MELANOPHORE RESPONSE AND BEHAVIOR OF THE MARINE ISOPOD SPHAEROMA QUADRIDENTATUM IN THE PRESENCE OF A COMMON ESTUARINE PREDATOR SPECIES

An Undergraduate Research Scholars Thesis

by

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Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

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This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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Melanophore Response and Behavior of the Marine Isopod Sphaeroma Quadridentatum in the Presence of a Common Estuarine Predator Species

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Sphaeroma quadridentatum (Say 1818) is a coastal species of marine isopod common in the US East and Gulf coasts that can be found in the TAMUG Small Boat Basin. *S. quadridentatum* has been shown to employ a melanophore response mechanism which changes the isopod's coloration in response to changes in light intensity, circadian rhythm, and substrate coloration. The aim of this study is to examine and observe the presence of a melanophore response system in *S. quadridentatum* as a response mechanism in the presence of *Callinectes sapidus* (Rathbun 1896) and to explore new alternative ways by which to quantify melanophore dispersal across a range of metazoan phyla. In similarity to many aquatic animals, *S. quadridentatum* employ a melanophore dispersal mechanism as a method for color change. Previous research examining the melanophore response in these isopods has found that the color change mechanism can be triggered in response to a variety of physiochemical cues, such as changes in salinity and lighting. For example, *S. quadridentatum* melanophore dispersal changes with the coloration of the substrate the isopod is placed on. These changes in coloration may

facilitate camouflage which, in turn, decrease predation pressure and facilitates survival. In addition, other isopod species have demonstrated behavioral changes in response to predatory olfactory cues. I hypothesize that the melanophore response of *S. quadridentatum* is affected by the presence of olfactory cues from a predator. *Callinectes sapidus* (Rathbun 1896) was chosen as the predator due to its frequency in estuarine habitats and the opportunistic nature of its foraging behavior. To test for the presence of a melanophore response mechanism to predatory cues, isopods were collected and cultured in the laboratory. Isopods were exposed to four treatments: 1) exposure to predatory cues on white substrate, 2) exposure to predatory cues on black substrate, 3) control without predatory cues on white substrate, and 4) control without predatory cues on black substrate. Isopods were photographed before and after exposure to treatments. Mean melanophore dispersal was quantified and variance analyzed. Melanophore dispersal response was variable across experimental and control isopods on both white and black substrates. Total mean melanophore data before and after treatment varied insignificantly but may suggest the presence of a melanophore response to hydrodynamic changes. Analysis of variance demonstrated that variations in substrate coloration are significant predictors of melanophore response in isopods relative to predatory olfactory cues. This represents a possible continuous and adaptable melanophore response adaptation which may enhance predator avoidance. Further research could focus in investigating the potential of a melanophore response to hydrodynamic cues and behavior modifications as a predator avoidance strategy.

DEDICATION

To my friends, family, instructors, and peers who supported me throughout the research process.

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NOMENCLATURE

TAMUG	Texas A&M University at Galveston
ANOVA	Analysis of Variance

1. INTRODUCTION

1.1 Background Information

Sphaeroma quadridentatum is a species of marine isopod which primarily inhabits shallow water habitats of the Eastern and Gulf coasts of the United States. The species has been observed in the Mississippi and Texas Gulf coasts where individuals usually inhabit a variety of solid substrates in intertidal zones (Clark 1978). *S. quadridentatum* tends to associate with oysters and barnacles in such natural or artificial solid substrate habitats (Leboeuf and Howe 1981). Moreover, *S. quadridentatum* is particularly abundant in the northern Texas coast where the isopod species is adapted to the highly dynamic nature of regional intertidal waters. In the solid substrate habitats, the isopod species can be differentiated based on the absence of dorsal tubercles and the presence of four indentations found in the exopods (Clark 1978). Although little is known about the feeding ecology of *S. quadridentatum*, it is likely that nutrition is dependent upon microphagous filter feeding and consumption of detritus as they traverse substrates (Si et al. 2002). Likely developed as a predator avoidance adaptation to camouflage in its substrate range of color, individual *S. quadridentatum* isopods demonstrate differences in color intensity and patterning. Such variation is consistent with the range of color of detritus and substrate in structures inhabited by S. *quadridentatum* (Leboeuf and Howe 1981).

Previous research done to study color change mechanisms employed by *S. quadridentatum* has highlighted the presence of active color change responses in sample isopods. Direct observations of the species suggest that melanophores are used as the primary method of color change across individual isopods, where the most abundant concentrations seem to be located across dorsal somites and the dorsal surface of the pleotelson, Previous studies have

