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Assessing Variation in Visual Abilities in Daphnia as a Result of Disparity in Eye Sizes

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THESIS SUMMARY

The successful survival and reproduction of an animal relies on its ability to gather information from the environment and make decisions accordingly. One common and often dominant sense that animals rely on is vision. Previous theoretical work has outlined the fact that, all else being equal, larger eyes should provide better vision for an animal (Land and Nilsson, 2012). The work presented here tests this hypothesis using several species of the microcrustacean *Daphnia* by evaluating the difference in visual ability among *Daphnia* species and within clonal populations as a result of differences in size between the functional units of their eye, known as ommatidia (Young and Downing, 1976). Visual ability is measured using a visually guided behavior known as the optomotor response. The behavior can be scored using an established set of criteria in order to make quantitative comparisons among individuals and populations.

The results of this study provide mixed evidence for the notion that increases in ommatidia size provides significant improvement in vision for *Daphnia*. Results across species showed a strong correlation between larger ommatidia size and increased swimming behavior in response to the optomotor stimulus; however, results within clonal populations showed no such pattern. It is suggested that one factor that may have an effect is motivational differences among species. That is to say, the difference may not be in how different *Daphnia* detect motion in their environment but how they chose to respond to that motion, and this difference may have to do with differences in the sizes of their ommatidia. It is equally likely that there may be other morphological factors associated with species identity, some that may involve temporary changes in the eye, that explain why the expected results of the experiment are only seen at a species level.

ABSTRACT

The functionality of an eye is affected by a number of structural factors, and altering any of these factors often involves inevitable tradeoffs between resolution and sensitivity. However, it has been theoretically reasoned, and tested empirically, that increasing the size of an eye allows for both of these central features of vision to be improved due to corresponding increases in lens size. This study examines the relevance of this hypothesis for the vision of *Daphnia* in both interspecific and intraspecific contexts. The visual capabilities of six different species of *Daphnia* are tested using the optomotor response—a visually mediated behavior that our lab developed a scoring method for so that quantitative comparisons among species and individuals can be made. The main prediction we test is that increased ommatidia diameter will correlate with significant increase in optomotor behavior as an indirect measure of sensitivity and resolution, both across species and within populations. Our study offers evidence of significant optomotor responses in five species of *Daphnia* and demonstrates that there are significant differences in behavior among species. We provide evidence suggesting that ommatidia diameter is associated with increased visual capacity and performance between species; however, this correlation is not demonstrated within any of the six clonal populations. Consequently, we propose that there must be other visual traits, possibly associated with species identity and habitat differences, which may have influenced these results and warrant further investigation.

INTRODUCTION

The ability for animals to gather information from their environment and make decisions based on that information is fundamental to their success in survival and reproduction. Sensory information directs the vital activities and behaviors of an animal: finding suitable mates, avoiding predators, and locating resources. Visual sensation is a particularly interesting sensory strategy because it is present in a diverse array of taxa living in dissimilar ecosystems. Moreover, the eyes animals have evolved vary greatly in the complexity of their structure and utility. Establishing a comprehensive understanding of an animal's visual system therefore includes ecological and morphological information. That is to say, it must be understood what ecological tasks the animal uses visual strategies to accomplish and what mechanisms associated with the eye and visual system allow the organism to implement these visual strategies (Dusenbury, 1992). Studying the morphological factors that affect an animal's vision has the potential to explain how visual capabilities in the organism are determined and limited, as well as suggest what features of the visual environment are important for the animal.

One way to test the visual capabilities of an organism is to study its optomotor response (OMR). The optomotor response is an innate reflex involving head, eye, or body movements that an organism exhibits in response to perceived movement in its environment (Anstis et al., 1998; Cronin et al. 2014). The OMR can be elicited by placing an animal in a stationary arena around which a series of vertical stripes are rotated. It can be tested using a variety of spatial frequencies and speeds and under various light conditions, making it a powerful experimental tool for defining the bounds of an animal's vision. The OMR has been previously studied across a range of distinct taxa, including guppies (Anstis et al., 1998), bees (Srinivasan et al., 1996), and mice (Abdeljalil et al., 2005). The response is normally studied as being either present or absent under different conditions; however, it was first quantified in a study using *Daphnia* (Hathaway & Dudycha, unpubl. data).

Daphnia are microcrustaceans (~3mm long) that live in freshwater lakes or ponds. They have long been a valuable model organism, partly due to their ecological importance and ease of study in the lab and field (Rudstam et al 1993). Previous studies on *Daphnia* have thoroughly characterized the structure of its eye (Ringelberg, 1999; Young and Downing, 1976). *Daphnia* have a single, pigmented apposition eye—a type of compound eye in which each rhabdom receives information from just one optical unit as opposed to multiple optical units in a superposition eye (Young and Downing, 1976; Cronin et al., 2014). *Daphnia* have only 22 of these optical units, known as ommatidia, and they are widely spaced out on the eye (Young and Downing, 1976). This is a very low number compared to other animals, such as dragonflies with 60,000 ommatidia, and so it is assumed that *Daphnia* have relatively coarse resolving capabilities (Cronin et al. 2014; Young and Downing, 1976; Frost, 1975).

However, recent OMR tests on *Daphnia pulex* revealed significant behavioral responses at a number of speeds and spatial frequencies (Hathaway & Dudycha, unpubl. data). This is the first evidence that *Daphnia* exhibit an OMR and so there are now novel opportunities for structural variation in the *Daphnia* eye to be studied in relation to a quantifiable visual behavior. Variation in *Daphnia* ommatidia, in particular, is likely to be connected with visual capabilities since they are associated with both fundamental aspects of vision: spatial resolution and sensitivity (Ringleberg, 1999; Rutowski, 2003;

Cronin et al. 2014). Spatial resolution is the ability for an animal's eye to distinguish details or patterns in its visual field (Land and Nilsson, 2012; Rutowski, 2003). It is a function of how densely the visual field is sampled and therefore tends to increase with ommatidia number (Rutowski, 2003; Cronin et al. 2014). Sensitivity, on the other hand, is a measure of how many photons are captured by each receptor (Rutowski, 2003). Sensitivity tends to increase with larger ommatidia diameter (Cronin et al. 2014).

There is an inherent tradeoff for any apposition eye between larger ommatidia with greater sensitivity and packing in a larger number of ommatidia for better spatial resolution (Rutowski, 2003; Cronin et al. 2014). Yet, it has been reasoned that poorly resolving systems with broad acceptance angles—a factor that increasingly limits resolution as ommatidia diameter increases (Cronin et al., 2014; Land and Nilsson, 2012)—may still be well suited for detecting motion because increased sensitivity allows for improved responses to changes in light intensity (Land and Nilsson, 2012). Larger ommatidia may also directly provide increased resolution due to decreased effects associated with diffraction issues with the lens aperture (Land and Nilsson, 2012). Thus, there are identifiable mechanisms by which *Daphnia* may be able to demonstrate significant motion detection capabilities and show variation in visual capabilities among individuals and species.

In combining this theoretical work with results of visual tests on *Daphnia pulex*, it is apparent that the OMR could be a valuable tool for characterizing the visual capabilities of *Daphnia*. The purpose of this study is to assess the amount of functional visual variation in *Daphnia* as a result of structural variation in the ommatidia. Since OMR performance by an animal is influenced by the spatial resolution and sensitivity of its eyes, an animal's behavior can be affected by a number of structural and environmental factors: the angle between the optical axes of adjacent ommatidia (known as the interommatidial angle), the diameter of the photoreceptors, the diameter of each lens, ambient light levels, and motion of the target (Rutowski, 2003; Land and Nilsson, 1997).

