Stable Isotope Trophic Ecology

Project Summary: The aim of our data expedition was to give students an introduction to stable isotopes and how the data can be used to understand trophic dynamics. Within a 3-hour lab students were introduced to methane seeps and the difference between photosynthetic and chemosynthetic carbon, before working through an analysis of data from deep-sea red crabs. Students were then introduced to data on Atlantic and Mediterranean fin whale populations and how diet is shown to vary between populations using stable isotopes. By exploring stable isotope data in R, students practiced coding skills and learned how to create publication-quality figures as preparation for their independent class projects.

Themes and Categories: Stable Isotope Ecology, Isotopic Mixing Models, Figures Design.

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Course: ENV 273 Marine Ecology (Cross Listing: BIOLOGY 273, EOS 374)
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1. Introduction to R
Previous experience with statistical programming varied in the class and so we began with a broad introduction to R. We started with a basic discussion of the components of a programming language and then introduced R and RStudio. We discussed vectors and an explanation of how variables are stored in and recalled from memory. Next, students explored the built in statistical functions of R and wrote multiple versions of their own *mean* function.

2. Plotting Basics
Using the *iris* dataset that is built into R, the class worked through code to transform basic plots into publication-quality figures using the *ggplot2* package. Students plotted both a scatterplot and boxplot, and learned how to control each element of the plot (e.g., grid-lines, legend, axis, labels, colors). Students were provided with working code that they could take away and use to create figures for their own datasets.

3. Deep-Sea Red Crab Diet Analysis
Question: How much chemosynthetic carbon (i.e., carbon originating from methane seeps) is present within the diets of deep-sea red crab? How does this vary between our two sites?

Students had prepared for class by reading the introductory chapter of Stable Isotope Ecology by Brian Fry[1]. In class, students were introduced to methane seeps and the difference between photosynthetic and chemosynthetic carbon with a brief presentation. Together, we created a basic scatterplot of the $\delta^{13}C$ data and discussed apparent patterns. The class then used the $\delta^{13}C$ data to calculate the proportion of each individual crab's diet that originated from chemosynthetic productivity, this data was presented using a basic boxplot and the differences between the two sites were discussed.

4. Atlantic and Mediterranean Fin Whale Diets

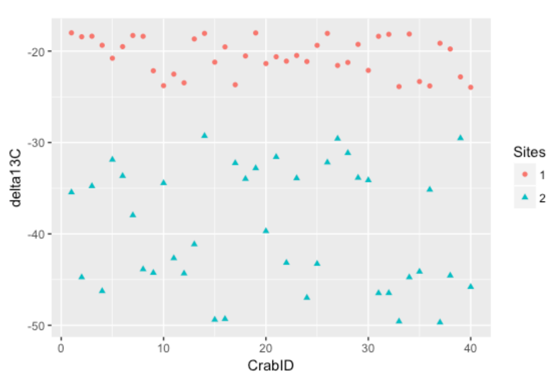
Question: How can we make inference about populations with stable isotopes where clear end-members do not exist?

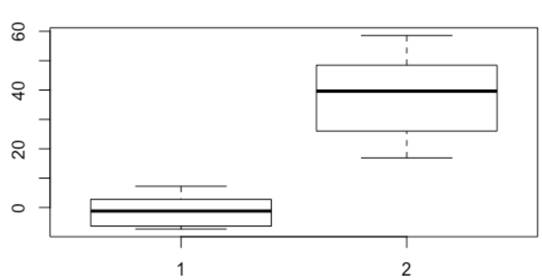
Students were introduced to a cetacean system, fin whales, where stable isotope analysis can identify population level differences even in the absence of clear end-members as in the deep-sea red crab example. Students were given a dataset and example code to import and clean the data and make some basic graphs.

5. Homework

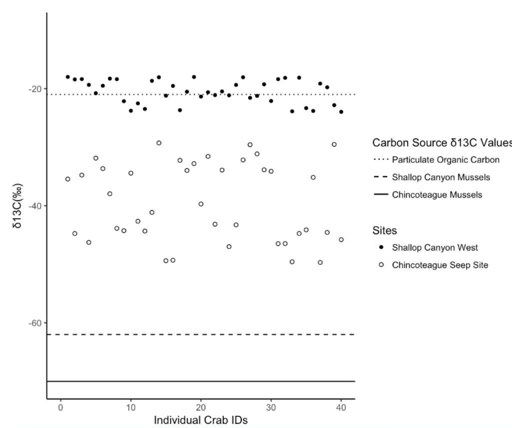
At the end of class, we asked students to practice the covered topics in a short homework. Using the plotting techniques illustrated in class students were asked to create publication-quality figures for the deep-sea red crab diet analysis and to explain the results in terms of trophic ecology. We pointed students to several stable isotope journal articles to assist in their interpretation. An example of the figures created by the students is below:

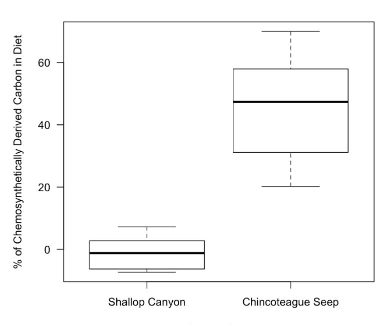
Figures created in class





Example student's homework figures





6. Follow up

Phillip and William both met with students in person and over email after class. We addressed specific questions about the data expeditions homework and using R in their independent class projects to generate figures and conduct statistical analysis.

Datasets

The homework dataset includes 80 $\delta^{13}C$ values from two sites (i.e., 40 samples per site), which represent muscle tissue samples from individual deep-sea red crab. This data is randomly generated but is based on a smaller dataset collected by Phillip Turner as part of his doctoral thesis, which investigates the role of methane seeps in supporting the deep-sea red crab fishery. Data represents crabs collected from Chincoteague seep (1040m depth) and Shallop Canyon seeps (360m depth). To calculate the proportion of diet originating from chemosynthetic productivity a dataset is also provided with *end-member* values (i.e., those representing a diet of 100% chemosynthetic and 100% photosynthetic productivity).

A second dataset was given to the students of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for Atlantic and Mediterranean fin whale baleen. Atlantic fin whale baleen data was generated by William Cioffi as part of his doctoral work and the Mediterranean fin whale baleen data has been previous published in Bentaleb et al. (2011)[2]. This data consists of 633 rows and the following columns: whale id, sample id, $\delta^{13}C$, $\delta^{15}N$, species, ocean of origin. This data represents multiple samples along the growth axis of fin whale baleen plates (n = 23 whales).

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[1] Fry, B. (2006). *Stable isotope ecology* (Vol. 521). New York: Springer.

[2] Bentaleb et al. (2011). Foraging ecology of Mediterranean fin whales in a changing environment elucidated by satellite tracking and baleen plate stable isotopes. *Marine Ecology Progress Series*, *438*, 285-302.