Freshwater zooplankton coloration as a possible evolutionary adaptation

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Abstract

Many zooplankton species have evolved an overall transparent anatomy, which is generally recognized as an antipredator solution to the problem of concealment against their aquatic background. Although this would suggest that clearer bodies are favorable in clear environments, scientists have also considered darker colorations to correlate with protection from harmful UV radiation, which usually penetrates more into clearer water habitats. Thus, it's currently unclear which factors provoke diverse coloration patterns in freshwater zooplankton populations. Because these organisms are such important drivers for many ecosystem processes, a thorough understanding of how these adaptations influence their survival is crucial. Zooplankton coloration has been studied in marine systems, but rarely in freshwater, and in general is much less understood than their other adaptations like cyclomorphosis and diel vertical migration. In this experiment, I studied how freshwater zooplankton coloration varies with respect to diverse environmental conditions, and what potential adaptation mechanisms are driving coloration. Several lakes and bogs were sampled for zooplankton and surveyed for water clarity, light availability, and food-web structure. We found that all zooplankton taxa were significantly clearer in clearer lakes, thus, the hypothesis for predator-avoidance is supported. This means that freshwater zooplankton coloration was observed to represent an antipredator adaptation.

Introduction

In order to survive, organisms must be able to adapt to their respective environments. Each ecosystem is comprised of many organisms constantly striving to compete for limited resources and to reproduce. These pressures have selected populations into producing a variety of adaptations for prey to avoid predators, and for predators to find and feed on prey. For example, some small, aquatic crustaceans called zooplankton have evolved strategies for evading predation while still managing to obtain food from their environment.

Numerous species of zooplankton are able to alter their bodies in an annual modification pattern called cyclomorphosis. This adaptive response includes changing their size to smaller configurations for defense against visual predators, such as fish, and growing spines, neck teeth, and helmets for defense against tactile predators, like small invertebrates (Dodson 1988). Moreover, these crustaceans participate in a migratory process called diel vertical migration (DVM), which enables them to avoid predators during the day by drifting deeper into the water

column where less light is able to penetrate, and to acquire resources during the night by returning to shallower waters where nutrients are more abundant. Recent studies suggest that in addition to predator avoidance, DVM also enables crustaceans to avoid ultraviolet radiation (UV) from the sun. UV light has been shown to be an important driver of DVM in *Daphnia*, a type of zooplankton (Leach et al. 2015). Although both cyclomorphosis and DVM have been well studied, many species of zooplankton display a third, less examined adaptive characteristic: coloration.

The biological use of colors as means for inducing "evolutionary progress" is commonplace throughout most of the planet's ecosystems. From bright colors that represent warning, to schematic camouflages for concealment, to underwater almost-transparent complexions, organisms have developed many advantageous uses of color that aid their survival. Many zooplankton species have evolved an overall transparent anatomy, which is now generally recognized as an antipredator solution to the problem of concealment against their aquatic background (Johnsen and Widder 1998, Kerfoot 1982, Vestheim and Kaartvedt 2006). Although this would suggest that a clearer zooplankter body is favorable in a clear environment, scientists have also considered darker colorations (or higher concentrations of carotenoids) to correlate with protection from harmful UV radiation, which usually penetrates more into clearer water environments (Kerfoot 1982, Scheider et al. 2012). Thus, it is currently unclear which factors provoke the diverse coloration patterns in freshwater zooplankton populations.

These organisms play very important roles in aquatic habitats. For instance, they serve as a link between lower trophic levels, such as algae and phytoplankton, and higher trophic levels like small fish. Furthermore, they can control the abundance of algae through grazing, and have a direct influence in nutrient cycling. Because these organisms are such important drivers for many

ecosystem processes, a thorough understanding of how these adaptations influence their survival is crucial. Zooplankton coloration has been studied in marine systems (Johnsen and Widder 1998, Vestheim and Kaartvedt 2006), but rarely in freshwater, and in general is most certainly much less understood than their other adaptations like cyclomorphosis and DVM.

In this experiment we sought to address this knowledge gap by studying zooplankton coloration in lake and bog habitats at the University of Notre Dame Environmental Research Center (UNDERC). The particular hypothesis being tested is whether the variability in zooplankton coloration in freshwater lakes is correlated with the color of the water, the presence of specific predators, protection from UV radiation, or other environmental factors.

Materials and Methods

Field Sampling

Sampling was conducted during the summer of 2016 at the UNDERC property, located in the Upper Peninsula of Michigan and northern Wisconsin. Ten lakes and three bogs were sampled for several taxa of zooplankton (*Chaoborus*, *Daphnia*, and *Holopedium*) using a 153 µm Wisconsin zooplankton net. The nets were towed vertically through the water column 2-3 times starting at the deepest part of the lake without disturbing the sediment, and the obtained samples were stored in capped jars prior to photomicrography analysis. All of the photomicrography analyses were done during the same day that the samples were taken. The samples were taken during June and July, with daytime sampling taking place from 10:00 a.m. to 3:00 p.m.

The lakes and the dates in which the samples were taken were: Cranberry Lake (June 13, June 29), Crampton Lake (June 29, July 12), Hummingbird Lake (June 13, June 29), Morris Lake (June 14), Paul Lake (June 8, June 16), Peter Lake (June 8, June 16), Tuesday Lake (June

7, June 17), East Long Lake (June 17), West Long Lake (June 17), Tenderfoot Lake (June 27), Tenderbog (June 27), Crystal bog (July 1), and Northgate bog (July 1).

Measuring water clarity

Light profiles were collected using a LI-COR Biosciences LI-1000 Datalogger with a spherical LI-COR Quantum Sensor, and were used to calculate the light extinction coefficient (k). Surface water was filtered through 25mm Whatman GF/F filters and the absorbance was measured at 440 nm with a Thermo Scientific Evolution 60 spectrophotometer. These measurements were used to calculate the lake color ($g440\ m^{-1}$), an indicator of its terrestrial dissolved organic matter (DOM). These values (light extinction coefficient and color) were used as indicators of lake water clarity and light availability.

Using the microscope and camera

In order to isolate the *Chaoborus*, large *Daphnia*, and large *Holopedium* from other biotic components, I sieved the contents of the jars through a filter cup with a 1 mm mesh, and flushed the zooplankton that were trapped in the mesh into a large beaker using deionized (DI) water.

