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The effects of ocean acidification on the food location behavior and locomotion of *Pagurus longicarpus*

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Summary

Although the mechanism is unknown, recent studies have indicated that seawater with reduced pH may affect the behavior of marine species. Ocean acidification resulting from increasing amounts of carbon dioxide released into the atmosphere from car exhausts, factories, and other sources may therefore affect a marine animal's food location ability and locomotion. In this experiment, the effect of seawater at different pHs on the food location behavior and locomotion of the long-clawed hermit crab (*Pagurus longicarpus*) was assessed. The hermit crabs were exposed for 5 and 19 days to seawater with pH levels predicted to be equivalent to the worldwide average pH in approximately 100 and 150 years (pH 7.85 and 7.75, respectively), as well as to seawater at the worldwide pH found today (approximately pH 8). On average, the hermit crabs traveled significantly faster in tests with food present than in tests with no food available. There was no statistically significant effect of lowered pH water on hermit crabs' average time to reach a piece of food, their speed, or their path length. Hermit crabs kept in lowered pH water for 5 days did take longer to reach a piece of food than their counterparts kept in untreated seawater, although the differences were not significant. Thus, it cannot be concluded that decreased pH has an effect on hermit crabs' food location behavior and locomotion. This is not surprising since they tend to inhabit intertidal zones and estuaries where they withstand a wide range of environmental conditions. Further studies, including those with longer treatment durations and a wider range of pHs, are needed to determine if and how ocean acidification will alter hermit crabs' food location abilities.

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Introduction

Today, global warming is of great concern as carbon dioxide emissions are released into the atmosphere and subsequently trap the sun's heat. Carbon dioxide emissions will likely have a huge effect on the oceans as well. As atmospheric carbon dioxide is absorbed by the oceans, a series of reactions occurs, which results in an increase in the concentration of hydrogen ions ([H+]) in the water and thus decreases the pH of the water (1).

As ocean acidification progresses, marine animals will have varying degrees of success in living with the change in pH. Studies have already shown that pH negatively affects calcification in mollusks and corals (2). While very little is currently known about the potential effects of ocean acidification on animals' senses and behavior (1), recent studies have begun to provide insight on the subject. For example, a 2010 study showed that the behavior of clownfish larvae, a tropical animal that has a very low tolerance for pH change, is affected by decreased pH (3).

Gathering information from its environment is a critical ability for any animal. Many aquatic animals depend upon their olfactory sense (*i.e.*, chemoreception) to gather vital information, such as food location. Chemoreception is especially important for aquatic animals because of the difficulty of detecting visual signals in murky water (1). If the olfactory senses of aquatic animals were impaired by a reduced pH, it could have a major effect on the organisms' ability to locate their food.

The long-clawed hermit crab, *Pagurus longicarpus*, lives in waters from within the intertidal zone to as deep as 200 m in the ocean along the east coast of North America from Texas to Nova Scotia (4). It is a common,

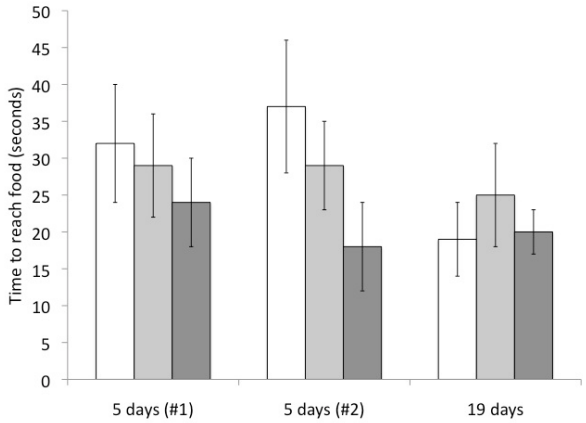

Figure 1: The mean time (± standard error (SE)) after release taken by hermit crabs from each set to reach the food at the other end of the flume. The white columns indicate the groups of hermit crabs exposed to water of pH 7.75; the light gray columns indicate the groups of hermit crabs exposed to water of pH 7.85; the dark gray columns indicate the groups of hermit crabs exposed to untreated water.

