

Abstract

Collective navigation is the process whereby individuals take directional and positional cues from nearby animals. It has been theorized that the presence of other conspecifics may improve navigational accuracy; however, behavioral experiments on the effectiveness of collective navigation have been done almost exclusively with pigeons. The goal of this research is to assess how collective navigation may assist in the orientation of *Megalorchestia sp.*, a genus of talitrid amphipods native to the eastern North Pacific. Talitrid amphipods are ideal candidates for an invertebrate model system because they orient strongly towards the wrack line of coastal beaches. Their navigational abilities are based on a robust set of cue types, including visual, celestial, and magnetic. Additionally, talitrid amphipods are plentiful and easy to access in the intertidal zone. For this study, we conducted a behavioral orientation assay to test how well *Megalorchestia* orients towards the beach in three different group sizes: individually, in groups of five, and in groups of ten. Contrary to our expectations, we found that the largest group size ($n = 10$) was significantly slower, less directed towards the beach, and started moving earlier in the trial than the individual animals. The intermediate group size ($n = 5$) also started moving significantly earlier in the trial, but otherwise exhibited no differences in orientation from the individual animals. Our results provide the first evidence for beachward orientation in *Megalorchestia*. Overall, our results support the idea that collective navigation may not be beneficial for all organisms or in all situations.

Introduction

Navigational accuracy is key to the survival and fitness of many organisms. Migrating to breeding grounds (Holland, 2014), returning to nests (Collett et al., 1998), and finding cached food (Gould et al., 2010) are all examples of when successful navigation has significant rewards and survival implications. Navigational mistakes, in turn, can have drastic consequences. For example, it has been proposed that a 1994 mass stranding of sperm whales in the North Sea may have been due to navigational errors during foraging (Jauniaux et al., 1998). The senses that sperm whales and other animals use for navigation are never perfectly accurate. Some specific navigational modalities such as magnetoreception, have behavioral features that suggest that the transduction of the underlying sensory cue is quite noisy (Johnsen et al., 2020). For example, turtles use magnetoreception to travel large distances to return to their natal beaches, yet laboratory experiments show substantial variability in headings when orienting relative to magnetic cues (Irwin & Lohmann, 2005). It is currently unknown how animals can use such noise-susceptible sensory modalities to achieve accurate migrations.

One phenomenon that might explain how animals compensate for noise and uncertainty is collective navigation (i.e., flocking). In this paper, we define collective navigation as the process whereby individuals take directional and positional cues from nearby animals. In many ways, traveling with other animals can potentially improve individual navigational accuracy. The simplest principle behind the benefits of collective navigation is the idea of the “wisdom of the crowd,” where the average of a group’s decisions is generally better than that of any individual member of the group (Simons, 2004). For example, if a group of people attempt to guess the weight of a pig in a pig-weighing competition, most individuals will have relatively inaccurate guesses; however, the guesses tend to vary in a statistically normal manner and the average of

everyone's guesses is often close to the actual weight of the pig. The same principle can be applied to a group that has many individual directional headings; the average group heading is likely more accurate than one individual's heading. Moving together would then theoretically benefit the group, but not necessarily benefit each animal. Beyond "wisdom of the crowd," another concept underlying the study of collective navigation is the "many wrongs" theory (Simons, 2004). It states that the standard error of a group's mean heading will decrease as the group size increases. Under this theory, every individual would experience reduced navigational error.

It has also been proposed that animals traveling in a group can take advantage of different levels of navigational experience. For instance, experienced navigators can lead the group, enabling naive navigators to reach the location successfully while also learning the correct route or cues. In turn, naive navigators can benefit the experienced navigators through their introduction of random error, potentially leading to the creation of new, improved routes (Berdahl et al., 2018).

Many theoretical papers have supported the idea that traveling in a group is helpful for navigation. For example, computer models have shown that long-distance navigation can be more accurate when poor navigators move together (Berdahl et al., 2016) and only small numbers of informed navigators are needed for large groups to move in the correct direction (Couzin et al., 2005). Most recently, Granger et al. (2022) showed through an agent-based model that collective navigation may be the best explanation for how certain animals are able to precisely navigate long distances to highly defined locations using a noisy sensory modality, like magnetoreception.

Behavioral experiments on collective navigation, however, have been limited. The majority have focused on flocks of homing pigeons. Flocks show improved navigational efficiency over solo flights in terms of higher speeds, straighter flight paths, and decreased time needed to rest (Dell'Araccia et al., 2008; Pettit et al., 2015). A small amount of research has been done on collective navigation in other birds and several species of fish (reviewed in Berdahl et al., 2018), and work in invertebrates is limited to leadership studies in honeybees (Schultz et al., 2008). Swarms of bees moving to new hives are composed almost entirely of naïve navigators, but video analysis has revealed that the swarms are able to successfully navigate by following the lead of the “scout bees,” who have previous knowledge of the new location for the hive and who signal to the rest of the swarm by making rapid, directed flights in the correct direction.

Collective navigation remains a difficult phenomenon to study empirically. The lack of accessible, economical, and lightweight tracking devices has impeded the field for many years. For example, early papers argued that flocking did not help homing pigeons with their navigation; however, this research was based only on “vanishing bearings,” defined as the bearing of the pigeons as they vanish from the researcher's sight (Berdahl et al., 2018; Keeton, 1970). With the recent advent of long-distance tracking systems, such as GPS tags, researchers are now able to study the entirety of a pigeon's movement. These sorts of opportunities to study wide-ranging animals have become available at relatively moderate expense (Luschi, 2013). Although many technological barriers have been overcome, care and handling of the animals themselves remains an issue. As the species that are known to collectively navigate are almost entirely vertebrates, any research on collective navigation currently requires significant time, permitting, and financial resources.

In this study, we propose an invertebrate model for collective navigation: beach hoppers in the *Talitridae* family. This family consists of terrestrial and marine amphipods that have been well-studied for their orientation behavior. The marine species of talitrid amphipods spend most of their lives burrowed in the dense sand of the wrack line on marine and brackish beaches. The “wrack line” is the area of the beach where logs, algae, and other debris are deposited at high tide. This means that foraging can sometimes be very efficient for talitrid amphipods, as algae is their preferred diet. However, when the tide has receded and the algae does not reach the wrack line, they are forced to leave the safety of their burrows and venture closer to the ocean. While on the surface, they are vulnerable to predation and desiccation, and so once they have finished foraging, they must be efficient and accurate at returning to their burrows in the wrack line (Williamson, 1951). This selection pressure likely led to the evolution of talitrid amphipods’ most well-studied feature: their ability to orient themselves perpendicular to the water. In other words, they are always able to turn themselves back towards the wrack line. Talitrid amphipods use a wide range of cues to guide this orientation, including the local landscape (Williamson, 1951), the position of the sun and moon in the sky (Pardi & Papi, 1952; Papi, 1960), the incline of the beach (Craig et al., 1973b), the salinity of any water they encounter (Scapini, 1979), and the Earth’s magnetic field (Van den Bercken et al., 1967; Arendse et al., 1978). Additionally, talitrid amphipods are found across the globe in dense populations. For example, the European species *Talitrus saltator* can be found in densities of over 9,000 individuals per square meter (Ruiz-Delgado et al., 2016). Their orientation behavior and gregariousness make talitrid amphipods a potentially ideal model system for testing the presence and possible benefits of collective navigation.

There has only been one study on talitrid amphipods' ability to orient in a group setting; Scapini et al. (1981). This study concluded that there did not appear to be a difference in the orientation accuracy of individual *Talitrus saltator* as compared to groups of five. The two metrics used by this study were the animals' orientation directions and their "dispersion," a measurement of the variance in headings. Their analyses were hampered by the technological and statistical methods available at the time. Most notably, the authors were unable to analyze the entire path of each animal and instead had to choose one moment of each trial upon which to base their conclusions. Because of Scapini's results, many *Talitridae* experiments have used varying numbers of individuals during each trial of their orientation experiments, from one (Edwards, 1987) to ten (Rothsey et al., 2016) to twenty five individuals at a time (terHorst, 2011). They have treated each animal as an independent data point and ignored possible inter-individual effects. However, if the presence of conspecifics does indeed affect group orientation, then experiments using one animal per trial would yield different results than those that used multiple animals per trial. Additionally, the movement of one animal could no longer be considered truly "independent" from all the other animals in the experiment, as the other animals specifically in that animal's trial may be impacting that individual's movement.

