Bioenergetics Lab Exercise

Some introductory notes on bioenergetic modeling

Bioenergetics is the study of the flow and transformation of energy in and between living organisms, and between living organisms and their environment. Energy is used for many tasks such as basic metabolism (lumping all physiological functions together), digestion and processing of food, activity and locomotion, reproduction, and somatic growth. The total inputs and the total outputs must balance (*Energy in = Energy out*). Because bioenergetics is fundamentally based on this balance, we can use a set of equations to represent different features of a fish's energy budget. The general equation for consumption is given as

Consumption = Metabolism + Wastes + Growth.

These general figures can be broken into several components:

$$C = (R + A + SDA) + (F + U) + (\Delta B + G), \text{ where:}$$

Consumption	*Metabolism*	*Waste*	*Growth*
C = consumption	R = respiration	F = egestion	ΔB = change in biomass (somatic growth)
	A = active metabolism	U = excretion	G = gonadal production
	SDA = specific dynamic action (cost of digestion)		

Because this is an equation we can calculate each component as long as we know the values of the other components. In many cases we're interested in calculating potential growth given a certain ration and temperature (say in an aquaculture setting to determine yield or when investigating habitat carrying capacity in natural environments). Then we simply solve the equation for growth:

Growth = consumption – metabolism – wastes

Figure 1 shows the chronology of consumption. First, some energy from consumption is first lost as unassimilated faeces (egestion, *F*), then after assimilation, waste products (e.g. urea) are eliminated through excretion (*U*). Next, energy is allocated to sustain metabolic demands. Surplus energy is then used for somatic growth or reproduction (Figure 1). In juvenile fish there is clearly no gonadal (reproductive tissue) production so all the energy is allocated towards somatic growth.

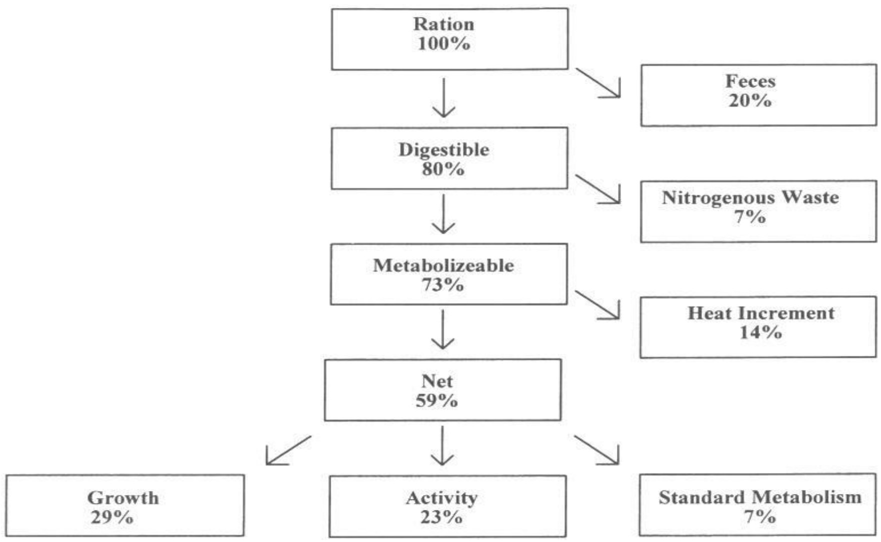


Figure 1. the energy budget for an average carnivorous fish. From Brett and Graves (1979)

Factors influencing the fish's physiology and hence its growth rate include

- species (sets the outer bounds of physiological constraints),
- ontogenetic state (e.g. juvenile or adult),
- body size (determines energy needs and assimilation efficiency),
- water temperature (determines energy expenditures and potential for energy intake),
- the energy density of the fish (fat content, think about the energy required to produce one kg of fatty herring {25% body fat} vs. cod {3.5 % body fat}),
- the activity level of the fish (upstream migration vs. rearing), and
- the energy density of its prey (high protein and fat yields more energy than diet items with lots of fiber and structure).

In general, carnivorous fishes allocate a higher percentage of their consumed energy towards growth than do herbivorous fishes because their diet is more energy dense (think of eating cheeseburgers and donuts vs. carrots and celery).

Normalized Percentages	*Consumption*	*Respiration*	*Waste*	*Growth*
Carnivore	100 =	44 +	27 +	29
Herbivore	100 =	37 +	43 +	20

The model developed at the University of Wisconsin is a commonly used bioenergetic model. Underlying are the above equations and data from lab experiments of fish physiology. This provides easy-to-use applications where the investigator can evaluate how different biotic and abiotic conditions within ecosystems will influence fish growth.

Background data and today's assignment

Today's assignment focuses on juvenile steelhead. We therefore don't need to worry about species differences or ontogenetic differences. We have specified all 25 parameters that go into the bioenergetics program, so for the purpose of this lab, *body size and temperature* are the two main factors that influence growth in juvenile steelhead. Temperature will be the primary abiotic factor that affects differences in energy expenditure, and body size will be the primary biotic factor pertaining to appetite and energy expenditure.

Your lab assignment for this week is to complete the bioenergetics worksheet found in BBLearn. As we complete each exercise, be sure to copy the graphs you make in Excel into the bioenergetics worksheet, as it will save you time later.

