Diet Manipulations and Color Changes in *Pisaster ochraceus*

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Introduction:

If you have spent any time in the intertidal, or tidepools, here on the West Coast of the US, you have no doubt noticed the species, *Pisaster ochraceus*, or the Ochre Sea Star. *Pisaster ochraceus* has been observed from Inside Passage, AK to Pt. Fermin, CA (Raimondi 2007). They are a very common and easily identified species in the intertidal because they are brilliantly colored and large compared to most other intertidal animals. They are the dominant predator of the intertidal, and a keystone species for their food web (Menge et al 1994). The taxonomic group that *Pisaster ochraceus* belongs to, Echinodermata, is the brightest colored group of all marine animals (Perumal 2006).

It has been observed and documented that *Pisaster ochraceus* has very striking colors, but the reasons behind the coloration are still unknown. It has been suggested that coloration may be derived from diet, especially from caroteniod input from feeding on *Mytilus californianus*, or California Mussels. *Mytilus californianus* accumulates carotenoids to aid in protecting egg and sperm when the mussels are out of water at low tide (Petes et al 2007). This large region of the Pacific Coast has revealed some differences among color frequencies from North to South with purple coloration becoming more dominant to the North (Harley 2006). It has also been speculated from regional studies that coloration may be a genetic polymorphic factor, or a result of diet, since frequency of *Mytilus californianus* decreases Northward (Harley 2006).

Common prey for *Pisaster ochraceus* are *Mytilus californianus,* the California Mussel, *Mytilus trossulus*, a smaller mussel species, *Pollicipes polymerus*, the Gooseneck Barnacle, *Semibalanus cariousus, Balanus gladula*, and *Chthalamus dali*, acorn barnacle species, and a variety of other sessile and mobile invertebrates such as limpets, whelks and even small crabs (Harley 2006). They are a low to mid intertidal feeders and have significant impacts on the location of mussel bed limits in the mid intertidal in wave exposed sites (Menge et al 1994).

Pisaster ochraceus is a well studied species with many studies have been done on their role as a keystone predator, (Menge et al 1994), regional color frequencies (Raimondi 2007), prey preferences, (Landenberger 1968) feeding patterns and movement (Garza and Robles 2010) (Paine 1976) and feeding rates (Sanford 2002), but nothing conclusive has been published

regarding coloration. There has been one study on polymorphism and genetic relatedness among Northeast Pacific populations (Harley 2006), but no data specifically regarding pigmentation was analyzed or even tested during the study.

The study that I conducted was aimed at shedding more light on the mystery of *Pisaster ochraceus* color. The three color categories I used for this experiment are: orange, maroon and purple, although there are many shades from orange to purple that have been observed in the field. This study was a feeding trial approach to investigate the effects of diet on coloration in *Pisaster ochraceus*. The null hypothesis for this experiment was: Diet manipulation will have no visible effect on the coloration of *Pisaster ochraceus* over a 6 week period. The alternative hypothesis for this is experiment was: Diet manipulation will have a visible effect on coloration in *Pisaster ochraceus* over a 6 week period. I created these hypotheses after learning about the input of carotenoids in a sea star by its food, and wondered if the carotenoids induced a orange coloration much like if you eat too many carrots your skin will turn orange, but in a sea star.

Since *Pisaster ochraceus* reproduce in late spring (Mauzey 1966) and feed more frequently as water temperatures rise (Sanford 2002), I expected that the six week period from April 22nd to June 2nd would be an ideal time to see an induced color change.

Materials and Methods:

This study involved 30 hand collected individuals from the *Pisaster ochraceus* population at Strawberry Hill with the assistance of Kat Delf. Individuals were selected based on coloration, 10 orange, 10 maroon, and 10 purple and were roughly the same size. The individuals were brought back to the Hatfield Marine Science Center (HMSC) and held in 6 outdoor tanks pumped constantly with sea water from the HMSC sea water system sourced in Yaquina Bay. Each tank received hand collected and cleaned prey often enough to simulate "unlimited prey". Each coloration group was divided in half and fed different prey: five orange sea stars received *Mytilus californianus* and five orange sea stars were fed *Pollicipes polymerus*, and the same methods were applied to each of the two maroon tanks and two purple tanks.