Here we aim to build upon the work done by Scapini et.al. to study the impacts of nearby-conspecifics on the orientation behavior of *Megalorchestia*, a genus of littoral talitrid amphipod native to the Eastern North Pacific. Like other genera in the family *Talitridae*, they are found in high densities on sandy coastal beaches. Previous research on this genus has focused only on topics unrelated to their orientation, such as their rapid colonization of algal patches and the effect of shoreline armoring (such as seawalls) on their abundances (Jaramillo et al., 2021; Pelletier et al., 2011). The aims of this study were to: 1) investigate the ability of *Megalorchestia*

to orient toward the wrack line and 2) test whether collective navigation improves the accuracy of this behavior. We investigated this by conducting experiments where animals were either released alone, in a group of five, or in a group of ten. We expanded upon the experiments of Scapini et al. (1981) with larger sample sizes, full-path tracking techniques, and additional metrics to assess their orientation performance. Based on what has been observed in other taxa who engage in collective navigation, we hypothesized that the presence of additional conspecifics would improve talitrid amphipods' ability to orient toward the wrack line by reducing the variation in their direction of travel, straightening their paths, and increasing their traveling speeds.

Methods

Our orientation experiments were conducted at Discovery Park in Seattle, WA (47.6633, -122.4316). Discovery Park is located on the shore of Puget Sound, an inlet of the Pacific Ocean. Within the park, I chose a sandy beach with a clearly defined wrack line and a high density of *Megalorchestia*.

The first step of each orientation experiment was collecting the animals. For trials in the early morning, I gathered them from the beach surface; however, for trials that were later in the day, the animals had buried themselves and required me to dig into the sand of the wrack line for collection. The animals were then combined into a plastic cup which was placed (upside down) in the arena. I collected all the animals required for a trial simultaneously so that each trial could occur immediately after collection and would represent individuals that naturally occurred on the beach at the same time. My group sizes ("GS") were 1, 5, and 10, which I refer to as GS1, GS5, and GS10 respectively.

Each arena consisted of a circular, clear plastic dish that was approximately 1 inch deep, with a diameter of 9 inches, and was held 15 inches away from the ground by PVC pipes (Fig. 1). A camera rested on the arena's base and pointed upward towards the dish, allowing me to film the animals' movement within the dish. The camera was oriented in the same direction relative to the arena from trial to trial to allow us to test for orientation relative to the arena itself. Before the introduction of animals, the entire arena was leveled with a hand-held level and the dish was wrapped in white paper to prevent any passerby or other inconsistent visual cues from affecting the animals' orientation.

The arenas were placed several feet above the wrack line to prompt the animals to travel back in the direction of the wrack line (Fig. 1). For talitrid amphipods, the area above the wrack line is somewhere they would rarely go; they would only enter this area through passive displacement, such as a storm, or because of a navigational mistake. It contains the same risks of predation and desiccation as below the wrack line, but without the potential benefits of foraging. Therefore, talitrid amphipods who find themselves above the wrack line are strongly motivated to return to the wrack line. Our placement of the arenas in this area is consistent with the vast majority of fieldwork on talitrid amphipod orientation (Pardi & Ercolini, 1986).



Figure 1. Left: layout of the experimental setup with regards to the overall beach. Right: close-up view of the composite parts of the arena.

Before the animals were placed into the arena, I recorded several pieces of information: the group size, trial number, cloud cover, temperature, direction of magnetic north, and direction of the wrack line. I visually determined the cloud cover as either “Sunny” or “Cloudy” based on the weather present at the start of each trial. The temperature at my location was obtained from the Apple iOS Weather app. Finally, I showed the camera the direction of north with an arrow that was aligned in accordance with a compass and the direction of the wrack line with an arrow that was pointed perpendicular to the water.

After this information was recorded, I placed the animals’ cup onto the arena. I secured the cup with a rock on top and left the animals to acclimate to the arena for 2 minutes. When that time had elapsed, I lifted the cup, releasing the animals, and then quickly placed a transparent lid over the arena to prevent them from jumping out (Fig. 2). The animals were allowed to move freely in the arena for 30 minutes before I ended the trial. All movement was recorded by the camera below the dish. At the end of each trial, I removed the animals, cleaned out any sand, and

wiped down the arena with a damp paper towel to eliminate potential scent cues left by the previous animals.

In total, I completed 108 trials from July 17 to August 17, 2021. The trials were conducted between 8 am and 12:40 pm and the temperature ranged from 57 to 79°F.

The videos of each trial were then filtered for quality. I excluded any video with the incorrect number of animals, videos where the entire arena was not in frame, or videos where any animals besides talitrid amphipods were accidentally trapped within the collection cup. This left me with 86 total trials: 30 for GS1, 29 for GS 5, and 27 for GS10.

The videos of each trial were digitized with the software DLTdv8a (Hedrick, 2008). This involved manually tracking each animal's location across the bottom of the dish (Fig. 3). Due to the time-intensive nature of the digitizing process, we digitized the path of each animal only until all animals in the trial had reached the edge of the arena. This decision was additionally supported by our observation that the animals' movement after they reach the wall of the arena appeared to no longer be directed towards the wrack line; instead, the animals would begin to circle the dish, presumably looking for a method of escape rather than attempting to continue towards the wrack line. Along with the animals' tracks, we also digitized the direction of the wrack line based on the arrow that I showed at the beginning of each video. All digitization was conducted by me (43 videos), Jesse Granger (18 videos), Ada Zhang (17 videos), and Maaz Ahmed (8 videos) from September 2021 to December 2022.

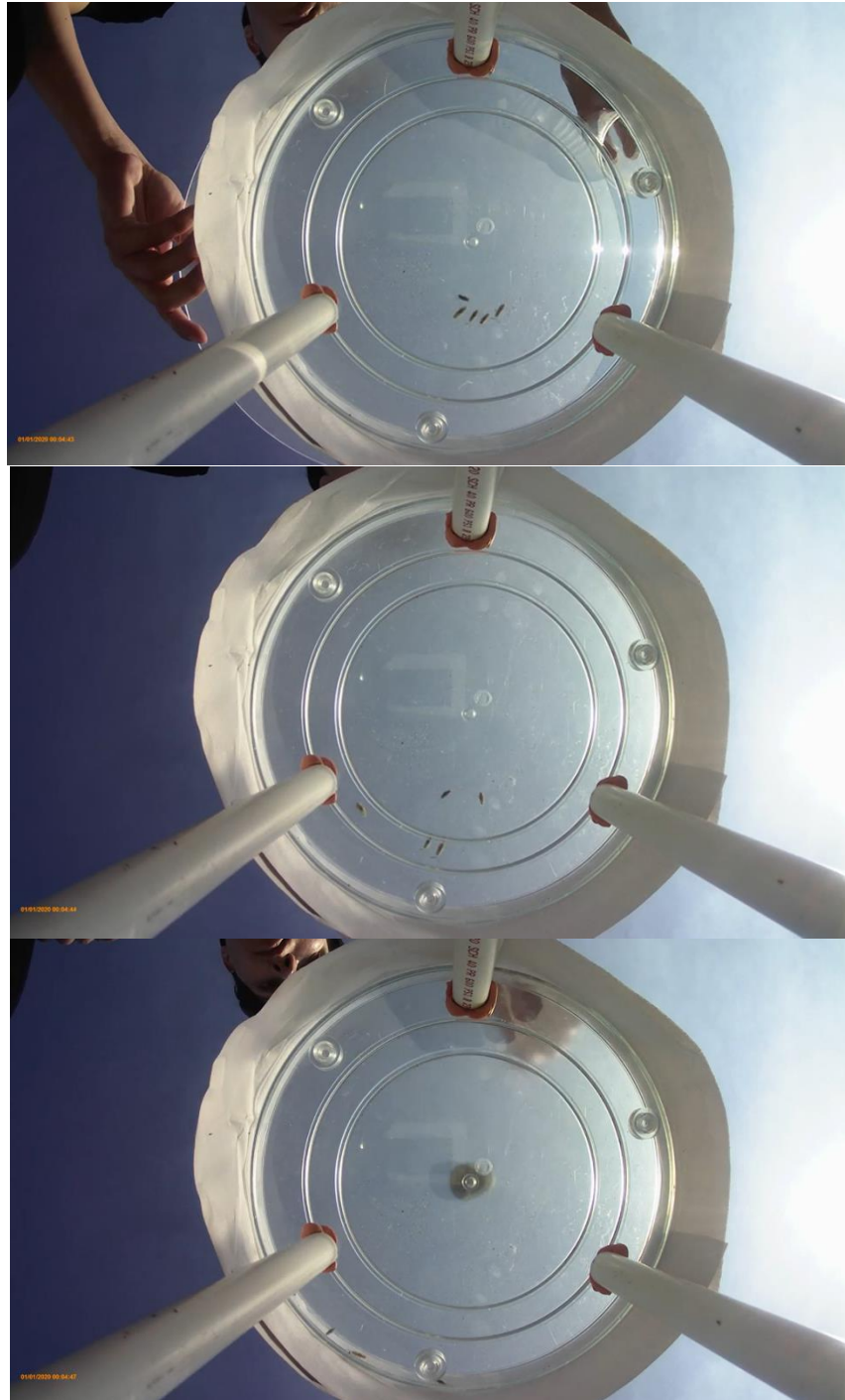


Figure 2. Progression of a GS5 trial as seen by the camera. In order, the photos show the animals just after release (top), mid-path (middle), and reaching the wall of the arena (bottom). Two of the animals are obscured by the left-most pole in the final photo.