determined that mechanisms of dispersal and aggregation control the melanophore response system in *S. quadridentatum* (Leboeuf and Howe 1981). It is likely that the underlying melanophore response system of the isopod species is controlled via the release of a chromatophotropic hormone regulated by a neuromodulator, similar to other crustacean species (Fingerman 1985). Such observations have demonstrated that increases in melanophore dispersal result in the expression of darker colors. The melanophore mechanism in *S. quadridentatum* is activated as a response to many physiochemical factors, such as incident lighting and substrate coloration. As isopods traverse the substrate, melanophore dispersal is induced as a response to darker substrate colors while melanophore aggregation is induced as a response to lighter substrate color (Leboeuf and Howe 1981). The response mechanism results in the expression of darker colors in the presence of darker incident substrate color, allowing *S. quadridentatum* to adjust its coloration to a variety of substrates. Other isopods demonstrate similar coloration responses to changes in incident light intensity (Leboeuf and Howe 1981). Therefore, the response mechanism is likely used as a predator avoidance strategy, as adjusting coloration enables individual isopods to conceal themselves in the surrounding environment. As *S. quadridentatum* demonstrates color change responses to various physiochemical cues and other isopod species demonstrate changes in behavior as a response to chemical cues from predators, it remains to be investigated whether melanophores from *S. quadridentatum* respond to chemical cues from a predator (Leboeuf and Howe 1981; Hegarty and Kight 2014).

1.2 Objective

The following study was performed to 1) examine the role predatory cues may play in regulating melanophore response in *S. quadridentatum* and 2) explore alternative, new ways by which to quantify melanophore dispersal across a range of metazoan phyla. The existence of a

melanophore dispersal mechanism in response to predatory olfactory cues can be inferred from previous studies outlining melanophore response mechanisms in isopods to other physiochemical cues (Leboeuf and Howe 1981). The prospect of a melanophore response mechanism was considered especially likely considering the beneficial influence camouflage could have on individual survival (Hultgren and Mittelstaedt 2015). To examine the validity of this hypothesis, microscope imagery was used to observe and record melanophore behavior in *S. quadridentatum* before and after specimens were exposed to chemical predatory cues. *Callinectes sapidus* (Rathbun, 1896) was chosen as the subject predator due to its abundance in estuarine habitats across the Gulf Coast and the observed opportunistic nature of its foraging behavior (Mansour 1992).

The other focus of the present study was to employ new methodology in assessing melanophore dispersal and behavior to better quantify and analyze visual data. Color change measurements in aquatic animals have previously been made using various methods. Methodologies in the past have employed the use of bioassays, reflectometry, and a visual approximation index to denote melanophore dispersal according to previously established scale systems (Logan et al. 2006). One such scale system, the melanophore index, was employed to study color change in *S. quadridentatum* (Hogben, Lancelot; Slome 1930; Leboeuf and Howe 1981). The present study recognizes the constraints associated with visual approximations to denote melanophore dispersal and therefore employs common image analysis methods as an alternative methodology to quantify melanophore dispersal and behavior across time.

2. METHODS

S. quadridentatum specimens were collected from the TAMUG Small Boat Basin at 29.3132, -94.8177 (Figure 2.1) between October and December 2020. Fouling communities were scraped from the side of the plastic floating docks and collected in a plastic container. Biofouling material was sifted and sorted under a dissecting microscope. Any observed isopods were collected and transported into a 30 cm x 30 cm x 15 cm culture. The culture container contained a diagonally placed plastic insert to enable isopods to easily access dry artificial substrate, in similarity to its habitat of origin. In addition to the plastic insert, 2 air stones were placed inside the isopod refugium at opposite ends of the container to maximize aeration and water movement. The culture was maintained at 30 ppt salinity under a natural light regime and were fed fish flakes on a weekly regimen. *C. sapidus* specimen was collected in the TAMUG T-Dock at 29.3130, -94.8182 using a crab trap on 4 February 2021, after which the specimen was transported to a 45 cm x 8 cm x 32 cm refugium (Figure 2.1). The refugium was maintained at 30 ppt salinity under a natural light regime. The specimen was fed frozen krill every other day. A calibrated refractometer was used to ensure the salinities of both enclosures would remain within the 30 ppt salinity parameter. The *C. sapidus* enclosure contained a sheltered side to minimize stress. The *C. sapidus* refugium water was aerated and cycled using a canister filter.

Figure 2.1: Relative locations of collection sites for S. quadridentatum and C. sapidus specimens. Note that Collection Site A and Collection Site B correspond to S. quadridentatum and C. sapidus collection sites, respectively.

2.1 Data Collection

24-well tissue culture dishes were converted into two treatment types: dark substrate and light substrate. Black and white construction papers were taped to the bottom of the well plate to simulate dark and light substrate treatment types, respectively. Isopod specimens were separated into individual wells in 24-well tissue culture plates to limit movement for increased photographic quality. Each tissue culture dish was divided in half and the subdivisions, containing 12 isopods each, were designated as either experimental or control groups. Each well was filled with 30 ppt salinity seawater to decrease reflective glare and to minimize disturbance. 30 ppt water from the *C. sapidus* refugium was pipetted into tissue culture wells containing isopods in experimental light and dark substrate treatment types. A mercury thermometer was used to ensure temperatures remained constant across all experimental and control groups.

Transfer of water from the *C. sapidus* refugium to the well plates was performed underneath a dissecting microscope under constant lighting. Isopods were photographed before and 1 minute and 30 seconds after the transfer of water to allow for maximum melanophore response, producing 1 photograph before the water transfer and 1 photograph taken after the water transfer. The central portion of the dorsal somites on each isopod were photographed at maximum magnification using a Celestron 5-megapixel digital microscope imager. A total of 6 melanophores were photographed per individual isopod. The photographed melanophores were used as a representative of melanophores across all dorsal somites. A metric ruler was included in the first photograph to determine the scale of each remaining photograph taken.