For each feeding, prey was cleaned to ensure only one species of prey was placed in the tanks, and was weighed before feeding and from the previous feeding (after feeding weight) to capture in weights and out weights to ensure feeding occurred.
Before each feeding, individuals were photographed with a Canon Power Shot A1100 IS on the automatic setting without flash. The sea stars were always placed on the North end of the middle bench of lab 31 at HMSC, and all the lights were always turned on during photographing. The photos were analyzed with the computer software GNU Image Manipulation Program that quantifies color shade, and percentage of red, green and blue colors present. A color pinpoint was taken from each ray and the center of the sea star and then averaged to create an overall value of the sea star's red, green and blue coloration each week.

The straightest ray on the sea star each week was also measured to help track the sea star and its growth. Measurements and weights were taken with tape measures and the scale in Lab 31 at HMSC. Prey was always collected by hand at Strawberry Hill on an "as needed" basis. Daily measurements for the sea water system salinity and temperature were taken once a day in late morning to early evening from the monitor display in HMSC to track the water quality of the outdoor tanks as well.

After all the data was collected, it was analyzed in Excel and Minitab. Figures 1 through 6 were created by averaging all the pinpoints from each sea star, and then averaging all of those values together to create one value for each tank on the photograph date. Figures 7 through 9 represent the same averages with the tanks from each color shade combined to show a better comparison of how different prey influenced red saturation.

All the figures were statistically analyzed with two way ANOVA tests in Excel except for the orange tank data because it was unbalanced, due to six missing data points. Shortly before the last photographing, Sea Star 9 died, so the ANOVA in Excel was unable to analyze the data. Instead I used Minitab and ran a general linear model so that the unbalanced data would still yield statistics.

I used these statistics to confirm the variability of the red saturation in the sea stars over time. I also used the descriptive statistics function in Excel to generate standard error bars for the figures (see appendix). The P values obtained from the ANOVA analysis are reported in Tables 1 and 2.

I also created Figure 10 by calculating the proportion of change from April 22nd to June 2nd of each tank of sea stars using the averages calculated earlier. I subtracted the value of red saturation from June 2nd from the value of red saturation on April 22nd.

Results:

Based on the data analyzed and collected, I have discerned the following results. The group of orange sea stars feeding on mussels trend toward losing red coloration over 6 weeks (Figure 1 and Figure 10), whereas the orange sea stars feeding on barnacles trend toward gaining red coloration over 6 weeks (Figure 2 and Figure 10). The group of maroon sea stars feeding on mussels shows a slight trend toward gaining red coloration over 6 weeks (Figure 3 and Figure 10) and the group of maroon stars feeding on barnacles shows a minute trend toward gaining red color over 6 weeks as well (Figure 4 and Figure 10). Both purple tanks show an increase in red coloration over 6 weeks also (figures 5 and 6)

The ANOVA analysis of the effect of prey over time shows that the orange and purple data is not statistically significant with P values reported in Table 1. The analysis did show that the values for the maroon tanks were statistically significant with very low P values reported in Table 1. The ANOVA of the variance due to prey on color did not yield statistically significant P values as reported in Table 2.

Discussion:

Based on the results, the null hypothesis cannot be rejected because diet manipulation did have an effect on the coloration of *Pisaster ochraceus,* but was not completely statistically significant. The only set of data that is significant after analysis is the relationship in the maroon color group between the prey and time. All other figures have much more variable data.

This does not mean that this data is worthless, there are trends showing effects of diet on the coloration. Red saturation did change over time, although not as expected. I expected the orange sea stars feeding on mussels to either remain constant, or become more saturated in red coloration, and the orange sea stars feeding on barnacles to lose red saturation. The exact opposite happened over these past six weeks (see Figure 7 and Figure 10).