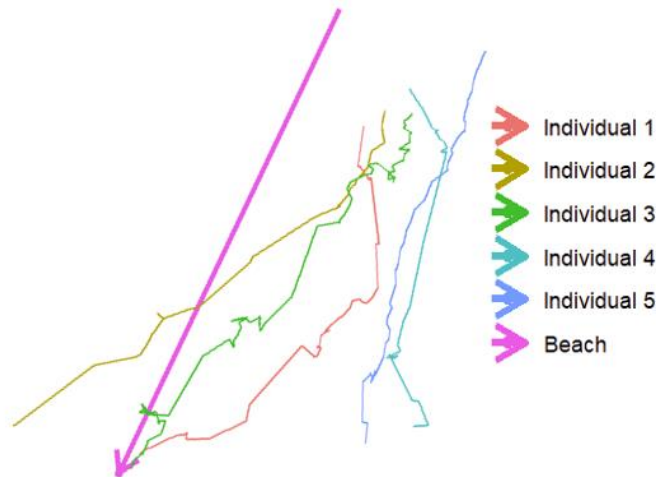


Figure 3. Digitization of the same trial as Figure 2. The pink arrow shows the direction of the wrack line, and each color represents the path of an individual animal. The center of the arena is located at the top of the figure and the closest edge of the arena is located at the bottom.

For our analysis, we used five metrics to assess orientation. The first was latency, which refers to the time that elapsed between when I lifted the cup and when the animal started moving. The second metric was speed, which we found by measuring the length of the animal's entire path and then dividing by the time between when the animal started moving and when it reached the wall of the arena. The third metric was sinuosity, which is a measure of the straightness of the animal's movement. A high sinuosity indicates a straighter path while a low sinuosity indicates a curved path. The fourth metric was the heading of each animal as it hit the wall of the arena. We chose that moment because we expect the animals to be orienting themselves as they move across the arena; by the time they reach the wall, we predict that they should be heading in the direction of the wrack line. The fifth and final metric was the mean resultant vector of each trial. This is a metric from circular statistics which quantifies how concentrated the animals in each trial are around their average heading. In other words, it is a measure of the variance of the group's headings. For example, a low mean resultant vector would mean that the animals in each

trial had headings that were very different from each other, while a high mean resultant vector would indicate that the group's headings were close together.

The lack of independence of our data points prevented us from using traditional statistical tests. For example, we couldn't treat the speed of each animal in a GS10 trial as independent because the animals may be affecting each other's movement. Instead, we used a nonparametric bootstrapping method to create a null distribution of our GS1 animals to compare our other group sizes against. See Granger et al. 2020 for more details on this statistical method. In short, we used the GS1's to create an underlying distribution for the animal's behavior when there were no group effects to compare with the results from when there were group effects.

To begin, we randomly resampled (with replacement) GS1 trials and took the mean of each of our metrics (latency, speed, sinuosity, final heading, and mean resultant vector) across these subsamples. We sampled five trials when comparing to the GS5 trials and ten when comparing to the GS10 trials. We then repeated this 1,000 times. This created "null" distributions for each of our metrics (i.e., the average of each metric for a comparable group size when we know there were no inter-individual interactions). We could then compare the averaged metrics from the GS5 and the GS10 trials, to these null distributions. For example, we sampled five GS1 trials (with replacement), took the average of their speed, and then repeated that process 1,000 times to create a distribution of the average speed for a sample of five individuals with no interindividual interactions. When we had that null distribution, we then took the average of the speeds of all the GS5 animals and compared that mean to the GS1 null distribution. Our "p value" was a measure of how likely it is how likely it is that the GS5 average came from the null distribution. In other words, a p-value of 0.05 means that there was only a 5% chance that the GS5 value came from the same underlying distribution as the null. This was calculated as $1 -$

$\frac{1 + \sum(\text{Null Distribution} \geq \text{mean}(GS5))}{1 + \sum(\text{Null Distribution})}$ or, the sum of the values from the null distribution which were

greater than or equal to the value of the mean of the comparison distribution.

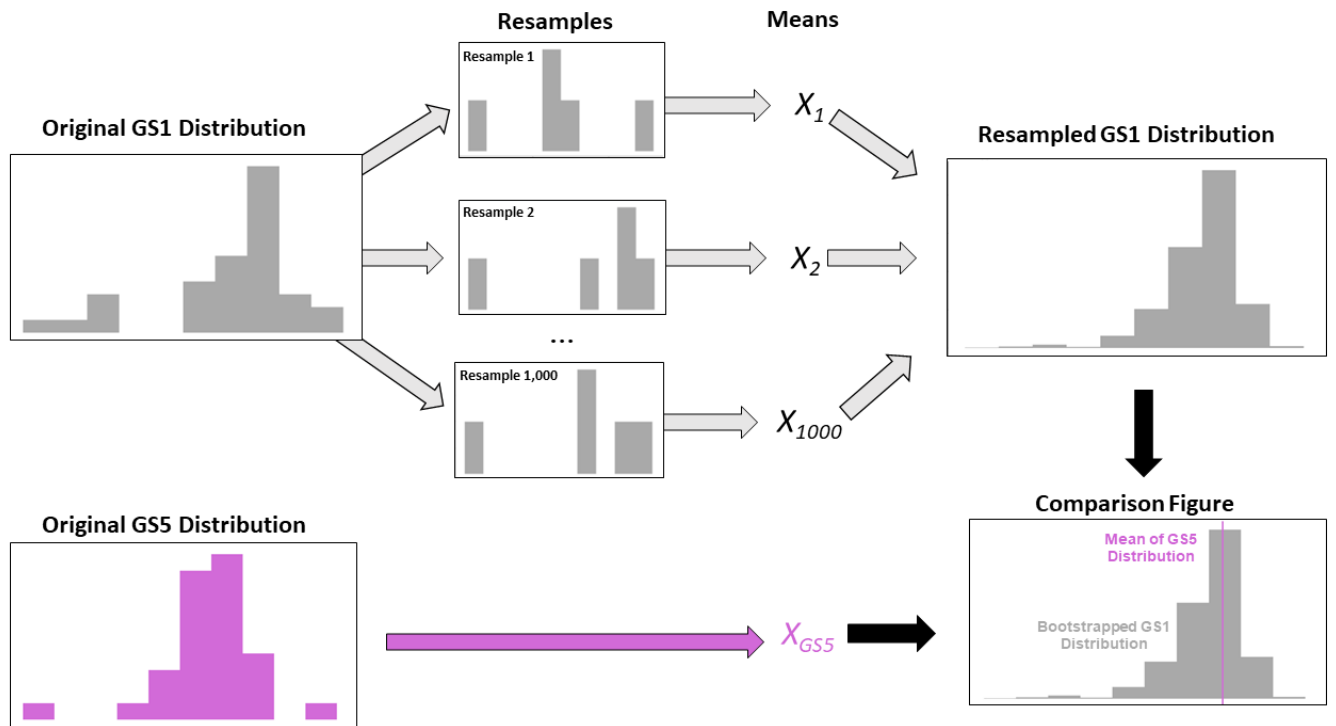


Figure 4. Overview of the resampling procedure that was used to compare GS1 metrics to GS5 and GS10. This is not meant to represent the data for any specific metric but instead illustrates the general process. Note that the methods of analyzing GS5 and GS10 were the same.

The comparison of the GS10 to the GS1 trials was conducted in the same way, with the only difference being that ten GS1 trials were subsampled instead of five. This was then repeated for each variable of interest (speed, sinuosity, direction, variance, and latency).

Based on the potential impact of weather, a second round of analysis was done only with trials that were conducted during “Sunny” weather. The outcomes are discussed briefly in the Results section and are shown fully in the Appendix.

Conceptualization of this statistical method was done by Jesse Granger with the assistance of Dr. Robert Fitak. All code for the statistical analyses was written by me and Jesse Granger in R Statistical Software (v4.2.3, R Core Team 2023).

Results

In our trials, *Megalorchestia* showed significant evidence of unimodal distribution in their final headings across every group size (Rayleigh test, $p < 0.001$ for all GS). The direction of the wrack line (“0” radians) was included within the 95% confidence interval of the mean headings for all group sizes (Fig. 5).