Assuming all *Daphnia* species have the same number of ommatidia and the same interommatidial angle, it is reasonable to predict that larger ommatidia diameter should directly correlate with increased resolution and sensitivity since issues with diffraction are reduced and more light is collected by each ommatidia. Consequently, it is predicted that larger ommatidia will correlate with a quantifiable increase in OMR behavior. This hypothesis is tested for *Daphnia* in both interspecific and intraspecific contexts. The species used in this study occupy a range of morphological sizes that vary greatly among each other, and so it is considered likely that there will be sizeable interspecific differences in OMR performance corresponding with disparities in eye morphology. Intraspecific differences are expected to follow this pattern as well, although the relationship may be less substantial since variation within populations is expected to be smaller than variation across species.

MATERIALS AND METHODS

Daphnia Husbandry

Six *Daphnia* clonal lines were used for this study with one clonal population of each species: *D. pulicaria*, *D. pulex*, *D. obtusa*, *D. dentifera*, *D. ambigua*, and a *D. pulex-pulicaria* hybrid. Populations were raised in 250 mL beakers filled with 200 milliliters of filtered lake water and kept at 20-22°C at all times. *Daphnia* were fed an algae solution every other day.

Experimental Procedure

Daphnia were tested in the OMR apparatus shown in Fig. 1. The machine allows a central 20 cm diameter water tank containing an individual *Daphnia* to remain stationary while a cylinder containing alternating black and white stripes rotates around the tank at a constant speed. The outside cylinder is attached to a DC motor with six different speed settings, ranging from 1.33 rpm to 4.29 rpm. There are also different stripe widths that can be attached to the inside of the rotating cylinder. For this experiment, all trials were conducted using the speed and stripe combination that elicited the most pronounced OMR in *Daphnia pulex*, 40° stripes at a speed of 2.72 rpm (Hathaway and Dudycha, unpubl. data). The angular size of the stripes is measured from the center point of the inner dish.

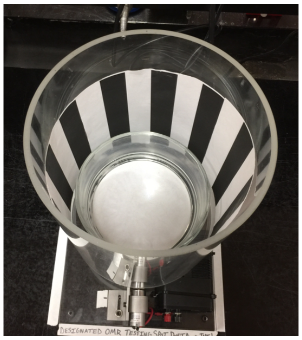

Fig. 1 Optomotor apparatus set up with 40° stripes.

To begin an experimental trial, a single *Daphnia* was placed in the inner dish. The *Daphnia* is allowed two minutes to acclimate to the tank without the cylinder in motion. The motor is then turned on and the response is scored during the third minute. Swimming behavior was observed during the fourth minute, but not scored. During the fifth minute, with the motor still on, the response was once again scored. The aforementioned study on *D. pulex* (Hathaway and Dudycha, unpubl. Data) only scored the fifth minute of the trial, using the third and fourth minutes as a second acclimation period once the motor is turned on; however, because the behavior is a physical reflex in response to motion it was predicted that the behavior may be most strongly exhibited when motion of the surrounding environment first starts. Therefore, both the third and fifth minute of the trial were scored and compared to each other in order to determine whether the intensity of the response degrades over time.

OMR tests usually score responses as either present or absent (Anstis et al., 1998; Abdeljalil et al. 2005). The scoring system used here is based on a scale from 0 to 60, which corresponds to the number of seconds that an individual's swimming behavior can indicate an OMR during the one-minute scoring period. There are two behaviors that count towards this total: optomotor circling and the compass reaction (Anstis et al., 1998). Optomotor circling refers to movement of the entire body in the same direction and speed as the moving stripes. The compass reaction refers to rotation around an individual's vertical axis that matches the speed and direction of the moving stripes. Both behaviors show responses to motion that correspond with environmental motion and are therefore considered as evidence of an OMR (Anstis et al., 1998; Abdeljalil 2005).

These responses cannot occur at the same time so the maximum score for the OMR is 60. A score of 60 indicates a full minute of swimming that matches stripe movement, a full minute of rotation matching the stripes, or a combination of both. Intermediate scores can be the sum of both if the individual switches behavior during the trial. A score of 0 indicates no movement during the observation period or movement in the opposite direction of the stripes, which cannot be considered evidence of an OMR (Anstis et al., 1998).

Control Procedure

The capability for the *Daphnia* to detect the motion of the black and white stripe pattern is lost if the stripes begin to move too fast or if the spatial frequency of the stripes is too high to be noticeable, a limitation known as maximum spatial frequency (Land and Nilsson 2012; Rutowski 2003). Once the maximum spatial resolution of an organism's eye has been surpassed, the black and white stripes will blur together and show no discernable motion. The same is true for if the stripes move too fast for the animal to detect the pattern. Therefore, controls for the experiment are conducted using the OMR apparatus fitted with a solid gray background. The control condition allows for swimming behavior to be scored in a "stationary" environment to grasp how much of an individual's swimming behavior naturally mimics the OMR in the experimental apparatus. It is also used to account for *Daphnia* responses to mechanical cues associated with the operation of the OMR machine. Individuals in the control group were scored, photographed, and measured using the same procedure as the other *Daphnia* in the study.

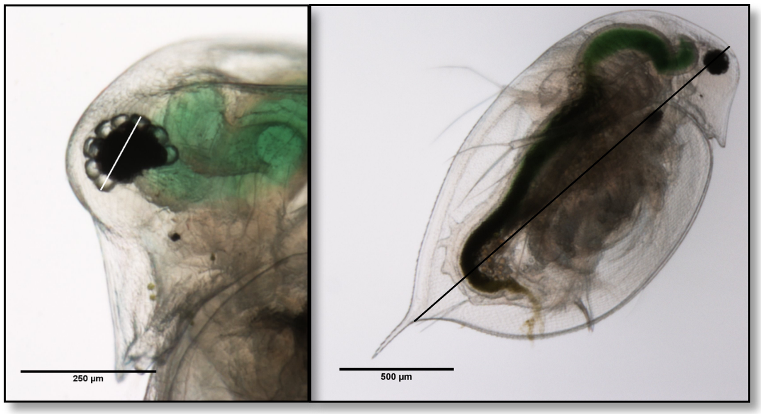

Fig. 2 Example photographs used for measurements in this study (on left: *D. pulicaria*; on right: a *D. pulicaria-pulex* hybrid). The white line represents eye diameter measurement and the black line represents body length measurement.

Morphological Measurements

Daphnia were sacrificed in 0.25 M KCl soon after being tested in the OMR arena. Individuals were photographed and measured using the same procedure described in Brandon and Dudycha (2014), with minor modifications. Due to the variation in body sizes of the species used, body pictures were taken at magnifications ranging from 20x to 40x. All eye and ommatidia pictures were still taken at 112.5x magnification in accordance with Brandon and Dudycha (2014). Body diameter, eye diameter, and ommatidia diameter were then all measured in ImageJ (Schneider et al. 2012).

Statistical Analysis

Preliminary data analysis was conducted on scores from the repeated measures (minute 3 and minute 5) so that it could be determined which score, or if a combination of both, would be used for further comparisons. Mean scores for both measures within populations were all tested separately using a series of paired t tests.

Two other aspects of the results that are important to initially determine are if all of the *Daphnia* species exhibited an OMR and whether species showed any significant differences in their OMR behavior. To test the significance of OMR behavior, a series of t-tests were used to determine if there were significant differences between scores from the experimental group of each species and its corresponding control group. A one-way ANOVA was then used to test if the experimental means of all species were significantly different. Post-hoc analysis of the ANOVA was then conducted using a Tukey HSD test to identify which specific species means were disparate.