2.2 Data Analysis

Scales were made using ImageJ image analysis software using a metric ruler as reference. Since all photographs were taken under maximum magnification, the scale used for the sample photograph was applied to all images taken. Photographs were analyzed using the tracing tool included in ImageJ image analysis software. Trace lines were superimposed on photographed melanophores and the lengths added to calculate melanophore dispersal (Figure 2.2).

Figure 2.2: Sample tracing of melanophore aggregation unit (not to scale). Trace length is added together to estimate melanophore dispersal.

Mean melanophore dispersal of each isopod was calculated by dividing the total melanophore trace dispersal sum by the number of individual melanophores measured (Equation 2.1). Mean melanophore dispersal by treatment type was calculated by dividing the sum of the mean melanophore dispersals by the total number of isopods in the given treatment type (Equation 2.2).

$$Mean\ Melanophore\ Dispersal_{Isopods} = \frac{Total\ Melanophore\ Trace\ Dispersal\ Sum}{Melanophore\ Units\ Measured} \quad (2.1)$$

$$Mean\ Melanophore\ Dispersal_{Treatment} = \frac{Sum\ of\ Melanophore\ Dispersals}{Isopods} \quad (2.2)$$

Two-factor ANOVA with replication was run using the data analysis toolkit in Microsoft Excel. Two-factor ANOVA with replication was run on the acquired experimental and control group melanophore dispersal data to evaluate the significance of sources of variation. The standard alpha value of 0.05 was used as the measure to denote significance in variation. P-values calculated below the determined alpha value in association with any observed source of variation was considered significant, while any P-values larger than the determined alpha value consistent with any source of variation was considered non-significant.

3. RESULTS

3.1 Black Substrate Treatments

Isopods on black substrate treatment exposed to water from the *C. sapidus* enclosure demonstrated variability in melanophore dispersal across the time frame of the experiment. However, melanophore aggregations in most isopods dispersed slightly after exposure to predatory olfactory cues. Melanophore dispersals in isopods 1, 3, 4, 6, 7, and 8 increased in response to treatment, with the highest dispersal increase observed in isopod 1. Isopods 2, 5, and 9 experienced decreases in melanophore dispersal after exposure to treatment, with the largest decreases observed in isopods 2 and 9. Despite the higher amount of melanophore dispersals compared to recorded aggregations, most changes in dispersal rates were relatively slight (<0.05 mm). Melanophore dispersal states varied more across individual isopods than across treatment time frame (Figure 3.1).

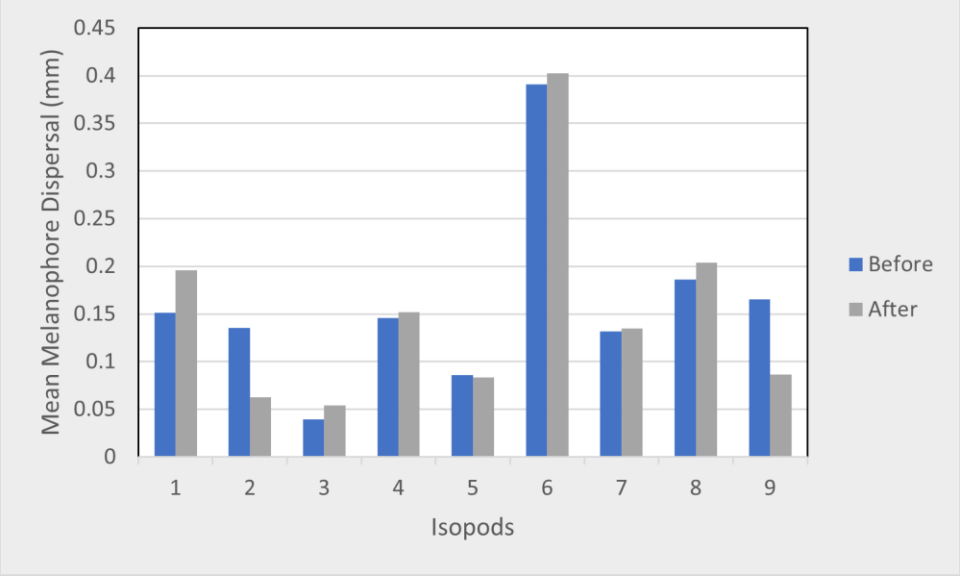

Figure 3.1: Mean melanophore dispersal of isopods under black substrate experimental treatment

Isopods observed under the control black substrate treatment demonstrated similar variability in melanophore dispersal measurements across the time frame of the experiment. In addition and in similarity to the experimental group, melanophore dispersal varied more across individual isopods than across control treatment exposure (>0.025 mm). All isopods except one in the black substrate control group demonstrated increased melanophore dispersal after exposure to four drops of sterile seawater. Only isopod 1 demonstrated an increase in melanophore aggregation after treatment. Isopod 2 experienced the largest dispersal increase. The melanophores of isopod 9 were observed to be completely dispersed before and after control treatment. Because of the difficulty in measuring the mean dispersal of completely dispersed melanophores, data for isopod 9 was not included in the results of the study (Figure 3.2).

