 400  $\mu\text{E m}^{-2}\text{s}^{-1}$ )
- Aluminum foil

<sup>1</sup>10 L DI Water, 917 mg CaCl<sub>2</sub>, 960 mg MgSO<sub>4</sub>, 584 mg NaHCO<sub>3</sub>, 154 mg KHCO<sub>3</sub>

<sup>2</sup>Aerate with N<sub>2</sub> gas for 10 minutes

- Stir bars / stir plate
- Sieves (fine mesh)
- Forceps
- Drying boats (aluminum)
- Analytical balance (capable of 0.001 g)
- Drying oven

Oxygen production varies with the intensity of light, but reaches saturation as light intensity increases (Fig. 2.2). Since different organisms display different light-response curves, we need to saturate the photosystems with high light levels to prevent a potential confounding variable in our experiment.



**FIGURE 2.2:** A photosynthesis light-response curve illustrates that as light intensity increases, dissolved oxygen (D.O.) production eventually becomes saturated. In the dark, photosynthesis shuts off, and respiration causes the rate of D.O. production to fall below 0 (red circle on the y-axis).

### 2.3.1 Methods

1. Each group will be responsible for **one** of the two species. Fill 9 BOD bottles with photosynthesis solution (fill to brim - the idea with BOD bottles is for the glass stopper to push any excess water out of the seal the stopper creates).
2. Carefully transfer a representative sample of your organism into 8 of the BOD bottles. Add a small stir bar to each bottle, and place the glass stopper and plastic cap on the bottle to seal. Use

- the D.O. probe to measure oxygen concentration in the remaining bottle (your control bottle).
3. You will have 4 replicates for the dark treatment, and 4 for the light. Wrap the dark treatment bottles in aluminum foil, and place the light treatment bottles under the light source. Record the intial times for these samples.
  4. While you wait (at least 1.5 hours)...
    - Observe samples of these organisms under a dissecting/compound microscope and note differences in morphology. Note differences in the proportion of support tissues (i.e. stems) vs. photosynthetic tissues for each organism.
  5. After at least 1.5 hours, measure D.O. concentrations in the light bottles. Make sure to record the end time each time you take a D.O. measurement.
  6. After 2 hours, measure the D.O. concentrations in the dark bottles. Make sure to record the end time each time you take a D.O. measurement.
  7. Carefully empty the BOD bottle into a fine-mesh sieve to separate the sample from the photosynthesis solution. Collect/scrape the sample into a labeled aluminum drying tin, and place tins into a drying oven for 24 hrs at 105°C.

## 2.4 Data analysis

You can directly determine  $P_{net}$  (from the light treatment bottles) and  $R$  (from the dark treatment bottles) by calculating the change ( $\Delta$ ) in dissolved oxygen (DO) concentrations:

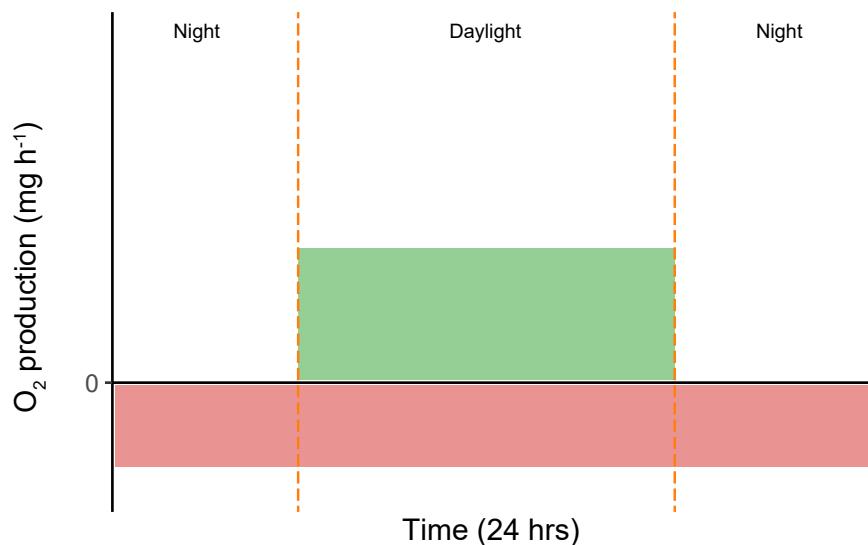
$$\Delta DO = DO_{final} - DO_{initial} \quad (2.2)$$