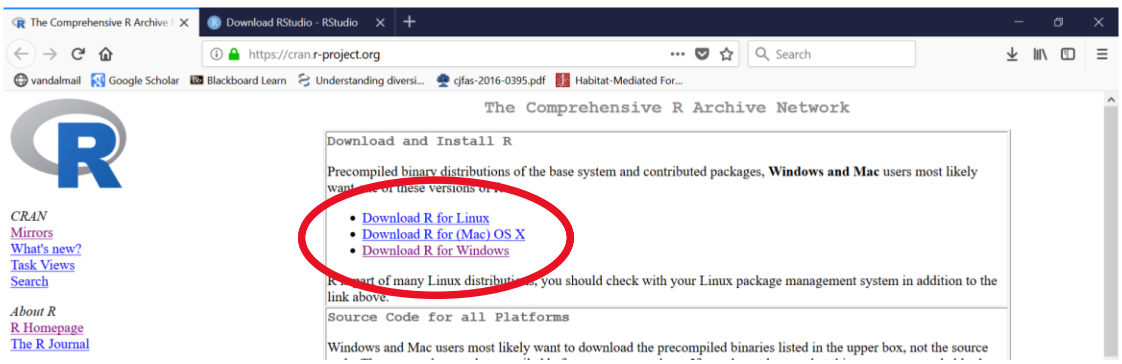
Installing bioenergetics software

We will use Bioenergetics 4.0 to complete Friday's lab exercise. You will need to bring your computer to lab and complete the installation process of Bioenergetics 4.0 during our lab meeting. If your computer operates outside of Windows, Linux or Mac operating systems or do not have access to a laptop, notify me prior to lab. Bioenergetics 4.0 uses R, RStudio, and Shiny to run. If you do not already have R and RStudio on your computer, follow the Install R instructions. If you already have R and RStudio on your computer, follow the Update R instructions.

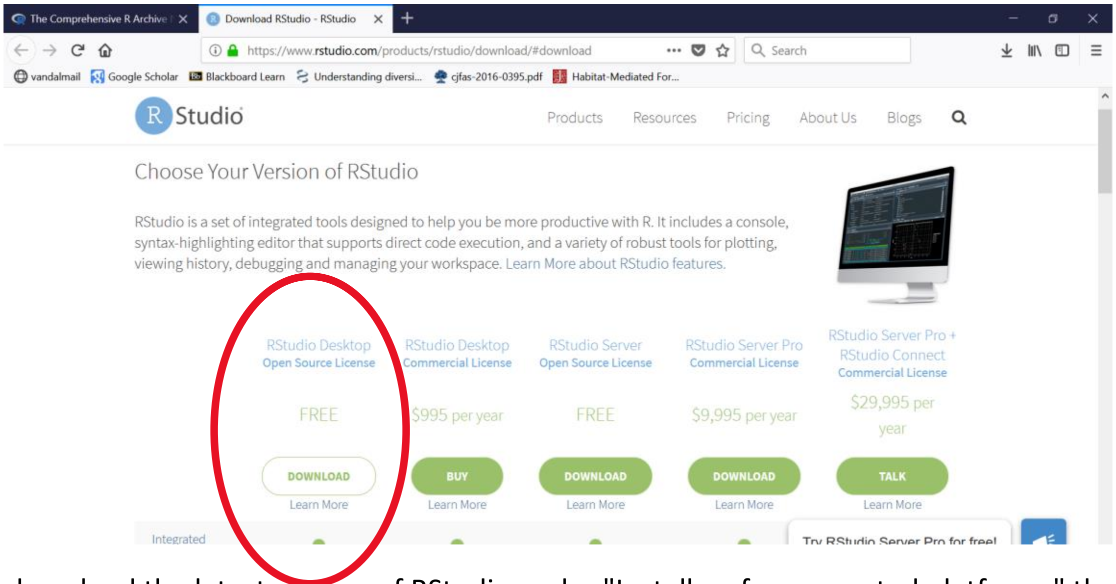
After installing/updating R and RStudio, follow the instructions for running Bioenergetics 4.0 and Shiny. Then download the exercise files in the Bioenergetics folder in BBLearn. This process should only take 15-30 minutes to complete. See me before lab to help you foresee any issues.

Install R and RStudio:

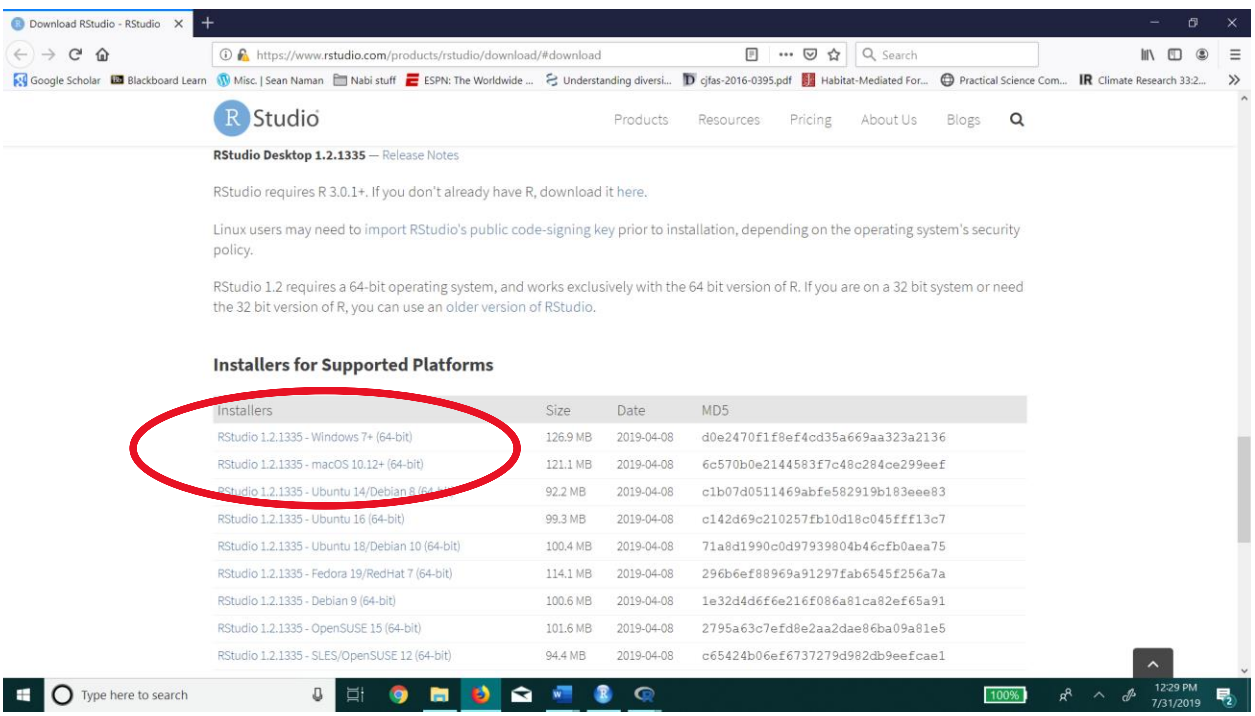
Go to https://cran.r-project.org/ and download and install the latest version of R that is compatible with your computer (Windows or Mac). Be sure to install R before you install RStudio.



Next, go to http://www.rstudio.com/products/rstudio/download/, click on the button to download the free RStudio Desktop version.



Then download the latest version of RStudio under "Installers for supported platforms" that is compatible with your operating system.



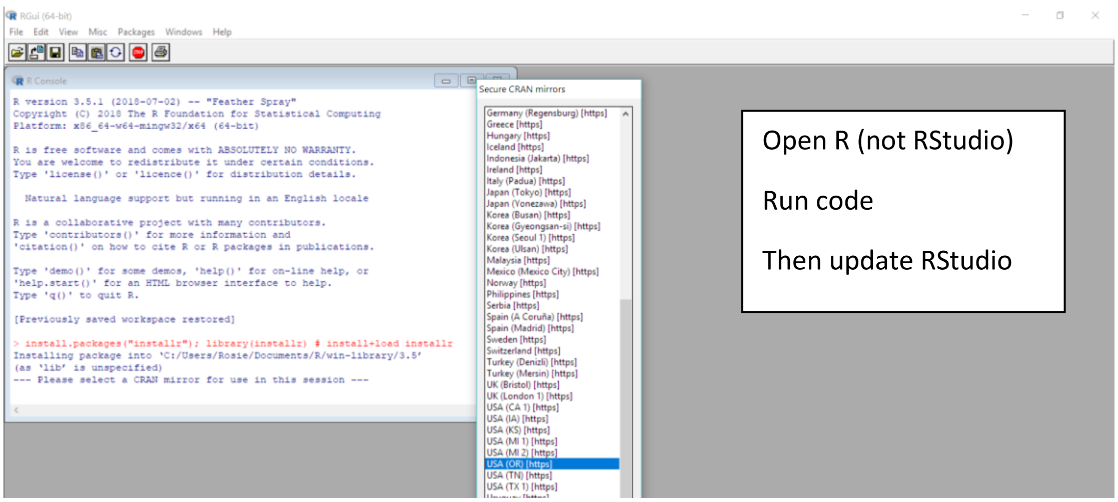
Update R and RStudio:

R updates quite frequently and does not automatically update. To ensure R packages run smoothly, you will need to ensure you are using the most recent version on R.

To update R simply, run the following code in R (**not RStudio**):

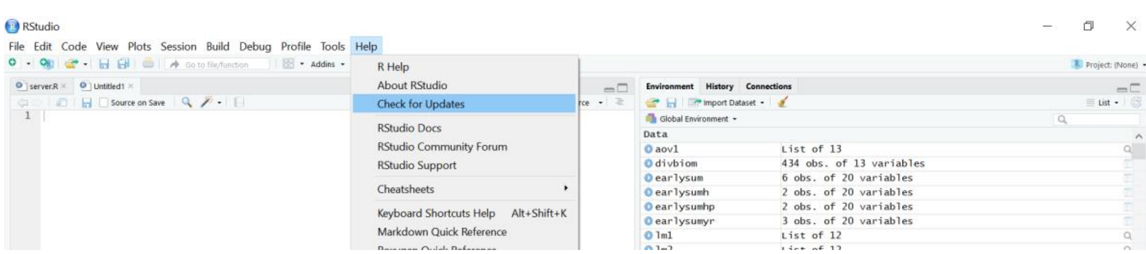
```r
install.packages("installr"); library(installr) # install+load installr

updateR() # updating R.
```

Select a USA CRAN mirror when prompted and give permissions for R to install new software.



