**Your (Reviewer) Name:** Nicole Kester

**Name of Author:** correct cardinal

**Summary**

Overall, I think that your statistical test and interpretations of the results are correct. I am not sure how your hypothesis would be evolutionary, I get that you are testing HNA and NO2 levels in different seasons throughout the years 2003-2012, but what about this is evolutionary? With minor changes throughout the paper and changing it from first to third person I think that it will greatly help your paper read more scientifically. The main things that I would focus on is trying to pick a side on your hypothesis and then explain how your results support or do not support it. Also mention how what your testing is evolutionary, but after tying all of these things together I think that your paper will be very strong!

**General Comments**

Try not to use “I” or “myself” in the paper, this is first person and scientific papers should be third person. So any of these places I would reword the sentences to remove anything that is personal and make it more matter of fact and stick to stating the results.

Why are you also testing the difference between seasons? Is this what makes it evolutionary, if so, how? Are you saying that you think that seasonal changes of nitrogen dioxide will increase or decrease the biomass of HNA? Choose a side and then further on in your discussion talk about the results and how it either supports or does not support your hypothesis.

Your first paragraph is good on background knowledge. In the second paragraph I would move the part about what data is being collected and put it in the materials and methods. I would then reword the sentence so that it does not say that you assume. I would also choose a side on your hypothesis instead of just saying that the hypothesis tests this I would try to pick a side like stating that the amount of HNA bacteria biomass will fluctuate depending on the amount of NO2.(….. I would change it to something along the lines of “Data collected from 2003-2012 looking at relative biomass size of HNA throughout the years was analyzed to see the impact of temperature and nitrogen. According to the TSR rule, an increase in temperature would result in a decrease of bacteria biomass. Looking at the distribution of HNA throughout different times among the years will show if seasonal changes of nitrogen dioxide impact the biomass of high nucleic acid.”)

In materials and methods, I would talk a little bit about how they collected the data that you are looking at. Also convert it to third person. I would keep the part describing what you are testing but would reword it. I would also consider including the implications of an ANOVA test and state what assumptions this test follows. I think its very good that you selected the largest seasonal temperature differences.

Results: I would place the figures in this section. I would also put the t-test results into a table and possibly reword the last sentence in the results section just to make it a little easier to read. You also did not mention figure 3 at all in the results so I would try to add in some results from this figure.

Discussion: connect the results of the study mentioned to the results that you found. I would also discuss more about the variation in the first figure and what this implies regarding the hypothesis. Did it support the hypothesis or not? Does this make it evolutionary? What new implications are found with the t-test? What exactly does the Welch t-test sample and is there a reason you picked the highest and lowest temperatures to test? Discuss some reasons that the hypothesis may not be true, what could have happened that made it seem not evolutionary. (could be insufficient sample size, could this certain location alter the effects/would the results be different located somewhere else?)

**Specific Comments**

For the t-test results I would put the information into a table instead of inserting a picture of the R-code.

I would put the figures in the results section instead of listing them at the end of the document.

In the first paragraph you mention that higher abundance is related to smaller size, what size is this looking at? Is it the size of biomass? If not, what does size have to do with what you tested?

In the discussion, “In another study….” Is vague, describe the study a little more and say like in a study that measured prokaryotic cell biomass they found….. (instead of describing the experiment I would talk more about the results that they found in the study and how it relates to what you found)

Consider changing the x and y axis in your graphs to make the graphs more appealing and easier to read.