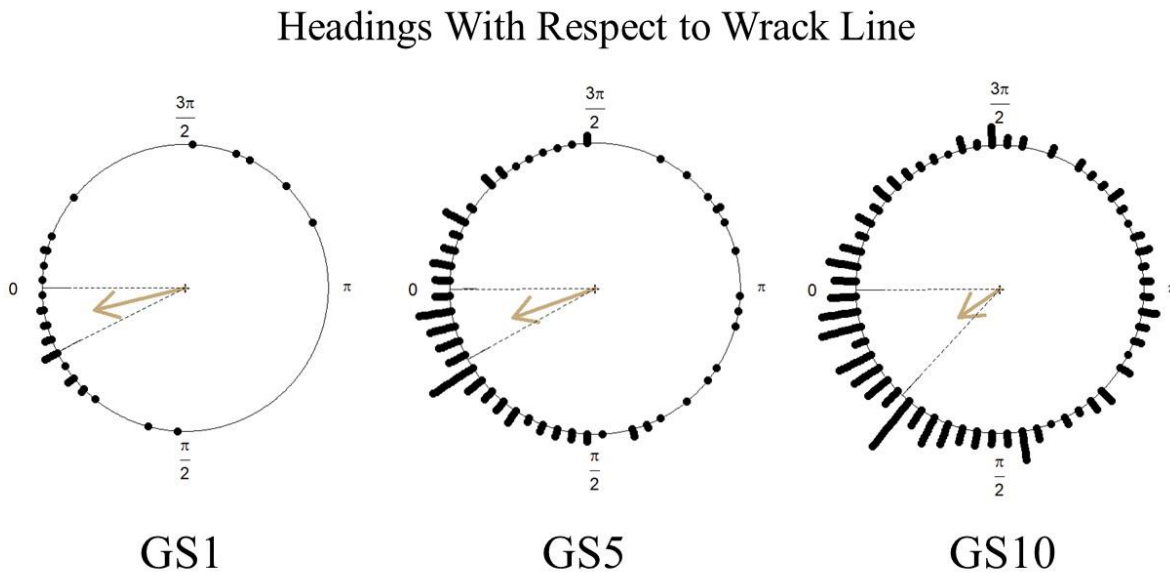


Figure 5. Circular distribution of the final headings (when they hit the wall of the arena) with respect to the wrack line. In this figure, “0” is the direction of the wrack line. The brown arrows point towards the mean heading of each group size and the lengths of the arrows represent the mean resultant length (a measure of concentration) of each distribution. The dotted lines are the bounds of the 95% confidence interval of the mean headings for each group size.

We determined that both GS1 and GS5 were not orienting towards any feature of the arena by ensuring that their distribution with respect to the arena was not significantly different

from a uniform circular distribution (Rayleigh test, $p > 0.05$). However, this was not true for the GS10 animals (Rayleigh test, $p = 0.003$).

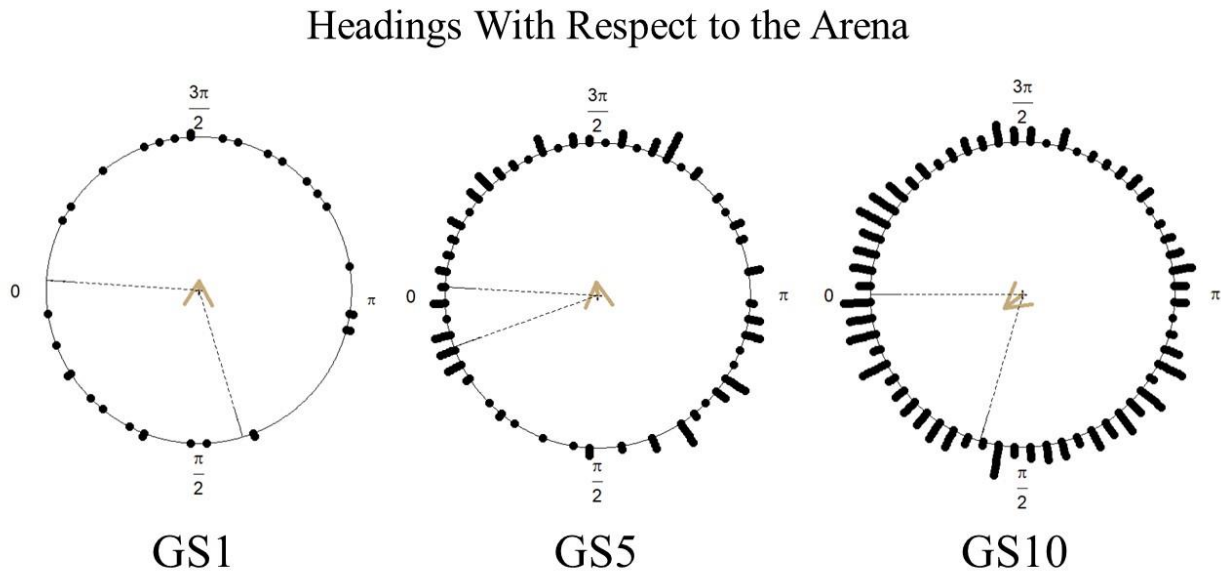


Figure 6. Circular distribution of the final headings with respect to the arena. “0” is the same point on the arena. Note that the “0” is not the same “0” as Figure 5 since the arena was rotated each day. The brown arrows point towards the mean heading of each group size and the lengths of the arrows represent the mean resultant length (a measure of concentration) of each distribution. The dotted lines are the bounds of the 95% confidence interval of the mean headings.

We observed significant differences in latency time between GS1 and GS5 ($p < 0.001$), as can be seen in Figure 7A. The mean latency time of GS5 was below the latency time of any of the bootstrapped GS1 samples. For our comparisons between GS1 and GS10, we saw the same pattern. The mean latency time of the GS10 trials were significantly lower than that of the bootstrapped GS1 samples ($p < 0.001$).

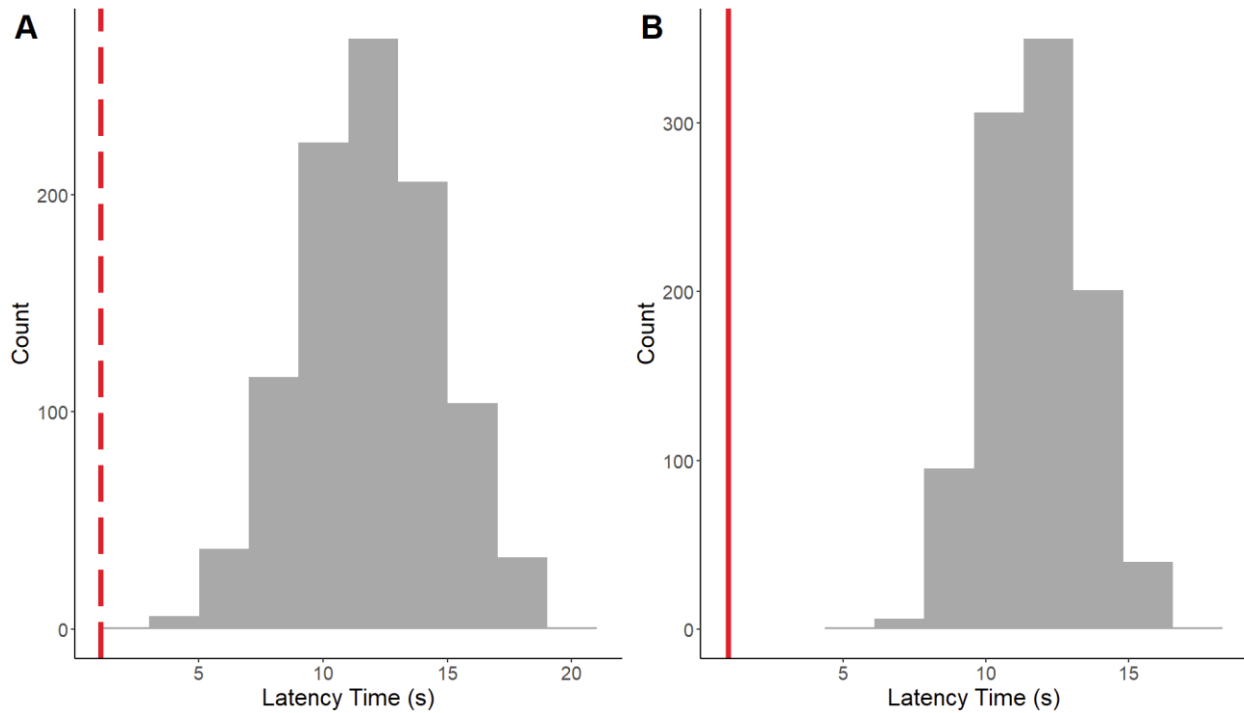


Figure 7. A) Comparison of the bootstrapped GS1 latency time distribution (gray histogram) with the mean GS5 latency time (red dotted vertical line). B) Comparison of the bootstrapped GS1 latency time distribution (gray histogram) with the mean GS10 latency time (red solid vertical line).