***If you have an old version of R that does not allow you to update R as shown above, go to Packages->Install package…->installr. Then go to Packages->Load package->installr. Installr will show up on the top menu bar next to Help. Under the installr menu, select update R and update R packages. Sometimes you need to update your packages before updating R if your version is especially old. If neither of these update methods work, you can always download the newest version of R and then install R as if it were never on your computer (which is often the easiest way to update R if you are running an obsolete version).

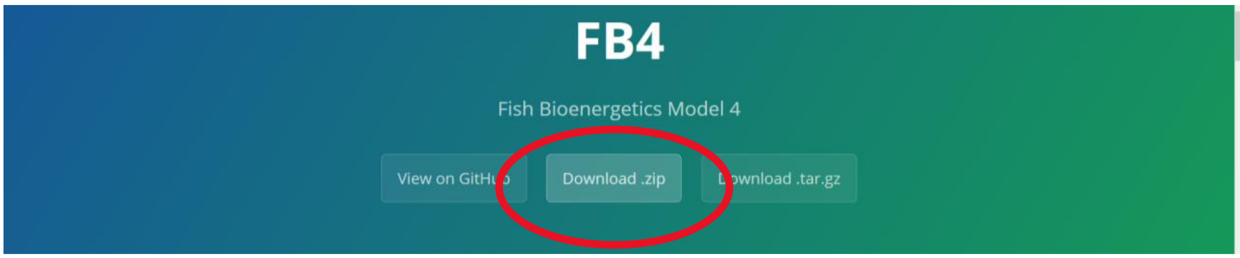
Next, open RStudio and check for updates (Help->Check for Updates). If RStudio is not up to date, follow the update prompts.



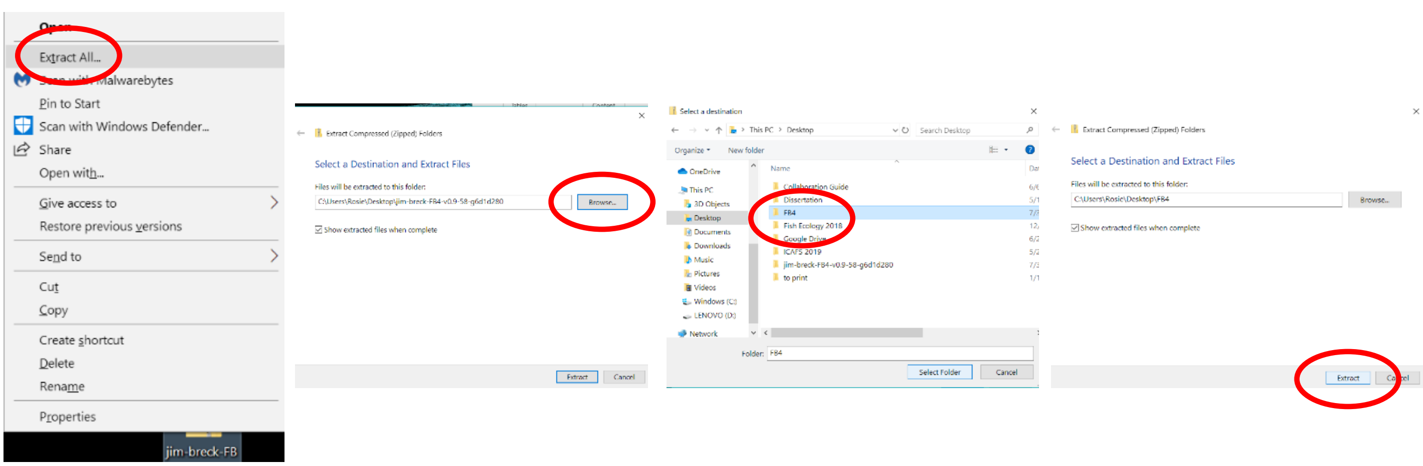
Install Bioenergetics 4.0

Create a file on your desktop named FB4.

Go to http://fishbioenergetics.org/ and download the .zip file on the top of the page. Select save file when prompted.