The main objective of the study was to determine if increased ommatidia size influenced OMR score across species and within populations. Across species, mean ommatidia diameter for each species was plotted against the corresponding mean OMR score for the species. Least squares regression was then used to analyze this relationship and the correlation was also tested for significance. The correlation between ommatidia diameter and OMR score within each population was then also evaluated and tested for significance in order to see if ommatidia size within populations is predictive of OMR performance. All data analysis was performed in R (R Core Team 2013)

RESULTS

Repeated OMR Measures

The composite OMR score for an individual is the sum of its circling behavior and compass reaction. Repeated composite measures from minute three and minute five were analyzed for each species separately. The only population that showed significant variation in behavior between time points was *D. pulex* (p= .000108). *D. pulex* showed a stronger average response during the 3rd minute. However, the difference was not consistently demonstrated among individuals and there was higher variance in composite 1 scores (scores from minute three). Select individuals with remarkably high third minute scores mostly drove the difference in averages. Given that all other populations exhibited consistent behavior in both scored minutes, and that there is no clear reason to choose either of the *D. pulex* composite scores over the other, it was considered reasonable to average composite scores for each species together as a standard score for further analysis.

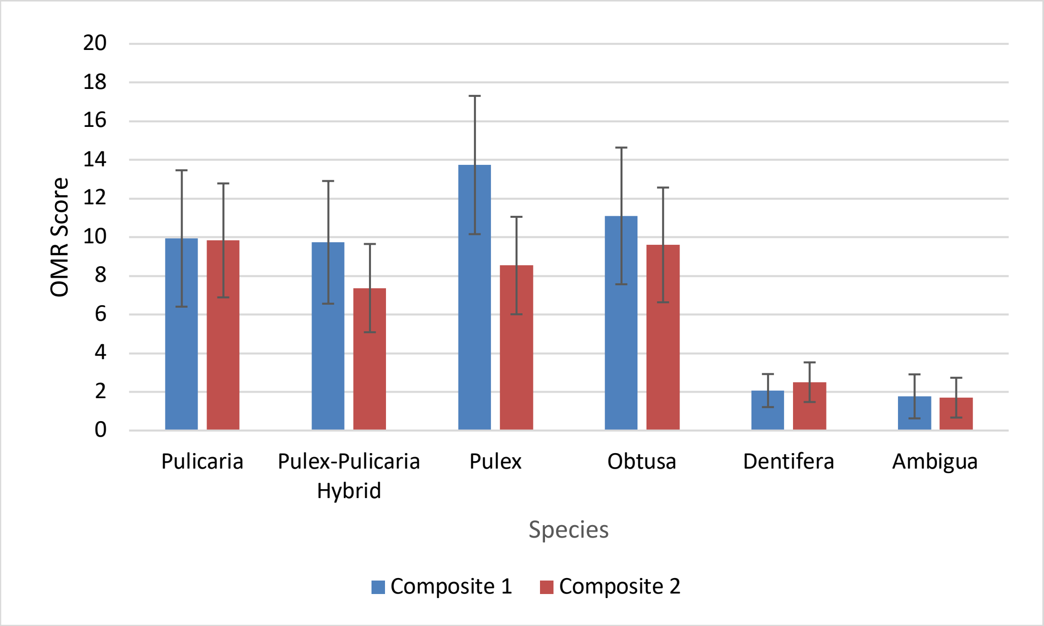

Fig. 3 Means of repeated measures of OMR performance for each population, shown with 95% confidence intervals. Paired t-tests for scores within populations reveals that the only species that exhibits significant difference between minutes is *D. pulex* (*p*= 0.000108), while repeated measures in other species are all significantly consistent (Max *p*= 0.9586 in *D. pulicaria*, Min *p*= 0.3323 in *D. dentifera*).

Species Responses to Motion

All *Daphnia* species, with the exception of *D. dentifera*, demonstrated significantly more OMR behavior in experimental conditions than control conditions (Fig. 3). This was true even for *D. ambigua*, which scored very low in both treatments. The result for an analysis of variance of experimental species means was highly significant ($p = 2.9 \times 10^{-14}$), indicating that although most species exhibited an OMR, differences existed in OMR behavior across species. *Post hoc* analysis (Table 1) identified that while *D. pulicaria*, *D. pulex*, *D. obtusa*, and the *D. pulex-pulicaria* hybrid did not exhibit significantly different OMR scores compared to each other, they did significantly outperform *D. dentifera* and *D. ambigua*. The analysis also determined that the difference in OMR behavior between *dentifera* and *ambigua* was not significant.

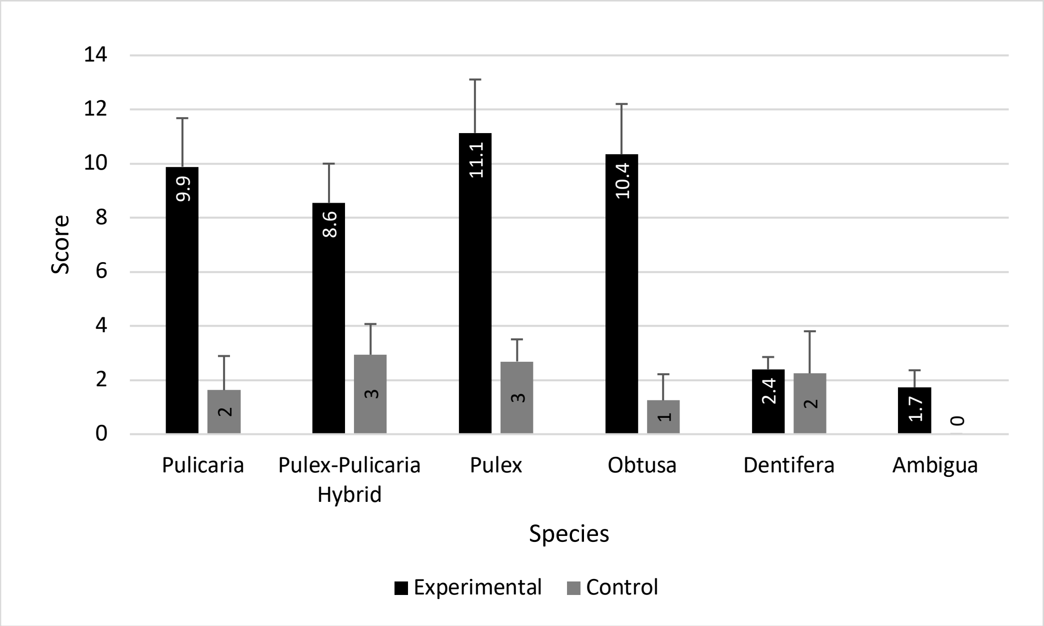

Fig. 4 Comparisons of OMR performance in striped (40°) and uniform gray environments, shown with 95% upper confidence interval (*n*= 30 for all experimental groups; *n*= 8 for all control groups). All experimental treatments were significantly different than controls (*p*< .05) except for in *D. dentifera*, which exhibited no significant difference in behavior between treatments (*p*= 0.9048). Difference in OMR performance among species was then tested using a one-way ANOVA, which revealed a high degree of significant difference (*p*= 2.9 x 10^{-14}).