We did not find significant differences in speed between GS1 and GS5 ($p = 0.28$).

Dissimilar to latency time, the mean speed of GS5 was clearly contained within the bootstrapped GS1 distribution (Fig. 8A). However, we did see a significant difference between the speeds of GS1 and GS10. The mean speed of the GS10 trials were significantly lower than that of the bootstrapped GS1 samples ($p < 0.001$).

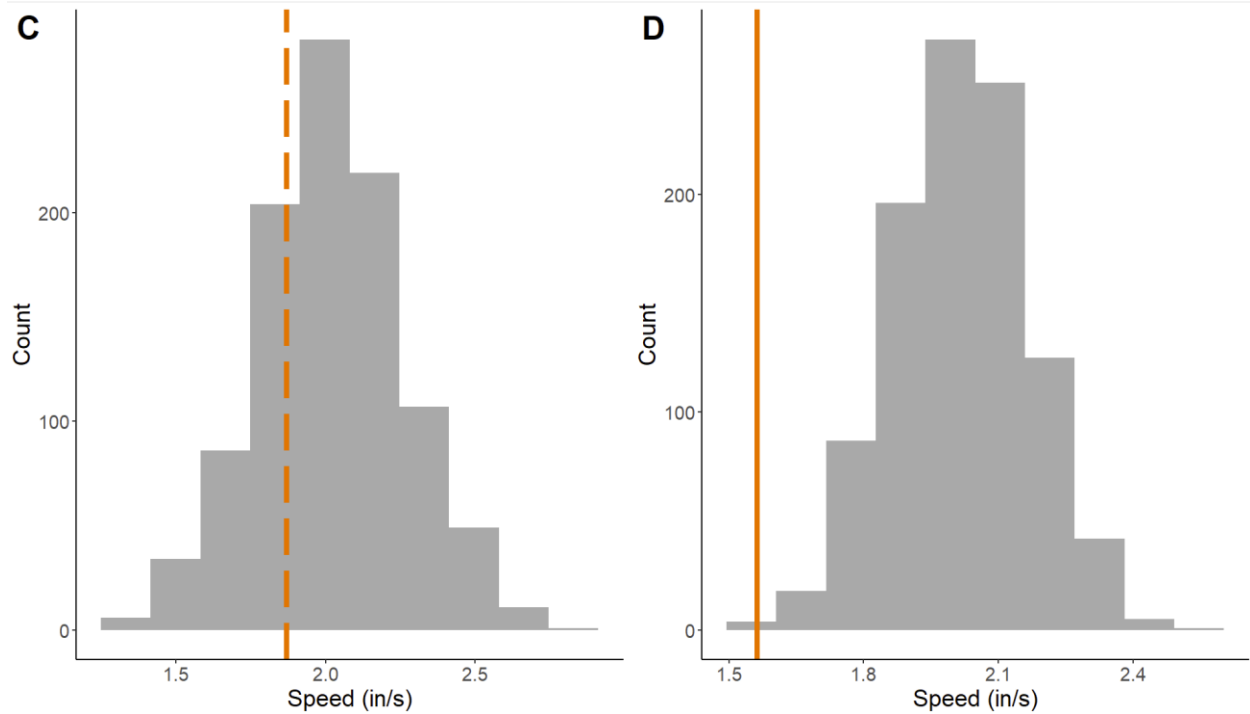


Figure 8. A) Comparison of the bootstrapped GS1 speed distribution (gray histogram) with the mean GS5 speed (orange dotted vertical line). B) Comparison of the bootstrapped GS1 speed distribution (gray histogram) with the mean GS10 speed (orange solid vertical line).

We did not find significant differences in sinuosity between GS1 and GS5 ($p > 0.05$).

Like with speed, the mean sinuosity of the GS5 trials was located within the bootstrapped GS1 distribution (Fig. 9A). We also did not see a significant difference between the sinuosity of GS1 and GS10, though our reported p-value was on the verge of significance ($p = 0.051$).

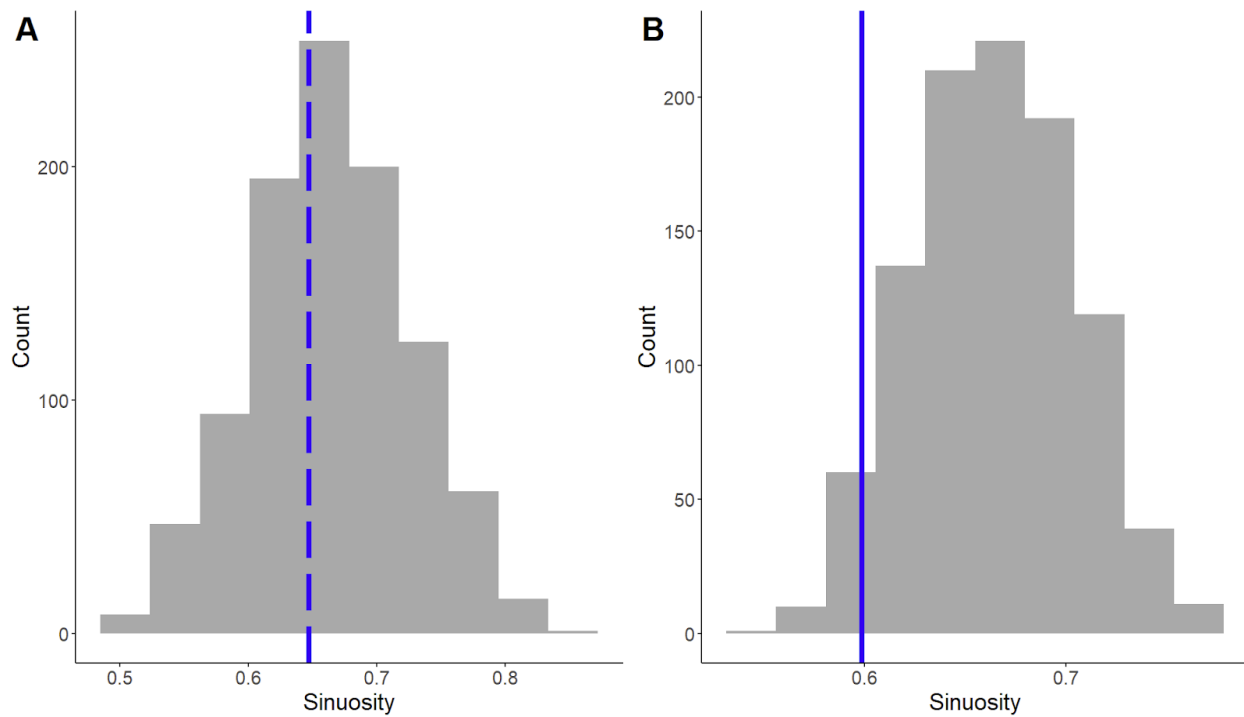


Figure 9. A) Comparison of the bootstrapped GS1 sinuosity distribution (gray histogram) with the mean GS5 sinuosity (blue dotted vertical line). B) Comparison of the bootstrapped GS1 sinuosity distribution (gray histogram) with the mean GS10 sinuosity (blue solid vertical line).

We did not find significant differences in the final headings between GS1 and GS5 ($p = 0.36$), as the mean heading of the GS5 trials was contained within the bootstrapped GS1 distribution (Fig. 10A). However, we did see a significant difference between the final headings of GS1 and GS10 ($p = 0.046$). The mean GS10 final heading was located within the “larger” end of the bootstrapped GS1 distribution; as the heading is measured in radians, and so in circular space, “higher” indicates a heading that is further away from the direction of the wrack line (0 radians).