Using a MACRO-SET pipette, I transferred randomly-selected groups of the zooplankton from the beaker into an unscratched, plastic 50 x 9 mm Petri dish (dish A) with DI water. When necessary, I added 1-2 mL of carbonated water to dish A as a source of CO_2 for use as an anesthetic to avoid flexion of the body during photographing. After adding the carbonated water, I waited 5 minutes or until they stopped moving. This method of anesthesia does not alter the color of the specimens, nor does it kill them (Vestheim and Kaartvedt 2006).

I then randomly selected one of the largest zooplankter from the group in dish A, transferred it into a second 50 x 9 mm Petri Dish (dish B) with water, and placed the second dish

under the microscope. I used a Leica WILD MZ8 microscope connected to a Windows PC and took 2048 x 1536 color photographs of the animals using the Leica Application Suite software (version 3.6.0, Build: 488). I took photographs of one zooplankter at a time with the microscope set to 0.63x on the manual zoom drive for the *Chaoborus* photographs, and 2.00x for *Daphnia* and *Holopedium* photographs. The camera was set to: automatic exposure at 100 %, automatic white balance, 1.0x gain, 2.50 saturation, and 1.00 gamma. After photographing, I moved the zooplankter from dish B to a third, clean Petri dish (dish C) with water; this was done before moving another zooplankter from the dish A to dish B.

Measurements and image processing

I used the GIMP for Mac (version 2.8) software for the image processing. I measured the length of the organisms in pixels using the "Measure Tool" inside GIMP. *Chaoborus* length was measured with a line that went from the back of the head, through the middle axis of the body, to the end of the penultimate segment. *Daphnia* length was measured with a straight line from top of the head to the base of the tail. *Holopedium* length was measured using a straight line from the meeting point between the head capsule and the start of the dorsal carapace to the end of the posterior carapace tip.

Then, I measured the magnitude of coloration of the organisms by changing the pictures to grayscale and calculating the mean value of all the pixels inside a 50 x 50 pixel square selection on one of their body segments. *Chaoborus* coloration was measured in one of the middle-body segments while avoiding the gut. *Daphnia* coloration was measured at the base of the tail (individuals with an obstructed view of the base of their tails were not considered for this experiment). *Holopedium* coloration was measured in the triangle-shaped dorsal carapace behind the head.

In order to account for the differences detected in the mean gray values in the background areas surrounding the animals, I also measured the mean gray values in a 50 x 50 pixel square selection on each side of the organism. The average of the values of these two selections was defined as white (gray value = 255), and each mean gray value measured on the animal's body segment was multiplied by a calibrating factor (CF) as following: CF = 255/ (ΣMean gray value on each side of the animal/2) (Vestheim and Kaartvedt 2006). An overall higher mean value represents a generally lighter coloration of the organism.

Statistical analysis

All statistical analyses were done with a Mac computer using R (version 3.3.0, GUI 1.68, Mavericks build) and RStudio (version 0.99.902). In order to determine if zooplankton in different lakes have different coloration, we ran a simple Kruskal Wallis test (the nonparametric equivalent of ANOVA) with zooplankton coloration as the dependent variable and the lakes as the independent variable. This test was used because the variance of zooplankton coloration was not normally distributed (Shapiro-Wilk test, all p-values > 0.05). After this, a Nemenyi post hoc test was used for pairwise comparisons.

To establish if zooplankton coloration was related to water clarity or light availability, we regressed zooplankton color against the two proxies for water clarity (lake color and light extinction coefficient), and based on the Quantile (Q-Q) plots of the residuals, these data were log-transformed to make the residuals more normal.

A Kruskal-Wallis test was used in order to compare zooplankton coloration (response) to the food-web structure of each lake (treatment). This test was used because of non-normality of the residuals and an unbalanced number of individuals in the treatment groups. Food-web structure categories were defined as piscivore-dominated, planktivore-dominated, and fishless. A

Nemenyi post hoc test was used for pairwise comparisons. Lastly, we used a linear regression to investigate if zooplankton coloration changes depending on their body size.

Results

Zooplankton coloration varied significantly among lakes for *Chaoborus* (Figure 1a, Kruskal-Wallis test, H = 50.074, df = 10, p-value = 2.586e-07) and *Holopedium* (Figure 1c, Kruskal-Wallis test, H = 32.074, df = 7, p-value = 3.936e-05), but not for *Daphnia* (Figure 1b, Kruskal-Wallis test, H = 10.17, df = 6, p-value = 0.1177). *Chaoborus* coloration in Tenderbog was significantly darker than in Crampton, East Long, Hummingbird, Morris, Paul, and Tuesday Lakes (Nemenyi post hoc test, all p-values < 0.05), and for *Holopedium*, coloration in Hummingbird Lake was significantly darker than in Crampton and Paul Lakes (Nemenyi post hoc test, p-values < 0.05), while coloration in Northgate bog was significantly darker than in Crampton Lake (Nemenyi post hoc test, p-value = 0.0009). Altogether, all zooplankton coloration was significantly darker in darker lakes (Figure 2, log-transformed linear regression, all p-values < 0.05). As the light extinction is similar to water color, only the water color data are presented here although the results using either variable were the same.

With respect to food-web structures, zooplankton coloration was significantly different for *Chaoborus* (Figure 3a, Kruskal-Wallis test, H = 29.281, df = 2, p-value = 4.383e-07) and *Holopedium* (Figure 3c, Kruskal-Wallis test, H = 16.292, df = 2, p-value = 0.0002898), but not for *Daphnia* (Figure 3b, Kruskal-Wallis test, H = 4.0128, df = 2, p-value = 0.1345). *Chaoborus* coloration in fishless locations was significantly darker than in planktivore-dominated and piscivore-dominated locations (Nemenyi post hoc test, p-values < 0.05). For *Holopedium*, coloration in fishless and planktivore-dominated locations was significantly darker than in piscivore-dominated locations (Nemenyi post hoc test, p-values < 0.05).

Finally, the relationship between zooplankton coloration and the individual lengths was not significant for *Chaoborus* and *Daphnia* (Figure 4a and 4b, linear regression, p-values > 0.05). However, a significant relationship between *Holopedium* color and size was observed, where smaller individuals are darker and more variable (Figure 4c, linear regression, p-value < 2e-16).

Discussion

In this study, I sought to investigate how freshwater zooplankton coloration varies with respect to diverse environmental conditions, and what potential adaptation mechanisms are driving coloration. Several, distinct lake and bog habitats were sampled for zooplankton and surveyed for various limnological characteristics including water clarity, light availability, and food-web structure, thereby furthering understanding of how zooplankton color relates to these ecological characteristics.