Table 1 Results of Tukey HSD comparison for experimental means
The analysis identifies which specific species exhibited significantly different mean OMR scores from each other. The results identify two clusters of species which show significantly similar means to each other but significantly disparate means from species in the other group. Note: Significant differences (*p*< .05) between means are noted in bold

Species	Mean Diff	Lower Bound	Upper Bound	Adjusted P
Dentifera-Ambigua	0.65	-3.247986	4.547986	0.996783861
Hybrid-Ambigua	6.8166667	2.918681	10.714653	**0.000017074**
Obtusa-Ambigua	7.4833333	3.585347	11.381319	**0.000001697**
Pulex-Ambigua	9.4	5.502014	13.297986	**0.000000001**
Pulicaria-Ambigua	8.15	4.252014	12.047986	**0.000000146**
Hybrid-Dentifera	6.1666667	2.268681	10.064653	**0.000139301**
Obtusa-Dentifera	6.8333333	2.935347	10.731319	**0.000016147**
Pulex-Dentifera	8.75	4.852014	12.647986	**0.000000014**
Pulicaria-Dentifera	7.5	3.602014	11.397986	**0.000001599**
Obtusa-Hybrid	0.6666667	-3.231319	4.564653	0.996374316
Pulex-Hybrid	2.5833333	-1.314653	6.481319	0.399655774
Pulicaria-Hybrid	1.3333333	-2.564653	5.231319	0.921984189
Pulex-Obtusa	1.9166667	-1.981319	5.814653	0.716715358
Pulicaria-Obtusa	0.6666667	-3.231319	4.564653	0.996374316
Pulicaria-Pulex	-1.25	-5.147986	2.647986	0.939856987

Morphological Variation and OMR Variation

Although this study is focused on the ommatidia in particular, the body diameter and eye diameter of tested individuals were plotted (Fig. 5) in order to show variation in related morphological traits. Clusters of population ranges for both traits can be recognized, as well as areas of overlap among species. The cluster of *D. ambigua* individuals is notable due to its isolation from other species as well as its relatively low amount of variation in comparison to other populations.

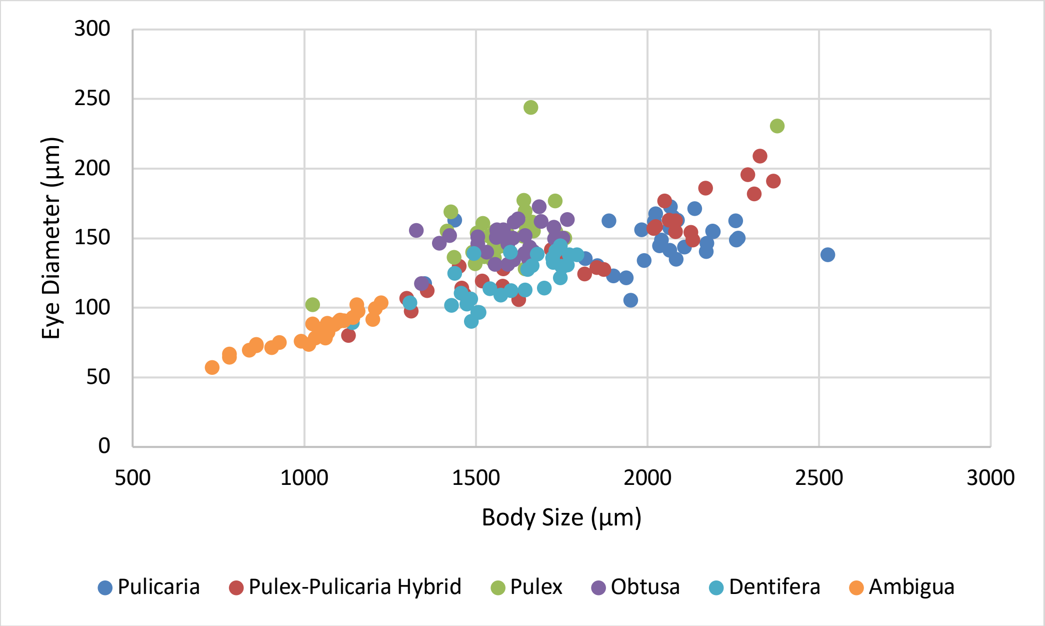

Fig. 5 *Daphnia* body size in relation to eye diameter across species. Ordinary least squares regression reveals a positive relationship ($R^2 = 0.5896$, $p = 2.2 \times 10^{-6}$).

Across species, ommatidia diameter proved to be a marginally significant predictor of OMR score ($p = 0.05758$). Analysis of the average ommatidia size for each species in relation to the population's average OMR score showed a strong positive relationship (Fig. 6; $R^2 = 0.6404$). However, no such pattern existed within clonal populations (Fig. 7). Intraspecific correlations were all very low and were determined not to be significant.

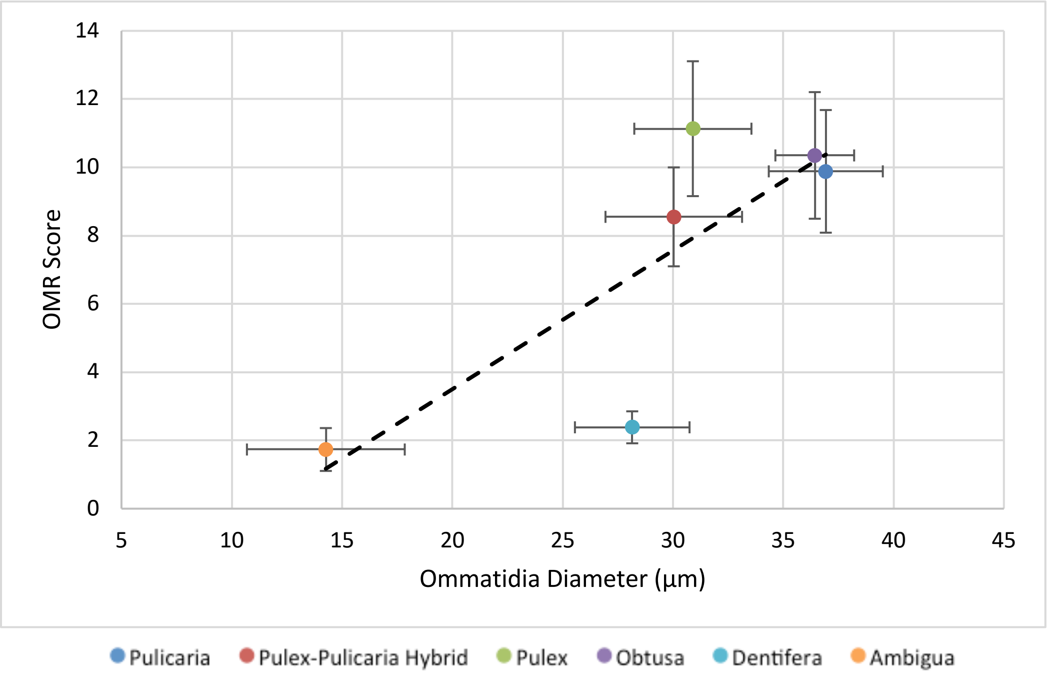

Fig. 6 Mean ommatidia diameter for each species in relation to OMR performance shown with 95% confidence intervals for both measures. Ordinary least squares regression reveals a moderately strong, positive relationship. The correlation is just above the normal threshold for significance but can be considered marginally significant ($R^2 = 0.6404$, $p= 0.05758$).