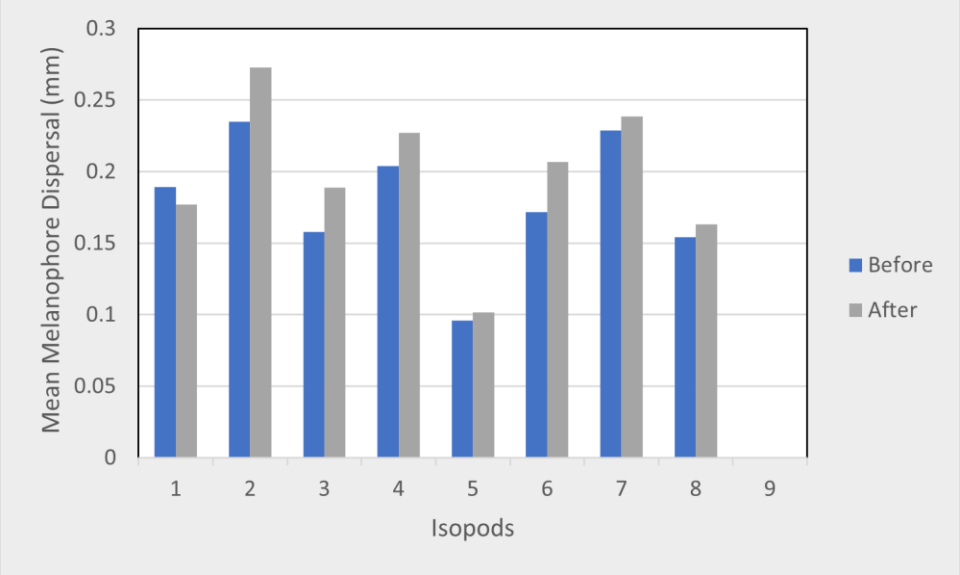

Figure 3.2: Mean melanophore dispersal of isopods under black substrate control treatment

Mean melanophore dispersal data demonstrated differing trends across experimental and control black substrate groups. Isopods exposed to water from the *C. sapidus* refugium experienced an average decrease in melanophore dispersal after exposure. For the experimental group, representative melanophores aggregated 0.006 mm on average relative to dispersal states

before treatment exposure. Unlike isopods from the experimental group, isopods exposed to sterilized seawater experienced an average increase in melanophore dispersal. For the control group, representative melanophores dispersed 0.017 mm on average relative to dispersal states before treatment exposure. Representative melanophores from isopods used for the experimental group were, on average, more aggregated than representative melanophores observed in isopods belonging to the control group. It should be noted that mean melanophore dispersals recorded before and after treatment across both groups lay partially or entirely within margins of errors calculated for each dataset (Figure 3.3).

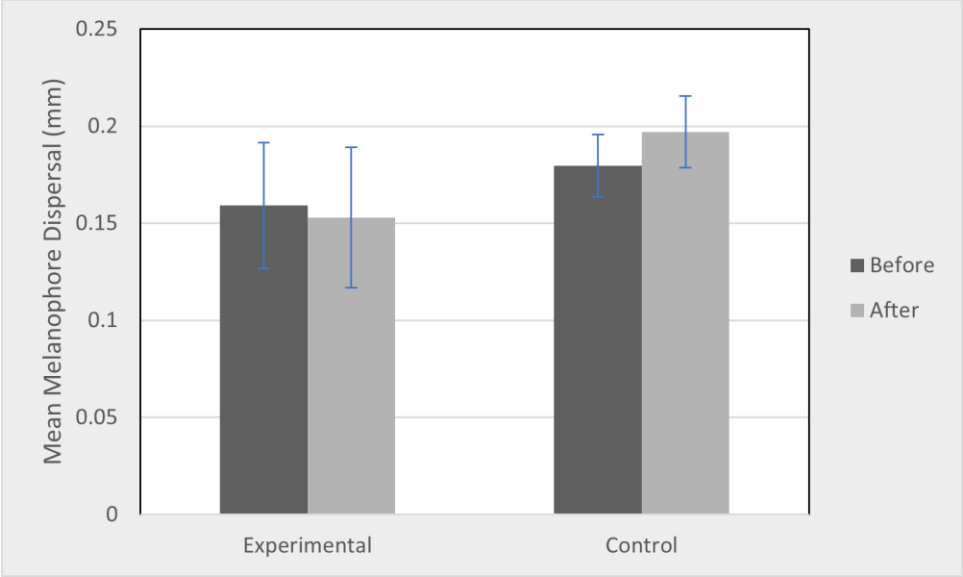

Figure 3.3: Total mean melanophore dispersal of experimental and control groups under black substrate treatment.