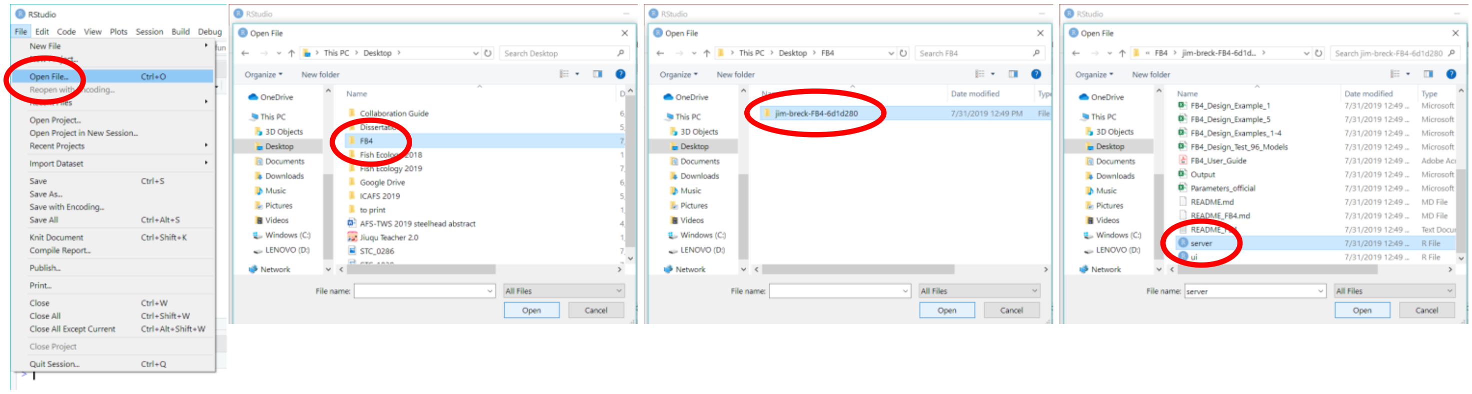


Then copy this folder to your desktop. Next you will need to decompress the files. Find the file that you just downloaded (either called jim-breck-FB4… or FB4) and extract all items to the newly created FB4 file on your desktop.

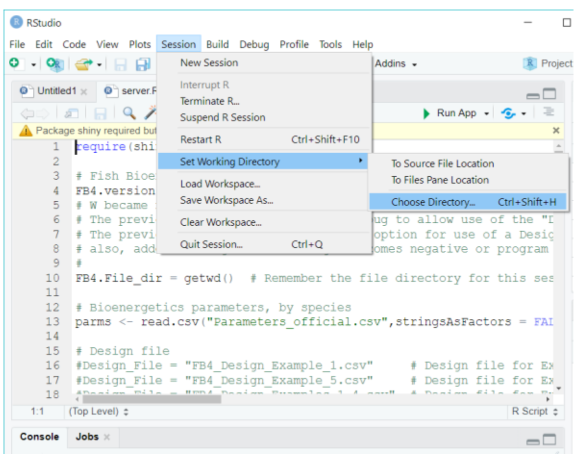


Open RStudio.

In RStudio, select File->Open File… and select server.R in the FB4 file on your desktop.



Next go to Session->Set Working Directory->Choose Directory and select the FB4 folder on your desktop.



Install Bioenergetics 4.0

Next go to Tools->Install Packages… and type shiny under Packages. Make sure install dependencies is checked and click on install.

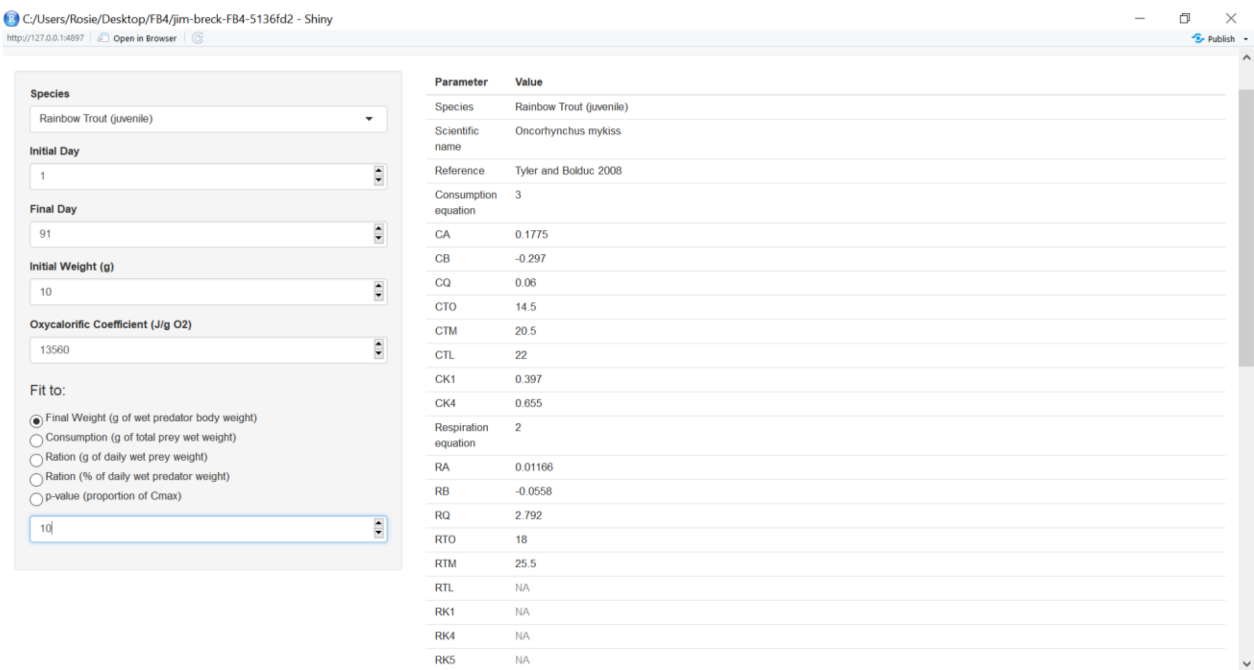
Download Exercise Files

We will be modifying the main input files from the default files in FB4. Download the Exercise 1 and 2 files from the Bioenergetics file in BBLearn and place them on your desktop.