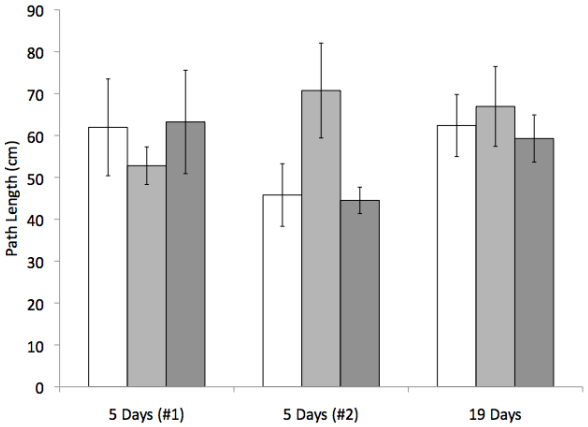

Figure 2: The mean length (± SE) of the hermit crabs' paths taken after release in tests with food in flume-square-sides (approximately 1.9 cm). The white columns indicate the groups of hermit crabs exposed to water of pH 7.75; the light gray columns indicate the groups of hermit crabs exposed to water of pH 7.85; the dark gray columns indicate the groups of hermit crabs exposed to untreated water.

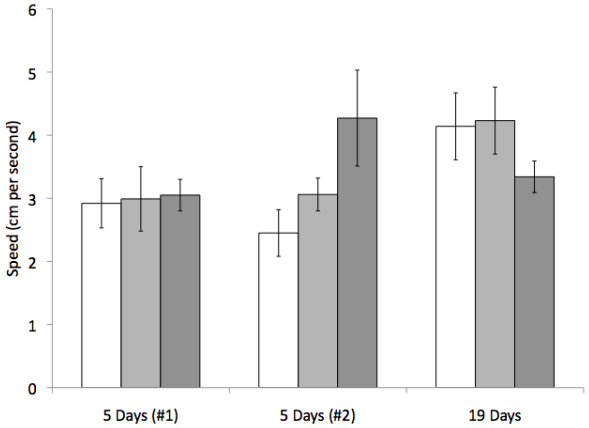

Figure 3: The mean speed (± SE) of hermit crabs after release in tests with food in flume-square-sides (approximately 1.9 cm) per second. The white columns indicate the groups of hermit crabs exposed to water of pH 7.75; the light gray columns indicate the groups of hermit crabs exposed to water of pH 7.85; the dark gray columns indicate the groups of hermit crabs exposed to untreated water.

easily hand-collected, scavenging decapod crustacean. Like all hermit crabs, *P. longicarpus* is dependent on the chemoreceptors on its two pairs of calcified antennae to detect water-borne chemicals from distant food sources (1). *P. longicarpus* is able to tolerate a wide range of changes in temperature, salinity, and pH (4) and can survive in confined areas with minimal water circulation, such as tide pools, where respiration can result in an increased concentration of carbon dioxide in the water. Due to its ability to cope with large daily fluctuations in pH, *P. longicarpus*, along with its fellow intertidal organisms, may be among the marine organisms best prepared to handle the decrease in pH that comes with ocean acidification. However, the effect of a long-term decrease in pH on *P. longicarpus* is not known, and ocean acidification may impair the crabs' food location abilities despite the crabs' ability to survive in water that exhibits quick fluctuations in pH (6).

This experiment examined the effects of 5- and 19-day exposure to decreased pH levels, which mimics ocean acidification, on the food location ability and locomotion of *P. longicarpus*. The mean time to reach the food, the mean path length, and the mean speed were compared between 3 treatment sets. The results indicated that the decreased pH levels of the treatment groups did not have a statistically significant effect on the hermit crabs' food location behavior and locomotion.

Results

The pH level appeared to affect the time that it took for the hermit crabs to reach the food (**Figure 1**). For the two 5-day treatment sets, the time to reach the food increased as the pH decreased, and crabs kept in untreated water (pH 8.10) took the least amount of time to reach the food. However, the differences were not statistically significant ($p > 0.05$). Furthermore, the pH did not seem to affect the crabs' time to reach the food for the 19-day treatment sets.

The pH level did not appear to have a consistent effect on mean path length (**Figure 2**). While there was a reverse trend between the two 5-day treatment sets, no group was significantly different from the rest (*i.e.*, all $p > 0.05$). However, there was a consistent difference between the path length of each group with and without food for the 5-day treatment sets (data not shown). With the exception of two 19-day groups, the hermit crabs traveled significantly farther without food than with food.

The pH level also did not have an effect on mean speed when food was present (**Figure 3**). Although crabs from the second 5-day treatment set followed a more extreme pattern than the first 5-day treatment set, no group in either set exhibited a statistically significant effect on mean speed when food was present ($p > 0.05$). There was also no obvious pattern between tests with food and tests without food. However, the hermit crabs from each group always traveled faster with food than without food (**Figure 4**).