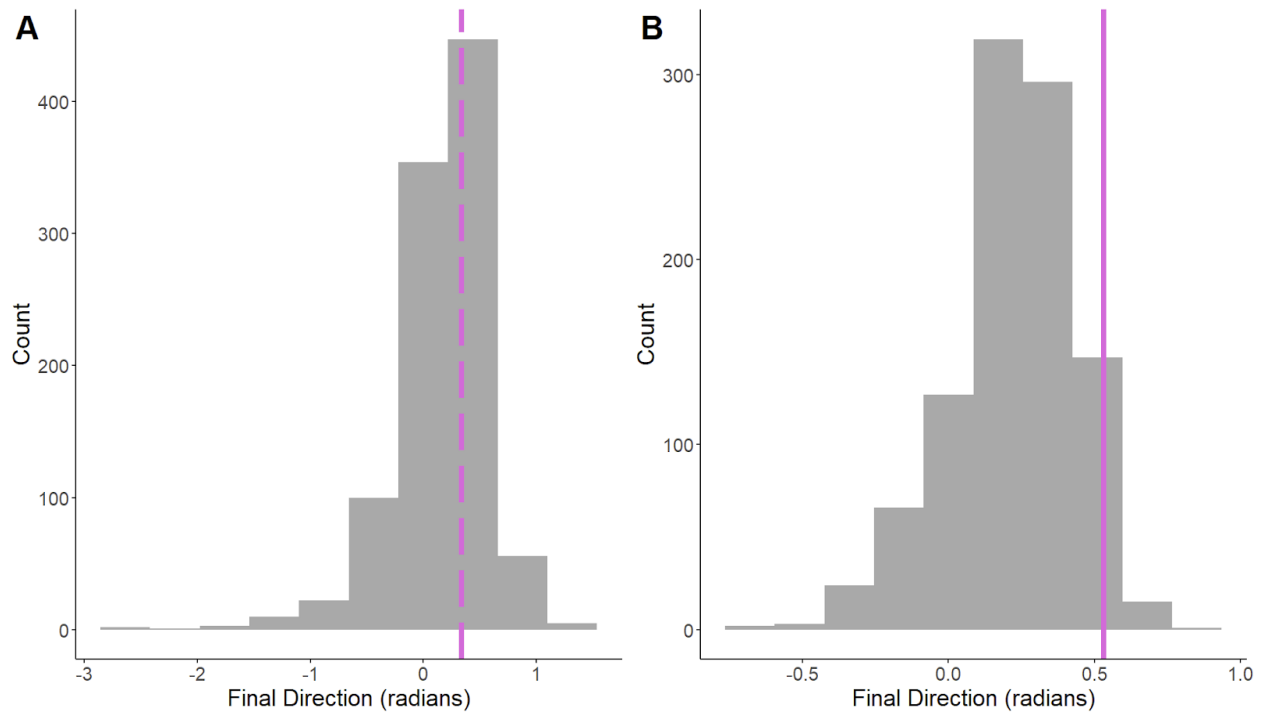


Figure 10. A) Comparison of the bootstrapped GS1 final heading distribution (gray histogram) with the mean GS5 final heading (pink dotted vertical line). B) Comparison of the bootstrapped GS1 final heading distribution (gray histogram) with the mean GS10 final heading (pink solid vertical line).

We did not find significant differences in the mean resultant vectors between GS1 and GS5 ($p = 0.58$) or between GS1 and GS10 ($p = 0.32$). The means of both the GS5 and the GS10 trials were clearly contained within the bootstrapped GS1 distributions (Fig. 11A).

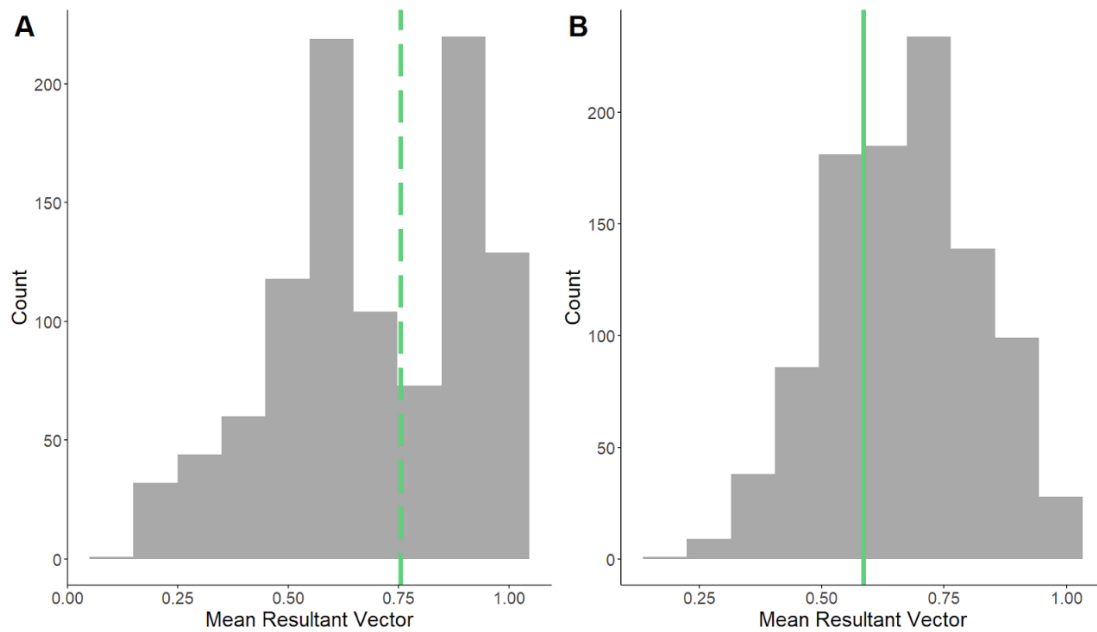


Figure 11. A) Comparison of the bootstrapped GS1 mean resultant vector distribution (gray histogram) with the average GS5 mean resultant vector (green dotted vertical line). B) Comparison of the bootstrapped GS1 mean resultant vector (gray histogram) with the average GS10 mean resultant vector (green solid vertical line).

After initial analysis, we discovered that the majority of our GS10 trials were conducted on cloudy days (Fig. 12A). This was distinct from the GS1 and GS5 trials, most of which were done on sunny days. Based on knowledge of talitrid amphipods' sun compass, we decided to investigate the effect of weather on our results. Overall, we saw that cloudy weather was generally detrimental to the animals' orientation. For example, we found that animals tended to be slower on cloudy days than on sunny days (Fig. 12B). Therefore, we decided to repeat our analyses with only the trials that were conducted on sunny days.

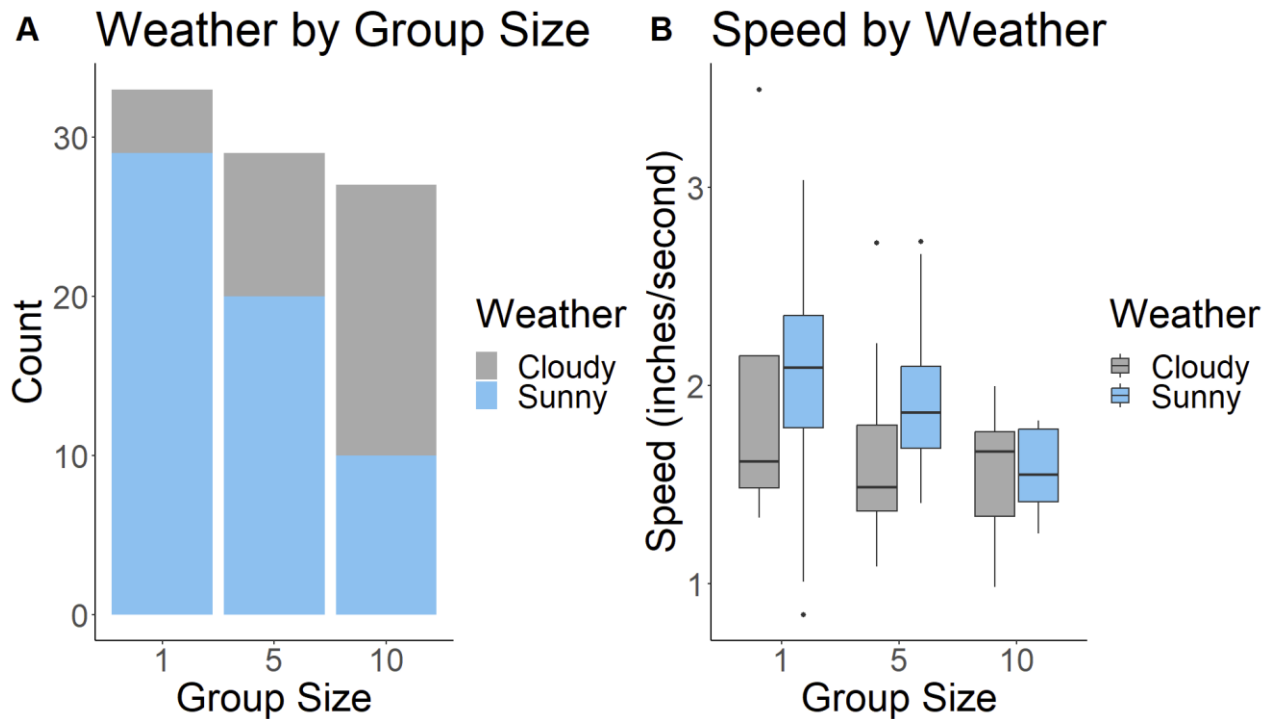


Figure 12. A) Distribution of the weather when trials were conducted (across group size). B) Boxplot of the average speed of each trial across group size and weather.