Some of this can be attributed to the lighting and capabilities of the camera. With more even artificial lighting, and a better camera would probably produce better results. Also more data points from each sea star would give a fuller perspective of the actual color of each individual. Another possible factor for these results is due to the stress that the sea stars experienced during the six weeks of the experiment, which was made obvious at the end of the trial by the death of Sea Star 9 who had been dwindling for weeks. All the other sea stars appeared healthy at the time of release, and had been feeding during the experiment, so Sea Star 9 could have died from another cause, but the stress of captivity was not a positive effect and could have caused the animals to lose color.

More analysis on the saturation of other colors may yield more insight into how color shifts when diet is manipulated. To fully understand this process and address this question, a highly replicated study in a low stress environment with better equipment should be carried out. Sea stars are long lived and have infinite different color shades, so more study is necessary to really quantify the effects of diet.

Another concern to address is the meaning on red saturation as a standard for measurement. All three color groups for this experiment have different levels of red saturation in them, but none of them are truly red. Many other shades and colors come into play when evaluating the actual color of orange, maroon, or purple. Using a different color for comparison and analysis may produce different results and more accurate results. Also, selecting individuals for experimentation that are closer in color at the beginning of the experiment could produce better results in replication.

This is definitely an area for further study, and is not fully understood. The evidence of diet having an effect on color is a good starting point in the study of the coloration of *Pisaster ochraceus*, and will hopefully launch further investigation to answer this question.

Literature Cited:

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Appendix:

Figure 1

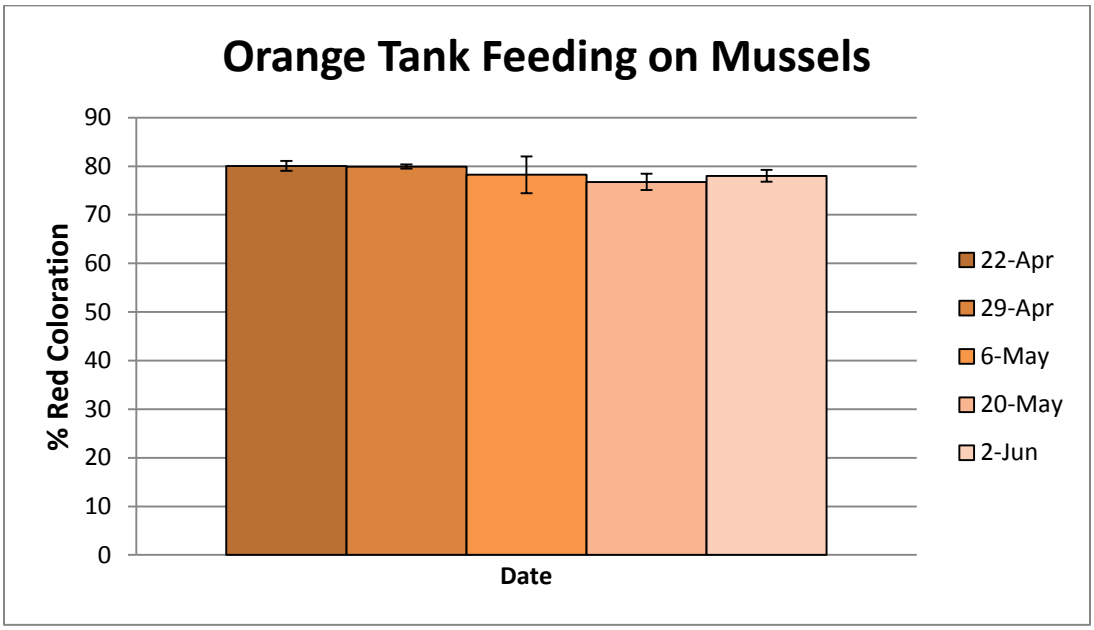

Figure 2

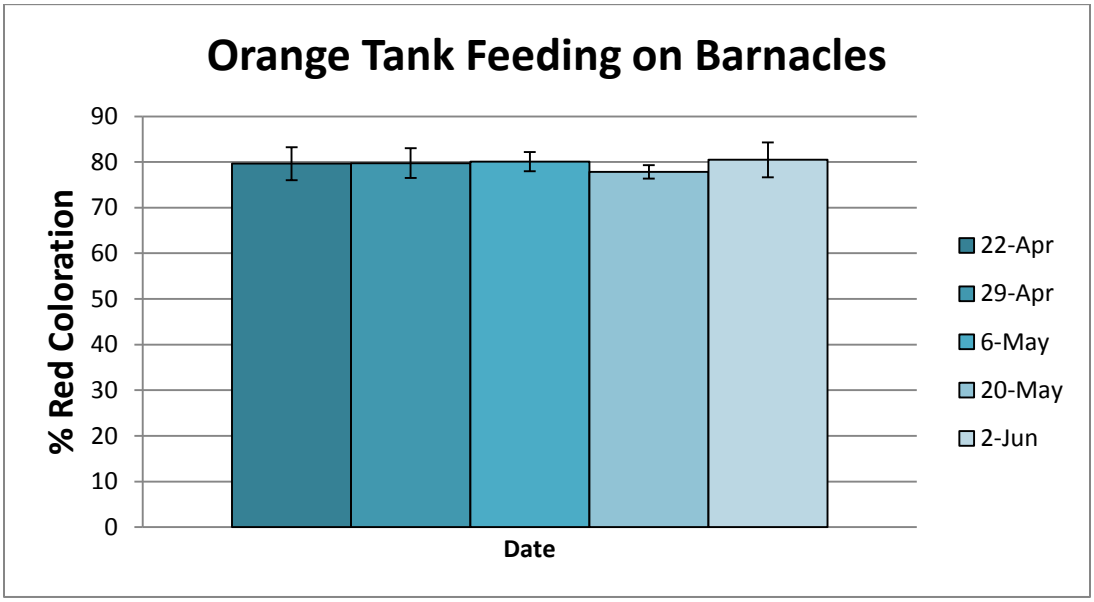

Figure 3

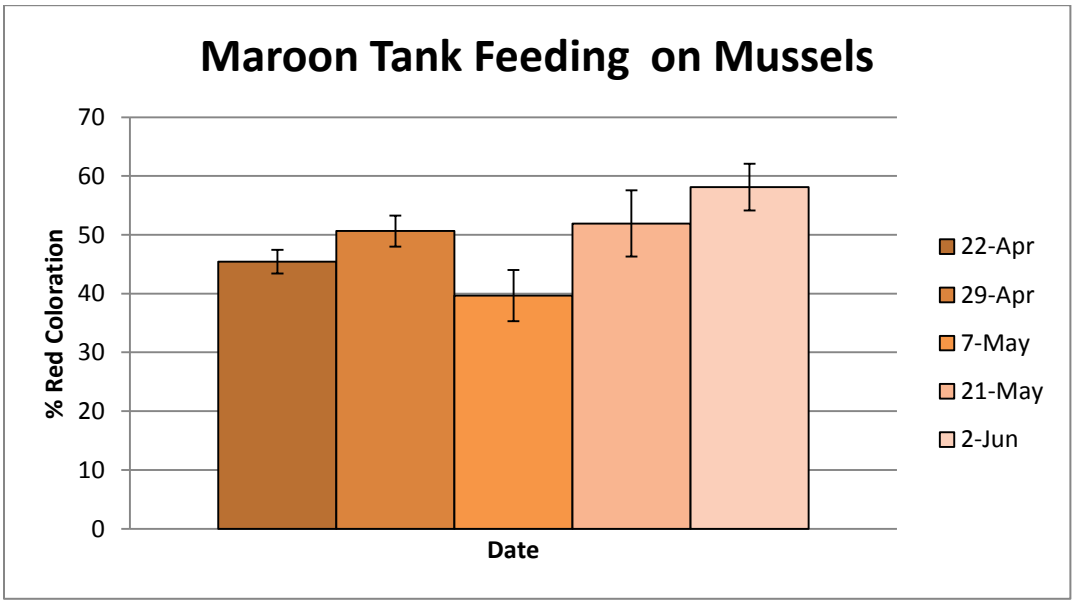