Of the three taxa, *Holopedium* had the most variable pattern of coloration, having significant differences between more lakes than the other genera. Coloration of *Holopedium* populations in Hummingbird Lake were significantly darker than in Paul Lake and Crampton Lake, while the Crampton Lake population was also significantly clearer than the Northgate bog one (Figure 1c). These differences correlate with the water clarity and light availability of these lakes. For instance, both Hummingbird Lake and Northgate bog had light extinction coefficients three times higher and lake colors over ten times darker than Crampton and Paul Lakes. This relationship is also shown when plotting the coloration of *Holopedium* versus the color of all lakes (Figure 2c). In can therefore be concluded that *Holopedium* coloration is darker in more colored lakes.

When analyzing the coloration of the *Daphnia* populations, there was not a significant difference among the sampled lakes. It is important to note that *Daphnia* had the lowest abundance and were found in the least amount of sites compared to the other taxa. Due to this low abundance, we only were able to capture one *Daphnia* from Tenderbog for which we could measure its coloration due to it being the only one having an unobstructed view at the base of its tail. That noted, this individual was darker than all other individuals. Despite this, the relationship between *Daphnia* coloration and lake color (Figure 2b) shows the same downward pattern as the *Holopedium* analysis: where there is darker water color and less light availability, zooplankton coloration is darker. *Chaoborus* coloration was also less variable among lakes than *Holopedium*. The only meaningful difference was that the Tenderbog population was significantly darker than most of the other lake samples (Figure 1a). When comparing the *Chaoborus* v. measured color of all lakes-(Figure 2a) to the *Holopedium* and *Daphnia* data, once more the same relation is observed: *Chaoborus* populations are darker in darker lakes compared to the ones in clearer lakes.

This establishes an important reasoning concerning the distribution of coloration throughout the different lake ecosystems. Therefore, the hypothesis for predator-avoidance is supported. This means that the observed phenomena in this investigation represent an adaptation for inconspicuousness towards predators. In clearer lakes, visual predators are more easily able to find prey, hence, the zooplankton communities are strongly selected for more overall transparent complexions. There may be various reasons why the populations that live in darker bodies of water tend to stay dark rather than clear. In darker lakes there could be less evolutionary pressure for transparency and as a result the less-transparent anatomy can be a “good-enough” camouflage in locations with predators that rely heavily on vision. Or it may

simply be that these colorations are not eliminated from the gene pool in fishless locations because of benefits associated with being darker. If the water that is incorporated into the zooplankton bodies is originally dark, then removing the coloration from their bodies can be a waste of energy since they are not strongly pressured towards having clear physical makeups. Also, the darker zooplankton colors may indicate the presence of organic compounds, like carotenoids, which protect against UV radiation (Kerfoot 1982, Scheider et al. 2012) or help them survive in the harsher, low-pH conditions that the dark-colored lakes and bogs are associated with. Nonetheless, other possible causes for the relationship may also have to be contemplated.

For example, even though all taxa in this experiment were generally darker in fishless locations (Figure 3), *Chaoborus* coloration was considerably clear in Cranberry, which is a fishless lake. In this case, other factors may be affecting coloration. Although Cranberry is currently a fishless lake, it would be a good idea to investigate for how long this has been the case. If this lake had a different food-web structure several decades or even centuries ago, it would be possible that it previously had a strong selective pressure towards clearer coloration in *Chaoborus*.

In conclusion, the pattern is clear: the sampled communities of freshwater zooplankton are darker in darker lakes and vice-versa. For these populations, the main driver of coloration seems to be predation. Fish are usually less prevalent in darker, boggy habitats because of nutrient deficiency and the low-pH conditions, and thus clearer zooplankton bodies are not necessarily selected for in these already-dark aquatic ecosystems. The evolutionary implications of freshwater zooplankton coloration resemble those of marine environments and gives an insight into how this adaptation can be further studied. For example, in order to better understand

if zooplankton have diverse coloration in different lakes during times when light availability is equal, taking samples during night and comparing the coloration of those individuals to samples taken during the day is encouraged.

Acknowledgements

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Figures

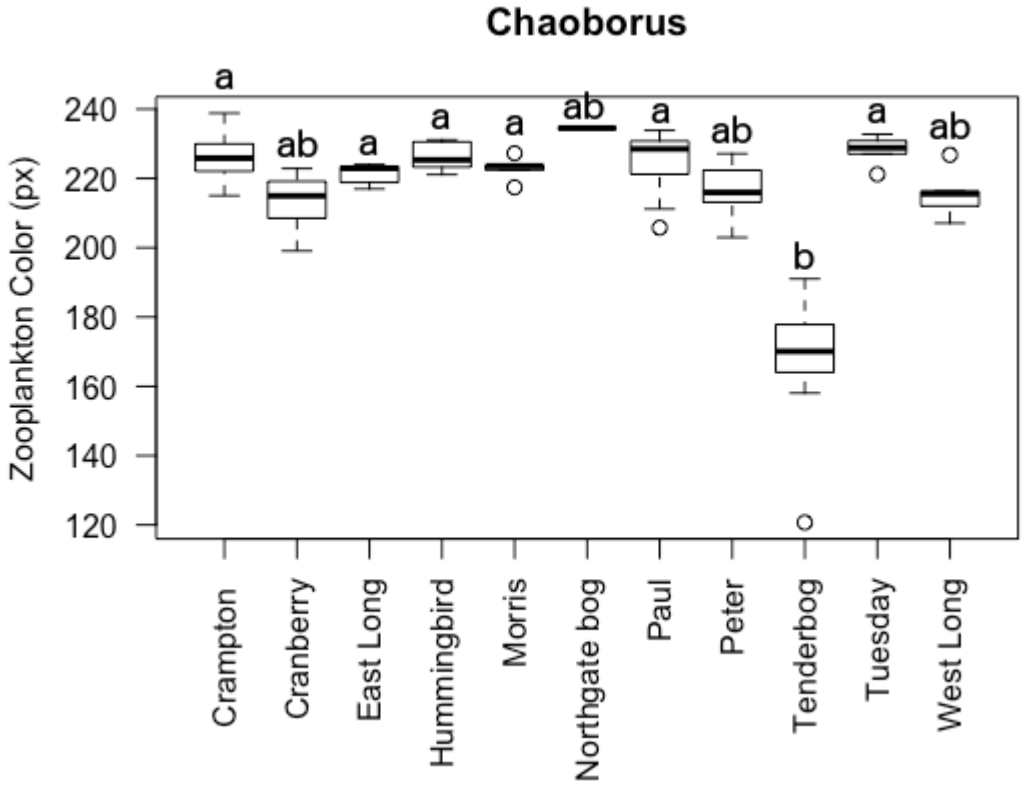

Figure 1a. *Chaoborus* coloration across lakes. *Chaoborus* coloration was not equal among lakes (Kruskal-Wallis test, n = 79, H = 50.074, df = 10, p-value = 2.586e-07). Coloration in Tenderbog was significantly darker than in Crampton, East Long, Hummingbird, Morris, Paul, and Tuesday lakes (Nemenyi post hoc test, all p-values < 0.05). Note: any two lakes sharing one or more letters are not significantly different from each other. Lakes not sharing letters are significantly different.