3.2 White Substrate Treatments

Isopods on white substrate treatment exposed to water from the *C. sapidus* enclosure also demonstrated variability in melanophore dispersal in congruence with exposure to treatment. Melanophore aggregations in most isopods dispersed slightly after exposure to predatory olfactory cues, similar to black substrate treatment. Melanophore dispersals in isopods 1, 4, 6, 7, and 9 increased in response to treatment, with the highest dispersal increase observed in isopod

6. Isopods 2, 3, 5, and 8 experienced decreases in melanophore dispersal after exposure to treatment, with the largest decrease observed in isopod 3. Most changes in dispersal rates were relatively slight (<0.05 mm), with the exception of isopod 3 which demonstrated a more than 0.1 mm melanophore aggregation. Despite periodic drastic changes, melanophore dispersal states remained varied across individual isopods more than across treatment time frame, similar to black substrate isopod groups (Figure 3.4).

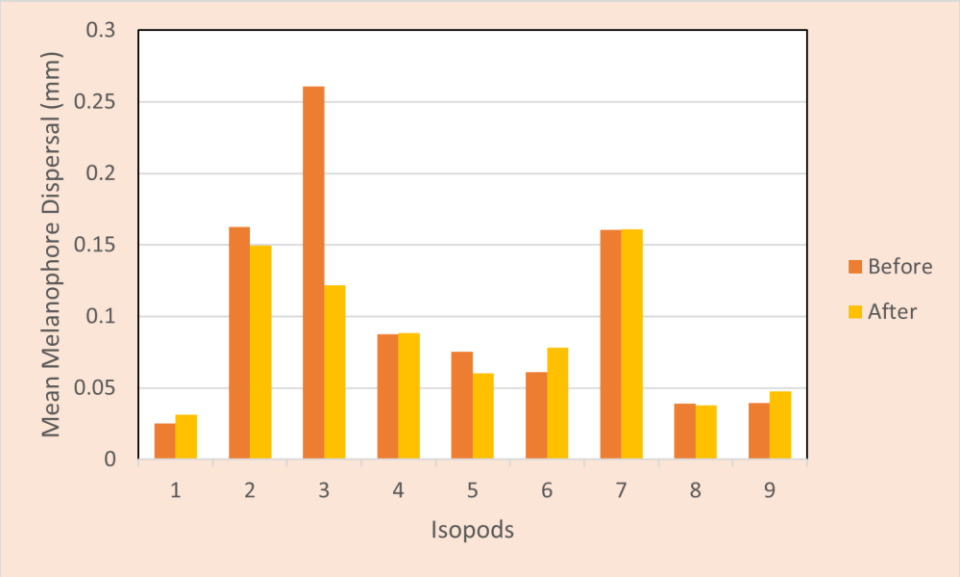

Figure 3.4: Mean melanophore dispersal of isopods under white substrate experimental treatment

Isopods observed under the control white substrate treatment demonstrated similar patterns of variability in melanophore dispersal measurements across the time frame of the experiment. Unlike all other experimental and control groups, melanophore dispersal varied comparably across individuals in the white control group and in correlation with control treatment exposure. 4 isopods (Isopods 5, 6, 8, and 9) demonstrated aggregation after exposure to sterilized seawater. In isopods demonstrating decreased dispersal after treatment, aggregation approached 25% reduction in dispersal. In addition, 4 isopods demonstrated slight increases in melanophore dispersal while the remaining isopod maintained a relatively similar dispersal state.

Overall, melanophore aggregation in isopod 9 was recorded to be the largest dispersal change in isopods belonging to the white substrate control group (>50% decrease in melanophore dispersal). Meanwhile, isopod 6 demonstrated the highest melanophore dispersal state both before and after treatment exposure. (Figure 3.5).

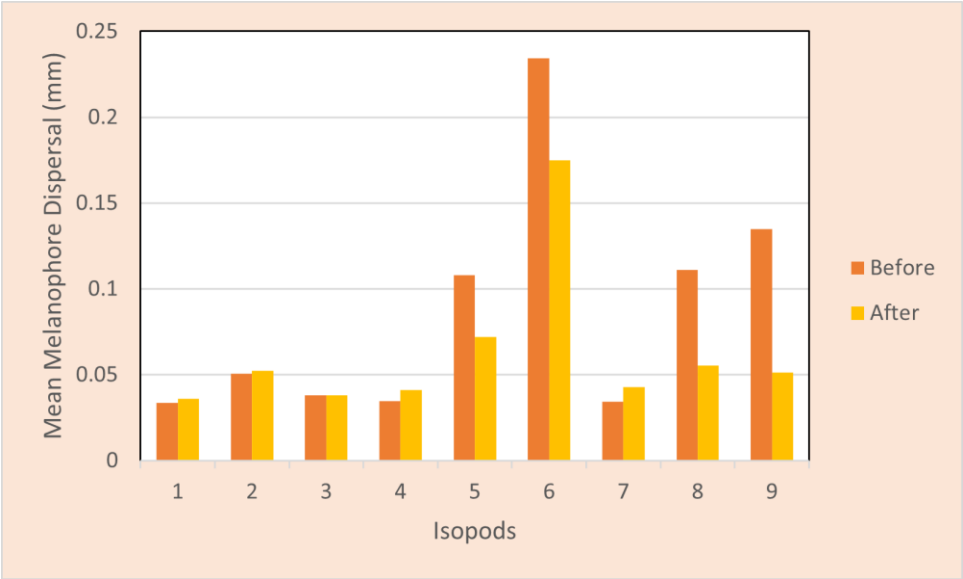

Figure 3.5: Mean melanophore dispersal of isopods under white substrate control treatment

In general, isopods exposed to predatory olfactory cues demonstrated increased melanophore dispersal states before and after treatment in comparison to isopods belonging to the control group. On average, both groups experienced reductions in melanophore dispersal. However, isopods belonging to the control group experienced a larger mean reduction above 0.02 mm. In similarity to mean melanophore data produced from the black substrate treatment, it should be noted that observed changes in melanophore data in correspondence with exposure to treatment were not statistically significant (Figure 3.6).