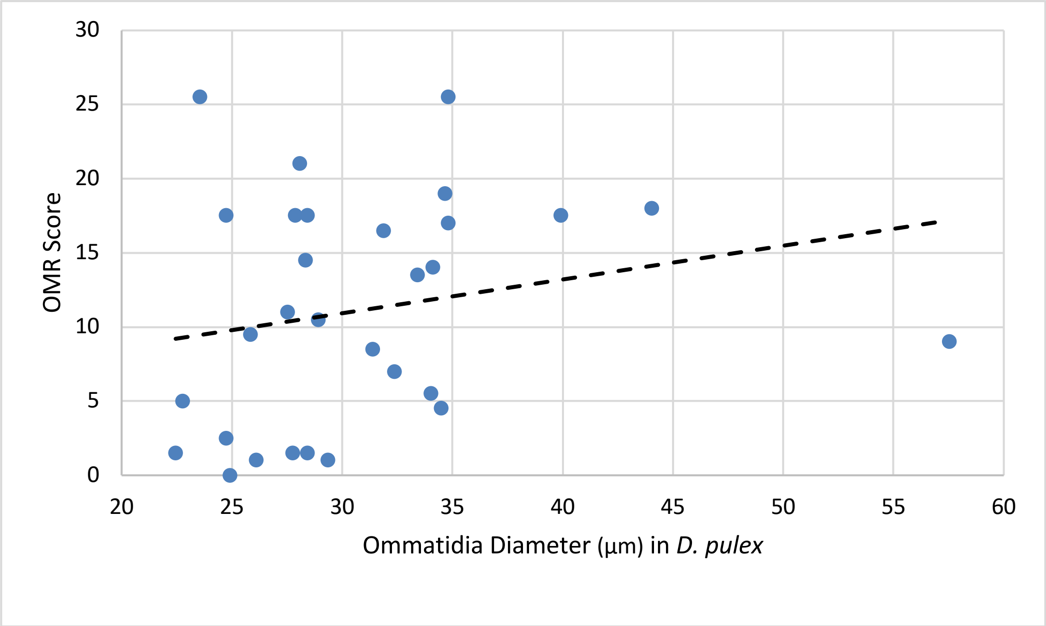

Fig. 7 Ommatidia diameter in relation to OMR performance for *D. pulex* individuals. Ordinary least squares regression shows no significant relationship in *pulex* or in any other species (**(**$p= 0.2614$, $R^2 = 0.04482$; Max $R^2= 0.0585$ in *D. obtusa*, Min $R^2= 0.0022$ in *D. pulicaria*).

DISCUSSION

The results of this study provide evidence of an OMR in multiple species of *Daphnia*; even in *D. ambigua*, which was morphologically distinct in its small size compared to the other species that significantly demonstrated OMR behavior (Fig. 4; Fig.5). It can be concluded from the ANOVA (p= $2.9 \, x \, 10^{-14}$) that OMR behavior does vary significantly among species, with the apparent pattern being that the smaller species (*D. dentifera* and *D. ambigua*) display significantly less OMR behavior than larger species (Fig. 4; Table 1). Given the relationship between increasing ommatidia diameter in each species and higher OMR scores (Fig. 6), it seems probable that these differences are a direct result of disparities in ommatidia diameter since both sensitivity and resolution are theoretically enhanced by larger ommatidia.

Yet whether disparity in ommatidia diameter is what actually separates visual capabilities among *Daphnia* species is still unclear. Patterns of OMR behavior in relation to ommatidia size within clonal populations (Fig. 7; see appendix) provide evidence that complicates the assumed effect ommatidia diameter has on visual function and OMR. It was predicted that the relationships within populations would not be as strong as the overall relationship across species, but the fact that there is convincing evidence of no correlation between visual morphology and OMR performance within each population creates an inconsistency in the results that may require additional information. Moreover, despite a large amount of morphological difference between certain species, ranges of variation within populations were large and most populations displayed morphological ranges that overlap with individuals of other species that exhibited significantly different scores (Fig. 7; see corresponding graphs for other clonal populations in appendix).

Due to the fact that individual *Daphnia* in different clonal populations show different behavior despite having practically identical eye sizes, the other explanatory variable in these results may therefore be species identity. Species identity itself would not be the actual mechanism driving differences, but rather it could be a result of variation in the environmental factors that characterize the different habitats *Daphnia* occupy (Holt, 1987). *Daphnia* species and intraspecific populations inhabit a wide range of habitats that can vary in terms of light availability, resource density, and predator presence (Brandon and Dudycha, 2014; Ebert, 2005). These factors provide different patterns of selection on the *Daphnia* visual system that are ultimately represented by variation in their visual morphology (Brandon et al. 2015; Holt, 1987).

Since *Daphnia* are often a predominant food source for planktivorous fish, species distributions and habitat ranges are closely linked with the presence of predators (Ebert, 2005). Larger, more detectable species, such as *D. pulicaria* and *D. pulex*, cannot survive intense predation pressure; however, in water bodies without predators they are able to outcompete smaller *Daphnia* species (Ebert, 2005). Consequently, smaller species such as *D. ambigua* and *D. dentifera* are usually only found in habitats with significant predator presence (Tessier et al. 2001; Tessier and Welser, 1991). Different size *Daphnia* species often cohabit the same lake using stratified layers of water to separate populations and decrease competition (Leibold and Tessier, 1991). Smaller *Daphnia* spend more time exploiting the high-light and nutrient rich (and predator dense) surface waters of lakes and ponds; lager *Daphnia* spend most of their time in deeper, darker waters and use diel vertical migration and shore flight to avoid encounters with predators (Tessier et al. 2001; Leibold and Tessier, 1991; Ringelberg, 1999).

In general, the *Daphnia* eye seems to show a structure that favors sensitivity over resolution, a pattern that is normal for animals in aquatic habitats such as ponds where dissolved particles can significantly absorb and scatter incoming light (Cronin et al. 2014). For larger *Daphnia* that reside in deeper, murkier waters, this loss of available light may be especially important for determining their ability to fully use their vision. The exact mechanisms associated with species identity proposed here are therefore adaptations for sensitivity in lager *Daphnia* species as a result of size-dependent differences in habitat selection and average light availability. In experimental tests that keep light availability constant, larger species with distinct adaptations for sensitivity may show superior visual capabilities despite similar eye sizes.

Sensitivity adaptations can manifest as either temporary or permanent changes in optical anatomy. Temporary adaptations for sensitivity include changes in aperture diameter, temporal summation, or spatial summation (Land and Nilsson, 2012), all of which were not examined in this study. Increasing ommatidia diameter in tandem with increasing eye size or decreasing the number of ommatidia in order to increase ommatidia diameter without a corresponding increase in eye size are both permanent adaptations for sensitivity. The former permanent adaptation is tested in this study using the measurements of ommatidia diameter but other adaptations may also be explanatory.

In light of these possibilities, it is probable that ommatidia diameter itself does not give a complete enough picture of a *Daphnia*'s visual morphology and how its vision is explicitly limited under different conditions. Rather, ommatidia diameter determines the upper limits to certain sensitivity adaptations, such as temporary aperture size changes. As a result of experiencing more restrictive and fluctuating environmental limits on vision, larger *Daphnia* may show more flexibility in their visual morphology that allows them to use larger ommatidia more effectively and under a larger range of light conditions. This may explain why improved OMR scores are dependent on a combination of increased ommatidia diameter and species identity, and why species that inhabit less visually restrictive habitats, such as *D. dentifera* and *D. ambigua,* demonstrate notably different ommatidia sizes but not significantly different visual performance (Table 1; Fig.6). Ommatidia size ranges within clonal populations may therefore not be biologically relevant enough by itself to predict OMR performance.

There may be yet another mechanism associated with species identity that may impact species results but diminish differences among individuals of the same clonal population. It is worth mentioning here that the strictness of the criteria for scoring the OMR was one of the main factors that affected OMR scores in *D. dentifera*. Their swimming under experimental conditions was generally chaotic, characterized by rapid swimming in small circles of alternating directions. The more composed, direct swimming that *dentifera* individuals showed during controls suggests that their erratic swimming behavior may be an alternative response to detecting motion in their environment. Even so, it does not correspond to the motion and speed of the stripes and cannot be quantified using OMR criteria. As a result, it is left unaccounted for in the data.