Discussion

The range of pH treatments (pH 7.75 – pH 8.10) was well within the pH range to which *P. longicarpus* is exposed in its natural habitat. In July 2011, pH levels of 7.1 – 8.5 were recorded by monitoring buoys at the Waquoit Bay National Estuarine Research Reserve in Waquoit Bay in Falmouth, MA (6), a site with a large *P. longicarpus* population. The length of the treatment

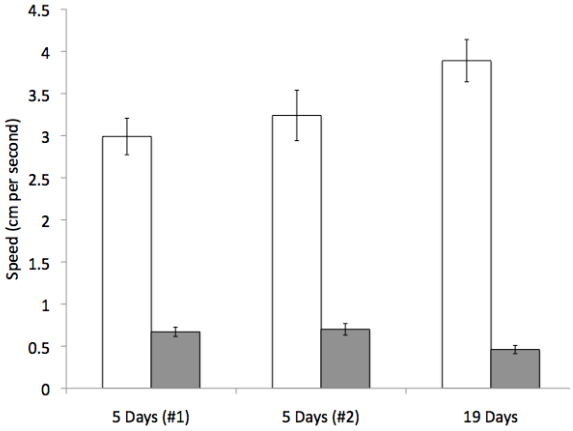

Figure 4: The mean speed (± SE) of hermit crabs after release in cm per second. The white columns indicate hermit crabs' speeds in tests without food; the gray columns indicate hermit crabs' speeds in tests with food.

trials (5 or 19 days) represents a small fraction of *P. longicarpus*'s potential lifespan of several years (7).

There were no obvious effects of pH on the distance that the hermit crabs traveled or the speed at which they traveled, with or without food. There were slight differences between the first and second 5-day treatment sets but these can be attributed to uncontrolled variables, such as the size of the crabs, the sex of the crabs, and slight unintentional variations in the handling method. However, there is quite a significant difference between the crabs' speed with food and their speed without food. Each group's average speed was always far higher with food than without food, which was expected and indicates that the crabs were likely attracted by the smell of the food. Thus, the food trials actually tested the crabs' food location ability rather than their behavior in general as was intended. The statistical analysis of the data does not indicate a discernible trend in the effect of the treatment pH levels on the food location behavior and locomotion of *P. longicarpus*.

The data do seem to suggest that decreased pH has an influence on the time it takes the hermit crabs to find the food. In both treatment sets of hermit crabs kept for 5 days, the hermit crabs' time to reach the food increased as the pH decreased. In both sets, the crabs kept in untreated "control" seawater were, on average, the quickest of the three groups to locate the food, while the crabs kept in seawater treated to pH 7.75 were the slowest to locate the food. However, there was no statistically significant difference between the groups with the conditions used in this set of experiments. Thus, additional experiments need to be performed to conclusively determine the effect of decreased pH levels on the crabs' food location ability.

Furthermore, decreased pH did not appear to affect the hermit crabs kept for 19 days. In the case of those groups, there was no obvious pattern. This may have been caused by a lack of control of several variables, including the ambient light, sex, and size of the crabs. Alternatively, the 19-day crabs may have simply adjusted to the new pH given that they were exposed for almost quadruple the length of time as the 5-day crabs. However, upon further inspection, only one group within the 19-day set was truly out of the ordinary. The crabs from the groups of pH 7.85 and untreated water took approximately the same average time to reach the food as their counterparts in the two 5-day treatment sets, and the decrease in time from the pH 7.85 group to the control group followed the same pattern as in the 5-day sets. The only group that did not follow the same pattern was the pH 7.75 group from the 19-day data set. This group had the quickest average time to reach the food of its set, and the time was much lower than either of the other pH 7.75 groups in the 5-day sets. The cause of the results for this outlying group is unknown.

The variability of the data may also reflect sources of error in the experiment, including the uncontrolled size and sex of the crabs and the location of the crabs' bins. Subsequent experiments would control for these factors, involve larger sample sizes, and use a computer program to accurately analyze the video. In addition, given that *P. longicarpus* is an intertidal crustacean and thus adapted to a wide variation in pH, it may take a more extreme drop in pH to effect its behavior. A recent study showed that the antennular flicking rate (*i.e.*, "sniffing") of P. bernhardus, an intertidal hermit crab in the UK, was significantly reduced at pH 6.8 compared to those in untreated seawater (1).