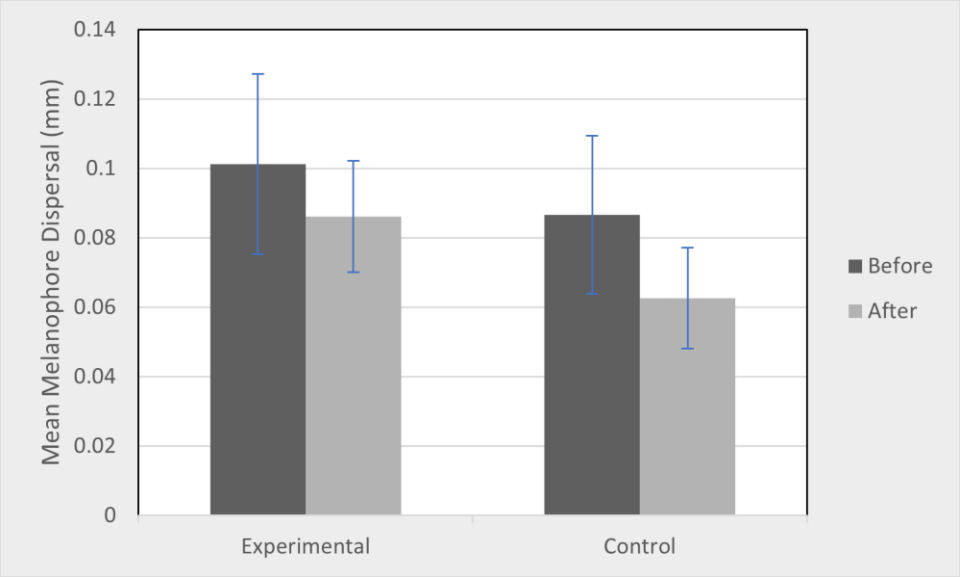

Figure 3.6: Total mean melanophore dispersals of experimental and control groups under white substrate treatment

3.3 Statistical Data

P-values calculated from two-factor ANOVA of melanophore dispersal data of experimental groups are less than the determined alpha value ($P<0.05$) when pertaining to substrate color as a source of variation. P-values are larger than the determined alpha value ($P>0.05$) in correspondence with all other sources of variation (Table 3.2).

Table 3.1: Two-factor ANOVA with replication of experimental group melanophore dispersal data

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Substrate Color	0.034876	1	0.034876	4.709004	0.037538	4.149097
Treatment	0.001026	1	0.001026	0.138504	0.712226	4.149097
Interaction	0.000177	1	0.000177	0.023904	0.8781	4.149097
Within	0.236997	32	0.007406			
Total	0.273075	35				

In similarity to statistical results computed for melanophore dispersal data in the experimental groups, P-values calculated from two-factor ANOVA of melanophore dispersal data of control groups are less than the determined alpha value ($P<0.05$) when pertaining to

substrate color as a source of variation. P-values calculated for other sources of variation are larger than the determined alpha value (P>0.05; Table 3.2).

Table 3.2: Two-factor ANOVA with replication of control group melanophore dispersal data

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Substrate Color	0.107576	1	0.107576	36.21836	1.74E-06	4.195972
Treatment	1.51E-06	1	1.51E-06	0.000508	0.982175	4.195972
Interaction	0.002296	1	0.002296	0.772966	0.386784	4.195972
Within	0.083166	28	0.00297			
Total	0.19304	31				

4. CONCLUSION

4.1 Black Substrate Experiments

In a total of 13 out of 17 isopods (experimental and control combined), melanophore dispersal increased after treatment with *C. sapidus* water (experimental) and sterilized seawater (control), including 6 out of 9 experimental group isopods and 7 out of 8 control group isopods. This result is consistent with expectations as melanophore dispersal is expected to increase after exposure to predatory cues on darker substrates. The response is hypothesized to decrease chances of detection by predators as the darker coloration, resulting from a dispersal response, is observed to enhance camouflage on darker substrates (Leboeuf and Howe 1981). However, the hypothesis that predatory olfactory cues affect melanophore dispersal could not be confirmed, as there was no statistically significant difference between the total mean melanophore data of the experimental and control group. The ability to conclusively suggest an underlying mechanism in black substrate treatment isopods is limited further by the variability of the melanophore response across specimens as overall dispersal decreased after treatment in experimental isopods and overall dispersal increased after treatment in control isopods.