Mechanisms associated with additional visual adaptations in certain species assume that OMR behavior directly corresponds to visual ability; however, it may be possible that each species of *Daphnia* is able to see the motion of the stripes relatively clearly but every species does not process and respond to that visual information in the same way. A limitation of using the OMR to try and quantify visual capabilities is that it is being used as a proxy for actual visual ability. The experiment can measure one specific visually mediated response, but there is still a gap that exits in using that response to determine if the visual system is capable of resolving the moving pattern.

Since larger *Daphnia* avoid predators through migratory behaviors that do not require the complexity that their eyes possess (Ringelberg, 1999), it seems likely that their vision is used to accomplish more involved tasks, such as identifying nutrient rich feeding patches in the dim light of deeper waters and nocturnal surface waters. It is also reasonable to predict that the most important visual function for smaller *Daphnia* is predator detection since they spend more time exposed to roaming predators. Generally speaking, *Daphnia* with larger body sizes may therefore show a more positive response to motion than smaller *Daphnia*, which may exhibit swimming that is indicative of trying to physically evade, or avoid detection by, a looming presence in its visual field (O'Keefe et al. 1998; Liaw and Arbib, 1993).

The extent to which these generalizations hold true may vary depending on how much *Daphnia* populations use other sensory information, such as fish kairomone detection, which has been shown to mediate predator avoidance and predator–induced defenses (Winder et al. 2004). However, it is possible that these innate differences in responses to motion may more directly explain overall differences in OMR among species and why two different species could exhibit vastly different behavior with eyes of the same size. The important caveat to this conclusion is whether or not the OMR stimulus is essentially neutral or if the stripes may convey some kind of ecologically relevant information beyond simply a moving environment given the specific spatial frequency used.

CONCLUSION

This project provides varying levels of insight into each of the questions that guided it. It can be confidently concluded that the OMR is present in multiple *Daphnia* species and that there is significant variation in the behavior among species. Variation of the behavior is also notably clustered, with species of similar sizes not showing significant differences between each other. The extent to which interspecific differences in behavior are explained by morphological mechanisms is still not conclusively understood. It could be that the relationship is more complicated than expected, and that it involves morphological factors and temporary visual changes that were not measured in this study. It is also considered probable that interspecific differences may have explanatory components associated with species identity and that, although these differences may be associated with ommatidia size, the influence of ommatidia size disparity alone is not biologically relevant within clonal populations.

The results of this experiment also identify a possible limitation of quantifying the OMR to assess visual variation among species. They do not, however, discount the value of using the OMR for other species-specific experiments or even for comparative assessments since there are still structural and environmental factors that require further investigation. Moving forward, there are a few directions that could prove immediately fruitful for understanding *Daphnia* vision and the OMR as a tool.

The cluster of larger sized *Daphnia* (*D. pulex, D. pulicaria, D. obtusa* and the *D. pulex-pulicaria* hybrid) that did not show significantly different results did not likely have the limits of their resolution or sensitivity tested using 40° stripes and ambient light levels. It would be interesting to not only keep testing these species and establish the limits of their vision but to keep doing so comparatively to see if easily measured morphological variation proves important in more demanding experimental conditions for each species.

The OMR responses of *D. dentifera* and *D. ambigua* both warrant further testing under different conditions. Attempting to find a different way to quantify the swimming behavior of *D. dentifera* under the same experimental conditions used here could be useful. Comparing that to swimming behavior across multiple spatial frequencies and speeds could be even more insightful. Although *D. ambigua* did show significant difference in OMR swimming between experimental and control conditions, this difference was still very slight. Testing *D. ambigua* at slower speeds could be important since speed seemed to be the more limiting criteria for *D. ambigua* individuals. For both populations, it would also be worth testing them using the same experimental combination used here but providing them with more light since ambient light levels may also be more limiting than expected compared to their usual environment.

Finally, studies more explicitly focused on larger-scale variation in visual morphology could prove valuable for the species used here, either by itself or as part of a behavioral experiment. In the literature surveyed here, only *D. pulex* and *D. pulicaria* have published ommatidia counts. Ommatidia counts for other species and some kind of assessment of ommatidia distribution in each species would be very useful in understanding any kind of comparative visual differences.

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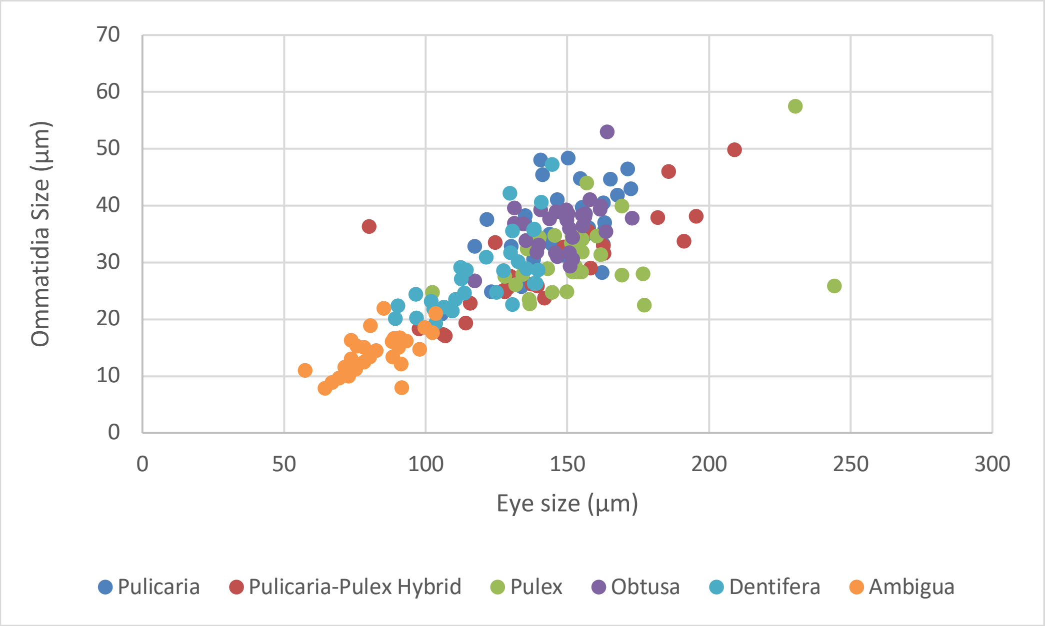
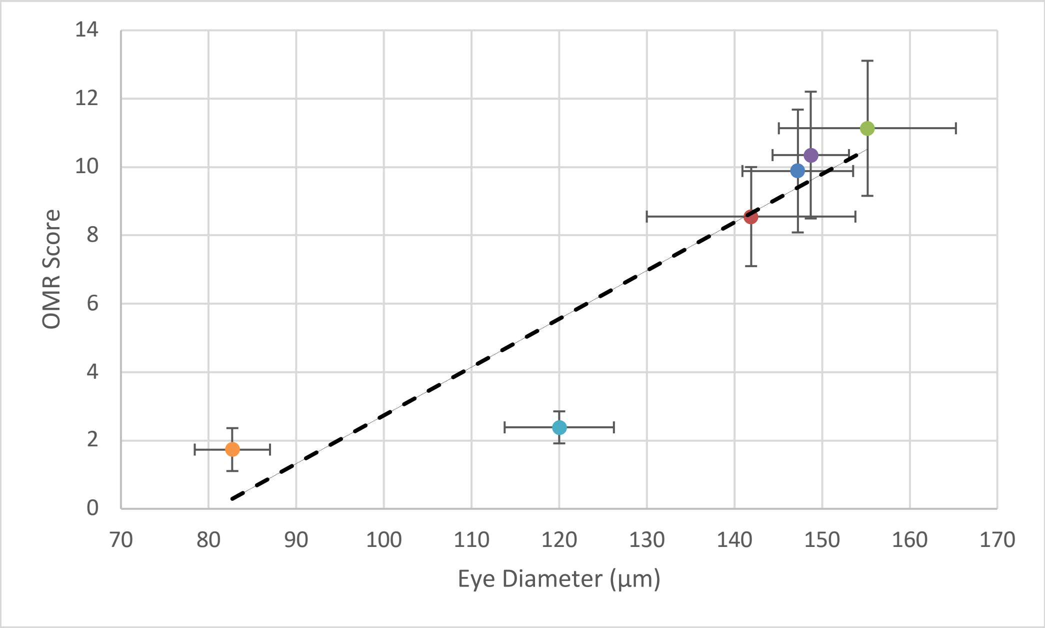
APPENDIX: ADDITIONAL GRAPHS