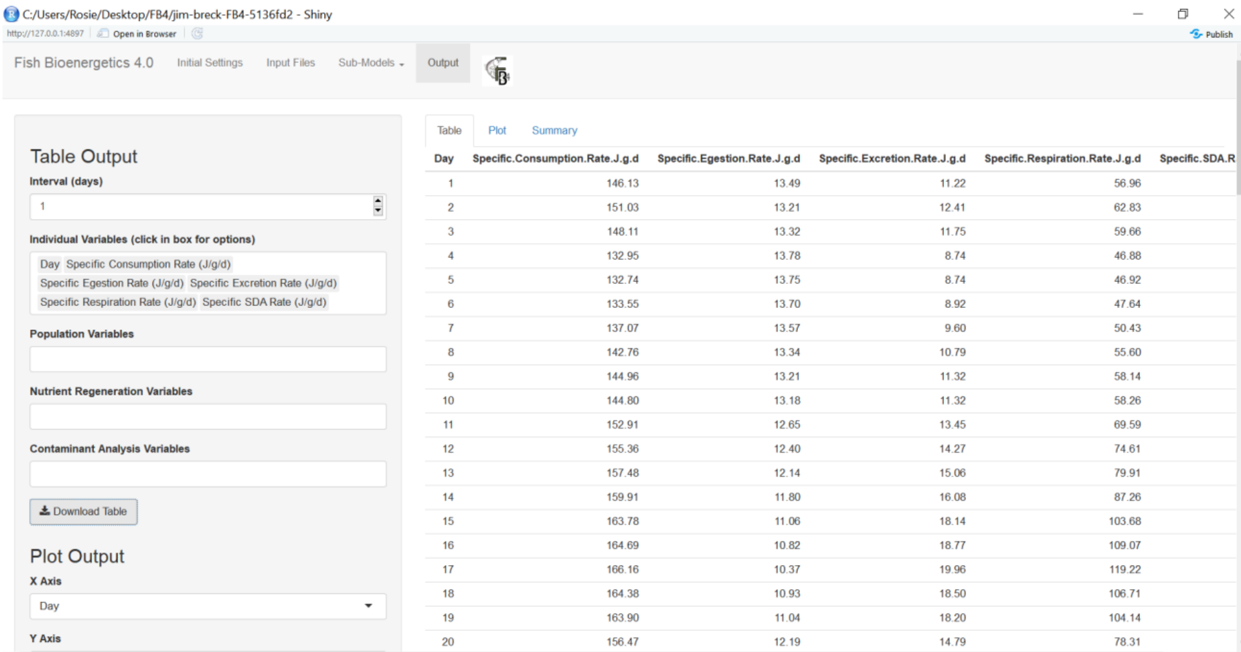
Question 1. Energy budgets

First, we will investigate the relative sizes of the different components of an energy budget. Close RStudio if it was opened and copy the files from the Exercise 1 folder into the Main Inputs folder in FB4. Be sure the names of the files that you place in the Main Inputs file are the exact same as the previous files. Specified in this file is the temperature regime in site UWM in Webb Creek from May 1 (day 1) to July 31 (day 91) with a typical diet of invertebrates averaging 4324 J/J fish/day. Reopen RStudio and select Run App.

For the purpose of this question we will keep growth at zero, i.e. start weight = end weight. This allows us to more easily investigate how the various components are distributed. Specify the species as Rainbow Trout (Juvenile), Initial day = 1, Final day = 91, and Initial weight = 10g. Select Fit to: Final Weight and in the empty box enter 10. Once all information is entered, select the Output tab.



Next, we will generate the data needed to create an energy budget graph. Within the individual variables box select Day and the following mass-specific rates (J/g/d): consumption rate, egestion, excretion, respiration, and SDA. Select Download Table and save this file as exercise1.csv to your desktop.



Day	Specific.Consumption.Rate.J.g.d	Specific.Egestion.Rate.J.g.d	Specific.Excretion.Rate.J.g.d	Specific.Respiration.Rate.J.g.d	Specific.SDA.R
1	146.13	13.49	11.22	56.96	
2	151.03	13.21	12.41	62.83	
3	148.11	13.32	11.75	59.66	
4	132.95	13.78	8.74	46.88	
5	132.74	13.75	8.74	46.92	
6	133.55	13.70	8.92	47.64	
7	137.07	13.57	9.60	50.43	
8	142.76	13.34	10.79	55.60	
9	144.96	13.21	11.32	58.14	
10	144.80	13.18	11.32	58.26	
11	152.91	12.65	13.45	69.59	
12	155.36	12.40	14.27	74.61	
13	157.48	12.14	15.06	79.91	
14	159.91	11.80	16.08	87.26	
15	163.78	11.06	18.14	103.68	
16	164.69	10.82	18.77	109.07	
17	166.16	10.37	19.96	119.22	
18	164.38	10.93	18.50	106.71	
19	163.90	11.04	18.20	104.14	
20	156.47	12.19	14.79	78.31	

Open the exercise1.csv file and graph the energy budget as a line graph.

a) Graph the energy budget by defining day of the year as the independent variable (x-axis) and selecting the following mass-specific rates (J/g/d) as dependent variables (y-axes): consumption rate, egestion, excretion, respiration, and SDA. Growth is zero, so we do not need to graph that. Copy this graph to your worksheet.
b) What can you read from the graph? Are the rate proportions the same throughout the simulation?
c) What do you think is causing all the rates to increase over the simulation?