4.2 White Substrate Experiment

In a total of 8 out of 18 isopods, mean melanophore dispersal decreased when placed in a white background after exposure to treatment, including a total of 4 out of 9 experimental isopods and 4 out of 9 control isopods. The experiment demonstrated melanophore dispersal responses inconsistent with expected results as melanophore dispersal increased in the majority of specimens after exposure to treatment. The response is in contrast with the expected results as decreases in melanophore dispersal are hypothesized to decrease chances of detection by

predators and improve camouflage (Leboeuf and Howe 1981). Despite the melanophore dispersal increase observed in the majority of experimental isopods, the mean total melanophore dispersal data demonstrates a statistically insignificant decrease in melanophore dispersal after exposure to treatment. Therefore, the total mean melanophore dispersal measurements of experimental white substrate treatment reflect a variability consistent with the expected results. Despite any observed trends, the lack of statistical significance, the variability of responses observed across specimens, and the similar melanophore response in both treatments suggest the presence of predatory olfactory cues is not a significant predictor of melanophore dispersal on isopods residing on lighter substrate coloration. This suggests that water disturbance, rather than the presence of predatory olfactory cues, may be a predictor of melanophore dispersal as water disturbance was a common effect of both treatments. The melanophore dispersal response to water disturbance may still represent a predator avoidance strategy as hydrodynamic changes may indicate the presence of a predator moving through the water column. This could be investigated in the future as a potential mechanism, as mechanoreception and a coupled predator avoidance response have been observed in other crustacean taxa, namely, copepods (Buskey et al. 2012). Despite the consideration, this hypothesis lies beyond the scope of this study and will have to be investigated further in future research.

4.3 ANOVA

Two-factor ANOVA of mean melanophore data acquired for each isopod present substrate coloration to be a significant predictor of variation in melanophore dispersal in both experimental and control group isopods. The statistically significant dispersal of melanophores in isopods residing on black substrate in contrast to white substrate upholds previous findings in studies of color change in *S. quadridentatum*. The observed trend suggests the presence of a

melanophore response to changes in background substrate coloration as previously discovered by Leboeuf and Howe (1981). The significance of substrate coloration as a predictor of melanophore dispersal response relative to exposure to predatory olfactory cues suggests the presence of a more environmentally dependent and continual melanophore response mechanism independent of predatory cues. Such a response could still be considered a predator avoidance adaptation as changes in coloration to match the resident surface likely reduce detection by predators. The mechanism may be of particular importance when considering the benefit isopods may reap from a more continual, adaptable pigment response when in quiescent states (Leboeuf and Howe 1981). Immediate responses to predatory cues may, therefore, be preserved for traits which could provide temporary protections against predators but are too costly when used beyond a temporary timeframe. Such traits would likely include temporary behavioral modifications to avoid predators which have been previously observed in some terrestrial and aquatic isopod species (Holomuzki and Short 1988; Zaguri et al. 2018).

4.4 Summary

To summarize, the experiments showed that melanophore dispersal is a function of the background coloration, as predicted. However, the hypothesis that melanophore dispersal is affected by olfactory cues from a predator could not be confirmed. Future research could focus on investigating behavioral modifications in *S. quadridentatum* as a response to various predatory cues and the possibility of a behavioral response to hydrodynamic cues.

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APPENDIX: RAW DATA

Table A.1: Individual melanophore dispersal states of experimental group isopods under black substrate treatment before exposure to C. sapidus.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.16	0.239	0.189	0.19	0.062	0.067	0.151166667
2	0.247	0.11	0.133	0.097	0.079	0.145	0.135166667
3	0.072	0.055	0.02	0.016	0.037	0.036	0.039333333
4	0.192	0.199	0.1	0.191	0.128	0.065	0.145833333
5	0.086	0.132	0.128	0.079	0.065	0.026	0.086
6	0.471	0.197	0.339	0.557	0.413	0.369	0.391
7	**0.119**	**0.109**	**0.163**	**0.121**	**0.138**	**0.142**	0.132
8	0.325	0.204	0.167	0.15	0.153	0.116	0.185833333
9	0.64	0.044	0.055	0.096	0.081	0.076	0.165333333
Mean Melanophore Dispersal of Treatment							0.159074074

Table A.2: Individual melanophore dispersal states of experimental group isopods under black substrate treatment after exposure to C. sapidus.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.235	0.287	0.148	0.245	0.104	0.156	0.195833333
2	0.154	0.027	0.062	0.093	0.016	0.023	0.0625
3	0.057	0.031	0.023	0.074	0.087	0.054	0.054333333
4	0.204	0.177	0.089	0.219	0.181	0.04	0.151666667
5	0.122	0.083	0.128	0.08	0.05	0.036	0.083166667
6	0.527	0.217	0.343	0.585	0.36	0.382	0.402333333
7	**0.138**	**0.118**	**0.164**	**0.127**	**0.134**	**0.129**	0.135
8	0.282	0.19	0.225	0.242	0.122	0.164	0.204166667
9	0.095	0.088	0.072	0.1	0.062	0.102	0.0865
Mean Melanophore Dispersal of Treatment							0.152833333

