How does seasonal and altitudinal temperature variation affect UFA: SFA ratio and unsaturation index in *Osmia*?

Part of my dissertation research seeks to understand the effects of seasonal and altitudinal temperature variation on fatty acid (FA) composition of native bees of Wyoming. In this project, I am studying how the “homeoviscous adaptation (HVA) hypothesis” holds in bees. According to this hypothesis, organisms can maintain membrane function at different environmental temperatures by altering the ratio of unsaturated to saturated fatty acids.

To study HVA in bees, I had collected about 250 bees representing four different genera, from an altitudinal gradient in Grand Teton national park (GTNP), in summer of 2012. The bees were collected from June through August from two sites; the low elevation site was at 2020 m and the high elevation site at 3310 m above sea level. The bees were then identified and processed in lab for fatty acid (FA) composition analysis using gas chromatography and the signals were detected using flame ionization detector. To be able to quantify FAs later during data analysis, I had added a known amount of internal standard (IS) in the tube in which bees were processed. The FA types were identified with comparison against the standards and I now have a raw dataset consisting of bee id, genus, collection date, collection site, amount of internal standard and area represented by the peak of individual FA.

After importing the data in R as a .csv file, I checked its structure to ensure the variables are in appropriate format. As per needed, the columns were changed from one class to another (e.g. ‘numerical’ to ‘factor’) and the time was changed to ‘strptime’ and data for month was extracted and stored in new column. To proceed with the analysis, I calculated the total amount of FA in milligrams using the formula given below:

\[
\text{FA (mg)} = \frac{\text{Amount of IS (mg)} \times \text{Area of FA}}{\text{Area of IS}}
\]

To be able to compare the FA composition across individuals and genera, I converted the calculated amount into concentration (amount per mg dry mass) as:
Amount of FA per mg dry mass = \frac{FA (mg)}{Dry mass (mg)}

All these calculated values (FA content as well as concentration) were stored in separate columns. Finally, I created three new columns to store the summed concentration values of unsaturated fatty acids (UFA) in first column and that of saturated FA (SFA) in second column using “apply” function and the ratio of UFA: SFA in the third one. This ratio was then used for final analyses, to study the effects of seasonal and altitudinal temperature variation in fatty acid composition of native bees.

In addition to the ratio of UFA: SFA, I also looked into the seasonal and altitudinal variation in unsaturation index (UI) in the same bees. Unsaturation index accounts not only for UFAs but also for how many double bonds UFAs have. To calculate the UI, I grabbed those columns containing the concentration of fatty acids using 'grep' function. The UI was then calculated for all samples using the formula given below, and stored in a new column named “UI”:

UI = \sum \text{Weight percent of individual FA} \times \text{no. of double bonds}

where, \text{Weight percent of individual FA} = \frac{\text{concentration of individual FA}}{\sum \text{concentration of FAs}}

Because I only have a complete data set for genus *Osmia* from GTNP, I used this genus for further analyses.

**UFA: SFA ratio**

To have an idea on general distribution of values, I looked into the histogram of the data (UFA: SFA; figure 1). I then performed two-way ANOVA test to determine whether either altitude, or season or their interaction has any effect in UFA: SFA ratio. Only altitude was found to have a significant impact \((p = 0.0236)\). I plotted the UFA: SFA values in *Osmia* against altitude for June and July and jittered the points for clarity (figure 2).
Figure 1: Histogram showing the frequency distribution of UFA:SFA values in *Osmia* collected from GTNP

Figure 2: Seasonal and altitudinal variation in UFA:SFA ratio in *Osmia* collected from GTNP. Point color indicates collection month and points are jittered for clarity. Relatively larger and darker points represent mean values with error bars and the lines join the mean values across altitude for July and August.
**Unsaturation Index**

Similar to UFA: SFA rations, I prepared a histogram showing frequency distribution of UI (figure 3) and made a plot similar to figure 2 (figure 4). I also performed two-way ANOVA test for the UI as a function of season and altitude. These variables together didn't seem to have much effect, so I performed one-way ANOVA for both the variables separately too. Based on the results obtained, altitude seemed to have somewhat significant impact in UI ($p = 0.055$) but not the season.

![Histogram showing the frequency distribution of UI values in *Osmia* collected from GTNP](image)

**Figure 3:** Histogram showing the frequency distribution of UI values in *Osmia* collected from GTNP
Figure 4: Seasonal and altitudinal variation in UI in *Osmia* collected from GTNP. Point color indicates collection month and points are jittered for clarity. Relatively larger and darker points represent mean values with error bars.