Copepod Adaptation to Crude Oil

The copepod *Eurytemora affinis* is a small planktonic crustacean that is dominant in the Gulf of Mexico. Copepods are essential components of food webs in all aquatic ecosystems. They form the largest biomass of all animals in coastal ecosystems, constitute the main grazers of algae, and are a major food source for larger carnivores. In addition to their ecological importance, copepods are ideal organisms for studying rapid evolutionary responses due to their large effective population sizes and short generation times. Previous studies have shown that copepods can adapt to changes in salinity in the environment. However, it is unknown whether copepod populations possess the necessary genetic variation for tolerance to crude oil, allowing natural selection to act.

To make these comparisons, a common garden experiment was designed to compare two populations of copepods collected at different times from the same location in Blue Hammock Bayou, off of Four League Bay in Louisiana, near New Orleans. This area was impacted by the Deep Horizon Oil Spill, the largest offshore oil spill in US history, in which oil from an underwater uncapped well gushed into the sea for months, beginning on April 20, 2010. Individuals from one population of copepods were collected in 2006, four years before the spill, while a second population was collected in 2013, nearly three years after the spill. Both populations are maintained in the Lee lab. The copepods disappeared from the water column for multiple years after the spill: an attempt to collect copepods failed in 2011. About one third of the oil released still remains in the region, and any copepod populations after the spill may still be affected by oil toxicity.

The primary goal of the experiment is to determine the extent to which populations of *E. affinis* could evolve in response to crude oil. Has there been a shift in tolerance to crude oil toxicity? Does the *after* population show increased ability to survive in conditions with high levels of oil present? If so, can you quantify the changes in various measures of fitness?

The study seeks in addition to quantify the potential evolutionary tradeoffs in response to adaptation to crude oil toxicity. Evolution in response to a stressful environmental factor can be costly, and could involve evolutionary tradeoffs. For example, adaptation to low salinity occurs at the cost of high salinity tolerance and also entails higher metabolic rates and slower growth rates. As a result of adaptation to crude oil toxicity, tradeoffs in fitness might occur, such as decreased hatching rates and increased development times. For instance, tolerance of crude oil might entail more energy for detoxification, as exemplified by the increased activity of the detoxification enzyme cytochrome P-450 330A1 observed in the copepod *Calanus finmarchicus* in response to crude oil. While evolution of tolerance to crude oil toxicity might allow the populations to avoid extinction and persist, tradeoffs in fitness might make the population vulnerable to demographic declines.

These goals can be addressed by comparing populations from *before* and *after* the Deep Horizon Gulf Oil Spill. Fitness (hatching rate, survival rate, and development times) can be compared between the *before* and *after* populations across three concentrations crude oil: zero (control), 75% water soluble fraction of crude oil, and 100% water soluble fraction of crude oil. If the *after* population shows higher fitness in the oil treatments compared to the *before* population, an evolutionary response to oil has occurred. In addition, if the *after* population shows reduced fitness in their native environment of no oil, relative to the *before* population, there might be costs to adaptation that could be quantified.

Experimental Design.— The experiment includes eight clutches of eggs from each population. Clutches come from different parents and are genetically distinct. Each clutch is divided into six samples of 4–6 eggs each and each sample is placed in a vial. There are two replicates, A and B, for each clutch for each of the three treatment conditions (crude oil levels). Each vial is observed every two days and the number of individuals at each developmental stage is observed. After hatching, a copepod larva is called a nauplis; it has no body segments. After metamorphosis, the nauplis becomes a juvenile, identifiable by the first body segment. Adulthood and the completion of development is defined by obtaining wings for females or differential thickness of antennae for males. The number of days spent at each stage are inferred and assume that the first individuals to reach each stage of development will continue to do so, assuming survival. Development times may be off by a day as observations were not daily.

Data.— The data is in the text file `oil.csv` with a row for each egg. As not all individuals survive to each stage, some columns have no data for some individuals.

Variables:

Concentration: one of 0, 75, or 100.

Population: either *before* or *after*.

Clutch: id for clutch within population.

Replicate: A or B, one of two replicates for each clutch and treatment.

Hatched: 1= yes, 0 = no.

Metamorphosis: 1 = yes, 0 = no.

Adult: 1 = yes, 0 = no.

DevTimeHM: days from hatching to metamorphosis.

DevTimeMA: days from metamorhosis to adulthood.

DevTimeHA: days from hatching to adulthood (sum of previous two variables).

Sex: female or male, only determined for individuals that survive to adulthood.

Research Questions:

1. How do measures of fitness (hatching rate, survival, development time) compare between the two populations across the different oil levels? Is there evidence that the *after* population has evolved to adapt to an environment with oil present, and if so, by how much and in which ways?

2. Has adaptation had a cost? Is there evidence that fitness of the *after* population under control conditions is less than in the *before* population? Can you quantify the changes?

Questions for Fall 2015: Use Dropbox under Assignments in Learn@UW to turn in a document that addresses the following questions.

1. How does hatching rate compare between the two populations across the different oil levels?
2. How does development time from hatching to adulthood, given hatching has occured, compare between the two populations across the different oil levels?

For each question, do the following.

1. Create a key graphic that shows information in the data that addresses the question.
2. Describe an appropriate model to analyze the data.
3. Provide a summary of key estimates and appropriate measures of uncertainty for each question of interest.
4. Write a summary that explains to the client the results of the analysis and interprets the results in the scientific context.