Question 2. Nuts and bolts of bioenergetics

Now it is time to jump into the model behavior, and in particular investigate how temperature and body mass affect consumption rates. For this question we keep growth at zero, i.e. start weight = end weight to more clearly understand how the primary abiotic and biotic factors affect basic metabolic needs.

Close RStudio (save workspace) and replace the Temperature file in the Main Inputs file in FB4 with the Temperature file from the Exercise 2 folder in BBLearn. Reopen RStudio and select Run App.

a) Specify the species as Rainbow Trout (Juvenile), Initial day = 1, Final day = 49, and Initial weight = 10g. Select Fit to: Final Weight and in the empty box enter 10. Once all information is entered, select the Output tab. Within the individual variables box select Temperature and the following mass-specific rates (J/g/d): consumption rate, egestion, excretion, respiration, and SDA. Select Download Table and save this file as exercise2a.csv to your desktop.
    i. Graph consumption rate (J/g/d) as a function of temperature (i.e. temperature on the x-axis). Copy this graph to your worksheet and describe the pattern.
    ii. Add egestion, excretion, respiration, and SDA to the y-axis. Copy this graph to your worksheet. How do these rates relate to each other and to the independent variable?

b) Go back to the Initial Settings Tab and specify Initial Weight and Final Weight = 50 g. Once all information is entered, select the Output tab. Within the individual variables box select Temperature and consumption rate (J/g/d). Select Download Table and save this file as exercise2b.csv to your desktop.
    i. Graph consumption rate (J/g/d) as a function of temperature (i.e. temperature on the x-axis) for both the 10 g and 50 g fish in one graph. Copy this graph to your worksheet and describe the pattern.
    ii. What happens to the pattern and the magnitude of the consumption rate?

c) Repeat this process for fish that are 5, 20, and 100 g.
    i. Graph specific consumption rates for fish that are 5, 10, 20, 50 and 100 g in one graph. You will need to combine the tables created into a single file. Copy this graph to your worksheet and describe the pattern.
    ii. How do the mass-specific rates compare to the absolute rates (i.e. in total Joules)?

Question 3. Individual level variation in growth

Below is a table with individual records for juvenile steelhead caught in Webb Creek (at site UWM) in August (day 107), and then again in September (day 138). We'll use the temperature file from Exercise 1 for this question. Close RStudio (save workspace) and replace the Temperature file in the Main Inputs file in FB4 with the Temperature file from the Exercise 1 folder in BBLearn. Reopen RStudio and select Run App.

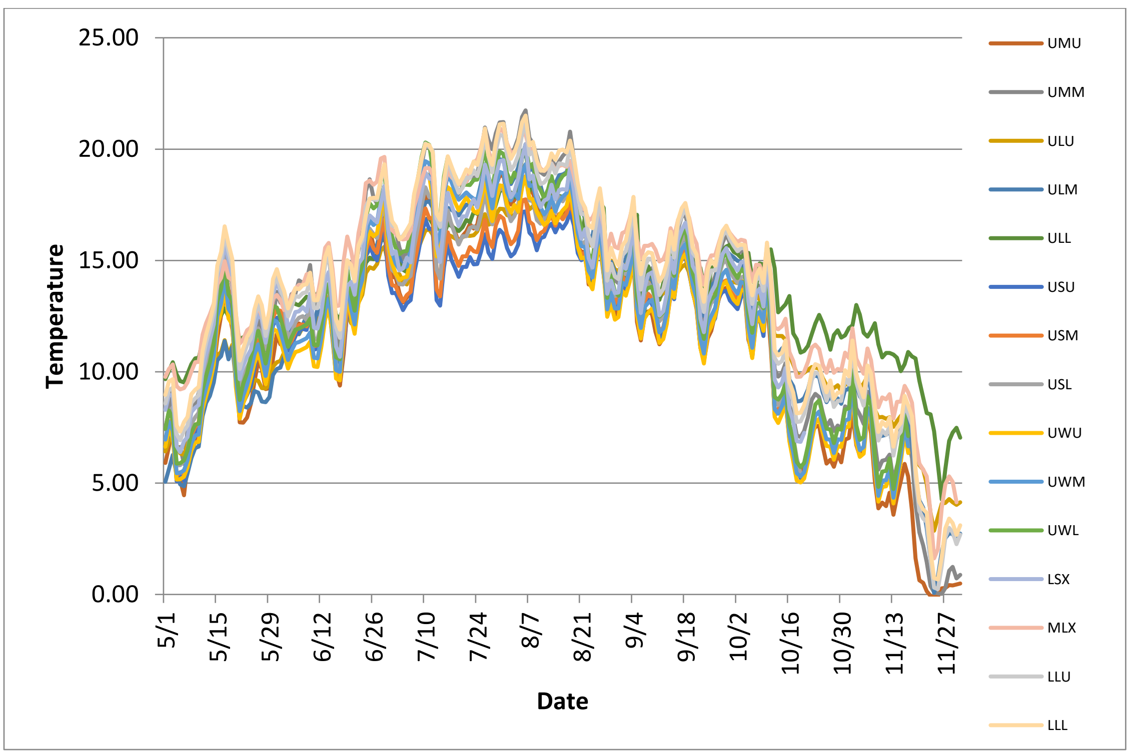
PIT tag #	Length1	Weight1	Length2	Weight2
985121020475978	86	7.0	92	10.1
985121020481883	93	9.3	100	11.3
985121020520475	85	7.0	95	8.9
985121020439990	73	4.3	82	6.3
985121020549504	85	7.5	97	10.6
985121020445181	75	4.6	86	6.7
985121020516982	76	5.4	89	7.9
985121020513372	70	4.0	82	6.3
985121020429310	69	3.5	82	5.9
985121020545793	74	5.3	90	8.6