Eye Size-Ommatidia Relationship

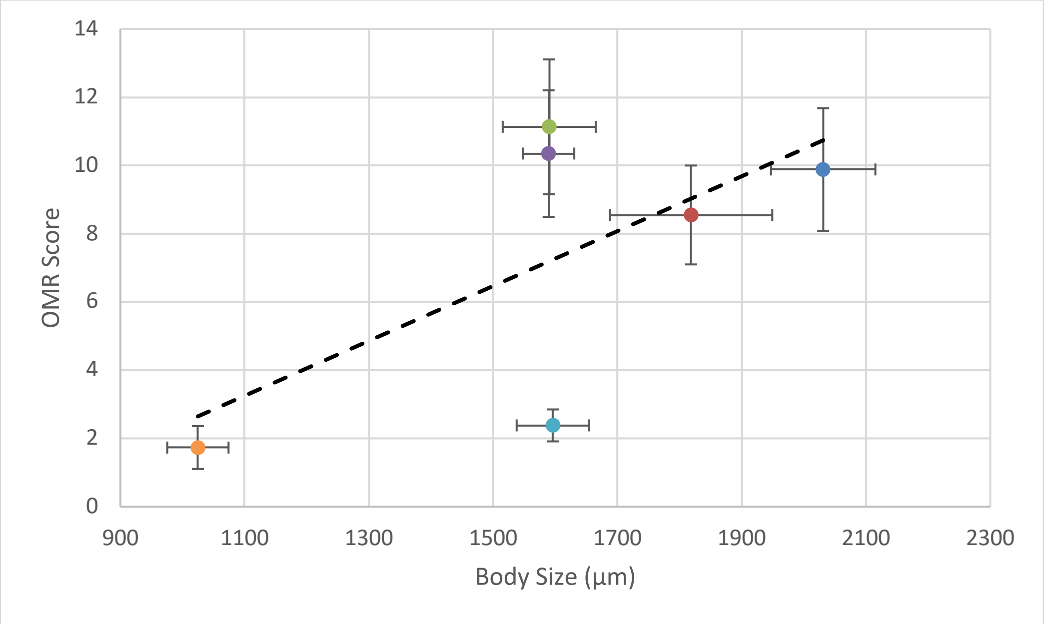


p= 2.2 x 10^{-16}, R² = 0.6134

Morphology-OMR relationships across species

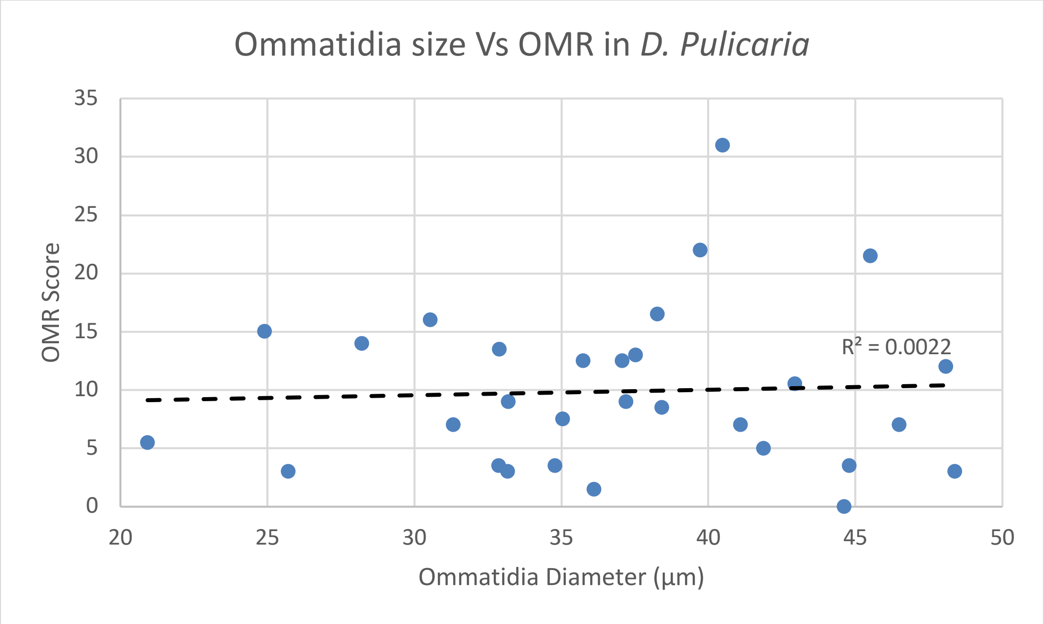


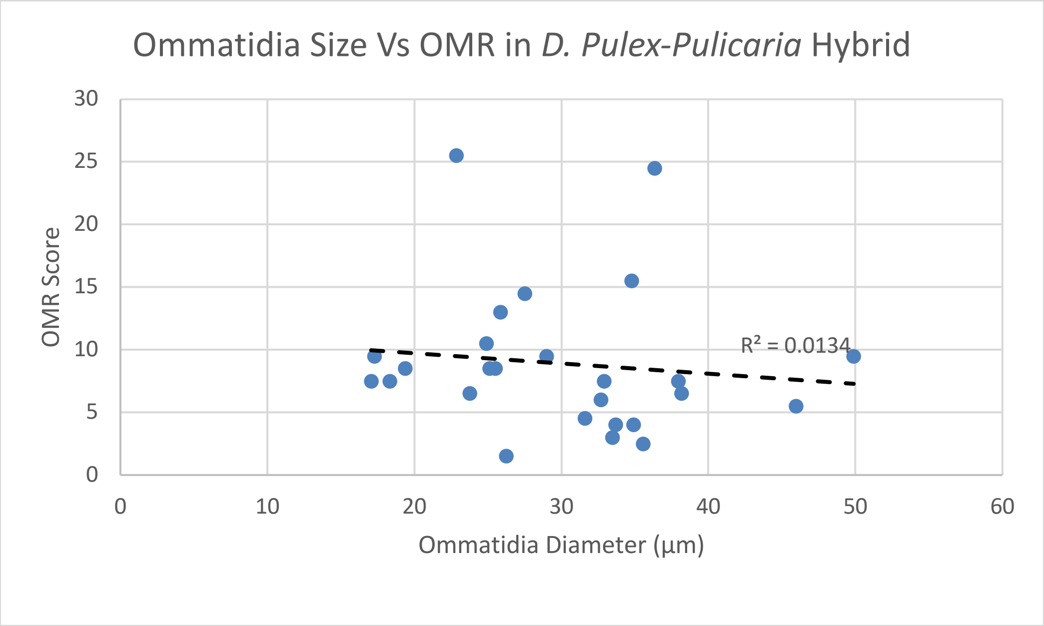
p= 0.009201, R² = 0.84755

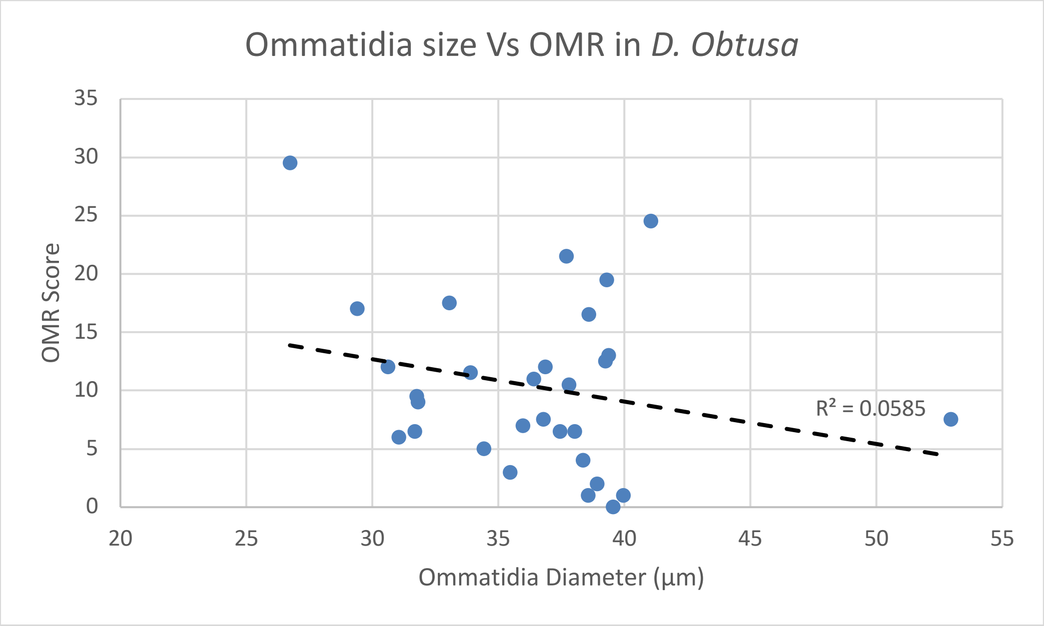


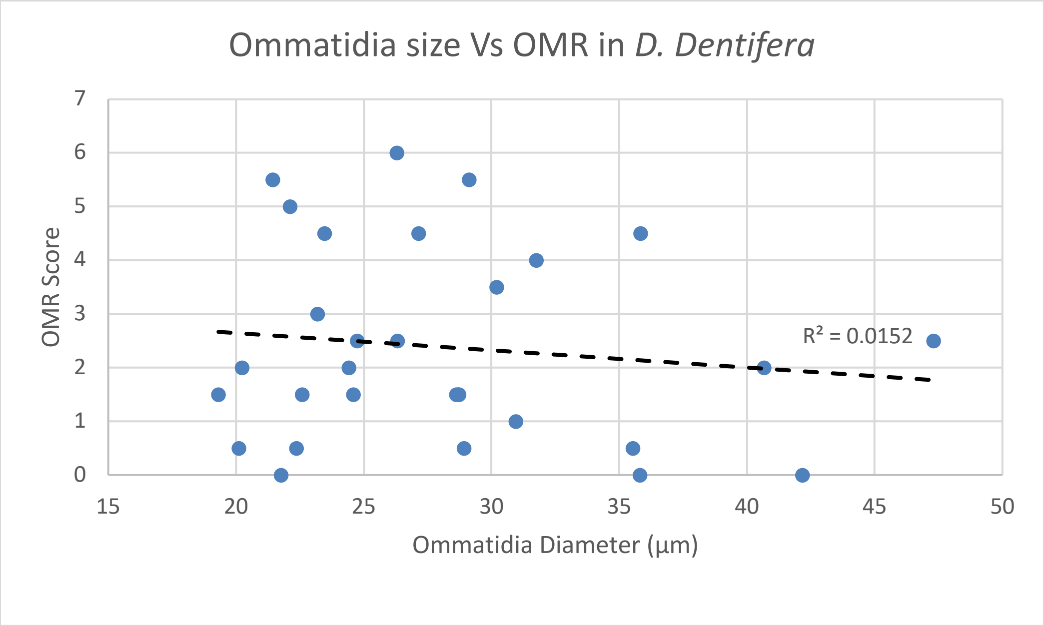
$p = 0.1658$, $R^2 = 0.41737$

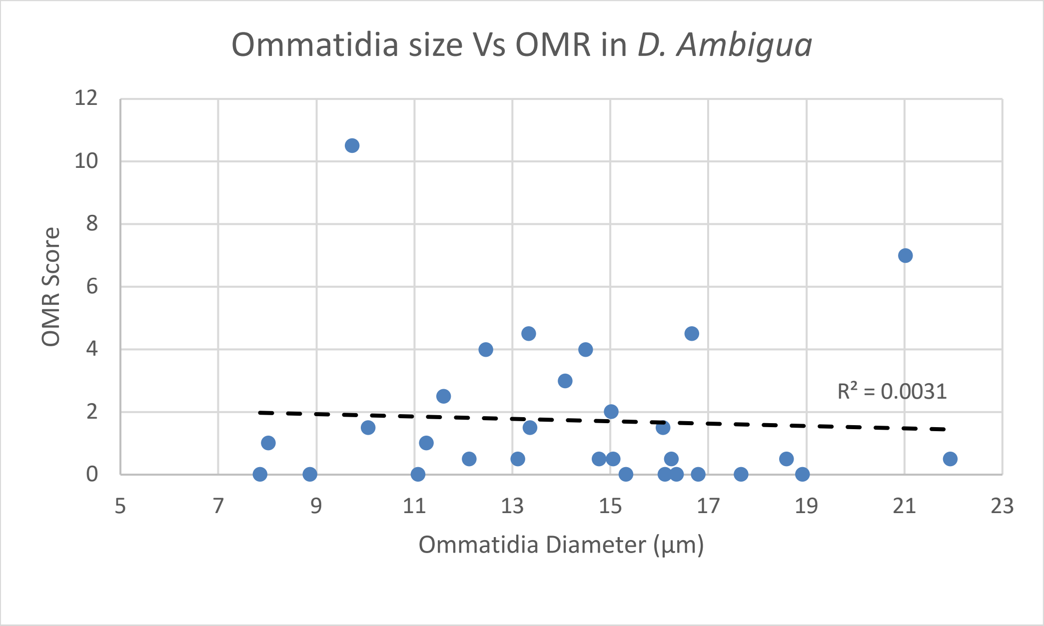
Ommatidia size-OMR relationships within clonal populations




Ommatidia Size Vs OMR in *D. Pulex-Pulicaria* Hybrid
OMR Score
0
5
10
15
20
25
30
0
10
20
30
40
50
60
Ommatidia Diameter (µm)
R² = 0.0134


Ommatidia size Vs OMR in *D. Obtusa*
OMR Score
0
5
10
15
20
25
30
35
20
25
30
35
40
45
50
55
Ommatidia Diameter (µm)
R² = 0.0585


Ommatidia size Vs OMR in *D. Dentifera*
OMR Score
0
1
2
3
4
5
6
7
15
20
25
30
35
40
45
50
Ommatidia Diameter (µm)
R² = 0.0152


Ommatidia size Vs OMR in *D. Ambigua*
OMR Score
0
2
4
6
8
10
12
5
7
9
11
13
15
17
19
21
23
Ommatidia Diameter (µm)
R² = 0.0031

Other morphology-OMR relationships in *pulex*