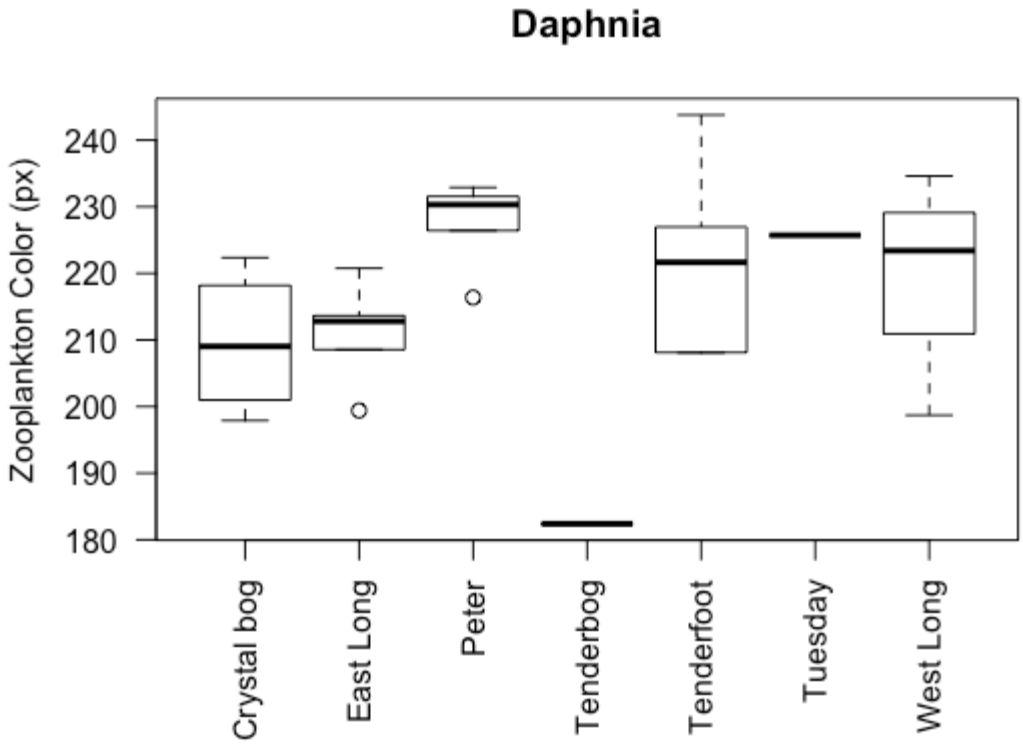

Figure 1b. *Daphnia* coloration across lakes. Coloration did not vary significantly among lakes (Kruskal-Wallis test, n = 26, H = 10.17, df = 6, p-value = 0.1177). Note: because of the low abundance of *Daphnia* in Tenderbog and Tuesday Lake, we only were able to capture one *Daphnia* for each of these sites for which we could measure its coloration due to it being the only one having an unobstructed view at the base of its tail.

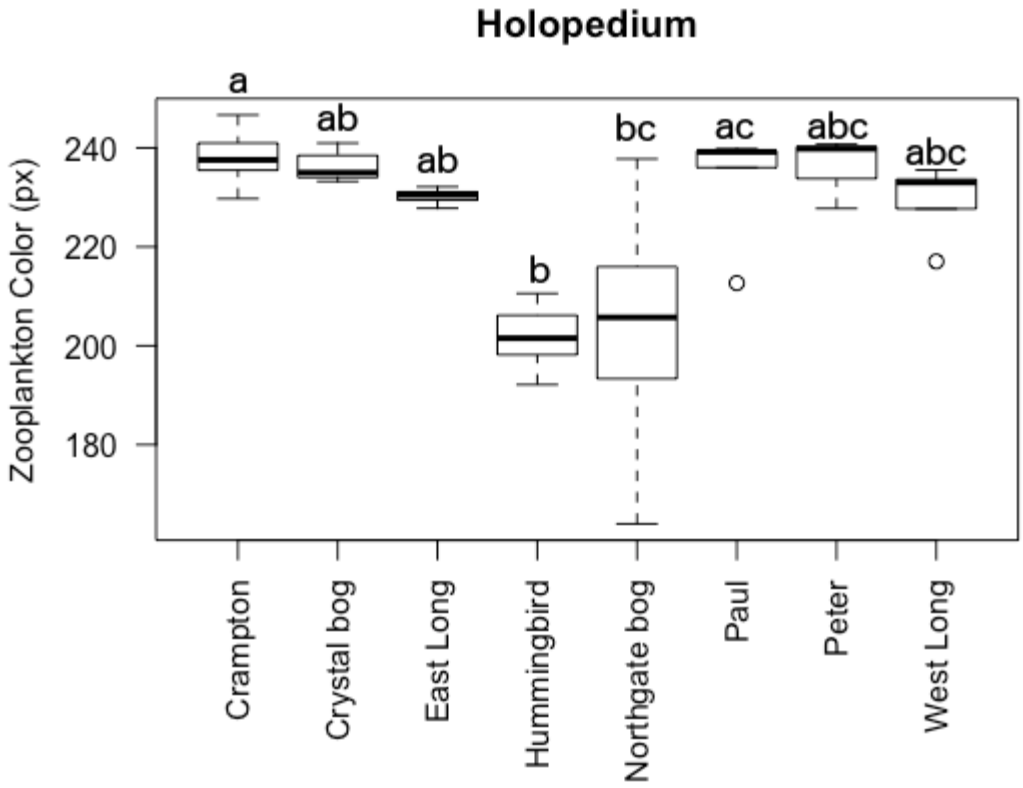

Figure 1b. ***Holopedium*** **coloration across lakes.** Coloration varied significantly among lakes (Kruskal-Wallis test, n = 52, H = 32.074, df = 7, p-value = 3.936e-05). The Hummingbird Lake population was significantly darker than that of Crampton and Paul Lakes (Nemenyi post hoc test, p-values < 0.05), and the Northgate bog population was significantly darker than that of Crampton Lake (Nemenyi post hoc test, p-value = 0.0009). Note: any two lakes sharing one or more letters are not significantly different from each other. Lakes not sharing letters are significantly different.