You also recorded the elapsed incubation time( $\Delta h$ ), the volume of the BOD bottles (0.330 L), and the mass of the sample (in dry weight, g). Using equation (2.1), after getting  $\Delta DO$  normalized to volume (L) and dry weight (g), you can then calculate  $P_{gross}$ , or the total oxygen produced by photosynthesis per unit biomass.

For the 1<sup>st</sup>, and 2<sup>nd</sup> questions, a two-sample t-test comparing the rates of each process ( $P_{gross}$  and  $R$ ) will tell you if there are significant differences between each organism. Bar-graphs (with error-bars display the standard error of the mean) are a good way of displaying this data visually.

The 3<sup>rd</sup> question requires you to construct a simple **model**. A model is a way

to represent a natural process using mathematics. Some models are simple (like the one you will construct), and some are incredibly complex. What assumptions, which may be overly simplistic, can we make to tackle question 3? If we assume that these organisms respire for 24 hours a day, and only photosynthesize when the sunlight is out (10 hours a day), we can use these values as a foundation for our model (Fig. 2.2).



**FIGURE 2.3:** A photosynthesis light-response curve illustrates that as light intensity increases, dissolved oxygen (D.O.) production eventually becomes saturated. In the dark, photosynthesis shuts off, and respiration causes the rate of D.O. production to fall below 0 (red circle on the y-axis).

There are no associated statistical tests for question 3, since you should calculate daily values using mean  $P_{\text{gross}}$  and mean  $R$  (no replicate values for this question). You can present the results of your model in the text of your results section.

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## 2.5 Lab report specifics

1. Introduction
  - Importance of photosynthesis
  - Morphological adaptations of aquatic photosynthesizers
  - Objectives
  - Hypotheses
2. Methods
  - Oxygen change method (light/dark treatments)
  - Experimental design
  - Calculations / statistics / model explanation
3. Results
  - Graphs/statistics for questions 1 and 2
  - Results for question 3
4. Discussion
  - Hypotheses rejected/supported
  - Provide a coherent explanation for the patterns you see in the photosynthesis and respiration data (use your observations of morphology)

BOD No.	Organism	Treatment	Initial time	Initial D.O. (mg/L)	Final time	Final D.O. (mg/L)	Dish ID	Weight (g)
	Algae	Light						
	Algae	Light						
	Algae	Light						
	Algae	Light						
	Algae	Dark						
	Algae	Dark						
	Algae	Dark						
	Algae	Dark						
	Algae	Control						
	Macrophyte	Light						
	Macrophyte	Light						
	Macrophyte	Light						
	Macrophyte	Light						
	Macrophyte	Dark						
	Macrophyte	Dark						
	Macrophyte	Dark						
	Macrophyte	Dark						
	Macrophyte	Control						

BOD No.	Organism	Treatment	Elapsed time (h)	Delta D.O. (mg/L)	Delta D.O. (mg)	Net O <sub>2</sub> Rate (mg/(g*h))	Rate (mg/(g*h))	Daily (mg/(g*h))
	Algae	Light						
	Algae	Light						
	Algae	Light						
	Algae	Light						
	Algae	Dark						
	Algae	Dark						
	Algae	Dark						
	Algae	Dark						
	Algae	Control						
	Macrophyte	Light						
	Macrophyte	Light						
	Macrophyte	Light						
	Macrophyte	Light						
	Macrophyte	Dark						
	Macrophyte	Dark						
	Macrophyte	Dark						
	Macrophyte	Dark						
	Macrophyte	Dark						
	Macrophyte	Control						

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