We found the same results as reported previously. Latency time was significantly different between GS1 and GS5 as well as between GS1 and GS10; GS10 was significantly slower than GS1; and the final headings of GS1 and GS10 were significantly different from each other (see Appendix). Noticeably, there was no significant difference between the sinusities of GS1 and GS10. This implies that our previous result of near significance between the sinusities of GS1 and GS10 (Fig. 8B) was likely driven by the weather when the GS10 trials were conducted, and not by a true difference between the group sizes.

I have chosen to focus my interpretation on the initial round of analysis due to the limited sample size in the secondary round (n=9 for GS10) and the overall consistency of our results between the two rounds.

Discussion

Our results are the first evidence of wrack-line orientation in the genus *Megalorchestia*. This adds *Megalorchestia* to the growing list of talitrid amphipods that have documented abilities to orient along the beach. Our results are focused on the genus's ability to orient in a group, but our results leave open the possibility for future research to further explore their orientational abilities, such as how it may change with varying levels of human disturbance to the beach.

We found that our GS10 animals exhibited some potential orientation towards the arena. However, this is likely an artifact of many GS10 trials being conducted on the same days as each other and the fact that I did not rotate the arena between trials on the same day. Therefore, the observed orientation "towards the arena" would be driven by their orientation towards the wrack line. In future work, I will ensure that the arena is rotated between every trial to prevent a similar outcome.

We observed that *Megalorchestia* orients slower and in a different direction when they are among other conspecifics versus when they are alone. This is the first evidence of differences between individual and group orientation in any talitrid amphipod. Our results conflict with Scapini et al. (1981), implying that our expanded list of orientational metrics and improved technology are able to uncover details of orientation in ways that were not possible when Scapini et al. was conducted. This is similar to previous research on pigeons where analysis of vanishing bearings alone was unable to show group influence, but studying the entire track of the animals provided the additional data to prove flocking was beneficial.

It is important to note that Scapini et al. used the species *Talitrus saltator*, which is not a member of our study genus *Megalorchestia*; it is unknown whether we have uncovered a difference between these two taxa or whether our results would hold consistent across genera. Therefore, a similar experiment to ours should be conducted in other talitrid amphipods before our conclusions about the differences between individual and group orientation are assumed to be true across the taxa. If our results prove to be broadly applicable, this has implications for all talitrid amphipod orientation research. The current standard research protocol is to use ten individuals in one trial and analyze each of their orientations as independent; however, our study implies that the orientations of these animals are not truly independent. Future researchers should use our results to judge carefully whether their research question would be better suited to trials with individual or group trials.

In terms of collective navigation, we did not observe the pattern that we had expected. Using the examples of pigeons and other animals, we had predicted that the groups of *Megalorchestia* would orient more successfully than the individuals. We had based our definition of “successful” orientation on the ways we have seen flocks of pigeons improve: increased speed, straighter paths, and more directed flights (Pettit, 2015). Instead, we saw no difference between the individual animals and our intermediate group, while our largest group exhibited decreased speed and orientations that were the furthest away from the wrack line. The reasons behind this break with our expectations are unclear.

It should first be noted that the largest group’s orientation away from the wrack line may not truly be evidence for *worse* orientation. GS1, GS5, and GS10 all exhibited directional orientations that were not pointed directly towards the wrack line. Instead, they were pointed slightly towards the “left” side of the beach (as seen when looking towards the water). We saw

this to the largest degree in the GS10 trials. Our current theory to explain this phenomenon is that the animals were orienting towards the shadows of the arena (as well as towards the wrack line). Based on the timing of all our trials, the “left” side of our arenas was slightly more shaded than the “right” side of the arenas. The animals may be attempting to simultaneously return to the wrack line and protect themselves from the desiccating effects of the sun. Therefore, the direction of the GS10’s orientation may not be evidence for *worse* orientation; instead, it is potential evidence for arguably *better* orientation towards the combination of shade and wrack line. However, our results only demonstrate that the orientational directions of large group sizes are *different* from individual animals; future research is needed to explore the exact form that this difference takes. For example, a study could conduct all their trials when the sun is directly overhead, removing the potential impact of the shadows.

The results pertaining to speed are less complex than those of the orientational direction. We clearly saw that the largest group size moved slower than the individual animals. It is difficult to explain why this is the case, as the field of collective navigation has focused almost entirely on the potential benefits of moving in a group and has spent very little effort exploring the potential downsides. As the most comprehensive review of collective navigation writes: “we note that for the most part we, and the field in general, focus particularly on [the] positive outcomes” of collective navigation (Berdahl et al., 2018). The resulting literature on the downsides of collective navigation is sparse. There is some evidence that small or intermediate-sized groups should theoretically be better navigators than both individuals and groups with too many members (Kao and Couzin, 2014), but that does not appear to be applicable to our case, as our intermediate-sized group (GS5) did not outperform our individual animals. Anecdotally, we observed that there were several “outlier” animals in our GS10 trials who would head in a

slightly different direction and with slower speeds than the rest of the group. Our heading and speed results may be partially caused by these animals. It is unknown why they may choose to break with the group; they may be worse orientators, or perhaps there are social dynamics at play that I was unable to recognize. Altogether, future research should attempt to understand the driving forces behind the slower speeds of our large group.

Our latency time results may also play a role in the differences in speed that we saw. We observed that the individual animals took much longer to start moving at the beginning of the trial than our groups, but then were faster once they started moving. It is possible that the individual animals are taking this time to orient themselves and they are able to move confidently and efficiently in the correct direction once they have made up their mind. In contrast, the groups that started moving immediately may be more uncertain about the direction of the wrack line and therefore take more time. This theory potentially links our speed and latency time results. However, there are many other explanations for our differences in latency times. The animals are contained in a small space (the cup) for the two minutes prior to release, and so the constant interactions with other animals in this space may be more stimulating than the experience of the animals that are alone. Alternatively, there may be social differences (such as age, sex, or size) driving them to distance themselves from each other as fast as possible. Lastly, this could be a predator avoidance strategy. Individual talitrid amphipods may rely more on staying still and camouflaging than a group who has safety in numbers. Although I have given several potential explanations for our latency results, this is not an exhaustive list. Future research is needed to further explore the drivers of this behavior.

It is important to keep in mind the limits and potential sources of error of our study. First, as this is a manipulative study, it is possible that we may have skewed our results through our

capturing of animals. We attempted to randomly sample from the available population on the beach, but it is unknown whether our trials truly represented the demographic makeup (such as age or sex) of animals that would normally orient together. Additionally, it is possible that we may have unintentionally sampled more than one taxon. The genus *Traskorchestia*, another talitrid amphipod, is known to sometimes cohabitate with *Megalorchestia* on the beaches of central Puget Sound. From our videos, it is impossible to obtain enough resolution to confidently identify the genus of all our animals. This potential mixture of genera is a possible explanation for why we did not see the orientation results we expected; however, if we do have some animals from *Traskorchestia*, it still may not be the entire explanation of our results. Nothing is known about how these genera interact with each other, and so we do not know if mixing them in our trials would lead to worse orientation. Instead, their simultaneous presence on the beach may mean that the two genera are in fact able to use each other to help in their orientation. To answer these queries, future field work should preserve all individuals so that their species and sex can be definitely determined.

With regards to our data analysis, our current approach renders us unable to directly compare our GS5 distributions to our GS10 distributions. Based on the fact that the GS1 and GS5 groups showed no differences from each other, we can infer that comparing the GS5 and GS10 would show the same results as comparing GS1 and GS10. However, this may not be the case for future results, and so we will continue to explore potential alternatives. Additionally, our analysis thus far has focused only on the path of the animals prior to when they hit the wall of the arena. As we move forward with the project, we intend to expand our analyses to include the later part of their movement. This will enable us to answer questions such as whether the increasing threat of desiccation over the course of the entire trial led the animals to resume their

movement towards the wrack line. We also have yet to analyze how the time of day affected our trials; similar to the length of trials, the increased sun and heat of midday may have impacted the motivation of the animals to return to their burrows.

Above, I have identified several future courses of study, both for ourselves and for other researchers. We intend to continue this project by adding to our sample size of trials, especially by supplementing the number of GS10 trials conducted on sunny days. This will help us definitively separate out the effects of weather from group size on the orientation behavior of talitrid amphipods. Overall, our study provides the first evidence for orientation in *Megalorchestia* and a difference in orientational abilities between individuals and groups in talitrid amphipods.