Figure 4

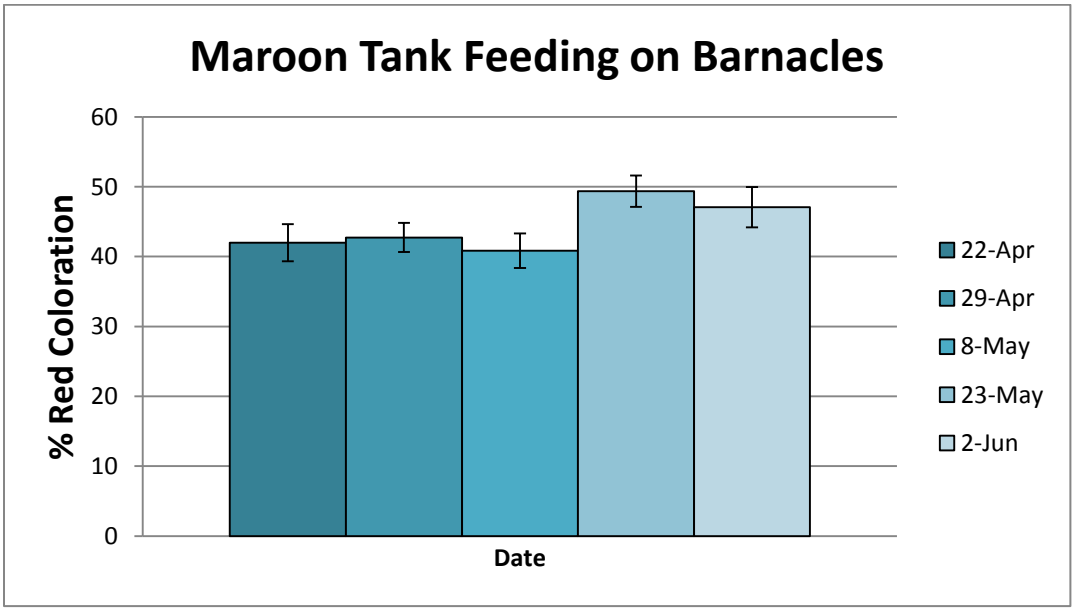

Figure 5

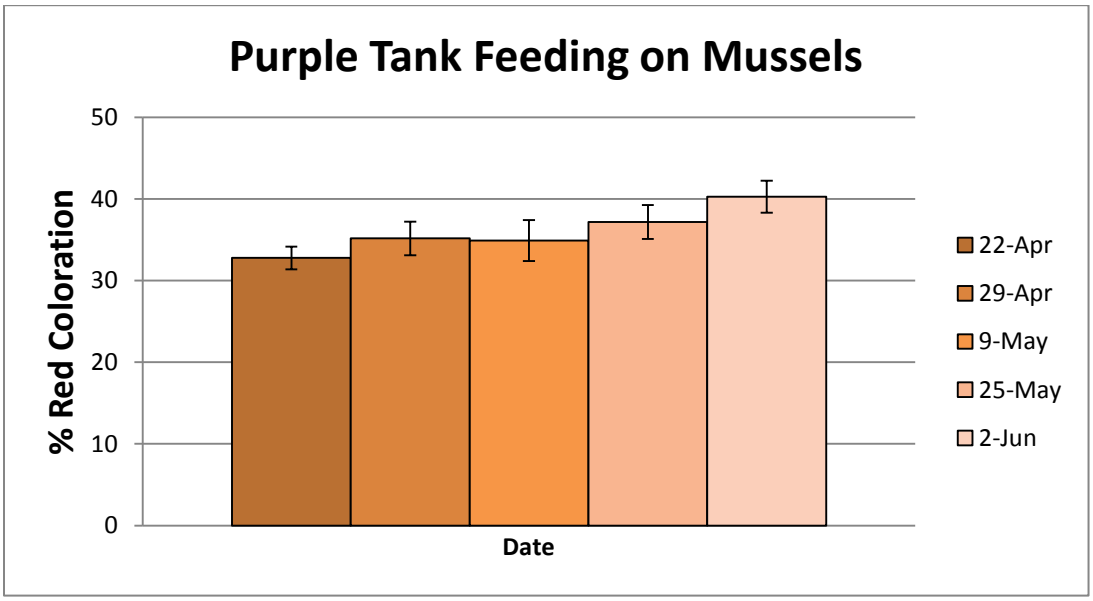

Figure 6

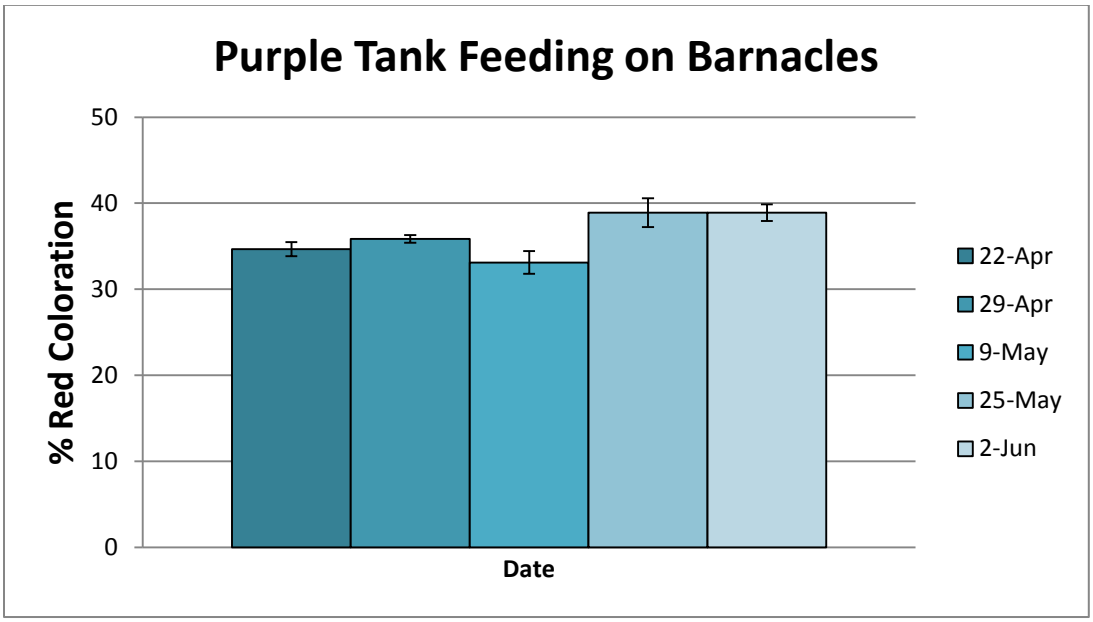