The exact method by which pH may affect animal senses is not known. However, there are many possible ways in which decreased pH could affect the food location abilities of *P. longicarpus*. Reduced pH could affect the food or the chemical signal itself, and the crab would be unable to detect an unfamiliar type of signal (1). It could also damage the hermit crab's chemoreceptors, preventing chemical signals from binding to the sites. The chemoreception mechanism of *P. longicarpus*, along with that of most other decapods, consists of the smaller of its two pairs of antennae. To 'sniff,' *P. longicarpus* flicks these antennules back and forth quickly through the water, enabling waterborne chemical cues to attach to the antennules' chemoreceptory sites (1). Nearly all crustaceans share this same chemoreceptory technique, and therefore, the crabs' reactions to decreased pH could be representative of other crustaceans. In addition, *P. longicarpus* is a calcifying organism, and thus, its exoskeleton formation may be affected by ocean acidification, which, in turn, may impair its locomotion or antennular flicking, a possibility that other studies have revealed (1). However, higher quality video than was used in this study would be needed to accurately determine

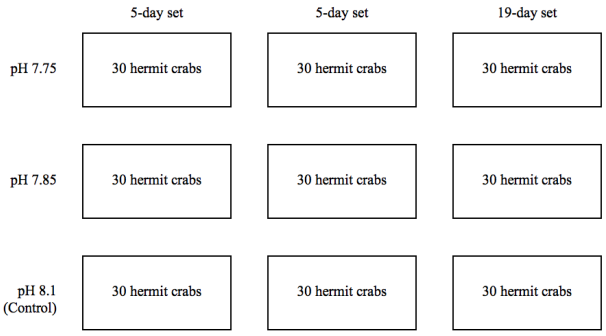

Figure 5: A basic diagram of the hermit crabs' bins.

the antennular flicking rate. Also, a reduction in pH could affect the crab's metabolism and activity level, thus influencing its ability to locate an odor source. Marine crustaceans are capable of regulating their body pH, but it is a process that requires a lot of energy that might normally have been used to locate an odor source (1). Since the crabs were not affected by the pH treatment levels examined in this study, the likely method by which pH may affect the crabs' food location behavior should be a topic for further research.

If decreased pH, such as that which would come with ocean acidification, does affect hermit crabs' food location behavior and locomotion, it could have serious ramifications on the survival of the crabs. However, the lack of a statistically significant trend seems to indicate that *P. longicarpus*, with its wide tolerance for daily fluctuations in pH, may place it and its fellow intertidal residents among the marine organisms best prepared to handle the decrease in pH that may eventually come with ocean acidification.

Methods

Long-clawed hermit crabs (*P. longicarpus*) were collected by hand from the intertidal zone just off the Woods Hole Yacht Club beach on Vineyard Sound in July 2011.

Crabs were chosen at random, with all but a few occupying Littorina littorea shells. After being collected, 270 crabs were kept in 3 buckets with a bubbler in each for approximately 3 hours. Then they were placed in their experimental bins at the Environmental Systems Laboratory at the Woods Hole Oceanographic Institution's Quissett campus in Woods Hole, MA.

The bins were small, lidless Tupperware containers with holes drilled in both ends to allow for seawater to flow through and drain out. They were secured in a concrete drainage trough in the floor of the laboratory. To ensure an adequate experimental supply in case of crab casualties, 30 hermit crabs were put in each of the 9 bins at the beginning of the experiment. Each bin was covered with fine plastic netting and placed under a metal grate. Pipes secured to the tops of the bins carried filtered seawater pumped from Vineyard Sound. On its way to the bins, the seawater passed through one of three inflatable plastic pools. In two of the pools, the water was treated with a Tunze carbon dioxide bubbler, which lowered the pH to the 2 different treatment levels. The bubbler measured the pH, and using that information, bubbled a certain amount of carbon dioxide into the water to maintain a set pH. The pH for the bins of treated water was checked and recorded every day during the experiment, along with the water temperature.

The crabs' bins were divided into 3 sets of 3 bins each (**Figure 5**). The water that ran through 3 bins, 1 bin from each set, was left untreated, maintaining a pH of approximately 8.10. Another bin from each set was treated to pH 7.85 using a carbon dioxide bubbler. The water that ran through the last 3 bins was treated to pH 7.75 with a carbon dioxide bubbler. The hermit crabs in 2 sets of bins were kept for 5 days, then tested; the hermit crabs from the other set were kept for 19 days, then tested.