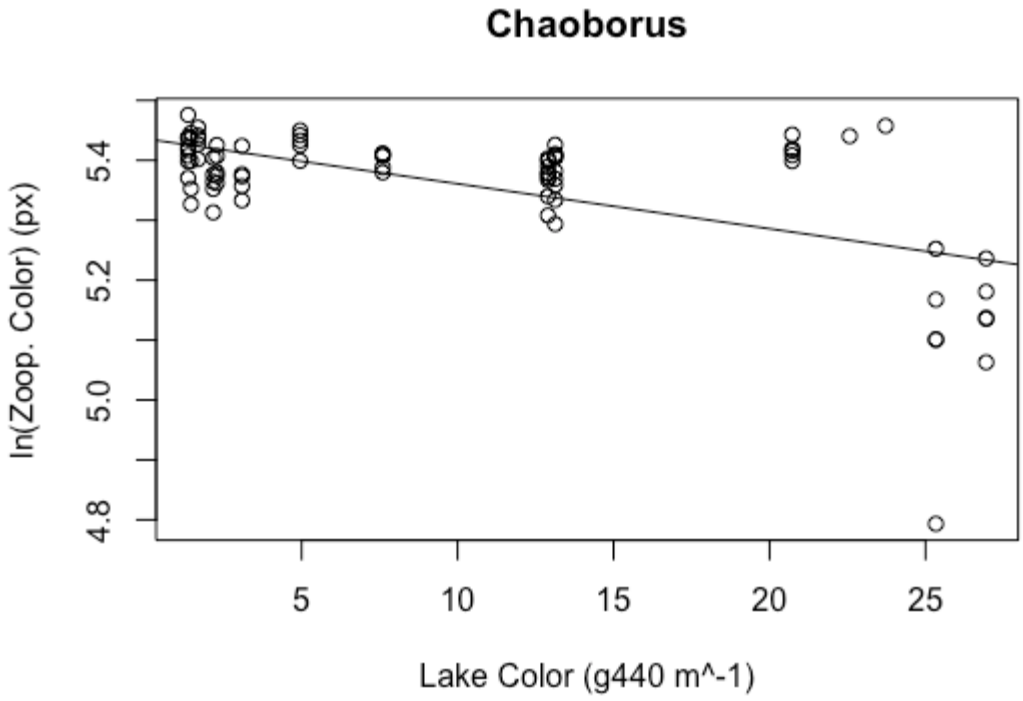

Figure 2a. *Chaoborus* coloration v. lake color. *Chaoborus* showed a significant negative relationship with lake color, meaning that individuals were darker-colored in darker-colored lakes (log-transformed linear regression, n = 79, R^2 = 0.3643, df = 77 p-value < 2e-16).

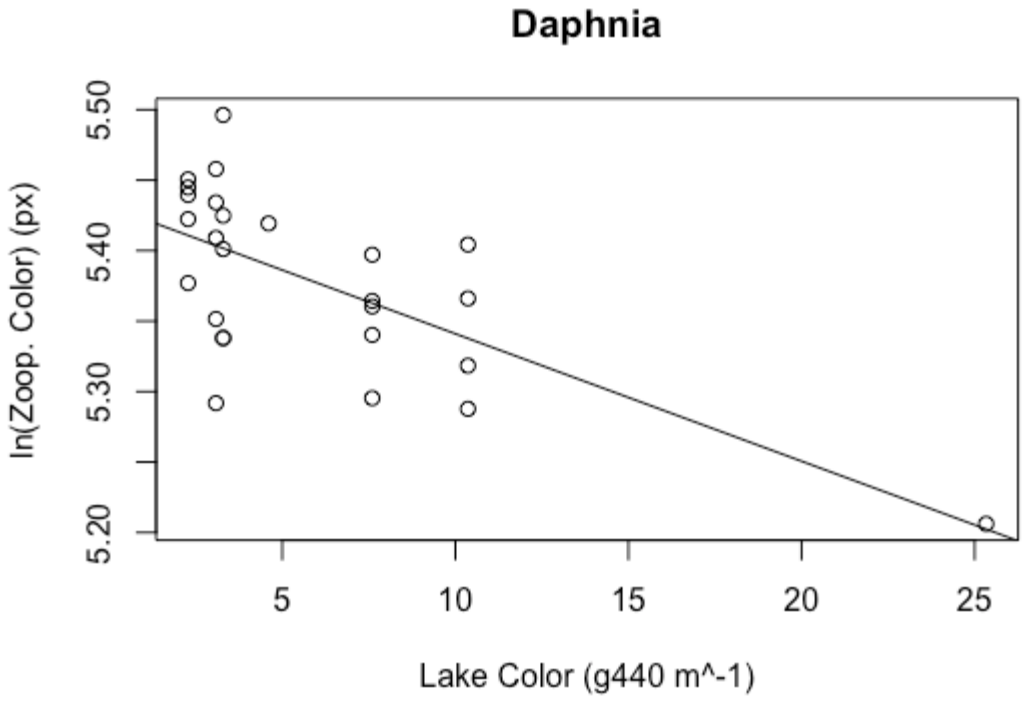

Figure 2b. *Daphnia* coloration v. lake color. *Daphnia* showed a significant negative relationship with lake color, meaning that zooplankton coloration was darker in darker lakes (log-transformed linear regression, n = 26, R^2 = 0.469, df = 24, p-value < 2e-16). Note: a significant negative relationship was still observed after removing the Tenderbog datum point at the bottom-right side of the plot.