Figure 7

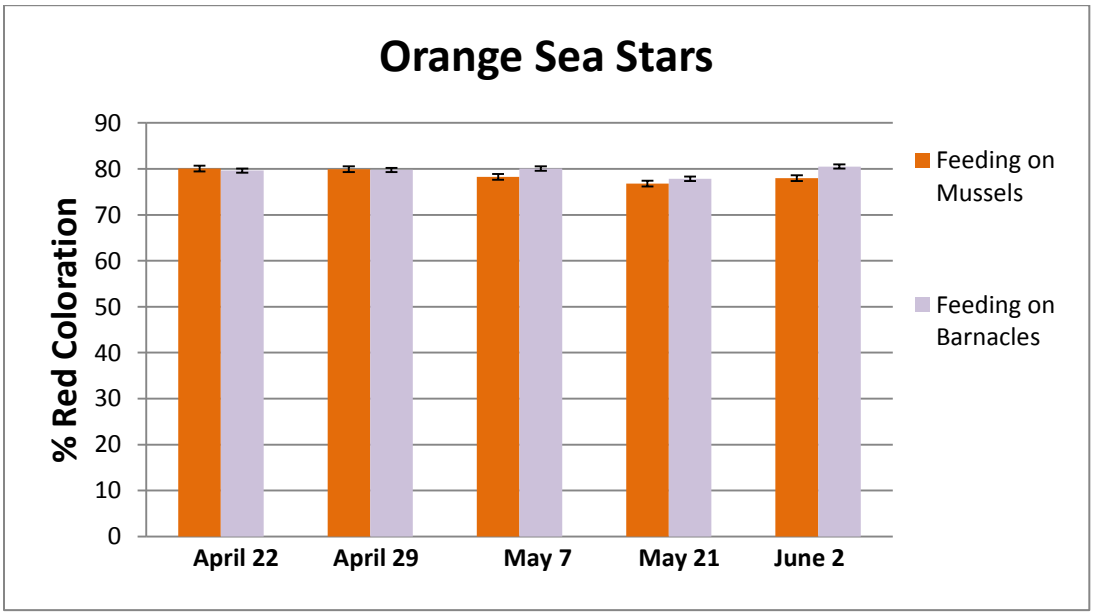

Figure 8

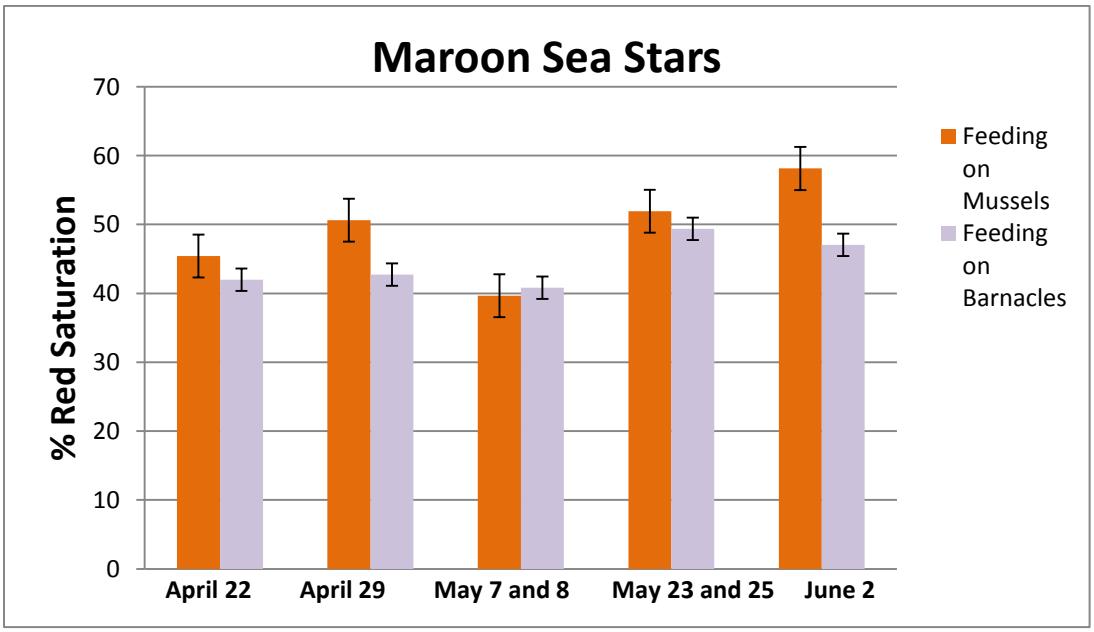

Figure 9