a) Calculate their growth rates (based on their weights) in Excel expressed as % change in body mass per day. To calculate daily growth rate over a period of 31 days, use the following equation: (Ln(final wt)-Ln(initial wt))/31*100.
b) Calculate the P-values of the slowest growing and fastest growing individuals, and graph their energy budgets. To do this, input the Initial Day=107, Final Day=138, Initial Weight=August weight, and Fit to Final Weight=September weight. Select the Output tab, then Summary. The P-value is the top listed parameter. Generate the data needed to create an energy budget following the instructions in Exercise 1a. List the fastest and slowest growing individuals and their corresponding P-values and energy budget graphs.
c) If these fish (the fastest and slowest growing individuals) were to achieve no growth, what would their P-values be? By means of the P-value, how much of their consumption did these fish allocate towards growth?
d) Based on drift samples, researchers found that there is about 400g of invertebrates flushing downstream through the reach each day during late summer (i.e. days 107-138). How many individuals can be supported by this drift? Here we simply assume that they all start out at 6g and end at 10g. Show your calculations. Discuss briefly two reasons why this kind of abundance calculation might be too simplistic.

Question 4. Variation in space: Differences in bioenergetic constraints over a stream system

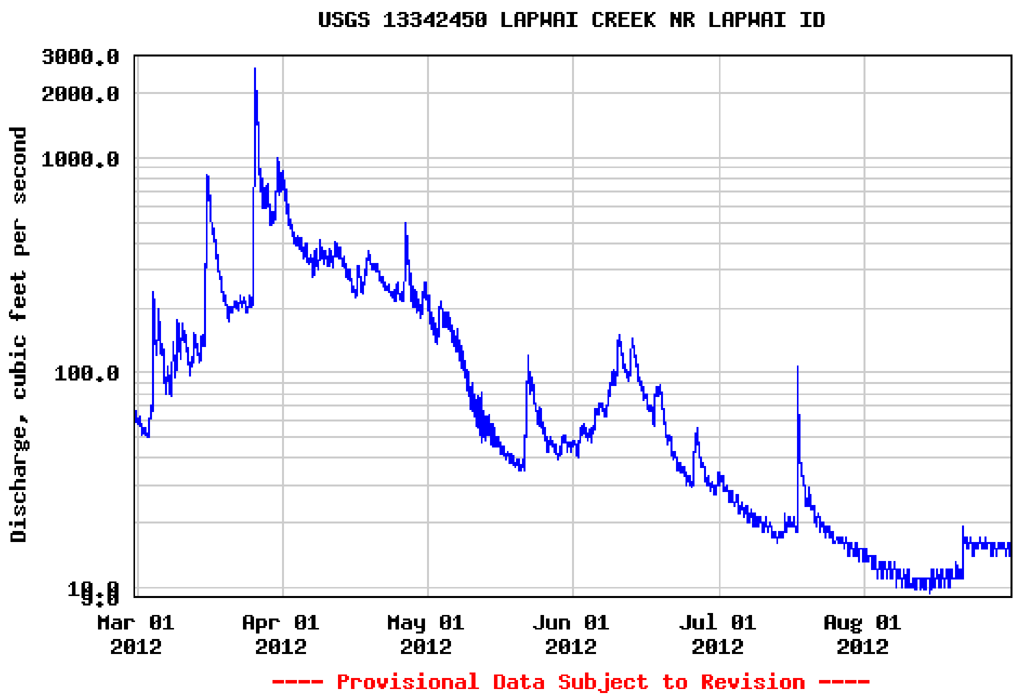
Across a watershed such as Lapwai there are gradients in underlying geology, topography, land cover, and land use. Depending on the location in the watershed, this physical setting affects the conditions in the stream (temperature, flow, substrate etc.), and in turn, these conditions control how suitable the stream is for organisms such as steelhead. These conditions vary within a single stream (from headwaters to the mouth) as well as among streams, so there can be large differences among discrete sites within a relatively small watershed. For this question, we are going to build on what you have learned in the previous questions, synthesize some key aspects of bioenergetics, and integrate with population dynamics. No specific analyses are necessary to answer the questions.

The following figure shows the temperature regimes across different sites in the Lapwai watershed. The figure is difficult to read so there is no point in following individual sites, just refer to warm or cool sites.



a) When are the most energetically demanding conditions occurring? Give some examples on energy expenditures based on the temperatures shown above and the relationships you established in question 2.

Steelhead in tributaries to the Lower Clearwater River, such as Lapwai Creek, spawn in late March to mid April, and their eggs hatch in mid-late May. Spring runoff in Lapwai is usually over by June 1, and spring rain usually recedes by mid-June. The hydrograph usually shows a marked drop at that point:



b) Speculate how the emergence of fry, smaller stream area (corresponding to discharge), and energetically demanding conditions affect population size in the early summer. How do you think it varies across the watershed?