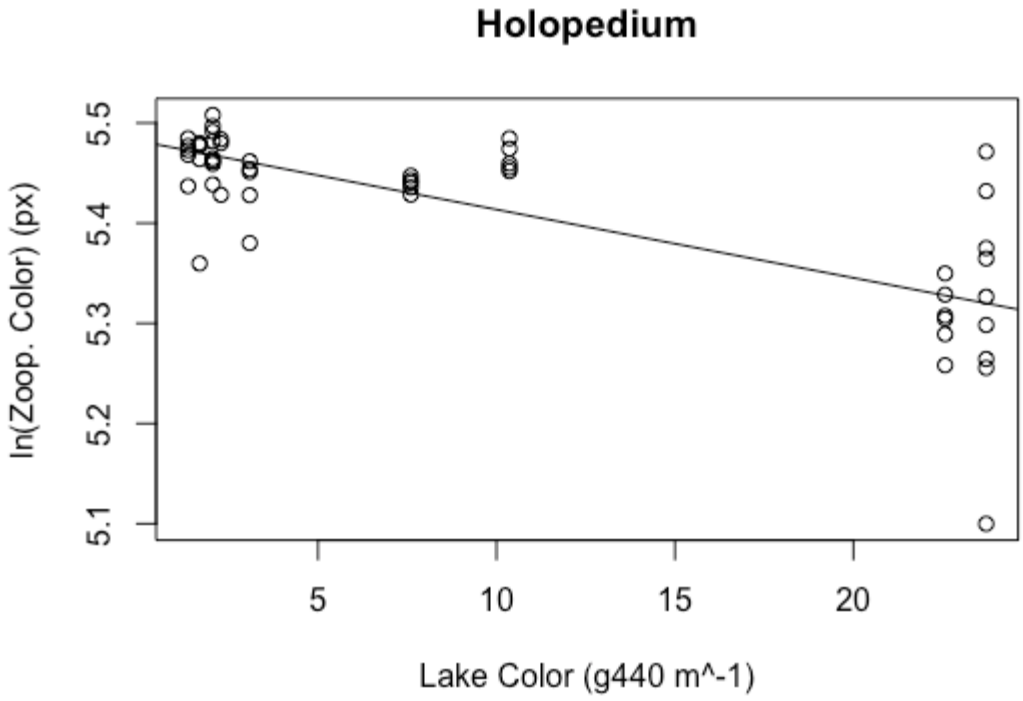

Figure 2c. *Holopedium* coloration v. lake color. *Holopedium* showed a significant negative relationship with lake color, meaning that individuals were darker-colored in darker-colored lakes (log-transformed linear regression, n = 52, R^2 = 0.5787, df = 50 p-value < 2e-16).

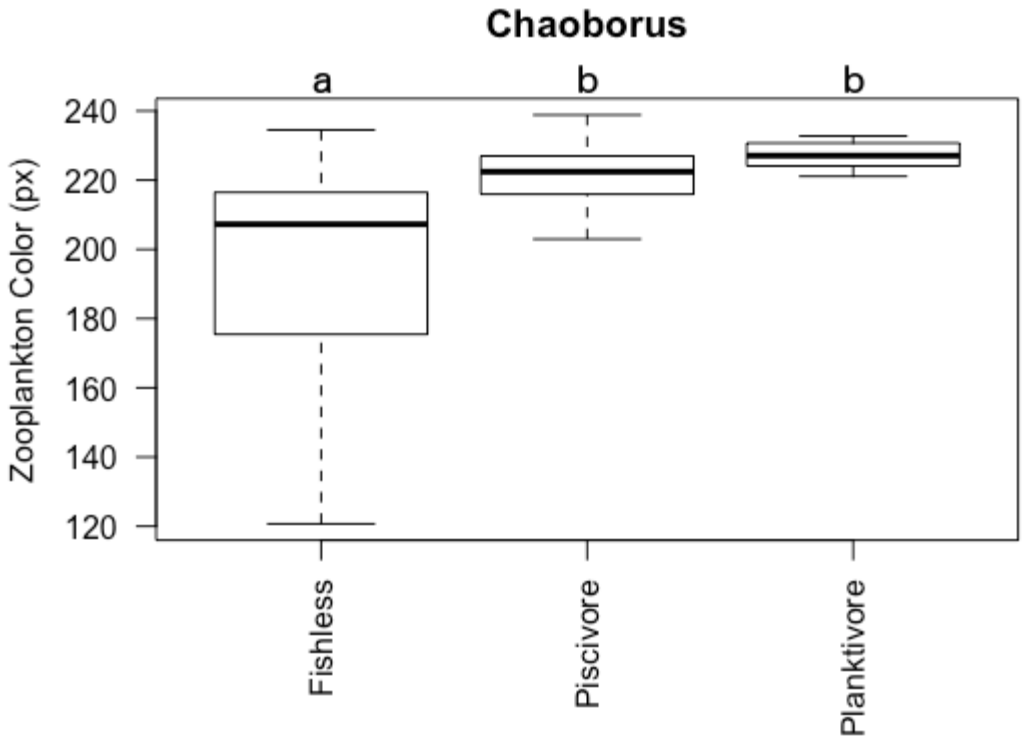

Figure 3a. *Chaoborus* coloration across food-web structures. 25 individuals were sampled from fishless sites, 43 were from piscivore-dominated sites, and 11 were from planktivore-dominated sites. *Chaoborus* coloration was not equal among food-web structure locations (Kruskal-Wallis test, n = 79, H = 29.281, df = 2, p-value = 4.383e-07). Coloration in fishless locations was significantly darker than in planktivore-dominated and piscivore-dominated locations (Nemenyi post hoc test, p-values < 0.05). Note: any two lakes sharing one or more letters are not significantly different from each other. Lakes not sharing letters are significantly different.