Table A.3: Individual melanophore dispersal states of control group isopods under black substrate treatment before exposure to added sterilized seawater.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.16	0.09	0.25	0.23	0.206	0.2	0.189333333
2	0.356	0.299	0.2	0.254	0.13	0.171	0.235
3	0.133	0.181	0.291	0.099	0.139	0.105	0.158
4	0.175	0.153	0.238	0.337	0.082	0.239	0.204
5	0.093	0.078	0.091	0.076	0.099	0.138	0.095833333
6	0.222	0.141	0.145	0.042	0.376	0.104	0.171666667
7	0.27	0.31	0.32	0.135	0.243	0.095	0.228833333
8	0.219	0.106	0.13	0.184	0.156	0.129	0.154
9							
Mean Melanophore Dispersal of Treatment							0.179583333

Table A.4: Individual melanophore dispersal states of control group isopods under black substrate treatment after exposure to added sterilized seawater.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.189	0.098	0.189	0.196	0.282	0.107	0.176833333
2	0.509	0.298	0.278	0.11	0.232	0.211	0.273
3	0.153	0.19	0.18	0.31	0.18	0.12	0.188833333
4	0.341	0.16	0.263	0.207	0.233	0.158	0.227
5	0.078	0.091	0.105	0.078	0.085	0.172	0.1015
6	0.308	0.175	0.055	0.17	0.432	0.1	0.206666667
7	0.302	0.353	0.414	0.114	0.149	0.1	0.238666667
8	0.258	0.111	0.138	0.167	0.154	0.151	0.163166667
9							
Mean Melanophore Dispersal of Treatment							0.196958333

Table A.5: Individual melanophore dispersal states of experimental group isopods under white substrate treatment before exposure to C. sapidus.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.023	0.018	0.025	0.03	0.018	0.038	0.025333333
2	0.144	0.123	0.128	0.211	0.205	0.163	0.162333333
3	0.131	0.158	0.98	0.059	0.137	0.1	0.260833333
4	0.062	0.072	0.125	0.064	0.084	0.117	0.087333333
5	0.054	0.079	0.068	0.063	0.12	0.069	0.0755
6	0.066	0.062	0.036	0.046	0.066	0.091	0.061166667
7	0.169	0.118	0.219	0.204	0.186	0.067	0.1605
8	0.034	0.053	0.038	0.034	0.042	0.032	0.038833333
9	0.043	0.034	0.043	0.043	0.043	0.031	0.0395
Mean Melanophore Dispersal of Treatment							0.101259259

Table A.6: Individual melanophore dispersal states of experimental group isopods under white substrate treatment after exposure to C. sapidus.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.025	0.024	0.025	0.033	0.041	0.039	0.031166667
2	0.162	0.118	0.13	0.143	0.178	0.166	0.1495
3	0.11	0.186	0.081	0.066	0.154	0.133	0.121666667
4	0.065	0.085	0.093	0.058	0.101	0.127	0.088166667
5	0.057	0.065	0.059	0.042	0.045	0.093	0.060166667
6	0.061	0.081	0.041	0.049	0.114	0.123	0.078166667
7	0.165	0.113	0.234	0.226	0.155	0.072	0.160833333
8	0.034	0.046	0.042	0.037	0.036	0.033	0.038
9	0.051	0.047	0.049	0.043	0.052	0.044	0.047666667
Mean Melanophore Dispersal of Treatment							0.086148148

Table A.7: Individual melanophore dispersal states of control group isopods under white substrate treatment before exposure to added sterilized seawater.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.048	0.031	0.034	0.03	0.026	0.032	0.0335
2	0.04	0.066	0.03	0.06	0.053	0.055	0.050666667
3	0.041	0.032	0.042	0.034	0.04	0.04	0.038166667
4	0.023	0.026	0.048	0.03	0.038	0.042	0.0345
5	0.093	0.033	0.179	0.142	0.033	0.169	0.108166667
6	0.291	0.237	0.253	0.104	0.378	0.143	0.234333333
7	0.027	0.058	0.021	0.03	0.028	0.041	0.034166667
8	0.048	0.075	0.099	0.033	0.041	0.37	0.111
9	0.093	0.052	0.043	0.059	0.5	0.063	0.135
Mean Melanophore Dispersal of Treatment							0.086611111

Table A.8: Individual melanophore dispersal states of control group isopods under white substrate treatment after exposure to added sterilized seawater.

Isopod	Individual Melanophore Dispersal Lengths (mm)						Mean Melanophore Dispersal (mm)
1	0.051	0.023	0.029	0.045	0.027	0.041	0.036
2	0.036	0.062	0.0107	0.096	0.039	0.07	0.052283333
3	0.046	0.034	0.038	0.04	0.038	0.033	0.038166667
4	0.051	0.047	0.035	0.034	0.038	0.041	0.041
5	0.066	0.059	0.047	0.088	0.091	0.08	0.071833333
6	0.255	0.185	0.333	0.124	0.103	0.05	0.175
7	0.069	0.035	0.035	0.042	0.05	0.026	0.042833333
8	0.044	0.094	0.065	0.049	0.043	0.037	0.055333333
9	0.068	0.053	0.065	0.034	0.041	0.047	0.051333333
Mean Melanophore Dispersal of Treatment							0.062642593