The crabs were kept primarily in natural light and temperature conditions (ambient water temperature

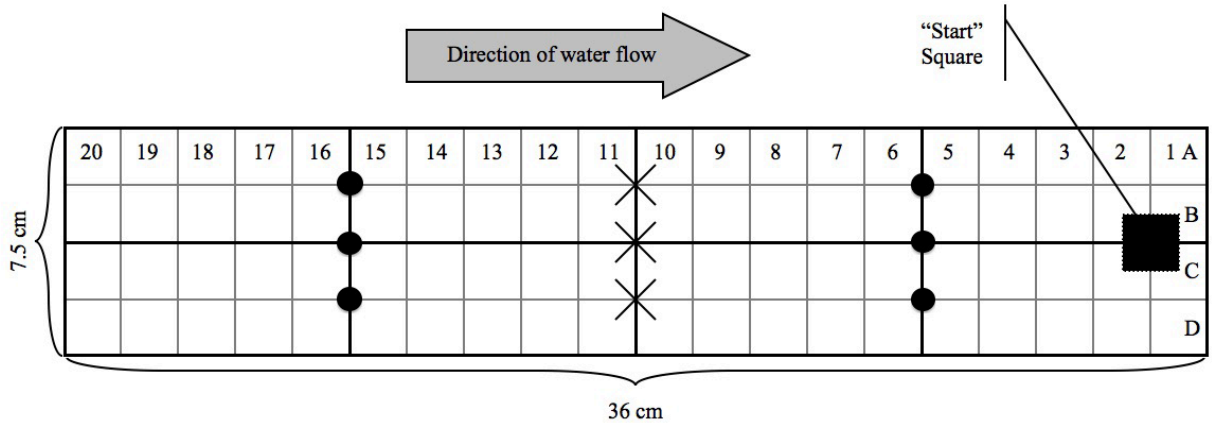

Figure 6: The bottom of the flume. For easy recognition of the quarter- and half-way points along the flume, they were marked with dots and Xs, respectively.