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Appendix

Sunny-Only Analysis

Table of Results

Metric	Comparison	P-Value
Latency Time	GS1 v GS5	< 0.001
	GS1 v GS10	< 0.001
Speed	GS1 v GS5	0.270
	GS1 v GS10	0.001
Sinuosity	GS1 v GS5	0.367
	GS1 v GS10	0.600
Final Heading	GS1 v GS5	0.396
	GS1 v GS10	0.038
Mean Resultant Vector	GS1 v GS5	0.622
	GS1 v GS10	0.557

ANOVA Results

I conducted my initial data analysis using one-way ANOVAs (or the nonparametric versions) and step-down tests to compare several metrics across group sizes. To account for lack of independence between animals in each trial, I took the average metric of all animals in each trial and used those data as the input to my tests. This approach had the advantage of allowing me to compare GS5 directly to GS10. Although this was not the final statistical method that we chose, I report these results as proof that I could reach the same conclusions through multiple different avenues.

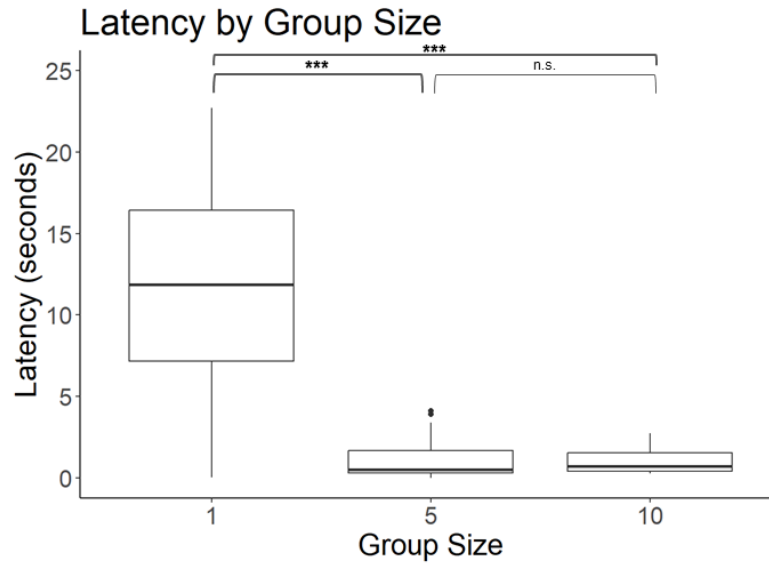


Figure S1. A boxplot of the average latency times for each trial (across group size). According to a Kruskal-Wallis test, the median latency times were significantly different between groups ($p=0.00$), and step-down Wilcoxon rank sum tests concluded that the medians of the GS1 vs GS5 ($p=0.00$) and GS1 vs GS10 ($p=0.00$) were significantly different from each other. The median latency times for GS5 vs GS10 were not significantly different from each other ($p=0.42$).

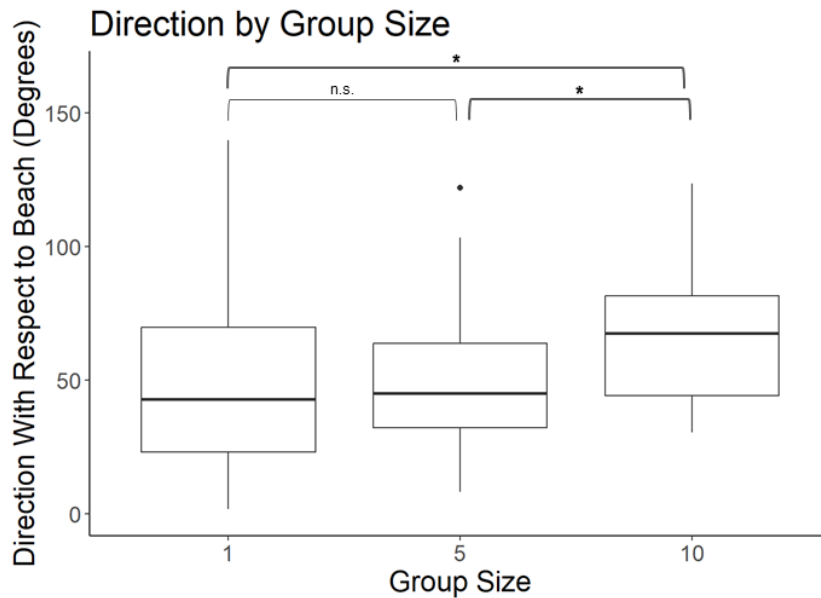


Figure S2. A boxplot of the average headings with respect to the wrack line for each trial (across group size). According to a Kruskal-Wallis test, the median headings were significantly different between groups ($p=0.02$), and step-down Wilcoxon rank sum tests concluded that the medians of the GS5 vs GS10 ($p=0.03$) and GS1 vs GS10 ($p=0.03$) were significantly different from each other. The median headings for GS1 vs GS5 were not significantly different from each other ($p=0.69$).

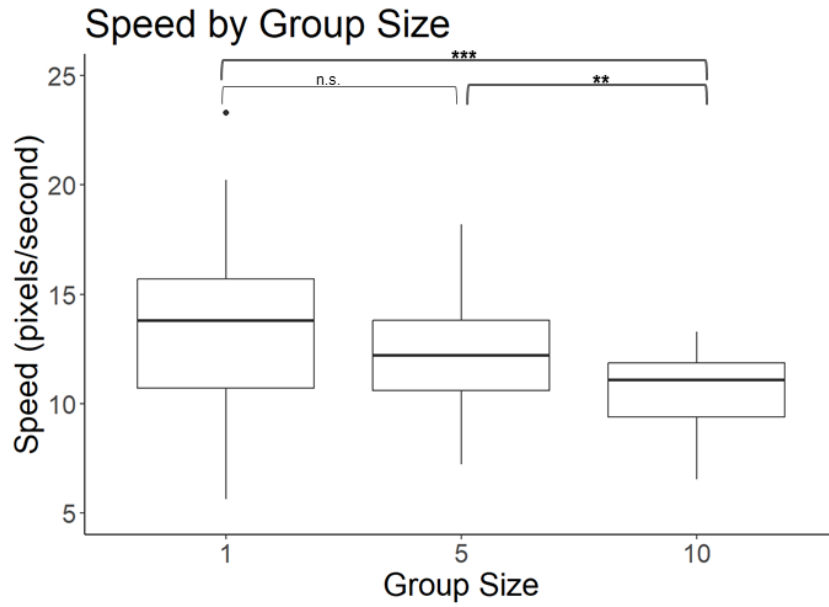


Figure S3. A boxplot of the average speeds for each trial (across group size). According to a Welch ANOVA, the mean speeds were significantly different between groups ($p=0.00$), and step-down Games Howell tests concluded that the means of the GS1 vs GS10 ($p=0.00$) and GS5 vs GS10 ($p=0.01$) were significantly different from each other. The average speeds for GS1 vs GS5 were not significantly different from each other ($p=0.50$).

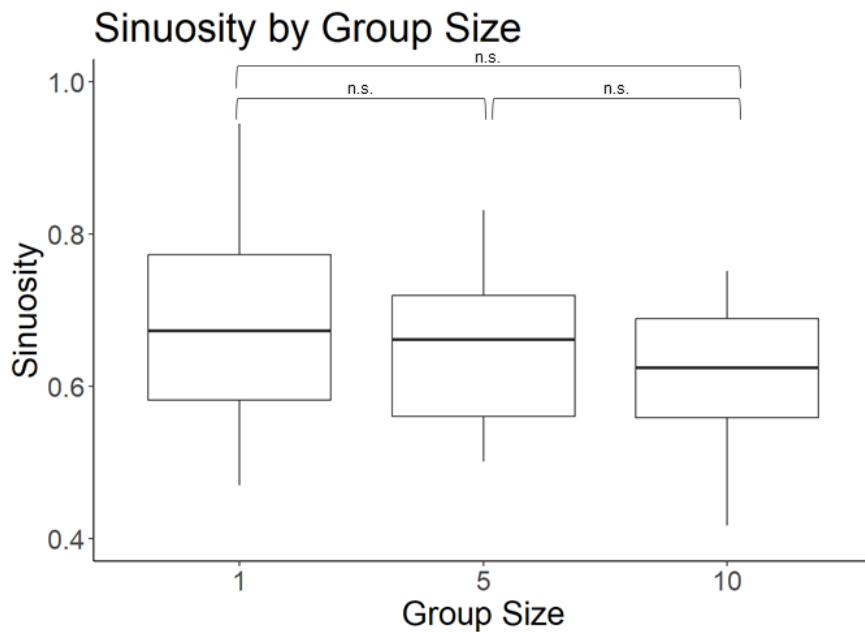


Figure S4. A boxplot of the average sinuosities for each trial (across group size). According to a one-way ANOVA, the mean sinuosities were not significantly different between groups ($p=0.11$).

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