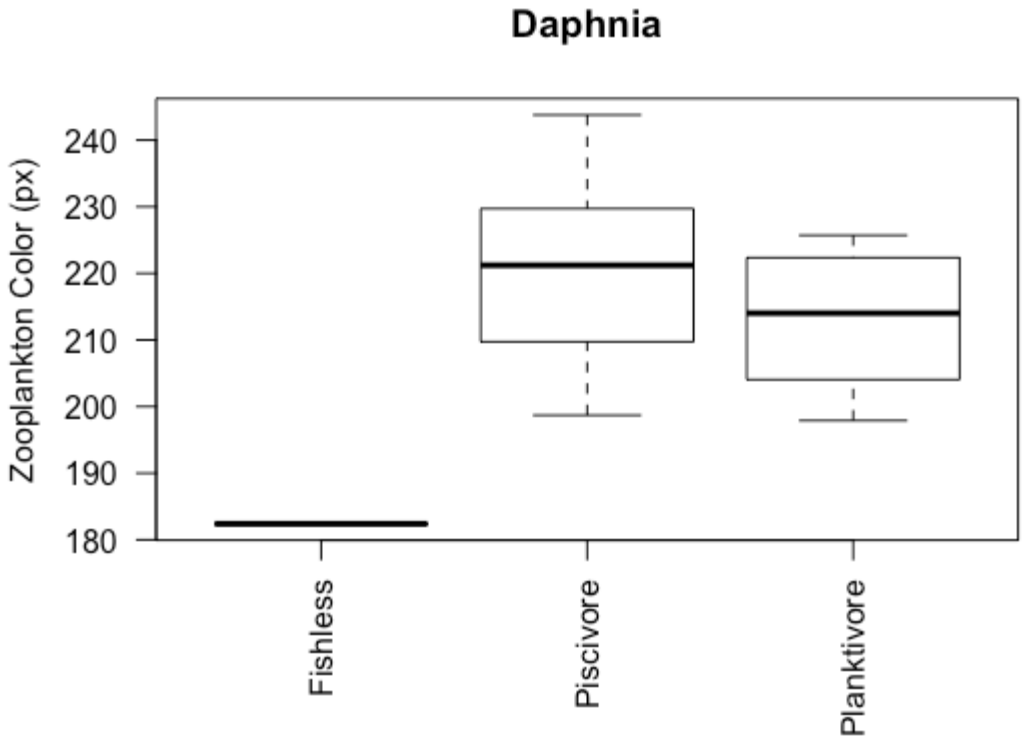

Figure 3b. *Daphnia* coloration across food-web structures. 1 individual was sampled from a fishless site, 20 were from piscivore-dominated sites, and 5 were from planktivore-dominated sites. Coloration did not vary significantly among food-web structure locations (Kruskal-Wallis test, n = 26, H = 29.281, df = 2, p-value = 4.383e-07). Note: because of the low abundance of *Daphnia* in Tenderbog, we only were able to capture one *Daphnia* from a fishless location for which we could measure its coloration due to it being the only one having an unobstructed view at the base of its tail.

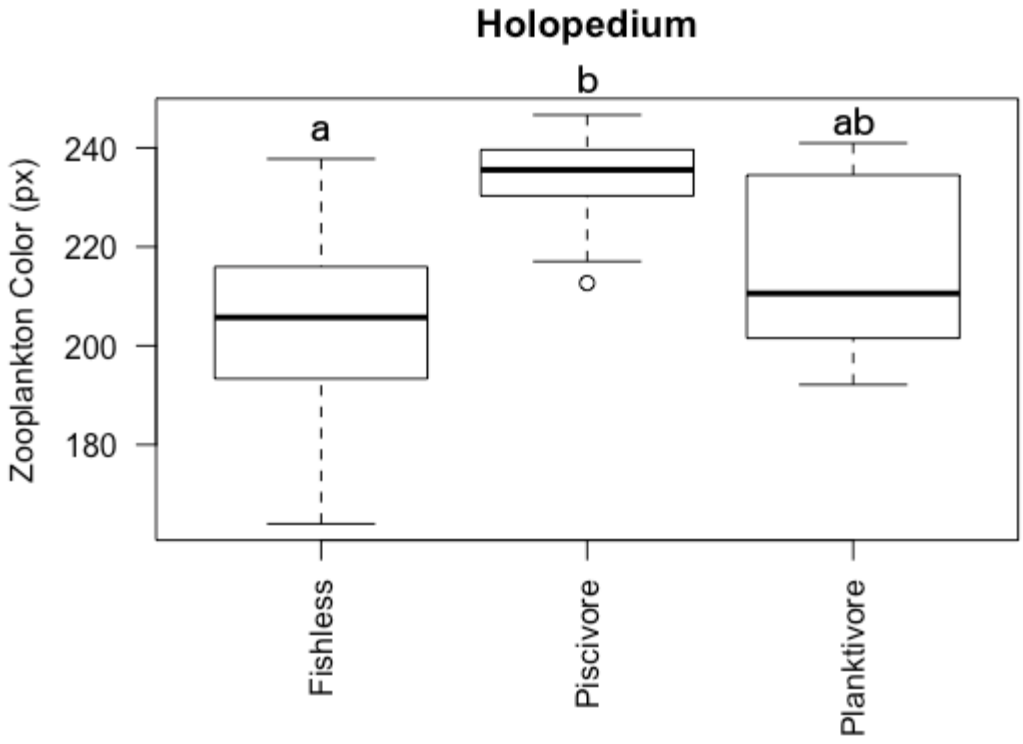

Figure 3c. *Holopedium* coloration across food-web structures. 9 individuals were sampled from fishless sites, 32 were from piscivore-dominated sites, and 11 were from planktivore-dominated sites. Coloration varied significantly among food-web structure locations (Kruskal-Wallis test, n = 52, H = 16.292, df = 2, p-value = 0.0002898). Coloration in fishless and planktivore-dominated locations was significantly darker than in piscivore-dominated locations (p-values < 0.05). Note: any two lakes sharing one or more letters are not significantly different from each other. Lakes not sharing letters are significantly different.