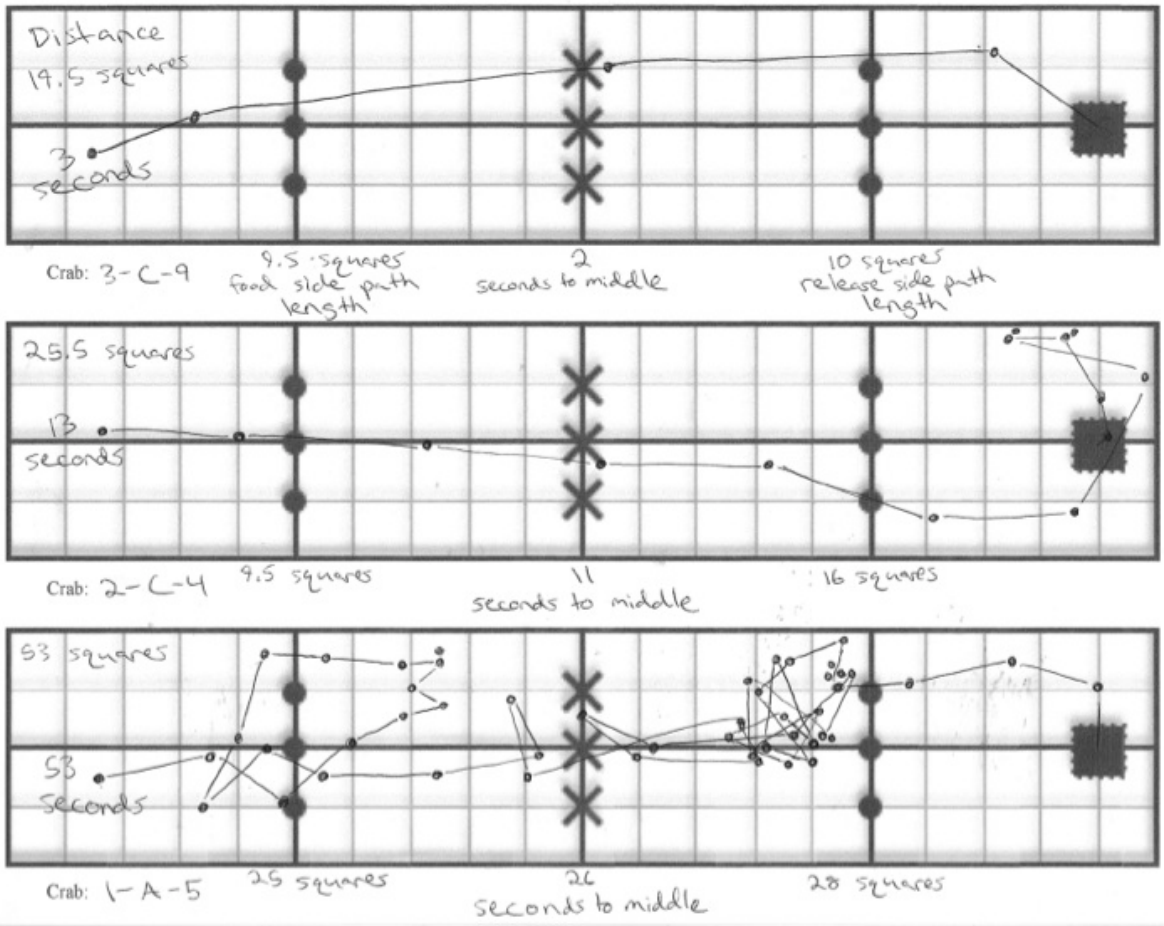

Figure 7: Examples of hermit crabs' paths. The top path is an example of a hermit crab that took a very short time to reach the food, and travelled along a short, straight path. The middle path is an example of a crab that took an average time to reach the food, travelling along a slightly longer and more crooked path. The bottom path is an example of a crab that took a very long time to reach the food, and travelled along a very long, complicated path with many changes in direction.

ranged from 22-23 °C). Each bin of crabs was fed 2.5 g of chopped, frozen clam every other day. The crabs were never fed the day before they were tested.

After the hermit crabs had been in the bins for their set treatment length (*e.g.*, 5 or 19 days), 15 hermit crabs were removed, one by one, from each bin, to be tested first without, then with, food. For the trial without food, the hermit crab was placed on a starting square at the downstream end of a Plexiglas flume. A plastic cup was immediately placed over the hermit crab. Water flowed through the flume at a rate of 2.5 cm per second, as determined by injecting dye into the flume prior to experimenting. The water flowing through the flume was of the same pH at which the hermit crab had been kept (**Figure 6**). After 15 seconds, the hermit crab was released from the cup and allowed to roam freely around the flume for 100 seconds. During this test, the hermit crab was recorded by an overhead video camera, as well as monitored manually; monitoring consisted of noting the hermit crab's position in the flume every five seconds and verbally relaying the information to a recorder. After 100 seconds, the hermit crab was returned to the starting square and covered with the cup in preparation for trial with food.

For the trial with food, a chopped-up silverside head of 0.8 grams was wrapped in plastic netting and then placed at the upstream end of the flume. After 15 seconds, the hermit crab was released from the cup and allowed to move freely again until it contacted the food, while being recorded by the camera and monitored manually, with a special notation made when the crab reached the food. If, after 100 seconds, the hermit crab did not contact the food, the trial was halted (this applied to only 7 of the 135 crabs tested). At the completion of each trial, the crab was removed from the flume, to be released later, and the flume was emptied and refilled. The food was replaced after every 5 crabs.

The manually recorded data was used to determine the time at which each hermit crab reached the food. The first time at which each crab was recorded feeding was taken as the amount of time that it took for the hermit crab to reach the food. Hermit crabs that never found

the food were assigned a time of 100 seconds for their time to reach the food. To find the path length for each hermit crab, the distance between each point at which the hermit crab was recorded was measured, and all the distances were added (**Figure 7**). To find the speed at which each hermit crab traveled, the time that the crab spent out of the cup in the flume was divided by the length of its path.

The videos of the crabs with food were used to determine the crabs' times to food, path lengths, and speeds with much more accuracy. The video of each hermit crab with food was watched, and the hermit crab's position was marked on a graph every second. The time to reach the food was determined to the nearest second, and the path length was measured with far more accuracy. The main results are based from this data collection. The data were then analyzed using an ANOVA test with the application JMP.

No crabs were injured during this experiment, except the occasional victim of cannibalism while the crabs were in their bins. Any instances of cannibalism were most likely due to the sudden increase in population density upon the crabs' removal from their relatively sparsely populated natural habitat. The pH values to which the water was adjusted are within the normal tolerances of these crabs, which typically experience much fluctuation in the water chemistry of their natural intertidal environment; our own pH measurements at the crabs' collection site indicated a daily pH range of approximately 7.8 to 8.2. The pH values used represent only levels that can reasonably be expected to be reached within the next two centuries.

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