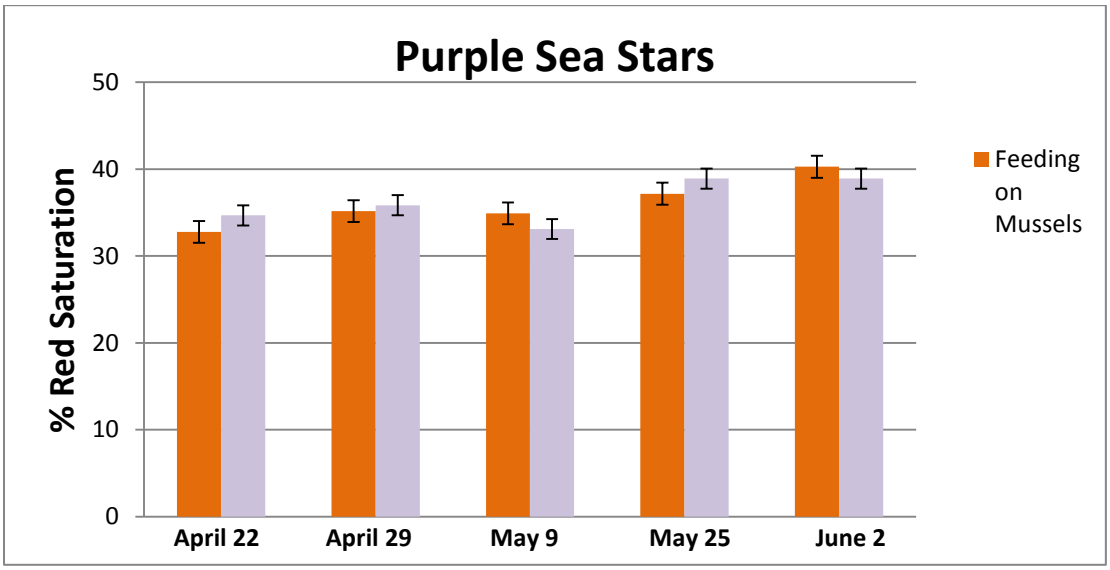

Figure 10

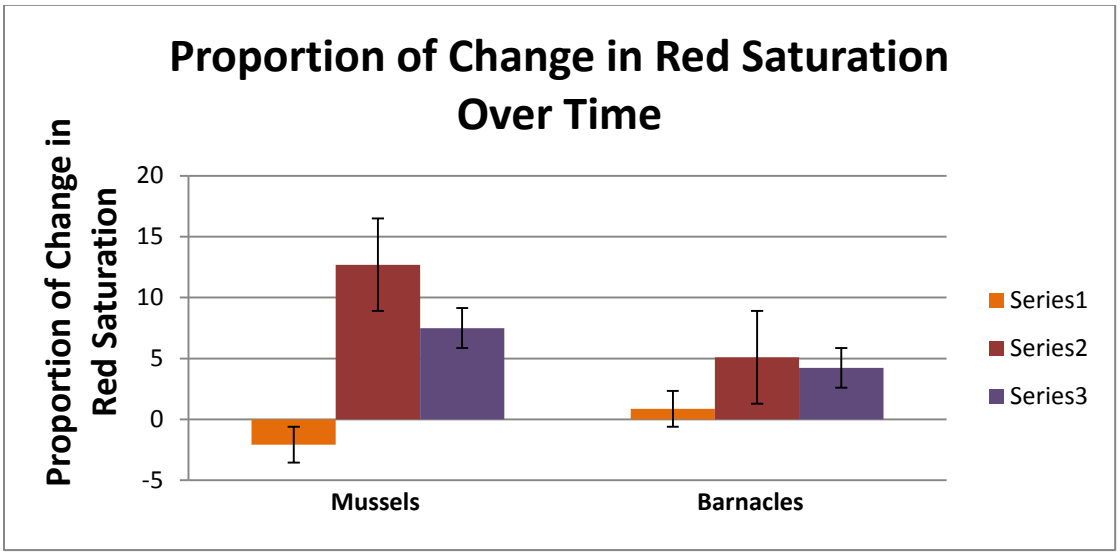

Table 1

ANOVA Analysis P Values: Prey versus Time	Orange	Maroon	Purple
Date	.417	.000632	.157299
Prey	.326	3.31E-10	.290929

Table 2

ANOVA Analysis P Values: Prey versus Color	
Prey	0.229349
Color	0.478145745035767