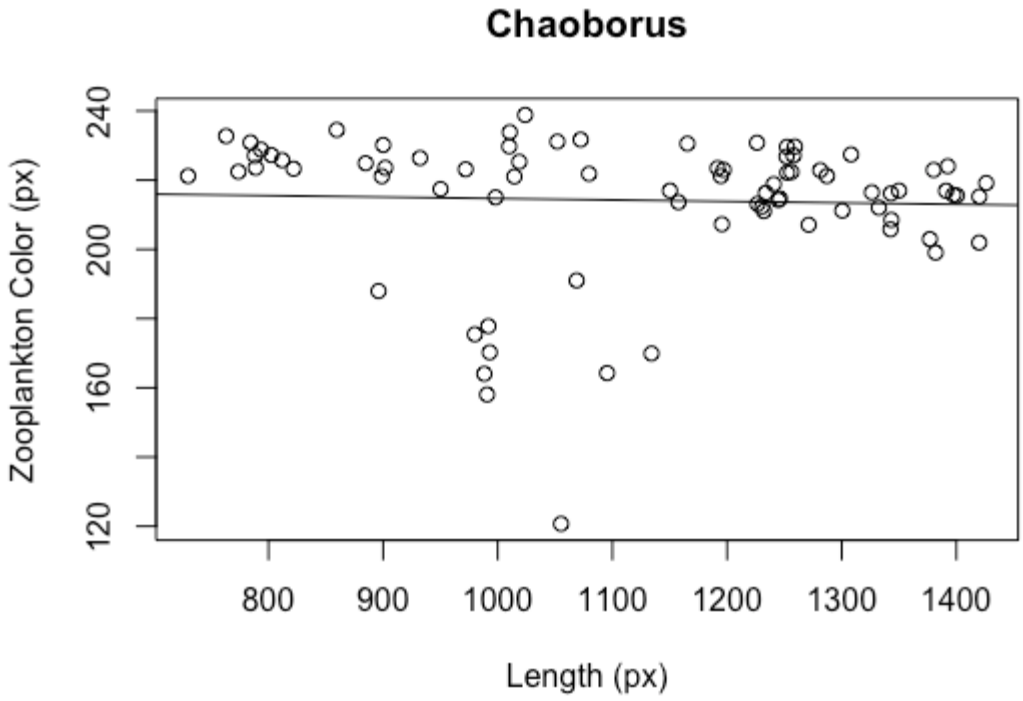

Figure 4a. *Chaoborus* coloration v. length. The relationship between *Chaoborus* coloration and size was not significant (linear regression, n = 79, R^2 = 0.001774, df = 77, p-value = 0.7125).

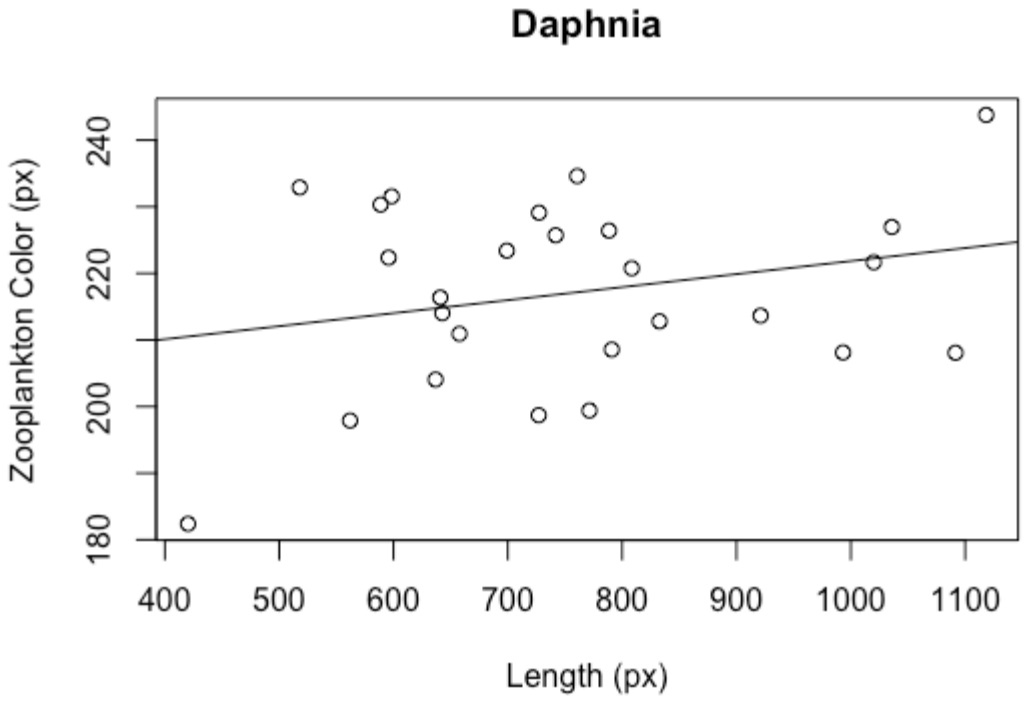

Figure 4b. *Daphnia* coloration v. length. The relationship between *Daphnia* coloration and size was not significant (linear regression, n = 26, R^2 = 0.06498, df = 24, p-value = 0.2088).

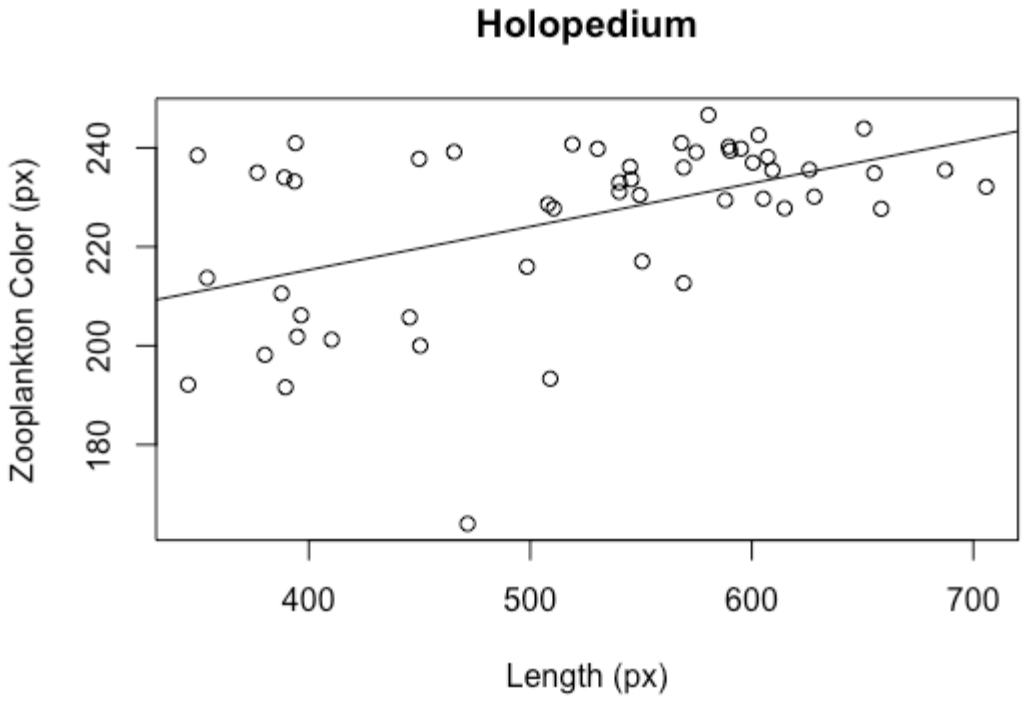

Figure 4c. *Holopedium* coloration v. length. Smaller *Holopedium* individuals were significantly darker-colored and more variable than larger individuals (linear regression, n = 52, $R^2 = 0.242$, df = 50, p-value = 0.0002119).